

ANTIOXIDANT ABILITIES COMPARISON OF LIGNINS WITH THEIR HYDROTHERMAL LIQUEFACTION PRODUCTS

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Black liquor alkaline lignin and magnesium lignosulfonate were liquefied at 320 °C. The antioxidant abilities of the liquefaction products were compared with the raw materials. Results showed that the total phenol content and unit antioxidant power of both alkaline lignin liquefaction products (ALLP) and magnesium lignosulfonate liquefaction products (MLLP) were improved, and ALLP had a larger increase than MLLP. The influence of reaction time and temperature on oil yield, total phenol content, and antioxidant power of ALLP was evaluated. The total phenol content was found to have certain relationships with the antioxidant abilities. These results explore a new approach for further studies and applications of liquid antioxidant from lignins.

Keywords: Alkaline lignin; Lignosulfonate; Antioxidant; Hydrothermal; Liquefaction

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INTRODUCTION

Black liquor alkaline lignin and lignosulfonates are common components of pulp and paper wastewaters, and they are hazardous environmental pollution sources. Lignins are polyphenolic compounds that contain phenolic groups, which possess antioxidant characteristics. Antioxidant effects of lignins have been reported in rubber, plastic, thermomechanical pulp, medicines, and dietary products (Xin and Saka 2009). Recent studies have revealed the efficiency, as antioxidants in different composite materials, of lignins isolated from wood by different methods of delignification, including alkaline pulping and some lignin-related monomeric and dimeric compounds (Dobelet et al. 2009). Bhat et al. (2009) have explored the antioxidant potential of lignin isolated from black liquor of oil palm waste, and they found that the extracted lignin exhibited rich antioxidant/free radical scavenging potential. Ugartondo et al. (2008) found that lingo-sulfonates have a high antioxidant capacity over a range of concentrations, and the suitability of these lignosulfonates was assessed for new commercial applications in cosmetics and pharmaceuticals.

These reports suggest that lignins from pulp and paper wastewater can be a source for antioxidant production. However, good solubility and mobility are important factors affecting the stabilisation properties of antioxidants. Lignins are polar polymers, and thus exhibit a very poor solubility in some apolar media, and lignosulfonates are insoluble in common organic solvents. These factors may limit their antioxidant reactivity and then limit their applications. Also, the high molecular weight and polydispersity of lignins are factors that decrease radical scavenging activity (Pan et al. 2006). Lignins in

hydrothermal conditions can be degraded to low molecular weight products, mainly phenolic compounds. Such phenolic compounds possess antioxidant properties and better solubility in most apolar or polar media. However, almost all the research in hydrothermal conditions has focused on conversion of lignins into value-added products such as bio-oil or energy gas. By contrast, the present work investigates and compares the antioxidant abilities of alkaline lignin and magnesium lignosulfonate with that of their hydrothermal liquefaction products.

EXPERIMENTAL

Materials

Alkaline lignin was obtained from Wuhan East China Chemical Co., Ltd., China. Magnesium lignosulfonate was obtained from The Jiangmen Sugarcane Chemical Factory (Group) Co., Ltd., China. 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals (97%) and 2,4,6-tri-2-pyridyl-s-triazin (TPTZ) were obtained from Tokyo Chemical Industry Co., Ltd., Japan. Folin–Ciocalteu phenol reagent was obtained from Guangzhou Qiyun Biotechnology Co., Ltd., China.

Liquefaction Method

Hydrothermal liquefaction experiments were conducted in a 250 mL stainless steel 316 autoclave with 1.5 kW heating power. The autoclave was loaded with 5.0 g alkaline lignin (or magnesium lignosulfonate) and 60 mL water. Then the reactor was purged 5 times with nitrogen to remove the inside air. After that the autoclave was under an initial nitrogen pressure of 2.0 MPa. The reactants were stirred at 100 rpm. When the temperature reached the setting values, the reaction time was recorded as zero, and thereafter the reaction time was recorded. The liquid and solid products were separated after reaction. Oil 1 was the extracted oil phase from liquid products with 250 mL CH₂Cl₂ used 5 times, and oil 2 was the extracted liquid phase of solid products obtained via soxhlet extractor with 60 mL CH₂Cl₂. Then oil 1 and oil 2 were mixed, and the mixture was distilled at 45 °C to exclude CH₂Cl₂. Then the remaining material consisted of the alkaline lignin liquefaction products (ALLP) (or magnesium lignosulfonate liquefaction products (MLLP)). The extracted solid products (residues) were dried and weighed. For the subsequent total phenol content and antioxidant power tests, the ALLP, MLLP, alkaline lignin, and lignosulfonate with the same concentrations, 0.53g/L were prepared.

Total Phenol Content Assay

The Folin-Ciocalteu method was used to test total phenol content. Results were expressed in g gallic acid (GA)/100g. The absorbance was measured at 760 nm.

DPPH Method

The antiradical activities of various antioxidants were determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (Brand-Williams et al. 1995). 0.2 mL samples (0.53g/L) were mixed with DPPH radical ethanol solutions, and then kept in the shade for 30 min, the absorbance at 517 nm was recorded A_i . As the method of measuring A_i , the

absorbance of the solution containing all the reagent beside sample was recorded as A_0 , The DPPH radicals scavenging percentages were calculated as $[(A_0 - A_i)/A_0]$.

Ferric Reducing Antioxidant Power (FRAP)

The FRAP method was used to test antioxidant power (Castro et al. 2008), with absorbance tested at 593 nm. An aqueous solution of known Fe^{2+} conc. (in range 0.05 to 1.2 mmol/L) was used for calibration. 0.2 mL samples were mixed with 3 mL water and 2 mL FRAP reagent. Greater reduction of Fe^{3+} to Fe^{2+} means better antioxidant power.

Fourier Transform Infrared Spectroscopy (FT-IR) Analysis

The raw material and their liquefaction products were analyzed with a Vector33 FT-IR spectrophotometer (Bruker Co., Ltd., Germany).

GC-MS Analysis

The composition of ALLP produced at 320 °C, 30 min were analyzed using a Shimadzu QP 2010 Plus equipped with Rxi-5ms column (30 m*0.25 mm*0.25 μm). The temperature of the injector was set at 280 °C. Temperature program: 40 °C (hold 2 min) \rightarrow 180 °C (3 °C/min) \rightarrow 280 °C (20 °C/min, hold 5 min). The compounds in the ALLP were identified by means of the NIST08 and NIST08s mass spectral data libraries.

RESULTS AND DISCUSSION

The oil and residue yields are shown in Fig. 1. Both the oil and residue yields of alkaline lignin were less than that of magnesium lignosulfonate, which may be explained by the difference in composition. The alkali in alkaline lignin is an effective catalyst, which may inhibit char formation (possibly through decomposition of HCHO) and promote gas formation (through water gas shift reaction) (Watanabe et al. 2003). The char formation and alkaline effects mechanism are shown in Fig. 2 (Watanabe et al. 2003; Osada et al. 2006). In the alkaline catalyst system, lignins are easier to decompose and more gases are produced. On the other hand, the alkali can be removed from the liquefaction products due to its water solubility during the products separation.

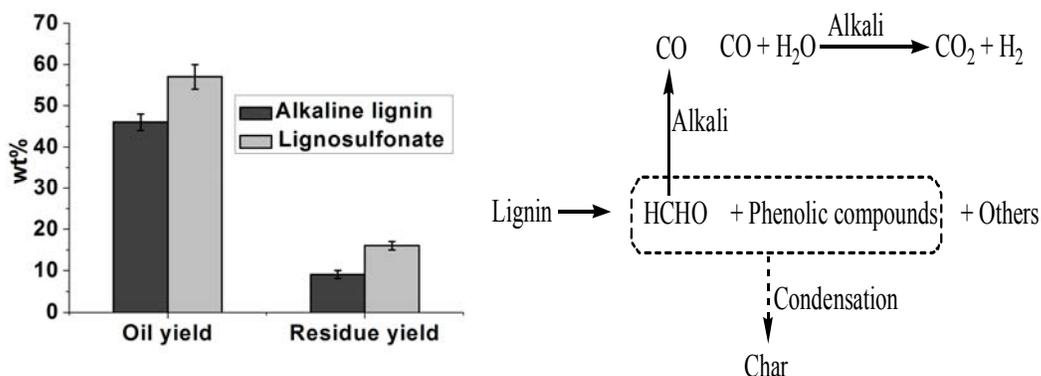


Fig. 1. (left) Oil and residue yield after liquefaction at 320 °C, 30 min

Fig. 2. (right) Char formation and alkaline effects mechanism

The alkaline lignin and ALLP were characterized by FTIR in the region of 4000 to 500 cm^{-1} , as shown in Fig. 3A. Both spectra show most of the characteristic bands of lignin, including 1510 and 1600 cm^{-1} (aromatic ring vibrations) and 1460 cm^{-1} (CH deformation and aromatic ring vibrations). The absorbance peak of O-H stretching vibrations between 3300 and 3600 cm^{-1} are due to alcoholic or phenolic components. The peaks around 1260 and 1220 cm^{-1} indicate the possible presence of phenols and esters, showing the O-H deformation vibration and C=O stretching. The peaks around 690, 740, and 810 cm^{-1} indicated the presence of monosubstituted and polysubstituted aromatic groups. The FTIR spectra change of MLLP with liginosulfonate is similar to that of ALLP with alkaline lignin, as shown in Fig. 3B.

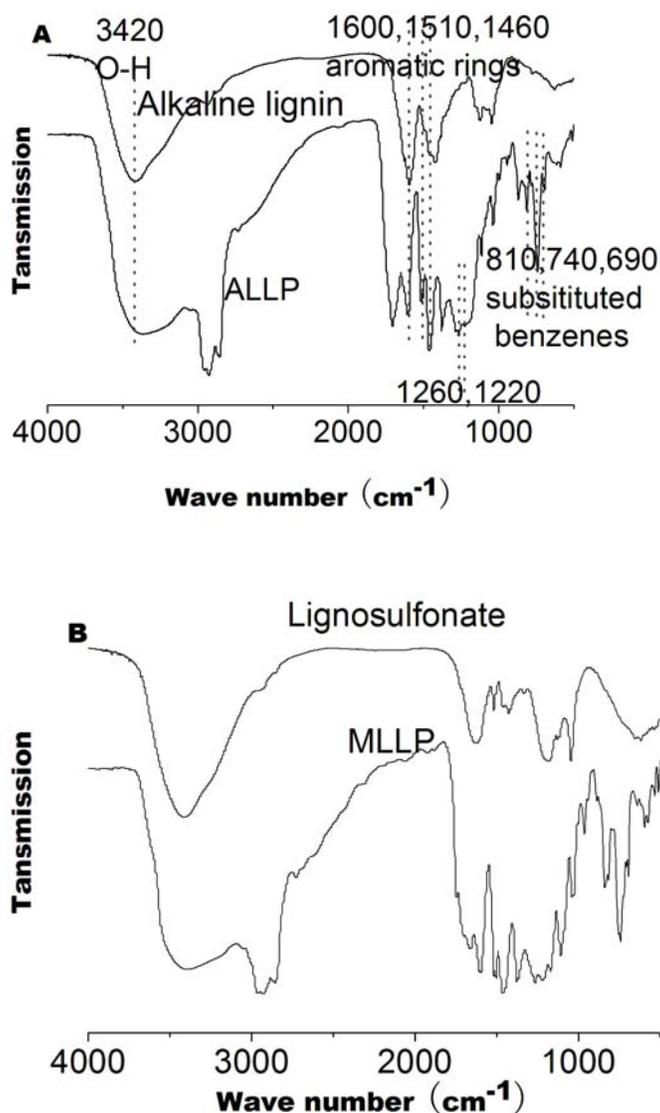


Fig. 3. FT-IR spectra: (A) alkaline lignin with ALLP; (B) liginosulfonate with MLLP. The ALLP and MLLP were obtained at 320 °C, 30 min

The FTIR spectra indicate that alkaline lignin was decomposed to some low molecular weight phenolic compounds. This finding was confirmed by GC-MS. As shown in Fig. 4 and Table 1, phenols were the main products in ALLP. However, the decomposition of alkaline lignin and lignosulfonate hardly changed the distribution of the functional groups, which means that the liquefaction products have the potential to possess antioxidant properties. Moreover, it has been reported that low molecular weight lignin showed high antioxidant activity (Pan et al. 2006).

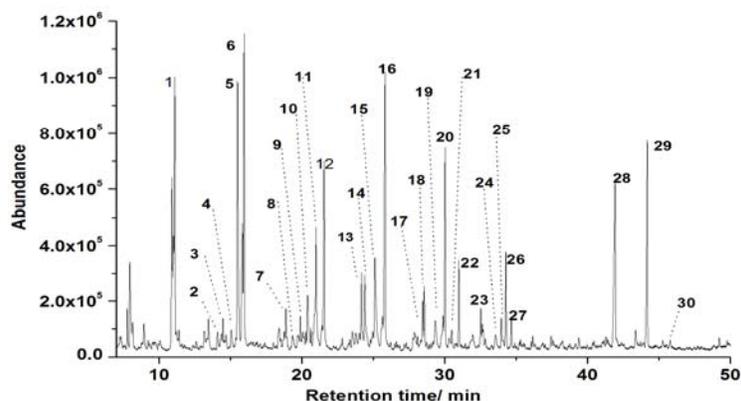


Fig. 4. GC-MS analysis of ALLP obtained at 320 °C, 30min.

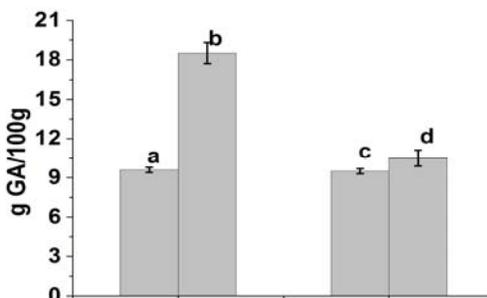
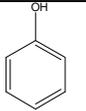
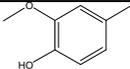
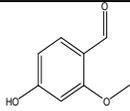
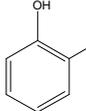
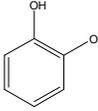
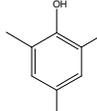
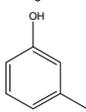
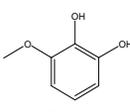
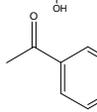
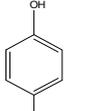
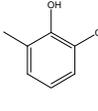
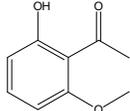
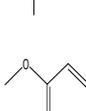
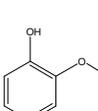
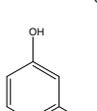
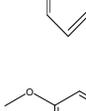
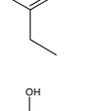
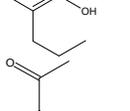
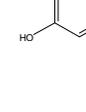
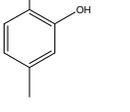
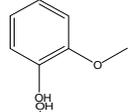
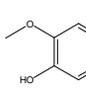
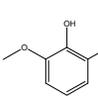
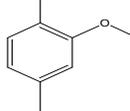
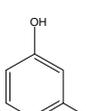
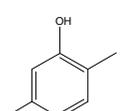
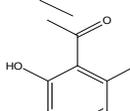
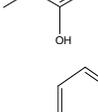
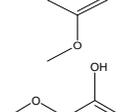


Fig. 5. Total phenol content, **a**: alkaline lignin; **b**: ALLP; **c**: lignosulfonate; and **d**: MLLP. The ALLP and MLLP were obtained at 320 °C, 30min.

The total phenol content (per 100g) of ALLP increased greatly after liquefaction, and it nearly doubled that of alkaline lignin. In contrast, the total phenol content increased relatively little (by only 9.5%) after the liquefaction of magnesium lignosulfonate, as shown in Fig. 5. Compared with the liquefaction products, the alkaline lignin and magnesium lignosulfonate were found to contain a small quantity of water, water-soluble salt, cellulose, and xylan. Water and water-soluble salts were removed or reduced in the liquefaction products. Also, the gasification ratios of cellulose and xylan were much higher than lignin at high temperature (Yoshida et al. 2001, 2004; Izumizaki et al. 2005). Much more cellulose and xylan were converted to gas at higher temperature, and the liquefaction products of cellulose and xylan in ALLP and MLLP may be relatively low. So liquefaction of alkaline lignin is conducive to the increase of phenol content. The mechanism that may account for the relatively low increase of phenol content in MLLP after magnesium lignosulfonate liquefaction is not clear; though one possible reason is

that more phenolic compounds in MLLP result in char formation due to the condensation in the absence of alkali.

Table 1. Phenolic Compounds Detected by GC/MS Analysis as in Fig. 4.

No.	Chemical structure	% Similarity	No.	Chemical structure	% Similarity	No.	Chemical structure	% Similarity
1		96	11		88	21		89
2		92	12		96	22		90
3		92	13		92	23		93
4		89	14		90	24		82
5		92	15		82	25		86
6		97	16		96	26		94
7		85	17		90	27		84
8		87	18		90	28		80
9		92	19		90	29		96
10		91	20		92	30		78

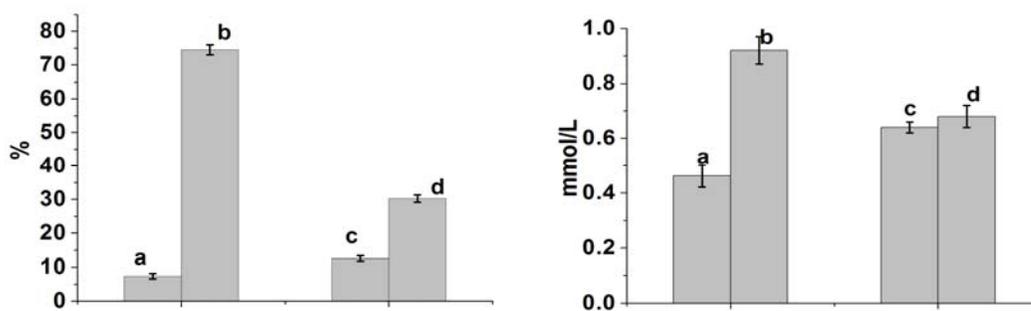


Fig. 6. (left). DPPH radicals scavenging percentages; the test condition was that 0.2 mL samples were mixed with 5 mL 5×10^{-5} mol/L DPPH radical ethanol solutions. **a:** alkaline lignin; **b:** ALLP; **c:** magnesium liginosulfonate; and **d:** MLLP. The ALLP and MLLP were obtained at 320 °C, 30min.

Fig. 7. (right). The detected concentrations of Fe²⁺ by FRAP method. **a:** alkaline lignin; **b:** ALLP; **c:** magnesium liginosulfonate; and **d:** MLLP. The ALLP and MLLP were obtained at 320 °C, 30min.

In Fig. 6, compared with alkaline lignin, the DPPH radicals scavenging percentage was increased from 7.3% to 74.5% by ALLP; and compared with magnesium liginosulfonate, the DPPH radicals scavenging percentage was increased from 12.6% to 30.2% by MLLP. In Fig. 7, the higher concentration of Fe²⁺ implies stronger the reducing power. Compared with alkaline lignin, the concentrations of Fe²⁺ with ALLP addition by FRAP method was nearly doubled, while compared with magnesium liginosulfonate, the concentrations of Fe²⁺ with MLLP addition increased only a little. The reasons for increase of antioxidant power after liquefaction can be explained as due to the higher phenol content, lower molecular weights, and better mobility. Comparing Fig. 5 with Fig. 6, the change degree of the DPPH radicals inhibition was more sensitive than that of Fe²⁺ concentration, which indicates the enhancement in the ability to capture radicals is stronger than that of reduction capacity after liquefaction. Also the results confirm that phenolic compounds as antioxidants mainly depend on their abilities to capture free radicals. Also, the results from Fig. 5-7 demonstrated that ALLP has higher increased antioxidant power than MLLP. So alkaline lignin and DPPH method were selected to test the influence of reaction time and temperature.

The effects of temperature and reaction time on oil yield, total phenol content, and DPPH radicals scavenging percentages of ALLP are shown in Figs. 8 and 9, respectively. The oil yield at 320 °C, 30 min achieved a maximum value, suggesting that a longer residence time and higher temperature are not necessary for a higher yield of the oil production. Two possible reasons to account for the level-off of the oil yields at a prolonged reaction time and elevated temperature would be: (a) cracking of the liquid products to gases and (b) formation of char by condensation, cyclization, and re-polymerization of the liquid products (Xu and Etcheverry 2008; Tymchyshyn and Xu 2010). From Figs. 8 and 9, the total phenol content increased from 280 to 300 °C, and then decreased with further increase of temperature from 300 to 350 °C at a reaction time of 30 min, while the total phenol content decreased markedly with increased reaction time at 320 °C. The results are likely due to that the decomposition degree of alkaline lignin to

phenolic compounds was not high enough at relative low temperature (280 °C), and the longer reaction time and elevated temperature are attributed to the enhanced condensation reactions of the phenolic products to form char, which results in low total phenol content in ALLP. Tymchyshyn and Xu (2010) have found the char formed by phenolic/neutral oils increased with increasing temperature by hydrothermal liquefaction of lignin within the range 250 to 350 °C.

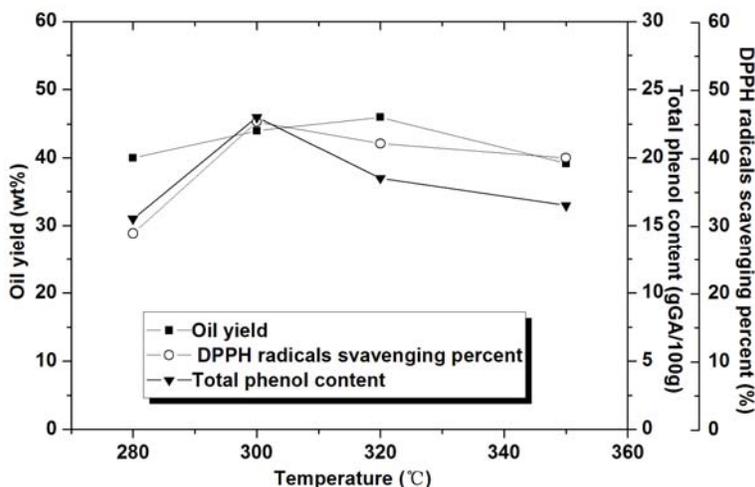


Fig. 8. Oil yield, total phenol content, and DPPH radicals scavenging percentages of ALLP at various temperatures at 30 min. In the DPPH method test condition 0.2 mL samples were mixed with 6 mL 1×10^{-4} mol/L DPPH radicals ethanol solutions.

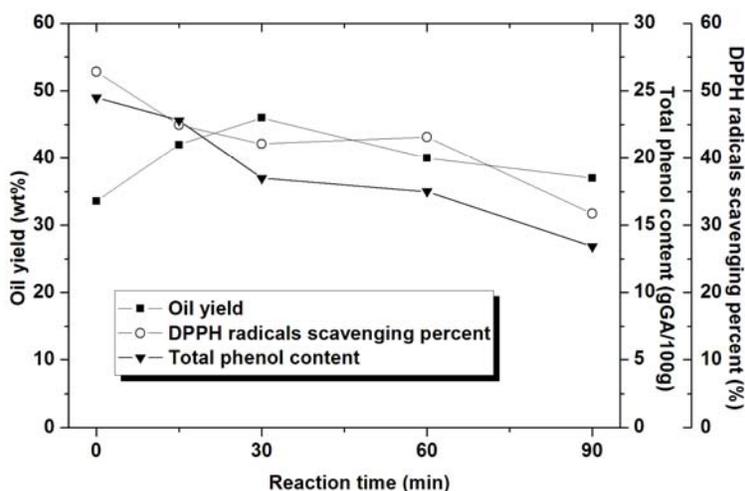


Fig. 9. Oil yield, total phenol content, and DPPH radicals scavenging percentages of ALLP at various reaction time at 320 °C. In the DPPH method test condition 0.2 mL samples were mixed with 6 ml 1×10^{-4} mol/L DPPH radicals ethanol solutions.

The change trends of DPPH radicals scavenging percentages with reaction time and temperature were similar to that of the total phenol content. Considering the oil yield, total phenol content and antioxidant ability, the process parameters at 300 °C, 30 min or 320 °C, 15 min are suitable conditions for antioxidant ALLP production. For all these values in Fig. 8 and 9, there is certain correlation between total phenol content and DPPH radicals scavenging percentages (linear correlation $R^2 = 0.7756$). However, it is difficult to explore the specific mechanism of the antioxidant power with the ALLP: (1). There are some low molecular carbohydrates in the liquid products, the aliphatic hydroxyl groups in these carbohydrates can decrease antioxidant activity since their polar groups may hydrogen bond with phenolic groups (Ugartondo et al. 2008). (2). In the ALLP, there may be some other antioxidant compounds, such as flavonoids. (3). There are various molecular weights and great diversity phenols in the ALLP. It has been reported that phenolic interactions can positively or negatively affect the antioxidant activity of natural mixtures (Iacopini et al. 2008).

Compared with the lignins, the hydrothermal liquefaction products greatly improved the unit antioxidant power. Due to this ability, together with their liquid nature, these liquefaction products may have the potential to serve a broader range of applications and achieve advantages in many liquid systems, such as in bio-diesel and lubricants, where phenolic antioxidants are often added.

CONCLUSIONS

Hydrothermal liquefaction products retained the basic functional groups of lignins. Compared with the raw materials, both ALLP and MLLP improved the unit antioxidant power, while ALLP showed better antioxidant abilities as measured by the DPPH method and FRAP method. The total phenol content had certain relationships with the antioxidant abilities. ALLP have the potential to become used as liquid antioxidants, suitable conditions for alkaline lignin hydrothermal liquefaction are at 300 °C, 30 min or 320 °C, 15 min.

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