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ORIGINAL PAPER

Fermented Cassava Residue Lignin Prepared by Sequential Acid Steam-Explosion and Hot-Alkaline Treatment and Its Antioxidant Properties

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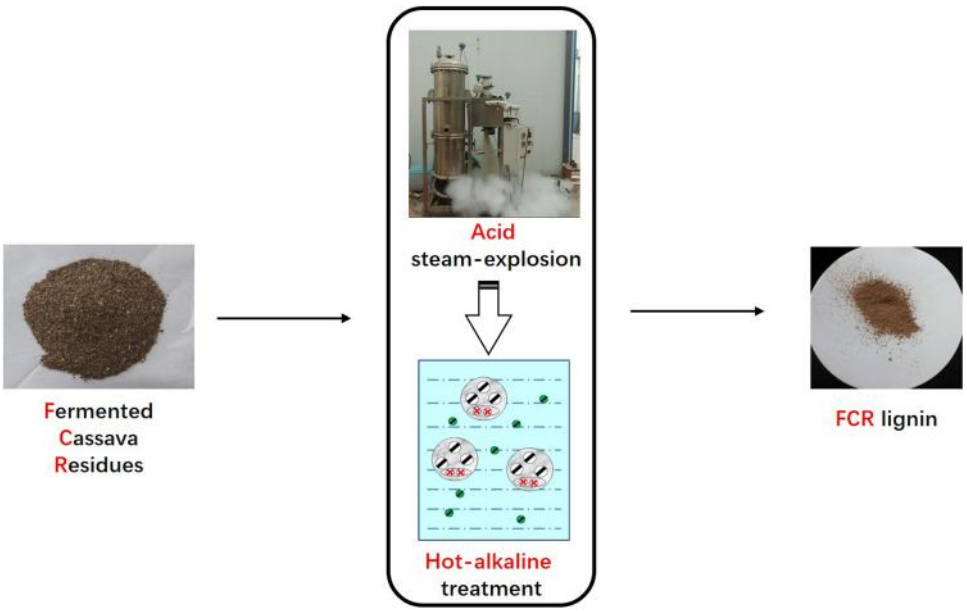
Abstract

Fermented cassava residue (FCR) is a solid waste generated in cassava-based ethanol production that constitutes a major environmental challenge and wastes a natural resource. In the present study, a process involving sequential acid steam-explosion and hot-alkaline treatment was developed to prepare an antioxidant lignin in high yield and purity from FCR. Two-dimensional NMR analysis indicates that FCR lignin belongs to a guaiacyl/syringyl/hydroxyphenyl (G/S/H) class of lignin. ^{31}P NMR analysis, after hydroxyl group phosphorylation, indicates that phenolic hydroxyl in FCR lignin attributable to mostly G-OH. One FCR lignin (No. 3) possessed the antioxidant activity similar to that of butylated hydroxytoluene. This FCR lignin may have potential applications as a food antioxidant or in other industrial processes as an antioxidant.

Graphic Abstract

The present study provided an efficient process of sequent acid steam-explosion and hot-alkaline treatment to improve the added value of fermented cassava residue (FCR).



Keywords Fermented cassava residue · Lignin · Steam-explosion · Antioxidant activity

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Statement of Novelty

Fermented cassava residue (FCR) lignin was prepared in an efficient process involving sequential acid steam-explosion and hot-alkaline treatment to improve the added value of FCR. FCR is the solid waste, generated from cassava-based ethanol production. The FCR lignin described in present paper showed high antioxidant activity similar to that of BHT (butylated hydroxytoluene), suggesting its potential application as a food antioxidant or in other industrial processes as an antioxidant.

Introduction

Lignin, a natural phenolic polymer, is the most abundant aromatic in nature and is also the major component of lignocellulose. In black spruce wood, the number of free phenolic groups in isolated lignin varies from 6–12 to 15–30 per 100 C_9 lignin units [1]. The polyphenolic structures within lignin are expected to exhibit antioxidant properties due to the presence of free radical scavenging of phenolic groups that can reduce oxygen radicals and prevent oxidation reactions [2, 3]. The inclusion of l to 10% lignin in a rat's diet results in a 50 to l00% increase retinol deposition, as compared to cellulose-fed control animals, and the phenolic fragments of Kraft lignin have been used as an effective antioxidant in vitamin E oil [4]. Further research indicates that lignin can also reduce lipid peroxidation [5], protect HeLa cells against colon cancer [3], and retain the structural stability under UV irradiation [5]. Cytotoxicity studies show that lignin exhibits cytotoxic effects only at very high concentrations [3]. All these observations suggest the potential application of lignin as an antioxidant in the food, feed, pharmaceutical and cosmetic industries [6]. In addition, lignin can also stabilize polymers from environmental oxidation, including photo oxidation [7] and thermal oxidation [8]. Different experiments have been investigated on the preparation of lignin from lignocellulose, using acid, alkali, and organic solvents, coupled with various operating conditions (time, temperature, etc.) [9]. The results indicate that different methods under the appropriate conditions can impact the chemical structure of the resulting lignin (molar mass, functionality, cross-linking and density) and its bioactivities [10]. The hydroxyl, methoxyl, carbonyl and carboxyl groups of lignin are important for conferring its antioxidant activity. Consequently, differences in the origin and method for preparing lignin can significantly impact its antioxidant properties [9].

During the past several decades, cassavas have been used at industrial scale to produce bio-ethanol in Europe, the United States and Asia, to relieve the emerging energy crisis and the environmental problems caused by the use of fossil fuels [11]. Fermented cassava residue (FCR) is a solid waste, generated from cassava-based ethanol production that consists of protein, polysaccharides, lignocellulose and other components [11]. Approximately 0.7 tons of FCR is produced for each ton of cassava ethanol produced in China, thus, the annual amount of FCR produced is more than 300,000 tons [12, 13]. FCR from cassava ethanol becomes moldy within a week, constituting a major environmental challenge [12]. Different technologies are currently being developed to add value to FCR by converting cassava residue into useful new compounds [14]. In the present study, the lignin component of FCR was recovered using sequential acid steam-explosion and hot-alkaline treatment and its antioxidant properties were characterized with the goal of developing a high-value added product from FCR.

Material and Methods

Material

FCR was collected from Fengyuan Co., Ltd in Anhui province, China. The dried FCR was mechanically milled into a powder and stored at room temperature for further experiments.

Starch Analysis of FCR

FCR (100 g powder) was mixed with 2 L of the enzyme-hydrolyzing solution (pH 6.5, 0.1 M $CaCl_2$, 10% (v/v) Novozyme Liquozyme Supra) and hydrolyzed at 80 °C for 2 h. Subsequently, the pH of the reaction system was adjusted to 4.5 with 37% (m/v) citric acid and 2 mL Amylase AG 300L (Novozyme) was added. Enzymatic hydrolysis was carried out for a further 3 h at 50 °C. Finally, the glucose levels of the hydrolyzed samples were determined by high performance liquid chromatography (Hitachi, Tokyo, Japan) equipped with refractive index detector and an Aminex HPX 87H column (BIO-RAD, USA). The starch mass was calculated by the following equation:

$$m_{starch} = m_{glucose} \times 0.9$$

Protein Analysis of FCR

Protein analysis was carried out using the Kjeldahl digestion method [15]. FCR was dried in a cross-flow oven at 50 °C overnight before all experiments. FCR (1 g of 60 mesh) was added to the digestion flask and a blank digestion flask was used as a control. K_2SO_4–$CuSO_4$ (0.02 g), 2 mL H_2SO_4 (98%) and 1 mL H_2O_2 (30%) was added to

each digestion flask. The digestion flasks were boiled for 1 h and then cooled to room temperature. After the digestion, the protein in sample solution was determined with titration method with 0.01 mol/L HCl. The protein percentage in FCR samples was calculated with the following equation:

$$X = \left[\left(V_1 - V_2\right) \times N \times 0.014\right] \div \left[m \times (10 \div 100) \times F\right] \times 100\%$$

X The mass percentage of protein in the FCR sample; V_1 The consumed HCl volume in sample reaction; V_2 The consumed HCl volume in the blank reaction; N The normal concentration of HCl solution; m The mass of FCR samples; F Protein and nitrogen conversion factors. (F = 6.25).

Cellulose/Hemicellulose/Lignin/Ash Analysis of FCR

The chemical compositions of FCR lignocellulose samples were analyzed using the National Renewable Energy Laboratory (NREL, USA) method [16]. Dried sample (300 mg) was hydrolyzed in a thick-wall flask using 3 mL of sulfuric acid (72%) for 30 min in a water bath at 30 °C with intermittent shaking every 5 min. After adding 84 mL water to dilute the 72% H_2SO_4 to 4%, the sample was hydrolyzed at 121 °C for 60 min in an autoclave. The hydrolyzed liquid was separated by air pump filtration. The filter cake was dried at 105 °C for 5 h and weighed. A portion of the filter cake was placed in a muffle furnace (Zhonghuan, Tianjin, China) at 575 °C to determine the ash content. The acid insoluble lignin (AIL) was calculated. The hydrolysate was analyzed by UV spectrophotometer (Shimadzu UV2450, Japan) to determine acid soluble lignin (ASL). Carbohydrate in the hydrolysate was measured to determine the cellulose and hemicellulose content by high performance liquid chromatography (Hitachi, Tokyo, Japan) equipped with refractive index detector and an Aminex HPX 87H column (BIO-RAD, USA).

FCR Lignin Preparation

The procedure of FCR lignin preparation is provided in Fig. S1. The No. 1 FCR lignin sample was prepared only with 0.5% NaOH extraction at 70 °C for 4 h and was used as the control. No. 2 and 3 samples were subjected to sequential acid steam-explosion and hot-alkaline treatment for FCR lignin preparation.

Prior to steam-explosion, 100 g of FCR was immersed in 0.3% or 0.6%, sulfuric acid solution at the solid/liquid ratio of 1:6 for 10 h. The FCR was then drained to obtain a solid with about 60% absorbed water content. Acid steam-explosion was carried out in the steam-explosion equipment according to Zhang [17]. The drained FCR was put into the steam-explosion equipment and heated to 1.2 MPa, corresponding to 188 °C. The steam pressure was held for 8 min (No. 2) or 10 min (No. 3), and the pressure was then reduced abruptly to standard atmospheric pressure. The effect of acid steam-explosion on FCR was described by the combined severity factor (CS) [18, 19], which was calculated by the following equation:

$$CS = \log\left[t \times \exp\left(\frac{T - 100}{14.75}\right)\right] - pH$$

t The retaining time of acid steam-explosion; T The temperature of acid steam-explosion.

The pH value was measured according to Zoulikha [18]. The CS values of different acid steam-explosions are provided in Table S1.

After acid steam-explosion, the treated residue was subjected to 0.5% NaOH treatment at 70 °C for 4 h. The NaOH hydrolysate was then centrifuged to collect supernatant and this was adjusted to pH 5.5 with 6 M HCl. After being concentrated by vacuum distillation, 3 volumes of 95% ethanol were added to the concentrated solution to precipitate hemicellulose. After the removal of hemicellulose residue by centrifugation, the supernatant was adjusted again to pH 2.0 with 6 M HCl to precipitate the lignin. The precipitated lignin was freeze-dried and kept in vacuum dryer at room temperature for further experiments.

Gel Permeation Chromatography (GPC)

The molecular weight of the lignin was determined using a Waters GPC system (Milford, Massachusetts, USA) equipped with UV detector and TSKG3000PWxl column (TOSOH, Japan). Different molecular weight (210, 4300, 6800, 10,000, 17,000, 32,000, 150,000) polystyrene sulfonates (Sigma-Aldrich) were used as standards. Sodium nitrate solution (0.1 mol/L) was used as the mobile phase. Other operating parameters was flow rate 1 mL/min, column temperature 30 °C, wavelength 280 nm. The resulting data were analyzed with Waters Breeze software.

2D-NMR Spectral Analysis

Acetylated lignin (80 mg) was dissolved in 0.5 mL DMSO-d_6 and sonicated for 5 min in ultrasonic cleaner to fully dissolve the sample. A Bruker AV 600 nuclear magnetic resonance instrument was used to collect the spectra using Bruker's "hsqcetgpsisp2.2" pulse sequence with spectral widths of 5387 Hz (from 9.5 to 0.5 ppm) and 28,674 Hz (from 203 to 13 ppm) for the ^{1}H- and ^{13}C dimensions. The number of collected complex points was 2048 for the ^{1}H-dimension with a relaxation delay of 1.8 s. Data were analyzed by Bruker Topspin software.

^{31}P NMR Spectroscopic Analysis

^{31}P NMR spectroscopy experiments were carried out according to Faix [20] and Wen [21]. FCR lignin (20 mg)

was dissolved in 500 µL pyridine-$CDCl_3$ (1.6:1, v/v), and then 100 µL cyclohexanol (10.85 mg/mL) was added to above solution as an internal standard (IS). Additionally, 100 µL chromium (III) acetylacetonate solution (5 mg/mL in pyridine-$CDCl_3$ (1.6:1, v/v)) was added as relaxation reagent. Finally, above solution was mixed with 100 µL of 2-chloro-1,3,2-dioxaphospholane as phosphorylating reagent. A Bruker AV 600 nuclear magnetic resonance instrument was used to collect the spectra. The spectrum was acquired using an inverse-gated decoupling pulse sequence, 90° pulse angle, 25 s pulse delay, and 128 scans. Data were analyzed by Bruker Topspin software.

Antioxidant Activity Analysis

The DPPH assay for the antioxidant activity of FCR lignin was carried out according to Azadfar [22] and it was described as the percentage of inhibition of 1,1-diphenyl-2-picrylhyrazyl (DPPH) radicals. The FCR lignin fractions and BHT were prepared at 0.4 mg/mL in 90% 1,4-dioxane (*v/v*). Sample solution (0.64 mL) was mixed with a 2.36 mL methanolic solution of DPPH (25 µg/mL). The absorbance variation of above mixture within 16 min was determined at 520 nm by a UV–Vis spectrophotometer (Shimadzu, Japan). The antioxidant activity of the samples was described by the following equation:

$$\text{Inhibition } \% = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\%$$

where $A_{control}$ is the absorbance in blank tube (antioxidant was omitted) and A_{sample} is the absorbance in the sample tube. The results were presented as mean ± standard error.

The ABTS assay was carried out according to previous papers and modified [23, 24]. ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, 7 mmol/L) and potassium persulfate (140 mmol/L) were mixed for the formation of $ABTS^{\cdot+}$ radical at 25 °C in the dark for 12 h. Then, the $ABTS^{\cdot+}$ radical solution was diluted with ethanol to OD = 0.7. Subsequently, 30 µL of FCR lignin ethanol solution were mixed with 3.0 mL of the diluted $ABTS^{\cdot+}$ radical solution at 30 °C in the dark for 6 min, immediately followed by the measurement of absorbance of mixture at 734 nm. Pure ethanol was used as a blank control. The antioxidant activity of the samples was described as the DPPH assay.

Results and Discussion

Effect of Acid Steam-Explosion on the FCR Lignin

FCR is generated from cassava-based ethanol production and contains different biomolecules, including proteins, polysaccharides, and lignocellulose [11]. The chemical composition of the FCR used in the present study contains starch 18.1%, cellulose 22.6%, hemicellulose 14.6%, lignin 22.0%, ash 13.9%, and protein 9.0% (Table 1).

In previous studies in our laboratory, an effective method was developed to prepare lignin from corncob by sequential acid steam-explosion and hot-ethanol treatment [25, 26]. However, this method was not effective for FCR. Acid steam-explosion barely removed most hemicellulose from FCR (data not shown) and, after steam-explosion treatment the FCR became very sticky, possibly the result of the complex composition of FCR. No lignin could be collected by hot-ethanol extraction. Given the effective nature of alkaline extraction for lignin preparation, a new method was developed in which acid steam-explosion was followed by hot alkaline extraction instead of hot ethanol extraction. And then, three volumes of ethanol were then added to the alkaline solution for the hemicellulose removal and finally lignin was precipitated from the supernatant by adjusting the pH to 2. With this modified method, lignin was prepared from FCR at large scale. In contrast to the non-acid-steam-explosion sample (No. 1), acid steam-explosion treatment (No. 2 and 3) significantly improved the lignin purity (the sum of acid soluble lignin and acid insoluble lignin) and yields (Table 2). The lignin purity was improved from 73.5% to 80.1% and 92.2%, and

Table 1 Composition of fermented cassava residue (FCR)

Component	Starch	Cellulose	Hemicellulose	Lignin	Ash	Protein
Percentage (%)	18.1	22.6	14.6	22.0	13.9	9.0

Table 2 The chemical composition analysis of different FCR lignin fractions

Number	CS	Cellulose (%)	Hemicellulose (%)	Acid-insoluble lignin (%)	Acid-soluble lignin (%)	Ash (%)	Yield (%)
1	0	7.92	7.02	66	7.52	6.9	19.4
2	1.05	–	2.75	75.3	4.78	5.8	35.9
3	2.18	–	2.11	87.9	4.27	5.1	73.9

yield from 19.4% to 35.9% and 73.9% (Table 2). Steam explosion severity has been shown to increase lignin purity and yield. In corncob, the steam explosion treatment improved the lignin purity from 29.3% to 93.6%, and yield from 6.8% to 40.9% [25]. The higher purity and yield of lignin could be attributed to the physical crushing ability of acid steam-explosion, which offers more accessible surface area for sodium hydroxide extraction of the lignin [25, 26]. Subsequent ethanol precipitation and pH adjustment also helped to purify lignin effectively, which was demonstrated by the low percentage content of cellulose and hemicellulose, and no detectable protein and starch in the lignin samples (Table 2).

2D-HSQC NMR Spectral Analysis of FCR Lignin

Lignin samples were analyzed using 2D-HSQC NMR spectroscopy to better understand the structural features of FCR lignin. The side-chain (δ_C/δ_H 50–90/2.5–6.0) and the aromatic (δ_C/δ_H 100–135/5.5–8.5) regions of the HSQC spectra are provided in Fig. 1, and the typical substructures in lignin are presented in Fig. 2.

Lignin's aromatic regions and the cross-peaks from syringyl (S), guaiacyl (G) and *p*-hydroxyphenyl (H) units could be observed in FCR lignin. The G unit shows signals for the C_6–H_6 correlation at δ_C/δ_H 119.0/6.79 and the oxidized G unit (G′) had signals for the C_6–H_6 correlation

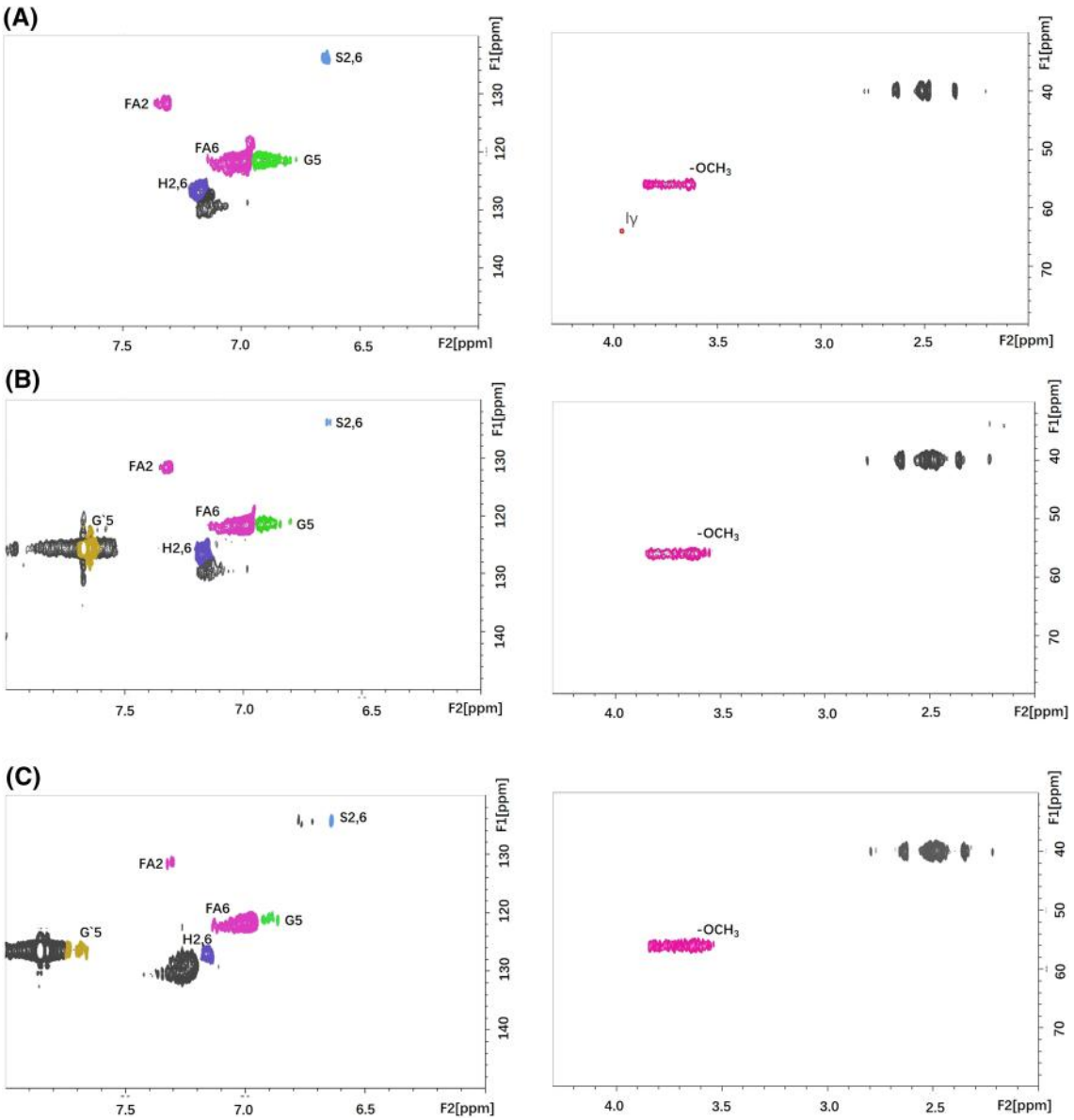

Fig. 1 Aromatic (left column) and side chain regions (right column) in 2D HSQC NMR spectra for FCR lignin. **a** No. 1 sample; **b** No. 2 sample; **c** No. 3 sample

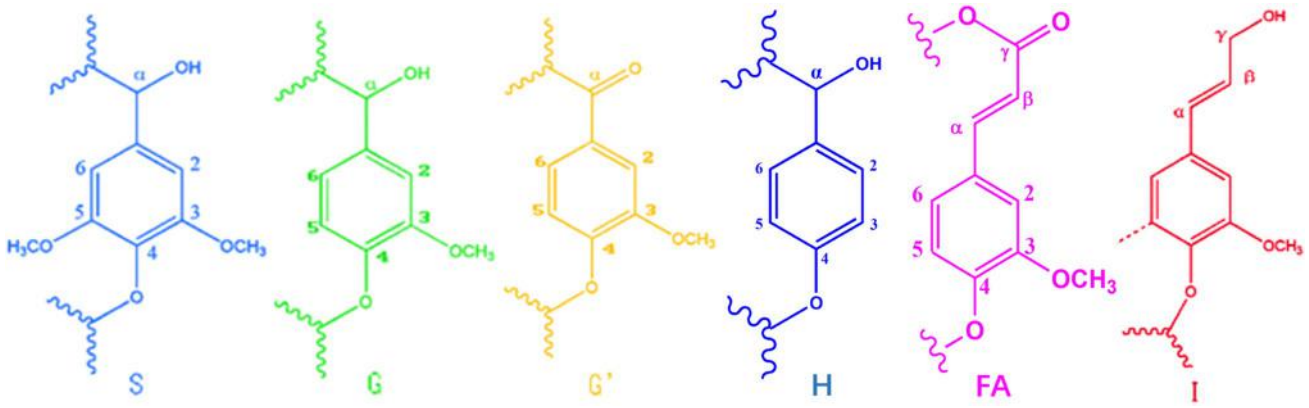

Fig. 2 Main classical aromatic subunits and side-chain linkages of FCR lignin detected by HSQC NMR spectrum: (S) syringyl units; (G) guaiacyl units; (G′) oxidized guaiacyl units with a α-ketone; (H) *p*-hydroxyphenyl units; (FA) ferulic acid; (I) *p*-hydroxycinnamyl alcohol end groups

Table 3 The molecular weights of different FCR lignin fractions

Number	CS	Mw	Mn	PDI
1	0	7320	1550	4.7
2	1.05	5050	1380	3.7
3	2.18	3510	1250	2.8

at δ_C/δ_H 123.3/7.60. The $C_{2,6}$–$H_{2,6}$ aromatic correlation signal from H unit could be observed at δ_C/δ_H 127.1/7.16. The normal S units exhibit signals for $C_{2,6}$–$H_{2,6}$ correlation at δ_C/δ_H 104.1/6.67, however, signals of S units were far weaker than those of G and H units. The identification of G, S, H units indicates that the cassava lignin was the G/S/H type lignin. Ferulic acid (FA) can also be identified in the 2D-HSQC spectra of the FCR lignin [27]. The cross-signals of FA were identified at C_2–H_2 with δ_C/δ_H 111.1/7.27 [27]. In the side-chain region of the lignin, the cross-signals for the methoxyl groups (δ_C/δ_H 55.6/3.71) were the most prominent and *β-O*-4 substructures were barely detectable. This suggests that the fermentation process, acidolysis and alkali treatment destroyed most *β-O*-4 linkages [28]. In addition, *p*-hydroxycinnamyl alcohol end groups (I_γ) were identified in No. 1 sample, but not in No. 2 and 3 samples, indicating that *p*-hydroxycinnamyl alcohol end groups were more vulnerable to the acid steam-explosion.

Effect of Acid Steam-Explosion on Lignin Molecular Weight from FCR

Acid steam-explosion depolymerized lignin to lower-weight molecules and the increase of combined severity factor (CS) (Table S1) enhanced this lignin depolymerization based on the weight-average (M_w) and number-average (M_n) molecular weights provided in Table 3. This decrease in lignin size results from the breakage of *β*-ether bonds between lignin units, as they account for 55% to 60% chemical linkages in natural lignin [29]. The polydispersity index (PDI) of the FCR lignin also became narrower with the increase of CS (Table 3; Table S1). The change of Mw, Mn and PDI with steam-explosion severity was consistent with those previously reported for organosolv methanol lignin [30] and organosolv ethanol lignin [31]. Depolymerizing lignin molecules can also be re-polymerized through a condensation reaction between aromatic and benzylic carbons, increasing the molecular weight of the resultant lignin [32]. However, in the present study the molecular weight of lignin decreased with increased CS, which might be due to a lower opportunity for the depolymerizing lignin to be condensed under these experimental conditions. Taken together, sequential acid steam-explosion and hot alkaline extraction could be used to effectively prepare lignin with higher purity and in higher yield from FCR. The increased CS under the conditions used also decreased the molecular weight of FCR lignin. For the purpose of preparing antioxidants, the characterization and antioxidant activity of FCR lignin were next analyzed.

^{31}P NMR Spectral Analysis and Antioxidant Activity of FCR Lignin

Since the phenolic hydroxyl groups have a critical impact on the antioxidant activity of lignin, FCR lignin was further characterized after phosphorylation using quantitative ^{31}P NMR spectroscopy to determine the amount of phenolic hydroxyl groups (S-OH, G-OH, P-OH). The phenolic hydroxyl groups were first phosphorylated using 1,3,2-dioxaphospholanyl chloride after which ^{31}P-NMR was used to determine the content of the different hydroxyls (aliphatic OH and phenolic OH) in lignin [20]. Quantification was carried out by peak integration using cyclohexanol as an internal standard (IS). Three standard substances

(sinapic acid, ferulic acid, *p*-coumaric acid) were first used to confirm the positions of phenolic S-OH, G-OH, P-OH units in the ^{31}P NMR spectrum (Fig. S2), which located at 131.0 ppm, 129.3 ppm and 127.8 ppm, respectively. In addition, the IS was located at 133.2 ppm, situated between α-OH and primary OH groups, and aliphatic OH calculated based on earlier reports [21, 27].

The phenolic and aliphatic hydroxyl content of the different FCR lignin fractions determined based on ^{31}P NMR spectra (Fig. 3) and the aliphatic and phenolic hydroxyl contents are provided in Table 4. On the basis of Table 4, most phenolic hydroxyl content was attributed to G-unit, the H-unit making the secondary contribution and the phenolic hydroxyl from S-unit was barely detectable. These results are in agreement with the results of HSQC (Fig. 1). Also, the constitution of the phenolic hydroxyl further confirmed that the FCR lignin belongs to the G/S/H class of lignin, just as the 2D-NMR results suggest. The esterified ferulic acid (FA) at γ position (primary OH) of *β-O*-4 bond could be hydrolyzed and released after acid steam-explosion and alkaline treatment [27], and then the free FA could be precipitated with the lignin by adjusting the pH. Consequently, free FA may contribute to the content of phenolic hydroxyl, which is included in the G-unit phenolic hydroxyl. The content of phenolic OH increased after acid steam-explosion, and when the combined severity factors of acid steam-explosion increased from 1.050 to 2.180 (Table S1), the content of phenolic OH in FCR lignin changed from 235 to 289 nmol/g (Table 4). These results suggest that the chemical bonds in FCR lignin (especially *β-O*-4 bond) can be broken by acid steam-explosion to form phenolic hydroxyl [33, 34], which agrees with the observed decrease in the molecular weight of FCR lignin on acid steam-explosion (Table 2).

In the case of the aliphatic OH groups, the content of primary OH groups are decreased by acid steam-explosion and an increase in combined severity factor can further decrease the primary OH content (Table 4; Table S1). When the combined severity factor was changed from 1.050 to 2.180, the primary OH decreased from 736 to 582 nmol/g. Since the primary OH group is mainly attributed to the γ-OH of *β-O*-4 bond that is active chemically, higher temperatures and oxygen concentrations, or extreme pH might destroy its structure [35, 36]. Additionally, when the CS increased from 0 to 1.050, the *α*-OH content of FCR lignin increased from 133 to 186 nmol/g. However, the further increase in severity to 2.720 results in a decrease of *α*-OH content to 159 nmol/g. Usually, the *α*-OH can be etherified in lignin. Acid steam-explosion and alkaline treatment can break the ether-bond to form C_α carbocation [37]. Under the condition of lower CS, the C_α carbocation is prone to form the C_α hydroxyl; while, under the higher CS conditions the C_α carbocation is prone to generate the C_5–C_5 bond. These different reactions might explain the *α*-OH content variation.

The antioxidant activity of FCR lignin increases with increased combined severity factors from 0 to 2.180 (Table S1). Since the enhancement of combined severity factors can increase the content of phenolic hydroxyl, the changes of antioxidant activity of FCR lignin might be attributable to an increase of phenolic hydroxyl content. It is notable that the antioxidant activity of No. 3 was similar to that of BHT (butylated hydroxytoluene), an extensively used antioxidant agent (Table 5), which suggests the potential application of FCR lignin as an antioxidant agent. The bamboo lignin, prepared with the aid of microwave-assisted extraction, shows excellent antioxidant activity, which is higher than that of BHT [9, 38]. It has been demonstrated that the antioxidant activity of lignin is due to the scavenging action of its phenolic structures on oxygen-containing reactive free radicals [9]. Many studies have demonstrated the antioxidant activities of lignin [2, 4, 9], lignin and their related monomeric and dimeric fragments (lignans) have been used as antioxidants in polymeric materials, medicines, cosmetics, feeds, and dietary products [9].

Since cassava is an important crop and the fermented cassava residues are collected from the ethanol-producing fermentation, FCR lignin might be suitable for the food antioxidant. Lignin has been reportedly applied in food oil to prevent oxidant reactions to increase shelf-time [4, 27] and it is considered to be safe for human beings [5]. Given the huge amount of FCR generated annually, FCR lignin could also be considered for application in polymeric materials, medicines, cosmetics, and feeds. Nevertheless, it should be noticed that the inherent hydrophobicity and complex structure of lignin make it less miscible and soluble when blending with other substances or solubilizing into a solution. Consequently, further studies are still needed on the chemical modification of FCR lignin for its practical application in future.

Conclusions

In the present paper, sequential acid steam-explosion and hot-alkaline treatment was developed to prepare lignin from fermented cassava residue. This provides an effective method to convert fermented cassava residue into a valuable compound. This process can generate the lignin in a yield of 74% with a purity of 88% under severity conditions of CS 2.180. The 2D-NMR analysis indicates that FCR lignin belongs to the G/S/H lignin class. The FCR lignin possesses antioxidant activity and in particular, the No. 3 lignin shows antioxidant activity similar to BHT. The antioxidant activity of FCR lignin can be attributed to the phenolic OH groups on the basis of ^{31}P NMR analysis. FCR lignin represents a good antioxidant agent that might be potentially applied

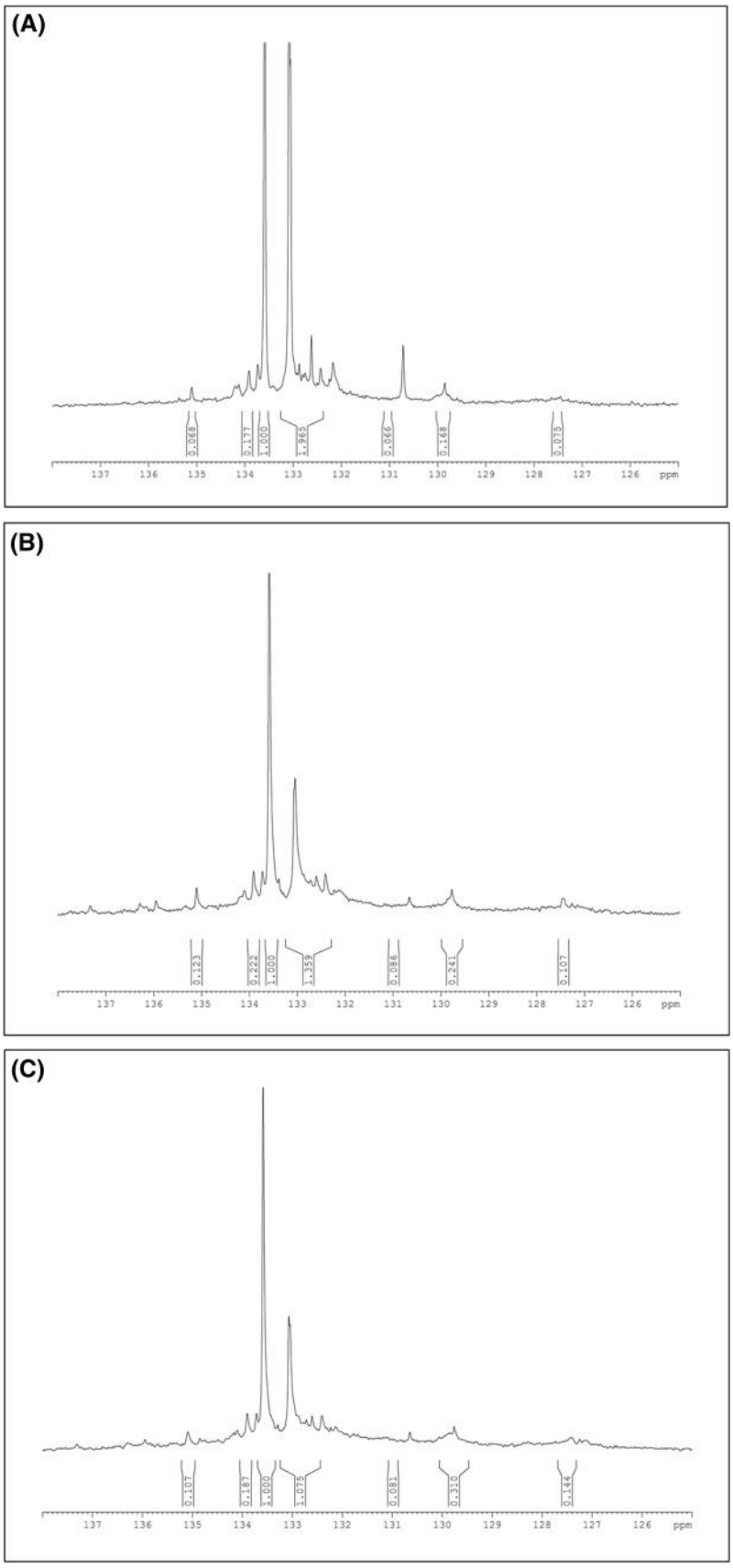

Fig. 3. ^{31}P NMR spectra of the FCR lignin. **a** No. 1 sample, **b** No. 2 sample, **c** No. 3 sample

Table 4 The aliphatic and phenolic hydroxyl contents of FCR lignin fraction determined by ^{31}P NMR

	Lignin fraction		
	1	2	3
Aliphatic OH (nmol/g)	1197	922	741
α-OH	133	186	159
Primary-OH	1064	736	582
Phenolic OH (nmol/g)	167	235	289
S-OH	36	47	44
G-OH	91	130	18
H-OH	41	58	78

Table 5 The antioxidant activity of FCR lignin fractions

	Lignin fraction			
	1	2	3	BHT
DPPH	51.3 ± 0.21	70.64 ± 0.32	84.95 ± 0.25	86.09 ± 0.34
ABTS	40.2 ± 1.40	64.64 ± 2.00	75.95 ± 0.93	83.09 ± 1.34

in food antioxidant or other industrial processes as an antioxidant.

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Compliance with Ethical Standards

Conflict of interest The authors have no conflict of interest.

Research Involving Human and Animal Rights This article does not contain any studies with human participants or animals performed by any of the authors.

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