Journal of Current Science and Technology, January - March, 2025 Vol. 15 No. 1, Article 86

ISSN 2630-0656 (Online)

Cite this article: Klaykruayat, B., Vuthiganond, N., & Chitichotpanya, P. (2025). Optimization of ultrasound-assisted anthocyanin extraction from black rice bran for simultaneous coloring, UV protection, and antioxidant silk finishes. *Journal of Current Science and Technology*, *15*(1), Article 86. https://doi.org/10.59796/jcst.V15N1.2025.86

Journal of Current Science and Technology

Journal homepage: https://jcst.rsu.ac.th

Optimization of Ultrasound-assisted Anthocyanin Extraction from Black Rice Bran for Simultaneous Coloring, UV Protection, and Antioxidant Silk Finishes

Benjamas Klaykruayat, Nattaya Vuthiganond, and Pisutsaran Chitichotpanya*

Division of Materials and Textile Technology, Faculty of Science and Technology, Thammasat University, 99 Phahonyothin Road, Klong Luang, Pathum Thani 12120, Thailand

*Corresponding author; E-mail: pisut_c@tu.ac.th

Received 13 June 2024; Revised 2 September 2024; Accepted 24 September 2024; Published online 24 December 2024

Abstract

This study examined the use of ultrasound to extract anthocyanin from agricultural byproduct black rice bran as a natural colorant and a multifunctional finishing agent for silk fabrics, taking into account the various health advantages linked to anthocyanins. The study employed response surface methodology to identify the optimal extraction process that would yield the highest extraction efficiency for total anthocyanin content. The optimal conditions were 30 Hz ultrasonic power, a liquor-to-material ratio of 21, 60°C an ultrasound temperature, and 30 minutes of an ultrasound time, yielding 173.25 mg/L anthocyanin. Silk fabrics were dyed in a reddish purple tone, and the mordant dyeing method produced a 34% higher color strength value than direct dyeing while displaying good colorfastness (grade ≥ 4) to washing and crocking. The dyed silks also provided excellent UV protection (UPF > 40+) and antioxidant activity, with a DPPH scavenging rate over 80%. Anthocyanins derived from black rice bran could thus be used as a bioactive functional colorant in medical and health-related textiles.

Keywords: *black rice bran; silk; bioactive dye; antioxidant activity; UV-protection; ultrasound; response surface methodology*

1. Introduction

The use of natural dyes on textiles has become increasingly important as environmental and health concerns have grown. Furthermore, throughout the synthetic colorant process, an extensive variety of solvents and chemical intermediates are employed. These hazardous chemicals contribute significantly to environmental pollution and cause harm to living organisms (Ardila-Leal et al., 2021). Bio-based colorants, primarily derived from natural plants, have become popular in the textile industry due to their non-toxic, biodegradable, and environmentally friendly properties (Benkhaya et al., 2020; Yadav et al., 2023). In addition to the environmental advantages, extensive research has also verified the diverse capabilities of natural dyes, including UV-protection, antibacterial activity, antioxidant activity, and anti-inflammatory activity. These dyes hold great potential for use in the production of medical and healthcare textiles.

Black rice, scientifically known as *Oryza sativa L.*, is a unique variety of rice that has been extensively consumed in several Eastern Asian nations since ancient times (Das et al., 2023; Ito, & Lacerda, 2019). It is considered a nutritious food because of its high concentration of anthocyanins. Many studies have demonstrated that anthocyanins possess potent antioxidant properties and provide protection against ultraviolet (UV) radiation (Kim et al., 2024). Black rice colorant, which contains cyaniding-3-glucoside as a major constituent (Figure 1), is a member of the anthocyanin pigment family and is found primarily in black rice seed coat (Ito, & Lacerda, 2019). Anthocyanin extract has been employed in medicine, food, and cosmetic industries because of its non-toxic, nutrient-rich nature, pharmacological activities, and water-solubility (Tan et al., 2022). It has been reported that black rice bran contains 6-9 times more anthocyanin than edible black rice (Chen et al., 2023). Black rice bran is a byproduct of the black rice milling process that makes up approximately 10% of the total weight of wholegrain black rice. Nevertheless, black rice bran has been primarily used as animal feed without being effectively utilized. The usage of agricultural byproducts for bio-based colorants, following biorefinery principles, is becoming increasingly popular due to their environmentally benign and non-toxic properties, as well as their contribution to resource sustainability (Koul et al., 2022).

Natural anthocyanin extraction is usually accomplished using classic procedures such as volatile solvent extraction, enzymolysis extraction, ethanol extraction, and aqueous biphasic extraction (Alappat, & Alappat, 2020). Nevertheless, these processes are limited by their low extraction efficiency, long-time consumption, as well as challenges related to separation. Advanced extraction techniques, such as ultrahigh-pressure, supercritical fluid, and subcritical water extractions, have been employed to successfully extract anthocyanins (Shen et al. 2023). However, these advanced techniques have considerable drawbacks due to their pricey equipment and severe operating circumstances. Emerging methods including ultrasound and microwave extractions are being investigated as potential alternatives to conventional methods for extracting anthocyanin (Kim et al., 2024). Aqueous ultrasound-assisted extraction (UAE) is a very effective and eco-friendly extraction technique that utilizes water as the extraction solvent (Baig et al., 2021; Kumar et al., 2021; Sadeghi-Kiakhani et al, 2021). The extraction process is influenced by the simultaneous presence of thermal, mechanical, and cavitation impacts of ultrasound waves (Lei et al. 2021). Ultrasound with a frequency range of 20-100 kHz can be used to strengthen and improve the extraction process (Tan et al., 2022). Backes et al., (2018) performed a comparative analysis of three extraction techniques (heat, ultrasound, and microwave) to recover anthocyanin pigments and optimize the extraction conditions from fig peel. The UAE demonstrated superior efficiency, yielding 19.4 mg of anthocyanin per gram of residue weight. The following steps were microwave and heat extractions, in that order. Similar results have been reported in other research that have conducted optimization studies with various natural plants (Albuquerque et al., 2017; Caleja et al., 2017). The effectiveness of conventional solid-liquid extraction was compared to that of UAE by Garcia-Castello et al., (2015). The researchers discovered that UAE produced 50% more total phenolic content and 66% more total antioxidant activity at lower temperatures and extraction times. Therefore, the aqueous UAE can assist natural anthocyanin extraction economically and practically by reducing extraction time, eliminating the need for volatile solvents, increasing extraction efficiency, and improving extract quality (Thakur et al., 2022). Das et al., (2017) optimized the extraction of anthocyanins from black rice bran using the UAE process. The ideal conditions were as follows: a pH of 2.52, a concentration of 24% ethanol, and an ultrasound bath temperature of 36°C for 23 minutes. These conditions yielded a total anthocyanin content (TAC) of 30.40 mg/l. Leonarski et al., (2024) investigated the extraction of anthocyanins from black rice bran using ultrasound. The optimum conditions were a frequency of 380 W, a temperature of 50°C, and a solvent of 60:40 (v/v) citric acid 0.1 M ethanol, yielding 2.44 mg/g of anthocyanin from black rice bran.

Silk, a natural macromolecular protein fiber generated by Bombyx mori silkworms, has long been recognized as one of the most preferred textile materials for the production of high-end textile products due to its superior wearing comfort and luxurious appearance (Pereira et al., 2015). However, silk fabric lacks certain characteristics such as UV protection and antioxidant activity, which limits its applicability in medical textile field (Jia et al., 2017). It is known that textiles with UV protection and antioxidant activity are effective in preventing UV light and free radicals from damaging the skin of the body, and hence can be classified as healthy textile (Attia et al., 2022; Ayesh et al., 2022). Improving the functional properties of silk materials is critical for expanding their applications in medical and healthcare textile products. Thus, the purpose of this study is to investigate the UAE of anthocyanin, a bioactive colorant generated from the agricultural byproduct black rice bran, as a functionalizing dye for silk finishes. Response surface methodology (RSM) was used to optimize the practical outcomes of UAE settings, such as liquor-to-material (L:M) ratio, ultrasound temperature, and ultrasound duration, as well as their combined effect. The extracted anthocyanin was then used to achieve multifunctional treatments on silk fabrics (Garcia-Castello et al., 2015; Yin et al., 2017; Lei et al., 2021; Sadeghi-Kiakhani et al., 2021). Validation of the optimal conditions determined by RSM in practice was conducted through performance assessments. The evaluations encompassed assessments for UV protection and antioxidant properties, along with color strength and color fastness, following standard testing protocols.

2. Objectives

This study explored the use of ultrasound to extract anthocyanin from black rice bran, an agricultural byproduct, as a natural colorant and multifunctional finishing agent for silk fabrics. Evaluations included UV protection, antioxidant properties, color strength, and color fastness, following standard protocols. The study contributes to promoting agricultural byproducts in textile dyeing through advanced techniques and enhancing the value of Thai silk as a health-beneficial textile.

3. Materials and Methods

3.1 Materials

The black rice bran powder was acquired at a local market in the province of Patumthani. A silk fabric with a plain weave structure and a density of 95 g/m^2 was acquired from Nakhon Ratchasima province. To remove contaminants from the fabric, a scouring procedure was conducted using a nonionic detergent with a concentration of 2.5 g/L at a temperature of 50°C for a duration of 30 minutes. Afterward, the fabric was washed and allowed to dry naturally in the air. Aluminum potassium sulfate ($AlK(SO_4)_2$) of analytical reagent (AR) quality was obtained from Sigma-Aldrich Co. Ltd.

3.2 Methods

3.2.1 UAE of Anthocyanin from Black Rice Bran

Anthocyanin extraction from black rice bran powder was performed by using the UAE method in an ultrasonic bath operating at a maximum frequency 35 kHz and a capacity 3 L (Sonorex DIGITEC DT 102 H-RC). The UAE settings, including ultrasound time, ultrasound temperature, and L:M ratio was determined based on Box-Behnken design (BBD) coupled with RSM. BBD refers to a second-order RSM class that is derived from incomplete 3^3 factorial designs (Box, & Wilson, 1951). The BBD method employs a reduced number of tests to construct response surfaces of higher order compared to traditional factorial techniques, resulting in more efficiency than three-level complete factorial designs (Khazaei et al., 2016). Initial screening tests were conducted to determine the ideal range of attributes associated with UAE conditions. The ultrasound time ranged from 25 to 35 minutes, the ultrasound temperature from 50 to 70°C, and the L:M ratio from 10 to 30 mL/g. Subsequently, the extracted solutions were subjected to filtration using Whatman No. 1. Then, the total anthocyanin content (TAC) of the extracts from black rice bran was determined using the pH differential method.

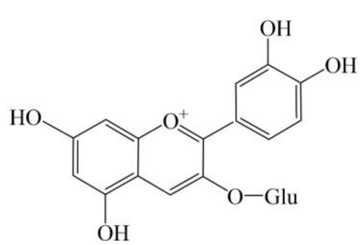


Figure 1 Chemical structure of cyaniding-3-glucoside

3.2.2 Determination of Total Anthocyanin Content

The measurement of TAC was conducted using the pH differential method, as described in reference (Yin et al., 2017). This technique measures the absorbance at pH 1.0 and 4.5 to detect any changes in the molecular structure of anthocyanin compounds. Two separate standard buffer solutions were prepared. The pH of the solution was adjusted to 1 by combining 25 mL of a potassium chloride solution with a concentration of 0.2 mol/L and 67 mL of a hydrochloric acid solution with a concentration of 0.2 mol/L. A second solution with a pH of 4.5 was created by mixing 6 mL of hydrochloric acid with a concentration of 1 mol/L, 10 mL of sodium acetate with a concentration of 1 mol/L, and 9 mL of deionized water. Equation (1) shows the TAC, which was determined and quantified based on the cyanidin 3-glucoside equivalent.

$$\text{TAC (mg/L)} = \frac{\text{A x Mw x DF x 1000}}{\text{E x L}} \quad (1)$$

The absorbance value (A) is calculated using the formula $[(A_{\lambda max} - A_{700})_{pH1.0} - (A_{\lambda max} - A_{700})_{pH4.5}]$, where $A_{\lambda max}$ represents the absorbance at the maximum wavelength. The molar mass (M_w) of cyanidin 3-glucoside is 449.2 g/mol. $\mathcal{E}$ represents the molar absorptivity, which is 26,900. L represents the path length of the cuvette, and DF represents the dilution factor.

3.2.3 Optimization of UAE Process

To investigate the impact of UAE process parameters on TAC, the experiment design included 16 experiments, as shown in Table 3. Each experiment had two duplicates, whereas the center point had four repetitions. The Minitab software (version 20) was implemented for data analysis. A response surface map in RSM visually depicts the mathematical relationship between experimental variables (X) and a response variable (Y) using a quadratic regression model, as expressed by equation (2).

$$Y = c_0 + \sum_{i=1}^{3} c_i X_i + \sum_{i=1}^{3} c_{ii} X_i^2 + \sum_{i=1}^{3} c_{ij} X_i X_j \quad (2)$$

Where Y is the estimated response or dependent variable, X_i and X_j are independent variables, c_0 is constant, c_i, c_{ij}, and c_{ii} are linear, interaction, and quadratic coefficients, respectively.

The statistical significance of each experimental variable in the predictive model was evaluated using analysis of variance (ANOVA). A *p*-value beneath the 0.05 threshold was considered statistically significant, indicating a 95% confidence level. Subsequently, the regression model equation was solved and the response surface contour plots were examined to determine the optimal parameters for the UAE process.

3.2.4 Dyeing Procedure for Silk Fabric

Scoured silk samples were dyed with obtained anthocyanin extract utilizing infrared dyeing equipment for 90 minutes at 65°C using an exhaust approach with a material-to-liquor ratio of 1:30. Both simultaneous mordanting and direct dyeing methods were applied. In the simultaneous mordanting dyeing procedure (Figure 2), the dyebath was supplied with potassium alum ($AlK(SO_4)_2$) at a concentration of 4% on weight of fabric (owf). Subsequently, the processed samples were washed with distilled water and allowed to dry naturally.

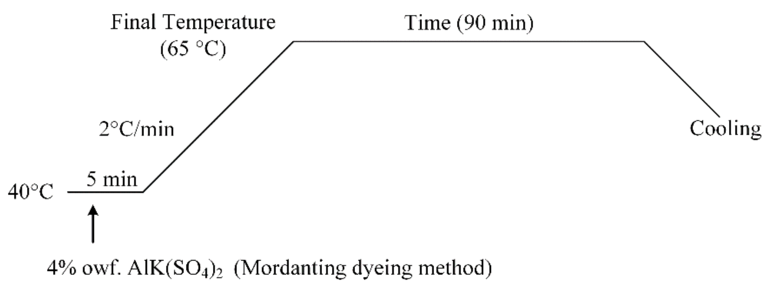


Figure 2 Schematic diagram of mordanting dyeing procedure

3.2.5 Color Measurement and Color Fastness Testing

The fabric samples were analyzed using a spectrophotometer (GretagMacbeth LLC, USA) to measure their colorimetric qualities. The spectrophotometer was equipped with an illuminant D65, which included specular and UV components, along with a 10° standard observer. The experiment was conducted in triplicate, and the average value was reported. The Kubelka-Munk equation (Eq. 3) was employed to compute the color strength (K/S).

$$\frac{K}{S} = \frac{(1-R)^2}{2R} \quad (3)$$

Where K, S, and R represent the absorption coefficient, scattering coefficient, and reflectance at the greatest absorption wavelength respectively.

The colorimetric values are denoted in the CIELAB color space, specifically $L*$, $a*$, $b*$, $h°$, and $C*$. In this approach, the variable $L*$ represents the level of brightness, where a value of 100 represents white and a value of 0 represents black. The green-red axis is represented by the $a*$ coordinate, with negative values indicating green and positive ones indicating red. The $b*$ coordinate represents the blue-yellow axis, with negative values indicating blue and positive ones indicating yellow. Furthermore, $C*$ and $h°$ denote chroma and hue angle, correspondingly.

Color fastness against crocking and washing was evaluated according to AATCC 8-2007 and ISO 105-C01:2013, respectively. The fastness rating was assessed by comparing it to the Grey Scale Standard ISO 105-A03. The variations in shades were correlated with the usual Grey Scale assessment (with a grading scale of 1-5; 1 indicating poor and 5 indicating excellent).

3.2.6 UV Protection Efficiency and Antioxidant Activity

The UV protection of untreated and dyed samples was assessed by measuring the percent transmittance of ultraviolet radiation (UVR) and calculating the ultraviolet protection factor (UPF) using a CamSpec spectrophotometer (Spectronic CamSpec Ltd., England) following the guidelines of the AS/NZS 4399:1996 test standard. In accordance with the AS/NZS 4399:1996 test procedure, textile items with a UPF value between 15 and 24 are classified as having "Good UV Protection", those with a value between 25 and 39 are classified as having "Very Good UV Protection", and those with a value between 40 and 50+ are classified as having "Excellent UV Protection". Additionally, the effectiveness of the dyed fabric's UV protection is deemed good if the UV transmittance is below 5%.

The antioxidant activity of dyed samples was measured utilizing the DPPH (2, 2-Diphenyl-1-picrylhydrazyl) radical scavenging method, according to previous report (Sadeghi-Kiakhani et al., 2021). DPPH has a single electron with a prominent absorption peak at 517 nm, with a violet hue in methanol solution. The presence of free radical scavengers would reduce or eliminate the absorption peak of the DPPH solution due to electron pairing. The fluctuation in absorbance of the DPPH solution is directly linked to the quantitative measurement obtained using a UV-vis spectrophotometer. For this evaluation, a fabric sample weighing 500 mg was submerged in a 30 mL solution containing 0.10 mM DPPH methanol. The sample was then kept in darkness and incubated for a duration of 30 minutes. The change in color of the solution, transitioning from a deep violet hue to a light yellow shade, was observed. Additionally, the absorbance of the solution at a wavelength of 517 nm was measured using a UV spectrometer. As a positive control, this study utilized ascorbic acid. The percentage of antioxidant activity was calculated using Equation (4).

$$\%\text{Antioxidant activity} = \frac{A_{ctrl} - A_{spl}}{A_{ctrl}} \times 100 \quad (4)$$

Where A_{ctrl} and A_{spl} represent the absorbance values of dark-incubated solutions for the untreated and treated specimens, respectively, at 517 nm.

4.Results and Discussion

4.1 RSM Optimization of UAE Conditions

To optimize the three individual factors in the Box-Behnken experimental design, which were used for extracting the natural colorant anthocyanin using ultrasound assistance from black rice bran, a total of 16 trials were conducted. The coded process factors and experimental results are displayed in Table 1. The experimental results varied from 123.72 to 172.32 mg/L, suggesting a correlation between the TAC (response variable) and the independent variables using RSM with BBD. Through the utilization of multiple regression analysis on the experimental data, the predicted response for the yield of the TAC can be represented by a second-order polynomial equation as shown below.

$$TAC\ (mg/L) = -1077.45 + 43.393*X_1 + 17.221*X_2 + 4.492*X_3 - 0.731*X_1^2 - 0.137*X_2^2 - 0.058*X_3^2 + 0.021*X_1*X_2 - 0.054*X_1*X_3 - 0.007*X_2*X_3 \quad (5)$$

Where, X_1 is ultrasound time, X_2 is ultrasound temperature, and X_3 is L:M ratio

If the model does not accurately fit the data, optimizing a fitted response surface can result in misleading outcomes. Therefore, it was crucial to verify the accuracy of the model equation. This study employed ANOVA to evaluate the adequacy of the quadratic regression model (Table 2). The regression model is fitted when the *p*-value is less than 0.05, indicating statistical significance at a 95% confidence level. Also, the statistical analysis indicated that the variables conformed well to the regression equation, as evidenced by a *p*-value below 0.05. In addition, the correlation coefficient (R^2) for Equation (5) demonstrated a satisfactory value of 0.9642, indicating that 4% of the variability could not be explained by the current model. A regression model with an R^2 value closer to 1 indicates higher accuracy and quality.

The values of the regression coefficients for Equation 5 are provided in Table 3. *P*-values are employed to assess the statistical significance of each coefficient. The data in Table 3 demonstrates that the linear coefficients (X_1 , X_2 , and X_3), quadratic coefficients (X_1^2 , X_2^2 , and X_3^2), and interaction coefficients (X_1X_2 and X_2X_3) all had statistically significant impacts on TAC yields, with a *p*-value of less than 0.05. The *F*-value indicates that the independent variables for the UAE of TAC yield were statistically significant in the following order: ultrasound temperature > ultrasound time > L:M ratio. Overall, the regression analysis reveals that the main, interaction, and square effects of the independent factors are statistically significant.

Table 1 BBD for UAE from black rice bran

Run	Independent variables			TAC
	Time	Temperature	L:M ratio	
	(X_1, min)	**(X_2,°C)**	**(X_3, mL/g)**	**(mg/L)**
1	30 (0)	60 (0)	20 (0)	172.23
2	30 (0)	70 (1)	30 (1)	165.86
3	30 (0)	50 (-1)	30 (1)	141.20
4	30 (0)	60 (0)	20 (0)	172.32
5	35 (1)	70 (1)	20 (0)	152.54
6	25 (-1)	70 (1)	20 (0)	153.16
7	25 (-1)	50 (-1)	20 (0)	128.72
8	25 (-1)	60 (0)	30 (1)	152.64
9	30 (0)	60 (0)	20 (0)	171.89
10	25 (-1)	60 (0)	10 (-1)	144.84
11	30 (0)	70 (1)	10 (-1)	164.82
12	35 (1)	60 (0)	10 (-1)	147.96
13	30 (0)	50 (-1)	10 (-1)	137.14
14	35 (1)	50 (-1)	20 (0)	123.72
15	35 (1)	60 (0)	30 (1)	144.84
16	30 (0)	60 (0)	20 (0)	172.20

Table 2 ANOVA analysis of UAE from black rice bran

Source	Sum of Square	Degree of freedom	Mean Square	*F*-value	*P*-value
Model	3239.45	9	359.89	5559.43	0.000
Linear	1364.60	3	454.68	7067.80	0.000
Square	1839.75	3	613.17	9531.41	0.000
Interaction	35.45	3	11.82	183.69	0.000
Residual	0.92	5	0.18		
Lack of fit	0.69	3	0.23	1.71	0.389
Pure error	0.23	2	0.12		
Total	32.39	14			
	$R^2 = 0.9642$				

Table 3 Regression analysis of UAE from black rice bran

Variable	Coefficient estimate	Standard error	*F*-value	*P*-value
X_1	-1.262	0.090	198.13	0.000
X_2	12.944	0.090	480.45	0.000
X_3	1.202	0.090	178.63	0.000
X_1X_2	1.071	0.127	71.32	0.000
X_1X_3	-2.684	0.127	1.85	0.250
X_2X_3	-0.739	0.127	34.00	0.002
X_1^2	-18.275	0.132	191.68	0.000
X_2^2	-13.685	0.132	23.86	0.000
X_3^2	-5.806	0.132	71.76	0.000

4.2 RSM Optimization of UAE Factors

The predicted model equation was visualized using a three-dimensional response surface plot and a two-dimensional contour plot. The three-dimensional response surface plot was used to depict the correlation between the response variable and the independent factors. The two-dimensional contour plot enabled the clear visualization of the shape of the response surface and aided in identifying the optimal values for optimization. Typically, circular contour plots demonstrate the individual impact of process factors on the response variable. Nevertheless, the elliptical contour plot clearly demonstrates that the interactions between the associated variables are significant. Figures 3-5 display three response surface plots along with their corresponding contour plots. The response surface plots illustrate two independent factors within the experiment range, while the other factor is held constant at a given level. Figure 3 illustrates that TAC yields increased as the ultrasound temperature increased, while ultrasound time remained constant. Furthermore, the TAC yields increased as the ultrasound time increased. Elevating the ultrasound temperature enhances the absorption of black rice bran, facilitating the penetration and dispersion of the solvent within the microstructure of the plant. The efficacy of plant extraction is enhanced by two factors: heightened susceptibility of cell walls and reduced viscosity of solvents at elevated temperatures (Kim et al., 2021). The ultrasound temperature and time were both found to affect TAC extraction. The contour plot also indicates that the optimal ultrasound temperature is between 61 and 69°C, and the optimal time is between 28 and 32 minutes. Under the specific temperature and time conditions in the UAE process, black rice bran demonstrated a greater yield in extracting anthocyanin. The Figure 4 and 5 illustrate the interactive correlation between the L:M ratio and the other factors. The TAC yield exhibits a positive correlation with the rise in L:M ratio, reaching its maximum level at the threshold value of 1:21. At a higher level, the TAC yield slightly decreases. By establishing a suitable L:M ratio, it is possible to achieve a greater TAC yield through modifications in the remaining factors. During the UAE process of the TAC extraction, water, serving as the extractant, primarily performed two functions. One functioned as a medium for the ultrasonic mechanical effect, while the other served as a source of cavitation, creating an environment for ultrasonic damage to the cell wall. The mass transfer power in the extraction process was determined by the L:M ratio. Once the ratio reached the threshold level, the diffusion and dissolution of the TAC reached the state of equilibrium. It is shown that as time, temperature, and L:M ratio increase, the TAC yields also increase. The contour plot in Figure 4 suggests that the ideal duration for ultrasound treatment falls within the range of 28 to 32 minutes, while the optimal L:M ratio lies between 14 and 27. Similarly, Figure 5 demonstrates that the optimal temperature ranges from 61 to 69°C, with the same optimal L:M ratio of 14 to 27.

The optimal conditions for extracting anthocyanin from black rice bran with the assistance of ultrasound were determined using Minitab software. The best result was obtained by using a 30-minute duration for the ultrasound, a temperature of 60°C, and a L:M ratio of 21. The given conditions resulted in a forecasted TAC of 174.80 mg/L. Afterward, the extraction experiment was conducted under the ideal conditions, which yielding an average TAC of 173.25 mg/L ± 1.2 (n = 3). This confirms the accuracy of the RSM model. Thus, the obtained anthocyanin colorants were applied to finished silk fabrics to assess their colorfastness and multifunctional properties.

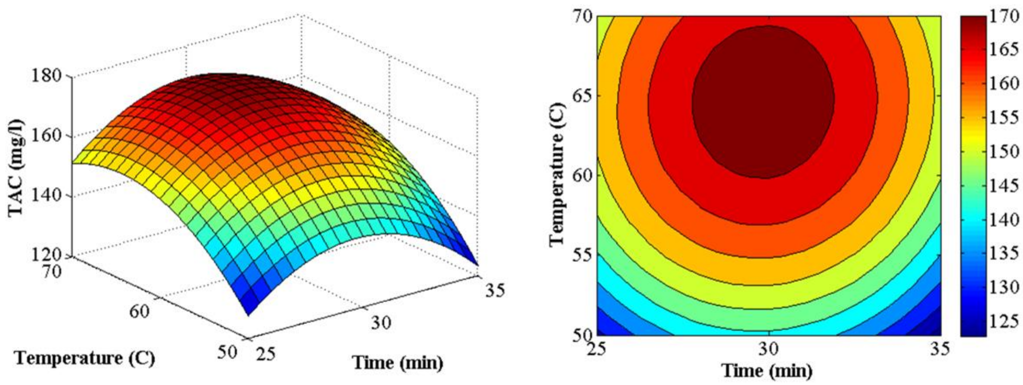


Figure 3 3D and contour plot showing effect of time and temperature on TAC

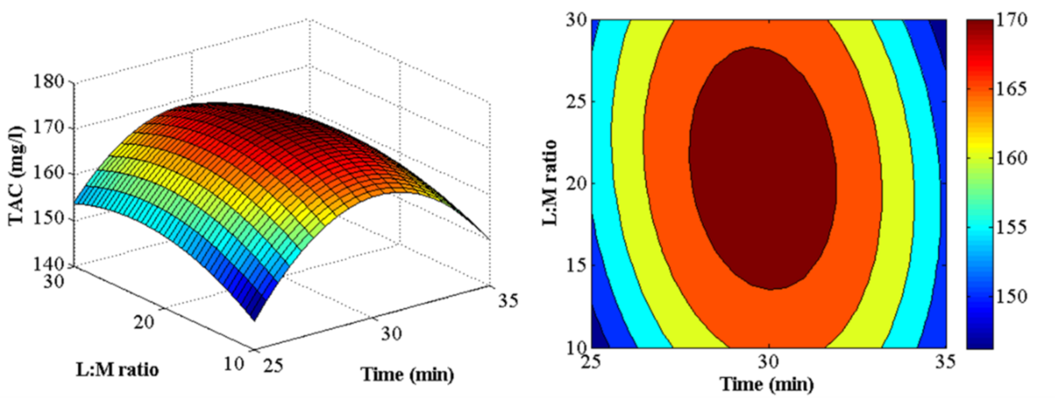


Figure 4 3D and contour plot showing effect of time and L:M ratio on TAC

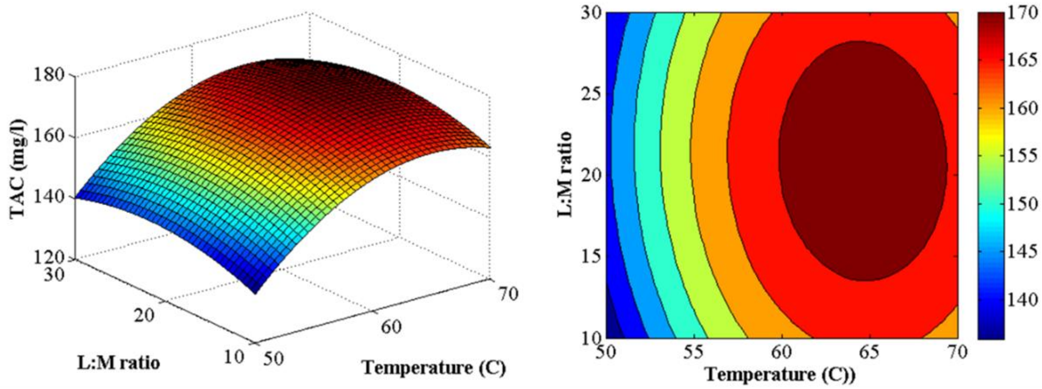
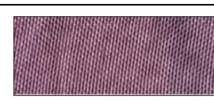


Figure 5 3D and contour plot showing effect of temperature and L:M ratio on TAC

Table 4 Color strength and colorimetric values

Dyeing method	K/S	Colorimetric values					Image
		L*	***a****	***b****	***C****	***h°***	
Untreated	0.17	91.74	0.66	9.38	9.41	86.00	
Direct dyeing	1.77	62.25	6.99	-1.71	7.20	346.27	
Mordant dyeing	2.37	47.77	16.53	-3.71	16.94	347.37	

4.3 Dyeing and Colorfastness Properties

The extracted anthocyanin from black rice bran was then employed to dye silk fabrics using direct and mordant dyeing methods. The K/S, colorimetric value, and colorfastness of the dyed silk fabrics were measured as presented in Tables 4 and 5. The value of L^* is indicative of lightness, with lesser value indicating darker shade. The color attributes are represented by the chromaticity coordinates a^* and b^*, which are set to $-a^*$ as greenness, $+a^*$ as redness, $-b^*$ as blueness, and $+b^*$ as yellowness. There are substantial differences observed in the L^* values of silk fabric treated with mordant dyeing compared to direct dyeing. However, the observed decrease in lightness in the mordant dyeing method can be attributed to a higher level of dye absorption onto the fabric, resulting in a more intense shade compared to the direct dyeing method. The a^* and b^* values slightly altered the impression of redness and blueness during the transition from the direct dyeing method to the mordant dyeing method. One advantage of using alum as a mordant in natural anthocyanin dyeing is that it increases the intensity of the color on the fabric without changing the original hue of the dye. Consequently, our silk fabric dyed with alum-anthocyanin maintains its original natural color. The treated silk fabrics exhibited K/S values of 2.37 and 1.77 for mordant dyeing and for direct dyeing, respectively. The application of mordant dyeing led to a 33.9% improvement compared to the direct dyeing approach.

The colorfastness assessment is used to determine the color stability of the treated fabrics. The mordant dyeing procedure resulted in a color change and color straining on multifiber fabrics that were assessed as good (grade: 4) and good to excellent (grade: 4-5), respectively (Table 5). The color change exhibited a one-grade enhancement in comparison to treated fabric using the direct dyeing method. Additionally, the crocking fastness of treated fabric using the mordant dyeing method was evaluated as fair (grade: 3) when wet and good (grade: 4) when dry, indicating a one-grade improvement compared to the direct dyeing method (Table 6). This occurs because a polar solvent (water) enhances the likelihood of color loss in treated fabrics when rubbed, especially when wet. The results of our study showed that the utilization of alum, a non-toxic metal mordant, improved the colorfastness of silk dyed with anthocyanin extract when subjected to washing and rubbing. The presence of aluminum ions in alum mordant facilitates the bonding between -OH groups in anthocyanin and $–NH_2$, -C=O, and $-CH_3$ groups in silk fibers (Figure 6). This bonding allows anthocyanin to attach to silk fabrics, improving colorfastness. Overall, the colorfastness properties were rated as good, indicating that natural anthocyanin, when combined with alum as a mordant, is suitable for dyeing silk, producing both intense color and satisfactory color fastness.

Table 5 Color fastness to washing (ISO 105-C01:2013)

Dyeing method	Color change	Color staining					
		Cotton	Acetate	Wool	Nylon	Acrylic	Polyester
Direct dyeing	3	4-5	4-5	4-5	4-5	4-5	4-5
Mordant dyeing	4	4-5	4-5	4-5	4-5	4-5	4-5

Table 6 Color fastness to crocking (AATCC 8-2007)

Dyeing method	Crocking fastness	
	Dry	Wet
Direct dyeing	4	3
Mordant dyeing	4-5	4

Table 7 Antioxidant activity and UV protection performance

Samples	Antioxidant Activity (%)	Transmittance (%)		UPF	UPF protection class
		UV-A	UV-B		
Untreated	-	15.9	10.3	9.4	No class
Direct dyeing	85.2	2.2	2.3	45.6	Excellent
Mordanting dyeing	80.3	1.9	1.7	48.4	Excellent

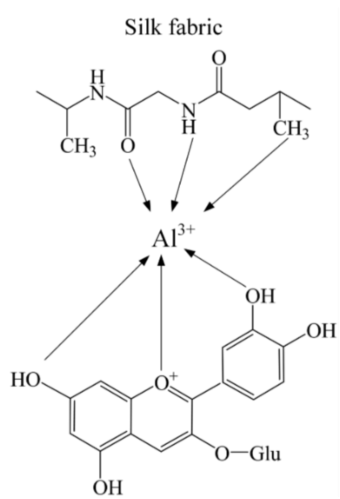


Figure 6 Proposed Al^{3+} mordant interaction between anthocyanin colorant and silk fabric

4.4 UV Protection Performance and Antioxidant Activity

In this study, the effectiveness of UV protection provided by dyed silk fabrics containing extracted anthocyanin is assessed by determining the percentage of UVR transmitted through the fabric and calculating the UPF. The percentage of transmittance measures the amount of UVR that reaches the skin, while UPF is a rating system that evaluates how well a fabric protects against UV rays. A higher UPF value signifies that the fabric provides superior protection against UVR. Table 7 displays the UPF value, as well as the percentage of UV-A (315-400 nm) and UV-B (290-315 nm) transmittance, for both dyed silk and pristine fabrics. The results demonstrate a significant disparity between silk textiles that have been dyed and those that have not been treated. The pristine silk fabric has a UPF of 9.4. Because untreated silk lacks carbon-carbon double bonds or UV-absorbing compounds in its fiber molecular structure, it cannot provide adequate UV protection unless properly treated. Silk fabrics dyed with anthocyanin possessed functional groups (polyphenol and flavonoid) that absorb UV radiation, offering superior UV protection to untreated fabrics. Additionally, certain natural dyes with an aromatic ring or a polymerized polyphenol structure such as anthocyanin, tannin, melanoidin, etc., can act as UV absorbers, offering improved UV protection (Mongkholrattanasit et al., 2021; Chitichotpanya et al., 2024). This is corroborated by decreased UV transmission rates and higher UPF values. The dyed silk also had the highest UPF (40+) rating, suggesting excellent UV protection. Furthermore, using a metal mordant (Al mordant) increases color depth by improving dye-fiber affinity, resulting in a higher UPF value (Vuthiganond et al., 2020; Chitichotpanya et al., 2023).

The presence of antioxidants in fabrics that come into close contact with the human body has sparked interest in developing health-promoting apparel. Anthocyanin has been shown to be effective at radical scavenging, implying that it has the potential to be used as a natural antioxidant to increase textile antioxidant activity. Thus, our study aimed to evaluate the antioxidant potential of silk fabric dyed with anthocyanin extract from black rice bran using the DPPH test method. The treated silk fabric exhibited 85.2% antioxidant activity. The capacity of the anthocyanin extract to scavenge radicals can be attributed to the presence of active hydrogen groups in its polyphenolic components (Suleria et al., 2020). The antioxidant qualities of dyed silk treated with mordant were shown to be decreased compared to dyed silk without mordant. This could be attributed to a reduction in free hydroxyl groups caused by the interaction between silk fiber molecules, metal ions, and dye molecules (Borah et al, 2023). Our findings align with the study conducted by Jia et al., (2017).

5. Conclusion

The aqueous UAE is a highly effective technique for extracting anthocyanins from black rice bran, while also being environmentally friendly. RSM optimized UAE settings with 30 Hz ultrasonic power to achieve the highest TAC yield, resulting in a TAC of 173.25 mg/L at a L:M ratio of 21, an ultrasound temperature of 60°C, and an ultrasound time of 30 minutes. Black rice is a healthful food high in anthocyanin, a type of water-soluble vacuolar pigment having medicinal properties. Therefore, it is expected that the simultaneous coloring and functionality can be achieved with silk fabrics dyed using anthocyanin extracted from black rice bran. Silk fabrics were colored in a reddish-purple shade through the dyeing process. The use of the mordant dyeing method resulted in a 34% higher K/S value compared to direct dyeing, with washing and crocking fastness grades of at least 4 (good). Furthermore, the dyed silk fabrics had exceptional UV protection (UPF > 40+) and antioxidant activity, with a DPPH scavenging rate of over 80%. These properties could be related to the biological activity of the phenolic hydroxyls found in natural anthocyanins. Due to its appealing properties and economic value, silk treated with natural anthocyanin derived from the agricultural byproduct black rice bran can be utilized in the production of medical and health-oriented textile goods.

6. Acknowledgements

The authors gratefully acknowledge the financial support provided by the Faculty of Science and Technology, Thammasat University, Contract No. SciGR 18/2567.

7. References

Alappat, B., & Alappat, J. (2020). Anthocyanin pigments: beyond aesthetics. *Molecules*, *25*(23), Article 5500. https://doi.org/10.3390/molecules25235500

Albuquerque, B. R., Prieto, M. A., Barreiro, M. F., Rodrigues, A., Curran, T. P., Barros, L., & Ferreira, I. C. (2017). Catechin-based extract optimization obtained from Arbutus unedo L. fruits using maceration/microwave/ultrasound extraction techniques. *Industrial Crops and Products*, *95*, 404-415. https://doi.org/10.1016/j.indcrop.2016.10.050

Ardila-Leal, L. D., Poutou-Piñales, R. A., Pedroza-Rodríguez, A. M., & Quevedo-Hidalgo, B. E. (2021). A brief history of colour, the environmental impact of synthetic dyes and removal by using laccases. *Molecules*, *26*(13), Article 3813. https://doi.org/10.3390/molecules26133813

Attia, N. F., Osama, R., Elashery, S. E. A., Kalam, A., Al-Sehemi, A. G., & Algarni, H. (2022). recent advances of sustainable textile fabric coatings for uv protection properties. *Coatings*, *12*(10), Article 1597. https://doi.org/10.3390/coatings12101597

Ayesh, M., Horrocks, A. R., & Kandola, B. K. (2022). The impact of atmospheric plasma/UV laser treatment on the chemical and physical properties of cotton and polyester fabrics. *Fibers*, *10*(8), 66. https://doi.org/10.3390/fib10080066

Backes, E., Pereira, C., Barros, L., Prieto, M. A., Genena, A. K., Barreiro, M. F., & Ferreira, I. C. F. R. (2018). Recovery of bioactive anthocyanin pigments from Ficus carica L. peel by heat, microwave, and ultrasound based extraction techniques. *Food Research International*, *113*, 197-209. https://doi.org/10.1016/j.foodres.2018.07.016

Baig, U., Khatri, A., Ali, S., Sanbhal, N., Ishaque, F., & Junejo, N. (2021). Ultrasound-assisted dyeing of cotton fabric with natural dye extracted from marigold flower. *The Journal of the Textile Institute*, *112*(5), 801-808. https://doi.org/10.1080/00405000.2020.1779907

Benkhaya, S., Rabet, S., & Harfi, A. E. (2020). A review on classifications, recent synthesis and applications of textile dyes. *Inorganic Chemistry Communications*, *115*, Article 107891. https://doi.org/10.1016/j.inoche.2020.107891

Borah, S., Bhuyan, P. M., Sarma, B., Hazarika, S., Gogoi, A., & Gogoi, P. (2023). Sustainable dyeing of mulberry silk fabric using extracts of green tea (Camellia sinensis): extraction, mordanting, dyed silk fabric properties and silk-dye interaction mechanism. *Industrial Crops and Products*, *205*, Article 117517. https://doi.org/10.1016/j.indcrop.2023.117517

Box, G. E. P., & Wilson, K. B. (1951). On the experimental attainment of optimum conditions. *Journal of the Royal Statistical Society: Series B*, *13*, 1-45. https://doi.org/10.1111/j.2517-6161.1951.tb00067.x

Caleja, C., Barros, L., Prieto, M. A., Barreiro, M. F., Oliveira, M. B. P., & Ferreira, I. C. F. R. (2017). Extraction of rosmarinic acid from Melissa officinalis L. by heat-, microwave- and ultrasound-assisted extraction techniques: A comparative study through response surface analysis. *Separation and Purification Technology*, *186*, 297–308. https://doi.org/10.1016/j.seppur.2017.06.029

Chen, T., Xie, L., Wang, G., Jiao, J., Zhao, J., Yu, Q., Chen, Y., Shen, M., Wen, H., Ou, X., & Xie, J. (2024). Anthocyanins-natural pigment of colored rice bran: Composition and biological activities. *Food Research International*, *175*, Article 113722. https://doi.org/10.1016/j.foodres.2023.113722

Chitichotpanya, P., Vuthiganond, N., Chutasen, P., & Inprasit, T. (2023). Green production of simultaneous coloration and functional finishing on hemp textiles through dyeing with Diospyros mollis Griff. extract. *Journal of Metals, Materials and Minerals*, *33*(2), 108–119. https://doi.org/10.55713/jmmm.v33i2.1697

Chitichotpanya, P., Vuthiganond, N., Inprasit, T. & Chutasen, P. (2024). Bioactive and multifunctional wool textiles finishing with diospyros mollis griff. extract. *Journal of Current Science and Technology*, *14*(1), Article 3. https://doi.org/10.59796/jcst.V14N1.2024.3

Das, A. B., Goud, V. V., & Das, C. (2017). Extraction of phenolic compounds and anthocyanin from black and purple rice bran (*Oryza sativa* L.) using ultrasound: A comparative analysis and phytochemical profiling. *Industrial Crops and Products, 95,* 332-341. https://doi.org/10.1016/j.indcrop.2016.10.041

Das, M., Dash, U., Mahanand, S. S., Nayak, P. K., & Kesavan, R. K. (2023). Black rice: A comprehensive review on its bioactive compounds, potential health benefits and food applications. *Food Chemistry Advances*, *3*, Article 100462. https://doi.org/10.1016/j.focha.2023.100462

Garcia-Castello, E. M., Rodriguez-Lopez, A. D., Mayor, L., Ballesteros, R., Conidi, C., & Cassano, A. (2015). Optimization of conventional and ultrasound assisted extraction of flavonoids from grapefruit (Citrus paradisi L.) solid wastes. *LWT-Food Science and Technology*, *64*(2), 1114-1122. https://doi.org/10.1016/j.lwt.2015.07.024

Ito, V. C., & Lacerda, L. G. (2019). Black rice (Oryza sativa L.): A review of its historical aspects, chemical composition, nutritional and functional properties, and applications and processing technologies. *Food Chemistry, 301*, Article 125304. https://doi.org/10.1016/j.foodchem.2019.125304

Jia, Y., Jiang, H., Liu, Z., & Wang, R. (2017). An innovative approach to the preparation of coloured and multifunctional silk material with the natural extracts from chestnut shell and black rice bran. *Coloration Technology*, *133*(2), 262-270. https://doi.org/10.1111/cote.12276

Khazaei, K. M., Jafari, S. M., Ghorbani, M., Kakhki, A., Sarfarazi, M. (2016). Optimization of anthocyanin extraction from saffron petals with response surface methodology. *Food Analytical Methods, 9,* 1993–2001. https://doi.org/10.1007/s12161-015-0375-4

Kim, S., Son, H., Pang, S. Y., Yang, J. J., Lee, J., Lee, K. H., ... & Yoo, H. Y. (2021). Optimization of major extraction variables to improve recovery of anthocyanins from elderberry by response surface methodology. *Processes*, *11*(1), Article 72. https://doi.org/10.3390/pr11010072

Kim, M. J., Park, E., Lee, W., Na, H. S., Choi, Y. H., & Lee, J. H. (2024). Exploring the Potential of Anthocyanins for Repairing Photoaged Skin: A Comprehensive Review. *Foods, 13*(21), Article 3506.https://doi.org/10.3390/foods13213506

Koul, B., Yakoob, M., & Shah, M. P. (2022). Agricultural waste management strategies for environmental sustainability. *Environmental Research*, *206*, Article 112285. https://doi.org/10.1016/j.envres.2021.112285

Kumar, K., Srivastav, S., & Sharanagat, V. S. (2021). Ultrasound assisted extraction (UAE) of bioactive compounds from fruit and vegetable processing by-products: A review. *Ultrasonics Sonochemistry*, *70*, Article 105325. https://doi.org/10.1016/j.ultsonch.2020.105325

Lei, T., Pan, Y., Zhang, B., Liu, R., & Pan Y. (2021). Optimisation of ultrasonic-assisted extraction of natural dyes from pomegranate rind using response surface methodology and its characterisation. *Coloration Technology*, *137*(2), 59–271. https://doi.org/10.1111/cote.12528

Leonarski, E., Kuasnei, M., Santos, E.H., Benvenutti, L., Moraes, P. A. D., Cesca, K., Oliveira, D., & Zielinski, A. A. F. (2024). Ultrasound and microwave-assisted extractions as green and efficient approaches to recover anthocyanin from black rice bran. *Biomass Conversion and Biorefinery*, 1-14. https://doi.org/10.1007/s13399-024-05479-4

Mongkholrattanasit, R., Nakpathom, M, & Vuthiganond, N. (2021). Eco-dyeing with biocolorant from spent coffee ground on low molecular weight chitosan crosslinked cotton. *Sustainable Chemistry and Pharmacy*, *20*, Article 100389. https://doi.org/10.1016/j.scp.2021.100389

Pereira, R. F., Silva, M. M., & de Zea Bermudez, V. (2015). Bombyx mori silk fibers: an outstanding family of materials. *Macromolecular Materials and Engineering*, *300*(12), 1171-1198. https://doi.org/10.1002/mame.201400276

Sadeghi-Kiakhani, M., Tehrani-Bagha, A. R., Safapour, S., Eshaghloo-Galugahi, S., & Etezad, S. E. (2021). Ultrasound-assisted extraction of natural dyes from Hawthorn fruits for dyeing polyamide fabric and study its fastness, antimicrobial, and antioxidant properties. *Environment, Development and Sustainability*, *23*, 9163–9180. https://doi.org/10.1007/s10668-020-01017-0

Shen, L., Pang, S., Zhong, M., Sun, Y., Qayum, A., Liu, Y., ... & Ren, X. (2023). A comprehensive review of ultrasonic assisted extraction (UAE) for bioactive components: Principles, advantages, equipment, and combined technologies. *Ultrasonics Sonochemistry*, *101*, Article 106646. https://doi.org/10.1016/j.ultsonch.2023.106646

Suleria, H. A. R., Barrow, C. J., & Dunshea, F. R. (2020). Screening and Characterization of Phenolic Compounds and Their Antioxidant Capacity in Different Fruit Peels. *Foods*, *9*(9), Article 1206. https://doi.org/10.3390/foods9091206

Tan, J., Han, Y., Han, B., Qi, X., Cai, X., Ge, S., & Xue, H. (2022). Extraction and purification of anthocyanins: A review. *Journal of Agriculture and Food Research*, *8*, Article 100306. https://doi.org/10.1016/j.jafr.2022.100306

Thakur, R., Gupta, V., Dhar, P., Deka, S. C., & Das, A. B. (2022). Ultrasound-assisted extraction of anthocyanin from black rice bran using natural deep eutectic solvents: Optimization, diffusivity, and stability. *Journal of Food Processing and Preservation*, *46*, Article e16309. https://doi.org/10.1111/jfpp.16309

Vuthiganond, N., Nakpathom, M., & Mongkholrattanasit, R. (2020). Azoic deep dyeing of silk and uv protection using plant polyphenols and diazonium coupling. *Fibers and Polymers*, *21*, 1052–1060. https://doi.org/10.1007/s12221-020-9057-y

Yadav, S., Tiwari, K. S., Gupta, C., Tiwari, M. K., Khan, A., & Sonkar, S. P. (2023). A brief review on natural dyes, pigments: Recent advances and future perspectives. *Results in Chemistry*, *5*, Article 100733. https://doi.org/10.1016/j.rechem.2022.100733

Yin, Y., Jia, J., Wang, T., & Wang, C. (2017). Optimization of natural anthocyanin efficient extracting from purple sweet potato for silk fabric dyeing. *Journal of Cleaner Production*, *149*, 673-679. https://doi.org/10.1016/j.jclepro.2017.02.134