Structure, Composition, and Thermal Properties of Cellulose Fibers from *Pueraria lobata* Treated with a Combination of Steam Explosion and Laccase Mediator System

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Cellulosic fibers from the bast of *Pueraria lobata* (*P. lobata*) vine were separated using a "green" and efficient method that combined steam explosion (SE) and a laccase mediator system (LMS). The chemical components, structure, and thermal alterations in the fibers were evaluated. The SE performed at 180 °C for 10 min did not change the chemical composition of *P. lobata*; however, SE did alter the fiber structure and rendered its surface more accessible to the laccase enzyme. Treated and untreated samples were characterized by scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), X-ray diffractometry (XRD), thermogravimetric analysis (TGA), and chemical methods. The cellulose content of the processed fibers was approximately 68.2%, and the lignin content was 11.8%, which was much lower than the 22.98% lignin content of the raw material. The cellulose fibers exhibited higher cellulose crystallinity and thermal stability compared with the untreated samples. This combined treatment approach may be useful for the isolation of cellulose fibers for composites, textiles, and other industrial applications.

Keywords: Pueraria lobata; Steam explosion; Cellulose fibers; Laccase

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INTRODUCTION

Growing environmental problems along with energy shortages are serious concerns to the global community. It is necessary to develop renewable, sustainable, and environmentally compatible energy resources. Natural fibers represent renewable, cost-effective, and readily available biomass. *Pueraria lobata* (*P. lobata*), also known by the common name kudzu, is a wild plant that is known for its roots in traditional Chinese medicine, which have been used for thousands of years to treat fever, diabetes, as well as cardiovascular and cerebrovascular diseases (Wong *et al*. 2014). As one of the largest sources of natural fibers, the wild *P. lobata* vine has been used as a textile material since 6000 B.C. During the Tang dynasty, the *P. lobata* vine was replaced by other plant fibers, such as hemp and ramie. Like other lignocellulosic biomass, the bast of the *P. lobata* vine is primarily composed of cellulose, hemicelluloses, and lignin. Of these biopolymers, cellulose has been used extensively as a raw material for the production of biocompatible and biodegradable materials. However, the cellulose fibers are positioned at the inner core of the structure, where lignin acts as an external crosslink binding cellulose and

hemicellulose (Moniruzzaman and Ono 2013; Iroba *et al*. 2014). Because lignocellulosic biomass matrix forms a tight and compact structure, it is difficult to separate the *P. lobata* fibers (Hu and Zhang 2013; Li and Qi 2013). Thus, *P. lobata* was abandoned and replaced by other plant fibers.

Several methods are used to fractionate biomass components, such as chemical (Asadieraghi and Daud 2014; Kim *et al*. 2014), physical (Mood *et al*. 2013), physicochemical (Sun *et al*. 2014a, b), and biological methods. Most of these methods require high temperatures as well as highly concentrated chemicals for the cooking process (Moniruzzaman and Ono 2013). Unlike physical or chemical processes, the bio-based processes are considered more environmentally friendly and sustainable. Laccase (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) contains multiple copper moieties and is a common ligninolytic enzyme. The laccase mediator system (LMS) increases the fermentability of lignocellulosic materials for biofuel production by degrading the lignin (Kudanga and Le Roes-Hill 2014) and enhances pulp quality by removing lignin-derived products (Couto and Herrera 2006). However, one disadvantage of enzymes for bioprocessing is their low effectiveness, which is related to the low accessibility of the enzyme penetrating the biomass (Grethlein 1985; Martin-Sarnpedro *et al*. 2011). Therefore, a chemical, physical, or physicochemical pretreatment of the biomass prior to the LMS treatment is necessary. Currently, the steam explosion (SE) treatment is a promising pretreatment to facilitate enzyme penetration (Han *et al*. 2010; Li and Chen 2014; Sabiha-Hanim *et al*. 2015). In this process, the biomass is treated with high pressure steam at high temperature, which is followed by an immediate depressurization that separates fibers and ruptures the fiber walls. This explosive force during depressurization opens the structure of the biomass, which increases the accessibility of the biomass to chemical and biological attack.

With technological developments and the demand for natural fiber products, the fibers from *P. lobata* have received more attention. The *P. lobata* vine grows rapidly and is widely spread in China; it also hinders the growth of other plants. Due to the structure of *P. lobata* bast and the need for an environmentally sustainable pretreatment, the less time and chemical consuming method (the combination of SE and LMS) was established to separate *P. lobata* fibers in this study. The combined SE and LMS treatment has not been studied previously with *P. lobata*. The effect of this fractionation process on the structure, composition, and thermal properties of the cellulose fibers was evaluated. This information will prompt further optimization of the process and the rational utilization of *P. lobata*.

EXPERIMENTAL

Materials

Pueraria lobata vines were collected from Shandong Province, China. The bast of the vines were peeled by hand and then cut to an average length of 10 cm, and air-dried. The component analysis was performed in accordance to Chinese national standard GB 5889-86 (1986). All of the chemicals used were of reagent grade. The laccase and 1-hydroxybenzotriazole (1-HBT) used in enzymatic treatments were obtained from Sigma-Aldrich (Tokyo, Japan).

Steam Explosion Treatment

The SE pretreatment was performed in a stainless steel vessel designed in-house for processing lignocellulosic biomasses. Before the steam explosion treatment, the chips were separated into 5 batches (200 g per batch). All batches were immersed in distilled water at 25 °C for 24 h and squeezed samples with 50% water content. The reactor was heated with saturated steam to reach 180 °C. After these conditions had been reached, different retention time (3 min, 5 min, and 10 min) were applied. At the end of the steaming reaction, the vessel was instantaneously depressurized to stop the reaction and to discharge the exploded feedstock. The defiberized feedstock was collected and washed with water.

The severity factor, S_0, for each treatment was calculated according to the equation given by Overend *et al.* (1987), which is a function of temperature (T (°C)) and treatment time (t (min)):

$$S_0 = \log\left(e^{\frac{T-100}{14.75}} \times t\right) \quad (1)$$

Table 1. Summary of Conditions Used in Steam Explosion of *P. lobata* Samples

Steam explosion condition	Severity factor (S_0)
180 °C, 3 min	2.82
180 °C, 5 min	3.04
180 °C, 10 min	3.34

Enzymatic Treatment

The SE samples were further treated with a laccase mediator system. Enzyme treatment was performed in a 500-mL flask with an air sparger; the flask was heated in a temperature-controlled shaker that operated at 130 rpm. The LMS consisted of the laccase enzyme (200 U of the laccase per g of dry SE sample) and the 5 mM 1-hydroxybenzotriazle (1-HBT) mediator. The temperature, time, and pH of the treatment were 50 °C, 18 h, and 5 (100 mL phosphate buffer), respectively.

Characterization of Untreated and Treated Samples

Chemical characterization of the samples

The chemical components of the untreated *P. lobata* samples were analyzed according to the Chinese national standard GB 5889-86 (1986). After SE and LMS treatment, the main components (*i.e.*, cellulose, hemicelluloses, and lignin) samples were determined by the standard analytical procedure of the National Renewable Energy Laboratory (NREL) method (Sluiter *et al.* 2011). Briefly, the *P. lobata* samples were dewaxed with benzene/ethanol (2:1, *v/v*) in Soxhlet extractor for 6 h, and the dried dewaxed samples were divided into two parts. One part was extracted by water, ammonium oxalate (5 g/L), and sodium hydroxide (20 g/L) consecutively. And then, after each step, the samples were dried and weighed for water soluble substances, pectin and hemicellulose content. For another dewaxed part was used to determine the lignin content.

Morphology of treated and untreated P. lobata samples

The morphologies of the samples were characterized using a scanning electron microscope (SEM) (S-4300, Hatchi Ltd., Tokyo, Japan). Before the SEM analysis, the samples were coated with gold to avoid sample charging under the electron beam.

Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of the samples were recorded using a Nicolet NEXUS 670 FTIR spectrometer (ThermoFisher Scientific, Madison, WI, USA) in the range of 4000 to 400 cm^{-1}. The raw material and SE samples, as well as samples treated by SE and LMS combined treatment, were milled with KBr (analytical grade); the milled mixtures were pressed into a pellet disk prior to recording FTIR spectra. Spectra were recorded in transmittance mode as a function of wavenumber.

X-ray diffraction (XRD)

Crystal structures of the untreated and treated *P. lobata* materials were examined by a XRD-6100 diffraction system (Shimadzu, Tokyo, Japan). The 2θ range was between 4° and 60°. The crystallinity of all samples was determined, and the crystallinity index (*CrI*) was calculated using the empirical formula from the XRD analysis (Segal *et al.* 1959),

$$CrI\% = \left(\frac{I_{002} - I_{am}}{I_{002}} \right) \times 100 \quad (2)$$

where I_{002} is the maximum intensity of the [002] lattice diffraction peak and I_{am} is the intensity of scattered by the amorphous part of the sample.

Thermal characterization

Thermogravimetric analysis was performed to compare the degradation characteristics of the treated and untreated *P. lobata* materials. The thermal stability of each sample was determined using a Dyntherm thermogravimetric analyzer (Rubotherm, Bochum, Germany). A 10 mg sample was heated in a platinum pan at a rate of 10 °C/min under a nitrogen atmosphere.

RESULTS AND DISCUSSION

Component Analysis

The components analysis of the raw material was determined in accordance to GB 5889-86 (1986) and compared with other plant fibers (Table 2).

Table 2. Chemical Component Contents of Raw *P. lobata* and Other Plant Fibers

Material	Wax (%)	Water-soluble substances (%)	Pectin (%)	Hemicellulose (%)	Lignin (%)	Cellulose (%)
P. lobata	1.28	9.22	5.43	20.04	22.98	41.05
Kenaf*	1.50	10.76	7.50	14.87	9.31	56.06
Flax*	2.00 - 4.00	1.0 - 2.0	1.0 - 4.0	8.0 - 11.0	1.5 - 7.0	70.0 - 80.0
Jute*	0.20 - 1.00	6.5 - 9.0	3.5 - 4.5	12.0 - 15.0	0.5 - 2.0	70.0 - 77.0
Hemp*	1.00 - 1.20	10.0 - 13.0	3.6 - 3.8	20.0 - 25.0	6.3 - 9.3	55.0 - 67.0
*Data from Zhang *et al*. (2014)						

Cellulose, hemicelluloses, and lignin are the primary constituents of plant cell walls; cellulose fibers are embedded in a cross-linked matrix of lignin and hemicelluloses. Compared with the other raw plant materials, the lignin content of *P. lobata* was as high as 22.98%, which indicated that *P. lobata* has a higher content of lignin and lower amounts of cellulose and hemicelluloses. The chemical compositions were determined in water pre-impregnated samples treated by different steam explosion conditions as well as the combined SE and LMS treatments (Fig. 1).

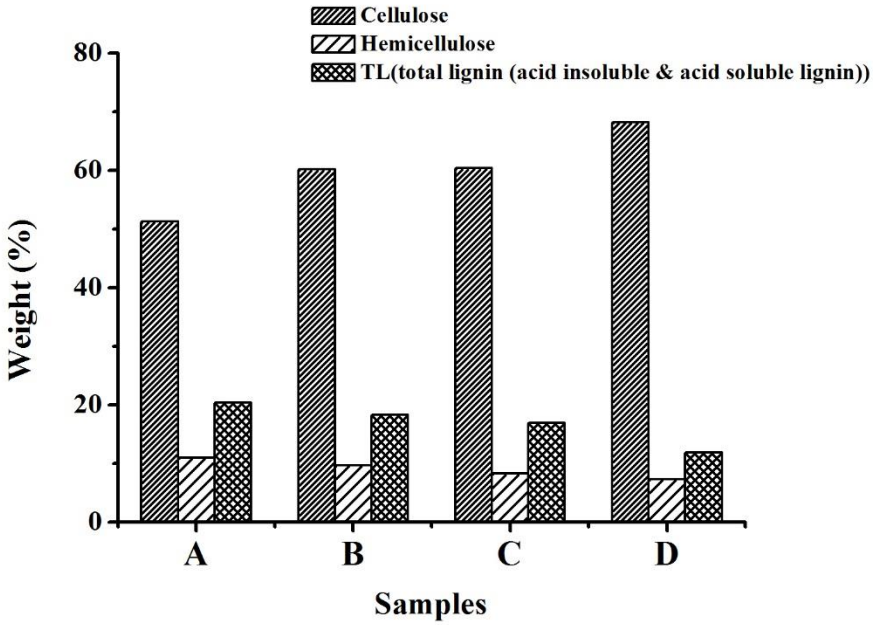

Fig. 1. Chemical compositions of the steam-exploded (SE) *P. lobata* samples: A. SE *P. lobata* (S_0 = 2.82); B. SE *P. lobata* (S_0 = 3.04); C. SE *P. lobata* (S_0 = 3.34); and D. combined SE and LMS *P. lobata* (S_0 = 3.34).

As compared to the original *P. lobata* raw material, the amounts of hemicelluloses and lignin in all the steamed exploded samples decreased. As shown in Fig. 1, the amount of cellulose in sample C (60.36%) was higher than in the other two SE samples, while the amounts of hemicelluloses and lignin (8.25% and 16.86%, respectively) were lower. This observation could be ascribed to the impact of steam explosion pretreatments. Hemicelluloses are thought to be hydrolyzed (autohydrolysis) by the acetic and other organic acids derived from acetyl groups at high temperatures (Chen and Qiu 2010). In addition, water itself also possesses certain acid properties at high temperature, which contributes to the hydrolysis of the hemicelluloses during steam explosion. Furthermore, the amount of cellulose in sample D (69.27%) was the higher and the amount of hemicellulose and lignin was the lower (7.11% and 11.6%, respectively) compared with sample C. This is because some lignin was further removed during the LMS treatment.

SEM Analysis

The physical structures of the untreated and SE samples under different conditions, as well as the samples treated with the combined SE and LMS treatment, were studied by SEM. Similar to other plant fibers, the basts of the *P. lobata* vines were covered with a layer of hemicelluloses, pectin, and other substances. The elementary fibers in the bast are glued together into a bundle (Le Troedec *et al*. 2008), which is shown in Fig. 2. The

samples treated with different steam explosion are shown in Fig. 3. After steam explosion treatments, the morphologies of the *P. lobata* changed dramatically (Fig. 3). As the severity factor (S_o) increased, the raw materials were loosened, and the fibers were separated as the cementing materials, such as lignin and hemicelluloses, were degraded and transformed.

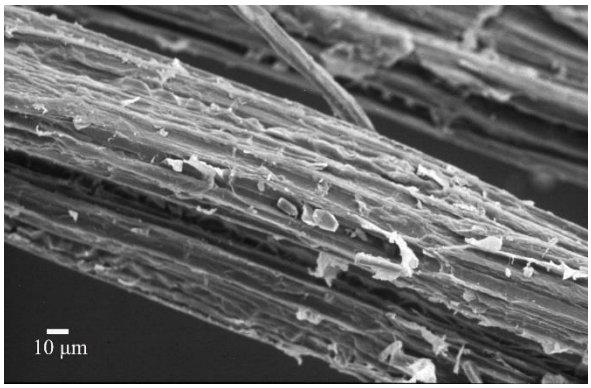

Fig. 2. SEM picture of the raw *P. lobata* sample

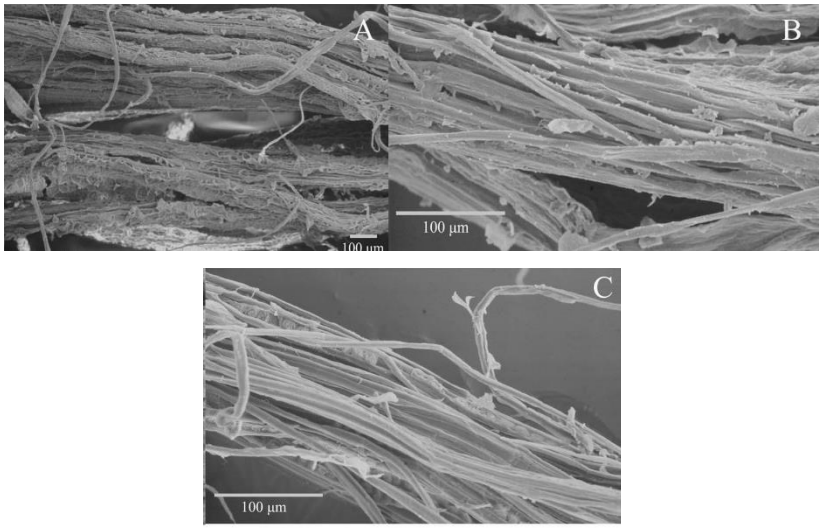

Fig. 3. SEM pictures of the steam exploded samples: A. steam-exploded *P. lobata* (S_0 = 2.82) B. steam-exploded *P. lobata* (S_0 = 3.04); and C. steam-exploded *P. lobata* (S_0 = 3.34)

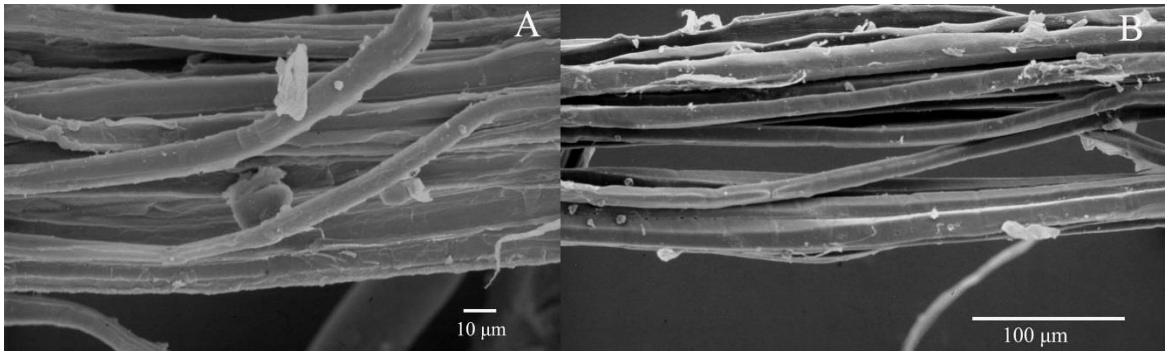

Fig. 4. SEM pictures of LMS treated samples: A. the raw *P. lobata* treated with only LMS; and B. the raw *P. lobata* treated with the combined SE and LMS (S_0 = 3.34)

The morphologies of the raw *P. lobata* sample treated with only LMS and samples treated with LMS followed by the steam explosion (1.0 MPa, 10 min) are shown in Fig. 4. Although the LMS was effective in degrading the lignin (Chen *et al*. 2012), the efficacy of these lignin-degrading enzymes when used directly on the raw material is constrained (Fig. 4(A)). Because of a complex formation of cellulose, hemicellulose, and lignin, the lignocellulosic biomass matrix is resistant to enzymatic treatment (Iroba *et al*. 2014). In the samples treated by LMS after steam explosion, however, the elementary fibers were further loosened. As shown, the size range of a single fiber was in the range 10 to 30 μm (Fig. 4 (B)). The steam explosive treatment opens the biomass structure and increases enzyme accessibility (Sun *et al*. 2014a, c). During the steam explosion, the *P. lobata* structure is steamed under high-pressure, which is followed by rapid decompression to atmospheric pressure. The sudden decrease of pressure during decompression (*i.e.*, the "explosion") causes fiber separation and physical rupture of the fiber walls (Fig. 3). The structure of the samples is opened, which increases the accessibility of the laccase and the mediator to penetrate the *P. lobata* (Martin-Sarnpedro *et al*. 2011b).

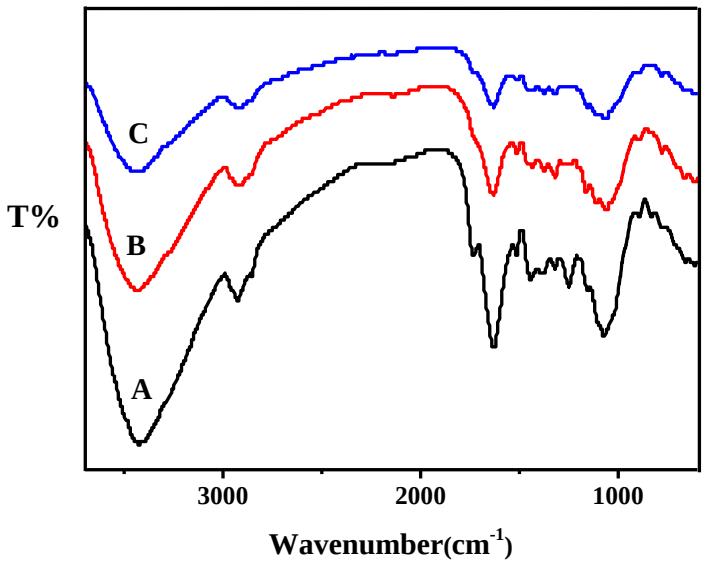

Fig. 5. FTIR spectra of the untreated/treated *P. lobata* samples: A. the raw *P. lobata*; B. the steam-exploded sample (S_0=3.34); and C. combined SE and LMS *P. lobata* (S_0=3.34)

FTIR Study

The FTIR spectra of *P. lobata* bast, the SE samples, and the samples treated with the combination of SE and LMS are shown in Fig. 5. The absorbance peaks in the 3300 to 3400 cm^{-1} region in all the samples corresponded to the free stretching and bending vibration of the OH groups in cellulose (Wang *et al*. 2007; Karimi *et al*. 2014), which means the SE treatment and the combination of SE and LMS did not change the cellulose structure of *P. lobata*. The absorption peak at 1737 cm^{-1} was observed in the bast, which was related to the stretching of C=O in hemicelluloses (Zhang *et al*. 2010; Sun *et al*. 2014a). The absorption peak at 1737 cm^{-1}, however, disappeared after treatment (spectra B and C), which indicated that hemicelluloses were extracted by this method. This result was ascribed to the effect of the SE treatment. During the SE treatment, the steam penetrates the plant cell wall, whereby acetic acid is cleaved from acetylated hemicelluloses; the released acetic acid catalyzes further hydrolysis of hemicelluloses. Furthermore, water possesses certain

acid properties at high temperature, which also contributes to hemicellulose hydrolysis (Weil *et al*. 1997; Xu and Huang 2014). The band at 1642 cm^{-1} was attributed to the joint vibration of carbonate inorganics and lignin in raw material (Jiang *et al*. 2013; Asadieraghi and Daud 2014), which decreased appreciably in treated samples (spectra B and C). This was due to the high amounts of inorganics being removed (Asadieraghi and Daud 2014). Meanwhile, the characteristic vibration of lignin at 1254 cm^{-1} (C=O stretching) and 837 cm^{-1} (C-H out of plane vibration) were also found in the raw material (Wang *et al*. 2007; Asadieraghi and Daud 2014; Ramadoss and Muthukumar 2014; Sun *et al*. 2014b). After the steam treatment (spectra B), the disappearance of these peaks was attributed to the acidolysis of lignin and to the cleavage of *β-O*-4 linkages (Xu and Huang 2014). By comparing the spectra of B and C, some differences were observed. The absorbance peak at 1119 cm^{-1}, as well as at 1166 cm^{-1}, belonged to lignin in the SE sample (Sun *et al*. 2014b). The 1166 cm^{-1} peak was reduced after the steam exploded samples were further treated with LMS. This observation suggested that the accessibility of the exploded samples increased, which could improve the diffusion of laccase and mediator. During the LMS treatment, some low molecular weight lignin fractions and/or small lignin fragments attached to carbohydrates were further solubilized into the aqueous solution.

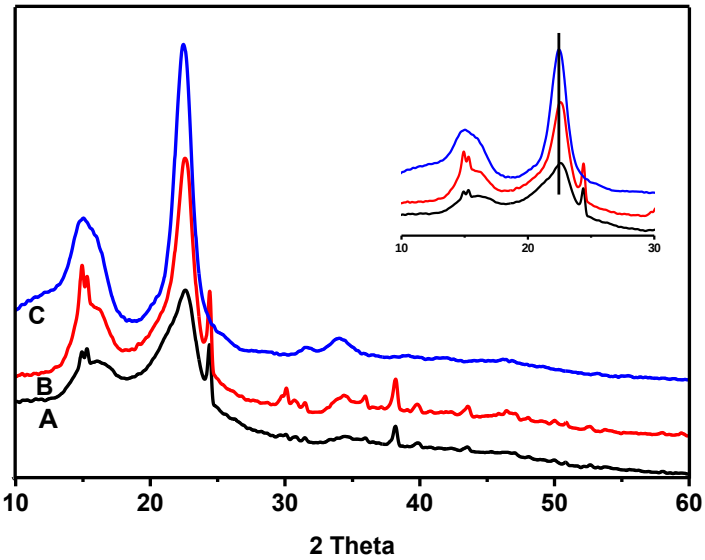

Fig. 6. XRD spectra of the untreated/treated *P. lobata* samples: A. the raw *P. lobata*; B. the steam-exploded sample (S_0 = 3.34); C. combined SE and LMS *P. lobata* (S_0 = 3.34)

X-ray Diffraction Study

X-ray diffractometry analysis was performed to determine the crystallinity of the fibers and the influence of each treatment on the processed material. All samples contained cellulose I structures; the diffraction peaks at 2θ of 15°, 22.5°, and 34° were assigned to the [101], [002], and [040] crystalline planes of cellulose I, respectively (Fig. 6). As expected, the magnitudes of these diffraction peaks increased in the treated samples *versus* the raw material. The crystallinity index (*CrI*) of the raw material was 45%; however, the *CrI* value of the SE sample increased to 65%. This observation was attributed to the amorphous hemicelluloses being solubilized and the softening of the lignin by SE treatment, which increased the *CrI* value (Li and Chen 2014). Meanwhile, the *CrI* values of the SE samples that were subsequently treated by LMS were slightly higher (*i.e*., 67.5%).

The plausible explanation is that the SE treatment facilitated laccase and mediator penetration, and some low molecular weight lignin fractions and/or small lignin fragments attached to carbohydrates were further solubilized in the aqueous solution (Martin-Sampedro *et al*. 2011a).

TGA Analysis

Thermogravimetric analysis (TGA) was performed to examine the mass loss of the material when heated due to the volatilization or charring. The thermogravimetric and differential thermal curves as a function of temperature for the starting material, SE samples, and combined SE and LMS treated samples are presented in Fig. 7. The TGA curves show that charring takes place in two steps. The first degradation phase occurs from 250 to 370 °C, which is mainly attributed to cellulose and hemicelluloses charring. The second degradation phase from 400 to 500 °C is attributed to the charring of lignin (Wang *et al*. 2009; Deepa *et al*. 2011; Martin-Sampedro *et al*. 2011a).

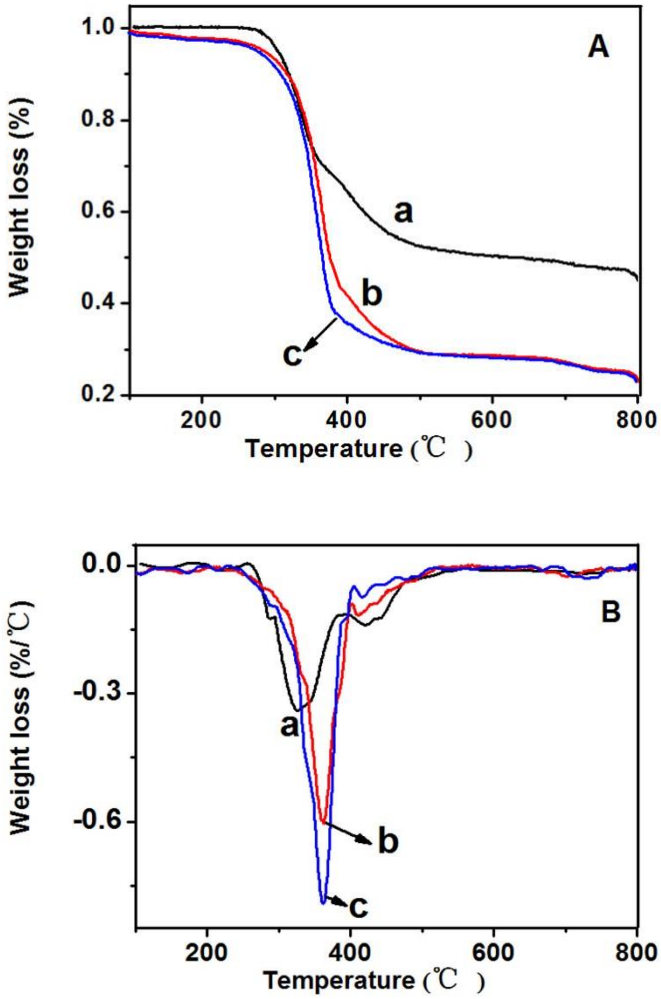

Fig. 7. (A) TG and (B) DTG curves of the untreated and treated *P. lobata* samples. (a) the raw *P. lobata*, (b) the steam-exploded sample (S_0 = 3.34), and (c) combined SE and LMS *P. lobata* (S_0 = 3.34)

Cellulose and hemicelluloses are aliphatic structures, and the higher degradation temperature for lignin is ascribed to its aromatic structure. A shoulder around 287 °C was observed in the DTG curve of the raw material, which is attributed to the presence of hemicelluloses (Martin-Sampedro *et al*. 2011a). After the treatment, it was removed for all treated samples. These results agreed with the previous chemical composition and FTIR analyses. The DTG curve of the raw *P. lobata* fibers showed a peak at 322 °C (*ca*. 50% mass loss), which was assigned to the thermal decomposition of cellulose. After the treatment (Fig. 7B; curves b and c), the decomposition peak of cellulose in the treated *P. lobata* fibers shifted to 360 °C (*ca*. 50% mass loss). These observations suggested that the steam exploded fibers and fibers treated with combined SE and LMS treatments had a higher thermal stability than the starting material. The removal of non-cellulosic constituents from the raw fiber during each treatment helped to refine and concentrate the amount of cellulose compared with raw material, which increased the degradation temperature of the treated samples.

CONCLUSIONS

1. The combined steam explosion (SE) and LMS treatment is a highly effective and clean method for the isolation of cellulose fibers from the bast of *P. lobata*.
2. After steam explosion at 180 °C for 10 min, the chemical compositions, cellulose crystalline structures, and thermal stabilities of *P. lobata* samples were altered. SE increased the accessible surface area of *P. lobata* samples, which lead to enhanced laccase delignification.
3. The approach used in this study may be a promising treatment for isolating cellulose fibers for composites, textiles and other industrial applications.

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