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ORIGINAL ARTICLE

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Effects of chromated-phosphate treatment process on the green color protection of ma bamboo (*Dendrocalamus latiflorus*)

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Abstract Ma bamboo (*Dendrocalamus latiflorus* Munro) treated with chromated phosphate (CP) exhibits an excellent green color. To understand the effects of the treatment sequence of CrO_3 and H_3PO_4 and their interactions regarding green color protection, CrO_3–H_3PO_4 and H_3PO_4–CrO_3 two-step treatments plus a H_3PO_4–CrO_3–H_3PO_4 three-step treatment were carried out in this study. Results revealed that the treatment sequence of CrO_3 and H_3PO_4 definitely affects the effectiveness of bamboo color protection. Green color protection of ma bamboo culm could not be achieved by treating it with CrO_3 or H_3PO_4 alone or with the H_3PO_4–CrO_3 two-step treatment. Only by treating it with the CrO_3–H_3PO_4 two-step treatment or the H_3PO_4–CrO_3–H_3PO_4 three-step treatment did ma bamboo exhibit an excellent green color. The results indicated that bamboo reacts first with CrO_3 and then forms an insoluble complex with H_3PO_4, which produces the green color on its epidermis. Chlorophyll analyses demonstrated that chlorophyll is not a key factor for green color protection. The green pigment was also formed when chlorophyll-free bamboo was treated with 2% CP at 60°C for 3 h.

Key words Chromated phosphate (CP) · *Dendrocalamus latiflorus* Munro · Green color protection · Chlorophyll

Introduction

Bamboo exhibits a fascinating greenish skin thanks to the abundant chlorophyll in its epidermis. After drying, storage, or some other utilization processes, however, the green color on the bamboo culm fades, thereby reducing the economic value of bamboo products. Numerous studies have reported that light, oxygen,[1–3] and enzymes play important roles in chlorophyll degradation[4] in vitro and in vivo. Similar to other green plants, bamboo loses its green color as a result of the deterioration of chlorophyll when exposed at ambient conditions.

To overcome this problem and to encourage the bamboo industry to explore potential utilization to increase the economic value of bamboo products using green culms, green color protection of bamboo had been widely studied during the last decade. In previous investigations, several inorganic salts, including chromates, nickel salts, and copper salts, were used as protectors.[5,6] In addition to these traditional chemicals, chromated copper phosphate (CCP) and chromated phosphate (CP) have proven to be effective green color protectors for the ma bamboo (*Dendrocalamus latiflorus* Munro) and moso bamboo (*Phyllostachys pubescens* Mazel).[7,8]

Although there have been studies on the effects of alkali pretreatment on green color protection of bamboo[9] and the effects of environmental factors (e.g., oxygen and light) on the color variations in CrO_3-treated bamboo,[10] the mechanisms of green color protection of bamboo remains unclear. So far, it has been known only that chromium and phosphorus from CP were located in the epidermis and cortical parenchyma of bamboo epidermal tissue after CP treatment, that CP oxidized the chemical components on the bamboo surface, and consequently that a large number of carbonyl groups and radicals were generated.[11] To gain a better understanding of the mechanisms behind green color protection of bamboo, the effects of CrO_3 and H_3PO_4 interactions (both are components of CP) during treatment on green color protection and the role of chlorophyll were investigated in this study.

Materials and methods

Materials

Three-year-old ma bamboo (*Dendrocalamus latiflorus* Munro) was obtained from the experimental forest of

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National Taiwan University. The fresh bamboo was cut into 4.0 × 1.5 × 0.4 cm pieces and stored at 4°C in the dark prior to use. CP (50% CrO_3/50% H_3PO_4) was used as a protector when treating the samples.

Pretreatment

Results of our previous studies indicated that before treating bamboo culms with inorganic salts alkali pretreatment was required to achieve green color protection.[7] Therefore, the bamboo specimens were pretreated at 80°C in 4% potassium carbonate containing 1% surfactant for 30 min to remove the wax layer on the outer surfaces; they were then carefully rinsed with distilled water.

Chemical treatments

After alkali pretreatment, a set of samples were treated with 2% aqueous solution CP at 60°C for 3 h or 6 h as described previously.[7] To understand the influence of components of CP on green color protection, two-step and three-step treatments were conducted. These treatment conditions were summarized in Table 1. In brief, during the two-step treatment the alkali-pretreated samples were first treated with 1% H_3PO_4 or 1% CrO_3 and then with the other component of CP (1% CrO_3 or 1% H_3PO_4). For the three-step treatment, the alkali-pretreated bamboo specimens were successively treated with 1% H_3PO_4, 1% CrO_3, and 1% H_3PO_4. Each treatment step was carried out at 60°C for 3 h, and all treated specimens were oven-dried at 60°C for 12 h before color and spectral analyses.

In addition, to clarify the influence of chlorophyll on green color protection, alkali-pretreated bamboo culms were extracted with EtOH/toluene (1:2, v/v) in a Soxhlet apparatus for 72 h to remove chlorophylls; then the chlorophyll-free bamboo specimens obtained were treated with CrO_3–H_3PO_4 or H_3PO_4–CrO_3 using the two-step processes. Similarly, each treatment step was carried out at 60°C for 3 h, and all treated specimens were oven-dried at 60°C for 12 h before color measurement.

Table 1. Treatment conditions for green color protection of ma bamboo

Treatment[a]	Chemical solutions	Treatment period (h)
Single process	2% CP	3
		6
	1% CrO_3	3
		6
	1% H_3PO_4	3
		6
Two-step process	1% CrO_3–1% H_3PO_4	3, 3
	1% H_3PO_4–1% CrO_3	3, 3
		3, 6
Three-step process	1% H_3PO_4–1% CrO_3–1% H_3PO_4	3, 3, 3

CP, Chromated phosphate
[a] All the processes were carried out at 60°C

Chlorophyll extraction and analysis

Chlorophyll of bamboo culm was extracted using the ultrasonic method established by Chang et al.[12] Scraped bamboo epidermis chips were ground to powder (particle diameter <0.7 mm) with a Wig-L-Bug grinder (Cresent Co., USA). Then 25 mg of bamboo epidermis powder was added to a sample vial containing 25 ml acetone; and chlorophyll was extracted by an ultrasonicator. After filtering the bamboo powder, the filtrates (chlorophyll solutions) were analyzed with an ultraviolet-visible (UV-VIS) spectrophotometer (Jasco V-550, Japan).

Measurement of surface color

The color of bamboo epidermis was measured by a color and color difference meter (Dr. Lange Co., Germany) under a D_{65} light source. The tristimulus values X, Y, and Z of all specimens were obtained directly from the colorimeter. Based on these data the L^* (value on the white/black axis), a^* (value on the red/green axis), and b^* (value on the blue/yellow axis) color coordinates were calculated as established by the Commission Internationale de Enluminure (CIE) in 1976.[7]

Diffuse reflectance UV-VIS spectral analysis

The diffuse reflectance UV-VIS spectra were obtained using an UV-VIS spectrophotometer (Jasco V-550, Japan) equipped with an integrating sphere. Specimens were placed directly into the integrating sphere sample holder such that the UV-VIS light beam impinged on the surface of the specimens. The scanning wavelength ranged from 300 to 800 nm.

Results and discussion

The CP-treated ma bamboo exhibited not only excellent green color protection but also good color fastness, as reported previously.[7,10] Table 2 shows the color variations of ma bamboo treated with CP and each component of it. The results revealed that the CP-treated ma bamboo displayed an excellent green color with $a^* = -6.1$ after 3 h of treatment, and $a^* = -14.0$ after 6 h of treatment. Green color protection was not observed after treatment with CrO_3 or H_3PO_4 alone. After treating for 3 h, the a^* values of CrO_3-treated and H_3PO_4-treated bamboo specimens were 17.6 and 11.4, respectively. Even after increasing the treatment period, the color of bamboo culms remained unchanged. This indicates that interactions of CrO_3 and H_3PO_4 play an important role in green color protection. To clarify further their relations and the influence of the two components of CP on the color of ma bamboo, a two-step or three-step treatment procedure was carried out.

Table 2. Color variations of ma bamboo treated with each component of chromated phosphate for 3 h or 6 h

Treatment	CIE Lab (3 h)			CIE Lab (6 h)		
	L^*	a^*	b^*	L^*	a^*	b^*
Control[a]	32.7 ± 0.2	−5.1 ± 0.5	17.1 ± 0.8	–	–	–
2% CP	46.0 ± 0.3	−6.1 ± 0.4	24.6 ± 0.4	47.8 ± 0.6	−14.0 ± 0.8	20.0 ± 0.6
1% CrO_3	28.7 ± 1.1	17.6 ± 0.6	9.5 ± 1.3	31.2 ± 0.5	17.4 ± 1.2	12.4 ± 0.4
1% H_3PO_4	38.5 ± 1.0	11.4 ± 1.0	20.3 ± 0.4	41.9 ± 2.0	9.4 ± 0.5	19.6 ± 1.1

Results are presented as means ± SD (n = 6)
[a] Fresh ma bamboo

Table 3. Color variations of ma bamboo during CrO_3–H_3PO_4 two-step treatment

Treatment[a]	CIE Lab		
	L^*	a^*	b^*
1% CrO_3 (1st step)	28.7 ± 1.1	17.6 ± 0.6	9.5 ± 1.3
1% H_3PO_4 (2nd step)	51.8 ± 1.5	−6.1 ± 1.6	29.1 ± 0.4

Results are presented as means ± SD (n = 6)
[a] Each treatment step was carried out at 60°C for 3 h

Table 4. Color variations of ma bamboo during H_3PO_4–CrO_3 two-step treatment

Treatment	CIE Lab		
	L^*	a^*	b^*
1% H_3PO_4 (1st step)[a]	38.5 ± 1.0	11.4 ± 1.0	20.3 ± 0.4
1% CrO_3 (2nd step)[a]	29.0 ± 0.6	17.7 ± 1.3	9.6 ± 1.0
1% CrO_3 (2nd step)[b]	33.3 ± 0.3	17.1 ± 0.9	14.6 ± 0.7

Results are presented as means ± SD (n = 6)
[a] Treatment time is 3 h
[b] Treatment time is 6 h

Effects of two- and three-step treatments on green color protection

After being treated with 1% CrO_3, ma bamboo epidermis became dark brown. The CIE Lab color parameters L^*, a^*, and b^*, as shown in Table 3, were 28.7, 17.6, and 9.5, respectively. However, the brown epidermis turned green after being treated with 1% H_3PO_4 during the second step of treatment, and the a^* value changed from 17.6 (first step of treatment) to −6.1. Variations in color were examined by diffuse reflectance UV-VIS spectrophotometer. Figure 1 shows that the reflectance on CrO_3-treated bamboo epidermis occurs only at wavelengths above 650 nm. After treatment with H_3PO_4, reflectance could also be observed at wavelengths of 450–600 nm. It demonstrated that ma bamboo treated first with 1% CrO_3 followed by 1% H_3PO_4 (two-step treatment) could achieve effective green color protection similar to that treated with 2% CP.

With a change in the treatment sequence of CrO_3 and H_3PO_4 (i.e., ma bamboo treated first with 1% H_3PO_4 followed by 1% CrO_3) the color of bamboo epidermis became quite different from that treated with the above-mentioned CrO_3–H_3PO_4 two-step treatment. The a^* values of specimens after each step of treatment (60°C, 3 h) were 11.4 and 17.7, respectively. When the treatment time of the second step was increased from 3 h to 6 h, the a^* value remained almost the same (Table 4). Moreover, Fig. 2 shows that neither H_3PO_4-treated nor H_3PO_4–CrO_3-treated bamboo has a distinguishable reflectance curve on the blue-green band. Thus, the green color of CP-treated ma bamboo may be the result of the CrO_3–H_3PO_4 two-step sequential reaction.

If this hypothesis is true, the H_3PO_4–CrO_3-treated bamboo should turn green after being treated with H_3PO_4 again. Accordingly, the three-step treatment was employed to evaluate the color change in specimens after each step of treatment. Results displayed in Table 5 revealed that when ma bamboo was treated with 1% H_3PO_4, 1% CrO_3, and 1% H_3PO_4 sequentially, the a^* value was 11.4, 17.7, and −6.0,

Table 5. Color variations of ma bamboo during H_3PO_4–CrO_3–H_3PO_4 three-step treatment

Treatment[a]	CIE Lab		
	L^*	a^*	b^*
1% H_3PO_4 (1st step)	38.5 ± 1.0	11.4 ± 1.0	20.3 ± 0.4
1% CrO_3 (2nd step)	29.0 ± 0.6	17.7 ± 1.3	9.6 ± 1.0
1% H_3PO_4 (3rd step)	53.7 ± 1.3	−6.0 ± 0.4	29.7 ± 1.1

Results are presented as means ± SD (n = 6)
[a] Each treatment step was carried out at 60°C for 3 h

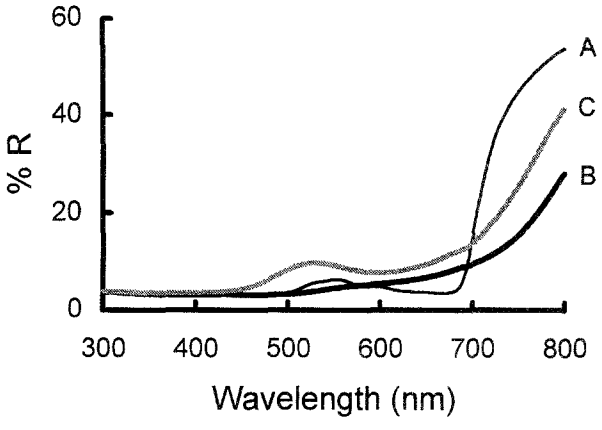

Fig. 1. Diffuse reflectance ultraviolet-visible (UV-VIS) spectra of ma bamboo after CrO_3–H_3PO_4 two-step treatment. *A*, pretreated; *B*, CrO_3-treated; *C*, CrO_3–H_3PO_4-treated

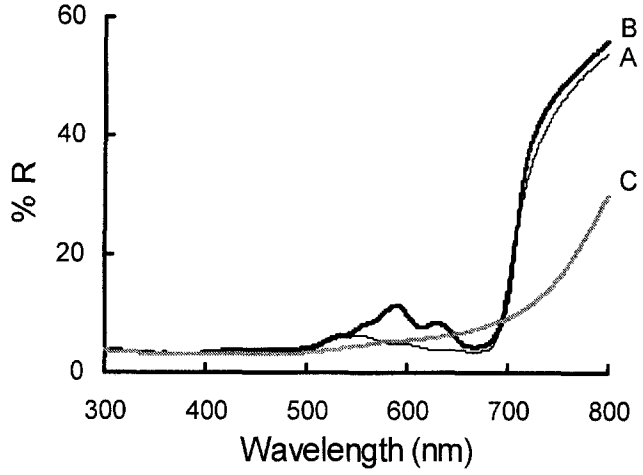

Fig. 2. Diffuse reflectance UV-VIS spectra of ma bamboo after H_3PO_4–CrO_3 two-step treatment. *A*, pretreated; *B*, H_3PO_4-treated; *C*, H_3PO_4–CrO_3-treated

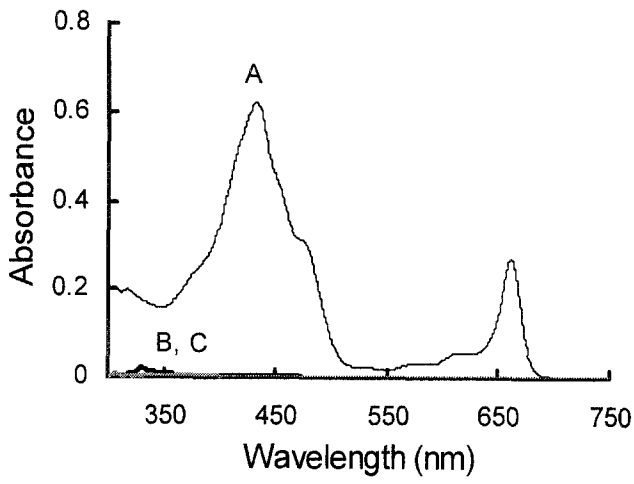

Fig. 3. Ultraviolet-visible spectra of chlorophyll extracted with acetone from the treated bamboo by ultrasonics. *A*, pretreated; *B*, CrO_3-treated; *C*, CrO_3–H_3PO_4-treated

respectively. Hence, green color protection of bamboo can be achieved definitely by the H_3PO_4–CrO_3–H_3PO_4 three-step-treatment. These results demonstrate that the CP treatment sequence has an impact on green color protection of bamboo.

Effect of two-step treatment on chlorophyll of bamboo epidermis

It is well known that standing culm of bamboo has an attractive green color owing to the chlorophyll in its epidermis. However, CrO_3-treated bamboo, which originally appears dark brown, turns green after H_3PO_4 treatment. This seems to imply that chlorophyll of bamboo epidermis is not a key or indispensable factor for green color protection. To study the changes in chlorophyll of bamboo epidermis during each step of chemical treatment, chlorophyll was extracted from bamboo and then analyzed by a UV-VIS spectrophotometer. Characteristic absorption peaks for chlorophyll a (662 and 431 nm) and chlorophyll b (642 and 452 nm) are recognized in the spectrum of fresh bamboo, as shown in Fig. 3. In contrast, no characteristic signals were detected in the spectra of CrO_3-treated specimens (Fig. 3B) or of CrO_3–H_3PO_4 two-step-treated specimens (Fig. 3C). Thus, it revealed that chlorophyll was degraded or an insoluble chlorophyll complex was formed on bamboo epidermis after the CrO_3–H_3PO_4 two-step treatment. On the other hand, the absorption spectra of the H_3PO_4–CrO_3 two-step treatment are shown in Fig. 4. Results revealed that when ma bamboo was treated with 1% H_3PO_4 during the first step of treatment the bathochromic and hypsochromic shift occurred at the red band and blue band, respectively, indicating a change in the structure of chlorophyll. After being treated with 1% CrO_3 during the second step of treatment, the bamboo epidermis turned green, but no chlorophyll absorption signal from the extracted solution was observed.

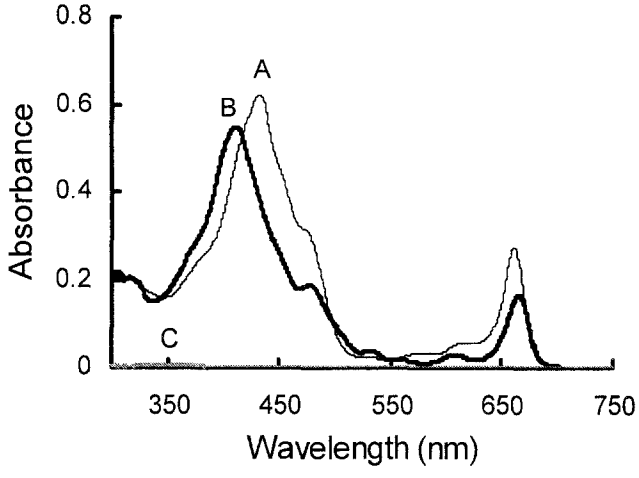

Fig. 4. Ultraviolet-visible spectra of chlorophyll extracted with acetone from the treated bamboo by ultrasonics. *A*, pretreated; *B*, H_3PO_4-treated; *C*, H_3PO_4–CrO_3-treated

To clarify further the influence of chlorophyll on green color protection, the chlorophyll-free bamboo culms were used as specimens in the study. After EtOH/toluene extraction, indeed, no chlorophyll absorption signal was observed in the spectrum of acetone extracts of bamboo epidermis (data not shown here). However, green color protection was obtained by treating the chlorophyll-free bamboo with 2% CP at 60°C for 3 h. Its L^*, a^*, and b^* values were 45.3, −6.6, and 20.9, respectively. Furthermore, the a^* values of chlorophyll-free bamboo treated by the CrO_3–H_3PO_4 and H_3PO_4–CrO_3 two-step treatments were −5.7 and 17.3 (Table 6), respectively, which are similar to those of the bamboo specimens without EtOH/toluene extraction, as shown in Tables 3 and 4. Accordingly, these results indicate that the chlorophyll of bamboo culm is not a key factor for green color protection.

Table 6. Color variations of chlorophyll-free ma bamboo after two-step treatment

Treatment	CIE Lab		
	L^*	a^*	b^*
CrO_3–H_3PO_4	55.9 ± 1.0	−5.7 ± 1.2	28.2 ± 0.9
H_3PO_4–CrO_3	30.8 ± 1.6	17.3 ± 1.2	11.9 ± 1.4

Results are presented as means ± SD (n = 6)

Conclusions

The standing culm of bamboo has an attractive green color owing to the chlorophyll in its epidermis. However, the chlorophyll does not play an important role on green color protection for CP-treated bamboo. The green color can be obtained by treating chlorophyll-free bamboo with CP. Furthermore, the reaction sequence of CrO_3 and H_3PO_4 in CP significantly affects the effectiveness of bamboo color protection. The green color can be obtained only by treating bamboo with CrO_3 and then with H_3PO_4, whereas a brown color is obtained by subjecting it to the H_3PO_4–CrO_3 two-step treatment. This indicates that bamboo reacts first with CrO_3 and then forms an insoluble complex with H_3PO_4 during CP treatment, which gives the green color on its epidermis.

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