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Exploring the antioxidant potential of lignin isolated from black liquor of oil palm waste

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Abstract

This study was conducted to evaluate the potential antioxidant activity of lignin obtained from black liquor, a hazardous waste product generated during the extraction of palm oil. Antioxidant potential of the extracted lignin was evaluated by dissolving the extracted samples in 2 different solvent systems, namely, 2-methoxy ethanol and DMSO. Results revealed high percent inhibition of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical in the lignin sample dissolved in 2-methoxy ethanol over DMSO (concentration range of 1–100 μg/ml). Lignin extracted in 2-methoxy ethanol exhibited higher inhibition percentage (at 50 μg/ml, 84.2%), whereas a concentration of 100 μg/ml was found to be effective in the case of the DMSO solvent (69.8%). Fourier transform infrared (FTIR) spectrometry revealed that the functional groups from the extracted lignin and commercial lignin were highly similar, indicating the purity of the lignin extracted from black liquor. These results provide a strong basis for further applications of lignin in the food industry and also illustrate an eco-friendly approach to utilize oil palm black liquor. ***To cite this article: R. Bhat et al., C. R. Biologies 332 (2009).***

Keywords: Lignin; Antioxidant; Black liquor; FTIR

1. Introduction

Malaysia is one of the leading producers of palm oil and has over 2.5 million hectares of oil-palm plantations distributed extensively throughout the country. During the oil extraction process, fruits and nuts are stripped from the fruit bunches, leaving behind the empty fruit bunch as waste. Mohamad Ibrahim et al. [1] have estimated that more than 8 million tones of empty fruit bunches are produced as waste annually in Malaysia alone. The by-product of the extraction process include the black liquor, an aqueous solution of lignin and hemicellulose residues, along with the inorganic chemicals used during processing, which pose a threat to the environment. If these by-products remain untreated for long, they lead to fouling of the environment and could be hazardous to human health [2,3].

Since the 1990s, the health-promoting potential of antioxidants obtained from natural plant products has received tremendous attention from researchers. Epidemiological studies have shown that consumption of certain plant produce might reduce the risk of chronic

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diseases related to oxidative stress via the plants' antioxidant compounds [4,5].

Lignin is a nontoxic, complex, natural phenolic polymer obtained from plant sources that possesses certain qualities that have made it increasingly important in many industrial applications (e.g., as a binder, dispersant, or emulsifier). Mitjans and Vinardell [6] reported that plant-derived lignin possesses certain health benefits, such as antibacterial, antiparasitic, antitumoral, antiviral, and immunopotentiating activities. Lignin isolated from different plant sources has been reported to posses' antioxidant properties and has been shown to be an effective free radical scavenger [7–9]. Although a few reports [9,10], discuss the application of lignin obtained from black liquor, to our knowledge no research exploring the antioxidant capacity of lignin obtained from oil palm waste has been reported. Thus, the goals of this study were to evaluate the antioxidant properties of lignin extracted from black liquor, and to characterize and compare the functional group with that of commercial lignin. Our results might pave way for further studies and applications of lignin from black liquor in the food industry.

2. Material and methods

2.1. Isolation and purification of lignin

Oil palm black liquor was collected from the Division of Bioresource, Paper and Coatings Technology, University Sains Malaysia, Penang, Malaysia. We used the method described by Rohella et al. [11] with some minor modifications to isolate and purify lignin from black liquor. In brief, lignin was precipitated by lowering the pH to 2.0 with 4.84 M H_2SO_4. The precipitate was filtered (Whatman #42), washed with distilled water until neutralized, and then dried in an oven at 55 °C. The dried lignin was treated by successive chemical extractions to remove impurities: first with di-ethyl ether to remove fats and fatty acids, and subsequently with an alcohol-benzene mixture (1:2) to remove dyes and coloring. The extracted lignin was dried (55 °C), powdered, and treated with 72% H_2SO_4 for 30–45 min. The contents were diluted to 3% strength of the acid, filtered, and washed 3–4 times with hot water. This residue was dried to obtain pure lignin, which was used for further analysis.

2.2. Determination of antioxidant activity

The antioxidant capacity of the lignin was studied by evaluating its free radical scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical following the method of Dizhbite et al. [7] and De Ancos et al. [12] with slight modifications. In brief, a known aliquot (320 µl) of the extract (lignin solution, 1 µg ml^{-1} to 100 µg ml^{-1}) in 2 different solvents, namely, 2-methoxy ethanol and dimethyl sulfoxide (DMSO) (90%) was mixed with 1180 µl of 6.1×10^{-5} mol/l DPPH (pre dissolved in 80% methanol solution) at 25 °C for 16 min. The mixture was thoroughly vortex-mixed and the absorbance was measured at 515 nm against a blank. Results expressed as percentage of inhibition of the DPPH radical, was calculated according to the following equation:

$$\%\ \text{inhibition of DPPH} = \left(\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}}\right) \times 100$$

where Abs control is the absorbance of the DPPH solution without the lignin extract.

The inhibition concentration of isolated lignin samples was expressed based on a varied concentration range (between 1 and 100 µg/ml).

2.3. FTIR analysis

We used Fourier transform infrared (FTIR) spectrometry (System 2000, Perkin Elmer, Wellesly, MD, U.S.A.) to determine the functional groups present in the isolated lignin and compared this lignin with a commercial lignin sample (lignin alkali with low sulfonate content, Sigma Aldrich, Batch, 04414PE, CAS 8068-05-1, Sigma, St. Louis, MO, U.S.A.). The lignin samples used for FTIR were in powder form and the analysis were performed in replicates ($n = 5$). The obtained spectra of the samples (on KBr pellets) were in the frequency range of 4000–400 cm^{-1}.

3. Results and discussion

3.1. Free radical scavenging activity

In the present study, isolation of lignin yielded a total mass of 4.63 (±0.03) g/500 ml of the black liquor used. However, this yield might vary from batch to batch and the final yield depends entirely on the initial raw material used.

Lignins are a rich source of antioxidants, mainly due to the scavenging action of their phenolic structures on oxygen-containing reactive free radicals. Antioxidant activity of naturally occurring phenolic compounds has been characterized using DPPH as a reactive free radical, and this method has been widely recognized as the

most reliable technique for measuring antioxidant activity [7,13]. Percent inhibition of the DPPH free radicals was higher in the lignin samples dissolved in 2-methoxy ethanol solvent over those of DMSO (Fig. 1). Lignin, depending on the mode of extraction, is either insoluble in organic solvents or is soluble only polar solvents which form strong hydrogen bonds with lignin. Results on the effectiveness of the dissolving power, is an indication that lignin is more readily soluble in 2-methoxy ethanol than any other organic solvents, and this finding might prove useful for further isolation studies. Scalbert and Monties [14] previously reported that due to extensive cross-linking, lignin generally is insoluble in solvents and degrades only by physical or chemical treatments.

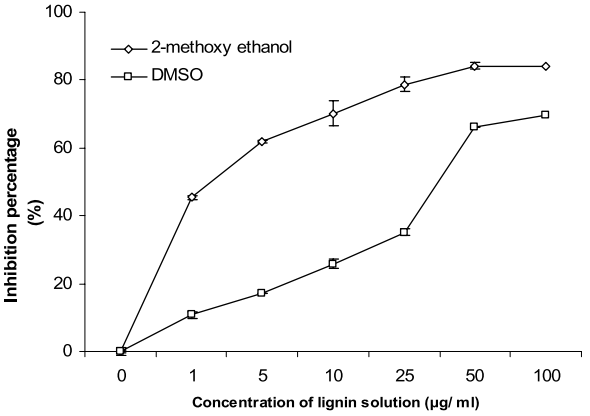

Fig. 1. Inhibition percentage versus the concentration of lignin solution (µg/ml).

In the present study, it was observed that different concentrations of lignin yielded different results. For example, 50 µg/ml of the lignin extract in 2-methoxy ethanol exhibited a high percentage of inhibition, whereas a concentration of 100 µg/ml was effective in the DMSO solvent alone (Fig. 1). Our results on the percentage inhibition of DPPH radical in the isolated lignin were comparable to the earlier reports on lignin extracted from other plant sources [9,15]. These results definitely show that lignin obtained from oil palm waste has wide potential for use as an antioxidant compound and its use should be explored commercially.

3.2. FTIR analysis

Enhancing the industrial application of lignin will require not only improving the lignin extraction processes, but also determining its precise structure and the functional groups, to develop new applications [16]. Gosselink et al. [17] reported that the most important chemical functional groups in lignin include the hydroxyl, methoxyl, carbonyl, and carboxyl groups in various numbers and proportions, depending on origin and extraction processes.

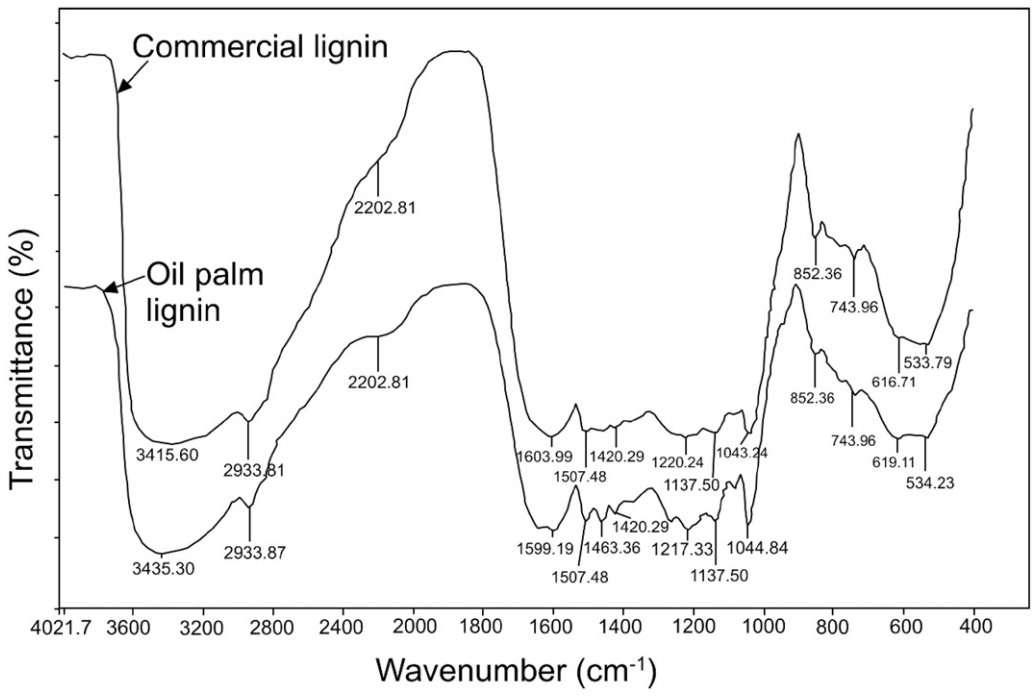

Fig. 2. FTIR spectra of commercial lignin and lignin isolated from oil palm waste.

Fig. 2 shows the infrared spectrum of lignin isolated from black liquor compared with that of commercial lignin. The FTIR spectra of the lignin samples showed that bands in the range of 3414–3435 cm^{-1} could be assigned to the OH group of the phenolic compounds present in the samples. The medium intensity band at 2933 cm^{-1} was assigned to the vibration of the methoxy ($–OCH_3$) group, and bands in the strong intensity range of 1463, 1507, and 1603 cm^{-1} were assigned to the aromatic skeleton or the aromatic ring/aromatic methyl group vibration. The band at 1420 cm^{-1} was due to the C–H group (i.e., the bending vibration of the aromatic group). The bands at 1217–1220 and 1137 cm^{-1} might have been due to the presence of syringyl ring breathing with C–O stretching and aromatic C–H plane deformation. The other weak intensity bands in the range of 1044, 852, and 743 cm^{-1} could have been due to the presence of a guaiacyl group, C–H out-of-plane deformation ($–CH_2{=}CH_2$), and C–H deformation and ring vibration. The other weak intensity bands at 616–619 and 533 cm^{-1} could have be due to C–S stretching and to the C–C=O band, respectively. These FTIR results are on par with some previous reports about lignin obtained from other plant sources [18–21].

In general, the FTIR results revealed high resemblance between commercial lignin and the lignin isolated from oil palm black liquor waste. This similarity indicates the purity of the sample and thus provides a strong basis for its commercial exploitation in the food industry.

4. Conclusions

Due to their health promoting activities, antioxidant compounds from various plant sources are being explored extensively. In this study, we isolated lignin from black liquor – a waste product of oil palm extraction that poses a serious risk to the environment as a pollutant – to evaluate its potential antioxidant activity. We were able to extract pure lignin and show that it exhibited rich antioxidant/free radical scavenging potential. Future studies are warranted to evaluate the effects of this lignin in *in vivo* experiments, to determine its antimicrobial effects, and to characterize its structures for commercial exploitation.

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