Measurement of Microfibril Angle Using X-Ray Diffraction and Light Microscope on 5-year-old Super and Conventional Teak Wood

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Abstract

The long harvest time causes the processing industry manufactured from teak wood (*Tectona grandis* L. f.) has experienced a lot of declines. To overcome this problem, currently, in Indonesia many types of fast-growing teak have been developed, one of which is Jati Unggul Nusantara (JUN). Trees that are accelerated in growth, are likely to cause changes in their anatomical structure. The anatomical structure of wood is one of the basic properties that greatly influences the use of wood as a raw material. Even small changes in cell shape and size can change the properties of wood as a raw material. One of the anatomical structures of wood, namely the ultramicroscopic structure that affects the quality of wood, is the microfibril angle (MFA). The purpose of this study is to determine the MFA of JUN at the planned cutting age of 5 years, compared to conventional teak at the same age. There are two methods used, namely using X-Ray Diffraction (XRD) and measuring the elongation of the pit apertures slope of the fiber cells using a light microscope, which was obtained from the maceration process. As a result, JUN's MFA is 22.09°, smaller than the conventional teak of 25.29°. This is because JUN was developed from top cuttings so although still young, it already reflects the characteristics of mature teak. The results of the MFA measurements on JUN using two methods (XRD and light microscope), resulted different values. It is different from the MFA measurement results on conventional teak. It is recommended to measure the MFA in JUN wood by using XRD, because possibly, due to accelerated growth, simple pits with an oval shape turn into circular. This difference causes the results of the JUN MFA measurement using a light microscope based on the pit apertures slope to be inconsistent, subjective, and different results in other pits contained in the fiber even though they are closely associated. However, in conventional teak, measurements using a light microscope are possible because the shape of the pit is oval so that the slope of the elongation of the pit aperture can be determined easily, and is more consistent with more uniform values in the same individual fiber.

Keywords: Anatomical structure, JUN, MFA, XRD, light microscope

Introduction

Currently in Indonesia, many types of fast-growing teak distribute under various trade names, such as golden teak, super teak, superior teak, prima teak, and monfori teak, all of which are developed through tissue culture. Similarly, the type of teak developed by the Research and Development Center of Perum Perhutani is known as a Plus Teak Tree (Jati Plus Perhutani/ JPP) (BBPBPTH 2008; Sumarni and Muslich 2008).

From the plant stem of JPP, currently derivatives have been made with various improvements in their characteristics. PT. Setyamitra and the Wanabakti Nusantara Housing Cooperative (Koperasi Perumahan Wanabakti Nusantara/ KPWN) have succeeded in inducing their roots to become stilt roots so that the roots are stronger and the stems grow quicker but do not easily collapse. The superior teak seeds were then given the name of Jati Unggul Nusantara/JUN (Soeroso and Poedjowadi 2008).

Trees accelerated in growth, allowing changes to the anatomical structure due to the growth of the length of the initial cambium cells being constrained and the production of cells with the maximum length is being delayed. The anatomical structure of wood is one of the basic properties that greatly influences the use of wood as a raw material. Panshin et al. (1964), Pandit (2006), and Pandit and Kurniawan (2008) stated that even small changes in the shape and size of cells will cause changes in the properties of wood as a raw material.

For teak wood that has a ring-porous, the earlywood formation produced from rapid growth will result shorter cells. The purpose of developing JUN was provide a teak plantation that can be harvested at a relatively young age. According to Panshin *et al.* (1964), in the first year of stem growth, after the formation of the vascular cambium, the rate of pseudo transverse division is very fast with a very large percentage of survival. This causes the average length of the initial cells of the cambium and its derivate cells to be short, which is known as the juvenile period.

There are at least four levels of structure in a wooden structure that can be identified, where the smaller the size the more advanced equipment is needed. The four levels are macroscopic structure, microscopic structure, nano/ultrastructure structure, and molecular structure level (Booker and Sell 1998).

The ultramicroscopic structure that affects wood quality is the microfibril angle (MFA) (Stuart and Evans 1994). Furthermore, Bendtsen and Senft (1986), in Barnett and Jeronimidis (2003) stated that MFA of the cellulose on the second secondary wall (S2) is a determining factor for the mechanical properties of wood. Microfibrils are the smallest components in the cell wall structure with a diameter of about 3-4 nm and consist of groups of cellulose molecules (protofibrils) covered by hemicellulose sheets. While the MFA is the direction of the slope of the cellulose microfibrils on the secondary wall with the long axis of the fiber or tracheid (Barnett and Bonham 2004, Stuart and Evans 1994). MFA size ranges from 5-34° in Angiosperms (Barnett and Bonham 2004).

The MFA of the cellulose fiber is defined as the angle of the winding that form the spirals of the cellulose chains in the cell wall structure to the fiber axis or the angle formed by the orientation of most of the cellulose microfibrils the long axis of the cell (Stuart and Evans 1994; Barnett and Jeronimidis 2003). Some terms that are also used *include helical angle, spiral angle, and micellar angle.*

The orientation of the cellulose structural unit of the fiber affects the physical and mechanical properties of the fiber, especially density, tensile strength, stiffness, and shrinkage, all of which determine the properties of the paper produced. Small changes in the degree of MFA result in changes in fiber properties (Stuart and Evans 1994).

The MFA can be determined by several techniques, including measuring the alignment of iodine crystals, examining the cell wall, the angle of inside pit aperture, using a confocal microscope, and by using X-Ray Diffraction (XRD) (Barnett and Jeronimidis 2003). The method of measuring the angle of microfibrils on an individual fiber, for example, using a light microscope, takes a very long time. A technique in determining the direction of the microfibril angle automatically is XRD analysis. This method is fast but requires more expensive costs. This study aims to determine the MFA of JUN at the planned harvesting age of 5 years, compared to conventional teak at the same age to estimate the properties of the wood and to determine the most appropriate method in determining MFA in teak.

Materials and Methods

Sampling Site

Sampling was carried out in September 2009. JUN samples were collected from Srengseng Village, Balapulang District, Tegal Regency, Central Java, while the conventional teak samples were from Dukuh Satir Village, Kuta Mendala District, Tonjong Regency, Brebes, Central Java. Laboratory research was carried out from December 2009 to March 2010 at the Plant Anatomy Laboratory and Integrated Instruments and Proximate Center Laboratory of Forest Products Research and Development, Bogor.

Sample Preparation

Samples for ultramicroscopic structure observation were taken from a 5 cm thick disc from the bottom part of the tree. For the measurement of MFA, two methods were used, namely XRD measurement and light microscope.

Sampling was carried out from the pith to the bark according to the amount of incremental growth from each stem. The sample size with a length of 12 mm, a width of 15 mm, and a thickness of 0.5 ~ 1 mm were taken in the tangential section, where from each growth increment the samples were distinct from the earlywood and latewood so that the two samples were obtained from each growth increment. Due to the limitations, radial section sampling cannot be done. However, this condition certainly will not affect the resulting data, because according to Stuart and Evans (1994), samples in the radial and tangential section have relatively the same results.

Microfibril Angle Measurement Using X-Ray Diffraction (XRD)

The MFA was measured by using XRD from Shimadzu with measurement conditions as follows: Cu-Kα radiation (λ= 1.54060 Å), voltage 40 kV and current 30 mA. The auto slit is not used, gap width distribution and receiver are 1 mm and 0.3 mm. Continuous scanning process, Theta/2 Theta, and scan region 0 ~ 360°. Scan speed 90°/min. In data processing, no smoothing of the curve is carried out and the peak of the curve is determined automatically. Shooting is carried out in two orientations, vertical (transmission) and horizontal (reflection). Figure 1 shows the scanning process for measuring the microfibril angle. To calculate the MFA, Cave formula is used: MFA = 0.6 T (Meylan 1976 in Stuart and Evans 1994).

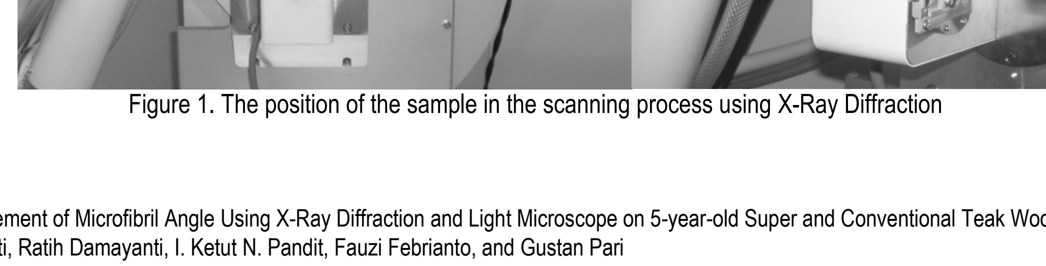

Figure 1. The position of the sample in the scanning process using X-Ray Diffraction

Microfibril Angle Measurement Using a Light Microscope

Measurement of MFA using a light microscope was carried out based on Krisdianto (2008). The fibers that have been decomposed from the maceration process (Tesoro 1989) were observed with a magnification of 500x. The microfibril angle was measured based on the direction of the inclination pit aperture of fiber cell to its long axis using the measurement program available on the Axio Imager Microscope (Zeiss). At each growth increment, the earlywood and latewood were distinct, and for each sample, the measurements were repeated 10 times. How to measure the size of the microfibril angle is presented in Figure 2.

Furthermore, the ultrastructure of wood was observed using a Scanning Electron Microscope (SEM) brand ZEISS type EVO 50. For observations using SEM, the sample size was used with the same cutting pattern as the sample for the MFA measurement. Since this tool does not require coating, there is no prior sample preparation.

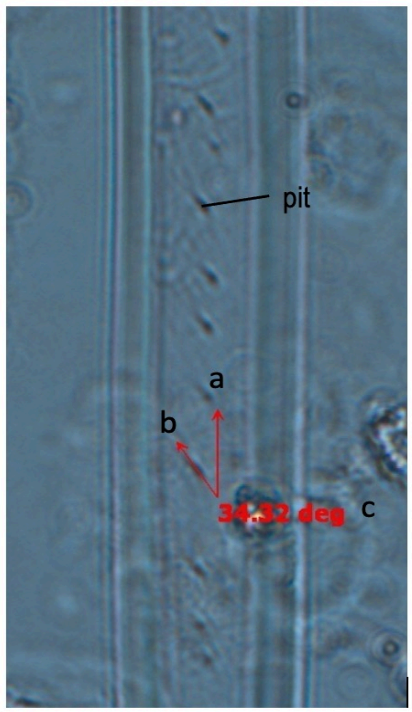

Figure 2. Procedure to measure microfibril angle in one individual fiber cell based on pit orientation (500x magnification). Description: (a) Long axis of fiber cell; (b) Long axis of pit aperture; (c) The size of the microfibril angle.

Data Analysis

MFA data of JUN and conventional teak obtained from measurements using XRD and light microscope were tested using t-student statistics and processed with Excel software. Previously, the F test was performed on the data to determine the diversity of the two samples. Based on a review of various literature, JUN as a species with accelerated growth will have a higher MFA than conventional teak. The hypotheses were:

$H_0 : \mu_1 = \mu_2$

$H_1 : \mu_1 > \mu_2$ or $\mu_1 < \mu_2$;

where $\overline{X_1}$ = average MFA of JUN and $\overline{X_2}$ = average MFA of conventional teak

The MFA values for the two methods used should have the same value, so to compare the results of the two methods, the following hypothesis was developed:

$H_0 : \mu_1 = \mu_2$

$H_1 : \mu_1 \neq \mu_2$;

where $\overline{X_1}$ = average MFA using XRD and $\overline{X_2}$ = average MFA using light microscope

Results and Discussion

The data from the MFA measurement using X-Ray Diffraction (XRD) and light microscope are presented in Table 1.

Table 1. Average data of microfibril angle of super teak Jati Unggul Nusantara (JUN) and conventional teak aged 5 years

Types of Teak/ Growth Increment	Microfibril Angle			
	5-year-old JUN		5-year-old conventional teak	
Earlywood	XRD	Light Microscope	XRD	Light Microscope
1	18,48	25,77	30,54	23,63
2	23,28	20,40	19,74	26,30
3	22,26	28,66	29,94	30,92
4	22,62	28,44	20,28	26,78
5	24,87	22,30	20,28	26,94
Mean	22,30	25,11	24,16	26,91
Latewood				
1	20,22	29,64	28,92	24,35
2	21,00	22,95	24,00	31,16
3	18,07	29,22	21,72	33,77
4	15,89	31,02	28,26	26,78
5	22,18	24,67	29,22	18,31
Mean	19,47	27,50	26,42	26,87

Many techniques have been used to measure the MFA value, including a polarizing microscope, light microscope based on the slope of pit aperture and cracks or tears in the cell wall which generally follow the direction of the microfibril slope, iodine infiltration, NIR (Near Infrared Spectroscopy), and others, but the most widely used and the most accurate result at present is using XRD (Barnett and Bonham 2004). Although the measurement of MFA based on the elongation of the pit aperture using a light microscope is the simplest method, it has the disadvantage that it is less effective because it takes time to measure it, and it is less consistent because the direction of pit aperture in one individual fiber cell can be different results of a review by Barnett and Bonham (2004). In addition, the results are doubtful if it is being measured in wood with simple and circular pit.

In this study, the microfibril angle values used mainly in JUN were calculated using XRD. Teak wood fibers have simple to minutely bordered pits, but in JUN, probably due to accelerated growth, it caused a change in simple pits with an oval shape to simple pit that were circular (Figure 3). This difference caused the results of the JUN MFA measurement using a light microscope based on the slope of the pit aperture to be inconsistent, subjective, and different results in another pit in the fiber even though they were closely associated.

In contrast to the MFA measurements of conventional teak, the measurement by using light microscope were easier because the shape of the pit was oval so that the slope of the elongation of the pit aperture can be determined easily and more consistent with more uniform values on the same individual fiber. From Table 1, it can be seen that the results of the MFA measurements on JUN by using two methods (XRD and light microscope based on the elongation of the pit aperture) produced different values, unlike the results of the MFA measurements on conventional teak, which was also supported by the results of statistical analysis.

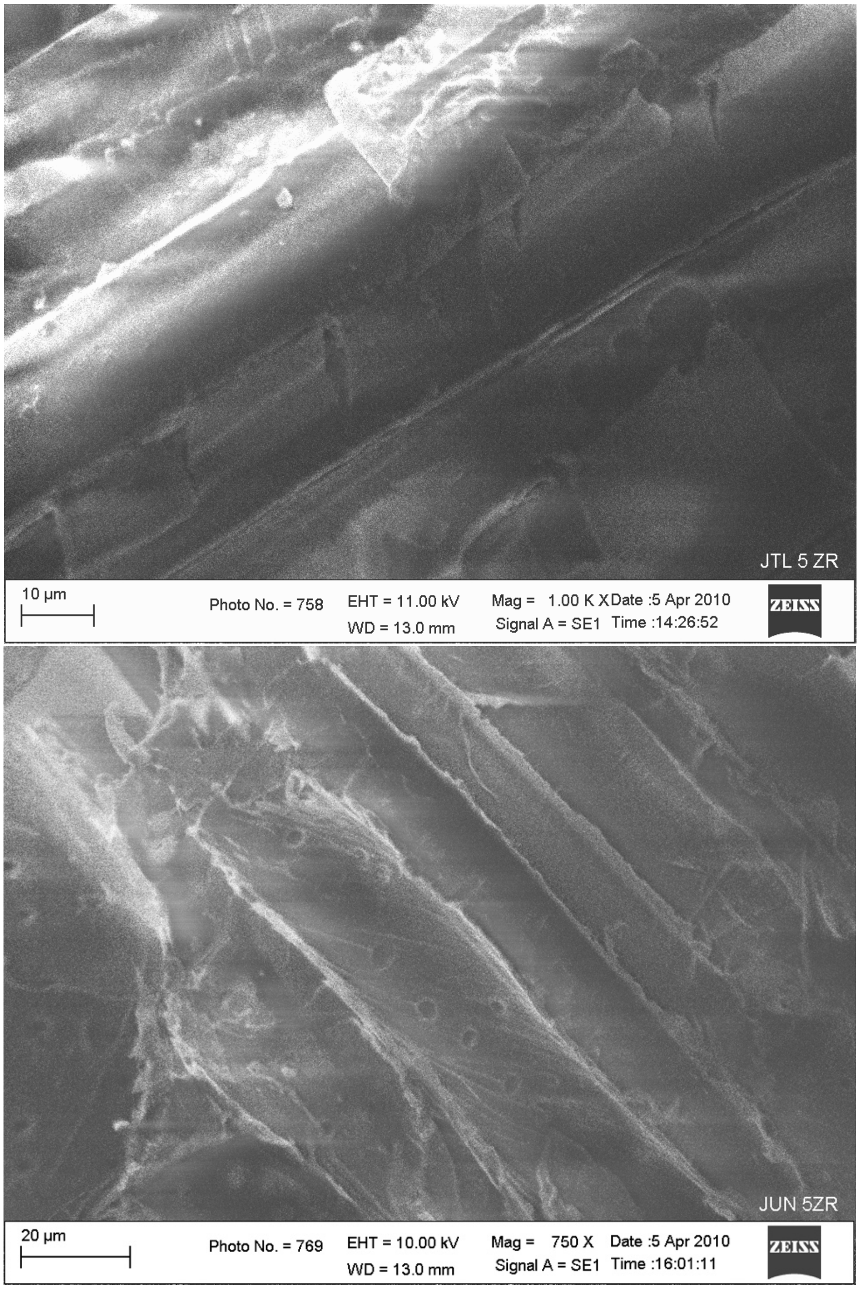

Figure 3. Conventional teak pits are oval (1,000x) and JUN wood pits are circular (750x)

The average MFA of JUN was 22.09° and the MFA of conventional teak was 25.29°. The results of statistical tests showed that the MFA of the two kind of wood was different, whereas the MFA of JUN was smaller than that of conventional teak. The MFA range of JUN wood was 22.30° for earlywood and decreased in the latewood, which was 19.47°. Meanwhile, the MFA of conventional teak wood was 24.16° for the earlywood and became larger for the latewood, which was 26.42°. This result is different from the research conducted by Krisdianto (2008) which carried out measurements using a light microscope on superior teak developed from tissue culture with conventional teak, which was both 7 years old, where the MFA of super teak was 23.29°, greater than that of conventional teak which was 22.05°. The explanation of the results was mentioned because of genetic factors as environmental conditions were considered the same.

Herman et al. (1999) in Barnett and Bonham (2004) stated that fast-grown wood with a large increment width will produce wood with a larger MFA. This shows the relationship that a large growth rate will result in shorter cells so that MFA becomes larger, whereas according to

Pandit (2006), cell length is negatively correlated with MFA. Although JUN wood has a faster growth rate, due to the longer JUN wood fiber (where these properties appear because it is inherited or because it was developed from shoot cuttings so that the young JUN immediately has a fiber structure like mature teak), the MFA value of JUN became smaller. In addition, the slenderness ratio of JUN wood was 41.46 which was higher than that of conventional teak at 32.05, where the results were statistically different. Similar to the fiber length factor, the degree of slenderness was also negatively correlated with MFA.

However, Wahyudi (2000) showed a different pattern where the growth rate had no effect on the amount of MFA, as was the case with fertilization treatment. The value of JUN MFA which is smaller than conventional teak gives different results to the hypothesis that was made earlier. This fact is in line with Donaldson (1996) in Barnett and Bonham (2004) who obtained lower MFA values in young trees derived from shoot cuttings produced from mature trees, compared to young trees of the same type developed from seeds as a control. This is sufficient to explain why MFA JUN is smaller, namely because the use of shoot cuttings causes the characteristics of young JUN to reflect the properties of mature teak. JUN seedlings are shoot cuttings derived from selected old teak trees, where superior parental traits are directly passed on to their breed and produce plants with superior characteristics and appear even though the tree is still young (Purwanto 2005; Wibowo 2005b).

Bendtsen and Senft (1986) in Barnett and Jeronimidis (2003) stated that the angle of the cellulose microfibrils on the wall is a determining factor for the mechanical properties of wood. In Rowell (2005), microfibrils are likened to steel bars to strengthen the concrete structure. The orientation of the cellulose structural unit in this fiber affects the physical and mechanical properties of the fiber, especially density, tensile strength, stiffness, and shrinkage. Small changes in the degree of microfibril angle result in changes in fiber properties (Stuart and Evans 1994). The main characteristic of wood that is affected by the size of the MFA is the shrinkage in the longitudinal direction, where the shrinkage in the longitudinal direction will increase with the increase in the MFA, but has a non-linear relationship (Barnett and Jeronimidis 2003). Likewise, the value of the Modulus of Elasticity (MOE), the larger the angle of the microfibril, the smaller the MOE value so that the wood is only suitable for low-value applications. There is currently no standard that shows what the minimum MFA value is required for wood to be used as a construction material.

Although the MFA value tends to have an effect on longitudinal shrinkage, the smaller JUN MFA compared to conventional teak at the same age will most likely cause the shrinkage (T/R ratio) of JUN wood to be smaller and the wood to be more stable (as evidenced by the results of physical research) so that it will be more profitable when it will be manufactured for veneer, furniture, and other products; higher tensile strength and stiffness, as well as a straighter fiber direction (observable in the longitudinal plane of JUN wood), so as a consequence, less energy is used to process this wood because it is easier to work with.

The structure of the fiber cell walls or tracheids in trees is designed so that the trunk and branches are able to withstand both from external and internal pressures, such as the weight of the tree trunk, the weight of the canopy, and external pressures such as wind and gravity. A large MFA in young trees or seedlings is needed to make the tree more flexible and bend easily without breaking in the wind. Wood formed at the beginning of growth with a large microfibril angle refers to the properties of young wood (Barnett and Bonham 2004). The large MFA value in the young wood area causes the section to become weak with a smaller and less stable MOE value. However, as the tree grows, the trunk becomes stiffer to support the added mass of the trunk and crown, and the lower MFA value in the outer wood allows the tree to do that (Barnett and Jeronimidis, 2003; Barnett and Bonham 2004).

As previously mentioned, naturally trees will form wood with larger MFAs in the early stages of growth. JUN and conventional teak were still very young (5-years age), moreover with the juvenile content is 100%. Besides being developed from shoot cuttings, another possibility that causes the MFA value of JUN to be smaller than conventional teak is thought to be due to the faster growth of the stems with a wide crown shape, so that JUN must adapt to form small microfibril angles. Even though it has a smaller MFA value, JUN will succeed in becoming a tall tree, but not easy to collapse if it is supported by a solid foundation. The weaknesses of superior teak wood are that it grows sideways or collapses quickly once the tree begins to grow, but not with JUN wood. The existence of a stilt root form allows the tree made stiffer by forming small microfibril angles, but does not harm the tree when exposed to internal and external pressures, where this structure is not possessed by conventional teak or other superior teak woods (Figure 4).

Figure 4. The form of taproots in teak growing from seeds, adventitious roots from shoot cuttings and tissue culture, as well as stilt roots in JUN

A small MFA value is one of the parameters chosen in tree breeding. The breeding aim is to reduce the proportion of young wood that has a large MFA so that the properties of the wood will be better and its value will increase economically. However, because the demand for wood is very large, this is a problem because we currently use a lot of fast-grown wood from short rotation cropping (Barnett and Bonham 2004). We can make efforts to minimize the MFA, but it must be ensured that these efforts will not harm the trees. One of the efforts that was quite successful in JUN was by modifying the roots to become stilt roots.

Conclusions

Microfibril angle (MFA) JUN was 22.09°, smaller than the conventional teak 25.29°. The lower value of MFA JUN was due to the fact that the wood was developed from shoot cuttings so that although it was young, it reflected the characteristics of mature teak. Technological input in the form of stilt roots allows JUN to have a small MFA without endangering tree growth due to pressure from inside or outside.

The results of MFA measurements on JUN by using two methods, namely XRD and light microscope, had different values. It was different from the results of MFA measurements on conventional teak. The MFA value used in JUN was proposed to be measured by using X-Ray Diffraction because it is possible that due to accelerated growth, a simple pit with an oval shape turns into circular. This difference causes the results of the JUN MFA measurement using a light microscope based on the slope of the pit aperture to be inconsistent, subjective, and different results in other pits contained in the fiber even though they are closely associated. However, in conventional teak, measurements using a light microscope are possible because the shape of the pit is oval so that the slope of the elongation of the pit aperture can be determined easily, and it is more consistent with more uniform values in the same individual fiber.

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