

Structural Properties of the Purified Lignins of Cornstalk in the Cooking Process with a Solid Alkali

Qiulin Yang,^{a,b,*} Dan Huo,^{a,d} Jianbin Shi,^{b,e} Lu Lin,^{c,*} Qiujuan Liu,^a Qingxi Hou,^a Hongjie Zhang,^a and Chuanling Si^a

The process of cooking with solid alkali is a novel and efficient technology. In the present work, milled wood lignin (MWL) and water-soluble milled wood lignin (WMWL) were used as the raw materials to research their properties when cooking with MgO and active oxygen. Before and after cooking, the lignin content of the samples was estimated using attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR), heteronuclear single-quantum coherence-nuclear magnetic resonance (HSQC-NMR), and gel permeation chromatography (GPC). The results showed that the properties of the MWL and WMWL were different. The G unit and A' structure in WMWL were more easily degraded than that in the MWL, where the G unit could be oxidized into a novel G' unit. The D structure in the MWL with a weak signal could be thoroughly degraded, and the P structure was only present in WMWL and could be generated during the cooking process.

Keywords: Lignin; solid alkali; Active oxygen; Cooking

Contact information: a: Tianjin Key Laboratory of Pulp & Paper, Tianjin University of Science & Technology, Tianjin 300457, China; b: State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou 510640, Guangdong province, China; c: School of Energy Research, Xiamen University, Xiamen 361005, Fujian Province, China; d: Research Institute of Agro-Products Processing and Nuclear-Agricultural Technology, Hubei Academy of Agricultural Sciences, Wuhan 430064, Hubei Province, China; e: Jiangsu Province Biomass Energy and Materials Laboratory, Institute of Chemical Industry of Forest Products, CAF, Nanjing, 210042, China; *Corresponding authors: qiulinyang@tust.edu.cn; lulin@xmu.edu.cn

INTRODUCTION

Lignin, the most abundant natural aromatic polymer, has a highly branched three-dimensional phenolic structure including three main phenylpropane units, namely p-hydroxyphenyl, guaiacyl, and syringyl units (Lora and Glasser 2002; Demirbas 2009; Fu *et al.* 2013). Normally, lignin is commercially available as a byproduct of the pulp and paper industry, and it is generated in large quantities. This supply is often used by paper mills as an energy source. Moreover, lignin can be used for the production of many products, so it can be regarded as a potential feedstock for biorefinery.

Solid alkali is a type of substrate that can accept protons or donate electrons and can be categorized according to super alkali, medium alkali, and weak alkali strengths. So far, the solid alkalis such as metal oxides, zeolite, supported alkali metal ions, clay minerals, non-oxide, and alkaline ion exchange resin are commonly found in manufacturing biodiesel and organic chemicals synthesis (Tanabe and Hölderich 1999). The super alkali is defined as having an *H*-value (Hammett acidity function) of greater than 26.0, and usually contains alkaline metal oxide, alkaline earth metal oxide/hydroxide, supported alkaline metal, alkaline metal hydroxide, *etc.* (Hattori 1995, 2001). Magnesium oxide

(MgO) which is produced by direct heating of magnesium carbonate or magnesium hydroxide has weak basic strength and solubility in water (Zabeti *et al.* 2009). Currently, magnesium oxide is commonly found in food, medicine, pulping, and other fields but is rarely used for biorefinery purposes.

The cooking technology using a solid alkali as a pretreatment for biorefinery is a novel and efficient process. In the cooking process, the MgO or magnesium dioxide (Mg(OH)₂) is used as a solid alkali, which provides an alkaline environment for the cooking process and exhibits a protective effect for carbohydrate stabilization especially for the cellulose (Guay *et al.* 2000). The MgO was partially dissolved into the cooking liquor, which could provide a relatively stable alkaline conditions for the cooking. When a conventional alkali such as NaOH is used in the cooking instead of the MgO, a great many of the cellulose could be degraded and dissolved into the cooking liquor. Reagents, such as hydrogen peroxide (H₂O₂) and O₂, are used as an active oxygen or a source of active oxygen, which has a high delignification effect on the biomass during the cooking (Yang *et al.* 2012). Currently, oxygen delignification is commonly utilized in the pulp and paper industry because an oxygen molecule containing two unpaired electrons is intensely reactive with organic substrates and can react with the phenolic and ring conjugate structures of the lignin compound (Yang *et al.* 2003). Hydrogen peroxide is often used in the bleaching process of mechanical pulps as it is a weak oxidant that can react with the chromophoric groups of the lignin (Gierer 1986). In addition to protect the carbohydrate, the magnesium ions have the ability to stabilize the hydrogen peroxide (Sun *et al.* 1998).

In our previous research, it was found that the delignification rate and dissolution sequences of the acid-soluble and acid-insoluble lignins in the cooking process with solid alkali were different, where 96.2% of the acid-soluble lignin and only 82.9% of the acid-insoluble lignin were removed from the raw material (Yang *et al.* 2014). However, the specific reactions taking place during the cooking of the two types of lignins are not yet understood, and the structural changes of lignins are also need further research. In the present work, milled wood lignin (MWL) and water-soluble milled wood lignin (WMWL) were extracted from cornstalk raw material to analyze the characteristics and reaction mechanism of the cooking process with solid alkali.

EXPERIMENTAL

Materials

Cornstalk raw material was provided by the BBKA Group Co., Ltd. (Bengbu, China). Magnesium oxide powder, with the purity of greater than 98%, was obtained from Tianjin Kermel Reagents Co., Ltd. (Tianjin, China). Deuterated dimethyl sulfoxide (DMSO-*d*₆, 99.9% purity) and deuterated water (D₂O, 99.9% purity) were obtained from Sigma-Aldrich Co. (Shanghai, China). All other reagents were of analytical grade and purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). The 1,4-dioxane was distilled prior to use for removing impurities.

Preparation of MWL and WMWL from Cornstalk

The cornstalk raw material was powdered using a plant miniature crusher (FZ102, Shanghai Keheng Industrial Development Co. Ltd., Shanghai, China), and extracted for 8 h in a Soxhlet extractor (Sichuan Shubo (Group) Co., Ltd., Chongzhou, China) with a benzene/ethanol (2/1, v/v) solution to remove the organic solvent extracts. The samples

were subsequently dried before being ground in a water-cooled vibratory ball mill (VS-1, Jnie Shokai Co., Ltd., Japan) with zirconia balls for 72 h (Björkman 1956).

For the preparation of the MWL and WMWL, the Björkman method was used (Björkman 1956). The samples were extracted with dioxane/water (96/4, v/v) for 24 h, and then the operation was repeated twice. The resulting supernatant was centrifuged, concentrated, and freeze-dried to obtain the crude milled wood lignin. The purification process of the crude milled wood lignin was described in more detail in previous research by others (Bu *et al.* 1991; Hage *et al.* 2009). These references describe consecutively treating the crude milled wood lignin with acetic acid, distilled water, a dichloroethane/ethanol solution, and petroleum ether. Finally, the crude milled wood lignin was separated into two parts, the milled wood lignin (MWL) and the water-soluble milled wood lignin (WMWL), and stored for later use.

Cooking Process

The cooking process was conducted in a stainless steel pressurized reactor (5500 Series, Parr Instrument Co., St. Moline, IL, USA), with an effective volume of 100 mL. Exactly 0.5 g of the lignin sample was placed into the pressurized reactor, and 50 mL of deionized water and 1.25 g of MgO powder was added. The reactor was sealed and fed O₂ to raise the pressure to 1 MPa at room temperature. Then, the reactor was heated to 165 °C at a rate of 1 °C/min and was stabilized at this temperature for 2 h.

Lignin Separation from the Cooking Liquors

After the reactor was cooled to room temperature, the liquors from the cooking process using the MWL and WMWL were centrifuged (3K15, Sigma-Aldrich Co., Shanghai, China) at 10,000 rpm for 10 min to remove the solid alkali, respectively. Then the samples were freeze-dried to obtain the cooked milled wood lignin (CMWL) and the cooked water-soluble milled wood lignin (CWMWL).

Characterization of the Lignins

Fourier transform infrared (FTIR) spectra were obtained on an attenuated total reflectance (ATR)-FTIR spectrophotometer (Bruker Tensor 27, Germany) using potassium bromide (KBr) disks containing 1% (w/w) of the finely ground lignin samples. The spectra were recorded over a wavenumber range of 4000 to 400 cm⁻¹ at a resolution of 2 cm⁻¹ in the transmission mode.

The reagent, DMSO-*d*₆, was used as a nuclear magnetic resonance (NMR) solvent for the MWL and WMWL samples, whereas the CMWL and CWMWL samples were dissolved with D₂O because of their enhanced solubility in water. When DMSO-*d*₆ was used as the solvent, chemical shifts were referred to as the residual DMSO peak at $\delta_{\text{H}}/\delta_{\text{C}}$ 2.50/40.0 ppm. All of the 2D heteronuclear single quantum coherence (HSQC)-NMR spectra for the samples were obtained using a Bruker AV 600 MHz NMR spectrometer (Buker Co., Germany) at 25 °C. The spectral widths were 8000 and 25,000 Hz for the ¹H- and ¹³C-dimensions, respectively. The acquisition time per scan was 0.0639 s, whereas the relaxation delay time was 1.5 s, and the pulse width was 12.1 Hz.

The molecular weights of the lignins were estimated using a gel permeation chromatography (GPC) device equipped with a high-performance liquid chromatography (HPLC) system (625LC system, Waters Corp., Milford, MA, USA). The MWL and WMWL samples were acetylated with a pyridine/anhydride mixture (v/v, 1/1) prior to the

molecular detection, which was described in detail in previous research (Scholze *et al.* 2001; Wang *et al.* 2010).

The acetylated lignin samples were dissolved in tetrahydrofuran (THF, 1 mg/mL). The THF was used as an eluent, with a flow rate of 1 mL/min. Shodex KF-G 802, KF-G 803 columns (Showa Denko, Japan) and polystyrene standards were used, and the column temperature was maintained at 35 °C. The CMWL and CWMWL samples were directly dissolved into monopotassium phosphate (KH₂PO₄, 0.02 M), due to the fact that they had a poor solubility in organic solvents and alkaline solutions. The KH₂PO₄ was used as an eluent with a flow rate of 0.6 mL/min. TSK G-5000 PW × L, G-3000 PW × L columns and glucan standards were used for size exclusion chromatography (SEC), and the column temperature was maintained at 35 °C.

RESULTS AND DISCUSSION

Molecular Structures of Lignins with ATR-FTIR Analysis

Before and after cooking, the lignin samples were characterized using ATR-FTIR to elucidate the structural changes and possible reactions. The spectra are shown in Fig. 1, and the assignments of the peaks or the absorption at specific wavenumber are given in accordance with the indications of previous research (Faix 1991; Jahan *et al.* 2007; Minu *et al.* 2012; Monteil-Rivera *et al.* 2013).

As shown in Fig. 1, the bands at 2925 cm⁻¹ and 2850 cm⁻¹ were assigned to C-H stretching in methyl and methylene groups, where the absorbance intensity of the CMWL and CWMWL was much weaker than that of the MWL and WMWL. The band at 1720 cm⁻¹ was assigned to the carbonyl stretching in unconjugated ketones and carbonyl groups, with strong absorbance intensity in the WMWL, indicating that it had been completely changed after cooking. The band at 1635 cm⁻¹ was assigned to the C=O stretching in conjugated p-substituted aryl ketones, which only appeared in the MWL, because of the degradation in the cooking process. The band at 1600 cm⁻¹ in all of the four types of lignin samples was assigned to the aromatic skeletal vibrations plus C=O stretching. The band at 1510 cm⁻¹ in the MWL and WMWL was assigned to aromatic skeletal vibrations coupled with C-H in plane deformations, which has been completely changed after cooking. The band at 1460 cm⁻¹ was related to the C-H deformations (asymmetric in methyl, methylene, and methoxyl groups), which only appeared in the MWL and WMWL. The bands at 1260 cm⁻¹ and 1160 cm⁻¹ were respectively assigned to the signal of the G (guaiacyl) unit and C=O stretching in ester group (conj.), which also only appeared in the MWL and WMWL. The band at 1120 cm⁻¹ was typically associated with the S (syringyl) unit and secondary alcohol and C=O stretching, which was present in all of the lignin samples. In addition, the absorbance intensity of the CMWL and CWMWL in 3400 cm⁻¹ was obviously higher than that in the MWL and WMWL. In the cooking with solid alkali and active oxygen, a proportion of the MgO was not dissolved into the cooking liquor and could become converted into Mg(OH)₂, so the strong signal intensity of the CMWL and CWMWL in 3400 cm⁻¹ was mainly related to the Mg(OH)₂. Based on the above analysis, it can be concluded that the structures of the MWL and WMWL changed during the cooking process with solid alkali, and the reactions mainly were related to the carbonyl carbon in several groups.

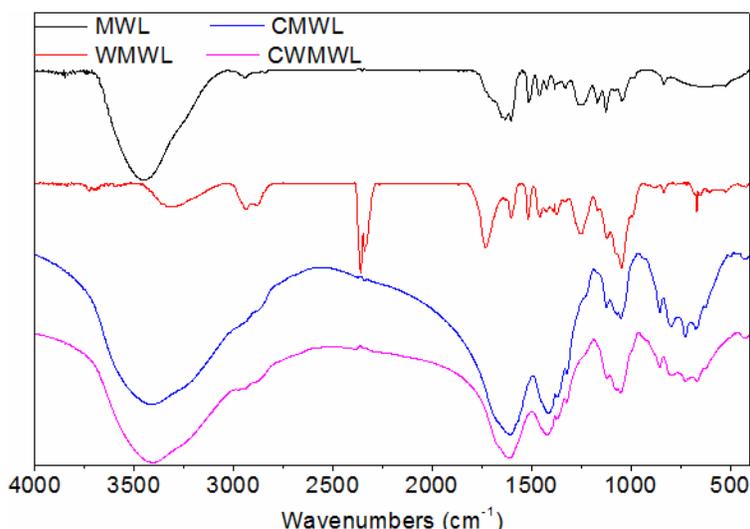


Fig. 1. ATR-FTIR spectra of the lignins

Substructures of the Lignins with HSQC NMR Analysis

To further elucidate the potential reactions of the two types of lignins in the cooking, 2D HSQC NMR technology was used. The interpretation and utilization of 2D spectra of lignin have been described in previous research (Liitia *et al.* 2003; Lu and Ralph 2003; Martinez *et al.* 2008; Rencoret *et al.* 2009; Wen *et al.* 2014). To increase the accuracy of the analysis results, a low concentration of the MWL and WMWL were added into the reactor to ensure they were completely dissolved in the cooking liquor. In addition, specific reactions of substructures of the molecules that were not clear could not be confirmed because of the complex structure of the lignin. The main substructures of the lignin assigned in NMR spectra are listed in Fig. 2, and the NMR spectra are shown in Fig. 3a-d.

As one of the three main basic structural units, the S unit and its deformation structures, S' and S'' units, were normally found in the MWL and WMWL; however, some of the structures were absent in the two types of cooking liquors (CMWL and CWMWL). In the cooking process with MgO and active oxygen, the reactivities of the S unit and its deformation units (S', S'') were higher, which was in agreement with the previous research (Ning and Chen 1987). In alkaline oxygen delignification, molecular oxygen generated hydroxyl radicals (HO·), and the benzylic alcohol group of the lignin molecule could be oxidized into a carbonyl group by the hydroxyl radical (Gierer 1997; Yang *et al.* 2003). Meanwhile, the carbonyl moieties were readily attacked by oxygen, and the cleavage of C α -C β bond in etherified structures containing a carbonyl group could generate conjugated acids (Gierer 1985). Hence, the S unit with a benzylic alcohol group, the S' unit, and the S'' unit with a ring-conjugated structure (carbonyl group at C α) respectively, could be degraded by oxygen. This suggested that the S, S', and S'' units present in the two types of lignins were changed thoroughly in the cooking process using the solid alkali. The G unit with a benzylic alcohol group existed in the MWL, WMWL, and CMWL, which was not found in the CWMWL, and could be oxidized into a carbonyl group using oxygen. The G' unit (δ_H/δ_C , 7.25/126.6 ppm) with a carbonyl group was only found in the CMWL and CWMWL, meaning that the G unit could be oxidized by oxygen to generate a G' unit. However, a proportion of the G unit with weak signal intensity was found in the CMWL, and did not appear in the CWMWL, suggesting that the reactivities of the G unit in the MWL and WMWL were different. It was probably related to the properties of the lignin,

where the MWL had a weaker solubility in water, while the WMWL was capable of thoroughly dissolving in water.

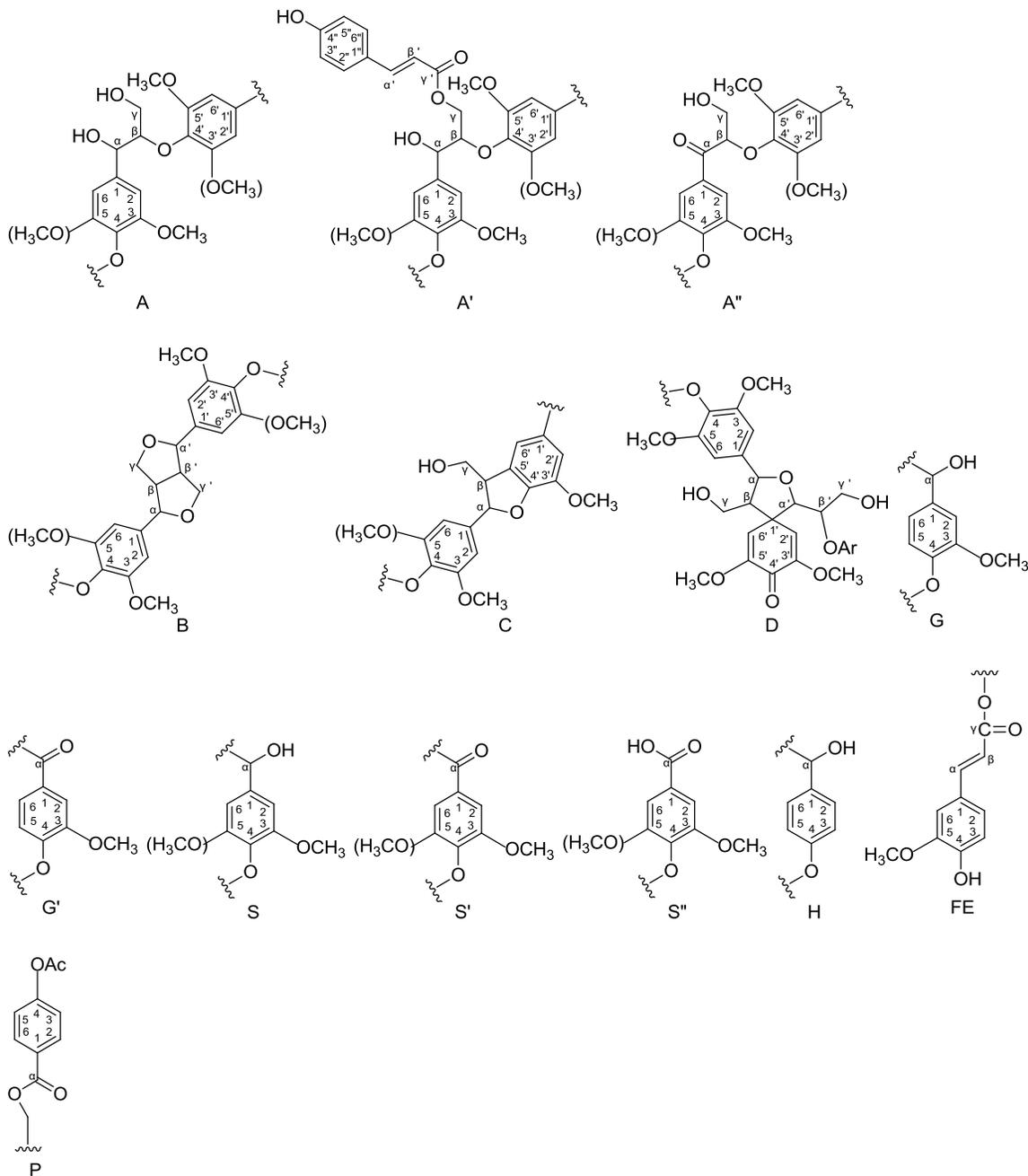
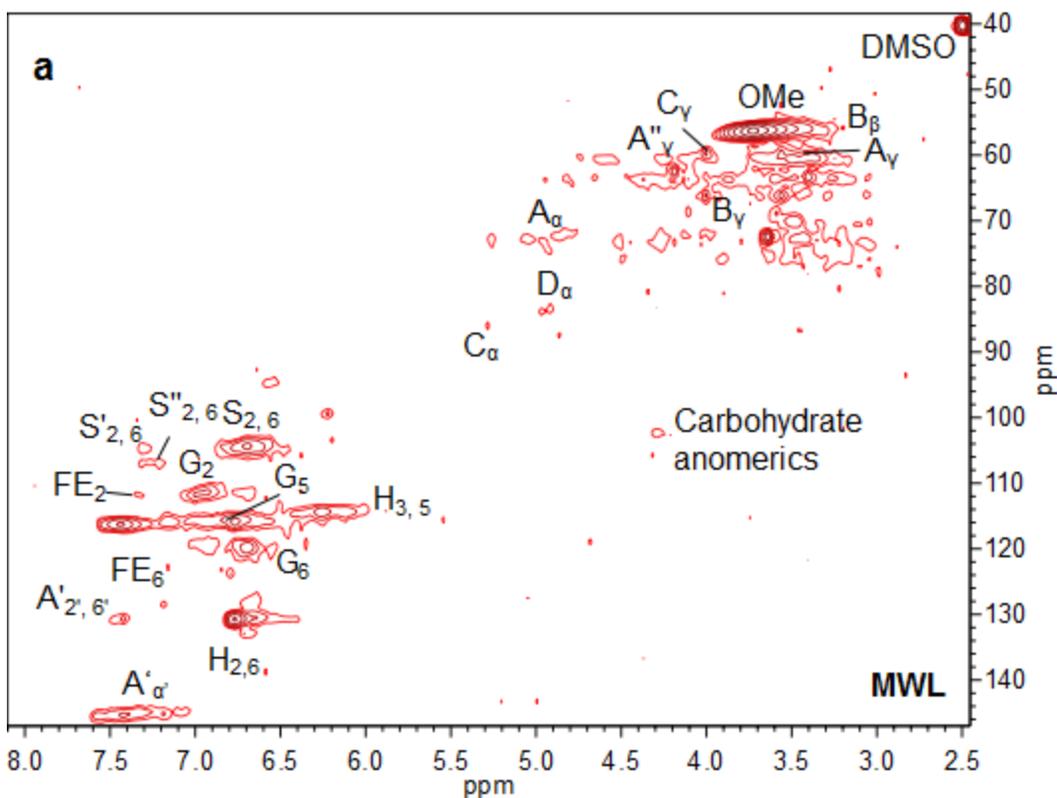
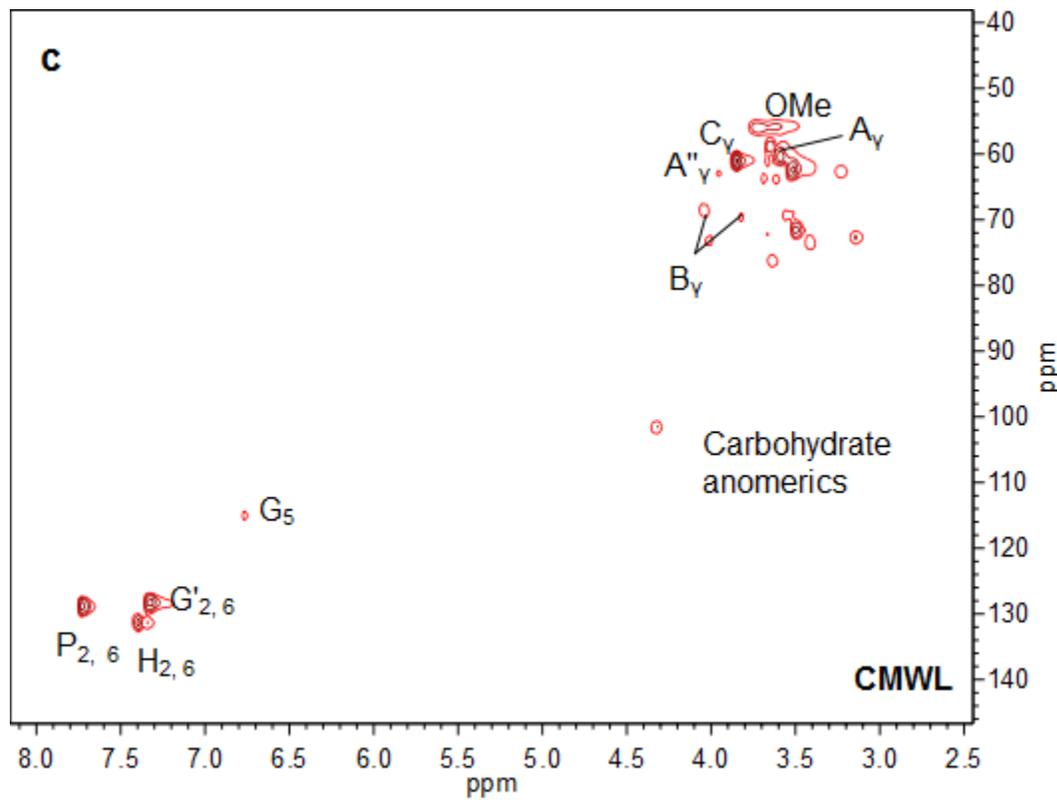
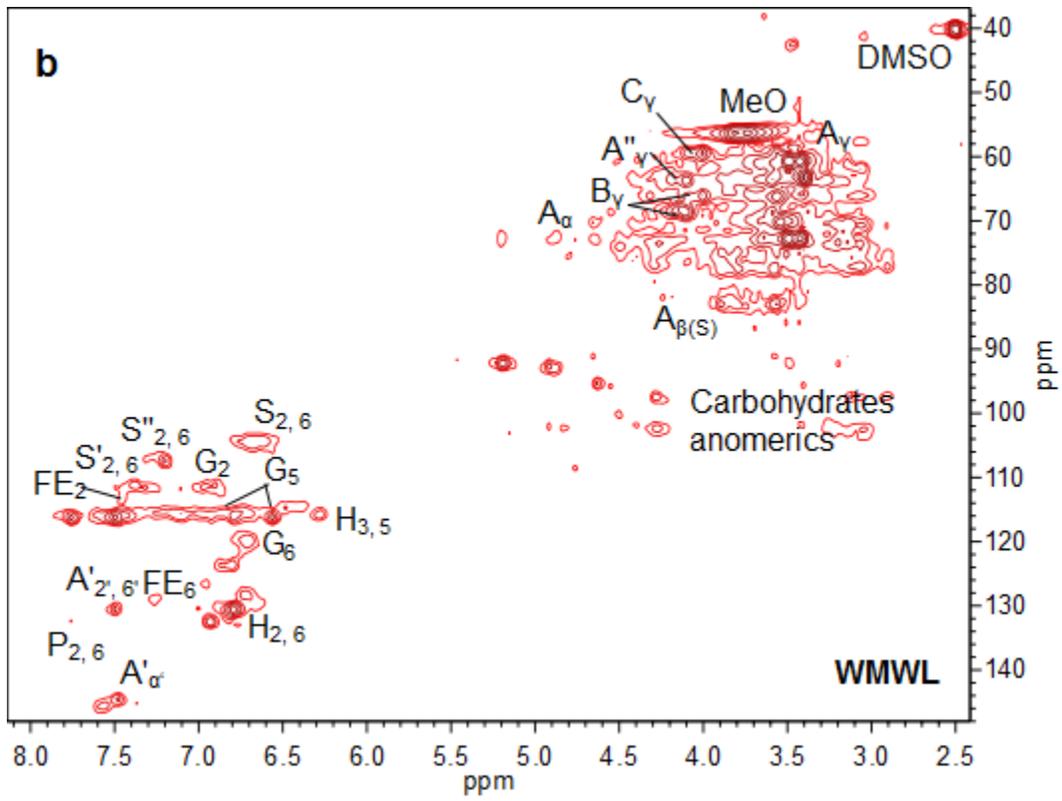


Fig. 2. Main substructures of the lignins assigned on the basis of 2D HSQC NMR: (A) β -O-4' linkages; (A') β -O-4' linkages with p-coumaroylated -OH at γ -carbon; (A'') β -O-4' linkages with a carbonyl group at C_{α} ; (B) resinol structures formed by β - β' / α -O- γ' / γ -O- α' linkages; (C) phenylcoumaran structures formed by β -5'/ α -O-4' linkages; (D) spirodienone structures formed by β -1'/ α -O- α' linkages; (G) guaiacyl unit; (G') guaiacyl unit with a carbonyl group at C_{α} ; (S) syringyl unit; (S') oxidized syringyl unit with a carbonyl group at C_{α} ; (S'') oxidized syringyl unit with a carboxyl group at C_{α} ; (H) p-hydroxyphenyl unit; (FE) esterified ferulic acid structure; (P) esterified p-acetoxybenzoic acid structure

In our previous research, a similar result was found when cornstalk was used as a raw material; a small portion of the G unit was still present in the lignin after the raw material was cooked (Yang *et al.* 2012). Based on these analyses, it was concluded that the G unit in the MWL could be partially degraded, and the G unit in the WMWL was completely changed in the cooking process. The H unit emerged in all of the four types of lignins, which could dissolve into the cooking liquors, and was a stable structure in the cooking process.

For the side-chain linkages, the β -O-4' linkages in A, A', and A'' structures were found in the MWL and WMWL, which were the main side-chain linkages in the lignin. The A structure was found in the two types of cooked lignins, and the A'' structure was only found in the CMWL, while the A' structure was not found in the two types of cooked lignins. The phenolic structures of the A, A', and A'' could be oxidized by oxygen in alkaline conditions (Johansson and Ljunggren 1994; Gierer 1997), so that the A, A', and A'' structures present in the cooked lignin were mainly the non-phenolic proportion. However, the A' structure with a benzylic alcohol group was not found in the two types of cooked lignins, which could be oxidized by the oxygen, indicating that it had been thoroughly changed in the cooking process. The A'' structure with a carbonyl group at C α was only found in the CMWL; however, no signal was found in the CWMWL, suggesting that the reactivity of the non-phenolic proportion of the MWL and WMWL was different in the cooking process. It was concluded that the non-phenolic proportion of the A structure in the two types of lignins was stable in the cooking process. However, the non-phenolic proportion of the A' structure in the MWL and WMWL, and the non-phenolic A'' structure in the WMWL, were completely changed, and the non-phenolic A'' structure in the MWL was partially changed in the cooking process.





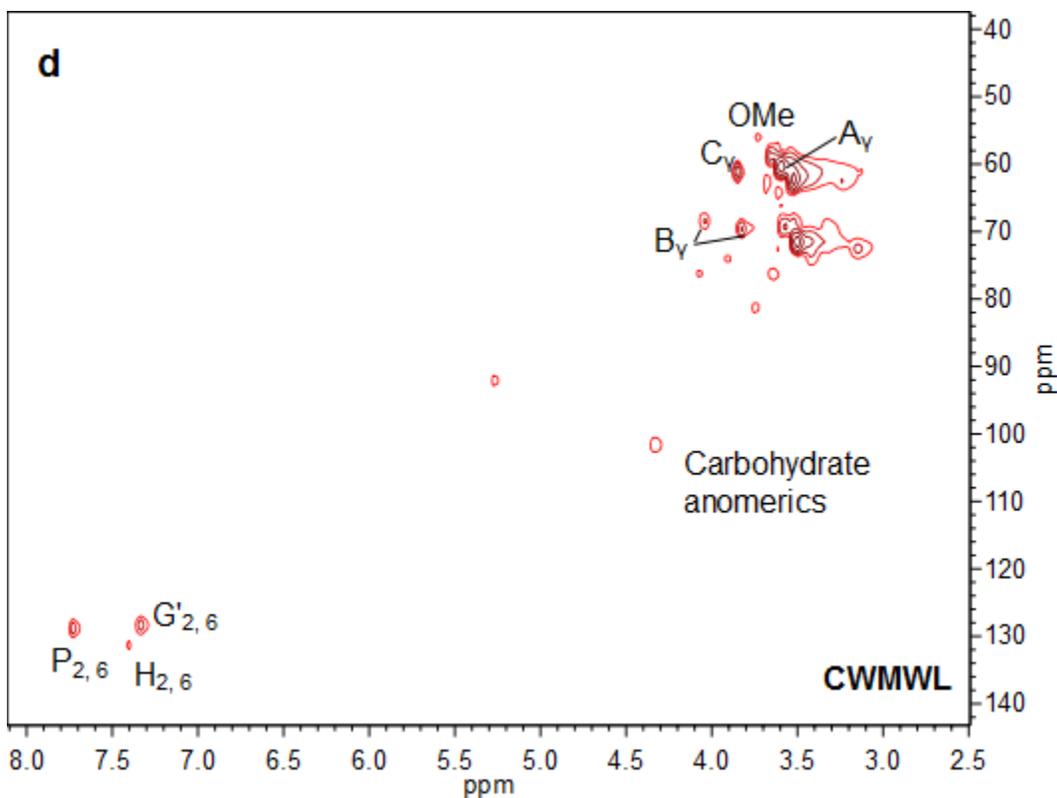


Fig. 3. 2D HSQC NMR spectra of the lignins: (a) MWL; (b) MWL; (c) CMWL; (d) CWMWL

For the B and C structures, it was found in all of the four types of lignins, considering their phenolic portion could react with oxygen, that their non-phenolic structures were stable in the cooking process. The D structure was complex and was found only in the MWL, and this reaction was probably related to the intramolecular nucleophilic attack by peroxide anions (Gierer 1986). This demonstrated that the D structure had been thoroughly degraded in the cooking process and its reaction was only related to the MWL. The FE structure was only present in the MWL and WMWL, which exhibited a classic phenolic structure, and could be completely degraded in the cooking process. The P structure was present in the WMWL, CMWL, and CWMWL only. Moreover, the P structure had stronger signal intensity in the CMWL and CWMWL than the WMWL, demonstrating that the P structure could be generated in the two cooking processes.

Molecular Weight of the Lignins with GPC Analysis

Before and after cooking, the weight-average (M_w), number-average (M_n) and Z-average (M_z) molecular weights of the four types of lignins were detected using GPC. The results are shown in Table 1.

It is important to point out that a small amount of the MgO and its reaction products could dissolve in the cooking liquor, which could have slightly decreased the molecular weights of the CMWL and CWMWL. After cooking, the molecular weights of the CMWL and CWMWL decreased. The M_w and distribution index of molecular weight M_w/M_n values of the MWL decreased more than that of the WMWL, indicating that the degradation activity of the MWL was greater in the cooking process.

Table 1. Molecular Weight of the Lignins

Samples	M_w	M_n	M_z	M_w/M_n
MWL	8569	5143	14770	1.67
WMWL	4541	3254	6124	1.39
CMWL	2106	1794	2519	1.17
CWMWL	2650	2055	3538	1.29

CONCLUSIONS

1. Cooking with solid alkali could degrade most of the substructures of the milled wood lignin (MWL) and water-soluble milled wood lignin (WMWL). In the cooking process, the p-hydroxyphenyl (H) unit was stable, while the syringyl (S) unit and its deformation structures were completely changed. The guaiacyl (G) unit could be oxidized into a G' structure, and the reactivities of which in the two types of lignin were different.
2. The reactions of the β -O-4' linkages in A, A', and A'' were also different, where the non-phenolic A structure was stable, the A' structure was completely changed, and the non-phenolic A'' structure in the MWL was partially degraded.
3. The non-phenolic resinol (B) and phenylcoumaran (C) structures were consistently stable in the cooking process, whereas the spiridienone (D) structure in the MWL and the FE structure in the MWL and WMWL were absolutely degraded. Moreover, the esterified P structure, which was only found in the WMWL, could be generated in the two cooking processes.

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