Structural Characterization of Lignin in the Process of Cooking of Cornstalk with Solid Alkali and Active Oxygen

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ABSTRACT: A novel, efficient, and environmentally friendly technology is used in cornstalk cooking, active oxygen (O_2 and H_2O_2) cooking with solid alkali (MgO). After the cooking, the milled wood lignin in the raw material and pulp and the water-soluble and insoluble lignin in the yellow liquor were all characterized by attenuated total reflectance Fourier transform infrared spectroscopy and two-dimensional heteronuclear single-quantum coherence NMR. The results showed that the cooking procedure with solid alkali and active oxygen had a high selectivity for delignification, which could remove 85.5% of the lignin from the raw material. The syringyl (S/S′/S′) units could be dissolved preferentially because of their high reactivity, and a novel guaiacyl unit with a carbonyl group (G′) was generated in the cooking process. Moreover, during the cooking, the β-O-4′ (A/A′/A″) structures as the main side-chain linkages in all the lignins could be partly broken and the β-O-4′ (A′) with a ring-conjugated structure was readily attacked by oxygen, whereas the H unit and β-5′ and β-β′ structures were found to stay stable without characteristic reaction.

KEYWORDS: *solid alkali, active oxygen, cornstalk, lignin*
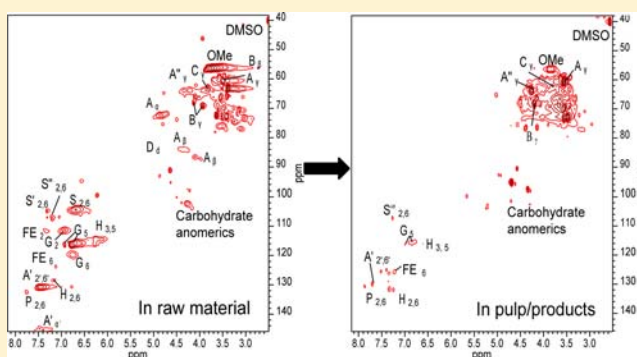
INTRODUCTION

Agricultural residues are a type of cellulose resource with rich reserves for the potential utilization in the papermaking and biomass refinery industry. Cornstalk, as one of the most widely distributed agricultural residues, has a huge production all over the word. However, because of its special structure, most of the cornstalk is used as animal feed or burned directly as fuel; only rarely are parts used as industrial raw material for producing composite products, pulp and paper, chemicals, and so on.[1,2] Therefore, how to efficiently make use of the cornstalk is of great significance.

A novel procedure of cooking with solid alkali and active oxygen has been reported by our laboratory.[3] Active oxygen is mainly composed of oxygen and hydrogen peroxide, which is widely used in the pulping and bleaching stage of the paper industry. Solid alkali, especially MgO, is a type of inexpensive industrial raw material, slightly soluble in water, which could be reused by calcinations after recycling from the cooking effluent. Reports of solid alkali when used in research for manufacturing of biodiesel or organic synthesis have been published commonly; these solid alkalis include metal oxides, zeolite, supported alkali-metal ions, clay minerals, non-oxide and alkaline ion-exchange resin, etc.[4,5] However, no published paper about active oxygen cooking with solid alkali has been found until now, except the research of our laboratory. In the research of our laboratory it was found that more than 83% of the lignin in the cornstalk was removed from the raw material

at optimum dosage of the solid alkali and active oxygen during the cooking process, which was a high delignification rate.[3] When compared with conventional kraft cooking, no malodorous gases such as sulfides were produced and the process was environmentally friendly.

In the traditional cooking process, inorganic ions such as Na^+ and S^{2+} would be introduced into the reaction system and dissolved into black liquor. In the process of oxygen delignification such as oxygen–alkali cooking and oxygen bleaching, the formation of hydroxyl radical ($HO^•$) could be catalyzed by the transition-metal ions, and the degradation of carbohydrate could be promoted by the $HO^•$, so a Mg^{2+} protective agent was added as one method to protect the carbohydrate.[6,7] In our present research, Mg^{2+} as the only ion is brought into the cooking system, not only as an alkali factor reacting with O^{2-}, but also as a protective agent for carbohydrate during the cooking process. The introduction of the MgO is different from the traditional cooking system, which may influence the delignification rate and ratio. So far, research reports about delignification in pulping and bleaching have been commonly found, such as oxygen delignification and hydrogen peroxide bleaching, the mechanisms of which have

been extensively acknowledged. However, the characteristics of the structural changes of lignin in the cooking process with solid alkali and active oxygen (O_2, H_2O_2) has not been understood yet. Whereas it is of significance to make clear reasonably and efficiently the characteristics of the lignin during the cooking process with solid alkali and active oxygen to further improve this procedure, for example, a higher yield of the pulp and utilization of the cooking yellow liquor is expected.

In our present research, the cornstalk lignin was separated into three parts: residual lignin in the pulp and water-soluble and insoluble lignin in the yellow liquor after the cooking process, where the removed lignin was mainly present in water-soluble lignin. The lignin in raw material and the separated lignins were all characterized to explore the structural changes during their reactions with solid alkali and active oxygen in the process of cooking.

■ MATERIALS AND METHODS

Materials. Cornstalk was provided by BBCA Group Co., Ltd. (Bengbu, China) and had been fragmented into pieces 10−100 mm in length. Solid alkali was magnesium oxide powder with a purity of over 98.0%, obtained from Tianjin Kermel Reagents Co. Ltd., China. Deuterated dimethyl sulfoxide (DMSO-d_6) and deuterated water (D_2O) with a purity of over 99.9% were obtained from Sigma-Aldrich (Shanghai, China). Other reagents were all of analytical grade purchased from Sinopharm Chemical Reagent Co. (Shanghai, China), with 1,4-dioxane being distilled before use for removing the impurity.

Cooking Process with Solid Alkali and Active Oxygen. Cornstalk raw material was placed into a pressure cooker with a liquid/solid ratio of 6.0 (w/w), adding 3% H_2O_2 and 15% solid alkali (all based on oven-dried (od) raw material). The cooker was sealed, fed O_2 to bring the pressure to 1 MPa, heated to 165 °C with a heating rate of 1 °C/min, and kept at that temperature for 2 h. After cooling, the resulted products in the cooker were all filtrated with an 80-mesh screen for recovering the liquor and pulp. The recovered liquor was yellow compared with the black liquor from the conventional kraft pulping process. The pulp was washed with 1 L of deionized water and filtrated with an 80-mesh screen, repeating the washing and filtrating two times.

Characteristics of the Raw Material and Pulp. The acid-insoluble Klason lignin was determined by the TAPPI method (T222 om-98) with a two-stage hydrolysis procedure (72% H_2SO_4/20 °C and 3% H_2SO_4/reflux), and lignin was left as insoluble residue recovered by filtration. The hydrolysis solution from the Klason lignin assay was determined by a spectrophotometer at 205 nm with an absorptivity of 110 L/g·cm for calculating the content of water-soluble lignin.[8] Total lignin was the sum of these two parts. The determination of holocellulose was according to Wises's sodium chlorite method,[9] and for cellulose Kurschner−Hoffer's nitric acid method was referenced,[10] where the hemicellulose content was obtained by subtracting the cellulose content from the holocellulose content. The yield of the pulp was calculated as the quality ratio of the pulp and raw material (od).

Preparation of MWL. Cornstalk raw material and pulp were separately smashed into powder by a plant miniature crusher, and the powders were extracted for 8 h in a Soxhlet extractor with benzene/ethanol (2/1, v/v) solution. After the samples absolutely dried, they were ground for 72 h in a water-cooled vibratory ball mill (VS-1, Jnie Shokai Co., Ltd., Japan) with zirconia balls.

For the preparation of the MWL, the BjÖrkman method[11] was used. The samples were extracted three times with dioxane/water (96/4, v/v) for 24 h, and the supernatant was centrifuged, concentrated, and freeze-dried for obtaining raw material milled wood lignin (RMWL, a) and pulp milled wood lignin (PMWL, b).

Isolation of Lignin in the Yellow Liquor. The yellow liquor from the cooking process was centrifuged at 5000 rpm for 10 min, and the supernatant was freeze-dried to generate water-soluble lignin

(WSL, c). The solid precipitate was sufficiently washed and centrifuged for removing water-soluble lignin, then freeze-dried, and extracted three times with dioxane/water for 24 h, and the supernatant was concentrated and freeze-dried to obtain water-insoluble lignin (WIL, d).

Characteristics of Lignins. All the lignin samples were dried sufficiently and detected with an ATR-FTIR spectrometer (Bruker Tensor 27, Germany).

DMSO-d_6 was used as the NMR solvent for RMWL, PMWL, and WIL detection, and the WSL was dissolved with D_2O because it had a weak solubility in DMSO. When DMSO-d_6 was as the solvent, chemical shifts were referenced to the residual DMSO at δ_H/δ_C 2.50/40.0 ppm. The detection of all the samples was performed with 2D HSQC NMR technology on a Bruker AV 600 MHz NMR spectrometer (Germany).

■ RESULTS AND DISCUSSION

Delignification of the Cornstalk during the Cooking Process. After cooking, the yield of the pulp was calculated as 50.2%. The specific components ash, benzene/ethanol extractive, holocellulose, and lignin in the raw material and pulp were also characterized, and the loss of these components in the cooking was calculated. The results are shown in Table 1.

Table 1. Characteristics of Cornstalk Raw Material and Pulp

		content in raw material, %	content in pulp, %	loss, %
ash		7.95	9.02	
benzene/ethanol extractive		4.22	1.09	87.0
holocellulose	cellulose	39.12	68.96	11.5
	hemicellulose	23.96	12.72	73.4
	total	63.08	81.68	35.0
lignin	acid-insoluble	17.13	5.92	82.9
	acid-soluble	4.60	0.35	96.2
	total	21.73	6.27	85.5

It was found that the ash content of the pulp was higher than that of the raw material. During the experiment, the pulp was washed efficiently with 1% sulfuric acid and deionized water to get the acidic ash content, which was only 2.2%, far below the original acidic ash content, meaning that a part of the solid alkali or its reaction products was absorbed or chelated onto the fiber surface. The lignin presented in the pulp only accounted for 6.27%, which revealed that 85.5% of the lignin in cornstalk was removed from the raw material in the process of cooking. The acid-insoluble lignin was primarily in the raw material, which accounts for 78.8% of the total lignin. However, 82.9% of the acid-insoluble lignin was removed and introduced to the yellow liquor, so the delignification of the cornstalk in the cooking process was mainly for acid-insoluble lignin. Though the content of the acid-soluble lignin was low, the rate of removal was higher than that of the acid-insoluble lignin, which was 96.2%. In addition, it was found that the loss of cellulose was lower than that of hemicellulose during the cooking process. However, the loss of the lignin was obviously higher than that of both the cellulose and hemicellulose, which indicated that the solid alkali and active oxygen cooking had a high selectivity of effect on delignification, and the Mg^{2+} could probably protect the carbohydrate components of the cellulose, especially in the cooking process.

Changes of the Molecular Structure of Lignins with ATR-FTIR Analysis. To elucidate the structure of lignins and their changes during the cooking process and investigate the

differences of the four kinds of lignins, ATR-FTIR spectra were recorded and are given in Figure 1, and the assignments are given in Table 2 in accordance with the indications of previous reports.[12−14]
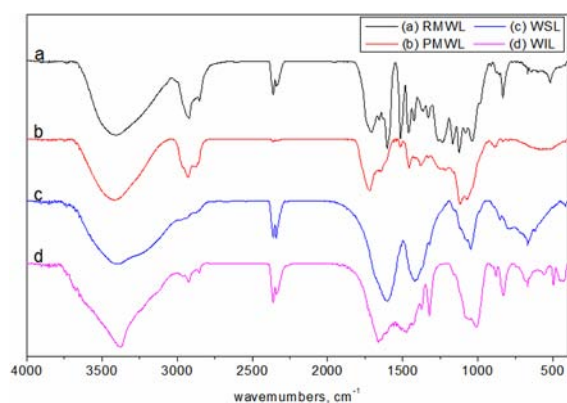

Figure 1. ATR-FTIR spectra of lignins.

As shown in ATR-FTIR spectra, the band at 2925 cm^{-1} was assigned to methyl and methylene groups; its absorbance intensity in WSL was obviously lower than those of the other three types of lignins. The band at 1710 cm^{-1} was assigned to carbonyl stretching in unconjugated ketones, which were only present in RMWL and PMWL. The band at 1650 cm^{-1} was assigned to conjugated carbonyls which can be mainly attributed to the coumaryl ester group, emerging in RMWL, PMWL, and WIL. The band at 1600 cm^{-1} in RMWL and WSL was assigned to aromatic skeletal vibrations, and that at 1510 cm^{-1} in RMWL and PMWL was assigned to aromatic skeletal vibrations coupled with C−H in-plane deformations. The band at 1460 cm^{-1} was related to C−H deformations (asymmetric in methyl, methylene, and methoxyl groups), which appeared in RMWL, PMWL, and WIL. The band at 1260 was the signal of guaiacyl ring (G), which only existed in RMWL. The strong intensities of the band at 1120 cm^{-1} were typically associated

with the syringyl (S) unit, which was present in RMWL and PMWL. The structure difference between the RMWL and PMWL is just in several absorption peaks, such as 1600, 1260, 1160, and 1030 cm^{-1}; however, the structure of WSL is obviously distinct from that of the MWL, indicating that the lignin structure change in the cooking process mainly occurred in WSL, which accounts for the vast majority of the removed lignin.

Changes of the Monolithic Structures of Lignins with 2D HSQC NMR Analysis. 2D HSQC NMR technology, which has high resolution and sensitivity, can solve the overlap problem of one-dimensional carbon and hydrogen spectra and is widely used in analysis of polymer structures such as lignin. The interpretation and utilization of 2D spectra of lignin have been described in more detail previously,[15−20] and the main substructures of the lignin assigned in NMR spectra are given in Figure 2. The 2D HSQC NMR spectra of cornstalk lignins mainly showed two regions, δ_H/δ_C 2.5−5.5/50.0−95.0 ppm corresponding to the oxygenated aliphatic region and δ_H/δ_C 5.5−8.0/95.0−150.0 ppm attributed to the aromatic region. It was found that all the lignin samples could contain certain carbohydrate structures due to close-knit bonds between lignins and carbohydrates. The results are shown in Figure 3.

The syringyl (S/S′/S″), guaiacyl (G/G′), and p-hydroxyphenyl (H) are the basic structural units of the lignin. In Figure 3a, the signal intensity of the S and G units was obviously higher compared to that of the H unit in the RMWL spectrum, and the S unit showed two other formations, an S′ unit (δ_H/δ_C 7.30/105.0 ppm) and an S″ unit (δ_H/δ_C 7.20/107.3 ppm). The S and S′ units were not found in pulp residual lignin (Figure 3b) nor in WSL (Figure 3c) and WIL (Figure 3d), and the S″ unit was only found in PMWL with a weak signal intensity. The inexistence of the syringyl (S/S′/S″) units was also confirmed by the fact that the signal intensity of the methoxyl group relative to other structures in PMWL, WSL, and WIL spectra was obviously lower than in RMWL. Therefore, the S and S′ units had been removed completely from the raw material and transformed distinctly; the S″ unit was also largely broken. In addition, the syringyl (S/S′/S″)

Table 2. Assignment of ATR-FTIR Spectra of Lignins

peak, cm^{-1}	assignment	wavenumber, cm^{-1}			
		a	b	c	d
3400	O−H stretching	3401	3420	3392	3376
2925	C−H stretch in methyl and methylene groups	2920	2928	2927	2923
2850		2851	2868		2852
1710	C=O stretch in unconjugated ketone, carbonyl, and ester groups	1711	1718		
1650	C=O stretch in conjugated para-substituted aryl ketones	1655	1647		1662
1600	aromatic skeleton vibrations plus C=O stretching (S > G, G$_{condensed}$ > G$_{etherified}$	1604		1603	
1510	aromatic skeleton vibration (G > S)	1515	1517		
1460	C−H deformations (asym in −CH$_3$ and −CH$_2$−)	1461	1457		1468
1420	aromatic skeleton vibration combined with C−H in-plane deformations	1424	1419	1423	1427
1370	aliphatic C−H stretching in CH$_3$ and phenolic OH	1365	1377		1377
1320	condensed S and G ring (G ring bound via position 5)	1325	1329	1325	1320
1260	G ring plus C + O stretching	1263			
1240	C−C + C−O + C=O stretching (G$_{condensed}$ > G$_{etherified}$)	1237	1246		
1160	typical for HGS lignins: C=O in ester groups (conjugated)	1168			
1120	typical of S unit, also secondary alcohol and C=O stretching	1124	1119		
1080	C−O deformation in secondary alcohol and aliphatic ether	1082	1074	1076	1067
1030	aromatic CH in-plane deformation (G > S) plus C−O deformation, in primary alcohols plus C−H stretching (unconjugated)	1035		1039	1021
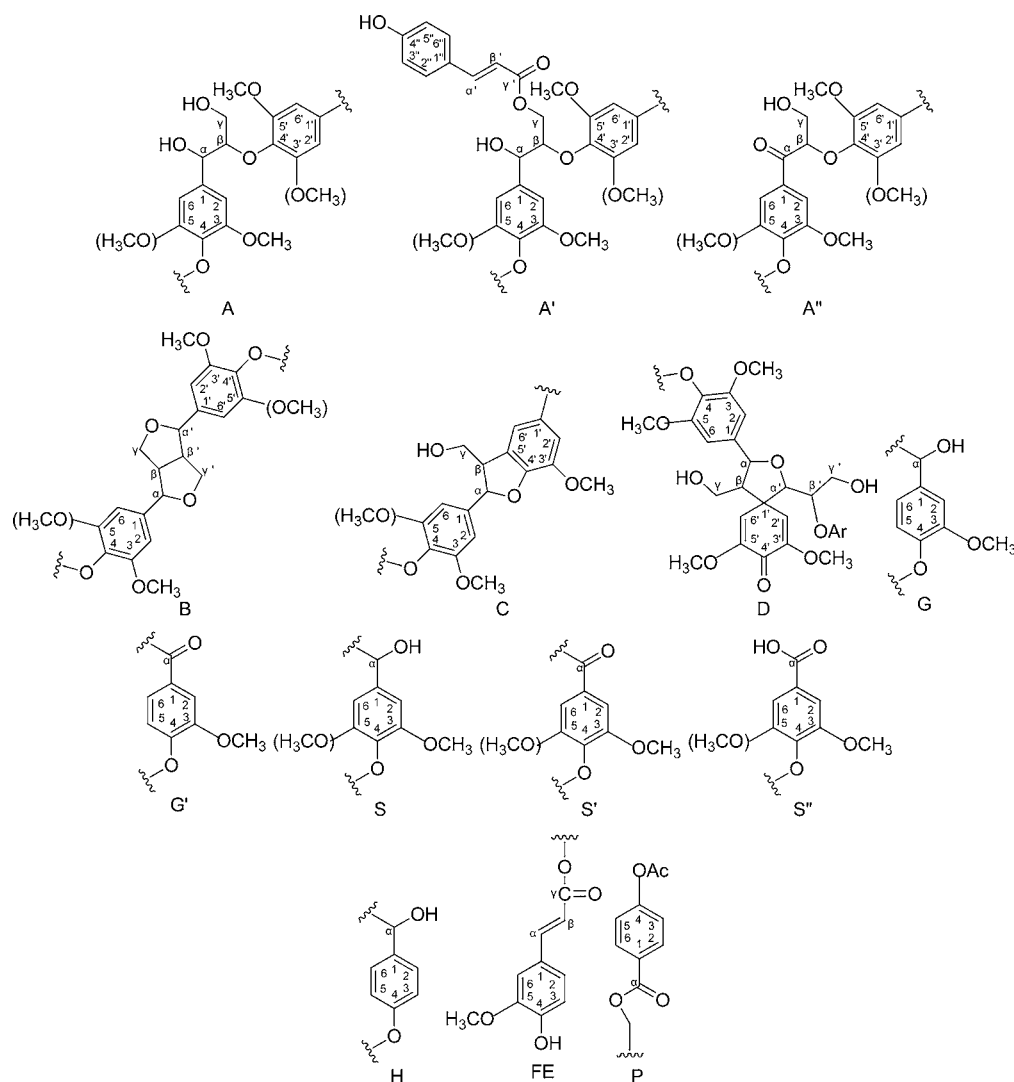
Figure 2. Main substructures of lignin assigned on the basis of 2D HSQC: (A) β-O-4 linkages; (A') β-O-4 linkages with p-coumaroylated −OH at the γ-carbon; (A″) β-O-4 linkages with a carbonyl group at C_α; (B) resinol structures formed by β-β'/α-O-γ'/γ-O-α' linkages; (C) phenylcoumaran structures formed 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.

group could be dissolved preferentially due to its lower degradation temperature, which was confirmed in kraft and NaOH-AQ pulping by Fengshou.[21] Hence, it is considered that all the syringyl (S/S'/S″) units have a high reactivity in the cooking process with solid alkali and active oxygen. The H unit was present in the four kinds of lignins, which implied that the H unit was partly removed from the raw material and did not even react in the cooking process due to its stable structure. The G unit existed in RMWL and PMWL, and the G' (δ_H/δ_C 7.25/126.6 ppm) unit with a carbonyl group at C_α was only found in WIL, not in RMWL, which suggested that the G' unit was generated in the cooking process. In alkali conditions of oxygen delignification, molecular oxygen could generate hydroxyl radicals (HO$^\bullet$), and lignin units with a benzylic alcohol group could be oxidized to a carbonyl group by the hydroxyl radical.[22,23] During the cooking process with the solid alkali and active oxygen, oxygen and hydrogen peroxide were employed, which could provide hydroxyl radicals, and the solid alkali could provide an alkaline environment for the cooking process. For these reasons, it is determined that the formation

of the G' unit is related to the oxidizing reaction of the active oxygen and G unit.

From Figure 3, it is shown that most original structures identified in RMWL are still present in pulp and yellow liquor lignins, although the relative proportions varied among them after the cooking process. The dominant side-chain linkages present in all samples are β-aryl ether (β-O-4', A/A'/A″), resinol (β-β', B) and phenylcoumaran (β-5', C) structures. The β-O-4' (A/A'/A″) structures as the main side-chain linkages turned up in the spetra of all four types of lignin samples, although the signal intensity was decreased in WSL and WIL in the yellow liquor relative to RMWL. The reason was that the oxygen could react with phenolic lignin structures in alkali conditions,[22] so the β-O-4' (A/A'/A″) structures which were partly related to the phenolic structure could be broken in the cooking process with solid alkali and active oxygen. In addition, only the A and A' structures were present in all four types of lignins, but the A″ structures were not found in WSL. The A″ (δ_H/δ_C 4.28/64.3 ppm) structure with a carbonyl group at C_α was different from the G' unit, which contained a ring-conjugated structure. It is
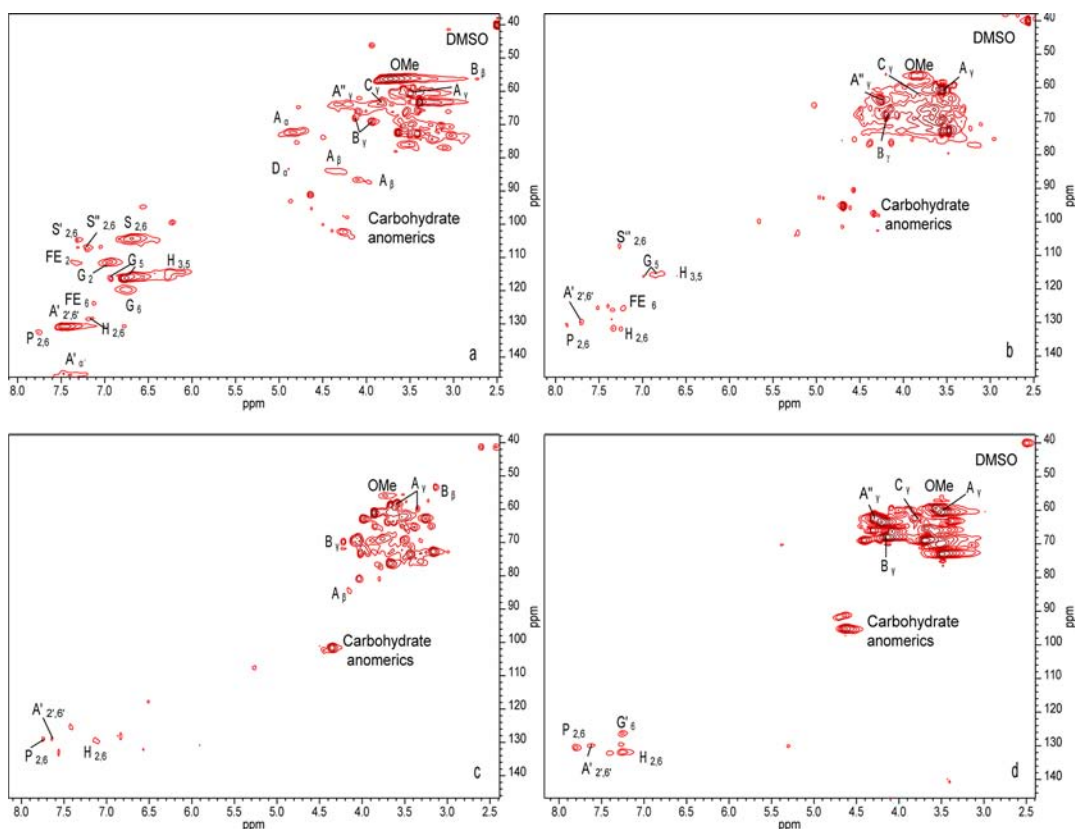
Figure 3. 2D NMR HSQC spectra of lignins.

deduced from previous reports that, in alkali conditions, the carbonyl moieties were readily attacked by oxygen, and the cleavage of the C_α–C_β bond in etherified structures containing an α-carbonyl group could generate conjugated acids. Another reaction was the oxidative attack of oxygen on phenolic moieties in lignin, which caused aromatic ring-opening and generation of muconic acids.[23,24] Therefore, the A″ structure could be removed from the raw material and was partly present in WSL, but the structure was changed completely by the attack of oxygen. The β-β' (B) structure was also found in the spectra of all four types of lignins. The signal intensity of the β-β' (B) structure in PMWL, which was also stable, was obviously increased in relation to those of the other structures. The β-5′ (C) structure was present in three types of lignins with a weak signal intensity, except in WSL, thus indicating the β-5′ (C) (phenolic) structure had been changed in alkaline pulping, while the solid alkali could not provide a strong alkaline environment in the cooking process. The pH of the yellow liquor only was 8.0 by our detection; therefore, it was indicated that the stable β-5′ (C) structure present in WIL could be removed from the raw material. In addition, the β-1′ (D) structure with a weak signal intensity was only found in RMWL, meaning that it had been thoroughly changed in the cooking, while the P structure was stable and was found in all four types of lignins.

Through the above analysis, it is found that the delignification of cornstalk by the cooking process with solid alkali and active oxygen is mainly related to oxygen delignification. The solid alkali can provide an alkaline environment for delignification, which also prevents the carbonization of cellulose in the cooking process. The presence of the hydrogen peroxide could promote the effects of delignification on the cornstalk and destroy the reaction of quinoid structures forming in the cooking process, which are the chromophoric groups in the yellow liquor from the cooking process.

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Notes
The authors declare no competing financial interest.

■ ABBREVIATIONS
MWL, milled wood lignin; DMSO-d_6, deuterated dimethyl sulfoxide; D_2O, deuterated water; ATR-FTIR, attenuated total reflectance Fourier transform infrared spectroscopy; HSQC, heteronuclear single-quantum coherence; RMWL, raw material milled wood lignin; PMWL, pulp milled wood lignin; WSL, water-soluble lignin; WIL, water-insoluble lignin

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