PREPARATION AND ANTIMICROBIAL ACTIVITY OF CAPRYLIC ACID EMULSION FOR FOOD COATING

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การเตรียมและฤทธิ์ต้านจุลชีพของอิมัลชันกรดคาไพรลิกเพื่อเคลือบผิวอาหาร

น.ส.ระวีวรรณ บรรยงวิมลณัฐ

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การเคลือบผิวอาหารด้วยสารต้านจุลชีพเป็นวิธีที่น่าสนใจในการถนอมอาหารโดยเฉพาะพวกผักผลไม้ที่เน่าเสียได้ง่าย การศึกษาก่อนหน้านี้พบว่ากรดคาไพรลิกมีฤทธิ์ต้านจุลชีพทั้งแบคทีเรียและเชื้อรา อย่างไรก็ตาม งานวิจัยเกี่ยวกับการนำกรดคาไพรลิกมาใช้ประโยชน์ในรูปแบบของการเคลือบผิวอาหารมีเพียงเล็กน้อยเท่านั้น ดังนั้นการศึกษาครั้งนี้จึงมีวัตถุประสงค์เพื่อศึกษาสภาวะที่เหมาะสมสาหรับการเตรียมอิมัลชันกรดคาไพรลิกและประเมินฤทธิ์ต้านจุลชีพของอิมัลชัน รวมถึงผลของอิมัลชันกรดคาไพรลิกในการเคลือบผิวอาหารต่อคุณสมบัติทางกายภาพและการยับยั้งจุลชีพในอาหาร โดยออกแบบการทดลองเตรียมอิมัลชันกรดคาไพรลิกที่ความเข้มข้นร้อยละ 4 โดยน้ำหนักต่อปริมาตร และมีส่วนผสมของสารลดแรงตึงผิวในความเข้มข้นต่างๆ กำหนดอัตราส่วนโดยน้ำหนักของสารทำอิมัลชันคือ polysorbate 80 ต่อ monocaprylin ที่ 1: 1 ศึกษาปัจจัยที่เหมาะสมสาหรับการเตรียมอิมัลชันด้วยวิธีพื้นที่ผิวตอบสนองแบบ Box-Behnken design ผลการศึกษาพบว่า สูตรการเตรียมอิมัลชันกรดคาไพรลิกที่เหมาะสมที่สุดเมื่อกำหนดการตอบสนอง ได้แก่ อนุภาคขนาดเล็ก ค่าความต่างศักย์ไฟฟ้าสูง และค่าการกระจายตัวของอนุภาคน้อย พบว่าต้องใช้สารทำอิมัลชันความเข้มข้นร้อยละ 3.6 โดยน้ำหนักต่อปริมาตร และเตรียมโดยการใช้คลื่นเสียงที่มีความถี่สูงเป็นเวลา 120 วินาที และแอมพลิจูดร้อยละ 30 โดยอิมัลชันสูตรที่ได้นี้มีความเข้มข้นต่ำที่สุดของกรดคาไพรลิกในการฆ่าแบคทีเรีย/เชื้อราที่ร้อยละ 0.25 โดยน้ำหนักต่อปริมาตร และมีความคงตัวตลอดการเก็บที่อุณหภูมิ 30 ± 2 °C และ 40 ± 2 °C ในสภาวะที่มีความชื้นสัมพัทธ์ร้อยละ 75 ± 5 เป็นเวลา 28 วัน เมื่อศึกษาผลของสารเคลือบผิวอาหารประเภทอิมัลชันที่มีกรดคาไพรลิกความเข้มข้นร้อยละ 0.25 โดยน้ำหนักต่อปริมาตร โดยใช้อัลจิเนตที่ความเข้มข้นร้อยละ 2 โดยน้ำหนักต่อปริมาตร เป็นสารก่อฟิล์มร่วมกับกลีเซอรอลและแคลเซียมคลอไรด์ที่ความเข้มข้นร้อยละ 1.5 และ 2 โดยน้ำหนักต่อปริมาตร ตามลำดับ พบว่าตัวอย่างอาหาร (แก้วมังกรหั่นชิ้น) ที่เคลือบด้วยอิมัลชันกรดคาไพรลิก เก็บในตู้เย็นเป็นเวลา 12 วัน มีการเปลี่ยนแปลงลักษณะทางกายภาพ ได้แก่ สี น้ำหนักที่ลดลง และความแน่นเนื้อ ไม่แตกต่างอย่างมีนัยสำคัญ ($p>0.05$) เมื่อเทียบกับกลุ่มควบคุมที่ไม่เคลือบผิวอาหาร ในขณะที่การเจริญเติบโตของแบคทีเรียและเชื้อรามีการลดลงอย่างมีนัยสำคัญ ($p<0.0001$) โดยสรุปอิมัลชันกรดคาไพรลิกสามารถนำมาประยุกต์ใช้เป็นสารเคลือบอาหารที่มีฤทธิ์ต้านจุลชีพได้ ทั้งนี้งานวิจัยที่จะดำเนินการต่อควรเป็นการประเมินคุณภาพทางประสาทสัมผัส เพื่อใช้ในการพัฒนาด้านการถนอมอาหารต่อไป

สาขาวิชา	อาหารเคมีและโภชนศาสตร์ทางการแพทย์	ลายมือชื่อนิสิต .....
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5976114733 : MAJOR FOOD CHEMISTRY AND MEDICAL NUTRITION

KEYWORD: CAPRYLIC ACID, FOOD COATING, ANTIMICROBIAL ACTIVITY, EMULSION

Raweewan Bunyovimonnat : PREPARATION AND ANTIMICROBIAL ACTIVITY OF CAPRYLIC ACID EMULSION FOR FOOD COATING. Advisor: Assoc. Prof. WARANGKANA WARISNOICHAROEN, Ph.D.

Antimicrobial food coating is the interesting method for food preservation, especially in fruits and vegetables. Previous studies have been reported that the caprylic acid had the antimicrobial activity against a variety of microorganisms. However, little research has been conducted on the use of caprylic acid in the form of food coatings. Therefore, this study aims to determine the optimum conditions for the caprylic acid emulsion preparation and to evaluate the antimicrobial activity of the emulsion, including the effect of food coatings with caprylic acid emulsion on physical properties and microbial growth inhibition. Emulsions containing 4% w/v caprylic acid (oil) and various concentration of surfactant mixture (polysorbate 80: monocaprylin at 1: 1 weight ratio) were prepared. The response surface methodology, Box-Behnken design, was used to optimize the factors for emulsion preparation which were surfactant concentration, sonication time and amplitude. The study showed that the optimized formula, which had small particle size, high values of zeta potential and low polydispersity index, could be prepared using 3.6% w/v surfactant mixture, sonication time of 120 sec and 30% sonication amplitude. The emulsion had the minimum bactericidal/fungicidal concentration at 0.25% w/v caprylic acid and was stable after storage at 30 ± 2 °C and 40 ± 2 °C, relative humidity (RH) 75 ± 5% for 28 days. Food coating formula contained caprylic acid (0.25% w/v) emulsion using 2% w/v alginate as a film-forming compound with 1.5% w/v glycerol and 2% w/v calcium chloride. Food sample (pitahaya pieces) coated with caprylic acid emulsion stored in the refrigerator for 12 days had statistically insignificant (p>0.05) change in color, weight loss and firmness compared with the uncoated food, whereas the growth of bacteria and fungi of coated samples was significantly reduced (p<0.0001). In conclusion, the caprylic acid emulsion could be used as antimicrobial food coating. Ongoing research should be conducted on sensory evaluation for further development in food preservation.

Field of Study:	Food Chemistry and Medical Nutrition	Student's Signature .....
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Raweewan Bunyovimonnat

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LIST OF ABBREVIATIONS

% v/w	percent volume by weight
% w/v	percent weight by volume
% w/w	percent weight by weight
°C	degree celsius
*a**	red to green color channels
*b**	yellow to blue color channels
*C**	chroma
*h**	hue angle
*L**	lightness
ΔE*	total color difference
cm	centimeter(s)
g	gram(s)
h	hour(s)
kg	kilogram(s)
kHz	kilohertz
mg	milligram(s)
min	minute(s)
ml	milliliter(s)
mm	millimeter(s)
mm^2	square millimeter(s)
mV	millivolt(s)
N	Newton
nm	nanometer(s)
psi	pound per square inch
R^2	coefficient of determination
RH	relative humidity
rpm	revolutions per minute
sec	second(s)
µl	microliter(s)

μm	micrometer
ADI	acceptable daily intake
ANOVA	analysis of variance
ATCC	American type culture collection
BBD	Box–Behnken design
CA	caprylic acid
CFR	code of federal regulations
CFUs	colony forming units
CIE	the international commission on illumination
D	desirability
DLS	dynamic light scattering
DRBC	Dichloran Rose Bengal chloramphenicol
FDA	food and drug administration
GRAS	generally recognized as safe
HLB	hydrophile-lipophile balance
LD_{50}	lethal dose 50
MBC	minimum bactericidal concentration
MFC	minimum fungicidal concentration
MHA	Mueller-Hinton agar
MHB	Mueller-Hinton broth
MIC	minimum inhibitory concentration
MTT	thiazolyl blue tetrazolium bromide
PCA	plate count agar
PDI	polydispersity index
RSM	response surface methodology
SD	standard deviation
SDA	Sabouraud's dextrose agar
SDB	Sabouraud's dextrose broth
SPSS	statistical package for the social sciences
TPA	texture profile analysis
WI	whiteness index

O/W	oil-in-water
O/W/O	oil-in-water-in-oil
W/O	water-in-oil
W/O/W	water-in-oil-in-water

CHAPTER I

INTRODUCTION

1.1 Background and rationale

Food spoilage causes unpleasant changes in food products such as texture, taste, and odor changes. The spoilage is mainly caused by the growth of microorganisms in food (Gram *et al.*, 2002). To enhance the shelf life of food for storage, edible coating is one of the methods of food preservation in the food industry. Edible coatings can prevent food from dehydration and water loss by acting as a moisture barrier; moreover, the coatings are expected to reduce atmospheric changes by acting as a gas barrier. Edible coatings can also improve textural properties and flavor loss (Montero-Calderón, Rojas-Graü, and Martín-Belloso, 2008; Azarakhsh *et al.*, 2014). Edible coatings, which are made from natural substances, are safe for consumers and environmental friendly (Yousuf, Qadri, and Srivastava, 2018).

The properties of the coating are different depending on type of compounds. Hydrophilic coatings, which are polysaccharides or proteins, may deliver good mechanical and structural properties but rather poor moisture barrier. Hydrophobic coatings, which are lipids, are excellent moisture barrier but poor mechanical properties and opaque characteristics. Therefore, composite coating containing both hydrophilic and hydrophobic compounds is of interest in order to reduce the limits

and enhance the advantages of each compound (Palou, Valencia-Chamorro, and Pérez-Gago, 2015; Yousuf *et al.*, 2018). Composite coating can be obtained as emulsions; however, preparation of composite coating may have a problem from instability of emulsion such as gravitational separation, flocculation, and coalescence (McClements, 2011). These problems can be solved by adding appropriate surfactants and homogenization in process of emulsion formation before adding hydrocolloid compound to prepare coatings (Galus and Kadzińska, 2015). In order to prevent the food spoilage, antimicrobials can be contained in the coating formulation (Yousuf *et al.*, 2018).

Recently, natural antimicrobial agents have been studied for coating components. Antimicrobial agents from plant are mainly represented by essential oils such as oregano and thyme essential oils, olive leaves extract, and garlic oil that contain various chemicals such as phenolics, terpenes, aliphatic alcohols, aldehydes, ketones, organic acids, saponins, thiosulfinates, and glucosinolates. Antimicrobial agents of animal sources are largely present in antimicrobial peptides like pleurocidin, lactoferrin, defensins, and protamine. Some polysaccharides and lipids from animals also display antimicrobial activity such as chitosan, fatty acids and monoglycerides. In addition, antimicrobial agents of microbial sources are nisin, natamycin, and reuterin (Pisoschi *et al.*, 2018).

Caprylic acid is the medium-chain fatty acids (octanoic acid, C8:0) present in milk and coconut oil (International Union of Pure and Applied Chemistry (IUPAC), 2001;

Lemarie *et al.*, 2015). Caprylic acid was reported to have antimicrobial activity against *Streptococcus agalactiae*, *S. dysgalactiae*, *S. uberis*, *Staphylococcus aureus*, *Escherichia coli*, *Vibrio parahaemolyticus*, *V. harveyi*, and *Shigella* species (Doores, 2005; Nair *et al.*, 2005; Immanuel, Sivagnanavelmurugan, and Palavesam, 2010). Furthermore, caprylic acid was studied as food coatings in frozen breaded chicken products, which could significantly reduce *Salmonella* when compared to uncoated products (Moschonas *et al.*, 2012).

However, little research has been done on caprylic acid in the form of emulsion-based coatings. This study is aimed to formulate emulsions containing caprylic acid and to evaluate their antimicrobial activity. The stable and effective formulation will be selected to prepare the coating in food samples, which will be studied for physical properties and microbial determination.

1.2 Objectives of the study

1. To study factors influencing preparation and physicochemical properties of caprylic acid emulsions
2. To evaluate antimicrobial activity of caprylic acid emulsions
3. To study the effect of emulsion-based food coating on physical properties and microbial inhibition of food samples

1.3 Benefits of the study

This study provides information on factors affecting the preparation, physicochemical properties, and antimicrobial activity of caprylic acid emulsions. In addition, emulsion-based coatings can be applied to food samples in order to preserve the quality of food.

CHAPTER II

LITERATURE REVIEW

In this study, food coating containing caprylic acid emulsion with antimicrobial activity was studied. The literature review was covered in the emulsion-based coating, microbial determination as well as evaluation of food properties. In addition, the experimental design using response surface methodology (RSM) was also reviewed.

2.1 Emulsion

2.1.1 Types of emulsion

Emulsion is a colloidal solution of small particles dispersed in other immiscible liquid. Emulsion can be classified based on the dispersion of droplets in a continuous phase into two main groups: simple and multiple emulsions. Oil-in-water (O/W) emulsions **(Figure 1A)** and water-in-oil (W/O) emulsions **(Figure 1B)** are simple emulsions. Multiple emulsions are simple emulsions dispersed in other liquid phase, such as water-in-oil-in-water (W/O/W) emulsions **(Figure 1C)**, and oil-in-water-in-oil (O/W/O) emulsions **(Figure 1D)**.

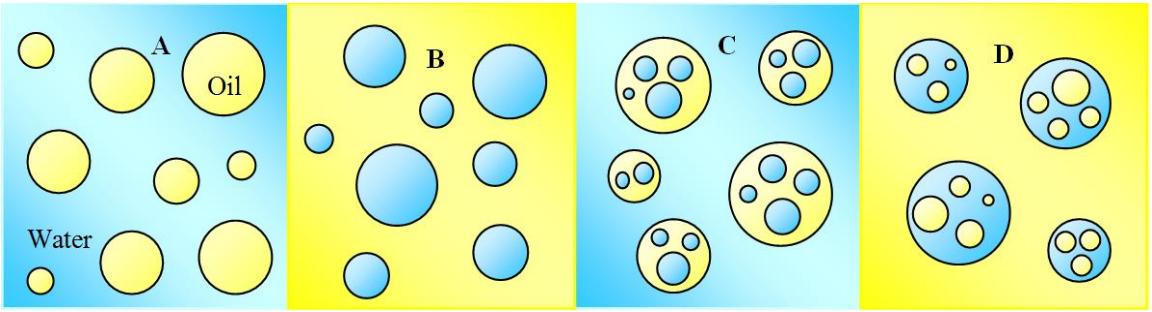


Figure 1 Type of emulsions (Prichapan and Klinkesorn, 2014)

2.1.2 Instability of emulsion

Emulsions are an unstable thermodynamic system due to the variety of physical mechanisms that tend to change the physicochemical properties of emulsions over time, as shown in **Figure 2**. Gravitational separation may occur as sedimentation or creaming. Sedimentation is the droplet movements onto downwards, which results from the droplet density higher than the continuous phase. On the other hand, creaming has droplet density lower than the continuous phase, which affects the droplet movements onto move upwards. Flocculation and coalescence are mechanisms that occurs when two or more droplets combine to form droplet aggregation. The difference between the two mechanisms is that the flocculation still maintains the integrity of each droplet, while coalescence merges each droplet into a single large droplet. Ostwald ripening is a mechanism in which the size of droplets in the emulsion increases from small to large droplets over time. However, all mechanisms lead to phase separation of the emulsion in the final (McClements, 2005; McClements, 2011).

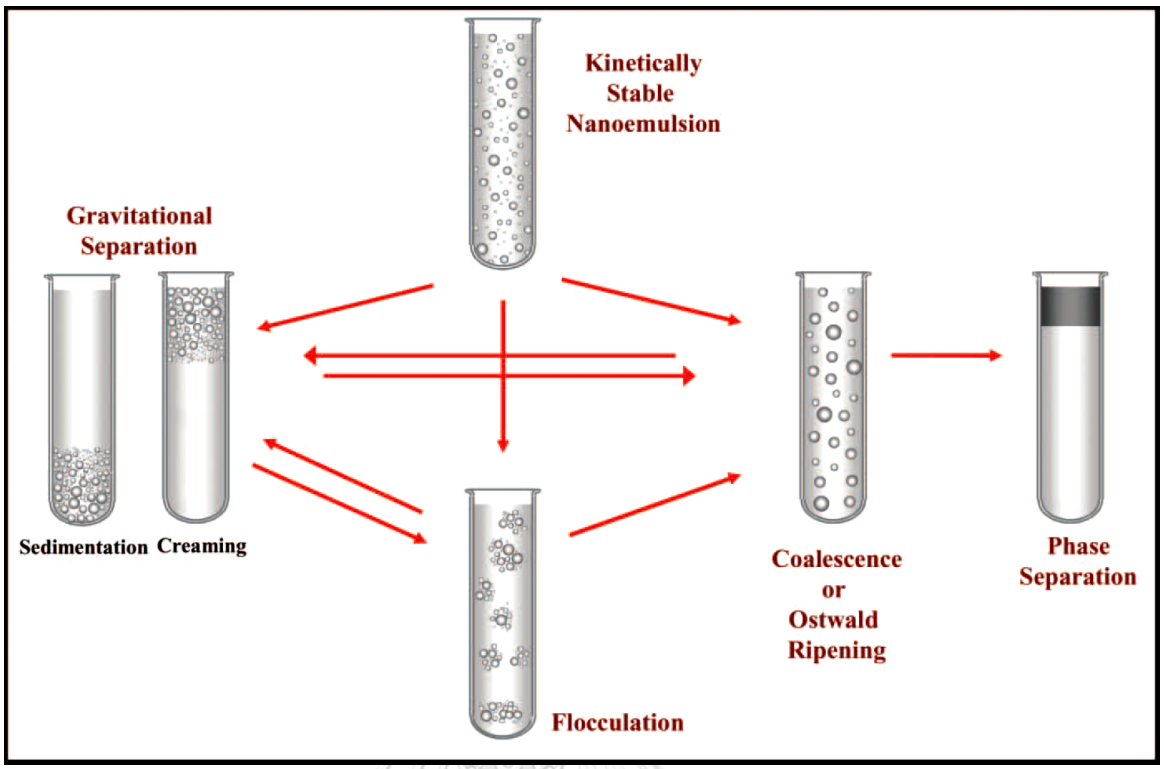


Figure 2 Instability mechanisms of emulsion (McClements and Rao, 2011)

2.1.3 Emulsion preparation

Preparation of emulsions relates to the mixing of hydrophilic and hydrophobic compounds. Emulsification process of oil phase in water phase is a significant process for stable emulsion. The important factor for stability of the emulsion is related to the size of oil droplets, which also affects the viscosity and other properties of the emulsion. Adding surfactants or emulsifiers improves emulsion stability by interacting at an oil-water interface resulting in decrease a surface tension of each phase. Different homogenization techniques affect droplet sizes. For example, Rotor-stator homogenizers, which are often used to prepare the emulsion, may be able to achieve a particle size about 1 μm. Microfluidization and ultrasonication can produce smaller

droplet sizes than the rotor-stator homogenizers, which may have reached the size of the nanoemulsions by shearing force of ultrahigh pressure homogenization. The particle size may reduce by increasing the pressure and duration of microfluidization or increasing time and amplitude of ultrasonication. A combination of homogeneous techniques are often used to obtain a stable emulsion by the first step with a rotor-stator homogenizer and the next step with a microfluidizer or an ultrasonicator (Galus and Kadzińska, 2015).

Ultrasound emulsification has two-stage mechanisms for droplet formation. The action of the interfacial wave of the acoustic field leads to the eruption of the oil phase into the aqueous phase to form large droplets. After that, the transformation and distribution of these large droplets are smaller due to the impact of cavitation-induced shock waves occurring near the interface when under an acoustic field, as shown in **Figure 3** (Manickam, 2013).

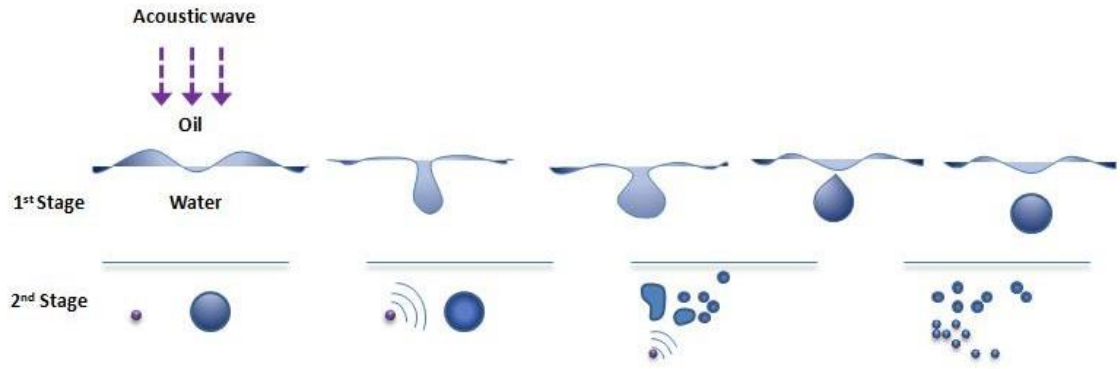


Figure 3 Droplet formation of ultrasound emulsification (Manickam, 2013)

2.1.4 Emulsion components

2.1.4.1 Caprylic acid

Caprylic acid, which is the chemical name for octanoic acid, is the eight-carbon saturated fatty acids commonly found in milk and coconut oil. The compound has a slightly rancid taste and poor water solubility (Jay, 1995). The oral lethal dose (LD_{50}) of caprylic acid in rat was 1,283 mg/kg (Alfa Aesar, 2012). Caprylic acid is permitted in flavoring agents, food additive and antifoaming agent, which has an acceptable daily intake (ADI) of acceptable (JECFA, 2004). In addition, caprylic acid has been approved by Food and Drug Administration (FDA) 21 CFR 184.1025 for use in foods at different maximal levels such as 0.013% w/w for baked goods, 0.04% w/w for cheeses, 0.005% w/w for fats and oils, 0.016% w/w for snack foods and 0.001% w/w or less for all other food categories (FDA, 2017). The structure of caprylic acid is shown in **Figure 4**.

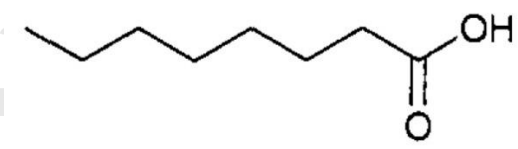


Figure 4 Chemical structure of caprylic acid (Milne, 2005)

Caprylic acid is of interest due to the beneficial effects of its antimicrobial activity. Previous antimicrobial activity studies are shown in **Tables 1 and 2**.

Table 1 Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of caprylic acid (CA).

Author	Microorganism	MIC	MBC/MFC
Batovska *et al.* (2009)	*Staphylococcus aureus*	>0.5 mg/ml	-
	Corynebacterium diphtheriae	>0.5 mg/ml	-
	Bacillus cereus	>0.5 mg/ml	-
	Listeria monocytogenes	0.25 mg/ml	-
	Streptococcus pyogenes	>0.5 mg/ml	-
Brandt *et al.* (2011)	*Listeria monocytogenes*	0.025 mg/ml	-
Grilli *et al.* (2013)	*Campylobacter jejuni*	9 mg/ml	-
Huang *et al.* (2010)	*Eurotium herbariorum*	0.5 mg/mg	-
	Empetrum rubrum	0.5 mg/mg	-
	Aspergillus flavus	0.5 mg/mg	-
	Aspergillus niger	0.5 mg/mg	-
	Penicillium roqueforti	0.5 mg/mg (sodium salt of CA, pH 5.0, 0.85 a_w)	-
Hulánková and Bořilová (2011)	*Salmonella enteritidis*	0.004 ml/ml	-
	Escherichia coli O157:H7	0.004 ml/ml	-
	Staphylococcus aureus	0.003 ml/ml	-
	Listeria monocytogenes	0.002 ml/ml	-
Kollanoor *et al.* (2007)	*Edwardsiella ictaluri*	1.1 mg/ml	1.4 mg/ml
	Edwardsiella tarda	1.1 mg/ml	1.4 mg/ml
	Streptococcus iniae	1.4 mg/ml	2.2 mg/ml
	Yersinia ruckeri	1.1 mg/ml	1.4 mg/ml

Table 1 Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of caprylic acid (CA) (continued).

Author	Microorganism	MIC	MBC/MFC
Nakai and Siebert (2004)	*Listeria innocua*	2.8 mg/ml	-
	Listeria ivanovii	2.8 mg/ml	-
	Pseudomonas aeruginosa	22.4 mg/ml	-
	Oenococcus oeni	0.89 mg/ml (pH 5.25)	-
Nobmann *et al.* (2010)	*Staphylococcus aureus*	1.4-2.9 mg/ml	-
Oshima *et al.* (2012)	*Cronobacter sakazakii*	8 mg/ml (2%Tween 80)	-
Skrivanova *et al.* (2006)	*Escherichia coli*	2 mg/ml	-
	Salmonella enteritidis	3 mg/ml	-
	Salmonella infantis	3 mg/ml	-
	Salmonella typhimurium	3 mg/ml	-
	Clostridium perfringens	2 mg/ml	-
Souza *et al.* (2014)	*Candida albicans*	0.6 mg/ml	1.2 mg/ml
	Candida parapsilosis	1.2 mg/ml	1.2 mg/ml
	Candida famata	0.6 mg/ml	1.2 mg/ml
	Candida glabrata	1.2 mg/ml	1.2 mg/ml
	Candida lipolytica	2.5 mg/ml	2.5 mg/ml
	Candida tropicalis	2.5 mg/ml	2.5 mg/ml
Sun, O'Connor and Roberton (2002)	*Escherichia coli*	<0.29 mg/ml (pH 4.6)	-
	Klebsiella pneumoniae		
	Enterococcus casseliflavus		
	Enterococcus faecium		
	Enterococcus faecalis		

Table 2 Antimicrobial activity of caprylic acid (CA) in food testing.

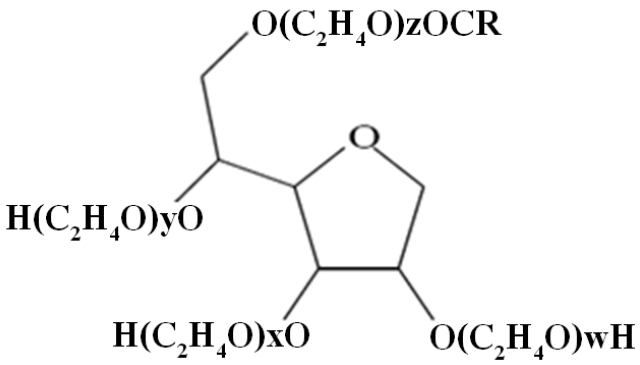
Author	CA conc.	Composition	Food testing	Microorganism	Controls
Hulankova, Borilova and Steinhauserova (2013)	0.5% v/w	-	Minced beef	Lactic acid bacteria, psychrotrophic bacteria and *L. monocytogenes*	Sterile distilled water
Moschonas *et al.* (2012)	1.0% v/w	Sodium chloride (1.2% w/w), Sodium tripolyphosphate (0.3% w/w)	Breaded chicken	*Salmonella* spp.	Sterile distilled water
Nair *et al.* (2005)	0.72% w/v, 1.44% w/v	Dimethyl sulfoxide (DMSO; 2% v/v)	Raw milk	*E. coli* *S. aureus* *S. agalactiae* *S. dysgalactiae* *S. uberis*	Milk containing DMSO or no DMSO
Wang *et al.* (2018)	1.0% w/w	Polysorbate 80 (0.5% w/w), Carvacrol (0.5% w/w), Chitosan (1% w/v)	Pacific white shrimp	Mesophillic bacteria	Sterile distilled water

2.1.4.2 Surfactant

Surfactant (abbreviation from surface active agent) is generally used in the emulsion preparation for the stabilization of emulsions. Surfactant can lower the interfacial tension of immiscible liquids and prevent droplet aggregation by formation of micelles. Surfactant selection depends on the balance in size and strength between hydrophilic and lipophilic parts of a surfactant, which is called hydrophile-lipophile balance (HLB) system. Lower HLB surfactants (values 1-10) are lipophilic surfactants and form W/O emulsions. Higher HLB surfactants (values >10) are hydrophilic surfactants and form O/W emulsions (Imperial Chemical Industries (ICI) Americas Inc, 1980). The classification of surfactant can be in 4 types depending on the characteristic of electric charges in hydrophilic parts: anionic surfactant, cationic surfactant, non-ioinic surfactant and amphoteric surfactant, which has both negative and positive charges (Cullum, 1994; Salager, 2002).

Polysorbate 80 or polyoxyethylene (20) sorbitan monooleate or Tween 80 is a non-ioinic surfactant derived from esters of sorbitol and oleic acid, reunite with approximately 20 moles of ethylene oxide per mole of sorbitol and its anhydrides as shown in **Figure 5**. The HLB value of polysorbate 80 is 15, which is suitable for forming O/W emulsions. Polysorbate 80 is permitted in food additive and emulsifier, which has ADI of 0-25 mg/kg body weight (JECFA, 1973). Furthermore, polysorbate 80 is used for

non-food applications such as pharmaceuticals, cosmetics and industrial applications (European Food Emulsifier Manufacturers' Association (EFEMA), 2015).



where w + x + y + z is approximately 20 and RCO- is the oleic acid moiety.

Figure 5 Chemical structure of polysorbate 80 (EFEMA, 2015)

2.1.4.3 Co-surfactant

Co-surfactant is used with a surfactant to reduce surface tension between oil-water interfaces and improve the stability of the emulsion. The system of micelle formation is shown in **Figure 6**. The typical co-surfactants are short chain alcohols, medium chain alcohols, glycols, amines or acids. The role of co-surfactant includes adjusting HLB value to within the optimum range for emulsion formation, increasing the fluidity of the interface, and protecting the formation of emulsion by destroying liquid crystalline or gel structure (Muzaffar, Singh, and Chauhan, 2013).

Monocaprylin, which has synonyms such as monoctanoin, monocaprylate and glyceryl monocaprylate, is produced by esterification and molecular distillation process of caprylic acid and glycerol. Structure of monocaprylin is shown in **Figure 7**.

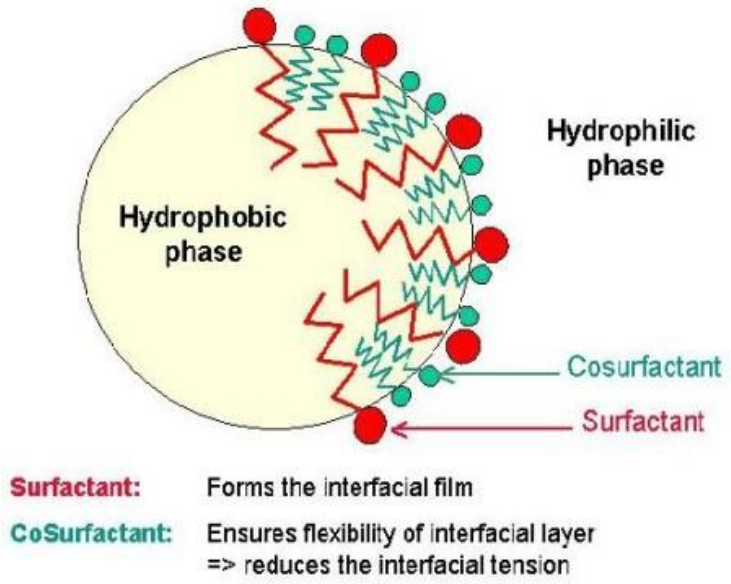


Figure 6 Oil-in-water emulsion system (Srikanth *et al.*, 2012)

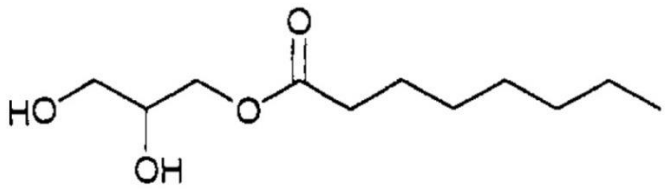


Figure 7 Chemical structure of monocaprylin (Milne, 2005)

Monocaprylin is often used as solubilizer, dispersant, plasticizer, lubricant, penetration enhancer, plasticizer and co-surfactant (Milne, 2005; Zargar-shoshtari, Wen, and Alany, 2010). The HLB value is 6.6 (Textron, 2007). For toxicological information, the oral LD_{50} of monocaprylin in rat was greater than 2,000 mg/kg (Cremer Oleo Division, 2012). Monocaprylin is permitted in food additive and emulsifier, which has ADI of 0-25 mg/kg body weight (JECFA, 1989). Moreover, monocaprylin is generally recognized as safe (GRAS) and can function as a preservative in food because of its

antimicrobial activity (Hyldgaard *et al.*, 2012). Previous antimicrobial activity studies of monocaprylin are shown in **Table 3**.

Table 3 MIC, MBC and MFC of monocaprylin

Author	Microorganism	MIC	MBC/MFC
Batovska *et al.* (2009)	*Staphylococcus aureus*	>0.5 mg/ml	-
	Corynebacterium diphtheriae	>0.5 mg/ml	-
	Bacillus cereus	>0.5 mg/ml	-
	Listeria monocytogenes	>0.5 mg/ml	-
	Streptococcus pyogenes	>0.5 mg/ml	-
Kollanoor *et al.* (2007)	*Edwardsiella ictaluri*	0.5-1.1 mg/ml	1.1 mg/ml
	Edwardsiella tarda		
	Streptococcus iniae		
	Yersinia ruckeri		
Nobmann *et al.* (2010)	*Staphylococcus aureus*	0.5-1.1 mg/ml	-
Oshima *et al.* (2012)	*Cronobacter sakazakii*	3 mg/ml (2%Tween 80)	-
United States Patent (1990)	*Bacillus cereus*	0.5 mg/mg	-
	Bacillus subtilis	0.5 mg/mg	-
	Bacillus coaglans	0.5 mg/mg	-
	Bacillus licheniformis	0.5 mg/mg	-
	Bacillus megaterium	0.5 mg/mg	-
	Staphylococcus aureus	1.0 mg/mg	-
	Leuconostoc mesenteroides	0.5 mg/mg	-
	Lactobacillus casei	0.5 mg/mg	-
	Streptococcus faecalis	0.5 mg/mg	-

2.2 Emulsion-based antimicrobial coating

Emulsion-based edible coatings have been used to prevent loss of moisture, especially in fresh fruits and vegetables. These coatings are made from edible components that directly coated on the food (Galus and Kadzińska, 2015). Currently, the development of emulsion-based edible antimicrobial coatings can be used as an alternative to maintain food products by delay spoilage from the growth of microorganisms in food. Adding antimicrobial agents directly to the food system may reduce antimicrobial activity during storage due to rapid diffusion within foods and possible interaction with food ingredients. The use of edible coatings for the entrapment of antimicrobial agents reduces the diffusion of antimicrobial agents onto the surface of food and maintains concentration at inhibitory level for microbial growth during prolonged storage, as shown in **Figure 8** (Aloui and Khwaldia, 2016).

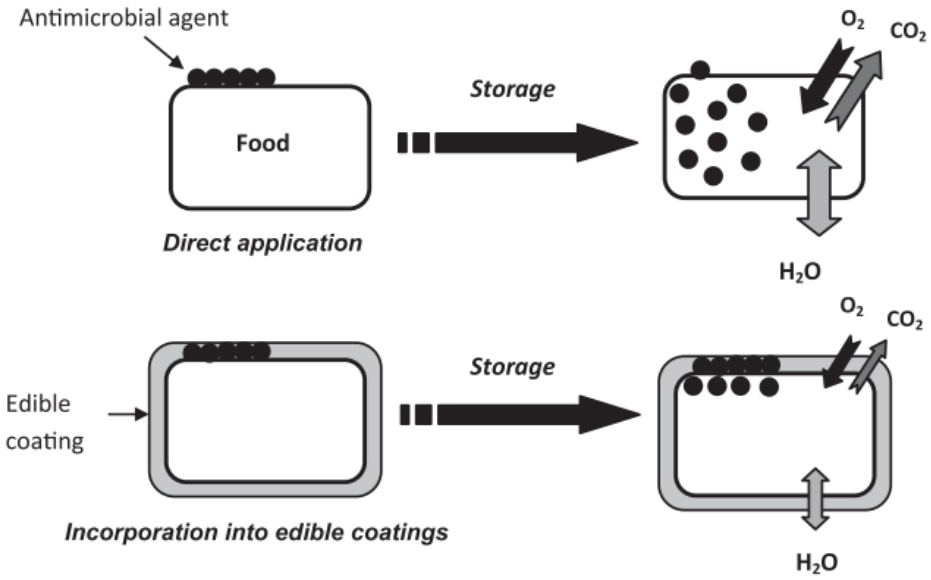


Figure 8 Functional properties of edible antimicrobial coating (Aloui and Khwaldia, 2016)

Emulsion for use in edible antimicrobial coatings is generally formulate a as O/W emulsion adding antimicrobial in oil phase and coating material in aqueous phase. In this study, the coating was prepared from caprylic acid emulsion and hydrocolloid solution with plasticizer.

2.2.1 Caprylic acid emulsion

Caprylic acid has been previously used as a component in emulsion formulation as shown in **Table 4**.

2.2.2 Hydrocolloid

Basicly, hydrocolloids of edible coatings are polysaccharides, proteins and lipids. Polysaccharides, which have highly polar polymers with hydroxyl groups in chemical structure, can form a cohesive and continuous matrix by linking through the hydrogen bonding of polymer chains. Due to hydrophilic properties, polysaccharides are a good oxygen barrier at low relative humidity, but poor moisture barrier. Proteins have hydrophilic properties similar to polysaccharides. Therefore, proteins are a good gas barrier, but low moisture barrier. The ability to form edible coatings depends on molecular characteristics of different proteins: molecular weight, flexibility, conformation, electrical properties, and thermal stability. Some people may be allergies or protein intolerances, so manufacturers should be cautious to inform consumers of the type of protein used in the coating. Lipids, which are hydrophobic

substances, are an excellent moisture barrier. However, disadvantage of lipid-based coatings are poor mechanical properties and opaque characteristics. (Palou *et al.*, 2015).

Alginates are a water-soluble polysaccharide, which are extracted from brown algae (*Laminaria digitata* and *Ascophyllum nodosum*). The oral LD_{50} of alginic acid sodium salt in rat was greater than 5,000 mg/kg (Sigma-aldrich, 2016). Sodium alginate is permitted in food additive, emulsifier, gelling agent, stabilizer, and thickener, which has ADI of not specified (JECFA, 1992). The structure of alginates consists of 1-4-linked β-D-mannuronate (M) and α-L-guluronate (G) monomers, as shown in **Figures 9 and 10**. Type of alginate chain conformation is shown in **Figure 11**. The physical properties of alginate depend on the structure of the chain. The G-block chains are more gel strength than the M-block chains and the MG-block chains determine the solubility of alginates in acid. Alginates are generally used in many applications due to being non-toxic, biodegradable, biocompatible and inexpensive. In addition, alginate can produce a strong film called “eggbox” from adding divalent ions such as calcium, as shown in **Figure 12** (Tavassoli-Kafrani, Shekarchizadeh, and Masoudpour-Behabadi, 2016).

Table 4 Formulation of emulsion containing caprylic acid and derivatives.

Author	Oil phase	Stabilizers	Continuous phase	Mean droplet size (nm)	Method of emulsification
Biradar, Dhumal and Paradkar (2009)	Caprylic/capric propylene glycol diesters	Polysorbate 80 (1:1 of oil) and glyceryl caprylate/caprate (20-60% w/w of oil: surfactant mixture)	Water	~ 100	Spontaneous emulsification
Jaworska, Sikora and Ogonowski (2014)	Caprylic/capric triglyceride Caprylic/capric/ linoleic triglyceride	Polysorbate 40 and buthanol (1:1)	Water	116-264	Phase inversion method
Jaworska *et al.* (2015)	Caprylic/capric propylene glycol diesters	Polysorbate 40, 60 or 80 and butanol (1:1)	Water	137-196	Spontaneous emulsification
Kadri *et al.* (2017)	Caprylic acid/ sunflower oil	Polysorbate 80 (1%, 8% w/w)	M9 media	170-650	High shear mixer homogenizer and probe sonicator
Khuwijitjaru *et al.* (2004)	Caprylic acid	Polysorbate 20 (0.042% w/v) or decaglycerol monolaurate (0.083% w/v)	Water	≤100	High-pressure-resistant vessel
Osborn and Akoh (2004)	Caprylic acid/ canola oil	Whey protein isolate and sucrose stearate (0.5% w/w)	Water	260-2,690	Packed bed bioreactor and high-pressure valve homogenizer

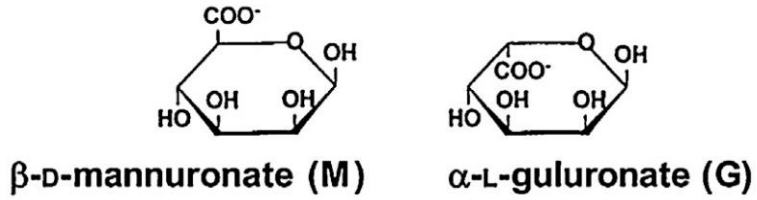


Figure 9 Structure of alginate monomers (Tavassoli-Kafrani et al., 2016)

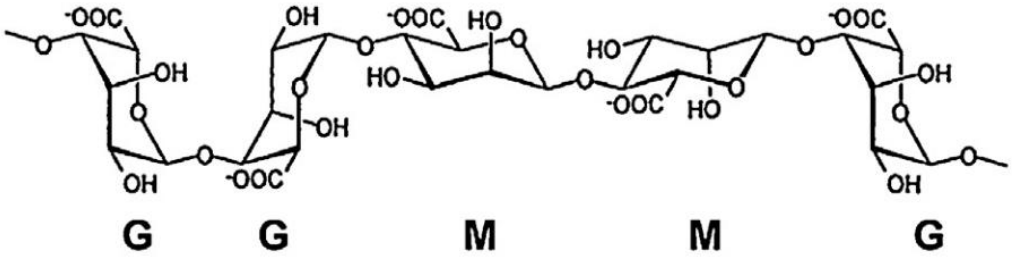


Figure 10 Structure of alginate chain formation (Tavassoli-Kafrani et al., 2016)

Figure 11 Type of alginate chain conformation (Tavassoli-Kafrani et al., 2016)

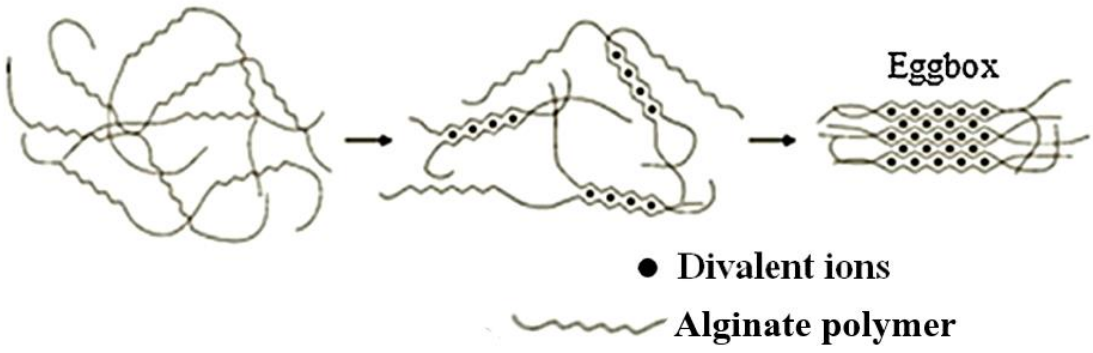


Figure 12 Formation of gelation by "eggbox" model
(Adapted from Tavassoli-Kafrani et al., 2016)

2.2.3 Plasticizer

Plasticizer is a substance or material that is incorporated in a material to modify polymer characteristics by increasing its flexibility, resistance to fracture and dielectric constant, together with reducing the tension of deformation, hardness, viscosity, density and electrostatic charge of polymers. The plasticizers can be divided into hydrophilic and hydrophobic plasticizers. Hydrophilic plasticizers are soluble in aqueous medium, which can increase water diffusion in the polymer at high concentrations. Conversely, hydrophobic plasticizers decrease in water uptake by close the micro-voids in the film; however, they may cause a phase separation that leads to the formation of discontinuity or loss of flexibility during film drying (Vieira *et al.*, 2011).

Glycerol, which is also known as glycerin, is a water-soluble, viscous, colorless, odorless, and sweet. Glycerol is synthesized from polypropylene or sucrose. Chemical structure of glycerol is shown in **Figure 13**. The molecules have 3 hydroxyl groups (-OH), which cause hydroscopic properties and dissolve well in water. The oral LD_{50} of glycerol in rat was 12.6 mg/kg (CDH, 2019). Glycerol is permitted in flavoring agent and food additive, which has ADI of not specified (JECFA, 1976). Glycerol is widely used in many applications such as food, medicines, cosmetics and soaps. Glycerol is commonly added in film-forming solutions to prevent brittle film (The Soap and Detergent Association (SDA). 1990; Gooch, 2011; Vieira *et al.*, 2011).

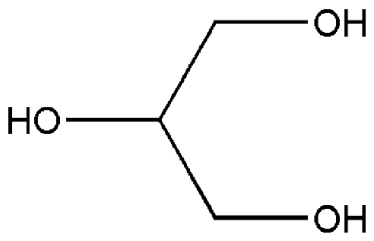


Figure 13 Chemical structure of glycerol (Gooch, 2011)

2.3 Food quality evaluation

2.3.1 Microbial determination

2.3.1.1 Microorganisms in food spoilage

Microbiological food spoilage is the main cause of food spoilage. Factors affecting microbial growth in foods include five categories of ecological determinants: (1) intrinsic factors are the inherent nature of the food such as pH, water activity, nutrient content, redox potential, and antimicrobial agents, which are naturally present in foods; (2) extrinsic factors are related to the characteristics of food storage environments such as temperature, atmosphere and relative humidity; (3) implicit factors are related to microbial content in food products; (4) processing factors are associated with the processing procedures by food manufacturers, which varies according to the type and amount of microorganisms, including composition and properties of each food; and (5) interaction between factors may be antagonism or synergism for microbial growth. These factors affect the formation of specific microorganisms and the growth of microbial populations that may occur (Hamad, 2012;

Lianou, Panagou, and Nychas, 2016). The microorganisms associated with spoilage of food are many species. Some food spoilage microorganisms also cause food-borne pathogens. People may gain food-borne disease when eating food that is spoiled. Major spoilage microorganisms can be classified into 3 major groups: bacteria, yeasts, and molds (Petruzzi *et al.*, 2017). The most common microorganisms are shown in **Table 5**.

Furthermore, bacteria can be classified according to temperature for growth, as shown in **Figure 14**. For example, psychrophilic bacteria are cold-tolerant bacteria that can grow at low temperature with minimum temperature for growth at 0 °C or below (Moyer, Collins, and Morita, 2017). These bacteria can cause spoilage of refrigerated products. Mesophilic bacteria are bacteria that prefer moderate temperature. The optimum temperature is in the range of 30-45 °C (Willey *et al.*, 2008). The major causes of microbial spoilage and almost all pathogenic pathogens are in this group. Thermophilic bacteria can grow at high temperatures, which is the cause of spoilage in foods in canned or sealed containers (Rawat, 2015).

Table 5 The most common microorganisms in food spoilage

	Description
S. aureus (Collins *et al.*, 2004; Hait and Bennett, 2012; Todd, 2014)	
- Classification and morphology	Round shape, non-spore forming, catalase-positive, and gram positive bacteria
- Enterotoxins	Heat-resistance enterotoxins
- Symptoms	Nausea, vomiting, diarrhea, abdominal pain In severe cases: headache, dehydration, muscle cramping, and temporary changes in blood pressure and pulse rate
- Common sources	Meat and meat products; poultry products; egg products; seafood products; pasta; milk and dairy products such as cheese; and ready-to-eat processed foods such as ham
B. cereus (Tallent and Bennett, 2012; Todd, 2014)	
- Classification and morphology	Rod shape, spore forming, *facultative anaerobic,* and gram positive bacteria
- Enterotoxins	Heat-stable emetic type and heat-labile diarrheal type
- Symptoms of food poisoning	Nausea and vomiting, diarrheal type: abdominal cramps and diarrhea
- Common sources	Rice products, potato, pasta and cheese products; diarrheal type: meats, milk, vegetables, and fish
E. coli (Collins *et al.*, 2004; Feng, 2012; Todd, 2014)	
- Classification and morphology	Round shape, non-spore forming, facultative anaerobic, and gram negative bacteria
- Pathogenic groups	Enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), and enterohemorrhagic *E. coli* (EHEC)
- Enterotoxins	Both heat-stable and heat-labile enterotoxins, EHEC: Shiga toxin

Table 5 The most common microorganisms (continued)

	Description
E. coli **(continued)**	
- Symptoms	ETEC: diarrhea in travelers from developing countries, infants and children living in developing countries; EPEC and EIEC: infantile diarrhea in developing countries; EHEC: mild diarrhea to severe illness such as bloody diarrhea, blood clots, kidney failure, and death
- Common sources	ETEC: brie cheese, turkey, crabmeat, mayonnaise, salad, and deli food; EPEC: raw beef, chicken, mayonnaise, lettuce and pickles; EIEC: no specific foods; EHEC: many foods; for example, ground meats, fermented sausages, raw milk, yogurt, mayonnaise, cheeses, unpasteurized fruit juices, sprouts, spinach, lettuce, and commercially made frozen cookie dough, raw or undercooked ground beef
P. aeruginosa (Kubota and Liu, 1971; Liao, 2006; Wu *et al.*, 2015; Lianou *et al.*, 2016)	
- Classification and morphology	Round shape, non-spore forming, and gram negative bacteria
- Pigment production	Pyocyanin (blue-green), pyorubin (red-brown), and pyoverdine (yellow-green)
- Enterotoxins	Heat-labile enterotoxins
- Symptoms	Diarrheal conditions, opportunistic infections in hospitals, especially immunocompromised patients
- Common sources	Poultry, meat, and seafood products, especially dairy products such as raw milk and cheese rind

Table 5 The most common microorganisms (continued)

	Description
C. albicans (Hocking and Faedo, 1992; Lianou *et al.*, 2016)	
- Classification	Yeast
- Symptoms	Superficial infections, such as oral or vaginal candidiasis, and life-threatening infections in immunocompromised patients
- Common sources	Soft drinks, syrups, dips, and salad dressing; dairy products such as butter, cheese and yogurt; apple and apple products such as apple juice and apple cider
A. niger (Plascencia-Jatomea *et al.*, 2014; Lianou *et al.*, 2016)	
- Classification	Generally, non-pathogenic mold and black spores production
- Symptoms	No obvious symptoms
- Common sources	Postharvest fruit and vegetable products, including apples, pears, peaches, citrus, grapes, guavas, strawberries, mangoes, melons, figs, cassava, potatoes, cucumbers, carrots, tomatoes, onions, garlic, and yams; grains and oil seeds, especially corn, barley, soybeans, rapeseed, sunflower seed, chickpeas and pigeon peas, stored and parboiled rice

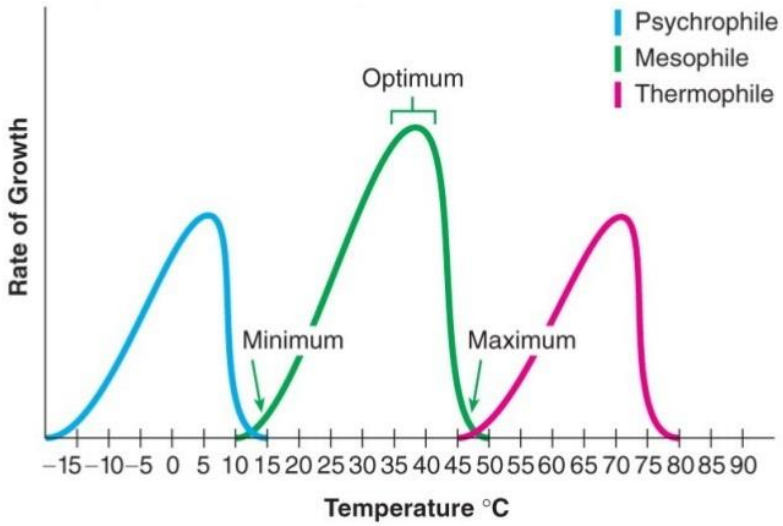


Figure 14 Bacterial growth curve temperature (Willey *et al.*, 2008)

2.3.1.2 Plate counting method

Plate counting method is one of the microbiological techniques used to count the number of living microorganisms in the sample. For accurate results, the operation procedure must use aseptic technique to avoid contamination of the sample. In addition, the colony counting should be in the range of 25-250 colonies per plate because outside the appropriate range may result in erroneous results.

The procedure, as shown in **Figure 15**, begins with diluting the sample by ten times dilution. After that, the sample (1 ml) of each dilution is filled on agar plate. Then, all plates are incubated in suitable temperature for microbial growth. Finally, the colonies of microorganisms that grow on the agar surface are counted. The first plate with less than 250 colonies was used to calculate the microbial count in the original sample (FDA, 1998; Tortora, Funke, and Case, 2016).

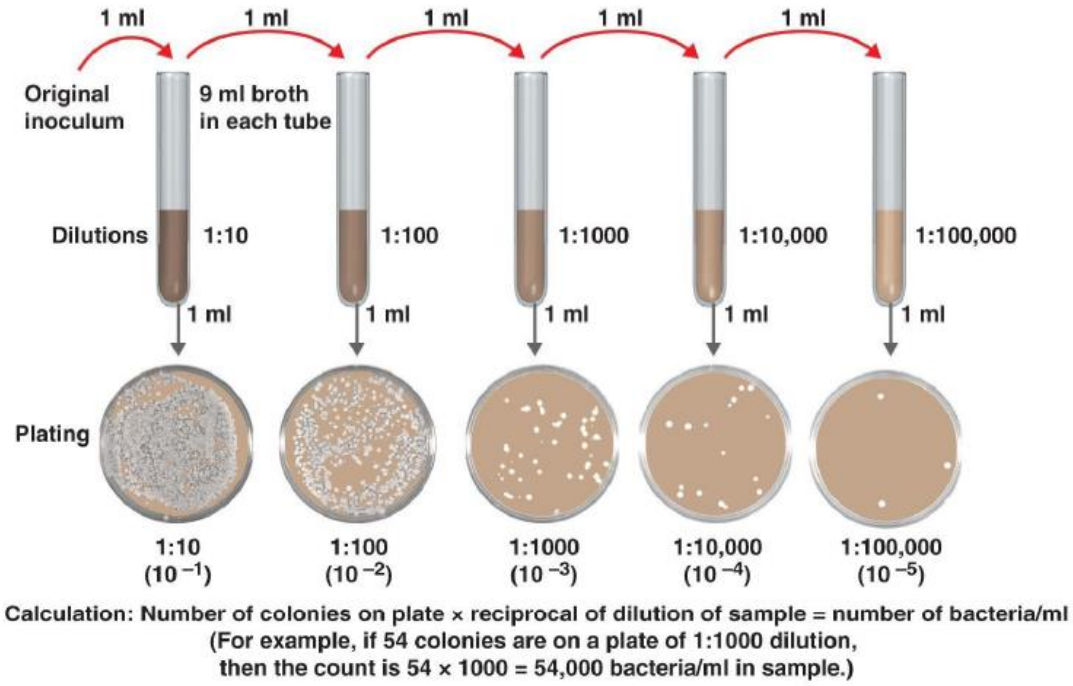


Figure 15 Serial dilutions and plate counts (Tortora *et al.*, 2016)

2.3.2 Color

The food appearance has a strong influence on the opinions of consumers about food quality in accepting or rejecting products. The color of food is one of the most important quality parameters that are evaluated by consumers. Color features in fruits and vegetables are derived from natural pigments, for example, carotenoids (yellow, orange, and red), flavonoids (yellow), betalains (red), anthocyanins (red, blue), and chlorophylls (green). The pigments of fruits and vegetables can be used as indicators of freshness of the product.

The color of the object can be explained by a variety of color systems. Some of the most popular systems are HunterLab L, a, b and the international commission

on illumination (CIE) system. The two systems are collectively referred to as the CIELAB color scales, as shown in **Figure 16**.

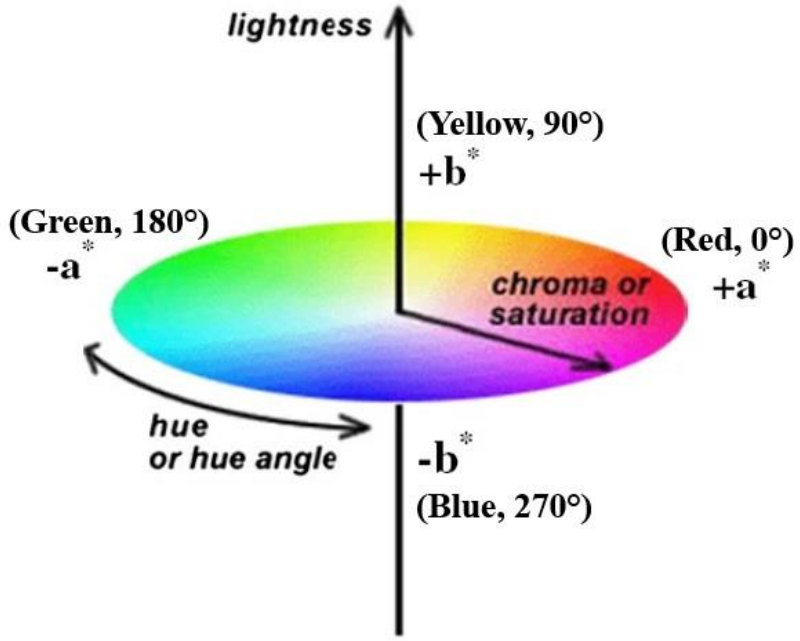


Figure 16 CIELAB color scales (adapted from Pathare, Opara, and Al-Said, 2013)

*L** is a measure of lightness, which is in the gray range between white and black. The parameter of *a** is red (positive value) and green (negative value), while *b** is yellow (positive value) and blue (negative value).

Chroma (*C**) is intensity of color, which is used to define the degree of hue difference compared with the same lightness. *C** was calculated using the equation below:

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad \textbf{(1)}$$

Hue angle (*h**) is used to determine the color difference referring to the same lightness. The angles of 0° (360°), 90°, 180° and 270° are red, yellow, green and blue, respectively. The *h** value can be calculated using the following equation:

$$h^* = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad \textbf{(2)}$$

In addition, color changes can be measured as the difference between the default color and the final color. Total color difference (*ΔE**) can be calculated as follows:

$$\Delta E^* = \sqrt{\Delta a^{*2} + \Delta b^{*2} + \Delta L^{*2}} \quad \textbf{(3)}$$

If the value of *ΔE** is less than 1.5, the color change is almost undifferentiated (Pathare, Opara, and Al-Said, 2013).

Moreover, the *L**, *a**, and *b** values can be calculated as whiteness index (WI) (Salvia-Trujillo *et al.*, 2015). The equation is calculated as follows:

$$WI = 100 - \sqrt{(100 - L^*)^2 + (a^{*2} + b^{*2})} \quad \textbf{(4)}$$

2.3.3 Firmness

Firmness is a value that indicates the softness or crispness of the fruit (Mitcham, Cantwell, and Kader, 1996). Loss of firmness is one factor that affects the sensory characteristics, resulting in decreased quality and consumer acceptance (Cock, Valenzuela, and Aponte, 2012). There are two basic methods for measuring fruit firmness: destructive method and non-destructive method (Blahovec and Kutílek,

2002). Destructive method can use various tests to evaluate the firmness of fruits such as compression, puncture, and texture profile analysis (TPA) tests (Olivas and Barbosa-Canovas, 2005). The force detection is used to calculate as force (Newton; N) or stress (N/mm^2) and create a deformation curve as shown in **Figure 17**.

The strain is ratio of the difference between the length that changes with the original length, which does not have a unit because it is the ratio of length in the same unit (Tipler and Walker, 1995). The strain can be calculated as $\Delta L / L$ when ΔL is length difference and L is original length. In addition, deformation can be calculated as %strain by multiplying the strain with 100. The maximum force before the deformation is reported as the firmness of fruit.

Non-destructive method can detect from the vibration signals of fruit by laser Doppler vibrometer, which based on the resonant theory. The index for fruit firmness is usually from the second resonance peak. This method is applied to hard fruit such as watermelon, avocado and pumpkin (Blahovec and Kutílek, 2002).

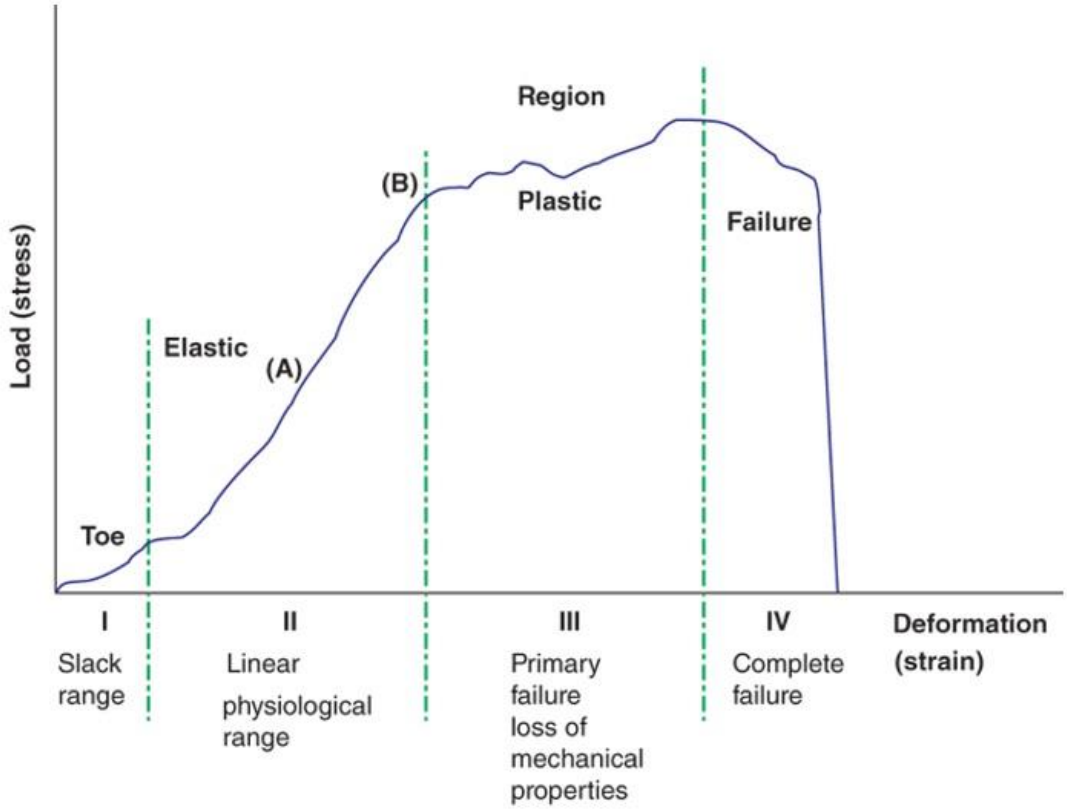


Figure 17 Deformation curve (Dutton, Ivey, and Smith, 2019)

2.4 Response surface methodology (RSM)

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

$$y = f(x_1, x_2, \dots, x_k) + \varepsilon \quad \textbf{(5)}$$

where ε is the error value of the response y from the experiment. The response surface is the surface represented by $f(x_1, x_2, \dots, x_k)$, 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 18**. The contour plot of three or more variables is only possible when one or more variables are constant. If the response model has a linear relationship with the independent variables, the appropriate equation used to evaluate the correlation of the response is follows:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \varepsilon \quad (6)$$

where k is the number of variables, β_0 is the constant value, β_i presents the coefficients of the linear parameters, x_i presents the variables, 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:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{1 \le i \le j}^{k} \beta_{ij} x_i x_j + \varepsilon \quad (7)$$

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

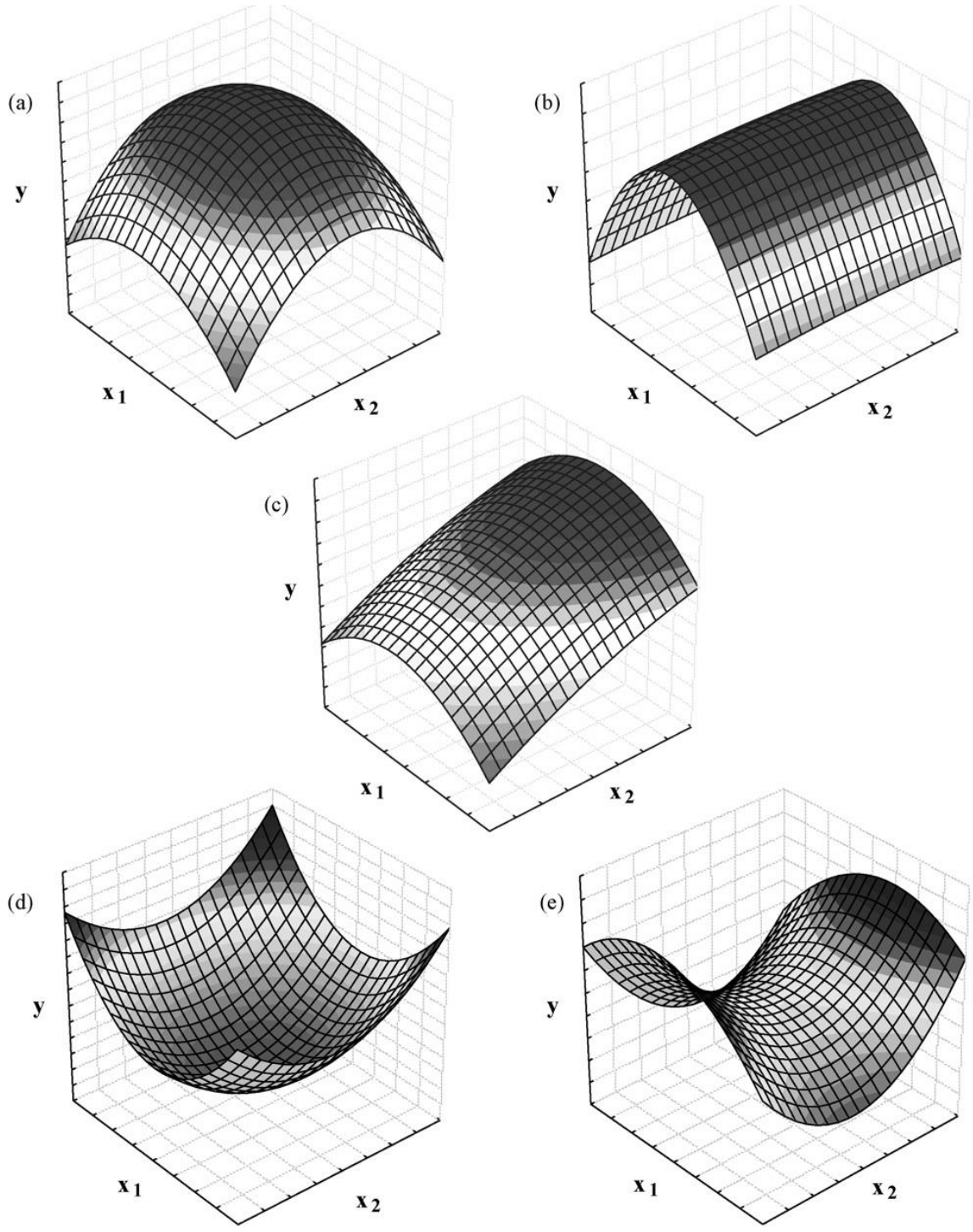


Figure 18 Some profiles of the surface response plots (x_i = variables, y = response) (a) maximum, (b) plateau, (c) maximum outside the experimental region, (d) minimum, and (e) saddle surfaces (Bezerra *et al.*, 2008)

The RSM requires to select a suitable experimental design before analysis, which has linear and quadratic models such as three-level factorial, Box-Behnken, central composite, and Doehlert designs. In Box-Behnken design, all factor levels are adjusted on three levels (-1, 0, +1) with equal intervals between these levels. The 13 experimental points are located on a hypersphere equidistant from the central point, as shown in **Figure 19** (Bezerra *et al.*, 2008; Myers, Montgomery, and Anderson-Cook, 2009).

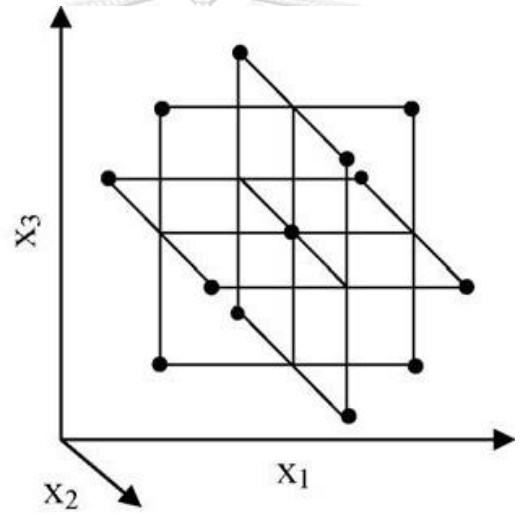


Figure 19 The study of three variables (x_i) of Box-Behnken design (Adapted from Bezerra et al., 2008)

CHAPTER III

MATERIALS AND METHODS

3.1 Chemicals and reagents

Caprylic acid with purity of more than 98% was obtained from Alfa Aesar, UK. Polysorbate 80 is a non-ionic surfactant, and thiazolyl blue tetrazolium bromide (MTT) were provided from Acros Organics, Switzerland. Monocaprylin with a minimum content of 88%, a co-surfactant, was supplied from IOI Oleo GmbH, Germany. Alginic acid sodium salt from brown algae was derived from Sigma, USA. Glycerin and calcium chloride were purchased from Daejung, South Korea.

3.2 Culture media

Nutrient broth, Mueller-Hinton broth (MHB), and Mueller-Hinton agar (MHA) were derived from BD, USA. Sabouraud's dextrose broth (SDB), Sabouraud's dextrose agar (SDA), plate count agar (PCA), and Dichloran Rose Bengal chloramphenicol (DRBC) agar were supplied from Himedia, India. Media were prepared as described (BD, 2009; Himedia, 2015) and sterilized with autoclaving (121 °C, 15 psi, 15 min). In addition, sodium chloride solution (Merck, Germany) and peptone water (Himedia, India) were used to adjust the concentration of microorganisms.

3.3 Microorganisms

Six microorganisms from Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University were used to evaluate the antimicrobial activity of test samples. Two gram-positive bacteria were *Staphylococcus aureus* (*S. aureus*) ATCC 25923 and *Bacillus cereus* (*B. cereus*) ATCC 11778. Two gram-negative bacteria were *Escherichia coli* (*E. coli*) ATCC 25922 and *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 27853. For fungi, yeast was *Candida albicans* (*C. albicans*) ATCC 10231 and mold was *Aspergillus niger* (*A. niger*) ATCC 16404.

3.4 Experimental design

Emulsions were prepared with sonication method. Particle size, size distribution, and zeta potential of emulsions were determined. The emulsion formulation was optimized by response surface methodology (RSM), Box-Behnken design (BBD). The optimal emulsion was used to study minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC). The MBC/MFC concentration was selected to formulate a food coating formulation. Coated fruits were stored in the refrigerator and analyzed for color, weight loss, firmness, and microbial counts. The experimental design is shown in **Figure 20**.

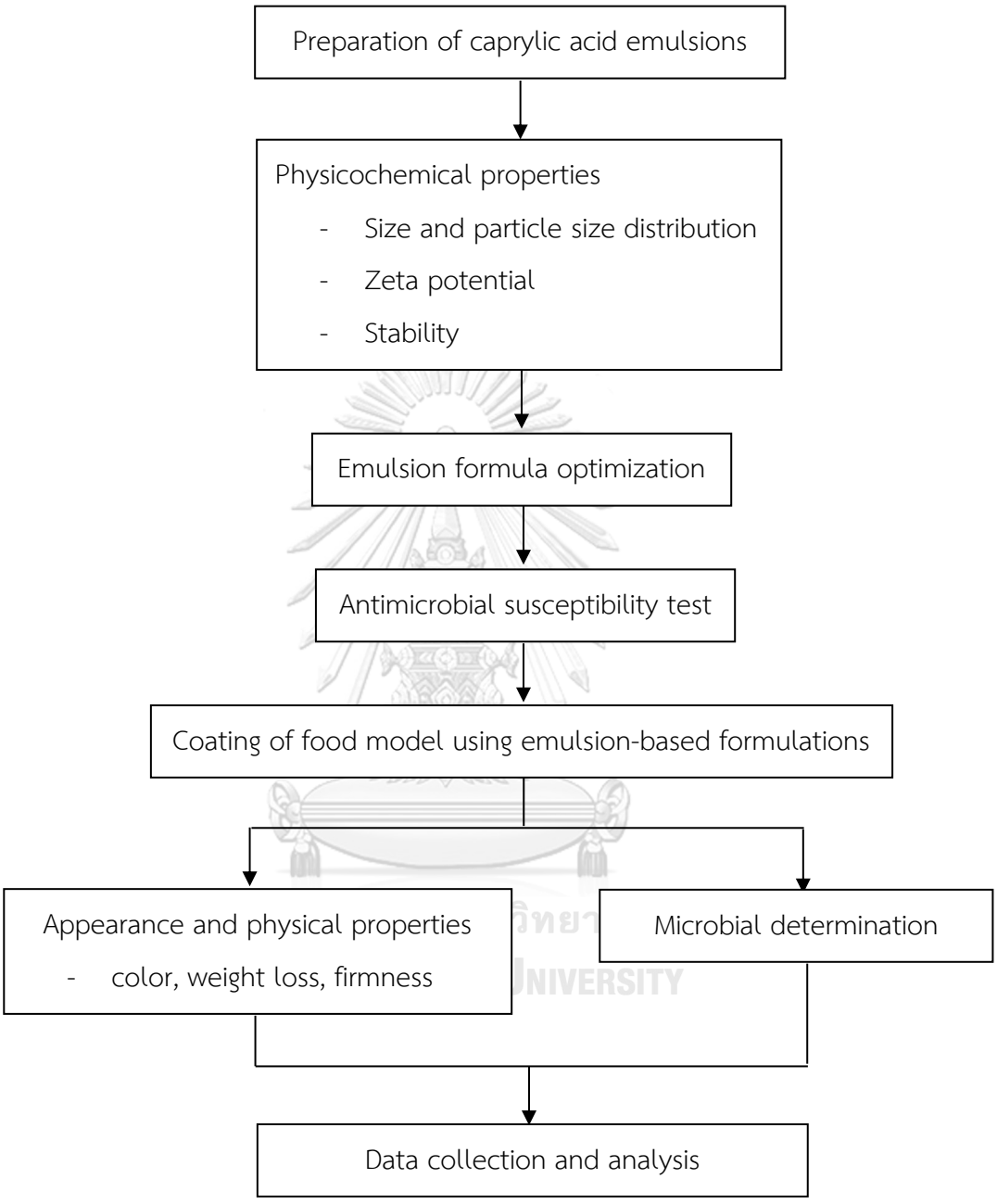


Figure 20 Diagram of the experimental design

3.5 Methods

3.5.1 Preparation of caprylic acid emulsions

Oil-in-water emulsions were formulated using 4% w/v caprylic acid as an oil. Notably, caprylic acid concentration was set at 4% w/v because this concentration was greater than the MIC of previous studies **(Table 1)**, which should have antimicrobial activity against all microorganisms. The surfactant phase used was polysorbate 80 and monocaprylin at a weight ratio of 1: 1 because this ratio had previously been reported to be suitable for emulsion preparation (Jaworska *et al.*, 2014). The coarse emulsions were prepared using different concentrations of ingredients. Oil phase (mixture of caprylic acid and surfactants) and aqueous phase (ultrapure water) were heated up to 70-75 ℃ for about 10 min. Oil phase was poured into aqueous phase and homogenized using an Ultra-Turrax T25 homogenizer (IKA, Germany) at 8000 rpm for 2 min. The coarse emulsions were emulsified using high intensity ultrasonic processor VC/VCX 750 (Sonic, USA) which parameters set in the range of 30% to 70% of amplitude and sonication time from 60 to 120 sec. The diameter of the ultrasonic probe tip was 13 mm. The emulsions were kept in sterile containers at 30 ± 2 ℃ / 75 ± 5% RH and 40 ± 2 ℃ / 75 ± 5% RH in constant climate chambers (Binder, Germany).

The formulations were prepared according to a 3-factor, 3-level BBD as shown in **Tables 6 and 7**. Before determining the level of each factor, the caprylic acid emulsions were prepared in a wide range of various factors and then, the results to a narrower level of each factor were selected, which could be future use in design

experiment. The effects of factors, surfactant concentration (X_1), sonication time (X_2), and amplitude (X_3), on the responses, particle size (Y_1), zeta potential (Y_2), and polydispersity index (Y_3), were studied.

3.5.2 Physicochemical properties of emulsions

3.5.2.1 Size and particle size distribution

The particle size and polydispersity index (PDI) of emulsions were measured using dynamic light scattering (DLS). The experiment was performed on the Zetasizer Nano ZS (Malvern Instruments, UK) equipped with helium-neon laser operating at a wavelength of 633 nm, 173° at 25 °C. The samples were diluted to no more than 1% w/w of the surfactant concentration before measurement in order to avoid the interparticulate interaction and multiple scattering (Warisnoicharoen, Lansley, and Lawrence, 2000). The scattered light was measured the diffusion speed of particles and scattering intensity at a specific angle that fluctuated with time and analyzed the data to determine the particle size and the size distribution (Malvern, 2018).

Table 6 Factors used in BBD for emulsion preparation

Symbol	Factors	levels		
		-1	**0**	**1**
X_1	Surfactant (% w/v)	3	5	7
X_2	Sonication time (sec)	60	90	120
X_3	Amplitude (%)	30	50	70

Surfactant = 1: 1 polysorbate 80: monocaprylin

Table 7 Coding and decoding factors used in BBD for emulsion formulation

Experiment	Coding			Decoding		
Order	**X_1**	**X_2**	**X_3**	**X_1**	**X_2**	**X_3**
1	-1	-1	0	3	60	50
2	+1	-1	0	7	60	50
3	-1	+1	0	3	120	50
4	+1	+1	0	7	120	50
5	-1	0	-1	3	90	30
6	+1	0	-1	7	90	30
7	-1	0	+1	3	90	70
8	+1	0	+1	7	90	70
9	0	-1	-1	5	60	30
10	0	+1	-1	5	120	30
11	0	-1	+1	5	60	70
12	0	+1	+1	5	120	70
13	0	0	0	5	90	50
14	0	0	0	5	90	50
15	0	0	0	5	90	50

3.5.2.2 Zeta potential

The zeta potential of emulsions was measured by Zetasizer Nano ZS, which measured electrophoretic mobility of oil using laser doppler electrophoresis and then calculated to values of zeta potential using Henry's equation (Kaszuba *et al.*, 2010). The measurements were done in triplicates at 25 °C using folded capillary cells.

3.5.2.3 Stability

Stability of emulsions was determined by measuring the change in visual appearance, particle size, size distribution, and zeta potential after storage for 0, 7, 14, 21 and 28 days at 30 ± 2 °C / 75 ± 5% RH and 40 ± 2 °C / 75 ± 5% RH (International Conference on Harmonisation (ICH), 2003).

3.5.3 Selection of optimized emulsion

The effects of factors (X_1 = surfactant concentration, X_2 = sonication time, and X_3 = amplitude) on the responses (Y_1 = particle size, Y_2 = zeta potential, and Y_3 = polydispersity index) were analyzed to optimize emulsion formulation by RSM using Design-Expert® Software version 11.0 (Stat-Ease, USA). The experimental model used was BBD. This program calculated by considering the statistical significance at 95% confidence level. The adequacy of the models was statistically analyzed from the model analysis, lack of fit test, and coefficient of determination (R^2) analysis. After that, equations indicating the significant relationship between each factor and response

were obtained. In addition, response surface plots were used to indicate the trend of factors that affect the response. These data were then used to evaluate the desirability of the optimal formula. 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 (Phoa and Chen, 2013).

3.5.4 Antimicrobial susceptibility test

3.5.4.1 Preparation of microorganisms

Each of microbial strains was cultured onto MHA (bacteria) or SDA (fungi). The microbial incubation was set at 37 °C for 24 h (bacteria), 30 °C for 24 h (yeasts) and 30 °C for 48 h (molds) using temperature controlled incubator (Memmert, Germany). Then, a single colony from MHA or SDA was diluted in sodium chloride solution and adjusted microbial concentration to 0.5 McFarland standard turbidity (1-2 x 10^8 CFUs/ml for bacteria and 1-5 x 10^6 CFUs/ml for yeast) (Balouiri, Sadiki, and Ibnsouda, 2016). The mold spores were dissolved with 0.05% w/v polysorbate 80 in sodium chloride solution for preventing coagulation of spores. The bright-line hemacytometer was used to count spore in suspension, which was adjusted to 1 x 10^6 spores/ml before antimicrobial susceptibility test (National Committee for Clinical Laboratory Standards (NCCLS), 2002; European Committee for Antimicrobial Susceptibility Testing (EUCAST), 2003).

3.5.4.2 Broth microdilution test

Broth microdilution method was used to determine MIC, which is the lowest concentration of microbial inhibition. The samples were diluted to different concentrations in the culture media and observed the growth of microorganisms (Clinical and Laboratory Standards Institute (CLSI), 2012; Balouiri *et al.*, 2016). The sample was the optimized emulsion containing caprylic acid, polysorbate 80 and monocaprylin. In addition, monocaprylin, polysorbate 80 solution, and caprylic acid P-80 emulsion containing caprylic acid and polysorbate 80 were also tested for comparison.

Broth microdilution test was done on 96-well microplates. Each well contained 50 µl of the different concentrations of the sample prepared by two-fold dilutions and 50 µl of microbial suspension. Each microbial suspension was prepared by adding microbial stock solution, which was adjusted to 0.5 McFarland standard turbidity, into media (MHB for bacteria and SDB for fungi) at a ratio of 1: 150. Blank control wells contained 50 µl of the sample and 50 µl of media without microbial. Positive control wells contained 50 µl of sterile ultrapure water and 50 µl of microbial suspension. Negative control wells contained 50 µl of sterile ultrapure water and 50 µl of media without microbial. The microplates were then incubated at 37 °C for 24 h (bacteria) and at 30 °C for 24 h (yeasts) or 48 h (molds).

After incubation period, 10 µl of MTT at a concentration of 0.5 mg/ml (bacteria) or 5 mg/ml (fungi) was added to each well and the microplates were incubated for

another 30 min (bacteria) and 48 h (fungi). MTT can detect dehydrogenase activity of living microorganisms by the occurrence of purple formazan crystal (Dewanjee *et al.*, 2015). The minimal concentration of wells contained the original color of MTT (yellow) was the MIC of the microorganisms.

MBC and MFC, which are the lowest concentrations that can kill microbe of 99.9%, were further analyzed by a plate method. The samples with no growth well from microplates were transferred to the plate containing MHA (bacteria) and SDA (fungi) and were incubated under appropriate conditions of each microorganism. MBC and MFC are the lowest concentrations that show absence of colony growth.

3.5.5 Coating of food model using emulsion-based formulations

Emulsion concentration selected for food coating was determined from MBC/MFC. Food coating was prepared by mixing 4% w/v caprylic acid emulsion with the solution containing 2% w/v alginate and 1.5% w/v glycerol at a volume ratio of 1: 16. The final concentration of caprylic acid in the coating formula was 0.25% w/v. For the solution preparation, 2% w/v of alginate was gradually dissolved in water containing 1.5% w/v of glycerin heated at 70-75 °C. Glycerol was used as a plasticizer for film coating.

Food samples (pitahaya or dragon fruit (*Hylocercus undatus* (Haw) Brit. & Rose.)) from local supermarket (Loei province, Thailand) were washed, peeled, and cut into cubic pieces about 1.4 x 1.4 x 1.4 cm (3-4 g, each). After that, the food samples were

dipped in emulsion-based coating solution for 2 min, dipped off for 1 min, and dipped in 2% w/v calcium chloride solution for 2 min then dipped off for 1 hr, respectively (Salinas-Roca *et al.*, 2016). Calcium chloride is used for food coating in order to ionically cross-link with alginate to form a thin film (Lee and Mooney, 2012). Uncoated samples (dipped in sterile water 2 min then dipped off for 1 hr) and samples coated with solution of alginate and glycerol without added emulsion and 2% w/v calcium chloride solution were used for comparison. All tested samples were kept in sterile bags in a refrigerator at 4 to 8 °C for 12 days prior to analysis.

3.5.6 Study on properties of coated-food samples

After storage for 0, 3, 7 and 12 days, coated samples were studied for any changes in color, weight loss and firmness.

3.5.6.1 Appearance and physical properties

3.5.6.1.1 Color

The color changes of food after storage were determined by UltraScan Pro (HunterLab, USA), which measures lightness (*L**), color channels (*a**, and *b**) of samples. Color measurements were repeated three times. The *L**, *a**, and *b** results of coating solutions were calculated as whiteness index (WI), while the *L**, *a**, and *b**

results of food were calculated as hue angle (*h**), chroma (*C**) and color difference (*ΔE**) (Pathare *et al.*, 2013; Salvia-Trujillo *et al.*, 2015).

3.5.6.1.2 Weight loss

Weight loss of food was evaluated by comparing the sample weight after storage with the initial weight (day 0). The measurement was done on a digital balance. The results were calculated as a percentage of weight loss (Azarakhsh *et al.*, 2014).

3.5.6.1.3 Firmness

Firmness of food, reported as maximum force before food deformation with the unit in Newton (N), was assessed with universal testing machine model EZ-S (Shimadzu, Japan). The machine was set cylindrical plate with a diameter of 3 cm, a 50 N load cell, at a speed of 10 mm/min, until the sample was compressed to 30% of the initial height (Cock *et al.*, 2012). Then, the maximum force before food deformation was recorded.

3.5.6.2 Microbial determination

Microbial population of the coated food was measured by plate count method (FDA, 1998; Rojas-Graü, Tapia, and Martín-Belloso, 2008). Mesophilic bacteria, psychrophilic bacteria, and yeasts/molds growth were counted amounts. Food sample (10 g) was aseptically weighed into a sterile bottle. Sterile peptone water (0.1% w/v)

90 ml was added into a bottle. The bottle was treated with an ultrasonic bath (Bandelin DT156, Germany), which operated with frequency of 35 kHz at 25 °C for 1 min (Benitez-Cabello *et al.*, 2015). The sample was diluted by tenfold dilution (i.e. sample 1 ml per 0.1% w/v peptone water 9 ml) until microbial population of less than 250 colonies per plate was obtained (FDA, 1998).

The sample 1 ml of each dilution was filled on agar plate and dispersed with sterile glass spreader. All plates were incubated at desired temperature. Mesophilic bacteria cultured with PCA was incubated at 35 °C for 24 h. Psychrophilic bacteria cultured with PCA was incubated in a refrigerator at 4 to 8 °C for 14 days. Yeasts and molds cultured with DRBC agar containing 100 mg chloramphenicol/liter were incubated at 25 °C for 5 days. After incubation, the results were the concentration of each specie (log CFUs/g of food) from the first plate having less than 250 colonies as calculated from the number of colonies on the plate multiplied by dilution.

3.5.7 Statistical analysis

The measurements were done in triplicates. The data of each group were presented as mean ± standard deviation (SD). Statistical package for the social sciences (SPSS) version 13.0 (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 emulsion, the data were analyzed for the statistical difference ($p < 0.05$) with Scheffe test 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

The caprylic acid emulsion was developed for food coating purpose. The food coating was prepared by adding the emulsion into the glycerol and alginate solution, which was used for film formation. For emulsion preparation, caprylic acid was an internal oil phase and surfactant phase contained polysorbate 80 and monocaprylin. Monocaprylin was used as a co-surfactant for increasing the stability of the emulsion by possibly optimizing the hydrophile-lipophile balance (HLB) of the system, reducing interfacial tension between oil-water interfaces and promoting the occurrence of spherical oil-in-water emulsion particles (Muzaffar *et al.*, 2013).

4.1 Formulation of caprylic acid emulsion

The factors used for emulsion preparation in this study were surfactant concentration, sonication time, and amplitude. Previous studies reported that these three factors had an effect on particle size, which affected the emulsion stability. The smallest particle size of the emulsion could occur when using the appropriate surfactant concentration (Kim *et al.*, 2014). The longer sonication time and higher amplitude could create the smallest particle size (Ngan *et al.*, 2014; Hashtjin and Abbasi, 2015).

The 15 formulas of caprylic acid emulsions were prepared according to the Box-Behnken design (BBD) of model experiment used, as seen in **Table 7**. The concentration of caprylic acid was given at 4% w/v since this concentration showed antimicrobial activity (Nakai and Siebert, 2004). A weight ratio of 1: 1 surfactant to co-surfactant was previously reported to be appropriate for emulsion preparation and was used in this study (Jaworska *et al.*, 2014). The parameters used to determine the responses including particle size (Y_1), zeta potential (Y_2), and polydispersity index (Y_3) for the 3 factors used namely, surfactant concentration (X_1), sonication time (X_2), and amplitude (X_3). The results of the particle size, zeta potential, and PDI of emulsions prepared within 24 h are shown in **Table 8**.

4.1.1 Optimization of emulsion formula

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 9**.

The sequential *p*-value was used to evaluate the suitability of the model. If this value is significant ($p<0.05$), this model can be used to evaluate the response. In contrast, *p*-value of "lack of fit" is the number of model predictions that are erroneously observed. If this value is significant ($p<0.05$), this model is not suitable to be used to evaluate responses (Stat-Ease, 2018).

Table 8 Results of particle size, zeta potential, and PDI of 4% w/v caprylic acid emulsions

Experiment order	Factors			Responses		
	Surfactant* (% w/v)	Sonication time (sec)	Amplitude (%)	Particle size (nm)	Zeta potential (mV)	PDI
1	3	60	50	198	-25.1	0.23
2	7	60	50	206	-15.3	0.18
3	3	120	50	216	-28.9	0.26
4	7	120	50	223	-14.8	0.24
5	3	90	30	205	-29.8	0.28
6	7	90	30	202	-15.3	0.21
7	3	90	70	221	-26.8	0.26
8	7	90	70	230	-15.5	0.23
9	5	60	30	180	-19.7	0.25
10	5	120	30	180	-18.6	0.20
11	5	60	70	192	-19.1	0.19
12	5	120	70	210	-21.4	0.27
13	5	90	50	202	-18.7	0.23
14	5	90	50	209	-19.1	0.24
15	5	90	50	205	-19.4	0.24

*Fixed weight ratio of polysorbate 80: monocaprylin = 1: 1

Table 9 Model analysis, lack of fit test and coefficient of determination (R^2) analysis of particle size, zeta potential, and PDI

Model	Sequential *p*-value	Lack of Fit *p*-value	Adjusted R^2	Predicted R^2	Suggestion
Particle size					
Linear	0.0686	0.0668	0.3155	-0.1435	
Quadratic	0.0012	0.4345	0.9274	0.6979	Suggested
Cubic	0.4345		0.9426		Aliased
Zeta potential					
Linear	< 0.0001	0.0347	0.8829	0.8215	
Quadratic	0.0352	0.0834	0.9643	0.8058	Suggested
Cubic	0.0834		0.9950		Aliased
PDI					
Linear	0.0736	0.0361	0.3060	-0.1607	
Quadratic	0.2321	0.1173	0.8443	0.1716	Suggested
Cubic	0.1173		0.9690		Aliased

R^2 is a statistical measurement 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, which is a modified version of R^2, has been adjusted for the number of predictors in the model. It is more suitable to use to interpret results better than R^2 value. The predicted R^2

indicates how well the model predicts the response for new observations (Minitab Blog, 2013).

For particle size and zeta potential, the quadratic model was found to meet the desired conditions as sequential *p*-value was significantly different (particle size = 0.0012 and zeta potential = 0.0352) and *p*-value of "lack of fit" is insignificant (particle size = 0.4345 and zeta potential = 0.0834). Hence, the quadratic model had enough of the variables to be used to predict the results of both responses correctly. For PDI, sequential *p*-value of all models were 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, only the *p*-value of "lack of fit" of quadratic model was insignificant, therefore, the quadratic model could be used to evaluate the PDI. The results showed that quadratic models were suitable to predict responses of particle size, zeta potential, and PDI with adjusted R^2 equal to 0.9274, 0.9643, and 0.8443, respectively. The predicted R^2 of particle size, zeta potential, and PDI were 0.6979, 0.8058, and 0.1716, respectively. The higher adjusted R^2 and predicted R^2 represent the higher the accuracy of prediction.

ANOVA of the coefficients of particle size (Y_1), zeta potential (Y_2), and PDI (Y_3) with quadratic models is shown in **Tables 10-12**. The *p*-values of models for particle size, zeta potential, and PDI were 0.0019, 0.0003 and 0.0118, respectively, which were statistically significant ($p<0.05$) confirming the adequacy of the quadratic model.

The effect of each factor on each response can be determined by the coefficients of polynomial equation, which is approximately the difference between the average response of the experiments with the positive sign (synergetic effect) and the negative sign (antagonistic effect) (Candioti *et al.*, 2014; Owolabi, Usman, and Kehinde, 2018). The second-order polynomial equation can be written as follows:

$$Y_i = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{33}X_3^2 \quad \textbf{(8)}$$

where X_1, X_2 and X_3 are factors influencing the response of Y_i; 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.

The significance of independent factor affect the response. A smaller *p*-value and larger *F*-value of the coefficients (β) indicates an even more important impact on the response. The factors influencing on the particle size (Y_1) were the linear term of sonication amplitude (X_3) (*p*=0.0005), followed by the quadratic term of surfactant (X_1^2) (*p*=0.0007), the linear term of sonication time (X_2) (*p*=0.0048), the quadratic term of sonication time (X_2^2) (*p*=0.0052) and amplitude (X_3^2) (*p*=0.0344). The quadratic equation of the particle size could be shown below.

$$Y_1 = 205.57 + 2.69X_1 + 6.61X_2 + 10.83X_3 - 0.30X_1X_2 + 2.93X_1X_3 + 4.42X_2X_3 + 14.79X_1^2 - 9.56X_2^2 - 5.83\ X_3^2 \quad \textbf{(9)}$$

Table 10 ANOVA of the coefficients for quadratic model of particle size (Y_1)

Variables	Coefficient	*F*-value	*p*-value
Model		20.88	0.0019*
β_0	205.57		
Linear			
β_1	2.69	3.83	0.1077
β_2	6.61	23.19	0.0048*
β_3	10.83	62.14	0.0005*
Interaction			
β_{12}	-0.30	0.02	0.8833
β_{13}	2.93	2.27	0.1924
β_{23}	4.42	5.19	0.0717
Quadratic			
β_{11}	14.79	53.55	0.0007*
β_{22}	-9.56	22.36	0.0052*
β_{33}	-5.83	8.33	0.0344*

* Significant (*p*-value < 0.05)

In term of the zeta potential (Y_2), the linear term and the quadratic term of surfactant (X_1 and X_1^2) had significant effects (p<0.0001 and p=0.0083, respectively). The equation of the zeta potential was shown below.

$$Y_2 = -19.07 + 6.21X_1 - 0.56X_2 + 0.08X_3 + 1.07X_1X_2 - 0.80X_1X_3 - 0.85X_2X_3 - 2.05X_1^2 + 0.10X_2^2 - 0.73X_3^2 \quad \textbf{(10)}$$

Table 11 ANOVA of the coefficients for quadratic model of zeta potential (Y_2)

Variables	Coefficient	*F*-value	*p*-value
Model		43.00	0.0003*
β_0	-19.07		
Linear			
β_1	6.21	352.94	< 0.0001*
β_2	-0.56	2.89	0.1497
β_3	0.08	0.05	0.8296
Interaction			
β_{12}	1.07	5.28	0.0699
β_{13}	-0.80	2.93	0.1478
β_{23}	-0.85	3.30	0.1288
Quadratic			
β_{11}	-2.05	17.81	0.0083*
β_{22}	0.10	0.039	0.8517
β_{33}	-0.73	2.24	0.1944

* Significant (*p*-value < 0.05)

The factors significantly affected on the PDI (Y_3) of emulsion were the interaction between sonication time and amplitude (X_2X_3) (*p*=0.0022), followed by the linear term of surfactant (X_1) (*p*=0.0034), and the linear term of sonication time (X_2) (*p*=0.0153), respectively. The PDI was calculated with the following equation.

$$Y_3 = 0.2367 - 0.0204X_1 + 0.0141X_2 + 0.003X_3 + 0.0068X_1X_2 + 0.0095X_1X_3 + 0.032X_2X_3 + 0.0028X_1^2 - 0.0127X_2^2 + 0.004X_3^2 \quad \textbf{(11)}$$

Table 12 ANOVA of the coefficients for quadratic model of PDI (Y_3)

Variables	Coefficient	*F*-value	*p*-value
Model		9.44	0.0118*
β_0	0.2367		
Linear			
β_1	-0.0204	27.23	0.0034*
β_2	0.0141	13.08	0.0153*
β_3	0.0030	0.59	0.4770
Interaction			
β_{12}	0.0068	1.49	0.2761
β_{13}	0.0095	2.96	0.1460
β_{23}	0.0320	33.58	0.0022*
Quadratic			
β_{11}	0.0028	0.24	0.6477
β_{22}	-0.0127	4.89	0.0780
β_{33}	0.0040	0.49	0.5133

* Significant (*p*-value < 0.05)

4.1.1.2 Response surface plot

The response surface plot of each response (axis Y) shows the results of two factors under the range of factors used in the experiment (axis X), while another factor is defined at the middle value (zero level) (Hashtjin and Abbasi, 2015). The response surface plot shows the extent of interaction between each factor and makes it easier to visualize the trend of each factor towards the response (Ngan *et al.*, 2014).

4.1.1.2.1 Particle size

The response surface plots of particle size are shown in **Figures 21-23**. The particle size gradually decreased when increasing the concentration of surfactant, but the particle size was increased with the highest surfactant concentration **(Figures 21 and 22)**. This result presented that the smallest particle size required appropriate surfactant concentration. The low surfactant concentration contributes to incomplete surface covering of the oil droplets, which leads to increased particle size. On the other hand, higher surfactant concentration reduces the interfacial tension, which results in the Laplace pressure to decrease and causes disproportionation (Ngan *et al.*, 2014).

In addition, increasing of sonication time resulted in particle size to gradually increase, but the highest sonication time was likely to reduce the particle size. The results of this study were consistent with previous study, which reported that higher sonication time increases force from prolonged periods of operation for ultrasonic waves, allowing the distribution of oil droplets and making up a smaller particle size (Ngan *et al.*, 2014). However, the previous study of Cucheval and Chow (2008) showed that the particle size unchanged when increasing the duration to a certain sonication time.

Furthermore, increasing sonication amplitude increased particle size. It was probably because increasing the amplitude of sonication would increase the ultrasonic wave force, leading to increased rates of droplet coalescence (Hashtjin and Abbasi, 2015).

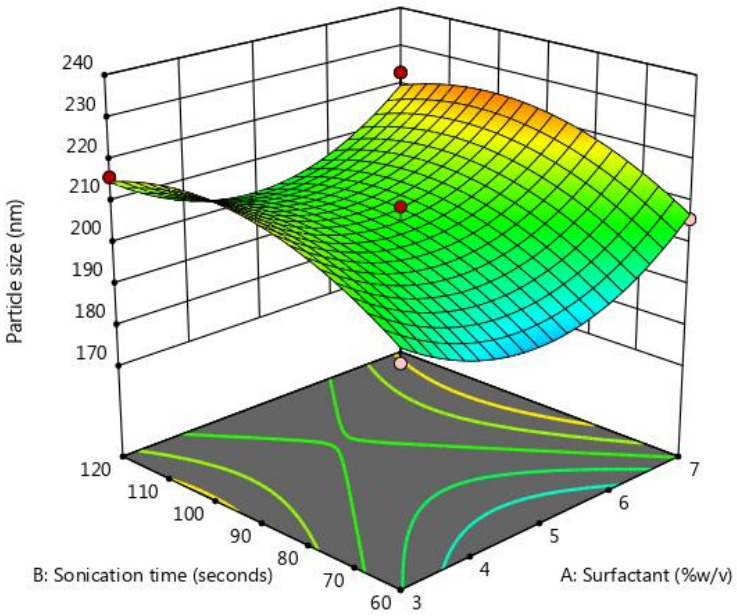


Figure 21 Response surface plot of particle size as a function of surfactant concentration (% w/v) and sonication time (sec) with amplitude of 50%

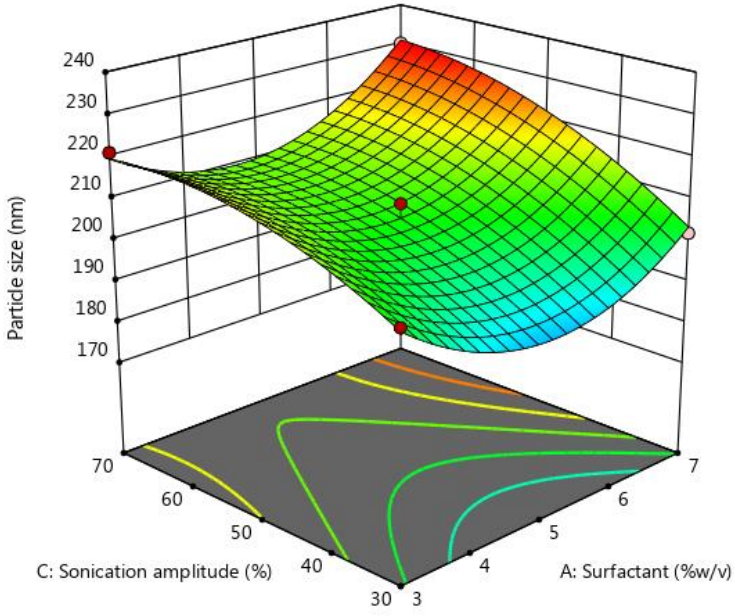


Figure 22 Response surface plot of particle size as a function of surfactant concentration (% w/v) and sonication amplitudes (%) with sonication time of 90 sec

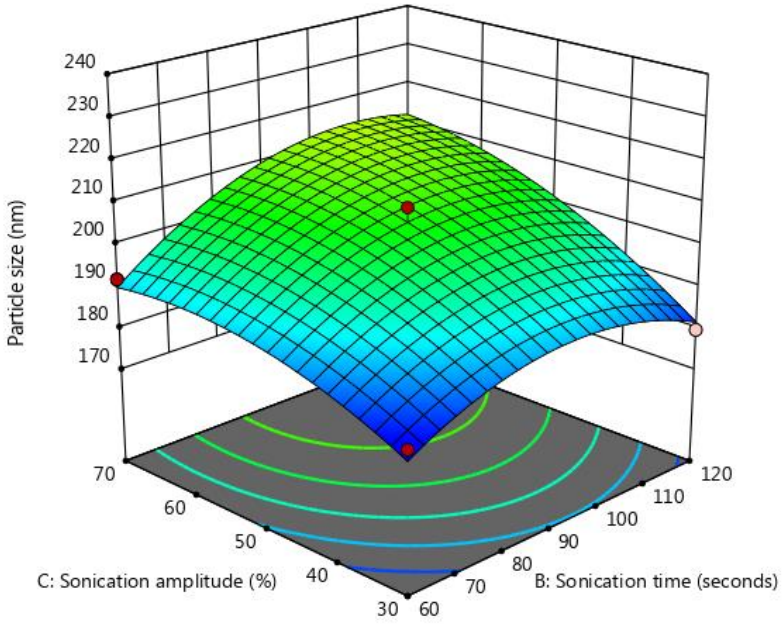


Figure 23 Response surface plot of particle size as a function of sonication time (sec) and amplitudes (%) with surfactant concentration of 5% w/v

4.1.1.2.2 Zeta potential

The response surface plots of zeta potential are shown in **Figures 24-26**. The results represented that the zeta potential was highest (more negative charge) at lowest surfactant concentration (3% w/v) **(Figures 24 and 25)**. The sonication time and amplitude did not affect the change in zeta potential as shown in **Figure 26**. The zeta potential is the charge that appears on the surface of oil droplets. The high negative or positive charges can resist aggregation and flocculation resulting in the sample more stable (Malvern, 2018). Caprylic acid has negative charges from the carboxyl group in the structure. The oil droplets were coated with molecules of non-ionic surfactants (polysorbate 80 and monocaprylin). The hydrophobic part of the

surfactants should strongly adsorb on the hydrophobic caprylic acid surface whereas the hydrophilic part of the surfactants provide steric stabilization by extending into aqueous phase. Therefore, increasing the concentration of surfactants might reduce the zeta potential by the formation of micelle layer (Sis and Birinci, 2009).

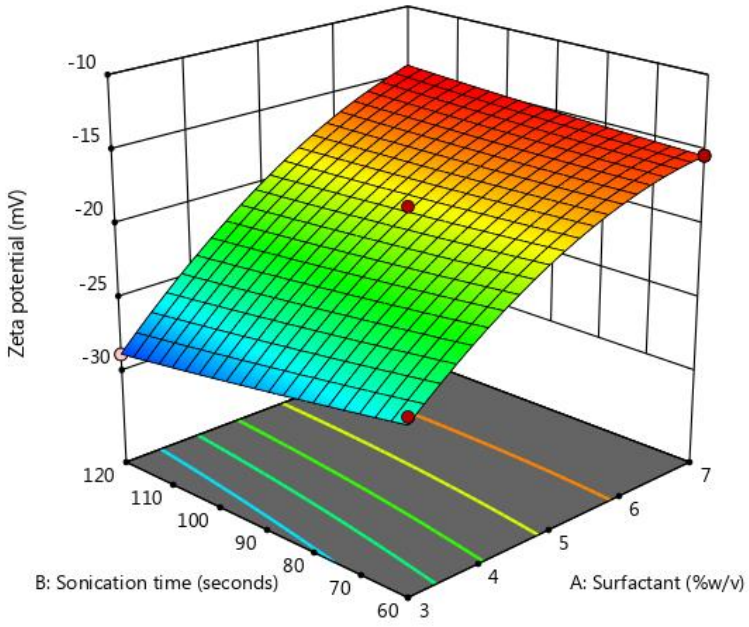


Figure 24 Response surface plot of zeta potential as a function of surfactant concentration (% w/v) and sonication time (sec) with amplitude of 50%

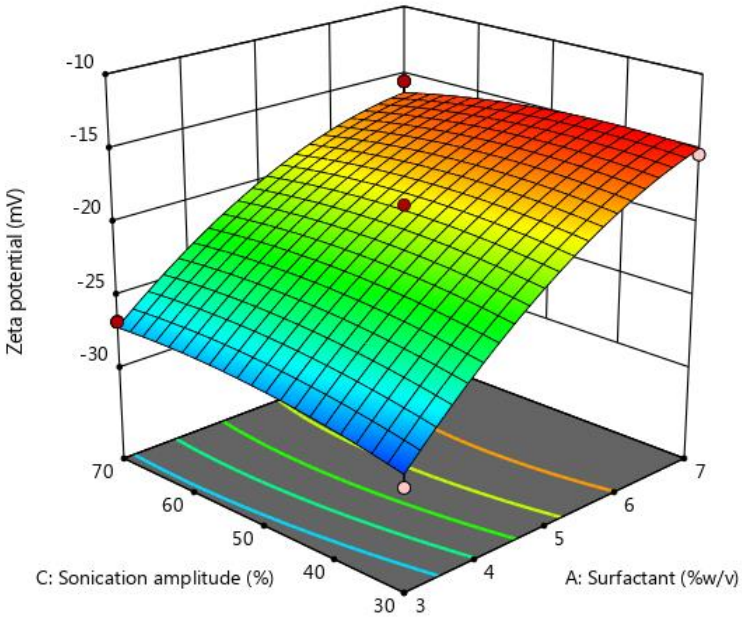


Figure 25 Response surface plot of zeta potential as a function of surfactant concentration (% w/v) and sonication amplitudes (%) with sonication time of 90 sec

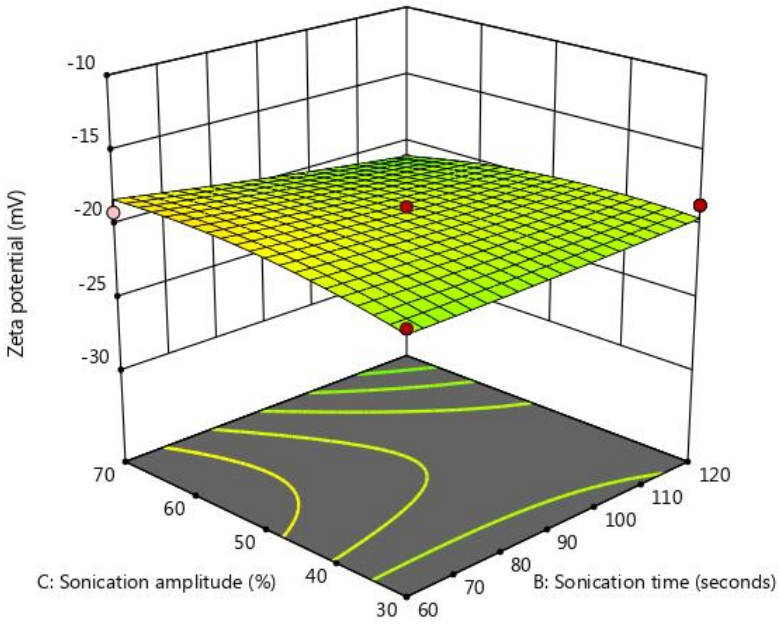


Figure 26 Response surface plot of zeta potential as a function of sonication time (sec) and amplitudes (%) with surfactant concentration of 5% w/v

4.1.1.2.3 Polydispersity index (PDI)

The response surface plots of PDI are shown in **Figures 27-29**. The results appeared that increasing the surfactant concentration, sonication time, and sonication amplitude led to a slight decrease of PDI values. PDI is a measure of the heterogeneity of the particle sizes in the emulsion. Less PDI values indicate that the particles are more similar in size, which is monodisperse (Urbina-Villalba *et al.*, 2004). If PDI values are greater than 0.7, the sample is polydisperse (Malvern, 2011). In this study, PDI values of emulsion were in the range of 0.19-0.28 indicating the monodispersity oil droplet.

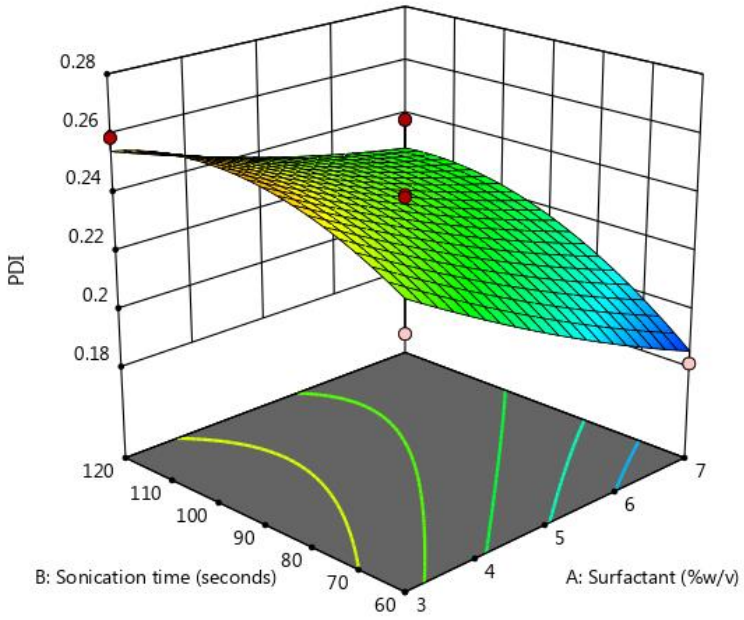


Figure 27 Response surface plot of PDI as a function of surfactant concentration (% w/v) and sonication time (sec) with amplitude of 50%

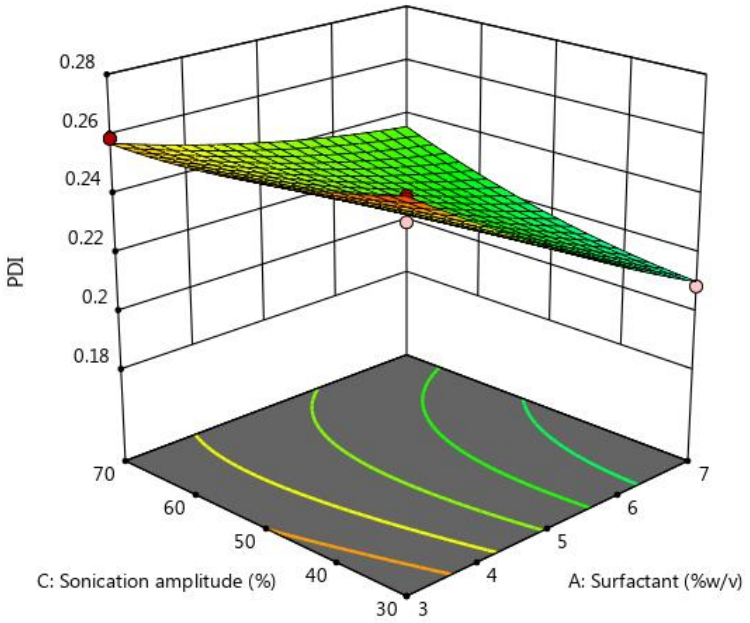


Figure 28 Response surface plot of PDI as a function of surfactant concentration (% w/v) and sonication amplitudes (%) with sonication time of 90 sec

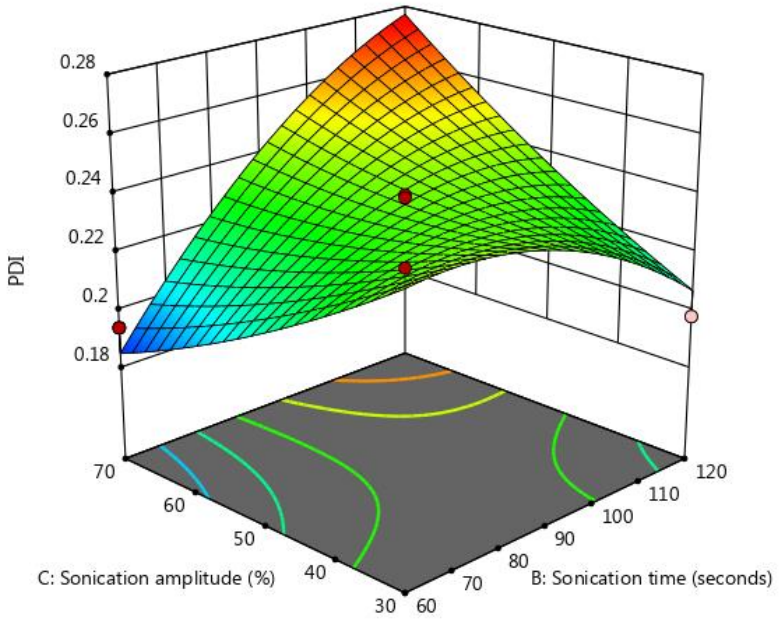


Figure 29 Response surface plot of PDI as a function of sonication time (sec) and amplitudes (%) with surfactant concentration of 5% w/v

4.1.1.3 Preparation of optimized emulsion

The selection of the optimized emulsion preparation (surfactant concentration, sonication time and amplitude) was set on the responses which were smallest particle size, highest zeta potential (negative) and lowest PDI because this responses tended to produce a stable emulsion. The optimal value of factors was determined by desirability value (D) calculated from the program, which 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 0.69 was obtained and the optimized values of surfactant concentration, sonication time and amplitude were 3.6% w/v, 120 sec, and 30 %, respectively.

The optimized formula was experimentally prepared to test the adequacy of the anticipated responses as shown in **Table 13**. Values of particle size, zeta potential, and PDI from the experiment were not significantly different (p>0.05) from the predicted values obtained from the program. Excellent consistency between experimental results and predictive results showed that the quadratic model was accurate and suitable for predicting of particle size, zeta potential, and PDI of emulsion

Table 13 The predicted and experimental values of 4% w/v caprylic acid emulsion

Factor	Values	Responses	Predicted values	Experimental values
Surfactant (X_1)	3.6 %w/v	Particle size (Y_1)	189 ± 4 nm	185 ± 1 nm
Sonication time (X_2)	120 sec	Zeta potential (Y_2)	-26.2 ± 0.9 mV	-25.5 ± 0.8 mV
Amplitude (X_3)	30 %	PDI (Y_3)	0.22 ± 0.01	0.23 ± 0.01

Fixed ratio surfactant = polysorbate 80: monocaprylin = 1: 1

4.1.2 Stability of emulsion

The stability of caprylic acid emulsions stored at controlled conditions of 30 ± 2 ℃ / 75 ± 5% RH and 40 ± 2 ℃ / 75 ± 5% RH for 28 days was evaluated. Overall results of the appearance, particle size, zeta potential, and PDI of emulsions are shown in **Appendix A**. The physical appearance of 15 emulsion formulas after 28-day storage did not appear signs of change or instability. However, the data based on particle size indicated that particle size of emulsions containing 7% w/v surfactant gradually increased after 14 days of storage at both temperatures indicating that the emulsion became unstable and could be separated later. In addition, the optimized emulsion formula (4% w/v caprylic acid, 1.8% w/v polysorbate 80, and 1.8% w/v monocaprylin prepared with sonication time of 120 sec and 30% amplitude) was found to be stable throughout the storage for 28 days on both temperatures **(Table 14 and Figure 30)**. Furthermore, the optimized emulsion should be further studied the antimicrobial activity of the emulsion after storage.

For determining the expiration date of the product, the proposed shelf life should be based on the stability studies, which cover the physical, chemical, biological, microbiological, and biopharmaceutical quality characteristics of the dosage form to determine a tentative shelf-life. WHO (1996) has suggested a tentative shelf-life of 24 months in products after storage test for 6 months in an accelerated state (40 ± 2 ℃/ 75 ± 5% RH) when the stability studies showed no significant changes in the active ingredient and the specifications for appearance and physical properties. After that,

the manufacturer will conduct real-time studies until covering the proposed shelf-life. Therefore, the data of this study may not be enough for shelf-life determination of the optimized emulsion formula.

Table 14 The particle size, zeta potential, and PDI of the optimized emulsion formula

Temperature and humidity	Day	Particle size (nm)	Zeta potential (mV)	PDI
30 ± 2 ℃ /	0	185 ± 1	-25.5 ± 0.8	0.23 ± 0.01
75 ± 5% RH	7	202 ± 2	-22.9 ± 1.0	0.24 ± 0.00
	14	197 ± 4	-21.4 ± 0.6	0.23 ± 0.01
	21	200 ± 1	-20.7 ± 0.2	0.23 ± 0.01
	28	202 ± 2	-20.2 ± 0.1	0.23 ± 0.00
40 ± 2 ℃ /	0	185 ± 1	-25.5 ± 0.8	0.23 ± 0.01
75 ± 5% RH	7	205 ± 6	-24.6 ± 0.9	0.24 ± 0.01
	14	196 ± 1	-23.1 ± 0.9	0.24 ± 0.01
	21	197 ± 1	-23.6 ± 0.4	0.19 ± 0.02
	28	193 ± 3	-21.7 ± 0.8	0.22 ± 0.00

(A)  (B)  (C) 

Figure 30 Physical appearance of the optimized emulsion formula
(A) day 0 (B) 30 ± 2 ℃ / 75 ± 5% RH at day 28 and (C) 40 ± 2 ℃ / 75 ± 5% RH at day 28

4.2 Antimicrobial susceptibility test

The microbial concentration was adjusted to 0.5 McFarland standard turbidity, as shown in **Table 15**. The results show that the tested microorganisms were in the concentration range according to 0.5 McFarland standard turbidity.

Table 15 Concentration of microorganisms by plate count method (0.5 McFarland)

Microorganisms	Concentration
Staphylococcus aureus ATCC 25923	1.04 - 1.36 x 10^8 CFUs/ml
Bacillus cereus ATCC 11778	0.65 - 0.81 x 10^8 CFUs/ml
Escherichia coli ATCC 25922	0.65 - 0.79 x 10^8 CFUs/ml
Pseudomonas aeruginosa ATCC 27853	0.95 - 1.17 x 10^8 CFUs/ml
Candida albicans ATCC 10231	3.10 - 3.80 x 10^6 CFUs/ml
Aspergillus niger ATCC 16404	1.90 - 2.20 x 10^7 spores/ml

The antimicrobial activity of the optimized emulsion (4% w/v caprylic acid, 1.8% w/v polysorbate 80 and 1.8% w/v monocaprylin) prepared with sonication time of 120 sec and 30% amplitude, was determined from MIC using broth microdilution method and MBC/MFC using a plate method. The other formulations were also prepared in the same way for comparison, which were caprylic acid P-80 emulsion (4% w/v caprylic acid and 1.8% w/v polysorbate 80), 1.8% w/v monocaprylin and 1.8% w/v polysorbate 80 solutions. The results showed that polysorbate 80 had no antimicrobial activity. The MIC, MBC and MFC results of optimized emulsion, caprylic acid P-80 emulsion, monocaprylin solution are presented in **Table 16** and **Appendix B**. The least MIC and MFC were against *C. albicans* and highest MIC and MBC were against *P.*

aeruginosa. The result might be due to the different structure of the microbial cell wall. *C. albicans* is unicellular fungi (Mehak, 2019) while *P. aeruginosa* is a gram-negative bacteria, which has a complex cell wall with a barrier antimicrobial permeability (Hayat, 2013). Therefore, the treatment of *P. aeruginosa* requires a higher concentration than other microorganisms.

Table 16 MIC, MBC and MFC (mg/ml) of caprylic acid in emulsions and monocaprylin

Sample	Optimized emulsion		Caprylic acid P-80 emulsion		Monocaprylin solution	
Microorganisms	MIC (mg/ml)	MBC/MFC (mg/ml)	MIC (mg/ml)	MBC/MFC (mg/ml)	MIC (mg/ml)	MBC/MFC (mg/ml)
S. aureus	0.63	1.25	0.63	1.25	1.13	2.25
B. cereus	0.63	1.25	1.25	1.25	2.25	2.25
E. coli	0.63	1.25	1.25	1.25	2.25	2.25
P. aeruginosa	1.25	2.50	1.25	2.50	>9	>9
C. albicans	0.08	0.16	0.08	0.16	0.14	2.25
A. niger	0.63	1.25	0.63	1.25	1.13	>9

The MIC results of caprylic acid was previously studied. For example, the MIC against *S. aureus* was 1.4-2.9 mg/ml (Nobmann *et al.*, 2010). *B. cereus* was greater than 0.5 mg/ml (Batovska *et al.*, 2009). *E. coli* was 2 mg/ml (Skrivanova *et al.*, 2006). *P. aeruginosa* was 1.4-22.4 mg/ml (Nakai and Siebert, 2004). *C. albicans* was 0.6 mg/ml (Souza *et al.*, 2014) and *A. niger* was 0.5 mg/ml (Huang *et al.*, 2010). The current results were lower than previous studies, which might be due to different experimental conditions. However, the results showed that caprylic acid had antimicrobial activity in

the same way as previous studies. Moreover, Marounek *et al.* (2012) had studied the antimicrobial activity of caprylic acid compared with sorbic acid, which is a natural organic compound used as a food preservative. The results showed that caprylic acid had more antimicrobial activity against *Cronobacter sakazakii* and *Cronobacter malonaticus* than sorbic acid. Therefore, caprylic acid may be interesting to use as an alternative to sorbic acid in food preservation.

In addition, monocaprylin also has antimicrobial activity. In this study, MIC of monocaprylin against *S. aureus* and *B. cereus* were 1.13 and 2.25 mg/ml, respectively, which were similar to the previous studies (>0.5 mg/ml) (Batovska *et al.*, 2009; Nobmann *et al.*, 2010). Interestingly, MIC of the optimized emulsion was lower than caprylic acid P-80 emulsion in *B. cereus* and *E. coli*. This might be the synergistic effect from the antimicrobial activity of monocaprylin and caprylic acid in form of emulsion. It was noted that the caprylic acid P-80 emulsion was unstable (after storage for 7 days). Hence, monocaprylin was necessary for preparation of caprylic acid emulsion.

Furthermore, broth microdilution method in this study use MTT assay. This dye has limitations that may result in errors if the test substance has a phenolic group in the structure. Phenolic compounds prevent against mitochondria injury and increase the succinate dehydrogenase activity. This may result in reduced cell injury and increased formazan production (Wang, Henning, and Heber, 2010). At present, several colorimetric methods is preferable to use other types of dyes such as resazurin (Balouiri *et al.*, 2016).

4.3 Coating of food model using emulsion-based formulations

From the antimicrobial test **(Table 16)** the optimized emulsion diluted to contain 0.25% w/v (2.5 mg/ml) caprylic acid in food coating formulation was selected. This concentration was the highest MBC/MFC against all six strains used in the test. Food coating formula was freshly prepared by mixing 4% w/v caprylic acid emulsion into the mixture of 2% w/v alginate and 1.5% w/v glycerol solution at a volume ratio of 1: 16.

The food sample for coating test were selected fresh-cut fruit, which has a short shelf life because peeling and cutting increase metabolic activities and lead to quality deterioration such as softening, browning, and microbial growth (Azarakhsh *et al.*, 2014). In this study, food sample used fresh-cut pitahaya for preliminary study in food coating. Food samples (fresh-cut pitahaya) were dipped in emulsion-based coating solution and then dipped in 2% w/v calcium chloride solution to perform film coating. Samples were kept in a refrigerator at 4 to 8°C and were studied at 0, 3, 7 and 12 days after storage. The results of samples coated with emulsions were compared with uncoated samples (dipped in sterile water) and samples coated with alginate solution (without emulsion added).

Notably, for food coating, alginate and glycerol were not added as components in the emulsion preparation due to the incompatibility (data shown in **Appendix C**). The emulsion prepared by adding alginate as an ingredient were separated after 7 days storage at 30 ± 2 °C / 75 ± 5% RH. Since alginate is a water-soluble polysaccharide, it

may interfere with the HLB of the system causing the phase separation of the emulsion. In addition, the structure of alginate contains negative charges from carboxyl group (Tavassoli-Kafrani *et al.*, 2016) while caprylic acid contain negative charges from carboxyl group (Milne, 2005). Therefore, the instability of formula may be due to the repulsion of the same negative charge resulting in the phase separation at last. Furthermore, glycerol has 3 hydroxyl groups (-OH), which cause hydroscopic properties and dissolve well in water (Gooch, 2011). It may affect the emulsion system, resulting in phase separation. Therefore, food coating was freshly prepared by adding the emulsion into a mixture of glycerol and alginate solution (Miladi *et al.*, 2013).

4.3.1 Physical properties

4.3.1.1 Appearance

The physical appearance of coated pitahaya after storage in the refrigerator is shown in **Table 17**. The sample gradually became darker and changed to red obviously on day 12, especially in the control group.

Table 17 Characteristics of coated pitahaya stored in a refrigerator (4-8 °C)

Sample	Day 0	Day 3	Day 7	Day 12
Control (uncoated)				
Alginate coated				
Emulsion coated				

Control = uncoated pitahaya, **Alginate** = coated pitahaya with 2% w/v alginate/1.5% w/v glycerol solution and 2% w/v calcium chloride solution, **Emulsion** = coated pitahaya with 0.25% w/v caprylic acid in 2% w/v alginate/1.5% w/v glycerol solution and 2% w/v calcium chloride solution

4.3.1.2 Color

The whiteness index (WI) of coating solutions is shown in **Figure 31** and **Appendix D**. The whiteness index is a value that indicates the transparency of the solution. If this value is less, the substance is more transparent. The transparent coating affects the color of the food less than the opaque coating (Acevedo-Fani *et al.*, 2015). The WI values of water, optimized emulsion (4% w/v caprylic acid), a mixture of alginate and glycerol were 3.82, 99.81, and 35.71 indicating the degree of transparency of the samples. For emulsion mixed with alginate-glycerol solution, its WI value was 86.76 indicating the rather opaque in color.

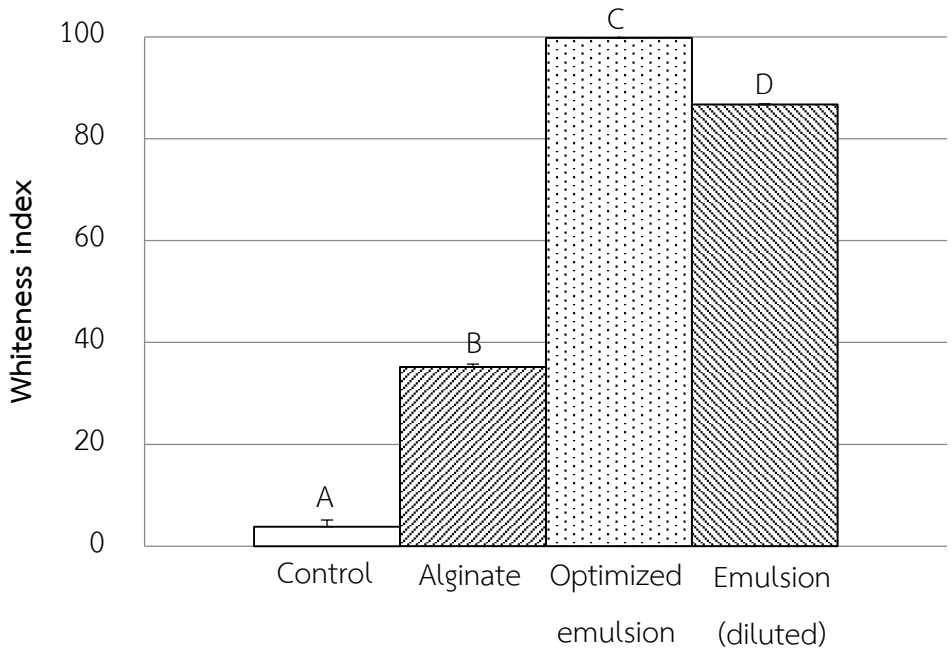


Figure 31 Whiteness index (WI) of the test samples

Control = ultrapure water, **Alginate** = 2% w/v alginate and 1.5% w/v glycerol solution, **Optimized emulsion** = 4% w/v caprylic acid, 1.8% w/v polysorbate and 1.8% w/v monocaprylin emulsion**, Emulsion (diluted)** = 0.25% w/v caprylic acid emulsion-based coating solution by mixing the optimized emulsion in 2% w/v alginate and 1.5% w/v glycerol solution (volume ratio 1:16). Means with the different letters are significantly different by Bonferroni test ($p < 0.05$)

The color changes of coated pitahaya after storage in refrigerator for 12 days are displayed in **Figures 32-35** and **Appendix D**. Color change is one of the major changes that directly affects customer acceptance (Olivas and Barbosa-Canovas, 2005). During the period of storage, lightness (*L**) and hue angle (*h**) of all groups were significantly reduced, especially on day 7. A decrease in *L** represents a darker sample. The reduction of *h** presents the color of the sample that changes red. The appearance of low *L** and *h** made the food unappetizing. The overall color intensities, chroma

(*C**), were not significantly different (p>0.05) when compared among three groups. For the color difference (*ΔE**), the values from samples coated with different coatings were not significantly different (p>0.05) when compared at the same storage time. The *ΔE** of pitahaya coated with emulsion had a significant change (p=0.04) after day 3 and day 7. However, the *ΔE** values of coated sample were less than those of the uncoated sample, as shown in **Figure 35**. Hence, the coated food might be better than uncoated food.

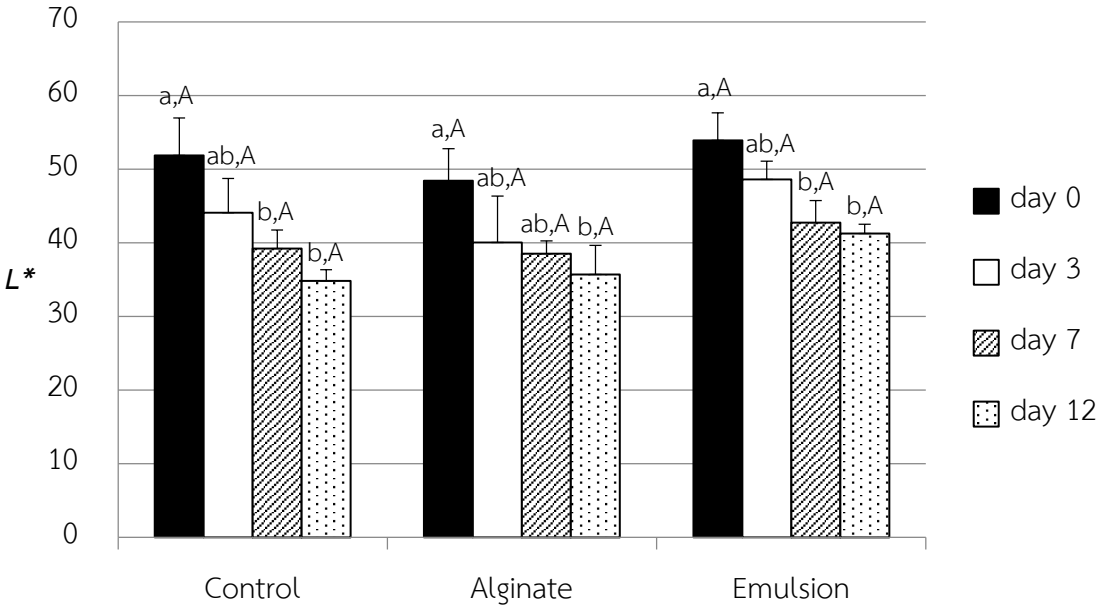


Figure 32 Lightness (*L**) of coated pitahaya with different coatings after different storage times compared to control (uncoated)

Control = uncoated pitahaya, **Alginate** = coated pitahaya with 2% w/v alginate/1.5% w/v glycerol solution and 2% w/v calcium chloride solution, **Emulsion** = coated pitahaya with 0.25% w/v caprylic acid in 2% w/v alginate/1.5% w/v glycerol solution and 2% w/v calcium chloride solution. 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).

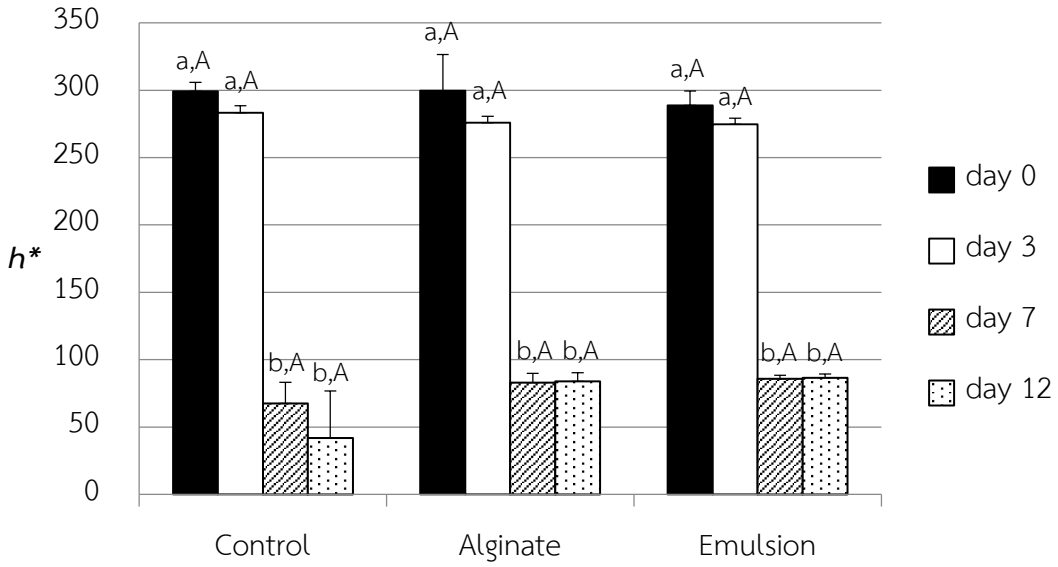


Figure 33 Hue angle (*h**) of coated pitahaya with different coatings after different storage times compared to control (uncoated)

Control = uncoated pitahaya, **Alginate** = coated pitahaya with 2% w/v alginate/1.5% w/v glycerol solution and 2% w/v calcium chloride solution, **Emulsion** = coated pitahaya with 0.25% w/v caprylic acid in 2% w/v alginate/1.5% w/v glycerol solution and 2% w/v calcium chloride solution. 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 ($p < 0.05$).

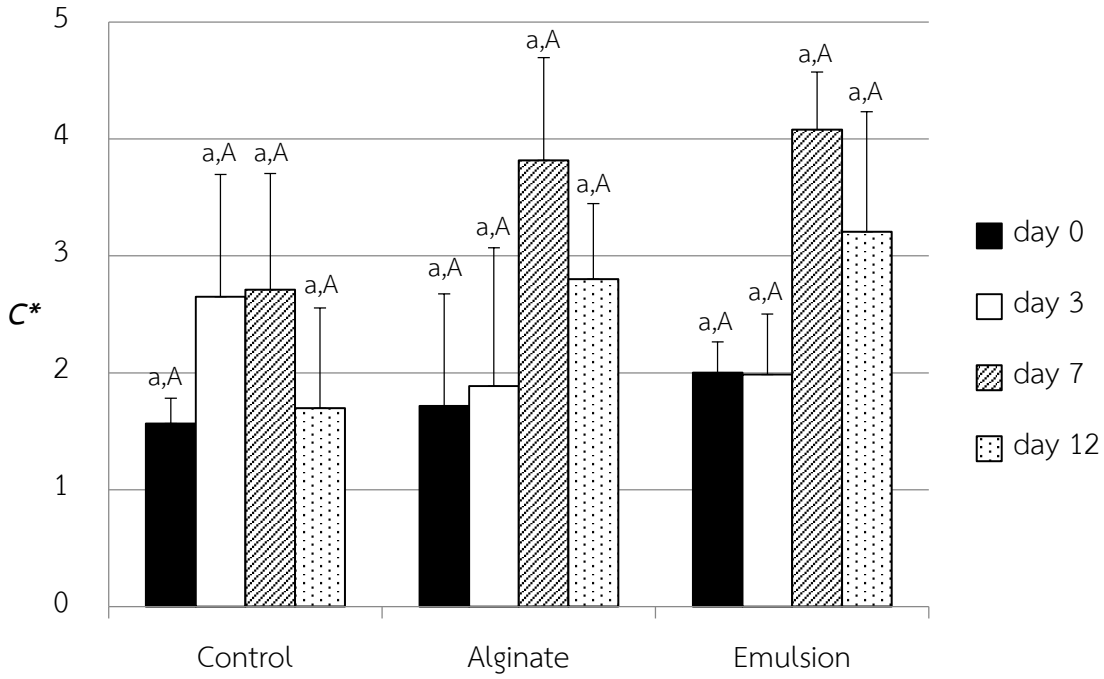


Figure 34 Chroma (*C**) of coated pitahaya with different coatings after different storage times compared to control (uncoated)

Control = uncoated pitahaya, **Alginate** = coated pitahaya with 2% w/v alginate/1.5% w/v glycerol solution and 2% w/v calcium chloride solution, **Emulsion** = coated pitahaya with 0.25% w/v caprylic acid in 2% w/v alginate/1.5% w/v glycerol solution and 2% w/v calcium chloride solution. 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 ($p < 0.05$).

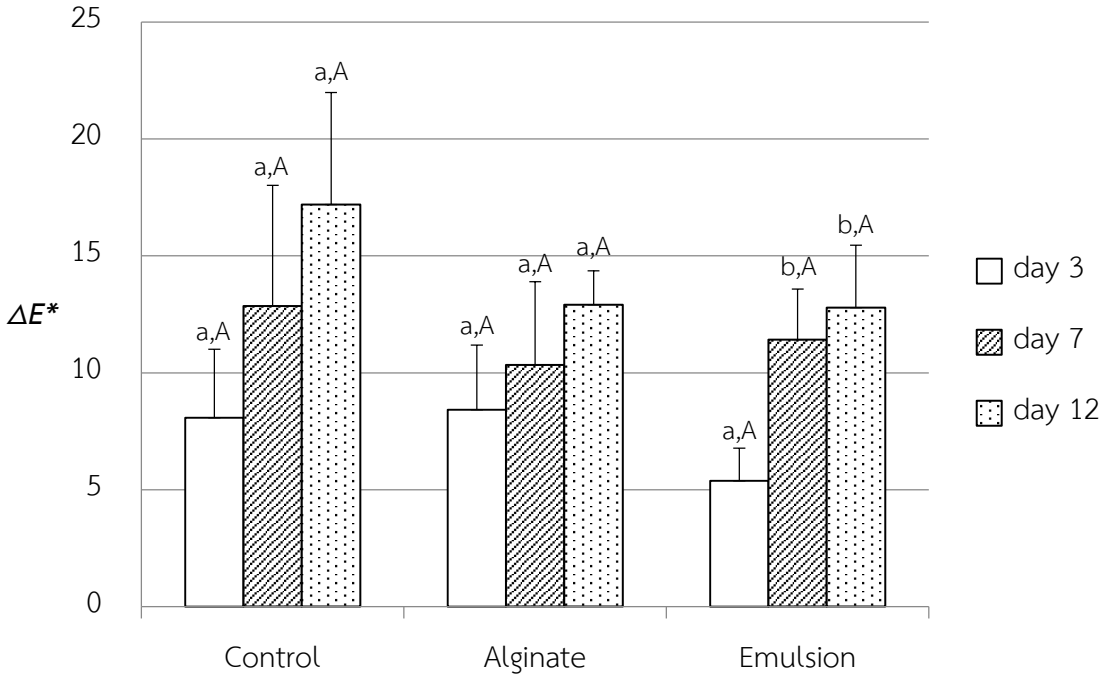


Figure 35 The color difference (*ΔE**) of coated pitahaya with different coatings after different storage times compared to control (uncoated)

Control = uncoated pitahaya, **Alginate** = coated pitahaya with 2% w/v alginate/1.5% w/v glycerol solution and 2% w/v calcium chloride solution, **Emulsion** = coated pitahaya with 0.25% w/v caprylic acid in 2% w/v alginate/1.5% w/v glycerol solution and 2% w/v calcium chloride solution. 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 ($p < 0.05$).

4.3.1.3 Weight loss

Food coating can prevent loss of moisture in fresh fruits and vegetables (Galus and Kadzińska, 2015). The results of weight loss are shown in **Figure 36** and **Appendix E**. The weight of all sample groups gradually decreased following the storage periods. When comparing between groups on the same day, the coated samples had

insignificantly less weight loss (p>0.05) than the uncoated control group. Food coatings with emulsion or alginate might slow down the loss of moisture as seen from previous studies using hydrocolloid coating (Azarakhsh *et al.*, 2014; Wang *et al.*, 2018).

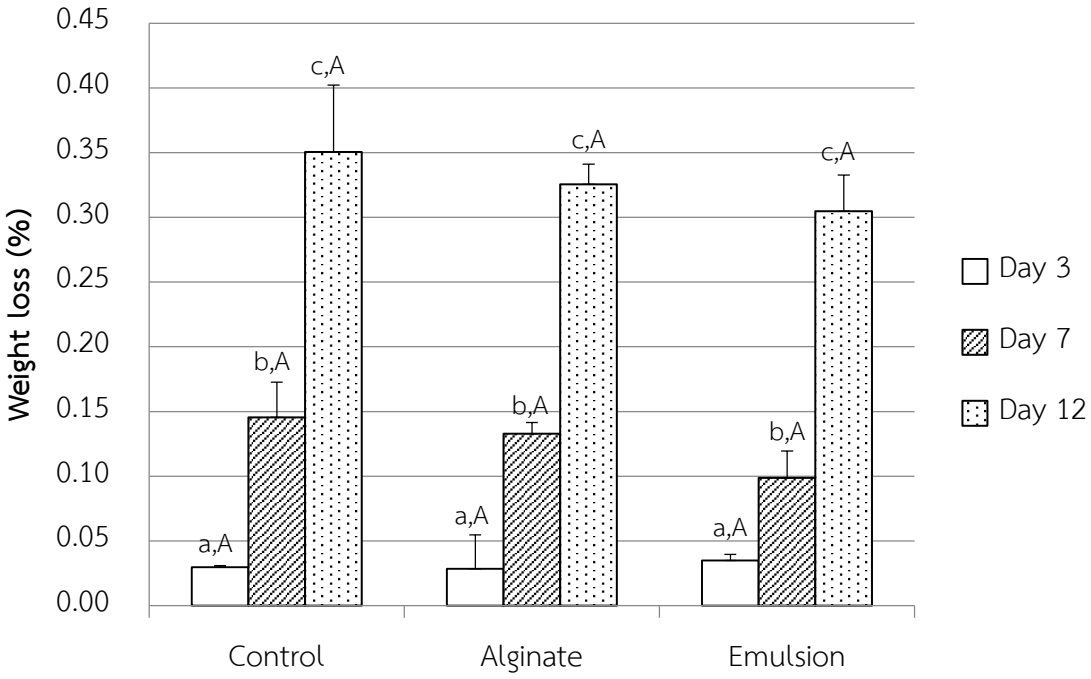


Figure 36 Weight loss (%) of coated pitahaya with different coatings after different storage times compared to control (uncoated)

Control = uncoated pitahaya, **Alginate** = coated pitahaya with 2% w/v alginate/1.5% w/v glycerol solution and 2% w/v calcium chloride solution, **Emulsion** = coated pitahaya with 0.25% w/v caprylic acid in 2% w/v alginate/1.5% w/v glycerol solution and 2% w/v calcium chloride solution. 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 ($p< 0.05$).

4.3.1.4 Firmness

A compression test was used to observe changes in firmness of the coated pitahaya. The results of firmness are presented in **Figure 37** and **Appendix F**. The uncoated sample had the lowest firmness. In contrast, the alginate-coated sample had the highest firmness, which was significantly different compared to the control group at day 0, 3 and 12 (p = 0.01, 0.01 and 0.06, respectively). Alginate can increase maximum stress because it can produce a strong film from adding calcium ions (Tavassoli-Kafrani *et al.*, 2016). For emulsion coating, the firmness was in between the control group and alginate group. Due to the oil droplets inserted in alginate, the coating film had lower strength and decreased firmness of the sample. This study had the same findings of Azarakhsh and coworkers (2014), who studied the effect of lemongrass essential oil in alginate coating on the firmness of fresh-cut pineapple. However, the sample coated with emulsion had no statistical difference (p>0.05) in firmness when compared to the control group. This result showed that coating with emulsion does not produce the texture of the food different from uncoated group.

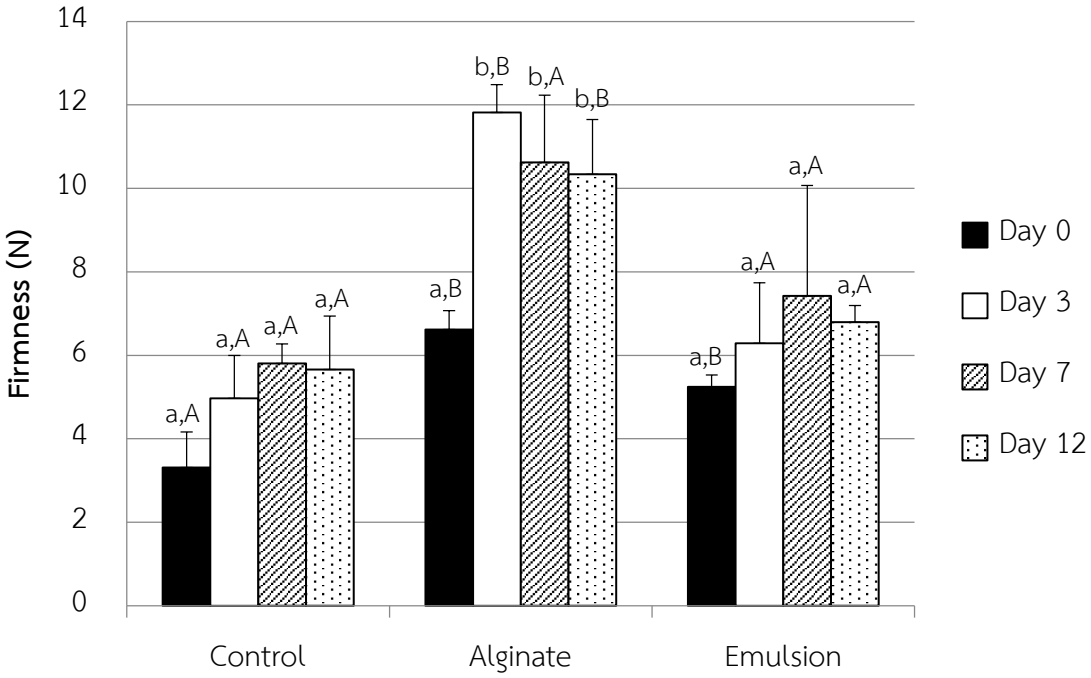


Figure 37 Firmness (N) of coated pitahaya with different coatings after different storage times compared to control (uncoated)

Control = uncoated pitahaya, **Alginate** = coated pitahaya with 2% w/v alginate/1.5% w/v glycerol solution and 2% w/v calcium chloride solution, **Emulsion** = coated pitahaya with 0.25% w/v caprylic acid in 2% w/v alginate/1.5% w/v glycerol solution and 2% w/v calcium chloride solution. 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 ($p < 0.05$).

4.3.2 Microbial determination

Fresh-cut fruit has a large cutting surface area which provides a good environment for microbial growth (Azarakhsh *et al.*, 2014). In this study, the emulsion coating group had significant reduction ($p<0.0001$) in microbial amount compared to

the alginate coating and control groups at day 12 of storage as shown in **Figures 38-40** and **Appendix G**.

Caprylic acid was classified as food additives at 0.001% w/w or less for all other food categories (FDA, 2017). However, the use of substances in a food contact article such as food-packaging, food coating or food-processing equipment are excluded in a regulation for use as food additives (FDA, 2018). Due to the LD_{50} of caprylic acid in rat was 1,283 mg/kg (Alfa Aesar, 2012), the concentration of 0.25% w/v caprylic acid (2.5 mg/ml) used for food coatings should not result in toxicity.

The potent antimicrobial activity might come from caprylic acid. For previous studies on the antimicrobial activity of caprylic acid in food, the breaded chicken products with caprylic acid (0.5% v/w) significantly reduced the growth of mesophilic bacteria after grinding, baking and frozen storage compared to products without caprylic acid (Moschonas *et al.*, 2012). In addition, Wang *et al.* (2018) found that chitosan-carvacrol coating with caprylic acid reduced the amount of mesophilic bacteria in Pacific white shrimp. Moreover, Hulankova *et al.* (2013) reported that minced beef contained 0.5% v/w caprylic acid had less psychrophilic bacteria growth than the control group after 10 days storage. Since the previous studies have never been done on using film coating containing caprylic acid in fruits, therefore, this study is the first investigation for the film coatings in fruits. The obtained information may be a guideline for further development of the film coating in other fruits.

According to the announcement of the Department of Medical Sciences on microbiological quality criteria of food and food contact containers No. 3, microbial analysis method is used according to bacteriological analytical manual of FDA (กรมวิทยาศาสตร์การแพทย์, 2557). The amount of microorganisms in cut fruit and vegetables should be less than 1 x 10^6 CFUs/g. Yeast content should be less than 1,000 CFUs/g and mold content should be less than 500 CFUs/g (กรมวิทยาศาสตร์การแพทย์, 2560). The emulsion coated-pitahaya contained microorganisms, yeast and mold less than the regulations after storage in the refrigerator for 12 days. For control and alginate coated groups, the growth of microorganism was extensively found after storage. This result showed that emulsion-based coating at 0.25% w/v caprylic acid could control microbial growth in fresh-cut pitahaya.

In addition, formulation development for business realization should take into account the changing taste after coating and production costs. In this study, food sample used pitahaya, which is a common fresh-cut fruit found in several food such as salad. However, pitahaya is an inexpensive fruit. The coating of food before eating to preserve the shelf life may not be worth the production cost. Therefore, implementation should be done in high value export fruits such as mango, durian, and grapes. Especially, further studies on sensory evaluation should be conducted to determine consumer satisfaction before developing the formula.

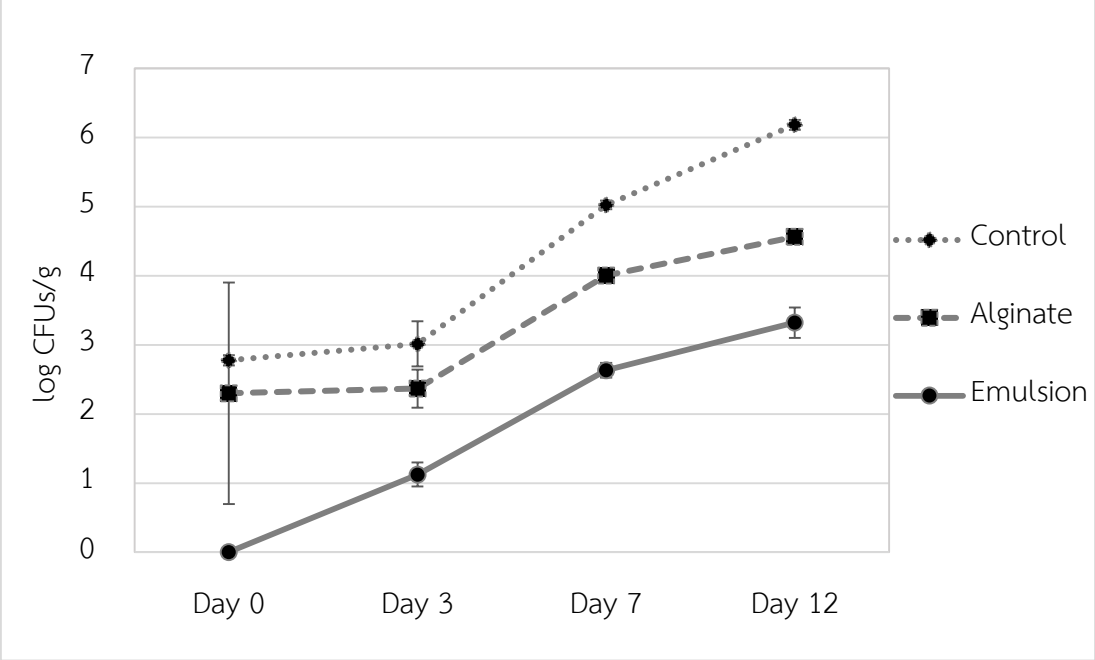


Figure 38 Mesophilic bacteria of coated pitahaya with different coatings after different storage times compared to control (uncoated)

Control = uncoated pitahaya, **Alginate** = coated pitahaya with 2% w/v alginate/1.5% w/v glycerol solution and 2% w/v calcium chloride solution, **Emulsion** = coated pitahaya with 0.25% w/v caprylic acid in 2% w/v alginate/1.5% w/v glycerol solution and 2% w/v calcium chloride solution

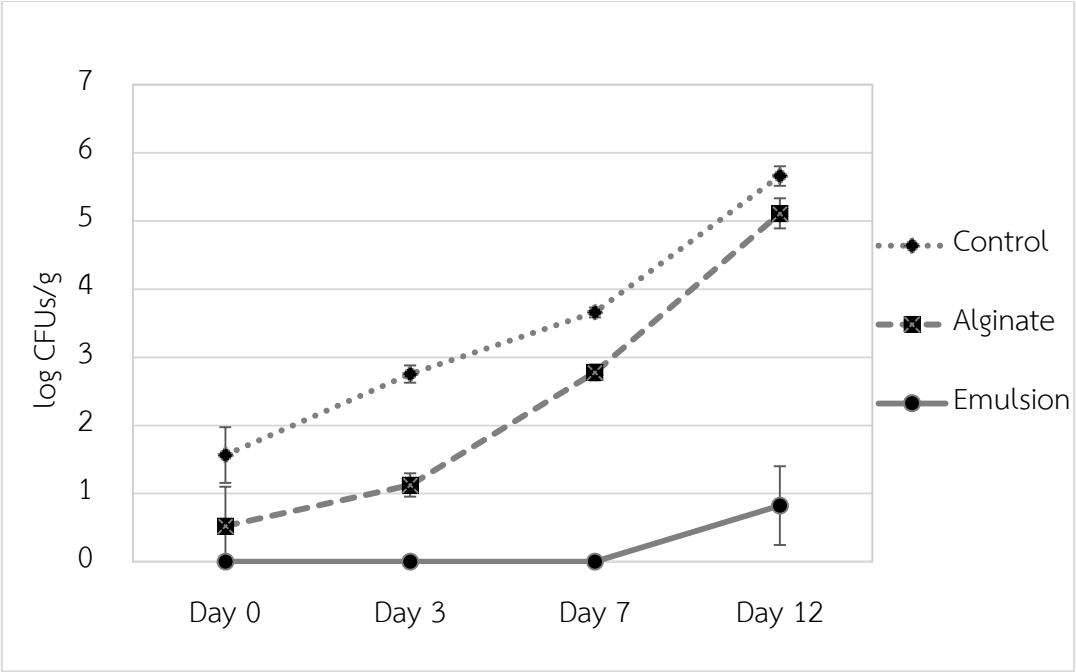


Figure 39 Psychrophilic bacteria of coated pitahaya with different coatings after different storage times compared to control (uncoated)

Control = uncoated pitahaya, **Alginate** = coated pitahaya with 2% w/v alginate/1.5% w/v glycerol solution and 2% w/v calcium chloride solution, **Emulsion** = coated pitahaya with 0.25% w/v caprylic acid in 2% w/v alginate/1.5% w/v glycerol solution and 2% w/v calcium chloride solution

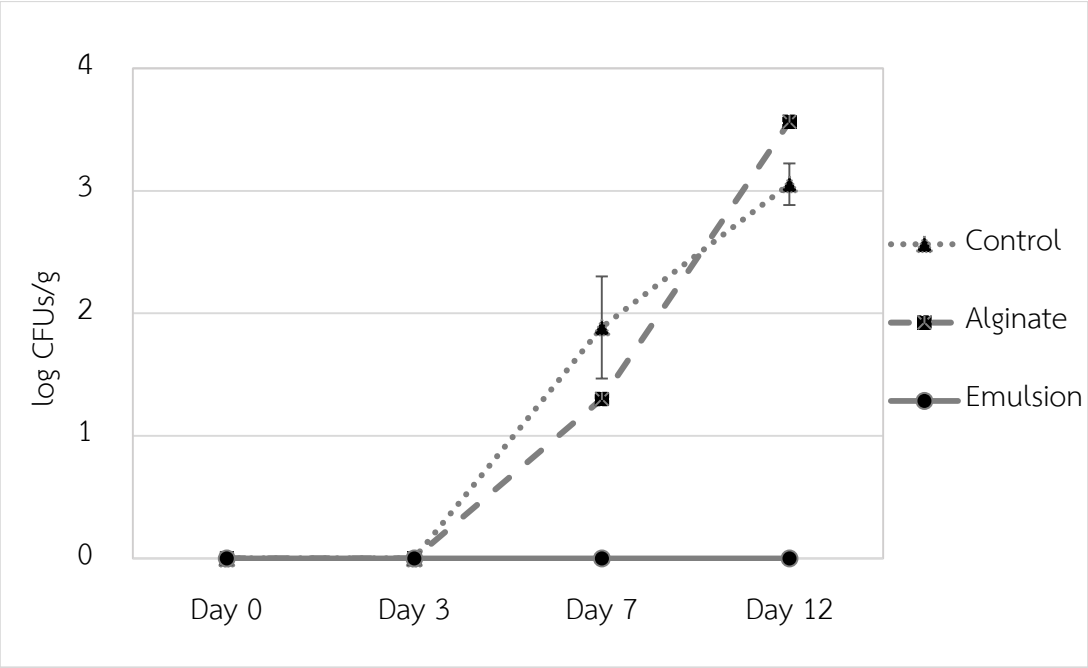


Figure 40 Yeasts and molds of coated pitahaya with different coatings after different storage times compared to control (uncoated)

Control = uncoated pitahaya, **Alginate** = coated pitahaya with 2% w/v alginate/1.5% w/v glycerol solution and 2% w/v calcium chloride solution, **Emulsion** = coated pitahaya with 0.25% w/v caprylic acid in 2% w/v alginate/1.5% w/v glycerol solution and 2% w/v calcium chloride solution

CHAPTER V

CONCLUSION

Factors influencing preparation and physicochemical properties of caprylic acid emulsions was evaluated with response surface methodology (Box–Behnken design). Determined factors were surfactant concentration, sonication time and amplitude with responses of particle size, zeta potential, and PDI. The criteria were the production of small particle sizes, high zeta potential (negative), and low PDI. The optimized formulation (desirability of 0.69) was the emulsion containing surfactant concentration (polysorbate 80 and monocaprylin at 1: 1 weight ratio), sonication time and amplitude of 3.6% w/v (1.8% w/v of each polysorbate 80 and monocaprylin), 120 sec, and 30%, respectively. The optimized emulsion could be stored at 30 ± 2 ℃ / 75 ± 5% RH and 40 ± 2 ℃ / 75 ± 5% RH for at least 28 days without changes in physiochemical properties

The optimized formula of caprylic acid emulsion had the MBC/MFC for all microorganism at 0.25% w/v (2.5 mg/ml) caprylic acid concentration. Caprylic acid emulsion-based coating for food sample (pitahaya) was then prepared by adding 4% w/v caprylic acid emulsion into the 2% w/v alginate and 1.5% w/v glycerol solution at a volume ratio of 1: 16 (0.25% w/v caprylic acid in the coating). Food coated with emulsion did not have significant (p>0.05) changes in physical properties compared to

the uncoated samples. However, food coated with emulsion had significantly (p<0.0001) lower in a number of microorganisms than the uncoated food at day 12 after storage in refrigerator. The findings suggest that emulsion-based coating could be used for control of microbial growth in food. This study is the first study for food film coatings containing caprylic acid emulsion. In addition, the further study should be done on antimicrobial activity of emulsion after storage and sensory evaluation.

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APPENDICES

APPENDIX A

Properties of emulsion

The physical appearance of caprylic acid emulsions, in order from left to right **(Figure 1A-3A)** according to the experiment order 1 to 15 as shown in **Table 7** (Chapter III Material and methods) and simply tabulated in **Table 1A**. **Tables 2A and 3A** shows the physiochemical properties of emulsions after storage.

Table 1A Experiment order of each emulsion formulation

Experiment order	Surfactant concentration (% w/v)	Sonication time (seconds)	Sonication amplitude (%)
1	3	60	50
2	7	60	50
3	3	120	50
4	7	120	50
5	3	90	30
6	7	90	30
7	3	90	70
8	7	90	70
9	5	60	30
10	5	120	30
11	5	60	70
12	5	120	70
13	5	90	50
14	5	90	50
15	5	90	50

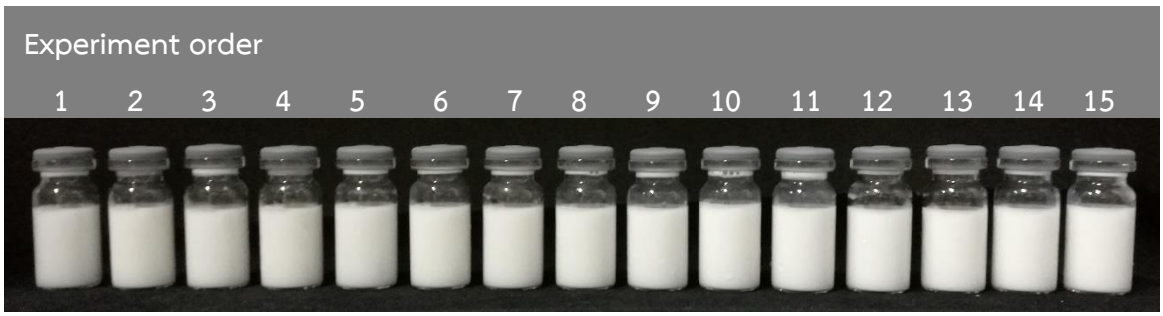

Figure 1A Emulsions stored in 30 ± 2 ℃ and 75 ± 5% RH at day 0

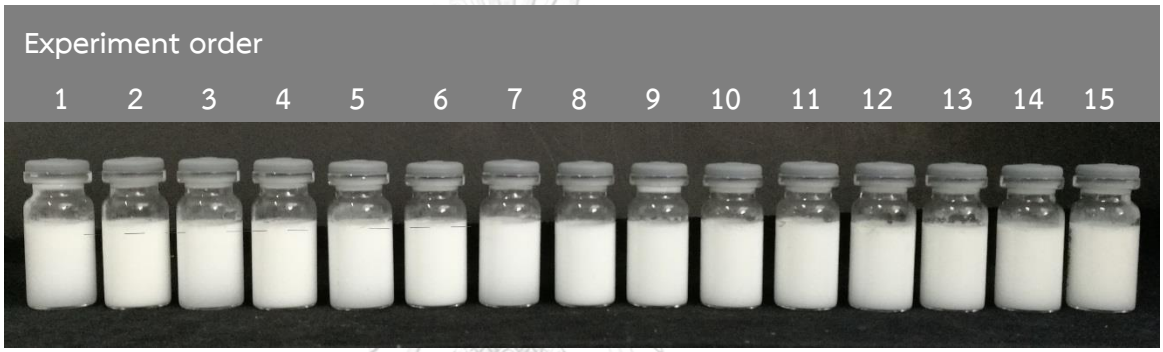

Figure 2A Emulsions stored in 30 ± 2 ℃ and 75 ± 5% RH at day 28

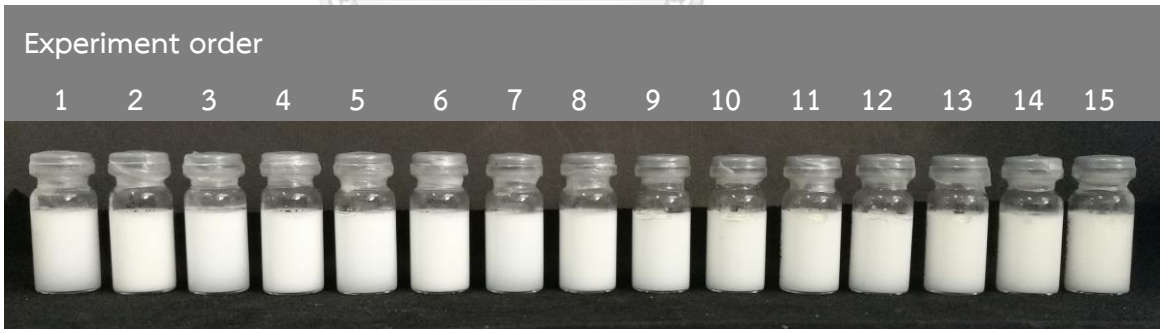

Figure 3A Emulsions stored in 40 ± 2 ℃ and 75 ± 5% RH at day 28

Table 2A The particle size, zeta potential, and PDI of 4% w/v caprylic acid emulsions stored in 30 ± 2 ℃ and 75 ± 5% RH

Experiment order	Day	Mean ± SD		
		Particle size (nm)	Zeta potential (mV)	PDI
1	0	198 ± 2	-25.1 ± 0.8	0.23 ± 0.01
	7	209 ± 2	-27.4 ± 0.8	0.25 ± 0.01
	14	209 ± 2	-26.7 ± 0.7	0.29 ± 0.02
	21	207 ± 1	-23.1 ± 0.2	0.29 ± 0.03
	28	202 ± 3	-23.1 ± 0.3	0.26 ± 0.02
2	0	206 ± 1	-15.3 ± 0.2	0.18 ± 0.01
	7	210 ± 1	-14.3 ± 0.7	0.19 ± 0.01
	14	208 ± 2	-15.4 ± 0.8	0.15 ± 0.01
	21	226 ± 3	-14.1 ± 1.0	0.17 ± 0.02
	28	236 ± 3	-16.0 ± 0.9	0.21 ± 0.02
3	0	216 ± 2	-28.9 ± 1.0	0.26 ± 0.01
	7	220 ± 2	-26.1 ± 0.5	0.30 ± 0.02
	14	225 ± 1	-25.6 ± 1.4	0.31 ± 0.04
	21	226 ± 3	-25.0 ± 0.9	0.34 ± 0.01
	28	211 ± 3	-25.1 ± 0.8	0.30 ± 0.02
4	0	223 ± 0	-14.8 ± 0.3	0.24 ± 0.01
	7	230 ± 3	-14.5 ± 0.3	0.21 ± 0.02
	14	290 ± 1	-15.5 ± 0.5	0.26 ± 0.01
	21	369 ± 9	-14.6 ± 0.4	0.34 ± 0.05
	28	460 ± 17	-15.7 ± 0.6	0.48 ± 0.02
5	0	205 ± 1	-29.8 ± 0.6	0.28 ± 0.02
	7	199 ± 2	-26.5 ± 0.5	0.25 ± 0.01
	14	209 ± 2	-25.1 ± 1.1	0.28 ± 0.02
	21	213 ± 3	-25.2 ± 1.6	0.32 ± 0.01
	28	204 ± 3	-24.6 ± 1.2	0.30 ± 0.03

Table 2A The particle size, zeta potential, and PDI of 4% w/v caprylic acid emulsions stored in 30 ± 2 °C and 75 ± 5% RH (continued)

Experiment order	Day	Mean ± SD		
		Particle size (nm)	Zeta potential (mV)	PDI
6	0	202 ± 2	-15.3 ± 1.3	0.21 ± 0.01
	7	205 ± 1	-16.0 ± 0.2	0.17 ± 0.01
	14	318 ± 7	-14.9 ± 0.2	0.26 ± 0.03
	21	405 ± 5	-14.3 ± 0.3	0.39 ± 0.05
	28	451 ± 11	-16.9 ± 0.2	0.77 ± 0.04
7	0	221 ± 2	-26.8 ± 1.1	0.26 ± 0.02
	7	224 ± 1	-25.7 ± 0.9	0.30 ± 0.03
	14	228 ± 2	-26.7 ± 0.4	0.33 ± 0.00
	21	227 ± 0	-25.6 ± 0.4	0.33 ± 0.03
	28	241 ± 4	-25.2 ± 0.6	0.34 ± 0.02
8	0	230 ± 1	-15.5 ± 0.2	0.23 ± 0.00
	7	282 ± 4	-15.9 ± 0.9	0.25 ± 0.00
	14	447 ± 5	-14.8 ± 0.2	0.43 ± 0.04
	21	545 ± 24	-14.9 ± 0.4	0.86 ± 0.05
	28	1148 ± 177	-18.9 ± 0.5	0.86 ± 0.04
9	0	180 ± 3	-19.7 ± 0.2	0.25 ± 0.02
	7	181 ± 1	-19.4 ± 0.2	0.26 ± 0.00
	14	176 ± 2	-19.1 ± 0.8	0.25 ± 0.00
	21	185 ± 1	-19.0 ± 0.8	0.26 ± 0.02
	28	178 ± 1	-18.4 ± 0.6	0.22 ± 0.01
10	0	180 ± 2	-18.6 ± 0.8	0.20 ± 0.02
	7	178 ± 1	-19.6 ± 0.7	0.21 ± 0.01
	14	180 ± 1	-18.5 ± 0.3	0.20 ± 0.01
	21	184 ± 2	-17.8 ± 0.4	0.22 ± 0.01
	28	185 ± 3	-19.5 ± 0.5	0.19 ± 0.01

Table 2A The particle size, zeta potential, and PDI of 4% w/v caprylic acid emulsions stored in 30 ± 2 ℃ and 75 ± 5% RH (continued)

Experiment order	Day	Mean ± SD		
		Particle size (nm)	Zeta potential (mV)	PDI
11	0	192 ± 0	-19.1 ± 0.7	0.19 ± 0.01
	7	189 ± 2	-18.6 ± 0.5	0.21 ± 0.01
	14	191 ± 1	-17.7 ± 0.4	0.20 ± 0.00
	21	183 ± 2	-16.0 ± 1.2	0.20 ± 0.02
	28	192 ± 2	-18.5 ± 0.8	0.18 ± 0.03
12	0	210 ± 1	-21.4 ± 0.2	0.27 ± 0.01
	7	213 ± 2	-20.0 ± 0.6	0.26 ± 0.02
	14	213 ± 3	-18.4 ± 0.6	0.27 ± 0.01
	21	210 ± 4	-17.9 ± 0.2	0.27 ± 0.01
	28	212 ± 4	-17.6 ± 0.4	0.25 ± 0.00
13	0	202 ± 1	-18.7 ± 0.5	0.23 ± 0.01
	7	203 ± 1	-19.2 ± 0.2	0.24 ± 0.01
	14	199 ± 1	-17.3 ± 0.4	0.24 ± 0.01
	21	199 ± 2	-16.1 ± 0.6	0.22 ± 0.00
	28	213 ± 5	-17.7 ± 0.4	0.31 ± 0.00
14	0	209 ± 2	-19.1 ± 0.6	0.24 ± 0.00
	7	211 ± 2	-19.2 ± 0.9	0.26 ± 0.01
	14	208 ± 3	-18.1 ± 0.5	0.25 ± 0.01
	21	210 ± 6	-17.8 ± 0.9	0.23 ± 0.01
	28	208 ± 2	-17.4 ± 0.3	0.25 ± 0.02
15	0	205 ± 3	-19.4 ± 0.8	0.24 ± 0.01
	7	206 ± 1	-19.8 ± 0.3	0.25 ± 0.02
	14	202 ± 2	-17.7 ± 0.4	0.24 ± 0.02
	21	205 ± 1	-17.8 ± 0.9	0.25 ± 0.02
	28	209 ± 0	-17.5 ± 0.4	0.27 ± 0.01

Table 3A The particle size, zeta potential, and PDI of 4% w/v caprylic acid emulsions stored in 40 ± 2 ℃ and 75 ± 5% RH

Experiment order	Day	Mean ± SD		
		Particle size (nm)	Zeta potential (mV)	PDI
1	0	198 ± 2	-25.1 ± 0.8	0.23 ± 0.01
	7	192 ± 1	-25.9 ± 0.1	0.22 ± 0.01
	14	214 ± 2	-24.9 ± 0.2	0.26 ± 0.01
	21	204 ± 2	-26.2 ± 0.7	0.24 ± 0.02
	28	208 ± 2	-26.1 ± 0.6	0.25 ± 0.01
2	0	206 ± 1	-15.3 ± 0.2	0.18 ± 0.01
	7	203 ± 0	-14.6 ± 0.2	0.17 ± 0.01
	14	197 ± 2	-15.8 ± 0.5	0.10 ± 0.03
	21	185 ± 1	-14.3 ± 0.7	0.12 ± 0.01
	28	200 ± 1	-14.9 ± 0.6	0.17 ± 0.02
3	0	216 ± 2	-28.9 ± 1.0	0.26 ± 0.01
	7	229 ± 4	-26.0 ± 1.1	0.29 ± 0.01
	14	228 ± 6	-24.6 ± 0.9	0.30 ± 0.04
	21	227 ± 6	-23.2 ± 0.9	0.31 ± 0.01
	28	228 ± 1	-26.8 ± 0.4	0.31 ± 0.03
4	0	223 ± 0	-14.8 ± 0.3	0.24 ± 0.01
	7	222 ± 4	-14.9 ± 0.8	0.19 ± 0.02
	14	488 ± 2	-14.5 ± 0.6	0.46 ± 0.01
	21	603 ± 73	-17.2 ± 0.7	0.70 ± 0.06
	28	611 ± 64	-18.1 ± 0.5	0.87 ± 0.11
5	0	205 ± 1	-29.8 ± 0.6	0.28 ± 0.02
	7	207 ± 4	-28.2 ± 0.3	0.27 ± 0.02
	14	206 ± 2	-26.8 ± 0.5	0.30 ± 0.02
	21	190 ± 2	-26.3 ± 0.7	0.25 ± 0.00
	28	205 ± 4	-22.7 ± 0.6	0.26 ± 0.02

Table 3A The particle size, zeta potential, and PDI of 4% w/v caprylic acid emulsions stored in 40 ± 2 °C and 75 ± 5% RH (continued)

Experiment order	Day	Mean ± SD		
		Particle size (nm)	Zeta potential (mV)	PDI
6	0	202 ± 2	-15.3 ± 1.3	0.21 ± 0.01
	7	206 ± 1	-15.3 ± 0.6	0.20 ± 0.01
	14	514 ± 1	-13.8 ± 0.2	0.45 ± 0.02
	21	610 ± 26	-19.3 ± 0.6	0.72 ± 0.04
	28	1247 ± 66	-19.3 ± 0.8	0.97 ± 0.03
7	0	221 ± 2	-26.8 ± 1.1	0.26 ± 0.02
	7	227 ± 2	-27.0 ± 0.3	0.34 ± 0.00
	14	232 ± 3	-24.2 ± 1.1	0.32 ± 0.04
	21	229 ± 3	-23.8 ± 0.5	0.28 ± 0.02
	28	229 ± 5	-24.3 ± 0.7	0.31 ± 0.04
8	0	230 ± 1	-15.5 ± 0.2	0.23 ± 0.00
	7	229 ± 0	-14.9 ± 0.9	0.23 ± 0.02
	14	547 ± 23	-15.8 ± 1.5	0.82 ± 0.04
	21	1361 ± 26	-19.9 ± 0.2	0.90 ± 0.01
	28	1912 ± 265	-17.6 ± 0.6	1.00 ± 0.00
9	0	180 ± 3	-19.7 ± 0.2	0.25 ± 0.02
	7	179 ± 2	-19.5 ± 0.7	0.27 ± 0.03
	14	185 ± 3	-17.8 ± 0.3	0.25 ± 0.01
	21	181 ± 1	-18.0 ± 0.5	0.23 ± 0.01
	28	193 ± 2	-21.0 ± 0.8	0.19 ± 0.01
10	0	180 ± 2	-18.6 ± 0.8	0.20 ± 0.02
	7	179 ± 1	-18.1 ± 0.1	0.19 ± 0.02
	14	178 ± 1	-19.5 ± 0.6	0.19 ± 0.01
	21	179 ± 1	-19.1 ± 0.2	0.19 ± 0.01
	28	195 ± 2	-18.2 ± 0.5	0.12 ± 0.02

Table 3A The particle size, zeta potential, and PDI of 4% w/v caprylic acid emulsions stored in 40 ± 2 °C and 75 ± 5% RH (continued)

Experiment order	Day	Mean ± SD		
		Particle size (nm)	Zeta potential (mV)	PDI
11	0	192 ± 0	-19.1 ± 0.7	0.19 ± 0.01
	7	191 ± 0	-18.6 ± 0.3	0.19 ± 0.01
	14	194 ± 2	-18.3 ± 0.9	0.20 ± 0.02
	21	187 ± 3	-16.1 ± 0.4	0.20 ± 0.00
	28	214 ± 4	-18.6 ± 0.4	0.18 ± 0.01
12	0	210 ± 1	-21.4 ± 0.2	0.27 ± 0.01
	7	197 ± 1	-18.2 ± 0.3	0.25 ± 0.00
	14	193 ± 1	-17.5 ± 0.2	0.17 ± 0.02
	21	212 ± 2	-18.4 ± 0.2	0.26 ± 0.00
	28	209 ± 2	-17.1 ± 0.2	0.25 ± 0.01
13	0	202 ± 1	-18.7 ± 0.5	0.23 ± 0.01
	7	203 ± 2	-18.8 ± 0.7	0.25 ± 0.01
	14	206 ± 4	-17.2 ± 0.1	0.23 ± 0.01
	21	196 ± 2	-17.6 ± 0.2	0.22 ± 0.01
	28	205 ± 5	-18.9 ± 0.8	0.22 ± 0.01
14	0	209 ± 2	-19.1 ± 0.6	0.24 ± 0.00
	7	210 ± 1	-19.2 ± 0.5	0.26 ± 0.02
	14	208 ± 2	-17.5 ± 0.3	0.26 ± 0.00
	21	209 ± 1	-18.6 ± 0.2	0.25 ± 0.01
	28	235 ± 2	-17.4 ± 0.8	0.25 ± 0.01
15	0	205 ± 3	-19.4 ± 0.8	0.24 ± 0.01
	7	208 ± 3	-19.0 ± 0.4	0.25 ± 0.01
	14	211 ± 4	-18.1 ± 0.4	0.25 ± 0.01
	21	209 ± 3	-18.6 ± 0.7	0.25 ± 0.01
	28	207 ± 1	-18.2 ± 0.3	0.22 ± 0.01

APPENDIX B

Microbial susceptibility test

Table 1B The concentration (mg/ml) of substance in each well in 96-well plate

Emulsion / solution	Column / Row	1	2	3	4	5	6	7	8	9	10	11	12	Media
Optimized emulsion	A	20	10	5.00	2.50	1.25	0.63	0.31	0.16	0.08	0.04	0.02	Blank	Bacteria or fungi media
Caprylic acid P-80 emulsion	B	20	10	5.00	2.50	1.25	0.63	0.31	0.16	0.08	0.04	0.02		
Monocaprylin solution	C	9	4.5	2.25	1.13	0.56	0.28	0.14	0.07	0.04	0.02	0.01		
Polysorbate 80 solution	D	9	4.5	2.25	1.13	0.56	0.28	0.14	0.07	0.04	0.02	0.01		
Optimized emulsion	E	20	10	5.00	2.50	1.25	0.63	0.31	0.16	0.08	0.04	0.02	Blank	Sterile media
Caprylic acid P-80 emulsion	F	20	10	5.00	2.50	1.25	0.63	0.31	0.16	0.08	0.04	0.02		
Monocaprylin solution	G	9	4.5	2.25	1.13	0.56	0.28	0.14	0.07	0.04	0.02	0.01		
Polysorbate 80 solution	H	9	4.5	2.25	1.13	0.56	0.28	0.14	0.07	0.04	0.02	0.01		

Optimized emulsion = 4% w/v caprylic acid, 1.8% w/v polysorbate and 1.8% w/v monocaprylin emulsion,

Caprylic acid P-80 emulsion = 4% w/v caprylic acid and 1.8% w/v polysorbate emulsion,

Monocaprylin solution = 1.8% w/v monocaprylin solution,

Polysorbate 80 solution = 1.8% w/v polysorbate 80 solution,

Blank = sterile ultrapure water

Figure 1B MIC result of *S. aureus*

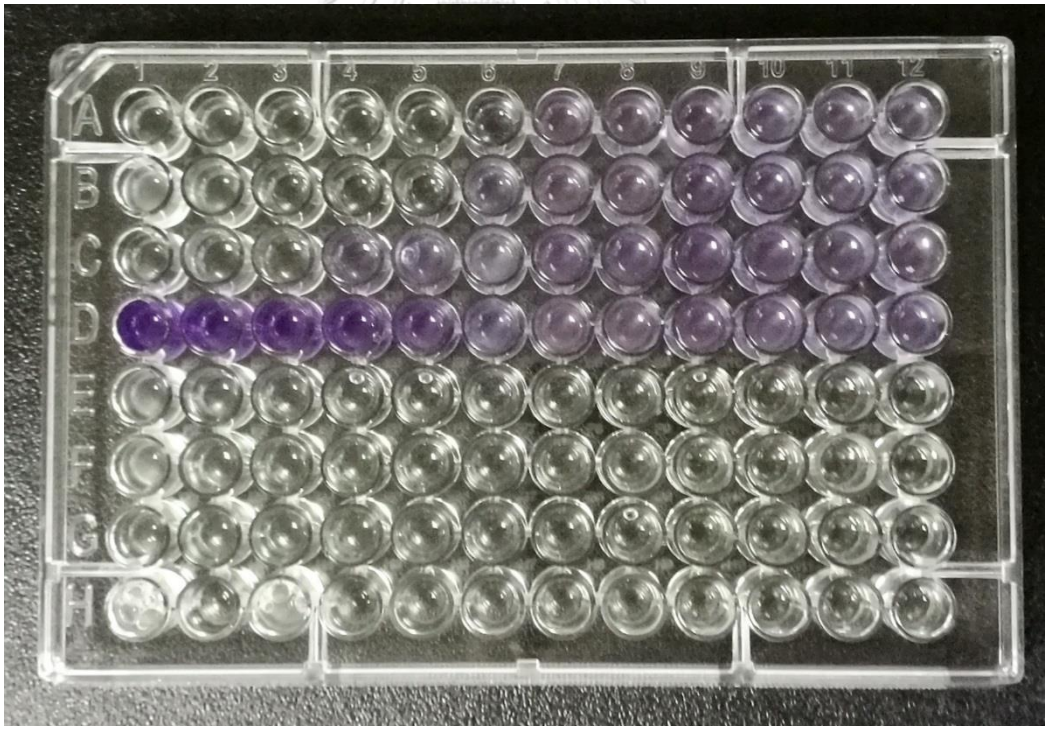

Figure 2B MIC result of *B. cereus*

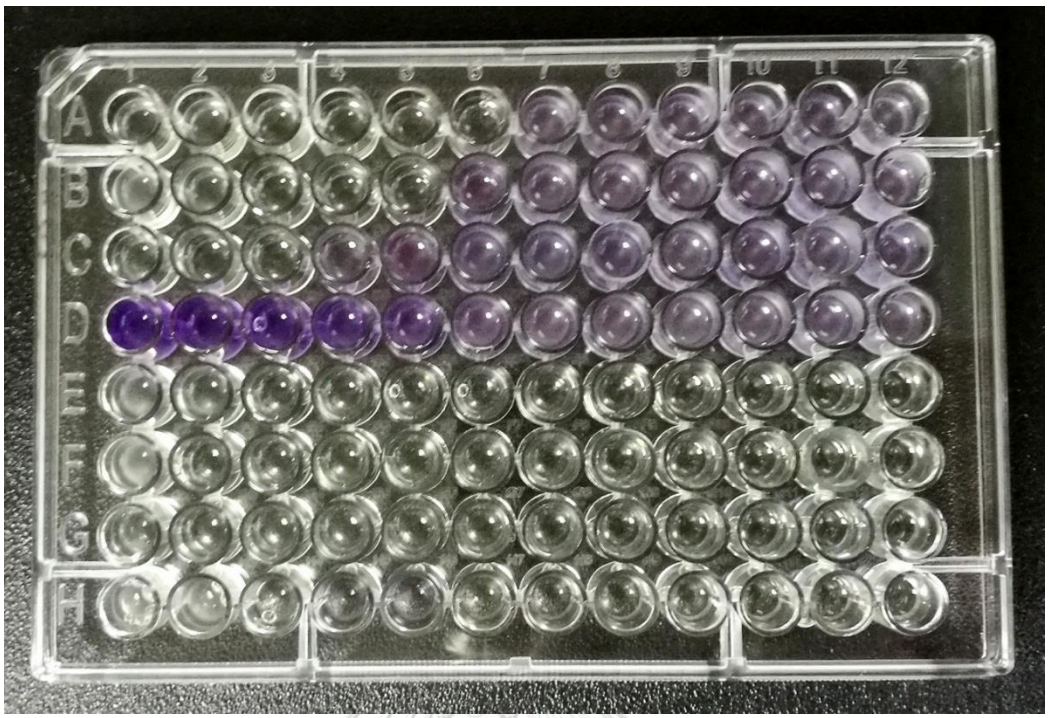

Figure 3B MIC result of *E. coli*

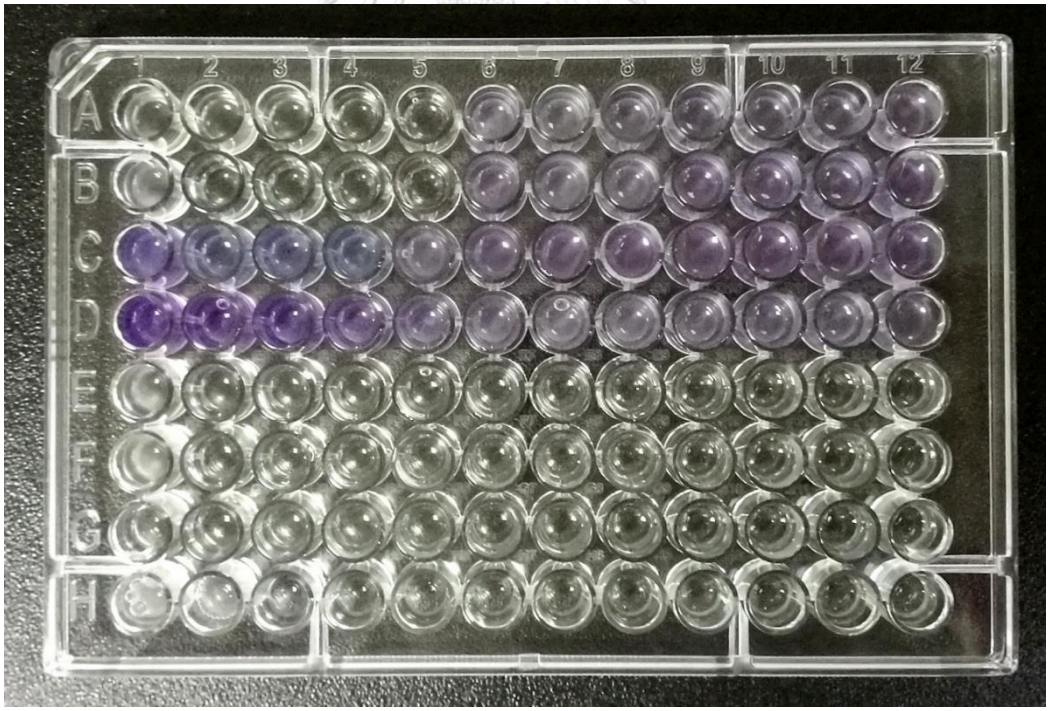

Figure 4B MIC result of *P. aeruginosa*

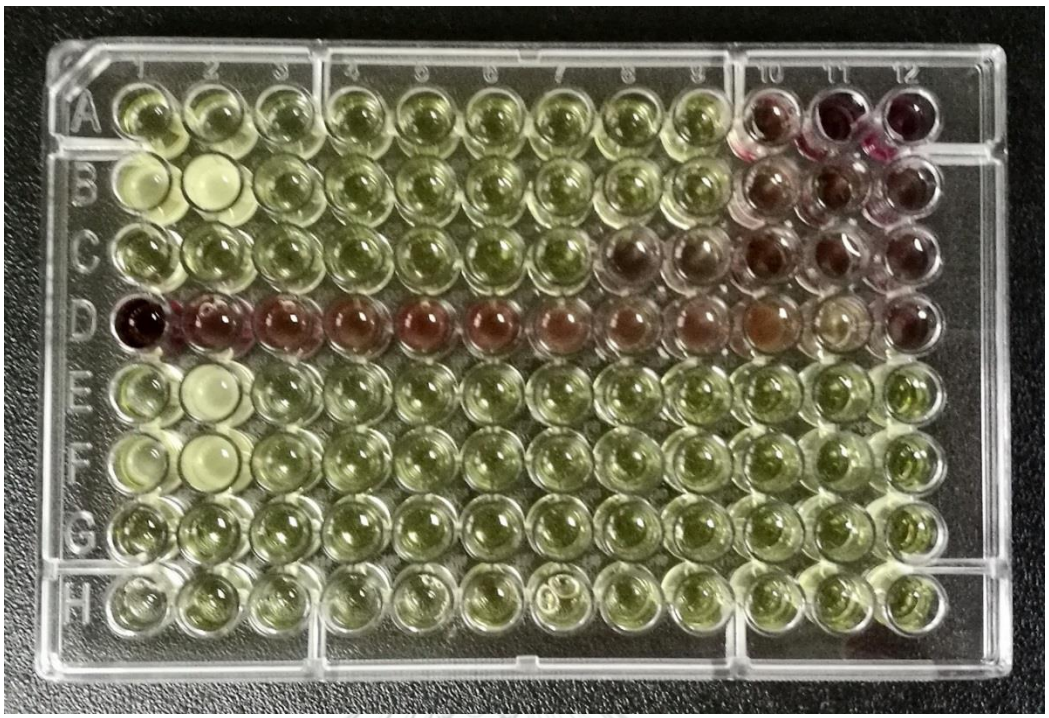

Figure 5B MIC result of *C. albicans*

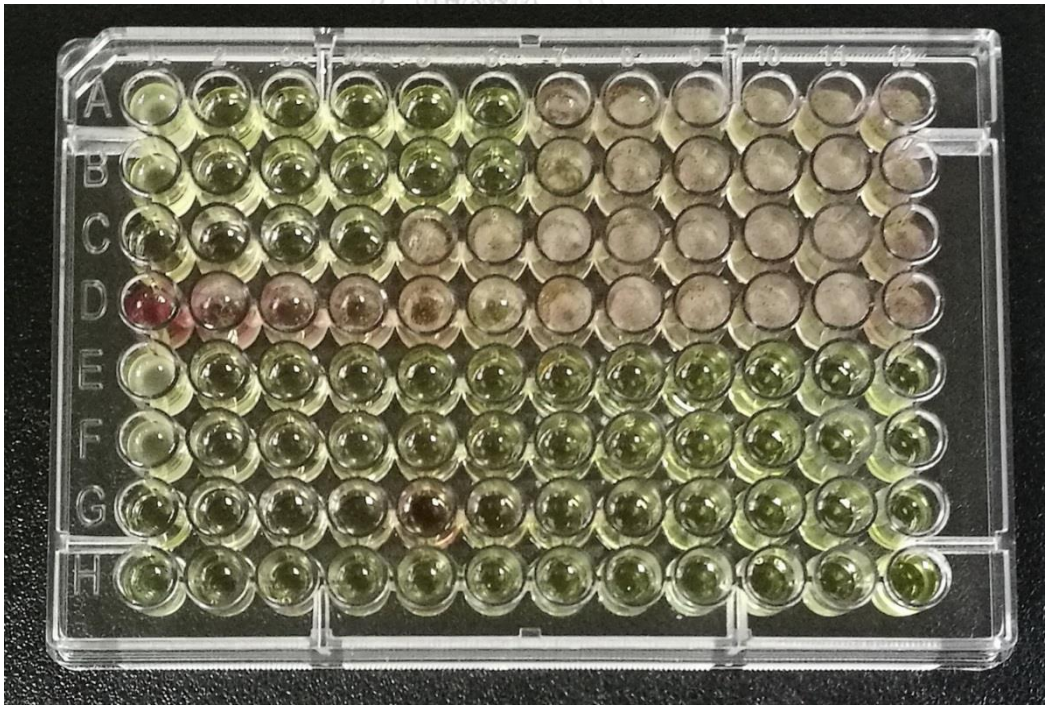

Figure 6B MIC result of *A. niger*

APPENDIX C

Compatability test of food coating formula

Table 1C The compatibility of the substance tested for food coating

Substance (emulsion/solution)	Compatibility
(A) C1T5	No (separated)
(B) C1T2.5M2.5	Yes
(C) C1T2.5M2.5G1.5	No (separated)
(D) C1T2.5M2.5G1.5A1	No (separated)
(E) T2.5M2.5	Yes
(F) T2.5M2.5G1.5	Yes
(G) T2.5M2.5G1.5A1	No (separated)
(H) G1.5A1	Yes

C1 = 1% w/v caprylic acid, T2.5/T5 = 2.5/5% w/v polysorbate 80, M2.5 = 2.5% w/v monocaprylin, G1.5 = 1.5% w/v glycerol, and A1 = 1% w/v alginate

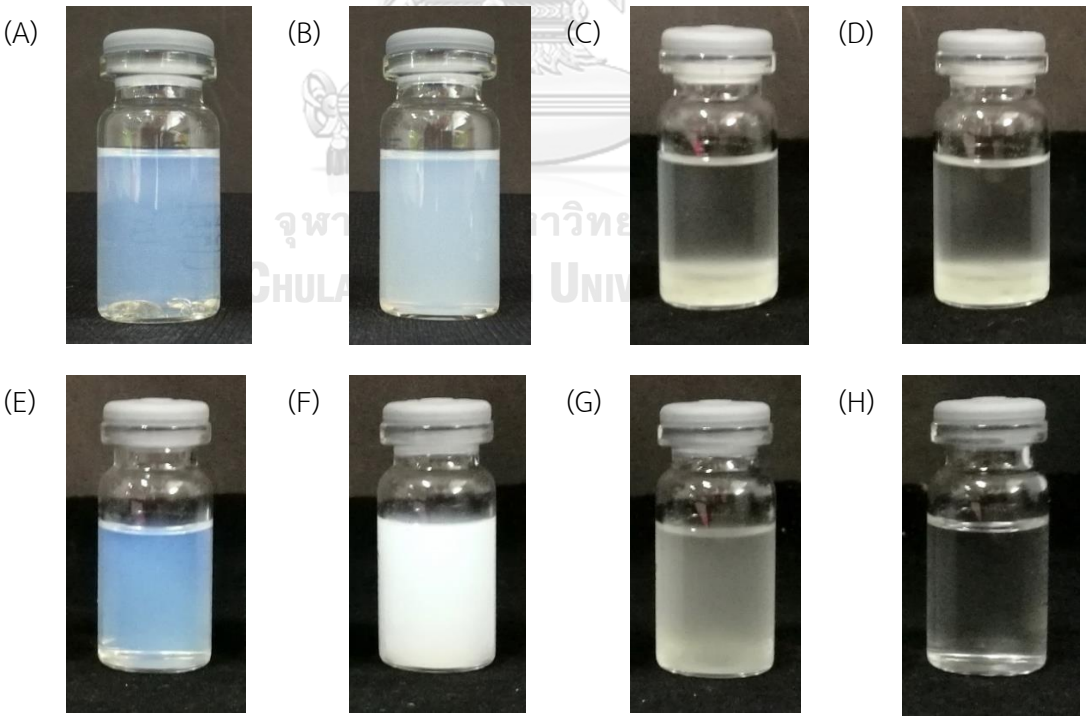

Figure 1C Sample appearances after 7 days storage at 30 ± 2 ℃ / 75 ± 5% RH

(A) C1T5 (B) C1T2.5M2.5 (C) C1T2.5M2.5G1.5 (D) C1T2.5M2.5G1.5A1 (E) T2.5M2.5 (F) T2.5M2.5G1.5 (G) T2.5M2.5G1.5A1 (H) G1.5A1

APPENDIX D

Whiteness index and color test

Table 1D Whiteness index (WI) of coating solution

Sample	Mean ± SD
Control	3.82 ± 0.007^{A}
Alginate	35.17 ± 0.165^{B}
Optimized emulsion	99.79 ± 0.010^{C}
Emulsion (diluted)	86.76 ± 0.012^{D}

Control = ultrapure water

Alginate = 2% w/v alginate and 1.5% w/v glycerol solution

Optimized emulsion = 4% w/v caprylic acid, 1.8% w/v polysorbate and 1.8% w/v monocaprylin emulsion

Emulsion (diluted) = 0.25% w/v caprylic acid emulsion-based coating solution by dissolving the optimized emulsion in 2% w/v alginate and 1.5% w/v glycerol solution (ratio 1:16)

WI = $100 - ((100 - L^*)^2 + (a^{*2} + b^{*2}))^{0.5}$

Means with the different letters are significantly different by Bonferroni test ($p < 0.05$)

Table 2D Color of coated pitahaya with different coatings after different storage times compared to control (uncoated) stored in refrigerator (4-8 °C)

Color measurement	Days	Mean ± SD of color		
		Control	Alginate	Emulsion
Lightness (*L**)	0	51.89 ± 5.10^{a,A}	48.45 ± 4.37^{a,A}	53.92 ± 3.74^{a,A}
	3	44.09 ± 4.68^{ab,A}	40.04 ± 6.32^{ab,A}	48.61 ± 2.49^{ab,A}
	7	39.24 ± 2.51^{b,A}	38.55 ± 1.74^{ab,A}	42.75 ± 3.00^{b,A}
	12	34.84 ± 1.52^{b,A}	35.70 ± 3.95^{b,A}	41.26 ± 1.30^{b,A}
Hue angle (*h**)	0	299.16 ± 6.59^{a,A}	299.63 ± 26.82^{a,A}	288.72 ± 10.75^{a,A}
	3	283.17 ± 5.27^{a,A}	275.93 ± 4.84^{a,A}	274.74 ± 4.60^{a,A}
	7	67.48 ± 15.67^{b,A}	82.97 ± 6.85^{b,A}	85.88 ± 2.65^{b,A}
	12	41.88 ± 34.83^{b,A}	83.91 ± 6.54^{b,A}	86.46 ± 2.94^{b,A}
Chroma (*C**)	0	1.57 ± 0.21^{a,A}	1.72 ± 0.96^{a,A}	2.00 ± 0.26^{a,A}
	3	2.65 ± 1.05^{a,A}	1.89 ± 1.18^{a,A}	1.99 ± 0.52^{a,A}
	7	2.17 ± 1.00^{a,A}	3.82 ± 0.88^{a,A}	4.08 ± 0.49^{a,A}
	12	1.70 ± 0.86^{a,A}	2.80 ± 0.65^{a,A}	3.21 ± 1.03^{a,A}
Color difference (*ΔE**)	3	8.07 ± 2.93^{a,A}	8.42 ± 2.77^{a,A}	5.38 ± 1.39^{a,A}
	7	12.85 ± 5.17^{a,A}	10.34 ± 3.55^{a,A}	11.42 ± 2.16^{b,A}
	12	17.19 ± 4.80^{a,A}	12.91 ± 1.44^{a,A}	12.79 ± 2.66^{b,A}

Control = uncoated pitahaya

Alginate = coated pitahaya with 2% w/v alginate/1.5% w/v glycerol solution and 2% w/v calcium chloride solution

Emulsion = coated pitahaya with 0.25% w/v caprylic acid in 2% w/v alginate/1.5% w/v glycerol solution and 2% w/v calcium chloride solution

Hue angle (*h)** = arctan (b^*/a^*)

Chroma (*C)** = $(a^{*2} + b^{*2})^{0.5}$

The color difference (*ΔE)** = $((L^*-L_0)^2+(a^*-a_0)^2+(b^*-b_0)^2)^{0.5}$

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 E

Weight loss determination

Table 1E Weight loss of coated pitahaya with different coatings after different storage times compared to control (uncoated) stored in refrigerator (4-8 °C)

	Mean ± SD of Weight loss (%)		
	Control	**Alginate**	**Emulsion**
Day 3	$0.03 \pm 0.001^{a,A}$	$0.03 \pm 0.026^{a,A}$	$0.03 \pm 0.005^{a,A}$
Day 7	$0.15 \pm 0.027^{b,A}$	$0.13 \pm 0.009^{b,A}$	$0.10 \pm 0.021^{b,A}$
Day 12	$0.35 \pm 0.052^{c,A}$	$0.33 \pm 0.016^{c,A}$	$0.30 \pm 0.028^{c,A}$

Control = uncoated pitahaya

Alginate = coated pitahaya with 2% w/v alginate/1.5% w/v glycerol solution and 2% w/v calcium chloride solution

Emulsion = coated pitahaya with 0.25% w/v caprylic acid in 2% w/v alginate/1.5% w/v glycerol solution and 2% w/v calcium chloride solution

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

Firmness determination

Table 1F Firmness of coated pitahaya with different coatings after different storage times compared to control (uncoated) stored in refrigerator (4-8 °C)

	Mean ± SD of maximum force (N)		
	Control	**Alginate**	**Emulsion**
Day 0	$3.31 \pm 0.856^{a,A}$	$6.61 \pm 0.456^{a,B}$	$5.25 \pm 0.283^{a,B}$
Day 3	$4.97 \pm 1.027^{a,A}$	$11.82 \pm 0.661^{b,B}$	$6.29 \pm 1.449^{a,A}$
Day 7	$5.80 \pm 0.467^{a,A}$	$10.62 \pm 1.609^{b,A}$	$7.42 \pm 2.643^{a,A}$
Day 12	$5.66 \pm 1.281^{a,A}$	$10.34 \pm 1.312^{b,B}$	$6.79 \pm 0.398^{a,A}$

Table 2F Modulus of elasticity of coated pitahaya with different coatings after different storage times compared to control (uncoated) stored in refrigerator (4-8 °C)

	Mean ± SD of modulus of elasticity (N/mm^2)		
	Control	**Alginate**	**Emulsion**
Day 0	$0.121 \pm 0.011^{a,A}$	$0.274 \pm 0.009^{a,B}$	$0.222 \pm 0.016^{a,C}$
Day 3	$0.146 \pm 0.025^{a,A}$	$0.325 \pm 0.021^{b,B}$	$0.214 \pm 0.079^{a,AB}$
Day 7	$0.173 \pm 0.014^{a,A}$	$0.302 \pm 0.006^{ab,A}$	$0.223 \pm 0.012^{a,C}$
Day 12	$0.173 \pm 0.018^{a,A}$	$0.315 \pm 0.017^{b,B}$	$0.198 \pm 0.043^{a,A}$

Control = uncoated pitahaya, **Alginate** = coated pitahaya with 2% w/v alginate/1.5% w/v glycerol solution and 2% w/v calcium chloride solution, **Emulsion** = coated pitahaya with 0.25% w/v caprylic acid in 2% w/v alginate/1.5% w/v glycerol solution and 2% w/v calcium chloride solution
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 ($p < 0.05$).

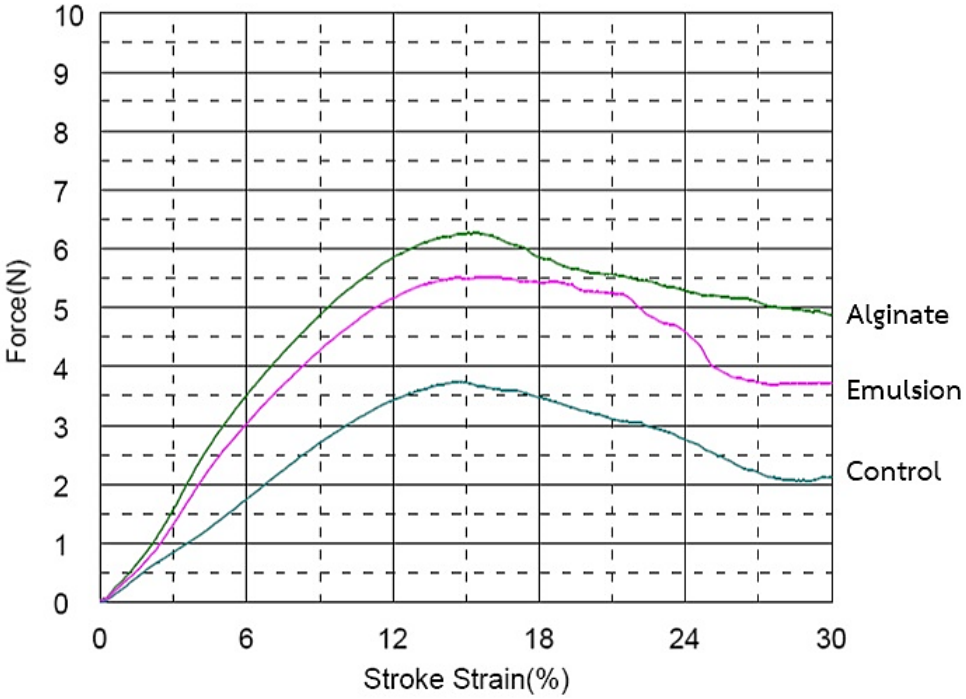

Figure 1F Compression test of coated pitahaya with different coatings compared to control (uncoated) at day 0

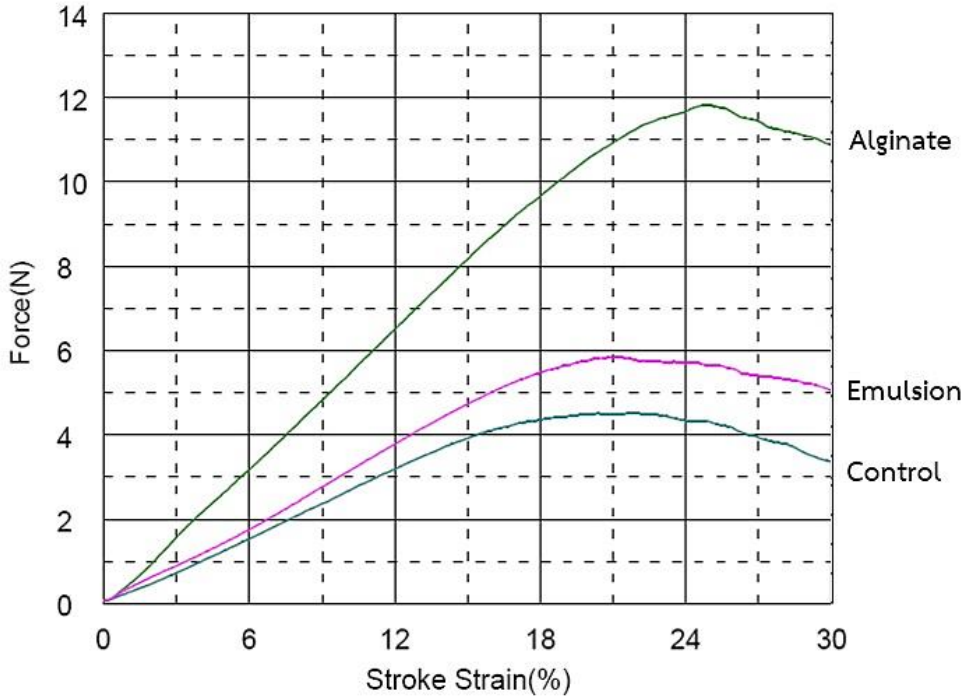

Figure 2F Compression test of coated pitahaya with different coatings compared to control (uncoated) at day 3

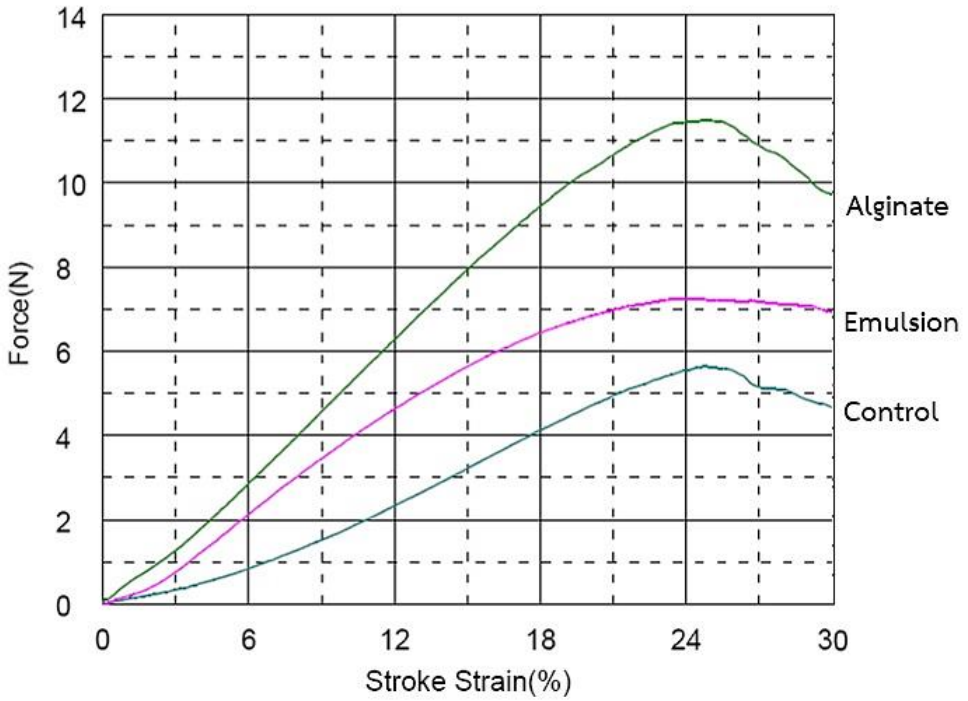

Figure 3F Compression test of coated pitahaya with different coatings compared to control (uncoated) at day 7

Figure 4F Compression test of coated pitahaya with different coatings compared to control (uncoated) at day 12

APPENDIX G

Microbial determination of coated-food

Table 1G Microbial growth of coated pitahaya with different coatings after different storage times compared to control (uncoated) stored in refrigerator (4-8 °C)

		Mean ± SD of log CFUs/g		
Microorganisms	**Days**	Control	Alginate	Emulsion
Mesophilic	0	2.78 ± 0.07^{a,A}	2.30 ± 1.60^{a,AB}	<1.00 ± 0.00^{a,B}
bacteria	3	3.01 ± 0.33^{a,A}	2.37 ± 0.28^{ab,A}	1.22 ± 0.17^{b,B}
	7	5.03 ± 0.06^{b,A}	4.00 ± 0.01^{b,A}	2.63 ± 0.11^{c,B}
	12	6.19 ± 0.07^{c,A}	4.56 ± 0.07^{b,B}	3.32 ± 0.22^{d,C}
Psychrophilic	0	1.56 ± 0.41^{a,A}	0.52 ± 0.58^{a,AB}	<1.00 ± 0.00^{a,B}
bacteria	3	2.75 ± 0.13^{b,A}	1.12 ± 0.17^{a,B}	<1.00 ± 0.00^{a,C}
	7	3.66 ± 0.07^{c,A}	2.78 ± 0.12^{b,B}	<1.00 ± 0.00^{a,C}
	12	5.66 ± 0.14^{d,A}	5.11 ± 0.22^{c,A}	0.82 ± 0.58^{a,B}
Yeasts and	0	<1.00 ± 0.00^{a,A}	<1.00 ± 0.00^{a,A}	<1.00 ± 0.00^{a,A}
molds	3	<1.00 ± 0.00^{a,A}	<1.00 ± 0.00^{a,A}	<1.00 ± 0.00^{a,A}
	7	1.88 ± 0.42^{b,A}	1.30 ± 0.00^{b,A}	<1.00 ± 0.00^{a,B}
	12	3.05 ± 0.17^{c,A}	3.56 ± 0.02^{c,B}	<1.00 ± 0.00^{a,C}

Control = uncoated pitahaya

Alginate = coated pitahaya with 2% w/v alginate/1.5% w/v glycerol solution and 2% w/v calcium chloride solution

Emulsion = coated pitahaya with 0.25% w/v caprylic acid in 2% w/v alginate/1.5% w/v glycerol solution and 2% w/v calcium chloride solution

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 ($p < 0.05$).

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