Cell Wall Structure of Various Tropical Plant Waste Fibers*1

H. P. S. Abdul Khalil*2†, M. Siti Alwani*2, and A. K. Mohd Omar*2

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 haveconfirmed 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 (S₁), middle layer (S₂), and inner layer (S₃) 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-

^{*1} Received on August 25, 2006; accepted on November 23, 2006.

^{*2} School of Industrial Technology, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia.

Corresponding author: Abdul Khalil H. P. Shawkataly (akhalil@usm.my)

cies, plant tissues, cells within tissues, and even between different morphological regions of a cell wall (Grunwald *et al.*, 2002).

Major tropical plant waste (oil palm, coir, banana, pineapple, etc), have attracted worldwide attention as a potential as raw material for value-added products for food (animal feed, microcrystalline cellulose, chemical products, and derivatives) and non-food (conventional composites, polymer composites, charcoal, filters, solvents, activated carbon, pulp and paper, etc.) industry. The easy availability as a renewable (resource), easy processability, light weight, reactive surface chemistry, non-hazardous, recyclables, and bio-friendly characteristics offers a number of opportunities in forms of optimized performance, cost effectiveness, controlled biodegradability, and environmental considerations of plant wastes products.

The economic utilization of these fibers will be beneficial because these plant wastes cancreate great environmental problems. In Malaysia, with such large area of plantation oil palm (4 million ha), coconut (140 thousands ha.), banana (34 thousands ha.) and pineapple (15 thousands ha) (MOA 2006), a large quantity of cellulosics and non-cellulosic raw material generated during harvesting. The fundamental aspects considered in theprevious literature, with other fibers have been reported extensively (Balashov et al., 1956; Fengel & 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 & Ahmad 1991; Cordeiro et al., 2004; Mishra et al., 2004; John et al., 2005). Until now, however, no research reported to evaluate the cell-wall structure of these major plant waste fibers (oil palm, coir, banana stem, pineapple leaf) in Malaysia until now.

The objective of this research is to analyze the cell wall structure of six different types of plant waste fibers (empty fruit bunch, oil palm frond, oil palm trunk, coir, banana stem, and pineapple leaf) by studying the differences of these fibers in terms of structure, size, and cell wall form. These structures are very important to study on the connections and contributions of them towards the strength of these plants.

2 MATERIALS and METHODS

Small samples either fresh from living trees or from previously dried material were taken from oil palm [Elaeis guineensis] fibers (empty fruit bunch, oil palm frond & oil palm trunk), coir (Cocos nucifera L.) fiber, banana (Musa paradisiaca) from family Musaceae stem and pineapple (Ananas cosomus) leaf. After appropriate washing, all samples were not fixed in any fixative as they were dehydrated in a graded ethanol series and embedded in epoxy resin (Epon). Ultrathin sections of the epoxy-embedded samples were cut transversely with Sorvall ultra microtome (MT 500) and stained with 2% uranyl acetate and Reynolds lead citrate followed by observation with transmission electron microscopy (TEM)[Phillips CM12]. The thickness of cell wall layer was measured using Image Analyzer (SONY model SSC-DC398P).

3. RESULTS and DISCUSSION

3.1. Multilayered Structure of Fibre Cell Wall

Cell wall structure of plant fibres [empty fruit bunch (EFB), oil palm frond (OPF), oil palm trunk (OPT), coconut husk (coir), banana stem (BS) and pineapple leaf (PALF)] can be seen clearly and comprehensively using transmission electron microscope (TEM). The electron micro-

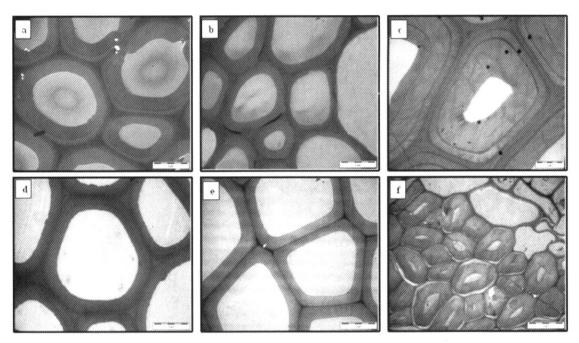


Fig. 1. Transmission electron micrograph of ultrathin sections of various types of plant fibers. a) EFB (3400×); b) OPF (3400×); c) OPT (2600×); d) Coir (3400×); e) BS (3400×); f) PALF (3400×). a~f (5 μm).

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 $0.11 \sim 0.47~\mu m$. 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-

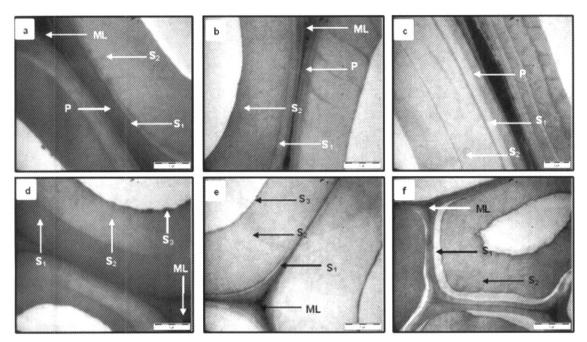


Fig. 2. Ultrathin sections showing primary and secondary wall layering. a) EFB (17 000×) (1; b) OPF (17 000×); c) OPT (6000×); d) Coir (17 000×); e) BS (17 000×); f) PALF (17 000×). a, b, d, e, & f (1 μ m); c (2 μ m). P = primary wall; ML = middle lamella; S₁ S₂ & S₃ = outer, middle, and inner layers of secondary wall.

tion in primary wall is not very clear. Primary wall that is next to middle lamella is difficult to be separated because these two layers have maybe the same chemical composition (Eames & MacDaneils 1974).

3.3. Secondary Wall

Secondary wall is a thick layer deposited inside the primary wall. It consists of S_1 , S_2 and S_3 layers. S_1 layercan be seen clearly in all TEM micrographsfor all types of fibre. Generally S_1 wall is known as the brightest layer of the wall (Fig. 2e). This is because this layer is believed to have the lowest lignin concentration in comparison with other layers. Micrographs acquired shows that S_1 layer in all types of fibre can be recognised and differentiates from S_2 layer (Fig. $2a \sim 2f$). The S_1 layer is approx-

imately $0.08 \sim 1.59~\mu m$ in width and OPT fibre have the thickest S_1 layer. Then, OPT fibre is believed to have resistance towards transwall fracture unlike other fibre. S_1 layer will limit maximum shear forces in middle lamella during axial compression by restricting the maximum diameter increase of the S_2 layer. This layeralso acts as effective protection layer towards intrawall cracksalong the border between middle lamella and S_1 layer (Booker & Sell 1998).

The S_2 layer is reinforced by microfibrils that usually lie from $5 \sim 30$ degrees to the axis and about forty times thicker than any other layers (Sjostrom 1993). Among all the plant fibre studied, OPT fibre is found to have the thickest S_2 layer that is 3.43 μ m. While coir fibre is found to have the thinnest S_2 layer. OPT fibre is estimate to have the highest strength. This is becausethe fibre strength is dependent on the cel-

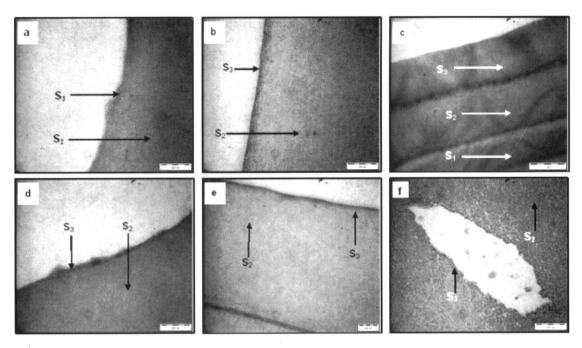


Fig. 3. Transverse section of a multi-layered fiber wall at high magnification showing secondary wall layers a) EFB (75 000×); b) OPF (75 000×); c) OPT (18 000×); d) Coir (75 000×); e) BS (45 000×); f) PALF (75 000×). a, b, d, e, & f (200 nm); c (1 μ m). S₁ S₂ & S₃ S₃ = outer, middle, and inner layers of secondary wall.

lulose microfibril that is aligned parallel to fibre axis on S_2 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_3 layer plays an important role in a few processes such as pulpand 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_3 layer in plant fibre (Fig. $3a \sim 3f$). TEM micrographs show that the different plant fibre has different S_3 thickness. Previous study has reported that *P. radiata* tracheid also shows variability in the thickness of S_3 layer ($0.06 \sim 0.3$ µm) (Singh *et al.*, 2002). They suggested that an irregular thickness of S_3 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 S₃ layer (2.37 µm) compared to other plant fibre (Fig. 3c). Therefore, this fibreis 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. S₃ layer is said to have microfibril angle perpendicular to S2 microfibril angle so this layer can minimize the effect of tension towards S₂ layer and all walls during the water transportation. Being the innermost layer, the S₃layer also can be a barrier to some chemical treatment process such as pulping, preservation, and modification (Blanchette et al., 1990).

4. CONCLUSIONS

Research on ultrastructure of plant fibre can be concluded as such:

- i) From the electron micrographs acquired can be stated that all cell wall structure of plant fibre (EFB, OPF, OPT, Coir, BS, PALF) have the same ultrastructure with wood fibre that is composed of primary (P) and secondary wall $(S_1, S_2 \text{ and } S_3)$.
- ii) Tropical plant fibre shows quite high variety in terms of size, shape, and cell wall structure. All fibre structure is formed in almost round shape except for cell wall structure of banana stemthat is polygonal in shape.
- iii) OPT fiber consists of the thickest primary and secondary wall layers.

ACKNOWLEDGEMENTS

The authors would like to thank Universiti Sains Malaysia, Penang for the offer of the Short Term Research Grant (304/PTEKIND/636070) that has made this research work possible.

REFERENCES

- Bai S. L., R. K. Y. Li, L. C. M Wu, H. M. Zheng, and Y. W. Mai. 1998. Tensile failure mechanisms of sisal fibers in composites. Journal of Materials Science Letters. 17(21): 1805~1807.
- Balashov V., R. D. Preston, G. W. Ripley, and L. C. Spark. 1956. Structure and mechanical properties of vegetable fibres. I. The influence of strain on the orientation of cellulose microfibrils in sisals leaf fibre. Proc. Roy. Soc. B. 146: 460~ 468.
- Blanchette, A. R., T. Nilsson, G. F. Daneil, and A. Abad. 1990. Biological degradation of wood. In: Advances in Chemistry Series. Archaeological Wood: Properties, Chemistry and Preservation (ed. by R. M. Rowell and R. J. Barbour.).

- pp. $141 \sim 174$. Washington D. C.
- 4. Booker, R. E. and J. Sell. 1998. The nanostructure of the cell wall of softwoods and its functions in a living tree. Holz-als-Roh-Und Werkstoff 56: $1\sim8$.
- Cordeiro, N., M. N. Belgacem, I. C. Torres, and J. C. V. P. Moura. 2004. Chemical compositition and pulping of banana pseudo-stem. Industrial Crops and Products 19: 147~154.
- Dickison, W. 2000. Integrative plant anatomy, New York, Harcourt Academic Press.
- Donaldson, L. A. 1992. Lignin distribution during latewood formation in *Pinus radiata* D. Don. IAWA Bull. 13: 381~387.
- Donaldson L. A. 1996. Determination of lignin distribution in agricultural fibres. Wood Processing Division, New Zealand Forest Research Institute. 4418: 1~25.
- Eames, A. J. and L. H. MacDaniels. 1974. An Introduction to Plant Anatomy. New York, Mac-Graw Hill Book Company.
- Fengel D. and X. Shao. 1984. A chemical and ultrastructural study of the bamboo species *Phy-llostachys makinoi* Hay. Wood Sci. Technol. 18: 103~112.
- Grunwald, C., K. Ruel, and U. Schmitt. 2002.
 Differentiation of xylem cells in rolC transgenic aspen trees: a study of secondary cell wall development. Ann. For. Sci. 59: 679~685.
- 12. Harada, H. 1964. Ultrastructure and organization of gymnosperm cell walls. In: Proceedings of the Advanced Science Seminar Pinebrook Conference Center (ed. by W. A. Cote). pp. 215~234. Syracuse University Press, New York.
- John, V. M., M. A. Cincotto, C. Sjostrom, V. Agopyan, and C. T. A. Oliveira. 2005. Durability of slag mortar reinforced with coconut fibre. Cement & Concrete Composites 27: 565~574.
- Koch, G. and G. Kleist. 2001. Application of scanning UV microspectrophotometry to localise lignins and phenolic extractives in plant cell walls. Holzforschung 55: 563~567.
- Mansor H. and A. R. Ahmad. 1991. Chemical composition of the oil palm trunk. Proc. Seminar Oil Palm Trunk & Other Palmwood Utilization, PORIM, Kuala Lumpur, pp. 335~342.
- 16. McNeil M., A. G. Darvill, S. C. Fry, and P.

- Albershiem. 1984. Structure and function of the primary cell walls of plants. Ann. Rev. Plant Physiol. $53:625\sim663$.
- Mishra, S., A. K. Mohanty, L. T. Drzal, M. Misra, and G. Hinrichsen. 2004. A review on pine-apple leaf fibers, sisal fibers and their biocomposites, Macromol. Mater. Eng., 289: 955~974.
- MOA (Ministry of Agriculture) Hectareage of Industrial Crops by Types, Malaysia. 2006. [Online]. [Accessed on 18 January 2006]. Available on website: http://www.doa.gov.my/doa/main.php ?Content=articles&ArticleID=5.
- Morvan, C., C. Andeme-Onzighi, R. Girault, D. S. Himmelsbach, A. Driouich, and D. F. Akin. 2003. Building flax fibres: More than one brick in the walls. Plant Physiology and Biochemistry 41: 935~944.
- 20. Sjostrom, E. 1993. Wood chemistry, fundamentals and applications. New York, Academic Press.
- 21. Singh, A., G. Daniel, and T. Nilsson. 2002. High variability in the thickness of the S_3 layer in *Pinus radiata* tracheids. Holzforchung 56: 111 \sim 116.