Influence of Steaming Pressure on Steam Explosion Pretreatment of Lespedeza stalks (*Lespedeza cyrtobotrya*). II. Characteristics of Degraded Lignin

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ABSTRACT: Lignin fractions obtained by steam explosion pretreatment and subsequent alkaline ethanol solution post-treatment from *Lespedeza cyrtobotrya* stalks were studied in terms of chemical characteristics, to reflect the influence of elevating steam pressure from 15 to 25 kg/m^2. Because of the remarkable selectivity with respect to lignin, the post-treatment with 60% ethanol solution containing 1% NaOH yielded 8.3, 13.0, 16.0, 16.4, and 17.8% lignin fractions from the samples steam-exploded at 15, 17.5, 20, 22.5, and 25 kg/m^2 for 4 min, respectively, comparing to 7.7% lignin removal from the raw material. Steam explosion pretreatment, not only obviously cleaved the linkage between carbohydrates and lignin resulting in the significantly decrease of the associated hemicelluloses in lignin fractions, but also broke the β-*O*-4 bond between lignins to some degrees. In particular, slightly more guaiacyl moieties than syringyl units were affected. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 116: 1617–1625, 2010

Key words: lignin; steam explosion; steaming pressure; fractionation; *Lespedeza cyrtobotrya*

INTRODUCTION

As the era of oil changes to the biomass age, new important industrial production opportunities have emerged, such as bio-ethanol production for replacement of transportation fuel of fossil origin. Indeed, for producing bio-fuels at levels as requested, it is unavoidable to use the carbohydrates in lignocellulosic resource. In this case, lignin is a non-negligible part of the raw material that cannot be transformed into sugars. Lignin is a three-dimensional phenylpropanoid polymer mainly linked by ether bonds between monomeric phenylpropane, and the main function is to cement the cellulose fibers in plants. Traditionally, lignin has been viewed as a waste material or a low value by-product of pulping so that its utilization predominantly limited to be a fuel to fire the pulping boilers. However, just due to the unique structure, lignin has many applications to replace many existing problematic products in life science industries, such as lignin-based polyesters and polyurethanes, carbon-fibers, plastics, surfactants, elastomer-reinforcing agents, adhesives, and so on. Thereby, for achieving economic viability, the fractionation of lignocellulosic material for the simultaneous production of hemicelluloses, cellulose and lignin preparations has become one of the most important key factors for success.

Among the different existing pretreatment methods, steam explosion is one of the most commonly used processes for the fractionation of biomass components, which can weaken the lignocellulosic structure and increase its chemical reactivity and enzyme accessibility. Steam explosion, without the addition of any mineral acids (prehydrolysis), is a process that pressurized steam is firstly applied to cellulosic biomass in a pressure actor, and after a set reaction time, some of the steam is vented to quickly reduce the pressure in the reactor, the disruption of cell walls was induced by the decompression of water in the biomass. Compared with alternative pretreatment methods, the advantages of steam explosion include a significantly lower environmental impact, lower capital investment, and less hazardous process chemicals.[1] During the steam explosion,

hemicelluloses are partially degraded to the soluble sugars in the present of the organic acids, mainly acetic acid derived from the acetylated polysaccharides present in wood. However, two types of reactions would occur for lignin fraction under this condition. Initially, the fast depolymerization reactions of the native lignin by acidic hydrolysis predominates the pretreatment process, accounting for the solubility of the lignin in the solvent. As heating continues, the condensation/repolymerization reactions take over, resulting in the increasing amounts of insoluble residual lignin.[2] Under acidic condition without any added nucleophile, the predominant reactions in lignin are fragmentation by acidolysis of β-O-4 linkages and polymerization by acid-catalyzed condensation between the aromatic C_6 or C_5 and a carbonium ion, normally located at C_α of the side chain.[3] The chemical changes which take place during autohydrolysis in the three main constituents of wood are very much dependent on the temperature and the time at temperature.

The purpose of this present work was to characterize the lignin fractionations released both from the raw and steam-exploded *Lespedeza cyrtobotrya* stalks by 60% alcohol solution containing 1% NaOH post-treatment, with a view to elucidating the influence of steaming pressure and the chemical structure of the lignin fractions that could be converted to high-valued products. The lignin fractions obtained were subjected to characterization by both the degraded method such as nitrobenzene oxidation and nondestructive techniques, e.g., ultraviolet (UV), Fourier transform infrared (FTIR), nuclear magnetic resonance spectroscopy (NMR), and gel permeation chromatography (GPC).

EXPERIMENTAL

Materials

Lespedeza stalks (*Lespedeza cyrtobotrya*) used in this experiment was obtained from the experimental farm of Beijing Forest University. The stalks were air-dried and cut into an average size of 50 mm × 30 mm × 20 mm. The chemical composition of the stalks was cellulose 44.6%, hemicelluloses 29.3%, lignin 17.0%, and wax 4.8%. The deviations of these contents from their respective means were all less than 3%.

Steam explosion pretreatment and alkaline ethanol solution post-treatment

The steam explosion experiments were carried out in a flash hydrolysis laboratory pilot unit (7.5 L reactor) designed especially for the processing of lignocellulosic materials. All experiments were carried

out on the same amount (300 g) of air-dried Lespedeza stalks. The reactor was heated with saturated steam, at the end of the steaming, the pressure was instantaneously released to stop the reaction, and the sample was blown in the receiver, then the exploded material was collected and oven-dried at 55°C for 12 h. In this article, we report the results of five experiments performed at 15, 17.5, 20, 22.5, and 25 kg/m^2 for 4 min, respectively. After steam experiment, all the samples were fractionated as described in Figure 1. The exploded samples were post-treated with 60% alcohol solution containing 1% NaOH at 78°C for 3 h with a shrub to water ratio of 1 : 20 (g/mL), and then filtered in a 100% polyester cloth to separate the insoluble rich-in-cellulose fraction. Afterwards, the filter liquor was neutralized to pH 5–6 with 6M HCl, mixed with three volumes of 95% ethanol, precipitated for 12 h, and filtered through filter paper to isolate the degraded hemicelluloses. The dissolved lignin was finally centrifuged by acidification at pH 2.0 with adding 6M HCl into the supernatant solution after evaporation of ethanol. The lignin fractions were washed with acidified water, freeze-dried, and labeled L_1, L_2, L_3, L_4, and L_5, respectively, corresponding to the steaming pressure 15, 17.5, 20, 22.5, and 25 kg/m^2. L_0, which represents the lignin fraction released by alkaline ethanol solution extraction from the raw material, was taken as a comparison. All the samples were kept in a desiccator at room temperature before further analysis. All experiments were performed at least in duplicate, and analyses were carried out at least three times for each of the sample. Yields of the lignin fractions are given on a dry weight basis related to the starting materials.

Physicochemical characterization of lignin fractions

The hemicelluloses associated with the lignin fractions were determined by hydrolysis with 1M H$_2$SO$_4$ for 2.5 h at 105°C, and the liberated neutral sugars were analyzed by the high-performance anion-exchange chromatography (HPAEC) system (ICS3000, Dionex, USA) with pulsed amperometric detector and an ion exchange Carbopac PA-1 column (4 mm × 250 mm). The molecular-average weights of the lignin fractions were determined by gel permeation chromatography on a PLgel 5 m Mixed-D column. The samples were dissolved in tetrahydrofuran at a concentration of 0.2%, and 20 μL sample in solution was injected. The column was operated at 30°C and eluted with tetrahydrofuran at a flow rate of 1 mL/min. To calibrate the column, monodisperse polystyrene of known molecular was used as the standard for the molecular weight of lignin. The chemical composition of phenolic acids/aldehydes was measured by alkaline nitrobenzene
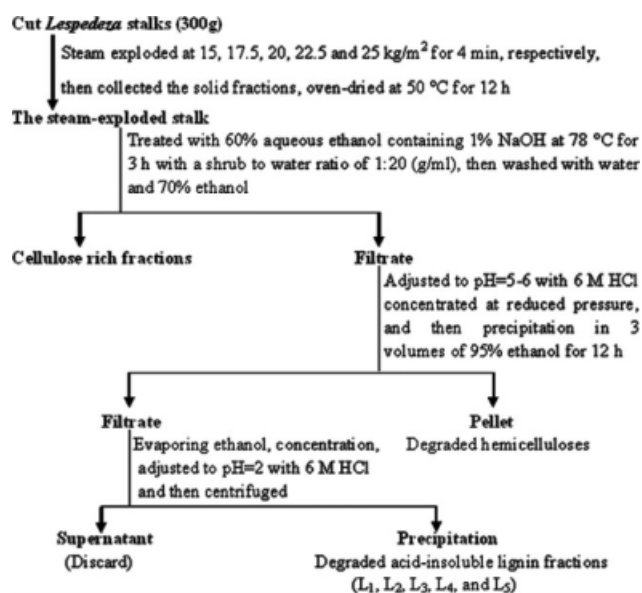
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Cut Lespedeza stalks (300g)
    │ Steam exploded at 15, 17.5, 20, 22.5 and 25 kg/m² for 4 min, respectively,
    │ then collected the solid fractions, oven-dried at 50 °C for 12 h
The steam-exploded stalk
    │ Treated with 60% aqueous ethanol containing 1% NaOH at 78 °C for
    │ 3 h with a shrub to water ratio of 1:20 (g/ml), then washed with water
    │ and 70% ethanol
    │
    ├─────────────────────────┐
Cellulose rich fractions      Filtrate
                              │ Adjusted to pH=5-6 with 6 M HCl
                              │ concentrated at reduced pressure,
                              │ and then precipitation in 3
                              │ volumes of 95% ethanol for 12 h
                    ┌─────────┴─────────┐
                 Filtrate              Pellet
                 Evaporing ethanol, concentration,   Degraded hemicelluloses
                 adjusted to pH=2 with 6 M HCl
                 and then centrifuged
          ┌──────────┴──────────┐
      Supernatant            Precipitation
      (Discard)              Degraded acid-insoluble lignin fractions
                             (L₁, L₂, L₃, L₄, and L₅)
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Figure 1 Scheme for isolation of lignin fractions from steam-exploded *Lespedeza cyrtobotrya* stalks.

oxidation method, which was performed at 145°C for 2.5 h, and determined by high-performance liquid chromatography (HPLC) on a ZORBAX Eclipse XDB-C$_{18}$ HPLC column of dimensions 250 mm × 4.6 mm (1200 series, Agilent Technologies, USA). The identification of the individual compounds was detected at 280 nm by computer comparison of the retention times and peak areas with the authentic phenolics.

The measurements of UV and FTIR spectra were described in a previous paper.[4] The ^{1}H- and ^{13}C-NMR spectra of the lignin fractions were recorded on a Bruker MSL-300 spectrometer (Germany) at 74.5 MHz. Sample concentrations of ∼ 25 mg in 1.0 mL DMSO-d_6 for ^{1}H-NMR and 250 mg in 1.0 mL DMSO-d_6 for ^{13}C-NMR were placed in 5 mm i.d. glass tubes and run at a temperature of 25°C for 2 h and 24 h, respectively. The acquisition time was 1.2 s and pulse was 80° (7 μs). The instrument was set in proton inverse gated decoupled mode and the delay between scans was 1.2 s.

RESULTS AND DISCUSSION

Fractional yield and molecular weight

In 1982, Marton and Granzow[5] patented a method for producing wood pulp using a mixture of ethanol and sodium hydroxide. Alcohol delignification is a complex process involving the breakdown of the lignin-carbohydrate complex, solvation of the breakdown products, and repolymerization and/or redeposition of the breakdown products on the solids. It has been suggested that ethanol swells the wood structure where the extent of swelling increases with

the polarity of the solvent and acts as a solvent for the lignin,[6] and reduces the surface tension of the pulping liquor at high temperature promoting the penetration of the alkali into the chips and the diffusion of the breakdown products of lignin from the chips to the liquor. This ensures that the lignin can be extracted without extensive modification of its structure. Simultaneously, alcohol also degrades lignin and prevents it from condensing. The presence of sodium hydroxide prevents or reduces the pentosan removal/hydrolysis,[6,7] and improves the delignifying ability of ethanol. Likewise, the mild alkaline treatment is not prone to cause much chemical modification beyond the saponification of ester bonds and cleavage α-ether linkages between lignin and polysaccharides. El-Sakhawy et al.[8] have studied the pulping of cotton stalks with aqueous ethanol and alkaline ethanol processes. The alkaline ethanol method produced pulps with acceptable quality under mild conditions while the aqueous ethanol process required the use of a catalyst. Alkaline ethanol pulping of wheat straw can be significantly improved by an acid catalyzed prehydrolysis.[9]

The yield of lignin fractions are listed in Table I. As can be seen, the combination of steam explosion pretreatment and alkaline ethanol solution posttreatment permitted the extraction of high yields of lignin fractions under the considerably mild condition. More specifically, the yield of lignin fractions released by post-treatment with 60% alcohol solution containing 1% NaOH at 78°C for 3 h was 7.7% from the raw material, and gradually increased from 8.3, to 13.0, to 16.0, to 16.4, and to 17.8% (% dry starting material) as the increment of steam pressure from 15, to 17.5, to 20, to 22.5, and to 25 kg/m^2, respectively, for a fixed steaming time (4 min). Fernández-Bolaños et al.[10] steam-exploded olive seed husks and whole stone without acid at 22–28 kg/m^2 for 2 min, the recovery yields of lignin released by 2% aqueous alkaline-extraction increased from 9.0% to 15.2% and from 10.9% to 29.4%, respectively. Li et al.[3] reported the autohydrolysis of aspen wood at 10–16 kg/m^2 for 5 min, and which yielded 10.2–16.0% lignin fractions extracted with 90% dioxane. As the steam explosion resulted in the chemical modification (autohydrolysis of the major part of the hemicelluloses and a small part of lignin/cellulose) and the physical modification (defibrillation), it is suggested that higher steam pressure profited more extraction of lignin fraction. The probable reason is that the vapor enters into the deeper position of the cell wall and then induces more cleavage of the ether linkages between hemicelluloses and lignin at higher pressure. On the other hand, comprehensive repolymerizations resulted in an increase in molecular size and a more heterogeneous lignin structure during steam explosion process. However, as the data

TABLE I
The Yield (% dry matter), Weight Average (M_w), Number Average (M_n), and Polydispersity (M_w/M_n) of the Lignin Fractions Obtained by Post-treatment of the Corresponding Steam Exploded Lespedeza stalks Residues with 60% Aqueous Ethanol Containing 1% NaOH at 78°C for 3 h, Respectively

	Lignin fractions[a]					
	L_0	L_1	L_2	L_3	L_4	L_5
Yield[b]	7.7	8.3	13.0	16.0	16.4	17.8
M_w	1453	900	1461	1601	1758	1791
M_n	909	705	980	1057	1106	1109
M_w/M_n	1.6	1.3	1.5	1.5	1.6	1.6

[a] L_0 represents the lignin fraction released by alkaline ethanol solution extraction from raw Lespedeza stalks, while L_1, L_2, L_3, L_4, and L_5 represent the lignin fractions obtained by posttreatment with 60% aqueous ethanol containing 1% NaOH at 78°C for 3 h with a shrub to water ratio of 1:20 (g/mL) from the steam exploded samples, corresponding to the steam explosion conditions at 15, 17.5, 20, 22.5, and 25 kg/m² for 4 min, respectively.
[b] % Dry matter of Lespedeza stalks

shown, the extraction yield at 25 kg/m² for 4 min constantly increased to 17.8% regardless of the higher molecular weight (1791 g/mol, shown in Table I). These results stated that the alkaline ethanol solution could efficiently fractionate the lignins from the steam-exploded samples.

The weight-average (M_w), number-average (M_n) molecular weights, and polydispersity value (M_w/M_n), calculated from the GPC curves (relative values related to polystyrene), and the molecular weight distribution curves of all the six lignin fractions are given in Table I and Figure 2. It is worth mentioning that the weight-average molecular weight (1453 g/mol) of lignin fraction (L_0) released from the original Lespedeza cyrtobotrya stalks is almost the truth value due to the well maintenance of lignin structure under the mild alkaline organsolvent condition. The delignification process under aqueous alkaline solution could reduce the cleavage of aryl-ether and ester bond, and yield relatively lower weight-
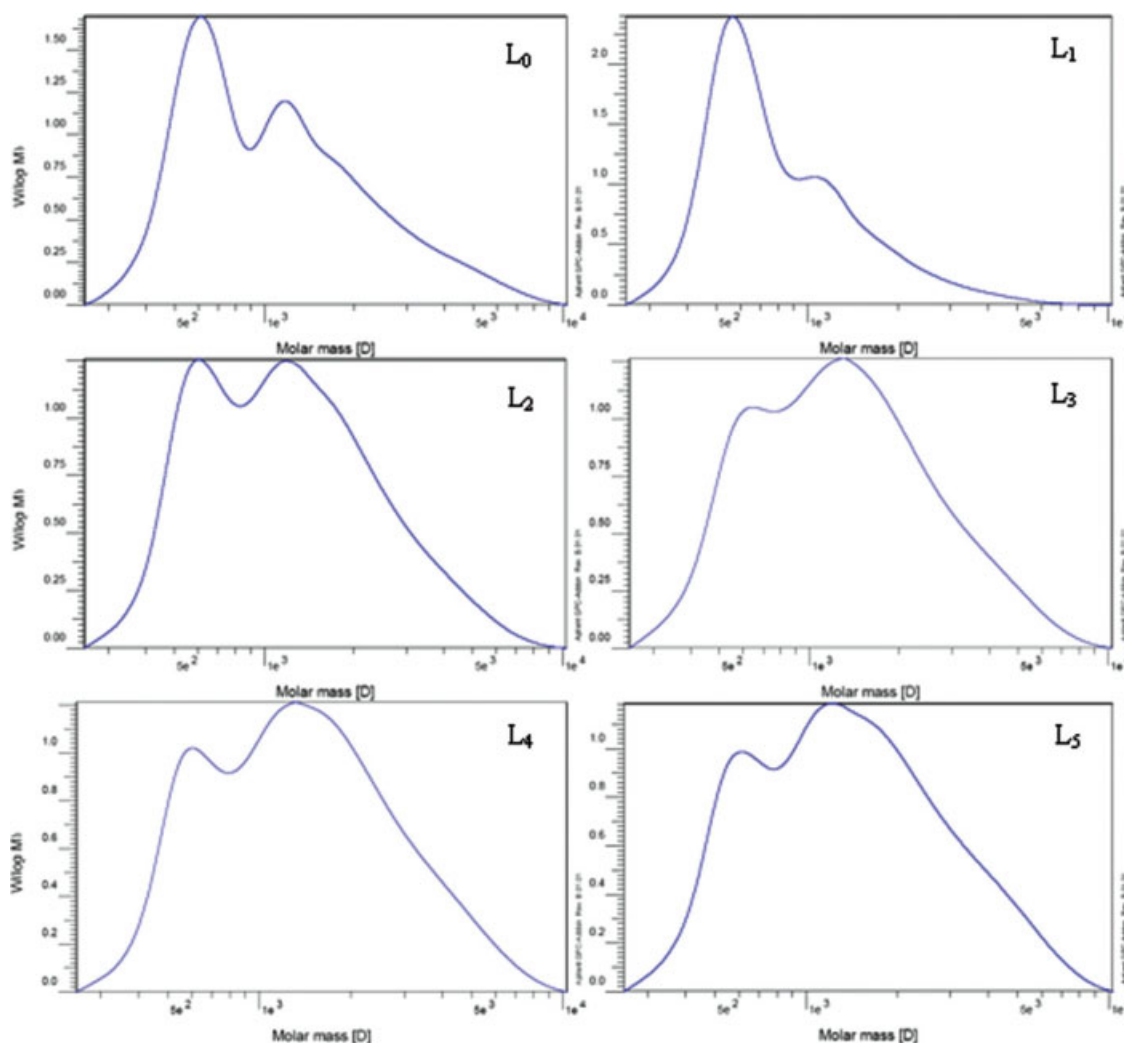

Figure 2 Molecular weight distribution curves of lignin fractions L_0, L_1, L_2, L_3, L_4, and L_5. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
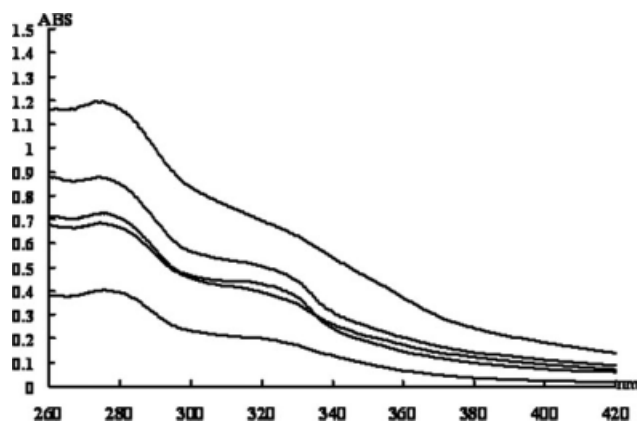
Figure 3 UV spectra of lignin fractions L_0, L_1, L_2, L_3, L_4, and L_5.

average molecular weight, which was not used to reflect the real value. As can be seen from Figure 3, most chromatograms took the form of a bimodal curve. The first peak was due to the low molecular weight portion less than 1000 g/mol, and the second peak was assigned to the high molecular weight portion above 1000 g/mol. Combining Figure 3 and Table I, it is indicated that at the lowest severity of the pretreatment there was a reduction in the yield of high molecular weight portion (900 g/mol), resulting from the depolymerization reactions. As the pressure rose above 17.5 kg/m², the molecular weight increased to 1461 g/mol and the $_w$ peak showed a clear shift to the higher molecular weight region, demonstrating that the comprehensive repolymerization reactions took the predominant position. When the pressure was higher than 20 kg/m², the molecular weight leveled off (1758 g/mol for L_4, 1791 g/

mol for L_5), where the balance between depolymerizaton and repolymerization reactions was formed.

Content of phenolic acids and aldehydes

The yield of alkaline nitrobnenzene oxidation products and the components of phenolic acids and aldehydes are given in Table II. The predominant oxidation products were found to be roughly equal amounts of syringaldehyde and vanillin, which together represented 65.5, 63.4, 62.9, 55.0, 58.3, and 68.8% of the total phenolic compounds in L_0, L_1, L_2, L_3, L_4, and L_5 fractions, respectively. In addition, small amounts of vanillic acid, syringic acid, p-hydrozybezonic acid, and p-hydroxybenzaldehyde, together with the trace of acetosyringone and acetovaillone were identified in all the six lignin fractions. It is suggested that Lespedeza cyrtobotrya stalks lignin contains approximately near quality of noncondensed guaiacyl and syringyl units with relatively fewer p-hydroxyphenyl units, just as the components of phenolic acids and aldehydes in wheat straw lignin.[11] Ferulic acid is known to be esterified with hemicelluloses and etherified with lignin. Further studies confirmed that all of the esterified ferulic acid was also ether linked at the β-position of coniferyl alcohol.[12] p-Coumaric acid, on the other hand, is known to be extensively esterified at the γ-position on the side chain of lignin monomers.[13] Occurrence of noticeable amounts of p-coumaric acid and small quantities of ferulic acid in the steam-exploded lignin fractions indicated that these two hydroxycinnamic acids are closely linked with lignins in the cell wall of Lespedeza cyrtobotrya stalks.

TABLE II
The Yield of Phenolic Acids and Aldehydes (% Lignin Sample, w/w) Obtained by Alkaline Nitrobenzene Oxidation of the Degraded Lignin Fractions

Phenolic acids and aldehydes	Lignin fractions[a]					
	L_0	L_1	L_2	L_3	L_4	L_5
p-Hydrozybezonic acid	0.3	0.4	0.3	0.3	0.3	0.1
p-Hydroxybenzaldehyde	0.5	0.4	0.3	0.3	0.2	0.1
Vanillic acid	0.8	0.9	0.8	0.9	0.8	0.3
Vanillin	5.0	3.6	3.5	2.6	3.0	2.1
Acetovaillone	T[b]	0.1	T	T	T	T
Syringic acid	1.4	1.2	1.1	1.2	1.1	0.8
Syringaldehyde	6.4	5.6	5.3	4.0	4.7	3.2
Acetosyringone	0.3	0.3	0.4	0.4	0.5	T
p-Coumaric acid	2.4	2.0	2.1	2.0	2.4	1.1
Ferulic acid	0.3	0.2	0.2	0.2	0.2	ND[c]
Total	17.4	14.5	14.0	12.0	13.2	7.7
S/G[d]	1.4	1.5	1.6	1.6	1.7	1.7

[a] Corresponding to the lignin fractions in Table I.
[b] T = trace.
[c] Not detectable.
[d] G represents the total moles of vanillin, vanillic acid, and acetovaillone; and S represents the total moles of syringaldehyde, syringic acid and acetosyringone.

The composition and quantity of the aromatic acids and aldehydes reflect the structural features of the side chain and the extent of carbon–carbon linkages present in the lignin. On the basis of an extensive study of aspen lignins by steam explosion, Chua and Wayman[14] reported that the decrease in aromatic aldehyde yield is attributed mainly to the condensation reactions involving the formation of new carbon–carbons and only to a minor extent to modification of the side chain of the lignin, resulting in a lignin structure which is much less amenable to oxidation to phenolic aldehydes. As the data shown in Table II, the total yield of phenolic acids and aldehydes decreased from 17.4% in the raw material lignin fraction (L_0) to 14.5(L_1), to 14.0 (L_2), to 12.0 (L_3), to 13.2 (L_4), and to 7.7% (L_5) in the steam-exploded lignin fraction as the steam pressure increased from 15 to 25 kg/m^2. The majority of the new formed carbon–carbon bonds come from the linkages between lignins. From studies of the reactivity of the aromatic sites in lignin,[14,15] it has been established that acid catalyzed condensation reactions processed most rapidly at the C-6 position. Because of the additional methoxyl group at the C-5 position, the syringyl units would represent a configuration sterically hindered to condensation at C-6. Therefore, more uncondensed syringyl than guaiacyl, and conversely preferential condensation of guaiacyl units present in the lignin fraction. The data in this study was agreed with aforementioned conclusion. From Table II, it is clear that the ratio of S/G increased from 1.4 in L_0, to 1.5 in L_1, to 1.6 in L_2 and L_3, and finally up to 1.7 in L_4 and L_5. The new formed carbon–carbon bonds can also be partly attributed to the physical or chemical incorporation of the nonlignin materials such as carbohydrate degradation products. It is probable that these reaction intermediates, namely furfural and its precursors, participate in the lignin condensation reactions, resulting in the formation of so-called pseudolignin.[16,17] From the aforementioned analysis, it is concluded that the increment of steam pressure given in our study could obviously enhance the extent of repolymerization/condensation reactions, and the uncondensed guaiacyl units could ever more react with the reactive intermediates forming new carbon–carbon bonds than the uncondensed syringyl units.

Composition of the associated polysaccharides

The composition of the associated hemicelluloses in the steam-exploded lignin fractions was determined by their contents of neutral sugars, and the analytical results are given in Table III. Obviously, all the lignin fractions contained rather low amounts of bound polysaccharides as shown by a 0.80–1.13% neutral sugar content, indicating that the two-stage

process under the conditions used significantly cleaved the ether bonds between lignin and hemicelluloses in the cell walls of *Lespedeza cyrtobotrya*, in addition to partial saponfication of hydroxycinnamic esters such as between *p*-coumaric acid and lignin/polysaccharides or between ferulic acid and hemicelluloses. It is clear that xylan-lignin linkage, which takes arabinose and galactose as bridge, was the most important connection in lignin-carbohydrate complexes (LCC). As 1, 3/6-linked galactan structures had a role in restricting lignin removal from the secondary fiber wall,[18] the content of galactose was kept around 0.13% despite of the severity. As expected, the increment of steam pressure from 15 to 25 kg/m^2 led to a gradual decrement in the content of associated polysaccharides from 1.13% to 0.80% in the lignin fractions. Similar results have been reported by Fernández-Bolaños et al.[10] The authors demonstrated that the content of carbohydrate decreased from 1.20% to 0.20% and from 2.22% to 0.70% in olive whole stone and seed husks, respectively, as the function of elevating steam pressure from 22 to 30 kg/m^2.

UV absorption

The five lignin fractions exhibited the basic UV spectrum of typical lignin with a maximum at about 280 nm originated from the nonconjugated phenolic groups (aromatic ring), which is known to be characteristic of guaiacyl-syingyl lignin (Fig. 3). However, on closer examination, this absorption revealed a shift towards lower wavelength from L_0 to L_5, resulting from steam explosion and elevating steam pressure. As syringyl units exhibit the bands at somewhat shorter wavelengths, it is indicated that a relatively higher content of syringyl units in the steam-exploded lignins under more severities, which is accordance with the increment of S/G ratio by analyzing the alkaline nitrobenzene oxidation products.[19] The second maximum at around 320 nm is mainly attributed to the carbonyl functions existed in the lignin fractions.[20] Moreover, a higher absorption of L_1, L_2, L_4, and L_5 fractions than L_0 fraction indicated a higher purity of the lignin fractions as the function of steam explosion.

FTIR spectra analysis

Figures 4 and 5 showed the FTIR spectra of all the six lignin fractions. The modification of the side chain group of lignin (α, β-unsaturated aldehydes and α-carbonyl groups) from the changes of the carbonyl absorption region might enable the evaluation of the effects of the steam explosion pretreatment. In the spectrum L_0, the small band at 1712 cm^{-1} is originated from the unconjugated carboxyl acid/ester

TABLE III
The Composition of Neutral Sugars (% Lignin Sample, w/w) in the Degraded Lignin Fractions

Sugars	Degraded lignin fractions[a]				
	L_1	L_2	L_3	L_4	L_5
Rhamnose	0.06	0.06	ND[b]	ND	ND
Arabinose	0.12	0.11	0.07	0.06	ND
Galactose	0.13	0.15	0.16	0.13	0.13
Glucose	0.11	0.11	0.12	0.09	0.10
Mannose	ND	ND	0.05	ND	ND
Xylose	0.71	0.68	0.62	0.59	0.57
Total	1.13	1.11	1.02	0.87	0.80

[a] Corresponding to the lignin fractions in Table I.
[b] Not detectable.

groups or β-ketone carbonyl groups, and the intensive band at 1666 cm^{-1} is assigned to the conjugated aryl carbonyl groups in lignin. In contrast, the remarkable increase of unconjugated carboxyl/carbonyl absorption and the decrease of conjugated carbonyl intensity with increasing steaming pressure were observed in the steam-exploded lignin fractions. Li et al.[3] stated that under acidic conditions without any added nucleophile, the predominant reactions in lignin are fragmentation by acidolysis of β-O-4 linkages and polymerization by acid-catalyzed condensation between the aromatic C_6 or C_5 and a carbonium ion, normally located at C_α of the side chain. Both of the reactions occurred more or less simultaneously. In the depolymerization reaction, the β-ketone structures are formed together with a new phenolic end group, whereas the latter gives rise to a new stable carbon–carbon linkage between two lignin units. Lora and Wayman[21] have reported that heating of aspen lignin at low pH increased the absorption intensity of the infrared band attributable to unconjugated β-ketone and carboxylic acid groups in the lignin. Similar phenomenon of bond-breakage
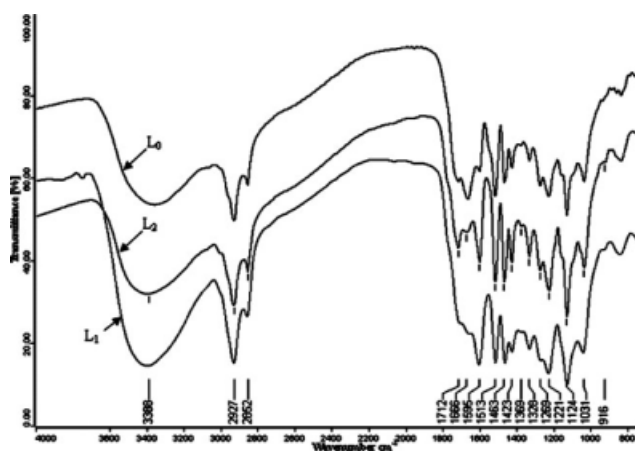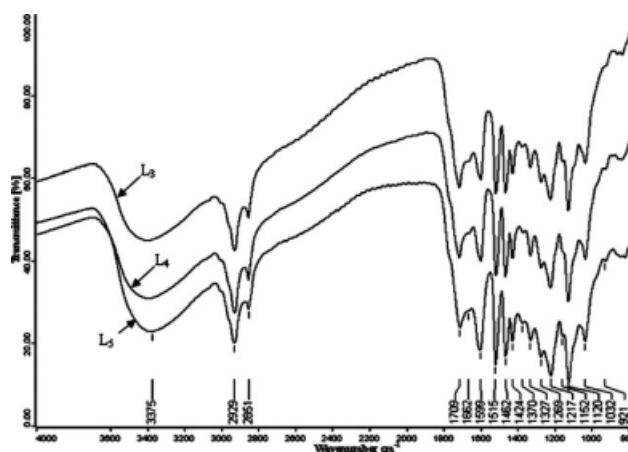

Figure 4 FT-IR spectra of the lignin fractions L_0, L_1 and L_2.

Figure 5 FT-IR spectra of the lignin fractions L_3, L_4 and L_5.

or bond-rearrangement has been reported in the steam exploded aspen[2,22] and wheat straw[14] lignins.

^{13}C- and ^{1}H-NMR analysis

To obtain further precise knowledge of the composition and structural features, two lignin fractions (L_1 and L_5) corresponding to the lowest and highest pretreatment severity were investigated by both ^{13}C- and ^{1}H-NMR spectroscopy. The signals in the spectra were assigned primarily in accordance with those of Scalbert et al.,[23] Sun et al.,[24] and Ralph et al.[25] for straw and wood lignins. In the ^{13}C spectra (Fig. 6), the intensive signals at 172–176 and 59 ppm are presumed due to C-6 in methyl urinates and 4-O-methoxyl group of glucuronic acid residue,[26] respectively. It is suggested that the uronic acids are closely associated with lignin macromolecules in the cell wall and the steam explosion pretreatment could not induce the cleavage of this linking bonds. In the region of the aromatic part of the lignin, the guaiacyl (G) units were identified by signals at 149 (C-3, G etherified), 148 (C-4, G etherified), 135 (C-1, G etherified), 119 (C-6, G), 115 (C-5, G) and 111 ppm (C-2, G). The syringyl (S) units were verified by signals at 152 (C-3/C-5, S), 148 (C-3/C-5, S nonetherified), 134 (C-1, S etherified), and 104 ppm (C-2/C-6, S). The p-hydroxyphenyl (H) units were detected at 128 ppm (C-2/C-6, H). These signals confirmed that the lignin in *Lespedeza cyrtobotrya* stalks could be justified as S-G-H lignin. It is clear that the intensities of signals for the syringyl units are higher than those of the guaiacyl units, indicating that the lignin fractions contained a higher proportion of syringyl moieties. Furthermore, as the steam pressure increased from 15 kg/m^2 to 25 kg/m^2, the signals for guaiacyl units had relative weaker intensities, and conversely, the signals for syringyl units exhibited obviously stronger intensities. The S/G ratio, thereby, was

notably increased, which is accordance with the result from components and UV spectra analysis. The sharp peak at 130 ppm (C-2/C-6, PC ester) was characterized by the presence of the esterified p-coumaric acid, indicating that the lignin fractions (F_1 and F_5) contained noticeable amounts of esterified p-coumaric acid and elevating steam pressure can break the linkage between lignin and p-coumaric acid. The signals below 104 ppm are the resonance of aliphatic carbons. Of them, signals at 85–86, 72, and 61 ppm belong to the resonances of C-β, C-α and C-γ in β-O-4 structure, respectively. The slight decreased intensities of these signals implied the partial cleavage of the β-O-4 linkages under the conditions given. However, as shown from the diagrams, they were still the major linkage between the lignin structural units. Besides, the common carbon–carbon linkage, β-β (C-γ in β-β units, 71 ppm; C-β in β-β units, 54 ppm) was also present and almost kept the same intensity regardless of the severity. This result suggested that steam explosion under the conditions used here may not attack the β-aryl ether structure to significant extent. Additionally, in the spectrum of L_5, the signals at 63–66 (C-5 in β-D-Xylp units), and 76–77 (C-2 in β-D-Xylp units) ppm, which are assigned to the associated hemicelluloses, are almost absent. It is implied that the completely breaking the linkages between lignin and polysac-
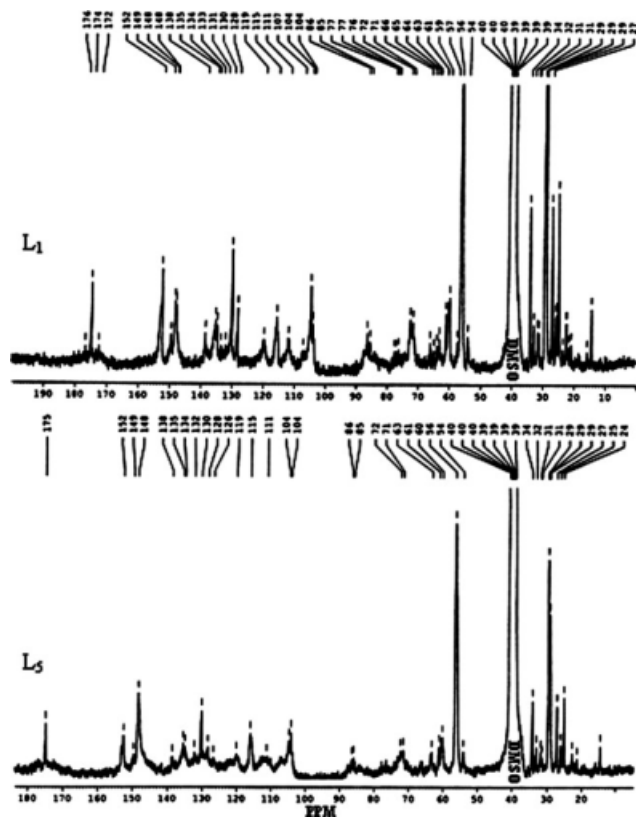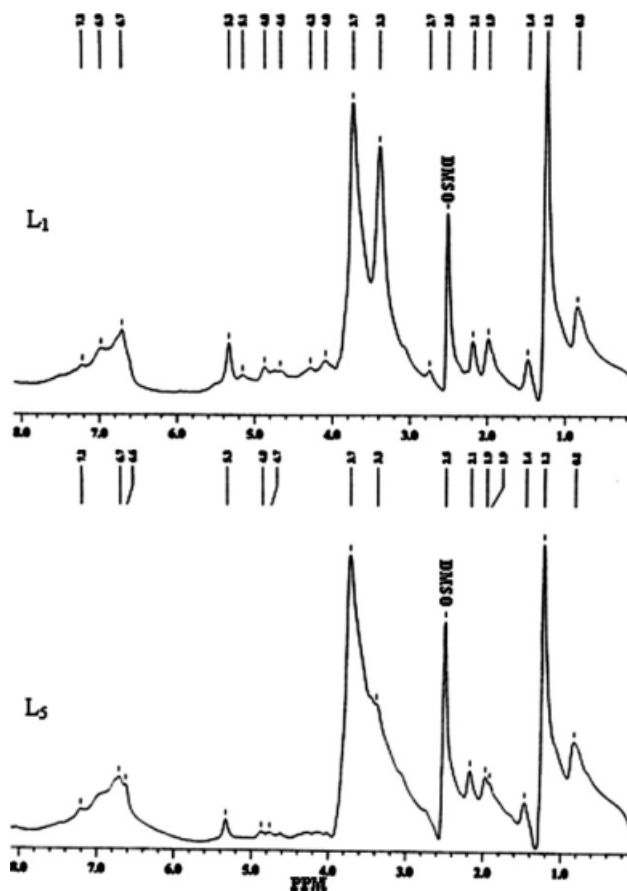

Figure 7 ^{1}H-NMR spectra of lignin fractions L_1 and L_5.

charide occurred as the steam pressure increased to 25 kg/m^2. A sharp signal at 56 ppm belongs to the —OCH$_3$ group in syringyl and guaiacyl units. The signals representing the γ-methyl and β- and α-methylene groups in n-propyl side chains gave the signals between 14 and 34 ppm.

The examination of the proton NMR spectra (Fig. 7) of these two lignin fractions further confirmed the aforementioned results in detail. The integral of all signals between 6.7 and 7.2 ppm belongs to aromatic protons in G and S units, whereas those between 0.8 and 1.4 ppm are due to the aliphatic moiety in lignin.[27] In particular, the signals at 6.9–7.2 and 6.6–6.7 ppm are attributed to the aromatic protons in G and S units,[28] respectively. It is noteworthy that a distinct peak at 5.3 ppm is observable in both spectra, supporting the ideal that phenyl coumaran structures are still present in these lignin fractions. Methoxyl protons (—OCH$_3$) give a strong signal at 3.7 ppm. A sharp signal at 2.5 ppm is due to the protons in DMSO. The peaks at 1.9 and 2.1 ppm arose from the methyl protons adjacent to double bonds or carbonyl groups. In comparison, one of the most striking characteristics of these two spectra is the decreasing intensities of the signals, which represent the associated

Figure 6 ^{13}C-NMR spectra of lignin fractions L_1 and L_5.

polysaccharides (5.1 ppm for the anomeric protons of the reducing end terminal xylose and 3.3 ppm for H-3 in the 1→4 linked β-D-Xyl residues), resulting from elevating steam pressure. The other is the rather weaker intensities in spectrum L_5 than in spectrum L_1 at the signals assigned to the β-O-4 structure (4.8 ppm for H-β in β-O-4 structure and 4.2 ppm for H-γ in β-O-4 structure). These results further confirmed the conclusion from the ^{13}C-NMR spectra analysis above.

CONCLUSIONS

Briefly, the results obtained showed that steam explosion and elevating steam pressure induced the chemical and physical modification of lignin fractions. It was found that the average molecular weight of lignin fractions firstly fell to 900 g/mol at the weakest condition (15 kg/m^2 for 4 min), and then gradually increased to 1791 g/mol at the severest condition (25 kg/m^2 for 4 min), which was probably connected with the formation of balance between depolymerization and repolymerization reactions at different stages. Because of the steric hindrance from the methoxyl group at C-5 position, less syringyl units than guaiacyl units condensed during steam explosion, resulting in the increment of S/G ratio. The result of NMR signals confirmed that the β-O-4 bond was obviously cleaved, even though it was still the main connection between lignin units.

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