ISOLATION AND PHYSICO-CHEMICAL CHARACTERIZATION OF LIGNINS FROM ULTRASOUND IRRADIATED FAST-GROWING POPLAR WOOD

Tong-Qi Yuan,[a] Shaoni Sun,[a] Feng Xu,[a,]* and Run-Cang Sun [a,b,]*

Ultrasonic irradiation with organic solvents and alkaline extractions were carried out on a fast-growing poplar wood, triploid of *Populus tomentosa* Carr., in an attempt to develop efficient lignin isolation procedures. Four organosolv and three alkaline lignin fractions were successively isolated and comparatively characterized by sugar analysis, alkaline nitrobenzene oxidation, gel permeation chromatography (GPC), Fourier transform infra-red spectroscopy (FT-IR), quantitative ^{13}C, and 2D HSQC nuclear magnetic resonance (NMR) spectroscopy, as well as thermogravimetric analysis (TGA). The results showed that the ultrasonic treatments and sequential extractions with three different concentrations of NaOH led to a release of 90.9% of the original lignin. The four organosolv lignin preparations obtained under the ultrasound-assisted extractions were degraded significantly and contained more carbohydrate and non-condensed syringyl units when compared to the three alkaline lignin preparations. Furthermore, the analyses confirmed that L_5, the lignin preparation with the highest yield (44.6% of the original lignin), was partially acylated at the γ-carbon of the side-chain preferentially over syringyl units. The percentage of lignin acylation of β-*O*-4' linkages was about 14%. The amount of β-*O*-4', β-β', and $–OCH_3$ were estimated to be about 0.31/Ar, 0.06/Ar, and 1.73/Ar, respectively. The ratio of S/G was calculated to be 2.0.

Keywords: HSQC; Lignin; Poplar wood; Quantitative ^{13}C NMR; Ultrasound

*Contact information: a: Institute of Biomass Chemistry and Technology, Beijing Forestry University, 100083,Beijing, China; b: State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, 510640, Guangzhou, China;*Corresponding author: xfx315@bjfu.edu.cn (F. Xu), rcsun3@bjfu.edu.cn (R.C. Sun)*

INTRODUCTION

In order to improve the ability to meet the increasing demand for paper and cardboard, the cultivation of fast-growing forestry is now the subject of accelerating interest and awareness. The use of poplar wood for the production of paper and paperboard is increasing at a significant rate, especially in the more temperate regions (Bose et al. 2009). For instance, the National Development and Reform Commission of China promulgated the National Forestry-Paper Integration Project Construction "15" and the 2010 Specific Plan in 2004 (http://wwwold.sdpc.gov.cn/l/l200402251.htm). In this project, fast-growing poplar wood plays a very important role because of its high pest resistance, high survival rate, high quality, and short rotation length. Besides, due to increasing environmental concerns, high oil prices, and the instability/uncertainty of petroleum reserves, there is a growing need to find alternatives to crude oil as the primary

feed stock for the chemicals and fuel industries. Ethanol has been demonstrated to be a viable alternative (Rostrup-Nielsen 2005; Ragauskas et al. 2006; Himmel et al. 2007; Kunkes et al. 2008), and meanwhile poplar has been considered to be a potential biorefinery feedstock for ethanol production (Davison et al. 2006; Balan et al. 2009).

Unfortunately, lignin, a major component of the cell wall of vascular plants, has long been recognized for its negative impact on papermaking and bioethanol production (Li et al. 2008). Generally, lignin is considered as being formed by the dehydrogenative polymerization of three *p*-hydroxycinnamyl alcohol precursors: *p*-coumaryl, coniferyl, and sinapyl alcohols (Higuchi 1997). Each of these monolignols gives rise to a different type of lignin unit called H (*p*-hydroxyphenyl), G (guaiacyl), and S (syringyl) units, respectively. However, it is now widely accepted that other monomers also participate in coupling reactions that give rise to the lignin macromolecule, such as *p*-hydroxycinnamaldehydes, ferulic acid, or 5-hydroxyconiferyl alcohol (Ralph 2007; Vanholme et al. 2008). Furthermore, lignin is a network polymer, which has many kinds of linkages between units, such as β-*O*-4', β-β', β-5', 5-5', and 4-*O*-5' linkages. The S:G ratio of hardwood lignin has long been identified as a significant parameter in delignification processes, and more recent results have shown that it is also important in determining the amount of ethanol that can be obtained from fermentation of hydrolyzed wood (Govender et al. 2009). The rate of kraft delignification is believed to be enhanced by a high S:G ratio. The lower the lignin content and the proportion of condensed structures in lignin, the less energy and reagents consumption are needed for the chemical pulping and bleaching processes. Moreover, the presence of lignin in plant cell walls impedes the breakdown of cell wall polysaccharides to simple sugars and the subsequent conversion of these sugars to usable fuel (Weng et al. 2008). Currently, the major by-product of the bioconversion of lignocellulosic resources to ethanol and in the industrial production of paper is lignin, which at present is considered predominantly for use as a process fuel. However, because of its polyphenolic chemical structure, lignin can be used in a variety of nonspecific and novel applications (Yuan et al. 2009). For instance, lignin can be depolymerized into liquid bio-oil, which is suitable as a blending component to be combined with conventional fossil fuels for motor fuel applications (Kleinert and Barth 2008). It is obvious that conversion of this low-value by-product into high-value co-products will help to offset the costs of both papermaking and bioethanol production.

The mechanochemical effect of ultrasound is believed to accelerate the extraction of organic compounds from plant materials due to disruption of cell walls and enhanced mass transfer of the cell contents (EI'Piner 1964). Recently, sonication has been reported to extract phenolic compounds from coconut (*Cocos nucifera*) shell (Rodrigues et al. 2008) and wheat bran (Wang et al. 2008), and xyloglucan from apple pomace (Fu et al. 2006). Different polysaccharides were successively extracted from ultrasonically irradiated *Salvia officinalis* L., corn bran, buckwheat hulls, and the roots of valerian (*Valeriana officinalis L.*) (Hromádková et al. 1999; Ebringerová and Hromádková 2002; Hromádková et al. 2002; Hromádková and Ebringerová 2003). In the present study, to explore an efficient method to isolate lignin, ultrasound-irradiated organic solvents and alkali extractions were carried out on a fast-growing poplar wood, one of the primary lignocellulosic feedstock being investigated for the production of ethanol and paper (Ballesteros et al. 2004; Yang et al. 2006). The structural features and physico-chemical

properties of the fractionated lignins were thoroughly investigated with a view to obtain valuable information for the industries of papermaking, bioethanol production, and value-added utilization of lignin.

EXPERIMENTAL

Materials

Triploid *Populus tomentosa* Carr., a fast-growing poplar tree, 7 years old, was harvested from the experimental farm of Beijing Forestry University. Poplar is known to consist mainly of three groups of organic compounds – cellulose (41.8%), hemicelluloses (32.3%), and lignin (21.5%) – on a dry weight basis (Yuan et al. 2010). The outer and inner layers of bark were peeled off, and the wood was cut into small pieces and then dried at 60 ^{o}C in an oven for 16 h. The wood sample was then ground to pass through a 0.8 mm size screen. The material, which passed through a 60-mesh screen and was retained on a 100-mesh screen, was collected for subsequent experimentation.

Isolation of Lignin

A scheme for ultrasound-assisted organic solvents and alkaline solutions extraction of lignin is shown in Fig. 1. The dried sample was first extracted with toluene-ethanol (2:1, v/v) in a Soxhlet apparatus for 6 h. The dewaxed material (14 g) was then soaked in 95% ethanol with a 1:15 material to liquor ratio (g/ml). The mixture was treated with ultrasound at 25 ^{o}C for 30 min in a 500 mL beaker using the Sonic system Scientz-IID (NingBo, 20–24 kHz) provided with a horn at a sonic power of 570 W. To avoid any chemical modification introduced by the increasing temperature during the ultrasonic treatment, the beaker was placed in a water bath, which could control the temperature at around 25 ^{o}C. After filtration, the residue was washed thoroughly with 95% ethanol and then dried in an oven at 60 ^{o}C for 16 h. The filtrate was collected and concentrated at reduced pressure for isolation of solubilized lignin. This lignin fraction was named L_1. The residue was successively treated with methanol, dioxane, and dimethyl sulfoxide under the same conditions. It should be noted that during ultra-sound-assisted extraction with dimethylsulfoxide, the filtrate was first concentrated at reduced pressure and then precipitated in 3 volumes of 95% ethanol. A pellet rich in hemicelluloses was recovered by filtering, washing with 70% ethanol, and freeze-drying. After evaporation of ethanol, the dimethyl sulfoxide-soluble lignin was obtained by precipitation at pH 1.5 to 2, which was adjusted by 6 M HCl. These three organosolv lignin fractions were named L_2, L_3, and L_4, respectively. After these steps, the ultra-sound-pretreated material was successively extracted with 70% ethanol containing 1% NaOH, 3% NaOH, and 6% NaOH at 75 ^{o}C for 3h. In each step, the residue was filtered off in a Büchner funnel and washed with distilled water until the filtrate was neutral and then dried in an oven at 60 ^{o}C for 16 h. Each of the supernatant fluids was neutralized to pH 5.5 with 6 M HCl, and the solubilized hemicelluloses were isolated by precipitation of the concentrated filtrates with 3 volumes of 95% ethanol. The acid-insoluble lignin prep-arations were obtained by the same method as precipitation of dimethyl sulfoxide-soluble lignin except for washing with acidified water (pH 2.0) before freeze-drying. Accordingly these three alkaline liginin fractions were titled L_5, L_6, and L_7, respectively.

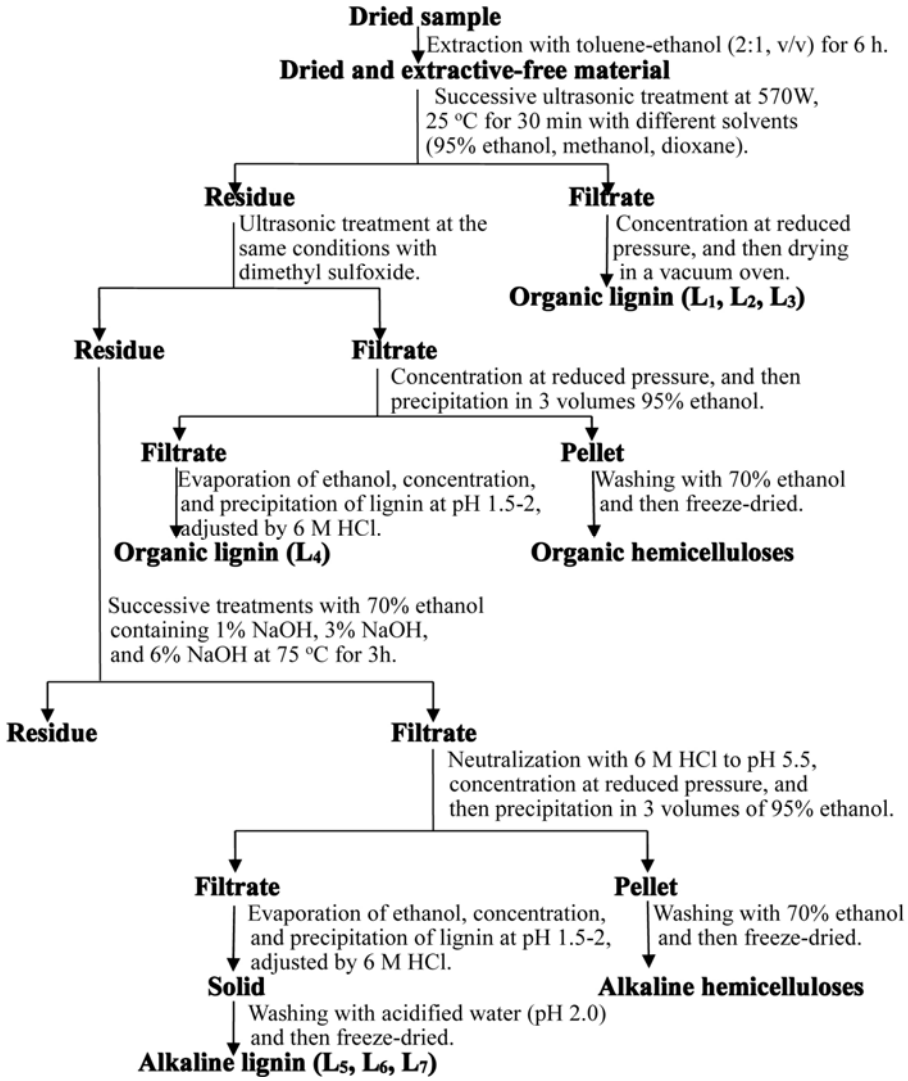

Fig. 1. Scheme for extraction of ultrasound-assisted organosolv and alkaline lignins

Analytical Methods

The carbohydrate moieties associated with the seven lignin preparations were determined by hydrolysis with dilute sulfuric acid according to the procedure described in a previous report (Yuan et al. 2010). After hydrolysis, the mixture was filtered and diluted 50-fold, and analyzed by a high-performance anion-exchange chromatography (HPAEC) system (Dionex ICS3000, U.S.) with pulsed amperometric detector, AS50 autosampler, the CarbopacTM PA-20 column (4 × 250 mm, Dionex), and the guard PA-20 column (3 × 30 mm, Dionex). The analysis of sugar composition in the present study was run in duplicate, and average values were calculated for all seven lignin preparations.

The monomeric composition of the non-condensed monomeric units of the lignin preparations was characterized by alkaline nitrobenzene oxidation and analyzed for the resulting aromatic aldehydes and acids by high-performance liquid chromatography (HPLC), as previously reported (Yuan et al. 2009). One change was that in the present

study alkaline nitrobenzene oxidation of the seven lignin preparations was carried out at 170 °C for 3h in a sealed Teflon-lined autoclave. The analysis of the resulting aromatic aldehydes and acids was run in duplicate, and average values were calculated for all seven lignin preparations.

The weight-average (M_w) and number-average (M_n) molecular weights of the lignin preparations were determined by gel permeation chromatography (GPC) on a PL-gel 10 mm Mixed-B 7.5 mm ID column. A 4 mg sample was dissolved in 2 mL of tetrahydrofuran, and a 10 μL sample in solution was injected. The column was operated at ambient temperature and eluted with tetrahydrofuran at a flow rate of 1 mL/min. Monodisperse polystyrene was used as the standard for the molecular weight of lignin.

The FTIR spectra of the lignin preparations were recorded from a KBr disc containing 1% finely ground samples on a Tensor 27 FTIR spectrophotometer in the range of 4000 to 400 cm^{-1}. Thirty-two scans were taken for each sample with a resolution of 2 cm^{-1} in the transmission mode.

The NMR spectra were recorded at 25 °C on a Bruker AVIII 400 MHz spectrometer. The lignin sample (180 mg for ^{13}C) was dissolved in 1 mL DMSO-d_6 (99.8% D). The quantitative ^{13}C NMR spectrum was recorded in the FT mode at 100.6 MHz. The inverse gated decoupling sequence, which allows quantitative analysis and comparison of signal intensities, was used with the following parameters: 30° pulse angle; 2 s relaxation delay; 64K data points, and 30000 scans. The central solvent (DMSO) peak was used as an internal chemical shift reference point (δ_C 39.5; δ_H 2.49 ppm).

Two-dimensional NMR spectra were recorded at 25 °C on a Bruker AVIII 400 MHz spectrometer. About 90 mg of lignin was dissolved in 0.5 mL of DMSO-d_6 (99.8% D). 2D-NMR spectra were recorded in HSQC experiments. The spectral widths were 5000 Hz and 20000 Hz for the ^1H- and ^{13}C-dimensions, respectively. The number of collected complex points was 1024 for ^1H-dimension with a recycle delay of 1.5 s. The number of transients was 64, and 256 time increments were always recorded in the ^{13}C-dimension. The $^1J_{CH}$ used was 145 Hz. Prior to Fourier transformation, the data matrixes were zero filled up to 1024 points in the ^{13}C-dimension. Data processing was performed using standard Bruker Topspin-NMR software. HSQC cross-signals were assigned by combining the results of the different experiments and comparison with the literature (del Río et al. 2008; Lu and Ralph 2003; Lu et al. 2004; Martínez et al. 2008; del Río et al. 2009; Rencoret et al. 2009; Villaverde et al. 2009). A semiquantitative analysis of the intensities of the HSQC cross-signal was performed according to the method given in previous reports (del Río et al. 2008, 2009; Villaverde et al. 2009). In the aliphatic oxygenated region, the relative abundance of the different inter-unit linkages were estimated from C_α-H_α correlations to avoid possible interference from homonuclear ^1H-^1H couplings, and the relative abundance of side-chains involved in the different interunit linkages were calculated. In the aromatic region, $C_{2,6}$-$H_{2,6}$ correlations from S units and C_2-H_2 correlation from G units were used to estimate the S/G ratio of lignin.

Thermal stability determinations of the lignin preparations were performed using thermogravimetric analysis (TGA) (DTG-60, Shimadzu, Japan). Samples of approximately 10 mg weight were heated in an aluminum crucible to 600 °C at a heating rate of 10 °C/min while the apparatus was continually flushed with a nitrogen flow of 20 ml/min. Prior to evaluation, all the samples were oven-dried at 105 °C for 2 h.

RESULTS AND DISCUSSION

Yield and Purity

In the present experiments, the dewaxed material was first partially delignified under successive ultrasonic treatments at 570 W, 25 °C for 30 min with 95% ethanol, methanol, dioxane, and dimethyl sulfoxide. The yield of lignin (% original lignin) extracted by sonication with organic solvents and alkaline solutions is given in Table 1. It must be mentioned that calibration of the yield of lignin has been carried out based on the results of sugar analysis. During these processes a total of about 34.1% of the original lignin was extracted with the four organic solvents used in succession. This data suggest that, under the ultrasonic treatments given, some linkages between lignin and polysaccharides were broken. It is known that during the alkaline treatment of the dewaxed and partially delignified fast-growing poplar wood, some alkali-labile linkages between lignin molecules, or between lignin and polysaccharides, might be broken by alkali. Acidic moieties such as carboxylic or phenolic groups, ionized in the alkaline solution, might also promote the solubilization of the hemicelluloses and residual lignin, either by increasing the solubility of individual fragments or by inducing the swelling of the cell wall (Scalbert et al. 1986). Therefore, after these steps, the ultrasonically pretreated material was successively extracted with 70% ethanol containing 1% NaOH, 3% NaOH, and 6% NaOH at 75 °C for 3h, and 44.6%, 9.8%, and 2.4% of the original lignin were obtained, respectively. In summary, in the present experiments, 90.9% of the original lignin could be extracted from the fast-growing poplar wood.

Table 1. Yield of Lignin (% Original Lignin) Released in the Treatment of Ultrasonic Irradiation with Organic Solvents and Alkaline Solutions

Lignin Preparation[a]	Organosolv Lignin				Alkaline Lignin		
	L_1	L_2	L_3	L_4	L_5	L_6	L_7
Yield	14.4	5.8	2.3	11.6	44.6	9.8	2.4
Total	34.1				56.8		

[a] Corresponding to the lignin preparations in Fig. 1.

To characterize composition of the ultrasound-assisted organic solvents and alkaline solutions extracted lignin, the seven lignin preparations were prepared for determination of their carbohydrate content, and the results are given in Table 2. As can be seen, all seven lignin preparations contained associated carbohydrate. In the four organosolv lignin preparations, the carbohydrate associated with the lignin preparations amounted to 13.11%, 13.15%, 4.62%, and 12.11%, respectively. The lignin preparation L_3, which was extracted with dioxane under the given experimental conditions, contained the lowest carbohydrate among the four organosolv lignins. However, it should be noted that glucose amounted to a great proportion of the sugar and uronic acids, from 48.4% to 89.8%. These data indicated that cellulose was the main component of the carbohydrate associated with the lignin preparations. Therefore, the results suggest that the macromolecules of cellulose in the cell walls might be degraded during the treatments by ultrasonic irradiation with organic solvents under the conditions given. Besides, the high proportion of glucose in the sugar analysis of the four organosolv lignin preparations may also be attributed to the incorporation of xyloglucan. On the contrary, the data showed

that the three alkaline lignin preparations contained relatively low amounts of associated carbohydrate, in which hemicelluloses were the main component. In comparison of the associated hemicelluloses in the three alkaline lignin preparations L_5-L_7, it was clear to note that an increase in the concentration of NaOH from 1% to 3%, and to 6% resulted in a decrease in the hemicelluloses content from 1.21% to 0.50%, and to 0.19%, respectively. This phenomenon revealed that more α-ether linkages between lignin and hemicelluloses would be cleaved during the successive extractions with an increasing of the concentration of NaOH. This was in line with the results of Sun et al. (2000) when studying the purity of lignin extracted from fast-growing poplar wood by an increase in the concentration of NaOH.

Table 2. The Content of Neutral Sugars and Uronic Acids (% Dry Sample, W/W) in the Isolated Lignin Preparations

Sugar/Uronic acids	Lignin Preparation[a]						
	L_1	L_2	L_3	L_4	L_5	L_6	L_7
Rhamnose	0.10	0.19	0.05	0.39	0.08	0.02	ND[b]
Arabinose	0.10	0.35	0.10	2.12	0.46	0.13	0.06
Galactose	0.64	1.47	0.18	1.51	0.16	0.05	0.03
Glucose	11.78	10.38	4.08	5.86	0.17	0.06	0.04
Mannose	0.03	0.35	0.01	1.29	ND	ND	ND
Xylose	0.06	0.14	0.07	0.58	0.10	0.24	0.06
Uronic acids	0.40	0.27	0.13	0.36	0.24	ND	ND
Total	13.11	13.15	4.62	12.11	1.21	0.50	0.19

[a] Corresponding to the lignin preparations in Fig. 1.
[b] ND = not detectable.

Content of Phenolic Acid and Aldehydes

Among the methods to deduce their structural features and degree of condensation of protolignin as well as isolated lignins, alkaline nitrobenzene oxidation of lignins is a simple but by no means perfect method for the characterization of lignins. However, this method is a useful approach in combination with other characterization methods, such as FTIR and NMR spectroscopy. Therefore, in the present study alkaline nitrobenzene oxidation was applied to determine whether a significant structural difference exists between these lignin fractions. Table 3 shows the relative yields of phenolic acids and aldehydes in each of the lignin preparations. As can be seen from Table 3, the predominant oxidation products were found to be vanillin, syringic acid, and syringaldehyde, which together comprised 60.0 to 81.7% of the total nitrobenzene oxidation products and resulted from the degradation of non-condensed guaiacyl units and non-condensed syringyl units. However, it is interesting to note that the relative yields of *p*-hydroxybenzaldehyde (8.1 to 32.1%) and *p*-hydroxybenzonic acid (1.2 to 6.0%) were higher when compared to the results of alkaline nitrobenzene oxidation of alkaline lignin preparations studied by Sun et al (2000). This phenomenon might be interpreted as being due to the incorporation of non-condensed *p*-hydroxyphenyl units into the lignin or *p*-coumaric acid ester linked to it. Both of these moieties are oxidized to *p*-hydroxybenzaldehyde at high yields during the alkaline nitrobenzene oxidation process.

The incorporation of *p*-coumarate and *p*-hydroxyphenyl units was further confirmed by the NMR spectroscopy subsequently. This observation also indicated that treatments of fast-growing poplar wood with organic solvents under ultrasonic irradiation and alkaline solutions can only result in partial cleavage of these esterified linkages.

The S:G ratio of hardwood lignin is a significant parameter in both delignification and fermentation of hydrolyzed wood processes. In the present study, the relative molar ratios of S (the relatively total moles of syringaldehyde and syringic acid) to G (the relatively total moles of vanillin and vanillic acid) appeared to be 2.5:1 in L_1, 1.5:1 in L_2, 1.4:1 in L_3, 1.2:1 in L_4, 0.3:1 in L_5, 0.6:1 in L_6, and 0.4:1 in L_7. Recently, Bose et al. (2009) proposed a corrected nitrobenzene oxidation method to determine the S:G ratio of 13 poplars, and the values ranged from 1.01 to 1.68. Therefore, it could be concluded that there were considerable structural variations among the seven lignin preparations. The four organosolv lignin preparations were dominated by non-condensed syringyl units, while the three alkaline lignin preparations were comprised of a large proportion of non-condensed guaiacyl units.

Table 3. The Yield (Relative Mol %) of Phenolic Acids and Aldehydes from Alkaline Nitrobenzene Oxidation of the Lignin Preparations

Phenolic Acids and Aldehydes	Lignin Preparation[a]						
	L_1	L_2	L_3	L_4	L_5	L_6	L_7
p-Hydroxybenzonic Acid	5.3	3.3	6.0	4.3	3.7	4.7	1.2
p-Hyroxybenzaldehyde	23.2	17.4	32.1	11.3	8.1	12.1	14.9
Syringic Acid	22.8	28.4	22.4	36.0	19.7	25.7	20.8
Syring Aldehyde	28.2	19.7	13.3	9.9	2.7	4.4	2.9
Vanillic Acid	3.7	3.4	1.8	4.9	6.5	9.4	7.0
Vanillin	16.8	27.7	24.3	33.5	59.3	43.7	53.2
Mole Ratio of S/G[b]	2.5:1	1.5:1	1.4:1	1.2:1	0.3:1	0.6:1	0.4:1

[a] Corresponding to the lignin preparations in Fig. 1.
[b] S represents the relatively total moles of syringaldehyde and syringic acid, G represents the relatively total moles of vanillin and vanillic acid.

Molecular Weight Distribution

The values of the weight-average (M_w) and number-average (M_n) molecular weights, calculated from the GPC curves (relative values related to polystyrene), and the polydispersity (M_w/M_n) of the seven lignin preparations are given in Table 4. It should be noted that tetrahydrofuran was used as eluant and all the seven lignin preparations were soluble in this solvent without derivatization. As shown in Table 4, all the seven lignin preparations exhibited low molecular-average weights, ranging from 910 to 2770 g/mol. In particular, the molecular-average weights of the four organosolv lignin preparations (910 to 1390 g/mol) were much lower than the three alkaline lignin preparations (2000 to 2770 g/mol). These molecular-average weights were rather lower when compared to the results obtained by Sun et al. (2000) (4520–6900 g/mol) during the study of extraction of lignins from fast-growing poplar wood with different concentrations of NaOH. This obvious difference for the four organosolv lignin preparations was undoubtedly due to the effects of ultrasonic irradiation under the given conditions. The β-*O*-4' linkages between the lignin precursors under a relatively longer sonication period would be cleaved.

Besides, we could note that the molecular-average weights of the alkaline lignin preparations in our previous studies were much higher than the alkaline lignin preparations obtained in the present study. One explanation for this phenomenon might be that the mechanochemical effect of ultrasound disrupted the cell walls and enhanced mass transfer of the cell contents (EI'Piner 1964). Therefore, the extraction of lignin from the ultrasonically pretreated material was easier than from that without ultrasonic pretreatment. Consequently, one could significantly avoid the condensation of lignin during the treatments at 75 ^{o}C for 3h with an increase in the concentration of NaOH. In addition, all the seven lignin preparations exhibited relatively narrow molecular weight distributions, as shown by $M_w/M_n < 3$. The polydispersities of the four organosolv lignin preparations (1.63 to 1.90) were lower than the three alkaline lignin preparations (2.18 to 2.92). These data indicate that extraction of lignin under alkaline conditions led to the dissolution of lignin fractions with a broader molecular weight distribution when compared to the ultrasound-assisted organic solvents extraction in the present study.

Table 4. Weight-Average (M_w) and Number-Average (M_n) Molecular Weights and Polydispersity (M_w/M_n) of the Isolated Lignin Preparations

	Lignin Preparation[a]						
	L_1	L_2	L_3	L_4	L_5	L_6	L_7
M_w	910	960	1390	1030	2000	2770	2310
M_n	560	570	730	620	690	950	1060
M_w/M_n	1.63	1.68	1.90	1.66	2.90	2.92	2.18

[a] Corresponding to the lignin preparations in Fig. 1

FTIR Spectra

To trace the structural changes of lignin during the ultrasound-assisted organic solvents and alkaline solutions extractions, FTIR spectra of three organosolv lignin preparations (L_1, L_2, and L_4) and three alkaline lignin preparations (L_5, L_6, and L_7) are shown (Fig. 2). It was clear to note that the spectra in Fig. 2 (a) and (b) were distinct in terms of the locations and intensities of the absorption bands. The bands at 1597, 1509, and 1420 cm^{-1}, corresponding to aromatic skeletal vibrations and the C-H deformation combined with aromatic ring vibration at 1462 cm^{-1}, are present in Fig. 2 (b). In Fig. 2 (a), the three organosolv lignin preparations have rather similar FT-IR spectra, however, not typical of hardwood lignin. These obvious differences proved that the aromatic structure of the three organosolv lignin preparations had been degraded significantly when compared to the three alkaline lignin preparations. This was in accordance with the results obtained from GPC, which indicated molecular weights of 910 to 1030 g/mol for the three organosolv lignin preparations in Fig. 2 (a), while 2000 to 2770 g/mol for the three alkaline lignin preparations in Fig. 2 (b). Another reason might be the high content of carbohydrate in the three organosolv lignin preparations, ranging from 12.11% to 13.15%, which could affect the absorption bands of lignin. In Fig. 2 (a), the absorptions at around 1726 cm^{-1} in all the three spectra are assigned to C=O stretching of unconjugated ketone, carbonyl, and ester groups. The small band at 950 cm^{-1} in the spectra of L_2 and L_4 might arise from the absorption of xyloglucan (Kačuráková et al. 2000). This was in line with the results obtained by sugar analysis. A weak band at 1637 cm^{-1} in the spectrum of L_1 is most likely derived from the ring-conjugated α,β unsaturated bond (Kihara et al. 2002). Other characteristic absorption peaks of lignin would be discussed in

detail in Fig. 2 (b).

As shown in Fig. 2 (b), a wide absorption band at 3353 cm^{-1} originated from the O-H stretching vibration in aromatic and aliphatic OH groups, while the bands at 2936 and 2849 cm^{-1} arise from the C-H asymmetric and symmetrical vibrations in methyl and methylene groups, respectively. A significant difference between the three alkaline lignin preparations is the strong absorption at 1709 cm^{-1} in the spectrum of L_5, corresponding to non-conjugated carbonyl stretching, which disappears in L_6 and L_7. In the meantime, the intensity of the absorption at 1667 cm^{-1} increasing from L_5 to L_7, represents the carbonyl stretching in conjugated *p*-substituted aryl ketones. Syringyl and condensed guaiacyl absorptions are obviously seen at 1328 cm^{-1}, and guaiacyl ring breathing with C=O stretching appears at 1265 cm^{-1}. The strong band at 1226 cm^{-1} is due to the C–C, C–O and C=O stretching. The maximum absorption band at 1124 cm^{-1} in Fig. 2 (b) indicated that all the three alkaline lignin preparations are GS lignins (Faix 1991). Besides, the band at 1032 cm^{-1} is indicative of the aromatic C-H in-plane deformation. According to the lignin classification system of Faix (1991), the intensity ratio of 1509 cm^{-1} /1462 cm^{-1} increasing from L_5 to L_7 indicated that the content of S units in the three alkaline lignin preparations decreased from L_5 to L_7. Furthermore, the A_{1462}/A_{1597} ratio in Fig. 2 (b) directly related to the amounts of methoxyl groups (Sarkanen et al. 1967), was smaller in L_7 than in L_5 and L_6. This result also revealed that L_7 contained fewer amounts of S units than L_5 and L_6.

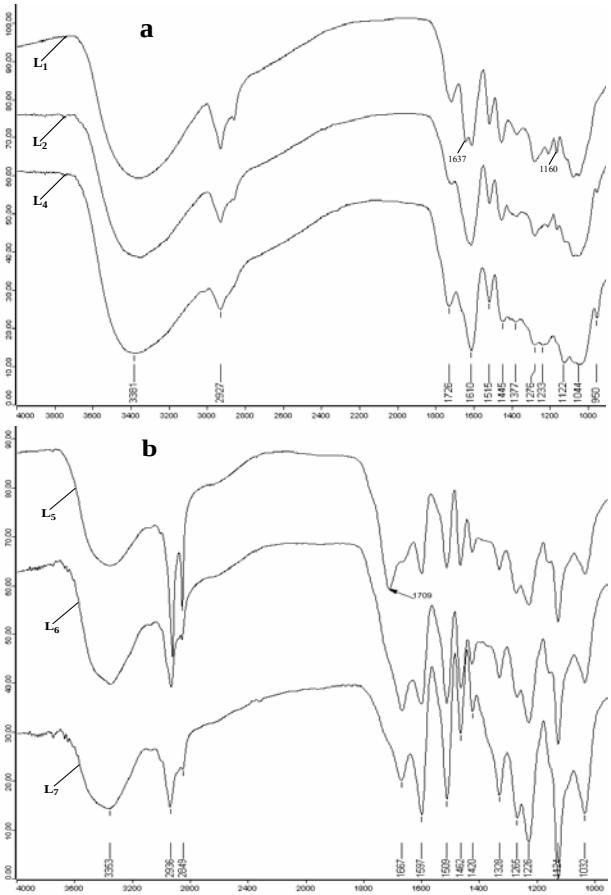

Fig. 2. FTIR spectra of three organosolv lignin preparations (a, L_1, L_2, and L_4) and three alkaline lignin preparations (b, L_5, L_6, and L_7)

Quantitative ^{13}C NMR Spectrum

To further investigate the structural features of the lignin preparations, the highest yield lignin preparation L_5, extracted from the ultrasonically pretreated material with 70% ethanol containing 1% NaOH at 75 ^{o}C for 3 h, was investigated with quantitative ^{13}C NMR spectrometry. The quantitative ^{13}C NMR spectrum of L_5 is shown in Fig. 3. Most of the observed signals have been previously assigned in wood lignin spectra (Lapierre et al. 1982; Capanema et al. 2005). The integral at 160 to 102 ppm was set as the reference, assuming that it includes six aromatic carbons. All structural moieties were expressed per one aromatic ring (Ar). The absence of signals between 90 and 102 ppm indicates a low concentration of residual sugars in this lignin preparation. This was in line with the result obtained by sugar analysis, just 1.20% of associated hemicelluloses in L_5. The strong peak at 174.4 ppm and the weak peaks between 171 and 173 ppm are attributed to carbon in carbonyl and carboxyl groups, which may originate from aliphatic carboxyl and aliphatic esters. In the aromatic region (153 to 103 ppm), the syringyl (S) units are detected by signals at 152.7 and 152.1 ppm (C-3/C-5, etherified) and 147.5 ppm (C-3/C-5, non-etherified), 138.1 ppm (C-4, etherified), 134.8 and 134.3 ppm (C-1, etherified), and 104.3 ppm (C-2/C-6). The guaiacyl (G) units produce signals at 149.2 ppm (C-3, etherified), 147.5 ppm (C-4, etherified), 145.4 ppm (C-4, non-etherified), 134.8 and 134.3 ppm (C-1, etherified), 115.2 ppm (C-5), and 111.2 ppm (C-2). The *p*-hydroxyphenyl (H) units appear as two signals at 128.0 and 129.2 ppm (C-2/C-6). The syringyl to guaiacyl ratio (S/G) was about 1.5:1, which was calculated on the basis of the number of carbons per aromatic ring in C-2/C-6 of syringyl unit and C-2 of guaiacyl unit (Capanema et al. 2005). Furthermore, the signals arising from the esterified *p*-hydroxybenzonic acid should be noted. This structural moiety is made evident by signals at 167.2 ppm (C=O), 161.8 ppm (C-4), 131.4 ppm (C-2/C-6), 121.3 ppm (C-1), and 114.9 ppm (data not shown, C-3/C-5). The sharp signals of this minor component suggested that they could be assigned to *p*-hydroxybenzoic units more mobile than the syringyl and guaiacyl ones constitutive of the lignin core (Lapierre et al. 1982). All these signals showed that this lignin preparation contained a certain amount of esterified *p*-hydroxybenzonic acids.

In the oxygenated and non-oxygenated inter-unit linkages region of lignin, the β-*O*-4' linkages were detected by signals at 86.0 (C_β in S β-*O*-4' erythro), 85.1(C_β in G β-*O*-4' threo), 72.2 (C_α in β-*O*-4' G and S erythro), 71.4 (C_α in β-*O*-4' G and S threo), 60.7, and 59.6 (C_γ in β-*O*-4' G and S threo and erythro) ppm. The strong signal at 55.9 ppm is attributed to the $-OCH_3$ groups in S and G units. A small signal at 53.8 ppm belongs to the C_β in β-β' structure. According to Capanema et al. (2005) the integral at 54 to 53 ppm could be used to estimate the amount of β-β' structure as ca. 0.06/Ar. The cluster at 87.5 to 82.5 ppm embodies moieties with the total amount of β-*O*-4', β-β', and β-5 structures. In the spectrum the signals of β-5 structure were found to be trace. So subtracting the amount of β-β' structure from the integral value of this region gives the total amount of β-*O*-4' as ca. 0.31/Ar. Besides, the amount of $-OCH_3$ was estimated from the integral at 57 to 54 ppm as ca. 1.73/Ar. In addition, the signals between 13.9 and 33.7 ppm represent the γ-methyl, as well as the α- and β-methylene groups in *n*-propyl side chains of the lignin preparation.

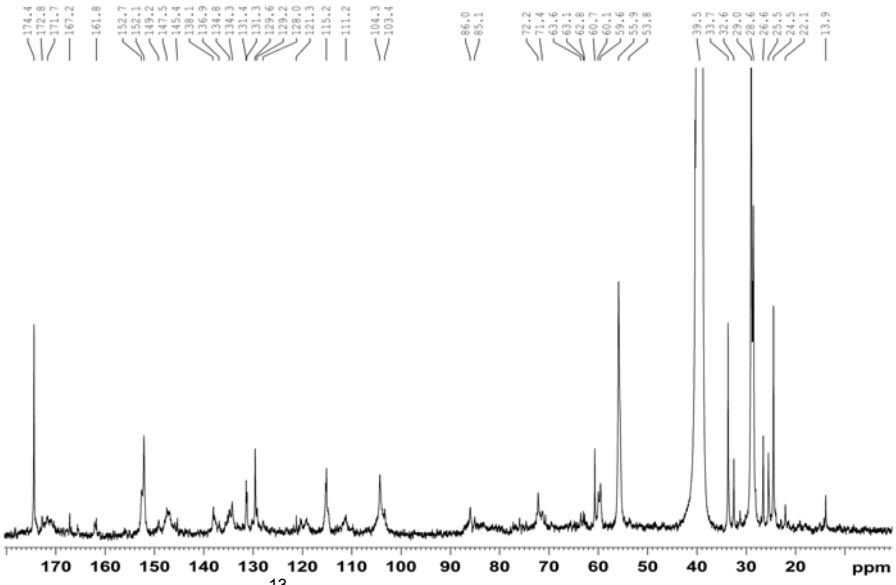

Fig. 3. Quantitative ^{13}C NMR spectrum of alkaline lignin preparation L_5

2D HSQC NMR Spectra

For a more complete structural characterization of the lignin preparation, the highest yield alkaline lignin preparation L_5 was subjected to 2D-NMR analysis. Generally, most NMR studies of lignins were carried out in acetylated samples to increase their solubility. However, this prevented the detection of natural acetylation. In the present study, the HSQC NMR spectrum of L_5 was acquired from underivatized sample. The HSQC NMR spectrum of L_5 showed three regions corresponding to aliphatic, side chain, and aromatic ^{13}C-^{1}H correlations. The aliphatic (nonoxygenated) region showed signals with no structural information and therefore is not discussed in detail. The side-chain (δ_C/δ_H 50–95/ 2.5–6.0) and the aromatic (δ_C/δ_H 95–145/5.5–8.5) regions of the HSQC spectrum of L_5 are shown in Fig. 4. The main lignin cross-signals assigned in the HSQC spectra are listed in Table 5, and the main substructures are depicted in Fig. 5.

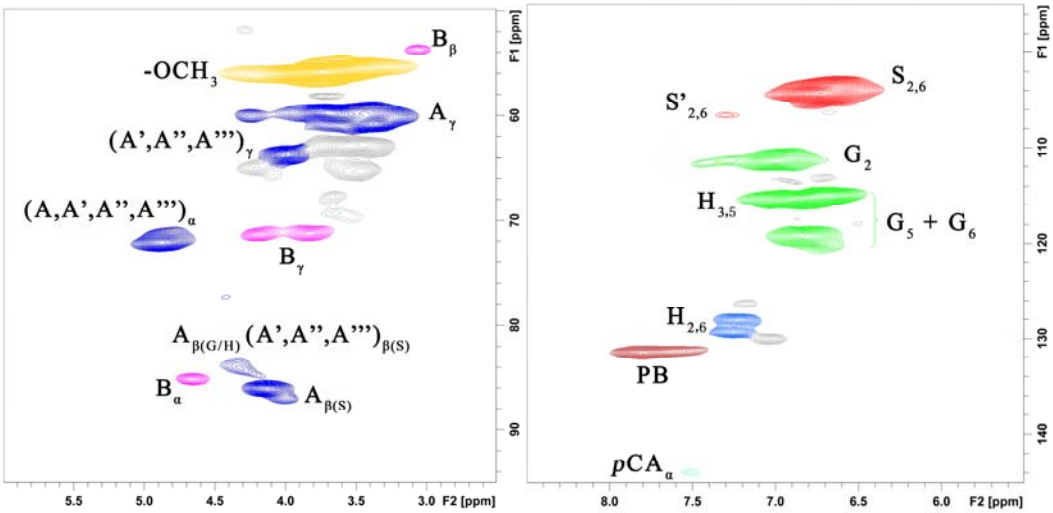

Fig. 4. Side-chain (left column) and aromatic regions (right column) in the 2D HSQC NMR spectrum: δ_C/δ_H 50–95/2.5–6.0 ppm and δ_C/δ_H 95–145/5.5–8.5 ppm, respectively. Symbols are taken from Fig. 5. See Table 5 for signal assignment.

Table 5. Assignment of Main Lignin ^{13}C-^{1}H Cross-Signals in the HSQC Spectrum of the Alkaline Lignin Preparation L_5

Labels	δ_C/δ_H (ppm)	Assignment
B_β	53.7/3.05	C_β-H_β in resinol substructures (**B**)
-OCH_3	55.9/3.73	C-H in methoxyls
A_γ	59.6-60.8/ 3.37-3.71	C_γ-H_γ in β-*O*-4' substructures (**A**)
$(A',A'',A''')_\gamma$	63.6/3.98	C_γ-H_γ in *γ*-acylated β-*O*-4' substructures (**A',A'',A'''**)
B_γ	71.3/4.18; 71.3/3.82	C_γ-H_γ in resinol substructures (**B**)
A_α	72.2/4.86	C_α-H_α in β-*O*-4' substructures (**A**)
$(A',A'',A''')_\alpha$	72.2/4.86	C_α-H_α in γ-acetylated β-*O*-4' substructures (**A',A'',A'''**)
$A_{\beta(G/H)}$	83.9/4.30	C_β-H_β in β-*O*-4' substructures linked to a G unit (**A**)
$(A',A'',A''')_{\beta(S)}$	83.9/4.30	C_β-H_β in γ-acylated β-*O*-4' substructures linked to a S unit (**A',A'',A'''**)
$A_{\beta(S)}$	86.0/4.11	C_β-H_β in β-*O*-4' substructures linked to a S unit (**A**)
B_α	85.1/4.66	C_α-H_α in resinol substructures (**B**)
C_α	87.1/5.51	C_α-H_α in phenylcoumaran substructures (**C**)
$S_{2,6}$	104.3/6.71	$C_{2,6}$-$H_{2,6}$ in etherified syringyl units (**S**)
$S'_{2,6}$	106.6/7.32	$C_{2,6}$-$H_{2,6}$ in oxidized (C_α=O) phenolic syringyl units (**S'**)
G_2	111.3/6.99	C_2-H_2 in guaiacyl units (**G**)
G_5	114.9-115.2/ 6.80-6.86	C_2-H_2 in guaiacyl units (**G**)
G_6	119.1/6.80	C_6-H_6 in guaiacyl units (**G**)
$H_{2,6}$	128.0-129.2/ 7.23-7.24	$C_{2,6}$-$H_{2,6}$ in *p*-hydroxyphenyl units (**H**)
$PB_{2,6}$	131.4/7.78	$C_{2,6}$-$H_{2,6}$ in sinapyl *p*-hydroxybenzoate substructures (**PB**)
pCA_α	143.9/7.50	C_α-H_α in *p*-coumarate substructures (***p*CA**)

In the side-chain region of the HSQC spectrum, cross-signals of methoxyls (δ_C/δ_H 55.9/3.73) and side-chains in β-*O*-4' aryl ether linkages were the most prominent in L_5. The C_α-H_α correlations in β-*O*-4' substructures were observed at δ_C/δ_H 72.2/4.86 (structures **A, A', A'',** and **A'''**). Likewise, C_β-H_β correlations were observed at δ_C/δ_H 86.0/4.11 for structure **A** linked to **S** lignin unit. These correlations shifted to δ_C/δ_H 83.9/4.30 in structure **A** linked to **G**/**H** lignin units and *γ*-acylated β-*O*-4' aryl ether substructures (**A', A'',** and **A'''**) linked to **S** lignin unit. The C_γ-H_γ correlations in β-*O*-4' substructures were observed at δ_C/δ_H 59.6–60.8/3.37–3.71 (structures **A**). Moreover, a weak signal was observed at δ_C/δ_H 63.6/3.98, which should be attributed to C_γ-H_γ correlations in *γ*-acylated lignin units (**A', A'',** and **A'''**). These signals indicate that the lignin from fast-growing poplar wood is partially acylated at the γ-carbon in β-*O*-4' aryl ether linkages of the side chains. However, some natural esterified groups present on lignin might have been hydrolyzed and removed when using the present isolation method. Therefore, further studies of milled wood lignin from the fast-growing poplar wood will be needed. It should be mentioned that γ-acylated **G** and **S** units have been detected in the lignins of many species. For instance, sinapyl acetate is implicated similarly as a monomer in lignification in kenaf bast fibers (Lu and Ralph 2002). The lignin of grasses is adorned with γ-*p*-coumarate substituents on a variety of lignin units.

In addition to β-*O*-4' substructures, other linkages were also observed. Strong signals for resinol (β-β'/α-*O*-γ'/γ-*O*-α') substructures (**B**) were observed in the spectrum, with their C_α-H_α and C_β-H_β correlations at δ_C/δ_H 85.1/4.66 and 53.7/3.05, respectively. However, the C_γ-H_γ correlations in these two substructures were disparate. The C_γ-H_γ correlations in substructures **B** were observed at δ_C/δ_H 71.3/4.18 and 71.3/3.82. Furthermore, a small signal of phenylcoumaran (β-5' linkages) may only be seen at lower contour levels (not shown). Its C_α-H_α correlation could be observed at δ_C/δ_H 87.1/5.51. It should be noted that this signal is too weak and could not be detected in the quantitative ^{13}C NMR spectrum.

In the aromatic region of the HSQC spectrum, cross-signals from syringyl (**S**) and guaiacyl (**G**) lignin units could be observed. The **S**-lignin units showed a prominent signal for the $C_{2,6}$-$H_{2,6}$ correlation at δ_C/δ_H 104.3/6.71, while the **G** units showed different correlations for C_2-H_2, C_5-H_5, and C_6-H_6 at δ_C/δ_H 111.3/6.99, 114.9/6.74-115.2/6.86, and 119.1/6.80, respectively. The double C_5-H_5 signal revealed some heterogeneity among the **G** units especially affecting the C_5-H_5 correlation, probably because it is due to different substituents at C_4 (e.g. phenolic or etherified in different substructures). Signals corresponding to $C_{2,6}$-$H_{2,6}$ correlations in C_α-oxidized S units (**S'**) (δ_C/δ_H 106.6/7.32) were present in the HSQC spectrum of L_5. These signals have also been found in the HSQC spectra of other lignin samples, such as the MWL from eucalypt wood and some nonwoody plants (del Río et al. 2008; Martínez et al. 2008; Rencoret et al. 2009).

Other significant signals in the aromatic region of the HSQC spectrum are assigned to *p*-hydroxyphenyl units (**H**) and sinapyl *p*-hydroxybenzoate substructures (**PB**). The $C_{2,6}$-$H_{2,6}$ aromatic correlations from *p*-hydroxyphenyl (**H**) units were clearly observed at 128.0-129.2/7.23-7.24 ppm, but the $C_{3,5}$-$H_{3,5}$ position correlations were overlapped with those from guaiacyl 5-positions. A strong signal for the $C_{2,6}$-$H_{2,6}$ correlations of sinapyl *p*-hydroxybenzoate substructures (**PB**) were also observed at δ_C/δ_H 131.4/7.78. This result was in line with the lignin structural analyses of poplar, aspen, willow, and palm, which have shown that γ-*p*-hydroxybenzoate esters are present (Smith 1955; Landucci et al. 1992; Sun et al. 1999; Meyermans et al. 2000; Morreel et al. 2004). Most importantly, Morreel et al. (2004) had found that only S units are γ-*p*-hydroxybenzoylated in poplar lignin and the sinapyl *p*-hydroxybenzoate is produced enzymatically and used as an authentic monomer for lignification in poplar. Furthermore, a weak signal for the C_α-H_α correlation of *p*-coumarate substructures (***p*CA**) was found at δ_C/δ_H 143.9/7.50.

The major lignin structural features were investigated for L_5, such as the percentage of the main inter-unit linkages (referred to as the total side-chains) and S/G ratio. As expected, the main substructures present in L_5 were the β-*O*-4' linked ones (**A, A', A'',** and **A'''**), which accounted for 83% of all side-chains, followed by the β-β' resinol substructures (**B**) that involved 16% of all side-chains. Besides, a trace amount of β-5' phenylcoumaran-type (1%) linkages was also detected in L_5. Moreover, the ratio of S/G was calculated to be 2.0.

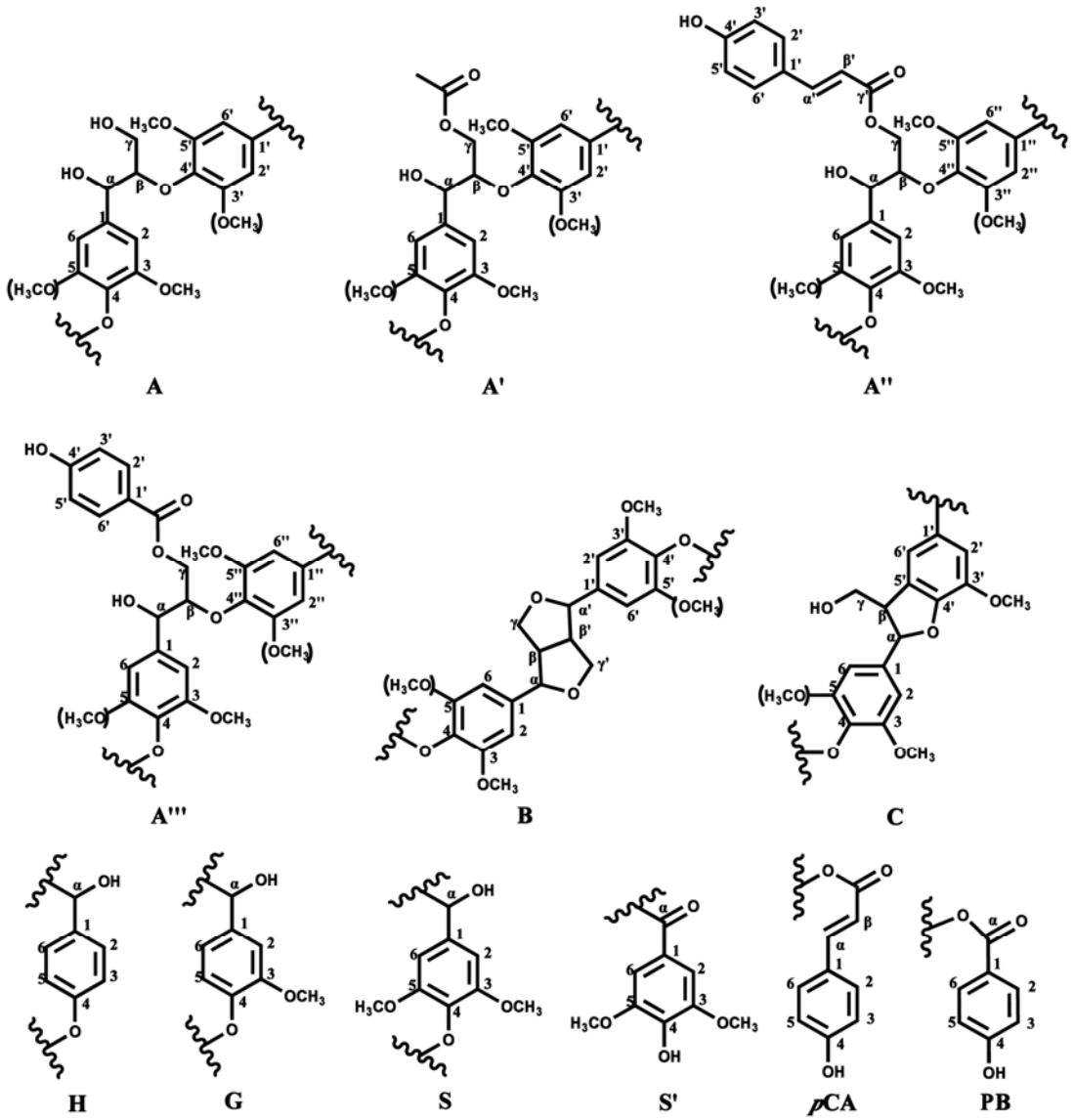

Fig. 5. Main classical and acetylated substructures, involving different side-chain linkages, and aromatic units identified by 2D NMR of alkaline lignin preparation L5: (**A**) *β-O-*4' aryl ether linkages with a free –OH at the *γ*-carbon; (**A'**) *β-O-*4' aryl ether linkages with acetylated -OH at *γ*-carbon; (**A"**) *β-O-*4' aryl ether linkages with *p*-coumaroylated -OH at *γ*-carbon; (**A'"**) *β-O-*4' aryl ether linkages with *p*-hydroxybenzoated -OH at *γ*-carbon; (**B**) resinol substructures formed by *β-β'*, *α-O-γ'*, and *γ-O-α'* linkages; (**C**) phenylcoumarane substructures formed by *β-5'* and *α-O-*4' linkages; (**H**) *p*-hydroxyphenyl unit; (**G**) guaiacyl unit; (**S**) syringyl unit; (**S'**) oxidized syringyl units with a C_α ketone; (***p*CA**) *p*-coumarate; (**PB**) *p*-hydroxybenzoate substructures.

Thermal stability

Lignins are utilized in a wide variety of applications, some of which involve brief exposure to moderately high temperatures, and in such applications the thermal stability is an issue (Domínguez et al. 2008). In the present study the thermal degradation of organosolv lignin preparation L_4 and alkaline lignin preparation L_5 have been

comparatively investigated using the techniques of thermogravimetric analysis (TGA) between room temperature and 600 ^{o}C. The thermogravimetric analysis (TGA) and 1st derivative thermogravimetric (DTG) curves are illustrated in Fig. 6. As shown in Fig. 6, the thermal degradation of both lignin preparations proceeded over a wide temperature range (100 to 600 ^{o}C). At 10% and 50% weight loss, the decomposition temperatures were determined to be 191 ^{o}C and 440 ^{o}C for L_4 and 265 ^{o}C and 520 ^{o}C for L_5, respectively. Furthermore, the DTG curves present the weight loss rates, while the DTG_{max} represents the maximum degradation rate, which can be used for the comparison of the thermal stability between the samples (Nadji et al. 2009). The DTG_{max} values were found to be 213 ^{o}C for L_4 and 281 ^{o}C and 516 ^{o}C for L_5. The differences in the inherent structures and chemical nature of the two lignin preparations possibly account for the different behaviors observed. The results from GPC indicated that the molecular-average weight of L_5 (2000 g/mol) was higher than that of L_4 (1030 g/mol). The thermal stability of the lignin preparations were found to increase with increasing molecular weight.

Recently, Yang et al. (2006) studied the pyrolysis behaviors of mixtures of hemicelluloses and lignin. They found that the temperature of DTG_{max} decreased with the increase in hemicelluloses content. In the present study, sugar analysis revealed that L_4 was much higher in hemicelluloses content than L_5, 12.1% for L_4 and just 1.2% for L_5. Furthermore, a previous study (Jakab et al. 1997) has shown that the ether bonds between syringyl units are easier to split than those between guaiacyl units. The results from alkaline nitrobenzene oxidation revealed that the mole ratios of S/G for L_4 and L_5 were 1.2:1 and 0.3:1, respectively. In other words, L_4 contained more non-condensed syringyl units than L_5. All these comparative analysis indicated that the alkaline lignin preparation L_5 had a higher thermal stability than the organosolv lignin preparation L_4 in the present experiment.

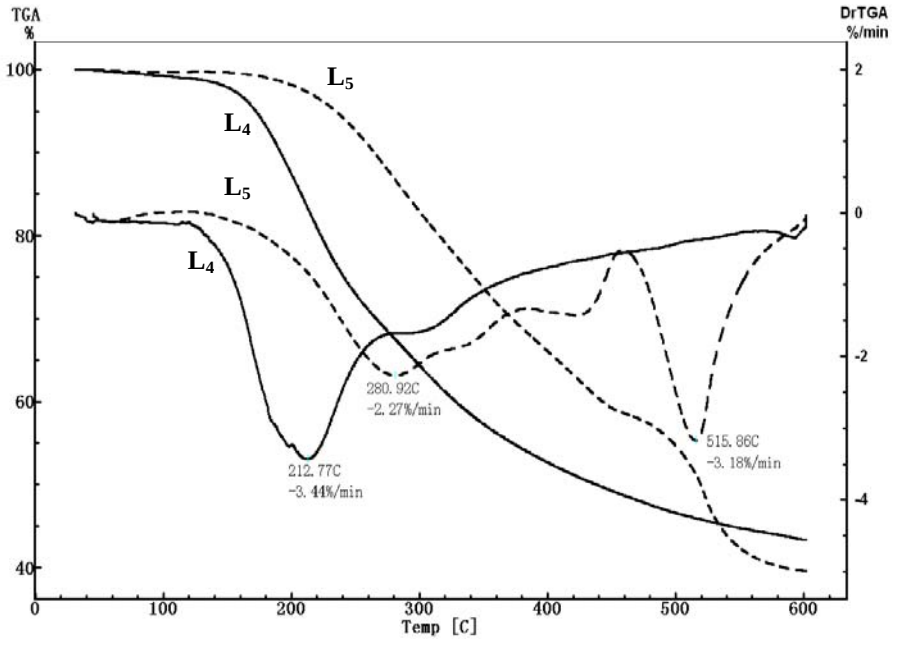

Fig. 6. TGA/DTG curves of organosolv lignin preparation L_4 and alkaline lignin preparation L_5

CONCLUSIONS

In the present study, it could be concluded that treatments with the combination of ultrasonically assisted organic solvents and alkaline solutions were an effective approach to remove lignin from the fast-growing poplar wood. Over 90% of the original lignin could be successively extracted from the ultrasound-pretreated material with a relatively low NaOH concentration. The yield, composition, physicochemical and thermal properties, and structural features of the obtained organosolv and alkaline lignin preparations were comparatively studied. Much significant structural information of the highest lignin-content preparation was obtained from quantitative ^{13}C and 2D HSQC NMR spectra. It was also found that the differences in the inherent structures and chemical nature of the lignin preparations play an important role in their thermal stability. In summary, we believe that the knowledge of the method to remove lignin from the cell wall and structural features of the lignin polymer of the fast-growing poplar wood will help to maximize the exploitation of this interesting wood for ethanol and paper production as well as the utilization of lignin for novel materials and chemicals.

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