Chemical and Process Engineering Research
ISSN 2224-7467 (Paper) ISSN 2225-0913 (Online)
Vol.57, 2018

Klason Method: An Effective Method for Isolation of Lignin Fractions from Date Palm Biomass Waste

Emmanuel Galiwango[1] Nour S. Abdel Rahman[1] Ali H. Al-Marzouqi[1*] Mahdi M. Abu-Omar[2]
Abbas A. Khaleel[3]
1.Department of Chemical and Petroleum Engineering, United Arab Emirates University, P.O.BOX 15551, Al Ain, UAE
2.Department of Chemistry and Biochemistry and Biochemistry, UC Santa Barbara
Santa Barbara, CA 93106-9510, USA
3.Department of Chemistry, United Arab Emirates University, P.O.BOX 15551, Al Ain, UAE

This work was funded by Emirates Center for Energy and Environment, UAE University.
[Grant No.: 31R107].

Abstract

Klason lignin extraction method is one of the robust techniques for isolation of lignin from lignocellulosic palm biomass waste for future production of High Value Chemicals (HVCs). To elucidate the mechanism of hemicellulose and cellulose glycosidic bond distraction, lignocellulose biomass of date palm; rachis, leaflet, fibers and mixture of the three parts was pretreated and characterized to assess the viability of the method towards lignin isolation.The differential thermogravimetric (DTG) results of Klason lignin extracts, showed only lignin decomposition regime in contrast to decomposition regimes of hemicellulose, cellulose and lignin exhibited in the original samples. SEM analysis revealed major morphological differences between original and Klason extracts. The unique helical chiral nematic ordering attributed to cellulose were not sighted after Klason treatment. The FTIR results after Klason treatment, further revealed the disappearance of β-1, 4- glycosidic bond cleavage of cellulose and dissolution of hemicellulose, thus leaving of lignin fractions.Klason method is a simple, cheap and fast method capable of recovering lignin from palm biomass waste. This study focused on whole non-edible date palm parts as opposed to many research works on one-part analysis, a clear indication that Klason treatment technique is not limited to specific lignocellulosic biomass. Lignin fractions from this Klason isolation will be used as a feed stock using biochemical conversion techniques to produce; Dihydroeugenol, DHE and 2,6-dimethoxy-4-propyl-phenol (DMPP), these are High Value Chemicals used in flavor and fragrance industry.

Keywords: Klason extraction, Lignocellulose Biomass, Lignin, Biomass Characterization

1. Introduction

Biomass and other renewable energy utilization are expected to increase in the future due to negative effects including; global warming, depletion of petroleum reserves and price fluctuations from fossil resource (Scarlat *et al.* 2015; Yat *et al.* 2008). To solve environmental and long-term economic un certainties, conversion of lignocellulosic biomass to biofuels and high value chemicals, presents a sustainable and viable option for future energy security and reducing green-house gases (Wyman 1999). Unlike fossil fuel that burn and add CO_2 in the atmosphere, lignocellulosic biomass is a cleaner-burning resource. For instance, ethanol from biomass has been reported to have a potential to reduce greenhouse gas emissions by almost 80% (Wang *et al.* 2007).

The huge potential in biomass has led to the current increase in research for the development of biomass utilization into high value chemical products and biofuels.

Lignocellulose biomass is a naturally occurring sustainable natural resource, comprising of; hemicellulose, cellulose and lignin. These fractions have a potential to produce high value chemicals. For example, Hemicellulose and cellulose have for a long time been used in textile and paper industry. Currently due to cellulose's durability and structural uniformity, cellulose Nano crystals act as a base for new polymer composite materials (Brinchi *et al.* 2013; Biagiotti *et al.* 2008).

Despite the need to fully utilize the three components of lignocellulose biomass, most recent research is focusing more on hemicellulose and cellulose utilization destroying lignin through developing various methods of delignification (Kumar *et al.* 2009). However, the breakthrough in the energy generation and future chemical reliance on lignocellulose biomass needs maximum biomass utilization. Lignin fractions, despite having a complex polymeric structure, they have a potential for production of high value chemicals (Klein *et al.* 2015).

Lignocellulose biomass is widely dispersed in many parts of the world including the sea, land and in adverse climates like dessert areas, making it a viable sustainable resource to contribute to future energy security and chemical industry. Despite the huge availability of date palm lignocellulose biomass in the Middle East, which is a precursor for HVC production, most of the waste from palm trees is currently turned into compost or burned to generate heat. UAE alone has over 30 million date palm trees and each palm tree, on average produces around 12-15 new leaves every year and the same amount is usually cut from each tree (Barreveld 1993), in total generating approximately 1.2 million tons of waste annually. Characterization and value addition to this waste instead of burning it, can be an opportunity to produce HVC and successively introduce the bio-refinery concept to UAE and the region known for petrol-refinery for decades.

Palm biomass is a lignocellulose component comprising of mainly; Cellulose, which is the main constituent of wood carbohydrates and is a polysaccharide of glucose units linearly inter-linking each other by β-,4-glycosidic bonds (Klemm *et al*. 2005); Hemicellulose, which is a hetero-polysaccharide with different structures in both soft and hard wood. Hemicellulose bind fibers to form inter-fiber bonding and due to low strength compared to cellulose, can easily be hydrolyzed by dilute acids or bases (Pauly *et al*. 2013); Lignin, is a polyphenolic polymer with a distinctive structure different from the other macro constituents of wood. Lignin polymer molecule is made of three dimensional networks of phenyl propane units with different phenolic groups of different substituents and its concentration in plant material is high mainly in the region of middle lamella (Alder 1977; Freudenberg & Neish 1968). Despite its complex structure, after valorization, lignin has a great potential to act as a precursor for production of bio-fuels and HVC. Production of HVC from lignin involves isolation of non-lignin fractions using classical methods such as Klason and other standard methods (Glennie & McCarthy 1962; López *et al*. 2010).

Although all kind of biomass in principle can be converted into fuels and HVCs, the use of edible crops for these applications cannot be justified in a world of increasing population whose demand for food is a priority in contrast to energy and chemicals. To prevent competition with the use of land and resources used to produce food, it is recommended to use second-generation biomass (those not in direct competition with human survival) to produce fuels and chemicals. The HVCs find their applications in food industry, fragrance and as fuel additives (Barreveld 1993). Lignocellulosic palm biomass provides an opportunity for future production of HVC in the gulf region. The huge abundance of palm trees in the region for decades, the increasing negative economic and environmental pressures facing the petroleum industry to date, is an opportunity for research to blossom in this new field of biomass utilization for green growth. Lignin remains a potential source of HVCs despite its complex molecular structure varying in different plant species (Evtuguin *et al.* 1998; Capanema *et al*. 2004; Chakar & Ragauskas 2004; Parlack *et al.* 2005). Therefore, lignin valorization is important to reduce its resistance to chemical and biodegradation. Despite the rich survival history and wide abundance in many regions worldwide, little or no information to our knowledge regarding different parts of *phoenix dicteylifera* date palm biomass lignin valorization studies using klason isolation technique and thermal decomposition studies has been reported before.

This study aims at isolation of lignin from leaflet, rachis, fibers and combination of the three parts (mixture) of date palm biomass using Klason method to recover lignin for downstream uses. Comparison of thermal analysis using thermogravimetric (TGA) for pyrolysis of date palm biomass before and after Klason lignin extraction was reported. TGA alone does not provide sufficient knowledge on biomass decomposition chemistry, thus there is a need for further techniques to provide a better understanding of the biomass prior to further processing (White *et al*. 2011). This study further presents different characterization techniques such as Fourier Transform Infrared (FT-IR) and Scanning Electron Microscopy (SEM) analysis, providing a more detailed understanding of palm biomass prior to being used for future production of target HVC.

2.0 Methods

2.1 Sample preparation and characterization

All chemicals used, unless stated otherwise, were supplied by sigma Aldrich and were of analytical grade for application during this study. The biomass samples were collected from adult local size (10-15 years old) date palm trees in Al-Ain, UAE (24122 N, 554441 E). Given the arid climate in the region, these lignocellulose biomasses were collected as naturally dried samples with less than 9 wt.% moisture contents. However, they were washed clean to remove any field contaminants and then dried under shade. Samples of 250-micron particle size were used in this work and American Standard test procedures were adopted for various physical-chemical analyses. The proximate analysis of the biomass samples was determined according to ASTM D3173 (inherent moisture content), where triplicate samples were dried under shade for 24 hours. Samples of 2 g in triplicates were weighed and kept in desiccator for 12 hours. The difference in weight before and after drying signified inherent moisture content. Volatile matter was determined according to ASTM D3175 where 1 g sample was weighed in an empty crucible and then placed in a furnace, set and operated at 950 ^{o}C for exactly 7 minutes. Loss in weight after cooling was calculated and the deduction of inherent moisture minus the weight loss, gave the amount of volatile matter. Each sample, 2 g in triplicates, was accurately weighed in a clean dry crucible and placed in a furnace at 575 ^{o}C for 3 hours, cooled under desiccator conditions for 1 hour and the difference in weight before and after, expressed as a percentage, signified ash content according to ASTM D3174 (ash). Fixed carbon content was determined according to ASTM D3172-07a (Fixed carbon) method, expressed as; [100-(moisture content + Ash + volatile matter)]**.** All the ASTM methods in this work can be accessed on ASTM website (ASTM compass 2018). The ultimate analysis of the sample to determine C, H, O, S and N contents were performed by the automatic elemental analyzer (Flash EA1112, CE Instruments).

The heating values of the samples were determined using bomb calorimeter model IKA C 2000 according to DIN 51900 method; where 0.5 g crushed biomass sample was pelletized and placed in a quartz crucible connected with a fuse wire (15 cm), the crucible was placed inside the bomb calorimeter and charged with oxygen to approximately 200 Psi for about 10 seconds. The heat of combustion was computed from the temperature observations before (T_o), during and after combustion (T_{max}), taking into care for thermochemical and heat transfer corrections. The calorimeter was calibrated by combusting a standard known mass, m, of benzoic acid with a known heat of combustion (26.453 MJ/kg). Calibration and analysis of samples were done in triplicates and the high heating value HHV of samples were calculated from a direct relationship of benzoic acid as follows:

$$HHV = (\mathrm{m} * 26.453)/(T_{max} - T_o) \qquad (1)$$

The bulk density was determined as mass per unit volume using 500 ml graduated measuring cylinder according to the reference method (Obernbereger & Thek 2004). Mixture in this work refers to composition of leaflet, rachis and fibers in 0.5:2:0.06 ratios, respectively according to their individual contribution in biomass bulk per unit length. During thermochemical conversion of this biomass, the mixture as a composite will be considered as feed stock in the reactor, thus its characterization was relevant in this study.

2.2 Thermogravimetric analysis

Thermogravimetric analysis of palm biomass in this study was carried out using TGA (Q500 series, TA Instrument). In each experiment, a sample weight of 6.0 ±1.0 mg was used. The heating rate was controlled at 10, 15, 20 and 25 °C/min from 25 to 900 °C, using nitrogen as a carrier gas at 20 ml/min. During the thermo-decomposition process, the initial weight was recorded continuously as a function of temperature and time. The derivative (DTG) curve showed the weight loss of sample per unit time against temperature.

2.3 Lignin extraction

The adopted method of lignin determination is commonly known as Klason lignin extraction (Carrier *et al.* 2011). However, this traditional method was by accelerated 2 hours heating at 47 ^{o}C during hydrolysis stage to completely hydrolyze and solubilize the carbohydrate from complex lignocellulosic palm biomass structure. Prior to isolation, the biomass was treated to make it extractive free (free of protein, waxes and resins) in accordance to TAPPI methods- T 204 cm-97 (TAPPI methods 1992); 5 ± 0.01 g biomass sample was transferred to a weighed thimble tube and extracted with 150 mL of ethanol-benzene solvent (1/2 v/v) for 5 hours. The residue extract was oven dried at 103 ± 2 ^{o}C for 1 hour, cooled in a desiccator and later weighed to determine the extractive free biomass to be used for klason lignin extraction.

The extractive free biomass (1± 0.1g) was treated with 72 % H_2SO_4 for 2 hours while stirring at 37 ^{o}C. The material was then diluted to 3 % H_2SO_4 and then refluxed at 80 ^{o}C for 4 hours. After filtration, the insoluble lignin was oven dried at 105 ± 1 ^{o}C for 1 hour and cooled in a desiccator to obtain constant weight; the difference in weight before and after oven drying gave the insoluble lignin. The soluble lignin fraction was determined according to TAPPI method UM 250 (TAPPI standards 1985), at absorbance 280 nm in UV spectrometer and calculated using the formula below;

$$Soluble\ Lignin = \frac{A}{110} \times \frac{dilution}{Original\ sample\ weight} 100\ \% \qquad (2)$$

Where; A is the absorbance at 280 nm, 110 is the absorptivity of soluble lignin measured in $Lg^{-1}cm^{-1}$.

2.4 Scanning Electron Microscopy

A JEOL /EO Scanning Electron Microscope (SEM) operated at 2 kV; spot size of 40 was used to image the samples before and after Klason lignin extraction. To improve conductivity and quality of image, samples were coated with Au/C using a vacuum sputter coater.

2.5 Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR)

ATR-FTIR analysis was performed to investigate the possible structural alteration between the samples before and after Klason lignin extraction. Palm biomass samples before and after Klason extraction were pressed uniformly against a diamond surface by a fixed sample holder anvil, spectra were observed using a Bruker optics vertex system with inbuilt diamond-germanium ATR single reflection crystal. Spectra were obtained over a range of 400 and 4000 cm^{-1} with 34 average numbers of scans and spectral resolution of 4 cm^{-1}.

3.0 Results and Discussion

3.1 Material Characterizations by Elemental Analysis

Elemental analysis on original biomass for palm biomass was carried out as shown in Table1. The results showed that calcium, magnesium and sodium were highest in fibers. On the other hand, leaflet and rachis were most rich in potassium and phosphorous, respectively.

Table 1. Composition of lignocellulose date palm biomass

Biomass	**Leaflet**	**Rachis**	**Fibers**	**Mixture**
Bulk Density (kg/m^3)	426±4 (411[a])	425±4 (635[a])	387±9 (209[a])	420±3
HHV (MJ/kg)	15.5±0.1(17.9[b])	15.5±0.1 (10.9[b])	14.8±0.1(16.4[c])	15.0±0.1
Element Analysis (ppm)				
K	33.0±0.6	28.9±0.6	30.8±0.0	26.3±0.4
P	10.9±0.2	16.7±1.8	6.7±1.6	12.0±0.4
Ca	196.5±8.0	158.2±20	305.9±28	166.6±12
Mg	24.0±2.2	21.4±3.0	46.6±8.0	21.2±2.0
Na	8.7±2.3	11.5±1.4	26.5±3.6	9.3±1.6
Hemicellulose (wt. %)	11.0±1.6	19.0±1.0	17.0±0.3	14.8±1.2
Cellulose (wt.%)	21.0±2.6	32.0±1.0	33.0±1.2	30.2±1.2
Insoluble Lignin (wt.%)	19.0±1.0	10.0±2.0	20.0±1.2	25.4±1.0
Soluble lignin (wt. %)	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0
Ethanol-Benzene Extractives (wt.%)	29.0±3.8	14.8±1.0	9.0±3.0	16.0±2.0

[a](Alim *et al.* 2015); [b](sait *et al.* 2012); [c](Asadullah *et al.* 2014)

However, when three palm parts were mixed in a ratio stated before to form a mixture sample, the elements composition in their decreasing order followed the trend; Ca>K>Mg>P>Na. These results confirm the claim from local farmers supplying our research group with raw biomass that "no artificial nutrient additives are added to the soil during palm tree cultivation period".

Taking a mixture sample as a reference sample, since it's a combination of the three palm parts considered in this study, the lignocellulosic composition experiments revealed that the sample had more cellulose (30.2 ± 1.2 wt.%) and lignin (26.4 ± 1.0 wt.%) than hemicellulose content (14.8 ± 1.2 wt.%). The observed trend was comparable to similar in the literature, where cellulose; 32.49-50.33 wt.%, lignin; 21.70-35.89 wt.% and hemicellulose; 22.97-23.94 wt.% were reported (*Abnisa* et al. 2013; Law et al. 2007; Kelly *et al.* 2007). The results of energy value, bulk density and extractives are also shown in Table 1. Extractive analysis was vital for this work as it is important in removing resins, carbohydrates and waxes prior to klason lignin extraction (Schwanninger & Hinterstoisser 2002; Moreira *et al.* 2014).

The proximate analysis of original palm biomass parts before Klason lignin extraction was conducted using corresponding standard ASTM methods and results were expressed in terms of ash content, volatile matter, fixed carbon and moisture content as shown in Table 2.

Table 2. Proximate analysis of date palm biomass

Biomass	**Moisture (wt.%)**	**Volatile matter (wt.%)**	**Ash content (wt.%)**	**Fixed carbon (wt.%)**
Leaflet	4.3±0.3	75.8±1.2	12.7±1.2	7.2±0.2
Rachis	5.7±0.2	84.6±1.8	6.1±1.0	3.6±1.0
Fibers	5.4±0.1	77.4±0.6	8.2±0.3	9.0±0.8
Mixture	3.5±0.1	80.8±1.6	8.7±0.4	9.6±0.4

Since the Klason method involved sulphuric acid that alters the material structural composition, ultimate analysis was performed as shown in Table 3, on before and after Klason lignin extracted samples.

Table 3. Ultimate analysis of palm biomass (as % on dry basis)

Biomass	**Leaflet**		**Rachis**		**Fibers**		**Mixture**	
	Original	Klason	Original	Klason	Original	Klason	Original	Klason
C	44.1±0.2	51.3±0.3	43.6±0.0	49.7±0.2	43.2±0.1	46.0±1.0	50.7±1.0	53.7±0.4
H	5.9±0.0	5.1±0.0	6.1±0.1	5.5±0.1	5.7±0.1	4.1±0.3	5.5±0.1	5.7±0.2
N	1.9±0.2	1.5±0.2	1.2±0.0	0.2±0.0	0.8±0.0	0.0	1.7±0.2	1.6±0.1
S	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
O	48.5±0.4	41.7±0.8	50.3±0.2	43.5±0.3	50.3±0.2	49.9±1.0	48.5±0.2	43.7±0.3
O/C	1.1±0.4	0.8±0.1	1.2±0.2	0.9±0.1	1.2±0.2	1.1±0.1	1.0±0.1	0.8±0.1
H/C	0.1±0.2	0.1±0.0	0.1±0.1	0.1±0.0	0.1±0.2	0.1±0.0	0.1±0.2	0.1±0.0

After acid hydrolysis, the carbon content increased, and oxygen content decreased; presumably sulphuric acid resulted in cleavage of the cellulosic glycosidic bond releasing some oxygen from the biomass structure. The following structural rearrangement with lignocellulosic material, could have resulted in the increase of carbon content. The decrease in nitrogen content was because of benzene-ethanol extraction that removes resins and other protein-like materials. Palm biomass was free of any sulphur content. Sulphur poisons catalysts which would have a negative impact on catalytic upgrading processes, thus its absence makes future catalytic upgrading more efficient.

3.2 Thermogravimetric Analysis

The results of thermogravimetric analysis before Klason lignin extraction for the mixture biomass at different heating rates are shown in Fig.1

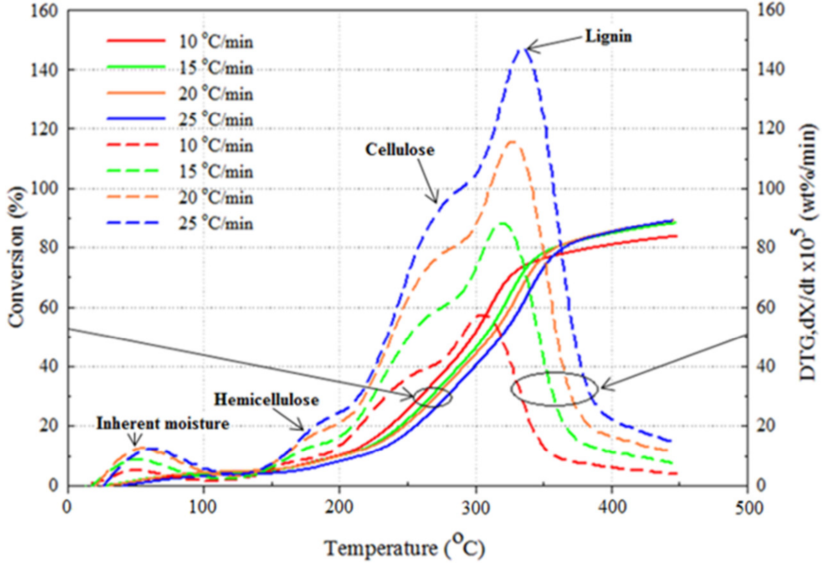

Figure 1. TGA and DTG curves for mixture of palm biomass at heating rates of 10, 15, 20 and 25 °C/min.

Increase in heating rate from 10 to 25 °C/min led to shift in temperature-conversion curves towards the right-hand side, which was attributed to increase in thermal energy and increased heat transfer between the sample and the surroundings thus causing slight decrease in conversion. Based on DTG analysis, the mixture showed four decomposition regimes for all the four heating rates considered. The first regime is below 100 °C which was attributed to the inherent moisture adsorbed on the samples and has less impact on physico-chemical nature of the sample as it can easily be removed by oven drying prior to reactor operations. Between 150-200 °C is the second regime and was assigned to decomposition of amorphous hemicellulose. In comparison to lignin and cellulose, hemicellulose is a random amorphous and thermally labile structure and hence easy to decompose at lower temperatures. The results of hemicellulose decomposition agreed with those reported in the literature (Lv *et al*. 2010), hence providing more proof about hemicellulose decomposition temperature profile. The third regime between 200-300 °C, was assigned to decomposition of cellulose. In contrast, cellulose is a long polymer of glucose units connecting one another by gylcosidic bond, which increases its thermal stability thus higher decomposition temperature compared to hemicellulose (Yang *et al*. 2006). The last regime between 300-380 °C was assigned to lignin that is an amorphous polymer made up of propane alcohol units making it more stable and thus requires more thermal energy for decomposition. After Klason lignin extraction, the decomposition trend changed as shown in Fig.2

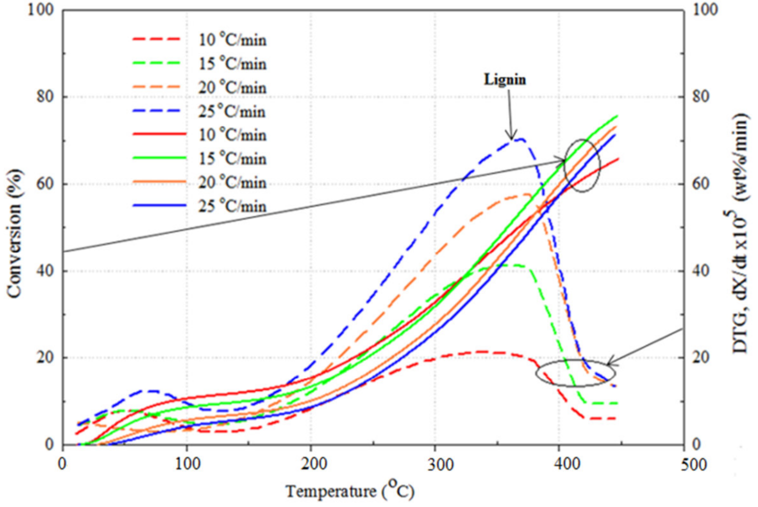

Figure 2. TGA and DTG curves of Klason lignin method for mixture of palm biomass at heating rates of 10, 15, 20 and 25 °C/min.

The conversion graphs maintained a similar trend with increasing heating rate but more skewed to the right compared to conversion graphs in Fig.1 before Klason extraction. For Klason lignin extraction, DTG analysis showed two decomposition regimes in contrast to the four regimes seen before extraction for the same mixture sample. The first regime below 100 °C was assigned to dehydration of inherent water adsorbed in the samples and the second peak had a wide decomposition temperature profile between 200-400 °C and was assigned to lignin decomposition. Lignin is believed to have a wide decomposition temperature profile depending on different kinds of biomass which can be in the range of 160-900 °C according to some literature reports (Liu *et al*. 2014). At this stage of research, we can only postulate that the wide decomposition trend from 200 °C for this palm biomass is presumably due to residual cellulose that remains after acid hydrolysis, or it is due to lignin wide range temperature decomposition profile. The biomass was then analyzed for morphological differences before and after Klason lignin extraction for different palm biomass parts.

3.3 Scanning electron microscopy

Fig. 3 (a)-(c) and (d)-(g) shows palm leaflet SEM images before and after klason lignin extraction, respectively.

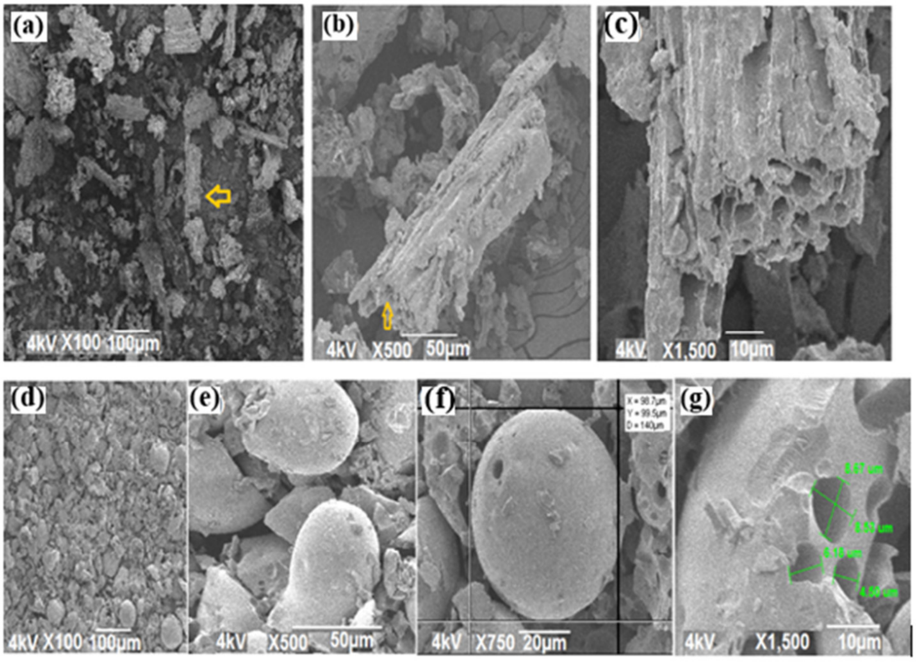

Figure 3. Leaflet SEM images: (a) to (c) for original sample and (d) to (g) after Klason lignin extraction.

Leaflet before extraction has irregular morphologies of different shapes at investigated magnifications. However, when focused in detail at elevated magnification X500 and X1500 in (b) and (c), respectively; concentrating on structure with yellow arrow, some particles are tubular-like, stacked together forming one definite cylindrical structure (Fig.3b) and when viewed much closer at X1500 these tubular-like stacks are hollow, separated from each other by walls and bound with fine strands on the outer surface. On contrast after Klason extraction, leaflet cylindrical morphologies were completely distorted to small regular spherical structures of measurable external diameter (140µm) and the broken samples were viewed in detail at X1500 where spheres had different internal diameters, ranging from 4.50 µm to 8.67 µm for smallest and biggest particles, respectively. In analogy to lignocellulose biomass, we presume that the cylindrical structure in Fig. 3(a)-(c) was a stack of, lignin walls and hemicellulose strands binding the cellulose together to form lignocellulose structure with all the three components and this presumption was supported by DTG results in Fig.1 that showed existence of three components making up lignocellulosic material. However, after Klason lignin extraction, the sulphuric acid dissolved the hemicellulose and cleaved the cellulose glycosidic bonds, thus weakening strong cellulose bonds while exposing lignin. A similar argument was supported by DTG results in Fig. 2 whereby after acid hydrolysis the hemicellulose and cellulose peaks disappeared.

Fig. 4 (a)-(c) and (d)-(g) show palm rachis SEM images before and after Klason lignin extraction, respectively.

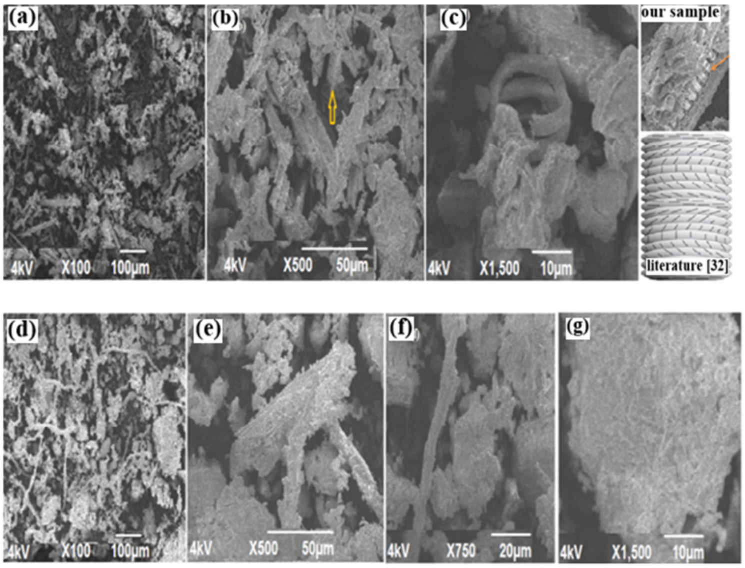

Figure 4. Rachis SEM images: (a) to (c) for original sample and (d) to (g) after Klason lignin extraction.

The lignocellulose morphology in rachis showed unique irregular fibrils (hemicellulose and lignin) and helical chiral nematic ordering (cellulose). These helical structures were like what was reported in the literature (Matheus & Heitor 2015) and can also be seen on right hand side of Fig. 4 (c). However, after Klason lignin extraction, the helical chiral nematic ordering disappeared, a clear indication, that either cellulose was completely hydrolyzed by H_2SO_4 or it was broken down from hard crystalline structure to soft amorphous fibril cellulose.

The palm fibers SEM images before and after klason lignin extraction, respectively, are shown by Fig.5 (a)-(d) and (e)-(h).

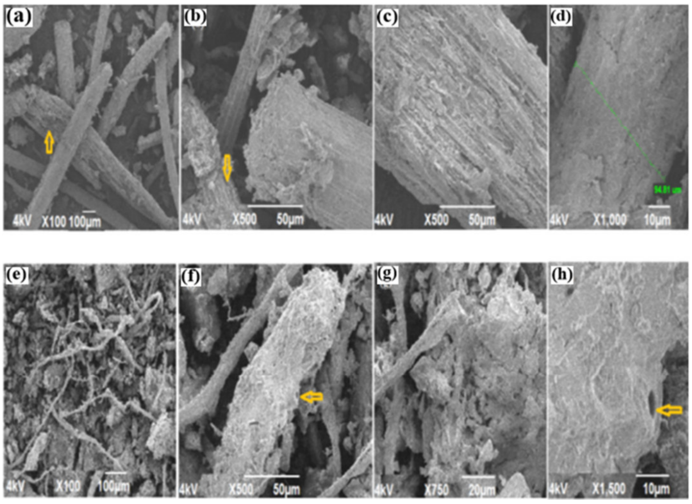

Figure 5. Fibers SEM images: (a) to (d) for original sample and (g) to (h) after Klason lignin extraction.

When viewed under different magnifications, fibers showed cylindrical closed tubes having regular shapes with measurable external diameters and when the broken parts were viewed at X500 concentrating on part showed by the yellow arrow, the inner surface was closely packed with fibers of irregular patterns. On the other hand, after Klason lignin extraction, the cylindrical shapes are deformed and spiral; hemicellulose binding cellulose and lignin disappeared, presumably it was dissolved by the sulphuric acid. The hollow-structures for fibers and leaflet Klason lignin extraction seem to suggest the morphologies of these two are close to each other than that of rachis.

When a mixture was taken from the three palm parts in each ratio, the mixture SEM images were analyzed as shown in Fig. 6 (a)-(c) and (d)-(g) for before and after Klason lignin extraction, respectively.

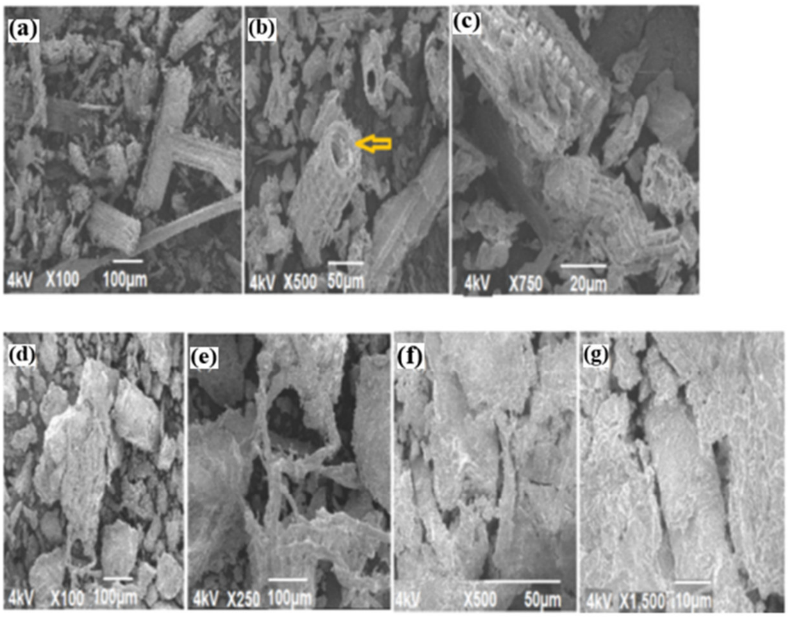

Figure 6. Mixture SEM images: (a) to (c) for original sample and (d) to (g) after Klason lignin extraction.

The morphologies of palm mixture before lignin extraction, showed structures of different sizes and shapes but like what was seen in Fig. 3, Fig. 4 and Fig.5 for leaflet, rachis and fibers, respectively, which was a confirmation of existence of the three palm biomass parts with their lignocellulose morphologies. In contrast after Klason lignin extraction, at all considered magnifications, palm mixture sample showed independent cross-linked one semi-fused structures. SEM results showed morphological differences before and after klason lignin extraction. However, further characterization was done to understand the chemical and structural changes using FTIR measurements.

3.4 Fourier transform infrared spectroscopy

The results of FTIR spectroscopy in this study were used basically to assess the chemical bond rearrangement and structural changes of palm lignocellulose biomass before and after Klason lignin extraction. The spectra in Fig.7 showed similar band trend for all samples before klason lignin extraction.

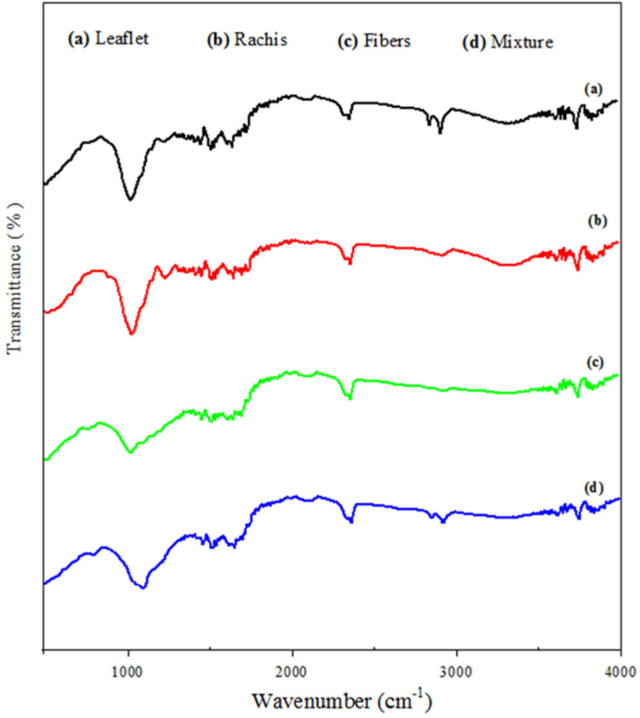

Figure 7. FTIR spectra of original leaflet, rachis, fibers and mixture palm biomass parts.

On the other hand, although all samples showed similar absorption bands after Klason treatment as shown in Fig. 8, there was a noticeable shift in peak positions after Klason lignin extraction in the region around 1000 and 1250 cm^{-1} for date palm leaflet, rachis, fibers and mixture samples. This is clear evidence that Klason lignin extraction altered the chemical and structural make up of palm biomass. The small band around 900 cm^{-1} representing glycosidic bond [β-(1→4)] in amorphous cellulose was observed in original sample, however was hardly seen in Klason lignin extracted sample and similar phenomena was reported in the literature (Pendey & Pitman 2003; Oh *et al.* 2005). This shows the effectiveness of Klason method towards cellulose and hemicellulose removal.

Strong peaks were observed around 1125 cm^{-1} for original sample and this was even stronger after Klason lignin treatment, observed around 1070 cm^{-1}.

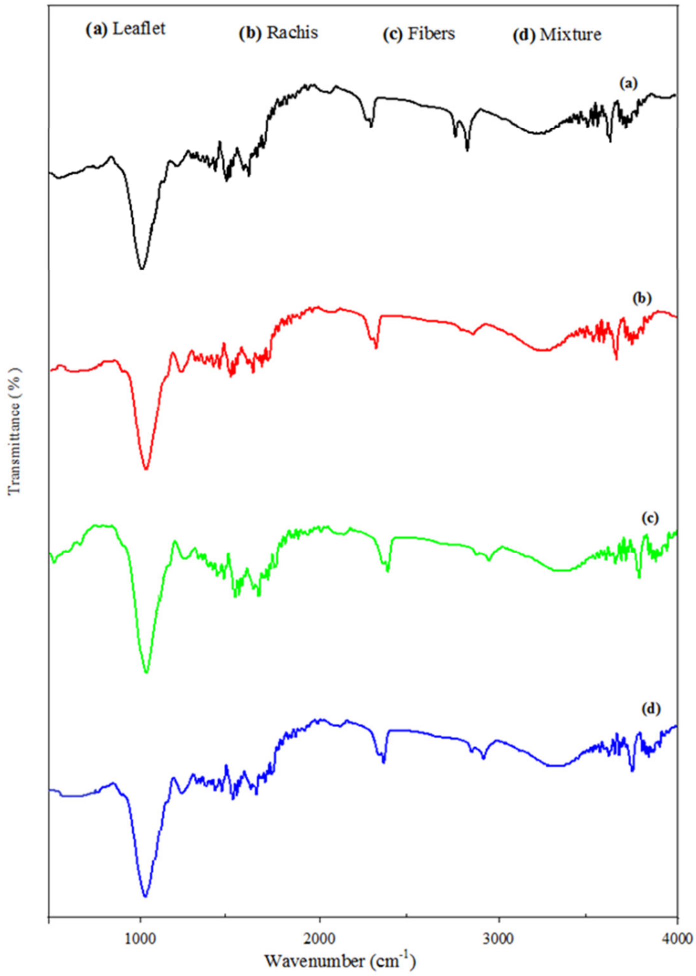

Figure 8. FTIR measurements for after Klason lignin extraction of leaflet, rachis, fibers and mixture palm biomass parts.

This change suggests an increase in lignin proportion in Klason lignin sample due to glycosidic bond cleavage in cellulose thus removing amorphous cellulose and dissolution of hemicellulose by sulphuric acid used during the Klason lignin extraction. For original sample, the wide band around 1200 and 1250 cm^{-1} converged into small peak around 1250 after Klason lignin extraction. This stronger exhibited peak after Klason treatment was referred to lignin (Pendey & Pitman 2003) after the removal of hemicellulose and cellulose, which is due to absorption by C-O bond stretching. Similar results emphasizing the existence of hemicellulose and cellulose in the region around 1035-1200 cm^{-1} and disappearance of these components after various treatment methods, was reported in the literature (Chandel *et al.* 2014). The region of wave length between 1300 to 1450 cm^{-1} exhibits high molecular coupling of complex compounds possibly of lignin and carbohydrate in nature (Ivanova & Korolok 1989). Ethanol- benzene extraction was carried out to extract carbohydrates and other resins, therefore, the vibrations in the preceding region were assigned to lignin. The band around 1458 cm^{-1} is where CH_2 and CH_3-lignin structures deform and all these effects in turn reduces lignin recalcitrant to chemical treatment processes. Literature has reported that bands around 1515 to 1605 cm^{-1} are due to stretching of C=C and C=O present in the lignin aromatic ring (Pandey 2005; Colom *et al.* 2003). The band for the range between 1595 to 2937 cm^{-1} is because of C=O, C-H, CH_2 stretching of unconjugated hemicellulose, symmetrical and unsymmetrical CH_2 bond stretching in cellulose and stretching of aromatic ring vibration in lignin (Ivanova &

Korolok 1989; Pandey 2005; Colom *et al* 2003; Li *et al* 2011). It was therefore presumed that the decrease in peak intensity in the preceding mentioned region was due to bond cleavage that reduced cellulose content and the sulphuric acid hydrolysis that dissolved the hemicellulose during Klason extraction process, leaving mainly lignin proportions. Moreover, the band trend never changed for samples before and after Klason lignin treatment because lignin bond vibration and stretching are greater than that of hemicellulose and cellulose in this region. The chemical changes in band position between 3250 to 3885 cm^{-1} is due to O-H stretching in methyl group of lignin (Li *et al*. 2011; Abnisa *et al*. 2013; Basma *et al*. 2016). Lignin has high absorption strength (Liu et *al*. 2008). In general, there was a clear distinction in chemical and structural orientation in the original samples after application of Klason lignin method.

4.0 Statistics

Statistical inferences about two mean groups; before klason extraction and after klason extraction, was carried out on carbon content and hydrogen content, as shown in the Minitab results in table 4. Carbon and hydrogen content form the base skeleton of lignocellulose biomass and are very crucial during polymerization and they carry energy value that is useful during downstream processing. Both carbon and hydrogen content, with 95% confidence interval, the hypothesis testing showed how two means were different before and after klason treatment with carbon and hydrogen content p-value of 0.000 and 0.005, both less than 0.05 significance level. There was an improvement in average carbon content from 45.4 to 50.18 and a decrease in hydrogen content from 5.78 to 5.12 for samples before and after klason treatment, respectively.

Table 4. Statistical data using Minitab software

```
Paired T-Test and CI: carbon content before klason, ... after klason treatment
Descriptive Statistics
 Sample                                N    Mean     StDev   SE Mean
 carbon content before klason          12   45.400   3.243   0.936
 Carbon content after klason treatment 12   50.175   2.962   0.855
Test
      Null hypothesis             H  : μ_difference = 0
      Alternative hypothesis      H₁ : μ_difference ≠ 0
 T-Value   P-Value
 -7.93     0.0000
μ_difference: mean of (carbon content before klason - Carbon content after klason
treatment)

Paired T-Test and CI: Hydrogen content before klason, ... t after klason treatment
Descriptive Statistics
 Sample                          N    Mean    StDev   SE Mean
 Hydrogen content before klason  12   5.775   0.267   0.077
 Hydrogen content after klason   12   5.117   0.642   0.185
Test
 Null hypothesis          H  : μ_difference = 0
 Alternative hypothesis   H₁ : μ_difference ≠ 0
 T-Value   P-Value
 3.49      0.005
μ_difference: mean of (Hydrogen content before klason - Hydrogen content after klason)
```

5.0 Conclusions

Despite date palm trees playing a major social and economic role in arid and semi-arid areas, its lignocellulose composition has not been utilized for value added products. Research has been done on valorization of cellulose and hemicellulose for application in bio-refinery; however, lignin due to its complex structure has not been given much attention. In this study, leaflet, rachis, fibers and a mixture of the three samples were studied for Klason lignin extraction method. Physicochemical characterization, extractive free determination, SEM and FTIR analysis were applied to original and Klason lignin extracted samples to evaluate the effectiveness of lignin isolation for future production of high value chemicals. After klason lignin extraction, the DTG curves only showed one peak regime that corresponds to decomposition of lignin. SEM results also showed morphological difference with samples like rachis displaying a unique helical chiral nematic ordering (cellulose), that later disappeared after Klason lignin treatment. The study from FTIR further revealed the digestibility of hemicellulose, the cleavage of cellulose glycosidic bonds and the exposure of lignin reducing its chemical recalcitrance tendencies. Therefore, results of this study have shown that Klason method is successful in isolating lignin fractions from different parts of date palm biomass. These results will be used in the next phase of this research for production of HVCs using them as a basis for reactor optimization and further understanding of date palm biomass characteristics.

Acknowledgement
Appreciation to Mr. Ismail Abdul Halim, Food Science Department UAEU and Dr. Hussain Awad El Sayed, Chemical & Petroleum Engineering Department UAEU, for their support during ICP analysis and permission to access restricted the analysis laboratories.

References

Scarlat, N., Dallemand, J., Monforti-Ferrario, F. & Nita, V. (2015), "The role of biomass and bioenergy in a future bioeconomy", Policies and facts 15, Environmental Development*, 3-34.*

Yat, SC., Berger, A. & Shonnard, DR. (2008) "Kinetic characterization of dilute surface acid hydrolysis of timber varieties and switchgrass", *Bioresource Technology* **99**, 3855–3863.

Wyman, CE. (1999), "Biomass ethanol: Technical progress, opportunities, and commercial challenges", *Annual Review of Energy and the Environment* **24**, 189– 226.

Wang, M., Wu, M. & Huo, H. (2007), "Life-cycle energy and greenhouse gas emission impacts of different corn ethanol plant types", *Environmental Research Letters* **2**, 1–13.

Brinchi, L., Cotana F., Fortunati, E. & Kenny, JM. (2013), "Production of nanocrystalline cellulose from lignocellulosic biomass: Technology and applications", *Carbohydrate Polymers* **94**(1), 154-169.

Biagiotti, J., Puglia, D. & Jose, MK. (2008), "A Review on Natural Fibre-Based Composites-Part I", *Journal of Natural Fibers*. 1:2, 37-68.

Kumar, P., Barrett, DM., Delwiche, MJ. & Stroeve, P. (2009),"Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production", *Industrial & Engineering Chemistry Research* **48**, 3713–3729.

Klein, I., Saha, B. & Abu-Omar, MM. (2014), "Lignin Depolymerization over Ni/C Catalyst in Methanol a Continuation: Effect of Substrate and Catalyst Loading", *Catalysis Science & Technology* **5**, 3242-3245.

Barreveld, WH. (1993), "Date Palm Products, in Food and Agriculture Organization of the United Nations Rome", *FAO Agricultural Services*.

Klemm, D., Heublein, B., Fink, H. & Bohn, A. (2005), "Cellulose: Fascinating biopolymer and sustainable raw material", *Angewandte Chemie International* **44**(22), 3358-3393.http://doi:10.1002/anie.200460587.

Pauly, M., Gille, S., Liu, L., Mansoori, N., Souza, Ad., Schultink, A. & Xiong, G. (2013), "Hemicellulose biosynthesis", Planta **238**(4), 627-642.

Adler, E. (1977), "Lignin chemistry - past, present and future", *Wood Science Technology* **11**, 169- 218.

Freudenberg, K. & Neish, AC. (1968), "The constitution and biosynthesis of lignin", *Springer Verlag, Berlin,* 47–122.

Glennie, DW. & McCarthy, JL. (1962), "Chemistry of Lignin. Libby (Ed.)", Pulp and Paper Science and Technology, McGraw-Hill Book Company, Inc, New York, 82–107.

López, M., Huerta Pujol, O., Martínez Farré, FX. & Soliva, M. (2010), "Approaching compost stability from klason lignin modified method: Chemical stability degree for OM and N quality assessment", *Resources, Conservation & Recycling* **55**(2), 171-181.http://doi:10.1016/j.resconrec.2010.09.005.

Evtuguin, DV., Pascoal Neto, C., Rocha, J. & Pedrosa de Jesus, JD. (1998), "Oxidative delignification in the presence of molybdovanadophosphate heteropolyanions: Mechanism and kinetic studies", *Applied Catalysis A: General* **167**(1), 123-139. http://doi:10.1016/S0926-860X(97)00306-2.

Capanema, EA., Balakshin, MY. & Kadla, JF. (2004), "A comprehensive approach for quantitative lignin characterization by NMR spectroscopy", *Journal of Agricultural and Food Chemistry* **52**(7), 1850-1860. http:// doi:10.1021/jf035282b.

Chakar, FS. & Ragauskas, AJ. (2004), "Review of current and future softwood kraft lignin process chemistry", *Industrial Crops & Products*. **20**(2), 131-141. http://doi:10.1016/j.indcrop.2004.04.016.

Parlack, RD., Wri5ght, LL., Turhollow, AF., Graham, RL. & Stroke BJ. (2005), "Biomass as Feedstock for a Bioenergy and Bioproducts Industry", Department of Energy.
www.eere.energy.gov/biomass/publications.html. [Accessed 29 December 2017].

White, JE., Catallo, WJ. & Legendre, BL. (2011), "Biomass pyrolysis kinetics: A comparative critical review with relevant agricultural residue case studies", *Journal of Analytical and Applied Pyrolysis* **91**(1), 1-33.
hhttp:// doi: 10.1016/j.jaap.2011.01.004.

ASTM compass. http://www.astm.org. [Accessed 05 March 2018].

Obernberger, I. & Thek, G. (2004), "Physical characterisation and chemical composition of densified biomass

fuels about their combustion behavior", *Biomass Bioenergy* **27**(6), 653-669.
http://doi:10.1016/j.biombioe.2003.07.006.

Carrier, M., Loppinet Serani, A., Denux, D., Lasnier, J., Ham Pichavant, F., Cansell, F. & Aymonier, C. (2011), "Thermogravimetric analysis as a new method to determine the lignocellulosic composition of biomass", *Biomass Bioenergy* **35**(1), 298-307.
http://doi:10.1016/j.biombioe.2010.08.067.

TAPPI Test Methods T 204 cm-97, Solvent extractives in wood and pulp. In: Tappi test methods. Atlanta, GA: Technical Association of the Pulp and Paper Industry. GA 1992.

TAPPI Standards, Acid-soluble lignin in wood and pulp, Tappi Method UM 250. Tappi Press, Atlanta, GA. 1985.

Almi, K., Benchabane, A., Lakel, S. & Kriker, A. (2015), "Potential utilization of date palm wood as composite reinforcement", *Journal of Reinforced Plastics and Composites* **34**, 231-1240.

Sait, HH., Hussain, A., Salema, AA. & Ani, FN. (2012), "Pyrolysis and combustion kinetics of date palm biomass using thermogravimetric analysis", *Bioresource Technology* **118**, 382-389.

Asadullah, M., Adi, AM., Suhada, N., Malek, NH., Saringat, MI. & Azdarpour, A. (2014), "Optimization of palm kernel shell torrefaction to produce energy densified bio-coal", *Energy Conversion and Management* **88**, 1086-1093.

Abnisa, F., Arami Niya, A., Wan Daud, WMA., Sahu, JN. & Noor, IM. (2013), "Utilization of oil palm tree residues to produce bio-oil and bio-char via pyrolysis", *Energy Conversion and Management* **76**, 1073-1082.

Law, KN., Daud, WRW. & Ghazali, A. (2007), "Morphological and chemical nature of fiber strands of oil palm empty-fruit-bunch (OPEFB)", *Bioresource Technology* **2**, 351-362.

Kelly Yong, TL., Lee, KT., Mohamed, AR. & Bhatia, S. (2007), "Potential of hydrogen from oil palm biomass as a source of renewable energy worldwide", *Energy Policy* **35**(11), 5692-5701.
http://doi:10.1016/j.enpol.2007.06.017.

Schwanninger, M. & Hinterstoisser, B. (2002), "Modification of Klason Lignin Determination", *Holzforschung* **56**: 161-166.

Moreira Vilar, FC., Siqueira Soares, RdC., Finger Teixeira, A., de Oliveira, DM., Ferro, AP., da Rocha, GJ. & Ferrarese Filho, O. (2014), "The acetyl bromide method is faster, simpler and presents best recovery of lignin in different herbaceous tissues than klason and thioglycolic acid methods", *PloS One* **9**(10), e110000.
http://doi:10.1371/journal.pone.0110000.

Lv, G., Wu, S. & Lou, R. (2010), "kinetic study for the thermal decomposition of hemicellulose isolated from corn stalk", *Bioresource* **5**(2), 1281-1291.

Yang, H., Yan, R., Chen, H., Zheng, C., Lee, DH. & Liang, DT. (2006), In-depth investigation of biomass pyrolysis based on three major components: hemicellulose, cellulose and lignin", *Energy Fuels* **20**(1), 388-393.
http://doi:10.1021/ef0580117.

Liu, C., Wang, H., Ayman, MK., Sun, J. & Wang, Y. (2014), "Catalytic Fast Pyrolysis of Lignocellulosic Biomass". Chemical Society Reviews **43**, 7594-7623.

Matheus, P. & Heitor, LOJ. (2015), "Cellulose - Fundamental Aspects and Current Trends", 282.
http://doi: 10.5772/59889.

Pandey, KK. & Pitman, AJ. (2003), "FTIR studies of the changes in wood chemistry following decay by brown-rot and white-rot fungi", *International Biodeterioration & Biodegradation* **52**,151–160.
http://doi: 10.1016/S0964-8305(03)00052-0.

Oh, SY., Yoo, DI., Shin, Y., Kim, HC., Kim, HY., Chung, YS., Park, WH. & Youk, JH. (2005), "Crystalline structure analysis of cellulose treated with sodium hydroxide and carbon dioxide by means of X-ray diffraction and FTIR spectroscopy", *Carbohydrate Research* **340**(15), 2376-91.

Chandel, AK., Antunes, FA., Anjos, V., Bell, MJ., Rodrigues, LN., Polikarpov, I. & da Silva, SS. (2014), Multi-scale structural and chemical analysis of sugarcane bagasse in the process of sequential acid–base pretreatment and ethanol production by Scheffersomyces shehatae and Saccharomyces cerevisiae", *Biotechnology for Biofuels* **7**; 63.
http://doi.org/10.1186/1754-6834-7-63.

Ivanova, NV. & Korolok, EV. (1989), "IR spectrum of cellulose", *Journal of Applied Spectroscopy* **51**,847–851.
http://doi: 10.1007/BF00659967.

Pandey, KK. (2005), "Study of the effect of photo-irradiation on the surface chemistry of wood", *Polymer Degradation and Stability* **90**, 9–20.
http://doi: 10.1016/j. polymdegradstab2005.02.009.

Colom, X., Carrillo, F., Nogués, F. & Garriga, P. (2003), "Structural analysis of photo-degraded wood by means of FTIR spectroscopy", *Polymer Degradation and Stability* **80**:543–549.
http://doi: 10.1016/S0141-3910(03)00051-X.

Li, C., Cheng, G. & Balan V. (2011), "Influence of Physico-Chemical changes on enzymatic digestibility of ionic liquid and AFEX pretreated corn Stover", *Bioresource Technology* **102**(13), 6928-6936.

Abnisa, F., Arami Niya, A., Daud, WMA. & Wan Sahu, JN. (2013), "Characterization of bio-oil and bio-char from pyrolysis of palm oil wastes", *BioEnergy Research* **6**(2), 830-840.
http://doi:10.1007/s12155-013-9313-8.

Basma, EKH., Margit, S. & Birgit, K. (2016), "Qualitative and Quantitative Analysis of Lignins from Different Sources and Isolation Methods for an Application as a Biobased Chemical Resource and Polymeric Material", *Analytical Techniques and Methods for Biomass*,15-44.
http:// 10.1007/978-3-319-41414-0_2.

Liu, Q., Wang, S., Zheng, Y., Luo, Z. & Cen, K. (2008), "Mechanism study of wood lignin pyrolysis by using TG–FTIR analysis", *Journal of Analytical and Applied Pyrolysis*. **82**(1), 170-177.
http://doi:10.1016/j.jaap.2008.03.007.