

# Fractionation and Characterization of Three Main Components from *Pennisetum sinense* Roxb. (*P. sinense*) by Microwave-assisted H<sub>2</sub>O<sub>2</sub>-NaOH Extraction

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Fractionation of lignocellulosic components is a prerequisite for maximizing valorization of plant biomass in an integrated biorefinery. In this study, microwave-assisted H<sub>2</sub>O<sub>2</sub>-NaOH extraction was developed for fractionation of *Pennisetum sinense* Roxb. (*P. sinense*), a highly productive energy crop. Different reaction conditions including solid-liquid ratio, NaOH concentration, H<sub>2</sub>O<sub>2</sub> concentration, temperature, and time were tested for their effects on *P. sinense* fractionation. The cellulose, hemicellulose, and lignin obtained under optimal fractionation conditions were characterized by Fourier transform infrared spectrometry (FT-IR) and nuclear magnetic resonance (NMR). The optimal fractionation conditions were a solid-liquid ratio of 1:25 using 0.5% H<sub>2</sub>O<sub>2</sub> and 4% NaOH at 75 °C for 4 h, which gave cellulose, hemicellulose, and lignin yields of 42.8%, 21.9%, and 15.2%, respectively. FT-IR and NMR analyses of the fractionated components clearly confirmed their structural integrity and representation. The work demonstrates the potential of the alternative approach for efficient fractionation of lignocellulosic biomass components for further valorization.

*Keywords:* Fractionation; Characterization; *P. sinense*; Hemicellulose; Lignin

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## INTRODUCTION

Lignocellulosic biomass is one of the most abundant renewable resources that can be transformed to fuels, chemicals, and materials to support sustainable development. The conversion of lignocellulosic biomass into liquid fuels and chemicals has received increasing public interest and some commercial implementations (Demirbas 2009; Naik *et al.* 2010). Future biorefineries may have a tremendous potential for producing chemicals and materials that are traditionally produced from petroleum.

The lignocellulosic biorefinery concept integrates renewable resources and necessary processes for their conversion to fuels, power, and value-added chemicals (Sheldon 2014). As biomass is a complex mixture of several different polymer constituents, it is difficult to use directly, and a transition from simple pretreatment processes to fractionation is needed. As part of an integrated lignocellulose biorefinery, fractionation of the raw material into its main lignocellulosic components, *i.e.* hemicellulose, cellulose, and lignin, maximizes the conversion of valuable feedstock streams to biofuels and chemicals (Grande *et al.* 2015; Imman *et al.* 2015).

Diverse techniques have been investigated for biomass fractionation including chemical, physicochemical, thermochemical, and biological methods (Lawther *et al.* 1995; Huijgen *et al.* 2010; Karp *et al.* 2014; Saito *et al.* 2014; Van den Bosch *et al.* 2015). The alkaline oxidative method is one of the most attractive strategies because it is versatile, low cost, nontoxic, and environmentally friendly (Azarpira *et al.* 2014). It effectively and selectively removes and depolymerizes lignin from wood pulp under relatively mild reaction conditions (Villar *et al.* 2001). Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) is an environmentally benign oxidizing agent. It is generally accepted that  $\text{H}_2\text{O}_2$  is unstable in alkaline conditions and readily decomposes to form hydroperoxide anion ( $\text{HOO}^-$ ), hydroxyl radicals ( $\text{HO}^\cdot$ ), and superoxide anion radicals ( $\text{O}_2^{\cdot-}$ ). These radicals may participate in the cleavage of some inter-unit bonds of biomass, eventually resulting in the dissolution of lignin and hemicelluloses (Pan *et al.* 1998). In an earlier paper, Sun *et al.* (2000) has been reported that  $\text{H}_2\text{O}_2$  in alkaline media could serve as a mild agent for solubilizing the macromolecular hemicelluloses besides its dual role in delignification and bleaching. Then, hemicelluloses from rice straw have been isolated and compared by alkali and hydrogen peroxide ( $\text{NaOH-H}_2\text{O}_2$ ) treatments by Sun *et al.* (2000), and they proposed that the treatment by  $\text{NaOH-H}_2\text{O}_2$  did not affect the overall structure of macromolecular hemicelluloses except for a noticeable degradation of cellulose and hemicelluloses when with more than 1.0%  $\text{H}_2\text{O}_2$ . Microwave-assisted heating applies an electromagnetic field to the molecular structure of the heated object to accelerate organic reactions (Tsubaki and Azuma 2013). It could increase specific surface area, decreases polymerization and crystallinity of cellulose, and causes lignin depolymerization (Peng *et al.* 2014). For this reason, it has been successfully applied to promote the dilute alkali and/or dilute acid pretreatment of biomass, causing swelling and fragmentation (Wu *et al.* 2010). Rossberg *et al.* (2015) explored the effects of different pulping procedures on lignin separation and composition, finding that microwave-assisted pulping allowed reduced pulping time while obtaining lignin with similar purity and composition. Diaz *et al.* (2015) proposed microwave-assisted pretreatment of lignocellulosic biomass immersed in alkaline glycerol and demonstrated that lignin removal from corn straw and rice husk were 30% and 12%, respectively. Recently, Jin *et al.* (2016) investigated microwave-assisted alkaline pretreatment of catalpa sawdust to remove hemicellulose and lignin and to enhance enzymatic saccharification. In summary, microwave-assisted alkaline treatment has been tested to remove and/or isolate one or two components in pulping or bioconversion, but there has not been much investigation on the fractionation of three main components as an integrated concept.

*P. sinense*, an energy crop, is a monocot  $\text{C}_4$  perennial grass that is a hybrid of *Pennisetum purpureum* and *Pennisetum americanum* (del Río *et al.* 2012). It is versatile and adaptable and can grow and thrive in a variety of harsh climates. Its high productivity ( $\sim 40 \text{ t ha}^{-1} \text{ y}^{-1}$ ) allows 3 to 4 harvests per year. The high productivity and low inputs for cultivation allow it to serve as a potential lignocellulosic feedstock for pulp and paper industries, textiles, bioethanol, and biofuels. Although *P. sinense* is an attractive alternative feedstock for biorefinery, it is largely underexploited.

This study evaluated the efficiency of microwave-assisted  $\text{H}_2\text{O}_2$ - $\text{NaOH}$  treatment on *P. sinense* fractionation and characterized the three main components obtained. The operating conditions of solid-liquid ratio,  $\text{NaOH}$  concentration,  $\text{H}_2\text{O}_2$  concentration, temperature, and time were optimized. Fourier transform infrared spectra (FT-IR) and nuclear magnetic resonance (NMR) spectra were used to determine the structural characteristics of the fractionated cellulose, hemicellulose, and lignin.

## EXPERIMENTAL

### Materials

*P. sinense* was obtained from Shandong Province, China, and air-dried at ambient temperature. The dried *P. sinense* was ground with a Wiley mill, and the fraction between 40- and 60-mesh was collected as feedstock. The initial composition of the *P. sinense* was determined based on Chinese National Standards GB/T2677.8-1994, GB/T10337-2008, GB/T10741-2008, and methods in reference (Shi and He 2003), and the results were as follows: 44.0% cellulose, 25.8% hemicellulose, 17.4% lignin, 4.3% ash, and 8.2% extractives as the components of unfractionated biomass

Sodium hydroxide, benzene, ethanol, hydrochloric acid, hydrogen peroxide (30 wt.%), and other chemicals were of analytical grade and used as received.

### Fractionation and Isolation

The overall process for fractionation of the three main components from *P. sinense* is summarized in Fig. 1. Briefly, *P. sinense* was first treated with benzene-alcohol mixture (2:1, v/v, 6 h) and hot water (85 °C, 3 h) to remove waxes and impurities. For each run, 10 g of dewaxed *P. sinense* and the necessary amount of liquid solution (with right concentration of NaOH and H<sub>2</sub>O<sub>2</sub> in deionized water) were loaded into a 500 mL flat-bottom flask. After pretreatment in a water bath with stirring at 50 °C for 30 min, the reactor was transferred into a microwave generator (MCR-3, Gongyi, China). Extraction time was counted when the temperature in the reactor reached the target extraction temperature. After the extraction was completed, the reactor was cooled to room temperature, and the solids and liquids were separated by vacuum filtration with fluted filter paper. The solid fractions were washed with deionized water until the pH was neutral and then oven-dried at 60 °C to retrieve the crude cellulose. The filtrate was acidified with 6 M HCl until the pH dropped to 11, and it was concentrated to 1/3 of the original volume. The concentrated filtrate was added to 3 volumes of 95% ethanol for precipitation and centrifuged at 8000 rpm for 10 min.

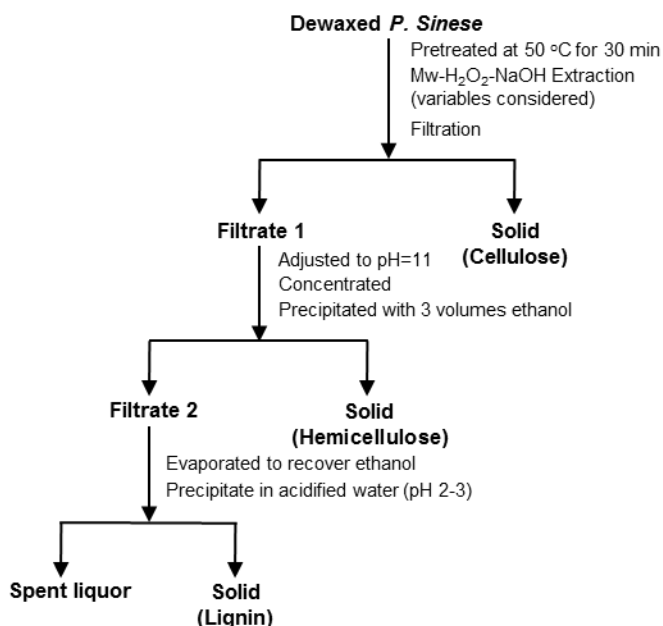


Fig. 1. Scheme for fractionation of cellulose, hemicellulose, and lignin from *P. sinense*

The precipitated hemicellulose solid was washed 3 times with 85% (v/v) ethanol, separated by centrifugation, and freeze-dried. The supernatant after ethanol precipitation was adjusted to pH 9 to 10 with 6 M HCl and rotary evaporated to recover ethanol. The supernatant was further acidified to pH 2 to 3 (drop-wise addition) to precipitate the lignin fraction. The lignin fraction was washed, freeze-dried, and quantified by weighing.

In this study, varying extraction parameters: solid-liquid ratio (1:15 to 1:35), NaOH concentration (1 to 5 wt.%), H<sub>2</sub>O<sub>2</sub> concentration (0 to 1 wt.%), extraction temperatures (55 to 95 °C), and extraction times (2 to 6 h) were investigated by single factor design method. At least two duplicate runs were performed for each condition to confirm the reproducibility of each test. The yields of crude cellulose ( $Y_{cell}$ ), hemicellulose ( $Y_{hemi}$ ), and lignin ( $Y_{lig}$ ) were calculated as shown below,

$$Y_{cell} (\%) = M_{cell}/M_i \times 100 \quad (1)$$

$$Y_{hemi} (\%) = M_{hemi}/M_i \times 100 \quad (2)$$

$$Y_{lig} (\%) = M_{lig}/M_i \times 100 \quad (3)$$

where  $M_{cell}$ ,  $M_{hemi}$ , and  $M_{lig}$  were the mass of fractionated crude cellulose, hemicellulose, and lignin respectively, and  $M_i$  was the mass of the initial dewaxed *P. sinense* loaded.

## Characterization

The fractionated cellulose, hemicellulose, and lignin were characterized by FT-IR and NMR. FT-IR spectra of the three samples were recorded on a VERTEX70 FT-IR spectrophotometer (Bruker, Karlsruhe, Germany) over 4000 to 400 cm<sup>-1</sup> using a KBr disc containing 1% finely ground samples.

The solid state CP/MAS <sup>13</sup>C-NMR spectra of cellulose was recorded using a Bruker Avance III 400 MHz spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) at 25 °C and 62.9 MHz for carbons. The MAS rate was 3 kHz. Each spectrum was obtained with an accumulation of 5000 scans. The delay time was 60 s, the proton 90° pulse width was 9 μm, and the contact time for cross polarization was 2 ms.

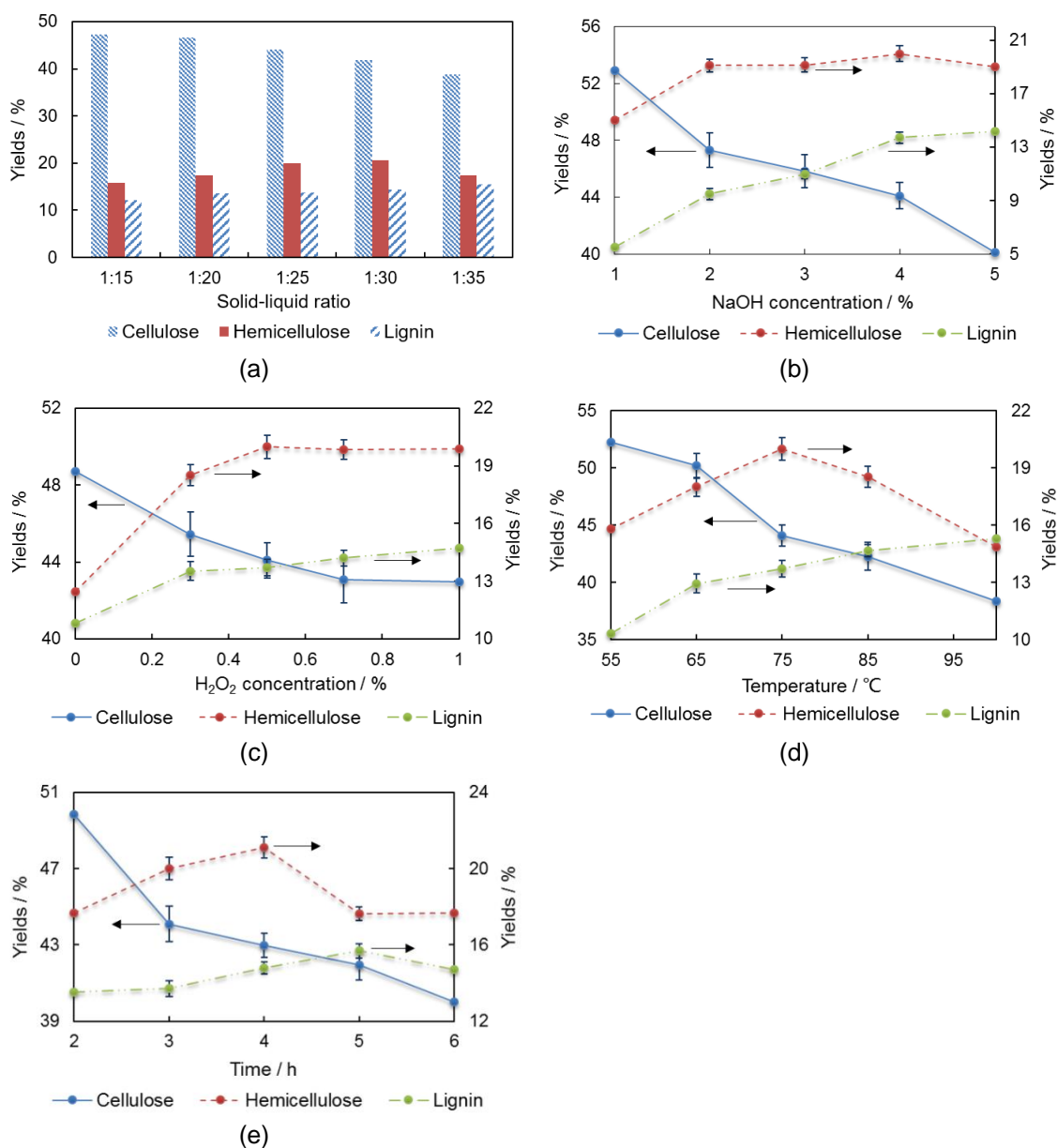
The solution-state <sup>1</sup>H-NMR and <sup>13</sup>C-NMR of hemicellulose and lignin were obtained on a Bruker AV 400MHz spectrometer operating in the FT mode at 100 MHz. The samples (20 to 100 mg) was dissolved in 1 mL DMSO-d<sub>6</sub> (99.8% D) before NMR analysis. The spectrum was recorded at 25 °C after 18,000 scans. The central DMSO solvent peaks were used as internal reference ( $\delta_H/\delta_C = 2.50/39.51$  ppm).

## RESULTS AND DISCUSSION

### Effect of Reaction Conditions on Fractionation

The effect of reaction conditions including solid-liquid ratio, NaOH concentration, H<sub>2</sub>O<sub>2</sub> concentration, temperature, and time on the yields of crude cellulose, hemicellulose, and lignin fractionated from *P. sinense* is shown in Fig. 2. The results in Fig. 2(a) indicated that the solid-liquid ratio was a factor affecting the yields of fractionated components. As the solid-liquid ratio increased, the yield of crude cellulose decreased and the yield of lignin increased, while the yield of hemicellulose increased first and then decreased. When the solid-liquid ratio was below 1:25, the yield of crude cellulose was more than 44.1%, while that of hemicellulose and lignin were no more than 20.0% and 13.7%, respectively, which indicated that insufficient loading of alkaline solution (low solid-liquid ratio) resulted in

impurities in the fractionated cellulose, such as hemicellulose and lignin. When the solid-liquid ratio was 1:35, the yields of crude cellulose, hemicellulose, and lignin were 38.8%, 17.5%, and 15.6%, respectively, showing rapid declines in the yield of cellulose and hemicellulose. Alkali has the greatest effect on breaking ester and ether bonds between hemicellulose and lignin (Ralph *et al.* 1994; Buranov and Mazza 2008). Increasing alkaline dosage favored removal of hemicellulose and lignin from raw material. However, when the alkaline dosage was beyond a certain point (*e.g.*, solid-liquid ratio > 1:25), the yield of hemicellulose was reduced.



**Fig. 2.** Effects of (a) solid-liquid ratio, (b) NaOH concentration, (c) H<sub>2</sub>O<sub>2</sub> concentration, (d) extraction temperature, and (e) extraction time on the yields of crude cellulose, hemicellulose, and lignin from *P. sinense* fractionation. Reaction conditions: (a) 75 °C with 0.5% H<sub>2</sub>O<sub>2</sub> and 4% NaOH for 3 h; (b) solid-liquid ratio of 1:25 with 0.5% H<sub>2</sub>O<sub>2</sub> at 75 °C for 3 h; (c) solid-liquid ratio of 1:25 with 4% NaOH at 75 °C for 3 h; (d) solid-liquid ratio of 1:25 with 0.5% H<sub>2</sub>O<sub>2</sub> and 4% NaOH for 3 h; (e) solid-liquid ratio of 1:25 with 0.5% H<sub>2</sub>O<sub>2</sub> and 4% NaOH at 75 °C.

In addition, varied dosage of H<sub>2</sub>O<sub>2</sub> may affect the lignocellulose components besides the alkalinity. Further peeling reaction and alkaline degradation resulting in the decline in the yield of cellulose. Figure 2(b) shows the effect of NaOH concentration on *P. sinense* fractionation. When the concentration of NaOH increased from 1% to 5%, the yield of crude cellulose decreased from 52.9% to 40.1%. The yield of crude hemicellulose and lignin increased dramatically when the concentration of NaOH increased from 1% to 2%, *i.e.*, both of which increased more than 5%. When the concentration of NaOH was 4%, the yield of crude hemicellulose reached maximum values of 20.0%. However, further reduction was observed when the concentration was raised to 5%. The yield of crude lignin increased with an increase of the NaOH concentration (1 to 5%).

The yield of cellulose, hemicellulose, and lignin was remarkably influenced by the concentration of H<sub>2</sub>O<sub>2</sub> (Fig. 2(c)). The yield of crude cellulose decreased while lignin increased slightly as H<sub>2</sub>O<sub>2</sub> concentration increased. The yield of hemicellulose increased at first and then decreased, with the maximum values at 0.5% H<sub>2</sub>O<sub>2</sub>. Thus, H<sub>2</sub>O<sub>2</sub> obviously improved the removal rate of lignin and hemicellulose in a certain concentration range.

As shown in Fig. 2(d), the yield of hemicellulose increased when the temperature was lower than 75 °C, but the yield was reduced at higher temperatures. For example, the hemicellulose yield decreased to 14.8% when the temperature was 100 °C. The lignin yield increased with increasing temperature, showing that a high temperature was beneficial to lignin removal. Below 100 °C, the yield of crude cellulose was decreased to 38.4%. The reduction in the yield of hemicellulose and cellulose was ascribed to peeling reaction and alkaline degradation, which was promoted at higher temperatures.

The effect of reaction time on the yield of three components is illustrated in Fig. 2(e). The yield of cellulose declined sharply as time increased, while the hemicellulose and lignin yields increased first and then decreased, with the maximum yields achieved at 4 h and 5 h respectively. Initially, the dissolution of hemicellulose and lignin were higher than the degradation, and thus, the yield increased. However, as the extracting time was increased to a certain value, the dissolution was far below the degradation, resulting in a downward trend. In addition, longer time led to further alkaline oxidative degradation of lignin to mono-aromatics and organic acids.

## Structural Characterization

### *FT-IR analysis*

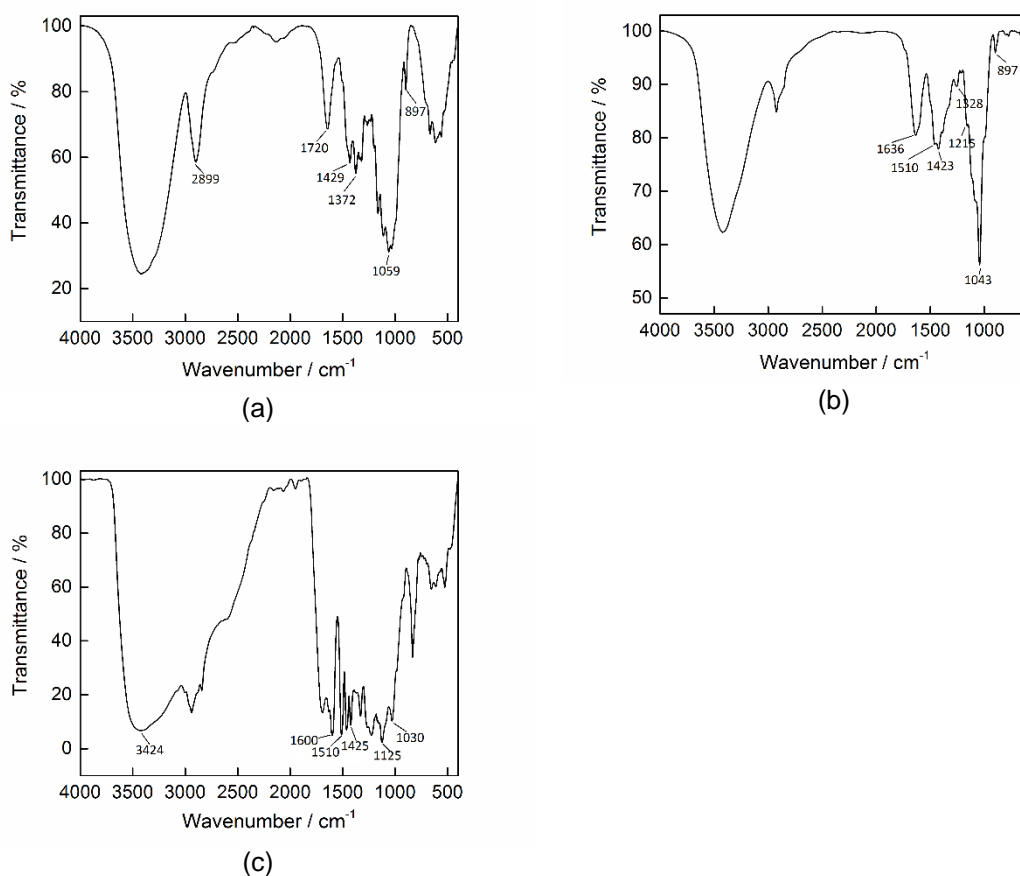
The above observations suggested that tuning reaction conditions was crucial to maintaining a high yield of the fractionated components while avoiding excessive carbohydrate degradation. The optimal fractionation conditions were obtained temporarily, *i.e.*, solid-liquid ratio of 1:25, 0.5% H<sub>2</sub>O<sub>2</sub>, 4% NaOH, 75 °C, and 4 h. The resulting fractionated cellulose, hemicellulose, and lignin were used for structural characterization. The optical photographs of cellulose, hemicellulose, and lignin obtained under optimal conditions are shown in Fig. 3, and their FT-IR spectra are shown in Fig. 4. The IR signal was assigned based on previous publications (Lawther and Sun 1996; Sun *et al.* 1998).

In the cellulose IR spectra (Fig. 4(a)), the absorption bands located at 2899 cm<sup>-1</sup>, 1429 cm<sup>-1</sup>, 1372 cm<sup>-1</sup>, 1310 cm<sup>-1</sup>, 1059 cm<sup>-1</sup>, and 897 cm<sup>-1</sup> are related to cellulose characteristics. The absorption at 2899 cm<sup>-1</sup> is due to the C-H stretching. The band at 1429 cm<sup>-1</sup> is associated with the CH<sub>2</sub> symmetric bending. The absorbances appearing at 1372 and 1310 cm<sup>-1</sup> originate from the O-H bending and C-H bending. The band at 1059 cm<sup>-1</sup> represents the C-O-C pyranose ring skeletal vibration (Lan *et al.* 2011). In addition, the

absorption band at  $1720\text{ cm}^{-1}$  is attributed to carbonyl groups, which may occur due to the presence of residual hemicellulose (Lawther and Sun 1996).



**Fig. 3.** The optical photographs of (a) cellulose, (b) hemicellulose, and (c) lignin prepared from *P. sinese* by microwave-assisted  $\text{H}_2\text{O}_2$ -NaOH extraction. Reaction conditions: solid-liquid ratio of 1:25, 0.5%  $\text{H}_2\text{O}_2$ , 4% NaOH,  $75\text{ }^\circ\text{C}$ , and 4 h.

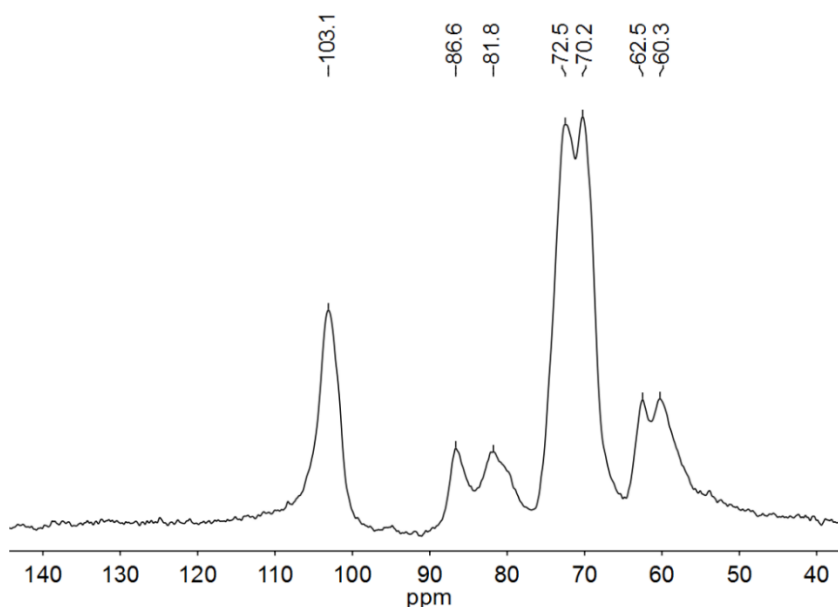


**Fig. 4.** FT-IR spectra of (a) cellulose, (b) hemicellulose, and (c) lignin from *P. sinese* by microwave-assisted  $\text{H}_2\text{O}_2$ -NaOH extraction. Reaction conditions: solid-liquid ratio of 1:25, 0.5%  $\text{H}_2\text{O}_2$ , 4% NaOH,  $75\text{ }^\circ\text{C}$ , and 4 h.

Figure 4(b) shows the FT-IR spectra of hemicellulose fractionated from *P. sinese* under optimal conditions. The absorption band between  $1636$  and  $800\text{ cm}^{-1}$  originated from

the typical absorption of hemicellulose. Specifically, the absorbance band at  $1423\text{ cm}^{-1}$  is the characteristic absorption of alkyl and xylan structure. The strong sharp band at  $1043\text{ cm}^{-1}$  originated from C-O and C-OH, which reflects the xylan structures. The absorption peak near  $897\text{ cm}^{-1}$  is due to  $\beta$ -glycosidic linkages between sugar units of hemicellulose (Lawther *et al.* 1995; Sun *et al.* 1998). There are inevitably some lignin contaminants in isolated hemicellulose because of their intricate connections. The peaks at  $1510\text{ cm}^{-1}$ ,  $1328\text{ cm}^{-1}$ , and  $1215\text{ cm}^{-1}$ , which corresponding to C-O stretching vibrations from aromatic, syringyl, and guaiacyl units of lignin, were inconspicuous. These suggested that the hemicellulose isolated by microwave-assisted  $\text{H}_2\text{O}_2$ -NaOH extraction has a good structural representation.

The FT-IR spectra (Fig. 4(c)) demonstrated that *P. sinense* has a typical GSH-type lignin. The absorbance peaks at  $1600$ ,  $1510$ , and  $1425\text{ cm}^{-1}$  indicate aromatic skeleton vibrations (Lawther and Sun 1996). The band at  $3424\text{ cm}^{-1}$  was assigned to the OH stretching of hydroxyl functional groups. Moreover, the peaks at  $1329$  and  $1125\text{ cm}^{-1}$  associated with the presence of dialkyl ethers represent syringyl (S) structures; the band at  $1030\text{ cm}^{-1}$  identified with alkyl aryl ether bond as well as the absorption peak located at  $1267\text{ cm}^{-1}$  represent the guaiacyl (G) structure. The small band found between  $1124$  and  $1223\text{ cm}^{-1}$  indicates only minor amounts of p-hydroxyphenyl (H) unit in *P. sinense* lignin (Martínez and Gutiérrez 2012).



**Fig. 5.** CP/MAS  $^{13}\text{C}$ -NMR spectra of cellulose isolated from *P. sinense*. Reaction conditions: solid-liquid ratio of 1:25, 0.5%  $\text{H}_2\text{O}_2$ , 4% NaOH,  $75\text{ }^\circ\text{C}$ , and 4 h

#### NMR analysis

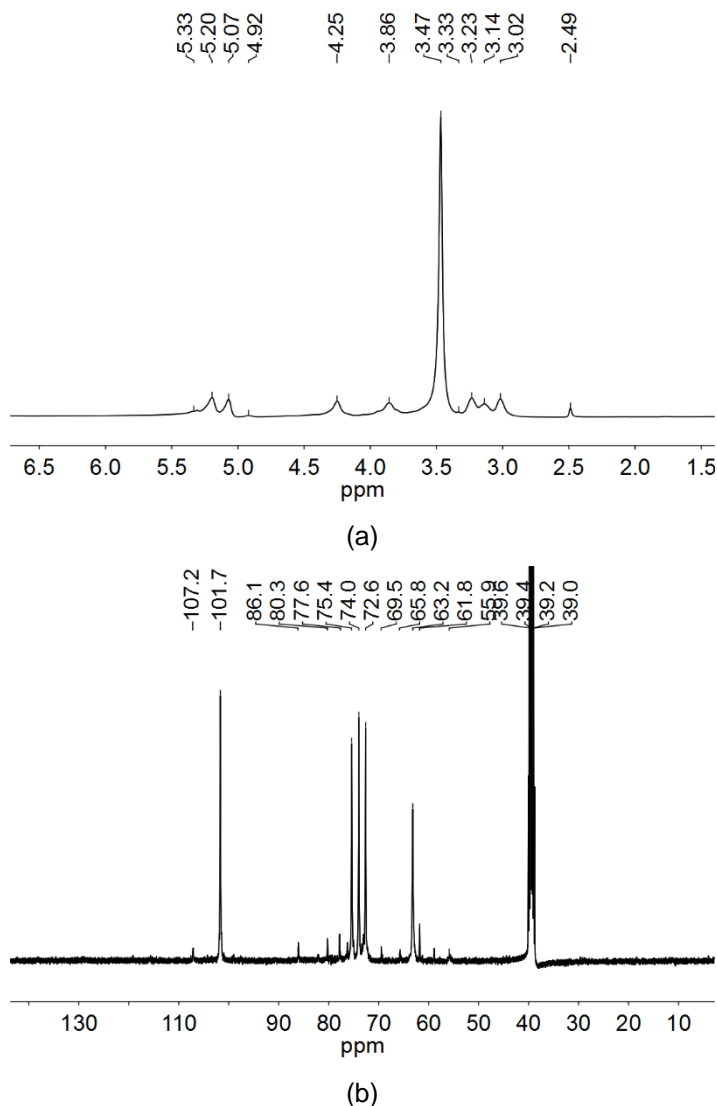
To confirm their structural integrity, the three components isolated under optimal reaction conditions were characterized using NMR, and the spectra peaks were assigned based on published values (Marita *et al.* 2001; Yang *et al.* 2011; Mansfield *et al.* 2012).

The CP/MAS  $^{13}\text{C}$ -NMR spectra of cellulose is illustrated in Fig. 5. The signals at 60 to 70 ppm corresponded to the chemical shift of C-6. The signals between 70 and 81 ppm were attributed to C-2, C-3, and C-5 of glucose units. The signals at 86.6 and 103.1 ppm represented to C-4 and C-1, respectively. The lack of signals near 122 and 98 ppm



indicated that the cellulose was relatively free from impurities such as lignin and hemicellulose, which is consistent with the FT-IR results (Fig. 4(a)).

The  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  spectra of hemicellulose are shown in Fig. 6. The four cross peaks at 4.25, 3.86, 3.47, and 3.14 ppm (Fig. 6(a)) were assigned to H-1, H-5/H-4, H-3, and H-2 of  $\beta\text{-D-Xylp}$  units, respectively. The small signal at 5.20 ppm was identified as alpha anomeric proton of arabinofuranose in hemicelluloses. The strong signals at 3.23 and 3.02 ppm were ascribed to 4-O-methyl- $\alpha\text{-D-glucuronic}$  acids. Further details can be seen from the  $^{13}\text{C-NMR}$  spectrum (Fig. 6(b)).

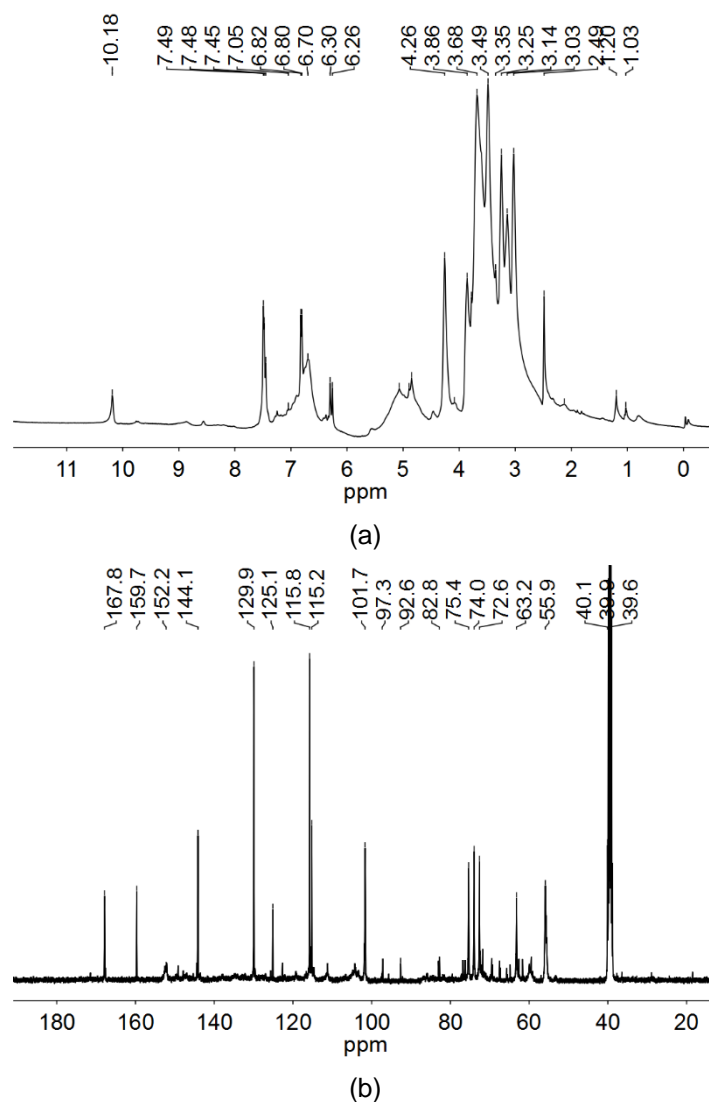


**Fig. 6.**  $^1\text{H-NMR}$  (a) and  $^{13}\text{C-NMR}$  spectra (b) of hemicellulose isolated from *P. sinense*. Reaction conditions: solid-liquid ratio of 1:25, 0.5%  $\text{H}_2\text{O}_2$ , 4%  $\text{NaOH}$ ,  $75^\circ\text{C}$ , and 4 h

The main 1,4-linked  $\beta\text{-D-Xylp}$  units were characterized by five strong signals at 101.7, 75.4, 74.0, 72.6, and 63.2 ppm, which were attributed to C-1, C-4, C-3, C-2, and C-5 of the  $\beta\text{-D-Xylp}$  units, respectively. In addition, the signals at 107.2, 86.1, 80.3, 77.6, and 61.8 ppm were assigned to C-1, C-4, C-3, C-2, and C-5 of  $\alpha\text{-L-arabinofuranose}$  units that linked to  $\beta\text{-D-xylans}$ . The signal at 55.9 ppm was ascribed to the carbonyl and the 4-O-methyl group of glucuronic acid residue in xylans. Thus, the hemicelluloses isolated with

H<sub>2</sub>O<sub>2</sub>-NaOH from *P. sinense* were mainly composed of 1, 4-linked  $\beta$ -D-xylopyranose as the main chain, with 4-O-methy glucuronic acid as the main side chain connected by  $\alpha$ -glycoside bond. In addition, some  $\alpha$ -L-arabinofuranose residues linked to main chain at C-2 and C-3 (Lan *et al.* 2011).

The <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra of lignin are presented in Fig. 7. As shown in Fig. 7(a), the <sup>1</sup>H-NMR of isolated lignin presented a wide spectrum of peaks and overlapped proton signals. This may be due to the complex three-dimensional structure of lignin as well as the steric hindrance of the lignin molecule.



**Fig. 7.** <sup>1</sup>H-NMR (a) and <sup>13</sup>C-NMR spectra (b) of lignin isolated from *P. sinense*. Reaction conditions: solid-liquid ratio of 1:25, 0.5% H<sub>2</sub>O<sub>2</sub>, 4% NaOH, 75 °C, and 4 h

The integral of all signals between 6.26 and 9.00 ppm is a typical indication of GSH lignin. Among them, the syringyl unit (S), guaiacyl unit (G), and p-hydroxyphenyl unit (H) from *P. sinense* lignin were characterized by three signals at 6.26 to 6.82, 6.82 to 7.49, and 7.49 to 9.00, respectively. In addition, peaks from 1.60 to 2.16 were attributed to aliphatic acetyl and diphenyl-o-acetyl protons, whereas the signals at 2.16 to 2.50 were

due to the acetyl protons of aromatic ring. The strong peaks at 3.49-4.09 ppm originated from methoxyl protons ( $-\text{OCH}_3$ ), indicating that methoxyl is one of the most important functional groups in lignin.

The structural features explored with  $^{13}\text{C}$ -NMR are shown in Fig. 7(b). The syringyl residues were identified by signals at 152.2 (C-3/C-5 in syringyl units) and 104.2 ppm (C-2/C-6 in syringyl units). The signals at 149.4 (C-3 in G units), 144.5 and 144.1 (C-1 in G units), 115.8 (C-5 in G units), 115.2 and 111.4 ppm (C-2 in G units) originated from the guaiacyl units, while hydroxyphenyl propane unit produced a signal at 129.9 ppm (C-2/C-6 in H units). Signals corresponding to the C- $\gamma$ , C-4, and C-1 of the *p*-coumarate unit were observed at 167.8, 159.7, and 125.1 ppm respectively. The C-2 and C-6 of the ferulate moieties were thought to be indicated by weak signals at 111.0 and 123.3 ppm (Sun *et al.* 2005; del Río *et al.* 2012). Furthermore, the signals showing the linkages between lignin phenylpropane units are also obvious in Fig. 7(b). The C $_{\beta}$ , C $_{\alpha}$ , and C $_{\gamma}$  in  $\beta$ -O-4 linkages were identified by three signals at 82.8, 72.6, and 59.8 ppm, respectively. The condensed substructures such as  $\beta$ - $\beta'$  and  $\beta$ -5' presented their typical signals near 71.8 and 62.6 ppm.

## CONCLUSIONS

1. Microwave-assisted  $\text{H}_2\text{O}_2$ -NaOH extraction was found to be an efficient way to fractionate three components from *P. sinense*. The yields of fractionated crude cellulose, hemicellulose, and lignin were dependent on the reaction conditions including solid-liquid ratio, NaOH concentration,  $\text{H}_2\text{O}_2$  concentration, temperature, and time. Tuning reaction conditions produced high yields of fractionated components while avoiding excessive degradation of carbohydrates. The yields of cellulose, hemicellulose, and lignin obtained under optimal fractionation conditions, *i.e.*, solid-liquid ratio of 1:25, 0.5%  $\text{H}_2\text{O}_2$ , 4% NaOH, 75 °C, and 4 h, were 42.8%, 21.9%, and 15.2%, respectively.
2. FT-IR and NMR analyses of the fractionated components clearly confirmed their structural integrity and representation. The structural features of the isolated cellulose under optimal reaction conditions was free from contaminants. The *P. sinense* hemicellulose was mainly 4-O-methyl-D-glucuronoxylans with some  $\alpha$ -L-arabinofuranose residue linked to main chain. Isolated lignin from *P. sinense* was characterized as a typical GSH-type lignin that consisted of three basic structural units: syringyl (S), guaiacyl (G), and *p*-hydroxyphenyl (H). The fractionated components could be converted to valuable products by further chemical conversion and modifications.

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