Antioxidant Activity of Organosolv Lignin Degraded Using SO_4^{2-}/ZrO_2 as Catalyst

Shengming Zhang, Yan Zhang, Liang Liu, and Guizhen Fang*

Organosolv lignin degradation was carried out through hydrogenolysis routes with SO_4^{2-}/ZrO_2 as the catalyst. Structural characterization and antioxidant activity of organosolv lignin samples were investigated; the antioxidant activity of the organosolv lignin was compared with that of soda lignin. Results showed that the active functional groups contents of organosolv lignin were increased, and the antioxidant activity of organosolv lignin was improved. The total hydroxyl and phenolic hydroxyl group contents of organosolv lignin were increased by 15.81% and 26.55%, respectively, and the M_w was decreased from 5739 g/mol to 4499 g/mol. The IC_{50} of organosolv lignin on DPPH radicals scavenging rate, $ABTS^+$ radicals scavenging rate, and reducing power were decreased by 18.91%, 18.08%, and 8.48%, respectively. The catalyzed organosolv lignin can be used as a natural antioxidant for functional food or in cosmetic and polymeric materials.

Keywords: Organosolv lignin; SO_4^{2-}/ZrO_2; Hydrogenolysis; Degradation; Antioxidant

*Contact information: College of Material Science and Engineering, Northeast Forestry University, Harbin 150040, China; *Corresponding author: fanggz_0@163.com*

INTRODUCTION

Lignins are now considered a main aromatic renewable resource. Large quantities of lignin are available yearly from numerous pulping processes, but its utilization in industrial production is low (Azadi *et al.* 2013; Laurichesse and Averous 2014). For researchers, to obtain the high added value utilization of lignin has been an important issue over a long-term period. Lignin consists of phenylpropane units and contains some active functional groups such as hydroxyl, carbonyl, and carboxyl. According to the structural characteristics of lignin, one likely usage is as a type of polymer antioxidant. The antioxidant activity of a polymer is dependent on the phenolic hydroxyl content and polymer environment (Ge *et al.* 2014).

Currently the main source of lignin is from the pulp and paper industries (Laurichesse and Averous 2014). Conventional pulping processes result in lignin structures that are seriously destroyed and have many impurities. In that frame, these types of lignin are not suitable as antioxidants. Therefore, choosing an appropriate type of lignin and improving its chemical reactivity are key points to large-scale usage for application as antioxidants.

Recently, the method of hydrogenolysis has been shown to be an effective way to improve the activity of lignin (Zhang *et al.* 2014). Fang *et al.* (2005, 2007) and Zhang *et al.* (2014) have developed a series of carbon-based metal catalysts and acid catalysts in order to improve the activity of lignin. Acid catalysis is an important method of hydrogenation reaction. SO_4^{2-}/ZrO_2, as a kind of environmentally friendly solid acid, has many advantages, such as high acid strength, small causticity for equipment, easy

separation, and the capacity to be used repeatedly (Yu *et al.* 2009). In some chemical reactions, Lewis acid sites and Bronsted acid sites of SO_4^{2-}/ZrO_2 provide the activation center (Salomatina *et al.* 2007). Zhang *et al.* (2014) used SO_4^{2-}/ZrO_2 to degrade soda lignin and obtained good effects. After catalysis, the phenolic hydroxyl group content of soda lignin was increased, and the antioxidant activity of soda lignin was improved. Soda lignin was extracted from black liquor. Lots of impurities such as Na, S, and ash were contained in the lignin, and the contents of active functional groups were low. Organosolv lignin, which was extracted directly from wheat straw, proved to be more advantageous compared with soda lignin and was much more suitable for use as a natural antioxidant for functional food or in cosmetic and polymeric materials.

In this work, organosolv lignin was catalyzed with SO_4^{2-}/ZrO_2. The structural characteristics of lignin were identified by means of chemical analysis, elemental analysis, UV spectroscopy, Fourier transform infrared spectroscopy (FT-IR spectra), and gel permeation chromatography (GPC). The antioxidant activity of lignin was evaluated using DPPH radicals scavenging, $ABTS^+$ radicals scavenging, and reducing power measures. The relationships between the contents of hydroxyl groups, molecular weight, and antioxidant activity were also discussed.

EXPERIMENTAL

Materials

The organosolv lignin used in this study was obtained from College of Materials Science and Technology of Beijing Forestry University, China. It was extracted from wheat straw using an organic solvent and low concentration sodium hydroxide solution. The elemental analysis result showed that the content of C, H, N, S, and O were 64.58%, 6.24%, 1.90%, 0%, and 27.28%, respectively. In organosolv lignin, the content of acid-insoluble lignin, acid-soluble lignin, total sugars, and ash were 94.68%, 1.64%, 3.22%, and 0.46%, respectively. The organosolv lignin had purities of $>$97.9%.

Soda lignin, as a contrast sample, was obtained from black liquor dry matter (Shandong Tralin Paper Co., China) through the acid-precipitation method (Zhang *et al.* 2014). In soda lignin, the content of C, H, N, S, and O were 56.72%, 5.78%, 3.48%, 2.49%, and 26.87%, respectively. The content of acid-insoluble lignin, acid-soluble lignin, total sugars, and ash were 89.26%, 2.88%, 3.20%, and 4.66%, respectively. The soda lignin had purities of $>$92.14%.

SO_4^{2-}/ZrO_2 was prepared using the method by Zhang *et al.* (2014) and Salomatina *et al.* (2007). In brief, zirconium hydroxide was prepared from an aqueous solution containing 13% zirconium oxychloride by adding dropwise an ammonium hydroxide solution up to pH = 9-10. After washing and drying at 110 ºC, the hydroxide was impregnated with 1 mol/L sulphuric acid for 12 h and dried at 110 ºC. The sample was then calcined at 600 ºC in an air stream for 3 h.

The acid strength of SO_4^{2-}/ZrO_2 was measured using the Hammett indicator method, and H_0=14.06 (Zhang *et al.* 2014). The elemental analysis results showed that the content of S was 4%. The BET surface area and microporous surface area of the SO_4^{2-}/ZrO_2 catalysts were 36.27 m^2/g and 2.26 m^2/g, respectively.

Methods

Degradation of organosolv lignin

The depolymerization reactions were conducted in a 1-L reaction kettle (Dalian-Controlled Plant Co., China). The reaction condition of soda lignin degradation was used the method that Zhang *et al.* (2014) described. A certain amount of lignin was dissolved in the dioxane-water (9:1, v/v) solution, and the amount of 5 wt% catalyst was added to the mixture; then the mixture was added to the reactor. The reaction conditions were as follows: the system was pressured into the initial pressure of 3.0 MPa with hydrogen and heated at 100 ºC for 4 h with a stirring speed of 400 rpm. After reaction, the catalyst was separated from the mixture through decompression filtration. The product was dried from the mixture through a reduced pressure distillation.

Characterization of products

FT-IR spectra were recorded on a Persee FTIR-650 spectrometer (Tianjin Gangdong Sci. & Tech. Development Co., China) from 4000 to 500 cm^{-1} with 16 scans at a resolution of 2 cm^{-1}. The total hydroxyl content of lignin was measured using chemical titration method that Zhang *et al.* (2014) described. In brief, lignin was acetylated using acetic anhydride, pyridine and dioxane mixture, and then titrated with sodium hydroxide solution. The content of total hydroxyl was calculated. The content of phenolic hydroxyl group was determined through ultraviolet-spectroscopy (Lai and Funaoka 1993). This method is based on the difference in absorptions at 300 nm and 360 nm between the phenolic units in the neutral and alkaline solutions. The content of ionizing phenol hydroxyl groups can be quantitatively evaluated through comparing the $\Delta\varepsilon$ values of the substance studied at certain wavelengths to the values of $\Delta\varepsilon$ of the respective model compounds. The number-average molecular weight (M_n), weight-average molecular weight (M_w), and polydispersity degree (M_w/M_n) of acetylated lignins were measured using GPC on an Agilent 1100 high-performance liquid chromatography apparatus (Agilent Technologies Inc., USA) with a UV Detector (254 nm). Tetrahydrofuran was used as an eluent with a flow rate of 1.0 mL/min. The injected sample volume was 50 μL, and the column was kept at 30 ºC. The columns were calibrated using polystyrene standards in the range of 580–504500 g/mol.

Antioxidant activity

The DPPH radical (DPPH•) scavenging assay was performed using the method that Aadil *et al.* (2014) and Zhang *et al.* (2014) described, with some modifications. Various concentration lignin samples (1 mL) in ethanol were added to 4 mL of a 0.2 mmol/L DPPH in ethanol. The reaction mixture was shaken and incubated in the dark for 30 min at room temperature, and then the absorbance was measured at 517 nm. The scavenging activity was calculated as follows (Eq. 1),

$$\text{Scavenging activity}(\%) = (A_{blank} - A_{sample} \div A_{blank}) \times 100\% \quad (1)$$

where A_{blank} is the absorbance of the control and A_{sample} is the absorbance of the sample. The results were expressed in terms of IC_{50}, the concentration of each lignin required to quench 50% of the initial DPPH•. A commonly used antioxidants butylated hydroxytoluene (BHT) was chosen as a reference.

For the $ABTS^+$ radical ($ABTS^+$•) scavenging assay, the method that Thana *et al.* (2008) and Zhang *et al.* (2014) described was followed with some modifications. Briefly,

the $ABTS^{+}$• was produced through reacting 7.4 mM of ABTS stock solution with 2.6 mM of potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. A volume of 1 mL of $ABTS^{+}$• solution was diluted with the phosphate buffer to A_{734}=0.700 (±0.050) and equilibrated at 25 ºC with the solvent that was used for assay. The sample solution (0.1 mL) was mixed with 4.9 mL of the $ABTS^{+}$• solution in the cuvette. The absorbance at 734 nm was read at ambient temperature after 10 min. The phosphate buffer was measured as a blank sample. The scavenging activity was calculated as described in DPPH assay.

The reducing power of lignin was determined according to the method of Lu *et al.* (2012). The lignin sample solution (1 mL) was mixed with 2 mol/L sodium phosphate buffer (2.5 mL) and 1% potassium ferricyanide solution at 50 ºC and incubated for 20 min. After 10% trichloroacetic acid was added, the mixture was centrifuged for 10 min. The supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and 0.1% $FeCl_3$ (0.5 mL), then the absorbance was measured at 700 nm. The absorption value was recorded at 0.5 as IC_{50}, and the scavenging activity was calculated as described in the DPPH assay.

RESULTS AND DISCUSSION

Characterization of Products

FT-IR spectra of organosolv lignin (OL) and catalyzed organosolv lignin (Catalyzed-OL) are shown in Fig. 1. For the two samples, the peaks located at 1327 and 1128 cm^{-1} were considered to correspond to the characteristic vibrations of the syringyl units (Zhou *et al.* 2012). Ring breathing with C-O stretching of both the syringyl and guaiacyl structures was associated with 1224 cm^{-1} (Gonzalez *et al.* 2009). The peaks at 1265 and 1045 cm^{-1} were attributed to C-O stretching of guaiacyl structures (Li *et al.* 2014).

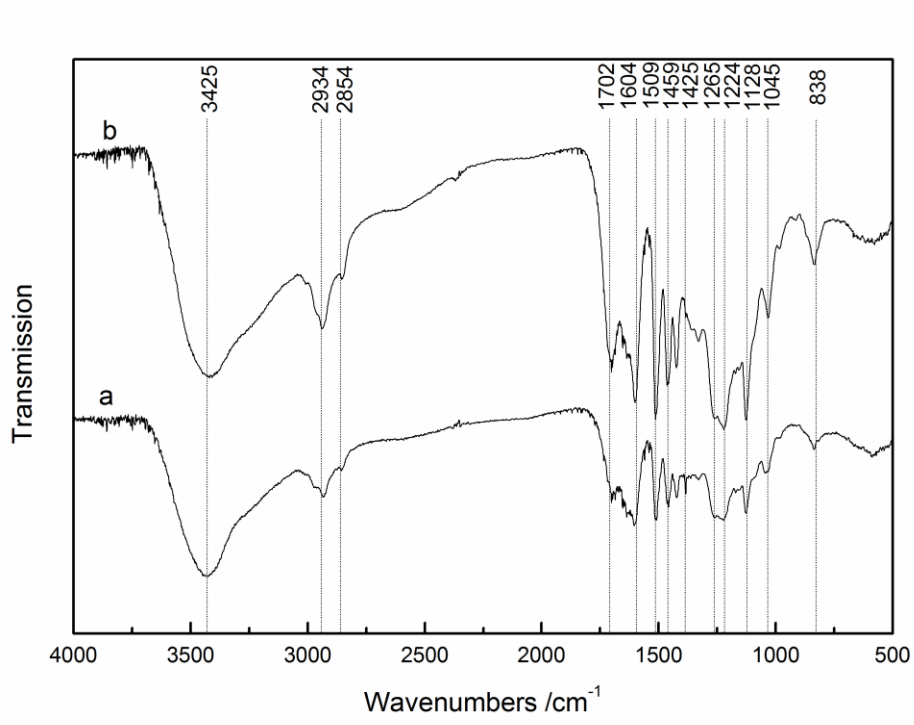

Fig. 1. FT-IR spectra of OL (a) and Catalyzed-OL (b)

The peaks at 1160 cm^{-1} were attributed to the conjugated C=O in the ester groups of the typical for G-S-H (Guaiacyl, Syringyl, and *para*-hydroxy-phenyl lignin) (Faix 1991). The peak at 838 cm^{-1} was attributed to C-H stretching of syringyl and all positions of H units (Li *et al.* 2014). The organosolv lignin was G-S-H typical. The peak at 3425 cm^{-1} was assigned to the stretch of OH groups. The peaks at 2934 and 2854 cm^{-1} in the spectra were due to the CH stretch in CH_2 and CH_3 groups. The characteristic stretching vibration of the carbonyl was regarded to be 1702 cm^{-1}.

The bands at 1604, 1509, and 1425 cm^{-1} corresponded to the aromatic ring vibrations of phenyl-propane (C_9) skeleton and the C-H deformation combined with aromatic ring vibration at 1459 cm^{-1} was observed in all spectra (Faix 1991). Although the intensity of the bands differs, the aromaticity of different samples remained stable and lignin did not change dramatically with the reaction.

The hydroxyl functional group contents of all lignin samples are mentioned in Table.1. The phenolic hydroxyl content of organosolv lignin (OL) was higher than soda lignin (SL) and catalyzed organosolv lignin (Catalyzed-OL) was higher than catalyzed soda lignin (Catalyzed-SL). Through the catalysis of hydrogenolysis, the total hydroxyl and phenolic hydroxyl contents of Catalyzed-OL were increased by 15.81% and 26.55% compared with OL. Using the same catalytic method, the total hydroxyl and phenolic hydroxyl contents of Catalyzed-SL were increased by 55.15% and 13.89%, compared with SL.

The phenolic hydroxyl content of Catalyzed-OL was the highest in all samples. Hydroxyl functional groups are the important active functional groups of lignin. Some literature has reported (Kang *et al.* 2011; Ge *et al.* 2014) that antioxidant activity and phenolic hydroxyl groups have a positive correlation. Through the catalysis, the activity of both organosolv lignin and soda lignin were improved. The degradation method of hydrogenolysis using SO_4^{2-}/ZrO_2 as a catalyst is effective for lignin.

Table 1. Functional Group and Average Molecular Weight of Lignin Samples

	Total hydroxyl groups (%)	Phenolic hydroxyl groups (%)	M_w (g•mol^{-1})	M_n (g•mol^{-1})	M_w/M_n
OL	5.44	2.90	5739	3617	1.59
Catalyzed-OL	6.30	3.67	4499	2210	2.04
SL	6.02	2.88	7652	4847	1.58
Catalyzed-SL	9.34	3.28	5816	3164	1.84

Weight-average molar mass (M_w), number-average molar mass (M_n), and molar mass dispersities (M_w/M_n) of lignin samples are shown in Table. 1. The M_w and M_n values of OL and SL were reduced in various degrees after hydrogenation and activation. The M_w and M_n of OL were decreased from 5739 g/mol to 4499 g/mol and 3617 g/mol to 2210 g/mol, respectively. The M_w and M_n of SL were decreased from 7652 g/mol to 5816 g/mol and 4847 g/mol to 3164 g/mol, respectively.

The data for molar mass dispersities (M_w/M_n) indicated that the homogeneity of OL and SL were changed to a broad range after catalysis. The results showed that the OL and SL were degraded after catalysis.

Antioxidant Activities

The DPPH• scavenging activity of lignin samples are shown in Fig. 2. After catalysis, the DPPH• scavenging activity of OL and SL both were improved. IC_{50} is the half maximal inhibitory concentration of free radicals. A higher antioxidant activity resulted in a lower IC_{50} value (Ge *et al.* 2015). The IC_{50} of lignin samples are shown in Table 2. The lowest IC_{50} value of lignin samples was observed in the Catalyzed-OL, which decreased by 18.91% compared with OL. It has been reported that the DPPH• scavenging activity of lignin could be due to the phenolic hydroxyl groups and molecular weight (Aadil *et al.* 2014). Furthermore, the antioxidant activity and phenolic hydroxyl groups have a positive correlation, and high molecular weight enhances the heterogeneity of lignin, which leads to a decreased free radical scavenging activity (Dizhbite *et al.* 2004; Ugartondo *et al.* 2008). In the experiment, the high DPPH• scavenging activity and low IC_{50} value of Catalyzed-OL may be ascribed to the high phenolic hydroxyl groups and low molecular weight. Meanwhile, these explanations were demonstrated in functional group tests and GPC analysis. In the following research, the relations between the contents of hydroxyl groups, molecular weight, and antioxidant activity will be discussed in detail.

Table 2. Antioxidant Activity of Lignin Samples

Sample	IC_{50}, μg/mL, in the tests with:		
	DPPH•	$ABTS^{+}$•	Reducing power
SL	97.1	208.2	300.2
Catalyzed-SL	90.3	167.6	284.1
OL	88.3	178.1	290.0
Catalyzed-OL	71.6	145.9	265.4
BHT	31.6	79.6	60.9

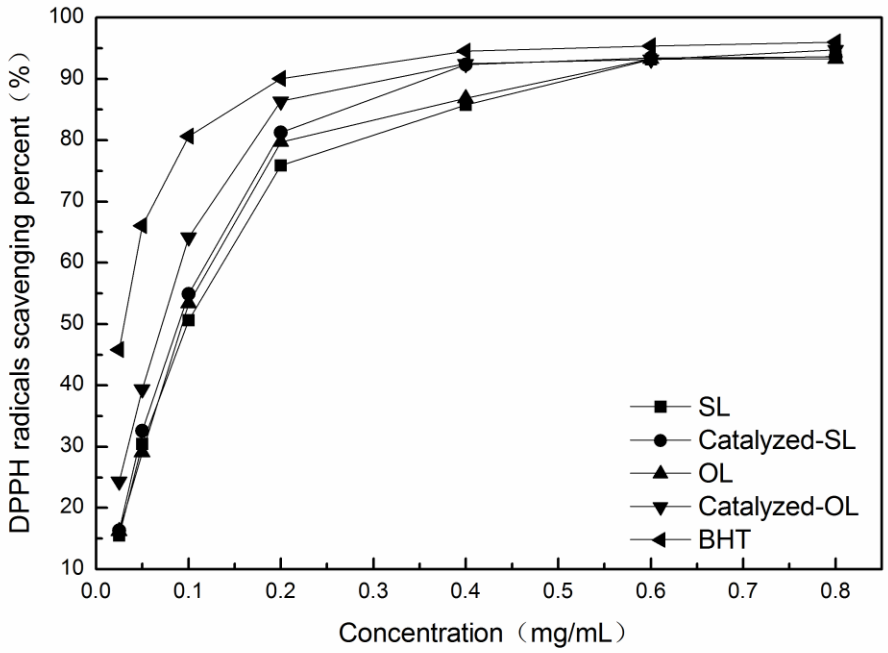

Fig. 2. DPPH radicals scavenging percent of lignin samples

The $ABTS^{+}$• scavenging activity of lignin samples are shown in Fig. 3. The highest $ABTS^{+}$• scavenging activity of lignin samples was observed in Catalyzed-OL.

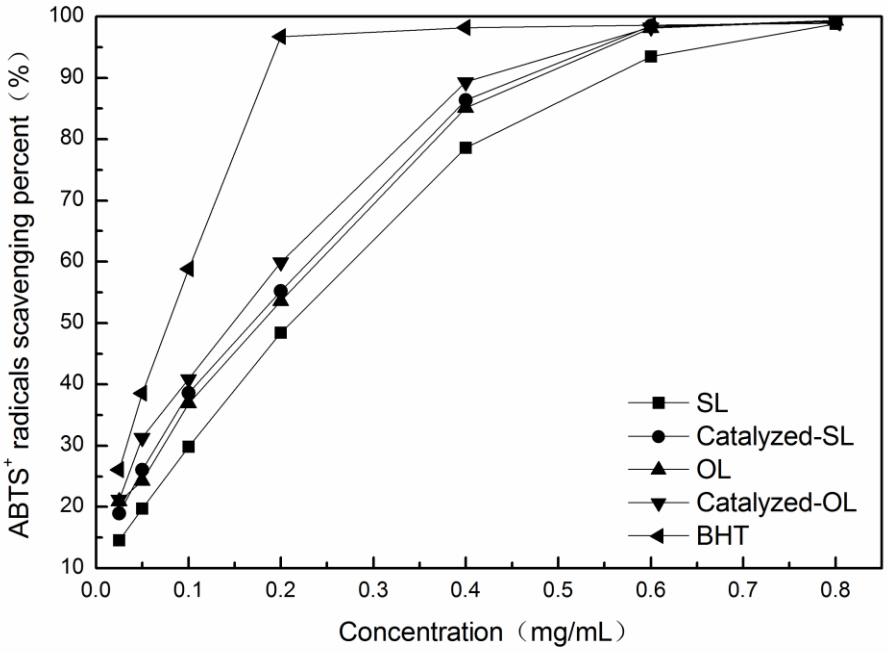

Fig. 3. $ABTS^{+}$ radicals scavenging percent of lignin samples

The IC_{50} values of lignin samples are shown in Table 2. The lowest IC_{50} value of lignin samples was observed in the Catalyzed-OL followed with the Catalyzed-SL, OL, and SL. Catalyzed-OL exhibited the lowest IC_{50} value 145.9 μg/mL, which was less than OL by 18.08%. Some literature on the lignin's antioxidant activity provided evidence that the scavenging activity of lignin on $ABTS^+$ radical is mainly due to the electron or proton transfer mechanism (Arshanitsa *et al.* 2013; Aadil *et al.* 2014). The high scavenging activity of Catalyzed-OL for $ABTS^{+}$• was possibly due to the high phenolic hydroxyl content.

The reducing power of lignin samples are shown in Fig. 4, and the IC_{50} values of lignin samples are shown in Table 2. The lowest IC_{50} value of lignin samples 265.4 μg/mL was observed in the Catalyzed-OL, which was decreased by 8.48% compared with OL. The reducing power assay is based on the mechanism of electro donating activity, which is the main mechanism of phenolic antioxidant action (Aadil *et al.* 2014). The strong reducing power of Catalyzed-OL may be because the sample had more phenolic hydroxyl groups than others. The functional groups test agreed with these explanations.

In Fig. 5, the relation between the antioxidant activity, contents of hydroxyl groups and molecular weight of lignin samples can be evaluated. The strongest antioxidant activity of lignin samples was observed in the Catalyzed-OL followed by the Catalyzed-SL, OL, and SL. The total hydroxyls group content of Catalyzed-SL was the highest in all lignin samples, and the antioxidant activity of Catalyzed-SL was less than Catalyzed-OL. Compared with OL, the Catalyzed-SL much higher total hydroxyl group content was expected, and the antioxidant activity was similar. Meanwhile, there was no correlation between antioxidant activity and the total hydroxyl groups of all lignin samples (R^2=0.1227). In all samples, the antioxidant activity and phenolic hydroxyl groups had a positive correlation (R^2=0.9036). These results indicated that the antioxidant property of lignin mainly depended on the content of phenolic hydroxyl groups.

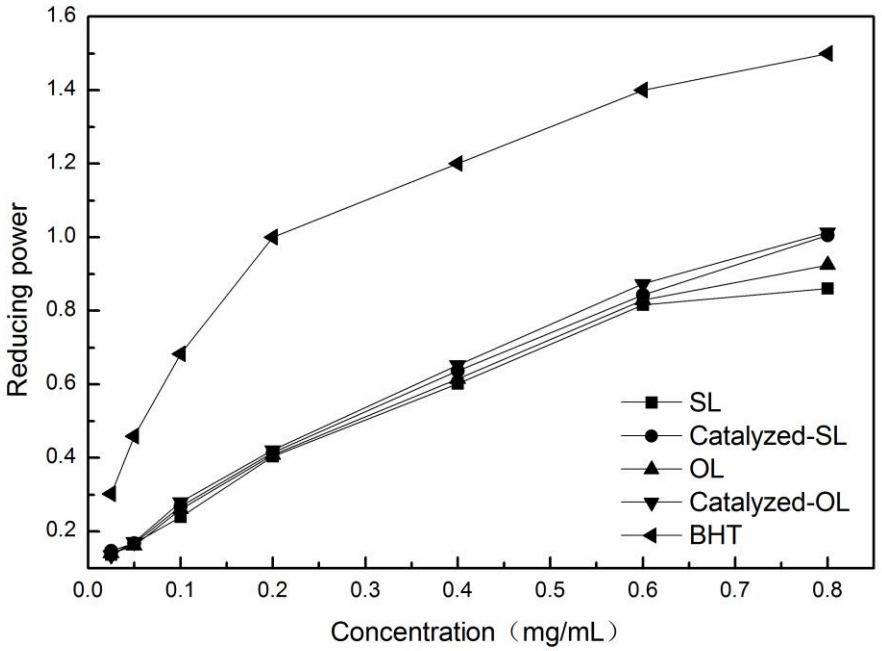

Fig. 4. Reducing power of lignin samples

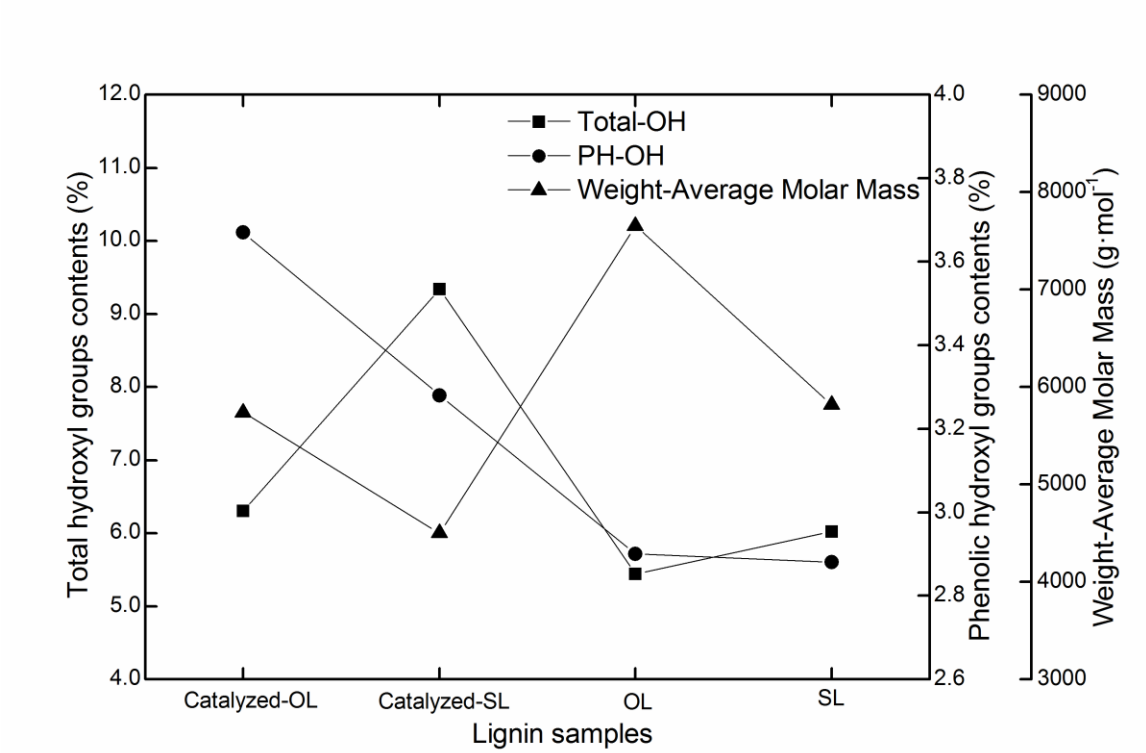

Fig. 5. Correlation between antioxidant activity, total hydroxyl, phenolic hydroxyl, and weight-average molar mass

For the same type of lignin as the OL and Catalyzed-OL, the low molecular weight sample showed good antioxidant activity. For different types of lignin such as the Catalyzed-SL and OL, the low molecular weight sample did not show good antioxidant activity. In all samples, there was no correlation between antioxidant activity and the weight-average molar mass (R^2=0.1131).

Various studies have reported that low molecular weight may have resulted from extensive depolymerization of the lignin, *i.e*., cleavage of ether linkages, which led to the formation of new phenolic hydroxyl groups, the center responsible for the trapping of radicals (Ge *et al.* 2014). So the higher hydroxyl group content is the most important factor for improving the antioxidant properties of lignin. The molecular weight of lignin can be only used as a reference condition.

In general, the antioxidant activity of organosolv lignin was improved after catalysis. The antioxidant activity of organosolv lignin was better than soda lignin. The butylated hydroxytoluene (BHT), which is one kind of commercially available antioxidant, was used as a reference in this study. Although the antioxidant activity of Catalyzed-OL was not better than BHT as a natural polymer compound, lignin may hopefully be used as a natural antioxidant for functional food or in cosmetic and polymeric materials.

CONCLUSIONS

1. Organosolv lignin was activated and degraded through hydrogenolysis routes using the SO_4^{2-}/ZrO_2 as catalyst. After catalysis, the average molecular weight of organosolv lignin was reduced, and the active functional groups content of organosolv lignin was increased.
2. Catalyzed organosolv lignin showed good antioxidant activity. The DPPH scavenging activity, $ABTS^+$ scavenging activity, and reducing power of organosolv lignin were improved by catalysis. A higher quantity of phenolic hydroxyl groups was observed to positively influence the antioxidant activity of lignin.
3. The catalyzed-organosolv lignin showed low impurities, harmlessness to the human body, and good antioxidant activity. And it can be used as natural additives for function food or in cosmetic and polymeric formulations.

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