

# The Role of pH and Acids in the Extraction and Precipitation of Lignin in Cashew (*Anacardium occidentale*)

Luke N. Ukiwe\* • Chris I. Nwoko • Chris O. Akalezi

Department of Chemistry, Federal University of Technology P.M.B 1526, Owerri, Nigeria

Corresponding author: \* luggil2002@yahoo.com

## ABSTRACT

The extent of extraction and precipitation of lignin was determined using inorganic and organic acids at different pHs (3.0, 4.0, 5.0). Oxalic acid (organic acid) precipitated the highest amount of lignin (14.85 g) at pH 3.0 while sulphuric acid (inorganic acid) precipitated the lowest amount (2.00 g) at pH 4.0. However, oxalic acid precipitated more lignin at pH 3.0, 4.0, and 5.0 than sulphuric acid at the same pH levels. At these three pH levels there were no visible differences in lignin extraction for any given acid. Overall, there was no advantage of one acid over another in precipitating lignin since both acids (inorganic and organic acid) were found to be effective in extracting lignin from cashew (*Anacardium occidentale*) at pH 3.0, 4.0 or 5.0 using the alkaline Kraft process. Infra-red spectrophotometry showed absorption bands which are characteristic of the lignin compound.

**Keywords:** acetic acid, coniferyl, infra-red, lignification methoxyl, phenolic

## INTRODUCTION

Lignin is a complex chemical compound most commonly derived from wood and an integral part of the cell walls of plants. In 1819, Candolle coined the word lignin, which is derived from the Latin word *lignum*, meaning wood. Lignin is the most abundant organic polymer on Earth after cellulose, employing 30% of non-fossil organic carbon (Boerjan *et al.* 2003) and constituting from a quarter to a third of the dry mass of wood. It is formed by the removal of water from sugars to create aromatic structures in an irreversible reaction.

Lignin reinforces the walls of certain cells in higher plants. It is mainly found in the vascular tissues, where its hydrophobicity waterproofs the conducting cells of the xylem and its rigidity strengthens the supporting fiber cells of both the xylem and phloem. It also plays a crucial role in defense against pathogen attack (Hawking *et al.* 1997). Lignin fills the spaces in the cell wall between cellulose, hemicellulose and pectin components, especially in tracheids, sclereids and xylem. It is covalently linked to hemicellulose and thereby crosslinks different plant polysaccharides, conferring mechanical strength to the cell wall and by extension the plant as a whole (Chabannes 2001). It is indigestible by mammalian and other animal enzymes, but some fungi and bacteria are able to biodegrade the polymer. The enzymes involved may employ free radicals for depolymerization reactions.

Lignin is a large, cross-linked, racemic macromolecule with molecular masses in excess of 10,000 (Davin and Lewis 2005). Lignin can be found in the liquor generated during commercial papermaking, which typically involves and reduction of wood (Fujimoto *et al.* 2004) to the fibrous mass through a series of cooking and bleaching operations using strong chemicals (a process known as pulping). The two principal chemical pulping methods are the alkaline Kraft process and the acidic sulphite process, each producing a different type of extractable lignin. It is relatively hydrophobic and aromatic in nature. In nature, during polymerization, the extent of polymerization appears extremely difficult to measure since the extracted fraction is fragmen-

ted and consist of molecules with various types of substructures appearing in repeated haphazard manner. Depending on the method of isolation different types of monolignol monomers exists which could be methoxylated to varying extents. These monomers include *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol (Ralph *et al.* 2006; Wagner *et al.* 2009).

The OH groups (either the alcoholic OH's on the chains or the phenolic OH's on the aromatic rings) can react with each other or with the aldehyde or ketone groups. When an OH reacts with another, an ether linkage is formed with the elimination of a water molecule. Lignin is a polymeric material composed of phenylpropanoid units derived from these three cinnamyl alcohols (monolignols).

In order to understand the behavior of lignin under steam explosion and treatment process, aspen wood and isolated lignin from aspen were subjected to steam treatment under various conditions. Using NMR and chromatography to analyze the lignin portion, the addition of a reactive phenol, 2-naphthol, inhibited the repolymerization reaction strongly, resulting in a highly improved delignification by subsequent solvent extraction and an extracted lignin of uniform structure (Li *et al.* 2006). In the present study, statistical analyses conducted on experimental data obtained show that the standard error of the mean weights of HCL extracts at pH 3.0, 4.0 and 5.0 is 0.00, while that of sulphuric at the same pH range is 0.307. However, the standard error of the mean weights of oxalic, benzoic, nitric and acetic acid extracts are 0.510, 0.01, 0.55 and 0.10, respectively. These values indicate no variation in the mean values due to random error.

A study on the use of lignin and lignin-based chars and activated carbons as adsorbents for the removal of substances from water showed that under appropriate condition of activation of the lignin materials, it is possible to obtain materials with surface areas and pore volumes approaching 2000 m<sup>2</sup>g<sup>-1</sup> and 1 cm<sup>3</sup>g<sup>-1</sup>, respectively and these materials have capacities for the aqueous phase adsorption of metallic pollutants that are comparable to those of commercial activated carbons (Suhana *et al.* 2006).

Comparison of various analytical methods for determini

ng functional groups in technical lignins of five different origins: Kraft (sulphate pulping), sulphite, soda/anthraquinone, organosolvent, and ethanol process lignins, indicated that non-aqueous potentiometry and aminolysis are the most reliable analytical methods for determining hydroxyl groups (phenolic and aliphatic) when the lignin extracts were characterized in terms of methoxyl, phenolic and aliphatic hydroxyl, carbonyl, carboxyl, and sulphonate groups (Noureddine and Salvado 2007). Lignin precipitation on the pulp fibers in the ethanol-based organosolvent pulping of eucalyptus wood chips and wheat straw revealed that the precipitation of dissolved lignin back onto the fibers in pulping and washing showed large amount of lignin particles present on the fibers after pulping and each stage of washing in a four-stage washing sequence, lignin precipitation occurs when the ethanol concentration of a lignin ethanol solution is reduced or the temperature drops (Xua *et al.* 2006).

Lignin plays an important role within the plant. It imparts strength to cell walls, facilitate water transport and due to its hydrophobicity impede the degradation of wall polysaccharides, thus acting as a major line of defense against pathogens, insects, etc. Highly lignified wood is durable and therefore a good raw material for many applications e.g. as fuel. Sulphite pulping removed sulphonates from wood pulp. These lignosulphonates can be used as: dispersants in high performance cement applications, water treatment formulations and textile dyes, etc.

In the present study, various inorganic and organic acids have been used to precipitate lignin extracted using the alkaline Kraft process with a view to determining which of the acids H<sub>2</sub>SO<sub>4</sub>, HCL, HNO<sub>3</sub>, benzoic, oxalic and acetic, would precipitate the larger amount of lignin extracted from cashew (*Anacardium occidentale*) between pH 3-5.

## MATERIALS AND METHODS

The cashew plant samples were gotten from the branch of the tree growing in the undergraduate project farm yard in the Federal University of Technology, Owerri. About 100 g of the wood sample was split into tiny splinters, weighed and placed into an autoclave (Akclave Ac064) containing 1000 ml of tap water (10% consistency) and 10 g of solid NaOH. This mixture was heated to 200°C for 1 hr. The autoclave was then turned off and the mixture was allowed to cool to ambient temperature. The mixture was decanted and filtered into a 1000-ml beaker. The extracted lignin-containing liquid in the beaker had a pH of 12.20 and this solution was precipitated by lowering the pH of the solution to a value of 3.0 using 10 ml 40% (v/v) H<sub>2</sub>SO<sub>4</sub>. The mixture was further decanted and filtered into a 250 ml beaker. The precipitate (lignin) was recovered and dried in a dessicator for 48 hrs and weighed. Three more repetitions were made and the mean weight determined. This procedure was repeated with H<sub>2</sub>SO<sub>4</sub> at pH 4.0 and 5.0 and also repeated for the acids, HNO<sub>3</sub>, HCL, benzoic, oxalic and acetic acid at pH 3.0, 4.0 and 5.0, respectively. Each of the five acids was used to extract lignin at pH 3.0, 4.0, and 5.0. These values are given in **Table 1**.

About 2 g of the acetic acid lignin precipitated was weighed and analyzed using an infra-red (IR) spectrophotometer (Unicam 1252-98). The resolution peaks of the IR spectrophotometry analysis of the various functional groups present in the lignin compound are shown in **Table 2**.

## Statistical analysis

Simple random sampling was adopted for sample collection. Data were reported as arithmetic mean and standard deviation. Analysis of variance (ANOVA) was employed to measure difference between mean weights of lignin extracts at various pH values. The standard error of the difference between mean weights of lignin extracts at various pH values and the generalized *t*-test were employed to test the significance of values obtained.

## RESULTS AND DISCUSSION

The preparation and performance of substances such as activated carbon is controlled by the extent of lignification of the wood from which it is prepared. Lignin plays a crucial part in conducting water in plant stems since it is present in all vascular plants thus enhancing the believe that its original function was restricted to water transport.

Certain aquatic plants such as water hyacinth (*Eichhornia crassipes*) rely on water uptake for their nutrients. Hence, it has been shown that the quantity of lignins increase particularly in monocotyledons plant where there is little wood (Ukiwe *et al.* 2008).

**Table 1** indicates values of mean weights of lignin extract precipitated with different inorganic and organic acids at different pH range. The highest amount of lignin precipitate was obtained with oxalic acid at pH 3.0 (14.848 g), while the least amount was obtained using sulphuric at pH 5.0 (1.108 g). Generally, oxalic acid extracts are highest at all pH levels while sulphuric acid extracts are lowest at all pH levels.

Lignification is the process of forming the collective of phenylpropanoid macromolecules termed lignin. Lignification encompasses the biosynthesis of monolignols, their transport to the cell wall, and polymerization into the final molecule. The amount and type of individual phenolics available at the lignification site and normal chemical coupling properties regulate lignin formation (Syrjanen *et al.* 2001). Much is not known about the digestion, solubilization and precipitation of lignin with acids. **Table 1** enables us to reason that solubilization, digestion and precipitation of lignin is effective with both inorganic and organic acids. But the low value obtained with H<sub>2</sub>SO<sub>4</sub> precipitation is a little confusing, but of course, this could be attributed to the partial lignin solubilisation in the H<sub>2</sub>SO<sub>4</sub> precipitates. Kenji *et al.* (1989) in a study to determine lignin in herbaceous plants by an improved acetyl bromide procedure indicated that the digestion with 25% acetyl bromide (AcBr) in acetic acid at 70°C was improved by including 4% perchloric acid in the digestion solutions. Addition of perchloric acid allows for more rapid, complete digestion.

IR spectra and nitrobenzene oxidation data for milled lignin samples gave the value of 20.0g<sup>-1</sup> Lcm<sup>-1</sup> for the specific absorption coefficient of AcBr-treated lignins, values which were consistent with nitrobenzene oxidation data, but higher than those obtained by the acid detergent lignin method and AcBr method proposed for grasses (Fujimoto *et al.* 2004).

The lower lignin values obtained in the latter methods are considered to be due to partial lignin solubilization in the sulphuric acid digestion.

**Table 2** shows the detailed list of peaks of the IR spectrum of the lignin monomer. The absorption bands at 3461 cm<sup>-1</sup> in the spectrum (due to O-H stretching of the intermolecular hydrogen bonded species) appear to decrease in intensity maybe due to dilution. The intensity of the aromatic (1415 and 1576 cm<sup>-1</sup>) [C-C multiple bond stretching vibrations], alkene (CH<sub>2</sub>), [1493 cm<sup>-1</sup>] (C-H bending vibrations) and the ether (1156 cm<sup>-1</sup>) [C-C stretch vibrations], absorptions appear to be most pronounced.

Hydrochloric and oxalic acids were used to show if

**Table 1** Mean weights of extracted lignins for various acids at pH 3.0, 4.0 and 5.0.

pH	3.0	4.0	5.0
Acids	Mean weight (g) ± SD × 10 <sup>-1</sup>	Mean weight (g) ± SD × 10 <sup>-1</sup>	Mean weight (g) ± SD × 10 <sup>-1</sup>
HCL	10.034 ± 1.8	10.214 ± 2.9	10.110 ± 1.8
H <sub>2</sub> SO <sub>4</sub>	2.061 ± 1.8	2.001 ± 1.8	1.108 ± 0.0
HNO <sub>3</sub>	4.300 ± 0.0	4.104 ± 0.0	4.222 ± 0.1
Oxalic	14.848 ± 2.9	14.012 ± 2.9	13.069 ± 1.8
Benzoic	4.916 ± 3.7	5.064 ± 0.0	5.501 ± 1.8
Acetic	3.392 ± 1.8	3.186 ± 2.4	3.033 ± 2.9

**Table 2** Peak positions and wavelength intensities of IR Spectrum of the lignin (acetic acid) extract.

Peak position (cm <sup>-1</sup> )	Wavelength intensity
594.14	9.086
619.00	8.911
703.40	10.416
968.73	16.316
1022.93	16.813
1131.03	17.778
1156.36	21.410
1345.00	26.549
1415.46	10.655
1473.42	12.550
1576.19	4.302
2149.95	32.688
2342.56	31.874
2360.07	31.525
3461.43	3.778

there was significance in the data for the inorganic and organic acids lignin extracts. For HCL, the standard error of the mean weights for lignin extracts at pH 3.0, 4.0, and 5.0 is 1.040, 1.676, and 1.040, respectively while, the standard error of the difference between mean weights at all three pHs is 1.972. The generalized *t*-test between these pH levels is 0.0912 and testing this value at 4 degrees of freedom, there was no significance at the 5% confidence level.

For oxalic acid, the standard error of the mean weights for lignin extracts at pH 3.0, 4.0, and 5.0 is 1.676, 1.676, and 1.040, respectively. The standard error of the difference between mean weights at pH 3.0/4.0 and 4.0/5.0 is 20.415 and 19.160, respectively. The *t*-test between these pH values set is 0.04 and testing these values at 4 degrees of freedom, no significance was also observed at the 5% confidence level.

Different types of lignin exist depending on the means of isolation. But much study is required to understand its biosynthesis (anabolism) and linkage specificity with a view to determine how well each fits based on experimental evidence.

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