

Combination of Low-pressure Steam Explosion and Alkaline Peroxide Pretreatment for Separation of Hemicellulose

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Low-pressure steam explosion (LPSE) combined with alkaline peroxide (AP) pretreatment was first employed to separate hemicellulose from *Lespedeza* stalks. The monosaccharide composition and molecular weight distribution of the obtained hemicellulose fractions were characterized in this study. The results show that the hemicellulose extracted from *Lespedeza* stalks consisted of xylose, glucose, galactose, and mannose, which was a mixture of arabinoxylans and xyloglucans or β -glucans. The yield of hemicellulose fractions after AP pretreatment ranged from 11.2% (2.5% hydrogen peroxide (H_2O_2), w/v for 12 h) to 12.2% (3.3% H_2O_2 , w/v for 72 h). The molecular weight of hemicellulose decreased from 2,458 g/mol to 1,984 g/mol after AP pretreatment, indicating its degradation reaction. The structure of hemicellulose was analyzed by Fourier transform infrared spectroscopy, proton nuclear magnetic resonance, and heteronuclear single quantum coherence. The AP pretreatment partially cleaved the backbone and the ether linkage between lignin and hemicelluloses. Also, branched-chain α -D-arabinofuranosyl in which β -D-xylose substituted at the C-3 position (monosubstituted) was removed, illustrating a partial debranching reaction. Therefore, the combination of low-pressure steam explosion and alkaline peroxide pretreatment (LPSE-AP) is an effective pretreatment method to separate hemicellulose from *Lespedeza* stalk.

Keywords: *Lespedeza stalk; Hemicelluloses; Combined pretreatment; Steam explosion; Alkaline peroxide*

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INTRODUCTION

Lignocelluloses have been studied extensively with the aim of converting them into ethanol, bio-based chemicals, and high value-added biomaterials to meet global energy and chemical needs. Hemicelluloses are usually defined as polymers that are solubilized from plant cell walls by alkali treatment (Darvill *et al.* 1980). Hemicelluloses have a backbone of β -1,4-D-pyranosyl residues in which O-4 is in the equatorial orientation (*e.g.*, glucose, mannose, and xylose) (Shallom and Shoham 2003). Because of their various functional properties, the potential applications of hemicelluloses are immense and span diverse fields (Chimphango and Görgens 2012; Doner and Hicks 1997). However, the natural structures of lignocellulosic materials make it hard for extraction of hemicelluloses. Not surprisingly, choosing an effective pretreatment method is important.

Low-pressure steam explosion (LPSE) pretreatment typically involves heating the cellulosic material from 150 °C to 190 °C for 30 to 300 s (Su *et al.* 2012). Applying this pretreatment can preserve valuable components used in various applications. Alkaline

peroxide (AP) post-treatment is one of the most effective methods used to treat biomass, especially removing part of the lignin and allowing more efficient enzymatic hydrolysis. Researchers have shown that pretreatment involving steam explosion with alkaline peroxide is a potential candidate approach for lignocellulosic raw materials (Su *et al.* 2012). However, the hemicellulose fractions extracted with alkaline peroxide treatment combined with low-pressure steam pretreatment has never been characterized and evaluated.

In China, *Lespedeza crytobotrya* is primarily used to prevent soil erosion and conserve soil moisture due to its well-developed root system. However, at present, stalks of *Lespedeza crytobotrya* are undervalued agricultural residues and thus are not used as industrial raw materials but rather burned in the field. Previous studies showed that *Lespedeza* stalks can be an appropriate substrate for bioconversion (Feng *et al.* 2011; Qi *et al.* 2011; Feng *et al.* 2012). Increasing attention has been paid to the exploitation of hemicelluloses as biopolymer resources in the last few years. A challenging aim of current research is to explore the comprehensive utilization of all biomass components.

The main purpose of this work was to separate the hemicellulose fractions from the *Lespedeza* stalks and to evaluate the effect of a combination of low-pressure steam pretreatment and alkaline peroxide post-treatment of *Lespedeza* stalks on the structure of hemicelluloses. The hemicelluloses were further characterized to assess the possibility of preparing certain chemicals and determine whether the product is marketable.

EXPERIMENTAL

Materials

Lespedeza stalks (*Lespedeza crytobotrya*) were obtained from the experimental farm of Beijing Forest University, China. They were chipped and screened to a relatively homogenous size of 50 mm × 30 mm. The main components of *Lespedeza* stalks were determined according to the literature (Su *et al.* 2012). It was found that the composition (w/w) of *Lespedeza* stalks was 46.34% cellulose, 29.20% hemicelluloses, and 17.08% lignin.

Methods

Steam explosion-alkaline peroxide (SE-AP) pretreatment

The steam pretreatment was carried out in batches of 300 g of dry stalk weight at 184 °C for 4 min in a reactor (7.5 L) heated by saturated steam. The steam pressure/temperature played a dominant role in the physical/chemical properties of degraded hemicelluloses obtained from steam-exploded *Lespedeza* stalks. Our previous work showed that a substantially higher recovery of sugars and minimization of the inhibition of the microorganisms used for fermentation can be achieved by “low-pressure steam treatment,” which typically involves heating the cellulosic material at a temperature that ranges from 150 °C to 190 °C for 30 to 300 s. Applying this treatment at 184 °C could preserve valuable components that can be separated and used in various applications. Therefore, 184 °C was selected for the present experiments (Wang *et al.* 2010). At the end of the steaming step, pressure was instantaneously released to quench the reaction. The steam-pretreated samples were stored in sealed plastic bags at 4 °C for later experiments. Different conditions used for alkaline peroxide pretreatment were compared in a preliminary experiment to obtain samples with lignin contents of 5%,

10%, and 15% (Table 1). Gould (1984) reported that a maximum of nearly 100% cellulose conversion was obtained when biomass was treated with H_2O_2 at pH 11.5. In order to study the H_2O_2 -dependent reaction in the absence of interfering, nonspecific alkaline effects, experiments were performed at pH 11.5, where effects of H_2O_2 were maximal and non-specific alkaline effects were minimal. All standard chemicals used in this study were of analytical grade.

Isolation of hemicelluloses

In order to study structural differences of the hemicelluloses present in the filtrate, hemicellulose fractions were isolated by sequential extractions according to the scheme in Fig. 1. The filtrate was neutralized to a pH range of 5 to 6 with 6 M HCl and precipitated in 3 volumes of 95% ethanol over a 12-h period. After filtration, the precipitated fractions were freeze-dried. The hemicellulose samples were labelled H_1 , H_2 , and H_3 , corresponding to samples obtained from processes I, II, and III, respectively (Table 1). All experiments were performed at least in duplicate, and analyses were carried out at least three times for each sample.

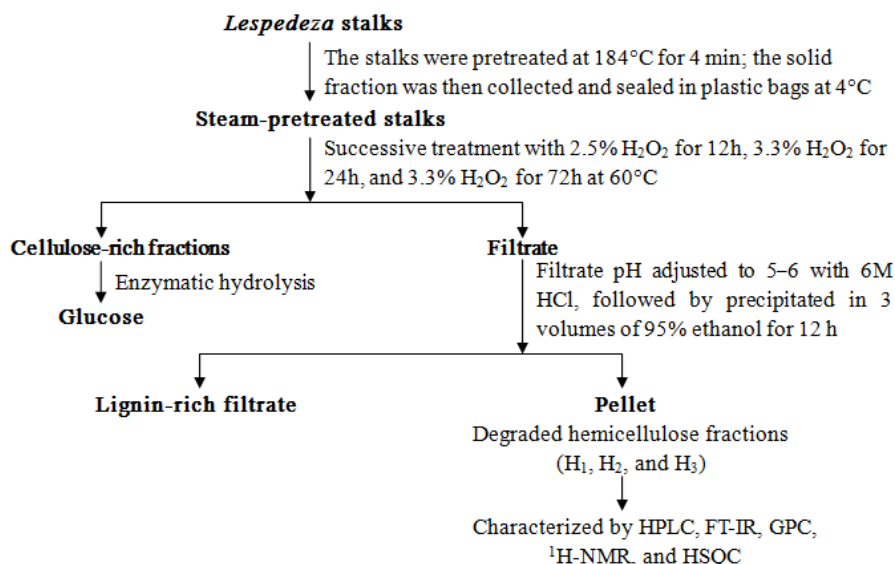


Fig. 1. Scheme for the isolation of hemicellulose fractions from steam-exploded *Lespedeza* stalks

Characterization of hemicellulose fractions

The neutral sugars in the hemicellulose fractions were obtained by hydrolysis with 87 mL of 4% sulfuric acid (H_2SO_4) at 121 °C for 1 h. The samples were then filtered and analyzed by high-performance liquid chromatography (HPLC; Waters 2695e, USA) using an Aminex HPX-87P column (Bio-Rad, USA) at 85 °C. The injection volume of the sample was 10 μL , and water was used as the eluent. The total analysis time was 50 min, and the flow rate was 0.6 mL min^{-1} . The analysis of neutral sugar composition was run in duplicate.

The measurement of the hemicellulosic molecular weights was determined by gel permeation chromatography (GPC) on a PL aquagel-OH mixed column (300 \times 7.5 mm, Agilent). The eluent was 0.02 M sodium chloride (NaCl) in 0.005 M sodium phosphate buffer, pH 7.5, with a flow rate of 0.1 mL min^{-1} . The column oven was maintained at 30 °C.

Table 1. Experimental Conditions Used for Alkaline Peroxide Treatment of the Steam-Pretreated Samples and the Yield (% Dry Weight of Raw Materials) of the Hemicellulose Fractions (H₀, H₁, H₂, and H₃)

Alkaline Peroxide Treatment	(H ₀)	I (H ₁)	II (H ₂)	III (H ₃)
Substrate Concentration (% w/v)	2.5	2.5	3.3	3.3
H ₂ O ₂ /substrate (% w/w)	0	0.4	0.6	1.0
Time, h	12	12	24	72
T, °C	60	60	60	60
pH	11.5	11.5	11.5	11.5
Hemicellulose yield %	6.2	11.2	11.9	12.2
Lignin content in cellulose-rich fraction	23.9	15.1	10.2	5.4
Hemicellulose content in cellulose-rich fraction	15.8	2.3	0.4	0

Fourier transform infrared (FT-IR) spectra were obtained on a FT-IR spectrophotometer (Tensor 27, Bruker, Germany) using a KBr disc containing 1% finely ground samples.

Solution-state proton nuclear magnetic resonance (¹H-NMR) spectra were obtained on a Bruker AVIII-400 MHz spectrometer (Germany) at 75.5 MHz. A sample concentration of approximately 10 mg in 1.0 mL of deuterium (D₂O) for ¹H NMR was placed in a 5 mm (i.d.) glass tube and run at a temperature of 25 °C for 2.0 h. A 60° pulse flipping angle, a 3.9-μs pulse width, and a 0.85-s delay time between scans were used. The heteronuclear single quantum coherence (HSQC) experiment was also recorded on a Bruker AVIII-400 MHz spectrometer after 128 scans with a 20-mg sample dissolved in 1.0 mL of D₂O. The spectral widths for HSQC were 5 and 20 kHz for the ¹H and ¹³C dimensions, respectively. The number of collected complex points was 1,024 for the ¹H dimension, with a cycle delay of 1.5 s. The number of scans was 128, and 256 time increments were always recorded in ¹³C dimension.

RESULTS AND DISCUSSION

Fractional Yield of Hemicellulose Fractions

The hemicellulose fractions obtained from steam-exploded *Lespedeza* stalks differed from each other in color, and the color of the hemicellulose fraction H₁ was lighter than that of H₀ obtained using alkaline extraction conditions in the absence of H₂O₂. The pigmentation associated with the hemicellulose fractions is likely due to the presence of components such as proteins or lignin. Proteins are very strongly associated with arabinoxylans *via* arabinose residues (Saulnier *et al.* 1995). The results corresponded well with the content of arabinoses obtained from the monosaccharide analysis. The amount of arabinose in the hemicellulose fraction H₀ (2.8%) was higher than that in the other fractions (1.6%) (Table 2). Apart from the above reasons, pigmentation possibly resulted from chromophores that were generated by alkali-catalyzed modification of reducing end groups on the polysaccharides at the elevated pH, especially at 60 °C (Doner and Hicks 1997). The color (data are not shown) of the H₂ and H₃ fractions was darker than that of H₁. In alkali-peroxide medium, long reaction time caused the complete peroxide decomposition and the consequent darkening of lignocellulose in the alkaline medium.

Information related to the yield of hemicellulose fractions is given in Table 1. All the values are expressed in terms of weight percent of the initial dry materials. During steam explosion, autohydrolysis occurred due to the presence of organic acids, mainly acetic acid, derived from acetylated hemicelluloses in the lignocellulosic materials and the hydronium ions produced from water autoionization (Wang *et al.* 2010a). In this study, steam explosion treatment of the *Lespedeza* stalks released hemicelluloses amounting to 6.2% of the initial *Lespedeza* stalks, which corresponds to 21.3% of the hemicelluloses initially contained in the raw material. It is apparent that in the presence of H₂O₂, yields increased sharply when using identical extraction conditions (time, temperature, and pH). A mild alkaline peroxide post-treatment process was developed for delignifying agricultural residues, with minimal concomitant loss of hemicelluloses (Gould 1984). In particular, the hemicellulose yields tended to increase, and this finding was undoubtedly due to enhancement of digestibility with an increase in treatment intensity.

Hemicellulose is cross-linked to other cell wall components by esterified ferulic and *p*-coumaric acids in various lignocellulosic biomass materials. There is a possibility that more severe conditions result in an increase in the hydrolysis of hemicelluloses and the cleavage of bonds between hemicelluloses and lignin. Ester-bound *p*-coumaric and ferulic acids are liberated under milder alkaline conditions as compared to those commonly used to extract hemicelluloses (Doner and Hicks 1997). Peroxide serves to oxidize lignin to low molecular weight (LMW) organic acids, thereby facilitating alkaline extraction of hemicellulose from the lignocellulosic fiber matrix (Doner and Hicks 1997). The hemicellulose yield after alkaline peroxide post-treatment ranged between 11.2 and 12.2% (based on the initial *Lespedeza* stalks), which means about 76.7 to 78.9% of the total released hemicelluloses. In addition, when considering the hemicellulose yield as well as color, the alkaline peroxide post-treatment process was judged to be more effective.

Neutral Sugar Composition

The composition of neutral sugars in the hemicellulose fractions is given in Table 2. Xylose (53.7%) and glucose (30.5%) were the predominant sugar constituents in the extracted hemicelluloses (H₀). As compared to the raw *Lespedeza* stalks, which have almost equal amounts of neutral sugars (Wang *et al.* 2010b), steam explosion-treated *Lespedeza* stalks were found to have a remarkably high xylose content. Samuel *et al.* (2011) reported that steam-treated (160 °C, 60 min) switchgrass was depleted of xylan. The results obtained were satisfactorily explained by the long duration (60 min) of steam explosion pretreatment. However, in the present work, steam explosion pretreatment led to partial dissolution of hemicelluloses due to the cleavage of glycosidic bonds in hemicelluloses and ester lignin-hemicelluloses linkages. Noticeable amounts of arabinose (2.8%), galactose (3.9%), and mannose (3.5%) were also identified as constituents. From these data, it can be speculated that the hemicellulose fractions, isolated by steam explosion of the raw material, contain a mixture of arabinoxylans, glucomannans, and β-glucans or xyloglucans.

With increasing treatment intensity, the content of xylose decreased gradually while the content of glucose increased in the hemicellulose fractions from alkaline peroxide post-treated *Lespedeza* stalks. This result can be attributed to the oxidative degradation of cellulose (amorphous cellulose) at long reaction times (H₂ and H₃).

Table 2. Sugar Content (Sugar/Hemicellulose, g/g, %) in Hemicellulose Fractions

	Hemicellulose fractions			
	H ₀	H ₁	H ₂	H ₃
Glucose	30.5	42.6	56.2	80.6
Xylose	53.7	40.7	32.3	12.7
Arabinose	2.8	1.6	ND ^a	ND
Galactose	3.9	2.6	2.7	1.8
Mannose	3.5	3.5	4.1	1.9
Ara/Xyl ratio	0.05	0.03	0	0

^a Below detectable limits

Arabinose-to-xylose (Ara/Xyl) ratios are indicative of the degree of linearity or branching of arabinoxylans (Wedig *et al.* 1987). The low Ara/Xyl ratio (0.05) of the original hemicellulose fraction indicated a high degree of polymerization with few linkages to other monosaccharide constituents. The Ara/Xyl ratio decreased from 0.05 to 0 in hemicellulose fractions H₂ and H₃ due to low-branched chains in these carbohydrates. Moreover, the content of mannose increased first and then decreased with increasing alkaline peroxide treatment intensity. According to the data, it is clear that the comprehensive hydrolysis of hemicelluloses led to partial losses of oligosaccharide. When the pretreatment intensity increased, hemicellulose fraction contained more glucose because of cellulose degradation.

Distribution of Molecular Weight

To investigate the extent of degradation that occurred during the process of steam explosion pretreatment and alkaline peroxide post-treatment, the four hemicellulose fractions were further analyzed by determining their weight-average (M_w) and number-average (M_n) molecular weights, allowing calculation of polydispersity (M_w/M_n), as summarized in Table 3. Compared with alternative pretreatments, the steam explosion pretreatment is more environmentally friendly and requires lower capital investment (Cara *et al.* 2006). However, the degradation of hemicelluloses in the high-pressure steam explosion process, as noted by other authors (Josefsson *et al.* 2002), is difficult to control.

Table 3. Weight-average (M_w), Number-average (M_n), and Polydispersity (M_w/M_n) of the Hemicellulose Fractions H₀, H₁, H₂, and H₃

	Hemicellulose fractions			
	H ₀	H ₁	H ₂	H ₃
M_w	14,090	11,948	11,648	9,062
M_n	2,458	2,908	2,623	1,984
M_w/M_n	5.67	4.11	4.44	4.57

Compared with high-pressure steam pretreatment, the low-pressure steam pretreatment has been shown to be very effective to control the degradation of hemicelluloses. The alkaline hemicellulose fraction H₀ showed a relatively high degree of polymerization with an M_w value of 14,090 g mol⁻¹, greater than that of the alkaline hemicelluloses fractions obtained by high-pressure (224 °C) steam pretreatment from *Lespedeza* stalks with an M_w value of 6,380 g mol⁻¹ (Wang *et al.* 2010b). The three alkaline peroxide hemicellulose fractions H₁ to H₃ displayed a lower degree of polymerization with M_w values between 9,062 and 11,948 g mol⁻¹. The M_w value

decreased gradually with increasing post-treatment intensity, demonstrating a significant degradation of hemicellulosic polymers during the post-treatment process. A high ratio (5.67) of steam-alkali-extractable *Lespedeza crytobotrya* hemicelluloses revealed their inherent polydispersity. The polydispersity value decreased to 4.11 under the conditions of 0.4 g H₂O₂ g⁻¹ substrate for 12 h, and then increased to 4.57 at the highest intensity (1.0 g H₂O₂ g⁻¹ substrate for 72 h), clearly manifesting significant structural modifications of the high-molecular-weight (HMW) molecules to smaller polymers at different intensities induced by alkaline peroxide. These noticeable changes are further reflected by the distribution profiles (Fig. 2). The molecular weight distribution curves of all hemicellulose fractions were significantly divided into 2 parts, with considerable amounts of molecules in the HMW (> 2,000 g mol⁻¹) and LMW (< 2,000 g mol⁻¹) regions. According to Wang *et al.* (2010b), with high-pressure (224 °C) steam pretreatment, the LMW fraction appeared as a sharp and symmetric peak and the HMW region fraction appeared as a lump. In the present work, both the LMW and HMW region fractions appeared as sharp symmetric peaks with the low-pressure (184 °C) steam pretreatment. Compared with the intensity of the LMW region of H₀, the intensity of the LMW region of H₁ was reduced slightly. However, the intensity of the LMW region of H₂ was higher than that of H₁, and the intensity of the LMW region clearly increased as the intensity of alkaline peroxide post-treatment increased. These results suggest that when the H₂O₂ treatment intensity increased, the HMW portion of the hemicelluloses degraded and the amount of LMW molecules increased, which resulted in hemicellulose dissolution. This trend was more clearly reflected in the hemicellulose fraction H₃; the intensity of the LMW peak was even higher than that of the hemicellulose fraction H₀.

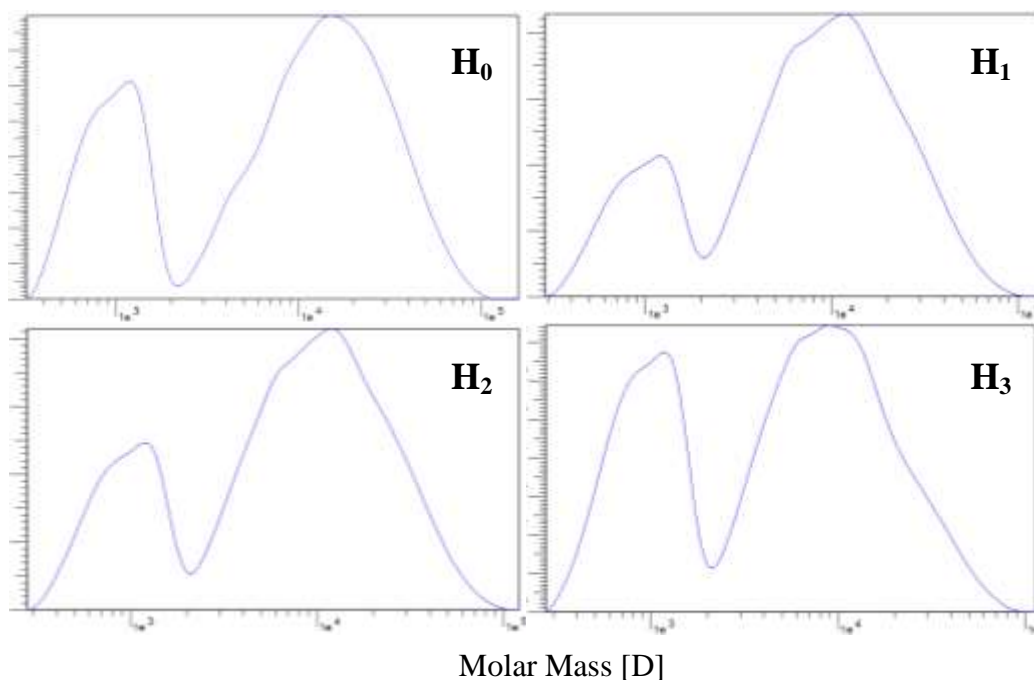


Fig. 2. Molecular weight distribution curves of the hemicellulose fractions H₀, H₁, H₂, and H₃

In addition, the intensity of the HMW region first increased (H₂) and then decreased (H₃). On the basis of this finding, it can be concluded that the depolymerization of hemicelluloses increased as the post-treatment intensity increased. In H₃, the LMW

ratio increased while the structure of the hemicelluloses changed greatly, which is indicative of degradation. This also indicates that a greater amount of hemicellulose was removed when the H_2O_2 treatment intensity increased.

FT-IR Spectra Analysis

The effect of the alkaline peroxide post-treatment conditions on the structure of the hemicelluloses was also demonstrated by FT-IR. As can be seen from Fig. 3, the FT-IR spectra of all 4 hemicellulose fractions showed typical signal patterns expected for the hemicellulosic moiety. The absorbance at 1,462, 1,420, 1,328, 1,268, 1,165, 1,121, 1,080, 1,049, 992, and 896 cm^{-1} are associated with hemicelluloses; among them, the four bands at 1462, 1420, 1328, and 1268 cm^{-1} represent the C-H and C-O bending or stretching frequencies. It is known that the absorption bands in the 1,200 to 800 cm^{-1} region may provide information about the polysaccharide types present (Hromadkova and Ebringerova 2003).

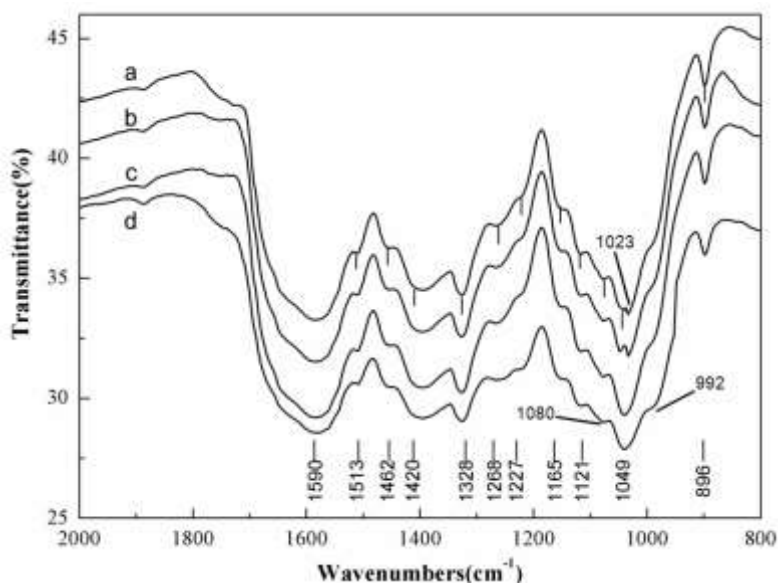


Fig. 3. FT-IR spectra of the hemicellulose fractions H_0 (spectrum a), H_1 (spectrum b), H_2 (spectrum c), and H_3 (spectrum d)

All four spectra exhibited a specific band maximum at 1,049 cm^{-1} , which is typical of xylans. This region is assigned to C-O and C-C stretching. The characteristic “anomeric region” absorption band for β -linkage (896 cm^{-1}), which is assigned to the C-1 group frequency or ring frequency, was observed as a small sharp peak in all spectra. Furthermore, this band is indicative of β -glycosidic linkages between the sugar units in the backbone structure of hemicelluloses (Geng *et al.* 2003). Shi *et al.* (2012) mentioned that the backbones and the ester linkages of hemicelluloses were heavily cleaved during the cooking process involving active oxygen (O_2 and H_2O_2) and solid alkali (magnesium oxide). As a consequence, alkaline peroxide post-treatment was shown to effectively aid in extracting hemicelluloses. The sharp peak at 1,023 cm^{-1} can be explained by the high xyloglucan content of hemicellulose fractions H_0 and H_1 , which is in accordance with the data obtained by sugar analysis. In all spectra, the band shape is influenced by the galactan moiety from the side chain, which has a band at 1,080 cm^{-1} (Kačuráková *et al.* 2000). The intensity shoulders at 1,165 cm^{-1} and 992 cm^{-1} in all hemicellulose fractions

are either indicative of the presence of β -(1 \rightarrow 4)-glucan or the presence of arabinosyl side chains (Ebringerova *et al.* 1992).

$^1\text{H-NMR}$ Spectra Analysis

To obtain further information about the degraded hemicellulose fractions, the four hemicellulose fractions H_0 , H_1 , H_2 , and H_3 were analyzed by $^1\text{H-NMR}$ spectrometry (Fig. 4) (H_1 and H_2 not shown).

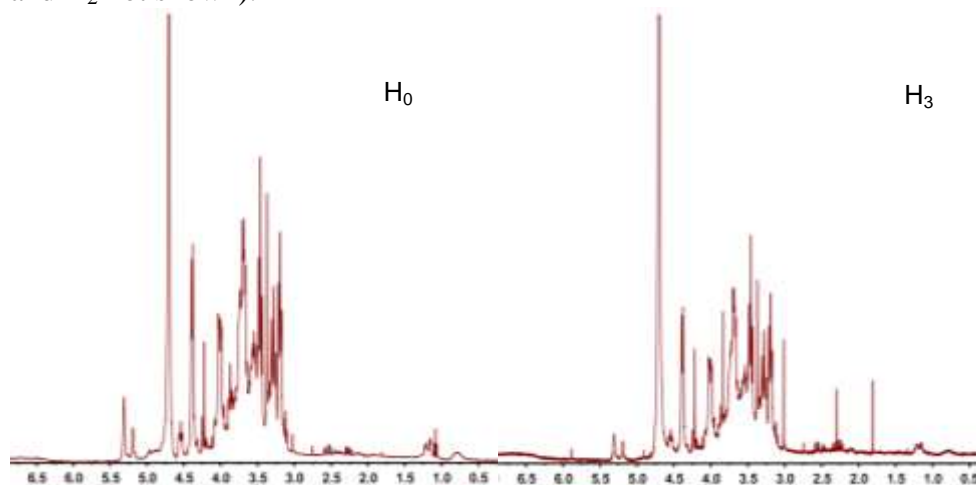


Fig. 4. $^1\text{H-NMR}$ spectrum of the degraded hemicellulose fractions H_0 and H_3

Anomeric protons of terminal α -D-arabinofuranosyl residues gave a shoulder at 5.2 ppm in the spectrums of H_0 and H_3 (Teleman *et al.* 2000). A signal was obtained at 4.4 ppm due to the anomeric protons of β -D-xylose substituted at the C-3 (monosubstituted) residues, since the region between 4.1 and 4.5 ppm corresponds to the β -configuration and the region at 4.9 to 5.6 ppm corresponds to the α -configuration (Kawagishi *et al.* 1990). This confirmed that the sugar units in the backbone structure of hemicelluloses were linked via β -glycosidic bonds, which agreed with the presence of the small sharp peak at 896 cm^{-1} in the FT-IR spectrum. Alkaline peroxide post-treatment decreased the intensities of the two signals, demonstrating that the arabinose and xylose content had decreased. These assumptions are well in accordance with the neutral sugar composition data. It also partially illustrated the debranching reaction, because the branched-chain α -D-arabinofuranosyl in β -D-xylose substituted at the C-3 (monosubstituted) was removed. It seems that the structure of an oxidation product depends on the reaction conditions (especially the concentration of the solution and the time) and the chemical structure of carbons being oxidized. The signals for other protons of glucose, arabinose, and xylose overlapped in the region of 3.2 to 4.3 ppm and centered at 3.3 to 3.4 ppm (Wang *et al.* 2010a).

$^1\text{H}/^{13}\text{C}$ NMR (HSQC) Spectrum Analysis

As shown in Fig. 5, the five predominant signals gave HSQC $^{13}\text{C}/^1\text{H}$ cross-peaks at 101.6/4.40, 72.5/3.22, 73.6/3.50, 75.8/3.70, and 62.9/4.05 + 3.32 ppm, which were assigned to C-1, C-2, C-3, C-4, and C-5 of (1 \rightarrow 4)-linked β -D-xylan, respectively. In addition, the cross-peaks at 99.5/5.32, 72.2/3.60, 73.2/3.88, 77.2/3.67, 71.4/3.59, and 60.5/3.71 ppm corresponded to C-1, C-2, C-3, C-4, C-5, and C-6 of the (1 \rightarrow 4)-linked α -D-glucan, respectively. The signal at 4.70 ppm was attributed to the residual solvent

hexanediol (HDO), while the strong signals at 36.4/2.21 and 170.8/8.39 ppm were assigned to the dimethylformamide (DMF) solution. Thus, from the results of NMR it can be concluded that the hemicellulose fraction H₃ was mainly composed of (1→4)-linked α -D-glucan and (1→4)-linked β -D-xylan.

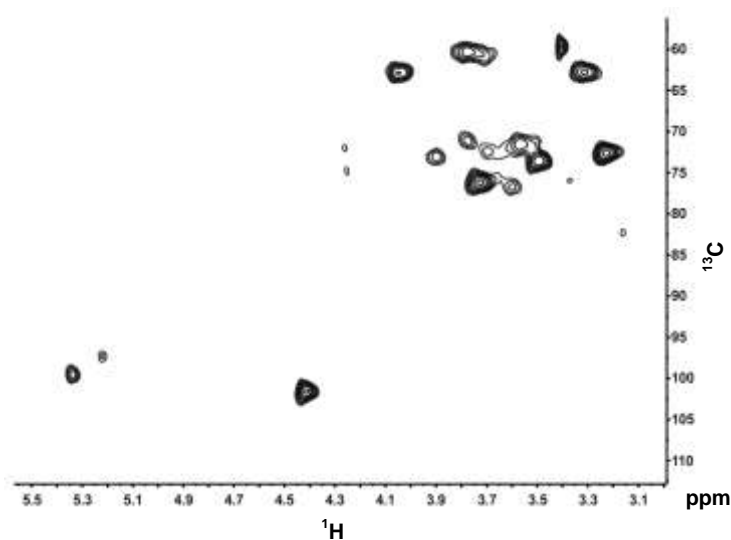


Fig. 5. $^1\text{H}/^{13}\text{C}$ -NMR (HSQC) spectrum of hemicellulose fraction H₃

CONCLUSIONS

1. The hemicellulose fraction H₀ of steam-exploded *Lespedeza* stalks is a mixture of arabinoxylans and xyloglucans or β -glucans. The recovery rate of the hemicellulose fractions increased from 6.2% to 12.2% by alkaline peroxide post-treatment.
2. A small amount of branched chains and significant degradation of hemicellulosic polymers were noted during alkaline peroxide post-treatment.
3. Analysis by FT-IR revealed that the post-treatment destroyed, in part, the linkage between lignin and hemicelluloses.
4. Analytical investigations by ^1H -NMR and HSQC indicated that the hemicellulose fraction H₃ is mainly composed of (1→4)-linked α -D-glucan and (1→4)-linked β -D-xylan.

ACKNOWLEDGMENTS

The authors are grateful for the financial support of this research from the Fundamental Research Funds for the Central Universities (BLYJ201417) and the China Ministry of Science and Technology (2014DFG32550). The authors are also grateful for the kind support from the Committee of the 4th Conference on Biorefinery towards Bioenergy (ICBB2013) in Xiamen, China.

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Article submitted: January 11, 2014; Peer review completed: April 4, 2014; Revised version received and accepted: April 16, 2014; Published: April 22, 2014.