ผลของสารเสริมสภาพพลาสติกต่อสมบัติฟิล์มสตาร์ชถั่วเขียวและการประยุกต์ใช้

นางสาวอรจิรา ร่มโพธิ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเทคโนโลยีทางอาหาร ภาควิชาเทคโนโลยีทางอาหาร
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2557

EFFECT OF PLASTICIZER ON PROPERTIES OF MUNG BEAN STARCH FILM AND ITS APPLICATION

Miss Onjira Rompothi

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Food Technology
Department of Food Technology
Faculty of Science
Chulalongkorn University
Academic Year 2014

Thesis Title	EFFECT OF PLASTICIZER ON PROPERTIES OF MUNG BEAN STARCH FILM AND ITS APPLICATION
By	Miss Onjira Rompothi
Field of Study	Food Technology
Thesis Advisor	Assistant Professor Pasawadee Pradipasena, Sc.D.
Thesis Co-Advisor	Theeranun Janjarasskul, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

....Dean of the Faculty of Science
(Professor Supot Hannongbua, Dr.rer.nat.)

THESIS COMMITTEE

....Chairman
(Associate Professor Jirarat Anuntagool, Ph.D.)
....Thesis Advisor
(Assistant Professor Pasawadee Pradipasena, Sc.D.)
....Thesis Co-Advisor
(Theeranun Janjarasskul, Ph.D.)
....Examiner
(Assistant Professor Chaleeda Borompichaichartkul, Ph.D.)
....External Examiner
(Nuttawee Niamsiri, Ph.D.)

อรจิรา ร่มโพธิ์ : ผลของสารเสริมสภาพพลาสติกต่อสมบัติฟิล์มสตาร์ชถั่วเขียวและการประยุกต์ใช้ (EFFECT OF PLASTICIZER ON PROPERTIES OF MUNG BEAN STARCH FILM AND ITS APPLICATION) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร.พาสวดี ประทีปะเสน, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: อ. ดร.ธีรนันท์ เจนจรัสสกุล, หน้า.

วัตถุประสงค์ของงานวิจัยนี้คือศึกษาผลของความเข้มข้นของสตาร์ช (ร้อยละ 3.5-5.0 โดยน้ำหนัก) และผลของชนิดและความเข้มข้นของสารเสริมสภาพพลาสติก ได้แก่ ซอร์บิทอล (ร้อยละ 10-60 โดยน้ำหนัก) และกลีเซอรอล (ร้อยละ 10-30 โดยน้ำหนัก) ต่อสมบัติของฟิล์มจากสตาร์ชถั่วเขียว ได้แก่ ความหนา ความต้านทานแรงดึง ร้อยละการยืด ร้อยละการละลายน้ำ การซึมผ่านของไอน้ำ การซึมผ่านของออกซิเจน ความแข็งแรงของรอยผนึก และสมบัติทางความร้อน อีกทั้งเพื่อหาความเข้มข้นของสารเสริมสภาพพลาสติกที่ทำให้ฟิล์มสามารถม้วนพับได้โดยไม่เกิดการแตกหัก การขึ้นรูปฟิล์มทำโดยการเตรียมสารละลายผสมของสตาร์ช สารเสริมสภาพพลาสติก และน้ำ แล้วให้ความร้อนที่ 85 องศาเซลเซียส เป็นเวลา 30 นาที จากนั้นนำแป้งเปียกมาเทลงบนแม่พิมพ์อะคริลิกแล้วทำให้แห้งในตู้อบลมร้อนที่อุณหภูมิ 35 องศาเซลเซียส เป็นเวลา 20 ชั่วโมง ฟิล์มจากสตาร์ชถั่วเขียวที่ได้มีลักษณะโปร่งแสง จากผลการทดลองพบว่า สำหรับฟิล์มที่ควบคุมชนิดและความเข้มข้นของสารเสริมสภาพพลาสติก เมื่อเพิ่มความเข้มข้นของสตาร์ชมีผลทำให้ความหนาเพิ่มขึ้น และการซึมผ่านของไอน้ำลดลงอย่างมีนัยสำคัญ ($p \leq 0.05$) แต่ไม่ส่งผลต่อความต้านทานแรงดึง ร้อยละการยืด ร้อยละการละลายน้ำ และความแข็งแรงของรอยผนึก อย่างไรก็ตาม ฟิล์มที่แต่ละความเข้มข้นของสตาร์ช การเพิ่มความเข้มข้นของสารเสริมสภาพพลาสติกทำให้ความต้านทานแรงดึงลดลง แต่ความหนา ร้อยละการยืด ร้อยละการละลายน้ำ การซึมผ่านของไอน้ำ และความแข็งแรงของรอยผนึกมีค่าเพิ่มขึ้นอย่างมีนัยสำคัญ ($p \leq 0.05$) แต่ไม่ผลต่อการซึมผ่านของออกซิเจน ในขณะที่ทุกความเข้มข้นของสตาร์ชพบว่าความเข้มข้นเริ่มต้นของกลีเซอรอลที่ทำให้ความต้านทานแรงดึง และร้อยละการยืดของฟิล์มเปลี่ยนแปลงอย่างมีนัยสำคัญ ($p \leq 0.05$) นั้นมีค่า ≥ ร้อยละ 10 และ 20 โดยน้ำหนัก ตามลำดับ ในขณะที่ความเข้มข้นเริ่มต้นของซอร์บิทอลที่ทำให้ความต้านทานแรงดึงและร้อยละการยืดของฟิล์มเปลี่ยนแปลงนั้นมีค่า ≥ ร้อยละ 30 และ 40 โดยน้ำหนัก ตามลำดับ จากสมบัติเชิงกลของฟิล์มแสดงว่ากลีเซอรอลเป็นสารเสริมสภาพพลาสติกที่มีประสิทธิภาพมากกว่าซอร์บิทอล สำหรับฟิล์มที่มีซอร์บิทอลพบว่ามีค่าความต้านทานแรงดึงมากกว่า แต่มีค่าความหนา ร้อยละการยืด การซึมผ่านของไอน้ำ การซึมผ่านของออกซิเจน และความแข็งแรงของรอยผนึกน้อยกว่าฟิล์มที่มีกลีเซอรอล ในขณะที่ความเข้มข้นของสารเสริมสภาพพลาสติกเท่ากับร้อยละ 30 โดยน้ำหนักฟิล์มที่มีซอร์บิทอลมีค่าร้อยละการยืด และความแข็งแรงของรอยผนึกน้อยกว่าฟิล์มที่มีกลีเซอรอล จากการศึกษาสมบัติทางความร้อนพบว่าฟิล์มจากสตาร์ชถั่วเขียวมีพีคดูดพลังงานที่มีอุณหภูมิเริ่มต้นในช่วง 48.00-63.50 องศาเซลเซียส และมีอุณหภูมิที่จุดพีคในช่วง 59.35-70.05 เซลเซียส ฟิล์มที่มีกลีเซอรอลหรือซอร์บิทอลที่ความเข้มข้น ≤ ร้อยละ 10 และ 30 โดยน้ำหนัก ตามลำดับ มีลักษณะเปราะ ดังนั้นเพื่อขึ้นรูปให้ได้ฟิล์มที่สามารถม้วนพับได้โดยไม่เกิดการแตกหักควรใช้ความเข้มข้นของกลีเซอรอลหรือซอร์บิทอลที่มีค่า ≥ ร้อยละ 20 หรือ 40 โดยน้ำหนัก ตามลำดับ จากนั้นศึกษาการนำไปใช้โดยบรรจุพริกป่นในถุงจากฟิล์มสตาร์ชถั่วเขียว (สตาร์ชร้อยละ 5 โดยน้ำหนัก และซอร์บิทอลร้อยละ 40 โดยน้ำหนัก) และถุงโพลิโพรพิลีนโดยดูผลของสภาวะการเก็บต่อสมบัติทางกายภาพ (ปริมาณน้ำอิสระ ความชื้น และการเปลี่ยนแปลงสี) ปริมาณฟีนอลิกทั้งหมด และฤทธิ์ต้านออกซิเดชันด้วยวิธี DPPH และ FRAP ของพริกป่นโดยเก็บที่ความชื้นสัมพัทธ์ ร้อยละ 50 ในอุณหภูมิ 35, 45 และ 55 องศาเซลเซียส เป็นเวลา 50 วัน พบว่า เมื่อระยะเวลาการเก็บเพิ่มมากขึ้น ปริมาณน้ำอิสระ ความชื้น และค่าการเปลี่ยนแปลงสีของพริกป่นมีค่าเพิ่มขึ้นอย่างมีนัยสำคัญ ($p \leq 0.05$) ที่ทุกอุณหภูมิ โดยการเปลี่ยนแปลงทางจลนพลศาสตร์ของปริมาณน้ำอิสระและความชื้นเป็นไปตามกฎอัตราการเกิดปฏิกิริยาอันดับหนึ่ง ในขณะที่ค่าการเปลี่ยนแปลงสี ปริมาณฟีนอลิกทั้งหมด และฤทธิ์ต้านออกซิเดชันเป็นไปตามกฎอัตราการเกิดปฏิกิริยาอันดับศูนย์ เมื่อเปรียบเทียบระหว่างถุงโพลิโพรพิลีนและถุงจากฟิล์มสตาร์ชถั่วเขียว พบว่า ถุงโพลิโพรพิลีนมีสมบัติต้านการซึมผ่านของไอน้ำดีกว่าถุงจากฟิล์มสตาร์ชถั่วเขียว นอกจากนี้ค่าคงที่ของอัตราการเปลี่ยนแปลงสี ปริมาณฟีนอลิกทั้งหมด และฤทธิ์ต้านออกซิเดชันของพริกป่นที่บรรจุในถุงจากฟิล์มสตาร์ชถั่วเขียวมีค่าต่ำกว่าที่บรรจุในถุงโพลิโพรพิลีนยกเว้นการเปลี่ยนแปลงฤทธิ์ต้านออกซิเดชันของพริกป่นที่เก็บที่ 55 องศาเซลเซียส ซึ่งวิเคราะห์ด้วยวิธี DPPH นอกจากนี้การเพิ่มอุณหภูมิการเก็บส่งผลให้ปริมาณฟีนอลิกทั้งหมด และฤทธิ์ต้านออกซิเดชันด้วยวิธี DPPH และ FRAP ของพริกป่นมีค่าลดลงอย่างมีนัยสำคัญ ($p \leq 0.05$) โดยสรุป ถุงจากฟิล์มสตาร์ชถั่วเขียวมีศักยภาพในการบรรจุพริกป่นดีกว่าถุงโพลิโพรพิลีน

ภาควิชา เทคโนโลยีทางอาหาร

สาขาวิชา เทคโนโลยีทางอาหาร

ปีการศึกษา 2557

ลายมือชื่อนิสิต ______________________________

ลายมือชื่อ อ.ที่ปรึกษาหลัก ______________________________

ลายมือชื่อ อ.ที่ปรึกษาร่วม ______________________________

5572170723 : MAJOR FOOD TECHNOLOGY

KEYWORDS: MUNG BEAN STARCH / GLYCEROL / SORBITOL / MECHANICAL PROPERTIES / BIOPOLYMER FILM

ONJIRA ROMPOTHI: EFFECT OF PLASTICIZER ON PROPERTIES OF MUNG BEAN STARCH FILM AND ITS APPLICATION. ADVISOR: ASST. PROF. PASAWADEE PRADIPASENA, Sc.D., CO-ADVISOR: THEERANUN JANJARASSKUL, Ph.D., pp.

This research determines the effects of starch concentration (3.5-5.0 %w/w) and plasticizer type and concentration [sorbitol (10-60 %w/w) or glycerol (10-30 %w/w)] on thickness, tensile strength (TS), percent elongation (%E), % solubility, water vapor permeability (WVP), oxygen permeability (OP), seal strength and thermal properties of mung bean starch (MBS) films and to determine the onset of the plasticizer concentration at which film can be formed without cracking. Films were prepared by mixing starch, plasticizer and water and heating at 85 ºC for 30 min. The paste was poured over the acrylic plate, and dried in a hot air oven at 35 ºC for 20 hr. MBS films were translucent. In general, increasing starch concentration significantly increased thickness, decreased % solubilityand WVP but did not affect TS, %E and seal strength of the films at controlled plasticizer type and concentration ($p \leq 0.05$). At controlled starch concentration, increasing the plasticizer concentration decreased TS and OP but increased thickness, %E, % solubility, WVP and seal strength ($p \leq 0.05$) of films. In this research, glycerol showed better plasticizer efficiency than sorbitol as shown by the onset plasticizer concentration that significantly change TS and %E of MBS film. The onset glycerol concentration for TS and %E were > 10 and > 20 %w/w at all controlled MBS concentration, respectively. The onset sorbitol concentration for TS and %E were > 30 and > 40 %w/w, respectively. Films containing sorbitol had higher TS but lower thickness, %E, WVP, OP and seal strength than films containing glycerol. At 30 %w/w plasticizer concentration, film containing sorbitol had lower %E and seal strength than film containing glycerol. According to differential scanning calorimetry study, MBS films exhibited a single endothermic peak with onset temperature (T_o) ranged between 48.00-63.50 ^{o}C and peak temperature (T_p) between 59.35-70.05 ^{o}C. Films having the glycerol or sorbitol concentration ≤ 10 or ≤ 30 %w/w were brittle. The glycerol or sorbitol concentration of ≥ 20 and ≥ 40 %w/w, respectively, was required to form flexible film for wrapping without breaking. To study the effects of storage condition on physical properties [water activity (a_w), moisture content (%MC) and total color differrence (ΔE)] and antioxidant capacity[total phenolic content (TPC), 2,2-diphenyl-1-picrylhydrazyl(DPPH) radical scavenging activity and ferric reducing antioxidant power (FRAP)], dried chili powder which packed inside MBS sachet (5 %w/w starch : 40 %w/w sorbitol) and polypropylene (PP) sachet were stored at 50 % RH and 35, 45 and 55 °C for 50 days. The a_w, %MC and ΔE of dried chili powder in all sachets tended to increase over storage time at all temperatures. The kinetics of changes in a_w and % MC followed the first-order rate law, while the kinetics of changes in other properties were best fitted by the zero-order model. PP sachet showed a higher moisture barrier ability compared to MBS sachet. The rate constants (k) for changes in color, TPC, DPPH and FRAP of dried chili powder packed in MBS sachets were lower than those packed in PP sachets, except for DPPH at 55 °C. For the effect of storage temperature, TPC, DPPH and FRAP of dried chili powder packed in MBS and PP sachets decreased as the temperature increased. Overall, MBS sachet was a better package for dried chili powder than PP sachet.

Department:	Food Technology	Student's Signature	
Field of Study:	Food Technology	Advisor's Signature	
Academic Year:	2014	Co-Advisor's Signature	

ACKNOWLEDGEMENTS

I would like to express my great appreciation to my advisor, Assist. Prof. Dr. Pasawadee Pradipasena and my co-advisors, Dr. Theeranun Janjarasskul for their suggestions, support and encouragement during the planning, execution and writing of this research. I would also like to thank my thesis examiners, Assoc. Prof. Dr. Jirarat Anuntagool, Assist. Prof. Dr. Chaleeda Borompichaichartkul and Dr. Nuttawee Niamsiri for their valuable guidance.

This research was funded by The 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund) and National Research Council of Thailand (under the project entitled “Functional Biodegradable Film from Natural Polymers”).

Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University is also acknowledged for permeability measurements.

I would like to thank all staffs and friends at Department of Food Technology, Faculty of Science, Chulalongkorn University for their help and support.

My special gratitudes are extended to my father, mother and sisters for their care, morale and encouragement.

CONTENTS

Page

Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER I
INTRODUCTION

Development of film from polymers is one of the most interesting research topics in the food packaging area. Since most of biopolymers are nontoxic, renewable and biocompatible. Casting of biopolymer films is considered as green process and biopolymer films are completely biodegradable.

The starch-based films have been reported to be low in oxygen permeability (especially film casted from high amylose starch) and cost effective (Liu, 2005). The unplasticized and plasticized films casted from cereal and root starches have been developed and studied for their properties extensively, while studies on films casted from legume starches and their properties are limited. Mung bean (*Vigna radiata*) starch has a high amylose content of 30-45 % and can forms good gel, which in turn gives good film-forming properties (Bae *et al.*, 2008; Hoover *et al.*, 1997; Li *et al.*, 2011; Sandhu and Lim, 2008; Thao and Noomhorm, 2011). The gelatinized mung bean starch gives a transparent gel upon cooling resulting in a transparent film. The brittleness of starch film can be improved by plasticizing with polyols. The hydrophilic nature of starch and polyols results in high water solubility and permeability to water vapor (Janjarasskul and Krochta, 2010b). To develop a safe food contact and heat sealed wrapping film from mung bean starch (MBS film), this research aims to determine

1. the effects of starch concentration and plasticizer type and concentration on the tensile strength (TS), % elongation (%E), % solubility, seal strength, water vapor permeability (WVP), oxygen permeability (OP) and thermal properties of mung bean starch films,
2. the onset of the plasticizer concentration at which film can be formed without cracking,
3. the ability of MBS film to prolong antioxidant activity of dried chili powder (a model for antioxidant food ingredients) by determination the effects of storage time and temperature on

a. physical properties: water activity (a_w), moisture content (%MC) and color (ΔE),
b. total phenolic content (TPC), and
c. antioxidant activity: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and ferric reducing antioxidant power (FRAP)

of dried chili powder packed in sachet of MBS film compared to those packed in polypropylene (PP) sachet.

CHAPTER II
LITERATURE REVIEW

2.1 Biopolymer

As defined by the council of the IUPAC (International Union of Pure and Applied Chemistry), biopolymers are macromolecules (including proteins, nucleic acids and polysaccharides) formed by living organisms. Like any polymers, biopolymers contain monomeric units that are covalently bonded to form larger structures. Biopolymers can be classified according to the monomer type into 7 categories as following (Elias, 1997; Guilbert *et al.*, 1996; Karlsson and Albertsson, 1998; Vieira *et al.*, 2011):

1) Nucleic acid is from nucleotide monomers linked by phosphate diester linkage. Ribonucleic acids (RNA) and deoxyribonucleic acids (DNA) are nucleic acid exist in all living organisms.

2) Protein is from amino acid monomers linked by peptide bonds. Protein consists of one or more long chains of amino acid residues. Casein, whey and collagen/gelatin are examples of protein from animals. Zein, soya and gluten are common plant derived proteins.

3) Lignin, which is commonly derived from wood, is a complex polymer of aromatic alcohol or monolignol.

4) Polysaccharide is from monosaccharides linked by glycosidic bonds. For examples, starch and pectin are plant polysaccharides, while chitosan is polysaccharide derived from shell fishes. Polysaccharide can be homoglycan or heteroglycan. Homoglycan composes of only one kind of monosaccharide such as starch is polymer of D-glucose monomer. Heteroglycan composes of two or more different kinds of monosaccharide such as konjac glucomannan is polymer of D-mannose monomer and D-glucose monomer.

5) Polyester is a polymer with ester and lactone monomers. Poly(hydroxybutyrate) or PHB is polyhydroxyalkanoates (PHA) which is polyester produced by bacteria. Polylactic acid or polylactide (PLA) is also polyester derived from agro-products such as starch or sugarcane.

6) Polyisoprene or natural rubber is from isoprene monomer and is produced by plants.

7) Lipid, from biological origin, is entirely or in part from two types of biochemical monomers, ketoacyl monomer and isoprene monomer. Therefore, lipids may be classified into 2 classes. One is derived from condensation of ketoacyl monomer including fatty acids, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids, and polyketides. Another is derived from condensation of isoprene monomer including sterol lipid (e.g. cholesterol) and prenol lipid (e.g. carotenoid).

Chemical structures of monomers for these biopolymers are presented in Figure 2.1. All biopolymers are biodegradable polymers from renewable resources (Nussinovitch, 2013). Biodegradable polymer is defined by IUPAC as "polymer susceptible to degradation by biological activity, with the degradation accompanied by a lowering of its molar mass". Biological activities for degradation of polymers can be the action of microorganisms and/or enzymes. In this case polymers change into environmentally acceptable substances. A complete degradation results in either fully oxidized or reduced simple molecules such as CO_2, water, methane and some biomass residue (Steuteville, 1993). Some petroleum-based polymers, whose both monomers and polymers are produced by chemical synthesis from fossil resources are also biodegradable polymers (Vieira *et al.*, 2011). These are polycaprolactones (PCL), polyesteramides (PEA), aliphatic co-polyesters [e.g. poly(butylene succinate)-co-(butylene adipate) or PBSA] and aromatic co-polyesters [e.g. poly(butylene adipate-co-terephthalate) or PBAT]. Thus, biopolymers are often used as film forming materials for biodegradable films. The obtained films are called biopolymer films to indicate that they are not made from the petroleum-based biodegradable polymers. Antimicrobials, antioxidants, nutrients, colorants are easy to be added into biopolymer films, and thus further enhancing their functions (Bourtoom, 2008). Biopolymer films from polysaccharides (such as starch and pectin) and proteins (such as casein and gelatin) are edible and are referred to as 'edible film' (Janjarasskul and Krochta, 2010b). Most synthetic films from petrochemical-based polymers are non-biodegradable leading to environmental pollution and serious ecological problems except for which are made from the aforesaid petroleum-based biodegradable polymers (Tharanathan, 2003).

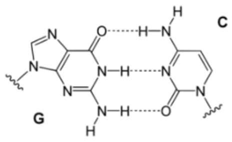
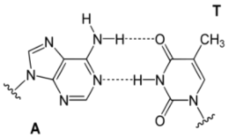
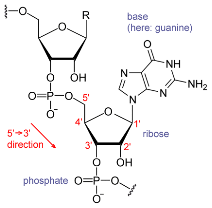
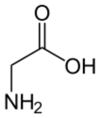
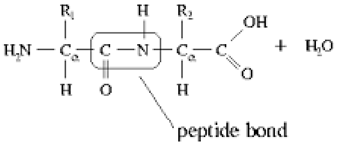
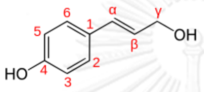
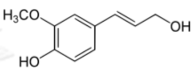
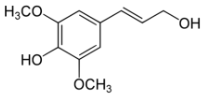
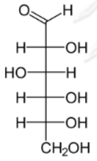
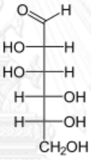
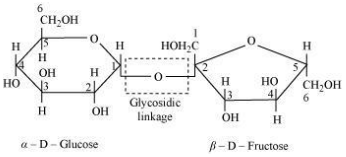
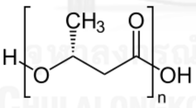
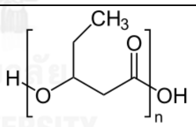
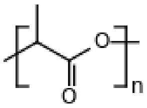
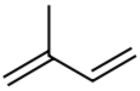
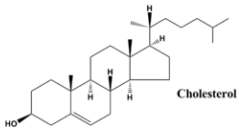
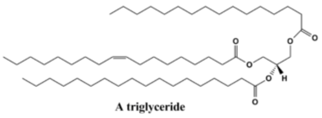
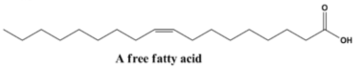
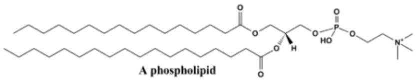
Biopolymers	Monomer Chemical Structures
Nucleic acid	 GC base pair  AT base pair  RNA
Protein	 Glycine  Peptide bond
Lignin	 Paracoumaryl alcohol  Coniferyl alcohol  Sinapyl alcohol
Polysaccharide	 D-Glucose  D-Mannose  Glycosidic bond
Polyester	 Poly-3-hydroxybutyrate  Polyhydroxyvalerate  Polylactic acid
Polyisoprene	 Isoprene
Lipid	 Cholesterol  A triglyceride  A free fatty acid  A phospholipid

Figure 2.1 Chemical structures of some monomers for biopolymer.
Source: Nussinovitch (2013)

2.2 Preparation of films from biopolymers

Biopolymer films can be formed via either a “wet/solvent process” or a “dry process” (Nussinovitch, 2013). A “wet/solvent process”, also called “casting”, is often used for making biopolymer films (Wolff *et al.*, 1951). For this process, biopolymers (film forming materials) and other ingredients (such as plasticizers, crosslinking agents, and emulsifiers) are solubilized or homogeneously dispersed in solvents to obtain the film-forming solution. For biopolymer, solvents are water, alcohol and mixtures of them (Vieira *et al.*, 2011). Then film-forming solution is applied to surfaces by pouring/spreading, brushing, spraying or dipping. Next, solvent is eliminated by drying and a film structure forms (Gontard and Guilbert, 1994). For “dry process”, the solvent free film-forming materials are heated in compression mold, injector or extruder to obtain temperature above their melting point, so they flow. The film-forming materials that can be used in dry process have to have the thermoplastic behavior (Guilbert *et al.*, 1997). The biopolymer with thermoplastic behavior is a polymer that becomes pliable or moldable above a specific temperature and solidifies upon cooling. Some proteins (such as casein and soy protein) and polysaccharide (such as starch) exhibit this behavior at low moisture content (Guilbert and Gontard, 2005; Nussinovitch, 2009; Nussinovitch, 2013; Vartiainen *et al.*, 2014).

Wet process is often used for making biopolymer film. Generally for this process, phase separation of incompatible ingredients from the film-forming solution should be avoid to obtain homogeneous film for desirable appearance and properties, except for the phase separation is intentionally designed for the formation of multilayer film structure. The solvent compatibility of these ingredients is very important to homogeneously dissolve or disperse in the solvent and to develop homogeneous film. For making edible and biodegradable films, the appropriate solvents are only water, ethanol and their mixtures. During drying step, the solvent evaporates, the biopolymer chains interpenetrate and gel forms. Interpenetration of biopolymer chains occurs at a specific concentration which is the reciprocal of their intrinsic viscosity in the solvent used (Han, 2014). The longer chains in solvent, which biopolymers dissolve better, result in the higher intrinsic viscosity. Therefore, this concentration, that the biopolymer chains interpenetrate, is lower. Thus, gel forms faster. Further drying results in forming of film structure. For starch-based film-forming solution, it starts to form gel upon cooling after starch gelatinization (Leloup *et al.*, 1992). The rate of solvent evaporation is critical parameter in the film formation process. Very fast evaporation rate can cause a skin to form on the top of surface of the film-forming solution, thus hindering evaporation of the solvent below (Felton, 2013). It can also cause cracking and warping of the film as well as leave concentric drying marks on the films (Liu, 2005). Factors influencing rate of solvent evaporation are temperature, atmospheric pressure, air movement and in the case of water, relative humidity (Urban and Takamura, 2002). Over-drying causes the films from becoming too brittle (Liu, 2005).

Interactions between the interpenetrating biopolymer chains produce strong cohesive and less flexible films. Plasticizers are required to a decrease in intermolecular forces along polymer chains, which enhances flexibility, decreases brittleness and avoids shrinking of the films (Bordes *et al.*, 2009; Guilbert *et al.*, 1996; Markarian, 2008). In contrast, formation of chemical linkages between biopolymer chains is required to enhance network structure and mechanical properties of films. In this case, crosslinking agents are added to the film-formation solution. For production of antimicrobial films, emulsifiers are essential film additive for the formation of a stable oil-in-water film-formation emulsion when oil-soluble antimicrobial agents are added in the film-formation solution (Krochta, 2002).

2.3 Plasticizer

Plasticizer, defined IUPAC is a “substance or material incorporated in a material (usually a plastic or an elastomer) to increase its flexibility, workability, or distensibility”. Plasticizers used in this study are categorized as external plasticizers, which are low volatility compounds added in film-forming solution. They do not chemically react but interact with film-forming material. For biopolymer films, both hydrophilic plasticizers and hydrophobic plasticizers can be used. Using hydrophilic plasticizer in high concentration can increase water diffusion through biopolymer films. Hydrophobic plasticizers decrease water uptake of biopolymer film as they can close the micro-voids in the film. Plasticizer concentration exceeding its compatibility limit with the biopolymer can cause phase separation. Compared to hydrophilic plasticizer, hydrophobic plasticizer is easier to cause phase separation resulting in losses of flexibility and continuity of films (Vieira *et al.*, 2011; Wojciechowska, 2012). Criterions of the plasticizer selection for biopolymer films are non-toxicity, compatibility with film-forming material, no taste and odor, effect on mechanical properties of film, high boiling point, biodegradable, leaching or migration during use or aging, cost and availability. For edible film, it has to be FDA approved food additive (Lacroix, 2009; Sothornvit and Krochta, 2005). For biopolymer films, polyols, monosaccharides, disaccharides, oligosaccharides, lipids, lipid derivatives and citrate ester are used as plasticizers (Embuscado and Huber, 2009). The most often used of polyols are glycerol, sorbitol, ethylene glycol (EG), propylene glycol (PG), diethylene glycol (DEG), triethylene glycol (TEG), and polyethylene glycol (PEG) (Wojciechowska, 2012).

Four theories have been proposed for plasticizing mechanism (Mekonnen *et al.*, 2013; Wojciechowska, 2012).

1. The lubricity theory

   The plasticizer acts as a lubricant to reduce intermolecular friction between polymer chains and facilitates mobility of polymer chains, consequently more flexible, softener and distensible polymer network.

2. The gel theory

   This theory suggests that a plasticizer attaches along the polymer chain and reduces polymer-polymer interaction (hydrogen bonds, van der Waals or ionic forces, etc.). These result in an increase in the space between polymer chains, thus increasing the flexibility of polymer gel.

3. The free volume theory

   The free volume is determined from the internal space available in a polymer for the movement of chains and is the difference between the observed volume at absolute zero and the volume measured at a selected temperature. Plasticizer increases the free volume of polymer material, thus lowering the glass transition temperature.

4. The mechanistic theory

   Plasticizers are not bound permanently to the polymer. Plasticizers are free to self-associations and to associate with polymer chain at certain sites and then disassociate. These interactions are weak, therefore, there is a dynamic exchange process whereby, as one plasticizer molecule becomes attached at a site and is rapidly detached, then is replaced by another. At low plasticizer concentration, plasticizer-polymer interactions are the dominant interactions thus causing antiplasticization. Plasticizer-plasticizer interactions predominate at high plasticizer concentration. Furthermore, a white residue developed on the surface of film can referred to as ‘‘blooming’’ or ‘‘blushing’’. This occurs when the plasticizer concentration above a critical concentration which exceed the compatibility limit with the biopolymer, and phase separation with plasticizer exclusion is usually observed (Muscat *et al.*, 2012; Talja *et al.*, 2007; Yang and Paulson, 2000).

2.4 Properties of biopolymer films

The use of films depends mainly on their mechanical properties, strength and flexibility, and barrier properties, water vapor and oxygen permeabilities (Guilbert *et al.*, 1996; Urban and Takamura, 2002). Films are subjected to force when they are used. These forces can deform (elongate) and break films. Film should be flexible and not crack during handling and wrapping. Cracking of the films leads to loss of barrier properties. Therefore, knowing how films elongate and break as a function of applied force (force-deformation or stress-strain behavior) is required for their applications. Force-deformation behavior (stress-strain curve) can be determined by applying force to pull film and measuring an increase in the corresponding length of the film. This test can be carried out using Universal Testing Machine as shown in Figure 2.2. Figure 2.3 shows the typical stress-strain curve for tensile testing. According to ASTM (2002), ultimate tensile strength (often shortened to tensile strength, TS) is the maximum stress that the film can withstand while being pulled before breaking. While % elongation (%E) is the % strain at the point when the film broken.

Figure 2.2 Instron Universal Testing Machine
Source: Gharagozlou (2014)

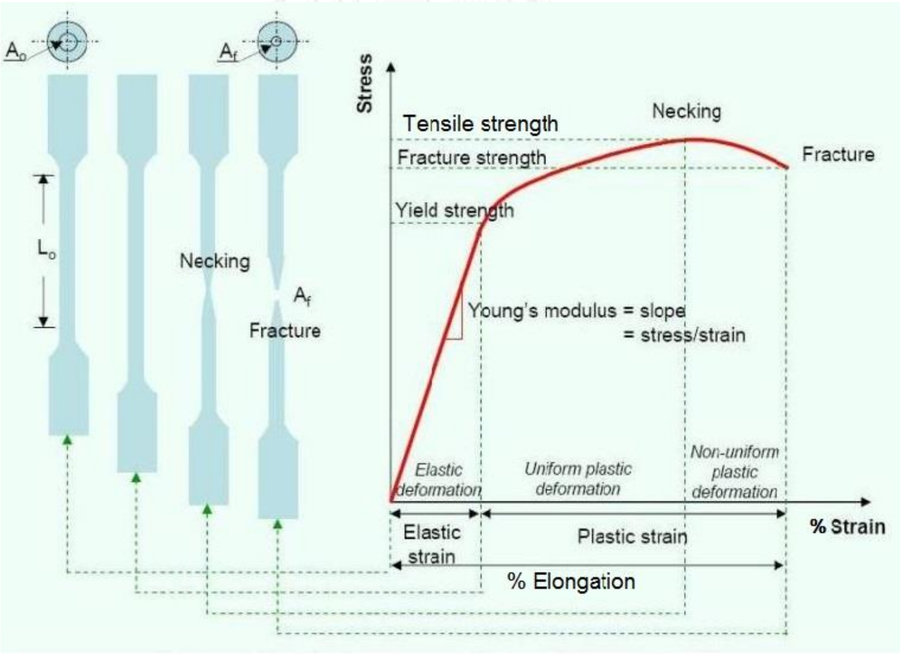


Figure 2.3 Stress-strain curve
Source: modified from Shah (2008)

For food industries, the main application of film is to prolong shelf life of food by maintaining transmission rate of various gases and liquids and preventing contamination (Wojciechowska, 2012). Mass transfer phenomena, such as moisture absorption and oxygen invasion, cause deterioration of food qualities (Kester and Fennema, 1986; Krochta, 2002; Miller and Krochta, 1997). For crispy food, a minor increase in moisture content leads to loss of crispness. Long term moisture absorption causes an increase in water activity, thereby enhancing the growth of micro-organisms. Oxygen penetration into foods enhances oxidation of food ingredients (Wareing *et al.*, 2011). These phenomena can be prevented by wrapping food with film. Thus, water vapor permeability and oxygen permeability are very important properties of films. Water vapor permeability (WVP) is defined as the time rate of water vapor transmission through unit area of flat material of unit thickness induced by unit vapor pressure difference between two specific surfaces, under specified temperature and humidity conditions (ASTM, 1995b). Oxygen permeability (OP) is defined as the time rate of oxygen transmission through unit area of flat material of unit thickness induced by oxygen partial pressure difference between two specific surfaces, under specified temperature and humidity conditions (ASTM, 1995a; Hong and Krochta, 2003).

From Table 2.1, film should have TS of ≥ 1 MPa, E of ≥ 1 %, WVP of ≤ 10 g mm/m^2 d kPa and OP of ≤ 1000 cm^3 μm/m^2 d kPa. TS of some protein-based films containing glycerol, such as whey protein isolate (WPI) and zein (Z), was 10 to 100 MPa which was comparable to that of propolypropylene and polystyrene. While some polysaccharide-based films, such as methylcellulose (MC), hydroxypropyl methylcellulose (HPMC) and amylose, had TS > 100 MPa, therefore, they are classified in the same class of oriented polypropylene (OPP), polyvinylidene chloride (PVDC) and polyvinylidene chloride (PET) (Han and Gennadios, 2005). For unplasticized starch films, a continuous increase in tensile strength (30-70 MPa) and elongation (4-6%) was observed as amylose increased from 0 to 100% (Lourdin *et al.*, 1995). It was found that %E of these protein-based and amylose-based films was 10-100 %, which was similar to that of OPP and PET. Protein- glycerol films from egg white protein (EWP), WPI, Z and soy protein isolate (SPI), and starch-based films from corn and potato had WVP > 1 g mm/m^2 d kPa (Muscat *et al.*, 2012; Talja *et al.*, 2007). The water vapor permeability of protein or polysaccharide-based films is generally higher than that of synthetic films. These are due to the hydrophilic nature of proteins and polysaccharides. Protein- glycerol films from Z, WPI and EWP, had

OP of 100 to 10 $\mathrm{cm^3\ \mu m/m^2}$ d kPa, which is similar to polyester. Starch-glycerol film from high-amylose pea starch was reported to have OP < 10 $\mathrm{cm^3\ \mu m/m^2}$ d kPa like ethylene vinylalcohol and PVDC. Therefore, starch films can be excellent oxygen barriers (Han and Gennadios, 2005).

Table 2.1 Classification of films according to their properties.

Properties	Classification			
	Poor	Marginal	Good	Superior
Mechanical				
• Tensile in MPa	< 1	1-10	10-100	> 100
• Elongation in %	< 1	1-10	10-100	> 100
Barrier				
• WVP in g $\mathrm{mm/m^2}$ d kPa (in mg mm/ $\mathrm{m^2}$ hr Pa)	> 10 (> 0.42)	10-1 (0.42-0.042)	1-0.1 (0.042-0.0042)	< 0.1 (< 0.0042)
• OP in $\mathrm{cm^3\ \mu m/m^2}$ d kPa	> 1000	1000-100	100-10	< 10

Source: modified from Han and Gennadios (2005)

It can be concluded that starch-based films possess good mechanical and gas barrier properties (Baldwin, Nisperos-Carriedo, *et al.*, 1995) also, they provide an efficient barrier against oils and lipids but poor against water vapor as an effect of their hydrophilic functional structure (Janjarasskul and Krochta, 2010a). Starch-based films are sensitive to humidity, and are soluble in water (Vachon *et al.*, 2000). As for good barrier properties, film should exhibit low water-solubility. However, high water-solubility biopolymer films can be degraded by biological activity. Polymer–polymer interactions via hydroxyl and carbonyl (H-bonds or covalent reaction) moieties can enhance film insolubility (Embuscado and Huber, 2009; Salmieri and Lacroix, 2006; Wang *et al.*, 2001).

Sealing of film is also required to prevent water vapor and oxygen transfer. Heat is widely used to join two films presented together between heated plates or dies (Mueller *et al.*, 1998). The surface of the crystalline polymer melts during heating. With applied pressure during heating, interfacial interactions occur across joint surfaces to seal adjoining pieces of film. Upon cooling, polymers recrystallize resulting in a formation of heat-sealed joint (Kim and Ustunol, 2001). Seal strength is a measure of the ability of a film seal to resist separation. According to Kim and Ustunol (2001), hydrogen bonds may be the main forces responsible for sealed joint formation in whey protein isolate films. (Embuscado and Huber, 2009)

Relative humidity (RH) is also an important variable affecting the mechanical properties of biopolymer films. At higher RH, their physical strength is lower than that at lower RH, since absorbed moisture functions as a plasticizer (Guilbert *et al.*, 1997). Oxygen permeability increases as RH increases. Therefore, it is very important to maintain low RH environments to maximize the effectiveness of biopolymer films as oxygen barriers (Guilbert *et al.*, 1997; Maté and Krochta, 1998). At high RH, starch has high swelling capacity and high chain mobility resulting in more water sorption and diffusion in starch matrix, thus water diffusion rate was higher (Wittaya, 2012).

Temperature is also an important factor affecting the mechanical and barrier properties of biopolymer films. The physical strength of materials dramatically decreases as temperature increases above the glass transition temperature (Guilbert *et al.*, 1996; Miller and Krochta, 1997; Wu *et al.*, 2002). Futhermore, Temperature is also an important factor of migration (Guilbert *et al.*, 1997; Wu *et al.*, 2002). An increase in temperature provides more energy to the migrating substances as a result the permeability increases.

Starch-based films are often transparent or translucent, odorless, tasteless, and colorless (Lourdin *et al.*, 1995; Myllärinen *et al.*, 2002; Rindlav-Westling *et al.*, 2002; Wolff *et al.*, 1951). They have been utilized as food packaging because of their excellent oxygen barrier property (Mark *et al.*, 1966). For water-soluble films, starch-based films are considered as the most effective films in terms of performance, adaptability to products, and production operations and costs (Daniels, 1973). Therefore, this research aimed at developing of heat sealable starch-based film that had acceptable mechanical and barrier properties.

2.5 Starch-based films

Starch is one of the most abundant, inexpensive, renewable and biodegradable biopolymer (Muscat *et al.*, 2012). Starch composes of two types of α-1,4 glucan, amylose and amylopectin. Amylose and amylopectin structures are shown in Figure 2.4. Amylose is considered as a linear polymer of α-1,4 anhydroglucose units, with a molecular weight of 10^5–10^6 (Durrani and Donald, 1995; Galliard and Bowler, 1987). Amylopectin is a highly branched polymer consisting of short α-1,4 glucosidic backbone with α-1,6 glucosidic branching points occurring every 25–30 glucose units. Amylopectin is a huge molecule with a molecular weight of 10^7–10^9 (Durrani and Donald, 1995; Galliard and Bowler, 1987). There are many hydroxyl groups on starch chains. The hydroxyl groups can participate in the formation of hydrogen bonds (Lu *et al.*, 2009). The amylose content in starch from different botanic origins is different (Gerard *et al.*, 2001; Shannon and Garwood, 1984; Yoshimoto *et al.*, 2000). Starch from waxy corn and rice contain 1-3 % amylose, while those from normal rice and corn contain 15-30 % amylose. Starch from high amylose corn (amylomaize) has amylose content of 50-70 %. Tapioca starch contain approximately 17 % amylose. Starches from legumes have amylose content of 30-40 % (Chang *et al.*, 2006; Colussi *et al.*, 2014; Li *et al.*, 2011; Liu, 2005; Lourdin *et al.*, 1995). Amylose content in starch is very important factor affecting the mechanical and barrier properties of starch-based films (Galliard and Bowler, 1987; van Soest, 1996). A linear structure of amylose holds responsible for the ability of its chain to interact by hydrogen bonds to a higher extent than the branched structure of amylopectin (Rindlav-Westling *et al.*, 1998). Consequently, amylose films are denser and stronger than amylopectin films (Lourdin *et al.*, 1995).

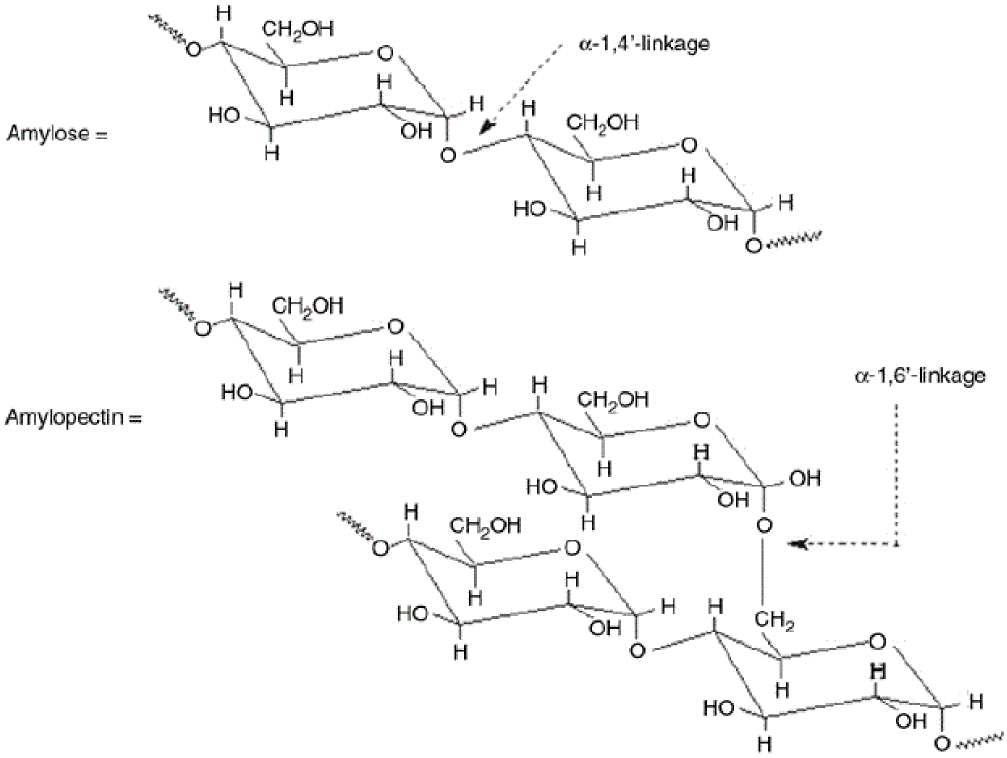


Figure 2.4 Amylose and amylopectin structures.
Source: Lu *et al.* (2009)

For examining the effect of amylose content on the mechanical properties of starch-based films, the purified amylose from pea starch and purified amylopectin from waxy maize starch were used as film-forming materials. Amylose, amylopectin and amylose-amylopectin films in the absence and the presence of 20 % glycerol (on dry starch basis**)** were wet-casted. For unplasticized films, a continuous increase in TS and %E was found as amylose content increased (Figure 2.5). For plasticized films, an increase in TS with a decrease in %E was only apparent for amylose content increased from 0 to 40 % (Figure 2.6). These two properties of films remained constant between 40 and 100 % amylose. Figure 2.7 clearly depicts that amylopectin-glycerol film had characteristic of a ductile material, while amylose-glycerol film exhibited typical of brittle behavior (Lourdin *et al.*, 1995).

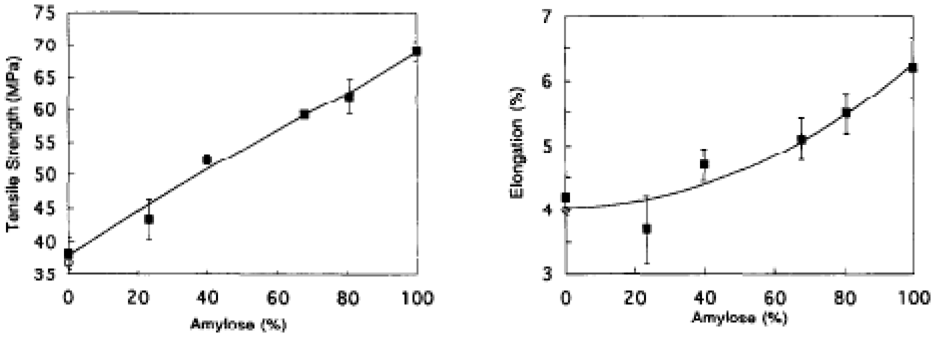


Figure 2.5 The relationship between amylose content and mechanical properties of the unplasticized starch-based film.

Source: Lourdin *et al.* (1995)

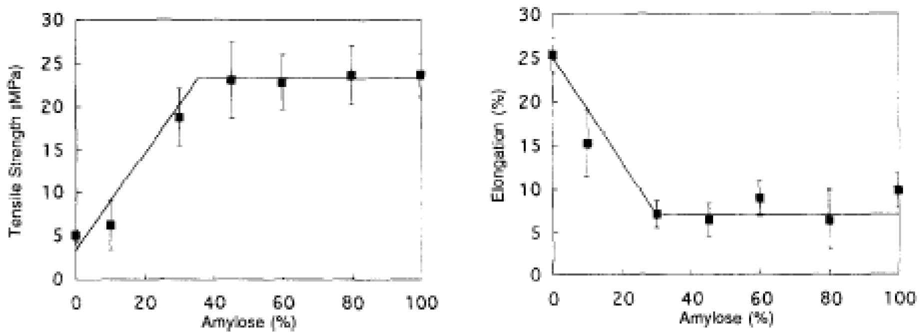


Figure 2.6 The relationship between amylose content and mechanical properties of the starch-based film plasticized with 20% glycerol on dry starch basis.

Source: Lourdin *et al.* (1995)

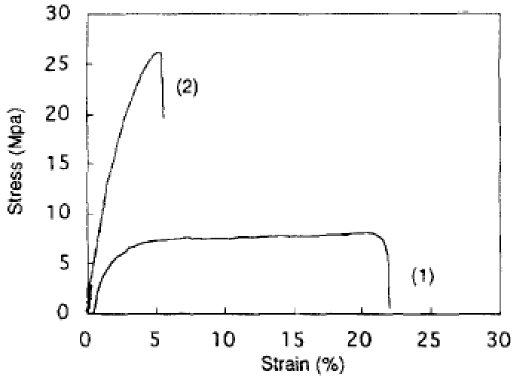


Figure 2.7 Typical stress-strain behavior of the amylopectin (curve 1) and amylose films (curve 2) plasticized with 20% glycerol on dry starch basis.

Source: Lourdin *et al.* (1995)

Film casted form potato amylose had a relative crystallinity of about 30 %, whereas film casted from granular amylopectin potato starch was entirely amorphous (Rindlav-Westling *et al.*, 2002). The relative crystallinity of the amylose-granular amylopectin potato starch films did not increase linearly with amylose content. The relative crystallinity of amylose-granular amylopectin potato starch films was considerably higher than that predicted from a theoretical blend of amorphous amylopectin and 33 % relative crystalline amylose (Lourdin *et al.*, 1995). This could be explained by co-crystallization between amylose and amylopectin and possibly by crystallization of amylopectin in the amylose-granular amylopectin potato starch films (Wittaya, 2012).

The TS and %E of starch-based films made from starches having various amylose content were listed in Table 2.2. It can concluded that the films made from starches having higher amylose content (26 and 33 %) gave the higher TS than those made from starches having the lower amylose content (<1 and 19 %). Except for film made from waxy maize starch which has the lowest amylose content (<1 %), %E decreased as amylose content increased. The proximity of starch chains induced by higher amylose contents could facilitate the formation of a starch network with more polymer content per area during drying (Alves *et al.*, 2007). Furthermore, the long linear amylose chains can easily entangle, and the granular structure retained in high amylose films may act as self-reinforcement (Wittaya, 2012).

Table 2.2 Tensile strength and % elongation of starch-based films with varied amylose content. Starch concentration in film-forming solution was 1.5 %w/w and moisture content of films was 15.7 % weight/total weight.

Starch	Amylose content (%)	Tensile strength (MPa)	Elongation (%)
Waxy maize	<1	38	4.2
potato	19	38	5.7
wheat	26	48	5.4
pea	33	46	4.9

Source: Lourdin *et al.* (1995)

The WVP was 0.59 and 0.52 g mm/m^2 h kPa for the film made from starch having low amylose content (≥ 25 %) and high amylose content (>70 %), respectively (Muscat *et al.*, 2012). Mark *et al.* (1966) reported that the unplasticized films produced from high amylose corn starch (71 % amylose) had no detectable oxygen permeability at RH < 100 %. This result suggested that absorption of water by hydrophilic polymers increased polymer chain mobility and generally led to an increase in gas permeability (Banker, 1966).

From the reported researches, amylose content at least 30-40 % gave homogenous film, and an increase in amylopectin content increased the possibility of phase separation (Dureja *et al.*, 2011). Mung bean (*Vigna radiata* L.) starch has been reported to have amylose content of 30-45 % (Bae *et al.*, 2008; Hoover *et al.*, 1997; Li *et al.*, 2011; Sandhu and Lim, 2008; Thao and Noomhorm, 2011). The gelatinized mung bean starch gives a transparent gel upon cooling. Noodle manufactured from mung bean starch is transparent, smooth, pliable and have good cooking quality (Abdel-Rahman *et al.*, 2008; Hoover *et al.*, 1997). Mung bean starch is expected to be proper film-forming material to make a homogenous film with good mechanical properties and oxygen barrier (Bae *et al.*, 2008; Hoover *et al.*, 1997). Thus, mung bean starch was used in this research.

2.6 Effect of plasticizer on properties of films

It has been known that starch it forms a brittle film. A plasticizer is required to improve film properties. For starch-based films, the preferred plasticizers are polyols such as glycerol, xylitol and sorbitol (Figure 2.8), which are hydrophilic compounds containing hydroxyl group similar to starch structure (Han, 2005; Sothornvit and Krochta, 2005). Polyols have been reported to reduce the molecular attraction between the starch chains by forming hydrogen bonds between polyols and starch molecules. Reduction of the formation of hydrogen bonds between the starch chains allows greater flexibility (higher %E) and subsequently decreases TS of film (Lourdin, Bizot, *et al.*, 1997; Mathew and Dufresne, 2002; Turhan and Sahbaz, 2004). This does not only affect mechanical properties but crystallinity and barrier properties of films (Gaudin *et al.*, 1999; Lourdin, Coignard, *et al.*, 1997; Myllärinen *et al.*, 2002). Relative crystallinity of film has been used to indicate degree of interaction between starch chains. Higher relative crystallinity indicates higher degree of interaction between starch chains, and thus film has higher TS along with lower %E.

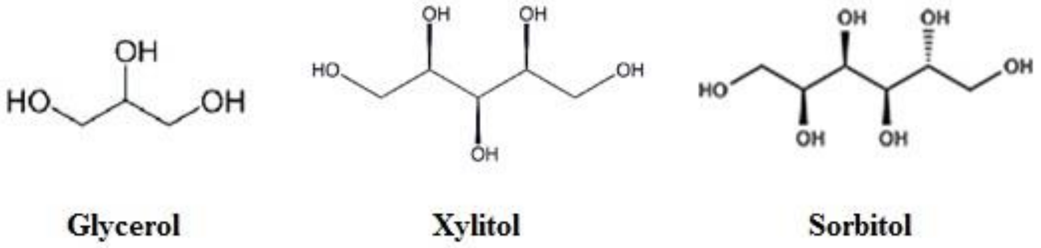


Figure 2.8 Polyols structures**.**
Source: Yang *et al.* (2009)

It was reported that type and concentration of polyols affected the properties of starch-based films differently (Alves *et al.*, 2007; Dai *et al.*, 2010; Muscat *et al.*, 2012; Talja *et al.*, 2007; Wittaya, 2012). An effectiveness of polyols as plasticizer has been reported to be related to the number of hydroxyl groups (OH groups) in their molecules. Lower relative crystallinity of granular potato starch film was found as polyols containing more OH groups were used during gelatinization (Smits *et al.*, 2003). Xylitol (5 OH groups) was reported to be a more effective plasticizer than glycerol (3 OH groups) for low amylose maize starch film (Adhikari *et al.*, 2010). It was explained that the larger molecular size xylitol could form stronger hydrogen bonds than the smaller molecular size glycerol. Muscat *et al*. (2012) found that glycerol acted as an antiplasticizer, while xylitol acted as plasticizer for low amylose maize starch at plasticizer concentration of 15 %w/w on dry solid basis. However, at plasticizer concentration of 20 and 30 %w/w on dry solid basis, glycerol was more effective to decrease TS along with increase %E than xylitol for both low amylose and high amylose maize starch films. The starch-polyols and starch-water and water-polyols interactions in these unplasticized and plasticized films were analyzed from their fourier transform infrared spectra. For low amylose maize starch film, the spectra indicated that starch-glycerol hydrogen bonds were stronger and more stable than intra- and intermolecular hydrogen bonds in starch with water at glycerol concentration of 15 %w/w. This resulted in a decrease in the free volume available for mobility if starch chains, hence, the film plasticized with glycerol of 15 %w/w was more rigid than the unplasticized film. As glycerol concentration increased from 15 to 30 %w/w, the spectra indicated that the intermolecular force of OH groups between starch-glycerol and starch-water and water-glycerol decreased. This suggested that glycerol may prevent the reformation of hydrogen bonds during drying as glycerol remained and solvated between starch chains, and the starch and glycerol were competing for hydrogen bonding with water as glycerol concentration increased. For film of low amylose maize starch, the spectra indicated a decrease in intermolecular hydrogen bonds in the presence of 20 %w/w xylitol, compared to unplasticized film. At 30 %w/w xylitol, spectra indicated that phase separation and physical exclusion of xylitol from film occurred. The spectra of films from high amylose maize starch suggested that new and strong glycerol-starch hydrogen bonds formed and replaced starch-starch interaction in the presence of 20-30 %w/w glycerol. While that of xylitol plasticized film at 20-30 %w/w suggested hydrogen bonds in film were weaken by xylitol that remained and solvated between starch chains during drying.

For potato starch film plasticized with polyols (glycerol, xylitol or sorbitol), it was found that plasticizer concentrations required to plasticize film or caused phase separation increased with their OH groups (Table 2.3). Phase separation of glycerol produced a sticky surface of film. Phase separation of xylitol and sorbitol caused crystallization in the film, which decreased plasticization effect in film and probably increased starch crystallization (Talja *et al.*, 2007).

Table 2.3 Effect of type and concentration of polyols**.**

Polyols	Concentration of polyols (%w/w on dry solid basis) that caused		
	Antiplasticization	Plasticization	Phase separation
Glycerol (3 OH groups)	< 12	20-30	> 27
Xylitol (5 OH groups)	≤ 20	> 30 - < 40	40
Sorbitol (6 OH groups)	≤ 30	> 40 - < 60	60

Source: Talja *et al.* (2007)

WVP increased with concentration of polyols used. At same concentration of polyols used, WVP of potato starch-based edible films decreased with increasing molecular weight of polyol (Talja *et al.*, 2007). Overall, increasing the plasticizer concentration always increases OP through biopolymer films, because of the higher free volume in the film network (Forssell *et al.*, 2002; Sothornvit and Krochta, 2005). Forssell *et al*. (2002) reported that OP of the amylopectin film increased with increasing glycerol concentration above 10 % glycerol. For amylose film, OP was practically independent of glycerol concentration and had a value of about 10^{-16} cm^2/Pa s (Sothornvit and Krochta, 2005).

For sago starch film at the same plasticizer concentration, glycerol-plasticized films were thicker than sorbitol-plasticized films (Abdorreza *et al.*, 2011). Compared to sorbitol, glycerol had lower molecular weight, which in turn governed the higher number of molecule and occupied molecular space. Glycerol-plasticized films exhibited markedly lower seal strength compared to sorbitol-plasticized films. As sorbitol concentration increased, seal strength significantly increased. The variable seal strength exhibited by the different starch films could have been influenced by the thickness of the films. An increase in film thickness decreases heat transfer; consequently, the temperature of the joint surface of the thick films to be lower than that of the thin films, as a result seal strength decreases. Another factor that could have influenced the seal strength was the hydroxyl groups in the plasticizers as hydrogen bonds may be the main forces responsible for the sealed joint formation of the starch-based films plasticized with polyols. (Abdorreza *et al.,* 2011). Thermal transition temperatures (onset temperature, T_o and peak temperature, T_p), which were typically used to determine sealing temperatures of polymers, can be determined using Differential scanning calorimetry, DSC (Hernandez, 1997). A significant difference between T_o and T_p is important to achieve good sealing. If the T_o and T_p values were very close, the film would nearly completely melt during sealing (Tanner *et al.*, 2003). Sago starch films plasticized with sorbitol or glycerol had endothermic peaks in the range between 110 °C and 190 °C. Compared to unplasticized film, sorbitol plasticized films had lower onset temperature and vice versa for glycerol plasticized films. The onset temperature of sorbitol plasticized films was close to the heat-sealing temperature, resulting in a better seal (Abdorreza *et al.*, 2011).

An increase in concentration of sago starch increased the TS along with decreased E, solubility, WVTR and OP of film (Polnaya *et al.*, 2012). Sothornvit and Pitak (2007) found that an increase in concentration of banana flour also reduced OP. This was due to the fact that an increase in starch/flour concentration caused an increase in the number of hydrogen bonds so that it formed rigid film. The decrease in WVTR caused by the increase in water soluble solids (in this case starch/flour) in the film. Increasing starch concentration significantly decreased OP by increasing the solid (starch/flour) concentration which in turn producing a smaller pore size.

2.7 Dried chili powder storage stability

Starch-based film is excellent oxygen barrier therefore it can be used to pack antioxidant compounds to protect their antioxidant activity. Dried chili powder containing capsaicin, an important phytochemical with antioxidant activities, is one of major seasoning packed with instant noodles (Wangcharoen and Morasuk, 2009). Owing to chemical oxidation of capsaicin during storage, its antioxidant activity decreases. Thus, dried chili powder was used in this research as a model antioxidant for testing how well sachet from MBS film can prolong antioxidant activity of capsaicin compared to polypropylene, also known as polypropene (PP), sachet. PP is thermoplastic polymer made from the monomer propylene and used in a wide variety of applications and. PP has a melting point of 171 °C and oxygen permeability of 580-970 cc µm/m^2 day kPa (Guilbert *et al.*, 1996). During storage at 3 temperatures, TPC dried chili powder was determined by the Folin–Ciocalteu reaction and antioxidant activity of dried chili powder in these two sachets was monitored by DPPH free radical scavenging activity assay and FRAP assay (Wangcharoen and Morasuk, 2009). Then, the shelf life of dried chili powder packed in these two sachets was estimated by accelerated shelf life test (ASLT).

2.8 Shelf life

Shelf life is the time between the packaging of a product and the point at which the product first becomes unacceptable to consumers under defined environmental conditions and selected packaging. Storage tests which maintain product in a static facility and evaluating product quality with time are used to determinate shelf life. One of the storage tests is accelerated shelf life test (ASLT). Products are stored in cabinet or room maintains high temperature to speed product degradation. Product quality data are obtained at high temperature for at least 3 temperatures. Applications of chemical kinetic principles allow to estimate of shelf life at lower typical/average temperature (Janjarasskul, 2014)

Table 2.4 shows rate law for kinetics of product quality changes. Figure 2.9 represents concentration of quality factor versus time for the zero-order reaction and the first order reaction.

Table 2.4 Kinetics (rates) of product quality changes.

	Zero-Order	First-Order
Rate Law	$-\frac{d[A]}{dt} = k$	$-\frac{d[A]}{dt} = k[A]$
Integrated Rate Law	$[A] = [A]_0 - kt$	$[A] = [A]_0 e^{-kt}$
Units of Rate Constant (k)	$\frac{M}{s}$	$\frac{1}{s}$
Linear Plot to determine k	[A] vs. t	ln ([A]) vs. t
Half-life	$t_{1/2} = \frac{[A]_0}{2k}$	$t_{1/2} = \frac{\ln(2)}{k}$

$[A]_0$ = concentration of quality factor, [A] = concentration of degradation product, k = rate constant at given T, t = time

Source : (Janjarasskul, 2014)

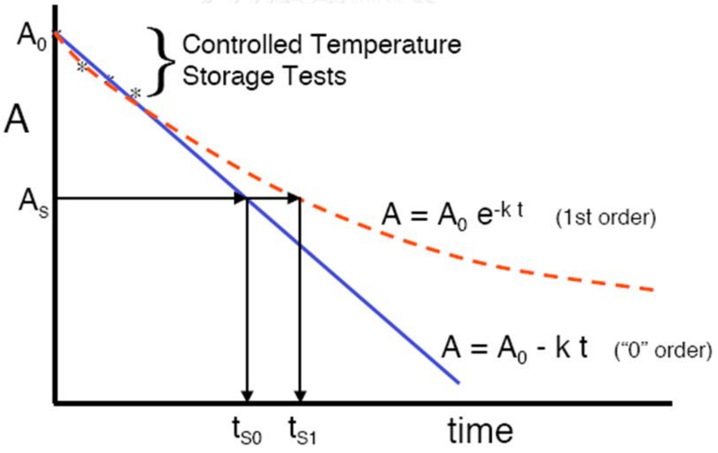


Figure 2.9 Zero-order and first-order reactions.
Source: (Janjarasskul, 2014)

Equation 2.1 and 2.2 are Arrhenius equations which show that log of rate constant is a reciprocal function of temperature (1/T). If the rate constants at 3 temperatures fall in a straight line on log k VS (1/T) plot (Figure 2.10), the Arrehnius relationship applies. The rate constants of the reaction at any other temperature can be predicted by interpolation and extrapolation of this curve (Janjarasskul, 2014).

$$k = k_0 e^{-E/RT} \quad [2.1]$$

$$\text{Log } k = \text{Log } k_0 - E/2.3RT \quad [2.2]$$

k = rate constant at T (°K)

k_0 = Arrhenius constant

E = activation energy

R = ideal gas constant

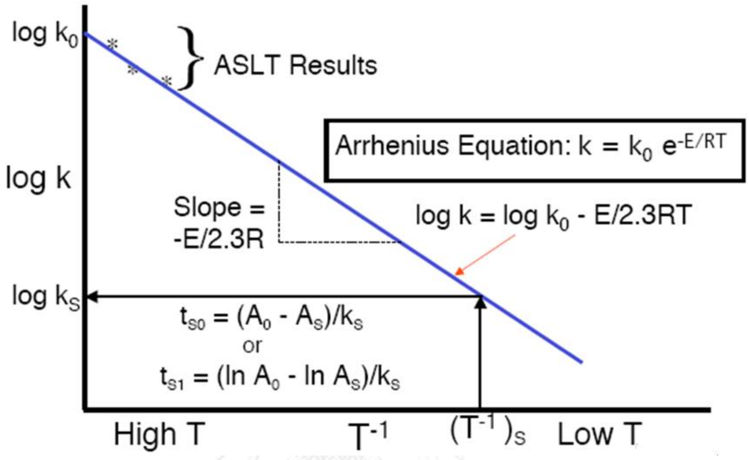


Figure 2.10 Arrhenius plot

Source: (Janjarasskul, 2014)

CHAPTER III
MATERIALS AND METHODS

3.1 Proximate analysis of mung bean starch

Composition of mung bean starch (Pine brand, Sitthinan Co., Ltd., Bangkok, Thailand), raw material. Its moisture, ash, crude fiber, protein, fat and carbohydrate contents were determined using the methods of Association of official Analytical Chemist (AOAC, 2010) as followed:

- Moisture by air oven method AOAC 925.10,
- Ash by direct method AOAC 923.03,
- Crude fiber by fritted glass crucible method AOAC 978.10,
- Protein by Kjeldahl method AOAC 991.20,
- Fat by acid hydrolysis method AOAC 922.06, and
- Carbohydrates by subtraction the sum of protein, fat, moisture, and ash contents from 100 g.

3.2 Film preparation

Mung bean starch (Pine brand, Sitthinan Co., Ltd., Bangkok, Thailand) was dispersed in distilled water. Then plasticizer, sorbitol (Fukuoka Qingdao Chemicals Trading Co., Ltd., Qingdao, China) or glycerol (Fukuoka Qingdao Chemicals Trading Co., Ltd., Qingdao, China), was added into the starch suspension. Composition for 100 g total suspension was shown in Table 3.1. Starch used in Table 3.1 was weighted of moist starch required to obtain weight of dry starch needed. The mixture was heated (85 ºC, 750 rpm, 30 min) on hot plates (MS7-H550-S, SCILOGEX, LLC., Rocky Hill, Connecticut, USA), and then cooled to 50±2 ºC. The paste was sonicated for 20 min using bath sonicator (DE-78224, Elma Hans Schmidbauer GmbH & Co. KG, Singen, Germany) to remove bubbles. Then, the paste (40 ml) was poured over a 15x15 cm acrylic plate, and dried in a hot air oven (YEO HENG Co., LTD., Bangkok, Thailand) at 35 ºC for 20 hr. The film was peeled and stored in a desiccator containing magnesium nitrate (52.9 % RH at 28±2 ºC) for 48 hr prior to analysis. All films were prepared in triplicate.

Table 3.1 Composition for 100 g total suspension

Suspension		Composition for 100 g suspension		
Starch concentration (%w/w)	Plasticizer concentration (%w/w)	Starch (g)	Plasticizer (g)	Water (g)
3.5	0	3.8335	0	96.1665
	10	3.8335	0.3834	95.7831
	20	3.8335	0.7667	95.3998
	30	3.8335	1.1500	95.0165
	40	3.8335	1.5334	94.6331
	50	3.8335	1.9168	94.2497
	60	3.8335	2.3001	93.8664
4.0	0	4.3812	0	95.6188
	10	4.3812	0.4381	95.1807
	20	4.3812	0.8762	94.7426
	30	4.3812	1.3144	94.3044
	40	4.3812	1.7525	93.8663
	50	4.3812	2.1906	93.4282
	60	4.3812	2.6287	92.9901
4.5	0	4.9288	0	95.0712
	10	4.9288	0.4929	94.5783
	20	4.9288	0.9858	94.0854
	30	4.9288	1.4786	93.5926
	40	4.9288	1.9715	93.0997
	50	4.9288	2.4644	92.6068
	60	4.9288	2.9573	92.1139
5.0	0	5.4764	0	94.5236
	10	5.4764	0.5476	93.9760
	20	5.4764	1.0953	93.4283
	30	5.4764	1.6429	92.8807
	40	5.4764	2.1906	92.3330
	50	5.4764	2.7382	91.7854
	60	5.4764	3.2858	91.2378

3.3 Characterization and Testing

3.3.1 Thickness

Thickness of film in mm was measured with a digital micrometer (Model ID-C112, Mitutoyo Manufacturing Co., Kanagawa, Japan) at 15 random positions of each test film and then calculated an average. Three replications were determined for each treatment.

3.3.2 Mechanical properties

Film was cut into strips of 30 mm wide (W) and 120 mm long (L), then kept in a controlled temperature and relative humidity (RH) chamber at 28±2 ºC and 52.9 % RH for 48 hr. Thickness of film in mm was measured then converted to m (l, m). Tensile strength (TS, MPa) and percent elongation (%E, %) were determined according to the standard test method for tensile properties of thin plastic sheeting, D882-02 (ASTM, 2002) using Instron Universal Testing Machine (Model 5565, Instron, Norwood, USA) equipped with 5 kg load cell. The initial distance between the grips (initial gauge length, L_i) was 50 mm. The cross-head speed was 5.0 mm/sec. The maximum load (F, N) and the extension at the moment of rupture of the test film strip (ΔL, mm) were determined, then TS and %E were calculated from Equation 3.1 and 3.2, respectively. A total of 9 measurements were made for each film replicate. Three replications were determined for each treatment.

$$\mathrm{TS} = \frac{\mathrm{F}}{\mathrm{Wl}} \qquad [3.1]$$

$$\%\mathrm{E} = \frac{100\Delta\mathrm{L}}{L_i} \qquad [3.2]$$

3.3.3 % Solubility

The 2x2 cm film was soaked in 10 ml distilled water at room temperature (28±2 ºC, 250 rpm, 24 hr) on a platform shaker (INNOVA 2050, Eppendorf, AG, Hamburg, Germany). The film residues were filtrated and dried in a hot air oven at 105 ºC until constant weight was obtained. The solubility was calculated from Equation 3.3. Where W_i is the initial dried weight (g) and W_f is the final dried weight (g). A total of 3 measurements were done for each film replication.

$$\%\ \text{Solubility} = 100\left(\frac{W_i - W_f}{W_i}\right) \qquad [3.3]$$

3.3.4 Water vapor permeability

The water vapor permeability (WVP) was determined using desiccant methods described by the standard method for water vapor transmission of materials, E96-95 (ASTM, 1995b). A permeation cells (31.4 mm internal diameter and 68.8 mm height) containing 20 g of dry siliga gel (24 mm height) were sealed by the test film (0.000777 m^2 film area, A) using parafilm. Then, the cells were placed in the desiccators containing distilled water to maintain a 100 % RH. After the sample reached steady state conditions, the cells were weighted at 12 hr intervals over a 7 days period. Weight change (G, g) of the cells were recorded to the nearest 0.0001 g and plotted as a function of time (t, hr). The slope of the straight line was determined by linear regression ($R^2 > 0.99$). The rate of water vapor transmission (WVT, g/ m^2 hr) was calculated from Equation 3.4. WVP was calculated from Equation 3.5. Where l is thickness of film (mm), p_1 is water vapor partial pressure at the film inner surface (kPa), and p_2 is water vapor partial pressure at the film outer surface (kPa). Three measurements were made for each film replicate.

$$\text{WVT} = \frac{\text{G}}{(t)(A)} \qquad [3.4]$$

$$\text{WVP} = \frac{(\text{WVT})(\text{l})}{\text{p}_1 - \text{p}_2} \qquad [3.5]$$

3.3.5 Oxygen permeability

Films were coated with polyethylene (PE) and covered with an aluminum foil mask with an open testing area of 25.5 cm^2. Oxygen transmission rate (OTR, cc / m^2 day) through MBS-coated PE films was determined according to standard method ASTM D3985-05 (ASTM, 1995a) using an OX-TRAN® 2/21 ST modular system (MOCON Inc., Minneapolis, Minnesota, U.S.A.) The system was programmed to have a 10 hr waiting period to allow the films to achieve equilibrium with the controlled temperature at 23 °C and 0 % RH. Oxygen permeability (OP, cc µm / m^2 day kPa) was calculated from Equation 3.6 where P_o is the oxygen partial pressure and l is thickness of film (µm). The MBS-coated PE film is a laminated structure consisting of a base PE film layer. The equations 3.7 and 3.8 were used for the 2-layer model. Where the subscripts 1 and 2 represent the PE layer and the MBS layer coated on the surface of PE films, respectively. Two measurements were done for each film replication. The analysis was replicated three times.

$$OP = \frac{OTR\,l}{P_o} \qquad [3.6]$$

$$l = l_1 + l_2 \qquad [3.7]$$

$$\frac{l}{OP} = \frac{l_1}{OP_1} + \frac{l_2}{OP_2} \qquad [3.8]$$

3.3.6 Thermal properties

Thermal properties were determined using a differential scanning calorimeter (DSC, NETZSCH DSC 204F1 Phoenix 240-12-0322-L, Selb, Germany). The diameter of film disc was 0.5 cm and the weight was about 10 mg. The film dise was hermetically sealed in a aluminium pan. The samples were heated from 25 ºC to 95 ºC at a rate of 10 ºC / min. Nitrogen gas was used at a rate of 30 ml/min. The DSC was calibrated using pure indium and pure ice. An empty aluminium pan was used as a reference. The onset temperature (T_o, °C) and peak temperature (T_p, °C) were determined.

3.3.7 Seal strength

Films were cut into strips of 2.54 cm wide (W) and 7.62 cm long (L). Two films strips were placed on top of one another, and an area of 1.5 cm wide and 2.54 cm long (at the edge of the film) was heat-sealed with semi-auto impulse sealer (Glory-Pack PHS 450 10D, Korea International Trade Association, South Korea). Heating time was 1 sec and dwell time was 3 sec. Heating temperature ranged from 68 to 85 ºC. All sealed film samples were kept in chamber at 28±2 ºC and 52.9 % RH for 48 hr. Seal strength (γ, N/m) was determined according to the standard test method for seal strength of flexible barrier material, E88-07a (ASTM, 2007) using an Instron universal testing machine (Model 5565, Instron, Norwood, USA). Each leg of the sealed film was clamped to the machine. Each end of the sealed film was aligned perpendicularly to the direction of the pull. The distance between clamps was 50 mm and the rate of loading was set at 5.0 mm/sec. Seal strength was calculated from Equation 3.9. Where F_p is the maximum force required to peel off the seal (N). A total of 5 measurements were done for each film replication.

$$\gamma = \frac{F_p}{W} \qquad [3.9]$$

3.4 Effect of storage time and temperature on physical and chemical properties of dried chili powder

3.4.1 Sample preparation

MBS films were cut into sheets of 9.2 cm wide and 9.2 cm long. Two films sheets were placed on one another with their bottom sides faced each other. Three edges of the films were heat-sealed with semi-auto impulse sealer at 70 ºC to form sachet. Sachets were kept in chamber at 28±2 ºC and 52.9 % RH for 48 hr. Forty grams of dried chili powder (red bird chili, *Capsicum frutescens* Linn.) (Raitip brand, Thai Cereals World Co., Ltd., Nonthaburi, Thailand) were packed in sachet of MBS film or PP sachet. PP sachet having the same size as a sachet of MBS film was used as a control sachet. The initial oxygen capacity was calculated by % oxygen in the air and the difference between the capacity of sachet and dried chili powder which was contained. Oxygen absorber (20 cc, Best KEPT type M-20, Alpine Foods Co., Ltd., Bangkok, Thailand) was put in each sachet for setting the initial oxygen capacity to zero. The sachet was heat-sealed and stored in desiccator containing magnesium nitrate (52.9 % RH) at 35, 45 and 55 ºC for 50 days, in order to take part in properties measurements during storage.

3.4.2 Sample extraction

Sample extraction method of Kim *et al.* (2003) was modified for sample preparation. The extraction solvent (80 %v/v of 95 % ethanol) was prepared by mixing of distilled deionized water and 95 % ethanol. Ten grams of dried chili powder were weight into 250 ml erlenmeyer flask, and 100 ml of a solvent was added. The suspension was shaken in water bath shaker (Julabo Shake Temp SW23, UNITED INSTRUMENT Co., Ltd., Germany) at 30±2 ºC and 150 rpm for 20 min. The suspension was filtered through Whatman filter paper no.1 (Whatman International Limited, Kent, England) using a vacuum filtration. The residue *was extracted repeatedly* with 100 ml of solvent using the same conditions. The two filtrates were combined and transferred into 1 L round bottom flask. The solvent was evaporated using a rotary evaporator (Stuart RE300, Keison International Ltd., England) at 40 ºC for 1 hr. The concentrated extract was diluted with distilled deionized water in 100 ml volumetric flask.

3.4.3 Moisture and water activity determination

Moisture was determined with moisture analyzer (HB43-S Halogen, Switzerland). Measures were performed at regular time intervals (every 3 or 7 days) during storage.

Water activity was measured at the same intervals of time by means of the water activity meter (Aqua Lab series 3 TE, Decagon Devices Inc., USA) 0.5 g of powder was inserted in the device and left for 1 min at 25 °C before recording the value. All measures were conducted in triplicate.

3.4.4 Color measurements

Color of dried chili powder was measured by a Chroma meter (Model CR-400, Minolta Co., Ltd., Tokyo, Japan) to determine the color values (L^*, a^* and b^* values). The L^* (Lightness), a^* (ranging from – a^* = greenness to + a^* = redness) and b^* (ranging from – b^* = blueness to + b^* = yellowness) values were averaged from five random readings, for each sample. The measured values were compared with those of the same sample prior to storage ($L_0^*, a_0^*\ and\ b_0^*$). The color values were used to calculate the total color degradation (ΔE) from Equation 3.10 and 3.11. Three measurements were done for each sample replication. The analysis was replicated three times.

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \qquad [3.10]$$

The values of ΔL^*, Δa^* and Δb^* were calculated by,

$$\Delta L^* = L^* - L_0^*, \quad \Delta a^* = a^* - a_0^* \quad \text{and} \quad \Delta b^* = b^* - b_0^* \qquad [3.11]$$

3.4.5 Total phenolic content

The Folin-Ciocalteau micro method of Waterhouse (2005) was used to estimate total phenolic content (TPC). The extract from 3.4.2 (100 µl) was added to a 10 ml volumetric flask containing 7 ml of distilled deionized water, 500 µl Folin-Ciocalteau reagent was added, and then the mixture was shaken. After 4 min, 1.5 ml of a 20 %w/v sodium carbonate solution was added and mixed. The solution was then immediately diluted to the volume of 10 ml with distilled deionized water and mixed thoroughly. The solution was incubated at 25±2 ºC for 2 hr in the dark cabinet and the absorbance at 765 nm was measured using spectrophotometer (Spectronic 20 Genesys model 4001/4, Thermo Fisher Scientific Inc., USA). Distilled deionized water was used as blank. Gallic acid (0 - 2,000 mg/l) was used as a standard (Appendix D), and results were reported as mg gallic acid/g dried chili powder weight. Analysis was conducted in triplicate.

3.4.6 DPPH free radical scavenging activity

The method of Brand-William *et al.* (1995a) was used with modification. DPPH radical solution (0.12 mM) in 95 % ethanol was prepared. Absorbance of DPPH radical solution was adjusted to 1.1 ($A_{d,initial}$) with 95 % ethanol. The extract of 250 µl (from 3.4.2) was mixed with 4.75 ml DPPH solution. The solution was incubated at 25±2 ºC for 30 min in the dark cabinet and the absorbance of the solution ($A_{d,final}$) at 515 nm was measured using spectrophotometer. Ethanol was used as blank. Absorbance difference ($A_{d,diff}$) was calculated from Equation 3.12. With calculated $A_{d,diff}$, DPPH values was read from the trolox standard curve (Appendix D), and results were reported as µmol trolox/g dried chili powder weight. Analysis was conducted in triplicate.

$$A_{d,diff} = A_{d,initial} - A_{d,final} \qquad [3.12]$$

3.4.7 Ferric reducing antioxidant power

The FRAP assay was conducted according to Benzie and Strain (1996) with modification. The FRAP reagent was prepared by mixing 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM hydrochloric acid (HCl), 20 mM ferric chloride ($FeCl_2$) solution and 0.3 M acetate buffer at volume ratio of 1:1:10. The extract of 250 µl (from 3.4.2) was mixed with 4.75 ml of FRAP reagent which was prepared daily. The solution was incubated at 25±2 ºC for 30 min in the dark cabinet. The absorbance of FRAP reagent ($A_{f,initial}$) and the solution ($A_{f,final}$) at 593 nm were measured using spectrophotometer. Distilled deionized water was used as blank. Absorbance difference ($A_{f,diff}$) was calculated from Equation 3.13. With calculated $A_{f,diff}$, FRAP values was read from the trolox standard curve (Appendix D) and results were reported as µmol trolox/g dried chili powder weight. Analysis was conducted in triplicate.

$$A_{f,diff} = A_{f,initial} - A_{f,final} \qquad [3.13]$$

3.5 Statistical analysis

An analysis of variance and Duncan’s multiple comparison range test were analyzed with SPSS software version 17.0 (SPSS Inc., Chicago, Illinois, USA).

CHAPTER IV
RESULTS AND DISSCUSSION

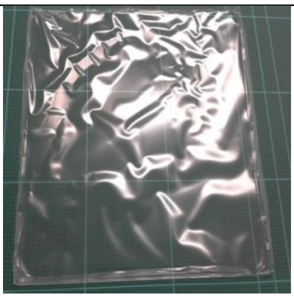
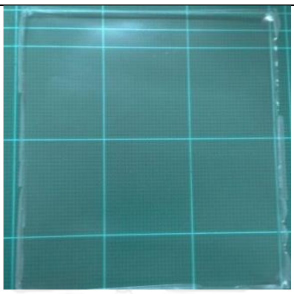
4.1 General physical properties of MBS films

Figure 4.1 shows the appearance of MBS-based films with varied starch concentration as well as plasticizer type and content. Overall, MBS were good film-formers, yielding strong films due to the linear structure of the polymer backbone. MBS films were glossy without apparent bubbles and pinholes. The bottom side, which was in contact with the acrylic casting plate, was slightly shinier than the top side, which had contact with the air. The shinny bottom side was the mirror-image of the smoothed surface acrylic mold served as a sufficient restraint to prevent the starch gel and film from shrinking. All MBS films were translucent indicating that solution-making process could adequately gelatinize, disintegrate and solubilize MBS.

The proper starch concentration for preparation of starch solutions was determined in order to insure both the continuity of films and the ease of casting process. The optimum range of starch content for casting MBS films were found to be in between 3.5 – 5.0 %w/w. The testing films obtained from starch concentration of 3.5 - 5.0 %w/w had good visual and handling properties. On the other hand, the film casted from 2.5 %w/w MBS was very thin and broke easily over peeling. The solution of completely solubilized starch hardly gels causing problems in forming a continuous film of sufficient thickness (Liu, 2005; Protzman *et al.*, 1967). On the other hand, when the starch concentration was too high, e.g. 6.0 % MBS, the solution was too viscous to cast evenly resulting in film with wrinkle surface.

It was possible to cast non-plasticized MBS films, however the films tended to become brittle and hard to handle at low relative humidity. Thus, common polyol plasticizers were explored to reduce brittleness, impart flexibility and toughness for films. To study the plasticizer effect of glycerol and sorbitol on the MBS film, the maximum concentrations for each type of plasticizer were defined. The result showed that film beyond the maximum plasticizer concentration (> 30 %w/w glycerol or > 60 %w/w sorbitol) had greasy and wrinkle surface. The excess plasticizer tended to form aggregates and/or migrate to surface of the film upon solvent evaporation and storage.

Therefore, the starch concentration of 3.5 - 5.0 %w/w and the plasticizer concentration of ≤ 30 %w/w glycerol or ≤ 60 %w/w sorbitol were studied in this research.

MBS films without plasticizer at starch concentration of		
2.5 %w/w	3.5-5.0 %w/w	6.0 %w/w
		

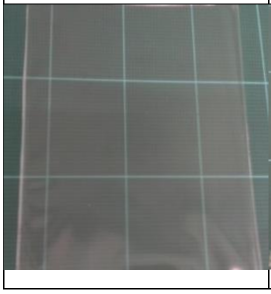
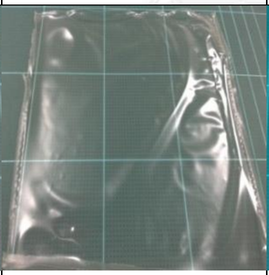
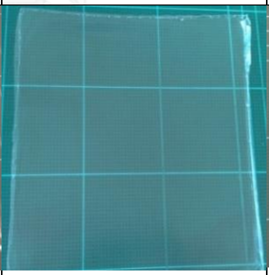
MBS films obtained from 5.0 %w/w starch at plasticizer concentration of			
Glycerol		Sorbitol	
≤ 30 %w/w	>30 %w/w	≤ 60 %w/w	> 60 %w/w
			

Figure 4.1 Edible mung bean starch (MBS) film with or without plasticizers (glycerol or sorbitol).

4.2 Thickness

Overall, casted MBS film had thickness ranged from 0.0462 mm to 0.0828 mm. The result showed that film thickness significantly ($p < 0.05$) and linearly (R^2 = 0.92-1.00) increased as starch concentration increased when examined at the same plasticizer type and concentration (Figure 4.2, Table 4.1). Figure 4.3 shows the effect of plasticizer type and concentration on MBS film thickness. In general, addition of plasticizer gave a rise to thickness of testing film having the same starch concentration. Figure 4.3 also shows that the thickness of the film fabricated from casting solution with starch concentration ≥ 4.5 %w/w was not significantly affected by addition of sorbitol above 40 %w/w. The thicker film as a result of increasing either starch and/or plasticizer content was due to the increased total solid in a controlled volume of film-forming solution casted in a controlled casting space. The film thickness increased linearly (R^2 = 0.84-0.97) as a function of total solid in casting solution (Figure 4.4, Table 4.2, Appendix A).

It was found that sample plasticized with glycerol were thicker than sorbitol-plasticized films at the onset plasticizer concentration at 30, 30, 20 and 10 %w/w plasticizer at 3.5, 4.0, 4.5 and 5.0 %w/w starch concentration, respectively. This was probably due to the fact that glycerol had lower molecular weight (MW = 92) than sorbitol (MW= 182) which in turn governed the higher number of molecules and occupied molecular space than sorbitol at the same plasticizer concentration (w/w) in film-forming solution.

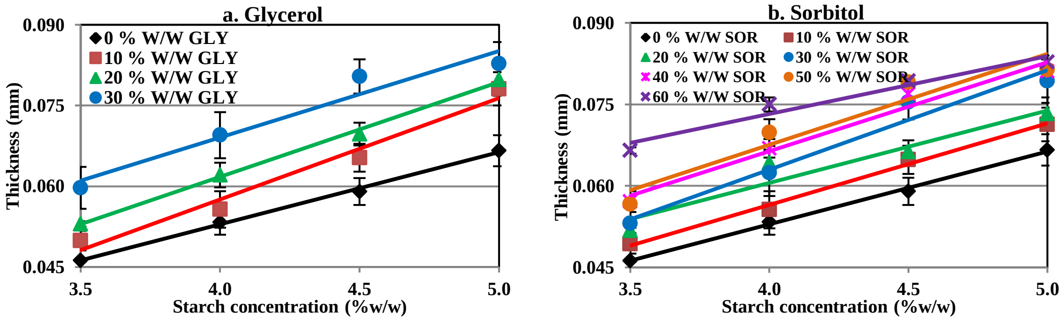


Figure 4.2 Effect of starch concentration and plasticizer type and concentration; glycerol (a) or sorbitol (b) on thickness (mm) of mung bean starch films.

Table 4.1 Linear relationship between mung bean starch concentration ([St], %) and thickness (l, mm) of films.

Plasticizer concentration (%w/w)	Glycerol plasticized MBS film		Sorbitol plasticized MBS film	
	Linear equation	R^2	Linear equation	R^2
0	l = 0.0134[St] - 0.0006	1.00	l = 0.0134[St] - 0.0006	1.00
10	l = 0.0188[St] - 0.0178	0.97	l = 0.0150[St] - 0.0037	0.99
20	l = 0.0176[St] - 0.0087	1.00	l = 0.0133[St] + 0.0073	0.92
30	l = 0.0160[St] + 0.0049	0.95	l = 0.0183[St] - 0.0103	0.96
40	-		l = 0.0164[St] + 0.0009	0.97
50	-		l = 0.0167[St] + 0.0006	0.92
60	-		l = 0.0106[St] + 0.0307	0.95

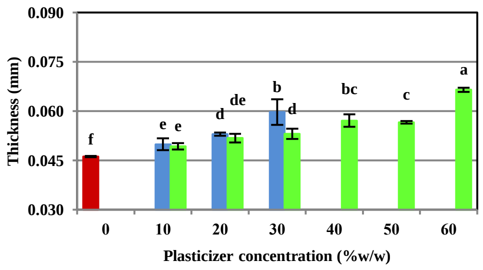
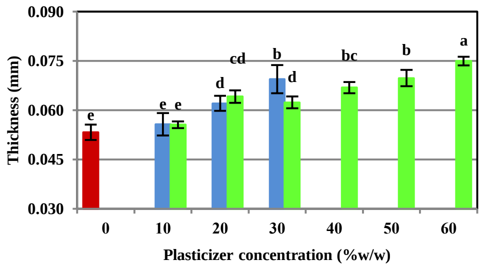
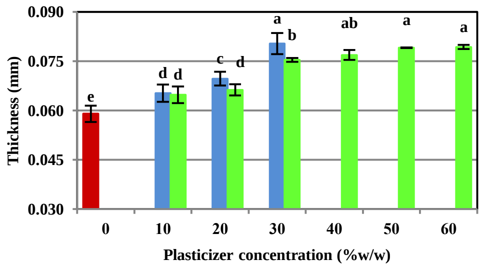
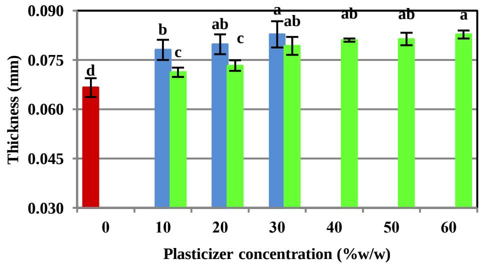
Starch Concentration (%w/w)	🟥 **Plasticizer-free** 🟦 **Glycerol** 🟩 **Sorbitol**
3.5	
4.0	
4.5	
5.0	

Figure 4.3 Effect of starch concentration and plasticizer type and concentration; glycerol or sorbitol on thickness (mm) of mung bean starch films.

Each data point represents the average value. Error bars show standard deviations. Different letters (a, b, ..) indicate significant differences between samples ($p \leq 0.05$).

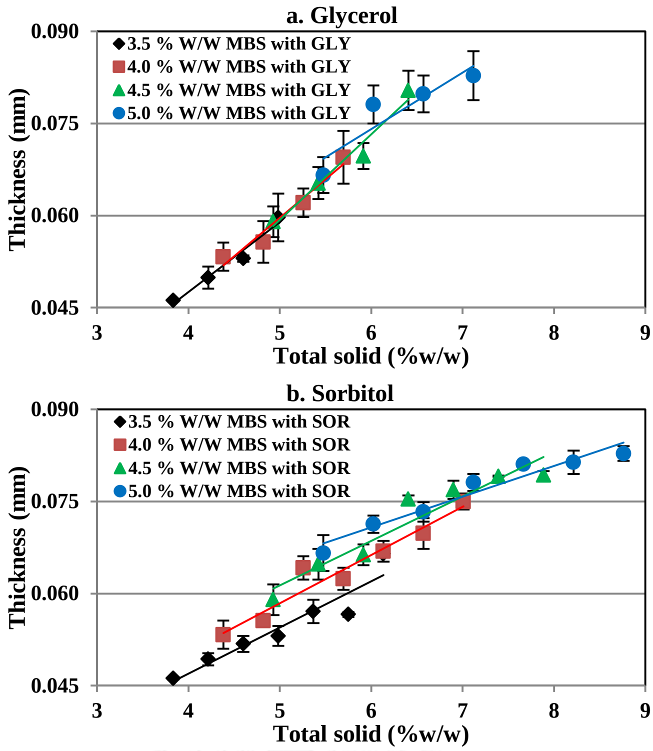


Figure 4.4 Effect of total solid (%w/w) in casting solution with glycerol (a) or sorbitol (b) on thickness (mm) of mung bean starch films.

Table 4.2 Linear relationship between total solid in casting solution of mung bean starch (MBS) film (T, %) and thickness (l, mm) of the film.

starch concentration (%w/w)	Glycerol plasticized MBS film		Sorbitol plasticized MBS film	
	Linear equation	R^2	Linear equation	R^2
3.5	$l = 0.0114T + 0.0021$	0.97	$l = 0.0075T + 0.0169$	0.90
4.0	$l = 0.0126T - 0.0031$	0.96	$l = 0.0078T + 0.0192$	0.94
4.5	$l = 0.0139T - 0.0103$	0.97	$l = 0.0073T + 0.0251$	0.91
5.0	$l = 0.0092T + 0.0190$	0.84	$l = 0.0050T + 0.0408$	0.94

4.3 Mechanical properties

Edible MBS film exhibited a good mechanical property with a wide range of tensile strength of 7.14 ± 0.95 to 46.30 ± 3.09 MPa and elongation of 2.46 ± 0.21 to 56.95 ± 4.34 %. The high mechanical strength of MBS film was by virtue of extensive attractive hydrogen bonding between film polymer molecules creating a strong cohesive network. Relatively, MBS based films were mechanically stronger than protein based films, e.g. wheat gluten (Cherian *et al.*, 1995), corn zein (Ryu *et al.*, 2002), sodium caseinate (Siew *et al.*, 1999), lactic acid casein (Chick and Ustunol, 1998) and whey protein isolate (McHugh and Krochta, 1994). Depending on type and amount of plasticizer, MBS films had similar mechanical properties to other carbohydrate biopolymer films, e.g. methyl cellulose (Donhowe and Fennema, 1993; Park *et al.*, 1993), high amylose corn starch (Ryu *et al.*, 2002), konjac glucomannan (Leuangsukrerk *et al.*, 2014).

The experimental TS and %E results of MBS films were within the literature range of starch films with the same range of amylose content (Lourdin *et al.*, 1995). The content of amylose in MBS varies from approximately 40 – 42 %, depending on varieties and seeds processing characteristics (Li *et al.*, 2011; Thao and Noomhorm, 2011). The proximate analysis revealed composition of MBS to be mainly carbohydrate (90.73 %) and moisture (8.70 %). The rest were trace amount of fat, crude fiber, protein and ash (Appendix B). Amylose is considered the key component involved in water adsorption, swelling and gelation (Wittaya, 2012), which in turn governs the properties of starch films.

The effect of starch concentration on tensile strength and % elongation of MBS films are illustrated in Figure 4.5 and Figure 4.6, respectively. In general, addition of starch concentration did not significant affect tensile strength and % elongation of MBS films at controlled plasticizer type and concentration. Although, there were some significant exceptions, for example 4.5 % MBS film was 1.1 times higher TS than those of other films at 10 % GLY level, the trends were random and the differences were relatively small. It was hypothesized that the optimized starch concentration (3.5 – 5.0 %w/w) to insure both the continuity of the films and the ease of casting process were too low and narrow to cause significant different in mechanical properties.

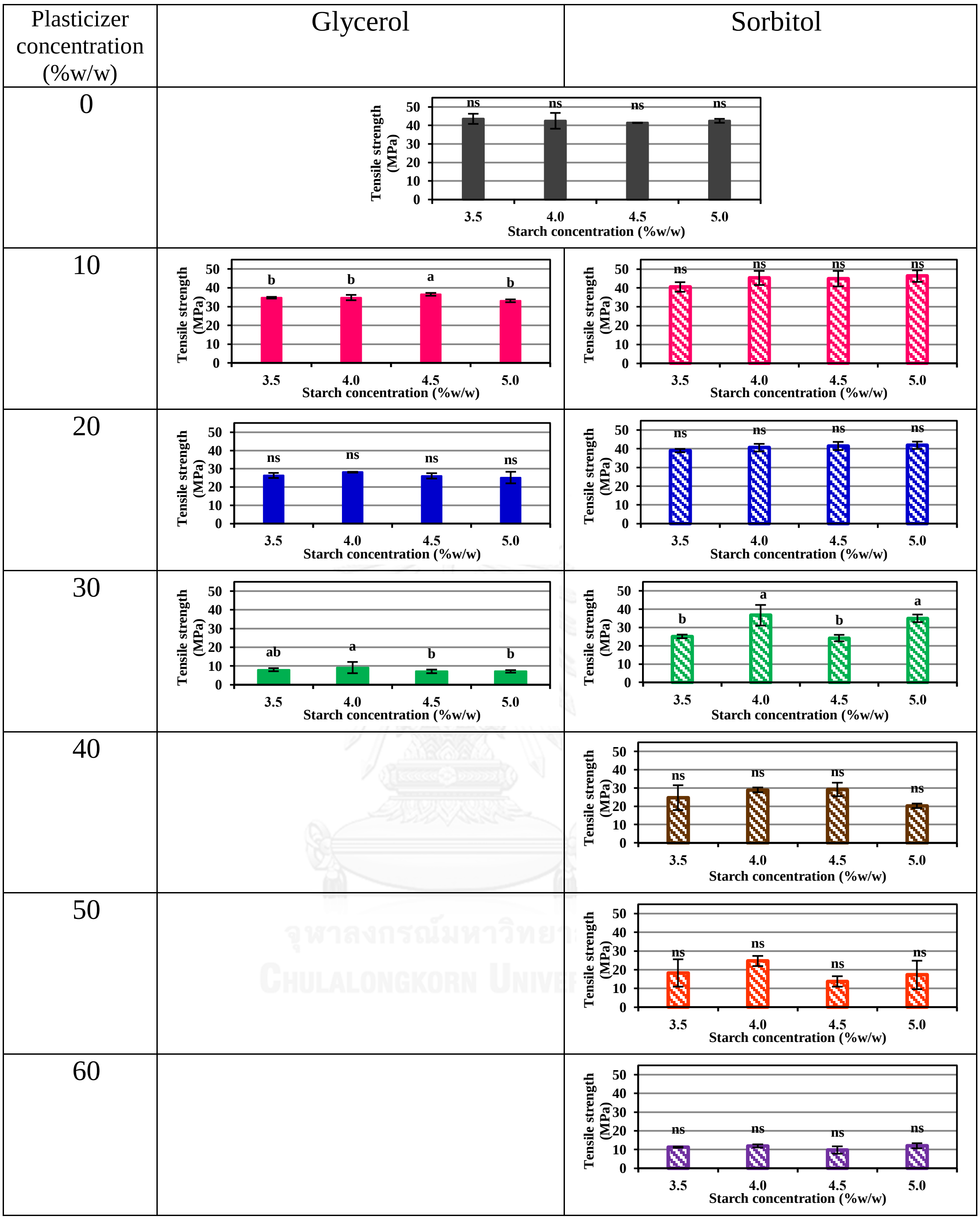


Figure 4.5 Effect of starch concentration and plasticizer type and concentration; glycerol or sorbitol on tensile strength of mung bean starch films.

Each data point represents the average value. Error bars show standard deviations. Different letters (a, b, ..) indicate significant differences between samples ($p \leq 0.05$) and (ns) means non significantly difference.

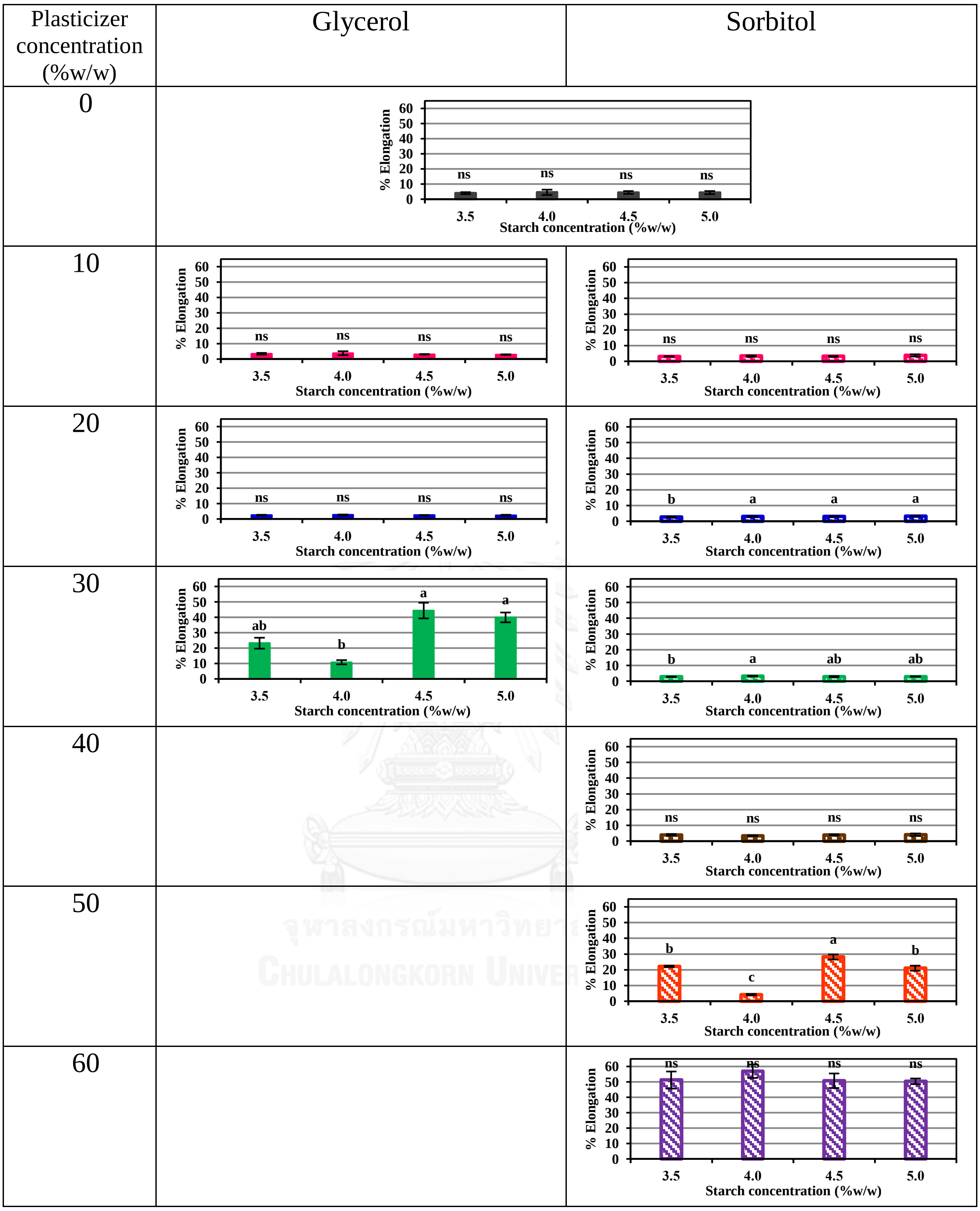


Figure 4.6 Effect of starch concentration and plasticizer type and concentration; glycerol or sorbitol on % elongation of mung bean starch films.

Each data point represents the average value. Error bars show standard deviations. Different letters (a, b, ..) indicate significant differences between samples ($p \leq 0.05$) and (ns) means non significantly difference.

Figure 4.7 and Figure 4.8 show the effect of type and concentration of plasticizers on mechanical properties of MBS films. It was found that inclusion of plasticizer tended to lower TS and elevate %E when compared to non-plasticized MBS film, regardless of starch concentration in film casting solution. The finding was consistent with previous reports (Baldwin, Nisperoscarriedo, *et al.*, 1995; Chang *et al.*, 2006; Muscat *et al.*, 2012; Talja *et al.*, 2007; Vieira *et al.*, 2011; Wojciechowska, 2012). Muscat et al., 2012 found that glycerol and/or xylitol decrease TS and increase %E of low and high amylose starches.

The highest TS and lowest %E values were observed in the unplasticized MBS film. The low flexibility film was attributed to the cohesive forces of numerous hydrogen bonds formed between amylose chains in the film network. Plasticizer was incorporated into film matrix to overcome brittleness by competing for attractive intermolecular forces resulting from polymer chain-to-chain interaction, thus increasing chain mobility and free volume (Sothornvit and Krochta, 2005; Turhan and Sahbaz, 2004). The increased plasticizer concentration also further improved film flexibility and toughness.

In this research, glycerol showed better plasticizer efficiency than sorbitol as shown by onset plasticizer concentration that significantly change TS and %E of MBS film. The onset glycerol content for TS and %E were ≥ 10% and ≥ 20 % at all controlled MBS concentration, respectively. Lourdin et al. (1995) reported the properties of plasticized films were not improved by the presence of 20 % (w/w) glycerol when amylose content was ≥ 40 %. The onset sorbitol content for TS and %E were ≥ 30% and ≥ 40 %, respectively. Furthermore, the comparisons at the same % MBS and % plasticizer showed that glycerol-plasticized films had lower TS and higher %E than those of film plasticized with sorbitol. Table 4.3 showed the linear relationship between the incorporated plasticizer and changed TS. The higher efficiency of glycerol as plasticizer for MBS films, than sorbitol, was indicated by the steeper slopes at all controlled MBS concentrations. This observation is accordance with previous report in starch based films (Wittaya, 2012).

The effectiveness of glycerol was most likely due to its smaller size (lower molecular weight) than sorbitol. A smaller molecule of glycerol allows it to insert in between the starch chains, interrupts hydrogen bonds and influence film mechanical properties more readily than sorbitol. In addition, the higher TS of sorbitol-plasticized films might have also been partially due to the fact that the total number of glycerol molecules was about double the number of sorbitol molecules, at an equal percentage of concentration (w/w) in film-forming solution.

The higher number of glycerol molecules consequently exerted more plasticizing influence on the mechanical properties than the larger sorbitol molecule. Furthermore, glycerol had higher viscosity (551.05 ± 2.47 cP) than sorbitol (143.35 ± 0.21 cP) at room temperature (25 ± 5 °C).

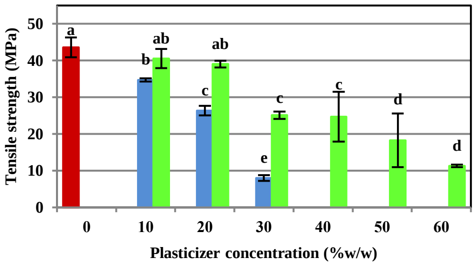
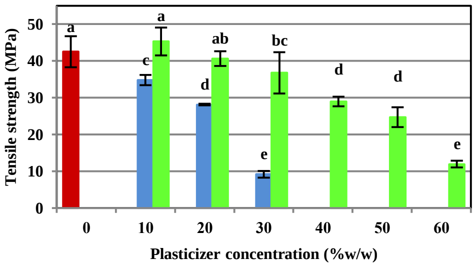
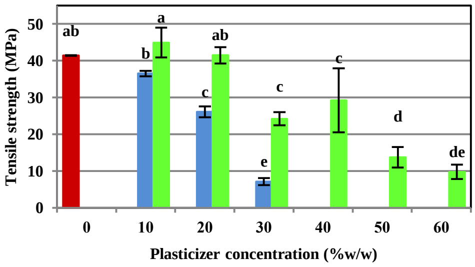
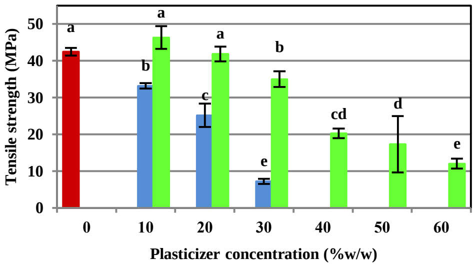
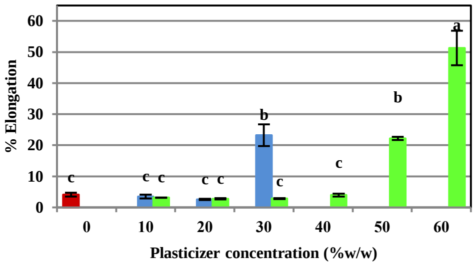
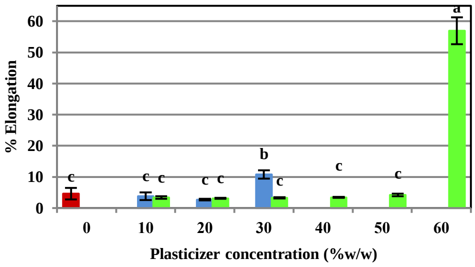
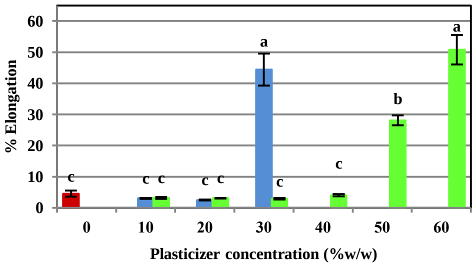
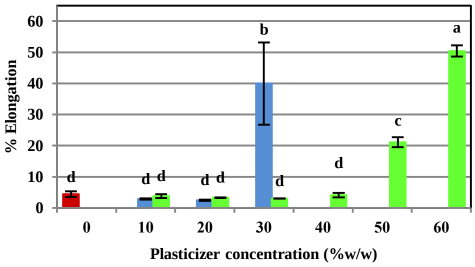
Starch concentration (%w/w)	🟥 **Plasticizer-free** 🟦 **Glycerol** 🟩 **Sorbitol**
3.5	
4.0	
4.5	
5.0	

Figure 4.7 Effect of starch concentration and plasticizer type and concentration; glycerol or sorbitol on tensile strength of mung bean starch films.
Each data point represents the average value. Error bars show standard deviations. Different letters (a, b, ..) indicate significant differences between samples ($p \leq 0.05$) and (ns) means non significantly difference.

<table>
<tr><th>Starch concentration (%w/w)</th><th>🟥 Plasticizer-free 🟦 Glycerol 🟩 Sorbitol</th></tr>
<tr><td>3.5</td><td>


</td></tr>
<tr><td>4.0</td><td>


</td></tr>
<tr><td>4.5</td><td>


</td></tr>
<tr><td>5.0</td><td>


</td></tr>
</table>

Figure 4.8 Effect of starch concentration and plasticizer type and concentration; glycerol or sorbitol on % elongation of mung bean starch films.

Each data point represents the average value. Error bars show standard deviations. Different letters (a, b, ..) indicate significant differences between samples ($p \leq 0.05$) and (ns) means non significantly difference.

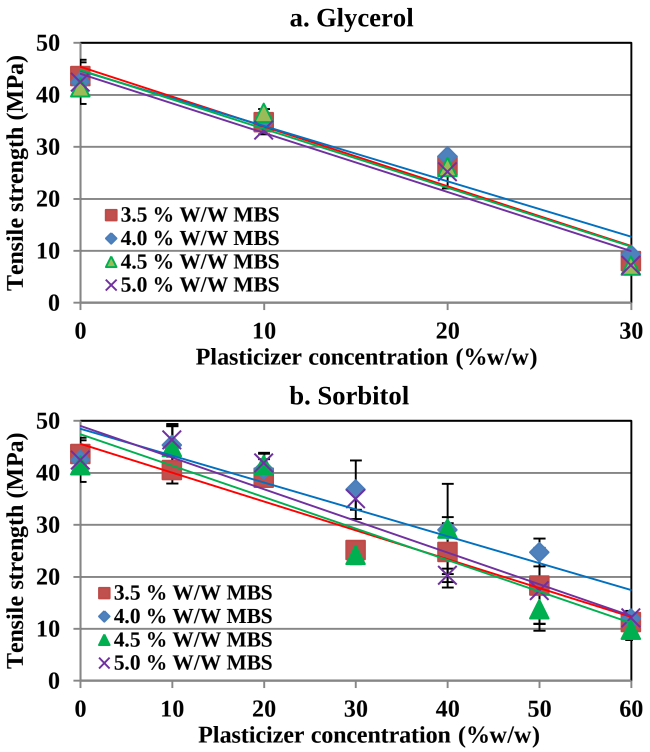


Figure 4.9 Effect of plasticizer type and concentration; glycerol (a) or sorbitol (b) on tensile strength of mung bean starch films.

Table 4.3 Linear relationship between tensile strength of mung bean starch film (TS, MPa) and incorporated plasticizer concentration ([P], % w/w).

Starch concentration (%w/w)	Glycerol plasticized MBS film		Sorbitol plasticized MBS film	
	Linear equation	R^2	Linear equation	R^2
3.5	TS = -1.1506[P] + 45.411	0.96	TS = -0.5561[P] + 45.609	0.95
4.0	TS = -1.0652[P] + 44.636	0.93	TS = -0.5164[P] + 48.459	0.89
4.5	TS = -1.1321[P] + 44.767	0.93	TS = -0.6054[P] + 47.411	0.86
5.0	TS = -1.1374[P] + 44.053	0.96	TS = -0.6102[P] + 49.046	0.90

4.4 % Solubility

The effect of starch concentration on the % solubility of MBS films is shown in Figure 4.10. The result showed that solubility of the films with 0 % plasticizer and 10 % glycerol was not affected by starch addition. For the other films, % solubility appeared to be significantly declined as a result of starch concentration. The relationship between % solubility of the plasticized film and starch concentration was fitted by empirical equation. The experimental data was best fitted with second order polynomial equation with $R^2 \geq 0.94$. The equation coefficients and coefficient of determinations are shown in Table 4.4. The decreased solubility was owing to the increase of possible chain association of glucose chain in starch solution with a higher starch concentration. As a result, concentrated aqueous solution of high amylose starch gelled with higher inter- and intrachain interactions upon cooling and form denser film with higher degree of cohesion upon evaporation. The result is consistent with previous reports in other carbohydrate systems (Laohakunjit and Noomhorm, 2004; Polnaya *et al.*, 2012; Tongdeesoontorn *et al.*, 2011).

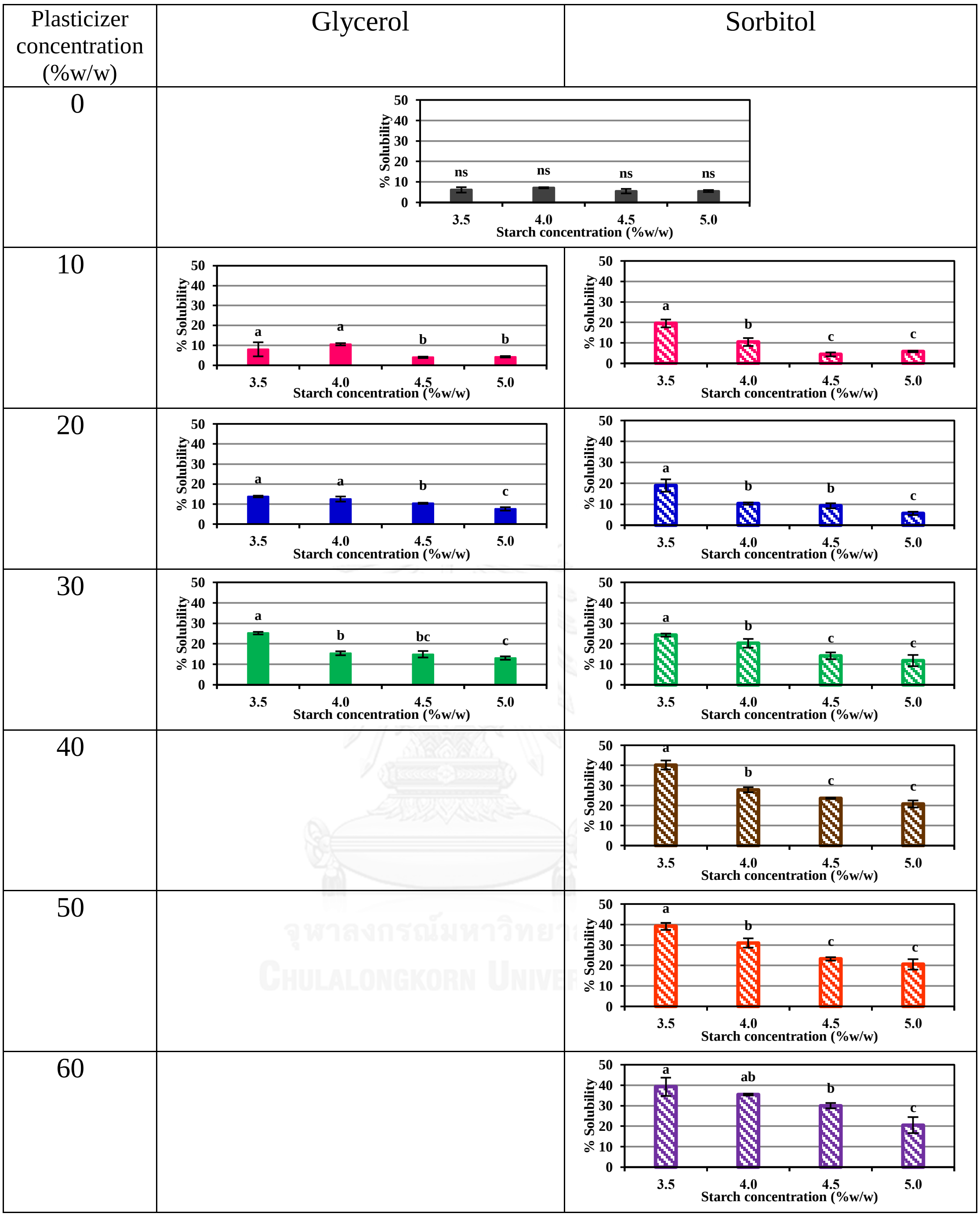


Figure 4.10 Effect of starch concentration and plasticizer type and concentration; glycerol or sorbitol on % solubility of mung bean starch films.

Each data point represents the average value. Error bars show standard deviations. Different letters (a, b, ..) indicate significant differences between samples ($p \leq 0.05$) and (ns) means non significantly difference.

Table 4.4 The equation coefficients of the effect of starch concentration [St] and plasticizer type and concentration on % solubility of mung bean starch films.

Plasticizer concentration (%w/w)	% solubility = a + b[St] + c$[St]^2$							
	Glycerol				Sorbitol			
	a	b	c	R^2	a	b	c	R^2
0	-	-	-	-	-	-	-	-
10	-	-	-	-	237.07	-98.97	10.54	0.99
20	0.03	9.56	-1.61	1.00	134.89	-50.84	5.02	0.95
30	189.52	-74.85	7.93	0.94	83.25	-22.43	1.62	0.98
40	-	-	-	-	250.88	-93.72	9.55	0.99
50	-	-	-	-	181.01	-59.91	5.55	0.99
60	-	-	-	-	-17.29	36.04	-5.69	1.00

The effect of plasticizer type and concentration on % solubility of MBS films was evaluated (Figure 4.11). The onset plasticizer concentrations giving significantly increased solubility compared to non-plasticized film were 10-20 % at 3.5 % MBS, 10 % at 4.0 % MBS, 20 % at 4.5 % MBS, and 30 % at 5.0 % MBS. The soluble matters were hypothesized to be mainly plasticizer and limited amount of starch. This is probably due to the fact that both types of hydrophilic plasticizer explored in this research had much greater water solubility than starch. Nevertheless, at concentration below the onset plasticizer concentration, plasticizers were hypothesized to interact and bind to starch chains. Furthermore, the onset plasticizer concentration was raised when film forming solution had higher starch concentration. It was hypothesized that the plasticizer migration was more difficult when film matrix was more compact with higher degree of cohesive forces between starch chains.

Figure 4.11 showed that glycerol-plasticized film tended to have significant lower % solubility than the sorbitol-plasticized film when compared at the same plasticizer concentration at low starch concentration, i.e., 10-20 % plasticizer at 3.5 % MBS and 30 % plasticizer at 4.0 % MBS. However, the effect of plasticizer type was not observed at higher level of starch concentration. This is most likely because both plasticizers exploited in this research were liquid and miscible with water.

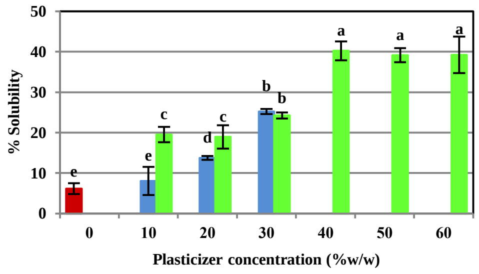
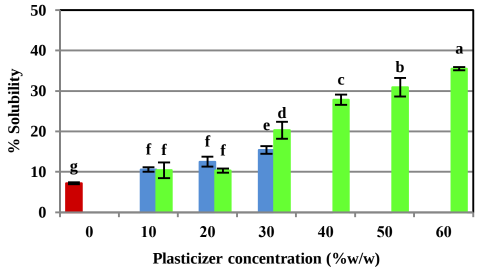
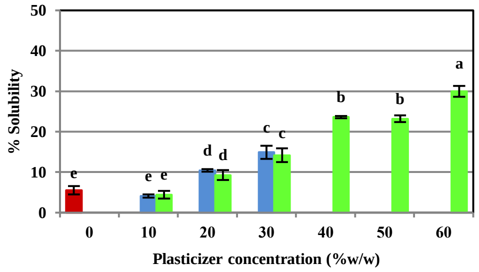
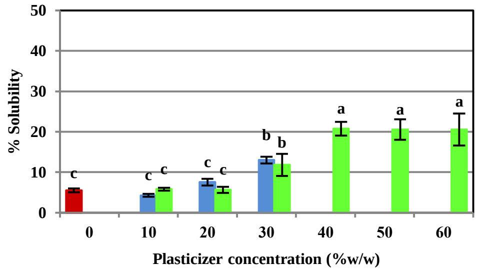
<table>
<tr><th>Starch Concentration (%w/w)</th><th>🟥 Plasticizer-free 🟦 Glycerol 🟩 Sorbitol</th></tr>
<tr><td>3.5</td><td>
</td></tr>
<tr><td>4.0</td><td>
</td></tr>
<tr><td>4.5</td><td>
</td></tr>
<tr><td>5.0</td><td>
</td></tr>
</table>

Figure 4.11 Effect of starch concentration and plasticizer type and concentration; sorbitol or glycerol on % solubility of mung bean starch films.

Each data point represents the average value. Error bars show standard deviations. Different letters (a, b, ..) indicate significant differences between samples ($p \leq 0.05$).

4.5 Water vapor permeability

The experimental result showed that MBS films were not good moisture barriers. Similar to other carbohydrate films, MBS films are characteristically hydrophilic because of numerous amounts of the functional hydroxyl groups on the polymer chains and thus exhibit poor water barrier properties. WVP of MBS films ranged from 0.200 ± 0.011 to 0.467 ± 0.023 mg·mm/Pa·hr·m^2. These values are relatively high comparing to WVP of common plastics such as polyethylene terephathalate 0.050 mg·mm/Pa·hr·m^2, low density polyethylene 0.038 mg·mm/Pa·hr·m^2 and high density polyethylene 0.010 mg·mm/Pa·hr·m^2 (Delassus, 1997).

Experimental result of WVP of MBS films significantly decreased with addition of starch concentration ($p \leq 0.05$), compared at the same type and constant concentration of plasticizer (Figure 4.12). A second order polynomial function was fitted to the WVP experimental data of plasticized MBS films at increasing starch concentration ($R^2 = 0.87 - 1.00$). The equation coefficients are shown in Table 4.5.

The decrease of WVP of MBS film as a result of increase starch concentration was due to the increased dissolved solids and amylose molecule in film-forming solution. The proximity of starch chain during gelling and drying induced by higher amylose content could facilitate the formation of denser matrix with more polymer content per area. The higher inter- and intra-molecular attractions between the starch chains were favorable allowing formation of compact structure which subsequently improved WVP of MBS films.

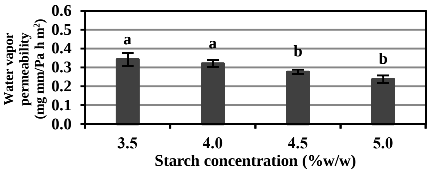
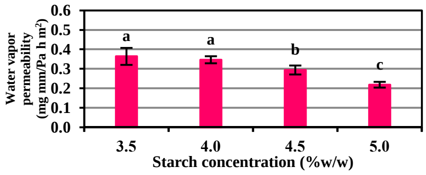
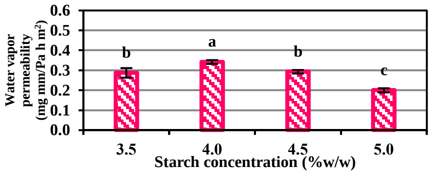
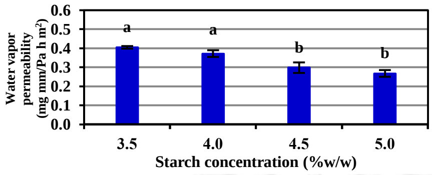
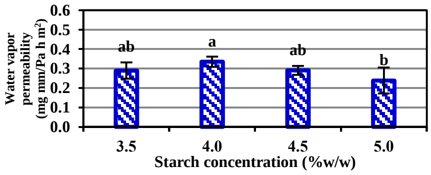
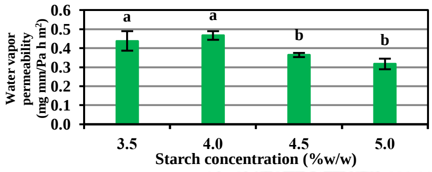
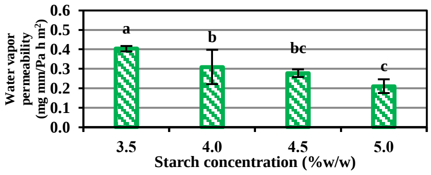
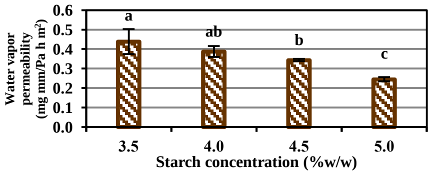
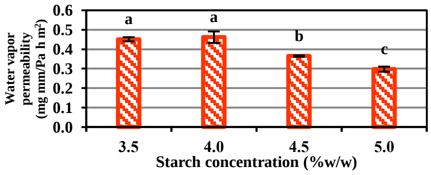
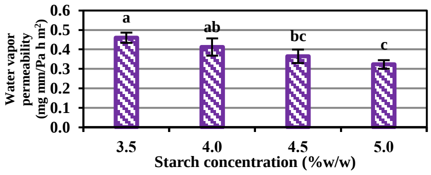
<table>
<tr><th>Plasticizer concentration (%w/w)</th><th>Glycerol</th><th>Sorbitol</th></tr>
<tr><td>0</td><td colspan="2">
</td></tr>
<tr><td>10</td><td>
</td><td>
</td></tr>
<tr><td>20</td><td>
</td><td>
</td></tr>
<tr><td>30</td><td>
</td><td>
</td></tr>
<tr><td>40</td><td></td><td>
</td></tr>
<tr><td>50</td><td></td><td>
</td></tr>
<tr><td>60</td><td></td><td>
</td></tr>
</table>

Figure 4.12 Effect of starch concentration and plasticizer type and concentration; glycerol or sorbitol on water vapor permeability (mg·mm/Pa·hr·m^2) of mung bean starch films.

Each data point represents the average value. Error bars show standard deviations. Different letters (a, b, ..) indicate significant differences between samples ($p \leq 0.05$).

Table 4.5 The equations coefficients of the effect of starch concentration [St] and plasticizer type and concentration on water vapor permeability (mg·mm/Pa·hr·m^2) of mung bean starch films.

Plasticizer concentration (%w/w)	$WVP = a + b[St] + c[St]^2$							
	Glycerol				Sorbitol			
	a	b	c	R^2	a	b	c	R^2
0	0.27	0.09	-0.02	0.99				
10	-0.31	0.40	-0.06	1.00	-2.08	1.20	-0.15	0.98
20	0.78	-0.11	0.00	0.97	-1.27	0.79	-0.10	0.93
30	-0.57	0.56	-0.08	0.87	1.30	-0.35	0.03	0.98
40	-	-	-	-	0.04	0.28	-0.05	0.99
50	-	-	-	-	-0.50	0.54	-0.08	0.95
60	-	-	-	-	0.88	-0.14	0.01	1.00

The effect of incorporation of glycerol or sorbitol at varied concentration into edible MBS films as a plasticizer on water vapor barrier was determined (Figure 4.13). Increasing concentration of plasticizer leaded to significantly increased WVP ($p \leq 0.05$). These polyol plasticizers, e.g. glycerol and sorbitol, were hypothesized to plasticize effectively because multiple hydroxyl groups on the molecules had ability to compete for active sites along polymer chains hence reducing intermolecular hydrogen bonding between neighboring polymer chains (Jesus *et al.*, 2012). As a result of increasing free volume and chain mobility in film matrix, water diffused through swelled structure with less difficulty. Similar trends have been reported in films with hydrophilic nature including carbohydrate-based matrices (Bertuzzi *et al.*, 2007) and protein-based films (Sothornvit and Krochta, 2000).

Sorbitol exhibited a lower plasticization efficiency compared to glycerol at the same concentration (Figure 4.13). The smaller plasticity of sorbitol was attributable to a larger molecule and lesser hygroscopic than glycerol. Moisture sorption of hygroscopic plasticizers plays an important role in affecting film properties because attracted water molecules can also function as supplementary plasticizers (Sothornvit and Krochta, 2005). The addition of plasticizers and absorption of water molecules by the hydrophilic plasticizers can increase polymer chain mobility and gas permeability (Jesus *et al.*, 2012). However, water is easily gained or lost due to adsorption or dehydration in high or low humidity environment which in turn fluctuate film properties.

The increased water vapor permeability of sorbitol-MBS film started to plateau at 40-60 % sorbitol (Figure 4.13). It was hypothesized that above these concentrations, excess plasticizer molecules that were not attached to polymer started to form aggregated plasticizers domains (Sothornvit and Krochta, 2001). These domains of plasticizer acted as lubricant to facilitate the movement of polymer chains over each other (Debeaufort and Voilley, 1997).

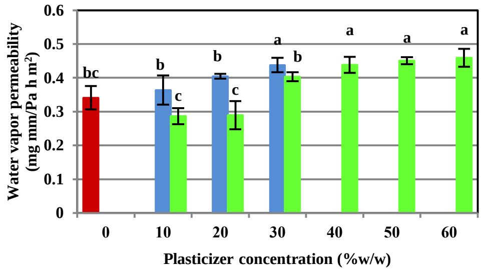
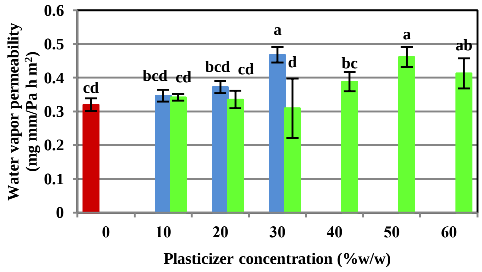
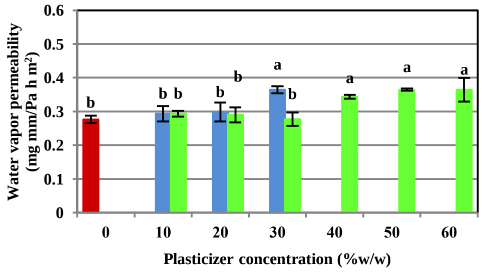
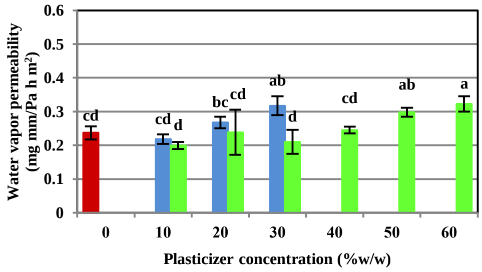
Starch Concentration (%w/w)	🟥 **Plasticizer-free** 🟦 **Glycerol** 🟩 **Sorbitol**
3.5	
4.0	
4.5	
5.0	

Figure 4.13 Effect of starch concentration and plasticizer type and concentration; sorbitol or glycerol on water vapor permeability (mg·mm/Pa·hr·m^2) of mung bean starch films.

Each data point represents the average value. Error bars show standard deviations. Different letters (a, b, ..) indicate significant differences between samples ($p \leq 0.05$).

4.6 Oxygen permeability

Although MBS-based films are hydrophilic and exhibited poor water vapor barrier property, MBS films were excellent barriers against oxygen at low-intermediate relative humidity. The oxygen permeability of non-plasticized and plasticized edible film prepared from 5 %w/w MBS ranged between 0.2397 ± 0.0365 to 1.1520 ± 0.1782 cc·µm/m^2·day·kPa. The OP values of MBS were much lower than common plastics such as low density polyethylene 970-1400 cc·µm/m^2·day·kPa, high density polyethylene 390-780 cc·µm/m^2·day·kPa and polyethylene terephthalate 12-16 cc·µm/m^2·day·kPa (Delassus, 1997). The barrier ability of MBS based film was comparable to high oxygen barrier plastics such as polyvinylidene chloride and ethylene vinyl alcohol copolymer (Delassus, 1997; Finnigan, 2009; Salame, 1986) at low to intermediate relative humidity.

The effect of plasticizers on oxygen permeability was less pronounced than other properties (Figure 4.14). From the result, it appears that addition of 20 - 30 % glycerol did not significantly increase OP of MBS film ($p \leq 0.05$). On the other hand, sorbitol film (20 – 40 %) had significantly lowered OP compared to non-plasticized film. Furthermore, sorbitol-plasticized MBS film exhibited lower oxygen permeability values than glycerol films, even when sorbitol concentration was higher. Similar trend was reported in other hydrophilic film matrices (McHugh and Krochta, 1994).

The addition of plasticizer deformed rigidity of the starch film network (Sears and Darby, 1982) causing film matrix to swell thus increasing free volume and chain movement (Daniels, 1989). However, incorporation of hydrophilic component into film matrix can improve barrier to incompatible hydrophobic permeant such as dioxygen.

The result suggested that the ability of incorporated glycerol and attracted water molecules to reduce intermolecular H-bonding along starch strands were cancelled out with their hydrophilicity in term of oxygen barrier ability of MBS films. On the other hand, the effect of sorbitol concentration on solubility was greater than that on diffusivity, thus oxygen barrier of sorbitol MBS films were improved.

This finding is beneficial to develop edible plasticized MBS films possessing favorable mechanical properties without compromising excellent barrier property the control of oxidation and respiration of food systems at dry to intermediate relative humidity.

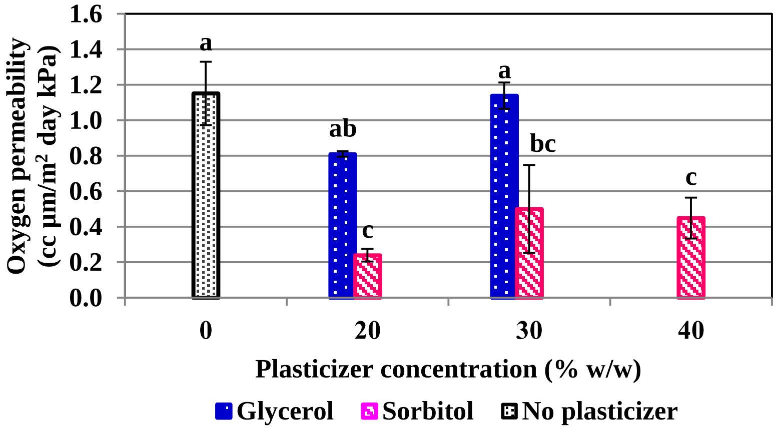


Figure 4.14 Effect of plasticizer type and concentration; sorbitol or glycerol on oxygen permeability (cc ·µm/m^2 ·day ·kPa) of mung bean starch (MBS) films prepared using 5 %w/w MBS.

Each data point represents the average value. Error bars show standard deviations. Different letters (a, b, ..) indicate significant differences between samples ($p \leq 0.05$).

According to the classification in Table 2.1, Mung bean starch films had TS of 7.14 ± 0.95 to 46.30 ± 3.09 MPa, therefore, they can be classified as marginal or good followed in TS values (TS of 1-100 MPa) and they are in the same class of low-density polyethylene (LDPE), high-density polyethylene (HDPE), polypropylene (PP) and polystyrene (PS). %E of MBS films was 2.46 ± 0.21 to 56.95 ± 4.34 which was comparable to that of oriented polypropylene (OPP) and polyvinylidene chloride (PET). WVP of MBS films ranged from 0.200 ± 0.011 to 0.467 ± 0.023 mg mm/Pa hr m^2 which can be classified as poor. OP of non-plasticized and plasticized MBS film prepared from 5 %w/w MBS ranged between 0.2397 ± 0.0365 to 1.1520 ± 0.1782 cm^3 $\mu m/m^2$ day kPa that can be classified as superior (< 10 cm^3 $\mu m/m^2$ d kPa) and they are in the same class of ethylene vinylalcohol (EVOH) and polyvinyl chloride (PVDC) (Han and Gennadios, 2005).

4.7 Thermal property

Thermal transitions of MBS-based films were explored by differential scanning calorimetry (DSC). For non-plasticized film, the endotherm with onset temperature (T_o) at 60.50 ± 3.25 ^oC and peak temperature (T_p) at 68.60 ± 2.97 ^oC was detected. Glycerol-plasticized MBS based film (10-30 % w/w) exhibited T_o from 53.60-63.50 ^oC and T_p from 69.15-70.05 ^oC. On the other hand, sorbitol-plasticized MBS films (10-40% w/w) showed T_o from 48.00-59.30 ^oC and T_p from 59.35-67.20 ^oC. All non-plasticized and plasticized MBS films exhibited a single endothermic peak with onset temperature (T_o) ranged between 48.00-63.50 ^oC and peak temperature (T_p) between 59.35-70.05 ^oC. Delville *et al.* (2003) also found the presence of endothermic peak at 70 ^oC for wheat starch based films. They reported that the endotherm represented the melting of amylopectin. The starch thermal events generally show two endotherms. The first endotherm, occurring between 50 - 120 ^oC, was attributed to the melting of amylopectin. The second transition, occurring between 120 – 170 ^oC was due to the melting of amylose (Silverio *et al.*, 1996). The thermograms of MBS films appear to correspond to the melting of amylopectin. It was hypothesized that the thermal transition involved an amylopectin specific macromolecular retrogradation or reorganization of starch molecules during storage. The melting endotherm at lower temperature was characteristic of lower ordered zones which were less organized than crystallites melting at higher temperature (De Meuter *et al.*, 1999; Delville *et al.*, 2003). Incorporation of 10 – 30 % glycerol or sorbitol did not significantly affect transition temperatures of MBS films. It was not until adding up to 40% w/w sorbitol that the T_o and T_p of MBS film started to show significant decrease.

4.8 Seal strength

Heat sealability of MBS-based films was determined near the existence of endothermic peak determined by DSC. Testing films were heat-sealed using an impulse heat-sealing technique. The important sealing variables affecting seal strength including impulse time, jaw pressure and cooling time were optimized and kept constant. The result showed that the MBS films could be optimally heat sealed at 70 ^{o}C. Above this optimal temperature, the seal distorted and became opaque. On the other hand, the seals made below optimum temperature delaminated easily. It was hypothesized that, the heat melts retrogradated structures on the surfaces of two MBS films pressed together between heated plates of impulse-sealer’s jaws during the heat sealing process. The interfacial interactions formed across the joint surface due to the application of pressure. A heat-sealed joint was produced due to re-crystallization of the starch chains during dwell time. All MBS films were heat sealable to a variable extent (Figure 4.15 and Figure 4.16). Although all MBS films were heat sealable, the seal strength of the non-, 10 % glycerol- and 10-20 % sorbitol-plasticized films could not be determined due to the associated failure mode of the seal. The sealed test strips of these film samples broke at sealing edge before the sealed area could be separated from each other by the load to yield “peeled seal” (ASTM, 2007). It was hypothesized that the heat-sealed area was stronger than the film sample causing material to break prior to peeled seal.

The result showed that change in seal strength with starch concentration had no specific trend (Figure 4.15). It was hypothesized that increase concentration of starch in film forming solution would result in decreased seal strength. The discrepancies were attributable to the increased film thickness and cohesive strength of film matrix as a result of increasing starch concentration. Films with higher thickness and/or cohesive forces typically required a longer impulse time to achieve a heat seal. Abdorreza *et al.* (2011) reported the markedly increased seal strength associated with increased sorbitol content in sago starch films.

Figure 4.16 shows the effect of plasticizer on seal strength of MBS films. As the amount of plasticizer increased, seal strength significantly increased. It appeared that plasticizer increased the mobility of the polymer chains and lower endothermic peak associated with retrograded structure of starch molecules. The result also shows that at the level of 30 % plasticizer, glycerol film showed significantly higher seal strength than sorbitol film. This was probably due to the fact that glycerol was considered more efficient plasticizer than sorbitol in MBS as previously discussed.

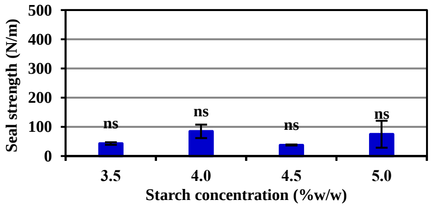
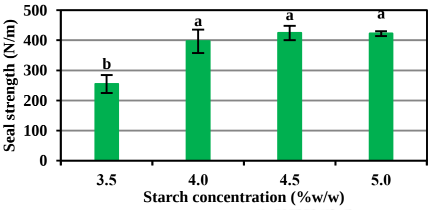
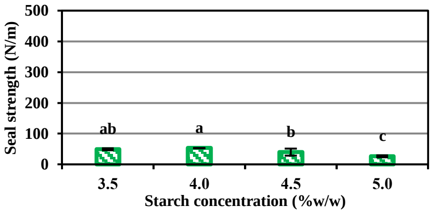
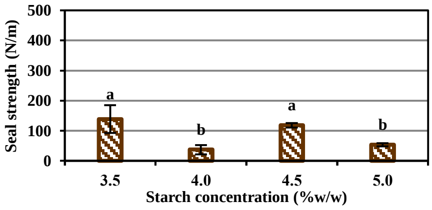
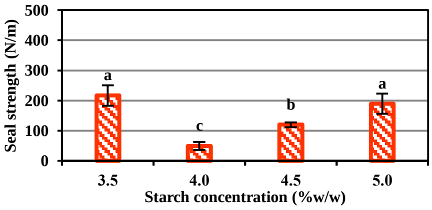
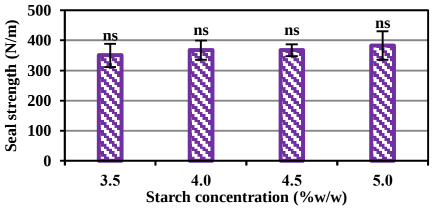
<table>
<tr><th>Plasticizer concentration (%w/w)</th><th>Glycerol</th><th>Sorbitol</th></tr>
<tr><td>20</td><td>
</td><td></td></tr>
<tr><td>30</td><td>
</td><td>
</td></tr>
<tr><td>40</td><td></td><td>
</td></tr>
<tr><td>50</td><td></td><td>
</td></tr>
<tr><td>60</td><td></td><td>
</td></tr>
</table>

Figure 4.15 Effect of starch concentration and plasticizer type and concentration; sorbitol or glycerol on seal strength (N/m) of mung bean starch films. Each data point represents the average value. Error bars show standard deviations. Different letters (a, b, ..) indicate significant differences between samples ($p \leq 0.05$) and (ns) means non significantly difference.

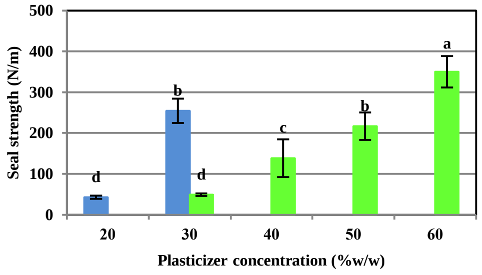
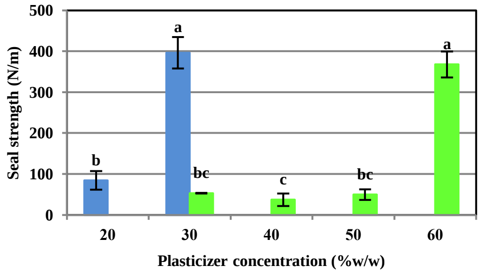
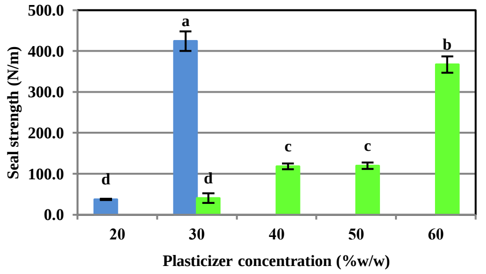
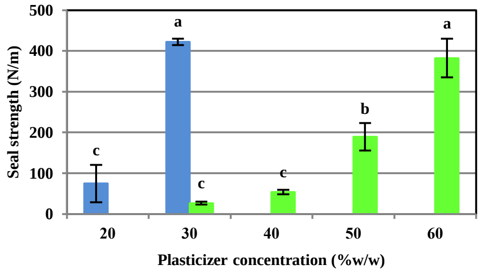
Starch Concentration (%w/w)	🟥 Plasticizer-free 🟦 Glycerol 🟩 Sorbitol
3.5	
4.0	
4.5	
5.0	

Figure 4.16 Effect of starch concentration and plasticizer type and concentration; sorbitol or glycerol on seal strength (N/m) of mung bean starch films. Each data point represents the average value. Error bars show standard deviations. Different letters (a, b, ..) indicate significant differences between samples ($p \leq 0.05$) and (ns) means non significantly difference.

4.9 Effect of storage time and temperature on physical properties and antioxidant capacity of dried chili powder packed in mung bean starch (MBS) and polypropylene (PP) sachets during storage

To demonstrate the ability of MBS film to extend shelf life of dried bird chili powder (Capsicum frutescens Linn.) aka “Prik Khee Nu” as oxygen-sensitive food model at 50 % RH, at 35-55 oC, four-side-seal pouch was made from MBS film fabricated from film formating solution containing 5 % (w/w) starch and 40 % (w/w) sorbitol. This film formulation was chosen based on the physical properties as well as oxygen barrier and heat sealability abilities. Dried chili sample packaged in four-side-seal sachet made of polypropylene (PP), a widely used flexible plastic for commercial packaged dried chili powder, was prepared and storaged for comparison.

4.9.1 Water activity and Moisture content

The effect of time and temperature on water activity (a_w) and moisture content (%MC) of dried chili powder packed inside MBS and PP sachets are shown in Figure 4.17 and Figure 4.18, respectively. The result showed the correspondent increases of a_w and %MC of packaged dried chili powder in both type of sachets over storage time at all three temperatures in accelerated storage test. The increasing trends of of a_w over storage can be fitted by either zero-order (R^2 = 0.82-1.00) or first-order reaction models (R^2 = 0.82 - 1.00) as shown in Table 4.6. Additionally, those of %MC can be fitted by either zero-order (R^2 = 0.79 - 0.99) or first-order reaction models (R^2 = 0.78 - 0.98) in like manner, as shown in Table 4.7. The moisture absorption of dried chili powder was hypothesized to be responsible for the raise of a_w and %MC of packaged samples, regardless of the packaging type. Although dried samples were packaged in flexible pouches, these polymeric films were not total barriers to gases and vapors. Over time, the dried chili powder equilibrated with moisture in the headspace, which in turn equilibrated with environment though water vapor permeation across the films.

The rates of raising a_w and %MC of MBS samples tended to increase at higher storage temperatures (Table 4.6-Table 4.7). This result indicated that water vapor barrier of MBS film was temperature-dependent in this elevated storage temperature range. Nonetheless, such changes did not follow Arrhenius behavior. On the other hand, changes of those values of PP samples tended to be independent of storage temperature. This was because changes in a_w and %MC of packaged dried chili powder in the pouches were in fact a result of a complex phenomenon of moisture equilibration between dried chili powder and headspace as well as mass transfer of water vapor across polymeric film. Both of these underlying causes were affected by temperature on the different rate.

Comparison between a_w and %MC of dried chili powder packaged in MBS and PP pouches showed that MBS sample absorbed moisture and significantly gained a_w and %MC at a faster rate than dried chili powder in PP pouch. Utilizing the predicted zero-order kinetics and target $a_w = 0.6$, the calculated shelf lives of dried chili powder packaged in MBS pouch at 35, 45 and 55 ^{o}C, were approximately 61, 60 and 44 days, respectively. On the other hand, calculated shelf lives of chili sample in PP pouch at 35, 45 and 55 ^{o}C, using the same criteria, were approximately 155, 113 and 201 days. PP is one of the best water barrier polyolefin with water vapor permeability (WVP) as low as 0.0017 mg·mm/Pa·hr·m^2 (Delassus, 1997). The water vapor transmission rate (WVTR) of testing PP film was estimated to be 0.045 mg/Pa·hr·m^2. Our result showed that WVP and WVTR of hydrophilic MBS film casted from film forming solution of 5 % starch and 40 % sorbitol was approximately 0.2451 mg·mm/Pa·hr·m^2 and 3.022 mg/Pa·hr·m^2 respectively.

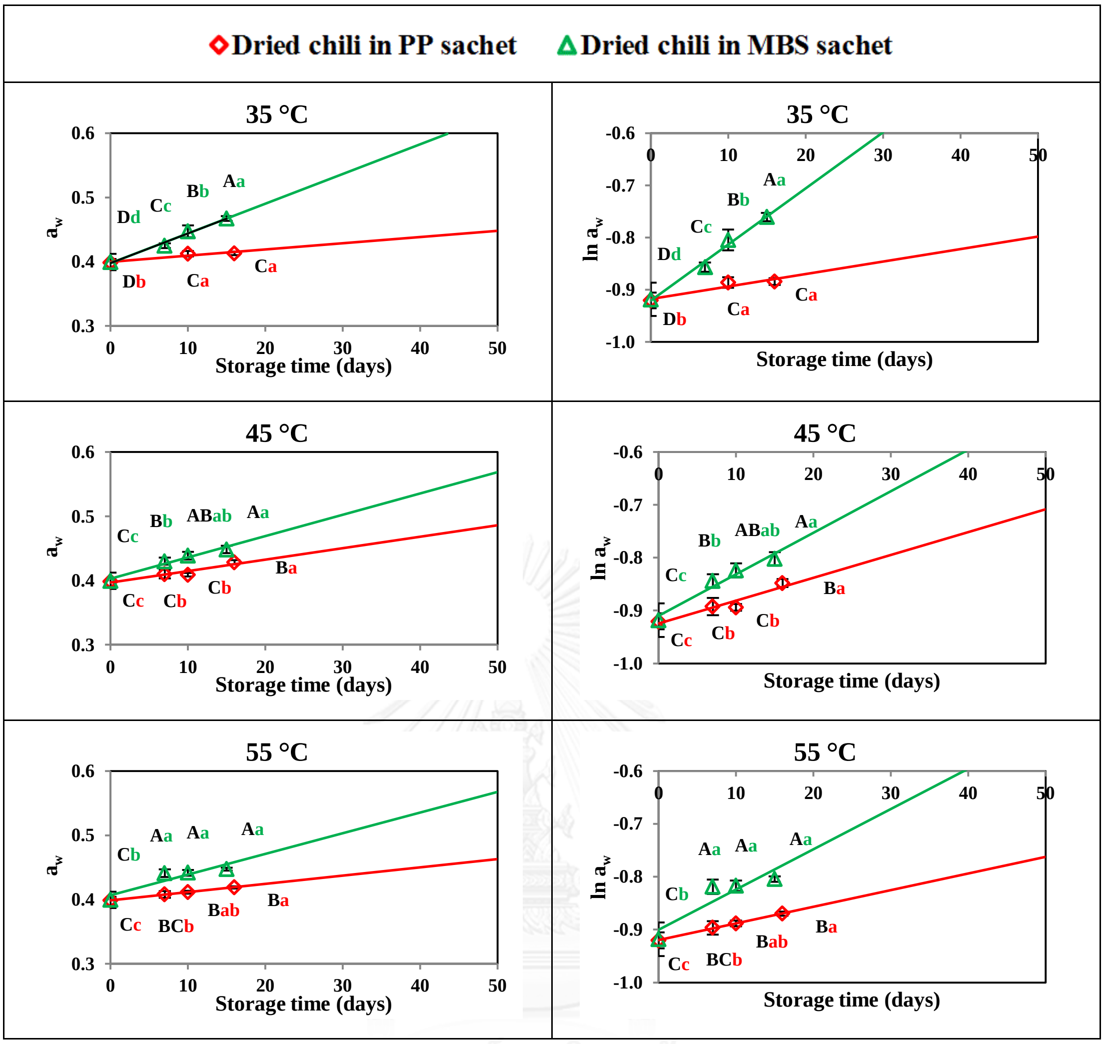


Figure 4.17 Effect of time and temperature on water activity (a_w) of dried chili powder packed in mung bean starch (MBS) and polypropylene (PP) sachets during storage.

* Each data point presents average value. Error bar shows standard deviation.

** Different small letters (a–d) indicate significant differences of packaged dried chili powder among the same sachet type during storage ($p \leq 0.05$).

*** Different capital letters (A-D) indicate significant differences between all packaged dried chili powder among the same storage temperature ($p \leq 0.05$).

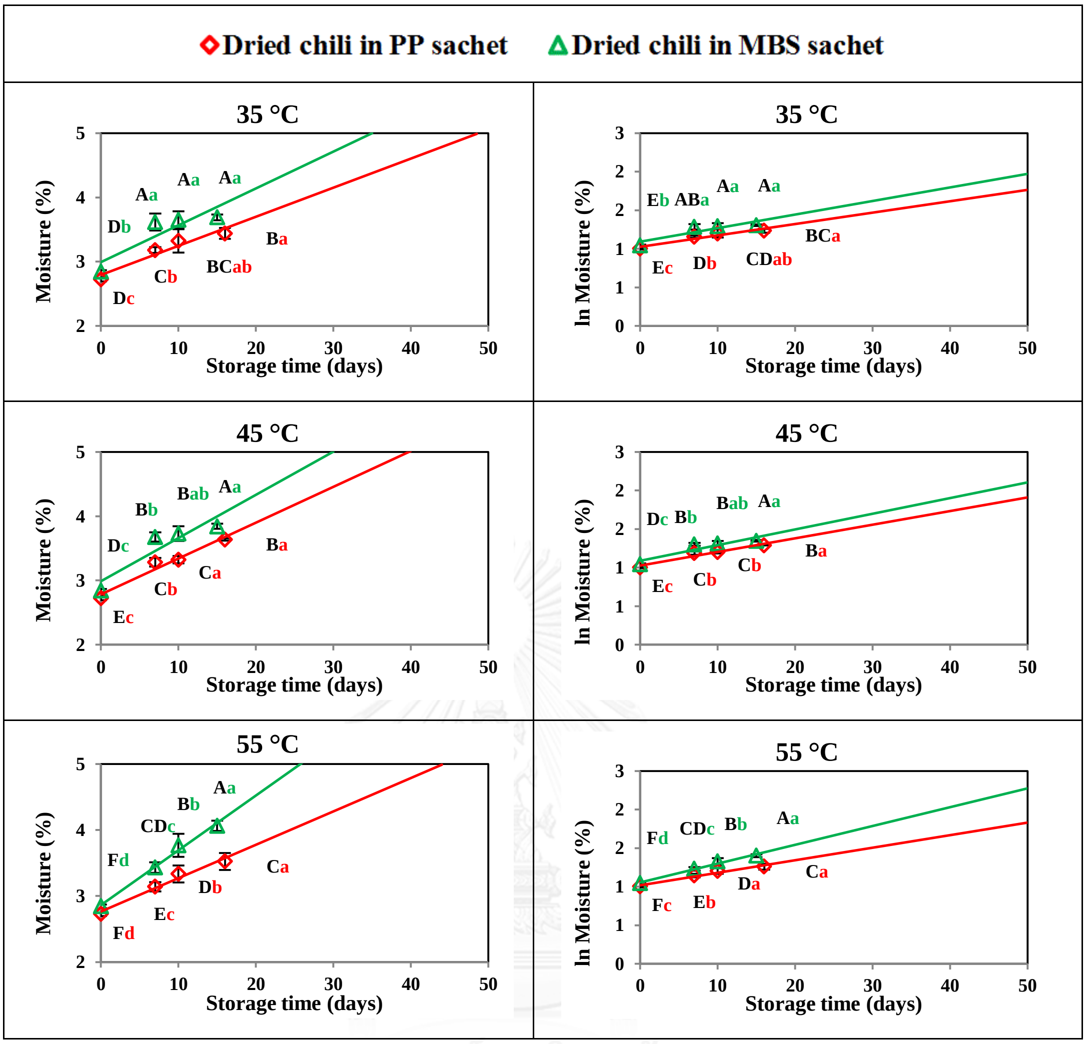


Figure 4.18 Effect of time and temperature on moisture content (%MC) of dried chili powder packed in mung bean starch (MBS) and polypropylene (PP) sachets during storage.

* Each data point presents average value. Error bar shows standard deviation.

** Different small letters (a–d) indicate significant differences of packaged dried chili powder among the same sachet type during storage ($p \leq 0.05$).

*** Different capital letters (A-F) indicate significant differences between all packaged dried chili powder among the same storage temperature ($p \leq 0.05$).

Table 4.6 Linear-model kinetics parameters for water activity (a_w) changes of dried chili powder packed in mung bean starch (MBS) and polypropylene (PP) sachets over storage time (t).

Storage Temperature (^{o}C)	Pouch	Water activity (a_w)			
		Zero-order kinetics model		First-order kinetics model	
		$a_w = a_{wo} - kt$	R^2	$\ln a_w = a_{wo} - kt$	R^2
35	MBS	$a_w = 0.4066 + 0.0032t$	0.82	$\ln a_w = - 0.9007 + 0.0076t$	0.82
	PP	$a_w = 0.3985 + 0.0013t$	1.00	$\ln a_w = - 0.9198 + 0.0032t$	1.00
45	MBS	$a_w = 0.4026 + 0.0033t$	0.96	$\ln a_w = - 0.9100 + 0.0078t$	0.95
	PP	$a_w = 0.3966 + 0.0018t$	0.91	$\ln a_w = - 0.9244 + 0.0043t$	0.91
55	MBS	$a_w = 0.3976 + 0.0046t$	0.98	$\ln a_w = - 0.9210 + 0.0108t$	0.99
	PP	$a_w = 0.3995 + 0.0010t$	0.89	$\ln a_w = - 0.9176 + 0.0024t$	0.89

Table 4.7 Linear-model kinetics parameters for moisture content (%MC) changes of dried chili powder packed in mung bean starch (MBS) and polypropylene (PP) sachets over storage time (t).

Storage Temperature (^{o}C)	Pouch	Moisture content (%MC)			
		Zero-order kinetics model		First-order kinetics model	
		$\%MC = \%MC_0 - kt$	R^2	$\ln \%MC = \%MC_0 - kt$	R^2
35	MBS	%MC = 2.9912 + 0.0573t	0.79	ln %MC = 1.0913 + 0.0176t	0.78
	PP	%MC = 2.7943 + 0.0452t	0.93	ln %MC = 1.0274 + 0.0147t	0.91
45	MBS	%MC = 2.9850 + 0.0673t	0.85	ln %MC = 1.0900 + 0.0203t	0.83
	PP	%MC = 2.7826 + 0.0557t	0.96	ln %MC = 1.0251 + 0.0177t	0.95
55	MBS	%MC = 2.8614 + 0.0831t	0.99	ln %MC = 1.0565 + 0.0243t	0.98
	PP	%MC = 2.7633 + 0.0506t	0.98	ln %MC = 1.0182 + 0.0163t	0.97

4.9.2 Color measurements

The effect of storage temperature on color of dried chili powder packaged in MBS and PP sachets were evaluated. The declining changes of L* (L* = 0 yield black and L* = 100 indicates diffuse white) and b* (negative values indicate blue and possitive values indicate yellow) values were found insignificant (data not shown). However, a* value (negative values indicate green while positive values indicate magenta) of all packaged dried chili powder decreased at all controlled storage temperatures (Figure 4.19). These decreasing trends in a* value can be fitted by zero-order reaction models ($R^2 = 0.92 - 0.99$) as shown in Table 4.8.

The total color difference (ΔE) of dried chili powder samples packed inside MBS and PP sachet were calculated to access overall color change of the samples (Figure 4.20). Corresponding to the changing trend of a* values, the ΔE of all samples increased linearly at all testing temperatures. Zero-order reaction models showed the best fit with $R^2 = 0.84$-0.97 (Figure 4.9).

The insignificant decrease of L* and b* suggested that maillard reaction was responsible to the color change of dried chili to a limited extent. Despite that fact that chili contain both amino acids and reducing sugars, the rate of non-enzymatic browning was relatively minor in lower range of a_w. The resulting color changes were hypothesized to mainly be caused by combination of pigment degradations. In *Capsicum,* fruit color is mainly determined by the composition and concentration of carotenoids in pericarp. Red fruited chili accessions accumulated six major carotenoid pigments; capsanthin, β-cryptoxanthin, β -carotene, capsorubin, zeaxanthin and antheraxanthin, at variable relative amounts (Lightbourn *et al.*, 2008; Wahyuni *et al.*, 2011). Thus, the loss of a* value was probably due to collective oxidative degradations of reddish pigment, e.g. carotenoids, in dried chili powder.

The result showed that storage temperature had significant effect on a* value and ΔE of dried chili powder packaged in MBS and PP films. Increasing temperature accelerated the degradation of chili reddish color. The change of a* value and ΔE did not follow Arrhenius equation.

It was found that the changes of a* value and ΔE were higher in dried chili powder packaged with PP than MBS samples as indicating by the higher slopes at all three storage temperatures (Table 4.8, Table 4.9). The calculated shelf lives of dried chili powder packaged in MBS pouches based on threshold detectable by human eye of $\Delta E > 3$ at 35, 45, 55 ^{o}C were 45, 40, 19 days, respectively. The correspondent calculated shelf lives of PP samples at 35, 45, 55 ^{o}C were 27, 21, 17 days, respectively. This was mainly due to the fact that PP film was not a good oxygen barrier with oxygen permeability range between 580-970 $cc\cdot\mu m/m^2\cdot day\cdot kPa$ (Delassus, 1997). Factoring in the thickness of testing PP, the oxygen transmission rate were approximately 15.5-25.9 $cc/m^2\cdot day\cdot kPa$. Our result showed that MBS film fabricated from casting solution with 5 % starch and 40 % sorbitol had a greater oxygen barrier with approximate oxygen permeability of 0.4493 $cc\cdot\mu m/m^2\cdot day\cdot kPa$ or oxygen transmission rate of 0.0055 $cc/m^2\cdot day\cdot kPa$.

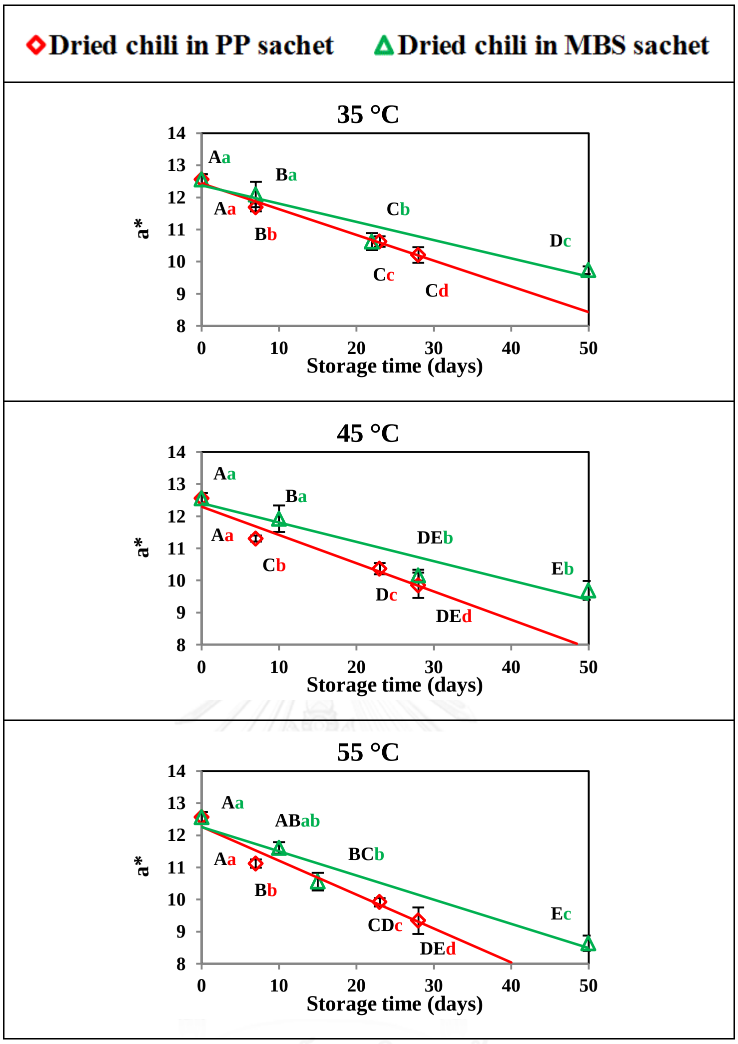


Figure 4.19 Effect of time and temperature on color values (a*) of dried chili powder packed in mung bean starch (MBS) and polypropylene (PP) sachets during storage.

* Each data point presents average value. Error bar shows standard deviation.

** Different small letters (a–d) indicate significant differences of dried chili powder packed inside among the same sachet type during storage ($p \leq 0.05$).

*** Different Capital letters (A-E) indicate significant differences between all dried chili powder packed inside the sachet among the same storage temperature used ($p \leq 0.05$).

Table 4.8 Linear-model kinetics parameters for color values (a*) changes of dried chili powder packed in mung bean starch (MBS) and polypropylene (PP) sachets over storage time

Storage Temperature (^oC)	Dried chili powder packed in	Color values (a*)	
		Zero-order kinetics model	
		$a^* = a^*_0 - kt$	R^2
35	MBS	a* = 12.377-0.0569t	0.93
	PP	a* = 12.432-0.0800t	0.99
45	MBS	a* = 12.408-0.0602t	0.92
	PP	a* = 12.295-0.088t	0.95
55	MBS	a* = 12.251-0.0752t	0.95
	PP	a* = 12.267-0.1057t	0.96

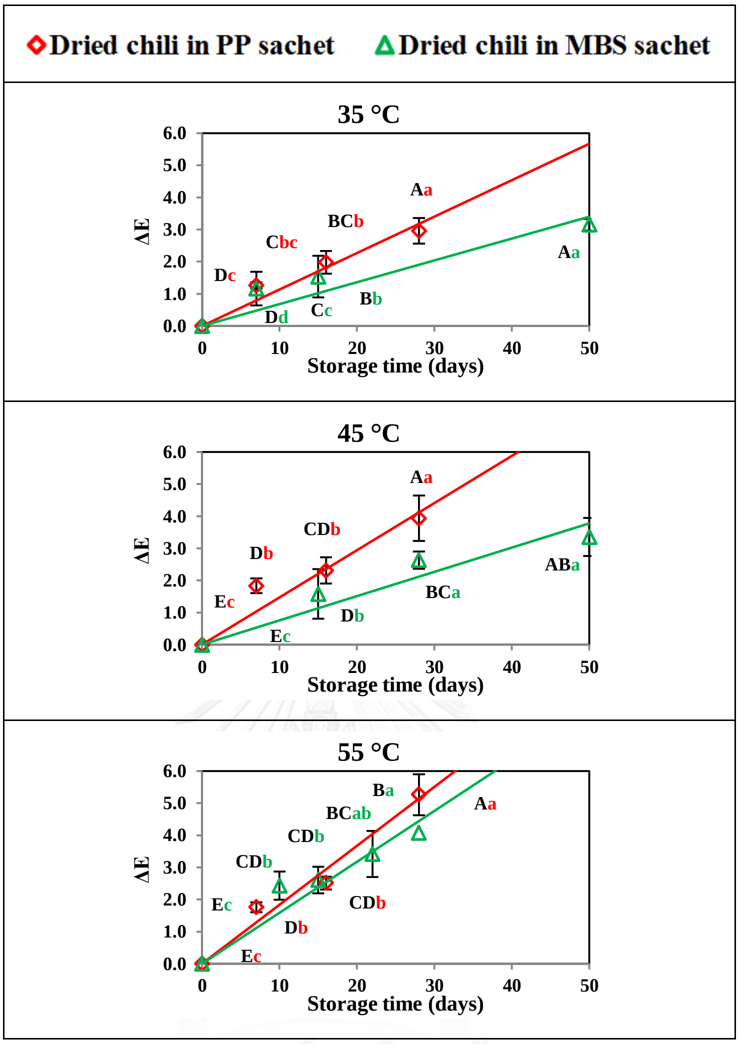


Figure 4.20 Effect of time and temperature on total color difference (ΔE) of dried chili powder packed in mung bean starch (MBS) and polypropylene (PP) sachets during storage.

* Each data point presents average value. Error bar shows standard deviation.

** Different small letters (a–d) indicate significant differences of packaged dried chili powder among the same sachet type during storage ($p \leq 0.05$).

*** Different capital letters (A-E) indicate significant differences between all packaged dried chili powder among the same storage temperature ($p \leq 0.05$).

Table 4.9 Linear-model kinetics parameters for total color difference (ΔE) changes of dried chili powder packed in mung bean starch (MBS) and polypropylene (PP) sachets over storage time (t).

Storage Temperature (^oC)	Dried chili powder packed in	Total color difference (ΔE)	
		Zero-order kinetics model	
		ΔE = kt	R^2
35	MBS	ΔE = 0.0680t	0.84
	PP	ΔE = 0.1134t	0.94
45	MBS	ΔE = 0.0755t	0.90
	PP	ΔE = 0.1469t	0.91
55	MBS	ΔE = 0.1586t	0.85
	PP	ΔE = 0.1835t	0.97

4.9.3 Antioxidant capacity

Chili is well-known as the rich source of vitamin C, vitamin E, provitamin A, carotenoids, flavonoids, capsaicin and other phenolic compounds which varied depending on many factors, e.g. varieties, stage of harvest, process (Navarro *et al.*, 2006; Perucka and Materska, 2007; Wahyuni *et al.*, 2011; Wangcharoen and Morasuk, 2009). To investigate the ability of MBS film to prevent chili phytochemicals from chemical oxidation, total phenolic content (TPC) and antioxidant activity were analyzed by two methods; determination of 2,2-diphenyl-1-picrylhydrazyl radical scavening activity (DPPH) and ferric ion reducing antioxidant power (FRAP).

The effect of storage time and temperature on TPC, DPPH and FRAP of dried chili powder packaged in MBS and PP sachet are shown in Figure 4.21**,** Figure 4.22, Figure 4.23, respectively. The result showed that, TPC of dried chili powder in all sachets significantly decreased over storage time at all three testing temperatures. The linearly decreased trends of TPC, DPPH and FRAP of packaged dried chilli powder were best mathematically fitted by zero models in Table 4.10, Table 4.12 and Table 4.14, respectively. The decreasing trends and good correlation between TPC and antioxidant activity were expected as a result of oxidative degradations of the dietary antioxidants in chili, e.g. ascorbic acid, lycopene, β-carotene, quercetin and phenolic compounds, over time.

The result showed that only the degradations of TPC of PP sample and DPPH of MBS- and PP-packaged dried chili powder were found obeyed Arrhenius relationship with $R^2 > 0.95$ (Table 4.11, Table 4.13 and Table 4.15). The discrepancies from the Arrhenius behavior of the other samples were attributable to the fact that the decreases of TPC and antioxidant activity of packaged chili was a empirical result of collective complex reactions and phenomena, e.g. development or chemical oxidation of variable oxidizable substrates by different oxidation mechanisms, as well as ingress mass transport of water vapor and oxygen through polymeric film. Water vapor and oxygen permeations of polymeric film are generally raised as a result of increase molecular mobility at elevated temperatures. To complicate the outcome, unlike stable petroleum-based plastics, vapor and gas permeabilities of biopolymer films, especially edible and biodegradable films, were typically hypothesized to change as a result of variable reactions and phenomena including, but not limited to morphological changes, molecular rearrangement and microbial growth and activities, over storage time.

The degradation rates of TPC and antioxidant activity of MBS sample were found lower than those of dried chili powder packaged in PP pouch at all storage temperatures. The better protection of MBS film against oxidation than PP film was hypothesized to be mainly due to the lower oxygen permeability.

The different mechanisms of analytical methods were explained the different results of the antioxidant capacity assessment of the studied chili as determined by FRAP and DPPH. FRAP measures the ability to reduce a ferric tripyridyltriazine (Fe^{3+}-TPTZ) to a ferrous form (Fe^{2+}-TPTZ) of samples (Leong and Shui, 2002). DPPH is based on the reduction of DPPH free radicals of samples (Brand-William *et al.*, 1995b; Wangcharoen and Morasuk, 2007). DPPH and FRAP are widely used to determine the antioxidant capacity in chili extracts due to their simplicity, stability and reproducibility (Reddy *et al.*, 2010). Relatively poor correlations was found between DPPH and FRAP. A major determinant of the DPPH is steric accessibility which might probably result in this discrepancy between the results obtained with DPPH and FRAP. Small molecules have better access to the radical site, they show higher apparent antioxidant capacity with DPPH (Lu *et al.*, 2011; Prior *et al.*, 2005).

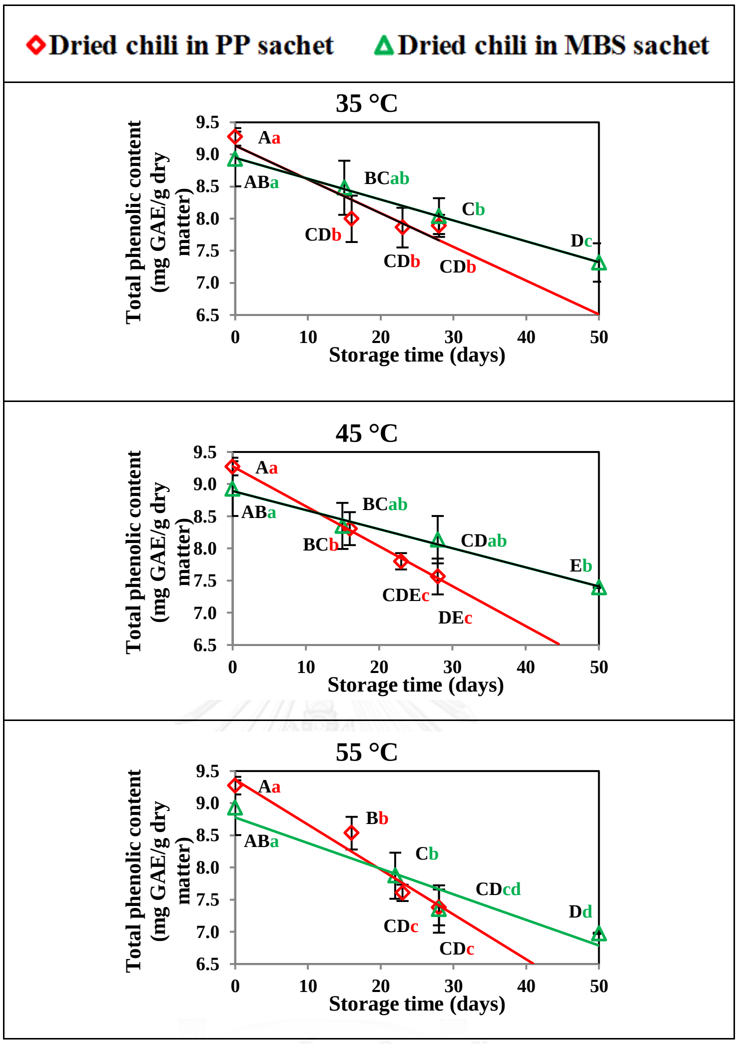


Figure 4.21 Effect of time and temperature on total phenolic content (TPC) of dried chili packed in mung bean starch (MBS) and polypropylene (PP) sachets during storage.

* Each data point presents average value. Error bar shows standard deviation.

** Different small letters (a–d) indicate significant differences of packaged dried chili among the same sachet type during storage ($p \leq 0.05$).

*** Different capital letters (A-D) indicate significant differences between all packaged dried chili among the same storage temperature ($p \leq 0.05$).

Table 4.10 Linear-model kinetics parameters for total phenolic content (TPC) changes of dried chili packed in mung bean starch (MBS) and polypropylene (PP) sachets during storage.

Storage Temperature (^{o}C)	Pouch	Total Phenolic content (TPC)	
		Zero-order kinetics model	
		$TPC = TPC_0 - kt$	R^2
35	MBS	TPC = 8.9449 - 0.03244t	1.00
	PP	TPC = 9.1329 - 0.05249t	0.88
45	MBS	TPC = 8.8932 - 0.02968t	0.99
	PP	TPC = 9.2733 - 0.06196t	1.00
55	MBS	TPC = 8.7762 - 0.03972t	0.93
	PP	TPC = 9.3656 - 0.06973t	0.95

Table 4.11 Arrhenius parameters for total phenolic content (TPC) changes of dried chili packed in mung bean starch (MBS) and polypropylene (PP) sachets over storage time (t).

Kinetic model	Pouch	Total Phenolic content (TPC)			
		$\log k = -(E/2.3RT) + \log k_0$	Arrhenius constant (k_0)	Ea (kJ/mol)	R^2
Zero-order	MBS	log k = -435.26(1/T) – 0.1029	1.12	8323.13	0.44
	PP	log k = -623.92(1/T) + 0.7485	2.11	11930.72	0.99

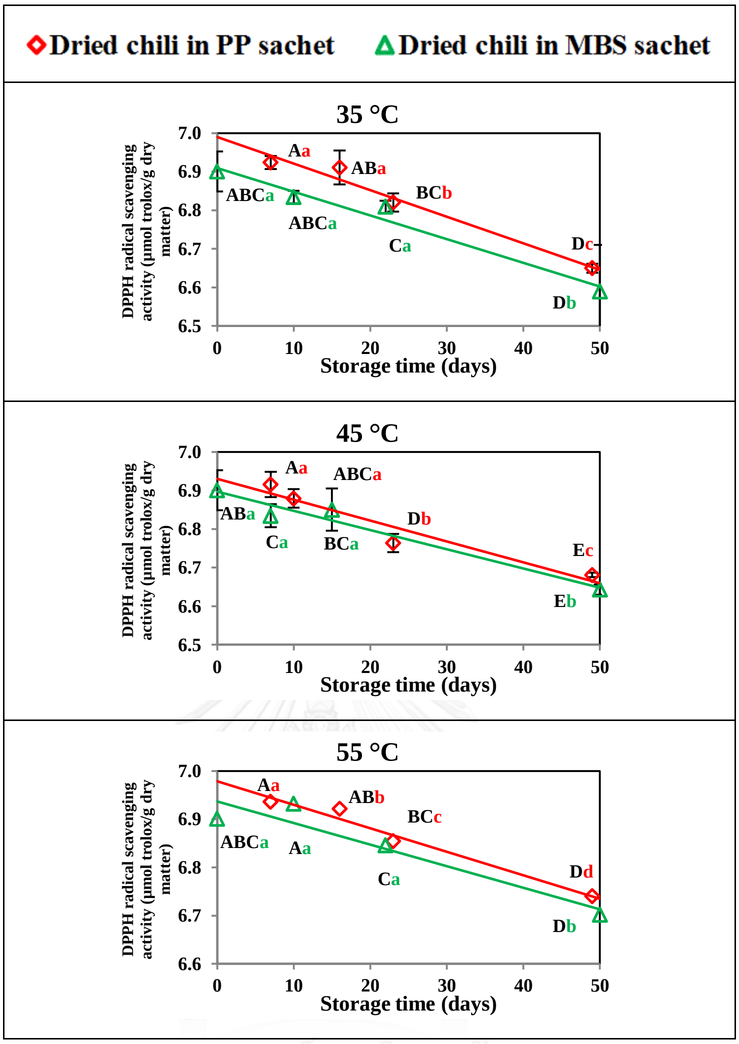


Figure 4.22 Effect of time and temperature on DPPH radical scavenging activity (DPPH) of dried chili packed in mung bean starch (MBS) and polypropylene (PP) sachets during storage.

* Each data point presents average value. Error bar shows standard deviation.

** Different small letters (a–d) indicate significant differences of packaged dried chili among the same sachet type during storage ($p \leq 0.05$).

*** Different capital letters (A-D) indicate significant differences between all packaged dried chili among the same storage temperature ($p \leq 0.05$).

Table 4.12 Linear-model kinetics parameters for DPPH radical scavenging activity (DPPH) changes of dried chili packed in mung bean starch (MBS) and polypropylene (PP) sachets over storage time (t).

Storage Temperature (^oC)	Pouch	DPPH radical scavenging activity (DPPH)	
		Zero-order kinetics model	
		$DPPH = DPPH_0 - kt$	R^2
35	MBS	DPPH = 6.9093-0.00614t	0.97
	PP	DPPH = 6.9898-0.00689t	0.97
45	MBS	DPPH = 6.8968-0.00498t	0.96
	PP	DPPH = 6.9302-0.00541t	0.93
55	MBS	DPPH = 6.9365-0.00447t	0.90
	PP	DPPH = 6.9783 – 0.00490t	0.97

Table 4.13 Arrhenius parameters for DPPH radical scavenging activity (DPPH) changes of dried chili packed in mung bean starch (MBS) and polypropylene (PP) sachets during storage.

Kinetic model	Pouch	DPPH radical scavenging activity (DPPH)			
		$\log k = -(E/2.3RT) + \log k_0$	Arrhenius constant (k_0)	Ea (kJ/mol)	R^2
Zero-order	MBS	log k = 698.46(1/T) – 4.486	88.77	13356.09	0.97
	PP	log k = 750.73(1/T) – 4.608	100.33	14355.61	0.95

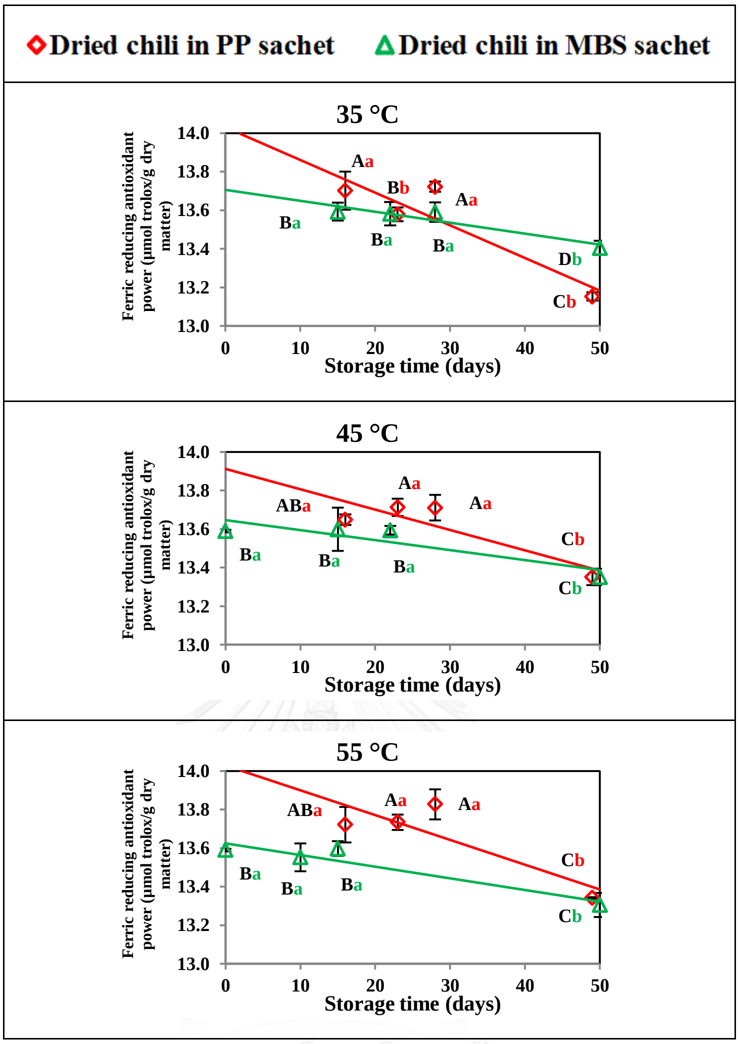


Figure 4.23 Effect of time and temperature on ferric reducing antioxidant power (FRAP) of dried chili packed in mung bean starch (MBS) and polypropylene (PP) sachets during storage.

** Different small letters (a–d) indicate significant differences of packaged dried chili among the same sachet type during storage ($p \leq 0.05$).

*** Different capital letters (A-D) indicate significant differences between all packaged dried chili among the same storage temperature ($p \leq 0.05$).

Table 4.14 Linear-model kinetics parameters for ferric reducing antioxidant power (FRAP) changes of dried chili packed in mung bean starch (MBS) and polypropylene (PP) sachets during storage.

Storage Temperature (^oC)	Pouch	Ferric reducing antioxidant power (FRAP)	
		Zero-order kinetics model	
		$FRAP = FRAP_0 - kt$	R^2
35	MBS	FRAP = 13.706 - 0.00570t	0.88
	PP	FRAP = 14.029 - 0.01692t	0.82
45	MBS	FRAP = 13.646 - 0.00516t	0.79
	PP	FRAP = 13.911 - 0.01053t	0.76
55	MBS	FRAP = 13.625 - 0.00606t	0.90
	PP	FRAP = 14.029 - 0.01287t	0.72

Table 4.15 Arrhenius parameters for ferric reducing antioxidant power (FRAP) changes of dried chili packed in mung bean starch (MBS) and polypropylene (PP) sachets during storage.

Kinetic model	Pouch	Ferric reducing antioxidant power (FRAP)			
		$\log k = -(E/2.3RT) + \log k_0$	Arrhenius constant (k_0)	Ea (kJ/mol)	R^2
Zero-order	MBS	log k = -128.32(1/T) - 1.8459	6.33	2453.76	0.13
	PP	log k = 615.51(1/T) - 3.8167	45.45	11769.91	0.35

CHAPTER V
CONCLUSION

5.1 Conclusion

The increasing starch concentration significantly increased thickness, decreased % solubility and WVP but did not affect TS, %E and seal strength of the films at controlled plasticizer type and concentration ($p \leq 0.05$). At controlled starch concentration, increasing the plasticizer concentration increased thickness, %E, % solubility, WVP and seal strength, decreased TS and OP of films ($p \leq 0.05$). The onset of the glycerol or sorbitol concentrations of ≥ 20 and ≥ 40 %w/w, respectively, was required to form a flexible film for wrapping without breaking. At the same starch and plasticizer concentrations, sorbitol-plasticized films had lower thickness, %E, WVP, OP and seal strength, and higher TS than those of glycerol-plasticized films.

The ability of MBS film to prolong antioxidant activity of dried chili powder, sachet of 5% %w/w starch and 40 %w/w sorbitol-plasticized MBS film was a better package to protect antioxidant activity compared to PP sachet.

5.2 Suggestion

1) To develop the properties of mung bean starch film, the effect of combined plasticizers should be investigated

2) Moisture sorption isotherm should be investigated for helping to develop the body of knowledge necessary in order for the food industry and the customer to fully benefit from the application of mung bean starch film.

REFERENCES

Abdel-Rahman, E.-S. A., El-Fishawy, F. A., El-Geddawy, M. A., Kurz, T., and El-Rify, M. N. (2008). Isolation and physicochemical characterization of mung bean starches. *International Journal of Food Engineering, 4*(1).

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 Hydrocolloids, 25*(1), 56-60.

Adhikari, B., Chaudhary, D. S., and Clerfeuille, E. (2010). Effect of plasticisers in the moisture migration behaviour of low-amylose starch films during drying. *Drying Technology, 28,* 468–480.

Alves, D. V., Mali, S., Beleia, A., and Grossmann, E. M. (2007). Effect of glycerol and amylose enrichment on cassava starch film properties. *Journal of Food Engineering, 78*(3), 941-946.

AOAC. (2010). Cereal foods. In W. Horwitz andG. W. Latimer (Eds.), *Official methods of analysis of AOAC international* (18th ed.): Gaithersburg, M. D., AOAC International.

ASTM. (1995a). Standard test method for oxygen gas transmission rate through plastic film and sheeting using a coulometric sensor. D3985-95 *Annual Book of American Standard Testing Methods* (pp. 784). Philadelphia, Pa: American Society for Testing and Materials.

ASTM. (1995b). Standard test methods for water vapor transmission of materials. E96-95 *Annual Book of American Standard Testing Methods* (pp. 785-792). United States of America: American Society for Testing and Materials.

ASTM. (2002). Standard test method for tensile properties of thin plastic sheeting. D882-02 *Annual Book of American Standard Testing Methods* (pp. 1-10). United States of America: American Society for Testing and Material.

ASTM. (2007). Standard test method for seal strength of flexible barrier materials. F 88-07 *Annual Book of American Standard Testing Methods* (pp. 768-777). United States: American Society for Testing and Materials.

Bae, H. J., Cha, D. S., Whiteside, W. S., and Park, H. J. (2008). Film and pharmaceutical hard capsule formation properties of mungbean, waterchestnut, and sweet potato starches. *Food Chemistry, 106*(1), 96-105.

Baldwin, E., Nisperos-Carriedo, M., and Baker, R. (1995). Edible coatings for lightly processed fruits and vegetables. *HortScience, 30*(1), 35-38.

Baldwin, E. A., Nisperoscarriedo, M. O., and Baker, R. A. (1995). Edible coatings for lightly processed fruits and vegetables. *Hortscience, 30*(1), 35-38.

Banker, G. S. (1966). Film coating theory and practice. *Pharmaceutical Sciences, 55*(1), 81-89.

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. *Analytical Biochemistry, 239*(1), 70-76.

Bertuzzi, M. A., Vidaurre, E. F. C., Armada, M., and Gottifredi, J. C. (2007). Water vapor permeability of edible starch based films. *Journal of Food Engineering, 80*(3), 972-978.

Bordes, P., Pollet, E., and Averous, L. (2009). Nano-biocomposites: Biodegradable polyester/nanoclay systems. *Progress in Polymer Science, 34*(2), 125-155.

Bourtoom, T. (2008). Factors affecting the properties of edible film prepared from mung bean proteins. *International Food Research Journal, 15*(2), 167-180.

Brand-William, W., Cuelier, M., and Berset, M. E. (1995a). Use of free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft und -Technologie, 28*, 25-30.

Brand-William, W., Cuelier, M., and Berset, M. E. (1995b). Use of free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft und -Technologie, 28*, 25-30.

Chang, Y. P., Abd Karim, A., and Seow, C. C. (2006). Interactive plasticizing–antiplasticizing effects of water and glycerol on the tensile properties of tapioca starch films. *Food Hydrocolloids, 20*(1), 1-8.

Cherian, G., Gennadios, A., Weller, C., and Chinachoti, P. (1995). Thermomechanical behavior of wheat gluten films: effect of sucrose, glycerin, and sorbitol. *Cereal Chemistry, 72*(1), 1-6.

Chick, J., and Ustunol, Z. (1998). Mechanical and barrier properties of lactic acid and rennet precipitated casein-based edible films. *Journal of food science, 63*(6), 1024-1027.

Colussi, R., Pinto, V. Z., Halal, S. L. M. E., Zavareze, E. D. R., and Dias, A. R. G. (2014). Physical, mechanical, and thermal properties of biodegradables films of rice starch. *Current Agricultural Science and Technology, 20*, 1-9.

Dai, H., Chang, P. R., Geng, F., Yu, J., and Ma, X. (2010). Preparation and properties of starch-based film using N,N-bis(2-hydroxyethyl) formamide as a new plasticizer. *Carbohydrate Polymers, 79*(2), 306–311.

Daniels, C. A. (1989). *Polymers: structure and properties*. Lancaster, U.S.A.: CRC Press.

Daniels, R. (1973). *Edible coatings and soluble packaging*. Park Ridge, NJ: Noyes Data Corporation.

De Meuter, P., Amelrijckx, J., Rahier, H., and Van Mele, B. (1999). Isothermal crystallization of concentrated amorphous starch systems measured by modulated differential scanning calorimetry. *Journal of Polymer Science Part B: Polymer Physics, 37*(20), 2881-2892.

Debeaufort, F., and Voilley, A. (1997). Methylcellulose-based edible films and coatings: 2. Mechanical and thermal properties as a function of plasticizer content. *Journal of Agricultural and Food Chemistry, 45*(3), 685-689.

Delassus, P. (1997). Barrier polymers. In A. L. Brody andK. S. Marsh (Eds.), *The Wiley encyclopedia of packaging technology* (pp. 71-77). New York: Wiley.

Delville, J., Joly, C., Dole, P., and Bliard, C. (2003). Influence of photocrosslinking on the retrogradation of wheat starch based films. *Carbohydrate Polymers, 53*(4), 373-381.

Donhowe, I. G., and Fennema, O. (1993). The effects of plasticizers on crystallinity, permeability, and mechanical properties of methylcellulose films. *Journal of Food Processing and Preservation, 17*(4), 247-257.

Dureja, H., Khatak, S., Khatak, M., and Kalra, M. (2011). Amylose rich starch as an aqueous based pharmaceutical coating material review. *International Journal of Pharmaceutical Science and Drug Research, 3*(1), 8-12.

Durrani, C. M., and Donald, A. M. (1995). Physical characterisation of amylopectin gels. *Polymer Gels and Networks, 3*, 1-27.

Elias, H. G. (1997). *An introduction to polymer science*. Weinheim (Federal Republic of Germany) and New York, USA: VCH Verlagsgesellschaft mbH and VCH Publisher.

Embuscado, M. E., and Huber, K. C. (2009). *Edible films and coatings for food applications*. New York: Springer Science+Business Media.

Felton, L. A. (2013). Mechanisms of polymeric film formation. *Int J Pharm, 457*(2), 423-427.

Finnigan, B. (2009). Barrier polymers. In K. L. Yam (Ed.), *The Wiley encyclopedia of packaging technology* (pp. 103–109). New York: Wiley.

Forssell, P., Lahtinen, R., Lahelin, M., and Myllarinen, P. (2002). Oxygen permeability of amylose and amylopectin film. *Carbohydrate Polymers, 47*, 125-129.

Galliard, T., and Bowler, P. (1987). Morphology and composition of starch. In T. Galliard (Ed.), *Starch: properties and potential* (pp. 55–78). New York, NY: John Wiley & Sons.

Gaudin, S., Lourdin, D., Le Botlan, D., Ilari, J. L., and Colonna, P. (1999). Plasticization and mobility in starch–sorbitol films. *Journal of Cereal Science, 29*(3), 273-284.

Gerard, C., Barron, C., Colonna, P., and Planchot, V. (2001). Amylose determination in genetically modified starches. *Carbohydrate Polymers, 44*, 19-27.

Gharagozlou, Y. (2014). Universal testing systems. Retrieved January 9, 2015, from http://www.instron.us

Gontard, N., and Guilbert, S. (1994). Biopackaging technology and properties of edible and/or biodegradable material of agricultural origin. In M. Mathlouthi (Ed.), *Food Packaging and Preservation* (pp. 159-181). Glasgow: Blackie Academic and Professional.

Guilbert, S., Cuq, B., and Gontard, N. (1997). Recent innovations in edible and/or biodegradable packaging materials. *Food Additives and Contaminants Part a-Chemistry Analysis Control Exposure & Risk Assessment, 14*(6-7), 741-751.

Guilbert, S., and Gontard, N. (2005). Agro-polymers for edible and biodegradable films: review of agricultural polymeric materials, physical and mechanical characteristics. In J. H. Han (Ed.), *Innovations in food packaging* (pp. 263-275). London: Academic Press.

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 Science and Technology, 29*(1–2), 10-17.

Han, J. H. (2005). *Innovations in food packaging*. London: Academic Press.

Han, J. H. (2014). *Innovations in food packaging* (2nd ed.). London: Academic Press.

Han, J. H., and Gennadios, A. (2005). Edible films and coatings: a review. In J. H. Han (Ed.), *Innovations in food packaging* (pp. 239-259). London: Academic Press.

Hernandez, R. J. (1997). Polymer properties. In A. L. Brody andK. Marsh (Eds.), *The Wiley encyclopedia of packaging technology* (2 ed., pp. 758-765). New York: John Wiley and Sons Inc.

Hong, S. I., and Krochta, J. M. (2003). Oxygen barrier properties of whey protein isolate coatings on polypropylene films. *Journal of Food Science: Food Engineering and Physical Properties*, *68*, 224-228.

Hoover, R., Li, Y. X., Hynes, G., and Senanayake, N. (1997). Physicochemical characterization of mung bean starch. *Food Hydrocolloids*, *11*(4), 401-408.

Janjarasskul, T. (2014). *Stability of packaged food products.* Chulalongkorn University.

Janjarasskul, T., and Krochta, J. M. (2010a). Edible Packaging Materials. *Annual Review of Food Science and Technology*, *1*(1), 415-448.

Janjarasskul, T., and Krochta, J. M. (2010b). Edible packaging materials. *Annu Rev Food Sci Technol*, *1*, 415-448.

Jesus, R. d., Avena-Bustillos, R. D., and Mchugh, T. H. (2012). Role of edible film and coating additives. In E. A. Baldwin, R. Hagenmaier, and J. Bai (Eds.), *Edible coatings and films to improve food quality* (2nd ed., pp. 157-184). New York, NY: CRC Press: Taylor and Francis Group.

Karlsson, S., and Albertsson, A.-C. (1998). Biodegradable polymers and environmental interaction. *Polymer Engineering and Science*, *38*(8), 1251-1253.

Kester, J. J., and Fennema, O. R. (1986). Edible biofilm and coatings: a review. *Food Technology*, *40*, 47-59.

Kim, S. J., and Ustunol, Z. (2001). Thermal properties, heat sealability and seal attributes of whey protein isolate/lipid emulsion edible films. *Journal of food science*, *66*(7), 985-990.

Krochta, J. M. (2002). Proteins as raw materials for films and coatings: definitions, current status, and opportunities. In A. Gennadios (Ed.), *Protein-based films and coatings* (pp. 1-32). London: CRC Press.

Lacroix, M. (2009). Mechanical and permeability properties of edible films and coatings for food and pharmaceutical applications. In M. E. Embuscado andK. C. Huber (Eds.), *Edible films and coatings for food applications* (pp. 347-366). New York: Springer Science+Business Media.

Laohakunjit, N., and Noomhorm, A. (2004). Effect of plasticizers on mechanical and barrier properties of rice starch film. *Starch - Stärke*, *56*(8), 348-356.

Leloup, V. M., Colonna, P., Ring, S. G., Roberts, K., and Wells, B. (1992). Microstructure of amylose gels. *Carbohydrate Polymers*, *18*, 189-197.

Leong, L. P., and Shui, G. (2002). An investigation of antioxidant capacity of fruits in Singapore markets. *Food Chemistry*, *76*, 69-75.

Leuangsukrerk, M., Phupoksakul, T., Tananuwong, K., Borompichaichartkul, C., and Janjarasskul, T. (2014). Properties of konjac glucomannan-whey protein isolate blend films. *LWT-Food Science and Technology*, *59*, 94-100.

Li, W., Shu, C., Zhang, P., and Shen, Q. (2011). Properties of starch separated from ten mung bean varieties and seeds processing characteristics. *Food and Bioprocess Technology*, *4*(5), 814-821.

Lightbourn, G. J., Griesbach, R. J., Novotny, J. A., Clevidence, B. A., Rao, D. D., and Stommel, J. R. (2008). Effects of anthocyanin and carotenoid combinations on foliage and immature fruit color of Capsicum annuum L. *Journal of Heredity*, *99*, 105–111.

Liu, Z. (2005). Edible films and coatings from starches. In J. H. Han (Ed.), *Innovations in food packaging* (pp. 318-332). UK: Acadamic Press.

Lourdin, D., Bizot, H., and Colonna, P. (1997). Antiplasticization in starch–glycerol films? *Journal of Applied Polymer Science, 63*(8), 1047–1053.

Lourdin, D., Coignard, L., Bizot, H., and Colonna, P. (1997). Influence of equilibrium relative humidity and plasticizer concentration on the water content and glass transition of starch materials. *Polymer, 38*(21), 5401–5406.

Lourdin, D., Valle, G. D., and Colonna, P. (1995). Influence of amylose content on starch films and foams. *Carhohydrurv Polymers, 27*, 261-270.

Lu, D. R., Xiao, C. M., and Xu, S. J. (2009). Starch-based completely biodegradable polymer materials. *Journal of Polymer Science Part C: Polymer Letters, 3*, 366–375.

Lu, M., Yuan, B., Zeng, M., and Chen, J. (2011). Antioxidant capacity and major phenolic compounds of spices commonly consumed in China. *Food Research International, 44*, 530-536.

Mark, A. M., Roth, W. B., Mehltretter, C. L., and Rist, C. E. (1966). Oxygen permeability of amylose maize starch films. *Food Technology, 20*, 75-77.

Markarian, J. (2008). Biopolymers present new market opportunities for additives in packaging. *Plastics, Additives and Compounding, 10*(3), 22-25.

Maté, J. I., and Krochta, J. M. (1998). Oxygen uptake model for uncoated and coated peanuts. *Journal of Food Engineering, 35*(3), 299-312.

Mathew, A. P., and Dufresne, A. (2002). Plasticized waxy maize starch: effect of polyols and relative humidity on material properties. *Biomacromolecules, 3*(5), 1101–1108.

McHugh, T. H., and Krochta, J. M. (1994). Sorbitol-vs glycerol-plasticized whey protein edible films: integrated oxygen permeability and tensile property evaluation. *Journal of Agricultural and Food Chemistry, 42*(4), 841-845.

Mekonnen, T., Mussone, P., Khalil, H., and Bressler, D. (2013). Progress in bio-based plastics and plasticizing modifications. *Journal of Materials Chemistry A, 1*(43), 13379.

Miller, K. S., and Krochta, J. M. (1997). Oxygen and aroma barrier properties of edible films: A review. *Trends in Food Science & Technology, 8*(7), 228-237.

Mueller, C., Cappacio, G., Hiltner, A., and Baer, E. (1998). Heat sealing of LLDPE: relationships to melting and interdiffusion. *Journal of Applied Polymer Science, 70*(11), 2021-2030.

Muscat, D., Adhikari, B., Adhikari, R., and Chaudhary, D. S. (2012). Comparative study of film forming behaviour of low and high amylose starches using glycerol and xylitol as plasticizers. *Journal of Food Engineering, 109*(2), 189-201.

Myllärinen, P., Partanen, R., Seppälä, J., and Forssell, P. (2002). Effect of glycerol on behaviour of amylose and amylopectin films. *Carbohydrate Polymers, 50*, 355–361.

Navarro, J. M., Flores, P., Garrido, C., and Martinez, V. (2006). Changes in the contents of antioxidant compounds in pepper fruits at different ripening stages, as affected by salinity. *Food Chemistry, 96*, 66-73.

Nussinovitch, A. (2009). Biopolymer films and composite coatings. In S. Kasapis, I. T. Norton, and J. B. Ubbink (Eds.), *Modern biopolymer science: bridging the* *divide between fundamental treatise and industrial application* (pp. 295-298). London: Academic Press.

Nussinovitch, A. (2013). *Handbook of biopolymers and biodegradable plastics properties, processing, and applications* (S. Ebnesajjad Ed.). 225 Wyman Street, Waltham, MA 02451, USA: William Andrew.

Park, H. J., Weller, C. L., Vergano, P. J., and Testin, R. F. (1993). Permeability and mechanical properties of cellulose-based edible films. *Journal of food science, 58*(6), 1361-1370.

Perucka, I., and Materska, M. (2007). Antioxidant vitamin contents of Capsicum annuum fruit extracts as affected by processing and varietal factors. *Acta Scientiarum Polonorum Technologia Alimentaria, 6*(4), 67-74.

Polnaya, F. J., Talahatu, J., Marseno, H. W., and Marseno, D. W. (2012). Properties of biodegradable films from hydroxypropyl sago starches. *Asian Journal of Food and Agro-Industry, 5*(3), 183-192.

Prior, R. L., Wu, X., and Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry, 53*(10), 4290-4302.

Protzman, T. F., Wagoner, J. A., and Young, A. H. (1967). US Patent No. 3344216.

Reddy, C. V. K., Sreeramulu, D., and Raghunath, M. (2010). Antioxidant activity of fresh and dry fruits commonly consumed in India. *Food Research International, 43*(1), 285-288.

Rindlav-Westling, A., Stading, M., and Gatenholm, P. (2002). Crystallinity and morphology in films of starch, amylose and amylopectic blends. *Biomacromolecules, 3*, 84-91.

Rindlav-Westling, A., Stading, M., Hermansson, A. M., and Gatenholm, P. (1998). Structure, mechanical and barrier properties of amylose and amylopectin films. *Carbohydrate Polymers, 36*, 217–224.

Ryu, S. Y., Rhim, J. W., Roh, H. J., and Kim, S. S. (2002). Preparation and physical properties of zein-coated high amylose corn starch film. *Lebensmittel-Wissenschaft und -Technologie, 35*, 680-686.

Salame, M. (1986). Barrier polymers. In M. Baker (Ed.), *The Wiley encyclopedia of packaging technology* (pp. 48-54). New York: Wiley.

Salmieri, S., and Lacroix, M. (2006). Physicochemical properties of alginate/polycaprolactone-based films containing essential oils. *Journal of Agricultural and Food Chemistry, 54*, 10205-10214.

Sandhu, K. S., and Lim, S.-T. (2008). Digestibility of legume starches as influenced by their physical and structural properties. *Carbohydrate Polymers, 71*(2), 245-252.

Sears, J. K., and Darby, J. R. (1982). *Mechanism of plasticizer action*. New York, NY: John Wiley & Sons.

Shah, K. P. (2008). The hand book on mechanical maintenance. Retrieved from http://praticalmaintenance.wordpress.com website:

Shannon, J. C., and Garwood, D. L. (1984). Genetics and physiology of starch development. In R. L. Whistler, J. N. BeMiller, and E. F. Paschall (Eds.), *Starch: chemistry and technology* (pp. 25–86). New York, NY: Academic Press.

Siew, D. C. W., Heilmann, C., Easteal, A. J., and Cooney, R. P. (1999). Solution and film properties of sodium caseinate/glycerol and sodium caseinate/polyethylene glycol edible coating system. *Journal of Agricultural and Food Chemistry, 47*, 3432–3440.

Silverio, J., Svensson, E., Eliasson, A.-C., and Olofsson, G. (1996). Isothermal microcalorimetric studies on starch retrogradation. *Journal of Thermal Analysis and Calorimetry, 47*(5), 1179-1200.

Smits, A. L. M., Kruiskamp, P. H., van Soest, J. J. G., and Vligenthart, J. F. G. (2003). The influence of various small plasticiser and malto-oligosaccharides on the retrogradation of (partly) gelatinised starch. *Carbohydrate Polymers, 51*, 417-424.

Sothornvit, R., and Krochta, J. M. (2000). Water vapor permeability and solubility of films from hydrolyzed whey protein. *Journal of food science, 65*(4), 700-705.

Sothornvit, R., and Krochta, J. M. (2001). Plasticizer effect on mechanical properties of beta-lactoglobulin (β-Lg) films. *Journal of Food Engineering, 50*(3), 149-155.

Sothornvit, R., and Krochta, J. M. (2005). Plasticizers in edible films and coatings. In J. H. Han (Ed.), *Innovations in food packaging* (pp. 403-433). UK: Academic Press.

Steuteville, R. (1993). Biodegradable polymers forge ahead. *Biocycle, 34*(9), 72-74.

Talja, R. A., Helén, H., Roos, Y. H., and Jouppila, K. (2007). Effect of various polyols and polyol contents on physical and mechanical properties of potato starch-based films. *Carbohydrate Polymers, 67*(3), 288-295.

Tanner, K. E., Getz, J. J., Burnett, S., Youngblood, E., and Draper, P. R. (2003). Film forming compositions comprising modified starches and iota-carrageenan and methods for manufacturing soft capsules using same, US Patent 6-582-727.

Thao, H. M., and Noomhorm, A. (2011). Physiochemical properties of sweet potato and mung bean starch and their blends for noodle production. *Journal of Food Processing & Technology, 2*(1).

Tharanathan, R. N. (2003). Biodegradable films and composite coatings: past, present and future. *Trends in Food Science & Technology, 14*(3), 71-78.

Tongdeesoontorn, W., Mauer, L. J., Wongruong, S., Sriburi, P., and Rachtanapun, P. (2011). Effect of carboxymethyl cellulose concentration on physical properties of biodegradable cassava starch-based films. *Chemistry Central Journal, 5*(6), 1-8.

Turhan, K. N., and Sahbaz, F. (2004). Water vapour permeability, tensile properties and solubility of methylcellulose-based edible films. *Journal of Food Engineering, 61*, 459–466.

Urban, D., and Takamura, K. (2002). *Polymer dispersions and their industrial applications*. Weinheim: Wiley-VCH Verlag GmbH.

Vachon, C., Yu, H. L., Yefsah, R., Alain, R., St-Gelais, D., and Lacroix, M. (2000). Mechanical and structural properties of milk protein edible film cross-linked by heating and gramma irradiation. *Journal of Agricultural and Food Chemistry, 48*, 3202-3209.

van Soest, J. J. G. (1996). *Starch plastics: structure–property relationships*. Utrecht, The Netherlands: Utrecht University.

Vartiainen, J., Vähä-Nissi, M., and Harlin, A. (2014). Biopolymer films and coatings in packaging applications-a review of recent developments. *Materials Sciences and Applications, 05*(10), 708-718.

Vieira, M. G. A., Silva, M. A. d., Santos, L. O. d., and Beppu, M. M. (2011). Natural-based plasticizers and biopolymer films: a review. *European Polymer Journal, 47*(3), 254-263.

Wahyuni, Y., Ballester, A.-R., Sudarmonowati, E., Bino, R. J., and G., B. A. (2011). Metabolite diversity in pepper (Capsicum) fruits of thirty-two diverse accessions: variation in health-related compounds and implications for breeding. *Phytochemistry, 72*, 1358–1370.

Wang, L., Khor, E., and Lim, L. Y. S. (2001). Chitosan-alginate-$CaCl_2$ system for membrane coat application. *Journal of Pharmaceutical Sciences, 90*, 1134-1142.

Wangcharoen, W., and Morasuk, W. (2007). Antioxidant capacity and phenolic content of some Thai culinary plants. *Maejo International Journal of Science and Technology, 1*(2), 100-106.

Wangcharoen, W., and Morasuk, W. (2009). Antioxidant capacity changes of bird chili (*capsicum frutescens* Linn.) during hot air drying. *Kasetsart Journal (Nat. Sci.), 43*, 12-20.

Wareing, P., Stuart, F., and Fernandes, R. (2011). Factors affecting the growth of microorganism in foods. *Royal Society of chemistry*, 437.

Waterhouse, A. (2005). *Determination of total phenolics* (Vol. 2): Hoboken: John Wiley and Sons.

Wittaya, T. (2012). Rice starch-based biodegradable films: properties enhancement. In A. A. Eissa (Ed.), *Structure and function of food engineering* (pp. 103-134). Croatia: InTech.

Wojciechowska, P. (2012). *Recent advances in plasticizers* M. Luqman (Ed.) *The effect of concentration and type of plasticizer on the mechanical propoties of cellulose acetate butyrate organic-inorganic hybrids* (pp. 141-164).

Wolff, I. A., Davis, H. A., Cluskey, J. E., Gundrum, L. J., and Rist, C. E. (1951). Preparation of films from amylose. *Industrial and Engineering Chemistry, 43*(4), 915-919.

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 *Advances in food and nutrition research* (Vol. 44, pp. 347-394): Academic Press.

Yang, L., and Paulson, A. T. (2000). Mechanical and water vapour barrier properties of edible gellan films. *Food Research International, 33*(7), 563-570.

Yang, S. P., Huang, L., Martin, S. M., Villanueva, J., Greene, S., Arehart, K., Sayre, C., and Johnson, R. B. (2009). Xylitol for treatment of vaginal infections: Google Patents.

Yoshimoto, Y., Tashiro, J., Takenouchi, T., and Takeda, Y. (2000). Molecular structure and some physicochemical properties of high-amylose barley starches. *Cereal Chemistry, 77*, 279–285.

APPENDIX

Appendix A

Effect of total solid on thickness of MBS films

Table A.1 Effect of total solid (T,%w/w) in casting solution on thickness (l, mm) of mung bean starch (MBS) films.

Starch : Plasticizer	Composition for 100 g casting solution			Total solid (T, % w/w)	Glycerol plasticized MBS film		Sorbitol plasticized MBS film	
	Starch (g)	Plasticizer (g)	Water (g)		Thickness (mm)	SD	Thickness (mm)	SD
3.5 : 0	3.8335	0.0000	96.1665	3.8335	0.0462	0.0002	0.0462	0.0002
3.5 : 10	3.8335	0.3834	95.7831	4.2169	0.0499	0.0018	0.0493	0.0010
3.5 : 20	3.8335	0.7667	95.3998	4.6002	0.0530	0.0005	0.0518	0.0013
3.5 : 30	3.8335	1.1500	95.0165	4.9835	0.0597	0.0039	0.0531	0.0016
3.5 : 40	3.8335	1.5334	94.6331	5.3669	n/a	n/a	0.0571	0.0019
3.5 : 50	3.8335	1.9168	94.2497	5.7503	n/a	n/a	0.0566	0.0004
3.5 : 60	3.8335	2.3001	93.8664	6.1336	n/a	n/a	0.0665	0.0006
4.0 : 0	4.3812	0.0000	95.6188	4.3812	0.0533	0.0023	0.0533	0.0023
4.0 : 10	4.3812	0.4381	95.1807	4.8193	0.0557	0.0034	0.0556	0.001
4.0 : 20	4.3812	0.8762	94.7426	5.2574	0.0621	0.0023	0.0642	0.0019
4.0 : 30	4.3812	1.3144	94.3044	5.6956	0.0695	0.0043	0.0624	0.0018
4.0 : 40	4.3812	1.7525	93.8663	6.1337	n/a	n/a	0.0669	0.0017
4.0 : 50	4.3812	2.1906	93.4282	6.5718	n/a	n/a	0.0698	0.0025
4.0 : 60	4.3812	2.6287	92.9901	7.0099	n/a	n/a	0.0750	0.0013
4.5 : 0	4.9288	0.0000	95.0712	4.9288	0.0590	0.0025	0.0590	0.0025
4.5 : 10	4.9288	0.4929	94.5783	5.4217	0.0653	0.0026	0.0648	0.0025
4.5 : 20	4.9288	0.9858	94.0854	5.9146	0.0697	0.0021	0.0663	0.0017
4.5 : 30	4.9288	1.4786	93.5926	6.4074	0.0804	0.0032	0.0754	0.0006
4.5 : 40	4.9288	1.9715	93.0997	6.9003	n/a	n/a	0.0769	0.0015
4.5 : 50	4.9288	2.4644	92.6068	7.3932	n/a	n/a	0.0791	0.0001
4.5 : 60	4.9288	2.9573	92.1139	7.8861	n/a	n/a	0.0793	0.0007
5.0 : 0	5.4764	0.0000	94.5236	5.4764	0.0666	0.0029	0.0666	0.0029
5.0 : 10	5.4764	0.5476	93.976	6.024	0.0781	0.0031	0.0713	0.0014
5.0 : 20	5.4764	1.0953	93.4283	6.5717	0.0798	0.003	0.0733	0.0016
5.0 : 30	5.4764	1.6429	92.8807	7.1193	0.0828	0.004	0.0781	0.0014
5.0 : 40	5.4764	2.1906	92.333	7.6670	n/a	n/a	0.0811	0.0005
5.0 : 50	5.4764	2.7382	91.7854	8.2146	n/a	n/a	0.0814	0.0019
5.0 : 60	5.4764	3.2858	91.2378	8.7622	n/a	n/a	0.0828	0.0012

* Each data represents average value. SD is standard deviation.

Appendix B

Composition for mung bean starch

Table B.1 Composition for 100 g mung bean starch.

Composition	**(g/100g)**
Moisture	8.70
Ash	0.044
Crude Fiber	0.34
Protein	0.17
Fat	0.36
Carbohydrate	90.73

(AOAC, 2010)

Appendix C

a_w of sorbitol or glycol-plasticized films

Table C.1 a_w of sorbitol or glycol-plasticized films at the same plasticizer concentration.

Starch concentration (%w/w)	Plasticizer concentration (%w/w)	Plasticizer type	a_w	SD
4.5	10	Glycerol	0.549^{ns}	0.003
		Sorbitol	0.544^{ns}	0.002

Starch concentration (%w/w)	Plasticizer concentration (%w/w)	Plasticizer type	a_w	SD
4.5	20	Glycerol	0.544^{a}	0.005
		Sorbitol	0.540^{b}	0.002

Starch concentration (%w/w)	Plasticizer concentration (%w/w)	Plasticizer type	a_w	SD
4.5	30	Glycerol	0.543^{ns}	0.000
		Sorbitol	0.540^{ns}	0.004

Different letters (a, b, ..) indicate significant differences between samples ($p \leq 0.05$) and (ns) means non significantly difference.

Appendix D

Standard curve for total phenolic content and antioxidant activity

Table D.1 Data of standard curve for total phenolic content.

Gallic acid concentration (mg/l)	A_{765}
0	0.0530
50	0.0985
200	0.2740
500	0.5730
1000	1.1280
2000	1.9680

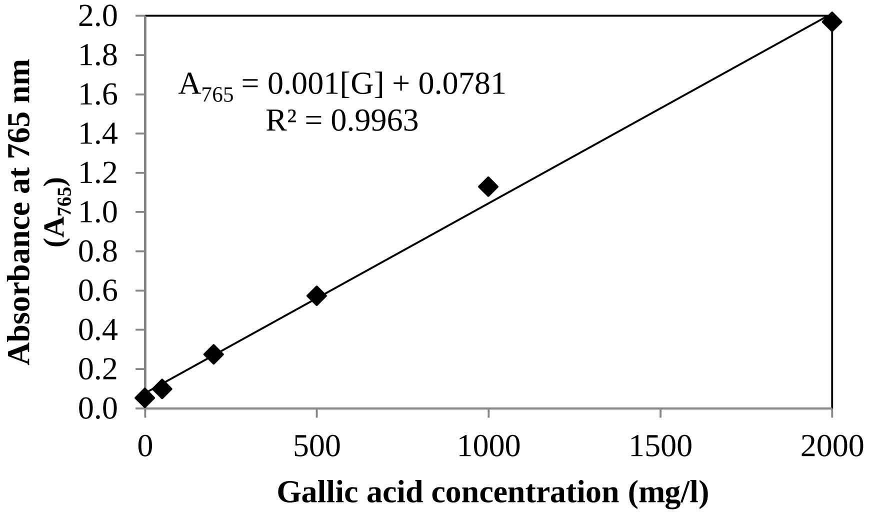


Figure D.1 Standard curve for total phenolic content.

Table D.2 Data of trolox standard curve for DPPH free radical scavenging activity.

Trolox concentration (µM)	$A_{diff,\ 515\ nm}$
123	0.2055
185	0.3310
278	0.4425
417	0.6435
625	0.8805

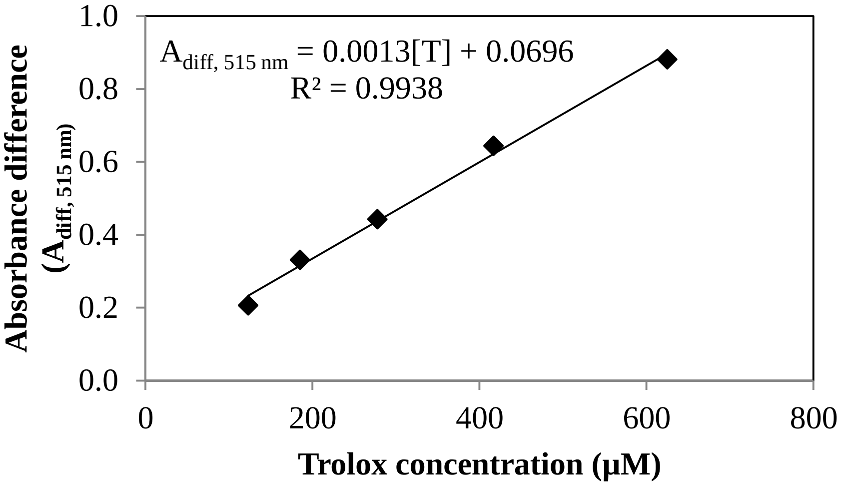


Figure D.2 Trolox standard curve for DPPH free radical scavenging activity.

Table D.3 Data of trolox standard curve for ferric reducing antioxidant power.

Trolox concentration (µM)	$A_{diff,\ 593\ nm}$
82	0.1885
123	0.2720
185	0.4535
278	0.6615
417	0.9935
625	1.3860

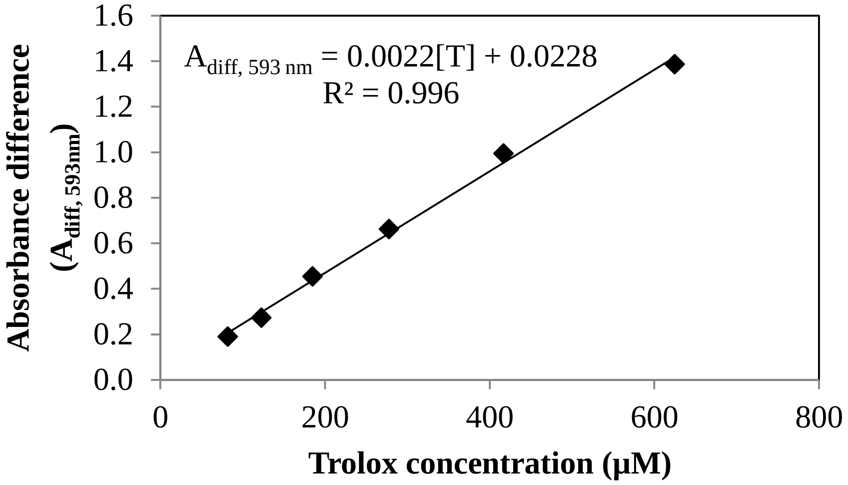


Figure D.3 Trolox standard curve for ferric reducing antioxidant power.

Appendix E

Thickness of PP and MBS films

Table E.1 Thickness (mm) of PP and MBS films.

Film	Thickness (mm)
PP	0.0375 ± 0.0035
MBS (5 %w/w starch, 40 %w/w sorbitol)	0.0811 ± 0.0005

VITA

Miss Onjira Rompothi was born on November 26, 1989 in Samutprakarn, Thailand. She obtained a Bachelor degree of Food Technology, Faculty of Science, Chulalongkorn University in 2011. In 2012, she enrolled the master degree program at Department of Food Technology, Faculty of Science, Chulalongkorn University.

Poster presentation at the 2nd CAS National and International Conference 2014 (CASNIC 2014) in College of Asian Scholars, Khon Kaen in the topic “Physical properties of mung bean starch-glycerol films”.

Poster presentation at the 12th International Hydrocolloids Conference (IHC) in Howard International Conference Center, Taipei, Taiwan in the topic “Physical properties of mung bean starch-sorbitol films”