Chemical and Anatomical Properties of *Dendrocalamus giganteus* Sheaths as Pulp Fiber

Hui Zhan Zhao-Hui Niu Mao-biao Li
Chang-ming Wang Shu-guang Wang

Abstract

Bamboo sheaths are typical, lignocellulosic, forest and agricultural residues in bamboo stands. This study investigated the potential for use of *Dendrocalamus giganteus* sheaths as the raw material for paper making, based on their morphological and chemical properties at various growth stages. Undifferentiated and differentiated vascular bundles of sheaths were studied, and phloem sheaths were found to have more fiber than protoxylem sheaths. The parenchymal cells adjoining the differentiated vascular bundles showed decomposition and formed air cavities. *D. giganteus* sheaths contained medium-sized fibers, and both the slenderness ratio and the Runkel ratio showed high suitability as pulp fiber. The chemical composition demonstrated moderate differences at various growth stages. Overall, *D. giganteus* sheaths had a low ash content and lignin and holocellulose contents that were comparable to those of traditional pulp fibers. Therefore, *D. giganteus* sheaths have a promising potential as pulp fiber.

Lignocellulosic forest and agricultural residues are composed primarily of holocellulose and lignin. To alleviate shortages, the pulp and paper industry has focused increasing attention on woody raw materials from forest and agricultural residues, because those fibers are annually or perennially renewable, abundant, and cost-effective (Nasser et al. 2015, Tutus et al. 2015). Moreover, the use of forest and agricultural residues in paper production can mitigate environmental pollution and enhance social and economic returns. Studies have examined a wide variety of forest and agricultural residue as alternative fiber sources; these include wheat and rice straw (Rousu et al. 2002); sorghum, canola, and corn stalks (Abdul Khalil et al. 2006, Enayati et al. 2009); banana pseudostems (Cordeiro et al. 2004); oil palm fronds, coconut and pineapple leaves, and Napier grass (Tran 2006, Daud et al. 2014); and tea waste (Tutus et al. 2015). Those studies have had encouraging results, suggesting that nonwood raw materials could become a principal resource for the pulp and paper industry and for new applications and could also mitigate the environmental hazards associated with plant waste disposal. Forest or agricultural residues are produced throughout the year and generally contain lower levels of lignin (Cordeiro et al. 2004), and delignification of such residues is more efficient and has milder and faster cooking conditions than wood-fiber materials have (Cordeiro et al. 2004).

During the last decade, bamboo plantations have been established in large areas of China to supply bamboo culms to the pulp and paper industry, producing large quantities of bamboo sheaths as the typical residue of the bamboo forest, as alternatives to depleting timber resources. Bamboo shoots are usually wrapped tightly in overlapping sheaths to protect and support their elongating nodes and internodes. When internode elongation is complete, the sheaths dry out and eventually fall off. Most sheaths are scattered in situ and decompose to enrich soil fertility or are collected for use as firewood by local farming households. An estimated 15.7 million tons of bamboo sheaths are available annually in China (Zhao et al. 2013). Yunnan Province has China's greatest diversity of bamboo species and is very interested in making the most of its bamboo resources. No studies, to our knowledge, have been conducted on whether bamboo sheaths would be an effective and appropriate source of

The authors are, respectively, Assistant Professor, Southwest Forestry Univ., and Key Lab. of Biodiversity Conservation in Southwest China, China's State Forestry Admin., Yunnan, Kunming, People's Republic of China (zhanhui_99@163.com); and Assistant Professor (920344870@qq.com), Associate Professor (benjamin@swfu.edu.cn), Professor (1563036678@qq.com), and Professor (stevenwang1979@126.com [corresponding author]), Southwest Forestry Univ., Yunnan, Kunming, People's Republic of China.
This paper was received for publication in January 2017. Article no. 17-00002.

Forest Prod. J. 67(7/8):474–480.
doi:10.13073/FPJ-D-17-00002

cellulosic fibers for paper making, and little is known about their basic properties.

Dendrocalamus giganteus is a sympodial bamboo species found largely in southern and southeastern Asian countries (Cheng et al. 2015). In China, *D. giganteus* plantations are among the largest of all forest crops, especially in south and southwest Yunnan Province, and such plantations are expanding rapidly (Cheng et al. 2015). Those plantations generate large and increasing quantities of sheath residues each year. Traditionally, this bamboo species is the most abundant local resource for construction wood, edible bamboo shoots, and highly elastic bamboo strips for weaving of household and farm tools. Wang et al. (2008) studied the anatomical and chemical properties of 10 major bamboo culms in Yunnan Province and suggested that *D. giganteus* culms would be the most suitable as pulp fiber. Little is known about the anatomical and chemical properties of its sheaths, however, and their potential for industrial use as raw pulp material has yet to be explored.

Knowledge about anatomical and chemical properties is fundamental to identifying ideal raw materials and residues for paper making. In this study, we characterized the anatomical and chemical properties of *D. giganteus* sheaths in various growth stages and evaluated their potential as pulp fiber.

Materials and Methods

Materials

D. giganteus sheaths were collected in August 2015 from a bamboo plantation at the nursery center of the Xinping County, Forestry Bureau, in Yunnan Province, China (24°04′89″N, 101°98′33″E; altitude, 1,605 m). August is the prime season for *D. giganteus* shoots. Sheaths from three growth stages were collected, including (1) shoot sheaths, which are tender, light purple or brown, and tightly enclose 2-week-old shoots; (2) culm sheaths, which are hard, fragile, brown with black hair, and embrace internodes of 1- to 3-month-old culms; and (3) shedding sheaths, which are hard, dark brown, and have fallen to the ground from 12-month-old culms. The bamboo attains its maximal culm height in about 3 months, after which the sheaths gradually dry up but still contain the internodes when they fall off. Sheath samples were chipped into strips (2 by 50 mm) and fixed in formalin–acetic acid alcohol (45% ethanol, 0.25% acetic acid, and 1.85% formaldehyde).

Methods

Determination of anatomical properties.—To study the vascular bundles, samples were preserved in a mixture of 50 percent ethanol, 10 percent glycerin, and 40 percent water for maceration (Lybeer et al. 2006). Because the bamboo sheaths are hard and fragile, they were difficult to cut with a rotary or sliding microtome if the samples were embedded in paraffin or polyethylene glycol, so Technovit 7100 resin was used instead. After dehydration in a graded series of ethanol (50%, 70%, 80%, 90%, and 100%), the sheath samples were permeated with 100 mL of base liquid plus 1 g of hardener I for 12 hours and then embedded in 15 mL of preparation solution with 1 mL of hardener II. When the embedded sheath samples were dry and hard, transverse sections were cut to 3 to 5 μm thick with a conventional rotary microtome (Leica RM 2165). Sections were then stained with Toluidine Blue O solution for 30 seconds and mounted in Canada balsam (*Abies balsamea*) medium. The sections were observed and photographed with a video camera linked to an inverted fluorescent microscope (Nikon E400) and a Lenovo desktop computer.

To measure fiber dimensions, sheath samples were macerated with Jeffrey's solution (a 1:1 mixture of 10% nitric acid and 10% chromic acid; Jeffrey 1917) for 36 to 72 hours. Then, the samples were washed five times in distilled water. A minimum of 150 intact and straight fibers from each sample, totaling 451 fibers, were measured with a microscope (Phoenix PH100-3B41L-IPL) to obtain data on fiber length and width, wall thickness, and lumen diameter with an objective lense at ×10, ×20, and ×40, respectively. The slenderness ratio (fiber length/fiber width) and the Runkel ratio [(2 × fiber cell wall thickness)/fiber lumen diameter] were calculated according to the fiber dimensions measured.

Determination of chemical properties.—The sheath samples were oven-dried at 60°C for 24 hours and then ground with a Wiley mill. Ground material was passed through a 40-mesh sieve but retained on a 60-mesh screen for subsequent chemical analyses. We conducted chemical analyses based on the methods from the Chinese National Standards for Testing and Materials (CNSTM). Each test was performed in triplicate. Following the standard for ash—GB/T 2677.3-93 (CNSTM 1993)—samples were carbonized in a porcelain crucible on an electric stove, ignited at 575°C ± 25°C for 2 hours in a muffle furnace, and then weighed. Ash samples were removed from the muffle furnace and cooled to room temperature. Then, following the standard for silicon dioxide (SiO_2)—GB/T 7978-87 (CNSTM 1987)—5 mL of HCl (6 mol/L) was added and evaporated in a steam bath. After three repetitions, the residue was rinsed in distilled water, filtered, moved to the muffle furnace for heating at 575°C ± 25°C for 2 hours, and then weighed. Following the standard for toluene–alcohol extractives—GB/T 10741-89 (CNSTM 1989)—samples were packaged with filter paper and put in a Soxhlet extractor for 6 hours for water-bath extraction with a benzene–alcohol solution (2:1) and weighed.

After the 6-hour water-bath extraction, samples were moved into a conical flask per the standards for lignin—GB/T 2677.8-94 (CNSTM 1994) and GB/T 10337-2008 (CNSTM 2008)—and 15 mL of sulfuric acid (72%) was used for a 2-hour water bath. Then, distilled water was added to 560 mL, and after a 4-hour water bath, the solution was filtered, rinsed with distilled water, dried, and weighed for acid-insoluble lignin. The filtrate of the acid-insoluble lignin was collected and measured. The absorbance at 205 nm was determined by ultraviolet spectrophotometer (752 N Hengping) as acid-soluble lignin.

The holocellulose standard—GB/T 2677.10-95 (CNSTM 1995)—was followed after the 6-hour water-bath extraction. Samples were moved to a conical flask, and 65 mL of distilled water, 0.5 mL of acetic acid, and 0.75 g of sodium chlorite were added. After a 1-hour water bath at a constant temperature of 75°C, 0.5 mL of acetic acid and 0.75 g of sodium chlorite were added. The procedure was repeated four times until the material turned white. The solution was then filtered and washed with distilled water until the filtrate was not acidic. Finally, the residue was washed three times with acetone, dried, and weighed.

Data analysis.—We conducted the statistical analyses for the experiments and compared one-way analyses of variance using the least-significant difference method to determine the level of significance at $P \leq 0.05$.

Results and Discussion

Vascular bundles

The leathery culm sheath of *D. giganteus* is among the largest of the bamboo species (Fig. 1A) and is reported to be 42 to 45 cm long and 50 to 55 cm wide (Sarma and Pathak 2004). Abundant longitudinal and transversal vascular bundles can be observed between the adaxial and abaxial epidermis (Fig. 1B). After internode elongation is complete, the sheaths of *D. giganteus* quickly dry and easily fall off (Yi et al. 2008); therefore, bamboo stands can produce large quantities of sheaths each year.

In bamboo plants, the anatomical structure of their vascular bundles is reported to be correlated with the mechanical properties of their bamboo culms (Mohmod 1993). Therefore, the anatomical characteristics of vascular bundles in the culm sheaths of *D. giganteus* were studied. A transverse section of a *D. giganteus* sheath showed two vascular bundle types—undifferentiated and differentiated—from the abaxial to the adaxial epidermis (Figs. 1B and 1C), based on the classifications of Wen and Chou (1984). Some of the undifferentiated vascular bundles contained

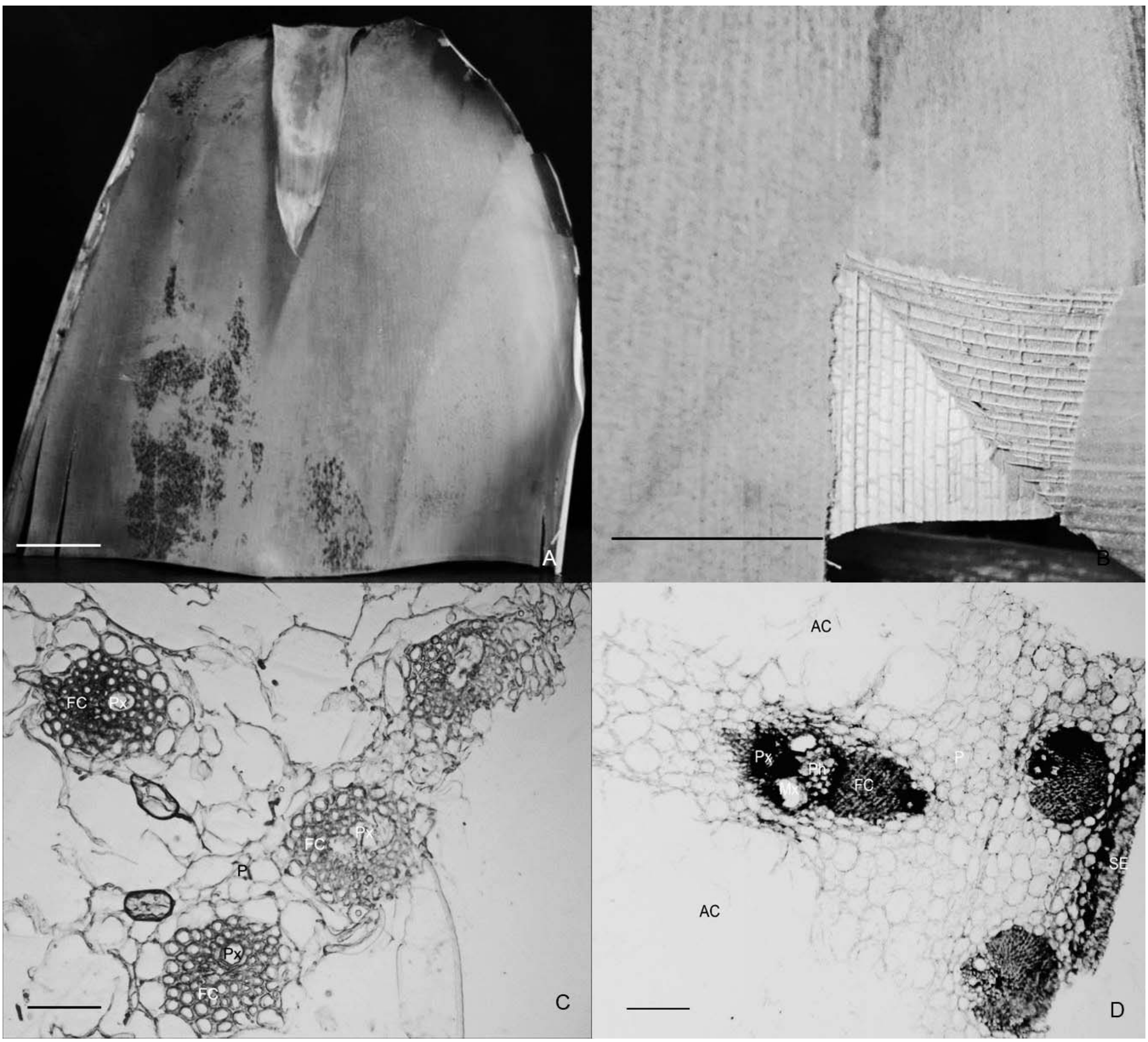

Figure 1.—Morphological characteristics of Dendrocalamus giganteus *sheaths. (A) Culm sheath of* D. giganteus. *Bar = 6 cm. (B) Internal structure of a culm sheath, showing the longitudinal and transversal vascular bundles. Bar = 2 cm. (C) Undifferentiated vascular bundle with only protoxylem transport tissues but no phloem and metaxylem xylem in the transverse section. Bar = 100 m. (D) Undifferentiated and differentiated vascular bundles in the transverse section. The vascular bundles that are close to the abaxial epidermis are undifferentiated with protoxylem transport tissues only. Parenchymal cells between the differentiated vascular bundles were usually decomposed and formed air cavities. More fibers were closer to phloem than to protoxylem. Bar = 100 m. Ph = phloem; Mx = metaxylem; Px = protoxylem; P = parenchyma; FC = fiber cell; AC = air cavity; SE = sheath epidermis.*

only protoxylem transport tissues (Fig. 1B). Differentiated vascular bundles were largely observed in the middle of the culm sheaths, whereas the undifferentiated ones were primarily close to the abaxial epidermis (Fig. 1C). More fibers were observed in phloem sheaths than in protoxylem sheaths. In addition, parenchymal cells adjoining differentiated vascular bundles were usually decomposed and formed air cavities (Fig. 1D).

Fiber dimensions

Fiber morphology has an important effect on the physical properties of pulp. The fiber morphological characteristics of the *D. giganteus* sheaths and their comparisons with other common paper-making fibers are summarized in Table 1.

Fiber length has a significant effect on the tearing and tensile strength of paper (Wang et al. 2008, Yang et al. 2008). The fiber length for the *D. giganteus* sheaths ranged from 0.37 to 5.10 mm (Table 1), and its mean value was 1.10 mm. According to the provisions of the International Association of Wood Anatomists, fiber lengths less than 0.90 mm are defined as short fiber, those greater than 1.60 mm are long fibers, and those in between are medium fibers. As such, *D. giganteus* sheaths produce medium-length fibers (Yang et al. 2008). The frequency distribution of fiber lengths is used to determine the matched rate of different types of pulp materials and is an important indicator for evaluating the quality of raw materials. Fibers largely distributed in the longer ranges with higher frequencies suggest pulps of high quality. The frequency histogram of *D. giganteus* sheath fiber lengths showed most fibers were concentrated in a range from 0.75 to 1.75 mm (Fig. 2). The mean fiber length was 1.10 mm, and lengths of 1.00 mm or greater accounted for more than half of the total.

The *D. giganteus* sheath fibers were shorter than those of its culms (2.72 mm) but longer than those of wheat straw (0.74 mm) and cotton stalk (0.83 mm) and were comparable to canola stalks (1.17 mm; Table 1).

Variability in fiber length among the sheaths at the three growth stages is shown in Table 2. The longest fibers were found in culm sheaths, and these fibers were significantly longer than those of the shoot ($P = 0.000$) and shedding sheaths ($P = 0.020$). Fiber width varied by stage of growth, however, with shoot sheaths being wider than shedding sheaths, which were wider than culm sheaths. The width value of culm sheaths was significantly smaller than that of shoot ($P = 0.000$) and shedding sheaths ($P = 0.002$).

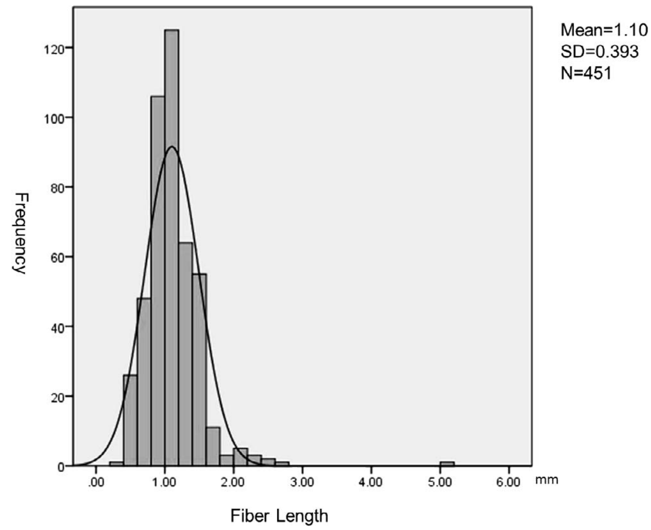

Figure 2.—Frequency histogram of the fiber lengths of Dendrocalamus giganteus *sheaths.*

Statistically significant differences were observed in the slenderness ratio of the three sheath growth stages ($P = 0.000$). Culm sheaths had the highest slenderness ratio at 101.73, whereas shoot sheaths had the smallest value at 61.11. High slenderness ratios enhance the strength of paper sheets, and fibers with higher slenderness ratios are longer, thinner, and more resistant to tearing (Nasser et al. 2015). In general, acceptable values for the slenderness ratio of pulp fibers are those greater than 33 (Rudi et al. 2016), and good pulp fiber ratios need to be greater than 100: the greater the ratio value, the better for paper strength (Wang et al. 2008; Yang et al. 2008). The slenderness ratio of the culm sheath from *D. giganteus* was well suited as a raw material for pulp. Moreover, the mean value of the slenderness ratio of the *D. giganteus* sheath was greater than those from other traditional farm crop residues, such as canola stalks (50.83), wheat straw (56.06), and cotton stalk (42.35; Table 1).

Overall, the fiber wall of the *D. giganteus* sheath (2.66 μm) was thinner than those of the culm (7.87 μm), canola stalk (5.26 μm), wheat straw (4.59 μm), and cotton stalk (3.40 μm; Table 1). The fiber wall of shoot sheaths (2.91 μm) was thicker than the fiber wall at either the culm (2.54 μm) or shedding stage (2.51 μm; Table 2), which might be

Table 1.— Fiber basic index for Dendrocalamus giganteus *sheaths at various growth stages, with a comparison to* D. giganteus *culms and other common pulp fibers.*

Parameters and comparisons	Length (mm)	Width (μm)	Slenderness ratio	Wall thickness (μm)	Lumen diameter (μm)	Runkel ratio
Maximum	5.10	31.20	245.00	7.80	20.80	3.00
Minimum	0.37	5.20	20.50	0.65	2.60	0.14
Mean	1.10	14.05	82.80	2.66	8.74	0.69
Culm[a]	2.72	26.37	103.20	7.87	2.16	3.64
Canola stalk[b]	1.17	23.02	50.83	5.26	12.50	0.84
Wheat straw[c]	0.74	13.20	56.06	4.59	4.02	2.28
Cotton stalk[d]	0.83	19.60	42.35	3.40	12.80	0.53

[a] From Wang et al. (2008).
[b] From Enayati et al. (2009).
[c] From Deniz et al. (2004).
[d] From Ververis et al. (2004).

Table 2.—Fiber characteristics of Dendrocalamus giganteus *sheaths at three different growth stages.*[a]

Stage	Length (mm)	Width (μm)	Slenderness ratio	Wall thickness (μm)	Lumen diameter (μm)	Runkel ratio
Shoot sheaths	0.85 A	14.67 A	61.11 A	2.91 A	8.86 A	0.69 A
Culm sheaths	1.27 B	13.14 B	101.73 B	2.54 B	8.06 B	0.75 B
Shedding sheaths	1.18 C	14.33 A	85.43 C	2.51 B	9.31 A	0.57 B
Mean	1.10	14.05	82.80	2.66	8.74	0.69

[a] Values followed by the same letter within the same column are not significantly different at $P < 0.05$ probability.

due to decreased water content or some decomposing and reuse of the components of the fiber walls. Shedding sheaths had the widest mean for the fiber lumen diameter (9.31 μm), and the difference between the shedding and shoot sheaths and the culm sheaths was significant ($P = 0.000$). A Runkel ratio is one of the criteria for assessing the suitability of fibrous materials for the paper industry (Wu 1997, Wang et al. 2008). Higher Runkel ratios have lower paper strength, especially for burst, tear, and tensile indices (Nasser et al. 2015). Fibers with a Runkel ratio less than 1 are considered to be ideal for paper making, because they are more flexible and collapse easily so that sheets can be produced with enhanced bonding of the fibers (Nasser et al. 2015). The largest Runkel ratio for the *D. giganteus* sheaths was observed in culm sheaths (0.75), and the smallest was for shedding sheaths (0.57). The mean Runkel ratio for *D. giganteus* sheaths was 0.69; accordingly, *D. giganteus* sheaths were found to be good pulp fiber.

Chemical properties

The average proportions of the main chemical constituents measured for the *D. giganteus* sheaths were 3.21 percent ash, 1.50 percent SiO_2, 2.66 percent toluene–alcohol extracts, 1.72 percent acid-soluble lignin, 21.65 percent acid-insoluble lignin, and 70.17 percent holocellulose (Table 3). The chemical compositions of sheaths showed significant variability among the different growth stages.

The ash and SiO_2 contents were greater in the sheaths than in culms, implying a greater consumption of pulping chemicals and more difficulties in recovering the cooking liquor (Nasser et al. 2015). However, the ash content was still much less than that of other forest or agricultural residues reported in literature (Table 3). Ash content showed great variability among the three sheath age groups. Shoot sheaths had significantly greater ash content (4.32%), whereas shedding sheaths had relatively less (2.41%). Low ash content indicates high pulp yield from the pulping process (Lopez et al. 2004). Shoot sheaths had relatively lower levels of SiO_2 (1.36%), whereas culm sheaths had the most SiO_2 (1.63%).

The toluene–alcohol extractable content of the shoot sheaths was the highest (3.46%) and was significantly higher than that of the culm sheaths ($P = 0.027$). Higher extractives contents are advantageous for resistance to decay and provide good strength in fiber processing (Abdul Khalil et al. 2006).

Acid-insoluble lignin content was also lower in shoot sheaths (21.53%), culm sheaths (21.15%), and shedding sheaths (22.28%) than in bamboo culms (23.70%). The total lignin content was also lower in sheaths (23.70%) than in tea waste (36.94%) but higher than in canola stalks (17.30%), wheat straw (15.30%), pineapple leaves (4.28%), corn stalks (7.30%), and Napier grass (10.8%; Table 3). Lignin functions as an adhesive to bind cellulose together in the fiber. Lower lignin content is commonly found in nonwood fibers, making the fiber strength greater and harder to break (Tran 2006). The lignin content was greatest for shedding

Table 3.—Mean values (%) of major chemical constituents of Dendrocalamus giganteus *sheaths at various growth stages and comparisons with* D. giganteus *culms and other common pulp fibers.*[a]

Stages and comparisons	Ash	SiO_2	Toluene–alcohol extracts	Acid-soluble lignin	Acid-insoluble lignin	Total lignin	Holocellulose
Shoot sheaths	4.32 A	1.36 A	3.46 A	2.17 A	21.53 AB	23.70 A	66.31 A
Culm sheaths	2. 91 B	1.63 B	1.72 B	1.40 B	21.15 A	22.55 B	76.98 B
Shedding sheaths	2.41 C	1.52 AB	2.78 AB	1.58 B	22.28 B	23.86 A	67.21 A
Mean	3.21	1.50	2.66	1.72	21.65	23.37	70.17
Culm[b]	1.33	0.66	4.90	—	23.70	—	46.90
Canola stalk[c]	8.20	—	—	—	—	17.30	73.60
Wheat straw[d]	4.70	—	—	—	—	15.30	74.50
Pineapple leaf[e]	4.50	—	—	—	—	4.28	85.7
Corn stalk[e]	24.90	—	—	—	—	7.30	82.1
Napier grass[e]	14.6	—	—	—	—	10.8	80.4
Tea waste[f]	4.53	—	15.22	—	—	36.94	60.81

[a] Means followed by the same letter within the same column are not significantly different at $P < 0.05$ probability.
[b] From Wang et al. (2008).
[c] From Enayati et al. (2009).
[d] From Deniz et al. (2004).
[e] From Daud et al. (2014).
[f] From Tutus et al. (2015).

sheaths (23.86%), followed by shoot sheaths (23.70%) and then culm sheaths (22.55%). Lignin is an undesirable polymer, and its removal in pulping requires higher amounts of energy and chemicals (Abdul Khalil et al. 2006). Therefore, more attention should be given to lignin removal during pulping. Overall, the mean lignin content (23.37%) places *D. giganteus* sheaths in the range (11% to 27%) reported for other nonwoody biomass and close to the ranges for North American softwoods (24% to 37%) and hardwoods (17% to 30%; Li et al. 2007).

The mean holocellulose content of *D. giganteus* sheaths (70.17%) was comparable to those of canola stalks (73.60%), and wheat straw (74.50%) but slightly lower than those of pineapple leaves (85.7%), corn stalks (82.1%), and Napier grass (80.4%). Culm sheaths contained the most holocellulose (76.98%), followed by shedding sheaths (67.21%) and then shoot sheaths (66.31%). Holocellulose is a combination of cellulose and hemicellulose. Greater holocellulose content produces better-quality paper (Daud et al. 2014). The holocellulose content of *D. giganteus* sheath was comparable to those of other forest or agricultural residues found in the literature (Table 3).

The chemical properties of *D. giganteus* sheaths suggest they have good potential as alternative cellulosic fibers for pulp and paper-making industries.

Conclusions

Undifferentiated and differentiated vascular bundles were observed in *D. giganteus* sheaths. More fibers were contained in the phloem sheaths than in the protoxylem sheaths. The parenchyma cells adjoining the differentiated vascular bundles showed decomposition and formed air cavities.

Fiber morphology at various growth stages of the *D. giganteus* sheaths demonstrated moderate differences. The fibers can be classified as short- and medium-sized fibers. Both the slenderness ratio and Runkel ratio showed the sheaths are well suited as a raw material for paper making.

The chemical composition at the various growth stages also demonstrated some differences. The ash and SiO_2 contents in *D. giganteus* sheaths were greater than those in culms but were still lower than those in other nonwood pulp fiber resources. The lignin and holocellulose contents were comparable in culms and other typical pulp fiber resources.

D. giganteus sheaths have a promising potential as pulp fiber or for making high-quality paper when mixed with culm pulp. Bamboo sheaths can be used as an alternative to fulfill the demand for pulp by the paper industry to help prevent the depletion of forest resources.

Acknowledgments

The research was funded by Yunnan Provincial Key Disciplines (Biology) Construction Project (50097505), China National Science Foundation (31560196 and 31460169), Yunnan Provincial Science Foundation (2015FB150), and PhD Candidate Innovation Project supported by Forestry First Discipline Degree program of Southwest Forestry University.

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