Preparation and Property Improvement of Biodegradable Rice Starch Films with Oil Palm Empty Fruit Bunch's Lignin

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ชื่อวิทยานิพนธ์ การเตรียมและการปรับปรุงสมบัติของฟิล์มสตาร์ชย่อยสลายได้ด้วยลิกนินจากทะลายปาล์มเปล่า
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บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อปรับปรุงสมบัติของฟิล์มย่อยสลายได้จากสตาร์ชข้าวเจ้าด้วยการเติมลิกนินที่สกัดจากทะลายปาล์มเปล่า (oil palm empty fruit bunch’s lignin, OPEFB’s lignin) โดยการศึกษาเริ่มต้นจากการสกัดและวิเคราะห์คุณลักษณะของสตาร์ชข้าวเจ้าและ OPEFB’s lignin พบว่าองค์ประกอบหลักของสตาร์ชข้าวเจ้าคือคาร์โบไฮเดรตซึ่งมีร้อยละ 85.79 และส่วนประกอบอื่นๆ จำนวนเล็กน้อยเช่น โปรตีน ไขมัน ความชื้นและเถ้า (ร้อยละ 0.43, 0.51, 13.04 และ 0.23 ตามลำดับ) ขณะที่ ลักษณะของ OPEFB’s lignin ที่สกัดได้มีลักษณะเป็นผงสีน้ำตาลเข้มและเมื่อวิเคราะห์หมู่ฟังก์ชันด้วยเครื่อง FTIR พบว่า มีหมู่ฟังก์ชันที่เหมือนกับลิกนินทางการค้า และผลการศึกษาความสามารถในการต้านเชื้อจุลินทรีย์ของ OPEFB’s lignin พบว่า OPEFB’s lignin สามารถยับยั้งการเจริญเติบโตของเชื้อจุลินทรีย์ทั้ง 3 ชนิดคือ *Staphylococcus aureus, Escherichia coli* O175:H7 และ *Listeria monocytogenes* โดยมีค่าความเข้มข้นต่ำสุดที่สามารถทำลายจุลินทรีย์ข้างต้น (minimal bactericidal concentration, MBC) เท่ากับ 3.12, 25 และ 25 มิลลิกรัม/มิลลิลิตร ตามลำดับ

จากการศึกษาการเติม OPEFB’s lignin ในปริมาณร้อยละ 1, 3, 5, 7 และ 10 ของน้ำหนักสตาร์ชต่อสมบัติทางกายภาพ สมบัติเชิงกลและสมบัติการขวางกั้นไอน้ำของฟิล์มสตาร์ชข้าวเจ้าพบว่า ค่าการต้านทานแรงดึงสูงสุด ค่าการต้านทานการซึมผ่านไอน้ำ ค่าความขุ่นและความเป็นสีน้ำตาลของฟิล์มเพิ่มขึ้นเมื่อเติมและปริมาณ OPEFB’s lignin เพิ่มขึ้นจนถึงร้อยละ 5 ขณะที่ค่าการละลายของฟิล์มมีค่าลดลงอย่างมีนัยสำคัญ ($p<0.05$) นอกจากนี้การเติมและการเพิ่มปริมาณของ OPEFB’s lignin ยังส่งผลให้สมบัติเชิงความร้อนของฟิล์มสตาร์ชข้าวเจ้าเพิ่มขึ้นด้วย อย่างไรก็ตามการเติม OPEFB’s lignin มากกว่าร้อยละ 5 ของน้ำหนักสตาร์ชส่งผลให้สมบัติของฟิล์มที่ได้มีสมบัติตรงกันข้าม การเติมและปริมาณ OPEFB’s lignin ไม่ส่งผลต่อค่าการยืดตัวเมื่อขาดของฟิล์มอย่างมีนัยสำคัญ ($p>0.05$) เมื่อพิจารณาความสามารถในการต้านเชื้อจุลินทรีย์ของฟิล์มสตาร์ชข้าวเจ้าที่เติม OPEFB’s lignin พบว่าฟิล์มสตาร์ชข้าวเจ้าที่เติม OPEFB’s lignin ร้อยละ 10 สามารถยับยั้งการเจริญเติบโตของเชื้อ *S. aureus* แต่ไม่สามารถยับยั้งการเจริญเติบโตของเชื้อ *E. coli* และเชื้อ

L. monocytogenes ซึ่งจากผลการทดลองข้างต้นสามารถกล่าวได้ว่า OPEFB’s lignin ร้อยละ 5 ให้สมบัติเชิงกลและสมบัติการต้านทานการซึมผ่านไอน้ำเหมาะสมที่สุด

การปรับปรุงสมบัติความไม่ชอบน้ำของ OPEFB’s lignin โดยการสังเคราะห์เป็นอนุพันธ์ของ OPEFB’s lignin (OPEFB’s lignin derivatives) ซึ่งเป็นการนำ OPEFB’s lignin มาทำปฏิกิริยากับน้ำมันปาล์มและน้ำมันถั่วเหลือง (อัตราส่วนระหว่าง OPEFB’s lignin และน้ำมันเท่ากับ 1:1) จากการศึกษาผลของของการเติม OPEFB’s lignin derivatives ในปริมาณต่างๆ (ร้อยละ 1, 3, 5, 7 และ 10 ของน้ำหนักสตาร์ช) ต่อสมบัติทางกายภาพ สมบัติเชิงกล สมบัติการต้านทานไอน้ำ มุมสัมผัส และความสามารถในการต้านการเจริญเติบโตของเชื้อจุลินทรีย์ พบว่าค่าการต้านทานแรงดึงสูงสุด ค่าการต้านทานไอน้ำ ค่าความขุ่น ค่ามุมสัมผัส และความเป็นสีน้ำตาลของฟิล์มเพิ่มขึ้นเมื่อเติมและปริมาณ OPEFB’s lignin derivatives เพิ่มขึ้นเป็นร้อยละ 5 ขณะที่ค่าการละลายของฟิล์มและค่าการยืดตัวเมื่อขาดมีค่าลดลงอย่างมีนัยสำคัญ ($p<0.05$) เมื่อเปรียบเทียบผลของการเติม OPEFB’s lignin และ OPEFB’s lignin derivatives ในฟิล์มสตาร์ชข้าวเจ้าในปริมาณเท่ากัน ฟิล์มสตาร์ชข้าวเจ้าที่เติม OPEFB’s lignin derivatives มีค่าการยืดตัวเมื่อขาด ค่าการต้านทานการซึมผ่านไอน้ำ ค่ามุมสัมผัส และความเป็นสีน้ำตาลของฟิล์มสูงกว่าฟิล์มที่เติม OPEFB’s lignin นอกจากนี้ลักษณะพื้นผิวและความสม่ำเสมอของฟิล์มสตาร์ชข้าวเจ้าที่เติม OPEFB’s lignin derivatives ดีกว่าการเติม OPEFB’s lignin ในส่วนของความสามารถในการต้านเชื้อจุลินทรีย์ของฟิล์มที่เติม OPEFB’s lignin derivatives พบว่า ฟิล์มที่เติม OPEFB’s lignin derivatives ร้อยละ 10 สามารถยับยั้งการเจริญเติบโตของเชื้อ *S. aureus* แต่ไม่สามารถยับยั้งการเจริญเติบโตของเชื้อ *E. coli* and *L. monocytogenes* ได้ และจากผลการทดลองข้างต้นการเติม OPEFB’s lignin derivatives ร้อยละ 5 ให้สมบัติเชิงกลและสมบัติการต้านทานการซึมผ่านไอน้ำเหมาะสมที่สุด

ความชื้นสัมพัทธ์ส่งผลต่อสมบัติของฟิล์มสตาร์ชข้าวเจ้าที่เติม OPEFB’s lignin และ OPEFB’s lignin derivatives อย่างมีนัยสำคัญ ($p <0.05$) โดยเมื่อความชื้นสัมพัทธ์เพิ่มขึ้นส่งผลให้สมบัติของฟิล์มด้อยลง อย่างไรก็ตามฟิล์มสตาร์ชข้าวเจ้าที่เติม OPEFB’s lignin derivatives ให้สมบัติเชิงกลและสมบัติการต้านทานการซึมผ่านไอน้ำดีกว่าฟิล์มที่เติม OPEFB’s lignin และเมื่อศึกษาการประยุกต์ใช้ฟิล์มสตาร์ชข้าวเจ้าที่เติม OPEFB’s lignin และ OPEFB’s lignin derivatives (ดัดแปรโดยน้ำมันถั่วเหลือง) เพื่อยืดอายุการเก็บรักษาผลิตภัณฑ์อาหาร (เนื้อปูเทียมและแผ่นขนมปังปอนด์) พบว่า ผลิตภัณฑ์อาหารที่ใช้ฟิล์มสตาร์ชข้าวเจ้าที่เติม OPEFB’s lignin และ OPEFB’s lignin derivatives มีการเปลี่ยนแปลงของค่า TBAR, a_w, ค่าสี และค่า TVC ต่ำกว่ารวมถึงมีความสามารถในการชะลอการเน่าเสียของอาหารได้ดีกว่าผลิตภัณฑ์อาหารที่ใช้ฟิล์มพอลิเอทิลีนและผลิตภัณฑ์ที่ไม่มีการใช้ฟิล์ม ซึ่งจากผลการทดลองสามารถกล่าวได้ว่าเราสามารถพัฒนาและ

ประยุกต์ใช้ฟิล์มสตาร์ชข้าวเจ้าที่เติม OPEFB's lignin และ OPEFB's lignin derivatives สำหรับเป็นบรรจุภัณฑ์อาหารได้

จากการศึกษาความสามารถในการย่อยสลายฟิล์มสตาร์ชข้าวเจ้าที่เติม OPEFB's lignin และ OPEFB's lignin derivatives โดยการฝังดิน พบว่า หลังจากการฝังดิน 24 วัน ฟิล์มสตาร์ชข้าวเจ้าที่ไม่เติม OPEFB's lignin มีอัตราการย่อยสลายเร็วกว่าฟิล์มสตาร์ชข้าวเจ้าที่เติม OPEFB's lignin และ OPEFB's lignin derivatives ตามลำดับ อย่างไรก็ตามเมื่อเวลาการฝังดินเพิ่มขึ้นเป็น 1 เดือน ไม่พบการเหลืออยู่ของฟิล์มทั้งหมดเนื่องจากเกิดการย่อยสลายกลายเป็นเนื้อเดียวกับดินนั่นเอง

Thesis Title Preparation and Property Improvement of Biodegradable Rice Starch Films with Oil Palm Empty Fruit Bunch's Lignin
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ABSTRACT

The purpose of this research was to improve the properties of biodegradable rice starch films by cooperating with lignin extracted from oil palm empty fruit bunches (OPEFB's lignin). The study was beginning with extraction and analyzing the characteristics of rice starch and OPEFB's lignin. It was found that carbohydrates are the main component of starch at 85.79% and a little amount of protein, fat, moisture and ash (0.43, 0.51, 13.04, and 0.23, respectively). While, the appearances of OPEFB's lignin are a powder and brown-black color and the characteristics of functional groups are the similar to commercial lignin observed by FTIR spectra. OPEFB's lignin showed inhibition the growth of *Staphylococcus aureus, Escherichia coli* O175:H7 and *Listeria monocytogenes*, the minimal bactericidal concentration (MBC) were 3.125, 25 and 25 mg/mL, respectively.

The physical properties, mechanical properties, water vapor barrier properties, thermal properties and antimicrobial properties of rice starch films reinforced with 1, 3, 5, 7 and 10 % (w/w) of OPEFB's lignin were investigated. The tensile strength, water vapor barrier, transparency value (opacity) and brownness of rice starch films increased with addition and increasing of OPEFB's lignin content up to 5% whereas films solubility was decreased($p<0.05$). The thermal properties of rice starch films were increased when OPEFB's lignin content increased. However, an inverse trend was observed when the OPEFB's lignin content was higher than 5%. The results showed that addition of OPEFB's lignin into rice starch films did not significantly ($p>0.05$) affected on the elongation at break of resulted films. In addition, the results showed that the inhibitory effect of rice starch films on *S. aureus* containing OPEFB's lignin was observed when 10% of OPEFB's lignin was applied but did not show any inhibition on *E. coli* and *L. monocytogenes*. The suitable content of OPEFB's lignin which provided a good mechanical properties and water vapor barrier properties was 5%.

The improvement of hydrophobic properties of OPEFB's lignin by synthesizing OPEFB's lignin derivatives modify with palm oil and soy bean oil (lignin and vegetable oils at the proportions of 1:1) was prepared. The effect of OPEFB's lignin derivatives on physical properties, mechanical properties, water vapor barrier properties, surface contact angle and antimicrobial properties of rice starch films reinforced with 1, 3, 5, 7 and 10 % (w/w) of OPEFB's lignin derivatives were determined. Tensile strength, water vapor barrier, transparency value (opacity) and brownness of rice starch films increased with addition and increasing of OPEFB's lignin derivatives content up to 5% whereas films solubility and elongation at break were decreased($p<0.05$). The surface contact angle of rice starch films were increased when OPEFB's lignin derivatives content increased. When compare between rice starch films contained OPEFB's lignin and OPEFB's lignin derivatives at the same content found that rice starch films contained OPEFB's lignin derivatives presented better elongation at break, water barrier property, brownness and surface contact angle better than using OPEFB's lignin. In addition, the surface of rice starch films cooperated with OPEFB's lignin derivative provided smoother and homogeneous better than rice starch films cooperated added with OPEFB's lignin. The results showed that the inhibitory effect of rice starch films containing OPEFB's lignin derivatives on *S. aureus* was observed when 10% of OPEFB's lignin derivatives was applied but did not show any inhibition on *E. coli* and *L. monocytogenes*. The suitable content of OPEFB's lignin derivatives which providing a good mechanical properties and water barrier properties of resulted rice starch films was 5%.

Relative humidity levels showed significantly affected ($p < 0.05$) on the properties of the rice starch films contained OPEFB's lignin and OPEFB's lignin derivatives. Relative humidity increased resulted in poorer properties of rice starch films. Rice starch films cooperated with OPEFB's lignin derivatives showed mechanical properties and water vapor barrier property better than OPEFB's lignin. Applications of rice starch films containing with OPEFB's lignin and OPEFB's lignin derivatives (modified with soybean oil) for shelf life extension of food products (imitated crab meat and bread) was also tested. The tested products using rice starch contained OPEFB's lignin and OPEFB's lignin derivative showed slower changed in TBAR, a_w, color and TVC values than food products packed in PE films and control sample (no films). The results showed that food products using rice starch films contained OPEFB's lignin and OPEFB's lignin derivatives can retard the deterioration of the food products better than PE films. This study suggests that rice starch films contained OPEFB's lignin and OPEFB's lignin derivatives can be developed and applied for food packaging applications.

Soil burial degradation of rice starch films, rice starch films contained OPEFB's lignin and OPEFB's lignin derivatives were investigated. The results showed that after 24 days of incubation, the rice starch films degraded faster than rice starch films contained OPEFB's lignin and OPEFB's lignin derivative, respectively. However, over time approximately a month all specimens were not able to tested because the films decomposed into homogeneous soil.

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Burin Meethip

CONTENTS

Contents **Page**

CONTENTS (CONTINUED)

Chapter **Page**

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

LIST OF FIGURES (CONTINUED)

Figure **Page**

LIST OF FIGURES (CONTINUED)

Figure **Page**

CHAPTER 1

INTRODUCTION

The environmental pollution caused by using synthetic plastic produced by petroleum chemistry has been recently recognized as a serious problem. In addition, synthetic plastic able to protect food from contamination and spoilage but may be the source of substances migration into the food (Rish, 1988). To make these problems go away, various methods have been used. These include: the restriction of the excessive use of goods and packaging materials made from the synthetic plastics, the burial and combustion of plastic wastes and using natural polymer derived from animal or plant sources. The increasing awareness of environmental conservation and protection has promoted the presentation of biopolymers produced from renewable sources as an alternative to synthetic polymers for selected industrial application (Muñoz *et al.*, 2003). Biodegradable or edible films from biopolymers have currently received increasing attention from both academic and industrial points of view. Due to their biodegradability, they can be used to replace the synthetic packaging films which mainly cause the environmental problems. Furthermore, natural polymer that added some kind of extract from natural can to resist the growth of microorganisms called "Antimicrobial substances". The natural polymers have attracted interest and have been widely studies due they can be readily decomposed in the environment during their lifetime (Yoon *et al.*, 1996).

Biodegradable polymer or edible films are no meant to totally replace synthetic packaging films, but to limit moisture, aroma, and lipid migration between food components where traditional packaging cannot function. The edible films have received a consideration attention in the recent years because of their advantage over the synthesis films. The advantage of biodegradable polymer films or edible films over other traditional synthetic such as they can be consumed with the package products. The films can function as carriers for antimicrobial and antioxidant agents. In addition, biodegradable and edible films can be used for versatile food products to reduce loss of moisture, to restrict absorption of oxygen, to lessen migration of lipids, to improve mechanical handling properties, to provide physical protection, or to offer an alternative to the commercial packaging materials (Bourtoom *et al.*, 2008). Even though, biopolymer based films are not the ultimate replacement to petroleum-based plastics, it is expected that the packaging material developed can be an efficient barrier to moisture, gas, and reduce lipid peroxidation along with retaining natural aroma of a food commodity (Siracusa *et al.*, 2008).

The development of food packaging may include material with antimicrobial properties. These packaging technologies could play a role in extending shelf-life of food to reduce the risk form pathogens (Appendini and Hotchkiss, 2002). Antimicrobial packaging is a promising form of active food packaging, since microbial contamination of food occurs primarily at the surface, due to post-processing handling; attempts have been made to improve safety and to delay spoilage. The application of antimicrobial packaging could extend the shelf-life of product and provides microbial safety for consumers. It acts to reduce, inhibit or retard the growth of pathogen microorganisms in packed foods and packaging material (Vermeiren *et al.*, 1999). For this reason, the search for antimicrobial from natural source has received much interesting to replace synthesis ones. Several compounds have been proposed for antimicrobial activity in food packaging, including organic acids and natural antimicrobial compounds such as lignin (Dong *et al.*, 2011). These compounds carry mostly antimicrobial and some antioxidant properties.

Starch is important biopolymer that is a new choice for production of biodegradable plastics. Starch is low cost material in comparison to most synthetic plastics and is readily available. Starch is the end product of photosynthesis in plants. It is a natural carbohydrate based polymer that is globally available from various natural sources including wheat, rice, corn and potato. Starch consists of two types of polysaccharides, namely amylose and amylopectin (Alves *et al.*, 2006). Amylose is a linear molecule with a few branches, whereas the amylopectin molecule has many branches. Therefore, the amylose content contributes to film strength and branched structure of amylopectin generally leads to film with low mechanical properties (Mali *et al.*, 2005). The relationship between the amylose content and film forming ability is quite good. This is because starches with high amylose content (approximately 30%

amylose) have excellent film forming properties compared to starches with low amylose content (Mali *et al.*, 2002). When compared to the common thermoplastics, biodegradable products based on starch (Lourdin *et al.*, 1995) unfortunately, still reveal many disadvantages such as low mechanical properties and in efficient barrier against low polarity compounds (Kester and Fennema, 1986). The disadvantages mainly attributed to the highly hydrophilic character of starch polymers (Santayanon and Wootthikanokkhan, 2003). To cope with these problems while preserving the biodegradability of the materials, one approach is the use of lignin as reinforcement for the starch.

Lignin is a useful ‘‘waste material’’ that possesses tremendous potential to be used as a base material for various materials applications (Stewart, 2008). Lignin, a natural renewable polymer is abundantly present in oil palm black liquor wastes. Lignin has been reported to that environmental and industrially useful property such as: high modification options, easy biodegradability, adhesiveness, adsorption, and solution properties, along with good compatibility with various non-toxic chemicals (Ciobanu *et al.*, 2004). Lignin is complex racemic aromatic hetero-polymers with the substituent connected by both ether (C-O-C) and carbon–carbon (C-C) linkages. Lignin has the right chemistry – small particle size, hydrophobic behavior, and ability to form stable mixtures (Narapakdeesakul *et al.*, 2013). The hydrophobic nature of lignin has been also shown to produce strong reduction of water absorbency and transparency in starch-based films (Ban *et al.*, 2007). In addition, the structure of lignin will ingredient of complex phenolic compound which can inhibit the growth of microorganisms (Dong *et al.*, 2011) and antioxidant (Dizhbite *et al.*, 2004). The radical scavenging activity of lignin is influenced by structural features, such as the presence of phenolic hydroxyl groups, methoxy groups, π-conjugation systems as well as the molecular weight, heterogeneity and polydispersity (Dizhbite *et al.*, 2004).

From that reason, it has the possibility to use lignin as the composition for improvement the properties of starch based biodegradable films. Hence, this research was to study the effect of oil palm empty fruit bunch’s lignin (OPEFB’ lignin) on mechanical, physical properties, degradability and antimicrobial efficiency of rice starch films. Furthermore, the improvement of biodegradable rice starch films with modified OPEFB' lignin by using soy bean oil and palm oil were also determined. Addition, using of rice starch films incorporated with OPEFB' lignin for shelf-life extension of food products was also studied.

Review of Literature

1. Starch

Starch is a common raw material that has economic and commercial values in both food and non-food industry. Starch is widely distributed in seeds, roots, tubers, stems, leaves, fruits and even pollen (Kaur *et al.*, 2004). The use of starch as biopolymer can be interesting solution because this polymer is quite cheap, rapidly available, renewable, biodegradable and edible (Mali and Grossman, 2003). Starch consists of two major types of molecule, primarily linear amylose and highly branched amylopectin. Normal starch consists of about 75% amylopectin and 25% amylose; waxy starches consist of mainly amylopectin and 0-8% amylose; and high-amylose starches consist of 40-70% amylose. Amylose is composing of D-glucose molecules, which are linked in a α-1, 4 conformations. The glucose monomers therefore form a linear straight chain polymer. Amylose is a key component involved in water absorption, swelling and gelation of starch in food and processing of material (Hoseney, 1986).

Amylopectin is the major component of most starches, and consists of a large number of shorter chains that are bound together at their reducing end side by a α-1, 6 linkage (Hoseney, 1986). Amylopectin is therefore highly branched as the α-1, 4 linear chains are punctuated with the α-1, 6 linkages. The α-1, 6 constitute about 5% of the structure of amylopectin and gives rise to branching. The amylopectin molecule is much larger than the amylose molecule. Minor components, such as lipids, phospholipids and phosphate monoester derivatives, are found in starch and have profound effects on the properties of starch. Lipids and phospholipids are found in cereal starches. Normal cereal starches contain up to 1% lipids, and the level of lipid content is proportional to the amylose content of the normal starch (Morrison, 1995). Starches of different botanical origins consist of different species of lipids. For example, normal maize starch consist of mainly free fatty acids, glycerides and little phospholipids; normal rice starch contains substantial amount of phospholipids and some free fatty acids; and wheat, barley, rye and triticale starches consist exclusively of phospholipids (Morrison, 1995; Kasemsuwan and Jane, 1996). Cereal waxy starches contain few lipids, whereas high-amylose starches contain substantially more lipids. Root and tuber starches contain very little lipids and no detectable phospholipids (McPherson and Jane, 1999).

Rice is the most widely consumed basic food in the world. Each year over 500 million tons of rice is harvested, providing sustenance to many countries and people throughout the world. The unique properties of rice starches are found in its many varieties. Due to different climates, soil characteristics and cultures, over 240,000 registered varieties of rice exist in the world. These varieties lead to a wide range of rice starches with many different characteristics including: different onset gelatinization temperatures; textures; processing stability and viscosities. Rice starch with high amylose is an attractive raw material for use as barriers in packaging materials. They have been used to produce biodegradable films to partially or entirely replace plastic polymers because of the low cost and renewability, as well as processing good mechanical properties. However, compared to the common thermoplastics, biodegradable rice starch products still reveal many disadvantages. These include low mechanical properties and lack of efficient barrier against high polarity compounds. The disadvantages are mainly attributed to the highly hydrophilic character of rice starch polymers. To cope with these problems while preserving the biodegradability of the materials, the improvement of rice starch film properties has been investigated to meet suitable applications (Wittaya, 2012).

1.1 The structure of starch

1.1.1Amylose

Amylose is essentially a linear molecule containing α-1, 4 linked glucose units with a small number of branches showed in Figure 1 (Greenwood, 1964; Takeda and Hizukuri, 1987). The side chains on those molecules that are branched are few and so long that they act similarly to unbranched molecules. With the linear chemical structure, amylose has the ability to change its conformation. With many hydroxyl groups, there is a high hydrogen bonding capability, with strong internal forces that permit these changes. A helical conformation is common for amylose and a double helix form when different helices pack together. An open channel in the center of a helix permits complexing with other molecular species, such as iodine. The linear fraction displays an intense blue color with iodine, while the pure branched fraction shows only a weak violet-red color. This color difference is attributed to helical conformation of the linear molecules, with iodine in the center of the helix. The particular electronic resonance of this system gives rise to the blue color (Pomeranz, 1991). The amylose content of starch is estimated by in of many variations of the classical reaction between amylose and iodine to form a stable complex, which is measured either spectrophotometrically or by potentiometric titration. The reaction is not an absolute indicator of amylose content because of interference from amylopectin and intermediate materials. e.g., long-chain amylopectin have been reported in some maize mutant genotypes.

Although the molecular composition of starch can be described approximately as a mixture of branched molecules of amylopectin (average molecular weight of at least 108) and amylose (average molecular weight between 105 and 106), the molecule structure of these components are quite complex. For example it has been shown that about one-half of the amylose molecules in maize starch are branched (Takeda *et al.*, 1988). In addition, some amylopectin such as in amylose extender-waxy maize starch contains long chains that bind iodine in a manner similar to amylose and contribute to apparent amylose content. It is suggested that the degree and size of branched, as well as their relative distribution pattern, might be more useful predictors of starch behavior.

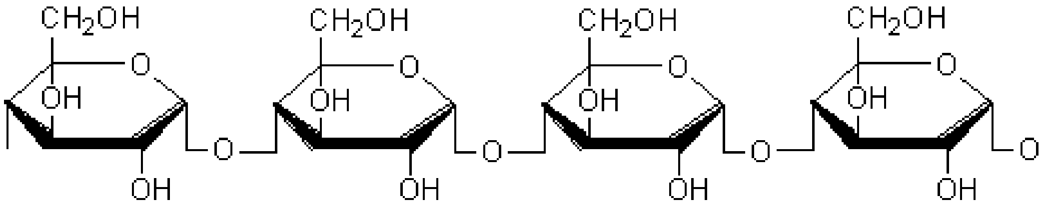


Figure 1 Amylose Structure

Source : French (1984)

1.1.2Amylopectin

Amylopectin is a branched molecule with (α-1, 4)-linked glucose units in linear chains and (α-1, 6)-linked branched points showed in Figure 2 (Greenwood, 1964; Lineback, 1984). Reported molecular weight values for amylopectin run into the millions (Hoseney, 1986). Amylopectin is less prone to gelation, retrogradation and syneresis because of the branched structure. There is no convenient method for directly determining amylopectin. Amylopectin is commonly estimated by subtraction of amylose. The amylopectin model of Manners (1985) shows how branched molecules can be involved in the formation of alternating crystalline and amorphous regions of the granule. The packing together of clustered branched would provide crystallinity, while the branched points are considered to be in the amorphous regions. The crystalline regions are more resistant to enzymatic and chemical action and to penetration by water than are amorphous regions in starch granules.

The prominence given to amylose in starch studies is understandable, since retrogradation and its consequences are relatively easy to identify and follow; however, studies had revealed crystallinity in the branched amylopectin fraction. Crystallinity data indicated that native waxy starch (100% amylopectin) had 40% crystallinity, while high-amylose starch has about 15% (Zobel, 1992). Imberty *et al.* (1991) presented a geometric arrangement where limited disruption of the original double helix would occur; further indicating that a branch point could facilitate crystallite formation. Amylose can be leached from starches, leaving the amylopectin as well as granule crystallinity largely intact. Such findings have led to the conclusion that amylose is mainly in the amorphous phase and that amylopectin is the main component of the crystalline fraction.

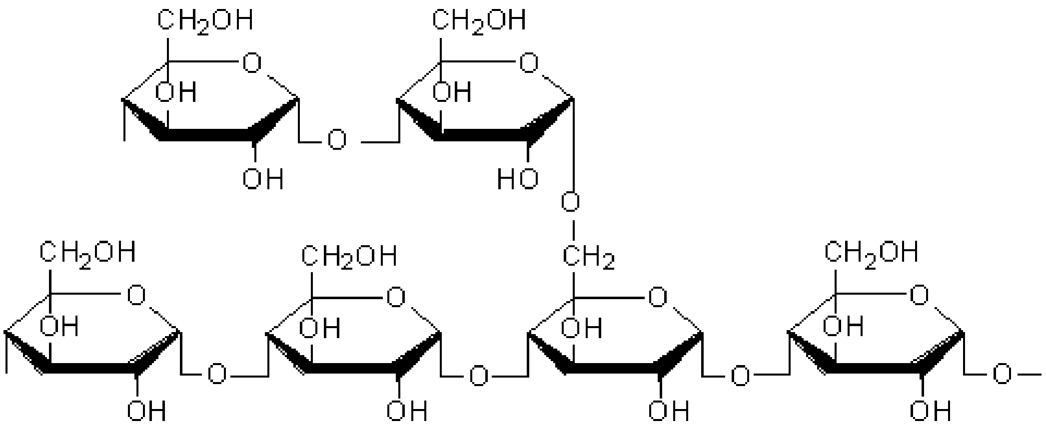


Figure 2 Amylopectin Structure
Source : French (1984)

1.1.3 Intermediate Material

Many reported have indicated the presence of a material having properties different from those of amylose and amylopectin so called the intermediate component (Lansky *et al.*, 1949, Wolff *et al.*, 1955). The intermediate component displayed iodine-binding affinity and by a beta-amylolysis limit between those of amylose and amylopectin. Lansky *et al.* (1949) reported that maize starch contained 5-7% of the intermediate component. French (1984) also reported the presence of a component with a lower molecular weight than amylopectin in waxy-maize starch. Many reports also suggest the presence of an intermediate component in high amylose starch (Bank *et al.*, 1974; Dias and Perlin, 1982; Colonna and Mercier, 1984). Dias and Perlin (1982) studied the intermediate component (amylopectin C) by using high field 13C NMR. They claimed that the intermediate component has a chain length that is 20-25% shorter than that of normal amylopectin.

1.1.4 Minor Component of Starch Granules

The interaction of the minor components, such as lipids and protein, with amylose and amylopectin, can also influence the properties and molecular architecture of the granule. Lipid-bound amylose is present within the amorphous regions of the granule (Morrison *et al.*, 1993) where the lipid (lysophospholipid and free fatty acid) is immobilized within the α-glucan helices. These amorphous regions are predicted to have a rubbery consistency, which acts as a shock absorber, if the granule is subjected to compression (Morgan *et al.*, 1995). Water absorption studies have also indicated lipid and amylose interaction within the granule contributing to inhibit of swelling when the granule is hydrated and heated (Taster and Morrison, 1990).

1.2 Properties of starch

1.2.1 Starch gelatinization

Starch molecules have a large number of hydroxyl groups, which also tend to attract each other. As a consequence hydrogen bonds are directly formed between adjacent starch molecules or indirectly formed with water. Even though the hydrogen bonding forces are weak, there are so many such bonds in a starch granule that starch does not dissolve in cold water below its gelatinization temperature. This is of importance since it enables an easy extraction of starch granules from plate source in aqueous system. Additionally, chemical modification can be performed in starch suspension, which is subsequently purified by filtration, washing with water and drying. Starch granules are generally insoluble in water below 50°C. But can imbibe water reversibly; that is, they can swell slightly, and then return to their original size on drying. When starch granules are heated in excess water, above 60% of the total wet weight (w.t.), beyond a critical temperature (55-80°C, depending on starch type). The hydrogen bonds which responsible for the structure integrity are weakening that allow the penetration of water which causes the hydration of the linear segment of the amylopectin. Starches from roots including the tapioca starch generally swell at lower temperature and to greater extent than the common cereal starches because of high amount of weak hydrogen bonds in the structure which easily hydrate and highly swell. The granules absorb a large amount of water and swell to times their original size until irreversible granule swelling was occurred. It undergoes an irreversible order-disorder transition called gelatinization as shown in Figure 3.

In the first stages of gelatinization the shorter micelles dissociate, while the longer micelles still persist to higher temperature and also gas bubbles develop in the central part of the starch granules and part of the amylose is solubilized and exuded (Sandstedt, 1955; Leach *et al.,* 1959). Upon raising the temperature of a starch suspension, each individual granule gelatinizes quite sharply, not all the granules in the same suspension gelatinize at the same temperature, but rather over a range of 8-10°C. That is to say, the gelatinization process of a starch suspension cannot be defined as to take place at certain temperature, but rather during a certain temperature range. On the whole, the smaller granules start to gelatinize at higher temperature (Whistler *et al.*, 1984). Then starch granules undergo more or less intense swelling depending on water availability. The swelling of granules and the concomitant solubilization of amylose and amylopectin gradually improve digestibility and induce the loss of granular integrity and then may burst or rupture, they giving rise to a viscous solution when gelatinization is achieved (Leach *et al.*, 1959).

Starch gelatinization is also affected by the solvent other than water, for example, liquid ammonia, formamide, formic acid, chloroacetic acids and dimethlysulfoxide. These solvents disrupt hydrogen bonding within the starch granule by forming soluble complexes with starch. Furthermore, some chemicals can decrease or increase starch gelatinization temperature. Salts, such as sodium chloride and sodium sulfate as well as sugar are used to raise the gelatinization temperature of starch being derivatized. In addition, the amylose-lipids complexes in cereal starches inhibit granule swelling that is increasing in pasting temperature (Whistler *et al.*, 1984).

Sodium sulfate is commonly used to stabilize starch granules during chemical reactions at an alkaline pH for manufacturing chemically modified starches (Wurzburg, 1986). Sodium sulfate increases the gelatinization temperature of starch granules through its structure-making effect and its large negative charge density (Jane, 1993). In the presence of a high concentration of sucrose, starch gelatinization temperature increase substantially (Ahmad and Williams 1999).

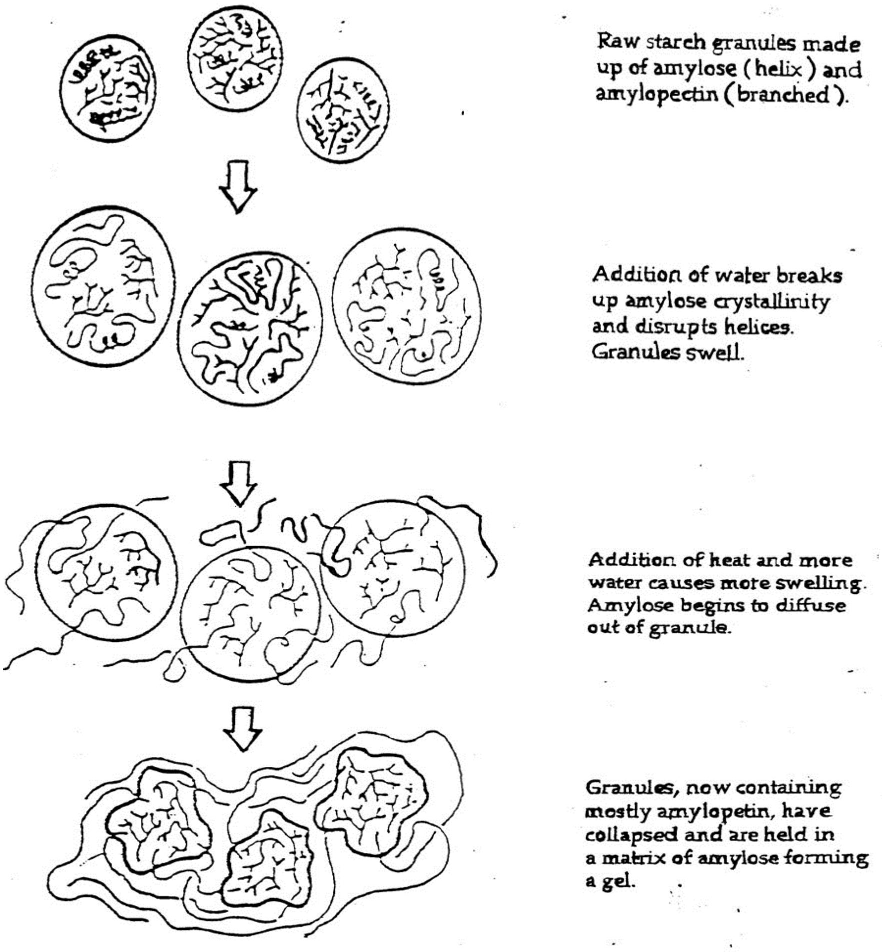


Figure 3 Scheme of starch gelatinization

Source : Remsen and Clark (1978)

1.2.2 Pasting properties

Continued heating of starch in excess water with stirring causes the granules to further swell, the amylose to leach more, and the granules to disintegrate, forming a viscous material called paste (BeMiller, 2007). Pasting occurs after or simultaneously with gelatinization. Pasting properties of starch are important indicators of how the starch will behave during processing (Figure 4) and are commonly measured using various viscometers like Brabender Viscometer and Rapid Visco Analyzer (RVA). Initially heating starch suspension results in swelling of starch granules. As heating continues, an increase in viscosity can be observed, which reflects the process of pasting. The temperature at the onset of viscosity increase is termed pasting temperature. Viscosity increase with continued heating, until the rate of granule swelling equals the rate of granule collapse, which is referred to as the peak viscosity (PV). PV reflects the swelling extent or water-binding capacity of starch and often correlates with final product quality since the swollen and collapsed granules relate to texture of cooked starch. Once PV is achieved, a drop in viscosity, or breakdown, is observed as a result of disintegration of granules. Break-down is a measure of the ease of disrupting swollen starch granules and suggests the degree of stability during cooking (Adebowale and Lawal, 2003). Minimum viscosity, also called hot paste viscosity, holding strength, or trough, marks the end of the holding stage at the maximum temperature of the RVA. Cooling stage begins and viscosity again rises (setback) which is caused by retrogradation of starch, particular amylose. Setback is an indicator of final product texture and is linked to syneresis or weeping during freeze-thaw cycles.

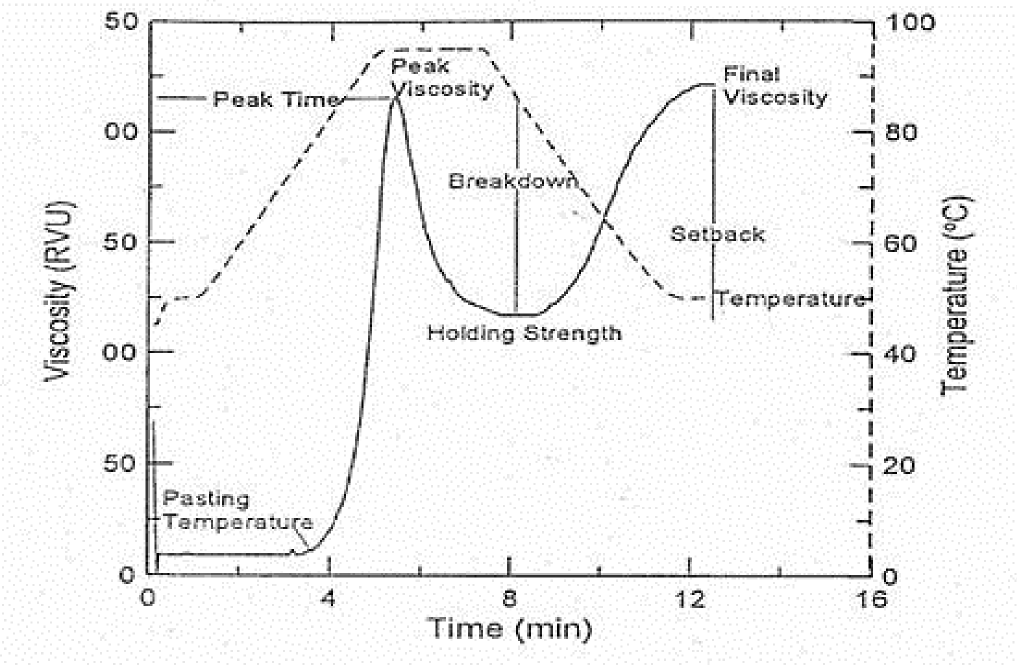


Figure 4 Viscosity profiles of starches
Source : Leach (1959)

2. Formation of rice starch-based biodegradable films

The formation of rice starch films requires the use of at least one constituent capable of forming a matrix with adequate continuity and cohesion. Generally, this is composed of polymer or compounds which, under the preparation conditions, are used to form continuous crystalline or amorphous products. In the case of coatings for which the films system can be applied directly to the product, two forces are relevant: that between the molecules of the coating material (cohesion) and that between the coating and the support structure (adhesion). The degree of cohesion produces the barrier and mechanical properties of the film. High structural cohesion is manifested by a reduction in flexibility, porosity and permeability to gases and solutes (Banker, 1966). The degree of cohesion depends on the chemical structure of the film material, the presence of plasticizing and crosslinking agents, the nature of the solvent used and its dilution, the method of application, the procedure used for removal of the solvent and the final thickness of the film.

The highest cohesion is generally obtained for ordered polar polymers with long chains which were precipitated in crystalline form. The preparation for average dilution which constitutes a compromise between the salvation and extension of polymer molecules and good initial viscosity is preferable (Banker, 1966). The cohesion of films generally increases in proportion to their thickness up to a threshold beyond which it remains constant. The speed of evaporation of the solvent and/or excessive temperature may be manifested by inadequate cohesion on account of the premature immobilization of the polymer molecular. Regarding the adhesion of the coatings to the food stuff, this is generally facilitated by hot application.

The formulation of films base on starch may require one of the following methods:

1. Casting, which is a process consisting of drying a solution or a gel is a simple method for producing films with controlled thickness. This technique is useful to mimic some industrial processes for forming free-standing starch films as is the case for dip-molding. In this method, used for food coatings as well as for non-food application, the gelled state is usually preferred to set hot solutions on a surface upon cooling.

2. Extrusion or the thermo pressing process is a process used to create objects of a fixed cross sectional profile. A material is pushed or drawn through a die of the desired cross section. The two main advantages of this process over other manufacturing processes are its ability to create very complex cross sections and work materials that are brittle, because the material only encounters compressive and shear stresses. It also gives finished parts an excellent surface finish.

3. Electrohydrodynamic atomization (EHDA), referred to as electrostatic atomization or electro-spraying. This is a process in which a liquid is forced through a capillary and a potential difference of the order of kilo volts is applied between the capillary and the collection electrode (Pareta and Edirisinghe, 2006). EHDA can conduct in various modes but the stable cone-jet mode is the most desirable as it provides near-mono dispersed droplets of a few micrometers in size. The droplets size can be

controlled by the flow rate and applied as appropriate (Clopeau and Prunet-Foch, 1990).

3. Factor affecting rice starch-based biodegradable films

3.1 Amylose and amylopectin content

Starch consists of two polysaccharides, the essentially linear amylose, and the branched amylopectin (Manners, 1989). The pure amylose structure is very stable, with strong molecular orientation, forming films denser and stronger than amylopectin films (Lourdin *et al*., 1995). The ability of amylose to produce self-supporting films has been known for a long time and this is attributed to the ability of its linear chains to interact by hydrogen bonds to a higher extent than the branched amylopectin chains. Amylopectin films, on the other hand, are rather frail due to the higher degree of entanglement caused by the extensive branching and the short average chain length (Rindlav-Westling *et al*., 1998). Rindlav-Westling *et al*. (2002) prepared films from potato starch, amylose, and amylopectin and blends by solution casting. Results showed that amylose films had a relative crystallinity of about 30% whereas amylopectin films were entirely amorphous. The blending of amylose and amylopectin resulted in films with a considerably higher degree of crystallinity than could be predicted. This is explained by co-crystallization between amylose and amylopectin and possibly by crystallization of amylopectin. The crystallized material gave rise to an endothermic detected with differential scanning calorimetry. The enthalpy and peak temperature of the transition also increased as the amylose content decreased. When the amylose proportion in the blends was low, separate phases of amylose and amylopectin were observed by light microscopy. At higher amylose proportions, however, the phase separation was apparently prevented by amylose gelation and the formation of continuous amylose network. Addition, the amylose network in the films, observed with transmission electron microscopy, consisted of stiff strands and open pores and became less visible as the amylose proportion decreased. Alves *et al*. (2006) studies the effect of amylose enrichment on cassava starch films properties. This study showed the mechanical and barrier properties of cassava films were influenced by the amylose contents. The amylose enrichment originated stronger films and this could be explained because during drying of film-forming solutions, water evaporates, allowing the formation of starch network. During this stage the proximity of starch chains induced by higher amylose contents could facilitate the formation of matrix with more polymer content per area.

Ming *et al.* (2011) characterized the biodegradable films from corn starch with different amylose content. They concluded that amylose content had significantly affected the mechanical and thermal properties of the biodegradable starch-based films. The high amylose starch films exhibited better mechanical properties, such as higher modulus and tensile strength, and very high impact strength. The reasons for this include not only the easy entanglement of long linear amylose chains, but also the retained granular structure in high amylose films, which may act as self-reinforcement.

Muscat *et al.* (2012) studies the effect of low and high amylose starches on film forming behavior. They found that, films with high amylose content showed higher glass transition temperature, tensile strength and modulus of elasticity values and lower elongation values than low amylose starch films. There was an increase in thermal and mechanical properties of high amylose starch films. This could be because of what happens when the drying of film-forming solutions, water evaporates, and allowing the formation of starch network takes place. During this stage, the proximity of starch chains induced by higher amylose contents could facilitate the formation of a matrix with more polymer content per area as well (Alves *et al.*, 2006).

3.2 Type and content of plasticizers

Native starch films are brittle compared with synthetic polymers such as polyethylene, and technically need to be plasticized. A plasticizer is substance that is incorporated into rigid materials to increase its flexibility, workability, and dispensability. By reducing the glass transition temperature and increasing chain lubricity, plasticizer could also improve processing and extrusion characteristics. They could also reduce the minimum required processing temperature, reduce the plastic’s hardness and improve low temperature flexibility. Generally, two types of plasticizers are distinguished. Internal plasticization is a result of modifications to the chemical structure of polymer. External plasticization is obtained by adding an agent which modifies the structure and energy within the tree-dimensional arrangement of the film polymer (Banker, 1966). It is second method which, on the basis of the type of materials and the technology used, is mainly used for biodegradable packaging. The addition of a plasticizer to a film produces a film which is less likely to break and is more flexible and stronger.

Basically, the plasticizers should be generally compatible to the structure of the polymer that they plasticize and the permeability is present within the solvent-polymer system and under the conditions used. To be compatible, it must be compatible with the polymer, which results in the inter-molecular reaction. It is important to note that the formulation of the whole film system (polymer, solvent, plasticizer and other additives) has a direct effect on the nature and characteristics of the film produced. As a result, the polymer and the plasticizer must not only be compatible, but must also have similar solubility in the solvent used. A soluble plasticizer will generally be sought for the development of soluble coating and an insoluble plasticizer (or a dispersible one) for an insoluble coating or for a slow solubilization.

The permanence of a plasticizer is also of prime importance since this influence the physical and mechanical stability of the film. The plasticizer should not be volatile (or not only very slightly volatile) and its degree of retention by the film should be high. Other properties, such as its chemical stability, hygroscopicity, color, flavor, and so on, are also more or less important depending on the type of film under consideration. In addition, the content of plasticizer necessarily varies from 10-60% (dry basis) according to the nature and type of film and the method of application. The plasticizers that are most usually used in the field of rice starch films are mono-, di-, and oligosaccharides, polyols and lipids and its derivatives. The molecular size, configuration and total number of functional groups of the plasticizer as well as its compatibility with the polymer, could affect the interaction between the plasticizer and the polymer (Yang and Paulson, 2000).

Bourtoom and Chinnan (2008) determined plasticizer effect on the properties of biodegradable blend film from rice starch-chitosan. The results of these studies demonstrated that sorbitol plasticized films provided the films with highest mechanical resistance, but the poorest film flexibility. In contrast, glycerol and polyethylene glycol plasticizer films exhibited flexible structure; however, the mechanical resistance was low, while inversely affecting the water vapor permeability.

The effectiveness of glycol in biodegradable blend films from rice starch-chitosan is most likely due to its small size which allows it to be more readily inserted between the polymer chains. It consequently exerts more influence on the mechanical properties than the larger polyethylene glycol molecule. In addition, at an equal percentage of concentration, the total number of glycerol molecules in the film solution is greater than that of the higher molecular weight polyethylene glycol. Therefore glycol has more functional groups (-OH) than polyethylene glycol which should promote the plasticizer-polymers interactions in the films.

As a result of the glycerol, plasticized films provided the films with higher water vapor permeability than polyethylene glycol, and sorbitol should be the result of the high hydrophilicity of the glycerol molecule, which is favorable to the adsorption of water molecules and could also contribute to the increase in the film water vapor permeability. In addition, at high glycerol concentration, glycerol could cluster with itself to open the polymer structure, enhancing the permeability of the film to moisture (Lieberman and Gilbert, 1973). An increase in inter chain spacing due to the inclusion of glycerol molecules between the polymer chain may promote water vapor diffusivity through the film and hence accelerate the water vapor transmission (Yang and Paulson, 2000).

Dai *et al.* (2010) reported that type and content of plasticizer affected the properties of corn starch films. Increasing the plasticizer content resulted in increasing water vapor permeability of the resulting film. These results would be relates to structural modifications of the starch network brought about by the plasticizer concomitant with the hydrophilic character of plasticizer, which favored the absorption and desorption of water molecule.

Plasticizers reduce intra- and inter-molecular forces in starch. In addition, plasticizers could extend, dilute and soften the structure effectively; then the starch chain mobility would be increased.

3.3 Relative humidity

Relative humidity is a term used to describe the amount of water vapor in a mixture of air and water vapor. It is defined as the ratio of the partial pressure of water vapor in the air water mixture to the saturated vapor pressure of water at those conditions. The relative humidity of air depends on the temperature and pressure of the system. Relative humidity is often used instead of absolute humidity in situations where the rate of water evaporation is important, as it takes into account the variation in saturated vapor or pressure. Biodegradable starch films genteelly provide a good barrier against oxygen at low and intermediate relative humidity, and have good mechanical properties, but their barrier against water vapor is poor due to their hydrophilic nature (Kester and Fennema, 1986). When starch films are exposed during storage time in certain environmental conditions, it is possible to obtain both physical and mechanical changes in their nature. Physical changes may be those such as polymer recrystallization (the retrogradation) and those due to the migration of low molecular weight components, such as plasticizers or water contained in film formulation. This migration of additives can be considered the most important cause of physical instability of starch films. In order to diminish film fragility and increase film flexibility and manageability, plasticizers are added into film formulation (Guilbert, 1986). Water also acts as a plasticizer in hydrophilic films; the plasticizing effect of water is based on the weakening of hydrogen bonds and the dipole-dipole intra and intermolecular interactions due to shielding of these attracting forces by the water molecule. As a consequence, free volume increases which affects their mechanical properties. Stading *et al*. (2001) studies the effect of relative humidity on amylose and amylopectin film properties. They found that when the relative humidity of surrounding films increase this yielded increasing water content and oxygen permeability. However, the storage modulus and transition temperature (Tg) showed an inverse effect. The change in surrounding relative humidity affected the water content of the films. When the water content in the films increase this provides an increasing mobility of molecule in the network allows swelling with resulting heterogeneous network structure. Hence, sharply decreased storage modulus and glass transition temperature and increased the oxygen permeability of the resulting films.

Masclaux *et al.* (2010) reported that relative humidity affected the properties of starch nanocomposite films. They found that at high relative humidity, the water diffusion rate showed higher in the starch nanocomposite films. According to these results, it seemed that it was more water sorption and diffusion in starch matrix due to its initially high swelling capacity and high chain mobility. Besides, the results demonstrated that the oxygen permeability coefficient slightly increased in the range of relative humidity between 30-45% and greatly increased at higher relative humidity.

4. Active packaging

Active packaging is an innovative concept that can be defined as a type of packaging that changes the condition of the packaging to extend shelf-life or improve safety or sensory properties while maintaining the quality of the food. Active packaging technologies includes with gas absorbing/emitting packaging, selective permeable films (Kester and Fenenma, 1986), microwave subsectors. Controlled release packaging is a group of technologies that uses a packaging as a delivery system to release active compounds such as antimicrobial, antioxidant, enzyme, flavor and nutraceuticals. Most attention in this group has been focused on antimicrobial packaging (Appendini and Hotchkiss, 2002) and antioxidant packaging (Kester and Fenenma, 1986). Active packaging is one of the innovative food packaging concepts that have been introduced as a response to the continuous changes in current consumer demands and market trends. It has been defined as "a type of packaging that changes the condition of the packaging to extend shelf-life or improve safety or sensory properties while maintaining the quality of the food". The definition of active packaging was chosen for the European FAIR-project CT 98-4170 (Gennadois *et al.*, 1993).

Active packaging can be classified into active-releasing systems (emitters) which actively add compounds to the packaged food such as carbon dioxide, water, antimicrobials aromas or preservatives and active-scavenging systems (absorbers), which remove undesired compounds such as oxygen, radicals, water, ethylene, carbon dioxide, taints and other specific compounds. Ethylene absorbers and oxygen scavengers are the most used and patented of all active packaging technologies (Brody, 2001). Oxygen removers are developed to avoid oxidation process. However, radicals mainly oxo, hydroxyl and superoxide are originated from oxygen and they are the main initiators of oxidation. Thus, a different approach can be considered: by eliminating radicals as soon as they are formed, the propagation of the oxidation reaction cannot take place and consequently, the concentration of molecular oxygen is not important, but the presence of radicals. In general, active food packaging can provide several functions that do not exist in conventional packaging systems. The active functions may include scavenging of oxygen, moisture or ethylene, emission of ethanol and flavors, and antimicrobial activity.

Microbial contamination reduces the shelf-life of foods and increases the risk of food bone illness. Traditional methods of preserving foods from the effect of microbial growth include thermal processing, drying, freezing, refrigeration, irradiation, modified atmosphere packaging and adding antimicrobial agents or salts. Unfortunately, some of these techniques cannot be applied to some food products, such as fresh meats and ready-to-eat products.

4.1 Antimicrobial packaging

Antimicrobial packaging is system that can kill or inhibit the growth of microorganisms and thus extend the shelf-life of perishable products and enhance the safety of packaged products. Antimicrobial packaging can kill or inhibit target microorganisms (Han, 2000). Among many applications such as oxygen-scavenging packaging and moisture-control packaging, antimicrobial packaging is one of the most promising innovations of active packaging technologies. It can be constructed by using antimicrobial packaging materials and/or antimicrobial agents inside the package space or inside foods. Most food packaging systems consist of the food products, the headspace atmosphere and the packaging materials. Any one of these three components of food packaging systems could possess an antimicrobial element to increase antimicrobial efficiency.

Antimicrobial packaging research generally started with the development of antimicrobial packaging materials that contain antimicrobial chemicals in their macromolecular. However, without the use of alternative packaging materials, common packaging materials can be utilized for antimicrobial packaging systems there is antimicrobial activity in packaged foods or in the in-package atmosphere. Edible antimicrobial agents can be incorporated into food ingredients, while antimicrobial resources can interleaved in the in-package headspace in the form of sachets, film, sheets or any in-package supplements, to generate antimicrobial atmospheres.

Besides the use of antimicrobial packaging materials or antimicrobial inserts in the package headspace, gaseous agents have been used to inhibit the growth of microorganisms. Common gases are carbon dioxide for modified atmosphere packaging, sulfur dioxide for barrier, and ethanol vapor for confection. These gases are injected into the package headspace or into palletized cases after shrink-wrapping of a unit load on a pallet. Vacuum, nitrogen-flushing and oxygen-scavenging packaging which were originally designed for preventing the oxidation of packaged foods, also possess antifungal and antimicrobial properties against aerobic bacteria as a secondary function, since these microorganisms are restrictively aerobic (Brody, 2001). However, these technologies, which control the low oxygen concentration to inhibit the growth of aerobic microorganisms, could cause the onset of anaerobic microbial growth. Controlling anaerobic bacteria in modified atmosphere packaging is a very important issue in maintaining the quality and safety of the products.

4.2 Antimicrobial agents

Various antimicrobial agents could be incorporated into conventional food packaging systems and materials to create new antimicrobial packaging systems. Table 1 shows potential antimicrobial agents and food-grade preservatives. They can generally be classified into three groups; chemical agents, natural agents, and probiotics. For the purpose of food preservation, all packaging ingredients should be food-grade additives. The chemical agents can be mixed with food ingredients, incorporated into packaging additives or inserted into the headspace atmosphere. The antimicrobial agents are in contact with and consumed with the food products in these applications (Han, 2000).

Table 1 Examples of potential antimicrobial agents for antimicrobial food packaging systems.

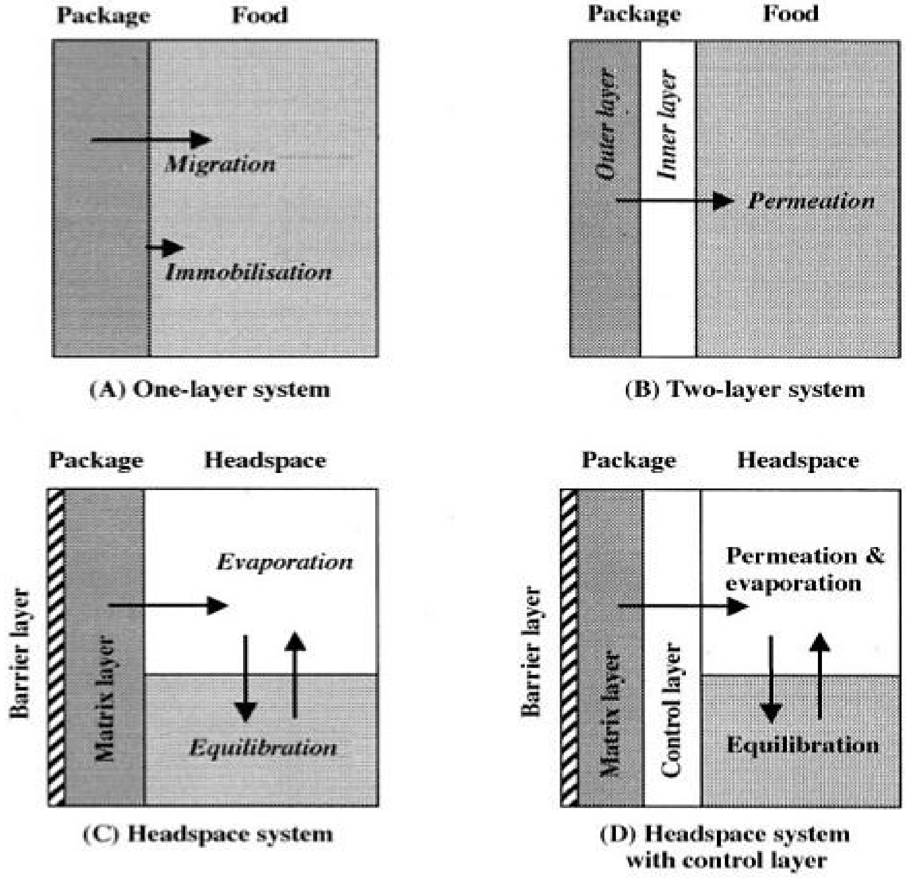
Classification	Antimicrobial agents
Organic acids	Acetic acid, benzoic acid, lactic acid, Citric acid, malic acid, probionic acid,sorbic acid, succinic acid, mixture of organic acids.
Acid salts	Potassium sorbate, sodium benzoate
Alcohol	Ethanol
Bacteriocins	Nisin, pediocin, subtillin, lacticin
Fatty acids	Lauric acid, palmitoleic acid
Chelating agents	EDTA, citrate, lactoferrin
Enzymes	Lysozyme, glucose oxidase, Lactoperoxidase
Antioxidants	BHA, BHT, TBHQ, iron salts

Antibiotic	Natamycin
Sanitizing gas	Ozone, chlorine dioxide, carbon Monoxide, carbon dioxide
Sanitizers	Cetyl pyridinium chloride, Acidified NaCl
Polysaccharide	Chitosan
Phenolic compound	Catechin, cresol, hydroquinone, lignin
Plant volatiles	Allyl isothiocyanate, cinnamaldehyde,eugenol, linalool, terpineol, thymol, cavacrol, pinene
Plant/spice extracts	Lignin extracted from Oil Palm Empty Fruit Bunch, hotbeta acid, Brassica erucic

Modified from Han (2000)

4.3 Shapes and compositions of antimicrobial packaging systems

Packaging is a system use to contain and protect enclosed products, which consists of a product, a package and the in-package atmosphere. Antimicrobial agents may be incorporated in the non-food pasts of the packaging system, which are the package of the in-package atmosphere. Antimicrobial agents can be incorporated directly in packaging materials in the form of films, over coating on the film, sheets, trays, and containers, or in the in-package space in the form of inserts, protecting the coated foods from microbial quality degradation (Han, 2000). Figure 5 illustrates the possible forms of antimicrobial packaging systems.



(A) One-layer system

(B) Two-layer system

(C) Headspace system

(D) Headspace system with control layer

Figure 5 Antimicrobial packaging systems

Source : Han (2000)

5. Plant extract

Antimicrobial agent can be extracted from various sources such as spices and their essential oil, food plants and antimicrobial peptide produced by bacteria (Beuchat and Doyle, 1995). Plant secondary metabolites, such as essential oils and plant extracts had antimicrobial activities and most essential oils derived from plants are known to possess insecticidal, antifungal, acaricidal, antibacterial and cytotoxin activities (Tepe *et al.*, 2004). Therefore, they are intensely screened and applied in the fields of pharmacology, pharmaceutical botany, medical and clinical microbiology, phytopathology and food preservation. The antimicrobial activities of the essential oils against the tested bacteria differed, depending on location and seasonal variations (Wang *et al.*, 2006). Kabuki *et al.* (2000) reported that mango seed kernel ethanol extract was composed of 79.5% polyphenol and had a broad spectrum antimicrobial activity. The extract was more active against gram-positive than gram-negative bacteria including some food borne pathogen. The essential oil of *Lippia orgiganoides* inhibits the growth of several microorganisms (bacteria; *S. aureus*, *L. casei, S.mutans* and fungi; *Candida spp., C. Neoformans*) (Kabuki *et a*l., 2000). The inhibitory effect of several terpenoids on microbial oxygen uptake and oxidative phosphorylation has also been demonstrated (Kabuki *et al.*, 2000).

Phenolic and non-phenolic alcohols in *L. origanoides* exhibited the strongest inhibitory effect, followed by aldehydes and ketones. The monoterpene hydrocarbons were less active and it has been suggested that this behavior depends on the free hydroxyl group from the alcohols. Wang *et al.* (2006) suggested that basil, clove, garlic, horseradish, marjoram, oregano, rosemary, and thyme exhibited antimicrobial activities. Those herbs can be practical for protecting seafood from the risk of contamination by *V. parahaemolyticus* (marine pathogenic bacteria) and also can be used in hurdle technology with low temperature. Moreover, these spices and herbs, as well as essential oils have been well known to have inhibitory effects against a variety of bacteria including Gram-negative bacteria and *E. coli*. Carom seed, ginger, Japanese pepper, sage, spearmint and turmeric were additionally found to exhibit antimicrobial activities against *V. parahaemolyticus.*

Benkeblia (2004) studied the effect of concentrations (50, 100, 200, 300 and 500 mL/L) of essential oil extracts from three type of onions (green, yell and red) and garlic against two bacteria, *S. aureus, S. enteritidis,* and three fungi, *A. niger, P. cyclopium* and *F. oxysporum.* The essential oil (EO) extracts of these *Allium* plants (garlic and onions) exhibited antibacterial activity, with garlic showing the highest inhibition and green onions the lowest. Comparatively, 50 and 100 mL/L concentrations of onions extracts were less inhibitory than 200, 300 and 500 mL/L concentrations. However, with garlic extract, high inhibitory activity was observed for all tested concentration. *S. aureus* showed less sensitivity towards EO extracts inhibition; however *S. enteritidis* was strongly inhibited by red onion and garlic extracts. The fungus *F. oxysporum* showed the lowest sensitivity towards EO extracts, whereas *A. niger* and *P. cyclopium* were significantly inhibited particularly at low concentration.

Seydim and Sarikus (2006) reported that antimicrobial properties of spice extracts are well known. According to these researched, antimicrobial properties of whey protein isolate (WPI) films containing 1-4% (w/v) ratio of oregano, rosemary and garlic essential oils were tested against *E. coli* O157:H7 (ATCC 35218), *S. aureus* (ATCC 43300), *S. enteritidis* (ATCC 13076), *L. monocytogenes* (NCTC 2167) and *L. plantarum* (DSM 20174). Ten milliliters of molten hard agar was inoculated by 200 ml of bacteria cultures (colony count of 1×10^8 CFU/mL) grown overnight in appropriate medium. Circular discs of WPI films containing spice extracts, prepared by casting method, were placed on a bacterial lawn. Zones of inhibition were measured after an incubation period. The film containing oregano essential oil was the most effective against these bacteria at 2% level than those containing garlic and rosemary extracts ($p<0.05$). The use of rosemary essential oil incorporated into WPI films did not exhibit any antimicrobial activity whereas inhibitory effect of WPI film containing garlic essential oil observed only at 3% and 4% level ($p<0.05$). The results of this study suggested that the antimicrobial activity of some spice extracts were expressed in a WPI based edible film.

6. Oil palm empty fruit bunches

Oil palm (*Elaeis guineensis* Jacq.) is a native of West Africa. From its home, the oil palm has spread throughout the tropics and is now grow in 16 or more countries for its oil-producing fruit (Ibrahim*et al.*, 2004). However, the major center of production is in South East Asia (SEA) with Malaysia, Indonesia and Thailand, which produce about 80% of world's palm oil in 2001 (Chavalparit, 2006). In Thailand, the oil palm was introduced in 1968, and become one of the major agro-industries in the south (Prasertsan *et al.,* 1996). The palm oil industry plays an important role in the economic development of the country and in enhancing the economic welfare of the population (Chavalparit, 2006). In 2010, the oil palm plantations in Thailand increased to 4.4×10^6 Rais (about 1.7×10^6 acres) and a large quantity of fresh fruit bunches (FFB) were harvested approximately about 8.2×10^6 tons (Phitthayaphinant*et al.*, 2010). Palm oil occupies 70% of the Thai vegetable oil market, and is estimated to be worth 40,000 million Baht per annum and has known an average annual growth rate of 15% during the last decade. As the climatic conditions in the south are suitable for palm trees, the oil palm plantation area has expanded ever since (Chavalparit, 2006). Palm oil production generates large amount of solid residues that are fibers, shells and empty fruit bunches. For every 100 tons of fresh fruit bunches entered to the oil extraction processes, about 25 tons of oil palm empty fruit bunches (OPEFB) as solid wastes will be produced.

Generally, the OPEFB contains about 40–50% cellulose, 20–30% hemicellulose, 20–30% lignin and 10–15% moisture content (Ahmadzadeh*et al.*, 2007). Abdul Khalil *et al.* (2006) revealed that OPEFB contain highest composition of hemicellulose compared to coir, pineapple, banana, and even soft and hardwood fibers. Lignin which is also responsible for tough and stiffness properties of the fiber was lower than coir fibers, but still higher than other fibers. In addition, the OPEFB contains minor components of arabinose, xylose, mannose, galactose, silica, copper, calcium, manganese, iron, and sodium. Law *et al.* (2007) found that OPEFB fiber contains about 1.5–2.5% of ash with carbon (45–50%) and oxygen (44–48%) as main constituents following the silica of about 5–6% and others.

In general, palm oil industry has to dispose about 1.1 ton of OPEFB per every ton of oil produced (Karina*et al.*, 2008). Some quantity of this cellulosic material is currently used in the preparation of fertilizers or as mulching material (Singh*et al.*, 2003). However, a major proportion is left in mill premises itself. When left in field, these waste materials create great environmental problems (Law *et al.,* 2007). A view of OPEFB wastes piled up for disposal in a palm oil mill is shown in Figure 6. Furthermore, these wastes also result in forfeiture of substantial economic value, which would have been induced by their suitable applications. Hence, palm oil producing countries, in particular, can generate revenue out of this waste product which till date is considered to be challenging. The sustainable, non-hazardous, non-carcinogenic, eco-friendly, biodegradable product developed from these cellulosic wastes will surely benefit the human kind across the globe in broad-spectrums (Hassan *et al.,* 2010).

Figure 6 Illustrative picture of OPEFB wastes piled up in a palm oil mill premise
Source: Shinoj *et al.* (2001)

From the previous study, composition of oil palm empty fruit bunches consists of approximately 20 – 30% lignin. Besides, other source and different type of lignin in the starch films such as lignin from residue of corn stover to ethanol production (Dong *et al.*, 2011), wheat straw and sugar cane bagasse (Lepifre *et al.*,

2004) were investigated. Lignins are complex racemic aromatic hetero polymers with the substituents connected by both ether (C-O-C) and carbon–carbon (C-C) linkages. Lignin has the right chemistry – small particle size, hydrophobic behavior, antimicrobial properties and ability to form stable mixtures.

7. Lignin

7.1 General information

Lignin is one of the most abundant natural non-carbohydrate organic substrate on the earth, accounting for approximately 30% of the organic carbon in the biosphere (Antonsson *et al.*, 2008). The importance of lignin in plants should be considered from different aspects, i.e. it is crucial for structure integrity of the cell wall and stiffness and strength of the stem (Zahedifar, 1996; Chabannes *et al.*, 2001). From the nutritional point of view, lignin has always been blamed as an important barrier to polysaccharide utilization (Van Soest, 1994). In addition, lignin waterproofs the cell wall, enabling transport of water and solute through the vascular system, and plays a role in protecting plants against pathogens (Rencoret *et al.*, 2011).

7.2 Chemistry

Lignin has a number of chemical groups such as aromatic rings, phenolic and aliphatic alcohol groups and methoxy groups that are possible sites for chemical modifications. Lignins are complex racemic aromatic hetero polymers with the substituents connected by both ether (C–O–C) and carbon-carbon (C–C) linkages (Figure 7). It is derived mainly from three hydroxycinnamyl alcohol monomers differing in their degree of methoxylation: p-coumaryl alcohol (p-hydroxyphenyl propanol), coniferyl alcohol (guaiacyl propanol) and sinapyl alcohol (syringyl propanol) (Figure 8) (Hermansson *et al.*, 1996). The lignin content of softwoods ranges from about 25–35%, which is greater than the lignin content of hardwoods, 18–25%. Softwood lignin is made up of approximately 80% coniferyl, 14% p-coumaryl and 6% sinapyl alcohols. In contrast, hardwood lignin is composed of 56% coniferyl, 4% p-coumaryl and 40% sinapyl alcohols (Jeffries**,** 1994). The dominant linkage in softwood lignin is the β–*O*–4 linkage (Higuchi, 1990). This linkage is often modeled by guaiacylglycerol-β-guaiacylether dimers (Figure 9).

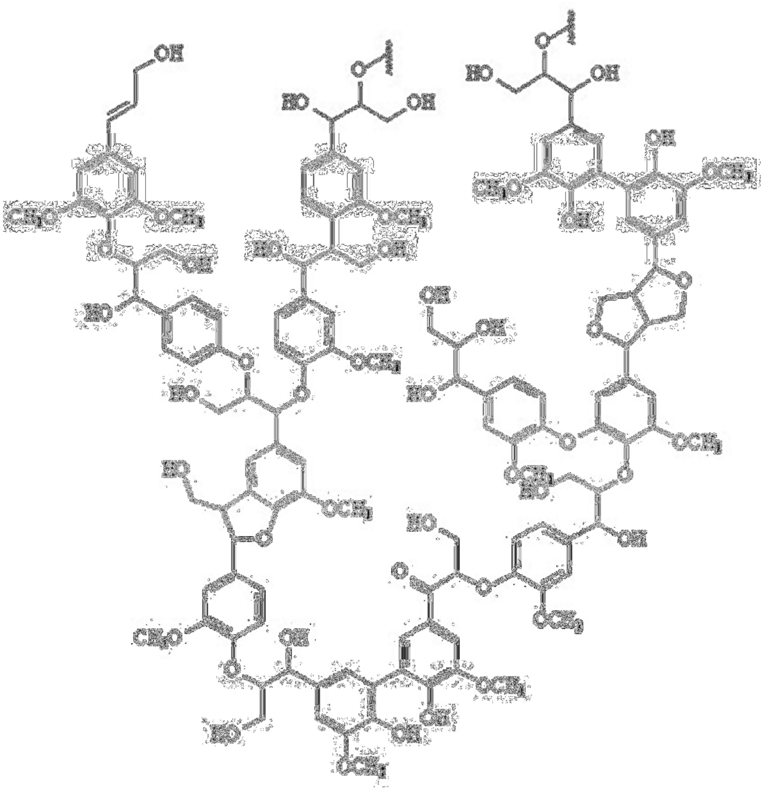


Figure 7 Chemical structure of lignin

Source: Zahedifar (1996)

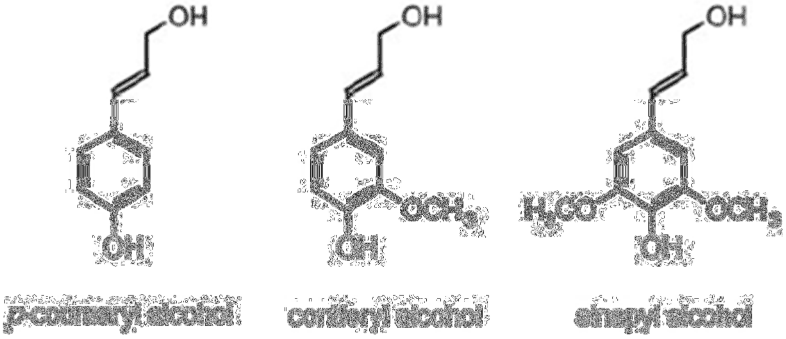


Figure 8 Major monolignols found in natural plant lignin

Source: Hermansson et al. (1996)

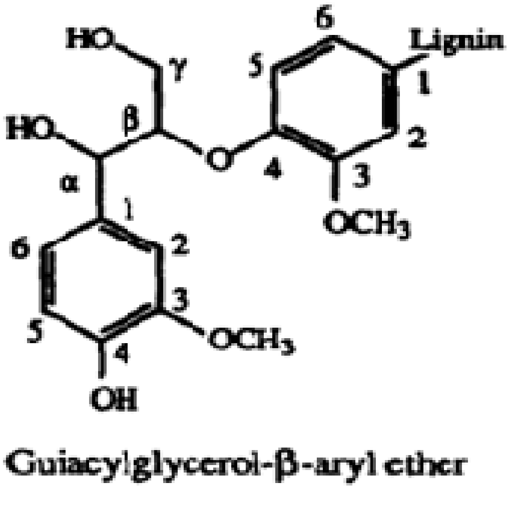


Figure 9 β-aryl ether lignin models
Source: **Jeffries** (1994).

Lignin has been reported to possess certain environmental and industrially useful properties such as high modification options, easy biodegradability, better adhesiveness, adsorption, and solution properties, along with good compatibility with various non-toxic chemicals (Ciobanu *et al.*, 2004). Due to their complex polyphenolic nature, lignin can exert antioxidant (radical scavenging capacities) (Dizhbite *et al.*, 2004; Lu *et al.*, 1998; Satoh *et al.*, 1999; Ugartondo *et al.*, 2008) and antimicrobial properties (Dong *et al.*, 2011), thus opening up the possibility of new potential applications. In addition, the hydrophobic nature of lignin has been also shown to produce strong reduction of water absorbency and transparency in starch-based films (Ban *et al.*, 2007). In composites prepared using adipic acid-modified starch microparticles within a corn-starch matrix, addition of lignin produced higher tensile strength and lower elongation capacity (Spiridon *et al.,* 2011). Similarly, lignin acted as a reinforcing agent with cellulose (Rohella *et al.*, 1996) or polyethylene oxide (Kadla and Kubo, 2003), in all cases providing adequate miscibility with the polymer. Addition, lignin is a valuable phenolic polymer that gives woods its characteristic brown color, density and mass. It has been estimated that 40 percent of the weight of the world's forests is lignin. Lumber is essentially composed of dead xylem cells that have dried out. The dead tissue is hard and dense because of lignin in the thickened secondary cell walls. In order to make paper, logs and wood chips must be converted into pulp. Several methods are used to convert wood into pulp, including the ground wood process, sulfite process and the sulfate process. In addition to chemically digesting the wood until it is reduced to its component fibers, the lignin must also be removed in fine quality papers. Cardboard containers and supermarket shopping bags (kraft paper) are stiff and brown because they still contain lignin.

Several works have been reported about biopolymer/lignin blends. Some authors showed that the addition of lignin into thermoplastic starch matrix (TPS), improved mechanical properties, increasing the tensile strength. Thermal stability also increased and a reduction of moisture absorption was achieved (Kaewtatip *et al.*, 2013). Other study, also confirmed that the use of lignin as a filler in TPS improved the mechanical and thermal properties; however a decrease in water vapor permeability was observed (Bhat *et al.*, 2013).

Núñez-Flores *et al.* (2013) studies the mechanical properties of fish gelatin films incorporated with lignin. From the results found that lignin produced an evident plasticizing effect, as deduced from significant decreases in both tensile strength (TS) and breaking force (BF) in the composite films, together with a marked increase in elongation at break (EAB) as well as in breaking deformation (BD). It has been reported as a plasticizing agent in composite films with starch (Baumberger *et al.,* 1997) or with soy protein isolates (SPI) (Huang *et al.*, 2003). In contrast, lignin acts as a reinforcing agent with cellulose (Rohella *et al.*, 1996) or polyethylene oxide (Kadla and Kubo, 2003). A significant increase in TS has been also reported for SPI-lignin blends (Huang *et al.*, 2003). However, it should be noted that SPI films were considerably less resistant than the gelatin films obtained in this study, in accordance to a previous work performed by Cao *et al.* (2007).

Bhat *et al.* (2013) studied the incorporating lignin from oil palm black liquor waste into the food packaging. In this study, food packaging films were prepared by casting method from sago starch (as film matrix with 30% w/w glycerol as plasticizer) by adding lignin isolated from empty fruit bunch, as a reinforcing material (1, 2, 3, 4 and 5% v/w). Results showed that incorporation of isolated lignin showed the improvement of barrier properties and heat seal strength. The observed decrease in water vapor permeability (WVP) in lignin incorporated films is an indication of improved permeability of films towards water vapor. Improvement in WVP in starch/lignin films can also be attributed to strong intermolecular interactions occurring between starch and lignin molecules. Heat seal strength of sago starch/isolated lignin films were found to be significantly improved corresponding to increase in lignin content. However, the sago starch films incorporated with isolated lignin decreased the brightness of the films on increasing lignin contents, which might be due to color of lignin (black or dark brown). The increasing values of a* indicates films to be more reddish, while increasing values of b* indicates yellow color of the films.

Chauhan *et al.* (2014) reported that when lignin content in polymer increased affected the contact angle increased. The resulting surface was considered to be relatively more hydrophobic which is attributed to the decrease of –OH groups in the blends. In addition, the initial slope of stress–strain curves increased with increasing lignin content. The elongation at break of films was reduced by ~15% at 5 wt% lignin additions and ~87% at 15 wt% lignin content respectively. Because of this, the film with high lignin content was rigid and may chip off in pieces. Thus, its use is limited for bonding of stiff materials. It was also noted that the energy to break of films was maximum at 5 wt% lignin content. The optimal properties of blend were achieved at 5–7 wt% lignin content probably due to its inter-mixed type of phase morphology.

Furthermore, lignin has been investigated as compatibilizer between natural fibers and polymer matrix (Graupner, 2008). The effective use of lignin in blends with various synthetic polymers such as poly (propylene) (Pouteau *et al.*, 2003), poly (ethylene terephthalate) (Canetti *et al.*, 2007), poly (vinyl chloride) (Mishra *et al.*, 2007), low-density polyethylene and linear low-density polyethylene (Alexy *et al.*, 2000) has been also reported in literature. Ciobanu *et al.* (2004) studied mechanical properties of polyurethane films blends with lignin. The mechanical properties blends with lignin are increased as compared with those of the pure polyurethane homopolymer. The higher strengths of lignin–polyurethane blends could be explained by a positive effect of lignin on the molecular order of the polyurethane network. This behavior is in good agreement with results obtained on lignin–cellulose blends (Glasser *et al.*, 1996) and suggests a phase-separated morphology for lignin–polyurethane, which is often responsible for increasing strength. However, the mechanical behavior of lignin–polyurethane films indicates that lignin can improves the polyurethane properties only when is incorporated in limited extent (no more than 10%, and preferably up to 5%). On the other hand, the environmental concerns and a shortage of petroleum resources have driven efforts on the preparation of bioplastics made from renewable materials.

8. Phenolic compounds

Phenolic compounds are commonly found in both edible and nonedible plants, and they have been reported to have multiple biological effects, including antioxidant activity. Crude extracts of fruits, herbs, vegetables, cereals, and other plant materials rich in phenolic are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufacturers, and consumers as the trend of the future is moving toward functional food with specific health effects (Scalbert, 1991).

Phenolic compounds composed of one or more aromatic benzene rings with one or more hydroxyl groups (C-OH). This enormous class includes numerous plant compounds that are chemically distinct from terpenes. Although the essential oils are often classified as terpenes, many of these volatile chemicals are actually phenolic compounds, such as eucalyptol from (*Eucalytus globulus*), citronellal from and clove oil from *Syzygium aromaticum*. Like the terpenes, many phenolic compounds are attached to sugar molecules and are called glucosides or glycosides, depending on the type of sugar. Most vanilla flavorings sold in markets are synthetic vanillin containing artificial food coloring and preservatives. Vanillin is a single-ring phenolic compound derived from the breakdown of lignin, a complex phenolic polymer that gives seasoned wood its color, hardness and mass. Natural vanilla flavoring also comes from vanillin plus several other aromatic compounds in the seed capsules of the vanilla orchid (*Vanilla fragrans*). The double-ring phenolic compounds called coumarin imparts the distinctive sweet smell to newly-mown hay. Coumarin is also an anticoagulant that represses the synthesis of prothrombin, a plasma protein produced in the liver in the presence of vitamin K. Prothrombin is the precursor of the enzyme thrombin which catalyzes the conversion of fibrinogen to fibrin in the clotting process. Threads of fibrin wind around blood platelets in the damaged area of a blood vessel and provide the framework of a blood clot. Coumarin is converted into the anticoagulant dicoumarin during the improper curing of sweet clover hay from species of Melilotus. Hemorrhaging and death may occur in cattle that eat spoiled sweet clover hay, depending on the amount consumed. Dicoumarin and related drugs are used in human medicines as blood thinners and are commonly used in rodent poisons such as Decone, which literally cause rats to bleed to death. Potential sources of antioxidant compounds have been searched in several types of plant materials such as vegetables, fruits, leaves, oilseeds, cereal crops, barks and roots, spices and herbs, and crude plant drugs. Flavonoids and other plant phenolics, such as phenolic acids, stilbenes, tannins, lignans, and lignin, are especially common in leaves, flowering tissues, and woody parts such as stems and barks (Campos *et al.*, 2003). They are important in the plant for normal growth development and defense against infection and injury. Flavonoids also partly provide plant colors present in flowers, fruits, and leaves. They generally occur as glycosylated derivate in plants, although conjugations with inorganic sulfate or organic acid as well as malonylation are also known (Wang and Weller, 2006). The antioxidant activity of phenolic is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. In addition, they have a metal chelation potential (Campos *et al.*, 2003).

9. Application of lignin extracts as antimicrobial and food applications

The antimicrobial properties of lignin have been reported previously in the literature, both in model system and in experimental animals, for example, from hydrolysates of several lignocellulosic materials (ethyl acetate extracts) (Cruz *et al.*, 2001), lignin-related structures from alkaline extractions (Oh-Hara *et al.*, 1990), kraft-lignins (Dizhbite *et al.*, 2004) and to a lesser extent from lignosulphonates (Núñez-Flores *et al.*, 2012). The origin of lignin might influence their antimicrobial properties. Thus, Oh-Hara *et al.* (1990) reported that the antimicrobial activity induced by commercial lignin was much lower than that induced by fractions of pine cone extracts obtained by successive alkaline extractions (and then recovered as acid precipitates at pH 5). By comparing the spectra, these authors found that some pine cone extract include more alkenic double bonds and fewer OCH_3 than commercial alkali-lignin, while a coumaryl type of lignin structure could be responsible for the antimicrobial activity.

Dong *et al.* (2011) studied the antimicrobial activities of lignin from residue of corn stover to ethanol production compare with lignin extract and commercial lignin. *Staphylococcus aureus, Listeria monocytogenes*, *Salmonella enterica Serovar Enteritidis, E. coli O157:H7* and *Candida lipolytica* were tested. The results showed there was no antimicrobial activity on Gram-negative bacteria (*E. coli* O157:H7 and *S. Enteritidis*). But, all the tested materials (lignin extract, lignin residue and commercial lignin) had inhibitory effect on Gram-positive bacteria (*L. monocytogenes* and *S. aureus*) and yeast (*C. lipolytica*). From the results, lignin extract exhibited strong inhibitory effect, but it was lower than that of commercial lignin. Lignin residue had minimal inhibitory effect. Commercial lignin possessing extraordinary antimicrobial effect may due to its high pH (10.5) and good solubility in trypticase soy broth (TSB) while the extracted lignin had approximately neutral pH and was not soluble in TSB. Efficiency of lignin for antimicrobial properties could be explained that as a complex phenolic polymer, lignin possesses antimicrobial properties (Lu *et al.*, 1998; Dizhbite *et al.*, 2004). In addition, Slavikova and Kosikova (1994) said that lignin samples from delignification of softwoods and hardwoods also demonstrated antimicrobial properties again a series of yeast, and the oxidation of lignin decreased its antimicrobial activities.

Núñez-Flores *et al.* (2013) studied effect of lignin on the antimicrobial activity of active fish gelatin films was determined by the disk diffusion method in agar against 26 microbial strains (including Gram-positive and Gram-negative bacteria, yeast and molds). The lignin showed antimicrobial activity against the 26 microbial strains studied. The pH of lignin (in this assay the lignin was tested at neutral and basic pH) could be partly responsible for this lack of activity, although the antimicrobial effectiveness of polyphenolic compounds from other sources (green tea) was similar in a pH range of 4.0-7.0 (von Staszewski *et al.*, 2011). Some authors suggested that the inhibition of microbial growth by phenolic acids, among which includes the p-coumaric, increased with decreasing pH (Wen *et al.*, 2003). Lignin have been shown to be effective antimicrobial at concentrations that are not harmful to normal human cells, thus furthering their possible use in the formulation of active food packaging biomaterials (Ugartondo *et al.*, 2008).

Furthermore, lignin also another important feature is antioxidant (radical scavenging capacities). The radical scavenging activity of lignin is influenced by structural features, such as the presence of phenolic hydroxyl groups, methoxy groups, π-conjugation systems as well as the molecular weight, heterogeneity and polydispersity (Dizhbite *et al.*, 2004). Results from the molecular complexity of lignin, it becomes difficult to assign the antioxidant efficacy to specific structural components, compared to the activities of chemically defined tannins and flavonoids (Sakagami *et al.*, 2005). Dizhbite *et al.* (2004) reported that lignin is a rich source of antioxidants, mainly due to the scavenging action of their phenolic structures on oxygen-containing reactive free radicals. Antioxidant activity of naturally occurring phenolic compounds has been characterized using DPPH as a reactive free radical, and this method has been widely recognized as the most reliable technique for measuring antioxidant activity. Recent works, have confirmed that lignin can be used in cosmetics and pharmaceutical preparations without harmful effects in human cells. These studies have also demonstrated that the origin of the lignin strongly influences their antiradical activity (Ugartondo *et al.*, 2008).

Objectives

1. To prepare and compositional analysis of rice starch and the oil palm empty fruit bunch's lignin (OPEFB's lignin).
2. To study the antimicrobial properties of OPEFB's lignin.
3. To study the effects of OPEFB's lignin and modified OPEFB's lignin on the properties of biodegradable rice starch films.
4. To study the effect of relative humidity on the properties of biodegradable rice starch films reinforced with OPEFB's lignin.
5. To study the potential application of biodegradable rice starch films reinforced with OPEFB's lignin for shelf- life extension of food products.
6. To determine the degradation of rice starch films reinforced with OPEFB's lignin.

CHAPTER 2

MATERIALS AND METHODS

1. Materials

1.1 Raw materials

The oil palms empty fruit bunches (OPEFB) were supplied by the Virgin Vegetable Oil Co., Ltd., Songkhla, Thailand. Chiang rice grain was purchased from a local rice mill in Phattalung, Thailand. The rice grain was stored in an ambient temperature until used. *Staphylococcus aureus, Escherichia coli O175:H7* and *Listeria. monocytogenes* were obtained from Food Safety Lab, Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Hat-Yai, Songkhla. Cultures were streak-plated once a week and cultures for experiments were inoculated into media from a single colony and incubated overnight under the appropriate media and atmospheric conditions.

1.2 Chemical reagents

Sorbitol and sodium benzoate were purchased from the Vidyasom (Bangkok, Thailand). Ethanol 95%, sulfuric acid and hydrochloric acid were purchased from J.T. Baker (Center Valley, PA, USA). Analytical grade (AR) magnesium chloride, magnesium nitrate, sodium nitrite, sodium chloride, ammonium sulfate and potassium nitrate for saturated salt solutions (at 33% RH, 50% RH, 60% RH, 75% RH, 79% RH and 90% RH, respectively), sodium hydroxide, potassium sulphate, acetone, pentane, sodium carbonate, trypticase soy broth (TSB), trypticase soy agar (TSA) and plate count agar (PCA) were purchased from High Science Co. Ltd. (Thailand). Folin-Ciocalteu reagents were purchased from Merck Co. Ltd. (Thailand). Gallic acid and commercial lignin (alkali process) were supplied by Sigma-Aldrich Co. Ltd. (Thailand).

2. Instruments

Table 2. List of equipment used in this work.

Instruments	Model	Company/City/Country
Magnetic stirrer	C-M A G HS 7	IKA LABORTECHNIK, Stanfen, Germany
Stirrer	R W 20n	IKA LABORTECHNIK, Stanfen, Germany
Homogenizer	T 25	Ultra Turrax, Malaysia
Water bath	W 350	Memmert,Schwabach, Germany
Spectrophotometer	LIBRA S21-S22	Biochrom Ltd, Cambridge,UK
Universal testing machine	LR 30K	Lloyd Instruments Ltd, Hampshire, UK
Balance	TE124S	Sartorius, MA, USA
CIE colorimeter	Color Flex	HunterLab Reston, Virginia, USA
Rotary digester	RDB-D352	Namasiam Intertrade Co., Ltd.
Refrigerated Centrifuge	Z 513 K	Wehingen, Germany
Differential scanning calorimeters	Perkin-Elmer DSC-7	Norwalk, Conn. U.S.
Thermogravimetric analyzer	Perkin-Elmer TGA-7	USA
Fourier transform infrared Spectrometer	Bruker Model EQUINOX 55	Bruker Co., Ettlingen, Germany
Contact angle measurement	OCA 15 EC	Data Physics Instruments GmbH, Germany

3. Methods

3.1 Preparation of rice starch

Rice starch was isolated by the alkaline method following the procedures of Sawai and Morita (1968) and Detduangchan (2012). The rice grain was soaked in 4 times the volume of distilled water for 24 h, followed by wet grinding with a rice grinding machine. The slurry was isolated by centrifuging at 3,000 xg for 30 min and referred rice flour. Then the flour was extracted with 2 times the volume of 5% NaCl for 2 h (globulin extract) and centrifuged at 3000 xg for 30 min. The flour was then extracted for the prolamin with 2 volumes of 70% ethanol for 2 h and followed by glutelin extraction with 1 time the volume of 0.35% NaOH and continuously stirred. After 16 h, the starch was adjusted to pH 7 with 1 M HCl and isolated by centrifuging (3,000 xg, 30 min), and wash with 4 times the volume of distilled water for 3 times (with albumin extract and other chemical reagents). The starch was dried at 50 ^{o}C for 24 h. After then, take to blend thoroughly, screen by mesh No.200.

Compositional analysis of rice starch
- Protein (AOAC, 2000)
- Fat (AOAC, 2000)
- Moisture (AOAC, 2000)
- Ash (AOAC, 2000)
- Carbohydrate (AOAC, 2000)
- Soluble amylose (Shanthy *et al.* (1980))

3.2 Preparation of oil palm empty fruit bunch’slignin (OPEFB’s lignin)

Palm fruit bunches were washed with clean water for 3-5 times, chopped into small pieces and then dried in hot air oven at least 72 hours (14-15% moisture content). Next, suspension (1:15 w/v of chopped palm fruit bunches in 20% NaOH solution) was prepared at 170 °C for 120 min in rotary digester (Model RDB-D352, from Nanasiam Intertrade Co., Ltd.). After that, the oil palm empty fruit bunches pulp was separated from black liquor with a strainer, and the obtained liquid was storage in ambient temperature for next experiment. The soluble lignin in black liquor was precipitated with 4M sulfuric acid drop wise added until the pH value of liquid reduced to 5 (this procedure was performed under the airflow to remove odorous compounds created during precipitation) and the obtained colloid was then storage in ambient temperature for 24 hours (Narapakdeesakul *et al.*, 2013). After that, the precipitated lignin was filtered through Whatman™ no.4 filter paper, and then washed with clean water for 3 times, the remaining left-over solution in precipitated lignin was evaporated in vacuum oven at 70 °C for 48 hours. The dried lignin was grinded in a mortar and sieved with 200 mesh strainer, and then storage in ambient temperature avoiding the daylight.

Analysis of OPEFB's lignin
- Moisture content (AOAC, 2000)
- Structural analysis of extracted lignin by FTIR
- Color by CIELAB system
- Total phenolic content
- Minimum bactericidal concentration (MBC)

3.3Analytics of rice starch and OPEFB's lignin

3.3.1 Compositional analysis of rice starch

The proximate composition such as protein, fat, moisture, ash and carbohydrate of rice starch was determined following standard methods of AOAC (2000). Soluble amyloses were measured using iodine binding procedures following method of Woggum (2014).

3.3.2 Structural of lignin by FTIR

FTIR analysis was performed by an Equinox 55 infrared spectrometer, from Bruker Corporation, Germany. Each spectrum was recorded in a frequency range of 400-4000 cm^{-1}. The lignin fractions were analyzed by using KBr pellet technique.

3.3.3 Total phenolic content of OPEFB's lignin

The TP content of the lignin extract were modified according to the previously reported method of Siripatrawan and Harte (2010), involving the Folin–Ciocalteu reagent and gallic acid as standard with some modifications. Shortly, 0.05 g of lignin extract sample was dissolved in 2 mL of distilled water. After, precipitate 10 h, then lignin extract solution (0.4 mL), distilled water (7 mL), and Folin–Ciocalteu reagent (0.7 mL) for 2 mL were mixed and kept at room temperature for 4 min, after which 1.6 mL sodium carbonate 75 g/L of distilled water. The mixture was stirred thoroughly and allowed to stand for 2 h at room temperature prior to an absorbance reading at 765 nm in a spectrophotometer (Shimadzu UV-VIS 1601, Japan). The concentration range of gallic acid is 0-500 mg/L (0-0.5 mg/mL). The results were expressed as mg gallic acid equivalents (GAE) per 100 mg of dried weights according to the following equation:

$$T = (C.V) / M$$

Where T is total content of phenolics compound (milligram per gram dried lignin, in GAE), C is the concentration of gallic acid obtained from the calibration curve (milligram per milliliter), V is the volume of lignin extract (milliliter) and M is the weight of dried lignin (milligram).

3.3.4 Preparation of inoculums and minimum bactericidal concentration (MBC) of OPEFB's lignin

- *L. monocytogenes*, *E. coli* and *S. aureus* were cultured into 5 mL of Trypticase Soy Broth (TSB), and incubated in a shaker incubator at 35^{o}C for 18-24 h. The optical density (OD) of the bacteria was adjusted to the standard of McFarland NO. 0.5 with 0.85-0.9 g sodium chloride/100 mL sterile solution to achieve a concentration of approximately 10^{8} CFU/mL. The final concentration of the cell number of approximately 10^{5}-10^{6} CFU/mL was obtained by diluting 100-1000 times with sterile sodium chloride solution.

- Minimum bactericidal concentrations (MBC) from lignin inhibiting the growth of *L. monocytogenes*, *E. coli* and *S. aureus* were analyzed by modification of the method used by Canillac and Mourey (2011). The lignin was diluted in to suitable concentration (mg/ml) of each bacterial, in trypticase soy broth (TSB). The medium was inoculated with 0.1 mL of a preculture in Trypticase soy agar (TSA) at 37°C. The cells of the inoculum were in exponential growth phase after 2-3 h of static incubation or in stationary phase after 17-19 h of static incubation. The final concentration of bacteria, determined with plate count method was of the order of 10^5 CFU/mL. The MBC is the lowest concentration of lignin which no growth was detected after 48 h at 37°C.

3.4 Effect of OPEFB's lignin content on the properties of rice starch films

3% starch solution (w/v) were prepared by adding rice starch in distilled water with vigorous stirring and heating the slurry to 85°C (gelatinization temperature) and held at this temperature for 10 min. Then the slurry was cooled to 50±5°C, sorbitol (plasticizer) was added as 50% of dry wt. starch, stirred for 2 min. and then lignin (1, 3, 5, 7 and 10% w/w of dry wt.) were added. Lignin was stirred for 5 min for completely dispersed in the starch solution. The mixtures were casted onto flat non-stick trays to set. After that the tray were held overnight at 55°C for 18-20 hours. The resulting films were kept in desiccators at 55% RH for 72 hours before irradiation as described in following section. All treatments were made in triplicate.

Testing of rice starch films reinforced with OPEFB's lignin

- Tensile strength and elongation at break by ASTM D882-91 (ASTM, 2000)
- Water vapor permeability by ASTME96-92 (McHugh *et al.* (1993))
- Film solubility (Jangchud and Chinnan, 1999)
- Degradation Temperature, T_d by Thermogravimetric analysis (TGA)
- Glass Transition Temperature, T_g by Differential Scanning Calorimetry (DSC)
- Film color by CIELAB system
- Film transparency value (Han and Floros, 1997)
- Morphological properties (distribution of lignin)
- Antimicrobial properties (modified from Pranoto *et al.* (2005))

3.5 Synthesizing of OPEFB's lignin derivatives (Hydrophobicity improvement)

The procedure for synthesizing the OPEFB's lignin derivatives was applied from Narapakdeesakul *et al.* (2013). OPEFB's lignin dissolving in acetone (9:90 g/mL) was combined with a mixture of oil (palm oil and soy bean oil) and concentrated sulfuric acid (45:0.3 g/mL). The mixing proportion used was 1:1 w/w. The mixtures were stirred in three neck round flask at 70°C for 2.5 h under a nitrogen flow. After the reaction finished, the mixtures were cooled to room temperature than washed in pentane solution for remove the excess oil from the lignin derivatives. The derivatives obtained were then dried in a vacuum oven for 48 h to evaporate the excess pentane solution. After that, the derivatives was mashed in a mortar, sieved through a mesh no.200 strainer, and then stored in an ambient temperature in the absence of daylight.

3.6 Effect of OPEFB's lignin derivatives content on the properties of rice starch films

3% starch solution (w/v) were prepared by adding rice starch in distilled water with vigorous stirring and heating the slurry to 85°C (gelatinize temperature) and held at this temperature for 10 min. Then the slurry was cooled to 50±5°C, sorbitol (plasticizer) was added as 50% of dry wt. starch, stirred for 2 min and then lignin derivatives (1, 3, 5, 7 and 10% w/w of dry wt.) were added. Starch and lignin derivative were stirred for 5 min for completely dispersed in the starch solution. The mixtures were cast onto flat, leveled, non-stick trays to set. After that the tray were held overnight at 55°C for 18-20 hours. The resulting films were kept in desiccators at 55% RH for 72 hours before irradiation as described in following section. All treatments were made in triplicate

Testing of rice starch films reinforced with OPEFB's lignin derivatives

- Tensile strength and elongation at break by ASTM D882-91 (ASTM, 2000)
- Water vapor permeability by ASTME96-92 (McHugh *et al.* (1993))
- Film solubility (Jangchud and Chinnan, 1999)
- Film color by CIELAB system

- Film transparency value (Han and Floros, 1997)
- Surface contact angle measurement
- Structural of lignin by Fourier Transform Infrared Spectroscopy (FTIR) method
- Morphological properties (distribution of lignin)
- Antimicrobial properties (modified from Pranoto *et al.* (2005))

3.7 Effect of relative humidity on the properties of rice starch films reinforced with OPEFB's lignin

Moisture sorption isotherms were determined by placing rice starch films into a controlled humidity environment at a constant temperature until equilibrium. Select the optimum conditions from section 4 and 6 for testing. The films were placed into environments of various relative humidity over salt solution and constant temperature at 27±2^{o}C in desiccators. The relative humilities were 33% RH (magnesium chloride), 50% RH (magnesium nitrate), 60% RH (sodium nitrite), 75% RH (sodium chloride), 80% RH (ammonium sulfate) and 90% RH (potassium nitrate). The films were cut to size of the tested (2.54 x 12 cm for tensile strength and elongation at break and diameter 7 cm for water vapor permeability) stored in desiccator for 72 h before testing, the films were tested by tensile strength, elongation at break and water vapor permeability.

3.8 Application the rice starch films reinforced with OPEFB's lignin in food products (imitated crab meat and bread)

Commercial imitated crab meat and bread were tested by wrapped and placed between the sheet of food products, respectively by rice starch films reinforced with 10% lignin and lignin derivative followed the section 4 and 6. For imitated crab meat kept at 4-6^{o}C tested every 7 day for 28 day and bread kept at room temperature tested every 2 day for 6 day. Polyethylene (PE) films were used as references for imitated crab meat. The food products were sampled for testing of physical quality, chemical quality and microorganism as followed;

- Sensory evaluation (Appearance, Odor and Texture)
- a_w value by Thermoconstanter (ASTM, 1995)
- Color value by CIELEB system
- TBAR value (Buege and Aust, 1978)
- Total viable count by method of Standard Method (BAM, 2001)

Before testing of sensory quality, physical quality, chemical quality and microorganism, the films were completely removed from food products.

3.9 Biodegradation tested

The soil burial degradation was carried out followed the method of Franco *et al* (2004). The soil burial degradation test started on May 2, 2014 and ended on May 30, 2014. The test was carried out in soil at Faculty of Agro-Industry, Prince of Songkla University, Thailand. Biodegradability of the samples was studied by evaluating weight loss of films over time in a soil environment. Indoor soil conditions were tested. Select the optimum conditions from section 4 and 6 for testing. The films samples were cut into 2 cm x 5 cm pieces dried in an oven until it attains the constant weight. Then the dried film samples were buried at the depth of 8 cm from the surface of the soil. The soil was kept moisture by sprinkling water at a regular time interval to maintain 30–50% humidity. The degradation of the specimen was determined at a regular time interval (4 days) by taking the specimen carefully from the soil and washing it gently with distilled water to remove the soil. The specimen was dried in an oven until a constant weight was obtained. Weight loss (WL) during soil burial was measured according to Rafiemanzelat *et al* (2012). The mass of each sample was weighed before and after degradation and weight loss of each film sample was obtained using the following formula:

$$\text{Weight loss (\%)} = \frac{\mathrm{M0-M1}}{\mathrm{M0}} x100$$

Where *M*o is the pre-degraded dry weight of the film and *M1* is the dry weight of the sample after degradation.

4. Determination of film properties

4.1 Conditioning

All films were conditioned prior to subjecting them to permeability and mechanical tests according to the Standard method, D618-61 (ASTM, 1993a). Films used for testing water vapor permeability (WVP), tensile strength (TS), and elongation (E) were conditioned at 60% RH and 27$\pm$2^{o}C by placing them in desiccators over a saturated solution of $Mg(NO_3)_2 .6H_2O$ for 72 h or more. For other tests, film samples were transferred to plastic bags after peeling and placed in desiccators.

4.2 Film thickness

Thickness of the films was measured with a precision digital micrometer (Digimatic Indicator, Mitutoyo Corporation, Japan) to the nearest 0.0001 (±5%) at five random locations on the film. Mean thickness values for each sample were calculated and used in water vapor permeability (WVP) and tensile strength (TS) calculations.

4.3 Mechanical properties

Before to the measurement of the mechanical properties, the films were conditioned for 72 h in a ventilated oven at 25^{o}C and 55±5% RH. The tensile strength (TS) and elongation at break (%E) of the films were determined as described by Detduangchan (2012) with a slight modification. This was done using a Universal Testing Machine (LLoyd Instruments, Hampshire, UK) equipped with tensile load cell of 100 N (ASTM, 1991). Twenty samples (2.54 cm x 12 cm) with an initial grip length of 10 cm were used for testing. The samples were clamped and deformed under a tensile load with a cross-head speed of 50 mm/min until the samples were broken. The TS was calculated by dividing the maximum force by the initial specimen cross-sectional area, and the %E was calculated as follows:

$$\%E = 100 \text{ x } (d_{\text{after}} - d_{\text{before}}) / d_{\text{before}}$$

Where, *d* was the distance between grips holding the specimen before or after the breaking of the specimen.

4.4 Water vapor permeability

The gravimetric modified cup method based on ASTME96-92 (McHugh *et al*., 1993) was used to determine the WVP of the films. The test cups were filled with 20g of Silica gel (the desiccant) to produce a 0% RH below the films. A sample was placed between the cup and the ring cover of each cup coated with silicone sealant (high vacuum grease, Lithelin, Hanau, Germany) and held with four screws around the cup's circumference. The air gap was at approximately 1.5 cm between the film surface and the desiccant. The water vapor transmission rates (WVTR) of each film were measured at 55±5% RH and 25±2^{o}C. After taking the initial weight of the test cup, it was placed in a growth chamber with an air velocity rate of 135 m/min (Model KBF115, Contherm Scientific, Lower Hutt, New Zealand). The weight gain measurements were taken by weighing the test cup to the nearest 0.0001 g with an electronic scale (Sartorious Corp.) every 3 h for 18 h. A plot of the weight gained versus time was used to determine the WVTR. The slope of the linear portion of this plot represented the steady state amount of water vapor diffusing through the film per unit time (g/h). The WVTR was expressed in gram units per square meter per day. The steady state over time (slope) yielded a regression coefficient of 0.99 or greater. Six samples per treatment were tested. The WVP of the film was calculated by multiplying the steady WVTR by the film thickness and dividing that by the water vapor pressure difference across the film.

4.5 Film solubility

A modified method from Jangchud and Chinnan (1999) was used to measure film solubility. Film pieces, 20 mm x 20 mm, were dried at 70 °C in a vacuum oven for 24 h and then weighed to the nearest 0.0001 g for the initial dry mass. Films were immersed in 20 ml of distilled water in 50 ml screw cap tubes containing 0.01 g/100 g sodium benzoate. The tubes were capped and placed in a shaking water bath for 24 h at 25 ±2 °C. A portion of the solution was removed and set aside for later use in protein solubility tests as described below. The remaining solution and film pieces were poured onto quality filter paper (Whatman™ no 1). These were rinsed with 10 ml distilled water, and dried at 70 °C in a vacuum oven for 24 h to determine the dry mass of the film. Five measurements were taken for each treatment. The total soluble matter was calculated from the initial gross mass and the final dry mass using the following equation:

$$\%\ \mathrm{FS\ (db)}\ = \frac{(\text{film mass before test} - \text{film mass after test})}{\text{film mass before test}}\, x\ 100$$

4.6 Thermal properties by thermogravimetric analysis (TGA)

Thermogravimetric analysis was performed to study the degradation characteristic of the films. Thermal stability of each sample was determined using a (Perkin-Elmer, TGA7) with a heating rate of 10°C/min in a nitrogen environment. It has a weighing capacity of 1 g. Sample were heated range of 50°C to 70°C.

4.7 Differential scanning calorimetry (DSC)

Thermal properties of the rice starch biodegradable films reinforcing with oil palm fruit bunch's lignin were analyzed with a Perkin-Elmer DSC-7 (Norwalk, Conn., U.S.A.) equipped with an intra-coolant Thermal Analysis Controller TAC7/DX (Perkin-Elmer). Samples (approx. 20 mg each, db) were weighed into stainless steel pans (Perkin-Elmer) designed to withstand high pressures. The stainless pan was sealed with an O-ring, and allowed to reach equilibrium of moisture for overnight. An empty DSC pan was used for a reference pan. The heating rate was programmed by holding at -50°C for 1 min, followed by ramping the temperature range of -50°C to 180°C at a rate of 10°C/min, and holding at 180°C for 1 min. Measurements were made at least in duplicate for each treatment.

4.8 Fourier transform infrared spectrophotometry (FTIR)

Before to the analysis, the films were conditioned in a desiccator containing dried silica gel for 7 days at room temperature to obtain the most dehydrated films. The films were scanned with a Bruker Model Equinox 55 FTIR spectrometer (Bruker Co., Ettlingen, Germany). The samples were measured in a horizontal ATR Trough plate crystal cell (45° ZnSe; 80 mm long, 10 mm wide and 4 mm think) (PIKE Technology Inc., Madison, WI). The spectra were performed in the 4000 – 400 cm^{-1} regions to resolve overlapping bands.

4.9 Contact angle measurement

Surface contact angle of films was measured using a contact angle measuring tool (Model OCA 15 EC from Data Physics Instruments GmbH, Germany). A specimen was fitted to the tool and then the water was dripped on the specimen surface. An angle of the water drop was recorded at 1 s after dripping.

4.10 Color and Transparency

Color of film was determined using a CIE colorimeter (Hunter associates laboratory, Inc., Reston, Virginia, USA), working with D_{65} (day light). The color parameters were expressed as L*, a* and b* values [(L* = 0 (black) to 100 (white); a* = -60 (green) to +60 (red); and b* = -60 (blue) to +60 (yellow)]. The standard plate (calibration plate CX0384, L* = 92.82, a* = -1.24 and b* = 0.5) was used as a standard. Color (means of five measurements at different locations on each specimen) was measured on 10 cm x 10 cm segment of films.

The transparency of films was determined using a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). The film samples were cut into rectangles and placed on the internal side of the spectrophotometer cell. The transmittance of films was determined at 600 nm as described by Han and Floros (1997). The transparency of the films was calculated as follows:

$$\text{Transparency} = -\log (T_{600} / x)$$

Where T_{600} is the transmittance at 600 nm and x is the film thickness (mm)

Before to taking color and transparency measurements, films specimens were precondition at 55±5% RH and 25±2°C for 72 h.

4.11 Antimicrobial activity of rice starch films reinforced with OPEFB's lignin.

Antimicrobial activity testing of the rice starch films reinforced with OPEFB's lignin according to Pranoto *et al.* (2005). The films were cut 16 mm diameter discs and treated with UV- light for 2 h, then placed on Mueller Hinton agar plates, with had been previously seeded with 0.1 mL inoculums containing approximately 10^5-10^6 CFU/mL of tested bacteria. The plates were then incubated at 37°C for 24 h. Observation on the diameter of the inhibitory zone surrounding film discs and content area of lignin films based on rice starch with agar surface were measured. Experiments were done in triplicate.

5. Statistical analysis

All the films properties were investigated with at least eight replicates of the samples tested. Means with standard deviations were calculated and reported. Analysis of variance (ANOVA) with Duncan's Multiple Range Test (DMRT) was performed on the data obtained to assay the significant differences between treatments used. All significant values were expressed at 95% confidence level.

CHAPTER 3

RESULTS AND DISCUSSION

1. Chemical composites of rice starch

Table 3 show the different component of rice starch from Chaing rice (Phattalung). Carbohydrates are the main component of starch at 85.79% and a little amount of protein, fat, moisture, and ash (0.43, 0.51, 13.04, and 0.23 respectively). Starch is composed of a mixture of two polymer are amylose and amylopectin. Amylose is an essentially linear polysaccharide and amylopectin is a highly branched polysaccharide. From the experiment soluble amylose content of starch is 19.60±0.25%. In this case, does not measure amylose equivalent value because a soluble amylose will be value than accurate. However, from other research found that the amylose equivalent is higher value than the soluble amylose that cause from inclusion of iodine into long B chains of amylopectin molecules (Khunae *et al.*, 2007). Therefore, it makes not true amylose content.

Table 3 Chemical composite of rice starch

Compositions	Amount (%)
Protein (%wb)	0.43±0.03
Fat (%wb)	0.51±0.02
Moisture (%wb)	13.04±0.11
Carbohydrate (%wb)	85.79±0.18
Ash (%wb)	0.23±0.04
Soluble amylose (%db)	19.60±0.25

Note: Each value is mean of triplicate ± SD.

2. Characteristics of lignin extracted

2.1 Physical properties

Figure 10 showed the OPEFB's lignin, appearance is small particle size and dark brown powder. The dark color of OPEFB's lignin powder is acquired from the chromopholic groups in the lignin structure such as quinones, carboxylic acid, carbonyl groups, phenolic hydroxyl groups and hydroperoxy radicals from distillation, which are all soluble in alkaline medium (pH = 5) (Mussatto *et al.*, 2007). The OPEFB's lignin were moisture content was 9.393±0.16 which different from commercial lignin a little (8.323±0.09). Moreover, the % yield of lignin from the resulting was about 14.52 %.

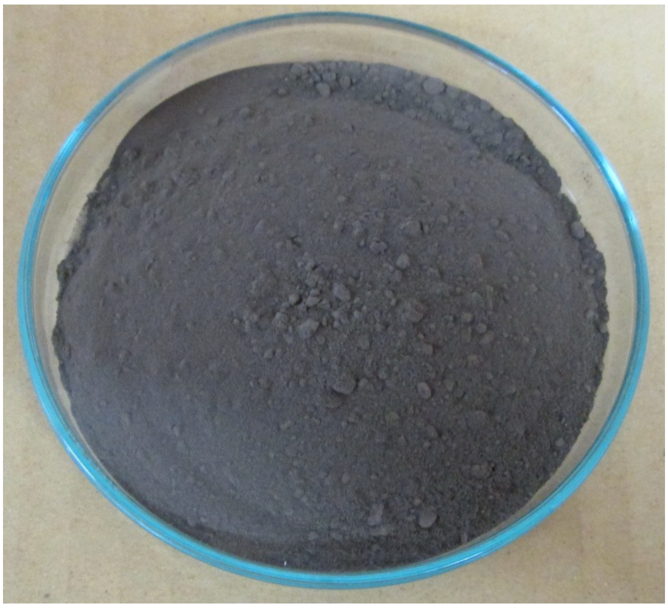


Figure 10 Lignin extracted from oil palm empty fruit branch

2.2 Fourier transforms infrared spectrophotometry (FTIR)

Figure 11 showed the FTIR spectra of OPEFB's lignin and commercial lignin. The FT-IR spectra of OPEFB's lignin and commercial lignin show range between 4000 cm^{-1} to 500 cm^{-1}. From experimental results were compared between OPEFB's lignin and commercial lignin which that have come out the same. In this case, show the absorption value of lignin extract only. All absorption bands are reported to be in according with previous studies (Oihana *et al.*, 2014, Ibrahim *et al.*, 2011). From the results, a band of carbonyl stretching vibration of lignin appeared at ~1700 cm^{-1}. A wide absorption band appeared in a range ~3419 cm^{-1} that can be predicted to aromatic and aliphatic O-H groups. Clear peaks of the band at ~2919 cm^{-1} show the C-H stretching vibration of methoxyl groups ($–CH_3$). A small peak appearing at about 2851 cm^{-1} show band peak of C-H bending of vibration in methoxy group. An absorption band found at ~1607 cm^{-1} presented the C=C (aromatic rings) of aromatic skeletal vibration. At ~1462 cm^{-1} indicated the C-H deformation in $–CH_3$ and $-CH_2-$. Next, the vibration of aromatic ring with C-H was observed at ~1426 cm^{-1}. At peak ~1329 cm^{-1} presented the vibration of syringyl ring breathing with C-O stretching. A clear band found at ~1329 cm^{-1} was predicted to the aromatic C-H deformation of the syringyl unit. The clear peak at ~1214 cm^{-1} was attributed to the C-C, C-O, and C=O stretching. The last, the aromatic C-H in the plane deformation of guaiacyl lignin show a strong band at ~1044 cm^{-1}.

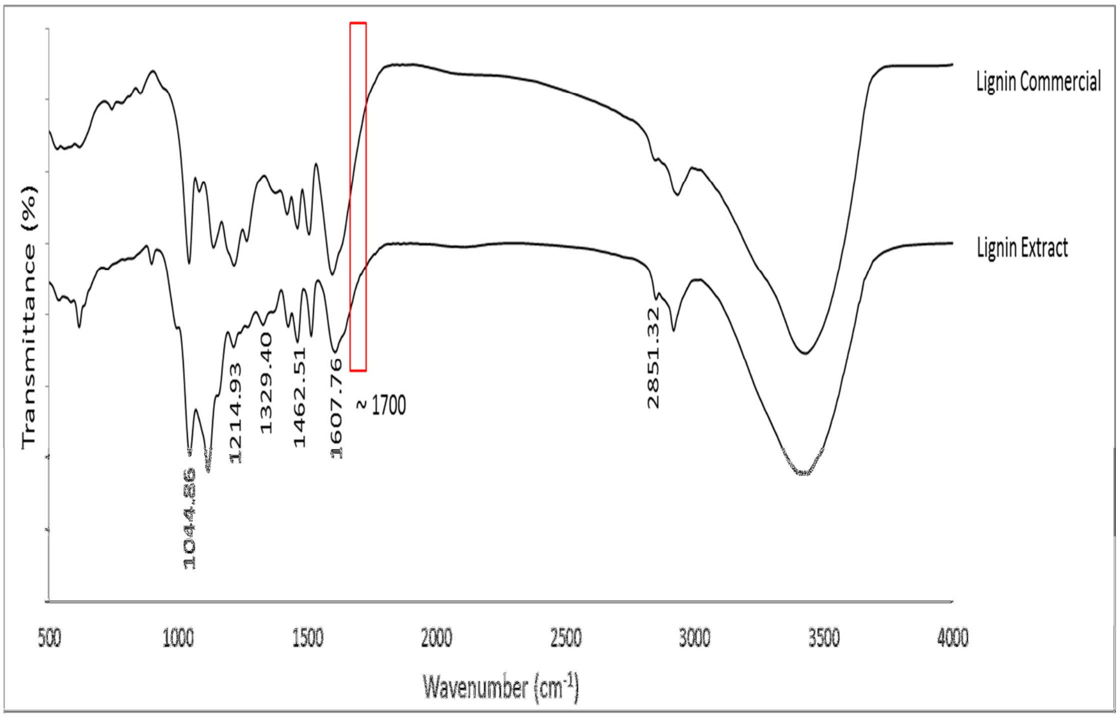


Figure 11 FTIR spectra of lignin extract and commercial lignin.

2.3 Color value of OPEFB's lignin and commercial lignin.

Table 4 present the color value by compared between OPEFB's lignin and commercial lignin. From the results, OPEFB's lignin had lightness (L^*), redness (a^*) and yellowness (b^*) value more than commercial lignin. The color of OPEFB's lignin was 40.23, 5.10 and 13.61 of L^*, a^* and b^* value, for the color of commercial lignin was 22.12, 4.84 and 7.39 of L^*, a^* and b^* value, respectively. Due to purity of OPEFB's lignin poorer than commercial lignin.

Table 4 The color value of extract lignin and commercial lignin.

Lignin	color value		
	L^*	a^*	b^*
Extract lignin	40.23±0.04	5.10±0.15	13.61±0.79
Commercial lignin	22.12±0.06	4.84±0.09	7.39±0.75

2.4 Minimal Bactericidal Concentration (MBC) of OPEFB's lignin.

The different concentration of OPEFB's lignin presented different levels of growth inhibition against *L. monocytogenes, S. aureus* and *E. coli* using the broth dilution method. The growth of *L. monocytogenes, S. aureus* and *E. coli* was inhibited by lignin extract at 25, 3.125 and 25 mg/mL, respectively (Figure 12 and Table 5), which delayed the lag phase and lowered growth rate and final cell concentration of the microorganism. The mechanism of action responsible for antimicrobial activity of phenolic compounds showed in herbaceous and woody plant has not been fully defined. From the experimentation, total phenolic content of OPEFB's lignin contained phenolic compounds about 4.687±0.15 mg GAE/100mg dry weight samples. The antimicrobial activities of OPEFB's lignin as shown above could be apply to develop lignin-based choice products of antimicrobial agents (Mussatto and Roberto, 2004). Cox *et al.* (2000) said that the growth inhibition of microorganisms from phenolic compounds in the extracts to inhibit respiration and cause leakage of cytoplasm of bacteria. In addition, ability to inhibit microorganisms of phenolic compounds caused inhibition of the enzyme activity of extract from phenolic compounds, which are enzymes involved in energy within production of microorganisms including caused sedimentation or loss condition of protein in the cell culture (Prindle and Wright, 1997). In this research, OPEFB's lignin had inhibitory and killing effect both on gram-negative and gram-positive bacteria in different concentration of each bacterium. These results pointed out that OPEFB's lignin have potential as natural antimicrobial agents.

Table 5 Minimal Bactericidal Concentration (MBC) of lignin extracts against *L. monocytogenes, S. aureus* and *E. coli.*

Bacteria type	Lignin extracts concentration (mg/mL)	Positive/Negative
L. monocytogenes	6.25	-
	12.5	-
	25	+
	50	+
	100	+
	200	+
S. aureus	0.39	-
	0.78	-
	1.56	-
	3.125	+
	6.25	+
	12.5	+
E. coli	6.25	-
	12.5	-
	25	+
	50	+
	100	+
	200	+

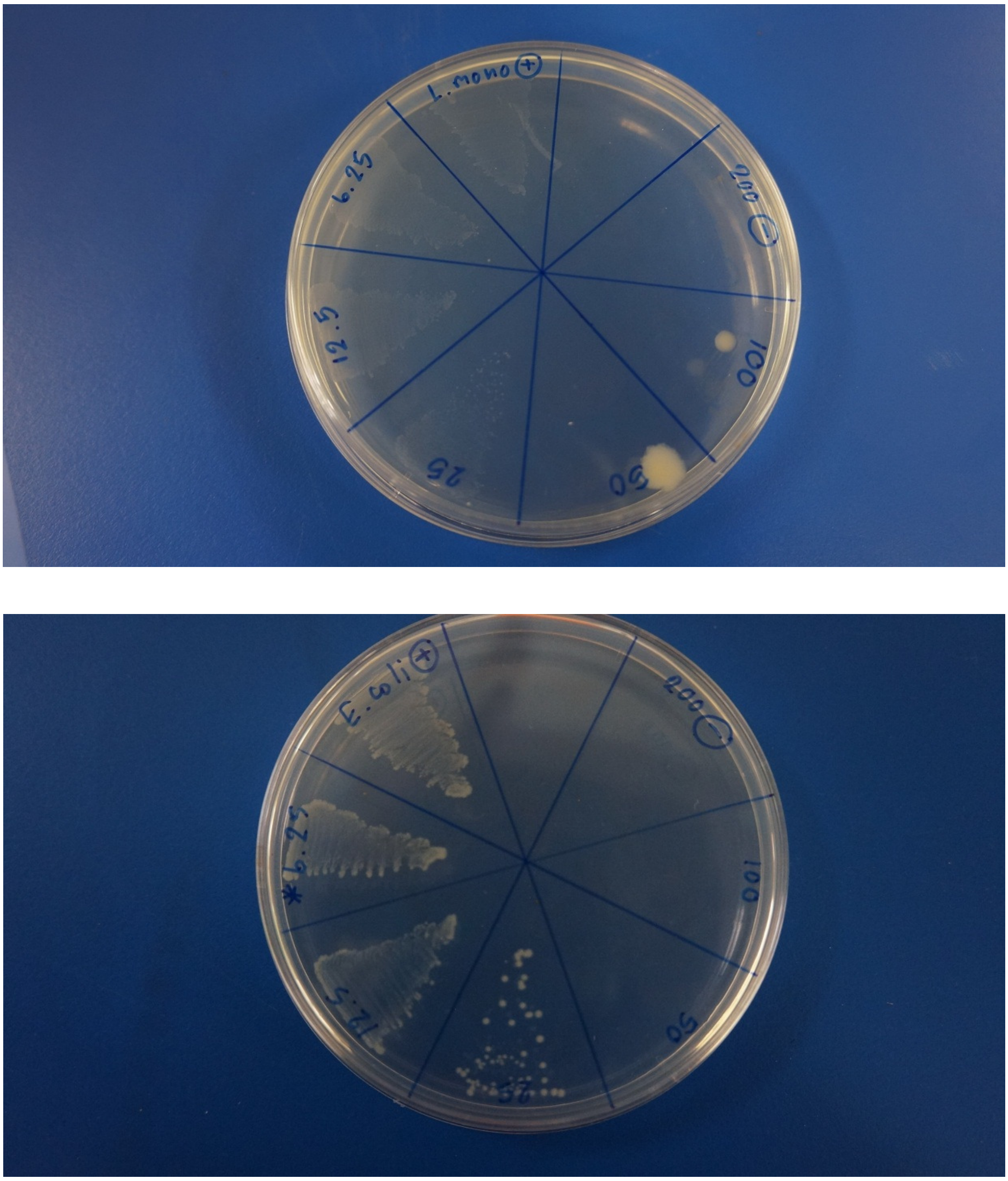


Figure 12 Minimal Bactericidal Concentration (MBC) of inhibitory zone of lignin extracts for *L. monocytogenes* and *E. coli.*

3. Effect of OPEFB's lignin content on the properties of rice starch films.

3.1Mechanical properties

Tensile strength is a measure of the resistivity of materials under tension to breaking. This depends on the strength, length and surface area of films, and also the bonding strength between them (Rabinovitch, 2003). Generally, a high tensile strength is required, but deformation values must be adjusted according to the intended application of the films. That is, whether it is undeform able material to provide structural integrity or to reinforce structure of the food (Gontard *et al*., 1992). The tensile strength (TS) and elongation at break (%E) of the rice starch biodegradable films reinforcing with OPEFB's lignin is depicted in Figure 13. The TS of biodegradable rice starch films were improved by the addition and content of OPEFB's lignin. The result showed that, TS remarked increase as content of lignin increase from 0 to 5% indicating that a high compatibility occurs between starch matrix and OPEFB's lignin. Due to lignin is a three-dimensional polyphonic macromolecule which has a very complex structure. That in the lignin structure composes of many subunits, such as phenols, aromatic rings and methoxy groups, joined together with both C-C and C-O-C linkages (Narapakdeesakul *et al*., 2013). Addition, at lower OPEFB's lignin content, dispersion of lignin is very well, and increases in TS were occurred. However, the TS showed decrease when higher (>5%) OPEFB's lignin was applied. Similar results were observed by Unnikrishnan and Thomas (2007) and Sangthong *et al* (2009). When lignin content up to 5% the films look like increase the hard and brittle due to complexity of lignin structure and unstable distribution affected TS value was decrease. The content of fillers also affected the TS of the biodegradable rice starch films. According to the results, the TS of the films was not statistically different ($p>0.05$) when 3% (5.554 MPa) and 5% (5.806 MPa) of OPEFB's lignin was used (Figure 13A). The elongation at break (%E) indicates the flexibility and stretch of the film, which is determined at the point when the film breaks under tensile testing. The %E of the biodegradable rice starch films reinforced with OPEFB's lignin content is shown in Figure 13B. The results showed that addition and increases in lignin content yielded decrease in %E from 35.75% to 23.87%. This might be due to lignin is non-elastic biopolymer and complex racemic aromatic heteropolymers (Narapakdeesakul *et al*., 2013). The experiments shown that TS and %E of biodegradable rice starch films is almost inversely related. Because the increase brittleness of the composite material induces a decrease of %E. The decrease in %E with the rigid filler addition is a well-known phenomenon that is related to the differences in the rigidity between matrix and fillers. Because of the rigid nature of the fillers, most of the system deformation under high strain comes from the polymer. Consequently, the actual deformation experienced only by the polymeric matrix is much greater than the measured deformation of the sample. Thus, the polymer reaches the failure strain limit at a lower total deformation (Wetzel *et al*., 2003). In conclusion, filling the lignin might act like reinforce in the films. Bringing together reinforced and starch induced formation causes starch-reinforced interactions that are detrimental to starch-starch interactions. Lignin has provided sufficiently compatibility with different polymers. It has been reported as a plasticizing agent in composite films with starch (Baumberger *et al*., 1997) but in the latter case only when incorporated at medium concentrations. As the result of the density of intermolecular interactions in the material decreased and free volume between polymer chains increase. The effect of lignin in the biodegradable rice starch films is most likely due to its small size. This allows it to be more rapidly inserted between the polymer chains, with a resulting influence on the mechanical properties.

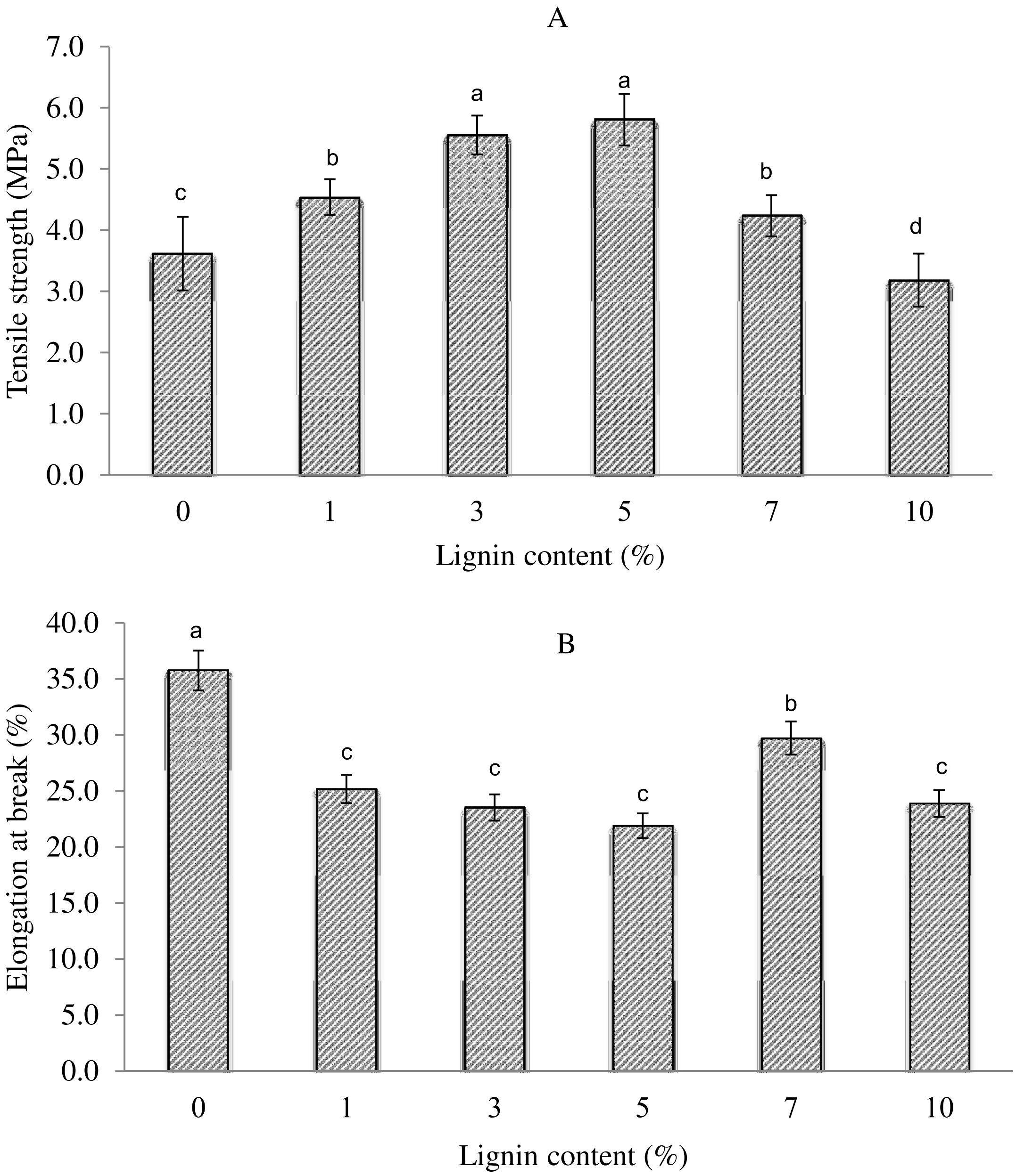


Figure 13 Effect of oil palm fruit bunch's lignin content on tensile strength (A) and elongation at break (B) of rice starch films.

3.2 Water vapor permeability (WVP) and Film solubility (FS)

As a food packaging, films is often required to avoid or at least to decrease moisture transfer between the food and the surrounding atmosphere, and water vapor permeability should be as low as possible (Ma *et al*., 2008). Water vapor permeability (WVP) is a proportional constant assumed to be independent of the water vapor pressure gradient applied across the films. However, hydrophilic materials, such as starch films, deviate from this ideal behavior due to interactions of permeating water molecules with polar groups in the film's structure (Hagenmaier and Shaw, 1990). The films ability to retard moisture loss from the product is an important characteristic that affects product quality. While, film solubility (FS) is an important property of edible films for application as food protection where water activity is high, or when the film must be in contact with water during processing of the coated food (e.g. to avoid of fresh or frozen products) (Gontard *et al.,* 1992). Generally, higher solubility would indicate lower water resistance. However, a high solubility may an advantage for some applications (Stuchell and Krochta, 1994).

The content of OPEFB's lignin on WVP of biodegradable rice starch films is presented in Figure 14. The WVP of the films decreased with the addition and increasing of lignin up to 5% (4.95 to 3.54 g.mm/m^2.day.kPa) and the highest value occurred when no lignin and 10% of lignin was applied (4.95 and 6.63 g.mm/m^2.day.kPa, respectively). By the reason of the water resistance of lignin was better than that of the rice starch matrix and lignin dispersed well in the matrix of starch provided fewer paths for water molecules to pass through the composites. Polysaccharides are able to combine with phenolic compounds via C–O or C–C bonding. This in turn can enhance the compatibility and hydrophobicity of starch/lignin combinations resulting in reduction in diffusion of water vapor across the films (Lepifre *et al*., 2004). The hydrophobic characteristics of lignin have been assumed as a major factor in improving the water resistivity of films. Generally, the structure of lignin comprises of some non-polar unit, such as phenols and benzene rings, which are hydrophobic (Narapakdeesakul *et al*., 2013). After the casting process, lignin particles are retained on the film surfaces and attached to the rice starch films. Addition, at a suitable of filler, lignin dispersed well in the rice starch matrix, and blocked the water vapor. However, superfluous filler was easy to congregate and increased porous on films, which actually decreased the effective content of lignin and facilitated the water vapor permeation (Ma *et al*., 2008)

The biodegradable rice starch films reinforced with OPEFB's lignin were clearly not dispersed without visual loss of integrity after a 24 h immersion in water. It was found that OPEFB's lignin content increase the film solubility was decrease (Figure 15). It could be hastily concluded that lignin reduce film solubility in water. The dry matter solubilized in water is likely to be constituted mainly by the rice starch and plasticizers. An increase in the polymer network interaction density due to the lignin presence was thus associated with this decrease in solubility properties. The lowest film solubility of biodegradable rice starch films was obtained when 10% lignin was applied, while the highest film solubility of the films was noticed when 1 % of lignin was used. It could be explained that, with higher content of lignin, there are more molecules of lignin hindered mobile of the solution.

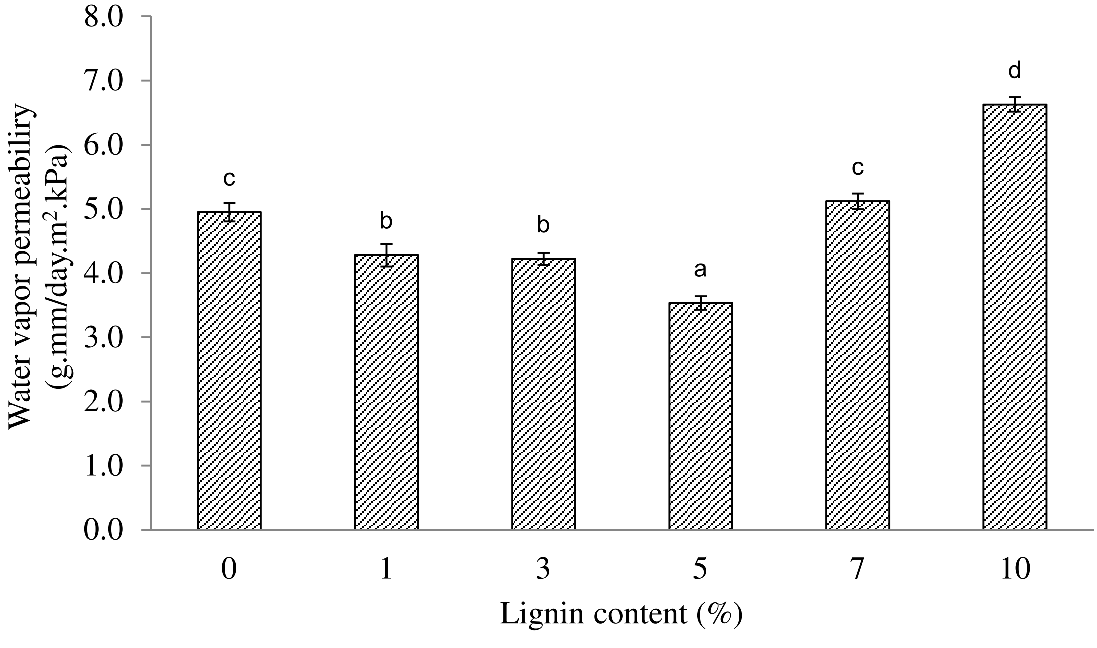


Figure 14 Effect of oil palm fruit bunch's lignin content on the water vapor permeability of rice starch films

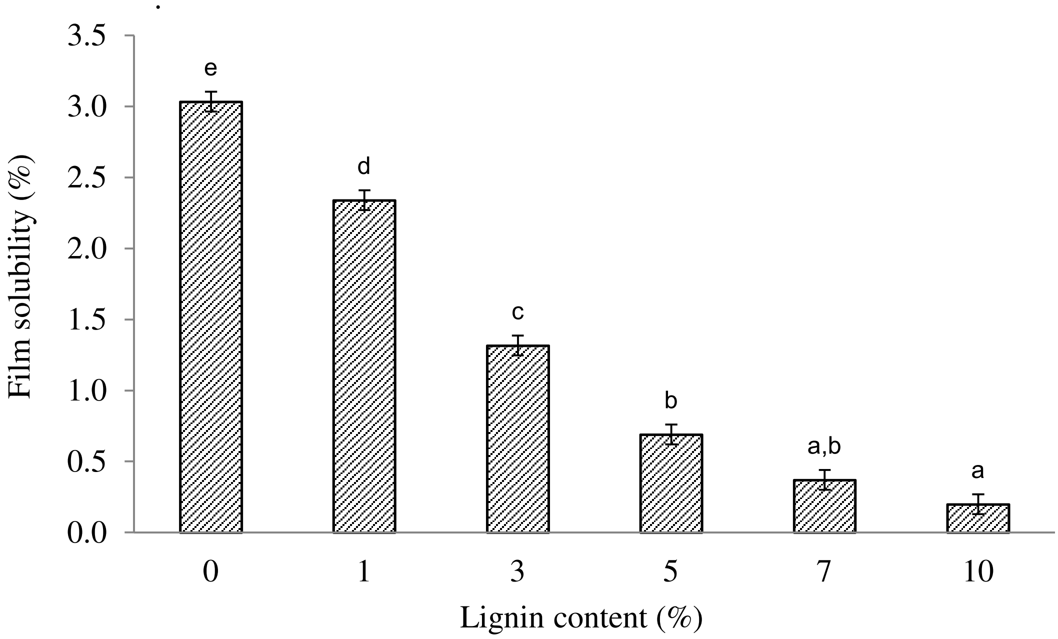


Figure 15 Effect of oil palm fruit bunch's lignin content on the film solubility of rice starch biodegradable films

3.3 Thermogravimetric analysis (TGA)

TGA results (Figure 16) showed three steps during samples degradation. The first one corresponded to the moisture removal; the second one was related by several authors (Dominguez *et al.*, 2008) with the degradation of lignin (between 250-330^{o}C) that can be present in lignin samples. The weight loss in this temperature range may be contributed to the moisture releasing and degradation of hydroxyl in alkali lignin. Therefore, the results indicated that copolymerization had rendered the lignin less hydrophilic and less prone to degradations (Yongchao *et al.*, 2014). The last step occurred progressively from 200 up to about 500^{o}C. The residue obtained after thermal degradation was also related with the structural complexity of the lignin molecule and its grade of linkages. The behavior of the char yield curves was similar in the composites. This can be attributed to degradation of the cellulose and hemicellulose attached to the structure of lignin. Ibrahim *et al.* (2011) found that the soda lignin extract from oil palm empty fruit bunches mainly degraded at temperatures between 300-310^{o}C. At above 350^{o}C, the mass loss rate of lignin fractions was very slow. The degradation in the region up to 400°C was due to the reaction of methyl-aryl ether bonds. Aromatic structures were decomposed at above 400°C though the cleavage of the C-C bonds of the rings. Methoxy groups were cracked at about 400-600°C. Further heating to above 600°C led to the cracking of the C-O-C and C=O bonds (Narapakdeesakul *et al.*, 2013). The TGA thermo grams and char yields of rice starch film and rice starch films reinforced with starch OPEFB's lignin(in the temperature range 50 to 700°C under nitrogen) are shown in Figure 16 and Table 6. The results demonstrated that the thermal stability and char yield of rice starch film was enhanced as OPEFB's lignin filler content increased from 0 to 10% of the starch. The char yield of rice starch films were lesser (10.6%) than rice starch films reinforced with lignin (11.26-17.07%) resulting from the great thermal stability of the lignin. These results indicated that addition of OPEFB's lignin increased the thermal stability of rice starch films. According to previous studies, thermal degradation of lignin occurs slowly under a wide temperature range from ambient to 800 °C (Yang *et al.*, 2007). This phenomenon results from the very complex structure of lignin, especially the presence of $C\alpha$-$C\beta$-$C\gamma$ side chains on the aromatic ring and several types of functional groups (Wang *et al.*, 2008). In addition, the high thermal stability of lignin is due to the presence of complex phenylpropanoid units, which consist of aromatic phenyl groups. These aromatic structures are very stable mainly due to the overlapping of p-orbitals which allows complete delocalization of the π electrons. Moreover, the presence of several hydroxyl groups also contributes to stability since the unpaired electrons also come into resonance, increasing the stability of the aromatic structure and impeding its break, which occurs only at high temperatures (Morandim-Giannetti *et al.*, 2012). Thus, blends with lignin in rice starch films exhibited higher temperatures than composites with no blends lignin in rice starch films. Therefore, addition of lignin into the rice starch films can be improved the thermal stability of rice starch films.

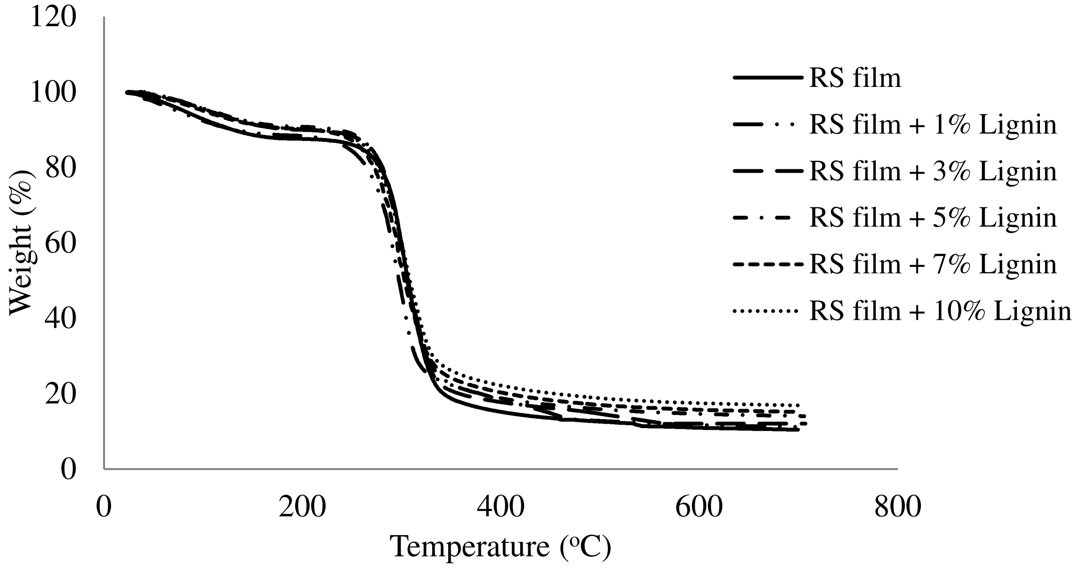


Figure 16 Effect of oil palm fruit bunch's lignin content on the TGA of rice starch films.

Table 6 Char yield of oil palm empty fruit bunch's lignin content on the TGA of rice starch films

Starch Films	Char yield (%)
Control	10.56
Rice starch films+ 1%Lignin	11.26
Rice starch films+ 3%Lignin	12.03
Rice starch films+ 5%Lignin	14.05
Rice starch films+ 7%Lignin	15.18
Rice starch films+ 10%Lignin	17.07

3.4 Differential scanning calorimetry (DSC)

Amorphous polymers such as lignin undergo a transition from a glassy state to a rubbery state at some temperature. This temperature is referred to as glass transition temperature or T_g. It is one of the significant characteristics of a polymer as it relates its application and processing (Steven, 1999). DSC analysis is an established method for determining glass transition temperature of polymer. Figure 17 shows the

DSC curves in the temperature region of -50°C to 180°C of rice starch films reinforced with OPEFB's lignin. The results demonstrated that the presence of OPEFB's lignin increases the T_g of the matrix. No endothermic peaks, assigned to the glass transition temperature (T_g) of rice starch films. The T_g of lignin is often difficult to detect due to the complex structure of this polymer (Cachet *et al.*, 2014). However, can be observed from the increasing endothermic heat flow, transition temperature (T_o, T_p and T_c) and enthalpy energy (ΔH) when lignin were added into rice starch films (Table 7). With the increase of lignin content, the endothermic heat flow, transition temperature and ΔH were increased gradually, might be attributed to the occurrence of intermolecular interaction occurred between starch and lignin, which reduces the flexibility of molecular chair of starch (Lu *et al.*, 2006). From result, the endothermic heat flow increased from 1.66 to 4.23 (W/g at 120°C) and the ΔH increase from 11.442 to 23.779 J/g when increasing the OPEFB's lignin content from 0 to 10% base on starch films. This behavior was already observed with different polysaccharides reinforced with lignin. The glass transition could be described by such activation energies through the use of free volume. The chains in lignin initially vibrate when energy was supplied. Adding some more energy, the lignin chains rotate and create free volume. Fox *et al.* (2010) reported that it is not possible to detect a clear T_g transition for lignin. Nevertheless, DSC analysis can provide useful information with regards to the chains mobility of the materials.

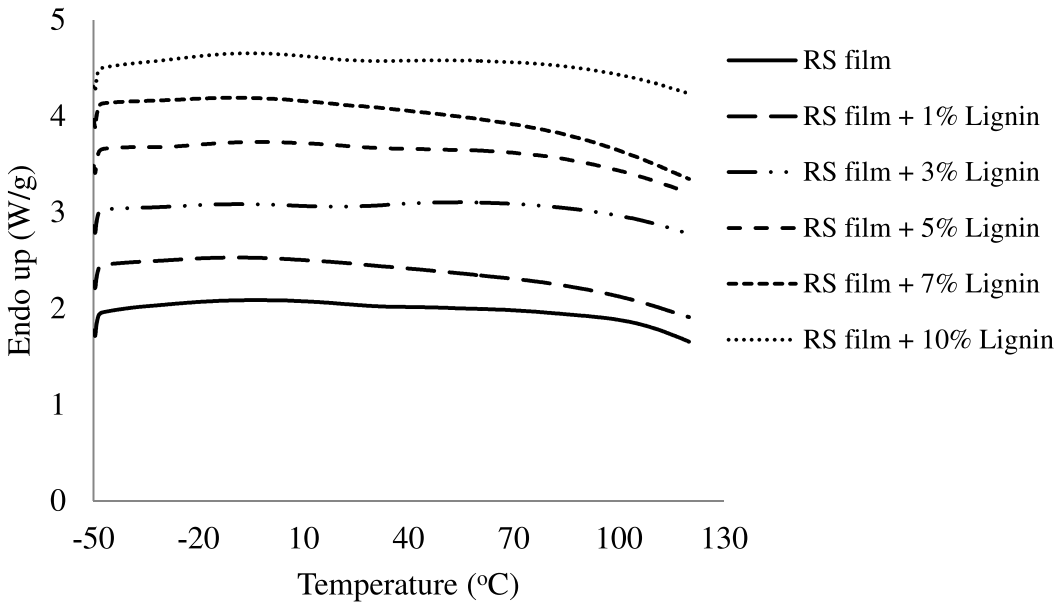


Figure 17 Effect of oil palm fruit bunch's lignin content on the DSC of rice starch biodegradable films.

Table 7 Energy used of oil palm empty fruit bunch's lignin content on the DSC of rice starch films

Rice starch films (RS films)	Temperature (°C)			ΔH (J/g)
	T_o	T_p	T_c	
Control (without lignin)	-31.390	0.500	20.105	11.442
RS films + 1%Lignin	-32.239	2.334	24.750	12.391
RS films + 3%Lignin	-30.817	3.831	25.508	15.347
RS films + 5%Lignin	-27.634	5.167	28.801	18.804
RS films + 7%Lignin	-27.253	5.584	29.747	22.443
RS films + 10%Lignin	-23.313	5.886	33.369	23.779

3.5 Color and Transparency value

Color of the packaging is an important factor in terms of general appearance and consumer acceptance (Srinivasa *et al.*, 2007). The result of the measurements performed on the biodegradable rice starch film's color were expressed in accordance with CIELAB system and the rectangular coordinates (L^*, a^* and b^*) were calculated. The rice starch films reinforced with OPEFB's lignin was characterized by an increasing tendency to both redness (a^* value) and yellowness (b^* value), as compared to the control film. In addition, the films with add lignin was characterized by a more intense brownish color than control film. Table 8 depicted the effect of OPEFB's lignin content on color (L^*, a^* and b^*) and transparency of biodegradable rice starch films. The results showed that addition and the content of lignin significantly ($p<0.05$) affected the color and transparency of rice starch films. The a^* and b^* values increased as content of lignin increased from 0 to 10% of starch concomitant with decreased in L^* values. This indicated the decreasing lightness (L^*) and increasing yellowness (b^*) and redness (a^*) of rice starch films, possibly due to the lignin have brownish color. These results also suggest that it could be claimed that the addition of lignin may change the lightness of rice starch films. Addition of OPEFB's lignin into the rice starch films resulted in increase their transparency value (low transparent). Rice starch films without lignin showed the highest transparent. However, the higher transparency value of the films was noticed when a greater amount of lignin incorporated. The decrease in transparent could possibly arise from the light scattering from the retarding of light transmission of the lignin and rice starch/lignin films. At high level of lignin, the rice starch films demonstrated lower transparency value than lesser lignin incorporation. The rice starch films with the addition of OPEFB's lignin to lose such a colorless and transparent appearance to them. Since, the chromophoric nature of lignin has highly capabilities of protecting against UV light (Pereira *et al.*, 2007).

Table 8 Effect of oil palm fruit bunch's lignin content on L*, a* and b* values and transparency values of rice starch films.

Rice starch films	Color			Transparency value
	L*	a*	b*	
Control (without lignin)	90.83 ± 0.12^{a}	-1.19 ± 0.07^{f}	2.33 ± 0.16^{e}	2.263 ± 0.32^{a}
Lignin 1%	80.71 ± 0.12^{b}	0.50 ± 0.02^{e}	11.77 ± 0.18^{d}	3.422 ± 0.35^{b}
Lignin 3%	69.03 ± 0.57^{c}	3.13 ± 0.15^{d}	21.24 ± 0.71^{c}	4.195 ± 0.27^{c}
Lignin 5%	59.85 ± 0.76^{d}	6.03 ± 0.61^{c}	25.73 ± 1.16^{b}	5.613 ± 0.37^{d}
Lignin 7%	44.24 ± 1.27^{e}	9.53 ± 0.54^{b}	27.83 ± 2.25^{a}	5.445 ± 0.59^{d}
Lignin 10%	40.42 ± 1.32^{e}	11.39 ± 0.23^{a}	21.66 ± 1.22^{c}	6.412 ± 0.53^{e}

Mean values with different letter are significantly different ($p<0.05$).

3.6 Morphology of rice starch films reinforced with OPEFB's lignin

Morphology of rice starch films reinforced with OPEFB's lignin at 0-10% of starch is depicted in Figure 18. At lower lignin content (1%) dispersion of lignin is poor than higher content. However, when lignin content higher 7% resulted in as clustering, then the properties of the films were poorer. The results showed that the suitable dispersion affected the properties of rice starch films because reinforcing filler can make force distribution all areas and regularly. The hydrophobic properties of lignin and hydrophilic properties of starch makes lignin distribution on the surface of rice starch film and cannot homogeneous in films. According to Huang *et al* (2003), immiscibility between hydrophobic groups of lignin and hydrophilic groups in soy protein isolate (SPI) has shown that to prevent interaction between SPI molecules causing micro phase separation in the corresponding blends. The lignin has a smaller particle size can be well dispersed in a suitable amount. It can protect and improve the properties of the films. From the result, optimal condition of rice starch films reinforced with lignin is 5% lignin content.

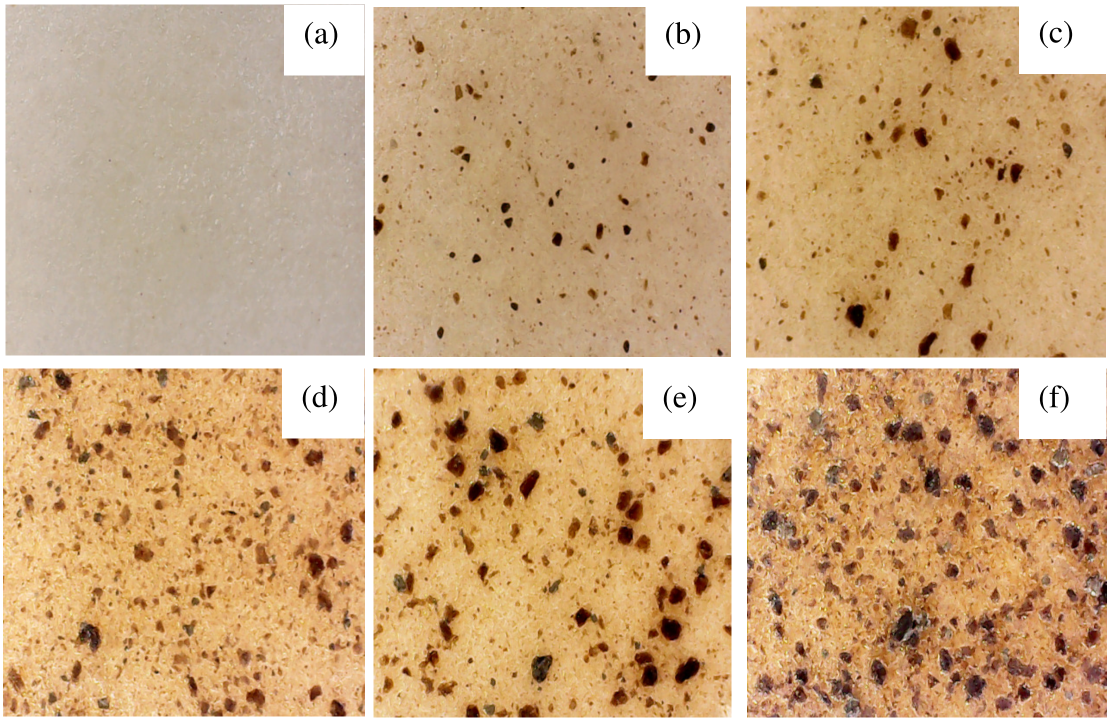
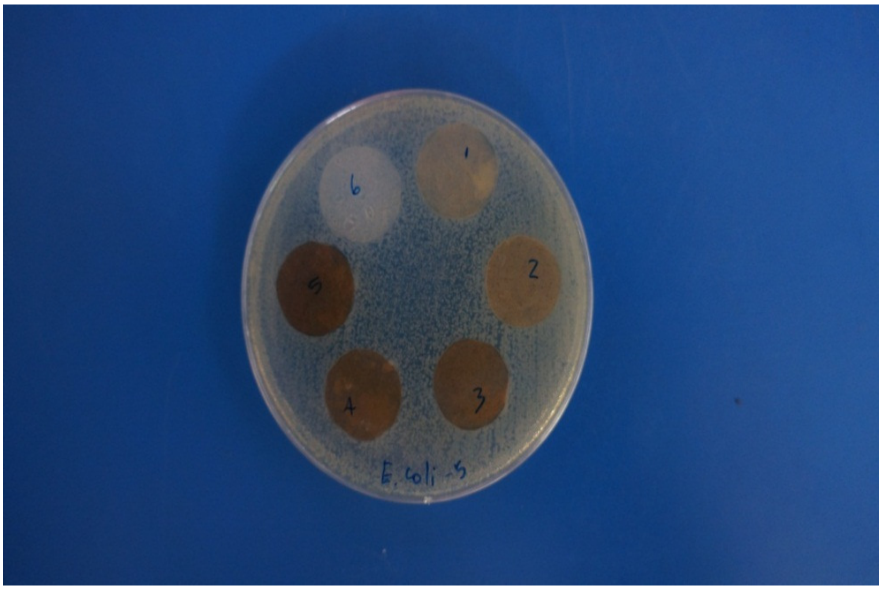


Figure 18 Surface of rice starch films and rice starch reinforced with oil palm fruit bunch's lignin at various content; (a) 0%, (b) 1%, (c) 3%, (d) 5%, (e) 7%, (f) 10% based on rice starch.

3.7 Antimicrobial properties

The growth of inhibition zones measured by using agar diffusion assay method. OPEFB's lignin exhibited different inhibition levels against *L. monocytogenes, S. aureus* and *E. coli* as shown in Figure 19. The inhibitory activity was measured based on clear zone surrounding circular film strips. Before testing, treat by UV 2 h for sterilized. Measurement of clear zone diameter included diameter of film strips, therefore, the values were always higher than the diameter of film strips (16 mm) whenever clearing zone was presented. If there is no clear zone surrounding, we predicted that there is no inhibition zone, and furthermore, the diameter was valued as zero. In the test, OPEFB's lignin content added into the rice starch films was 1,3,5,7 and 10% w/v. If added OPEFB's lignin more than 10% w/v provided the films look rigid and brittle, cannot be tested. From the result, antimicrobial properties showed inhibition zone only of lignin for *S. aureus* at 10% lignin content. However, the antimicrobial properties were not shown inhibition of lignin for *L. monocytogenes* and *E. coli* (Figure 19). The antimicrobial properties of lignin could explain that lignin has a complex structure and lignin might influence their antimicrobial properties. Oh-Hara et al. (1990) reported that the antimicrobial activity induced by commercial lignin was much lower than that induced by fractions of pine cone extracts obtained by successive alkaline extractions (and then recovered as acid precipitates at pH 5). Some authors suggested that the inhibition of microbial growth by phenolic acids, among which includes the p-coumaric, increased with decreasing pH (Delaquis *et al*., 2005). In the additional, it is reported that polyphenols inhibit the growth of microorganisms by forming complexes with their enzymes and proteins and can dissolve the microbial membrane or wall, thus polyphenols can penetrate the cell, where they interaction and disruption the permeability barrier of microbial membrane structures (Daglia, 2012). Summary, lignin from natural source of each species can inhibit the growth of microorganisms.



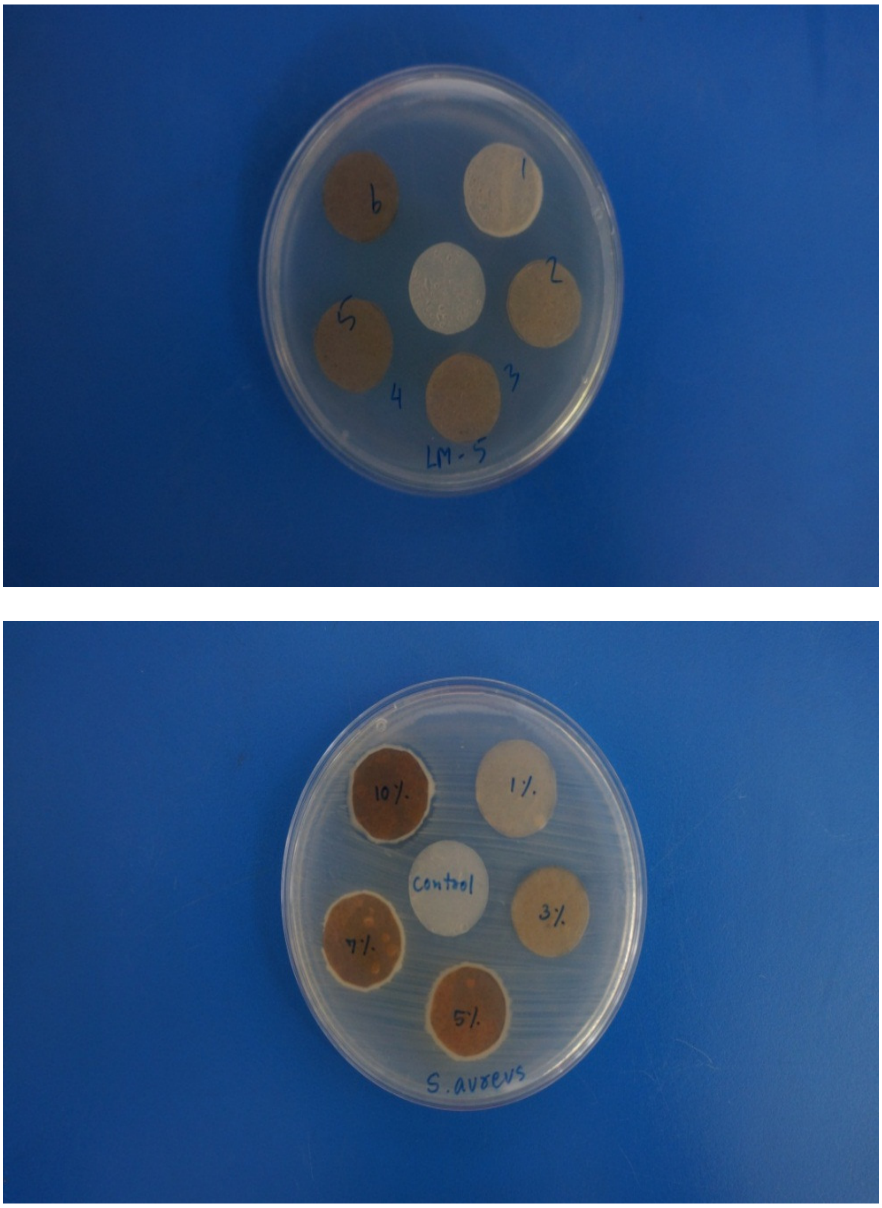


Figure 19 Inhibitory zone of rice starch films reinforced with oil palm fruit bunch's lignin for antimicrobial properties.

4. Effect of OPEFB's lignin derivatives content on the properties of rice starch films

4.1 Mechanical Properties

Bio-polymer films are mainly characterized by their mechanical properties, which includes: tensile strength (TS) and elongation at break (E). Mechanical properties are an indication of the integrity of films on subjecting to stress conditions. TS is a measurement of film strength and is calculated by dividing the maximum force needed to break the films by the cross-sectional area of the films (Han *et al.*, 2006) whereas, E-value is an indication of flexibility of films. Results on the mechanical properties of starch/OPEFB's lignin derivative films produced in this study are shown in Figure 20. On overall comparison with the control (without lignin), an increase in TS with was recorded in films produced by incorporating OPEFB's lignin derivative both modified by palm oil and soy bean oil. In all cases, the mechanical properties of rice starch films with both OPEFB's lignin and OPEFB's lignin derivative are increased as compared with those of the rice starch films without added lignin but decrease when lignin content higher 5%, the better results being obtained at 5% lignin base on rice starch films (Figure 20A). While compared during OPEFB's lignin and OPEFB's lignin derivatives found that rice starch films contained OPEFB's lignin showed higher TS value than rice starch films contained OPEFB's lignin derivative. However, TS of OPEFB's lignin derivatives that modified with soy bean oil and palm oil demonstrated the same values when the same content of oil was applied. The results showed that rice starch films without lignin presented TS value about 3.612 MPa, while TS of the films were 5.806, 4.493 and 4.545 MPa when 5% of OPEFB's lignin and OPEFB's lignin derivative modified from palm oil and soy bean was used, respectively. Elongation at the break (E) is an indication of the films flexibility and stretchability (extensibility), which is determined at the point when the film breaks under tensile testing and is expressed as the percentage of change of the original length of the specimen between the grips of a film to stretch (extend). According to the results reveal that rice starch films contained OPEFB's lignin derivative modified with palm oil showed higher E than rice starch films contained OPEFB's lignin derivative modified with soy bean oil and OPEFB's lignin, respectively (Figure 20B). The higher E of rice starch films was observed when OPEFB's lignin derivative and OPEFB's lignin was used could be explained that addition of lignin with filling plasticizer, as deduced from significant decreases in tensile strength (TS) in the composite films, together with a marked increase in elongation at break (E) as well as in breaking deformation (BD) (Núñez-Flores *et al.*, 2013). Thus, the apparent plasticizing effect observed in composite films could be attributed not only to a direct effect of lignin but also to the presence of water free molecules, causing a reduction in intermolecular attractive forces between polymer chains (Cuq *et al.*, 1997) and plasticizer is blended to alleviate the rigidity of films by reducing the hydrogen bonds and van der Waals forces in matrix (Azeredo *et al.*, 2010). Modification of lignin by using palm oil and soy bean oil significantly ($p<0.05$) affected the elongation at break of the films. The incorporation of hydrophobic substances (lipids, fatty acids, wax, oils, essential oils etc.), generally decreased TS and increase E value of films from various sources including porcine-hide gelatin (Andreuccetti *et al.*, 2009) and fish skin gelatin (Jongjareonrak *et al.*, 2006). Prodpran *et al.* (2007) reported that addition the palm oil reduced film strength but increased the flexibility, as indicated by decreased TS with the concomitant increase in E of films. The result suggested the plasticizing effect of palm oil in film matrix. Palm oil more likely hindered interaction in film network, leading to the discontinuity of film matrix. The reduced continuity and lack of cohesive structure integrity of film network therefore lowered the strength of films. Furthermore, longer carbon chains in soy bean oil may lead to new entanglement between soy bean oil and material chains (Broström *et al.*, 2004) resulting E value of rice starch films contained OPEFB's lignin derivative modified by soy bean oil showed lower than rice starch films contained OPEFB's lignin derivative modified by palm oil. In addition, the effect of the soy bean oil on the mechanical properties of the films was probably due to the interaction of the carboxylic groups of the fatty acids present in the oil (Brandelero *et al.*, 2011).

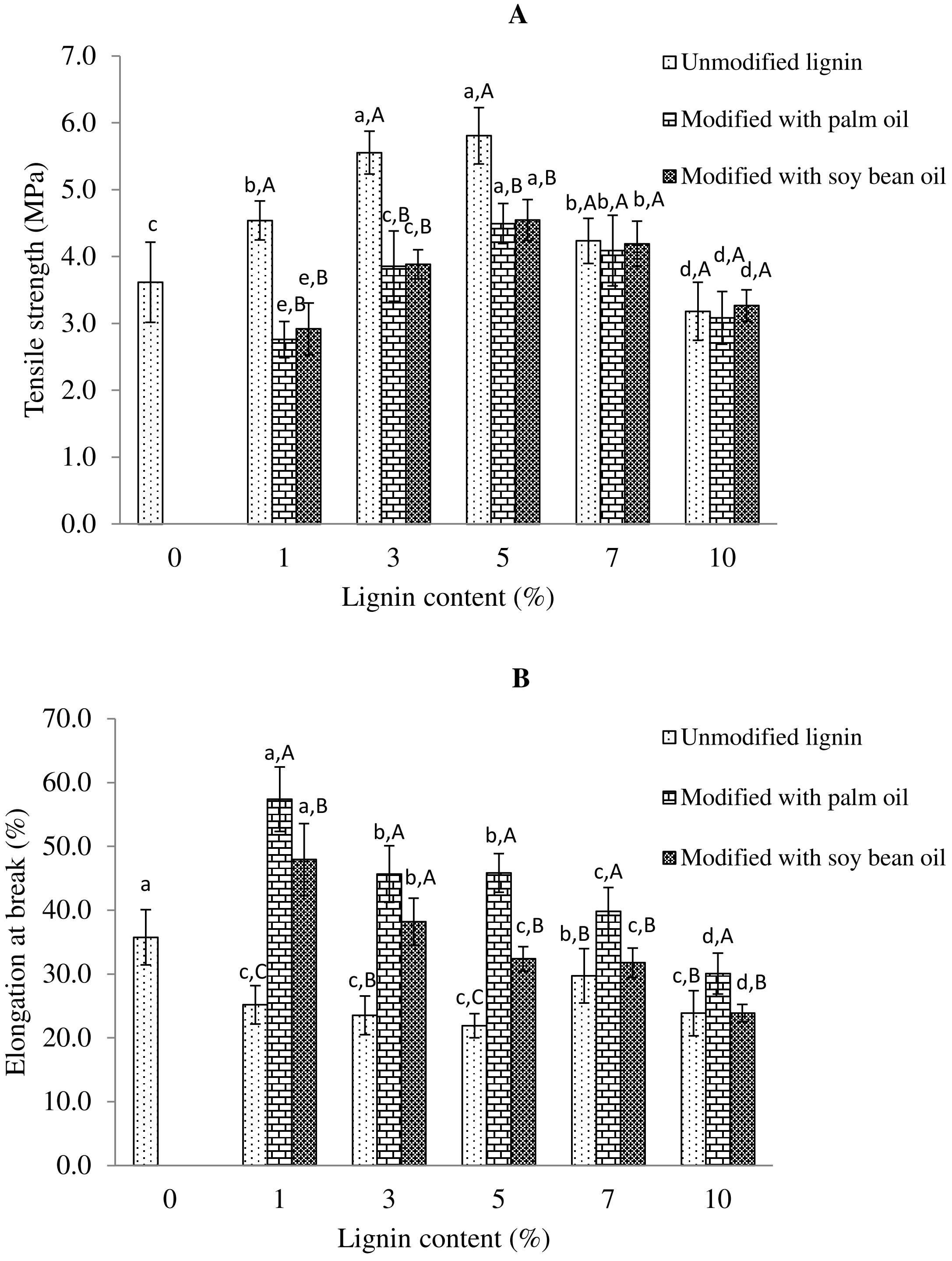


Figure 20 Effect of OPEFB's lignin derivative content on tensile strength (A) and elongation at break (B) of biodegradable rice starch films. Bars represent the standard deviation (n =3). Different lowercase letters on the bars within the same reinforcing filler indicate significant differences ($p<0.05$). Different uppercase letters on the bars within the same content indicate significant differences ($p<0.05$).

4.2Water vapor permeability (WVP)

WVP is a measure of the amount of water vapor passing through unit area of material per unit time. WVP is a proportionality constant that is assumed to be independent of the water vapor pressure gradient applied across the films. However, natural materials are hydrophilic materials with polar groups in their molecular structures, and the interactions of polar groups with permeating water molecules causes the WVP to depart from ideal behavior (Roy *et al.*, 2000). The deviation from ideal behavior is attributed to the variation of structure of the materials. The deviation was believed to be governed by free volume theory (Sablani *et al.*, 2002). Water increases the polymer free volume, allowing the polymeric chain segments to increase their mobility. Higher segment mobility results in higher WVP. Since a main function of a biodegradable or edible film is often to impede moisture transfer between food and the surrounding atmosphere, or between two components of a heterogeneous food product, water vapor permeability should be as low as possible.

The WVP of biodegradable rice films with different content of both OPEFB's lignin and OPEFB's lignin derivative (soy bean oil and palm oil) on rice starch films were examined at a vapor pressure difference of 0/55% across films. The results showed that the WVP of biodegradable rice films decreased with an adding an increase in the content of both OPEFB's lignin and OPEFB's lignin derivative. The observed decrease in WVP in OPEFB's lignin incorporated films is an indication of improved permeability of films towards water vapor. Generally, nature of lignin has hydrophobic behavior affected can reduction of water absorbency in starch-based films (Ban *et al.*, 2007). Narapakdeesakul *et al.* (2013) said that the structure of lignin comprises of some non-polar unit such as benzene rings (aromatic hydrocarbon) and phenol resulting lignin has the hydrophobic characteristics. The hydrophobic characteristics of lignin have been predicted as a major factor for improving the resistant of water for the films. In addition, lignin is known to have a low ability to absorb water compared to for example the hydrophilic cellulose. Previous publications demonstrate how lignin-containing bio based polymer composites can be used as films for improving the water resistance (Baumberger, 2002). However, WVP of the films contained OPEFB's lignin derivative demonstrated lower WVP than biodegradable rice films incorporated with OPEFB's lignin. Modification of lignin by added soy bean oil and palm oil able improved the barrier properties as well. Limpisophon *et al.* (2010) reported that improvement the water vapor barrier property, the incorporation of hydrophobic substances such as lipids and oils has been implemented to improve water vapor barrier property due to the hydrophobic nature of the oils. Thus, palm oil can serve as hydrophobic substances to improve the water vapor barrier property of edible/biodegradable films. Triglycerides in palm oil consist of a mixture of monounsaturated, polyunsaturated and saturated fatty acids (Jeirani *et al.*, 2013) but saturated fatty acid constitutes at higher content than others (Phan *et al.*, 2011). In addition, Yang *et al.* (2014) studies the effect of admixture soy oil into the ethyl cellulose films found that soy oil caused the WVP value was decrease. The non-polar and hydrophobic behavior of soy bean oil which abundant polyunsaturated fatty acid able retard the permeation of water vapor, resulting in a molecule of water penetrates through the film slowly. Rice starch films reinforced with OPEFB's lignin derivative modified with palm oil can improve the WVP value better than other treatment due to palm oil consist saturated fatty acid more than other components and structure have long linear chain of hydrocarbon. As a result, palm oil has non-polar properties and water resistant more than soy bean oil and unmodified lignin. Decrease in WVP is considered advantageous when producing food packaging materials requiring efficient barrier properties to minimize moisture transfer between food and outside packaging environment (Voon *et al.*, 2012). The results presented that incorporated of OPEFB's lignin and OPEFB's lignin derivative, at 5% trended to provide lower WVP than others contents (Figure 21). At 5 % content, consistent distribution throughout the films and not include the clusters on the films surface of OPEFB's lignin and OPEFB's lignin derivative resulting the ability to permeate through the film of water vapor is reduced which can be observed from Morphology (Figure 28).

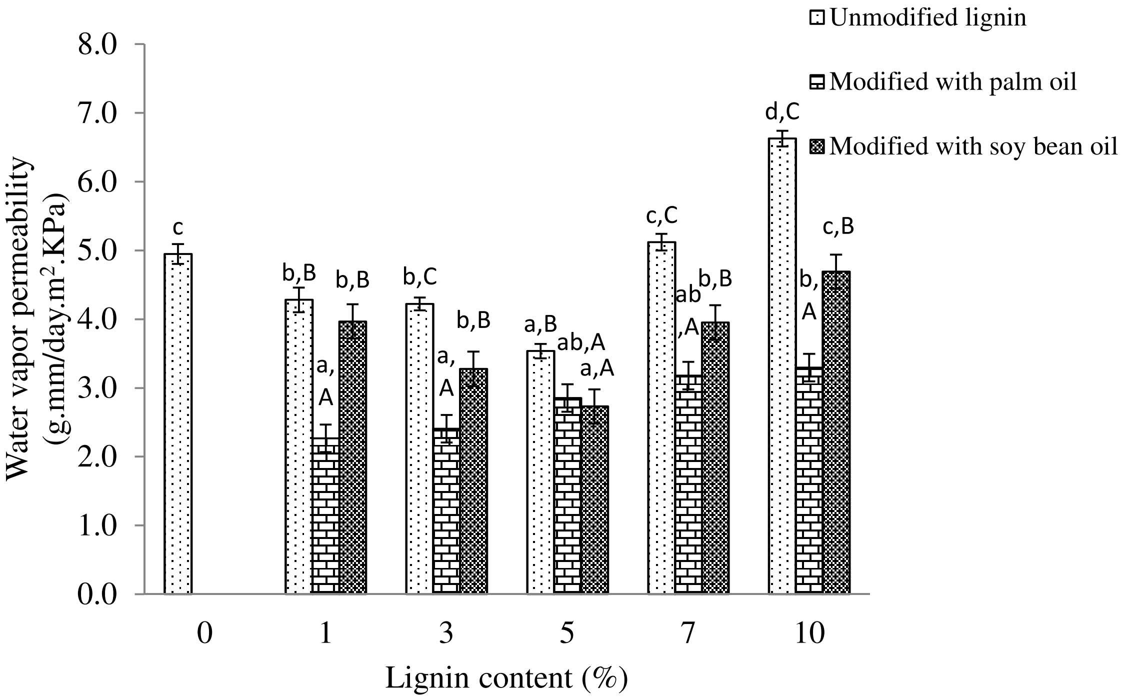


Figure 21 Effect of OPEFB's lignin derivative content on water vapor permeability of rice starch films. Bars represent the standard deviation (n =3). Different lowercase letters on the bars within the same reinforcing filler indicate significant differences ($p<0.05$). Different uppercase letters on the bars within the same content indicate significant differences ($p<0.05$).

4.3 Film solubility

Film solubility is advantageous in situations when the films will be consumed with a product that is heated prior to consumption and may also be an important factor that determines biodegradability of films when used as packaging wrap. Biodegradable film pieces produced from rice starch and rice starch contained lignin maintained their integrity (i.e., did not dissolve or break a part) even after 24 h of incubation with gentle motion. Figure 22 shows film solubility of the biodegradable rice starch films incorporated with OPEFB's lignin and OPEFB's lignin derivatives and without lignin. Generally, solubility of film decreased when with increasing both lignin and lignin derivative content. Film solubility of rice starch films, rice starch films reinforced with OPEFB's lignin and OPEFB's lignin derivatives modified by soy bean oil and palm oil were 3.03%, 0.20- 2.34%, 0.69-2.88% and 0.74-2.91% when 1-10% of lignin was applied, respectively. The results showed that, the loss in film solubility was mainly likely caused by interaction between starches, lignin and lipid leading to greater molecular weight cross-links affect the ability to dissolve is reduced. Additionally, sorbitol which is plasticizer would bind with lignin, especially at OH groups. As a consequence, sorbitol was most likely imbibed in the film matrix and could not be leached out. When compare between rice starch films contained OPEFB's lignin and OPEFB's lignin derivatives, it was found that rice starch films contained OPEFB's lignin and OPEFB's lignin derivatives showed lower film solubility than pure rice starch films. Addition, the content of OPEFB's lignin and OPEFB's lignin derivatives increase showed lower the solubility of resulted films. The effect of addition of OPEFB's lignin and OPEFB's lignin derivatives modified with soy bean oil and palm oil on film solubility were compared. The results presented that rice starch films reinforced with OPEFB's lignin showed lower film solubility than rice starch films reinforced with OPEFB's lignin derivatives. These resulted pointed out that OPEFB's lignin presented more hydrophobic properties than OPEFB's lignin derivative resulted in lower dissolutions of the films. Therefore, the addition of OPEFB's lignin was able to reduce solubility of rice starch films and retard the adsorption of water through the films. The synthesis of OPEFB's lignin derivative found that hydrogen bond was destroyed from catalyst and heat makes the coagulated loose structure was occurs. In addition, esterification reaction was obtained from lipid as a result OPEFB's lignin derivative can absorb and dissolve water better than OPEFB's lignin when long storage times. The polysaccharide films generally possessed weak water barrier properties (Sebastian *et al.*, 2006). If the films are cationic and strongly hydrophilic, water interacts with the polymer matrix and the dissolution for water is increased (Gocho *et al.*, 2001). Comparing between the effects of OPEFB's lignin derivatives modified with palm oil and soy bean oil found that rice starch films reinforced with OPEFB's lignin derivatives modified with soy bean oil showed lower film solubility than rice starch films reinforced OPEFB's lignin derivatives modified with palm oil. Due triglycerides in palm oil consist of a mixture of monounsaturated, polyunsaturated and saturated fatty acids (Jeirani *et al.*, 2013) but saturated fatty acid constitutes at higher content than others (Phan *et al.*, 2011) and palm oil have single covalent bond affected hydrophobicity behavior of palm oil lower than soy bean oil.

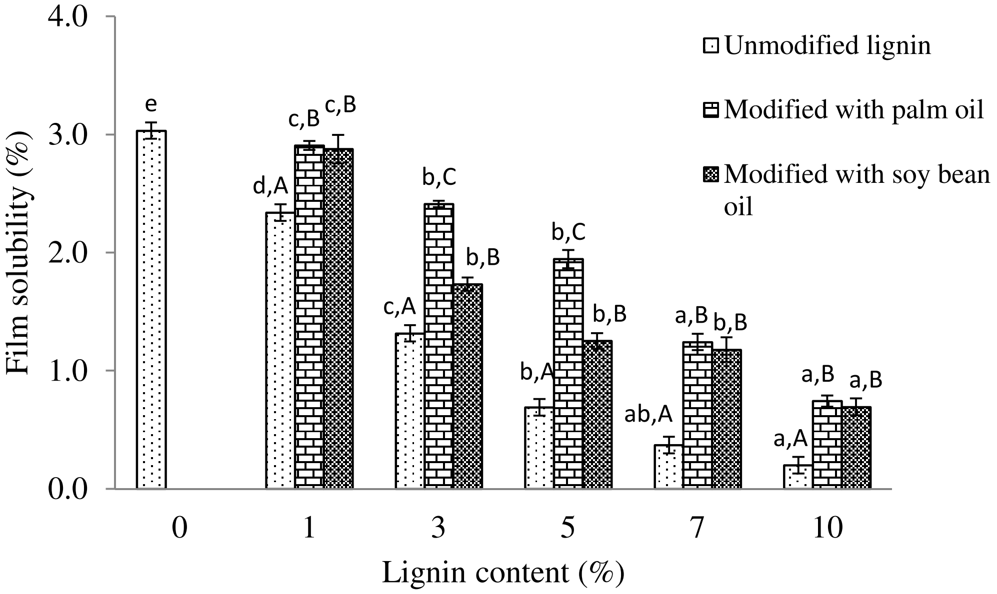
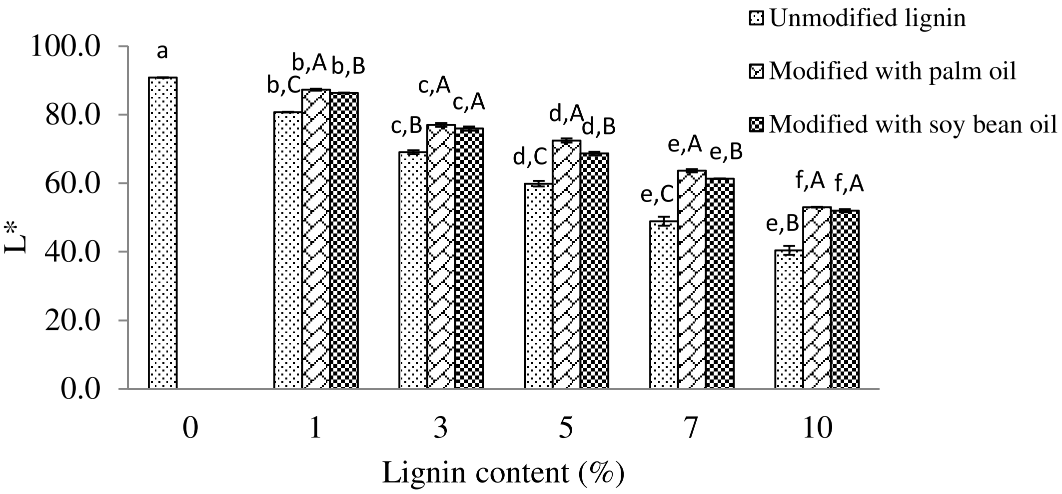
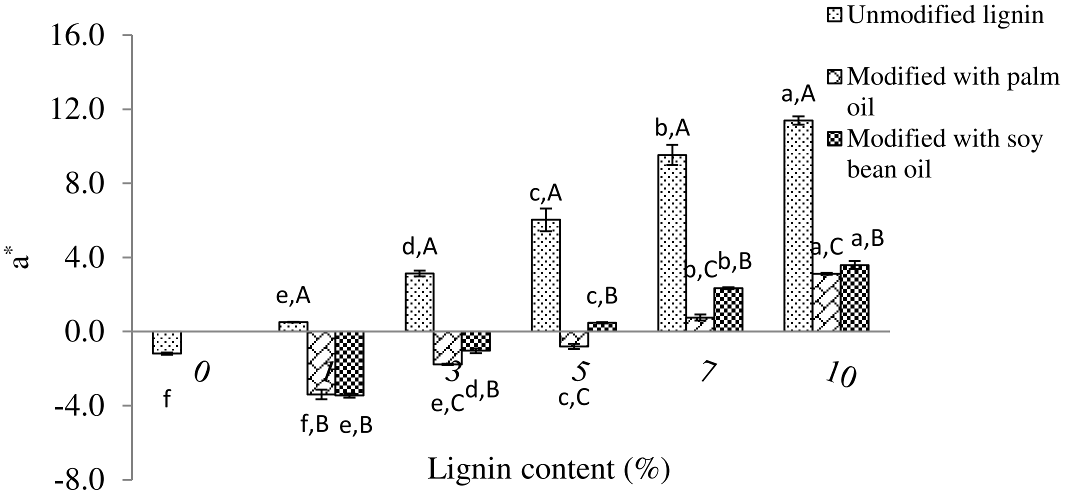


Figure 22 Effect of OPEFB's lignin derivatives content on film solubility of biodegradable rice starch films. Bars represent the standard deviation (n =3). Different lowercase letters on the bars within the same reinforcing filler indicate significant difference ($p<0.05$). Different uppercase letters on the bars within the same content indicate significant differences ($p<0.05$).

4.4 Color and film transparency

Color is one of the important parameters of packaging films, which tends to attract (visual appeal) and affect consumers' acceptability of packed food products. Packaging films with low opacity presume importance where in it can prevent light sensitive food products from deteriorating. Generally, 3 color parameters are measured for a biopolymer based film, which includes: L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness). Results compare on the color parameters of rice starch films produced by incorporating both OPEFB's lignin and OPEFB's lignin derivatives are depicted in shows Figure 23. From the results, addition and increasing the content of OPEFB's lignin and OPEFB's lignin derivatives provided decreasing of L^* value, while a^* and b^* values increased. Hence, addition of lignin significantly affects color of the rice starch films. Possibly due to the OPEFB's lignin have dark brown color. In term of L^* value, rice starch films incorporated with OPEFB's lignin derivatives modified with soy bean oil and palm oil showed higher L* value than rice starch films reinforced with OPEFB's lignin because when it's get heat and acids after the synthesis as a result, color of OPEFB's lignin derivative are brownish more than OPEFB's lignin. However, rice starch films incorporated with OPEFB's lignin derivatives modified with soy bean oil and palm oil presented did not different in L* value. The effect of lignin on a^* and b^* values of rice starch films were compared. Addition of OPEFB's lignin promoted the increasing of a^* and b^* values. The increasing values of a^* indicates films to be more reddish, while increasing values of b^* indicates yellow color of the films. Prodpran *et al.* (2007) reported that increasing of b^* value caused the browning via Maillard reaction that may be enhanced seeming by the increased yellowness. The results demonstrated that rice starch films contained OPEFB's lignin derivatives showed lower a^* and b^*values than rice starch films incorporated with OPEFB's lignin. By the reason of soy bean oil and palm oil in the OPEFB's lignin derivatives had yellowish color due to its coloring compounds or pigments. The pale yellow color in palm oil and soy bean oil were due to its high beta-carotene content (Manorama and Rukmini, 1992). Therefore, the color of palm oil and soy bean oil in lignin directly affected the color of resulting films. Transparency values of rice starch films incorporated with lignin and OPEFB's lignin derivatives was shown in Figure 24. The lowest transparency value was obtained in pure rice starch film, compared with those of films incorporated with lignin and OPEFB's lignin derivatives ($p < 0.05$). The transparency value of films containing both lignin and OPEFB's lignin derivatives increased as the amount of lignin increased ($p < 0.05$). However, not difference significant in transparency value was observed between rice starch films incorporated with lignin and OPEFB's lignin derivatives (soy bean oil and palm oil) at the same levels. The lower transparency value indicated that the films were more transparent. Lignin conferred a dark reddish-brown color as borne out by the L* value affect reduction of transparent. Reduction of transparency in rice starch/lignin and lignin derivative films produced by addition of lignin might also prove to be advantageous, as opaque packaging films can effectively inhibit UV radiation (Spiridon *et al.*, 2011).





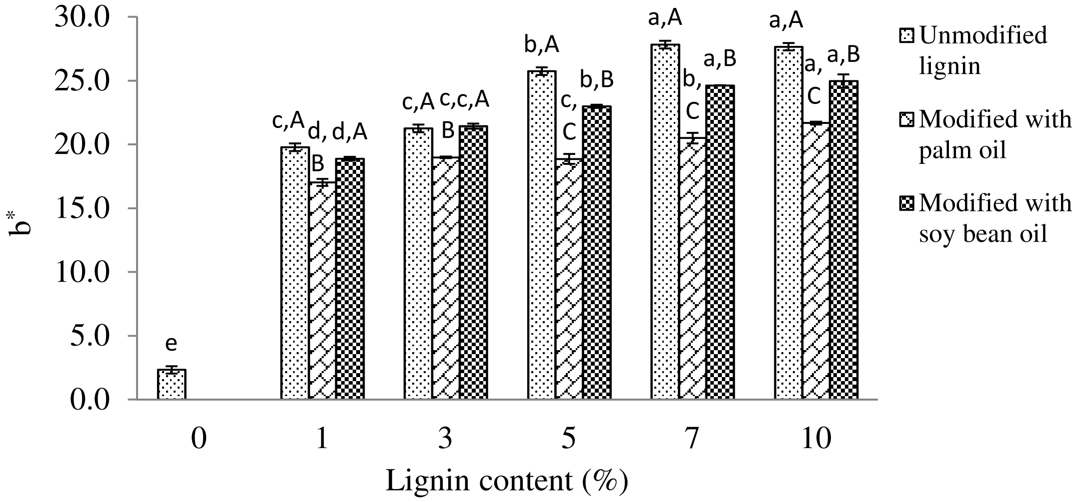


Figure 23 Effect of OPEFB’s lignin derivatives content on color values of biodegradable rice starch films. Bars represent the standard deviation (n =3). Different lowercase letters on the bars within the same reinforcing filler indicate significant differences (p<0.05). Different uppercase letters on the bars within the same content indicate significant differences (p<0.05).

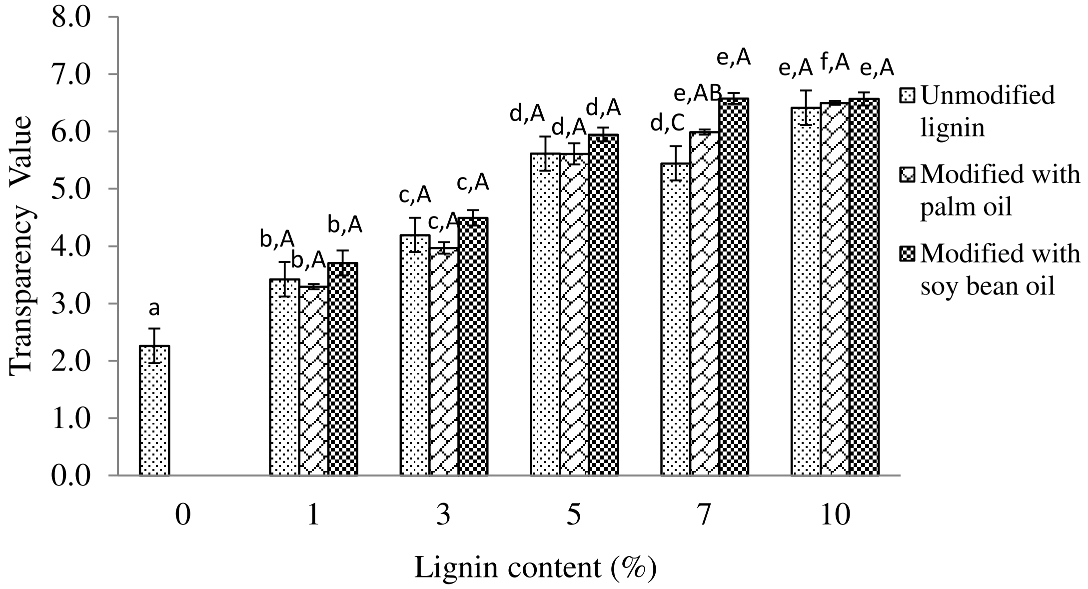


Figure 24 Effect of OPEFB’s lignin derivatives content on transparency values of biodegradable rice starch films. Bars represent the standard deviation (n =3). Different lowercase letters on the bars within the same reinforcing filler indicate significant differences (p<0.05). Different uppercase letters on the bars within the same content indicate significant differences (p<0.05).

4.5 Surface contact angle

Surface properties of the films (i.e., contact angle) give information about the phenomenon of wetting or non-wetting of a product surface by film forming dispersions and thus, about the uniformity of coating when applied to a particular solid surface (Vargas *et al*., 2009). Moreover, the contact angle method is a simple way to determine the superficial hydrophilicity of films since when using water or another polar solvent, contact angle will increase with increasing surface hydrophobicity (Hambleton *et al*., 2009). The overall interaction between a films surface and a wetting liquid depends on the degree of contact achieved and the magnitude of the intermolecular forces involved. The latter are affected by several factors, including film surface free energy, liquid surface tension, and roughness. Generally, higher measured contact angles result in a reduced potential interaction of the fluid with the film (Meiron and Saguy, 2007). Figure 25 shows the surface contact angle values of the rice starch films and rice starch films incorporated with lignin and OPEFB's lignin derivatives at the each level. The lignin content increased in the films, the surface contact angle of probe liquids on the resulting substrate increased. This suggested that tendency of wetting liquids to flow over the surface was unable to overcome the strong hydrophobic behavior of lignin and OPEFB's lignin derivatives; consequently, the wetting liquid contacted lesser surface area of the substrate. The resulting surface was considered to be relatively more hydrophobic which is attributed to the decrease of –OH groups in the forming. Increasing lignin content in the films, the polar component of surface-free energy of the resulting blends decreased significantly while their dispersive component affected contact angle value too. From the results, when lignin and OPEFB's lignin derivatives modified with soy bean oil and palm oil content increase provided the increasing of surface contact angle values of the films from 65.62^{o} to 116.64^{o}, 65.62^{o} to 116.47^{o} and 65.62^{o} to 126.33^{o} , respectively (Figure 26). The incorporation of nonpolar or hydrophobic substances with uniform distribution in film matrix could increase hydrophobicity of the film including lower adsorption and diffusion of wetting liquid through the film as evidenced by the higher contact angle value on the films. Hydrophobic nature of lignin including modification lignin by adding soy bean oil and palm oil then spread throughout the film more likely impeded the wetting liquid absorption and affect the contact of the wetting liquid that touch with the film surface was decreased. Therefore, the use of hydrophobic substance such lignin and OPEFB's lignin derivatives modified with palm oil and soy bean oil at an appropriate level could improve water barrier properties of rice starch films, which could be potentially used as an alternative packaging to biodegradable films.

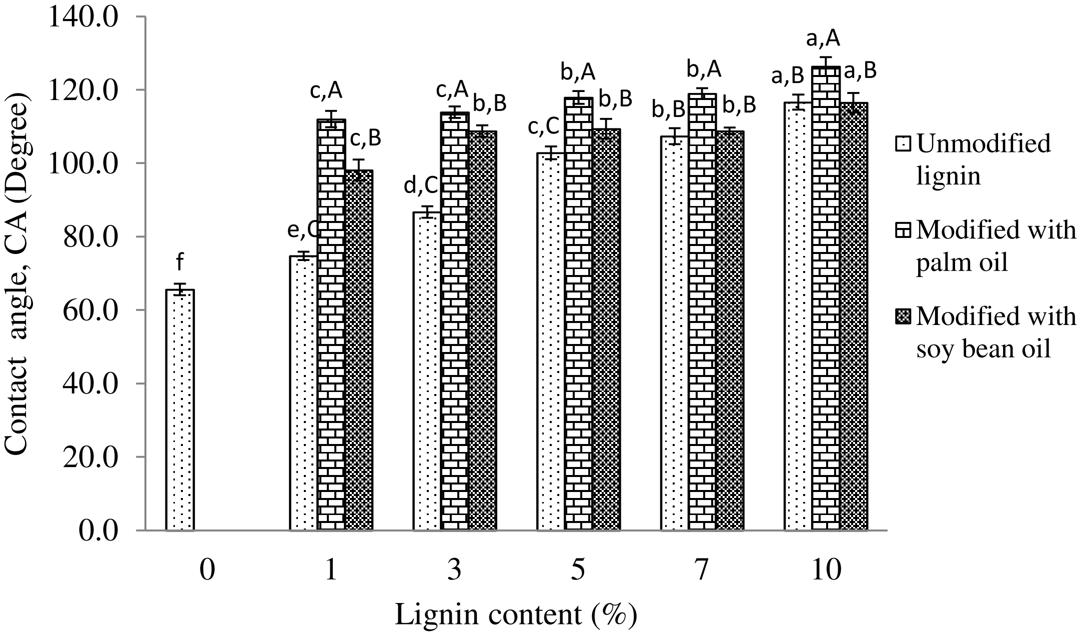


Figure 25 Effect of OPEFB's lignin derivatives content on surface contact angle values of biodegradable rice starch films. Bars represent the standard deviation (n =3). Different lowercase letters on the bars within the same reinforcing filler indicate significant differences ($p<0.05$). Different uppercase letters on the bars within the same content indicate significant differences ($p<0.05$).

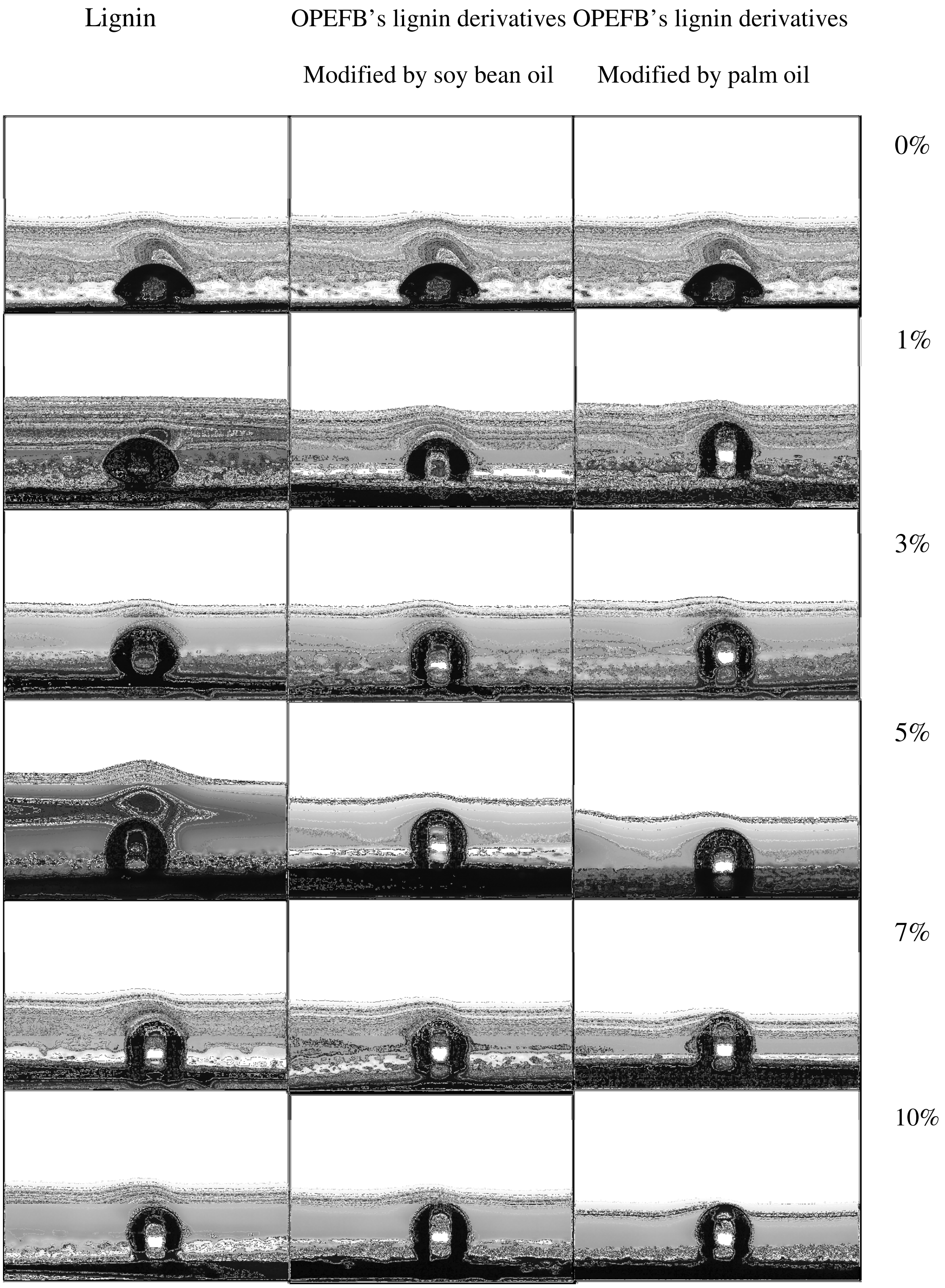


Figure 26 Illustration on surface contact angle of rice starch films and rice starch films incorporated with OPEFB's lignin and OPEFB's lignin derivatives at different content.

4.6 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy was used to determine the interactions between rice starch films, lignin and its derivatives. The infrared spectra of rice starch, lignin, lipid and their blends are shown in Figure 27. The assignments of the bands are reported according to other literature findings (Tejado *et al.*, 2007). The broad band of rice starch film about at 3300 cm^{-1} was the O-H stretching vibrations in aromatic and aliphatic O-H groups for lignin. The peaks at 1550 cm^{-1} are due to C-C of aromatic skeletal vibrations. Meanwhile, the signal of aromatic C-H out-of-plane deformation in G, S and H units appears at 860 cm^{-1}. The presence of syringyl (S) and guaiacyl (G) bands for lignin indicated that the lignin extracted from OPEFB is more similar to wood lignin than annual plant lignin, which is normally HGS lignin. This similarity was due to the absence of band of phydroxyl phenyl propane (H) about at 1160 cm^{-1} in the spectra (Zhao *et al.*, 2009). The presence of guaiacyl-type (G) unit in lignin confirmed that lignin had potential active site for polymerization. In phenol formaldehyde condensation reactions, phenol condenses initially with formaldehyde in the presence of alkaline to form methylolphenol (Pizzi, 1983). The changes in wave number and their amplitude peaks might be correlated with the interaction between the functional groups of polysaccharide, lignin and lipid. Peaks at wave numbers around 2870 cm^{-1} and 2950 cm^{-1} represent the methylene asymmetrical and symmetrical stretching vibration of the aliphatic C-H in CH_2 and CH_3 groups, respectively (Guillen and Cabo, 1997). Both methylene asymmetrical stretching bands at approximately 2870 cm^{-1} and methylene symmetrical stretching band near 2950 cm^{-1} were found in most lipids and hydrophobic substances (Guillen and Cabo, 2004). Both peaks had the increase in amplitude with adding lipid. Palm oil can be seen the peak more clearly than soy bean oil. Furthermore, the peak at wavenumber of about 1760 cm^{-1} was observed in films containing OPEFB’s lignin modified with palm oil. However, there was no peak at 1760 cm^{-1} found in the control films (rice starch films) and rice starch films incorporated with OPEFB’s lignin. The carbonyl absorption of triglyceride ester linkage was observed at 1746 cm^{-1} (Setiowaty *et al.*, 2000). Hien *et al.* (2011) reported that the higher intensity of the peak at 1745 cm^{-1}, representing C=O in ester, also demonstrated the increasing lipid concentration in the films and those in the 1100 cm^{-1} region were related to the C-O group, which were related to ester bonds. In addition, Eva-Lena *et al.* (2013) said that in the oil fatty acid lignin ester samples the appearance of two new peaks at 1739 cm^{-1} and 1762 cm^{-1} is clearly seen. They are assigned to aliphatic and aromatic ester bonds, respectively, and indicate esterification of lignin with fatty acid both via aliphatic and aromatic hydroxyl groups. A peak in the 720 cm^{-1} region was related to the asymmetric angular deformation of CH_2 groups, which is characteristic of long-chain hydrocarbons for lipid. According to the authors, this finding indicates the presence of an ester bond between the starch films and the plasticizer. Under the temperature and pressure conditions at the process, it is unlikely that a covalent bond occurred between lipid and the materials of the blend in films. The observed effect on the mechanical properties (tensile strength) was likely due to the presence of weak interactions between lipid in lignin substances and the starch films affected tensile strength value was decrease when compare with lignin that no modified by lipid.

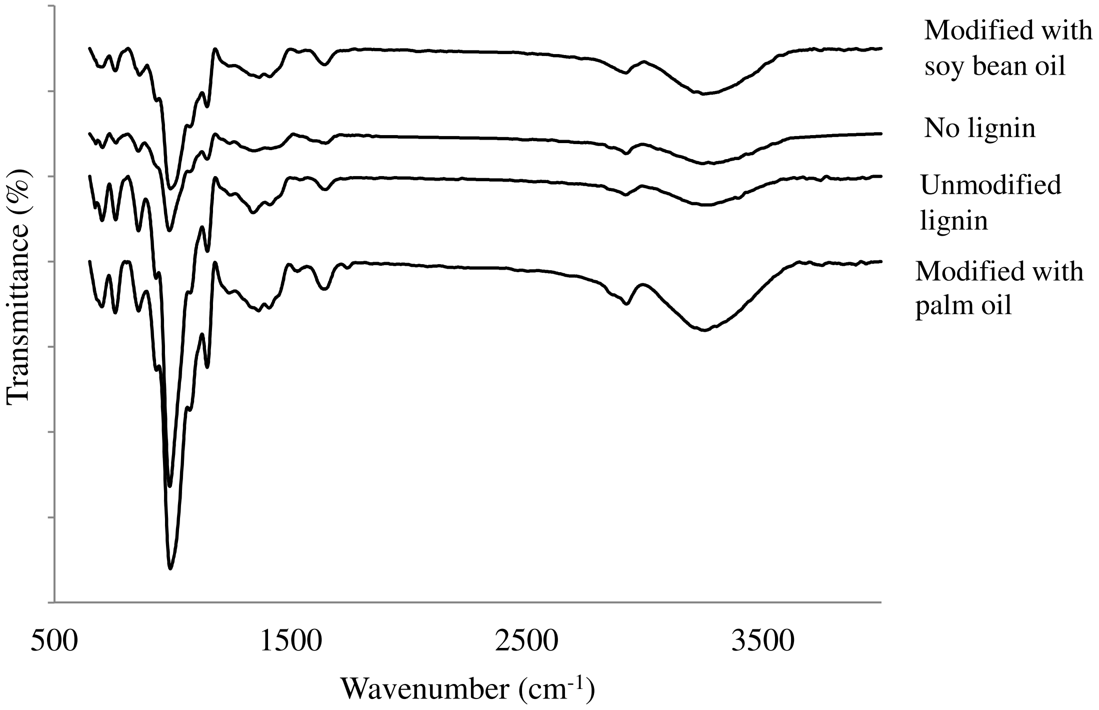


Figure 27 FTIR spectra of rice starch films incorporated with OPEFB's lignin and OPEFB's lignin derivatives.

4.7 Morphological properties

It was observed that the OPEFB's lignin derivatives was light brownish in color and more amorphous than the OPEFB's lignin derivatives. Morphology showed that the particles seem to be large; less agglomerated probably due to the surface modification effects and also represented a group of globules viewed at higher magnification shows in Figure 28. The control films (without lignin) had the smooth and continuous surface without grainy and porous structure. This indicated that films with ordered matrix were formed. The addition of both unmodified OPEFB's lignin and modified OPEFB's lignin provoked a strong disruption of the a little smooth and non-homogeneous structure of the rice starch films, inducing a partial laminar-like appearance with increased density. The incompatibility of lignin and starch denoted by the non-homogeneous appearance of the corresponding film forming, the bimodal microstructure in the composite films would indicate many phase segregation of the two components. A certain degree of immiscibility between hydrophobic groups of lignin and lipid and hydrophilic groups in starch has been shown to protect interaction between starch molecules causing microphase separation in the consistent blends (Huang *et al.*, 2003). Figure 28 shows micrographs of rice starch films contained 5% lignin which was the optimum content in this study. It can be observed that rice starch films contained both unmodified and modified OPEFB's lignin (palm oil and soy bean oil) presented aggregates due to incompatibility between starch films and lignin. Starch films-lignin contained both modified and unmodified OPEFB's lignin had a little rougher surface, probably owing to the hydrophobic nature of lignin and lipid, which meant that lignin and lipid were less compatible with rice starch films, which could be the result of the higher molecular weight and longer carbon chain of lipid or the benzene ring structure of lignin. As well as the Prodpran *et al.*, (2007) reported that for films added with palm oil; the surface of film had the irregular surface with the distribution of oil. In this case, modified OPEFB's lignin have smaller particles than OPEFB's lignin, hence, a greater compatibility between matrix (starch) and filler (lignin) and also a higher dispersion was occurred. The results showed that the lignin segments (brown point) were aggregated into spherical shapes. The great distributions were likely to be originated from the condensation of lignin molecules. Therefore, in reinforced films the chemical groups or long chains of fillers would have significant influences on the formation of hydrogen, van der Waals and dipole–dipole bonds. Molecular weight, hydrophobicity and non-polarity of fillers resulted in different surface configurations as shown in Figure 28. According to our results the film structure as well as the distribution of lipid in lignin might be associated with the properties of film, particularly water vapor permeability and contact angel value of resulting films.

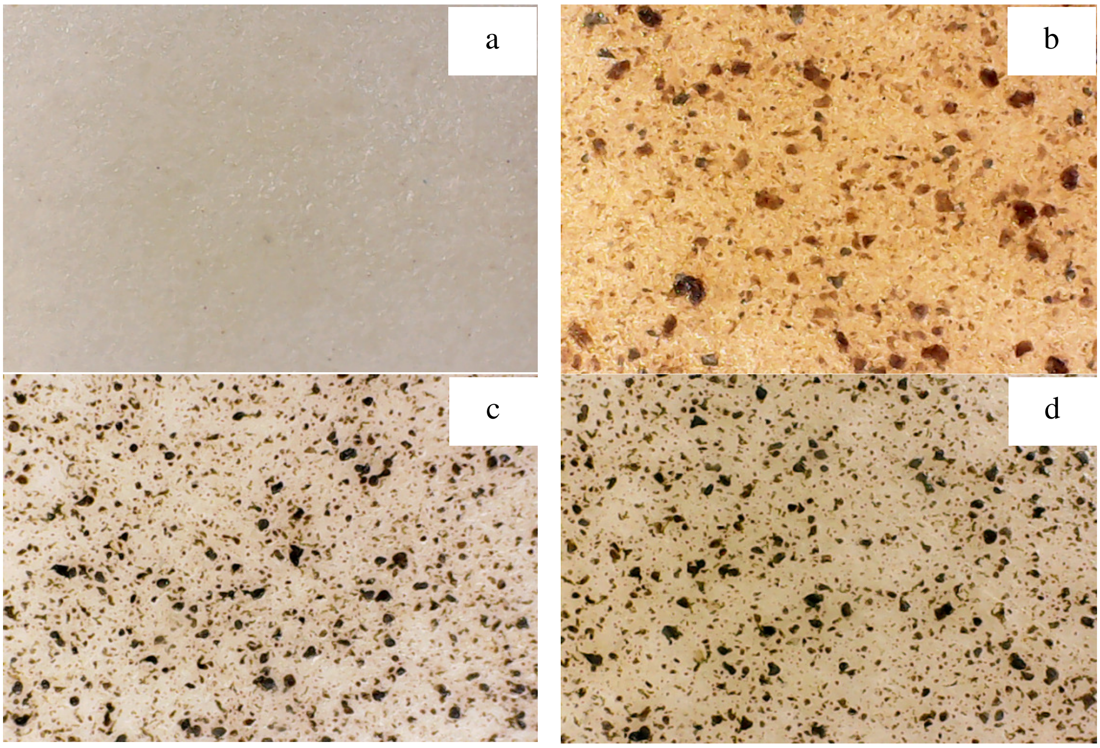


Figure 28 Morphology of rice starch films; without lignin (a), contained 5% unmodified OPEFB's lignin (b), contained 5% OPEFB's lignin modified with palm oil (c) and contained 5% OPEFB's lignin modified with soy bean oil (d).

4.8 Antimicrobial properties

The antimicrobial activity against screening microorganisms 3 types such as *L. monocytogenes*, *S. aureus* and *E. coli* of rice starch films fillers with OPEFB's lignin modified with palm oil and soy bean oil compared with unmodified lignin were tested. The results showed there was no antimicrobial activity on *L. monocytogenes* and *E. coli*, thus the data did not presents. To inhibit the growth of *L.*

monocytogenes and *E. coli* could requires concentration of OPEFB's lignin in high doses detected from the MBC results. At minimum concentration (lower 25 mg/mL of lignin) there was no antimicrobial activity of OPEFB's lignin against gram-negative bacteria (*E. coli*), these results was related with previous results of Nada *et al.* (1989). However, the inhibitory effect on gram-positive bacteria (*S. aureus*) at was occurred when 10% of both unmodified and modified lignin was applied (Fig.29). Hence, in this study it could be revealed that modification of lignin with lipids (soy bean oil and palm oil) had no effect on the inhibition of tested bacteria. As well known that lignin is an aromatic amorphous biopolymer which is expected to play an important role in the near future as raw materials for the production of bio-products. It is built up by oxidative coupling of three major C6–C3 (phenylpropanoid) units; p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol (She *et al.*, 2010). Lignin as well as other polyphenols are potent free radical scavengers and considered to be a valuable source of antioxidant phenolic. The applicability of lignin from different sources as potential antioxidants has been also successfully tested Urgatondo *et al.*, 2009 and El Hage *et al.*, 2012. Consequently, the antioxidant properties exhibited by lignin can give broader applications as antimicrobial, anti-aging agents and corrosion inhibitors. Due to the high content of diverse functional groups (phenolic and aliphatic hydroxyls, carbonyls, carboxyls, etc.) and its phenylpropanoic structure, lignin can act as an inhibitor for microorganism. Thus, as a complex phenolic polymer, lignin possesses antimicrobial properties (Dizhbite *et al.*, 2004). In addition, the increase of lignin which have hydrophobic behavior in composite due to lower content of hydroxyl groups affect can improvement in antimicrobial properties. Nada *et al.* (1989) studied the IR spectra of the different lignin precipitated from the pulping liquor and their antimicrobial activities towards some bacteria. The results showed that the prepared lignin had no antimicrobial effects against the gram-negative bacteria (*E. coli*), but was effective against the gram-positive bacteria (*B. subtilis* and *B. mycoids*). Moreover, Scalbert (1991) reported that the antibacterial properties of woody plant extracts are associated with its lipophilic components, leading to change in membrane potential and increase in permeability of the cytoplasm membrane for protons and potassium ions, including depletion of the intracellular ATP pool. The mechanism of action responsible for antimicrobial activity of phenolic compounds present in herbarceous and woody plants has not been fully defined, although activity has been attributed to inhibition of extracellular enzymes, deprivation of substrates required for growth, inhibition of oxidative phosphorylation or iron deprivation (Scalbert, 1991).

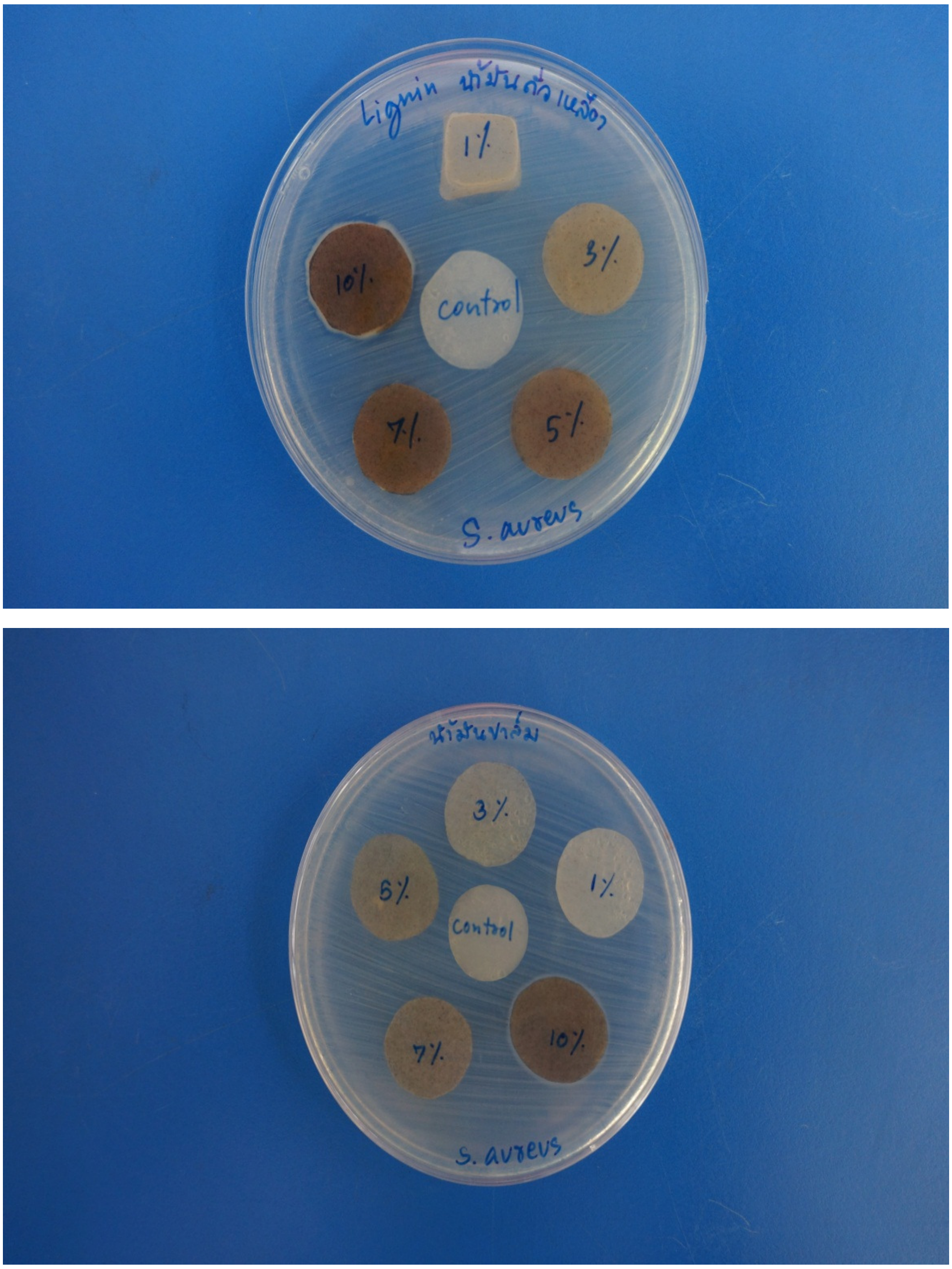


Figure 29 Illustration of the antimicrobial properties of OPEFB's lignin derivative at various content (0-10%).

5. Effect of relative humidity on the properties of rice starch films

Moisture sorption isotherms are relation between the effects of relative humidity (RH) on film properties. Adding or removal of water may cause phase transitions in the macromolecular structure. Relative humidity (RH) of a film's surroundings will affect its performance. As RH increases, the capacity of films to function as barriers to water vapor permeability and mechanical properties was decreases. These properties are dependent on the structure, behavior, chains interactions and film thickness, as well as being affected by the RH of the environment (Lourdin *et al.*, 1997). Therefore, data from experimental sorption isotherms are a useful tool to predicted the effect of the environmental conditions that surround the rice starch film on its properties; particularly considering that the stability of an biodegradable film is function of its mechanical and moisture barrier properties and both are strongly influenced by the presence of water, which is related to storage conditions of the films (Soazo *et al.*, 2011). Jangchud and Chinnan (1999) reported the water sorption isotherm of a material represents the equilibrium relationship between their moisture content and the water activity (a_w) at constant temperatures and pressures. The sorption isotherms obtained from experimental data result in an estimation of equilibrium moisture content, which is necessary to predict the properties of films in different environments pertinent to their application. Polysaccharides and protein films have overall suitable properties but when obtained the high moisture affect mechanical properties and water vapor barriers are very poor (Guilbert *et al.*, 1996). In addition, biodegradable rice starch films were the hydrophilic properties and low barrier to moisture. This study evaluates the effect of relative humidity on the interaction between moisture and OPEFB's lignin or modified OPEFB's lignin (palm oil and soy bean oil) based on rice starch films using relative humidity determined at 32.8, 50.0, 64.4, 75.4, 79.7 and 91.6% RH. Before testing the films were store in the desiccators according relative humidity levels at 25 ± 2^{o}C for 72 h. Then, the selected properties (mechanical properties and water vapor permeability) of the films were tested.

5.1 Mechanical properties

Biodegradable rice starch films contained 5% of OPEFB's lignin and 5% of OPEFB's lignin modified with palm oil and soy bean oil stored at various level of relative humidity were determined. Moisture adsorption was more rapid in the initial stages of moisture adsorption and lesser amount of moisture was adsorbed as adsorption time increased. Films stored at higher relative humidity required more time to reach their equilibrium. The moisture adsorption of rice starch films were increased with increasing the relative humidity. Figure 30 and 31 shows the TS and elongation at break of biodegradable rice starch films at different relative humidity. The results demonstrated that the TS of the rice starch films (no lignin) and rice starch films contained 5% of OPEFB's lignin and 5% of OPEFB's lignin modified with palm oil and soy bean oil were decreased as the relative humidity increased. At the lowest relative humidity (32.8%) providing the film with hard and brittle due at the low humidity environment looks dry as a result properties of the films decreased. While, relative humidity higher 64.4% the TS of the films was decreased. The results showed that rice starch films without lignin showed lower TS than rice starch films contained OPEFB's lignin and modified OPEFB's lignin when a high relative humidity was tested. It was due to rice starch films were strongly sensitive to the moisture, while the OPEFB's lignin and modified OPEFB's lignin have a good water resistance properties (hydrophobic properties). The results demonstrated using 64.4% relative humidity provided the suitable properties of the resulting films.

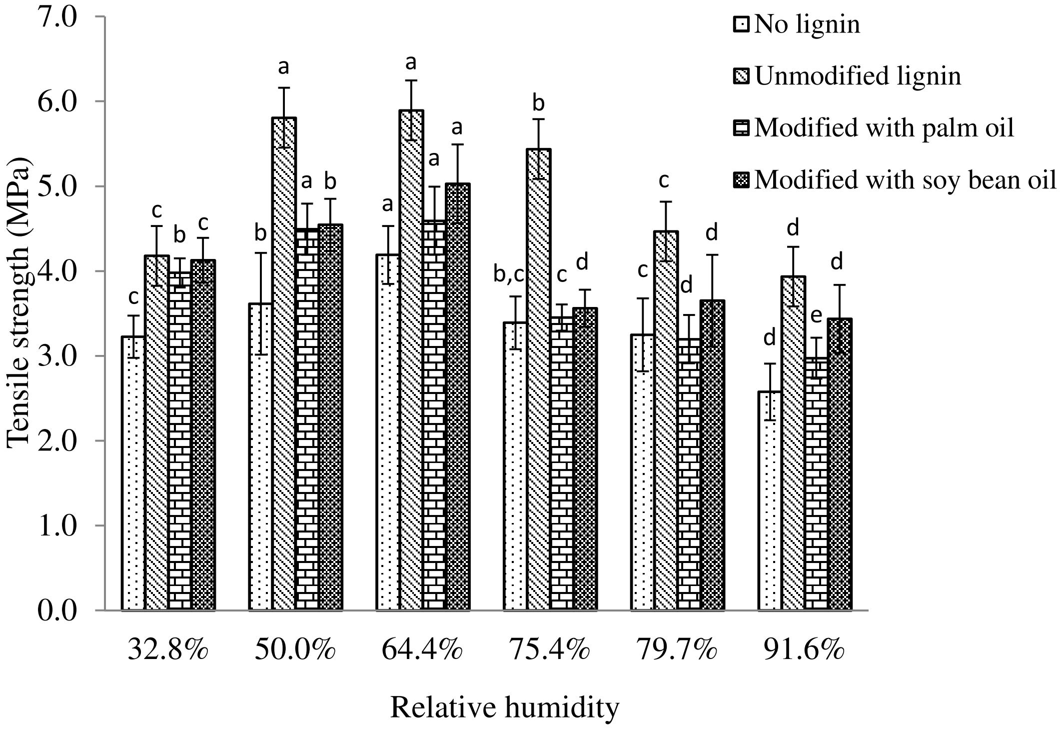


Figure 30 Effect of relative humidity on tensile strength of rice starch films contained OPEFB's lignin and modified OPEFB's lignin.

Figure 31 shows the effect of relative humidity on elongation at break of the rice starch films. The results showed that elongation at break of the films decreased when relative humidity increase from 32.8% to 75.4% due the amount of moisture that absorbed into the film influences the structure of the film is more flexible; resulted in an increasing in elongation at break. In addition, when relative humidity higher than 75.4% provided the films which poor and limp because too much moisture content causes the film properties deteriorate. The effect of OPEFB's lignin and modified OPEFB's lignin on elongation at break of rice starch was compared. It was found that rice starch films contained OPEFB's lignin showed the lowest elongation at break than rice starch films contained modified OPEFB's lignin and rice starch films without OPEFB's lignin, respectively. Generally, lignin is non-elastic biopolymer and complex racemic aromatic heteropolymers (Narapakdeesakul *et al.*, 2013). In addition, modification of lignin by increasing hydrophobic behavior could improve the elasticity of OPEFB's lignin as a results presented higher elongation at break of rice starch films than rice starch films contained only OPEFB's lignin.

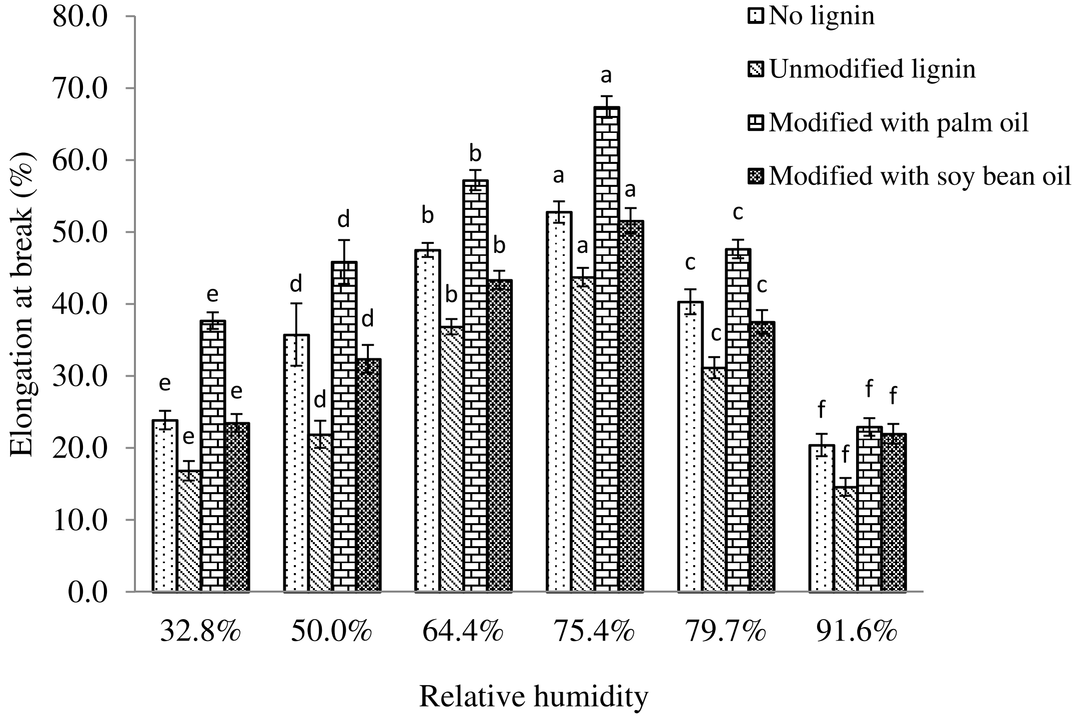


Figure 31 Effect of relative humidity on elongation at break of rice starch films contained OPEFB's lignin and modified OPEFB's lignin.

5.2 Water vapor permeability (WVP)

Permeability is a most importance related to the transmission rate of permeated via a resisting material which involves a multi-mode process. First, the permeate molecules dissolve in the film matrix on the high concentration side. After that, they spread through the film, driven by a permeate concentration gradient. Finally, they desorb and evaporate from the other side of the film (Kester *et al.*, 1986). The permeability of films is not a constant because it increases with RH gradient, opposed to the predicted sorption–diffusion model that describes permeability. This non-ideal behavior is generally recording to a structural modification of the film such as swelling due to the sorption of water vapors. This swelling it was noted by others for biopolymers based materials (Karbowiak *et al.*, 2006). Figure 32 show the water vapor permeability (WVP) of rice starch films with difference filler at various humidity levels. In this study, WVP data were in the range 2.56 – 11.69 g.mm/m^2.day.KPa, and were significantly affected by either OPEFB's lignin and modified OPEFB's lignin at various relative humidity levels. The results demonstrated that, when the relative humidity increases WVP was increase. At high relative humidity (91.6% RH) WVP was most increased due external relative humidity (chamber) rather than internal (aluminum cup) affect vapor penetration into the film more than at lower relative humidity levels. In addition, for higher relative humidity when a swollen network was noticed, it was predicted that a diffusion-controlling mechanism will be found since the polymer will be in the rubbery state and the chains will adjust structure so quickly is causing the presence of the infiltrate (Gontard *et al.*, 1996). When comparing at the same relative humidity levels, it was found that rice starch films contained OPEFB's lignin and modified OPEFB's lignin showed lower WVP than rice starch films without OPEFB's lignin. By the reason of the hydrophobic substances of lignin can decrease the hydrophilic section of the film decreasing its relationship for moisture at high relative humidity (Hernandez-Munoz *et al.*, 2004). Basically, polysaccharide films are rather moisture sensitive and this inherent hydrophilic nature makes them great barriers to non-polar substances such as oxygen or some aroma compounds but poor barriers to water vapor. Consequently, permeability of water vapor via these hydrophilic film increases a lot as the relative humidity to which the film is exposed is raised. This behavior has been mainly observed in polysaccharide films (Gontard *et al.*, 1993). Garcia *et al.* (2000a) said that permeability depends on many factors as the ratio between crystalline and amorphous zone, polymeric chain mobility and specific interaction between the functional groups of the polymers and the gases in the amorphous zone. Few differences of WVP values may be implicated to the difference of water molecule diffusion and hydrophilic-hydrophobic ratio. Although the values obtained of WVP in all condition was higher than for good water vapor barriers such as synthesis polymer but they are showed that this experiment can improve water resistance at medium relative humidity, and might be sufficient to protect food for short-term applications, which is consistent with the biodegradable properties of the film and its intended uses.

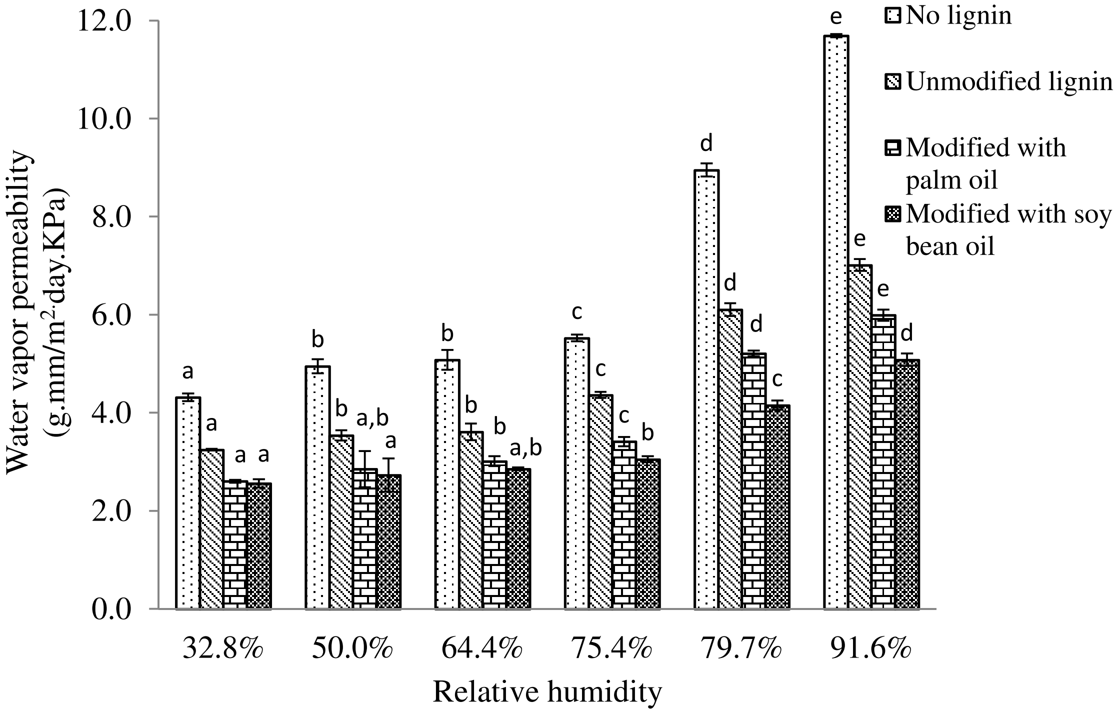


Figure 32 Effect of relative humidity on water vapor permeability of rice starch films contained OPEFB's lignin and modified OPEFB's lignin.

6. Application of rice starch films contained OPEFB's lignin for shelf life extension of selected foods (imitated crab meat and bread)

According to the OPEFB's lignin presented inhibitory on tested microorganism and also addition of OPEFB's lignin and OPEFB's lignin modified with soy bean oil at 10% (OPEFB's lignin modified with soy bean oil showed better properties than palm oil) provided a good in antimicrobial properties of the rice starch films. Hence, application of rice starch films containing OPEFB's lignin for shelf life extension of selected foods (imitated crab meat and bread) was studied and compared with polyethylene film.

6.1 Effect of biodegradable films on the sensory qualities of food products.

The sensory qualities of imitated crab and bread samples were evaluated in terms of appearance, odor and texture were shown in Table 8 and 9, respectively. The results showed that the color of imitation crab wrapped PE film did not change during storage, while the imitation crab color wrapped with rice starch films contained both OPEFB's lignin and OPEFB's lignin modified with soy bean oil changed to yellow-brown after 8 days of storage. It might be due to migrate of OPEFB's lignin from the films to food products. The effect of the films on odor of imitated crab was determined. The result showed that the off odor of imitated crab were detected at 7, 21, 21 and 21 days of storage when without films, PE film, rice starch films contained both OPEFB's lignin and OPEFB's lignin modified with soy bean oil were applied, respectively. In addition, the mucus on the product was noticed at 14 days of storage for without films, while other treatment did not show any mucus. Textural testing (juicy and callousness) of imitated crab wrapped with various films found that the callousness of imitated crab wrapped with PE films, rice starch films contained both OPEFB's lignin and OPEFB's lignin modified with soy bean oil and without films was detected at 21, 14, 14 and 7 day of storage.

From the result, in parts of odor and texture testing found that PE films showed product quality better than OPEFB's lignin and OPEFB's lignin modified with soy bean oil. Lignin can to protect the deterioration of product at a certain level. According to the physical appearance of the films, it was found that PE films were unchanged during storage while rice starch films added OPEFB's lignin and OPEFB's lignin modified with soy bean oil were changed such as swelling due to the hydrophilic behavior of the rice starch films causes absorption of water from the food product (table 9 and figure 35).

For the characteristics of bread during storage found that the color of bread stored in rice starch films contained OPEFB's lignin and OPEFB's lignin modified with soy bean oil did not showed any changes during storage, while the green and black of bread without films was noticed after 3 days of storage. It might be due to the migration of OPEFB's lignin from the films to food products then the antimicrobial properties of OPEFB's lignin were occurred. The effect of the films used on odor of bread was also determined. The result showed that the off odor of bread was detected at 4, 4 and 2 days of storage when rice starch films contained both OPEFB's lignin and OPEFB's lignin modified with soy bean oil and without films were applied, respectively. Addition, the textural properties (stickiness, softness) of bread was tested, it was found the stickiness and softness of bread stored with rice starch films contained both OPEFB's lignin and OPEFB's lignin modified with soy bean oil and without the films was detected at 4, 4 and 2 day of storage, respectively (table 10 and figure 36). According to the sensory qualities of tested food products, it would be pointed out that rice starch films contained with OPEFB's lignin and OPEFB's lignin modified with soy bean oil could extend the shelf-life of both imitated crab and bread better than PE films and without films, respectively. This may be attributed to lignin could act as antimicrobial activity, resulted in better spoilage retard of food product.

Table 9 Sensory evaluation of imitated crab wrapped with rice starch films contained with OPEFB's lignin, OPEFB's lignin modified with soy bean oil, PE films and without films during storage.

Days	Experiment	Appearance	Odor	Texture
0	Without film	On white and red side are usual	Fresh	Fresh, juicy
	RS films+Lignin	On white and red side are usual	Fresh	Fresh, juicy
	RS films+ Modified Lignin	On white and red side are usual	Fresh	Fresh, juicy
	PE films	On white and red side are usual	Fresh	Fresh, juicy
7	Without film	On white and red side are usual	Low Freshness	A little bit callous
	RS films+Lignin	On white and red side are usual	Fresh and lignin odor	Fresh, juicy
	RS films+ Modified Lignin	On white and red side are usual	Fresh and lignin odor	Fresh, juicy
	PE films	On white and red side are usual	Fresh	Fresh, juicy
14	Without film	Slime occurred	Low freshness	Callous
	RS films+Lignin	Red side is usual and white side is mind yellow-brown	Fresh and lignin odor	A little bit callous
	RS films+ Modified Lignin	Red side is usual and white side is mind yellow-brown	Fresh and lignin odor	A little bit callous
	PE films	On white and red side are usual	Fresh	Fresh, juicy
21	Without film	Slime occurred	-	Hard
	RS films+Lignin	Red side is usual and white side is yellow-brown	Low fresh and lignin odor	Callous
	RS films+	Red side is usual and white	Low fresh and	Callous

	Modified Lignin	side is yellow-brown	lignin odor	
	PE films	Red side is usual and white side is yellow	Low freshness	A little bit callous
28	Without film	-	-	-
	RS films+Lignin	Slime occurred	Low fresh and lignin odor	Hard
	RS films+ Modified Lignin	Slime occurred	Low fresh and lignin odor	Hard
	PE film	Slime occurred	Low fresh	Hard

Table 10 Sensory evaluation of bread placed between the sheets with rice starch films contained with OPEFB's lignin, OPEFB's lignin modified with soy bean oil and without films during storage.

Days	Experiment	Appearance	Odor	Texture
0	Without film	White yellow and brown edge	Bread smell	Sticky, soft
	RS films+Lignin	White yellow and brown edge	Bread smell	Sticky, soft
	RS films+ Modified Lignin	White yellow and brown edge	Bread smell	Sticky, soft
2	Without film	White yellow and brown edge	Low bread smell	Soft and a little dry
	RS films+Lignin	White yellow and brown edge	Bread smell and little lignin odor	Sticky, soft
	RS films+ Modified Lignin	White yellow and brown edge	Bread smell and little lignin odor	Sticky, soft

4	Without film	White yellow and brown edge, a many green and black dot at bread and edge	Off flavor and fungus smell	Hard
	RS films+Lignin	White yellow and brown edge	Low bread smell and little lignin odor	A little callous
	RS films+ Modified Lignin	White yellow and brown edge	Low bread smell and little lignin odor	A little callous
6	Without film	-	-	-
	RS films+Lignin	White yellow and brown edge, a little green and black dot at bread and edge	Off flavor, fungus smell and lignin odor	Hard
	RS films+ Modified Lignin	White yellow and brown edge, a little green and black dot at bread and edge	Off flavor, fungus smell and lignin odor	Hard

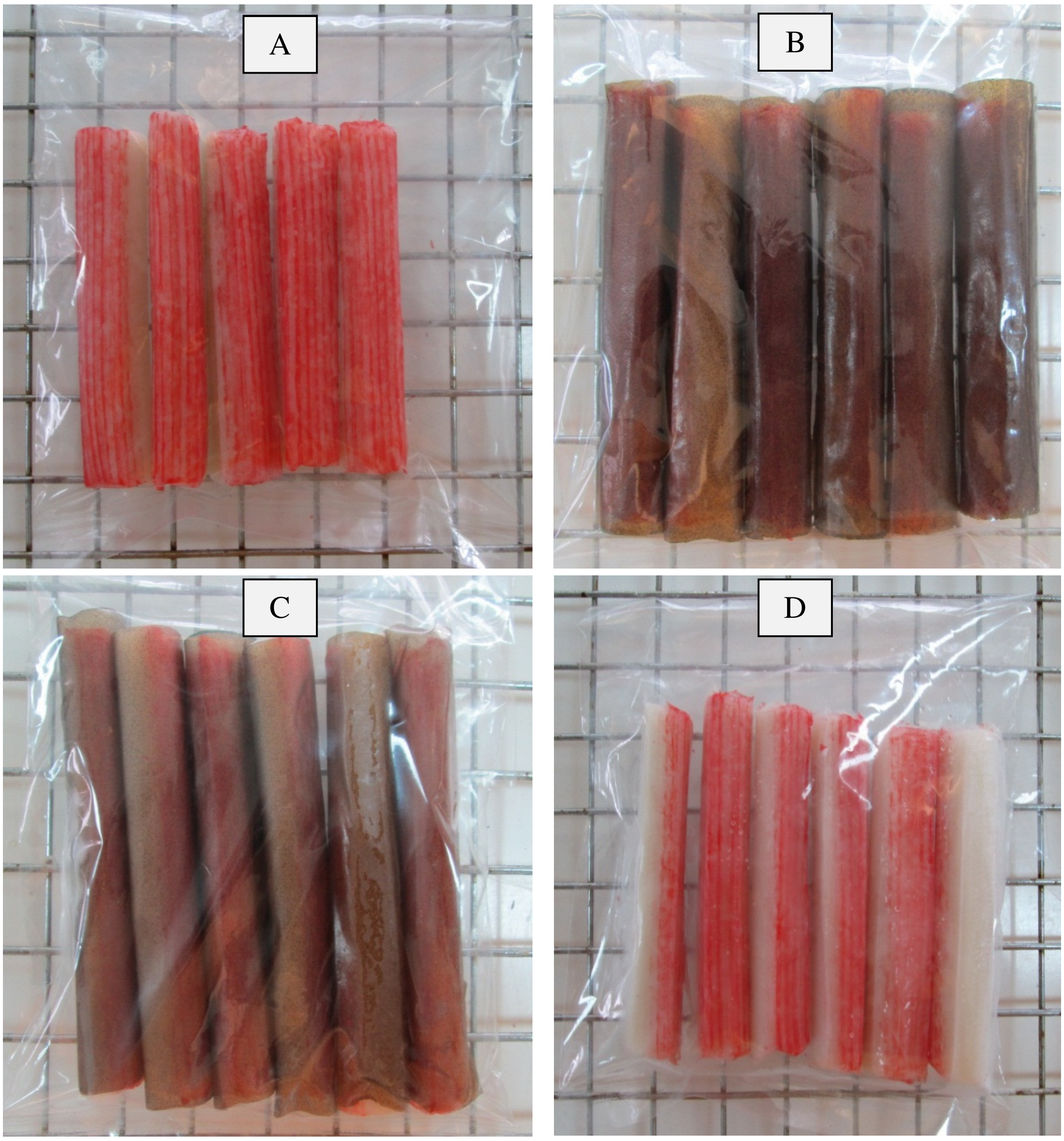


Figure 33 Illustrative of imitated crab wrapped with various type of the films. Without film (A), wrapped with rice starch films contained OPEFB's lignin (B), wrapped with rice starch films contained modified OPEFB's lignin (C) and wrapped with PE films (D).

Figure 34 Illustrative of sliced bread treated with various films and packed in PE bags. Without film (A), treated with rice starch films contained OPEFB’s lignin (B) and treated with rice starch films contained modified OPEFB’s lignin (C).

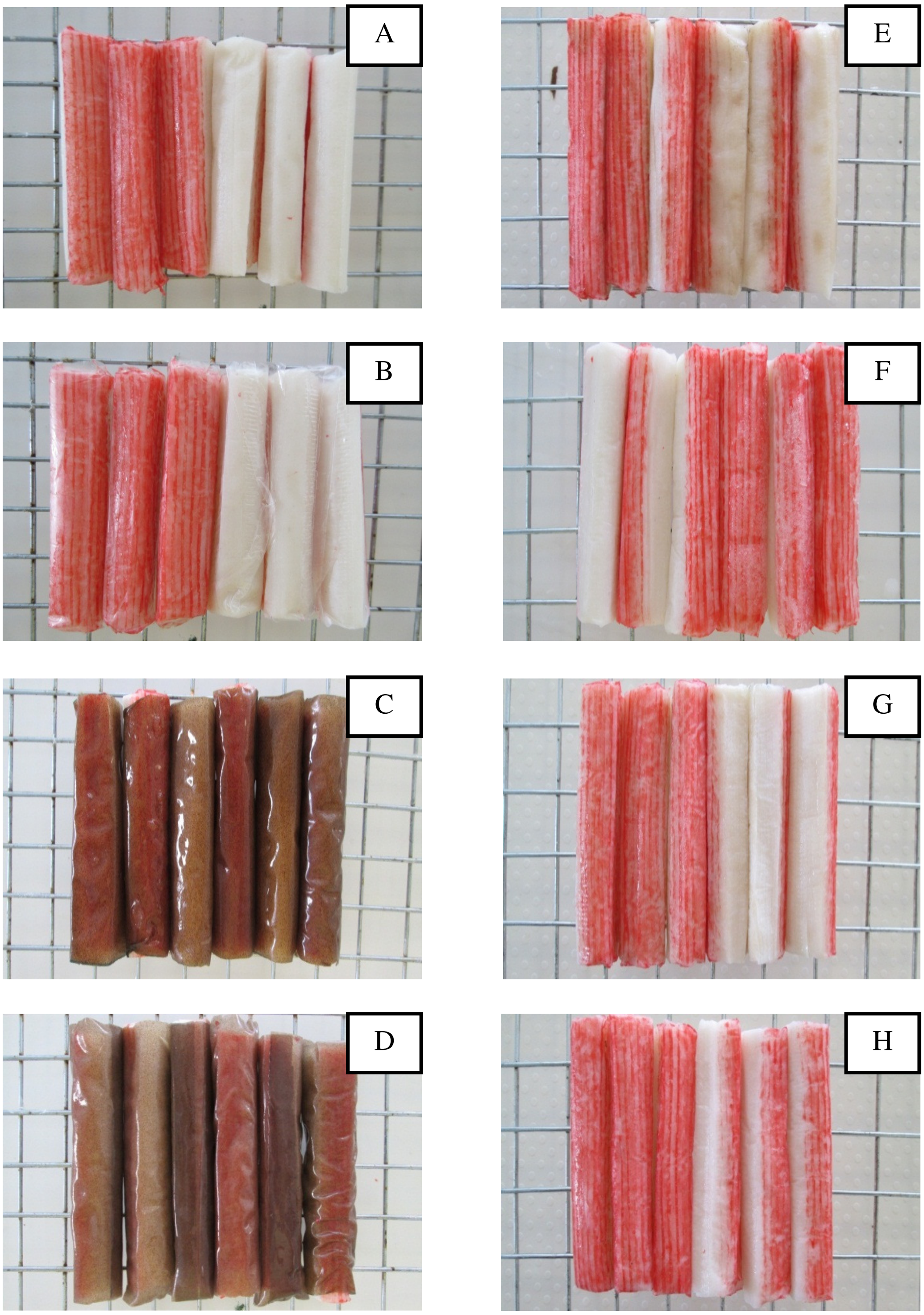


Figure 35 Illustrative pictures of imitated crab without films wrapping (A), imitated crab wrapped with PE films (B), imitated crab wrapped with rice starch films contained OPEFB's lignin (C), imitated crab wrapped with rice starch films contained modified OPEFB's lignin (D) at 0 day of storage; imitated crab without films (E), imitated crab wrapped with PE films (F), imitated crab wrapped with rice starch films contained OPEFB's lignin (G), imitated crab wrapped with rice starch films contained modified OPEFB's lignin (H) at 21 days of storage.

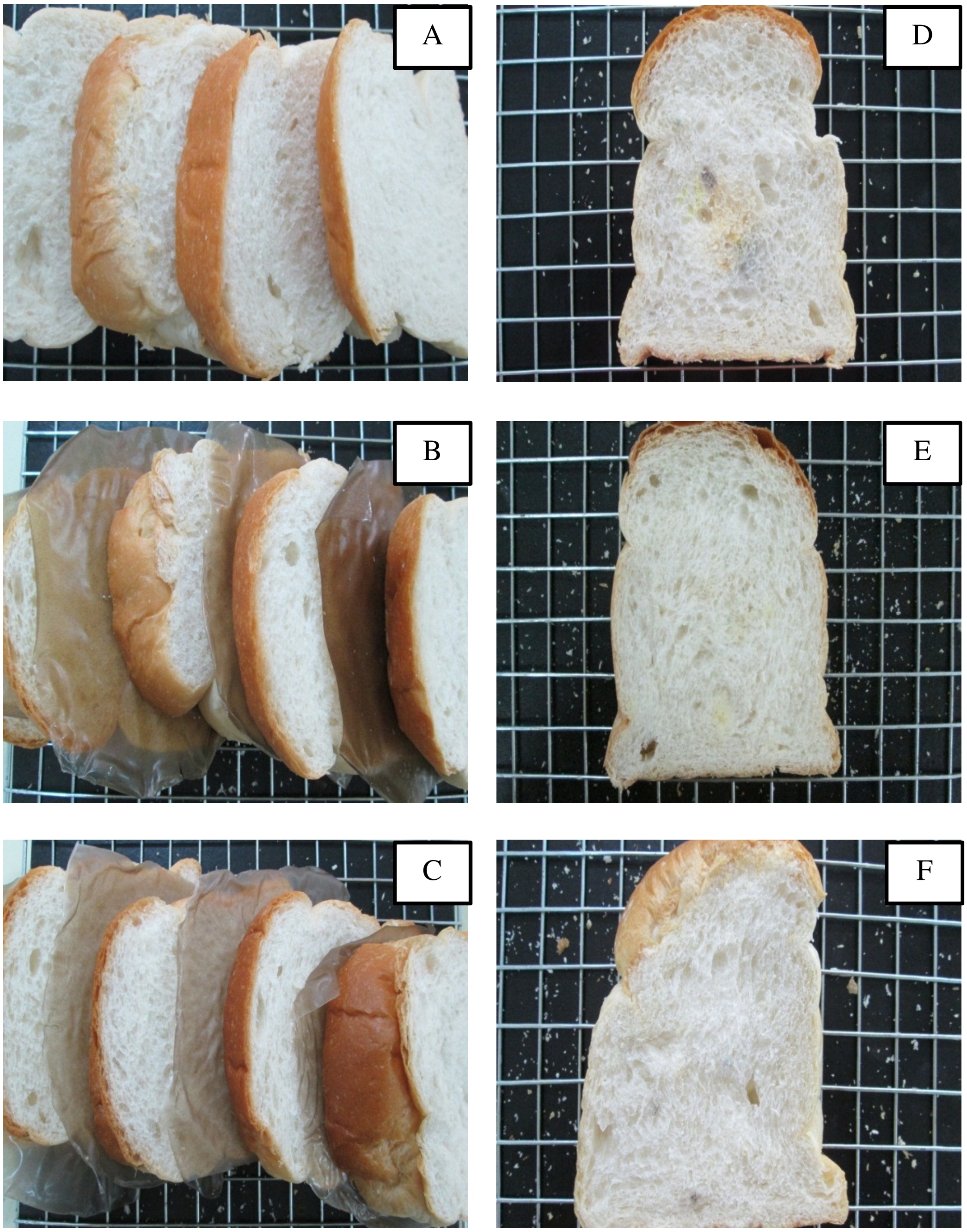


Figure 36 Illustrative of sliced bread treat with various films and packed in PE bags. Bread without films (A), bread treated with rice starch films contained OPEFB's lignin (B) and bread treated with rice starch films contained modified OPEFB's lignin (C) at 2 day of storage; bread without films (D), bread treated with rice starch films contained OPEFB's lignin (E) and bread treated with rice starch films contained modified OPEFB's lignin (F) at 6 day of storage.

6.2 Effect of biodegradable films on water activity (a_w) of food products.

Figure 37 showed the water activity of food products. Wrapping and placed between the sheet of each type films significantly ($p<0.05$) affected the water activity (a_w) of imitated crab meat and bread. When the storage time increased a_w was decreased both the imitated crab meat and bread. Both imitated crab meat and bread used rice starch films contained OPEFB's lignin and modified OPEFB's lignin showed higher a_w than without films. However, imitated crab, wrapped with PE films have a_w values higher than wrapped with rice starch films contained OPEFB's lignin and modified OPEFB's lignin. The results showed that at 6 days of storage, the a_w of bread did not analyze due to a little green and black spot on the bread was occurred. Our studies showed that a_w of products decreased when the storage time increased resulted in hardening of the products due to the moisture of products were lose to the surrounding atmosphere that absorb through the packaging. The decrease of water activity indicate that each type films can be prevent movement of water molecules was different. The non-polarity of PE films showed higher a_w of imitated crab meat and also hydrophobic behavior of lignin can make water penetrates out-in difficult. All of the above it can be concluded that OPEFB's lignin able protect the penetration of moisture and resulting product have a longer shelf life but still slightly poorer than PE films , however, disadvantages of PE films were sensitive lipids and air which can be easily permeable. Hence, products that sensitive to air when put in PE bags food quality will change rapidly.

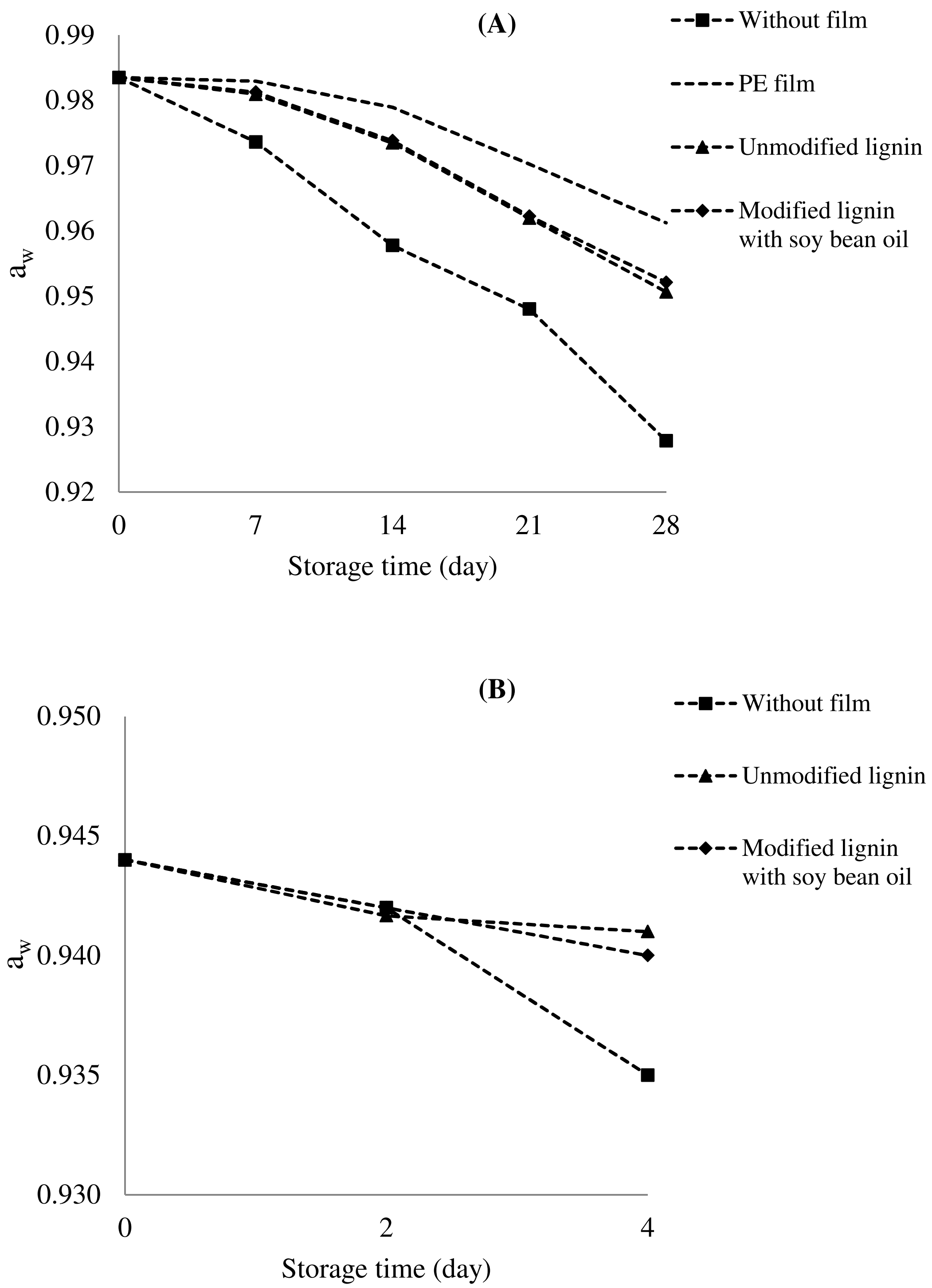
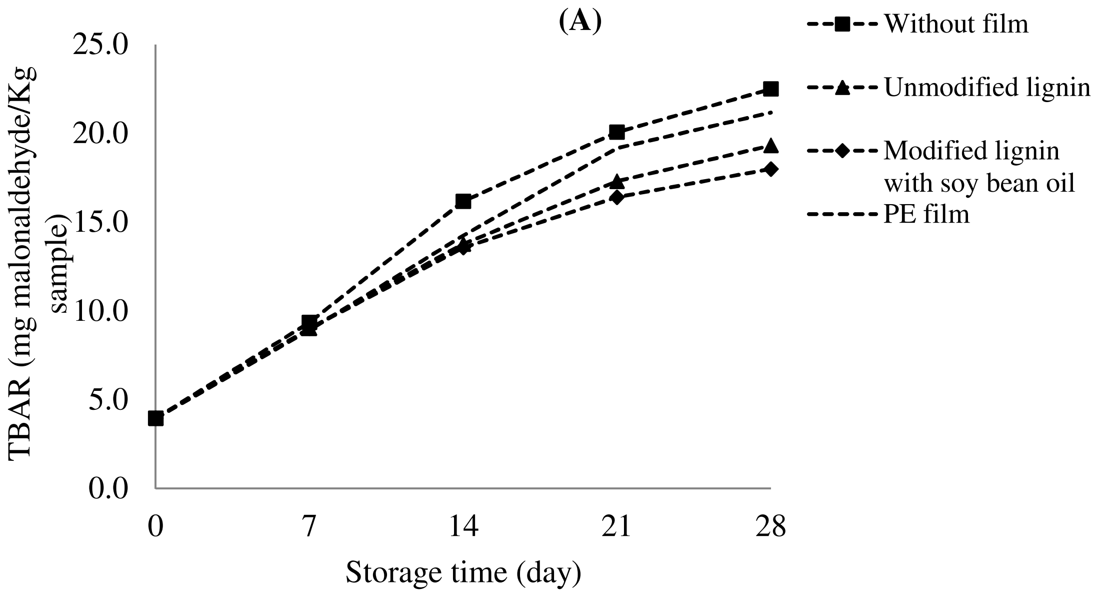


Figure 37 Effect of the wrap film type on water activity (a_w) of imitated crab meat (A) and bread (B) during storage at 4-6^{o}C and at room temperature, respectively.

6.3 Effect of biodegradable films on TBARS of food products.

TBARS is an index of lipid oxidation. Changes in TBARS value of imitated crab meat (wrapped) and bread (placed between the sheets) using difference films (PE films, rice starch films contained OPEFB's lignin and modified OPEFB's lignin) during the storage are show in Figure 38. TBARS values of all treatments increased continuously with increasing storage time ($p<0.05$). From the results the TBARS values of imitated crab meat within the rage 3.947-22.473, 3.947-21.149, 3.947-19.302 and 3.947-17.975 mg malonaldehyde/kg sample for without films, PE film, rice starch films contained OPEFB's lignin and modified OPEFB's lignin, respectively at 28 days of storage. While the TBARS values of bread were range 1.63-6.03, 1.63-4.22 and 1.63-4.00 mg malonaldehyde/kg sample for without films, rice starch films contained OPEFB's lignin and modified OPEFB's lignin, respectively for 6 days of storage. The results demonstrated that imitated crab meat wrapped with rice starch films contained modified OPEFB's lignin had lower TBARS values than rice starch films contained OPEFB's lignin, PE films and without films, respectively. For the bread, product samples placed between the sheets with rice starch films contained modified OPEFB's lignin had lower TBARS values than rice starch films contained OPEFB's lignin and without films, respectively as well. The high content of polyunsaturated fatty acid in the food product affect TBARS values was increase (Saunders, 1994). The double bonds in an unsaturated fatty acid are locked into position when oxygen reacts with the methylene group next to the double bonds cause lipid oxidation (Porter *et al.*, 1995). Lignin has the ability to resist free radicals (antioxidant) resulting can retard the reaction between oxygen and lipid (lipid oxidation), slowly down rancidity. Due phenolic groups in lignin structure are known to react in free radical reactions where they normally inhibit conventional free radical polymerization reactions (Minoura *et al.*, 1964). In addition, Salehi (2010) reported that phenol groups and conjugated double bonds of the lignin structure had the largest retardation effect on the onset time of oxidation. This experiment indicated that both rice starch films contained OPEFB's lignin and modified OPEFB's lignin can inhibit or retard lipid oxidation in imitated crab meat and bread better than PE films and without films resulting in a longer time shelf life and quality of the product.



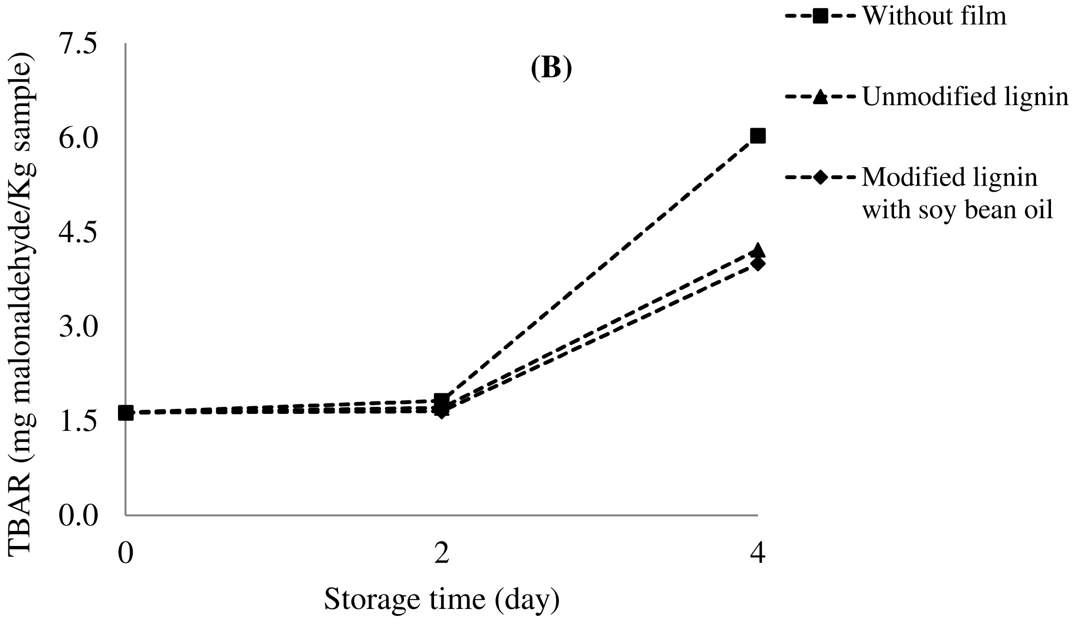


Figure 38 Effect of biodegradable films on TBARS of imitated crab meat (A) and bread (B) during storage at 4-6°C and at room temperature, respectively.

6.4 Effect of biodegradable films on color of food products.

Color was as an indication external appearance. In addition, it can also be predict of quality and product changes. Figure 39 and 40 showed the L^*, a^* and b^* value of imitated crab meat and bread, wrapped and placed between the sheet with difference type films, respectively. It was noted that the increase in a^* and b^* values and decrease in L^* value were observed in both imitated crab meat and bread throughout the retention period. From the experiment, the increasing of a^* and b^* values identified the formation of yellowish pigment, especially via maillard reaction, which might be associated with increasing moisture content in products. Color changes of antimicrobial films caused yellow-brown color of OPEFB's lignin mixed into films resulting color of OPEFB's lignin from films tough to external surface of products. The products wrapped and placed between the sheet with rice starch films contained OPEFB's lignin and modified OPEFB's lignin demonstrated higher lightness but lower redness and yellowness when compared with without films and PE films, as identified by higher L^* values and lower a^* and b^* value, respectively. These results suggested that contained OPEFB's lignin and modified OPEFB's lignin could retard the browning reaction, which might be associated with lipid oxidation.

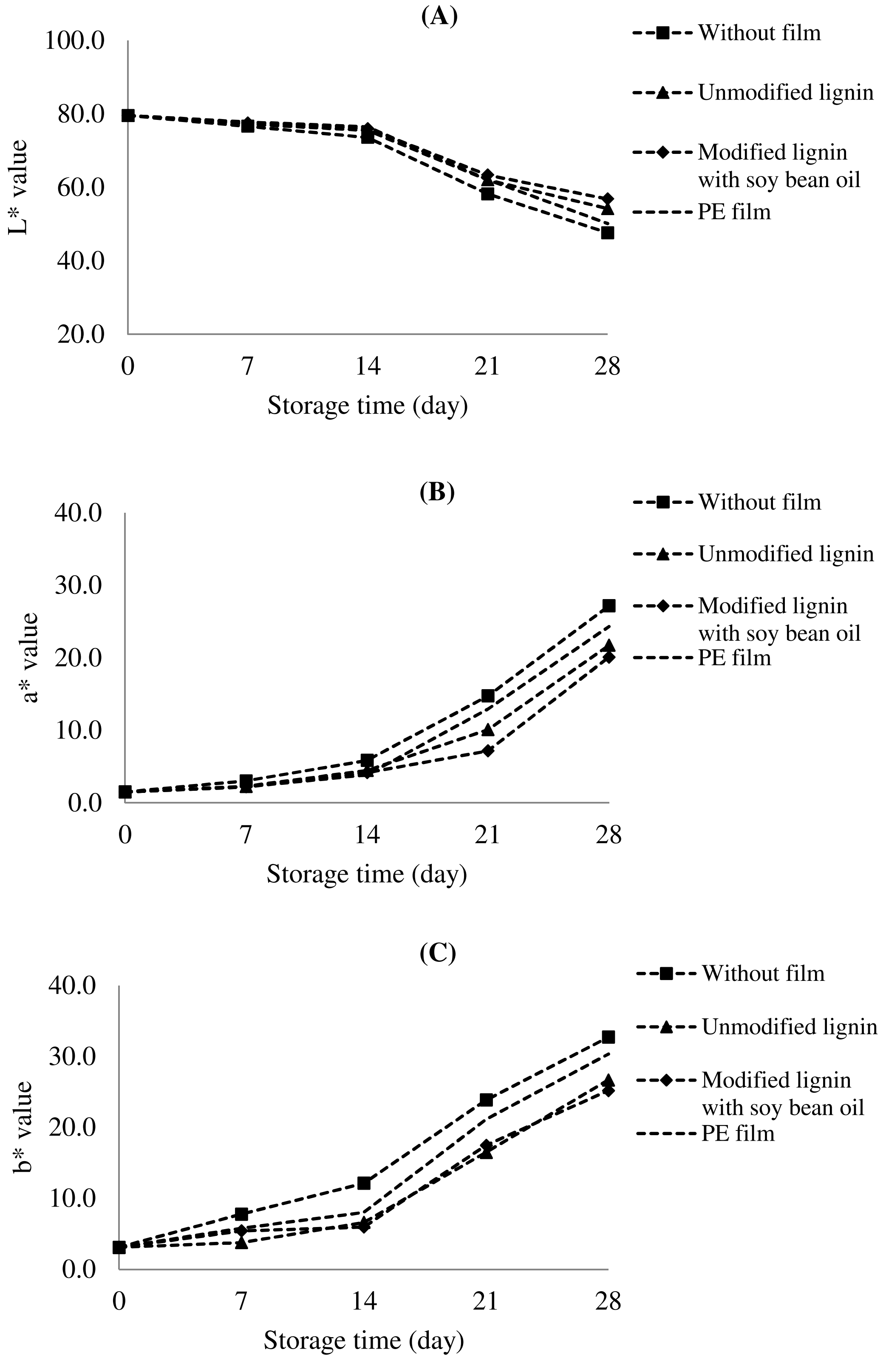


Figure 39 Effect of biodegradable films on L^* (A), a^* (B) and b^* (C) values of imitated crab meat during storage at 4-6°C.

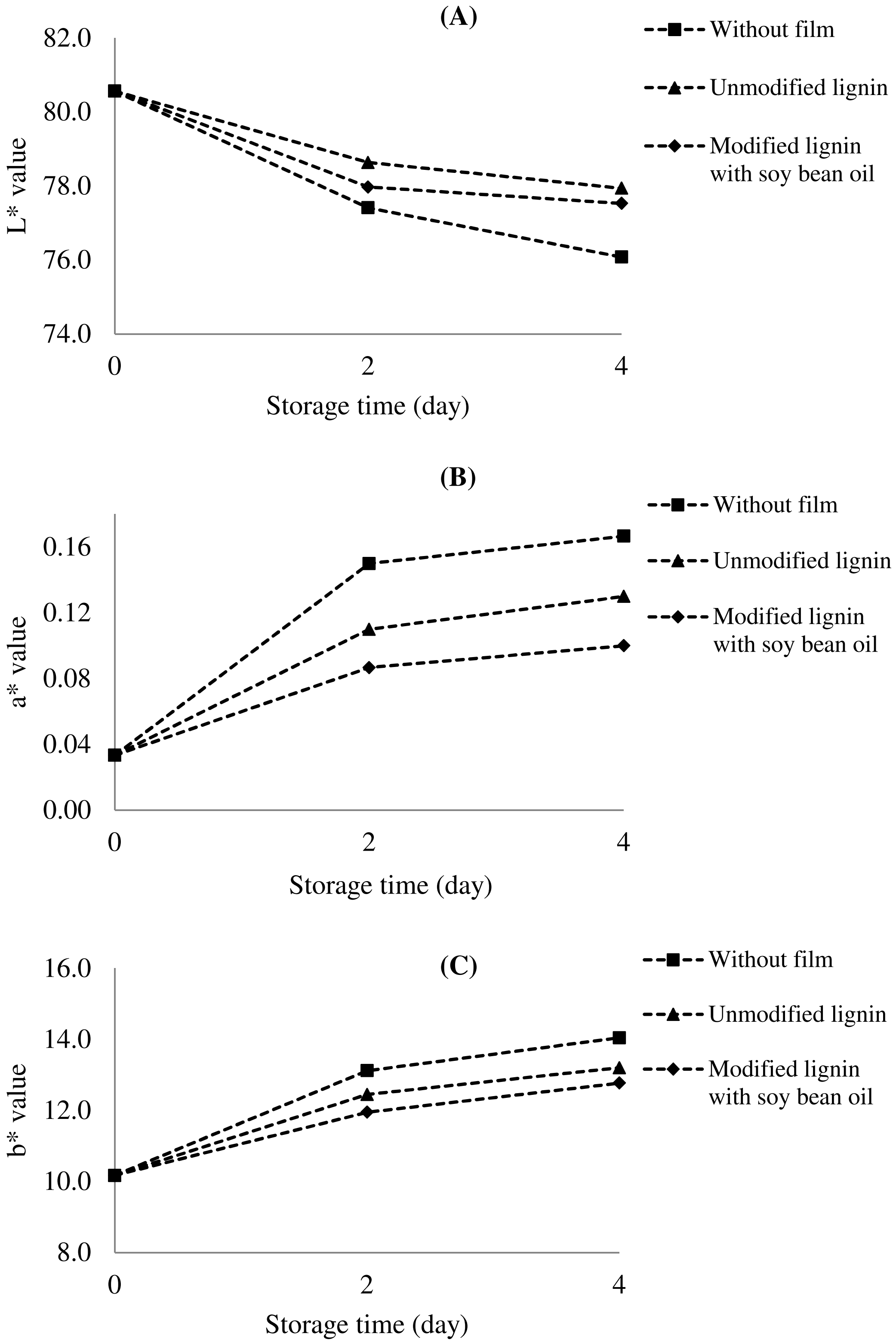


Figure 40 Effect of biodegradable films on L^* (A), a^* (B) and b^* (C) values of bread during storage at room temperature.

6.5 Effect of biodegradable films on microbiological property of food products.

Microorganism can be considered as the most serious factor affecting the quality and safety of food product and the number of microorganisms will directly affect the quality of food product. It has been recommended that the critical value in relation to spoilage, total viable counts (TVC) of imitated crab meat and bread have been defined 1x10^4 CFU/g. Lignin and lignin derivative based on rice starch films might be used to produce the food packaging which is important material to maintain the quality of foods during storage, transport and handing. It is necessary to protect food products from external influences and damage, to hold the food product together, to provide consumers with ingredient and nutrition information (Schou *et al.*, 2004).

Changes in total viable count (TVC) of imitated crab meat and bread wrapped and placed between the sheets with different type films sample during storage are shown in Figure 41. TVC showed a significant increased with increasing storage period. The results demonstrated that wrapped and placed between the films plays a significant role as a protection against TVC of both imitated crab meat and bread, respectively. From the experiment, wrapped and placed films between the sheets for imitated crab and bread with rice starch films contained OPEFB's lignin and modified OPEFB's lignin, the TVC values were lower than PE films and without films, respectively. Due to OPEFB's lignin and modified OPEFB's lignin have antimicrobial properties more than PE films. For imitated crab meat, the TVC values (28 days of storage) were range 1-8.61, 1-7.23, 1-6.43 and 1-6.15 log CFU/g for without films, PE films, rice starch films contained OPEFB's lignin and modified OPEFB's lignin, respectively. While, TVC values of bread (6 days of storage) were range 2.52-7.43, 2.52-6.52 and 2.52-6.11 log CFU/g for without films, rice starch films contained OPEFB's lignin and modified OPEFB's lignin, respectively. Generally, the structure of lignin will ingredient of complex phenolic compound which can inhibit the growth of microorganisms (Dong *et al.*, 2011). Plant derived lignin are non-toxic and are known to exhibit rich antioxidant, anti-fungal, anti-cancer, anti-viral activities (Bhat *et al.*, 2009). From other researchers reported that polyphenols inhibit the growth of microorganisms by forming complexes with their enzymes and proteins and can dissolve the microbial membrane or wall; thus polyphenols can penetrate the cell, where they interact and disrupt the permeability barrier of microbial membrane structures (Daglia, 2012; Sikkema *et al.*, 1995). In this connection, the use of natural antimicrobial compounds such as lignin could be a viable alternative to prevent some spoilage of food products.

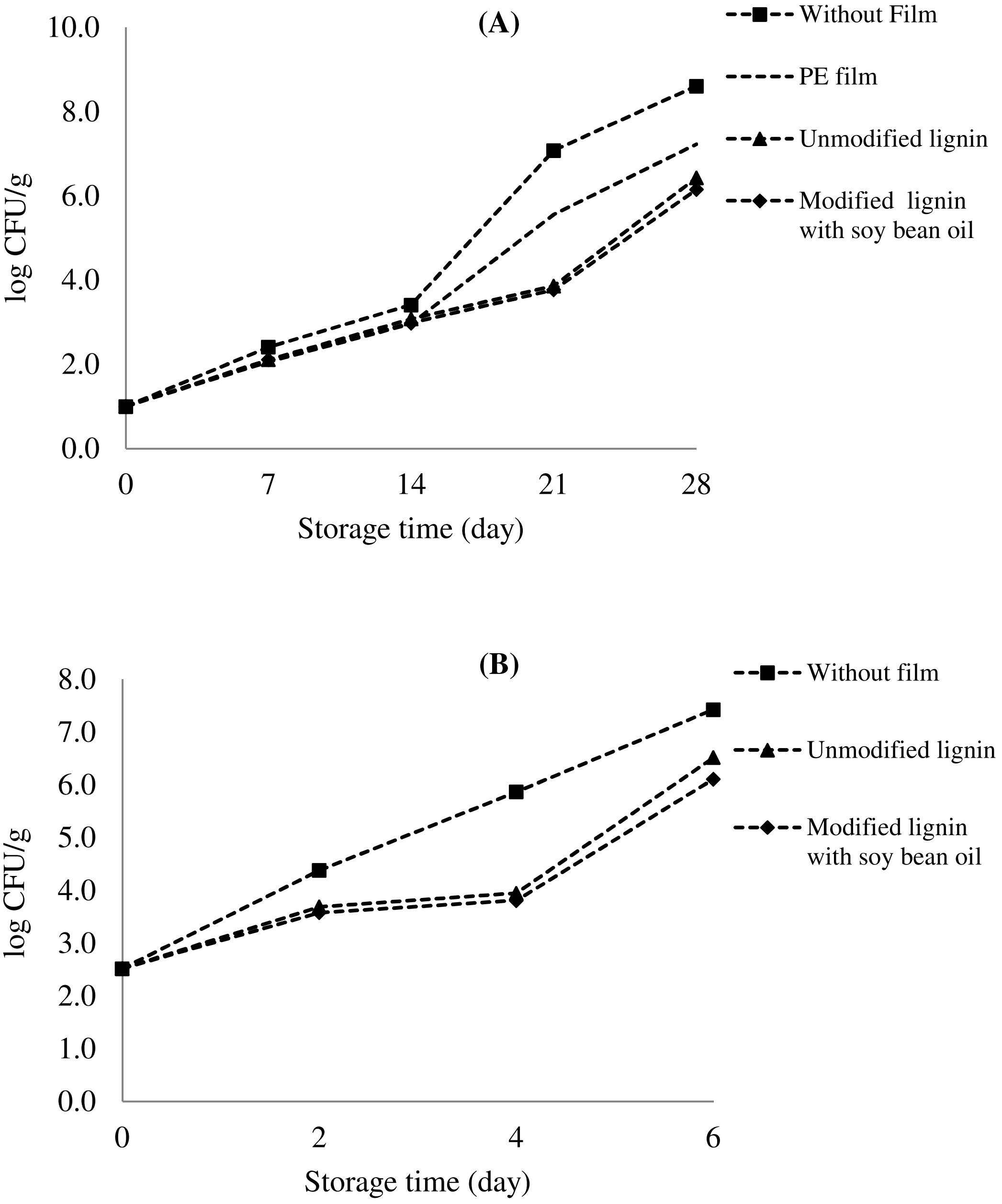


Figure 41 Effect of biodegradable films on TVC of imitated crab meat (A) and bread (B) during storage at 4-6°C and at room temperature, respectively.

7. Soil burial degradation

Biodegradation is a biochemical transformation of compounds by microorganisms and the propensity of a material to get breakdown into its constituent molecules by natural processes. Among various methods of degradation, soil burial method is one of the frequently used methods for the determination of biodegradability of biopolymer films (Yang *et al.*, 2005). Soil micro flora constitute a mixed microbial population (including bacteria, fungi and protozoa, among others) which may act synergistically during degradation and may also reproduce naturally occurring conditions. In addition, microorganisms in the soil produce many types of enzymes that are capable of reacting with exposed protein surfaces (Abrusci *et al.*, 2006). The experiment was carried out up to 28 days. However beyond the 24th day, samples could not be easily recovered due to their macroscopic deterioration presented in Figure 42. Weight loss of films in soil degradation could be taken as an indicator of degradation which shown in Figure 43. Maran *et al.* (2013) reported that weight loss of the films during soil degradation of films were increase because materials are hydrophilic, favors the absorption of water, increases the water activity of films and promotes the growth of microorganisms. The microorganisms which are present in the soil take advantage of the rice starch as a sole source of carbon resulting in partial degradation of films.

Form the results, no lignin, OPEFB's lignin and OPEFB's lignin derivatives at 5% base on starch films which optimum condition were investigated. Weight loss data were used herein only to qualitatively analyze the effect of microbial attack on the lignin-based on rice starch films. After 24 days incubation, the maximum weight loss showed the following tendency OPEFB's lignin derivative (palm oil) ≤ OPEFB's lignin derivative (soy oil) < OPEFB's lignin < no lignin. These results are more likely related to the water absorption capacity of the different films since biodegradation depends on transporting water from the surface to the bulk of the films (Gu, 2003). Control films (no lignin) degrade faster than OPEFB's lignin and OPEFB's lignin derivative based on rice starch films because lignin has the features hydrophobic. However, over time approximately a month all specimens were not able to tested because the films decomposed into homogeneous soil.

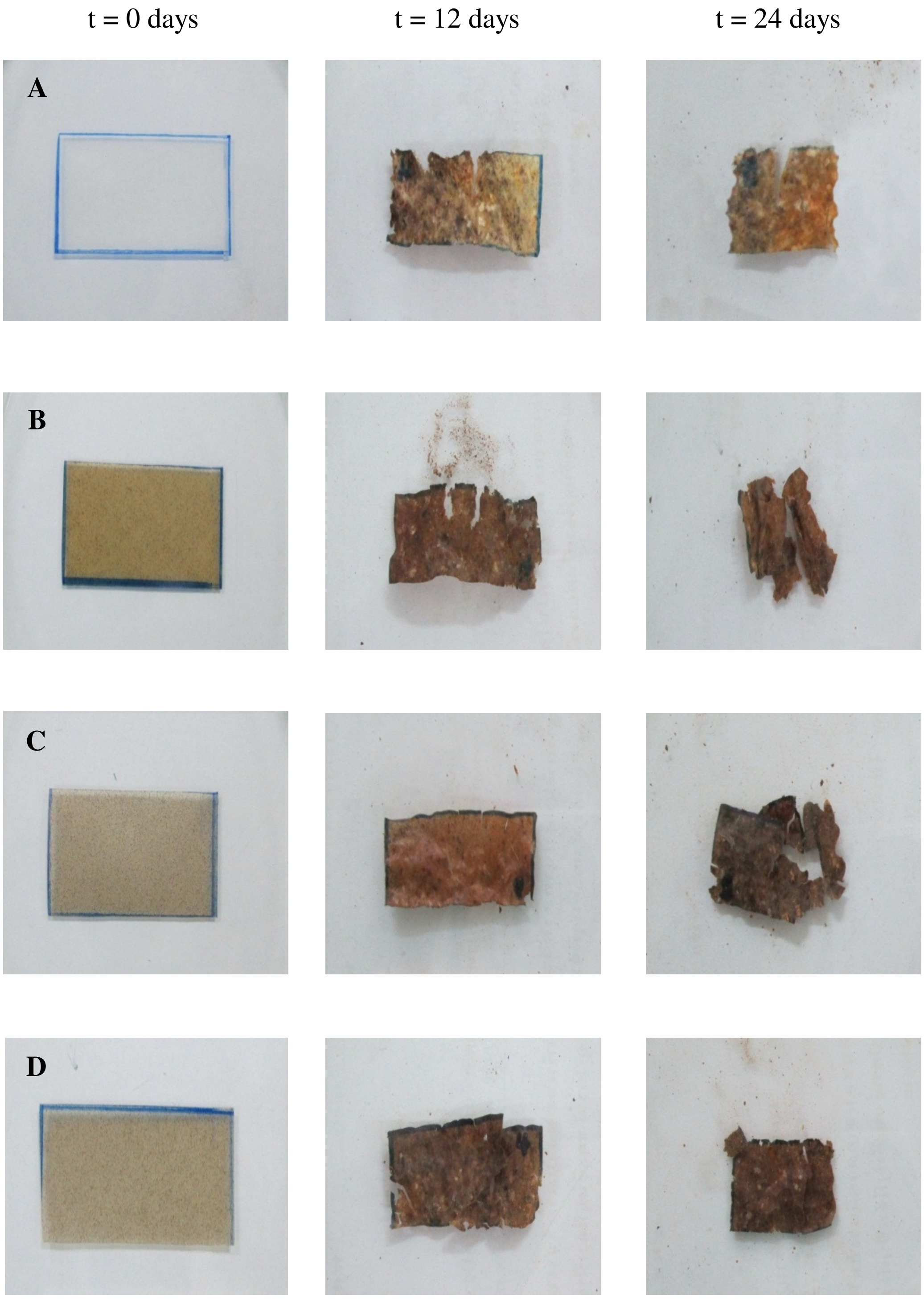


Figure 42 Macroscopic aspects of rice starch films (A=without OPEFB's lignin, B=contained OPEFB's lignin, C= contained OPEFB's lignin modified with palm oil), D= contained OPEFB's lignin modified with soy bean oil) at 0, 12 and 24 days of incubation in soil.

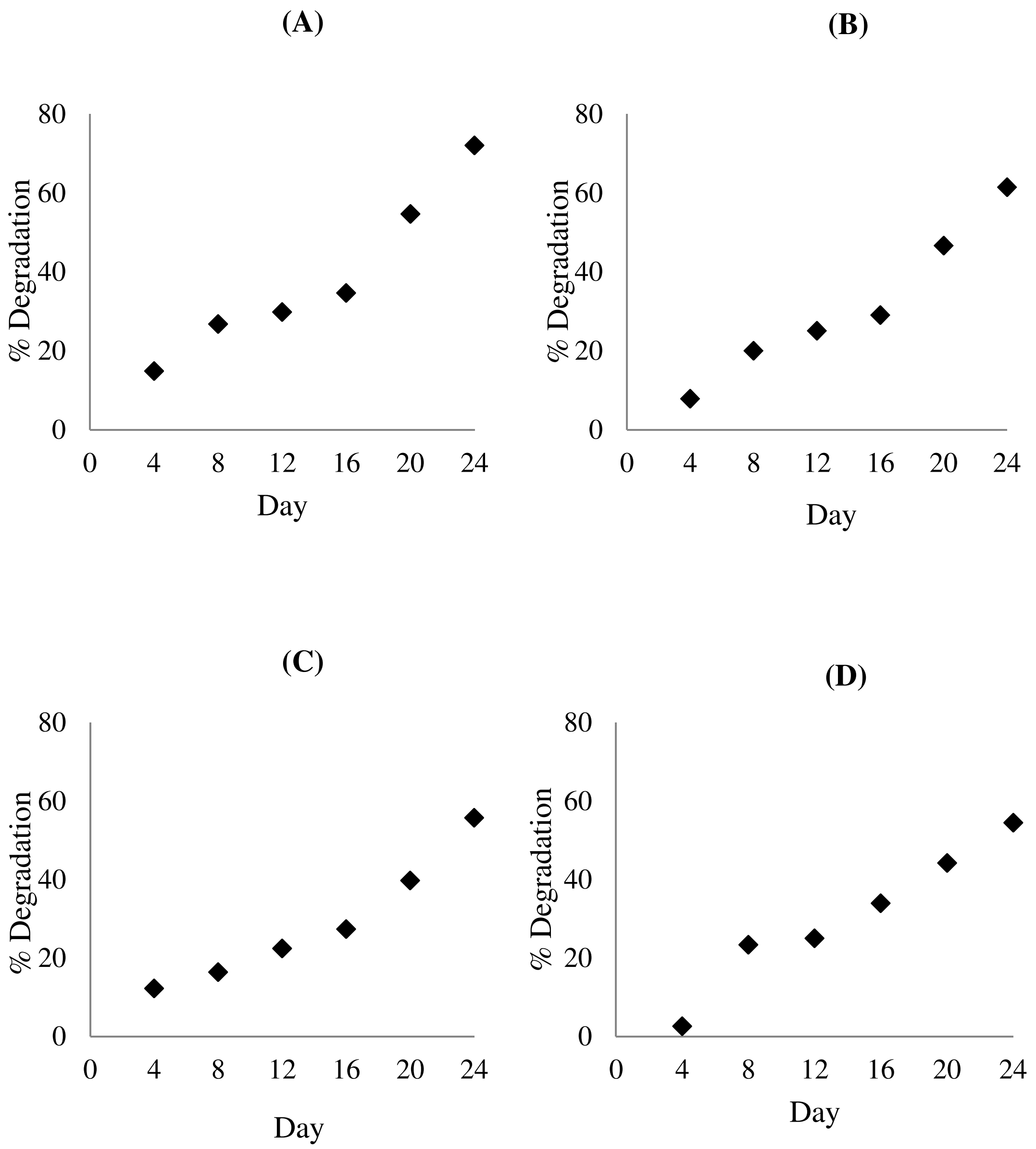


Figure 43 Weight losses in the degradation of rice starch films (A=without OPEFB's lignin, B=contained OPEFB's lignin, C= contained OPEFB's lignin modified with soy bean oil), D= contained OPEFB's lignin modified with palm oil) by soil burial method.

CHAPTER 4

CONCLUSIONS

1. The soluble amylose content of starch is 19.60±0.25% and moisture content is 13.04±0.11%. While OPEFB's lignin contained moisture content as 9.393±0.16 and the % yield of lignin about 14.52 %. The structural of lignin showed the same peaks between OPEFB's lignin and commercial lignin observed from FTIR spectra. OPEFB's lignin had lightness (L^*), redness (a^*) and yellowness (b^*) value more than commercial lignin.

2. The total phenolic content of OPEFB's lignin were about 4.687 ± 0.15 mg GAE/100mg dry weight samples which effected to antimicrobial properties. OPEFB's lignin had inhibitory and killing effect both on gram-negative and gram-positive bacteria in different concentration of each bacterium. The minimum bactericidal concentration (MBC) of OPEFB's lignin at which bacterial growth was completely inhibited was observed as 3.125, 25 and 25 mg/mL for *Staphylococcus aureus,* Escherichia coli O175:H7 and *Listeria monocytogenes*, respectively.

3. Adding of OPEFB's lignin showed significantly improved mechanical properties, thermal properties, film solubility and water vapor barrier of biodegradable rice starch films. However, addition and increasing of OPEFB's lignin content reduced the lightness and transparent of the resulted films. The suitable content of OPEFB's lignin which provided good properties of rice starch films was 5%. However, the antimicrobial properties which inhibit the growth of tested microorganism was noticed when 10% OPEFB's lignin was applied.

4. Adding of OPEFB's lignin derivative showed significantly improves mechanical properties, film solubility, water vapor barrier and contact angle of biodegradable rice starch films when compare with OPEFB's lignin. Addition of OPEFB's lignin derivative into the rice starch films presented a better distribution than OPEFB's lignin. OPEFB's lignin derivative modified with palm oil provided the elongation at break, WVP and contact angle values better than modified with soy bean

oil. However, OPEFB’s lignin derivatives showed similar affected antimicrobial properties with OPEFB’s lignin. The suitable content of OPEFB’s lignin derivative which provided a good mechanical properties and water vapor barrier properties was 5%.

5. Relative humidity affected both mechanical properties and water vapor permeability. Rice starch films reinforced with OPEFB’s lignin derivative had presented better elongation at break and water vapor barrier properties than of rice starch films reinforced with OPEFB’s lignin.

6. Both OPEFB’s lignin and OPEFB’s lignin derivative modified with soy bean oil can extend the shelf-life of imitated crab meat and bread. Rice starch films reinforced OPEFB’s lignin and OPEFB’s lignin derivative modified with soy bean oil can retain quality of imitated crab meat poorer than PE films. Moreover, the OPEFB’s lignin and OPEFB’s lignin derivative modified with soy bean oil can retard the growth of microorganisms at a certain level.

7. Rice starch films, rice starch films reinforced with OPEFB’s lignin and OPEFB’s lignin derivative can degrade by soil burial method in approximately 1 month.

8. According to our results, it could be pointed out that rice starch films reinforced with OPEFB’s lignin and OPEFB’s lignin derivative are a new choice for the application in food packaging.

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APPENDIX

ANALYTICAL METHODS

1. Determination of moisture content (AOAC, 2000)

Method

1. Dry the empty dish and lid in the oven at 105°C for 3 h and transfer to desiccator to cool (30 min). Weigh the empty dish and lid.

2. Weigh about 3 g of sample to the dish. Spread the sample to the uniformity.

3. Place the dish with sample in the oven. Dry for 3 h at 105°C.

4. After drying, transfer the dish with partially covered lid to the desiccator to cool. Reweigh the dish and its dried sample.

Calculation

$$\text{Moisture content (\%)} = \frac{(W1 - W2)}{W1} \text{ x } 100$$

Where W_1 = weight (g) of sample before drying

W_2 = weight (g) of sample after drying

2. Determination of protein content (AOAC, 2000)

Reagents

- Kjedahl catalyst: Mix 9 past of potassium sulphate (K_2SO_4) with 1 past of copper sulphate ($CuSO_4$)
- Sulfuric acid (H_2SO_4)
- 0.2 N HCl solution
- 40% NaOH solution (w/v)
- 4% H_3BO_3 solution (w/v)
- Indicator solution: Mix 100 ml of 0.1% methyl red (in 95% ethanol) with 200 ml of 0.2% bromocresol green (in 95% ethanol)

Method

1. Place sample (0.5 – 1.0 g) in digestion flask.
2. Add 5 g Kjedahl catalyst and 20 ml of conc. H_2SO_4
3. Prepare a tube containing the above chemical except sample as blank. Place flask in inclined position and heat gently unit frothing ceases. Boil briskly until solution clears.
4. Cool and add 60 ml distilled water cautiously.
5. Immediately connect flask to digestion bulb on condenser and with tip of condenser immersed in standard acid and 5-7 indicator in receiver. Rotate flask to mix content thoroughly, and then heat until all NH_3 is distilled.
6. Remove receiver, wash tip of condenser and titrate excess standard acid distilled with standard NaOH solution.

Calculation

$$\text{Protein content (\%)} = \frac{(A-B) \text{ x } N \text{ x } 14.007 \text{ x } 5.95}{W}$$

Where A = volume (ml) of 0.2 N HCl used sample titration

B = volume (ml) of 0.2 N HCl used in blank titration

N = normality of HCl

W = weight (g) of sample

14.007 = atomic weight of nitrogen

5.95 = the protein – nitrogen conversion factor for starch and it's by-products

3. Determination of fat content (AOAC, 2000)

Reagents

-Petroleum ether

Method

1. Place the bottle and lid in the incubator at 105°C overnight to ensure that weight of bottle is stable.
2. Weigh about 3-5 g of sample to paper filter and wrap.
3. Take the sample into extraction thimble and transfer into soxhlet.
4. Fill petroleum ether about 250 ml into the bottle and take it on the heating mantle.
5. Connect the soxhlet apparatus and turn on the water to cool them and then switch on the heating mantle.
6. Heat the sample about 14 h (heat rate of 150 drop/min).
7. Evaporate the solvent by using the vacuum condenser.
8. Incubate the bottle at 80-90°C until solvent is completely evaporated and bottle is completely dried.
9. After drying, transfer the bottle with partially covered lid to the desiccator to cool. Reweigh the bottle and its dried content.

Calculation

$$\text{Fat content (\%)} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \text{ x } 100$$

4. Determination of ash content (AOAC, 2000)

Method

1. Place the crucible and lid in the furnace at 550°C overnight to ensure that impurities on the surface of crucible are burned off.

2. Cool the crucible in the desiccator (30 min).

3. Weigh the crucible and lid to 3 decimal places.

4. Weigh about 5 g sample into the crucible. Heat over low Bunsen flame with lid half covered. When fumes are no longer produced, place crucible and lid in furnace.

5. Heat at 550°C overnight. During heating, do not cover the lid. Place the lid after complete heating to prevent loss of fluffy ash. Cool down in the desiccator.

6. Weigh the ash with crucible and lid when the sample turns to gray. If not, return the crucible and lid to the furnace for further ashing.

Calculation

$$\text{Ash content (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

5. Determination of carbohydrate content

Carbohydrate content (%) = 100 – (Moisture + Protein + Fat + Ash)

6. Determination of amylose content (Iodine method)

Reagents

- Ethanol
- 1 N HCl
- 0.2% Iodine solution
- Standard amylose (Sigma Chemical Co, USA)

Instruments

- Water bath
- Spectrophotometer

Soluble amylose (Shanthy *et al*. (1980))

1. Weigh 100 mg rice starch into 100 ml volumetric flask.
2. Add 1 ml of 95% ethanol and 50 ml of distilled water.
3. Heat the sample at 95°C for 20 min and cool it at 25±2°C and adjust the volume to 100 ml
4. Filter the sample through No.4 filter paper.
5. Add 5 ml from the filtrate into 50 ml distilled water and neutralize with 1 N HCl.
6. Add 2 ml of 0.2% (w/v) iodine solution and make up to 100 ml with distilled water and left at 25±2°C for 20 min.
7. The absorbance at 630 nm for each sample is read against the blank using a spectrophotometer (Ultrospec 4050, LKB Biochrom, UK).
8. Treat the standard amylose similarly to the starch sample, but use 1 ml of dilute solution instead of 5 ml to develop color.

Calculation

$$\text{Soluble amylose (\%)} = \frac{R \times a \times 20}{A \times r}$$

Where R = absorbance of sample

A = absorbance of standard amylose

r = weight of sample (mg)

a = weight of standard amylose (mg)

7. Determination of thiobarbituric acid substance (TBARs) (Buege and Aust, 1978)

Reagents

- TBA solution: 0.375 g of thiobarbituric acid, 15 g of trichloroacetic acid and 0.875 ml of HCl were mixed thoroughly in 100 ml of distilled water.

Method

1. Mix sample (0.5 g) with 2.5 ml of TBA solution.
2. Heat the mixture for 10 min in a boiling water bath (95-100°C) to develop pink color and cool with tap water and centrifuge at 5500 rpm for 25 min.
3. Read the absorbance of the supernatant at 532 nm.
4. Prepare a standard curve with malonaldehyde (dimethyl acetal) at concentration ranging from 0.5 – 8 µg/ml.
5. Calculate the quantity of TBARs in each sample using standard curve as mg of malonaldehyde / kg sample.

8. Total viable count (TVC)

Method

1. Using separate sterile pipets, prepare decimal dilutions of 10^{-2}, 10^{-3}, 10^{-4} and others as appropriate, of food homogenate for sample preparation by transferring 10 ml of previous dilution to 90 ml of diluent.

2. Avoid sampling foam. Shake all dilutions 25 times in 30 cm arc within 7 s.

3. Pipet 1 ml of each dilution into separate, duplicate, appropriately marked petri dishes. Reshake dilution bottle 25 times in 30 cm arc within 7 s if it stands more than 3 min before it is pipetted into petri dish.

4. Add 12-15 ml plate count agar (cooled to 45±1°C) to each plate within 15 min of original dilution. For milk samples, pour an agar control, pour a dilution water control and pipet water for pipet control.

5. Add agar to the latter two for each series of samples. Add agar immediately to petri dished when sample diluent contains hygroscopic materials. e.g., flour and starch. Pour agar and dilution water control plates for each series of samples.

6. Immediately mix sample dilutions and agar medium thoroughly and uniformly by alternate rotation and back-and-forth motion of plated on flat level surface.

7. Let agar solidify. Inverts solidified petri dishes, and incubate promptly for 48±2 h at 35°C. Do not stack plates when pouring agar or when agar is solidifying.

9. A standard curve of malonaldehyde (dimethyl acetal).

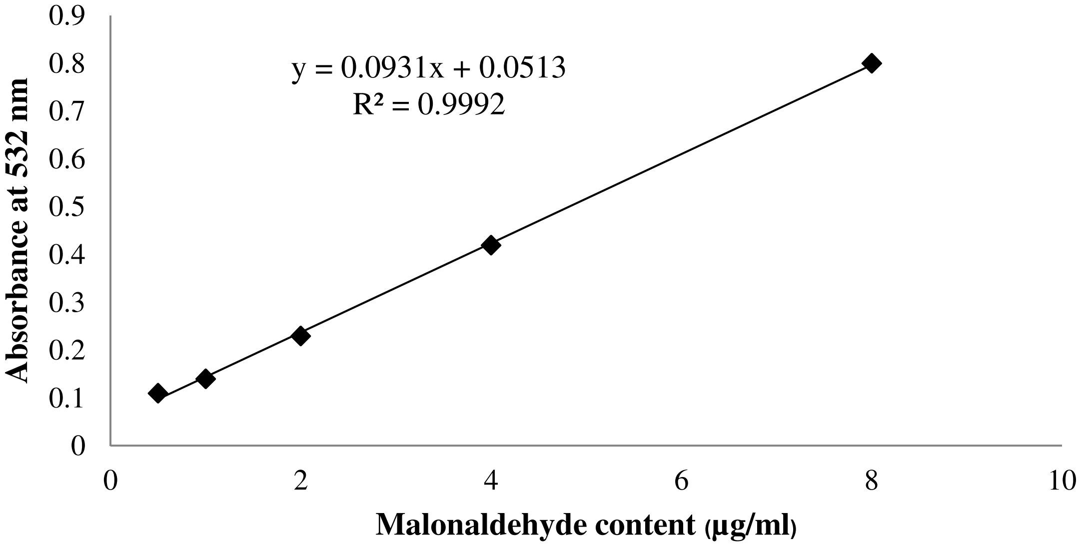

Figure 44 A standard curve of malonaldehyde per ml.

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List of Publication and Proceedings

Proceedings

1. Meethip, B and Wittaya, T. 2014. Properties of biodegradable rice starch films reinforced with oil palm empty fruit bunch’s lignin. The 16th Food Innovation Asia Conference 2014. BITEC Bangna, Bangkok, Thailand. 12-13 June 2014. P. 818-831.