ไมโครเอนแคปซูเลชันของยูจินอลในสารเชิงซ้อนพอลิอิเล็กโทรไลต์ของไคโตซานและแอลจิเนต

นางสาวชไมพร ศุภเชษฐพันธ์

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MICROENCAPSULATION OF EUGENOL IN POLYELECTROLYTE COMPLEXES OF CHITOSAN AND ALGINATE

Miss Chamaiporn Supachettapun

A Thesis Submitted in Partial Fulfillment of the Requirements
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Thesis Title	MICROENCAPSULATION OF EUGENOL IN POLYELECTROLYTE COMPLEXES OF CHITOSAN AND ALGINATE
By	Miss Chamaiporn Supachettapun
Field of Study	Petrochemistry and Polymer Science
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ชไมพร ศุภเชษฐพันธ์ : ไมโครเอนแคปซูเลชันของยูจินอลในสารเชิงซ้อนพอลิอิเล็กโทรไลต์ของไคโตซานและแอลจิเนต (MICROENCAPSULATION OF EUGENOL IN POLYELECTROLYTE COMPLEXES OF CHITOSAN AND ALGINATE) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ศ. ดร.นงนุช เหมืองสิน, 94 หน้า.

ยูจีนอลเป็นที่นิยมใช้อย่างแพร่หลายเช่น ถูกนำมาใช้ทำเป็นแพคเกจจิ้งเพื่อใช้ยืดอายุผักและผลไม้ เนื่องมาจากว่าประสิทธิภาพของยูจีนอลนั้นสามารถยับยั้งเชื้อราและเชื้อแบคทีเรียได้ดี แต่อย่างไรก็ตามยูจีนอลง่ายต่อการสลายตัวได้ง่าย ส่งผลให้ประสิทธิภาพในการใช้งานลดลง ในงานวิจัยนี้สนใจที่จะเตรียมบีดแอลจิเนต-ไคโตซานบรรจุและควบคุมการปลดปล่อยโดยอาศัยวิธีไอออนิกเจลเลชั่น อีกทั้งยังศึกษาปัจจัยของความเข้มข้นของยูจีนอลตั้งแต่ 0.1-0.5 กรัม ความเข้มข้นของแคลเซียมคลอไรด์ 0.5- 8 และสารลดแรงตึงผิว 2-10 เปอร์เซ็นต์ โดยน้ำหนักต่อปริมาตร จากการทดลองแสดงให้เห็นว่าที่ยูจีนอล 0.1 กรัม แคลเซียมคลอไรด์ 2 และ สารลดแรงตึงผิว 4 เปอร์เซ็นต์ โดยน้ำหนักต่อปริมาตรให้ผลการกักเก็บที่ดีที่สุด การพิสูจน์เอกลักษณ์เชิงสัญฐานวิทยาของบีดแอลจิเนต-ไคโตซานที่อัตราส่วน 1:1 บีดมีรูปร่างเป็นทรงกลม และบีดมีขนาดประมาณ 1 มม. FTIR แสดงให้เห็นถึงอันตรกิริยาระหว่างหมู่คาร์บอกซิลิกกรุ๊ปของแอลจิเนตและหมู่เอมีนกรุ๊ปของไคโตซาน ผลการศึกษาการปลดปล่อยพบว่าบีดแอลจิเนต-ไคโตซานให้การปลดปล่อยได้ดีกว่าบีดแอลจิเนตเพียงอย่างเดียว นอกจากนั้นสตอเบอร์รีถูกใช้เป็นผลไม้ที่จะนำมาศึกษาประสิทธิภาพการยับยั้งเชื้อราและเชื้อแบคทีเรียของบีดแอลจิเนต-ไคโตซาน จากผลการทดลองแสดงให้เห็นว่าบีดแอลจิเนต-ไคโตซานสามารถที่จะยืดอายุและคงซึ่งความสดของสตอเบอร์รี่ได้นานขึ้น 12 วัน การศึกษาคุณภาพของสี การสูญเสียน้ำของผลไม้ และปริมาณของแข็งที่ละลายระหว่างเก็บไว้ที่เวลา 0, 4, 8 และ 12 วัน สามารถยืนยันได้ว่าสตอเบอร์รีที่ใช้บีดแอลจิเนต-ไคโตซานให้ผลที่ไม่แตกต่างกัน แต่ในขณะที่ผลการศึกษาความแน่นของผลไม้มีค่าเพิ่มมากขึ้นซึ่งเป็นผลมาจากบีดดูดซับความชื้นภายในกล่องซึ่งเป็นปัจจัยสำคัญที่เกี่ยวข้องกับความแน่นของเนื้อผลไม้

สาขาวิชา ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์ ลายมือชื่อนิสิต .....

ปีการศึกษา 2560 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

5771951523 : MAJOR PETROCHEMISTRY AND POLYMER SCIENCE
KEYWORDS: ALGINATE / CHITOSAN / POLYELECTROLYTE / EUGENOL

CHAMAIPORN SUPACHETTAPUN: MICROENCAPSULATION OF EUGENOL IN POLYELECTROLYTE COMPLEXES OF CHITOSAN AND ALGINATE. ADVISOR: PROF. NONGNUJ MUANGSIN, Ph.D., 94 pp.

Eugenol is widely used in several applications such as food activity packaging to prolong shelf life of fruits and vegetables because of its antimicrobial activity. However, eugenol is easy to decompose, leading to reduce its activity. In this work, we prepared eugenol loaded alginate-chitosan microparticles in order to solve this problem and controlled release essential oil using ionic gelation technique. The effect of concentration of eugenol (0.1-0.5 g), calcium chloride (0.5 – 8 %W/V) and surfactant (2-10% W/V) was investigated. The results showed that the optimum condition was eugenol 0.1 g, calcium chloride 2% W/V and surfactant 4% W/V. The SEM image of alginate-chitosan microparticles as ration 1:1 showed a regular distribution and spherical shape with the size of 1 mm and after loading eugenol into the beads, it was still spherical in shape. FTIR spectrum showed the interaction between carboxylic group of alginate and amine group of chitosan. According to the release profiles, it was found that release rate of eugenol loaded alginate-chitosan microparticles was higher than that of eugenol loaded alginate microparticles. In addition, strawberries were selected as a fruit model for evaluation of antimicrobial properties of eugenol loaded alginate-chitosan microparticles. The results showed that eugenol loaded alginate-chitosan microparticles could inhibit microbial in strawberries and prolong shelf-life up to 12 days. The measurements of color, lightness (*L**), hue angle (*h*°), weight loss, and total soluble solid during storage at 0, 4, 8 and 12 days confirm that there are no significantly difference between treatment group of strawberries and control. However, the firmness of treatment group increased, which might be due to the beads could adsorb moisture, which is a major factor related to their firmness.

Field of Study: Petrochemistry and Polymer Science Student's Signature .....
Advisor's Signature .....

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CONTENTS

Page

Page

Page

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ALG	Alginate
CS	Chitosan
ALG-CS	Alginate-Chitosan
EE	Encapsulation efficiency
%	Percentage
hr	Hour
mg	Milligram
rpm	Round Per Minute
DD	Degree of Deacetylation
mL	Milliliter
EAC	Eugenol loaded Alginate-Chitosan
kDa	Kilodalton
SEM	Scanning Electron Microscope
GC	Gas Chromatography
FT-IR	Fourier Transform Infrared Spectrophotometer
TPP	Sodium tripolyphosphate
W/W	Weight by Weight
W/V	Weight by Volume
V/V	Volume by Volume
S.D.	Standard Deviation
cm^{-1}	Unit of wave number
$CaCl_2$	Calcium chloride

CHAPTER I
INTRODUCTION

1.1 Introduction

Global fresh soft fruits such as strawberries, blueberries, raspberries or cherries have one common limitation. The limitation of soft fruits is short shelf life due to microbe such molds and bacterial. Fresh soft fruits are highly perishable product with fast ripening which cause losses during transport and stored. Current technology for preservation of fresh soft fruits in the market such as waxing or radiation are limited by non-effective, high set up cost and the extend shelf life by only 4-5 days. Nowadays, essential oils are usually used for prolonging shelf life of fresh fruits and vegetables due to essential oils consist of phenolic compounds that have effect to inhibit microbe, the effect of essential oils as antimicrobial attract the outer membrane of microbe. Essential oils have been reported to use in food packaging such as oregano, rosemary, carvone thymol or eugenol etc.

Cloves are the aromatic dried flower buds of a tree belonging to the family of Myrtaceae, '*Syzygium aromaticum*' [1]. Eugenol (4-allyl-2-methoxyphenol) as shown in Figure1. Eugenol comprises 72-90% of the essential oil extracted from cloves and is responsible for the aroma of the clove. Eugenol is widely used as a perfume cosmetic pharmaceutical, food industrial, active packaging applications and exhibits pharmacological effects on almost all system. It possesses significant anti-microbial, anti-viral, anaesthecit, anti-fungal properties, eugenol oil for toothache, antiseptic and antioxidant [2].

However, the main drawback of using eugenol is that it is a volatile compound which easily evaporates and decomposes during food processing, drug formulation and

preparation of antimicrobial film, etc., owing to direct exposure to heat, pressure, light or oxygen [3].

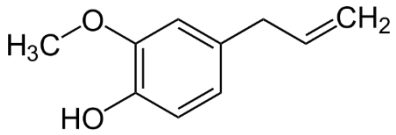

Figure 1. The chemical structure of eugenol

Encapsulation is a technique to encapsulate active agents within a carrier material which one material or a mixture of materials is coated with another material. The coated material is called core material, and the coating material is called shell, encapsulant or wall material [4]. There are many techniques for encapsulation such as extrusion [5], spray dry [6], fluid bed coating [7] or vacuum freeze-drying [8].

Ionic gelation method is a technique that drops droplets of an aqueous solution (most often use concentration of polymer) into a gelling bath (active for crosslinking such as calcium chloride for crosslink alginate, sodium tripolyphosphate for crosslink chitosan). This technique can be easily to prepare a pipette, a syringe, a spraying nozzle [9].

Nowadays, polymers become an alternative material in the food industrial, cosmetic or pharmaceutical. A number of polymer is widely used for the microparticle such as cellulose, pactin, chitosan, carrageenan or alginate. Alginate is anionic polysaccharide. It is easysimplicity to form microparticle by adding polymer solution into a calcium chloride solution. Chitosan is cationic polysaccharide. It can be form microparticle by adding chitosan solution into the sodium troipolyphosphate solution. However, this method was found the grug-loss during the microparticles arrangement. This problem can prevent by blending with other polymer such as chitosan[10], gelatin[11]. Alginate and chitosan are biopolymers that were obtained lots of attention and have been widely study for such use.

Alginate-Chitosan (ALG-CS) polyionic complexes are formed through the ionic gelation via interaction between the carboxylic groups of alginates and the amine

groups of chitosan. Alginate-chitosan microparticles are formed by dropping alginate solution into chitosan solution containing crosslinking agent calcium chloride. The microparticles protect the encapsulant, its biocompatibles and have limitations the release of encapsulant which more effective than either alginate or chitosan because alginate solution is simple to formed alginate microparticle but it not good for release.

Alginate is a natural polysaccharide extracted of brown seaweeds and marine algae. Alginate is a water-soluble linear polysaccharide consisting of alternating blocks 1-4linked α-L-guluronic (G-block) and β-D-mannuronic acid (M-block) residues as shown in Figure 2. The carboxylic groups on these units attribute negative charge of alginate, and thus can interact electrostatic ally with the positively charged molecule to from microparticles. Alginate can be easily cross-linked with non-toxic reactants, especially with divalent cations such as Ca^{2+}, Sr^{2+}, Zn^{2+}, among which Ca^{2+} is the most investigated one

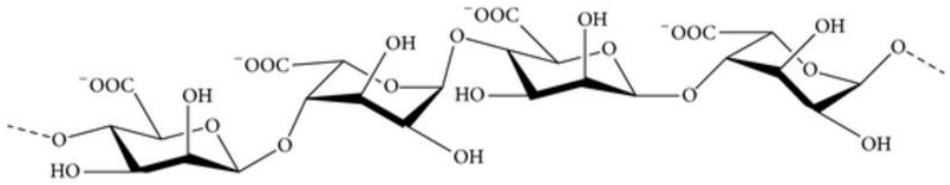

Figure 2. Structure of alginate[12].

Chitosan is a cationic natural linear binary copolymer that consists of β-(1→4) linked 2-acetoamido-2-deoxy-β-D-glucopyranose (Glc-NAc; A-unit) and 2-amino-2-deoxy-β-D-glucopyranose (Glc-N; D-unit). The A- and D-type residues are randomly distributed along the chitosan chain, the structure of chitosan as shown in Figure 3. The chitosan is widely used in the agriculture filed because of its polymeric cations character, good biocompatibility, non-toxic material and biodegradability that manifests antibacterial properties. Chitosan is frequently reacted with tripolyphosphate in the preparation of nano/microparticles.

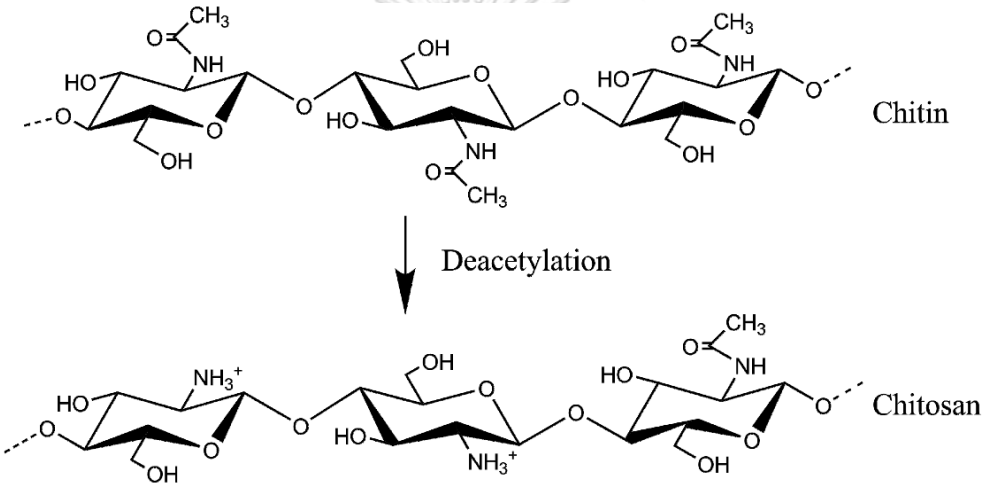

Figure 3. Structure of chitosan[13]

1.2 The objectives of this research

The aim of this present work is to prepare microparticles of eugenol, using the matrix polymer consisting of alginate, chitosan as a retarding material, by ionic gelation method. The detail of these objectives was described as follows:

1) To evaluate the effect of alginate concentration on the alginate-chitosan microparticles.

2) To prepare eugenol loaded alginate-chitosan microparticles by using ionic gelation technique

3) To study the release profile of alginate-chitosan microparticles in soft skin fruit during storage such as strawberries.

4) To study antimicrobial profile in soft skin fruit such as strawberries with the microparticles.

5) To study general quality parameter of soft skin fruit

1.3 The scope of research

The scope of this research was carried out by stepwise investigating as follows:

1) Part I: Preparation of the alginate-chitosan microparticles

a. Study alginate-chitosan microparticles with parameters of alginate concentration and chitosan concentration

b. Characterization of obtained microparticles in terms of morphology and chemical reaction of microparticles by using SEM and FTIR

2) Part II: To evaluate the conditions for preparation alginate-chitosan microparticles loaded eugenol

a. To study the effect of calcium chloride concentration to prepare eugenol loaded alginate-chitosan microparticles by using ionic gelation technique

b. To study the effect of surfactant concentration to prepare eugenol loaded alginate-chitosan microparticles by using ionic gelation technique

c. To Study the effect of eugenol concentration to eugenol loaded alginate-chitosan microparticles by using ionic gelation technique

d. Evaluation of eugenol encapsulation efficiency

e. To study releasing profile of eugenol loaded alginate-chitosan microparticles in soft skin fruits

3) Part III: Study properties of eugenol loaded alginate-chitosan microparticles

a. Study antimicrobial profile of eugenol loaded alginate-chitosan microparticles

b. Study general quality parameter of soft skin fruits such as color, firmness, weight loss

CHAPTER II
BACKGROUND AND LITERATURE REVIEWS

Clove plant comprises of clove oil. The main components of clove oils were eugenol, it is extract manufactured by solvent extraction from clove, nutmeg, cinnamon, basil, and bay leaf. In clove contains the highly of eugenol (90%). Eugenol has several properties such as antioxidant, anti-inflame, antiseptic, antioxidant including activity against fungi.

In agriculture, post-harvest handling is the stage of crop production including cooling, cleaning and packaging. The most important of post-harvest handling is post-harvest technology involve all treatments or process that occur from time of harvesting until the fruits reaches to final customer such as keeping fruits and vegetables the product cool, to avoid moisture loss by using chemical such as waxing, avoiding physical damage example bruising or to delay spoilage by using irradiation technique or using essential for prolong shelf life of fruits and vegetables such as essential oil blending with edible film, using essential oil incorporated with packaging and slowly release essential oil to modified atmosphere packaging (MAP) [14] or using polysaccharide to encapsulated essential oil and control release essential oil to inhibit microbial in fruits and vegetables.

2.1 Fresh Soft fruits

Fresh soft fruits give essential components such as vitamin mineral carbohydrate and fiber that important for human. Moreover, soft fruits such as strawberries, blueberries, raspberries and blackberries are high antioxidant and also have no cholesterol, but the limitation of soft fruits are softening and short shelf life. The strawberries are spoiled fruits due to high respiration rate, softening and short shelf life so that management post-harvest is important either transportation or commercialization. Nowadays, the postharvest handing of soft fruits is abundant such as waxing, modified atmosphere packaging (see in Figure 4), radiation (see in Figure 5)

and edible film or used essential oils for inhibit mold and extended shelf life of soft fruits.

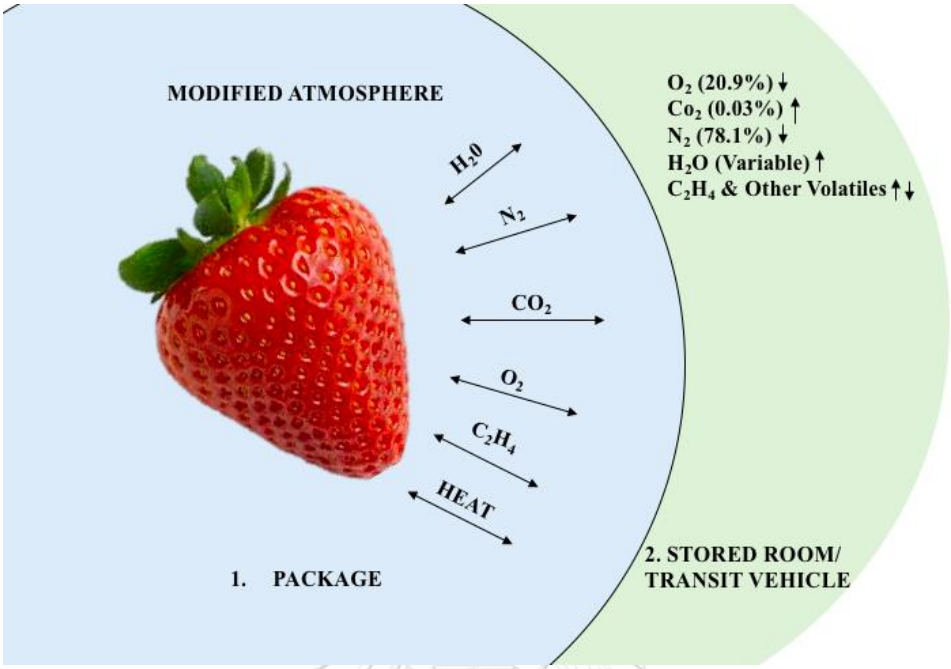

Figure 4. Modified atmosphere packaging (MAP)

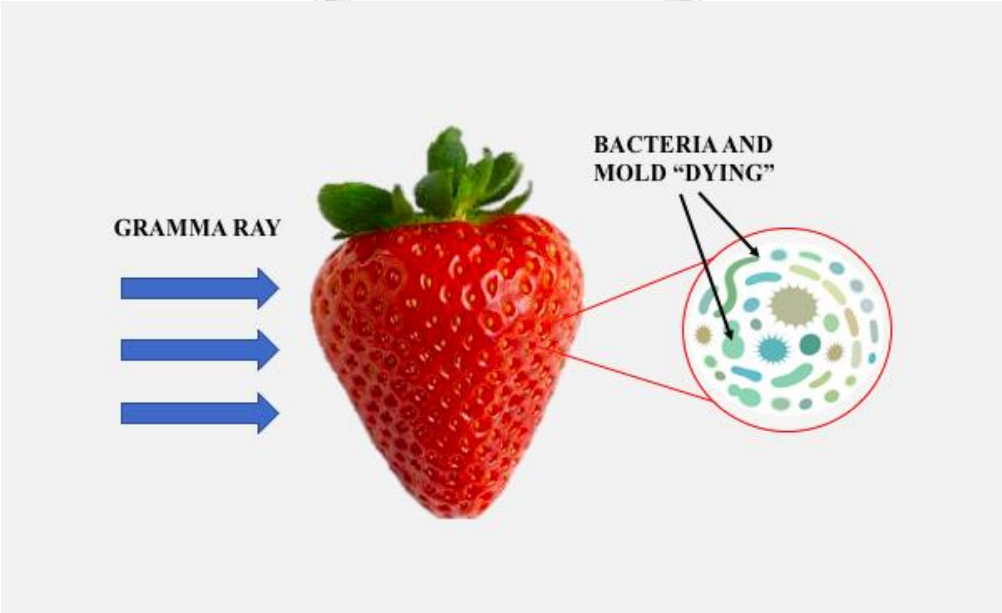

Figure 5. Radiation for extended shelf life of fruits

2.2 Essential oils

Essential oils are aromatic oily liquids extracted from aromatic plants by using hydro distillation, steam diterpenstillation or dry distillation technology from buds, flower, herbs leaves, root and wood [15]. Essential oils have several complexes compositions. The great of components in essential oils, terpenes with monoterpenes include allyl- and propenylphenols (penylpropanoids) are important compounds in essential oils. The essential oils are easy to volatile, lower density than water and colorless. Several properties of essential oils have been showed in the other applications such as anti-viral, anti-cancer, anti-inflammatory, anti-septic, anti-oxidant and anti-microbial.

2.2.1 Eugenol

Eugenol is a major compound of clove oils (approximately 90%) Eugenol, which is widely used in several applications such as food active packaging [16] for prolong shelf life of fruits and vegetables because of its antimicrobial activity. The activity of eugenol for inhibit bacteria and fungi were showed in Figure 6 and the chemical structure of eugenol as shown below in Figure 7.

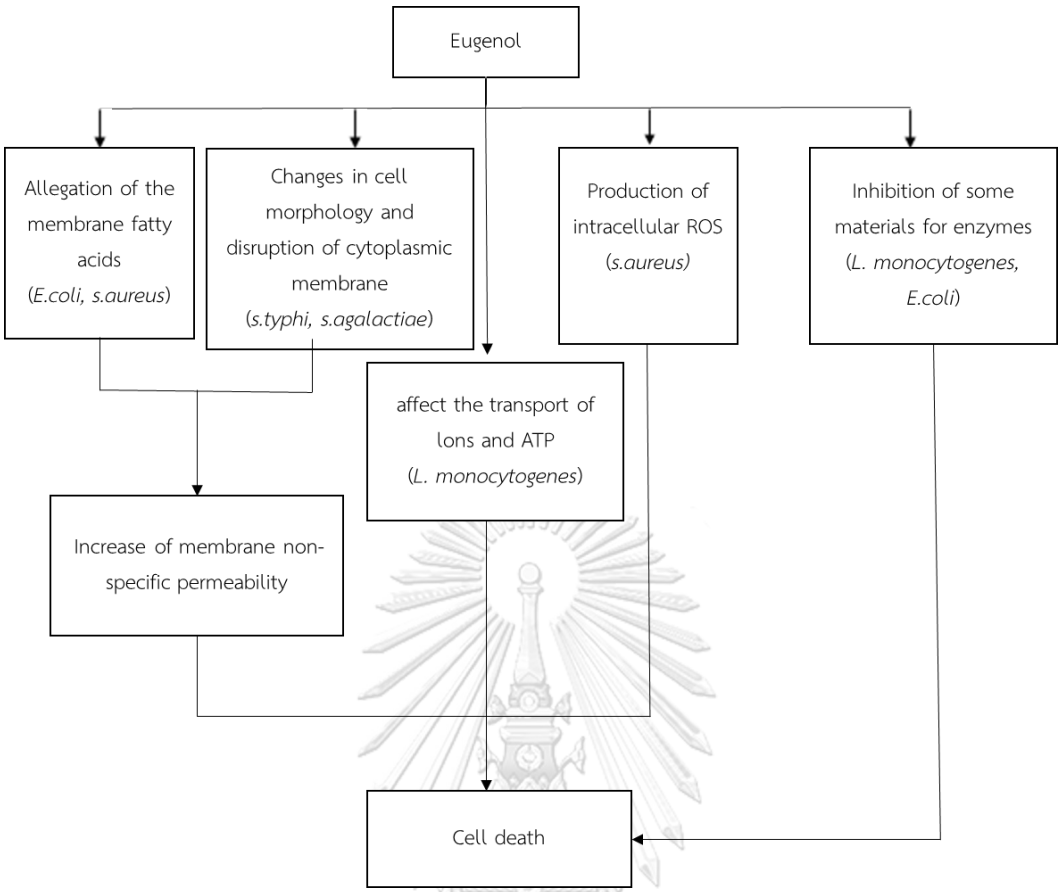

Figure 6. Effects of eugenol against bacteria and fungi *[17]*

2.2.2 Physical properties

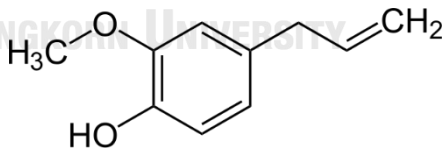

Figure 7. Chemical structure of Eugenol

IUPAC name: 4-allyl-2-methoxyphenol

Molecular formula: $C_{10}H_{12}O_2$

Molecular weight: 164.2 g/mol

Melting point: -7.5 °C

Boiling point: 254 °C

Density: 1.06 g/cm^3

Solubility (water): less than 1 mg/ml at 20 °C

Physical description: Clear colorless pale yellow or amber-colored liquid. Odor of cloves. Spicy pungent taste.

Solvent solubility: eugenol is slightly soluble in water and soluble in organic solvent

2.3 Microencapsulation

Microencapsulation is a process by which solid particles or liquid solution are enclosed by an integral shell. The method has core and shell materials that select to prepared microparticles to improve delivery of bioactive e.g. antioxidants or easy to evaporate [18]. The microencapsulation is widely used in many applications such as pharmaceutical [19], agriculture [20], cosmetic [21] or food application [22]. Materials are usually designed as protective encapsulates. Mostly, this material should be a biodegradable food-grade polymer and also acts as barrier between the internal layer and outer layer. Materials are the most widely used for encapsulation in many applications especially in food application [23]. Among different types of polymer, carrageenan, chitosan, alginate, gelatin, and pectin. There commonly used as an outer layer because of non-toxic, safe, and especially its antimicrobial activity.

There are many techniques for encapsulations. Since encapsulating is a liquid form, many technologies based on drying like spray drying, fluid bed coating, spray cooling are available to encapsulate the active agent. Emulsification methods is a case of water solution agents and there are two unifications of emulsions such as water/oil/water, water/oil or oil/water and it can be dried by several techniques.

Ionic gelation method is comprising of two parts dropping droplets of an aqueous solution of polymer and active agent into gelling bath. The dropping tool are technically easy to use such as a syringe, a pipette, a vibrating nozzle, a spraying nozzle or atomizing disk. Ionic gelation method is especially effective for product of small particles.

Food applications of microencapsulation

- Prevent vaporization of volatile compounds such as aroma or organic molecules
- Prevent probiotics, probiotics are highly sensitive to surrounding [24].
- Used to mask unpleasant such as bitter taste and other compounds that high antioxidant activity [25].
- Used to enzymes immobilize cells or yeast in food processing [26].

A main advantage of microparticles provides an efficiency method to entrap an active agent that protect by wall materials. Moreover, the microparticles can be controlled by slow release characteristics.

2.4 Controlled release system

Controlled release is a technique for active agent by using polymer. This technique has existed for over three decades in application of pharmaceutical and agriculture industrials.

Controlled release system is comprised of active agent such as volatile compounds, drug molecule or enzymes that combined with polymeric system in such a way active agent is released from the polymeric in predesigned manner.

2.4.1 Controlled release mechanism

In application of microencapsulation, controlled release system is an application that important. Controlled release system can be released by 3 mechanisms.

2.4.1.1 Diffusion Controlled Release

Diffusion Controlled Release occurs when the drug passes from the polymer matrix to the external environment. As the release continuous, its rate will be depending on type of system. The mechanism of diffusion controlled release as shown in Figure 8 [27].

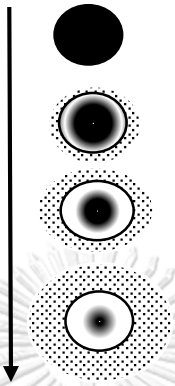

Figure 8. Presentation of diffusion controlled release

2.4.1.2 Swelling Controlled Release

Swelling controlled release is a technique that the swelling of the carrier increases the solvent content within the polymer matrix, enabling the drug to diffuse thought the swollen network into the external environment [28]. Most of materials used are based on hydrogel. The swelling of hydrogels has ability to swell in water, aqueous, solvents, temperature, pH, etc. the mechanism of swelling controlled release as shown in Figure 9.

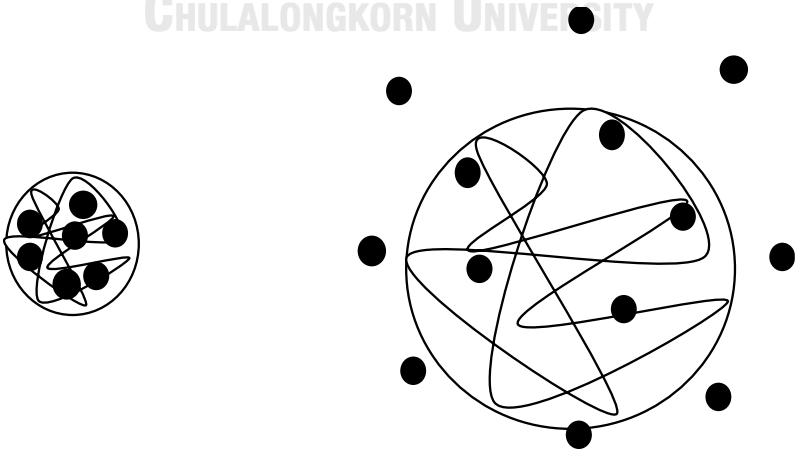

Figure 9. Presentation of Swelling controlled release

2.4.1.3 Erosion Controlled Release

The drug can be release from the matrix by erosion controlled release system [29], which can be classified 2 type [30].

3.1 Surface Erosion: The material degrades from the outer surface. The density of the matrix remains constant while the volume decreases the mechanism as shown below in Figure 10.

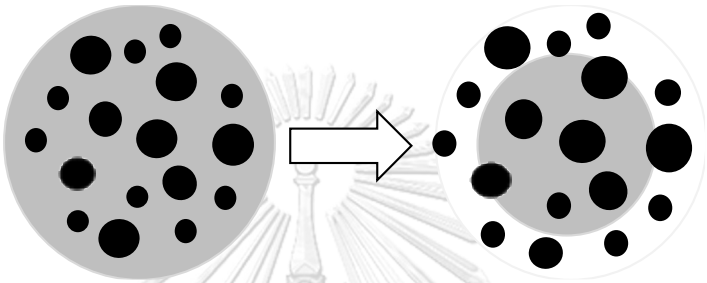

Figure 10. Presentation of erosion controlled release-surface erosion

3.2 Bulk Erosion: The material degrades throughout the bulk of the materials. The density of the matrix decreases while the volume remains constant the mechanism as shown below in Figure 11.

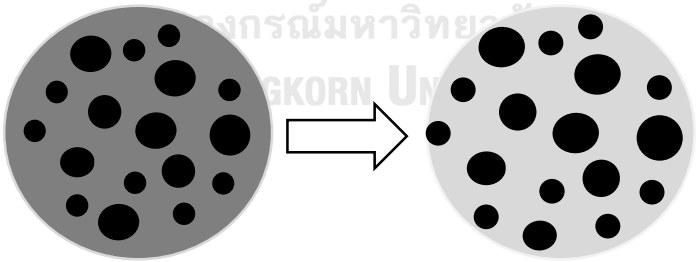

Figure 11. Presentation of erosion controlled release-bulk erosion

2.5 Preparation of polymeric particles

The main types of preparation methods of micro/nanoparticles, are as follow;

1. Ionic gelation
2. Emulsion cross-linking
3. Coacervation method
4. Spray-drying
5. Electrospray ionization method
6. Inclusion complexes

Various methods for preparation of micro/nanoparticles have been reported such as, complexes coacervation of chitosan/k-carrageenan, water in oil emulsification, gelification and Ionic gelation.

This work focuses on a technique for preparation of eugenol loaded alginate-chitosan microparticles using ionic gelation.

2.6 Polyelectrolyte complexes (PECs)

Polyelectrolyte complexes are macromolecules containing ionizable groups. They can form polyelectrolyte complexes (PECs) with opposite charged molecules or polymer through intermolecular interactions. They can be divided into two classes, i.e. chemical and physical or reversible hydrogel. In chemical hydrogel, it different charged of polymeric are handled by covalent crosslinks, but in physical hydrogel the opposite charged polymer or molecule are through internal interaction, i.e. van der Waals force, ionic bonds, hydrogen bonding such as alginate and chitosan can be used together to form polyelectrolyte complexes in many applications such as encapsulate proteins or enzyme, biomaterials, removal heavy metal from industry waste waters and encapsulated volatile compound in application of food industry [31].

2.6.1 Chitosan-alginate polyelectrolyte complexes (PECs)

Alginate is a polysaccharide consisted of 1,4-linked β-D-mannuronic acid [M] and α-L-guluronic acid [G] monomer in varying propositions. The negative charged

carboxylic group of mannuronic acid or guluronic in alginate interaction electrostatic with the positive charged amino groups of chitosan to form polyelectrolyte complexes [32]. The mechanism of alginate and chitosan as shown below in Figure 12. Currently the most common crosslinks used for chitosan-alginate complexes in form of film membranes or beads by several research or technique applications. For example, chitosan-alginate microspheres were developed to encapsulated coriander essential oil by external gelation method and study release rate of coriander essential oil with microsphere [33].

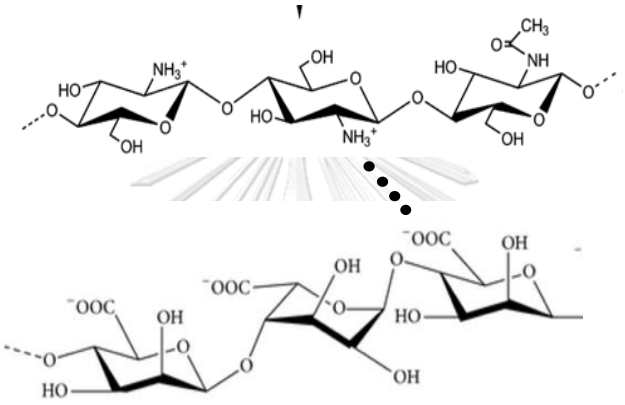

Figure 12. Schematic chemical structure of chitosan-alginate

2.6.2 Chitosan

After cellulose and chitin, chitosan are polysaccharides with similarity with chitin, chitin is obtained from exoskeletons (crabs and shrimp) after demineralization, deproteinization and decoloration of cellulose. Chitosan is obtained after deacetylation of chitin [34]. The chemical structure of cellulose, chitin and chitosan as shown in Figure 13.

Chitosan is cationic natural linear polysaccharide consisting of copolymer of D-glucosamine and N-acetyl-D-glucosamine units linked by β-(1-4)-glycosidic linkages. Chitosan is soluble in dilute acidic solution. The degree of deacetylation (%DD) has a significant effect on the solubility and properties of polymer. The degree of deacetylation is one of the more important chemical characteristics of chitosan. They are several methods for checking the removal of acetyl groups in chitosan such as UV spectrophotometry, IR spectrometry, gas spectrometry and dye adsorption [35].

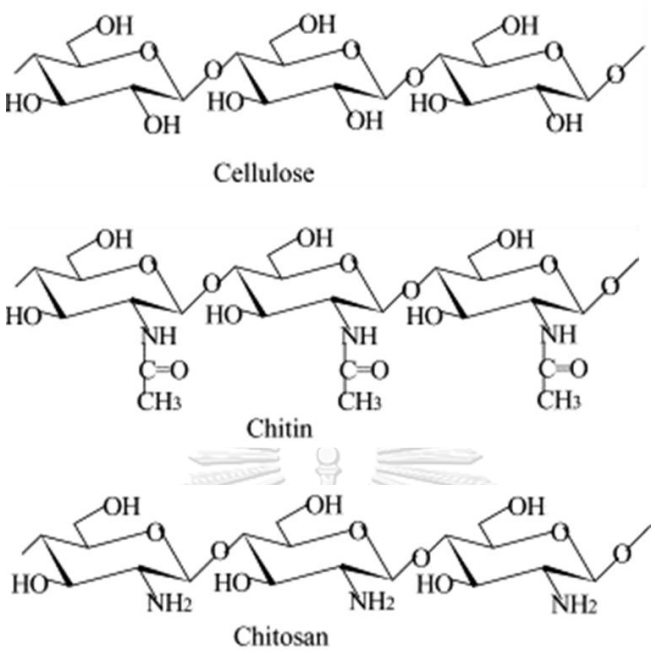

Figure 13. Chemical structure of cellulose, fully acetylated chitin and fully deacetylated chitosan

2.6.2.1 Properties of chitosan

2.6.2.1.1 Biocompatibility and biodegradability

Chitosan is a natural polysaccharide that has been studied in biomedical and found that it is highly biocompatible and also biodegradable [36].

2.6.2.1.2 Mucoadhesive

Chitosan is amino polysaccharide, which soluble in aqueous solution at pH<6.The cationic natural polymer of chitosan showed strong electrostatic with negative charge mucosal surface. More specifically, chitosan shows the ability to adhere gastric mucosa *in vitro*. Therefore, it could be used in site-specific drug delivery [37].

2.6.2.1.3 pH sensitiveness

Chitosan exhibits a pH-sensitive in a weak polybase due to the amino group on its structure. Chitosan dissolves easily at low pH but poorly in higher pH. The mechanism of pH sensitive swelling associates with the protonation of amine groups of chitosan under low pH. This protonation diffuses proton and counter ion together with water inside the gel [38].

2.6.2.1.4 gelation

Chitosan solution can be formed to a gel by adding cross-linking agent. One of the commonly used cross-linking agent for chitosan by ionic gelation is sodium tripolyphosphate (see in Figure 14.). Sodium tripolyphosphate (TPP) is multivalent anions and nontoxic. It can form gel by interaction between positive charge of chitosan and negative charge of TPP. The crosslink properties help increase reinforce of chemical and mechanism of chitosan [39].

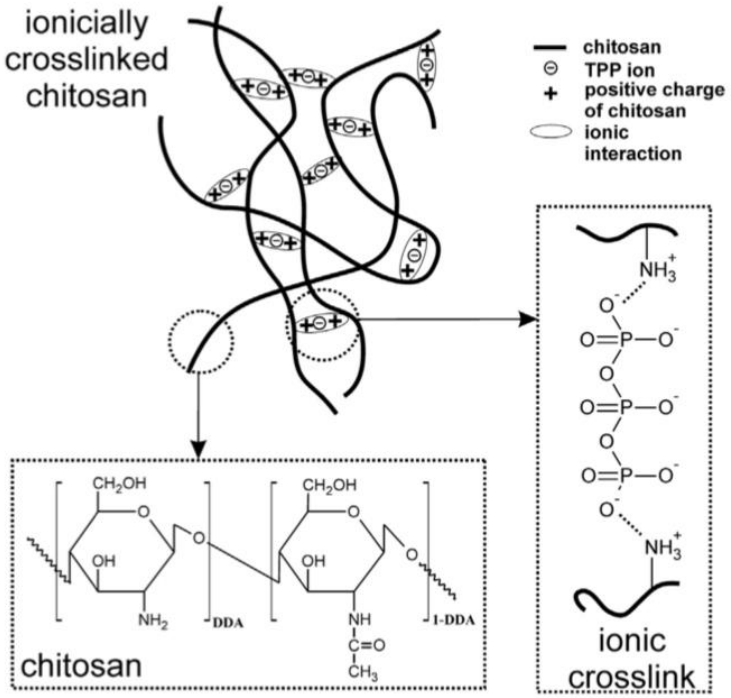

Figure 14.Structure of chitosan crosslink with sodium tripolyhosphate (TPP)

2.6.2.2 Chitosan used

2.6.2.2.1 Agriculture

In agriculture, chitosan is a polysaccharide that commonly used in application of preservative for extend shelf life of fruits and vegetables, during the storage. As you know chitosan is a biopolymer, low potential to be harmful, environmental safe and also it is a good antimicrobial agent. For example, chitosan as coating materials for preservative in storage of organic for reduce respiration rate, ethylene production and inhibit mold, bacteria [40].

2.6.2.2.2 Cosmetics and pharmaceutical

In cosmeceutical industry, chitosan is commonly used due to their unique biological properties. For examples in pharmaceutical, chitosan can be used in difference part such as in fields of dentistry chitosan can be preventive dentistry, conservative dentistry and orthodontics [41]. In cosmetics, chitosan is commonly used in skin care fields such as application of UV protection, skin conditioning and emollient, moisturizing.

2.6.2.2.3. Environmental treatment

In environmental treatment, chitosan is highly stable materials and difficult to degrade materials. One of the application is study ability of chitosan, as an effectiveness of major environmental treatment. Chitosan could remove metal ion from waste water [42].

2.6.3 Alginate

Alginate is a natural biopolymer extracted form marine brown. It is polysaccharides consisting of 1,4-linked β-D-mannuronic acid and α-L-guluronic acid monomer in varying proportions (see in Figure 15). Alginate is used as a biopolymer for many applications due to its good biodegradability, bioadhesiveness, film and gelation properties [43].

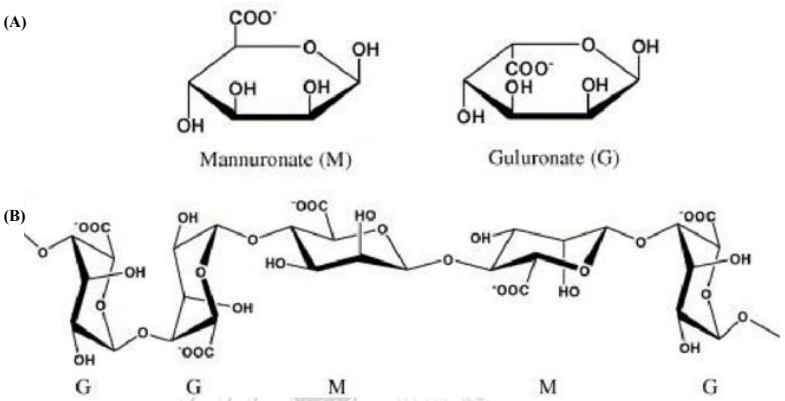

Figure 15. (A) structure of alginate monomer and (B) chain of alginate [44]

2.6.3.1 Gel formation

The gelation of alginate that commonly used crosslinked alginate to form gels are divalent cations such as Ca^{2+}, Sr^{2+} or Ba^{2+}. The gelation and cross-linking of this polymer can be increased the technologist properties. The gelation of this polymers is mainly by the exchange sodium ions from structure with the divalent cations and their polymer to from the characteristic egg-box structure [45] shown in Figure 16.

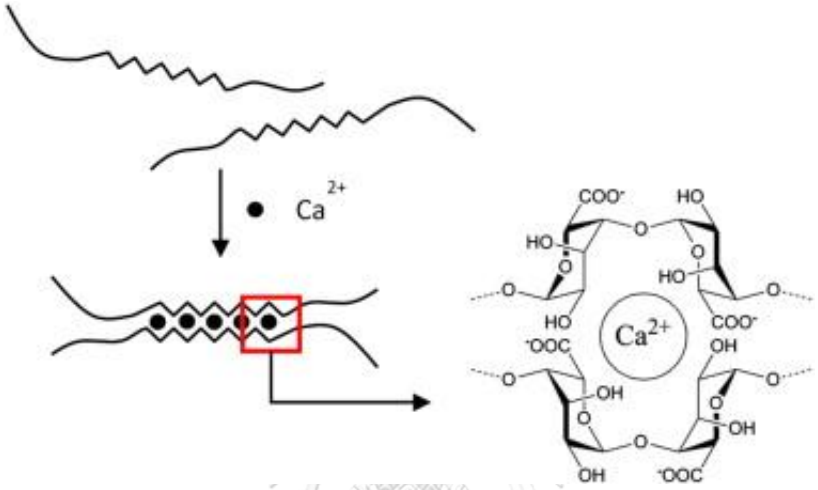

Figure 16. Egg-box structures of alginate and formation of alginate gel by Ca^{2+}ions

2.6.3.2 Properties of Alginate

2.6.3.2.1 Biodegradability

Alginate is used extensively for food industry as a stabilizer, edible film, emulsifier and a thickener. Alginate is polysaccharides that included in a group of compounds which are generally regarded as safe (GRAS) by the FDA [46].

2.6.3.2.2. Bioadhesive

Alginate is an anionic polymer that have carboxyl end groups, it's a good mucoadhesive agent, alginate showed that the highest mucoadhesive as a compared with polymer such as chitosan, carboxymethylcellulose and polystyrene. Due to the adherence of alginate microparticles to the mucosal tissues, protein transit time is delayed, and the drug is localized to the adsorptive surfaces. It improved drug effectiveness and bioavailability [47].

2.6.3.2.3. pH sensitive

Alginate is a polysaccharide that have carboxyl end group, in theoretical the most commonly pH-responsive functional groups are carboxyl and pyridine groups. The carboxyl groups are functional groups that consist of carbonyl and hydroxyl. The pH-sensitive is a membrane can be divided non-porous and porous membranes for non-porous when barrier swelling can be change the permeability and selectivity and porous membranes layer of grafted functional polymer can be reversibly changes permeability or selectivity [48]. The application of pH-sensitive membranes has been widely used in the applications of drug control release or control water flux.

2.6.3.3 Alginate uses

The uses of alginate are based on many properties such as the first properties, when dissolved alginate in the water, the results solution was viscous and thickened or the seconds properties alginate can be forms gel with calcium chloride salt by chemical reactions, the calcium will be displacing the sodium from alginate, each alginate molecules will hold together, and a gel is the results.

2.6.3.3.1. Food

In food, alginate is used in food because it's powerful thickening, stabilizing and gel forming agent. Some food such as ice-cream, fruits-filled snacks, salad dressings are including alginate as an ingredient.

Additionally, the application of alginate is widely for preservative fresh food, fresh fruits and fresh vegetables [49]. For example, alginate have been used to help preservation of fresh fruits by using alginate as an edible coating with essential oils for extend shelf life fruits storage, the results edible coating of alginate and essential oils could be preservative quality of parameter such as sensory test and reduce microbials spoilage [50].

2.6.3.3.2. Textile printing

In textile printing, sodium alginates are used as thickener for the paste containing the dye [51]. There paste may be applied to the fabric by screen or roller printing equipment. Sodium alginate is an important thickener in reactive dye printing system due to it has absence of primary hydroxyl groups. Also, under alkaline conditions, the carboxyl group of alginates eliminate the dye anions and then decreasing the dye thickener reactivity. In textile printing the application of thickness is used with dye to be washed off the fabric chemical or mechanical finishing.

2.6.3.3.3. Pharmaceutical and medical uses

The fiber of calcium alginate is widely used in wood dressings [52]. They have very good wood properties and it can be absorbed by body fluids due to the calcium in the alginate is exchange with the sodium from the body fluid and give a soluble sodium alginate, this also easy to remove these dressings from the large open woods.

2.7 Surfactant

Polysorbates for example Tween 20, 60 and 80 are widely used in agriculture food industries [53] and pharmaceutical [54]. For agriculture application, polysorbates are useful in emulsifying agent to disperse essential oils for inhibit microbial and extended fresh fruits and vegetables. Moreover, in food application due to polysorbates are emulsifiers. It was added to ice-cream to make smoother, easy to handle and prevent melting.

Polysorbate 80 or Tween 80 is a nonionic surfactant and emulsifier often used in several application such as food, cosmetic, pharmaceuticals or agriculture. Polysorbate 80 is from polyethoxylated sorbitan and oleic acid. In structure of polysorbate 80 comprised of two groups, (i) hydrophilic groups of polyether (polyoxyethylene groups) and (ii) hydrophobic groups of oleic acid, which refers to ability to dissolve in non-polar agent such as fat, oils or lipids [55]. The structure of polysorbate or tween 80 as shown in Figure 17.

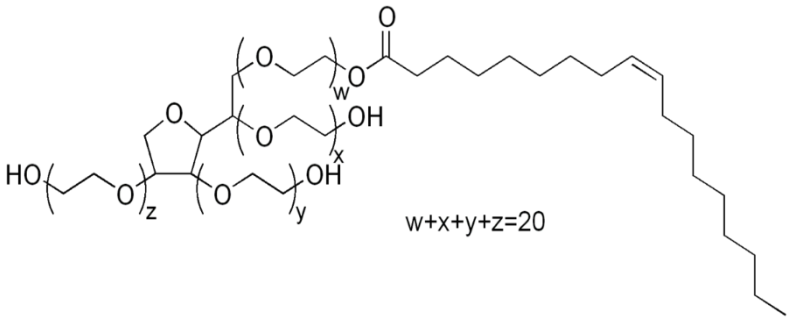

Figure 17. Structure of polysorbate 80 (Tween 80)

The aim of this work is to prepare eugenol essential oils-loaded alginate-chitosan microparticles using calcium chloride and TPP as a cross-linked agent by ionic gelation technique. The eugenol and alginate-chitosan solutions cloud be agglutinate by surfactant (polysorbate 80). The mechanism as shown in Figure 18.

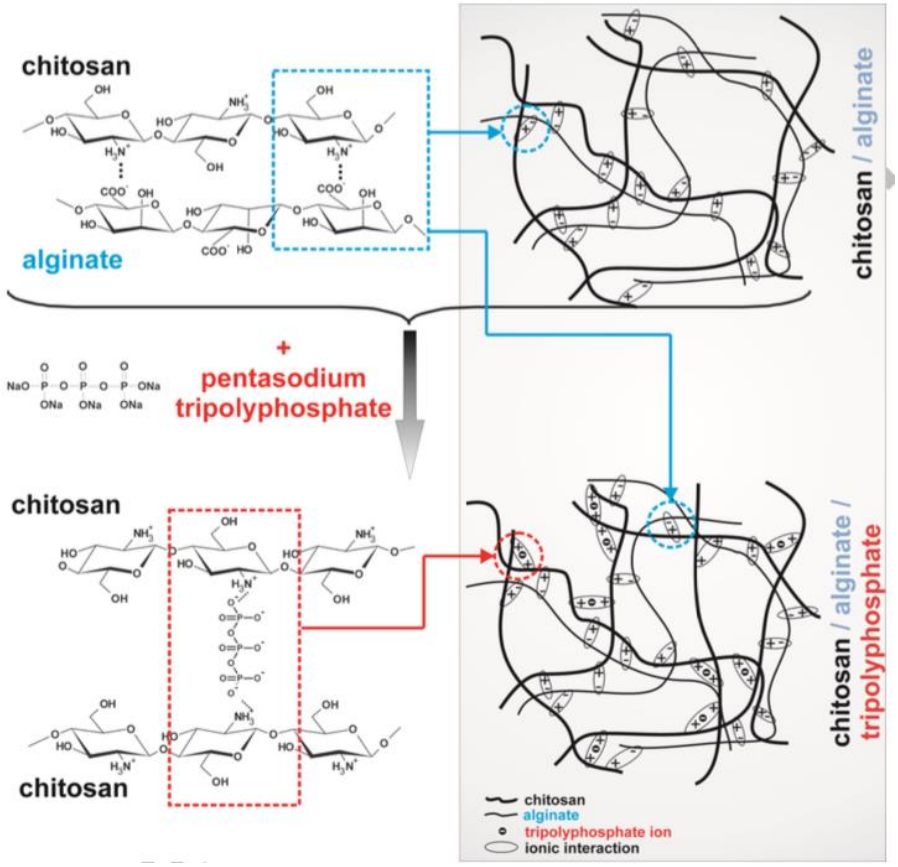

Figure 18. The model of alginate-chitosan microparticles/ alginate-chitosan and TPP

2.8 Literature reviews

Mangal Nagarsenker and coworker[56] were studied microencapsulation of eugenol using gelatin-sodium alginate complexes coacervation. The eugenol were evaluated for surface characteristics oil loading and encapsulation efficiency. The results showed that the percent loading and encapsulation efficiency increased with increased percent of core: core ration

Dima and coworker[57] used alginate and chitosan as shell and coriander essential oils as core substance by using emulsification external gelation method with in the concept of encapsulated coriander essential oils in polymer. They studied results of encapsulation efficiency, swelling degree and release rate. The content of difference of chitosan and alginate microspheres were showed the release rate is influenced by the chitosan-alginate. So that, the content of chitosan increases the release rate is grow up as pH 4 and decrease as pH 5. Moreover, temperature is effect to the release profile of coriander essential oils due to some morphology of this application modified that easily diffuse to the microspheres pores.

Maria G.C. Miguel and coworker[58] were developed edible coating for increase shelf life of some horticultural product such as strawberries, in this research studied edible coating based on alginate and pectin merge with essential oils such as citral and eugenol. The results showed that during stored 0, 7 and 14 day, the edible coating were showed the best for preserving strawberries. Moreover, they were studied quality parameter color (L^*, h°) firmness, weight loss and soluble solid content and no significant difference between group treatment and control.

Yi-Ru Jing and coworker[59] were prepared electrospun of polyvinyl alcohol with cinnamon essential oil and β-cyclodextrin for active food packaging. Moreover, they studied antimicrobial and quality parameter such as color value, appearance, taste, brightness and odor during stored at 0-18 day in Figure 19.

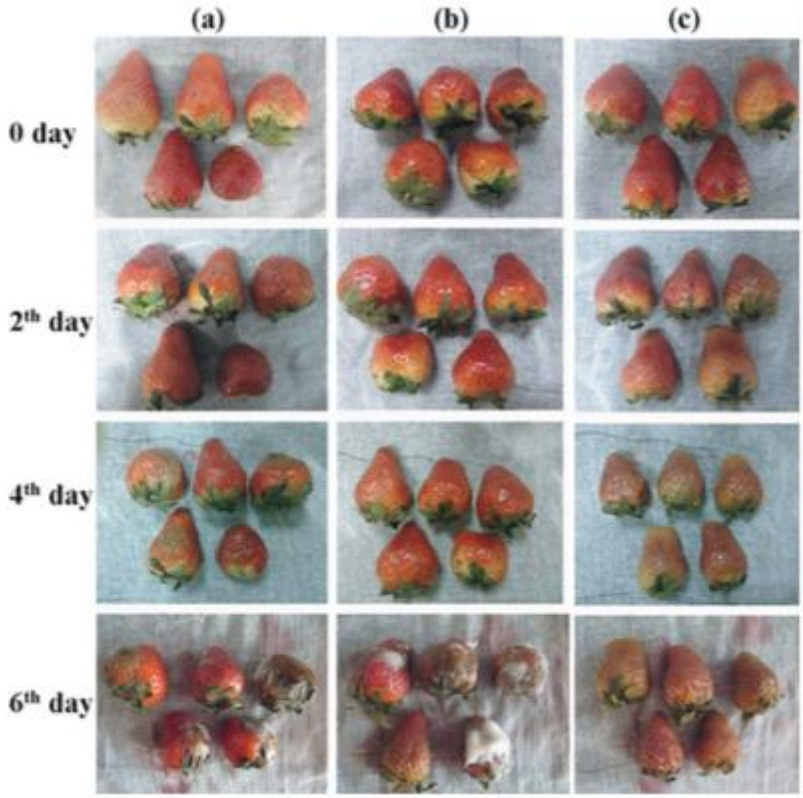

Figure 19. Appearance of strawberries at 21 °C (A) control (B) packed with keep film (C) packed with PVA/CEO/β-CD nanofilm

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

3.1.1 Active components

Eugenol were purchased from Chemipan Corporation (Food grade)

3.1.2 Polymer

- Sodium alginate (Food grade), average molecular weight is from 14,000 to 132,000 were purchased from Union Chemical 1986

- Chitosan with an average molecular weight (Food grade), MW, of 500 kDa and a degree of deacetylation (DD) of 81% from Sea fresh

3.1.3 Chemicals

- Calcium chloride (Union Chemical 1986)

- Tween 80, Food grade (Union Chemical 1986)

- Ethanol (Carlo Erba Reactifs SA)

- Acetic acid glacial grade (Carlo Erba Reactifs)

- Sodium tripolyphosphate (Union Chemical 1986)

3.1.4 Instruments

The instruments used in this study are listed in Table 1.

Table 1. Instrument

Instruments	**Manufacture**	**Model**
Scanning electron microscope	Philips	XL30CP
Fourier transform spectrometry	Nicolet	6700
Magnetic stirrer	LMS	C-MAG HS 7
Gas chromatrography	Varian	CP-3800 Gas chromatography
Micropipettes (100-1000 µL)	Mettler Toledo	Volumate
Fruit penetrometer	Hangzhou Chincan Trading	GY-3
Portable refractometer	Advance Research Instruments Company	PR-32

3.2 Preparation of microparticles

3.2.1 Preparation of alginate-chitosan microparticles

Alginate-chitosan microparticles were prepared by ionic gelation method using alginate as a gel core. The alginate solution was prepared by dissolving ratio of sodium alginate in 100 ml of distilled water. A gelling bath was prepared by mixing 100 ml of of chitosan solution dissolved in 1% (V/V) acetic acid and 100 mL of 2% (W/V) calcium chloride solution. The alginate solution was extruded into 200 mL of a gelling bath. The microspheres were stirred 2 hr. The particles were cross-linked by adding 5 mL of 2% (W/V) of sodium tripolyphosphate (TPP) around 30 min before washing with distilled water and dried at room temperature. As shown In Figure 20. The method for prepared the alginate-chotosan microparticles as a difference ration of chitosan and alginate.

Table 2. The ratios of chitosan and alginate for preparation alginate-chitosan microparticles

Ratio of chitosan and alginate (w/w)	Total solid (chitosan and alginate) 4 g	
	Chitosan (g)[a]	Alginate (g)[b]
1:0.1	3.64	0.36
1:0.3	3.08	0.92
1:0.5	2.70	1.30
1:1	2.00	2.00

[a]: dissolved in 100 mL 0f 1% acetic acid

[b]: dissolved in 100 mL 0f distilled water

The ratios of chitosan-alginate microparticles in this study were 1:0.1, 1:0.3, 1:0.5 and 1:1 (total solid 4 g), as shown in table 2. In addition, alginate-chitosan microparticles ration 1:1 cross-linked with TPP, studies of the release profile of essential oil.

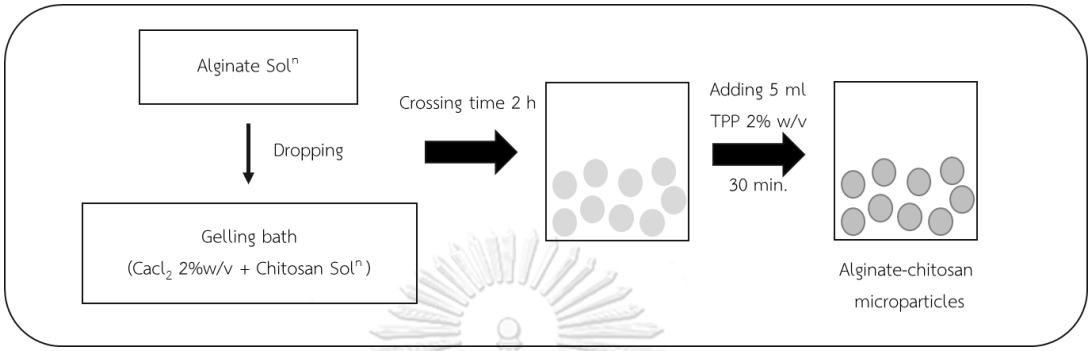

Figure 20. Preparation of alginate-chitosan microparticles

3.2.2 Preparation of eugenol-loaded alginate-chitosan microparticles

The microparticles were prepared by using a chitosan: alginate: eugenol essential oil mass ratios 1:1:0.1-1:1:0.5. Eugenol essential oil was mixed with surfactant tween 80 ratios 2, 4, 6, 8 and 10% (W/V), (EAC2-1 – EAC2-5, EAC4-1 – EAC4-5, EAC6-1 – EAC6-5, EAC8-1 – EAC8-5 and EAC10-1 – EAC10-5) and then added in 100 mL of 2% (W/V) alginate solution under IKA RW20 digital at 400 rpm for 30 min. This solution was extruded into 200 mL of a gelling bath (100 mL of 2% (W/V) chitosan and 100 mL (W/V) of 2% calcium chloride solution) using a syringe needle. The microparticle were stirred for 2 hr. and then the particles were cross-linked by adding 5 mL of 2% (W/V) sodium tripolyphosphate (TPP) around 30 min before washing with distilled water and dried at room temperature as shown in Figure 21.

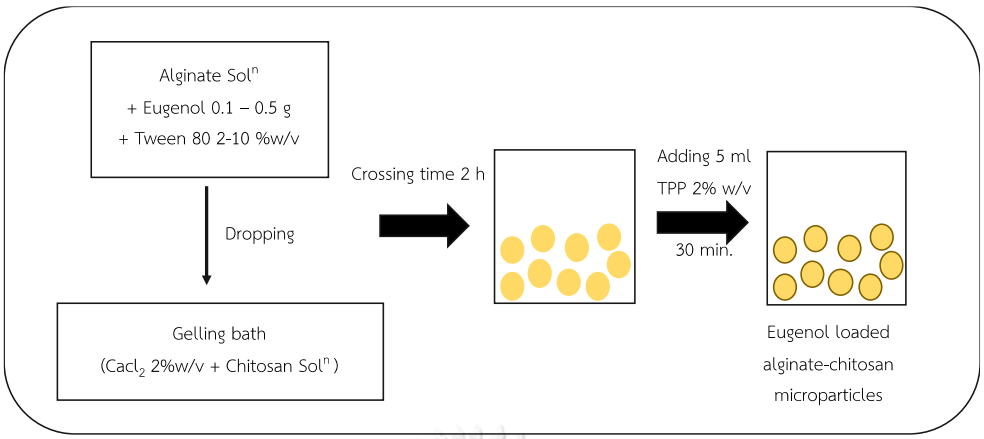

Figure 21. Preparation of eugenol-loaded alginate-chitosan microparticles.

3.3 Characterization of alginate and chitosan microparticles

3.3.1 Fourier transform spectrometry (FTIR-ATR)

FTIR spectra of dried samples were acquired with a Nicolet 6700 FTIR spectrometer in the region over the 4000-400 cm^{-1}. FTIR is a method of measuring infrared absorption and emission spectra. The spectra of pure alginate, pure chitosan and alginate-chitosan microparticles were reported in wavenumber (cm^{-1}) and FTIR information was assessed with the OMNIC software.

3.3.2 Scanning electron microscopy (SEM)

SEM morphologies of sample were mounted on an aluminum stub using double-sided carbon adhesive tape and coated with gold-palladium. The microspheres were investigated by SEM using Philips XL30CP and performed under high vacuum and ambient temperature with beam voltage of 60 KV.

3.4 Instrumentation and Analytical Conditions

A gas liquid chromatography with flame ionization detector (CP-3800 Gas chromatography) was used for the determination of eugenol. Instrument is coupled with a split/split less injector, operation in a split mode and used FID is a detector. The computer software has been used to control the gas chromatography and DB-5 capillary column (cross bond 5%-phenyl/95% methylpolysiloxane) with an internal

diameter of 0.25 nm and a length of 30 meters was used throughout this study. The GC-FID parameters used in the method development were based on the boiling point of the essential oil. Eugenol has a boiling point about 254 ºC. The injection port and detector temperature were set to 225 ºC and 280 ºC, respectively. The split injection of approximately 1 µL sample was performed at an inlet temperature of 225 ºC. The detector temperature was set to 280 ºC. After injection, the oven temperature was increased quickly from 110 ºC and then programmed within 8 min to 280 ºC at a rate at 15 ºC per min for 2 min. Nitrogen at a flow rate of 1.18 mL/min was used as a carrier gas.

3.5 Determination of eugenol content and encapsulation efficiency (EE)

3.5.1 Standard curve of eugenol by using gas chromatography

A stock solution of 3000 mg/L was prepared by dissolving in 150 mg of standard eugenol in ethanol and adjust the volume to be 50 ml in volumetric flask. This stock solution was individually pipetted 10, 6.7, 5, 3.4, 2, 1.67, 1.34, 1, 0.66 0.33, 0.23 and 0.17 mL respectively, into 10 mL volumetric flask and adjust the volume with ethanol. The final concentrations of each solution were 3000, 2000, 1500, 1000, 600, 500, 400, 300, 200, 100, 70 and 50 mg/L., respectively.

3.5.2 Determination of encapsulation efficiency (EE) and loading

3.5.2.1 Encapsulation efficiency

The encapsulation efficiency of eugenol in this study was done. The weight encapsulation amount of microparticles 500 mg was poured into 10 mL of ethanol and then stirring for 24 hours. Then, solution was filtered thought nylon filter (0.20 µm, Nylon, Syringe Filters) and determined eugenol content by Gas chromatography. The actual amount of eugenol content was determined by using data from 3.5.1 and the eugenol content from gas chromatography. The amount of essential oils was calculated from pattern calibration curves for essential oils.

$$\text{Encapsulation efficiency} = \frac{\textbf{Quantity of loaded EO (mg)}}{\textbf{Initial quantity of EO (mg)}} \times 100 \quad \ldots\ldots (1)$$

3.6 Release studies

The eugenol release was carried out in condition box of fruits. The microparticles absorb the moisture from box of fruits. The microparticles were swollen and then slow releasing essential oil to outer. The mechanism release of eugenol loaded alginate-chitosan microparticles as shown in Figure 22.

Alginate-chitosan microparticles of 2 g were placed in box of fruits. The sample keep at 10 ºC at the time intervals of 0-12 days. After finished each day transferred the microparticles 500 mg was poured into 10 mL of ethanol and stirring for 24 hrs. Then, solution was filtered thought nylon filter (0.20 μm, Nylon, Syringe Filters) and determined eugenol content by gas chromatography. The amount of eugenol was calculated from the calibration curve in the table A1 and Figure A1 (Appendix A). The percentage of cumulative eugenol release at time intervals was calculated from the following equation.

$$\% \text{ Cumulative release} = \frac{\text{Amount of eugenol after releasing}}{\text{Amount of eugenol before releasing}} \times 100 \quad \text{.....(2)}$$

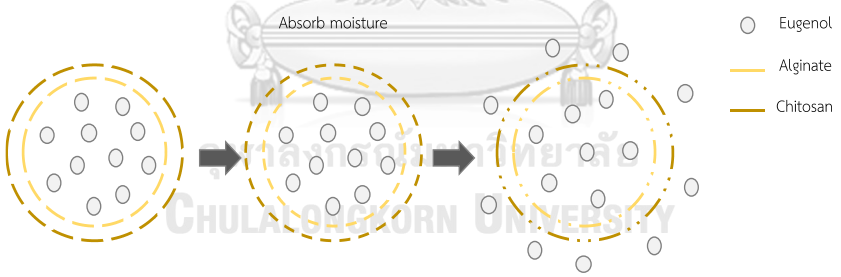

Figure 22. Mechanism release profile of eugenol

3.7 General Quality Parameter analysis

Firstly, 2 g of alginate-chitosan microparticles (EAC4-1) was deposited on the box of soft fruits for 2 groups of strawberries. The sampling tested strawberries 1 treatment and 1 non-treatment were stored at 10 °C. 2 groups of strawberries were

periodically weighed throughout the stored period and include studied color of fruits, firmness and % Brix of strawberry.

The Fruit weight loss was measured at every sampling time in the same fruits and weight loss was present as the percentage of the initial weight.

Weight loss (%) = $\frac{W0-Wt}{W0} \times 100$.....(3)

Where $W0$ is a initial weight and Wt is a weight of sample at difference time.

The color parameter of strawberries was measured by calculating the RGB of fruits by using digital camera. The parameter of digital camera were shown in Table 3.

Table 3. The parameter of digital camera

Detail	**Status**
Focus distance	20 cm.
Image size	2048×1536 pixels
Flash	No.
White balance	Automatic
Aperture	2.4

The firmness of strawberries was measured by fruit penetrometer with a GY-3 using a piston cylinder of 3.5 mm diameter at a depth of 5 mm.

The determination of soluble solid content (%) in soft fruits was used a digital refractometer PR-32 with the juice of fruits.

CHAPTER IV
RESULTS AND DISCUSSION

4.1 Effect of Alginate Concentration

The difference rations of chitosan and alginate; 1:0.1, 1:0.3, 1:0.5 and 1:1 were used to prepare alginate-chitosan microparticles. The SEM micrographs and the average size of these microparticles were shown in Figure 23 and Table 4. respectively, at chitosan and alginate ratio of 1:0.1, it could not be formed the spherical bead and at the other rations the particles were form and the size of microparticles increased by increasing the alginate concentration. The results can be explained that when alginate solution was added dropwise into a coagulation fluid, both the positive charged of chitosan and calcium ions are competing with the negative charged on the surface of the alginate core. At low concentration of alginate, the binding degree between alginate and chitosan/calcium chloride was quite low and not enough to from spherical shape. However, chitosan-alginate microparticles of 1:1 gave the best morphology, spherical shape and smooth surface.

Table 4. The average size of alginate-chitosan microparticles with difference ratios of chitosan and alginate Average size

Ratio of ALG:CS (W/W)	Average size (W/W) (μm)
0.1:1	933±45.21
0.3:1	1196±15.63
0.5:1	1262±19.33
1:1	1314±11.04

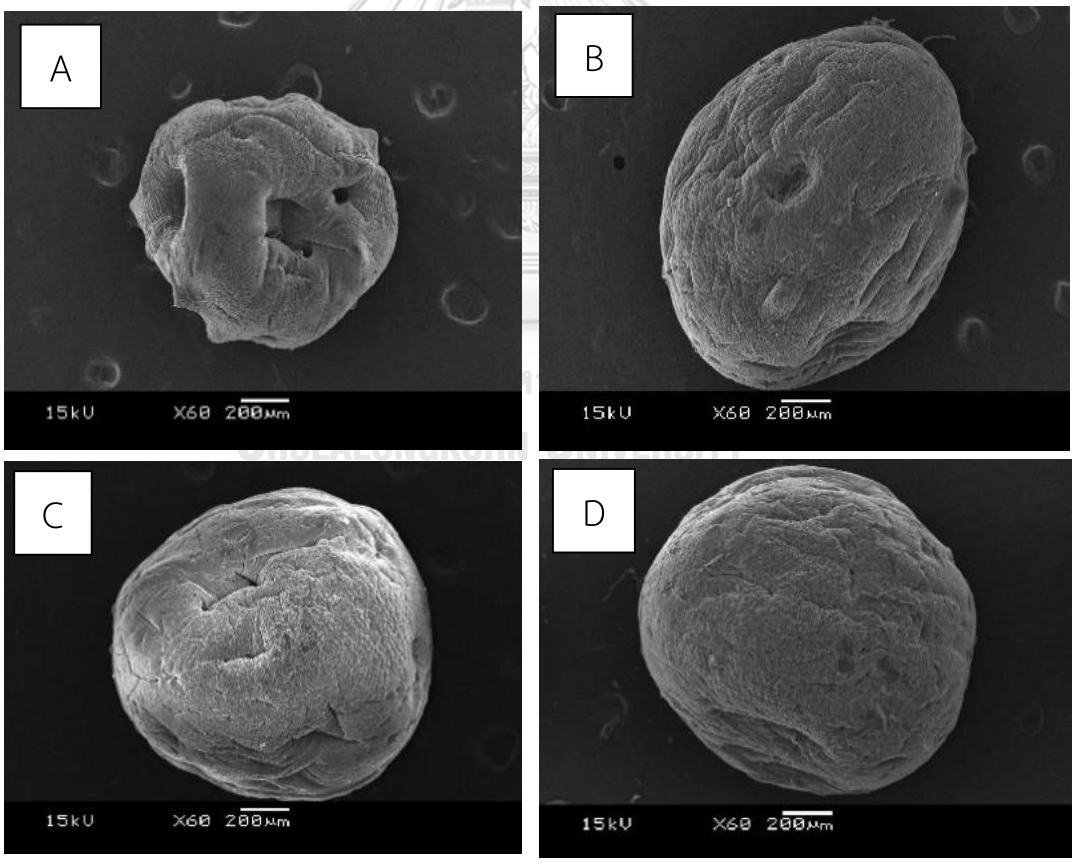

Figure 23. SEM image of the microparticles at CS: ALG ratio of (A) 1:0.1 (B) 1:0.3 (C) 1:0.5 and (D) 1:1

4.2 Fourier transform infrared spectroscopy (FT-IR)

FT-IR Spectroscopy was used to determine the chemical reaction of the sample (see in Figure. 24) The IR spectrum of pure alginate (Figure 24A.) showed the characteristic absorption bands at 1592 cm^{-1},1414 cm^{-1} and 1026 cm^{-1} attributed to the asymmetric and symmetric stretching vibrations of -COO and the stretching of C-O-C, respectively. The IR spectrum of chitosan (Figure 24B.) showed the characteristic peaks at 892 cm^{-1} and 1027cm^{-1}assigned to the saccharine structure and a strong characteristic amino peak at around 1591cm^{-1}. The peak at 1591 cm^{-1} was attributed to the amide of N- acylated chitosan. The IR spectrum of the alginate-chitosan microparticles (Figure 24C.) showed a band at 1589 cm^{-1}, which was attributed to the formation of NH^{3+} and indicative of the complexation between the amino groups of chitosan and the carboxylic groups of alginates.

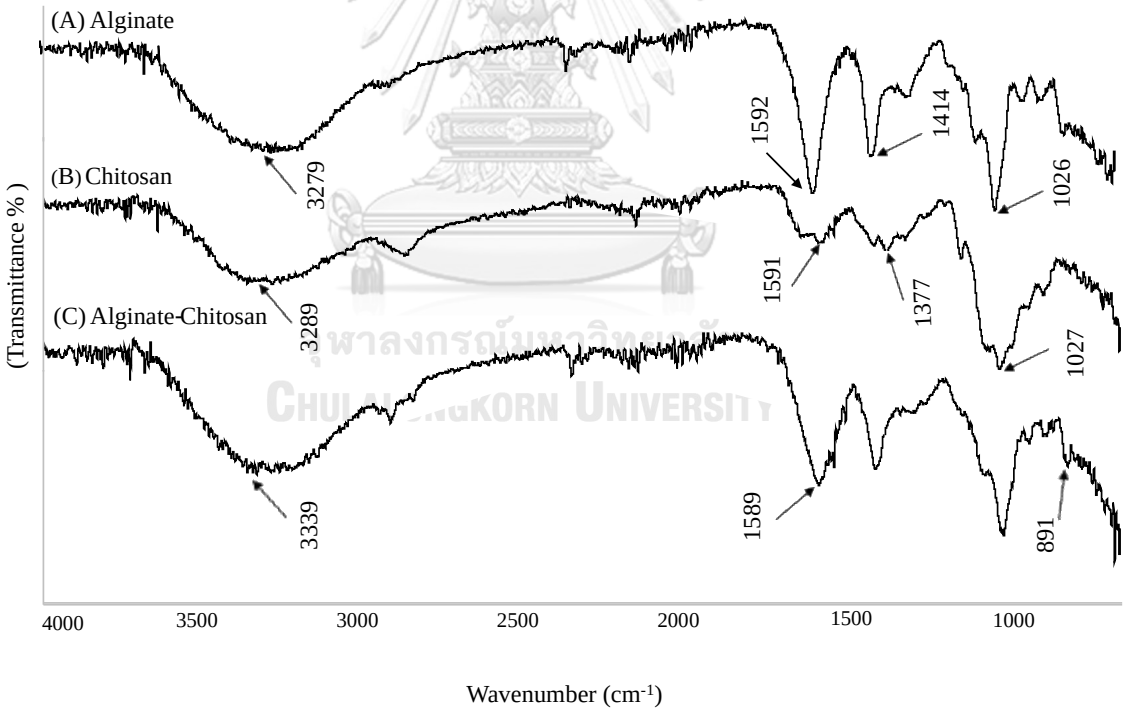

Figure 24. IR spectra of (A) Alginate, (B) Chitosan and (C) alginate chitosan microsphere

4.3. Effect of calcium chloride concentration

Cross-linking agent concentration on the affecting factor of encapsulation efficiency was studied by using various concentrations of calcium chloride (0.5%-8%) with alginate-chitosan ratio 1:1 and crosslink time 2 hr. and the results of this study were shown in Table 5. These results indicated that the increase of calcium chloride concentration from 0.5% to 2.00 % W/V the drug entrapment efficiency was found from 37.39% to 46.39%. The Increasing of calcium chloride concentration which means the amount of Ca^{2+} ions are increased for crosslink between alginate chains and alginate chains. While the decrease of the encapsulation efficiency of alginate-chitosan microparticles with increasing the amount of calcium chloride over 2% could be clarify on the general basic crosslink between alginate chains and alginate chains.

Table 5. Encapsulation efficiency (%EE) of eugenol loaded alginate-chitosan microparticles at calcium chloride 0.50-8.00 % (W/V)

Ratios of chitosan: alginate (W/W)	Calcium Chloride (%W/V)	Encapsulation efficiency (%EE)
1:1	0.50	37.39±3.74
1:1	1.00	33.88±1.78
1:1	1.50	36.06±0.53
1:1	2.00	46.39±1.67
1:1	4.00	19.95±0.11
1:1	8.00	19.09±0.19

Moreover, the concentration of calcium chloride (0.5%-0.8%) affect to the morphology of microparticles at cross-linking 2 hours as shown in Figure 25. This result showed that with increasing the concentration of calcium chloride, the color of beads changed from pale yellow to darker yellow, while the shape and size are still the same.

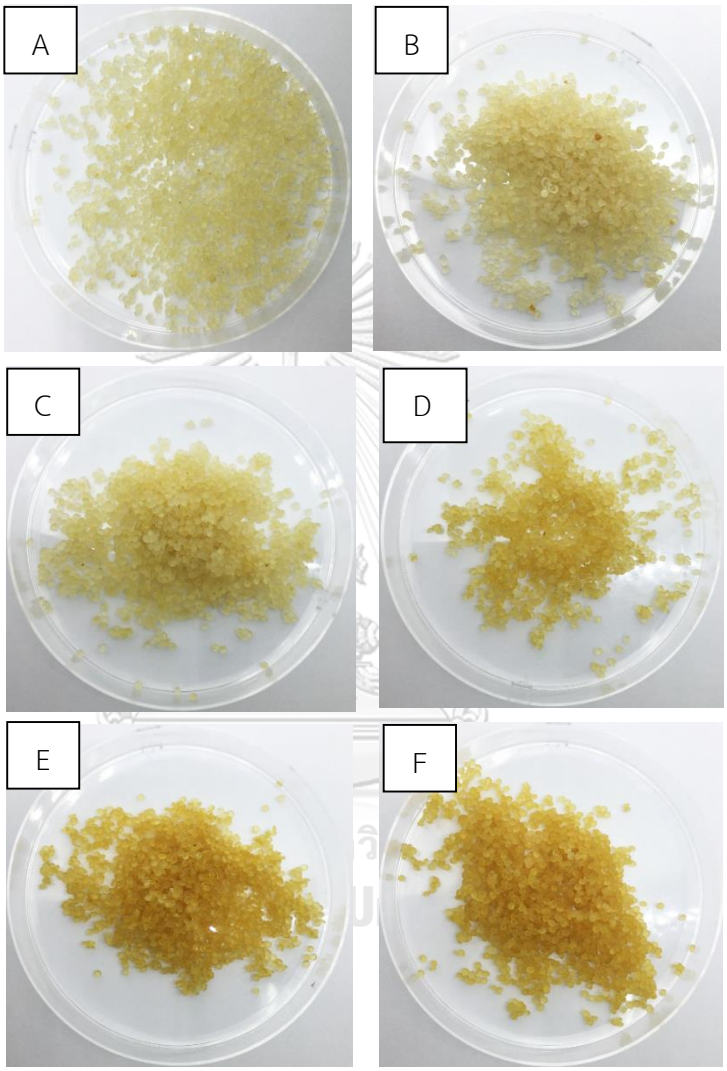

Figure 25. Effect of calcium chloride concentration on the color of microparticles A) 0.5% of $CaCl_2$, B) 1.00% of $CaCl_2$, C) 1.50 % of $CaCl_2$, D) 2.00 % of $CaCl_2$, E) 4.00 % of $CaCl_2$ and F) 8.00% of $CaCl_2$

4.4 Effect of surfactant with encapsulation efficiency (EE)

The effect of surfactant concentration as an affecting factor on the encapsulation efficiency was studied by using various concentrations of surfactant (2%-10%) with preserving alginate-chitosan at ration 1:1, amount of eugenol (0.1-0.5 g), cross-linking agent 2% W/V. The results of this study were showed in table 6-10.

Table 6. Encapsulation efficiency (%EE) of eugenol loaded alginate-chitosan microparticles between tween 80 2.00 (%W/V)

Conditions	Ratios of chitosan: alginate (W/W)	Amount of eugenol (g)	Amount of Tween 80 (%W/V)	Encapsulation efficiency (%EE)
EAC2-1	1:1	0.1	2.00	42.85±0.30
EAC2-2	1:1	0.2	2.00	45.99±1.48
EAC2-3	1:1	0.3	2.00	45.19±0.70
EAC2-4	1:1	0.4	2.00	41.91±0.46
EAC2-5	1:1	0.5	2.00	38.81±0.45

Table 7. Encapsulation efficiency (%EE) of eugenol loaded alginate-chitosan microparticles between tween 80 4.00 (%W/V)

Conditions	Ratios of chitosan: alginate (W/W)	Amount of eugenol (g)	Amount of Tween 80 (%W/V)	Encapsulation efficiency (%EE)
EAC4-1	1:1	0.1	4.00	53.01±1.51
EAC4-2	1:1	0.2	4.00	42.23±0.26
EAC4-3	1:1	0.3	4.00	30.73±0.93
EAC4-4	1:1	0.4	4.00	30.39±1.18
EAC4-5	1:1	0.5	4.00	26.55±0.18

Table 8. Encapsulation efficiency (%EE) of eugenol loaded alginate-chitosan microparticles between tween 80 6.00 (%W/V)

Conditions	Ratios of chitosan: alginate (W/W)	Amount of eugenol (g)	Amount of Tween 80 (%W/V)	Encapsulation efficiency (%EE)
EAC6-1	1:1	0.1	6.00	43.69±0.69
EAC6-2	1:1	0.2	6.00	24.79±0.24
EAC6-3	1:1	0.3	6.00	25.96±0.58
EAC6-4	1:1	0.4	6.00	24.11±0.96
EAC6-5	1:1	0.5	6.00	5.32±0.13

Table 9. Encapsulation efficiency (%EE) of eugenol loaded alginate-chitosan microparticles between tween 80 8.00 (%W/V)

Conditions	Ratios of chitosan: alginate (W/W)	Amount of eugenol (g)	Amount of Tween 80 (%W/V)	Encapsulation efficiency (%EE)
EAC8-1	1:1	0.1	8.00	21.81±0.15
EAC8-2	1:1	0.2	8.00	19.95±0.17
EAC8-3	1:1	0.3	8.00	21.41±0.18
EAC8-4	1:1	0.4	8.00	19.05±0.06
EAC8-5	1:1	0.5	8.00	5.47±0.11

Table 10. Encapsulation efficiency (%EE) of eugenol loaded alginate-chitosan microparticles between tween 80 10.00 (%W/V)

Conditions	Ratios of chitosan: alginate (W/W)	Amount of eugenol (g)	Amount of Tween 80 (%W/V)	Encapsulation efficiency (%EE)
EAC10-1	1:1	0.1	10.00	15.16±0.20
EAC10-2	1:1	0.2	10.00	20.94±0.43
EAC10-3	1:1	0.3	10.00	17.81±0.26
EAC10-4	1:1	0.4	10.00	15.00±0.17
EAC10-5	1:1	0.5	10.00	14.44±0.18

The amount of eugenol loaded alginate-chitosan beads was determined by using gas chromatography. 10 g of beads crushed in ethanol 10 ml the standard curve with the equation of the linear regression (y=336.17x-5162.5 where y and x are the peak area of eugenol concentration (ppm)) as showed in the Table A1 and Figure A1 (Appendix A). The table 6-10 showed that when increased the amount of tween 80 %W/V between EAC2-1 to EAC4-1, % encapsulation efficiency increased 42.85% to 53.01%, while increased the amount of tween 80% from EAC4-1 to EAC10-1, the encapsulation efficiency decreased from 53.01% to 15.16%. Moreover, studied encapsulation efficiency of amount of eugenol. The results showed that when increased the amount of eugenol from 0.1 to 0.5 g. the EE of 0.1% eugenol loading to alginate-chitosan microparticles was higher than another amount of eugenol. The EE of 0.1 g of eugenol showed 68.78%

It suggested that eugunol loaded alginate-chitosan microparticles of 0.1 g and surfactant 4% W/V is the optimum condition for encapsulation in alginate-chitosan micropartocles.

4.5 Release essential oils

The release profile of EAC4-1 comparison with EA4-1 at 10℃ during 12 days compares strawberries boxes that during time 0-12 days at 10 °C in refrigerator between eugenol loaded alginate-chitosan beads (EAC4-1) and eugenol loaded alginate microparticles (EA4-1) containing surfactant 4% w/v. This suggested that the most effective condition to inhibit microbials was eugenol-loaded alginated-chitosan mirocparticles. The release of eugenol from two formulations showed in Figure 26. The release rate of EAC was 4.96% after 2 days, while the release from eugenol loaded alginate microparticles was 3.07%. However, eugenol loaded alginate-chitosan microparticles showed higher release rate and amount of eugenol than eugenol loaded alginate microparticles. Moreover, the release profile of eugenol loaded alginate-chitosan microparticles and eugenol loaded alginate microparticles were showed cumulative eugenol release between 0-12 days from 0 - 15.82 % and 0 - 6.92%

respectively. Both of release profile are very lower due to in boxes of fruits the beads have to absorb moisture in boxes and after that the beads swollen and released eugenol to outer.

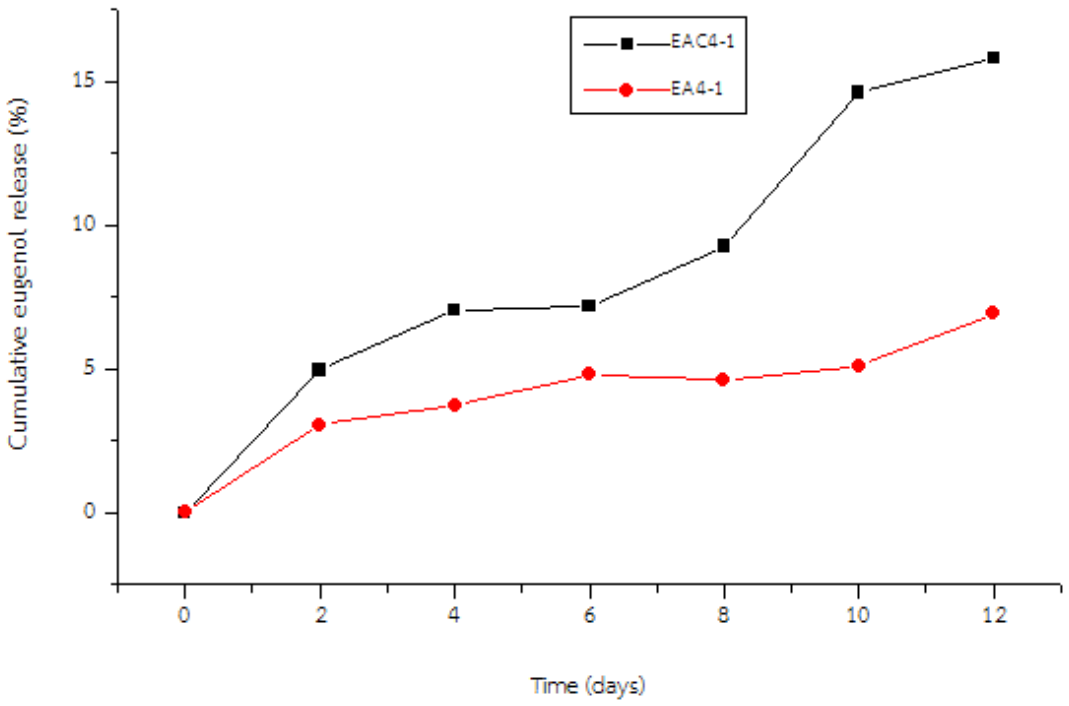

Figure 26. Release profile of eugenol from alginate-chitosan micropartiparticle (EAC4-1) compared with eugenol loaded alginate microparticles (EA4-1)

4.6 Antimicrobial Activity Profile of Encapsulated Eos and quality of soft fruits

The activity profile of sample control and eugenol loaded alginate-chitosan (EAC4-1) with strawberries in refrigerator temperature at 10 °C were taken study in the difference times 0-12 days. According to Figure 27, encapsulated beads inhibited the growth of microbial until 8-12 days, which cannot visually observe any microbials. The fresher color of treated fruits was observed comparison with control, indication that the encapsulated bead could be effective antimicrobial material.

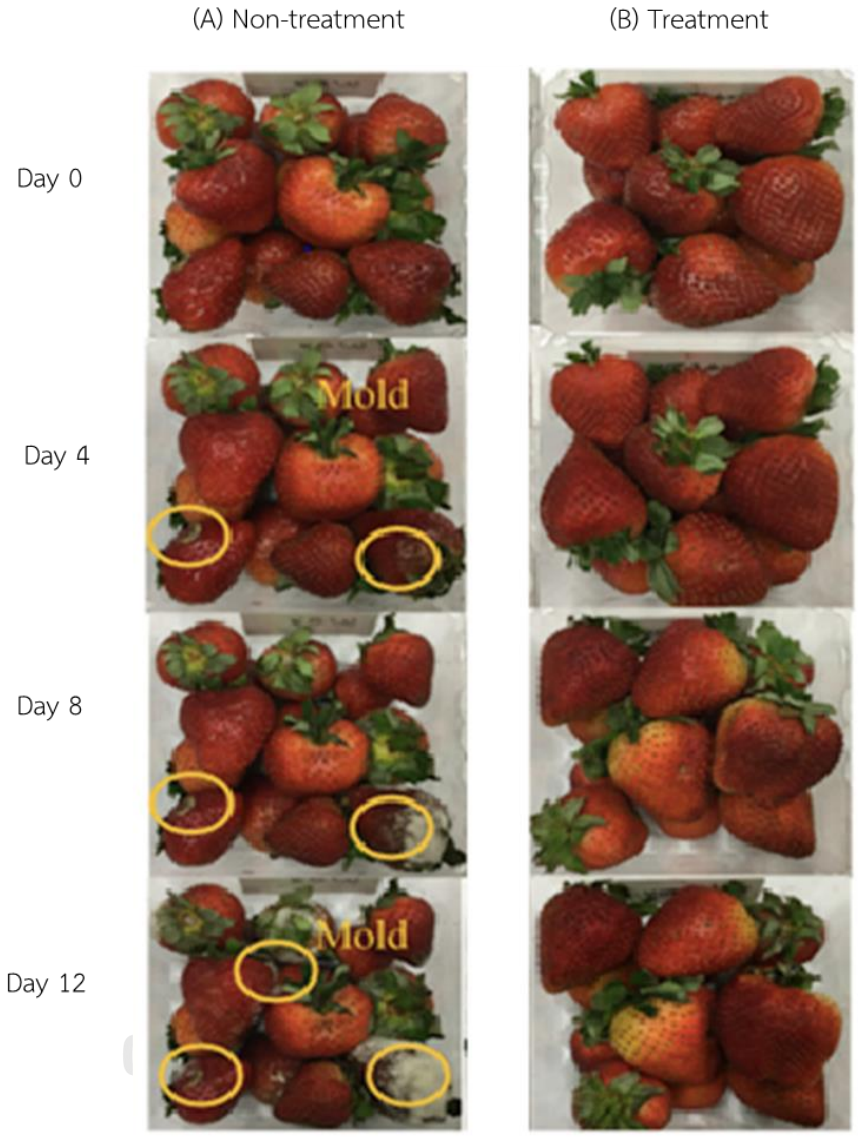

Figure 27. Appearance changes of strawberries stored at refrigerator temperature

(A) Non-treatment and (B) Treatment with eugenol loaded alginate-chitosan microparticles (EAC4-1)

Table 11. Color parameter (L* and h°), of strawberries with alginate-chitosan microparticles (EAC4-1) during storage at 10 °C. Parameters were evaluated at harvest (before treatment) and after (treatment) between 0, 4, 8 and 12 days stored.

	Day	Non-treatment	Treatment
Lightness (*L**)	0	36.85±2.11	38.98±1.58
	4	30.15±2.20	30.79±1.59
	8	26.44±1.12	25.74±0.48
	12	21.46±0.64	22.41±0.37
Hue angle (*h* ๑)	0	347.13±4.73	349.87±1.07
	4	348.40± 0.92	343.30±1.23
	8	340.13±3.47	345.60±4.94
	12	350.67±3.23	346.00±4.25

Table 12. Firmness, weight loss and %Brix of strawberries with alginate-chitosan microparticles (EAC4-1) during storage at 10 °C. Parameters were evaluated at harvest (before treatment) and after (treatment) between 0, 4, 8 and 12 days stored

	Day	Non-treatment	Treatment
Firmness	0	1.03±0.06	0.97±0.06
	4	1.03±0.06	1.03±0.06
	8	0.83±0.06	1.27±0.12
	12	0.63±0.06	1.30±0.10
Weight loss (%)	0	0.00±0.00	0.00±0.00
	4	11.79±0.88	12.07±1.54
	8	23.50±2.94	24.51±1.36
	12	34.77±2.57	34.41±3.47
Brix (%)	0	6.37±0.55	6.93±0.12
	4	7.07±0.12	7.17±0.29
	8	8.63±.0.12	8.13±0.12
	12	9.07±0.12	10.07±0.12

Table 11, showed the parameters of strawberries that were tested between non-treatment (without encapsulated beads) and treatment (with encapsulated beads). The results indicated Lightness *(L*)* of the color (lightness variation between 0 = black and 100 = white). The strawberries showed changes of color at 10 °C, 0-12 day from 38.98 decreased to 22.41. For non-treatment the results showed lightness of strawberries from 36.85-21.46. Strawberries stored at 10 °C for 0-12 day had no significant difference between treatment and non-treatment. The results showed similar for hue color value.

For hue angle (h^{9}) value, strawberries stored at 10 ^{o}C for 8 day become darkness but hue angle (h^{9}) value did not change during stored. There were no significant difference between non-treatment and treatment

Firmness of fresh fruits is an important quality parameter. The results showed firmness of strawberries (Table 12.) decreased during stored from 1.3 to 0.63 of non-treatment, as a results of cell wall degradation and the fruit are rotten. The application of treatment of encapsulated beads improved the firmness of strawberries compared with non-treatment. The encapsulated beads during stored 12 day showed the higher firmness of strawberries than strawberries with non-treatment (without encapsulated beads) due to the encapsulated beads absorb the moisture in a box of fruits.

The weight loss decreased during stored in non-treatment including treatment (Table 12.). The weight loss of all sample did not significantly difference from non-treatment. It is known that losing water of fresh fruits to environmental is the major cause of weight loss in fruits stored and spoilage of fruits. In non-treatment during stored, it is losing of water from fruits to environment and fruits were spoilage during stored at day 4. Fruits spoilage is a major cause of moisture in box of fruits. In treatment with encapsulated beads during stored, it is also losing of water from fruits to environment, but the fruits were still fresher than non-treatment due to the encapsulated beads absorb the moisture in box of fruits.

The soluble solid content in strawberries (%Brix) increased through storage in all sample (Table 12.) with the fruit stored during day 0 to day 12. There were no significances.

Moreover, the results of the encapsulated bead tested with other soft fruits such as blueberries, raspberries, cherry and soft vegetables such as tomato, are shown in Figure 28-31. Furthermore, other soft fruits, including blueberries and cherries were tested between treatment and non-treatment. The treatment group could extend shelf life to 8 days, while non-treatment blueberries and cherries got spoilage in day 4 and showed spoilage in day 5, respectively at 10 ^{o}C during storage as shown in Figure 28 and 29.

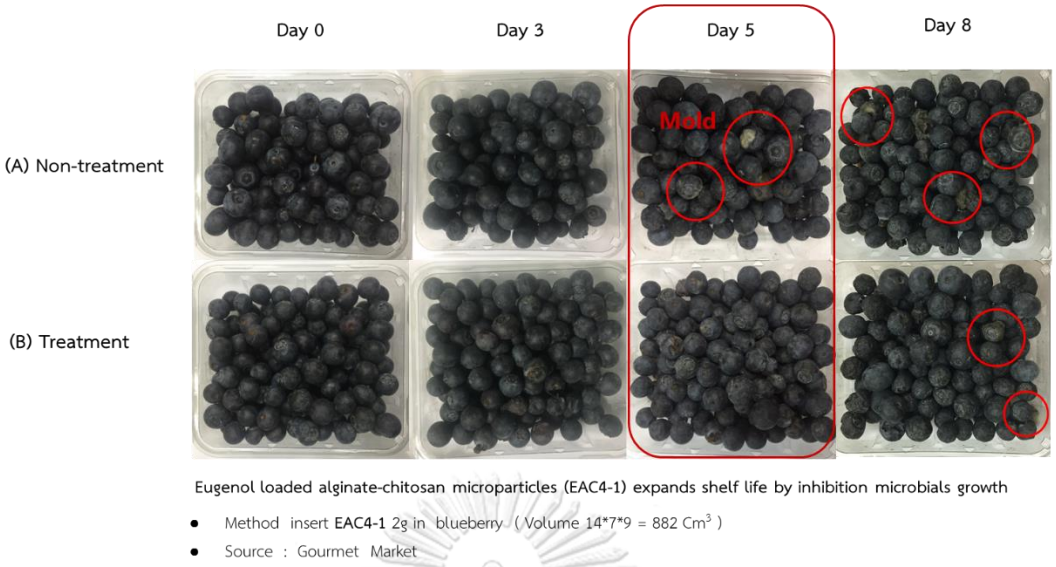

Figure 28. Appearance changes of blueberries stored at refrigerator temperature (A) Non-treatment and (B) Treatment with eugenol loaded alginate-chitosan microparticles (EAC4-1)

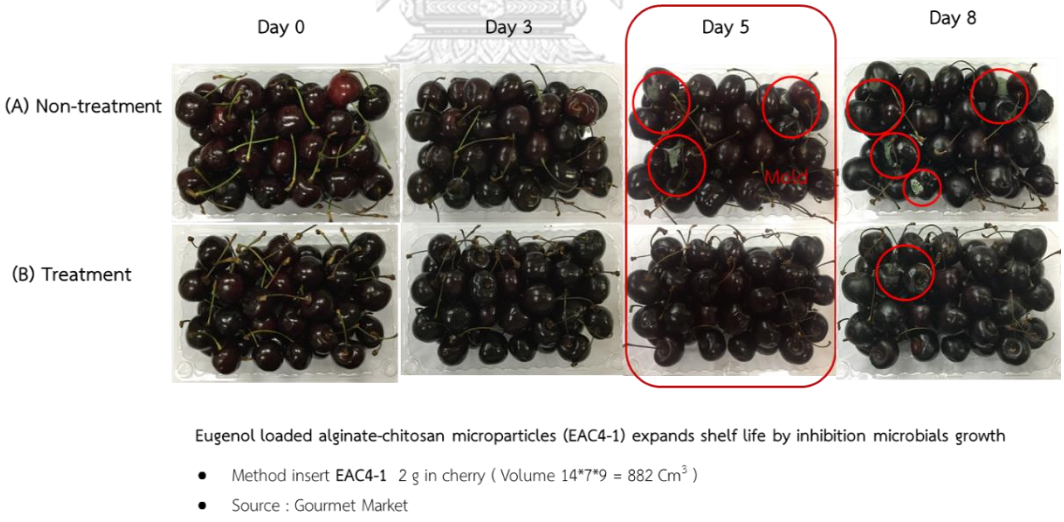

Figure 29. Appearance changes of cherry stored at refrigerator temperature (A) Non-treatment and (B) Treatment with eugenol loaded alginate-chitosan microparticles (EAC4-1)

According to Figure 30, raspberries were tested between treatment and non-treatment, the treatment group could extend shelf life to 5 days, while non-treatment got spoilage in day 3 and showed spoilage in day 5 at 10 °C during storage.

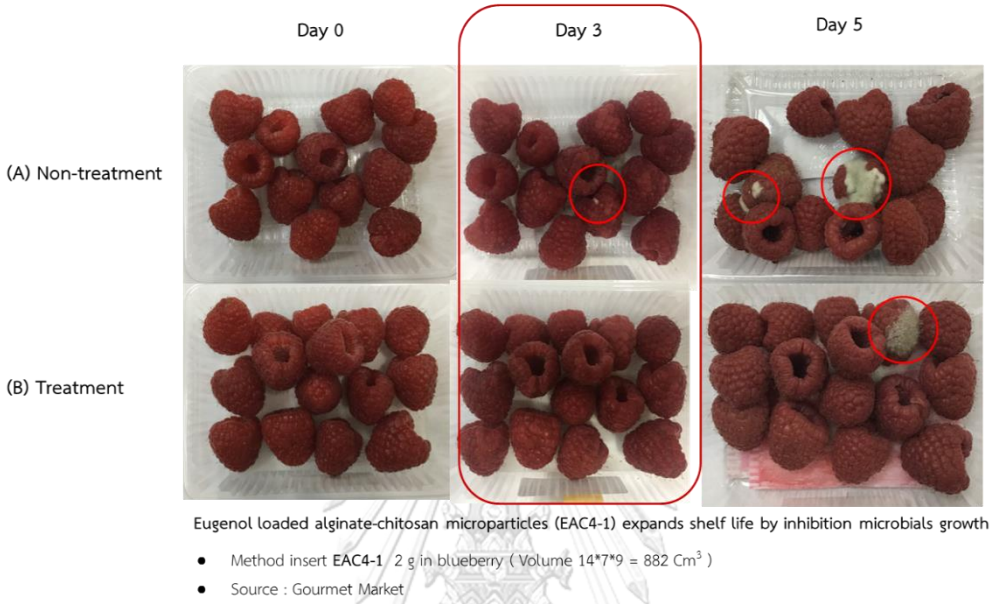

Figure 30. Appearance changes of raspberries stored at refrigerator temperature (A) Non-treatment and (B) Treatment with eugenol loaded alginate-chitosan microparticles (EAC4-1)

According to Figure 31, tomato were tested between treatment and non-treatment, the treatment group could extend shelf life to 9 days, while non-treatment got spoilage in day 9 and showed spoilage in day 9 during storage.

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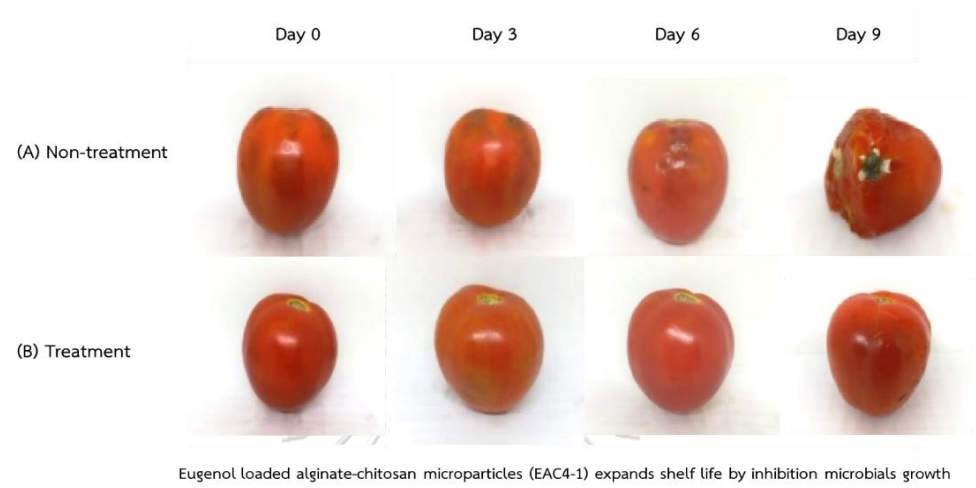

Figure 31. Appearance changes of tomato stored at refrigerator temperature (A) Non-treatment and (B) Treatment with eugenol loaded alginate-chitosan microparticles (EAC4-1)

CHAPTER V
CONCLUSION

5.1 Conclusion

In this work, the alginate-chitosan microparticles were successfully prepared by the combination of the ionic gelation method in order to controlled release of eugenol for inhibit mold and bacterial in fresh soft fruits (strawberries, blueberries, raspberries, cherries and tomato. First the microparticles were formed with difference ratio of both polymers. SEM image showed the ALG:CS at mass ration 1:1 were the best morphology which smooth surface and spherical shape. Moreover, the FT-IR of alginate-chitosan microparticles showed the formation of NH^{3+} and indicative of the complexation between the amino groups of chitosan and the carboxylic groups of alginates.

Eugenol loaded alginate-chitosan microparticles were successfully by using calcium chloride concentration at 2% w/v as the crosslinking agent. The highest encapsulation efficiency were around 46.39±1.67%. Moreover, eugenol loaded alginate-chitosan microparticles at 2% w/v with difference amount of surfactant (Tween 80) 2% - 10% w/v and difference amount of eugenol oils 0.1 g – 0.5 g was investigated. The result showed that with a ratio of 1:1 alginate-chitosan microparticles, calcium chloride 2% w/v, surfactant 4% w/v and eugenol oils 0.1 g (EAC4-1) had the highest encapsulation efficiency of 53.01±1.51%.

For the controlled release profile eugenol loaded alginate-chitosan microparticles (EAC4-1) was deposited in the box of soft fruits at 10°C refrigerator during stored 0-12 days compared with eugenol encapsulated alginate (EA4-1). The results showed eugenol loaded alginate-chitosan microparticles could release higher than eugenol encapsulated alginate microparticles during stored from 0 - 15.82% and 0 – 6.92% respectively,

Moreover, the antimicrobial activity profile and quality of soft skin fruits were tested with strawberries at 10°C refrigerator during stored 0-12 days compared between non-treatment (without encapsulated beads) and treatment (with encapsulated beads). The results exhibited that encapsulated beads could extend shelf life of strawberries more than 12 days, whereas nontreated strawberries got spoilage in day 4 during storage. Subsequently, the appearance of strawberries with and without was studied, including lightness *(L*)*, hue angle *(h °)*, weight loss, soluble solid content and firmness with quality parameter during stored.

REFERENCES

[1] Hu, Q., Gerhard, H., Upadhyaya, I., Venkitanarayanan, K., and Luo, Y. Antimicrobial eugenol nanoemulsion prepared by gum arabic and lecithin and evaluation of drying technologies. Int J Biol Macromol 87 (2016): 130-40.

[2] Xie, Y., Yang, Z., Cao, D., Rong, F., Ding, H., and Zhang, D. Antitermitic and antifungal activities of eugenol and its congeners from the flower buds of Syzgium aromaticum (clove). Industrial Crops and Products 77 (2015): 780-786.

[3] Turek, C. and Stintzing Florian, C. Stability of Essential Oils: A Review. Comprehensive Reviews in Food Science and Food Safety 12(1) (2013): 40-53.

[4] Madene, A., Jacquot, M., Scher, J., and Desobry, S. Flavour encapsulation and controlled release – a review. International Journal of Food Science & Technology 41(1) (2005): 1-21.

[5] Belščak-Cvitanović, A., et al. Encapsulation of polyphenolic antioxidants from medicinal plant extracts in alginate–chitosan system enhanced with ascorbic acid by electrostatic extrusion. Food research international 44(4) (2011): 1094-1101.

[6] Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A., and Saurel, R. Applications of spray-drying in microencapsulation of food ingredients: An overview. Food Research International 40(9) (2007): 1107-1121.

[7] Dewettinck, K. and Huyghebaert, A. Fluidized bed coating in food technology. Trends in Food Science & Technology 10(4) (1999): 163-168.

[8] Desobry Stephane, A., Netto Flavia, M., and Labuza Theodore, P. Comparison of Spray-drying, Drum-drying and Freeze-drying for β-Carotene Encapsulation and Preservation. Journal of Food Science 62(6) (2006): 1158-1162.

[9] Fàbregas, A., et al. Impact of physical parameters on particle size and reaction yield when using the ionic gelation method to obtain cationic polymeric chitosan–tripolyphosphate nanoparticles. International Journal of Pharmaceutics 446(1) (2013): 199-204.

[10] Altenhofen, S.M., Thie, I.B., Hiromi, T.M., and Guenter, K.T. Evaluation of the Antimicrobial Potential of Alginate and Alginate/Chitosan Films Containing Potassium Sorbate and Natamycin. Packaging Technology and Science 26(8) (2013): 479-492.

[11] Marzieh, K.S. and Masoud, R. Antimicrobial Effectiveness of Gelatin–Alginate Film Containing Oregano Essential Oil for Fish Preservation. Journal of Food Safety 35(4) (2015): 482-490.

[12] Lee, K.Y. and Mooney, D.J. Alginate: properties and biomedical applications. Prog Polym Sci 37(1) (2012): 106-126.

[13] Pillai, C.K.S., Paul, W., and Sharma, C.P. Chitin and chitosan polymers: Chemistry, solubility and fiber formation. Progress in Polymer Science 34(7) (2009): 641-678.

[14] Kahraman, T., Issa, G., Bingol, E.B., Kahraman, B.B., and Dumen, E. Effect of rosemary essential oil and modified-atmosphere packaging (MAP) on meat quality and survival of pathogens in poultry fillets. Braz J Microbiol 46(2) (2015): 591-9.

[15] Cassel, E., Vargas, R.M.F., Martinez, N., Lorenzo, D., and Dellacassa, E. Steam distillation modeling for essential oil extraction process. Industrial Crops and Products 29(1) (2009): 171-176.

[16] Suppakul, P., Miltz, J., Sonneveld, K., and Bigger, S.W. Antimicrobial Properties of Basil and Its Possible Application in Food Packaging. Journal of Agricultural and Food Chemistry 51(11) (2003): 3197-3207.

[17] Ghosh, V., Mukherjee, A., and Chandrasekaran, N. Eugenol-loaded antimicrobial nanoemulsion preserves fruit juice against, microbial spoilage. Colloids and Surfaces B: Biointerfaces 114 (2014): 392-397.

[18] Venkatesan, P., Manavalan, R., and Valliappan, K. Microencapsulation: a vital technique in novel drug delivery system. Journal of Pharmaceutical Sciences and Research 1(4) (2009): 26-35.

[19] Weinbreck, F., Minor, M., and De Kruif, C. Microencapsulation of oils using whey protein/gum arabic coacervates. Journal of microencapsulation 21(6) (2004): 667-679.

[20] Alikhani, M. and Daraei Garmakhany, A. Effect of microencapsulated essential oils on storage life and quality of strawberry (Fragaria ananassa cv. Camarosa). Quality Assurance and Safety of Crops & Foods 4(2) (2012): 106-112.

[21] Martins, I.M., Barreiro, M.F., Coelho, M., and Rodrigues, A.E. Microencapsulation of essential oils with biodegradable polymeric carriers for cosmetic applications. Chemical Engineering Journal 245 (2014): 191-200.

[22] Drusch, S. Sugar beet pectin: A novel emulsifying wall component for microencapsulation of lipophilic food ingredients by spray-drying. Food Hydrocolloids 21(7) (2007): 1223-1228.

[23] Ribeiro, C., Vicente, A.A., Teixeira, J.A., and Miranda, C. Optimization of edible coating composition to retard strawberry fruit senescence. Postharvest Biology and Technology 44(1) (2007): 63-70.

[24] Marques, H.M.C. A review on cyclodextrin encapsulation of essential oils and volatiles. Flavour and fragrance journal 25(5) (2010): 313-326.

[25] Betz, M., et al. Antioxidant capacity of bilberry extract microencapsulated in whey protein hydrogels. Food Research International 47(1) (2012): 51-57.

[26] Cheong, S.H., Park, J.K., Kim, B.S., and Chang, H.N. Microencapsulation of yeast cells in the calcium alginate membrane. Biotechnology techniques 7(12) (1993): 879-884.

[27] Brannon-Peppas, L. Controlled release in the food and cosmetics industries. in: ACS Publications, 1993.

[28] Rao, K.R. and Devi, K.P. Swelling controlled-release systems: recent developments and applications. International Journal of Pharmaceutics 48(1-3) (1988): 1-13.

[29] Rao, K.R., Devi, K.P., and Buri, P. Influence of molecular size and water solubility of the solute on its release from swelling and erosion controlled polymeric matrices. Journal of Controlled Release 12(2) (1990): 133-141.

[30] von Burkersroda, F., Schedl, L., and Göpferich, A. Why degradable polymers undergo surface erosion or bulk erosion. Biomaterials 23(21) (2002): 4221-4231.

[31] Thünemann, A.F., Müller, M., Dautzenberg, H., Joanny, J.-F., and Löwen, H. Polyelectrolyte complexes. in Polyelectrolytes with defined molecular architecture II, pp. 113-171: Springer, 2004.

[32] Lankalapalli, S. and Kolapalli, V. Polyelectrolyte complexes: A review of their applicability in drug delivery technology. Indian journal of pharmaceutical sciences 71(5) (2009): 481.

[33] Dima, C., Gitin, L., Alexe, P., and Dima, S. Encapsulation of coriander essential oil in alginate and alginate/chitosan microspheres by emulsification external gelation method. in Inside food symposium, pp. 1-6, 2013.

[34] Koide, S. Chitin-chitosan: properties, benefits and risks. Nutrition research 18(6) (1998): 1091-1101.

[35] Hamed, I., Özogul, F., and Regenstein, J.M. Industrial applications of crustacean by-products (chitin, chitosan, and chitooligosaccharides): A review. Trends in Food Science & Technology 48 (2016): 40-50.

[36] Riva, R., Ragelle, H., des Rieux, A., Duhem, N., Jérôme, C., and Préat, V. Chitosan and chitosan derivatives in drug delivery and tissue engineering. in Chitosan for biomaterials II, pp. 19-44: Springer, 2011.

[37] Sogias, I.A., Williams, A.C., and Khutoryanskiy, V.V. Why is chitosan mucoadhesive? Biomacromolecules 9(7) (2008): 1837-1842.

[38] Yang, J., Chen, J., Pan, D., Wan, Y., and Wang, Z. pH-sensitive interpenetrating network hydrogels based on chitosan derivatives and alginate for oral drug delivery. Carbohydrate polymers 92(1) (2013): 719-725.

[39] Gierszewska, M. and Ostrowska-Czubenko, J. Chitosan-based membranes with different ionic crosslinking density for pharmaceutical and industrial applications. Carbohydrate polymers 153 (2016): 501-511.

[40] Devlieghere, F., Vermeulen, A., and Debevere, J. Chitosan: antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables. Food microbiology 21(6) (2004): 703-714.

[41] Kumar, M.R., Muzzarelli, R.A., Muzzarelli, C., Sashiwa, H., and Domb, A. Chitosan chemistry and pharmaceutical perspectives. Chemical reviews 104(12) (2004): 6017-6084.

[42] Saifuddin, M. and Kumaran, P. Removal of heavy metal from industrial wastewater using chitosan coated oil palm shell charcoal. Electronic journal of Biotechnology 8(1) (2005): 43-53.

[43] Lee, K.Y. and Mooney, D.J. Alginate: properties and biomedical applications. Progress in polymer science 37(1) (2012): 106-126.

[44] Park, J., Ye, M., and Park, K. Biodegradable polymers for microencapsulation of drugs. Molecules 10(1) (2005): 146-161.

[45] Paques, J.P., van der Linden, E., van Rijn, C.J., and Sagis, L.M. Preparation methods of alginate nanoparticles. Advances in colloid and interface science 209 (2014): 163-171.

[46] Jeon, O., Powell, C., Ahmed, S.M., and Alsberg, E. Biodegradable, photocrosslinked alginate hydrogels with independently tailorable physical properties and cell adhesivity. Tissue Engineering Part A 16(9) (2010): 2915-2925.

[47] Shefy-Peleg, A., Foox, M., Cohen, B., and Zilberman, M. Novel antibiotic-eluting gelatin-alginate soft tissue adhesives for various wound closing applications. International Journal of Polymeric Materials and Polymeric Biomaterials 63(14) (2014): 699-707.

[48] Mukhopadhyay, P., Chakraborty, S., Bhattacharya, S., Mishra, R., and Kundu, P. pH-sensitive chitosan/alginate core-shell nanoparticles for efficient and safe oral insulin delivery. International journal of biological macromolecules 72 (2015): 640-648.

[49] Azarakhsh, N., Osman, A., Ghazali, H.M., Tan, C.P., and Adzahan, N.M. Lemongrass essential oil incorporated into alginate-based edible coating for shelf-life extension and quality retention of fresh-cut pineapple. Postharvest Biology and Technology 88 (2014): 1-7.

[50] Aloui, H., et al. Alginate coatings containing grapefruit essential oil or grapefruit seed extract for grapes preservation. International journal of food science & technology 49(4) (2014): 952-959.

[51] Wang, L., Li, R., Shao, J., and Wang, Z. Rheological behaviors of carboxymethyl tamarind gum as thickener on georgette printing with disperse dyes. Journal of Applied Polymer Science 134(26) (2017).

[52] Liakos, I., Rizzello, L., Scurr, D.J., Pompa, P.P., Bayer, I.S., and Athanassiou, A. All-natural composite wound dressing films of essential oils encapsulated in sodium alginate with antimicrobial properties. International journal of pharmaceutics 463(2) (2014): 137-145.

[53] Khan, T.A., Mahler, H.-C., and Kishore, R.S. Key interactions of surfactants in therapeutic protein formulations: a review. European journal of pharmaceutics and biopharmaceutics 97 (2015): 60-67.

[54] Weiszhár, Z., Czúcz, J., Révész, C., Rosivall, L., Szebeni, J., and Rozsnyay, Z. Complement activation by polyethoxylated pharmaceutical surfactants: Cremophor-EL, Tween-80 and Tween-20. European Journal of Pharmaceutical Sciences 45(4) (2012): 492-498.

[55] Gierszewska, M., Ostrowska-Czubenko, J., and Chrzanowska, E. pH-responsive chitosan/alginate polyelectrolyte complex membranes reinforced by tripolyphosphate. European Polymer Journal 101 (2018): 282-290.

[56] Shinde, U. and Nagarsenker, M. Microencapsulation of Eugenol by Gelatin-Sodium Alginate Complex Coacervation. Indian Journal of Pharmaceutical Sciences 73(3) (2011): 311-315.

[57] Dima, C., Gitin, L., Alexe, P., and Dima, S. Encapsulation of coriander essential oil in alginate and alginate/chitosan microspheres by emulsification external gelation method. 2013.

[58] Guerreiro, A.C., Gago, C.M.L., Faleiro, M.L., Miguel, M.G.C., and Antunes, M.D.C. The effect of alginate-based edible coatings enriched with essential oils constituents on Arbutus unedo L. fresh fruit storage. Postharvest Biology and Technology 100 (2015): 226-233.

[59] Wen, P., Zhu, D.-H., Wu, H., Zong, M.-H., Jing, Y.-R., and Han, S.-Y. Encapsulation of cinnamon essential oil in electrospun nanofibrous film for active food packaging. Vol. 59, 2016.

APPENDIX A

CALIBRATION CURVE

Table A1. Peak area of standard eugenol in ethanol determined by gas-chromatography

Concentration (ppm)	Peak area
50	11130
70	15967
100	31028
200	60835
300	97876
400	131651
500	161340
600	211038
1000	325799
1500	493938
2000	647423
3000	1018078

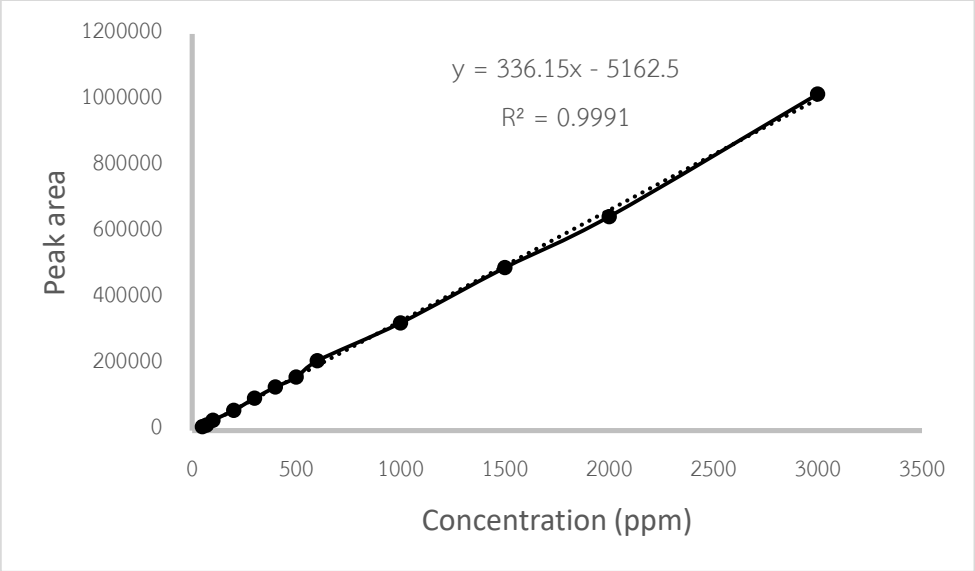

Figure A1. Peak area of standard eugenol in ethanol determined by gas-chromatography

APPENDIX B

ENCAPSULATION EFFICIENCY

Table B1. Effect of calcium chloride concentrations on encapsulation efficiency (%EE)

Concentration of $CaCl_2$ (%W/V)	Encapsulation efficiency (mg)			Encapsulation efficiency (%EE)			Average	
	1	2	3	1	2	3	Encapsulation efficiency (mg)	Encapsulation efficiency (%EE)
0.50	36.03	41.13	43.99	33.36	38.08	40.74	40.38±4.03	37.39±3.74
1.00	37.89	37.50	34.38	35.09	34.72	31.83	36.59±1.92	33.88±1.78
1.50	39.15	38.61	39.60	35.75	35.75	36.67	39.12±0.50	36.06±0.53
2.00	50.36	51.75	48.18	46.63	47.92	44.61	50.10±1.80	46.39±1.67
4.00	21.64	21.42	21.58	20.04	19.83	19.98	21.55±0.11	19.95±0.11
8.00	20.38	20.74	20.74	18.87	19.21	19.20	20.62±0.21	19.09±0.19

Table B2. Effect of amount of eugenol on encapsulation efficiency (%EE) at 2 % surfactant.

Conditions	Encapsulation efficiency (mg)			Encapsulation efficiency (%EE)			Average	
	1	2	3	1	2	3	Encapsulation efficiency (mg)	Encapsulation efficiency (%EE)
EAC2-1	50.86	50.66	50.18	43.10	42.93	42.52	50.57±0.35	42.85±0.30
EAC2-2	95.46	94.76	93.91	47.68	45.34	44.94	94.71±0.78	45.99±1.48
EAC2-3	150.64	153.40	148.73	45.10	45.93	44.53	150.92±2.35	45.19±0.70
EAC2-4	168.42	165.53	168.95	42.11	41.38	42.24	167.63±1.84	41.91±0.46
EAC2-5	193.07	197.49	196.19	38.31	39.18	38.93	195.58±2.27	38.81±0.45

Table B3. Effect of amount of eugenol on encapsulation efficiency (%EE) at 4% surfactant.

Conditions	Encapsulation efficiency (mg)			Encapsulation efficiency (%EE)			Average	
	1	2	3	1	2	3	Encapsulation efficiency (mg)	Encapsulation efficiency (%EE)
EAC4-1	53.01	54.51	51.50	53.01	54.51	51.5	53.01±1.51	53.01±1.51
EAC4-2	92.52	91.90	93.01	42.25	41.96	42.47	92.48±0.56	42.23±0.26
EAC4-3	93.10	98.04	98.31	29.65	31.22	31.31	96.48±2.93	30.73±0.93
EAC4-4	116.36	125.62	122.75	29.09	31.4	30.69	121.58±4.74	30.39±1.18
EAC4-5	139.09	137.85	137.24	26.75	26.51	26.39	138.06±0.94	26.55±0.18

Table B4. Effect of amount of eugenol on **e**ncapsulation efficiency (%EE) at 6% surfactant.

Conditions	Encapsulation efficiency (mg)			Encapsulation efficiency (%EE)			Mean	
	1	2	3	1	2	3	Encapsulation efficiency (mg)	Encapsulation efficiency (%EE)
EAC6-1	47.49	47.90	48.89	43.08	43.55	44.44	48.09±0.72	43.69±0.69
EAC6-2	50.89	50.09	49.97	25.07	24.68	24.62	50.32±0.50	24.79±0.24
EAC6-3	76.80	77.57	78.86	25.35	26.5	26.03	77.74±1.04	25.96±0.58
EAC6-4	94.54	101.83	95.91	23.4	25.2	23.74	97.43±3.87	24.11±0.96
EAC6-5	26.78	25.97	27.18	5.34	5.18	5.43	26.64±0.62	5.32±0.13

Table B5. Effect of amount of eugenol on encapsulation efficiency (%EE) at 8% surfactant.

Conditions	Encapsulation efficiency (mg)			Encapsulation efficiency (%EE)			Mean	
	1	2	3	1	2	3	Encapsulation efficiency (mg)	Encapsulation efficiency (%EE)
EAC8-1	25.88	25.53	25.80	21.93	21.64	21.86	25.74±0.18	21.81±0.15
EAC8-2	40.41	41.07	40.59	19.81	20.13	19.9	40.69±0.34	19.95±0.17
EAC8-3	68.02	67.22	68.36	21.46	21.21	21.57	67.87±0.59	21.41±0.18
EAC8-4	79.35	78.98	79.43	19.07	18.98	19.09	79.25±0.24	19.05±0.06
EAC8-5	28.78	27.64	28.45	5.57	5.35	5.5	28.29±0.59	5.47±0.11

Table B6. Effect of amount of eugenol on encapsulation efficiency at 10 % surfactant.

Conditions	Encapsulation Efficiency (mg)			Encapsulation efficiency (%EE)			Mean	
	1	2	3	1	2	3	Encapsulation efficiency (mg)	Encapsulation efficiency (%EE)
EAC10-1	16.86	16.75	16.42	15.32	15.23	14.93	16.68±0.23	15.16±0.20
EAC10-2	43.91	45.74	44.79	20.52	21.37	20.93	44.81±0.92	20.94±0.43
EAC10-3	54.99	53.43	54.50	18.03	17.52	17.87	54.31±0.80	17.81±0.26
EAC10-4	62.02	62.66	63.41	14.84	14.99	15.17	62.70±0.70	15.00±0.17
EAC10-5	73.79	75.62	74.55	14.27	14.63	14.42	74.65±0.92	14.44±0.18

APPENDIX C

PERCENTAGE OF DRUG RELEASE

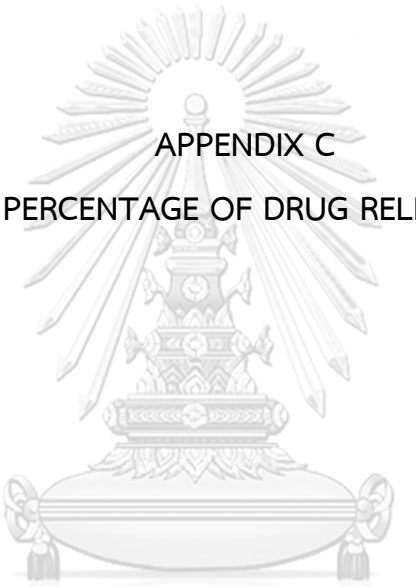

Table C1. Cumulative of eugenol loaded alginate-chitosan microparticles (EAC4-1) during stored 0-12 day at 10°C

	Time (Day)	% Cumulative eugenol release				
		1	2	3	Mean	SD
EAC4-1	0	0.00	0.00	0.00	0.00	0.00
	2	3.74	6.60	4.54	4.96	1.48
	4	5.82	7.91	7.39	7.04	1.09
	6	5.74	8.48	7.36	7.19	1.38
	8	7.58	10.58	9.65	9.27	1.54
	10	14.34	16.02	13.50	14.62	1.28
	12	14.67	17.14	15.64	15.82	1.24

Table C2. Cumulative of eugenol loaded alginate microparticles (EA4-1) during stored 0-12 day at 10°C

	Time (Day)	% Cumulative eugenol release				
		1	2	3	Mean	SD
EA4-1	0	0.00	0.00	0.00	0.00	0.00
	2	2.49	3.97	2.76	3.07	0.79
	4	3.96	4.54	2.73	3.74	0.92
	6	4.54	5.78	4.15	4.82	0.85
	8	4.34	5.60	3.97	4.64	0.85
	10	4.53	6.03	4.76	5.11	0.81
	12	6.99	7.59	6.19	6.92	0.70

APPENDIX D

QUALITY PARAMETER OF FRUITS

Table D1. Weight loss of strawberries at different time during stored 0-12 days at 10 °C compared between non-treatment and treatment as the percentage of the initial weight

	Time (Day)	Initial weight (W_0)			Weight of sample at different time (w_t)			% weight loss			
		1	2	3	1	2	3	1	2	3	Mean±SD
Non-treatment	0	32.23	29.87	34.4	32.23	29.87	34.4	0.00	0.00	0.00	0.00±0.00
	4	32.23	29.87	34.4	28.69	26.39	30.02	10.99	11.66	12.73	11.79±0.88
	8	32.23	29.87	34.4	23.64	23.64	26.5	26.66	20.86	22.97	23.50±2.94
	12	32.23	29.87	34.4	20.099	20.1	22.72	37.65	32.71	33.95	34.77±2.57
Treatment	0	29.53	26.16	26.99	29.53	26.16	26.99	0.00	0.00	0.00	0.00±0.00
	4	29.53	26.16	26.99	25.95	23.50	23.23	12.12	10.16	13.93	12.07±1.54
	8	29.53	26.16	26.99	22.68	19.89	19.87	23.20	23.96	26.38	24.51±1.36
	12	29.53	26.16	26.99	20.52	16.62	16.94	29.53	36.46	37.24	34.41±3.47

Table D2. Percent of solid content of strawberries at different time during stored 0-12 days at 10 °C compared between non-treatment and treatment determined by portable refractometer (PR-32)

	Time (Day)	%Solid content (Brix)			
		1	2	3	Mean±SD
Non-treatment	0	6.00	6.10	7.00	6.37±0.55
	4	7.00	7.20	7.00	7.07±0.12
	8	8.50	8.70	8.70	8.63±0.12
	12	9.00	9.00	9.20	9.07±0.12
Treatment	0	6.80	7.00	7.00	6.93±0.12
	4	7.00	7.50	7.00	7.17±0.29
	8	8.00	8.20	8.20	8.13±0.12
	12	10.00	10.00	10.20	10.07±0.12

Table D3. Color parameter showed RGB, *L** and h^{o} of strawberries at different time during stored 0-12 days at 10 ^{o}C compared between non-treatment and treatment by using digital camera

	Time (Day)		R (RED)	G (GREEN)	B (BLUE)	*L**	h^{o}	Mean±SD	
								*L**	h^{o}
Non-treatment	0	1	143	66	60	37.95	350.30	36.85±2.11	347.13±4.73
		2	139	51	58	34.42	341.70		
		3	144	66	61	38.18	349.40		
	4	1	125	31	33	27.98	349.40	30.15±2.20	348.40±0.92
		2	129	39	41	30.1	347.60		
		3	129	51	49	32.38	348.2		
	8	1	106	41	47	26.32	339.70	26.44±1.12	340.13±3.47
		2	107	36	40	25.38	343.80		
		3	107	46	53	27.62	336.90		
	12	1	95	25	25	20.82	349.60	21.46±0.64	350.67±3.23
		2	98	25	27	21.47	348.10		
		3	99	28	22	22.09	354.30		

Table D3. Color parameter showed RGB, L^* and h^o of strawberries at different time during stored 0-12 days at 10 ^oC compared between non-treatment and treatment by using digital camera

	Time (Day)		R (RED)	G (GREEN)	B (BLUE)	L^*	h^o	Mean	
								L^*	h^o
Treatment	0	1	140	77	70	40.12	350.80	38.98±1.58	349.87±1.07
		2	135	68	62	37.18	350.10		
		3	138	76	71	39.63	348.70		
	4	1	121	41	44	28.99	344.70	30.79±1.59	343.30±1.23
		2	126	51	55	31.99	342.40		
		3	125	49	53	31.38	342.80		
	8	1	104	38	42	25.25	342.6	25.74±0.48	345.60±4.94
		2	108	39	43	26.20	342.9		
		3	108	37	41	25.78	351.3		
	12	1	94	33	30	22.16	350.30	22.41±0.37	346.00±4.25
		2	92	37	40	22.83	341.80		
		3	94	33	34	22.24	345.90		

Table D4. Firmness of strawberries at different time during stored 0-12 days at 10 °C compare between non-treatment and treatment determined by refractometer (PR-32)

	Time (Day)	Firmness			Mean
		1	2	3	
Non-treatment	0	1.10	1.00	1.00	1.03±0.06
	4	1.00	1.00	1.10	1.03±0.06
	8	0.90	0.80	0.80	0.83±0.06
	12	0.70	0.60	0.60	0.63±0.06
Treatment	0	1.00	0.90	1.00	0.97±0.06
	4	1.00	1.10	1.00	1.03±0.06
	8	1.20	1.20	1.40	1.27±0.12
	12	1.20	1.30	1.40	1.30±0.10

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1. Chamaiporn Supachettapun and Nongnuj Muangsin on the topic of "Microencapsulation of Eugenol in Polyelectrolyte Complexes of Chitosan and Alginate" Proceeding of the 23rd PPC Symposium on Petroleum,Petrochemicals and Polymers and The 8th Research Symposium on Petrochemical and Materials Technology at Pathumwan Princess Hotel, Bangkok,Thailand on May 23,2017

2. Chamaiporn Supachettapun and Nongnuj Muangsin on the topic of "Microencapsulation of Eugenol in Chitosan and Alginate: Formation and Evaluation of Antimicrobial activity" Proceeding of The 43rd Congress on Science and Technology of Thailand (STT43) at Chamchuri 10, Chulalongkorn University, Bangkok, Thailand on 17-19 October, 2017