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Dyeing Behavior of Silk Dyed with Indigo Leaf Powder Using Reduction and Nonreduction Dyeing and Its Relationship with the Amount of Indigotin and Indirubin Adsorbed in Silk

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Abstract

Dyeing behavior of indigo leaf powder was examined in regards to the effect of the amount of pigments on color and dye adsorption for silk dyed by reduction and nonreduction dyeing. The amount of indigotin and indirubin pigments adsorbed in dyed silk was examined by HPLC-DAD analysis. The color of dyed silk showed 7.7BG - 2.7B hue when silk was dyed at 50°C, and 3.5G - 4.9BG when dyed at 70°C. Blue (b*) and green (a*) color decreased as the pH of dyebath increased. When silk was dyed using nonreduction, R (red) and RP (red purple) hue and R hue was more apparent in samples dyed at 90°C. In reduction dyeing, amount of indigotin detected from silk exceeded the amount that was initially contained in the input dye. The amount of indirubin was lower than indirubin that was initially in the powder. In nonreduction dyeing, silk showed a higher amount of indirubin adsorption compared to silk dyed by reduction. The amount of indigotin adsorbed in silk was lower than the amount initially contained in the input dye. The amount of indigotin and indirubin adsorption was primarily dependent upon the dyeing method-reduction or nonreduction along with dyeing temperature and the pH of dyebath.

Key words: Indigo leaf power, Silk dyeing, Reduction, Nonreduction, Indirubin

I. Introduction

Indigo plant is most often used as a source of natural indigo dye in the form of fermented indigo paste or powder. Fresh leaves of indigo plant are used in dyeing but dyeing practice is only possible if it is done at locations close to the plant cultivation and during the right season. Indigo leaf powder is the powder of indigo leaves which is obtained by drying and grinding the whole leaves of indigo plant such as *Indigofera tinctoria* L. (IndiaMART, 1996-2019). Commercial products of indigo leaf powder are easily obtainable from the markets. Manufacturing process of indigo leaf powder is simple and environment friendly because unlike fermented indigo powder, indigo leaf powder is made without the complicated vat process involving strong alkali and a large amount of water (Vuorema, 2008). Indigo leaf powder as indigo dye is free of geographic and seasonal limitations.

Within the leaf powder, there are coloring substances which are blue indigotin ($C_{16}H_{10}N_2O_2$, m.w. 262.27 g/mol) and red indirubin ($C_{16}H_{10}N_2O_2$, m.w. 262.27 g/mol). Considering that indirubin is a red pigment, opposed to indigotin the blue pigment, it is expected that the amo-

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unt or content ratio of the two pigments in a particular indigo leaf powder would affect the color of the dyed textiles. As it is widely known, indigotin must be reduced into soluble leuco form in order to dye the fiber (Poulin, 2007). It was found that indirubin showed a relatively higher dye adsorption and deeper color through nonreduction dyeing (Yoo, 2017; Yoo & Ahn, 2017).

Extensive research was conducted by Shin et al. (2009a, 2009b) on the dyeing behavior of indigo leaf powder regarding different powder manufacturing processes and storage time. Silk dyed with indigo powder prepared by freeze-drying indigo raw juice after deep freezing, freeze-drying fresh indigo leaf, or freeze-drying indigo leaf after deep freezing were used to dye silk without reduction (Shin et al., 2009a). Silk dyed with all three types of powder showed B hue of Munsell color system when fresh powder was used, but silk dyed with freeze-dried indigo leaf powder after 4 or 30 days of storage showed PB hue with little change in the K/S values (Shin et al., 2009a). Shin et al. (2009a) suggested that the PB hue of silk dyed with freeze-dried indigo leaf powder by nonreduction method was due to the transformation of indoxyl to indirubin during the storage of leaf powder. In another study, Shin et al. (2009b) found out that the color of silk dyed by room temperature dried powder showed the hue of 7.9B and the silk dyed by hot air dried powder showed 6.2B. When silk was dyed using sodium hydrosulfite with no alkali at pH 6.04 - 6.36, the color of silk showed the hue range of 4.7BG to 6.9B, shifting from BG to B with the increase in concentration of sodium hydrosulfite (Shin et al., 2009b).

While previous literature provided information on the color and the K/S values of the silk dyed with indigo leaf powder and the UV-Vis absorption behavior of indigo leaf powder as dye, the question still remains on how indigotin and indirubin actually contribute to the color of fiber dyed by indigo leaf powder and whether their contribution behavior changes when dyeing condition changes.

Yoo and Ahn (2018) examined whether the color of silk dyed using fermented indigo powder by reduction and nonreduction dyeing methods was affected by the amount of indigotin and indirubin fixed on the silk by dyeing. Results showed that the silk dyed with fermented indigo powder using reduction method showed PB hue when the adsorption of indigotin was low whereas the adsorption of indirubin was relatively high (Yoo & Ahn, 2018). It was found that basically indirubin had much lower adsorption rate than indigotin, however more redness was shown in silk when the silk was dyed by nonreduction dyeing than reduction dyeing, and under higher degree of alkalinity regardless of dyeing method (Yoo & Ahn, 2018). Amount of indigotin adsorbed in the fiber was dependent on the dyeing temperature and the pH of dyebath (Yoo & Ahn, 2018). When the dyeing behavior of silk dyed with indigo/indirubin mixed dye was examined, higher proportion of indigotin resulted in a higher K/S value while the proportion of indirubin had low or negative contribution to the K/S value (Yoo & Ahn, 2017). Higher amount of indirubin in silk resulting in a darker PB color, and a shift to P color with a further increase of indirubin content (Yoo & Ahn, 2017).

In this research, indigo leaf powder was selected as the source of indigo dye to examine how indigotin and indirubin actually contribute to the color of dyed fiber and whether their contribution changes by dyeing conditions. For the purpose, a commercial product of indigo leaf powder with Indian origin was purchased and used to dye silk through reduction and nonreduction methods using different dyeing temperature and pH. Amount of indigotin and indirubin in the leaf powder, and the adsorption of the two pigments on silk was analyzed using high performance liquid chromatography equipped with a diode array detector (HPLC-DAD). Adsorption of indigotin and indirubin pigments was evaluated by the amount of each pigment detected by the HPLC analysis from the dye extracted from dyed silk. K/S value and CIELAB, Munsell HVC values of dyed silk were examined using a spectrocolorimeter. In this research, the term 'indigotin' was used to refer to the pigment compound detected in the dye and the dyed silk and the term 'indigo' was used to refer to the dye or dye solution.

II. Materials and Methods

1. Materials

Indigo leaf powder (100% leaves) produced in India was purchased from Art & Crafts (Korea). Synthetic indigo (CAS 482-89-3, 262.26 g/mol) and indirubin (CAS 479-41-4, 262.27 g/mol) were purchased from Sigma-Aldrich (U.S.A.) and were used as standard indigotin and standard indirubin for the HPLC analysis. Silk fabric used for dyeing was purchased from Sombe (Korea). Specifications of the fabric were; density 122 ×117/inch^2, thickness 0.10 mm, and weight 0.50 g/100 cm^2. Methanol (HPLC grade) and dimethyl sulfoxide (DMSO, HPLC grade) were purchased from J. T. Baker (U.S.A.). Sodium hydrosulfite and sodium hydroxide were purchased from Junsei Chemical (Japan). Acetic acid and hydrochloric acid were purchased from Daejung Chemical (Korea). Water was doubly distilled using a water purification system by Human Power (Korea).

2. Methods

1) Preparation of Dyebath and Dyeing

For reduction dyeing, dyebaths were prepared by adding 600 mg/L of indigo leaf powder and 100 mg/L of sodium hydrosulfite to water with the liquor ratio of 1:200 each. The pH of dyebaths was adjusted to pH 7, 8, 9, 10, 11, and 12 using sodium hydroxide than were reduced for 30 minutes in room temperature. A 4 cm × 5 cm sized silk sample (100 mg) was dyed in each dyebath at 50°C and 70°C for 30 minutes. Then, dyed silk samples were oxidized in the air for 2 minutes, and washed in running cold water for 2 minutes to develop color.

For nonreduction dyeing, dyebaths were prepared by adding 600 mg/L of indigo leaf powder to water with the liquor ratio of 1:200 each. Sodium hydrosulfite was not added to the dyebaths. The pH of dyebaths were adjusted to pH 3 - 12 using acetic acid or sodium hydroxide. A 4 cm × 5 cm sized silk sample was dyed in each dyebath at 50°C, 70°C, and 90°C for 30 minutes. The dyed silk fabric was oxidized in the air for 2 minutes, and washed in running cold water for 2 minutes to develop color.

2) Color Measurement of Dyed Silk

Color of the dyed silk samples was measured using a spectro-colorimeter (Color i5, X-rite, U.S.A.) under the D_{65} light source and 10° observer. From the measurement, K/S value was used as the measure of dye adsorption. Munsell H (hue), V (value), C (chroma) values and the CIELAB L* (lightness), a* (redness to greenness), b* (yellowness to blueness) values were used to evaluate the color of the dyed samples.

3) HPLC Analysis of the Amount of Pigments in Leaf Powder

(1) Preparation of Standard Calibration Graphs of Indigotin and Indirubin Pigments

Standard dye solutions of indigotin and indirubin and their standard calibration graphs were prepared by the following procedure. A 10 mg/L each of synthetic indigo and natural indirubin was dissolved separately in dimethyl sulfoxide (DMSO) to prepare the stock solutions of indigo standard dye and indirubin standard dye. Each standard dye solution was diluted by the fraction of 2, 4, 8, 16, and 32 times. As a result, the concentration range of standard solutions was 10 mg/L to 0.3125 mg/L. Each fraction was analyzed using the HPLC analysis. From the HPLC data, relative abundance values at 604 nm and 540 nm wavelengths were used to produce the standard calibration graphs of indigotin and indirubin, respectively. The regression equations generated from the standard graphs were used to calculate the amount of indigotin and indirubin in the dye solution and the dyed silk.

(2) Analysis of the Pigments in indigo Leaf Powder and Dyed Silk Samples

A 1 mg of indigo leaf powder was dissolved in 100 mg of DMSO. Each solution was filtered using a 0.45 μm nylon syringe filter (Alltech, U.S.A.) and subjected to HPLC-DAD analysis. To extract the dye from the dyed silk samples, a 0.5 cm × 0.5 cm size of the dyed silk and 0.4 mL of a mixed solution of hydrochloric acid:

methanol: water (2:1:1, v/v/v) were added in a small beaker. The beaker was then placed in an oven at 110°C for about 10 minutes to evaporate the liquid. After removing the beaker from the oven, 1.5 mL of DMSO was added to the beaker. Remaining solution was filtered through a 0.45 μm nylon syringe filter and analyzed using the HPLC.

(3) HPLC Condition

An Ultimate 3000 HPLC (Thermo Dionex, U.S.A.) equipped with a diode array detector (DAD) was used in the HPLC analysis. Column used was VDSpher C-18 column (VDS Optilab, Germany 4.6 × 250, 5 μm), flow rate was 1 mL/min, and the oven temperature was 30°C. Mobile phase was buffer A (0.5% formic acid) and buffer B (acetonitrile), and the gradient mode was 0 - 10 minutes A:B 75:25, 10 - 16 minutes A:B 10:90, and 16 - 20 minutes A:B 75:25 during the total runtime 20 minutes. Detection wavelength for the DAD was set for 288 nm, 540 nm, 604 nm with the total scanning done in 190 - 800 nm range.

III. Results and Discussion

1. HPLC Analysis of Standard Dyes and Formulation of Standard Graphs

In order to analyze the amount of indigotin and indirubin pigments in indigo leaf powder and dyed silk samples, indigo and indirubin standard dyes were analyzed using the HPL-DAD instrument. Calibration graphs of detected indigotin and indirubin were generated based on the HPLC data.

Two different HPLC data were collected based on the 2 sets of HPLC run. The chromatogram of the 1st HPLC run showed major peaks at (R_t) 9.15 min, 9.58 min, and the chromatogram of the 2nd HPLC run showed major peaks at 11.02 min, 11.46 min (Fig. 1). UV-Vis spectrum of the peaks at 9.15 min (1st run) and 11.02 min (2nd run) showed the maximum absorbance (λ_{max}) at 604 nm and the peaks at 9.58 min (1st run) and 11.46 min (2nd run) showed the maximum absorbance at 540 nm. Based on the literatures, the peaks' maximum UV-Vis absorbance of 604 nm and 540 nm confirmed that the peaks represent indigotin and indirubin, respectively (Ahn et al., 2015; Shin et al., 2009a).

A 10 mg/L solution each of indigo and indirubin standard dye in DMSO solution was diluted by the fraction of 2, 4, 8, 16, and 32 times and analyzed using the HPLC instrument. <Fig. 2> shows the chromatograms of the dilutions around the retention times (R_t) 9.15 min and 9.58 min based on the 1st HPLC run and the standard graphsof indigotin and indirubin pigments generated from the peaks. Using the known concentrations of the standard dilutions and the relative abundance of the peak of each dilution obtained from the HPLC analysis, standard graphs were generated for indigotin and indirubin pigments (Fig. 2). From the graphs, 2 different regression equations were obtained; $y = 0.9822x - 0.0169$ ($R^2 = 1.0000$) for indigotin and $y = 0.9765x - 0.0148$ ($R^2 = 0.9999$) for indirubin where x is the concentration and y is the relative abundance of the peak obtained from HPLC analysis. These regression equations were used to calculate the amount of indigotin and indirubin pigments inindigo leaf powders.

By the same manner, the results of 2nd HPLC run of standard dilutions were used to generate the standard graph and corresponding regression equations. As a result, the regression equation $y = 0.9432x - 0.1144$ ($R^2 = 0.9999$) for indigotin and $y = 0.9570x - 0.0057$ ($R^2 = 1.0000$) for indirubin were obtained. These regression equations were used to calculate the amount of indigo tin and indirubin pigments adsorbed in the dyed silk samples.

2. HPLC Analysis on the Amount of Pigment in Natural Indigo Leaf Powder Used in the Study

HPLC analysis was conducted on the indigo leaf powder selected for this study. It was confirmed that the indigo leaf powder contained indigotin pigment through a peak at R_t 9.15 min and its UV-Vis spectrum with maximum absorbance (λ_{max}) at 604 nm, and also contained indirubin pigment through a peak at R_t 9.58 min and its UV-Vis spectrum with maximum absorbance at 540 nm (Fig. 3).

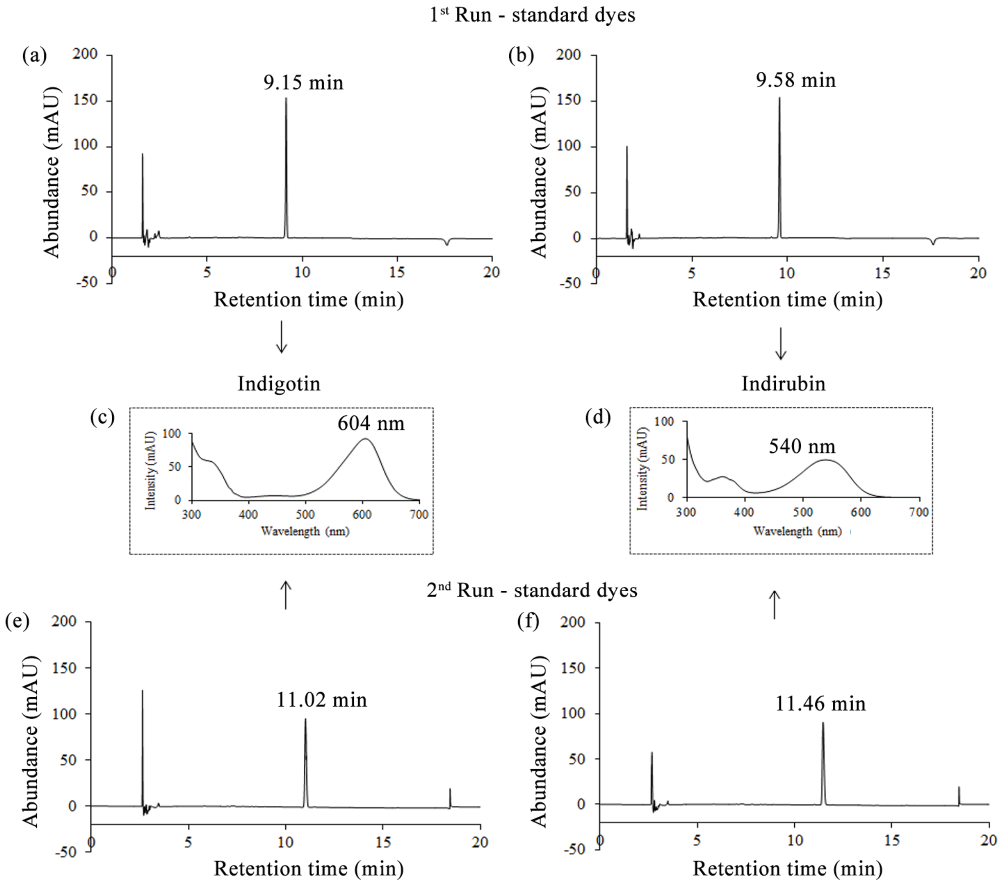


Fig. 1. The 1st and 2nd HPLC results of indigo and indirubin standard dyes. HPLC chromatograms of standard indigo (a), (e) and indirubin (b), (f) dye, UV-Vis spectrum of peaks at retention time 9.15 min/9.58 min and 11.02 min/11.46 min indicating indigotin (c) and indirubin (d).

<Table 1> shows the amount of indigotin and indirubin pigments contained in the dye solution of indigo leaf powder used in the study. The amount of indigotin was 0.083 mg/L and indirubin was 0.037 mg/L using the equations $y = 0.9822x - 0.0169$ ($R^2 = 1.0000$) for indigotin and $y = 0.9765x - 0.0148$ ($R^2 = 0.9999$) for indirubin. As a result, the amount of the total pigment contained in the indigo leaf powder used in the study was 0.120 mg/L.

Kukula-Koch et al. (2015) reported that the percent concentration of indigotin and indirubin in the extracts of indigo leaf powder from 3 different harvests of indigo plant ranged indigotin 0.24 - 1.47% and indirubin 0.15 - 0.54% when 4 different solvent systems were used. When these numbers were converted to the percent ratio between the 2 pigments, the content ratios of indigotin:indirubin were such as 69.39:30:61, 78.61:21.39, 85.09:14.91 etc., which indicate a higher ratio of indigotin over indirubin. Oh and Ahn (2013) reported that the content ratio of indigotin and indirubin in 2 different indigo leaf powder products were 49:51 and 8:91 while the ratio of the two pigments in 2 different fermented indigo powder products were 69:31 and 76:24. The percent ratio of indigotin and indirubin in the indigo leaf powder used in this study was similar to that of some of the powders used by Kukula-Koch et al. (2015)

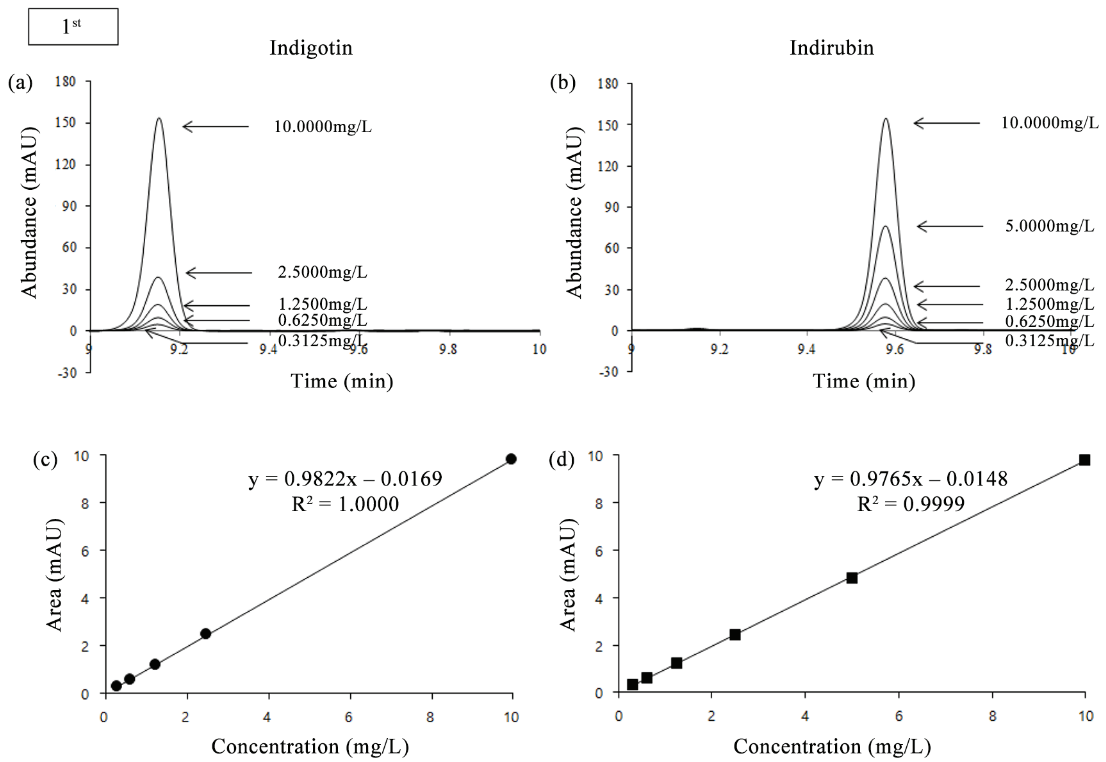


Fig. 2. Chromatograms of 1st HPLC run of standard indigo (a) and indirubin (b) dyes and standard graph of indigotin (c) and indirubin (d) generated from the abundance of each peak in (a) and (b).

and that of the fermented indigo powder used by Oh and Ahn (2013).

3. Color Analysis of Silk Dyed with Indigo Leaf Powder by Reduction Dyeing

<Table 2> shows the visual color and <Table 3> shows the color measurements of silk dyed with indigo leaf powder by reduction dyeing using dyeing temperature 50°C and 70°C and dyebath pH from 6 to 12. By dyeing temperature 50°C, silk dyed with pH 6 - 11 dyebaths using reduction dyeing showed hue value ranging from 7.7BG (blue green) - 2.7B (blue) (Table 3). When 70°C dyeing temperature was applied the hue values of silk dyed with pH 6 - 11 dyebaths ranged 3.5G (green) - 4.9BG (blue green). When the pH of dyebath was 12, the color of silk dyed showed a Y (yellow) hue (3.0Y - 3.4Y) at both dyeing temperatures. In all cases, chroma (C) values of the samples were in the range of 1.2 - 2.5. When L*a*b* values of the CIELAB color space were examined, the color of silk dyed at 50°C by reduction showed negative b* (negative: blue, positive: yellow) values (–4.61 - –1.42) representing a bluer color and negative a* values (negative: green, positive: red) (–10.82 - –9.72) representing a greener color (Table 3). As the dyebath became more alkaline (pH 6 - 11) blueness and greenness gradually decreased. When the silk was dyed at 70°C with pH 6 - 11 dyebath, samples showed greener color (–15.02 - –7.81) and yellower color (1.12 - 4.37) than those dyed at 50°C. Generally, with the increase in alkalinity of dyebath (pH 6 - 11) redness and yellowness increased. When the pH was 12, blueness decreased dramatically with high positive b* values (8.00 - 9.51) for samples dyed at both dyeing temperatures. Higher

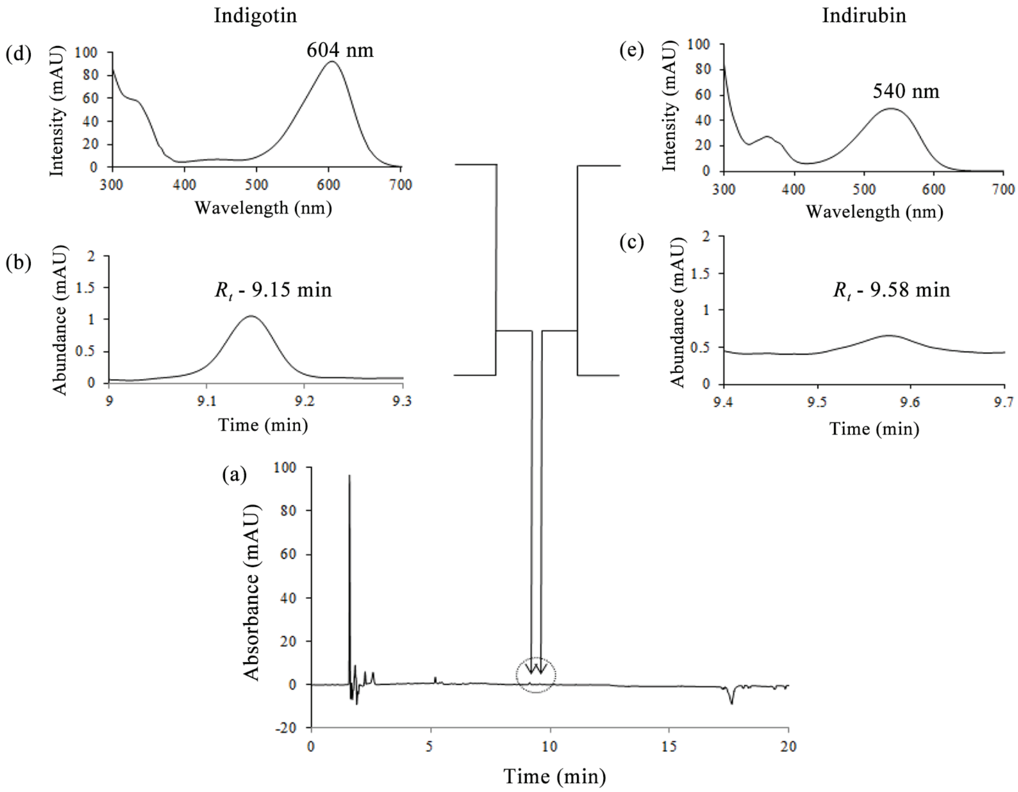


Fig. 3. HPLC results of indigo leaf powder used in the study. HPLC chromatogram of indigo leaf powder (a), close-up of the chromatograms at 9.15 min (b) and 9.58 min (c), UV-Vis spectra of the peak at 9.15 min indicating indigotin (d) and peak at 9.58 indicating indirubin (e).

Table 1. Amount of indigotin and indirubin in the dye solution of indigo leaf powder used in the study

Concentration of indigo leaf powder (mg/L)	Pigment	Amount of pigment (mg/L)	Total pigment (mg/L)	Pigment in natural dye (%)	Percent ratio (%)
100	Indigotin	0.083	0.120	0.083	69.17
	Indirubin	0.037		0.037	30.83

Table 2. Color of silk dyed with indigo leaf powder by reduction dyeing

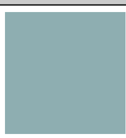
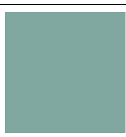
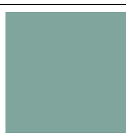
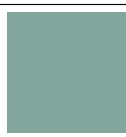
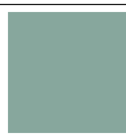
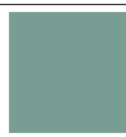
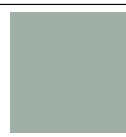
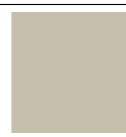
Dyeing temp.	pH 6	pH 7	pH 8	pH 9	pH 10	pH 11	pH 12
50°C							
70°C							

Table 3. Color measurement of silk dyed with indigo leaf powder by reduction using different dyeing temperature and pH

Dyeing temp.	pH	K/S	λ_{max} (nm)	ΔE	L*	a*	b*	Munsell value		
								H	V	C
Control		-	-	-	92.34	–0.10	0.93	4.6Y	9.1	0.1
50°C	6	0.76	620	24.84	70.63	–10.82	–4.61	2.7B	6.9	2.4
	7	0.89	620	26.66	69.00	–11.85	–4.35	1.5B	6.7	2.5
	8	0.81	620	25.65	69.60	–11.21	–3.19	0.2B	6.8	2.2
	9	0.75	620	24.76	70.19	–10.55	–2.67	9.9BG	6.8	2.1
	10	0.75	620	24.97	69.75	–10.44	–1.56	7.9BG	6.8	1.9
	11	0.76	620	24.88	69.52	–9.72	–1.42	7.7BG	6.8	1.8
	12	0.20	620	15.86	78.21	1.26	8.00	3.0Y	7.7	1.2
70°C	6	1.06	620	29.57	66.84	–15.02	–0.10	4.9BG	6.5	2.6
	7	1.09	620	30.16	66.14	–15.03	1.12	3.4BG	6.4	2.6
	8	1.08	620	30.05	65.91	–14.36	1.68	2.6BG	6.4	2.5
	9	0.96	620	28.78	66.93	–13.48	2.72	0.5BG	6.5	2.3
	10	1.35	620	33.28	62.99	–15.69	2.57	1.3BG	6.1	2.7
	11	0.56	620	22.73	71.24	–7.81	4.37	3.5G	7.0	1.3
	12	0.20	620	16.87	77.86	1.05	9.51	3.4Y	7.7	1.3

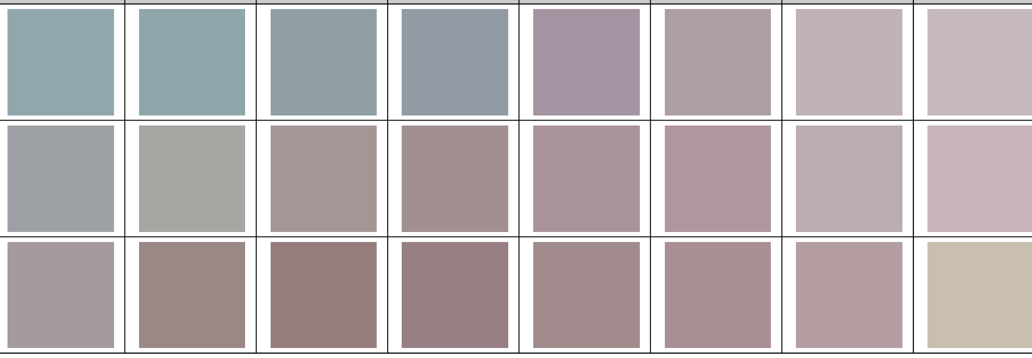
K/S values (1.35 - 0.20) were obtained when the silk was dyed at 70°C than 50°C, among which the sample dyed with pH 10 dyebath showed the highest K/S (1.35). Lowest K/S was observed when the silk was dyed using the pH 12 (Table 3).

The result of present investigation was not comparable to Shin et al. (2009b) primarily since the two research applied different dyeing temperatures. However, considering that the color of silk samples of this research showed a shift of hue from 2.7B→3.5G with the shift of dyeing temperature from 50°C→70°C and the pH from 7→11, and those of Shin et al. (2009b) showed hue values of 6.8B→7.0B when dyeing temperature was 22°C and the pH shifted from 5.46→11.76, and hue values of 6.8B→6.0B with the increase in dyeing temperature from 50°C→60°C, it is conjectured that the increase in dyeing temperature together with the increase of pH may shift the hue from blue (B) to green (G), exhibiting a counterclockwise continuity of hue in the Munsell color space. Present results on the dye adsorption showed higher K/S value when dyeing temperature was 70°C than when it was 50°C. This result was very different from Shin et al. (2009b) who said that the K/S of the silk dyed at 22°C in pH 5.46 was notably higher than those of any other sample, and that the K/S value was the higher when dyeing temperature was 50°C than when it was 60°C.

4. Color Analysis of Silk Dyed with Indigo Leaf Powder by Nonreduction Dyeing

<Table 4> shows the visual color of silk dyed with indigo leaf powder by nonreduction dyeing using dyeing temperature 50°C, 70°C, and 90°C and dyebath pH from 3 to 12. <Table 5> shows the results of Munsell hue and CIELAB L*a*b* values on the silk dyed by nonreduction. A majority of the samples resulted in the hue value of R (red) and RP (red purple) type. When dyeing temperature was 50°C, P (purple) type appeared in the samples of dyebath pH 9 and above, and RP and R type appeared with dyeing temperature 70°C and dyebath pH 7 and above. When the dyeing temperature was 90°C, RP and R type appeared in all samples except for the one dyed in pH 12 dyebath. Chroma values

Table 4. Color of silk dyed with indigo leaf powder by nonreduction dyeing using different dyeing temperature and pH



Dyeing temp.	pH 3	pH 5	pH 7	pH 8	pH 9	pH 10	pH 11	pH 12
50°C								
70°C								
90°C								

Table 5. Color measurement of silk dyed with indigo leaf powder by nonreduction using different dyeing temperature and pH

Dyeing temp.	pH	K/S	λ_{max} (nm)	ΔE	L*	a*	b*	Munsell value		
								H	V	C
Control		-	-	-	92.34	–0.10	0.93	4.6Y	9.1	0.1
50°C	3	0.76	620	25.56	68.48	–7.07	–5.00	6.4B	6.6	2.0
	5	0.83	620	26.40	67.73	–7.59	–4.98	5.3B	6.6	2.1
	7	0.85	620	27.69	65.58	–5.64	–3.48	5.6B	6.4	1.5
	8	0.80	590	28.49	64.48	–2.61	–4.45	3.0PB	6.2	1.5
	9	0.87	560	30.22	63.50	6.65	–5.01	8.5P	6.2	2.7
	10	0.64	550	26.41	67.02	6.59	–2.44	1.8RP	6.5	2.4
	11	0.34	550	18.74	74.45	5.23	–0.66	4.0RP	7.3	2.0
	12	0.27	550	16.49	76.90	5.09	–1.65	2.0RP	7.5	2.2
70°C	3	0.65	580	26.09	66.56	–0.85	–2.98	7.1PB	6.5	1.1
	5	0.54	570	24.43	67.96	–0.66	2.18	2.2Y	6.6	0.2
	7	0.80	550	29.01	63.81	4.88	2.54	3.1R	6.2	1.6
	8	0.98	550	31.65	61.27	5.87	0.36	6.4RP	6.0	1.9
	9	0.85	550	29.88	63.38	7.19	0.22	6.2RP	6.2	2.3
	10	0.76	540	28.46	65.22	8.43	–0.29	5.4RP	6.4	2.7
	11	0.43	550	21.27	72.18	6.52	–0.50	4.5RP	7.1	2.4
	12	0.33	550	18.21	75.51	6.76	–0.14	5.0RP	7.4	2.5
90°C	3	0.73	560	27.61	65.03	3.52	–0.83	2.7RP	6.3	1.5
	5	1.20	550	34.22	58.60	4.79	3.92	6.4R	5.7	1.6
	7	1.56	550	38.09	55.20	8.08	3.05	1.7R	5.4	2.4
	8	1.45	550	37.11	56.26	8.53	1.23	8.0RP	5.5	2.5
	9	1.11	540	33.53	59.72	7.52	2.10	0.1R	5.8	2.2
	10	0.97	540	31.71	62.19	9.70	1.34	8.0RP	6.1	2.9
	11	0.61	540	25.65	67.93	7.75	1.60	8.8RP	6.6	2.5
	12	0.20	550	16.58	78.28	0.46	9.69	2.3Y	7.7	1.3

were low with the range of 1.1 - 2.9. In CIELAB color space, positive a* values were observed in the silk dyed in pH 9 - 12 dyebath when the dyeing temperature was 50°C. When dyeing temperature was 70°C and 90°C the positive a* values resulted in a wider range of dyebath pH and the value increased with increased alkalinity. Overall, significantly less blue color, represented by positive b* values, was observed in these silk compared to the silk dyed by reduction process.

5. HPLC Verification of Indigotin and Indirubin in the Silk Dyed with Indigo Leaf Powder

HPLC analysis was conducted on the dye extracted from silk dyed with indigo leaf powder using reduction and nonreduction method to verify that dyed silk contained indigotin and indirubin pigments. <Fig. 4>–<Fig. 5> show HPLC chromatograms of the dye extracted from silk samples dyed with indigo leaf powder by reduction and nonreduction, respectively. From the chromatograms, major peak was observed at R_t 11.01 min and R_t 11.46 min in all silk dyed with different combinations of dyeing temperature and dyebath pH. The maximum absorbances of the peaks appeared at 604 nm and 540 nm of their UV-Vis spectra, which represent the peaks of indigotin (604 nm) and indirubin (540 nm).

6. Analysis of the Amount of Pigments Detected from the Dyed Silk

HPLC analysis was conducted on selected silk samples to examine the adsorption behavior of indigotin and indirubin in the silk dyed by reduction dyeing (Table 6). The adsorption of indigotin and indirubin was evaluated by the amount of each pigment detected from the dye extracted from silk after dyeing. The highest amount of indigotin was adsorbed when the silk was

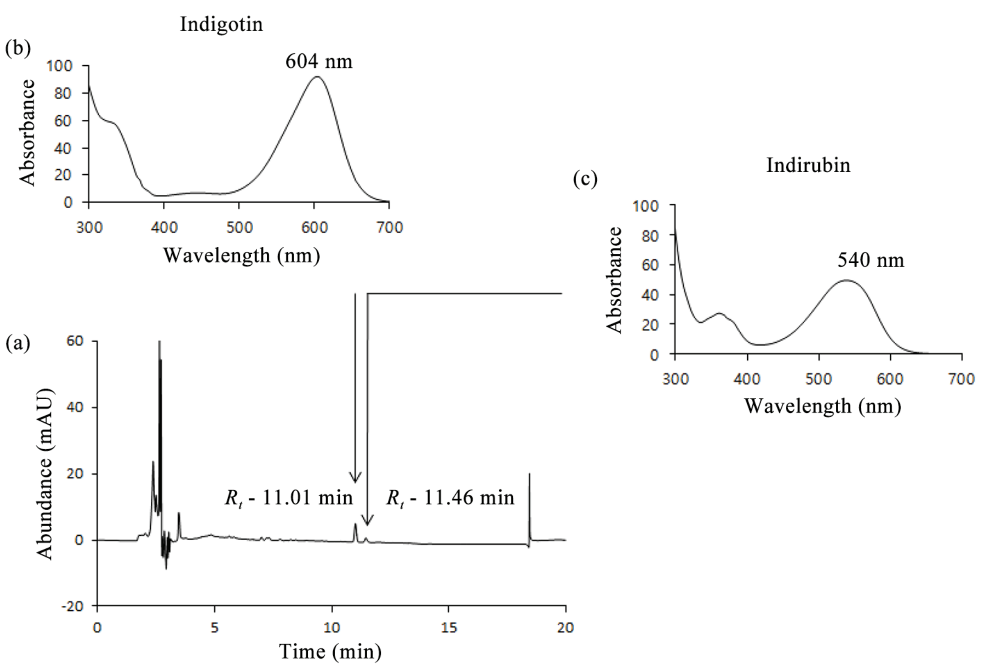


Fig. 4. HPLC chromatogram of dye extracted from the silk dyed with indigo leaf powder by reduction using 50°C dyeing temperature and pH 7 dyebath. HPLC chromatogram (a), UV-Vis spectrum of the peak of 11.01 min and 11.46 min indicating indigotin (b) and indirubin (c).

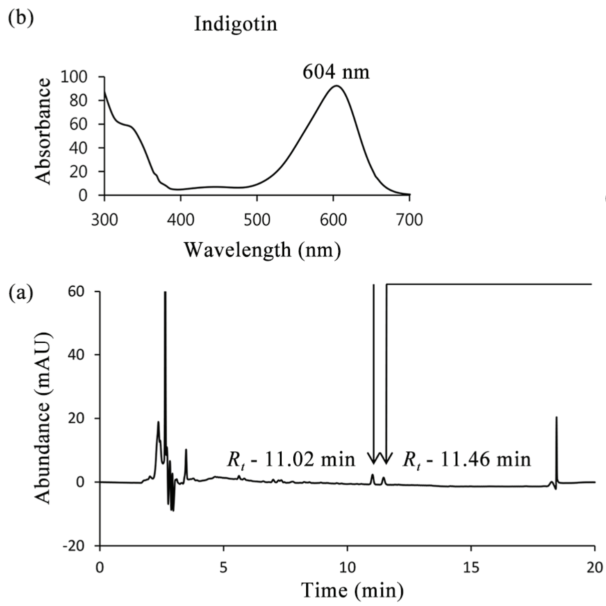


Fig. 5. HPLC chromatogram of dye extracted from the silk dyed with indigo leaf powder by nonreduction using 50°C dyeing temperature and pH 3 dyebath. HPLC chromatogram (a), UV-Vis spectrum of the peak of 11.01 min and 11.46 min indicating indigotin (b) and indirubin (c).

Table 6. Amount of pigments detected from silk dyed with indigo leaf powder by reduction

Amount of natural dye used in dyeing (mg/L)	Dyeing temp. (^{o}C)	pH	Pigment	Amount of pigment in natural dye* (A) (mg/L)	Amount of dye detected from silk (B) (mg/L)	Amount fixed in silk relative to A (B/A) × 100
600	50	7	Indigotin	0.50	0.72	144.58
			Indirubin	0.22	0.16	72.07
600	50	11	Indigotin	0.50	0.51	102.41
			Indirubin	0.22	0.16	72.07
600	70	7	Indigotin	0.50	0.72	144.58
			Indirubin	0.22	0.10	45.45
600	70	11	Indigotin	0.50	0.27	54.00
			Indirubin	0.22	0.08	36.36

*: Amount of pigment was calculated based on the amount of indigotin and indirubin detected from each indigo leaf powder which is shown in <Table 1>.

dyed in pH 7 dyebath, whether the dyeing temperature was 50^{o}C or 70^{o}C. When the amount of pigments adsorbed in silk was compared to the initial pigments contained in the input leaf powder (amount calculated based on the data of <Table 1>), the amount of indigotin detected from silk exceeded the amount that was initially contained in the input dye. This phenomenon was evident especially when the silk was dyed in pH 7 dyebath regardless of dyeing temperature. Different from indigotin, indirubin detected from silk dyed by reduction was consistently lower than indirubin that was contained in the leaf powder used in dyeing.

HPLC analysis was conducted on selected silk samples to examine the adsorption behavior of indigotin and indirubin in silk dyed by nonreduction dyeing (Table 7). The adsorption of indigotin and indirubin was evaluated by the amount of each pigment detected from the dye extracted from silk after dyeing. The results were quite different from those obtained from the silk dyed by reduction. Overall, higher amount of indirubin was adsorbed in all samples compared to the silk dyed by reduction. In many cases, indirubin adsorbed in the silk dyed by nonreduction exceeded the amount that was initially contained in the input dye. Adsorption of indigotin was overall much lower in the silk dyed by nonreduction than those dyed by reduction. Amount of indigotin adsorbed in silk was always lower than the amount that was initially contained in the input dye.

7. Discussion

In reduction dyed silk, K/S value was directly related to the amount of indigotin but its relationship with indirubin was relatively low (Table 8). In nonreduction dyeing, K/S value had some relationship with the amount of indigotin and the amount of indirubin, but the rela-

Table 7. Amount of pigments detected from silk dyed with indigo leaf powder by nonreduction

Amount of natural dye used in dyeing (mg/L)	Dyeing temp. (°C)	pH	Pigment detected	Amount of pigment in natural dye* (A) (mg/L)	Amount of dye detected from silk (B) (mg/L)	Amount fixed in silk relative to A (B/A) × 100
600	50	3	Indigotin	0.50	0.44	88.35
			Indirubin	0.22	0.26	117.12
600	50	7	Indigotin	0.50	0.40	80.32
			Indirubin	0.22	0.28	126.13
600	50	11	Indigotin	0.50	No detection	No detection
			Indirubin	0.22	0.19	85.59
600	50	12	Indigotin	0.50	No detection	No detection
			Indirubin	0.22	0.19	85.59
600	90	3	Indigotin	0.50	0.40	80.32
			Indirubin	0.22	0.31	139.64
600	90	7	Indigotin	0.50	0.35	70.28
			Indirubin	0.22	0.17	76.58
600	90	11	Indigotin	0.50	0.14	28.11
			Indirubin	0.22	0.39	175.68
600	90	12	Indigotin	0.50	No detection	No detection
			Indirubin	0.22	0.04	18.02

*: Amount of pigment was calculated based on the amount of indigotin and indirubin detected from each indigo leaf powder which is shown in <Table 1>.

Table 8. Rank comparison of K/S value, hue and the amount of pigments adsorbed in silk dyed by reduction dyeing of indigo leaf powder

Dyeing condition	Munsell hue	Rank of K/S value	Rank of amount of indigotin	Rank of amount of indirubin
50°C / pH 7	1.5B	2	1	1
50°C / pH 11	7.7BG	3	3	1
70°C / pH 7	3.4BG	1	1	3
70°C / pH 11	3.5G	4	4	4

tionship was not as clear as in reduction dyeing (Table 9). As far as the color goes, higher amount of indigotin and higher amount of indirubin in reduction dyeing was related with B hue. G hue was related to the least K/S value and also to the least amount of indigotin or indirubin. In nonreduction dyeing, higher amount of indigotin and low amount of indirubin resuted in B hue. Overall, R hue was produced when the amount of indigotin was low.

Relationship between the a* ((–)greenness - redness (+)) and b* ((–)blueness - yellowness(+)) values of CIELAB color space and the amount of pigments examined by the HPLC method is illustrated in <Fig. 6>. It can be seen that a* values are spread in larger scale (from –15 to +10) than the b* value (from –5 to +5) depending on the amount of indigotin or indirubin. This indicates that a* values are more affected by a small change in the amount of indigotin and indirubin than the b* values.

Indigotin and indirubin reside in the fresh leaves in the form of glycoside indican (Kokubun et al., 1998). When leaves are pounded or damaged, indican is hydrolyzed into glucose and indoxyl by the action of β-glucosidase enzyme in the leaf (Minami et al., 1997). Indoxyl spontaneously dimerize to form the insoluble blue indigotin when in contact with oxygen (Kokubun et al., 1998; Minami et al., 1997). Indoxyl also oxidizes to form isatin by spontaneous reaction and isatin and free indoxyl dimerize to form the red indirubin (Kokubun et al., 1998). In fermented indigo paste or powder, indican is already transformed into insoluble indigotin (Wenner, 2017). Since indigotin is the major pigment of fermented indigo powder, indigotin plays a major role when dyeing and therefore the powder must be reduced in order to dye the fiber. Reduction process forms leuco-indigotin, and this soluble form penetrates the pores of fiber during the dyeing process (Poulin, 2007). Within the fiber, leuco-indigotin converts back to insoluble indigotin when the dyed fabric is dried in the air.

In fresh indigo leaves, hydrolysis of indican to indoxyl occurs during the dyeing process when the leaves are crushed in water (Minami et al., 1997). So, it is indoxyl which penetrates the fiber during the dyeing of fresh indigo leaves. When fabric is dried in the air, oxygen in the air oxidizes indoxyl to form indigotin and with abundant oxygen indirubin would also form inside the fiber. Since water-soluble indoxyl is adsorbed in the fiber and not indigotin, dyeing with fresh indigo leaves does not require reduction process. Similar dyeing method can be apply in indigo leaf powder dyeing since it contains indigotin which was made during the grinding process and also indican which still exists in the undamaged sections of the leaf within the powder. Therefore, both reduction and nonreduction dyeing method can be applied when dyeing with indigo leaf powder.

There was also an occurrence of Y hue on the samples dyed in pH 12 dyebath. However, since K/S values of the samples were lower than 0.2, adsorption of pigments was very low when strong alkaline condition was used. It is highly probable that the expression of G (green) hue in the silk samples dyed by reduction of

Table 9. Rank comparison of K/S value, hue and the amount of pigments adsorbed in silk dyed by nonreduction dyeing of indigo leaf powder

Dyeing condition	Munsell hue	Rank of K/S value	Rank of amount of indigotin	Rank of amount of indirubin
50°C / pH 3	6.4B	3	1	4
50°C / pH 7	5.6B	2	2	3
50°C / pH 11	4.0RP	6	6	5
50°C / pH 12	2.0RP	7	6	5
90°C / pH 3	2.7RP	4	2	2
90°C / pH 7	1.7R	1	4	7
90°C / pH 11	8.8RP	5	5	1
90°C / pH 12	2.3Y	8	6	8

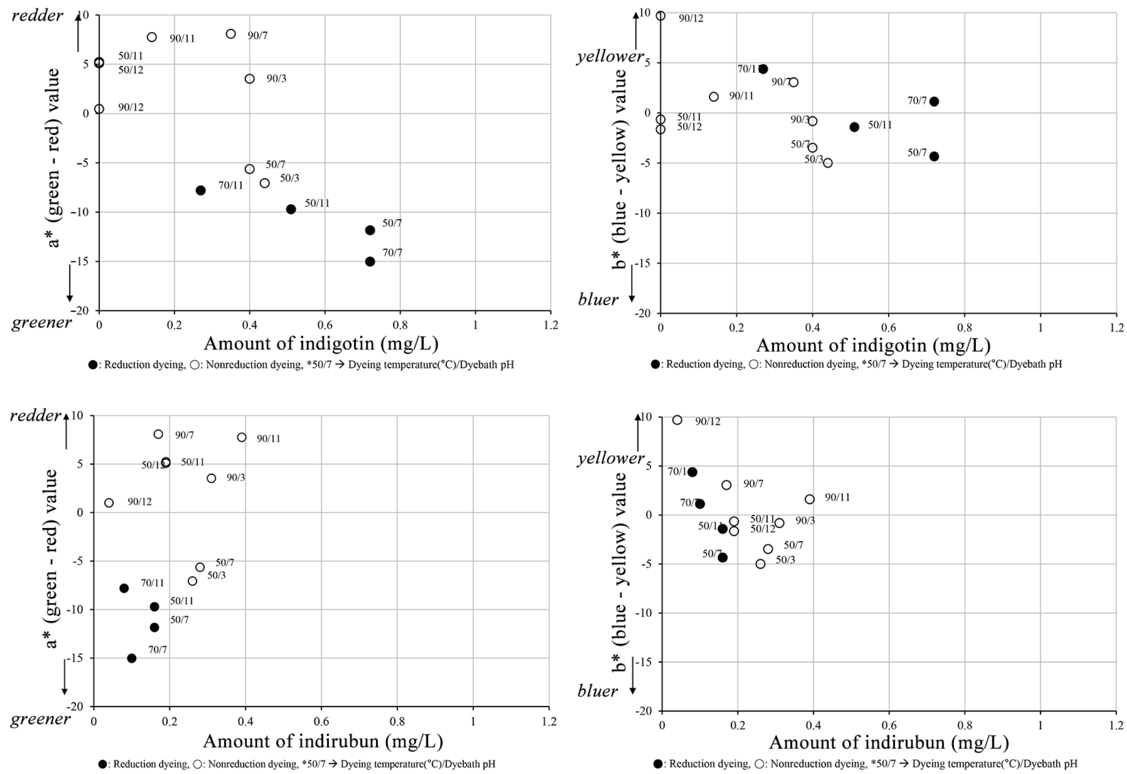


Fig. 6. Relationship between a* (green - red) and b* (blue - yellow) values of CIELAB and the amount of indigotin (a, b) and indirubin (c, d) pigments in the dyed silk samples.

indigo leaf powder was due to the effect of chlorophyll in the leaves considering that indigo leaf powder is the powder of whole dry leaves themselves (Shin et al., 2009b).

IV. Conclusions

The purpose of this research was to examine the dyeing behavior of indigo leaf powder in regard to how indigotin and indirubin pigments within the leaf powder contribute to dye adsorption and color of dyed fiber. The results of this study are summarized as follows.

1. By reduction dyeing using sodium hydrosulfite and alkali, silk samples dyed with indigo leaf powder showed BG (blue green) hue especially when silk was dyed at 70°C. 57.0% of the samples showed BG hue, and 1 sample showed G hue, indicating that 64.2% exhibited some degree of G hue in their colors.

2. By nonreduction dyeing, silk exhibited RP hue and some R hue while there were a few B (blue), PB, and P hue as well. 50.0% of the samples showed RP hue and 16.7% showed R hue, resulting in 66.7% of the samples that expressed some degrees of R hue in their colors.

3. Dye adsorption (K/S value) was overall low in all samples with the highest K/S value of 1.56 observed when silk was dyed at 90°C and pH 7 using nonreduction method. In reduction dyeing, the highest K/S value resulted when silk was dyed at 70°C and pH 10.

4. K/S value was directly related to the amount of indigotin but with much lesser degree related to the amount of indirubin when silk was dyed by reduction. In nonreduction dyeing, relationship between K/S value and the amount of indigotin and/or indirubin was not clear.

5. In reduction dyeing, B hue appeared when there was higher amount of both indigotin and indirubin adsorbed in silk. G hue appeared when there was least amount of indigotin and/or indirubin adsorbed in silk and when the K/S value was very low.

6. In nonreduction dyeing, higher amount of indigotin and low amount of indirubin resulted in B hue. R hue resulted when the amount of indigotin was low.

7. Indigo leaf powder produced red hue in silk through nonreduction dyeing using dyeing temperature above 70°C, or using 50°C with pH of dyebath higher than 9. Red hue was due to indirubin pigment in indigo plant and its active adsorption when silk was dyed by nonreduction method.

While natural indigo dyeing is most often related with B or PB colors, present investigation indicated that by varying the dyeing methods a wider range of colors were obtained by using indigo leaf powder and this phenomenon resulted from the preferential adsorption of indigotin and indirubin pigments by different dyeing conditions.

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