Estimation of the Ratio of Vascular Bundles to Parenchyma Tissue in Oil Palm Trunks using NIR Spectroscopy

Hisashi Abe,[a,*] Yoshinori Murata,[b] Satoshi Kubo,[a] Ken Watanabe,[a] Ryohei Tanaka,[a] Othman Sulaiman,[c] Rokiah Hashim,[c] Sitti Fatimah Mhd Ramle,[c] Chunhua Zhang,[a] Shuichi Noshiro,[a] and Yutaka Mori [b,d]

In order to use oil palm trunks more effectively, a new method was investigated to estimate the weight-based ratio of vascular bundles (VB) to parenchyma tissue (PT) in study materials taken from oil palm trunks, by using near infrared (NIR) spectroscopy based on chemical analyses of the composition. The VB and PT were carefully separated by hand from oil palm trunks using a polarizing microscope to ensure purity, and then they were mixed at certain ratios. As the VB ratio was increased, extractives, lignin, hemicellulose, and starch contents decreased, while the alpha-cellulose content increased. By using NIR spectroscopy coupled with partial least squares regression analysis, we could predict the ratio of VB to PT with an accuracy of R^2 = 0.99. Absorption peaks significantly affecting estimation were observed at 1929, 2104, 2276, and 2335 nm, which were assigned to the chemical compositions of cellulose and starch. The NIR absorbance is considered to reflect the ratio of VB to PT, according to the compositions of cellulose and starch in oil palm trunks.

Keywords: Oil palm; Vascular bundle; Parenchyma tissue; Chemical composition; Cellulose; NIR

*Contact information: a: Forestry and Forest Products Research Institute, 1 Matsunosato, Tsukuba, Ibaraki 305-8687, Japan; b: Japan International Research Center for Agricultural Sciences, 1-1, Owashi, Tsukuba, Ibaraki 305-8686, Japan; c: School of Industrial Technology, Universiti Sains Malaysia, 11800, Penang, Malaysia; d: Department of Global Agricultural Sciences, University of Tokyo, 1-1-1, Yayoi, Bunkyo 113-8657, Japan; *Corresponding author: abeq@affrc.go.jp*

INTRODUCTION

The oil palm tree (*Elaeis guineensis*), which originated in tropical Africa, has recently been widely planted for its edible oil in such Southeast Asian countries as Malaysia and Indonesia (Corley and Tinker 2007). The oil is mainly used as food in related industries, as well as a raw material for various products, such as detergents and cosmetics. However, a decline in palm oil productivity with increasing age of the tree means that oil palm trees are being replanted at an interval of approximately 25 years. Consequently, the accumulated stock of felled trunks has became a vast biomass resource in the palm oil industry, and numerous studies have been conducted on the development and utilization of this resource.

According to Baker *et al*. (2008), oil palm wood has minimal natural durability due to its high concentration of sugar and starch, which hinders the utilization of its trunks as solid wood. Oil palm sap in the trunks reportedly contains approximately 11% sugars, of which about 90% has sucrose as a major component (Eze and Ogan 1988).

Yamada *et al*. (2010) found that the sugar content in the sap of felled trunks increases during post-logging storage, suggesting that oil palm trunks may be a promising source of sugars following proper post-logging treatment, and that its sap could be an effective feedstock for bio-ethanol (Kosugi *et al*. 2010; Yamada *et al*. 2010). Conversely, as oil palm trunk fibers have been considered to be useful materials for pulp and paper, the fiber properties have been intensively investigated (Tomimura 1992; Abdul Khalil *et al*. 2008; Wan Rosli and Law 2011). However, the chemical compositions of VB and PT have not been well studied (Tomimura 1992).

Oil palm trunks are mainly comprised of vascular bundles (VB) and parenchyma tissue (PT). Fibers useful for materials occur in VB, while living cells containing sugars and starch useful for energy and livestock foods mainly exist in PT. Their physical properties differ significantly, as VB are dense and fibrous, while PT is sparse and spongy (Lim and Khoon 1986; Lim and Fujii 1997; Baker *et al*. 2008). Hence, the separate utilization of such tissues is preferable. Due to the variable ratio of VB to PT within a trunk (Baker *et al*. 2008), a difference that strongly affects the yield of each component, it is important to estimate the ratio in terms of the materials taken from oil palm trunks, in order to ensure their effective utilization as biomass resources.

Anatomical studies of oil palm trunks have clarified the existence of inner and outer vascular systems, as in other monocots (Tomlinson 1961, 1990; Baker *et al*. 2008). The outer region of living tissue is differentiated into a narrow cortex from the wide central cylinder. In the central cylinder, VB are concentrated in the outer region and scattered in the inner region (Tomlinson 1961, 1990; Baker *et al*. 2008). Although some anatomical studies have investigated the ratio of VB in the trunks of palms on a volume basis (Sudo 1980; Baker *et al*. 2008), it is very difficult to estimate the ratio of VB to PT based on their weight.

An NIR spectrum (800 to 2500 nm), on the other hand, mainly consists of the overtone and combination bands of fundamental stretching vibrations of the O-H, N-H, and C-H functional groups. NIR spectroscopy is a promising technique that can be used to rapidly estimate the physical and chemical properties of materials, and it has been employed in the scientific fields of agriculture, forestry, and forest products (So *et al*. 2004; Yeh *et al*. 2004; Tsuchikawa 2007; Shenk *et al*. 2008).

In the field of forest products, NIR spectroscopy has also proved useful in estimating chemical composition, wood quality, and moisture content (Tsuchikawa 2007). Thus its ability to estimate the ratio of VB and PT in oil palm trunks is highly anticipated, despite there having been no previous reports on such estimation. In this study, we investigated the applicability of NIR spectroscopy for estimating the ratio of VB and PT in the weight base of wood meal obtained from oil palm trunks.

EXPERIMENTAL

Materials

Oil palm trees aged 25 years (height: 12 m; diameter: 36 to 41 cm) were logged in Johor, Malaysia. The oil palm trunks were roughly shredded by a machine, and the shredded sample was dried in a dry oven at 50˚C for two days.

Sample Preparation

The dried sample was put through a mesh to screen VB based on the difference in the fragility of VB and PT, whereupon PT still attached to the VB was removed by hand. We then observed PT and VB under cross Nicol with a light polarizing microscope (ECLIPSE 80i, Nikon, Tokyo, Japan) in order to confirm the purity of both tissues. This is because only VB have intensive birefringence in their secondary cell walls (Fig. 1), in which cellulose microfibrils are oriented in a certain direction (Tomlinson *et al*. 2011). The sample was then completely separated into PT and VB by using a horizontally rotating sieve (SKH-01; AS ONE, Osaka, Japan) while changing the mesh size from 1 mm to 106 μm. Next, PT and VB were finely ground with a speed rotor mill (Pluverisette 14, FRITSCH, Idar-Oberstein, Germany). To prepare the reference samples for chemical analysis and NIR spectroscopy, VB and PT were mixed at ratios of 0:100, 30:70, 50:50, 70:30, and 100:0, respectively.

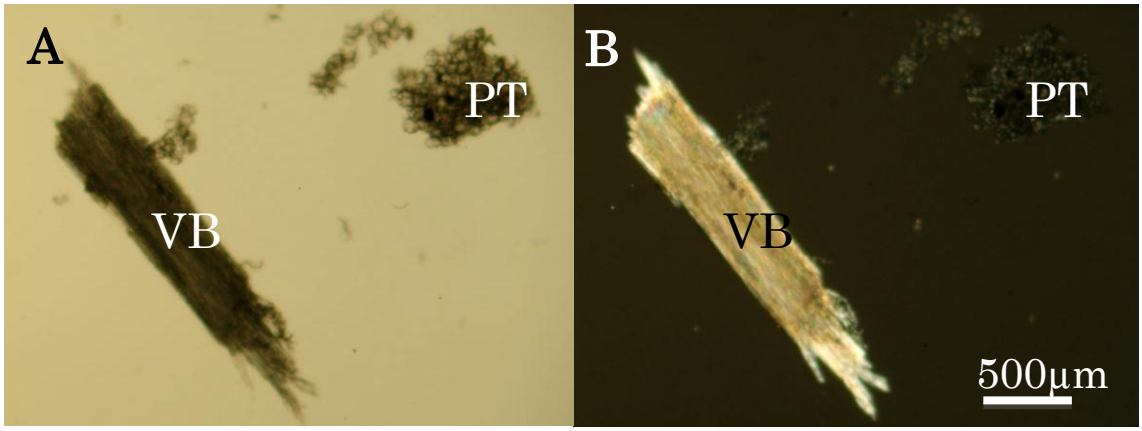

Fig. 1. Photographs of the light microscopy (A), and the polarizing microscopy (B) of the same region. VB: Vascular bundle, PT: Parenchyma tissue

Chemical Analysis

The reference samples were extracted with ethanol/benzene (1/2, v/v) for 6 h using a Soxhlet extractor. Klason lignin, α-cellulose, and hemicellulose (holocellulose - α-cellulose) contents were measured following the standard procedure. Klason lignin content was not corrected with acid-soluble lignin, as the molar UV absorbance had not been evaluated for oil palm samples. Hollocellulose content was measured by using the Wise method. The delignification process using sodium chlorite was repeated three times in order to completely remove lignin. The α-cellulose content was determined as the alkaline (17.5% aq. NaOH) insoluble fraction of holocellulose prepared using the Wise method. The concentration of starch was determined using a total starch kit (Megazyme Co., Wicklow, Ireland).

NIR Measurement

Approximately 0.3 g of the reference sample was placed in a glass tube having an inner diameter of 15 mm. Five replicates were prepared per ratio of VB to PT, with 25 samples consequently being used for NIR measurement. Diffuse reflectance spectra on a spot diameter of approximately 3 mm were collected at 4 cm^{-1} intervals over the range 12000 to 4000 cm^{-1} (830 to 2500 nm) by using a MATRIX-F spectrometer (Bruker

Optics, Ettlingen, Germany) equipped with an NIR fiber optic probe. For the measurement, the tip of the probe was placed into contact with the upper surface of the sample, and a piece of commercial resin Spectralon was used as the reference material. Fifteen scans were accumulated and averaged into a single spectrum. The samples were divided into a calibration set consisting 15 samples (three replicates for each ratio of VB to PT), and a validation set consisting of 10 samples (two replicates for each ratio of VB to PT). Three spectra were collected from each sample and then further averaged into one spectrum. The calibration set was used to develop a calibration model for estimating the ratio of VB to PT, while those in the validation set were used for validating the model.

Multivariate Analysis

Prior to multivariate analysis the diffuse reflectance spectra were converted to absorbance, and a calibration model for the ratio of VB to PT was developed using Unscrambler software, version 9.8 (CAMO, OR, USA). Partial least squares (PLS) regression analysis in full cross-validation was conducted with the spectra in the calibration set. The optimal number of factors (NF) used in the models was determined by observing the response of residual variance with the added NF. When the additional NF did not substantially decrease the residual variance, iterations were terminated, whereupon the model was used to estimate the ratio of samples in the validation set, and the quality of the model was evaluated by comparing the predicted and measured values. The standard error of prediction (SEP) was used to measure the extent to which the calibration model predicted the parameters of interest for a set of unknown samples. The ratio of performance to deviation (RPD), that is, the ratio of standard deviation of the reference data to the standard error of prediction, provides further assessment of the calibration model (Williams and Sobering, 1993). Note that an RPD greater than 8 is good for process control, development, and applied research; moreover, values of 5 to 8 are adequate for quality control, and values of 2.5 to 5 are satisfactory for screening.

RESULTS AND DISCUSSION

Chemical Composition

Table 1 lists the chemical compositions and equilibrium moisture content under the conditions of NIR measurement (temperature of 25˚C, humidity of 70%) of the reference samples containing a mixture of VB and PT. As the ratio of VB was increased, extractives, lignin, hemicellulose, and starch contents decreased, while the α-cellulose content increased. There were also positive and negative correlations between each chemical composition (Table 2). In particular, the content of α-cellulose of VB (42.51%) was four times higher than that of PT (9.03%). Chemical analysis of oil palm VB revealed that the major components were α-cellulose (42.51%), hemicellulose (37.62%), and lignin (16.11%). Tomimura (1992) reported a similar value of lignin content of VB (15.7%). VB are composed of fibers, vessels, protoxylem, sieve tubes, axial perencyma, stegmata, and companion cells, where the fibers are the main components (Tomlinson 1961, 1990; Lim and Fujii 1997; Baker *et al*. 2008). Abdul Khalil *et al*. (2008) reported 73.06% holocellulose, 41.02% α-cellulose, and 24.51% lignin in the fibers of oil palm trunks. These results are relatively similar to those obtained in our studies. Conversely, the chemical compositions of PT have not been well studied, as PT is not as useful as

fibers. Surprisingly, α-cellulose accounted for only 9.03% of PT in oil palm trunks. Tomimura (1992) reported the lignin content of PT as 20.0%, which is comparable to our result (21.36% lignin). In the case of dicotyledonous wood, the lignin content is higher in the primary cell wall than in the secondary cell wall (Saka 1992). The lignin concentration in PT is higher than that in VB, and this may be attributed to the parenchyma cells of oil palm trunks not having a secondary wall (Fig. 1). The starch concentration was 6.01% in VB and 16.49% in PT (Table 1), thereby suggesting that more starch is distributed in PT than in VB, as stated in a previous report (Tomimura 1992). The equilibrium moisture content decreased with a higher ratio of VB, which may affect NIR analysis. This may be due to the higher porosity of PT relative to VB (Abe *et al*. 2012).

Table 1. Chemical Composition and Equilibrium Moisture Content of the Mixture of VB and PT in Different Ratios

Ratio of VB	Extractives (%)	Lignin (%)	Hemicellulose (%)	α-cellulose (%)	Starch (%)	Moisture content (%)
100	2.54	16.11	37.62	42.51	6.01	7.92
70	2.73	17.05	37.29	37.45	8.83	8.15
50	3.22	18.67	37.01	35.70	8.79	8.46
30	3.31	19.12	48.67	22.75	12.81	9.00
0	3.86	21.36	55.70	9.03	16.49	10.92

Table 2. Correlation Coefficient (r) between each Chemical Composition and Moisture Content

	Ratio of VB	Extractives (%)	Lignin (%)	Hemi-cellulose (%)	α-cellulose (%)	Starch (%)
Ratio of VB	1	-	-	-	-	-
Extractives (%)	-0.98**	1	-	-	-	-
Lignin (%)	-0.99**	1.00***	1	-	-	-
Hemicellulose (%)	-0.87	0.86	0.87	1	-	-
α-cellulose (%)	0.95*	-0.94*	-0.95*	-0.98**	1	-
Starch (%)	-0.97**	0.93*	0.95*	0.95*	-0.99***	1
Moisture content (%)	-0.91*	0.93*	0.94*	0.94*	-0.97**	0.95*

*P < 0.05 **P < 0.01 ***P < 0.001

NIR Analysis of the Estimated Ratio of VB in Oil Palm Trunks

Figure 2 shows the relation between the ratio measured in the laboratory and the NIR-estimated ratio. The calibration model showed a strong correlation with R^2 of 0.99, SEP of 4.42%, and RPD of 8.12, thereby demonstrating that the NIR method is useful for estimating the weight base ratio of VB to PT in ground wood meal obtained from oil palm trunks.

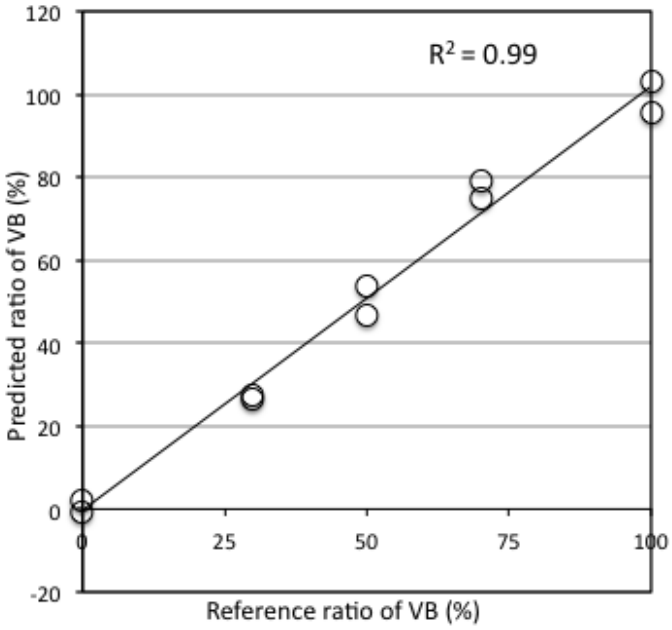

Fig. 2. Relation between reference and predicted values of the ratio of VB. NF of 3 was used in the calibration model

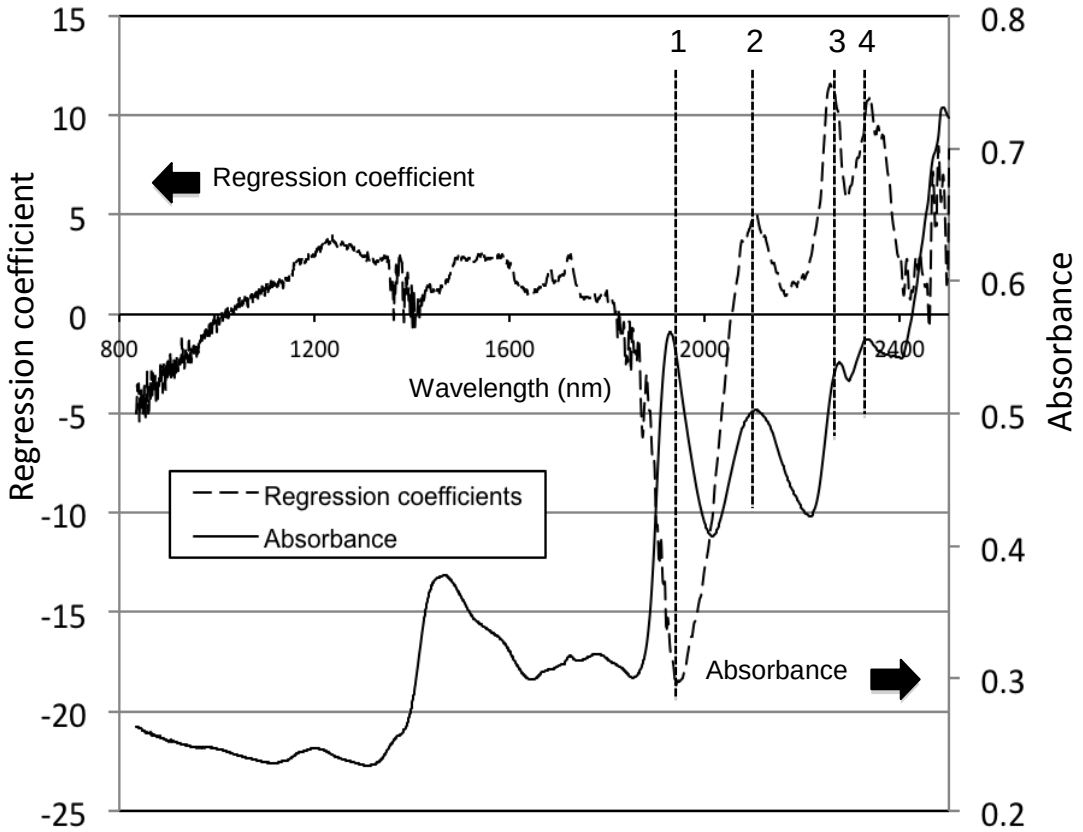

Fig. 3. Raw spectrum measured from a 50% VB reference and regression coefficients from PLS regression used for prediction

The calibration model is expected to have an average estimation error up to the SEP value within the calibrated range of the ratio (0 to 100%). Figure 3 shows a typical absorbance spectrum of the reference sample with a ratio of 50% and the spectral plots of regression coefficients in PLS analysis. Regression coefficients can be used to determine important spectral regions that are responsible for the correlation with the ratio of VB. It is obvious in the absorbance spectrum that at least four peaks exist at wavelengths of 1929, 2104, 2276, and 2335 nm, respectively.

Table 3. Assignment of Absorption Bands in the NIR Region

Peak number*	Wavelength (nm)	Bond vibration	Remarks
1	1929	OH str./HOH def. combination	Cellulose Starch
2	2104	OH bend CO str. combination	Starch
		Asym. COO str. 3rd overtone	Cellulose
3	2276	OH str./CO str. combination	Cellulose
		CH str./CH_2 deformation	Starch
4	2330	CH str./CH_2 def. combination	Starch
4	2335	CH str./CH deformation	Cellulose

* Numbers in the first column correspond to those in Fig. 3.

The absorption bands for these wavelengths can be assigned to chemical components, namely cellulose and starch (Table 3) (Shenk *et al*. 2008). A highly negative impact and a positive impact on the calibration model were found at these wavelengths, indicating that their absorbance was highly correlated with the ratio of VB to PT. In Table 2, cellulose and starch contents are negatively correlated to each other, and their absorption bands closely overlap at these wavelengths. Therefore, both components have positive and negative impacts on the calibration model, whereas positive regression coefficients were found at wavelengths of 2104, 2276, and 2335 nm. This can be explained by the higher amount of cellulose in the given sample than that of starch, as listed in Table 1. Since the ratio of VB is more strongly dependent on the cellulose content than on the starch content, the composition of cellulose has relatively higher impact on the calibration model, as is reflected on the positive regression coefficients at wavelengths of 2104, 2276, and 2335 nm.

Negative regression coefficients were conversely found in the wavelength region around 1929 nm, probably because of the strong absorption band due to water, which had a negative correlation with the ratio of VB (Table 2). In order to eliminate the effect of moisture content on predicting the ratio of VB, another calibration model was developed, excluding the absorption bands due to water (1425-1475, 1765-1815, 1915-1965 nm) (Shenk, 2008). The calibration model shows a sufficiently high level of quality for prediction (R^2 = 0.99, SEP = 3.75%, RPD = 9.57) with NF of 4.

It was reported that the concentration of starch within a trunk of oil palm shows seasonal and regional variations (Legros *et al.* 2009). Thus, it is necessary to consider the influence of the variation of starch concentration for the practical estimation of the ratio of VB to PT using NIR spectroscopy. As NIR measurement is a relatively simple procedure used to obtain spectra, it is available in laboratories, in the field, and in

factories for estimating the ratio of VB to PT in materials taken from oil palm trunks. Thus, it allows us to consider more effective utilization of oil palm trunks as a biomass resource.

CONCLUSIONS

The absorption peaks significantly affecting estimation were assigned to chemical components, namely, cellulose and starch. NIR absorbance is considered to reflect the ratio of vascular bundles (VB) to parenchyma tissue (PT), according to the compositions of cellulose and starch in oil palm trunks. The results suggest that NIR spectroscopy combined with PLS regression analysis has the potential to estimate the VB to PT ratio for more effective utilization of oil palm trunks.

ACKNOWLEDGMENTS

This study was supported by a Grant-in-Aid for Scientific Research (No. 23580463, 21300332) from Japan's Ministry of Education, Culture, Sports, Science and Technology.

REFERENCES CITED

Abe, H., Zhang, C., Tanaka, R., Sulaiman, O., Hashim, R., Kosugi, A., and Mori, Y. (2012). "Anatomical study on the change in the trunk tissues of an oil palm tree during storage in the atmosphere of tropical climate," *JIRCAS Working Report* 73, 131-134.

Abdul Khalil, H. P. S., Siti Alwani, M., Ridzuan, R., Kamarudin, H., and Kharil, A. (2008). "Chemical composition, morphological characteristics, and cell wall structure of Malaysian oil palm fibers," *Polymer-Plastics Technology and Engineering* 47(3), 273-280.

Baker, E. S., Sahri, M. H., and H'ng, P. S. (2008). "Anatomical characteristics and utilization of oil palm wood," In: *The Formation of Wood in Tropical Forest Trees – A Challenge from the Perspective of Functional Wood Anatomy*, T. Nobuchi and M. H. Sahari (eds.), Penerbit Universiti Putra Malaysia, Serdang, 161-178.

Corley, R. H. V., and Tinker, P. B. (2007). *The Oil Palm*, Fourth Ed., 2, Blackwell Science Ltd. Oxford, UK.

Eze, M. O., and Ogan, A. U. (1988). "Sugars of the unfermented sap and the wine from the oil palm, *Elaeis guinensis*, tree," *Plant Foods Human Nutrients* 38, 121-126.

Kosugi, A., Tanaka, R., Magara, K., Murata, Y., Arai, T., Sulaiman, O., Hashim, R., Hamid, Z. A. A., Yahya, M. K. A., Yusof, M. N. M., Ibrahim, W. A., and Mori, Y. (2010). "Ethanol and lactic acid production using sap squeezed from old oil palm trunks felled for replanting," *J. Bioscience and Bioengineering* 110(3), 322-325.

Legros, S., Mialet-Serra, I., Clement-Vidal, A., Caliman, J. P., Siregar, F. A., Fabre, D., and Dingkuhn, M. (2009). "Role of transitory carbon reserves during adjustment to

climate variability and source-sink imbalances in oil palm (*Elaeis guineensis*)," *Tree Physiology* 29(10), 1199-1211.

Lim, S. C., and Khoon, K. C. (1986). "Characteristics of oil palm trunk and its potential utilization," *Malaysian Forester* 47(1), 3-11.

Lim, S. C., and Fujii, T. (1997). "A note on the structure of oil palm trunk by scanning electron microscopy," *J. Tropical Forest Products* 3(1), 105-109.

Saka, S. (1992). "Electron microscopy," In *Methods in Lignin Chemistry*," S. Y. Lin and C. W. Dence (eds.), Springer-Verlag, Berlin, Heidelberg, 133-145.

Shenk, J. S., Workman, J. J. Jr., and Westerhoaus, M. O. (2008). "Application of NIR spectroscopy to agricultural products," In *Handbook of Near- Infrared Analysis third edition*, D. A. Burns and E. W. Ciurczakeds (eds.), CRC Press, Taylor & Francis Group, Boca Raton, 347-386.

So, C. L., Via, B. K., Groom, L. H., Schimleck, L. R, Shupe, T. F, Kelley, S. S., and Rials, T. G. (2004). "Near infrared spectroscopy on the forest products industry," *Forest Products J.* 54(3), 6-16.

Sudo, S. (1980). "Variation in some important anatomical properties and density in the stem of coconut palm (*Cocos nucifera*)," *Bull. of the Forestry and Forest Products Research Institute* 312, 81-101.

Tomimura, Y. (1992). "Chemical characteristics and utilization of oil palm trunks," *JARQ* 25, 283-288.

Tomlinson, P. B. (1961). *Anatomy of the Monocotyledons. II. Palmae*, Oxford University Press, London, UK.

Tomlinson, P. B. (1990). *The Structural Biology of Palms*, Oxford University Press, New York, USA.

Tomlinson, P. B., Horn, J. W., and Fisher, J. B. (2011). *The Anatomy of Palms*, Oxford University Press, New York, USA.

Tsuchikawa, S. (2007). "A review of recent near infrared research for wood and paper," *Applied Spectroscopy Review* 42, 43-71.

Wan Rosli, W. D., and Law, K. N. (2011). "Oil palm fibers as papermaking material: Potentials and challenges," *BioResources* 6(1), 901-917.

Williams, P.C., and Sobering, D.C. (1993). Comparison of commercial near infrared transmittance and reflectance instruments for analysis of whole grains and seeds. *J Near Infrared Spectroscopy* 1, 25-32.

Yamada, H., Tanaka, R., Sulaiman, O., Hashim, R., Hamid, Z. A. A., Yahya, M. K. A., Kosugi, A., Arai, T., Murata, Y., Nirasawa, S., Yamamoto, K., Ohara S., Yusof, M. N. M., Ibrahim, W. A., and Mori, Y. (2010). "Old oil palm trunk: A promising source of sugars for bioethanol production," *Biomass and Bioenergy* 34, 1608-1613.

Yeh, T. F., Chang, H. M. and Kadla, J. F. (2004). "Rapid prediction of soil wood lignin content using transmitted near-infrared spectroscopy," *J. Agricultural and Food Chemistry* 52, 1435-1439.

Article submitted: February 2, 2012; Peer review completed: April 22, 2012; Revised version received and accepted: January 29, 2013; Published: February 4, 2013.