

# Preparation and Characterization of Polyhydroxybutyrate-Bamboo Lignophenol Biocomposite Films

Shuang Qian, Xin Dai, Yangling Qi, and Hao Ren \*

Four kinds of lignophenols (Lps) were derived from native bamboo (*Sinocalamus affinis*) lignin through phase separation system by using four kinds of phenols (*p*-cresol, catechol, resorcinol and pyrogallol) as derivatives and 72% concentrated sulfuric acid as catalyst. The resulting lignophenols were characterized by <sup>1</sup>H-NMR, FT-IR, and GPC analysis. Then they were blended with polyhydroxybutyrate (PHB) to cast thin biocomposite films. The mechanical properties, water-absorbing qualities, and thermal properties of films were tested and discussed. The results indicated that phase separation treatment can effectively improve phenolic hydroxyl contents of lignins. The lignin macromolecule was significantly reduced to small size and well-soluble polymers. The best amounts of added Lps in composite films depended upon the kind of phenols. In present study, the mechanical properties, water-absorbing qualities, and thermal properties of biocomposite films showed good results at less than 10% Lps' addition. This provides a possibility that a new kind of biodegradable films can be made up of engineering plastics and lignin.

*Keywords:* Bamboo (*Sinocalamus affinis*); Lignophenol; Polyhydroxybutyrate (PHB); Biocomposite Films; Mechanical properties

*Contact information:* Jiangsu Provincial Key Lab of Pulp and Paper Science and Technology, Nanjing Forestry University, 159 Longpan Rd, Nanjing 210037, China;

\* Corresponding author: renhaomie@hotmail.co.jp (Hao Ren)

## INTRODUCTION

The development of commercially viable “green products” based on natural resources for both matrices and reinforcements for a wide range of applications is on the rise (Satyanarayana *et al.* 2009). Lignin, as the second most abundant renewable biopolymer in nature and an inexpensive and currently underutilized waste product from papermaking and biorefinery industries, has been a candidate for renewable materials; however, it is often difficult to blend with other biopolymer due to its complex and irregular structure. So it is necessary to modify lignin.

Phenol chemical modification is one kind of method to modify lignin. Recently a class of lignin-based functional polymers, the lignophenols, has originally been designed and their synthesis process from native lignin has been developed. This process includes the phase separation reaction system composed of phenol derivatives and concentrated acid. In the process, native lignin is modified by selectively grafting phenol derivatives to benzyl position. The separated lignin derivatives have several structural characteristics, for example being highly phenolic, very light colored (pinkish white) and highly stable, which is in contrast to conventional lignin (Funaoka *et al.* 1989).

Polyhydroxybutyrate (PHB) is accumulated by a wide variety of micro-organisms as an intracellular storage source of organic carbon and chemical energy. PHB has mechanical strength, elastic modulus, thermal moldability, and biocompatibility. It has attracted much attention as a biocompatible and biodegradable thermoplastic polymer, but its application has often been limited by its brittleness. In recent years, fillers from renewable source have been increasingly used in the preparation of PHB-based biocomposites.

Bamboo encompasses 1,250 species within 75 genera, most of which are relatively fast-growing, attaining stand maturity within five years, but flowering infrequently (Ribas *et al.* 2012). As an industrial raw material, the successful application of bamboo cases are currently focused on bamboo fibers and pulps (Sood *et al.* 2005), bamboo charcoal and vinegar (Cai *et al.* 2011), *etc.* However, there has not yet been large-scale development based on the lignin content of bamboo.

In this study, four kinds of lignophenols (Lps) were derived from bamboo (*Sinocalamus affinis*) lignin through a phase separation system (Funaoka 1998) by using *p*-cresol, catechol, resorcinol, and pyrogallol as phenolic derivatives. These products were named lignocresol, lignocatechol, lignoresorcinol, and lignopyrogallol, respectively. The structural characteristics of lignophenols are shown in Fig. 1. The upper left structure of Fig. 1 will be changing with the introduced phenols species. It can be observed from Fig. 1 that the content of hydroxyl groups in lignophenols was higher than in industrial lignin, especially the content of phenolic hydroxyl groups. The structural characteristics of lignophenols were analyzed by <sup>1</sup>H-NMR, FT-IR, and GPC. Based on that, the four kinds of Lps were blended with polyhydroxybutyrate (PHB) respectively to cast thin biocomposite films at ordinary temperatures and pressures. Then the mechanical and thermal properties of the obtained composites were tested by tensile strength instrument and thermogravimetry (TG). The results indicated that four kinds of lignophenols all had low molecular weights and high phenolic hydroxyl group contents. The mechanical strength, water absorbency, and thermal properties of composite films were increased with the addition of proper lignocresol content. And the elongation at break of these two kinds of composites were more than three times of pure PHB, which indicated that lignocatechol and lignoresorcinol were more suitable to develop composite films. However, it's still critically important to further modify side chains of lignocresol and lignodiphenol for the subsequent development of lignophenol-PHB composite film.

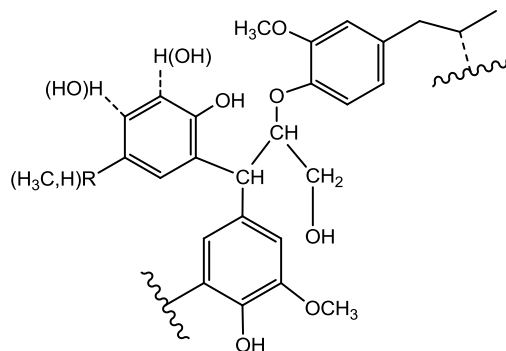


Fig. 1. Structural characteristics of lignophenols (Funaoka 1998)

## EXPERIMENTAL

### Materials and Chemical Reagents

Bamboo (*Sinocalamus affinis*) was grown in Ganzhou, Jiangxi, China. Internodal pieces of bamboo were mechanically liberated from the epidermis, cut into short rings (about 1 cm in length), and reduced to small pieces. Air-dried bamboo chips were ground successively in a Wiley mill and a vibrational mill to pass a 60-mesh screen, and extracted with ethanol-benzene (1:2, v/v) for 48 h. The moisture content of the sample was 8.12%. According to the wood chemistry analysis method (Ren and Funaoka 2007), the chemical components were as follows: Klason lignin: 23.95%; pentosan: 17.26%;  $\alpha$ -cellulose: 46.87%; ash: 1.32%; alcohol-benzene extractives: 4.40%. Benzyl-alcohol extracted bamboo meals were used as raw materials in the phase separation system.

Cresol, resorcinol, catechol, pyrogallol, and sulfuric acid were supplied by Sinopharm Chemical Reagent Co. Ltd, China.

Polyhydroxybutyrate (PHB) was from Solvay Advanced Polymer. The MFI for PHB was between 2 and 3 g/10 min (170 °C, 2.16 kg).

### Synthesis of Lignophenols

Synthesis of ligno-*p*-cresols was carried out according to the one-step process described in the preliminary literature report (Funaoka 1998). In order to save the amount of organic solvent, in the case of polyphenols (catechol, resorcinol, and pyrogallol), the two-step process method (process II) in the same publication (Funaoka 1998) was used. The 3 mol/C<sub>9</sub> (C<sub>6</sub>-C<sub>3</sub> basic unit of lignin) of polyphenols were dissolved in acetone and allowed to penetrate within the cell wall, after which the solvent was evaporated; this was followed by treatment with 72% concentrated sulfuric acid. The resulting lignophenol derivatives were separated from the carbohydrates by using acetone extraction from the water-insoluble fractions.

All kinds of lignophenols were collected by centrifugation and then vacuum dried to constant weight. The yields of lignophenols were calculated by using over dried weight of bamboo meals and Klason lignin, respectively.

### Physicochemical Characterization Analysis of Lignophenols

Gel permeation chromatography (GPC) of lignophenols was determined on a PL-GPC50 plus Integrated GPC System (Varian Inc. Company) equipped with Waters 2410 RID detector. Sepax Mono-GPC columns (100 Å, 300 Å, 500 Å, 10 mm ID×300 mm) were connected in series and tetrahydrofuran (THF) used as an eluent [flowing rate: 1.0 mL/min].

Calibration for weight-average molecular weight ( $M_w$ ), number-average molecular weight ( $M_n$ ), and polydispersity ( $M_w/M_n$ ) was performed using standard polystyrene. <sup>1</sup>H-NMR analyses of lignocresols were acquired with a BRUKER AVANCE III FT-NMR600 System. The amounts of combined cresols were calculated based on the signal intensity of cresolic methyl protons (1.6 to 2.4 ppm) against aromatic protons (7.8 to 8.4 ppm) of internal standard (*p*-nitrobenzaldehyde) on <sup>1</sup>H-NMR spectra. FT-IR spectra were obtained on Bruker VERTEX80 Spectroscopy using KBr discs. The spectra were recorded in the range from 400 to 4000 cm<sup>-1</sup> with a resolution of 4 cm<sup>-1</sup> over 32 scans.

### Preparation of Composite Films

PHB and lignophenols were dissolved in chloroform at 90 °C. Then the solution was cast on the teflon plate, and the solvent was evaporated at room temperature. Lignocresol content in the film was 0%, 3%, 6%, 9%, 12%, respectively. Three kinds of lignopolyphenols content were 3%, which were used to compare with the 3% lignocresol.

### Physical and Thermal Properties of Composite Films

The test specimens ([GB ISO 527-3: 1995 (E)], thickness of 1-1.5 mm, length of 33 mm and width of 6 mm) were prepared from the composite films. The tensile strength and elongation ratio were measured at the rate of 10 mm/min using EZ Test (CMT4303) at room temperature.

Thermogravimetry (TG) of the composite films was investigated using TG/DTA 6200 (SEIKO Instruments Inc.). TG was run at a heating rate of 10 °C/min in nitrogen, heating temperature range: 0 to 600 °C.

### Water Absorbency Test of Composite Films

Three pieces of small squares (1 cm × 1 cm) were cut from the films mentioned in preceding section. The initial weight ( $W_0$ ) was recorded. These small squares were immersed in deionized water at room temperature ( $25 \pm 2$  °C) for 4 h, and the samples were drained for 30 min for the elimination of excess nonabsorbed water. Then the weight of the swollen samples ( $W_s$ ) was recorded. The water absorbency was calculated using the following equation:  $Q$  (g H<sub>2</sub>O/g sample) =  $[(W_s - W_0) / W_0]$

## RESULTS AND DISCUSSION

### Yield of Lignophenols

The yields of lignophenols are shown in Table 1. The yields of four kinds of lignophenols relative to oven-dried (105 °C) bamboo meal were 14.0%, 16.0%, 14.7%, and 8.3% respectively, which relative to Klason lignin were 58.5%, 66.8%, 61.3%, and 34.6%. The lignophenol yield of bamboo was lower than softwood and hardwood under the same processing conditions (Funaoka 1998). The differences are attributable to the differences in frequency of condensed structures between bamboo and woody biomass (Ren and Funaoka 2007). Compared with the perennial wood, lignification of bamboo was lower. A large amount of low molecular weight lignophenols are thought to be lost during the multiple steps of organic solvent refining as solvent-soluble fractions. The yield of lignopyrogallol was much lower than others. This is based on better solubility in ether and aqueous solution, contributing to more phenolic hydroxyl groups (Akao *et al.* 2004). Therefore, the color of ether was darkest from the process of refining lignopyrogallol.

**Table 1.** Yield of Lignophenols

Sample	Yield (to bamboo meal, %)	Yield (to Klason lignin, %)
Lignocresol	14.0	58.5
Lignocatechol	16.0	66.8
Lignoresorcinol	14.7	61.3
Lignopyrogallol	8.3	34.6

## FTIR Spectra of Lignophenols

Four kinds of lignophenols and one sample of alkaline lignin were compared by FTIR analysis (Fig. 2). The alkaline lignin was obtained from black liquor of bamboo sulfate pulping. There were peaks at 1325, 1220, and 1130  $\text{cm}^{-1}$ , which belong to vibrations of the syringyl group, as well as 1270 and 1040  $\text{cm}^{-1}$ , which belong to the guaiacyl group in all of spectra. A distinct C=O vibration belonging to carboxyl or ester group at 1730  $\text{cm}^{-1}$  was found in all the lignophenols' spectra, which is attributed to the esterified coumaric acid in bamboo lignin (Ren 2007). In the FTIR spectra of alkaline lignin, the big peak at 1500 to 1800  $\text{cm}^{-1}$  was due to aromatic structure. Other observed peaks were wider than corresponding peaks in lignophenols; the overlapped areas are thought to be caused by impurities. The introduced phenols peaks were observed at 815  $\text{cm}^{-1}$  for lignocresol and 800 to 1000  $\text{cm}^{-1}$  for the other three lignophenols.

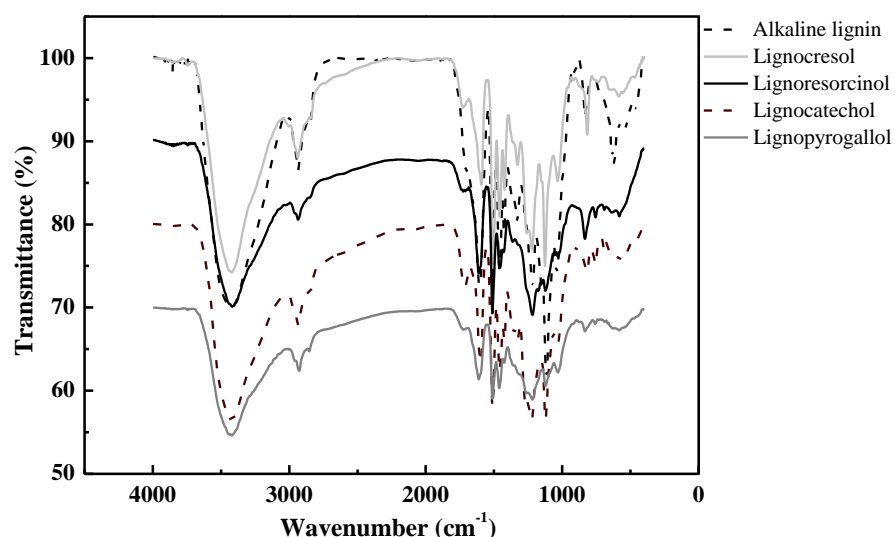


Fig. 2. FTIR Spectra of Lignophenols

## Molecular Weight Distribution of Lignophenols

Molecular weight distribution of lignophenols was determined using gel permeation chromatography. The results are shown in Table 2. The weight-average molecular weight of lignophenol obtained by phase separation system was 2500 to 4000 g/mol, and the polydispersity was 1.6 to 2.3. These results indicated that during the phase separation treatment, the random molecular structure of native lignin had been cleaved regularly. The molecular size and structure of lignophenols showed regularity.

Table 2. Molecular Weight Distribution of Lignophenols

Sample	$\bar{M}_n$ (g/mol)	$\bar{M}_w$ (g/mol)	$\bar{M}_w/\bar{M}_n$
Lignocresol	1800	2884	1.60
Lignocatechol	2450	4050	1.66
Lignoresorcinol	1117	2569	2.30
Lignopyrogallol	1964	3507	1.79

### Contents of Combined *p*-Cresol and Hydroxyl Groups of Lignophenols

The combined *p*-cresol content was calculated based on the reported literature (Funaoka and Fukatsu 1996). The content of hydroxyl groups was calculated depending on  $^1\text{H-NMR}$  spectra of lignocresols and acetylated lignocresols. The amounts of combined *p*-cresol, phenolic hydroxyl groups, and aliphatic hydroxyl group were 0.7 mol/C<sub>9</sub>, 1.2 mol/C<sub>9</sub> (Ca. 8.7%), and 1.0 mol/C<sub>9</sub> (Ca. 7.4%), respectively. The amounts of phenolic hydroxyl groups in ligno-*p*-cresol were 2 to 3 times larger than conventional lignins (Goldschmid 1954). The content of phenolic hydroxyl groups in cresol was 1.0 mol/mol, which is less than 2 or 3 mol/mol in catechol, resorcinol, and pyrogallol. Therefore, the amount of phenolic hydroxyl groups in lignopolyphenols would be larger than in lignocresol.

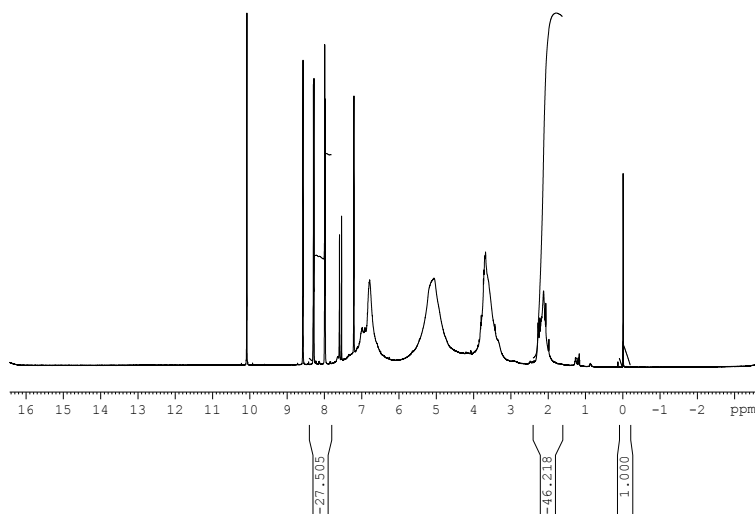


Fig. 3.  $^1\text{H-NMR}$  spectra of lignocresols

### Mechanical Strength Analysis of Composite Films

Polyhydroxybutyrate (PHB), as a kind of biodegradable polymeric materials, has mechanical strength, elastic modulus, thermal moldability, and biocompatibility, *etc.* However, its application on thermoplastic materials is limited due to high crystallinity (Furukawa *et al.* 2005). It is necessary to develop PHB based co-polymer or composites in order to meet industrial using purpose (Teramoto *et al.* 2004). In this study, lignophenols and PHB were mixed uniformly in chloroform then cast films at room temperature and pressure. Intermolecular interactions between lignophenol and PHB were expected to generate. A new kind of materials with better mechanical properties, lower crystallinity, and reduced brittleness was expected before experiments.

#### Tensile Strength

The tensile strength of lignocresol-PHB composite changing with lignocresol contents were shown in Fig. 4. The tensile strength of composite films presented the tendency of first increasing and then decreasing. When the lignocresol content was 3%, tensile strength was strongest. Then it weakened on further addition of lignocresols. However, tensile strengths of all composite films were stronger than pure polyhydroxybutyrate film. As expected, it was found to be beneficial to add lignocresol for improving mechanical strength of the composite films. Additionally, it was observed that the addition amount of lignocresol should be less than 15 to 20% during the

experimental process. When lignocresol content was over 12 to 15%, the process of casting films became difficult and the films began to be brittle. In conclusion, the optimum adding amount of lignocresol should be controlled less than 12%.

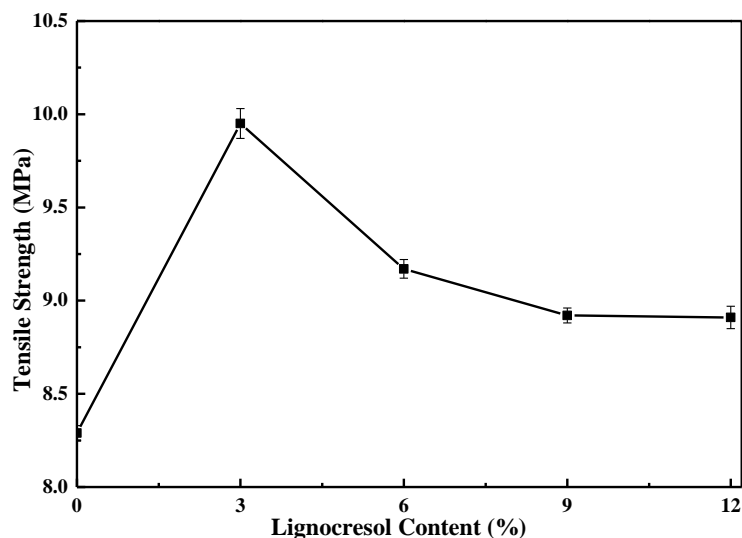


Fig. 4. Relationship between lignocresol content and tensile strength

The relationship between lignophenol species and tensile strength is shown as Fig. 5. Under the same addition amount of 3% lignophenols, lignopyrogallol-PHB film showed the weakest tensile strength. In comparison with the lignocresol case, tensile strengths of lignocatechol and lignoresorcinol cases were a little stronger but roughly the same. Although lignopyrogallol has more phenolic hydroxyl groups, the mechanical properties were worst among the four kinds. This indicated that the amount of phenolic hydroxyl group was not the only factor for improving composites properties. It is necessary to explore on increasing aliphatic hydroxyl groups content or lengthening side chains to further improve the mechanical properties of lignophenol-PHB composites.

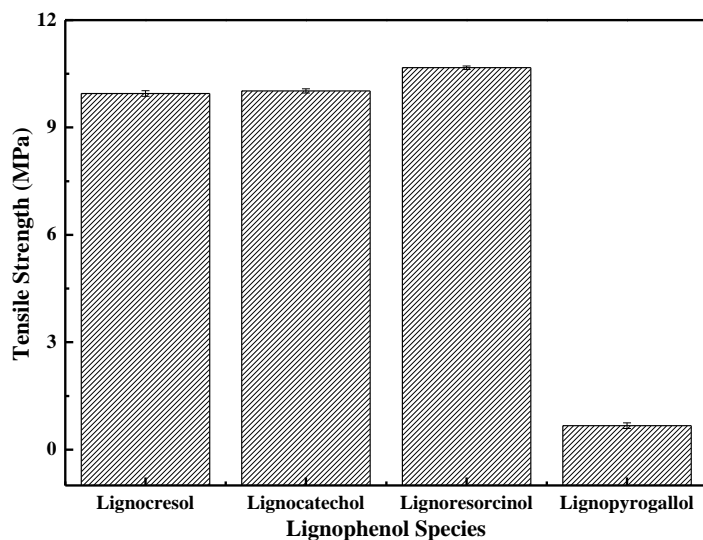
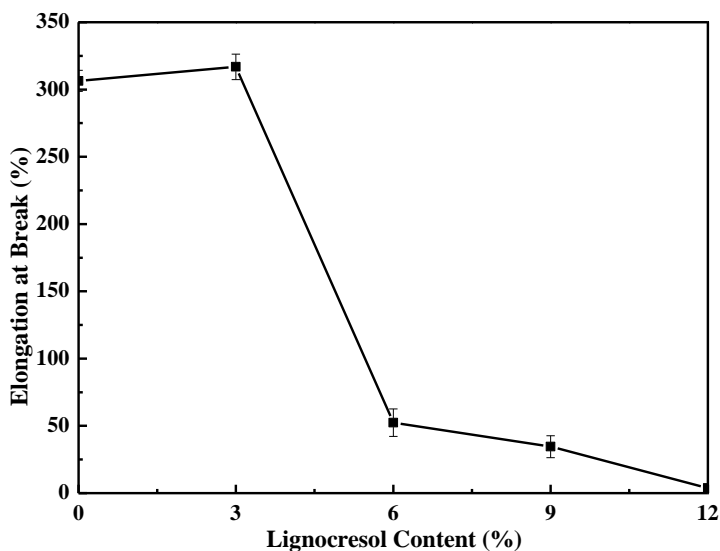


Fig. 5. Relationship between lignophenol species and tensile strength

### Elongation at break

The relationship between lignocresol content and elongation at break is shown as Fig. 6. The elongation at break of composite films increased within a narrow range on the addition of 3% lignocresol. When lignocresol content arrived at 6%, elongation at break decreased sharply. That means that the flexibility of biocomposite films will not be affected when lignocresol content is lower than 3%. The added amount of lignocresol could be controlled according to the application purpose.



**Fig. 6.** Relationship between lignocresol content and elongation at break

The relationship between lignophenol species and elongation at break is shown as Fig. 7. Under the same addition amount of 3% lignophenols, elongation at break of lignocatechol and lignoresorcinol cases were very strong, especially for the lignocatechol case, for which the elongation at break of composite films was more than three times that of pure PHB case. However, elongation at break of lignopyrogallol case was less than 70 times that of the pure PHB case. These data suggested that lignodiphenol is suitable for improving flexibility of composite materials, while lignotrisphenol is not suitable for improving both flexibility and strength. This may be a consequence of the change of the molecular stereo-structure of lignopyrogallol, which was caused by more hydroxyl groups, so that chemical or physical combination between PHB and lignopyrogallol was not formed. Thus, further modifying side chains of lignocresol and lignodiphenol will be a challenge for the subsequent development of lignophenol-PHB composite film.



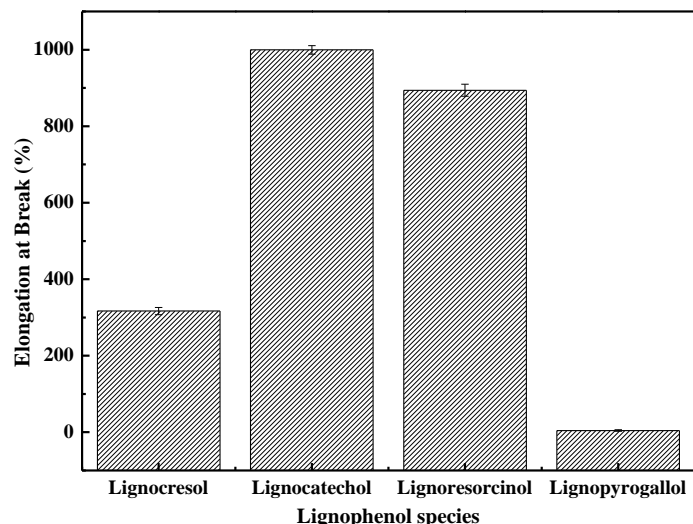


Fig. 7. Relationship between lignophenol species and elongation at break

### Water Absorbency of the Biocomposite Films

The relationship between lignocresol content and water absorbency is shown in Fig. 8. Water absorbency of composite films decreased with lignocresol content increasing at the range of 0 to 9%. This means that hydrophobicity of the composite films had been improved, which can be attributed to the introduction of hydrophobic lignophenols. This conclusion is very important to develop a new kind of hydrophobic composite material.

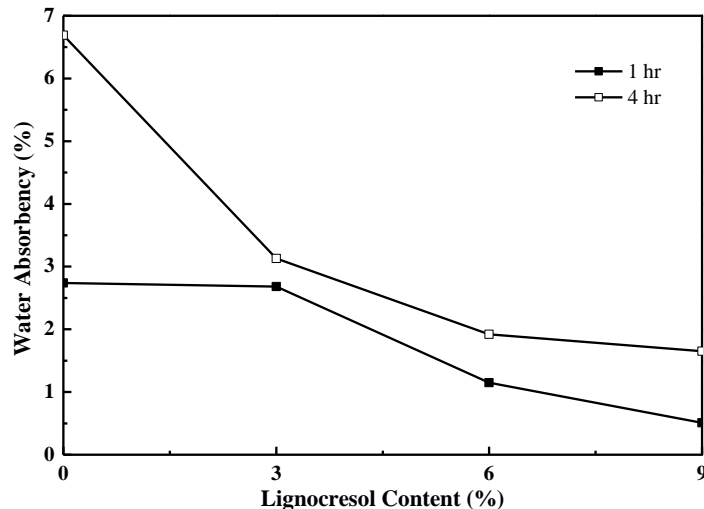


Fig. 8. Relationship between lignocresol content and water absorbency

### TG Analysis of the Composite Films

The thermal behaviors of lignocresol-PHB composite are shown in Fig. 9. The onset temperature of pure PHB is 248 °C. The onset temperature of composites was found to be very much influenced by the addition of lignocresol. From the curves, it can be observed that lignophenol can slow down the degradation of PHB. When lignophenol content was 9%, the degradation of PHB was the slowest, with an onset temperature of 390 °C.

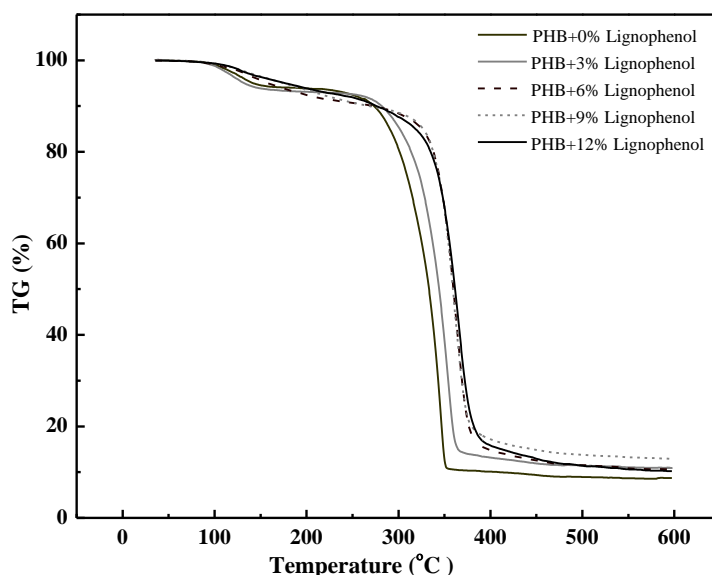


Fig. 9. Relationship between temperature and TG

Residual mass values of composite film also indicated that the addition of lignocresol reduced the degradation of PHB, with the 9% content lignocresol film showing the highest residual mass. Thus, the onset temperature values and residual mass indicate that on addition of lignocresol to the lignocresol-PHB composite became somewhat resistant to thermal action (An 2012; Nemoto *et al.* 2010).

## CONCLUSIONS

1. Four kinds of lignophenols were derived from bamboo through a phase separation system by using *p*-cresol, catechol, resorcinol, and pyrogallol. The resulting lignophenols were characterized by <sup>1</sup>H-NMR, FT-IR, and GPC analysis. They all showed typical lignin skeletal structure based on FTIR analysis and showed low molecular weights and high phenolic hydroxyl group contents.
2. Concerning the mechanical properties, the tensile strength of composite films was improved within 10% lignocresols' addition amount, where 3% addition showed the highest tensile strength. Lignocatechol, lignoresorcinol, and lignopylogallol were tested by 3% addition amount to compare with lignocresol. The elongation at break values of these two kinds of composites were more than three times of pure PHB, which indicated that lignocatechol and lignoresorcinol were more suitable to develop composite films.
3. Additionally, the water absorbency and thermal decomposition of lignocresol-PHB composites were tested. The results indicated that hydrophobicity of composite films was improved with the increase of lignocresol content. The thermal resistance was also improved by the addition of lignocresol.
4. All in all, lignophenols can be used to develop a new kind of composite film by blending with PHB in solvent, then casting.

## ACKNOWLEDGMENTS

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