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Structural elucidation and antioxidant activity of lignin isolated from rice straw and alkali oxygen black liquor

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ABSTRACT

Alkali oxygen cooking of lignocellulose offers lignin many structural properties and bioactivities for biorefinery. In this work, milled wood lignin (MWL) and alkali oxygen lignin (AOL) were isolated from rice straw and alkali oxygen black liquor, respectively. The lignin structure was characterized by spectroscopy and wet chemistry. Antioxidant activity of lignins was assessed by DPPH·and ABTS scavenging ability assay. Results showed the oxidization and condensation of lignin occurred during alkali oxygen cooking. The *p*-hydroxyphenyl was more easily removed from rice straw than guaiacyl and syringyl units. The ester or ether linkages derived from hydroxycynnamic acids, and the main interunit linkages, i.e. β–O–4′ bonds, were mostly cleaved. Lignin-xylan complex had high reactivity under alkali oxygen condition. Tricin, incorporated into lignin, was detected in MWL but was absent in AOL. Nitrobenzene oxidation showed MWL can well represent the protolignin of rice straw, and the products yield decreased dramatically after alkali oxygen cooking. AOL had higher radical scavenging ability than MWL indicating alkali oxygen cooking was an effective pathway for the enhancement of antioxidant activity of lignin.

1. Introduction

Black liquor, as a bioenergy resource, is the main byproduct of pulp and paper industry. It is the residue of lignin, few polysaccharides and spent chemicals produced during chemical cooking process in digesters [1]. Caustic alkali and kraft sulphate cooking, with the advantage of alkali recovery, are the common methods for wood pulp and bleached-printing, writing papers production [2]. Alkaline cooking induces the separation of carbohydrates and lignin. Simultaneously, this process is also accompanied by the degradation of carbohydrates while the lignocellulose is subjected to direct delignification using alkaline agents [3,4]. For decreasing the carbohydrates degradation and increasing the lignin removal, oxygen is often combined with alkaline cooking, which is considered to be more selective (carbohydrates yield/delignification) than kraft cooking [5]. However, the reactivity of lignocellulose especially the lignin increases in the alkali oxygen system, leading to the formation of various degradation products.

As a major constituent of black liquor and a natural aromatic polymer, lignin is an ideal biopolymer for producing chemicals and functional materials. Lignin is the most abundant aromatic polymer in lignocellulosic biomass, and is derived from three hydroxycinnamyl alcohols (*p*-coumaryl, coniferyl and sinapyl alcohols) [6]. The amorphous lignin is constituted by *p*-hydroxypenyl (H), guaiacyl (G) and syringyl (S) moieties cross-linked via β–O–4′, α–O–4′, β–5′, β–1′, 5–5′, 4–O–5′ and β–β' linkages. These structures give lignin many physicochemical properties, which can be used to produce biofuels or value-added products such as absorbents, dispersants, lignin-based resin, adhesive, gel and so on [7,8].

The structure of lignin is believed to change dramatically after alkali oxygen cooking. These structural changes may give lignin different structural properties and bioactivities for biorefinery. Isolation of lignin from black liquor is a frequently used method for structural analysis and biological activity assay. Many separation methods are reported such as acid precipitation [9], ultrafiltration [10], supercritical fluid extraction and solvent extraction [11], electrolysis [12]. In which, acid precipitation is the most common method to recover lignin because of the simple procedure and low cost.

The structure-bioactivity relationship of lignin plays an important role in the economic viability of lignocellulosic biorefinery or materials production. In order to clarify the effects of the structure of protolignin and chemical lignin on their bioactivities, in this work, the milled wood lignin (MWL) and alkali oxygen lignin (AOL) were isolated from rice

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straw and its alkali oxygen black liquor, respectively. The lignin preparations were structural characterized by spectroscopy and wet chemistry. The effects of structural changes on antioxidant activity of lignin were evaluated via determining the radical scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH·) and 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS).

2. Materials and methods

2.1. Materials

Rice straw (*Oryza sativa*) and alkali oxygen black liquor used in this work were obtained from a pulp mill in Jiangsu, China. The alkali oxygen black liquor and air-dried rice straw were stored in a refrigerator at 4 °C before use. The amount of lignin in rice straw was 20.6%, the polysaccharides glucan, xylan and arabinan accounted for 22.4%, 20.6 and 2.7%, respectively. The benzene-ethanol extractives and ash content was 2.5% and 9.3%, respectively. The information of alkali oxygen black liquor was as follows: pH, 10.0; solid content, 0.27 g/mL; density, 1.12 g/cm^3; residual alkali, 1.25 g/L; lignin, 66.7 g/L. The 1,4-dioxane for lignin extraction was purified with NaOH. Other chemicals were all analytical or reagent grade and used as received without further purification.

2.2. Lignin isolation

Lignin in black liquor was precipitated by adjusting pH to 2 with 2 mol/L H_2SO_4, and stirring for 1 h at room temperature. The crude lignin dissolved in 90% (*w*/w) acetic acid, and the soluble fraction was slowly introduced into deionized water. The precipitate was washed with deionized water (pH = 2, adjusted with 2 mol/L H_2SO_4) until the odor of acetic acid disappeared, and then was freeze-dried to obtain AOL.

The rice straw was ground in a Wiley mill. Particles between 40 mesh (0.425 mm) and 80 mesh (0.180 mm) were collected. The meals were extracted with ethanol/benzene (1:2, *v*/v) in a Soxhlet extractor for 8 h. The extractive-free sample was used for component analysis and MWL isolation. The MWL was isolated according to the procedure described by Björkman [13]. Extractive-free materials (4 g in each bowl) were milled in a planetary ball mill (Fritsch GMBH, Pulverisette 7 premium line, Idar-Oberstein, Germany) at a frequency of 10 Hz for 2 h. Two 80 mL zirconium dioxide bowls with 25 zirconium dioxide balls (1 cm diameter) in each bowl were used in the milling. An interval of 5 min was set between every 15 min of milling to prevent overheating. After ball milling, the straw powder was carefully collected and dried under vacuum. Ball-milled samples were suspended in 96% (*v*/v) 1,4-dioxane/water with a solid/liquid ratio of 1/15 (g/mL) at room temperature for 24 h. The extraction procedure was conducted in the dark and under a nitrogen atmosphere. The mixture was centrifuged and washed with 96% 1,4-dioxane/water until the filtrate was clear. Such operations were repeated thrice. The supernatants were combined and the solvent was recycled by vacuum evaporation. The crude lignin was purified by 90% acetic acid identical to the purified procedure of AOL. No further purification was performed for the preservation of the structural features of the lignin preparations.

Table 1
Main components of lignin preparations (%).

Samples	Polysaccharides			Lignin		Ash
	Glucan	Xylan	Arabinan	Klason	Acid-soluble	
MWL	1.3 ± 0.3	1.8 ± 0.4	0.1 ± 0.0	83.6 ± 0.6	3.9 ± 0.4	0.5 ± 0.0
AOL	0.8 ± 0.2	0.2 ± 0.1	0.5 ± 0.2	76.5 ± 0.4	9.1 ± 0.2	2.3 ± 0.0

Table 2
Elemental analysis of MWL and AOL (%).

Samples	C	H	N	S	O [a]
MWL	61.7	6.4	0.9	0.8	31.2
AOL	59.8	6.0	1.0	0.9	32.3

[a] The content of oxygen (O) element was calculated by the difference.

2.3. Analytical methods

The content of lignin and sugars in rice straw, MWL and AOL was analyzed according to the method described by Gu et al. [14]. Elemental analyser (Vario EL III, Elementar, Germany) was used to measure C, H, N and S content of MWL and AOL, and the O content was calculated by difference. The ultraviolet (UV) spectra of lignins were recorded on a UV spectrometer (TU-1810, Puxi, Beijing, China).

The two-dementional heteronuclear singular quantum correlation nuclear magnetic resonance (2D HSQC NMR), Fourier transform infrared spectroscopy (FTIR) spectra of lignins were recorded on an AVANCE III 600 MHz instrument (Bruker, Switzerland) and a VERTEX 80 V FTIR spectrometer (Bruker, Germany), respectively, according to the method described by our previous work [15].

Alkaline nitrobenzene oxidation (NBO) was applied to the extractive-free rice straw (40–80 mesh), MWL and AOL according to the procedure reported by Chen [16].

The DPPH· and ABTS· radical scavenging assay of MWL and AOL was performed using a spectrophotometric method. MWL and AOL was dissolved in 1,4-dioxane/water (9/1, *v*/v). The DPPH· was dissolved in anhydrous ethanol with the concentration of 6×10^{-5} mol/L. ABTS· was generated by reacting 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (7 mM) with 2.45 mM potassium persulfate ($K_2S_2O_8$) in ultrapure water and then letting the solution stand for 15 h in the dark at room temperature. The radical solution was adjusted to obtain an UV absorbance of 0.70 ± 0.02 at 517 nm and 734 nm for DPPH· and ABTS·, respectively. The lignin solution with different volume was diluted to 100 μL using 1,4-dioxane/water and then mixed with 100 μL DPPH· or ABTS·. The absorbance of tested samples was measured using a microplate spectrophotometer (Infinite M200, Kunshan, China). All measurements were performed in duplicate. The radical scavenging ability was calculated using the formula (1):

$$\text{Scavenging ability } (\%) = \left[1-(A_i-A_j)/A_0\right] \times 100\% \quad (1)$$

where A_i is the absorbance of the tested sample; A_j is the absorbance of the blank sample via 100 μL anhydrous ethanol replacing DPPH· or 100

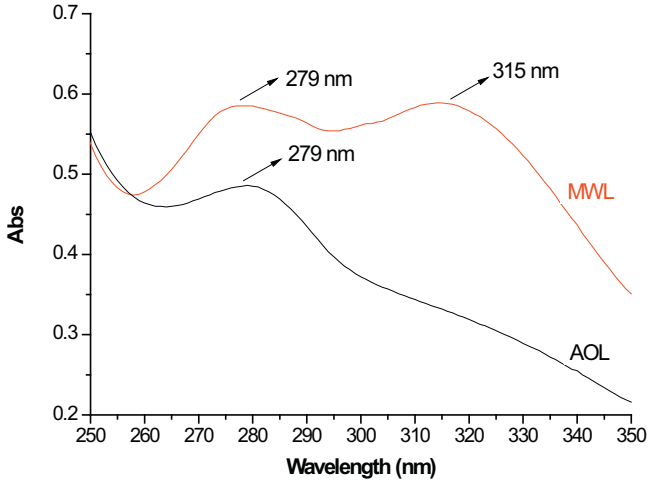

Fig. 1. UV spectra of MWL and AOL.