การเตรียมและการพิสูจน์เอกลักษณ์ทางเคมีกายภาพของแผ่นคาร์บอกซีเมทิลเซลลูโลสผสมแซนแทนกัมที่บรรจุน้ำมันหอมระเหย

นางสาวอติกานต์ ศรีชูศิลป์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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PREPARATION AND PHYSICOCHEMICAL CHARACTERIZATION OF ESSENTIAL OILS-LOADED CARBOXYMETHYLCELLULOSE/XANTHAN GUM BLEND SHEETS

Miss Atikan Srichoosilp

A Thesis Submitted in Partial Fulfillment of the Requirements
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อติกานต์ ศรีชูศิลป์ : การเตรียมและการพิสูจน์เอกลักษณ์ทางเคมีกายภาพของแผ่นคาร์บอกซีเมทิลเซลลูโลสผสมแซนแทนกัมที่บรรจุน้ำมันหอมระเหย (PREPARATION AND PHYSICOCHEMICAL CHARACTERIZATION OF ESSENTIAL OILS-LOADED CARBOXYMETHYLCELLULOSE/XANTHAN GUM BLEND SHEETS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร.พัฒทรา ธีรพิบูลย์เดช, 76 หน้า.

สามารถสร้างแผ่นฟิล์มแต่งกลิ่นน้ำมันหอมระเหยในรูปแบบใหม่ได้ด้วยกระบวนการหล่อขึ้นรูป ใช้สารก่อฟิล์มคือคาร์บอกซีเมทิลเซลลูโลสและแซนแทนกัมซึ่งนำมาผสมน้ำมันหอมระเหย 4 ชนิด ที่สกัดจากตะไคร้ ใบมะกรูด ข่า และเปลือกมะนาว ภาพจาก SEM สเปกตรัม FTIR และเทอร์โมแกรม TGA สามารถยืนยันการมีอยู่ของน้ำมันหอมระเหยภายในสายโซ่ของพอลิเมอร์ก่อฟิล์ม ความสามารถในการละลายน้ำและความสามารถในการกักเก็บน้ำมันหอมระเหยอยู่ที่ประมาณ 90-95% และ 15-26% ตามลำดับ สมบัติทางเคมีกายภาพของแผ่นฟิล์มน้ำมันหอมระเหยที่เก็บในสภาวะปิดผนึกแบบสุญญากาศและไม่สุญญากาศที่อุณหภูมิ 40 องศาเซลเซียสเป็นเวลา 120 วันได้ถูกตรวจสอบ ผลการทดลองพบว่าแผ่นฟิล์มน้ำมันหอมระเหยในสภาวะปิดผนึกแบบไม่สุญญากาศมีสมบัติที่ด้อยกว่าสภาวะซีลแบบสุญญากาศแต่ไม่มีความแตกต่างอย่างมีนัยสำคัญ นอกจากนี้ยังตรวจสอบสมบัติของแผ่นฟิล์มน้ำมันหอมระเหยที่เก็บในสภาวะเปิดที่ค่าความชื้นสัมพัทธ์ 70% ที่อุณหภูมิแวดล้อมเป็นเวลา 30 วัน ในสภาวะนี้สมบัติส่วนใหญ่ของแผ่นฟิล์มน้ำมันหอมระเหยมีการเปลี่ยนแปลงอย่างรวดเร็วเมื่อเทียบกับสภาวะปิดผนึก ซึ่งยืนยันได้ว่าพอลิเมอร์ก่อฟิล์มไม่สามารถป้องกันการสลายตัวของน้ำมันหอมระเหยสลายตัวจากออกซิเจนและแสงได้

สาขาวิชา ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์ ลายมือชื่อนิสิต ....

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ATIKAN SRICHOOSILP: PREPARATION AND PHYSICOCHEMICAL CHARACTERIZATION OF ESSENTIAL OILS-LOADED CARBOXYMETHYLCELLULOSE/XANTHAN GUM BLEND SHEETS. ADVISOR: ASST. PROF. PATTARA THIRAPHIBUNDET, Ph.D., 76 pp.

Novel flavor as an essential oil film was successfully fabricated by solvent casting method. Carboxymethyl cellulose and xanthan gum were film forming polymers incorporating with 4 kinds of essential oils which extracted from lemongrass, kaffir lime leaf, galangal and lime peel. The SEM images, IR spectra and TGA thermogram clearly confirmed the presence of essential oils in the network of film-forming polymers. The water solubility and loading capacity were about 90-95% and 15-26%, respectively. The physicochemical properties of the essential oil films kept in vacuum and non-vacuum seal conditions at 40°C for 120 days were investigated. The results showed that the properties of essential oil films in non-vacuum seal condition were lower than those in vacuum seal condition but not significantly different. Moreover, the properties of essential oil films which exposed to 70% RH at ambient temperature for 30 days were determined. In this condition, most properties of essential oil films were more rapidly changed in comparison to seal condition. These can be confirmed that the film forming polymers are not able to protect the degradation of essential oil from oxygen and light.

Field of Study:	Petrochemistry and Polymer Science	Student's Signature	....
		Advisor's Signature	....
Academic Year:	2016		

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LIST OF ABBREVIATIONS

%	percentage
°C	degree celsius
β	beta position
α	alpha position
cm^{-1}	unit of wavenumber (IR)
CMC	Carboxymethylcellulose
cP	centipoise
EE	entrapment efficiency
EO	essential oil
et al.	et alli, and other
FTIR	fourier transform infrared
g	gram
hr	hour
i.e.	that is
in	inch
kV	kilovolt
LC	loading capacity
µL	microliter
M	molar
mg	milligram
min	minute
mL	milliliter
mm	millimeter
nm	nanometer
pH	negative logarithm of the activity of the hydrogen ion in an aqueous solution
rpm	revolution per minute
s	second
S.D.	Standard deviation

SEM	Scanning electron microscope
w/w	weight by weight

CHAPTER I

INTRODUCTION

1.1 Motivation of research

Flavoring agents are food additives which used to enhance taste and aroma of foods. Natural flavoring substances are extracted from plants, herbs and spices. Essential oils (EOs) from herbs are used as flavoring agents in many kinds of foods [1]. However, most EOs are easily evaporated at room temperature and get lost during the food processing. Researchers try to solve this problem by encapsulation EOs in many matrices. The popular one is encapsulated by spray dry technique. This technique converts EOs or other flavors from liquid to be powder. However, this technique still uses high temperature which resulting in low content of flavor [2].

This research aims to develop a novel flavor in form of edible film. The flavors or EOs are entrapped in the network of film forming polymers and fabricated using solvent casting method. The EO film can slowly dissolve in water and release essential oil droplets which can disperse in the water and enhance smell of cooked food. Four EOs extracted from lemongrass, galanga, kaffir lime leaf, and lime peel are chosen to study. These EOs are the important smell of Thai food recipes, especially Tom-Yum. The film forming polymers in this study are carboxymethylcellulose and xanthan gum which were reported the potential in film fabrication [3, 4].

1.2 The Objectives of Research

The aims of this study are

1. To prepare EOs-loaded edible films by solvent casting method.
2. To characterize EOs-loaded edible films for their thickness, moisture content, water solubility, color, entrapment efficiency percentage and loading capacity percentage by gas chromatography, scanning electron microscope, fourier transform infrared spectroscopy, thermal gravimetric analysis and DPPH radical-scavenging activity.
3. To study stability of EOs-loaded edible films storage in vacuum and non-vacuum seal condition at 40°C in a period of 180 days.
4. To study stability of EOs-loaded edible films storage in expose condition in a period of 30 days.

CHAPTER II

THEORY AND LITERATURE REVIEWS

In this work, EO films were fabricated by solvent casting method. Lemongrass, kaffir lime leaf, galanga and lime peel essential oils were chosen to incorporate in the network of films. These EO films are aimed to be used for enhancing aroma in the instant food products as flavoring agents or instead of herbs and spices. This chapter presents the theory and literature reviews involved in this research.

2.1 Edible film [5]

Edible films are thin films prepared from edible materials that are mostly used to protect the product from environment. Film-forming polymers can be divided into three classifications: hydrocolloids, lipids, and composites. Hydrocolloids are proteins and polysaccharides, such as starch, cellulose derivatives, chitosan and alginate. Lipids are fatty acids and waxes. Composites are the combination of hydrocolloids and lipids. In this research, hydrocolloids were chosen to study due to their good water solubility property.

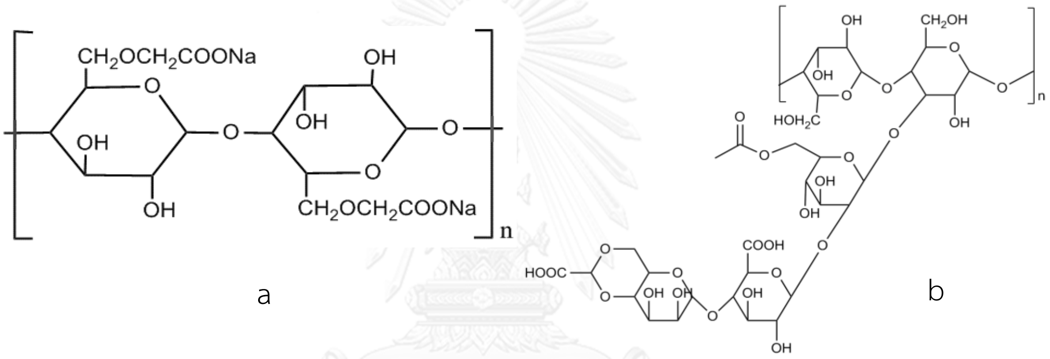
Hydrocolloids are hydrophilic polymers which had several hydroxyl groups on the polymer chains. Hydrocolloids are used as film-forming substances to control and achieve the flavor, quality and shelf-life of foods. Hydrocolloids can soluble in water and increase the viscosity of aqueous phase so they can be used as gelling agents or thickeners. Carboxymethylcellulose and xanthan gum are hydrocolloids which were chosen as film forming agents in this study.

2.1.1 Carboxymethylcellulose (CMC)

Carboxymethylcellulose (CMC) or sodium carboxymethylcellulose is an anionic long chain polysaccharide modified from natural cellulose. The carboxymethyl groups (-CH_2-COOH) bound to hydroxyl groups of the glucopyranose monomers in the cellulose backbone of CMC (Fig. 2.1). The water solubility of CMC depends on the degree of substitution and pH value. CMC solution stables in a wide pH range of 4-10 and have a maximum viscosity at pH 7-9. CMC has been widely used in different industries, such as detergents, paints, adhesives, textiles, papers, ceramics, foods and medicines. CMC is a good emulsion stabilizer and thickener, and it can improve the storage time of product. CMC films have a medium strength, resistant to oils and fats, flexible, transparent, colorless, flavorless, tasteless and moderate resistant oxygen which can be used as wrapping films [6, 7]. However, CMC films have lower barrier property because of the nature of the hydrophilic polysaccharide. The way to improve the barrier property achieved by a combination of hydrophobic components such as fatty acids in a composite film [8].

2.1.2 Xanthan gum [4]

Xanthan gum produced by the bacterium *Xanthomonas campestris* is a high molecular weight heteropolysaccharide and classified as a modified natural hydrocolloid. It is composed of pentasaccharide repeat units, comprising beta-D-glucose, alpha-D-mannose and alpha-D-glucoronic acid in a ratio of 2:2:1 attach to the cellulose backbone (Fig. 2.1). Xanthan gum can dissolve in cold and hot water. The viscosity of aqueous solution is generally not affected by pH value and temperature change and resists to enzymatic. Xanthan gum commonly used as a thickening agent and a stabilizer in various foods, like jellies, desserts and candies.



a b

Figure 2.1 Chemical structures of a. carboxymethylcellulose and b. xanthan gum.

2.2 Essential oils

Essential oils (EOs) are natural volatile aromatic liquids which were extracted from several parts of the plants such as root, stems, leaves, flowers and seeds by steam distillation. Generally, EOs contain more than 100 single substances and lead to the unique scent of each EO. EOs can be used in various applications which were cosmetic industry, food industry (fragrances and flavors), pharmaceutical industry (active components of medicines) and aromatherapy [9].

The use of EOs as flavoring agents in food is generally accepted as safe as Generally Recognized as Safe (GRAS) [10]. Flavoring agents are used to create flavors in foods such as snack that do not have flavors of their own. Moreover, flavoring agents can modify the taste and the aroma in instant food products which could have got lost due to food processing. However, EOs are unstable volatile compounds if they are not protected from external factors. The degradation occurs easily such as by oxidation, volatilization, heating and light. The protection could prolong their actions and provide a controlled release [11]. The common method to protect the EOs stability is the encapsulation techniques such as spray drying, freeze drying and extrusion. Each technique has difference principles which should be compromised to any types of flavors.

2.2.1 Spray drying technique [2]

Spray drying is an encapsulation technique based on conversion fluid or slurry of encapsulated emulsion to dry powder state by hot air continuous processing (Fig. 2.2). The encapsulated emulsion include carrier or wall material such as maltodextrin or modified starch mixing with the active ingredient such as flavors in the help of emulsifier. After that, the emulsion is fed out as small droplets and quickly dried with the hot air flowing. It makes the water in the emulsion droplets evaporate entirely. The products is in the form of a dry powder.

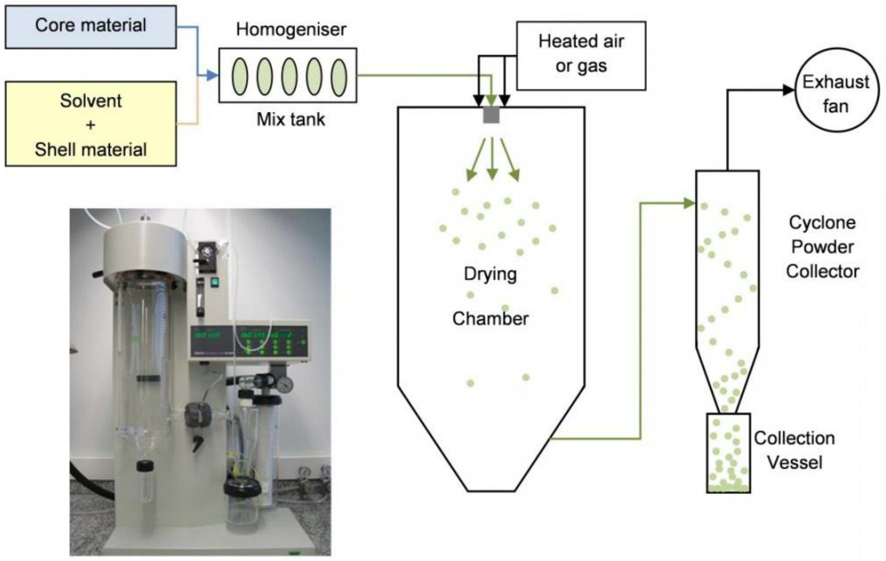


Figure 2.2 Spray drying technique [12].

2.2.2 Freeze drying technique [13]

Freeze drying process is suitable for encapsulated heat-sensitive substances because this process use low temperature to dry the encapsulated emulsion. The encapsulated emulsion should be oil in water emulsion in which the freezing water phase is removed under low temperature and pressure.

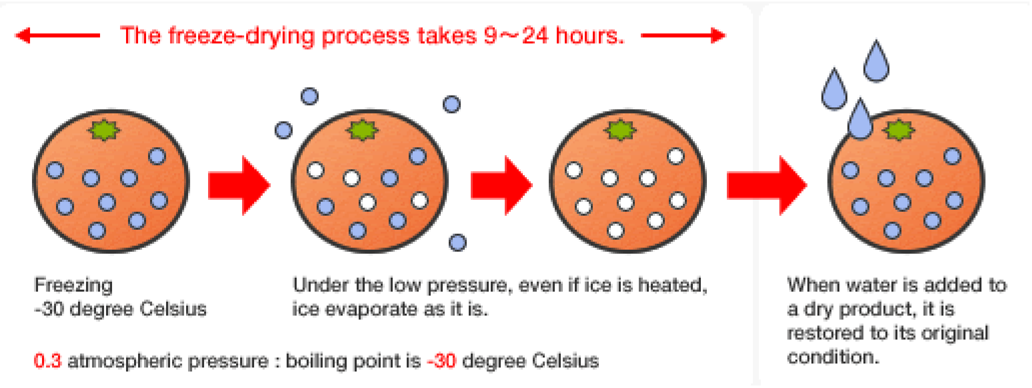


Figure 2.3 Freeze drying technique [14].

2.2.3 Melt extrusion technique [15].

Melt extrusion is an encapsulation technique which commonly uses for aroma encapsulation. The aroma is firstly mixed with a molten carbohydrates and then flushed out under high pressure through a die to form different shapes depending on the die geometry i.e. sheets, capsules or ropes. Finally, an amorphous or solid extrudates are obtained after rapid cooling by air or cold bath. The solid extrudates can be immediately cut into satisfy shapes or after the cooling step. Melt extrusion encapsulation often used in flavoring industry to manufacture of confectionery.

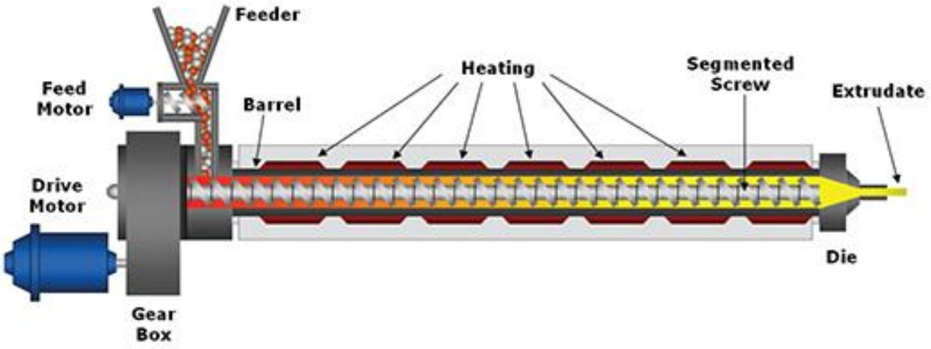


Figure 2.4 Melt extrusion technique [16].

Although these encapsulation techniques commonly use in food industry, these techniques and the encapsulated aroma products have some disadvantages. The cost of encapsulation by spray drying, freeze drying and melt extrusion are high due to the expensive apparatus and high energy consumption. Moreover, the high temperature in spray drying and melt extrusion techniques led to extreme loss of volatile aroma components. The caking problem of aroma powders obtained from spray drying and freeze drying methods is normally observed. In addition, the open porous structure of the aroma powders may allow oxygen to enter and cause oxidative degradation of aroma components [17]. In this study, we offer a new platform of aroma or flavor encapsulation as the aroma film using the solvent casting method (Fig. 2.5). The aroma is trapped by the film-forming polymers in an aqueous phase and casting in the plate before drying by the oven. This technique is simple and uses low energy consumption. The dry temperature can be adjusted and normally lower than 100°C. Moreover, the surface area per volume of the films is lower than that of the powders leading to slower degradation of the aroma.

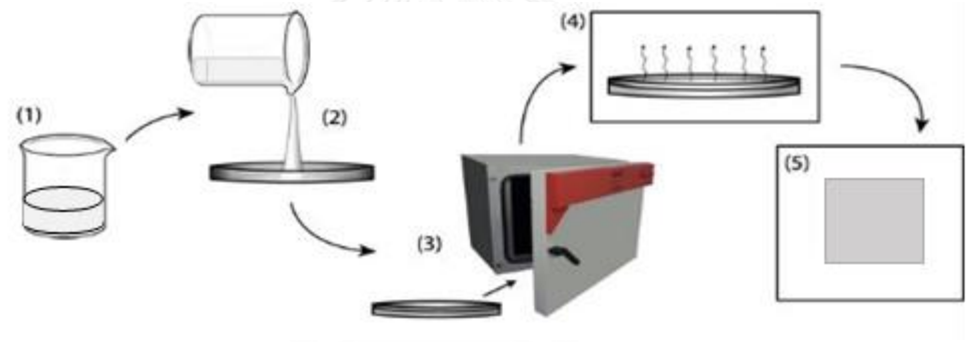


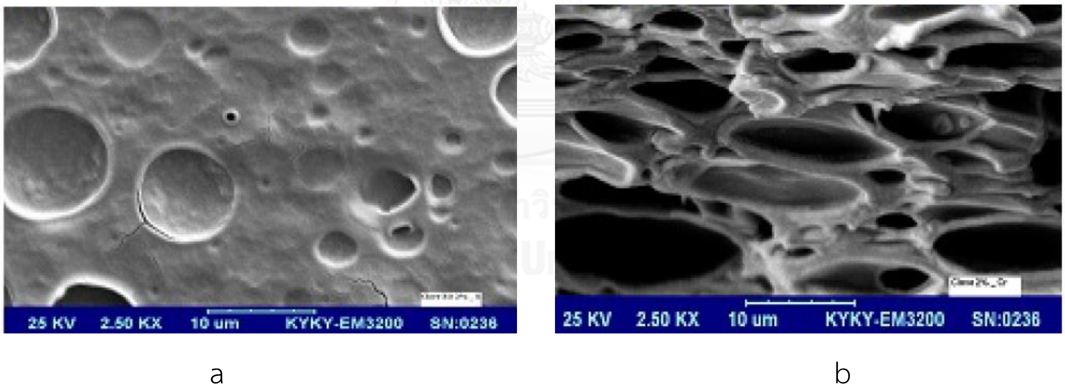
Figure 2.5 Solvent casting technique [18].

2.3 The essential oil film

There are many publications about EOs films. Most EOs films were use as film packaging to preserve foods due to the antimicrobial activity of EOs. There are many kinds of film-forming materials. However, in this study we interested CMC and xanthan gum to use as film forming agents. The following studies were some reports about fabrication of EOs films using CMC as food packaging and the role of xanthan gum as emulsifier.

In 2014, Dashipour and colleagues prepared CMC edible films incorporated with clove oil by casting method [3]. The surface and cross section of clove oil films were shown in Fig. 2.6. These films exhibited antimicrobial and antioxidant properties which can be used as the active biodegradable packaging films.

In 2014, Riveros and colleagues used CMC mixing with thyme and basil oils to coat the roasted sunflower seeds to improve the sensory and chemical stability [19]. They found that CMC with both oils can prevent lipid oxidation and the development of rancid flavors. However, thyme oil was more effective than basil oil. Thus, thyme oil is an effective natural antioxidant for the roasted sunflower seeds and probably also uses in other foods with similar physical and chemical characteristics.



a b

Figure 2.6 The a. surface and b. cross section of clove oil film [3].

In 2016, Chityala and colleagues used xanthan gum and enzyme-modified guar mixtures to enhance the oxidative stability of whey protein isolate by oil-in-water emulsion [20]. Xanthan gum acted as a stabilizer in the emulsion. The addition of XG/EMG gum mixtures significantly increased the emulsions viscosity, reduced creaming, and decreased depletion flocculation of oil droplets.

In 2015, Raeisi and colleagues studied the effect of carboxymethyl cellulose-based coatings incorporated with *Zataria multiflora* Boiss. essential oil (ZMEO) and grape seed extract (GSE) [21]. They found that CMC coating with ZMEO and GSE can extend the shelf life of rainbow trout fillets. Moreover, ZMEO and GSE at higher concentrations led to stronger antioxidant activities and sensorial scores.

In 2016, Hazirah and colleagues studied the effect of xanthan gum on the physical and mechanical properties of gelatin-carboxymethyl cellulose blend film [22]. They found that physical and mechanical properties of gelatin-CMC films were best improved with 5% xanthan gum added.

As above reports, most of them used surfactants and plasticizers to prepare EO films because those films were used as packaging film. However, high concentration of surfactant and plasticizer might be harmful to consumer. In this study, our EO films do not need any surfactants and plasticizers because we want our film to re-dissolve in water within a few minutes. We use food grade film-forming polymers and EOs extracted from plant by water distillation. Thus, our EO films are safe to consumer [23].

This research interested to prepare EO films from 4 EOs which were lemongrass, kaffir lime leaf, galanga and lime peel EOs. These EOs are the main aroma of Thai Tom-Yum recipe.

2.3.1 Lemongrass essential oil [24]

The scientific name of lemongrass is *Cymbopogon Citratus*. The stems of lemongrass contain high amount of aroma essential oil which is normally extracted by steam distillation. The main component of its essential oil is geranial (Fig. 2.7). Lemongrass smells like lemons but milder. Lemongrass commonly used in Chinese and Thai recipes and also used in beverages including tea, desserts and other forms of culinary creations as a flavoring agent.

2.3.2 Kaffir lime leaf essential oil [25]

The scientific name of kaffir lime is *Citrus hystrix*. Kaffir lime leaves are used as seasoning and flavor in many Thai dishes. The essential oil from kaffir lime leaf is extracted by steam distillation. The main component of its essential oil is citronellal (Fig. 2.7). Kaffir lime leaves EO was found to be effective in inhibiting tumors in the digestive tract and some microorganisms such as *Staphylococcus aureus*. In addition, kaffir lime leaves are the main source of β-carotene which has a good antioxidant property.

2.3.3 Galanga essential oil [26]

The scientific name of galanga is ***Alpinia officinarum*** in a family of *Zingiberaceae*. Galanga essential oil is extracted from the rhizomes by the steam distillation. The main component of its essential oil is eucalyptol (Fig. 2.7). The galangal rhizomes have many biological activities such as antitumor, antiulcer, antibacterial and antifungal properties.

2.4.4 Lime peel essential oil [27]

The scientific name of lime is ***Citrus aurantifolia***. Lime is a citric fruit used worldwide in cuisine and belongs to the Rutaceae family. Lime peel essential oil is commonly extracted by steam distillation. The main component of its essential oil is limonene (Fig. 2.7). Lime peel have antioxidant and antimicrobial properties. In addition, it has been used as flavoring agent since its organoleptic property like citric flavor.

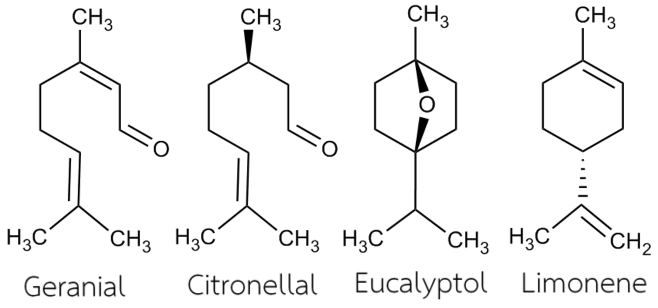


Figure 2.7 Chemical structures of the geranial, citronellal, eucalyptol and limonene

CHAPTER III

EXPERIMENTALS

3.1 Chemicals and Materials

Carboxymethyl cellulose (CMC; viscosity of 1% solution at 25°C = 2,160 cP) and xanthan gum (viscosity of 1% solution at 25°C = 1,500 cP) were purchased from Union Chemical 1986 Co., Ltd (Thailand). Lemongrass (LG) oil with 60-80% geranial, kaffir lime leaf (KLL) oil with 65% citronellal, galanga (G) oil with 40% eugenol, and lime peel (LP) oil with 60% eucalyptol were purchased from Thai-China Flavours *and Fragrances* Industry Co., Ltd (Thailand). Analytical grade organic solvents were used without further purification.

3.2 Preparation of films

Films were prepared as described by Shojaee-Aliabadi et al.[28] with some modifications. Film solutions were prepared by dissolving 9 g of CMC and 0.9 g of xanthan gum in 300 ml of distilled water. Then, a volume of 9 mL essential oil (EO) was incorporated using an homogenizer (IKAT25-Digital Ultra Turrax, Staufen, Germany) at 3,500 rpm for 5 min. The solution was cooled to 25°C for 10 min to exhaust air bubbles after homogenization. The final film forming solution was poured in a rectangular stainless steel plate (20x30 cm2) and dried at 60°C for about 14 h to provide a uniform thickness of 0.32±0.02 mm in all samples. The dry films were peeled off and stored in a desiccator with silica gel until further analysis. Film without any essential oils were also prepared as control film. Four types of EO films were prepared which were LG, KLL, G and LP films.

3.3 Film characterization

3.3.1 Thickness

Film thickness was determined with a digital electronic vernier caliper micrometer (Mituto, Tokyo, Japan) with a sensitivity of 0.01 mm. Each film sheet was measured at different points at least seven random locations and results reported as mean and standard deviation.

3.3.2 Moisture content

Films were weighed approximately 0.0100 g (W_o). Then, those films were oven-dried at 110°C until constant weight was reached (W_f). Moisture content was calculated according to the equation (1). Three replications of each film measurement were performed.

$$\%\ \text{Moisture content} = \frac{W_0 - W_f}{W_0} \times 100 \qquad (1)$$

Where W_o was the sample weight before drying, W_f was its weight after drying.

3.3.3 Water solubility

Water solubility is expressed as the percentage of the dry matter of the film that solubilizes after 24 h immersion in water using the method of Jutaporn et al. [29] with some modifications. Three film pieces of 0.25 cm^2 from each type of film were dried in an oven at 110 °C for 24 h to reach constant weight (W_i). Then, pieces were immersed, under constant agitation, into 50 ml of distilled water for 24 h at 30°C. Whatman filter paper NO. 41 was used to separate unsolved pieces film from the water. Filter papers and remained films on filter papers were dried in the oven at 110 °C until reaching constant weight (W_f). The water solubility of the film was calculated according to equation (2)

$$\%\ \text{Water solubility} = \frac{Wi - Wf}{Wi} \times 100 \qquad (2)$$

Where W_i is the initial dry weight of the film sample after reaching constant weight by drying at 110°C, and W_f is the dry weight of the unsolved film.

3.3.4 Color

The color of samples includes "L*" value, that indicates the lightness [black (L*=0) and white (L*=100)], "a" value that indicates redness–greenness [total red (a*=100) and total green (*a=-100)], and "b" value that indicates yellowness–blueness [total yellow (b*=100) and total blue (b*=-100)] were measured by the Minolta Chroma Meter CR-400 (Minolta Co., Ltd, Japan). The measurements were taken on white standard backgrounds (L = 93.49, a = -0. 25 and b = -0. 09). Total color difference (ΔE) was calculated using the equations 3.

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

Where L*, a*, and b* are the color parameter values of the film and L, a, and b are the color parameter values of the standard.

3.3.5 Entrapment efficiency percentage and loading capacity percentage

Gas chromatography (GC) analysis was used to evaluate the amount of essential oil trapped in film network. The calibration curve of each essential oil was plotted between the concentration of essential oil (X-axis) and peak area of main component in each essential oil (Y-axis). Geranial, citronellal, eucalyptol and limonene are main components of LG, KLL, G and LP oils, respectively.

Approximately 0.3 g of the essential oil film was completely dissolved in 20 mL of distilled water at room temperature with mild stirring. Hexane (20 mL) was added in the solution to extract the essential oil. The extraction was made three times and the hexane layers were combined together. The hexane extract was then adjust the volume to be 50 mL in a volumetric flask and consequently evaluated the concentration of essential oil by GC analysis. The entrapment efficiency (% EE) and loading capacity (% LC) were calculated using equations 4 and 5, respectively.

$$\%\ \mathrm{EE} = \frac{\text{Weight of essential oil in film}}{\text{Total essential oil loading}} \times 100 \qquad (4)$$

$$\%\ \mathrm{LC} = \frac{\text{Weight of essential oil in film}}{\text{Weight of film}} \times 100 \qquad (5)$$

GC analysis was performed on a Varian CP-3800 gas chromatograph (USA), using CP-Sil 8 CB fused silica capillary column (30 m × 0.25 mm with 0.25 μm film thickness). The temperature programing for detection was as follows: the temperature ramped at 2 °C/min from 45 to 100 °C and maintained for 5 min; and then ramped at 20 °C/min to 200 °C. The GC analysis conditions were as follows: the nitrogen carrier gas flow rate was 25 mL/min, hydrogen flow to the detector was 30 mL/min, air flow was 300 mL/min and the flow of nitrogen makeup gas was 45 mL/min. Injection temperature was 250 °C and detection temperature was 250 °C. The peak area was identified using GC ChemStation software. Standards of main component in each essential oil were used to identify the peaks

3.3.6 Scanning electron microscopy (SEM)

The microstructure of film surface and cross-section was observed by scanning electron microscopy (SEM; JSM-6480LV, JEOL, Japan). Film samples were attached to double side adhesive tape and gold coated under vacuum. Film samples were captured with an accelerating beam voltage of 15 kV.

3.3.7 Fourier transform infrared spectroscopy (FTIR)

The functional groups of control and essential oil films were determined using thermo Nicolet Avatar 6700 Fourier Transform Infrared Spectrophotometer (Thermo Electron Scientific, Madison, WI). The spectrometer was equipped with an attenuated total reflection (ATR) accessory with a germanium crystal. The sample was put on a germanium crystal and infrared light was passed through the sample with a frequency ranging from 4000 to 800 cm^{-1}.

3.3.8 Thermogravimetric analysis (TGA)

The thermal stability of CMC, xanthan gum and essential oil films was measured with a Mettler Toledo SDTA 851e TGA instrument. The measurements were recorded in a nitrogen gas atmosphere at a flow rate of 10 mL min^{-1}. The sample mass was about 3-5 mg and it was heated from 30 to 500 °C at a heating rate of 10 °C min^{-1}.

3.3.9 DPPH radical-scavenging activity

The antioxidant activity of the essential oil films was measured by the method of Mehdizadeh et al. [30]. This test is based on the hydrogen atom or electron donation abilities, and is evaluated by measuring the colorimetric changes (from deep-violet to light-yellow) on methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). Briefly, 0.3 g of essential oil film was completely dissolved in 10 mL of distilled water by continuous stirring, and 20 μL of this solution was added to 100 μL of the DPPH solution (0.1 mM methanol solution) and incubated in a dark place at ambient temperature for 60 min. The absorbance was measured against methanol (blank) at 517 nm and the percentage of DPPH radical scavenging activity was achieved by following equation:

$$\%\ \text{DPPH scavenging activity} = \frac{\text{A}_{blank} - \text{A}_{sample}}{\text{A}_{blank}} \times 100 \quad (6)$$

Where A_{blank} is the absorbance of blank solution and A_{sample} is the absorbance of sample.

3.4 Shelf stability

The stability of essential oil films in vacuum and non-vacuum seal condition at 40°C for 180 days were studied according to Table 3.1.

Table 3.1 Characterization of EO films in vacuum and non-vacuum seal condition

Characterization	Storage time (day) in 40°C				
	0	30	60	90	180
Thickness	√	√	√	√	√
Moisture content	√	√	√	√	√
Solubility in water	√	√	√	√	√
Color	√	√	√	√	√
%EE and %LC	√	√	√	√	√
SEM	√	-	-	√	√
TGA	√	-	-	-	√
DPPH scavenging activity	√	√	√	√	√

√ = Evaluated - = Not evaluated

3.5 Properties of EO film in exposed condition

Weight loss percentage, color and DPPH scavenging activity of LG, KLL, G and LP films which stored in 70% relative humidity (RH) at ambient temperature were evaluated at the interval time; 1, 2, 4, 6, 8, 10, 15, 20 and 30 days. Moreover, the %LC of KLL oil in KLL film was determined at 4, 8, 10, 20 and 30 days. The method for determine color, DPPH scavenging activity and %LC of this section were the same procedure as described in section 3.3.4, 3.3.5 and 3.3.9, respectively.

3.5.1 Weight change percentage

Control and essential oil films were weighed approximately 0.0100 g (W_i). Then, these films were weight at the interval times (W_f). Weight change percentage was calculated according to the equation (7). Three replications of each film measurement were performed.

$$\%\ \text{Weight change} = \frac{W_f - W_i}{W_i} \times 100 \qquad (7)$$

3.6. Statistical Analysis

Quantitative data were reported as means ± standard deviations, where indicated. Statistical analysis was performed using a one-way Anova analysis, followed by the Turkeys HSD for multiple comparisons. A p-value <0.05 was considered statistically significant.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Film thickness, moisture content and water solubility

The thickness of control and EO films were measured by a digital micrometer and presented in Table 4.1. The thickness of the control film (0.21 mm) was statistically significant different from thicknesses of EO films in the range of 0.30 to 0.33 mm ($p<0.05$). This indicated that incorporation of EO into the film forming matrix was able to increase the thickness of film.

The moisture content and the water solubility of the control and the EO films were shown in Table 4.1. It is clearly show that the moisture content of the EO films was lower whereas the water solubility was higher than those of control film. This might be because EO destroyed or interrupted the hydrogen bond interaction between water molecules and the functional groups (-COOH and –OH) of CMC and xanthan gum [3]. The EO droplets repelled the water molecules in the network of film forming led to the decrease of moisture content. Moreover, the weak interaction between polymer chains of EO film resulted in the easier re-dissolving and dispersion in the water [30].

Table 4.1 Film thickness, moisture content and water solubility of the EO films.

Sample	Thickness (mm)	Moisture content (%)	Water solubility (%)
Control	0.21±0.01	16.67±2.01	85.39±2.04
LGF	0.35±0.04	12.42±2.45	95.09±2.15
KLLF	0.30±0.03	12.18±1.59	94.66±3.87
GF	0.32±0.04	12.73±1.50	89.31±3.69
LPF	0.32±0.03	12.42±1.65	90.6±1.71

Note: Data reported are average values ± standard deviations. Values within first rows are significantly different ($p<0.05$).

4.2 Appearance, color and thickness

The appearances of the dried EO films were shown in Fig. 4.1. The control and the EO films were transparent and had rough surfaces. The colors of the EO films depended on the color of incorporated EOs. The control film had more bubbles than the EO films. In the solution preparation step, we noticed that the film forming solution of the control film had more air bubbles than those of EO solution. This might be that the EO droplets repelled the bubbles and resulted in decrease the bubble in the film forming solution.

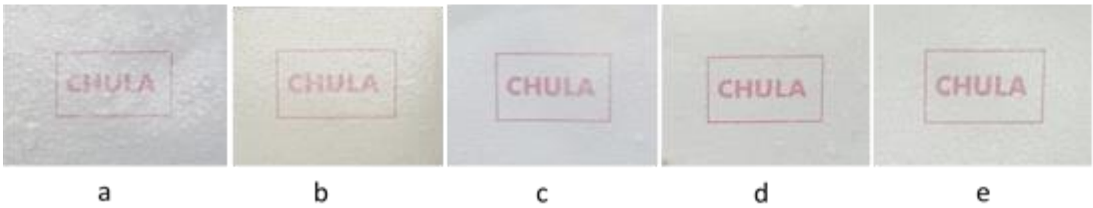


Figure 4.1 Appearance photos of a. control, b. LG, c. KLL, d. G, and e. LP films.

Hunter Lab color values ("L", "a" and "b"), and total color difference (ΔE) values were evaluated to express the optical properties and the results are shown in Table 4.2. The control film was colorless, clear and has a transparent appearance (highest L* value), but the incorporation of the essential oils in the films led to the decrease of clarity and transparency [3]. The L* and ΔE values of control film were significantly different from those of EO films (p<0.05) whereas the positive a* (greenness) and negative b* (yellowness) values were not.

Table 4.2 The optical properties of EO films

Sample	L*	a*	b*	ΔE
Control	47.38±1.98	1.36±0.11	-3.21±0.31	50.51±1.58
LGF	43.19±0.93	0.89±0.23	-2.79±0.46	54.63±2.30
KLLF	42.98±0.79	1.22±0.06	-4.81±0.12	55.05±1.52
GF	43.65±0.85	1.16±0.12	-3.98±0.24	54.29±1.04
LPF	43.6±0.69	1.15±0.14	-4.28±0.26	54.37±0.76

Data reported are average values ± standard deviations. L* and ΔE values at first row are significantly different (p<0.05).

4.3 Films microstructure and dispersion of EO particles

The SEM images of the control film and the EO films are shown in the Fig. 4.2. The surface of the control film was smoother than that of the EO films. The cross section micrographs clearly revealed that the EO films contained different sizes of small oil droplets dispersed in the polymer matrix. In this case, EO films were prepared without any emulsifier such as tween 80. The small EO droplets were created by the homogenize speed and were stabilized by the hydrophilic property of polymer chain to inhibit the agglomerate of EO droplets. The EO entrapment in the network of polymer was proposed as depicted in Fig. 4.3.

Moreover, after dissolving the EO film in water, we found that the EO droplets can well disperse in the water which was different from EO that is immiscible in the water as show in Fig. 4.4. The EO droplets were surrounded with CMC chain and xanthan gum. Xanthan gum is an amphiphilic substance and can acts as an emulsifier. In the solution, xanthan gum stays at the interface between the dispersed phase of EO droplets and the continuous phase of water. Thus, xanthan gum can help EOs and CMC droplets to disperse in the water.

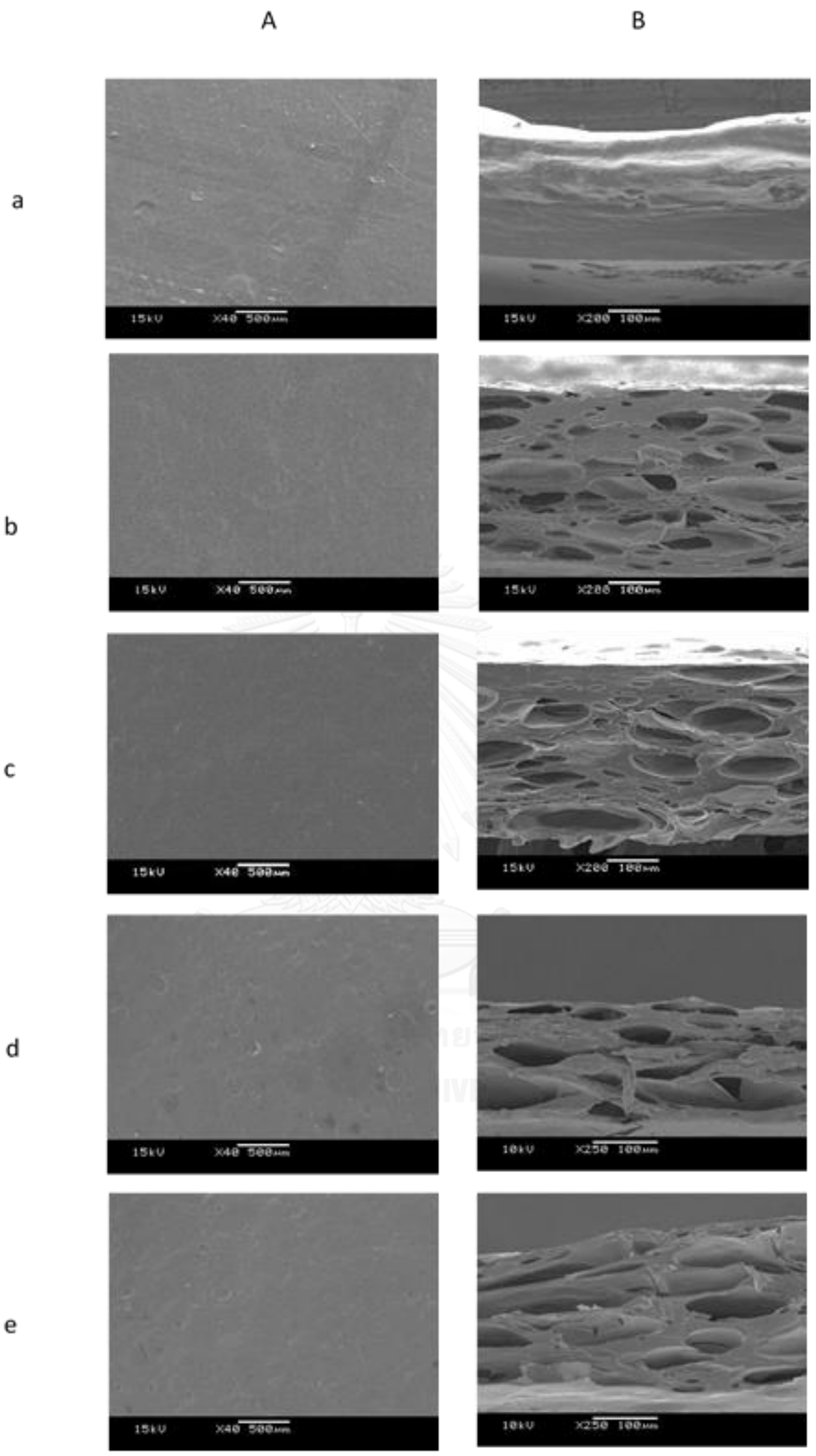


Figure 4.2 SEM micrographs of the surfaces (left, A) and cross-sections (right, B) of the a. control films, b. LG, c. KLL, d. G, and e. LP film.

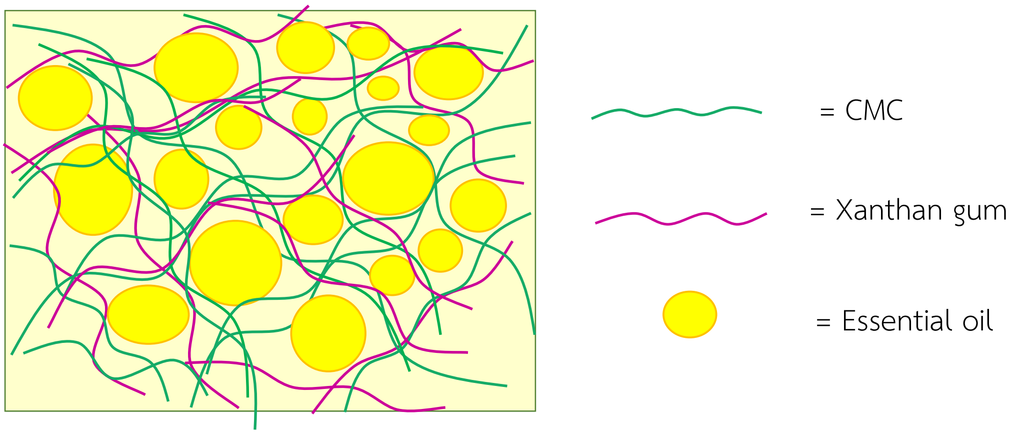


Figure 4.3 The scheme of EO entrapment in the network of polymers.

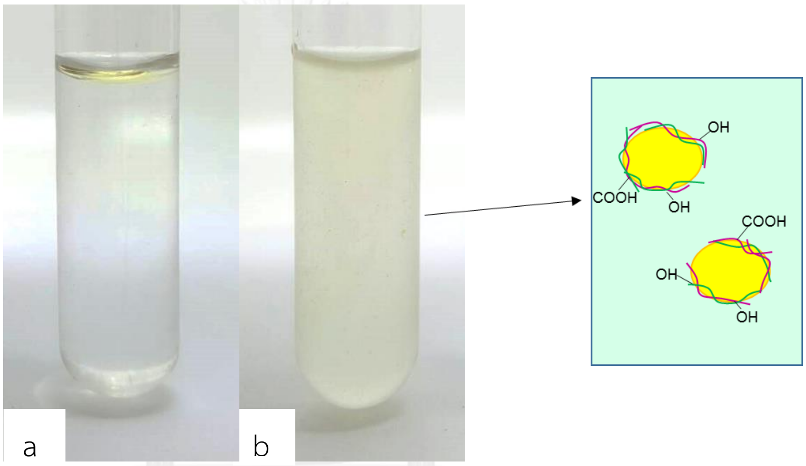


Figure 4.4 The a. EO in water and b. EO film dissolve in the water.

4.4 Fourier-transform infrared (FTIR) spectroscopy

FTIR spectra of the control film and EO films were illustrated in Fig. 4.5. Generally, the FTIR spectra of the control film, and the film containing EO exhibited the similar major peaks but the characteristic peaks of the EO films are quite different from the polymers.

The IR spectra of the control film (Fig. 4.5a) showed the emblematic absorptions of the cellulose backbone of CMC and xanthan gum which is the OH stretching broad band at 3296 cm^{-1} , the adsorption of COO- at 1585 – 1589 cm^{-1} [..], the C-O-C asymmetry bridge stretching band at 1017 cm^{-1} and the C-H stretching band at 2850-2950 cm^{-1} [31]. The characteristic multiple peaks in the range of 1300-1450 cm^{-1} were different in any cellulose.

The FTIR spectra of EOs were shown in Fig. 4.6 in which most of them showed the strong saturated hydrocarbon C-H stretching absorptions at 2800-3000 cm^{-1}. The spectra of LG and KLL EOs showed a sharp C=O stretching peak of geranial and citronellal at 1674 and 1726 cm^{-1} [32, 33], respectively. The C=O stretching peak also found in the spectra of G and LP EOs which might be belong to minor compounds in oils. The FTIR of the EO films (Fig. 4.5b, c, d and e) confirmed the presence of EOs in the films by the incidence of the small peaks of C=C adjacent at 1374, C=O stretching at ~1700 cm^{-1} and saturated hydrocarbon C-H stretching absorptions of EOs [34, 35].

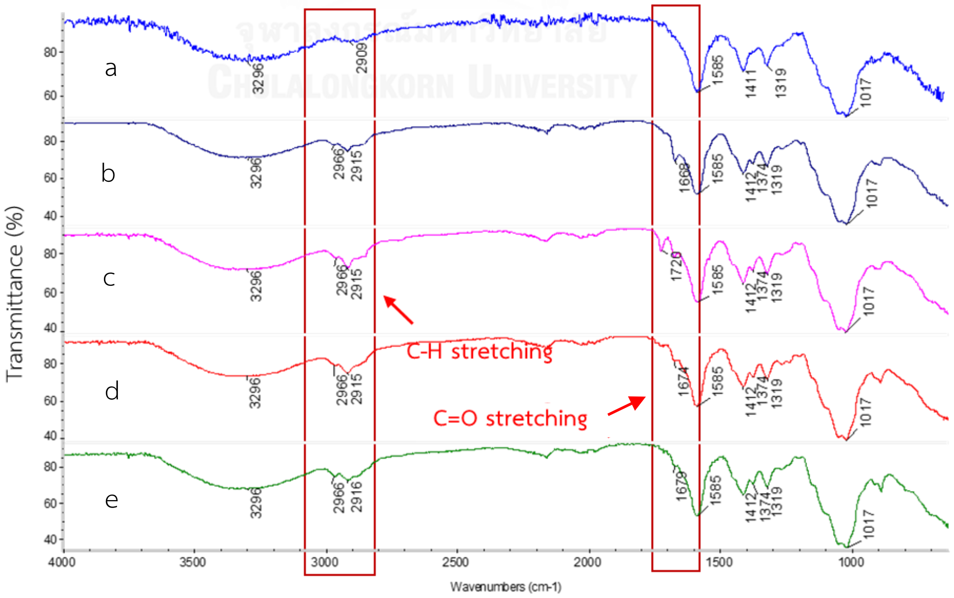


Figure 4.5 FTIR spectra of a. conrol film, and b. LG, c. KLL, d. G and e. LP films.

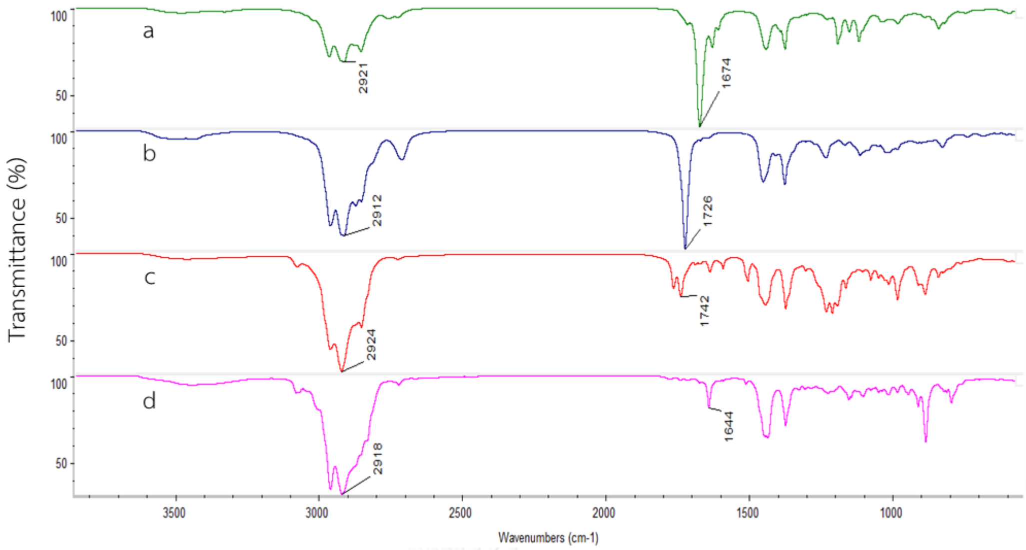


Figure 4.6 FTIR spectra of a. LG, b. KLL, c. G and d. LP EOs.

4.5 Thermal stability

Thermogravimetric analysis (TGA) is a process to determine the decompose temperature by heat of each composition in material. The TGA curves and their derivatives are presented in Fig. 4.7

The TGA thermograms of CMC and xanthan gum powder (Fig. 4.7a and 4.7b) showed the evaporated temperature of the bound water in the first step at 100°C and the degradation temperature side chain and the loss of carboxyl group of CMC and xanthan gum in the second step at 290 and 300°C, respectively [36]. Moreover, the control film (Fig. 4.7c) had a degradation temperature in a combination manner of individual CMC and xanthan gum at 280 and 310 °C. The EOs in films (Fig. 4.7d-g) showed pyrolysis and decomposed temperature at 250-260°C (depend on kinds of EO) with about 20-30% weight loss in the TG curves corresponding to %LC.

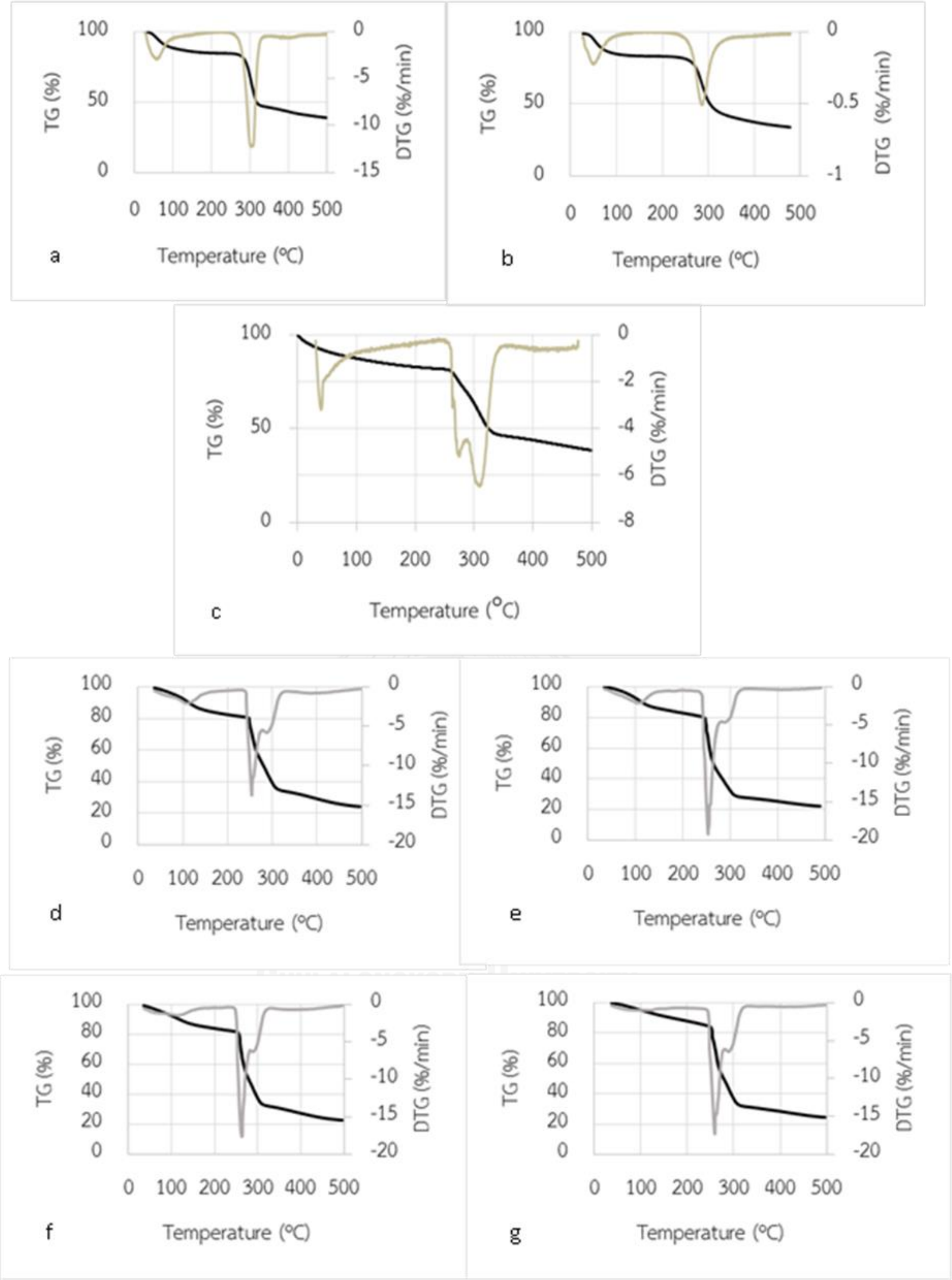


Figure 4.7 TG and DGT curve of a. CMC, b. xanthan gum, c. control, d. LG, e. KLL, f. G and g. LP film

4.6 Loading capacity and entrapment efficiency percentages

Natural essential oil contains more than 100 compounds which lead to the unique scent of each essential oil. To evaluate the amount of the EO incorporated in the films, only the major component was identified by gas chromatography (GC) spectrometry. The main compounds of LG, KLL, G, and LP oils are geranial, citronellal, eucalyptol and limonene, respectively. The results of the loading capacity (LC) and entrapment efficiency (EE) percentages are shown in Table 4.3. The different LC percentages might be caused by the different boiling points of each EO. The loss rate of EO during oven drying depend on the temperature and drying time. The higher temperature will spend shorter drying time. But the decomposition of EO at high temperature should be conscious.

Table 4.3 Loading capacity and entrapment efficiency percentage of EO in films.

Sample	Major volatile of EO	Boiling point (ºC)	%LC	%EE
LGF	Geranial	229	25.9 ± 1.7	43.2 ± 2.8
KLLF	Citronellal	208	23.1 ± 2.6	38.5 ± 4.4
GF	Eucalyptol	177	14.2 ± 2.1	23.6 ± 3.6
LPF	Limonene	176	20.7 ± 6.8	34.5 ± 11.3

4.7 DPPH scavenging activity

The DPPH scavenging assay was used to determine the antioxidant activity of the EOs in films. The results in Table 4.4 showed that the control film has no antioxidant activity whereas the EO in films and the free EO have similar power of the antioxidant activity in the range of 12-31% at the EO concentration of 4-8 mg/mL. The different concentration of each EO was used to determine the activity due to the different LC percentage of each EO in film (∼0.3 g).

Table 4.4 Percentage of DPPH scavenging activity of EOs in films

Sample	Concentration of EO (mg/mL)	DPPH scavenging activity (%)	
		Free EO	EO in film
LGF	7.5	19.7±0.1	19.0±5.7
KLLF	6.9	12.5±0.1	12.3±8.0
GF	4.3	31.3±0.1	30.8±5.7
LPF	6.2	27.0 ±0.1	26.4±3.9

4.8 Shelf-life stability

The EO films were kept in aluminum foil sealing bags with vacuum and non-vacuum conditions at 40°C. The EO films were followed up their morphology and physicochemical properties at the interval time; 30, 60, 90 and 180 days, except for 90 and 180 days for SEM analysis and 180 days for TGA analysis.

4.8.1 Appearance, thickness and color

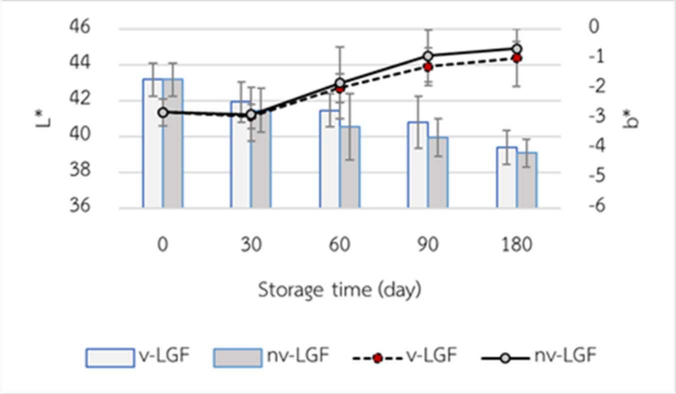
The photograph images and the thickness of the stored EO films were summarized in Table. 4.5. The transparency of most EO films were not significantly changed which was consistent to their L* values in Fig. 4.8**.** The reduction of L* values indicated the increase of turbidity which L* values of most films were decreased 3-4 unit within 180 days. However, it was obviously noticed that the yellowish color of LG films remarkably increased compared to other EO films. This result also confirmed by the b* value investigation shown in Fig. 4.8. The more positive b* value means more yellowish color. The b* values of 180-day vacuum and non-vacuum LG films significantly changed from -2.79 to -0.96 and -0.68, respectively, while those of other EO films were less changed.

The thickness of all the vacuum and non-vacuum EO films significantly decreased at $p<0.5$ when were stored for 180 days.

Table 4.5 Appearance and thickness of EO films

Sample	Storage time (day)									
	0		30		60		90		180	
	Apperance	Thickness (mm.)	Apperance	Thickness (mm.)	Apperance	Thickness (mm.)	Apperance	Thickness (mm.)	Apperance	Thickness (mm.)
v-LGF		0.35±0.04		0.32±0.02		0.31±0.01		0.27±0.02		0.25±0.01
nv-LGF				0.31±0.02		0.29±0.02		0.27±0.03		0.24±0.02
v-KLLF		0.30±0.03		0.29±0.01		0.28±0.01		0.27±0.02		0.25±0.02
nv-KLLF				0.29±0.01		0.28±0.02		0.27±0.03		0.23±0.03
v-GF		0.32±0.04		0.31±0.03		0.29±0.01		0.27±0.03		0.25±0.02
nv-GF				0.30±0.03		0.27±0.01		0.26±0.02		0.25±0.02
v-LPF		0.32±0.03		0.31±0.03		0.30±0.02		0.28±0.01		0.26±0.02
nv-LPF				0.30±0.03		0.29±0.01		0.27±0.02		0.25±0.03

a


L*
b*
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40
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Storage time (day)
v-LGF
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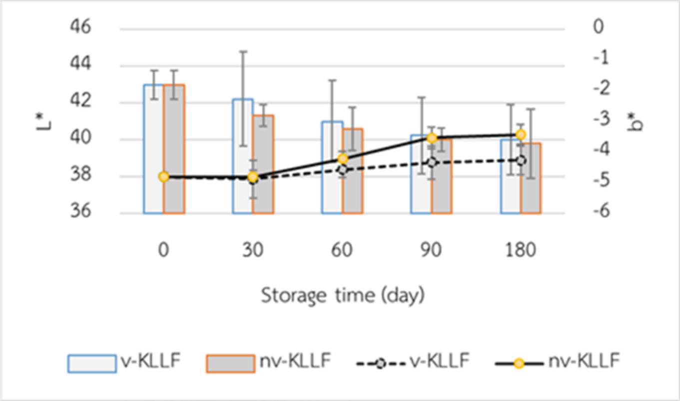
nv-LGF

b


L*
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Storage time (day)
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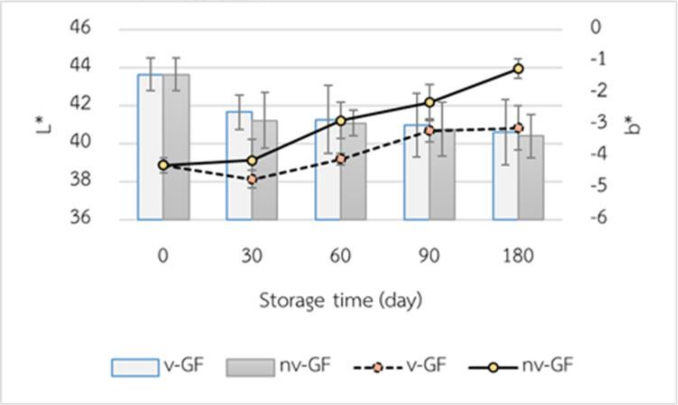
c


L*
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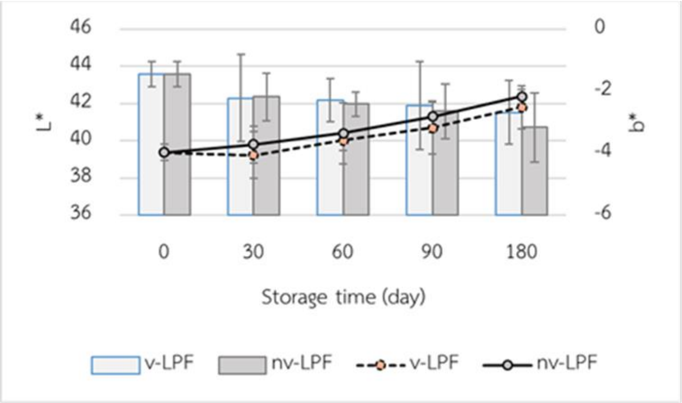
L*
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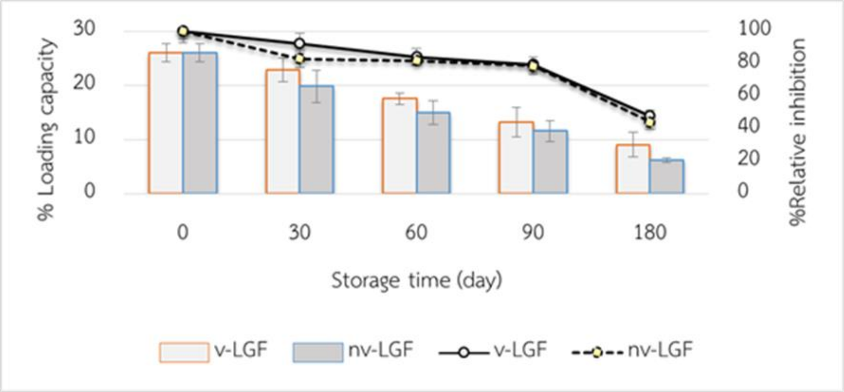
Storage time (day)
v-LPF
nv-LPF
v-LPF
nv-LPF

Figure 4.8 L* value (bar graph) and b* value (line graph) of a. LG film in vacuum (v-LGF) and non-vacuum (nv-LGF) conditions, b. KLL film in vacuum (v-KLLF) and non-vacuum (nv-KLLF), c. G film in vacuum (v-GF) and non-vacuum (nv-GF) and d. LP film in vacuum (v-LPF) and non-vacuum (nv-LPF).

4.8.2 Loading capacity percentage, DPPH scavenging activity and SEM analysis

The loading capacity and DPPH scavenging activity of vacuum and non-vacuum EO films within 180 days were shown in Fig 4.9. The EO amount entrapped in film was obtained by the peak area of the major compound in GC chromatogram. The results revealed that EO content in vacuum and non-vacuum EO films was gradually decreased with the closer rate. The loss of the EO content might be caused by degradation of the major compound to others. However, the degraded compounds could not be observe in GC chromatogram due to very less amount or still in aqueous phase during extraction. The decrease of %LC was corresponded to the decrease of %relative DPPH scavenging activity.

a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%Relative inhibition
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Storage time (day)
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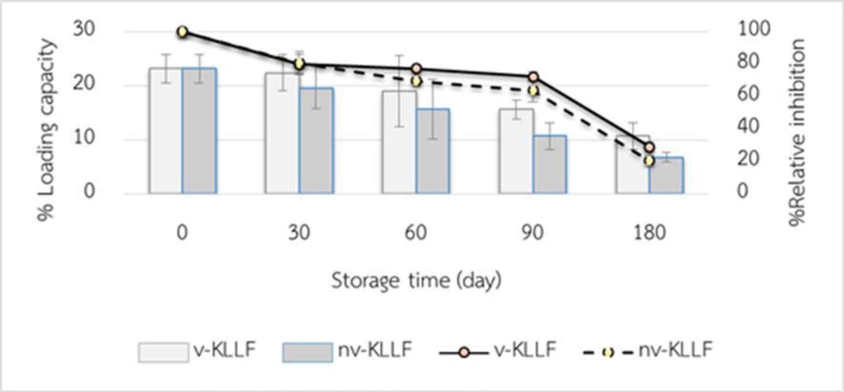
v-LGF
nv-LGF

b


% Loading capacity
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%Relative inhibition
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Storage time (day)
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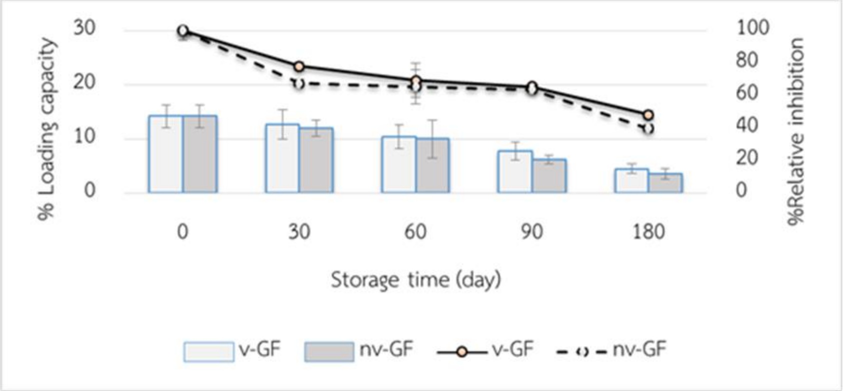
nv-KLLF
v-KLLF
nv-KLLF

c


% Loading capacity
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%Relative inhibition
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Storage time (day)
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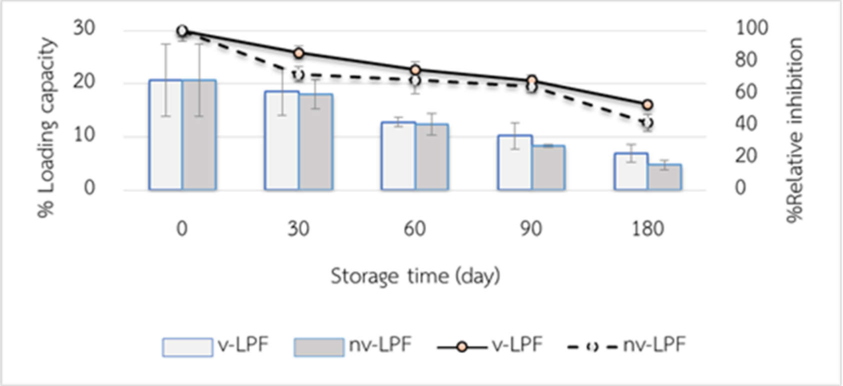
d


% Loading capacity
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%Relative inhibition
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80
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30
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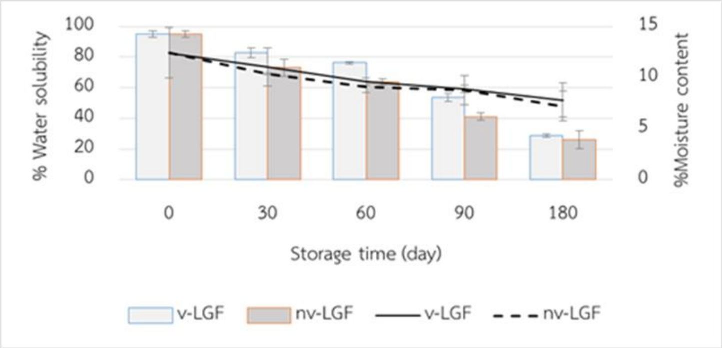
180
Storage time (day)
v-LPF
nv-LPF
v-LPF
nv-LPF

Figure 4.9 The %loading capacity (bar graph) and %relative DPPH inhibition (line graph) of a. LG film in vacuum (v-LGF) and non-vacuum (nv-LGF) conditions, b. KLL film in vacuum (v-KLLF) and non-vacuum (nv-KLLF), c. G film in vacuum (v-GF) and non-vacuum (nv-GF) and d. LP film in vacuum (v-LPF) and non-vacuum (nv-LPF).

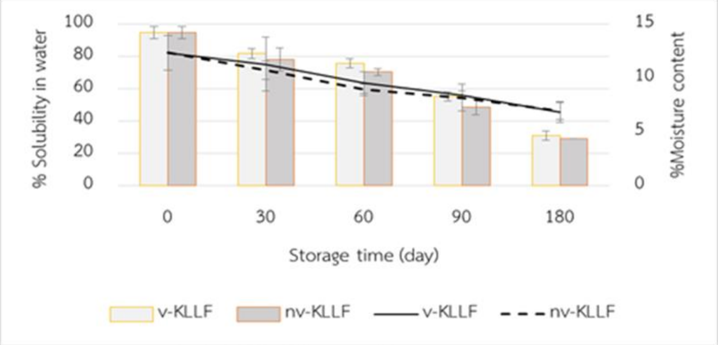
4.8.3 Moisture content and water solubility

The moisture content and the water solubility of the vacuum and the non-vacuum EO films within 180 days were shown in Fig. 4.10. The moisture content and water solubility of films gradually decreased during the storage by a function of time but not significantly different between the vacuum and the non-vacuum EO films. The moisture content of the EO film is the sum total of water and EO contents in film. According to the decrease of %LC in section 4.8.2, the loss of EO content affected the moisture content. Moreover, the lessening of the water solubility of the EO films might be caused by the collapse of network since polymer chains mobility is restricted by the extension of the intra- and inter molecular hydrogen bonds network between the cellulose chains [37].

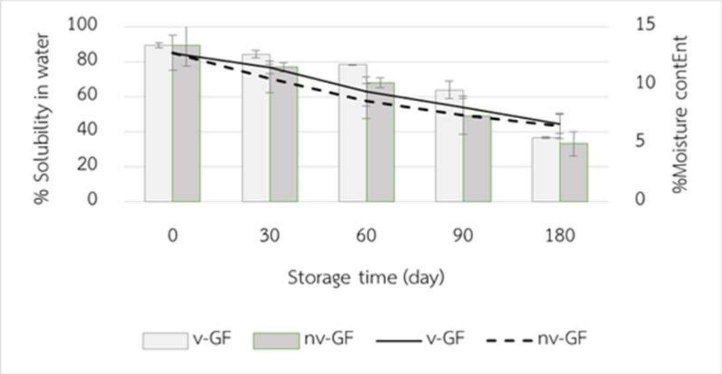
a


% Water solubility
100
80
60
40
20
0
15
10
5
0
%Moisture content
0
30
60
90
180
Storage time (day)
v-LGF
nv-LGF
v-LGF
nv-LGF

b


% Solubility in water
100
80
60
40
20
0
15
10
5
0
%Moisture content
0
30
60
90
180
Storage time (day)
v-KLLF
nv-KLLF
v-KLLF
nv-KLLF

c


% Solubility in water
100
80
60
40
20
0
15
10
5
0
%Moisture contEnt
0
30
60
90
180
Storage time (day)
v-GF
nv-GF
v-GF
nv-GF

d

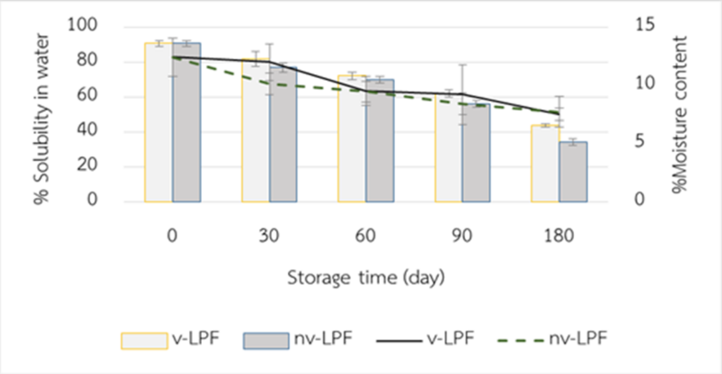

% Solubility in water
100
80
60
40
20
0
15
10
5
0
%Moisture content
0
30
60
90
180
Storage time (day)
v-LPF
nv-LPF
v-LPF
nv-LPF

Figure 4.10 The %water solubility (bar graph) and %moisture content (line graph) of a. control film, b. LG film in vacuum (v-LGF) and non-vacuum (nv-LGF) conditions, c. G film in vacuum (v-GF) and non-vacuum (nv-GF) and d. LP film in vacuum (v-LPF) and non-vacuum (nv-LPF).

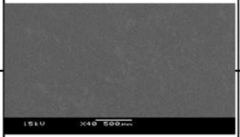
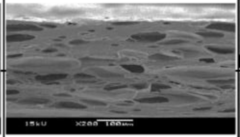
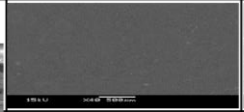
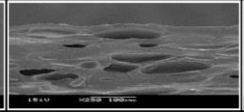
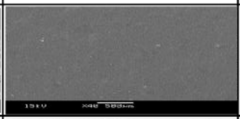
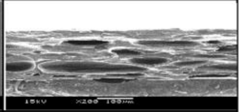
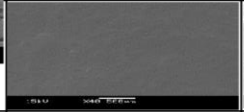
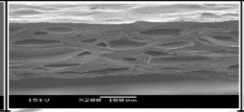
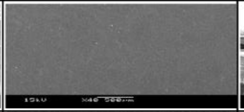
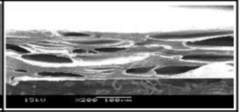
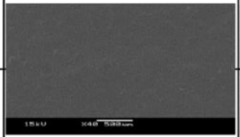
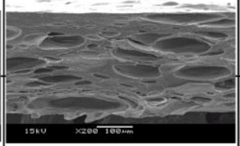
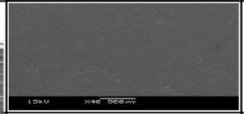
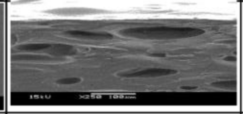
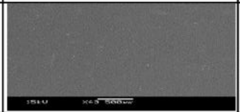
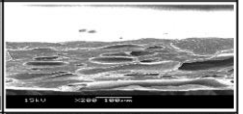
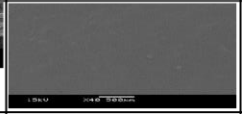
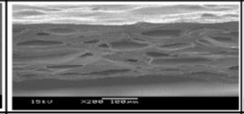
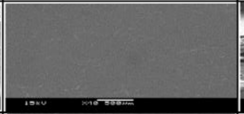
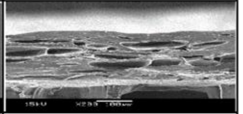
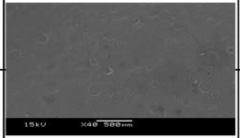
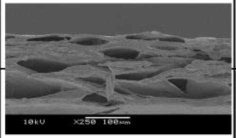
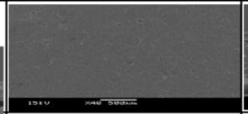
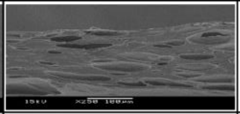
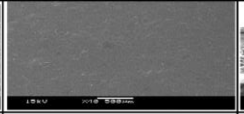
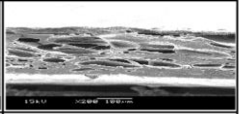
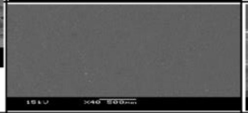
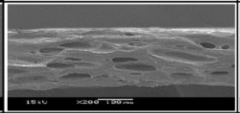
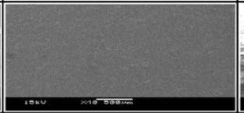
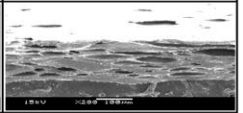
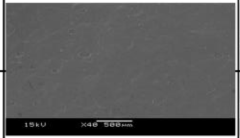
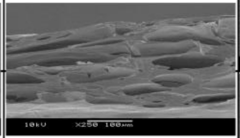
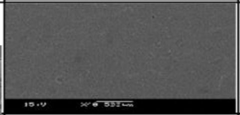
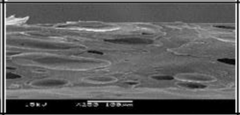
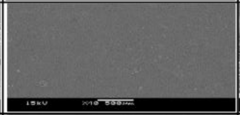
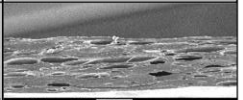
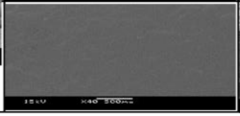
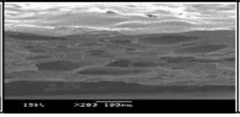
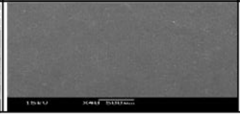
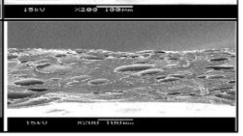
4.8.6 Films Microstructure

The SEM image of EO films at 0, 90 and 180 days were shown in the Table 4.6. The surface of vacuum and non-vacuum EO films at each time were not changed. However, the cross-section micrographs of those films showed that most EO droplets collapsed and led to the decline of film thickness.

4.8.7 Thermal stability

The TGA thermograms of EO films at 0 and 180 days were shown in the Fig. 4.11. The degradation pattern of EO films did not changed whereas the %weight loss (%TG) changed depend on the decline of EO content during storage for 180 days. The %weight loss of non-vacuum EO film was slightly lower than that of vacuum EO film which correlated to %LC.

Table 4.6 SEM micrographs of the surfaces and cross-sections of the EO films

Sample	Storage time (day)					
	0		90		180	
	Surface	Cross section	Surface	Cross section	Surface	Cross section
v-LGF						
nv-LGF						
v-KLLF						
nv-KLLF						
v-GF						
nv-GF						
v-LPF						
nv-LPF						

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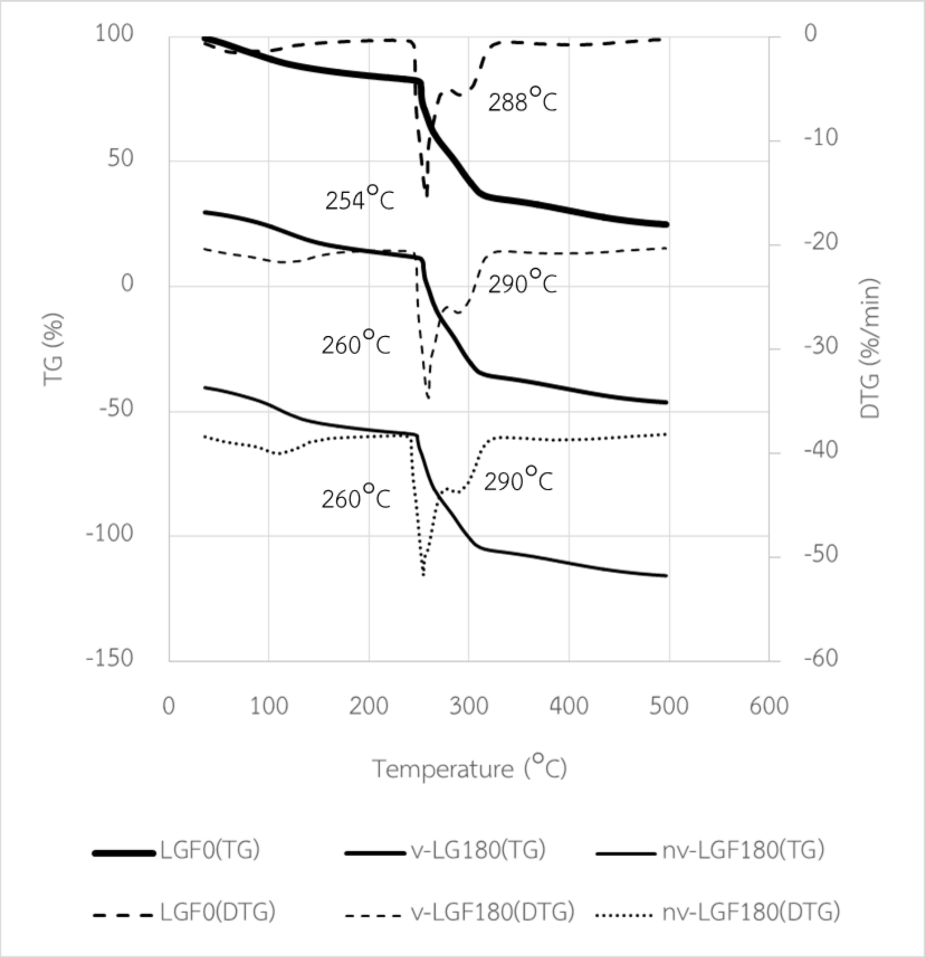
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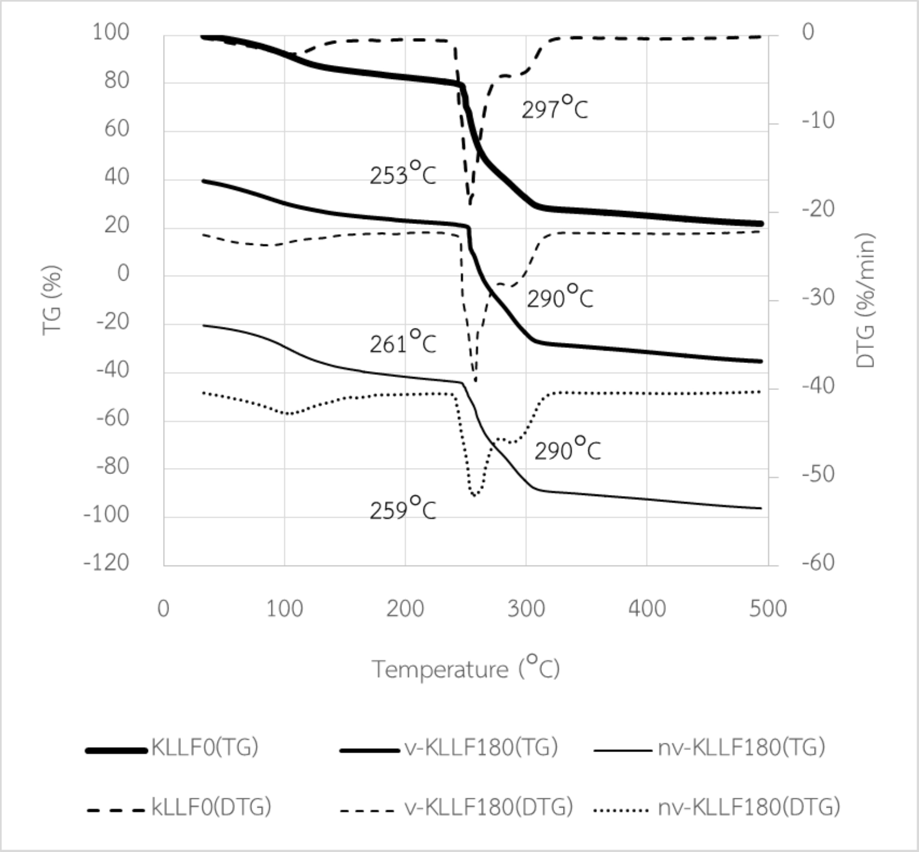
50
0
-50
-100
-150
TG (%)
0
-10
-20
-30
-40
-50
-60
DTG (%/min)
0
100
200
300
400
500
600
Temperature (°C)
288°C
254°C
290°C
260°C
290°C
260°C
LGF0(TG)
v-LG180(TG)
nv-LGF180(TG)
LGF0(DTG)
v-LGF180(DTG)
nv-LGF180(DTG)

b


TG (%)
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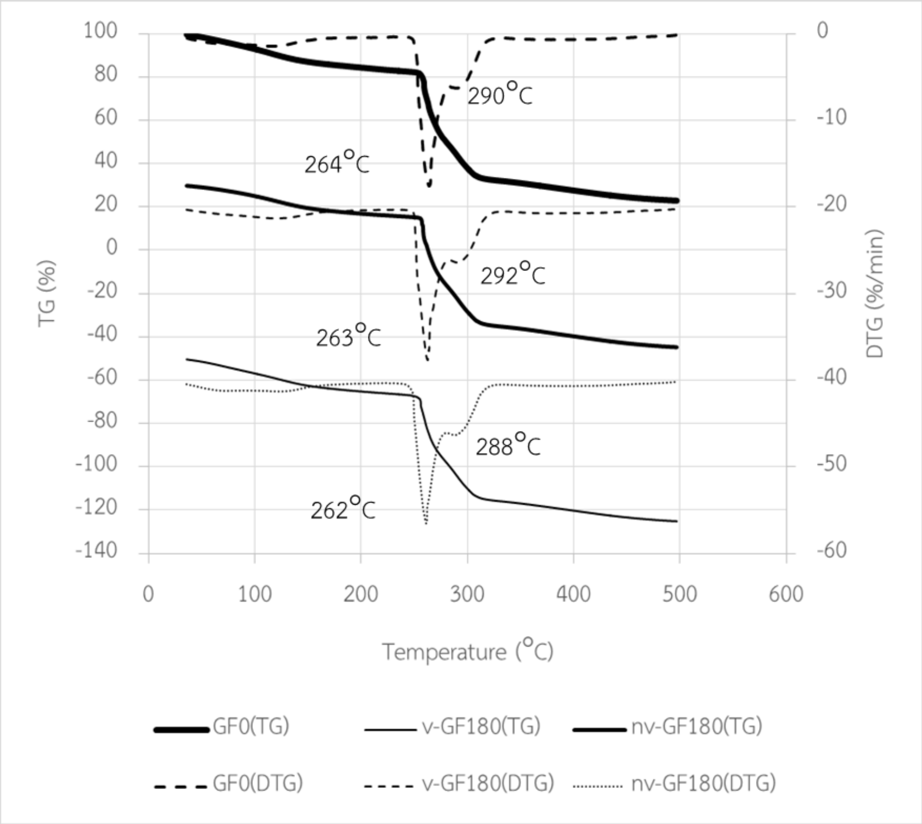
80
60
40
20
0
-20
-40
-60
-80
-100
-120
DTG (%/min)
0
-10
-20
-30
-40
-50
-60
0
100
200
300
400
500
Temperature (°C)
297°C
253°C
290°C
261°C
290°C
259°C
KLLF0(TG)
v-KLLF180(TG)
nv-KLLF180(TG)
kLLF0(DTG)
v-KLLF180(DTG)
nv-KLLF180(DTG)

c


100
80
60
40
20
0
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-40
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-100
-120
-140
TG (%)
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-10
-20
-30
-40
-50
-60
DTG (%/min)
0
100
200
300
400
500
600
Temperature (°C)
290°C
264°C
292°C
263°C
288°C
262°C
GF0(TG)
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nv-GF180(TG)
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v-GF180(DTG)
nv-GF180(DTG)

d

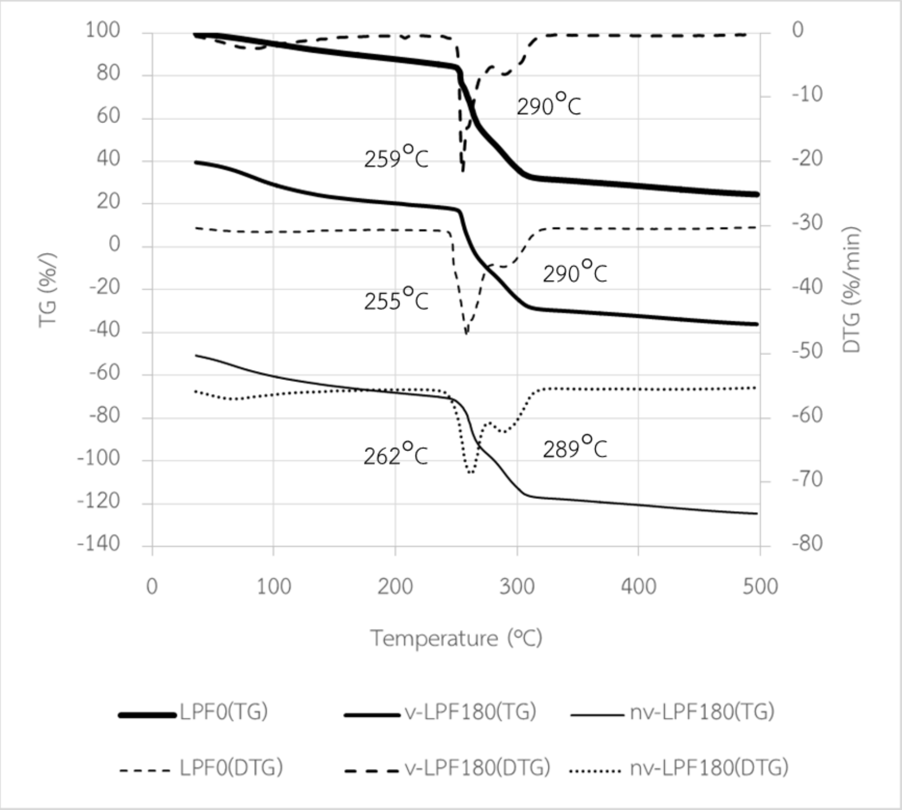

Figure 4.11 The TG and DTG thermogram of a. LG film in vacuum (v-LGF) and non-vacuum (nv-LGF) conditions, b. KLL film in vacuum (v-KLLF) and non-vacuum (nv-KLLF), c. G film in vacuum (v-GF) and non-vacuum (nv-GF) and d. LP film in vacuum (v-LPF) and non-vacuum (nv-LPF).

4.9 Properties of EO film in exposed condition

The EO films were kept in a desiccator at ambient temperature for 30 days. This experiment was studied in the control humidity which was 70% RH. This condition simulated the normal humidity in Thailand. All EO films were followed up their %weight loss, color and DPPH scavenging activity at the interval time; 1, 2, 4, 6, 8, 10, 15, 20 and 30 days. And %LC of KLL film were determined at 4, 8, 10, 20 and 30 days by GC analysis.

4.9.1 %Weight change

EO films were taken out from desiccator and weighed to determine %weight change during 30 days which showed in Fig. 4.12. The %weight change of the control film was increased only 4 percentages within 30 days. This indicated that this film scarcely absorbed moisture in the expose condition. In contrast, the %weight change of all EO films gradually increased about 3-5% in the 8 days and gradually decreased after that until lower than the initial weight. The weight change of EO film depended on two factors which were the rate of EO loss and the rate of moisture absorption. During the first 8 days, there was exchange equilibrium between moisture absorption and EO evaporation on the surface of film. And the rate of moisture absorption was higher than the rate of EO evaporation resulted in the upsurge of %weight change. After 8 days, the rate of moisture absorption was lower than that of EO evaporation. We noticed that the film surface was dried and led to the collapse of the film after 8 days. This phenomena might disrupt the moisture absorption and EO evaporated of the EO films.

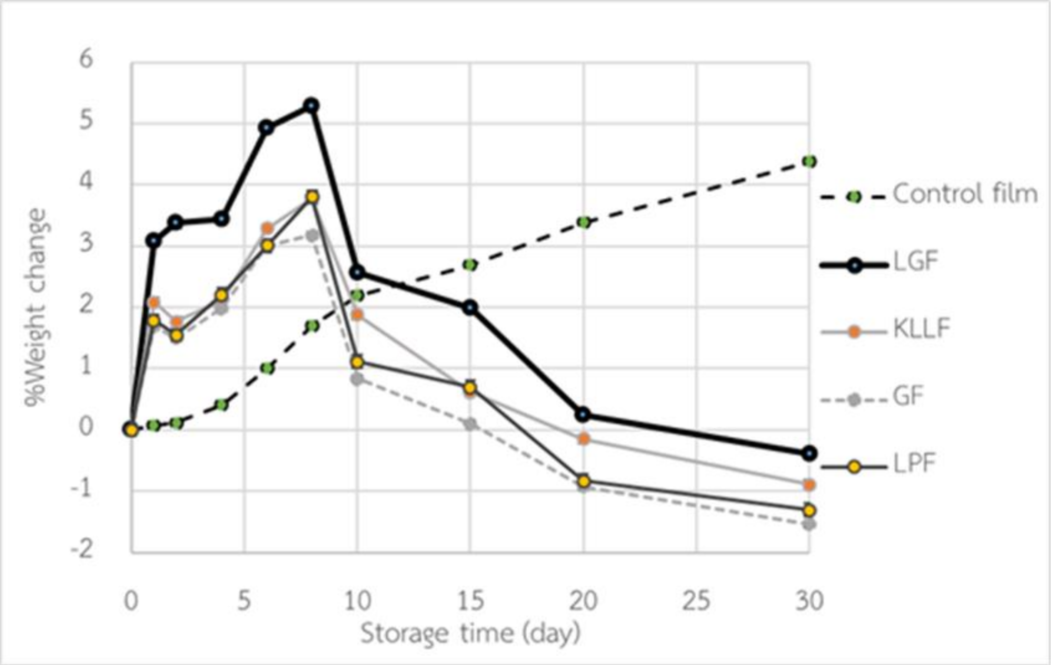

Figure 4.12 %Weight change of EO films

4.9.2 The color of EO film

The transparency (L* value) and color (b* value) of EO films within 30 days were shown in Figs. 4.13-4.14. The results of both experiments indicated the instant increase of turbidity and yellowish of EO films. The L* value of 2-day films and the b* value of 8-day films in expose condition were nearest to those of 180-day film storing in seal foil bags (data in section 4.8.1). These results represented that oxygen and light significantly affected the properties of the EO in the film and both CMC and xanthan gum could not good protect the EO from oxygen and light.

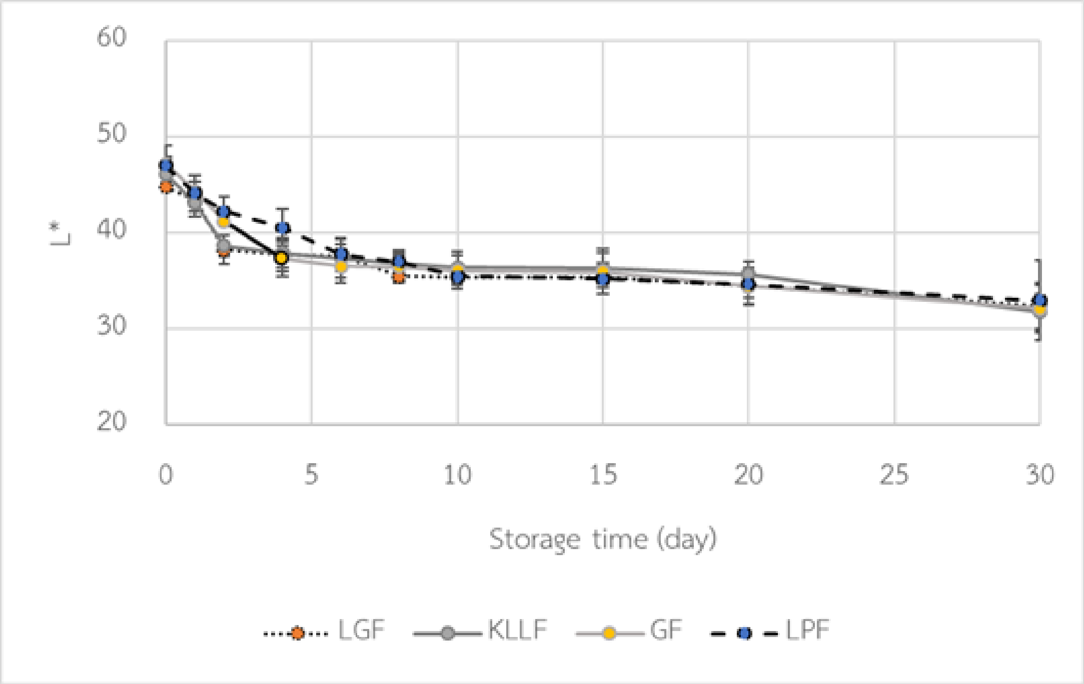

Figure 4.13 L* value of EO films

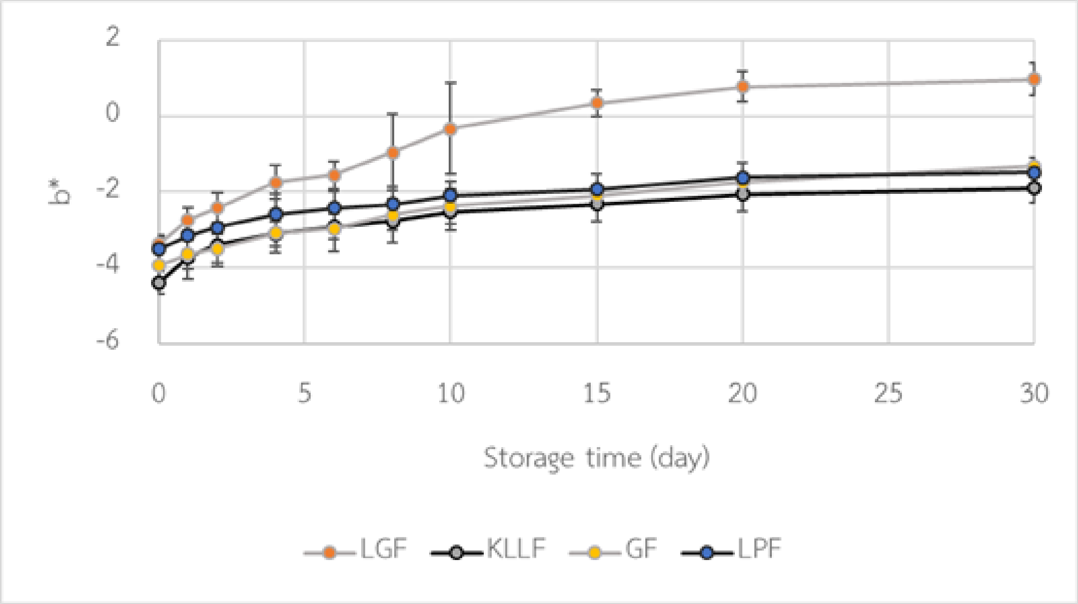

Figure 4.14 b* value of EO films

4.9.3 DPPH scavenging activity

The %relative inhibition of DPPH scavenging activity of EO films in the expose condition within 30 days were shown in Fig 4.14 Most EO films exhibited the steady decline of activity. The 20-day EO films in the expose condition possessed the same activity as the 180-day film storing in seal foil bags (data in section 4.8.2). This might be imply that some active components in EO were not degraded by oxygen and light.

4.9.4 Loading capacity percentage

The %LC of KLL film in expose condition within 30 days were shown in Fig 4.15 The results indicated that EO content in KLL films was gradually decreased and obviously exhausted in the 30th day. The 10-day KLL films in the expose condition possessed the same %LC as the 180-day film storing in seal foil bags (data in section 4.8.2).

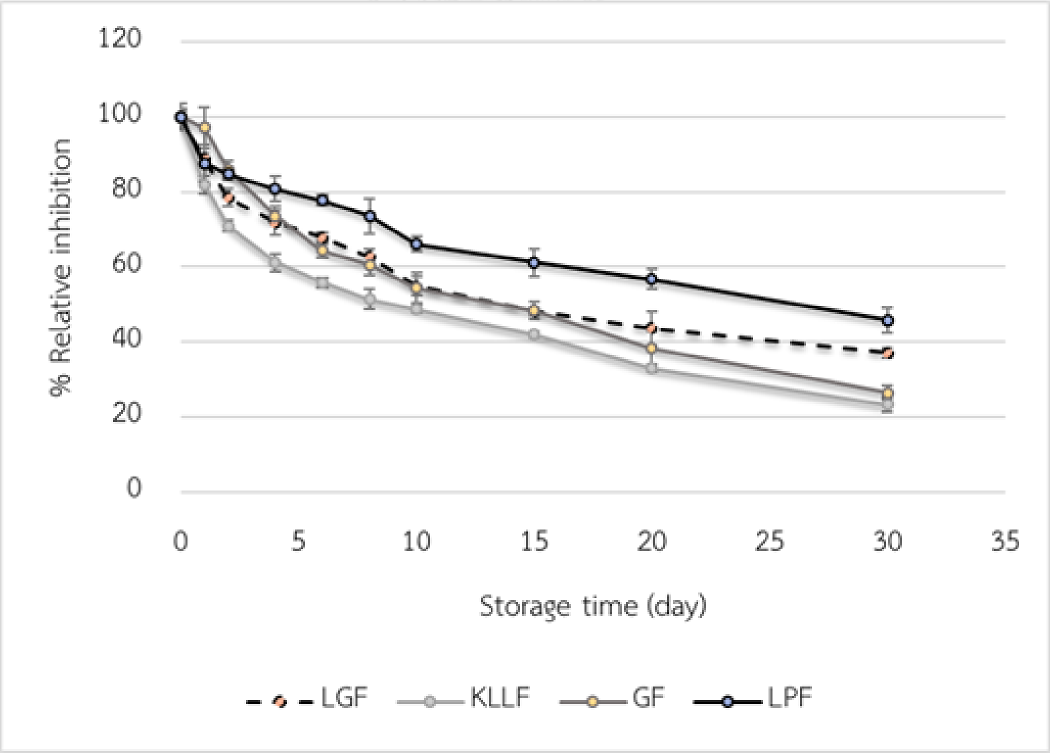

Figure 4.15 %Relative inhibition of EO films

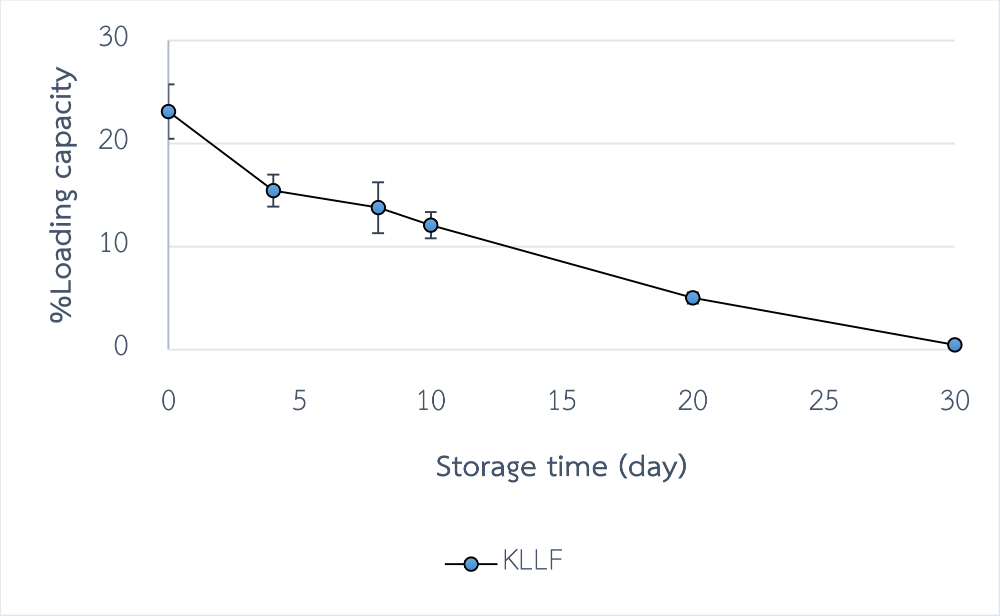

Figure 4.16 %LC of KLL film

CHAPTER V

CONCLUSION

In this research, EO films were successfully prepared by solvent casting method. Carboxymethyl cellulose and xanthan gum were film forming polymers incorporating with 4 kinds of the EOs which extracted from lemongrass, kaffir lime leaf, galangal and lime peel. The average thicknesses of the EO films were 0.32 mm. The SEM images showed the entrapment of the EO droplets in the network of polymers. The IR spectrum and TGA thermograms of the EO films clearly confirmed the presence of EO in the films. The %moisture content and %water solubility of the EO films were about 12-13% and 90-95%, respectively. The %LC and %EE were about 14-26%, 24-44%, respectively.

EO films were kept in vacuum and non-vacuum seal conditions at 40°C for 180 days and determined their physicochemical properties which were %moisture content, %water solubility, color, %LC , SEM, TGA and DPPH radical-scavenging activity. The %moisture content and %water solubility gradually decreased whereas turbidity and yellowish of films increased by a function of time. The amount of EO slowly decreased which were verified by %LC, SEM, TGA and DPPH radical-scavenging activity analysis. However, there were no significant difference between vacuum and non-vacuum EO films.

Moreover, we studied the properties of the EO films in an expose condition. The EO films were exposed to 70% RH in the close system at ambient temperature for 30 days. This system was simulated system of Thai humidity and study in close system was the easy way to control fluctuated factors. The results showed that the EO film properties were rapidly changed comparing to opaque seal condition. These results represented that oxygen and light significantly affected to most properties of EOs in film and both CMC and xanthan gum could not protect EOs from oxygen and light.

In conclusion, we are successful to fabricate a new flavor in the form of EO film. This film is prepared without any surfactants and plasticizers which is safe for consumers. Besides, the production of this film also require less energy consumption in comparison with spray drying technique. The study of shelf life stability can conclude that EO films should be avoid from oxygen and light to protect from degradation of EOs. Opaque condition can extend the properties of EO film about 20 times compare to in expose condition. Therefore, more research are needed in order to enhance stability of EOs by adding some additives or modifying process.

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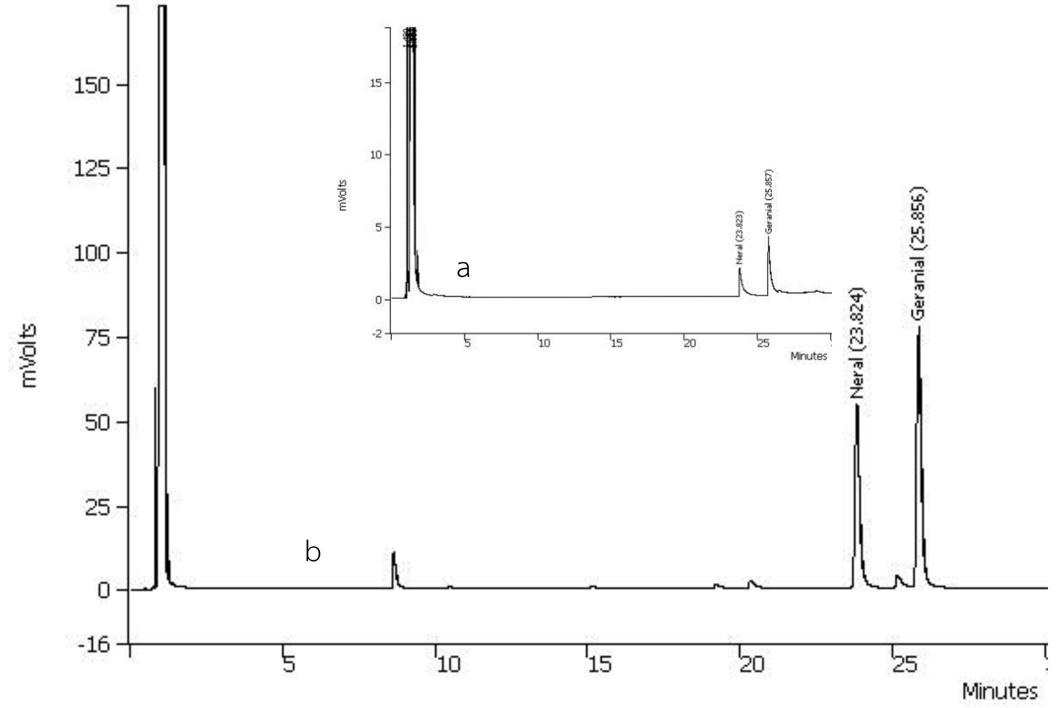
APPENDIX

Appendix A

GC

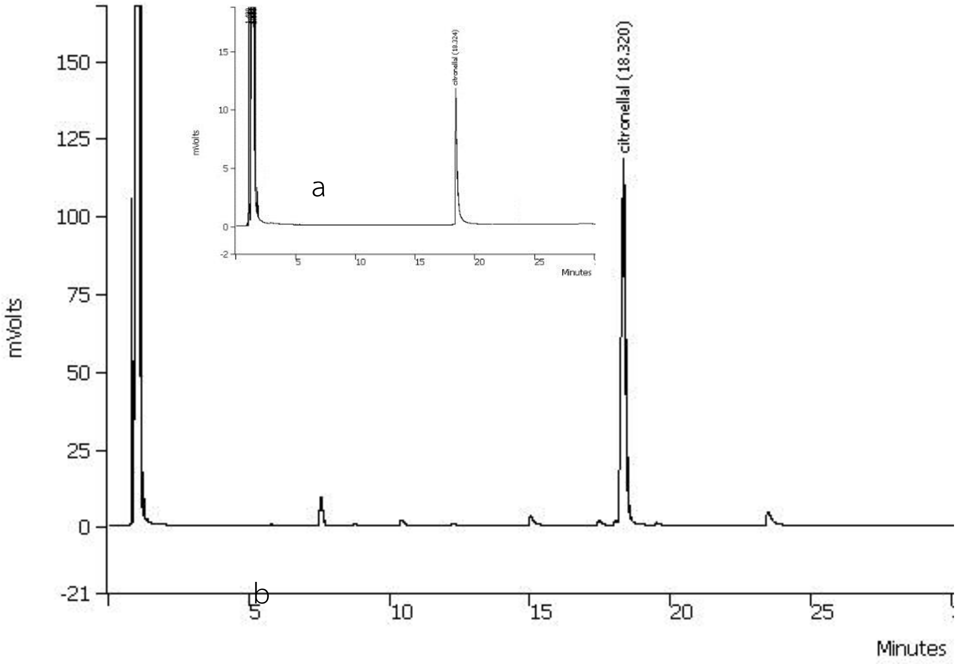
GC Chromatogram of EO films

0 day



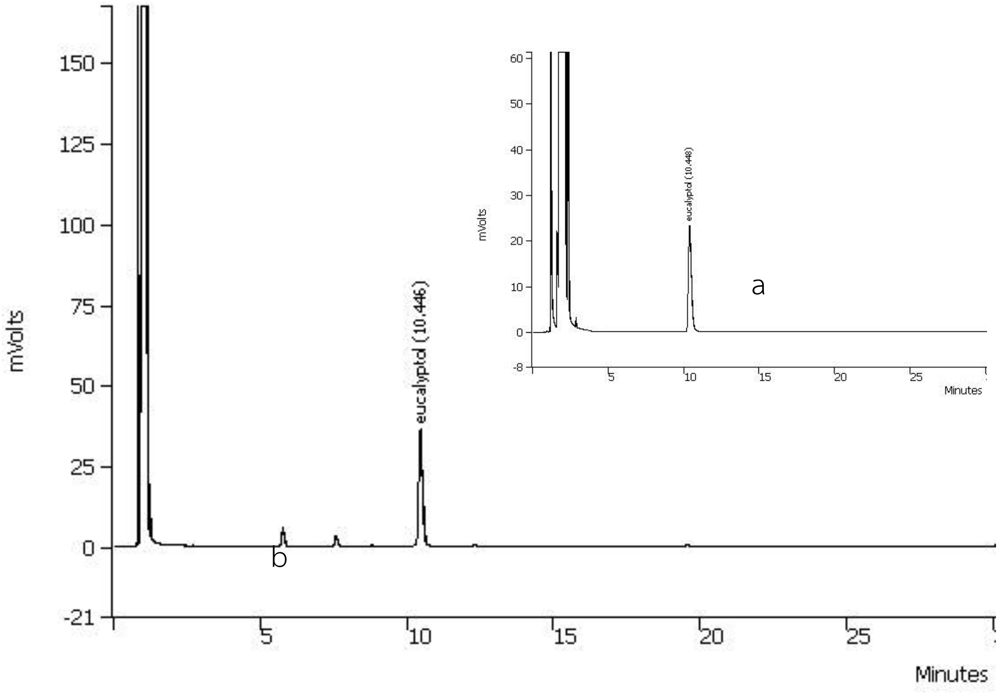
Peak No	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Rel Ret Time	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	Neral	93.0736	23.824	-0.107	0.00	623970	BV	9.9	C	0
2	Geranial	131.0563	25.856	-0.302	0.00	888589	VB	10.2	C	0
	Totals	**224.1299**		**-0.409**		**1512559**				

Figure A1 GC Chromatograms of a. geranial and b. LG film



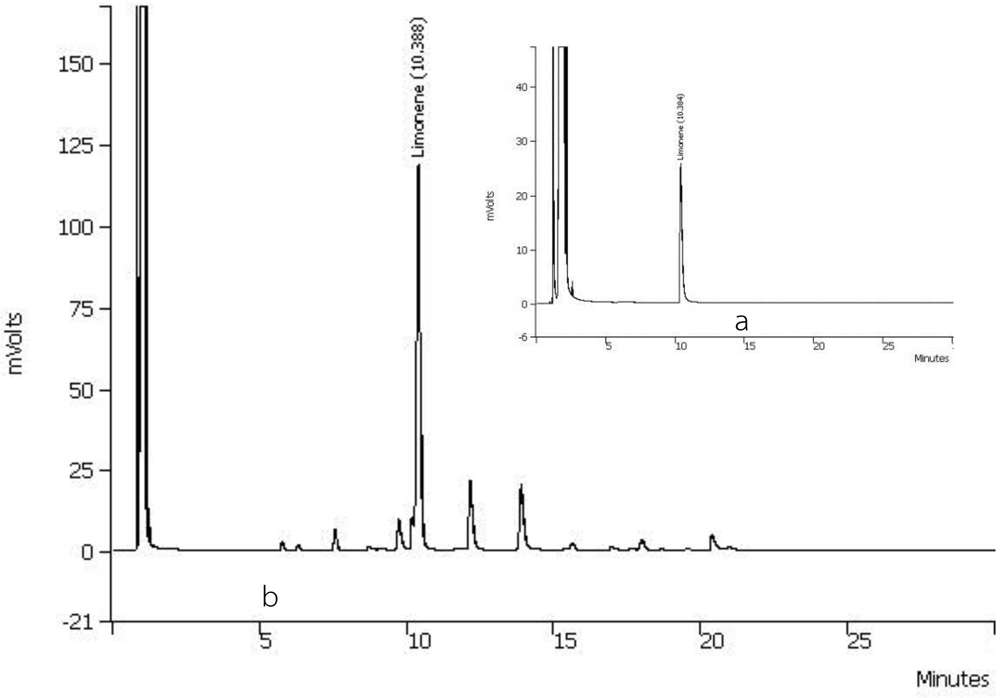
Peak No	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Rel Ret Time	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	citronellal	191.7721	18.320	0.008	0.00	1285649	VB	9.9	C	0
	Totals	**191.7721**		**0.008**		**1285649**				

Figure A2 GC Chromatograms of a. citronellal and b. KLL film



Peak No	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Rel Ret Time	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	citronellal	46.7243	24.029	-0.184	0.00	313242	VB	6.3	C	0
	Totals	**46.7243**		**-0.184**		**313242**				

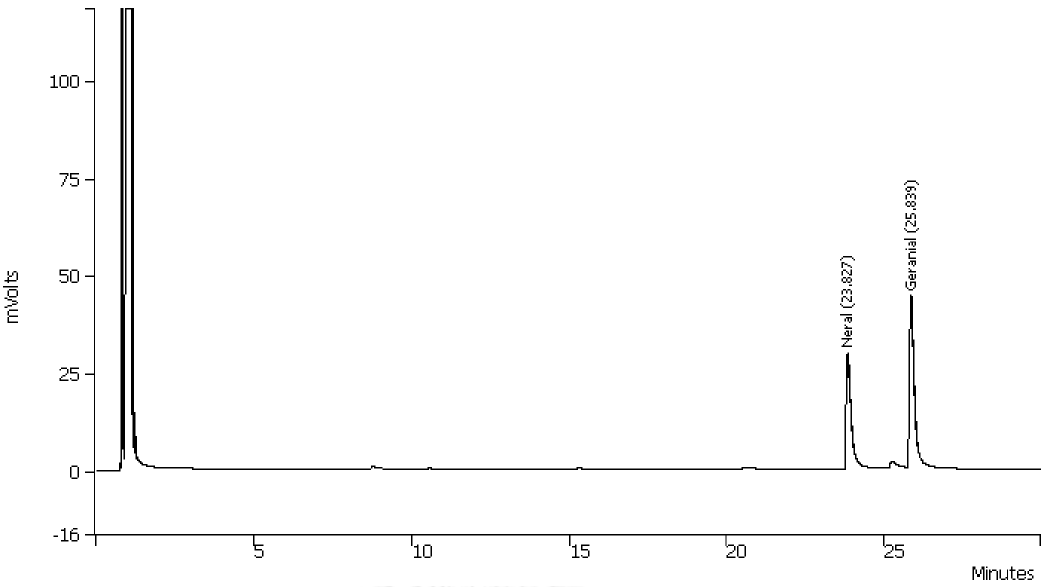
Figure A3 GC Chromatograms of a. eucalyptol and b. G film



Peak No	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Rel Ret Time	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	Limonene	152.5391	10.388	-0.064	0.00	1022629	VB	7.7	C	0
	Totals	**152.5391**		**-0.064**		**1022629**				

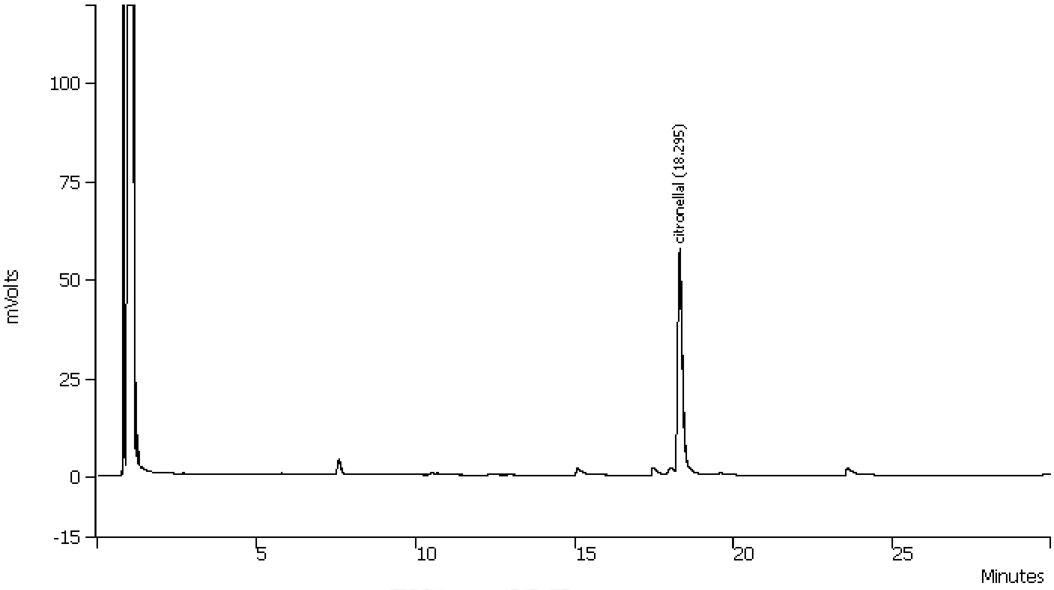
Figure A4 GC Chromatograms of a. limonene and b. LP film

180 day vacuum seal condition



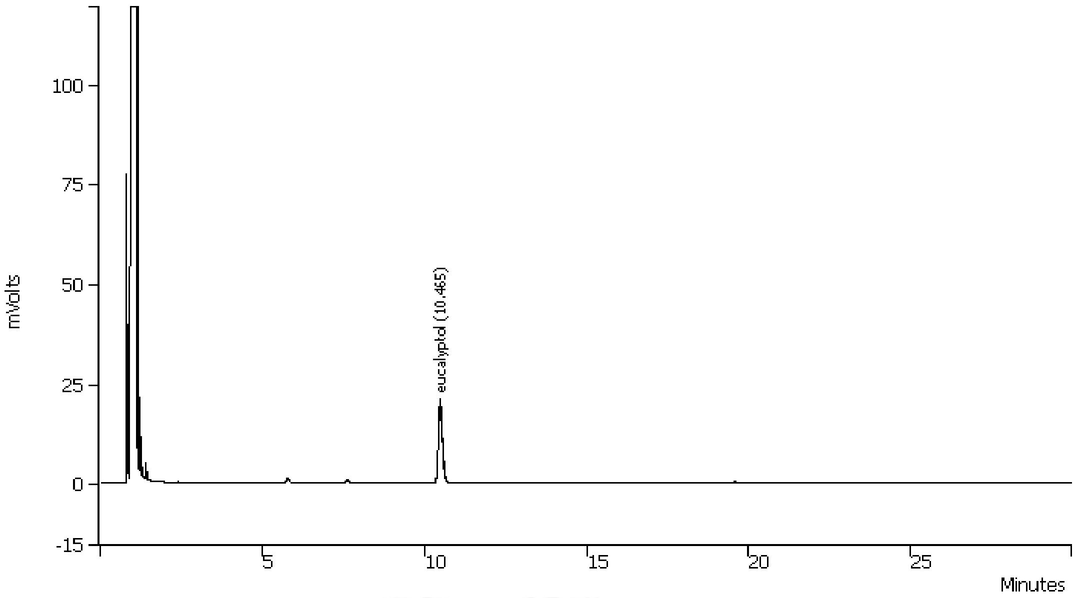
Peak No	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Rel Ret Time	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	Limonene	34.4111	10.385	-0.016	0.00	230694	VB	7.9		0
	Totals	**34.4111**		**-0.016**		**230694**				

Figure A5 GC Chromatograms of v-LG film



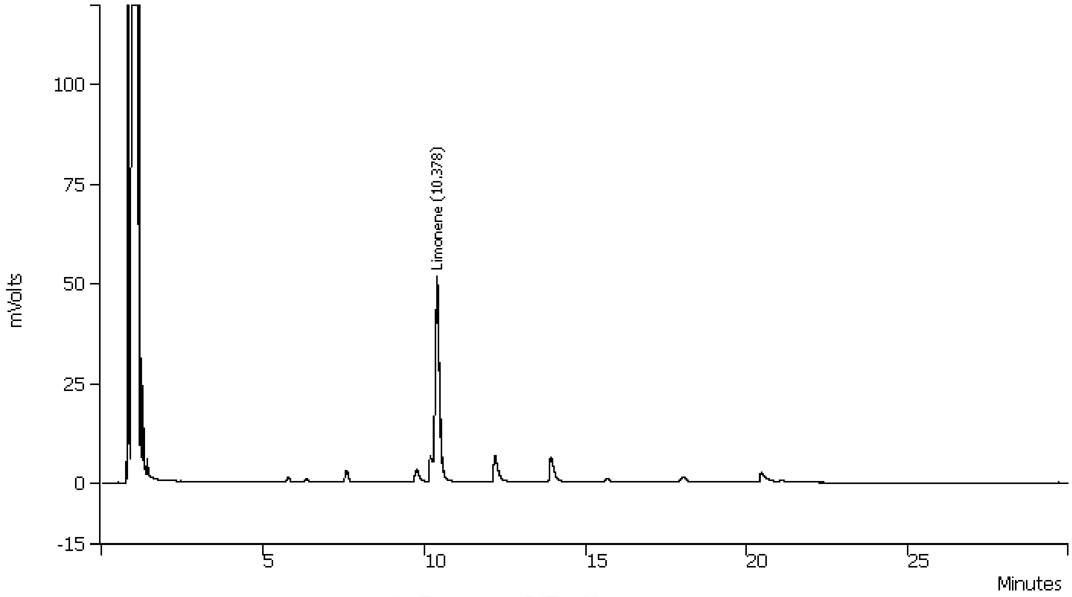
Peak No	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Rel Ret Time	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	citronellal	91.7271	18.295	-0.196	0.00	614943	VB	9.3	C	0
	Totals	**91.7271**		**-0.196**		**614943**				

Figure A6 GC Chromatograms of v-KLL film



Peak No	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Rel Ret Time	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	eucalyptol	27.0431	10.465	-0.242	0.00	181298	BB	8.0		0
	Totals	**27.0431**		**-0.242**		**181298**				

Figure A7 GC Chromatograms of v-G film

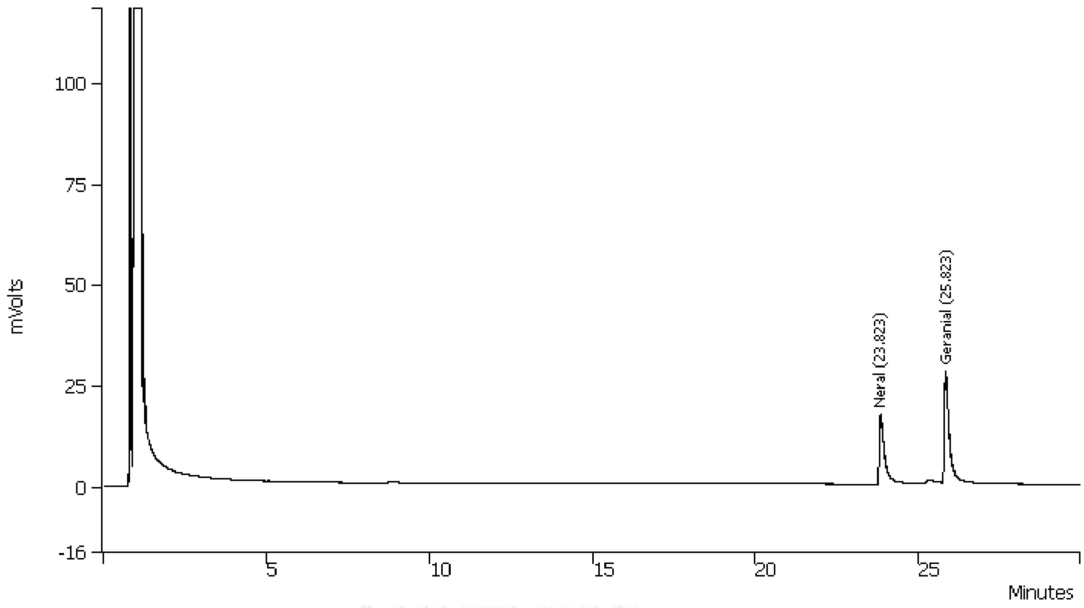


Peak No	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Rel Ret Time	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	Limonene	68.9392	10.378	-0.023	0.00	462172	VB	7.7	C	0
	Totals	**68.9392**		**-0.023**		**462172**				

Status Codes:
C - Result out of calibration range

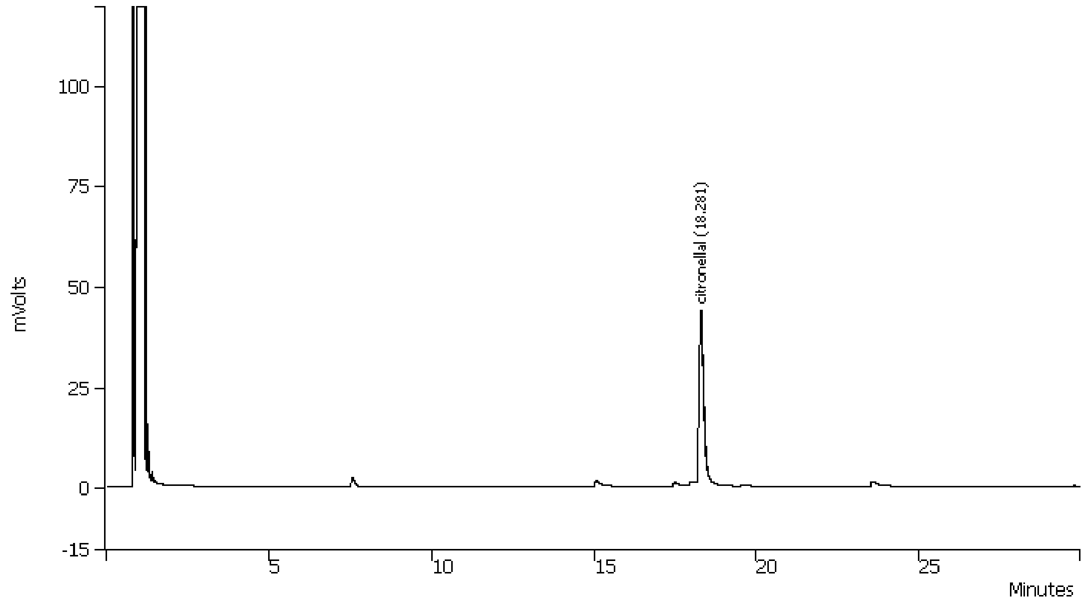
Figure A8 GC Chromatograms of v-LP film

180 day non vacuum seal condition



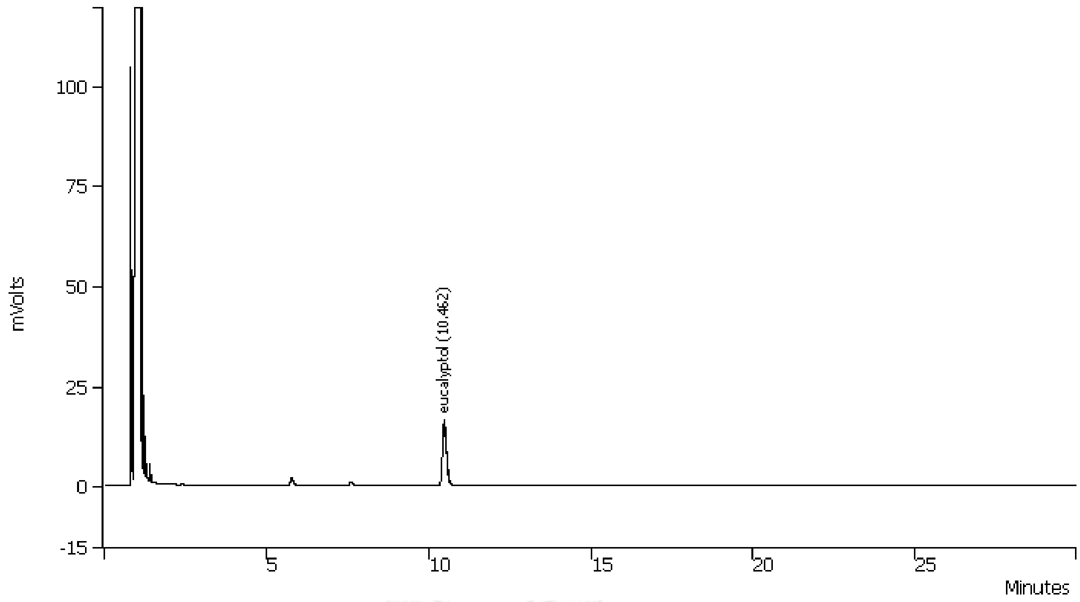
Peak No	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Rel Ret Time	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	Neral	32.1229	23.823	-0.177	0.00	215353	BV	9.2		0
2	Geranial	48.5971	25.823	-0.235	0.00	329498	VB	9.3	C	0
	Totals	**80.7200**		**-0.412**		**544851**				

Figure A9 GC Chromatograms of nv-LG film



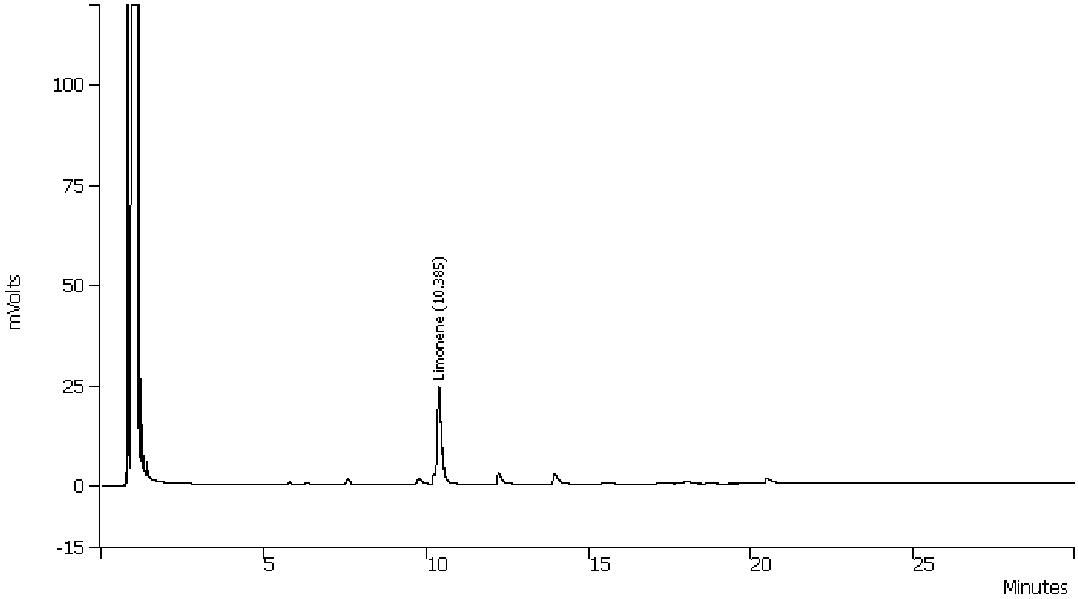
Peak No	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Rel Ret Time	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	citronellal	71.1285	18.281	-0.032	0.00	476849	VB	9.4	C	0
	Totals	**71.1285**		**-0.032**		**476849**				

Figure A10 GC Chromatograms of nv-KLL film



Peak No	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Rel Ret Time	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	eucalyptol	21.2848	10.462	-0.245	0.00	142694	BB	8.0		0
	Totals	**21.2848**		**-0.245**		**142694**				

Figure A11 GC Chromatograms of nv-G film



Peak No	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Rel Ret Time	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	Limonene	34.4111	10.385	-0.016	0.00	230694	VB	7.9		0
	Totals	**34.4111**		**-0.016**		**230694**				

Figure A12 GC Chromatograms of nv-LP film

Appendix B

Calibration curve of EO films

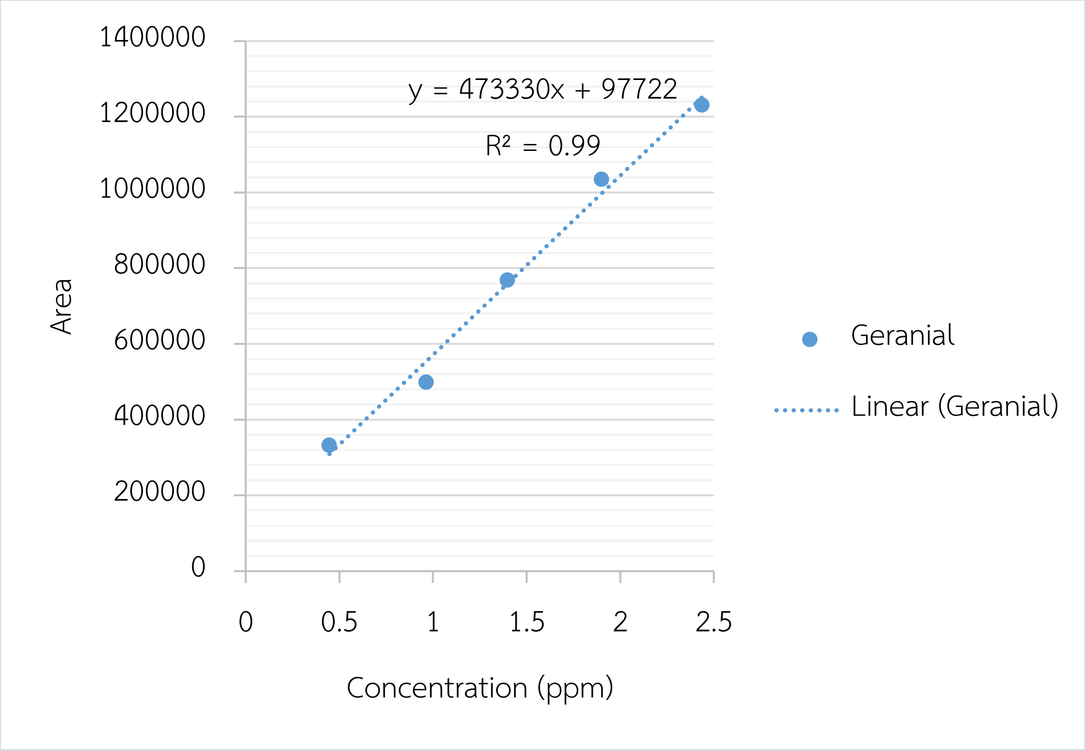


Figure B1 Calibration curve of LG film

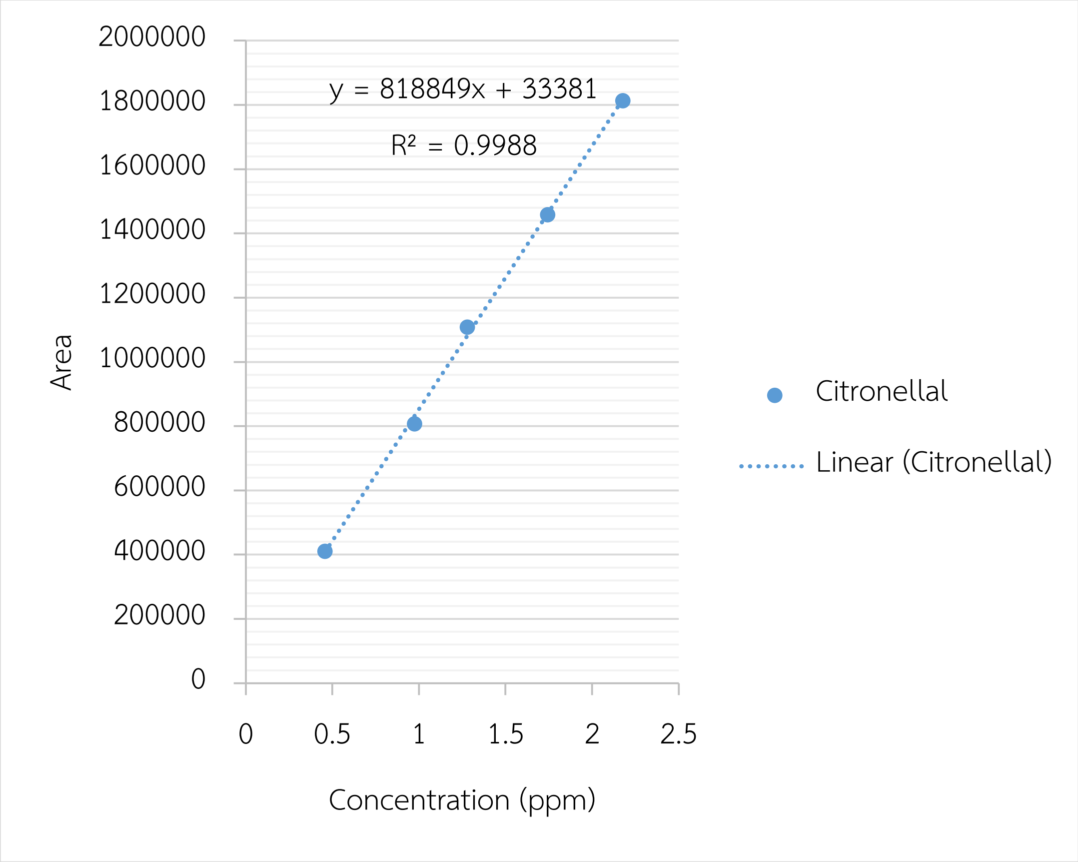


Figure B2 Calibration curve of KLL film

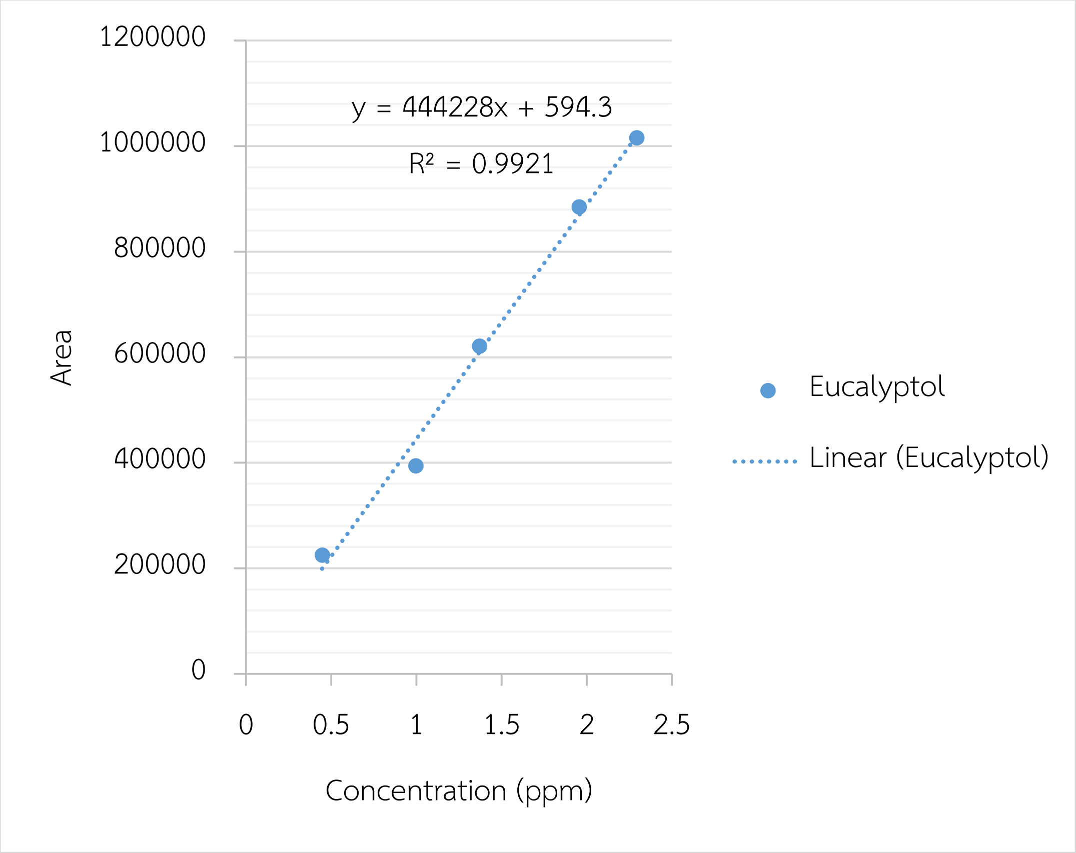


Figure B3 Calibration curve of G film

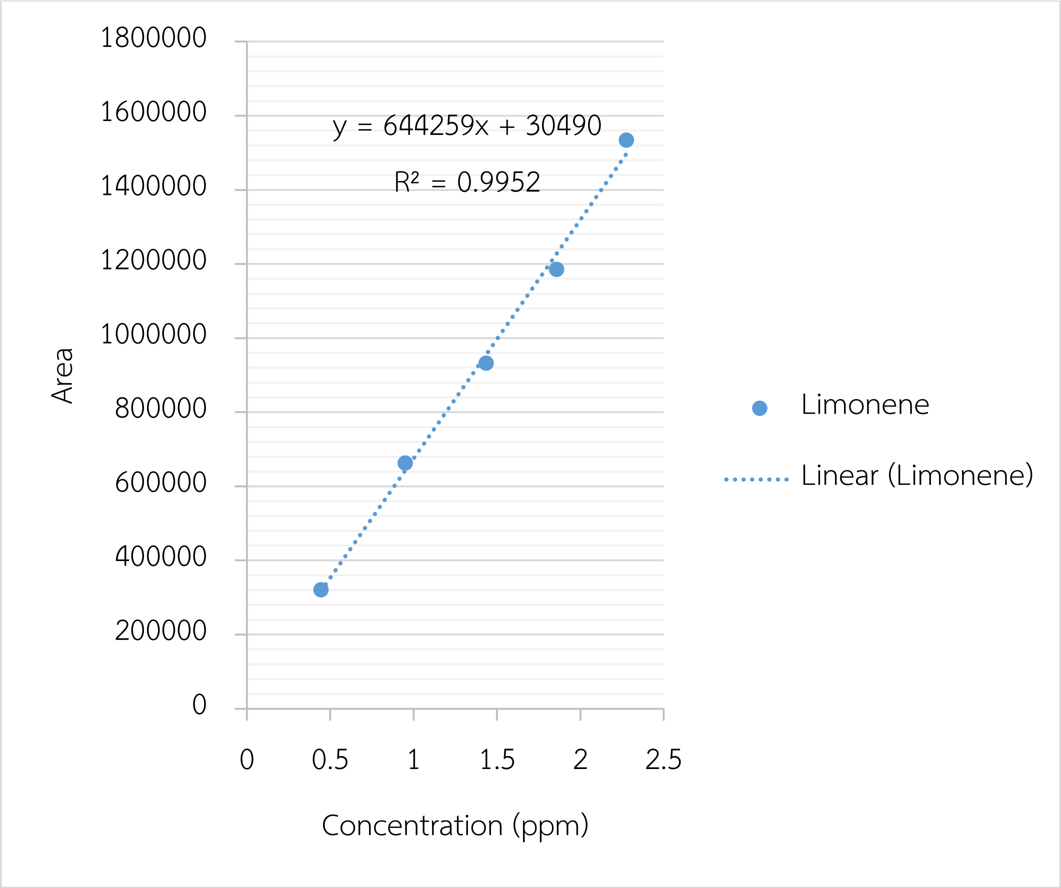


Figure B4 Calibration curve of LP film.

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