# CHEMICAL CHANGES WITH MATURATION OF THE BAMBOO SPECIES *PHYLLOSTACHYS PUBESCENS*

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**LI, X. B., SHUPE, T. F., PETER, G. F., HSE, C. Y. & EBERHARDT, T. L. 2007. Chemical changes with maturation of the bamboo species *Phyllostachys pubescens*.** Bamboo chemical properties were measured at three different heights from one-, three- and five-year-old plants and at three different radial positions from the three-year-old culms of *Phyllostachys pubescens* grown in south-eastern USA. Small but significant increases in holocellulose and α-cellulose contents were detected from the base to the top of the culm at all three ages. In contrast, no significant differences in Klason lignin and ash were detected. The increases in carbohydrate content with age correlated with increases in specific gravity. Alcohol-toluene extractive content increased from the base to the top of the stem in the three-year-old and five-year-old bamboo and showed a continuous increase with age. The epidermis had the highest extractive and ash contents and the lowest holocellulose and α-cellulose contents. The outer layer of the stem had the highest holocellulose, α-cellulose and Klason lignin contents and the lowest extractive and ash contents. The chemical content and specific gravity of bamboo stabilizes at about three years of age, which is an appropriate time for harvesting.

Keywords: Anatomy, ash, cellulose, chemical analysis, extractives, lignin

**LI, X. B., SHUPE, T. F., PETER, G. F., HSE, C. Y. & EBERHARDT, T. L. 2007. Perubahan kimia semasa pematangan spesies buluh *Phyllostachys pubescens*.** Sifat kimia buluh *Phyllostachys pubescens* yang berusia satu tahun, tiga tahun dan lima tahun yang ditanam di tenggara Amerika Syarikat disukat pada tiga ketinggian yang berbeza. Selain itu, bagi pokok yang berusia tiga tahun, sukatan juga dibuat pada tiga kedudukan radial yang berlainan. Peningkatan kecil tetapi signifikan dalam jumlah holoselulosa dan α-selulosa dikesan dari dasar ke bahagian atas kulma buluh yang berlainan usia itu. Sebaliknya tiada perbezaan signifikan direkod bagi jumlah lignin Klason dan abu. Kandungan karbohidrat yang meningkat mengikut usia berkait rapat dengan peningkatan graviti tentu. Kandungan ekstrak alkohol-toluena meningkat dari dasar ke bahagian atas batang buluh yang berusia tiga tahun serta lima tahun dan menunjukkan peningkatan yang berterusan mengikut usia. Epidermis mempunyai kandungan ekstrak serta abu yang paling tinggi dan kandungan holoselulosa serta α-selulosa yang paling rendah. Lapisan luar batang mempunyai kandungan holoselulosa, α-selulosa dan lignin Klason yang tertinggi tetapi kandungan ekstrak dan abu yang terendah. Kandungan kimia serta graviti tentu buluh menjadi stabil pada usia tiga tahun, iaitu ketika buluh sesuai dituai.

## INTRODUCTION

In recent decades, bamboo has attracted more attention as an alternative to timber due to its short (3–5 years) rotation and favourable mechanical properties for solid bamboo, and also medium density fibreboard and pulp and paper products (Janssen 1995, Ahmad 2000, Hammett *et al.* 2001). It is well established that the chemical composition of bamboo is similar to that of wood with cellulose, hemicellulose and lignin accounting for over 90% of the total mass. In general, the α-cellulose content in bamboo is 40–50%, which is comparable with the reported α-cellulose contents of softwoods (40–52%) and hardwoods (38–56%) (Fengel & Wegener 1984, Dence 1992). Cellulose contents in this range make bamboo a suitable raw material for the pulp and paper industry. The lignin content (20–26%) places bamboo at the high end of the normal range (11–27%) reported for non-woody biomass (Bagby *et al.* 1971) and close to the ranges for North American softwoods (24–37%) and hardwoods (17–30%). The high

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lignin content of bamboo contributes to its high heating value and its structural rigidity with the latter making it a valuable building material (Scurlock *et al.* 2000).

High ash contents for some bamboo species can adversely affect tool knife wear during machining operations and processing into pulp (Abd. Latif 1993). The ash content of bamboo is mostly silica, along with metals such as calcium and potassium. Internodes of bamboo have significantly higher ash, 1% NaOH, alcohol-toluene and hot-water solubles than the nodes (Mabilangan & Estudillo 2001). However, differences between major chemical constituents of node and internode fractions of bamboo are small (Scurlock *et al.* 2000). Therefore, neither the number of nodes, nor the length of internode segments, would be critical to the utilization of bamboo for energy conversion, chemical production or as a building material.

It is well established that the mechanical properties of bamboo culm improve with age (Li *et al.* 2004, Liese 1985, Liese & Weiner 1996, 1997). As a monocot, bamboo species grow to their full height and diameter in about one year. The culm matures in two to four years. The time it takes for maturation limits the commercial harvest age. Improved mechanical properties in mature bamboo are correlated with increased specific gravity, which is due to anatomical changes in the vascular bundles (Abd. Latif *et al.* 1993). The number of vascular bundles per unit area of the stem remains unchanged during maturation; however, the number of fibre cells with thickened secondary walls increases within the vascular bundles.

Age dependent changes in chemical composition are less well characterized. It was thought that lignification was completed within the first growing season (Itoh 1990, Itoh & Shimajii 1981). In contrast, Lin *et al.* (2002) showed that lignification continues after the first year's growth, especially in the parenchyma cell walls; fibre and most parenchyma cell walls thicken during stem growth to form a poly-lamellate structure and the lignification process of these cells may last up to seven years. Fujii (1985) showed fibres continued wall thicken well into the second year. Mohd Nor *et al.* (1992) studied the chemical constituents of one-, two-, and three-year-old *Gigantochloa scortechinii* and found that holocellulose, α-cellulose and lignin contents increased slightly from one to three years of age. Lybeer and Koch (2005a, b) found that lignin in the epidermal cell wall did not increase after one month whereas the lignin content of fibres and ground parenchyma cells increased during the first year, but not afterwards. The chemical composition of bamboo appears to vary with age. It remains to be determined if chemical composition would differ with height and radial position within a given culm. It is likely that any of these variations may be correlated with variations in the physical and mechanical properties. Accordingly, the objective of this study was to determine the effects of age, height and radial position on the chemical composition of bamboo culms.

## MATERIALS AND METHODS

Bamboo culms for this study were collected in June 2003 from the Kisatchie National Forest near Pineville, Louisiana, USA. The bamboo originated from a clonal clump planted in the 1960s. The age of culms was estimated based on visual inspection (i.e. colour, sheaths in culms and surface lichen growth) by experienced field personnel familiar with the history of the clump. Two representative bamboo culms for each age group, namely, one, three and five years old were harvested. Details for the sampling method were previously reported by Li (2004). The following is a brief description. Internodes were consecutively numbered from bottom to top for each culm which was then divided into three sections, i.e. bottom, middle and top, each with an equal number of internode sections. Selected internodes from each section (2nd for bottom, 12th for middle and 22nd for top) and age group were cut into small strips with a razor blade. The strips were dried in an oven (40 °C, 8 hours) and then ground in a Wiley Mill equipped with a No. 20 mesh screen. The ground material was placed in a shaker and particles that passed through a No. 40 mesh sieve but retained on a No. 60 mesh sieve were used for subsequent chemical analyses.

Bottom sections of the three-year-old bamboo culms were used to determine differences in chemical composition for the different radial layers. The epidermis of each strip was first removed with a fine blade and analyzed independently. The remaining material was

divided evenly based on volume into inner, middle and outer layers along the radial direction. The grinding and sieving processes were the same as described above. All chemical analyses were conducted based on standard methods from the American Society for Testing and Materials (Table 1). Toluene was used instead of benzene and reported as alcohol-toluene extractives. The amounts were expressed on a percentage basis of the starting oven-dry mass.

Vascular bundle concentration was measured in 45 micron thick cross (transverse) sections cut with a sliding microtome. Each slice was dried in an oven (40 °C, 8 hours) and viewed at 5× magnification under a light microscope equipped with a digital camera. Images were analyzed with Image Pro-Plus® software. The cross section of each sample was divided evenly horizontally into five layers and the number of vascular bundles counted. The cross-sectional areas for the bundles were also measured.

Effects of age, height and layer on chemical properties were evaluated by analysis of variance at $\alpha = 0.05$ using SAS software. The Tukey pairwise comparison method was used to compare mean values for the different layers and ages.

## RESULTS AND DISCUSSION

The average air-dry specific gravity (0.66 g/cm$^3$) of *P. pubescens* grown in the south-eastern USA was similar to that found for many other bamboo species grown in the tropics of Asia (Narayanamurty & Mohan 1972). The average relative proportions of the main chemical constituents, holocellulose (71.4%), α-cellulose (47%), Klason lignin (22.8%), alcohol-toluene soluble extractives (5%) and ash (1.5%) measured for *P. pubescens* were also similar to those observed in other species of bamboo (Higuchi 1957, Fengel & Wegener 1984, Dence 1992).

### Changes in chemical composition, specific gravity and anatomy with age

Chemical compositions for the one, three- and five-year-old culms appeared to show relatively small differences (Table 2). However, when compared by analysis of variance, all the components measured, except for α-cellulose, varied significantly with age. The one-year-old culms had lower holocellulose and Klason lignin contents than the three- and five-year-old culms, which were similar (Table 2). The alcohol-toluene extractives showed the largest variation, with average values for each age ranging from 3.2–6.9%. Alcohol-toluene extractives increased significantly with age, with the five-year-old bamboo having the highest average content at 6.9% (Table 2). Ash content of the one-year-old bamboo was significantly higher than that of three- and five-year-old bamboo (Table 2). Elevated ash content in one-year-old stems may be explained by the higher proportions of silica-rich epidermal tissue and metabolically active vascular tissues that function to mobilize inorganic nutrients to other regions of the plant. This is analogous to differences in ash content between living sapwood and dead (metabolically inactive) heartwood in trees, with the latter having a lower and more constant ash content. Three- and five-year-old bamboo showed no significant difference in ash content. With respect to culm specific gravity, a significant increase was observed with increasing age (Table 3). Specific gravity increased dramatically between year one and three and only increased slightly from year three to five. This agrees with the positive correlation between age and fibre dimension from age one to age three years old reported previously (Abd. Latif *et al.* 1994). Values for vascular bundles per unit area did not change with age. Changes in specific gravity with age are consistent with changes in chemical composition with age.

**Table 1** Standard methods used for chemical analysis of bamboo culms

| Property | Replicates | Standard |
|---|---|---|
| Alcohol-toluene solubility | 3 | ASTM D 1107-56 (Reapproved 1972) |
| Klason lignin | 3 | ASTM D 1106-56 (Reapproved 1977) |
| Holocellulose | 3 | ASTM D 1104-56 (Reapproved 1978) |
| α-Cellulose | 3 | ASTM D 1103-60 (Reapproved 1978) |
| Ash content | 3 | ASTM D 11084 (Reapproved 1990) |

**Table 2** Mean chemical compositions of bamboo culms at three different ages

| Age (Year) | Location[1] | Ash (%) | Alcohol-toluene extractives (%) | Klason lignin (%) | Holocellulose (%) | α-Cellulose (%) |
|---|---|---|---|---|---|---|
| One | Bottom | 1.82(a)[2] | 3.32(a) | 21.98(a) | 68.92(c) | 46.52(b) |
| | Middle | 1.94(a) | 2.86(b) | 22.11(a) | 70.84(b) | 47.30(ab) |
| | Top | 1.95(a) | 3.48(a) | 21.26(a) | 71.95(a) | 47.51(a) |
| Three | Bottom | 1.30(b) | 4.17(b) | 23.21(b) | 68.58(b) | 46.21(a) |
| | Middle | 1.36(ab) | 4.38(b) | 23.95(a) | 72.69(a) | 46.82(a) |
| | Top | 1.41(a) | 5.21(a) | 23.71(a) | 73.82(a) | 46.99(a) |
| Five | Bottom | 1.26(a) | 6.61(b) | 22.93(a) | 69.94(b) | 46.08(b) |
| | Middle | 1.30(a) | 6.81(b) | 22.97(a) | 72.50(a) | 47.65(ab) |
| | Top | 1.35(a) | 7.34(a) | 23.02(a) | 73.65(a) | 47.91(a) |

[1]The bottom portion of three-year-old bamboo was used to determine the effect of horizontal layer on the chemical constituent of bamboo.
[2]Means with similar letters for a particular test, location, and age are not significantly different at $\alpha = 0.05$ using the least significant difference test.

**Table 3** Air-dried specific gravity (SG) (g/cm$^3$) and vascular bundle concentration (VBC) (bundles/cm$^2$) of bamboo[1]

| Age (Year) | Location | Mean | | Outer[2] | | Middle | | Inner | |
|---|---|---|---|---|---|---|---|---|---|
| | | SG | VBC | SG | VBC | SG | VBC | SG | VBC |
| One | Bottom | 0.41 | 208 | 0.61 (0.05)[3] | 308 | 0.32 (0.03) | 174 | 0.29 (0.06) | 105 |
| | Middle | 0.42 | 243 | 0.63 (0.08) | 323 | 0.33(0.05) | 232 | 0.29 (0.04) | 153 |
| | Top | 0.43 | 301 | 0.63 (0.06) | 392 | 0.34 (0.02) | 297 | 0.32 (0.04) | 214 |
| Three | Bottom | 0.66 | 191 | 0.81 (0.06) | 292 | 0.60 (0.02) | 155 | 0.56 (0.02) | 126 |
| | Middle | 0.66 | 242 | 0.82 (0.04) | 378 | 0.61 (0.06) | 213 | 0.55 (0.07) | 135 |
| | Top | 0.66 | 295 | 0.84 (0.02) | 467 | 0.60 (0.04) | 256 | 0.55 (0.04) | 163 |
| Five | Bottom | 0.68 | 197 | 0.81(0.03) | 298 | 0.66 (0.02) | 175 | 0.58 (0.04) | 117 |
| | Middle | 0.69 | 236 | 0.82 (0.04) | 369 | 0.66 (0.01) | 193 | 0.59 (0.03) | 146 |
| | Top | 0.68 | 300 | 0.84 (0.03) | 458 | 0.63 (0.02) | 295 | 0.58 (0.02) | 148 |

[1]The samples had an average moisture content of 12%.
[2]The cross section was divided evenly into three layers along the radial direction.
[3]Standard deviations shown in parentheses.

Although statistically significant differences could be found in the Klason lignin and holocellulose contents between year one and years three and five, these differences were relatively small when compared with the increase in culm specific gravity (Table 3). This increase in specific gravity was due to the increase in number of cells in the vascular bundles with secondary thickenings. Minor changes in chemical composition suggested that lignification continued to occur during years two and three while more cells were forming secondary walls; otherwise the lignin content would remain equal or decrease. Several studies found that bamboo fibres can retain their living protoplasts over several growing seasons as cell walls continue to thicken, acquiring multiple layers and lignifying (Liese 1998, Murphy & Alvin 1997a, b). It has been reported that fibre cell wall thickness increased during the first year of growth and from year one to year three (Gritsch *et al.* 2004). The data in our study showed that after three years the bamboo appeared to achieve its maximum degree of lignification (Table 2). This appears to coincide with the improvement in mechanical properties that also occur up to three years of age (Li 2004). The increase in extractive contents in bamboo is analogous to that in trees where extractives deposition can also increase with age. Like wood, this may impart greater decay resistance. Thus, for applications that do not require natural decay resistance, there may be little advantage to harvesting later than three years. In summary, the age-dependent changes in holocellulose and α-cellulose contents are consistent with age-related changes in physical and mechanical properties of the bamboo. Together these results support the practice of harvesting bamboo at about three years.

## Changes in chemical composition, specific gravity and anatomy with height

The differences in holocellulose and α-cellulose with height were statistically significant (Tables 2). Their contents increased gradually from the bottom to the top of the culm (Table 2). In contrast, Klason lignin content did not appear to vary significantly with height. Although the differences in alcohol-toluene extractive and ash contents seemed small, the statistical analysis suggested that they varied significantly with height. The top section of the culm had significantly higher extractive and ash contents than the middle and bottom sections which had similar amounts. The results showed that with the exception of one-year-old bamboo, alcohol-toluene extractive content increased from the bottom to the top of the culms (Table 2). Specific gravity and vascular bundles per unit area did not vary significantly with height (Table 3). In contrast to trees which have more mature wood at the bottom and juvenile wood at the top with different properties, bamboo has similar chemical and anatomical properties up and down the stem.

## Radial differences in chemical composition, specific gravity and anatomy

Three-year-old culms were chosen to determine the distribution of chemical components from the inside to the outside of the culm because no significant difference was found between ages three and five. Also the basal internode was chosen because there was no significant difference with height. Basal internodes were divided into four radial sections, namely, epidermal, outer, middle, and inner layers for chemical analyses. Compared with the inner layers, the epidermis had three times more ash (Table 4). It has been suggested that higher ash content in the epidermis is due to high levels of silica; the level of silica in the rest of the culm is relatively low (Satish *et al.* 1994). Several common wood species have ash contents ranging from 0.43% (aspen) to 0.87% (white oak) (Misra *et al.* 1993). It is clear that the bamboo in this study had significantly higher ash content than these common wood species. The α-cellulose and holocellulose contents were highest in the outer layer and lowest in the epidermis. Low contents of α-cellulose and holocellulose in the epidermis is partly due to high extractive and ash contents. Previous research also showed that the epidermis consists of an outer and inner layer. The inner layer appears to be highly lignified while the outer cutinized layer is composed of cellulose and pectin (Liese 1987).

Wax like material attached to the inner layer was observed and may have contributed to the higher alcohol-toluene extractive content relative to the middle and outer layers. However, the exact nature of this material remains to be verified. The outer and middle layers had the same ash content as the inner layer, but differed significantly in extractives, holocellulose and α-cellulose between all three layers (Table 4). Holocellulose content decreased from the outer to the inner radial layer, but was not significantly different between the middle and inner layers. α-cellulose differed greatly across radial layers of the culm in the basal portion of the three-year-old bamboo (Table 4) and consistently decreased from the outer to inner layer. Klason lignin content was the most similar in the different layers, although still significantly different by analysis of variance. Specific gravity and vascular bundle content per unit area increased significantly from the inner to the outer layer at all three heights (Table 3). The specific gravity doubled and the number of vascular bundles nearly tripled between the inner and outer layers.

**Table 4** Mean chemical compositions of different radial layers of three-year-old bamboo culms

| Location | Ash (%) | Alcohol-toluene extractives (%) | Klason lignin (%) | Holocellulose (%) | α-Cellulose (%) |
|---|---|---|---|---|---|
| Epidermis | 4.09(a)[1] | 5.99(a) | 22.41(b) | 63.14(c) | 41.71(c) |
| Outer | 0.54(b) | 3.15(c) | 24.30(a) | 69.94(a) | 49.02(a) |
| Middle | 0.65(b) | 4.25(b) | 21.79(b) | 65.84(b) | 45.08(b) |
| Inner | 0.88(b) | 5.78(a) | 22.57(b) | 64.54(c) | 42.84(c) |

[1]Means with similar letters for a particular location are not significantly different at α = 0.05 using the least significant difference test.

Higher Klason lignin and α-cellulose contents in the outer layer are consistent with this layer being the most important in determining mechanical properties. α-Cellulose is the principal determinant of mechanical properties of bamboo and wood (Janssen 1981). Klason lignin content of bamboo stabilized after about three years. As the lignification process proceeds and fibres thicken from juvenility to maturity, we can expect that the amount of ash within a stem remains approximately stable while the total dry material increases, thus lowering the ash content. The variation in ash content with age was greater than with height. Overall, holocellulose varied significantly with age and height. α-Cellulose varied significantly with height but not with age. In contrast, Klason lignin did not vary significantly with height but did with age.

## CONCLUSIONS

The objective of this study was to determine the effects of age, height and radial position on the chemical composition of bamboo stems. From this research, we have found small but significant increases in holocellulose and α-cellulose content from the base to the top of the culm at all three ages. Bamboo age correlated significantly with specific gravity. Alcohol-toluene extractive content increased from the base to the top of the stem in the three- and five-year-old bamboos and showed a continuous increase with age. The epidermis had the highest extractive and ash contents and the lowest holocellulose and α-cellulose contents. The outer layer of the stem had the highest holocellulose, α-cellulose and Klason lignin contents and the lowest extractive and ash contents.

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