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Structural characterization of lignin: A potential source of antioxidants *guaiacol* and *4-vinylguaiacol*

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ABSTRACT

The structure of lignin obtained from the ozone and soaking aqueous ammonia pretreatment of wheat straw has been characterized utilizing chemical analytical methods in order to reveal its antioxidant characteristics, including attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR), pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS), pyrolysis/tetramethylammonium hydroxide-gas chromatography/mass spectrometry (Py/TMAH-GC/MS), gel permeation chromatography (GPC), ultra violet–visible spectroscopy (UV–vis), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) antioxidant evaluation assay. The results demonstrated that the isolated lignin is a *ρ*-hydroxyphenyl- guaiacyl-syringyl (H-G-S) lignin, with S/G ratio of 0.35 and significant amounts of phenol 2-methoxy (guaiacol) and phenol 2-methoxy-4-vinyl (4-vinylguaiacol). The Py-GC/MS and Py/TMAH-GC/MS pyrograms indicated that the major units in this lignin are derived from hydroxycinnamic acids. The GPC results revealed the molecular weight of the lignin was considerably low and also the FTIR analysis showed that the lignin possessed hydroxyl and methoxy functional groups; the factors led to the extracted lignin having a comparable antioxidant activity to that of currently used commercial antioxidants. The UV–vis and DPPH antioxidant assay results suggested a percentage of inhibition of the DPPH radicals in the following order: guaiacol (103.6 ± 1.36) > butylated hydroxytoluene (103.3 ± 1) > ferulic acid (102.6 ± 0.79) > pretreated lignin (86.9 ± 0.34).

1. Introduction

Developing valuable products from lignin can improve the economics of a biorefinery using lignocellulosic biomass. Lignin is present in significant amounts in plants, accounting for 15–25% (w/w) of herbaceous biomass. To date however, lignin has little high-value practical use [1]. As a heteropolymeric aromatic compound, the lignin structure is composed of an aromatic ring with hydroxyl and methoxy functional groups and a propanoid chain. This configuration has many properties that could give rise to the possibility of using lignin for producing value-added products. However, the heterogeneity of the lignin structure makes it difficult to break down and isolate into a targeted product. Wheat straw possesses three mono-lignols (*ρ*-coumaryl, coniferyl and sinapyl alcohols) in significant amounts [1,2]. It has been found that wheat straw lignin polymer contains, esterified, etherified and carbon–carbon bonds which link guaiacyl (G), syringyl (S), *ρ*-hydroxyphenyl (H) units [2]. Due to the complexity of the lignin structure and the effect of pretreatment processes on it, identifying and extracting chemicals from lignin requires extensive characterization to understand the lignin's polymeric properties, linkages, and properties of the functional groups connected to the aromatic ring. Nonetheless, the features [3] existing in the lignin macromolecule and its characterization suggests the predominant presence of antioxidant monomers in the lignin.

Antioxidants are chemicals that inhibit the oxidation process. The oxidation reaction typically follows three main steps including initiation where free radicals are being produced, propagation where free radicals are created through a chain reaction involving a series of molecules, and finally termination, during which two free radicals interact with each other to end the reaction.

Abbreviations: ATR-FTIR, attenuated total reflectance-Fourier transform infrared spectroscopy; Py-GC/MS, pyrolysis-gas chromatography/mass spectrometry; Py/TMAH-GC-MS, pyrolysis/tetramethylammonium hydroxide-gas chromatography/mass spectrometry; GPC, gel permeation chromatography; UV–vis, ultra violet–visible spectroscopy; DPPH, 1,1-diphenyl-2-picrylhydrazyl.

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When acting as an antioxidant, lignin-derived units have the ability to break the oxidation propagation reaction through hydrogen donation which occurs primarily due to the presence of phenolic hydroxyl groups [4]. Lignin compounds that contain more phenolic hydroxyl groups, fewer aliphatic hydroxyl groups, have a low molecular weight, and narrow polydispersity are reported to have higher antioxidant activity [3]. The antioxidant properties of phenolic compounds from different origins and processes were reported previously; however, the efficacy of the emersion of monomers derived from wheat straw by the ozone and soaking aqueous ammonia (OSAA) pretreatment on the antioxidant activity of the isolated lignin macromolecule has not yet been studied.

In the present work, we studied the structure of the lignin isolated by an acidic precipitation method from a basic effluent produced by the OSAA pretreatment of wheat straw in order to characterize the lignin macromolecule antioxidant characteristics. In this regard, a series of chemical analytical techniques were employed, including attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR), pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) with and without tetramethylammonium hydroxide (TMAH), ultra violet–visible spectrophotometer (UV–vis), gel permeation chromatography (GPC), and the radical scavenging activity was measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay.

The ozone and soaking aqueous ammonia (OSAA) pretreatment process is a chemical pretreatment that is designed to effectively remove the lignin of herbaceous biomass [5,6]. Its process requires only a low temperature and ambient pressure. The process takes advantage of the synergy inherent in the sequential treatment of biomass by ozone and ammonia. The ozone treatment increases the hydrophilicity of the biomass and breaks the physical barrier that prevents diffusion of the ammonia into the biomass. The ammonia is then able to enter the biomass and cleavage the lignin linkages and subsequently dissolve the lignin into the effluent. The ATR-FTIR was employed to study the functionality of lignin structure isolated from the pretreatment effluent. The Py-GC/MS and Py/TMAH-GC/MS were used to analyze the structure of lignin-derived compounds. Pyrolysis combined with gas chromatography (Py-GC) is a useful tool for synthetic and natural polymer analysis. The lignin weight-average (M_w) and number-average (M_n) molecular weights was determined using GPC [7]. Eventually, the UV–vis and DPPH assay provided data pertinent to antiradical activity of lignin in comparison with other commercial antioxidants (i.e., guaiacol, butylated hydroxytoluene (BHT), and ferulic acid). The knowledge obtained on the structural features of the lignin will aid to establish processes to convert the lignin into value-added chemicals and provide economics benefit for biorefinery.

2. Materials and methods

2.1. Materials

The lignin sample was isolated from the ammonia effluent of the sequential ozone and soaking aqueous ammonia pretreatment of wheat straw by acetic acid precipitation method. The chemicals 2-methoxy phenol (guaiacol), trans-ferulic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), tetramethylammonium hydroxide (TMAH), acetic acid, and 2,6-di-tert-butyl-4-methoxyl phenol (BHT) were purchased from Sigma-Aldrich Inc (Milwaukee, US).

2.2. Ozone and soaking aqueous ammonia (OSAA) pretreatment

The wheat straw was first soaked in water to bring the moisture content of the mass up to 45% (w/w) water, and then subjected to ozone pretreatment. The ozonation reaction was performed with 5.3% ozone concentration (5.3%, w/w) at oxygen flow rate of 2 L/min for 10 min [5]. Following the ozone pretreatment, the wheat straw was flushed to soaking aqueous ammonia pretreatment, where 20% ammonia solution was used to treat the biomass for 4 h at a 1:4 biomass to aqueous ammonia loading, including heating time [6]. The ammonia solution was recovered through the use of a two step washing process with E-pure water, and the obtained effluent was gathered for lignin precipitation step. The process flowchart is shown in Fig. 1.

2.3. Precipitation of lignin

The presence of non-lignin components could influence antiradical activity of lignin. Hydrogen bonding between carbohydrate admixture polar groups and lignin phenolic groups could be formed and results in a reduction of antioxidant activity [8–11].

An acetic acid precipitation method which does not contain sulfur and uses an environmentally-friendly reagent for precipitating lignin in effluent was employed [12,13]. Before precipitation, the ammonium in effluent needs to be removed as much as possible. In order to evaporate the ammonium hydroxide, the measured effluent was put in a vented oven for 1 h at 75 °C. The precipitation of lignin performed by adding 20 ml acetic acid:water (9:1, v/v) into

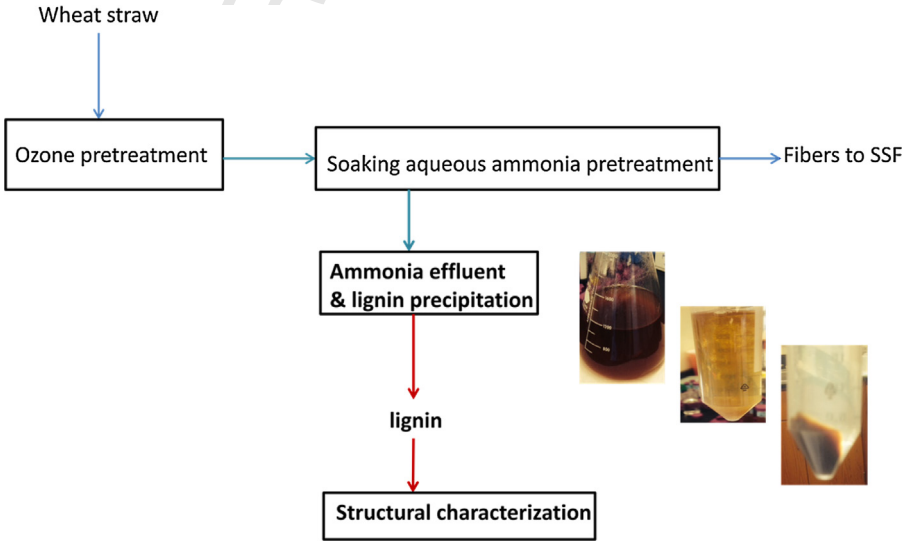

Fig. 1. Process flowchart.

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