DEVELOPMENT OF BIOPLASTIC COMPOSITE FILMS WITH IMPROVED ANTIMICROBIAL PROPERTIES: A COMPARATIVE STUDY BETWEEN BLOWN FILM EXTRUSION, DOCTOR BLADING AND SPRAY COATING

Mr. Atiwit Singhapan

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การพัฒนาฟิล์มพลาสติกชีวภาพเชิงประกอบที่มีสมบัติต้านเชื้อจุลินทรีย์โดยศึกษาเปรียบเทียบระหว่างกระบวนการอัดรีดแบบเป่าถุง การปาดด้วยใบมีด และการพ่นเคลือบ

นายอติวิชญ์ สิงหพันธ์

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By	Mr. Atiwit Singhapan
Field of Study	Chemical Engineering
Thesis Advisor	Professor ANONGNAT SOMWANGTHANAROJ, Ph.D.

Accepted by the FACULTY OF ENGINEERING, Chulalongkorn University in Partial Fulfillment of the Requirement for the Master of Engineering

.... Dean of the FACULTY OF ENGINEERING
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.... Chairman
(Professor SARAWUT RIMDUSIT, Ph.D.)

.... Thesis Advisor
(Professor ANONGNAT SOMWANGTHANAROJ, Ph.D.)

.... Examiner
(Professor SUTTICHAI ASSABUMRUNGRAT, Ph.D.)

.... External Examiner
(Assistant Professor Wanchai Lerdwijitjarud, Ph.D.)

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พอลิ(3-ไฮดรอกซีบิวทิเรต-โค-4-ไฮดรอกซีบิวทิเรต) เป็นพลาสติกชีวภาพที่ได้รับความสนใจอย่างมากในฐานะทางเลือกที่เป็นไปได้สำหรับพลาสติกที่ผลิตจากปิโตรเคมี เนื่องจากสมบัติต้านการซึมผ่านและเสถียรภาพทางความร้อนที่ดีจึงถูกนำมาใช้เป็นฟิล์มบรรจุภัณฑ์ อย่างไรก็ตามพอลิ(3-ไฮดรอกซีบิวทิเรต-โค-4-ไฮดรอกซีบิวทิเรต)ไม่มีสมบัติอย่างอื่นเพิ่มเติม ทางผู้วิจัยจึงเติมผงลิกนินเพื่อเพิ่มสมบัติยับยั้งแบคทีเรียซึ่งเป็นอีกหนึ่งสมบัติที่จำเป็นอย่างยิ่งในฟิล์มบรรจุภัณฑ์ ในงานวิจัยนี้ฟิล์มพลาสติกชีวภาพเชิงประกอบระหว่างพอลิ(3-ไฮดรอกซีบิวทิเรต-โค-4-ไฮดรอกซีบิวทิเรต) และผงลิกนิน ถูกจัดเตรียมได้สำเร็จ โดยขึ้นรูปผ่านกระบวนการอัดรีดแบบเป่าถุง ปาดด้วยใบมีด และการพ่นเคลือบ เพื่อให้ฟิล์มมีพลังงานพื้นผิวที่สูงและสามารถยึดติดกับสารละลายลิกนินได้เป็นอย่างดี พื้นผิวของฟิล์มได้ทำการปรับสภาพพื้นผิวด้วยโคโรนาก่อนทำการพ่นเคลือบ นำฟิล์มพลาสติกชีวภาพเชิงประกอบไปศึกษาสมบัติทางกายภาพ สมบัติทางกล มุมสัมผัสน้ำที่ผิว สมบัติต้านการซึมผ่านของก๊าซและของเหลว สมบัติการต้านรังสียูวี และสมบัติการยับยั้งแบคทีเรีย ด้วยเครื่องมือการวิเคราะห์ต่าง ๆ ผลของลิกนินที่ทำการเติมลงไปนั้นสามารถช่วยปรับปรุงสมบัติต้านการซึมผ่านของออกซิเจนและไอน้ำได้เป็นอย่างดี ในส่วนของสมบัติต้านรังสียูวีพบว่าลิกนินแสดงสมบัติกันยูวีในช่วงความยาวคลื่น 300 ถึง 400 นาโนเมตร เมื่อศึกษาสมบัติการยับยั้งแบคทีเรียพบว่าฟิล์มเชิงประกอบสามารถยับยั้งแบคทีเรียแกรมบวกได้ดี เมื่อเทียบกับแบคทีเรียแกรมลบที่ไม่สามารถแสดงสมบัติการยับยั้งแบคทีเรียออกมาได้ดีมากนัก เมื่อเปรียบเทียบสมบัติโดยรวมพบว่าฟิล์มพลาสติกชีวภาพเชิงประกอบที่ทำการขึ้นรูปด้วยกระบวนการอัดรีดแบบเป่าถุงแสดงสมบัติในด้านการใช้เป็นฟิล์มบรรจุภัณฑ์ได้ดีเยี่ยมเมื่อเทียบกับการปาดด้วยใบมีด และการพ่นเคลือบ

สาขาวิชา	วิศวกรรมเคมี	ลายมือชื่อนิสิต ....
ปีการศึกษา	2565	ลายมือชื่อ อ.ที่ปรึกษาหลัก ....

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KEYWORD: poly(3-hydroxybutyrate-co-4-hydroxybutyrate), lignin powder, blown film extrusion, doctor blading, spray coating

Atiwit Singhapan : DEVELOPMENT OF BIOPLASTIC COMPOSITE FILMS WITH IMPROVED ANTIMICROBIAL PROPERTIES: A COMPARATIVE STUDY BETWEEN BLOWN FILM EXTRUSION, DOCTOR BLADING AND SPRAY COATING. Advisor: Prof. ANONGNAT SOMWANGTHANAROJ, Ph.D.

Poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (P3HB4HB) is a bioplastic that has gained attention as a potential alternative to petroleum-based plastics in film packaging due to its good barrier properties and thermal stability. However, poly(3-hydroxybutyrate-co-4-hydroxybutyrate) has no outstanding properties. Therefore, the researcher added lignin powder to improve its antimicrobial properties, another essential property of the packaging film. This work successfully prepared a composite bioplastic film between poly(3-hydroxybutyrate-co-4-hydroxybutyrate) and lignin powder by forming through a blown film extrusion, doctor blading, and spray coating. In order to give the film a high surface energy and enable it to adhere to the lignin solution on the surface, the film's surface underwent corona treatment before the spray coating process. The composite films were studied with analytical instruments for morphological, mechanical, contact angle, barrier, anti-UV, and antimicrobial properties. The effect of added lignin greatly improves the oxygen and water vapor permeability properties of the film. Regarding anti-UV properties, lignin exhibited UV blocking in the wavelength range of 300 to 400 nm. When studying the antimicrobial property, it was found that the composite film was able to inhibit gram-positive bacteria well. Compared to gram-negative bacteria, which cannot show very good antimicrobial properties. Corresponding to the overall properties, the bioplastic composite film formed by blown film extrusion showed excellent properties as a packaging film compared to the doctor blading and spray coating.

Field of Study:	Chemical Engineering	Student's Signature ....
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Atiwit Singhapan

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Chapter 1

INTRODUCTION

1.1 General introduction

Food spoilage is a big issue in today's economy, as much food is wasted due to microbiological decomposition. Many people may not be aware of how much food spoilage brought on by microorganisms affects us. In addition to microorganisms causing food to spoil and taste unpalatable, some spoilage can be caused by pathogenic bacteria that can have serious health consequences. Most microorganisms can grow in various conditions, where microbial growth results in reduced food quality and a shorter shelf life [1]. Therefore, we are looking for innovations that prevent food spoilage and preserve shelf life.

Packaging films are used in the food industry as they play a critical role in food preservation [2, 3]. Choosing a suitable packaging film for food can improve the food's shelf life, safety, and sustainability. Products such as vegetables, fruits, milk, meat, and seafood are highly perishable. These products require a flexible, sealed barrier that extends shelf life and protects against external factors [4-8]. Most consumers are interested in food that is minimally processed, free of toxins, and safe. This resulted in the development of non-toxic antimicrobial packaging films by combining antimicrobial additives with biopolymer films.

In the food packaging industry, polymer materials are mainly used as the main components in production. Researchers are interested in poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (P3HB4HB), a biodegradable polymer being studied and developed a lot right now. It is appropriate for use as a packaging film and extrusion process due to its biocompatibility and ability to be adjusted from highly crystalline plastic to elastic rubber by adjusting the ratio of 4HB to 3HB. However, poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (P3HB4HB) has no antimicrobial properties. Therefore, the researcher is interested in improving the antimicrobial property of food packaging by using an antimicrobial filler in the packaging film. Lignin is an attractive choice due to its antimicrobial properties and various benefits, such as oxygen barrier and UV protection. Moreover, lignin is a readily available natural biomass, resulting in great interest in its use as an additive in food packaging [9].

Most polymeric materials are hydrophobic and have low surface adhesion properties [10]. For this reason, it is difficult to use polymeric materials for applications where there is a need for adhesion, such as coating, and this limitation makes improving the surface of the polymer before coating very necessary. One of the most widely used pretreatment techniques in the plastics industry to improve surface adhesion is corona treatment, which is superior to other techniques due to its friendliness with the environment and does not affect the internal polymer (bulk polymer). After the polymer material undergoes corona treatment, it will make the surface hydrophilic, and the adhesion ability will increase because polar functional groups are formed on the surface [10]. Therefore, this method seems feasible for efficiently coating substances onto polymeric materials.

As for the film-forming method, the researcher is interested in using blown film extrusion, doctor blading, and spray coating, as these are popular processes for forming packaging films [11]. The antimicrobial properties and other properties of the films formed by these three processes were compared to see what effects the forming process had on their properties.

From various problems, this made the researcher interested in developing poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (P3HB4HB) film to have antimicrobial properties by mixing lignin powder with poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (P3HB4HB), resulting in the prepared composite film that can resist microorganisms. This research hopes to prepare composite films from blown film extrusion, doctor blading, and spray coating processes with antimicrobial properties and other properties important for food preservation.

1.2 Objectives of the research

1.2.1 To develop composite films as films with antimicrobial properties.

1.2.2 To study the effects of different processing methods between blown film extrusion and doctor blading on the properties of the resulting P3HB4HB composite films.

1.2.3 To study the antimicrobial properties of different processing methods between blown film extrusion, doctor blading, and spray coating.

1.3 Scopes of the research

1.3.1 Prepare a composite film between P3HB4HB and antimicrobial agent at appropriate ratios and PET film coated with antimicrobial agent.

1.3.2 Composite films and Coating films were prepared through three processing techniques, blown film extrusion, doctor blading, and spray coating.

1.3.3 Corona Discharge Treatment is used to increase the surface energy of composite films.

1.3.4 Morphological, contact angle, gas barrier, mechanical, anti-UV and antimicrobial properties of all prepared P3HB4HB composite films were studied.

Chapter 2

THEORY AND LITERATURE REVIEW

2.1 Poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (P3HB4HB)

Polyhydroxyalkanoates, often known as PHAs, are biodegradable aliphatic polyesters created by microorganisms without harmful byproducts. PHAs have outstanding processability, biocompatibility, flexibility, and barrier properties. These characteristics make PHAs appropriate for use as films for food packaging. PHAs display a wide range of different physical and mechanical properties, which are dependent on the copolymer composition.

PHAs are classified into three types based on the number of carbon atoms on the monomer side chain: short chain length PHAs (scl-PHAs) (1 to 3 carbon atoms), medium chain length PHAs (mcl-PHAs) (4 to 9 carbon atoms), and long chain length PHAs (lcl-PHAs) (more than 10 carbon atoms) [12, 13]. Depending on the monomer composition and the chain length, PHAs exhibit a wide range of physicochemical properties, from rigid to flexible thermoplastic elastomers. For example, scl-PHAs tend to be hard and brittle, while mcl-PHAs tend to be soft and flexible, with little to no crystallinity [12].

One of the most extensively researched PHAs for use in medical applications and food packaging is poly(3-hydroxybutyrate, or P3HB for short. As a replacement for traditional polymers, P3HB has limited utility due to its brittleness and thermal instability during standard melt processing [14]. An intriguing alternate is poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (P3HB4HB), which is made by copolymerizing P3HB with 4-hydroxybutyrate (4HB) monomers. In its semicrystalline plastic form, P3HB4HB has a 4HB concentration of less than 10 mol%, whereas in its fully amorphous elastomer form, it can reach a 4HB content of more than 40 mol% [15]. When 4HB units are present, the processability and elongation at break tend to increase. However, the 4HB units inhibit the crystallization so that the decrease of the crystallinity degree reduces barrier properties of P3HB4HB [16, 17]. The chemical structure of P3HB4HB is shown in Figure 2.1.

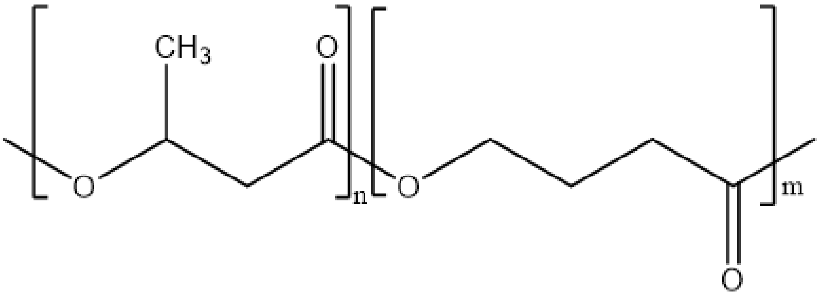

Figure 2.1 Chemical structure of P3HB4HB.

2.1.1 P3HB4HB in food packaging

In addition to its role in the storage of food, the primary purpose of food packaging is to ensure the quality and safety of the food it contains. This means that food packaging must prevent the physical, chemical, and microbial spoilage of food at every stage of its lifecycle, from production to consumption. The properties required for food packaging are listed on Figure 2.2.

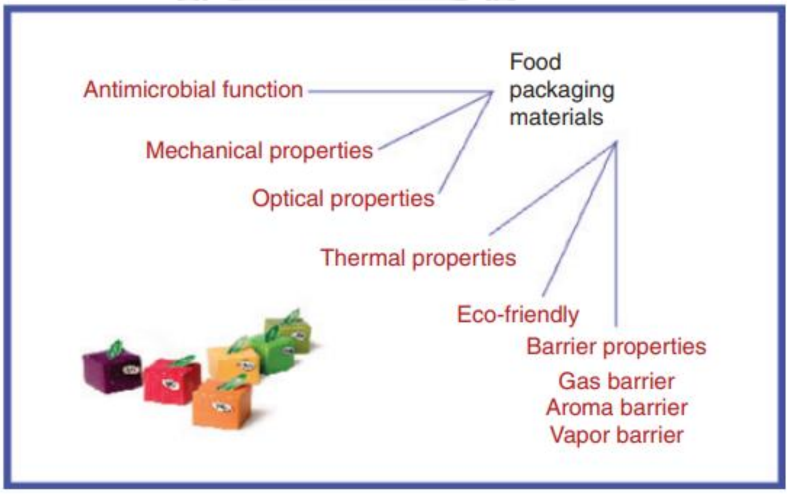

Figure 2.2 Key highlights of biodegradable food packaging [18].

Poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (P3HB4HB) is a type of biodegradable polymer that has gained attention for its potential applications in packaging. Some advantages of P3HB4HB as a packaging material include the following:

1. Biodegradability: P3HB4HB is a biodegradable polymer, which means it can naturally break down into harmless substances by microorganisms in the environment. This property makes it an environmentally friendly option for packaging, as it can reduce the accumulation of plastic waste and reduce pollution.

2. Renewable source: P3HB4HB can be produced from renewable sources such as plant-based feedstocks or waste materials from agricultural or food processing industries. This makes it a sustainable alternative to petroleum-based plastics that are derived from fossil fuels.

3. Barrier properties: P3HB4HB has good barrier properties against oxygen, moisture, and other gases, which can help to protect the packaged contents from spoilage, degradation, and contamination. This makes it an effective option for packaging perishable products or sensitive materials.

4. Mechanical properties: P3HB4HB has good mechanical properties, including flexibility and toughness, which make it suitable for packaging that requires durability and resilience. It can withstand handling, transportation, and storage without compromising the integrity of the packaged contents.

Aversa, Clizia, et al. [19] have studied the effect on barrier properties of the PLA blends containing P3HB4HB, as shown in Table 2.1.

Table 2.1 Composition of the PLA /P3HB4HB blends studied [19].

Sample	PLA (wt%)	P3HB4HB (wt%)
CL10	90	10
CL20	80	20
CL30	70	30
CL40	60	40
CL50	50	50

The oxygen transmission rate (OTR) and water vapor transmission rate (WVTR) analysis results of this work are presented in Table 2.2. The OTR value decreased when the content of P3HB4HB was varied. The improvement of the oxygen barrier could be attributed to the presence of P3HB4HB, which inherently features lower permeability and to the crystallization effect caused by P3HB4HB in PLA. Also, the WVTR value of the plastic films displays a decreasing trend with increasing P3HB4HB content. Likewise, the decrease in WVTR could be attributed to the increased crystallinity and hydrophobic of P3HB4HB.

Table 2.2 The barrier properties test results.

Sample	OTR [cm3 /(m^2.24 h)]	WVTR [g/(m^2.24 h)]
CL10	40.59	9.62
CL20	22.17	4.03
CL30	17.84	3.41
CL40	13.39	2.93

2.2 Lignin

Lignin is a type of aromatic heteropolymer that is cross-linked, and it is one of the components of the plant cell wall, along with cellulose and hemicellulose. The oxidative radical polymerization of coniferyl, sinapyl, and p-coumaryl alcohol, which is involved in the biosynthesis of lignin, is driven by a sequence of enzymes that includes laccases and peroxidases as main actors. This polymerization occurs during the formation of lignin. [20, 21].

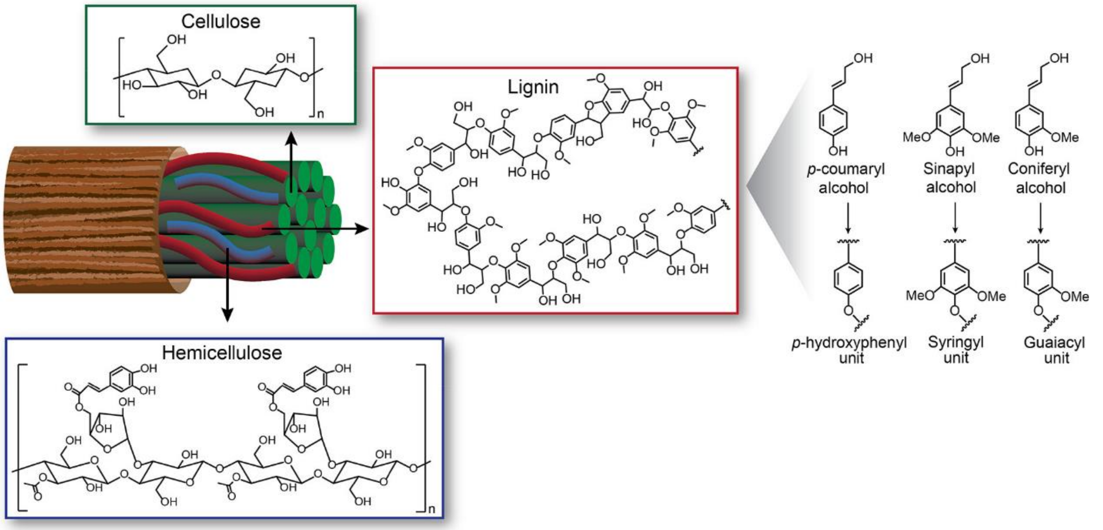

Figure 2.3 A overview of the lignocellulosic biomass structural components [9].

The terms guaiacyl, syringyl, and p-hydroxyphenyl units are used to refer to these structural units once they have been incorporated into the lignin polymer [21]. Figure 2.3 shows the structure of lignin as well as the different building blocks that it is composed of. Because lignin is produced through coupling reactions between phenolic radicals, the molecular weight distribution and concentration of this biopolymer are highly heterogeneous and can vary considerably depending on the plant species [22].

2.2.1 Lignin in food packaging

Incorporating lignin can improve the mechanical and barrier properties of food packaging films, two of the primary drawbacks of biodegradable polymers. In addition, the integration of lignin can give the products and packaging additional features, such as antioxidant and UV-barrier activities, which are extremely important for food preservation (Figure 2.4).

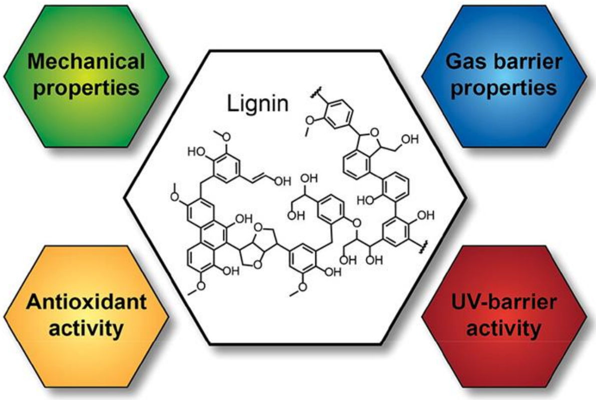

Figure 2.4 Properties of lignin in a biodegradable polymer film [9].

Excellent lignin dispersion and compatibilization efficiency generally enhance the material's mechanical properties. Lignin esterification, cross-linkers, and polymer surface modification are all potential strategies that might be implemented to prevent phase separation and improve the compatibility of lignin with the polymer matrix. [23-26].

One of the primary reasons why food spoilage is because its lipids and proteins have been oxidized. This process alters the appearance, flavor, and odor of food, and it can also result in the production of harmful aldehydes [27]. It is possible to avoid the oxidation of food by incorporating antioxidant chemicals into the materials used for food packaging. These compounds work as radical scavengers and delay the processes caused by radicals. Lignin is an effective antioxidant, which makes it an appealing alternative because it is a safe chemical that is friendly to the environment and natural, making it a great option. The phenolic functional group in the lignin structure, which can act as radical scavengers, is the reason for lignin's ability to possess antioxidant properties. Several studies have shown that the antioxidant activity of lignin is increased when it has a higher phenol content, a lower molecular weight, and narrower dispersities [28]. Lignin can absorb light in the UV range of 200–400 nm because of chromophores inside the structure [29]. This provides various advantages for the food packaging industry, one of which is the ability to protect food from the damaging effects of UV radiation. Because of the brown hue of the lignin, it is essential to keep in mind that the polymer film will lose some of its visual transparency due to the UV protection provided by the lignin. Therefore, optimizing the lignin content and dispersion inside the polymeric matrix is always essential.

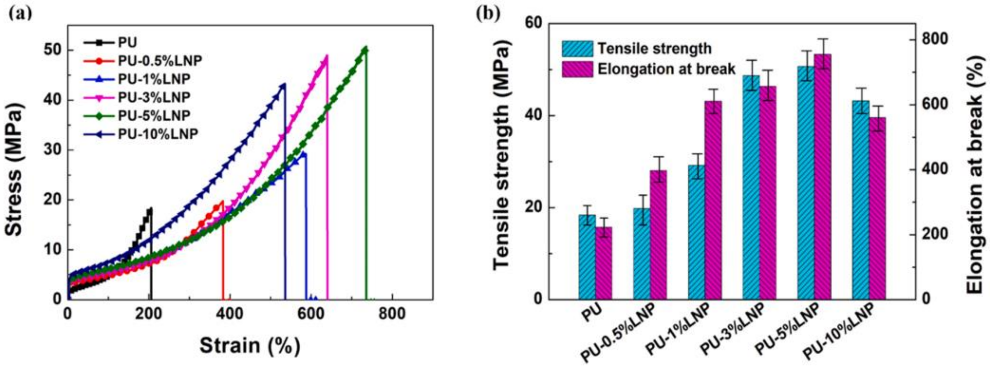

Figure 2.5 (a) Stress-strain curves and (b) tensile strength and elongation at break of PU and PU composite films.

Wu, Liran, et al. [30] have investigated the impact that adding lignin has on the characteristics of the PU film. In order to prepare the lignin nanoparticle (LNP), γ-valerolactone (GVL) was used as the solvent. After that, the casting process produced the composite film. The experimental results in Figure 2.5 (b) showed that, when compared to PU, both the tensile strength and the elongation at break were much more than usual. The tensile strength and the elongation at break in the composite films containing 5%LNP reached their maximum compared to others at 50.7 MPa and 755.8%, respectively.

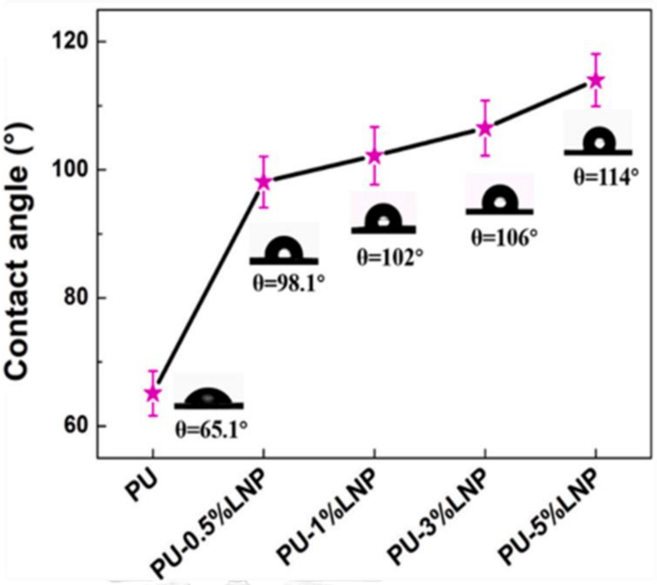

Figure 2.6 Contact angle of PU and composite films.

The surface wettability of the film was studied by measuring water contact angle (WCA). As can be seen in Figure 2.6, the pure PU film has a WCA value of 65.1° compared to the composite film, which significantly increased to 98.1° when 0.5% LNP was added. Additionally, the water contact angle of composite films increased slowly with increased LNP content as the composites were developed. The composite film with a 5% LNP component obtained the highest WCA value of these composites. These results show that lignin could enhance the hydrophobicity properties of the film.

Also, this research discovered that the PU film had a high level of transparency in the UV light region; however, when LNP was added to the PU film, it could block most of the UV spectrum. Based on the results, the functionality of PU-LNP composite films steadily improved gradually.

Boarino, Alice, et al. [31] have investigated the effect of lignin, which improves the mechanical and barrier properties of PLA. They compare and assess three distinct approaches to the formation of a film: PLA blends which contain lignin; lignin nanoparticles; and PLA-grafted lignin nanoparticles (PLA-LNPs).

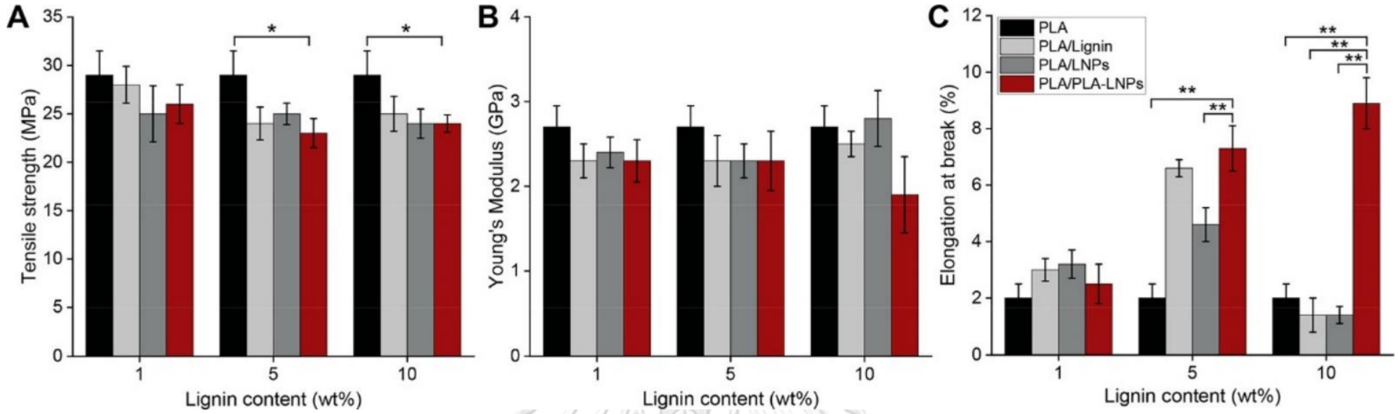

Figure 2.7 Mechanical properties of pure PLA and composite films with different lignin content: (A) tensile strength, (B) Young’s modulus, and (C) elongation at break.

As shown in Figure 2.7, when compared to that of neat PLA, both the tensile strength and Young's modulus of the blends were slightly reduced. The elongation at break of the composite films increased when 1 wt% lignin was introduced, and it grew even further when the lignin content was raised to 5 wt% across all three types of blend films. Despite this, increasing the lignin to 10 wt% decreased elongation at break for both the PLA/lignin and PLA/lignin nanoparticle blends. This was most likely due to phase separation and the low integration between the lignin and PLA. In contrast, PLA-LNPs showed further elongation at break when the lignin content was increased from 5 to 10 wt%. This can be explained by the existence of grafted PLA chains, which serve as the basis of compatibilizers in the material.

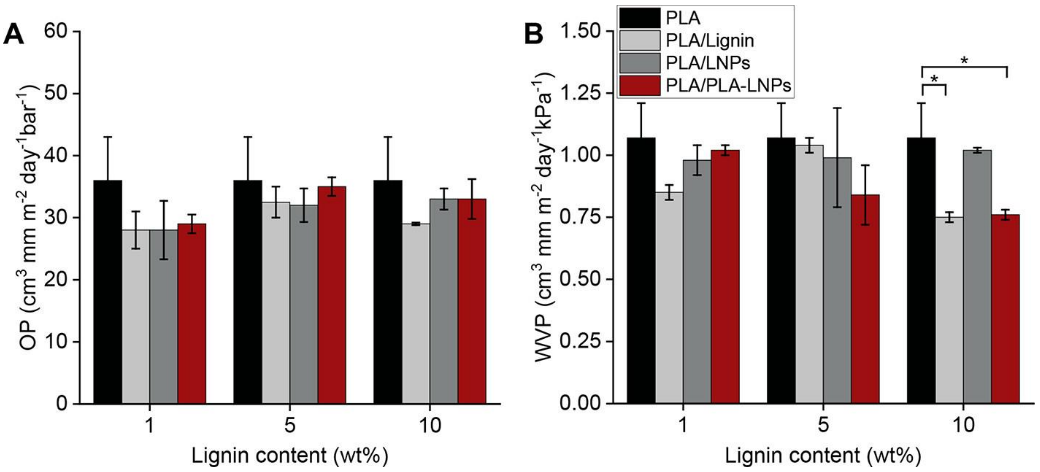

Figure 2.8 (A) oxygen permeability (OP) and (B) water vapor permeability (WVP) of pure PLA and composite films.

A comparison was made between the oxygen and water vapor permeability (Figure 2.8) of the various PLA/lignin blend films and the permeability of pure PLA. These results, provided in Figure 2.8 (A) demonstrate that the addition of lignin did not substantially impact the oxygen permeability of the PLA films. In contrast, the results of a comparison of the water vapor permeability show that blends have a somewhat lower water vapor permeability when compared to pure PLA. However, there is a substantial decrease in water vapor permeability for films that include lignin or PLA-grafted nanoparticles when the lignin component is 10 wt%.

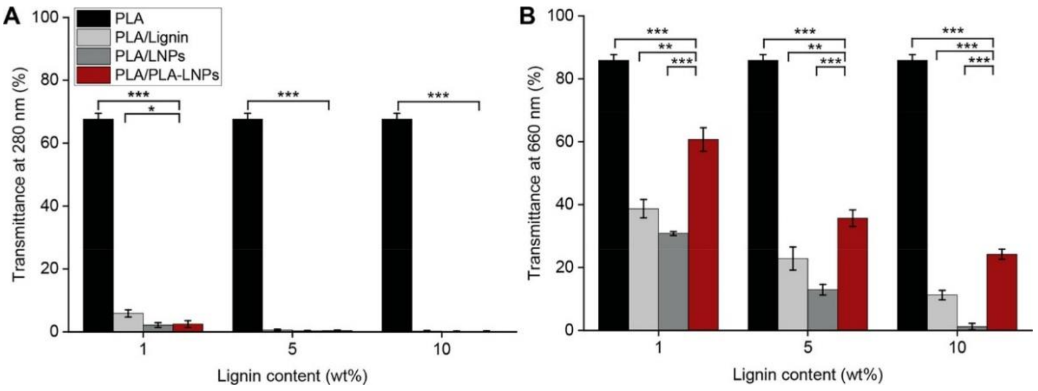

Figure 2.9 Transmittance (%) of pure PLA and composite films with different lignin content, recorded at (A) 280 nm and (B) 660 nm.

The transmittance of PLA blend film was studied in two wavelengths of light at 280 nm and 660 nm (Figure 2.9). According to these spectra, PLA is highly transparent in UV and visible light regions, with high transmittance at wavelengths above 280 nm. In contrast, as shown in Figure 2.9 (A), mixing PLA with 1 wt % lignin affects the UV light region at 280 nm. Increasing the lignin concentration of the blends to 10 wt% almost completely blocks the UV region. In Figure 2.9 (B), PLA and PLA/PLA-LNPs showed more transparency in the visible range than PLA/LNPs or PLA/lignin, which reflects the uniform distribution of the PLA-LNPs in the PLA matrix.

2.2.2 Lignin as antimicrobial Agent

Lignin has recently acquired a lot of attention due to the fact that it is easy to acquire, does not cost very much, and demonstrates some fascinating antimicrobial functions. The natural ability of lignin to shield plants from the attack of pathogens is the source of this compound's antibacterial effect [32].

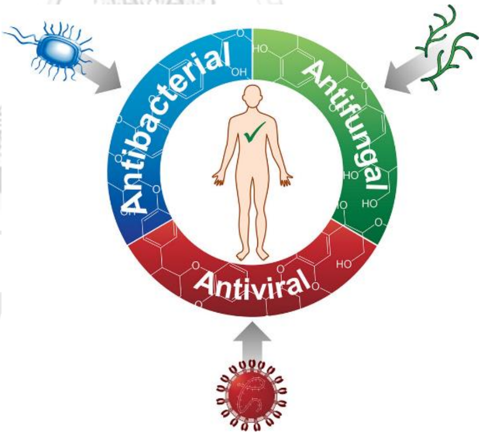

Figure 2.10 Lignin antimicrobial action [9].

Antimicrobial, antifungal, and antiviral properties have been attributed to the technical lignin that have been extracted from lignocellulosic biomass and used in various investigations in the fields of biology and medicine (Figure 2.10). Table 2.3 provides some examples of research that has been conducted to explore the antimicrobial activity of different lignin when they are dissolved in a solvent.

Table 2.3 Examples of research examining the antibacterial effects of lignin in solution [9]

Types of lignin	Solvent	Conc. (mg/mL)	Microorganisms	Reference
Bacteria				
1. kraft lignin	DMSO	15	E. coli, S. aureus, Pseudomonas, Salmonella and Bacillus	[33]
2. pyrolytic lignin	DMSO	5	E. coli and S. aureus	[34]
Fungi				
1. organosolv/ Kraft lignin	DMSO	1–20	A. niger	[35]
2. organosolv lignin	DMSO	0.48–0.025	Candida species and Aspergillus species	[36]
3. organosolv lignin	DMSO	0.5, 5, 10	Candida species and Aspergillus species	[37]

Lignin's antimicrobial activity is almost always related to the phenolic hydroxyl groups, which can cause damage to the bacterial cell membrane and ultimately result in the lysis of the bacteria [38, 39]. The effectiveness of lignin as an antimicrobial agent varies with and is dependent on the kind of bacteria being targeted.

Bužarovska, A.,et al. [40] studied the antimicrobial properties tested for gram-positive and gram-negative bacterial strains using PLA/alkali lignin films prepared from the solution casting technique as a sample. As can be seen in Figure 2.11, The PLA containing lignin carried moderate antimicrobial activity against gram-positive bacteria such as *S. aureus* and *E. faecalis*. The PLA with 10% lignin greatly reduced the viability of gram-positive bacteria. However, gram-negative bacteria have a different type of cell wall when compared with gram-positive bacteria, so the films did not demonstrate antibacterial activity when tested on gram-negative bacterial strains.

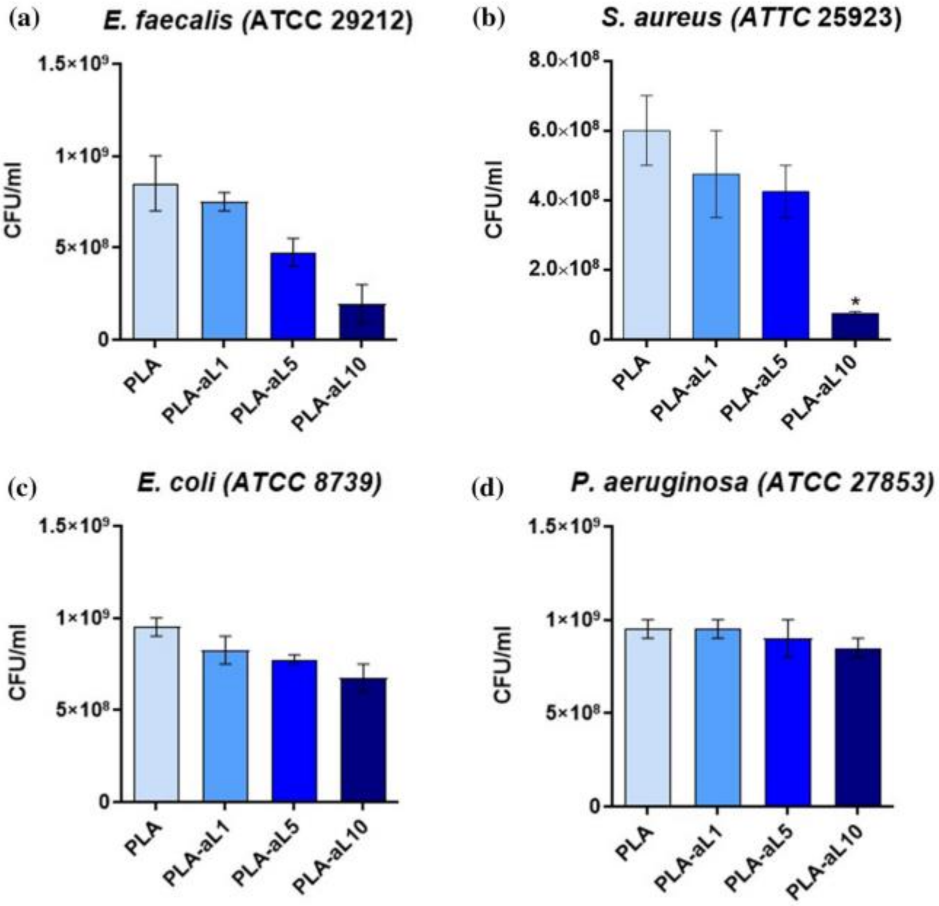

Figure 2.11 Comparison of the antimicrobial activity against (a) E. faecalis (b) S. aureus (c) E. coli (d) P. aeruginosa.

2.3 Surface modification

Most plastic is chemically inert and will not react chemically with other substances due to its low surface tension. As a result, the plastic is unable to adhere well to various coatings, adhesives, and inks. Therefore, surface modification is very important when coating or printing for better adhesion properties. In most cases, surface treatment is often used in coatings, laminates, etc. There are currently three ways of surface modification [41-43].

1. Corona treatment
2. Flame treatment
3. Plasma treatment

2.3.1 Corona treatment

The use of corona (corona treatment) is a simple process, and it is a continuous production process. Using a high voltage and a frequency of about 1-100 kHz, the adhesion efficiency depends on the surface energy. The plastic's surface energy must be higher than the surface energy of the substance to be coated to make the adhesion occur better [41, 42].

The corona is caused by ionizing air that is ionized by electricity. The corona charge is discharged to the surface of the polymer or plastic, which improves the chemical bonding on the surface. However, surface modification using corona only occurs on the surface of the plastic, about 0.01 microns in depth, which will not affect the strength of the plastic.

2.3.2 Flame treatment

This method will increase the bonding ability of the material by oxidizing the surface through the mechanism of free radicals. As a result, the chain was cut short. and bonding links oxidation produces hydroxyl groups, carbonyl, and amide groups at a depth of 4–9 nm. In addition, this method resulted in higher surface tension and better wettability.

2.3.3 Plasma treatment

It is a method to increase the adhesion to the surface of a material by bombarding the particles on the surface with gas ions such as argon (Ar), helium (He), nitrogen (N_2), and oxygen (O_2) at low pressure. As a result, the surface of the material has the ability to wet the surface more and facial skin is more sensitive to reactions.

Chapter 3

EXPERIMENTS

3.1 Materials

To improve the antimicrobial properties of P3HB4HB, lignin, organosolv was supplied from Chemical Point in powder form. For P3HB4HB polymer, Ecomann Thermoplastics Resin EM10080 (density 1.26, melt flow rate (MFR) of 2.5 g/10, melting point 150 °C) was supplied from Ecomann. Chloroform was supplied by RCI Labscan. γ-valerolactone (GVL) was purchased from Sigma-Aldrich. All materials were consumed as received. Deionized (DI) water was also used in all the experiments.

3.2 Preparation of Lignin powder

This study used commercially available powders. Lignin powder was granulated by lightly grinding with an alumina mortar and pestle and sieving with a mesh of 53 microns in a sieve shaker. After that, Lignin powder was dried in an oven at 80 °C for 12 h before being characterized in the next step.

3.3 Lignin powder characterizations

3.3.1 Morphology analysis

The surface morphology of lignin powder was observed by field emission scanning electron microscopy (FE-SEM) (Quanta 250 FEG, FEI, Czech Republic). The samples were prepared onto carbon tape and coated with gold, operating at 15 kV. Digital images were captured at 2000x.

3.3.2 Thermogravimetric analysis (TGA)

The thermal stability of lignin powder was analyzed by a thermogravimetric analyzer (TGA 1, Mettler-Toledo, Thailand). The mass of the lignin powder is about 14 mg, and it was analyzed with a temperature range of

25 to 800 $^{\circ}$C at a heating rate 10 $^{\circ}$C/min under a nitrogen atmosphere with a constant N_2 purge gas flow (50 mL/min).

3.3.3 Differential scanning calorimetry (DSC)

The thermal behavior of lignin powder was analyzed by a differential scanning calorimeter (DSC 1, Mettler-Toledo, Thailand). The DSC was carried out with 5 mg of lignin powder under a nitrogen atmosphere with a constant N_2 purge gas flow (50 mL/min). The temperature program contained three stages: heating from 25 to 250 (heating rate 10 $^{\circ}$C/ min), quenching from 250 to -10 $^{\circ}$C (heating rate 10 $^{\circ}$C/min), and finally heating from -10 to 250 °C (heating rate 10 $^{\circ}$C/min).

3.4 Preparation of P3HB4HB/Lignin blends

P3HB4HB pellets and lignin powder were dried at 80 °C, for 12 h before use. P3HB4HB/Lignin blends were fabricated through a melt processing technique using a twin-screw extruder (diameter [D] = 40 mm; length to diameter ratio [L/D] = 26; LabTech Engineering Co. Ltd., model LE40-26, Thailand) with a temperature profile of 130, 130, 140, 140, 145, 145, 150, 150, 145 and 145 °C from the feeder to the die. The screw speed was 50 rpm. After that, it was pulled through the water to be cut with a pelletizer. The polymer blends were prepared with different amounts of lignin, as shown in Table 3.1.

Table 3.1 Compositions under study.

Sample name	P3HB4HB (wt%)	Lignin (wt%)
P3HB4HB	100	0
P3HB4HB/L0.5	99.5	0.5
P3HB4HB/L1	99	1

3.5 Preparation of P3HB4HB/Lignin composite films

3.5.1 Blown film extrusion

In this step, the polymer blend is formed into a film. The first step was to put the prepared polymer blend pellets into an oven at 80 °C for 12 h. Then the polymer blend pellets were blown into a film using a single screw extruder (Collin, Blown film line BL 180/400E, Germany) with the temperature in the barrel set to 130, 140, 145, 150, 130 and 130 °C. The screw speed was 80 rpm. The composite films were rolled through the roll, and the thickness of all collected films was 70 microns.

3.5.2 Doctor Blading

3.5.2.1 Preparation of P3HB4HB/Lignin solution

First, P3HB4HB pellets and lignin powder were dried overnight at 80 °C for 12 h before use. Then, P3HB4HB pellets were dissolved in chloroform at 10 wt % and stirred at room temperature for 12 h. Meanwhile, lignin was also stirred in chloroform (concentration 10 mg/mL) for 12 h. The solutions were then mixed and stirred overnight. The composite films' lignin content varied by adjusting the volumes of the P3HB4HB solution and the lignin dispersions.

3.5.2.2 Preparation of composite films

In this step, the composite films were prepared by doctor blade technique using a P3HB4HB/Lignin solution. The composite films were fabricated by a doctor blade film coater (Tmax Battery Equipments, model TMAX-MS-ZN320B, China) at a speed of 7 cm/min with a fixed doctor blade gap of 0.25 mm at room temperature. The films were left to dry for 24 h at room temperature under the fume hood and finally for 24 h in a vacuum oven at 50 °C. A film with 70 microns in thickness was obtained.

3.5.3 Spray coating

3.5.3.1 Preparation of lignin solution

First, lignin powder was dried overnight at 80 °C for 12 h before use. Then, lignin was dissolved in γ-valerolactone (GVL) at 10 mg/mL for 12 h. After that, the lignin solution was stored in a Duran bottle for use in the next step.

3.5.3.2 Corona treatment

Since plastic films generally have a low surface tension, the plastic cannot adhere well to the coating. Therefore, surface modification is very important in film coating. In this step, the surface of the PET films is pretreated by using a corona treatment which was carried out under room temperature conditions. Before pretreatment, the PET films were wiped clean with distilled water and then dried at room temperature to remove all contaminants. The PET films were placed on the plane anode electrode, setting the gap between the plane and the pointed cathode electrode at 1.5 cm. The pointed cathode electrode was connected with a dc power supply (Matsusada Precision Inc., 0-30 kV adjustable, maximum current 1.33 mA). After that, The PET films were treated by a corona discharge machine (Nuclear Engineering, Chulalongkorn University, Thailand) with an applied voltage of 12 kV and current of 0.30 mA) for about 3 min.

3.5.3.3 Preparation of PET films coated with lignin

In this step, the lignin solution from above was poured into a Flairosol spray bottle. Once the solution was ready, the lignin solution was sprayed four times from a Flairosol spray bottle, distributing the aerosols into the surface of the films. After that, the films were left drying for 24 h at room temperature under the fume hood before being characterized in the next step.

3.6 Film characterizations

In this study, P3HB4HB/Lignin composite films and PET films coated with lignin were analyzed to compare the properties resulting from different processes. All the film's characterization techniques are shown in Table 3.2.

Table 3.2 Composite and coated films characterization techniques.

	Blown Film Extrusion			Doctor Blading			Spray Coating	
	Pure	L0.5	L1	Pure	L0.5	L1	Uncoated	Coated
1. FE-SEM	✓	✓	✓	N/A	✓	✓	N/A	N/A
2. Contact angle	✓	✓	✓	✓	✓	✓	✓	✓
3. OTR	✓	✓	✓	N/A	✓	✓	N/A	N/A
4. WVTR	✓	✓	✓	N/A	✓	✓	N/A	N/A
5. Mechanical	✓	✓	✓	✓	N/A	N/A	N/A	N/A
6. Anti-UV	✓	✓	✓	N/A	✓	✓	✓	✓
7. Antimicrobial	N/A	N/A	✓	N/A	N/A	✓	N/A	✓

3.6.1 Morphology analysis

The surface morphology of composite films was observed by field emission scanning electron microscopy (FE-SEM) (Quanta 250 FEG, FEI, Czech Republic). The samples were prepared onto carbon tape and coated with gold, operating at 5 kV. Digital images were captured at 5000x.

3.6.2 Water contact angle analysis

The surface wettability of composite films was measured using a contact angle instrument (DMe-210, Kyowa, Japan). The samples were cut into 3 cm x 3 cm and placed on a glass slide. Then, 3 μL of deionized water was dropped on the surface of the films. The contact angle was analyzed using

FAMAS software. All surface contact angle values of composite films were reported as the average values of at least five measurements at different positions on the sample surface.

3.6.3 Barrier properties analysis

The oxygen transmission rate (OTR) of composite films was measured by using oxygen permeation analyzer (OX-TRAN 2/21, Mocon, USA) following the ASTM D3985 standard. The test was performed at 23 °C and 0% relative humidity (RH), using a 40 cm^3/min oxygen flow rate. The samples were cut into a circle mold of equipment with a 5 cm^2 surface area. In order to achieve the precise value, four films were tested.

The water vapor transmission rate (WVTR) of composite films was measured by using water vapor permeation analyzer (PERMATRAN-W Model 398, Mocon, USA) at 23 °C and 0% relative humidity (RH) according to ASTM E-398. The samples were cut into a circle mold of equipment with a 5 cm^2 surface area. In order to achieve the precise value, four films were tested.

3.6.4 Mechanical properties analysis

The composite films thicknesses were measured at five points using a Digimatic thickness gauge at random locations on the film sample. The tensile test of all P3HB4HB/Lignin composite films was measured according to ASTM D882 using Universal Testing Machine (Instron 5567, NY, USA) with 70 microns thick film samples cut in the machine direction (MD). For each composition, 10 specimens were prepared and tested.

3.6.5 Anti-UV activity analysis

A UV-Vis Spectrophotometer (SP-UV 300, Spectrum Instruments, Perkin Elmer company, U.K) determined the anti-UV activity of composite films. The samples were cut into 4 cm × 2 cm and placed directly in a spectrometer test cell; the transmittance was measured from 200 to 800 nm. In addition, the film was cut to a size of 2 cm × 3 cm and placed over Chulalongkorn University (CU) logo to observe the transparency of the composite film with the naked eye.

3.6.6 Antimicrobial activity analysis

The antimicrobial activity of composite films was analyzed in a quantitative way by modifying the test for antimicrobial activity and efficacy (JIS Z 2801:2006) of the samples. In brief, the control and sample materials were cut into squares 5 cm x 5 cm and sterilized by UV exposure for 2 h. The tested side was inoculated with 0.4 mL inoculum and placed in a covered petri dish. The samples were incubated at 37 °C and 90% RH for 24 h. After incubation, the colonies on each of the dishes were counted to calculate the number of viable cells. The CFU/mL of each replica was determined, and their mean values were recorded. Each sample was repeated 3 times. The testing method and microorganisms that be used are shown in Table 3.3.

Table 3.3 Testing Method of JIS Z 2801.

Microorganisms	Method	Incubation condition	Inoculum amounts/ Samples size
Staphylococcus aureus *(S. aureus)*	JIS Z 2801 : 2006	37 °C and 90% RH for 24 h	0.4 mL/ 5 cm x 5 cm
Bacillus subtilis *(B. subtilis)*	JIS Z 2801 : 2006	37 °C and 90% RH for 24 h	0.4 mL/ 5 cm x 5 cm
Escherichia coli *(E. coli)*	JIS Z 2801 : 2006	37 °C and 90% RH for 24 h	0.4 mL/ 5 cm x 5 cm
Pseudomonas aeruginosa *(P. aeruginosa)*	JIS Z 2801 : 2006	37 °C and 90% RH for 24 h	0.4 mL/ 5 cm x 5 cm
Candida albicans	JIS Z 2801 : 2006	37 °C and 90% RH for 24 h	0.4 mL/ 5 cm x 5 cm
Aspergillus niger	JIS Z 2801 : 2006	37 °C and 90% RH for	0.4 mL/ 5 cm x 5 cm

(A. niger)		24 h	

Calculation

$$\% \text{ Reduction} = \left(\frac{B - A}{B}\right) \times 100$$

Where:

B = Number of viable microorganisms before test

A = Number of viable microorganisms after test

Noted:

- (% Reduction) = Number of viable test microorganisms after test higher than number of viable test microorganisms before test

Chapter 4

RESULTS AND DISCUSSION

4.1 Characterization of lignin powder

4.1.1 Morphology

The morphology of lignin powder was analyzed by field emission scanning electron microscopy (FE-SEM) at 2000x magnification. It can be seen from Figure 4.1 that lignin powder consists of large agglomerates, which are composed of fine lignin particles and have a polygonal shape. The FE-SEM micrograph of lignin powder shows a particle size distribution of approximately 10 to 30 microns.

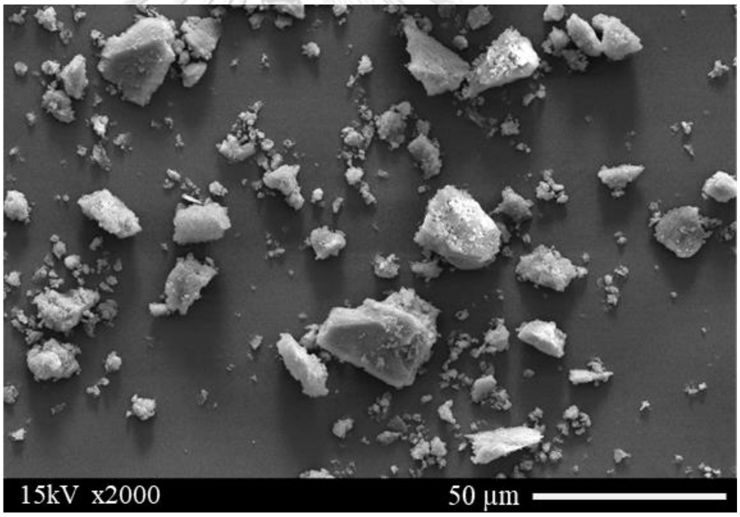

Figure 4.1 SEM image of lignin powder at 2000x magnification.

4.1.2 Thermal stability

The thermal stability of lignin powder was analyzed by thermogravimetric analysis (TGA), and the thermogram is shown in Figure 4.2. The thermal degradation of lignin powder shows two steps of degradation. The first step is in a temperature range of 100 to 125 °C due to the evaporation of absorbed and intermolecular H-bonded water [44]. After 125 °C, the mass remains constant up to 210 °C. However, The second step is the main degradation step in a temperature range of around 210 to 500 °C, which was attributed to the release of aliphatic linkages, aromatic rings, aryl ethers, and esters [45]. After 500 °C, the mass loss continued gradually but weakly compared with the previous steps.

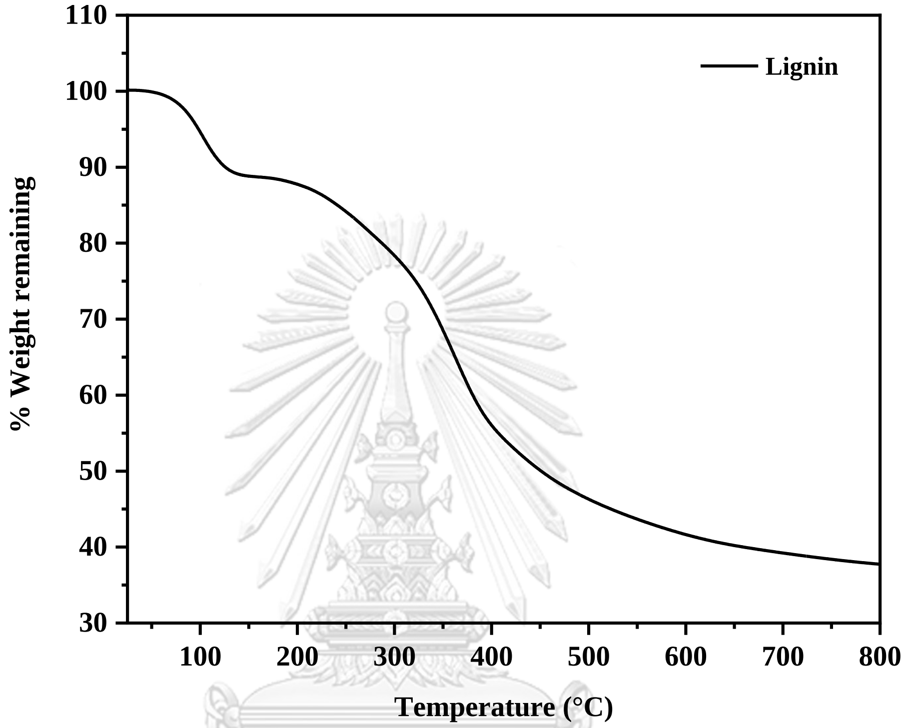

Figure 4.2 TGA thermogram of lignin.

4.1.3 Thermal behavior

The thermal behavior of lignin powder was analyzed by Differential scanning calorimetry (DSC), and the thermogram is shown in Figure 4.3. DSC analysis was used to determine the glass transition temperature (Tg) of lignin, which is needed for the blending of thermoplastic in the extrusion process. This Tg value indicated that this temperature corresponded to the change from a glassy state to a rubbery state. From the thermogram, the glass transition temperature determined for the lignin is 135.3 °C.

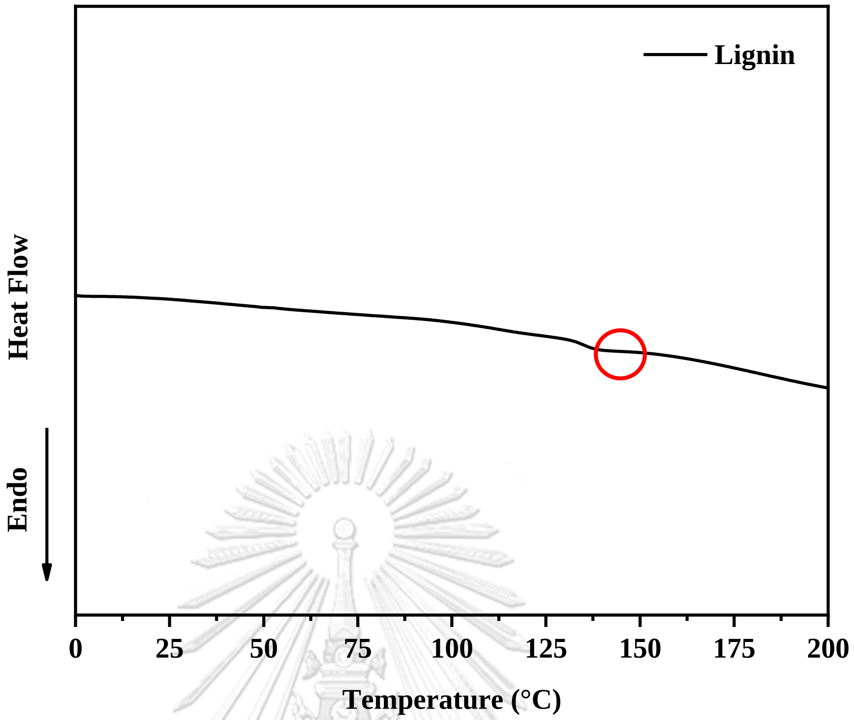

Figure 4.3 DSC thermogram of lignin.

4.2 Characterization of composite film

The characterization results in this work will have four different processes. The researcher will set the films named B-P3HB4HB/LX, C-P3HB4HB/LX, and D-P3HB4HB/LX, where B represents the blown film extrusion, C represents the cast film extrusion, D represents the doctor blading, and X represents the weight percentage of lignin (0.5% and 1%) in the P3HB4HB film.

4.2.1 Morphology

Morphology analysis can be used to investigate how lignin content affects the miscibility of lignin with P3HB4HB. The cross-sectional morphology of P3HB4HB and composite films in the machine direction were analyzed by field emission scanning electron microscopy (FE-SEM) at 4000x and 5000x magnification. It can be seen from Figure 4.4 that when compared between blown film extrusion and doctor blading, the image of P3HB4HB film clearly defined a rough, layered surface with particles that are distributed across the surface. In the doctor blading process, the particles are large ellipsoids distributed over the surface because of blade movement. However, the blown film process makes the particles smaller and more spherical. These spherical particles were found to be less with a decrease in P3HB4HB content, respectively. This is because the material is subjected to high shear forces during the extrusion process due to the movement of the screw or the extrusion die. These shear forces can cause mechanical breakdown and fragmentation of the particles present in the material. The extrusion process also involves heating the material to a molten state, making it more susceptible to particle size reduction. When lignin is added to P3HB4HB, the fracture surfaces of FE-SEM images of P3HB4HB/L0.5 and P3HB4HB/L1 of both blown film and doctor blading look the same. There were no detectable voids or indications of separation of the phase into 2 phases. The results indicate that the low lignin content was well distributed in P3HB4HB.

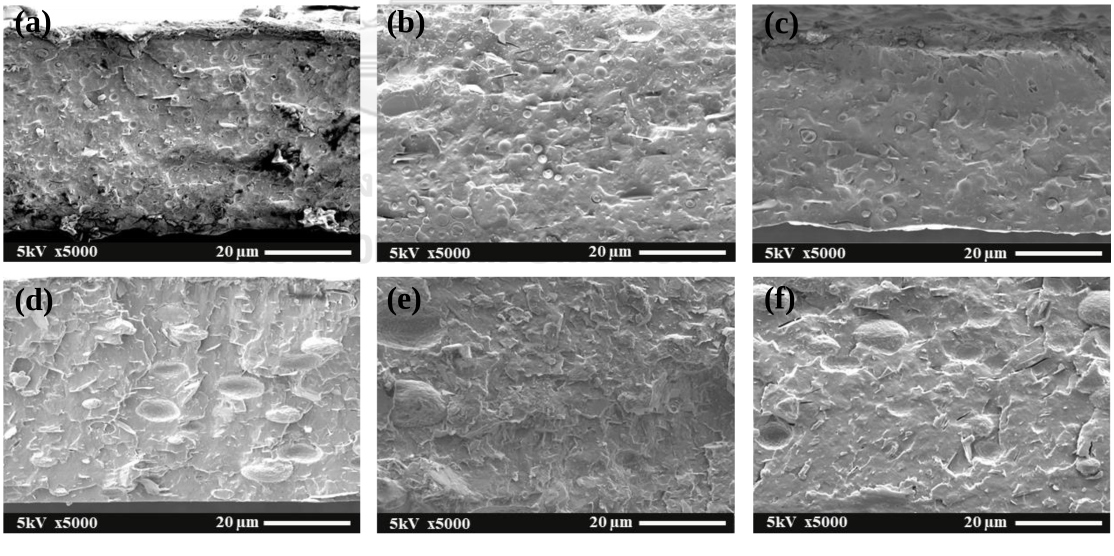

Figure 4.4 SEM images of P3HB4HB and composite films: (a) B-P3HB4HB (b) B-P3HB4HB/L0.5 (c) B-P3HB4HB/L1 (d) D-P3HB4HB (e) D-P3HB4HB/L0.5 (f) D-P3HB4HB/L1

4.2.2 Water contact angle

The surface wettability of P3HB4HB and composite films were determined by measuring the water contact angle (WCA), which was also used to estimate the hydrophilicity or hydrophobicity of a film surface. The results are displayed in Table 4.1, as the WCA value of pure P3HB4HB film that formed from the blown film extrusion process was only 97.4° when compared with the doctor blading process because blown film extrusion had higher surface energy. As the film is stretched during the process, the polymer chains within the material align and become oriented in a specific direction. This molecular orientation increases the surface energy because the aligned chains create a more ordered and exposed surface. The WCA value of pure P3HB4HB from the doctor blading process was 103.5°, which can be attributed to chloroform making the films more hydrophobic. The WCA value of the composite film increased significantly in both processes after L0.5 and L1 were added into the film. These findings demonstrated the composite film's improved hydrophobic performance, primarily due to the lignin in the P3HB4HB film.

Table 4.1 WCA of P3HB4HB and composite films compares with different processes.

Samples	WCA (°)	
	Blown Film Extrusion	Doctor Blading
P3HB4HB	97.4 ± 3.2	103.5 ± 1.5
P3HB4HB/L0.5	98.4 ± 1.6	104.7 ± 1.0
P3HB4HB/L1	101.5 ± 1.8	105.3 ± 2.3

In the next part, when comparing PET film treated and untreated as a result of Table 4.2, it can be seen that PET treated by corona discharge has a WCA value that gradually decreased from 72.4° to 56.0°, because the surface energy of the film increased due to surface modification from corona treatment and also improved the hydrophilicity of the PET film, This is to make the surface of the film to be coated with lignin have good adhesion. When lignin was sprayed onto the film surface, the composite film's WCA value improved in all untreated and treated films, similar to the previous two processes above.

Table 4.2 WCA of PET film both uncoated and coated with lignin solution.

Samples	**WCA (°)**	
	Uncoated	**Coated lignin**
PET untreated	72.4 ± 1.7	75.8 ± 2.0
PET treated	56.0 ± 2.6	80.0 ± 2.7

4.2.3 Barrier properties

Essential for extending the shelf life of food by delaying oxidation and spoilage are barrier properties that prevent the entry of water vapor and oxygen. The results of the oxygen transmission rate (OTR) of P3HB4HB and composite films are shown in Figures 4.5. It can be seen that pure P3HB4HB film from blown film extrusion has an OTR value less than doctor blading due to the stretching and aligning its polymer chains in the machine and transverse direction [46]. This alignment reduces the gaps and pathways through which gases or liquids can pass, resulting in lower permeability. As a result, the film becomes more effective at blocking the passage of oxygen and moisture. When L0.5 and L1 were incorporated into the film, the OTR value of composite film that formed blown film extrusion was a similar decrease from 3532.77 to 1259.74 and 1251.69 cc/m².day, respectively. Also, in the doctor blading process, the OTR value of the composite film was significantly decreased because the lignin particles that made the diffusing oxygen molecules followed more tortuous pathways to diffuse through the composite films [47]. As a result, a significant increase in gas barrier performance occurred.

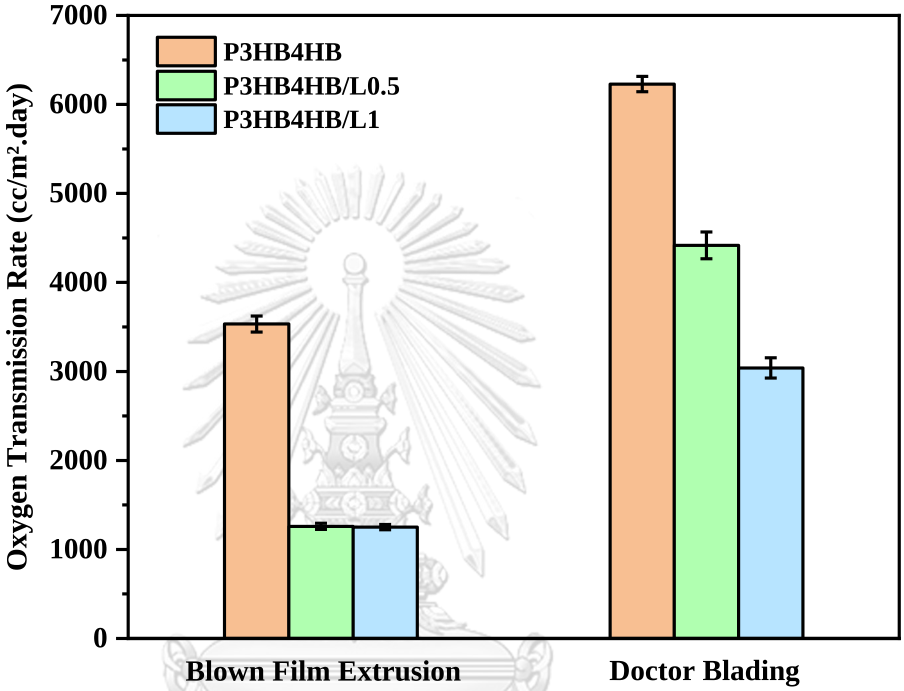

Figure 4.5 Oxygen transmission rate (OTR) of P3HB4HB and composite films compares with different processes.

Next, the results of the water vapor transmission rate (WVTR) of P3HB4HB and composite films are shown in Figures 4.6. It can be seen that pure P3HB4HB film from doctor blading process has a WVTR value lower than blown film extrusion because of the film's hydrophilicity, which affects its interaction with water molecules. Hydrophilic materials tend to have a higher WVTR as they attract and absorb water vapor more readily, allowing it to pass through the material. In contrast, hydrophobic materials repel water vapor and exhibit lower WVTR. As a result, in Table 7, doctor blading has the highest WCA value compared to blown film extrusion. When L0.5 and L1 were incorporated into the film, the WVTR value of composite film formed by blown film extrusion and doctor blading process decreased because lignin has many OH functional groups, the OH functional groups usually cause increased water absorptivity (WA), while WA is proportional to WVTR [48]. The decreased WVP values with increased lignin in the result shown in Figure 16 might be because water molecules are trapped due to hydrogen bonding with OH groups [49].

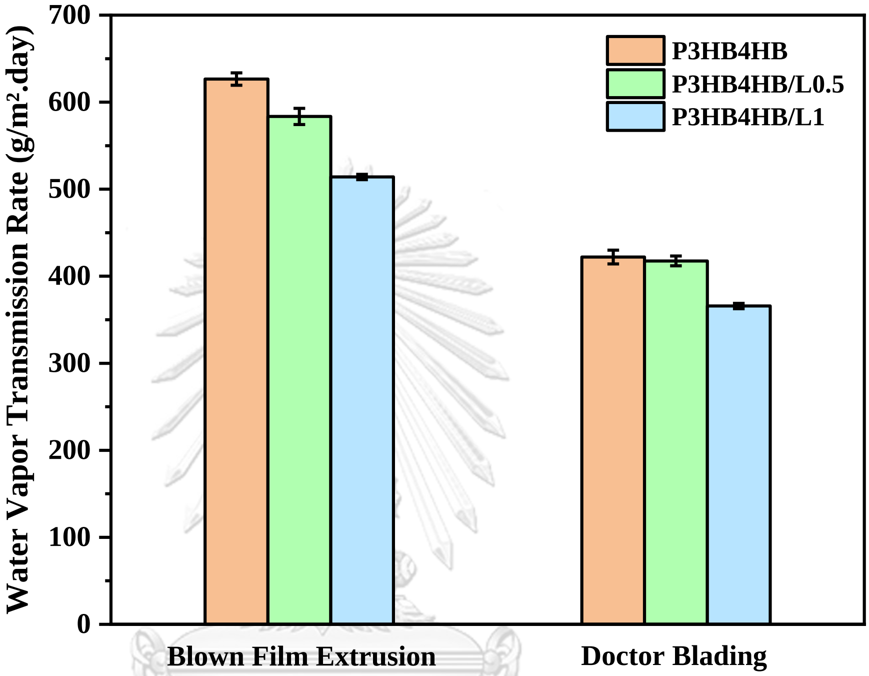

Figure 4.6 Water vapor transmission rate (WVTR) of P3HB4HB and composite films compares with different processes.

4.2.4 Mechanical properties

Mechanical characterization of P3HB4HB and composite films were conducted on samples in the machine direction, and the mechanical properties are presented in Figure 4.7. As shown in Figure 4.7 (a), the stress-strain curves of the P3HB4HB exhibited an elastic region, clearly indicating the ductility of the materials. When L0.5 and L1 were incorporated into the film, the behavior of the composite film changed to more brittle. In Figure 4.7 (b), the composite film's modulus and tensile strength significantly decreased with the increase of lignin content from 71.19 to 49.96 and 5.70 to 3.63, respectively. The elongation at break slightly decreased from 69.92 to 37.60

when 0.5 wt % filler was incorporated and even further upon increasing the lignin content. This also resulted in toughness due to lignin obstructing chain mobility and reducing flexibility. Therefore, adding low lignin content induces weaker mechanical properties than the pure polymer matrix because lignin hinders the long-range ordering of P3HB4HB molecules.

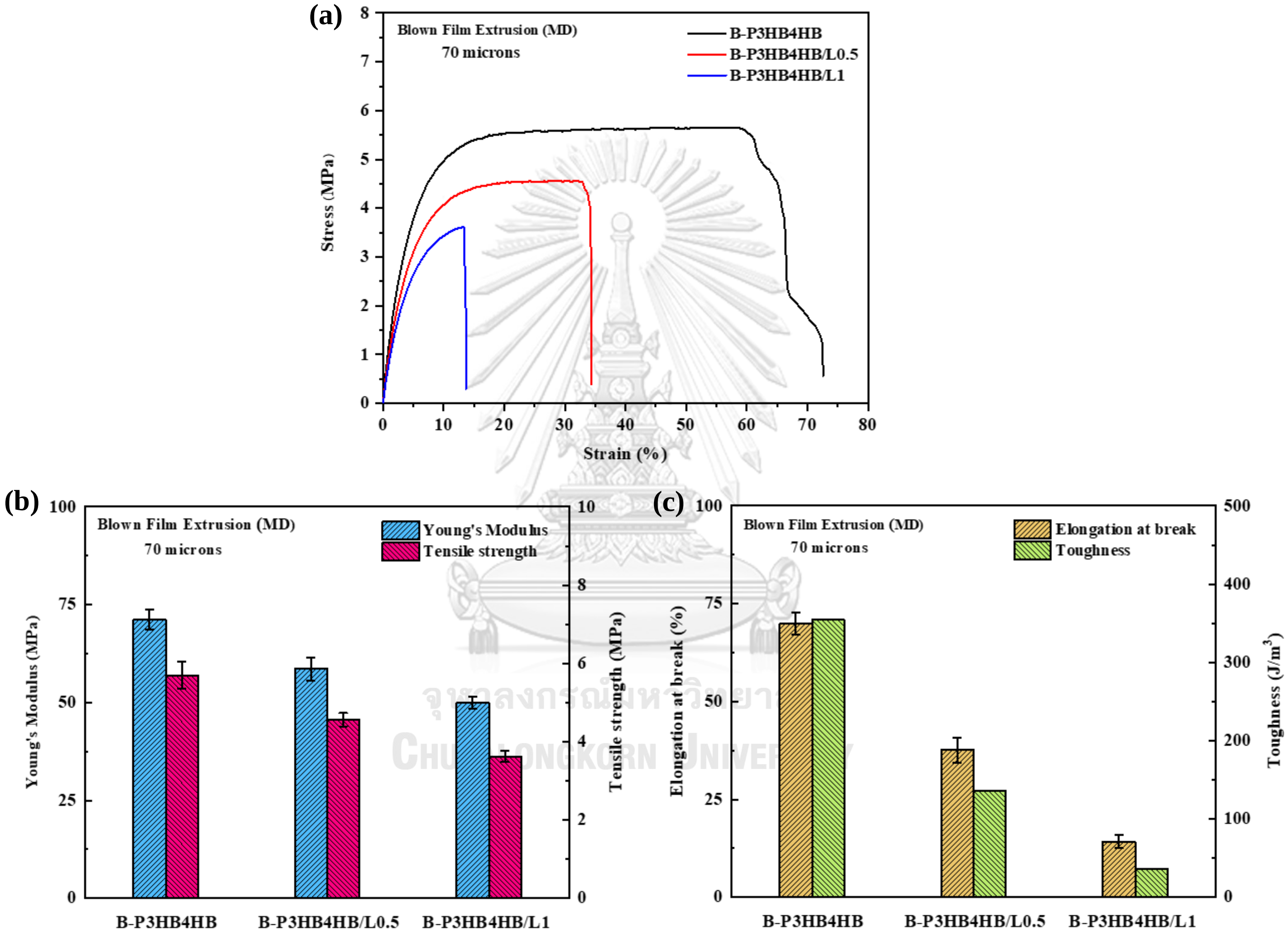

Figure 4.7 Tensile test results (a) Stress–strain curves (b) Young's modulus and tensile strength (c) Elongation at break and toughness of P3HB4HB and composite films.

As a result of Figure 4.7 (c), when lignin incorporates into the composite film, elongation at break and toughness will decrease with the same as tensile modulus and tensile strength it also because of lignin hinder the arrangement ordering of P3HB4HB molecules and in nature lignin relatively brittle and lacks the ductility make the toughness of P3HB4HB decrease.

4.5.5 Anti-UV activity

Exposure to UV light can reduce the food shelf life, so the film packaging should provide UV protection to the food while maintaining the product visible to the stripped eye. The anti-UV activity of P3HB4HB and composite films was investigated using UV-vis spectroscopy by measuring the transmittance in two wavelength ranges: UV (250-400 nm) and visible light (400-800 nm). As a result of Figure 4.8 (a-b), the pure P3HB4HB film showed a prevented transmittance in the UV light region, whereas the addition of lignin to the P3HB4HB film could protect a small part of the UV spectrum at 300-400 nm. The outstanding anti-UV performance was attributed to the abundant phenolic hydroxyl groups in the lignin, which showed intense UV absorption.

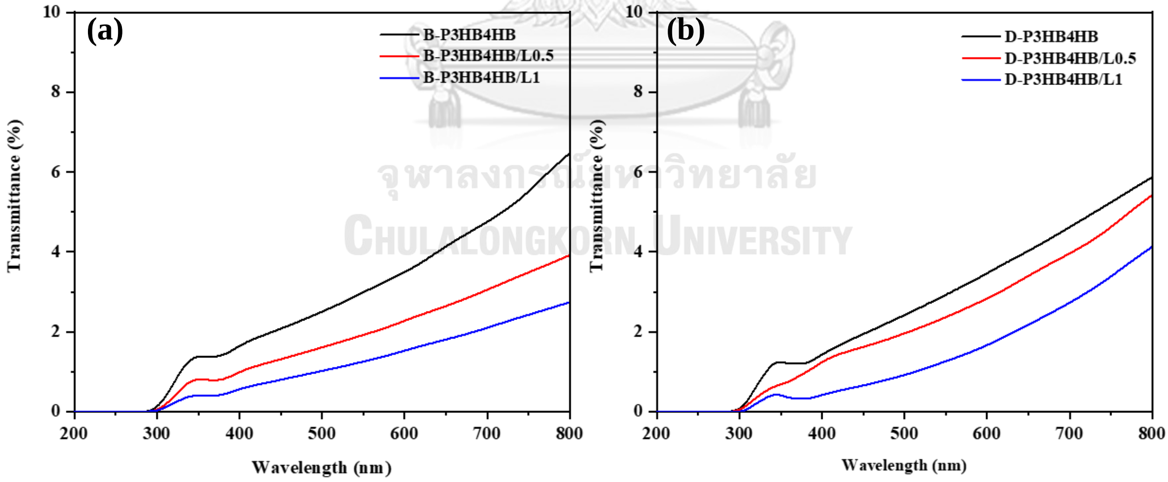

Figure 4.8 UV–vis transmittance spectra of P3HB4HB and composite films (a) blown film extrusion (b) doctor blading

Figure 4.9 shows that the pure PET film has a transmittance of about 80% in the UV region. When spraying lignin on the PET film, it could protect a small part of the UV spectrum at 300–400 nm. Like the previous result, the outstanding anti-UV performance was attributed to the abundant phenolic hydroxyl groups in the lignin, which showed intense UV absorption.

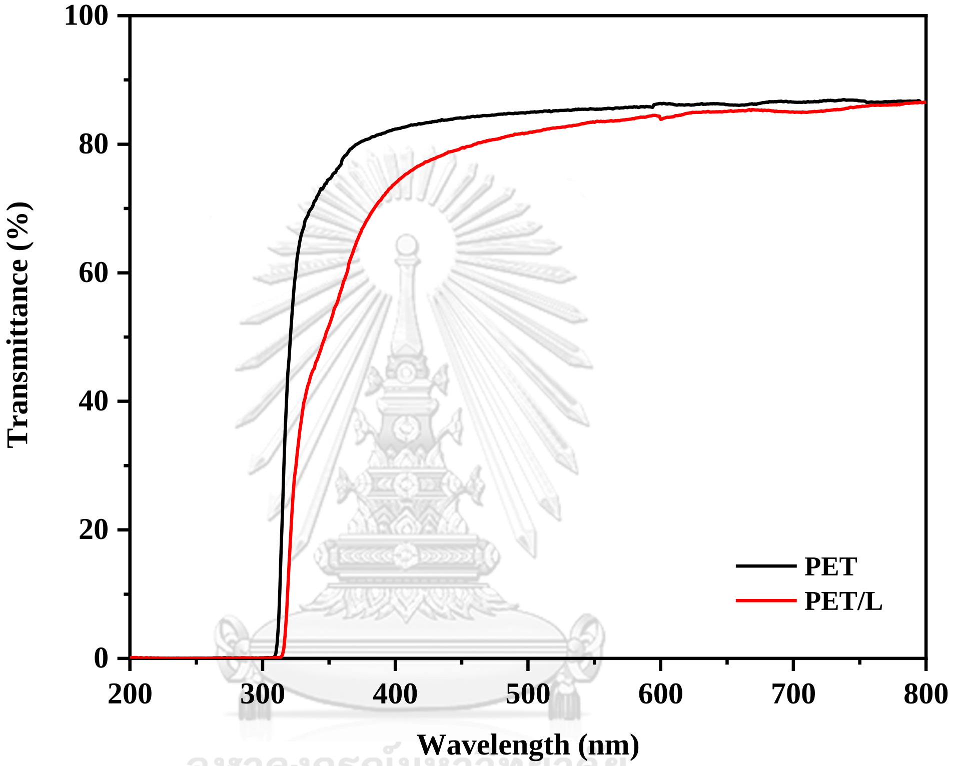

Figure 4.9 UV–vis transmittance spectra of PET film both uncoated and coated with lignin solution.

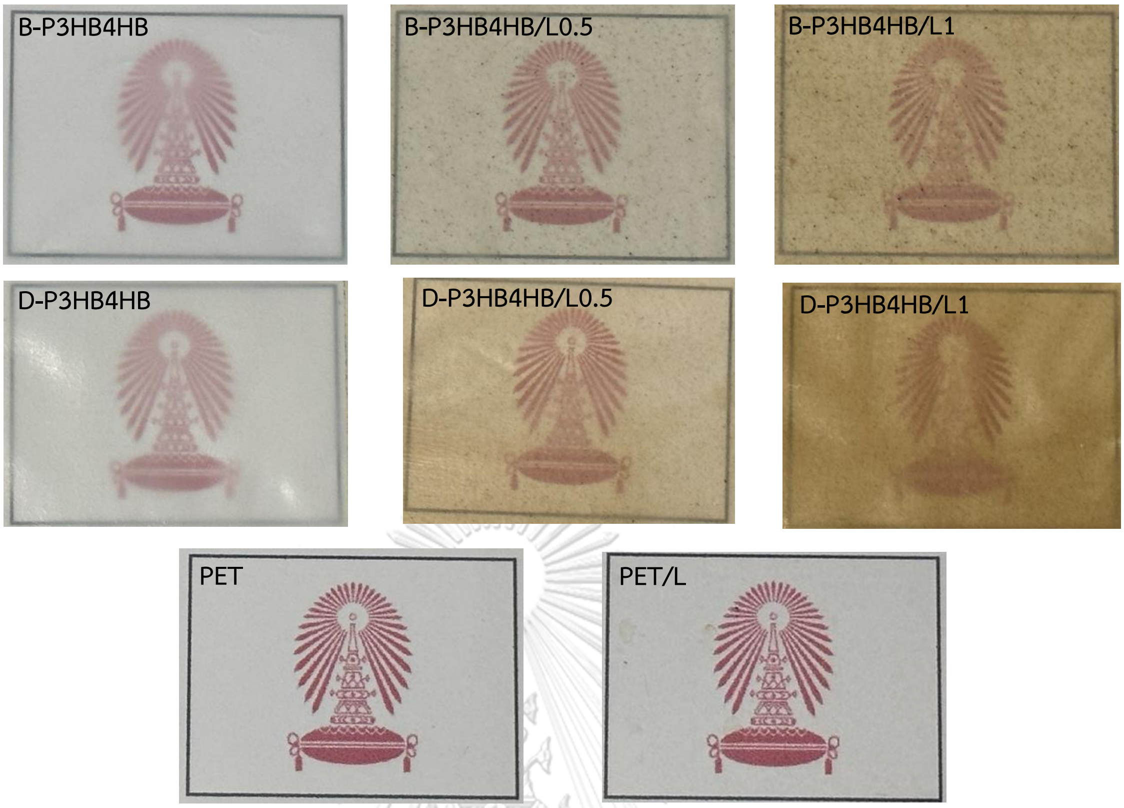

Figure 4.10 Photographs of composite films on the CU logo.

4.5.6 Antimicrobial activity

From the antimicrobial activity analysis of composite films shown in Figure 4.11, it was found that both P3HB4HB and PET containing lignin show good inhibition performance against gram-positive bacteria (*S. aureus* and *B. Subtilis*) by reducing bacteria viability due to gram-positive bacteria having a thinner cell wall, which is easily destroyed by lignin's phenolic hydroxyl groups, inhibiting bacterial growth [50]. On the other hand, PET/L did not exhibit antimicrobial activity on *B. subtilis* because the viable cells were increased after 24 h. Next, the film result does not show good antimicrobial activity in gram-negative bacterial strains (*E. coli and P. aeruginosa*), probably due to the differences existing in the bacterial cell wall. In the fungi species, B-P3HB4HB/L1 show an excellent antimicrobial performance against *Candida albicans and A. niger* when compared to other films. Most films are effective against *Candida albicans*, but only the composite film from doctor blading can't inhibit it.

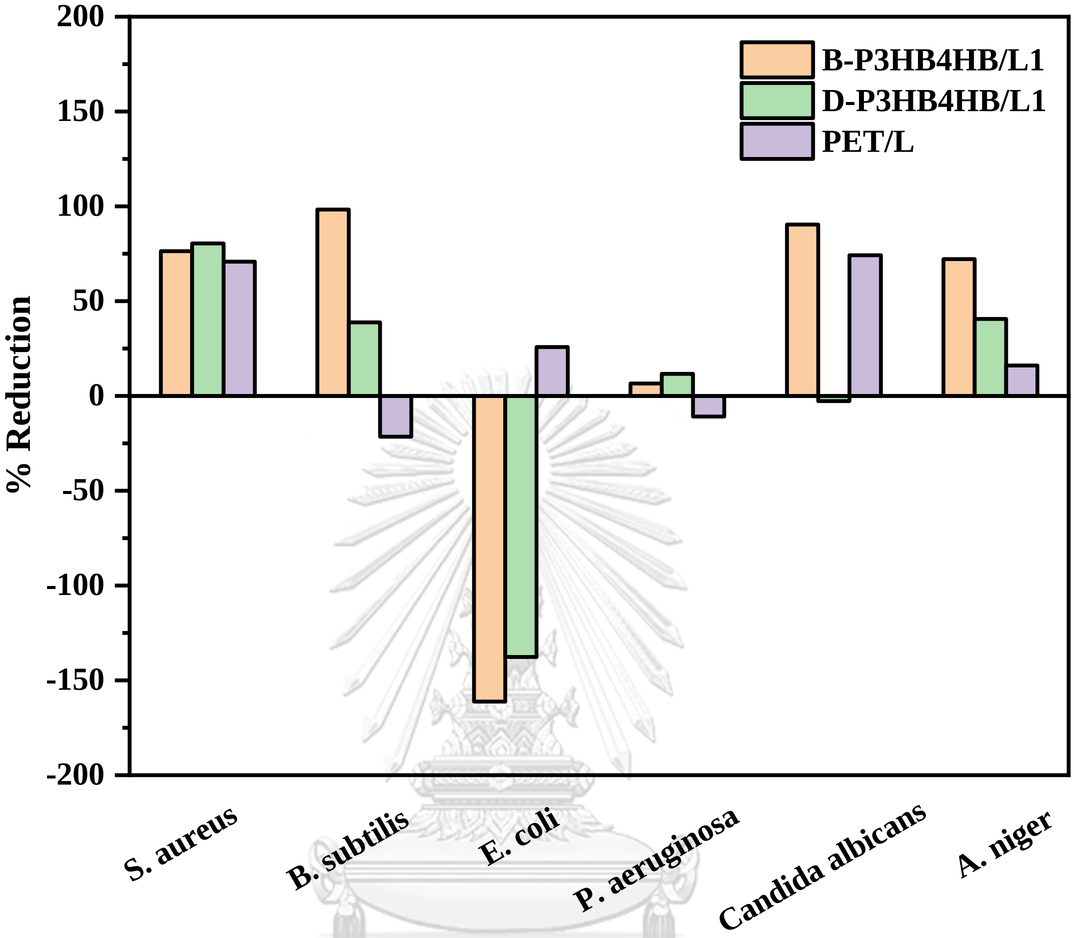

Figure 4.11 Antimicrobial activity of composite and coated films.

Chapter 5

CONCLUSIONS AND SUGGESTIONS

5.1 Conclusions

The researcher successfully prepared bioplastic composite films between P3HB4HB and lignin powders through blown film extrusion, doctor blading, and spray coating. The effect of lignin was studied in terms of morphological, mechanical, contact angle, barrier, anti-UV, and antimicrobial properties.

From the morphological analysis of the fracture surface of blown film extrusion and doctor blading, visible voids or another phase in the polymer matrix were not found. The results indicate that the lignin was well distributed in the P3HB4HB matrix. From the contact angle analysis of blown film extrusion, doctor blading, and spray coating, the results show that the WCA value of the composite film was increased across the board for the three processes when L0.5 and L1 were added to the film.

The barrier properties of composite films are essential for extending the shelf life of food. The results show that the composite films with a lignin filler decrease both OTR and WVTR from blown film extrusion and doctor blading process. It can be concluded that lignin dramatically increases the barrier properties of composite films.

From the mechanical properties analysis, it was found that when lignin was added, most of the mechanical properties of the composite films were reduced, and the properties of the film changed from ductile to brittle. It is because lignin hinders the long-range ordering of P3HB4HB molecules in the polymer matrix.

For the anti-UV properties of composite films, the exceptional anti-UV performance of the lignin was determined to be due to the abundance of phenolic hydroxyl groups inside the lignin, which demonstrated significant UV absorption.

In terms of antimicrobial activity analysis, it was shown that most composite films show excellent antimicrobial activity when against gram-positive bacteria and Fungi. A %reduction higher than 90 was found in blown film extrusion when against *B. subtilis* and *Candida albicans*, which shows the ability almost completely to inhibit the growth of bacteria.

5.2 Suggestions

5.2.1 The mixing of lignin powder with poly(3-hydroxybutyrate-co-4-hydroxybutyrate) should be mixed as homogeneously as possible to reduce the deviation from the analysis of various properties of the composite film.

5.2.2 The thickness of composite films between poly(3-hydroxybutyrate-co-4-hydroxybutyrate) and lignin powder from blown film extrusion and doctor blading process should have the same consistency for both techniques.

5.2.3 In the spray coating technique, the solution of lignin sprayed should be uniform and distributed over the film's surface.

5.2.4 The density of the composite film should be analyzed to support the results of the analysis.

5.2.5 The blown film extrusion process should be preferred in packaging film applications as it provides better properties in each aspect than other processes.

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APPENDIX

1. Preparation of P3HB4HB/Lignin blends

P3HB4HB pellets and Lignin powder were dried at 80 °C, for 12 h before use. P3HB4HB/Lignin blends were fabricated through a melt processing technique using a twin-screw extruder (diameter [D] = 40 mm; length to diameter ratio [L/D] = 26; LabTech Engineering Co. Ltd., model LE40-26, Thailand) with a temperature profile of 130, 130, 140, 140, 145, 145, 150, 150, 145 and 145 °C from the feeder to the die. The screw speed was 50 rpm. After that, it will be pulled through the water to be cut with a pelletizer. The polymer blends were prepared with different amounts of lignin, as shown in Table 3.1.

2. Preparation of P3HB4HB/Lignin composite films

2.1 Cast film extrusion

In this step, the polymer blend is formed into a film. The first step was to put the prepared polymer blend pellets into an oven at 80 °C for 12 h and then form them in a twin-screw extruder (Thermo Haake Rheomex, Germany) with the temperature in the barrel set to 130, 130, 140, 140, 145, 145, 150, 150, 145 and 145 °C. The screw speed was 90 rpm, and a sheet ribbon die was used. The composite films were rolled through the chill roll at 25 °C, and the thickness of all collected films was 30 microns.

3. Characterization of composite film

3.1 Water contact angle

The surface wettability of P3HB4HB and composite films were determined by measuring the water contact angle (WCA), and it was also used to estimate the hydrophilicity or hydrophobicity of a film surface. The results are displayed in Table 5.1 as it can be seen that the WCA value of pure

P3HB4HB film that formed from the cast film extrusion process was 93.1°. However, the result from Table 5.1 shows that the blown film has a higher WCA value than the cast film because the surface roughness of the blown process is higher than that of the casting process. Adding L0.5 and L1 to the film raised the WCA value of the composite film. These findings demonstrated the composite film's improved hydrophobic performance, primarily due to the lignin in the P3HB4HB film.

Table 5.1 WCA of P3HB4HB and composite films from cast film extrusion.

Samples	**WCA (°)**
P3HB4HB	93.1 ± 1.5
P3HB4HB/L0.5	97.0 ± 2.1
P3HB4HB/L1	98.1 ± 2.8

3.2 Mechanical properties

Mechanical characterization of P3HB4HB and composite films were conducted on samples in the machine direction, and the mechanical properties are presented in Figure 5.1. Figure 5.1 (a-c) shows the tensile test results similar to the blown film process. It can be seen that when lignin incorporates into the composite film, all of the mechanical properties were decreased when compared with tensile strength, and elongation at break cast film has better properties than blown film. Still, when compared to modulus, blown film has a higher value than cast film because the molecular orientation of the machine direction of the blown film is better than cast film.

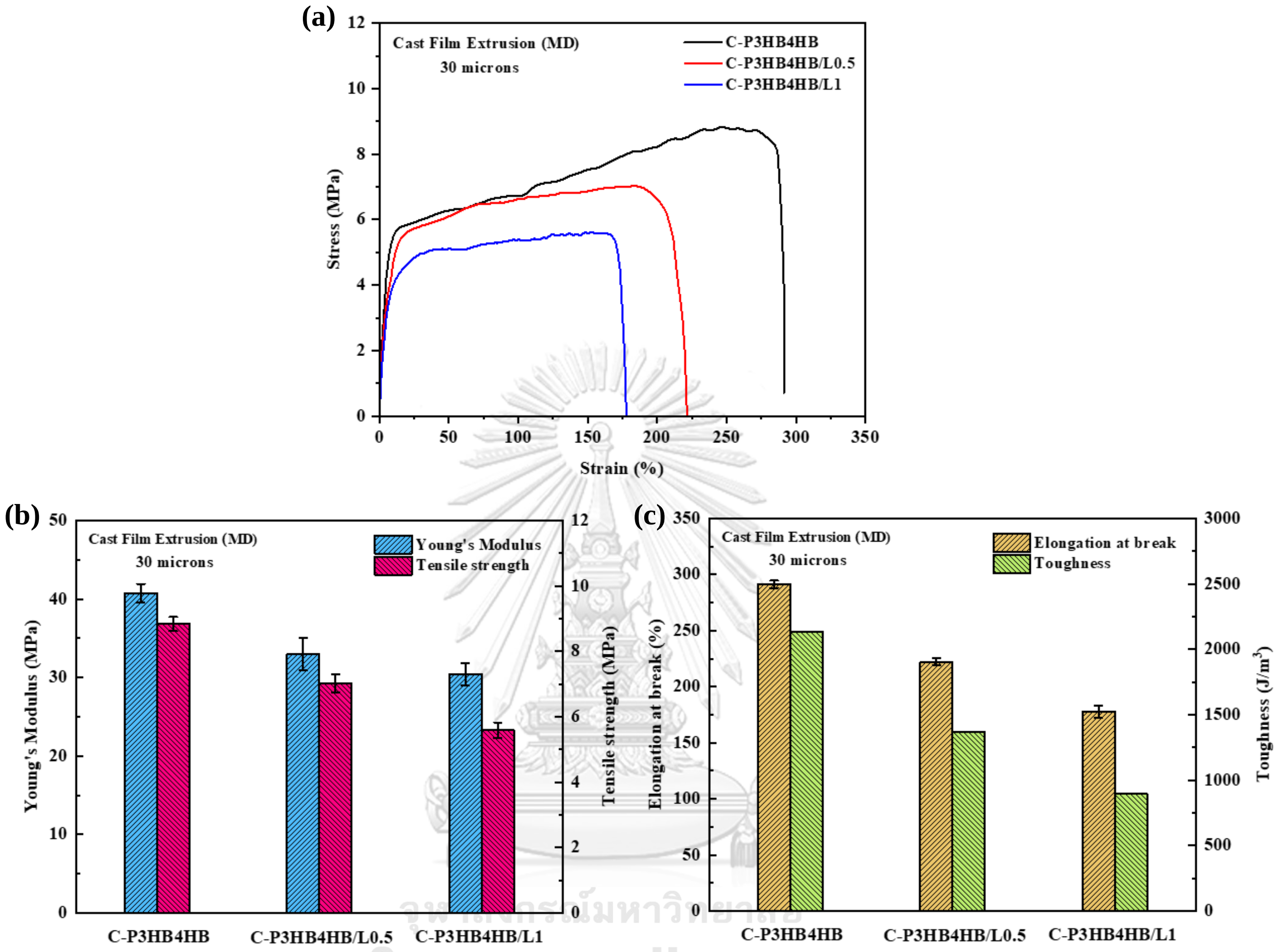

Figure 5.1 Tensile test results (a) Stress–strain curves (b) Young's modulus and tensile strength (c) Elongation at break and toughness of P3HB4HB and composite films.

VITA

NAME	Atiwit Singhapan
DATE OF BIRTH	16 November 1998
PLACE OF BIRTH	Bangkok, Thailand
INSTITUTIONS ATTENDED	Engineering, Kasetsart University, Bangkok, Thailand
HOME ADDRESS	7/85 The Rich Village, Moo 3, Thapma Rd, Noen Phra, Mueang, Rayong 21000