



RESEARCH ARTICLE

RELATIONSHIP BETWEEN CHEMICAL COMPOSITIONS AND ANATOMICAL STRUCTURE ON THE MATURITY OF 4-YEAR-OLD CULMS *SCHIZOSTACHYUM BRACHYCLADUM* KURZ

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ABSTRACT

The chemical composition and anatomical structure on mature *Schizostachyum brachycladum* Kurz investigated. Harvested four-year-old bamboo culms segregated into the bottom, middle and top portions. The samples then undergo the Scanning Electron Microscopy (SEM) to determine their structure such as vascular bundle, parenchyma, and sclerenchyma, and Fourier Transform Infrared Spectroscopy (FTIR) to detect and quantify the changes of chemical composition of bamboo under the different variables which were extractive and the free extractive. The anatomical structures and the chemical compositions of the bamboo culms such as the extractive, holocellulose, alpha cellulose, hemicellulose, and lignin contents were analyzed. Standards by the Technical Association of the Pulp and Paper Industry (TAPPI) followed. Results show the chemical composition especially the extractive, and alpha cellulose were higher at the bottom portion around 5% and 54% respectively, while top portions shows higher in the value of the holocellulose and hemicellulose at 90% and 51%, respectively. The lignin content was higher in the middle portions at 20%. The SEM viewed cell wall structure of vascular bundle, parenchyma, sclerenchyma, and vessel. The different part of mature *S. brachycladum* Kurz which are top, middle and bottom at the 50X magnification viewed. The FTIR spectroscopy which are applied for the determination, quantify, and detected of chemical composition, lignin distribution, changes in wood properties during wood composites manufacture, wood density and discrimination of wood and non-wood from various species. The O-H stretching absorption bands (around 3600-3200 cm⁻¹) and C-H absorption bands (around 2927 cm⁻¹) have contributions from all these chemical components.

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INTRODUCTION

Bamboo, the most significant member of grass family of *Gramineae* consisting of more than 1250 species with 75 genera recorded in the worldwide (Mudoj et al., 2013). About 70 species of these are found growing in Malaysia which comprises about 7% of the lowland and highland forests areas. These bamboos consisted of 25 cultivated and 45 indigenous species (Bahari and Krause, 2016; Wahab, 1998). Others, bamboo also is a fast-growing species and can mature within 3 to 4 years after cultivating (Wahab et al., 2018). The primary chemical composition in the bamboo culms mainly in the cell wall consisting of cellulose, hemicelluloses, lignin, and extractive. According to Wahab et al., (2013 and 2005), the percentages indicate that cellulose, hemicellulose, and lignin

were dominant content in cell wall structure with the composition 90%-98% while extractive consists at about 2-10%. The other chemical compositions are the lignin and hemicelluloses (each amounted to about 20-30%), pentosans (20-25%), and holocellulose (60-70%). Additionally, the waxes, tannins, resins and inorganic salts considered as the minor constituents. Anatomically, the whole culms of bamboo comprise 40% fibers, which consisted of vascular bundle and sclerenchyma. The remaining 60% of total culm are considering of parenchyma (Wahab et al., 2015). The main chemical constituents in bamboo vary depending on the species, age, positions in the culms length and habitat where they are found. Studies in the bamboo culms chemical constituents undertaken by researchers so far have confined to species with thick culms wall. No previous studies have ever been recorded on the bamboo with thin culms wall. Thin culms wall bamboo species such as *S. brachycladum* Kurz has been used for making flooring and panels by the rural communities as they easy to process and work with (Anokye et al., 2014).

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MATERIALS AND METHODS

The four-year-old culms of *S. brachycladum* Kurz randomly selected and harvested from the forest areas in Jeli, Kelantan. Bamboo culms chose with diameters in the range from 5 to 6 cm. They were segregated into portions bottom, middle and top. The culms were cut approximately 30 cm from ground level. The portions were later grounded into powdered form and screened to collect the comparable size of the sample for subsequent studies. Samples for the structure of cell wall prepared separately for the Scanning Electron Microscopic (SEM) and Fourier Transform Infrared Spectroscopy (FTIR).

Qualitative analysis of Extractive for *S. brachycladum* Kurz: The extractive content determined by TAPPI T 264 om-97, (1997). The powder form of bamboo weighed at 10 gram. The samples then put in the extraction thimble which size 125 mm x 30 mm. Ethanol-benzene (at ratio 2:1) was as a solvent in the extraction process. The amount of the solvent used in this extraction process was 300 ml for 5-7 hrs. The solvent contained extractive was evaporated using Rotary Evaporator machine to remove all the solvent. In the next stage, the extractive dried in an oven at $103 \pm \text{two } ^\circ\text{C}$ for 24 hours, and the percentage of extractive content calculated.

Determination of Holocellulose for *S. brachycladum* Kurz: Holocellulose content determined according to Wahab (1998), Salim *et al.*, (2008) and Mustafa *et al.*, (2017) method. Approximately 5 gram air-dried free extractive powder used as a sample for holocellulose analysis. Then, total of 9-gram Sodium Chloride (NaClO_2), 30ml 10% acetic acid and 100 ml distilled water mixed with a free extractive sample for the period (within 5 hours). Next procedure, the residue was heated and stirred at 70°C . Lastly, the sample dried and the percentage of holocellulosecalculated.

Determination of Alpha Cellulose *S. brachycladum* Kurz: The alpha cellulose determined according to TAPPI 203 os-74, (1997) method. Approximately 2-gram holocellulose powder form used as a sample for alpha cellulose content analysis. A total of 75 ml 17.5% NaOH in stirring condition for 15 minutes were mixed slowly with holocellulose powder. In the stage, 100 ml of cold distilled water was added to the mixture and continuously stirred. After 30 minutes, the sample filtrated and washed with 8.3% NaOH and 2N acetic acid. The sample left to dry and weighed to measure the percentage of alpha cellulose.

Determination of Lignin Content for *S. brachycladum* Kurz: Lignin content determined according to TAPPI 222 om-88, (2002) standard. The dry air of extractive-free sample was used to determine the lignin content the part of bamboo species. Firstly, 1 gram extractive free sample mixed with 25 ml of 72% sulphuric acid (H_2SO_4) then the mixture slowly stirred for 2 hours in cold condition. Next stage, 560 ml distilled water added and heated at 180°C for 4 hrs. The mixture then filtrated and dried before weighed.

Anatomical structure analysis using Scanning Electron Microscopy (SEM): *S. brachycladum* Kurz visualized under Scanning electron microscopy (SEM) to characterize their morphology structure such as vascular bundle, parenchyma fiber, vessel and structure of sclerenchyma (Wahab *et al.*, 2002). The SEM micrographs taken from the cross-section of samples into 5mm x 5mm size of samples block.

The samples with the dry condition were coated with gold by an ion sputter coater (Polaron SC515, Fisons Instruments, and United Kingdom). Then, the samples visualized by Scanning Electron Microscope LEOSUPRA 55 VP, Field Emission SEM, Carl-Zeiss, Oberkochen, Germany.

Determination of Fourier Transform Infrared Spectroscopy (FTIR) on *S. brachycladum* Kurz: The functional group present determined using Fourier Transform Infrared (FTIR) spectroscopy with Perkin Elmer spectrum. Perkin Elmer spectrum 100 was used to analyze the spectra for each sample. About 0.1 gram sample from extractive, extractive free and natural bamboo used for examining sample on spectra. The sample was placed into spectra laser using Attenuated Total Reflexion mode (ATR) were obtain in the range of 4000 cm^{-1} to 600 cm^{-1} . Spectral output recorded in the transmittance followed by wave number.

RESULTS AND DISCUSSION

The major chemical compositions found in the 4-year-old of *S. brachycladum* Kurz were the extractive, holocellulose, alpha cellulose, hemicellulose and lignin content (Figure 1). Firstly, the extractive content indicated that the higher value on bottom portion with 4.52% then followed by the middle and top portions which were 4.08% and 3.59%, respectively. These because the bottom portions had a more significant surface area of parenchyma fiber (Figure 2) that believe acts as extractive content (Liese, 1985; Wahab *et al.*, 2005). The top of *S. brachycladum* Kurz had the higher content of holocellulose and hemicellulose with 89.69% and 50.93%. According to Sulaiman *et al.*, (2016) and Wahab *et al.*, (2014) study, the holocellulose content was collected using the process of pre-treatment to removes lignin, and it proofed by these study which was higher of holocellulose content proportionally lower of lignin content on the bottom portion. Besides, the middle and bottom of them indicated which were 87.82% and 85.30% for the holocellulose while 43.33% and 31.56% for the hemicellulose content. Moreover, the alpha cellulose content of matured *S. brachycladum* Kurz indicated the higher value on the bottom, then followed by middle and top which were 53.74%, 44.63%, and 38.76%, respectively. Nonetheless, the lignin of matured *S. brachycladum* Kurz shown the higher content on the middle portion with 20.36%. Besides, followed by the top and bottom portion with were 17.93% and 16.39%.

The statistical analysis of ANOVA in Table 1 highlighted that the significant difference ($P \leq 0.05$) between portions on extractive, holocellulose, alpha cellulose and hemicellulose content which were 0.000, 0.001, 0.000, and 0.000, respectively. Also, for lignin content, the result indicated that the significant difference ($P \leq 0.05$) with 0.003. But then, Figure 1 shows no significant difference between the bottom and top portion. The Scanning Electron Microscopy (SEM) was viewed cell wall structure of vascular bundle, parenchyma, sclerenchyma, and vessel. Figure 2 indicated that the different part of mature *S. brachycladum* Kurz which are top, middle and bottom at the 50X magnification viewed. Based on the figure, the top part had the more massive and complex structure of vascular bundle compared to the middle and bottom part. According to Liese, (1985) and Wahab *et al.*, (2010) the vascular bundles were smaller and more numerous at the peripheral zone of the culm while it was more significant and fewer in the inner parts (Figure 2 and Table 2).

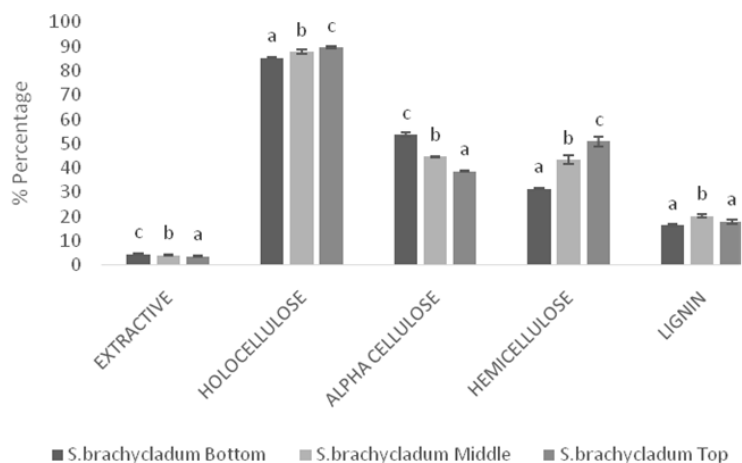


Figure 1. Percentage of major chemical compositions for the 4-year-old *S. brachycladum* Kurz

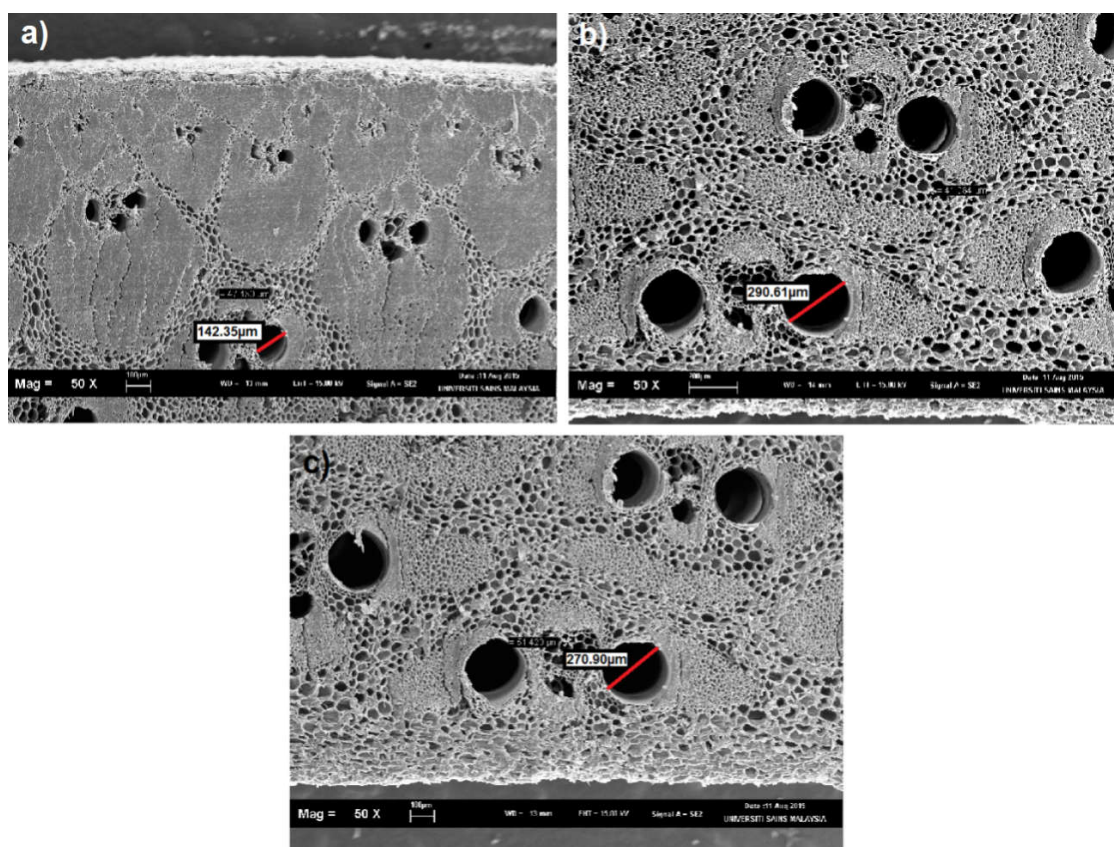


Figure 2. Scanning Electron Microscopy (SEM) at 50 x magnification were visualized in a different part of the 4-year-old *S. brachycladum* Kurz transverse cross-section which was the top (a), middle (b) and bottom (c).

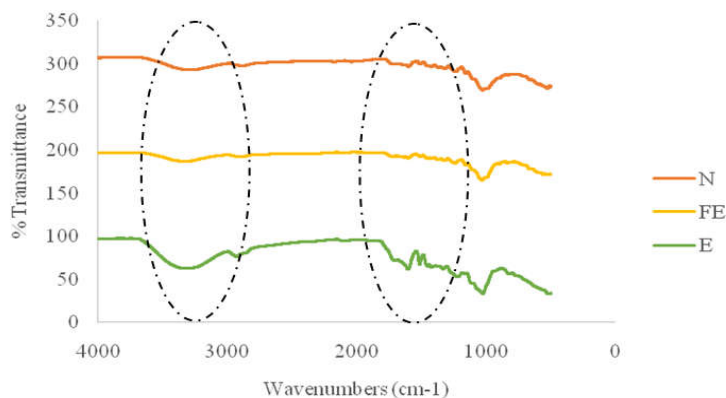


Figure 3. FTIR examined in the 4-year-old *S. brachycladum* Kurz for the natural sample (N), free-extractive (FE) and extractive (E); (sources: FE offset to 100% of transmittance and N offset to 200% of transmittance)

Table 1. Analysis of Variance (ANOVA) for chemical composition in the 4-year-old *S. brachycladum Kurz*

		Sum of square	Mean square	F
Extractive	Between groups	1.354	0.677	81.252*
	Within groups	0.050	0.008	
Holocellulose	Between groups	29.165	14.583	33.116*
	Within groups	2.642	0.440	
Alpha cellulose	Between groups	341.871	170.936	706.767*
	Within groups	1.451	0.242	
Hemicellulose	Between groups	571.753	285.877	124.747*
	Within groups	13.750	2.292	
Lignin	Between groups	24.086	12.043	18.775*
	Within groups	3.849	0.641	

*indicated the significant level at 99%.

Table 2. Sizes of vessel and parenchyma fiber on Scanning Electron Microscopy (SEM)

Location	Vessel (μm)	Parenchyma (μm)
Top <i>S.brachycladum</i>	142.35	47.13
Middle <i>S.brachycladum</i>	290.61	41.08
Bottom <i>S.brachycladum</i>	270.90	51.42

Besides, Figure 2 also indicated that bottom part had the complex structure of vascular bundle and parenchyma fiber with a proportionally content higher content of extractive. It is proof by reported from Espiloy, (1985) and Wahab *et al.*, (2010), the structure and thickness of cell wall, the width of a cell, and the relative proportions of different types of cells were affected an amount of extractive content. Others factor that affects the higher content of extractive is the presence of a larger area of parenchyma cell (Liese, 1985). Nevertheless, holocellulose is content the cellulose and hemicellulose. As shown in SEM figure, the top part consisted high amount of holocellulose which is cellulose content at 60% on the outside, and the inside of culm indicated to 40% (Janssen, 1985). The middle part had the bigger vessel (about to 290.61 μm), and that believe consists rich of lignin content (Table 2). Based on the previous study, the walls of metaxylem vessels of bamboo were characterized by a middle lamella and a primary wall together with a well-developed gap of the secondary wall into S1 and S2 (Liese, 1985 and Wahab 1998). While the secondary wall of bamboo fibers consisted of alternate wide and narrow layer with normally can reach 9 or even more number of cell wall layers. Based on that matter, the lignin content was richer in the narrow layer, and it has transverse cellulose microfibril angle (Li *et al.*, 2014). The Fourier transform infrared (FTIR) spectroscopy applied for the determination, quantify, and detected of chemical composition, lignin distribution, changes in wood properties during wood composites manufacture, wood density and discrimination of wood and non-wood from various species (Sulaiman, 2017; Wahab *et al.*, 2015a; Wahab *et al.*, 2015b; Zhou *et al.*, 2011; Pandey and Pitman, 2003; Pandey and Pitman, 2004; Li *et al.*, 2011; Shi *et al.*, 2012). Figure 3 shows the O-H stretching absorption bands (around 3600-3200 cm^{-1}), and C-H absorption bands (around 2927 cm^{-1}) have contributions from all these chemical components (Shi *et al.*, 2012). On the otherhand, the absorption bands around 1640 cm^{-1} and 1604 cm^{-1} region on an extractive sample of mature *S. brachycladum Kurz* shows a significant different of C-O stretching vibration of the alpha-keto carbonyl in the cellulose component compared to the extractive-free and natural bamboo (Wahab *et al.*, 2016 and 2014). Nevertheless, according to Wahab *et al.*, (2014) the absorptions bands in the region 1248-1049 cm^{-1} on mature *S. brachycladum Kurz* were attributed to C-O stretching

vibrations of aliphatic primary and secondary alcohols in cellulose, hemicellulose, and lignin. Although, more absorptions bands region could see in the extractive but distinctly different with extractive free and natural bamboo of te 4-year-old *S. brachycladum Kurz* at 1268 cm^{-1} , 1244 cm^{-1} , 1158 cm^{-1} , 1122 cm^{-1} , 1048 cm^{-1} and 898 cm^{-1} represented as guaiacyl ring breathing of C-O stretch in lignin, syringyl ring and C-O stretch in lignin and xylan, C-O-C vibration in cellulose and hemicellulose, aromatic skeletal and C-O stretch, C-O stretch in cellulose and hemicellulose, then C-H deformation in cellulose, respectively (Pandey and Pitman, 2003; Pizzo *et al.*, 2015; Shi *et al.*, 2012; El Oudiani *et al.*, 2009).

Conclusion

The bottom portions of the 4-year-old *S. brachycladum Kurz* having vessel sizes of 279.90 μm and parenchyma fiber diameters 51.42 μm contain the higher percentage of extractive at 4.54% compared to the middle and top portion. The alpha cellulose also shows higher content in bottom portions with 53.74%. The middle portions having vessel sizes of 290.61 μm and parenchyma fiber diameters 41.08 μm contain greater lignin at 20.36%. The top portions having vessel sizes of 142.35 μm and parenchyma fiber diameters 47.13 μm possesses higher amounts of holocellulose and hemicellulose at 89.69% and 50.95%, respectively.

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