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Expanding the Color Range of Natural Dyes on Cotton through Sequential Dyeing of Brazilein and Curcumin

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Abstract: Natural dyes are increasingly recognized as a sustainable and environmentally benign alternative to synthetic dyes in the textile industry. However, the practical application of natural dyes is severely limited by their narrow color range and relatively low color fastness. This study investigated the effects of chemical environment and dye application sequence on the color development of cotton fabrics dyed with brazilein and curcumin. Cotton samples were dyed using three methods: single-dye dyeing, blended dyeing, and sequential dyeing. All samples were tested under neutral, acidic, alkaline, and three metal mordant conditions, including Al^{3+}, Cu^{2+}, and Fe^{2+}. Color parameters were quantified using CIELAB coordinates and color difference values (ΔE), and UV–Vis absorption spectroscopy was used to characterize changes in dye molecular structures. The results revealed that brazilein was highly sensitive to metal ions, especially Fe^{2+}, which induced the most significant color change relative to the neutral control. Curcumin exhibited strong pH-dependent behavior, with the most dramatic color shift observed under alkaline conditions. Compared with blended dyeing, sequential dyeing produced a significantly wider and more diverse color palette. In particular, the sequence of brazilein followed by curcumin generated the most distinct and varied color outcomes across all chemical conditions. These findings demonstrate that sequential dyeing is an effective and sustainable strategy to expand the color range of natural dyes using only two plant-based colorants. This approach provides a simple, low-cost, and eco-friendly method to enhance the practicality of natural dyes for green textile manufacturing.

Author keywords: Natural dyes; sequential dyeing; brazilein; curcumin; cotton fabric

Introduction

The global textile industry relies heavily on synthetic dyes, which offer high color stability, bright hues, and low production costs. However, synthetic dyes are associated with serious environmental and human health concerns, including toxic wastewater discharge, carcinogenicity, and poor biodegradability.[1,2] In contrast, natural dyes extracted from renewable plant materials are non-toxic, biodegradable, and compatible with ecological textile production systems.[3,4] Natural dyes also support biodiversity conservation and reduce the carbon footprint of textile manufacturing.[5,6] Despite these advantages, natural dyes have not been widely adopted in industrial production due to two major limitations: a narrow available color gamut and insufficient color fastness, which lead to fading during washing, light exposure, and daily use.[7,8]

To address these challenges, mordants are commonly used to strengthen the binding between dye molecules and textile fibers, thereby improving color intensity and durability.[9,10] Metal mordants such as aluminum, copper, and iron ions can form coordination complexes with dye functional groups, enhancing fixation to cellulose fibers.[11] In addition, pH conditions strongly affect the molecular structure and chromogenic properties of many natural dyes, leading to changes in light absorption and visible color.[12]

Brazilin is the major active ingredient extracted from the heartwood of sappan wood (*Caesalpinia sappan* L.). It can be oxidized to the colorful formation brazilein, which is a historically important red natural colorant.[13] Brazilein contains multiple phenolic hydroxyl and carbonyl groups (Figs. 1A–1B), which enable coordination interactions with metal ions and confer high sensitivity to chemical environments.[14] It has been reported that brazilein, in combination with different metallic salts and pH conditions, can lead to a variety of colors of dyed fabrics.[15] Curcumin, the major color component of turmeric (*Curcuma longa* L.), is one of the very few natural products with promising prospects in both biology and chemistry.[16] It exhibits keto–enol tautomerism (Figs. 1C–1D), allowing it to chelate different metals and to form stable curcumin–metal complexes.[17] Meanwhile, curcumin shows a yellow-to-red color shift upon pH change.[18] Both dyes can bind to cotton cellulose through hydrogen bonding and are highly responsive to pH and metal ions, making them suitable model compounds for studying color modulation in natural dye systems.

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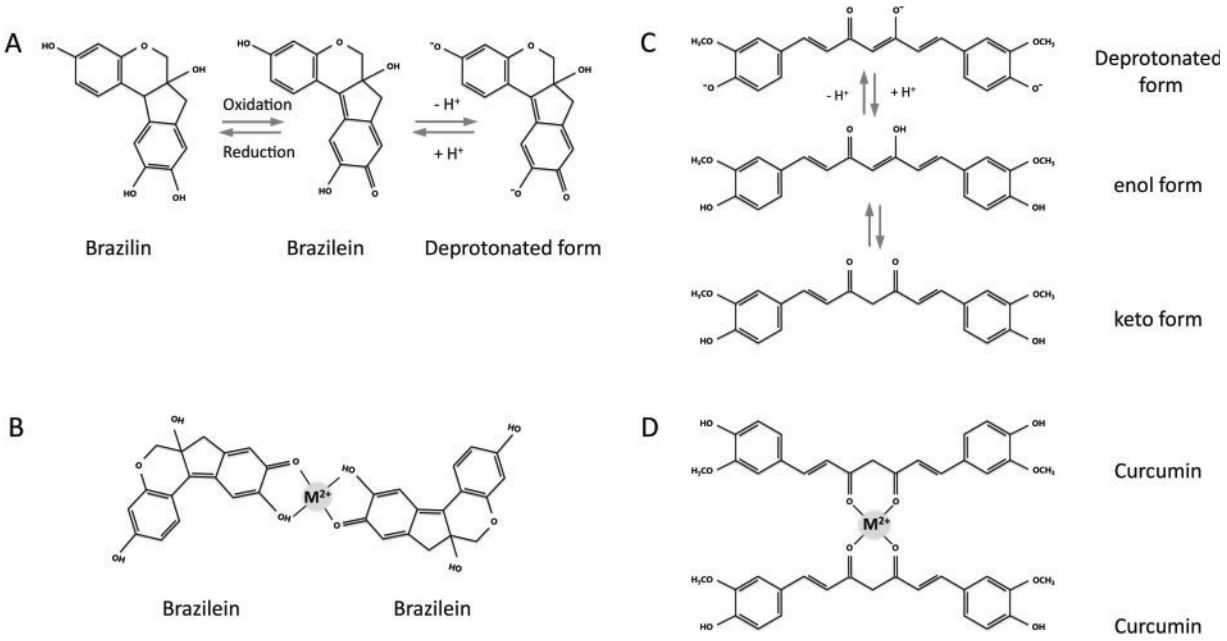

Figure 1. Scheme of chemical structure of (A) brazilin and brazilein, (B) 2:1 brazilein:metal complex, (C) keto–enol tautomerism of curcumin, and (D) 2:1 curcumin:metal complex

Most existing studies on natural dyes focus on single-dye systems or simple blended mixtures, where dyes are applied simultaneously. However, limited attention has been given to the effect of dyeing sequence on multiple color developments.[19,20] Sequential dyeing, in which dyes are applied in a controlled order, has the potential to introduce interfacial interactions between different dye molecules and the fiber substrate, leading to non-additive optical effects. Such interactions may enable the generation of new colors that cannot be achieved through single or blended dyeing systems.

Based on this hypothesis, this study investigates the influence of chemical environment and dye application sequence on the color behavior of brazilein and curcumin on cotton fabrics. The objectives are to (i) characterize the color responses of each dye under different pH conditions and metal ion mordants, (ii) compare color outcomes between blended dyeing and sequential dyeing, (iii) identify the optimal dyeing sequence for maximizing color diversity, and (iv) discuss the potential mechanism for color enhancement based on interfacial chromophore interactions. This work provides a simple, low-cost, and sustainable strategy to expand the color range of natural dyes without introducing additional dye sources, contributing to the development of environmentally friendly textile technologies.

Methodology

Materials

Cotton swatches (5 cm × 5 cm) were purchased from Alibaba. Acetic acid and $NaHCO_3$, as well as mordants including potassium aluminum sulfate (Al^{3+}), copper sulfate (Cu^{2+}), and ferrous sulfate (Fe^{2+}), were obtained from Sigma-Aldrich. Sappan wood and turmeric roots were purchased from Alibaba Health Pharmacy as dye sources. Deionized water was generated through filtration by a Milli-Q (Millipore) system and was used throughout all experiments.

Preparation for mordant solutions

Acetic acid, $NaHCO_3$, and the three metal mordant solutions (Al^{3+}, Cu^{2+}, and Fe^{2+}) were prepared at a concentration of 1% (w/v). Each solution was made by dissolving 1 g of the corresponding metal salt in 100 mL deionized water and stirring until fully dissolved.

Dye extraction

Dye extraction from sappan wood or turmeric roots was performed using a heating extraction method. For each dye, 10 g of ground plant material was added to 100 mL deionized water (10% w/v) and heated to boiling for 15 minutes. The mixture was then filtered through Whatman Grade 4 filter paper to remove solid residues, yielding clear dye liquors for subsequent dyeing.

Dyeing procedures

Single-dye dyeing

Pre-cut cotton fabric swatches (5 cm × 5 cm) were pre-wetted by soaking in deionized water for 15 minutes, then wrung out to remove excess water. The wet swatches were then immersed in the hot dye liquor at 80°C for 15 minutes, with occasional agitation to ensure even dye distribution. After dyeing, the swatches were thoroughly rinsed with running water to remove any unfixed dye. Following this, the dyed swatches underwent a post-dyeing mordanting process,

where they were immersed in each mordant solution, including acidic (acetic acid), alkaline ($NaHCO_3$), and metal ions (Al^{3+}, Cu^{2+}, and Fe^{2+}), for 15 minutes at room temperature. In the meantime, the dyed swatches were tested under neutral conditions (H_2O), which served as a control. Finally, the mordanted swatches were rinsed with deionized water and air-dried at room temperature.

Blended dyeing

Brazilein and curcumin liquors were mixed at a 1:1 volume ratio to prepare a blended dye solution. Cotton swatches were dyed and mordanted following the same procedure as single-dye dyeing.

Sequential dyeing

Sequential dyeing was carried out using two distinct application orders: Sequence 1 (brazilein followed by curcumin) and Sequence 2 (curcumin followed by brazilein). In each sequence, the fabric was first dyed with the initial dye, thoroughly rinsed, then dyed with the second dye, rinsed again, and finally given a mordanting treatment. This layered process enabled the stepwise adsorption and targeted interaction of each dye molecule on the surface of cotton fibers.

UV–Vis spectroscopy

Brazilein and curcumin crude extracts were pre-centrifuged to remove fine suspended particles and impurities before dilution. Brazilein solutions were diluted with deionized water at a ratio of 1:200, whereas curcumin solutions were diluted with 50% ethanol at the same 1:200 dilution ratio to obtain a clear, stable liquid. The prepared dye solutions were mixed with acetic acid, $NaHCO_3$, or metal solutions at a 1:1 (v/v) ratio. All resulting mixtures were centrifuged at 13,000 rpm for 5 min at room temperature prior to analysis. UV–Vis absorption spectra were recorded using a FlexA 200HT spectrophotometer over the wavelength range of 300–700 nm to evaluate changes in electronic structure and conjugation under different chemical conditions.

Color analysis

The color differences between the fabric swatches were quantified to provide an objective measure of perceptible color changes. The CIELAB color space was applied to calculate the difference between colors. CIELAB was created by the International Commission on Illumination (CIE), where the "CIE" refers to the French name of the organization: Commission Internationale de l'Éclairage. It is a device-independent, three-dimensional color space that measures and compares all perceptible colors using three values (L^*, a^*, b^*). In this space, numerical differences correspond to the degree of change humans can perceive. The CIEDE2000 formula (Eq. (1)) was then applied to calculate the color differences (ΔE). ΔE values ≥ 3 were considered perceptible to the human eye, while $\Delta E \geq 6$ indicated clearly distinct colors.

$$\Delta E = \sqrt{\left(L_2^* - L_1^*\right)^2 + \left(a_2^* - a_1^*\right)^2 + \left(b_2^* - b_1^*\right)^2} \quad (1)$$

where L^* measures whether the sample is light (high L^*) or dark (low L^*) , a^* (red/green index, with positive values indicating red and negative values indicating green) and b^* (yellow/blue index, with positive values indicating yellow and negative values indicating blue) represent the chromaticity (hue and chroma) of the sample.

The color of the fabric swatches was measured as described previously.[21] Digital images of the dyed swatches were captured with a smartphone (iPhone 17 Pro Max) under consistent lighting conditions. The open-source image analysis software ImageJ (NIH, USA) was then applied for the color conversion and CIE L^*, a^*, b^* measurement. Captured RGB images were converted into RGB stacks using ImageJ software. The average RGB values of each converted image with corresponding grey (white–black) intensity were extracted for assessment of individual chromatic hue in the CIELAB color space (L^*, a^*, b^*).

Results

Effect of chemical environment on brazilein staining

The influence of the chemical environment on brazilein dyeing behavior was evaluated using cotton fabrics and UV–Vis spectroscopy. In the single-dye dyeing procedure, fabrics exhibited a reddish color under neutral conditions (H_2O). Acidic (acetic acid) treatment resulted in pale yellow–brown color hues, while alkaline conditions ($NaHCO_3$) generated more saturated reddish-purple colors. Metal ions significantly altered the color outcome. Al^{3+} produced relatively light pink hues, whereas Cu^{2+} produced darker shades with a shift toward purplish-red hues. Fe^{2+} induced the most pronounced visual change, yielding khaki hues (Fig. 2B).

The color hues were then determined from quantitative color analysis (Figs. 2C–2D). Compared to the neutral conditions (H_2O), the red components (a^*) were reduced under both acidic and Fe^{2+} conditions. While more yellow/blue colors (b^*) were observed, they were in the opposite direction of yellow and blue in the acidic and Fe^{2+} conditions, respectively. The largest color difference relative to the neutral condition was observed under Fe^{2+} ($\Delta E = 143.27 \pm 8.09$), followed by acetic acid ($\Delta E = 107.53 \pm 9.77$). Moderate differences were observed for Al^{3+} ($\Delta E = 49.65 \pm 6.88$) and $NaHCO_3$ ($\Delta E = 21.71 \pm 2.41$), while Cu^{2+} showed a smaller but noticeable change ($\Delta E = 17.64 \pm 4.84$).

The UV–Vis absorbance spectra supported these observations (Fig. 3A). Under neutral conditions, brazilein exhibited a predominant absorption peak (~540 nm) with a small shoulder peak (~515 nm) in the visible region. Acidic treatment caused a hypsochromic shift to about 440 nm, while alkaline conditions led to the peak at 540 nm with an increase in intensity. The spectra represented the major forms of brazilein existing in the aqueous solutions, due to the protonation and deprotonation of the hydroxyl (-OH) group upon pH changes.[22] Metal coordination further modified the spectra. Al^{3+}, Cu^{2+}, and Fe^{2+} induced maximum absorption shifts to 525 nm, 555 nm, and 575 nm,

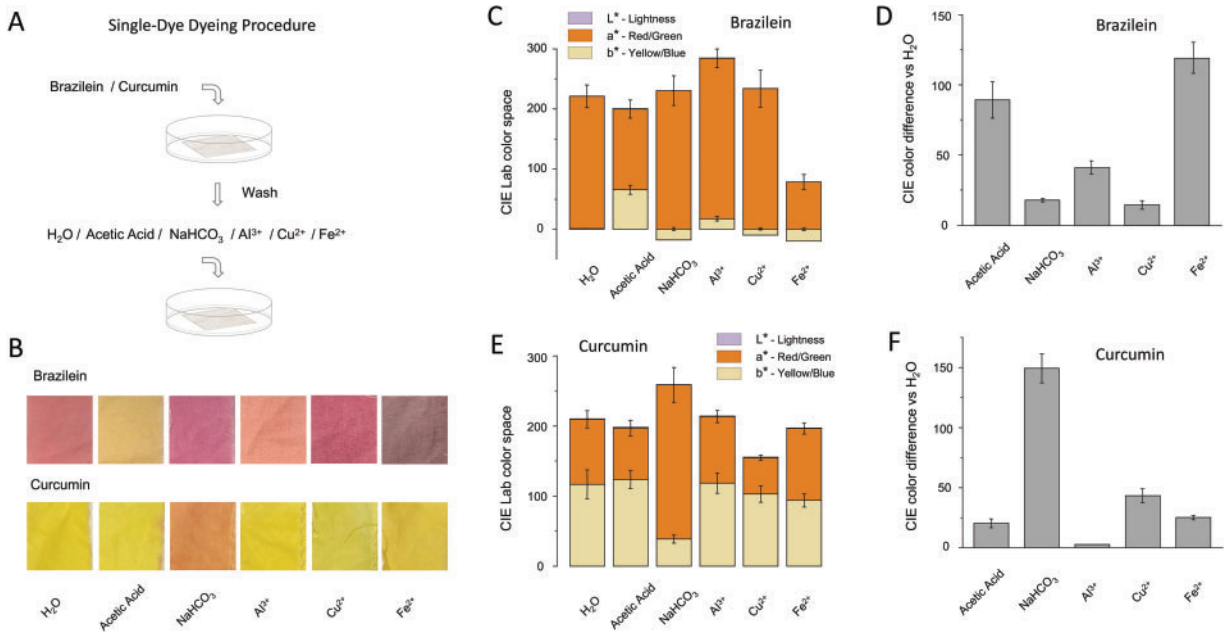

Figure 2. Effect of chemical environment on brazilein/curcumin staining. (A) Illustration of the single-dye dyeing procedure. (B) Samples of dyed cotton fabrics. (C) CIELAB color space analysis of brazilein-stained samples. (D) CIE color difference analysis of brazilein-stained samples. (E) CIELAB color space analysis of curcumin-stained samples. (F) CIE color difference analysis of curcumin-stained samples. Data were collected in three replicates and are presented as mean ± SEM

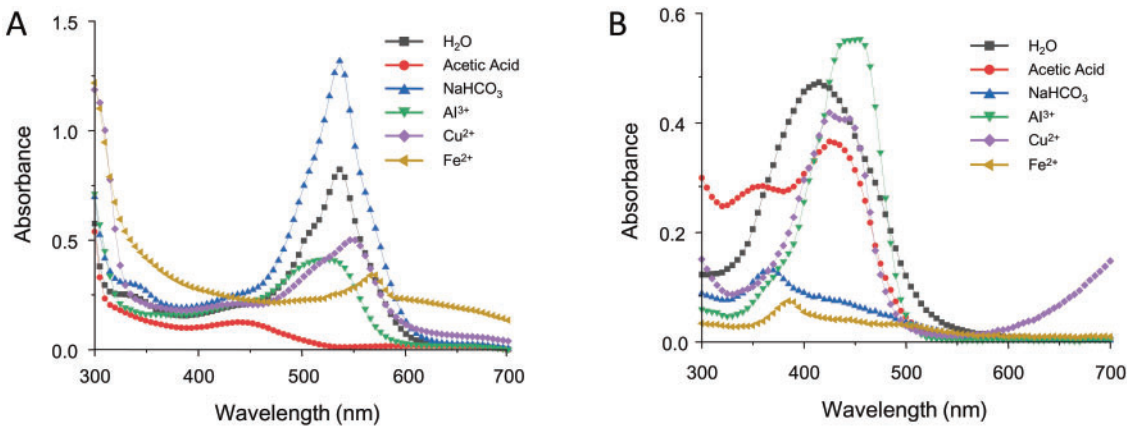

Figure 3. UV–Vis spectra under different chemical environments. (A) Brazilein and (B) curcumin

respectively, which correspond to the formation of a metal–brazilein complexation.

These results indicate that, in combination with brazilein, metal mordants impart more color variations to cotton fibers than that of pH changes.

Effect of chemical environment on curcumin staining

The effect of chemical environment on curcumin single-dyeing behavior is shown in Fig. 2B. Under neutral and acidic conditions, fabrics exhibited bright yellow coloration with minimal variation, indicating that curcumin remains stable in mildly acidic environments. In contrast, alkaline treatment ($NaHCO_3$) produced a pronounced color change toward darker orange-brown with light pink hues. Quantitative analysis (Figs. 2E–2F) revealed that more red components (a*) and the largest color difference occurred under $NaHCO_3$ ($\Delta E = 149.39 \pm 13.24$). Compared to brazilein, all metal ions (Al^{3+}, Cu^{2+}, and Fe^{2+}) had a weaker influence on the color change in curcumin, despite a little green color induced by Cu^{2+}.

UV–Vis spectra further support these observations (Fig. 3B). Under neutral conditions, curcumin showed a predominant absorption peak at about 415 nm in the visible region. In contrast, alkaline conditions led to reduced absorbance intensity and an altered spectral peak (~370 nm), while the main peak (~425 nm) and a small shoulder peak (~355 nm) were shown under acidic conditions. These are consistent with structural transformation of the chromophore, which is attributed to the $\pi \rightarrow \pi^*$ transition

of the keto–enol tautomeric form of curcumin.[23] Compared to neutral conditions, Al^{3+} and Cu^{2+} led to a bathochromic shift of the maximum absorption by 10–40 nm, while Fe^{2+} caused a hypsochromic shift of the absorbance peak to 385 nm. These shifts reflect the changes in intramolecular charge conjugation of the keto–enol moiety in curcumin, which may be caused by the formation of curcumin–metal complexation.

These results indicate that curcumin color variation is primarily produced by pH rather than metal coordination.

Color variation in blended and sequential dyeing

We next investigated how combining brazilein and curcumin, either by blending or sequential dyeing, influences the resulting color outcomes (Fig. 4). In the blended procedure, fabrics exhibited intermediate colors ranges compared to the brazilein- or curcumin-alone conditions, reflecting the combined contributions of both dyes (Fig. 4B). Neutral conditions enhanced light brown saturation, as indicated by increased yellow/blue (b^*) values, suggesting a stronger contribution from curcumin while brazilein maintained the red component. Under acidic conditions, a balanced yellowish-brown hue was observed, with moderate chromaticity (a^* and b^*) of the sample. In contrast, alkaline conditions produced darker and less saturated colors, showing reduced yellow components. Metal ions further influenced the blended procedure: Al^{3+} yielded relatively bright and vivid colors, whereas Cu^{2+} and Fe^{2+} resulted in darker hues. Red hues were predominant in all metal ion conditions, accompanied by a suppression of the yellow components, which were consistent with their effects in the brazilein-alone condition. The ΔE values (from 33.34 ± 3.52 to 79.69 ± 9.76) indicate moderate but clearly perceptible color differences across conditions.

Sequential dyeing with brazilein applied first, followed by curcumin, produced more pronounced and diverse color variations compared to the blended procedure. Under neutral conditions, fabrics exhibited stronger red hues with higher a^* values, indicating dominance of the initial color from the brazilein layer (Figs. 4B–4C). Similarly, alkaline conditions resulted in predominant red hues with diminished yellow contribution. Metal ions further amplified these effects: Cu^{2+} caused noticeable desaturation and induced the most pronounced color differentiation relative to the brazilein-alone condition, as exhibited by the highest ΔE value (138.39 ± 11.61). Fe^{2+} produced the most muted hues among all treatments. Consistently higher ΔE values compared to the brazilein-alone condition confirm greater color differentiation in this sequence (Fig. 4D), suggesting that the initial brazilein layer enhances subsequent interaction with curcumin and leads to more color variation.

In contrast, reversing the dyeing order (curcumin followed by brazilein) resulted in more limited color variation (Fig. 4B). Under neutral and acidic conditions, the colors shifted toward darker reddish-brown hues. However, fabrics retained similar background hues as seen in the curcumin-alone treatment, indicating that curcumin remained the dominant contributor. Metal ions induced some darkening, but the color variation remained less pronounced than in the brazilein-first sequence. Although color differences relative to curcumin alone were observed from ΔE values, the overall variations were consistent with the intrinsic behavior of curcumin in the test. These results indicate a reduced influence of brazilein when applied after curcumin, suggesting that the initial curcumin layer may limit further dye interaction.

Discussion

In this study, the experimental results demonstrate that the color variation of cotton fabrics dyed with brazilein–curcumin systems is determined by the dyeing sequencing. The sequential dyeing enables expanded color diversity beyond that achievable by either dye alone. These effects may arise from the associations between metal coordination, pH-responsive structural transformation, and interfacial chromophore interaction. The potential interactions between natural dyes, metal ions, and cotton fibers are proposed in Fig. 5

Cotton fiber is a cellulose fiber rich in hydrophilic hydroxyl groups. When brazilein is applied first, it is attracted to the fiber surface through hydrogen bonds and, where applicable, coordinative bonds with metallic mordants.[15,20] The hydrogen bridges are formed between phenolic -OH in the brazilein and the functional groups of cellulose (-OH) in the cotton fibers. During the mordanting process, $Fe^{2+}/Cu^{2+}/Al^{3+}$ interacts with these hydroxyl groups and forms the metal chelates instead. The metal–brazilein complexations not only enhance the adhesion of the brazilein dye with cotton cellulose but also introduce new shades to the dyed fabric. As a monobasic bidentate ligand, curcumin forms stable metal coordination upon subsequent dyeing through its enolic group. Meanwhile, the o-methoxy phenolic moiety of curcumin remains intact in these complexes, allowing it to interact with the hydroxyl groups in the cellulose to form hydrogen bonds.[18] The composite layer with modified chromophore interactions may generate greater color diversity. This behavior is consistent with previous findings that multi-component dyes effectively broadened the dyeing color spectrum.[20]

Interestingly, reversing the dyeing sequence results in a different outcome of dyed fabric. When curcumin is applied first, it forms a synergistic network of wide interactions with metal mordants and cotton fiber. These interactions include hydrogen bonds between the phenolic -OH or enolic -OH groups and cellulose -OH groups, van der Waals forces between the C=O group and cellulose -OH groups, and metal coordinations through the enolic groups and cellulose -OH groups, as well as π–π stacking interactions between aromatic C=C bands and cellulose -OH groups.[24,25] These combinations ensure the stable immobilization of curcumin on the cellulose surface. On the other hand, Puchtler et al. have reported the inadequate binding capacity of brazilein due to the lack of a second free phenolic -OH group in brazilein; i.e., one hydrogen bond is insufficient to anchor the dye to the tissues.[26] Consequently, when brazilein is introduced in the second dyeing procedure, the tight binding

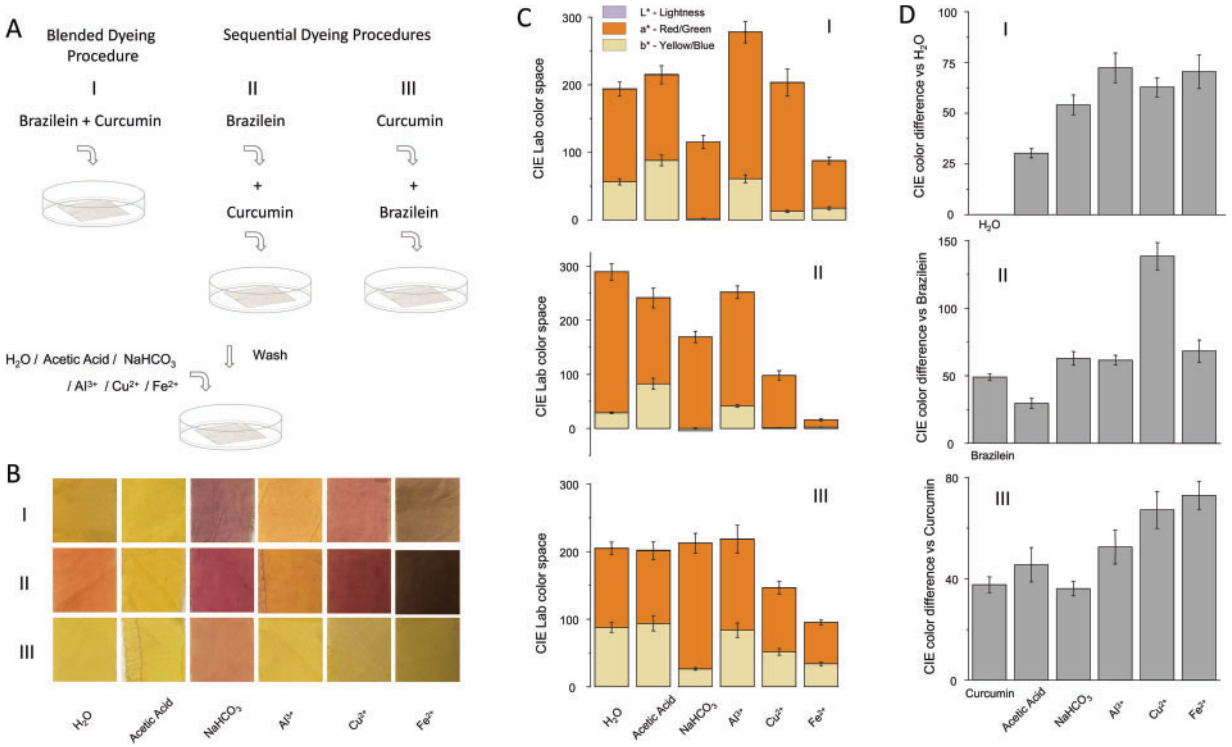

Figure 4. Effect of chemical environment on blended or sequential dyeing. (A) Illustration of the blended dyeing procedure (I), sequential dyeing procedure with brazilein followed by curcumin (II), and sequential dyeing procedure with curcumin followed by brazilein (III). (B) Samples of dyed cotton fabrics under different procedures. (C) CIELAB color space analysis of stained samples under different procedures. (D) CIE color difference analysis of stained samples under different procedures. Data were collected in three replicates and are presented as mean ± SEM

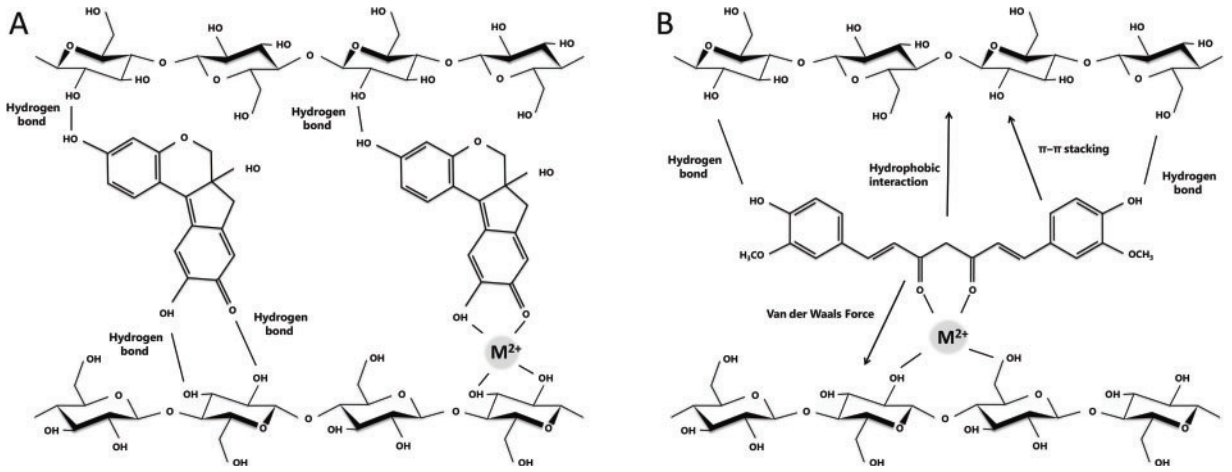

Figure 5. Illustration of the interactions between dye molecules, metal mordants, and cellulosic fibers. (A) Brazilein and (B) curcumin

of curcumin may affect the occupancy of brazilein in the cellulose interface. This could be an explanation for why staining patterns for the dyeing procedure of curcumin followed by brazilein are predominant in the yellow range.

The comparison between sequential and blended dyeing further highlights the color outcomes that are associated with dyeing order. In the blended procedure, both dyes interact simultaneously with the fiber, resulting in averaged optical properties and an intermediate color spectrum. In contrast, sequential dyeing establishes a directional interaction pathway, where the first dyeing modifies the crosslinking between dyes, metal ions, and the cellulosic interface through various kinds of interactions. This enables hierarchical interactions, leading to nonlinear optical behavior and enhanced color diversity, which produces color outcomes that cannot be achieved through simple additive mixing.

The narrow color spectrum is the major limitation of individual natural dyes. Using brazilein and curcumin as

examples of natural red and yellow dye, this study effectively expanded the color gamut and created more color tones through the approach of sequential dyeing with two natural dyes. By controlling the order of dye deposition, it is possible to modulate intermolecular interactions and broaden the accessible color space of natural dyes. Combined with optimized mordant and pH conditions, the color range of dyed fabrics could be enriched. This approach provides a simple and low-cost method to enhance the practicality of natural dyes for green textile manufacturing.

Conclusions

This study investigated the effects of chemical environment and dye application sequence on the color development of cotton fabrics dyed with brazilein and curcumin. The different staining patterns were observed among dyeing methods. Brazilein was highly sensitive to metal ions, while curcumin exhibited strong pH-dependent behavior. The blended system produced intermediate colors with moderate variation, reflecting simultaneous contributions of both dyes. In contrast, sequential dyeing generated more distinct and diverse color outcomes. Among these, the brazilein followed by curcumin sequence consistently yielded the largest color differences and the widest range of hues across all chemical conditions, particularly under metal ion treatments. The reverse sequence produced more limited variation, with colors largely governed by the initial curcumin layer. These results demonstrate that both dye combination and application sequence play critical roles in determining the final color of dyed fabrics. This provides a simple yet powerful strategy for enhancing the versatility of sustainable dyeing systems without introducing additional chemical complexity, aligning with current efforts toward environmentally responsible textile production.

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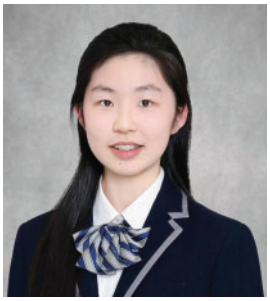
About the Author



Megan Li is an 11th-grade student at BASIS International School in Hangzhou, China. Her curiosity about chemistry began with a drop of Motrin she took for a childhood fever. She has since pursued diverse projects through independent research, including assessing heavy metal contamination in local waterways via colorimetric analysis and extracting natural dyes from sappan wood and turmeric to study coordination chemistry. Aiming to improve the efficiency and accuracy of sample collection, she also designed a water sampling device. Currently, she interns for a drug development project, assisting in an anti-influenza drug synthesis program and monitoring reactions by LC-MS. These experiences have shaped her interests in analytical chemistry and medicinal synthesis.