Fractionated Wheat Straw Lignin and Its Application as Antioxidant

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Three kinds of wheat straw black liquor were extracted by alkaline solution at 25 °C, 100 °C, and 165 °C, and were precipitated by the stepwise addition of acid to pH 10.5, 9.0, and 2.0, respectively. The corresponding three lignin fractions were isolated. The characteristics of these lignin fractions were investigated, and their impacts on anti-oxidant properties were evaluated. The pH 10.5 fractions with low lignin content, low phenolic hydroxyl content, and high lignin molecular weight showed very poor radical scavenging ability. The pH 9.0 and 2.0 fractions with high phenolic hydroxyl contents exhibited excellent radical scavenging ability. The major portion of the degraded lignin was precipitated in the pH 2.0 fraction, resulting in a lower molecular weight and higher phenolic hydroxyl content as compared to the pH 10.5 and pH 9.0 fractions. The high extraction temperature degraded more lignin and generated more phenolic hydroxyl groups. Therefore, the lignin fractions extracted at 165 °C exhibited the best radical trapping potential as compared to the lignin extracted at 100 °C and 25 °C. The coefficients of DPPH· removal for the lignin fractions were ordered in sequence by phenolic hydroxyl content, methoxyl content, molecular weight, E+T content, and NO yield of lignin. The lignin fraction extracted at higher temperature and precipitated at lower pH had the best radical scavenging ability.

Keywords: Wheat straw; Lignin; Phenolic hydroxyl group; Antioxidant; Radical scavenging

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INTRODUCTION

Lignin, as a representative polyphenol in the plant kingdom, is the second most abundant natural polymer after cellulose. Generally, lignin is treated as a waste product of the pulping industry and is often used as fuel for energy balance in the alkali recovery process (McCarthy and Islam 2000). Lignin contains several chemical functional groups, such as phenolic hydroxyl, methoxyl, carbonyl, and carboxyl groups (Gosselink *et al.* 2004b). These active functional groups could provide some new utilization for lignin. Studies on lignin model compounds have shown that the phenolic hydroxyl groups are essential for radical scavenging; they can trap radicals and become phenoxyl radicals (Barclay *et al.* 1997). Moreover, the methoxyl groups located in the ortho position to the hydroxyl groups can stabilize the phenoxyl radicals (Pan *et al.* 2006), which is also a step toward the capture of the radicals. Based on the radical-scavenging ability of phenolic hydroxyl and methoxyl groups, the influences of lignin and lignin-related phenolic polymers on oxidant-resistant properties have been studied in recent decades. The lignin and lignin-related compounds are very effective as antioxidants when used in poly-propylene (Pouteau *et al.* 2003) and cellulose composite materials (Schmidt *et al.* 1995),

as well as for medicines and dietary products (Sakagami *et al.* 1991; Toh *et al.* 2005; Bunzel and Ralph 2006).

Lignin is abundant in wood and cereal fibers. A large amount of lignin is generated in the pulping industry and it is mainly used as fuel in the alkali recovery process. In Asian countries, in particular in China and India, straw and other cereal fibers are being used as one of the most important raw materials for pulp and paper production due to the shortage of forest-based raw materials. However, the lignin of cereal fibers is different from wood lignin because of its phenylpropane unit structure and low lignin molecular weight (Dence and Lin 1992). It has been reported that up to 50% of Gramineae lignin can be readily solubilized by 1.5% aqueous soda solution at room temperature (Beckmann *et al.* 1923), 60 to 70% of the wheat straw lignin could be removed when cooking temperatures reached 100 °C, and 90% could be removed at 160 °C (Cheng 1993). In contrast, only 20% of the lignin is removed from spruce wood at 140 °C. The characteristics and structures of wheat straw lignin are different when dissolved at different temperatures, and their performances as antioxidants should be different as well.

On the other hand, the radical-scavenging activity of lignin depends not only on its phenolic hydroxyl groups, but also on other properties, such as molecular weight, polydispersity, and solubility in the aqueous phase (Dizhbite *et al.* 2004). Finding an effective and economical way to recover the degraded lignin from the pulping industry can be regarded as the first step towards the reuse of the lignin as antioxidants. The acid precipitation has been considered as a feasible approach (Dence and Lin 1992). For acid precipitated lignin, the chemical composition and molecular weight depends to a great extent on the terminal pH of the acidification process. Generally, neutralization of the phenolic hydroxyl groups in kraft lignin occurs in the pH range of about 10.5 to 11. Therefore, the lignin fraction precipitated at pH values higher than 10.5 contains few phenolic groups. Cereal fibers are characterized by their high silica content, and a major portion of the silica is dissolved in the cooking process. The dissolved silica could be precipitated as the pH is reduced from 10.5 to 9.0 (Mandavgane *et al.* 2006). Hence, pH 9.0 was selected to another acidizing point in the present study. The continuous decline of pH to 2.0 would practically precipitate all the lignin in black liquor. Therefore, to understand the antioxidant ability of degraded wheat straw lignin, the black liquor extracted at 25 °C, 100 °C, and 165 °C was fractionated to pH 10.5, pH 9.0, and pH 2.0 fractions by acid precipitation. The characteristics of these lignin fractions, including functional group content, lignin molecular weight, lignin aromatic rings, and side chain structures were investigated in this study, and their influences on the antioxidant capacity were also evaluated.

EXPERIMENTAL

Materials

The wheat straw (*Triticum aestivum* cv. Yang No.4) material was obtained from a local farm in Nanjing, China. Straws were cut to lengths of about 3 to 4 cm and washed with fresh water to remove impurities. Then, the air-dried wheat straw was stored for chemical analysis and alkaline extraction. The chemical composition of wheat straw material is shown in Table 1.

Table 1. Chemical Composition of Wheat Straw

Alcohol-benzene Extractives %	Ash content %	Holocellulose %	Pentosans %	Lignin (%)		
				Acid Soluble	Klason	Total
1.33±0.02	2.80±0.04	73.98±0.12	25.41±0.08	2.56±0.04	21.10±0.06	23.66±0.08

Alkaline Extraction of Wheat Straw

The air-dried wheat straw meals (40 to 60 mesh) were extracted by 1M NaOH with a liquor ratio of 1:8 at 25 °C for 48 h, and the collected black liquor were used for lignin fractionation. For the extracted black liquor at 100 °C and 165 °C, the wheat straw meals were firstly extracted at ambient temperature using 1M NaOH with a 1:8 liquor ratio for 30 min, and then the temperature was increased to 100 °C and 165 °C at a rate of 1.1 °C/min, respectively. After the reactor reached the set temperature, the temperature was maintained constant for another 30 min, and then the 100 °C and 165 °C black liquors were collected and used for lignin fractionation.

Lignin Fractionation

The fractionation process of these extracted lignin is shown in Fig. 1. Sequential fractionation was carried out to separate the lignin into three fractions according to their solubility at different pH.

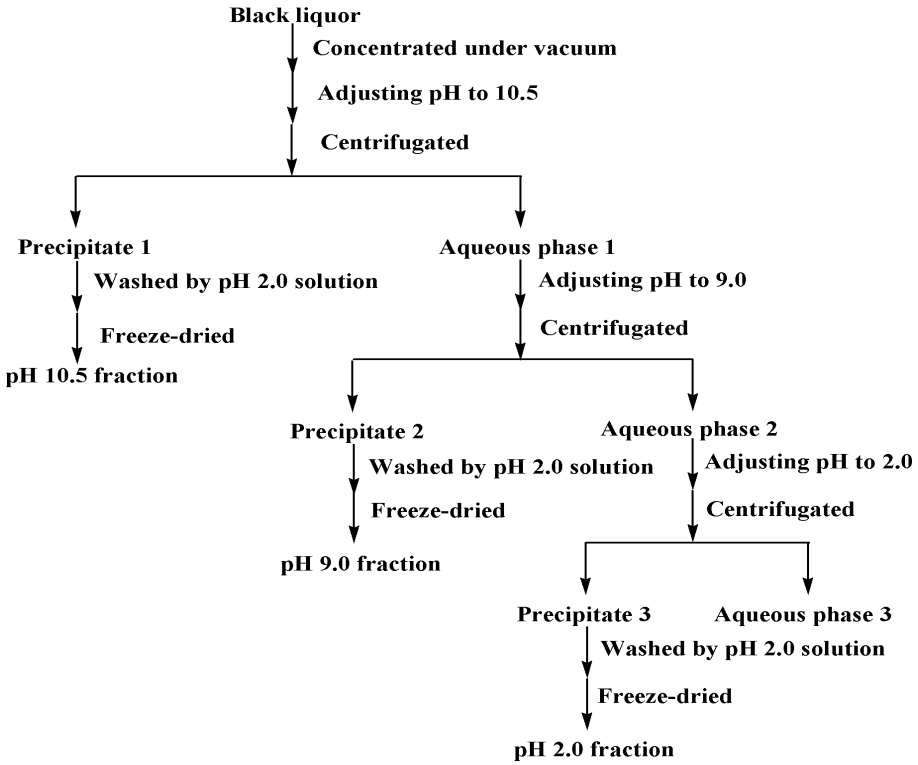

Fig. 1. Scheme of lignin fractionation from black liquor

For all three black liquor samples, the mixture was concentrated under reduced pressure firstly, and then the pH of the concentrated black liquor was adjusted to 10.5 using 0.1 M HCl. Then, the pH 10.5 mixture was transferred to a water bath at 65 °C for 30 min. The mixture was then centrifuged to separate the precipitated lignin (precipitate 1) and aqueous phase (aqueous phase 1). Precipitate 1 was repeatedly washed with 0.1 mol/L hot HCl solution to remove the residual degraded carbohydrates. The purified lignin was then freeze-dried, and the pH 10.5 fraction was obtained. Similarly, for the preparation of pH 10.5 lignin fraction, the aqueous phase 1 and 2 were adjusted to pH 9.0 and 2.0, respectively, and then were treated by the same fractionation and purification procedures as the pH 10.5 lignin fraction. Finally, the pH 9.0 and pH 2.0 lignin fractions were obtained, respectively.

Characterization of Fractionated Lignin

Klason lignin and acid-soluble lignin contents of the lignin fractions were determined according to TAPPI method T222 om-02 and TAPPI UM 250 um-83, respectively. The carbohydrate composition was measured by gas-liquid chromatography in accordance with TAPPI T 249 cm-09. Phenolic hydroxyl group content was measured using a non-aqueous potentiometric titration with tetra-n-butylammonium hydroxide (TnBAH), as described by Gosselink *et al.* (2004a). Methoxyl content of the residual lignin was determined by measuring the release of methyl iodide (CH_3I) using the Zeisel technique as described by Keppler *et al*. (2007). The molecular weight was analyzed by GPC (Gel Permeation chromatography, Waters150-C) based on the method described by Heitner *et al.* (2001). The lignin samples were also characterized by ^{13}C NMR (Nuclear Magnetic Resonance); ^{13}C NMR spectra of lignin samples were recorded on a Bruker Avance 500 MHz spectrometer from 100 mg of sample dissolved in DMSO-d6 (1.0 mL). The NMR spectrum was acquired by applying a 30° pulse width, a 1.36 s acquisition time, and a 1.89 pulse delay. In particular, 30,000 scans were used for the acquisition of ^{13}C NMR spectra of lignin samples. The lignin aromatic ring structure was detected by alkaline nitrobenzene oxidation in accordance with the technique described by Funaoka and Abe (1983). To determine the lignin side chain structure, ozonation with post-treatment was performed according to Akiyama *et al.* (2002) with modifications.

Evaluation of Antioxidant Properties

A method using DPPH· (1, 1-diphenyl-2-picrylhydrazyl) as a reactive free radical provides an opportunity to measure the radical-scavenging ability (Senba *et al.* 1999; Cotelle *et al.* 1996). A spectrophotometry method described by Brand-Williams *et al.* (1995) was utilized with a slight modification. Dioxane was used as a solvent instead of methanol in this study. In detail, a predetermined amount of fractionated lignin was added to 4 mL of 0.1g/L DPPH· dioxane. The mixture was placed in a dark environment for 30 min. Then, the sample was centrifuged, and the absorbance at 517 nm of the supernatant liquor was measured using a UV/VIS spectrophotometer immediately. The absorbance number was recorded as A_2. The fractionated lignin was placed into 4 mL of dioxane, and the absorbance number at 517 nm was recorded as A_1. Meanwhile, the absorbance number of 4 mL of 0.1g/L DPPH· dioxane solution without lignin at 517 nm was considered as the reference sample and was recorded as A_0. The ratio of DPPH· removal (R) was calculated using the following equation (Eq. (1)):

$$R = \left(1 - \frac{A_2 - A_1}{A_0}\right) * 100\ \% \quad (1)$$

RESULTS AND DISCUSSION

Chemical Composition

The chemical compositions of the acid-precipitated lignin fractions are shown in Table 2. For wheat straw, a portion of the lignin is connected with carbohydrates in the plant cell wall and forms a lignin-carbohydrate complex (LCC). In addition, 63% of lignin dissolves when wheat straw is extracted with 1 M NaOH for 48 h at ambient temperature and ~60% of the dissolved lignin exists as LCC (Liu and Lee 1991). Therefore, a large portion of carbohydrates were observed in all the lignin fractions, in particular for the pH 10.5 fractions, as shown in Table 2. The sugar contents of three pH 10.5 lignin fractions were over 70%. A similar phenomenon has been observed in a case where wheat straw was extracted with 1 M NaOH (Sun *et al.* 1996). In addition, the lignin contents of pH 9.0 and pH 2.0 fractions were much higher than that of pH 10.5 fractions at any given temperature. And interestingly, the ash contents of the three pH 9.0 fractions were 13.3%, 13.7%, and 16.3% respectively. This confirms the previous finding that portions of the dissolved inorganic materials were precipitated at pH 9.0 (Mandavgane *et al.* 2006). The amount of dissolved inorganic materials increased with ascending extraction temperature.

The extraction temperature also had different impacts on the chemical components. The lignin fractions extracted at 100 °C were distinct from those extracted at 25 °C and 165 °C. The pH 9.0 and pH 2.0 lignin fractions extracted at 100 °C had extremely higher lignin content and lower carbohydrates content, as compared to the pH 9.0 and 2.0 fractions extracted at 25 °C and 165 °C. Besides, the acid-soluble lignin contents of the lignin fractions extracted at 25 °C and 100 °C were lower than that extracted at 165 °C. This should be attributed to the extensive degradation of high-molecular weight lignin during pulping at higher temperature.

Table 2. Chemical Components of Lignin Fractions

		Ash (%)	Total Sugars (%)	Lignin, %		
				Klason	Acid Soluble	Total
25 °C	pH 10.5	2.58±0.03	74.7±0.17	20.8±0.08	1.97±0.03	22.8±0.08
	pH 9.0	13.3±0.08	16.4±0.08	67.4±0.16	1.89±0.02	69.3±0.18
	pH 2.0	0.28±0.01	21.0±0.08	76.2±0.18	2.53±0.03	78.7±0.20
100 °C	pH 10.5	4.26±0.05	71.1±0.16	22.8±0.10	1.86±0.02	24.7±0.10
	pH 9.0	13.7±0.07	1.79±0.02	82.6±0.20	1.86±0.02	84.5±0.20
	pH 2.0	0.21±0.01	13.7±0.10	83.6±0.20	2.46±0.03	86.1±0.22
165 °C	pH 10.5	5.82±0.06	70.9±0.18	20.9±0.10	2.46±0.03	23.4±0.12
	pH 9.0	16.3±0.08	10.5±0.05	70.8±0.18	2.43±0.03	73.2±0.18
	pH 2.0	0.15±0.01	18.1±0.07	77.3±0.18	4.43±0.04	81.7±0.20
Wheat Straw		2.80±0.02	73.6±0.18	21.1±0.08	2.56±0.03	23.7±0.10

Functional Groups

The phenolic hydroxyl and methoxyl groups play very important roles in radical scavenging. The low-molecular weight kraft lignin in black liquor with a high phenolic hydroxyl, exhibits a good radical-scavenging ability (Dence and Lin 1992). The phenolic hydroxyl and methoxyl group contents of the acid-precipitated lignin fractions are shown in Table 3. Generally, the β-O-4 and α-O-4 lignin structures were degraded during pulping, and more phenolic groups were generated. Therefore, it was proposed that the lignin fractions precipitated at lower pH had more phenolic hydroxyl groups. On the other hand, the methoxyl group contents increased with the decline of pH value. This was quite different from the finding as wood kraft black liquor was stepwise acid-precipitated (Dence and Lin 1992), but similar results were observed as wheat straw soda lignin was stepwise acid-precipitated (Chen *et al.* 1997).

The extraction temperature also affected the functional group contents. More lignin ether bonds were fractured as wheat straw was extracted at 165 °C, and more phenolic hydroxyl groups were generated. The phenolic hydroxyl contents of the pH 9.0 and pH 2.0 lignin fractions extracted at 165 °C were 7.68% and 9.42%, respectively. They were much higher than that obtained at 25 °C and 100 °C. In addition, the methoxyl contents of all the lignin fractions extracted at 165 °C were higher than that prepared at a lower temperature. The syringyl unit with two $–OCH_3$ is preferentially degraded at high temperatures, and this should be responsible for the higher methoxyl content of the lignin fractions extracted at 165 °C.

Table 3. Functional Group Contents of Lignin Fractions

	25 °C		100 °C		165 °C	
	Phenolic OH (%)	Methoxyl (%)	Phenolic OH (%)	Methoxyl (%)	Phenolic OH (%)	Methoxyl (%)
pH 10.5	3.46±0.06	13.7±0.12	2.23±0.04	13.5±0.11	2.40±0.04	13.6±0.13
pH 9.0	3.89±0.06	14.2±0.12	4.27±0.06	14.6±0.13	7.68±0.10	15.4±0.14
pH 2.0	4.07±0.06	15.6±0.13	8.36±0.12	16.0±0.14	9.42±0.12	16.9±0.15

* Functional groups contents were based on lignin phenylpropane units

Lignin Structure

According to the report by Leopold (1952), nitrobenzene oxidation (NO) can selectively degrade the uncondensed lignin nuclei to simple nitrobenzene oxidation products. For wheat straw material, NO gives G (vanillin plus vanillic acid) unit, S (syringaldehyde and syringic acid) unit, and H (4-hydroxybenzaldehyde plus 4-hydroxybenzoic acid) unit products. The NO yield (G+S+H) and the molar ratio of phenolic compounds (G:S:H) provide information on the minimal quantities and the relative amounts, respectively.

For the wheat straw lignin fractions in the present study, the NO yield increased with the decline of pH value, and the lignin fractions precipitated at lower pH (pH 9.0 and pH 2.0) had more uncondensed lignin units. The NO yield decreased as the extraction temperature increased at any given pH. More condensed lignin was formed at higher temperature; this should be responsible for the lower NO yield at 165 °C (Funaoka *et al.* 1990). G:S:H decreased with the reduction of pH at any given temperature in this study, and the lignin fraction precipitated at lower pH had fewer syringyl- and *p*-hydroxyphenyl units. In addition, the syringyl- and *p*-hydroxyphenyl units were easier to degrade at

higher temperature as compared to guaiacyl unit. This led to the lower G:S:H as the wheat straw was extracted at higher temperature in Table 4.

Table 4. Aromatic Ring Structure of Lignin Fractions

	25 °C		100 °C		165 °C	
	NO Yield (%,mol)	G:S:H	NO Yield (%,mol)	G:S:H	NO Yield (%,mol)	G:S:H
pH 10.5	43.6±0.20	1:1.13:0.17	40.8±0.19	1:1.11:0.10	26.7±0.16	1:1.01:0.14
pH 9.0	47.5±0.22	1:0.97:0.12	43.8±0.20	1:0.99:0.10	44.3±0.20	1:0.88:0.12
pH 2.0	59.5±0.24	1:0.88:0.12	56.2±0.24	1:0.85:0.11	49.2±0.22	1:0.85:0.10

The side chain structures of the lignin fractions are shown in Table 5. Ozonation is widely used to analyze the stereo structures of arylglycerol-β-aryl ether linkages and was selected to measure the lignin side chain structures in present study. The two products generated by ozonation were erythronic and threonic acids. The ratio of erythronic to threonic acids (E/T) gives the erythro/threo ratio of the β-O-4 structure, and the total yield of erythronic and threonic acids (E+T) gives information about the contents of this structure. For the lignin fractions extracted at any given temperature in present study, the (E+T) increased with the decline of pH, and the pH 2.0 fraction extracted at 25 °C possessed the highest β-O-4 lignin content. The extraction temperature also affected the (E+T). The (E+T) of pH 9.0 and 2.0 fractions dropped sharply when the extraction temperature increased from 25 °C to 165 °C, in particular for the pH 2.0 fraction.

Besides, the E/T of the lignin fraction obtained at different pH and temperature was quite different. The lignin fractions precipitated at a higher pH had a lower E/T, and the lignin fractions extracted at a higher temperature showed a lower E/T. This interesting phenomenon indicated that more etherified erythro β-O-4 structures were degraded than threo β-O-4 structures at higher temperatures (Miksche 1972).

Table 5. Side Chain Structure of Lignin Fractions

	25 °C		100 °C		165 °C	
	E+T (%, mol)	E/T (molar ratio)	E+T (%, mol)	E/T (molar ratio)	E+T (%, mol)	E/T (molar ratio)
pH 10.5	5.2±0.08	0.54±0.01	5.2±0.08	0.52±0.01	5.2±0.08	0.48±0.01
pH 9.0	11.4±0.14	0.75±0.02	8.5±0.10	0.71±0.02	7.2±0.10	0.62±0.02
pH 2.0	19.2±0.20	0.86±0.02	11.9±0.14	0.83±0.02	8.5±0.11	0.77±0.02

Lignin Molecular Weight

The acid precipitation of lignin is based on the solubility of lignin at different pH. Generally, lignin with high molecular weight is easily condensed and precipitated at high pH, and the lignin with low molecular weight can only be precipitated at very low pH. The lignin molecular weights of the fractionated lignins are shown in Table 6. The molecular weights of the three pH 10.5 fractions were higher than that of pH 9.0 and 2.0 fractions, and the pH 2.0 lignin fractions had the lowest molecular weight. In addition, the extraction temperature also generated different impacts on the molecular weight of lignin fractions. At a given pH, the molecular weight of the lignin fraction extracted at 25

°C was higher than that of 100 °C. The wheat straw lignin could be dissolved easily by 1 M NaOH at ambient temperature, and the dominant material of the dissolved lignin was LCC (Liu and Lee 1991). LCC was a very complex compound with high molecular weight; thus, it should be responsible for the higher molecular weight of the lignin fractions extracted at 25 °C. As wheat straw was extracted at 100 °C, parts of the soluble lignin were dissolved first, and then another portion of lignin was degraded and dissolved, leading to the lower molecular weight of the lignin fractions extracted at 100 °C. Furthermore, with an increase in temperature to 165 °C, parts of the residual lignin could be re-dissolved, rather than degraded from the pore cannels, which were opened at 100 °C (Jiang *et al.* 1989). This mechanism could explain the higher molecular weight of the lignin fractions obtained at 165 °C as compared to that at 25 °C and 100 °C.

Table 6. Lignin Molecular Weight of Fractionated Lignin Fractions

	25 °C			100 °C			165 °C		
	M_w	M_n	M_w/M_n	M_w	M_n	M_w/M_n	M_w	M_n	M_w/M_n
pH 10.5	10400	3000	3.47	9200	2900	3.17	11500	3200	3.59
pH 9.0	7900	2600	3.04	7200	2500	2.88	8400	2700	3.30
pH 2.0	4100	1500	2.73	3600	1400	2.64	4300	1500	2.87

^{13}C NMR Spectra

As described in Table 2, the pH 10.5 lignin fractions were rich in carbohydrates, and the ash contents of pH 9.0 fractions were very high. Therefore, the pH 2.0 fractions with high lignin content extracted at 25 °C, 100 °C and 165 °C were analyzed by ^{13}C NMR spectroscopy, as shown in Fig. 2, and the signals were assigned according to the literature reported by Sun *et al.* (2011), as shown in Table 7.

For wheat straw material, its striking characteristic is the presence of *p*-coumarate ester (C-γ, ~166.4 ppm; C-β, ~115.3 ppm) and etherified ferulates (C-γ, ~163.1 ppm) (Sun *et al.* 1996). Diferulate and *p*-coumarate ester in β-O-4 were also identified by two signals at 164.5 and 161.5 ppm, respectively, assigned to C-γ and C-4 (Bunzel *et al.* 2003). The relatively weak signals of *p*-hydroxyphenyl units, in particular for the lignin fraction extracted at 100 °C and 165 °C, indicated the degradation of *p*-hydroxyphenyl unit during alkaline extraction and acid precipitation.

The β-O-4 linked units of these three pH lignin fractions were located at 61.0 to 63.0 ppm (Fig. 2), assigned to C-γ in β-O-4 with Cα=O and CH2-γ in β--O-4. The intensities of signals for the three pH 2.0 lignin fractions at 60.2 were very strong, and in contrast to the signal intensity of lignin fractions extracted at 165 °C and 100 °C at 62.7 ppm, the signal intensity of lignin extracted at 25 °C was much higher. These phenomena proved the relative higher β-O-4 lignin content of pH 2.0 fraction extracted at 25 °C. This was consistent with the results obtained by NO in Table 4.

The characteristic tertiary carbon resonances from S units at 103 to 110 ppm (C-2, 6), G units at 110-125 ppm (C-2, 5, 6), and H units at 129-131 ppm (C-2, 6) could be used to assess the G:S:H of these three pH 2.0 lignin fractions. The signal intensities of these three pH 2.0 lignin fractions at 119 ppm (G) and 104 ppm (S) mean and the peak values were equivalent, and signals with weak intensity but equal peak value were also observed at 130.3 ppm (H). These interesting findings also verified the results of G:S:H for the three pH 2.0 lignin fractions determined by NO in Table 4.

In addition, the signal at 87.2 ppm was assigned to erythro β-O-4 lignin and the signal at 84.6 ppm was assigned to threo β-O-4 lignin. For the pH 2.0 lignin fraction extracted at 165 °C, the signal at 87.2 ppm could not be detected, and the signal at 84.6 ppm was weak. In turn, for the pH 2.0 lignin fraction extracted at 25 °C, the signal at 87.2 ppm was weak and the signal at 84.6 ppm could not been detected. This indicated that more erythro β-O-4 existed in the lignin extracted at lower temperature, and more etherified erythro β-O-4 structures could be degraded than threo β-O-4 structure as they were extracted at higher temperature (Miksche 1972).

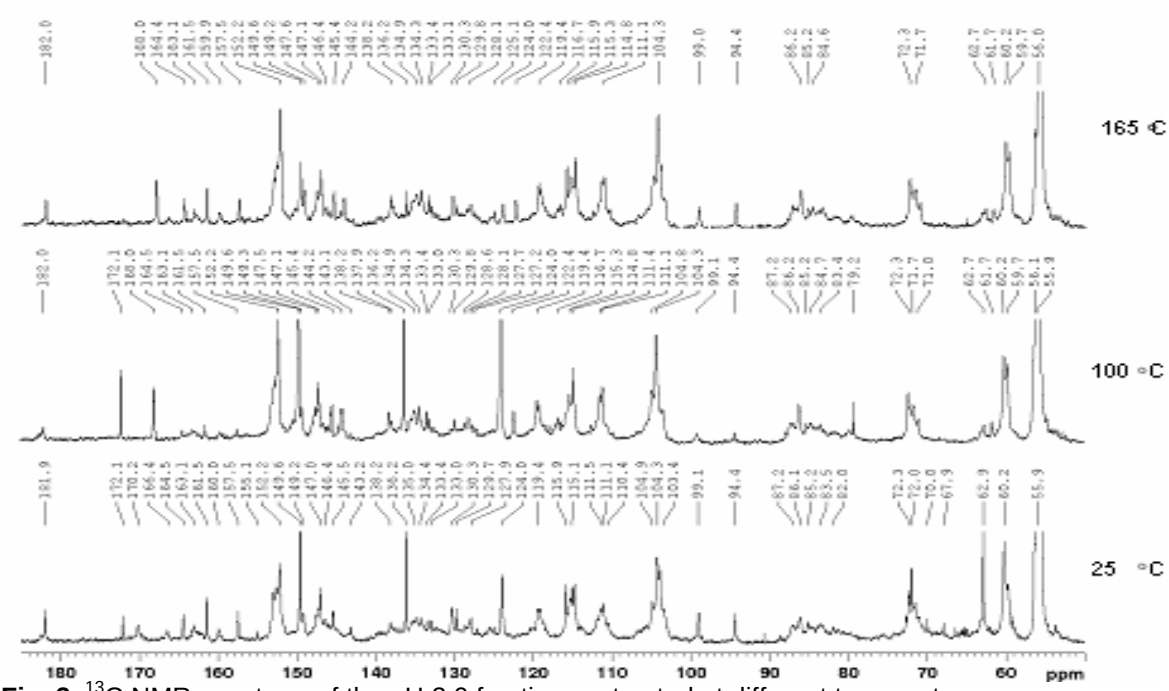

Fig. 2. ^{13}C NMR spectrum of the pH 2.0 fractions extracted at different temperature

Antioxidant Properties

The antioxidant properties of these fractionated lignins were evaluated by DPPH· scavenging ability. To evaluate their radical-scavenging capacity, butylated hydroxy-toluene (BHT), an antioxidant additive widely used in food, cosmetics, pharmaceuticals, petroleum products, and electrical transformer oil, was selected as the reference sample. The radical-scavenging ability of these lignin fractions are shown in Fig. 3. Apparently, in contrast to the lignins precipitated at pH 9.0 and pH 2.0, the radical scavenging abilities of pH 10.5 lignin fractions were very poor. The radical scavenging ability of pH 10.5 lignin fractions were much lower than that of BHT. The pH 2.0 fractions exhibited better DPPH· than pH 9.0 fractions, and much higher than BHT. This should be attributed to its higher phenolic hydroxyl content and lower molecular weight. On the other hand, for lignin fractions precipitated at any given pH, ascending extraction temperature would increase the DPPH· scavenging ability, in particular for the lignin fractions extracted at 165 °C . ～80% DPPH· was removed as 5 mg lignin/mg DPPH· of pH 9.0 or pH 2.0 lignin fraction extracted at 165 °C was applied. Similar results could be obtained when 25 mg lignin/ mg DPPH· of pH 9.0 or pH 2.0 lignin fraction extracted at 100 ^{o}C was utilized.

Table 7. Carbon Chemical Shifts (δ, ppm) in ^{13}C NMR of pH 2.0 Lignin Fractions Extracted at Different Temperature

Δc-ppm (Intensity)*			Assignments
165 °C	100 °C	25 °C	
181.9 (w)	182.0 (w)	182.0 (w)	Carboxyl group
172.1 (w)	172.1 (s)	172.1 (w)	Acetyl group in aliphatic chain
166.4 (w)	-	-	C-γ in *p*-coumarate ester, in γ-ester
164.5 (w)	164.5 (w)	164.4 (w)	C-γ in β-O-4 diferulate
163.1 (m)	163.2 (w)	163.1 (w)	C-γ in etherified ferulic acid
161.5 (m)	161.5 (w)	161.5 (w)	C-4 in *p*-coumarate ester, in β-O-4
160.0 (w)	-	159.9 (w)	C-4 in *p*-coumarate ester
157.5 (m)	157.5 (w)	157.5 (m)	C-4 in *p*-hydroxyphenyl units
152.2 (s)	152.6 (s)	152.2 (s)	C-3/C-5 in etherified syringyl unit
149.6 (s)	149.6 (s)	149.6 (m)	C-3 in etherified guaiacyl unit
147.5 (m)	147.5 (m)	147.6 (w)	C-3/C-5 in non-etherified guaiacyl unit
147.0 (m)	147.1 (m)	147.1 (m)	C-4 in etherified guaiacyl unit, in β-5
145.4 (w)	145.4 (w)	145.4 (w)	C-4 in non-etherified syringyl unit
138.2 (w)	138.2 (w)	138.1 (w)	C-4 in etherified syringyl unit
134.9 (s)	134.9 (s)	135.0 (w)	C-1 in etherified guaiacyl unit
134.3 (s)	134.3 (s)	134.4 (s)	C-1 in etherified syringyl unit
133.4 (w)	133.4 (w)	133.4 (w)	C-1 in non-etherified syringyl unit
130.3 (w)	130.3 (w)	130.3 (w)	CH-2/CH-6 in *p*-coumarate ester
128.1 (w)	128.1 (w)	127.9 (w)	CH-2/CH-6 in *p*-hydroxyphenyl units
125.1 (w)	125.1 (w)	125.1 (w)	C-1 in *p*-coumarate ester
119.4 (m)	119.4 (m)	119.4 (m)	CH-6 in guaiacyl units
115.9 (m)	115.9 (m)	115.9 (m)	CH-3/CH-5 in *p*-hydroxyphenyl units
115.3 (m)	116.7 (m)	115.1 (m)	C–β in *p*-coumarate ester
114.8 (w)	114.8 (w)	114.8 (w)	CH-5 in guaiacyl unit, in β-1units
111.1 (m)	111.1 (m)	111.1 (m)	CH-2 in guaiacyl unit
104.3 (s)	104.3 (s)	104.3 (s)	CH-2/CH-6 in syringyl units
99.0 (w)	99.1(w)	99.1(w)	CH-1 in MeGlcA
94.4 (w)	94.4 (w)	94.4 (w)	CH-1, in xylose, α-anomer
-	87.2 (w)	87.2 (w)	CH-β, in syringyl β-O-4 (erythro)
86.2 (w)	86.1 (w)	86.1 (w)	CH-α, in syringyl units
85.1 (w)	85.2 (w)	85.2 (w)	CH-α, in β-β'
84.6 (w)	84.7 (w)	-	CH-β, in guaiacyl l β-O-4 (threo)
-	-	82.6 (w)	CH-β, in β-O-4
-	79.2 (w)	-	CH-3 in arabinofuranose
72.3 (w)	72.3 (w)	72.3 (w)	CH-α, in β-O-4 (erythro) guaicyl
71.7 (w)	71.7 (w)	-	CH-γ, in β-β' units, and CH-α in β-O-4 (threo) guaicyl
-	71.0 (w)	-	CH-γ in *p*-hydroxyphenyl units
-	-	70.0 (w)	CH-4 in xylose non reducing end unit
62.7 (w)	62.7 (w)	62.7 (m)	CH_2-γ, in β-O-4 with Cα=O or β-1
60.2 (s)	60.2 (s)	60.2 (s)	CH2-γ, in β--O-4
56.0 (s)	55.9 (s)	55.9 (s)	OCH_3 in guaiacyl and syringyl units

***Intensity abbreviations: s-strong; m-mean; w-weak.

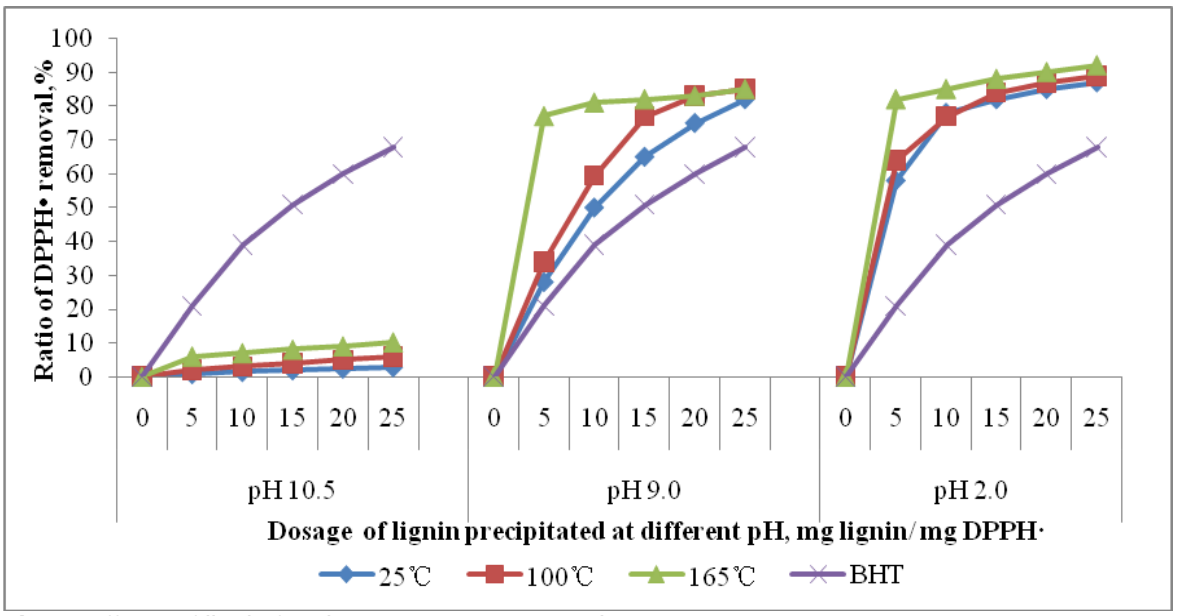

Fig. 3. Effects of lignin fractions on DPPH· removal

The lignin extracted at higher temperature and precipitated at lower pH showed the best DPPH· scavenging ability, and there is no doubt that higher phenolic hydroxyl content, higher methoxyl content, and lower lignin molecular weight had positive effects on radical scavenging. However, besides the factors mentioned above, the lignin structures, including aromatic ring and side chain structure, probably affect the radical scavenging ability as well. In addition, the coefficient sequence of these factors on radical scavenging ability have not been determined yet. Therefore, the effects and coefficients of these factors on DPPH· scavenging activity were analyzed by partial least squares (PLS) using Simca-P, as shown in Fig. 4. PLS is a method for constructing predictive models when the factors are many and highly collinear. Note that the emphasis is on predicting the responses and not necessarily on trying to understand the underlying relationship between the variables. The general idea of PLS is to try to extract the latent factors, accounting for as much of the manifest factor variation as possible while modeling the responses well. PLS now has became a standard tool for processing a wide spectrum of chemical data problems (Geladi and Kowalski 1986).

The coefficients of the various factors affecting DPPH· removal are shown in Fig. 4. Among these five factors, the phenolic hydroxyl content of the lignin played the dominant role on DPPH· scavenging, and its coefficient was 0.255. The methoxyl content of lignin was the second important one affecting radical scavenging. Its coefficient was 0.231, a slightly lower value than that of phenolic hydroxyl content. This should be attributed to its ability to stabilize the phenoxyl radicals (Pan *et al.* 2006; Barclay *et al.* 1997). The molecular weight of lignin was another important variable affecting its antioxidant property. The coefficient of lignin molecular weight on radical scavenging was -0.140. This reveals that the lower molecular weight of lignin is a positive factor for the catching of radicals. Generally, the better radical-scavenging ability of lignin with lower molecular weight is proposed to be close related to its high solubility and dispersity in solvent. The effect of lignin aromatic ring structure on DPPH· scavenging ability was quite low, with a coefficient of 0.042. The content of β-O-4 lignin exhibited a higher

coefficient than lignin aromatic ring structure, represented as 0.105. However, it should be pointed that the (E+T) showed a very wide amplitude variance. This means that high or low values of the (E+T) might show quite a distinct impact on radical scavenging ability of lignin.

In sum, the coefficients of DPPH· removal for the lignin fractions were ordered in sequence by phenolic hydroxyl content, methoxyl content, molecular weight, E+T content, and NO yield of lignin. The lignin extracted at higher temperature generated more phenolic hydroxyl groups, and the lignin with lower molecular weight only can be precipitated at very low pH value. This can explain the excellent radical scavenging ability of the lignin fraction extracted at higher temperature and precipitated at lower pH.

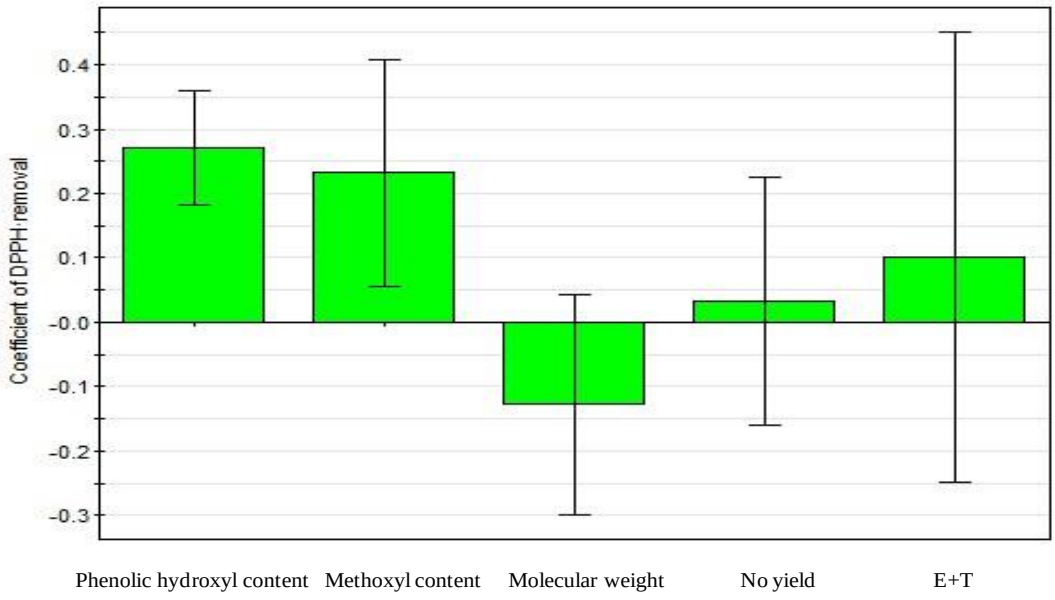

Fig. 4. The coefficient of variables affecting DPPH· scavenging

CONCLUSIONS

1. Wheat straw lignin was extracted with an alkaline solution at 25 ^{o}C , 100 ^{o}C, and 165 ^{o}C. The fractionated lignin was obtained by acid precipitation at pH 10.5, 9.0, and 2.0. The lignin contents of the pH 10.5 fractions were low, and their antioxidant properties were very poor. The pH 9.0 fractions were rich in ash, but the lignin content was much higher than that of the pH 10.5 fractions. The pH 2.0 fractions, with the highest contents of lignin and phenolic hydroxyl groups, had the best radical scavenging ability.
2. The high phenolic hydroxyl group content and low molecular weight of the pH 2.0 fraction could be responsible for its strong radical scavenging capacity. In contrast to the lignin fraction extracted at 25 ^{o}C, the pH 2.0 fractions extracted at 100 ^{o}C and 165 ^{o}C had higher phenolic hydroxyl group contents and lower molecular weights, resulting in excellent radical scavenging ability.

3. The coefficients of DPPH· removal for the lignin fractions were ordered in sequence by phenolic hydroxyl content, methoxyl content, molecular weight, E+T content, and NO yield of lignin. These factors should be responsible for the excellent radical scavenging ability of the lignin extracted at higher temperature and precipitated at lower pH.

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