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A Value-Added Utilization Method of Sugar Production By-Products from Rice Straw: Extraction of Lignin and Evaluation of Its Antioxidant Activity

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Abstract: To value-added utilization of the rice straw, two types of lignin were extracted from the by-products of sugar production. The ether-extracted lignin with a purity of 98.7% was extracted from the pretreatment filtrate with two times the concentrated filtrate volume of ether, where the lignin yield was 6.62 mg/g of the rice straw. The ball-milled lignin with a purity of 99.6% was extracted from the milled enzymatic hydrolysis residue with a 1,4-dioxane solution, where the revolution speed and grinding time were 300 rpm and 12 h, respectively. The yield of ball-milled lignin was 34.52 mg/g of the rice straw, which was 421.5% higher than that extracted from extract-free rice straw. In the process of rice straw pretreatment and lignin extraction, 76.43% by mass of phosphotungstic acid catalyst and approximately 98% by volume of 1,4-dioxane solution could be recycled and reused. Compared with the soda lignin extracted from papermaking black liquor, the scavenging rates of DPPH radical and $ABTS^+$ radical of ether-extracted lignin increased by 36.26% and 41.18%, respectively, while the above scavenging rates of ball-milled lignin increased by 30.22% and 37.75%, respectively. Moreover, the reducing power of the two extracted lignins was also stronger than that of soda lignin. The ether-extracted lignin and ball-milled lignin have the potential to be developed as natural macromolecular antioxidants.

Keywords: lignin; rice straw; ether extraction; ball-milling extraction; antioxidant activity

Citation: Zhang, S.; Ma, L.; Gao, S.; Zhu, C.; Yan, Y.; Liu, X.; Li, L.; Chen, H. A Value-Added Utilization Method of Sugar Production By-Products from Rice Straw: Extraction of Lignin and Evaluation of Its Antioxidant Activity. *Processes* **2022**, *10*, 1210. https://doi.org/10.3390/pr10061210

Academic Editor: Davide Dionisi

Received: 31 May 2022
Accepted: 16 June 2022
Published: 17 June 2022

1. Introduction

Lignin is the second most abundant component in biomass and a rare renewable aromatic polymer in nature [1,2]. Compared with carbohydrates, which can be relatively easily converted into sugar platform compounds and fine chemicals, the utilization value of lignin has not been fully developed [3–5]. Although it is possible to convert lignin to aromatic chemicals and hydrocarbon liquid fuels through biorefinery pathways such as pyrolysis and hydrogenolysis, the conversion conditions are often too harsh and limited by reactors [6–8]. Moreover, there are often problems such as low recovery rate of sugar platform molecules and difficulty in catalyst recovery [9,10]. Therefore, to improve the recycling value of lignocellulosic biomass, a conversion strategy that takes into account the effective utilization of lignin and carbohydrates should be developed [11,12].

Recently, the conversion of lignin into additives or processing aids has received extensive attention, including antioxidants [13–15]. Lauberte et al. [16] used ultrasonic and hydrodynamic cavitation methods to pretreat wheat straw, and they found that the low molecular weight lignin and its derivatives in the pretreatment solution had strong antioxidant activity. Tha et al. [17] found that the kraft lignin extracted from eucalyptus black liquor by the acid precipitation method had the function of scavenging DPPH (1,1-diphenyl-2-picrylhydrazyl) free radicals. In addition, the antioxidant activity of lignin could be enhanced by chemical modification or fractional extraction. Wei et al. [18] found that the low molecular weight eucalyptus kraft lignin fractionated with organic solvents had a stronger antioxidant activity than high molecular weight lignin. Su et al. [19] also demonstrated that the antioxidant activity of ball-milled wheat straw lignin catalyzed by a LiCl/DMSO solvent was improved. Moreover, studies have shown that the antioxidant activity of lignin was related to its supramolecular structure [20–22]. The antioxidant activity of lignin showed a positive correlation with its phenolic hydroxyl groups [23,24]. For lignins from the same source, the lignin with a lower molecular weight usually had a better antioxidant activity due to the possible formation of phenolic hydroxyl groups after β-O-4 bond cleavage [21,24].

The processing and extraction process also had a great impact on lignin's antioxidant activity. Industrial lignin undergoes complex degradation and polycondensation reactions during the acid-base treatment, resulting in a low content of active functional groups [25]. Even the DPPH and $ABTS^+$ (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate)) radical scavenging rates of chemical modified industrial lignin were improved by 17.2–22.1% and 14.2–30.4%, respectively; their antioxidant activity was still lower than that of the unmodified organic solvent lignin [24–26]. The chemical structure of ball-milled lignin was close to that of natural lignin. It contains more active functional groups such as phenolic hydroxyl group, alcoholic hydroxyl group, and carboxyl group, and these functional groups had a positive effect on the antioxidant activity of the samples [27]. However, the industrial application of ball-milled lignin was limited by the low extraction rate and long extraction time [28,29]. In the past, the main purpose of extracting ball-milled lignin was to analyze the chemical structure of the extracted products [30]. Some studies have shown that the extraction efficiency of lignin can be improved by combining chemical or biological methods with ball-milling extraction [31–33]. Yang et al. [27] found that lignin could be more easily extracted from the lignocellulose substrate after the moderate deconstruction of the LCC (lignin–carbohydrate) complex.

In the previous study, a combined process of liquid hot water (LHW) and phosphotungstic acid (PTA) pretreatment and enzymatic hydrolysis was used to produce fermentable sugars from rice straw [34]. The glucose yield was about 27.6% by the mass of air-dried straw. However, rice straw lignin was not extracted from sugar production by-products such as enzymatic hydrolysis residues and PTA treatment solution. In this study, the extraction methods of rice straw lignin from the PTA treatment solution and the enzymatic hydrolysis residue were carried out by ether extraction and ball-milling extraction, respectively. The chemical properties of extracted lignin were analyzed by the chemical analysis, GPC (gel permeation chromatography), and FT-IR (Fourier transform infrared) methods. The antioxidant activity of extracted lignin was evaluated by the tests of DPPH and $ABTS^+$ free radical scavenging rates and reducing power. The results will provide a method that can efficiently convert both lignin and carbohydrates, which is a positive sign for improving the full-component utilization values of biomass.

2. Materials and Methods

2.1. Materials

The air-dried rice straw (RS) was harvested from northeastern China (44°55′12″ N, 127°9′ E) and pulverized to 40–60 mesh powder by an FW-100 plant cell crusher (Tianjin Taisite Instrument Co., Tianjin, China). The RS powder contains 37.8% of cellulose, 18.4% of hemicellulose, 14.6% of lignin, 14.2% of ash, 3.7% of moisture, and 11.3% of ethanol

extracts. Soda lignin used as a control was extracted from the dried black liquor (Shandong Tralin Paper Co., Ltd., Liaocheng, China) with a purity of 92.2%, containing 89.3% of acid-insoluble lignin, 2.9% of acid-soluble lignin, 3.2% of sugars, and 4.6% of ash [26]. The phosphotungstic acid, complex enzyme (cellulase enzyme blend Cellic CTec2), and other major reagents were purchased from Sigma-Aldrich Co., LLC., (Shanghai, China) and Tianjin Kemiou Chemical Reagent Co., Ltd., (Tianjin, China).

2.2. Process for Producing Glucose from Rice Straw

The process of producing glucose from rice straw was referred to the method of our previous study with some modifications [34]. Briefly, approximately 15 g of RS powder was extracted with absolute ethanol by the Soxhlet extraction method for 24 h and vacuum dried to obtain the extract-free RS. A total of 8 g of extract-free RS and 160 mL of distilled water were added into a 200 mL hydrothermal reactor (Beijing Huotong Experimental Instrument Co., Ltd., Beijing, China) with a polytetrafluoroethylene liner. The hydrothermal reactor was transferred to a drying oven and heated at 180 °C for 90 min. After the initial hydrothermal reaction was completed, the solid residue obtained by vacuum filtration was dried at 105 °C to a constant weight, and then the dried power was subsequently treated with a 140 mL of 20 mM PTA solution at 130 °C for 60 min. After the followed PTA pretreatment was completed, the solid residue was separated by vacuum filtration while washing to neutrality with about 100 mL of distilled water. Approximately 240 mL of filtrate was concentrated to a volume of about 30 mL by rotary evaporation for further lignin extraction with ether. The solid residue was dried at 105 °C to a constant weight to obtain the LHW-PTA RS. Then, it was enzymatically hydrolyzed with a Cellic CTec2 enzyme at 50 °C for 72 h. The enzyme activity was 20 FPU/g of the substrate, the substrate concentration was 2% (w/v), and the pH value of the sodium acetate buffer solution was 4.8. After the enzymatic hydrolysis experiment was completed, the liquid fraction containing fermentable sugars was collected for further analysis. The solid residue was dried at 105 °C to a constant weight to obtain the enzymatic hydrolysis residue (EH residue) for further lignin extraction by the method of ball milling.

2.3. Lignin Extraction

2.3.1. Extraction of Lignin from PTA Pretreatment Concentrated Filtrate

About 30 mL of concentrate from the PTA treatment and 30 mL of diethyl ether were transferred to a separatory funnel and shaken well. After the mixture was allowed to stand for 10 min, the bottom mixture with a volume of about 25 mL was collected and mixed with an additional 30 mL of diethyl ether and allowed to stand for 10 min. The bottom mixture with a volume of about 25 mL was collected and vacuum filtered through a 0.22 μm filter membrane, while the solid matter on the filter membrane was washed to neutrality with approximately 10 mL of diethyl ether. About 30 mL of filtrate was transferred to a Petri dish and purged with air to remove diethyl ether. After the diethyl ether was evaporated, the PTA catalyst could be recovered and recycled. The washed solid matter was vacuum dried at 45 °C to a constant weight to obtain the ether-extracted lignin.

2.3.2. Extraction of Lignin from EH Residue

The effects of revolution speed and grinding time on the extraction of ball-milled lignin were carried out using a DECO-PBM-V-2L Planetary ball mill with four 250 mL zirconia grinding jars (Changsha DECO Instrument Equipment Co., Ltd., Changsha, China). A total of 15 g of EH residue and approximately 300 g of zirconia grinding balls (including 120 g of 10 mm grinding balls and 180 g of 5 mm grinding balls) were added to each zirconia grinding jar to start the operation. To prevent the EH residue from overheating, the ball mill was stopped for 5 min every 10 min of operation. The effect of the large plate revolution speed was studied at 250 rad/min, 300 rad/min, 350 rad/min, and 400 rad/min, where the revolution speed ratio of the large plate to the grinding jar was 1:2. The effect of total grinding time (including rest time) was studied at 4 h, 8 h, 12 h, 16 h, and 20 h. After the

operation was completed, 500 mL of 1,4-dioxane solution (1,4-dioxane: distilled water, v:v, 9:1) and 5 g of milled EH residue powder were mixed and stirred at 800 rpm at room temperature for 24 h. The mixture was vacuum filtered to collect the filtrate and solid residue. The solid residue was mixed with an additional 500 mL of 1,4-dioxane solution and stirred under the above conditions. Then, the vacuum filtration was performed on the mixture again. A total volume of about 990 mL of filtrate collected two times was concentrated to approximately 10 mL by rotary evaporation, and then the concentrated filtrate was mixed with 40 mL of diethyl ether and centrifuged at 4000 rpm for 5 min. After the precipitate was vacuum dried at 45 °C to a constant weight, the ball-milled lignin was obtained.

2.4. Analysis and Characterization

2.4.1. Chemical Composition Analysis

The contents of the sugars, carbohydrates, lignin, and ethanol extraction of rice straw samples were measured by the NREL (National Renewable Energy Laboratory) method [35,36]. In brief, the content of sugars was determined using an Agilent 1260 HPLC apparatus (Agilent Technologies Inc., Waldbronn, Germany) with a Bio-Rad Aminex HPX-87H column and a Micro-Guard Cation H guard column. The cellulose content was calculated by multiplying the glucose content by a correction of 0.90, and the hemicellulose content was obtained by multiplying the sum of xylose and arabinose by a correction of 0.88. The content of lignin was determined by a two-step hydrolysis method with sulfuric acid, where the acid-insoluble lignin was analyzed by a Persee T6 UV spectrometer (Beijing persee General Instruments Co., Ltd., Beijing, China). The purity of lignin was calculated based on the proportion of the total mass of acid-insoluble and acid-soluble lignin in the lignin sample.

2.4.2. Determination of Phenolic Hydroxyl Groups

The content of phenolic hydroxyl groups of lignin samples was determined by measuring the difference in absorption of phenolic units at 300 nm and 360 nm using a UV spectrometer, which can be found elsewhere [37].

2.4.3. Determination of Molecular Weight

The weight-average molecular weight (M_w), number-average molecular weight (M_n), and molecular weight distribution (M_w/M_n) of acetylated lignin samples were determined by the GPC method on an Agilent 1260 HPLC apparatus with a 79911GP-101 column and a 79911GP-104 column. Briefly, 50 mg of lignin sample and 1 mL of acetylation reagent (pyridine, acetic anhydride, and 1,4-dioxane mixed in a 1:1:1 volume ratio) were mixed and kept at 50 °C for 48 h to obtain the acetylated lignin. 10 mg of acetylated lignin was dissolved in 5 mL of tetrahydrofuran and then measured under the following conditions: the tetrahydrofuran was the mobile phase; the flow rate was 1 mL·min^{-1}; the column temperature was set to 30 °C [25].

2.4.4. FT-IR Analysis

The FT-IR spectra of lignin samples were scanned on a Nicolet iS50 spectrometer (Thermo Fisher Scientific Inc., Madison, USA). The number of scans and resolution were 32 times and 2 cm^{-1}, respectively. The scanning range was from 4000 cm^{-1} to 400 cm^{-1}.

2.4.5. Calculation Formulas and Data Analysis

The solid recovery of rice straw samples and the extraction rate, yield, and purity of lignin samples were calculated based on the following equations:

$$\text{Solid recovery } (\%) = \frac{\text{mass of solid residue after the treatment or enzymatic hydrolysis}}{\text{corresponding mass of RS before all treatments}} \times 100 \quad (1)$$

where treatment refers to absolute ethanol extraction, liquid hot water pretreatment, or PTA pretreatment.

$$\text{Extraction rate of ether-extracted lignin } (\%) = \frac{\text{mass of extracted lignin}}{\text{mass of lignin in RS}} \times 100 \quad (2)$$

$$\text{Extraction rate of ball-milled lignin } (\%) = \frac{\text{mass of extracted lignin}}{\text{mass of lignin in EH residue}} \times 100 \quad (3)$$

$$\text{Lignin yield } (\text{mg/g of the RS}) = \frac{\text{mass of extracted lignin} \times 1000}{\text{corresponding mass of RS before all treatments}} \quad (4)$$

$$\text{Lignin purity } (\%) = \frac{\text{mass of acid soluble lignin} + \text{mass of acid insoluble lignin}}{\text{mass of lignin sample}} \times 100 \quad (5)$$

The experiments were performed in triplicates. The data were analyzed statistically by the variance (ANOVA) of software SPSS 17.0 (IBM (China) Investment Co., Ltd., Shanghai, China) and presented as mean ± standard deviation.

2.5. Evaluation of Antioxidant Activity

The scavenging rates of DPPH and $ABTS^+$ radical of ether-extracted lignin, ball-milled lignin, and soda lignin were determined by measuring the absorbance at 734 nm and 517 nm with a UV spectrophotometer, as described in our previous study [24]. The reducing power of different lignin samples was determined based on the absorbance at 700 nm, which can be found in a previous study [23].

3. Results and Discussion

3.1. Effects of Treatment Process on Chemical Composition of Rice Straw

The changes in the chemical composition of RS before and after the absolute ethanol extraction, LHW-PTA pretreatment, and enzymatic hydrolysis are shown in Table 1. The results showed that the fat-soluble components accounting for 11.3% by mass of the RS were removed by absolute ethanol extraction, which could reduce the measurement error in the determination of chemical components. The content of carbohydrates and lignin determined by the NREL method was calculated based on the extract-free biomass [35,36]. In addition, the removal of fat-soluble components such as pigment and pectin was beneficial in improving the separation efficiency and purity of ball-milled lignin. After the LHW-PTA pretreatment was completed, 85.63% of hemicellulose and 18.66% of lignin were removed from the RS substrate, respectively, while 88.04% of cellulose was retained in the LHW-PTA RS. After the enzymatic hydrolysis was completed, 78.14% of cellulose and 93.27% of hemicellulose were removed from the RS substrate. A glucose recovery rate (the ratio of mass obtained to theoretical mass in RS) of 65.64% and a solid recovery of EH residue of 33.83% were obtained, respectively.

Table 1. The chemical composition of rice straw before and after the absolute ethanol extraction, pretreatment, and enzymatic hydrolysis.

Run	Sample	Solid Recovery (%)	Chemical Composition (%)				
			Cellulose	Hemicellulose	Lignin	Extract	Moisture
— [1]	RS	100	37.80 ± 0.08	18.39 ± 0.26	14.60 ± 0.16	11.34 ± 0.23	3.70 ± 0.10
Soxhlet extraction	Extract-free RS	85.02 ± 0.28	44.47 ± 0.13	21.64 ± 0.31	17.18 ± 0.20	N.D. [2]	N.D.
Pretreatment	LHW-PTA RS	60.38 ± 0.42	55.12 ± 0.21	4.38 ± 0.35	19.67 ± 0.31	N.D.	N.D.
Enzymatic hydrolysis	EH residue	33.83 ± 0.72	24.43 ± 0.86	3.66 ± 0.18	35.54 ± 0.51	N.D.	N.D.

[1]—indicates no operation was performed. [2] N.D.—indicates no value was detected or the detected value was close to zero.

3.2. Subsection

The ether-extracted lignin was extracted from the concentrated filtrate of pretreatment according to the method in Section 2.3.1. The extraction rate and the yield of ether-extracted lignin were 3.85 ± 0.77% and 6.62 ± 1.32 mg/g of the RS, respectively. Meanwhile, 76.43 ± 1.81% by weight of the PTA catalyst was recovered from the concentrated filtrate.

The effects of revolution speed and grinding time on the extraction rate and yield of ball-milled lignin are shown in Figure 1 (data in Supplementary Table S1). The effect of the revolution speed increasing from 200 rpm to 350 rpm was investigated at a total grinding time of 12 h. The highest extraction rate of 28.71% was obtained at the revolution speed of 300 rpm, where the lignin yield was 34.52 mg/g of the RS. Lignin and carbohydrates are present in the cell walls of biomass, and there are complex chemical and physical connections between them [32]. The extraction rate of lignin was lower during low-speed grinding, because the strength of physical grinding was not strong enough to destroy the crystallinity of cellulose in the cell wall. On the contrary, when the revolution speed was adjusted to above 300 rpm, the centrifugal force from the ball mill jar was larger than that from the main plate, and the grinding balls would run at the same speed along with the jar wall, thereby reducing the impact of the grinding balls on the material; therefore, the extraction rate of lignin was not high. The effect of grinding time increasing from 4 h to 18 h was studied using a revolution speed of 300 rpm. In the grinding time range from 4 h to 12 h, the grinding efficiency was significantly increased by increasing the grinding time. When the grinding time was over 12 h, the extraction rate of ball-milled lignin was no longer significantly improved. Previous studies showed that the extraction rate of ball-milled lignin can be improved by moderately prolonging the milling time [38,39]. The results of this study are consistent with previous studies. Therefore, the optimum ball milling conditions were determined with the revolution speed at 300 rpm and the grinding time at 12 h. Extract-free RS was used as a control for EH residue. After the extract-free RS was ground at 300 rpm for 12 h, a lignin extraction rate of 5.82 ± 1.13% and a lignin yield of 8.50 ± 1.64 mg/g of the RS were obtained.

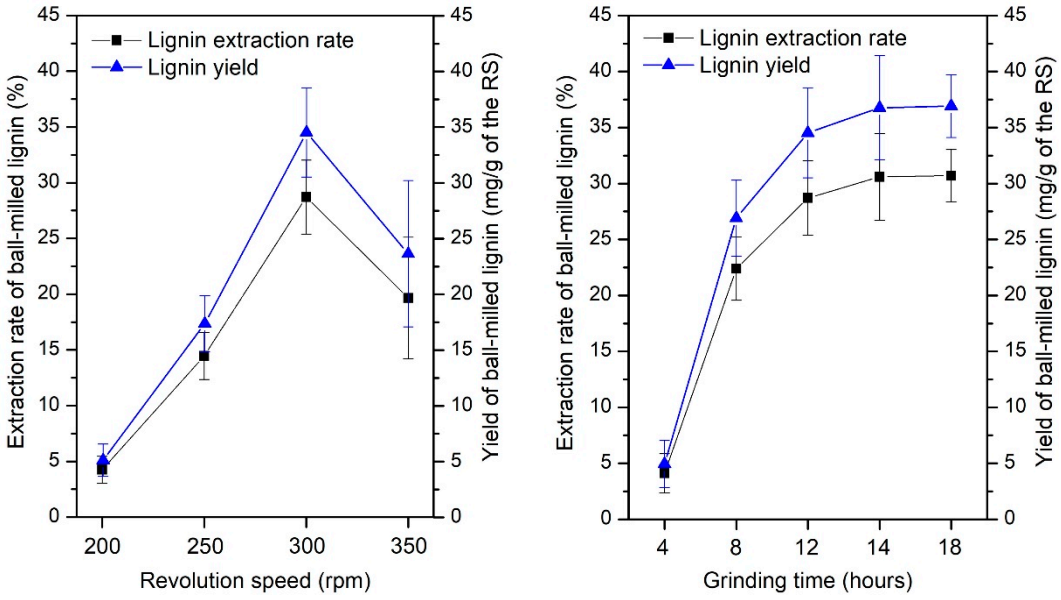

Figure 1. The effects of revolution speed and grinding time on the extraction rate and yield of ball-milled lignin.

Under the optimal ball milling conditions, the extraction rate and yield of ball-milled lignin from the EH residue were 645.7% and 421.5% higher than those from extract-free RS, respectively. This is because 78.14% of cellulose and 93.27% of hemicellulose in the rice straw were removed by the LHW-PTA pretreatment and enzymatic hydrolysis, which

resulted in the change of the physical structure of the rice straw substrate from dense to porous. Under the impact of the grinding ball, the cell wall was more easily destroyed. Moreover, the degradation of hemicellulose leads to the destruction of the chemical structure of the LCC complex (lignin–carbohydrate complex), and the cleavage of the covalent bonds between lignin and pentoses, such as ether bonds and ester bonds. This ultimately results in easier extraction of lignin by the 1,4-dioxane solution. Previous studies showed that the milling efficiency could be improved by pretreating biomass substrate with enzyme preparations or acid-base solutions [31,40]. Regrettably, although it was much easier to extract lignin from the EH residue than from the extract-free RS, there was still a part of the lignin remaining in the EH residue that was not extracted. Previous studies showed the extraction efficiency of ball-milled lignin can be improved by adding dilute alkali (NaOH) to dioxane solution or using DMSO/LiCl or ionic liquid instead of dioxane solution to extract lignin from biomass [32,41]. Chen et al. [33] and Wang et al. [42] demonstrated that the total extraction amount of lignin could be improved by the extraction process of secondary enzymatic hydrolysis. Future work could further optimize the solvent or refine the enzymatic hydrolysis process, thereby further improving the extraction rate of ball-milled lignin.

3.3. Characterization of Different Lignin Samples

The chemical composition, molecular weight, and molecular weight distribution of different lignin samples are shown in Table 2. The measurement errors of M_w and M_n were below 100 g·mol^{-1}. The content of impurities of ether-extracted lignin and ball-milled lignin was less than 1.3% and 0.4%, respectively, and the two lignin samples contained almost no ash. Compared with the soda lignin extracted from black liquor, the above two extracted lignin samples had a higher content of phenolic hydroxyl groups.

Table 2. The chemical composition of rice straw before and after the absolute ethanol extraction, pretreatment, and enzymatic hydrolysis.

Sample	Purity (%)	Phenolic Hydroxyl Groups (mmol·g^{-1})	M_w (g·mol^{-1})	M_n (g·mol^{-1})	M_w/M_n
Ether-extracted lignin	98.7 ± 0.3	2.40 ± 0.02	3200	1400	2.29
Ball-milled lignin	99.6 ± 0.1	2.21 ± 0.01	10,200	6800	1.50
Soda lignin	92.2 ± 0.2	1.70 ± 0.03	7700	4900	1.57

The FT-IR spectra of the ether-extracted lignin, ball-milled lignin, and soda lignin are depicted in Figure 2. The assignation of the peaks was determined by the description of Zhang et al. [26], Wang et al. [43], and Faxi [44]. In spectra (b) and (c), the peaks at 1654 cm^{-1}, 1361 cm^{-1}, and 819 cm^{-1} were assigned to the C=O stretching in conjugated ketone, symmetric bending deformation of the methyl group, and aromatic C-H deformation out of the plane. The above peaks in spectra (a) were significantly weaker or not observed. The high electron cloud density brought by the conjugated structure could adsorb free radicals. Therefore, the ether-extracted lignin and ball-milled lignin might exhibit better adsorption capacity for free radicals than that of soda lignin. In spectra (a), the peaks at 1331 cm^{-1} and 1216 cm^{-1} were assigned to the C-O vibration of syringyl and guaiacyl, respectively. In spectra (a) and (c), the peaks at 1268 cm^{-1} and 1031 cm^{-1} were assigned to the guaiacyl C-O units. In all spectra, the peaks at 1604 cm^{-1}, 1511 cm^{-1}, and 1421 cm^{-1} were attributed to the aromatic ring skeleton vibration of lignin. Other peaks commonly found in lignin samples, such as those at 2932 cm^{-1} (C-H stretching), 2846 cm^{-1} (C-H symmetric stretching), 1716 cm^{-1} (C=O stretching of carbonyl group), 1462 cm^{-1} (C-H deformation of methyl and methylene groups), 1119 cm^{-1} (aromatic C-H deformation in syringyl units), and 1078 cm^{-1} (C-O deformation in secondary alcohols and aliphatic ethers), were observed in all spectra. The above analysis showed that the structure of the three lignin samples was stable. The peaks at 978 cm^{-1} and 896 cm^{-1} were observed in

spectra (b), which might attribute to the −HC=CH stretching out of the plane. These peaks were not observed in spectrum (a) and spectrum (b). This might be because the degraded small molecule lignin in the PTA solution was polymerized into new esters. In spectra (c), the peak at 1167 cm^{-1} was observed, which indicated that the ball-milled lignin was typical HGS lignin.

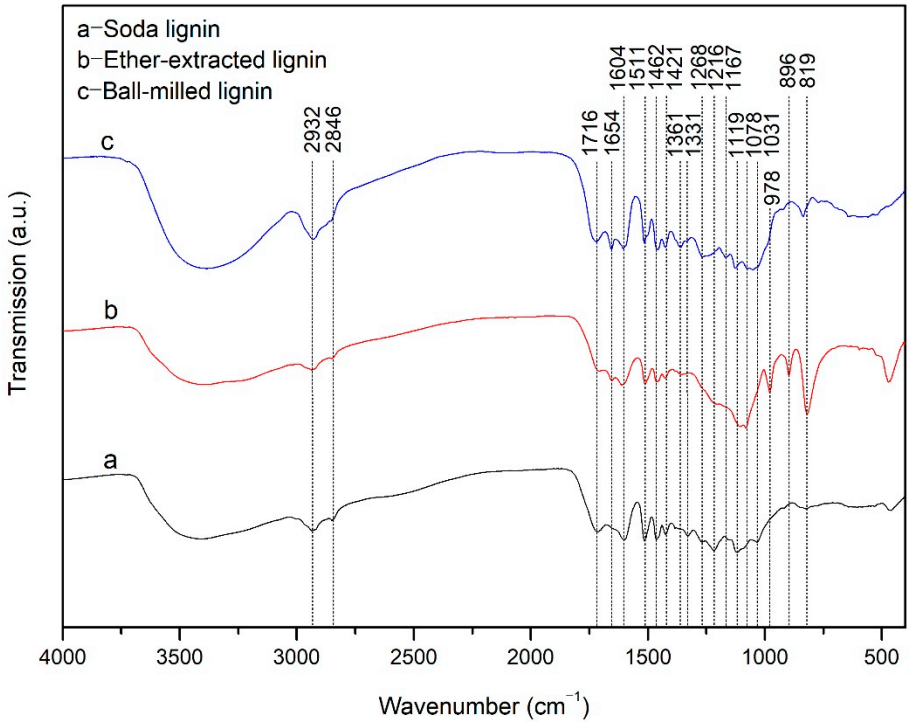

Figure 2. FT-IR spectra of ether-extracted lignin, ball-milled lignin, and soda lignin.

3.4. Antioxidative Activities of Different Lignin Samples

The antioxidant activities of ether-extracted lignin and ball-milled lignin are shown in Figure 3. The soda lignin and BHT were used as controls. The EC_{50} value represents the sample concentration corresponding to the free radical scavenging rate at 50% [26]. A smaller EC_{50} value indicates that the sample had a stronger antioxidant activity. The EC_{50} values of DPPH free radical scavenging rate of different samples were ranked as follows: BHT (0.029 mg·mL^{-1}) < ether-extracted lignin (0.116 mg·mL^{-1}) < ball-milled lignin (0.127 mg·mL^{-1}) < soda lignin (0.182 mg·mL^{-1}). The EC50 values of $ABTS^+$ free radical scavenging rate of different samples were ranked as follows: BHT (0.079 mg·mL^{-1}) < ether-extracted lignin (0.12 mg·mL^{-1}) < ball-milled lignin (0.127 mg·mL^{-1}) < soda lignin (0.204 mg·mL^{-1}). The maximum DPPH free radical scavenging rate of all three lignin samples could reach about 85%, where the corresponding concentrations of ether-extracted lignin and ball-milled lignin were both 0.4 mg·mL^{-1} and the corresponding concentration of soda lignin was 0.6 mg·mL^{-1}. The maximum $ABTS^+$ free radical scavenging rate for the three lignin samples was about 99%, where the corresponding concentrations of ether-extracted lignin and ball-milled lignin were 0.6 mg·mL^{-1}, while that of soda lignin was 0.8 mg·mL^{-1}. In the concentration range from 0.1 mg·mL^{-1} to 0.6 mg·mL^{-1}, the reducing power of different lignin and BHT samples increased with increasing concentration, and the reduction power of ether-extracted lignin was the strongest in lignin samples but weaker than that of BHT. As a result, the antioxidant activity of ether-extracted lignin and ball-milled lignin was weaker than that of the commercially available antioxidant BHT but stronger than that of soda lignin and the antioxidant activity of ether-extracted lignin was slightly stronger than that of ball-milled lignin.

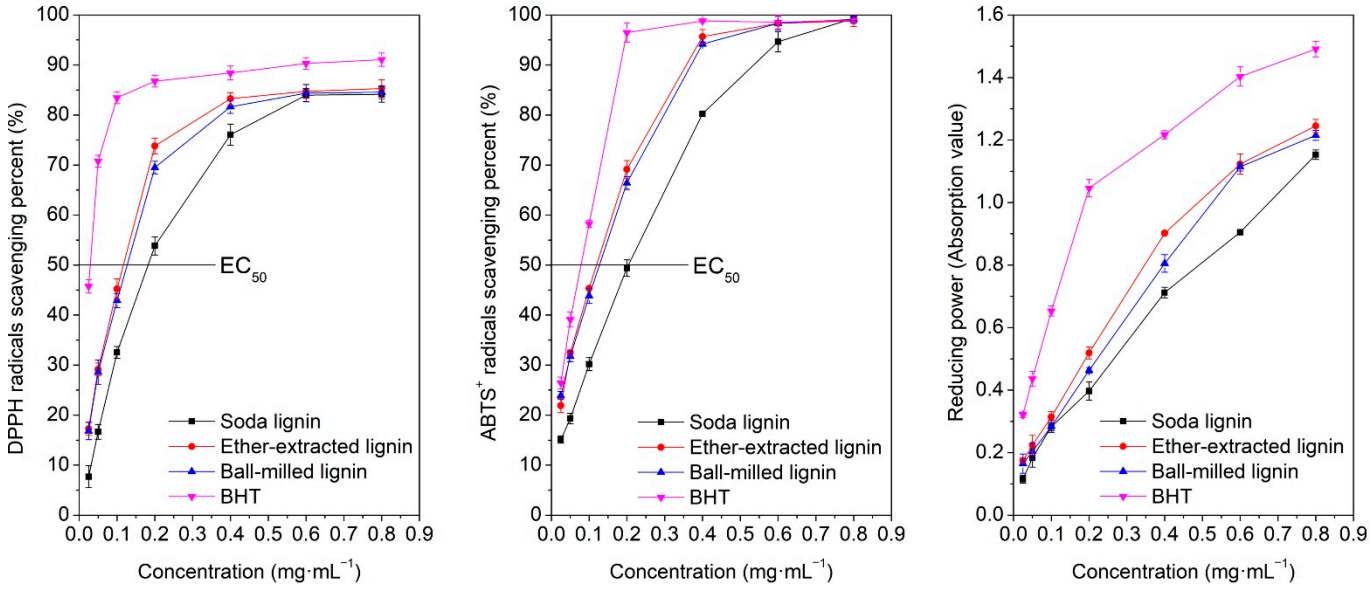

Figure 3. The antioxidant activities of different lignin and BHT samples.

The chemical analysis results in Section 3.3 showed that the order of the content of phenolic hydroxyl groups of the three lignin samples was as follows: ether-extracted lignin (2.4 mmol·g^{-1}) > ball-milled lignin (2.21 mmol·g^{-1}) > soda lignin (1.7 mmol·g^{-1}), which was consistent with the order of the antioxidant activity of the three lignin samples. Previous studies showed that the antioxidant activity of lignin was mainly contributed by phenolic hydroxyl groups [23]. Compared with the traditional inorganic acid-base pretreatment processes, the combined treatment conditions of LHW pretreatment, PTA pretreatment, and enzymatic hydrolysis were relatively milder. Since rice straw lignin did not undergo complex degradation and condensation processes, the extracted lignin contained higher phenolic hydroxyl functional groups, thus showing good antioxidant activity. Therefore, the ether-extracted lignin and ball-milled lignin showed better antioxidant activity than the soda lignin.

In previous studies, the antioxidant activity of soda lignin was improved by chemical modification methods such as ionic liquid-catalyzed hydrolysis [24] and $Pd/SO_4^{2-}/ZrO_2$ catalyzed reduction [26]. The EC_{50} values of DPPH and $ABTS^+$ radical scavenging rate of ionic liquid activated soda lignin decreased by 17.2% and 14.2%, respectively [24]. The EC_{50} values of $Pd/SO_4^{2-}/ZrO_2$-activated soda lignin for the above-mentioned free radical scavenging rate decreased by 20.6% and 32.6%, respectively [26]. In this study, compared with the soda lignin, the EC_{50} values of DPPH free radical scavenging rate of ether-extracted lignin and ball-milled lignin decreased by 36.26% and 30.22%, respectively, and their EC_{50} values of $ABTS^+$ free radical scavenging rate were also decreased by 41.18% and 37.75%, respectively. The results showed that the antioxidant activity of the two types of extracted rice straw lignin was stronger than that of the chemically modified soda lignin. The extracted lignin may be added to oil products as a phenolic antioxidant to prolong the shelf life by blocking the chain reaction of auto-oxidation of oil products; it may also be added to functional materials as additives to improve the stability of materials and prolong service life.

3.5. Mass Balance

The mass balance diagram based on 100 g of rice straw during the pretreatment, enzymatic hydrolysis, and lignin extraction is shown in Figure 4. An amount of 100 g of air-dried rice straw was extracted with absolute ethanol to obtain 85.02 g of extract-free RS. After the extract-free RS was pretreated with the LHW and PTA, 60.38 g of LHW-PTA RS and a certain volume of pretreatment filtrate were obtained. On the one hand, 0.66 g of

rice straw lignin could be separated from the filtrate by the ether extraction method, while the PTA catalyst with a mass fraction of 76.43% could be recovered. On the other hand, enzymatic hydrolysis was performed on the LHW-PTA RS to obtain 27.57 g of glucose and 33.83 g of EH residue. The glucose could be further converted to cellulosic ethanol through a fermentation process. 3.45 g of lignin was extracted from the EH residue by ball milling and 1,4-dioxane extraction, where the 1,4-dioxane solution with a volume fraction of about 98% could be recovered. The extraction yield of ball-milled lignin from the EH residue was 645.7% higher than that from the extract-free RS. In summary, 4.11 g of lignin with good antioxidant activity could be obtained from 100 g of air-dried rice straw, which could be developed as natural macromolecular antioxidants.

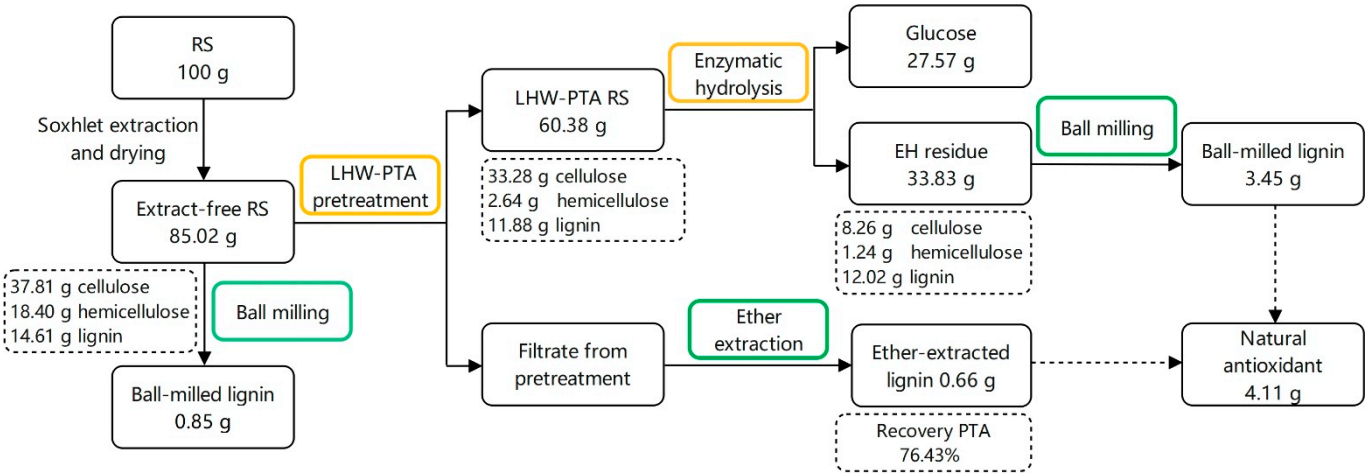

Figure 4. The mass balance diagram of the extraction process of rice straw lignin.

4. Conclusions

Two types of lignin, which accounted for 4.11% of the mass of rice straw, were extracted from sugar production by-products (pretreatment filtrate and enzymatic hydrolysis residue) by the methods of ether extraction and ball-milling extraction. The yields of ether-extracted lignin with a purity of 98.7% and ball-milled lignin with a purity of 99.7% were 6.62 mg/g of the RS and 34.52 mg/g of the RS, respectively. Since 78.14% of cellulose and 93.27% of hemicellulose were removed by the pretreatment and enzymatic hydrolysis from the RS substrate, the physical structure of the cell wall became porous and the chemical structure of the LCC complex was disrupted, resulting in an extraction rate of ball-milled lignin of 28.71%, which was 645.7% higher than that extracted from extract-free RS. Compared with the soda lignin extracted from papermaking black liquor, the antioxidant activity of ether-extracted lignin and ball-milled lignin was significantly stronger because of the higher content of phenolic hydroxyl groups in the above two types of lignin. Since the glucose accounting for 27.57% of the mass of rice straw can be obtained during lignin extraction, this study has a positive significance for improving the utilization value of the whole components of rice straw in the field of biorefinery.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr10061210/s1, Table S1: The extraction rate and yield of the ball-milled lignin samples.

Author Contributions: Conceptualization, S.Z.; methodology, S.Z., L.M. and S.G.; validation, Y.Y., X.L. and L.L.; formal analysis and investigation: S.Z., L.M. and C.Z.; writing—original draft preparation, review, and editing, S.Z.; visualization, S.Z.; supervision, H.C.; funding acquisition, S.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Natural Science Foundation of Heilongjiang Province, China (No. LH2020E001), the China Postdoctoral Science Foundation (No. 2019M651246), the Heilongjiang Postdoctoral Financial Assistant (No. LBH-Z19112), and the "Academic Backbone" Project of Northeast Agricultural University (No. 54911012).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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