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Detection of vascular bundles using cell wall birefringence on exposure to polarized light

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ABSTRACT

Vascular bundles (VBs) of oil palm trunks exhibited birefringence due to polarized orientation of their cell wall components, although another component of the trunks, parenchyma tissue (PT), did not. The intensity of the birefringence depended on the VB composition; therefore, it was possible to estimate the VB content readily using a polarizing microscope connected to an image analyzer. The method proposed in this manuscript should allow easier quantification of VBs in oil palm trunks than methods using X-ray diffraction. Both VBs and PT contained cellulose, hemicelluloses, lignin, and starch; however, the cellulose content of PT was less than half that of VBs. This difference in cellulose content between VB and PT may explain why VBs were detected under polarizing light.

1. Introduction

The oil palm (*Elaeis guineensis*) is one of the world's most rapidly expanding equatorial crops (Koh and Wilcove, 2008). Global palm oil production is increasing by 9% every year, and the number of oil palm plantations increasing largely in response to economic growth in Indonesia, India, and China (Fitzherbert et al., 2008). Oil palm trees have to be replanted about every 25 years because of a reduction in palm oil yield of older trees. During recultivation of oil palm trees, many palm trunks are simply cut down and go to waste (Ismail and Mamat, 2002). In order to utilize this waste efficiently, many studies have been conducted on using it as a raw material for useful wood-based products, including medium-density fiberboard, pulp and paper, and other products (Sulaiman et al., 2008). However, the heterogeneous structure of oil palm trunk has hindered its efficient utilization.

An oil palm trunk consists mainly of vascular bundles (VBs) and parenchyma tissue (PT). VBs support the oil palm body and transport water from the root to the top of the palm. To perform these functions, VBs are composed mainly of stiff tissues with highly developed secondary cell walls, such as vessels and fibers. VBs are raw materials for wood-based products and paper. On the other hand, PT consists mainly of parenchyma cells with metabolic and storage functions, and is soft tissue due to a absence of secondary cell walls. Since PT contains significant amounts of sugar and starch, it is suitable as a biofuel. For efficient ethanol production, the trunk of oil palm needed to be separated into the PT and the VBs (Prawitwong et al., 2012). The distribution of VBs and PT in the trunk varies in both the horizontal and vertical portions of the trunk, such as the center and outer regions, or the top and bottom. Therefore, it is necessary to develop methods to estimate the amount of VB and PT in a certain sample.

We examined the efficiency of near infrared (NIR) spectroscopy to estimate the ratio of VB in materials from oil palm trunks; however, we concluded that it would be preferable to develop other methods to estimate the VB ratio accurately in oil palm trunks due to interference by starch and carbohydrates in NIR spectroscopy of samples (Abe et al., 2013). VBs exhibit birefringence due to polar-

Abbreviations: NIR, near infrared; PT, parenchyma tissue; VBs, vascular bundles; XRD, X-ray diffraction.

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ization induced by the structure of their secondary cell walls when observed under crossed nicols with a light polarizing microscope (Lim and Fujii, 1999). In this study, we evaluated the use of the birefringence of VBs as a means of detecting them in a sample based on this polarization. Pure VBs and pure PT were prepared by hand, and standard mixtures of the PT and VBs were prepared at different ratios (while maintaining the total mass fraction constant). These standard mixtures were then used to estimate the amount of VB and the purity of PT. The intensity of birefringence due to polarization was observed for different standards, and the results obtained using birefringence were compared with those obtained using X-ray diffraction (XRD). In addition, the chemical components in the standards, such as α-cellulose, hemicellulose, lignin, and starch, were analyzed. It was found that the cellulose content correlated strongly with the intensity of the polarization from the VBs.

2. Materials and methods

2.1. Pretreatment of the residues compressed from oil palm trunks

Twenty-six entire oil palm (*Elaeis gunensis* 'Deli Dura' × 'Yangambi,' 'URTA', or 'URTC') trunks aged 25 years (5 m in height and up to 40 cm in diameter) were harvested from Johor province (1°28′0″N, 103°45′0″E), Malaysia. The palm trunk core (1.2 m in height, 20 cm in diameter), which was prepared by peeling off the bark and outer layers, was shredded into chips and then compressed using a mill in order to squeeze out the sap (Murata et al., 2013).

2.2. VB and PT preparation

The compressed residues were dried for 1–2 days at 50–60°C and then put through a mesh in order to screen out the VBs, because the friability and density of the PT are quite different from the VBs. The VBs and PT were then roughly separated from the compressed residues using a model SKH-01 horizontally rotating sieve (AS ONE, Osaka, Japan). Since the PT remained attached to the VBs after separation using this sieve, as much PT as possible was removed from the VB by hand. Pure VBs and pure PT were then separated from the roughly separated samples using the horizontally rotating sieve after changing the mesh size from 106 μm to 500 μm.

2.3. Observation using a polarizing microscope

To confirm the purity of both the VBs and PT, both were observed under crossed nicols using an Eclipse 80i light polarizing microscope (Nikon, Tokyo, Japan). The light conditions for this microscope were as follows: the module light on the halogen lamp was set to the middle level; an ND8 filter was used to reduce the light to one-eighth; and the diaphragm shutter under the stage was adjusted to 0.5%. Next, the purified VBs and purified PT were ground more finely using a Pluverisette 14 speed rotor mill (Fritsch, Idar-Oberstein, Germany). Standards were then prepared in order to estimate the VB quantity in mixtures of the VB and PT. For preparation of the standards, the VBs and PT were blended at a constant mass fraction (g) at ratios of 0:100, 30:70, 50:50, 70:30, and 100:0. Prior to observation, an equivalent mass from each of the standards (0.6 g) was boiled in 80% glycerol (15 cm^3) for 5 min to eliminate any bubbles. After cooling to room temperature, the standards were mixed violently to be equated. The constant volume (60×10^{-3} cm^3) was taken exactly from each mixed standard, and it (60×10^{-3} cm^3) was spread in rectangle area on slide glass (Fig. 1b). After that, the standards were observed under polarizing conditions using an Eclipse 80i microscope (Nikon).

Table 1
Standard curves determined from the polarization analysis.

VB quantity(%)	No. 1[a]	No. 2[a]	No. 3[a]	No. 4[a]	No. 5[a]	No. 6[a]	Ave.	SD
0	2.7	1.8	1.9	2.7	1.9	3.1	2.4	0.5
30	20.7	29.6	26.1	19.8	28.1	28.3	25.4	4.2
50	37.9	50.5	48.0	39.6	41.2	42.6	43.3	4.9
70	66.8	67.0	69.0	48.9	65.4	71.6	64.8	8.1
100	106.3	96.9	101.7	82.9	91.9	91.7	95.2	8.3

The numbers in the table indicate the different days for preparation and observation of the samples.

[a] Experiments were repeated six times.

2.4. Estimation of VBs based on microscope analysis

The mechanism of VB detection using a polarizing microscope is described in Fig. 1a. The analysis of image data was processed using Winroof ver.6.3 (MITANI corporation, Tokyo, Japan). In addition, the slight variation among the slide glasses was calibrated by the function of analysis software, Winroof ver. 6.3, when birefringence from polarization was detected by microscope. Each standard that was mounted on a glass slide was divided into 10 parts, each of which was observed under the non-polarized light and the polarized light using the polarizing microscope, and two images within 10 parts were shown in Fig. 1b. The bright areas were identified as VB areas, and the VB quantity was estimated for these areas. The VB areas for the 10 parts on the same slide were summed, and this value was assumed to be the VB quantity in that standard (Fig. 1c). Several lots of standards were prepared and the birefringence under polarization was measured independently at least six times. After image analysis, a calibration curve was plotted using the VB area (%) that was estimated from the birefringence and the quantity of VBs (% $w\,w^{-1}$) in the standards. The quantity of VBs in the samples was determined from the amount of birefringence for given VB area in the same samples.

2.5. XRD analysis

Each 100 mg standard (100% VB, 70% VB, 50% VB, 30% VB, and 0% VB) was pressed at 150 kg cm^{-2} by hand pressing machine for 1 min into a 10 mm diameter disk, and wide-angle XRD patterns were measured using nickel-filtered CuKα radiation produced by a Rigaku RINT-2550HF X-ray generator (Rigaku, Tokyo, Japan) with a 1 mm diameter pinhole collimator at 40 kV and 200 mA. The XRD patterns were recorded by transmission using a scintillation counter with a scanning speed of 0.5° min^{-1} and scanned through a range 2θ angles (i.e., $2\theta = 5^\circ - 40^\circ$). 2$\theta$ is defined as Bragg angle employed for measuring X-ray diffraction. We prepared standards 60 mg standards, and conducted XRD analysis on these standards to compare with 100 mg standards. Consequently, XRD were same pattern with 100 mg Standards, hence, we adopted the result of 100 mg standards here.

2.6. Chemical analysis

The standards were extracted with toluene for 6 h using a Soxhlet apparatus to quantify the amount of the extractive from standards. Klason lignin (acid-insoluble lignin) was determined in accordance with the cm-98 TAPPI standard (2007) (T204 cm-98, 2007). Holocellulose content was measured using the method of Wise et al. (1946). In this procedure, delignification was repeated five times. Cellulose content was determined as the alkaline (17.5% aq. NaOH) insoluble fraction of holocellulose. The starch content was determined using a total starch kit (Megazyme Co., Wicklow, Ireland). Elution of each chemical component was repeated at least twice independently, and the average values are shown in Table 1.