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Dual fermentation of silk yarn and *hibiscus rosa-sinensis* dye extract for enhanced colour strength and intensity in silk dyeing

Puteri Arina Amni Kamaruzzaman[1], Wan Azwin Idayu Wan Mohd Juhari[1], Wan Khartini Wan Abdul Khodir[1,2], Ahmad Farid Abdul Jalal[3] & Shafida Abd Hamid[1,2,a]

[1]Department of Chemistry, Kulliyyah of Science, International Islamic University Malaysia, Bandar Indera Mahkota 25200, Kuantan
[2]SYNTOF, Kulliyyah of Science, International Islamic University Malaysia, Bandar Indera Mahkota 25200, Kuantan
[3]Lembaga Muzium Negeri Pahang, Jalan Sultan Ahmad 26600, Pekan

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Natural dyes offer an environmentally friendly alternative to synthetic dyes, but their broader use in textiles is constrained by low dye uptake and poor colour fastness. The effect of fermenting both silk yarn and *Hibiscus rosa-sinensis* dye extract on colour performance has not been systematically studied. This study examines the effects of fermentation pre-treatment on the dyeing performance of silk yarn dyed with *H. rosa-sinensis* flower extract. Two strategies were employed: pre-treatment of silk yarn with yeast, alum, or a combination of both, and fermentation of the flower extracts using *symplocos* or alum immersion. Dyed samples were evaluated for colour intensity (C*) and colour strength (*K*/*S*) using spectrocolourimetric methods. The highest colour intensity (C* = 26.65) and colour strength (*K*/*S* = 15.03) were obtained using yeast-fermented silk yarn dyed with alum-assisted dye extract, indicating enhanced dye penetration and fixation. Post-dyeing fixation with tannin and *N*-cetyltrimethylammonium bromide (CTAB), although intended to improve fastness, reduced the colour intensity, likely due to competitive binding or electrostatic repulsion with anthocyanins. Fermentation appears to promote structural or chemical changes in both fibre and dye, improving dye affinity and stability. The combined fermentation of yarn and extract presents a promising method for enhancing the natural silk dyeing performance.

Keywords: Anthocyanin pigments, Dye–fibre interactions, Microbial fermentation, Natural colourants, Sustainable dyeing

1 Introduction

Natural dyes are frequently used in traditional dyeing. Berries, flowers, barks, and roots were among the earliest recognised sources of fibre dyes. However, despite being environmentally friendly, natural dyes face challenges such as poor binding affinity to fibres, inconsistent colour yield, and lower overall dyeing efficiency, which limit their widespread adoption. As a result, only a small number of natural dyes have demonstrated sufficient stability and durability to be considered viable alternatives to synthetic dyes in commercial textile production[1]. Moreover, since the early 19th century, the dominance of synthetic dyes such as French ultramarine, Cobalt blue, and Phthalo blue in the textile industry has led to the gradual replacement of natural dyes. This shift occurred due to limitations of natural dyes, including low yields, difficulty in achieving consistent colour replication, inefficient dyeing processes, and a limited range of available colours[1,2].

Chequer *et al.*[3] reported that over 700,000 tonnes of synthetic dyes are produced globally each year, with 10–50 % of these colourants being lost to the environment during the dyeing process. These discharged synthetic dyes in water bodies and into nearby aquatic ecosystems are of concern because many are non-biodegradable, chemically stable, and contain aromatic structures or heavy metal components that could lead to significant environmental consequences. Synthetic dyes can also block light penetration, disrupting photosynthesis in aquatic plants and causing fluctuations in biochemical oxygen demand (BOD), chemical oxygen demand (COD), total organic carbon (TOC), and suspended solids[4]. They have also been linked to adverse health effects, including allergies, dermatitis, skin irritation, and potentially carcinogenic effects in humans.

Hibiscus rosa-sinensis, commonly known as Red Hibiscus, Chinese Hibiscus, China Rose, and Shoe Flower, is a widely cultivated ornamental plant in tropical and subtropical regions, and valued for its large, vibrant red flowers. The pigmentation of this species is largely attributed to its anthocyanin content,

[a]Corresponding author.
E-mail: shafida@iium.edu.my

producing a spectrum of hues in hibiscus petals, ranging from shiny orange and pink to deep red, violet, and blue[5]. These colour variations result from the electronic delocalisation of the flavylium ion, which enables anthocyanins to exhibit pH-dependent colour shifts. According to Shukla and Vankar[6], the primary anthocyanin in *H. rosa-sinensis* is cyanidin-3-sophoroside, which plays a key role in the plant's pigmentation and its potential as a natural dye. The five auxochrome groups in cyanidins play a crucial role in enhancing the dye–fibre interactions. However, anthocyanins, including cyanidin derivatives, are unstable and prone to degradation upon exposure to light, pH fluctuations, and high temperatures, leading to significant colour fading[7]. To address these issues, various stabilisation techniques have been explored, including the use of different mordants to improve dye–fibre affinity. For example, Fazal-ur-Rehman *et al.*[8] demonstrated an eco-friendly bio-dyeing process for silk using *Alkanna tinctoria* (Alkannin) and bio-mordanted silk substrates, which shows that biological modifications can enhance the performance of natural dyes. However, previous studies indicate that traditional mordanting alone does not yield consistently satisfactory results in improving natural dye retention and fastness.

Fermentation technique can be applied as a pre-treatment strategy to enhance the colour intensity and strength of natural dyes. Previous studies have reported that enzymatic pre-treatment strategies can modify fibre surfaces by increasing porosity, loosening cuticular layers, and generating organic acids that enhance dye–fibre interactions, improving dye uptake and fastness performance[9-12]. Fermented dye extracts may also contain partially hydrolysed or structurally modified pigment molecules with higher affinity for protein fibres. Microbial natural pigments and bio-processing are increasingly used for textile colouration and can be integrated with fibre or dye fermentation strategies[13]. Recent reviews also highlight biomordants and bio-assisted processes as effective, lower-impact alternatives to conventional fixatives[14]. Given this context, the present study investigates the dual fermentation of both silk yarn and *H. rosa-sinensis* dye extract. This dual approach is anticipated to provide synergistic benefits, including enhanced dye penetration, stronger dye–fibre bonding, improved colour strength, and potentially better fastness by simultaneously modifying the fibre's receptivity and stabilising the anthocyanin dye molecules.

Although some studies have explored the microbial or fermentation-based production of pigments for textile dyeing[13,15,16], scientific reports on its direct influence on the dyed fabric or fibres remain limited. For example, only a few recent studies, such as the dyeing of Eri silk using a fermented banana trunk extract approach this concept[17]. In this work, the silk yarn was pre-treated using three methods: yeast-fermentation, alum-assisted immersion, and yeast–alum combined fermentation. In a separate process, hibiscus flowers were fermented with *symplocos* and alum (separately), and the resulting extracts were subsequently used to dye the silk yarn. Previous studies have explored microbial or alum-based modification of fibres or dyes separately, but to our knowledge, this is the first comparative study evaluating the dual fermentation of both silk yarn and dye extract from *H. rosa-sinensis* for enhancing anthocyanin-based textile dyeing. This study offers new insight into how fermentation influences fibre receptivity, anthocyanin stability, and dye–fibre interactions.

2 Materials and Methods

2.1 Plant Material and Chemicals

Hibiscus rosa-sinensis flowers were collected from various locations in Kuantan, Pahang, Malaysia. The flowers were dried in a dehydrator at 50 °C for 8–10 h, then stored in airtight containers with silica gel in the dark until further use. The silk yarn used in this study was 120/2, 100 % mulberry silk, purchased from TCB Batik & Songket Sdn. Bhd., Malaysia, corresponding to the standard silk typically used for textile dyeing applications. Aluminium potassium sulphate (alum, 99.7 %) was purchased from Bendosen, while *N*-cetyltrimethylammonium bromide (CTAB, 98.0 %) was purchased from R&M Chemicals. Methanol (99.9 %), citric acid and fructose (99.5 %) (99.7 %) were obtained from HmbG Chemicals. Sodium hydroxide was obtained from Merck, while soap nut, *symplocos*, and yeast were procured from local suppliers. All reagents were used without further purification.

2.2 Extraction of Dyes from *H. rosa-sinensis* Flowers

The dehydrated hibiscus petals underwent a maceration process in methanol with 4 % (w/v) citric acid for 24 h at room temperature in the dark, without

stirring. The mixture was then filtered using a strainer cloth to separate the liquid extract from the solid petals and other solid impurities. To ensure complete extraction, the remaining petals were rinsed with the same solvent (acidified methanol) until a clear solution was obtained. The filtrates were pooled together and concentrated by removing the methanol–citric acid solvent using a rotary evaporator under reduced pressure at 40 °C to prevent thermal degradation of anthocyanins. The resulting gel-like pigment concentrate was then stored in a sealed vial under controlled conditions until further use.

2.3 Degumming of Silk

The silk yarn was degummed using a soap nut solution for 30 min, prepared at a material-to-liquor ratio of 1:50. Eco-friendly degumming and surface modification steps increase fibre wettability and exposure of amino/carboxyl groups to enhance anthocyanin dyes uptake[18]. A higher liquor ratio was selected to ensure sufficient bath volume for fibre mobility and uniform exposure to the natural saponins released from the soap nut. The degummed silk yarn was treated in the soap nut solution for 30 min, thoroughly rinsed with tap water, and air-dried at room temperature for two days.

2.4 Fermentation

Two types of treatment processes were employed in this study: fermentation/treatment of silk yarn and fermentation of *H. rosa-sinensis* flowers. For silk yarn fermentation/pre-treatment, three different approaches were used: i) yeast-only fermentation, ii) alum-immersion treatment, and iii) a combination of yeast–alum fermentation. Meanwhile, for *H. rosa-sinensis* flower fermentation, the flowers were fermented with either i) *symplocos* or ii) alum.

2.4.1 Fermentation of Silk Yarn

A 30-mL solution was prepared in a beaker containing 10 % aluminium potassium sulphate (alum) and/or 50 % yeast, both calculated based on the weight of fabric (owf). When yeast was included, 50 % fructose owf was also added to the solution as a nutrient source. Distilled water (30 mL) was added to the solution to dissolve the alum and fructose with a material-to-liquor (MLR) ratio of 1:30. The prepared mixture was then stored in an airtight container in the dark and left to ferment for four days. After fermentation, the degummed silk yarn (1 g per trial) was pre-wetted and immersed in the fermented mixture for an additional four days to continue the fermentation process. The silk yarn was then thoroughly washed with water to remove any residual substances. A schematic overview of the silk yarn fermentation process is provided in Fig. 1.

2.4.2 Fermentation of Flowers

Dried *H. rosa-sinensis* flowers (3 g) were soaked in a 10 % *symplocos* or alum solution in distilled water (150 mL), maintaining a material-to-liquor ratio of 1:50. This volume was necessary to ensure complete submersion of the petals during fermentation. The mixture was then stored in an airtight container in the dark and allowed to ferment for five days. After the fermentation period, the solution was filtered through a strainer cloth to remove any solid residues. The pH of the extracted dye was adjusted to 3.0–3.5 by adding a 4 % citric acid solution. Figure 2 provides an overview of the *Hibiscus rosa-sinensis* dye extract fermentation process.

2.5 Mordanting

A pre-mordanting method was applied to the silk yarn before the dyeing process. The silk yarn was pre-wetted before being immersed in a 10 % owf alum mordant solution, prepared at a material-to-liquor ratio of 1:30. The mordant bath was then heated to

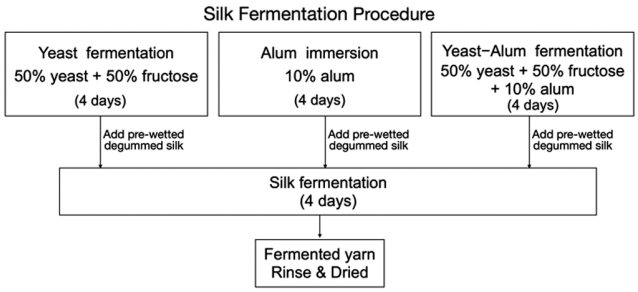

Fig. 1 — Flowchart of the three silk pre-treatment methods: yeast fermentation, alum immersion, and yeast–alum combined treatment

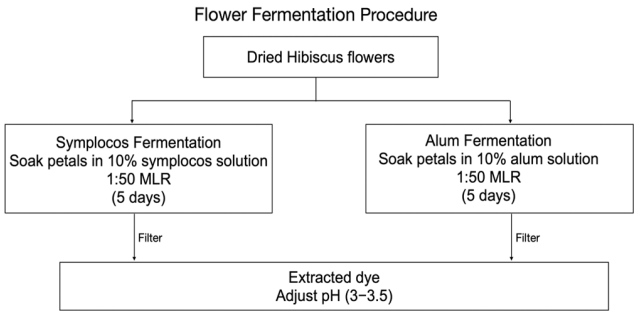

Fig. 2 — Flowchart showing the three dye extract preparation pathways: *symplocos* fermentation and alum fermentation

60 °C and maintained at this temperature for 30 min. The silk yarn was then thoroughly rinsed with clean water to remove any excess mordant and air-dried in a well-ventilated area, away from direct sunlight, before proceeding with the dyeing process.

2.6 Silk Dyeing

The dyeing process for the silk yarn was carried out using a material-to-liquor ratio of 1:30, i.e. for every 1 g of silk, 30 mL of dye solution was used in the dye bath. For fermentation dyeing, the fermented *H. rosa-sinensis* solution was used directly as the dye bath without concentration adjustment. For maceration dyeing, the concentrated *H. rosa-sinensis* extract 3 g was dissolved in 30 mL of distilled water, maintaining a material-to-liquor ratio of 1:30. The extract was stirred gently until a uniform dye solution was formed. The pre-wetted silk yarn was fully submerged in the dye bath and allowed to soak for 24 h at room temperature in the dark to prevent anthocyanin degradation. After dyeing, the silk yarn was thoroughly rinsed with cold water to remove unfixed dye molecules and then air-dried at room temperature.

2.7 Post-Treatment Dye Fixation

The post-treatment dye fixation process was carried out using two types of agents: *N*-cetyltrimethylammonium bromide (CTAB) and oak gall extract, which is commonly known to be rich in tannins. Although the presence of tannins in the extract was not verified in this study, the use of oak gall as a natural tannin source is well documented[19,20]. For CTAB dye-fixing, 1 % owf of CTAB was dissolved in 30 mL of distilled water, maintaining a material-to-liquor ratio of 1:30. For tannin dye-fixing, the tannin was extracted by boiling 10 % owf of oak gall in 30 mL of distilled water for one hour. The solution was then filtered to obtain a tannin-rich extract. The alum–treated silk sample (sample 2) was subjected to an oak gall extract, while alum-treated dye extract (samples 4) and yeast-alum-fermented silk (sample 7) underwent dye fixation with CTAB. The dyed silk samples were immersed in their respective fixing solutions: sample 2 in the tannin-rich solution, while samples 4 and 7 in the CTAB solution for 30 min at room temperature. After the treatment, the samples were rinsed with cold water to remove excess agents and then air-dried at room temperature. The dyeing methods used in this study are outlined in Fig. 3, which illustrates the three pre-treatment routes together with the control.

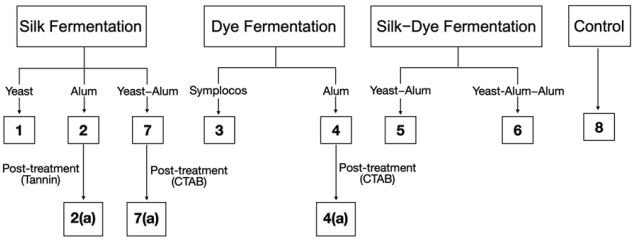

Fig. 3 — Flowchart summarising the silk yarn dyeing process illustrating the three pre-treatment pathways (silk fermentation/treatment, dye fermentation, and combined silk–dye fermentation) alongside the control. Each numbered sample represents a distinct treatment condition, with post-treatment variants (2(a), 4(a) and 7(a)) indicating tannin- and CTAB-fixed samples, respectively

2.8 Instrumentation

2.8.1 UV-Vis Spectrophotometer

The presence of anthocyanins in the dye bath was analysed using a double-beam UV-Vis spectrophotometer (Shimadzu Corp.) within the wavelength range of 400–700 nm. Each dye bath solution was diluted tenfold with distilled water, and the diluted samples were placed in cuvettes, with distilled water serving as the blank.

2.8.2 Spectrocolourimeter

The colour intensity of the dyed silk yarn was measured using a spectrocolourimeter (Model HS-410, Hangzhou CHNSpec Technology Co., China). This device operates within the visible spectrum (400–700 nm) and is specifically designed for reflectance measurements. To ensure accuracy, the spectrocolourimeter was calibrated using a white reference standard and a black trap before each set of measurements. The instrument provides colourimetric data in the CIE-Lab format, following the CIE (International Commission on Illumination) standards to ensure consistent and standardised colour analysis across all samples[21]. In this study, the non-fermented dyed silk yarn served as the reference standard. The colour intensity (Metric Chroma, C)* and colour strength (*K*/*S*) were determined using the CIELAB equations and the Kubelka-Munk formula, respectively, as calculated by the following equations:

$$\text{Metric Chroma, } C^* = \sqrt{(a*)^2 + (b*)^2} \quad \ldots (1)$$

$$\text{Colour Strength, } K/S = \frac{(1-R)^2}{2R} \quad \ldots (2)$$

where a*, b* is the chromaticity coordinated in the L*a*b* colour space; *K*, absorption coefficient; *S*,

scattering coefficient; and *R*, fractional reflectance value

3 Results and Discussion

Bright red dye pigment was extracted from *H. rosa-sinensis* by immersing the petals in 4% (w/v) citric acid in methanol for 24 h. Anthocyanins exhibit strong pH-dependent stability, with the red flavylium cation favoured under acidic conditions. Co-pigmentation and metal complexation (e.g., with Al^{3+}) further stabilise the colour expression[22]. Acidified alcoholic extraction and post-extraction acidification were also reported to improve anthocyanin yield and stability in *Hibiscus* extracts[23]. Hence, the extraction was conducted under acidic conditions to prevent anthocyanin degradation[5]. Citric acid was chosen as the acidifying agent due to its mild, non-corrosive nature and its ability to chelate metal ions, which helps to stabilise anthocyanins during extraction. This stabilisation not only preserves the anthocyanin integrity but also improves the yield of pigment. After solvent evaporation, the extracted dye formed a thick, gel-like concentrate (Fig. 4). This crude extract was subsequently used to prepare the dye bath solution for silk yarn dyeing.

3.1 Silk Yarn Fermentation/Treatment

Three fermentation conditions were applied to the silk fermentation/treatment process: fermentation with yeast only, treatment with alum only, and fermentation with both yeast and alum. After immersing the silk yarn in each fermentation solution for an additional four days, distinct differences were observed. The yeast-only solution developed a pronounced sour odour, with visible mould growth floating on the surface. In contrast, the alum-only and yeast-alum solutions remained odourless with no noticeable changes in appearance. This is likely due to alum's strong antimicrobial properties, which inhibit mould growth[24]. The pH values of the yeast-only, alum-only, and yeast-alum solutions were recorded as 5.1, 4.7, and 4.6, respectively. These variations suggest that the presence of alum contributes to a more acidic environment, which may further suppress microbial activity and enhance the stability of the fermentation process.

Fig. 4 — The dye pigment extract of *H. rosa-sinensis* from acidified methanol

3.2 Fermentation of *Hibiscus Rosa-Sinensis* Flower

The *H. rosa-sinensis* petals were fermented for five days using 10 % *symplocos* and 10 % alum solutions. Both fermentation mixtures developed a distinct purple colouration accompanied by visible mould growth. The initial pH for the *symplocos* and alum fermentations was 6.7 and 5.9, respectively. To enhance pigment stability, 4 % citric acid was added to each solution, lowering the pH to 3.4. This acidification induced a visible colour shift from purple to red, attributed to pH-dependent structural transformations of anthocyanins[5] (Fig. 5). At pH

Fig. 5 — The physical descriptions, colour, and pH *Hibiscus rosa-sinensis* dye extracts during *symplocos*- and alum-assisted fermentation, shown before filtration and after acidification. (a) 10 % *Symplocos*-fermented dye extract before filtration, (b) 10 % *Symplocos*-fermented dye extract after filtration and acidification, (c) 10 % Alum-assisted fermented dye extract before filtration, and (d) 10 % Alum-assisted fermented dye extract after filtration and acid addition

values below 3, anthocyanins predominantly exist in the red flavylium cation form. Between pH 2 and 4, deprotonation of the flavylium ion at one of the phenolic hydroxyl groups leads to the formation of a blue quinoidal base, while at neutral pH (~7), further deprotonation results in an anionic quinoidal base, causing a shift in hue and decreased pigment stability[25].

Although the ideal red colouration is typically achieved at pH values below 3, the addition of citric acid at pH 3.4 provided a buffering effect that stabilised the intermediate red hue. This stabilisation arises from the equilibrium between the flavylium cation and its quinoidal forms. Citric acid, a triprotic acid with a first dissociation constant (pKa_1) of 3.1, contributes to this buffering effect[26]. A stronger acid, such as hydrochloric acid, could have further reduced the pH, but citric acid was favoured for its milder, less corrosive nature[23].

3.3 UV-Vis Spectrophotometric Analysis

The UV-Vis spectra of the alum-assisted, *symplocos*-fermented, and non-fermented dye bath solutions are shown in Fig. 6. All three solutions exhibited a maximum absorbance (λ_{max}) at 514 nm, which is within the characteristic absorption range of anthocyanins (510–520 nm)[27]. This peak corresponds to the $\pi \rightarrow \pi^*$ electronic transitions within the conjugated aromatic ring systems of anthocyanin molecules. In addition, a secondary absorption hump observed between 400–450 nm is consistent with the typical spectral profile of anthocyanins, further confirming their presence in all dye solutions derived from *H. rosa-sinensis*. The absorption in the visible region (400–700 nm) supports the suitability of these solutions for textile dyeing applications, as it ensures effective colouration through the chromophoric activity of the anthocyanin's conjugated systems.

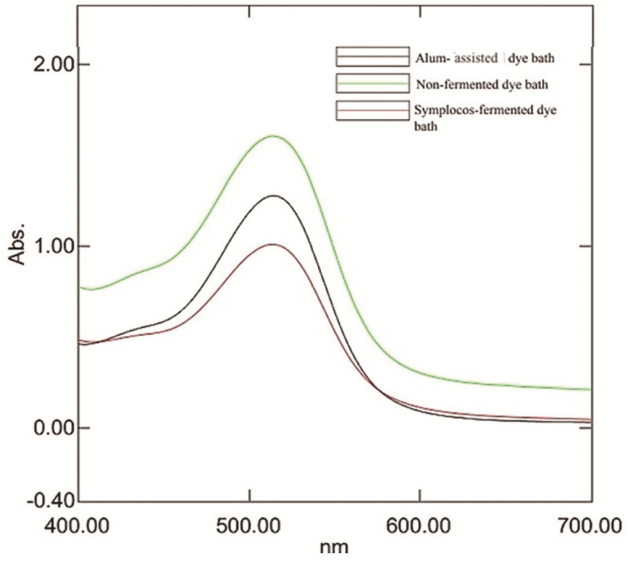

Fig. 6 — The UV-vis spectra of alum-assisted, *symplocos*-fermented, and non-fermented dye bath solutions

Among the three dye baths, the non-fermented solution exhibited the highest absorbance peak at 1.603 absorbance units, which suggests that anthocyanin compounds in the non-fermented extract remained largely in their original state, with minimal degradation or complexation. In contrast, the lower absorbance values observed in the alum-assisted and *symplocos*-fermented dye baths may reflect partial degradation or binding interactions during fermentation, affecting the anthocyanin concentration or stability.

3.4 Colour Intensity

The colour intensity of the dyed silk yarns was measured immediately after they were completely dried following the dyeing process. Measurements were based on the CIELab system, and the fermented/treated samples were compared with the non-fermented control to evaluate the effect of fermentation[28,29]. The L* (lightness) value for the control was 70.06, whereas fermented/treated samples ranged from 62.50 to 71.46, indicating a darker appearance of the fermented dyed samples compared to the control, except for the sample dyed with *symplocos*-fermented dye extract (sample 3), which recorded an L* value of 71.46, suggesting a lighter shade. The a* (red-green) values ranged from 14.17 to 26.63, with all values remaining positive, confirming the presence of red hues. Most fermented samples exhibited higher a* values than the control, suggesting that fermentation enhanced the red colour intensity. The b* (yellow–blue) coordinate for the non-fermented control (sample 8) was 11.22. Among the fermented/treated treatments, most showed positive b* values (9.74–11.07), indicating yellow hues; however, the alum-treated dye extract cases (samples 4–6) were slightly negative (-0.99 to -0.14), reflecting blue undertones. In contrast, the *symplocos*-fermented dye (sample 3) exhibited positive at 2.01, suggesting a faint yellowish tint.

Except for sample 3, all fermented/treated dye samples demonstrated higher colour intensity compared to the non-fermented dyed sample. However, the chroma (C*) values showed only minor variations among the different fermented silk yarns.

The most intense colour was observed in sample 5, which underwent yeast fermentation prior to dyeing, and was subsequently dyed using alum-treated dye extract, achieving a C* value of 26.65. This result was significantly higher than the non-fermented dyed silk yarn (C* = 18.07), demonstrating the enhancement in colour intensity due to fermentation. The colour parameters of all dyed silk yarn samples are summarised in Table 1.

3.4.1 Effect of Fermentation and Alum on Dye Uptake and Colouration

Fermentation of fabric prior to dyeing has been shown to increase cuticle permeability, thereby

Table 1 — The colour parameters of the dyed silk yarns under different fermentation conditions.

No.	Fermentation variable	L*	a*	b*	C*	K/S	Colour appearance
1	S: Yeast D: -	67.13	19.81	10.86	22.59	10.64	
2	S: Alum D: -	62.50	20.53	11.07	23.32	10.16	
2(a)*	S: Alum D: - (tannin)	75.85	9.76	7.91	12.57	13.72	
3	S: - D: *Symplocos*	71.46	17.17	2.01	17.29	12.83	
4	S: - D: Alum	69.45	21.71	-0.14	21.70	14.05	
4(a)*	S: - D: Alum (CTAB)	76.13	7.24	4.45	8.50	17.70	
5	S: Yeast D: Alum	65.34	26.63	-0.99	26.65	15.03	
6	S: Yeast-Alum D: Alum	67.00	22.49	-0.16	22.49	14.06	
7	S: Yeast-Alum D: -	67.30	20.77	9.74	22.94	11.42	
7(a)*	S: Yeast-Alum D: - (CTAB)	67.11	10.46	11.98	15.90	9.72	
8	S: - D: -	70.06	14.17	11.22	18.07	11.86	

Footnote: S = Silk fermentation; D = Dye bath fermentation
* = Sample underwent post-dyeing dye fixation

enhancing dye penetration[10]. This is consistent with the current findings, where fermented/treated silk samples, especially those treated with yeast, exhibited higher C* values, indicating more intense colouration. The alum-treated dye extract also contributed to the increased colour intensity in the dyed silk yarn. In this process, alum functioned as a pre-mordant, facilitating the formation of metal-anthocyanin complexes during the dyeing process. The blue hues observed in silk samples dyed with the alum-treated extract (samples 4–6) are attributed to the formation of metalloanthocyanin complexes between aluminium ions (Al^{3+}) and anthocyanins, which stabilise the anthocyanin structure and shift the chromatic equilibrium towards bluer tones[30,31].

Interestingly, some fermented dye baths, such as those prepared with alum or *symplocos* exhibited lower absorbance values in the UV–vis spectra than the non-fermented extract, but the dyed silk yarns still exhibited higher C* and *K*/*S* values. Although the dye baths were not concentration-normalised, this variation does not compromise the validity of the results. Table 1 clearly demonstrates that the enhanced colouration of fermented samples cannot be explained by dye concentration alone. For example, the alum-treated extract (sample 4) produced a higher *K*/*S* value (14.05) and chroma (21.70) compared to the non-fermented control (*K*/*S* = 11.86; C* = 18.07). Similarly, yeast-pre-treated silk dyed with alum-enriched extract (sample 5) achieved the highest overall colour performance (C* = 26.65; *K*/*S* = 15.03), far exceeding the control. The results clearly demonstrate that fermentation enhanced dye–fibre interactions and stabilised anthocyanins, highlighting the structural and chemical modifications induced by fermentation. Partial hydrolysis of glycosidic bonds during fermentation increases the binding affinity for silk, while organic acids lower the pH and stabilise the flavylium cation form[5,25]. In alum-treated dye baths, Al^{3+} ions further coordinate with anthocyanins (Fig. 7), while also interacting with functional groups in silk fibres such as carboxyl groups, forming molecular bridges that anchor and stabilise the dye molecules within silk microfibrils. This dual role enhances the dye uptake and improves colour stability[11,12,30].

3.4.2 Post-Treatment Dye Fixation and its Effect on Colour Intensity

Post-treatment dye fixation was applied to three dyed silk samples to evaluate its effect on colour intensity. Samples 2, 4, and 7 were selected because they represented the main fermentation conditions that produced comparatively strong initial colouration. Hence, they served as suitable representatives to assess whether fixation with tannin or CTAB could further enhance dye retention. The post-dyeing treatments aim to minimise colour bleeding or transfer during subsequent use or washing[32]. Sample 2(a) was treated with tannin, while samples 4(a) and 7(a) were treated with *N*-cetyl trimethyl ammonium bromide (CTAB). However, a comparison of colour intensity before and after fixation revealed substantial decreases across all treated samples. The C* value of sample 2 dropped from 23.32 to 12.57, while samples 4 and 7 showed a reduction from 21.70 to 8.50, and from 22.94 to 15.90, respectively. These reductions are likely due to competitive interactions between the fixing agents and the previously adsorbed anthocyanins for binding sites on the silk fibre. In the case of sample 2(a), competitive adsorption may occur between the tannin molecules and the anthocyanin dye for the limited active binding sites on the fibre surface. Although tannins are known as mordants, their application after dyeing may result in partial displacement of already-

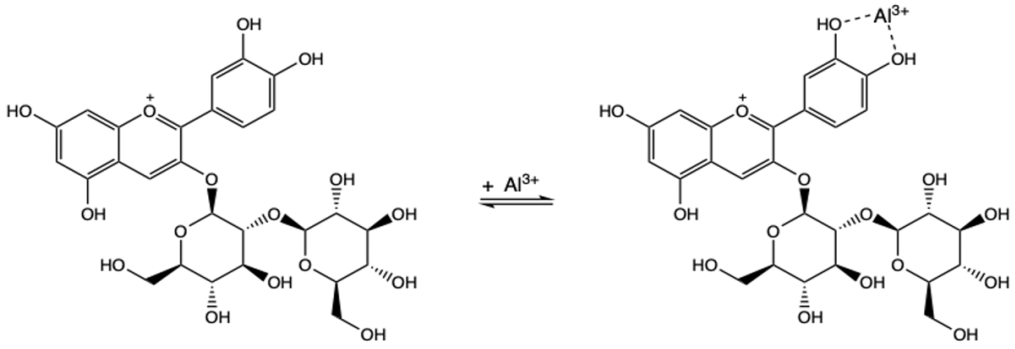

Fig. 7 — Proposed complexation of the cyanidin-3-sophoroside at positions 3' and 4' during the fermentation of *H. rosa-sinensis* flowers with alum

bound anthocyanin molecules, especially if the tannins exhibit stronger binding affinities to the silk protein.

In samples 4(a) and 7(a), CTAB, a cationic surfactant, interacts primarily through electrostatic attraction with oppositely charged dye species[33]. However, under acidic conditions where anthocyanins exist predominantly in their flavylium cation form, electrostatic repulsion between CTAB and anthocyanin molecules may occur. This repulsion may weaken the dye–fibre interaction, contributing to the observed decrease in colour intensity in the samples.

3.5 Colour Strength

The colour strength of the dyed silk yarns was quantitatively evaluated using Kubelka–Munk (K/S) values, which reflect the relative dye concentration on the fibre surface. Higher *K*/*S* values indicate greater dye uptake and colour depth[34]. The summary of *K*/*S* values for all samples is tabulated in Table 1. Among all samples, sample 4(a), which used alum-treated dye extract and then post-treated with CTAB, recorded the highest *K*/*S* value of 17.70, while sample 5, which combined yeast-fermented silk yarn and alum-treated dye extract, achieved the second-highest *K*/*S* value of 15.03, highlighting the positive impact of fermentation. In contrast, the control (sample 8) exhibited a significantly lower *K*/*S* value of 11.86, suggesting less effective dye penetration and fixation.

Post-treated samples showed a more complex behaviour. Although the colour intensity (C*) decreased after CTAB or tannin fixation, *K/S* increased for samples 2(a) and 4(a), demonstrating that fixation can darken the fibre and increase optical density even though the chroma is reduced. However, sample 7(a) displayed a decrease in colour strength (*K/S* = 9.72), likely due to the reduced dye retention resulting from post-treatment with CTAB. This reduction can be attributed to the dual-modified surface of yeast–alum–fermented silk, which carries both fermentation-induced porosity and alum-derived metal ion coordination. CTAB, being a cationic surfactant, may disrupt the alum–anthocyanin complexes or desorb the loosely bound dye molecules from the more modified fibre surface. This contrasts with samples 2 and 4, where only a single mode of pre-treatment (alum or fermentation) was applied. These findings demonstrate that fermentation generally enhances dye uptake and colour strength, but the extent of improvement is influenced by the specific combination of fermentation route, mordanting pathway, and post-treatment chemistry. This observation corresponds with previous findings in silk bio-dyeing, where biological treatments can modify dye–fibre interactions and influence dye uptake behaviour[8]. Overall, the present results indicate that post-dyeing fixation does not universally enhance dye retention and may be unnecessary for fermented samples.

Mondal *et al.* [35] reported an inverse correlation between the L* value (lightness) and *K*/*S* value, where an increase in lightness typically corresponds to a decrease in dye concentration. Interestingly, sample 4(a) showed both the highest colour strength (*K*/*S* = 17.70) and the highest lightness (L* = 76.13). This finding suggests that a high level of dye uptake does not necessarily correlate with lower lightness. The fermentation process may have altered the anthocyanin composition, promoting improved solubility and interaction with the silk fibre. Such modifications can result in effective dye penetration, while maintaining surface characteristics that reflect light, thus contributing to both high *K*/*S* and L* values. Furthermore, no direct correlation was observed between the *K*/*S* values and the chroma (C*) values of the dyed silk yarns, which reflects the perceived vividness or saturation of colour. Sample 5, which was dyed with alum-treated dye bath and underwent yeast fermentation, recorded the second-highest *K*/*S* value (15.03) and the highest chroma value, highlighting the positive impact of fermentation on both dye absorption and colour vividness. In contrast, although sample 4(a) achieved the highest *K*/*S* value, it showed the lowest chroma, further demonstrating that greater dye uptake does not necessarily result in higher colour intensity. These observations highlight the complex interplay between dye distribution, dye–fibre interactions, and the visual colour outcomes in natural dyeing applications.

4 Conclusion

This study demonstrates that fermentation enhances the dyeing performance of *H. rosa-sinensis* extracts on silk yarn, with the combination of yeast-fermented silk and alum-treated dye extract producing the most vibrant and intense colouration (C* = 26.65) and one of the highest colour strength values (*K*/*S* = 15.03). These findings suggest that microbial fermentation increases silk fibre receptivity through

biochemical and microstructural changes, while alum in the fermented dye bath facilitates dye fixation through metal-anthocyanin complexation. Although post-dyeing treatment with fixing agents such as CTAB and tannin was intended to improve colour fastness, it led to a noticeable decrease in colour intensity, possibly due to competitive interactions or electrostatic repulsion with anthocyanins. Fermentation of silk yarn increases fibre porosity, exposes additional amino and carboxyl functional groups, and is expected to promote deeper dye penetration, potentially reducing dye wash-off during laundering. In addition, fermentation of the dye extract, particularly in alum-containing media, may enhance the stability of anthocyanin–metal complexes and improve resistance to pH fluctuations, which can also contribute positively to washing, perspiration, and light fastness. Although fastness testing was not conducted in this study, these findings suggest that fermentation-assisted natural dyeing could offer advantages for the silk dyeing applications by improving colour retention, reducing bleeding, and supporting more sustainable colouration practices. Future work should incorporate standardised fastness assessments and spectroscopic techniques such as FTIR to validate these anticipated benefits and further elucidate the structural modifications occurring in fermented silk fibres. In summary, the dual-fermentation approach presents a promising strategy for improving the efficiency, stability, and overall performance of natural dyes in silk dyeing.

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