Variation in the Cell Wall Mechanical Properties of *Dendrocalamus farinosus* Bamboo by Nanoindentation

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The *in situ* imaging nanoindentation technique was used to investigate the effect of age, culm height, and radial position on the cell wall mechanical properties of bamboo (*Dendrocalamus farinosus*) along the longitudinal direction of culms. The results indicated that among our four-sampled culm ages, the fiber cell wall had average values for the elastic modulus (MOE) and hardness (H_L) of 18.56 GPa and 410.72 MPa, respectively. The ages of the culm had no significant effect on the observed MOE and H_L among the 2-, 3-, 4-, and 5-year-old *D. farinosus* test specimens, with similar results observed at three different culm heights and radial positions. Furthermore, longitudinal MOE and H_L values along the thickness of the cell wall were uneven, with average values for the middle lamella and the edge near the cavity only 20.97% to 29.78% and 9.22% to 31.71%, respectively, of the values found in the cell wall.

Keywords: Dendrocalamus farinosus; Nanoindentation; Cell wall mechanical properties; Variation

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INTRODUCTION

Numerous investigations of mechanical characteristics and potential commercial applications of bamboo have shown that their extraordinary mechanical properties originate primarily from the fiber components (Amada and Untao 2001; Lo *et al*. 2004). Concerning *Dendrocalamus farinosus*, an amphipodial bamboo species, there has been little discussion of its microscale mechanical properties, even the variation of bamboo cell wall mechanics. Bamboo is a typical natural biological material with hierarchical structures from macroscopic to nanometer scale and the corresponding interfaces, which makes it urgent and important to fully understand the mechanical behaviour of bamboo at the micrometer and nanometer scales to explore potential commercial applications.

Nanoindentation, a method used to study the hardness and elastic modulus at the micrometer and nanometer scales, has been widely used in the mechanical characterisation of soft and hard tissues, cones, and plant fibers (Rho and Pharr 1999; Turner *et al*. 1999). Wimmer and Lucas (1997) first introduced nanoindentation to wood science by estimating the mechanical properties of the secondary wall (S_2) and the cell corner middle lamella (CCML) of spruce tracheids. Their work found that there was no significant difference in the longitudinal hardness (H_L) between the S_2 layer and the CCML, though the former had a much higher elastic modulus (MOE). While there was a high correlation (r = 0.74) between both H_L and MOE and the CCML, no such correlation was observed for the S_2 layer. Subsequently, Gindl *et al*. (2002) and Gindl and Schöerl (2004) used this approach to study the relationship between the microfibril angle (MFA) and lignification to H_L and MOE in the S_2 layer of tracheids. Finally, Jiang *et al*. (2004) investigated the difference in MOE and H_L between the cell walls of early-wood and late-wood tracheids. In addition, the mechanical properties of other biomass materials, such as reed stalk, cotton stalk, and soybean stalk, were studied by nanoindentation (Wu *et al*. 2010; Wang *et al*. 2013).

Bamboo can be considered to be a natural fiber-reinforced composite that consists mainly of two components: (a) reinforcement (in this case the fibers) and (b) matrix (hemicellulose and lignin). It is generally known that the radial gradient distribution of fibers plays a critical role in determining the natural gradient of macro-mechanical properties of bamboo (Ray *et al*. 2005; Tommy *et al*. 2004). Recently, interest in bamboo fiber-reinforced composites has also led to more research classifying the micro-mechanical and cell wall mechanical properties of bamboo fibers (Takagi *et al*. 2003). Yu *et al*. (2007) applied the nanoindentation technique to measure differences in the longitudinal and transverse mechanical properties of Moso bamboo (*Phyllostachys pubescens*), a monopodial bamboo that is the most important commercial bamboo species in China.

This paper focused on *D. farinosus*, which is predominantly found in southwestern China. Due to a smaller diameter at breast height and thinner cell wall compared to Moso bamboo, *D. farinosus* is typically not used for industrially processed high value products. However, larger holocellulose and longer structural fiber components make this species a good source of biomass for pulping, papermaking, and fiber-reinforced composite materials. However, the authors were unable to find any publication of the microscale mechanical properties, even the variation of bamboo cell wall mechanics.

In the present study, samples were prepared from a block of bamboo fiber band sectioned on an ultramicrotome with a diamond knife. This approach avoided the need to use the resin embedding process, thereby greatly simplifying the preparation procedure; the nano-indenter incorporates a unique *in situ* imaging function that improves the location reliability of results. The samples were prepared to investigate the effects of bamboo culm age, height position, and radial position on *D. farinosus* cell wall mechanical properties, as well as to record the differences between the secondary wall, middle lamella, and edge of cavity sections. The load-depth curve method (Oliver and Pharr 1992) was used to determine the H_L and MOE values from nanoindentation measurements. This is the first time that nanoindentation testing has been conducted on an amphipodial bamboo. Therefore, apart from helping to establish the relationship of the macro scale mechanical responses of *D. farinosus* to its micro and nano scale mechanical properties, this is also an important first step to enhancing the utilisation of amphipodial bamboo, a major sub-set of bamboo.

MATERIALS AND METHODS

Sample Preparation

For the study, *Dendrocalamus farinosus* samples with ages of 2, 3, 4, and 5 years were collected from Zhuhai town, Sichuan province, China. The selected bamboo culms had an average external diameter of 65 mm and average length of 602 mm, and they had no obvious visible decay or defects. Bamboo blocks of approximately *t* (radial thickness) × 5 mm (tangential) × 5 mm (longitudinal) were cut from the base (approximately 1.5 m from the ground), middle (approximately 3.5 m from ground) and upper regions (approximately 5.5 m from ground) of the culm for each of the sampled ages.

To study the mechanical properties of the cell wall in the radial direction, the samples were prepared using a procedure similar to that suggested by Wimmer *et al*. (1997), neglecting their Spurr resin embedding process. This was based on the fact that the bamboo single fiber cell often had thicker wall and smaller cavity compared to spruce tracheids, which provided enough space to accurately hit the cell wall. In addition, we wanted to reduce the effect of embedded Spurr resin on the mechanical properties of the edge near the cavity and largely simplify the sample preparation. First, the blocks were softened to allow cutting with a sliding microtome (SM2000R, Leica, Germany) of an inverted pyramid along the culm in the longitudinal direction. A sample holder was used to fix and keep the polished surface of the sample vertical to the indenting direction. Next, a cross-section of the sample was polished with an ultra-microtome using a diamond knife to obtain a very smooth surface for indentation. Before testing, samples were left in the nanoindentation instrument at a temperature of 23 °C and relative humidity of 50% for a week to eliminate the potential influence of moisture on the cell wall mechanical properties. An image of prepared samples in the radial direction is given in Fig. 1.

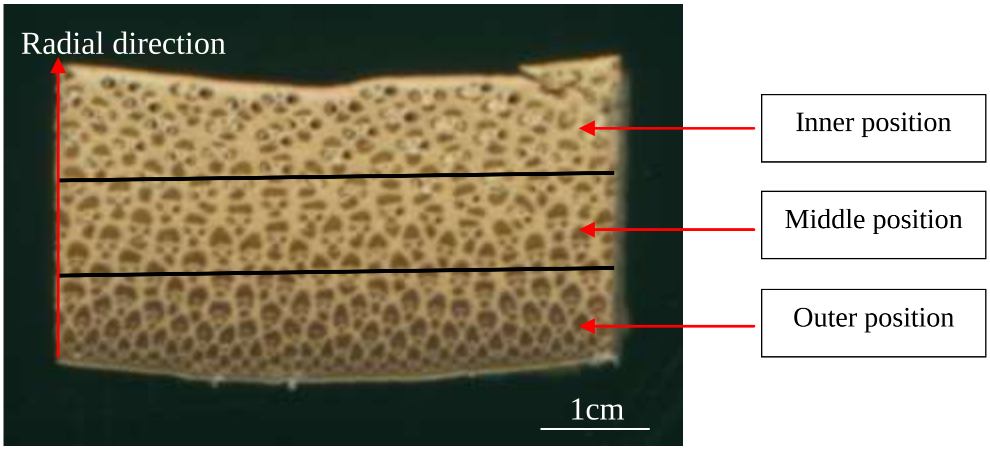

Fig. 1. A cross-section image of *D. farinosus* samples in the radial direction

Nanoindentation Testing and MFA Measurement

A triboindenter (Hysitron TI 950, USA) was used to determine the cell wall mechanical properties (hardness, elastic modulus, and residual deformation) of *D. farinosus* fibers using the *in situ* imaging nanoindentation technique. A Berkovich diamond tip with a radius of curvature of less than 100 nm was adopted for scanning and indentation.

Figure 2 shows the “advanced feedback force control mode”, which was applied to produce a more accurate control of a peak load of 250 µN. The loading rate and hold time used were 50 μNs^{-1} and 6 s, respectively. The holding segment was used to evaluate the creep rate of the cell wall and to minimise the creep component in the following unloading segment. The load and displacement resolutions were 1 nN and 0.01 nm, respectively.

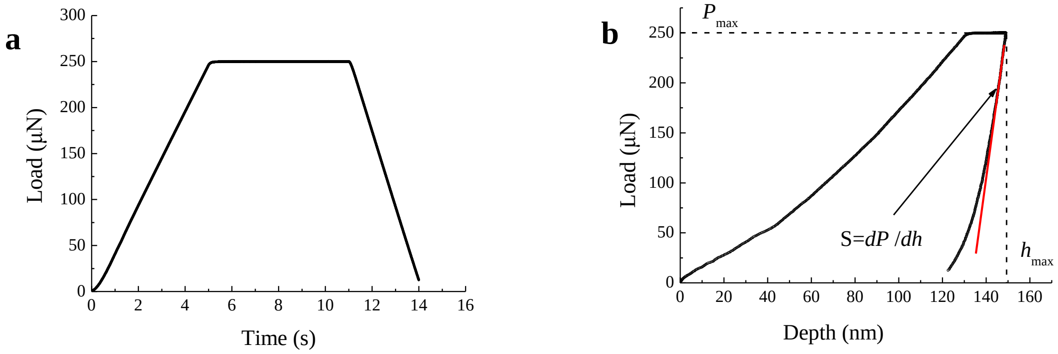

Fig. 2. (a) Load-time curve of a nanoindentation test (b) and typical loading and unloading curves and the relevant parameters

From the load-depth curve (Fig. 2b) recorded during the nanoindentation experiment, the key parameters, peak load (P_{max}) and depth at peak load (h_{max}), as well as the initial slope of the unloading curve (S) were determined. The elastic modulus and hardness of bamboo fiber cell wall were then calculated according to the slope (S) of the unloading curve using equations from Oliver and Pharr, as follows,

$$\frac{1}{E_r} = \frac{1-\upsilon^2}{E} + \frac{1-\upsilon_i^2}{E_i} , \tag{1}$$

$$E_r = \frac{\sqrt{\pi}}{2\beta h_c}\frac{S}{\sqrt{24.5}} , \tag{2}$$

where E_i and ν_i are, respectively, the elastic modulus and Poisson ratio of the tips. For diamond tips, E_i is 1141 GPa and ν_i is 0.07. E_r is called the reduced elastic modulus, a value that can be directly attributable to the instrument itself. E and ν are, respectively, the elastic modulus and Poisson ratio of the samples. For most materials, ν varies from 0.15 to 0.35, which results only in 5% error for E,

$$H = \frac{P_{max}}{A_c} , \tag{3}$$

$$h_c = h_{max} - \varepsilon\frac{P_{max}}{S} \tag{4}$$

where P_{max} is the peak load; A_c, the area of the indent, is determined based on the contact depth h_c, which depends on the indenter shape; and ε is a constant that depends on the geometry of the indenter (ε = 0.75 for a Berkovich indenter).

Furthermore, for data analysis of the residual indentations in the cell wall, it was necessary to determine whether they accurately hit the cell wall. There is a possibility that some indentations may hit the middle lamella or the edge of cavity, as is shown in Fig. 3.

One-way variance analysis on mechanical properties with age, culm height, radial position, and different cell wall layers of *D. farinosus* bamboo fiber was performed to evaluate their effects to the elastic modulus and hardness.

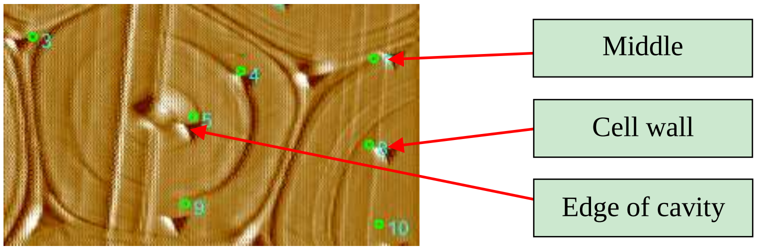

Fig. 3. Residual indentation shows the actual positions of the indentations

To determine the microfibril angle (MFA) variation associated with age, culm height, and radial position of bamboo fibers, specimens were cut from the outer position to the inner position in the tangential direction with dimensions of 1 mm thickness, 10 mm width, and 30 mm length. This was tested using a powder X-ray diffractometer (X'Pertpro, Panalytical, USA). The mean MFA of several hundred cells was determined by a diffraction pattern according to the method of Cave (1997), which was obtained with a radiation source of CuKα, a tube voltage of 40 kV, and a tube current of 40 mA.

RESULTS AND DISCUSSION

Effect of Bamboo Age on Cell Wall Mechanical Properties

The hardness values below 100 nm indentation were highly variable due to surface roughness. However, values from indentations above 100 nm had no significant influence on the stiffness and modulus (Tze *et al.* 2007; Wang *et al.* 2006; Jiang *et al.* 2004). Therefore, the authors adopted the average MOE and H_L from the 100 to 200 nm range of indentation for every indentation of the fiber cell wall. From the load-displacement curve (Fig. 2b), one can see that the bamboo cell wall mostly undergoes plastic deformation when the nano-indenter vertically contacts the cell wall of the bamboo fibers in the longitudinal direction. The residual deformation (120 nm) accounted for the maximum deformation (160 nm) of all the samples with a result of approximately 75%, while elastic deformation accounts for only 25%.

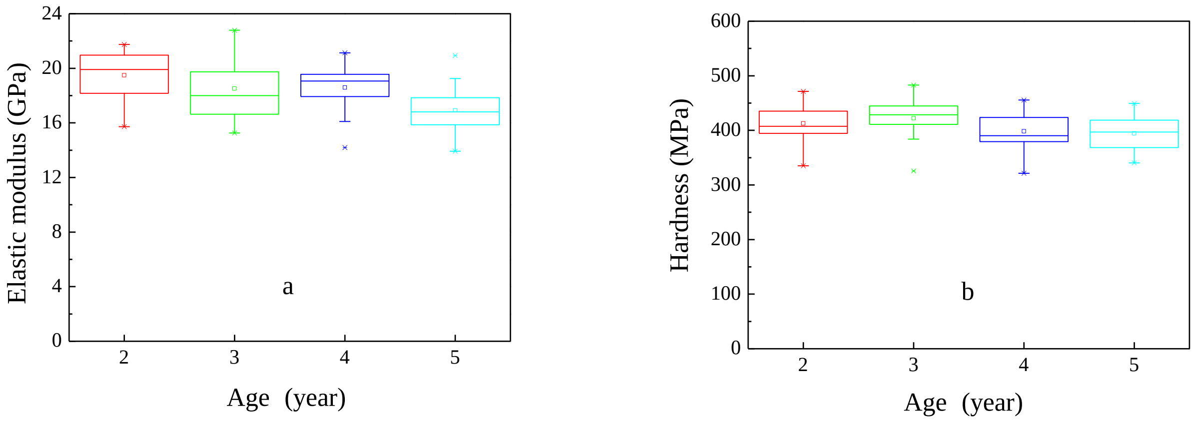

Fig. 4. Box-and-whisker plots of (a) elastic modulus and (b) hardness of bamboo fibers of different ages

Statistical analysis of 110 valid hits in the cell wall across the four sampled ages indicated that the average values of MOE and H_L for *D. farinosus* fiber cell walls were 18.56 GPa and 410.72 MPa, respectively. Figure 4 shows there was little difference in MOE and H_L among the four ages, except for a slightly lower MOE in 5-year-old samples and a higher H_L in 3-year-old samples. Analysis of variance indicated that there was highly significant difference ($p < 0.01$) between 2-, 3-, 4- and 5-year old bamboo with respect to MOE, and significant difference ($p < 0.05$) to H_L (Table 1). The mechanical properties of the fiber cell wall are affected by a number of factors, such as density, the moisture content (MC), chemical composition, MFA, *etc*. Cave *et al.* (1969) revealed that longitudinal elastic modulus is highly dependent on the microfibril angle, but no obvious differences were found in the microfibril angles of the four sampled ages (Fig. 5). That implied the MFA might not the major factor for the variation of the mechanics in the fiber cell wall. The cell wall of bamboo fiber continued to thicken, acquire multiple layers and lignify over growing years. Gindl and Gupta (2002a) determined that the lignification had an obvious effect on the mechanical properties of cell wall. Which meant the density or chemical composition changing with the bamboo ages affected the mechanical properties.

Table 1. P and Sig. of Variance Analysis on Mechanical Properties

	Age	Culm height	Radial position	Different layer
MOE	.008**	.039	.130	.000**
H_L	.020*	.322	.303	.000**

**means highly significant difference at $p < 0.01$

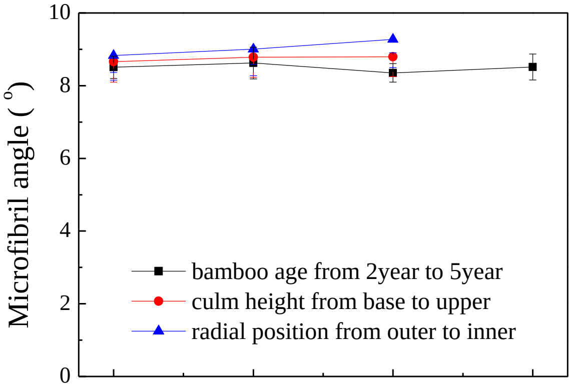

Fig. 5. Relationship of microfibril angle to bamboo age, culm height, and radial position

Effect of Positions on Cell Wall Mechanical Properties

Based on statistical analysis of 83 valid indentation data sets from the base, middle, and upper positions of the 4-year old *D. farinosus* bamboo fiber cell wall along the culm height, the authors observed average values of MOE and H_L of 17.87 GPa and 403.26 MPa, respectively. The cell wall MOE of 4-year-old bamboo from the base (18.58 GPa) to the upper position (17.41 GPa) along the bamboo culm showed a slight decrease, but no remarkable differences (Fig. 6a and Table 1). There was small or no remarkable difference observed for H_L of the *D. farinosus* cell wall among the three culm positions (Fig. 6b and Table 1).

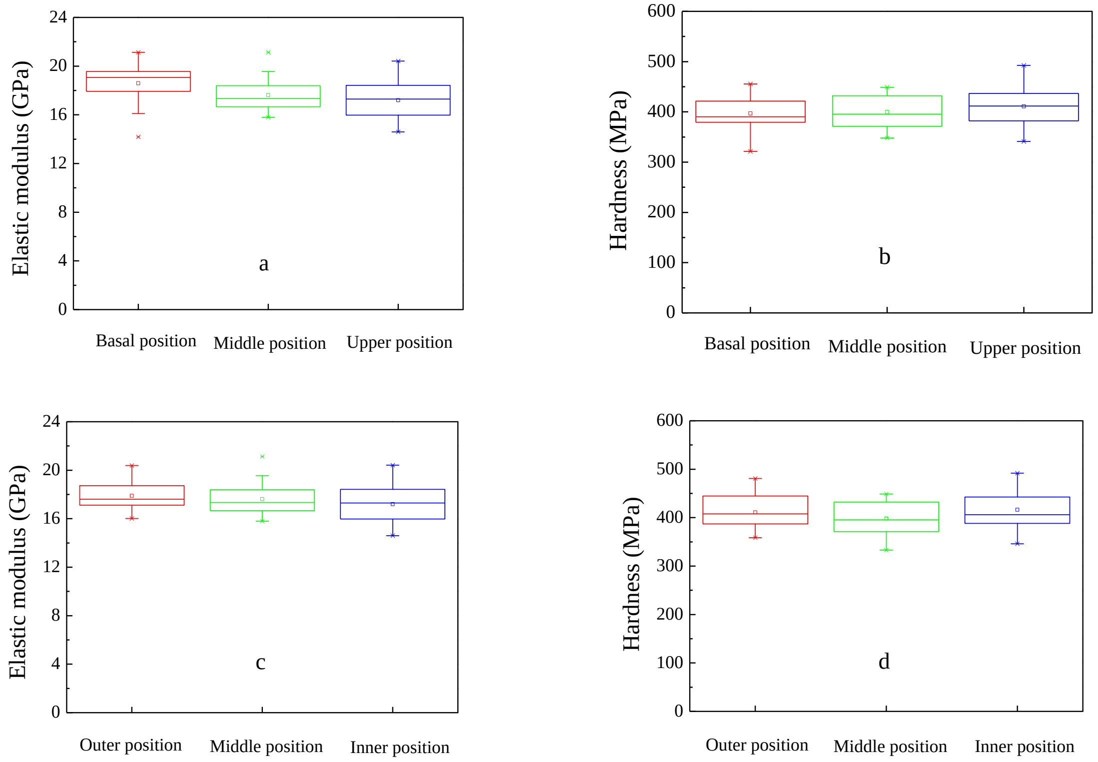

Fig. 6. Box-and-whisker plots of (a) elastic modulus and (b) hardness of bamboo fibers with culm heights; and (c) elastic modulus and (d) hardness with radial positions

In Fig. 6c and d, a statistical summary for 90 valid indentation data sets taken from 4-year old *D. farinosus* cell walls at the outer, middle, and inner positions in the radial direction are shown. The average MOE and H_L for this data set were 17.87 GPa and 403.26MPa, respectively. From the figure and variance analysis (Table 1), MOE and H_L in the cell wall showed no significant differences among the three radial positions, which is consistent with other studies that show a constant longitudinal MOE from the outer portion to the inner portion (Yu *et al*. 2007). Figure 5 also shows that there is a stable microfibril angle (8 ° to 9 °) along the height of the bamboo culm from the base to the upper position, as well as in the radial orientation from the outer to the inner position. This result conforms to the findings of Cave *et al*. (1969).

Longitudinal Mechanical Properties of Different Cell Wall Layers

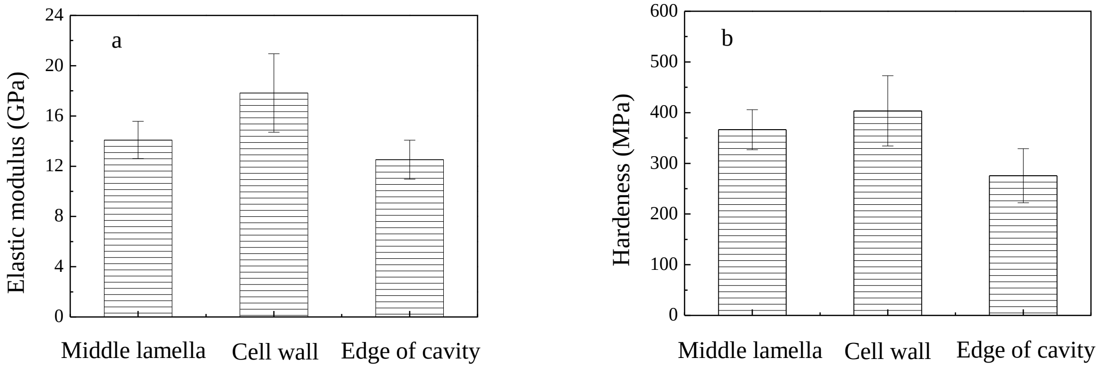

Fig. 7. (a) Longitudinal elastic modulus and (b) hardness of middle lamella, cell wall, and the edge of cavity of *D. farinosus* bamboo fibers

Residual indentations of numbers 3 and 7 hitting in the middle lamella, numbers 4, 8, and 9 hitting in the cell wall, and number 5 hitting the edge of cavity of *D. farinosus* are shown in Fig. 3. We also conducted statistical analysis for the longitudinal MOE, as shown in Fig. 7a and b. Indentations of the middle lamella and the edge of cavity were rare, occurring 19 and 10 times, respectively. These incidences occurred in regions that were less suitable for indentation. The distribution of longitudinal MOE and H_L along the thickness of the cell wall was uneven, with much lower values found in the middle lamella and the edge of cavity than in the cell wall layer. The rough nature of the surface may mean that there were more of the hemicellulose and lignin components in the middle lamella and the edge of cavity. Figure 7 indicates that the average values of the longitudinal MOE and H_L of middle lamella were lower by 20.97% and 9.22%, respectively, compared to those measured in cell wall layers. This can be explained by the fact that the longitudinal MOE of the cell wall in *D. farinosus* bamboo fibers was significantly larger than that of the middle lamella (Table 1). The hardness showed a significant difference between the cell wall and middle lamella (Table 1), supporting the results of Yu *et al*. (2006) for softwood tracheids. Average values of the longitudinal MOE and H_L of the edge of cavity are also lower by 29.78% and 31.71%, respectively, than those measured in the cell wall layer. Jakes *et al*. (2011) discussed that when indents were placed sufficiently close to the edge of fused silica and poly (methyl methacrylate), the modulus and hardness decreased, which confirmed above-mentioned results. This indicates that there are significant differences between the cell wall and the edge near the cavity with respect to both MOE and H_L.

CONCLUSIONS

1. The *D. farinosus* fiber cell wall had average values for the MOE and H_L of 18.56 GPa and 410.72 MPa, respectively, among the four sampled culm ages.
2. Longitudinal MOE and H_L of *D. farinosus* bamboo fibers were significantly different ($p<0.01$) among the four age groups, but not significantly different along the longitudinal and radial direction.
3. The distribution of longitudinal MOE and H_L along the thickness of the cell wall was uneven, with average values that were lower by 20.97% and 9.22%, respectively, at the middle lamella and 29.78% and 31.71%, respectively, at the edge near the cavity, compared with those measured in the cell wall layer.

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