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Chemical, Crystallinity and Morphological Properties of Oil Palm Trunk Parenchyma and Vascular Bundle

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***Abstract*— Parenchyma and vascular bundle of oil palm trunk were segregated manually and were evaluated for their holocellulose, α-cellulose, hemicelluloses, lignin and starch content accordingly. The functional groups, crystallinity and morphological characteristics were also studied using FTIR, XRD and scanning electron microscopy respectively. The results showed that oil palm trunks were found to be rich in the vascular bundle rather than in parenchyma. It was shown that the vascular bundle had a higher amount of α-cellulose compared to parenchyma which had a higher amount of lignin, extractives and starch. The XRD analyses reveal that the vascular bundle of oil palm trunk contain a high percentage of crystallinity index compared to parenchyma.**

***Keywords*—oil palm trunk, parenchyma, vascular bundle, chemical composition, starch, crystallinity**

I. Introduction

Oil palm industry produce a large quantity of biomass in the form of empty fruit bunches, palm kernel shells, fronds, trunks, fibre, leaves and effluent. The availability of the oil palm trunks was only when the economic life-span of the palm reached its maturity limits that are 25-30 years. After the oil palm becoming old, the palm tree will be cut down so that the replanting plan can be started. Throwing away the trunks give a slow breakdown of the material. The freshly felled trunks cannot be easily burnt due to the presence of the high amount of moisture in the trunks and leaving the trunks in the plantation area without further processing will hinder the replanting process. Oil palm trunk normally takes about five to six years to root and mineralize. At this time, the left trunks will become the habitat and breeding ground for the oil palm pest. Physical removal of these unwanted trunks to the dump sites is costly due to the weight of freshly felled trunk which is about 3 to 4 times its dry weight (Husin et al., 1986).

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The height of matured oil palm trunk tree measured between 7 m to 13 m and 45 cm to 65 cm in diameter and 1.5 meter above the ground level (Husin et al., 2000). As a monocotyledonous species, oil palm trunk does not have cambium, secondary growth, growth rings, ray cells, sapwood and heartwood or branches or knots. Thus, the growth and increase in diameter of the trunks is due to the overall cell division and cell enlargement in the parenchymatous ground tissues, together with the enlargement of the fibers of the vascular bundles (Sulaiman et al., 2012).

The trunk is divided into three main zones which are cortex, periphery and central. The outer part of the trunk is covered by a narrow cortex with approximately 1.5 -3.5 cm in wide. This part is largely composed of ground parenchyma with numerous strands of small and irregular shaped fibrous strands and vascular bundles (Killman and Lim, 1985; Sulaiman et al., 2012). In the periphery region, it consist of narrow layers of parenchyma and congested vascular bundles that generate rise to a sclerotic zone which provides the main mechanical support for the palm. The central region that covered with 80 % of the total area of the trunk is composed of vascular bundles.

The high moisture content of the trunks is due to the distribution of the parenchymatous cell which holds more moisture than vascular bundles. Mainly these tissues are more abundant towards the apex of the palm trunk as well as radially from the periphery towards the centre pithy region. There is a wide variation of density values at different parts of the oil palm trunk due to its monocotyledonous nature. The variation in density along the cross-section of the trunk indicates that the highest mean density is at peripheral zones and decrease gradually towards the central core. This is due to the high concentration of vascular bundles that can be found in the peripheral zones which decrease gradually towards the central zone (Lim and Khoo, 1986).

Vascular bundles are slightly larger and widely scattered. They are embedded in the thin-walled parenchymatous ground tissues. The size of the vascular bundles tends to increase and more widely scattered towards the core of the trunk. Killman and Lim (1985) and Abe et al., (2013) also recorded that the vascular bundle is less hygroscopic, dense and fibrous meanwhile the parenchyma cells are naturally spongy and high capacity in water absorption to store in the living cells

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containing sugars and starch useful for energy and livestock foods.

Characterization of oil palm trunk parenchyma and vascular bundle has not been elucidated clearly, and only small number of study had been done in this topic. Mhd Ramle et al., (2012) characterized the parenchyma and vascular bundle of oil palm trunk with respect to storage time of inner, middle and outer part of the trunk. Prawitwong et al., (2012) studied the efficient production of bioethanol from the separated parenchyma and vascular bundle. Meanwhile, Abe et al., (2013) focused on the ratio estimation of vascular and parenchyma using the Near Infrared (NIR) spectroscopy. The objective of this study therefore was to provide more information and data on the chemical, morphological, crystallinity and thermal characteristic of the vascular bundle and parenchyma of old oil palm trunk. The data collected will be useful and needed for a certain aspect of utilization such as in the isolation of micro/nano cellulose and bioethanol production.

II. Experimental

A. Preparation of samples

Samples of old oil palm trunks were obtained from a plantation in Kuala Selangor were sawn into a disc and the bark was removed. The samples were then chopped and dried before being segregated manually to obtain the vascular bundles and parenchyma. The percentage ratio of vascular bundle and parenchyma of oil palm was identified. About 25 g of oil palm trunk samples was weighed before being segregated to parenchyma and vascular bundle. They were then dried in an oven at a temperature of 105° C for 24 hr. Their weight was calculated based on oven-dried method. The parts were then segregated manually to obtain the vascular bundles and parenchyma.

B. Evaluation on chemical components

The chemical components of the oil palm trunks vascular bundles and parenchyma was investigated. Preparations of extractive free samples were done according to TAPPI 204 cm-97 (TAPPI, 1997) with a modification of the solvent ethanol-toluene ratio of 2:1. Lignin content was evaluated according to TAPPI 222 om-02 (TAPPI, 2002). The holocellulose content was performed by the method of Wise et al., (1946), meanwhile the cellulose content was extracted from the percentage of holocellulose with 17.5% sodium hydroxide. Total starch content was carried out using a total starch kit (Megazyme International Ltd, Bray, Ireland). Each measurement was conducted in triplicate, and the recorded data were found to be reproducible.

C. Microstructure study

The morphological properties of the vascular bundle and parenchyma cells were studied using scanning electron microscopy (SEM). The SEM micrographs of vascular bundle and parenchyma were viewed by a LEO Supra 50 Vp field emission scanning microscope (FESEM) with ultra-high resolution. All samples were gold-sputtered using sputter coater model Polaron SC 515 ± 20 nm to avoid charging.

D. Spectroscopic Study by Fourier Transform Infrared

The functional groups present in parenchyma cells and vascular bundle fibers were measured by FT-IR Spectroscopy. The pellets were prepared by mixing approximately 5 mg of particles samples and mixed with 95mg of finely ground KBr before pressed into a small disc about 1 mm thick. Spectrums were viewed using a Nicolet infrared spectrophotometer (Avatar 360 FT-IR E.S.P) machine where the samples will be run under infrared light. The spectra recorded are transmittance mode between wave numbers of 4000cm^{-1} and 500cm^{-1}.

E. X-ray Analysis

Structural and phase analyses of the samples were measured using an X-ray diffractometer with CuKα radiation (wavelength of 1.5406 Å) generated at operating voltage and current of 40 kV and 30 mA, respectively. The CuKα radiation was filtered electronically with a Ni-filter. A 2θ angle range from 5° to 50° in reflection mode was scanned at 2°/min. The crystallinity index was calculated according to Segal method (Segal et al. 1959). The crystallinity index (C_{Ir}) is defined by:

$$C_{Ir}\ (\%) = (I_{crytsalline} - I_{amorphous}) / I_{crystalline} \times 100 \qquad (1)$$

where $I_{crystalline}$ is the peak intensity corresponding to crystalline and $I_{amorphous}$ is the peak intensity of the amorphous fraction.

III. Result

A. Parenchyma and Vascular Bundles Percentage Ratio

Fig. 1 displayed the ratio of parenchyma to the vascular bundle. It can be seen that the oil palm trunk were high in the vascular bundle compared to parenchyma. From the figure, almost 62 % of oil palm trunk is composed of the vascular bundle with the remaining percentage is parenchyma. This is in accord with the findings of Imamura (1990) and Mhd Ramle et al, (2012), that parenchyma was detected higher in the inner part of the trunk followed by middle and outer part of the oil palm trunk. The vascular bundle was found to be randomly scattered and embedded in parenchymatous tissue. Since oil palm trunk taken was as a whole part which is a mixture of inner, middle and outer part, the percentage ratio obtained showed a similar pattern when compared to Mhd Ramle et al., (2012) findings.

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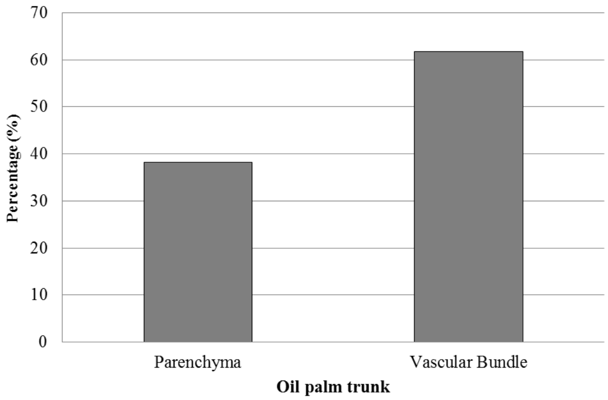

Figure 1. Percentage ratio of parenchyma to the vascular bundle

B. *Chemical Composition Analysis*

Table 2 showed the chemical compositions of the parenchyma cells and vascular bundle fibers. The parenchyma cells contain high extractives (19.05 %) and lignin content (20.71 %) compared to vascular bundle with 15.33 % of extractives and 11.91 % of lignin. Meanwhile, the vascular bundle was high in α-cellulose content that is 56.91 % and 25.47 % of hemicelluloses. As can be seen from Fig. 2, parenchyma contains high extractives, lignin and hemicelluloses compared to the vascular bundle which is high in α-cellulose content.

As the vascular bundle main component is fibers, vessels, protoxylem, sieve tubes, axial parenchyma, stegmata and companion cells (Abe et al., 2013), the α-cellulose is expected to be high in vascular bundle fibers. The vascular bundles provide the main mechanical support as well act as transportation system; therefore, α-cellulose content was found higher in vascular bundle compare to parenchyma. High cellulose content also gives strength and stability to vascular bundle cell walls. When pulled under a density, fiber containing high cellulose will create higher tensile strength.

TABLE I. CHEMICAL COMPOSITION OF PARENCHYMA AND VASCULAR BUNDLE OF OIL PALM TRUNK

Oil palm trunk	Extractives (%)	Lignin (%)	Holo-cellulose (%)	α-cellulose (%)	Hemi-cellulose (%)
Parenchyma	19.05 ±1.02	20.71 ±1.45	77.38 ±0.34	41.81 ±2.37	35.57
Vascular Bundle	15.33 ±0.63	11.91 ±0.54	82.38 ±0.21	56.91 ±0.99	25.47

Fig. 3 displayed amount of starch presence in parenchyma and vascular bundle of oil palm trunk. The starch accumulated high in parenchyma that is 17.71 % while in the vascular bundle only 7.14 %. The data collected is comparable and considered within the range with the other researchers (Hashim et al., 2010; Prawitwong et al., 2012) to the result by another researcher. These suggested that the starch can be found mainly in parenchyma rather than vascular bundle. It was supported by the SEM images in Figure 6 (a) and (b). Abundant amount of parenchymatous tissue that rich in starch accumulates in peripheral cortex that is the middle part of oil palm trunk.

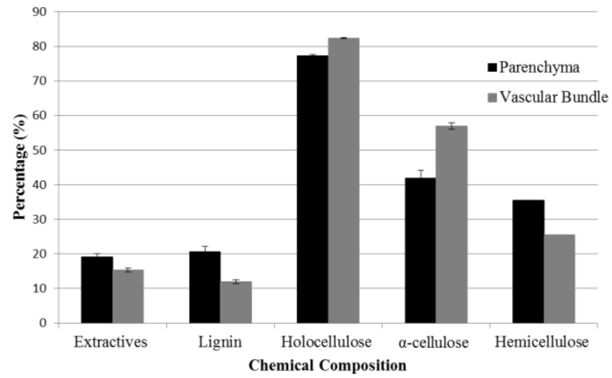

Figure 2. Chemical composition of parenchyma and vascular bundle of oil palm trunk

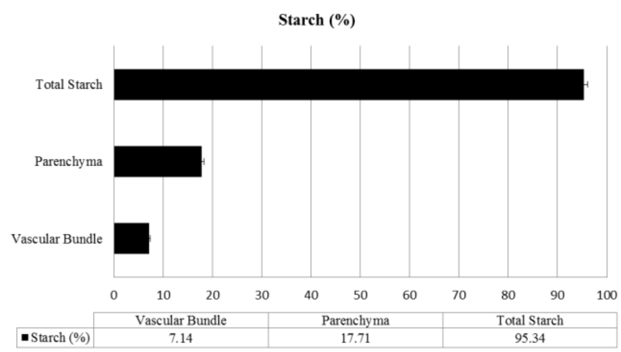

Figure 3. *Starch content of parenchyma and vascular bundle of oil palm trunk*

C. *Fourier Transform Infrared Analysis*

The spectra of the parenchyma and vascular bundle of oil palm trunk were displayed in Fig. 4. As a lignocellulosic material, oil palm trunk composed mainly of cellulose, hemicelluloses, lignin and starch. The three constitutes are mainly composed of alkanes, esters, aromatics, ketones and alcohols, with different oxygen-containing functional groups (Abraham et al., 2011). Parenchyma and vascular bundle presented two main transmittance regions. The band at 3407 and 3424 cm^{-1} represents the stretching vibrations of –OH group due to the moisture content of hydroxyl found in cellulose, hemicelluloses and lignin (Hsu, 1997).

The band at 2926 cm^{-1} of parenchyma and 2923 cm^{-1} of the vascular bundle represent the C-H stretching, which associated with methylene groups in cellulose (Hsu, 1997).

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The band present at 1730 -1740 cm^{-1} in the spectrum of parenchyma and vascular bundle corresponding to the C=O linkage that exist in lignin and hemicelluloses (Abraham et al., 2011). The –OH bending of adsorbed water was recorded at 1640 cm^{-1}. The peak of parenchyma shows an increase in intensity compared to the vascular bundle could be due to the increasing of –OH concentration in starch, hemicellulose and α-cellulose. The spectra recorded were similar with the previous research done by Mhd Ramle et al., (2012).

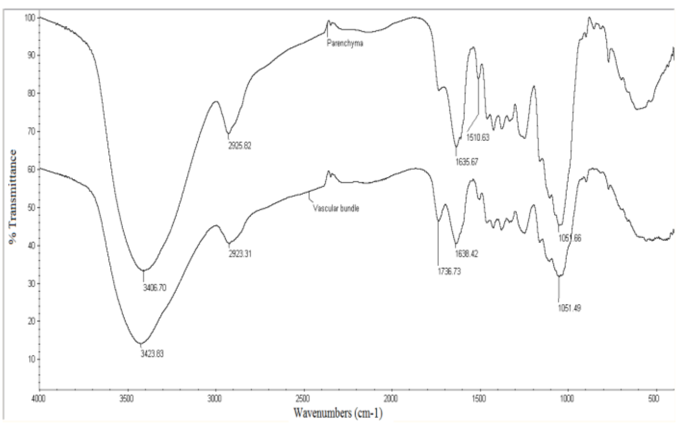

Figure 4. Infrared spectra of parenchyma and vascular bundle of oil palm trunk

D. *X-ray Diffraction Analysis*

Fig. 5 shows the X-ray diffraction analysis of the parenchyma and vascular bundle of oil palm trunk. The fiber constitutes crystalline and amorphous regions. According to Abraham et al., (2011), crystalline cellulose components are oriented in the matrix of lignin, hemicelluloses, pectin and others in the raw fiber. The XRD graph showed that the parenchyma and vascular bundle are crystalline nature. The cellulose present in parenchyma and vascular bundle are cellulose type 1 which is the crystal form of native cellulose.

Table 2 shows the values of crystallinity index obtained in the parenchyma and vascular bundle calculated based on the Equation 1. We can see that the crystallinity index percentage was high in the vascular bundle (76.78 %) compared to the parenchyma (69.70 %). This agrees with the values of cellulose content determined in these samples as shown in Table 1.

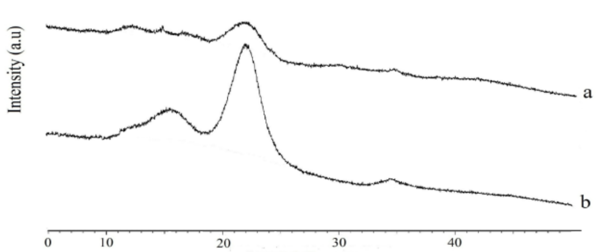

Figure 5. XRD curve of (a) parenchyma and (b) vascular bundle of oil palm trunk

TABLE II. THE CRYSTALLINITY INDEX OF PARENCHYMA AND VASCULAR BUNDLE OF OIL PALM TRUNK

Oil palm trunk	Crystallinity index (%)
Parenchyma	69.70
Vascular bundle	76.78

E. *Microscopic Study*

The SEM micrographs of parenchyma cells and vascular bundle fibers are displayed in Fig. 6. As a carbohydrate storage, the starch granules that are embedded in the lumen of the cells can be seen clearly in parenchyma in Fig. 6 (a) and (b). Most of the starch granules were in the oval shape laminated together in the lumen cells still intact. The impurities were found in the surface of the vascular bundle as shown in Fig. 6 (c) and (d). As can be seen in the micrographs, the parenchyma cells are very thin thus considered as feeble cells when compared to the thick walls of fiber cells. Past researchers suspected that the cell shape of parenchyma cell was a result of surface tension and/or pressure from surrounding cells (Mauseth, 1988; Pruyn, 2007). They also add that under total isolation, cells should assume a spherical shape or geometrically perfect, however shape cells can be flattened, elongate, curved or in stellate shapes without the influence of physical forces of pressure or surface tension.

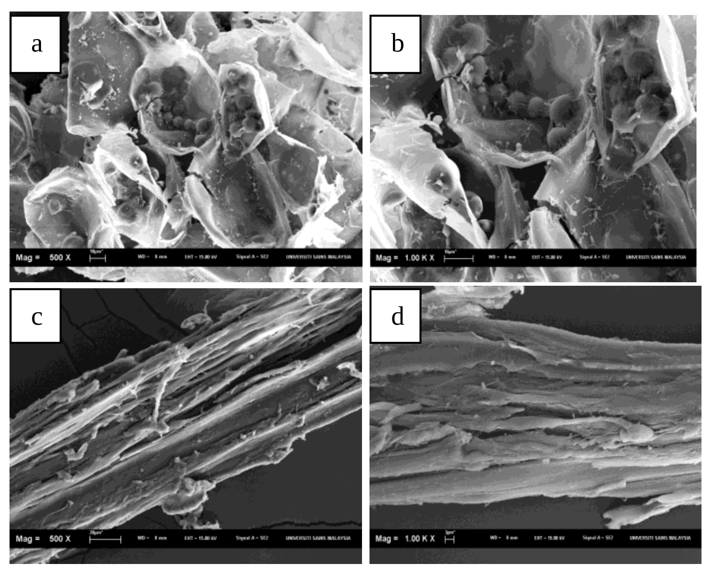

Figure 6. SEM micrograhs of (a and b) parenchyma and (c and d) vascular bundle surface of oil palm trunk

IV. Conclusion

Percentage ratio displays that oil palm trunk was high in the vascular bundle. The chemical composition showed that parenchyma is higher in extractives, lignin, hemicelluloses and

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starch content compared to the vascular bundle that is higher in α-cellulose. The FTIR analysis and morphological analyses support the chemical composition value of the parenchyma and vascular bundle. The XRD graph revealed that the vascular bundle had a higher percentage of crystallinity index compared to parenchyma that is supported with the cellulose content determined in the chemical composition studies.

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Junidah Lamaming is a postgraduate student in Universiti Sains Malaysia, Penang, Malaysia. Currently, she is doing her doctoral degree in field of micro/nano cellulose from oil palm trunk. The isolated cellulose later will be used as raw material for medical applications.