

Comparison of Variations in the Chemical Constituents of the Rhizome and Culm of *Phyllostachys pubescens* at Different Ages

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A systematic study was conducted on *Phyllostachys pubescens* by analyzing the chemical constituents of its rhizome and culm at different ages. Our results indicated that the ash contents of the rhizome and culm of *P. pubescens* at different ages showed the largest coefficient of variation (CV), followed by alcohol benzene extractives. The CVs of acid-insoluble lignin, holocellulose, HNO₃-C₂H₅OH cellulose, and pentosan were relatively small. Analysis of t-tests indicated that significant differences were found in the contents of extractives, acid-insoluble lignin, holocellulose, and ash of rhizome and culm ($p < 0.05$). The differences in contents of HNO₃-C₂H₅OH cellulose and pentosan were not significant. Analysis of multiplicity showed that the contents of HNO₃-C₂H₅OH cellulose, pentosan, and ash were not significantly different in bamboos at the ages examined. Likewise, the contents of lignin, alcohol benzene extractives, and holocellulose exhibited no significant difference between one-year-old and three-year-old bamboos. However, the differences in these parameters between five-year-old bamboos and one- and three-year-old bamboos were all statistically significant. Our results suggest that three-year-old *P. pubescens* is suitable for use as a raw material for papermaking. In addition, our findings provide a theoretical basis for effective utilization of *P. pubescens* and enhancement of its value.

Keywords: *Phyllostachys pubescens*; Rhizome; Culm; Bamboo age; Chemical constituent; Axial variation

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INTRODUCTION

As a commercial bamboo species in China, *Phyllostachys pubescens* is cultivated over a large region, mostly in subtropical areas such as the Qinling Mountains, and between the Hanjiang River Basin and the Yangtze River Basin (Jiang 2007; Geng and Wang 2008). The underground portion of *P. pubescens* is composed of rhizome, root, and bud, while the aboveground part consists of culm, branch, and leaf. The bamboo rhizome, or underground stem, is an important component of *P. pubescens*, with a huge potential for exploitation and utilization. It is well known the removal of rhizome appropriately is beneficial to the bamboo forest restoration and refreshment of soil, which can enhance the quality of afforestation (Mao *et al.* 2011). Also, the rational use of rhizome can improve the comprehensive utilization and high-added value of residue. The utilization of rhizome has a long history, and it is widely used in bamboo charcoal, bamboo pipe, and bamboo carvings. Nevertheless, existing research on the wood properties of *P. pubescens* has focused mostly on bamboo culm. With increasing demand for bamboo resources,

more scholars have begun to study ways to promote effective utilization of rhizome resources (Chen *et al.* 2001; Wang 2001; Li *et al.* 2002; Wang and Wei 2002; Han 2007; Zhang *et al.* 2007; Shi *et al.* 2008), However the properties and utilization of rhizome have been reported rarely. Two important factors, the resistance to insects and decay, affect the use of bamboos, and these in turn are determined by the chemical properties of the bamboo (Zheng 2001; Pu *et al.* 2002; Chilako *et al.* 2004; Guo *et al.* 2005; Lu *et al.* 2008; Fang *et al.* 2009; Yang *et al.* 2009; Fan *et al.* 2010; Dinesh *et al.* 2012). Accordingly, the chemical constituents of rhizome were compared with the culm of *P. pubescens*. The research focused mostly on the rhizome at different ages. Differences of chemical constituents were analyzed, and the variation features in the axial direction were identified to provide a basis for the utilization of whole bamboo and to increase the value derived from rhizome's utilization.

EXPERIMENTAL

Materials

Phyllostachys pubescens samples were collected from a northern slope (gradient 30° to 40°), where the east longitude and northern latitude were 118°01'22'' and 30°12'55'', respectively on 27th Sept, 2012 in Tangjiacun village, (Taiping) Jiaocunzhen town in Huangshan City, Anhui Province. The bamboos were II operation level with healthy growth and development status, but without the topping treatment (Liu and Zeng 2010). The average heights of culm and clear bole were 16.5 m and 7.1 m, respectively, and the average diameter at eyebrow was 10.96 cm. Bamboo age was determined based on the integrity or degree of decay of the surviving bamboo shoot's shell from mother bamboo, age marker of branchlet, and culm colour. One-year-old, three-year-old, and five-year-old *P. pubescens* samples including rhizome parts (30 cm each) were collected with three replicates each, and preserved indoors using pigsty stacking in a ventilated corridor for three months before the tests.

Methods

Sampling (30 cm) was carried out on different parts of the rhizome and culm (1.5 m, 3.5 m, 5.5 m, and 7.5 m) of bamboo. Culm top segments were taken from the top 2 m with culm diameter more than 4 cm (Wang and Pu 2000) following guidelines in GB/T 15780-1995 (1996). Three samples were taken from every part (rhizome, 1.5 m, 3.5 m, 5.5 m, 7.5 m, and the top) of bamboo at each age. For example, in the case of rhizome, three segments (5 cm each) were sampled, as shown in Fig. 1. Fresh samples were dried naturally until they reached air-dried moisture content, then sliced into thin sections (the size as match) by an axe, after which they were ground using a TL2020 high-speed pulverizer (Beijing DHS Life Science and Technology Co., Ltd.; Beijing). Sample powder was obtained that could pass through a 0.38-mm aperture (40 mesh), but failed to pass through a 0.25-mm aperture (60 mesh). Six indicators of chemical composition of bamboos, selected based on TAPPI standards (T 258 om-02 2004; T 211 om-02 2004; T 204 om-88 2004; T 222 om-02 2004; T 9 wd-75 2004) and on the work of Qu (1990) comprising alcohol benzene extractives, holocellulose, acid-insoluble lignin, cellulose (extracted by the HNO₃-C₂H₅OH method; Qu 1990), pentosan, and ash, were assayed. The extractives and the pentosan were detected by the alcohol-benzene extraction method (GB/T 2677.6 1995) and the dibromination method (Qu 1990), respectively.

Analysis of multiplicity is widely used for comparison between two groups of all independent multi-samples. Thereby the difference of the specific chemical constituent between different ages of bamboo can be obtained easily. A t-test was used to determine differences between rhizome and culm due to analysis of t-test can reflect the mean difference between two groups with independent sample.

Using analysis methods, *i.e.* variance, t-test, and multiplicity, the experimental data were analyzed with SPSS software (International Business 90 Machines Corp., New York), version 21.0.

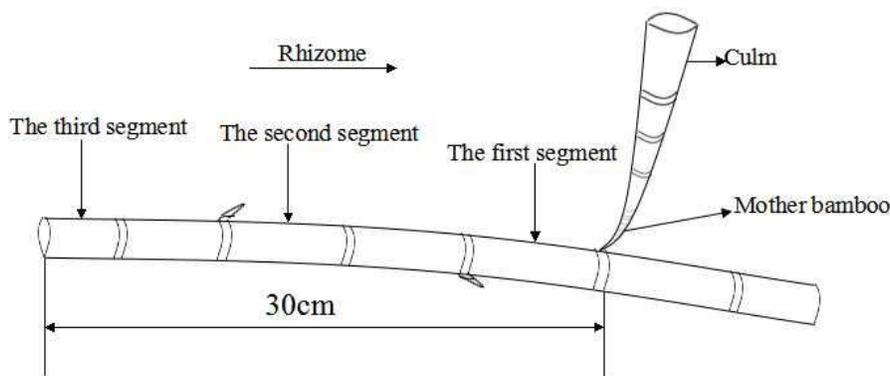


Fig. 1. Schematic diagram of sampling

RESULTS AND DISCUSSION

Analysis of Chemical Constituents of Rhizome and Culm of *P. pubescens*

The major chemical constituents of bamboo include cellulose, lignin, and hemicellulose, which are the structural materials of cell walls and used as the main parts for processing and utilization. Although extractives and ash are minor constituents, they also have an impact on the properties of the wood, as well as processing and utilization (Xu *et al.* 2013). As shown in Table 1, statistical analysis of six chemical parameters indicated that the contents of ash in the rhizome and culm of *P. pubescens* of different ages showed the largest coefficient of variation (CV), followed by the alcohol benzene extractives; the CVs of the contents of acid-insoluble lignin, holocellulose, HNO₃-C₂H₅OH cellulose, and pentosan were relatively small. The results supported the operative conclusion raised by the previous study that major chemical constituents, *i.e.* cellulose, hemicellulose and lignin of *P. pubescens* at six different locations, were less variable, while the extractives and ash showed relatively large fluctuation (Lin *et al.* 2010). This indicates that the major chemical constituents of the bamboos were relatively stable. However, the minor constituents, which affect insect prevention and decay resistance fluctuated, may be influenced by bamboo age, sampling segment, and growth status. Generally, the raw materials will perform better for pulping when several conditions are met, *i.e.* with higher content of cellulose and pentosan but lower content of extractives and ash (Cui 2010), as well as the fiber length more than 0.3 mm, the ratio of fiber length to width more than 35, and the content of mixed cells less than 50% (Guo 1989). So along with our previous study about the fiber characteristics of rhizome (Liu *et al.* 2014), we can conclude that the three-year-old rhizome is the most suitable for use as raw materials for papermaking.

Table 1. Descriptive Statistics of Chemical Composition of *P. pubescens* from Rhizome to Top at Different Ages

Parameters	Bamboo age	Rhizome (%)	1.5 m (%)	3.5 m (%)	5.5 m (%)	7.5 m (%)	Top of Bamboo (%)	Mean (%)	Standard Deviation (SD)	Coefficient of Variation (CV)
Alcohol	1	1.34	2.16	2.22	2.40	2.18	1.69	1.78	0.005	0.283
	3	1.24	2.02	1.96	2.03	2.00	2.11	1.70	0.005	0.279
	5	1.57	2.44	3.04	2.97	2.17	3.10	2.22	0.007	0.328
Benzene extractive	1	23.90	22.70	22.77	22.82	23.37	24.25	23.39	0.007	0.030
	3	25.03	24.12	24.42	24.73	23.81	24.29	24.49	0.006	0.026
	5	25.31	24.45	23.54	23.70	24.67	23.88	24.41	0.010	0.041
Acid-insoluble lignin	1	68.25	73.44	73.54	74.50	75.18	75.07	72.61	0.032	0.044
	3	68.59	73.57	74.62	74.04	72.27	70.98	71.81	0.028	0.039
	5	65.60	69.97	71.67	70.94	70.53	70.57	69.27	0.026	0.038
Holocellulose	1	37.09	37.71	42.39	41.81	39.81	39.55	39.72	0.028	0.069
	3	39.61	40.80	40.34	38.45	40.37	39.56	39.86	0.017	0.042
	5	38.46	41.09	41.50	41.31	39.24	38.68	40.05	0.015	0.037
HNO ₃ -C ₂ H ₅ OH Cellulose	1	18.71	20.27	20.37	20.49	20.58	21.29	20.06	0.010	0.050
	3	18.33	20.58	20.40	19.68	19.85	20.84	19.72	0.011	0.056
	5	16.90	20.46	19.75	20.40	21.19	21.07	19.52	0.020	0.099
Pentosan	1	1.43	1.02	0.99	1.03	1.03	1.05	1.14	0.005	0.463
	3	1.56	0.96	0.97	0.96	0.95	0.94	1.13	0.004	0.372
	5	1.63	0.79	0.84	0.82	0.89	0.93	1.08	0.005	0.455
Ash	1	1.43	1.02	0.99	1.03	1.03	1.05	1.14	0.005	0.463
	3	1.56	0.96	0.97	0.96	0.95	0.94	1.13	0.004	0.372
	5	1.63	0.79	0.84	0.82	0.89	0.93	1.08	0.005	0.455

Differences in Chemical Constituents of Rhizome & Culm of *P. pubescens*

To determine differences between the rhizome and the aboveground part of *P. pubescens*, the chemical constituents of the rhizome were compared with those of the segment at diameter eye-height (1.5 m of culm above the ground, abbr. D.E.H). The results are listed in Table 2. The contents of extractives, acid-insoluble lignin, holocellulose, and ash in rhizome and culm were significantly different ($p < 0.05$). In contrast, the contents of HNO₃-C₂H₅OH cellulose and pentosan showed no significant difference ($p > 0.05$). These differences may affect the process change of pulp and paper (Guo *et al.* 2005). So this result could reflect the material heterogeneity between the rhizome and the aboveground part of bamboo.

Analysis of the Significance of Variance in Chemical Constituents of *P. pubescens*

Table 3 shows the variability of chemical constituents at different bamboo ages. The contents of acid-insoluble lignin at different bamboo ages showed the largest variability ($F > F_{0.01}$, $P < 0.01$). Variation of the contents of extractives and holocellulose were also significantly different ($F > F_{0.05}$, $p < 0.05$). Alternatively, variations in the contents of HNO₃-C₂H₅OH cellulose, pentosan, and ash were relatively small, with no significant differences across bamboo ages ($F < 1$, $p > 0.05$). The result indicated that chemical constituents (especially in lignin, extractives and holocellulose) of bamboo have variability among different ages. The results agreed with the literature about influence of bamboo ages on the pulping performance of *P. pubescens* (Cui 2010; Dong 2010).

Table 2. Analysis of T-test on Differences in the Chemical Constituents of Rhizome and Culm of *P. pubescens*

		Levene's Test for Equality of Variances		The Difference t-test for Equality of Means			
		Sig	F	T	df	p	Mean Difference
Alcohol benzene extractive	Equal variances assumed	0.227	0.636	-8.961	46	0.001	-0.008
	Equal variances not assumed			-8.961	44.959	0.001	-0.008
Acid-insoluble lignin	Equal variances assumed	0.700	0.407	3.629	46	0.001	0.010
	Equal variances not assumed			3.629	42.815	0.001	0.010
Holocellulose	Equal variances assumed	1.844	0.181	-8.091	46	0.002	-0.048
	Equal variances not assumed			-8.091	45.146	0.002	-0.048
HNO ₃ -C ₂ H ₅ OH Cellulose	Equal variances assumed	2.321	0.135	-2.010	46	0.050	-0.013
	Equal variances not assumed			-2.010	39.777	0.051	-0.013
Pentosan	Equal variances assumed	3.002	0.090	1.528	46	0.133	0.002
	Equal variances not assumed			1.528	42.150	0.134	0.002
Ash	Equal variances assumed	16.263	0.000	2.176	46	0.035	0.030
	Equal variances not assumed			2.176	23.090	0.040	0.030

Note: The statistical significance was at the 95% confidence level.

Table 3. Analysis of Variance on Chemical Constituents of *P. pubescens*

	Alcohol benzene extractive	Acid-insoluble lignin	Holocellulose	HNO ₃ -C ₂ H ₅ OH Cellulose	Pentosan	Ash
Sample ID	162	162	162	162	162	162
Mean(%)	1.900	24.097	71.230	39.877	19.767	1.117
F	4.241	8.372	5.099	0.077	0.517	0.067
P	0.020	0.001	0.011	0.926	0.600	0.935
Std error	0.002	0.002	0.011	0.008	0.005	0.002

Note: $F_{0.05}(18, 36) = 1.90$; $F_{0.01}(18, 36) = 2.48$, Sample ID represents total test sample numbers.

Analysis of Axial Variation and Multiplicity of Chemical Constituents of *P. pubescens*

Because *P. pubescens* is a monocotyledon, it grows mostly in the longitudinal direction, and less radially. The variation curves in Fig. 2 show that the chemical constituents of *P. pubescens* vary with bamboo age and from the underground rhizome to different segments of culm.

As shown in Fig. 2, with increasing bamboo age, the contents of HNO₃-C₂H₅OH cellulose, lignin, and extractives increased gradually, while the contents of pentosan, holocellulose, and ash decreased.

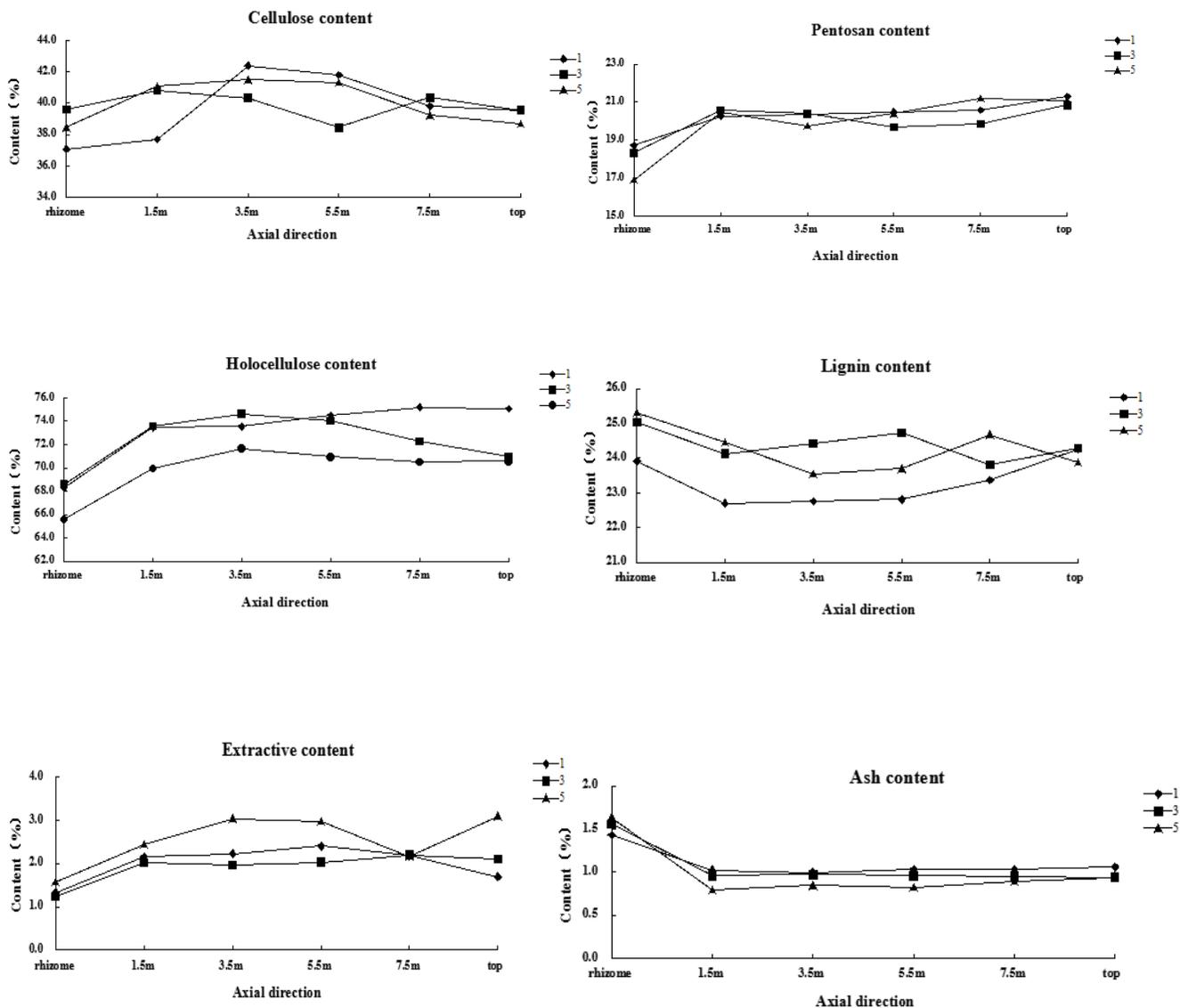


Fig. 2. Plots showing axial variation in chemical constituents of *P. pubescens* at different ages
Note: the number 1, 3, and 5 indicates one-year-old, three-year-old, and five-year-old bamboo, respectively

Along the axial direction from the rhizome to the top, the contents of HNO₃-C₂H₅OH cellulose, holocellulose, and extractives of bamboo increased at first, and then declined; this was observed across all ages. The pentosan content showed an increasing trend along the same axis. Furthermore, differences were observed in lignin contents at different bamboo ages. The lignin content decreased at first, and then increased from the rhizome to the top in one-year-old and three-year-old bamboos, while it fluctuated in five-year-old bamboos. For different ages of bamboos, the ash contents first decreased from the rhizome to the top, and tended to remain stable thereafter. In Table 4 the analysis of multiplicity indicated no significant differences in the contents of HNO₃-C₂H₅OH cellulose and pentosan of *P. pubescens* at different ages ($p > 0.05$).

Table 4. Multiplicity Analysis of Major Chemical Constituents of *P. pubescens* at Different Ages

	Age of bamboo	Age of bamboo	Difference of means	Standard error	Significance (P)	95% confidence interval	
						Lower bound	Upper bound
Acid-insoluble lignin	1	3	0.001	0.002	0.683	-0.003	0.005
		5	-0.004*		0.026	-0.008	-0.001
	3	1	-0.001		0.683	-0.005	0.003
		5	-0.0051*		0.009	-0.009	-0.001
	5	1	0.0041*		0.026	0.001	0.008
		3	0.0051*		0.009	0.001	0.009
Pentosan	1	3	0.0031	0.005	0.525	-0.007	0.014
		5	0.0051		0.322	-0.005	0.016
	3	1	-0.0031		0.525	-0.014	0.007
		5	0.002		0.718	-0.009	0.013
	5	1	-0.005		0.322	-0.016	0.005
		3	-0.002		0.718	-0.013	0.009
Cellulose	1	3	-0.001	0.008	0.874	-0.018	0.016
		5	-0.003		0.699	-0.020	0.014
	3	1	0.001		0.874	-0.0156	0.018
		5	-0.002		0.820	-0.019	0.015
	5	1	0.003		0.699	-0.014	0.020
		3	0.002		0.820	-0.015	0.019
Holo-cellulose	1	3	0.008	0.011	0.470	-0.014	0.030
		5	0.033*		0.004	0.011	0.056
	3	1	-0.008		0.470	-0.030	0.014
		5	0.025*		0.025	0.003	0.048
	5	1	-0.033*		0.004	-0.056	-0.011
		3	-0.025*		0.025	-0.048	-0.003

* Denotes statistical significance at the 95% confidence level

Likewise, no significant difference was found between the lignin contents of one-year-old and three-year-old bamboos ($p>0.05$). However, lignin content was significantly different between one-year-old and five-year-old bamboos, as well as between three-year-old and five-year-old bamboos ($p<0.05$). Although the difference in holocellulose contents between one-year-old and three-year-old bamboos was not significant ($p>0.05$), the differences between three-year-old and five-year-old bamboos and between one-year-old and five-year-old bamboos were statistically significant ($p<0.05$). As given in Table 5, analysis of multiplicity indicated that ash content did not differ across bamboos of different ages. Likewise, no significant difference was found in the contents of alcohol benzene extractives between one-year-old and three-year-old bamboos ($p>0.05$). However, the differences between one-year-old and five-year-old and between three-year-old and five-year-old bamboos were significant ($p<0.05$). Consistent with the previous study (Cui 2010; Dong 2010), also our results indicate the difference exist among different ages of bamboo regarding the chemical constituents of culms or rhizomes.

Table 5. Multiplicity Analysis of Minor Chemical Constituents of *P. pubescens* at Different Ages

Parameters	Age of bamboo	Age of bamboo	Difference of means	Standard error	Significance (P)	95% confidence interval	
						Lower bound	Upper bound
Alcohol benzene extractive	1	3	0.001	0.002	0.683	-0.003	0.005
		5	-0.004*		0.026	-0.008	-0.001
	3	1	-0.001		0.683	-0.005	0.003
		5	-0.005*		0.009	-0.009	-0.001
	5	1	0.004*		0.026	0.001	0.008
		3	0.005*		0.009	0.001	0.009
Ash	1	3	0.001	0.002	0.941	-0.003	0.004
		5	0.001		0.729	-0.003	0.004
	3	1	-0.001		0.941	-0.003	0.004
		5	0.001		0.785	-0.003	0.004
	5	1	-0.001		0.729	-0.004	0.003
		3	-0.001		0.785	-0.004	0.003

* Denotes statistical significance at the 95% confidence level

CONCLUSIONS

1. With increasing age of bamboo, the contents of $\text{HNO}_3\text{-C}_2\text{H}_5\text{OH}$ cellulose, lignin, and extractives gradually increased, while the contents of pentosan, holocellulose, and ash decreased.
2. Along the axial direction from the rhizome to the top, the contents of $\text{HNO}_3\text{-C}_2\text{H}_5\text{OH}$ cellulose, holocellulose, and extractives of bamboos all increased initially and then

declined across all ages. Interestingly, the contents of pentosan showed an increasing trend.

3. Significant differences in the lignin contents of bamboos at different ages indicated that it decreased initially, but increased from the rhizome to the top in one-year-old and three-year-old bamboos; however, the lignin contents from five-year-old bamboos fluctuated. Also, with age increasing, the ash contents first decreased from the rhizome to the top, and tended to remain stable thereafter.
4. Analysis of a T-test to investigate differences between the rhizome and the aboveground part of *P. pubescens* found that extractives, acid-insoluble lignin, holocellulose, and ash in the rhizome and the culm (D.E.H.) all showed significant differences, while HNO₃-C₂H₅OH cellulose and pentosan contents showed no significant difference.
5. Multiplicity analysis found that there were no significant differences ($p > 0.05$) in the contents of HNO₃-C₂H₅OH cellulose, pentosan, and ash in bamboos at the ages examined. Also, other contents including lignin, alcohol benzene extractives, and holocellulose exhibited no significant difference between one-year-old and three-year-old bamboos. However, the differences in these parameters between five-year-old bamboos and one- and three-year-old bamboos were all statistically significant.
6. Along with our previous study on the fiber characteristic of rhizome at different ages (Liu *et al.* 2014), the related results obtained in this paper show that the three-year-old rhizome is most suitable for use as a good raw material for papermaking. Also to some extent, the finding provide a theoretical basis for effective utilization of rhizome as well as enhancement of its value.

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