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Acidification treatment of lignin from sugarcane bagasse results in fractions of reduced polydispersity and high free-radical scavenging capacity

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ABSTRACT

Lignin constitutes up to one-third of the material found in plant cell walls and is considered the second most abundant natural polymer in the world. Despite unique characteristics of lignin, it is mostly used for low-value commercial applications. In this study, lignin obtained after alkaline treatment of steam-exploded sugarcane bagasse was submitted to an acidification process. The soluble fractions produced at different pH values were comprehensively characterized and *in vitro* antioxidant capacity against reactive oxygen ($ROO^{\bullet}$ and H_2O_2) and nitrogen ($ONOO^-$) species was evaluated. The soluble fraction obtained at pH 2 exhibited the highest scavenging capacities against all species tested (10.2 ± 0.7 mmol Trolox equivalent g^{-1} for $ROO^{\bullet}$, $IC_{30} = 14.9\ \mu g\ mL^{-1}$ for H_2O_2 and $IC_{50} = 2.3\ \mu g\ mL^{-1}$ for $ONOO^-$) and the lowest polydispersity value (1.2) compared to others fractions. According to the SAXS data, the soluble fractions obtained at pH 4 and pH 2 consisted of small nanometer-sized discs and low molecular weight polyphenolic clusters, while soluble fractions obtained at high pH predominated wide lignin nanoparticles and larger aggregates. Mass spectroscopy analysis revealed the presence of phenolic and non-phenolic compounds, well-known as efficient antioxidants, which were identified in all soluble fractions. Collectively, our results provided further demonstration that acidification treatment is a promising strategy to upgrade heterogeneous lignin-enriched stream from sugarcane bagasse, such as preparations with homogeneous compositions and high antioxidant activity.

Abbreviations: SCB, sugarcane bagasse; AT, alkaline treatment; SF, soluble fraction; ROS, reactive oxygen species; RNS, reactive nitrogen species; TPC, total phenolic compounds; LMW, low molecular weight; ORAC, oxygen radical absorbance capacity; FTIR, Fourier transform infrared spectroscopy; GPC, gel permeation chromatography; ^{1}H NMR, proton nuclear magnetic resonance; Mn, number-average molecular weight; Mw, weight-average molecular weight; SAXS, small-angle X-ray scattering; GC–MS, gas chromatography–mass spectrometry.

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1. Introduction

Sugarcane bagasse (SCB) is a renewable power generation source that provides energy to operate sugar and ethanol mills and bagasse-based bioelectricity, which is exported to the national grid (Bizzo et al., 2014). Furthermore, this lignocellulosic material has been considered promising resource to produce biofuel and others value-added products because of the relatively low cost, great abundance and low environmental impact (Mandelli et al., 2014; Chundawat et al., 2011).

The biofuel production from SCB usually requires pretreatment and delignification steps, to separate lignin and hemicellulose from cellulose and reduce the crystallinity and enzymatic recalcitrance of plant biomass polysaccharides (Martínez et al., 2015; Benjamin et al., 2013; Kuo and Lee, 2009). Most of the available SCB pretreatment technologies for ethanol production (e.g organosolv, hydrothermal, dilute acid and alkaline) often produce a large amount of lignin stream as major residue (Carvalho et al., 2015; Dias et al., 2009). During lignocellulose pretreatment, lignin is generally extracted under conditions in which is progressively broken down into lower molecular weight (LMW) fragments, resulting in considerable changes in physicochemical properties. Consequently, apart from the lignocellulosic biomass, lignin isolation procedure also affects the structure and purity of the final material (Doherty et al., 2011).

Lignin often constitutes up to one-third of the material found in plant cell walls and the second most abundant natural polymer in the world. This aromatic polymer is a complex polymeric and amorphous structure arising from the enzymatic dehydrogenative polymerization of coniferyl, sinapyl and *p*-coumaryl alcohol (Ayyachamy et al., 2013), although this proportion can vary depending on the source. Despite lignin's unique characteristics, it is mostly used for low-value commercial applications, such as combusted for energy production, and considered untapped biopolymers in biomass conversion technologies (Ayyachamy et al., 2013; Doherty et al., 2011).

There are several studies demonstrating that lignin can serve as renewable resource for aromatic compounds with antioxidant capacity (García et al., 2010; Vinardell et al., 2008; Pan et al., 2006). The recently literature has reported that the antioxidant capacity of lignin fractions from steam-exploded bamboo stems and SCB are higher than the synthetic antioxidant dibutylhydroxytoluene (BHT) (Kaur and Uppal, 2015; Sun et al., 2014).

Because of its high content of diverse functional groups (e.g., phenolic and aliphatic hydroxyls, carbonyls, and carboxyls) and phenylpropanoic structure, lignin can act as a neutralizer or inhibitor in oxidation processes and stabilize reactions that are induced by oxygen radicals and derivatives thereof (Randhir et al., 2004). Nevertheless, the antioxidant capacity of lignins significantly depends on the lignocellulosic material and isolation method employed (Ponomarenko et al., 2015; Li and Ge, 2012; Dizhibite et al., 2004).

A number of studies have shown that high chemical heterogeneity of lignin, including differences in lignin macromolecule chemical structure, functionality and molecular mass distribution, can turn impractical its applicability as antioxidant in targeted systems (Ponomarenko et al., 2015; Bikova et al., 2004). Recently, it was reported different strategies to obtain homogeneous lignin fractions with antioxidant activity, such as acid precipitation (dos Santos et al., 2014; Ma et al., 2013; Faustino et al., 2010) and sequential solvent fractionation (Ponomarenko et al., 2015; Cui et al., 2014; Li et al., 2012). The acid precipitation approach is based on differences in either solubility or molecular weight of lignins and it has been widely employed since 1960s for Kraft black liquor (Wada et al., 1962).

The expansion of commercial lignocellulosic biorefineries based on the enzymatic deconstruction of plant polysaccharides will increase the production of lignin-rich streams (Ragauskas et al., 2014). Based on projected scenarios for integrating first- and second-generation ethanol production processes in Brazil, with the electrical cogeneration system, lignin streams are currently considered fuel for boilers to supply the required energy power to run plants (Dias et al., 2013). Thus, the development of strategies for fully exploiting the potential of lignin is necessary to overcome the economic and sustainability challenges associated with the use of lignocellulose in the bio-product value chain.

This work focused on studying lignin derived from SCB after steam explosion and alkaline treatment (AT lignin), which is a consistent stream from the second-generation bioethanol pilot-scale study in Brazil (Rocha et al., 2012a,b). This AT lignin was submitted to a simple acidification method for dissolving the heterogeneous lignin mixture to generate soluble fractions with low polydispersity, similar phenolic compositions and high antioxidant capacity. In this study, the structural features of the soluble fractions were comprehensively investigated by different techniques, as well as, the *in vitro* antioxidant capacity against reactive oxygen ($ROO^{\bullet}$ and H_2O_2) and nitrogen ($ONOO^-$) species were also evaluated.

2. Methods

2.1. Raw material

Lignin extracted from steam-exploded SCB with NaOH 1% (w/v) was provided by The Engineering School of Lorena (EEL-USP) and the process is described in detail in Rocha et al. (2012b). The material was named in this study as AT lignin (Alkaline Treatment).

2.2. Purity analysis and acid-insoluble and acid-soluble lignin determination

The impurities in the AT lignin (starting material) were defined as ash content (inorganic material), sugars (cellobiose, xylose, glucose, arabinose and galactose), acetic acid furfuraldehyde and hydroxymethylfurfural content. For ash analysis **c**rucibles were pre-dried to constant weight in a muffle furnace at 575 °C. The AT lignin samples were weighed into the crucibles and heated to 100 °C to remove moisture. The crucibles were subsequently heated at 300 °C for 2 h and 800 °C for 2 h to constant weight (ASTM Methods, 1966). The weight of the remaining ash was calculated as a percentage of the original dry weight of the sample. For insoluble and soluble lignin determination, carbohydrates, acetic acid and furfuraldehyde/hydroxymethylfurfural determination, approximately 2 g of AT lignin was treated with 10 mL of 72% sulfuric acid under vigorous mixing for 7 min in a bath at 45 °C (Gouveia et al., 2009). The reaction was interrupted thought the addition of 275 mL of distilled water and the solution was autoclaved for 15 min at 1.05 atmand 121 °C to complete the oligomer hydrolysis. The hydrolyzed material was separated from solids through filtration using a paper filter (Nalgon, 18.5 cm diameter) previously weighed. The solid in the paper filter was washed with distilled water which was added in the hydrolysate stored for further analysis. The lignin retained in paper filter was dried in oven at constant temperature of 105 °C and the content was obtained by gravimetry. Soluble lignin in the hydrolysate was determined by UV spectroscopy as previously described (Rocha et al., 2012a). The results reported as a percentage of the original dry weight of the sample. Sugars, acetic acid and furfuraldehyde/hydroxymethylfurfural content in the hydrolysate were determined through high performance liquid chromatography (HPLC) as described in Rocha et al. (2012a) and reported as a percentage of the original dry weight of the sample.