Evaluation of the antioxidant activities of different bio-oils and their phenolic distilled fractions for wood preservation

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Abstract

This research investigated the antioxidant activities of raw bio-oils produced from pine and oak wood species. The study also included the effect of the pyrolysis parameters on the properties of the bio-oils produced. In addition to raw bio-oils, phenolic fractions were further isolated from the bio-oils at two different temperature ranges (180-220°C and 220-270°C) by using spinning band distillation columns. The antioxidant activities of raw bio-oils and phenolic fractions were evaluated using the Trolox equivalent antioxidant capacity (TEAC) assay. The concentrations of phenolic compounds were determined by gas chromatography/mass spectrometry (GC/MS). The results of the assay showed that the antioxidant activities of the phenolic fractions are much higher than those of the parent bio-oils. GC/MS analysis results showed that o-guaiacol and its derivatives (4-methylguaiacol, 4-ethylguaiacol, and 4-propylguaiacol), phenol, 3-methylphenol, 2,3-dimethylphenols, 3-ethylphenol and eugenol are the most

influential phenolic compounds on the antioxidant activity of the bio-oils and their distilled fractions.

Keywords:

Bio-oil; wood preservation; antioxidant activity; phenolic compounds

1. Introduction

Wood is one of the most important materials available to human beings. It is inexpensive, renewable and has unique properties compared with other materials that make it highly beneficial for residential, commercial, and industrial use. However, wood may be readily damaged and destroyed by a variety of biodegrading organisms and microbes such as fungi and bacteria, termites, insects and marine borers because of its organic chemical structure (Sundararaj et al., 2015). In general, wood degrading processes can cause billions of dollars loss every year to repair and replace the damaged wood structures (Goodell et al., 2003). Accordingly, treatment of wood with chemical preservatives to prevent its damage by these aggressive biodeteriogens is very important. The treatment has a dual advantage of extending the service life of the wooden material and conserving the forest resources (Schultz et al., 2007).

Traditionally, oil-borne organic solvent preservatives (creosote and penta) and the water-borne chromated copper arsenate (CCA) were the major three 1st-generation wood preservatives for many decades due to their long term effectiveness, low cost, and robust formulations that were easy to use in a treating facility (Schultz & Nicholas, 2008). In the last thirty years, health and environmental concerns associated with CCA, penta, and creosote arose rapidly all over the world and pressed for the development of

2nd-generation copper rich preservatives. However, the growing environmental concerns about disposal of toxic copper leaching chemicals directed wood preservation industry toward more environmentally benign alternatives that are effective and cheap as traditional wood preservatives (Ahn et al., 2008). The current state of art wood preservatives emphasis on using totally organic compounds and non-biocidal systems (3rd and 4th-generation) to protect wood (Schultz & Nicholas, 2008).

Termites and fungi are the two extremely harmful forces which can destroy any wood species. Wood decay fungi is of interest because without fungi wood would never decay, the two major decay fungi are brown-rot and white-rot fungi. Brown-rot fungi are the most destructive type of decay fungi especially for wood components in service (Cheng et al., 2008). This type of fungi attacks only the cellulose components of the cell wall in softwoods. On the other hand, white-rot fungi prefer hardwoods and attack both the lignin and cellulose components of the cell wall. The most acceptable mechanism for brown- rot decay is the production of hydroxyl radical (OH·) via the Fenton reaction or one-electron oxidation (Enoki et al., 1997; Highley & Flournoy, 1994). The OH· radical are the tool that fungi use for non-enzymatic attack to break though the walls of the wood cells (Schultz et al., 2005). It was also proposed that decay fungi use metal ions native to wood such as iron (II) or copper (I) for this reaction (Jensen et al., 2001):

Therefore, any compound that is capable of neutralizing the OH· radicals and/or chelating the metal ions, would be able to prevent this non-enzymatic, radical attack, and may be useful as wood preservative against fungal decay (Binbuga et al., 2005).

Focus in the current study was given to scavenging the free radicals and not to the metal chelation. It has been shown that effective radical-scavenging compounds are polyphenolic organic species, such as tannins and flavonoids (Boligon et al., 2009; Obanda et al., 2008). These compounds are able to rapidly neutralize free radicals, leaving stable, unreactive products (Aadil et al., 2014), and therefore may be useful as wood preservatives and fungal inhibitors. In the current study, a particular attention will be given to the phenolic compounds.

The last two decades have witnessed an increased interest in using renewable biomass feedstocks as a source for energy and chemical feedstocks. Bio-oil, a product of the pyrolysis of biomass, is considered to be an excellent source for environmentally benign, totally organic, polyphenolic compounds (Temiz et al., 2013) that are able to neutralize the free radicals. Bio-oils also add structural integrity to wood, have a pleasing appearance when applied, non-hazardous, and not only abundant in nature, but easily replenished and sustained (No, 2014). Bio-oils also cheaply produced, the cost to produce bio-oils are \$0.62-1.40/gal or \$0.16- 0.35/liter (Shemfe et al., 2015). Antifungal properties of several pyrolytic bio-oils and their lignin-rich fractions were tested previously and showed excellent decay protection in soil block tests (Mohan et al., 2008). In a recent study, bio-oil obtained from the fast pyrolysis poplar wood showed good decay resistance against wood-rot fungi. The phenolic compounds derived from lignin played an important role against fungal attack (Yen & Chang, 2008). Antifungal activity of liquefied wood and liquefied wood containing biocide were also tested against the selected wood degrading fungi in several studies (Hrastnik et al., 2013; Humar et al., 2011)

Determining major phenolic compounds in the bio-oil and their lignin-rich fraction that potentially have higher antioxidant activity is important. Identification of these chemicals will help to use them directly as antifungal agents for wood preservation. To the best of our knowledge, there are no previous studies on the identification of the major phenolic compounds that potentially have high antioxidant activity in the bio-oil. Therefore, the main objectives of this research were to measure the antioxidant activities for raw bio-oils and their phenolic distilled fractions and determine the phenolic compounds that greatly effect on the antioxidant capacity.

2. Materials and methods

2.1. Materials

In the current study, twenty four bio-oils were used, eight of them were obtained from the National Renewable Energy Laboratory (NREL) (Golden, CO) and sixteen were prepared at Mississippi State University (MSU) using an auger reactor.

2.2. Bio-oil preparation

The NREL bio-oils were prepared from oak and pine woods in a fixed-bed reactor. The preparation conditions and parameters (the type of wood species, temperature, and residence time) are summarized in Table 1. The MSU bio-oils were produced through the pyrolysis of oak wood, pine wood, oak bark, and pine bark in the auger reactor. The oak and pine wood feedstocks were supplied by Fiber Resources, Inc. (Pine Bluff, AR) in the form of wood pellets. The pine bark and oak bark were supplied by Packaging Corporation of America (PCA, Ackerman, MS), and Rives & Reynolds Lumber Co., Inc. (Louisville, MS), respectively. The pine bark and oak bark

111 samples were air-dried for 1–2 days to 8–10% moisture content. The oak and pine
112 wood pelletized samples were pyrolyzed at the moisture content received (6–8%
113 moisture). Prior to pyrolysis, each feed was ground in a Bauer mill (Bauer Brothers
114 Construction, Inc., Parker, CO) and sieved to a particle size of 2–4 mm. The pyrolysis
115 process was conducted in a stainless steel proprietary auger reactor with a feed rate of
116 ~1 kg/h. The parameters and conditions used to prepare MSU bio-oils are also
117 summarized in Table 1.

118 2.3. *Distillation of phenolic fractions*

119 The six bio-oils that showed the highest antioxidant activity were selected for
120 phenolic distillation. A B/R Instrument spinning band distillation system (Model 36/100,
121 B/R Instrument Corporation Co., Ltd.) was used to distill phenolic fractions from raw bio-
122 oil based on their boiling points (Figure 1). The distillation process was carried out in the
123 temperature range 180-270 °C. Two distilled fractions were collected. The first phenolic
124 distilled fraction was collected in the temperatures range 180-220 °C while the second
125 distilled fraction was collected in the temperature range 220-270 °C.

126 2.4. *TEAC assay for bio-oils*

127 The antioxidant activities of raw bio-oils were measured using the Trolox equivalent
128 antioxidant capacity (TEAC) assay according to a reported method by Re (Re et al.,
129 1999). In the TEAC assay, 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonate) (ABTS)
130 was allowed to react overnight with 5.0 g of potassium persulfate in 30.0 mL of de-
131 ionized water to produce the radical cation ABTS^{•+}. The radical cation solution was,
132 then, filtered and diluted with methanol to give an absorbance value of about 1.7 at 743

nm. This solution was used as the working solution. About 0.50 g of each bio-oil was diluted with methanol to a total volume of 50.0 mL to obtain a 1.0% w/v solution. Similarly, a 1.0% w/v Trolox standard solution was prepared in methanol. A 5.0 mL aliquot solutions of ABTS^{•+} were transferred into twenty six separate test tubes in order to test twenty four bio-oils, one Trolox standard solution, and a blank. One drop of each bio-oil and one drop of the standard solution were added to each of the properly labeled test tubes containing the ABTS^{•+} solution. The reactions between each bio-oil and Trolox with ABTS^{•+} were allowed to proceed for one hour. The absorbance values for all solutions were measured at 743 nm using a Varian UV-Vis spectrophotometer. The absorbance value for the ABTS^{•+}/Trolox mixture was subtracted from that of the ABTS^{•+} blank solution. The difference in absorbance is equal to one Trolox equivalency. The TEAC value for each bio-oil was calculated by subtracting the absorbance value for each bio-oil/ABTS^{•+} mixture from that of the ABTS^{•+} blank followed by dividing by the one Trolox equivalency number. Two replicate runs were performed for each experiment to minimize the experimental error within 5%.

2.5. TEAC assay for phenolic fractions

The TEAC assay for the phenolic fractions was performed and the TEAC values were calculated the same way as the assay was done for raw bio-oils but with a 0.10% w/v Trolox standard solution. The absorbance values were also recorded at 743 nm. The 1/10 dilution of the Trolox solution was necessary to get absorbance values within a useful range.

2.6. Gas chromatography/mass spectrometry (GC/MS) analysis

The bio-oils and phenolic distilled fractions chemical compositions were characterized by gas chromatography-mass spectrometry (GC/MS) using an HP 5890 gas chromatograph equipped with an HP 5971 mass spectrometer detector (Hewlett-Packard, Palo Alto, CA). The mass spectrometer was configured for electron impact ionization at 70 eV, with an interface temperature of 225 °C and a source temperature of 210 °C. Prior to the analysis, the spectrometer was mass calibrated and abundance tuned using perfluorotributylamine. The calibration method for determining the concentration of the bio-oil components was described previously (Hassan et al., 2009). Briefly, a representative sample (0.20 g) of each bio-oil was weighed to the nearest 0.1 mg and diluted to 10.00 mL with methanol. One mL of this solution was transferred to an auto-sampler vial and spiked with 10 µg/mL from the internal standard (US-108N, Ultra Scientific) just prior to the analysis. A 1.00 µL of the diluted sample was injected into a fused silica capillary column coated with 5% phenylmethylpolysiloxane (DB 5, J&W Scientific). The initial oven temperature of the GC was kept at 40 °C for 4 min. The temperature was then increased to 270 °C at a rate of 5 °C /min. The temperature was then held at 270 °C for 15 min. The injector and detector temperatures were 270 and 250 °C, respectively. The carrier gas was He of 99.99% purity. The *m/z* values of the fragment ions were recorded. Full scan mass spectra were acquired from a 35 to 550 *m/z* at a scan rate of 0.2 s per scan, with an inter-scan delay of 0.1 s. The total time for each injection run was 67 min. The chemical compositions of the bio-oil sample were determined by comparing the integrated peak areas of the characteristic compounds to the responses generated with the working standards.

3. Results and discussion

3.1. TEAC assay for raw bio-oils

Figure 2 shows the concentration of phenolic compounds and the antioxidant activities (TEAC value) for all studied raw bio-oils compared with the antioxidant activity of the Trolox solution (TEAC = 1). It is clear from the figure that the following six bio-oils (BO-2, BO-3, BO-5, BO-13, BO-17, and BO-22) showed relatively higher antioxidant activities than the rest. The TEAC values for the above six bio-oils range from 0.27-0.38 or were (27-83% as effective as Trolox). According to the bio-oil description shown in Table 1, the bio-oils that showed higher TEAC values are: NREL oak wood (high temp, long residence time); MSU oak bark (low temp, long residence time); MSU oak bark (high temp, long residence time); MSU pine bark (high temp, long residence time); MSU pine wood (high temp, long residence time); and MSU pine wood (low temp, long residence time). It can be noticed that, all of the above six bio-oils were pyrolyzed at relatively longer residence time (6 sec), and five bio-oils were produced at the relatively higher pyrolysis temperatures (450 °C for MSU bio-oil and 550 °C for NREL bio-oil). The above observation indicates that the pyrolysis time and temperature play a major role in determining the antioxidant activity of the bio-oils. A previous study suggested that phenolic compounds might be primarily responsible for the antioxidant activity of the bio-oils (Mohan et al., 2008). In the current investigation, the GC/MS analysis results for the different bio-oils showed that, the major fifteen phenolic compounds detected in the raw bio-oils are: phenol; 2-methylphenol; 3-methylphenol; 2-methoxyphenol; 2,6-dimethylphenol; 2,4-dimethylphenol; 3-ethylphenol; 2,3-dimethylphenol, 2-methoxy-4-methyl phenol; 4-ethyl-2-methoxyphenol; 2,6-dimethoxyphenol; eugenol; 2-methoxy-4-propylphenol; and cis- as well as trans-isoeugenol. The chemical structures for the

above phenolic compounds are shown in Figure 3. The total concentrations of phenolic compounds in each bio-oil are also presented also in Figure 2. It is obvious that, the six bio-oils of highest TEAC values are also associated with the highest concentration of phenolic compounds. This result means that, the antioxidant activities of raw bio-oils are directly related to the concentrations of phenolic compounds. This result in good agreement with previous reported studies and indicates the role of phenolic compounds in increasing the antioxidant activity of different wood species (Anis et al., 2012; Lamounier et al., 2012; Rosales-Castro et al., 2015; Willfoer et al., 2003). The ability of the phenolic compounds in improve the antioxidant activity arises from the ability of these compounds to react with the hydroxyl radicals ($\text{OH}\cdot$), thus, causing oxidation of the wood and accordingly impeding the attack of radicals to the wood (Mohan et al., 2008; Schultz et al., 2005).

3.2. TEAC assay and concentration of phenolic compounds in the raw bio-oils and their distilled fractions

To confirm the effect of phenolic compounds on the antioxidant activity, the six raw bio-oils with the highest antioxidant activities (BO-2, BO-3, BO-5, BO-13, BO-17, and BO-22) were further isolated from the bio-oils using spinning band distillation columns at different temperature ranges (180-220 °C and 220-270 °C). The above temperature ranges were chosen because the boiling points of most phenolic compounds determined by GC/MS fall in this range. Figure 4a shows the antioxidant activity (i.e., TEAC values) for the selected raw bio-oils and their distilled fractions. It is clear from the figure that, there is a substantial difference in the antioxidant activity between the raw

bio-oils and their derived phenolic fractions. The TEAC values of most of the selected raw bio-oils are in the 0.21 to 0.25 range indicating that these bio-oils are 21 to 25% effective as Trolox. On the other hand, the TEAC values of the distilled fractions of the above bio-oils are close to 0.60 or 60% as effective as Trolox. This means that, the antioxidant activity of the phenolic compounds increased to more than double that of the parent bio-oil.

Figure 4b shows the total concentration of phenolic compounds for the six selected raw bio-oils and their distilled fractions. Amazingly, it was found that the total concentrations of the phenolic compounds are almost in the same trend of the TEAC values. This result becomes more obvious when the TEAC values are plotted against the total concentration of the phenolic compounds (Figure 5). It can be easily concluded that there is a direct relationship between the concentration of the phenolic compounds and their TEAC values. The total concentrations of the phenolic compounds in the raw bio-oils are about 5%, and their TEAC values are in the 0.20-0.25 range. In the low boiling point (180-220 °C) distilled fractions, the total concentrations of the phenolic compounds increased from 15 to 20% and their corresponding TEAC values increased to 0.55 to 0.60. Similarly for the high boiling point (220-270 °C) distilled fractions, TEAC values of more than 0.65 are associated with higher phenolic concentrations of ~23%. Also, the TEAC values of all fractions distilled at the higher temperature range (220-270 °C) are slightly higher than that for fractions distilled at the lower temperature range (180-220 °C). This indicates that the phenolic compounds with high boiling points might have higher antioxidant activities than their analogs with low boiling points. In general, both the phenolic compounds and their distilled fractions have TEAC values that are more

than double that of the parent bio-oils. All of the above results strongly confirm the suggestions given in the literature (Ilnicka et al., 2014; Mohan et al., 2008) that the phenolic compounds extracted from pyrolysis oils possess significant antioxidant capability that occurs via a free radical scavenging mechanism.

3.3. Identification of the phenolic compounds

Figure 5 shows that the raw bio-oils that contain high percentage of the phenolic compounds and distilled fractions clearly have higher antioxidant activity than the rest of the bio-oils. To identify the specific phenolic compounds that greatly affect the antioxidant activity, the GC/MS analysis was performed on the six bio-oils that showed the highest antioxidant activity as well as their distilled fractions (Figure 6). It is obvious that the highest antioxidant activities in both distilled fractions arise from some and not all of the phenolic compounds identified by the GC/MS. The compounds that are most likely responsible for the bio-oils' antioxidant activities are: phenol, 2-methoxyphenol (*o*-guaiacol), 2,4-dimethylphenol, 3-ethylphenol, 2,3-dimethylphenol, 2-methoxy-4-methylphenol (4-methylguaiacol), 4-ethyl-2-methoxyphenol (4-ethylguaiacol), eugenol, and 2-methoxy-4-propylphenol (4-propylguaiacol). Phenolic compounds that exist in both distilled fractions with high yield such as *o*-guaiacol and its derivatives (4-methylguaiacol, 4-ethylguaiacol, and 4-propylguaiacol) are probably the most influential phenolic compounds in increasing the antioxidant activities. These results are in strong agreement with previously reported studies where phenolic compounds derived from lignin degradation showed a strong protective effect against fungal attack (Kim et al., 2012; Mourant et al., 2005; Suzuki et al., 1997). Other phenolic compounds such as phenol, 3-methylphenol, 2,3-dimethylphenols, 3-ethylphenol and eugenol also have

strong effect on the antioxidant activity of raw bio-oils and their distilled fractions. On the other hand, compounds such as 2-methylphenol, 2,6-dimethylphenol, 2,6-dimethoxyphenol (syringol), and cis- as well as trans-isoeugenol showed small effect on the antioxidant activity of raw bio-oils and their distilled fractions.

4. Conclusions

Bio-oils, in general, show a measurable amount of antioxidant activity, much of which is driven from their phenolic components. Six raw bio-oils showed higher antioxidant activities and phenolic concentrations than the rest of the bio-oils. The phenolic distilled fractions generally have higher antioxidant activity than their parent bio-oils. The GC/MS analysis showed that o-guaiacol and its derivatives (4-methylguaiacol, 4-ethylguaiacol, and 4-propylguaiacol) are the most influential phenolic compounds in increasing the antioxidant activities of the raw bio-oils and their distilled fractions. Phenolic compounds such as phenol, 3-methylphenol, 2,3-dimethylphenol, 3-ethylphenol and eugenol also have strong effect on the antioxidant activity.

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383

384 Figure 1.

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Figure 3.

394 **Figure 4.**

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397 **Figure 5.**

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Figure caption

Figure 1. A schematic diagram for the B/R spinning band distillation Instrument used in the study

Figure 2. TEAC values and concentrations of phenolic compounds for the 24 studied bio-oils.

Figure 3. Chemical structures of the major phenolic compounds identified in the raw bio-oils.

Figure 4. TEAC values and concentration of the phenolic compounds of raw bio-oils with higher antioxidant activities and their distilled fractions.

Figure 5. The relationship between the TEAC values and the total concentrations of the phenolic compounds of raw bio-oils with higher antioxidant activities and their distilled fractions.

Figure 6. Concentrations of different phenolic compounds in the raw bio-oils with higher antioxidant activities and their distilled fractions.

Table 1

Description of the different bio-oils used in this study

Bio-Oil (ID)	Bio-oil Description	Bio-oil Source
BO-1	Oak wood, low temperature, long residence time	NREL
BO-2	Oak wood, high temperature, long residence time	NREL
BO-3	Oak bark, low temperature, long residence time	MSU
BO-4	Oak bark, low temperature, short residence time	MSU
BO-5	Oak bark, high temperature, long residence time	MSU
BO-6	Oak bark, high temperature, short residence time	MSU
BO-7	Oak wood, low temperature, long residence time	MSU
BO-8	Oak wood, low temperature, short residence time	MSU
BO-9	Oak wood, high temperature, long residence time	MSU
BO-10	Oak wood, high temperature, short residence time	MSU
BO-11	Pine bark, low temperature, long residence time	MSU
BO-12	Pine bark, low temperature, short residence time	MSU
BO-13	Pine bark, high temperature, long residence time	MSU
BO-14	Pine bark, high temperature, short residence time	MSU
BO-15	Pine wood, low temperature, long residence time	MSU
BO-16	Pine wood, low temperature, short residence time	MSU
BO-17	Pine wood, high temperature, long residence time	MSU
BO-18	Pine wood, high temperature, short residence time	MSU
BO-19	Pine wood, high temperature, long residence time	NREL
BO-20	Pine wood, low temperature, short residence time	NREL
BO-21	Pine wood, high temperature, short residence time	NREL
BO-22	Pine wood, low temperature, long residence time	NREL
BO-23	Oak wood, low temperature, short residence time	NREL
BO-24	Oak wood, high temperature, short residence time	NREL

High temperature = 550 °C for NREL bio-oil and 450 °C for MSU bio-oil

Low temperature = 500 °C for NREL bio-oil and 400 °C for MSU bio-oil

Short residence time = 4 sec for both NREL and MSU bio-oil

Long residence time = 6 sec for both NREL and MSU bio-oil