H$_3$PW$_{12}$O$_{40}$/ZrO$_2$ and 1-Butyl-3-methylimidazolium Chloride: A Double-Effect Catalyst for the Degradation of Alkali Lignin

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ABSTRACT: Alkali lignin is a component of the waste black liquor produced by the paper-making industry that is difficult to degrade. In recent years, the biological activities of lignin, such as free-radical scavenging and antioxidant capacity, have received increasing attention. Here, we prepared H$_3$PW$_{12}$O$_{40}$/ZrO$_2$ and used this catalyst together with the ionic liquid 1-butyl-3-methylimidazolium chloride ([BMIM]Cl) as a double-effect catalyst for the degradation of alkali lignin. Single-factor and orthogonal tests showed that the best degradation conditions were as follows: reaction time, 2 h; reaction temperature, 100 °C; mass ratio of H$_3$PW$_{12}$O$_{40}$/ZrO$_2$ to lignin, 1:4; and substrate concentration, 2.5%. The phenolic hydroxyl group content of the lignin degradation product increased by 231.2% and the total hydroxyl group content increased by 337.1% when the double-effect catalyst was used rather than [BMIM]Cl alone. Analysis by gel permeation chromatography showed that both the weight-average molecular weight and the number-average molecular weight of the product were reduced and that the lignin was degraded into small-molecular-weight compounds by the macromolecule. The product after the catalytic degradation of lignin showed a markedly increased antioxidant capacity, which was similar to that of the commercial antioxidant, 2,6-diter-butyl-4-methylphenol. The study opens up a new direction for the better utilization of lignin.

INTRODUCTION

Heteropoly acids, which consist of water, organic molecules, and heteropoly anions and cations (protons, metal cations, organic cations), can be classified into several structural types: Keggin type (ratio of the central metal atom to heteroatoms is 1:12), Dawson type (ratio of central metal atoms to heteroatoms is 2:18), Waugh type (ratio of the central metal atom to heteroatoms is 1:9), Silver type, and Anderson type. The primary structure of a heteropoly acid refers to the structure of the heteropoly anion, for example [XM$_{12}$O$_{40}$]$^{n-}$ (X = P, Si, Ge, etc.; M = W, Mo, V, etc.), and describes the constituent elements of the heteropoly anion, their number, and the structure of the skeleton. The crystal structure of a combination of polyanions and counterions defines the secondary structure of a heteropoly acid, in which multiple secondary structural units form a heteropoly compound unit, which determines the acidity, redox properties, and polar properties of the heteropoly acid in solution.1,2 Solubility, catalytic activity, and selectivity in the medium are affected by the point charge, electronegativity, and radius. The tertiary structure of heteropoly acids refers to the aggregation of polyanions, counterions, and crystal water in three-dimensional space. H$_3$PW$_{12}$O$_{40}$·5H$_2$O is used as an example to introduce the concept of first-, second-, and third-order structures of heteropoly acids (Figure 1).

A Keggin-type space accumulation structure was first described for Na$_5$[PW$_{12}$O$_{40}$]·5H$_2$O (Figure 2), and Keggin-type structures remain the most frequently studied of the heteropoly acid structural types described above.

Because heteropoly acids contain both complexes and metal oxides, they have both acidic and redox properties and can, therefore, be used as acidic catalysts, redox catalysts, and dual-function catalysts.

Ionic liquids, such as 1-butyl-3-methylimidazolium chloride ([BMIM]Cl), which are organic salts that are liquids at room temperature or close to room temperature, have long been of great scientific and technological interest because of their unique properties. Ionic liquids are green solvents and have recently become increasingly popular for catalytic reactions.3

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In contrast to other organic solvents, there are no neutral molecules in ionic liquids, which are composed of anions and cations with almost the same radius. Ionic liquids thus have better thermal stability and electrical conductivity than conventional solvents and have a number of advantages for carrying out chemical reactions. These include faster reaction rates, high selectivity and conversion rates of reactions, and recyclability of the catalytic systems.

Alkali lignin is one of the large amounts of byproducts of the pulp and paper industry. If not handled properly, it will not only cause a wastage of resources but also pollute the environment. To expand the application of alkali lignin and increase its added value, the common method is catalytic degradation. In the present study, [BMIM]Cl was used alone to catalyze the degradation of lignin and, additionally, \([\text{BMIM}]\text{Cl} + \text{H}_3\text{PW}_{12}\text{O}_{40}/\text{ZrO}_2\) was used to optimize the experimental conditions for the degradation of lignin. The effects of time, temperature, and lignin concentration on the degradation reaction catalyzed by the ionic liquid alone were analyzed. The effects of reaction time, temperature, and loading of H₃PW₁₂O₄₀/ZrO₂ on the degradation reaction were then investigated during the degradation of lignin using the double-effect catalyst. The chemical structure of the lignin degradation products and the bond-breaking method were investigated by Fourier transform infrared (FT-IR) and ¹³C-NMR spectroscopy. The free-radical-scavenging activity and reducing power of the lignin degradation products were analyzed using diphenyl-2-trinitrophenylhydrazone (DPPH) and hydroxyl free radicals. The degradation product produced using the double-effect catalyst was found to have better free-radical scavenging and antioxidant activities.

**RESULTS AND DISCUSSION**

**Optimization of Reaction Conditions for the Catalytic Degradation of Alkali Lignin by Ionic Liquids.** The molar mass concentrations of degradation products at different temperatures and times are shown in Figure 3. The total amount of hydroxyl groups reached a maximum of 10.92 mmol/g at 3 h and 140 °C (Figure 3a). This is mainly because when the reaction time is short and the temperature is low, the \(\beta-O-4\) bonds in lignin are broken down and hydroxyl groups are formed. When the temperature rises and the reaction time increases, some of the newly generated hydroxyl groups decompose or polymerize with other compounds under the continuous high-temperature conditions, resulting in a significant decrease in the molar concentration of hydroxyl groups. The amount of phenolic hydroxyl groups in the degradation products showed a gradual increase with increasing time and temperature, reaching a maximum of 4.12 mmol/g at 140 °C for 12 h (Figure 3b).

The molecular masses of lignin degradation products formed with different reaction times and temperatures are shown in Table 1. When the reaction temperature was lower than 120 °C, the molecular weight of the degradation product gradually decreased with the prolongation of reaction time. When the reaction temperature was higher than 120 °C, the molecular weight of the degradation product first decreased and then slowly increased; the higher the temperature, the higher the rate of increase. The molecular weight of the degradation product was the smallest with a reaction time of 3 h and a temperature of 140 °C. Taking into account the trend for a reduced total hydroxyl content with prolonged reaction times at higher temperatures, it was concluded that a reaction...
temperature of 140 °C and a degradation time of 3 h are the best conditions for the catalytic degradation of alkali lignin using [BMIM]Cl.

In addition to the effects of the reaction temperature and time on the degradation of lignin, the substrate concentration also plays a role in determining the outcome of the reaction. The hydroxyl content after reaction at different lignin substrate concentrations, using the optimized reaction time of 3 h and temperature of 140 °C, is shown in Table 2. Both total hydroxyl groups and phenolic hydroxyl groups reached a maximum when the concentration of the substrate was 2.5%. At higher substrate concentrations, the hydroxyl content decreased, and so 2.5% was chosen as the best substrate concentration.

| substrate concentration (%) | phenolic hydroxyl groups (mmol/g) | total hydroxyl groups (mmol/g) | aliphatic hydroxyl groups (mmol/g) | weight average molecular weight (Mw) (g/mol) |
|-----------------------------|----------------------------------|-------------------------------|-----------------------------------|------------------------------------------|
| 1                           | 2.90                             | 7.66                          | 4.76                              | 6017                                     |
| 2.5                         | 3.47                             | 10.92                         | 7.45                              | 3978                                     |
| 5                           | 2.89                             | 8.21                          | 5.32                              | 4867                                     |
| 7.5                         | 2.67                             | 6.69                          | 4.02                              | 5814                                     |
| 10                          | 2.35                             | 4.62                          | 2.27                              | 6159                                     |

Degradation of Alkali Lignin Using the Double-Effect Catalyst \( \{H_3PW_12O_{40}/ZrO_2 + [BMIM]Cl\} \). Analysis of Degradation Conditions. The distribution of phenolic and aliphatic hydroxyl groups in the alkali lignin degradation products at different times and temperatures is shown in Figure 4. Both aliphatic and phenolic hydroxyl groups reached a maximum at 2 h and 100 °C at 8.52 and 6.69 mmol/g, respectively. At the beginning of the reaction, when the temperature is low, some of the carboxyl groups in the lignin are reduced, or C=O bonds are broken, and new hydroxyl groups are generated. With a prolonged reaction time and increasing temperature, however, the amount of hydroxyl groups in the degradation product is greatly reduced because some of the newly generated hydroxyl groups are decomposed or polymerize with other compounds at higher temperatures.4,11

Gel permeation chromatography (GPC) was used to determine the molecular weight of the lignin degradation products. Before analysis, both the alkali lignin raw material and the degraded solid product were acetylated to improve the solubility and reduce association and aggregation. The molecular mass of the lignin degradation products showed a trend of first decreasing and then increasing with increasing temperature and time (Figures 5 and 6). This is because two major reactions, depolymerization and condensation, compete during the degradation of lignin.9 In the depolymerization reaction, β-O-4 bonds are broken, generating α-carbon cations in the lignin structure. The formation of these α-carbon cations, however, promotes the condensation reaction. This means that competition between the cleavage of the β-O-4 bond and condensation at the α-position results in

Figure 4. Effect of reaction time on the content of phenolic hydroxyl groups (a) and the content of alcoholic hydroxyl groups (b) in alkali lignin degradation products.

Figure 5. Molecular weight distribution of alkali lignin (a) and degraded alkali lignin (b) (degradation conditions: 2 h at 100 °C).

Figure 6. Weight-average molecular weight of alkali lignin degradation products at different reaction times and temperatures.
changes in the relative molecular mass of the degradation products throughout the reaction. The relative molecular mass decreased from 10 600 to 3853 g/mol after 2 h at 100 °C and then gradually increased. Taking into account the effects of reaction time and temperature on the hydroxyl content, it was concluded that the optimal reaction temperature and time for the degradation of alkali lignin are 100 °C and 2 h, respectively.

The relationship between total hydroxyl groups and phenolic hydroxyl groups in the product after the degradation of alkali lignin catalyzed by different mass fractions of H₃PW₁₂O₄₀/ZrO₂ is shown in Table 3. Both total hydroxyl content reached a maximum value when the ratio of H₃PW₁₂O₄₀/ZrO₂ to lignin was 1:4. When the amount of H₃PW₁₂O₄₀/ZrO₂ is too small, not all of the active sites of H₃PW₁₂O₄₀/ZrO₂ to lignin increases beyond 1:4 and the degradation deteriorates.

### Table 3. Effect of H₃PW₁₂O₄₀/ZrO₂ Loading on the Hydroxyl Content

| ratio of H₃PW₁₂O₄₀/ZrO₂ and lignin | phenolic hydroxyl groups (mmol/g) | total hydroxyl groups (mmol/g) | aliphatic hydroxyl groups (mmol/g) | weight average molecular weight (Mₐ) (g/mol) |
|------------------------------------|----------------------------------|-------------------------------|-----------------------------------|---------------------------------------------|
| 1:2                                | 4.64                             | 11.66                         | 7.03                              | 3905                                        |
| 1:4                                | 6.69                             | 15.21                         | 8.53                              | 3853                                        |
| 1:8                                | 5.98                             | 10.59                         | 4.61                              | 4310                                        |
| 1:12                               | 4.27                             | 6.79                          | 2.52                              | 4210                                        |
| 1:16                               | 2.75                             | 5.22                          | 2.47                              | 3934                                        |

groups and phenolic hydroxyl groups first increased and then decreased with increasing amounts of the catalyst. The hydroxyl content reached a maximum value when the ratio of H₃PW₁₂O₄₀/ZrO₂ to lignin was 1:4. When the amount of H₃PW₁₂O₄₀/ZrO₂ is too small, not all of the active sites of lignin participate in the reaction, resulting in fewer hydroxyl groups. When the amount of H₃PW₁₂O₄₀/ZrO₂ exceeds a certain level, however, besides increasing the reaction rate, the increased surface area of the catalyst also promotes the oxidation of the newly generated hydroxyl groups. The hydroxyl content thus decreases as the ratio of H₃PW₁₂O₄₀/ZrO₂ to lignin increases beyond 1:4 and the degradation reaction deteriorates.

**Analysis of the Chemical Structure of Degradation Products.** The infrared spectra of alkali lignin before and after degradation are very similar (Figure 7). The strength of the broad absorption peak around 3400 cm⁻¹, which is attributed to the stretching vibration of O–H groups, increased after degradation and activation. The band at 2840 cm⁻¹ is due to the C–H stretching vibration of –OCH₃ groups,12,13 and the bands at 1600 and 1528 cm⁻¹ are due to benzene ring skeleton vibrations, and the bands at 1500 and 1368 cm⁻¹ are due to saturated C–H bending vibrations. The benzene ring structures were found before and after degradation, indicating that they mainly occurred on the side chain and the reactive functional groups on the benzene ring. Comparing the infrared spectra before and after degradation, a new peak appeared at 1718 cm⁻¹, which was attributed to a stretching vibration of C=O groups.

¹³C-NMR spectroscopy was used to further characterize the structural changes that occurred on the degradation of alkali lignin. The ¹³C-NMR spectra of alkali lignin (A) and the degradation product after dual-action catalytic degradation by [BMIM]Cl + H₃PW₁₂O₄₀/ZrO₂ (B) are shown in Figure 8. Assignments of individual peaks in the ¹³C-NMR spectra are provided in Table 4.14–16 After degradation, peaks attributed to –COOH groups in the fatty acids of alkali lignin disappeared and it can be inferred that a decarboxylation reaction occurred during degradation. The spectrum of the degradation product also lacked the characteristic peak at 153.08 ppm for the S-type β−O−4 bond, indicating that fragmentation had occurred during the degradation reaction. The spectra both before and after degradation show peaks at 148.32−149.28 ppm that are characteristic of a G-type structural unit, indicating that G-type β−O−4 bonds are not cleaved during degradation. Dual-action catalytic degradation by [BMIM]Cl + H₃PW₁₂O₄₀/ZrO₂ thus generates mainly S-type structures.

**Antioxidant Performance of Products of the Dual-Action Catalytic Degradation.** DPPH is a stable synthetic nitrogen-containing free radical with a lone pair of electrons. A solution of DPPH in methanol or ethanol is purple, with a maximum absorption peak at 517 nm. In the presence of free-radical scavengers, the lone pairs of electrons in DPPH are paired, and the purple color of the radical •DPPH fades and is replaced by the yellow color of the reduced nonradical form, DPPH-H. The amount of electrons donated by a free-radical scavenger can be quantified by the degree of fading of the purple color of a DPPH solution, allowing quantitative analysis of the antioxidant capacity of lignin by measuring changes in the absorbance of a solution of DPPH.17,18 Line graphs of the DPPH free-radical-scavenging capacity of alkali lignin as a function of the mass concentration before and after degradation can be quantified with those of the commercial antioxidant 2,6-ditert-butyl-4-methylphenol (BHT) in Figure 9. As the mass concentration increases, the capacity of degraded alkali lignin to remove •DPPH gradually increases. The antioxidant capacity of degraded alkali lignin is close to that of BHT at the same mass concentration and is significantly higher than that of untreated alkali lignin. Once the concentration of degraded alkali lignin reached 0.6 g/L, the curve gradually flattened and the clearance rate was 80%. The degradation of alkali lignin thus increases its free-radical-scavenging capacity.

The capacity of wheat straw alkali lignin, before and after degradation, to scavenge hydroxyl radicals (•OH) is compared with that of BHT in Figure 10. •OH radicals are formed from OH⁻ by the loss of one electron and are highly active oxidizing agents. •Phenanthroline reacts with ferrous ions to form an iron-•-phenanthroline complex, which is commonly used as a redox indicator. The complex has a maximum absorption at a wavelength of 536 nm, and when the ferrous iron-•-
Phenanthroline is oxidized by \( \cdot OH \) to a ferric iron-phenanthroline complex, the absorbance at 536 nm is reduced. In the presence of \( \cdot OH \) radical scavengers, oxidation of the ferrous complex is reduced and absorbance at 536 nm is relatively unchanged. When this system was used to measure the \( \cdot OH \)-scavenging activity of alkali lignin and degraded alkali lignin, the scavenging activity was shown to increase as the mass concentration increased until the lignin concentration reached 0.6 g/L, when the scavenging activity increased more slowly (Figure 10). Degraded alkali lignin has a slightly greater \( \cdot OH \)-scavenging ability than untreated alkali lignin, but its activity is much lower than that of the positive control BHT.

An analysis of the reducing capacity of alkali lignin is shown in Figure 11. The reducing capacity of antioxidants reflects their antioxidant activity; the stronger the reducing power, the stronger the antioxidant effect. The magnitude of the reducing capacity thus reflects the magnitude of the antioxidant effect. In this experiment, the ability of the antioxidants to reduce ferric ions to ferrous ions was determined. The reducing capacity of alkali lignin before and after degradation increased with increasing mass concentration but was less than that of the positive control BHT. The reducing power of the alkali lignin degradation product was, however, markedly higher than that of the raw material at the same mass concentration.

**CONCLUSIONS**

Based on the hydroxyl content and molecular weight of the products, the optimal reaction time for the degradation of alkali lignin by the ionic liquid \([BMIM]Cl\) was 3 h, the optimal reaction temperature was 140 °C, and the optimal substrate concentration was 2.5%. When \( H_{3}PW_{12}O_{40}/ZrO_{2} \) was used in the mass ratio of 1:4, the optimal reaction time for degradation was shortened to 2 h and the optimal reaction temperature was reduced to 100 °C. In the presence of \( H_{3}PW_{12}O_{40}/ZrO_{2} \), the total hydroxyl content in the degradation product increased by 123.4% compared with the ionic liquid alone and the relative molecular weight of the product was reduced by 3853 g/mol.

FT-IR and \(^{13}C\)-NMR spectroscopy were used to characterize the structures of the alkali lignin degradation products and to determine the bond-breaking mechanisms. The main bond-breaking mechanism was found to be rupture of the \( \beta-O-4 \) bond. The degradation product obtained by catalysis with \([H_{3}PW_{12}O_{40}/ZrO_{2} + [BMIM]Cl]\) showed good antioxidant properties and this degradation method thus provides a promising route for the future development of new applications for lignin.

**EXPERIMENTAL SECTION**

**Preparation of \( H_{3}PW_{12}O_{40}/ZrO_{2} \) and Alkali Lignin.**

Preparation of \( H_{3}PW_{12}O_{40}/ZrO_{2} \). A 10 M ammonia solution was added dropwise with stirring to a 0.2 M solution of zirconium chloride until the pH of the solution was 8. The precipitate was collected by filtration and washed with aqueous ammonia (pH = 8) until no chloride ions were detected by a silver nitrate test. The solid was dried at 120 °C for 12 h, and the resulting zirconia was ground to provide a 400 mesh powder. \( H_{3}PW_{12}O_{40} \) (1 g) was dissolved in absolute ethanol (16 mL), and zirconia (4 g) was added. The mixture was refluxed at 80 °C for 12 h, and excess ethanol was removed by rotary vacuum evaporation at 39 °C. The resulting solid was dried at 120 °C for 24 h and then activated by heating in a muffle furnace at 200 °C for 4 h.

**Alkali Lignin Pretreatment.** Crude alkali lignin was stirred with distilled water (1:9 lignin/distilled water) to rapidly dissolve the lignin. After standing for 12−24 h, insoluble impurities were removed by suction filtration. Aqueous hydrochloric acid (2 M) was then slowly added to the filtrate until the pH of the filtrate was 2. After standing for 12−24 h,
the precipitate was removed by suction filtration and the filter cake was washed with water until the washings were neutral.

The resulting refined alkali lignin was dried under vacuum at 50 °C for 48 h and ground to provide a 400 mesh powder.19

Catalytic Degradation of Alkali Lignin by [BMIM]Cl. [BMIM]Cl was heated to 80 °C with stirring in a 250 mL beaker until the solid had melted. Wheat straw lignin was added and the mixture was stirred rapidly until the lignin had completely dissolved. An aliquot (30 mL) of the solution was transferred to a 50 mL reaction kettle and heated under vacuum for several hours. The reaction kettle was then cooled to room temperature and the contents were transferred to a beaker. Distilled water (600 mL, \( V_{\text{distilled liquid}} / V_{\text{distilled water}} = 1:20 \)) was added and the resulting flocculent precipitate was separated by filtration through a membrane filter. The solid product was dried under vacuum at 45 °C for 24 h and the filtrate was collected.

Double-Effect Catalytic Degradation of Alkali Lignin by \((\text{H}_3\text{PW}_{12}\text{O}_{40}/\text{ZrO}_2 + [\text{BMIM}]\text{Cl})\). [BMIM]Cl was heated to 80 °C with stirring in a 250 mL beaker until the solid had melted. Wheat straw lignin was added to the ionic liquid and the mixture was stirred at 400 rpm for 2 h to completely dissolve the lignin. An aliquot (30 mL) of the above solution was transferred to a 50 mL microreactor and the \( \text{H}_3\text{PW}_{12}\text{O}_{40} / \text{ZrO}_2 \) catalyst was added. The mixture was heated to 45 °C with stirring in a 250 mL beaker until the solid had completely dissolved. An aliquot (30 mL) of the solution was transferred to a 50 mL reaction kettle and heated under vacuum for several hours. The reaction kettle was then cooled to room temperature and the contents were transferred to a beaker. Distilled water (600 mL, \( V_{\text{distilled liquid}} / V_{\text{distilled water}} = 1:20 \)) was added and the resulting flocculent precipitate was separated by filtration through a membrane filter. The solid product was dried under vacuum at 45 °C for 24 h and the filtrate was collected.

**Table 4. Chemical Shifts (δ, ppm) and Assignments of the \( ^{13} \text{C-NMR} \) Spectrum of Nonacetylated Lignin**

| ppm  | assignment                                      | ppm  | assignment                                      |
|------|------------------------------------------------|------|------------------------------------------------|
| 174.7| \(-\text{COOH}, \) aliphatic acids or esters    | 125.9| \( \text{C-5, nonetherified S-S} \)             |
| 166.5| \( \text{C-9 in P-coumarate (PCE)} \)            | 122.7| \( \text{C-1 and C-6 in Ar-C(=O)} \)            |
| 160.0| \( \text{C-4 in PCE} \)                          | 119.4| \( \text{C-6 in G units} \)                     |
| 144.7| \( \text{C-7 in PCE} \)                          | 118.4| \( \text{C-6 in G units} \)                     |
| 130.3| \( \text{C-2/C-6 in PCE} \)                      | 115.1| \( \text{C-5 in G units} \)                     |
| 125.1| \( \text{C-1 in PCE} \)                          | 114.7| \( \text{C-5 in G units} \)                     |
| 116.0| \( \text{C-3/C-5 in PCE} \)                      | 111.1| \( \text{C-2 in G units} \)                     |
| 115.0| \( \text{C-8 in PCE} \)                          | 110.4| \( \text{C-2 in G units} \)                     |
| 152.5| \( \text{C-3/S-5, etherified S units} \)        | 106.8| \( \text{C-2/C-6, S units with } \alpha-\text{CO} \) |
| 149.7| \( \text{C-3, etherified G units} \)            | 104.3| \( \text{C-2/C-6, S units} \)                   |
| 148.4| \( \text{C-3, G units} \)                        | 86.6 | \( \text{C-\( \beta \) in G-type } \beta-5 \text{ units} \) |
| 148.0| \( \text{C-3, G units} \)                        | 84.6 | \( \text{C-\( \beta \) in G-type } \beta-\alpha \text{ units (three) } \) |
| 146.8| \( \text{C-4, etherified G units} \)            | 83.8 | \( \text{C-\( \beta \) in G-type } \beta\text{-O-4} \text{-units (erythro) } \) |
| 145.8| \( \text{C-4, nonetherified G units} \)         | 74.1 | \( \text{C-\( \gamma \) in } \beta\text{-O-4} \) |
| 145.0| \( \text{C-4, etherified S-5} \)                 | 72.4 | \( \text{C-\( \gamma \) in } \beta\text{-O-4} \text{-units (threo) } \) |
| 143.3| \( \text{C-4, nonetherified S-5} \)              | 71.8 | \( \text{C-\( \alpha \) in G-type } \beta\text{-O-4} \text{-units (erythro) } \) |
| 137.2| \( \text{C-4, etherified S units} \)             | 71.2 | \( \text{C-\( \alpha \) in G-type } \beta\text{-O-4} \text{-units (threo) } \) |
| 135.6| \( \text{C-1, etherified S units} \); \( \text{C-1, etherified G units} \) | 63.2 | \( \text{C-\( \gamma \) in G-type } \beta\text{-O-4} \text{-units with } \alpha\text{-C=O} \) |
| 133.4| \( \text{C-1, nonetherified S units} \); \( \text{C-1, nonetherified G units} \) | 62.8 | \( \text{C-\( \gamma \) in G-type } \beta\text{-S, } \beta\text{-1 units} \) |
| 132.4| \( \text{C-5, etherified S-5} \)                 | 60.2 | \( \text{C-\( \beta \) in G-type } \beta\text{-O-4} \text{-units } \) |
| 131.1| \( \text{C-1, nonetherified S-5} \)              | 55.6 | \( \text{C in Ar-C(=O)CH} \)                     |
| 129.3| \( \text{C-\( \beta \) in Ar-CH=CH-CHO} \)       | 53.9 | \( \text{C-\( \beta \) in } \beta\text{-O-4} \text{-units } \) |
| 128.0| \( \text{C-\( \alpha \) and C-\( \beta \) in Ar-CH=CH-CHO} \) | 53.4 | \( \text{C-\( \beta \) in } \beta\text{-S units } \) |
| 128.1| \( \text{C-2/C-6, in H units} \)                 | 34.8 | \( \text{CH}_3 \text{ group in ketones (conjugated) or aliphatic side chains } \) |
| 124.1| \( \text{C-5/C-5', nonetherified S-S} \)        | 29.2 | \( \text{CH}_3 \text{ in aliphatic side chains } \) |
| 122.6| \( \text{C-1/C-6 in Ar-C(=O)C-C} \)             | 21.7 | \( \text{CH}_3/\text{CH}_2 \text{ group in saturated side chains } \) |

**Figure 9.** Clearance rates of DPPH free radicals by wheat straw alkali lignin before and after degradation compared with the clearance by BHT.

**Figure 10.** \( ^{\cdot} \text{OH} \) radical-scavenging activity of wheat straw alkali lignin, before and after degradation, compared with the activity of BHT.

**Figure 11.** Reducing capacity of wheat straw lignin before and after degradation compared with that of BHT.
Characterization of Alkali Lignin. Determination of Hydroxyl Groups. The molar mass of total hydroxyl groups in the lignin was determined using the acetylation–hydrolysis method,\(^{20,21}\) and the molar concentration of phenolic hydroxyl groups was determined using differential photometry.\(^{22,23}\) The aliphatic hydroxyl content was calculated as follows: total hydroxyl content—phenolic hydroxyl content.

FT-IR Spectroscopy. Infrared spectroscopic analysis of lignin and its degradation products was performed using an FT-IR-650 Fourier transform infrared spectrometer over the range of 400–4000 cm\(^{-1}\) at a resolution of 4 cm\(^{-1}\) with 64 scans.

Determination of the Molecular Weight of Lignin. Gel permeation chromatography (GPC) was carried out using an Agilent Series 1100 instrument (Agilent Technologies, Santa Clara, CA) to determine the molecular weight of the lignin. The lignin was acetylated using the following procedure: Lignin (0.08 g) was dissolved in the acetylation reagent (1.50 mL) and the solution was heated to 50 °C for 48 h. After cooling to room temperature, the solution was washed with ether to remove excess acetylating reagent. The solid product was removed by centrifugation and dried in a vacuum oven at 40 °C for 24 h.\(^{24}\) Acetylated lignin (20 mg) was added to tetrahydrofuran (10 mL) and the insoluble material was removed by filtration through a 0.45 μm filter. GPC was carried out using 79911GP-101 and 79911GP-104 (Tianjin Gangdong Technology Co., Ltd.) columns connected in series. The mobile phase was tetrahydrofuran, the flow rate was 1.0 mL/min, the column temperature was 25 °C, and the injection volume was 10 μL.

\(^{13}\)C-NMR Spectroscopy. \(^{13}\)C-NMR spectra were recorded using a Bruker 400 M superconducting nuclear magnetic resonance instrument (Bruker, Germany). Lignin (100 mg) was dissolved in dimethyl sulfoxide (DMSO)-d6 (0.6 mL), and spectra were recorded using a 30 pulse sequence, a sampling time of 1.36 s, a relaxation time of 1.89 s, and 30 000 accumulations. MestReNova software (Mestrelab Research SL) was used for subsequent data processing.

Antioxidant Properties of Alkali Lignin. 1-Diphenyl-2-trinitrophenylhydrazone (DPPH) Radical-Scavenging Ability. DPPH (4 mg) was dissolved in absolute ethanol and diluted to 100 mL in a volumetric flask. The solution was stored at 0–4 °C in the dark before use. Sample solutions (1 mL; 0.025, 0.05, 0.1, 0.2, 0.4, 0.6, and 0.8 g/L) were shaken well with the DPPH solution (4 mL) and placed in the dark for 30 min. Absolute ethanol was used as the blank and its absorbance was measured at 517 nm. 2,6-Ditet-butyl-4-methylphenol (BHT), which is commonly used as an antioxidant in food processing, was used as the positive control. The clearance rate was calculated by eq 1

\[
\text{clearance rate} = \left( \frac{A_0 - A_i}{A_0} \right) \times 100\% \quad (1)
\]

where \(A_0\) is the absorbance of the DPPH solution in anhydrous ethanol and \(A_i\) is the absorbance of the test solution.

Hydroxyl Radical (·OH)-Scavenging Ability. A phenanthroline solution (5 mM, 0.3 mL) was placed in a ground-glass tube, and pH 7.45 phosphate-buffered solution (0.8 mL) and deionized water (0.25 mL) were added sequentially. The mixture was shaken well; FeSO\(_4\) solution (7.5 mM, 0.3 mL) was added and the solution was mixed immediately. The sample solution (0.025, 0.05, 0.1, 0.2, 0.4, 0.6, and 0.8 g/L) was added to the test tube, followed by 1% H\(_2\)O\(_2\) solution (0.2 mL). After incubation at 37 °C for 60 min, the absorbance was measured at 536 nm. BHT was used as the positive control. The clearance rate was calculated by eq 2

\[
\text{clearance rate} = \left( \frac{A_{\text{sample}} - A_{\text{damage}}}{A_{\text{contrast}} - A_{\text{blank}}} \right) \times 100\% \quad (2)
\]

where \(A_{\text{sample}}\) is the absorbance of the lignin sample (Fe\(^{2+}\) + phenanthroline + H\(_2\)O\(_2\) + lignin), \(A_{\text{damage}}\) is the absorbance of damaged tube (Fe\(^{2+}\) + phenanthroline + H\(_2\)O\(_2\)), \(A_{\text{contrast}}\) is the absorbance of the control tube (Fe\(^{2+}\) + phenanthroline), and \(A_{\text{blank}}\) is the absorbance of the blank (Fe\(^{2+}\) + phenanthroline + lignin).

Measurement of Reducing Power. The sample solution (0.025, 0.05, 0.1, 0.2, 0.4, 0.6, and 0.8 g/L) was placed in a test tube and treated with pH 6.6 phosphate-buffered saline (2.5 mL) and 1% potassium ferricyanide solution. After shaking and mixing, the reaction mixture was stored at 50 °C for 20 min. Trichloroacetic acid solution (2.5 mL) was then added and the mixture was shaken. An aliquot (2.5 mL) of the supernatant was treated with deionized water (2.5 mL) and 0.1% FeCl\(_3\) (0.5 mL) solution, and the absorbance was measured at 700 nm.

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**Author Contributions**

S.R. and G.F. provided the idea and supported the experiments. X.L. and Y.Z. designed and performed the experiments and analyzed the data. G.Z. and J.Z. provided guidance for the degradation method. X.L. wrote this paper.

**Notes**

The authors declare no competing financial interest.

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