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Accelerated Solvent Extraction of Lignin from *Aleurites moluccana* (Candlenut) Nutshells

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Lignin from candlenut shells was isolated using an ethanol−water accelerated solvent extraction method. Yields (based on Klason lignin) increased from about 14 to 33% as temperature increased from 100 to 195 °C and were also influenced by the amount of aqueous acid used to precipitate lignin from the extraction liquor. These yields were higher than could be obtained using a conventional dioxane−water acidolysis method. The resulting lignin was characterized by IR, ^{31}P NMR, and ^{1}H−^{13}C HMQC NMR spectroscopic techniques. The lignin contained predominantly guaiacyl units, and both the total hydroxyl group content and phenolic hydroxyl group content were high.

KEYWORDS: Lignin; *Aleurites moluccana*; candlenut; accelerated solvent extraction; organosolv; biorefinery

INTRODUCTION

Biorefineries producing fuels, power, and fine chemicals from renewable sources are a pillar of a sustainable chemical enterprise (*1*). To be both economically viable and environmentally benign, a biorefinery must strive for zero waste and incorporate all fractions of its feedstocks into valuable products, as the petroleum industry has done. This challenge has focused research efforts on processing and utilizing lignocellulosic biomass (*2*, *3*). In East Africa, biodiesel is produced from nonfood oilseed crops including the candlenut tree (*Aleurites moluccana*). Typically, the candlenuts are mechanically cracked and the oil-rich kernel is manually separated from the shells, resulting in the accumulation of over 1 kg of nutshells for every 1 L of biodiesel produced. It is possible to convert the nutshell byproduct into biomass briquettes for cooking and heating. This low-cost, high-volume energy source is useful to the approximately 90% agrarian population of the region. However, there is also interest in producing high-value, low-volume chemicals to meet the needs of developing countries such as Uganda, a land-locked nation with negligible petroleum infrastructure (*4*). It has been previously reported that candlenut shells possess 60.1% Klason lignin, greater than the 15−30% lignin content typical of woody plant material (*5*). Although significant research efforts have been devoted to applications of nutshells, for example, as raw materials for activated carbon (*6*−*8*), pyrolysis oil (*9*, *10*), or adhesive resins (*11*), very little is known about the extraction or molecular properties of lignin from the nutshells (*12*). The abundance of candlenut shells as a waste product from fuel production as well as their exceptionally high lignin content inspired the present investigation into the extraction and analysis of candlenut nutshell lignin and its prospects as a valuable coproduct of the biodiesel enterprise in East Africa.

Lignin is an amorphous, three-dimensional heteropolymer composed of phenylpropanoid units; as such, it is nature's major source of aromatic carbon. Lignins are produced on the scale of millions of metric tons per year as a byproduct of the pulp and paper industry, where about 98% is incinerated for energy recovery (*13*). Present industrially relevant applications of lignin include incorporation into polyurethane foams (*14*), thermosetting polymers (*15*), phenolic resins, and biodispersants (*16*). Increasingly important is the production of small aromatic building blocks from lignin to satisfy the enormous and diverse industrial demand for aromatic carbon compounds (*1*).

If lignin platform chemicals and polymer materials are to play a role in supporting the economic and environmental sustainability of future biorefineries, the choice of lignin extraction process will be a critical factor. The use of "organosolv" techniques makes it possible to avoid the environmental impacts of odor, sulfur emissions, and acidic or basic aqueous waste streams from conventional wood pulp delignification processes. One of the more benign organosolv technologies is the Alcell process, which uses an ethanol−water solvent mixture without acid or alkali, making use of a renewable-resource-derived solvent and minimizing the use of hazardous chemicals (*17*). The present study reports on the use of an accelerated solvent extraction (ASE) system to extract lignin from candlenut shells in a convenient laboratory-scale approximation of the Alcell process (**Table 1**). The parameters of temperature, sample size, and solvent flush percentage (additional solvent used to separate analytes from the sample) were explored. The isolated lignin was characterized by FTIR, ^{31}P NMR, and HSQC NMR spectroscopic techniques and compared with lignin extracted from candlenut shells by a conventional dioxane−water acidolysis organosolv method.

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Table 1. Comparison of Alcell and ASE Extraction Parameters

	Alcell (*20*)	ASE
solvent composition (ethanol/water)	60:40	60:40
temperature	195−205 °C	100−195 °C
time	30−120 min	15 min

MATERIALS AND METHODS

Candlenut (*A. moluccana*; Euphorbiaceae) nutshells were obtained by the author (A.P.K.) in Kasese District, Uganda. The nutshells were rinsed in water to remove soil and debris and then air-dried at room temperature. The moisture content of these nutshells was determined to be 9.6% by drying to constant weight at 105 °C. The nutshells were ground in a hand mill and passed through standard sieves (ASTM E-11) to obtain particles of 250 μm−1 mm in diameter (60−18 mesh). All solvents and chemicals were of reagent grade or higher.

Lignin Extraction. *Klason Lignin Determination*. Extractive-free samples were prepared from the milled nutshells, and then Klason lignin content was evaluated following standard methods (*18*, *19*).

Accelerated Solvent Extraction (*ASE*) *Method*. An ASE 300 system (Dionex Corp., Sunnyvale, CA) was used to extract lignin from milled nutshells. The parameters were modeled on the Alcell process as described in the literature (*20*). The samples for extraction were placed in stainless steel ASE cells (33 mL capacity) using 5 or 10 g amounts of milled candlenut shells. The remaining space in each ASE cell was filled with inert sand to prevent compaction during extraction. The extraction with 60% (v/v) ethanol−water took place at 100, 150, or 195 °C using a flush percentage of 70, 100, or 130%, using three static cycles of 5 min each while pressure was maintained between 10.34 and 11.72 MPa (1500−1700 psi). The final purging of the cell took place for 200 s with nitrogen gas. The lignin was precipitated from the ASE liquor by adding 1 volume of ASE liquor to 2−3 volumes of aqueous acid depending on the amount of liquor removed (HCl, 16 mM). The lignin was recovered by centrifugation (12000 rpm for 10 min) and drying the pellet at 60 °C to constant mass.

Dioxane−Water Acidolysis Method. The procedure was adapted from the literature (*21*). Milled candlenut shells (49.26 g) were pre-extracted with cyclohexane−ethanol (1:1, v/v) and then deionized water to remove unwanted wood extractives. These steps removed 3.35 and 2.51 g of extractives, respectively. The resulting shells were dried at 60 °C and then treated with a solution of dioxane and 0.1 N aqueous HCl (8.5:1.5, v/v). The isolated lignin (2.56 g) was dried in vacuo over phosphorus pentoxide for 20 h.

Spectroscopy. *Fourier Transform Infrared* (*FTIR*) *Spectroscopy*. Finely powdered lignin was analyzed using a Nicolet 6700 infrared spectrometer (Thermo Fisher Scientific, Waltham, MA) equipped with a Thermo Smart Orbit diamond anvil ATR probe. Sixteen scans were recorded over the range of 1800−400 cm^{-1}.

HMQC NMR Spectroscopy. The procedure was adapted from the literature (*22*). The spectra were recorded at 25 °C in an Avance 500 MHz instrument (Bruker Biospin, Billerica, MA). One hundred milligrams of ASE−Alcell or dioxane−water acidolysis lignin was dissolved in 0.84 mL of dimethyl-d_6 sulfoxide (DMSO), and gradient-selected heteronuclear multiple bond coherence (HMQC) spectra were recorded, with decoupling during acquisition. A 12 ppm sweep width was used in ^{1}H, and a 200 ppm sweep width was used in ^{13}C, centered at 6 and 100 ppm, respectively. The spectra were acquired with 8 transients and a recycle delay of 1.5 s over 256 increments and 2048 data points. The $^{1}J_{CH}$ was 140 Hz. The spectra were processed with MNova software version 5.3.1 (Mestrelab Research S.L., Santiago de Compostela, Spain) using a 1024 × 1024 matrix and cosine-squared window functions in both dimensions.

^{31}P NMR Spectroscopy. Lignin was phosphitylated with 2-chloro-4,4,5,5-tetramethyl-1,2,3-dioxaphospholane (TMDP) and analyzed by ^{31}P NMR spectroscopy using inverse-gated decoupling. The sample preparation and acquisition parameters were based on the literature (*14*, *23*). Spectra were recorded at 25 °C in an Avance 500 MHz instrument (Bruker Biospin), with 32000 data points, 256 scans, a 62 ppm sweep width centered at 140 ppm, a 25 s relaxation delay, and a 75° pulse. The spectra were processed in MNova with 4 Hz line broadening. Chemical shifts were calibrated from the sharp ^{31}P NMR signal at 145.1 ppm arising from the reaction product between cyclohexanol and TMDP. Cyclohexanol (4.0 mg/mL) was used as the internal standard for the quantitative evaluations of the lignin structural elements.

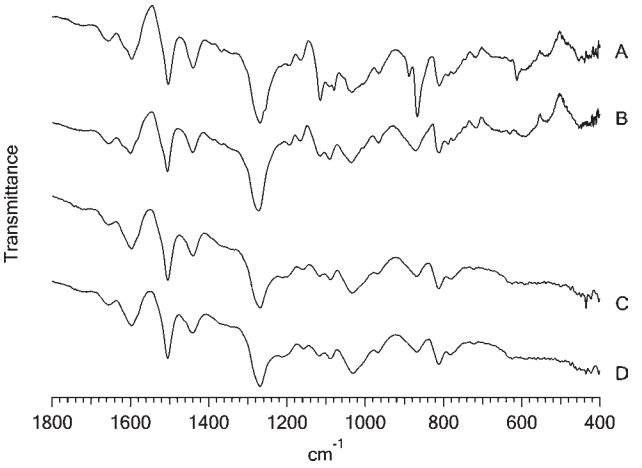

Figure 1. IR spectra of various lignins extracted from candlenut shells: (**A**) dioxane−water acidolysis lignin; (**B**) ASE lignin (100 °C); (**C**) ASE lignin (150 °C); (**D**) ASE lignin (195 °C).

RESULTS AND DISCUSSION

Lignin Extraction. The ASE system using an ethanol−water solvent mixture provided a rapid and high-throughput method to isolate lignin from candlenut shells. Lignin yield was calculated by comparing the amount of recovered lignin with total Klason lignin (59.3% of the nutshells by dry weight). The lignin yields ranged from 14 to 33% of Klason lignin (Supporting Information, Table A). As there are no prior reports of candlenut shell delignification, it is difficult to gauge the efficiency of the ASE method, although the ASE yields were higher than was obtained by a standard dioxane−water acidolysis extraction technique (8.84% of Klason lignin). The relatively low yield is not uncommon for that technique (*24*). Using the ASE system, temperature was strongly correlated with yield ($p < 0.0001$); the yield of lignin increased linearly as extraction temperature increased from 100 to 195 °C (Supporting Information, Figure A). The industrial Alcell process, which the ASE method in this study emulates, typically operates at 195 °C and is known to achieve increased delignification with temperature (*20*). The other ASE parameters tested (sample size and solvent flush percentage) had no statistically significant effect on lignin yield, considered individually or in combination with increased temperature. This finding indicates that the solvent was not saturated with dissolved lignin under the tested conditions. The amount of solvent ("liquor") found in collected extracts ranged from 55 to 110 mL, and these volumes were not correlated with ASE parameters or yield. However, in the subsequent isolation procedure, the ratio of added aqueous acid to liquor influenced yield ($p < 0.005$), and when this factor was considered in combination with temperature, it was a highly significant predictor of yield (Supporting Information, Table B). The purpose of the aqueous acid is to precipitate lignin from the liquor, and it is expected that yield would increase with increasing acid-to-liquor ratio and level off when precipitation is complete. The ratios of acid to liquor used in this study did not show this leveling-off trend, indicating that higher yields might be obtained by continuing to increase the amount of aqueous acid added. Higher yields might also be obtained by increasing the nutshell−solvent contact time, because the industrial Alcell process cooking cycle is generally longer than the present study conditions of three 5-min cycles (**Table 1**).

FTIR Spectroscopy. FTIR spectroscopy serves as a qualitative indicator of the purity and structural similarity of lignin samples (*25*). Representative FTIR spectra of ASE lignins extracted at 100, 150, and 195 °C, as well as dioxane−water acidolysis lignin, are displayed in **Figure 1**. The close similarity of the FTIR spectra suggests that these lignins have similar chemical structures.

The peaks are well-defined and match those expected for lignin, indicating that the extraction methods yielded lignin in high purity (*25*). The peaks at 710 and 605 cm^{-1} are stronger and sharper in the dioxane lignin, but these are in the fingerprint region and difficult to attribute to structural elements. More detailed structural information can be obtained using NMR spectroscopy.

Table 2. Quantification of Hydroxyl Groups by ^{31}P NMR Spectroscopy

	lignin sample (mmol/g)		
structure	ASE (100 °C)	dioxane−water acidolysis	integral region[a] (^{31}P NMR, δ)
aliphatic	6.34	5.76	149 to 146
condensed residues	0.77	0.85	(144 to 140 ppm−143 to 142 ppm)
syringyl	0.18	0.21	143 to 142
guaiacyl	7.4	5.47	140 to 138.4
p-hydroxyphenyl	0.54	0.54	138.4 to 137.2
acids	0.22	0.09	135.5 to 134

[a] Based on the literature (*23*).

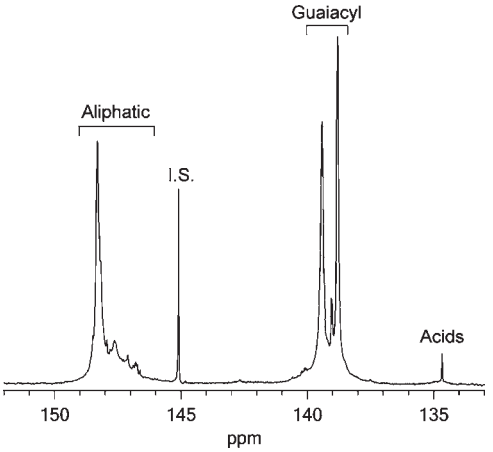

Figure 2. ^{31}P NMR spectrum of phosphitylated ASE lignin extracted at 100 °C. (I.S. = cyclohexanol internal standard.)

Hydroxyl Group Content (^{31}P NMR Spectroscopy). It is possible to measure the phenolic content of lignin in situ, without extraction techniques that degrade the native structure (*26*); however, in the interest of characterizing candlenut lignin as it might be encountered as a processed biorefinery product, the organsolv lignins were isolated and analyzed in the present study. The content of various hydroxyl groups was calculated using the integral regions given in **Table 2**. An example spectrum is shown in **Figure 2**. Both ASE and dioxin lignin samples contained very low levels of condensed residues, which is as expected for organosolv extraction methods. On the basis of the hydroxyl group values, the syringyl/guaiacyl/*p*-hydroxyphenyl ratio was calculated to be 1:41:3 for Alcell lignin and 1:26:3 for dioxane lignin. The predominance of guaiacyl residues is unexpected because *A. moluccana* is a hardwood species and hardwood lignins tend to have higher syringyl content. It has been previously reported that alcohol pulping processes remove syringyl lignins preferentially from hardwood, so it is unlikely that the low syringyl content can be attributed to the extraction technique (*27*). The low syringyl/guaiacyl ratio was confirmed by HMQC NMR spectroscopy as discussed below. Both the total hydroxyl group and phenolic hydroxyl group contents were significantly higher than was previously reported for a mixed hardwood Alcell lignin, by a factor of > 2 (*14*). Because both ASE and dioxane extraction methods yielded roughly the same results, the high hydroxyl group content appears to be characteristic of candlenut shell and not related to extraction conditions.

Composition and Interunit Linkages (HMQC NMR Spectroscopy). HMQC NMR spectroscopy is a powerful technique for direct detection of various lignin interunit linkages (*22*, *28*). In the aromatic region (**Figure 3**, left) signals from guaiacyl units were pronounced in the region 7.0−6.5/120−115 ppm. Signals from syringyl or *p*-hydroxyphenyl units were not apparent, in agreement with analysis of aromatic hydroxyl group content by ^{31}P NMR spectroscopy. Additional cross peaks at 7.32/117.3 and 7.22/123.6 ppm can be attributed to vanillin or vanillic acid moieties and signals at 7.56/153.7 and 6.67/127.1 ppm correspond to α and β carbons, respectively, in a cinnamyl-type aldehyde (*22*). In the phenylpropanoid side-chain region (**Figure 3**, right) it can be seen that β-*O*-4′ (**A**) and β-β resinol (**B**) linkages were the predominant structures found in ASE candlenut lignin. A signal

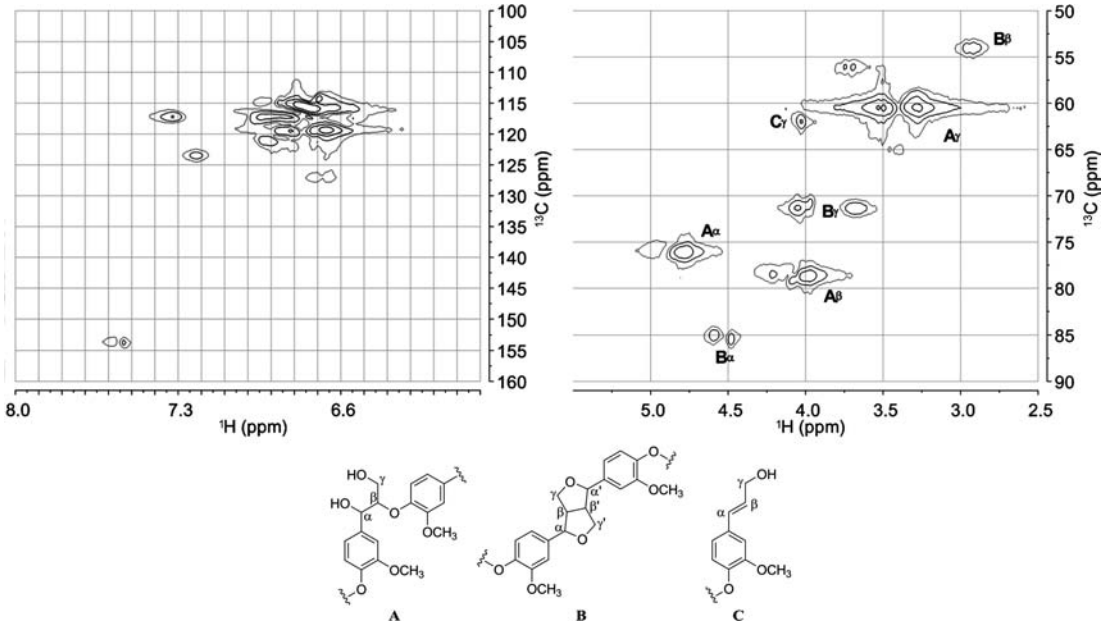

Figure 3. $^{1}H-^{13}C$ HMQC NMR spectrum of ASE lignin extracted at 100 °C, showing the aromatic and phenylpropanoid side-chain regions.

was also detected for cinnamyl-type alcohols (**C**). An additional cross peak at 3.71/56.2 ppm is characteristic of methoxy groups. These are all common structures in native lignins (*29*). In studies of the Alcell pulping process it was reported that β-*O*-4′-ether linkages were present in the cooking liquor in the early stages of the extraction, decomposing later (*30*). Because ASE samples in the present work were exposed to solvent for only 15 min total, the detection of β-ether linkages is expected.

The ASE ethanol−water extraction method yielded up to 33% of organosolv lignin from candlenut shells in 15 min of contact time. The use of benign solvents and reduced solvent dependence are among the environmental benefits of the ASE method compared to conventional extraction techniques. Candlenut lignin could be a substantial asset to a biorefinery, considering the candlenut shells' high lignin content and the abundance of the shells as a waste product of biodiesel production. In particular, the high hydroxyl group content provides an increased density of reactive sites for polyurethane synthesis or other polymer applications. Size exclusion chromatography would reveal to what extent the extracted lignin has been degraded into low molecular weight oligomers that might be useful as chemical building blocks. Future work in our laboratory will focus on further optimization of lignin yield and exploitation of the hydroxyl group functionality.

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Supporting Information Available: Lignin yield under various extraction conditions; statistical analysis of factors correlated with yield. This material is available free of charge via the Internet at http://pubs.acs.org.

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