

Effect of Lignin on Bamboo Biomass Self-Bonding During Hot-Pressing: Lignin Structure and Characterization

Dong-Li Li,^{a,#} Jian-Quan Wu,^{b,#} Wan-Xi Peng,^{a,c,*} Wen-Fei Xiao,^d Jian-Guo Wu,^e Jian-Yin Zhuo,^f Tong-Qi Yuan,^b and Run-Cang Sun^{b,*}

To achieve high value-added utilization of lignin extracted from the biorefinery process in the wood composite industry, binderless particle boards were manufactured by bamboo materials combined with alkaline lignin (AL) in various proportions under various hot-pressing conditions. To elucidate the reactivity and chemical transformations of lignin macromolecules during the hot-pressing process, lignin samples were isolated from the corresponding boards and characterized by high-performance anion-exchange chromatography (HPAEC), gel permeation chromatography (GPC), quantitative ³¹P-NMR, and 2D-HSQC NMR. The best bonding strength (1.36 MPa) of the binderless particle boards was obtained under the conditions of 180 °C, pressure 5 MPa, and lignin/bamboo mass ratio 0.4. The molecular weight of the lignin samples decreased from 3260 to 1420 g/mol during hot-pressing. The NMR results showed that the contents of β-O-4' and β-β' linkages were reduced and β-5' linkages were increased as the hot-pressing temperature rose. Simultaneously, the percentage of G-type and H-type lignins as well as the content of phenolic OH increased.

Keywords: Alkaline lignin; Bamboo biomass; Particle boards; Quantitative ³¹P-NMR; 2D-HSQC NMR

Contact information: a: School of Materials Science and Engineering, Central South University of Forestry and Technology, Changsha, 410004, China; b: Beijing Key Laboratory of Lignocellulosic Chemistry, Beijing Forestry University, Beijing, 100083, China; c: Laboratory of Biomaterials Science, Kyoto Prefectural University, Kyoto, Japan; d: Guangxi Nanning Luyuan Beilin Wood Industry Co. Ltd., Nanning, 530104, China; e: Xuzhou Feiya Wood Industry Co. Ltd., Xuzhou, 221003, China; f: Guzhang Zhuoliang Wood Industry Co. Ltd., Guzhang, 416300, China; #: Dong-Li Li and Jian-Quan Wu are co-first authors; *Corresponding authors: pengwanxi@163.com; rcsun3@bjfu.edu.cn

INTRODUCTION

In conventional processes, wood-based composites are made from lignocellulosic fibers combined with some synthetic adhesives, such as urea formaldehyde (UF) or phenol formaldehyde (PF), and then bonded together under heat and pressure (Hüttermann *et al.* 2001). However, the emission of formaldehyde vapors from the synthetic adhesive may cause environmental or health hazards (Liu *et al.* 2014b). On the other hand, because of rapidly rising oil prices, the higher price of non-renewable raw materials for UF and PF have become the main reason to restrict their broad application (Zhang *et al.* 2013; Liu *et al.* 2014a). Therefore, reduction of cost and substitution of petroleum-based materials by environmentally friendly raw materials are the most important motivations in the development of wood adhesives.

In recent decades, various techniques for using different kinds of lignocellulosic waste materials for wood composites production, especially lignin as a natural adhesive,

have been extensively investigated (Stewart 2008; Widsten and Kandelbauer 2008). Previous studies have demonstrated that lignin as a natural adhesive for boards can replace the conventional synthetic adhesives (Grávitis *et al.* 2010; Mancera *et al.* 2011). Anglès *et al.* (2001) attempted to use lignin as a natural adhesive in fiberboard manufacture by adding various types of technical lignins. Replacing up to 20% of the fibers with kraft lignin improved the water resistance and mechanical properties of the boards without significantly affecting their density. Rubberwood fibers mixed with organosolv lignins have also been pressed into medium-density fiberboard, which exhibited high mechanical strength and met the ASTM D1037-12 Standard (2012) requirements for internal bonding and modulus of rupture (Nasir *et al.* 2013).

However, commonly used technical lignins, such as kraft lignin and lignosulfonate, mostly come from the pulping and papermaking industry (Schorr *et al.* 2014). These lignin materials have undergone severe chemical reactions and possess low activity (Collinson and Thielemans 2010; Barakate *et al.* 2011). This presents a difficulty in industrial application. However, in the biochemical reaction, moderate reaction conditions cause the lignin to retain more hydroxyl and other active groups. In most biorefinery processes to produce ethanol, butanol, xylitol, and lactic acid, the cellulose and hemicelluloses are enzymatically hydrolyzed to monosaccharides and then subjected to fermentation with various bacteria (Amidon *et al.* 2008; Balat 2011; Zhang *et al.* 2013). Currently, large quantities of residues, which are rich in lignin, retaining high activity and purity, have been reserved, causing a waste of high-value bio-resources. For the United States alone, 223 million tons of biomass will be used annually, producing about 62 million tons of lignin by 2022 (Ragauskas *et al.* 2014). Without new product streams, the lignin produced would far exceed the current world market for lignin used in specialty products (Zhang 2008). Therefore, any value-added lignin-derived products would improve the economy of biomass conversion.

The full utilization of lignin extracted from the biorefinery process in the wood composite industry could be an intelligent double benefit solution. Fundamental research has historically focused on the process parameters of binderless boards manufactured using a mixture of lignin from the pulping and papermaking industry (Velásquez *et al.* 2003; Sun *et al.* 2014). Limited efforts have been seen in the investigation of the structural transformation of lignin macromolecules during the hot-pressing process. The self-bonding mechanism remains unclear. Fortunately, the extensive application of nuclear magnetic resonance (NMR) technology in lignin structural characterization (Sathitsuksanoh *et al.* 2014; Huijgen *et al.* 2014) provides a new analysis method for the study of the self-bonding behaviors of lignin macromolecules during the hot-pressing process.

In this study, an attempt was made to fabricate particleboard solely mixed with the lignin extracted from the biorefinery process, without the addition of synthetic adhesives. NMR technology was used to analyze the reactivity and chemical transformations of lignin macromolecules during the hot-pressing process. The binderless particle boards were manufactured using bamboo materials, which are widely planted in southern China, combined with alkaline lignin (AL) in various proportions under various hot-pressing conditions (temperature and pressure). It is expected that the structural evolution of lignin macromolecules after hot-pressing could help elucidate the self-bonding mechanism and optimize the hot-pressing conditions, hence improving the utilization of lignin biomass in the wood composite industry.

EXPERIMENTAL

Materials

Bamboo branches were obtained from Hunan Province, China. They were air-dried and cut into splinters smaller than 5 cm, then ground and sieved to obtain 40- to 100-mesh fractions. The major chemical composition of the initial material was 33.12% cellulose, 26.65% hemicelluloses, and 29.82% lignin. The alkaline lignin (AL), which was directly used in this study without any pretreatments, was a by-product of bio-ethanol production from corn cob at Shandong Longlive Bio-technology Co., Ltd., China. The AL consisted of 90.81% Klason lignin, 3.61% acid-soluble lignin, 0.63% sugars, 2.16% ash, and 2.79% others (Yang *et al.* 2014). Both bamboo and lignin samples were dried at 60 °C in an oven for 16 h and stored at 5 °C before use. All chemicals purchased were of analytical or reagent grade and used without further purification.

Methods

Preparation of binderless particleboards

Binderless particleboards were manufactured from bamboo particles mixed with AL in various proportions based on oven-dried weight. Then, the mixture (360 g) was shaped to a mat using a forming box, 300 mm in length × 300 mm in width, and a target thickness of 5 mm, controlled by a thickness gauge, with a target density of 0.8 g/cm³. After forming, the mat was loaded into a hot-press. Three single-factor experimental designs were used to determine the effect of temperature (130, 140, 150, 160, 170, and 180 °C), pressure (3, 5, 7, 9, 11, and 13 MPa), and lignin/bamboo (L/B) mass ratio (10%, 20%, 30%, 40%, 50%, and 60%) on the properties of the prepared boards. The pressing time was kept constant at 20 min. Each sample was prepared in triplicate.

Preparation of the lignin samples

Lignin samples were isolated from raw bamboo, particleboards formed under the conditions of 170 °C and L/B mass ratio 30%, and boards formed at 180 °C and L/B mass ratio 40%, with the same pressing time (20 min) and pressure (5 MPa). The boards were ground and sieved to obtain 80- to 120-mesh wood meal. The lignin was prepared from the wood meal according to the recent literature (Wu *et al.* 2015). Approximately 15 g of wood meal was milled (5 h) in a Fritsch planetary ball mill (Germany). The ball-milled sample was extracted with a dioxane/water solution (96:4, v/v) for 24 h. The mixture was filtered, and the filtrate was concentrated to *ca.* 30 mL. The obtained concentrated liquid was added dropwise into 10 volumes of acidified water (pH 2.0) adjusted with 6 M HCl. The precipitated lignin was obtained by centrifugation, washed with the same acidified water, and finally freeze-dried. The three wood meal samples underwent the same process, and the resulting lignin samples were labelled BL, PBL₁, and PBL₂, respectively.

Physical and mechanical properties of the boards

The boards were characterized using China National Standard GB/T 17657-2013 (2013). Prior to the evaluation of the mechanical and physical properties, the boards were conditioned at 25 °C and 65% relative humidity (RH) until the boards reached equilibrium moisture content. The specimen was held together with two clips by hot-melts and re-conditioned at 25 °C and 65% RH for 12 h. Six 50 mm × 50 mm specimens were cut from each board for internal bonding strength testing.

Characterization of the lignin samples

The carbohydrate moieties associated with all the lignin samples were determined by sugar analysis according to the procedure described in a previous paper (Yuan *et al.* 2011). The weight-average (M_w) and number-average (M_n) molecular weights of all lignin samples were determined by gel permeation chromatography (GPC, Agilent 1200 series, Agilent Technologies Inc. USA) with an ultraviolet detector (UV) at 280 nm according to a previous report (Wen *et al.* 2012). NMR spectra were recorded on a Bruker AVIII 400 MHz (Bruker, Germany) spectrometer at 25 °C in DMSO- d_6 . For the quantitative 2D-HSQC spectra, approximately 90 mg of lignin was dissolved in 0.5 mL of DMSO- d_6 . Quantitative ^{31}P NMR spectra were recorded according to published procedures (Yuan *et al.* 2011; Wen *et al.* 2012).

RESULTS AND DISCUSSION

Performance of the Boards

Lignin has a high reaction activity, which could play a crucial role in self-bonding during hot-pressing (Yang *et al.* 2014). To explore the effects of lignin on bamboo biomass self-bonding, the boards were formed using AL and bamboo biomass under the conditions listed in Table 1. The internal bonding strength (IBS) can be regarded as an important parameter among the mechanical characteristics. As shown in Table 1, the IBS of the boards showed a clear trend: with increasing temperature, the IBS improved. When the temperature rose to 160 °C, the IBS reached 0.66 MPa, which could satisfy the requirements of China National Standard GB/T 11718-2009 (2009). The best IBS (1.12 MPa) was obtained at 180 °C. The results show that the effect of temperature on the IBS became more evident as the temperature was increased.

Table 1. Results of Single-Factor Tests

Sample	T (°C)	P (MPa)	L/B	IBS (MPa)
1	130	5	0.3	0.02
2	140	5	0.3	0.18
3	150	5	0.3	0.26
4	160	5	0.3	0.66
5	170	5	0.3	0.91
6	180	5	0.3	1.12
7	180	3	0.3	0.0
8	180	5	0.3	1.32
9	180	7	0.3	0.15
10	180	9	0.3	0.08
11	180	11	0.3	0.23
12	180	13	0.3	0.0
13	180	5	0.1	0.09
14	180	5	0.2	0.24
15	180	5	0.3	0.8
16	180	5	0.4	1.36
17	180	5	0.5	1.32
18	180	5	0.6	0.12

Note: T-Temperature, P-Pressure, L/B= $W_{\text{lignin}}/W_{\text{bamboo}}$, IBS-internal bonding strength

Lignin would have melted well under high pressing temperature like a plastic material, therefore increasing the lignin fluidity, improving lignin distribution on the surface of fibers, and forming strong inter-fiber bonds. However, carbonization would gradually appear on the surface of the boards with increasing temperature. A different tendency was presented with increasing pressure. The maximal IBS was obtained when the single factor pressure reached 5 MPa. It may be concluded that AL and bamboo particles could not self-bond when the pressure was less than 3 MPa. When the pressure was higher than 13 MPa, the board was completely crushed. The L/B mass ratio also had an important effect on the value of IBS. If the amount of AL was not enough, then there was an obvious lack of adhesive to glue the particles. As the lignin powder itself did not possess enough mechanical strength, the addition of excess of lignin is expected to reduce the mechanical performance (Cao and Xiao 2011). As shown in Table 1, the optimal L/B mass ratio was 0.4. It could be inferred that the content of AL clearly had an effect on the self-bonding. For best self-bonding conditions, a temperature of 180 °C, pressure of 5 MPa, and L/B mass ratio of 0.4 is advised. However, the pattern of self-bonding could not be revealed just by the mechanical strength, but requires further analysis of the structure of the lignin macromolecules during the hot-pressing.

Carbohydrate Analysis of the Lignin Samples

Although all lignin samples had been purified and substantial amounts of carbohydrates had been removed, some carbohydrates remained in the purified lignin (Yuan *et al.* 2011). To determine the nature of associated carbohydrates existing in the lignin samples, four lignin samples were prepared. The results of the carbohydrate contents in these samples are given in Table 2. As can be seen, the AL showed the highest purity, with just 0.63% sugar content. The associated carbohydrate contents of BL, PBL₁, and PBL₂ amounted to 1.57%, 1.60%, and 13.17%, respectively. The higher sugar content in the PBL₂ was mostly due to the residues of glucose and xylose. This may be attributed to the sugar that precipitated out during the acidified water wash. This fact was also confirmed by the signals present in the following 2D-NMR spectra. Other monosaccharides, such as mannose, rhamnose, arabinose, and galactose, were observed in minor amounts. The yields of the BL, PBL₁, and PBL₂ lignin fractions were 6.57%, 43.36%, and 63.54%, respectively. It was no surprise that the yield of the BL sample was lower than that of PBL₁ and PBL₂. This result could be ascribed to the fact that a certain amount of AL had been incorporated into the board, causing the relative increase in lignin content.

Table 2. Yield and Contents of Associated Carbohydrates of the AL and Isolated Lignin Samples

Sample	Carbohydrate ^a content (%)						Total sugar content (%)	Yield (%) with sugars ^c
	Rhm	Ara	Gal	Glu	Xly	Man		
AL	ND	0.13	0.02	0.32	0.13	0.03	0.63	ND
BL	0.10	0.40	0.66	0.41	ND ^b	ND	1.57	6.57
PBL ₁	0.18	0.33	0.11	0.18	0.80	ND	1.60	43.36
PBL ₂	0.97	0.86	0.18	3.15	7.8	0.21	13.17	63.54

Note: ^a Ara, arabinose; Gal, galactose; Glc, glucose; Man, mannose; Xyl, xylose. Rhm, rhamnose; ^bND, Not detected. ^cBased on the dry mass of Klason lignin (% w/w).

Molecular Weight Analysis of the Lignin Samples

Changes in the molecular weight of lignin could provide important insights into lignin fragmentation or condensation reactions during hot-pressing. Generally, while cleavage of β -O-4' linkages could result in a decrease in the molecular weight of lignin, condensation reactions usually lead to a condensed and heterogeneous lignin structure with an increase in the molecular size (Yuan *et al.* 2011; Wen *et al.* 2013a). The weight-average (M_w) and number-average (M_n) molecular weights of the four lignin samples were analyzed by their GPC curves (relative to polystyrene), and the results are listed in Table 3. As can be seen, AL had the highest molecular weight among the four lignin samples. The other three lignin samples exhibited smaller molecular weight distributions, ranging from 1420 to 1670 g/mol. Specifically, the M_w of PBL₁ and PBL₂ decreased with increasing hot-pressing temperature. The polydispersity (M_w/M_n) determined for AL, BL, PBL₁, and PBL₂ was 1.44, 2.17, 2.00, and 2.03, respectively. This suggested that depolymerization reactions were dominant at the selected temperature conditions. Actually, depolymerization and recondensation are concerted reactions occurring during the hot-pressing process, which was confirmed by the following 2D-HSQC analysis. When hot-pressing under severe conditions, the cleavage of the dominant aryl ether linkages occurs, producing some “low-molecular” fractions, such as syringyl (S), guaiacyl (G), and p-hydroxyphenyl (H) oligomers (Fig. 2). The active sites (unsubstituted C-3 and/or C-5 positions in aromatic rings of phenolic aryl propane units) in these oligomers are important reactive sites for coupling reactions, forming the net structure needed to glue the adjacent fibers and thus increasing the bonding strength of the board produced (El Mansouri and Salvadó 2006).

Table 3. Weight-Average (M_w) and Number-Average (M_n) Molecular Weights and Polydispersity (M_w/M_n) of the AL, BL, and PBL Lignin Samples

Index	Lignin sample			
	AL	BL	PBL ₁	PBL ₂
M_w	3260	1670	1580	1420
M_n	2260	770	790	700
M_w/M_n	1.44	2.17	2.00	2.03

2D-HSQC NMR Spectra of the Lignin Samples

Two-dimensional ^{13}C - ^1H NMR (2D-HSQC) can allow the assignment of individual signals in crowded spectral regions and the quantification of various structural units. In the present study, the four lignin samples were characterized by 2D-HSQC NMR techniques to understand their detailed structures. The signal assignments were made according to previous studies (Wen *et al.* 2013b; Yuan *et al.* 2013). The 2D-HSQC NMR spectrum can be divided into three regions, corresponding to aliphatic, side chain, and aromatic ^{13}C - ^1H correlations. However, the aliphatic (non-oxygenated) region shows signals that will not provide any lignin structural information and therefore is not discussed here. The side-chain ($\delta_{\text{C}}/\delta_{\text{H}}$ 50-90/2.5-6.0) and aromatic ($\delta_{\text{C}}/\delta_{\text{H}}$ 100-135/5.5-8.5) regions of the HSQC spectra and the structural units of the identified lignin substructures in the analytical lignins are shown in Figs. 1 and 2, respectively.

In the side-chain regions, the prominent correlating signals observed in all four spectra were the β -O-4' ether linkages (substructure A). Specifically, the signals at $\delta_{\text{C}}/\delta_{\text{H}}$ 71.8/4.86, $\delta_{\text{C}}/\delta_{\text{H}}$ 83.4-86.7/4.00-4.38, and $\delta_{\text{C}}/\delta_{\text{H}}$ 59.9/3.35-3.80 belong to the C $_{\alpha}$ -H $_{\alpha}$, C $_{\beta}$ -

H_β , and C_γ - H_γ correlations of the β -O-4' ether substructures, respectively. β -5' (phenylcoumaran, C) linkages were found at relatively low levels, as shown by the signals at δ_C/δ_H 86.8/5.45, 53.1/3.46, and 62.3/3.76, corresponding to C_α - H_α , C_β - H_β , and C_γ - H_γ , respectively. β - β' (resinol B) and β -1' (spirodienones D) linkages could not be observed at this level.

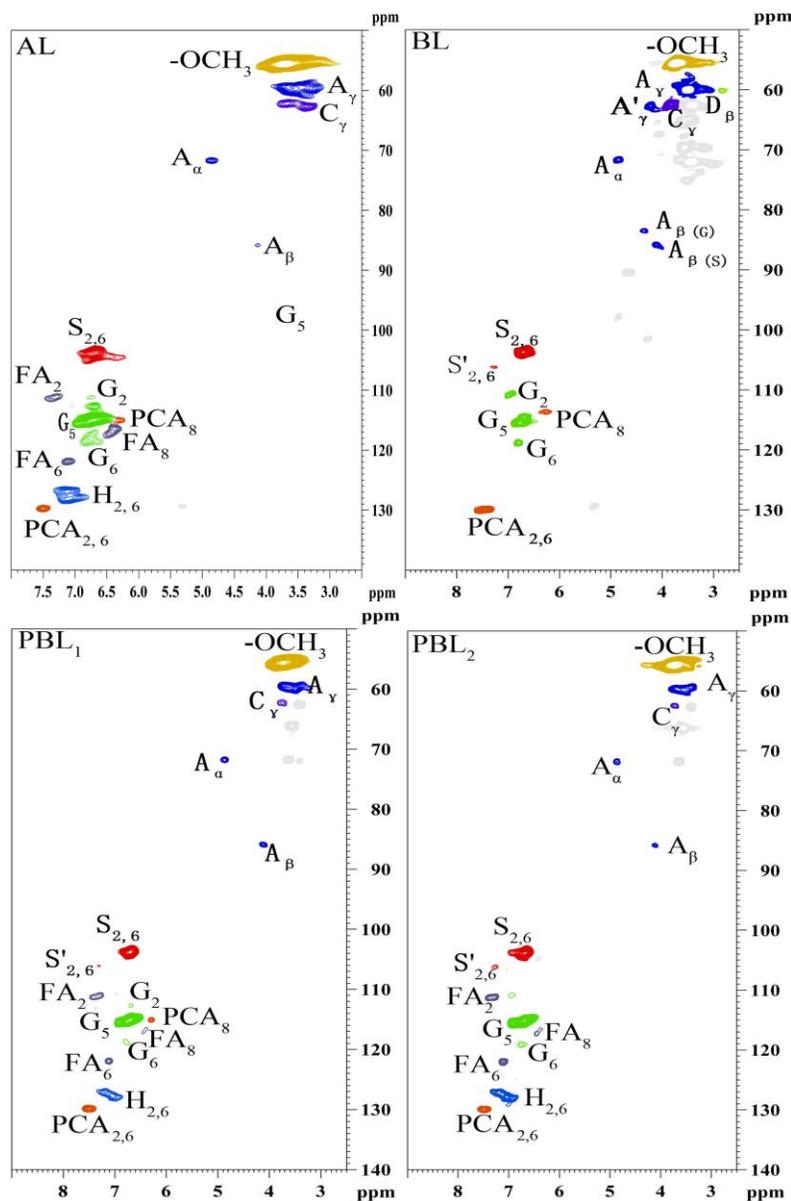


Fig. 1. The 2D HSQC NMR spectra of the AL, BL, PBL₁ and PBL₂ lignin samples

In the aromatic region, C-H correlations corresponding to S, G, and H units could be observed in all the spectra. The normal S-type lignin units showed a prominent signal for the $C_{2,6}$ - $H_{2,6}$ correlations at δ_C/δ_H 103.9/6.70, whereas the signals corresponding to $C_{2,6}$ - $H_{2,6}$ correlations in C_α -oxidized S' units shifted to δ_C/δ_H 112.6/7.40. However, 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 110.8/6.97, 114.5/6.70, and 119.0/6.78, respectively. The $C_{2,6}$ - $H_{2,6}$ correlations of H units were

observed at δ_C/δ_H 127.7/6.91-7.23. The esterified ferulic acid and *p*-coumaric acid at the γ -position of the lignin polymers were cleaved during the alkali treatment (Lu and Ralph 1999; Wen *et al.* 2013b). The typical signals of ferulate (FA) were observed at δ_C/δ_H 112.7/7.45, 123.1/7.10, and 117.0/6.42, corresponding to C₂-H₂, C₆-H₆, and C₈-H₈ correlations, respectively. The free *p*-coumarate (PCA) was confirmed by the obvious signals at δ_C/δ_H 130.2/7.58 and 113.7/6.34 of the C_{2,6}-H_{2,6} and C₈-H₈ correlations, respectively.

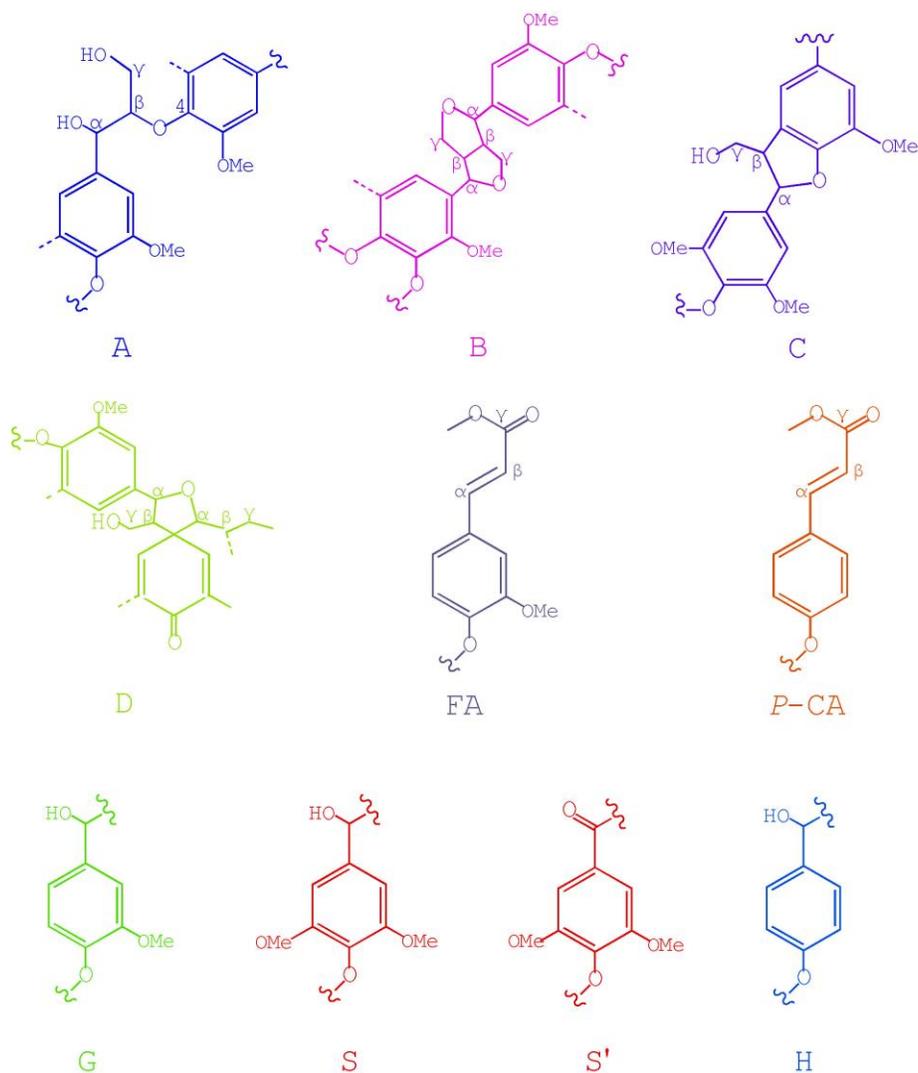


Fig. 2. Classic substructures involving different side-chain linkages, and aromatic units: (A) β -O-4' aryl ether linkages with a free -OH at the γ -carbon; (B) resinol substructures formed by β - β' , α -O- γ' , and γ -O- α' linkages; (C) phenylcoumaran substructures formed by β -5' and α -O-4' linkages; (D) spirodienones; (G) guaiacyl units; (S) syringyl units; (S') oxidized syringyl units with a C _{α} ketone. (H) *p*-hydroxyphenyl units; (P-CA) *P*-coumarate; (FA) ferulate

2D-HSQC NMR analysis provided evidence of the structural changes in lignin after hot-pressing. The main structural characteristics of the lignin framework, including basic composition (S, G, H, PCA, and FA units) and various inter-unit linkages, were observed in the 2D-HSQC spectra. The relative percentages of the each linkage bonds,

such as β -O-4', β - β ', β -5', and S/G/H ratios, are listed in Table 4. As shown, the AL mostly consisted of β -O-4' aryl ether and a small number of β -5' carbon-carbon linkages, which was in accordance with the high molecular weight. As the temperature was increased from 170 to 180 °C, the content of β -O-4' aryl ether in BL, which was 87.1%, decreased to 83.9% and 80.2% in PBL₁ and PBL₂, respectively. This suggested that the cleavage of β -O-4' aryl ether linkages occurred during hot-pressing. Simultaneously, the β - β ' linkages decreased from 12.2% to 7.6%, which also indicated depolymerization. However, the β -5' linkages presented a different tendency. The samples of PBL₁ and PBL₂, which were isolated from the particleboard manufactured by bamboo fractions mixed with AL after hot-pressing, had more β -5' linkages than AL and BL. This was probably attributable to unsubstituted C-3 and C-5 positions in the oligomers, coming from the cleavage of β -O-4' aryl ether and β - β ' linkages, as a result of the recondensation reaction (Kuroda and Nakagawa-izumi 2005). As shown in the following ³¹P-NMR spectra, the signals from the condensed S and G units increased with increasing hot-pressing temperature. This meant that a small proportion of the recondensation reaction of lignin occurred during hot-pressing.

Moreover, the change in the S/G/H ratio in lignin was another prominent structural alteration observed after hot-pressing. Bamboo biomass primarily consists of S-type and G-type lignin, as well as a small amount of H-type lignin (Lou *et al.* 2010; Wen *et al.* 2012, 2013a). In the present study, the BL sample was in overall agreement with published reports, as shown in Table 4. As compared to the BL, however, the proportion of G-type and H-type lignin was increased in the PBL₁ and PBL₂. There are two possible explanations for this result: (1) the AL (S/G/H, 43/25/32) includes a small amount of H-type lignin, which was one of sources for the PBL₁ and PBL₂ samples isolated from the compound board; or (2) the S-type and G-type lignin could become transformed into H units as a result of demethoxylation (Wen *et al.* 2012). A more interesting result was that the rate of demethoxylation was probably faster in S-type than G-type lignin at higher temperatures. Generally speaking, in lignin, G-type units have a free C-5 position and H-type units have both C-3 and C-5 positions free (ortho to the phenolic hydroxyl) in the ring, while in S-type units, both C-3 and C-5 positions are linked to a methoxy group, resulting in low reactivity (Del Río *et al.* 2002). From this point of view, lignin with G-type and H-type units as the principal structural units must be *a priori* more suitable for self-bonding. Another factor that must be considered is the existence of large quantities of phenolic hydroxyls in the lignin structure: they activate the free ring positions, making them susceptible to reacting with other lignin units (Feldman *et al.* 2001; Thring *et al.* 2004). Overall, G-type and H-type lignins possess additional active positions, which could further promote the formation of self-bonding (El Mansouri and Salvadó 2006).

Table 4. Quantification of the AL, BL, PBL₁, and PBL₂ Lignin Samples by 2D-HSQC NMR

Sample	β -O-4'	β - β '	β -5'	S/G/H
AL	94.4	ND	5.6	43/25/32
BL	87.1	12.2	0.7	62/35/3
PBL ₁	83.9	9.4	6.7	36/37/27
PBL ₂	80.2	7.6	12.2	32/37/31

³¹P-NMR Spectra of the Lignin Samples

³¹P-NMR spectroscopy is a facile and direct analysis tool for quantitating the major hydroxyl groups in lignin. To study the changes in various hydroxyl groups in these lignin samples, typical ³¹P-NMR spectra of phosphitylated lignin and the various hydroxyl group contents are shown in Fig. 3 and Table 5, respectively.

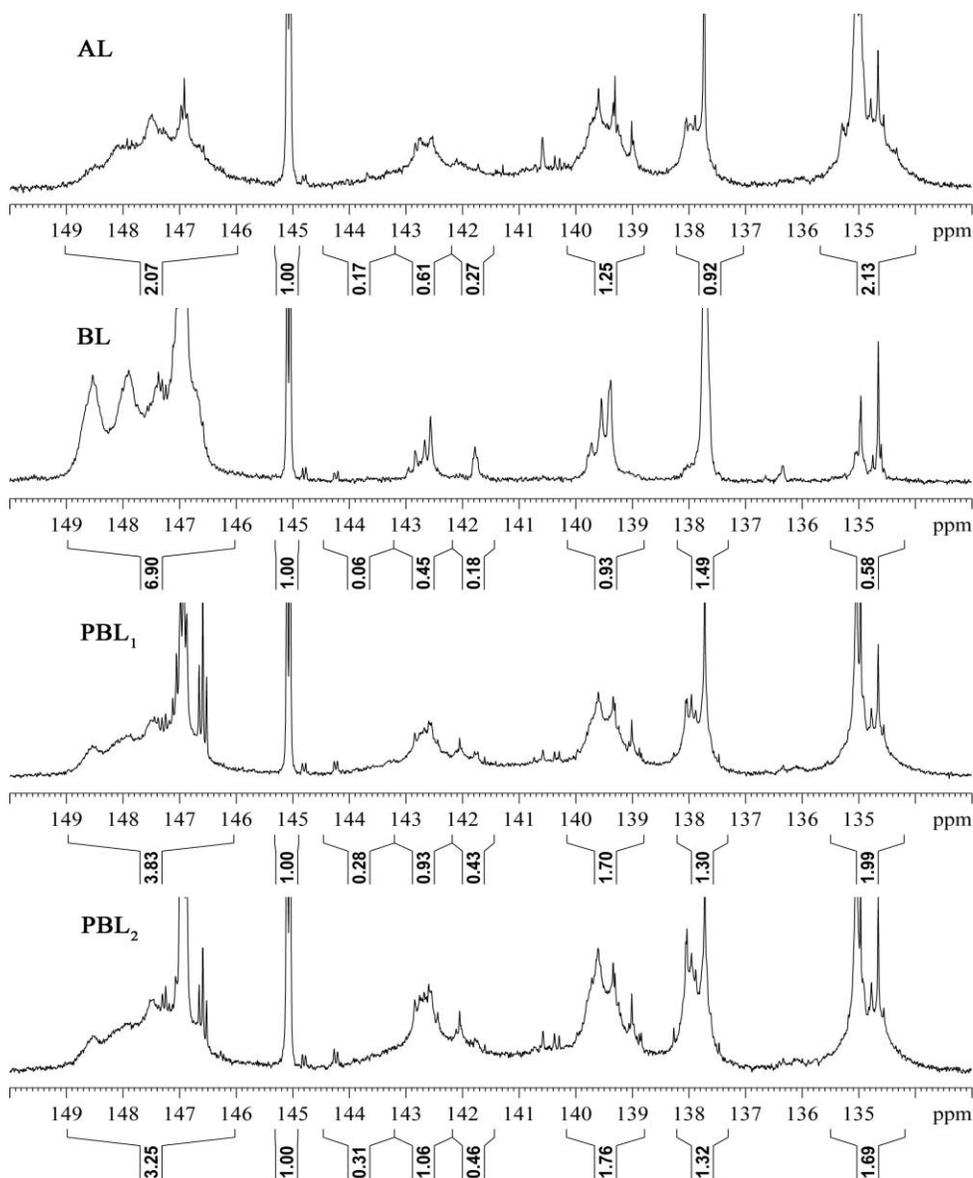


Fig. 3. Quantitative ³¹P-NMR spectra of the AL, BL, PBL₁, and PBL₂ lignins

The carboxylic groups increased from 0.31 to 1.08 mmol/g, primarily because of the high content of PCA and FA included in the AL. The content of aliphatic hydroxyl groups, mostly from the side-chains of lignin in PBL₁ and PBL₂, were lower than that of BL after hot-pressing. This phenomenon could be partly attributed to the fact that a certain amount of the isolated lignin came from the AL, which contains low amounts of aliphatic OH. Another possible reason was the dehydration reaction, which led to the reduced aliphatic hydroxyl content during the hot-pressing process (El Hage *et al.* 2010).

In contrast, the S-type and G-type phenolic OH increased after hot-pressing. As shown in Table 5, the content of S non-condensed (S-NC) phenolic OH was 0.50 and 0.57 mmol/g for PBL₁ and PBL₂, respectively, which was more than two times that of BL. The G non-condensed (G-NC) phenolic OH displayed the same tendency. This fact indicated that the cleavage of β -O-4' aryl ether linkages occurred during hot-pressing, producing some small lignin oligomers. These results are in agreement with the reduction in molecular weight shown by the GPC analysis. The content of H-type phenolic OH slightly increased, from 0.65 to 0.81 mmol/g. This may have been due to the demethoxylation at the 3-position of G units or the 3,5-position of S units at higher temperatures. Another reasonable explanation for this could be that bamboo is a gramineous biomass and a large amount of PCA is associated with its lignin (Wen *et al.* 2012), which was detected by the 2D-HSQC analysis. As a result, the total phenolic OH evidently increased, which was beneficial for the self-bonding of lignin.

Table 5. Functional Groups of the AL, BL, PBL₁, and PBL₂ Lignins as Determined by Quantitative ³¹P-NMR (mmol/g)

Sample	Aliphatic OH	Syringyl OH		Guaiacyl OH		<i>P</i> -hydroxy phenyl OH	Carboxylic group	Total phenolic OH
		C ^a	NC ^a	C	NC			
AL	1.77	0.10	0.55	0.22	0.42	0.65	1.09	1.94
BL	3.74	0.03	0.24	0.10	0.50	0.81	0.31	1.68
PBL ₁	2.07	0.15	0.50	0.23	0.92	0.70	1.08	2.51
PBL ₂	1.76	0.17	0.57	0.25	0.95	0.71	0.92	2.66

Note: ^a Abbreviation: C, condensed; NC, non-condensed

CONCLUSIONS

1. The comprehensive GPC and NMR results revealed that the content of β -O-4' aryl ether linkages and the molecular weights decreased after hot-pressing of particle board formed with self-bonding lignin. Simultaneously, the percentage of G-type and H-type lignins, as well as the content of phenolic OH, increased.
2. These transformations of aryl ether linkages and functional groups in the lignin backbone effectively increased the active sites of the lignin benzylic structure and can easily form carbon-carbon linkages, promoting self-bonding.
3. Depolymerization and recondensation reactions occurred simultaneously during the hot-pressing process.

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