Enhancing antioxidative property of LDPE by additional ethanol organosolv lignin (EOL) extracted from eucalyptus

Nutchapon Horakul and Navadol Laosiripojana

The Joint Graduate School of Energy and Environment, Center of Excellence on Energy Technology and Environment, King Mongkut's University of Technology Thonburi, 126 Pracha Uthit Rd., Bang Mod, Thung Khru, Bangkok 10140, Thailand

Abstract: Lignin, a biopolymer which can be found in lignocellulosic materials, is the second most abundant from cellulose. Unlike cellulosic compounds, lignin has an equivocal structure which is the limitation for the industrial applications. Since lignin has a great antioxidative property, it has a capability to incorporate with the conventional plastics for the purpose of shelf-life extension as packaging materials. In this work, Ethanol organosolv lignin (EOL), a sulfur-free lignin, was extracted from eucalyptus sawdust by using ethanol as an organic solvent. The profiling of EOL was done via gel permeation chromatography (GPC), Scanning electron microscopy (SEM), and Fourier transform infrared spectroscopy (FT-IR). EOL from eucalyptus was undergone the partial depolymerization via mild ethanolysis (160, 200 °C for 1 h) to homogenize the lignin by lowering molecular weight (MW) and increasing radical scavenging activity (RSA). Eucalyptus organosolv lignin (EL) has M_w of 1758 Da and 1.48 for P_d while the product at 160 °C (ESL160) and at 200 °C (ESL200) has M_w of 1643 Da, 1.42 for P_d and M_w of 1613 Da, 1.41 for P_d. Lignin samples including EL, ESL160, and ESL200 were then blended with LDPE and produced film, which was tested toward antioxidant activity. DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was used to evaluate the antioxidant activity of LDPE/lignin films and reported in the value of %RSA. It was found that, at 1%wt of lignin in LDPE, %RSA of LDPE/EL, LDPE/ESL160, and LDPE/ESL200 were 17.83, 27.65, and 27.54, respectively.

Keywords: Lignin, mild ethanolysis, depolymerization, LDPE, antioxidant activity.

1. Introduction

Lignin is an amorphous, highly branched polyphenolic macromolecule of complex structure with high molecular weight. The chemical structure of lignin is highly irregular and extremely challenging. Due to its complex nature and undefined chemical structure, the industrial applications of lignin are rather limited. The significant factor for integrating lignins more efficiently into polymeric materials is to establish compatibility between lignin and its petroleum-based counterparts. Lignin incorporation in polymers for application of biomaterials is not limited only by the reactivity of its various chemical composition and functional groups but rather by other complex factors such as solubility, molecular weight, rheology, dispersity, and morphology. Molecular weight or size distribution of different polymeric fragments in lignin impacts their performance during copolymerization [1].

Among various delignification processes, the type of lignin is determined by four main chemical pulping processes which are kraft, sulfite, soda, and organosolv. Focusing on the organosolv lignin, the term organosolv refers to the most alternative pulping process that utilizes mixtures of organic solvents with water as the pulping media. Ethanol is the most commonly used organic solvent in this process. The lignin contains considerable amounts of free phenolic hydroxyl groups as well as condensed units. Lignin recovered from this process is often free of carbohydrate contaminants. The sulfur-free lignin has higher purity with lower ash content and lower molecular weight than other lignin samples. [1] With these advantages, organosolv lignin could be a good choice for plastic filler. Several studies have been focusing on adding lignin to various types of plastic as it can enhance the UV-absorption ability and antioxidant activity which can be applied as food packaging materials [2-3].

Antioxidants are compounds that capture and stabilize the free radicals which are originally being generated in the presence of oxygen. Antioxidants have significant role in medical, food, and chemical industries [4]. In the present day, several commercial antioxidants take the main share in the market. However, natural antioxidants are taking more interests due to the nontoxicity [5]. Previously, several studies have confirmed that lignin has great potential on antioxidant activity because of the phenolic hydroxyl groups (Ph-OH), methoxyl groups (OMe), and carboxyl groups. In addition, this property of lignin can be enhanced via several lignin upgrading procedures. In order to have better antioxidative property, lignin need to have greater amount of phenolic hydroxyl groups (Phe-OH) and methoxyl groups (OMe), less aliphatic hydroxyl groups (Aliph-OH), lower molecular weight, and narrower polydispersity index (P_d) [6].

Partial depolymerization is one approach that is capable of producing lignin fragments with high homogeneity and better properties. Partial lignin depolymerization can be done in various methods. Yang et al. [7] have done partial depolymerization (selective β-O-4 cleavage) of lignin with the presence of concentrated lithium bromide (LiBr) and acid catalyst and successfully reduced the MW of lignin. Xin et al. [8] have successfully done partial depolymerization of lignin via mild hydrogenolysis in hydrogen atmosphere with the presence of Raney nickel catalyst. Lignin products have lower MW and higher Phe-OH groups. Zhao et al. [9] have also done the hydrogenolysis with the presence of formic acid and Pd/C catalyst that resulted in lignin with highly improved antioxidant activity. On the other hand, Li et al. [10] have done it via enzymatic depolymerization, with the presence of laccase. The lignin products have significantly improved as higher Phe-OH and lower MW were obtained but the retention time of this process was found to be up to 6 hours. Since all

processes needed catalysts of enzymes, Kim et al. [6] suggested the mild ethanolysis of soda lignin without any catalyst. The reason of using ethanol is that ethanol can directly facilitate the C-O cleavage while capable of inhibiting the repolymerization. This procedure was interesting because the reaction conditions were mild, and no catalyst was used. Lignin products are called solubilized lignin (SL) which can be recovered from the solvent after the reaction was completed. Surprisingly, the results were spectacular, SL have almost a half lower on M_w and a significantly narrower P_d while having antioxidant activity as great as ferulic acid and guaiacol. However, there is no information on partial depolymerization of EOL by ethanolysis and whether it can increase the total Phe-OH, OMe or only decrease molecular weight and narrow P_d. This needs to be further studied.

Since there has been no previous research focusing on the partial depolymerization of organosolv lignin to homogenize and enhance antioxidative properties via mild ethanolysis, these investigations were performed in this study. Higher antioxidative properties are expected to be obtained by incorporating less lignin, also minimizing changes in film color. In this work, Ethanol organosolv lignin (EOL), a sulfur-free lignin, was extracted from eucalyptus sawdust by using ethanol as an organic solvent. The EOL was then blended with LDPE to produce a film that was then tested toward antioxidant activity.

2. Materials and Method

2.1 Raw materials and chemicals

Eucalyptus wood chips were dried at 107 °C in hot air oven for 3 days then kept in dried place. Organosolv lignin (German grade) was provided from Chemical Point. Ethanol 99.9% (Analytical grade) was purchased from Qrec, New Zealand, ethanol 70% (Lab grade) was purchased from Peter Mediphar, Thailand, methanol (AR grade) was purchased from MERCK, DPPH was purchased from CTi&Science, methane sulfonic acid (MSA) was purchased from Sigma Aldrich. LDPE pellets (InnoPlus LD2426K) were received from PTTPM, Thailand.

2.2 Lignin Fractionation

Ethanol organosolv lignin (EL) is obtained by the reaction as follow. Eucalyptus wood chips (160 g) and 1120 mL of 50% ethanol (7:1 solid per solvent ratio) are loaded into 2 L Parr reactor. The reactor is heated up to 200 °C and hold for 50 min. Then, the reactor is rapidly quenched to a room temperature. The pulp is firstly separated from the black liquor by rough mesh then follow by vacuum filtration using Whatman filter paper No.4. To lower the pH of black liquor, 3360 mL of DI water is added to precipitate out the lignin. The suspension is leaved overnight in the closed container then vacuum filtrated using Whatman filter paper No.2. The filtrate is re-filtered until the clear filtrate is obtained. Lignin on the filter paper is dried in the oven at 50 °C until no weight change then kept in desiccator.

2.3 Partial Depolymerization

Partial depolymerization is done at 160 and 200 °C, 1 h for both types of lignin (CL and EL) in 300 mL Parr reactor. 4g of each lignin is loaded into the reactor and filled with 100 mL of ethanol (AR grade). After the reaction was completed, the reactor is rapidly quenched to a room temperature. The liquid product is collected and centrifuged at 3500 rpm for 20 min to remove the insoluble lignin. Then oven dried until no weight change. Lignin is ground into a powder and kept in desiccator.

2.4 LDPE/lignin films preparation
2.4.1 Pelletization

Lignin is melt-blended with LDPE pellets (2%wt of lignin) by a single screw extruder (HAAKE PolyLab OS Rheodrive

7 with Rheomix OS) equipped with standard metering screw 3:1, L/D: 25. Screw temperature ranges from 150 to 170 °C. Rod die with 3.0 mm nozzle is used and the die temperature is set at 170 °C. LDPE/lignin composite is extruded and then cut into pellets.

2.4.2 Blown Film Extrusion

LDPE/lignin pellets were mixed with LDPE pellets to make two different LDPE/lignin films (0.5%wt and 1%wt). Single screw extruder with similar operation parameters as pelletization process was used, annular slit die with 30 mm nozzle is used. Blow up ratio (BUR) is 2.75.
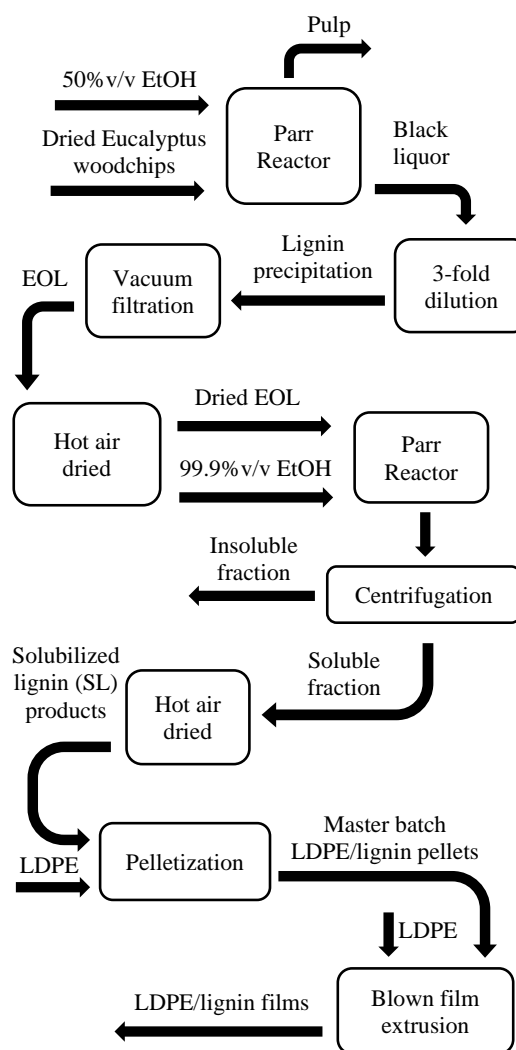

Figure 1. Overall process of EOL fractionation, partial depolymerization and film forming.

2.5 Structural Characterization and molecular weight distribution of lignin

Functional groups of lignin were determined by FTIR (PerkinElmer Spectrum One QP2010). Lignin was mixed with KBr at the ratio of 0.25%wt lignin to KBr. Pure KBr disc was used as a background. The scan was done from 450-2000 cm^{-1} with 64 scans and the resolution of 4 cm^{-1}.

SEM images were obtained by Hitachi Schottky FE-SEM SU5000 with the acceleration voltage of 3000 V. Lignin and film samples were gold coated. Three images were taken for each sample at 500x, 4000x, and 8000x.

The molecular weight of lignin samples was determined by gel permeation chromatography (GPC). Lignin samples were dissolved in tetrahydrofuran (THF) at the concentration of

0.2%w/v and filtrated with 0.2 μm nylon syringe filter before injected. The analysis was performed on Waters e2695 HPLC system equipped with PL gel 10 μm mixed B 2 columns operated at 35 °C, RI detector was used and operated similarly at 35 °C. THF was used as the eluent (1.0 mL/min), the injection volume was 100 μL. Polystyrene standard (MW 1250-920000) were used to calibrate the instrument.

2.6 Antioxidant activity of LDPE/lignin films

Antioxidant activity is reported in radical scavenging activity (RSA). The RSA of films is measured by using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). Firstly, 350 mg of films were placed in the 100 mL Erlenmeyer flask and 50 mL of methanol was added. All samples were leave at room temperature for 24 h. Then, 100 μL each sample was transferred to a 96 well plate and added with 100 μL of 0.08 mM DPPH/methanol solution. The samples were incubated in the dark for 30 min. The absorbance is determined at 517 nm. %RSA is calculated as follow

$$RSA\ (\%)=1-\left(\frac{A_{sample}}{A_{control}}\right) x\ 100$$

Where A_{sample} is the absorbance of the film samples in DPPH solution while $A_{control}$ is the absorbance of the DPPH solution without film samples.

3. Results and Discussion

3.1 Functional groups of lignin

The IR spectra of commercial organosolv lignin (CL) and eucalyptus ethanosolv lignin (EL) are shown in figure 2. These 2 lignins almost have the identical peaks. The 1710 cm⁻¹ peak generated by non-conjugated carbonyl, CL exhibits a significantly stronger band which may results from the source of lignin and fractionation process. CL also has stronger peak at 1601 cm⁻¹ which represents the aromatic ring in lignin structure. Slightly differences are observed in 1513 cm⁻¹, 1460 cm⁻¹, and 1423 cm⁻¹ absorption bands which is aromatic ring, C-H deformation in methyl and methylene, and aromatic ring stretching with in-plane C-H deformation, respectively. EL exhibits the strong band at 1330 cm⁻¹ which is the characteristic of hardwood lignin, this band is resulted from syringyl ring breathing with CO-stretching. Comparing to CL, EL shows a significantly stronger absorption band means higher S-unit. On the other hand, guaiacyl ring breathing with CO-stretching which is the peak at 1271 cm⁻¹ of CL is much stronger than EL. This indicates the larger amount of G-units in CL. Peak at 1120 cm⁻¹ which indicates aromatic C-H in-plane deformation of S-unit, EL has stronger peak.

For the partial depolymerization lignin, figures 3 and 4 are the IR spectra of CSL and ESL, respectively. Both CL and EL has no functional group changes during partial depolymerization process since the IR absorption band are identical for both CL, CSL160, CSL200 and EL, ESL160, ESL200.
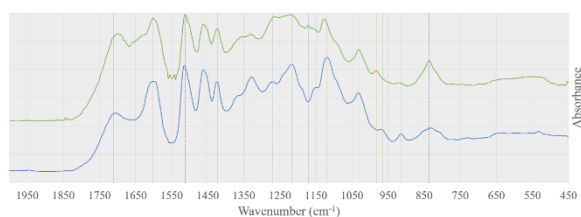
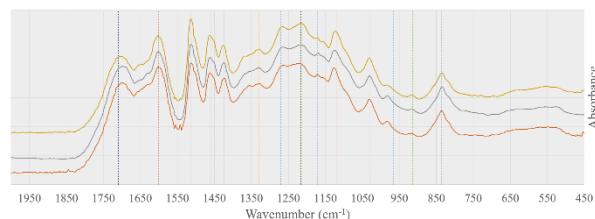**Figure 2.** IR absorption spectra of EL (blue) and CL (green).

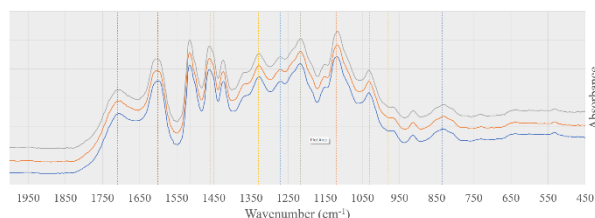**Figure 3.** IR absorption spectra of CL (blue), CSL160 (gray), and CSL200 (yellow).

Figure 4. IR absorption spectra of EL (blue), ESL160 (orange), and ESL200 (gray).

3.2 Surface morphology

SEM images for EL, ESL160, and ESL200 are shown as figure 5a, 5b, and 5c, respectively. From figure 4, lignin morphology is changed through the partial depolymerization process. Noticeably changes to physical structure of EL can be seen. The original EL has sphere and round morphological structure. After mild ethanolysis, the particle size is slightly reduced, and the porosity is increased due to the multi-edges shape. ESL seems to be more crystalline than the original EL.
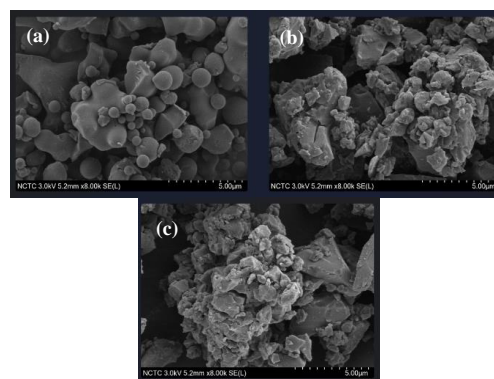
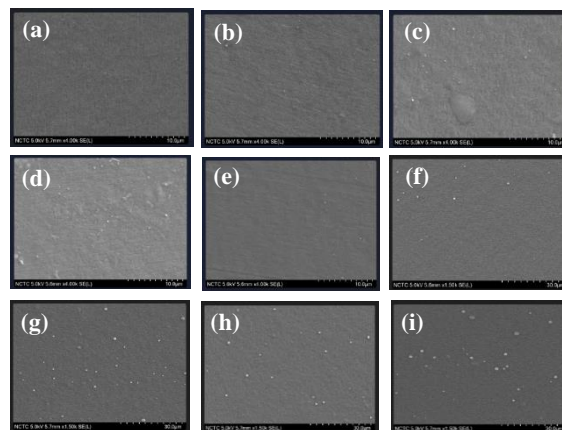**Figure 5.** SEM images of EL, ESL160, and ESL200 (a, b, and c, respectively).

Figure 6. SEM images of (a) Neat LDPE, (b) LDPE/CL-0.5, (c) LDPE/CL-1.0, (d) LDPE/EL-0.5, (e) LDPE/EL-1.0, (f) LDPE/ESL160-0.5, (g) LDPE/ESL160-1.0, (h) LDPE/ESL200-0.5, (i) LDPE/ESL200-1.0.

From figure 6, lignin shows great compatibility with LDPE given smooth film surfaces. Few more speckles appeared on the surface of LDPE with ESL blended because of the mild ethanolysis reduced thermal stability of lignin.

3.3 Molecular weight distribution

Weight-average molecular weight (M_w), number-average molecular weight (M_n), and polydispersity index (P_d) of lignin before and after partial depolymerization process are shows below in table 1. It can be clearly seen that EL has lower molecular weight and narrower P_d compared to CL. For CL increasing temperature results significantly lower values for all M_w, M_n, and P_d. Which means that mild ethanolysis is successfully breakdown CL into smaller molecules by mainly cleaved β-O-4 linkage and also the formation of OMe side chains [6]. For EL, M_w, M_n, and P_d are slightly decreased which be conclude that the mild ethanolysis for EL, the main reaction is the formation of OMe side chains rather than the β-O-4 cleavage. Lignin with a lower polydispersity index indicates a narrower distribution in molecular weight which favors the polymer-filler application because of the enhanced miscibility. Low Pd indicates great distribution of lignin in the polymers [11].

Table 1. GPC results of solubilized lignin.

Sample	M_n	M_w	P_d
CL	1758	3268	1.859
CSL160	1379	2097	1.520
CSL200	1204	1756	1.458
EL	1184	1758	1.484
ESL160	1159	1643	1.418
ESL200	1141	1613	1.413

3.4 Solubilized Lignin Yield

Table 2 shows the %yield of lignin after partial depolymerization. CSL160 and CSL200 have %yield of 53.75% and 50.75%, respectively. As CL has a high molecular weight thus, the solubility in ethanol is poor. Meanwhile, the solubilized lignin yield is low. On the other hand, ESL yields are much higher than that of CSL because of the lower molecular weight and its nature since the fractionation process is ethanol organic solvent makes it more soluble in pure ethanol. Increasing the reaction temperature may causes the degradation of lignin and results the lower yield of solubilized lignin. Similarly, ESL160 has higher yield than ESL200. For CSL by increasing the temperature from 160 °C to 200 °C, results 5.66% decrease in yield while 13.71% for ESL. This is because of the nature of lignin which effect the thermal stability. In case of optimization, several parameters must be further studied.

Table 2. Solubilized lignin yield.

Sample	%Yield
CSL160	53.75
CSL200	50.75
ESL160	80.25
ESL200	69.25

3.5 Antioxidant activity

The antioxidant activities of LDPE/lignin films were reported as radical scavenging activity (%RSA) in table 3. The %RSA is directly proportional to the antioxidant activity. Thus, a high %RSA means a greater antioxidant [property.

Table 3. Antioxidant activity of LDPE/lignin films.

Sample	%RSA (at 30 min)
LDPE	3.39 ± 0.12*
LDPE/CL0.5	6.09 ± 0.23*
LDPE/CL1.0	10.05 ± 0.46*
LDPE/EL0.5	9.71 ± 0.36*
LDPE/EL1.0	17.83 ± 0.75*
LDPE/EL160-0.5	20.43 ± 0.77*
LDPE/EL160-1.0	27.65 ± 1.22*
LDPE/EL200-0.5	12.42 ± 0.48*
LDPE/EL200-1.0	27.54 ± 1.07*

*Note: Statistical Error

Neat LDPE almost has relatively low radical scavenging activity. By incorporating lignin into LDPE, the composites show significantly improvement in radical scavenging activity. Comparing CL and EL at the same concentration, for 0.5%wt lignin incorporated, EL shows better result with 59.44% higher than that of CL while 77.41% better when the lignin content is high up to 1.0%wt. Several studies have information about the antioxidative property of lignin. Molecular weight and phenolic hydroxyl group (Ph-OH) are the contributor to the antioxidant activity [6,9,10]. EL has lower molecular weight than CL which can be concluded that lignin with lower molecular weight and narrower polydispersity index has higher Ph-OH groups [6,8,9]. Ph-OH groups scavenge free radicals through three main mechanisms: hydrogen abstraction transfer (HAT), proton coupled electron transfer (PCET), and sequential proton loss electron transfer (SPLET) [12,13]. The free radical scavenging activity of lignin is based on the hydrogen donating ability and the stability of the free radical intermediate form during the scavenging reaction [14]. Since the hydrogen donation of lignin is mainly from the Ph-OH groups, that is why Ph-OH content has a significant role in antioxidant activity of lignin [10].

Moreover, the amount of phenolic sub-units (H, G, and S) is also account for the antioxidant property of lignin. The different among these 3 sub-units is the number of methoxy groups (OMe). H-unit has no OMe group while G-unit has one, and S-unit has two. In radical scavenging mechanism, OMe group has to stabilize the Ph-OH radical after lignin has donated hydrogen from Ph-OH to capture DPPH molecules [6]. Antioxidant activity is mainly determined by the formation of Ph-OH radicals to capture the free radicals. More Ph-OH radicals would form if the bond dissociation enthalpy (BDE) was lowered. By having OMe group as a side chain especially in *ortho*-position, BDE can be significantly lowered [9]. Comparing the antioxidant activity of phenolic sub-units, S-unit is the main contributor followed by G-unit and H-unit [3]. As EL is hardwood lignin thus, higher S-unit than that of CL, the better antioxidant activity is obtained.

For antioxidant activity of ESL, significant improvement can be seen from the results. Comparing at the same lignin content (1.0%wt), ESL160 and ESL200 shows almost the same antioxidant activity and both of them are about 55% better than the EL. Even though, the molecular weight and polydispersity index of both ESL160 and ESL200 have not significantly decreased from the EL during the partial depolymerization process means only few β-O-4 bonds have been cleaved and produced more Ph-OH groups [6,9]. The possible reason to explain the results is the increasing of G and S-unit by the formation of OMe side chains which improve the antioxidant activity of the LDPE/lignin composites [6]. Further investigation such as ^{31}P-NMR or Pyrolysis-GC/MS must be done to confirm

the reason of better antioxidant activity through the mild ethanolysis partial depolymerization reaction.

4. Conclusions

Mild ethanolysis, did not significantly change the functional group of lignin. On the other hand, it significantly reduced the molecular weight and polydispersity index (P_d) especially the CL due to a higher initial molecular weight. However, it was still able to reduce the molecular weight of eucalyptus organosolv lignin which make it more applicable for blending with conventional polymers as P_d described how well it dispersed in the polymer composite. ESL has a higher yield than CSL because it was fractionated by using ethanol as a solvent. Eventually, with 1%wt of ESL, the antioxidant activity of film composite was improved about 55% when the mild ethanolysis temperature was 200 °C compared to the EL. However, ^{31}P NMR is suggested to demonstrate the effect of mild ethanolysis on lignin structure. Furthermore, tensile properties have to be done to find out how lignin affects the mechanical properties of the composite films.

Acknowledgements

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