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USE OF GLUCOMANNAN FILMS FOR EUGENOL ENCAPSULATION

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ฟิล์มบริโภคได้ที่สามารถกักเก็บสารที่มีประโยชน์เพื่อปรับปรุงคุณสมบัติการใช้งานของฟิล์มบริโภคได้กำลังเป็นที่สนใจอย่างกว้างขวาง สารที่สามารถนำมากักเก็บในฟิล์มบริโภคได้ เช่น สารให้กลิ่นรส สารต้านออกซิเดชัน และ สารต้านจุลชีพ เป็นต้น เพื่อชะลอการเปลี่ยนแปลงจากผลกระทบของสภาวะแวดล้อมหรือควบคุมการปลดปล่อยของสารดังกล่าว วัตถุประสงค์ของงานวิจัยนี้คือศึกษาผลของการกักเก็บยูจีนอลต่อฟิล์มคอนยัคกลูโคแมนแนน เช่น โครงสร้าง สมบัติทางกล สมบัติทางกายภาพ การละลาย สมบัติทางความร้อน และปริมาณของยูจีนอลที่ถูกกักเก็บไว้ในฟิล์ม ฟิล์มที่ขึ้นรูปได้ถูกนำไปวัดความหนา ค่าการต้านแรงดึง (TS) ค่าการยืดตัว (EB) ค่ามอดูลัสของยัง (YM) ค่าการซึมผ่านของไอน้ำ (WVP) ความโปร่งแสง ความสามารถในการละลายน้ำ ความสามารถในการกักเก็บยูจีนอล และความสามารถในการปลดปล่อยยูจีนอลในน้ำ นอกจากนี้ยังศึกษาภาพตัดขวางของฟิล์มโดยกล้องจุลทรรศน์อิเล็กตรอนชนิดส่องกราด (SEM) อันตรกิริยาภายในฟิล์มด้วย Fourier transform infrared spectrometer (FT-IR) และสมบัติทางความร้อนโดย differential scanning calorimeter (DSC) และศึกษาผลของเวลาการเก็บรักษาต่อสมบัติของฟิล์มกลูโคแมนแนนที่กักเก็บยูจีนอลและปริมาณยูจีนอลที่ถูกกักเก็บไว้ ผลการศึกษาพบว่าความหนาของฟิล์มที่ขึ้นรูปได้อยู่ในช่วง 25.80 μm ถึง 51.60 μm ฟิล์มจะมีความแข็งแรงเพิ่มขึ้นเมื่อความเข้มข้นของคอนยัคกลูโคแมนแนนมากขึ้น แต่จะมีการยืดตัวและความยืดหยุ่นเพิ่มขึ้นตามความเข้มข้นของยูจีนอล สอดคล้องกับผลการทดลองที่ได้จาก SEM FT-IR และ DSC เมื่อใส่ยูจีนอลและเพิ่มความเข้มข้นของยูจีนอลในสารละลายฟิล์ม ฟิล์มที่ได้จะมีความเป็นเนื้อเดียวกันลดลง การรวมตัวของยูจีนอลและคอนยัคกลูโคแมนแนนนั้นไม่มีการเกิดพันธะใหม่ระหว่างสารทั้งสอง ไอน้ำสามารถเคลื่อนที่ผ่านฟิล์มได้มากขึ้น ความโปร่งแสงของฟิล์มจะลดลงเมื่อความเข้มข้นของยูจีนอลภายในฟิล์มสูงขึ้น เมื่อนำฟิล์มไปละลายน้ำพบว่าเมื่อความเข้มข้นของคอนยัคกลูโคแมนแนนเพิ่มขึ้นจะทำให้การละลายของฟิล์มลดลง ในขณะที่เมื่อความเข้มข้นของยูจีนอลเพิ่มขึ้นการละลายของฟิล์มจะเพิ่มขึ้น การวัดความสามารถในการกักเก็บยูจีนอลภายในฟิล์มคอนยัคกลูโคแมนแนนมีค่าเพิ่มขึ้นตามความเข้มข้นของยูจีนอลที่ใส่ลงไปในสารละลายฟิล์มก่อนการขึ้นรูป ยูจีนอลสามารถถูกปลดปล่อยออกจากฟิล์มคอนยัคกลูโคแมนแนนในน้ำได้ภายใน 20-30 นาที ฟิล์มที่มีความเข้มข้นเริ่มต้นของยูจีนอลอยู่มากกว่าสามารถปลดปล่อยยูจีนอลออกมาในน้ำได้มากกว่าฟิล์มที่มียูจีนอลเริ่มต้นอยู่น้อยกว่า จากการทดลองข้างต้นได้เลือกฟิล์มที่มีความสามารถในการกักเก็บสูงที่สุดคือ KGM:EU 0.75%:1.50% (w/w) เพื่อทดสอบการเปลี่ยนแปลงของฟิล์มที่กักเก็บยูจีนอลเมื่อเก็บรักษาฟิล์มโดยการตรวจสอบการเปลี่ยนแปลงของฟิล์มด้วยเทคนิค thermogravimetric analysis (TGA) ผลการตรวจสอบพบว่าเมื่อเก็บฟิล์มไว้เป็นเวลา 60 วัน รูปแบบการเปลี่ยนแปลงของน้ำหนัก (derivative thermogram, DTG) ในช่วงอุณหภูมิที่ทำการทดลองใกล้เคียงกัน แต่สีของฟิล์มมีการเปลี่ยนแปลง และเมื่อทดสอบความสามารถในการกักเก็บยูจีนอลในฟิล์มเมื่อเก็บฟิล์มไว้เป็นเวลา 8 เดือน พบว่าปริมาณยูจีนอลลดลง 36.90% ของปริมาณยูจีนอลที่สามารถกักเก็บได้เริ่มต้น โดยสรุปฟิล์มคอนยัคกลูโคแมนแนนจึงสามารถกักเก็บยูจีนอล เพื่อลดการสูญเสียของยูจีนอลสู่สิ่งแวดล้อมและฟิล์มที่ได้มีความแข็งแรง สะดวกต่อการนำไปใช้งานเป็นบรรจุภัณฑ์

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5471927223 : MAJOR FOOD TECHNOLOGY
KEYWORDS: EDIBLE FILM / KONJAC GLUCOMANNAN / EUGENOL / ENCAPSULATION

JARUPAN VATEE: USE OF GLUCOMANNAN FILMS FOR EUGENOL ENCAPSULATION. ADVISOR: ASST. PROF. ROMANEE SANGUANDEEKUL, Ph.D., CO-ADVISOR: ASSOC. PROF. UBONRAT SIRIPATRAWAN, Ph.D., 125 pp.

Edible film encapsulated with active compound to improve its functional properties is gaining more attention. The active compounds which are usually encapsulated in edible film are flavors, odors, antioxidant agents, and antimicrobial agents. The purpose of encapsulation is to control the releasing rate and to protect active compounds from the environmental effect. The aim of this study was to analyze the effect of EU incorporation on the structural of KGM film, mechanical, physical, and thermal properties. In addition, the amount of EU remained in the film (encapsulation efficiency, EE) was also studied. The film properties including thickness, tensile strength (TS), elongation at break (EB), Young's modulus (YM), water vapor permeability (WVP), transparency value, solubility, encapsulation efficiency (EE) of EU, and EU releasing in water were determined. Moreover, cross-section of film was analyzed by scanning electron microscope (SEM). Structural interaction and thermal property of film were studied by Fourier transform infrared spectrometer (FT-IR) and differential scanning calorimeter (DSC), respectively. The changing of thermal stability of the films and EE of EU during storage for 8 months was monitored and reported as thermogravimetric analysis (TGA) thermograph and EE. The results showed that the thickness of film was in a range of 25.80 μm to 51.60 μm. The resistance to break of film increased with increasing KGM, while adding and increasing concentration of EU made the film more stretchable and flexible. These results agree with SEM photographs, FT-IR spectra, and DSC analysis. The results from FT-IR and DSC analysis showed that there was no interaction between KGM and EU. The heterogeneity of the film had the effect to increase WVP and decrease transparent. The solubility of film increased with EU concentration due to the heterogeneous structure but decreased with increasing KGM content. EE of EU encapsulated in KGM film was determined. Amount of EU which could be entrapped in the film depend on the initial EU concentration added in film forming solution. The releasing of EU from film in water reached the equilibrium in 20 - 30 minutes. The amount of EU released depended on the initial EU concentration added. The 0.75% KGM film encapsulated with 1.50% EU was selected to determine the changing of thermal stability of the film and amount of remain EU during storage for 8 months. The pattern of derivative thermogram (DTG) of the films during storage for 60 days were similar but the color of the films changed. In addition, EE (%) of the film decreased 36.90% of initial EE (%) when storage for 8 months. The result showed that KGM could be used for encapsulating EU and preventing the loss of EU to the environment. Moreover, KGM/EU films had high mechanical strength, convenience for use as packaging.

Department:	Food Technology	Student's Signature	.....
Field of Study:	Food Technology	Advisor's Signature	.....
Academic Year:	2013	Co-Advisor's Signature	.....

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NOMENCLATURE

ΔE^*	=	Total different in color
ΔH_d	=	Degradation enthalpy
ΔH_m	=	Melting enthalpy
ΔP	=	Vapor pressure differential across the film
A	=	Cross section area of film
a^*	=	Redness
a^*_0	=	Redness of white plate
A_{283}	=	Absorbance at 283 nm
ANOVA	=	Analysis of variance
b^*	=	Yellowness
b^*_0	=	Yellowness of white plate
CMC	=	Carboxymethyl cellulose
DSC	=	Differential scanning calorimetry
DTG	=	Derivative thermogram
EB	=	Elongation at break
EE	=	Encapsulation efficiency
EU	=	Eugenol
F	=	Force at maximum load
FCC	=	Food Chemical Codex
FT-IR	=	Fourier transform infrared spectroscopy
GRAS	=	Generally recognized as safe
HPMC	=	Hydroxymethylcellulose
KGM	=	Konjac glucomannan
KGM/EU	=	KGM encapsulated with EU
L	=	Length of the film at breaking point
L^*	=	Lightness

L^*_0	=	Lightness of white plate
L_0	=	Initial grip length
PO	=	Palm olein
SEM	=	Scanning electron microscope
t	=	Time
T_{600}	=	Transmittance measuring at 600 nm
T_d	=	Degradation temperature
T_g	=	Glass transition temperature
TG	=	Thermogram
TGA	=	Thermogravimetric analysis
T_m	=	Melting temperature
TS	=	Tensile strength
w	=	The gain weight
W_f	=	Final weight of the non-soluble KGM film sample after drying at 105°C for 24 hours
W_i	=	Initial weight of KGM film sample
WSC	=	Water sorptive capacity
WVP	=	Water vapor permeability
WVTR	=	Water vapor transmission rate
x	=	Thickness of film
YM	=	Young's modulus

CHAPTER I

INTRODUCTION

The applications of edible film have received much attention due to its biodegradable and edible. Edible film can be value-added when using as an active packaging to maintain product quality and safety, and to prolong shelf life of product by adding active compounds such as food additives, antimicrobial agents, or antioxidant agents. Moreover, edible film can be used to carry an active compound, such as flavoring agents, through encapsulation technique to protect active compound within polymer material. The materials, which are used to make edible film, are eco-friendly material from natural polymer, for instance proteins, polysaccharides and lipids. Proteins and polysaccharides, due to their high polarity, have good mechanical properties and are good barrier for gas and aroma. On the other hand, lipid molecule, having non-polar property, is good moisture barrier.

Aroma compounds are easy to evaporate because of high saturated vapor pressure. Encapsulation of aroma compounds in polymer matrix can prolong its shelf life. Thus, the edible film can be used to encapsulate the aroma compound and the film is reference as active packaging.

In this study, konjac glucomannan (KGM) films were used to encapsulate eugenol (EU). EU was selected as a model of active compound which was used as core material because this compound was widely distributed in many plant extracts such as cinnamon, basil, and nutmeg. It could be used as food flavoring agent, and as an additive in fragrances, cosmetics, and tobacco products (Zhang et al., 2000). Therefore, KGM was chosen as film matrix to encapsulate EU in order to prevent EU from evaporation, due to its good gas permeation barrier and good film forming property (Chen et al., 2008). The aim of this study was to analyze the effect of EU incorporation on the structural of KGM film, mechanical, physical, and thermal properties. In addition, the amount of EU remained in the film (encapsulation efficiency, EE) was also studied.

CHAPTER II

LITERATURE REVIEW

2.1 Edible film

Edible films or coatings are thin sheet materials that are edible and biodegradable. Investigation of an edible film as a packaging to wrap or coat food products are received much attention due to the concerning of environmental impact. The materials that are generally used to form edible film are natural materials such as proteins, carbohydrates, and lipids. They are used for food protection and to prolong shelf life of food products (Guilbert and Gontard, 1995). These materials can be used to prevent and control mass transfer (Karbowiak et al., 2007) such as control moisture transfer, limit gas transportation, retard oil and fat migration. They also prevent solute or flavor absorption. In addition, they can carry food additives such as antimicrobial agents or antioxidants and improve structural integrity of foods (Ozdemir and Floros, 2008).

Edible films can be used as active packaging by adding active compounds such as flavorings, colorings, sweeteners, antioxidants or antimicrobial agents. The addition of different active compounds depend on the final application of the edible film.

2.1.1 Classification of edible films and coatings

2.1.1.1 Polysaccharide films

There are many research that making film from polysaccharide polymer such as film from konjac glucomannan (KGM), alginate, pectin, carrageenan, starch hydrolysate, cellulose and cellulose derivatives (Cutter, 2006). Polysaccharids which

are normally used for casting film are long chain polymer and capable to be dissolved and dispersed in water (Rattanapitigorn, 1998). Polysaccharide films are brittle and compact. In spite of good mechanical property of polysaccharide edible films, they have low moisture barrier property because hydrophilic nature of polymers. Thus, they are not suitable for use as a moisture control packaging (Kester and Fennema, 1986).

2.1.1.2 Protein films

Proteins that are normally used for making an edible film are collagen, gelatin, egg white protein, casein, whey protein, wheat protein and soy protein. The mechanical and water barrier properties of protein film are almost similar to polysaccharide film but protein film have good gas barrier properties at low humidity (Rattanapitigorn, 1998; Cutter, 2006).

2.1.1.3 Lipid films

Lipid films are normally used as coating such as coating fruits. They are good moisture and oxygen barrier (Chantrai, 2000; Rattananin, 2011). They protect the agricultural product during the transportation, preventing browning reaction and the loss of moisture from products. Lipids that are normally used for coating are paraffin, beeswax, surfactant, fatty alcohol, glycerol monopalmitate, and glycerol monostearate (Kester and Fennema, 1986).

2.1.1.4 Composite films

Composite films are the film that composed of two or more polymer materials. Composite film can be emulsion film or bilayer film (McHugh, 1996). They are designed to improve some properties of single film. Many research have been done on composite film such as sodium caseinate mixed with acetylated monoglyceride and alginate (Wong et al., 1992a), sodium caseinate blended with

stearic acid (Avena-Bustillos et al., 1993), and whey protein isolated combind with coconut oil (Stuchell and Krochta, 1995).

Polysaccharide and protein films contained hydrogen bond. This bonding type facilitates the migration of water molecule through the films and oxygen molecules can pass through the film easily at high relative humidity too (Lehduwi, 2007). However, lipid films are not polymer structure but it is an arrangement of lipid crystal. Compact structure lipids have good vapor barrier property (Kester and Fennema, 1986). Furthermore, a hydrophobicity of lipid results in good gas barrier property of lipid film (Rattanapitigorn, 1998).

Both polysaccharide and protein films are brittle because of high strength of hydrogen bonding in film structure. Therefore, the plasticizers are usually added to the films to improve these properties (Brody and Marsh, 1999). Adding plasticizer decreases the strength of the linkage between polymer chain and increases the movement of polymer chain (Guilbert, 1986). In addition, plasticizer decreases glass transition temperature (T_g) of films and improves elasticity and strength of film (Brody and Marsh, 1999). They also enhance the migration of water through the film because of the disturbance in the matrix. Materials that are normally used as plasticizer are sucrose, glycerol, sorbitol, propylene glycol, polyethylene glycol, fatty acid, monoglycerol and water. Moreover, antioxidants, antimicrobial agents, food additives, odor, and colors can also be added to edible film (Brody and Marsh, 1999).

2.1.2 Production of edible films

The edible films can be produced by 3 procedures (Brody and Marsh, 1999).

2.1.2.1 Solvent casting

The film forming solution is poured or pressed on smooth surface and the solvent is evaporated. Moreover, there are many shapes of film that can be casted by solvent casting method, such as capsule or sheet, depend on a casting mold. The

samples of edible films using solvent casting method are methyl cellulose film, hydroxypropylmethylcellulose (HPMC) film, and gelatin capsule (Brody and Marsh, 1999; Lehduwi, 2007).

2.1.2.2 Molten casting

Molten casting is casting method which the film material is melted before casting or coating. Then, they are cooled for setting as sheet or coating at the surface. This method is usually used to produce lipid film. They can be casted into single layer film or multilayer film (composite film) on polysaccharide or protein films. Furthermore, coating lipid on polysaccharide or protein film can improve the moisture barrier property of those films.

2.1.2.3 Extrusion

The film forming solution is extruded through extruder. This casting method is used for making collagen film that used to wrap the sausages.

2.1.3 Properties of edible films

2.1.3.1 Thickness

Thickness is the distance between surfaces of two side of film. It is reported in micrometers or millimeters unit. This value is used to calculate tensile strength (TS), water vapor transmission rate (WVTR) and oxygen transmission rate of the film (Guilbert, 1986).

2.1.3.2 Mechanical properties

To measure the mechanical properties of film, the film is pulled at both ends of film by instrument until the film rupture. TS shows the strength of film as maximum force before the film was ruptured. This value depends on the strength of

film polymer and bonding of polymer in film matrix. Adding plasticizer disturbs the structure of film by decreasing the intermolecular force then decreases TS of the film (Guilbert, 1986).

Elongation at break (EB) is the extend distance of film when pulling the film by testing instrument, compares with the initial length of the film. High elongation shows high elasticity of film. Similar to TS, adding plasticizer disturbs the structure of film and decreases intermolecular force of polymer. It increases the movement of film polymer structure. So, the film with plasticizer has percent of elongation higher than film without plasticizer.

2.1.3.3 Water vapor transmission rate (WVTR)

WVTR displays the amount of water that pass through the film from one side to another at specified condition and expressed in g /m^2 day. It can be converted to water vapor permeability (WVP) by divided by thickness of the film (Guilbert, 1986).

2.1.3.4 Gas transmission rate

Gas transmission rate is the amount of gas that can be adsorbed from one side to another. The unit of gas transmission rate is cm^3/m^2·day·atm. Furthermore, gas transmission value can be expressed to gas permeability value by divided by thickness of film (Guilbert, 1986).

2.1.3.5 Solubility

The requirement for solubility of film depends on the application of film. The film should have high solubility property for using as ingredient in packaging that able to dissolve in water. On the other hand, the film should have low solubility for using as water barrier packaging (Lehduwi, 2007).

2.2 Konjac glucomannan (KGM)

Konjac or *Amorphousphallus konjac* is a member of the family of Araceae. KGM is high molecular weight polymer (molecular weight is in the range of 10^5 – 10^6 dalton) that generally found in the konjac tuber (Li and Xie, 2003). Konjac is abundantly found in tropical region of Asia, Africa and northeast of Australia to subtropical region in middle of China, Korea and Japan. Konjac is also known as elephant yam, elephant foot, elephant bread, sweet yam, and telinga potato.

Tuber and leaves of konjac composed of many compounds such as calcium oxalate, mucilage, starch, alkaloid and coniine (Kritsanapan, 1998), but the main compound that abundantly found in konjac tuber is KGM. KGM is polysaccharide that is the polymer of D-mannose and D-glucose at the ratio of 3:2 with β-1,4 glycosidic linkage (Figure 2.1) (Tye, 1991) . Polymer chain has a 5% – 10% acetyl group substituted (Takigami, 2000). There are random branching, with an approximate degree of branching of 8%, at the C-3 position of the D-mannoses and D-glucose.

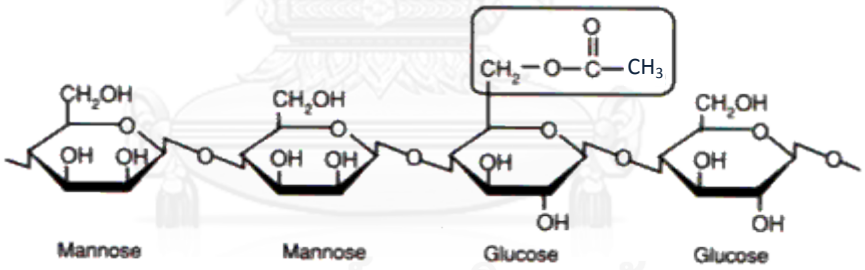


Figure 2.1 KGM structure

Source: modified from Tye (1991)

Kato and Matsuda (1970) studied the structure of konjac flour by digesting with sulfuric acid and cellulase. They disclosed that glucomannan have 2 types of repeating unit as shown in Figure 2.2.

Glucomannan A: -G-G-M-M-M-M-G-M-
Glucomannan B: -G-G-M-G-M-M-M-M-
when G is D-glucose
M is D-mannose
(-) is β-1,4 glycosidic linkage

Figure 2.2 Repeating unit of KGM

Source: Kato and Matsuda (1970)

2.2.1 Extraction of KGM

Konjac tuber is washed, sliced, dried and ground to konjac flour. The flour contains insoluble starch, cellulose, proteins and lipids. KGM can be extracted from konjac flour by washing with ethanol to remove the impurities which entrap with konjac particle. Other extraction techniques which can be applied are enzyme treatment, dialysis, washing with alcohol or a combination. Content of KGM in raw tuber varies in the range of 8 – 10g/100g raw tuber. After refined, 100 g of konjac flour contain 50 – 70 g of KGM and after purification, KGM content is more than 90 g/100 g KGM flour (Ohashi et al., 2000; Takigami, 2000; Fang and Wu, 2004; Tatirat and Charoenrein, 2011).

2.2.2 Properties of KGM

KGM is soluble fiber and has high water absorbency. The polymer structure has functional groups that can interact with water molecule. KGM solution is highly viscous and has pseudoplastic property (Nishinari et al., 1987). The viscosity of 1%(w/w), KGM solution is about 30,000 cps (Takigami, 2000). KGM is listed in the Food Chemical Codex (FCC) and is generally recognized as safe (GRAS) for use as a gelling, stabilizing, thickening, texturizing and film-forming agent (Cheng et al., 2002).

Gelation of KGM can be formed in two processes (Rattananin, 2011; Jimenez-Colmenero et al., 2013). Adding alkaline solution (pH 9 – 10) can be used for forming KGM gel (Cheng et al., 2002; Cheng et al., 2008; Jimenez-Colmenero et al., 2013).

Alkaline solutions remove acetyl group in KGM polymer, at C-3, which blocks gel formation of KGM (Nishinari et al., 1987). The alkaline solutions that always used are calcium hydroxide and potassium hydroxide. Moreover, adding hydrocolloid, such as xanthan gum or carrageenan (Tye, 1991), into KGM solution helps gel formation of KGM (Li et al., 2006; Chen et al., 2008; Jia et al., 2009). This type of gelation is related to the interaction of macromolecules with other molecules through van der waals interaction or dipole-dipole interaction (Whistler and Daneil, 1990). KGM and hydrocolloid, which assemble together more than 1 position, produce 3-D structure gel. The strength of gel depends on bond type and bond length.

KGM film can be prepared by dehydrating water from KGM solution. The KGM film is tough, flexible, stable in acid and base solution and cold or hot water even boiling for many hours. Adding plasticizer, such as glycerine, to KGM film forming solution, decreases film strength but increases film flexibility. The addition of other material might affects permeability of KGM film. The polarity of materials added with KGM increase water permeability of the film. Conversely, hydrophobic substances such as corn oil decrease amount of water that pass through the film, while hydrophilic compounds support the absorption of water molecule (Tye, 1991).

2.2.3 KGM film

KGM is normally used as a material to form the edible film, due to its good film forming ability and biodegradability. There are a few research studied properties of pure KGM film. It was normally blended with others materials to improve poor properties, such as mechanical, barrier, or thermal properties to appropriate potential applications. This section will reveal an improvement of pure KGM films to modified or blended KGM films and their properties.

The properties of pure KGM films were studied by Lai et al. (2006). Different amounts of glycerol and sorbitol were added, water sorption capacity (WSC), differential scanning colorimetry (DSC), and mechanical properties were observed. The incorporation of higher polyol concentration reduced WSC at a_w in the range of 0 to 0.60 but a_w more than 0.6 resulted in the increasing of WSC. In case of low a_w (less than 0.6) the polyol competed with the water active site of KGM (-OH group) and

hence reduced the water sorption of KGM film at the surface, whilst higher a_w enhanced hydrogen bonding between plasticizer and water molecule. Gontard et al. (1993) found that the higher moisture content reduced the interaction between plasticizer and polymer, enhanced free volume in film matrix and allowed water to penetrate through the film. The plasticizer type also affected WSC. Sorbitol exhibited lower to WSC more than glycerol. It was due to hygroscopic nature and lower molecular weight of glycerol affected to higher water absorption of water molecule at KGM film surface. DSC was used to study thermal properties of the film in this study. Pure KGM film exhibited onset temperature, peak temperature, and enthalpy at 93.2°C, 128.1°C, and 184.2 Jg^{-1}, respectively. At low moisture content, the increase of plasticizer concentration resulted in lower of melting enthalpy. On the other hand, at higher moisture content the increase of plasticizer revealed higher melting enthalpy of the film. The TS of plasticized film was in a range of 3.5 MPa to 5.5 MPa. Type of plasiticizer (glycerol and sorbitol) and a_w affected mechanical properties of the film. Glycerol had a greater plasticizing effect than sorbitol because of the smaller molecular size. Therefore, there was higher number of glycerol molecules in the film matrix when comparing with sorbitol at the same concentration. It promoted the water molecule to pass through the film. Thus, sorbitol plasticized film was stronger than glycerol plasticized film.

The film formed by using KGM had good mechanical properties and KGM is also used to enhance the properties of other polymer. Blending KGM formed strong interaction bond (hydrogen bond) with others materials. In case of incorporation with polysaccharide, such as carboxymethylcellulose (CMC), cassava starch, pea starch, or chitosan, KGM formed hydrogen bond and disturbed crystallinity in the film matrix. The occurrence of new interaction in the matrix could be observed by FT-IR. KGM film showed characteristic absorbance band of mannose at 807 – 810 cm^{-1} and 880 – 892 cm^{-1-}. The stretching vibration peak of C-H of methyl was at 2921 – 2925 cm^{-1} and 2894 – 2898 cm^{-1} and the peak of carbonyl were at 1727 – 1736 cm^{-1}, 990 cm^{-1}, and 924 cm^{-1}. At wavenumber of 3291 cm^{-1} and 1641 cm^{-1} showed the absorption peak of inter- and intra-molecular hydrogen bonding of hydroxyl groups of KGM, respectively (Xiao et al., 2001; Li et al., 2006; Chen et al., 2008). After blended with others materials, FT-IR spectrum of good compatible polymers was different from those pure polymers (Li et al., 2006). The peak of blended films with higher KGM content indicated broaden and shifted to higher wavenumber at absorption area which presented hydrogen bonding. Moreover, the peak at 1727 cm^{-1} (aceto group)

disappeared. It indicated the forming of hydrogen bond between acetyl group of KGM to other material (Xiao et al., 2001; Li et al., 2006; Chen et al., 2008). The disturbance of KGM molecule in the film matrix could be confirmed by thermal analysis. Melting temperature (T_m) and melting enthalpy (ΔH_m) decreased because the suppression of KGM to the retrogradation of starch and formed strong hydrogen bond with starch molecules (Chen et al., 2008; Nair et al., 2011). In case of blending with gelatin, DSC thermogram showed higher exothermic peak and enthalpy, while TGA showed higher onset temperature. These indicated the enhancement of thermal stability of two (or more) different materials. The production of strong interaction affected the mechanical properties, water barrier properties, and others properties of the films. TS and EM were increased with higher KGM added, related to stronger hydrogen interaction. At the same time, KGM had high water sorption tendency because of hydrophilic property. It promoted the penetrate of water vapor molecules through the film matrix (Xiao et al., 2001; Li et al., 2006; Chen et al., 2008; Jia et al., 2009; Nair et al., 2011).

Chemical solution could be used to modify the properties. Acid solution affected to lower molecular size of KGM. While alkaline solution (such as calcium hydroxide, sodium or potassium carbonate) modified the KGM structure through deacetylation reaction, hence it reduced the acetyl group which obstructed the orderliness of film matrix and a thermal stable gel was formed.

Cheng et al. (2007) studied the effects of acid modification on physical properties of KGM films. The modification of polymer by acid is a potentially useful method for improving the film properties. The longer time and higher concentration of acid added affected to lower KGM molecular weight. Pure KGM film was analyzed for the crystallinity by wide-angle X-ray diffraction which indicated the highly amorphous with low crystallinity structure due to the randomly acetyl group along the KGM molecule (Cheng et al., 2002). Hydrolysis resulted in shorter KGM polymer length and increased WSC and WVP (1.20×10^{-11} to 1.64×10^{-11} g Pa^{-1} s^{-1} m^{-1}) due to the increasing of the active sites (-OH group) that promoted the absorption of water molecules. The thermal properties of the films by DSC indicated the decreasing of enthalpy when adding higher acid concentration (lower molecular weight). This means less hydrogen bond in the matrix due to the formation of junction zone of small molecules (Yoshimura and Nishinari, 1999). KGM modified with 30 mL HCl

resulted in enhance of chain alignment with the highest enthalpy (253.9 Jg^{-1}). The changing of KGM molecular weight affected to the tensile properties. YM and TS increased but EB decreased due to the small molecular weight could be filled in the void and resulted in stiffer and denser films structure. The maximum YM and TS and minimum EB were found at 30 mL HCl/ 250 mL alcohol. The higher amount of acid added (50 mL and 70 mL) made YM and TS decreased and opposite in EB. This is the effect of the lower molecular weight which is more difficult to form entanglements between the chains. Nevertheless, too high modification level would decrease the viscosity of KGM solution and thus the film could not be formed.

Cheng et al. (2002) studied the microstructure and physical properties of KGM-based films which were modified by alkali and sodium carboxymethylcellulose (CMC). The films were flexible and easily peeled from the casting plate and hence did not need the addition of plasticizer in order to reduce the brittleness of the film. Pure KGM and KGM-KOH films displayed rougher surface than KGM-CMC and KGM-CMC-KOH. This indicated CMC enhanced the compatibility of alkaline deacetylated KGM molecules. The cross-section of pure KGM films presented stack structure oriented paralled to the film surface (Figure 2.3a) and became oriented perpendicular to the surface when adding KOH (Figure 2.3a, d). Chanzy et al. (1982) reported two different types of recrystallized glucomannan – mannan I and mannan II. The mannan I was the recrystallized of native glucomannan molecule. The KGM granular precipitate composed of lozenge shaped lamellae and rearranged to lamella plane. Mannan II was the recrystallized of alkaline treated KGM, the crystals were small square crystals which formed a pseudo-fibrillar precipitate and rearranged perpendicular to the surface. In the modification KGM with alkali (KOH), the crystallinity was improved so WSC and WVP decreased but TS of film increased. It was due to the higher intermolecular interaction (hydrogen bonding) of alkaline deacetylated of KGM. Adding CMC disturbed the crystallinity of native KGM enhanced the compatibility of alkaline deacetylated KGM. Moreover, CMC, which had hydrophilic nature, could absorb water molecules to the films easier. Thus, WSC and WVP increased. The lowest and highest WVP were 1.15×10^{-11} g Pa^{-1} s^{-1} m^{-1} and 1.92×10^{-11} g Pa^{-1} s^{-1} m^{-1}, while pure KGM film was 1.37×10^{-11} g Pa^{-1} s^{-1} m^{-1}. At water activity 0.40 – 0.84, the KGM-CMC-KOH film had the highest YM (about 0.36×10^{3} MPa) and TS (about 6.9 MPa) while KGM-CMC film had minimum EB (about 7.5%).

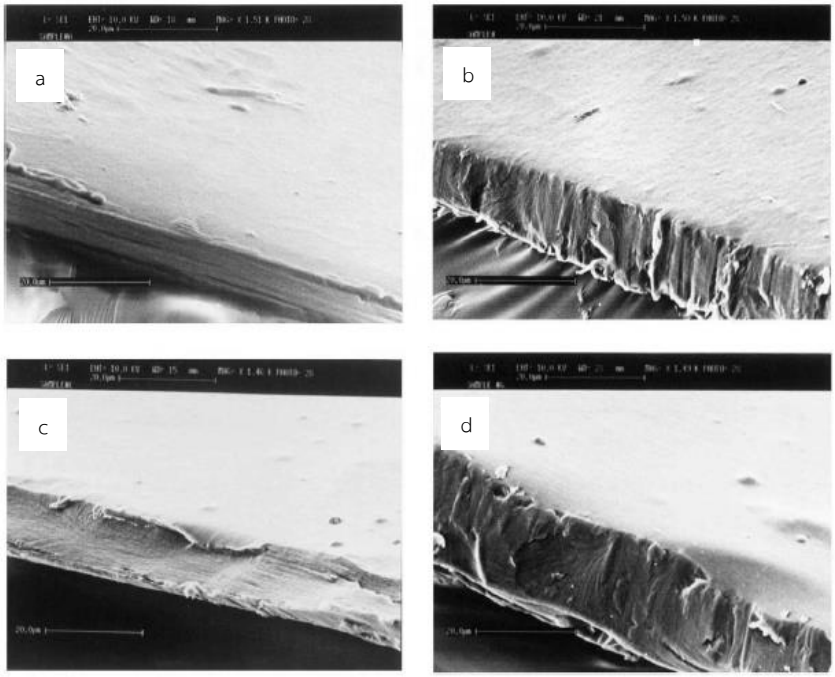


Figure 2.3 SEM photographs of KGM-based films: pure KGM (a), KGM-KOH (b), KGM-CMC (c), and KGM-CMC-KOH (d)

Source : Cheng et al. (2002)

Subsequently, Cheng et al. (2008) studied the composite film that made of KGM, KOH, CMC, and lipid. KOH modification made film structure compact and dense, while CMC made the film structure weaker in the same way with the research of Cheng et al. (2002). Adding Palm olein (PO) disturbed film structure dramatically when compared with KGM film of Cheng et al. (2002) except the homogeneously dispersion of PO in KGM film when blending with CMC and KOH which showed compact and dense film structure as shown in Figure 2.4d. In other words, CMC in alkaline-deacetylated KGM worked as emulsion enhancer. There were pores, voids, cracks, and channel in other film formulas of KGM film incorporated with PO, KGM-PO, KGM-CMC-PO, and KGM-KOH-PO, except KGM-CMC-KOH-PO. It was due to weak interaction of KGM and PO produced channeling in the film matrix because of different drying rate of PO globules creamed at the surface and KGM-PO lower layer. Moreover, they found that the mixing of KOH and KGM film with PO, the different rate of drying between PO globules creamed layer and KGM-KOH-PO layer also resulted in crack and craters in film matrix. In KGM-CMC-PO, it was found that CMC enhanced the stability of PO emulsion but weak interaction between KGM and CMC

resulted in the sponge-like structure. The heterogeneous structure of PO incorporation promoted the diffusion of water molecules as shown in Table 2.1 (Wong et al., 1992b; Torres, 1994). Therefore, the TS and YM decreased while EB, WVP, and WSC increased in film formula KGM-PO, KGM-KOH-PO and KGM-CMC-PO. It was due to the plasticizing effect of the incorporation of hydrophobic materials into hydrophilic matrix (Quezada Gallo et al., 2000). In addition, PO disturbed the intermolecular hydrogen bond of KGM matrix. The highest YM and TS were in KGM-CMC-KOH-PO film which were 0.31 × 10^3 MPa and 5.4 MPa at a_w 0.4, respectively and minimum EB was 10.75% in KGM-KOH-PO film at a_w 0.4, due to the stabilizing effect of CMC to deacetylated KGM with PO as mention previous.

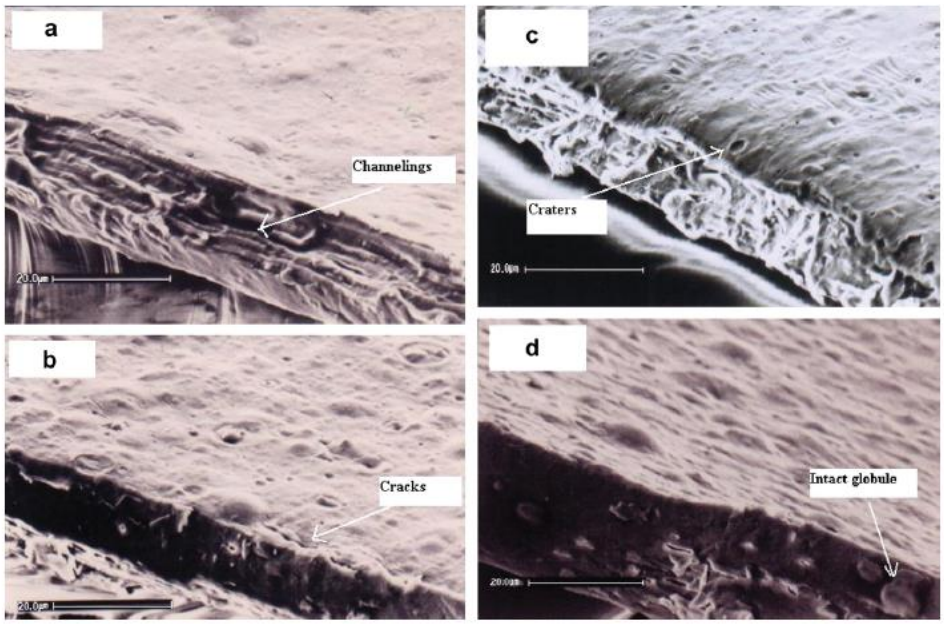


Figure 2.4 SEM photographs of KGM-based emulsion films with PO: KGM-PO (a), KGM-KOH-PO (b), KGM-CMC-PO (c), and KGM-CMC-KOH-PO (d)

Source : Cheng et al. (2008)

Table 2.1 WVP of KGM-based films with and without PO

Film type[A]	WVP* (10^{-11} g m^{-1} s^{-1} Pa^{-1})	Film type[B]	WVP* (10^{-11} g m^{-1} s^{-1} Pa^{-1})
KGM	1.37^{bc} ± 0.24	KGM-PO	2.15^{a} ± 0.05
KGM-KOH	1.15^{c} ± 0.10	KGM-KOH-PO	1.82^{b} ± 0.08
KGM-CMC	1.92^{a} ± 0.13	KGM-CMC-PO	1.76^{b} ± 0.15
KGM-CMC-KOH	1.53^{b} ± 0.30	KGM-CMC-KOH-PO	1.19^{c} ± 0.20

* Each value presents the mean±SD. Different superscript letters in the same column show a significant difference ($p \leq 0.05$).

[A] Data from Cheng et al. (2002)

[B] Data from Cheng et al. (2008)

Source : modified from Cheng et al. (2008)

2.3 Eugenol (EU)

EU (4-allyl-2-methoxyphenol) is a major phenolic compound that generally found in clove oil (*Eugenia caryophyllata*, Myrtaceae) (Figure 2.5). Other than clove, It can be extracted from cinnamon, basil, and nutmeg. EU is widely used in foods, pharmaceuticals, cosmetics, and active packaging materials (Sanla-Ead et al., 2012). There are many research studying the properties of EU. Its application are anti-imflammatory (Son et al., 1998), analgesic activity (Ohkubo and Shibata, 1997), anti-oxidation activity (Ou et al., 2006), anti-bacterial activity (Laekeman et al., 1990; Kalemba and Kunicka, 2003). It can be used as fragrant and favoring agent in food and cosmetic (Atsumi et al., 2001; Fujisawa et al., 2002; Ito et al., 2005). However, EU is phenolic compound, hence it is sensitive to light, oxygen and heat (Chalier et al., 2007; Choi et al., 2009; Coimbra et al., 2011) and poor water solubility (Choi et al., 2009). EU and clove oil have darker color and spicy flavor after storage for a long time (Thompson et al., 1989; Wongnimitkul et al., 2008).

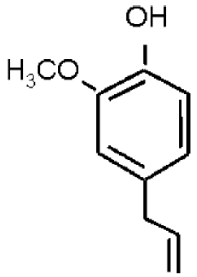


Figure 2.5 Structure of EU

Source: Atsumi et al. (2005)

EU is in a group of methoxyphenols compound which is sensitive to light. EU can be oxidized by an enzymatic or non-enzymatic reaction. The product of oxidized EU is phenoxyl radical which and finally changes to eugenol quinone methide as shown in Figure 2.6 (Suzuki et al., 1985; Thompson et al., 1989; Thompson et al., 1993; Jeng et al., 1994; Thompson et al., 1995; Thompson et al., 1998).

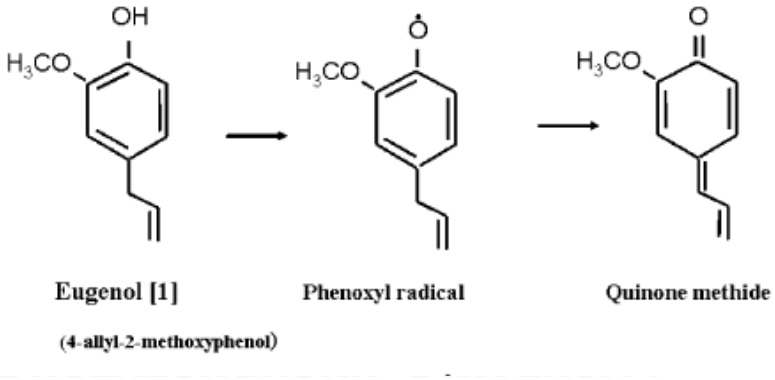


Figure 2.6 Chemical structure of EU and derivatives

Source: Modified from Atsumi et al. (2005)

Due to the fact that EU is highly sensitive to the environment, one method which can be used for preventing the decreasing amount of EU to the environment and oxidation reaction, and it is usually used in food industry, is the encapsulation technique. The encapsulation technique was used to protect the high sensitive plant

compound by using wall material to coat the active compounds from the environment (Choi et al., 2009). In addition, it can be used for control the releasing of the active compound (Fabra et al., 2012).

2.4 Encapsulation

Encapsulation is a technological process to entrap active compounds or living cells within coating materials. It is also identified as a packaging technology which maintains materials (active compound) in small capsules to control the releasing rate, protect the active compounds during processing (Desai and Park, 2005), store and prevent the undesirable interaction with surrounding in order to extend the shelf life. The active compounds or living cells which often used in food industry are antioxidant, minerals, vitamins, phytosterols, lutein, fatty acids, lycopene, or probiotic (Wandray et al., 2009; de Vos et al., 2010). Moreover, encapsulation can be used for control flavor, color, texture, or preservation properties.

2.4.1 Encapsulation materials

Coating materials must be food-grade, biodegradable, and able to form a barrier between the internal phase and environment. The materials which can be used in food industry should be GRAS. Polysaccharides, proteins and lipids are natural materials normally used as coating materials.

Some polysaccharides which have been studied were starch and starch derivatives (amylose, amylopectin, dextrins, maltodextrins, polydextrose, syrup and cellulose and cellulose derivatives), plant exudates and extracts (gum Arabic, gum tragacanth, gum karaya, mesquite gum, galactomannan, pectin and soluble soybean polysaccharides). Marine extracts such as carrageenans and alginate are also used. Dextran, chitosan, xanthan and gellan are microbial or animal polysaccharides which can be used too. Proteins which have been studied are caseins, gelatin, and gluten. Lipids are fatty acid and fatty alcohols, waxes (beeswax, carnauba wax, candellia wax), glycerides and phospholipids. Other materials which have been found in the

previous researches are polyvinylpyrolidone, paraffin, shellac and inorganic materials (Wandray et al., 2009).

2.4.2 Encapsulation technique

In food industry, there are many processes for encapsulation active compounds in wall materials. They are spray drying, fluid-bed coating, spray-chilling, spray-cooling or melt injection (Gibbs et al., 1999; Zuidam and Heinrich, 2009). Moreover, extrusion method, emulsification, vacuum and freeze-drying, and molecular inclusion can also be used (Nedovic et al., 2011).

2.4.2.1 Spray drying

Spray drying is a technique to produce encapsulation in particle or powder form by using the heat drying method. This method is widely used as encapsulation technique in food industry due to its high capacity production in short time. Around 80 - 90% of encapsulation products uses spray drying technique to produce encapsulated particles. The final products, from this process, are acceptable in sensorial and textural characteristics (Nedovic et al., 2011).

2.4.2.2 Extrusion method

Encapsulation by extrusion method is done by dropping the solution of polymer (such as sodium alginate) which composed of active compound using pipette, syringe, vibrating nozzle, spraying nozzle, jet cutter, or atomizing disk (Wandray et al., 2009) into the gelling bath (such as calcium-chloride solution). Extrusion by Jet Cutter is the best technology for large-scale production (Prüsse et al., 2008). This method is capable to produce very small particles (50 μm) in diameter by electrostatic extrusion. Furthermore, co-extrusion is an optional technique to produce hydrophobic core in a hydrophilic or hydrophobic shell spherical microbeads.

2.4.2.3 Emulsification

This method refers to the technique for making emulsion. The emulsion types are water-in-oil emulsion, oil-in-water emulsion, and water-in-oil-in-water emulsion. An emulsion film is the dispersion of lipid which is entrapped in protein or polysaccharide continuous matrix (Pérez-Gago and Krochta, 2005). One step process is an advantage of this technique (Pérez-Gago and Rhim, 2014). The emulsion was prepared and dried by drying technique (Zuidam and Shimoni, 2009). Protein and polysaccharide can be used as base for making emulsion film. However, polysaccharide is not as effective in forming emulsion as protein. So, emulsifier must be added to improve stability of the emulsion (Pérez-Gago and Rhim, 2014).

2.4.2.4 Spray-chilling or spray-cooling

Lipid-coated active compounds are produced by spray-chilling or spray-cooling method. The difference of these two methods is the temperature used in the process. In spray-chilling, the temperature is in the range of 34 – 42°C, while it is higher in spray-cooling technique. Active compounds which usually encapsulated by this technique were materials which can be dissolved in lipid, dry particles, or aqueous emulsion (Gouin, 2004; Zuidam and Shimoni, 2009).

2.4.2.5 Fluid bed coating

Fluid bed coating is used for produce encapsulated or coated core material, which is suspended by an air stream, by spraying with an atomized, wall material. Normally, the coating material might be an aqueous solution of cellulose, starch derivatives, proteins, and gums (Dewettinck and Huyghebaert, 1999).

2.4.2.6 Vacuum and freeze-drying

Vacuum drying and freeze-drying are similar drying method. However, there are different in time, cost, and temperature for the operation. Vacuum drying is fast and cheaper than freeze-drying for making encapsulation because it can be done at temperature above the freezing point of the solvent. Conversely, freeze-drying uses high energy and takes long processing time. The particles obtained from both methods have high porosity and may lose the protection properties between cores and wall materials (Zuidam and Shimoni, 2009).

2.4.2.7 Molecular inclusion

Molecular inclusion is the technique to carry active compound in cavity of substance that is used as wall material such as cyclodextrins or liposomal vesicles. This technique is expensive and less utilized (Nedovic et al., 2011).

2.4.3 Encapsulation active compounds in food

Many active compounds are used in food products such as volatile compounds, flavors, antioxidant agents, phenolic compounds, essential oils, antimicrobial agents, vitamins, micronutrients, fish oils, peptides, etc (Voilley and Etievant, 2006; Zuidam and Heinrich, 2009). Encapsulation of active compounds is designed to control the releasing rate, improve the stability during processing, storage before using and preventing the reaction with environment or other components in food (oxygen or water). Also, encapsulation can be used for the specific application depends on type of active compounds. It is used to mask unpleasant feeling, such as bitter taste and astringency of phenolic compounds (Bell, 2001).

From the above, there are many methods for encapsulation. The appropriate method for encapsulation different active compounds depends on property, stability and final product application. For examples, flavor can be encapsulated by spray-drying, spray-chilling, spray-cooling, spray bed drying and other (Zuidam and Heinrich,

2009). The spray-drying and vacuum-drying can be used for encapsulation of plant extracts (Schimidt, 1997).

2.4.4 Encapsulation active compounds in edible films

The utilization of edible films incorporated with active compounds, as active packaging, is an interesting research topic in packaging. The natural active compounds which usually incorporated in the film are essential oil extracted from herbs or plants (Giménez et al., 2013; Wang et al., 2013). Active packaging can be used as carrier of antioxidant agents, antimicrobial agents, or aroma agents (Li et al., 2006; Hambleton et al., 2009; Cerqueira et al., 2010; Salgado et al., 2012). Adding active compounds to the films affect the properties of films such as microstructure, appearances, mechanical properties, WVP or WVTR, solubility or etc. (Cerqueira et al., 2010; Arcan and Yemenicioğlu, 2011; Giménez et al., 2013; Wang et al., 2013).

Edible films from proteins and polysaccharides are normally used for encapsulation of active compounds. The effect of essential oils on the properties of the films depends on the interaction between essential oils and film materials. Furthermore, microstructure also affects the properties of the films. The following reviews showed the effect of essential oils or active compounds to the properties of polysaccharide films.

The active compounds can either interact with the polysaccharide or no interact occur between them. The occurrence of intermolecular interaction between the two components resulted in strong interaction considerably affected to the structure and the properties of the films. Research that reported the interaction of film polymers and active compounds were chitosan incorporated with tea polyphenol, cinnamon essential oil, and *Aloe vera* gel (Ojagh et al., 2010; Khoshgozaran-Abras et al., 2012; Wang et al., 2013). The appearance of strong intermolecular (especially crosslinking interaction) could be observed using SEM, FT-IR, and DSC. The cross-section of SEM photographs showed compact structure and the thickness of the films did not change (Ojagh et al., 2010). In addition, the occurrence of new interaction would indicate by the existence of new peak(s) or the

shift of absorption band to greater wavenumber of FT-IR spectrum. The incorporation of tea polyphenol to chitosan film revealed the new peak at wavenumber 1230 cm^{-1} which indicated CO group of tea polyphenol and the peak of –C-O-C- at 1020 cm^{-1} to 1025 cm^{-1} refer to the interaction of chitosan and tea polyphenol (Banerjee et al., 2002; Evans et al., 2008; Wang et al., 2013). Because of the occurrence of strong interaction, hence it affected to greater resistance of film against the rupture and barrier properties. Therefore, TS increased while EB and solubility decreased. The intermolecular interaction influenced the reduction of polymer mobility and limited the water vapor to pass through the film, hence WVP and solubility decreased. Moreover, the reduction of WVP could be the effect of essential oil's hydrophobicity. Thus, the incorporation of essential oil which interacted with polymer could be used to improve the potential application of films.

No interaction between essential oils and film polysaccharide polymers was another effect to the structure and properties of films. This type of effect was reported in the incorporation of garlic oil in alginate-based edible film (Pranoto et al., 2005), plant essential oil and oil compounds in alginate-apple puree film (Rojas-Graü et al., 2007), tea tree essential oil in hydroxypropylmethylcellulose (HPMC)-based film (Sánchez-González et al., 2009), thyme oil in chitosan film (Altiok et al., 2010), *Gleditsia triacanthos* seed extract in galactomannan film (Cerqueira et al., 2010), and oregano essential oil in alginate film (Benavides et al., 2012). From SEM analysis, the addition of essential oil affected the discontinuity of film structure comparing to film without essential oil added. Sánchez-González et al. (2009) described the separation of the film to two phases – continuous of the polymer and disperse phase of oil droplet. FT-IR analysis supported heterogeneous film structure by the presence of no new absorption peak (Altiok et al., 2010). In addition, the FT-IR spectrum showed the combination peak which appeared in essential oil and polymer, which indicated no interaction in the film matrix. No or weak interaction between two components caused the change of thickness and deterioration of mechanical properties. Cerqueira et al. (2010) and Benavides et al. (2012) showed the increasing of film thickness significantly. Moreover, Benavides et al. (2012) also found the greater thickness with increasing $CaCO_3$, which used as crosslinking agent of alginate polymer, and this finding agree with the increasing of solid content of film forming solution resulted in the increase of thickness of film of Han and Krochta (1999). The reduction of film resistance to rupture was the effect of essential oil incorporation. The discontinuity of film matrix decreased TS of film (Pranoto et al., 2005; Zivanovic et al., 2005; Rojas-

Graü et al., 2007; Sánchez-González et al., 2009; Altiok et al., 2010; Benavides et al., 2012), whilst EB had two different effects. Pranoto et al. (2005), Rojas-Graü et al. (2007), and Benavides et al. (2012) concluded the plasticizing effect of essential oil which promoted the mobility of the matrix and increased EB of the films. On the other hand, the EB could decrease. This was the effect of essential oil (thyme oil) to pore size and porosity of film that made the film easy to rupture (Altiok et al., 2010).

The increasing of WVP could be the result of hydrophilic, plasticizing properties of essential oil added, or the formation of porous structure of composite film (Pranoto et al., 2005; Altiok et al., 2010; Cerqueira et al., 2010). It was noted that incorporation of essential oil with no interaction to film polymer could reduce WVP, due to the hydrophobicity of essential oil that prevented water to penetrate through the film matrix (Rojas-Graü et al., 2007; Sánchez-González et al., 2009; Benavides et al., 2012).

The addition of essential oil affected the optical properties of the film whether the interaction with film polymer occurred or not. The incorporated film became opaque and darker (Pranoto et al., 2005; Sánchez-González et al., 2009; Cerqueira et al., 2010; Benavides et al., 2012; Wang et al., 2013). Sánchez-González et al. (2009) concluded that the changing of the optical property of the film with essential oil was the results of light scattering of oil droplet which had different refractive index with film polymer and oil droplet size.

Woranuch and Yoksan (2013a) and Woranuch and Yoksan (2013b) studied EU-loaded chitosan nanoparticles which indicated the thermal stability of the encapsulation EU in chitosan nanoparticles and added in bio-based plastics for active packaging. The thermal stability of EU-loaded chitosan nanoparticles was investigated by FT-IR, TGA, and DSC. In FT-IR spectrum, EU showed characteristic peaks were at 3522 cm^{-1} for -OH group, 2841 to 3004 cm^{-1} for C-H stretching, while 1511 cm^{-1}, 1611 cm^{-1}, and 1638 cm^{-1} for C=C aromatic ring (Dhoot et al., 2009). EU-loaded chitosan nanoparticles indicated EU encapsulation in nanoparticle by the increasing of the peak intensity at 2920 to 2925 cm^{-1} (Figure 2.7 c-g). In addition, they studied the intensity ratio of C-H stretching of EU at 2925 cm^{-1} and the pyranose band of chitosan at 891 cm^{-1} (I_{2925}/I_{891}) and found the higher intensity ratio than pure chitosan

nanoparticle, hence it showed the successful loading of EU (Figure 2.7). The improvement of thermal stability of EU in chitosan nanoparticle represented in TGA and DSC results. The TGA thermograph showed the EU decomposition peak temperature at 258°C. After encapsulation in chitosan nanoparticle, the decomposition temperature changed to the higher temperature in the range of 280 °C to 340°C. The oxidative stability of EU was also greater by the incorporation in chitosan nanoparticles. DSC analysis displayed the onset temperature of EU auto-oxidation at 159°C and became higher to 223 after incorporated with chitosan nanoparticle. Thus, using chitosan nanoparticle improved the thermal stability of EU and 1:1 ratio of chitosan:EU was the best, owing to the highest EE.

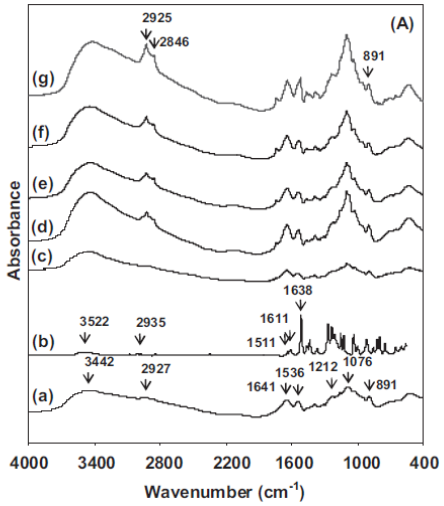


Figure 2.7 FT-IR spectra of chitosan nanoparticle (a), EU (b), and EU loaded chitosan nanoparticle prepared with 0.5% (w/v) tripolyphosphate at the ratio of chitosan to EU 1:0.25 (c), 1:0.50 (d), 1:0.75 (e), 1:1 (f), and 1:1.25 (g).

Source: modified from Woranuch and Yoksan (2013a)

The EU-loaded chitosan nanoparticles was also blended with thermoplastic flour (mixed flour cassava, rice and waxy rice flours in a weight ratio of 50:30:20) and formed the film (Woranuch and Yoksan, 2013b). They found that EU-loaded chitosan nanoparticles caused the reduction of thermal stability of thermoplastic flour film which indicated by the decreasing of degradation temperature (T_d) in TGA thermographs and melting enthalpy (ΔH_m) in DSC thermographs. They concluded that EU-loaded chitosan nanoparticle showed the immiscibility with starch polymer

and suppressed the retrogradation of starch, hence reduced the resistance of the film. The incorporation of chitosan nanoparticle caused the deterioration of tensile properties and reduced TS, YM, and EB. The barrier property of film also changed. The film incorporated with free EU had lower WVP than pure thermoplastic flour film, due to the hydrophobicity of EU. Furthermore, the film with EU-loaded chitosan nanoparticle and the film with chitosan nanoparticle presented fewer –OH group available and more tortuous path of water vapor to pass through, hence it showed the enhancement of barrier property (lower WVP). In the study of EE of EU in the film, they found the dependence of EE with higher concentration of EU added as shown in Table 2.2. In the film with free EU, the remaining amounts of EU could not be detected while the film with EU-loaded chitosan nanoparticles presented the increase amount of remaining EU 65.45% (w/w) with the increasing of EU concentration added from 0.35% (w/w) to 0.70% (w/w). Thus, thermoplastic flour was able to carry the EU-loaded chitosan nanoparticles.

Table 2.2 Remaining content of EU and EU-loaded chitosan nanoparticles in thermoplastic flour film

Additional additive	Nanoparticle content (%, w/w)	EU content (%, w/w)	Remaining content of EU* (mg/g)
Free EU	-	0.35	n.d.
Free EU	-	0.70	n.d.
EU-loaded chitosan nanopartcles	3	0.35	0.036 ± 0.005^{b}
EU-loaded chitosan nanopartcles	6	0.70	0.055 ± 0.005^{a}

n.d., not detected

* Each value presents the mean±SD. Different superscript letters in the same column show a significant difference ($p \leq 0.05$).

Source : modified from Woranuch and Yoksan (2013b)

KGM edible film could be modify to wider that potential applications of KGM by blending with others macromolecules or adding chemical solutions (refer to section section 2.2.3). The good mechanical properties were useful for extensive

potential applications. Korkiatithaweechai et al. (2011) used chitosan blended with oxidized KGM to encapsulate diclofenac. Sodium periodate performed as oxidizing reagent to change KGM to oxidized KGM. After that the obtained oxidized KGM was blended with chitosan and incorporated with diclofenac. Oxidized KGM and chitosan interacted with each other through crosslinking interaction (imine bond, -C=N-) between -CHO group of oxidized KGM and $-NH_2$ of chitosan. The interaction was monitored using FT-IR. They found the new absorption peak at about 1720 cm^{-1}, owing to C=N stretching vibration of imine group of a Schift base. Incorporation of diclofenac presented normal peaks that found in chitosan-oxidized KGM composite film and diclofenac. This pointed out that the composite matrix and diclofenac did not react with each other. The effect of chitosan, oxidized KGM, and diclofenac content were used to study EE. The greater chitosan and oxidized KGM content made EE increased. Although there was no interaction between the composite polymer and diclofenac, the crosslinking could help to entrap higher amounts of diclofenac. When focusing on the increasing of diclofenac concentration, 0.5 g to 1 g added, the greater EE was found. However, EE was lower when 2 g diclofenac added. This is owing to the reason that excess diclofenac could not be entrapped in the matrix. The pure oxidized KGM film indicated the fastest releasing (90% of EE), whilst they were slower in composite film at both pH 1.2 and pH 7.4. The increasing of diclofenac added, exhibited higher releasing up to 73%. blended film at 1:2:1 ratio of chitosan : oxidized KGM : diclofenac gave the highest EE and the lowest releasing and this formula should be used to study the controlled release drug model.

Moreover, there was the research that using KGM film to entrap the essential oil. Rattananin (2011) studied the properties of alkaline-treated KGM film which the incorporate with of galangal and galingale extract and use as active film to prevent deterioration of mango cv. Nam dok mai #4. However, they studied the mechanical properties and WVTR of the film also. They revealed that pure KGM film had the highest TS and EB (51.78 MPa and 37.29%, respectively), while the incorporation of higher plant extract caused the reduction of film strength and flexibility. It was due to the hydrophobicity of the extracts that could retard the orderliness of the film polymer and hence the structure of the film was heterogeneous and easy to rupture. Furthermore, the hydrophobic of extract oil suppressed the penetration of water vapor trough the film and reduced WVTR. This results corresponded to Sánchez-González et al. (2010) that hydrophobic of bergamot oil disturbed intermolecular interaction of chitosan polymer and caused the reduction of TS, EB, and WVTR.

Furthermore, the KGM film was able to carry the oil extract and prolong the shelf life of the mango during 30 days. Thus, this indicated that KGM could be used to encapsulate active compounds and act as active packaging.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

KGM powder was from Monkey King Food Co., Ltd. (Bangkok, Thailand) is white, fine (with 120 mesh particle size) with ≥ 90% purity, and viscosity of 1% ≥32000 mpa·s. EU 99% purity was from Tien Yuan Chemical (PTE) Ltd. (Singapore). Tween 20 was from Merck (Germany). 99% ethanol and 37% hydrochloric acid were from QRëc (New Zealand).

3.2 Methods

3.2.1 Films preparation

Film preparation was performed according to modified method of Cheng et al. (2002) and Cheng et al. (2008). EU (0.50%, 1.00% or 1.50% of film forming solution (w/w)) was homogenized by Ystral homogenizer model X10125 (Ystral GmbH maschinenbau+processtechnik Co., Ltd., Germany) at 25,000 rpm for 5 minutes in distilled water with Tween 20 (15% (w/w) of EU) for making EU emulsion. KGM powder (0.50%, 0.75% or 1.00% of film forming solution (w/w)) was dissolved in distilled water, then EU emulsion was added immediately. After mixing for 1 hour, film forming solution was weighed at 110 g then poured onto 28.4 cm × 12.2 cm porcelain plate and dried over night at room temperature. Dried films were peeled off and kept at 50±1% RH and 30°C cabinet before testing. The concentration of KGM and EU in each film formula was shown in Table 3.1.

Table 3.1 Concentration of KGM and EU of 12 film formula

Formula (% w/w)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
%KGM	0.50	0.75	1.00	0.50	0.50	0.50	0.75	0.75	0.75	1.00	1.00	1.00
%EU	0.00	0.00	0.00	0.50	1.00	1.50	0.50	1.00	1.50	0.50	1.00	1.50

3.2.2 Properties of KGM films

3.2.2.1 Thickness

Thickness of the films was measured by a digital micrometer model ID-C112 (Mitutoyo MFG Co., Ltd., Japan) at fifteen random positions per each film strip (30 mm × 120 mm).

3.2.2.2 Mechanical properties

Mechanical tests were performed according to American Society for Testing and Materials (ASTM, 2001), with some modification using Instron® universal material testing machine model 5565 (Instron, Norwood, MA). The 30 mm × 120 mm film sample was cut and examined. The test condition was 50 mm initial grip length, 5.0 mm/sec crosshead speed and 5 kg load cell. The evaluation data were shown as force at maximum load (gf) and length of film at breaking point (mm). Tensile strength (TS), elongation at break (EB, %), and Young's modulus (YM) were calculated using Eq. (1) to (3). The determination was done in three replications.

$$TS\ (Pa) = \frac{F}{A} \quad (1)$$

$$EB\ (\%) = \frac{L - L_0}{L_0} \times 100\% \quad (2)$$

$$YM\ (Pa) = \frac{TS}{EB} \quad (3)$$

where F is the force at maximum load (N), A is cross section area of film (m^2), L_0 is the initial grip length (50 mm) and L is the length of film at breaking point (mm).

3.2.2.3 Water vapor permeability (WVP)

WVP was determined using a modified method of ASTM that described by Shiku et al. (2004) with some modification. The films were cut to 60 mm × 60 mm dimension and sealed on a glass cup containing siliga gel (0% RH) using plastic band and parafilm. The cups were placed in the desiccator which saturated with distilled water and weighed every day for 7 days. The WVP values were calculated by using Eq. (4) (McHugh et al., 1993).

$$WVP = \frac{wx}{At\Delta P} \quad (4)$$

where w is the gain weight of cup with film sample (g), x is thickness of film (m), A is the area of exposed film (m^2), t is time of weight gaining (s) and ΔP is the vapor pressure differential across the film (4224.9 Pa) (Hoque et al., 2011). The determination was done in three replications.

3.2.2.4 Transparency

The transparency of film was measured by measuring the transmittance at wavelength 600 nm using Genesys 20 spectrophotometer model 4001/4 (Thermo Fisher scientific, Becthai Bangkok Equipment & Chemical CO., LTD. Bangkok, Thailand).

The transparency was calculated according to the equation reported in Han and Floros (1997) and Suppakul et al. (2006) as shown in Eq. (5).

$$\text{Transparency} = \frac{log\mathrm{T}_{600}}{\mathrm{x}} \quad (5)$$

where T_{600} is the transmittance of film measuring at wavelength 600 nm and x is the thickness of film (mm). The determination was done in three replications.

3.2.2.5 Scanning electron microscope (SEM)

The cross section morphology of pure KGM films and KGM encapsulated with EU (KGM/EU) films was observed by SEM model JSM-6400 (JEOL Ltd., Japan)**.** The film speciments of 5×3 mm film were mounted on copper stubs using double sided adhesive tape and coated with gold, in order to make the sample conductive, before testing. The samples were observed at the 1000 magnificant.

3.2.2.6 Solubility

The solubility of film in water was determined according to the method of Tunc et al. (2007) with some modification. The film samples in dimension of 40 mm × 40 mm were shaken in 50 mL distilled water at 250 rpm for 10 minutes. The non-soluble matters were dried at 105 °C for 24 hours. The film solubility (%) was calculated using Eq. (6).

$$\text{Solubility (\%)} = \frac{\mathrm{W_i - W_f}}{\mathrm{W_i}} \times 100\% \quad (6)$$

where W_i is the initial weight of film sample and W_f is the final weight of the non-soluble matter after drying at 105°C for 24 hours. The determination was done in three replications.

3.2.3 Effect of EU encapsulation to film structure

3.2.3.1 Fourier transform infrared spectroscopy (FT-IR)

Transmission infrared spectra of the film was recorded using FT-IR Spectrum One model (Perkin Elmer, Waltham, MA, USA) in the wavenumber range of 4000 – 400 cm^{-1}.

3.2.3.2 Differential scanning calorimetry (DSC)

DSC was performed according to the condition of Li et al. (2006) using DSC 204 F1 Phoenix model (Netzsch, Germany). The analysis condition is under a nitrogen atmosphere with a flow capacity of 25 ml/min from 30 to 400 °C at a heating rate of 10 °C/min by using aluminum sample pan. The determination was done in two replications.

3.2.4 Encapsulation efficiency (EE) of EU in KGM film

EE of KGM/EU films were measured by the method of Woranuch and Yoksan (2013b) with some modification. The film sample (0.01 g) was mixed with distilled water (1 mL) and hydrochloric acid solution (2.5 M, 5 mL). The sample was heated at 95°C for 30 minutes and cooled to the room temperature. The solution was added to of 95% ethanol (1 mL) and centrifuged at 4500 rpm for 4 minutes. The amount of EU in the supernatant fraction was determined by measuring the absorbance at wavelength 283 nm, which is a maximum absorption wavelength of EU (Woranuch and Yoksan, 2013a), by using Spectronic spectrophotometer model Genesys 10 UV

(Thermo Spectronic, Rochester, NY, USA) and calculated by Eq. (7). The EE(%) was calculated by Eq. (8) (Keawchaoon and Yoksan, 2011).

A_{283} = slope(weight of EU encapsulated, g); (R^2 = 0.994, Figure A.2) (7)

$$\text{EE (\%)} = \frac{\textbf{weight of EU encapsualted from Eq.(7)}}{\textbf{weight of initial EU (g)}} \times 100\% \quad (8)$$

where A_{283} is the absorbance of EU in sample measuring at wavelength 283 nm. The determination was done in three replications.

3.2.5 Releasing of EU from KGM/EU film

Releasing of EU from KGM films in water was determined by modified method of Tunc et al. (2007), Woranuch and Yoksan (2013a), and Woranuch and Yoksan (2013b). The film samples (0.2 g) were mixed with 100 mL distilled water and shake at 250 rpm. The solutions (1 mL) were taken every 10 minutes for 60 minutes and diluted in 9 mL distilled water. The amount of releasing EU was determined by measuring the absorbance at wavelength 283 nm and calculated by Eq. (9). The %releasing was calculated according to Eq. (10).

A_{283} = slope(weight of EU released, g); (R^2 = 0.997, Figure A.3) (9)

$$\text{Releasing (\%)} = \frac{\textbf{weight of EU released fom Eq.(9)}}{\textbf{weight of initial EU (g)}} \times 100\% \quad (10)$$

where A_{283} is the absorbance of EU in sample measuring at wavelength 283 nm. The determination was done in three replications.

3.2.6 Effect of storage time to KGM/EU films

KGM/EU film was analyzed during storage in plastic bag which kept at 50±1% RH and 30°C cabinet. The films were analyzed by thermogravimetric analysis (TGA) on 0, 20, 40, and 60 days. EE (%) of EU in KGM/EU film was determined by measuring the absorbance at wavelength 283 nm on 0, 210, and 240 days.

3.2.6.1 Thermal stability of the films during storage

TGA was used to analyze the storage stability of KGM/EU film by using Perkin Pyris 1 TGA (Perkin Elmer Ltd, USA). The films were investigated by injection of N_2 purge gas (80 mL/min). The condition was heating from 25°C to 550°C at a rate of 10 °C/min. The results were shown in two graph types: Thermogram (TG) and derivative thermogram (DTG). The determination was done in three replications.

Film color was measured using colorimeter (Chroma Meter CR-300, Minolta Camera Co., Osaka, Japan). The CIE color space was used. Color of the film was expressed as *L** (lightness/darkness), *a** (redness/greenness), and *b** (yellowness/blueness) values by using D65 as an illuminant. The total difference in color (ΔE^*) was calculated by Eq. (11). The determination was done in three replications.

$$\Delta E^* = \sqrt{(L_0^* - L^*) + (a_0^* - a^*) + (b_0^* - b^*)} \quad (11)$$

where L_0^*, a_0^*, and b_0^* are the color values of the white plate which using beneath the samples for testing, and L*, a*, and b* are the color values of tested samples. (L_0^* = 94.36, a_0^* = -2.14, and b_0^* = 2.91)

3.2.6.2 EE of EU in KGM/EU film during storage

EE (%) of EU which encapsulated in KGM films was analyzed following the method in section 3.2.4 to measure amount of EU and calculate as EE during storage. Before the measurement, the films were kept in 50±1% RH and 30°C cabinet and measured on 0, 7, and 8 months.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Film appearance

Pure KGM films and KGM/EU films were casted with different KGM concentration at 0.50%, 0.75% and 1.00% (w/w) and EU concentration at 0.50%, 1.00%, and 1.50% (w/w) as shown in Table 3.1. The highest concentration of KGM which could be casted as film was 1.00% (w/w). From observation, the higher concentration of KGM made the film more viscous and when the concentration of KGM was higher than 1% (w/w) it could not be spread on the plate to form film. Thus, 1.00% KGM was chosen as maximum concentration of KGM to cast the film in this study.

The concentration of EU 1.00% (w/w) was selected according to the result of Shinde and Nagarsenke (2011) and Woranuch and Yoksan (2013a). Shinde and Nagarsenke (2011) used gelatin-sodium alginate to encapsulate EU and found that the highest EE (%) was at the ratio of EU:gelatin-sodium alginate 2:1 (15.99%) and 1:1 (15.41%). Moreover, Woranuch and Yoksan (2013a) revealed the highest EE (%) of EU incorporated with chitosan nanoparticle at the ratio of chitosan:EU at 1:1 (w/w). Therefore, 1.00% (w/w) EU was chosen as middle concentration added in film forming solution in this study. The lower EU concentration (0.50% w/w) and the higher EU concentration (1.50% w/w) were selected to study the properties of the film when incorporated at different EU concentration compared with 1.00% (w/w) of EU.

In this study, two types of films were formed: pure KGM film and KGM/EU film. Pure KGM film was more difficult to peel off from porcelain plate than the KGM/EU films. The film had two sides – the upper side and the lower side. The upper side of the film, the surface which faced to air or environment during casting,

was rough and had grainy texture. The lower side or the film surface which faced to the casting plate during drying was smoother and glossy.

The difference of KGM and EU concentration in each film formula affected the appearance of the films. Pure KGM films were translucent. Higher KGM concentration made the films thicker and stronger. Moreover, adding EU affected the turbidity and color of the film. KGM/EU films were more opaque than pure KGM films. The films became more opaque with higher concentration of EU. The intensity of yellowness depended on the concentration of EU added. Both pure KGM films and KGM/EU films were not rigid and could be folded several times without film broken.

4.2 Properties of KGM films

4.2.1 Thickness

The thickness of the films was in the range of 25.80 μm to 51.60 μm as shown in Table 4.1. Increasing of KGM and EU concentration resulted in increasing the thickness of the films. The lowest thickness was 25.80 μm for KGM:EU at 0.50%:0.50% (w/w). This was the film with the lowest concentration if KGM and EU. However, it was not significantly different than the thickness of pure KGM film with the lowest concentration (27.45 μm). The increasing of KGM and EU content resulted in higher film thickness and reached the maximum at 51.60 μm for KGM:EU film at 1.00%:1.50% (w/w). Thus, the changing of KGM film's thickness depended on the combination effect of KGM and EU concentration.

With respect to the concentration of KGM, the thickness of the film was significantly increased with higher concentration of KGM (0.50%, 0.75%, and 1.00% of film forming solution). The results are consistent with Benavides et al. (2012) research. They found the slightly decease of thickness with increasing of $CaCO_3$ added in alginate to form film. This effect was similar to the dependent of thickness with whey protein concentration. They concluded more film's thickness was due to the higher solid content in the film forming solution (Han and Krochta, 1999).

With respect to the concentration of EU, the addition of EU to the film made the film thicker and the increasing of EU concentration (0.50%, 1.00%, and 1.50% of film forming solution) resulted in the increasing of the film thickness. Cerqueira et al. (2010) and Benavides et al. (2012) showed the increasing of films' thickness of the addition of seed extract in galactomannan film and oregano oil in alginate film, respectively and hence it made the heterogeneous film structure and thickness of the film increased. Therefore, it was possible that EU and KGM matrix in KGM/EU film was low compatibility. Thus, the thickness of films increased.

Table 4.1 Thickness (μm) of pure KGM films and KGM/EU films

%KGM	%EU	Thickness* (μm)
0.50	0.00	27.45^{g} ± 4.68
0.75	0.00	34.01^{f} ± 2.51
1.00	0.00	39.30^{de} ± 0.42
0.50	0.50	25.80^{g} ± 0.27
0.50	1.00	35.67^{ef} ± 0.88
0.50	1.50	35.75^{def} ± 0.58
0.75	0.50	40.71^{cde} ± 1.94
0.75	1.00	40.82^{cd} ± 3.13
0.75	1.50	44.78^{bc} ± 3.23
1.00	0.50	46.88^{ab} ± 3.13
1.00	1.00	48.38^{ab} ± 5.80
1.00	1.50	51.60^{a} ± 3.78

* Each value presents the mean±SD. Different superscript letters in the same column show a significant difference ($p \leq 0.05$).

4.2.2 Mechanical properties

Tensile strength (TS), elongation at break (EB, %) and Young's modulus (YM) are parameters that reflect the mechanical properties of the films (Cheng et al., 2007; Benavides et al., 2012; Woranuch and Yoksan, 2013b). They are presented in Table 4.2. TS was calculated as force at maximum load before the film break divided by cross-section area of the film (Gennadios et al., 1997). EB expressed the expandable of film before rupture comparing to initial length of the film (Krochta and De Mulder-Johnston, 1997). YM was calculated by dividing TS by EB or calculated from the slope between stress and strain. YM value indicates the rigidity of the film (Pelissari et al., 2009). In this research, TS and YM increased when KGM content increased but decreased when EU increased.

The tensile properties were shown in Table 4.2, the TS of the films were in the range of 15.16 MPa to 36.48 MPa. The highest and lowest TS were in samples with the ratio of KGM:EU at 1.00%:0.00% (w/w) and 0.50%:1.50% (w/w), respectively. The maximum TS was in the film with highest KGM concentration without the incorporation EU. This could indicate the high resistance of pure KGM films. When adding EU in film forming solution, the TS became lower and reached the minimum at 0.50%:1.50% of KGM:EU. This film formula was the lowest KGM concentration with the highest EU concentration.

Pure KGM film at 0.75% (w/w) KGM and 1.00% (w/w) KGM presented minimum EB (1.34%) and the opposite in YM (1.57×10^{-3} MPa). It might be due to the high resistance of the film at high KGM concentration and hence the reduction of expandability of the films. In the films incorporated with EU, the increasing of EB and the reduction of YM were found. This phenomenon was probably due to the effect of EU which could extend the mobility and increased the expandability of the KGM matrix (comparing to other films in this study) and reached the maximum EB (9.52%) and minimum YM ($0.27 \times 10^{-3-}$ MPa) at the concentration 0.50%:0.50% (w/w) of KGM:EU. Thus, the combination effect of KGM and EU affected the changing of mechanical properties of the films.

Table 4.2 TS, EB (%) and YM of KGM/EU films

%KGM	%EU	TS* (MPa)	%EB*	YM* × 10^3 (MPa)
0.50	0.00	19.99^{ef} ± 3.14	3.24^{e} ± 1.03	1.05^{b} ± 0.24
0.75	0.00	19.14^{efg} ± 1.24	1.34^{f} ± 0.14	1.57^{a} ± 0.17
1.00	0.00	36.48^{a} ± 2.67	2.81^{ef} ± 0.08	1.56^{a} ± 0.20
0.50	0.50	20.74^{de} ± 2.04	9.52^{a} ± 1.25	0.27^{e} ± 0.07
0.50	1.00	15.85^{fg} ± 2.52	7.01^{bc} ± 0.85	0.28^{e} ± 0.06
0.50	1.50	15.16^{g} ± 1.41	5.89^{bcd} ± 0.93	0.30^{e} ± 0.04
0.75	0.50	22.80^{bcde} ± 3.03	3.33^{e} ± 0.47	0.93^{b} ± 0.23
0.75	1.00	24.36^{bcd} ± 3.23	7.28^{bc} ± 1.85	0.43^{de} ± 0.08
0.75	1.50	21.88^{cde} ± 2.00	7.50^{b} ± 1.50	0.32^{e} ± 0.09
1.00	0.50	24.48^{bcd} ± 2.13	4.52^{de} ± 1.09	0.78^{bc} ± 0.17
1.00	1.00	25.53^{bc} ± 2.22	6.13^{bcd} ± 1.14	0.50^{de} ± 0.09
1.00	1.50	26.26^{b} ± 3.54	5.58^{cd} ± 0.89	0.56^{cd} ± 0.09

*Each value presents the mean±SD. Different superscript letters in the same column show a significant difference ($p \leq 0.05$).

In order to investigate the effect of KGM concentration, TS significantly increased with increasing KGM content. The highest TS was 36.48 MPa with pure KGM film at 1.00% (w/w). This result agrees with the research of Xu et al. (2007) and Chen et al. (2008) who studied the effect of KGM blend with gellan gum and pea starch, respectively. They found that increasing KGM content affected higher TS value. They concluded the presence of larger number of hydrophilic hydroxyl group of KGM which resulted in higher inter-molecular hydrogen bond between KGM molecules. Thus, TS value increased with increasing KGM concentration.

The addition of EU significantly reduced TS. This finding agrees with the study of Benavides et al. (2012). They reported that adding oregano essential oil had the effect to reduce TS of KGM film. This was due to heterogeneity and discontinuities of film structure that occurred with the incorporation of essential oil or extra-polymeric component which affected the reduction of TS value of films. The immiscibility between hydrophilic and hydrophobic molecules in the film might cause the decreasing of tensile properties (Pranoto et al., 2005; Zivanovic et al., 2005; Rojas-Graü et al., 2007; Sánchez-González et al., 2009; Altiok et al., 2010; Benavides et al., 2012; Woranuch and Yoksan, 2013b). Thus, it displayed that EU might have the function as plasticizer that could decrease the intermolecular interaction between KGM matrices then decreased the tensile properties and homogeneous structure of the film.

EB of KGM/EU films decreased with increasing KGM concentration and increased when EU concentration increased. As the above result, TS of the films increased with increasing KGM content because of the hydrogen bonding between KGM molecules which made the films rigid (Chen et al., 2008). Thus, EB of films decreased.

Incorporation of EU into KGM film resulted in higher EB of the film. As shown in Table. 4.2, the highest EB was 9.52% when the ratio of KGM:EU was 0.50%:0.50% (w/w), while the lowest EB value was 1.34% of pure KGM films at 0.75% (w/w). As refer above, EU might work as plasticizer which could interfere to the intermolecular interaction of KGM film and resulted in the increasing of EB. This phenomenon was similar to Pranoto et al. (2005), Rojas-Graü et al. (2007), and Benavides et al. (2012) reports. They concluded that the addition of essential oil resulted in greater movement of the film matrix and increased EB. Thus, the film expandability improved.

In this research, YM increased when KGM content increased but decreased when EU increased. The results were due to higher resistance of films when increasing KGM concentration and weaker interaction of EU that incorporated into the film (Pranoto et al., 2005; Zivanovic et al., 2005; Rojas-Graü et al., 2007; Xu et al.,

2007; Chen et al., 2008; Sánchez-González et al., 2009; Altiok et al., 2010; Benavides et al., 2012).

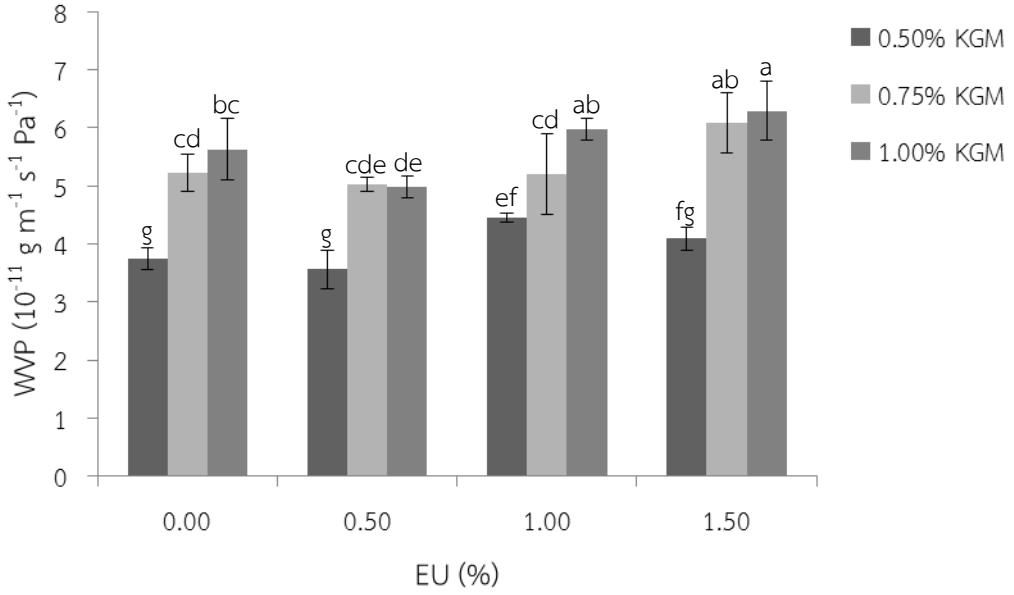
The mechanical properties in this study were different from KGM film in the research of Cheng et al. (2002), Lai et al. (2006), and Cheng et al. (2008). This was due to the greater plasticizing effect of polyol (glycerol and sorbitol) and PO compared to the plasticizing effect of EU in the film forming solution which reduced the rigidity and increased expandability. Therefore, TS and YM values were lower but EB values were higher. Moreover, the film obtained in this study had the mechanical properties close to other KGM-blended films (Cheng et al., 2002; Li et al., 2006; Cheng et al., 2007; Xu et al., 2007; Chen et al., 2008; Cheng et al., 2008; Jia et al., 2009; Nair et al., 2011).

Other polysaccharide films which incorporated with essential oils were investigated and assessed for TS, EB, and YM. The incorporation of garlic oil to alginate film reported TS and EB in the range of 38.67 MPa to 66.12 MPa and 2.73% to 4.05%, respectively (Pranoto et al., 2005). The addition of oregano and lemon grass oil to alginate apple puree films showed 2.47 MPa to 2.91 MPa of TS, 51.06% to 58.33% of EB, and 5.75 MPa to 6.86 MPa of YM (Rojas-Graü et al., 2007). Hydroxyproplymethylcellulose (HPMC) films incorporated with tea tree essential oil revealed 42 MPa to 59 MPa of TS, 0.09% to 0.11% of EB, and 0.96×10^{-3} MPa to 1.70×10^{-3} MPa of YM (Sánchez-González et al., 2009). Thyme oil was added to chitosan films, without the addition of glycerol, revealed TS, EB, and YM of the films in the range of 31.05 MPa to 51.20 MPa, 1.80% to 4.80%, and 19.32 MPa to 34.08 MPa, respectively (Altiok et al., 2010). The incorporation of cinnamon to chitosan films disclosed TS at 10.97 MPa to 29.23 MPa and EB at 3.58% to 24.73% (Ojagh et al., 2010). Oregano oil was added to alginate films which reported TS and EB in the range of 31.10 MPa to 71.00 MPa and 2.20% to 3.70%, respectively (Benavides et al., 2012). The wide range of mechanical properties values was due to the components of the film forming solution. In this study, pure KGM films and KGM/EU films revealed higher TS and YM but lower EB than reported in Rojas-Graü et al. (2007) research. It was due to high glycerol content (15% of film forming solution) added to alginate-apple puree film which made the film less resistance to rupture and greater flexibility. Furthermore, the films in recent study were flexible than the research of incorporation essential oil in HPMC and alginate film (Pranoto et al., 2005; Sánchez-

González et al., 2009; Benavides et al., 2012). It was due to the strong interaction of HPMC polymer and 3-dimension of alginate and calcium ion which resulted in film's high rigidity. However, pure KGM films and KGM/EU films in this study showed less TS and more EB than those study of chitosan film without adding plasticizer incorporated with thyme oil (Altiok et al., 2010). Thus, the pure KGM and KGM/EU in this study showed good potential application to use as packaging.

4.2.3 WVP

WVP is the barrier property of film against water vapor to pass through the films. WVP of pure KGM film and KGM/EU film were presented in Figure 4.1.



a, b, c, ... with different letters show a significant difference ($p \leq 0.05$).

Figure 4.1 WVP of KGM/EU films

The highest and lowest WVP values were 6.29×10^{-11} g m^{-1} s^{-1} Pa^{-1} and 3.56×10^{-11} g m^{-1} s^{-1} Pa^{-1}, respectively. The highest WVP was at KGM:EU of 1.00%:1.50% (w/w), which was the highest KGM and EU concentration used in this study. While the

lowest WVP was at KGM:EU of 0.50%:0.50% (w/w) which was the lowest KGM and EU concentration. WVP values of KGM/EU films at different ratio of KGM:EU were slightly different as shown in Figure 4.1.

WVP was significantly increased with increasing KGM and EU concentration. These results were probably due to hydrophilic property of KGM (Chen et al., 2008) and heterogeneous structure of the film that allowed water vapor to pass through easily. This finding agrees with the research of Pranoto et al. (2005). They reported that the WVP values increased after addition of 0.1% (v/v) to 0.4% (v/v) of garlic oil to 1% (w/v) of alginate-based edible film. Furthermore, the increasing of EU concentrations in KGM film increased WVP ($p \leq 0.05$). This is consistent with the research of Pranoto et al. (2005) and Altiok et al. (2010). The increasing of the essential oil concentration, such as 0.1% (v/v) to 0.4% (v/v) of garlic oil and 0.2% (v/v) to 1.2% (v/v) of thyme oil, might reduce the homogeneity of films and then increase WVP. Moreover, Cerqueira et al. (2010) disclosed that the hydrophobic properties of the extracts resulted in loss of intermolecular interactions of the structure matrix in the galactomannan films and support the increasing of WVP of the film. The adding of essential oil to the films had two effects. First, adding hydrophobic lipid to hydrophilic polymer film resulted in decrease WVP because hydrophobic property of lipid prevented polar molecule, such as water molecule, to pass through the film (Zivanovic et al., 2005; Rojas-Graü et al., 2007; Hosseini et al., 2009; Pelissari et al., 2009; Benavides et al., 2012). However, the addition of essential oil could increase WVP. It was due to the effect of hydrophobicity of oil which disturbed the microstructure of the emulsified film and contributed to extend intermolecular interactions of the film matrix and supported the passing of water molecule (Pranoto et al., 2005; Altiok et al., 2010; Cerqueira et al., 2010). Thus, WVP of KGM/EU films in this study resulted in the combination effect of the hydrophilic nature of KGM and the effect of EU.

The WVP of pure KGM films and KGM/EU films were higher than the research of Cheng et al. (2002) and Cheng et al. (2008) as shown in Table 2.1. WVP of pure KGM films and KGM-PO films were 1.37×10^{-11} g m^{-1} s^{-1} Pa^{-1} and 2.15×10^{-11} g m^{-1} s^{-1} Pa^{-1}, respectively. It might due to high porosity of the film which resulted from the inability to degas the highly viscous film forming solution and hence the water vapor molecule could diffuse though the matrix easier. However, WVP of pure KGM films

and KGM/EU films were lower than other essential oil added polysaccharide films. Pranoto et al. (2005) found WVP of alginate films incorporated with garlic oil in the range of 21.67 × 10^{-11} g m^{-1} s^{-1} Pa^{-1} to 35.75 × 10^{-11} g m^{-1} s^{-1} Pa^{-1}. Rojas-Graü et al. (2007) studied alginate-apple puree films incorporated with oregano and lemongrass oil and found WVP in the range of 121.40 × 10^{-11} g m^{-1} s^{-1} Pa^{-1} to 145.90× 10^{-11} g m^{-1} s^{-1} Pa^{-1}. Sánchez-González et al. (2009) revealed WVP of HPMC films incorporated with tea tree essential oil in the range of 50.00 × 10^{-11} g m^{-1} s^{-1} Pa^{-1} to 85.00 × 10^{-11} g m^{-1} s^{-1} Pa^{-1}. Chitosan films incorporated with thyme oil (without plasticizer added) of Altiok et al. (2010) showed WVP at 16.24 × 10^{-11} g m^{-1} s^{-1} Pa^{-1} to 20.85 × 10^{-11} g m^{-1} s^{-1} Pa^{-1}. Cerqueira et al. (2010) indicated WVP in the range of 5.02 × 10^{-11} g m^{-1} s^{-1} Pa^{-1} to 12.05 × 10^{-11} g m^{-1} s^{-1} Pa^{-1} of galactomannan films incorporated with seed extract. Ojagh et al. (2010) studied WVP of chitosan films incorporated with cinnamon oil and which were in the range of 10.03 × 10^{-11} g m^{-1} s^{-1} Pa^{-1} to 22.50 × 10^{-11} g m^{-1} s^{-1} Pa^{-1}. Benavides et al. (2012) presented WVP of alginate films incorporated with oregano oil at 270.00 × 10^{-11} g m^{-1} s^{-1} Pa^{-1} to 310.00 × 10^{-11} g m^{-1} s^{-1} Pa^{-1}. In most casted edible film, plasticizer was normally added to reduce rigidity in order to peel off easily. It might be the effect of hygroscopic and the structure interference of those plasticizers that promoted the water molecules to pass through the matrix and resulted in greater WVP. Thus, pure KGM films and KGM/EU films showed good barrier properties for the application as water barrier packaging film.

4.2.4 Transparency

Transparency of pure KGM films and KGM/EU films were displayed in Table 4.3. The transparency of the films relates to the appearance of the films and is important for its application. In this study the wavelength at 600 nm was used to determine the light transmission ability of the films. KGM:EU at 0.50%:0.00% (w/w) was the film formula which had the highest film transparency value. In addition, observation in the difference of components, pure KGM films were more transparent than KGM/EU films (Table 4.3) at the same KGM concentration and reached the least transparency at 1.00%:1.50% of KGM:EU.

With respect of pure KGM films, higher KGM concentration resulted in the reduction of film transparency. It was probably due to the increasing of KGM content

resulted in more heterogeneity of film matrix. Thus, the film with higher KGM concentration revealed low transparency.

Table 4.3 T_{600} (%) and transparency of pure KGM films and KGM/EU films

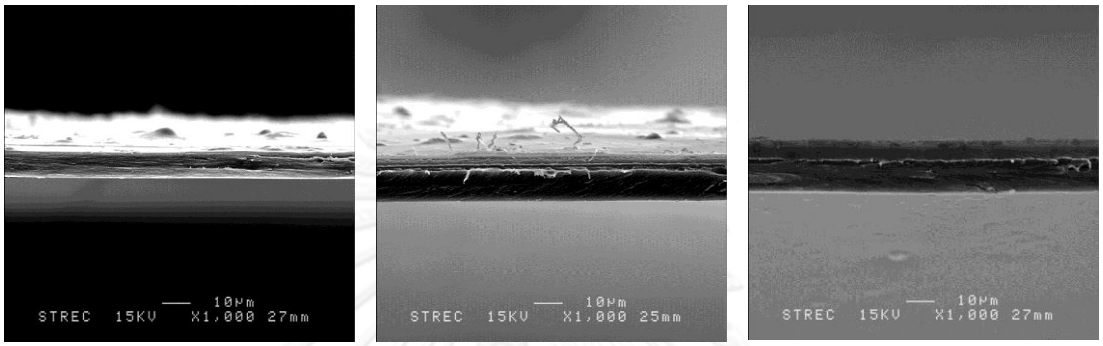
KGM (%, w/w)	EU (%, w/w)	$\%T_{600}$*	Transparency*
0.50	0.00	$88.57^{a} \pm 0.23$	$77.10^{a} \pm 7.26$
0.75	0.00	$88.42^{a} \pm 0.22$	$56.29^{b} \pm 7.44$
1.00	0.00	$87.56^{a} \pm 0.72$	$50.13^{bc} \pm 5.06$
0.50	0.50	$76.21^{cd} \pm 1.98$	$73.10^{a} \pm 2.40$
0.50	1.00	$62.16^{f} \pm 8.15$	$49.37^{bc} \pm 7.71$
0.50	1.50	$67.37^{ef} \pm 6.29$	$55.97^{b} \pm 6.70$
0.75	0.50	$82.00^{bc} \pm 3.17$	$55.17^{b} \pm 7.86$
0.75	1.00	$76.66^{c} \pm 2.52$	$44.11^{bcd} \pm 3.15$
0.75	1.50	$69.76^{e} \pm 2.11$	$41.67^{cd} \pm 5.52$
1.00	0.50	$76.98^{c} \pm 2.18$	$40.77^{cd} \pm 1.22$
1.00	1.00	$79.08^{c} \pm 0.89$	$42.54^{cd} \pm 2.94$
1.00	1.50	$70.28^{de} \pm 3.77$	$33.87^{d} \pm 3.14$

*Each value presents the mean±SD. Different superscript letters in the same column show a significant difference ($p \leq 0.05$).

The results also suggested that films' transparency decreased with encapsulation of EU, and the lowest transparency was found to be the ratio of KGM:EU at 1.00%:1.50% (w/w). Sánchez-González et al. (2009) reported that HPMC films incorporated with tea tree essential oil more than 1% were opaque than pure HPMC film. They concluded that it was due to the effect of lipid droplet (with different refractive index) which affected the light scattering of the film. Higher oil concentration developed more light scattering intensity. Moreover, the results were also resembled to the work of Wang et al. (2013) who disclosed the addition of tea polyphenol to chitosan films which made the films less transparent. Thus, it could be concluded that changes in transparency of the films was due to the difference of refractive index of KGM matrix and EU oil droplets.

4.2.5 SEM

Scanning electron photographs of cross-sections of KGM/EU films are shown in Figure 4.2 and 4.3.



a b c

Figure 4.2 SEM photographs of pure KGM films at the ratio of KGM%:EU%: 0.50:0.00(a), 0.75:0.00(b), 1.00:0.00(c)

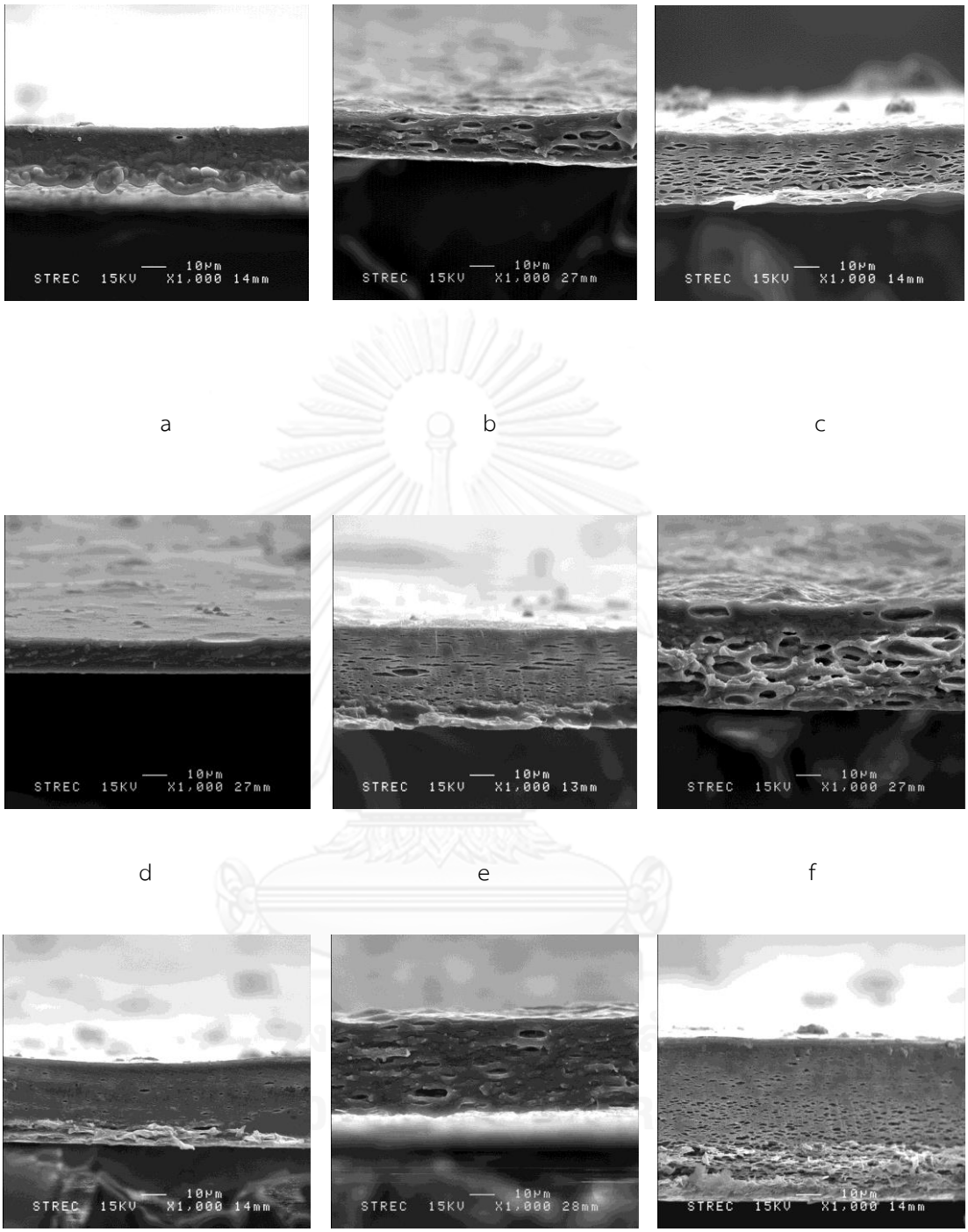


Figure 4.3 SEM photographs of KGM/EU films at different ratio of KGM%:EU%: 0.50:0.50(a), 0.50:1.00(b), 0.50:1.50(c), 0.75:0.50(d), 0.75:1.00(e), 0.75:1.50(f), 1.00:0.50(g), 1.00:1.00(h), 1.00:1.50(i)

The photographs of pure KGM films showed compact and stack-liked structure of the film (Figure 4.2). This stack layer structure was consistent with the report of Cheng et al. (2002) who studied the cross-sections of pure KGM film samples. They found that the presence of the layer structure might be due to the recrystallization of mannan I as shown in Figure 2.3(a). According to Chanzy et al. (1982) who studied polymorphs of recrystallized glucomannan of non-treated KGM solution and alkaline deacethylated KGM solution, mannan I was common polymorph which formed stack-liked matrix which parallel to the film surface, while mannan II was the polymorph of glucomannan when treated with alkali, which formed perpendicular matrix to the film surface. In this study, the KGM solutions were not treated by alkaline solution. Thus, the cross-section of the pure KGM film presented the compact and stack-liked structure.

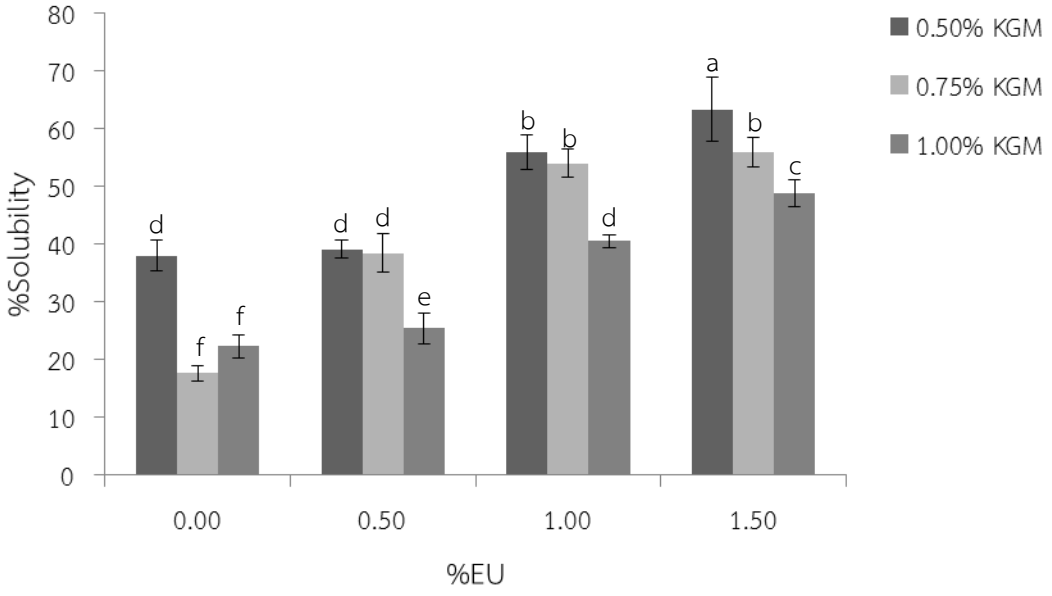
The cross-section of KGM/EU films are presented in Figure 4.3. The films were thicker and less homogeneous than pure KGM films (Figure 4.2). There were many pores inside the stack layer structure. This is similar to the study of Cheng et al. (2008) who found that KGM-PO film was heterogeneous than pure KGM film of Cheng et al. (2002), due to low miscibility of KGM and PO. Sánchez-González et al. (2009) also found the discontinuous structure when adding tea tree essential oil into HPMC film. The presence of tea tree essential oil caused the discontinuous structure because there was no interaction between two components. The films were separated into two phases: lipid droplets embedded in a continuous polymer network. Moreover, the essential oil which might evaporate during drying step that caused many pores in film matrix and increased the heterogeneity of film (Ahmad et al., 2012).

From SEM photographs, KGM/EU films were more heterogeneous with loose structure. The cavities which occurred in the KGM/EU films allowed the water vapor to pass through. Furthermore, the heterogeneity decreased TS, YS and EB of the film.

4.2.6 Solubility

The water solubility of film is the measurement of the resistance of film to water (Giménez et al., 2013). In this study, the film was shaken in distilled water for 10 minutes. The non-soluble film fragments were separated and dried. From Figure 4.4, the highest and lowest solubility were 17.65% (w/w) and 63.30% (w/w), respectively. The pure KGM film at KGM concentration 0.75% (w/w) showed the lowest solubility which was not significantly different from the solubility of KGM concentration 1.00% (w/w). The soluble matter was higher with more EU concentration and reached the maximum solubility at 0.50%:1.50% (w/w) of KGM:EU .

With respect to the concentration of KGM, higher KGM concentration resulted in less solubility of film. This phenomenon related to the high inter-molecular H-bond of KGM molecules which was the strong interaction and difficult to destroy (Chen et al., 2008). Thus, with higher KGM concentration, the film is less soluble.



a, b, c, ... with different letters show a significant difference ($p \leq 0.05$).

Figure 4.4 Solubility of KGM/EU films

The film became more soluble with the increasing concentration of EU added. From SEM photographs in section 4.2.5, the structure of KGM/EU films (Figure 4.3) was more heterogeneous than pure KGM films (Figure 4.2) because of the disturbance of EU to the films matrix. Thus, it might promote the solubility of the films in water. This finding agrees with the study of Wang et al. (2013) that the solubility of chitosan film increased when the films were incorporated with tea polyphenols. Pires et al. (2013) reported that adding essential oil (clove and thyme oil) increased the solubility of gelatin-chitosan film. Adding higher EU concentration made solubility of KGM film higher. This is agree with the research of Khoshgozaran-Abras et al. (2012). They disclosed that the solubility of chitosan films increased when adding *Aroe vera* gel and increased with increasing *A. vera* gel content. Moreover, this phenomenon agree with the study of Mahmoud and Savello (1993) which reported that higher amount of glycerol increased film solubility because of the effect of plasticizer which decreased polymer interaction in film matrix. Thus, EU might work as plasticizer which could disturb film structure. It resulted in weaker interaction between KGM molecules which was easier to be destroyed and solubility of the film increased. From the results, KGM/EU films were easy to soluble in water within in 10 minutes. Thus, this film might be suitable to carry the active compounds and release in water medium.

The solubility of pure KGM films and KGM/EU films in this study were higher than chitosan films incorporated with 0.4% - 2% (v/v) of cinnamon essential oil (Ojagh et al., 2010). In their study, the addition of essential oil increased the solubility of film in the range of 10.40% to 23.20%. The lower film solubility in their study is due to the crosslinking of chitosan and essential oil, while there was no interaction between KGM and EU. Moreover, in this study, the film was shaken at high speed (250 rpm). Thus, KGM/EU films revealed higher solubility in water.

4.3 Effects of EU encapsulation to film structure

4.3.1 FT-IR

IR spectra of the film was analyzed at the wavenumber range of 4000 – 400 cm^{-1}. This technique is used to study the interaction in the structure of the film (Li et al., 2006). The FT-IR spectra of pure KGM films and KGM/EU films are shown Figure 4.5 – 4.8.

IR spectra of the pure KGM films at different concentration was shown in Figure 4.5 and A.6-A.8. In all concentration of KGM, the spectra showed broad absorption band at 3428.00 – 3433.96 cm^{-1} which was due to the complex stretch vibration associated with free inter-molecular bonding hydroxyl group (Krochta and De Mulder-Johnston, 1997; Korkiatithaweechai and Umsarika, 2009). This supported the high strength of the film. KGM molecules in the film interacted together by hydrogen bond. There were other bands which also indicated the KGM structure. They were stretching peak of intra-molecular bonding of hydroxyl group at 1637.66 – 1638.75 cm^{-1} (Krochta and De Mulder-Johnston, 1997), Moreover, the peaks at 807 cm^{-1} and 894 cm^{-1}, 2890 and 2922 cm^{-1}, and 1731 cm^{-1} were also found which were similar to the characteristic absorption peaks of the mannose at 807 cm^{-1} and 892 cm^{-1}, the stretching peak of the C-H of methyl at 2898 cm^{-1} and 2925 cm^{-1}, and the carbonyl group at 1727 cm^{-1} of the KGM films in the research of Li et al. (2006), respectively.

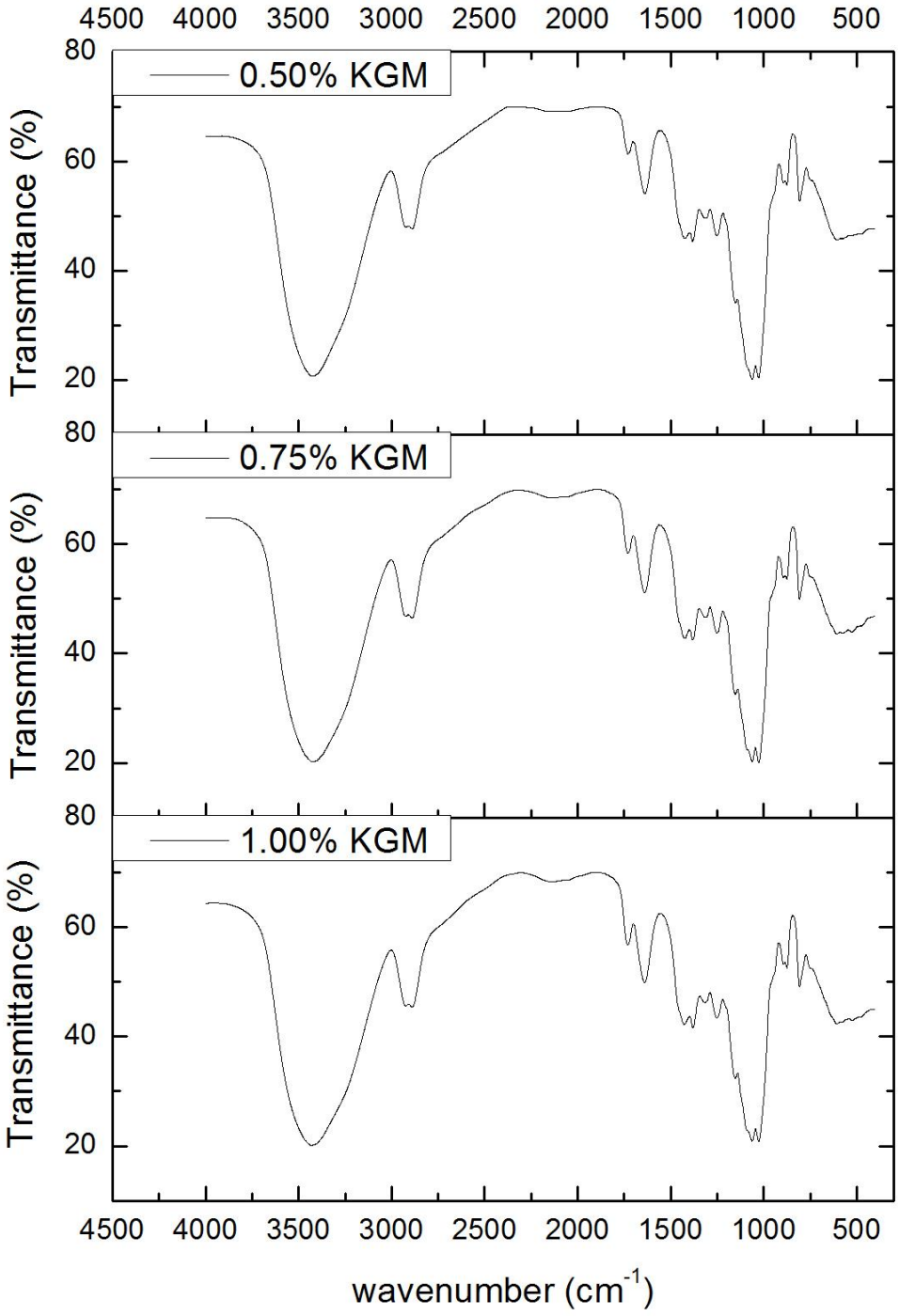


Figure 4.5 FT-IR spectra of pure KGM films

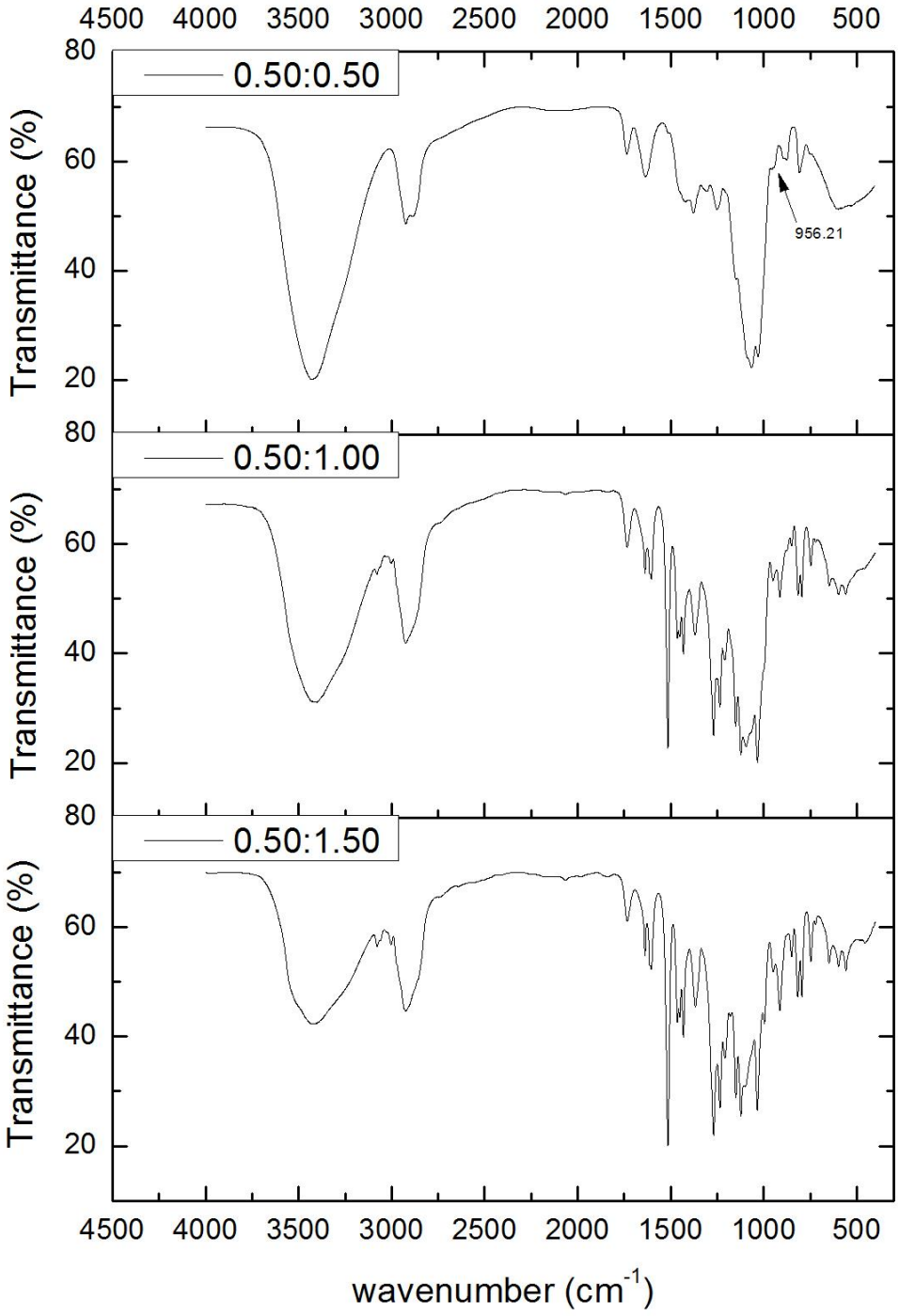


Figure 4.6 FT-IR spectra of 0.50% KGM films with 0.50%, 1.00%, and 1.50% EU

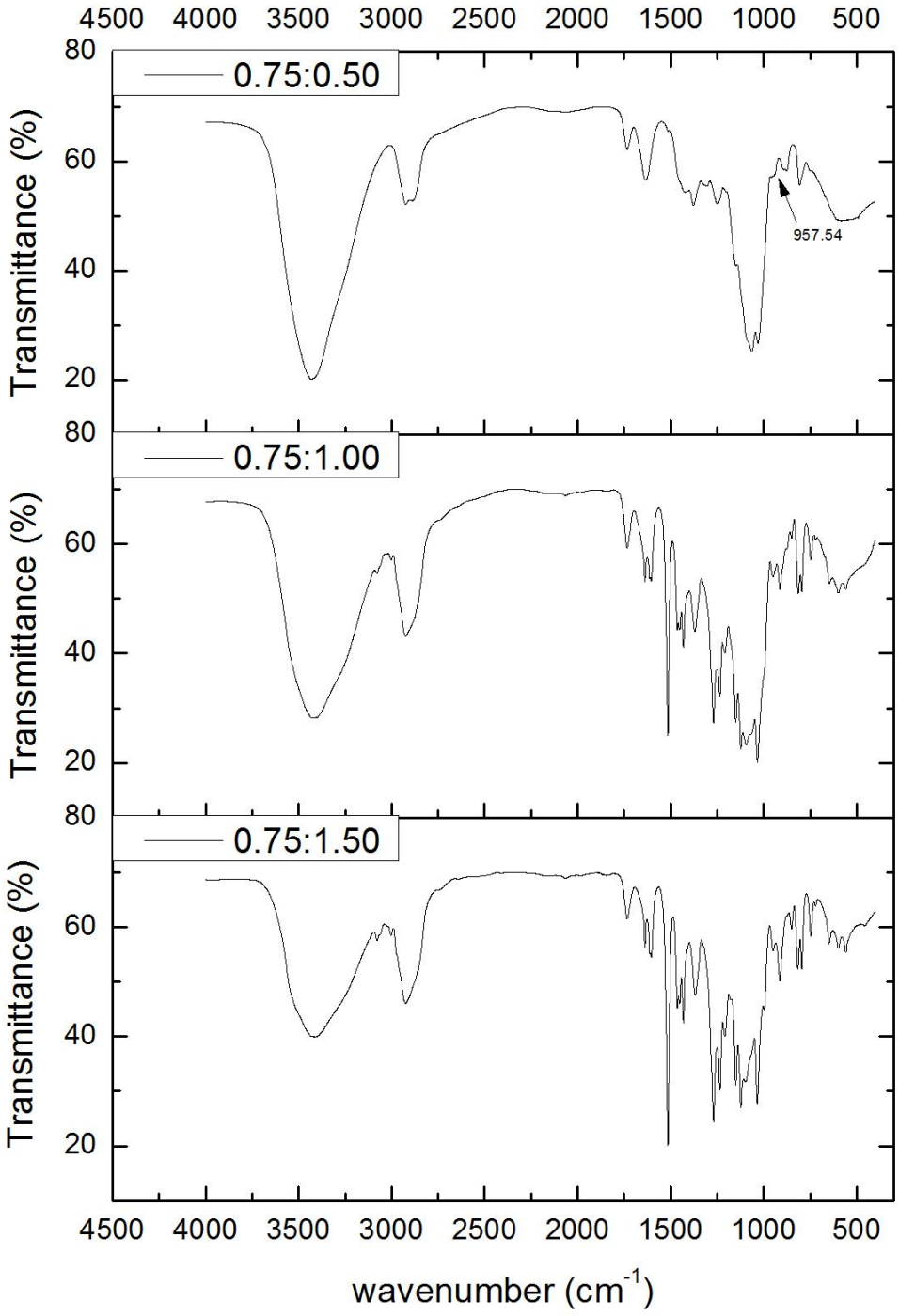


Figure 4.7 FT-IR spectra of 0.75% KGM films with 0.50%, 1.00%, and 1.50% EU

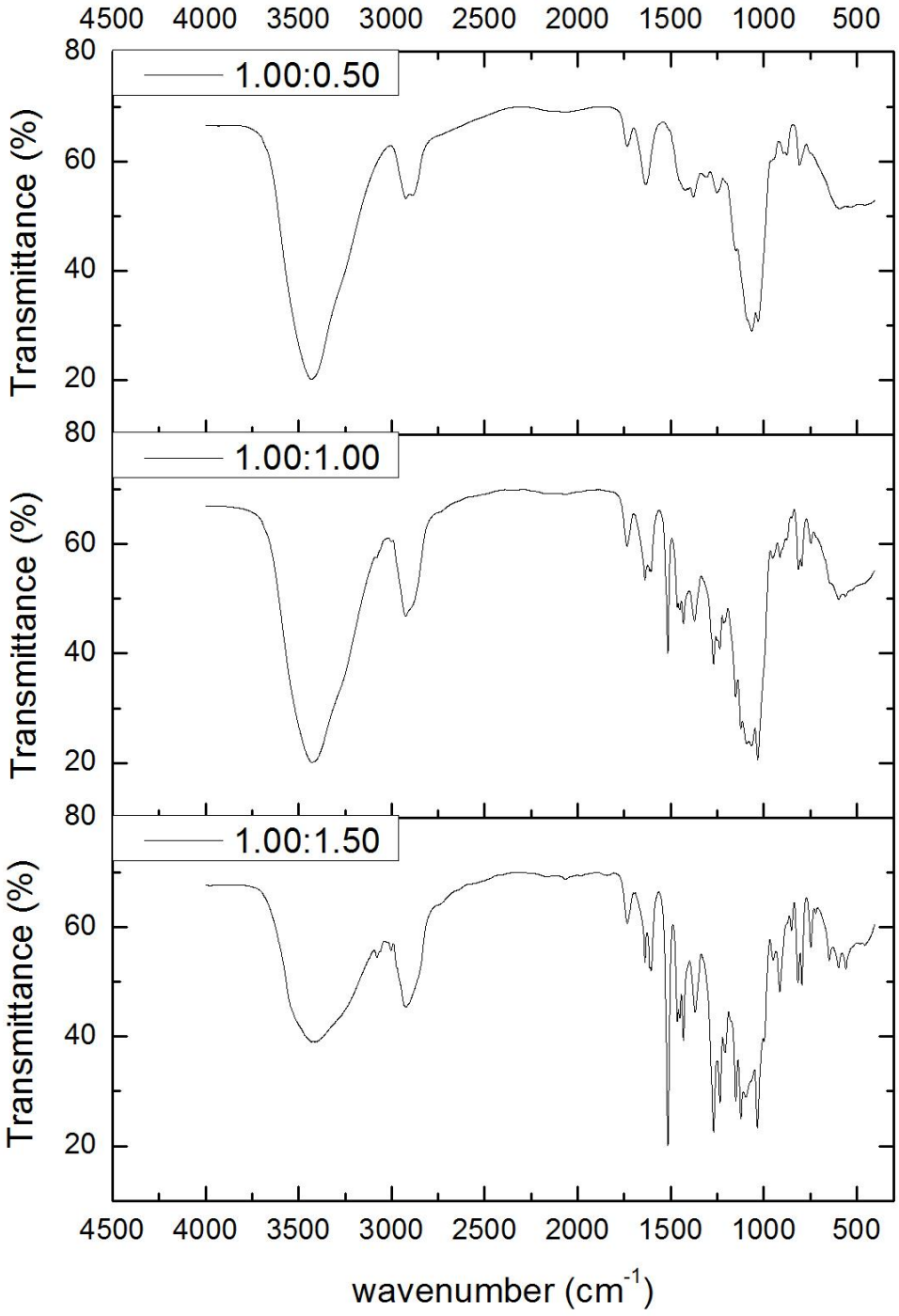


Figure 4.8 FT-IR spectra of 1.00% KGM films with 0.50%, 1.00%, and 1.50% EU

Nuchuchua et al. (2009) reported that the signature peaks of EU were in 720 – 1250 cm^{-1} which presented the C=C region and 1640, 1610, and 1510 cm^{-1} which presented C=C stretching of aromatic moiety of EU molecule. The FT-IR spectra of KGM/EU films were shown in Figure 4.6-4.8 and A.9-A.17. The FT-IR spectra of KGM films encapsulated with the lowest concentration of EU (0.50% w/w) presented an absorption peak at 956.21 – 957.54 cm^{-1}, which was different from the spectra of the pure KGM film (except at 1.00% KGM concentration). The absorption band at this region is in the signature peak of EU. Therefore, the results presented that there was EU encapsulated in KGM film. Moreover, FT-IR spectra of higher EU concentration (1.00% and 1.50% w/w of EU) in the KGM film presented many peaks at 720.82 – 1269.78 cm^{-1} which was in the region of the signature band of EU and C=C stretching of aromatic moiety (Dhoot et al., 2009; Nuchuchua et al., 2009; Woranuch and Yoksan, 2013a). The intensity of EU peak of the films containing 1.50% (w/w) EU was more than the films containing 1.00% (w/w) EU (as shown in Figure 4.6(b-c), 4.7(b-c), and 4.8(b-c)). Thus, by observation, there was more amount of EU in the film which had higher initial EU concentration.

The pattern of the peak of FT-IR spectra of the KGM/EU films which presented in Figure 4.6-4.8 were similar to that presented normally in FT-IR peak of pure KGM films (Figure 4.5) and pure EU (Figure 2.7b). It confirmed the presence of EU in KGM film matrix. In addition, no peak of new functional group was found. The results were consistent with the observation of Nuchuchua et al. (2009) and Korkiatithaweechai et al. (2011) which concluded that the two components did not react with each other because no peak of new functional group was found.

As in Figure 4.6-4.8, the FT-IR spectra with higher EU concentration showed the decreasing of the sharpness of the peak at 3428 cm^{-1} which attributed to the OH-stretching in KGM matrices (Xu et al., 2007). It was due to the decreasing of homogeneous structure of the film when incorporated with increasing concentration of EU.

4.3.2 DSC

The thermal properties of KGM/EU films were investigated by DCS analysis. This technique can be used to study the structure or crystallinity of the matrix (Li et al., 2006; Xu et al., 2007). The DSC data of the KGM/EU films were shown in Table 4.4. The thermal properties are presented as onset of temperature, peak temperature and enthalpy.

Table 4.4 DSC data of pure KGM films and KGM/EU films

%KGM	%EU	Temperature (°C)		Ehthalpy*[A] (J/g)
		Onset*	Peak*	
0.50	0.00	290.75[a] ± 2.05	303.30[c] ± 1.70	92.05[ab] ± 10.21
0.75	0.00	291.20[a] ± 0.57	303.90[bc] ± 0.28	87.15[bc] ± 4.66
1.00	0.00	247.65[d] ± 0.21	311.60[a] ± 0.57	104.18[a] ± 15.45
0.50	0.50	248.85[d] ± 1.20	278.95[c] ± 0.92	56.42[d] ± 8.11
0.50	1.00	285.75[ab] ± 2.33	305.05[bc] ± 0.35	-71.77[f] ± 8.61
0.50	1.50	284.25[b] ± 0.92	307.05[bc] ± 1.34	-135.25[i] ± 3.32
0.75	0.50	250.80[d] ± 0.14	279.00[c] ± 1.27	73.00[cd] ± 7.69
0.75	1.00	283.70[b] ± 4.38	304.40[bc] ± 0.71	-78.38[fg] ± 4.75
0.75	1.50	281.15[bc] ± 1.06	306.45[b] ± 1.06	-94.75[g] ± 2.24
1.00	0.50	250.50[d] ± 2.12	278.50[c] ± 3.82	80.28[bc] ± 6.46
1.00	1.00	277.25[c] ± 0.35	298.60[d] ± 1.56	-51.14[e] ± 7.91
1.00	1.50	283.25[bc] ± 7.99	306.00[bc] ± 2.83	-105.50[h] ± 0.42

*Each value presents the mean±SD. Different superscript letters in the same column show a significant difference ($p \leq 0.05$).

[A] The minus values show endothermic peak

From Table 4.4, pure KGM film showed onset temperature, peak temperature, and enthalpy in the range of 247.65°C to 291.20°C, 303.30°C to 311.60°C, and 87.15 J/g to 104.18 J/g, respectively. The higher KGM concentration revealed greater peak

temperature. Onset temperature was higher when KGM concentration increased from 0.50% to 0.75% (w/w) KGM and decreased at 1.50% (w/w) KGM. While enthalpy slightly increased. It was noted that the incorporation of EU in the film matrix slightly changed the thermal properties of the film. The enthalpy of pure KGM film showed the exothermic thermographs, while they became lower when incorporated with 0.50% (w/w) EU and changed to endothermic thermograph at the concentration of EU 1.00% (w/w) and 1.50% (w/w).

The pure KGM film showed the exothermic peak temperature at 303.30 – 311.60°C. This temperature range of DSC peak was due to the thermal degradation of KGM (Li et al., 2006; Xu et al., 2007). As mention previous, the acetyl group in KGM resulted in highly amorphous with low crystallinity of the film structure (Cheng et al., 2002), hence the observed KGM thermograms had only degradation temperature (T_d) and degradation enthalpy (ΔH_d). Peak temperature and enthalpy were slightly higher. It might be due to greater hydrogen bond interaction in the film matrix with higher KGM concentration (Chen et al., 2008).

The addition of EU resulted in lower the onset temperature or peak temperature compared with pure KGM film. It might relate to the less homogeneous structure of film when incorporated with EU which may work as plasticizer (section 4.2.2). This phenomenon was similar to the research of Chen et al. (2008). They pointed out that the decreasing of crystallinity structure of pea starch when blending with KGM resulted in decreasing peak temperature of DSC curve. The increasing of EU concentration resulted in higher onset and peak temperature. However, KGM/EU films at EU concentration 1.50% (w/w) presented the onset temperature at 281 – 285 °C which attributed to the onset temperature of boiling point of free EU in films (Choi et al., 2009). The decreasing of onset temperature of KGM/EU film, when compared with pure KGM films, supported the FT-IR results that KGM and EU did not produce new bond.

DSC thermograph of KGM/EU films at EU concentration 0.50% (w/w) presented the exothermic peak and gradually changed to endothermic peak at EU concentration 1.00% and 1.50% (w/w), Choi et al. (2009) reported the endothermic peak of pure EU at 282.8°C. These might be due to the disturbance of EU when

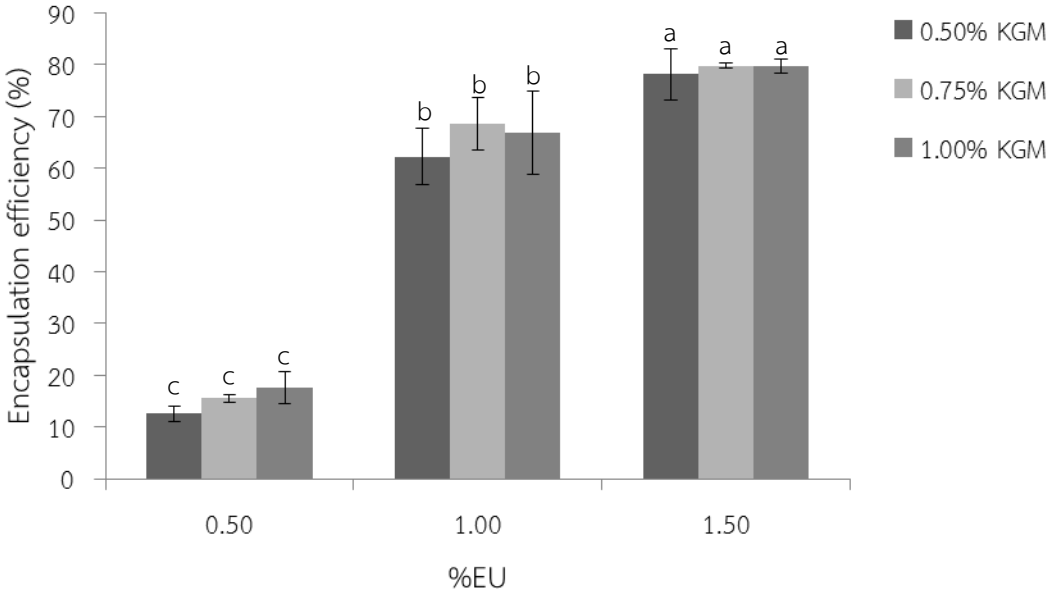
incorporated to KGM matrix. The peak gradually changed from exothermic peak to endothermic peak with increasing EU added. The greater endothermic enthalpy of KGM film with 1.00% (w/w) and 1.50% (w/w) of EU from the range of 51.14 J g^{-1} - 71.77 J g^{-1} to 94.75 J g^{-1} – 135.25 J g^{-1} as shown in Table 4.4. respectively, indicated higher EU being entrapped in the matrix.

From results above, the increase of EU concentration added in KGM film developed the heterogeneity of film matrix. Thus, it could support the increasing of thickness, EB, WVP, opacity and solubility while TS and YM decreased.

4.4 Encapsulation efficiency (EE) of EU in KGM film

EE was used to reveal the amount of active compounds which could be entrapped in coating materials. Figure 4.9 presents the information of EE (%) of KGM/EU films. The film samples were digested by 2.5N HCl for releasing EU encapsulated inside the matrix. The amount of EU which released out was determined by measuring the absorbance at 283 nm which was the maximum absorbance of EU (Woranuch and Yoksan, 2013a, b). EE (%) was calculated by comparing with initial EU concentration of each film formula.

The highest and lowest EE (%) were 79.78 (KGM:EU 0.75%:1.50%, w/w) and 12.62 (KGM:EU 0.50%:0.50%, w/w), respectively. In addition, EU at concentration 0.50%, 1.00%, and 1.50% (w/w) could be encapsulated in 0.50%, 0.75%, and 1.00% (w/w) KGM film. From a statistical point of view, there was difference of EE between different initial concentration of EU added, (p<0.05) but no significantly different between KGM concentration. This result agrees with the research of Korkiatithaweechai et al. (2011), Wang et al. (2013), Woranuch and Yoksan (2013a) and Woranuch and Yoksan (2013b). They disclosed that increasing initial EU concentration tended to increase EE in the range of investigation.



a, b, c, ... with different letters show a significant difference ($p \leq 0.05$).

Figure 4.9 EE (%) of KGM/EU films

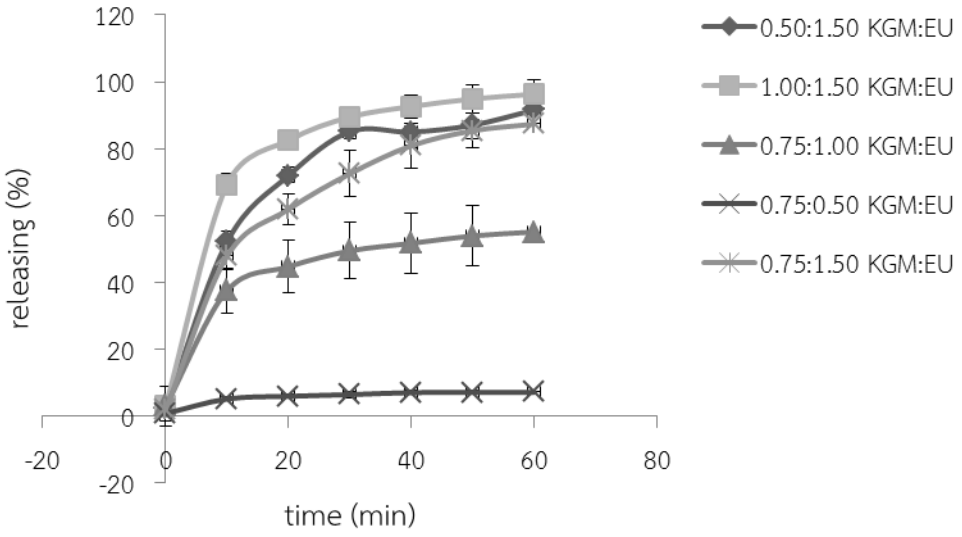
The physical and mechanical properties of KGM/EU film showed good film strength and easy to dissolve. The highest EE was at the ratio of KGM:EU at 0.75%:1.50% (w/w). Thus, this film formula was chosen for studying the releasing of EU from KGM film and the effect of storage time to EU in KGM/EU film.

4.5 Releasing of EU from KGM/EU film

The releasing of EU from KGM/EU films were measured by shaking pieces of films in distilled water at 250 rpm in order to study the fastest releasing ability. At every 10 minutes for 60 minutes, the solution was sampling to measure the releasing amount of EU in water. The releasing EU in water was determined at the absorbance 283.5 nm (Woranuch and Yoksan, 2013a, b). From EE results, the film formula 0.75% (w/w) KGM with 1.50% (w/w) EU was chosen to study the releasing of EU because it had the highest EE (%) (Figure 4.10). Other film formula, ratio of KGM:EU 0.75%:0.50%, 0.75%:1.00%, 0.50%:1.50%, and 1.00%:1.50% (w/w), were chosen to compare the

releasing ability of different concentration of KGM and EU in the film. The releasing of EU is presented in Figure 4.10.

As shown in Figure 4.10, the highest releasing (%) was at EU concentration 1.50% (w/w), and followed by 1.00% (w/w) and 0.50% (w/w). The highest and lowest EU releasing (%) were in the film formula at the ratio of KGM:EU 1.00%:1.50% and 0.75%:0.50% (w/w), respectively. In addition EU in KGM/EU film could release out from the film more than 46.52% of EE. The releasing reached the equilibrium in 20 - 30 minutes in every film formula.



a, b, c, ... with different letters show a significant difference ($p \leq 0.05$).

Figure 4.10 Releasing (%) of EU from KGM/EU films

The greatest EU releasing value (%) was 96.36% with 1.00% KGM film encapsulated with 1.50% EU, even though the solubility of this film was lower than other KGM film at different concentration (0.75% and 0.50%, w/w) with the same EU concentration (1.5%, w/w) as shown in section 4.2.6. It might be due to high porosity of the film matrix which promoted EU to release out from the matrix easier than 0.75% (w/w) and 0.50% (w/w) of KGM. Moreover, the releasing of EU in this study was higher when comparing with other research, such as the releasing of diclofenac from

chitosan-oxidized KGM (Korkiatithaweechai et al., 2011). The studies of Arora et al. (1991) who studied the encapsulation of compounds by low density polyethylene film disclosed that aroma compound could retain more in the matrix which had the similar polarity. In this study, EU was non-polar compound while KGM was polar compound. SEM photographs (section 4.2.5), FT-IR spectra (section 4.3.1), and DSC analysis (section 4.3.2) also supported that EU did not interact with KGM and made the film was heterogeneous structure. Therefore, EU could easily release out of KGM film. Thus, this film might be suitable for the application which preferred the high releasing ability of active compounds such as the encapsulation of foods and additives (Bertuzzi et al., 2007).

4.6 Effect of storage time to EU in KGM/EU films

4.6.1 Thermal stability of the films during storage

TGA is used for analyzing the change of mass of the sample as a function of temperature at constant condition (Woranuch and Yoksan, 2013a). TGA analysis is presented in thermogram (TG) and derivative thermogram (DTG). TG displays the weight loss (%) at different temperature, while DTG is the graph of the changing of mass as a function of time at different temperature. Figure 4.11 showed TG curves of KGM/EU films at the ratio of KGM:EU 0.75%:1.50% (w/w) when using N_2 as a purge gas to determine thermal stability of KGM/EU film during storage for 60 days.

Figure 4.11a was TG curve of pure KGM. It was not much different from KGM/EU films (Figure 4.11b-e). TG curves can be divided into 3 parts according to temperature range. The first part of temperature, approximately in the range of 30°C to 170°C, was an evaporation of water in the film. The second part, approximately in the range of 170°C to 370°C, might be due to the decomposition of EU and KGM because it was the maximum weight loss rate which can be defined as the decomposition temperature (Woranuch and Yoksan, 2013a). This supported the result of FT-IR and DSC that no interaction between KGM and EU to form new bond. The last part, approximately in the range of 370°C to 600°C, was probably due to a decomposition of the remaining KGM.

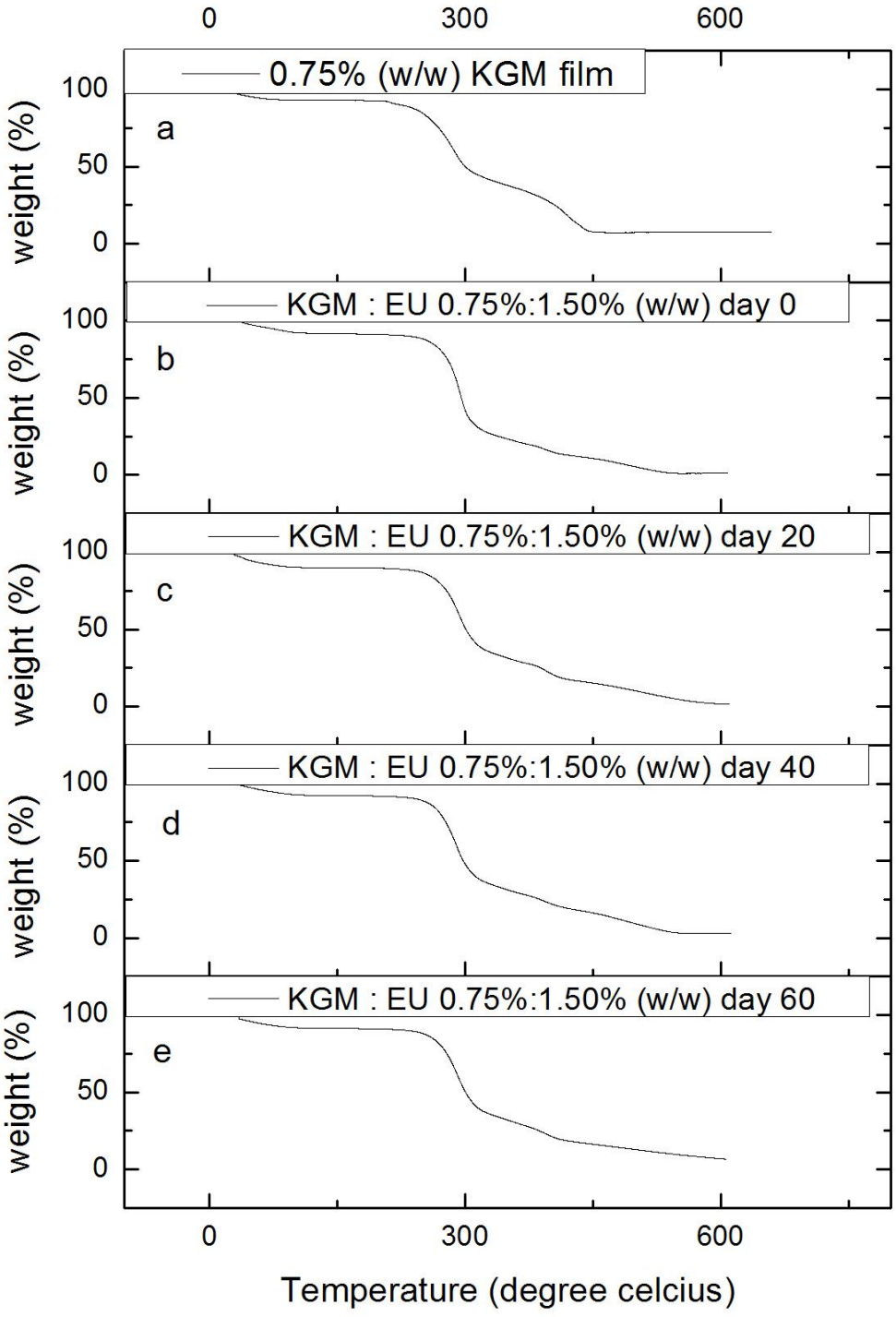


Figure 4.11 TG curves of pure KGM films and KGM/EU films using N_2 as purge gas

As shown in Figure 4.11, TG curves showed similar pattern of mass loss at day 0, 20, 40, and 60. It should be pointed out that the onset temperature of KGM/EU film was not lower. This implied the good stability of the film structure during storage for 60 days. Although, the structure of KGM/EU films did not change, there was changing of color during storage for 8 months. The film became more brownish in color. It revealed by the changing of *L** from 91.81 to 91.26., *a** from -0.71 to -0.18, and *b** from 4.55 to 9.00. This change in color agrees with the studies which reported that EU (light yellow) become darker after storage because it changed to EU quinone methide (Suzuki et al., 1985; Thompson et al., 1989; Thompson et al., 1993; Jeng et al., 1994; Thompson et al., 1995; Thompson et al., 1998). Thus, KGM/EU films indicated high stability of structure after storage which supported for the potential application as edible film even though the changing of EU color in the film might limit their application.

4.6.2 EE of EU in KGM/EU films during storage

EE of EU in KGM films in this part was determined after storage the film 0.75% KGM with 1.50% EU for 8 months. The determination method was the same as in experiment 3.2.4. The film was digested by 2.5M HCl and the amount of EU was determined by measuring the absorbance of EU at 283 nm. EE (%) of EU in KGM film in day 0 was the same as EE (%) of 0.75% (w/w) KGM with 1.50% (w/w) EU film formula in section 4.3. EE (%) of EU in KGM/EU film during storage for 8 months were showed in Table 4.5.

Table 4.5 EE of EU encapsulated in KGM films at 0 month and after storage for 8 months

Time (month)	EE remaining (%)	EU lost (%)
0	79.78^{a} ±0.55	-
7	52.46^{b} ±1.60	34.24
8	50.34^{b} ±1.47	36.90

*Each value presents the mean±SD. Different superscript letters in the same column show a significant difference ($p \leq 0.05$).

During 8 months, EE (%) decreased 36.90% from initial EE (79.78%). Comparing with EE of EU encapsulated in edible film of (Woranuch and Yoksan (2013b)) which added 0.35% (w/w) and 0.70% (w/w) EU to thermoplastic flour, they could not find any EU remained in the films after drying the film. This indicated the ability of KGM film to encapsulate EU and protect them from the evaporation to the environment during storage for at least 8 months.

CHAPTER V

CONCLUSIONS AND SUGGESTIONS

The results of this study suggested that incorporation of EU into KGM films had the potential for using as an edible film for carrying EU in the matrix. KGM/EU films had high mechanical strength. Films with higher KGM content were stronger but less expandable, due to higher intermolecular hydrogen bond in the matrices. The addition of EU to the films reduced film's strength and increased expandability. The SEM photographs showed less homogeneous film matrix with increasing EU concentration. As indicated by FT-IR and DSC, KGM and EU did not interact with each other. This affected on WVP and solubility of the films, they were higher when adding and increasing EU concentration to KGM films. The EE (%) and releasing (%) of EU of KGM/EU films increased with increasing EU concentration. The films prepared from 0.75% (w/w) KGM and 1.50% (w/w) EU had the highest EE and high solubility. Therefore, this film was used to study the thermal stability and EE (%) of EU entrapped in KGM films during storage for 8 months. It was found that the structure of the films did not change but the color became darker after 60 days storage. After storage for 8 months, 36.90% of EU was lost. Therefore, KGM films were efficient in preventing the evaporation of EU. Thus, the film prepared from 0.75% (w/w) KGM incorporated with 1.50% (w/w) EU was the best formula and had potential to be used as an active packaging.

Suggestions

The EE of EU in the films depended on the initial EU concentration added. Therefore, the future research should determine the higher EU concentration which can be entrapped in KGM films.

The releasing of EU from KGM/EU films reached the constant rate rapidly. If slow releasing film is desired, the films may be coated or blended with other material to reduce the releasing rate and reduce the chance to contact with environment.

Furthermore, the obtained EU encapsulated films should be investigated for other properties of EU such as antimicrobial and antioxidant properties.

REFERENCES

Ahmad, M., S. Benjakul, T. Prodpran, and T. W. Agustini. 2012. Physico-mechanical and antimicrobial properties of gelatin film from the skin of unicorn leatherjacket incorporated with essential oils. Food Hydrocolloids 28: 189-199.

Altiok, D., E. Altiok, and F. Tihminlioglu. 2010. Physical, antibacterial and antioxidant properties of chitosan films incorporated with thyme oil for potential wound healing applications. Journal of Materials Science. Materials in Medicine 21: 2227-2236.

Arcan, I., and A. Yemenicioğlu. 2011. Incorporating phenolic compounds opens a new perspective to use zein films as flexible bioactive packaging materials. Food Research International 44: 550-556.

Arora, D. K., A. P. Hansen, and M. S. Armagost. 1991. Sorption of flavor compounds by low density polyethylene film. Journal of Food Science 56: 1421-1423.

ASTM. 2001. Standard Test Method for Tensile Properties of Thin Plastic Sheeting Annual Book of ASTM Standard. p 162-170. American Society for Testing and Materials, Philadelphia.

Atsumi, T., S. Fujisawa, and K. Tonosaki. 2005. A comparative study of the antioxidant/prooxidant activities of eugenol and isoeugenol with various concentrations and oxidation conditions. Toxicology in Vitro 19: 1025-1033.

Atsumi, T., I. Iwakura, S. Fujisawa, and T. Ueha. 2001. Reactive oxygen species generation and photo-cytotoxicity of eugenol in solutions of various pH. Biomaterials 22: 1459-1466.

Avena-Bustillos, R. J., L. A. Cisneros-Zevallos, J. M. Krochta, and M. E. Saltveit. 1993. Optimization of edible coatings on minimally processed carrots using response surface methodology. Transactions of the American Society of Agricultural Engineers 36: 801-805.

Banerjee, T., S. Mitra, A. Kumar Singh, R. Kumar Sharma, and A. Maitra. 2002. Preparation, characterization and biodistribution of ultrafine chitosan nanoparticles. International Journal of Pharmaceutics 243: 93-105.

Bell, L. N. 2001. Stability testing of nutraceuticals and function foods. In: R. E. C. Wildman (ed.) Handbook of Nutraceuticals and Functional Foods. p 501-516. CRC Press, New York.

Benavides, S., R. Villalobos-Carvajal, and J. E. Reyes. 2012. Physical, mechanical and antibacterial properties of alginate film: Effect of the crosslinking degree and oregano essential oil concentration. Journal of Food Engineering 110: 232-239.

Bertuzzi, M. A., M. Armada, and J. C. Gottifredi. 2007. Physicochemical characterization of starch based films. Journal of Food Engineering 82: 17-25.

Brody, A. L., and K. S. Marsh. 1999. The Wiley Encyclopedia of Packaging Technology. 2nd ed. A Wiley-Interscience Publication Weinheim.

Cerqueira, M. A., B. W. S. Souza, J. T. Martins, J. A. Teixeira, and A. A. Vicente. 2010. Seed extracts of *Gleditsia triacanthos*: Functional properties evaluation and incorporation into galactomannan films. Food Research International 43: 2031-2038.

Chalier, P., A. B. Arfa, L. Preziosi-Belloy, and N. Gontard. 2007. Carvacrol losses from soy protein coated papers as a function of drying conditions. Journal of Applied Polymer Science 106: 611-620.

Chantrai, P. 2000. Improvement in water-resistant properties of edible cassava starch film and application, Master's Thesis, Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University.

Chanzy, H. D., A. Grosrenaud, J. P. Joseleau, M. Dubé, and R. H. Marchessault. 1982. Crystallization behavior of glucomannan. Biopolymers 21: 301-319.

Chen, J., C. Liu, Y. Chen, Y. Chen, and P. R. Chang. 2008. Structural characterization and properties of starch/konjac glucomannan blend films. Carbohydrate Polymers 74: 946-952.

Cheng, L. H., A. Abd Karim, M. H. Norziah, and C. C. Seow. 2002. Modification of the microstructural and physical properties of konjac glucomannan-based films by alkali and sodium carboxymethylcellulose. Food Research International 35: 829-836.

Cheng, L. H., A. Abd Karim, and C. C. Seow. 2007. Effects of acid modification on physical properties of konjac glucomannan (KGM) films. Food Chemistry 103: 994-1002.

Cheng, L. H., A. Abd Karim, and C. C. Seow. 2008. Characterisation of composite films made of konjac glucomannan (KGM), carboxymethyl cellulose (CMC) and lipid. Food Chemistry 107: 411-418.

Choi, M.-J., A. Soottitantawat, O. Nuchuchua, S.-G. Min, and U. Ruktanonchai. 2009. Physical and light oxidative properties of eugenol encapsulated by molecular inclusion and emulsion–diffusion method. Food Research International 42: 148-156.

Coimbra, M. et al. 2011. Improving solubility and chemical stability of natural compounds for medicinal use by incorporation into liposomes. International Journal of Pharmaceutics 416: 433-442.

Cutter, C. N. 2006. Opportunities for bio-based packaging technologies to improve the quality and safety of fresh and further processed muscle foods. Meat Science 74: 131-142.

de Vos, P., M. M. Faas, M. Spasojevic, and J. Sikkema. 2010. Encapsulation for preservation of functionality and targeted delivery of bioactive food components. International Dairy Journal 20: 292-302.

Desai, K. G. H., and H. J. Park. 2005. Recent developments in microencapsulation of food ingredients. Drying Technology 23: 1361-1394.

Dewettinck, K., and A. Huyghebaert. 1999. Fluidized bed coating in food technology. Trends in Food Science and Technology 10: 163-168.

Dhoot, G., R. Auras, M. Rubino, K. Dolan, and H. Soto-Valdez. 2009. Determination of eugenol diffusion through LLDPE using FTIR-ATR flow cell and HPLC techniques. Polymer 50: 1470-1482.

Evans, P. J., M. R. Bird, A. Pihlajamäki, and M. Nyström. 2008. The influence of hydrophobicity, roughness and charge upon ultrafiltration membranes for black tea liquor clarification. Journal of Membrane Science 313: 250-262.

Fabra, M. J., O. Chambin, A. Voilley, J. P. Gay, and F. Debeaufort. 2012. Influence of temperature and NaCl on the release in aqueous liquid media of aroma compounds encapsulated in edible films. Journal of Food Engineering 108: 30-36.

Fang, W., and P. Wu. 2004. Variations of Konjac glucomannan (KGM) from Amorphophallus konjac and its refined powder in China. Food Hydrocolloids 18: 167-170.

Fujisawa, S., T. Atsumi, Y. Kadoma, and H. Sakagami. 2002. Antioxidant and prooxidant action of eugenol-related compounds and their cytotoxicity. Toxicology 177: 39-54.

Gennadios, A., M. A. Hanna, and L. B. Kurth. 1997. Application of edible coatings on meats, poultry and seafoods: A review. LWT - Food Science and Technology 30: 337-350.

Gibbs, B. F., S. Kermasha, I. Alli, and C. N. Mulligan. 1999. Encapsulation in the food industry: a review. International journal of food sciences and nutrition 50: 213-224.

Giménez, B., A. López de Lacey, E. Pérez-Santín, M. E. López-Caballero, and P. Montero. 2013. Release of active compounds from agar and agar-gelatin films with green tea extract. Food Hydrocolloids 30: 264-271.

Gontard, N., S. Guilbert, and J. L. Cuq. 1993. Water and glycerol as plasticizers affect mechanical and water vapor barrier properties of an edible wheat gluten film. Journal of Food Science 58: 206-211.

Gouin, S. 2004. Microencapsulation: Industrial appraisal of existing technologies and trends. Trends in Food Science and Technology 15: 330-347.

Guilbert, S. 1986. Technology and application of edible protective films. In: M. Mathlouthi (ed.) Food Packaging and Preservation: Theory and Practice. Elsevier Applied Science Publishing Co., London.

Guilbert, S., and N. Gontard. 1995. Edible and biodegradable food packaging. In: P. Ackermann, M. Jägerstad and T. Ohlsson (eds.) Food and Packaging Materials-Chemical Interactions. p 159-168. Royal Society of Chemisty, Cambridge, UK.

Hambleton, A., M. J. Fabra, F. Debeaufort, C. Dury-Brun, and A. Voilley. 2009. Interface and aroma barrier properties of iota-carrageenan emulsion-based films used for encapsulation of active food compounds. Journal of Food Engineering 93: 80-88.

Han, J. H., and J. D. Floros. 1997. Casting antimicrobial packaging films and measuring their physical properties and antimicrobial activity. Journal of Plastic Film and Sheeting 13: 287-298.

Han, J. H., and J. M. Krochta. 1999. Wetting properties and water vapor permeability of whey-protein-coated paper. Transactions of the American Society of Agricultural Engineers 42: 1375-1382.

Hoque, M. S., S. Benjakul, T. Prodpran, and P. Songtipya. 2011. Properties of blend film based on cuttlefish (*Sepia pharaonis*) skin gelatin and mungbean protein isolate. International Journal of Biological Macromolecules 49: 663-673.

Hosseini, M. H., S. H. Razavi, and M. A. Mousavi. 2009. Antimicrobial, physical and mechanical properties of chitosan-based films incorporated with thyme, clove and cinnamon essential oils. Journal of Food Processing and Preservation 33: 727-743.

Ito, M., K. Murakami, and M. Yoshino. 2005. Antioxidant action of eugenol compounds: role of metal ion in the inhibition of lipid peroxidation. Food and Chemical Toxicology 43: 461-466.

Jeng, J. H., L. J. Hahn, E. Lu, Y. Wang, and M. Y. P. Kuo. 1994. Eugenol Triggers Different Pathobiological Effects on Human Oral Mucosal Fibroblasts 1. Journal of Dental Research 73: 1050-1055.

Jia, D., Y. Fang, and K. Yao. 2009. Water vapor barrier and mechanical properties of konjac glucomannan-chitosan-soy protein isolate edible films. Food and Bioproducts Processing 87: 7-10.

Jimenez-Colmenero, F., S. Cofrades, A. M. Herrero, M. T. Solas, and C. Ruiz-Capillas. 2013. Konjac gel for use as potential fat analogue for healthier meat product development: Effect of chilled and frozen storage. Food Hydrocolloids 30: 351-357.

Kalemba, D., and A. Kunicka. 2003. Antibacterial and antifungal properties of essential oils. Current Medicinal Chemistry 10: 813-829.

Karbowiak, T., F. Debeaufort, and A. Voilley. 2007. Influence of thermal process on structure and functional properties of emulsion-based edible films. Food Hydrocolloids 21: 879-888.

Kato, K., and K. Matsuda. 1970. Studies on the chemical structure of konjac mannan part II. Isolation and characterization of oligosaccharides from the partial acid hydrolysate of the mannan. Agricultural and Biological Chemistry 34: 532-538.

Keawchaoon, L., and R. Yoksan. 2011. Preparation, characterization and in vitro release study of carvacrol-loaded chitosan nanoparticles. Colloids and Surfaces B: Biointerfaces 84: 163-171.

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

Khoshgozaran-Abras, S., M. H. Azizi, Z. Hamidy, and N. Bagheripoor-Fallah. 2012. Mechanical, physicochemical and color properties of chitosan based-films as a function of Aloe vera gel incorporation. Carbohydrate Polymers 87: 2058-2062.

Korkiatithaweechai, S., and P. Umsarika. 2009. Controlled Release of Diclofenac from Matrix Polymer of Chitosan and Oxidized Konjac Glucomannan, Bachelor's Project, Department of Chemistry, Faculty of Science, Chulalongkorn University.

Korkiatithaweechai, S., P. Umsarika, N. Praphairaksit, and N. Muangsin. 2011. Controlled release of diclofenac from matrix polymer of chitosan and oxidized konjac glucomannan. Marine Drugs 9: 1649-1663.

Kritsanapan, W. 1998. Samunprai naroo. Chulalongkorn, Bangkok.

Krochta, J. M., and C. De Mulder-Johnston. 1997. Edible and biodegradable polymer films: challenges and opportunities. Food Technology 51: 61-74.

Laekeman, G. M. et al. 1990. Eugenol a valuable compound for in vitro experimental research and worthwhile for further in vivo investigation. Phytotherapy Research 4: 90-96.

Lai, H. C., A. A. Karim, and C. S. Chee. 2006. Effects of water-glycerol and water-sorbitol interactions on the physical properties of konjac glucomannan films. Journal of Food Science 71: E62-E67.

Lehduwi, N. 2007. Effects of composite on properties of edible modified rice starch film, Master's Thesis, Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University.

Li, B., J. F. Kennedy, Q. G. Jiang, and B. J. Xie. 2006. Quick dissolvable, edible and heatsealable blend films based on konjac glucomannan - gelatin. Food Research International 39: 544-549.

Li, B., and B. Xie. 2003. Study on gel formation mechanism of konjac glucomannan. Agricultural Sciences in China 2: 424-428.

Mahmoud, R., and P. A. Savello. 1993. Solubility and hydrolyzability of films produced by transglutaminase catalytic crosslinking of whey protein. Journal of Dairy Science 76: 29-35.

McHugh, T. H. 1996. Effects of macromolecular interactions on the permeability of composite edible films. In: N. Parris, A. Kato, L. K. Creamer and J. Pearce (eds.) Macromolecular Interactions in Food Technology. p 134-144. American Chemistry Society, Washington, D. C.

McHugh, T. H., R. Avena-Bustillos, and J. M. Krochta. 1993. Hydrophilic edible films: Modified procedure for water vapor permeability and explanation of thickness effects. Journal of Food Science 58: 899-903.

Nair, S. B., A. N. Jyothi, M. S. Sajeev, and R. Misra. 2011. Rheological, mechanical and moisture sorption characteristics of cassava starch-konjac glucomannan blend films. Starch/Staerke 63: 728-739.

Nedovic, V., A. Kalusevic, V. Manojlovic, S. Levic, and B. Bugarski. 2011. An overview of encapsulation technologies for food applications. Procedia Food Science 1: 1806-1815.

Nishinari, K., P. A. Williams, and G. O. Philips. 1987. Solution properties of konjac mannan. In: Abstracts of 2nd International Workshop on Plant Polysaccharides, Grenoble. p 23.

Nuchuchua, O. et al. 2009. Physicochemical investigation and molecular modeling of cyclodextrin complexation mechanism with eugenol. Food Research International 42: 1178-1185.

Ohashi, S., G. J. Shelso, A. L. Moirano, and W. L. Drinkwater. 2000. Clarified Konjac Glucomannan No. 6. p 906, United state.

Ohkubo, T., and M. Shibata. 1997. The selective capsaicin antagonist capsazepine abolishes the antinociceptive action of eugenol and guaiacol. Journal of Dental Research 76: 848-851.

Ojagh, S. M., M. Rezaei, S. H. Razavi, and S. M. H. Hosseini. 2010. Development and evaluation of a novel biodegradable film made from chitosan and cinnamon essential oil with low affinity toward water. Food Chemistry 122: 161-166.

Ou, H.-C., F.-P. Chou, T.-M. Lin, C.-H. Yang, and W. H.-H. Sheu. 2006. Protective effects of eugenol against oxidized LDL-induced cytotoxicity and adhesion molecule expression in endothelial cells. Food and Chemical Toxicology 44: 1485-1495.

Ozdemir, M., and J. D. Floros. 2008. Optimization of edible whey protein films containing preservatives for water vapor permeability, water solubility and sensory characteristics. Journal of Food Engineering 86: 215-224.

Pelissari, F. M., M. V. E. Grossmann, F. Yamashita, and E. A. G. Pined. 2009. Antimicrobial, mechanical, and barrier properties of cassava starch-chitosan films incorporated with oregano essential oil. Journal of Agricultural and Food Chemistry 57: 7499-7504.

Pérez-Gago, M. B., and J. M. Krochta. 2005. Emulsion and bi-layer edible films. In: J. H. Han (ed.) Innovations in Food Packaging. p 362 - 383. Elsevier, London.

Pérez-Gago, M. B., and J.-W. Rhim. 2014. Edible coating and film materials: lipid bilayers and lipid emulsions. In: J. H. Han (ed.) Innovations in Food Packaging (Second edition). p 325-350. Elsevier, London.

Pires, C. et al. 2013. Hake proteins edible films incorporated with essential oils: Physical, mechanical, antioxidant and antibacterial properties. Food Hydrocolloids 30: 224-231.

Pranoto, Y., V. M. Salokhe, and S. K. Rakshit. 2005. Physical and antibacterial properties of alginate-based edible film incorporated with garlic oil. Food Research International 38: 267-272.

Prüsse, U. et al. 2008. Comparison of different technologies for alginate beads production. Chemical Papers 62: 364-374.

Quezada Gallo, J. A., F. Debeaufort, F. Callegarin, and A. Voilley. 2000. Lipid hydrophobicity, physical state and distribution effects on the properties of emulsion-based edible films. Journal of Membrane Science 180: 37-46.

Rattananin, J. 2011. Production of edible film from konjac powder incorporated with Thai medicinal plants extracts for extending shelf life of mango cv. Nam Dok Mai # 4, Master's Thesis, Department of Food Technology, Faculty of Science, Chulalongkorn University.

Rattanapitigorn, P. 1998. Development of composite edible film for coating fish snack, Master's Thesis, Department of Food Technology, Faculty of Science, Chulalongkorn University.

Rojas-Graü, M. A. et al. 2007. Effects of plant essential oils and oil compounds on mechanical barrier and antimicrobial properties of alginate-apple puree edible films. Journal of Food Engineering 81: 634-641.

Salgado, P. R., M. E. López-Caballero, M. C. Gómez-Guillén, A. N. Mauri, and M. P. Montero. 2012. Exploration of the antioxidant and antimicrobial capacity of two sunflower protein concentrate films with naturally present phenolic compounds. Food Hydrocolloids 29: 374-381.

Sánchez-González, L., M. Cháfer, A. Chiralt, and C. González-Martínez. 2010. Physical properties of edible chitosan films containing bergamot essential oil and their inhibitory action on Penicillium italicum. Carbohydrate Polymers 82: 277-283.

Sánchez-González, L., M. Vargas, C. González-Martínez, A. Chiralt, and M. Cháfer. 2009. Characterization of edible films based on hydroxypropylmethylcellulose and tea tree essential oil. Food Hydrocolloids 23: 2102-2109.

Sanla-Ead, N., A. Jangchud, V. Chonhenchob, and P. Suppakul. 2012. Antimicrobial activity of cinnamaldehyde and eugenol and their activity after incorporation into cellulose-based packaging films. Packaging Technology and Science 25: 7-17.

Schimidt, P. C. 1997. Technological aspects of the development and production of plant extracts. Pharmazeutische Industrie 59: 69.

Shiku, Y., P. Y. Hamaguchi, S. Benjakul, W. Visessanguan, and M. Tanaka. 2004. Effect of surimi quality on properties of edible films based on Alaska pollack. Food Chemistry 86: 493-499.

Shinde, U., and M. Nagarsenke. 2011. Microencapsulation of eugenol by gelatin sodium alginate complex coacervation. Indian Journal of Pharmaceutical Sciences 73: 311-315.

Son, K. H., S. Y. Kwon, H. P. Kim, H. W. Chang, and S. S. Kang. 1998. Constituents from *Syzygium aromaticum* Merr. *et* Perry. Natural Product Sciences 4: 263-267.

Stuchell, Y. M., and J. M. Krochta. 1995. Edible coating on frozen king salmon: Effect of whey protein isolate and acetylated monoglycerides on moisture loss and lipid oxidation. Journal of Food Science 60: 28-31.

Suppakul, P., J. Miltz, K. Sonneveld, and S. W. Bigger. 2006. Characterization of antimicrobial films containing basil extracts. Packaging Technology and Science 19: 259-268.

Suzuki, Y., K. Sugiyama, and H. Furuta. 1985. Eugenol-mediated superoxide generation and cytotoxicity in guinea pig neutrophils. Japanese Journal of Pharmacology 39: 381-386.

Takigami, S. 2000. Konjac mannan. In: G. O. Philips and P. A. Williams (eds.) Handbook of Hydrocolloids. p 889-901. Woodhead Publishing Limited, New York.

Tatirat, O., and S. Charoenrein. 2011. Physicochemical properties of konjac glucomannan extracted from konjac flour by a simple centrifugation process. LWT - Food Science and Technology 44: 2059-2063.

Thompson, D. et al. 1989. Peroxidase-catalyzed oxidation of eugenol: Formation of a cytotoxic metabolite(s). The Journal of Biological Chemistry 264: 1016-1021.

Thompson, D. C., R. Barhoumi, and R. C. Burghardt. 1998. Comparative toxicity of eugenol and its quinone methide metabolite in cultured liver cells using kinetic fluorescence bioassays. Toxicology and Applied Pharmacology 149: 55-63.

Thompson, D. C., K. Perera, E. S. Krol, and J. L. Bolton. 1995. o-Methoxy-4-alkylphenols that form quinone methides of intermediate reactivity are the most toxic in rat liver slices. Chemical Reseach in Toxicology 8: 323-327.

Thompson, D. C., J. A. Thompson, M. Sugumaran, and P. Moldeus. 1993. Biological and toxicological consequences of quinone methide formation. Chemical-biological Interaction 86: 129-162.

Torres, J. A. 1994. Edible films and coatings from proteins. In: N. S. Hettiarachchy and G. R. Ziegler (eds.) Protein functionality in food systems. Mercel Dekker, New York.

Tunc, S. et al. 2007. Functional properties of wheat gluten/montmorillonite nanocomposite films processed by casting. Journal of Membrane Science 289: 159-168.

Tye, R. J. 1991. Konjac flour: properties and applications. Food Technology 45: 86-92.

Voilley, A., and P. Etievant. 2006. Flavor in Food. Woodhead Publishing Limited, Cambridge.

Wandray, C., A. Bartkowiak, and S. E. Harding. 2009. Materials for encapsulation. In: N. J. Zuidam and V. A. Nedovic (eds.) Encapsulation Technologies for Food Active Ingredients and Processing. p 31-100. Springer, Dordrecht, The Netherlands.

Wang, L., Y. Dong, H. Men, J. Tong, and J. Zhou. 2013. Preparation and characterization of active films based on chitosan incorporated tea polyphenols. Food Hydrocolloids 32: 35-41.

Whistler, R. J., and R. Daneil. 1990. Function of polysaccharides in foods. In: A. L. Branen, P. M. Davidson and S. Sailnen (eds.) Food Additives. p 395-423. Marcel Dekker, New York.

Wong, D. E., A. E. Parlath, and S. J. Tillin. 1992a. Edible double - layer coating for slightly processed fruits and vegetables. In: J. M. Krochta, E. A. Baldwin and M. O. Nisperos-Carriedo (eds.) Edible coating and films to improve food quality. p 73-76. Technomic, Pennsylvania.

Wong, D. W. S., F. A. Gastineau, K. S. Gregorski, S. J. Tillin, and A. E. Pavlath. 1992b. Chitosan-lipid films: Microstructure and surface energy. Journal of Agricultural and Food Chemistry 40: 540-544.

Wongnimitkul, N., B. Ouiha, and P. Reukruetairat. 2008. Production of edible film from konjac powder added with clove oil for extending shelf life tomato, Bachelor's

Project, Department of Food Technology, Faculty of Science, Chulalongkorn University.

Woranuch, S., and R. Yoksan. 2013a. Eugenol-loaded chitosan nanoparticles: I. Thermal stability improvement of eugenol through encapsulation. Carbohydrate Polymers 96: 578-585.

Woranuch, S., and R. Yoksan. 2013b. Eugenol-loaded chitosan nanoparticles: II. Application in bio-based plastics for active packaging. Carbohydrate Polymers 96: 586-592.

Xiao, C., Y. Lu, H. Liu, and L. Zhang. 2001. Preparation and characterization of konjac glucomannan and sodium carboxymethylcellulose blend films. Journal of Applied Polymer Science 80: 26-31.

Xu, X., B. Li, J. F. Kennedy, B. J. Xie, and M. Huang. 2007. Characterization of konjac glucomannan–gellan gum blend films and their suitability for release of nisin incorporated therein. Carbohydrate Polymers 70: 192-197.

Yoshimura, M., and K. Nishinari. 1999. Dynamic viscoelastic study on the gelation of konjac glucomannan with different molecular weights. Food Hydrocolloids 13: 227-233.

Zhang, R., K. A. Kulkarni, and A. P. Kulkarni. 2000. Oxidation of eugenol by purified human term placenta peroxidase. Placenta 21: 234-240.

Zivanovic, S., S. Chi, and A. F. Draughon. 2005. Antimicrobial activity of chitosan films enriched with essential oils. Journal of Food Science 70: M45-M51.

Zuidam, N. J., and J. Heinrich. 2009. Encapsulation of aroma. In: N. J. Zuidam and V. A. Nedovic (eds.) Encapsulation Technologies for Food Active Ingredients and Food Processing. Springer, Dordrecht, The Netherlands.

Zuidam, N. J., and E. Shimoni. 2009. Overview of microencapsulates for use in food products or processes and methods to make them. In: N. J. Zuidam and V. A. Nedovic (eds.) Encapsulation Technologies for Food Active Ingredients and Food Processing. Springer, The Netherlands.

APPENDIX

APPENDIX A

ADDITIONAL DATA

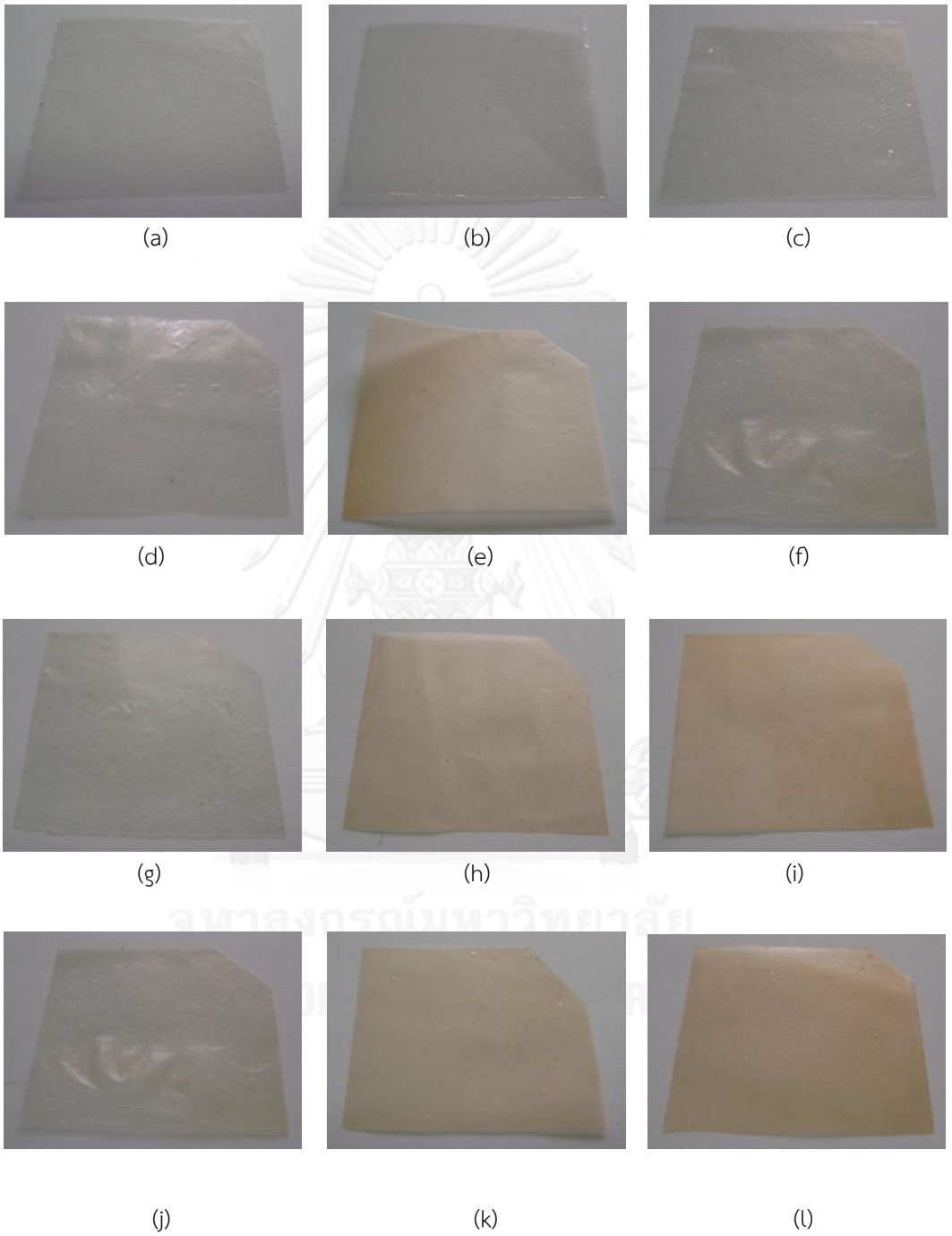


Figure A.1 Examples of KGM/EU films at different ratio of KGM%:EU% (w/w); 0.50:0.00 (a) 0.75:0.00 (b) 1.00:0.00 (c) 0.50:0.50 (d) 0.50:1.00 (e) 0.50:1.50 (f) 0.75:0.50 (g) 0.75:1.00 (h) 0.75:1.50 (i) 1.00:0.50 (j) 1.00:1.00 (k) 1.00:1.50 (l)

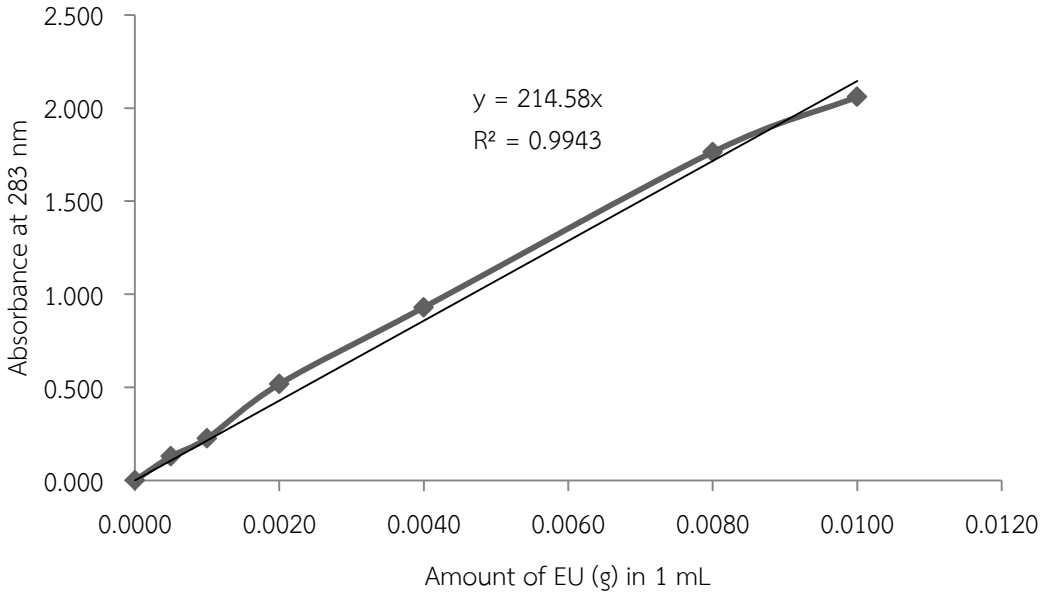


Figure A.2 EU standard curve for measuring EE

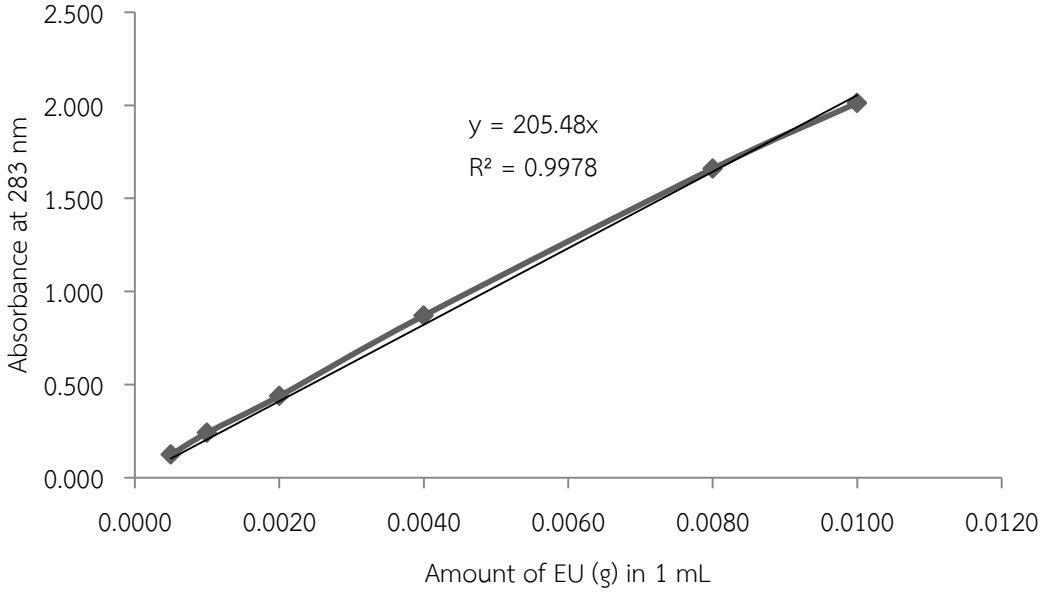


Figure A.3 EU standard curve for measuring releasing (%)

Figure A.4 Blower cabinet for film casting and drying

Figure A.5 Casting plate

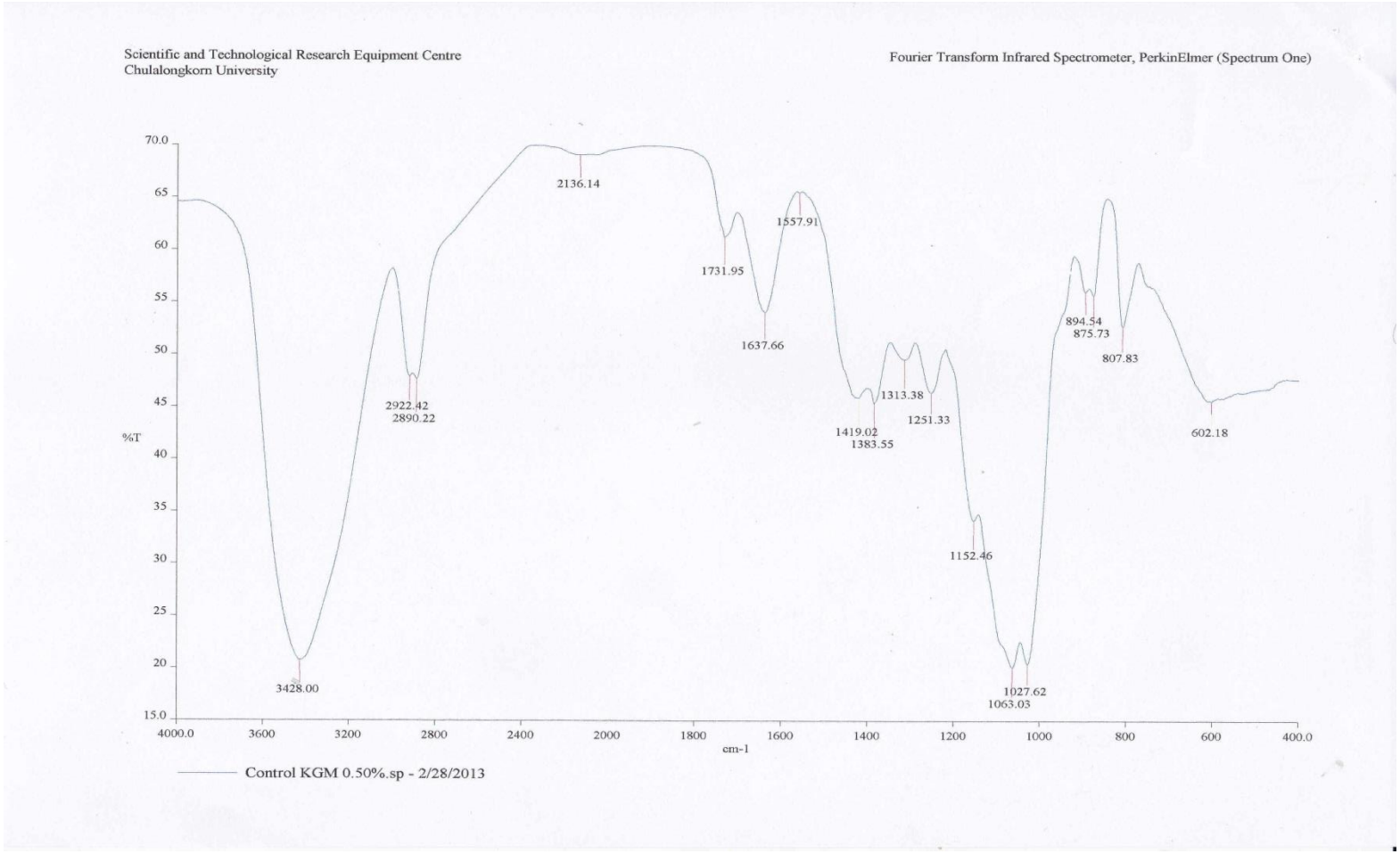


Figure A.6 FT-IR spectra of 0.50% (w/w) pure KGM film

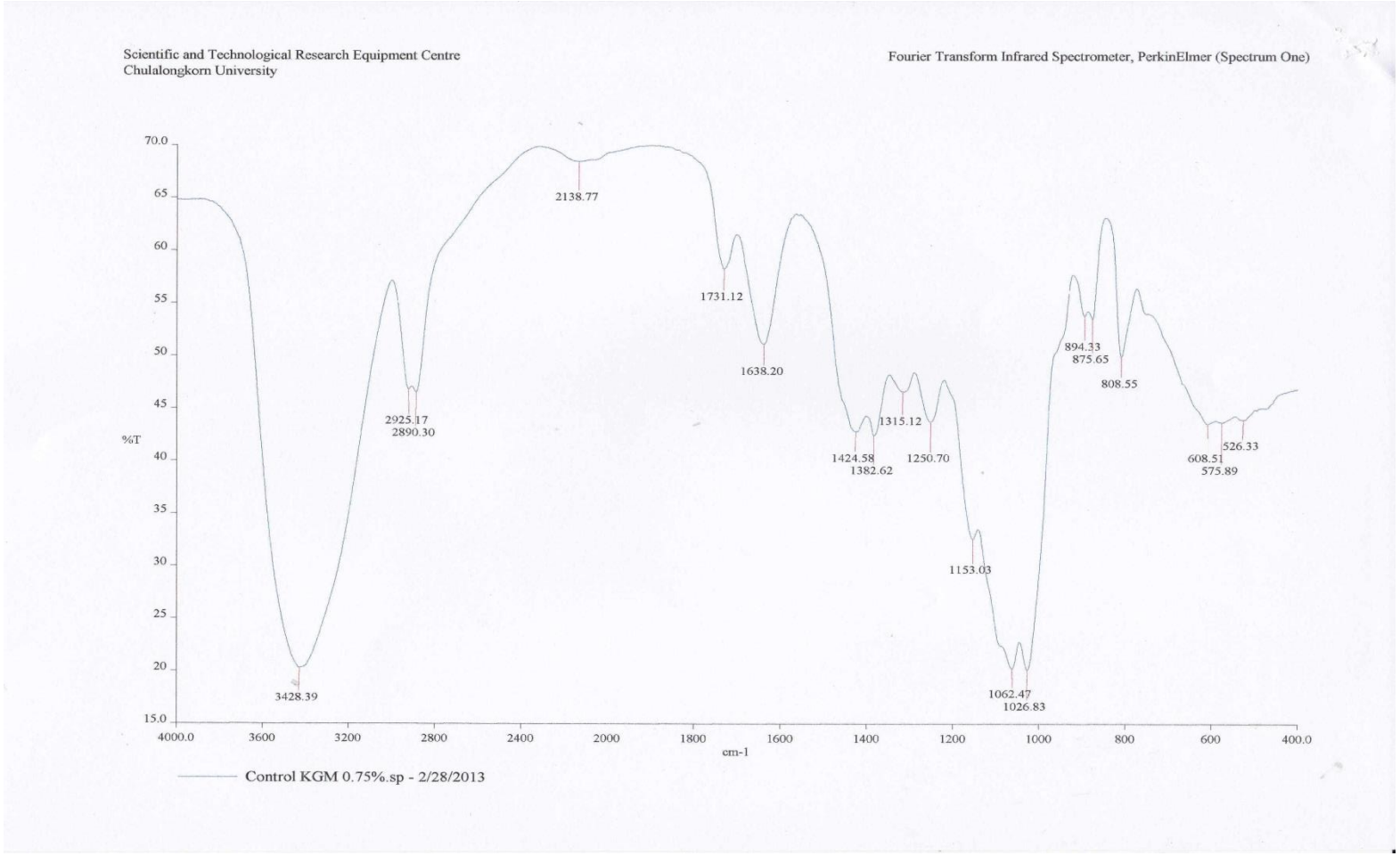


Figure A.7 FT-IR spectra of 0.75% (w/w) pure KGM film

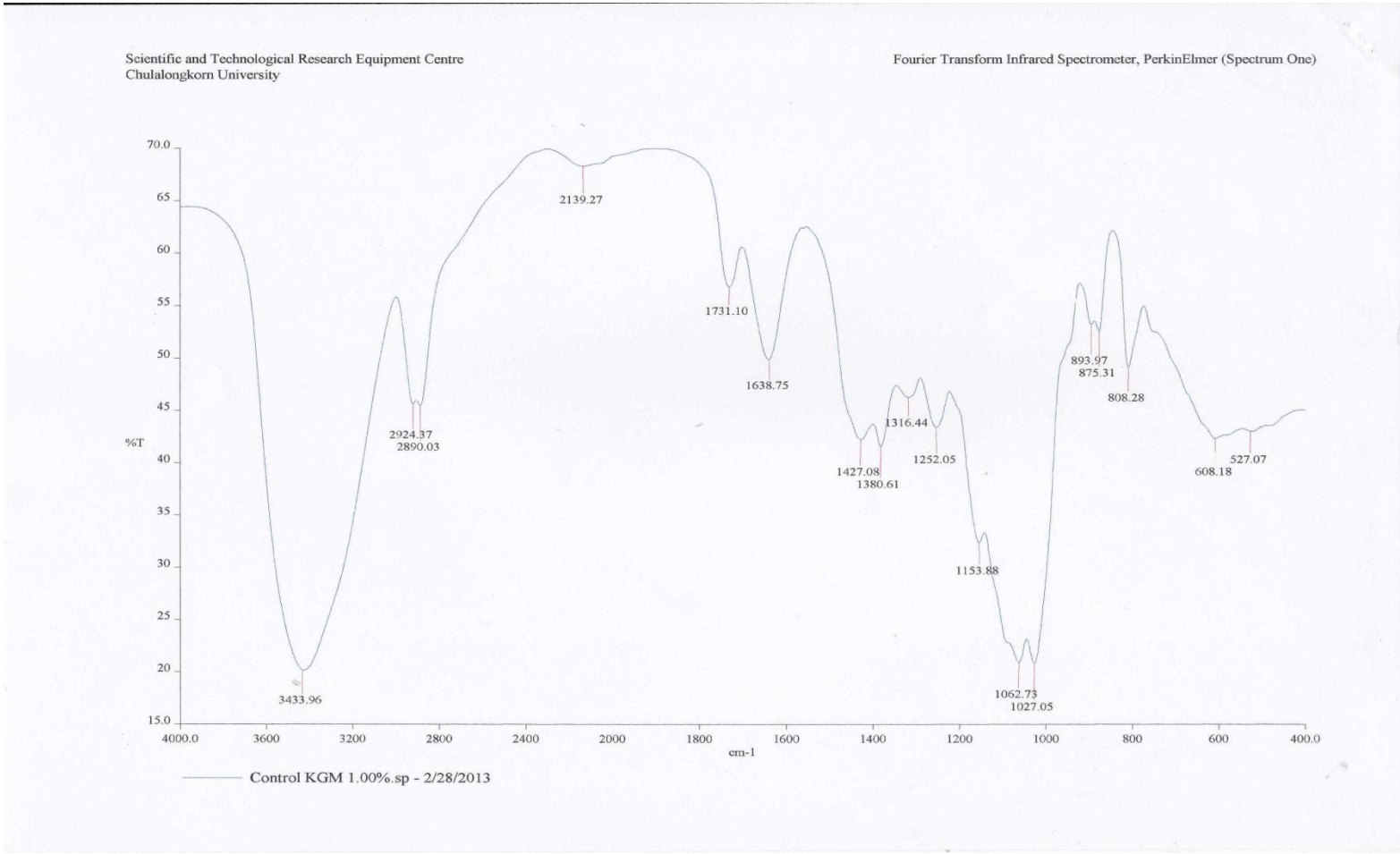


Figure A.8 FT-IR spectra of 1.00% (w/w) pure KGM film

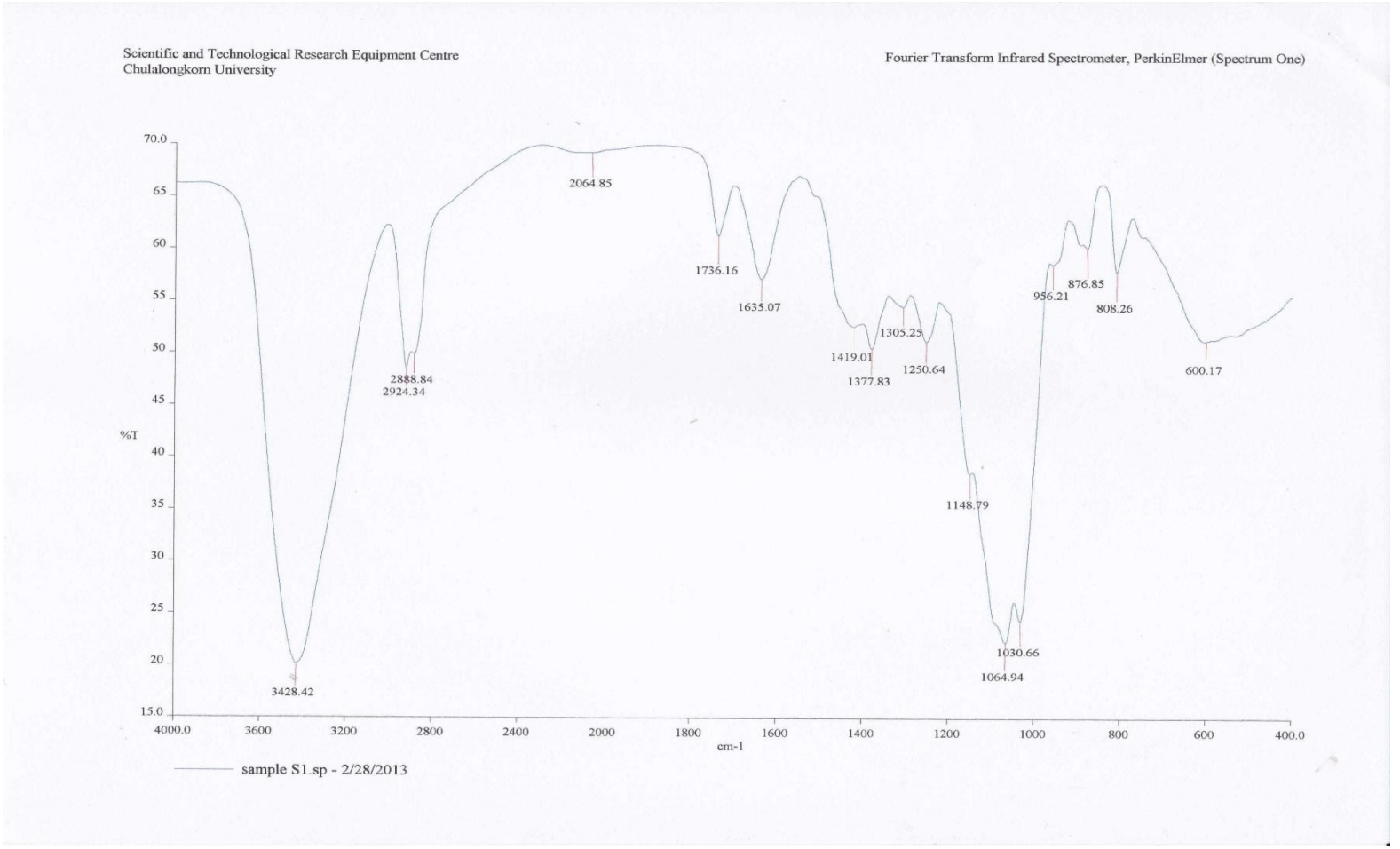


Figure A.9 FT-IR spectra of KGM/EU film in the ratio of KGM:EU 0.50%:0.50% (w/w)

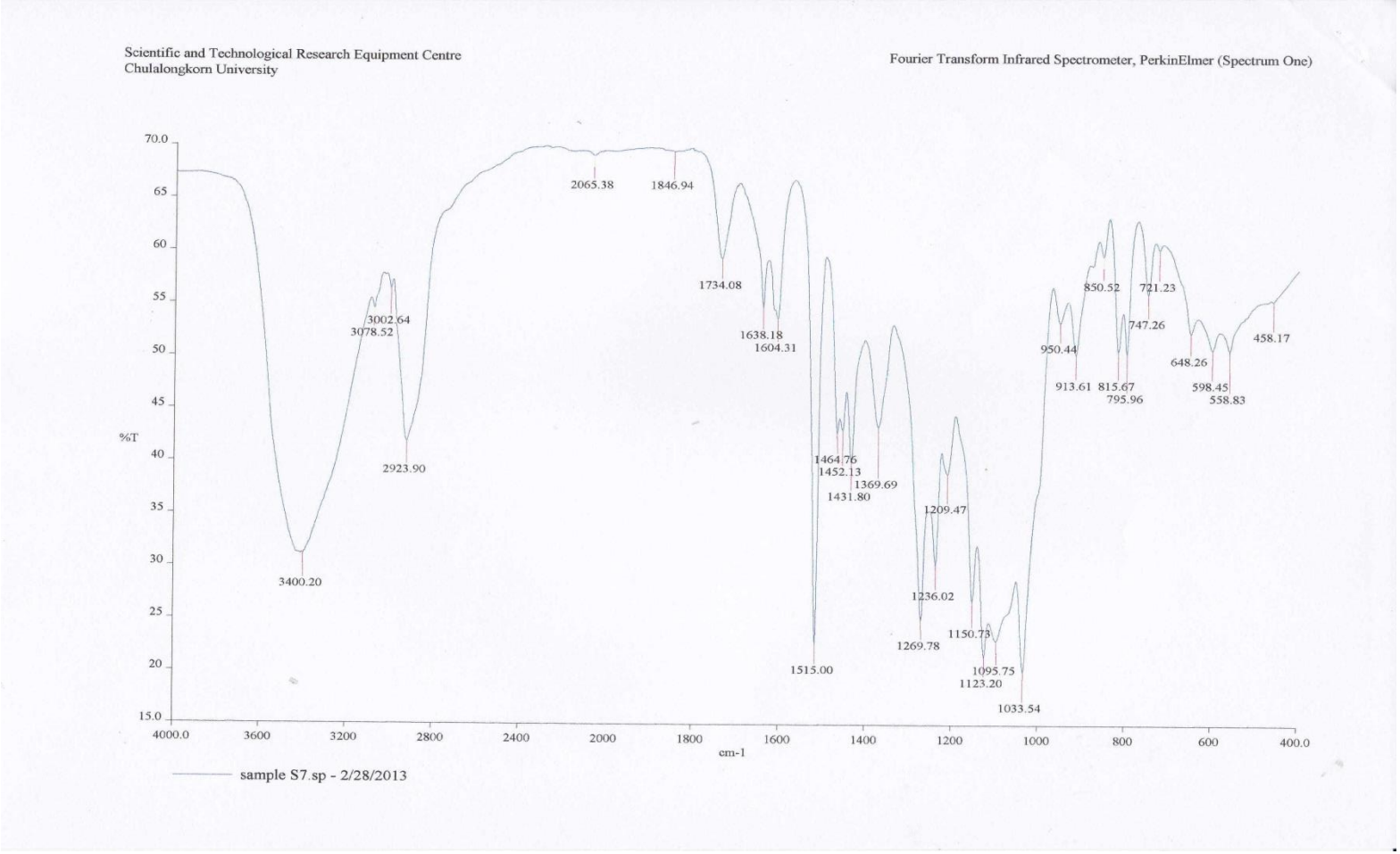


Figure A.10 FT-IR spectra of KGM/EU film in the ratio of KGM:EU 0.50%:1.00% (w/w)

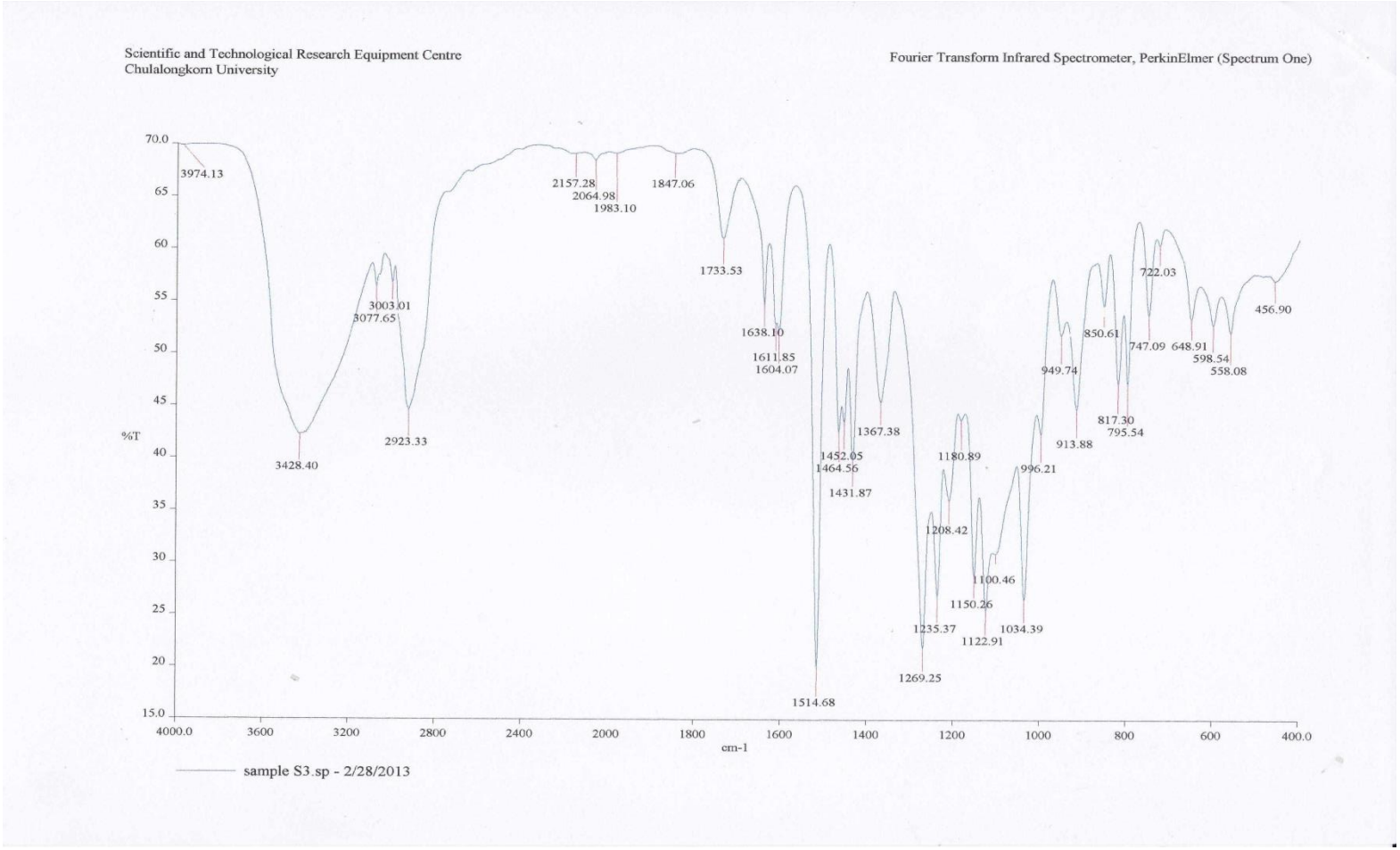


Figure A.11 FT-IR spectra of KGM/EU film in the ratio of KGM:EU 0.50%:1.50% (w/w)

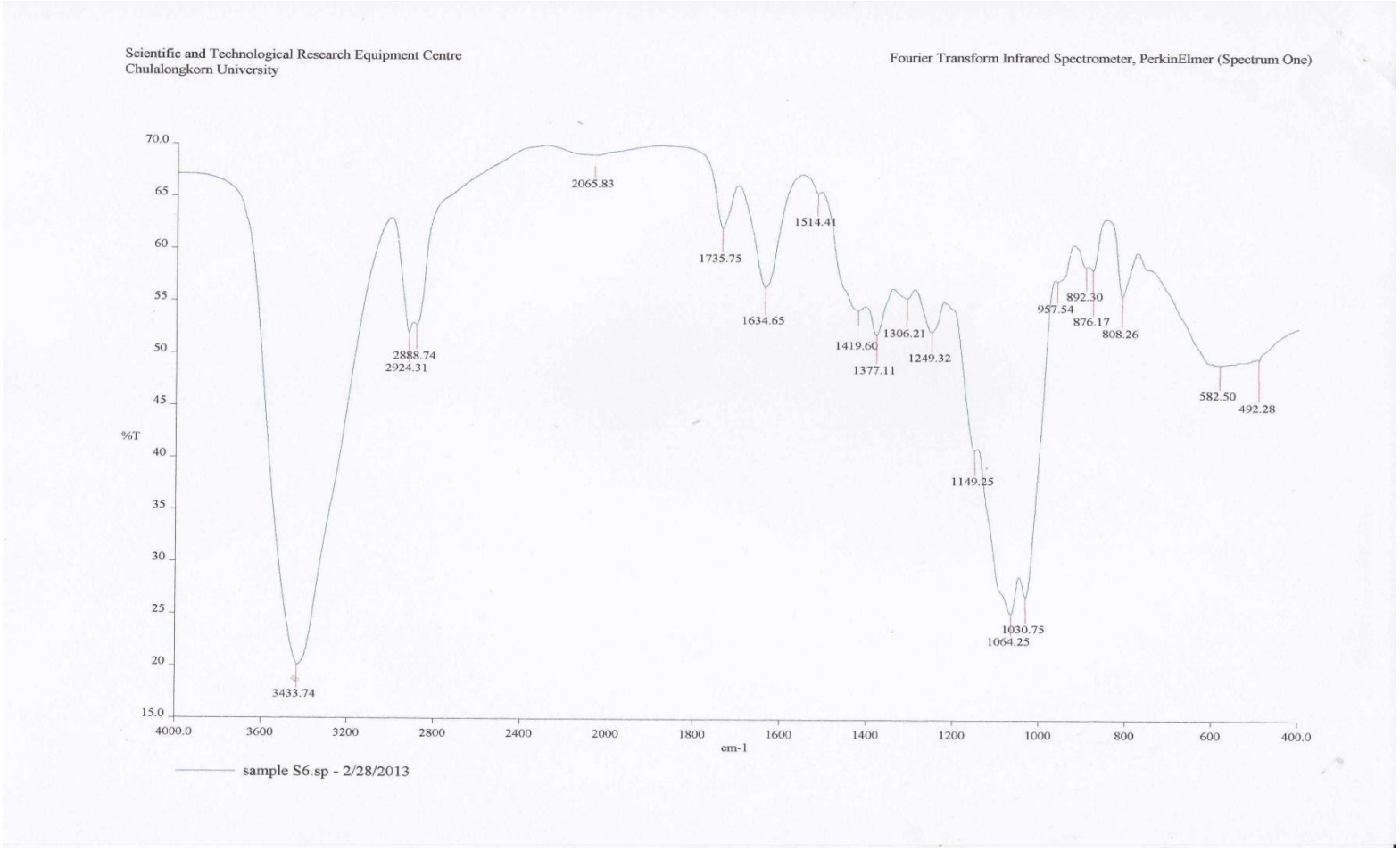


Figure A.12 FT-IR spectra of KGM/EU film in the ratio of KGM:EU 0.75%:0.50% (w/w)

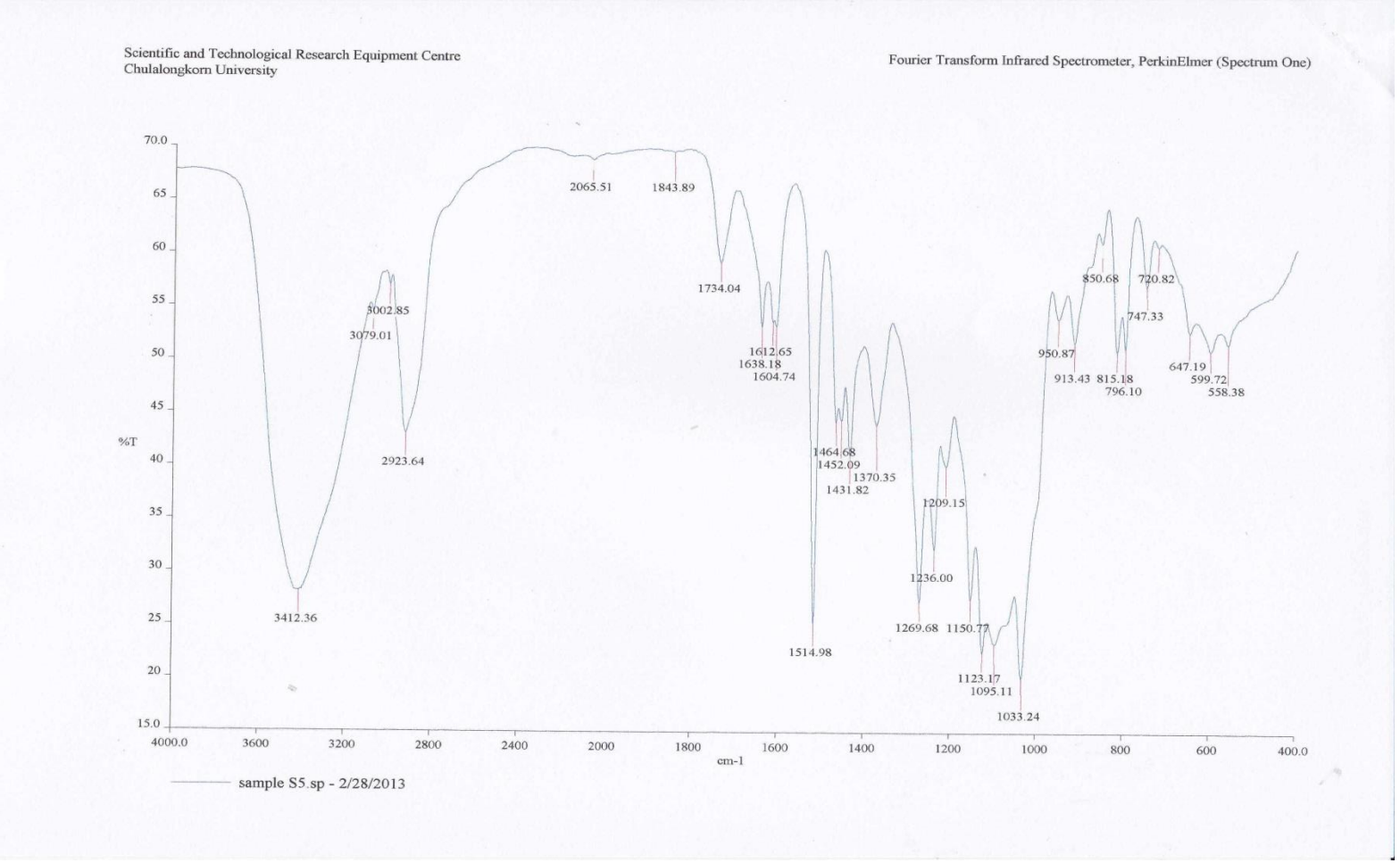


Figure A.13 FT-IR spectra of KGM/EU film in the ratio of KGM:EU 0.75%:1.00% (w/w)

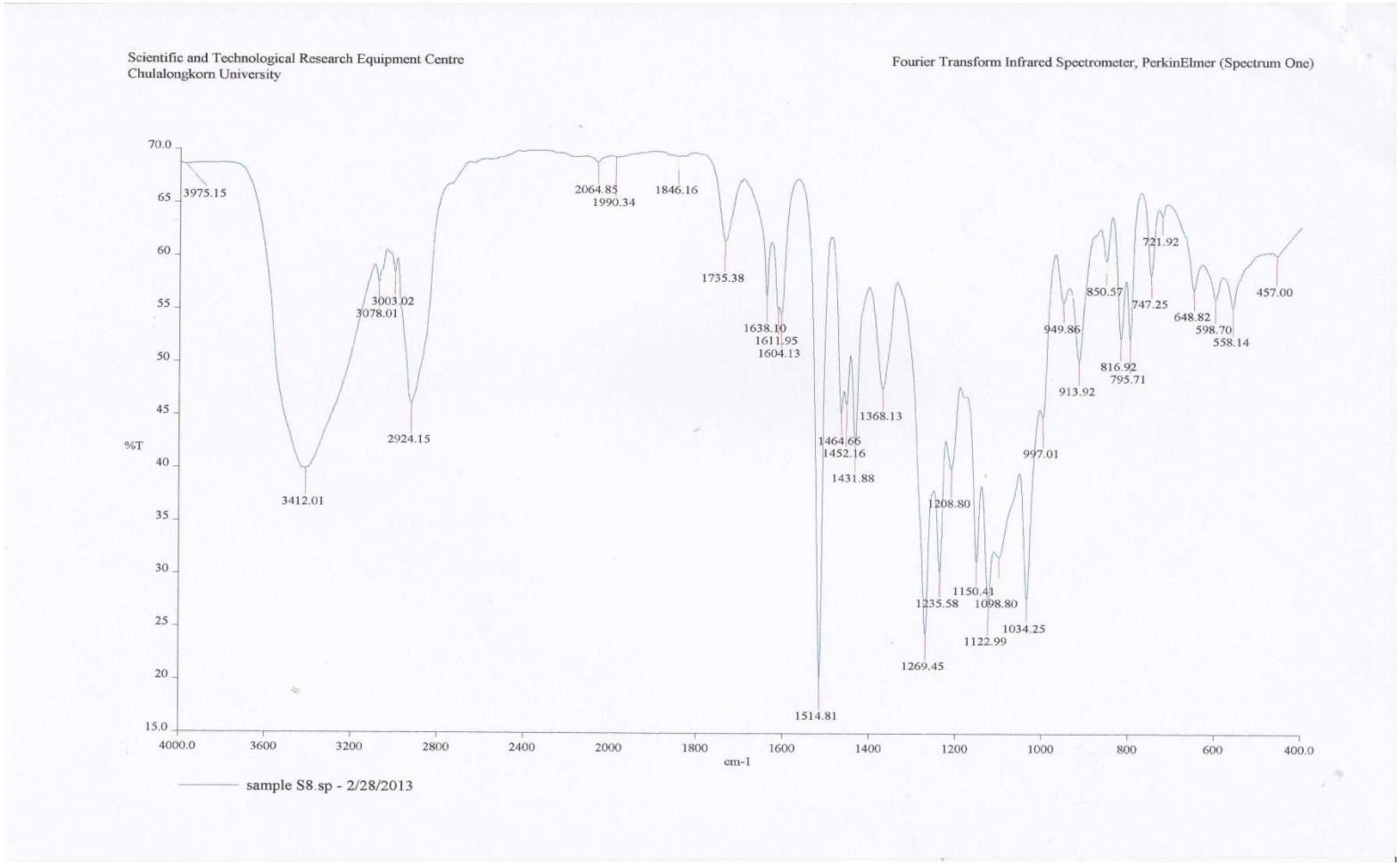


Figure A.14 FT-IR spectra of KGM/EU film in the ratio of KGM:EU 0.75%:1.50% (w/w)

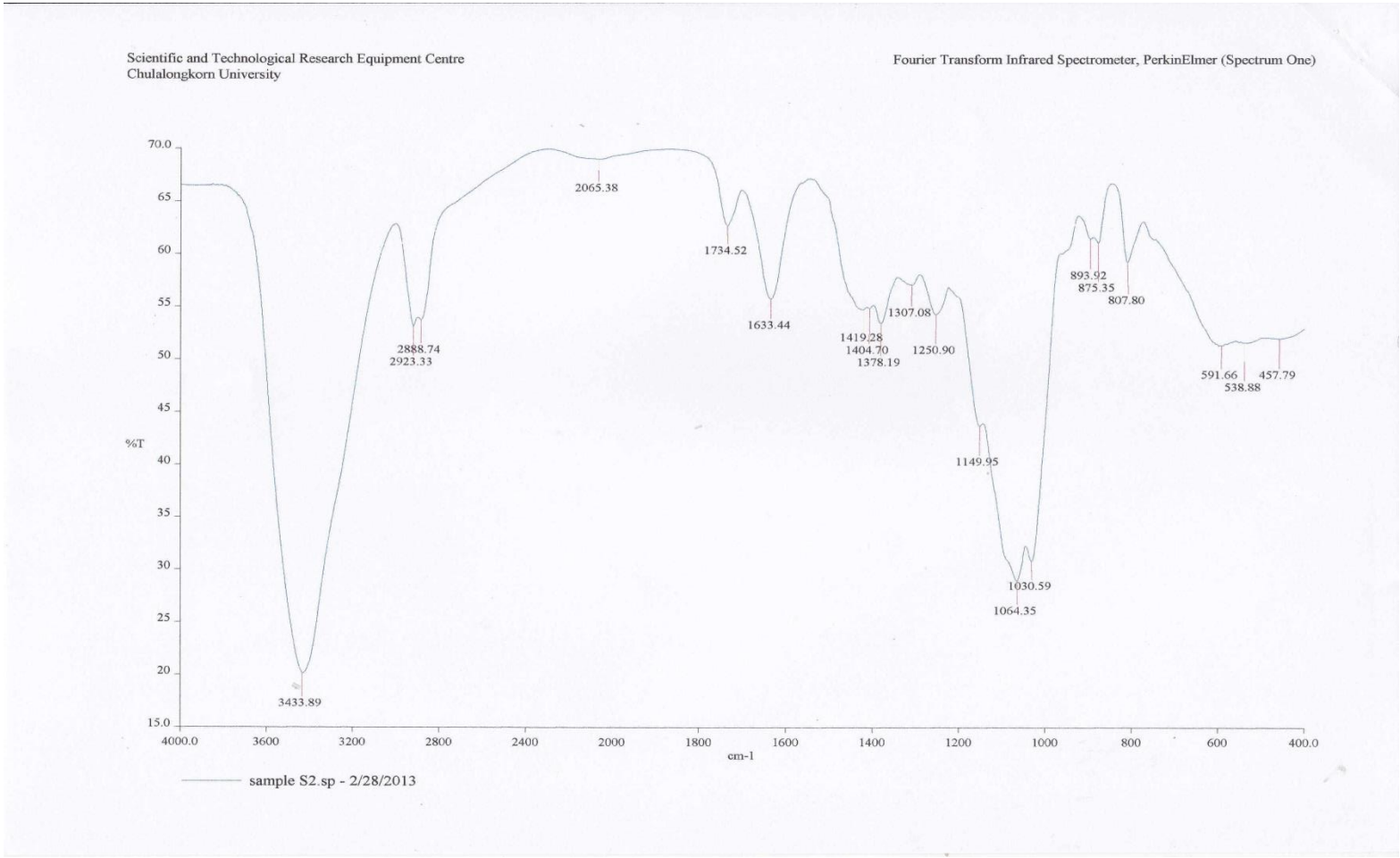


Figure A.15 FT-IR spectra of KGM/EU film in the ratio of KGM:EU 1.00%:0.50% (w/w)

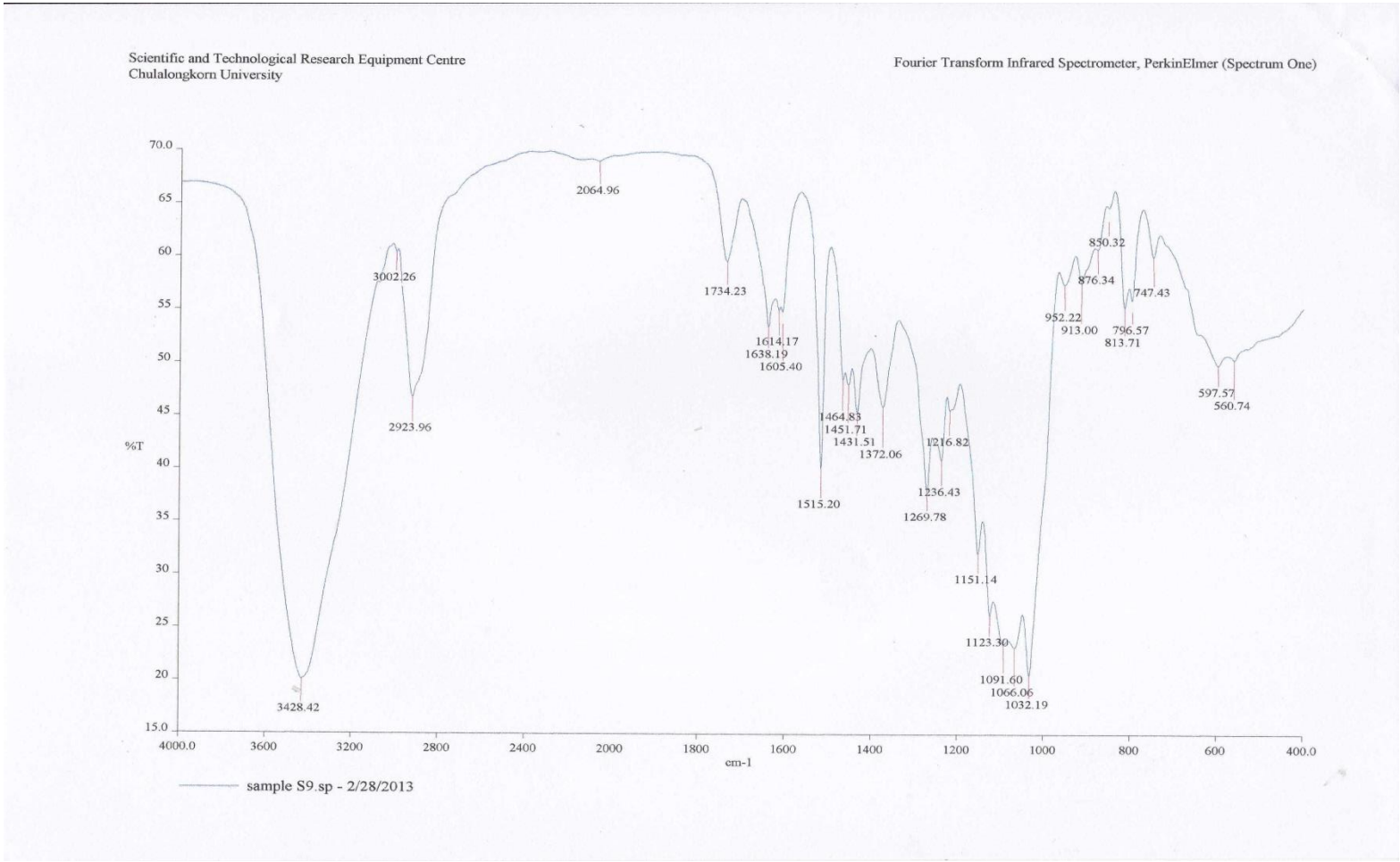


Figure A.16 FT-IR spectra of KGM/EU film in the ratio of KGM:EU 1.00%:1.00% (w/w)

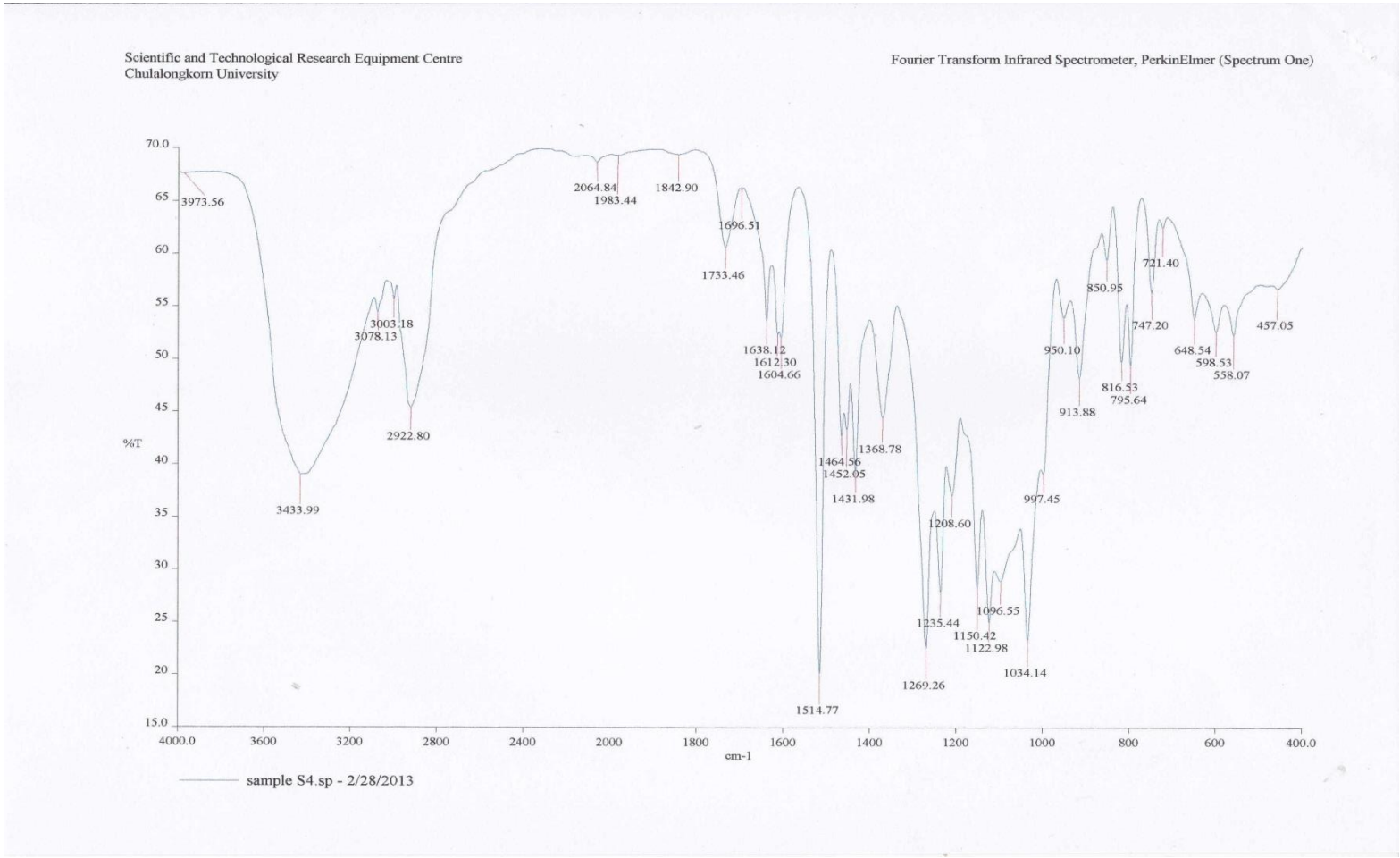


Figure A.17 FT-IR spectra of KGM/EU film in the ratio of KGM:EU 1.00%:1.50% (w/w)

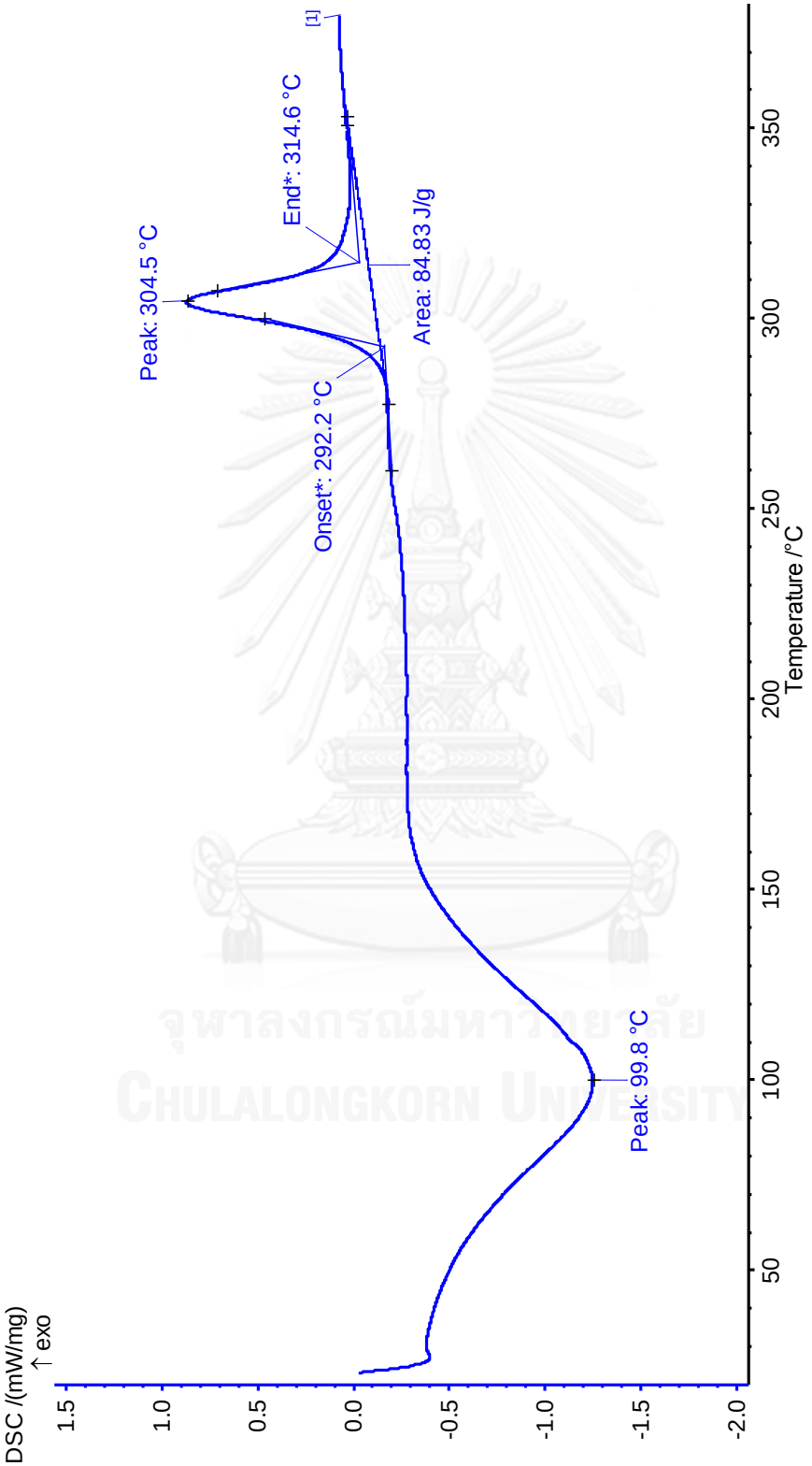


Figure A.18 DSC thermogram of 0.50% (w/w) pure KGM film

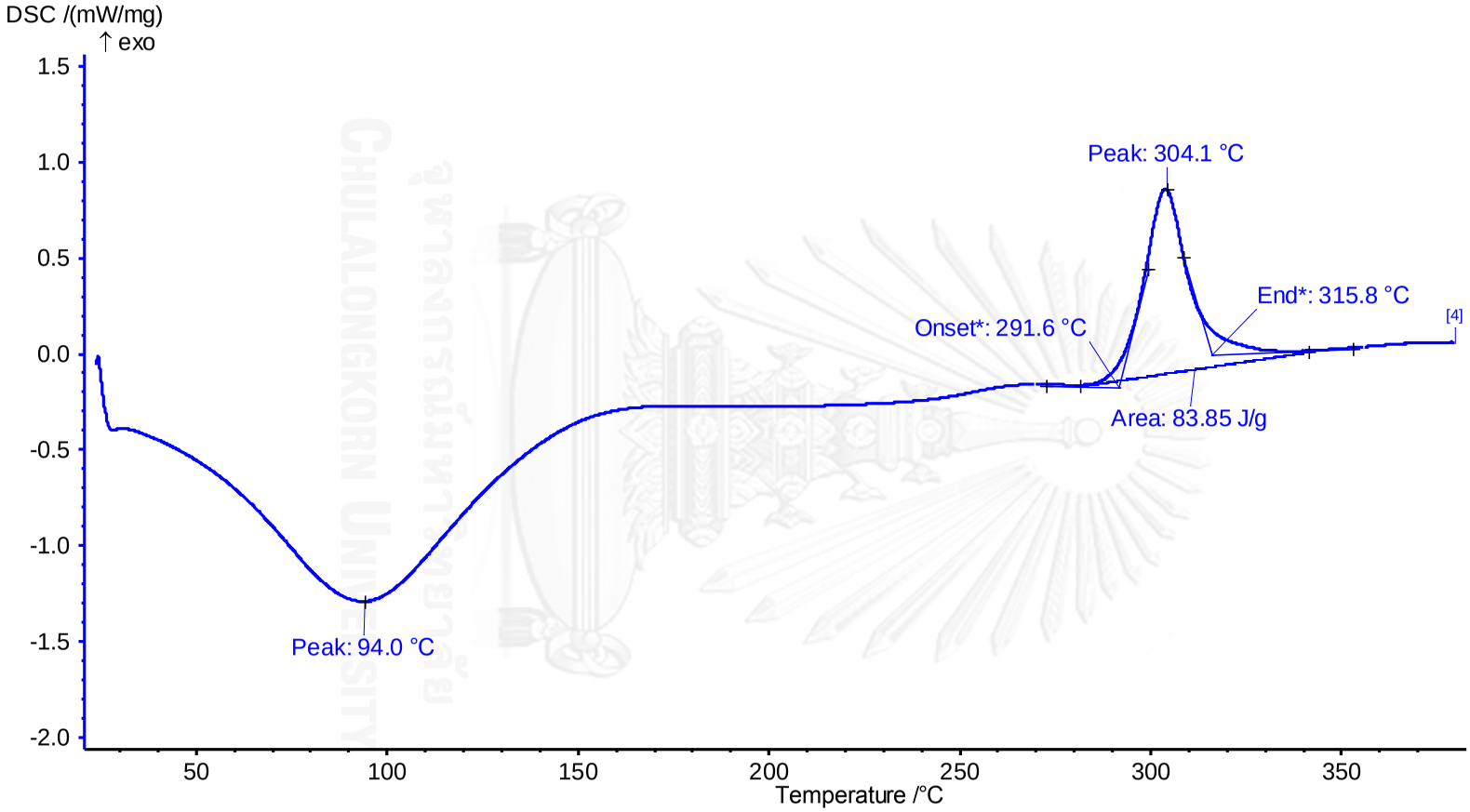


Figure A.19 DSC thermogram of 0.75% (w/w) pure KGM film

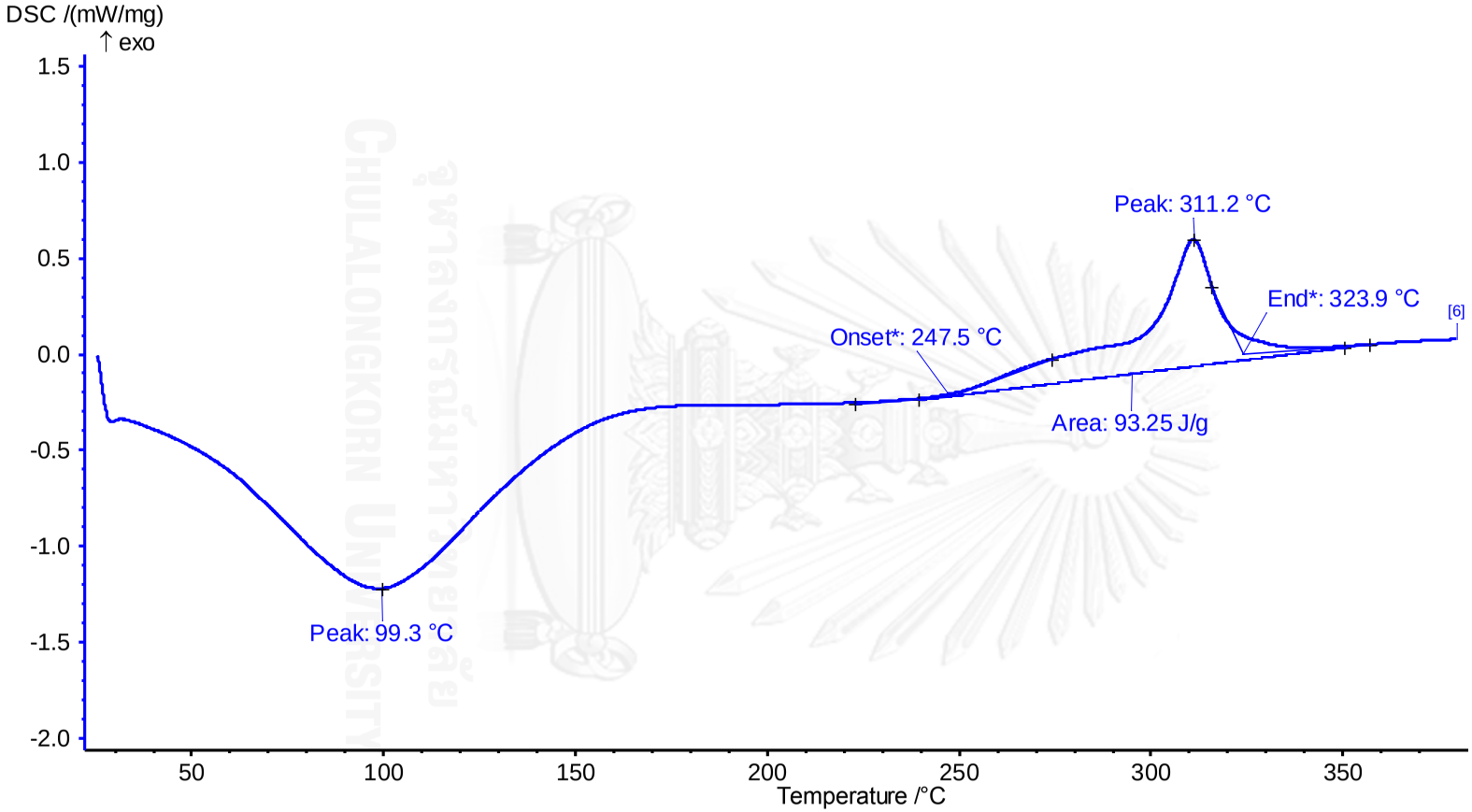


Figure A.20 DSC thermogram of 1.00% (w/w) pure KGM film

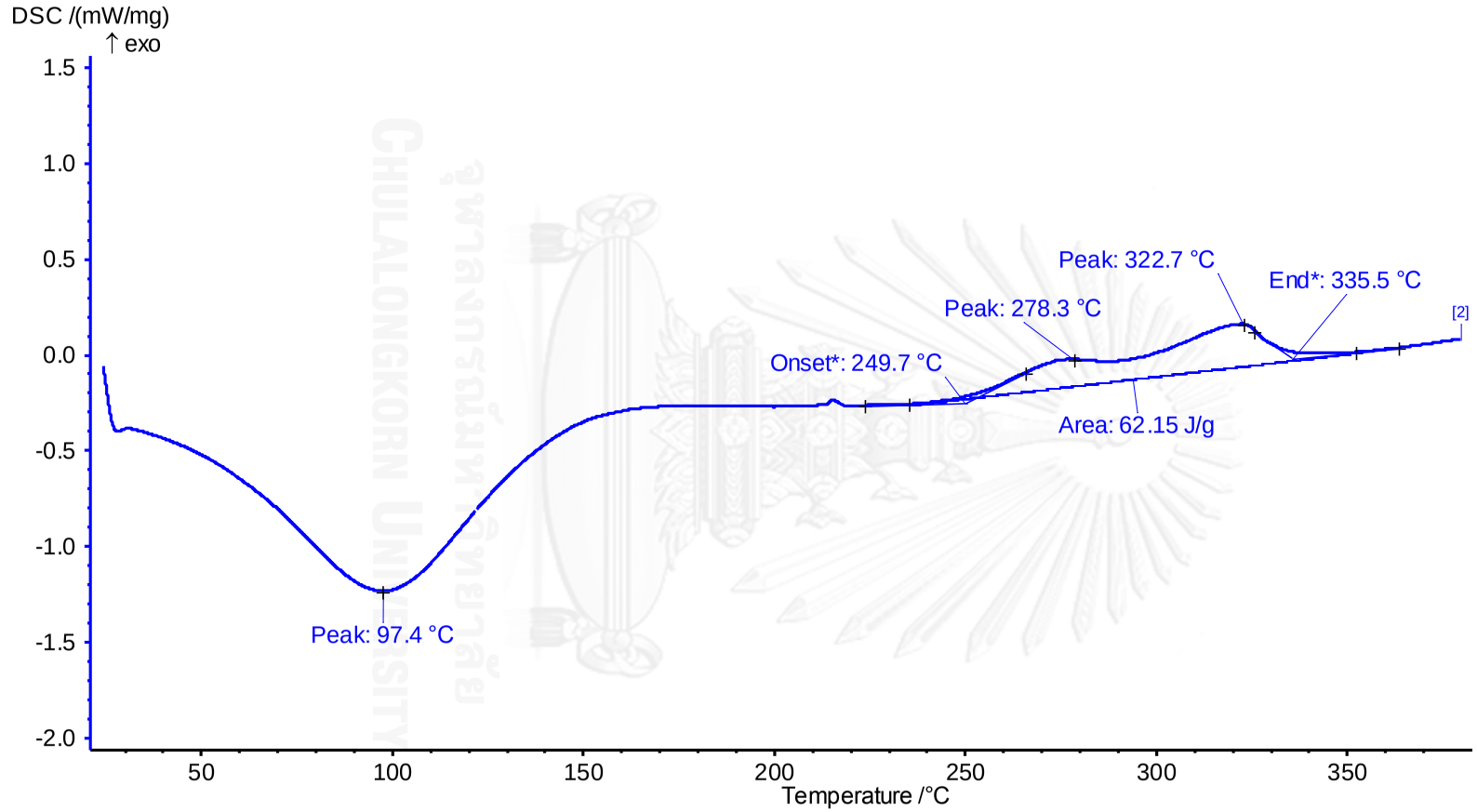


Figure A.21 DSC thermogram of KGM/EU film in the ratio of KGM:EU 0.50%:0.50% (w/w)

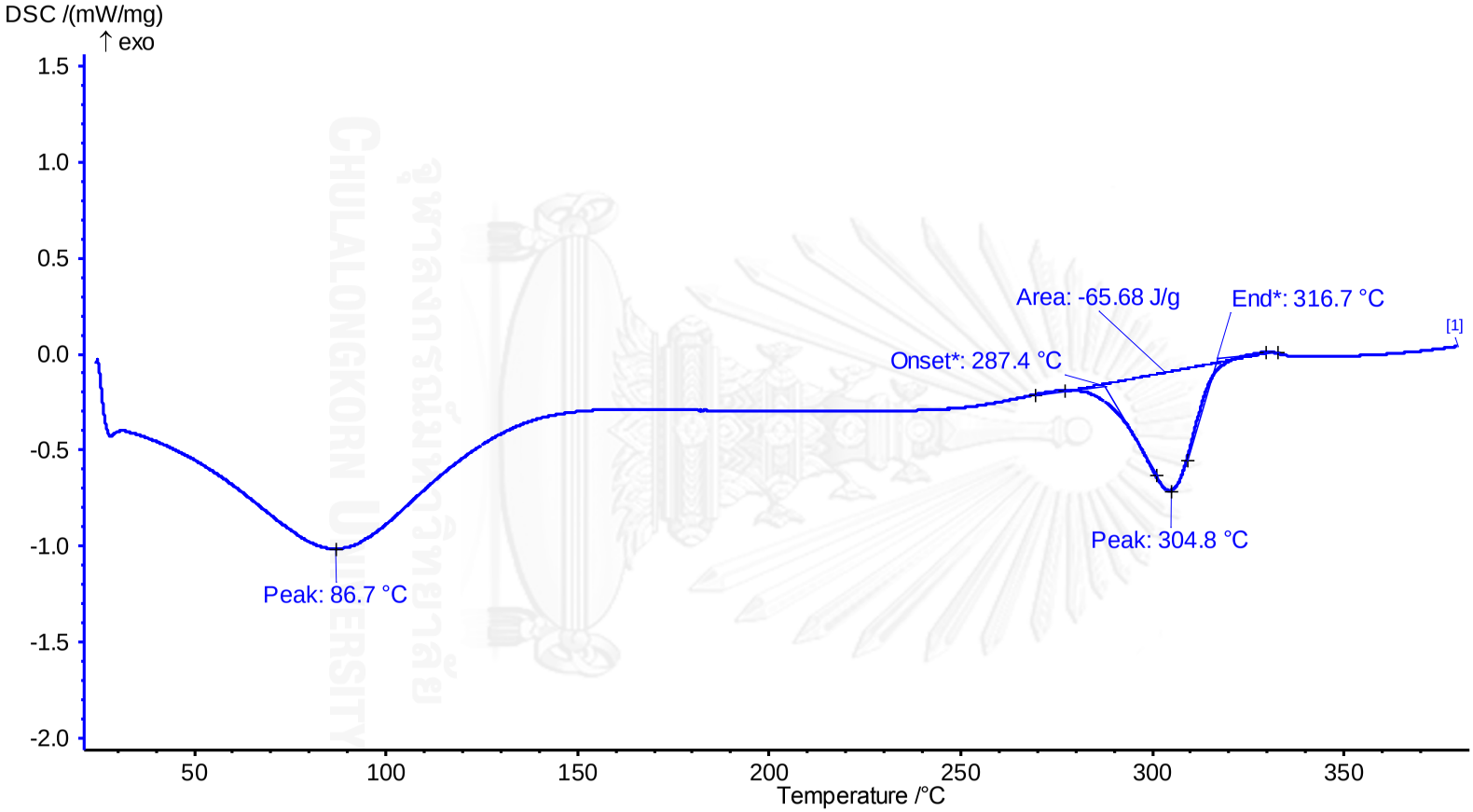


Figure A.22 DSC thermogram of KGM/EU film in the ratio of KGM:EU 0.50%:1.00% (w/w)

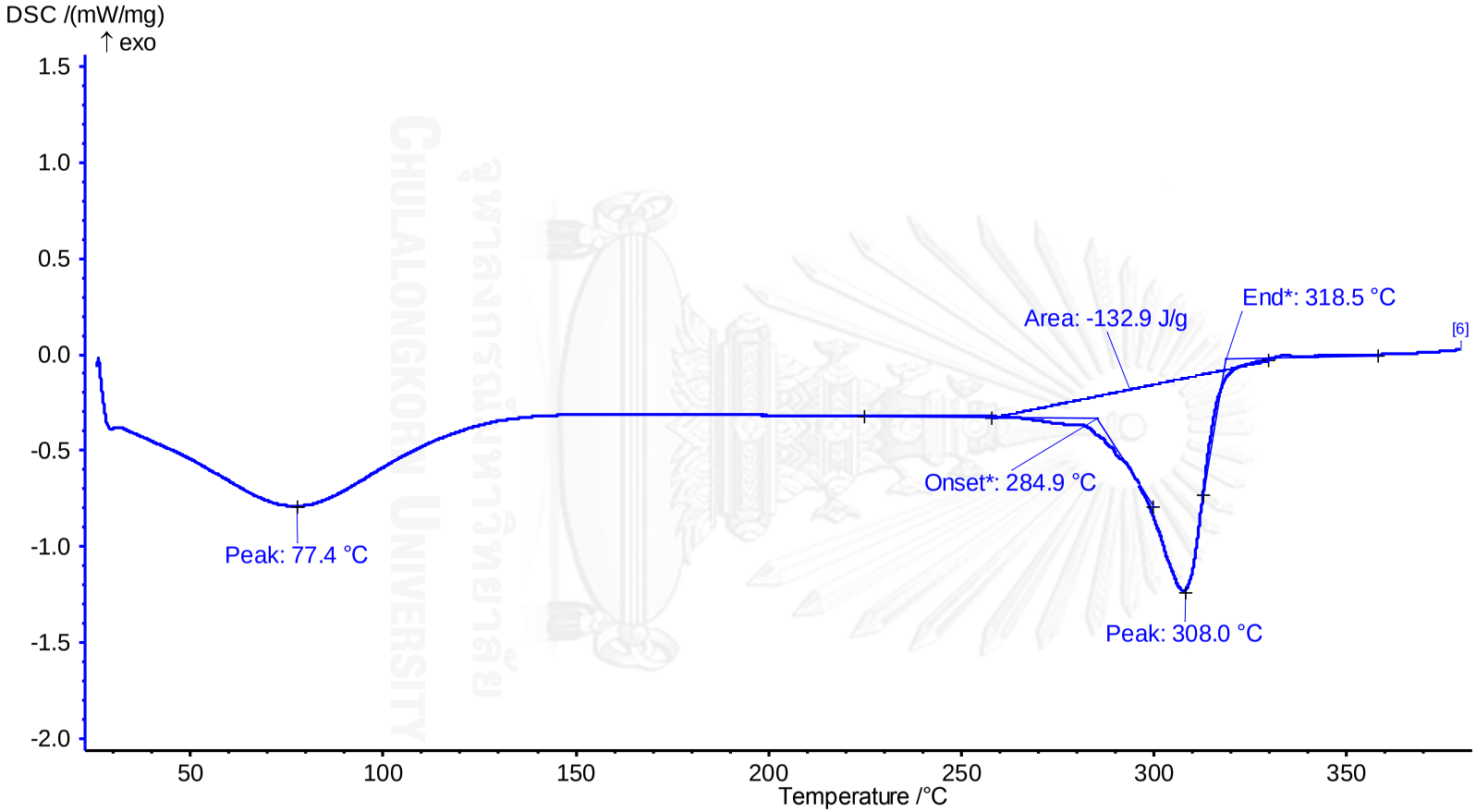


Figure A.23 DSC thermogram of KGM/EU film in the ratio of KGM:EU 0.50%:1.50% (w/w)

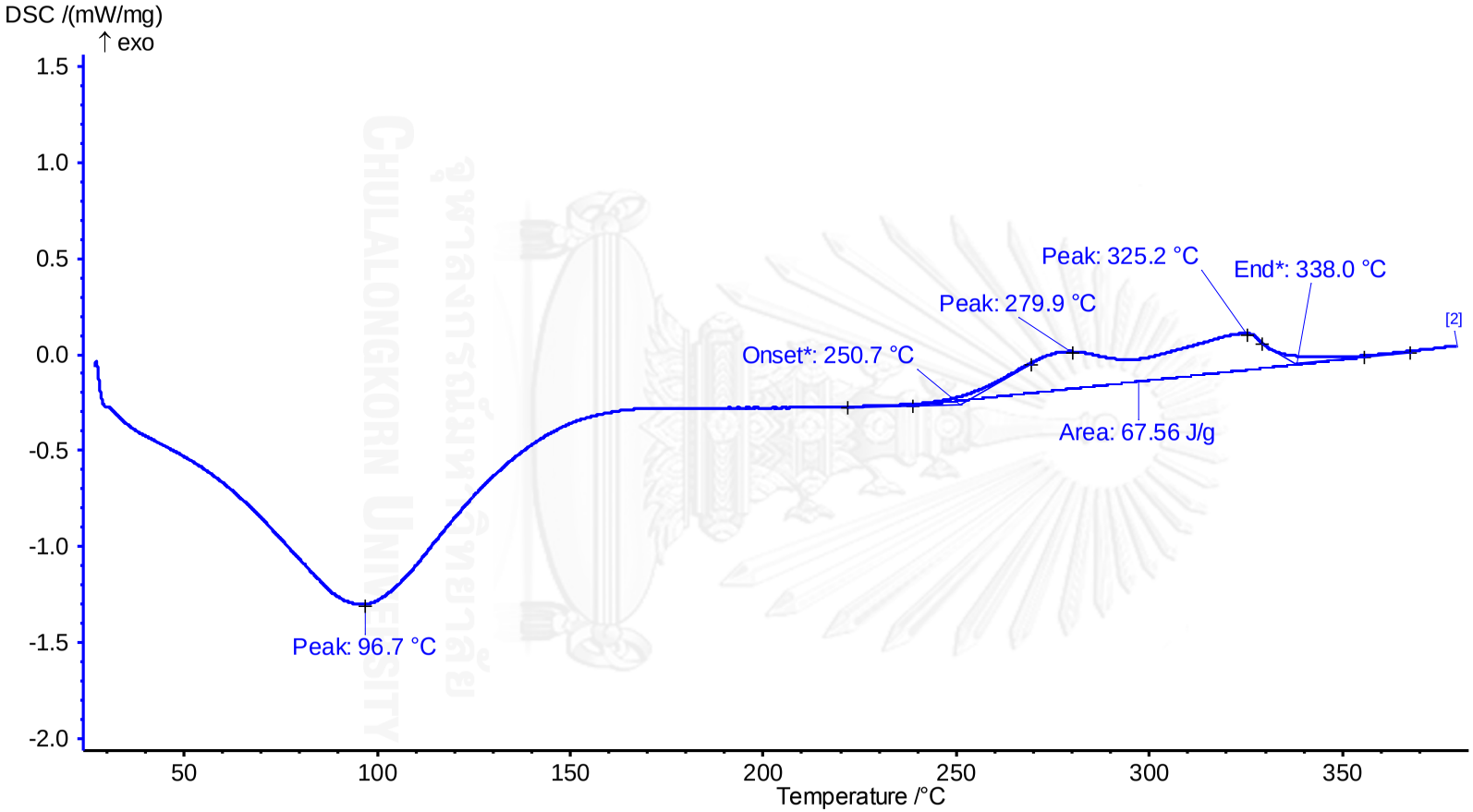


Figure A.24 DSC thermogram of KGM/EU film in the ratio of KGM:EU 0.75%:0.50% (w/w)

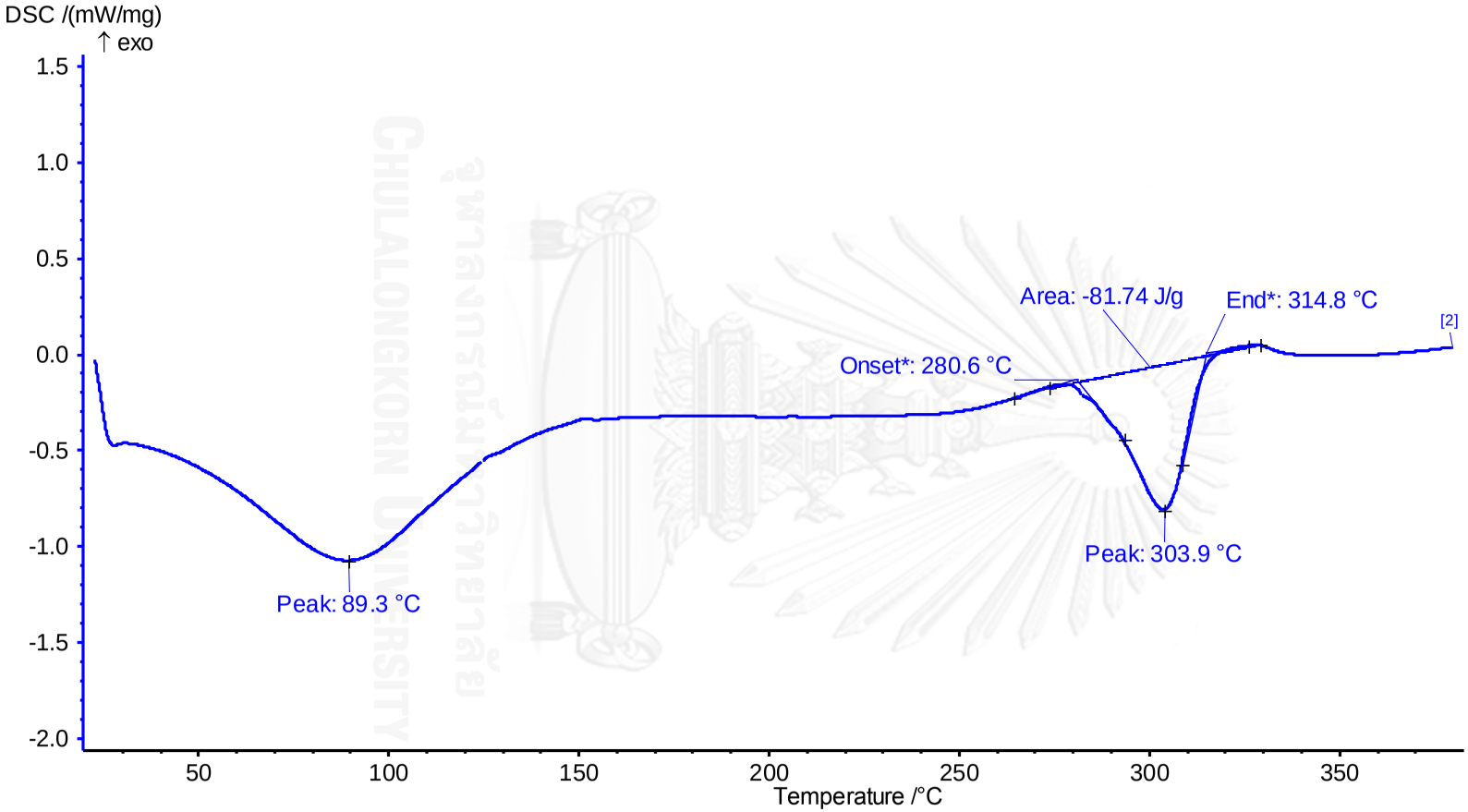


Figure A.25 DSC thermogram of KGM/EU film in the ratio of KGM:EU 0.75%:1.00% (w/w)

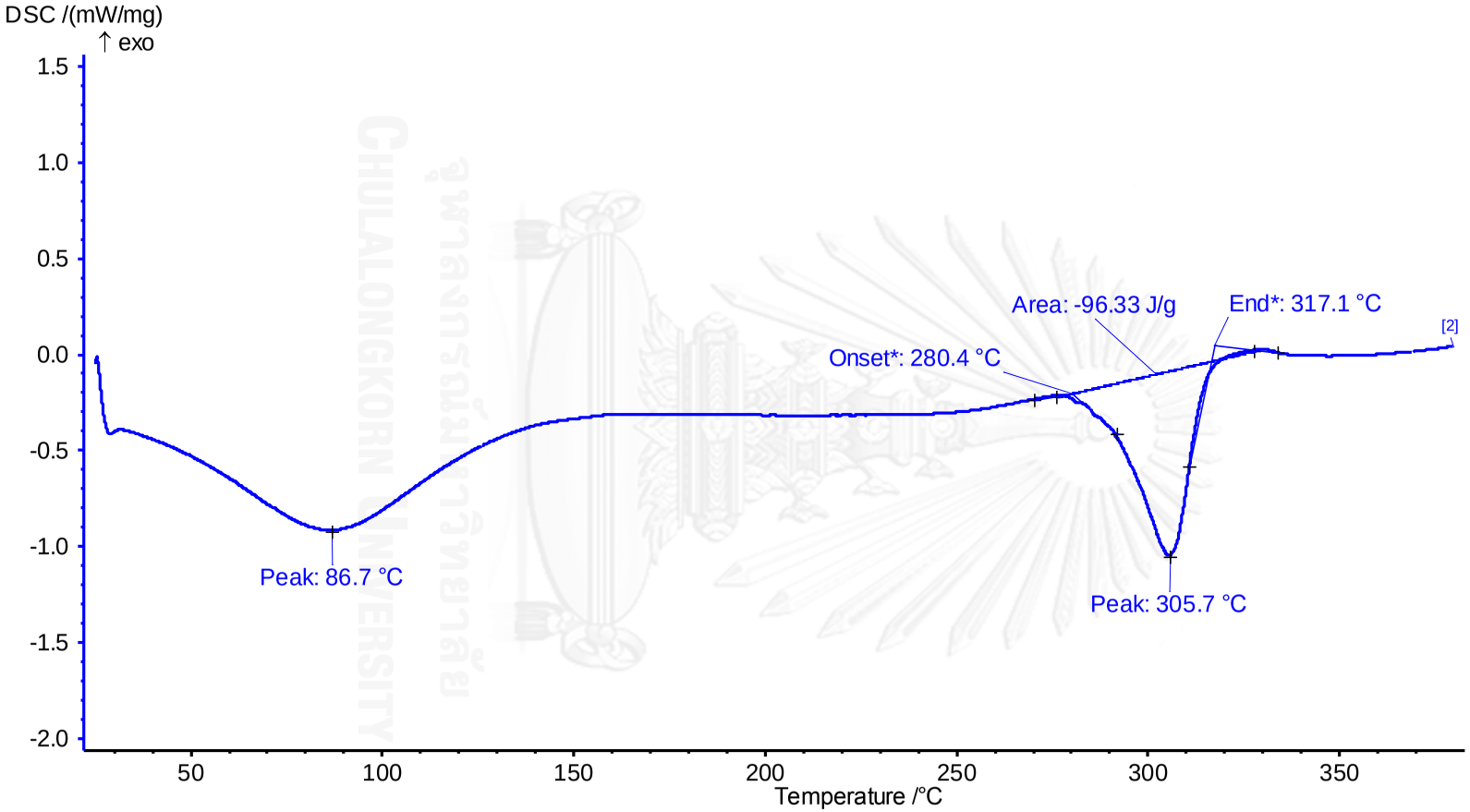


Figure A.26 DSC thermogram of KGM/EU film in the ratio of KGM:EU 0.75%:1.50% (w/w)

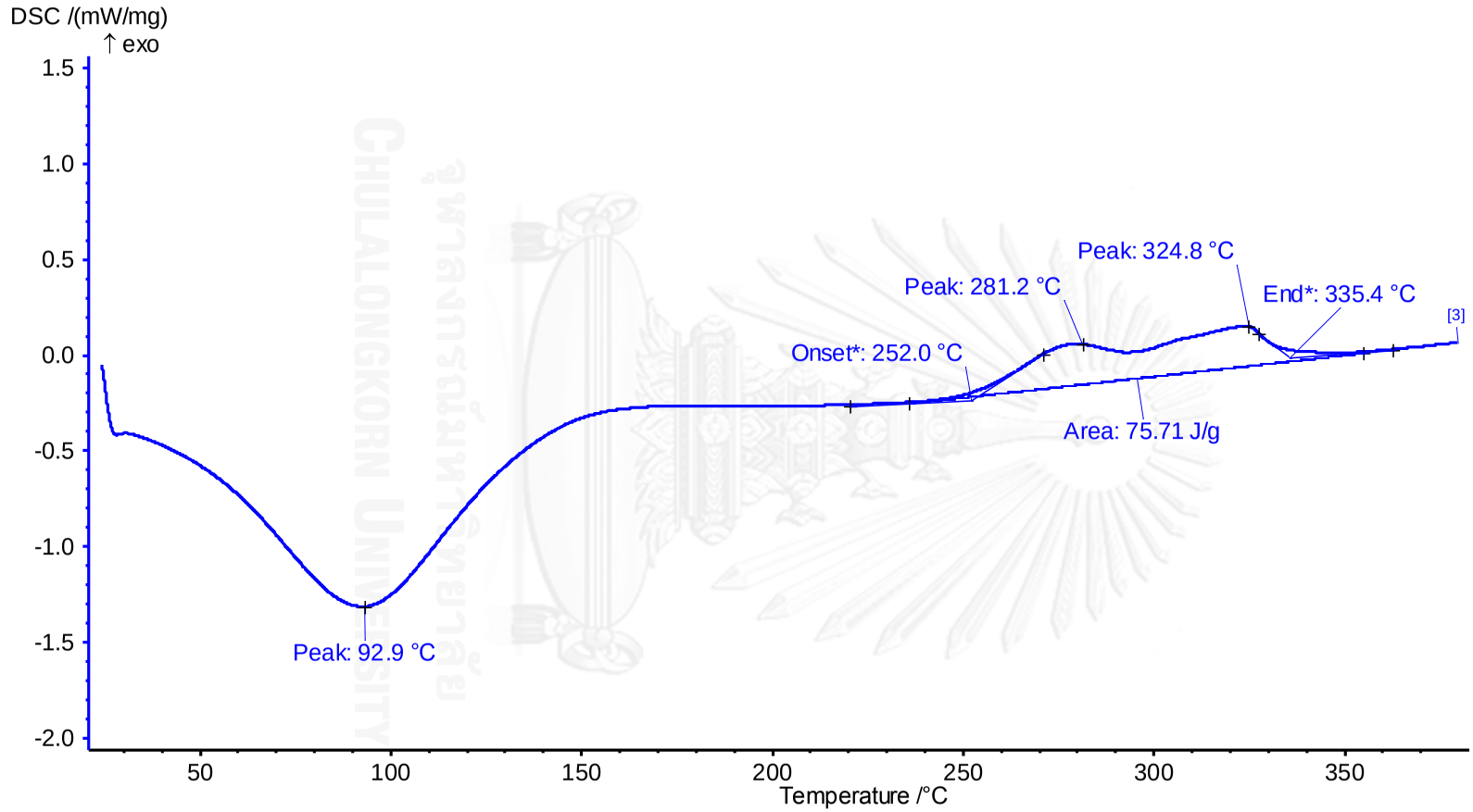


Figure A.27 DSC thermogram of KGM/EU film in the ratio of KGM:EU 1.00%:0.50% (w/w)

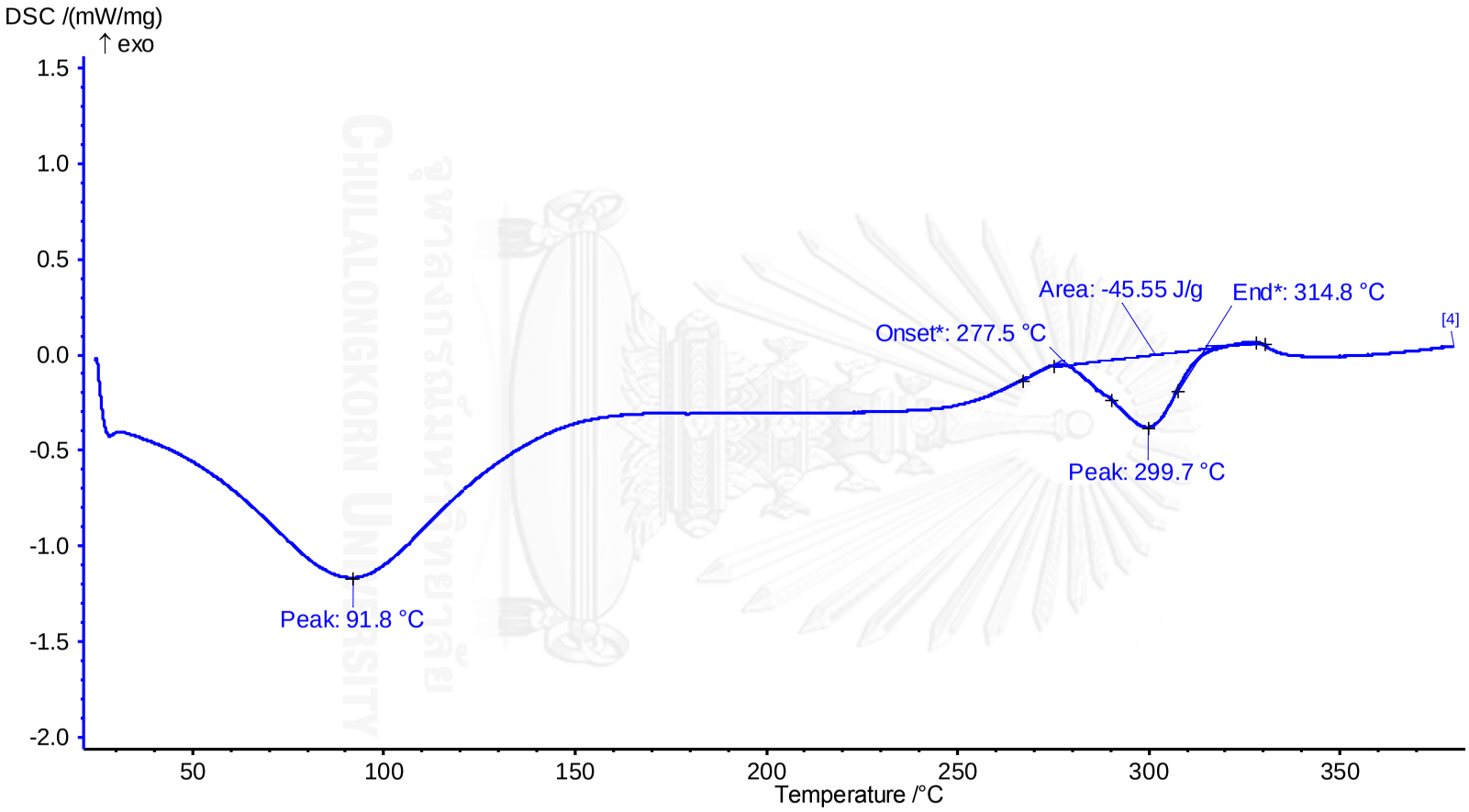


Figure A.28 DSC thermogram of KGM/EU film in the ratio of KGM:EU 1.00%:1.00% (w/w)

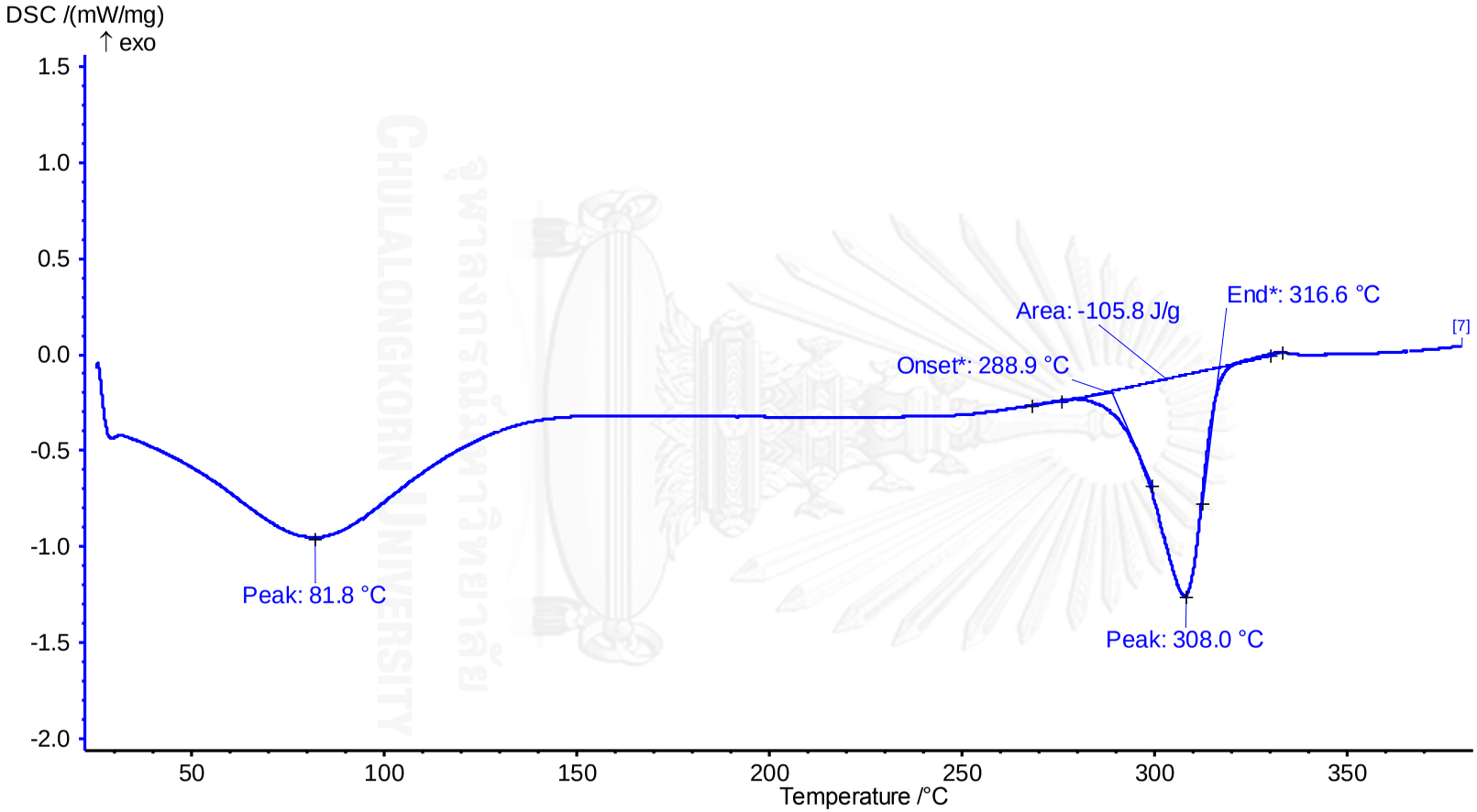


Figure A.29 DSC thermogram of KGM/EU film in the ratio of KGM:EU 1.00%:1.50% (w/w)

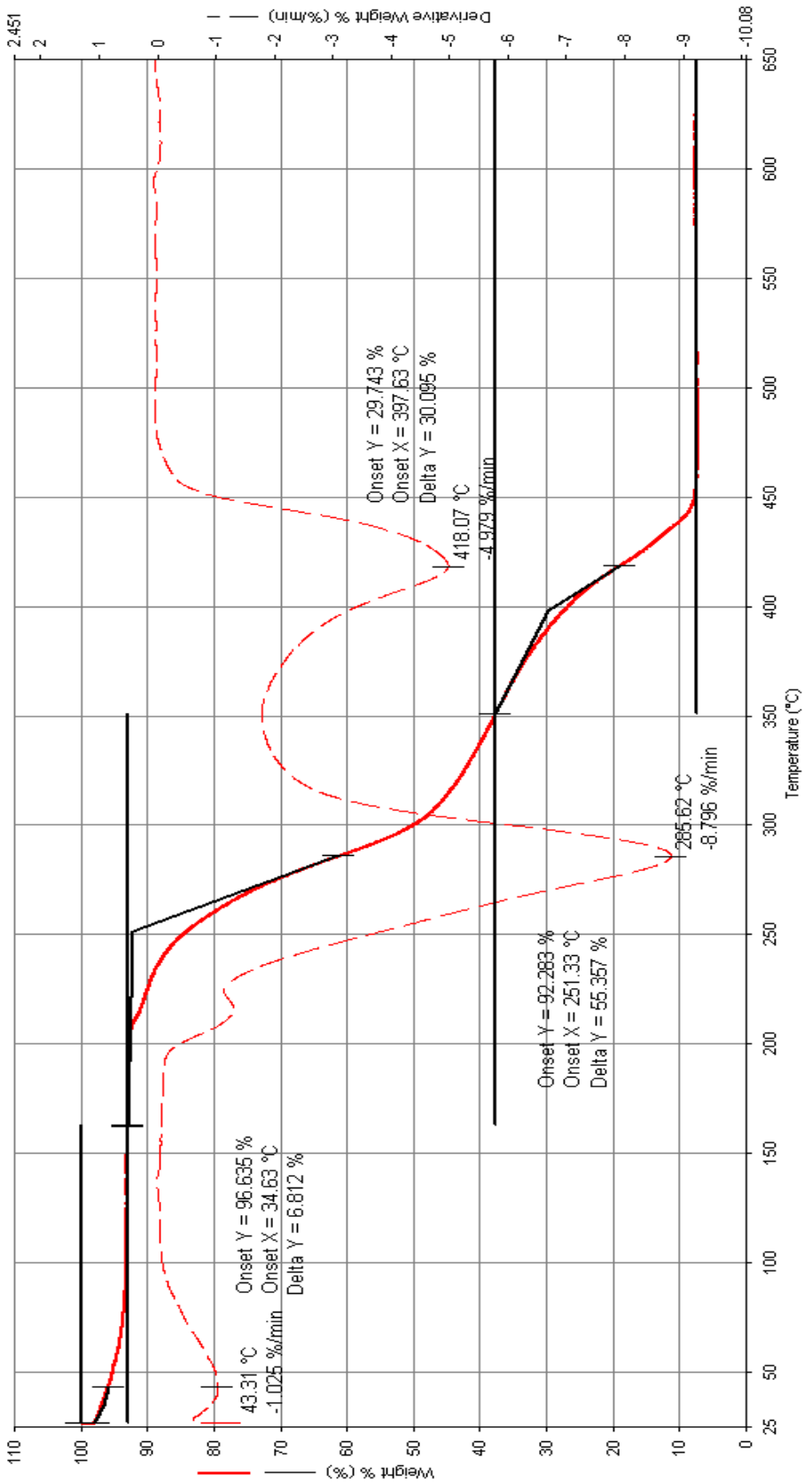


Figure A.30 TG and DTG curve of 0.75% (w/w) KGM film

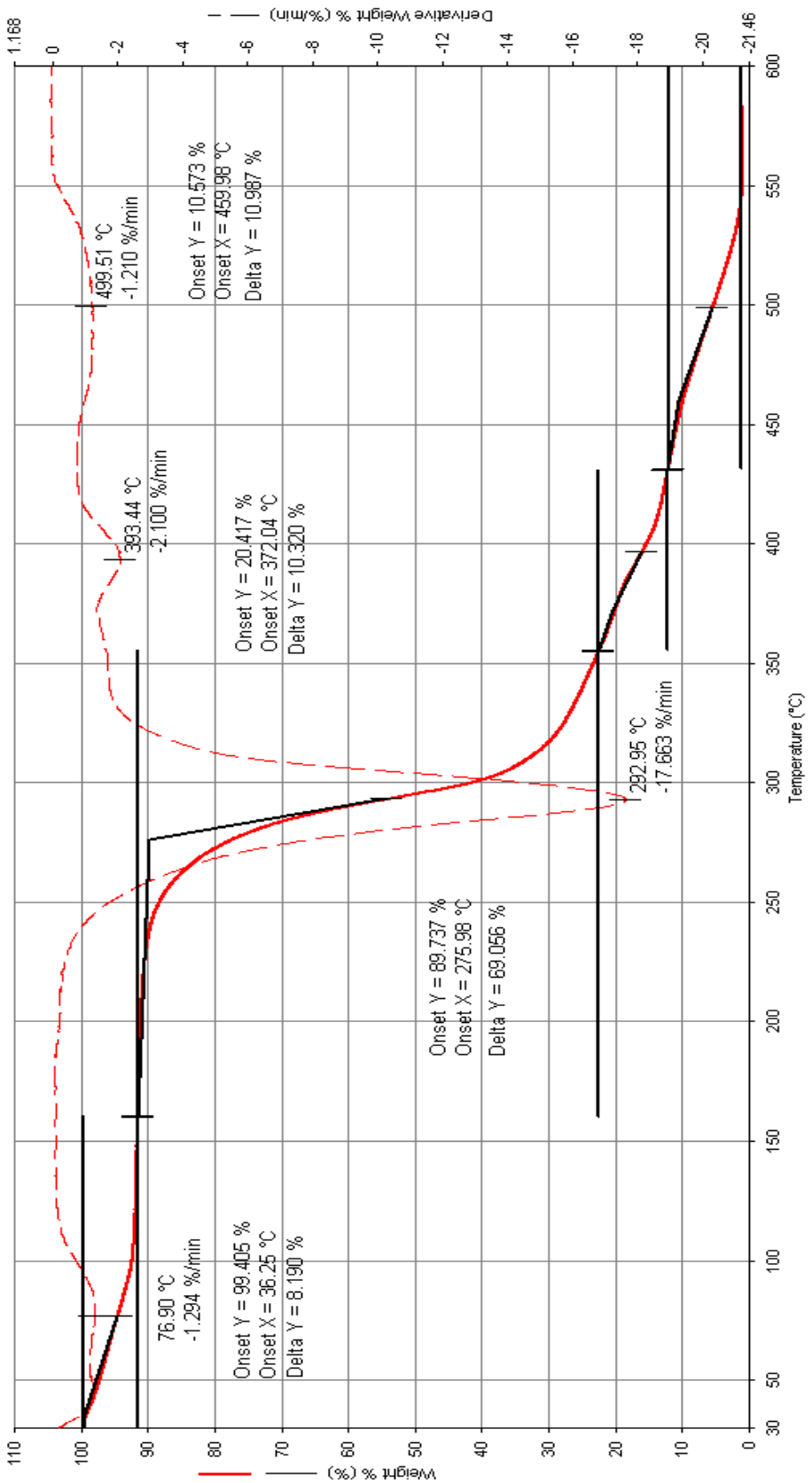


Figure A.31 TG and DTG curve of KGM/EU film in the ratio of KGM:EU 0.75%:1.50% (w/w) at day 0

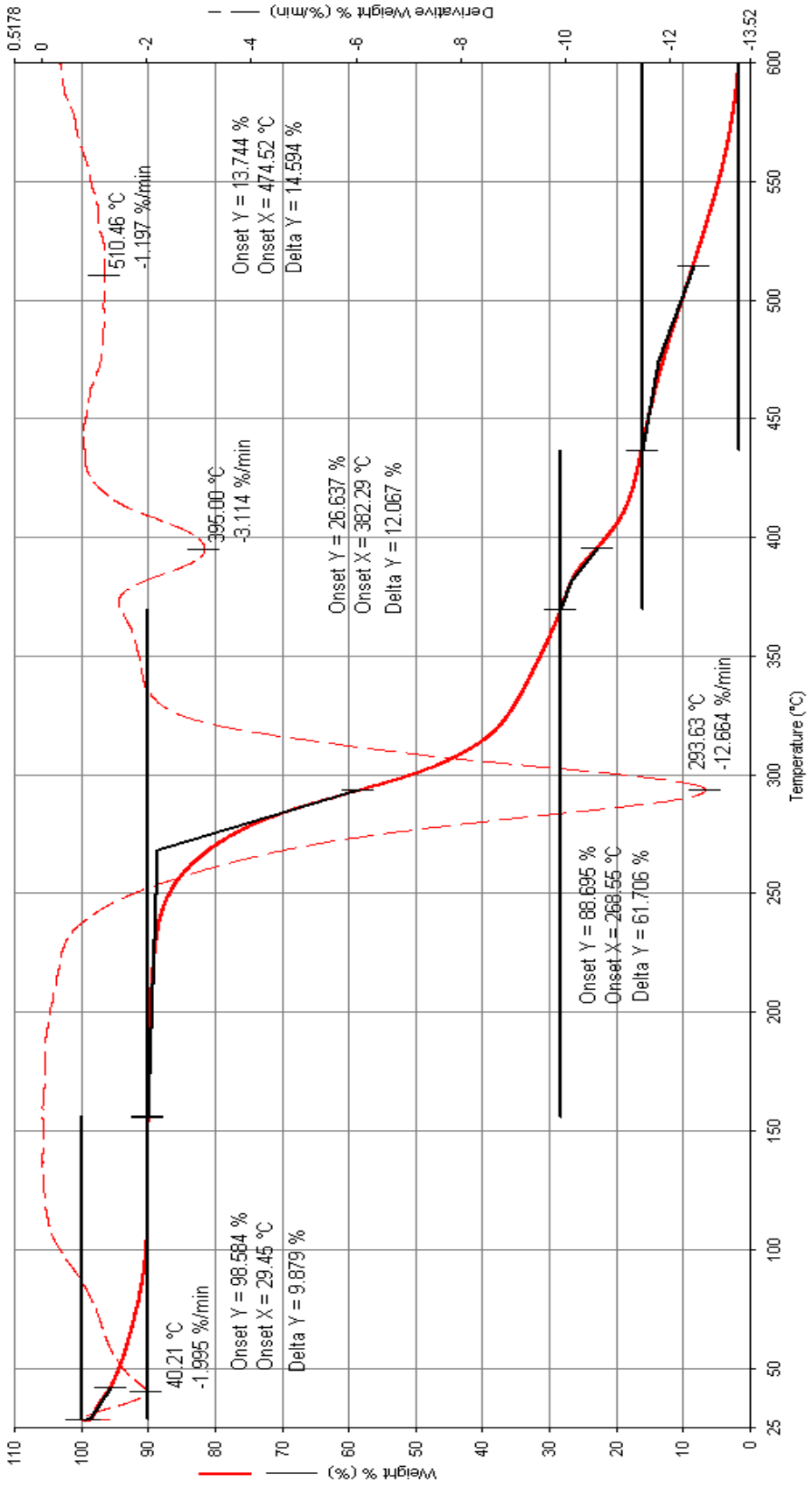


Figure A.32 TG and DTG curve of KGM/EU film in the ratio of KGM:EU 0.75%:1.50% (w/w) at day 20

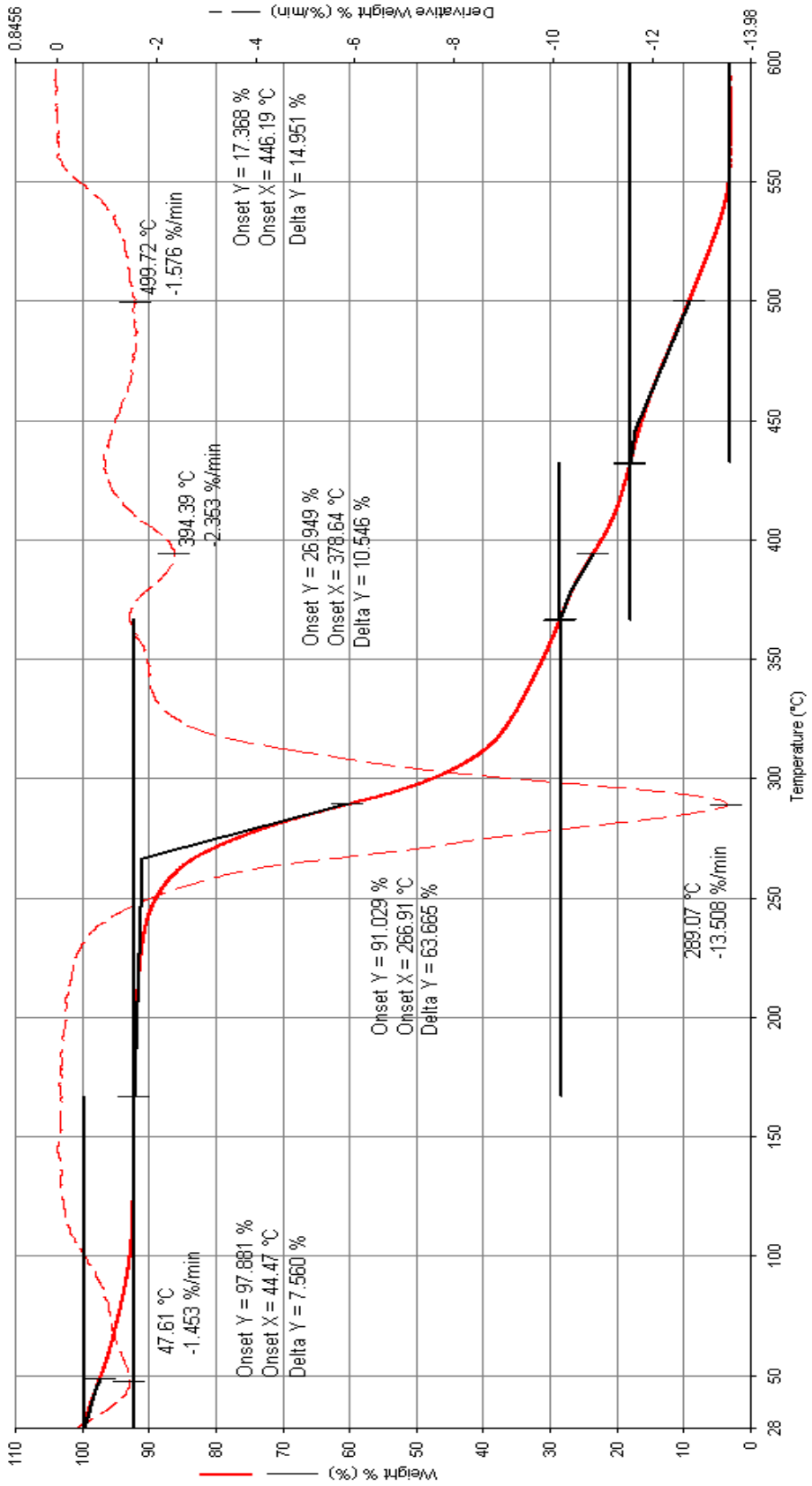


Figure A.33 TG and DTG curve of KGM/EU film in the ratio of KGM:EU 0.75%:1.50% (w/w) at day 40

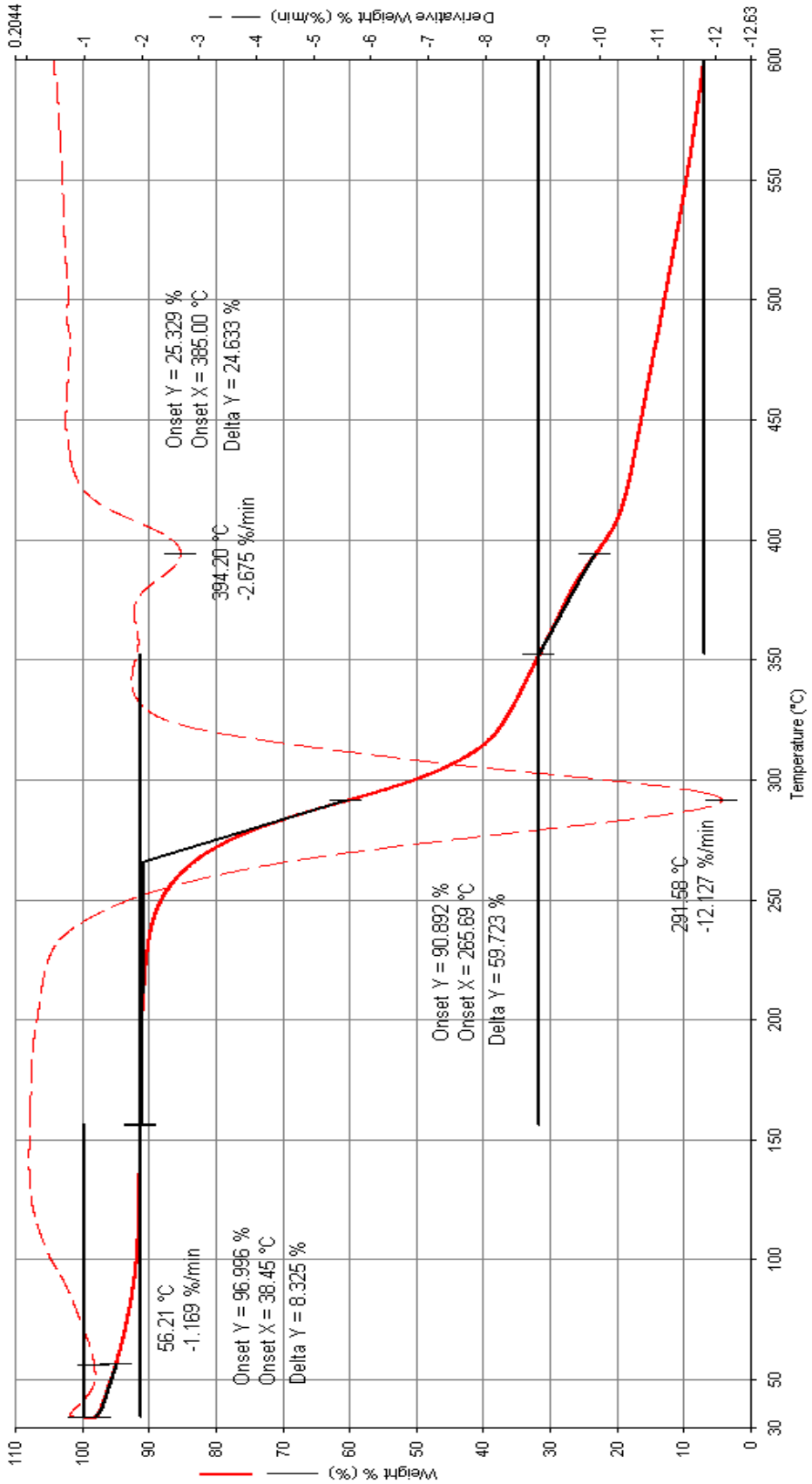


Figure A.34 TG and DTG curve of KGM/EU film in the ratio of KGM:EU 0.75%:1.50% (w/w) at day 60

APPENDIX B

STATISTICAL ANALYSIS

Table B.1 The ANOVA table showing effects of KGM and EU on thickness of KGM/EU films at $p \leq 0.05$

Dependent Variable: Thickness					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2105.705[a]	11	191.428	20.606	.000
Intercept	55498.722	1	55498.722	5974.187	.000
KGM	1430.365	2	715.183	76.986	.000
EU	564.725	3	188.242	20.263	.000
KGM * EU	110.615	6	18.436	1.985	.108
Error	222.954	24	9.290		
Total	57827.381	36			
Corrected Total	2328.659	35			

a. R Squared = .904 (Adjusted R Squared = .860)

Table B.2 The ANOVA table showing effects of treatment on thickness of KGM/EU films at $p \leq 0.05$

Dependent Variable: Thickness					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2105.705[a]	11	191.428	20.606	.000
Intercept	55498.722	1	55498.722	5974.187	.000
Treatment	2105.705	11	191.428	20.606	.000
Error	222.954	24	9.290		
Total	57827.381	36			
Corrected Total	2328.659	35			

a. R Squared = .904 (Adjusted R Squared = .860)

Table B.3 The ANOVA table showing effects of KGM and EU on TS of KGM/EU films at $p \leq 0.05$

Dependent Variable: TS					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1034.326[a]	11	94.030	14.702	.000
Intercept	18589.050	1	18589.050	2906.488	.000
KGM	639.013	2	319.507	49.956	.000
EU	85.161	3	28.387	4.438	.013
KGM * EU	310.152	6	51.692	8.082	.000
Error	153.497	24	6.396		
Total	19776.873	36			
Corrected Total	1187.823	35			

a. R Squared = .871 (Adjusted R Squared = .812)

Table B.4 The ANOVA table showing effects of treatment on TS of KGM/EU films at $p \leq 0.05$

Dependent Variable: TS					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1034.326[a]	11	94.030	14.702	.000
Intercept	18589.050	1	18589.050	2906.488	.000
Treatment	1034.326	11	94.030	14.702	.000
Error	153.497	24	6.396		
Total	19776.873	36			
Corrected Total	1187.823	35			

a. R Squared = .871 (Adjusted R Squared = .812)

Table B.5 The ANOVA table showing effects of KGM and EU on EB of KGM/EU films at $p \leq 0.05$

Dependent Variable: EB					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	183.588[a]	11	16.690	14.953	.000
Intercept	1028.592	1	1028.592	921.533	.000
KGM	20.594	2	10.297	9.225	.001
EU	104.420	3	34.807	31.184	.000
KGM * EU	58.573	6	9.762	8.746	.000
Error	26.788	24	1.116		
Total	1238.968	36			
Corrected Total	210.376	35			

a. R Squared = .873 (Adjusted R Squared = .814)

Table B.6 The ANOVA table showing effects of treatment on EB of KGM films encapsulated with EU at $p \leq 0.05$

Dependent Variable: EB					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	183.588[a]	11	16.690	14.953	.000
Intercept	1028.592	1	1028.592	921.533	.000
Treatment	183.588	11	16.690	14.953	.000
Error	26.788	24	1.116		
Total	1238.968	36			
Corrected Total	210.376	35			

a. R Squared = .873 (Adjusted R Squared = .814)

Table B.7 The ANOVA table showing effects of KGM and EU on YM of KGM/EU films at $p \leq 0.05$

Dependent Variable: YM					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	7.263E6[a]	11	660261.519	38.614	.000
Intercept	1.792E7	1	1.792E7	1048.252	.000
KGM	1161925.559	2	580962.779	33.976	.000
EU	5596915.423	3	1865638.474	109.108	.000
KGM * EU	504035.731	6	84005.955	4.913	.002
Error	410376.591	24	17099.025		
Total	2.560E7	36			
Corrected Total	7673253.303	35			

a. R Squared = .947 (Adjusted R Squared = .922)

Table B.8 The ANOVA table showing effects of treatment on YM of KGM/EU films at $p \leq 0.05$

Dependent Variable: YM					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	7.263E6[a]	11	660261.519	38.614	.000
Intercept	1.792E7	1	1.792E7	1048.252	.000
Treatment	7262876.712	11	660261.519	38.614	.000
Error	410376.591	24	17099.025		
Total	2.560E7	36			
Corrected Total	7673253.303	35			

a. R Squared = .947 (Adjusted R Squared = .922)

Table B.9 The ANOVA table showing effects of KGM and EU on WVP of KGM/EU films at $p \leq 0.05$

Dependent Variable: WVP					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	27.170[a]	11	2.470	17.448	.000
Intercept	909.525	1	909.525	6424.838	.000
KGM	20.919	2	10.460	73.887	.000
EU	4.734	3	1.578	11.147	0.000
KGM * EU	1.517	6	.253	1.786	.145
Error	3.398	24	.142		
Total	940.093	36			
Corrected Total	30.568	35			

a. R Squared = .889 (Adjusted R Squared = .838)

Table B.10 The ANOVA table showing effects of treatment on WVP of KGM/EU films at $p \leq 0.05$

Dependent Variable: WVP					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	27.170[a]	11	2.470	17.448	.000
Intercept	909.525	1	909.525	6424.838	.000
Treatment	27.170	11	2.470	17.448	.000
Error	3.398	24	.142		
Total	940.093	36			
Corrected Total	30.568	35			

a. R Squared = .889 (Adjusted R Squared = .838)

Table B.11 The ANOVA table showing effects of KGM and EU on transmittance at 600 nm of KGM/EU films at $p \leq 0.05$

Dependent Variable: Transmittance					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2449.095[a]	11	222.645	17.672	.000
Intercept	213917.042	1	213917.042	16978.906	.000
KGM	225.046	2	112.523	8.931	.001
EU	1871.096	3	623.699	49.504	.000
KGM * EU	352.953	6	58.826	4.669	.003
Error	302.376	24	12.599		
Total	216668.513	36			
Corrected Total	2751.471	35			

a. R Squared = .890 (Adjusted R Squared = .840)

Table B.12 The ANOVA table showing effects of treatment on transmittance at 600 nm of KGM/EU films at $p \leq 0.05$

Dependent Variable: Transmittance					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2449.095[a]	11	222.645	17.672	.000
Intercept	213917.042	1	213917.042	16978.906	.000
Treatment	2449.095	11	222.645	17.672	.000
Error	302.376	24	12.599		
Total	216668.513	36			
Corrected Total	2751.471	35			

a. R Squared = .890 (Adjusted R Squared = .840)

Table B.13 The ANOVA table showing effects of KGM and EU on transparency of KGM/EU films at $p \leq 0.05$

Dependent Variable: Transparency					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6916.512[a]	11	628.775	11.456	.000
Intercept	98959.528	1	98959.528	1802.942	.000
KGM	3724.505	2	1862.252	33.928	.000
EU	2237.985	3	745.995	13.591	.000
KGM * EU	954.031	6	159.005	2.897	.029
Error	1317.307	24	54.888		
Total	107193.356	36			
Corrected Total	8233.828	35			

a. R Squared = .840 (Adjusted R Squared = .767)

Table B.14 The ANOVA table showing effects of treatment on transparency of KGM/EU films at $p \leq 0.05$

Dependent Variable: Transparency					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6916.512[a]	11	628.775	11.456	.000
Intercept	98959.528	1	98959.528	1802.924	.000
Treatment	6916.521	11	628.775	11.456	.000
Error	1317.307	24	54.888		
Total	107193.356	36			
Corrected Total	8233.828	35			

a. R Squared = .840 (Adjusted R Squared = .767)

Table B.15 The ANOVA table showing effects of KGM and EU on solubility of KGM/EU films $p \leq 0.05$

Dependent Variable: Solubility					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	7385.137[a]	11	671.376	78.637	.000
Intercept	61492.427	1	61492.427	7202.536	.000
KGM	1465.877	2	732.938	85.848	.000
EU	5516.750	3	1838.917	215.390	.000
KGM * EU	402.510	6	67.085	7.858	0.000
Error	204.903	24	8.538		
Total	69082.467	36			
Corrected Total	7590.039	35			

a. R Squared = .973 (Adjusted R Squared = .961)

Table B.16 The ANOVA table showing effects of treatment on solubility of KGM/EU films at $p \leq 0.05$

Dependent Variable: Solubility					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	7385.137[a]	11	671.376	78.637	.000
Intercept	61492.427	1	61492.427	7202.536	.000
Treatment	7385.137	11	671.376	78.637	.000
Error	204.903	24	8.538		
Total	69082.467	36			
Corrected Total	7590.039	35			

a. R Squared = .973 (Adjusted R Squared = .961)

Table B.17 The ANOVA table showing effects of KGM and EU on DSC onset temperature of KGM/EU films at $p \leq 0.05$

Dependent Variable: DSC onset temperature					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6928.665[a]	11	629.879	74.733	.000
Intercept	1787713.335	1	1787713.335	212107.574	.000
KGM	821.118	2	410.559	171.350	.000
EU	4332.595	3	1444.198	171.350	.000
KGM * EU	1774.952	6	295.825	35.099	.000
Error	101.140	12	8.428		
Total	1794743.140	24			
Corrected Total	7029.805	23			

a. R Squared = .986 (Adjusted R Squared = .972)

Table B.18 The ANOVA table showing effects of treatment on DSC onset temperature of KGM/EU films at $p \leq 0.05$

Dependent Variable: DSC onset temperature					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6928.665[a]	11	629.879	74.733	.000
Intercept	1787713.335	1	1787713.335	212107.574	.000
Treatment	6928.665	11	629.879	74.733	.000
Error	101.140	12	8.428		
Total	1794743.140	24			
Corrected Total	7029.805	23			

a. R Squared = .986 (Adjusted R Squared = .972)

Table B.19 The ANOVA table showing effects of KGM and EU on DSC peak temperature of KGM/EU films at $p \leq 0.05$

Dependent Variable: DSC peak temperature					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3312.973[a]	11	301.179	105.369	.000
Intercept	2139409.307	1	2139409.307	748481.390	.000
KGM	.231	2	.115	.040	.961
EU	3175.423	3	1058.474	370.312	.000
KGM * EU	137.319	6	22.887	8.007	.001
Error	34.300	12	2.858		
Total	2142756.580	24			
Corrected Total	3347.273	23			

a. R Squared = .990 (Adjusted R Squared = .980

Table B.20 The ANOVA table showing effects of treatment on DSC peak temperature of KGM/EU films at $p \leq 0.05$

Dependent Variable: DSC peak temperature					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3312.973[a]	11	301.179	105.369	.000
Intercept	2139409.307	1	2139409.307	748481.390	.000
Treatment	3312.973	11	301.179	105.369	.000
Error	34.300	12	2.858		
Total	2142756.580	24			
Corrected Total	3347.273	25			

a. R Squared = .990 (Adjusted R Squared = .980)

Table B.21 The ANOVA table showing effects of KGM and EU on DSC enthalpy of KGM/EU films at $p \leq 0.05$

Dependent Variable: DSC enthalpy					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	188049.384[a]	11	17095.399	290.282	.000
Intercept	318.573	1	318.573	5.409	.038
KGM	1866.416	2	933.208	15.846	.000
EU	184575.426	3	61525.142	1044.706	.000
KGM * EU	1607.707	6	267.924	4.549	.012
Error	706.707	12	58.892		
Total	189074.665	24			
Corrected Total	188756.092	23			

a. R Squared = .996 (Adjusted R Squared = .993)

Table B.22 The ANOVA table showing effects of treatment on DSC enthalpy of KGM/EU films at $p \leq 0.05$

Dependent Variable: DSC enthalpy					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	188049.384[a]	11	17095.399	290.282	.000
Intercept	318.573	1	318.573	5.409	.038
Treatment	188049.384	11	17095.399	290.282	.000
Error	706.707	12	58.892		
Total	189074.665	24			
Corrected Total	188756.092	23			

a. R Squared = .996 (Adjusted R Squared = .993)

Table B.23 The ANOVA table showing effects of KGM and EU on EE (%) of KGM/EU films at $p \leq 0.05$

Dependent Variable: %EE					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	20585.137[a]	8	2573.142	148.522	.000
Intercept	77134.230	1	77134.230	4452.198	.000
KGM	81.842	2	40.921	2.362	.123
EU	20476.814	2	10238.407	590.962	.000
KGM * EU	26.481	4	6.620	.382	.818
Error	311.850	18	17.325		
Total	98031.216	27			
Corrected Total	20896.987	26			

a. R Squared = .985 (Adjusted R Squared = .978)

Table B.24 The ANOVA table showing effects of treatment on EE (%) of KGM/EU films at $p \leq 0.05$

Dependent Variable: %EE					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	20585.137[a]	8	2573.142	148.522	.000
Intercept	77134.230	1	77134.230	4452.198	.000
Treatment	20585.137	8	2573.142	148.522	.000
Error	311.850	18	17.325		
Total	98031.216	27			
Corrected Total	20896.987	26			

a. R Squared = .985 (Adjusted R Squared = .978)

Table B.25 The ANOVA table showing EE (%) in KGM/EU films at KGM:EU 0.75:1.50 at month 0, 7, and 8 at $p \leq 0.05$

Dependent Variable: %EE(storage)					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4156.741[a]	2	2078.371	344.610	.000
Intercept	75009.798	1	75009.798	12437.224	.000
Month	4156.741	2	2078.371	344.610	.000
Error	108.559	18	6.031		
Total	89116.630	21			
Corrected Total	4265.301	20			

a. R Squared = .975 (Adjusted R Squared = .972)

VITA

Miss Jarupan Vatee was born on November 3th, 1988 at Chulalongkorn Hospital, Bangkok, Thailand. She graduated Bachelor degree of Science in Food Technology in 2010 with first class honors from Chulalongkorn University.

In 2011, she applied the Graduate School at Chulalongkorn University and got the CHULALONGKORN UNIVERSITY GRADUATE SCHOLARSHIP TO COMMEMORATE THE 72nd ANNIVERSARY OF HIS MAJESTY KING BHUMIBOL ADULYADEJ and THE 90TH ANNIVERSARY OF CHULALONGKORN UNIVERSITY FUND (Rachadaphiseksomphot Endowment Fund).

In August 2013, she attended and had an oral presentation at The 5th International Conference on Fermentation Technology for Value Added Agricultural Products at Khon Kaen University, Thailand.