

CHARACTERISATION OF LIGNINS ISOLATED FROM BAMBOO ORGANOSOLV AND KRAFT BLACK LIQUOR

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ABSTRACT

Bamboo biomass was pulped using organosolv methods: Acetic acid and formic acid; hydrogen peroxide and formic acid; ethanol and water and kraft process with varying concentrations of the cooking chemicals. The properties of the isolated lignin were influenced by the black liquor pH as well as the pulping method. Highest pH (12,8) was recorded for kraft at 70:30 liquor concentration while lowest pH of 2,5 was recorded for both Aceticformic (70:30) and Ethanol/water (70:30 and 60:40). There were obvious differences in the characteristic colours, shapes and sizes of the lignin samples. Bamboo kraft lignin samples formed large pieces much easier than that of Organosolv. Aceticformic (70:30) had the best filtration properties (FC) while Ethanol/water had the poorest. Generally, kraft recorded the highest lignin yield while all the organosolv processes recorded reduced yield. Highest yield (119,25 g/L) was gotten from kraft (50:50) while Peroxyformic (60:40) had the lowest yield of 8,20 g/L. The results showed that higher liquor pH favours total dissolved solid as well as lignin precipitation. As the pH increases, total dissolved solid, yield, Klason and low molecular weight (LMW) lignin content increases while FC decreases. The Klason and LMW lignin content increased with increasing lignin yield.

Keywords: Bamboo, *Bambusa vulgaris*, filtration capacity, guaiacyl-syringyl units, low molecular weight lignin, total dissolve solid.

INTRODUCTION

After cellulose, lignin is not only the second most abundant polymeric organic substance on the planet but also the most abundant renewable aromatic bio-polymer resource in nature (Wang *et al.* 2021, Gellerstedt and Henriksson 2008) with complex molecular structure, containing cross-linked polymers of phenolic monomers mainly consisting of p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol (Inkrod *et al.* 2018, Soongprasit *et al.* 2020). Lignin is one of the most potential renewable and sustainable energy resource which is present in a huge amount of waste such as wood, maize, rice straw, corn stover, sugarcane bagasse accounting for 15-30 wt% in the dry lignocellulosic biomass (Wang *et al.* 2021). Lignins have been isolated from different biomass such as olive tree prunings (Erdocia *et al.* 2014), oil palm (Faris *et al.* 2017); rice husk (Singh and Dhepe 2016, Kim *et al.* 2021), baggass (Inkrod *et al.* 2018); palm oil empty fruit bunch (Hidayati *et al.* 2018); wood and bamboo (Wang *et al.* 2021). Fractionated lignins can be further used for various applications, including liquid fuel, composite materials, and production of low molecular weight aromatic or phenolic compounds (Inkrod *et al.* 2018) and other value-added chemicals, and functional materials due to its high-energy density and intrinsic aromatic-based structure (Jardim *et al.* 2022).

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Kraft process is the dominant alkaline pulping process and most used pulping process for wood and non-wood such as bamboo (Kamthai and Puthson 2005). Kraft process is a cheap conventional way of extracting lignin, however with negative health effects (Krief *et al.* 2008). The residual liquor gotten when wood is digested to pulp is called black liquor. Black liquor disposal constitute a major problem to the environment and a threat to human health. The black liquor consists of almost all the inorganic chemicals used in pulping and organics in the form of dissolved wood constituents (Hidayati *et al.* 2018). Most importantly, black liquor contains lignin which is about 46 % of its total solid (Hidayati *et al.* 2018). Lignin extraction from black liquor is an attractive option for pulp mills as it allows for increased production capacity without increasing recovery boiler load (Jardim *et al.* 2022). It also enables the industry to become a biorefinery by allowing for the manufacture of multiple products from biomass.

Although, Kraft liquor yields are encouraging, but environmental concern on the pulping process are discouraging. The organosolv process on the other hand has the advantage of using substances which are less harmful to human health and the environment compared to conventional processes (Kraft and Sulfite) that cause major problems of pollution (FAO 1996, UNEP 1996, Suhr *et al.* 2015, Gavrilescu *et al.* 2008, Ruiz *et al.* 2011). However, few papers present the utilization of Organosolv methods because they are mostly expensive. Organosolv pulping is a clean chemical process that uses organic solvents to aid the removal of lignin and hemicelluloses from lignocellulosic raw materials. The process involves the cooking of lignocellulosic biomass in a mixture of water and an organic solvent that leads to the deconstruction of both lignin and hemicelluloses and their dissolution in the cooking liquor (Sannigrahi *et al.* 2011). Lignin is typically retrieved as a precipitate by dilution of the liquor with water. The process provides facility for solvent recovery after pulping (Ruiz *et al.* 2011). The process has been successfully used as a pretreatment and fractionation process for a variety of lignocellulosic materials, such as agricultural, hardwoods and softwoods (Galbe and Zacchi 2002).

Separation of lignin from various cellulosic materials has been reported using different techniques, e.g., alkaline treatment, solvent extraction, organosolv pretreatment, ionic liquid extraction, and milled wood lignin isolation which resulted in isolation of lignin with variation in purity and chemical characteristics (Raita *et al.* 2017). Also, many methods have been employed to recover pure lignin from black liquor such as acidification, ultrafiltration and electrolysis (Do *et al.* 2020, Kihlman 2016). Research has also made progress in two techniques; LignoBoost and Sequential Liquid-lignin Recovery and Purification (SLRP) (Öhman *et al.* 2007, Tomani 2010, Velez and Thies 2013). However, the use of acid to precipitate lignin has been widely practised (Hubbe *et al.* 2019, Ma'ruf *et al.* 2017, Minu *et al.* 2012).

Lignin structure and physic chemical properties depends on isolation method and raw material source (Hidayati *et al.* 2018). The biomass source is the primary factor determining the lignin composition, especially the contents of the three structural units. Biomass source as well as extraction methods have been reported to affect the final structures of lignin, in terms of bond linkages, functionalities and structural units (Dominguez *et al.* 2018). Various methods have been developed to isolate lignin from lignocellulosic biomass. Organosolv lignin (Patwardhan *et al.* 2011) and milled wood lignin (Jiang *et al.* 2020) as well as acidolysis of cellulose and hemicellulose to obtain Klason lignin and hydrolyzed lignin have been investigated. Milled wood lignin and alkali lignin have been reported to generate more phenols owing to the abundant ether bonds and side chains because of their lower bond strength. While organosolv lignin produced more CO - O) and CO₂ in the non-condensate gas due to its high carbonyl (C-content (Wang *et al.* 2015). The organosolv lignin has chemical structure closer to native lignin. More importantly, the delignification process is more environment-friendly (Chen *et al.* 2019, Qin *et al.* 2021). However, studies on yield and other properties of lignin have been more on Kraft process. There is need to evaluate the concentration and pulping type that favours the best lignin in terms of yield and functionality from kraft and organosolv pulping processes using *Bambusa vulgaris* as biomass source. In Nigeria, bamboo is underutilized, lignin can be extracted from these bamboos for high value-added use.

MATERIALS AND METHODS

Biomass collection and preparation

Bamboo biomass wastes were collected during industrial bamboo processing at Forest Product Development Unit of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State. The bamboo wastes were dried to constant weight and there after screened with 40 mesh sieve to obtain homogenous particle size. All reagents used such as NaOH, Na₂S, H₂O₂, CH₂O₂, H₂SO₄, C₂H₅OH, were analytical grade.

Organosolv pulping of Bamboo wastes

Three organosolv pulping types were employed which were based on the type of reagents used.

Acetic-formic acid (PFA) treatment

In the acetic-formic treatment, the volume ratio of acetic acid to formic acid were varied. The ratios were 50:50, 60:40, and 70:30 respectively. The biomass was delignified at 90 °C for 90 minutes. Thereafter, the black liquor was filtered from the delignified cellulosic residues.

Ethanol/water (EW) treatment

Delignification procedures followed the procedures suggested by Zhao *et al.* (2009). In Ethanol/water process, the volume of ethanol to water was varied as 50:50, 60:40, and 70:30 respectively as was done for acetic-formic process. The reaction was catalysed by the addition of 40 ml H₂SO₄ at 90 °C for 90 minutes.

Peroxyformic acid (PFA) treatment

Bamboo biomass was delignified by reacting with a mixture of hydrogen peroxide and formic acid of varying concentrations following the procedures of Watkins *et al.* (2014). The volume of H₂O₂ to formic acid were 50:50, 60:40, and 70:30 respectively. After delignification, the spent liquor was filtered from the delignified fibre. Lignin precipitation was done as for the two previous processes.

Kraft pulping

The cooking liquor (white liquor) comprises of sodium hydroxide (NaOH) and sodium sulphite (Na₂S). The concentration of NaOH to Na₂S were varied as done for organosolv process; 50:50, 60:40, and 70:30 respectively. Delignification was carried out at a temperature of 120 °C for 2 h. After delignification, black liquor was filtered off the delignified fibre.

Lignin isolation

For the organosolv spent liquor, the black liquors were concentrated at 40 °C, the lignin in the black liquor were then precipitated by addition of water, five times the volume of the black liquor for 1 hour with constant agitation. The filter cake recovered after filtration, were then subsequently washed three times with 200 mL of the corresponding organic solvent-water (50/50, v/v) and finally washed severally with 300 mL of distilled water. Acid (60 % H₂SO₄) was used to precipitate lignin from kraft liquor. The precipitated lignin was filtered, washed with acidified water followed by de-ionised water. The filter cake was thereafter air dried and then further dried in an oven at 30 °C to constant weight.

Characterisation of the lignin

The total dry solids (TDS) content of the black liquor was determined according to the TAPPI T650 om-09 method (2009): 10 mL each of the black liquor samples was put in a ceramic cup and put into an oven with a temperature of 105 °C overnight. After constant weight was obtained, the total dissolved solid was determined gravimetrically, and the pH of the black liquor was measured with a digital pH meter. Ease of filtering (filtration property) which is the quantity of precipitated lignin that can be filtered per unit time. This was done by taking note of the time it took to filters 150 mL of mixture of the black liquor and precipitating agent. Yield was determined gravimetrically by measuring the quantity of lignin that was recovered from a certain quantity of spent liquor and thereafter estimated in g/L. The Klason lignin content was determined following TAPPI T222 om-02 method (2006).

The filtrate was analysed for residual lignin (low molecular weight lignin) using Atomic Absorption Spectrometry (AAS) at 270 nm. Briefly, the filtrate from Klason lignin was diluted to 100 mL in a volumetric flask. A solution that was 50 (for precipitated lignin) or 100 (for black liquor filtrate) times weaker was prepared for UV measurement. The concentration of acid-soluble lignin was measured based on the absorbance value determined by UV at a wavelength of 270 nm in a Specord 205, Analytik Jena.

Analysis of the functional groups

Fourier transform infrared spectroscopy (FTIR) was used to identify and determine the lignin content, the functional group and bond linkage contained in the lignin samples. The spectra were recorded on a Nicolet iS5 FT-IR Spectrometer. Isolated lignin sample was vacuum dried at room temperature for 24 h. The dried lignin sample (2 mg) was powdered and mixed with 300 mg of anhydrous KBr. The mixture was pressed under vacuum to obtain the pellets, and the spectrum between 4000 cm^{-1} and 500 cm^{-1} was measured. The background spectrum of air was subtracted from the spectra of the samples. The Organosolv and Kraft lignin samples were investigated using this technique.

Statistical analysis

The experiment was two factors experiment in a Completely Randomised Design (CRD) with five replications. Factor A: Pulping type (4 levels: Ethanol, Peroxyformic, Acetylformic and Kraft); Factor B: Liquor concentration (3 levels: 50:50, 60:40 and 70:30). Data were analysed by one-way analysis of variance (ANOVA). Significant difference among means were determined. Means were separated using Duncan Multiple Range test.

RESULTS AND DISCUSSION

Influence of liquor concentration and cooking type on the properties of isolated lignin

The properties of the black liquor and isolated lignin from organosolv and kraft lignin are presented on Table 1 and Table 2 as well as Figure 1, Figure 2 and Figure 3. The properties of the isolated lignin were influenced by the black liquor pH as well as the pulping methods. The ANOVA results (Table 2) showed significant statistical differences in the pH, TDS, FC, yield, Klason content, LMW and HMW lignin content. Liquor concentration and cooking type had significant influence on pH, TDS, FC, yield, Klason content and LMW lignin. However, HMW lignin concentration were not influenced by liquor concentration (Table 2).

Table 1: Delignification variables for the Organosolv and Kraft process.

Pulping type	Liquor Conc. (%)	Process Designation	Biomass (g)	Temperature ($^{\circ}\text{C}$)	Pulping Time (min)
$\text{CH}_3\text{COOH}:\text{CH}_2\text{O}_2$	70:30	Aceticformic 70:30 = AF1	125	80	60
$\text{CH}_3\text{COOH}:\text{CH}_2\text{O}_2$	60:40	Aceticformic 60:40 = AF2	125	80	60
$\text{CH}_3\text{COOH}:\text{CH}_2\text{O}_2$	50:50	Aceticformic 50:50 = AF3	125	80	60
$\text{C}_2\text{H}_5\text{OH}:\text{H}_2\text{O}$	70:30	Ethanol/water 70:30 = EW1	125	80	60
$\text{C}_2\text{H}_5\text{OH}:\text{H}_2\text{O}$	60:40	Ethanol/water 60:40 = EW2	125	80	60
$\text{C}_2\text{H}_5\text{OH}:\text{H}_2\text{O}$	50:50	Ethanol/water 50:50 = EW3	125	80	60
$\text{CH}_2\text{O}_2 : \text{H}_2\text{O}_2$	70:30	Peroxyformic 70:30 = PF1	125	80	60
$\text{CH}_2\text{O}_2 : \text{H}_2\text{O}_2$	60:40	Peroxyformic 60:40 = PF2	125	80	60
$\text{CH}_2\text{O}_2 : \text{H}_2\text{O}_2$	50:50	Peroxyformic 50:50 = PF3	125	80	60
$\text{NaOH} : \text{Na}_2\text{S}$	70:30	Kraft 70:30 = KL1	125	120	90
$\text{NaOH} : \text{Na}_2\text{S}$	60:40	Kraft 60:40 = KL2	125	120	90
$\text{NaOH} : \text{Na}_2\text{S}$	50:50	Kraft 50:50 = KL3	125	120	90

Table 2: Analysis of variance for the influence of liquor concentration and pulping type on the pH, total dissolved solid, filtration capacity, yield, Klason lignin, HMW and LMW Lignin.

Source of variation	Black liquor		FC (mL/s)	Yield (g/L)	Klason (%)	Lignin Concentration (nm)	
	pH	TDS				HMW	LMW
Liquor Conc.	7,877**	63,683**	7,189**	167,693**	229,099**	0,955ns	117,499**
Pulping Type	3,818E3**	702,896**	51,295**	1,712E4**	1,128E3**	45,233**	125,413**
Liquor Conc.* Pulping type	3,375**	63,101**	2,079**	133,579**	362,295**	15,081**	187,922**

** = Significant at (p≤ 0,05) probability level; ns = Not significant

The mean value for the properties of lignin from Organosolv and Kraft process are presented in Table 3 and Table 4. Highest pH (12,8) was recorded for kraft at 70:30 liquor concentration which was closely followed by 60:40 with pH value 12,4. Lowest pH of 2,5 was recorded for both AF (70:30) and EW (70:30 and 60:40). This indicates that kraft process is alkaline while all the Organosolv processes were acidic in nature. Also, the result further shows that Kraft (50:50) had the highest TDS of 394 % followed by kraft (60:40) with value of 288 % while AF (50:50 and 70:30) had the lowest 23 % (Figure 2 and Figure 3).

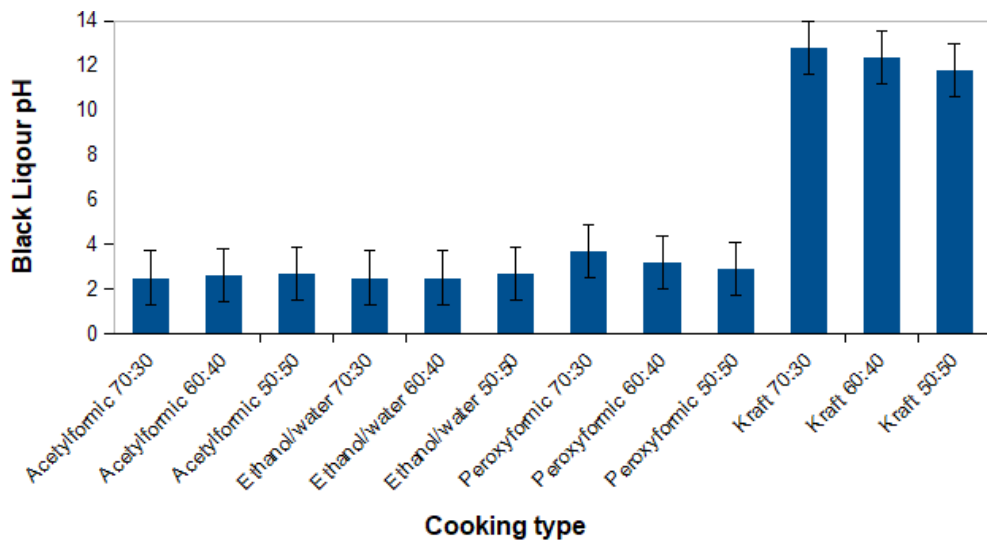


Figure 1: Influence of pulping method on the Black liquor pH.

The pulping processes and liquor concentration with the best filtration property was Aceticformic (70:30) which was filtered within 39 seconds. This was closely followed by Kraft (70:30) with filtration time of 60 seconds. All the Ethanol/water (EW) lignin had the poorest filtration (Figure 2 and Figure 3). Highest lignin yield (119,25 g/L) was gotten from kraft (50:50) closely followed by kraft (70:30) while Peroxyformic (PF) (60:40) had the lowest yield of 8,20 g/L. The result showed that higher liquor pH favours TDS as well as lignin precipitation. This is in line with the report of Hidayati *et al.* (2018) and Xu *et al.* (2013) where they reported that yield is influenced by different process condition, i.e, the pH, temperature and ion strength of black liquor.

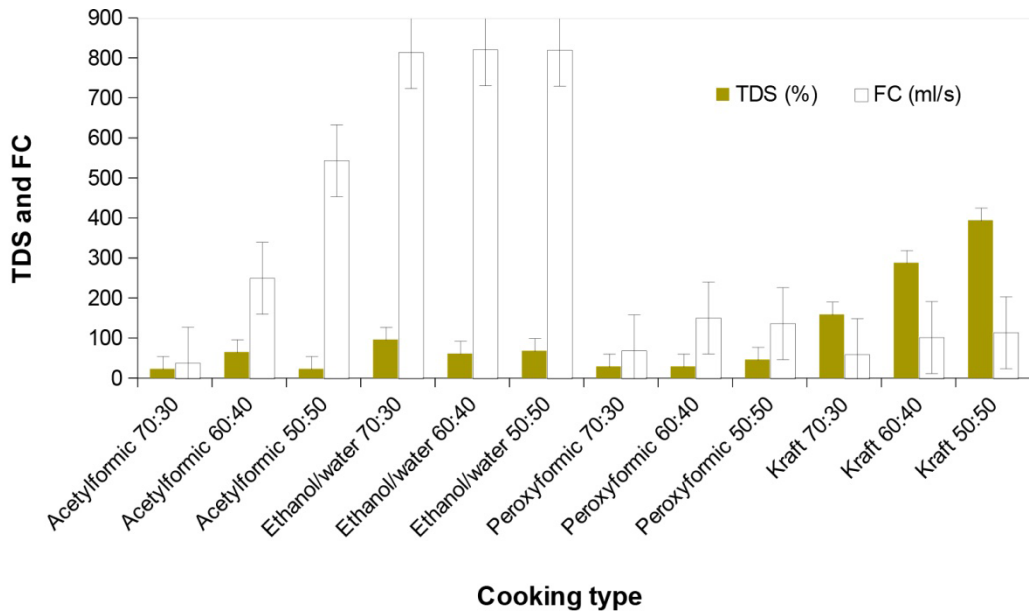


Figure 2: Influence of pulping method on the Total dissolve solid and filtration properties of the isolated lignin.

Generally, kraft process recorded the highest lignin yield while all the organosolv processes recorded reduced lignin yield. The reason for the low yield of organosolv lignin might be as a result of the volatility of the organic solvents compared to the inorganic chemicals which resulted in low delignification at low temperature. This result was in agreement with the study of Vazquez-Torres *et al.* (1992). Also, the higher the black liquor pH, the higher the lignin yield. This indicates that the lignin in the bamboo biomass was more solubilized at low pH than high pH. However, low pH favours lignin precipitation from Bamboo Kraft black liquor. This is because the lignin was more soluble at high pH than low pH, and therefore more low-molecular fragments of lignin were dissolved at higher pH such as 11,8 g/L, 12,4 g/L and 12,8 g/L than at low pH (Gellerstedt *et al.* 1994).

Klason lignin is the solid residual material that is obtained after a sample of BL has been subjected to hydrolysis treatment with 72 % H_2SO_4 . Klason lignin content of kraft (50:50) was highest with value of 49 % followed by kraft (70:30) while lowest (11,20 %) was recorded for AF at 50:50 liquor concentration. The content of acid-soluble lignin, the purity and the components of isolated lignin, was determined using UV spectroscopy. The absorbance of lignin was recorded at wavelength of 270 nm. The concentration of the High Molecular Weight (HMW) and Low Molecular Weight (LMW) lignin showed EW (60:40) and kraft (70:30) to be highest with values of 0,164 nm and 0,229 nm respectively while the lowest of 0,003 nm and 0,024 nm were recorded for HMW and LMW lignin respectively (Figure 4).

Higher liquor pH favours LMW lignin. Although, HMW lignin was not influenced by liquor pH but seemed that concentration of HMW lignin were higher for Organosolv than Kraft process. Although there were statistical variations in the properties of the black liquor and the precipitated lignin based on the liquor concentration and pulping type. However, the pH of Aceticformic and Ethanol as well as TDS of Aceticformic and Peroxyformic were statistically similar (Table 3). The Klason lignin content of Aceticformic and Ethanol/water were similar as well as LMW lignin concentration of Ethanol/water and Peroxyformic lignin (Table 4).

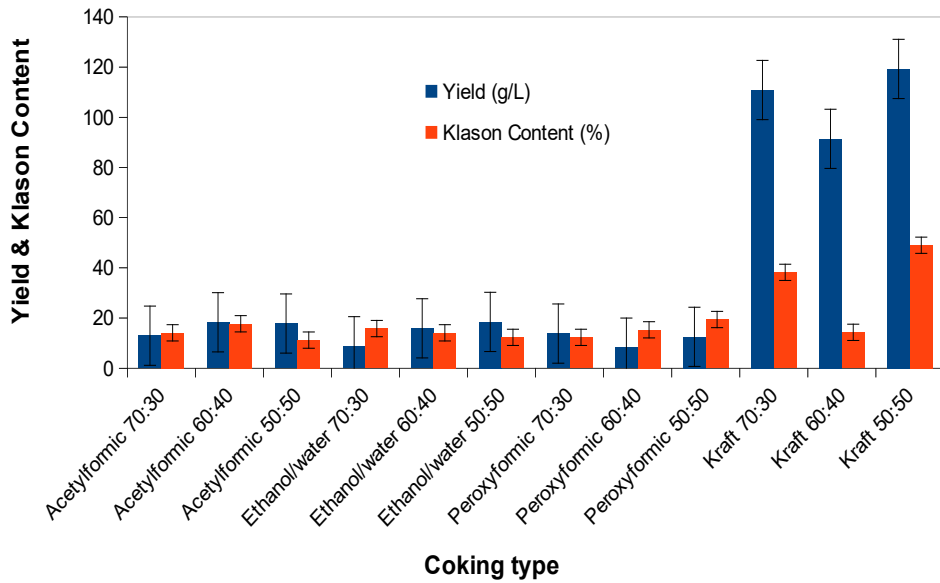


Figure 3: Influence of pulping method on the yield and Klason content of the isolated lignin.

Table 3: influence of cooking type and liquor concentration on the pH, total dissolved solid, filtration property and yield of technical lignin.

Source of variation	Levels	pH	TDS (%)	FC (ml/s)	Yield (g/L)
Cooking type	Aceticformic	2,60 ^a	37,22 ^a	278,22 ^b	16,35 ^c
	Ethanol/water	2,66 ^a	70,00 ^b	745,20 ^c	14,26 ^b
	Peroxyformic	3,24 ^b	34,50 ^a	126,38 ^a	11,23 ^a
	Kraft	12,33 ^c	271,22 ^c	92,78 ^a	106,64 ^d
Liquor Concentration	70:30	5,04 ^a	128,75 ^a	405,17 ^a	41,46 ^a
	60:40	5,35 ^b	74,33 ^b	246,67 ^b	33,60 ^b
	50:50	5,18 ^{ab}	109,58 ^c	331,58 ^c	36,55 ^c

*Values in the same column with the same superscript are not significantly different.

Table 4: Influence of cooking type and liquor concentration on the klason, high molecular weight lignin and low molecular weight lignin of technical lignin.

Source of variation	Levels	Klason content (%)	HMW (cm ⁻¹)	LMW (cm ⁻¹)
Cooking type	Aceticformic	14,47 ^a	0,08 ^a	0,14 ^c
	Ethanol/water	13,87 ^a	0,11 ^b	0,07 ^a
	Peroxyformic	16,09 ^b	0,18 ^c	0,07 ^a
	Kraft	33,66 ^c	0,31 ^a	0,08 ^b
Liquor Concentration	70:30	22,88 ^a	1002,00 ^a	0,075 ^a
	60:40	20,04 ^b	1128,00 ^b	0,081 ^b
	50:50	15,46 ^c	1156,00 ^c	0,123 ^c

*Values in the same column with the same superscript are not significantly different.

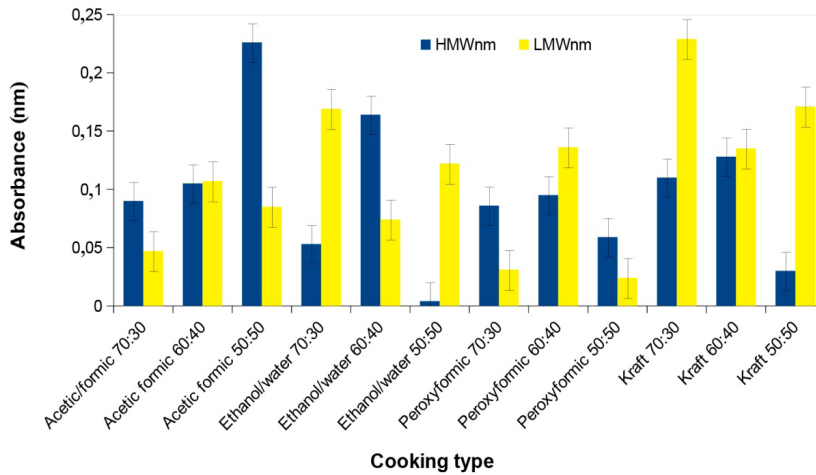


Figure 4: Influence of pulping method on the Concentration of HMW and LMW lignin.

According to the intrinsic structure of lignin, several absorptions attributed to different purity level were observed, as shown in Table 5. Based on the Lambert-Beer's Law, UV spectroscopy can be used for the semi-quantitative determination of the purity of lignin and its degradation product (Prozil *et al.* 2012). Because of the cross-linking structures of lignin with carbohydrates, cellulose, and hemicelluloses, the isolation of lignin is extremely difficult. The absorption values for the HMW lignins are arranged in decreasing order, indicating that the lignin purity increases with decreasing absorption value. Acetylformic (50:50) had the highest purity with absorbance of 0,226 nm followed by Ethanol/Water (60:40) while Ethanol/water (50:50) had the least purity with absorbance value of 0,004 nm (Table 5).

Table 5: Lignin purity based on the UV absorbance of HMW lignin.

Lignin type	UV absorbance (nm)	
	Yield (g/L)	Lignin purity
Acetic formic 50:50	17,80	0,226
Ethanol/water 60:40	15,90	0,164
Kraft 60:40	91,40	0,128
Kraft 70:30	110,85	0,110
Acetic formic 60:40	18,30	0,105
Peroxyformic 60:40	8,20	0,095
Acetic/formic 70:30	12,95	0,090
Peroxyformic 70:30	13,80	0,086
Peroxyformic 50:50	12,50	0,059
Ethanol/water 70:30	8,75	0,053
Ethanol/water 50:50	18,45	0,004
Kraft 50:50	119,25	0,003

Characteristic colour and forms of the isolated lignin

Due to different pH value, the colour of the isolated lignin varies significantly from cream to light brown and dark brown (Figure 5 and Figure 6). The colour changes from cream to dark brown with decreasing pH. In addition, there were obvious differences in the shape and size of lignin. Kraft lignin colour also varied from brown to dark brow with decreasing pH. In general, the precipitation yield of organosolv and Kraft lignin depended on several factors, for instance, the pH value of liquor and the cooking method. The comparison of organosolv and Kraft lignins according to different pH values are shown in Figure 5. As can be seen, for a certain type of process e.g. Peroxyformic, due to different pH value, the colour of the lignin varied from light brown to dark brown with pH value decreasing. In addition, there were obvious differences in the shape and size of

the lignins. Kraft lignin colour too varied from brown to dark brow with decreasing pH. Kraft lignin sample of bamboo formed large pieces much easier than that of Organosolv, and the process also filtered relatively faster than that of Organosolv during the precipitation process. This was related to the work of (Li *et al.* 2012) when he compared different Kraft lignins based on hardwood and softwood.

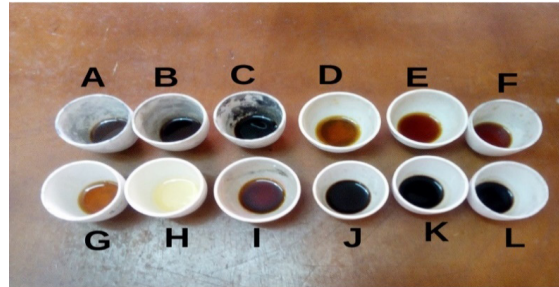


Figure 5: The comparison of organosolv and Kraft lignins according to different pH values
 A = AF1 = Acetylformic 70:30; B = AF2 = Acetylformic 60:40; C = AF 3 = Acetylformic 50:50;
 D = EW1 = Ethanol/water 70:30; E = EW2 = Ethanol/water 60:40; F = EW3 = Ethanol/water 50:50
 G = PFI = Peroxyformic 70:30; H = PF2 = Peroxyformic 60:40; I = PF3 = Peroxyformic 50:50
 J = KL1 = Kraft 70:30; K = KL2 = Kraft 60:40; L = KL3 = Kraft 50:50

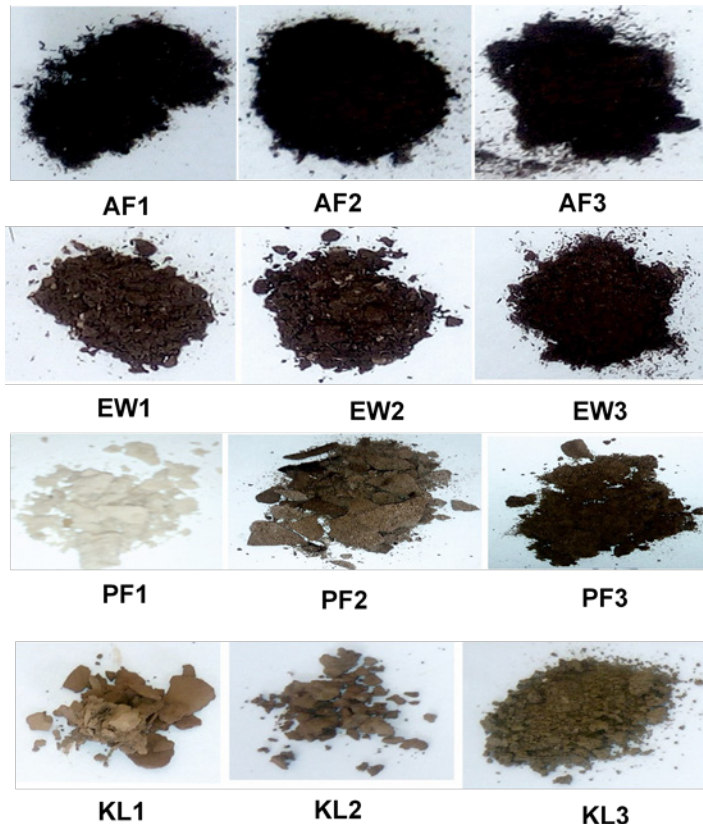


Figure 6: Samples of the Isolated lignins.

Characteristic colour and forms of the isolated lignin

Correlation analysis

The correlation between the liquor concentration and lignin parameters were presented in Table 6. There was strong positive correlation between pH and TDS, yield, Klason and low molecular weight lignin content. Only FC had strong negative correlation with pH. TDS had strong positive correlation with yield, Klason and

Low molecular weight lignin. Yield had significant positive correlation with Klason and Low molecular weight lignin content. Likewise, Klason lignin content showed significant positive and negative correlations with Low molecular and High molecular weight lignin content respectively (Table 6). This indicates that as the pH increases, the TDS, yield, Klason and low molecular weight lignin content increases while FC decreases and vice versa. Also, Klason and Low molecular weight lignin content increased with increasing lignin yield. In addition, the Klason lignin content had a considerable influence on the absorbance values of the HMW lignin. This agreed with the work of (Kallavus *et al.* 2015), who reported that the type and structure of lignin, solvent, and pH of the solution have a considerable influence on the UV spectra values.

Table 6: Correlation matrix of the liquor concentration and lignin properties.

Properties	Liquor conc.	pH	TDS (%)	FC (mL/s)	Yield (g/L)	Klason (%)	HMW (cm ⁻¹)	LMW (cm ⁻¹)
Liquor Conc.	1							
pH	-0,030	1						
TDS (%)	0,204	0,854**	1					
FC (mL/s)	0,208	-0,477**	-0,250	1				
Yield (g/L)	0,049	0,980**	0,883**	-0,413*	1			
Klason (%)	0,105	0,728**	0,726**	-0,375*	0,815**	1		
HMW (cm ⁻¹)	-0,046	-0,079	-0,275	0,074	-0,109	-0,335*	1	
LMW (cm ⁻¹)	-0,107	0,671**	0,577**	0,016	0,692**	0,618**	-0,133	1

** = Highly significant correlation; * = Significant correlation.

Fourier transform infrared spectroscopy (FTIR) analysis of the lignin

The FTIR spectra of the lignin isolated from the *B. vulgaris* contained most of the characteristic absorption bands for the different chemical structures. The lignin samples were analyzed based on the chemical structures and the peaks were identified by comparing their wavenumbers with literature data. The FTIR spectra obtained for both processes are shown in Figure 7. The characteristic bands between 3500 cm⁻¹ - 3000 cm⁻¹ indicate the existence of alcohol and carboxylic group in the samples. It was observed that Ethanol/Water, Peroxyformic, and Kraft lignin resonates at this wavelength. Which means Acetylformic did not provide information about the secondary structure of this functional group. The appearance of the band in the range of 1120 cm⁻¹- 1270 cm⁻¹ and 1330 cm⁻¹ - 1375 cm⁻¹ shows the existence of P-hydroxyl (H), Guaiacyl (G) and Syringyl (S) Unit in the bamboo lignin which are the basic precursor for lignins (Table 7).

Table 7: Band assignment for the isolated Lignin FTIR spectra.

S/N	Class of Compounds	Wave no cm ⁻¹	Intensity	Functional groups	AF	EW	PF	KL
1.	Alcohol & Carboxylic Acids	3500-3000	s	O-H stretch		✓	✓	✓
2.	Alkanes and Alkyls	2985-2900	s	C=H stretch	✓	✓	✓	✓
3.	Carbonyls	1700-2500	m-s	C=O stretch			✓	
4.	Alkenes	1600-1680	m-s	C-H bend	✓	✓	✓	✓
5.	Amides R-C(O)-NH-R	1500-1550	s	N-H bend	✓	✓		✓
6.	Nitro Compounds	1300-1400	s	N-O symmetric				✓
7.	P-hydroxyl (H), guaiacyl (G) & Syringyl (S) Unit	1200-1295	s	C-O-C stretch	✓	✓	✓	✓
8.	Alkyl halides	1019-400	m-s	C-Br stretch		✓	✓	✓

Apparently, this characteristics band were found to be present in all the lignin type. C-H stretching in Alkanes and Alkyls group is reflected in the band of 2901 cm⁻¹. All the lignin types exhibited this functional

groups. Band at 1600 cm^{-1} - 2500 cm^{-1} were attributed to Alkenes and Carbonyls group which are the characteristic bands found in the infrared spectra of Peroxyformic lignin (Table 7). The absorption band associated with all the lignin type at 1600 cm^{-1} - 1680 cm^{-1} are attributed to stretching vibrations of the C=H bond of the Alkenes. Absorption associated with the Amides R-C(O)-NH-R band at wave number between 1500 cm^{-1} - 1550 cm^{-1} leads primarily to bending vibrations of the N-H bond. Furthermore, C-Br stretching in Alkyl halides groups is reflected in the band of 1019 cm^{-1} - 400 cm^{-1} for Ethanol/Water, Peroxyformic and Kraft lignin.

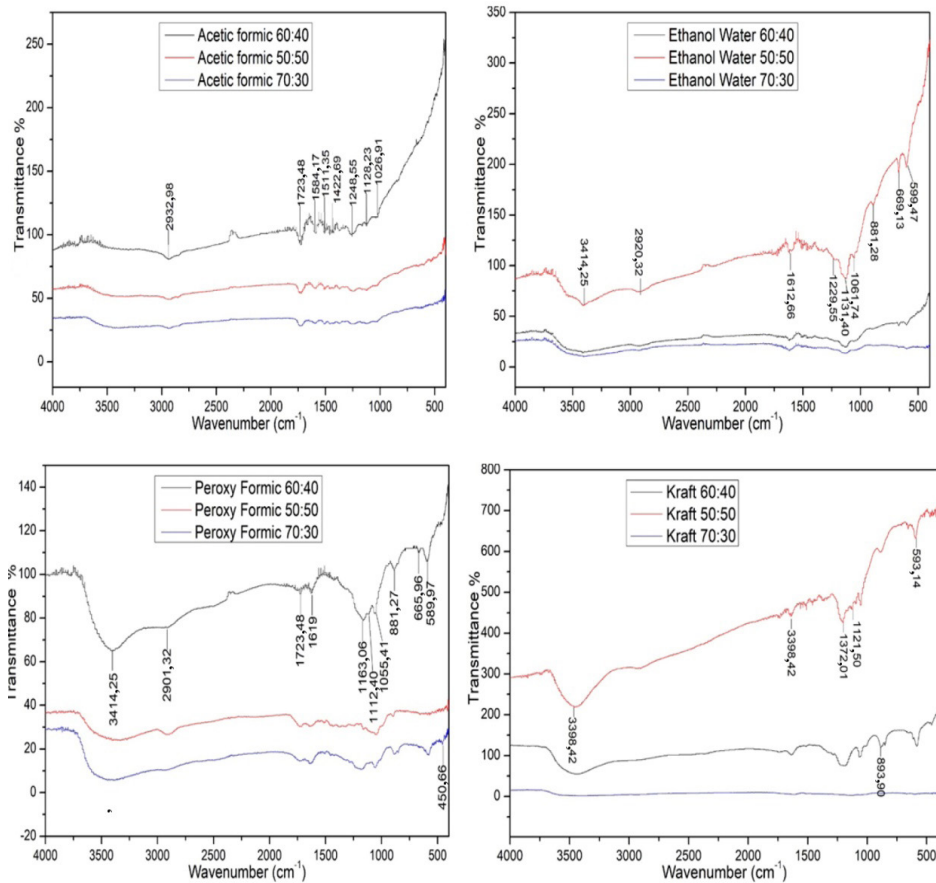


Figure 7: FTIR Spectra of the isolated lignins.

CONCLUSIONS

The highest lignin precipitated (119,25 g/L) was recorded for Kraft at 50:50 liquor concentration while the lowest (8,20 g/L) was recorded for Peroxyformic at 60:40 liquor concentration. Acetylformic (70:30) was the easiest to filter which was filtered within the shortest period of 39 seconds compared to Ethanol/water (60:40) that took 822 seconds before the filter cakes could be filtered off the residual filtrate. Generally, the precipitation yields of lignin decreased with increasing pH value. Ethanol/Water at 60:40 liquor concentration was found to have the highest purity judging from the UV spectrometric analysis. The cooking type and liquor concentrations have considerable influence on the concentration of the lignin. Functionality of organosolv and Kraft lignin showed that alcohol and carboxylic acids, alkanes and alkyls, carbonyls, alkenes, amide, and alkyl halides are functional groups present in the isolated lignin.

REFERENCES

- Chen, H.; Xu, G.H.; Xiao, C.Y.; Bi, Y.D.; Hu, J.L. 2019.** Fast pyrolysis of organosolv lignin: effect of adding stabilization reagents to the extraction process. *Energy Fuels* 33: 8676-8682. <https://doi.org/10.1021/acs.energyfuels.9b01486>
- Do, N.H.; Pham, H.H.; Le, T.M.; Lauwaert, J.; Diels, L.; Verberckmoes, A.; Do, N.H.N.; Tran, V.T.; Le, P.K. 2020.** The novel method to reduce the silica content in lignin recovered from black liquor originating from rice straw. *Scientific Reports* 10(21263): 1-10. <https://doi.org/10.1038/s41598-020-77867-5>
- Domínguez, J.C.; Santos, T.M.; Rigual, V.; Oliet, M.; Alonso, M.V.; Rodríguez, F. 2018.** Thermal stability, degradation kinetics, and molecular weight of organosolv lignins from *Pinus radiata*. *Industrial Crops and Products* 111: 889-898. <https://doi.org/10.1016/j.indcrop.2017.10.059>
- Erdocia, X.; Prado, R.; Corcuera, M.A.; Labidi, J. 2014.** Effect of different organosolv treatments on the structure and properties of olive tree pruning lignin. *Journal of Industrial and Engineering Chemistry* 20(3):1103-1108. <https://doi.org/10.1016/j.jiec.2013.06.048>
- Faris, A.H.; Rahim, A.A.; Ibrahim, M.N.M.; Hussin, M.H.; Alkurdi, A.M.; Salehabadi, A. 2017.** Investigation of oil palm based Kraft and auto-catalyzed organosolv lignin susceptibility as a green wood adhesives. *International Journal of Adhesion and Adhesives* 74: 115-122. <https://doi.org/10.1016/j.ijadh.2017.01.006>
- FAO. 1996.** Environmental impact assessment and environmental auditing in the pulp and paper industry. FAO forestry paper no. 129. Rome, Italy.
- Galbe, M.; Zacchi, G. 2002.** A review of the production of ethanol from softwood. *Applied Microbiology and Biotechnology* 59(1): 618-628. <https://doi.org/10.1007/s00253-002-1058-9>
- Gavrilescu, M.; Teodosiu, C.; Gavrilescu, D.; Lupu, L. 2008.** Strategies and practices for sustainable use of water in industrial papermaking processes. *Engineering in Life Sciences* 8(1): 99-124. <https://doi.org/10.1002/elsc.200720236>
- Gellerstedt, G.; Pranda, J.; Lindfors, E. 1994.** Structure and Molecular Properties of Residual Birch Kraft Lignins. *Journal of Wood Chemistry and Technology* 14(4): 467-482. <https://doi.org/10.1080/02773819408003108>
- Gellerstedt, G.; Henriksson, G. 2008.** Lignins: Major Sources, Structure and Properties. Chapter 9: 201-224. In *Monomers, Polymers and Composites from Renewable Resources*. Belgacem, N.; Gandini, A. (Eds.). Elsevier Science. <https://doi.org/10.1016/B978-0-08-045316-3.00009-0>
- Hidayati, S.; Zuidar, A.S.; Satyajaya, W.; Murhadi.; Retnowati, D. 2018.** Isolation and characterization of formacell Lignins from oil empty fruits bunches. IOP Conference Series: Materials Science and Engineering, Volume 344, The 3rd International Conference on Science, Technology, and Interdisciplinary Research (IC-STAR): University of Lampung, Indonesia. 18–20 September 2017, pp. 1-15. <https://iopscience.iop.org/article/10.1088/1757-899X/344/1/012006>
- Hubbe, M.A.; Alén, R.; Paleologou, M.; Kannangara, M.; Kihlman, J. 2019.** Lignin recovery from spent alkaline pulping liquors using acidification, membrane separation, and related processing steps: a review. *BioResources* 14(1): 2300-2351. <https://bioresources.cnr.ncsu.edu/resources/lignin-recovery-from-spent-alkaline-pulping-liquors-using-acidification-membrane-separation-and-related-processing-steps-a-review/>
- Inkrod, C.; Raita, M.; Champreda, V.; Laosiripojana, N. 2018.** Characteristics of Lignin Extracted from Different Lignocellulosic Materials via Organosolv Fractionation. *Bio Energy Research* 11: 277-290. <https://doi.org/10.1007/s12155-018-9895-2>
- Jardim, J.M.; Hart, P.W.; Lucia, L.A.; Jameel, H.; Chang, H.M. 2022.** The Effect of the Kraft Pulping Process, Wood Species, and pH on Lignin Recovery from Black Liquor. *Fibers* 10(2): 16. <https://doi.org/10.3390/fib10020016>

Jiang, B.; Chen, H.; Zhao, H.F.; Wu, W.J.; Jin, Y.C. 2020. Structural features and antioxidant behavior of lignins successively extracted from ginkgo shells (*Ginkgo biloba* L). *International Journal of Biological Macromolecules* 163: 694-701. <https://doi.org/10.1016/j.ijbiomac.2020.07.027>

Kallavus, U.; Kärner, K.; Kärner, K.; Elomaa, M. 2015. Rapid semi-quantitative determination of aspen lignin in lignocellulosic products. *Proceedings of the Estonian Academy of Sciences* 64. <https://doi.org/10.3176/PROC.2015.1S.06>

Kamthai, S.; Puthson, P. 2005. Effect of beating revolution on sweet bamboo (*Dendrocalamus asper* Backer) kraft pulp properties. *CMU Journal of Science* 4(2): 137-147. <https://www.thaiscience.info/Journals/Article/CMUJ/10325022.pdf>

Kihlman, J. 2016. The sequential liquid-lignin recovery and purification process: Analysis of integration aspects for a kraft pulp mill. *Nordic Pulp & Paper Research Journal* 31: 573-582. <https://doi.org/10.3183/npprj-2016-31-04-p573-582>

Kim, T.H.; Kwak, H.; Kim, T.H.; Oh, K.K. 2021. Reaction Characteristics of Organosolv-Fractionation Process for Selective Extraction of Carbohydrates and Lignin from Rice Husks. *Energies* 14: 686. <https://doi.org/10.3390/en14030686>

Krief, P.; Courtrot, D.; Conso, F. 2008. Occupational toxicological risk related to the exposure to MDF wood dust. *Archive des Maladies Professionnelles et de l' Environnement* 69(5-6): 665-666. <http://dx.doi.org/10.1016/j.admp.2008.09.007>

Li, M.F.; Sun, S.N.; Xu, F.; Sun, R.C. 2012. Formic acid based organosolv pulping of bamboo (*Phyllostachys acuta*): comparative characterization of the dissolved lignins with milled wood lignin. *Chemical Engineering Journal* 179(1): 80-89. <https://doi.org/10.1016/j.cej.2011.10.060>

Ma'ruf, P.B.; Pramudono, B.; Aryanti, N. 2017. Optimization of Lignin Extraction from Rice Husk by Alkaline Hydrogen Peroxide Using Response Surface Methodology. *Rasayan Journal of Chemistry* 10(2): 407-414. http://www.rasayanjournal.co.in/admin/php/upload/159_pdf.pdf

Minu, K.; Jiby, K.K.; Kishore, V.V.N. 2012. Isolation and purification of lignin and silica from the black liquor generated during the production of bioethanol from rice straw. *Biomass and Bioenergy* 39: 210-217. <https://doi.org/10.1016/j.biombioe.2012.01.007>

Öhman, F.; Wallmo, H.; Theliander, H. 2007. Precipitation and filtration of lignin from black liquor of different origin. *Nordic Pulp & Paper Research Journal* 22: 188-193. <https://doi.org/10.3183/npprj-2007-22-02-p188-193>

Patwardhan, P.R.; Brown, R.C.; Shanks, B.H. 2011. Understanding the fast pyrolysis of lignin. *ChemSusChem* 4(11): 1629-1636. <https://doi.org/10.1002/cssc.201100133>

Prozil, S.O.; Costa, E.V.; Evtuguin, D.V.; Lopes, L.P.C.; Domingues, M.R.M. 2012. Structural characterization of polysaccharides isolated from grape stalks of *Vitis vinifera* L. *Carbohydrate Research* 356 (1): 252-259. <https://doi.org/10.1016/j.carres.2012.02.001>

Qin, Z.; Yang, Q.L.; Cheng, X.C.; Liu, H.M.; Wang, X.D. 2021. Structural features, chemical composition, antioxidant activities of organosolv lignins extracted from black and white sesame capsules and stalks. *Industrial Crops and Products* 169: 113677. <https://doi.org/10.1016/j.indcrop.2021.113677>

Raita, M.; Denchokepraguy, N.; Champreda, V.; Laosiripojana, N. 2017. Effects of alkaline catalysts on acetone-based organosolv pretreatment of rice straw. *3 Biotech* 7(5): e340. <https://doi.org/10.1007/s13205-017-0969-1>

Ruiz, H.A.; Ruzene, D.S.; Silva, D.P.; Macieira da Silva, F.F.; Vicente, A.A.; Teixeira, J.A. 2011. Development and Characterization of an Environmentally Friendly Process Sequence (Autohydrolysis and Organosolv) for Wheat Straw Delignification. *Applied Biochemistry and Biotechnology* 164(1): 629-641. <https://doi.org/10.1007/s12010-011-9163-9>

Sannigrahi, P.; Kim, D.H.; Jung, S.; Ragauskas, A. 2011. Pseudo-lignin and pretreatment chemistry. *Energy & Environmental Science* 4(1): 1306-1311. <https://doi.org/10.1039/C0EE00378F>

Singh, S.K.; Dhepe, P.L. 2016. Isolation of lignin by organosolv process from different varieties of rice husk: understanding their physical and chemical properties. *Bioresource Technology* 221:310-317. <https://doi.org/10.1016/j.biortech.2016.09.042>

Soongprasit, K.; Sricharoenchaikul, V.; Atong, D. 2020. Phenol-derived products from fast pyrolysis of organosolv lignin. *Energy Reports* 6: 151-167. <https://doi.org/10.1016/j.egy.2020.08.040>

Suhr, M.; Klein G.; Kourtí, I.; Gonzalo, M.R.; Santonja, G.G.; Roudier, S.; Sancho, L.D. 2015. Best Available Techniques (BAT) Reference Document for the Production of Pulp, Paper and Board. Industrial Emissions Directive. 2010/75/EU (Integrated Pollution Prevention and Control). Institute for Prospective Technological studies, European Commission: Luxembourg. https://eippcb.jrc.ec.europa.eu/sites/default/files/2019-11/PP_revised_BREF_2015.pdf

TAPPI. 2006. Standard test methods for determining Acid Insoluble Lignin in Wood and Pulp. Reaffirmation of Tappi T222 om-02. TAPPI Press, Atlanta, GA.

TAPPI. 2009. Standard test methods for determining Solids content of black liquor 2009. Tappi T 650 om-09. TAPPI Press, Atlanta, GA.

Tomani, P. 2010. The lignoboost process. *Cellulose Chemistry and Technology* 44: 53-58. [https://cellulosechemtechnol.ro/pdf/CCT44,1-3%20\(2010\)/P.53-58.pdf](https://cellulosechemtechnol.ro/pdf/CCT44,1-3%20(2010)/P.53-58.pdf)

UNEP. 1996. Environmental management in the pulp and paper industry. United Nation Environment Programme, Industries and Environment: Stockholm, Sweden. Technical Report No. 4.

Vazquez-Torres, H.; Canchg-Escamilla, G.; Cruz-Ramos, C.A. 1992. Coconut husk lignin. 1. Extraction and characterization. *Journal of Applied Polymer Science* 45(1): 633-644. <https://doi.org/10.1002/app.1992.070450410>

Velez, J.; Thies, M.C. 2013. Solvated liquid-lignin fractions from a Kraft black liquor. *Bioresource Technology* 148: 586-590. <https://doi.org/10.1016/j.biortech.2013.08.097>

Wang, S.R.; Ru, B.; Lin, H.Z.; Sun, W.X.; Luo, Z.Y. 2015. Pyrolysis behaviors of four lignin polymers isolated from the same pine wood. *Bioresource Technology* 182: 120-127. <https://doi.org/10.1016/j.biortech.2015.01.127>

Wang, T.; Li, H.; Yuan, J.; Li, W.; Li, K.; Huang, Y.; Xiao, L.; Lu, Q. 2021. Structures and pyrolytic characteristics of organosolv lignins from typical softwood, hardwood and herbaceous biomass. *Industrial Crops and Products* 171:e13912. <https://doi.org/10.1016/j.indcrop.2021.113912>

Watkins, D.; Nuruddin, M.D.; Hosur, N.M.; Tcherbi-Narteh, A.; Jeelani, S. 2014. Extraction and characterization of lignin from different biomass resources. *Journal of Materials Research and Technology* 4(1): 26-32. <https://doi.org/10.1016/j.jmrt.2014.10.009>

Xu, F.; Yu, J.; Tesso, T.; Dowell, F.; Wang, D. 2013. Qualitative and quantitative analysis of lignocellulosic biomass using infrared techniques: a mini-review. *Applied Energy* 104(1): 801-809. <https://doi.org/10.1016/j.apenergy.2012.12.019>

Zhao, X.; Cheng, K.; Liu, D. 2009. Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis. *Applied Microbiology and Biotechnology* 82(1): 815-827. <https://doi.org/10.1007/s00253-009-1883-1>