



Microwave-assisted organic acid extraction of lignin from bamboo: Structure and antioxidant activity investigation

Ming-Fei Li^a, Shao-Ni Sun^a, Feng Xu^{a,*}, Run-Cang Sun^{a,b,*}

^a Institute of Biomass Chemistry and Technology, Beijing Forestry University, 100083 Beijing, China

^b State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, 510640 Guangzhou, China

ARTICLE INFO

Article history:

Received 26 January 2012

Received in revised form 25 February 2012

Accepted 7 March 2012

Available online 19 March 2012

Keywords:

Antioxidant activity

Microwave-assisted extraction

Organic acid

Bamboo

Lignin

HSQC

ABSTRACT

Microwave-assisted extraction in organic acid aqueous solution (formic acid/acetic acid/water, 3/5/2, v/v/v) was applied to isolate lignin from bamboo. Additionally, the structural features of the extracted lignins were thoroughly investigated in terms of C₉ formula, molecular weight distribution, FT-IR, ¹H NMR and HSQC spectroscopy. It was found that with an increase in the severity of microwave-assisted extraction, there was an increase of phenolic hydroxyl content in the lignin. In addition, an increase of the severity resulted in a decrease of the bound carbohydrate content as well as molecular weight of the lignin. Antioxidant activity investigation indicated that the radical scavenging index of the extracted lignins (0.35–1.15) was higher than that of BHT (0.29) but lower than that of BHA (3.85). The results suggested that microwave-assisted organic acid extraction provides a promising way to prepare lignin from bamboo with good antioxidant activity for potential application in the food industry.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Lignin is the third most abundant natural polymer after cellulose and hemicelluloses in the world. In addition to wood, it is widely distributed in the plant cell walls of a variety of vegetative by-products in food industries as well as industrial crops, such as straws of rice, wheat and corn, sugarcane bagasse, tea residue, bamboo culm, etc. Lignin is a radically coupled amorphous macromolecule with three fundamental monolignols, i.e., *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol, together with a minor amount of other monolignols, such as coniferyl aldehyde, acetylated coniferyl alcohol, ferulic acid, etc. Based on the degrees of contribution of monolignols, lignins are usually grouped into three types, guaiacyl (G), syringyl–guaiacyl (SG) and *p*-hydroxyphenyl–syringyl–guaiacyl (HSG) lignin, from softwood, hardwood, and grass, respectively. As a non-toxic and natural phenolic polymer, lignin is widely applied in many fields, due to its structural features. In the food industry, it is a very frequently used feedstock to produce vanillin as a food and beverage flavouring agent (Walton, Mayer, & Narbad, 2003). For instance, lignosulphonates, recovered from the spent liquor in sulphite pulp mill, can be oxidised to vanillin with yields ranging from 4.1% to 7.2% (Bjorsvik, 1999).

* Corresponding authors. Address: Institute of Biomass Chemistry and Technology, Beijing Forestry University, 100083 Beijing, China (R.-C. Sun). Tel./fax: +86 10 62336972.

E-mail addresses: xfx315@bjfu.edu.cn (F. Xu), racsun3@bjfu.edu.cn (R.-C. Sun).

Nevertheless, exploring its application in more valuable forms is attracted increasing attention. It has been reported that plant-derived lignin possesses certain health benefits, such as antibacterial, antiparasitic, antitumoral, antiviral, and immunopotentiating activities (Mitjans & Vinardell, 2005). Lignin isolated from different plant sources has antioxidant properties and can be used as an effective free radical scavenger (Balasundram, Sundram, & Samman, 2006).

The majority of lignin available on an industrial scale is the by-product from spent liquor in chemical pulping mills. Extraction is mainly performed with alkali or acid to delignify wood or grass for the production of pulp at elevated temperatures under high pressures. In addition, lignin is also produced in the pre-treatment process during bio-ethanol production. These pre-treatment methods include sulphuric acid pre-treatment, sodium hydroxide pre-treatment, steam explosion, and so on. Most of these operations are conducted at elevated temperatures, which are usually achieved by conductive heating from an external heat source for several hours. These long reaction times are not desirable because of high energy consumption, as well as multiple unwanted side reactions for lignin (Monteil-Rivera et al., 2012).

Microwave-assisted extraction has been recently explored as a potential technology in terms of the separation of components from lignocelluloses. It is a process using microwave energy, along with solvent, to extract target compounds from various materials. The extraction process shows advantages, including the reduction of process energy requirements, uniform and selective processing,

as well as the ability to start and stop the operation instantaneously. In terms of microwave-assisted extraction of components from plant materials, water is by far the most widely used medium since it is an environmentally friendly and readily available solvent. Microwave-assisted extraction with water is usually operated under high pressure in a closed vessel. In this case, the water with high temperature and pressure has a low dielectric constant and a high quantity of iron products which enables solubilisation of fewer polar substances. For instance, when tea residues were subjected to microwave irradiation treatment at 230 °C, 40.8–48.1% of the solids were dissolved, resulting in the production of 13.9–14.7% of carbohydrates together with 8.7–14.4% polyphenols (Tsubaki, Iida, Sakamoto, & Azuma, 2008; Tsubaki, Sakamoto, & Azuma, 2010). In essence, it is an acid-catalysed extraction process, since organic acids are produced at elevated temperatures. In addition, the extraction efficiency can also be enhanced by the addition of acids, alkalis, hydrogen peroxide, or alcohols, depending on the target products. Organic acids, especially formic acid and acetic acid, are easily recyclable organic solvents showing interesting features as agents for the separation of components from the plant cell wall (Rodriguez & Jimenez, 2008).

Due to the degradation and solubilisation of lignin and hemicelluloses in organic acid, cellulose pulp has been reported to be obtained from a variety of woody and non-woody lignocellulosic materials via organic acid fractionation or pulping. Previously, we have applied formic acid to pulp bamboo, a fast growing monocotyledonous plant with highly lignified structure, and acceptable delignification degrees (over 79.1%) have been achieved in 2–3 h under atmospheric pressure via conventional heating (Li, Sun, Xu, & Sun, 2012). Recently, extraction of degraded hemicellulosic product (xylose) from corn cob in formic acid has been reported, in which a higher xylose yield of 96.3% was obtained in the process of microwave heating, as compared to extraction by conventional oil-bath heating (71.7%) (Wang et al., 2011). However, to our knowledge, the combination of organic acid and microwave treatment aimed at extraction of lignin from lignocelluloses has not been reported.

In the present study, therefore, the combination of microwave heating and organic acid was investigated, aiming at a mild extraction of lignin from bamboo. The extraction was conducted under atmospheric pressure using easily recyclable solvents, i.e., mixtures of formic acid and acetic acid plus a small amount of HCl. The solubilisation behaviour of lignin in organic acids was studied, and the extracted lignin fractions were characterised in terms of elemental analysis, sugar analysis, molecular weights, FT-IR, ¹H NMR, and HSQC spectroscopy. In addition, the antioxidant ability was also evaluated, since it is closely related to the structure as well as potential application. The comprehensive structural characterisation and physicochemical evaluation of the extracted lignin will afford information to help explore its utilisation.

2. Materials and methods

2.1. Materials

Two-year-old bamboo (*Phyllostachys sulphurea* (Carr.) A. et C. Riv.) was freshly harvested from Yunnan province, in southwest China. Its leaves and branches were manually removed. The obtained stem was chopped into small pieces and then air-dried. The pieces of bamboo stem were ground to obtain a fraction of 40–60 mesh particle size. The obtained fraction was first submitted to Soxhlet extraction with toluene/ethanol (2:1, v/v), and then air-dried and stored in a hermetic polypropylene container before use. The chemical composition of the dewaxed bamboo was: carbohydrates 77.03% (arabinose 2.18%, galactose 0.35%, glucose 50.02%,

xylose 22.98%, and glucuronic acid 1.50%), lignin 21.24% and ash 1.27%, determined according to the literature (Li et al., 2012; Peng et al., 2009).

2.2. Microwave-assisted extraction of lignin from bamboo

Microwave-assisted treatment of bamboo was carried out using a microwave extractor (XH-100B, Beijing Xianghu, China) under various times (0–120 min) and temperatures (90 and 109 °C). A power of 700 W was applied to heat the materials inside the oven to the designated temperatures in a short time (109 and 90 °C within 2 and 1.5 min, respectively). In a typical run (e.g., microwave heating at 90 °C for 40 min), 2 g bamboo sample were immersed in 40 mL organic acid aqueous solution (formic acid/acetic acid/water, 3/5/2, v/v/v) with the addition of 6% HCl (based on the dewaxed bamboo) for 20 min. After that, the mixture was placed in the microwave oven under stirring, and the temperature was raised to 90 °C and kept for 40 min. After the extraction, the mixture was cooled to room temperature with tap water. Then the liquor was filtered and concentrated to ~5 mL under reduced pressure at 60 °C. Subsequently, 10 volumes of water were poured into the concentrated liquor to precipitate lignin. The crude lignin was separated by centrifugation and washed with water twice. The obtained solid residue was washed with 90% acetic acid five times, then washed thoroughly with ethanol and water, and dried at 75 °C. The yields of the solid residue and extracted lignin were determined gravimetrically. Solubilisation rate after microwave irradiation was calculated based on the equation:

$$\text{solubilisation rate(\%)} = 100 \times \frac{((\text{initial weight of dewaxed bamboo} - \text{weight of solid residue})}{\text{initial weight of dewaxed bamboo}}.$$

Scanning electron microscopy (SEM) analysis of the bamboo and the solid residue was performed using a Quanta 200F machine (FEI, Hillsboro, OR).

2.3. Compositional analysis of the extracted lignin

Sugar analysis (neutral sugars and uronic acids) was conducted by using high-performance anion exchange chromatography (HPAEC), as previously described (Sun, Li, Yuan, Xu, & Sun, 2012; Wen, Sun, Xu, & Sun, 2010). Elemental analysis was performed using a Vario EL III Elemental analyser (Elementar, Hanau, Germany) according to the literature (Li et al., 2012). Methoxyl content was determined according to Mousavioun and Doherty (2010). Molecular weights of the fractions were determined by gel permeation chromatography (GPC) after acetylation of lignins (Li et al., 2012). The GPC system was calibrated with polystyrene standards.

Fourier-transform infrared (FT-IR) spectroscopic measurements were performed on a Nicolet iN10 spectrophotometer with an MCT detector. The samples were scanned between 4000 and 650 cm⁻¹ at a resolution of 4 cm⁻¹. The solution-state NMR spectra of the samples were acquired on a Bruker AVIII 400 MHz spectrometer at 25 °C. The ¹H NMR spectra were recorded at 100 MHz using 15 mg of acetylated lignin fractions in 1 mL of CDCl₃ using *p*-nitrobenzaldehyde (NBA) as internal standard (Pan, Kadla, Ehara, Gilkes, & Sessler, 2006). For HSQC spectroscopic experiments, the data were acquired in HSQC GE experiment mode using 20 mg lignin in 1 mL DMSO-*d*₆. The parameters for data acquisition were set according to the literature (Li et al., 2012).

2.4. Antioxidant activity against DPPH radical

The antioxidant activity of the specimens was determined by a spectroscopic assay involving the consumption of the stable free radical originating from DPPH in ethanol solution. The colorimetric

assay was performed according to a modification of the method described by Blois (1958). Briefly, a lignin sample solution in 0.1 mL dioxane–water (9/1, v/v) was added to 3.9 mL of a DPPH solution (25 mg/L in ethanol) as the free radical source and kept for 30 min at room temperature. The decrease of the solution absorbance, due to proton-donating activity, was measured at 517 nm using a UV 2300 spectrometer (Shanghai Tianmei Science and Technology Corporation, China). The DPPH radical-scavenging activity was calculated using the following formula:

$$\text{DPPH radical scavenging activity(\%)} = (A_0 - A_1)/A_0$$

where A_0 is the absorbance of control, and A_1 is the absorbance of lignin sample. DPPH radical-scavenging activity was plotted as a function of the lignin concentration. From this graph, the lignin concentration needed to achieve DPPH radical-scavenging activity of 50% was defined as EC_{50} . Radical scavenging index (RSI) was defined as the inverse of EC_{50} . The analyses were performed in triplicate.

3. Results and discussion

3.1. Solubilisation of bamboo in microwave-assisted extraction

CIMV process, developed in recent years, is a technology based on the treatment of lignocelluloses with a mixture of formic acid/acetic acid/water (Delmas, 2008). This process has been successfully applied to various species, especially grasses, such as sugarcane, sweet sorghum, and alfa grass (Abdelkafi et al., 2011; Delmas, 2008), to produce pure cellulose, xylose and its derivatives together with value-added lignin. Generally, under the conventional conduction heating, a long time (3 h) is needed to achieve an efficient solubilisation of the raw material. In order to accelerate the extraction, microwave-assisted heating was adopted in the present study. In a preliminary investigation, extraction with a mixture of formic acid/acetic acid/water only resulted in slightly solubilisation of bamboo, whereas the addition of 6% HCl led to an ideal solubilisation. Therefore, a further examination of the effect of microwave-enhanced extraction was conducted based on the conditions above. The solubilisation profile of the bamboo at different temperatures is shown in Fig. 1a and the corresponding yield of the extracted lignin is illustrated in Fig. 1b. As seen, during the increase of temperature to the target temperature, a certain amount of the raw material had been released. A rise of temperature from 25 to 90 °C led to dissolution of 14.16% of the raw material and yielded 3.48% lignin fraction, whereas an elevation of temperature from 25 to 109 °C in 2 min resulted in dissolution of 28.86% of the raw material, accompanied by a yield of lignin fraction of 8.56%. At 90 °C, when the extraction time at the target temperature reached 10 min, 42.25% of the raw

material was dissolved; a further increase of the time to 60 min resulted in the release of 50.60% of the bamboo, and a prolongation of time to 120 min only resulted in a very slight increase of the solubilisation rate. Correspondingly, the yields of the lignin fractions were 15.16%, 17.98% and 18.51% for the extractions at 109 °C for 10, 60 and 120 min. Clearly, an increase of time from 60 to 120 min at both 90 and 109 °C did not induce a further enhancement of the solubilisation rate. Extraction at 109 °C for 60 min resulted in a degree of delignification of over 80%. The extraction efficiency in the present study was comparable to that reported by Monteil-Rivera et al. (2012), in which over 70% of the lignin in raw material was released in the microwave-assisted extraction of lignin from triticale straw under pressure (125 °C, 81% ethanol, 0.5 N H₂SO₄, 30 min, and microwave power 1600 W).

SEM observation of the original and treated bamboo indicated that the extraction induced physical changes of the raw material (Fig. S1). The solid residue fractions B_{M10} , B_{M60} , B_{H10} , and B_{H60} represent the treated bamboo samples for 10 min at 90 °C, 60 min at 90 °C, 10 min at 109 °C, and 60 min at 109 °C, respectively. Clearly, the untreated bamboo (B) has a completely fibrillar and compact morphology. For a short time, microwave treatment at a low temperature did not induce remarkable changes of the morphology of the bamboo (B_{M10}), and the image for the sample subjected to a treatment at a high temperature showed that many fibres were still bound together, indicating partially defibrillation (B_{H10}). When the irradiation time was prolonged, the disruption of the lignocellulosic structure became more pronounced. Samples B_{M60} and B_{H60} exhibited smaller diameter of particles owing to fundamental delignification. In addition, some cracks were also observed on their surface. The defibrillated solid residue with more accessible surface area was a good feedstock for further enzymatic hydrolysis. Similar structural change has also been reported for rice straw treated with microwaves in aqueous acetic acid for enzymatic hydrolysis (Gong, Liu, & Huang, 2010).

3.2. Chemical component analysis and molecular weight of the extracted lignin

To reveal the effect of the extraction on the structural changes of lignin, four typical lignin fractions L_{M10} , L_{M60} , L_{H10} , and L_{H60} , corresponding to the recovered lignin fractions prepared under microwave-assisted extraction for 10 min at 90 °C, 60 min at 90 °C, 10 min at 109 °C, and 60 min at 109 °C were further investigated. Elemental analysis and methoxyl contents determined for the studied lignins are shown in Table 1 along with the calculated C_9 formulae. No nitrogen was detected in any of the samples, indicating that the extracted lignin was not contaminated by protein. This

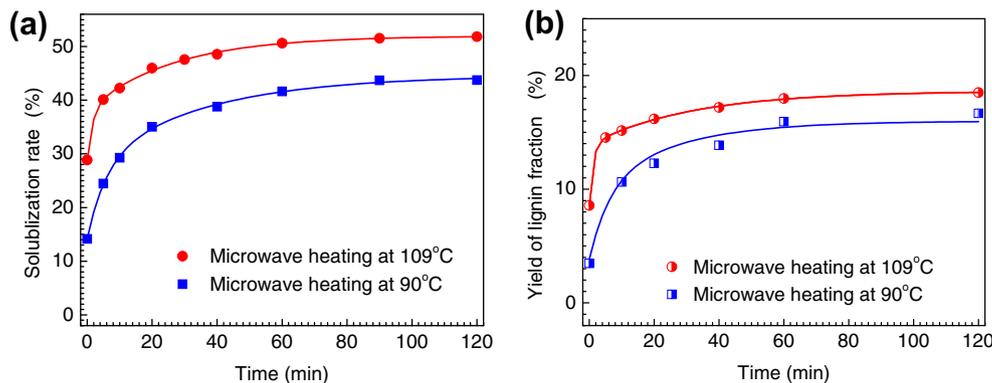


Fig. 1. Effect of microwave heating time on (a) solubilisation rate and (b) the yield of lignin fraction during microwave-assisted organic acid extraction.

Table 1
Elemental analysis, methoxyl content, and C₉ formula of bamboo lignins extracted by microwave heating in organic acids.

Lignin samples ^a	Elemental component (%)				C ₉ formula	C ₉ weight (g/mol)
	C	H	O	OCH ₃		
L _{M10}	60.82	5.62	33.57	19.18	C ₉ H _{7.20} O _{2.76} (OCH ₃) _{1.32}	200.48
L _{M60}	61.86	5.62	32.52	19.25	C ₉ H _{7.09} O _{2.60} (OCH ₃) _{1.29}	196.87
L _{H10}	62.21	5.56	32.23	18.89	C ₉ H _{7.13} O _{2.66} (OCH ₃) _{1.22}	195.70
L _{H60}	64.22	5.49	30.29	18.80	C ₉ H _{6.81} O _{2.38} (OCH ₃) _{1.16}	189.04

^a L_{M10}, L_{M60}, L_{H10}, and L_{H60} represent lignin fractions extracted by microwave heating for 10 min at 90 °C, 60 min at 90 °C, 10 min at 109 °C, and 60 min at 109 °C, respectively.

phenomenon was different from the case during alkaline extraction in which the lignin obtained had a relatively high amount of bound protein (Nadji et al., 2009). Apparently, there was an increase of carbon content together with a decrease of oxygen content with increased extraction temperature and time. In addition, both the increase of extraction temperature and time enhanced the release of OCH₃ groups in lignin, as indicated by the reduction of the number of OCH₃ groups in the C₉ formula. This was mainly due to the demethylation reaction under more drastic conditions. In fact, demethylation has been reported in the delignification process during formic acid pulping (Perez, Terrones, Grelier, Nourmode, & Castellan, 1998).

Sugar analysis results of the extracted lignin fractions are illustrated in Table S1. It was obvious that the extracted lignin fractions obtained at a low temperature of 90 °C had a relatively higher amount of sugars than those obtained at 109 °C. Comparatively, L_{H60} obtained under the severest conditions had the lowest amount of bound sugars. In all cases, xylose was the predominant sugar, followed by arabinose, glucuronic acid and glucose, together with a small amount of galactose. An increase of the severity, especially in the case of prolongation of the time from 10 to 60 min at 109 °C, resulted in a noticeable decrease of the xylose content, together with a relatively slight decrease of the arabinose content. In the plant cell wall of bamboo, arabinoxylan is the major carbohydrate which is closely linked with hemicelluloses via arabinose side chains of xylan by ether bonds (Buranov & Mazza, 2008; Wen et al., 2010). The results in the present study indicated that the glycosidic bonds between the sugar units in the backbone of the bound hemicelluloses were more sensitive to the severity of microwave heating than the ether linkages between lignin and arabinose.

The molecular weight distribution of the extracted lignin samples was analysed using gel permeation chromatography (GPC). As seen from Fig. S2, all the samples show unimodal patterns, with their major peaks observed at 6180, 6410, 5936, and 4950 g/mol in lignin fractions L_{M10}, L_{M60}, L_{H10}, and L_{H60}, respectively. The results of molecular weight for these samples can be compared because polystyrene standards were used for calibration (Sun et al., 2012). Based on the chromatograph, weight-average (\bar{M}_w) and number-average (\bar{M}_n) molecular weights and polydispersity (\bar{M}_w/\bar{M}_n) of the preparations were calculated and are presented in Table S2. As for the polydispersity, all lignin fractions possessed narrow molecular weight distributions ($\bar{M}_w/\bar{M}_n < 2$). When the microwave-assisted extraction was conducted at a low temperature, the extracted lignins had high molecular weights (9230–9400 g/mol). However, a significant reduction of the molecular weight and a narrower molecular weight distribution were observed in the lignin fraction L_{H60} (\bar{M}_w 6060 g/mol, \bar{M}_w/\bar{M}_n 1.61). It has been reported that there are two major reactions under acidic conditions: lignin degradation via acidolysis of ether bonds and polymerisation by acid-catalysed condensation (Vanderghem, Richel, Jacquet, Blecker, & Paquot, 2011). Obviously, the progressive decrease of the molecular weight at higher temperature indicated the preferential occurrence of degradation.

3.3. Spectroscopic analysis of the extracted lignin

To compare the structural changes that occurred during the extraction process, FT-IR spectra of the lignin fractions were recorded (Fig. S3). The FT-IR spectra of the lignin fractions presented typical HSG lignin absorption bands. A comparison of the fingerprint region between the extracted lignin fractions in the present study with the milled wood lignin of bamboo (Li et al., 2012), indicated the similarity of their major bands. Although their intensities differed, the presence of the bands confirmed that the “core” structure of lignin did not change dramatically after the extraction. The bands around 1596, 1505, and 1420 cm⁻¹ in all lignins, are characteristic of aromatic ring vibrations of phenylpropane groups. Absorptions at 1324 and 1111 cm⁻¹ correspond to syringyl groups and those around 1268 and 1161 cm⁻¹ to guaiacyl units. Asymmetric C–H deformation in methyl, methylene and methoxyl groups was observed at 1456 cm⁻¹. The peak at 833 cm⁻¹, a typical signal in herb plants, is attributed to C–H out-of-plane in positions 2 and 6 of S units, and in all positions of H units. In addition, a wide absorption band at 3426 cm⁻¹ is attributed to aromatic and aliphatic OH groups, whereas the peaks at 2942 and 2846 cm⁻¹ are assigned to C–H stretching in methyl, methylene and methoxyl groups. The strong signals at 1724 cm⁻¹ are ascribed to ester group C=O stretching, mainly due to *p*-hydroxycinnamate, carbohydrate esters, and acetate groups (Abdelkafi et al., 2011; Sun, Sun, & Xu, 2002). The acetate groups were natural or introduced during the extraction process in the organic acid medium. Similar spectrum behaviour has been observed in a CIMV process reported previously (Abdelkafi et al., 2011), in which alfa grass was delignified in acetic acid/formic acid/water via a conventional heating approach. Overall, the microwave-assisted extraction process did not induce significant changes in the structure of bamboo lignin.

¹H NMR spectra of the acetylated lignin samples are shown in Fig. S4. The spectra of the four lignin preparations show similar spectra indicating their analogous structure. Since there were signals overlapping in ¹H NMR spectra, the main typical signals were roughly identified and summarised as follows. The strong peak at 3.6 ppm was clear evidence of the methoxyl substituent of constitutive aromatic monolignols. The region between 7.5 and 6.0 ppm is related to aromatic protons, and inter-unit ether and C–C linkages between lignin units show signals at 6.1–4.0 ppm. Signals at 2.5–2.2 ppm are attributed to the protons in aromatic acetates whereas those at 2.2–1.6 ppm are related to the protons in aliphatic acetates. In addition, aliphatic groups were detected at 1.6 and 1.3 ppm. Quantitative analysis of phenolic and aliphatic hydroxyl groups of lignin was determined according to a previous paper (Pan et al., 2006), and the results are shown in Fig. 2. As seen, a high severity of microwave treatment resulted in higher amounts of both phenolic and aliphatic hydroxyl groups. The increase of phenolic hydroxyl content in lignin samples was contributed by the cleaving of alkyl–aryl ether linkages during the extraction process, which resulted in the formation of new phenolic end groups and thus increased the phenolic hydroxyl content in the sample.

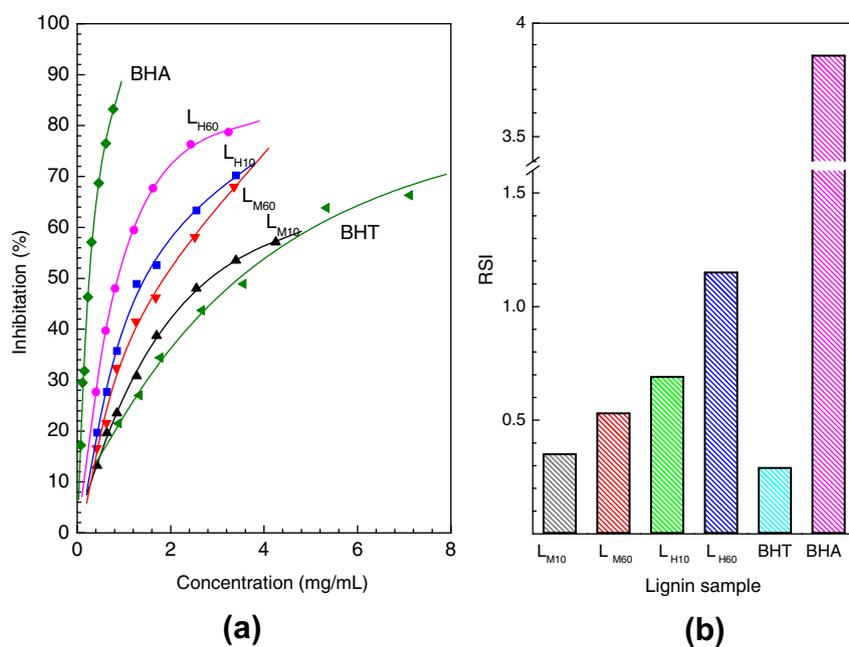


Fig. 4. Antioxidant activity against DPPH of bamboo lignins extracted by microwave heating in organic acids as compared to BHA and BHT. (a) DPPH inhibitory effect; and (b) RSI value.

were characterised by their correlations at 85.0/4.64 (B_{α}), 53.4/3.07 (B_{β}), 70.8/3.78 and 70.7/4.16 ppm (B_{γ}), and resinol substructures were also present with their signals at 87.1/5.44 (C_{α}), 53.0/3.46 (C_{β}), and 62.5/3.74 ppm (C_{γ} , can be seen in higher intensity of contour line). In the aromatic region, H, G, and S units were easily detected by their specific correlations at 128.5/6.96, 111.7/6.94, 1118.6/6.79, 103.6/6.68 ppm, corresponding to $H_{2/6}$, G_2 , G_6 and $S_{2/6}$ positions. The oxidised (α -ketone) structure of syringyl lignin (S') was identified at δ_C/δ_H 103.6/6.68 ppm. The presence of *p*-coumarate and ferulate esters was confirmed due to the correlation signals at 144.8/7.44 and 113.7/6.30 ppm. In addition, signals corresponding to coniferaldehyde groups were observed at 153.4/7.62 (J_{α}), 126.0/6.78 (J_{β}), 111.2/7.32 ($J-G_2$), 130.0/7.48 ($J-G_6$) ppm. Obviously, from L_{M10} to L_{H60} , the intensities of the signals of the linkages (based on the same intensities in the aromatic region) decreased with increased treatment severity. This phenomenon supported the increase of the cleaving of the linkages between lignin units with the increase of the microwave-assisted extraction time and temperature. The abundance of the linkages as well as the S/G ratio of the samples was estimated and the data are listed in Table 2. Overall, with increased severity, a marked decrease of the relative abundance of β -O-4 linkages was observed accompanying an increase of those of the phenyl coumaran and resinol substructures. This suggested that β -O-4 linkages were readily degraded with increasing severity of the microwave treatment. Additionally, there was a slight increase of S/G ratio from 1.00 in L_{M10} to 1.18 in L_{H60} , suggesting an increase of the relative content of the S units with increasing severity.

3.4. Antioxidant activity of the lignins

The antioxidant activity of the lignin samples was investigated in comparison with two typical commercial antioxidants, BHA and BHT, and the curves of the DPPH inhibitory effects of these specimens are shown in Fig. 4. Clearly, all lignin samples exhibited antioxidant activity, and their effect against DPPH increased with increasing concentration. The RSI values of L_{M10} , L_{M60} , L_{H10} , and

L_{H60} were 0.35, 0.53, 0.69, and 1.15, as compared to 0.29 for BHT, and 3.85 for BHA, respectively. The data suggested that all the lignin samples had higher antioxidant activity than BHT but lower than BHA. L_{H60} had DPPH inhibitory effect comparable to that of the acetic acid lignin extracted at 200 °C for 120 min in a previous report (Lu et al., 2012). Generally, the capacity of phenolic compound depended on its ability to form phenoxyl radical by donating H atom as well as the stability of the formed free radical. Overall, the increase of the antioxidant activity of the lignin samples in the present study was in agreement with the increase of the amount of phenolic hydroxyl groups. Carbohydrates bound with lignin had a negative effect on the antioxidant activity, since the formation of hydrogen bonding with lignin impeded the formation of free radical. In addition, it should be noted that although the mechanism has not been clearly confirmed, aliphatic hydroxyl groups did not contribute to the DPPH inhibitory effect, but a significantly high amount of aliphatic hydroxyl groups resulted in a decrease of the radical-scavenging index (RSI) value in lignin as reported previously (Pan et al., 2006). This may explain the fact that the lignin sample L_{H10} had higher amounts of aliphatic hydroxyl groups but exhibited a slightly lower DPPH inhibitory effect than L_{M60} , although they had comparable phenolic hydroxyl groups. It has been reported that soda lignin of alfa grass showed antioxidant activity but it was lower than BHT (Nadji et al., 2009). The results above indicated that microwave-assisted extraction in organic acid can produce lignin with relatively high antioxidant activity. Lignin from green tea waste has also been reported to have antioxidant effect against the autoxidation of linoleic acid (Toh, Yokoyama, Noda, & Yuguchi, 2010). The relatively high antioxidant of the lignin in the present study suggests that it may be a potential antioxidant of food oils and fats used for food preservatives, which can prevent the loss of food colour, flavour, and active vitamins content. The extracted lignin provided a natural and relatively safe source for antioxidant as compared to the costs and the relatively poor efficiency of natural tocopherols for synthetic antioxidants (e.g., BHA, BHT) (Faustino, Gil, Baptista, & Duarte, 2010). In addition, it has been demonstrated that lignin is not harmful to eye and skin, indicating that this would open new perspectives for its

additional application in cosmetic and topical formulations (Vinar-dell, Ugartondo, & Mitjans, 2008).

4. Conclusions

Bamboo lignin was successfully extracted by microwave heating in organic acid aqueous solution under mild conditions. An increase of the extraction temperature markedly increased the yield of the extracted lignin. Structural investigation of the extracted lignin indicated that there was an increase of the phenolic hydroxyl content in the lignin, due to the cleavage of the linkages between lignin units (mainly β -O-4 linkages). In addition, an increase of the severity of the microwave-assisted extraction resulted in a decrease of bound carbohydrate content and molecular weight of the lignin. Under the optimal conditions, i.e., microwave-assisted extraction at 109 °C for 60 min, a lignin yield of 17.98% was achieved. The obtained lignin fraction had a low bound sugar content (1.81%), together with a low weight-average molecular weight (M_w 6070 g/mol), and showed good antioxidant activity (RSI 1.15), which was higher than that of BHT but lower than that of BHA. This suggested that microwave-assisted organic acid extraction provided a promising way to isolate lignin from bamboo for potential application as a natural antioxidant. Nevertheless, safety testing is needed, since its natural origin and environmental-friendly preparation do not assure its non-toxicity in humans.

Acknowledgements

The authors wish to express their gratitude for the financial support from the National Natural Science Foundation of China (31110103902), Major State Basic Research Projects of China (973-2010CB732204), and the Ministry of Education (111).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2012.03.037>.

References

- Abdelkafi, F., Ammar, H., Rousseau, B., Tessier, M., El Gharbi, R., & Fradet, A. (2011). Structural analysis of alfa grass (*Stipa tenacissima* L.) lignin obtained by acetic acid/formic acid delignification. *Biomacromolecules*, *12*, 3895–3902.
- Balasundram, N., Sundram, K., & Samman, S. (2006). Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chemistry*, *99*, 191–203.
- Bjorsvik, H. R. (1999). Fine chemicals from lignosulfonates. 1. Synthesis of vanillin by oxidation of lignosulfonates. *Organic Process Research and Development*, *3*, 330–340.
- Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, *181*, 1199–1200.
- Buranov, A. U., & Mazza, G. (2008). Lignin in straw of herbaceous crops. *Industrial Crops and Products*, *28*, 237–259.
- Delmas, M. (2008). Vegetal refining and agrichemistry. *Chemical Engineering and Technology*, *31*, 792–797.
- Faustino, H., Gil, N., Baptista, C., & Duarte, A. P. (2010). Antioxidant activity of lignin phenolic compounds extracted from kraft and sulphite black liquors. *Molecules*, *15*, 9308–9322.
- Gong, G. F., Liu, D. Y., & Huang, Y. D. (2010). Microwave-assisted organic acid pretreatment for enzymatic hydrolysis of rice straw. *Biosystems Engineering*, *107*, 67–73.
- Li, M. F., Sun, S. N., Xu, F., & Sun, R. C. (2012). Formic acid based organosolv pulping of bamboo (*Phyllostachys acuta*): Comparative characterization of the dissolved lignins with milled wood lignin. *Chemical Engineering Journal*, *179*, 80–89.
- Lu, Q., Liu, W., Yang, L., Zu, Y., Zu, B., Zhu, M., et al. (2012). Investigation of the effects of different organosolv pulping methods on antioxidant capacity and extraction efficiency of lignin. *Food Chemistry*, *131*, 313–317.
- Mitjans, M., & Vinar-dell, M. (2005). Biological activity and health benefits of lignans and lignins. *Trends Comp. Biochem. Physiol.*, *11*, 55–62.
- Monteil-Rivera, F., Huang, G. H., Paquet, L., Deschamps, S., Beaulieu, C., & Hawari, J. (2012). Microwave-assisted extraction of lignin from triticale straw: Optimization and microwave effects. *Bioresource Technology*, *104*, 775–782.
- Mousavioun, P., & Doherty, W. O. S. (2010). Chemical and thermal properties of fractionated bagasse soda lignin. *Industrial Crops and Products*, *31*, 52–58.
- Nadji, H., Diouf, P. N., Benaboura, A., Bedard, Y., Riedl, B., & Stevanovic, T. (2009). Comparative study of lignins isolated from Alfa grass (*Stipa tenacissima* L.). *Bioresource Technology*, *100*, 3585–3592.
- Pan, X. J., Kadla, J. F., Ehara, K., Gilkes, N., & Saddler, J. N. (2006). Organosolv ethanol lignin from hybrid poplar as a radical scavenger: Relationship between lignin structure, extraction conditions, and antioxidant activity. *Journal of Agricultural and Food Chemistry*, *54*, 5806–5813.
- Peng, F., Ren, J. L., Xu, F., Bian, J., Peng, P., & Sun, R. C. (2009). Comparative study of hemicelluloses obtained by graded ethanol precipitation from sugarcane bagasse. *Journal of Agricultural and Food Chemistry*, *57*, 6305–6317.
- Perez, D. D., Terrones, M. G. H., Grelier, S., Nourmamode, A., & Castellan, A. (1998). Peroxyformic acid pulping of *Eucalyptus grandis* wood chips and sugar cane bagasse in one stage and characterization of the isolated lignins. *Journal of Wood Chemistry and Technology*, *18*, 333–365.
- Rencoret, J., Marques, G., Gutiérrez, A., Nieto, L., Jiménez-Barbero, J., Martínez, A. T., et al. (2009). Isolation and structural characterization of the milled-wood lignin from *Paulownia fortunei* wood. *Industrial Crops and Products*, *30*, 137–143.
- Rodríguez, A., & Jimenez, L. (2008). Pulping with organic solvents other than alcohols. *Afinidad*, *65*, 188–196.
- Sun, R. C., Sun, X. F., & Xu, X. P. (2002). Effect of ultrasound on the physicochemical properties of organosolv lignins from wheat straw. *Journal of Applied Polymer Science*, *84*, 2512–2522.
- Sun, S. N., Li, M. F., Yuan, T. Q., Xu, F., & Sun, R. C. (2012). Sequential extractions and structural characterization of lignin with ethanol and alkali from bamboo (*Neosinocalamus affinis*). *Industrial Crops and Products*, *37*, 51–60.
- Toh, K., Yokoyama, H., Noda, H., & Yuguchi, Y. (2010). Antioxidant capacity of lignin from green tea waste. *Journal of Food Biochemistry*, *34*, 192–206.
- Tsubaki, S., Iida, H., Sakamoto, M., & Azuma, J. I. (2008). Microwave heating of tea residue yields polysaccharides, polyphenols, and plant biopolyester. *Journal of Agricultural and Food Chemistry*, *56*, 11293–11299.
- Tsubaki, S., Sakamoto, M., & Azuma, J. (2010). Microwave-assisted extraction of phenolic compounds from tea residues under autohydrolytic conditions. *Food Chemistry*, *123*, 1255–1258.
- Vanderghem, C., Richel, A., Jacquet, N., Blecker, C., & Paquot, M. (2011). Impact of formic/acetic acid and ammonia pre-treatments on chemical structure and physico-chemical properties of *Miscanthus x giganteus* lignins. *Polymer Degradation and Stability*, *96*, 1761–1770.
- Vinar-dell, M. P., Ugartondo, V., & Mitjans, M. (2008). Potential applications of antioxidant lignins from different sources. *Industrial Crops and Products*, *27*, 220–223.
- Walton, N. J., Mayer, M. J., & Narbad, A. (2003). Vanillin. *Phytochemistry*, *63*, 505–515.
- Wang, L. L., Jiang, Y. J., Li, C. H., Li, X. T., Meng, L. Q., Wang, W., & Mu, X. D. (2011). Microwave-assisted hydrolysis of corn cob for xylose production in formic acid. In *Materials for Renewable Energy & Environment* (Vol. 2, pp. 332–335).
- Wen, J. L., Sun, Y. C., Xu, F., & Sun, R. C. (2010). Fractional isolation and chemical structure of hemicellulosic polymers obtained from *Bambusa rigida* species. *Journal of Agricultural and Food Chemistry*, *58*, 11372–11383.