

Surface Discoloration Analysis and Lignin Degradation Fragments Identification of UV-Irradiated Moso Bamboo (*Phyllostachys pubescens* Mazel)

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Color changes caused by artificial UV radiation of Moso bamboo (*Phyllostachys pubescens* Mazel) were recorded as a function of exposure time to obtain the maximum absorption trend by the Kubelka-Munk (K-M) spectra. Lignin photolysis into smaller molecules was evaluated using spectrophotometry and gas chromatography-mass spectrometry (GC-MS) analysis. Results showed that the K-M absorption peak increased in the yellow and red regions (360 to 500 nm) when compared with the untreated sample, which was in accordance with the yellow-red shift by visual observation. The maximum absorption of the K-M spectra from UVB phototreated bamboo was in the UV region. GC-MS analysis showed that benzene carbonyls, organic acid, and esters were the major types of photolyzed molecules of bamboo lignin, which were derived from the C-C bonds adjacent to the α -carbonyl. UV irradiation (295 to 400 nm) resulted in the breakdown of carbonyl and unsaturated C-C groups conjugated to aromatic ring at the C α , which partly contributed to the quick discoloration at the initial 100 h of UV irradiation.

Keywords: Bamboo; UV irradiation; Lignin; Photolysis; Kubelka–Munk; GC-MS

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INTRODUCTION

Bamboo is a type of highly lignified grass that has good mechanical properties. It is one of the fastest growing plants in the world and can be harvested in three to five years after planting. Bamboo is one of the most important substitutes for wood resources, especially in China; the total bamboo area in China is 6.7 million hm², of which moso bamboo (*Phyllostachys pubescens* Mazel) accounts for 70% (according to the *Eighth National Forest Resource Inventory* report) and accounts for 30% of the world's total bamboo resources (Jia 2013). Because of its good mechanical properties, bamboo has become a popular outdoor construction material, such as scaffolding, outdoor flooring, external tables, and chairs (Yu 2012). However, similar to wood, bamboo is susceptible to environmental-induced weathering, such as solar irradiation, moisture, temperature, and oxidation (Hon and Feist 1986). During its degradation, bamboo materials undergo complex chemical changes, which lead to its discoloration, decreased lightness, roughening, and cracking of surfaces. The environmental-induced weathering will result in reduced commercial value and shortened product life. Many studies have been conducted on the color, chemical, and microstructural changes in wood caused from artificial photo treatment exposure as analyzed by Fourier transform infrared

spectroscopy (FTIR) and scanning electron microscopy (Hembree and Smyrl 1989; Owen *et al.* 1993; Fischer *et al.* 1995; Evans *et al.* 2008; Huang *et al.* 2012, 2013). These results showed that lignin is susceptible to UV irradiation, and the formation of colored unsaturated carbonyl compounds (*e.g.*, quinones) that are the main causes for wood discoloration (Anderson *et al.* 1991; Pastore *et al.* 2004; Pandey 2005; Williams 2005; Oltean *et al.* 2008). The oxidation and condensation of phenolic extractives also cause part of the color changes that occur during UV irradiation (Jamalirad *et al.* 2012). Bamboo is a highly lignified grass and has quite different chemical composition when compared to wood. Qualitative studies on lignin photolysis products and their relationship to photo-discoloration are limited in bamboo. The present study was performed to characterize the UV-irradiated bamboo discoloration by Kubelka-Munk (K-M) absorption, and to identify the small soluble lignin degradation fragments by gas chromatography mass spectrometry (GC-MS) analyses. The results of this study could help determine the mechanism underlying the photo-degradation of lignin in bamboo.

EXPERIMENTAL

Materials

Moso bamboo (*P. pubescens* Mazel) samples that were 60 mm in diameter were cut 1.3 m from the root apex to obtain culms that were 800 mm in length. The samples were split into eighths with a final dimension of approximately 800 mm × 20 mm × 12 mm; these samples were then oven-dried at 50 °C to obtain a final moisture content of about 12%. After drying, the bamboo strips were planed to 4 mm thickness, to obtain smooth surfaces on both sides, and sawed to obtain 200 mm × 15 mm × 4 mm specimens.

UV Light Irradiation

The prepared specimens were exposed to UV light irradiation in a chamber according to Jamalirad *et al.* (2012). The irradiance level was 25 W/m² at a 310 nm wavelength to simulate a maximum of one year of solar UV irradiation in the southern area of China, which was calculated using the formula:

$$kJ / m^2 = \frac{W}{m^2} \times t \times \frac{1}{1000} \times 3600 \frac{s}{h} = \frac{W}{m^2} \times 3.6 \times t \quad (1)$$

Irradiation time (*t*) was conducted at 100, 200, or 300 h intervals.

Color Determination

The surface color change of the specimens exposed to UV irradiation was measured at intervals of 100, 200, and 300 h using a Konica Minolta spectrophotometer (model CM-3600d) with a measuring head of 10 mm in diameter. Equipment calibration was carried out with the standards provided by the manufacturer. The spectrum was the mean value of three measurements obtained at different positions on the bamboo surface. The K-M spectra were calculated according to the K-M equation (Hembree and Smyrl 1989):

$$F(\gamma_\lambda) = \frac{K}{S} = \frac{(1 - \gamma_\lambda)^2}{2\gamma_\lambda} \text{ with } \gamma_\lambda = \frac{\gamma_{(sample)}}{\gamma_{(standard)}} \quad (2)$$

where K is the absorption coefficient, S is the scattering coefficient at wavelength λ , and γ_λ is the ratio of sample reflectance ($\gamma_{(sample)}$) and Whatman cellulose paper (No. 42) reflectance ($\gamma_{(standard)}$) with a defined porosity (Pastore *et al.* 2004; Tereza *et al.* 2004).

Chemical Analysis of Lignin Degradation Components

Bamboo shavings scraped from 100 h, 200 h, and 300 h UV-irradiated bamboo surfaces were mixture and analyzed to identify lignin degradation compounds. The shavings were extracted using a Thermo Scientific accelerated solvent extraction system (model Dionex ASE 350) (Pecina *et al.* 1986) with acetone/methanol/water (3:2:1) mixture at the following conditions: 50 °C temperature, 100 bar pressure, and 7 min static equilibration treatment time. For qualitative separation by a GC-MS system, acetone, methanol, and water were evaporated to dryness with a rotary evaporator, and then dissolved in acetonitrile for analysis in the GC-MS system. Analyses were carried out in a GC-MS (Agilent 7890A) system using a 0.25- μ m HP-5MS column (30 m \times 0.25 mm \times 0.25 μ m). Helium was used as a carrier gas at a flow rate of 1 mL/min. The injector was maintained at 270 °C. The column temperature was programmed as follows: 50 °C (1 min), 5 °C/min to 240 °C (1 min), and 10 °C/min to 280 °C (10 min). MS (full scan mode) was conducted as the following parameters: EM voltage of 1306 V, MS source at 230 °C, and MS quad at 150 °C.

RESULTS AND DISCUSSION

Color Changes and Reflectance Spectra of Bamboo Exposed to UV Light

Figure 1 shows that the bamboo surface discolored quickly when subjected to 100 h of irradiation. The surface color changed from white to yellow-red, and the color deepened with prolonged UV exposure. This finding was similar to the results reported by Pecina *et al.* (1986) for the photolysis of wood. The wood color change was closely related to radiation with wavelengths below 396 nm, which primarily caused photo-yellowing.

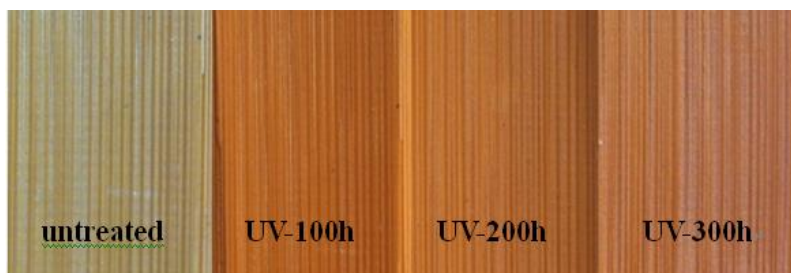


Fig. 1. Comparison of surface color of untreated and UV-treated bamboo strips

Wood and bamboo reflected most of the red-yellow wavelengths of light, which provides a warm color feeling. According to the color wavelength spectra, 400 nm was assigned to purple, 580 nm was assigned to yellow, and 700 nm was assigned to red.

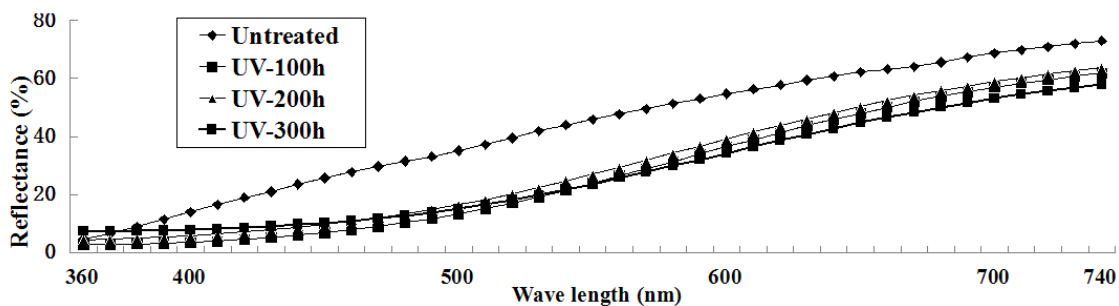


Fig. 2 Reflectance spectra of bamboo strip surface exposed to UV light irradiation

Figure 2 shows the reflectance spectra of the specimens after 100 h to 300 h of irradiation. The reflectance values decreased over the 380 nm to 540 nm wavelengths and showed a relative increasing over the 540 nm to 700 nm wavelengths compared with the untreated sample. Thus, the reflectance spectra were correlated with the yellow-red shift.

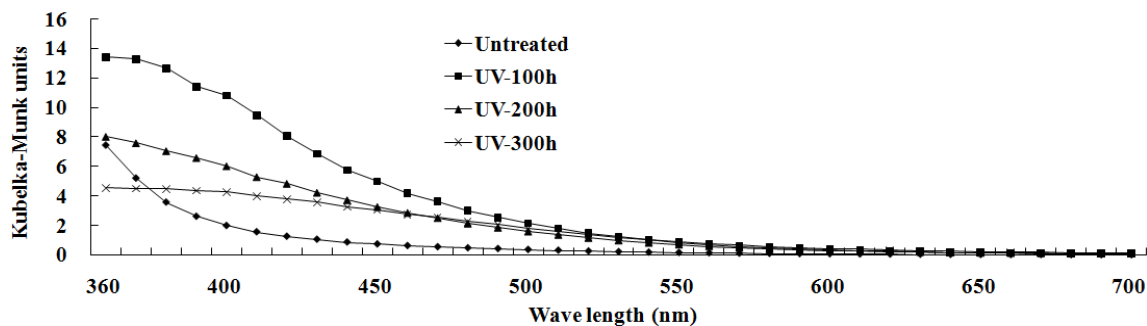


Fig. 3. Kubelka-Munk (K-M) spectra of bamboo strips exposed to UV light irradiation

Figure 3 plots the K-M function ($F(\gamma_\lambda)$; Eq. 2) based on the reflectance spectra of bamboo surfaces exposed to UV artificial weathering for 0 (*i.e.*, untreated), 100, 200, and 300 h. After UV irradiation, specimens exhibited a decrease in absorption from 360 nm to 500 nm when compared to the untreated sample. Samples exposed to UV irradiation for 100 and 300 h showed a maximum absorption near 360 nm, whereas the sample exposed to UV irradiation for 200 h did not present a maximum absorption peak over 360 nm to 700 nm range. This finding differed from previous studies of Huang (2012) and Tereza *et al.* (2004), which showed the maximum absorption occurred at 410 to 420 nm wavelengths for wood species. The difference in results can be explained by chemical and structural variations between wood species and bamboo. Visual observations correlated well with the changes in the K-M function since the darker samples had larger absorptions at the visible wavelengths of 380 to 500 nm (*i.e.*, UV-100 h > UV-200 h > UV-300 h).

GC-MS Analysis of Soluble Degradation Small Molecules

Lignin is a natural phenolic macromolecule that is present in the cell wall. It consists of three main phenylpropane units, which are bonded to each other by ether (C-O-C) or carbon-carbon (C-C) linkages. Natural lignin contains functional groups, such as methoxyl, phenolic, primary and secondary aliphatic hydroxyl, ketone, and aldehyde groups (Sarkanen and Ludwig 1971). The conjugation of carbonyl groups and

unsaturated C-C groups with the aromatic ring at the C α -position of the side-chain predominantly contribute to the color of wood (Hon 1981). The total amount of carbonyl groups in native wood lignin is approximately 0.21 per phenylpropane unit, among which 0.04 is aldehyde groups located at the C γ position and 0.17 is ketonic groups (Sarkanen and Ludwig 1971). These groups can absorb visible light and contribute to the color of wood; however, these groups are also vulnerable to degradation (George 2005). Figure 4 shows the main chromophoric groups found in lignin-rich mechanical pulps, along where their maximum absorption wavelengths occur (Paulsson and Parkas 2012).

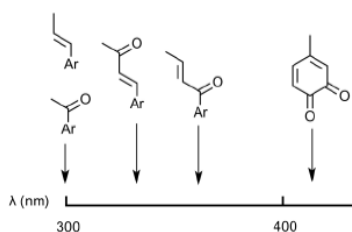


Fig. 4. Lignin-related chromophores in lignin-rich pulps and their approximate maximum absorption wavelengths (diagram adapted from Paulsson and Parkas (2012))

The photosensitivity of lignin in wood surfaces caused by photoirradiation has been extensively studied (Hon and Feist 1986; Pandey 2005; Wang and Ren 2009). Bamboo has higher levels of lignin and is speculated to be more susceptible to photodegradation, which is accompanied by the formation of new carbonyl groups that are observed at 1735 cm⁻¹ in the IR spectra (Wang 2009). The mechanisms behind photoyellowing of lignin-rich bamboo are complex, and several types of reaction pathways may coexist according to previous studies (Paulsson 2012). Photoyellowing proceeds *via* one initial fast phase and a slower following phase. The fast phase has been ascribed to oxidation of free phenolic groups and/or hydroquinones and catechols to photoproducts of mainly quinonoid character (Paulsson 2012). GC-MS analysis allowed for rapid qualitative analysis of lignin degradation compounds without derivatization (Brebua *et al.* 2013). The lignin degradation compounds in the UV-irradiated bamboo tissue was identified by GC-MS, and the results are shown in Fig. 5 and Table 1. Figure 5 shows the GC chromatogram of 16 types of small molecular degradation products, which have aromatic characteristics.

In Fig. 5 the absorption peaks of compounds in GC chromatograms can be identified by the fragment ion which comes from electron impact of mass spectrometry (MS). The samples total ion flow chromatogram were then compared by computer with the standard library, and the similarity was calculated according to peak position, intensity and molecular weight.

These degradation products could be classified into six categories: organic acids (peak 7, 8), aldehydes (peak 4, 5, and 10), phenols (peak 2, 3), ketones (peak 6, 9, and 11), esters (peak 12), and furans (peak 1) which are rich in carbonyls and carboxyl. Phenol products were also observed by Fischer *et al.* (1995) from photoinduced yellowing of high yield pulps. When excited by light, a carbonyl group can be quenched by oxygen (yielding singlet oxygen and ground-state carbonyl), which then undergoes C-C cleavage (Norrish Type I) (Lanzalunga and Bietti 2000). The carbonyl group acts as a photocatalyst and eventually leads to the formation of *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid end-groups (Paulsson 2012).

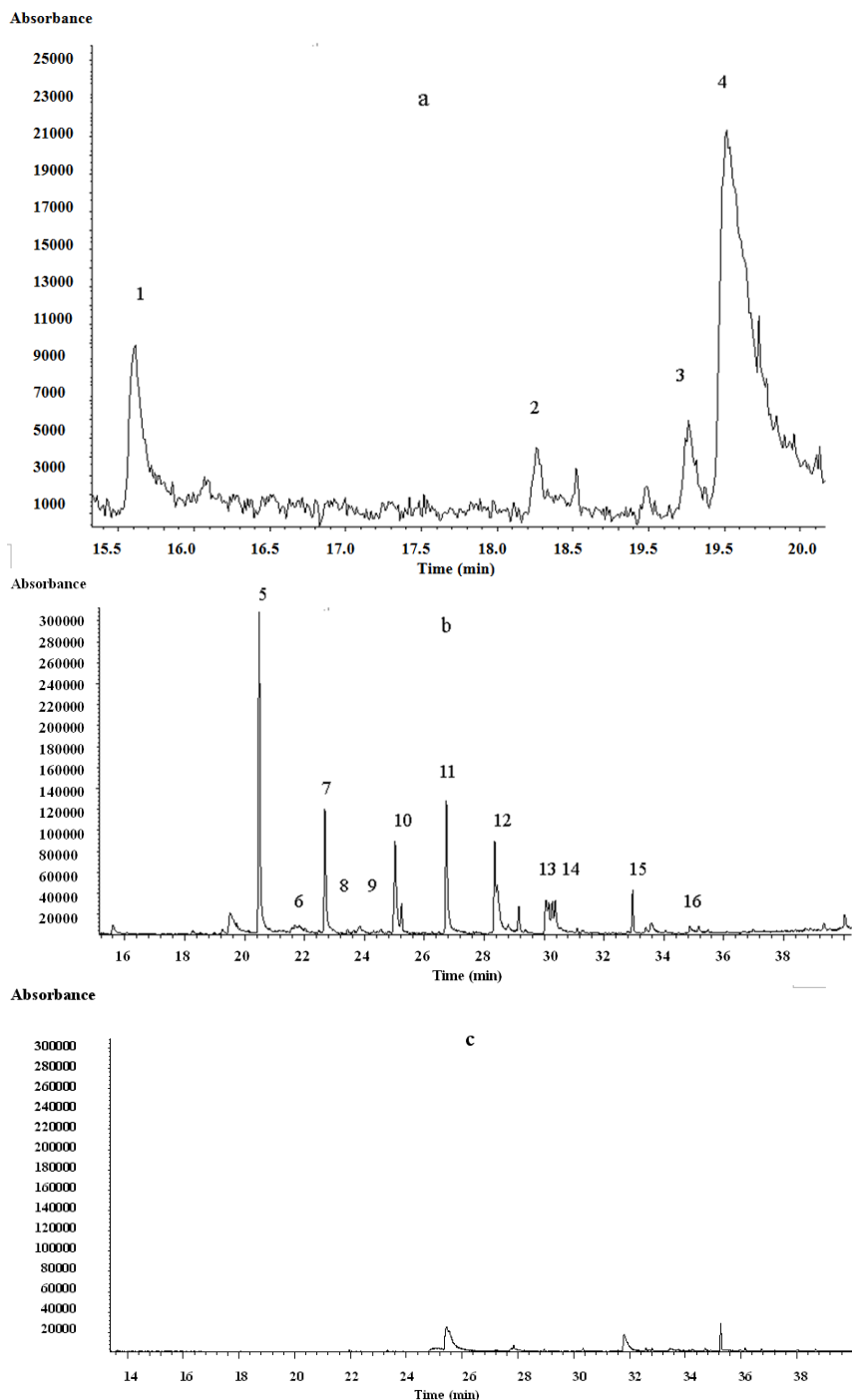
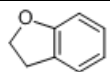
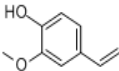
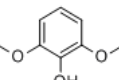
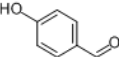
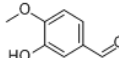
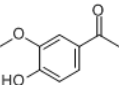
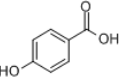
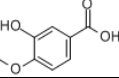
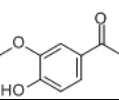
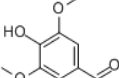
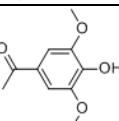
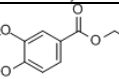
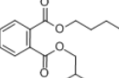
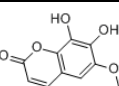
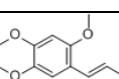
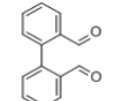


Fig. 5. GC-MS total chromatogram of soluble degradations of bamboo (a. UV-irradiated bamboo of retention time from 15 to 20 min; b. UV-irradiated bamboo of retention time from 20 to 35 min; c. Untreated bamboo)

Hon and Feist (1992) and Beatrice (2005) reported that light at wavelength of 295 to 400 nm can lead to the breakdown of carbonyl and unsaturated C-C groups conjugated to aromatic ring at the C_{α} . Peaks 2, 4 to 13, 15, and 16 all contained conjugated double bonds. These products were speculated to be lignin degradation fragments.

Table 1. Acetone/Methanol Extractives Identified by GC-MS

Peak No.	R.T. (min)	CAS No.	Structural formula	Similarity (%)	Peak area (%)
1	15.560	496-16-2		72	2.44
2	18.256	7786-61-0		91	0.52
3	19.240	91-10-1		90	0.52
4	19.451	123-08-0		96	5.28
5	20.488	621-59-0		97	13.48
6	22.688	498-02-2		97	3.30
7	23.121	99-96-7		95	4.87
8	24.496	645-08-9		96	1.60
9	25.035	498-02-2		49	3.08
10	26.748	134-96-3		97	10.51
11	28.366	2478-38-8		96	5.53
12	30.069	617-05-0		74	7.51
13	30.126	17851-53-5		98	2.31
14	31.942	574-84-5		83	1.35
15	33.411	494-40-6		52	0.75
16	34.870	1210-05-5		53	0.52

The main chromophoric groups (Fig. 4) in lignin-rich mechanical pulps (Paulsson and Parkas 2012), with approximate maximum absorption wavelength of 300 to 380 nm, partly contribute to quick discoloration at the initial 100 h of UV irradiation. However, given its small molecular size, this group was easily lost during the test, which partly

causes bleaching after 200 h of UV irradiation. Previous studies utilizing FTIR analysis ascribed the chief reason for wood discoloration after photoirradiation to the formation of quinones and quinone methides (Anderson *et al.* 1991). The present study showed that other carbonyl-conjugated aromatics partly contributed to bamboo discoloration.

From the degradation products (Fig. 5 and Table 1), it can also be observed that most of them have aromatic characteristics and are predicated to be degraded from the phenylpropane units of lignin (Ralph *et al.* 2004): *p*-hydroxyphenyl (H) type (peak 4 and 7), guaiacyl (G) type (peak 2, 5, 6, 8, 9, and 12) and syringyl (S) type (peak 3, 10, and 11). When calculated from the relative peak areas of Fig. 5, the degraded products from H-units account for 10.15%, from G-units account for 29.49%, and from S-units account for 7.56%. So it can be speculated that the G-units in lignin is more susceptible to light than H-units and S-units; this finding is in agreement with previous findings reported by Colom *et al.* (2003) and Fengel and Shao (1984).

CONCLUSIONS

After 100, 200, and 300 h of UV irradiation, the bamboo surface reflectance spectra decreased at wavelengths 360 to 540 nm and increased at wavelengths 540 to 700 nm; these changes in the spectra primarily contributed to the yellow-red shift. These results were in accordance with the visual observations. K-M spectra absorptions showed an increasing trend from 360 nm to 500 nm wavelengths when compared to the untreated sample (*i.e.*, control). The maximum absorption of phototreated bamboo was near 360 nm and possibly appeared in the UV region for UV-200 h sample, which was different from that of wood species. Visual observations correlated well with the changes in the K-M function since the darker samples had larger absorptions at the visible wavelengths of 380 to 500 nm (*i.e.*, UV-100 h > UV-200 h > UV-300 h).

Lignin photo-degraded small molecules such as benzene carbonyls, organic acids, and esters, and these were identified using GC-MS analysis. These products contributed to rapid discoloration after the initial 100 h of irradiation. Thus, conjugated aromatic carbonyls partly caused the discoloration at 295 to 400 nm wavelengths, which could be attributed to the breakdown of carbonyl and unsaturated C-C groups conjugated to aromatic rings at the C α position.

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