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Revolutionizing Indigo-Dyed Fabric Identification: A Novel Sulfonation Approach for Authenticity and Quality Assurance

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Abstract

This study explores a novel sulfonation method for analyzing blue-dyed fabrics containing reactive, direct and indigo dyes. The proposed method accurately distinguishes indigo fabrics by converting indigo blue into indigo carmine using concentrated H_2SO_4, resulting in distinct spectral analysis through UV-Visible spectrophotometry. Generally, UV-Visible spectroscopy shows absorption peak characteristics influenced by analyte and environmental properties, leading to broadening and wavelength shifts. Therefore, statistical analysis of peak characteristics, including center, centroid, Full Width at Half Maximum (FWHM), height and L/R ratio, was performed on the obtained spectra to facilitate comprehensive quality evaluation of indigo-dyed fabrics. The proposed method demonstrated good precision and accuracy, with %RSD values indicating excellent repeatability and reproducibility, contingent on consistent sample preparation, narrow analyte peaks and minimized background signals. Furthermore, this method was applied to analyze the collected indigo-dyed fabrics from Sakon Nakhon Province, revealing the center value (611.79 nm) as a precise marker for indigo dye, with a narrow control range (607.02 - 616.56 nm) and a low coefficient of variation (CV) of 0.26 %. Moreover, centroid, L/R ratio and FWHM significantly contributed to fabric classification and quality assessment. This research offers valuable insights to improve indigo fabric production in Sakon Nakhon Province, leading to more reliable authentication and quality control of the natural indigo-dyed industry.

Keywords: Indigo dyed fabric, Indigo carmine, Indigo blue, Sulfonation, Peak characteristics, Reactive dye, Direct dye

Introduction

The majority of textiles worldwide are composed of cellulose fibers, including cotton, hemp, bamboo, linen and rayon [1,2]. Dyeing these fibers for blue hues primarily employs 3 categories: Direct, reactive, and vat dyes. Each type possesses distinct dyeing processes and mechanisms for color attachment to the fiber. Consumer awareness regarding the textile industry environmental impact has surged in recent years [3,4]. Consequently, interest in naturally dyed textiles, particularly indigo-dyed fabrics, sourced from local producers has escalated [5]. Natural indigo-dyed fabric refers to textiles colored with the natural indigo dye extracted from the leaves of the Indigofera plant [6,7]. This centuries-old practice is renowned for producing vibrant, deep blue shades with distinctive characteristics [3].

The natural indigo-dyed fabric holds significant value and global recognition, with Sakon Nakhon Province being renowned for its expertise in producing such textiles [8]. However, the deceptive use of unrelated colors for dyeing, known as color mislabeling, significantly undermines the integrity of natural indigo-dyed fabrics. This deceptive practice can erode consumer trust, resulting in dissatisfaction and a loss of confidence in authentic products [9]. Building consumer confidence involves employing scientific methods to examine and verify distinctions between natural indigo and alternative blue dyes, ensuring the quality and authenticity of the products.

Identifying various dye types in textiles, particularly those present in indigo-dyed fabrics, relies on sophisticated instruments deeply rooted in the field of textile chemistry. UV-Vis spectrophotometry plays a pivotal role in scrutinizing how dye molecules absorb light across both ultraviolet and visible spectrums, thereby facilitating the differentiation of dyed fabrics [10-12]. High-performance liquid chromatography (HPLC) within chromatography efficiently separates dye components. Simultaneously, microscopy provides intricate insights into the structure and dispersion of dye particles within fibers. Fourier transform infrared spectroscopy (FTIR) proves instrumental in distinguishing the functional groups of each dye type [13,14]. Additionally, mass spectrometry ensures an accurate analysis of dye molecular structures [15]. However, the choice of a specific method depends on factors such as the type of dye under consideration, the desired level of detail and the available resources.

Identifying indigo blue in fabrics through sulfonation exploits a captivating chemical principle. Concentrated sulfuric acid, a potent oxidizing agent, achieves 2 crucial effects. Firstly, it aggressively attacks and cleaves the cellulose fibers in the fabric, transforming them into a water-soluble cellulose sulfate solution [16,17]. This step serves dual purposes: Exposing dye molecules for analysis and providing insights into the composition of fabric and potential adulteration with synthetic fibers. Secondly, concentrated sulfuric acid undergoes a unique reaction with indigo blue, the primary dye in these fabrics, converting it into a structurally similar compound called indigo carmine [11,18]. Importantly, unlike indigo blue, indigo carmine is readily soluble in water, making it easily quantifiable using UV-Visible spectrophotometry [19]. Moreover, UV-Visible peak analysis encounters challenges due to broader peak of band spectrum and susceptibility to solvent matrix interference, complicating precise identification of indigo carmine through conventional methods [41,42]. However, the statistical methods were employed in this research for integrating the UV-Visible peak analysis of indigo carmine, enhancing differentiation despite solvent matrix interferences [39,40]. Therefore, this research aims to use sulfonation to assess the quality of naturally indigo-dyed fabrics in Sakon Nakhon Province, contributing to decision-making, transparency, sustainable dyeing practices and empowering local communities by providing quality control tools for potential market benefits.

Materials and methods

Materials and chemicals

Cotton yarn with a count of 22/40 utilized was from Thai Spinning Company Limited (Thailand). The blue reactive dyes (RD 1 and RD 2) used were Indafix brand (KCB Textile Company Limited, Thailand). The blue direct dye 1 (DD 1) and commercial indigo dye 2 (CID 2) were obtained from Samphao Brand (Siwasumpan Company Limited, Thailand). The blue direct dye 2 (DD2) used was from Thep Phanom brand (Thailand). The commercial indigo dye 1 (CID 1) was purchased from Lazada (Thailand). The natural indigo dye (ND) was supplied by the Ban Don Koi weaving group in Phanna Nikhom District, Thailand. Sodium dithionite and calcium hydroxide were bought from Loba Chemie (India). Dimethyl sulfoxide (DMSO) and sulfuric acid (98 %w/w) were obtained from Carlo Erba (Italy). Indigo blue (IB) was purchased from Acros Organics (Belgium). Calcium hydroxide was bought from Loba Chemie (India). The 51 natural indigo-dyed fabric samples were supplied by the producer in Sakon Nakhon Province.

Instrumentation

A UV-Vis spectrophotometer employed to analyze the spectrum of the solution of blue-dyed fabrics was the Go Direct SpectroVis Plus model from Vernier Science Education (USA). The color parameters of the blue-dyed fabrics were measured using the MiniScan EZ 4000 portable spectrophotometer from HunterLab (USA).

The process of dyeing cotton with indigo dyes using a chemical reducing agent

The synthetic indigo dyeing was adapted from John and Angelini [20]. The cotton fabrics dyed with IB, CID 1 and CID 2 were named FIBC, FCID 1 and FCID 2, respectively. The process began with preparing a dye bath by dissolving 2 g of indigo dye, 5 g of calcium hydroxide and 6 g of sodium dithionite in 500 mL of warm water. The mixture was mixed by stirring until it acquired a characteristic yellowish-green color. Clean cotton yarn (10 g) was soaked in the dye bath for 10 min to ensure thorough dye absorption. Afterward, the yarn was squeezed and removed from the dye bath. Exposure to air induced the oxidation of the colorless leuco indigo in the fibers back into the vibrant indigo blue pigment, thereby permanently coloring the cellulose. The repetition of the dyeing cycle 5 times enhanced the resulting deep blue shade. Finally, the dyed fibers were thoroughly rinsed with clean water to remove any residual dye or chemicals, and a mild detergent wash further ensured their cleanliness. Once dry, these naturally indigo-dyed fibers were ready for further analysis. For the chemical reducing process used with the natural dye (ND), 80 g of indigo paste was used in the dye batch, following the same procedure as for other indigo dyes. The ND-dyed cotton fabric was labeled FNDC.

The process of dyeing cotton with natural indigo dye using a natural reducing agent

The process of natural indigo dyeing was demonstrated by Sunanta and Srinathom [21]. It was provided by mixing 80 g of indigo paste, 250 mL of lye (pH 13 - 14) and 250 mL of tamarind juice in a container. The mixture was added with 50 g natural sugarcane sucrose. Afterward, the mixture underwent fermentation at 40 °C until the indigo blue was reduced to indigo white, typically taking 1 - 2 h and resulting in a yellowish-green color. The cotton yarn (10 g) was cleaned in water for 10 min and then gently squeezed it. The fibers were then plunged into the indigo dye solution, allowing the indigo white to bind. Upon exposure to air, the indigo white oxidized and re-formed into indigo blue, permanently attaching to the fibers. This process imparted various shades of blue onto the cellulose fibers. The dyed-cotton yarn was repeated the dyeing process 5 times to achieve the dark blue shades. Finally, the dyed-cotton yarn was rinsed with clean water 3 times. Afterward, it was washed with detergent and dried prior to determination process. The cotton fabrics dyed with ND using the natural reducing agent was named FNDN.

The process of cotton dyeing with reactive dyes

The cotton yarn dyeing process with reactive dye was demonstrated by the KCB Textile Company Limited (Thailand) [22]. The cotton yarn (10 g) was pre-washed in warm water to remove impurities. Meanwhile, 5 g of reactive blue dye and 5 g of sodium chloride (NaCl) were accurately measured and dissolved in 500 mL of warm water (40 - 50 °C) in a beaker, stirring until completely dissolved. The prepared dye solution was then carefully poured into the designated dyeing container. The 10 g cotton yarn was immersed in the dye bath for optimal color absorption and stirred continuously for 15 min, followed by a 45-min dwell time. Finally, the dyed yarn was rinsed thoroughly to remove excess dye, washed with a mild detergent in warm water and air-dried in a shaded area. The cotton fabrics dyed with RD 1 and RD 2 were named FRD 1 and FRD 2, respectively.

The process of cotton dyeing with direct dyes

The of dyeing process of blue direct dye was demonstrated Siwasumpan Company Limited (Thailand) [23]. It involved dissolving 5 g of dye and 15 g of NaCl in 1 L of hot water. Following this, 10 g of cotton yarn were introduced into the dye bath and simmered for 30 min. After simmering, the yarn was twisted to remove excess dye and allowed to air-dry. Subsequently, the fibers were washed with detergent to remove any remaining chemicals and rinsed with clean water until the rinse water ran clear. Finally, the dyed-cotton fabric was tested with the sulfonation method. The cotton fabrics dyed with DD 1 and DD 2 were named FDD 1 and FDD 2, respectively.

DMSO extraction method

Indigo blue analysis the blue dyed-yarn samples were prepared by extraction with dimethyl sulfoxide (DMSO). This method is recognized to extract indigo blue from sample [11]. The procedure involved weighing 0.10 g of the blue dyed-yarn sample into an extraction glass bottle, adding 10 mL of DMSO and sonicating in an ultrasonic bath for 20 min. Subsequently, the extract solution was separated from the fiber. Afterward, the spectrum of the extract solution was analyzed using a UV-Visible spectrophotometer to characterize each type of blue dye present in the cotton yarn. Solutions with absorbance values above 0.80 were diluted with distilled water to bring them within the optimal range for analysis [19]. The resulting spectra were then used to identify and characterize the different types of blue dyes present.

Sulfonation method

The blue dyed-yarn samples were prepared by digesting with sulfuric acid (98 %w/v) [19]. The procedure was performed by weighing 0.10 the blue dyed-yarn sample into Erlenmeyer flask, added 5.0 mL of concentrated sulfuric acid (98 %w/w), and incubated in the fume hood for 10 min. After that, the digestant was poured into cold water in a beaker. The cool digestant solution wad added into a volumetric flask and adjusted the volume to 100 mL with distilled water. The sample solution was analyzed the spectrum with a UV-Visible spectrophotometer for characterizing each kind of blue dye which was dyed in the cotton yarn. The solutions with absorbance values above 0.80 were diluted with distilled water to bring them within the optimal range for analysis. The resulting spectra were then used to identify and characterize the different types of blue dyes present.

The analysis of 51 indigo dyed-fabric samples which were collected from the producers in Sakon Nakhon Province were similarly prepared with the sulfonation method. The spectrum of each indigo-dyed fabric sample was analyzed the peak characteristics. The data was provided the control chat for evaluating the quality of indigo-dyed fabric samples.

Method validation

The precision of the proposed method was evaluated using tests for repeatability and reproducibility. Repeatability was determined by preparing 10 replicates of a specific indigo-dyed fabric sample using the sulfonation method and recording their absorption spectra. Subsequently, reproducibility was assessed by creating 5 replicates of the sample using the sulfonation method on 3 different days. The relative standard deviation (RSD) was calculated for each measurement set to ascertain both precision parameters. The accuracy of the proposed method was assessed in by applying the sulfonation method to indigo carmine and indigo blue standards. The experiment involved treating samples as follows: A = 0.10 g cotton yarn, B = 23.3 mg (5 µmol) indigo carmine standard, C = 13.1 mg (5 µmol) indigo blue standard, D = 0.10 g cotton yarn with 23.3 mg indigo carmine standard and E = 0.10 g cotton yarn with 13.1 mg indigo blue standard. All samples underwent treatment using the sulfonation method, and their spectra were determined using

UV-Visible spectrophotometry to analyze the peak characteristics of indigo carmine. The peak characteristics of indigo carmine from all treatments were used to evaluate the accuracy of the data from the collected indigo-dyed fabrics. Furthermore, the presence of indigo carmine in the sample solution was confirmed by adjusting to basic conditions. This was achieved by transferring 2 mL of the sample solution into a test tube and adding 8 mL of 2 M NaOH. If indigo carmine is present, the solution will change to a yellow color [43,44].

Color analysis

The CIELAB color space method thoroughly evaluates blue-dyed fabric colors. Lightness (L^*) gauges brightness, with higher values indicating lighter shades. The green-red axis (a^*) and blue-yellow axis (b^*) reveal color components, respectively. Chroma (C^*) measures color intensity, higher values indicating vividness. Hue angle (h^*) identifies specific color tones. The MiniScan EZ 4000 portable spectrophotometer from HunterLab was used to analyze these parameters in blue-dyed fabric samples.

Spectrum characterization

The UV-Visible spectrum of a solution of blue-dyed fabric samples was analyzed using OriginPro software version 2019. Various technical terms relevant to spectrum characterization were employed, including center, centroid, FWHM, L/R ratio and peak height [24-26]. The center and centroid parameters helped identify the dominant wavelength, with center being suitable for symmetrical peaks and centroid offering greater accuracy for broader or asymmetrical ones. FWHM represents the width of a peak at half its maximum intensity, facilitated the differentiation of overlapping peaks and the estimation of peak purity. The L/R ratio (left half width/right half width of peak ratio) assessed peak symmetry, with values closer to 1 indicating higher symmetry. Peak height, denoting the maximum absorbance value, enabled the comparison of peak intensities.

The integration of these technical terms allowed for a comprehensive characterization and differentiation of peaks within UV-Visible spectrum of the blue-dyed fabric sample solution based on their position, width, symmetry and intensity. However, thorough spectrum characterization requires considering the specific application, individual peak characteristics and visual inspection.

Data analysis

The integration of spectrum was carried out by using OriginPro Version 2019 (OriginLab Corporation). The data analysis was carried out using SPSS version 19.0 (SPSS Inc.). For comparing the peak characteristics of different blue-dyed fabrics, the 1-way analysis of variance (1-way ANOVA) was used, followed by the Duncan's multiple comparison test at $p < 0.05$.

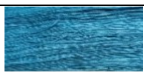
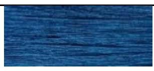
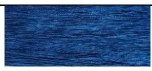
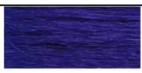
Results and discussion

Characterization of blue dyed-fabric with colorimetry

The CIELAB color space was used to scrutinize the color characteristics of a diverse array of blue-dyed fabrics, evaluating parameters such as lightness (L^*), the green-red axis (a^*), the blue-yellow axis (b^*), chroma (C^*) and hue angle (h^*) (**Table 1**). Noteworthy findings emerged across these parameters, revealing significant variations among dyed fabrics. FNDC exhibited the highest L^* value (49.56, indicative of relative lightness), while FRD 2 displayed the lowest (15.99). Generally, indigo vat-dyed fabrics demonstrated higher L^* values compared to reactive and direct-dyed fabrics. Concerning the a^*, fabrics, excluding FRD 2, clustered around 0, indicating a balanced presence of green and red hues. FRD 2 exhibited a positive a^* value (4.76), signifying a slight shift towards redness. Significant differences were noted in the b^*, with FDD 1 presenting the most negative b^* value (–30.06, denoting intense blue color). Indigo vat-dyed fabrics typically exhibited lower b^* values, indicating stronger blue chroma. The C^* values ranged from 19.26 to 27.14, indicating moderate to high saturation. The h^* values clustered around 260 - 270 °C, highlighting a dominant blue hue for most fabrics [27].

The results highlight the influence of dyeing methods on fabric color. Indigo-dyed fabrics generally have lighter shades and a more prominent blue color [28,29]. The fabrics dyed with indigo showed unique characteristics, possibly due to different dye formulations or dyeing processes [30]. Meanwhile, the reactive and direct dyed fabrics have distinct color profiles, indicating variations in color components or dyeing conditions. Despite these distinctions, all blue-dyed fabrics share a similar blue tone [29]. Categorizing each type of blue-dyed fabric cannot depend solely on colorimetry but requires integrating data from other tools [27].

Table 1 The color parameters of each blue dyed-fabrics.

Kinds of dye	**Dyed fabrics**	**Color parameters**				
		L*	**a^***	**b^***	**C^***	**h**
FIBC		23.51 ± 1.23^{c}	-0.011 ± 0.001^{e}	-20.15 ± 0.46^{de}	20.16 ± 0.45^{ab}	269.00 ± 0.92^{d}
FNDC		49.56 ± 1.27^{e}	-3.71 ± 0.08^{a}	-18.90 ± 0.69^{f}	19.26 ± 0.68^{a}	258.88 ± 0.52^{a}
FNDN		23.53 ± 1.14^{c}	-0.32 ± 0.01^{d}	-22.35 ± 0.20^{c}	22.35 ± 0.20^{c}	269.71 ± 0.70^{d}
FCID 1		48.58 ± 0.84^{e}	-3.48 ± 0.03^{b}	-20.82 ± 0.57^{d}	21.11 ± 0.56^{b}	260.50 ± 0.23^{b}
FCID 2		21.37 ± 0.30^{bc}	-0.011 ± 0.002^{e}	-21.21 ± 0.29^{cd}	21.21 ± 0.29^{bc}	270.02 ± 0.23^{d}
FRD 1		19.75 ± 0.68^{a}	-1.20 ± 0.07^{c}	-19.36 ± 0.36^{df}	19.39 ± 0.37^{a}	266.46 ± 0.16^{c}
FRD 2		15.99 ± 0.49^{a}	4.76 ± 0.20^{g}	-18.34 ± 0.49^{f}	18.95 ± 0.52^{a}	284.54 ± 0.36^{g}
FDD 1		32.79 ± 0.71^{d}	0.96 ± 0.02^{f}	-30.06 ± 0.54^{a}	30.07 ± 0.55^{e}	271.54 ± 0.29^{e}
FDD 2		19.99 ± 0.56^{b}	5.30 ± 0.10^{h}	-26.61 ± 0.26^{b}	27.14 ± 0.27^{d}	281.27 ± 0.15^{f}

Note: The letters a, b, c, d, e, f and g indicate statistically significant differences in column ($p < 0.05$).

Characterization of blue dyed-fabric with DMSO extraction method

DMSO extraction method is a recognized method for preparing indigo-dyed fabric for determining indigo blue with UV-Visible spectrophotometry and other methods [11,32]. The results showed that the color of the extract solutions of all blue-dyed samples were appeared similar blue shade color (**Figure 1**). This finding is difficult to classify the types of blue dye by observing with eyes. The analysis by UV-Visible spectrophotometry revealed that reactive and direct dyed fabrics exhibit broad peaks within the 500 - 650 nm wavelength range, lacking clear maximum absorption colors [12]. In contrast, indigo-dyed fabrics display narrow peaks centered at 618 nm, suggesting a deep blue color attributed to a sharp peak of indigo blue [31].

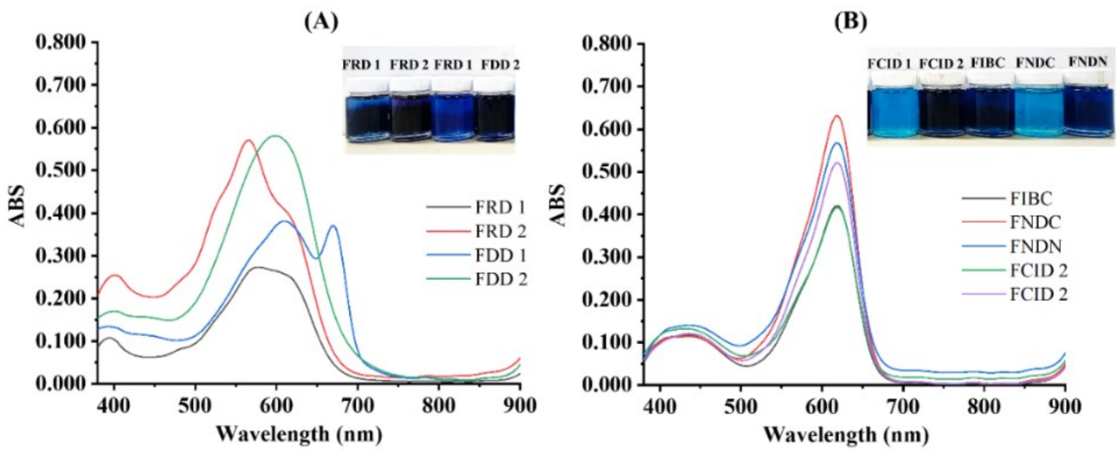


Figure 1 Spectrum of blue-dyed fabric solutions prepared by extracting with DMSO: (A) reactive and direct dyed fabrics and (B) indigo-dyed fabrics.

The analysis of spectra from blue-dyed fabrics, extracted using DMSO (**Table 2**), revealed significant variations in color characteristics associated with different dyeing methods. The indigo-dyed fabrics (FIBC, FNDC, FNDN, FCID 1 and FCID 2) statistically demonstrate higher center and centroid values around 618 nm (indigo peak in DMSO) compared to reactive and direct-dyed fabrics, excluding FDD peak 2 ($p < 0.05$).

Examination of the indigo blue peak revealed several noteworthy characteristics. The peak exhibited a broad absorption band ranging from approximately 510 to 680 nm with asymmetrical sides. Additionally, there was an elevation in the baseline ranging from 380 to 510 nm compared to the baseline ranging from 680 to 900 nm. This uneven baseline, it was combined with matrix effects in the sample solution. This can affect the distribution of the indigo blue peak and hinder the precise determination of the peak width at its base [42]. Therefore, the FWHM values was utilized to characterize the distribution of the indigo blue peak, providing a reliable measure of peak width even in the presence of baseline variations.

This characteristic enhances their effectiveness in accurately pinpointing the indigo peak, supported by their lower FWHM values. The right-skewed L/R ratios observed in indigo fabrics contrast with the left-skewed ratios found in others, and height values do not impact parameters. These finding indicated that the analysis of peak characteristics could be used to classify of blue dyes in the extract solution. The statistical data were carried out to evaluate the potential of this method for identifying the indigo blue in the blue-dyed fabric samples. However, the analysis of indigo blue with this method has several important requirements. First, all samples must be prepared under identical conditions. Second, the indigo blue peak must be narrow absorption band, with an intensity exceeding the baseline. Finally, minimal interference from sample matrices is crucial. While DMSO effectively emphasizes color differences, and aiding in fabric type identification [11,33]. It is crucial to consider factors such as its cost, potential toxicity and limitations in dye identification within the analytical process.

Table 2 Characteristics of the spectrum of blue dyed-fabrics prepared by extracting with DMSO.

Kinds of dyed-fabrics	Characteristics of peak				
	Center (nm)	Centroid (nm)	FWHM (nm)	L/R ratio	Height (Abs)
FRD 1	578.60 ± 0.87^{b}	583.35 ± 3.73^{b}	122.44 ± 2.41^{d}	0.84 ± 0.02^{a}	0.26 ± 0.01^{a}
FRD 2	565.35 ± 0.05^{a}	570.30 ± 1.53^{a}	126.09 ± 3.41^{e}	0.80 ± 0.05^{a}	0.55 ± 0.01^{g}
FDD 1 (peak 1)	610.15 ± 0.05^{c}	588.99 ± 0.63^{c}	105.19 ± 0.87^{c}	1.81 ± 0.02^{d,e}	0.37 ± 0.02^{c}
FDD 1 (peak 2)	669.94 ± 0.05^{e}	671.64 ± 0.09^{e}	43.68 ± 0.05^{a}	1.36 ± 0.01^{b}	0.36 ± 0.01^{b}
FDD 2	610.15 ± 0.05^{c}	588.99 ± 0.63^{c}	105.19 ± 0.87^{c}	1.81 ± 0.02^{c,d,e}	0.37 ± 0.01^{c}
FIBC	618.74 ± 0.05^{d}	604.89 ± 0.71^{d}	71.38 ± 0.93^{b}	1.75 ± 0.03^{c,d}	0.40 ± 0.01^{d}
FNDC	618.47 ± 0.40^{d}	604.34 ± 0.16^{d}	71.99 ± 0.80^{b}	1.77 ± 0.02^{c,d,e}	0.53 ± 0.01^{f}
FNDN	618.74 ± 0.05^{d}	603.53 ± 0.34^{d}	72.10 ± 1.12^{b}	1.82 ± 0.04^{e}	0.62 ± 0.01^{h}
FCID 1	618.74 ± 0.05^{d}	604.76 ± 0.16^{d}	71.08 ± 0.74^{b}	1.74 ± 0.02^{c}	0.39 ± 0.01^{d}
FCID 2	618.73 ± 0.04^{d}	604.31 ± 0.28^{d}	71.93 ± 0.72^{b}	1.77 ± 0.02^{c,d,e}	0.51 ± 0.01^{e}

Note: The letters a, b, c, d, e, f, g and h indicate statistically significant differences in column ($p < 0.05$).

Characterization of blue-dyed fabric with sulfonation method

The sulfonation method is different from the DMSO extraction method. It was developed for identifying the indigo-dyed fabric by derivatizing indigo blue to be indigo carmine [19]. **Figure 2** showed the distinct spectral signatures of different dye types. Fabrics dyed with reactive and direct dyes with reactive and direct dyes, prepared through the sulfonation method, commonly manifest a blue or violet color. This coloration is associated with the absorption of light, and spectrophotometric analysis typically indicates peak absorbance in the 544 - 595 nm range. Meanwhile, indigo-dyed fabrics exhibited peak absorbance at 610 - 614 nm, also within the blue range of indigo carmine [18,34].

Furthermore, the findings revealed that the solutions from indigo-dyed fabrics turned yellow upon NaOH treatment. On the other hand, the solutions from direct and reactive-dyed fabrics did not change to be a yellow color. Upon exposure to NaOH, indigo carmine undergoes deprotonation, transforming from its blue acidic form to a yellow salt form [43]. During this reaction, NaOH reacts with acidic hydrogen ions in the dye, giving water and the sodium salt of indigo carmine [44]. These outcomes authenticate the efficacy of the sulfonation method in converting indigo blue from indigo-dyed fabrics into indigo carmine, providing an alternative method to distinguish indigo-dyed fabrics from other blue-dyed fabrics.

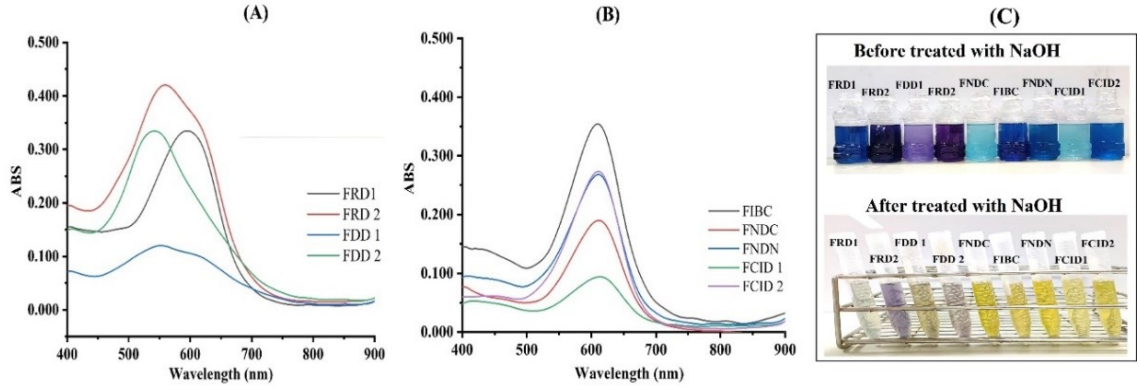


Figure 2 Spectrum of blue-dyed fabric solutions prepared using the sulfonation method: (A) reactive and direct dyed fabrics, (B) indigo carmine prepared from indigo-dyed fabrics, and (C) indigo carmine testing with NaOH.

Table 3 illuminates unique spectral characteristics associated with the indigo carmine peak in fabrics dyed using various indigo dye types, differentiating them from fabrics dyed with reactive and direct dyes. The indigo carmine maxima consistently exhibit a blue-shift across diverse sources (608.53 - 613.47 nm), distinctly differing ($p < 0.05$) from those observed with reactive and direct dyes (595.23 - 569.17 nm and 551.30 - 544.53 nm). This consistent blue shift signifies a hypsochromic shift in indigo dyes, compared to the more intricate structures of reactive and direct dyes [35,36].

The analysis revealed that there was no significant difference ($p > 0.05$) in the FWHM values of indigo carmine peaks across fabrics dyed with various types of indigo dye (ranging from 88.38 to 90.11 nm). However, the FWHM of the reactive and direct-dyed fabric solutions (ranging from 107.63 to 160.84 nm) was significantly broader than that of the indigo-dyed fabric solutions ($p < 0.05$). The asymmetry factor, calculated from the L/R ratio of the peaks, showed that the L/R ratio for indigo carmine peaks ranged from 1.15 to 1.28, representing a substantial increase compared to reactive and direct dyes (which ranged from 0.90 to 0.77). This signifies heightened asymmetry in the peak shape, characterized by a sharper ascent on the leading edge. These findings align with the intrinsic properties of indigo carmine and its distinct chromophore structure, distinguishing it from the complex structures found in reactive and direct dyes [18,35,36].

Accordingly, the sulfonation method shows promise in differentiating indigo-dyed fabrics from those dyed with reactive and direct dyes. The unique spectral attributes, such as a continual shift towards blue, reduced FWHM values and an elevated asymmetry factor, function as distinguishing indicators. The characterization of the indigo carmine peak can be used to identify indigo-dyed fabric. Furthermore, this method serves as a primary screening method through a simple reaction involving treatment with NaOH. It offers a cost-effective alternative to the DMSO extraction method. However, it requires proper conditions to minimize the effects of matrices in the solvent, similar to the DMSO extraction process.

Table 3 Characteristics of the spectrum of blue dyed-fabrics prepared with sulfonation method.

Kinds of dyed-fabrics	Characteristics of peak				
	Center (nm)	Centroid (nm)	FWHM (nm)	L/R ratio	Height (nm)
FRD 1	595.23 ± 0.40^{d}	601.36 ± 0.59^{c}	107.63 ± 1.42^{b}	0.90 ± 0.003^{b}	0.30 ± 0.018^{de}
FRD 2	569.17 ± 1.12^{c}	580.21 ± 0.84^{b}	146.49 ± 0.44^{c}	0.90 ± 0.003^{b}	0.36 ± 0.013^{f}
FDD 1	551.30 ± 0.87^{b}	583.71 ± 2.74^{b}	160.84 ± 6.59^{d}	0.77 ± 0.025^{a}	0.11 ± 0.003^{a}
FDD 2	544.53 ± 2.24^{a}	570.06 ± 0.85^{a}	140.57 ± 0.88^{c}	0.66 ± 0.034^{a}	0.30 ± 0.009^{de}
FIBC	610.20 ± 0.79^{e}	608.53 ± 0.79^{e}	90.01 ± 0.25^{a}	1.15 ± 0.032^{c}	0.33 ± 0.024^{ef}
FNDC	611.53 ± 0.75^{e}	607.41 ± 2.38^{e}	88.38 ± 1.55^{a}	1.24 ± 0.061^{cd}	0.18 ± 0.010^{b}
FNDN	611.03 ± 0.40^{e}	607.19 ± 1.35^{e}	89.56 ± 1.63^{a}	1.21 ± 0.032^{cd}	0.26 ± 0.007^{c}
FCID 1	613.47 ± 0.81^{e}	608.96 ± 1.14^{e}	90.11 ± 2.78^{a}	1.24 ± 0.061^{cd}	0.071 ± 0.0005^{a}
FCID 2	610.80 ± 1.20^{e}	604.29 ± 0.15^{e}	89.55 ± 0.01^{a}	1.28 ± 0.004^{d}	0.27 ± 0.010^{cd}

Note: The letters a, b, c, d, e and f indicate statistically significant differences in column ($p < 0.05$).

Method validation of the proposed method

The precision assessment of the sulfonation method for analyzing indigo-dyed fabric samples involved evaluating various peak characteristics and calculating their relative standard deviation (%RSD). **Table 4** presents results from both intra-day and inter-day analyses, including repeatability and reproducibility tests. The FWHM values were 100.76 ± 4.30 nm for intra-day and 102.59 ± 1.62 nm for inter-day analyses. The %RSD values for repeatability and reproducibility tests were 4.27 and 1.58 %, respectively. The center and centroid values demonstrated high precision, with %RSD values ranging from 0.10 to 0.87 %. Similarly, the R/L ratio and height measurements exhibited acceptable %RSD values, indicating good repeatability and reproducibility. These consistent and reliable findings highlight the reliability of the sulfonation method in precisely characterizing indigo carmine peaks from the indigo-dye fabric sample.

Table 4 Precision of the sulfonation method for analyzing the indigo-dye fabric sample.

Peak characteristics	Values		Precision (%RSD)	
	Intra-day analysis	Inter-day analysis	Repeatability	Reproductivity
FWHM (nm)	100.76 ± 4.30	102.59 ± 1.62	4.27	1.58
Center (nm)	613.05 ± 0.62	613.34 ± 0.95	0.10	0.15
Centroid (nm)	602.07 ± 0.93	608.05 ± 5.27	0.15	0.87
R/L ratio	134.35 ± 6.51	131.75 ± 2.33	4.84	1.77
Height (abs)	0.17 ± 0.0081	0.17 ± 0.0080	4.76	4.97

Figure 3 shows the spectral analysis of indigo carmine derived through the sulfonation method across various treatments (A = cotton yarn, B = indigo carmine standard, C = indigo blue standard, D = cotton yarn spiked with indigo carmine standard, E = cotton yarn spiked with indigo blue standard). It was observed that all treatments exhibited consistent spectra, emphasizing the reliability of the proposed method. Treatment with NaOH resulted in a yellow color in all solutions, confirming the successful

conversion of indigo blue to indigo carmine. This finding highlights the efficacy of the proposed method even with complex matrices in the solution [41,42].

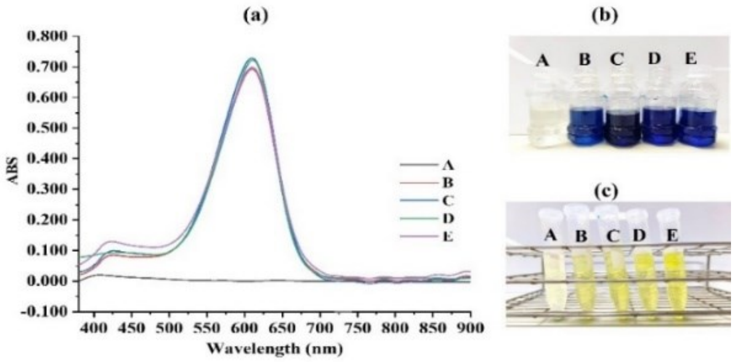


Figure 3 Accuracy verification of the proposed method: (a) spectra of indigo carmine derived from different substrates prepared using the sulfonation method, (b) indigo carmine solution before treatment with NaOH, and (c) indigo carmine solution after treatment with NaOH.

An intriguing observation from **Table 5** is the comparison of the FWHM of the indigo carmine standard (treatment A) peak with those resulting from different treatments (B, C and D). The observed discrepancies in peak width underscore the influence of matrix effects, a well-understood phenomenon in UV-Visible spectrophotometry. The sulfonation process, which involves treating cellulose with concentrated sulfuric acid, introduces sulfonic acid groups into the cellulose polymer chains. This chemical modification creates a more complex sample matrix during analysis, potentially broadening the peaks of the obtained indigo carmine compared to the pure standard. Despite the broadening effect, the measured center, R/L ratio and height values of indigo carmine peaks across all treatments remain statistically similar ($p > 0.05$).

Furthermore, the comparison of peak attributes from treatments B, C, D and E to those of indigo-dyed fabrics (**Table 6**), all falling within acceptable control ranges, further supports the method's reliability. However, variations in peak center shifts and width among fabric samples underscore the necessity of meticulous statistical evaluation for effective screening and quality assessment. To enhance accuracy, especially for suspected non-authentic fabrics, leveraging complementary techniques alongside UV-Visible spectrophotometry is advisable, ensuring comprehensive and reliable testing outcomes consistent with spectroscopic analysis principles [39,40].

Table 5 Accuracy of the sulfonation method for analyzing the indigo-dye fabric sample.

Treatments	Peak characteristics				
	FWHM (nm)	Center (nm)	R/L ratio	Center (nm)	Height (abs)
A	n.d.	n.d.	n.d.	n.d.	n.d.
B	86.69 ± 0.42^a	610.60 ± 0.20^a	1.25 ± 0.011^a	603.36 ± 1.04^a	0.70 ± 0.021^a
C	89.26 ± 0.53^b	610.14 ± 0.046^a	1.27 ± 0.021^a	604.23 ± 0.041^a	0.72 ± 0.009^a
D	89.76 ± 0.71^b	610.15 ± 0.036^a	1.27 ± 0.017^a	605.35 ± 0.091^a	0.71 ± 0.007^a
E	89.26 ± 0.53^b	610.14 ± 0.046^a	1.28 ± 0.015^a	605.23 ± 0.041^a	0.69 ± 0.010^a

Note: The letters a and b indicate statistically significant differences in column ($p < 0.05$) and n.d. = not detected.

Characterization of indigo dyed-fabric fabric samples

The examination of peak characteristics in meticulously prepared indigo-dyed fabrics by utilizing sulfonation and UV-Visible spectrophotometry was applied to study the quality of natural indigo-dyed fabrics (n = 51) from the producer in Skon Nakhon Province (**Table 6**). The results showed that the center value (611.79 nm) in the red spectrum is a precise marker for indigo dye, highlighted by its consistency shown through a narrow control range (607.02 - 616.56 nm) and low CV (0.26 %). This parameter distinguishes indigo colors and identifies specific types, such as indigo carmine (around 610 nm), from other blue dyes. However, slight variations in the center value may arise from differences in fabric matrices and dyeing processes [5,19].

The closely aligned centroid (609.31 nm) is highly sensitive to FWHM but maintains a narrow control range (601.11 - 617.52 nm) and low CV (0.45 %). This alignment reflects both color purity and adherence to rigorous analytical principles. The L/R ratio (1.17) suggests peak symmetry, further supported by the control range (0.98 - 1.36). However, a higher CV (5.45 %) indicates some variability, highlighting the importance of considering peak shape symmetry in analysis.

The crucial FWHM (94.71 nm) exhibits variability within the control range (84.28 - 105.13 nm) but boasts a low CV (3.67 %), emphasizing consistent width measurement. The independence of the method from color intensity is demonstrated by the fact that peak height does not affect the values of center, centroid, FWHM, or L/R ratio. This comprehensive approach empowers the classification and evaluation of indigo-dyed fabrics, providing valuable quality assessment across various color intensities.

Table 6 The statistical analysis of peak characteristics obtained from the spectra of indigo-dyed fabric samples.

Statistics parameters	**Characteristics of peak (n = 51)**			
	Center (nm)	**Centroid (nm)**	**FWHM (nm)**	**L/R ratio**
Average value ($\bar{X}$)	611.79	609.31	94.71	1.17
Standard deviation (σ)	1.59	2.73	3.47	0.06
CV (%)	0.26	0.45	3.67	5.45
Control range				
-Lower values ($\bar{X} - 3\sigma$)	607.02	601.11	84.28	0.98
-Upper values ($\bar{X} + 3\sigma$)	616.56	617.52	105.13	1.36
Correlation with peak height (r)	0.652	0.650	0.066	0.416

The data obtained through spectrum analysis forms the foundation for quality control assessments. Peak characteristics, including center, centroid, L/R ratio, and FWHM, are meticulously analyzed, and control charts for each parameter are provided for a comprehensive evaluation [24-26,37]. **Figure 4** illustrates that most indigo-dyed fabric samples conform to the defined control limits for center, centroid, FWHM and L/R ratio. However, a small number of samples deviate from these standards, as shown in **Figure 5**, suggesting the possible use of alternative dye colors. This enhanced method, empowered by sulfonation, effectively serves as an initial classifier for distinguishing types of indigo-dyed fabrics from blue-dyed fabrics. For samples outside the control limits, further verification using other techniques such as FT-IR and HPLC will be carried out.

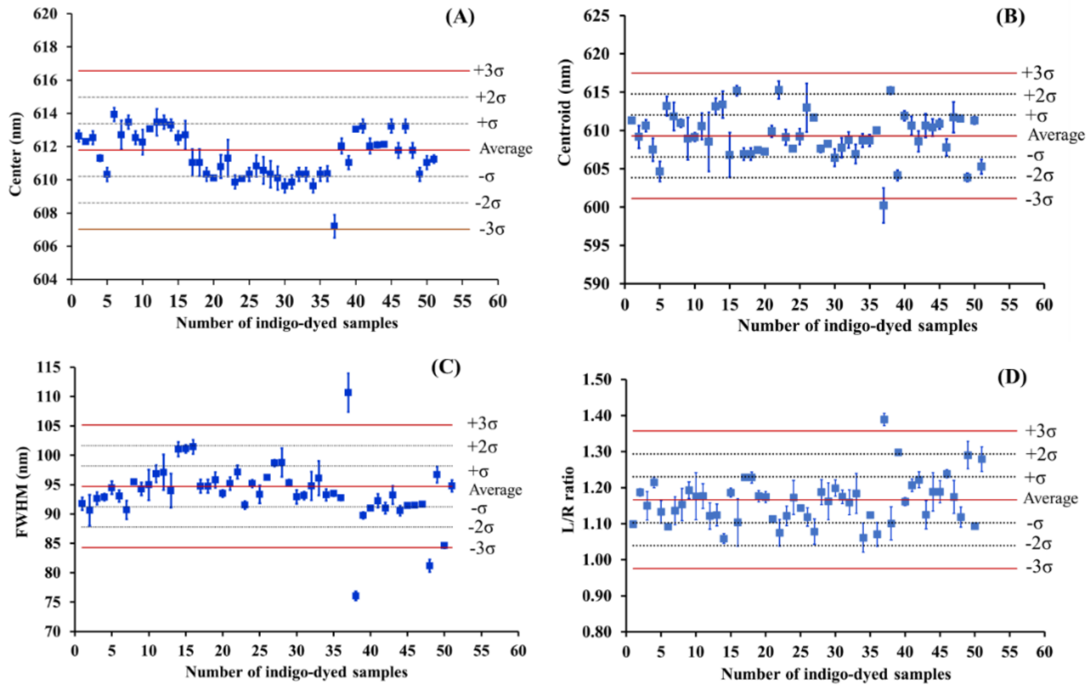


Figure 4 Control chart of (A) center, (B) centroid, (C) FWHM, and (D) L/R ratio of indigo-dyed fabric samples (n = 51).

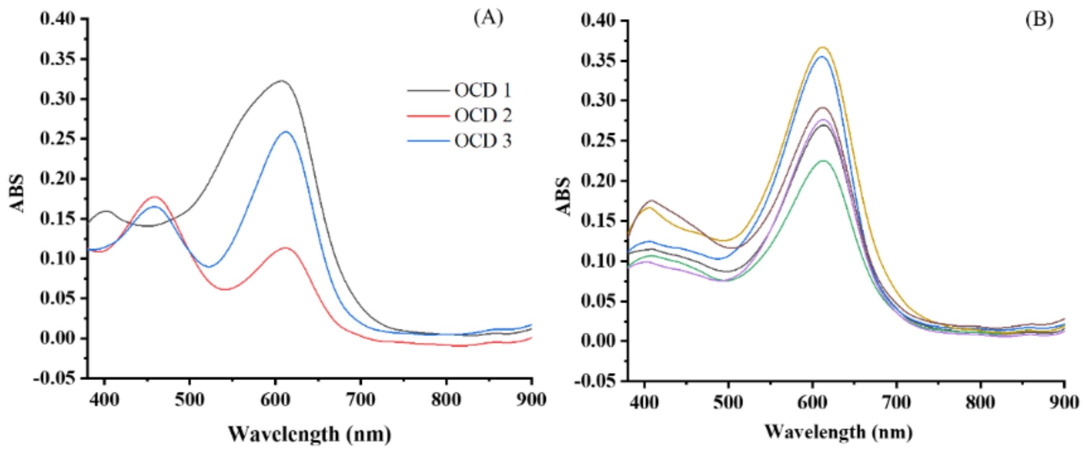


Figure 5 Spectrum of (A) the out-of-control value samples and (B) the in-control value samples.

Conclusions

The study introduces and evaluates a novel sulfonation technique tailored to differentiate blue-dyed fabrics containing reactive, direct and indigo dyes. This method efficiently derivatizes indigo blue into indigo carmine, leading to unique spectral features that are crucial for identifying indigo-dyed fabrics. UV-Visible spectrophotometry confirmed the effectiveness of this approach in spectral discrimination. The proposed method served as an initial quality control classifier, and statistical analysis of peak characteristics such as Center, Centroid, FWHM and L/R ratio established control charts for indigo fabrics, enabling comprehensive quality evaluation. The accuracy and reliability of this method depend on several factors, including consistent sample preparation, obtaining narrow and intense analyte peaks and minimizing background signals from sample matrices. Accordingly, the proposed method demonstrated good precision and accuracy. Deviations observed in some samples suggested the presence of alternative dyes, warranting further verification using complementary techniques. This research offers valuable insights into the production of indigo-dyed fabrics in Sakon Nakhon Province, providing promising methods for fabric authentication, provenance studies and quality control. The proposed method enhances authenticity, improves quality assurance and contributes to the overall success of the indigo-dyed fabric production chain.

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References

[1] V Gupta and S Gupta. *Cotton fiber and its sustainable alternatives*. Novel Sustainable Raw Material Alternatives for the Textiles and Fashion Industry. Springer Cham, Germany, 2023, p. 139-60.

[2] R Nayak, L Jajpura and A Khandual. *Traditional fibres for fashion and textiles: Associated problems and future sustainable fibres*. Sustainable Fibres for Fashion and Textile Manufacturing. Woodhead Publishing, Cambridge, 2023, p. 3-25.

[3] P Hopewell and S Harris. Blue dyed textiles in Early Iron Age Europe: accessible or exclusive? *Archaeol. Textil. Rev.* 2019; **61**, 24-37.

[4] C Gervais, MA Languille, S Reguer, C Garnier and M Gillet. Light and anoxia fading of Prussian blue dyed textiles. *Herit. Sci.* 2014; **2**, 26.

[5] N Tello-Burgos, AM López-Montes, J Ballesta-Claver, FJ Collado-Montero and MDRB García. Identification of indigo dye (*Indigofera tinctoria*) and its degradation products by separation and spectroscopic techniques on historic documents and textile fibers. *Stud. Conservat.* 2021; **66**, 7-22.

[6] AK Kabish, MT Abate, ZA Alemar and S Girmay. The importance of natural indigo dye and its revitalization and Ethiopian potential for indigo growing. *Adv. Mater. Sci. Eng.* 2023; **2023**, 2135014.

[7] FT Gbolarumi, KY Wong and ST Olohunde. Sustainability assessment in the textile and apparel industry: A review of recent studies. *In*: Proceedings of the 5th International Conference on Advanced Technology and Applied Sciences, Kuala Lumpur, Malaysia. 2020, p. 2-16.

[8] C Duangbubp, S Chantachon and N Pratumnet. Application of traditional knowledge to create indigo-dyed fabric products in Sakon Nakhon Province, Thailand. *Asia Pac. J. Multidisciplinary Res.* 2015; **3**, 6-10.

[9] P Upping and T Keson. Factors influencing the success of local products: A case study of Sakon Nakhon Province. *Univ. Thai Chamber Commerce J. Humanit. Soc. Sci.* 2019; **35**, 1-13.

[10] JJ Berzas, JR Flores, MJV Llerena and NR Fariñas. Spectrophotometric resolution of ternary mixtures of Tartrazine, Patent Blue V and Indigo Carmine in commercial products. *Anal. Chim. Acta* 1999; **391**, 353-64.

[11] VV Volkov, R Chelli, R Righini and CC Perry. Indigo chromophores and pigments: Structure and dynamics. *Dyes Pigments* 2020; **172**, 107761.

[12] D Mantzouris, I Karapanagiotis and C Panayiotou. Comparison of extraction methods for the analysis of *Indigofera tinctoria* and *Carthamus tinctorius* in textiles by high performance liquid chromatography. *Microchem. J.* 2014; **115**, 78-86.

[13] D Śmigiel-Kamińska, J Wąs-Gubała, P Stepnowski and J Kumirska. The identification of cotton fibers dyed with reactive dyes for forensic purposes. *Molecules* 2020; **25**, 5435.

[14] MD Hossain, MMR Khan and MZ Uddin. Fastness properties and color analysis of natural indigo dye and compatibility study of different natural reducing agents. *J. Polymer. Environ.* 2017; **25**, 1219-30.

[15] APS Crema, LDP Borges, GA Micke and NA Debacher. Degradation of indigo carmine in water induced by non-thermal plasma, ozone and hydrogen peroxide: A comparative study and by-product identification. *Chemosphere* 2020; **244**, 125502.

[16] J Zhang, N Jiang, Z Dang, TJ Elder and AJ Ragauskas. Oxidation and sulfonation of cellulosics. *Cellulose* 2008; **15**, 489-96.

[17] K Wang, J Jiang, X Liang, H Wu and J Xu. Direct conversion of cellulose to levulinic acid over multifunctional sulfonated humins in sulfolane-water solution. *ACS Sustain. Chem. Eng.* 2018; **6**, 15092-99.

[18] MC Pagnacco, JP Maksimović, NT Nikolić, DVB Bogdanović, MM Kragović, MD Stojmenović, SN Blagojević and JV Senćanski. Indigo carmine in a food dye: Spectroscopic characterization and determining its micro-concentration through the clock reaction. *Molecules* 2022; **27**, 4853.

[19] W Thosaikham and P Sakong. Indigo blue determination for quality evaluation by UV-Visible spectrophotometry method. *J. Sci. Tech. Mahasarakham Univ.* 2023; **42**, 20-30.

[20] P John and LG Angelini. *Indigo—Production and properties*. *In*: C Stevens, T Bechtold, A Manian and T Pham (Eds.). Handbook of natural colorants. John Wiley & Sons, New Jersey, 2023.

[21] C Sunanta and N Srinathom. 2021, Development of lye solution for natural indigo dyeing. Bachelor Degrees. Sakon Nakhon Ratjabhat University, Sakon Nakhon, Thailand.

[22] KCB Textile Company Limited, Available at: https://www.indafix.com/contact-us/, accessed February 2024.

[23] Siwasumpan Company Limited, Available at: https://www.sivasumpan.com/th, accessed February 2024.

[24] MJ Vowels, LM Vowels and ND Wood. Spectral and cross-spectral analysis - a tutorial for psychologists and social scientists. *Psychol. Meth.* 2021; **28**, 631-50.

[25] CT Chen, LM Chang and CH Loh. A review of spectral analysis for low-frequency transient vibrations. *J. Low. Freq. Noise Vib. Active Contr.* 2021; **40**, 656-71.

[26] MH Trauth. Spectral analysis in Quaternary sciences. *Quaternary Sci. Rev.* 2021; **270**, 107157.

[27] NT Burgos, A López-Montes, EM Valero, JL Nieves and MR Blanc. Characterization of the evolution of indigo blue by multispectral imaging. *Color Res. Appl.* 2022; **47**, 486-97.

[28] J Yang, S Chen and G Liu. Eco-friendly dual-chromophore functionalized polyvinylamine derivatives for clean and sustainable coloration of cotton fabric. *Dyes Pigments* 2022; **207**, 110792.

[29] H Iino, S Tokino, J Ryu, M Chen, M Suzuka and T Wakida. Change in color of dyed wool and nylon 6 fabrics by sputter etching. *J. Fiber Sci. Tech.* 1996; **52**, 213-9.

[30] N Meksi, M Kechida and MF Mhenni. New process of dyeing cotton textile by indigo with borohydride: Effect of the concentration of the reducing agent. *Int. J. Appl. Chem.* 2008; **4**, 1-14.

[31] E Frignani, V D'Eusanio, M Grandi, L Pigani and F Roncaglia. A continuous extraction protocol for the characterisation of a sustainably produced natural indigo pigment. *Life* 2024; **14**, 59.

[32] A Doménech-Carbó, S Holmwood, FD Turo, N Montoya, FM Valle-Algarra, HGM Edwards and MT Doménech-Carsbó. Composition and color of maya blue: Reexamination of literature data based on the dehydroindigo model. *J. Phys. Chem. C* 2019; **123**, 770-82.

[33] Z Ju, J Sun and Y Liu. Molecular structures and spectral properties of natural indigo and indirubin: Experimental and DFT studies. *Molecules* 2019; **24**, 3831.

[34] S Ammar, R Abdelhedi, C Flox, C Arias and E Brillas. Electrochemical degradation of the dye indigo carmine at boron-doped diamond anode for wastewaters remediation. *Environ. Chem. Lett.* 2006; **4**, 229-33.

[35] S Younis, HN Bhatti, SZ Bajwa, J Xu and S Jamil. Photodegradation of direct violet 51 dye using Bi_2 MoO_6/GO nanoflakes as promising solar light-driven photocatalyst. *Proc. Pakistan Acad. Sci. B* 2023; **60**, 113-24.

[36] PO Edlund, ED Lee, JD Henion and WL Budde. The determination of sulfonated azo dyes in municipal wastewater by ion spray liquid chromatography tandem mass spectrometry. *Biomed. Environm. Mass Spectrom.* 1989; **18**, 233-40.

[37] ÁG Aponte, MAL RamÍrez, YC Mora, JF Santa, MarÍn and RB Sierra. Cerium oxide nanoparticles for color removal of indigo carmine and methylene blue solutions. *AIMS Mater. Sci.* 2020; **7**, 468-85.

[38] H Zhang, H Yang, K Xie, A Hou and A Gao. Novel reactive dyes with intramolecular color matching combination containing different chromophores. *Dyes Pigments* 2018; **159**, 576-83.

[39] SG Muntean, Z Szabadai and L Halip. Investigation of aggregation behavior using computational methods and absorption spectra for trisazo direct dyes. *Struct. Chem.* 2016; **27**, 1049-59.

[40] M Reuschenbach, LL Hohrenk-Danzouma, TC Schmidt and G Renner. Development of a scoring parameter to characterize data quality of centroids in high-resolution mass spectra. *Anal. Bioanalytical Chem.* 2022; **414**, 6635-45.

[41] R Wahlen and C Wolff-Briche. Comparison of GC-ICP-MS and HPLC-ICP-MS for species-specific isotope dilution analysis of tributyltin in sediment after accelerated solvent extraction. *Anal. Bioanalytical Chem.* 2003; **377**, 140-8.

[42] L Fasolato, NA Andreani, RD Nardi, G Nalotto, L Serva, B Cardazzo, S Balzan, L Carraro, F Fontana and E Novelli. Spectrophotometric techniques for the characterization of strains involved in the blue pigmentation of food: Preliminary results. *Ital. J. Food Saf.* 2018; **7**, 6932.

[43] ME Ristea and O Zarnescu. Indigo carmine: Between necessity and concern. *J. Xenobiotics* 2023; **13**, 509-28.

[44] K Takeuchi and T Ibusuki. Quantitative determination of aqueous-phase ozone by chemiluminescence using indigo-5,5'-disulfonate. *Anal. Chem.* 1989; **61**, 619-23.