The Influence of pH, Temperature and Time on Dyeing of Silk Fabric by Black Bean Anthocyanin-rich Extract as Colorant

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ABSTRACT
Anthocyanins are natural dye substances that provide attractive colors from red to blue. However, large quantities of Anthocyanins have been found in wastewater from soaked black beans, resulting in environmental pollution. The utilization of theses wastewaters would therefore be an effective way of reducing environmental pollution, and the water could be used to dye silk fabric, displaying a more sustainable use of resources. In this paper, the effects of pH, temperature and time for dyeing of silk fabric were investigated. It was found that the optimum dyeing conditions were pH 3 at 60 °C for 60 min to achieve the best colors and the most effective color fastness on silk. Moreover, Anthocyanins were the potential to reduce 80% gram-negative and gram-positive bacteria. These results improved our understanding of dyeing conditions using Anthocyanins in the wastewater from soaked black beans, which plays an important role in reducing pollution, reducing costs and improving the quality of the related dyeing fabric families. Prog. Color Colorants Coat. 14 (2021), 179-186 © Institute for Color Science and Technology.

1. Introduction
Color is a vital aspect of the beauty of nature, and has been studied in a wide range of highly interdisciplinary scientific fields such as food coloring, cosmetics, pharmaceuticals, beverages, and textile materials [1, 2]. Colors are commonly revealed through dyes and pigments, which are created by chemical substances or synthetic and natural compounds, leading to their application in a broad range in products. Synthetic dyes play a role in many industries due to the extensive range of colors that can be achieved and their higher degree of reproducibility. However, some dyes are containing heavy metals and other toxic substances leading from the production process, also the pollution in wastewaters from the discharged of incomplete dye bath exhaustion process. These can have a serious impact on human health, causing cancers, skin damage, and lung diseases, as well as a negative impact to environment and to wildlife [3, 4]. In contrast, natural dyes are free from such substances and conform to the stringent environmental standards imposed by many countries, which are becoming increasingly aware of ecological safety and sustainable production in terms of coloration technologies. Accordingly, natural dyes are increasingly being researched to broaden their potential use and add differentiated competitive strategies for industry. This is especially true in dyeing and printing textiles as well as to meet niche markets in which consumers are becoming increasingly concerned about environmental factors and are looking towards

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responsible purchasing, which generally means that they are willing to pay more for products that are less harmful to the environment.

Recently, the study of characteristics and extraction processes of natural dyes from crops and plants such as Curcuma longa, Trigonella foenum graecum, Nerium oleander [3], Terminalia arjuna, Thespesia populnea [5], Croton urucurana Baill, bark [6], cranberry [7], eucalyptus [4], solanaceous vegetables [8], henna [9], berberine species [10] red onion [11], red sorghum [12], and Parthenocissus quinquefolia [13] have been studied, and some of the natural waste products from agricultural by-products as well as food and beverage industries were found to contain pigments that could be recycled for textile coloration. Researchers have expressed more attention in these by-products due to their biodegradability and the growth of environmental awareness. Research focused on dye extraction from the peel of black cardamom [14], eggplant peel [15], pomegranate peels [16, 17], red areca peel [18] and green walnut husks [17]. The natural dye extraction processes indicated specific levels of interest based on the bioactive compounds obtained by extracted dye properties of these natural products. The most common methodology was conventional extraction using water or organic solvents by simmering or boiling [3, 5, 6, 14, 15-19]. Less conventional methodologies use ultrasound [8, 18], liquid-pressurized processes, microwaves and supercritical fluids [20]. The most natural dyes (which include Curcumin, Henna and Quercus infectoria) display distinctive properties such as UV protection, antimicrobial functions and deodorized finishing [1, 15]. However, natural colorants were considered as indigo for the blues, Chlorophylls for the greens, Anthraquinone and Flavylium for the reds, Flavonoid and Carotenoid for the yellows and Anthocyanins for the purples to reds [21].

Anthocyanins are considered as potential substitutes for synthetic dyes, and represent one of the major groups of pigments in many plants including black chokeberry, elderberries, grapes, red cabbage and sweet potato. All these plants can provide colors in violet-red shades [21, 22]. Basically, Anthocyanins are the trans-structures of two aromatic benzene rings with an oxygenated heterocycle, and can be found in more than twenty structures, the six most common of which are Pelargonidin, Cyanidin, Delphinidin, Peonidin, Petunidin, and Malvidin [8, 13, 21]. Primary Anthocyanins are observed in Pelargonidin, Cyanidin, and Delphinidin, which have hydroxyl groups in their B-rings, main factor for describing the change of color [8]. Pelargonidin, Cyanidin, and Delphinidin showed orange-red, red-magenta and violet-blue hues, respectively. Meanwhile, Peonidin was transformed from Cyanidin along with Petunidin and Malvidin, provide violet color [8]. Black beans (Phaseolus vulgaris) are an interesting source of Anthocyanins due to the high concentration of Anthocyanins in the seed coat [23]. The concentration of Anthocyanins in black beans was found to be up to 44.5 mg/100 g [24]. However, the rich content of Anthocyanins is dissolved to the water in which the black beans are soaked, then holds the color, which is the key for use in textile dyeing processes.

In this work, the water in which the black beans were soaked was used to study the dyeing behavior, the influence of pH, dyeing temperature and dyeing time on coloring the silk fabric, and its protective antibacterial properties. These findings provide important clues for understanding the dyeing conditions that are best suited to use black bean water for dyeing the silk and related fabrics.

2. Experimental

2.1. Silk fabric preparation

100 % silk fabric with a double thread plain weave, 92.8 g/m² fabric weight and 72.1 CIE-Whiteness Index was purchased from a commercial store in Bangkok, Thailand, as the dyeing material. Specimens were treated in the preliminary bath with 2g/L soaping agent and 2 g/L sodium bicarbonate at 100 °C for 30 min to remove wax, sericin gum and any impurities from the material. Then, they were pressed in a Laboratory Pad-Mangle machine (Newave Lab Equipment, Taiwan), and dried at room temperature. The specimens of degummed silk fabric were used for the subsequent dyeing step.

2.2. Anthocyanins extraction

Anthocyanin solution from black beans was obtained from the Thanya Company, Thailand. In a typical synthesis process, 1kg of dried black beans was put into two liters of water for 12 h at room temperature. The solution was then passed through a polyester fabric filter to obtain an Anthocyanin solution with pH 6.9.
2.3. Dyeing procedure

The dyeing process was performed using Lab IR Dyeing Machines (Starlet DL-6000**, South Korea) with a liquor ratio of 50:1. The experiment was evaluated for the dyeing quality on specimens at various pH levels of anthocyanin solution, various dyeing temperatures, and dyeing times. The dyed specimens were then rinsed under running water, pressed and dried at room temperature.

The first factor examined was pH by dye maintaining at 60 °C for 60 min. Anthocyanin solutions were prepared and the accurate pH values were measured using a pH meter. The pH value was adjusted by buffer solutions such as acetic acid, sulfuric acid, sodium carbonate, and sodium hydroxide.

Afterward, the best results in terms of the pH effect, the dye adsorption was considered at different dyeing temperatures (50-100 °C) and times (30-240 min). Here, the absorption of the aqueous solution was recorded using a UVA-Vis spectrophotometer. The dyeing adsorption values were calculated using the following equation (Eq. 1):

\[
DA(\%) = \left( \frac{\text{ABS}_{\text{int}} - \text{ABS}_{\text{fin}}}{{\text{ABS}}_{\text{int}}} \right) \times 100
\]  

Where \(\text{ABS}_{\text{int}}\) and \(\text{ABS}_{\text{fin}}\) are the initial dye absorbance and the final dye absorbance, respectively. Tensile strength test was also performed to evaluate the breaking force (N) and percentage of apparent elongation using a HT 400 Pneumatic-H5KT, Tinius Olsen according to ASTM D 5035-06 (1-inch strip method).

2.4. Color quality measurement

The dyeing quality of the dyed samples was evaluated in terms of Colorimetric parameters (CIELab coordinate: L*α*β*) and color strength parameters (K/S) using a SpectroFlash SF600-CT spectrophotometer (Illuminant D65/10\(^0\) observer (with Colortools software (Datacolor International, USA)). L* is a measure of lightness/darkness of the fabric, which ranges from 100 (white) to 0 (black); \(\alpha^*\) is a measure of redness/greenness of the fabric, with positive (+) values indicating red and negative (-) values indicating green; and \(\beta^*\) is a measure of yellowness/blueness of the fabric, which positive (+) values indicating yellow and negative (-) values indicating blue.

The dyed specimens were tested for washing fastness using a James H. Heal Gyrowash according to ISO 105-C06. The color fastness to light was measured by the use of Atlas Alfa 150S test instrument according to ISO 105-B02, and the color fastness to rubbing was measured by a H. Heal 255 crockmeter according to ISO 105-X12. The evaluation of color fastness on samples was measured using a SpectroFlash SF600-CT spectrophotometer with a function-gray scale for change and staining.

2.5. Fourier transform infrared spectrometer (FT-IR) evaluation

Fourier transform infrared spectroscopy (Perkin Elmer, Spectrum One: USA) was carried out in the 400-4000 cm\(^{-1}\) region with Attenuated Total Reflectance (ATR) to investigate the chemical properties and structure of the normal silk fabric and the anthocyanin-dyed silk fabric at pH 3, 7, and 10.

2.6. Anti-bacteria activity

The untreated silk fabric control sample and the anthocyanin-dyed silk fabric at 60 °C for 60 min were tested against 2 bacteria strains using AATCC 100-2012 method. Klebsiella pneumoniae and Staphylococcus aureus were used as gram-negative and gram-positive bacteria, respectively. Anti-bacteria activity was shown in a % Reduction value according to the following equation (Eq. 2):

\[
%R = \frac{B - A}{A} \times 100
\]

where A and B are the amounts of colony-forming units (CFU) per sample before and after testing conditions, respectively.

3. Results and Discussion

3.1. Dyeing assessment

3.1.1. Influence of pH on color characteristics of the Anthocyanin-dyed silk

In order to study the stability of the anthocyanin solution, the soaked black beans were tested at different pH values in the range of acidic (pH 1-5), neutral or slightly acidic (pH 6-8), and alkaline (pH ≥ 9) conditions. It was noticeable that the highest color strength of Anthocyanin solution was noticeable in strongly acidic conditions (pH 1-2) with reddish-purple color, which gradually faded to a much paler reddish-
purple color by increasing the pH value (pH 3-5). It was obvious that the choice of color in anthocyanin solution depending on their co-pigment structure, Anthocyanin structure in the acidic condition showed the structural form of Flavylium as R-AH⁻ OH, which was a stable salt only in strong acidic conditions [11, 12]. Furthermore, the Anthocyanin solution showed a reddish color in neutral conditions (pH 6-8). The Anthocyanin structure was transformed into Quinonoidal bases in form of RA=O [10], which is an unstable pigment that rapidly bonded to water leading to the formation of a colorless compound called Carbinol pseudobase, caused the colorless material. Meanwhile, Anthocyanin solution exhibited violet/blue hue in alkaline conditions (pH > 8) due to Quinonoidal bases formed again in the form of RA=O⁻ [16] whereas the amount of Carbinol pseudobases decreased to form a color rapidly [10, 12]. Therefore, Anthocyanin dyes appeared to be a natural dye and a pH indicator [2]. The results of Anthocyanins dyeing at 60°C for 60 min and the measured tensile strength are shown in Table 1.

It was obvious that the color of the Anthocyanin-dyed silk fabrics was related to the anthocyanin solution in the dye bath. Basically, the silk fibers as protein fibers were a significant component of the repeating unit of polyamides, presented as amino and carboxylic groups in the protein polymer. They could be dyed without mordanting methods, and the dyeing schematic was present as follows (Eq. 3) [9]:

$$H\_2N^+ - \text{Silk} - COOH \xrightarrow{H^+} H\_2N^+ - \text{Silk} - COO^- \xrightarrow{OH^-} H\_2N - \text{Silk} - COO^-$$

Acidic pH<5 isoelectric pH 5.5 alkaline pH>8

(3)

Table 1: The influence of pH on color characteristics of Anthocyanin-dyed silk.

<table>
<thead>
<tr>
<th>pH</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>Color appearance</th>
<th>K/S</th>
<th>Tensile strength</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Breaking force; N</td>
</tr>
<tr>
<td>undyed</td>
<td>87.89</td>
<td>4.29</td>
<td>-1.33</td>
<td></td>
<td>0.38</td>
<td>325.00</td>
</tr>
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<td>19.00</td>
<td>2.35</td>
<td></td>
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<td>398.60</td>
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<tr>
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<td>16.88</td>
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<td>352.90</td>
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<tr>
<td>5</td>
<td>58.55</td>
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<td>1.02</td>
<td></td>
<td>2.10</td>
<td>347.20</td>
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<td>6</td>
<td>76.29</td>
<td>5.80</td>
<td>1.94</td>
<td></td>
<td>1.87</td>
<td>343.60</td>
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<td>7</td>
<td>76.67</td>
<td>7.54</td>
<td>7.01</td>
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<tr>
<td>8</td>
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<td>6.72</td>
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<tr>
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<tr>
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<td>6.11</td>
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<td></td>
<td>1.13</td>
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<td></td>
<td>0.83</td>
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</tr>
<tr>
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<td>5.23</td>
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<td>0.54</td>
<td>420.30</td>
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<td>14</td>
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<td>4.45</td>
<td>-0.94</td>
<td></td>
<td>0.48</td>
<td>425.20</td>
</tr>
</tbody>
</table>
The amino group takes the hydronium ion (H\(^+\)) from the anthocyanin solution and transforms the structure into a protonated amino group (NH\(_3^+\)) under acidic conditions, which is a key for promoting dye fixation onto the silk fiber. By increasing the pH value, the dye strength gradually fades, becoming colorless at around pH 5.5 due to the effect of iso-electric point [9]. Afterwards, this point was decreasing of number of protonated silk terminals could be substituted with hydroxyl ions (OH\(^-\)), which could be attributed to a weakly ionic interaction in which the dye molecules reduce the affinity in basic conditions. This means that the fibers could not be dyed, although the violet/blue hue was present in the dye bath.

Afterwards, dyed silk fabric was considered in terms of tensile strength, breaking force (N), and apparent elongation (%). Normally, silk is a semi-crystalline material, which tends to be oriented along axis of the fibers. The heat and chemical treatments especially in the strong condition were impacted on the fiber surface, which might be completely removed wax, oil, and other organic compounds covering on the external surface of the fiber cell wall. It was noted that the silk fabric became hydrolyzed in the chemical reaction, and the hydrolyzing process manifested in enhanced crystallinity values and surface roughness, meaning that the specimens became stronger and decreasing elongation [25]. The strong condition both in acidic and base condition affected on dyed silk fabrics, however base condition was more damaged than the acidic state. Consequently, the dyeing condition at pH 3 was acceptable due to explicit colorization and retained silk resiliency.

### 3.1.2. Influence of temperature and time on the color characteristics of Anthocyanin-dyed silk

The influence of temperature and time on the color of Anthocyanin-dyed silk at pH 3 was also investigated. Figure 1 (a) shows the correlation between the dye adsorption and temperature. At 50-60 °C, the dyeing adsorption process was increasing and get the highest peak at 30 min then desorption occurring and ran into a steady-state at 120 min, whereas 70-90 °C indicated 2 stages of the dyeing adsorption process. First, the adsorption lines were raising to the high level of dyeing adsorption percentage at that point desorbed and raising once time at 60 min to 120 min subsequently the dye adsorption was a steady-state after 120 min. Therefore, dye adsorption characteristics were shown in Figure 1 (b).

We can classify the dyeing absorption curves into three regions: (1) low dyeing absorption at 50-60 °C, (2) medium dyeing absorption at 70 °C, and (3) high dyeing absorption at 80-90 °C. It was found that the dye uptake into the silk fabric occurred by the exhaustion process at low dyeing absorption levels, giving a violet-red hue to the specimens at a dyeing equilibrium time of 60 min. Temperature also plays a significant role in the first stage of the dyeing exhaustion at medium and high regions, where the dyeing equilibrium time was 120 min. Here, the silk fabric color was red-brown at 70 °C and dark brown at 80 and 90 °C. Remarkably, the rise in temperature

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**Figure 1:** (a) the dyeing absorption at different times and temperatures and (b) color characteristics at different temperatures.
was an important cause of water evaporation from black bean seeds and at this point, the oxygen stopped entering the skin, resulting in damage to the anthocyanin structure. The degradation mechanism of anthocyanin structure is related to the oxidation of generated O-quinone enzymatic on the part of the O-diphenolic co-substrate. Meanwhile, the degradation reaction of Anthocyanins can be attributed to the loss of the red color and the change to the brown color in reactions, which generated Melanoidins from free carbonyl and amino groups. This simultaneously contributed to the condensation reactions with copigment reactions was Flavonols or Tannins [26-28].

Color fastness can be used as a useful factor for assessing the color resistance on the dyed silk fabric, as shown in Table 2. The results showed that dyeing at 50 and 60 °C yields the same violet-red hue. However, dyeing at 60 °C, the dyed fabric showed the better color fastness in washing, rubbing and light fastness. Remarkably, the increase in temperature turned the fabric from red brown to a darker brown hue, which represents the specific characteristics of tannins. Moreover, tannins is the significant to improve the light fastness of Anthocyanin-dyed fabric [21].

3.2. FTIR spectroscopy analysis
To investigate the structure change on silk fabrics, the FTIR spectra of all samples were collected as shown in Figure 2. The samples showed similar absorption bands for degumming silk fabric [29, 30], namely 1063 cm⁻¹ for the carbon nitrogen bond (C-N), 1227 cm⁻¹ for the carbon oxygen bond (C-O), 1443 cm⁻¹ for the carbon hydrogen bond (C-H), 1620 cm⁻¹ for carbonyl group (C=O), 2850 cm⁻¹ for the carbon hydrogen bond (C-H), 2960 cm⁻¹ for the carboxylic group (COOH), and 3391 cm⁻¹ for the hydroxyl group (OH). Moreover, the FTIR spectra of anthocyanin-dyed fabric at pH 7 and 10 are similar to the spectrum of undyed silk fabric, showing that none or only slight amounts of the pigments are attached to the fabric surface. On the other hand, the FTIR spectrum of Anthocyanin-dyed fabric at pH 3 showed peaks at 2607 and 2724 cm⁻¹, which can be assigned to aromatic and phenoxy structures, respectively, as discussed in Section 3.1.1 [7, 8, 24].

3.3. Anti-bacteria studies
The effects of bacteria on natural and Anthocyanin-dyed silk fabrics were also studied. Table 3 clearly demonstrated that the dyed silk fabrics with Anthocyanin solution were able to inhibit the growth of *Klebsiella pneumoniae* and *Staphylococcus aureus* bacteria more effectively than the natural silk fabric due to the fact that the anthocyanin solution contains Polyphenolic compounds such as Tannins, Quinone, Flavonoids, and Saponins, which play an important role in microbial growth inhibition [31, 32]. Moreover, Anthocyanin-dyed silk fabrics were a sustainable technique for value adding of waste into produce the products for various application fields (including protective clothing, home textiles, and industrial products.

![Figure 2: FTIR spectra of Anthocyanin-dyed fabric.](image-url)
Table 2: Color fastness variation of Anthocyanin-dyed fabric with temperature.

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Color fastness</th>
<th>Light</th>
<th>Rubbing</th>
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</thead>
<tbody>
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<td>CA</td>
<td>CO</td>
<td>PA</td>
<td>PES</td>
<td>PAN</td>
<td>Wo</td>
<td>Color change</td>
<td>Dry</td>
<td>Wet</td>
<td></td>
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<tr>
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<td>4</td>
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<td>3-4</td>
<td>4-5</td>
<td>3-4</td>
<td>3</td>
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<td>60</td>
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<td>4-5</td>
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</table>

Table 3: The effective anti-bacteria activities of samples.

<table>
<thead>
<tr>
<th>Bacteria type</th>
<th>Products</th>
<th>Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella pneumoniae</td>
<td>Silk fabric</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Dyed silk fabric</td>
<td>86.48</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Silk fabric</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Dyed silk fabric</td>
<td>85.21</td>
</tr>
</tbody>
</table>

4. Conclusions
Anthocyanin solution from soaked black bean water was an alternative choice of wastewater utilization which it applied for using in dyeing silk fabrics. It was found that the best dyeing procedure should be performed at 60 °C for 60 min in acidic conditions (pH 3), and the results showed good coloristic qualities and effective attachment to silk fabric. Moreover, Anthocyanins dyed onto silk fabrics inhibit bacteria growth (Klebsiella pneumoniae and Staphylococcus aureus bacteria) by more than 80 % according to AATCC100-2012 standards. This work helps support an alternative approach to develop niche market products from natural dyes.

5. References
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