DEVELOPMENT OF COMPOSITE FILM COATING FOR FREEZE-DRIED FRUIT SLICES

Miss Nichayarach Invichian

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การพัฒนาการเคลือบผิวชิ้นผลไม้ที่ผ่านการทำแห้งเยือกแข็งด้วยฟิล์มคอมโพสิท

น.ส.ณิชญารัชต์ อินวิเชียร

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
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By	Miss Nichayarach Invichian
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Thesis Advisor	Associate Professor WARANGKANA WARISNOICHAROEN, Ph.D.

Accepted by the Faculty of Pharmaceutical Sciences, Chulalongkorn University in Partial Fulfillment of the Requirement for the Master of Science in Pharmacy

..... Dean of the Faculty of Pharmaceutical Sciences

(Assistant Professor RUNGPETCH SAKULBUMRUNGSIL, Ph.D.)

THESIS COMMITTEE

..... Chairman

(Associate Professor SUYANEE PONGTHANANIKORN, Dr.P.H.)

..... Thesis Advisor

(Associate Professor WARANGKANA WARISNOICHAROEN, Ph.D.)

..... External Examiner

(Assistant Professor LINNA TONGYONK, D.Sc.)

..... External Examiner

(Assistant Professor Suchada Jongrungruangchok, Ph.D.)

ณิชญารัชต์ อินวิเชียร : การพัฒนาการเคลือบผิวชิ้นผลไม้ที่ผ่านการทำแห้งเยือกแข็งด้วยฟิล์มคอมโพสิท. (DEVELOPMENT OF COMPOSITE FILM COATING FOR FREEZE-DRIED FRUIT SLICES) อ.ที่ปรึกษาหลัก : รศ. ภญ. ดร.วรางคณา วารีสน้อยเจริญ

การเคลือบผิวอาหารด้วยฟิล์มที่บริโภคได้ก่อนผ่านกระบวณการทำแห้งเยือกแข็ง เป็นอีกวิธีที่น่าสนใจในการถนอมอาหาร โดยเฉพาะพวกผักผลไม้ที่มีความไวต่อความร้อนสูง การศึกษาก่อนหน้านี้พบว่าน้ำตาลทรีฮาโลสมีฤทธิ์ป้องกันผลึกน้ำแข็งในการทำแห้งเยือกแข็ง และมีคุณสมบัติเป็นน้ำตาลนอนรีดิวซ์และดูดความชื้นต่ำ อย่างไรก็ตามยังไม่พบงานวิจัยที่นำน้ำตาลทรีฮาโลสมาใช้ประโยชน์ในรูปแบบฟิล์มเคลือบผิวอาหาร ดังนั้นการศึกษาครั้งนี้จึงมีวัตถุประสงค์เพื่อพัฒนาฟิล์มคอมโพสิทที่ประกอบด้วยอัลจิเนตและทรีฮาโลสและประเมินคุณสมบัติทางกายภาพของผลไม้หั่นชิ้นเคลือบผิวด้วยฟิล์มคอมโพสิทที่ผ่านการทำแห้งเยือกแข็ง วิธีศึกษาทำโดยการเตรียมฟิล์มคอมโพสิทที่ประกอบด้วยทรีฮาโลส อัลจิเนต และแคลเซียมคลอไรด์ ที่มีความเข้มข้นร้อยละ 3.0-9.0 1.2-2.0 และ 0.2-0.6 โดยน้ำหนักต่อปริมาตร ตามลำดับ ศึกษาปัจจัยที่เหมาะสมสำหรับการเตรียมฟิล์มคอมโพสิทด้วยวิธีพื้นที่ผิวตอบสนองแบบ Box-Behnken design ผลการศึกษาพบว่า สูตรการเตรียมฟิล์มคอมโพสิทที่เหมาะสมเมื่อกำหนดการตอบสนอง ได้แก่ ความหนา ค่าความขุ่น ปริมาณความชื้น และความสามารถในการซึมผ่านของไอน้ำ ให้มีค่าน้อยที่สุด พบว่าต้องใช้ความเข้มข้นของทรีฮาโลส อัลจิเนต และแคลเซียมคลอไรด์ ร้อยละ 6.71 2.0 และ 0.4 โดยน้ำหนักต่อปริมาตร ตามลำดับ ซึ่งความเข้มข้นของทรีฮาโลสที่เพิ่มขึ้นมีแนวโน้มในการเพิ่มความหนาและความสามารถในการซึมผ่านของไอน้ำและลดปริมาณความชื้นของแผ่นฟิล์ม เมื่อศึกษาผลของฟิล์มคอมโพสิทที่เคลือบผิวผลไม้ (แอปเปิ้ล) หั่นชิ้นและผ่านกระบวณการทำแห้งเยือกแข็ง พบว่า อาหารกลุ่มที่เคลือบด้วยฟิล์มคอมโพสิทมีการสูญเสียมวลมากกว่ากลุ่มควบคุมที่ไม่ได้เคลือบผิวอย่างมีนัยสำคัญที่ระดับความเชื่อมั่นร้อยละ 95 (p=0.001) และค่าวอเตอร์แอคทิวิตี้ของผลไม้หั่นชิ้นที่เคลือบด้วยฟิล์มคอมโพสิทมีค่าน้อยกว่ากลุ่มควบคุมที่ไม่ได้เคลือบผิวผลไม้อย่างมีนัยสำคัญที่ระดับความเชื่อมั่นร้อยละ 95 (p=0.001) นอกจากนั้นสีของตัวอย่างอาหารที่เคลือบด้วยฟิล์มคอมโพสิท มีค่า hue angle (h^*) มากกว่ากลุ่มควบคุมอย่างมีนัยสำคัญที่ระดับความเชื่อมั่นร้อยละ 95 (p=0.047) ในขณะที่มีค่าดัชนีสีน้ำตาลน้อยกว่ากลุ่มควบคุมอย่างมีนัยสำคัญที่ระดับความเชื่อมั่นร้อยละ 95 (p=0.034) และเมื่อเก็บตัวอย่างอาหารในตู้ดูดความชื้นที่อุณหภูมิ 25 ± 5 องศาเซลเซียส เป็นเวลา 4 สัปดาห์ พบว่าตัวอย่างที่เคลือบด้วยฟิล์มคอมโพสิทมีความสามารถในการดูดน้ำคืนและความแน่นเนื้อมากกว่ากลุ่มควบคุมที่ไม่ได้เคลือบผิวผลไม้อย่างมีนัยสำคัญที่ระดับความเชื่อมั่นร้อยละ 95 (p=0.038 และ p=0.012 ตามลำดับ) ในขณะที่มีค่าปริมาณความชื้นน้อยกว่ากลุ่มควบคุมที่ไม่ได้เคลือบผิวผลไม้อย่างมีนัยสำคัญที่ระดับความเชื่อมั่นร้อยละ 95 (p=0.002) ผลการศึกษาที่ได้อาจเกิดจากโมเลกุลของทรีฮาโลสในขั้นตอนการทำแห้งแบบเยือกแข็งจะอยู่ในสภาวะเสมือนแก้วมากกว่าผลึก ซึ่งจะมีผลลดการเกิดผลึกน้ำแข็งขนาดใหญ่ในเนื้ออาหาร อีกทั้งยังไม่มีผลเหนี่ยวนำการเกิดปฏิกิริยาเมลลาร์ดด้วย จากผลการศึกษาสรุปได้ว่าการเคลือบผลไม้หั่นชิ้นด้วยฟิล์มคอมโพสิทจะรักษาคุณสมบัติที่ดีของอาหารหลังผ่านกระบวนการทำแห้งเยือกแข็งไว้ได้เมื่อเทียบกับผลไม้หั่นชิ้นที่ไม่ได้เคลือบฟิล์มคอมโพสิท ทั้งนี้ควรทำการศึกษาเพิ่มเติมเกี่ยวกับประสิทธิผลของฟิล์มคอมโพสิทเมื่อนำไปประยุกต์ใช้ในการเคลือบผลไม้ชนิดอื่นๆ ต่อไป

สาขาวิชา	อาหารเคมีและโภชนศาสตร์ทางการแพทย์	ลายมือชื่อนิสิต .....
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KEYWORD: freeze drying, trehalose, alginate, response surface methodology, Box-Behnken design

Nichayarach Invichian : DEVELOPMENT OF COMPOSITE FILM COATING FOR FREEZE-DRIED FRUIT SLICES. Advisor: Assoc. Prof. WARANGKANA WARISNOICHAROEN, Ph.D.

Edible film coating on food before freeze-drying process can be used for food preservation, especially in heat-sensitive food such as fruits and vegetables. Previous studies have been reported that trehalose had the cryoprotective effect and unique properties such as non-reducing sugar and low hygroscopic. However, no research has been conducted on the use of trehalose as film coatings. Therefore, this study aimed to develop a composite film consisting of alginate and trehalose and to evaluate physical properties of freeze-dried fruit slices coated with a composite film. The experiment was designed to prepare a composite film consisting of various concentrations of trehalose, alginate and calcium chloride (3.0-9.0% w/v, 1.2-2.0% w/v and 0.2-0.6% w/v, respectively). The response surface methodology, Box-Behnken design, was used to optimize the factors of composite film preparation which were concentrations of trehalose, alginate and calcium chloride. The study showed that the optimized formula, which gave the least in thickness, opacity, moisture content, and water vapor permeability, could be prepared using 6.71% w/v trehalose, 2.0% w/v alginate and 0.4% w/v calcium chloride. Higher concentrations of trehalose seemed to increase thickness and water vapor permeability of a composite film whereas the moisture content of a composite film was decreased. The effect of composite film coating on physical properties of freeze-dried fruit (apple) slices was determined. After freeze drying, the mass loss of composite film-coated samples was statistically significant higher than the uncoated (control) samples (p<0.05; p=0.001). The water activity of composite film-coated samples was statistically significant lower than the uncoated samples (p<0.05; p=0.001). Moreover, the color h^* values of composite film-coated freeze-dried samples were statistically significant higher than the uncoated samples (p<0.05; p=0.047), while the browning index was statistically significant lower than the uncoated group (p<0.05; p=0.034). For freeze-dried samples coated with a composite film stored in a desiccator at 25 ± 5 °C for 4 weeks, the rehydration capacity and firmness of film-coated samples were statistically significant higher than the uncoated samples (p<0.05; p=0.038 and p=0.012, respectively), while the moisture content of film-coated samples was statistically significant lower than the uncoated samples (p<0.05; p=0.002). The results were possibly due to ability of trehalose to form glassy state rather than crystal state during freeze drying and ability to inhibit ice crystal growth. Also, trehalose did not promote the Maillard reaction. In conclusion, the trehalose-alginate composite film could be used as film coating on fruit slices prior to freeze drying process in order to maintain appropriate food properties. Ongoing research should be done on the effectiveness of composite film coating on physical properties of a variety of fruits.

Field of Study:	Food Chemistry and Medical Nutrition	Student's Signature .....
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Nichayarach Invichian

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF ABBREVIATIONS

% w/v	percent weight by volume
% w/w	percent weight by weight
°C	degree Celsius
*a**	red to green color channels
a_w	water activity
*b**	yellow to blue color channels
*h**	hue angle
*L**	lightness
ΔE*	total color difference
cm	centimeter(s)
g	gram(s)
h	hour(s)
kPa	kilopascal(s)
m	meter(s)
m^2	square meter(s)
mg	milligram(s)
min	minute(s)
ml	milliliter(s)
mm	millimeter(s)
mm^2	square millimeter(s)

N	Newton
R^2	coefficient of determination
RC	rehydration capacity
RH	relative humidity
s	second(s)
µl	microliter(s)
ADI	acceptable daily intake
ANOVA	analysis of variance
BBD	Box-Behnken design
BI	browning index
CFUs	colony forming units
CIE	Commission Internationale de l'Eclairage
D	desirability
DRBC	Dichloran Rose Bengal chloramphenicol
FD	freeze drying
FDA	Food and Drug Administration
JECFA	Joint FAO/WHO Expert Committee on Food Additives
PCA	plate count agar
RC	rehydration capacity
RSM	response surface methodology
SA	sodium alginate

SD	standard deviation
SPSS	statistical package for the social sciences
Tre	trehalose
WVP	water vapor permeability
WVTR	water vapor transmission rate

CHAPTER I

INTRODUCTION

1.1 Background and rationale

Drying is the important process in the food industry to preserve food by preventing microbial contamination, reducing moisture-mediated deterioration, facilitating storage, and saving transportation costs (Delele, Weigler, and Mellmann, 2014). The heat-sensitive food such as vegetables, fruits and other biological products is selected for appropriate drying technologies. Freeze drying (FD) is considered to be the best method for keeping and maintaining the highest quality of food (Ochoa-Martínez et al., 2012). Freeze-drying process is based on sublimation, which helps to reduce structural changes and maintains nutrients or bioactive substances including flavors (Ceballos, Giraldo, and Orrego, 2012). The three main steps of FD are freezing product, removing the ice by direct sublimation under reduced pressure (primary drying) and releasing of unfrozen water by desorption (secondary drying) (Geidobler and Winter, 2013). Before FD process, the pretreatment procedure such as blanching has to be done. Blanching, using hot water or by stream, can inactivate the enzyme activity and remove the air inside the tissue to avoid oxidation. However, blanching may not be appropriate for heat-sensitive food such as fruits and vegetables (Wang et al., 2018). Therefore, using edible coating method with appropriate solutions for

pretreatment may be an alternative to blanching. Edible coating can reduce moisture loss, color change, unexpected chemical reactions, and microbial contamination (Rojas-Graü, Soliva-Fortuny, and Martín-Belloso, 2009).

Edible films and coatings can also offer a possibility to extend the shelf-life of fresh-cut food by providing a thin layer on the surface of a product as a barrier to gases, reducing respiration, controlling enzymatic browning, and protecting water loss (Perez-Gago, Serra, and Rio, 2006). Polysaccharide-based edible coating films have good physical and mechanical properties namely transparent, homogenous, flexible, and elastic. Among polysaccharide-based edible coatings, alginate, a polymer of D-mannuronic acid and L-guluronic acid extracted from brown algae, has an ability to form insoluble polymer or stable gel structure with multivalent cationic compounds such as calcium (Rojas-Graü, Tapia, and Martín-Belloso, 2008). Moreover, alginate is a food additive as thickener, stabilizer, suspending and gelling agents. It is considered to be non-toxic, biodegradable, and biocompatible (Tavassoli-Kafrani, Shekarchizadeh, and Masoudpour-Behabadi, 2016).

Also cryoprotectant is suggested to be added prior to FD process for maintaining the cell structure of the product during FD process. Trehalose (α-D-glucopyranosyl-(1⟶1)-α-D-glucopyranoside) is a non-reducing disaccharide. There are many studies on the use of trehalose as a cryoprotectant in food

application (Ma et al., 2015; Zhang et al., 2017; Stefanello et al., 2018). Trehalose is used to protect proteins and lipids in the membrane structure during stress conditions such as heat and freeze-thawing (Yoshiyama et al., 2015). The major advantage of trehalose compared to other sugars, such as sucrose and lactose, is its water-binding ability which can prevent the formation of intracellular and extracellular ice crystals (Costa et al., 2000). Moreover, trehalose is stable, colorless, odorless, slightly sweet and can prevent browning of the product during processing (Ohtake and Wang, 2011).

Notably, browning reaction of fresh-cut fruits results from both enzymatic and non-enzymatic reactions (Rocha and Morais, 2002). Polyphenol oxidase in fruit tissue needs oxygen for browning reactions; therefore, providing an oxygen barrier can be advantageous of browning prevention (Rocha and Morais, 2002). Alginate-based edible coating could provide low oxygen permeability (Perez-Gago et al., 2006; Rojas-Graü et al., 2008; Tavassoli-Kafrani et al., 2016). Non-enzymatic browning is mainly associated with degradation reactions of carbohydrate, such as the Maillard reactions and the oxidation of phenolic compounds (BeMiller and Whistler, 1996; Rocha and Morais, 2002). The color change of the dried fruit could be due to the browning formation, associated with the Maillard reaction (Baini and Langrish, 2009; Persic et al., 2017; Djekic et al., 2018).

Previous studies have been reported that coating fish, shrimp, pork, and scallops with sodium alginate biofilms extended shelf-life, reduced thawing loss, reduced weight loss, and maintained the functional properties during frozen storage (Yu et al., 2008; Song et al., 2011). During frozen storage of peeled shrimp, trehalose and alginate treatment had cryoprotective effect, by prevention of thawing loss, degradation of textural and color properties, and the physical damage caused by the formation of large ice crystals (Ma et al., 2015). In addition, few studies have shown that pretreatment with trehalose improved the reconstitution properties of dried sliced potato and carrot products compared with the dried products pre-treated with sucrose, which is generally used for osmotic dehydration (Aktas et al., 2007). Trehalose could also improve physical change (i.e. color, firmness, weight loss) of apple slices and litchi (Albanese, Cinquanta, and Dimatteo, 2007; Mahayothee et al., 2009).

However, no research has been done on using composite film of trehalose and alginate for sample requiring FD process. This study was aimed to formulate composite film solution using alginate and trehalose. The optimized formulation was selected for coating fruit slices prior FD process. The freeze-dried samples were determined for physical properties and microbial amount.

1.2 Objectives of the study

1. To develop the composite film containing alginate and trehalose.

2. To determine the effect of composite film coating on physical properties of freeze-dried fruit slices.

1.3 Benefits of the study

The study provides information on the preparation of composite films with appropriate physical characteristics and the properties of freeze-dried fruit slices coated with composite film. The research could be used for future application of composite film for freeze-dried food.

CHAPTER II

LITERATURE REVIEW

In this study, freeze-dried food coating with alginate-based film containing trehalose was studied. The literature review was covered in the alginate-based film, FD process, cryoprotectant as well as evaluation of food properties. In addition, using response surface methodology (RSM) as experimental design was also reviewed.

2.1 Freeze drying

Freeze-drying, also called lyophilization, is used for dry products of high quality and long-term preservation of heat-sensitive foods such as fruits and vegetables (Ochoa-Martínez et al., 2012). Freeze-dried products have some high quality characteristic compared to products of alternative drying process such as shape retention, high porosity, good rehydration, and good color change (Valentina et al., 2016). FD is a method of dehydration of frozen materials by sublimation under vacuum (Ciurzyńska and Lenart, 2011). The main procedures of FD can also be applied to foods, which include three stages: freezing, primary drying and secondary drying (Geidobler and Winter, 2013).

2.1.1 Freezing stage

Approximately 90% of the total water in the food, mainly all the free water and some of the bound water, is frozen until crystalline ice forms part of the water and the reminder of the product is freeze-concentration into a glassy state that the

viscosity is too high to allow further crystallization (Ceballos et al., 2012). Freezing has an important influence in the size, shape, and distribution of the ice crystals (Geidobler and Winter, 2013). According to Charoenrein and Owcharoen (2016), increasing the sizes of ice crystals has also increased negative effect on texture and microstructure of freeze-dried product.

2.1.2 Primary drying stage

In this stage, the ice (free water) in the product has been removed by direct sublimation under reduced pressure (Ciurzyńska and Lenart, 2011). The heat supplied in this process should be below the glass transition temperature (Tg) of the product so that the solution transforms into glassy state (Harnkarnsujarit, Kawai, and Suzuki, 2016). Glassy state has very high viscosity and low movement, leading to the reduction of water mobility (Patist and Zoerb, 2005; Liu, Chen, and Li, 2017). Some water molecules are hold tightly in glassy state; therefore, water molecules cannot interact with any component of the material, leading to the increased stability of the preserved product such as low water activity and moisture content (Patist and Zoerb, 2005).

2.1.3 Secondary drying stage

This stage is used for removal of bound water in the frozen product by desorption in which the remaining water is removed by heating the product under vacuum. This stage should be carefully designed to prevent the foods from

undesirable quality. The product should contain less than 1-3% residual water (Ciurzyńska and Lenart, 2011).

2.2 Edible coating

FD process, is well-known, that produces the highest-quality dried foods. However, a major limitation with lyophilization is the long drying time needed (Reyes, Mahn, and Huenulaf, 2011). Pretreatment of raw material can reduce the time for FD process (Ciurzyńska and Lenart, 2011). Thus, it is necessary to pretreat the food by physical and/or chemical methods before FD process. The use of pretreatments includes cleaning, peeling, and blanching. Blanching, using hot water or by stream, can inactivate the enzyme activity and remove the air inside the tissue to avoid oxidation. However, blanching may not be appropriate for heat-sensitive foods such as fruits and vegetables (Wang et al., 2018). Therefore, using edible coating method with appropriate solutions for pretreatment may be an alternative to blanching. Edible coating can reduce moisture loss, color change, unexpected chemical reactions, and microbial contamination (Rojas-Graü et al., 2009). The selection of coating solutions depends on the types of food and drying methods. For FD process, cryoprotectant, is suggested to be added in a coating solution for maintaining the cell structure of the products during FD process.

2.2.1 Alginate-based film

Polysaccharide-based edible coating films have good physical and mechanical properties as being transparent, homogenous, flexible, and elastic. Among polysaccharide-based edible coatings, alginate, a 1-4-linked polymer of β-D-mannuronic acid (M) and α-L-guluronic acid (G) extracted from brown algae (*Laminaria digitata* and *Ascophyllum nodosum*) (**Figures 1 and 2**) (Rojas-Graü et al., 2008). It has an ability to form insoluble polymer or stable gel structure with multivalent cationic compounds. The strong film called "eggbox" is formed when adding divalent cations such as calcium, as shown in **Figure 3** (Tavassoli-Kafrani et al., 2016). Joint FAO/WHO Expert Committee on Food Additives (JECFA) approved alginate in 1992, with an ADI (acceptable daily intake) as not specified. Alginate is used in food as thickener, stabilizer, suspending, and gelling agents. It is considered to be non-toxic, biodegradable, and biocompatible (Tavassoli-Kafrani et al., 2016).

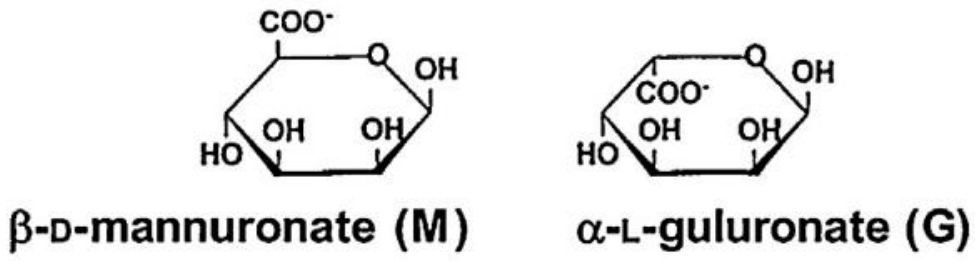


Figure 1 Structure of alginate monomers (Tavassoli-Kafrani et al., 2016)

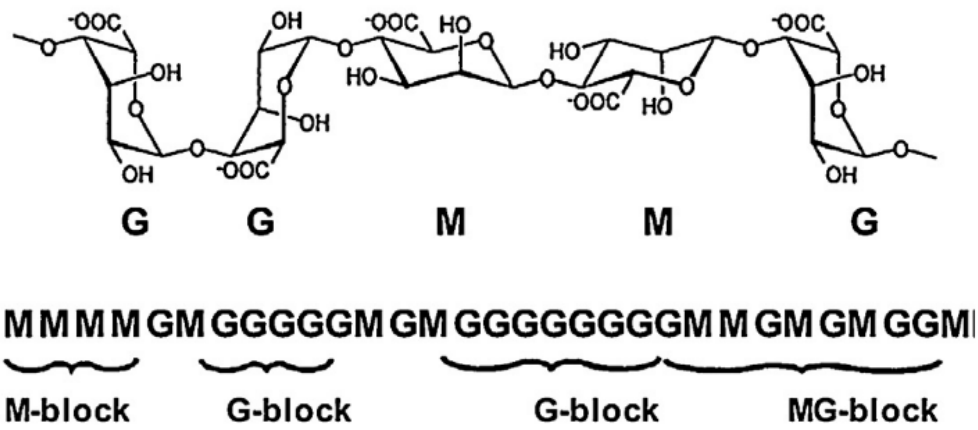


Figure 2 Structure of chain formation and block distribution of alginate polymer

(Tavassoli-Kafrani et al., 2016)

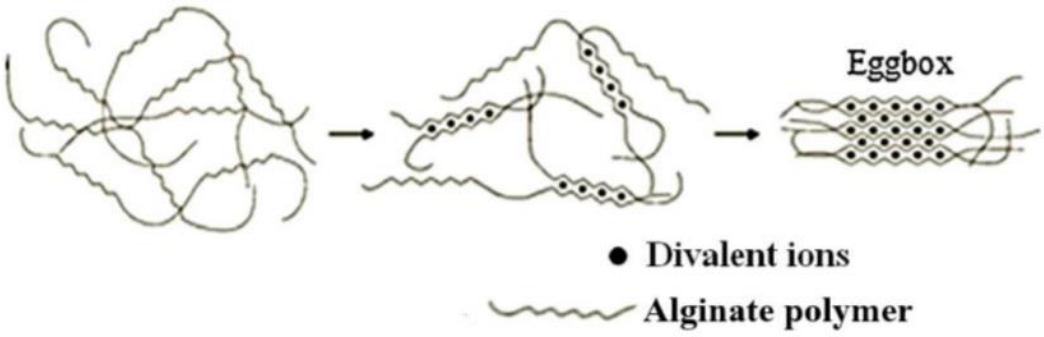


Figure 3 Formation of gelation by "eggbox" model of alginate

(Adapted from Tavassoli-Kafrani et al., 2016)

Alginate-based coatings can also offer a possibility to prolong the shelf-life of fresh-cut food by providing a thin layer on the surface of a product as a barrier to gases, reducing respiration, controlling enzymatic browning, and protecting water loss (Perez-Gago et al., 2006; Han, Yu, and Wang, 2018; Parreidt, Muller, and Schmid,

2018). Alginate-based film may not control water vapor removal. However, this film can be used for a sacrificial moisture agent in that moisture evaporates from the film instead of the food surface (Embuscado and Huber, 2009). Water in food is almost removed during FD process; however, food should still have moisture content as a suitable value. Hence, alginate-based film is the one choice of film coating in freeze-dried product.

Moreover, previous studies have reported that coating shrimp, fish, scallops, and pork with sodium alginate biofilms extended shelf-life, reduced weight loss, reduced thawing loss, and maintained the functional properties during frozen storage (Yu et al., 2008; Song et al., 2011).

2.2.2 Plasticizer

Plasticizer is a substance or material that is incorporated into a material to modify polymer characteristics by increasing its flexibility and reducing the tension of deformation (Parreidt et al., 2018). Glycerol is commonly added in film-forming solutions to prevent brittle film (Carneiro-da-Cunha et al., 2009; Santana and Kieckbusch, 2013). Glycerol, synthesized from polypropylene or sucrose, is a water-soluble, viscous, colorless, odorless, and sweet. The structure of glycerol is 3 hydroxyl groups (OH), which causes hydroscopic properties and dissolves well in water (Cerqueira et al., 2012). JECFA approved glycerol as food additive in 1976, with

an ADI as not specified. In addition, Mahayothee et al. (2009) reported the use of glycerol for pretreatment can improve the texture of reconstituted fruits.

2.2.3 Trehalose

Trehalose (α-D-glucopyranosyl-(1⟶1)-α-D-glucopyranoside) is a naturally occurring disaccharide composed of two glucose molecules bound by an α,α (1,1) glycosidic linkage as shown in **Figure 4** (Ohtake and Wang, 2011)**.** It is found in bacteria, fungi, plants, and in many invertebrates and is synthesized by enzymatic process as illustrated in **Figure 5** (Cai et al., 2018)**.** Several studies have shown that trehalose has a variety of beneficial properties, such as maintenance of texture and shape, modification of taste, and extension of shelf life (Mahayothee et al., 2009; O'Donnell, 2012; Aktas et al., 2013; Velickova, Winkelhausen, and Kuzmanova, 2013; Stefanello et al., 2018; Zhang et al., 2019).

In FD process, trehalose has been widely used as a cryoprotectant which plays an important role in preserving biological systems from injury caused by ice forming, or damage of cell membrane under stress condition (Patist and Zoerb, 2005). **Figure 6** shows the change in biological membrane (phospholipid bilayer) from the lamellar phase (**Figure 6A**) to gel phase during drying process (**Figure 6B**), leading to the membranes leaky (**Figure 6C**) (Patist and Zoerb, 2005). As shown in **Figure 7**, trehalose shows a direct interaction with the headgroups of phospholipid bilayer during drying, thus reducing the van der Waals interactions among the hydrocarbon

chains (**Figure 7B**). Upon rehydration the membrane integrity remains intact (**Figure 7C**) (Patist and Zoerb, 2005). Zhang et al. (2019) reported that trehalose could inhibit ice crystal size during freezing.

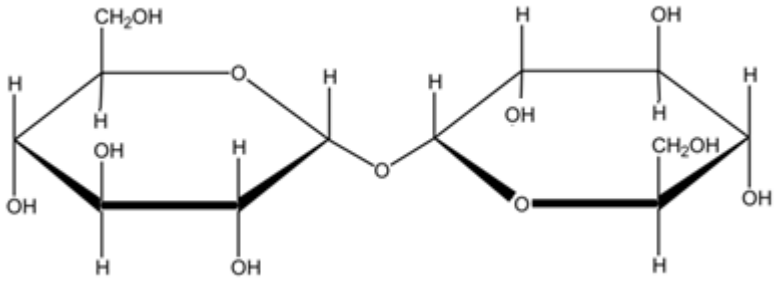


Figure 4 Structure of trehalose (Patist and Zoerb, 2005)

Physicochemical properties of trehalose are listed in **Table 1** (Cai et al., 2018). There are three main physical properties which make trehalose unique and beneficial in food systems. The first property is, α,α (1,1) glycosidic bond of trehalose which is very stable compared with other disaccharides. It is a non-reducing sugar and does not take part in Maillard reactions (Patist and Zoerb, 2005).

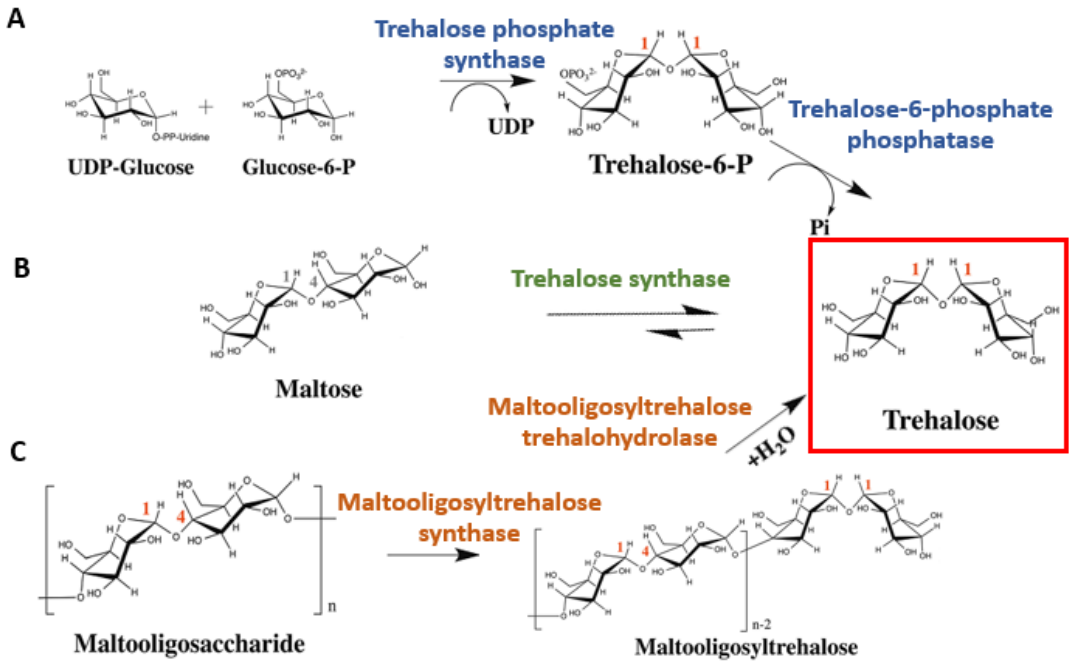


Figure 5 Biosynthesis pathways of trehalose production by trehalose phosphate synthase (A), trehalose synthase (B) and maltooligosyltrehalose synthase (C) (Adapted from Cai et al., 2018)

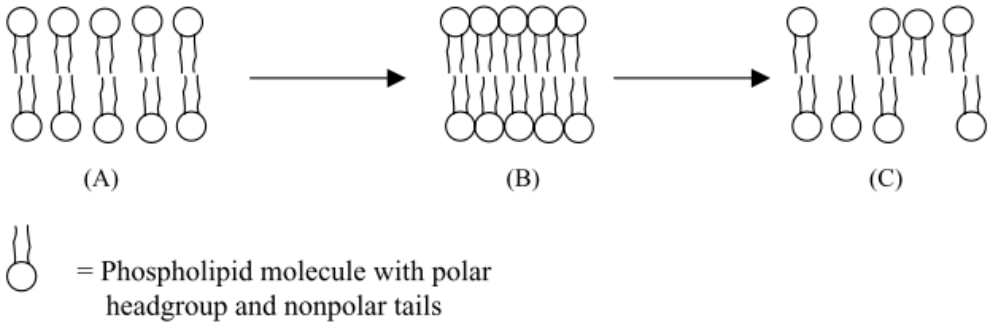


Figure 6 A change in biological membrane (phospholipid bilayer) in drying process (Patist and Zoerb, 2005)

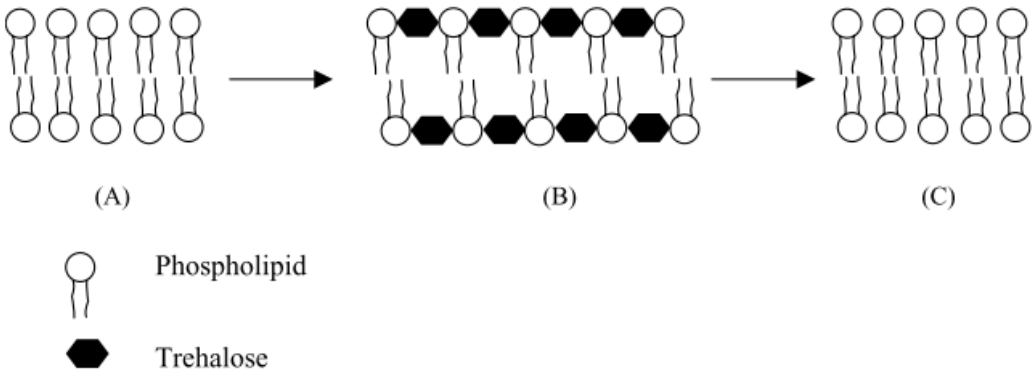


Figure 7 Proposed mechanism by which trehalose preserves phospholipid membrane (Patist and Zoerb, 2005)

The second property is that trehalose has only one intermolecular hydrogen bond and has more locales possible to form hydrogen bonds with water or other molecules (Liu et al., 2017). The mechanisms by which trehalose can protect biological systems in a dry condition have been proposed for the "water replacement theory", trehalose forms hydrogen bonds with biological substance instead of water, thus protecting it from denaturation from drying process. Another mechanism is called "water entrapment theory" in which trehalose traps a water layer between the biological system and a layer of trehalose (Patist and Zoerb, 2005; Liu et al., 2017).

Table 1 Physicochemical properties of trehalose

Properties	Trehalose
Molecular formula (anhydride)	$C_{12}H_{22}O_{11}$
Molecular weight (g/mol)	342.31 (anhydride)/ 378.33 (dihydrate)
Physical status	White orthorhombic crystals
Melting points (°C)	210.5 (anhydride)/ 97.0 (dihydrate)
Solubility in water	68.9 g/100 g at 20 °C
Glass transition temperature (Tg, °C)	115
Sweetness	45% of sucrose
Caloric effect	4 kcal/g
Maillard reaction	No
Toxicity	No
Digestibility	Digested and absorbed by the small intestine
pH stability of solution	>99% (pH 3.5-10, at 100 °C for 24 h)
Heat stability of solution	>99% (at 120 °C for 90 min)
Hygroscopicity	Non-hygroscopic under RH 90%

The third main property of trehalose is its high glass transition temperature (Tg) of 115°C compared to other disaccharides (**Table 2**) (Patist and Zoerb, 2005). This allows trehalose to maintain a glassy state without recrystallization under a wide range of condition. The glassy form has very high viscosity and low movement, leading to the reduction of molecular mobility and interaction. Thus, trehalose can be used to maintain proteins and lipids structure during freezing (Chang et al., 2005).

In addition, few studies have shown that dried sliced carrot and potato samples with trehalose pretreatment were improved the reconstitution properties compared with the dried products pre-treated with sucrose, which is generally used for osmotic dehydration (Aktas et al., 2007). Trehalose could also improve physical change (i.e. color, firmness, weight loss) of apple slices and litchi after drying (Albanese et al., 2007; Mahayothee et al., 2009).

In 2000, trehalose was approved as generally recognized as safe (GRAS) by the US Food and Drug Administration (US FDA) while JECFA reviewed and approved trehalose with an ADI as not specified (O'Donnell, 2012).

Table 2 Glass transition temperature of several sugars

Sugar	Tg (°C)
Trehalose	115
Maltose	84
Sucrose	60
Glucose	37
Fructose	5
Ribose	-22

2.3 Freeze-dried food quality evaluation

2.3.1 Water activity

Water activity (a_w) is the ratio of the equilibrium partial vapor pressure of pure water in the system to the equilibrium partial vapor pressure at the same temperature (Barbosa-Canovas et al., 2007). The a_w value is in range of 0-1. In the field of food science, a_w is a measurement of how tightly water is bound, structurally or chemically, in a food and hence it describes the availability of the water to take part in chemical and biochemical reactions (Barbosa-Canovas et al., 2007). The a_w value is also used to control the growth of microorganisms in foods (**Figure 8**). The food with a_w value of less than 0.6 can have no growth of any microorganisms especially pathogenic bacteria (Barbosa-Canovas et al., 2007).

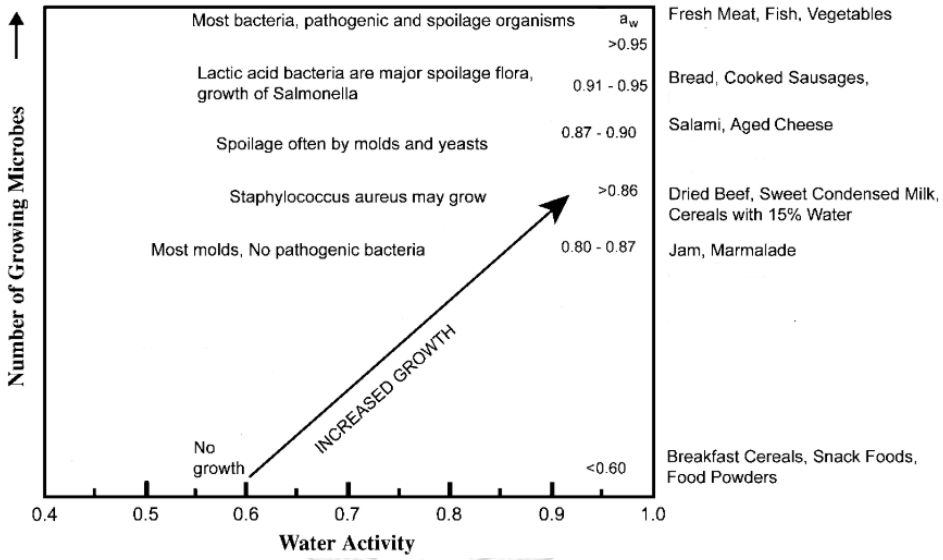


Figure 8 Water activity limits for growth of microorganisms in foods

(Adapted from Barbosa-Canovas et al., 2007)

2.3.2 Rehydration capacity

Rehydration capacity (RC) is another important quality parameter of dried foods (Lewicki and Wiczkowska, 2006). The good quality of dried food should have higher RC, high porosity of the dried products and ability to recover its original properties (Reyes et al., 2011). The freeze-dried food such as apple slices had higher porosity from the sublimation of smaller size of ice crystals (Cui et al., 2008). Charoenrein and Owcharoen (2016) showed that the large size of ice crystals formation during the freezing process damages cell membranes and brokedown the physical structure of the fruit more than small size of ice crystals.

2.3.3 Firmness

Firmness is a value that indicates the softness or hardness of the dried fruit (Antal and Kerekes, 2016). Firmness is considered to be one of the most important criteria concerning eating quality of dried fruit (Antal et al., 2015). The combination of cell structure integrity and tissue turgor referred to the firmness. During drying, cell wall structure is remodeled due to water loss, resulting in fruit firmness (Moreno et al., 2004). There are two basic methods for measuring food firmness or texture: destructive method and non-destructive method. Compression test is one of the most common destructive methods for dried fruit texture measurement (Chen and Opara, 2013). The force detection is used to calculate as force (Newton; N) or stress (N/mm^2) and creates a deformation curve as shown in **Figure 9**.

The deformation or strain is a ratio of the difference between the length (ΔL) that changes with the original length (L) (Li, Miao, and Andrews, 2017). The maximum force before the deformation is reported as the firmness of dried fruit (Antal et al., 2015).

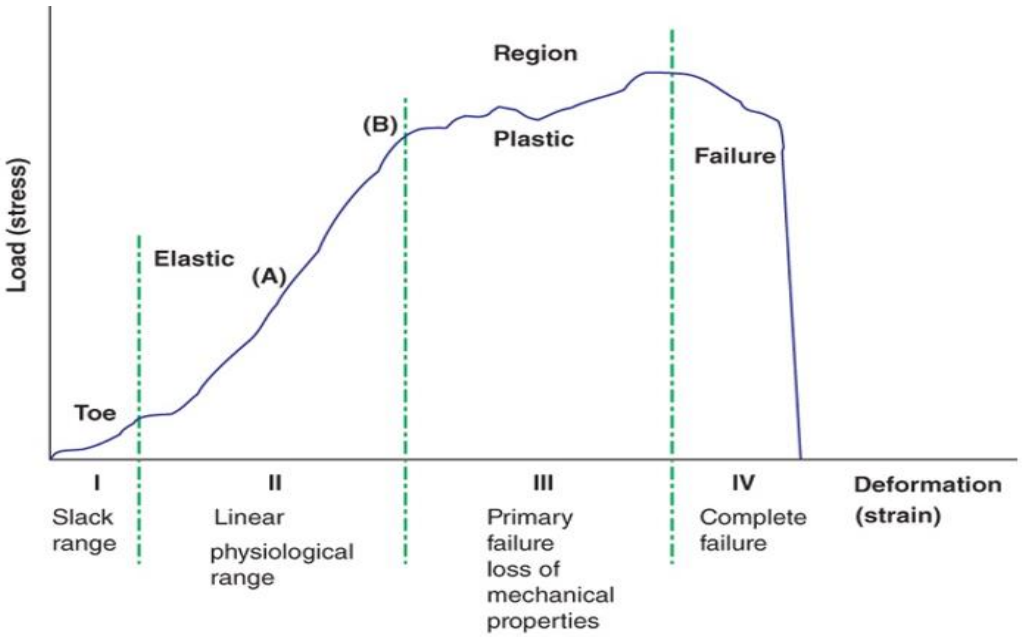


Figure 9 Deformation curve of compression test (Dutton, Ivey, and Smith, 2019)

2.3.4 Color

The color is one of the most important quality attributes of processed food since it influences consumer acceptability (Valentina et al., 2016). Color in fruits and vegetables is derived from natural pigments, for example, carotenoids (yellow, orange, and red), chlorophylls (green), flavonoids (yellow), betalains (red) and anthocyanins (red, blue) (Barrett, Beaulieu, and Shewfelt, 2010). Color features can be used to indicate defects in food product, such as the surface of fresh-cut apples and freeze-dried apples (Albanese et al., 2007; Antal, Figiel, et al., 2013).

The one of the most popular color coordinate systems is RGB (red, green, and blue), which is used in the Commission Internationale de l'Eclairage (CIE) *L* a* b** (Pathare, Opara, and Al-Said, 2012). As illustrated in **Figure 10**, CIE *L* a* b** concepts

are the combinations of three color receptors (red, green, and blue) of human eye and all colors.

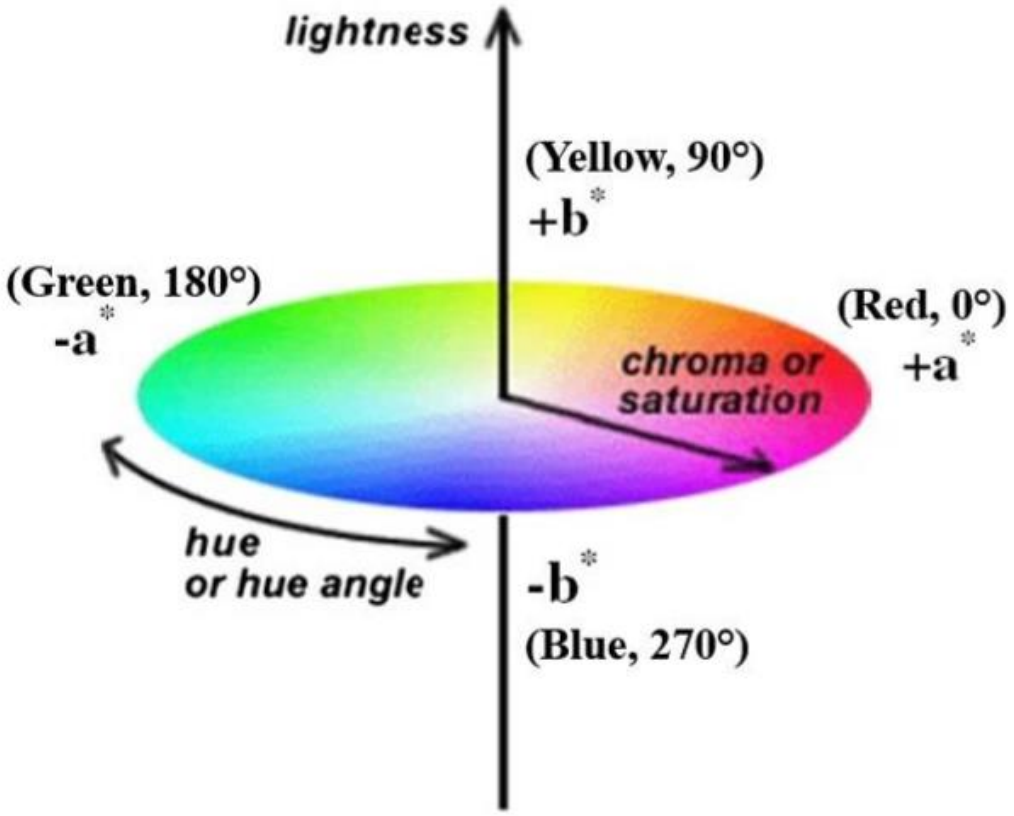


Figure 10 CIELAB color scale (Adapted from Pathare et al., 2012)

The parameter *a** shows positive values for red and negative values for green, whereas *b** shows positive values for yellow and negative values for blue. *L** parameter is a measure of lightness, which is in the grayscale between white and black (Granato and Masson, 2010).

Hue angle (h^*) is used to define the color difference with reference to the lightness. The angle of 0° or 360° are red, while the angle of 90°, 180°, and 270° are yellow, green, and blue, respectively. This parameter can be used for color evaluation in fruits and vegetables (Barrett et al., 2010). The equation of h^* value is calculated as follows:

$$h^* = \tan^{-1}(b^*/a^*) \quad \textbf{(1)}$$

Total color difference (ΔE^*) represents the color change between the initial color and final color. ΔE^* can be calculated using the following equation:

$$(\Delta E^*) = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5} \quad \textbf{(2)}$$

ΔE^* value can be analytically classified as very distinct ($\Delta E^* > 3$), distinct ($1.5 < \Delta E^* < 3$), and small difference ($1.5 < \Delta E^*$). If the ΔE^* value is less than 1.5, the change of color is almost not differentiated (Pathare et al., 2012).

Moreover, the browning index (BI) defines the browning of the fruit surface. It is also one of the most common indicators of browning in sugar-containing food products (Lunadei et al., 2011). BI can be calculated as follows:

$$BI = 100\,(x - 0.31)/\,0.172 \quad \textbf{(3)}$$

where $x = (a^* + 1.75L^*)/\,(5.645L^* + a^* - 3.012b^*)$

2.4 Response surface methodology

Response surface methodology (RSM) is a term applied to multivariate techniques (mathematical and statistical techniques) that are useful for modeling and research problem analysis to provide the optimal solutions of various physical and chemical processes. The response y depends on the independent factors that are a function of x. The equation can be written as follows:

$$y = f(x_1, x_2, ..., x_k) + \varepsilon \quad (4)$$

where ε is the experiment error of the response (y). The response surface is the surface represents by $f(x_1, x_2, ..., x_k)$, which can be presented in graphical form. The contour plot is written on x_1 and x_2 planes in order to better visualize the shape of the surface response, as shown in **Figure 11**. The contour plot of three or more factors is only possible when one or more factors are constant. If the response model has a linear relationship with the independent factors, the appropriate experiment can be described by a linear statistical model as follows:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \varepsilon \quad (5)$$

where k is the number of factors, β_0 is the constant value, β_i presents the coefficients of the linear terms, x_i presents the factors, and ε is the residual related to the experiments.

If the response model has a curve, the optimal equations are the polynomial

functions such as quadratic terms, as shown below:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{1 \le i \le j}^{k} \beta_{ij} x_i x_j + \varepsilon \quad \textbf{(6)}$$

where k is the number of factors, β_0 is the constant value, β_i presents the coefficients of the linear terms, β_{ii} presents the coefficients of the quadratic terms, β_{ij} presents the coefficients of the interaction terms, x_i and x_j present the factors, and ε is the residual related to the experiments

Before analysis, the RSM requires to choose a suitable experimental design, which has linear and quadratic models such as three-level factorial, Box-Behnken, central composite, and Doehlert designs. Box-Behnken design (BBD) takes three equally interval levels (-1, 0, +1) of all factors. All the experimental points are showed in the hypersphere form and are placed equidistant from the central point, as shown in **Figure 12** (Myers and Montgomery, 2002; Bezerra et al., 2008; Candioti et al., 2014).

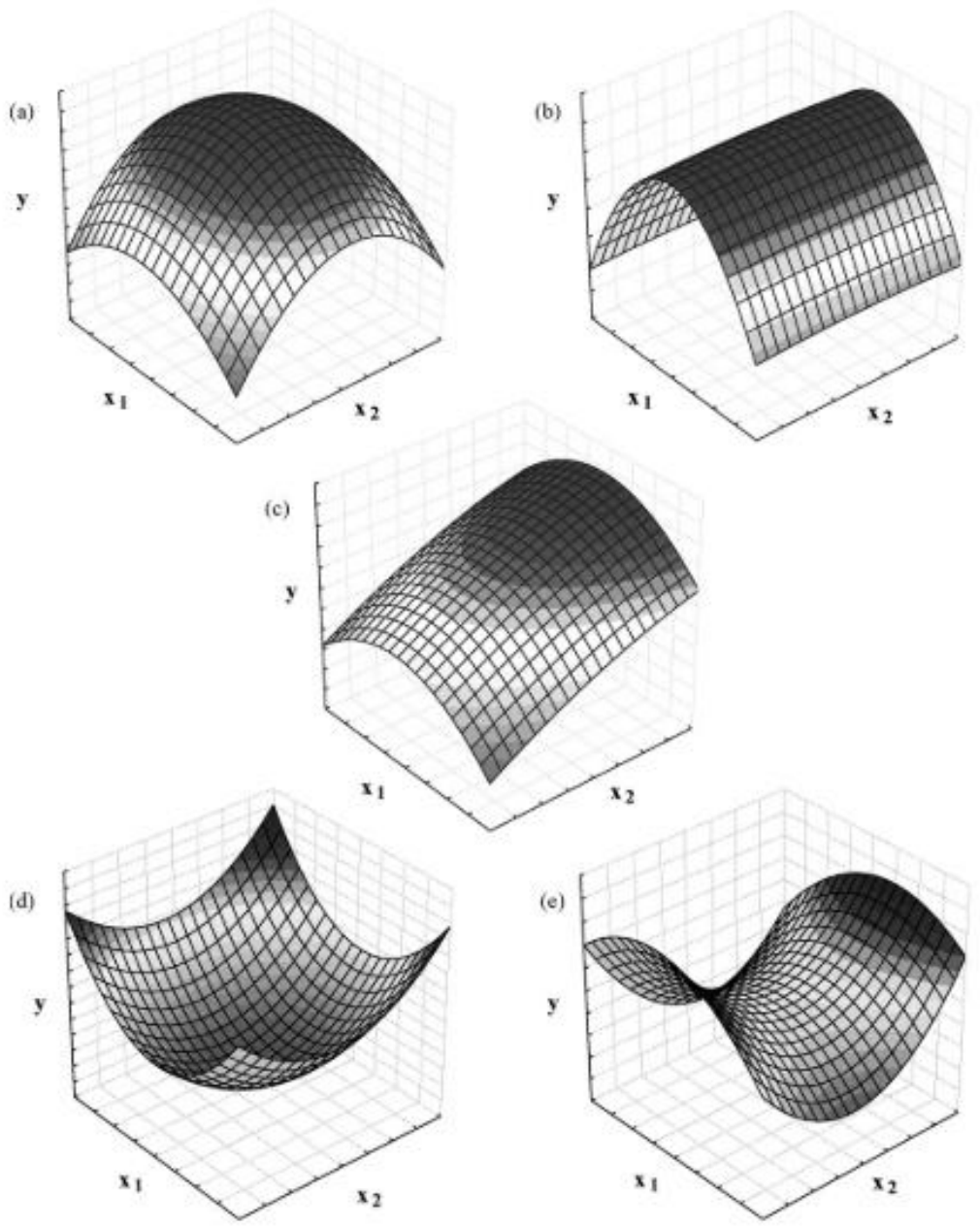


Figure 11 Some profiles of the surface response plots (x_i = variables, y = response)

(a) maximum, (b) plateau, (c) maximum outside the experimental region,

(d) minimum, and (e) saddle surfaces (Bezerra et al., 2008)

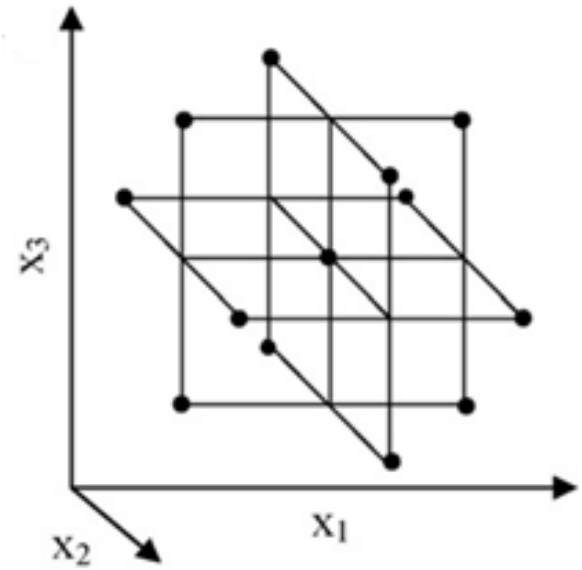


Figure 12 The study of three variables (x_1, x_2, x_3) of Box-Behnken design (Adapted from Bezerra et al., 2008)

CHAPTER III

MATERIALS AND METHODS

3.1 Chemicals and reagents

Trehalose (Tre) as dihydrate form was derived from Hayashibara, Japan. Sodium alginate (SA) was supplied from Sigma-Aldrich, USA. Glycerol and calcium chloride ($CaCl_2$) were purchased from Daejung, South Korea. Deionized water obtained from distillation using water purification system (Pacific TII 12 UV, Thermo Scientific, Hungary).

3.2 Experimental design

Films were prepared with different concentrations of Tre, SA and $CaCl_2$. Physical properties of film were determined by thickness, opacity, moisture content, and water vapor permeability (WVP). The film formulation was optimized by response surface methodology (RSM), Box-Behnken design (BBD). The optimal film formulation was selected to coat fruit slices for FD process. Freeze-dried fruit slices were stored in desiccator and analyzed for physical properties (moisture content, water activity, rehydration capacity, firmness, color, and mass loss) and microbial determination. The experimental design is shown in **Figure 13**.

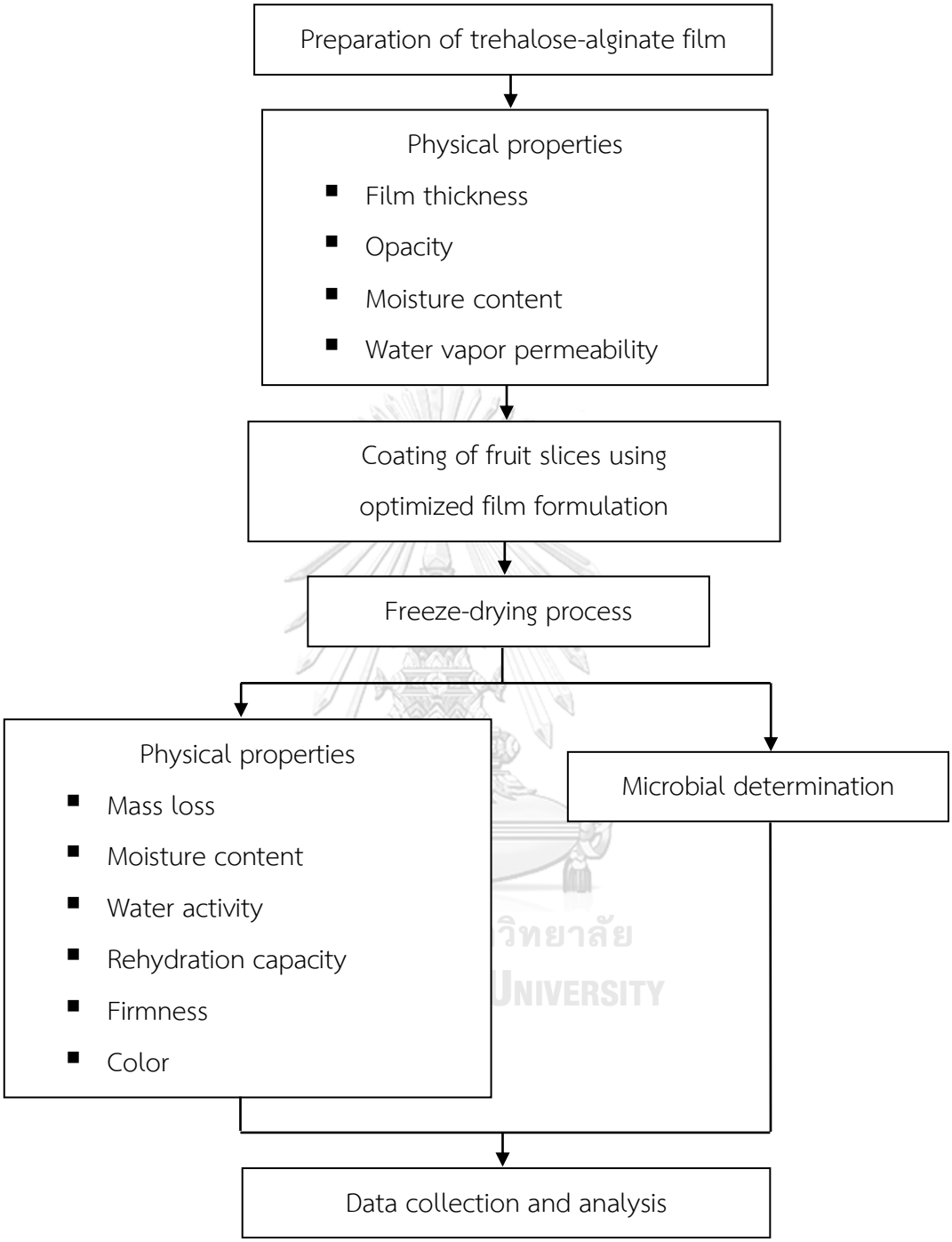


Figure 13 Diagram of the experimental design

3.3 Methods

3.3.1 Preparation of composite films

The formulations were prepared according to a 3-factor, 3-level BBD as shown in **Table 3. Table 4** shows the film formulations which were formed by various concentrations of factors (3-9% w/v Tre (X_1), 1.2-2.0% w/v SA (X_2), and 0.2-0.6% w/v $CaCl_2$ (X_3)). The effects of factors, Tre concentration (X_1), SA concentration (X_2), and $CaCl_2$ concentration (X_3) on the responses, thickness (Y_1), opacity (Y_2), moisture content (Y_3), and WVP (Y_4) were studied. Before determining the concentration of each factor, preliminary study was done and then the results to narrower level of each factor were selected (Ohtake and Wang, 2011; O'Donnell, 2012; Pérez et al., 2016; Han et al., 2018). The film forming solutions were prepared by dissolving SA (1.2, 1.6, 2.0% w/v) in distilled water with stirring using stirrer and heating (70ºC) (VELP Scientifica, Italy) to obtain a clear solution before adding glycerol (1% w/v) as a plasticizer. Tre at different concentrations (3, 6, 9% w/v) was then added to the mixtures. After complete solubilization, $CaCl_2$ at various concentrations (0.2, 0.4, 0.6% w/v) as a cross-linking agent was added and stirred continuously. The film coating solution was poured onto a petridish with Teflon sheet (INDY supply & service Ltd., Thailand) and kept for drying in an oven at 60ºC for 24 h. All films were stored in a desiccator for 6 h before analysis.

Table 3 Factors used in Box-Behnken design (BBD) for film preparation

Symbol	Factors	Levels		
		-1	**0**	**1**
X_1	Trehalose (% w/v)	3	6	9
X_2	Sodium alginate (% w/v)	1.2	1.6	2.0
X_3	Calcium chloride (% w/v)	0.2	0.4	0.6

3.3.2 Determination of film physical properties

3.3.2.1 Thickness

By using a digital vernier caliper (Intro TSC Co., Ltd., Thailand), the thickness of films was measured at three different areas on each film, and a mean value was calculated (Rangel-Marrón et al., 2013).

3.3.2.2 Opacity

The opacity based on the CIE *L* a* b** was measured for each film by using UltraScan XE colorimeter (Hunterlab, Inc., Reston, USA) which was calibrated with standard white and black backgrounds. Three measurements were performed for each film, and the mean values were determined for each parameter. The EasyMatch QC software version 4.62 (Hunterlab, Inc., USA) was automatically calculated for opacity.

Table 4 Coding and decoding factors used in Box-Behnken design (BBD) for film formulation

Experiment order	Coding			Decoding		
	X_1	X_2	X_3	X_1	X_2	X_3
1	-1	-1	0	3	1.2	0.4
2	1	-1	0	9	1.2	0.4
3	-1	1	0	3	2.0	0.4
4	1	1	0	9	2.0	0.4
5	-1	0	-1	3	1.6	0.2
6	1	0	-1	9	1.6	0.2
7	-1	0	1	3	1.6	0.6
8	1	0	1	9	1.6	0.6
9	0	-1	-1	6	1.2	0.2
10	0	1	-1	6	2.0	0.2
11	0	-1	1	6	1.2	0.6
12	0	1	1	6	2.0	0.6
13	0	0	0	6	1.6	0.4
14	0	0	0	6	1.6	0.4
15	0	0	0	6	1.6	0.4

3.3.2.3 Moisture content

Films were dried in oven at 105°C for 24 h then the moisture content was measured and calculated as the percentage of water removed from the film (Rhim et al., 2002)

3.3.2.4 Water vapor permeability

Water vapor permeability was determined as described in Rangel-Marrón et al. (2013). Developed films was cut into 2-cm diameter and sealed on top of the bottle with distilled water. The glass bottles containing 5 mL of distilled water, leaving air space between the water surface and the film was kept in a desiccator containing a saturated solution of $MgCl_2.6H_2O$ at 25°C/ 33% RH. The bottles were weighed and recorded every 60 min for 8 h, then water vapor transmission rate (WVTR) was calculated by using the slope of the linear regression of weight loss versus time (g h^{-1}) divided by the exposed area of film (m^2). WVP (g mm m^{-2} h^{-1} kPa^{-1}) was determined as follows;

$$WVP = L \times WVTR / (P_i - P_a) \quad \textbf{(7)}$$

P_i was the partial pressure (kPa^{-1}) of water vapor in the air and P_a was the partial pressure of water vapor in the air saturated to 25°C/ 33% RH. L was the average thickness (mm).

3.3.3 Selection of optimized composite film

The effects of factors (X_1 = Tre concentration, X_2 = SA concentration, and X_3 = $CaCl_2$ concentration) on the responses (Y_1 = thickness, Y_2 = opacity, Y_3 = moisture content, and Y_4 = WVP) were analyzed to optimize composite film formulation by RSM using Design-Expert® Software version 11.0 (Stat-Ease, USA). BBD was used as an experimental model. Parameters found to be significant at least the 95% confidence level were considered in the final prediction model (Candioti et al., 2014). The model analysis, lack of fit test, and coefficient of determination (R^2) analysis were performed to determine the adequacy of the models (Candioti et al., 2014). After that, equations indicating the significant relationship between each factor and response were obtained. Response surface plots were also used to show the trend of factors that affected the response. These data were then used to evaluate the desirability of the optimal formulation. The desirability (D) is the overall satisfaction value of the response and has a value between 0 and 1. If D is close to 1, the corresponding setting would be a good compromise among the responses (Candioti et al., 2014).

3.3.4 Coating of fruit slices using optimized film formulation

Fuji apples (*Malus domestica* Borkh. cv. Fuji) were purchased from local supermarket in Bangkok, Thailand (145-180 g per apple). Apples were washed, peeled, and cut into 10 mm x 10 mm x 10 mm (2-3 g per piece). After that, the sample was dipped in the optimized film coating solution (from 3.3.3) for 2 min,

dipped off for 1 min and dipped in $CaCl_2$ solution for 2 min then dipped off for 1 min, respectively (Salinas-Roca et al., 2016). The sample groups were dipped in solution coating Tre and alginate, the control group (dipped in water only) and the alginate-coated group (dipped in alginate solution without Tre) were used for comparison.

3.3.5 Freeze-drying process

The coated samples were frozen at -72ºC by using ultra-low temperature freezer (Thermo Electron Corporation, USA) for 24 h. After that, freeze-dried samples were performed by using a freeze-dryer (Labconco Freezone Plus 6, USA) for 60 h at -50 ± 5°C and a vacuum level between 0.01-0.22 mBar (Antal, Sikolya, and Kerekes, 2013). Then the samples were stored in a desiccator at 25 ± 5ºC. Non-freeze dried samples were analyzed before FD process.

3.3.6 Determination of properties of freeze-dried fruit slices

The freeze-dried samples were determined for the properties after storage at 25 ± 5ºC in desiccator for 0, 2 and 4 weeks.

3.3.6.1 Physical properties

The freeze-dried samples were determined for physical properties. All the measurements were performed in triplicate and the average values were reported.

3.3.6.1.1 Mass loss

Mass loss (ML) of sample was evaluated by comparing the sample weight after FD with the initial weight (before FD). The measurements of ML were carried out by using the following formula:

$$ML\ (\%) = m_0\text{-}m_1/m_0 \times 100\% \quad \textbf{(8)}$$

ML (%) was the percentage of mass loss in the sample during freezing, m_0(g) and m_1(g) were the weights of the sample before and after FD process, respectively (Wang et al., 2018).

3.3.6.1.2 Moisture content

Moisture content of samples was measured by using the oven drying method described in AOAC (2000), Method 934.06 (Salazar, Alvarez, and Orrego, 2017).

3.3.6.1.3 Water activity

Freeze-dried samples were measured by using LabMaster Neo (NOVASINA) at 25 ± 2ºC (Mahayothee et al., 2009).

3.3.6.1.4 Rehydration capacity

Samples (1 g) after FD were soaked in 100 mL distilled water at 25°C for 15 min. After that, samples were weighed and calculated for the rehydration capacity (RC) from the equation:

$$RC = m_f / m_o \quad \textbf{(9)}$$

m_f was the weight after immersion, and m_0 was the initial weight of the freeze-dried sample (Salazar et al., 2017).

3.3.6.1.5 Firmness

Firmness of freeze-dried samples, reported as a maximum force before food deformation with the unit in Newton (N), was assessed by compression test with universal testing machine model EZ-S (Shimadzu, Japan). The parameters that have been used were the following: 50 N of force load cell, 1 mms^{-1} of test speed, 2 cm in diameter of cylindrical probe. The maximum depth of penetration was 50% of the initial height of sample (Antal, Sikolya, et al., 2013). Then, the maximum force before food deformation was recorded.

3.3.6.1.6 Color

Samples were measured in CIE L*a*b* color space by using UltraScan XE colorimeter (Hunterlab, Inc., Reston, USA). The color of samples was measured before and after FD process. The parameters L^* (lightness), a^* (red-green dimension), and b^* (yellow-blue dimension) were determined (Pathare et al., 2012). The total color difference (ΔE^*) based on changes in values of L^*, a^* and b^* was calculated according to the following equation:

$$(\Delta E^*) = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5} \quad \textbf{(10)}$$

The values of a* and b* were used to calculate the hue angle (h*):

$$h^* = \tan^{-1}(b^*/a^*) \quad \textbf{(11)}$$

Browning of the fruit surface determined as browning index (BI) was calculated as follows:

$$BI = 100\,(x - 0.31)/\,0.172 \quad \textbf{(12)}$$

where $x = (a^* + 1.75L^*)/\,(5.645L^* + a^* - 3.012b^*)$

3.3.6.2 Microbial determination

Microbial population of coated freeze-dried samples was measured by plate count method (Food and Drug Administration, 1998). The amounts of psychrophilic bacteria, mesophilic bacteria, and fungi (yeasts and molds) were counted. Psychrophilic bacteria were cold-tolerant bacteria that can grow at low temperature with minimum temperature for growth at 0°C or below (Moyer, Eric Collins, and Morita, 2017). Mesophilic bacteria were bacteria that prefer moderate temperature of 30-45°C (Willey et al., 2008).

At day of analysis, all operation procedures required aseptic technique. Freeze-dried sample (10 g) was aseptically weighed into a sterile blender jar. Sterile peptone water (0.1% w/v) 90 mL was added into a blender jar. The blender mixed the sample into a homogeneous solution. A serial dilution was used to dilute sample until appropriate concentration at microbial population of 25-250 colonies per plate (Food and Drug Administration, 1998). The sample 0.1 mL of each dilution was filled

on agar plate and dispersed with sterile glass spreader. Mesophilic and psychrophilic bacteria were cultured with plate count agar (PCA). Mesophilic bacteria were incubated at 35°C for 48 h, and psychrophilic bacteria were incubated at 5°C for 5 days. Yeast and mold were cultured with Dichloran Rose Bengal Chloramphenicol (DRBC) agar and were incubated at 25°C for 5 days. After incubation, the results were the concentration of each specie (CFU/g of food), which was calculated from the number of colonies on the plate (Food and Drug Administration, 1998; Rojas-Graü et al., 2008; Mohammadi, Hashemi, and Hosseini, 2015).

3.3.7 Statistical analysis

The data of each group were presented as mean ± standard deviation (SD). Statistical package for the social sciences (SPSS) version 22.0 (IBM, USA) was used for calculated the variance in each group with homogeneity of variance test and the analysis of variance (ANOVA) for the statistical difference at *p* values of less than 0.05 with Bonferroni test.

For optimization of the composite film, the data were analyzed for the statistical difference ($p<0.05$) by using Design-Expert® Software version 11.0 (Stat-Ease, USA). The significance of the equation parameters for each response and the adequacy of the models by model analysis, lack of fit test and coefficient of determination (R^2) analysis were determined. These data were used to evaluate the appropriate preparation that achieved the desired responses.

CHAPTER IV

RESULTS AND DISCUSSIONS

Composite films containing alginate and trehalose was developed for freeze-dried fruit slices. The film coating was optimized by design of experiment (Box-Behnken design). The film-coated freeze-dried fruit slices were studied for their properties.

4.1 Formulation of film

The factors used for film preparation in this study were concentrations of Tre, SA, and $CaCl_2$. Previous studies reported that these three factors had an effect on physical properties of film such as thickness, opacity, moisture content, and WVP (Rangel-Marrón et al., 2013; Khairunnisa et al., 2018; Parreidt et al., 2018).

The 15 film formulations were prepared according to the Box-Behnken design (BBD) of model experiment used, as seen in **Table 4**. Notably, the concentrations of SA, Tre and $CaCl_2$ were chosen based on preliminary studies that the formation of homogeneous films was confirmed (Ohtake and Wang, 2011; O'Donnell, 2012; Pérez et al., 2016; Han et al., 2018). The dependent variables (responses) were thickness (Y_1), opacity (Y_2), moisture content (Y_3), and WVP (Y_4) which depended on the 3 factors used namely, Tre concentration (X_1), SA concentration (X_2), and $CaCl_2$ concentration (X_3). The results of the physical properties (Y_1-Y_4) of film are shown in **Table 5**.

4.1.1 Optimization of film formulation

4.1.1.1 Analysis of model fitting

The response data obtained from experimental BBD design were analyzed in order to find the optimal mathematic model fitting for each response. The statistical analysis of the suitability of each model is shown in **Table 6**.

The suitability of the model was evaluated by using the sequential p-value. The model can be used to evaluate the response when the sequential p-value is significant ($p<0.05$). In contrast, *p*-value of "lack of fit", the number of model predictions that are erroneously observed, is insignificant ($p>0.05$) indicating that the model is suitable to be used to evaluate responses (Stat-Ease, 2018).

R^2 is a statistical measure that represents the proportion of the variance for a dependent variable as described by an independent variable in a regression model. R^2 value is in the range from 0 to 1. The R^2 of 1 means that all dependent variables are completely explained by movements in the independent variables. However, R^2 only works as intended in a simple linear regression model with one explanatory variable (Hayes, 2019). For non-linear model, the adjusted R^2 is more suitable to interpret results better than R^2 value because the adjusted R^2 has been adjusted for a number of predictors in the model. The predicted R^2 indicates how well the model predicts the response for new observations (Minitab-Blog, 2013; Candioti et al., 2014).

Table 5 Results of thickness, opacity, moisture content, and water vapor permeability (WVP) of film

Experiment order	Responses			
	Thickness (mm)	Opacity	Moisture content (% w/w)	WVP ($g \cdot mm \cdot m^{-2} \cdot h^{-1} \cdot kPa^{-1}$)
1	0.06 ± 0.01	17.3 ± 0.1	20.68 ± 0.35	42.03 ± 0.04
2	0.12 ± 0.02	17.5 ± 0.1	12.23 ± 0.67	72.86 ± 0.21
3	0.13 ± 0.01	17.8 ± 0.4	17.18 ± 0.45	62.74 ± 0.39
4	0.17 ± 0.02	18.5 ± 0.3	10.75 ± 0.21	44.99 ± 0.29
5	0.07 ± 0.01	16.7 ± 0.1	19.11 ± 0.34	44.68 ± 0.18
6	0.09 ± 0.01	17.2 ± 0.1	11.4 ± 0.19	39.23 ± 0.23
7	0.16 ± 0.01	20.5 ± 0.1	19.04 ± 0.41	94.65 ± 0.36
8	0.20 ± 0.02	19.9 ± 0.2	11.75 ± 0.54	133.89 ± 0.19
9	0.04 ± 0.01	16.2 ± 0.2	13.4 ± 0.13	20.55 ± 0.37
10	0.08 ± 0.01	17.2 ± 0.2	12.05 ± 0.25	46.08 ± 0.31
11	0.18 ± 0.02	20.7 ± 0.2	14.19 ± 0.29	78.46 ± 0.44
12	0.20 ± 0.03	19.8 ± 0.2	13.62 ± 0.18	87.18 ± 0.39
13	0.08 ± 0.01	17.6 ± 0.1	13.48 ± 0.31	37.36 ± 0.30
14	0.09 ± 0.01	17.9 ± 0.1	13.42 ± 0.27	36.24 ± 0.25
15	0.09 ± 0.02	18.1 ± 0.1	12.87 ± 0.33	37.83 ± 0.36

Table 6 Model fitting, lack of fit test and coefficient of determination (R^2) analysis of thickness, opacity, moisture content, and water vapor permeability (WVP)

Model	Sequential *p*-value	Lack of Fit *p*-value	Adjusted R^2	Predicted R^2	Suggestion
Thickness					
Linear	< 0.0001	0.0565	0.8301	0.7878	
Quadratic	0.0515	0.0845	0.9160	0.5436	Suggested
Cubic	0.0845		0.9880		Aliased
Opacity					
Linear	< 0.0001	0.1440	0.8258	0.7232	
Quadratic	0.0425	0.3294	0.9443	0.7459	Suggested
Cubic	0.3294		0.9675		Aliased
Moisture content					
Linear	< 0.0001	0.0559	0.8338	0.7479	
Quadratic	0.0091	0.1697	0.9601	0.7950	Suggested
Cubic	0.1697		0.9884		Aliased
WVP					
Linear	0.0079	0.0515	0.5488	0.3321	
Quadratic	0.0281	0.1267	0.8658	0.2902	Suggested
Cubic	0.1267		0.9710		Aliased

For opacity, moisture content, and WVP, the sequential *p*-value of quadratic model was significant (opacity = 0.0425, moisture content = 0.0091, and WVP = 0.0281) and lack of fit *p*-value was insignificant (opacity = 0.3294, moisture content = 0.1697, and WVP = 0.1267). Hence, the quadratic model had enough of the variables to be used to predict the results of responses correctly. For thickness, the sequential *p*-value of quadratic model was insignificant. However, the significance of the model may not be necessary if the model can accurately describe the response of the data (Candioti et al., 2014). In addition, the lack of fit *p*-value of quadratic model was insignificant and adjusted R^2 value of quadratic model was more than the value of linear model, therefore, the quadratic model could be used to evaluate the thickness. The results showed that quadratic models were suitable to predict responses of thickness, opacity, moisture content, and WVP with adjusted R^2 equal of 0.9160, 0.9443, 0.9601, and 0.8658, respectively. The higher adjusted R^2 represents the higher the accuracy of prediction.

Table 7 ANOVA of the coefficients for quadratic model of thickness (Y_1)

Variables	Coefficient	*F*-value	*p*-value
Model			
β_0	0.087	17.97	0.0027*
Linear			
β_1	0.020	13.71	0.0140*
β_2	0.023	17.36	0.0088*
β_3	0.058	113.36	0.0001*
Interaction			
β_{12}	-0.0050	0.43	0.5416
β_{13}	0.0050	0.43	0.5416
β_{23}	-0.0050	0.43	0.5416
Quadratic			
β_{11}	0.019	5.81	0.0608
β_{22}	0.014	3.18	0.1348
β_{33}	0.024	9.24	0.0287*

*Significant (*p*-value < 0.05)

Table 8 ANOVA of the coefficients for quadratic model of opacity (Y_2)

Variables	Coefficient	*F*-value	*p*-value
Model			
β_0	17.87	27.39	0.0010*
Linear			
β_1	0.10	0.74	0.4294
β_2	0.20	2.95	0.1463
β_3	1.70	213.42	< 0.0001*
Interaction			
β_{12}	0.13	0.58	0.4818
β_{13}	-0.28	2.79	0.1556
β_{23}	-0.48	8.33	0.0343*
Quadratic			
β_{11}	0.0042	0.0006	0.9815
β_{22}	-0.096	0.31	0.6000
β_{33}	0.70	16.90	0.0093*

*Significant (*p*-value < 0.05)

Table 9 ANOVA of the coefficients for quadratic model of moisture content (Y_3)

Variables	Coefficient	*F*-value	*p*-value
Model			
β_0	13.26	38.47	0.0004*
Linear			
β_1	-3.74	287.76	< 0.0001*
β_2	-0.86	15.35	0.0112*
β_3	0.33	2.25	0.1942
Interaction			
β_{12}	0.51	2.63	0.1658
β_{13}	0.11	0.11	0.7497
β_{23}	0.20	0.39	0.5586
Quadratic			
β_{11}	1.98	37.39	0.0017*
β_{22}	-0.028	0.0076	0.9337
β_{33}	0.087	0.072	0.7998

*Significant (*p*-value < 0.05)

Table 10 ANOVA of the coefficients for quadratic model of water vapor permeability (WVP) (Y_4)

Variables	Coefficient	*F*-value	*p*-value
Model			
β_0	40.48	11.04	0.0083*
Linear			
β_1	5.86	2.38	0.1839
β_2	3.39	0.79	0.4139
β_3	30.46	64.18	0.0005*
Interaction			
β_{12}	-12.14	5.10	0.0734
β_{13}	11.17	4.32	0.0923
β_{23}	-4.20	0.61	0.4698
Quadratic			
β_{11}	17.61	9.91	0.0255*
β_{22}	-2.43	0.19	0.6818
β_{33}	20.02	12.81	0.0159*

*Significant (*p*-value < 0.05)

The coefficients of polynomial equation determine the effect of each factor on each response. A positive value in regression equation for a response represents an effect that is synergetic effect, while a negative value indicates an inverse relationship between the factor and the response (Candioti et al., 2014; Yolmeh and Jafari, 2017). For evaluation the relationship between the response and independent variables, the generalized polynomial equation can be written as follows:

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 \quad \textbf{(13)}$$

In this equation, Y_i is a calculated response. X_1, X_2 and X_3 are factors influencing the response of Yi; β_0 is the constant coefficient; β_1, β_2 and β_3 indicate linear coefficients; β_{12}, β_{13} and β_{23} represent interaction coefficients; and β_{11}, β_{22} and β_{33} present coefficients of quadratic term (Yolmeh and Jafari, 2017).

ANOVA of the coefficients (β) of thickness (Y_1), opacity (Y_2), moisture content (Y_3), and WVP (Y_4) with quadratic models is shown in **Tables 7-10**. The *p*-values of models for thickness, opacity, moisture content, and WVP were 0.0027, 0.0010, 0.0004, and 0.0083, respectively, which were statistically significant ($p<0.05$) confirming the adequacy of the quadratic model.

The significance of independent factors (X) has an effect on the response. A smaller *p*-value and larger F-value of the coefficients (β) represent more important impact on the response. The factors influencing thickness (Y_1) were the linear term of Tre concentration (X_1) (p=0.0140), SA concentration (X_2) (p=0.0088), and $CaCl_2$

concentration (X_3) (p=0.0001), followed by the quadratic term of $CaCl_2$ concentration (X_3^2) (p=0.0287). The quadratic equation of the thickness could be shown below.

$$Y_1 = \mathbf{0.087} + \mathbf{0.02X_1} + \mathbf{0.023X_2} + \mathbf{0.058X_3} - 0.005X_1X_2 + 0.005X_1X_3$$

$$- 0.005X_2X_3 + 0.019X_1^2 + 0.014X_2^2 + \mathbf{0.024X_3^2} \quad \textbf{(14)}$$

In term of the opacity (Y_2), the linear term and the quadratic term of $CaCl_2$ concentration (X_3 and X_3^2) had significant effects (p<0.0001 and p=0.0093, respectively). The interaction between SA and $CaCl_2$ concentration (X_2X_3) was significant (p=0.0343). The equation of the opacity was shown below.

$$Y_2 = \mathbf{17.87} + 0.10X_1 + 0.20X_2 + \mathbf{1.70X_3} + 0.13X_1X_2 - 0.28\ X_1X_3$$

$$\mathbf{- 0.48\ X_2X_3} + 0.0042X_1^2 - 0.096X_2^2 + \mathbf{0.70X_3^2} \quad \textbf{(15)}$$

The factors significantly affected on the moisture content (Y_3) of film were the linear term of Tre concentration (X_1) (p<0.0001) and SA concentration (X_2) (p=0.0112), followed by quadratic term of Tre concentration (X_1^2) (p=0.0017). The moisture content was calculated with the following equation.

$$Y_3 = \mathbf{13.26} - \mathbf{3.74X_1} - \mathbf{0.86X_2} + 0.33X_3 + 0.51X_1X_2 + 0.11X_1X_3$$

$$+ 0.20X_2X_3 + \mathbf{1.98X_1^2} - 0.028X_2^2 + 0.087X_3^2 \quad \textbf{(16)}$$

The factors influencing on WVP (Y_4) were the linear term of $CaCl_2$ concentration (X_3) (p=0.0005), followed by the quadratic term of Tre concentration

(X_1^2) (p=0.0255) and $CaCl_2$ concentration (X_3^2) (p=0.0159). The quadratic equation of the WVP could be shown below.

$$Y_4 = \mathbf{40.48} + 5.86X_1 + 3.39X_2 + \mathbf{30.46X_3} - 12.14X_1X_2 + 11.17X_1X_3$$

$$- 4.20X_2X_3 + \mathbf{17.61X_1^2} - 2.43X_2^2 + \mathbf{20.02X_3^2} \quad \textbf{(17)}$$

4.1.1.2 Response surface plot

The response surface plot is useful to study interaction effects of the factors on the responses and makes it easier to visualize the trend of each factor towards the response (Yolmeh and Jafari, 2017). Three-dimensional plots are useful in study of the effects of two factors on the response at one time, when the third factor is defined at the middle value (zero level) (Motwani et al., 2008).

4.1.1.2.1 Thickness

The film thickness is an important parameter that affects the use of film in the coating of food. Thickness can also affect the physical properties of film, such as opacity and WVP. For film coating, the thickness of the edible film must be adjusted to the type of food that will be coated (Vargas et al., 2008). The thicknesses of all the films were below 0.25 mm, which is within the generally acceptable value for films for food applications (Skurtys et al., 2014). The response surface plots of thickness are shown in **Figures 14-16**. The results showed that increasing the

concentrations of Tre, SA, and $CaCl_2$ increased the thickness of film. Rhim (2004) found that the addition of the crosslinking agent ($CaCl_2$) and an increase in the $CaCl_2$ concentration led to thicker films. For Tre and SA as hydrophilic compounds, Namwongsa, Wiset, and Poomsaad (2016) reported that the addition of hydrophilic compounds as sucrose in starch-based film led to an increase in thickness of film when compared to sorbitol and polyethylene glycol.

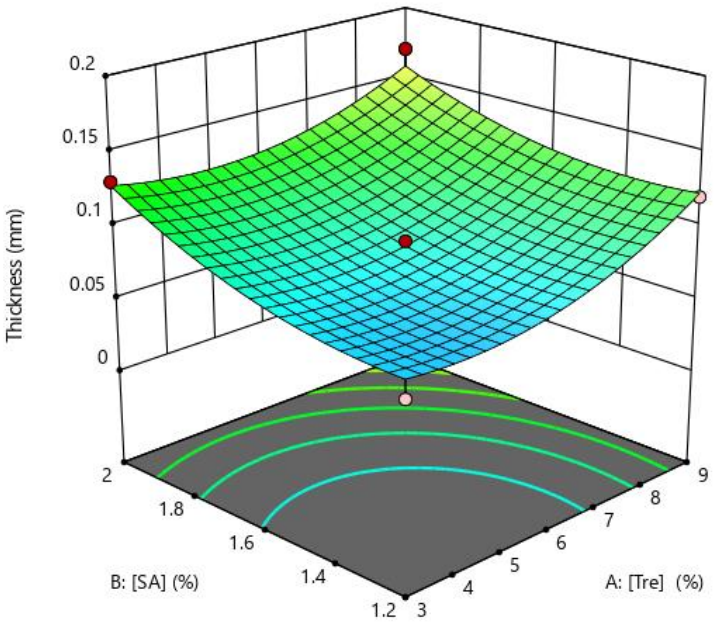


Figure 14 Response surface plot of thickness as a function of trehalose (Tre) concentration (%w/v) and sodium alginate (SA) concentration (%w/v) with 0.4% w/v of ($CaCl_2$) concentration

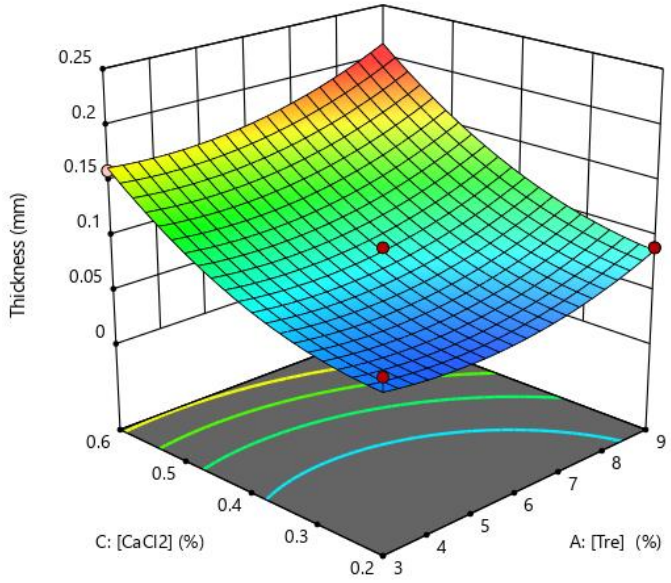


Figure 15 Response surface plot of thickness as a function of trehalose (Tre) concentration (%w/v) and $CaCl_2$ concentration (%w/v) with 1.6% w/v of sodium alginate (SA) concentration

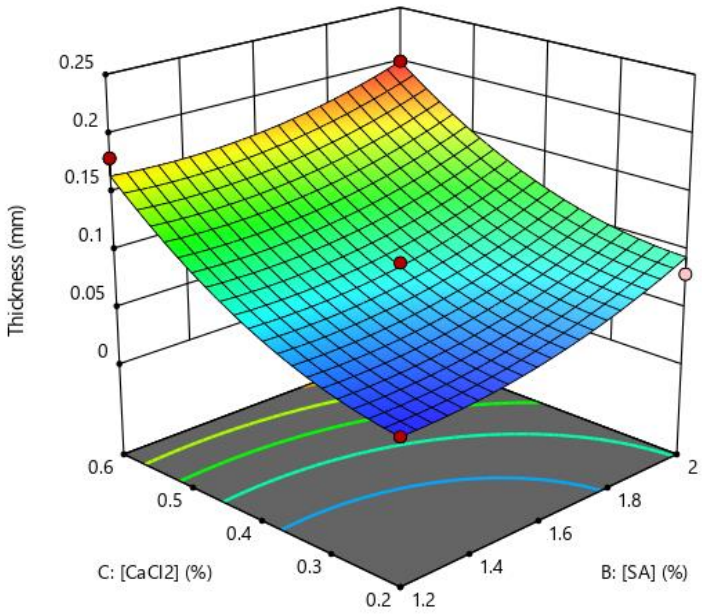


Figure 16 Response surface plot of thickness as a function of sodium alginate (SA) concentration (%w/v) and $CaCl_2$ concentration (%w/v) with 6.0% w/v of trehalose (Tre) concentration

4.1.1.2.2 Opacity

A low opacity is important for an acceptable coating. The film should have low coloring such that the original food features are not influenced when the coating is applied (Falguera et al., 2011). The response surface plots of opacity are shown in **Figures 17-19**. The results represented that the opacity value was higher when $CaCl_2$ concentration increased. The concentration of Tre and SA did not affect the opacity of film as shown in **Figure 17.** By increasing $CaCl_2$ concentration, the higher the thickness value of edible film decreased the diffusion of light so that the film appeared more turbid, resulting in higher opacity value (Rhim, 2004; Khairunnisa et al., 2018). Some studies showed that the crosslinking of alginate-based films with calcium led to higher values of film thickness. Calcium-induced gelation was from strong and specific interactions between Ca^{2+} with G blocks of alginate, resulting in the “egg-box” structure (Cathell and Schauer, 2007; Fu et al., 2011; Galus, Uchanski, and Lenart, 2013; Costa et al., 2018).

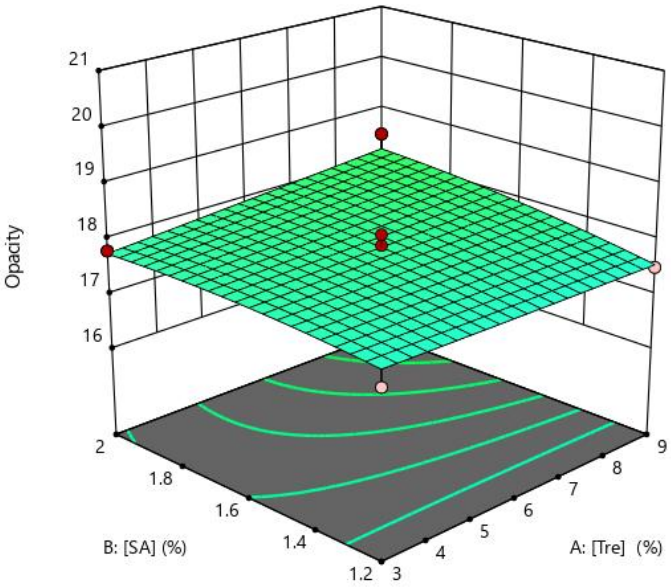


Figure 17 Response surface plot of opacity as a function of trehalose (Tre) concentration (%w/v) and sodium alginate (SA) concentration (%w/v) with 0.4% w/v of $CaCl_2$ concentration

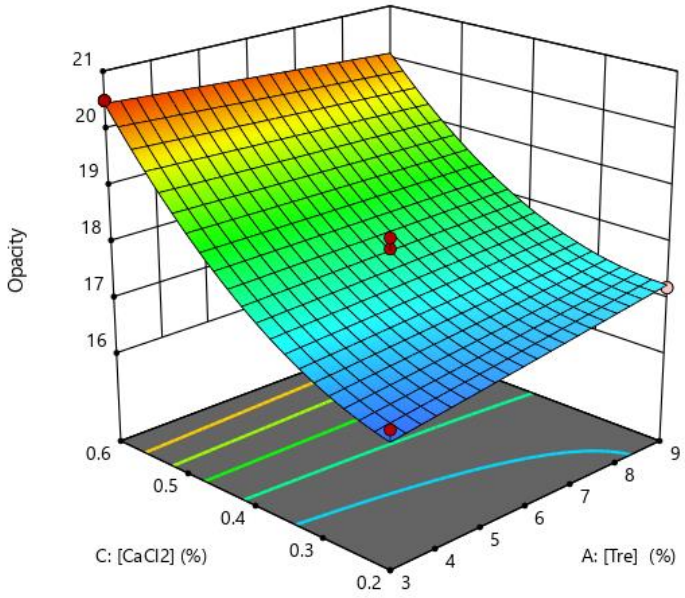


Figure 18 Response surface plot of opacity as a function of trehalose (Tre) concentration (%w/v) and $CaCl_2$ concentration (%w/v) with 1.6 %w/v of sodium alginate (SA) concentration

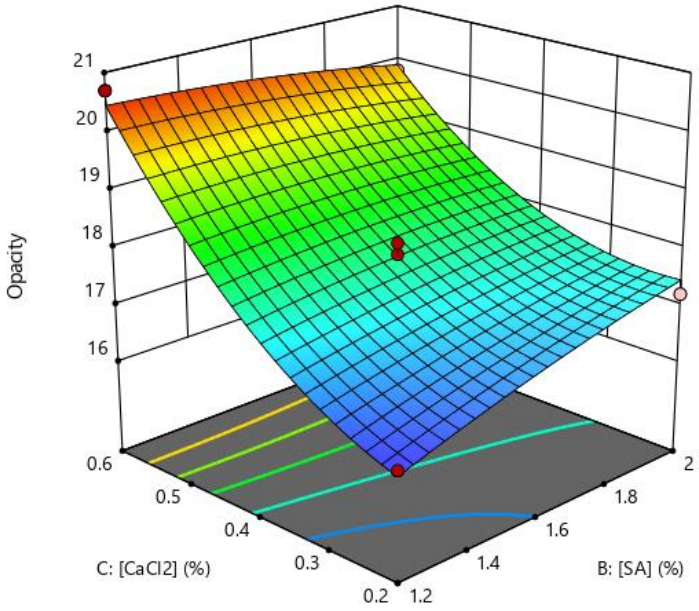


Figure 19 Response surface plot of opacity as a function of sodium alginate (SA) concentration (%w/v) and $CaCl_2$ concentration (%w/v) with 6.0% w/v of trehalose (Tre) concentration

4.1.1.2.3 Moisture content

The response surface plots of moisture content are shown in **Figures 20-22**. The results appeared that increasing the Tre concentration led to a decrease in moisture content. Pérez et al. (2016) reported that an increase of Tre concentration in edible films produced a decrease in moisture content. The reason may be related to the low hygroscopicity of the dihydrate crystal of Tre that does not adsorb water from surroundings even at high relative humidity (O'Donnell, 2012).

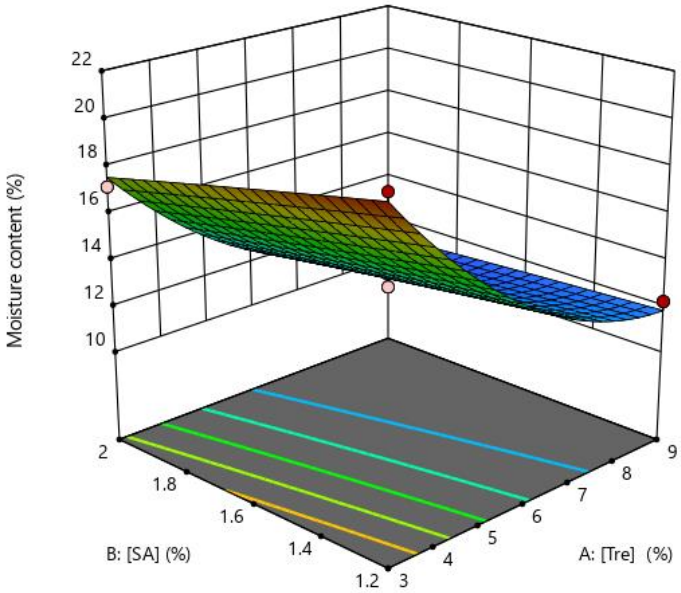


Figure 20 Response surface plot of moisture content as a function of trehalose (Tre) concentration (%w/v) and sodium alginate (SA) concentration (%w/v) with 0.4% w/v of $CaCl_2$ concentration

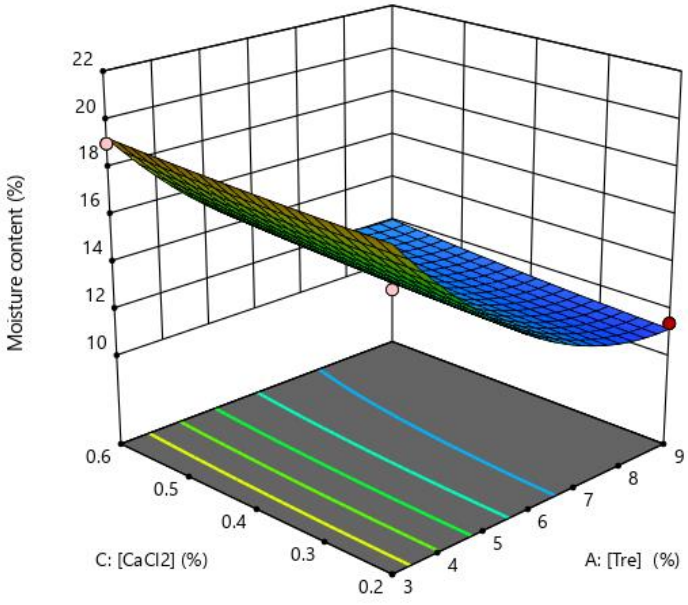


Figure 21 Response surface plot of moisture content as a function of trehalose (Tre) concentration (%w/v) and $CaCl_2$ concentration (%w/v) with 1.6% w/v of sodium alginate (SA) concentration

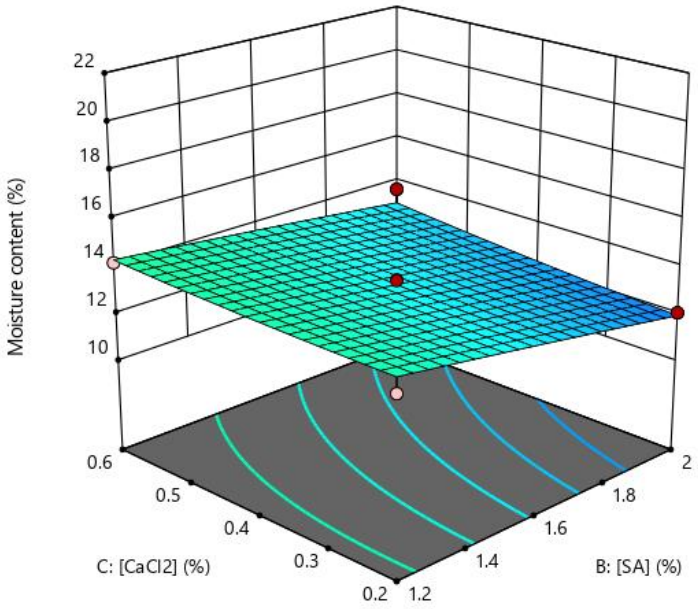


Figure 22 Response surface plot of moisture content as a function of sodium alginate (SA) concentration (%w/v) and $CaCl_2$ concentration (%w/v) with 6.0% w/v of trehalose (Tre) concentration

4.1.1.2.4 Water vapor permeability

The water vapor permeability is important in deteriorative reactions hence the WVP value should be low for food coating. However, the "poor" water vapor barrier may provide some benefits since it allows water vapor to pass through the film (Skurtys et al., 2014). Tanada-Palmu and Grosso (2003) showed that low WVP of edible films could avoid excess exudation of the coated food during processing. The response surface plots of WVP are shown in **Figures 23-25**. The results presented that increasing the concentrations of $CaCl_2$ and Tre led to increased WVP value. Hydrophilic compounds like Tre may increase WVP when incorporated into films and coating by reducing the intermolecular bonds between alginate polymer chain

(Ayranci and Tunc, 2004; Jost et al., 2014). For increasing $CaCl_2$ concentration, Costa et al. (2018) found that the higher Ca^{2+} ions mostly react with alginate (G block) and thus forming the "egg-box" formation which led to stronger films, the higher WVP value and increasing thickness (Olivas and Barbosa-Cánovas, 2008). In addition, some studies reported that the thickness of the film can influence the value of WVP. As the thickness increased, the film offered greater resistance to mass transfer through it, thus increasing the partial vapor pressure in the film's inner surface (Pranoto, Salokhe, and Rakshit, 2005; Olivas and Barbosa-Cánovas, 2008; Carneiro-da-Cunha et al., 2009; Cerqueira et al., 2012; Santana and Kieckbusch, 2013; Khairunnisa et al., 2018).

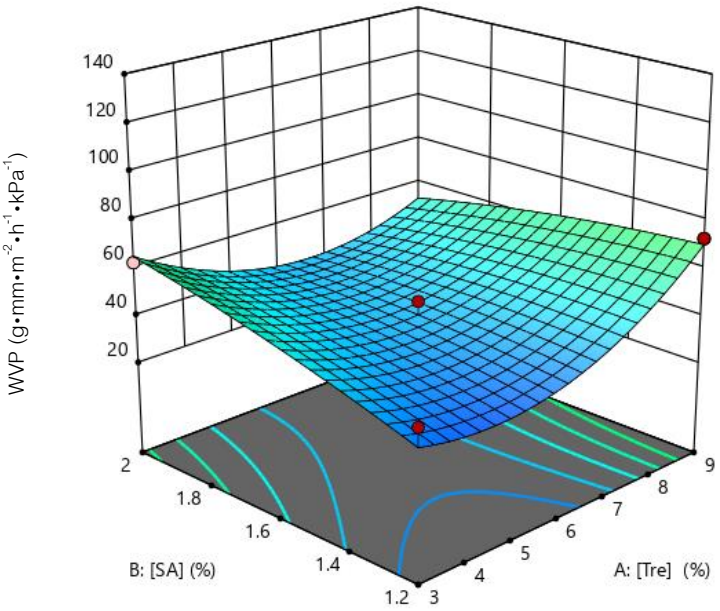


Figure 23 Response surface plot of water vapor permeability (WVP) as a function of trehalose (Tre) concentration (%w/v) and sodium alginate (SA) concentration (%w/v) with 0.4% w/v of $CaCl_2$ concentration

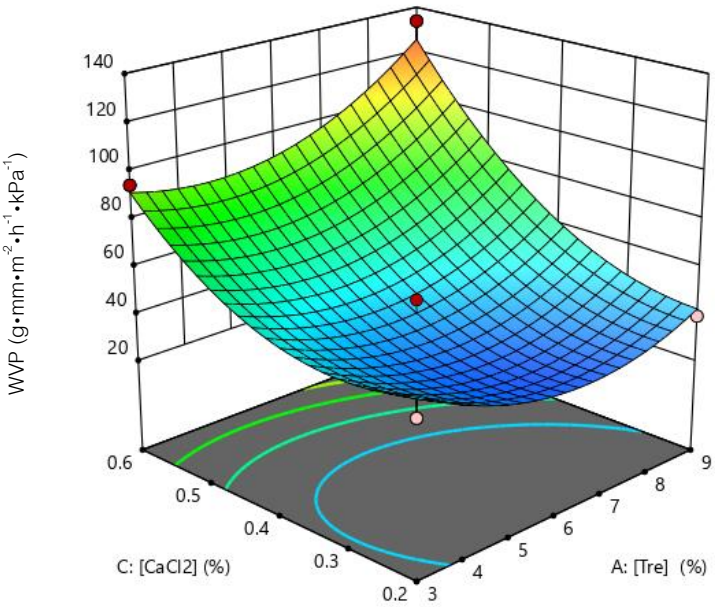


Figure 24 Response surface plot of water vapor permeability (WVP) as a function of trehalose (Tre) concentration (%w/v) and $CaCl_2$ concentration (%w/v) with 1.6% w/v of sodium alginate (SA) concentration

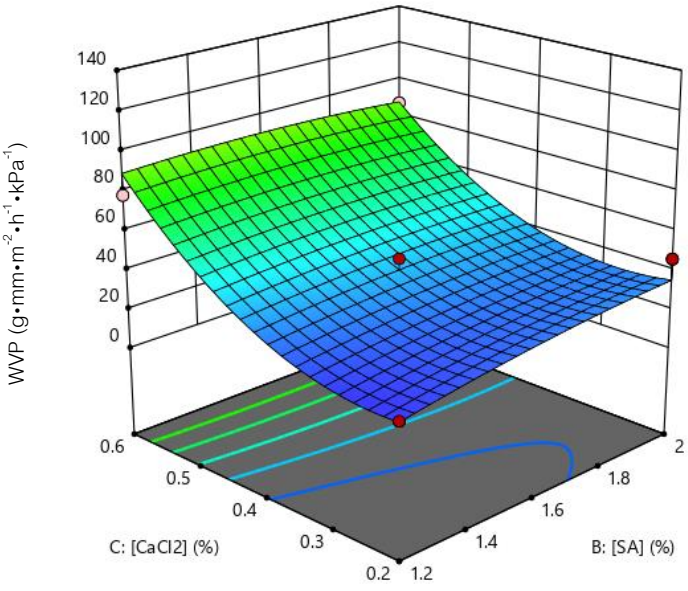


Figure 25 Response surface plot of water vapor permeability (WVP) as a function of sodium alginate (SA) concentration (%w/v) and $CaCl_2$ concentration (%w/v) with 6.0% w/v of trehalose (Tre) concentration

4.1.1.3 Preparation of optimized film

The optimization of film preparation (Tre concentration, SA concentration, and $CaCl_2$ concentration) was set on minimal value of thickness, opacity, moisture content and WVP because these responses tended to be useful for coating freeze-dried food (Falguera et al., 2011; Santana and Kieckbusch, 2013; Skurtys et al., 2014). The optimal condition was determined by desirability value (D) calculated from the program. D is the overall satisfaction value of the response and has a value between 0 and 1. If D is equal to 1, the response is completely satisfied (Phoa and Chen, 2013). In this study, the maximum D value of 0.84 was obtained and the optimized values of Tre concentration, SA concentration, and $CaCl_2$ concentration were 6.71% w/v, 2.0% w/v, and 0.4% w/v, respectively.

The optimized composition was then used to prepare the film. Values of thickness, opacity, moisture content and WVP of the film from the experiment were not significantly different ($p>0.05$) from the values predicted from the program (**Table 11**). The result indicated that the quadratic model was accurate and suitable for predicting physical properties of film.

Additionally, this study indicated that the optimized formulation of the film was prepared from a 5:1 weight ratio of SA to $CaCl_2$. The previous studies reported the weight ratio of SA to $CaCl_2$ of as 1:1 (Han et al., 2018) and 2:1 (Parreidt et al., 2018), however, Tre was not composed in the film.

Table 11 The predicted and experimental values of optimized film formulation

	Responses			
	Thickness (mm)	**Opacity**	**Moisture content (%w/w)**	**WVP ($g \cdot mm \cdot m^{-2} \cdot h^{-1} \cdot kPa^{-1}$)**
Predicted values	0.13 ± 0.02	18.0 ± 0.3	11.71 ± 0.62	40.92 ± 10.75
Experimental values	0.13 ± 0.01	17.6 ± 0.2	12.03 ± 0.17	41.02 ± 6.40

4.2 Freeze-drying of film-coated fruit slices

The fresh-cut apple slices were used as food samples for study the effect of coating on physical changes by enzymes and microbial (Putnik et al., 2017). The coated apple slices were subjected to freeze-drying which is the food preservation to maintain the quality of heat-sensitive products as fruits (Valentina et al., 2016). Before FD process, pretreatment with edible coating film is proposed to reduce moisture loss, color change, unexpected chemical reactions, and microbial contamination (Rojas-Graü et al., 2009). Moreover, the addition of cryoprotectant prior to FD process can prevent intracellular and extracellular ice crystals formation, leading to improve physical appearance of FD products (Patist and Zoerb, 2005). Ma et al. (2015) showed that Tre and alginate treatment provided cryoprotective effects in peeled shrimp during frozen storage such as preventing the thawing loss, the

change of color and textural properties, and the physical damage caused by the formation of large ice crystals in food.

4.2.1 Physical properties

4.2.1.1 Mass loss

The mass loss (water evaporation) represents the amount of moisture removal from apple slice after FD. High mass loss of freeze-dried food helps to reduce water-mediated deterioration in food (Wang et al., 2018). However, the parameter of quality of freeze-dried food is not only mass loss but also others such as water activity, firmness, color (Valentina et al., 2016). Only higher mass loss did not refer the good quality of food. The results of mass loss are shown in **Table 12**. When compared among groups, the mass loss of all groups was significantly different (alginate-control; p=0.001, TreAlg-control; p=0.001, alginate-TreAlg; p=0.001). The alginate-coated group had the lowest mass loss while the TreAlg-coated group had the highest mass loss. The result indicated that alginate coating could prevent mass loss of freeze-dried sample as seen from previous studies (Parreidt et al., 2018; Wang et al., 2018). On the other hand, the addition of Tre in alginate-coating solution increased mass loss of freeze-dried sample. It was possibly due to the osmotic dehydration of Tre that water flow from fruits to sugar solution. Aktas et al. (2007) reported that during pretreatment with Tre of sliced potato and carrot increased the mass loss.

Table 12 Mass loss of apple slices after freeze-drying (FD)

Sample groups	Mean ± SD of mass loss (%)
Alginate-coated	84.649 ± 0.347^{A}
TreAlg-coated	89.006 ± 0.402^{B}
Control	86.848 ± 0.179^{C}

Control = uncoated apple slices, **Alginate-coated** = coated apple slices with 2% w/v alginate/ 1% w/v glycerin/ 0.4% w/v $CaCl_2$, **TreAlg-coated** = coated apple slices with 6.71% w/v Tre in 2% w/v alginate/ 1% w/v glycerin/ 0.4% w/v $CaCl_2$, Means with the different letters are significantly different by Bonferroni test ($p<0.05$).

4.2.1.2 Moisture content

The moisture content is formally used to determine the quality or food from the announcement of the Thai Community Product Standard on dried fruits and vegetables, the moisture content of dried product should be less than 12% by weight (TISI, 2015) as determined by AOAC method (i.e. oven drying). The results of moisture content of dried samples are shown in **Figure 26** and **Appendix A**. The moisture content of all freeze-dried groups gradually increased following the storage periods. At week 4, moisture content of alginate-coated and TreAlg-coated groups were significantly lower than that of the control group ($p=0.001$ and $p=0.002$, respectively). However, the moisture contents of alginate-coated and TreAlg-coated groups were insignificantly different ($p>0.05$). The results indicated that the coating using alginate and Tre could prevent moisture accumulation in freeze-dried apple

slices during storage. Since, Tre was low hygroscopic, hence it was unlikely to accumulate water from the atmosphere even at high RH (Ohtake and Wang, 2011). Albanese et al. (2007) reported that apple slices with Tre treatment was effective in slowing down moisture increase during the storage period. Although, alginate was hygroscopic, alginate-coated fruit slice could reduce water loss and maintain moisture by moisture evaporating from the film instead of the fresh-cut surface of the fruit (Parreidt et al., 2019).

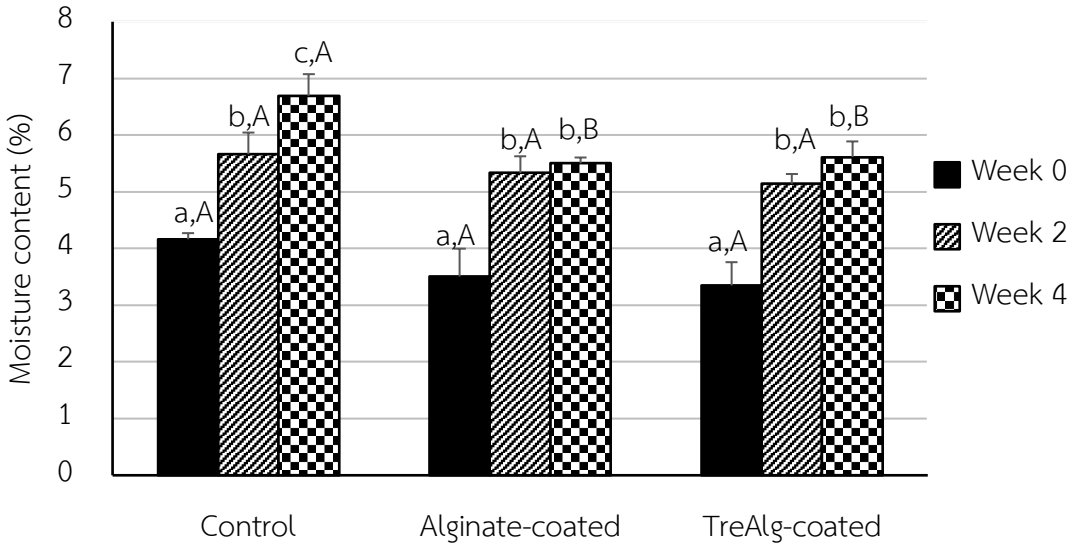


Figure 26 Moisture content of freeze-dried coated apple slices with different coatings after different storage times compared to a control (uncoated) kept at 25 ± 5ºC

Control = uncoated apple slices, **Alginate-coated** = coated apple slices with 2% w/v alginate/ 1% w/v glycerin/ 0.4% w/v $CaCl_2$, **TreAlg-coated** = coated apple slices with 6.71% w/v Tre in 2% w/v alginate/ 1% w/v glycerin/ 0.4% w/v $CaCl_2$, Means with the different letters (lowercase: the same coating group at different storage times; uppercase:

different coating groups at the same storage time) are significantly different by Bonferroni test (p<0.05).

4.2.1.3 Water activity

The water activity (a_w) is the measurement of how tightly water is bound structurally in food. It describes the availability of the free water to take part in chemical and biochemical reactions (Barbosa-Canovas et al., 2007). Moreover, this parameter is used to control the growth of microorganisms in foods. The a_w value of dried fruit by Thai Community Product Standard should be less than 0.6 for preventing any microbial growth (TISI, 2015). The results of a_w value are shown in **Figure 27** and **Appendix B.** During storage period, the a_w values of all freeze-dried groups slightly increased. At week 0, the a_w values of all groups were significantly different (alginate-control; p=0.006, TreAlg-control; p=0.001, alginate-TreAlg; p=0.001). TreAlg-coated group had the lowest value of a_w while alginate-coated group had the highest a_w value. Tre treatment was found to be more effective in preventing an increase of a_w. It was possibly due to the limitation of water mobility in the presence of the Tre which was thought to be in the glassy state rather than crystal state during freeze drying. At week 4, although the a_w values of all groups were insignificantly different (p>0.05), the value of a_w was slightly higher in the control group. Since all a_w values of freeze-dried samples were below 0.5, the growth of molds, yeast, and bacteria should not be found and enzymatic reactions were unlikely to appear (Barbosa-Canovas et al., 2007).

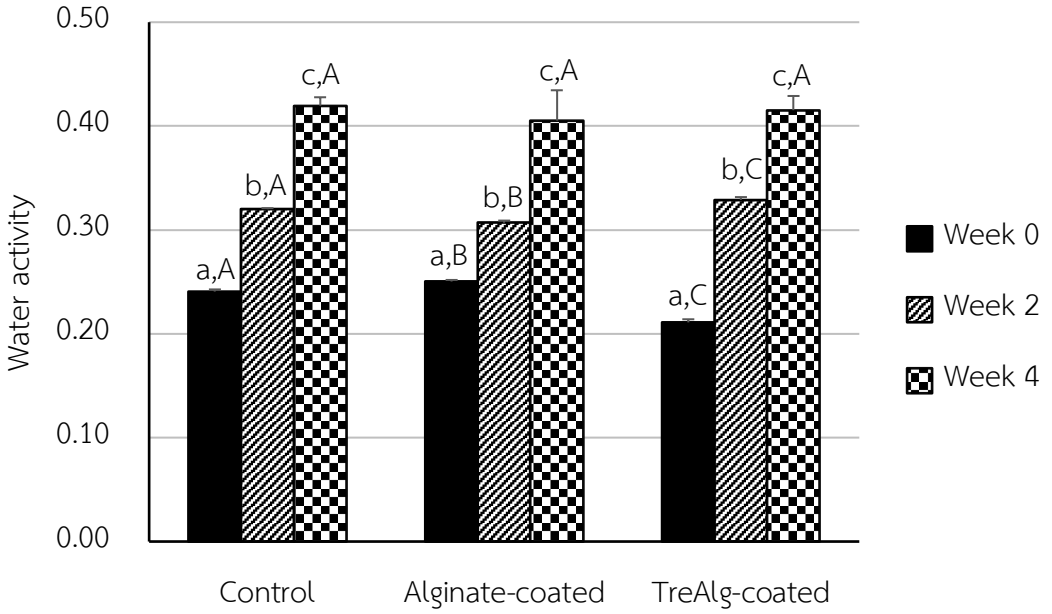


Figure 27 Water activity of freeze-dried coated apple slices with different coatings after different storage times compared to a control (uncoated) kept at 25 ± 5°C

Control = uncoated apple slices, **Alginate-coated** = coated apple slices with 2% w/v alginate/ 1% w/v glycerin/ 0.4% w/v $CaCl_2$, **TreAlg-coated** = coated apple slices with 6.71% w/v Tre in 2% w/v alginate/ 1% w/v glycerin/ 0.4% w/v $CaCl_2$, Means with the different letters (lowercase: the same coating group at different storage times; uppercase: different coating groups at the same storage time) are significantly different by Bonferroni test (p<0.05).

4.2.1.4 Rehydration capacity

Rehydration capacity (RC) is an important quality parameter of dried foods (Lewicki and Wiczkowska, 2006). RC value represents as the ratio of freeze-dried sample weight after water immersion and the initial weight of freeze-dried sample (Salazar et al., 2017). The good quality of dried fruits should have higher RC, the high

porosity of the dried products and ability to recover its original properties (Reyes et al., 2011). The high porosity in freeze-dried apple slices was caused by the ice crystals which sublimated and created small pores (Cui et al., 2008). The RC values of control freeze-dried apple slices (6.843 ± 0.052 to 7.659 ± 0.177) were similar to previous report (6-7) (Antal and Kerekes, 2016). The results of RC values are reported in **Figure 28** and **Appendix C.** At week 0 and 2, the RC values of alginate-coated and TreAlg-coated group were significantly different from the control group (at week 0; p=0.006 and p=0.044, respectively, at week 2; p=0.039 and p=0.005, respectively). The TreAlg-coated group had the significant higher RC value than those of alginate-coated and control groups. The results indicated that freeze-dried samples with Tre could improve quality of dried fruits. In previous study, dried banana slices with Tre easily rehydrated to yield soft fruit slice with texture of fresh banana slices (Colaco and Roser, 1994). Aktas et al. (2007) also reported that Tre improved reconstitution properties of dried fruit slices. It was possibly due to the fact that Tre can prevent the degradation of protein or cell structure by inhibiting ice crystal growth (Zhang et al., 2019). The large size of ice crystals formation during the freezing process may damage cell membranes and breakdown the physical structure of the fruit more than smaller size of ice crystals (Charoenrein and Owcharoen, 2016). In addition, Tre may fit more closely to surface of macromolecules resulting in protecting the cell structure during FD (Patist and Zoerb, 2005). These possible reasons might explain why the higher RC was observed in the TreAlg-coated group.

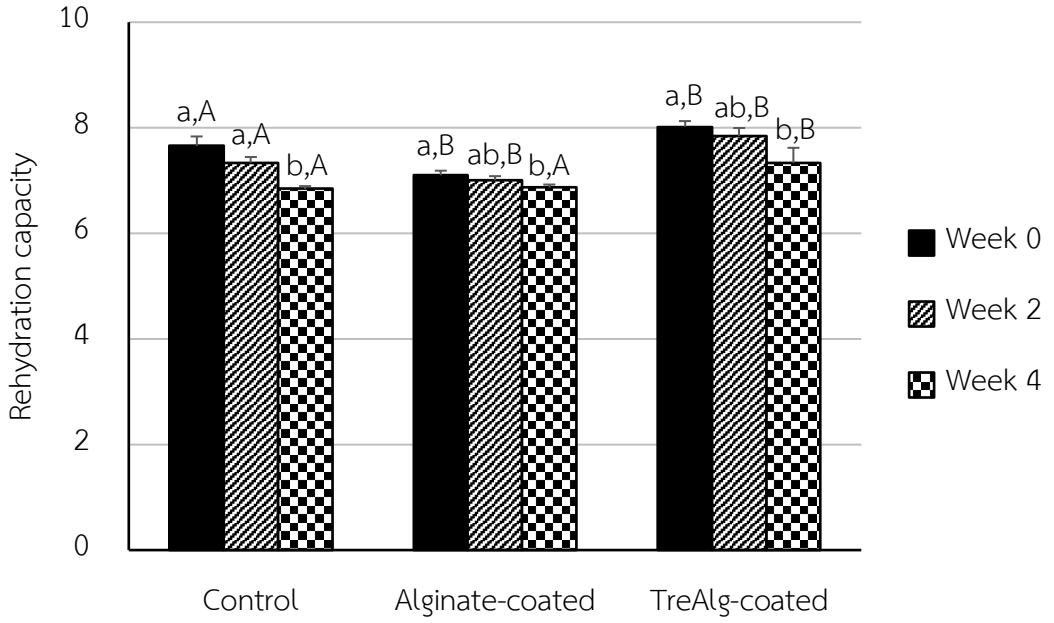


Figure 28 Rehydration capacity of freeze-dried coated apple slices with different coatings after different storage times compared to a control (uncoated) kept at 25 ± 5°C

Control = uncoated apple slices, **Alginate-coated** = coated apple slices with 2% w/v alginate/ 1% w/v glycerin/ 0.4% w/v $CaCl_2$, **TreAlg-coated** = coated apple slices with 6.71% w/v Tre in 2% w/v alginate/ 1% w/v glycerin/ 0.4% w/v $CaCl_2$, Means with the different letters (lowercase: the same coating group at different storage times; uppercase: different coating groups at the same storage time) are significantly different by Bonferroni test (p<0.05).

4.2.1.5 Firmness

Firmness is a combination of cell structure integrity and tissue turgor and is important for sensory characteristics (Antal et al., 2015). During freeze drying, cell wall structure can be remodeled due to water loss (Moreno et al., 2004). As shown in **Figure 29** and **Appendix D**, firmness of dried samples was decreased during

storage for all freeze-dried groups. At week 0, the firmness of alginate-coated group was significantly higher than the others (control; p=0.001, TreAlg-coated group; p=0.026). Rojas-Graü et al. (2008) showed that fresh-cut fruit coated by alginate solution increased firmness. The firmness of TreAlg-coated samples had also significantly higher than the control group (at week 0, 2, 4; p=0.001, p=0.035, p=0.012, respectively). Velickova, Tylewicz, et al. (2013) reported that texture of strawberry with Tre after freezing was harder than untreated with Tre and cell structure was preserved by Tre. The ice crystals during FD might destroy the texture and microstructure of freeze-dried product resulting in less firmness (Charoenrein and Owcharoen, 2016). Tre could inhibit of ice crystal growth, thus promoting the firmness of freeze-dried apple slices in TreAlg-coated group. In contrary Aktas et al. (2013) found that osmotic pretreated sample with Tre had softer texture than untreated dry samples. Moreover, the addition of Tre in alginate-base coating might effective in firmness of freeze-dried apple slices. However, the firmness values of alginate-coated and TreAlg-coated were insignificantly different at week 4 (p>0.05).

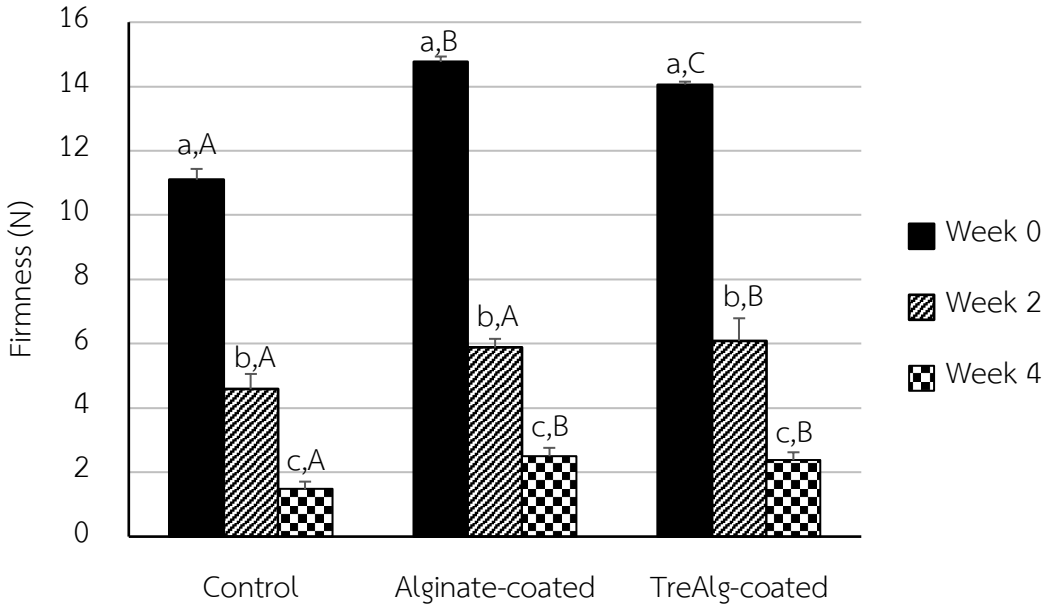


Figure 29 Firmness of freeze-dried coated apple slices with different coatings after different storage times compared to a control (uncoated) kept at 25 ± 5ºC

Control = uncoated apple slices, **Alginate-coated** = coated apple slices with 2% w/v alginate/ 1% w/v glycerin/ 0.4% w/v $CaCl_2$, **TreAlg-coated** = coated apple slices with 6.71% w/v Tre in 2% w/v alginate/ 1% w/v glycerin/ 0.4% w/v $CaCl_2$, Means with the different letters (lowercase: the same coating group at different storage times; uppercase: different coating groups at the same storage time) are significantly different by Bonferroni test (p<0.05).

4.2.1.6 Color

The results of color parameters in freeze-dried samples are demonstrated in **Figures 30-33** and **Appendix E**. The *L** color parameter indicates whiteness of a product (Pathare et al., 2012). After FD, the *L** values of all groups were significantly higher than the samples before FD (p=0.001) (**Figure 30**) which was similar to previous studies (Antal, Sikolya, et al., 2013; Antal and Kerekes, 2016; Link, Tribuzi,

and Laurindo, 2017). However, the *L** values of all freeze-dried groups were not significantly different after time storage ($p>0.05$).

A hue angle of 90° (*h**) represents yellowish of surface color in fruits in that more yellowish refers the less browning (Pathare et al., 2012). The *h** values of TreAlg-coated group before and after FD (88.18 ± 1.39 and 88.79 ± 0.77) was significantly higher than the control group (84.14 ± 0.41 and 85.06 ± 1.23) ($p=0.009$ and $p=0.047$, respectively) (**Figure 31**). The result was similar to Mahayothee et al. (2009) who showed that dried litchi with Tre treatment had higher *h** value compared to a control group.

The Δ*E** value, indicates the color difference between freeze-dried sample and the fresh apple slice (before FD). The low Δ*E** value refers to less color change in FD process and during time storage (Aktas et al., 2013). After FD (week 0), the Δ*E** values of control, alginate-coated, and TreAlg-coated group were 7.77 ± 0.86, 10.23 ± 1.30, 7.33 ± 1.53, respectively. The values were not significantly different among 3 groups ($p>0.05$). However, the Δ*E** value of TreAlg-coated group was likely to get lower value during storage.

BI value represents the brown color in fruit product due to enzymatic and non-enzymatic activities (Pathare et al., 2012). The color change of the dried fruit could be due to the formation of browning, associated with the Maillard reaction (Baini and Langrish, 2009; Djekic et al., 2018). An increase in *L** value may relate to

lowering BI value (Pathare et al., 2012). As shown in **Figure 33**, at week 0 and 2, the BI value of TreAlg-coated group (34.52 ± 2.03 and 28.74 ± 2.06, respectively) was significantly lower than that of control group (46.63 ± 6.67 and 40.29 ± 4.21, respectively) (p=0.034 and p=0.010, respectively). The result indicated that Tre can retard browning of freeze-dried apple slices which was similar to previous studies (Albanese et al., 2007; Mahayothee et al., 2009; Aktas et al., 2013). Since, Tre is a non-reducing sugar and resistant to chemical reaction like Maillard reactions (Ohtake and Wang, 2011), hence TreAlg-coated group could be expected to have lower BI value.

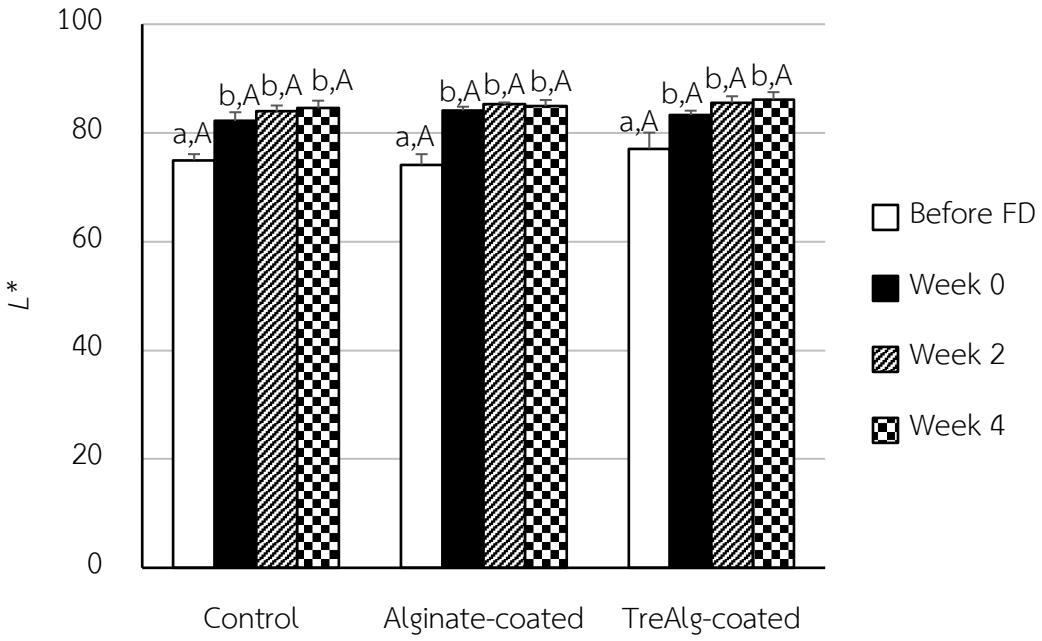


Figure 30 Lightness (*L**) of non freeze-dried and freeze-dried coated apple slices with different coatings after different storage times compared to a control (uncoated) kept at 25 ± 5°C

Control = uncoated apple slices, **Alginate-coated** = coated apple slices with 2% w/v alginate/ 1% w/v glycerin/ 0.4% w/v $CaCl_2$, **TreAlg-coated** = coated apple slices with 6.71% w/v Tre in 2% w/v alginate/ 1% w/v glycerin/ 0.4% w/v $CaCl_2$, **Before FD** = non-freeze dried apple slices, Means with the different letters (lowercase: the same coating group at different storage times; uppercase: different coating groups at the same storage time) are significantly different by Bonferroni test (p<0.05).

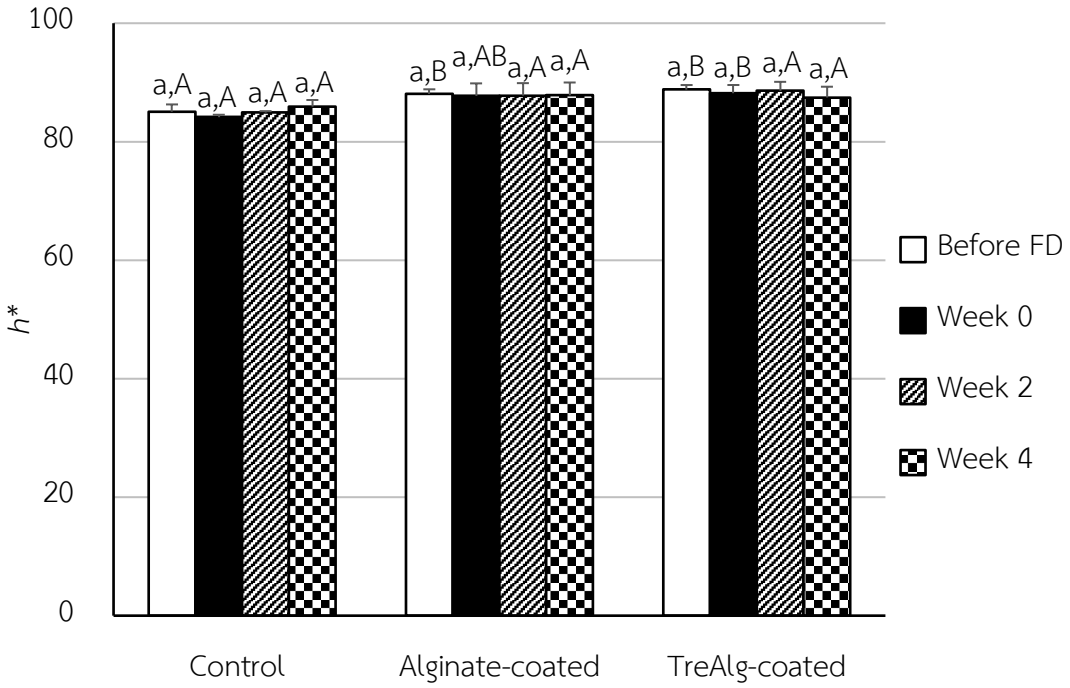


Figure 31 Hue angle (*h**) of non freeze-dried and freeze-dried coated apple slices with different coatings after different storage times compared to a control (uncoated) kept at 25 ± 5ºC

Control = uncoated apple slices, **Alginate-coated** = coated apple slices with 2% w/v alginate/ 1% w/v glycerin/ 0.4% w/v $CaCl_2$, **TreAlg-coated** = coated apple slices with 6.71% w/v Tre in 2% w/v alginate/ 1% w/v glycerin/ 0.4% w/v $CaCl_2$, **Before FD** = non-freeze dried apple slices, Means with the different letters (lowercase: the same coating group at different storage times; uppercase: different coating groups at the same storage time) are significantly different by Bonferroni test (p<0.05).

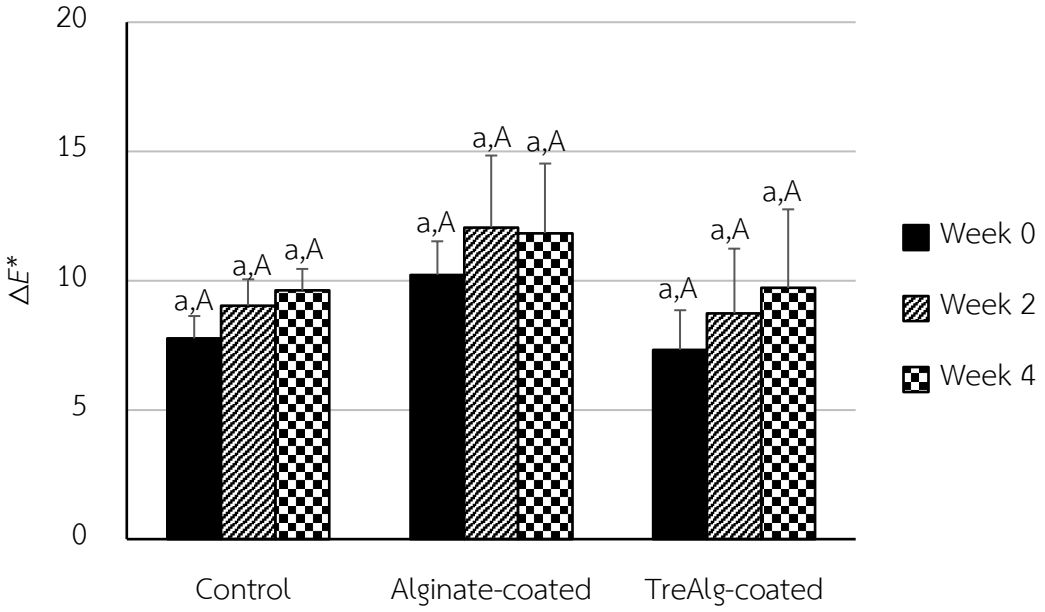


Figure 32 Color difference (Δ*E**) of freeze-dried coated apple slices with different coatings after different storage times compared to a control (uncoated) kept at 25 ± 5ºC

Control = uncoated apple slices, **Alginate-coated** = coated apple slices with 2% w/v alginate/ 1% w/v glycerin/ 0.4% w/v $CaCl_2$, **TreAlg-coated** = coated apple slices with 6.71% w/v Tre in 2% w/v alginate/ 1% w/v glycerin/ 0.4% w/v $CaCl_2$, Means with the different letters (lowercase: the same coating group at different storage times; uppercase: different coating groups at the same storage time) are significantly different by Bonferroni test (p<0.05).

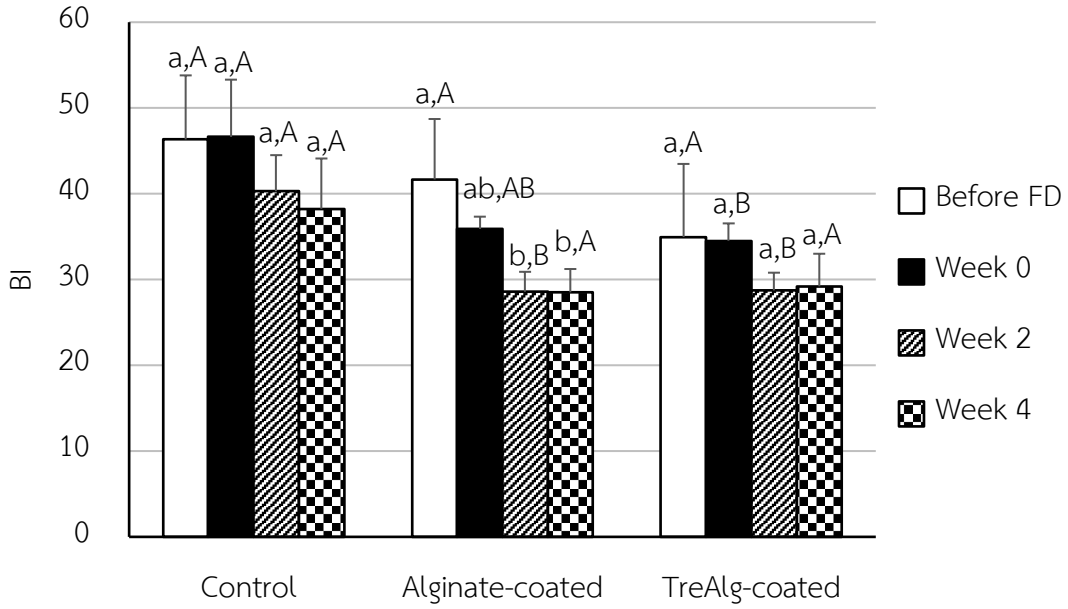


Figure 33 Browning index (BI) of non freeze-dried and freeze-dried coated apple slices with different coatings after different storage times compared to a control (uncoated) kept at 25 ± 5°C

Control = uncoated apple slices, **Alginate-coated** = coated apple slices with 2% w/v alginate/ 1% w/v glycerin/ 0.4% w/v $CaCl_2$, **TreAlg-coated** = coated apple slices with 6.71% w/v Tre in 2% w/v alginate/ 1% w/v glycerin/ 0.4% w/v $CaCl_2$, **Before FD** = non-freeze dried apple slices, Means with the different letters (lowercase: the same coating group at different storage times; uppercase: different coating groups at the same storage time) are significantly different by Bonferroni test (p<0.05).

4.2.2 Microbial determination

For the announcement of the Thai Community Product Standard on microbiological quality criteria of dried fruits and vegetables, microbial analysis is quantified according to FDA manual (TISI, 2015). The amount of total bacterial count in dried fruits should be less than $1x10^6$ CFUs/g (<6 log CFUs/g). Yeast and mold

content should be less than $1x10^3$ CFUs/g (<3 log CFUs/g) (TISI, 2015). As demonstrated in **Figure 34** and **Appendix F**, a number of mesophilic bacteria in all groups of freeze-dried samples ($<10^3$ CFUs/g) were not significantly different during storage ($p>0.05$). Additionally, a number of yeast and mold in all groups during storage (<10 CFUs/g) were also insignificantly different ($p>0.05$). A little growth of fungi was observed. Also, psychrophilic bacteria were not detected in all groups of freeze-dried samples. The results showed that all freeze-dried samples could be stored at 25 ± 5ºC without microorganism growth for at least 4 weeks.

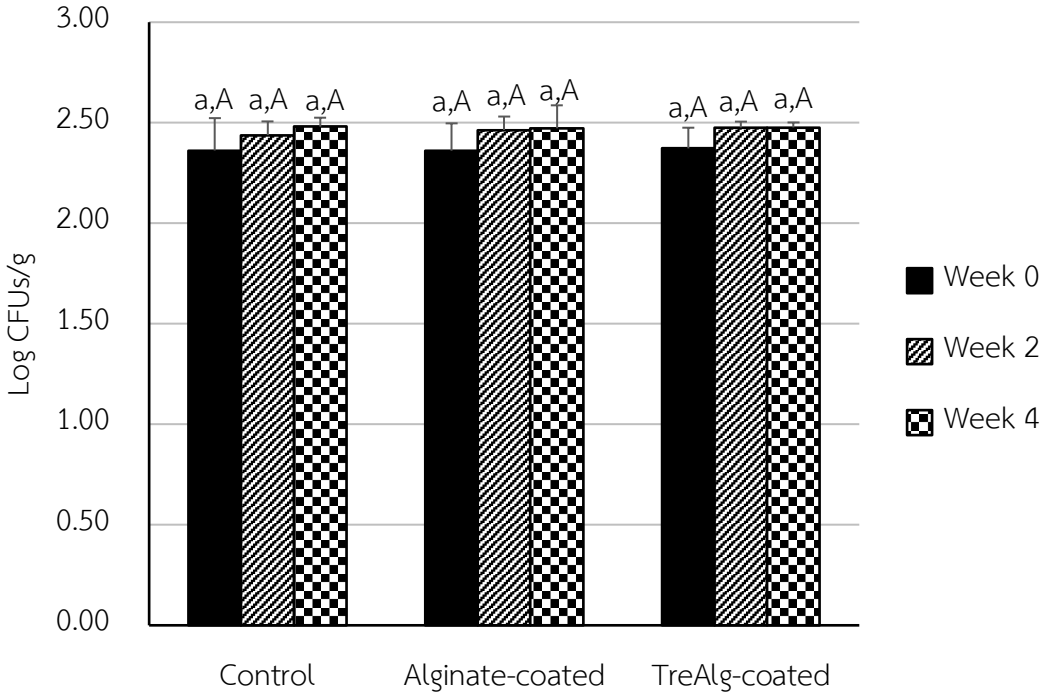


Figure 34 Mesophilic bacteria of freeze-dried coated apple slices with different coatings after different storage times compared to a control (uncoated) kept at 25 ± 5°C

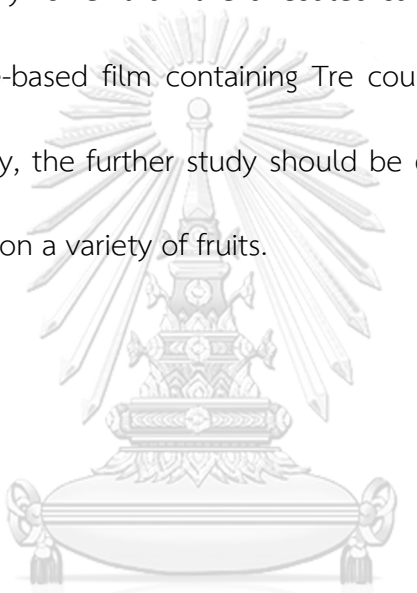
Control = uncoated apple slices, **Alginate-coated** = coated apple slices with 2% w/v alginate/ 1% w/v glycerin/ 0.4% w/v $CaCl_2$, **TreAlg-coated** = coated apple slices with 6.71% w/v Tre in 2% w/v alginate/ 1% w/v glycerin/ 0.4% w/v $CaCl_2$, Means with the different letters (lowercase: the same coating group at different storage times; uppercase: different coating groups at the same storage time) are significantly different by Bonferroni test (p<0.05)

CHAPTER V
CONCLUSION

In this study, factors influencing preparation and physical properties of composite film containing alginate and trehalose for food coating were evaluated with response surface methodology (Box-Behnken design). Determined factors were 3-9% w/v Tre concentrations, 1.2-2.0% w/v SA concentrations and 0.2-0.6% $CaCl_2$ concentrations with responses of thickness, opacity, moisture content, and WVP of the film. The criteria of film formation were minimal value of thickness, opacity, moisture content and WVP. The optimized formulation (desirability of 0.85) was the composite film containing Tre concentration of 6.71% w/v, SA concentration of 2.0% w/v, and $CaCl_2$ concentration of 0.4% w/v. The composite film had thickness of 0.13 ± 0.01 mm, opacity of 17.6 ± 0.2, moisture content of 12.03 ± 0.17 %w/w, and WVP of 41.02 ± 6.40 $g \cdot mm \cdot m^{-2} \cdot h^{-1} \cdot kPa^{-1}$. It was mentioned that a 5:1 weight ratio of SA to $CaCl_2$ was appropriate for composite film forming.

The effect of composite film coating on physical properties of freeze-dried fruit (apple) slices was determined. Pretreatment with composite film could improve physical properties of freeze-dried apple slices. After freeze drying, the water activity and the browning index of composite film-coated samples was significantly lower than the uncoated samples (p=0.001 and p=0.034, respectively), while the color h^* values of composite film-coated freeze-dried samples were significantly higher than

the uncoated samples (p=0.034). Browning on apple surface was retarded in composite film-coated samples. For freeze-dried samples coated with a composite film stored in a desiccator at 25 ± 5 °C for 4 weeks, the rehydration capacity and firmness of film-coated samples were significantly higher than the uncoated samples (p=0.038 and p=0.012, respectively), while the moisture content of film-coated samples was significantly lower than the uncoated samples (p=0.002). The findings suggested that alginate-based film containing Tre could be useful for freeze-dried food application. Finally, the further study should be done on the effectiveness of composite film coating on a variety of fruits.



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APPENDICES

APPENDIX A

Moisture content determination

Table 1A Moisture content of freeze-dried coated apple slices with different coatings after different storage times compared to control (uncoated) kept at 25 ± 5ºC

	Mean ± SD of moisture content (%)		
	Control	**Alginate-coated**	**TreAlg-coated**
Week 0	$4.163 \pm 0.108^{a,A}$	$3.507 \pm 0.491^{a,A}$	$3.347 \pm 0.412^{a,A}$
Week 2	$5.663 \pm 0.382^{b,A}$	$5.333 \pm 0.294^{b,A}$	$5.143 \pm 0.171^{b,A}$
Week 4	$6.693 \pm 0.197^{c,A}$	$5.503 \pm 0.102^{b,B}$	$5.613 \pm 0.276^{b,B}$

Control = uncoated apple slices, **Alginate-coated** = coated apple slices with 2% w/v alginate/ 1% w/v glycerin/ 0.4% w/v $CaCl_2$, **TreAlg-coated** = coated apple slices with 6.71% w/v Tre in 2% w/v alginate/ 1% w/v glycerin/ 0.4% w/v $CaCl_2$

Means with the different letters (lowercase: the same coating group at different storage times; uppercase: different coating groups at the same storage time) are significantly different by Bonferroni test ($p<0.05$).

APPENDIX B

Water activity determination

Table 1B Water activity of freeze-dried coated apple slices with different coatings after different storage times compared to control (uncoated) kept at 25 ± 5°C

	Mean ± SD of a_w		
	Control	**Alginate-coated**	**TreAlg-coated**
Week 0	$0.241 \pm 0.002^{a,A}$	$0.250 \pm 0.002^{a,B}$	$0.211 \pm 0.003^{a,C}$
Week 2	$0.320 \pm 0.001^{b,A}$	$0.307 \pm 0.002^{b,B}$	$0.329 \pm 0.003^{b,C}$
Week 4	$0.420 \pm 0.008^{c,A}$	$0.405 \pm 0.029^{c,A}$	$0.415 \pm 0.014^{c,A}$

Control = uncoated apple slices, **Alginate-coated** = coated apple slices with 2% w/v alginate/ 1% w/v glycerin/ 0.4% w/v $CaCl_2$, **TreAlg-coated** = coated apple slices with 6.71% w/v Tre in 2% w/v alginate/ 1% w/v glycerin/ 0.4% w/v $CaCl_2$

Means with the different letters (lowercase: the same coating group at different storage times; uppercase: different coating groups at the same storage time) are significantly different by Bonferroni test ($p<0.05$).

APPENDIX C

Rehydration capacity determination

Table 1C Rehydration capacity of freeze-dried coated apple slices with different coatings after different storage times compared to control (uncoated) kept at 25 ± 5ºC

	Mean ± SD of RC		
	Control	**Alginate-coated**	**TreAlg-coated**
Week 0	$7.659 \pm 0.177^{a,A}$	$7.100 \pm 0.088^{a,B}$	$8.019 \pm 0.108^{a,B}$
Week 2	$7.337 \pm 0.109^{a,A}$	$7.007 \pm 0.077^{ab,B}$	$7.846 \pm 0.150^{ab,B}$
Week 4	$6.843 \pm 0.052^{b,A}$	$6.869 \pm 0.057^{b,A}$	$7.335 \pm 0.286^{b,B}$

Control = uncoated apple slices, **Alginate-coated** = coated apple slices with 2% w/v alginate/ 1% w/v glycerin/ 0.4% w/v $CaCl_2$, **TreAlg-coated** = coated apple slices with 6.71% w/v Tre in 2% w/v alginate/ 1% w/v glycerin/ 0.4% w/v $CaCl_2$

Means with the different letters (lowercase: the same coating group at different storage times; uppercase: different coating groups at the same storage time) are significantly different by Bonferroni test ($p<0.05$).

APPENDIX D

Firmness determination

Table 1D Firmness of freeze-dried coated apple slices with different coatings after different storage times compared to control (uncoated) kept at 25 ± 5ºC

	Mean ± SD of maximum force (N)		
	Control	**Alginate-coated**	**TreAlg-coated**
Week 0	$11.100 \pm 0.337^{a,A}$	$14.762 \pm 0.170^{a,B}$	$14.061 \pm 0.090^{a,C}$
Week 2	$4.586 \pm 0.474^{b,A}$	$5.879 \pm 0.273^{b,A}$	$6.088 \pm 0.703^{b,B}$
Week 4	$1.476 \pm 0.234^{c,A}$	$2.501 \pm 0.259^{c,B}$	$2.379 \pm 0.245^{c,B}$

Control = uncoated apple slices, **Alginate-coated** = coated apple slices with 2% w/v alginate/ 1% w/v glycerin/ 0.4% w/v $CaCl_2$, **TreAlg-coated** = coated apple slices with 6.71% w/v Tre in 2% w/v alginate/ 1% w/v glycerin/ 0.4% w/v $CaCl_2$

Means with the different letters (lowercase: the same coating group at different storage times; uppercase: different coating groups at the same storage time) are significantly different by Bonferroni test ($p<0.05$).

APPENDIX E

Color measurement

Table 1E Color of non freeze-dried and freeze-dried coated apple slices with different coatings after different storage times compared to control (uncoated) kept at 25 ± 5°C

Color measurement	Weeks	Mean ± SD of color		
		Control	Alginate-coated	TreAlg-coated
Lightness (*L**)	Before FD	74.98 ± 1.11^{a,A}	74.16 ± 1.93^{a,A}	77.11 ± 2.95^{a,A}
	0	82.19 ± 1.61^{b,A}	84.14 ± 0.68^{b,A}	83.31 ± 0.78^{b,A}
	2	83.96 ±1.09^{b,A}	85.25 ± 0.35^{b,A}	85.55 ± 1.20^{b,A}
	4	84.55 ± 1.39^{b,A}	84.92 ± 1.15^{b,A}	86.11 ± 1.39^{b,A}
Hue angle (*h**)	Before FD	85.06 ± 1.23^{a,A}	88.08 ± 0.76^{a,B}	88.79 ± 0.77^{a,B}
	0	84.14 ± 0.41^{a,A}	87.73 ± 2.12^{a,AB}	88.18 ± 1.39^{a,B}
	2	84.94 ± 0.21^{a,A}	87.79 ± 2.10^{a,A}	88.60 ± 1.50^{a,A}
	4	85.92 ± 1.12^{a,A}	87.83 ± 2.16^{a,A}	87.46 ± 1.82^{a,A}
Browning index	Before FD	46.34 ± 7.46^{a,A}	41.67 ± 7.04^{a,A}	34.96 ± 8.52^{a,A}
(BI)	0	46.63 ± 6.67^{a,A}	35.90 ± 1.43^{ab,AB}	34.52 ± 2.03^{a,B}
	2	40.29 ± 4.21^{a,A}	28.58 ± 2.33^{b,B}	28.74 ± 2.06^{a,B}
	4	38.25 ± 5.86^{a,A}	28.51 ± 2.72^{b,A}	29.21 ± 3.80^{a,A}
Color difference	0	7.77 ± 0.86^{a,A}	10.23 ± 1.30^{a,A}	7.33 ± 1.53^{a,A}
(*ΔE**)	2	9.04 ± 1.02^{a,A}	12.06 ± 2.78^{a,A}	8.74 ± 2.50^{a,A}
	4	9.62 ± 0.83^{a,A}	11.84 ± 2.69^{a,A}	9.72 ± 3.04^{a,A}

Control = uncoated apple slices, **Alginate-coated** = coated apple slices with 2% w/v alginate/ 1% w/v glycerin/ 0.4% w/v $CaCl_2$, **TreAlg-coated** = coated apple slices with 6.71% w/v Tre in 2% w/v alginate/ 1% w/v glycerin/ 0.4% w/v $CaCl_2$

Before FD = non freeze-dried sample

Hue angle (*h)** = $\tan^{-1}(b^*/a^*)$

Browning index (BI) = 100 (x-0.31)/ 0.172 where x = $(a^* + 1.75L^*)/ (5.645L^* + a^* - 3.012b^*)$

Color difference (ΔE^*) = $[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5}$

Means with the different letters (lowercase: the same coating group at different storage times; uppercase: different coating groups at the same storage time) are significantly different by Bonferroni test ($p<0.05$).

APPENDIX F

Microbial determination

Table 1F Microbial growth of freeze-dried coated apple slices with different coatings after different storage times compared to control (uncoated) kept at 25 ± 5°C

Microorganisms	Weeks	Mean ± SD of log CFUs/g		
		Control	Alginate-coated	TreAlg-coated
Mesophilic	0	$2.36 \pm 0.16^{a,A}$	$2.36 \pm 0.14^{a,A}$	$2.37 \pm 0.10^{a,A}$
bacteria	2	$2.44 \pm 0.07^{a,A}$	$2.46 \pm 0.07^{a,A}$	$2.48 \pm 0.03^{a,A}$
	4	$2.48 \pm 0.04^{a,A}$	$2.47 \pm 0.11^{a,A}$	$2.48 \pm 0.02^{a,A}$
Psychrophilic	0	ND	ND	ND
bacteria	2	ND	ND	ND
	4	ND	ND	ND
Yeasts and	0	$<1.00 \pm 0.00^{a,A}$	$<1.00 \pm 0.00^{a,A}$	$<1.00 \pm 0.00^{a,A}$
molds	2	$<1.00 \pm 0.00^{a,A}$	$<1.00 \pm 0.00^{a,A}$	$<1.00 \pm 0.00^{a,A}$
	4	$<1.00 \pm 0.00^{a,A}$	$<1.00 \pm 0.00^{a,A}$	$<1.00 \pm 0.00^{a,A}$

ND = not detected, **Control** = uncoated apple slices, **Alginate-coated** = coated apple slices with 2% w/v alginate/ 1% w/v glycerin/ 0.4% w/v $CaCl_2$, **TreAlg-coated** = coated apple slices with 6.71% w/v Tre in 2% w/v alginate/ 1% w/v glycerin/ 0.4% w/v $CaCl_2$

Means with the different letters (lowercase: the same coating group at different storage times; uppercase: different coating groups at the same storage time) are significantly different by Bonferroni test ($p < 0.05$).

VITA

NAME	Miss Nichayarach Invichian
DATE OF BIRTH	6 January 1991
PLACE OF BIRTH	Phitsanulok, Thailand
INSTITUTIONS ATTENDED	Naresuan University, 2009-2014 Doctor of Pharmacy (Pharmaceutical Care)
HOME ADDRESS	298/103 Phayasue Rd, Tambon Nai Muang, Amphoe Muang Phitsanulok, Phitsanulok, Thailand 65000