

**LAPORAN AKHIR PROJEK PENYELIDIKAN JANGKA PENDEK**  
**FINAL REPORT OF SHORT TERM RESEARCH PROJECT**

Sila kemukakan laporan akhir ini melalui Jawatankuasa Penyelidikan di Pusat Pengajian dan Dekan/Pengarah/Ketua Jabatan kepada Pejabat Pelantar Penyelidikan

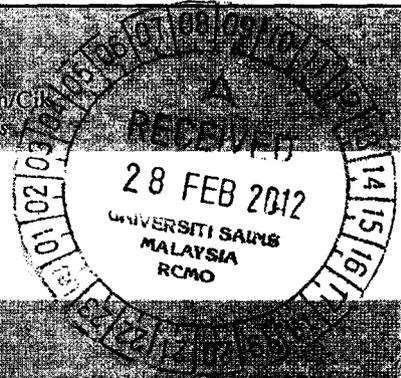
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4. Tajuk Projek: Fundamental Studies of Cell Wall Structure From Selected Agro-Wastes Fibers  
*Title of Project*

5. Ringkasan Penilaian/Summary of Assessment

Tidak  
 Mencukupi  
*Inadequate*

Boleh  
 Diterima  
*Acceptable*

Sangat Baik  
*Very Good*

1 2 3 4 5

i) Pencapaian objektif projek:  
*Achievement of project objectives*

ii) Kualiti output:  
*Quality of outputs*

iii) Kualiti impak:  
*Quality of impacts*

iv) Pemindahan teknologi/potensi pengkomersialan:  
*Technology transfer/commercialization potential*

v) Kualiti dan usahasama :  
*Quality and intensity of collaboration*

vi) Penilaian kepentingan secara keseluruhan:  
*Overall assessment of benefits*

8. Output dan Faedah Projek  
Output and Benefits of Project

(a) Penerbitan Jurnal  
Publication of Journals

(Sila nyatakan jenis, tajuk, pengarang/editor, tahun terbitan dan di mana telah diterbitkan/dicetak)  
(State type, title, author/editor, publication year and where it has been published/printed)

Abdul Khalil, H.P.S., Siti Alwani, M., Mohd Omar, A.K. (2006). Chemical Composition, Anatomy, Lignin Distribution and Cell Wall Structure of Malaysian Plant Waste Fibers. *BioResources* 1(2), 220-232

Abdul Khalil, H.P.S., Siti Alwani, M., Mohd Omar, A.K. (2007). Cell Wall Structure of Various Tropical Plant Waste Fibers. *Mokkehae Konghak* 35(2), 9-15

(b) Faedah-faedah lain seperti perkembangan produk, pengkomersialan produk, pendaftaran paten atau impak kepada dasar dan masyarakat.  
State other benefits such as product development, product commercialisation/patent registration or impact on source and society

Anatomy, chemical composition, fiber dimension, lignin distribution and cell wall ultrastructure properties and information are very important knowledge about the possibility of using agro-wastes as a material in biocomposite, pulp and paper, cellulose production, food additives, natural pharmaceutical products, paints and varnishes production and etc. These fundamental information of agro-wastes fiber are required and needed in this area to elucidate knowledge/information to food technologists, material scientist and polymer chemist for further applied research study.

(c) Latihan Sumber Manusia  
Training in Human Resources

i) Pelajar Sarjana  
Graduates Students

(Perincikan nama, ijazah dan status)  
(Provide names, degrees and status)

Siti Alwani Mohamed - MSc - Tamat  
Ireana Yusra - MSc - Tamat

ii) Lain-lain:  
Others

9. Peralatan yang Telah Dibeli:  
Equipment that has been purchased

Tiada



Tandatangan Penyelidik  
Signature of Researcher

28/02/12

Tarikh  
Date

## Cell Wall Structure of Various Tropical Plant Waste Fibers\*<sup>1</sup>

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### ABSTRACT

A comparative study of the structure and organization of the primary and secondary walls in different types of tropical plant waste fibers was carried out using transmission electron microscopy (TEM). The thickness of each layer was also measured using Image Analyzer. TEM micrographs have confirmed that cell wall structure of all six types of tropical plant waste fibers (empty fruit bunch, oil palm frond, oil palm trunk, coir, banana stem and pineapple leaf) has the same ultrastructure with wood fibre. The fibers consisted of middle lamella, primary and thick secondary wall with different thickness for different types of fibers. The secondary wall was differentiated into a  $S_1$  layer, a unique multi-lamellae  $S_2$  layer, and  $S_3$  layer.

*Keywords* : oil palm, banana stem, coir, pineapple leaf, cell wall structure, transmission electron microscopy

### 1. INTRODUCTION

One unique plant cell structure is in the cell walls that are formed from cellulose in fibril form. Cell wall is said as non living entity that supports protoplast and gives shape to the cells form other than strengthens the cells. Cell wall is dynamic structure that grows up and has the ability to change its composition and shape. This structure plays an important role in plant production, communication among cells, physiology and environment adjustment (Dickison 2000).

Woody cell walls are composed of cellulose,

hemicelluloses, lignin and pectic substances as main components. Each of these has been intensively studied for many years (Harada 1964; Donaldson 1992; Koch & Kleist 2001; Morvan *et al.*, 2003). All of these research agreed that the wood wall cell consisted of primary and secondary layers. It consists of outer layer ( $S_1$ ), middle layer ( $S_2$ ), and inner layer ( $S_3$ ) with different microfibril orientation. Nevertheless the knowledge of their biosynthesis, structure, and properties as well as their three dimensional assembly in the cell wall is not well understood. Research on this topic is difficult because of the diverse ultrastructure of cell walls between spe-

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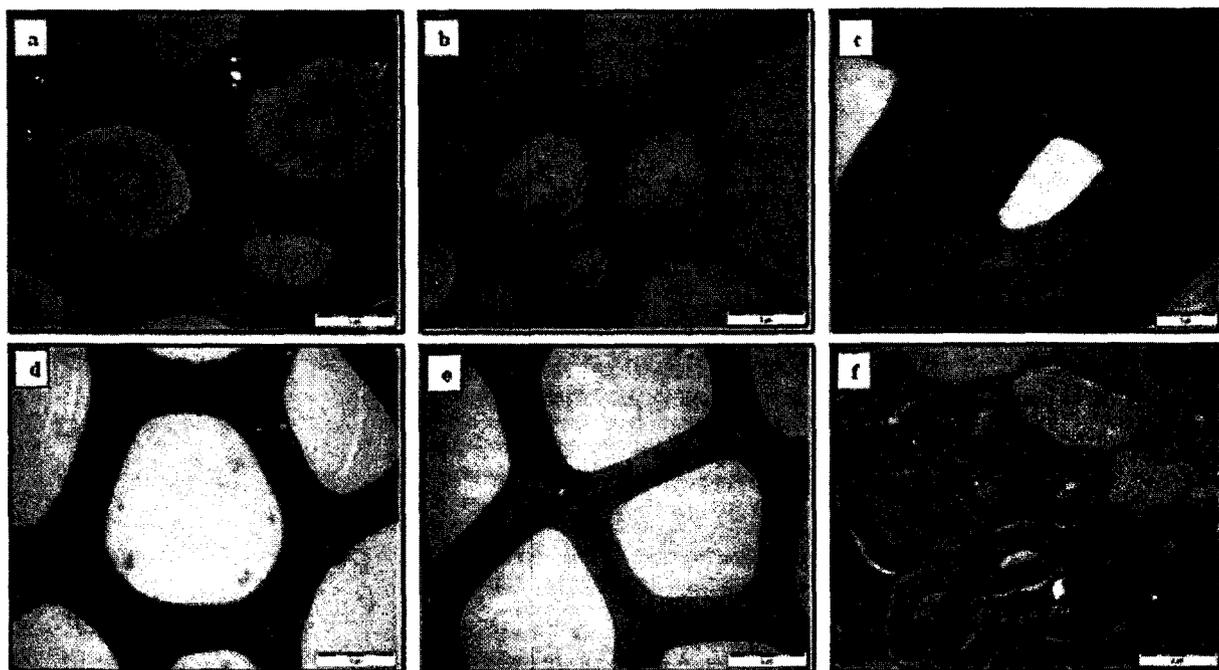


Fig. 1. Transmission electron micrograph of ultrathin sections of various types of plant fibers. a) EFB (3400 $\times$ ); b) OPF (3400 $\times$ ); c) OPT (2600 $\times$ ); d) Coir (3400 $\times$ ); e) BS (3400 $\times$ ); f) PALF (3400 $\times$ ). a~f (5  $\mu$ m).

scopic observations were restricted mainly to the walls of fibers within the vascular bundles. Generally, plant fibre shows great variability in size, shape, and cell wall structure (Fig. 1 and Fig. 2). All of these fibre structures are almost round in shape except for banana stem fibre with polygonal in shape (Fig. 1e). Fibre wall is very thick especially in OPT fibre (Fig. 1c).

In composite technology, these structures will also give high toughness cause by weak interface between cell wall layers that is support by Gordon-Cook Theory. Strengthening mechanism in certain composite depends on the stress transfer from the matrix substance (resin) to the fibre. According to this theory, in fiber reinforced composites, the interface area acts as stopping cracks that build the toughness of the composites.

### 3.2. Primary Wall

The primary cell wall is a thin layer produced by cell division and the subsequent growth of xylem mother cells. The wall layers (primary and secondary walls) clearly distinguish in ultrathin transverse sections of fibers. The primary wall of plant fibers appeared as a solid boundary of the cell. The middle lamella which glues the adjacent cells together showed clear transition to the adjacent primary wall layers (Fig. 2a, 2c & 2e). The thickness of the layer that has been measured randomly showing that thickness of primary wall has the range between 0.11~0.47  $\mu$ m. OPT fibre is found to have the thickest primary wall (Fig. 2c) while coir has the thinnest primary wall.

Certain primary wall can be differentiated clearly between middle lamella but there are some cells that have both cell layers that were hard to differentiate (Fig. 2d). Usually lamella-

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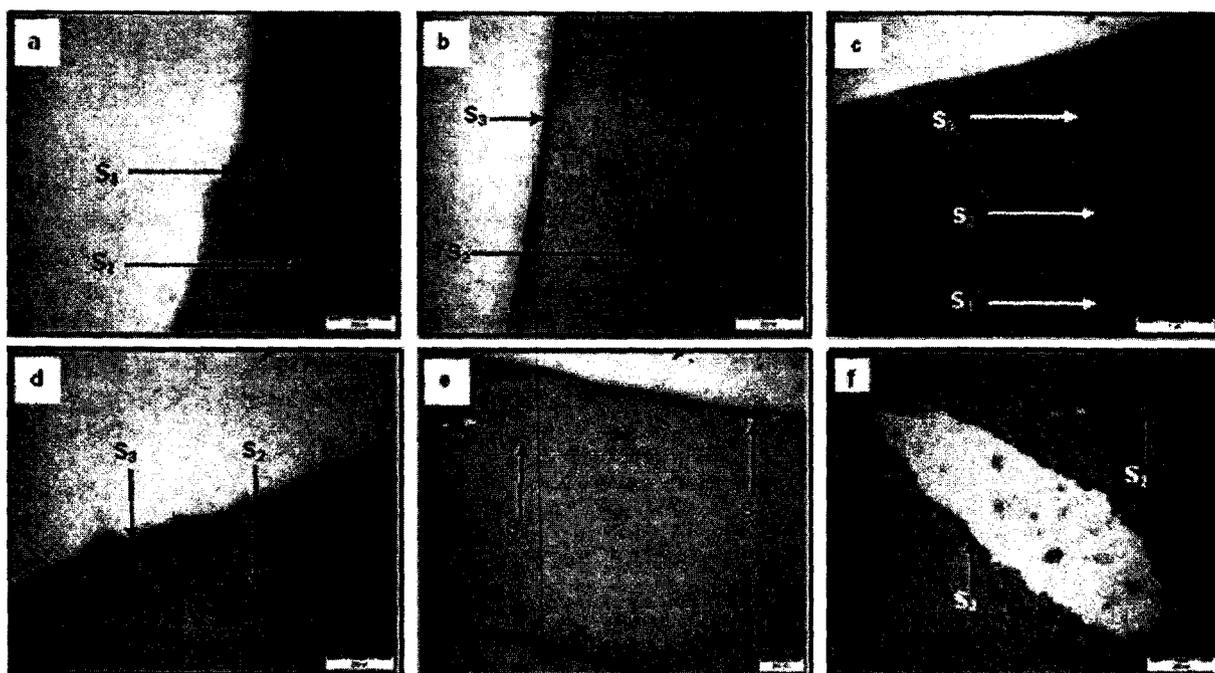


Fig. 3. Transverse section of a multi-layered fiber wall at high magnification showing secondary wall layers a) EFB (75 000 $\times$ ); b) OPF (75 000 $\times$ ); c) OPT (18 000 $\times$ ); d) Coir (75 000 $\times$ ); e) BS (45 000 $\times$ ); f) PALF (75 000 $\times$ ). a, b, d, e, & f (200 nm); c (1  $\mu$ m). S<sub>1</sub> S<sub>2</sub> & S<sub>3</sub> S<sub>3</sub> = outer, middle, and inner layers of secondary wall.

lulose microfibril that is aligned parallel to fibre axis on S<sub>2</sub> layer. This fibre strength is presumed in parallel with the stem's needs to support the weight and height of the tree plus to ensure the efficiency of water transportation and nutrient from the roots to the upper part of the tree.

S<sub>3</sub> layer plays an important role in a few processes such as pulpan and paper making and genetics engineering but this layer has received less attention because of the difficulties in studying them. Observation acquired in this research show proof of the existence of the different S<sub>3</sub> layer in plant fibre (Fig. 3a~3f). TEM micrographs show that the different plant fibre has different S<sub>3</sub> thickness. Previous study has reported that *P. radiata* tracheid also shows variability in the thickness of S<sub>3</sub> layer (0.06~0.3  $\mu$ m) (Singh *et al.*, 2002). They suggested that an irregular thickness of S<sub>3</sub> layer is maybe suited

to relieve the pressure of the axial compression force on the tracheid wall than that of uniform thickness. OPT fibre has the thickest S<sub>3</sub> layer (2.37  $\mu$ m) compared to other plant fibre (Fig. 3c). Therefore, this fibre is believed to have more resistance to collapse caused by water tension and buckling. Water from the ground that is taken by roots in trees has to reach certain height, especially in tall forests trees to reach the crown. The force needed to take water to these heights can produce tremendous tension in plant cell wall. S<sub>3</sub> layer is said to have microfibril angle perpendicular to S<sub>2</sub> microfibril angle so this layer can minimize the effect of tension towards S<sub>2</sub> layer and all walls during the water transportation. Being the innermost layer, the S<sub>3</sub> layer also can be a barrier to some chemical treatment process such as pulping, preservation, and modification (Blanchette *et al.*, 1990).

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harvesting (MAO, 2006; MPOB, 2001). The explosive expansion of plantation in this country has generated enormous amounts of vegetable waste, creating problems in replanting operations and tremendous environmental concerns.

When left on the plantation floor, these waste materials create great environmental problems (Sreekala et al. 1997; Reddy and Yang 2005). Therefore, economic utilization of these fibers will be beneficial. In spite of the abundance of published work dealing with the technological aspects of the agro-fibers applications, the bibliography covering comprehensive fundamental aspects of specific agro-fibers is quite scarce, disperse, and inadequate. In order to completely evaluate the potential of agro-wastes for new applications, a detailed and comprehensive study of fundamental properties is necessary.

The fundamental aspects considered in the previous literature, with other fibers, have been reported extensively (Balashov et al. 1956; Fengel and Shao 1984; McNeil et al. 1984; Bai et al. 1998; Donaldson 1996), except for oil palm, banana stem, and pineapple leaf fibers. Only the chemical composition and anatomy of these fibers have already been reported (Mansor and Ahmad 1991; Cordeiro et al. 2004; Mishra et al. 2004; John et al. 2005). However, no research has been reported to evaluate the cell-wall structure, lignin distribution, and physical properties of these major plant wastes fibers (oil palm, coir, banana stem, pineapple leaf) in Malaysia until now.

The main objective of the present study is, therefore, to make some observations on the fine structure of plant fibers, using light and electron microscopy. The research findings can lead to a better understanding and knowledge of chemical compositions, fiber dimensions, cell wall structure, and lignin distribution of the fibers. This information is very important to reduce the environmental and health hazards associated with the disposal of plant wastes such as oil palm, banana tree, and pineapple leaf fibers. Hence, this basic studies/knowledge can be used by the food technologist, material scientist, and polymer chemist for further applied research study.

## EXPERIMENTAL

For chemical composition determination, agro-fibers were ground, and 40-mesh fractions were selected. The procedures were performed according to TAPPI Method T 264 om-88. The samples were first submitted to Soxhlet extraction with ethanol/benzene [1:2 (v/v)] for 6 hours. The determination of alpha-cellulose, lignin, and ash content were performed following the standard methods T 212 om-93, T 203 os-74, and T 211 om-93, respectively. Holocelluloses were determined according to a previous study (Wise et al. 1946).

For cell wall structure and lignin distribution determinations, agro fibers were chosen randomly and cut into 2 × 3 mm blocks. Samples were then dehydrated in an ethanol series and embedded in Epoxy resin (Epon), which was polymerized for 24 hours at 60°C. Transverse sections (1 μm) were cut from embedded material, using a Sorvall ultra microtome (MT 500) and stained with 1% Toluidine Blue for lignin distribution determination. The sections were viewed under polarized microscope (Olympus BX50). Ultra-thin sections (0.1 μm) also were obtained from embedded samples, stained with 2%

Coconut palm (*Cocos nucifera* L.) (Fig. 1b) is one of the most important crops in Malaysia. Statistics showed that total area planted of coconut palm increased from 117 000 (1998) to 147,000 hectares in 2004 (MAO 2006). Coir fiber is obtained from coconut husk. Coir fiber is one of the hardest natural fibers, because of its high content of lignin.

The pineapple (*Ananas comosus*) (Fig. 1c) is one of the important tropical fruits in Malaysia, which was produced in economic quantities on about 14,042.9 hectares in 2001 (MAO, 2006). Pineapples are rosette-forming, herbaceous monocots. The stems are short and inconspicuous in the center of the rosette of long and linear leaves.

Bananas (Musaceae) (Fig. 1d) are produced in large quantities in tropical and subtropical areas. The total planted area of banana in Malaysia (2001) was 33,704.2 hectares (MAO, 2006). Banana plants range in height from 0.8 m to more than 15 m. Each contains a flattened, modified stem, called a pseudostem, consisting of concentric layers of leaf sheath and crown of large leaves (Ennos et al., 2000). After harvesting fruit, the pseudostem is traditionally wasted, as it usually left in the soil plantation to be used as organic materials.

### Chemical Composition

Table 1 shows the percentage of various chemical components present in OPF, COIR, PALF, and BS fiber. The data show that BS fibers exhibited the highest solubility in ethanol-benzene (10.6%), compared to other fibers. As for wood processing, higher ethanol-benzene extractive content in BS may be advantageous for decay resistance and will provide good strength in fiber processing, because of its higher specific gravity.

**Table 1.** Chemical composition of different lignocellulosic fibers

	Oil palm frond	Coconut	Pineapple leaf	Banana stem	Softwood*	Hardwood*
Extractive (%)	4.5	6.4	5.5	10.6	0.2-8.5	0.1-7.7
Holocellulose (%)	83.5	56.3	80.5	65.2	60-80	71-89
$\alpha$ -cellulose (%)	49.8	44.2	73.4	63.9	30-60	31-64
Lignin (%)	20.5	32.8	10.5	18.6	21-37	14-34
Ash (%)	2.4	2.2	2.0	1.5	<1	<1

\* Tsoumis 1996

Generally, coir fibers contained the highest percentage of lignin (32.8%), but the lignin content of coir fiber was still lower than that of wood fiber (14-37%) (Tsoumis 1991). Generally, the high content of lignin in coir fiber made the fiber tougher and stiffer, compared to other fibers. This was because lignin provides plant tissue and individual fibers with compressive strength and stiffens the cell wall of the fibers, to protect the carbohydrates from chemical and physical damage (Saheb and Jog 1999). Lignin is an undesirable polymer, and its removal during pulping requires high amounts of energy and chemicals. PALF fibers had the lowest lignin content, which suggests that this material can undergo bleaching more easily and with the utilization of lower amounts of chemicals than coir fibers.

### Anatomical Characteristics

OPF fibers contained various sizes of vascular bundles. The vascular bundles were widely imbedded in thin-walled parenchymatous ground tissue. Each bundle was made up of a fibrous sheath, vessels, fibers, phloem, and parenchymatous tissues (Fig. 2a). Xylem and phloem tissues are clearly distinguishable. Phloem was divided into two separate areas in each bundle. Some vascular bundles also contained several well-defined protoxylem elements. Protoxylem and metaxylem vessels in the bundle were separated by a layer of parenchyma cells (Fig. 3a). According to previous study, within the stem and leaves, proto- and metaxylem vessels are separated by at least one layer of live parenchyma cells, which form a living barrier to possible transfer of gas bubbles between proto- and metaxylem vessels (Tomlinson et al. 2001).

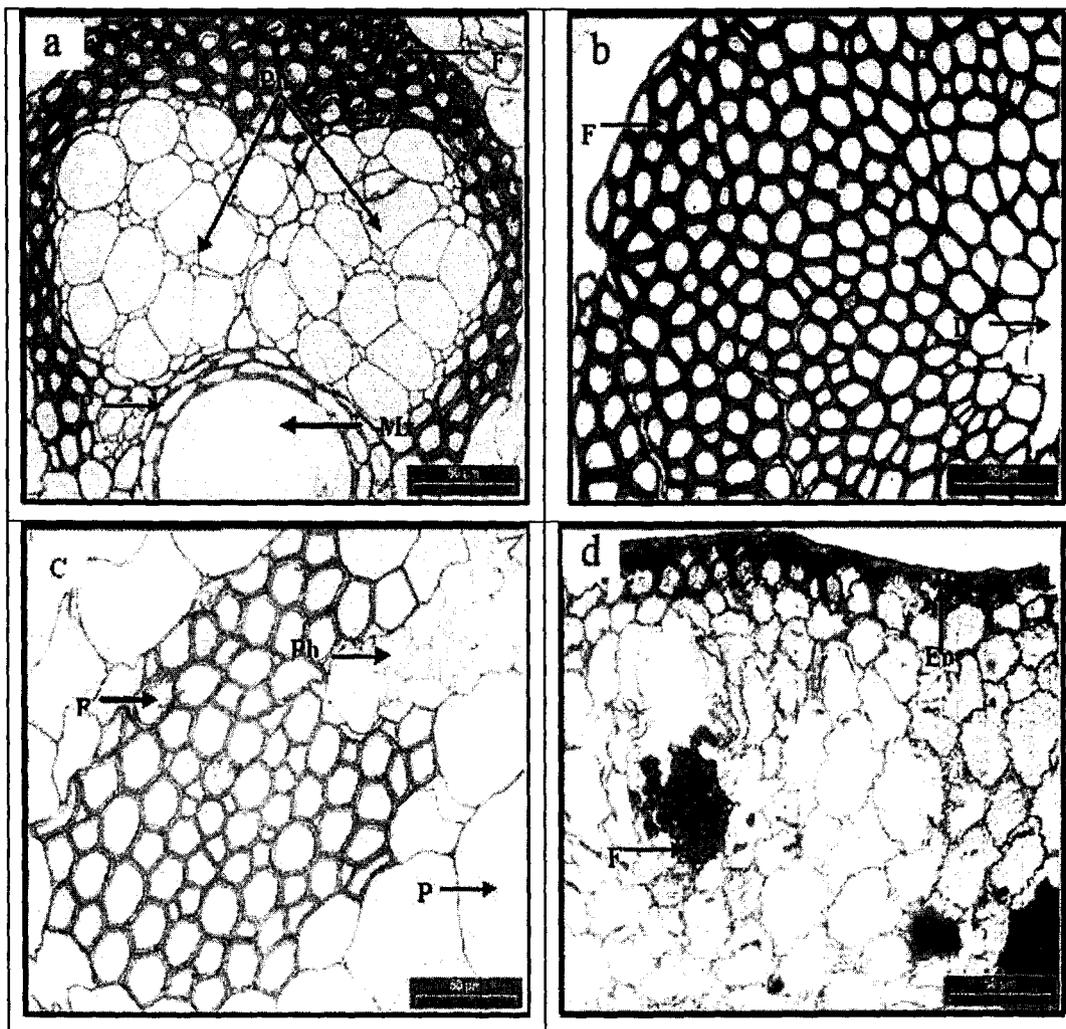


Fig. 3. Transverse section of different types of agro fibers at high magnification (20×). a) Oil palm frond (OPF) b) Coconut (COIR) c) Banana stem (BS) d) Pineapple leaf (PALF). F Fiber; P Parenchyma; Mx Metaxylem; Ph Phloem. F Fiber; L Lacuna; Ep Epidermis

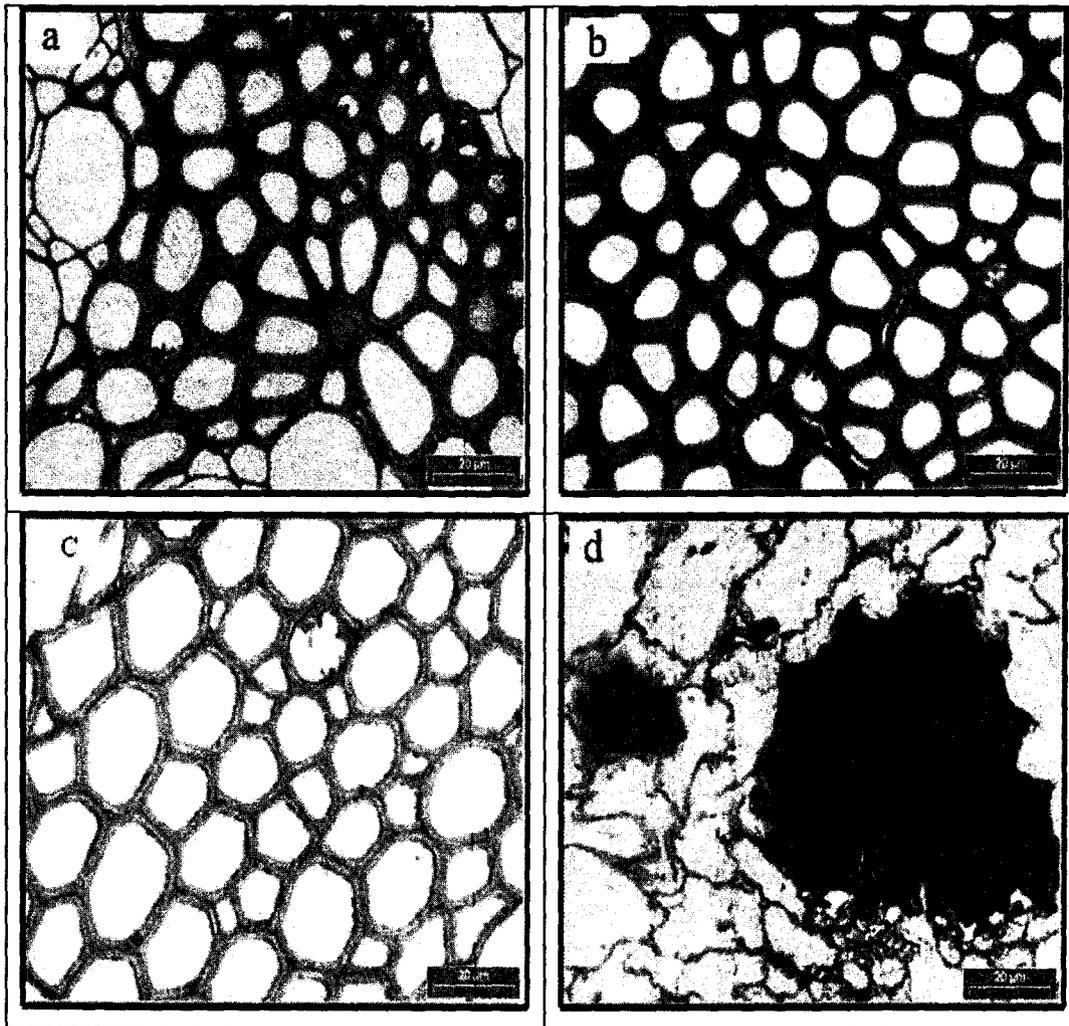
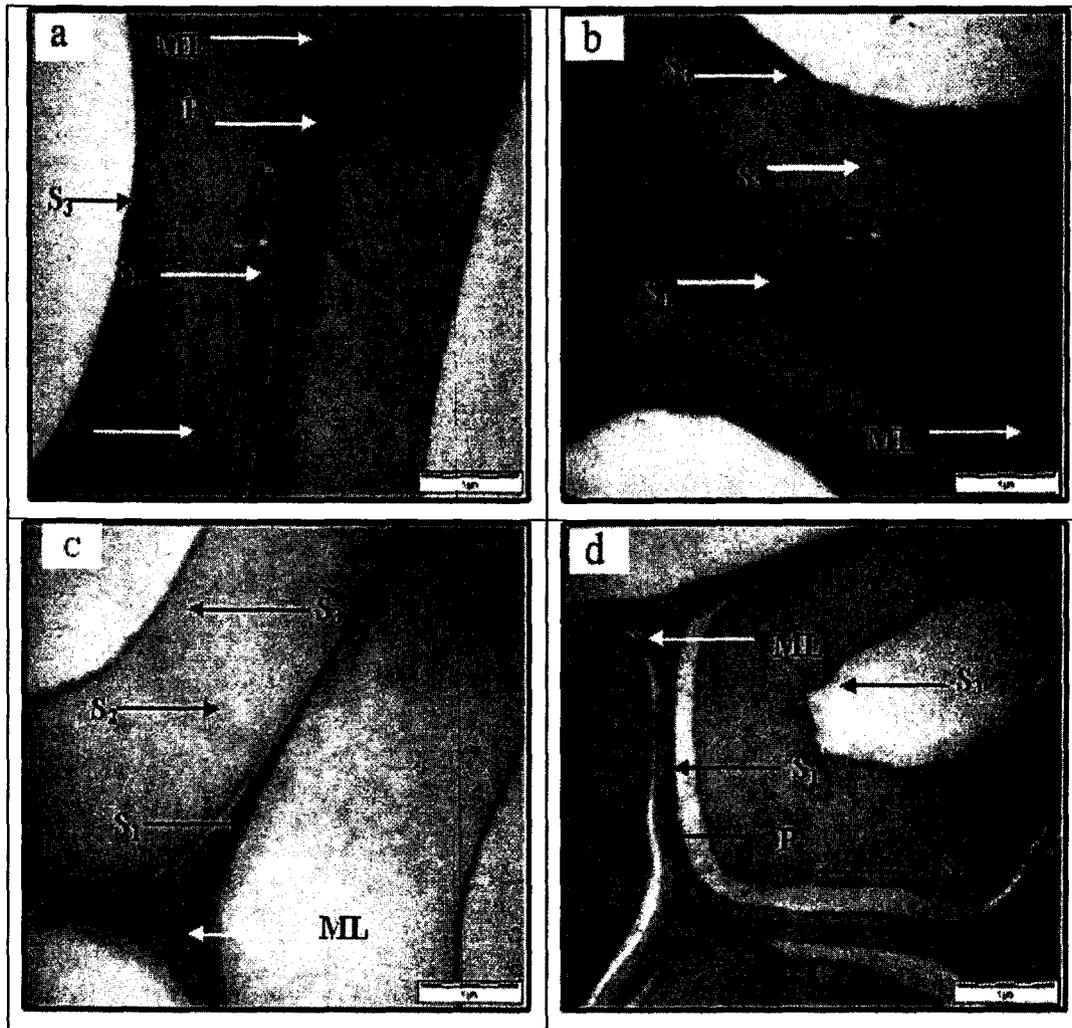


Fig. 4. Transverse section of agro fibers after stained with toluidine blue at high magnification (40 $\times$ ). a) Oil palm frond (OPF) b) Coconut (COIR) c) Banana stem (BS) d) Pineapple leaf (PALF).

Transmission electron microscopy (TEM) views of transverse sections of agro fibers are shown in Figs. 5-6. The electron microscopic observations were restricted mainly to the walls of fibers within the vascular bundles. Generally, all fibers showed great variability in size, shape, and structure of the cell wall (Fig. 5a-5d). The TEM electron micrographs have confirmed that the layered structure of OPF, COIR, PALF, and BS fiber wall contained primary (P) and secondary ( $S_1$ ,  $S_2$  and  $S_3$ ) wall layers (Fig. 6a-6d). This structure was similar to the wood cell wall structure that has been proposed by Harada and Cote (1985) and other lignocellulosic fibers such as flax, jute, *etc.* However, according to Liese (1985), bamboo showed different structure of the cell wall layer. Bamboo culm fiber has a polylamellated wall structure [middle lamella, primary and secondary wall ( $S_0$ ,  $S_{1-l}$ ,  $S_{2-t}$ ,  $S_{n-l}$ ,  $S_{n-t}$ )]. A typical tertiary wall is not present, but warts cover the innermost layer of the cell wall.

is about forty times thicker than any of the other layers (Booker and Sell, 1998). Cells with thick walls contain a large  $S_2$  layer, whereas thin-wall cells have a small  $S_2$  layer. The  $S_2$  layer of agro fibers occupied about 43-78% of the whole wall in thickness, and BS fibers exhibited the thickest  $S_2$  layer ( $1.57 \mu\text{m}$ ) (Fig. 6c).



**Fig. 6.** Transverse section of a multi-layered structure of agro fibers at high magnification (17000 $\times$ ). a) Oil palm frond (OPF) b) Coconut (COIR) c) Banana stem (BS) d) Pineapple leaf (PALF). ML Middle Lamella; P Primary Wall;  $S_1$   $S_2$  &  $S_3$  Secondary wall sub-layers

The observations using TEM micrographs also provide evidence for the presence of a distinct  $S_3$  layer in the cell wall of agro fibers. Agro fibers showed a great variability in the thickness of the  $S_3$  layer. COIR fibers (Fig. 6b) contained a well developed  $S_3$  layer, being the thickest  $S_3$  layers ( $0.089 \mu\text{m}$ ) among all fibers. COIR fibers are supposed to have more resistance against collapse due to water tension and buckling due to axial compression forces, as also proposed by Booker and Sell (1998). Previous studies have

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