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Biological activities and thermal behavior of lignin from oil palm empty fruit bunches as potential source of chemicals of added value

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ABSTRACT

The lignin is the most important renewable source of aromatic compounds on earth. It could represent up to 40% of dry matter in a biomass, however, its potential is underestimated being used as heat recovery source in industrial processes. Therefore, this work aimed to show the chemical feature of lignin isolated from sequential acid-alkaline pretreatment of oil palm empty fruit bunches. The extracted lignin was subjected to studies of this thermal behavior and biological properties as antioxidant, antimicrobial, and antidiabetic. The 2D HSQC spectroscopy analysis showed syringyl aromatic structure and presence of aromatic rings in lignin. The antioxidant assay showed that 2 mg of lignin were required to inhibit 50 wt.% DPPH, while the antimicrobial test inhibited the growth of *Escherichia coli*, *Salmonella enterica serovar thyphimurium*, *Bacillus subtilis* and *Staphyloccocus aureus*. The antidiabetic assay revealed inhibition of 20% of α-amylase activity. The thermogravimetric analysis gave out two peaks of decomposition at 230 °C and 350 °C and the glass transition temperature at 70 °C. These results showed the potential of lignin as precursor of chemicals of added value in a biorefinery process using as feedstock oil palm empty fruit bunches.

1. Introduction

Oil palm empty fruit bunches (OPEFB) represents one of the solid residues in the palm oil mill industries (Kim and Kim, 2013). Is estimated that by 100 tons of oil palm fresh fruit bunches (OPFFB) processed to oil extraction are produced 22 tons of OPEFB (Hosseini and Wahid, 2014). The molecular structure of this solid residue is composed generally of 30–40 wt% of cellulose, 20–25 wt% hemicellulose and 20–35 wt% of lignin, containing also some quantities of pectin, protein, extractives (nonstructural sugars, nitrogenous material, chlorophyll and waxes) and ash (Shinoj et al., 2011). The mass composition of OPEFB makes it an attractive feedstock in a biorefinery process based on lignocellulosic material for chemicals and fuels production (Taylor, 2008). However, at present OPEFB residues have not been optimally used, because they are wet, bulky, and voluminous which are unfavorable properties for transportation and handling. Therefore, it has been used as organic fertilizer and as a source of heat and energy recovery in oil palm mills, and the remaining ashes are used as fertilizers (Arrieta et al., 2007; Chiew and Shimada, 2013; de Souza et al., 2010).

To make use of OPEFB, different approaches have been developed and reported in literature, including the production of ethanol, methane, hydrogen, briquette and organic acids as the levulinic acid (Chin et al., 2015; Ibrahim et al., 2015; Jung et al., 2013; Tan et al., 2013). However, the uses of lignin have been left in the background. The lignin represents 30 percent of all the non-fossil organic carbon and is the most important renewable source of aromatic compounds on Earth. Nevertheless, the valorization of residual lignin as a co-product or precursor of chemical value added products is considered a challenge and the technology is less developed than those for polysaccharides, being its amorphous structure the main cause (Cotana et al., 2014).

The lignin is composed by crosslinked phenylpropanoid monomer structures of sinapyl, coniferyl and *p*-coumaryl alcohols, with a molecular weight distribution between 1000 and 2000 g mol^{-1}. Depending on the type of biomass and the process

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of extraction, the lignin presents different chemical structures. It means that more or less syringyl, guaiacyl or *p*-hydroxycoumaryl units are present, which impacts in different molecular weight distribution (Yang et al., 2016).

The isolation process of lignin can be classified in two groups as it is reported by (Azadi et al., 2013). Processes in which lignin is degraded into soluble fragments, and processes that selectively hydrolyze polysaccharides and leave lignin along with some carbohydrates deconstruction products as solid residue, being the lignin degradation processes the most commonly used.

Lignin residue has been traditionally burned in factories as a source of heat and energy, ignoring its potential as co-product (Mussatto et al., 2007). Because of recent growth of industries producing ethanol from lignocellulosic biomass, the availability of lignin as black liquor is huge. In 2010 the lignin production as a residue was 50 million tons, being only 2% of this amount used for low value-added products (Smolarski, 2012). Consequently, the valorization of lignin as a precursor of highly value-added products is an alternative to generate economic benefit and develop biorefineries from lignocellulosic biomass (Azadi et al., 2013; Doherty et al., 2011; Ghaffar and Fan, 2014).

Based on the fact that lignin would be a source of chemicals of added value, this work focused on the evaluation of biological activities and thermal performance of lignin extracted from OPEFB using sequential acid-alkaline treatment. The analyses of chemical structure were carried out using 2D HSQC NMR. The thermal behavior was studied through TGA and DSC analyses. The biological properties evaluated were antioxidant activity using DPPH and ABTS, antimicrobial activity against two Gram-negative bacteria: *Escherichia coli* and *Salmonella enterica serovar thyphimurium*, and two Gram-positive bacteria: *Bacillus subtilis* and *Staphylococcus aureus*, one filamentous fungus: *Aspergillus Niger*, and one yeast: *Candida albicans*. The potential uses as antidiabetic agent were evaluated by the α-amylase inhibition, and the toxicity was determined by lethality assay on the wild-type *Caenorhabditis elegans*.

2. Material and methods

2.1. Lignocellulosic biomass OPEFB

Oil palm empty fruit bunches (OPEFB) were obtained from Biopalm Vale factory, located in Mojú, in the Pará state of Brazil. The OPEFB was dried in a cross flow stove at 65 °C for 48 h, milled in hammer mill, sieved through a mesh 42 (0.350 mm) and used for all experiments. All experiments were carried out in triplicates.

2.2. Analysis of carbohydrates and total lignin

The composition of polysaccharides present in raw OPEFB was determined using the NREL analytical procedure reported by Sluiter et al. (2011), as was reported in our previous work (Coral Medina et al., 2015).

2.3. Lignin extraction from OPEFB

The residual lignin was obtained from OPEFB based on our previous work using sequential acid-alkaline pretreatment (Coral Medina et al., 2015). Briefly, the acid hydrolysis was carried out using 1 wt.% of H_2SO_4 at 121 °C by 60 min, following the procedure reported by Minu et al. (2012). Form the alkaline lignin extraction the time and temperature were fixed at 80 min and 121 °C respectively. The mass percentage of NaOH was varied from 0.5 to 5.5 wt.% in solution, whit increase of 1 wt.% in NaOH. The mass percentage of OPEFB in solution was 10 wt.%, and the balance of distilled water. Six fractions (L_1 to L_6) of lignin one by each concentration of NaOH used were obtained. After extraction, each fraction of lignin was washed three times with hot water at 70 °C to remove the residual sugars, then, vacuum dried at 50 °C by 8 h.

To perform analysis of biological properties as Total phenolic content (TPC), on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radical scavenging activity and antimicrobial assay, the lignin was solubilized using alkaline water at pH 11 using magnetic shuffler at 50 °C. After solubilization the pH of lignin solution was set at 6–7 using H_2SO_4 10% (v/v).

2.4. Two-dimensional 2D HSQC NMR spectroscopy

2D-NMR spectra of underivatized lignin were carried out in a Bruker spectrometer at 400 MHz. The preparation of samples is described below. About 80 mg of solid lignin was dissolved in 0.5 mL of DMSO-d_6. The chemical shift for ^{13}C NMR was calibrated with reference to DMSO-d_6, standard peak at 39.51 ppm. The spectrum was recorded keeping the following parameters constant: acquisition time of 0.68 s, frequency 100.6 MHz, receiver gain 182.08, sweep width 24,038.09 Hz and temperature 30 °C.

2.5. Determination of the total phenolic content (TPC)

The TPC of all lignin fractions were determined by Folin-Ciocalteu reagent method according to Singleton and Joseph (1965) and Rusaczonek et al. (2007) with modifications. Briefly, a lignin solution of 200 μg mL^{-1} was prepared, an aliquot of 500 μL of each sample were added to 2.5 mL of (1:10 v/v) Folin-Ciocalteu solution, after 5 min, 2 mL of (7.5% w/v) Na_2CO_3 was added. The mixture was maintained at room temperature in a dark environment by 1 h. The absorbance was read at 740 nm in a spectrophotometer (SP – 2000 UV) using quartz cuvette and distilled water as blank. Gallic acid was employed as calibration standard (0–200 μg mL^{-1}) to determine the phenol quantity in milligrams of Gallic acid equivalent. All experiments were carried out in triplicates.

2.6. Antioxidant assay as DPPH and ABTS radical scavenging activity

The antioxidant capacity of lignin was studied by evaluating its free radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, following the procedure reported by (Dizhbite et al., 2004) with modifications. A solution of 0.004% (w/v) of DPPH-methanol was prepared. A set of lignin solutions was prepared from 5 to 200 μg mL^{-1}, aliquots of 1 mL of lignin solution were added to 3.9 mL of DPPH-methanol solution. The reaction time was 30 min at room temperature. The absorbance was recorded at 517 nm. Inhibition of free radicals by DPPH in percentage was calculated according to Eq. (1).

$$Scavening\,(DPPH\ or\ ABTS)\,(\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \qquad (1)$$

where $A_{control}$ is the absorbance of the control reaction. A_{sample} is the absorbance of the test compound. The values of inhibition were calculated according to ascorbic acid as control in the interval of 5 to 200 μg mL^{-1}.

The fraction of lignin which presented the best results of DPPH inhibitions, its mean the lowest concentration for DPPH inhibition, was used to determine the 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radical scavenging activity. The ABTS assay was carried out based on the method reported by (Miller et al., 1993), with modifications. 2 mL of ABTS solution (7 mM) and 2 mL of potassium persulfate solution (2.45 mM) were mixed to make the stock solution (ABTS+) and placed in the dark for 6 h. Then, 50 μL of ABTS+ solution was added to 200 μL of lignin solution (using the same concentration employed in DPPH assay), the mixture was