

Oxidative Production of Vanillin from Industrial Lignin Using Oxygen and Nitrobenzene: A Comparative Study

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ABSTRACT: Vanillin production from lignin oxidation is a biomass-based process that employs a by-product of the pulp and paper industry. Lignins are phenolic polymers which are found in plant cell walls with a structure depending strongly on the source of lignin and the process condition, which should be adjusted for different samples. In this work, lignin was extracted from kraft cooking liquor of *Pinus eldarica*. The amount of extracted lignin was 27%, based on oven dry weight of wood. Comparative oxidation of *Pinus eldarica* lignin for producing vanillin was separately carried out using oxygen (batch reactor) and alkaline nitrobenzene. The FT-IR spectrum of vanillin was similar to standard. Finally, the vanillin concentration in product solution was analyzed by high performance liquid chromatography (HPLC). The result showed that the amount of vanillin in oxidation of lignin with oxygen was more than that of with nitrobenzene. The yield obtained from oxidation with oxygen and nitrobenzene was 4.2% and 1.2%, respectively.

Keywords: Vanillin, Lignin, FT-IR, HPLC, Nitrobenzene, *Pinus eldarica*

INTRODUCTION

Vanillin (4-hydroxy-3-methoxybenzaldehyde), (Fig. 1) is the major flavor constituent of vanilla. It has a wide range of applications in food industry as a flavor agent and in perfumery as an additive, other applications as antioxidant additive, antifoaming agent, vulcanization inhibitor and chemical precursor for pharmaceutical and agrochemicals industries (Borges et al., 2009). The natural source of vanillin is from the seeds of the vanilla plant, a member of the orchid family. However, the production of natural vanillin from vanilla pods covers only 0.2% of the market requirement, and its production cost is very high (Mohamad Ibrahim et al., 2009). Vanillin may also be obtained by chemical synthesis (Tarabanko et al. 1995), but the vanillin obtained in this way may not be called a natural flavoring. Synthesis of vanillin from renewable sources should result in a greener and more sustainable process. Vanillin was first synthesized from Eugenol (found in oil of clove) and later synthesized from lignin-containing sulfite liquor, a by-product of wood pulp processing in paper manufacture.

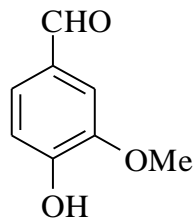


Figure 1. Vanillin structure

While some vanillin is still made from lignin wastes, one possible path to produce vanillin based on biomass is through controlled oxidation of lignin. Lignin is one of the most abundant of organic chemical material resources on the plant. It is recoverable and available in large quantities from black liquor widely generated by the pulp and paper industry. Currently, the vast majority of lignin is burned without any industrial utilization. Lignins are phenolic polymers (Fig. 2) found in plant cell walls, which are responsible, with cellulose, for the stiffness and rigidity of plant stems.

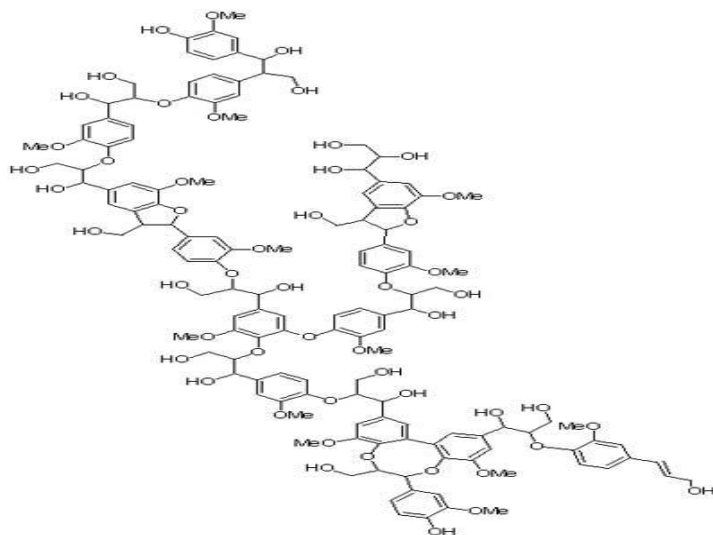


Figure 2. Lignin structure

The lignin (around 30% of wood composition) is a reticulated polymer composed by phenyl propane units synthesized by radical coupling of mainly three hydroxypropanoids; coumaryl alcohol, coniferyl alcohol and sinapyl alcohol (Fig. 3), where the monomers are connected by several ether (non condensed) and covalent carbon-carbon (condensed) bonds (Christiernin 2006). This polymer has two main functions in the tree, binding the fibers to each other and providing rigidity. Softwood lignin consists almost exclusively of guaiacyl type lignin derived from coniferyl alcohol. Hardwood lignin consists mainly of syringyl units, derived from sinapyl alcohol that has two methoxy-group, which results in lignin with a larger number of ether bonds. Hardwood lignin also contains on average 30% guaiacyl units.

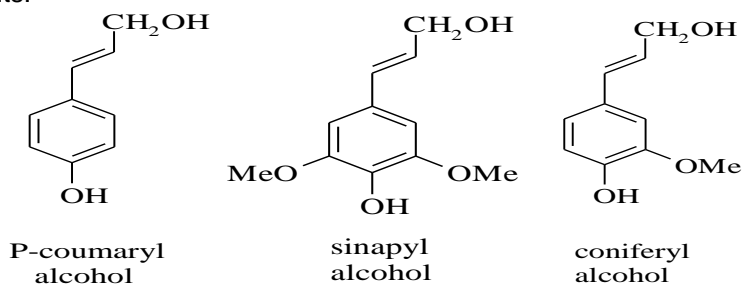


Figure 3. Monomers of the Lignin

The widely used chemical process to transform wood into pulp (kraft pulping or cooking) utilizes alkaline pulping liquor (NaOH and Na₂S). The main purpose of these chemicals is to dissolve the lignin in order to promote cellulosic fibres separation from the wood matrix (Baptista et al., 2006).

In this project lignin was extracted from kraft black liquor of *Pinus eldarica* and analyzed by FTIR. Then Lignin was oxidized with oxygen and also with nitrobenzene. In order to de-polymerization lignin to monomer the batch experiments of lignin oxidation have been performed in a jacketed reactor with injection of oxygen. The vanillin concentration in product solution with 2 ways were used an internal standard and analyzed with HPLC.

MATERIALS AND METHODS

All reagents and solvents for synthesis and analysis were commercially available and used as received without further purifications.

Preparation of pulp samples

The kraft cookings were performed in a batch reactor, taken directly from digester. The *Pinus* chips were cooked using the following constant conditions: active alkali charge, 25% (as NaOH); Sulfidity, 30%; liquor to wood ratio, 6:1; cooking temperature, 170°C (Ola and Ann-Soffi, 2006).

Preparation of lignin samples

The liquors with pH=14, were acidified with 2N H₂SO₄ until pH=1.5. The resulting precipitates were centrifuged, washed by distilled water, and finally dried at 40°C under vacuum over P₂O₅ (Baptista et al., 2008).

FT-IR spectroscopy

Infrared spectra were recorded using KBr disks on a FT-IR Perkin–Elmer spectrophotometer (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. 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 (Rajeev Bhat, 2009).

Preparation of Vanillin under oxygen condition

The first step to start the batch reactor experiments is to prepare the liquid mixture: The 500 ml of aqueous solution with 60 g/l of lignin and 80 g/l of sodium hydroxide was prepared. The thermostatic bath was already turned on with a 101°C set point, Nitrogen was admitted to the reactor and another valve was opened to remove air (oxygen) from the gas phase. After 10 minutes, the feed of nitrogen was stopped. At this time, the final pressure of the system was increased to 6.5 bar with the injection of oxygen, then both valves are closed and the system is ready to start the oxidation reaction. The heating step normally took 120 minutes to be completed, and in that time digester was hand shaken every 10 minutes. Finally the vanillin concentration in product solution was analyzed with HPLC (Araujo et al., 2010).

Preparation of Vanillin with Nitrobenzene

Approximately 0.2 g (oven dried) of wood sample with NaOH aqueous solution (7 ml; 2 mol L⁻¹) and nitrobenzene (0.5 ml) was loaded in to a 500 ml flask and heated up to 170°C and refluxed for 2.5 hours, with analyses repeated twice. The oxidized material was added (2.5 ml; 4 mol L⁻¹) to the aqueous phase. The combined organic phases were evaporated. The sample was transferred to a 50 ml volumetric flask and the volume completed with methanol/water solution (1:1 v/v). Following, the solution was filtered in a regenerate cellulose membrane, pore size 0.45 µm. The lignin oxidation products were then analyzed using high performance liquid chromatography (HPLC) (Claudio et al., 2008).

High-Performance Liquid Chromatography (HPLC)

The HPLC system consists of a Chem- Station for LC system (KNAUER, Germany) with a liquid chromatograph, a UV-visible detector, a quaternary pump, a vacuum degasser and a manual sampler. Chromatographic separation was performed on a Lichrospher 100 RP-18 column (250*4mm i.d., 5µm particle size) at room temperature; the mobile phase, at a flow rate of 1 mL min⁻¹, comprised a mixture of two solvents: 50% water, and 50% methanol. Elute was continuously monitored by UV detector at 280 nm (Yong-Hong et al., 2004).

RESULTS AND DISCUSSION

In this project the concentration of lignin was determined using ASTM E 1721-95 standard method. The amount of extracted lignin was 27% based on oven dry weight of wood.

FT-IR 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 (Boeriu et al., 2004). Gosselink et al. 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. 4 shows the infrared spectrum of lignin isolated from black liquor was similar to standard (Biljana et al., 2001). 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 ($-\text{OCH}_3$) 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 ($-\text{CH}_2=\text{CH}_2$), and C–H deformation and ring vibration. The other weak intensity bands at 616–619 and 533 cm^{-1} could have been 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 [sun et al. 1999, Hattalli et al. 2002 and Tejado et al. 2007]. In general, the FTIR results revealed high resemblance between commercial lignin and the kraft lignin isolated from *Pinus Eldarica* 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.

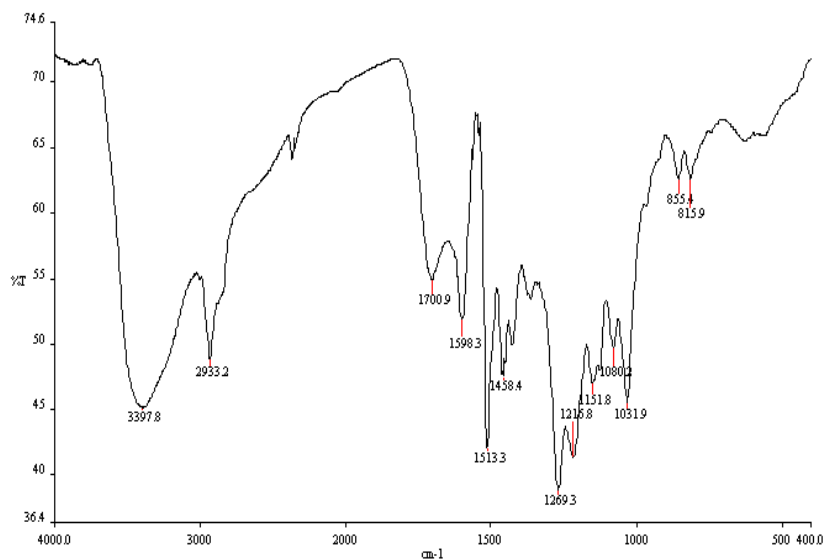


Figure 4. FT-IR spectra of kraft lignin extracted from *Pinus eldarica*

Amount of Vanillin

For the lignin that was oxidated under oxygen pressure, as it showed in Fig. 5, the spectra of vanillin standard was in 7-8 min and after injection of sample it appeared in 7.67 min (Fig. 6), and also for the lignin oxidized with nitrobenzene, the spectra of sample appeared in 7.43 min (Fig.7). The quantification of existence vanillin was performed using an internal standard. The yield of vanillin obtained from oxidation was around 4.2% (wt vanillin / wt

lignin) and oxidized with nitrobenzene was 1.2% (wt vanillin / wt lignin). These data were in good agreement with Gogotov et al.'s study in which they reported that nitrobenzene oxidation produced approximately equal amounts of vanillin and syringic aldehyde and only traces of p-hydroxy-benzaldehyde and Araujo Jose and et al. which reported that batch oxidation produced 3.5% to 7.6% vanillin yields with respect to the lignin. Interestingly, the concentration of vanillin in the oxygen condition (batch) oxidation products was much higher than that in nitrobenzene oxidation product.

It is assumed that the oxygen degradation breaks ether linkages between cinnamic acid and lignin (Fidalgo et al., 1993) and that oxygen is a milder oxidant than nitrobenzene for conversion ferulic acid into vanillin.

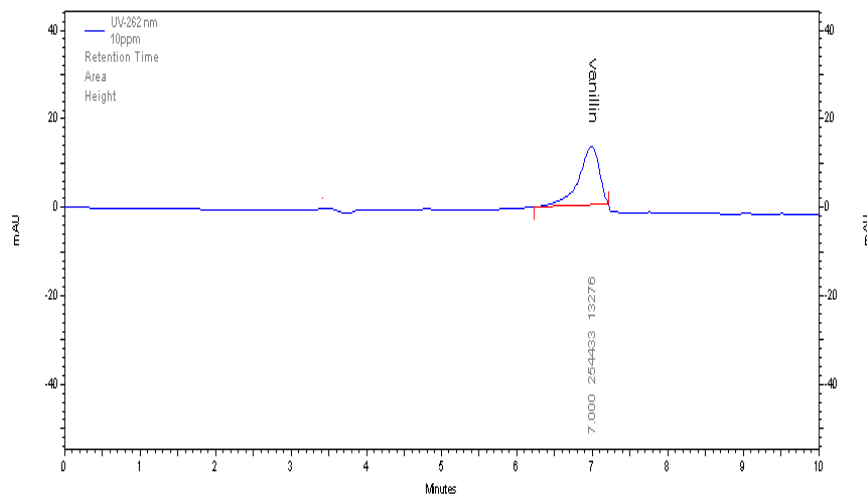


Figure 5. HPLC spectra of standard Vanillin

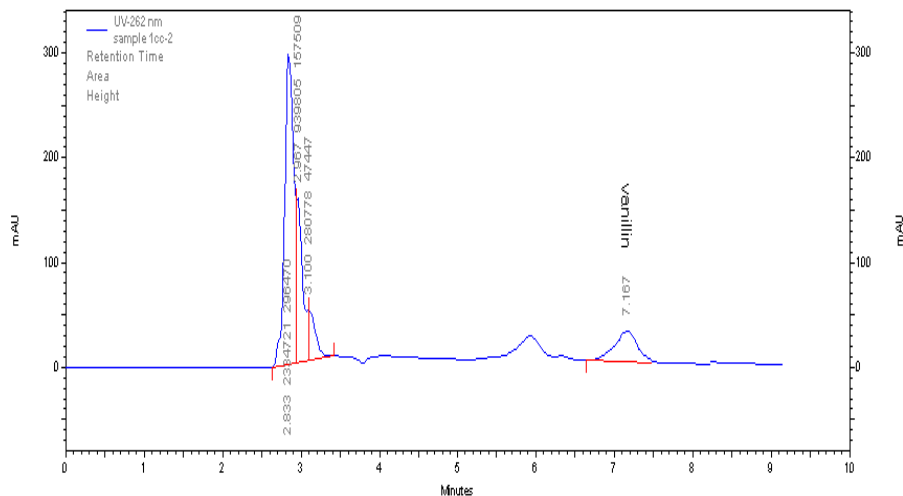


Figure 6. HPLC spectra of sample oxidized under pressure of oxygen

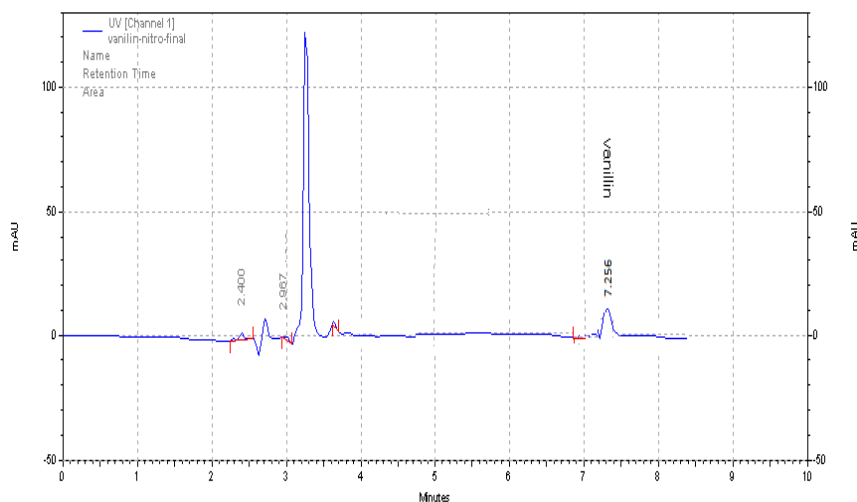


Figure 7. HPLC spectra of sample oxidized with Nitrobenzene

CONCLUSION

In this study, we isolated lignin from black liquor – a waste product of *Pinus eldarica* - extraction and then the controlled oxidation of lignin to produce vanillin in a batch reactor and also with nitrobenzene was studied. The reaction system involved a step where vanillin was formed from lignin and because of the similarity of coniferyl alcohol to the vanillin structure; this monomer was oxidized to vanillin. Other monomers were also oxidized but in this project, recognition of the vanillin was important. As explained, the yield of vanillin obtained from oxidation with oxygen (batch reactor) was more than that of with nitrobenzene.

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