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Research article

Preparation and characterization of antimicrobial laminated films based on poly(lactic acid)/chitosan via a lamination technique

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Abstract. Nowadays, there has been an increase in demand for using biodegradable food packaging due to its sustainable and environmentally friendly. Poly(lactic acid) is an interesting alternative. To increase the potential use of packaging, the development of a bioactive packaging by using a natural antimicrobial like chitosan has gained considerable attention due to its excellent antimicrobial activity and performance meanwhile, both layers have different surface properties that result in poor interfacial adhesion. So, this research aims to improve the interfacial adhesion between PLA and chitosan by grafting chitosan with 3-(trimethoxysilyl)propyl methacrylate (TMSPM) using ultrasonic vibration and to study the preparation of the laminated film via lamination technique. From the FTIR results, the characteristic peaks at a wavenumber of 1701 and 877 cm^{-1} corresponding to the C=O vibration in the methacrylate and Si–O stretching groups of TMSPM were observed. This indicates that the grafting reaction occurs completely. Interestingly, the PLA/CS_10%TMSPM laminated film revealed an improved interfacial adhesion and hydrophobicity. This was observed from the strength value that increased from 21.9 to 35.8 MPa and the water vapor permeability value that decreased from $14.6 \cdot 10^{-7}$ to $9.1 \cdot 10^{-7}$ (g·m)/(m²·Pa·s) when compared to the PLA/CS laminated film. In addition, the PLA/CS_10%TMSPM laminated film also showed excellent antimicrobial activity against *E. coli* as well.

Keywords: biodegradable polymers, poly(lactic acid), chitosan, silane coupling agent, lamination technique

1. Introduction

Nowadays, the food packaging industry is one of the fastest-growing industries, which can be seen from the amount of usage that is increasing each year. Most food packaging was made from plastics or polymers that are synthetically derived from petrochemical sources due to their lightweight, excellent physical properties, good processability, and relatively low cost [1]. Although plastic is considered to be a material that is convenient to use and has advantages over other materials, plastic still has disadvantages as well, which are difficult to decompose and deteriorate. As a result, plastic waste can remain in the environment for a long time and increase each year rapidly. In addition, it is also the cause of environmental problems that inevitably follow, including plastic can contaminate the food chain and pose a danger to human health. For example, when plastic expires some lifetime, it will disintegrate into microplastic, also known as microbead, which can penetrate onto the soil layer or contaminate water sources. Most importantly, disposing of plastic waste by burning methods results in

toxic fumes in the air and carbon dioxide that causes global warming as well [2, 3].

Bioplastics are one of the most suitable alternatives for instead of conventional plastics because they are the biodegradable and bio-based origin of plastics, which are derived from plants, microorganisms, or renewable materials, whereby the main advantages of using bioplastics are improved the potential environmental impacts and decreased the fossil fuel contents [4, 5]. Among the bioplastics, polyesters are the plastics that play the most important role because they have hydrolysable ester bonds along their polymer backbone [6]. Poly(lactic acid) (PLA) is linear aliphatic biodegradable polyester that has highly hydrolysable ester bonds and it is considered to be a hydrolytically degradable polymer that can degrade in high humid environments, including it's non-toxicity material and also shows some properties like polystyrene (PS) and poly(ethylene terephthalate) (PET) [7, 8]. Moreover, poly(lactic acid) is also a thermoplastic made from renewable sources that have an excellent strength or modulus, and it can yield articles for use in the packaging industry [9].

To increase the potential to use, the use of natural antimicrobial components in food packaging productions has gained considerable attention from both the consumers and the food industry to improve food quality by preventing food from spoiling too quickly [10]. For this, the natural antimicrobial component like chitosan is an interesting alternative. Chitosan (CS) has been used as a packaging material for the quality preservation of food due to its biodegradability, antimicrobial activity, non-toxicity, and versatile chemical and physical properties [11]. For the use of chitosan as an antimicrobial agent, there was previous research that studied chitosan-based nanocomposite films and coatings by blending chitosan with biopolymer via solution casting. From the results, it indicated that chitosan could prevent the growth of spoilage and could also improve food quality and extend the shelf life of food [12].

As mentioned above, both poly(lactic acid) and chitosan are biodegradable polymers with easy availability. The preparation as a multilayer polymer film via lamination technique could enhance the film's functional properties and is considered an interesting technique due to its ease, convenience, and low cost, while the poly(lactic acid) layer could serve as an oxygen or moisture barrier, and the chitosan layer may also be used to against bacteria and extend food

shelf life [13]. Previously, there was a lot of research involved in the preparation of laminated films for the production of food preservation packaging based on poly(lactic acid) and natural antimicrobial. However, there was very few research study on the preparation of the laminated film based on poly(lactic acid) and chitosan directly due to both materials have different surface properties resulting in poor interfacial adhesion [14]. Recently, there is some previous research by Fang *et al*. [15] investigated the preparation of composites materials between poly(lactic acid) and natural jute fiber by treating the fiber with a silane coupling agent due to both materials having different surfaces properties, and the composites were fabricated via the film stacking method. The results reported that the use of a silane coupling agent could significantly enhance the interfacial adhesion, including improving the performance of the composites as well .

In this research, the authors chose to use an above similar strategy to improve the surface adhesion of the two materials. Covalent grafting of silane coupling agent to chitosan is an interesting method to improve interfacial adhesion and enhance the performance of the laminated film through chemical bond formation. 3-(Trimethoxysilyl)propyl methacrylate) (TMSPM) is the most commonly used organofunctional silane coupling agent due to it can improve strength, enhance adhesion and increase the hydrophobicity of the materials. Furthermore, this silane is considered an environmentally friendly petroleum-derived hydrophobic material and nontoxic. This was confirmed by the Hazard Communication Standard of the US Occupational Safety and Health Administration. And the use of this silane coupling agent did not affect biodegradation significantly. But on the contrary, it can improve the performance and the durability of each material when applied [16–19].

So, the objective of this research is to study the preparation of the laminated film between PLA and chitosan by improving the interfacial adhesion via the grafting of chitosan with 3-(trimethoxysilyl)propyl methacrylate. And in this research, silane was grafted onto the chitosan structure via ultrasonic vibration. Ultrasonic vibrations are widely used to promote chemical or interface reactions by improving an adhesive bonding owing to its high energy [20, 21]. The vibration with high energy can improve the bonding stability and adhesion strength by using external

energy to induce the chemical reactions and to effectively facilitate the bonding [22]. Therefore, this method is an attractive alternative for silane grafting. Finally, the films were examined for their characteristic properties by using an attenuated total reflectance-Fourier transform infrared (ATR-FTIR), scanning electron microscopy (SEM), differential scanning calorimetry (DSC), tensile testing, water vapor permeability (*WVP*), dynamic mechanical analysis (DMA), contact angle, X-ray diffraction (XRD) and antimicrobial activity.

2. Material and methods
2.1. Materials
Poly(lactic acid) grade Ingeo® 4043D was purchased from Nature Works (Blair, USA). Seafresh chitosan powder 85% DAC, molecular weight ~550 000 g/mol was purchased from Seafresh Chitosan (Lab) (Chumphon, Thailand). 3-(trimethoxysilyl)propyl methacrylate 98%, M_w 248.35 g/mol was purchased from Sigma-Aldrich (St. Louis, USA), a superhydrophobic material acted as a silane coupling agent. Acetic acid glacial and chloroform were purchased from RCI Labscan (Bangkok, Thailand).

2.2. Preparation of the modified chitosan by grafting with TMSPM
The modified chitosan has been prepared in two steps. The first step is the preparation of the chitosan solution. For this, chitosan powder was dissolved (0.5 g) in 1% v/v acetic acid solution (100 ml) and stirred continuously for 24 h. Then, heated the chitosan solution at a temperature of 80 °C for 6 h and stirred at room temperature overnight under magnetic stirring until chitosan completely dissolved. The second step is the preparation of the modified chitosan. Added 3-(trimethoxysilyl)propyl methacrylate (2, 5, and 10 mol% substitutions of chitosan content) into the chitosan solution. Then, sonicated the solution for two hours to complete the dispersion of silane. Finally, the solution was stirred at room temperature for 24 h to achieve a complete reaction.

The grafting reaction of the modified chitosan is shown in Figure 1. Briefly, the possible grafting mechanism can be explained in two steps. The first step is the hydrolysis reaction of the methoxy group in TMSPM structure to form the silanol group under acid conditions. The second step is the reaction between the silanol group of TMSPM and the hydroxyl group
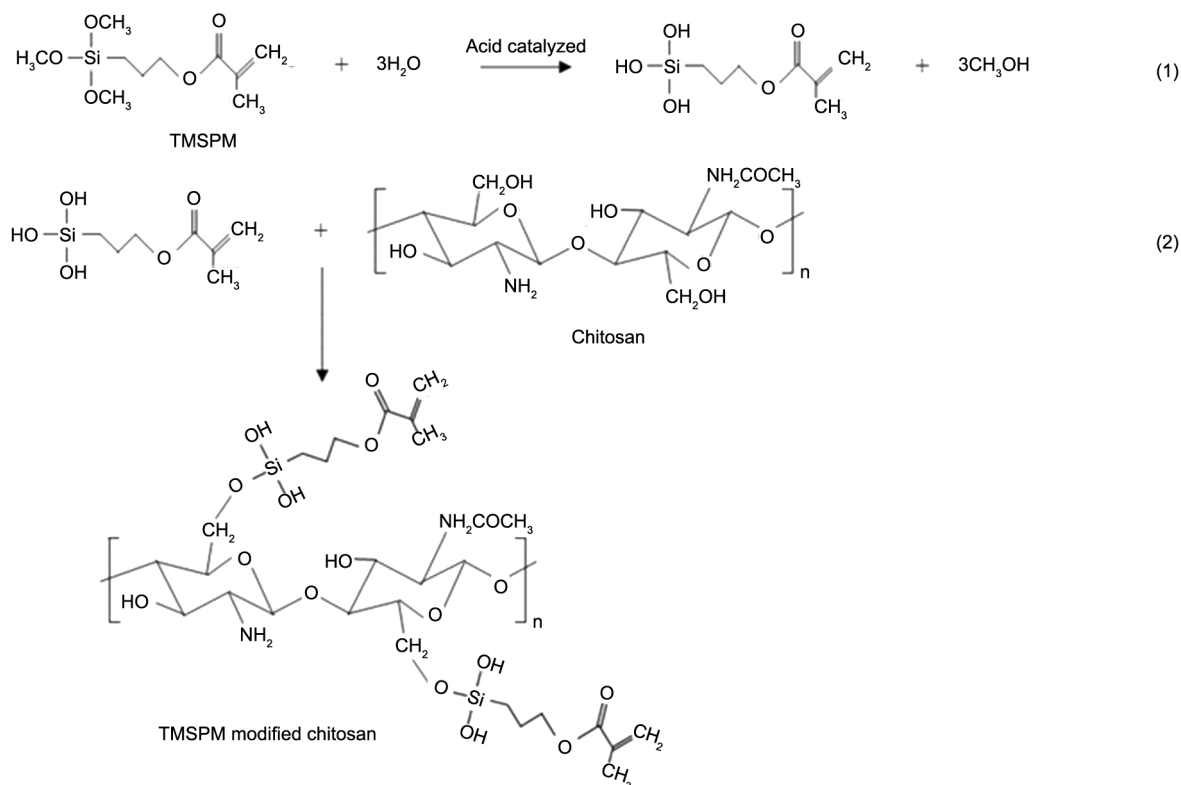

Figure 1. The grafting reaction of the TMSPM-modified chitosan.

at carbon-6 of chitosan whereby the bond between silicon and oxygen atom is the covalent bond [23].

2.3. Preparation of the laminated film between PLA and chitosan

The laminated film between PLA and the modified chitosan was carried out using a lamination technique (Table 1). Firstly, the PLA film was prepared through a solution casting by dissolved PLA pellets (2.5 g) in chloroform (100 ml), left at room temperature without stirring to prevent foaming until the PLA pellets were completely dissolved. The PLA solution was cast on a melamine tray and dried for 24 h at room temperature. Then, the solidified PLA film was laminated with the modified chitosan solution prepared according to steps 2.2, dried at 35 °C for 24 h, and stored in a desiccator with silica gel to prevent the moisture. Finally, compressed the laminated film at 150 °C for 1 min with a pressure of 10 bar. The neat PLA film and the laminated film between PLA and the unmodified chitosan were also prepared in the same condition. The experimental procedure is shown in Figure 2.

2.4. Characterization
2.4.1. Infrared spectroscopy (FTIR)
An attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy (TENSOR 27, Bruker, USA) was used to analyze the functional group of each film. Spectra of each specimen were collected at a wavenumber range of 4000–500 cm^{-1} with

averaging 32 scans and a resolution of 4 cm^{-1}. The results were repeated 3 times.

2.4.2. Scanning electron microscopy (SEM)
The cross-section images of the laminated films were analyzed with scanning electron microscopy by using a Quanta 400 (Thermo Fisher Scientific, Czech Republic). All films were sputtered with gold (Au). Furthermore, the samples were observed by using an accelerating voltage of 10 kV.

2.4.3. Differential scanning calorimetry (DSC)
The thermal properties of the films were investigated by using a PerkinElmer DSC-7 type differential scanning calorimeter (DSC) from PerkinElmer, Inc. (USA). Approximately 10 mg of each specimen was placed in an aluminum crucible and heated from 30 to 190 °C under a nitrogen atmosphere at a heating rate of 10 °C/min.

2.4.4. Tensile properties
Tensile properties of the films were determined by using an Instron universal testing machine (Instron 3365, USA) according to the standard ASTM D638 (Type V). The films were cut into a dumbbell shape with a gauge length of 10 mm by using a dumbbell die cutter. The machine was operated with a tensile load cell of 100 N and a speed of 5 mm/min.

2.4.5. Barrier properties
Water vapor permeability (*WVP*) was used to determine the vapor pressure of the PLA and PLA/chitosan laminated films by using a Payne permeability cup according to the ASTM E96-95 standard method. Firstly, 15 ml of distilled water was added to a *WVP* cup with a depth of 1.5 cm and diameter of 4.5 cm, and placed the film (8 cm×8 cm) on the test cup and sealed tightly. Then, the specimen was placed in a hot air oven (MEMMERT UF55, Germany) at 37 °C and 60% RH and weighed at the specified time. Water vapor transmission rate (*WVTR*) or moisture

Table 1. The sample codes and the compositions of the films.

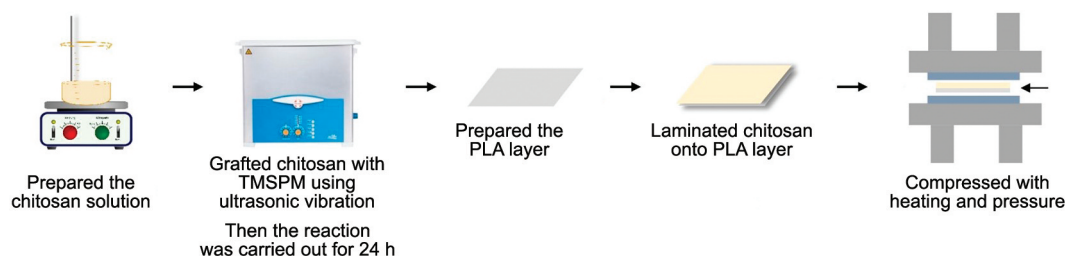| Sample	Compositions		
	PLA [g]	Chitosan [g]	TMSPM [mol%]
PLA	2.5	–	–
PLA/CS	2.5	0.5	–
PLA/CS_2%TMSPM	2.5	0.5	2
PLA/CS_5%TMSPM	2.5	0.5	5
PLA/CS_10%TMSPM	2.5	0.5	10

Figure 2. Schematic representation of the processing steps to obtain the laminated films between PLA and chitosan.

vapor transmission rate (*MVTR*) was determined from the slope of weight loss vs time and calculated as Equation (1):

$$WVTR\left[\mathrm{g}/\left(\mathrm{m}^2\cdot\mathrm{s}\right)\right] = \frac{\text{slope}}{\text{film area}} \qquad (1)$$

For *WVP* could calculate as the Equation (2):

$$WVP\left[\mathrm{g}\cdot\mathrm{m}/\left(\mathrm{m}^2\cdot\mathrm{Pa}\cdot\mathrm{s}\right)\right] = \frac{WVTR\cdot L}{\Delta P} \qquad (2)$$

where *L* is the film specimen thickness [m] and Δ*P* is the difference in partial pressure [Pa].

2.4.6. Dynamic mechanical analysis (DMA)
Dynamic mechanical analysis was used to analyze the mechanical-thermal behavior of the material as a function of temperature and time by using the dynamic mechanical analyzer (DMA8000, Perkin Elmer, USA). Each specimen was cut into a rectangle shape of size 5 mm×30 mm and carried out at temperatures of 25 to 140 °C and a rate of 3 °C/min. For this, will investigate only the storage modulus (*E′*) and tan delta (tan δ).

2.4.7. Contact angle (*CA*)
The contact angle was used to determine the surface wettability or surface energy of the films by using the optical contact angle analyzer (OCA 25, Dataphysics, Germany). The samples were cut into a rectangle with a size of 2.5 cm×7.5 cm. For this, the samples were tested with 3 different solutions like distilled water, dimethylformamide (DMF), and ethanol. Moreover, approximately 2 μl of the solution was used for each test, and the results are reported as the average of the contact angle values and surface energy.

2.4.8. X-ray diffraction analysis (XRD)
X-Ray Diffraction analysis was used for phase identification of a crystalline of the PLA and PLA/chitosan laminated films by using an X-ray diffractometer (Empyrean, PaNalytical, Netherlands) at a scan angle range of 5–40°.

2.4.9. Antimicrobial activity
The antimicrobial activity of the films was examined against *E. coli* (gram-negative) and *S. aureus* (gram-positive) according to ISO 22169. Firstly, *E. coli* and *S. aureus* were inoculated in nutrient broth (NB) at 37 °C for 24 h and sub-cultured in broths at 37 °C for 20 h, and the cultures must be diluted with normal saline solution to reduce the concentration before the test. The samples (50 mm×50 mm) and the covered plastic films (40 mm×40 mm) must be sterilized with UV light for 24 h. Then, the samples were added with the diluted culture of 0.4 ml, covered with the PET film, and incubated at 37 °C for 24 h. After incubation, leached the film surface with the soybean casein digest broth with lecithin and polyoxyethylene sorbitan monooleate (SCDLP). The total number of viable bacteria was measured by counting colonies on the plate count agar (PCA). Finally, the measurements were performed in triplicate.

3. Results and discussion
3.1. Effect of grafting on chemical and physical properties
3.1.1. Chemical analysis of the films
The FTIR spectra of the neat PLA, neat chitosan, and PLA/CS laminated films are shown in Figure 3. The neat PLA revealed the characteristic peak at a wavenumber of 1739 cm^{-1}, which was referred to as the ester (O=C–O) group in the structure of this biopoly-
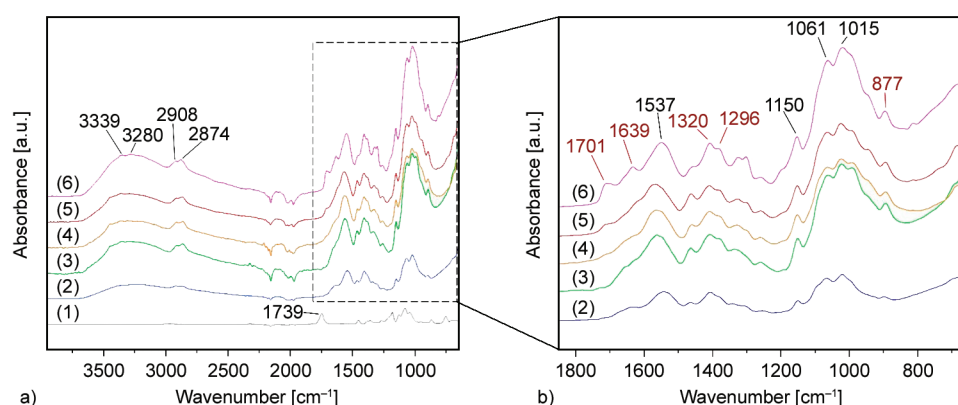

Figure 3. FTIR spectra of the (1) neat PLA, (2) neat CS, (3) PLA/CS, (4) PLA/CS_2%TMSPM, (5) PLA/CS_5%TMSPM, and (6) PLA/CS_10%TMSPM laminated films. a) Overview of spectral lines; b) enlargement of the characteristic peaks..

mer [24]. The FTIR spectra of chitosan showed the peaks at a wavenumber of 3339 and 3280 cm^{-1}, which was referred to O–H and N–H stretching, respectively. The peaks around 2908 and 2874 cm^{-1} can be attributed to the C–H symmetric and C–H asymmetric stretching of chitosan. A peak observed at 1537 cm^{-1} indicated C=O stretching of primary amine. Furthermore, chitosan also showed the absorption peak at 1150 cm^{-1} can be attributed to asymmetric stretching of the C–O–C bridge, and the peak at 1061 and 1015 cm^{-1} corresponds to C–O stretching [25]. Interestingly, the PLA film that was laminated with chitosan didn't represent any new peak. This indicated that there were no significant changes in the chemical structure between PLA and chitosan. But it may be that the PLA and chitosan have mutually physical interactions. And the grafting with 3-(trimethoxysilyl) propyl methacrylate onto chitosan structures of the PLA/CS laminated films was mainly visible by the absorbance increase of the functional group at a wavenumber of 1701 and 1639 cm^{-1}.

This indicated C=O and C=C vibrations in the methacrylate group of TMSPM, respectively. The peak at 1320 and 1296 cm^{-1} were assigned to C–O and C–C stretching vibrations of TMSPM [26]. In addition, the peak was found at a wavenumber of 877 cm^{-1}, corresponding to the Si–O stretching group of TMSPM [27]. In which the grafting reaction of the TMSPM-modified chitosan is described and shown in Figure 1.

Figure 4 reveals the intermolecular dipole-dipole interaction between the TMSPM-modified chitosan and PLA. It can be found that the dipole-dipole reaction that occurs may be caused by the carbonyl in the ester group of PLA reacting with the carbonyl in the methacrylate group of TMSPM [28–31].

3.1.2. Morphology
Figure 5 shows the cross-sections of the total laminated films. Figure 5a shows a cross-section of the PLA/CS laminated film; this film revealed the wide gap between PLA and chitosan layer at the interface
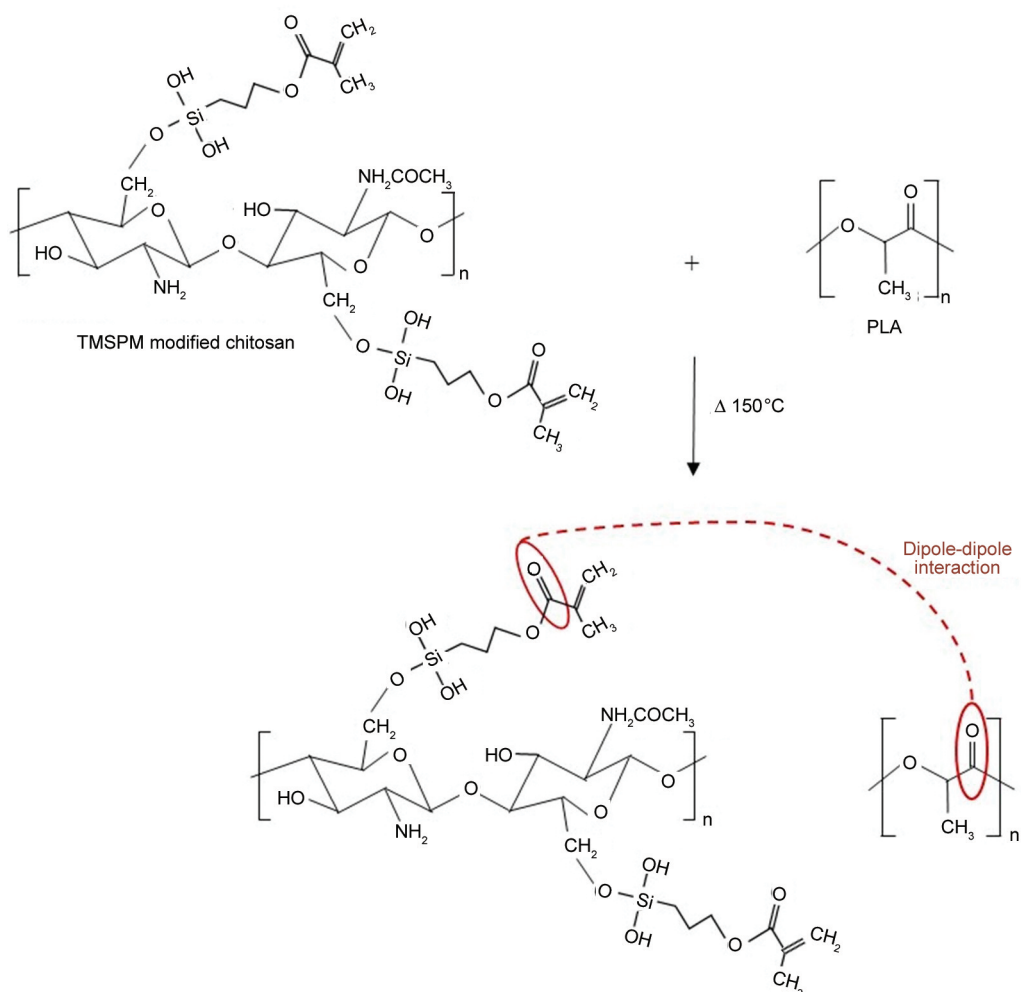

Figure 4. The dipole-dipole interaction between the TMSPM-modified chitosan and PLA.
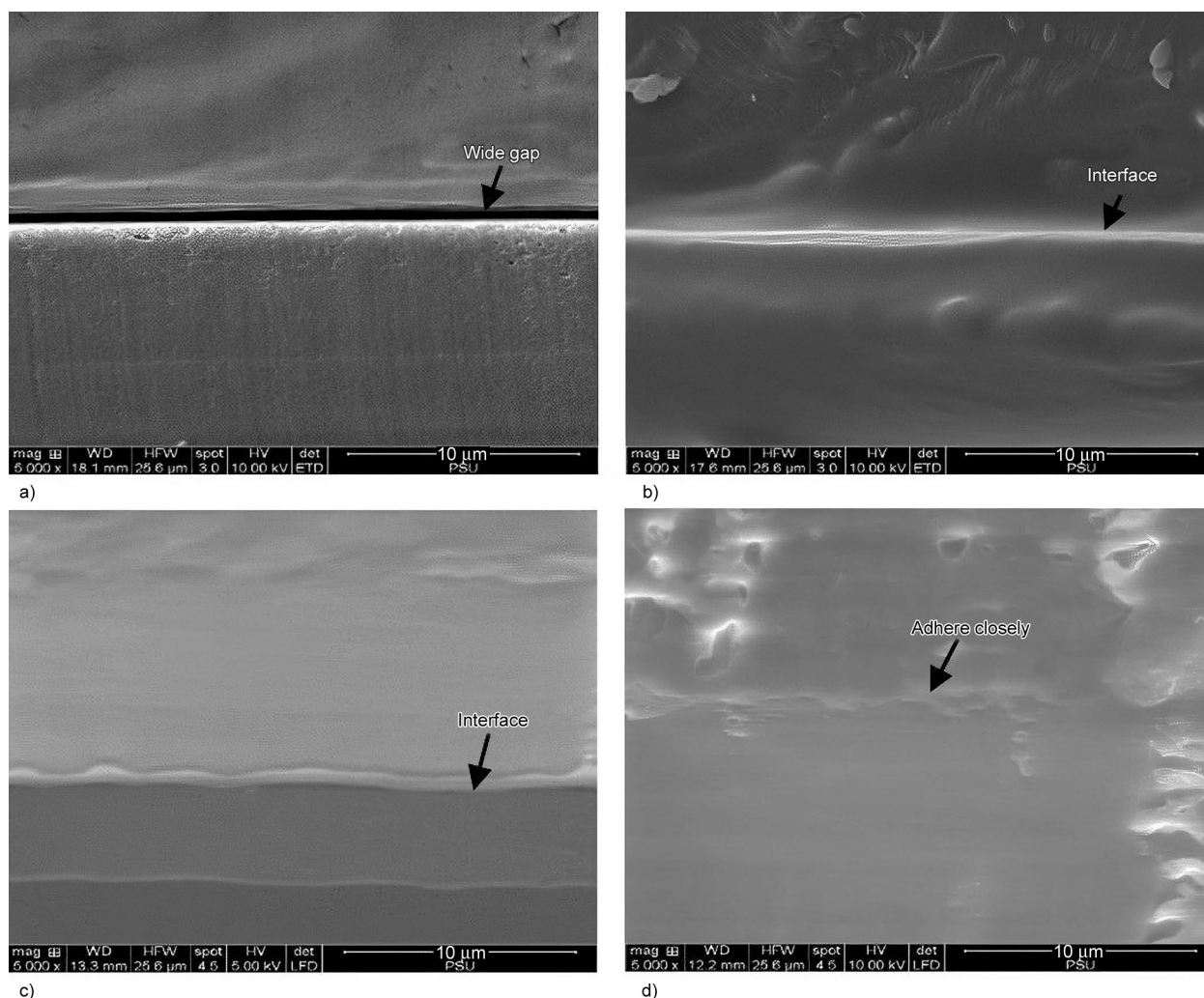
Figure 5. SEM cross-sections of the (a) PLA/CS, (b) PLA/CS_2%TMSPM, (c) PLA/CS_5%TMSPM and (d) PLA/CS_10%TMSPM laminated films; whereby the top is the chitosan layer, and the bottom is the PLA layer.

position. This indicated that both layers have poor interfacial adhesion due to both PLA and chitosan having different surface properties [32]. The case of the modified laminated films, as shown in Figure 5b–5d, respectively, revealed an improvement in the interfacial adhesion between PLA and the modified chitosan increased when TMSPM contents increased. At 10 mol% of TMSPM showed the best interfacial adhesion compared to 2 and 5 mol% of TMSPM whereby both layers adhere closely together and almost become homogeneous. The SEM results indicated that the grafting chitosan with TMSPM was good adhesion with the PLA layer. Thus, the cross-sections supported further the successful properties of the laminated films that will be discussed later.

3.1.3. Thermal properties

DSC analysis was used to measure the heat flow produced in the neat PLA and PLA/chitosan laminated
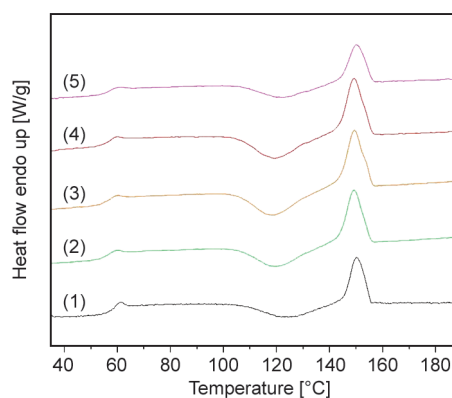

Figure 6. DSC thermogram of the (1) neat PLA, (2) PLA/CS, (3) PLA/CS_2%TMSPM, (4) PLA/CS_5%TMSPM and (5) PLA/CS_10%TMSPM laminated films.

films (Figure 6). The results are summarized in Table 2. The T_g, T_c, and T_m values of the neat PLA film were 58.4±0.1, 121.7±0.4, and 150.2±0.2 °C, respectively. When laminated the PLA film with the

Table 2 Thermal information and percent crystallinity of the neat PLA and PLA/chitosan laminated films.

Sample	T_g [°C]	T_c [°C]	T_m [°C]	ΔH [J/g]	Crystallinity [%]
PLA	58.4±0.1	121.7±0.4	150.2±0.2	23.5±0.2	25.2
PLA/CS	55.4±0.2	119.8±0.3	149.4±0.1	27.0±0.4	29.0
PLA/CS_2%TMSPM	55.9±0.2	118.7±0.7	149.4±0.1	32.0±0.1	34.4
PLA/CS_5%TMSPM	56.4±0.2	119.2±0.5	149.3±0.1	32.0±0.2	34.4
PLA/CS_10%TMSPM	56.0±0.3	120.9±0.2	150.2±0.2	20.7±0.4	22.2

Each value was presented as a mean ± standard deviation.

unmodified chitosan layer, the T_g noticeably decreased compared to the neat PLA. But there was several research that reported that chitosan didn't affect the T_g value of PLA [33]. In this research, the decrease in the T_g value of PLA may be due to the additional action of chitosan that probably increased hydrophilicity of the laminated films resulting in a higher amount of bound water, which acted as a plasticizer that could be increased the chain mobility of PLA. This was confirmed by the barrier performance of the neat PLA and PLA/CS film according to Table 4, which shows that the PLA/CS film has a higher water vapor permeability than the neat PLA meanwhile the T_c and T_m values of the PLA/CS film slightly decreased [34–36]. In addition, the grafting of chitosan with TMSPM didn't significantly change the T_g and T_m values relative to the unmodified. But the T_c of the modified laminated films slightly increased when TMSPM increased up to 10 mol%. This may be due to the modified laminated films having bulky groups at the backbone chain of chitosan that affects the chain mobility of PLA whereby these bulky groups will hinder the motion of the PLA chain.

The percent crystallinity of the neat PLA film was 25.2%. When laminated with the unmodified and modified chitosan layers, the percent crystallinity of the laminated films will increase due to the high crystalline nature of chitosan, including grafting with silane coupling agents can induce the modified chitosan and PLA to form the dipole-dipole reaction

through the covalent bind between Si and O or may be due to the facilitating to induce crystallization of the silane coupling agent.

3.1.4. Tensile properties

Tensile testing is a crucial property for the production and application of packaging. Figure 7 shows the stress-strain curves of the neat PLA and PLA/chitosan laminated films. The tensile strength (*TS*), Young's modulus (*EM*), and elongation at break (*EB*) values of the films calculated from the aforementioned stress-strain curves are shown in Table 3. The neat PLA film presented values of *TS* and *EM* of 14.6±0.6 and 485±12 MPa, respectively; compared to the PLA/CS laminated film, the *TS* and *EM* values of this film increased up to 21.9±0.9 and 546±13 MPa, respectively. In addition, the *TS* and *EM* values of
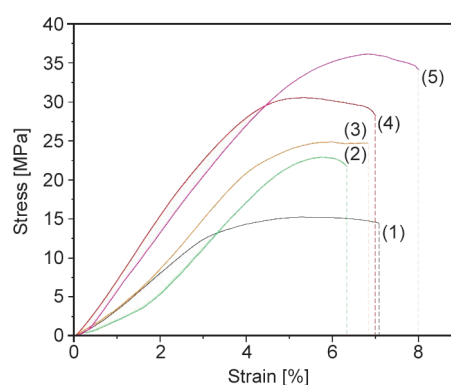

Figure 7. The stress–strain curves of the (1) neat PLA, (2) PLA/CS, (3) PLA/CS_2%TMSPM, (4) PLA/CS_5%TMSPM and (5) PLA/CS_10%TMSPM laminated films.

Table 3. The tensile properties of the neat PLA and PLA/chitosan laminated films.

Sample	Tensile strength [MPa]	Young's modulus [MPa]	Elongation at break [%]
PLA	14.6±0.6	485±12	7.1±0.1
PLA/CS	21.9±0.9	546±13	6.3±0.2
PLA/CS_2%TMSPM	23.6±1.5	584±20	6.8±0.2
PLA/CS_5%TMSPM	30.5±0.8	777±8	7.0±0.1
PLA/CS_10%TMSPM	35.8±1.1	935±11	8.0±0.2

Each value was presented as a mean ± standard deviation.

the laminated films noticeably increased by grafting with TMSPM up to 10 mol%. The improvements were attributed to the better dispersion of the silane and particularly to the reinforcement of interfacial bonds between PLA and chitosan. The EB or fracture strains of the neat PLA and PLA/CS film were 7.1±0.1 and 6.3±0.2%, respectively. It is worth noting that laminated with the unmodified chitosan layer led to a reduction of *EB* due to the interfacial interactions between the hydrophobic PLA and the hydrophilic chitosan were weak [37] or may be due to the nature of chitosan with a highly crystalline structure that could impede the chain mobility of PLA. At the same time, the EB of the laminated films increased by grafting with TMSPM up to 10 mol%, which could be attributed to the enhanced interfacial adhesion between the neat PLA and the modified chitosan or may be due to the synergistic effect of both layers.

3.1.5. Barrier properties

The barrier performance of the packaging is one of the most significant properties, especially for suitable to use. For this, the results are summarized in Table 4. The *WVP* of neat PLA was $6.1\pm0.6\cdot10^{-7}$ (g·m)/(m²·Pa·s), which indicated that the surface of PLA is strongly hydrophobic due to the subsistence of the methyl group (–CH₃), which is a non-polar and covalent bond [38]. When laminated with the unmodified chitosan layer, the *WVP* increased up to $14.6\pm0.8\cdot10^{-7}$ (g·m)/(m²·Pa·s). This suggested that the chitosan has a greater water affinity which could assist the transport of water molecules through the film due to its hydrophilic properties or expected to be caused by the water absorption and desorption of the PLA/CS laminated film [39, 40]. Interestingly, the *WVP* values of the laminated films decreased when the grafting with TMSPM up to 10 mol% compared to the unmodified, due to the improved hydrophobicity and adhesion between

Table 4. Water vapor permeability of the neat PLA and PLA/chitosan laminated films.

Sample	*WVP* $[10^{-7}$ (g·m)/(m²·Pa·s)]
PLA	6.1±0.6
PLA/CS	14.6±0.8
PLA/CS_2%TMSPM	10.6±0.5
PLA/CS_5%TMSPM	9.9±0.4
PLA/CS_10%TMSPM	9.1±0.4

Each value was presented as a mean ± standard deviation.

both layers, including TMSPM still acted as a super-hydrophobic material as well [41].

3.2. The influence of grafting on mechanical-thermal behavior

The mechanical-thermal behavior of the films was examined by dynamic mechanical analysis. Figure 8 shows the correlation between storage modulus and tan δ *versus* temperature. The modified laminated film has a higher storage modulus value than the unmodified film and the neat PLA. This indicated that the modified film has the greatest stiffness due to good interfacial adhesion between PLA and the modified chitosan. In addition, according to the tan δ profile, the glass transition temperature of the neat PLA was 62.9 °C. When laminated the unmodified chitosan layer on PLA, the glass transition temperature decreased to 58.4 °C. Interestingly, the modified laminated film showed a T_g value that is close to the neat PLA whereby the T_g of the modified film was 62.0 °C. This indicated that the neat PLA and the modified chitosan have good interfacial adhesion [42].

3.3. The influence of grafting on the surface wettability and surface energy

The contact angle, surface energy, dispersive, and polar component values of the PLA/chitosan laminated films and compositions are shown in Table 5. The neat PLA film revealed the contact angle that was tested with distilled water, DMF, and ethanol was 80.3±0.6, 28.4±0.5, and 21.9±0.3°, respectively. When compared with the neat chitosan film, the contact angle will increase due to the nature of chitosan with hydrophilicity. Interestingly, the influence of grafting with silane couplings resulted in a significantly reduced contact angle of the modified chitosan film. This indicated that the modified chitosan
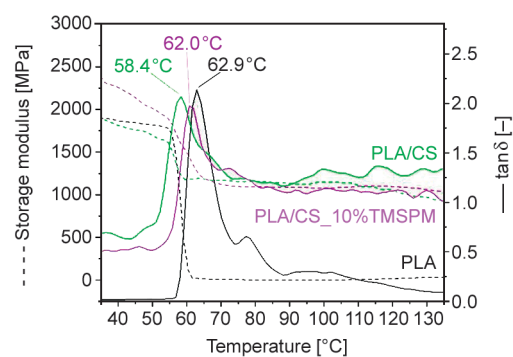

Figure 8. Dynamic mechanical analysis of the films. Whereby the black line is PLA, green is PLA/CS and purplei is PLA/CS_10%TMSPM laminated films.
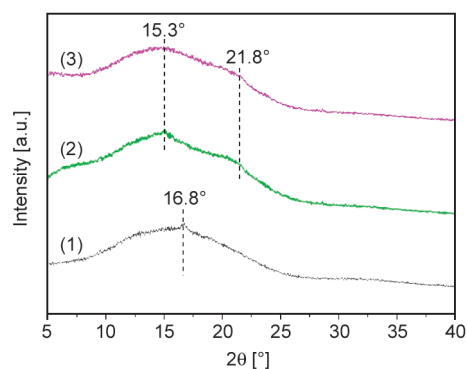
Table 5. Contact angle, surface energy (*SE*), dispersive (γ^D) and polar (γ^P) component values of the PLA/chitosan laminated films and compositions.

Sample	Contact angle [°]			*SE* [mN/m]	γ^D [mN/m]	γ^P [mN/m]
	Water	DMF	Ethanol			
PLA	80.3±0.6	28.4±0.5	21.9±0.3	27.0	17.1	9.89
CS	99.3±0.5	40.3±0.6	18.7±1.1	29.7	29.5	0.18
CS_10%TMSPM	90.9±0.3	28.9±0.2	14.2±1.1	28.6	26.6	2.02
PLA/CS	97.1±0.5	43.6±0.3	27.9±0.7	26.2	25.3	0.87
PLA/CS_10%TMSPM	96.6±0.2	41.2±0.7	25.2±0.9	27.0	26.1	0.85

had reduced hydrophilicity. In addition, it was observed that the contact angle of the PLA/CS laminated film that was tested with distilled water, DMF, and ethanol was 97.1±0.5, 43.6±0.3, and 27.9 ± 0.7°, respectively. The contact angle values of the laminated film will decrease slightly when grafting the chitosan layer with 10 mol% of TMSPM whereby the contact angle of the PLA/CS_10%TMSPM laminated film that was tested with distilled water, DMF, and ethanol was 96.6±0.2, 41.2±0.7 and 25.2±0.9°, respectively. This indicated that the modification of chitosan with TMSPM will reduce the polarity of the laminated film due to the hydrophobic nature of TMSPM and could be confirmed by the polar component value, which decreased from 0.87 to 0.85 mN/m [43]. Moreover, the grafting with TMSPM resulted in an increase in the surface energy and dispersive component values due to the grafting of chitosan with TMSPM was more compatible with the PLA layer and also decreased the hygroscopic property of the modified laminated film as well.

3.4. Assessment of the crystallinity of the films
X-ray diffraction was used to evaluate the crystallinity of the materials. The XRD profiles of the neat PLA, PLA/CS, and PLA/CS_10%TMSPM films are shown in Figure 9. According to the XRD pattern,

the neat PLA showed a broad diffraction peak at 16.8°. This indicated that this biopolymer has a rather amorphous structure [44]. When laminated with the unmodified chitosan layer, the film revealed 2 peaks at 2θ of 15.3 and 21.8°. These peaks correspond to the crystalline phase of chitosan [45]. Furthermore, the PLA/CS_10%TMSPM laminated film specimen didn't show any noticeable change compared to the PLA/CS laminated film.

3.5. Assessment of the viable bacterial counts after antimicrobial activity testing of the films
Antimicrobial activity of the neat PLA, PLA/CS, and PLA/CS0.1TMSPM films were tested with *E. coli* and *S. aureus* bacteria. The results were reported as the bacterial counts after testing according to Table 6. The PLA film that was laminated with the unmodified and modified chitosan showed better antimicrobial activity than the PLA film. This suggested that chitosan has an antibacterial activity to both *E. coli* and *S. aureus* [46]. In addition, each laminated film also showed better against *E. coli* than *S. aureus* due to chitosan being less active against gram-positive bacteria than gram-negative bacteria [47]. The antimicrobial mechanism of chitosan is caused by the positive charge of NH_3^+ (cationic amino group) on the chitosan molecule that reacted with the negative charge of each bacterial. As a result, the cell wall is damaged and unable to control the passage of nutrients causing

Figure 9. XRD pattern of (1) the neat PLA, (2) PLA/CS and (3) PLA/CS_10%TMSPM laminated films.

Table 6. The viable bacterial counts after testing the antimicrobial activity of the films against *E. coli* and *S. aureus* bacteria.

Sample	Bacterial counts [log (CFU/ml)]	
	E. coli	*S. aureus*
PLA	4.46	7.46
PLA/CS	3.34	4.39
PLA/CS_10%TMSPM	N.G.	4.39

N.G. – No growth was observed.

the cells to eventually die [48]. Moreover, it may also be caused by chitosan molecules when entering the bacterial cell, it will bind to DNA resulting in the inhibition of RNA and protein synthesis, or it can bind to metal ions and essential nutrients that cause the bacteria to starve and die [49]. Interestingly, the grafting of 3-(trimethoxysilyl)propyl methacrylate onto chitosan led to the film has an increase in antimicrobial activity compared to the neat PLA and PLA/CS film due to the hydrophobicity of the PLA/CS_10%TMSPM laminated film delayed bacterial growth. Furthermore, most bacteria commonly favor adhering and growing on the surface hydrophilicity [50].

4. Conclusions

This research revealed the preparation of the laminated films between PLA and chitosan by improving the interfacial adhesion between both layers via the grafting of chitosan with 3-(trimethoxysilyl)propyl methacrylate was successfully prepared. It was observed that the modified laminated films showed better characteristic properties compared to the unmodified laminated films. In this research, the PLA/CS_10%TMSPM showed the best properties. So, this confirmed that the grafting of 3-(trimethoxysilyl)propyl methacrylate could enhance the interfacial adhesion hydrophobicity, including improving the performance of the films. Importantly, the grafting also resulted in the prepared laminated film having an excellent antimicrobial activity against *E. coli* as well. Finally, this research is one of the interesting alternatives to developing food packaging in the future to replace petroleum-based food packaging.

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