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Extraction of Indigo from Some *Isatis* species and Dyeing Standardization Using Low-technology Methods

Nazan Comlekcioglu[1*], Lale Efe[2] and Sengul Karaman[1]
[1]Biology Department; Kahramanmaras Sutcu Imam University; Kahramanmaras – Turkey. [2]Crop Fields Deparment; Kahramanmaras Sutcu Imam University; Kahramanmaras - Turkey

ABSTRACT

Fresh leaves of four Isatis species culture form of I. tinctoria L and wild forms of I. buschiana Schischkin, I. candolleana Boiss. (endemic) and I. tinctoria L. subsp. corymbosa. (Boiss.) were used for indigo production. Dyes were extracted by fermentation and hot water application. The extracted dyes were optimized with different pH and reducing agents. Results showed that the dye from hot water application produced the desired dying quality at pH 11. Reducing agent concentrations had no significant effect on color quality. Dark blue and blue colors were obtained from I. tinctoria and I. candolleana extracts although I. tinctoria subsp. corymbosa and I. buschiana produced mostly yellow-gray colors. Light, dry and wet rubbing fastness values varied between 3 and 3/4 while washing fastness was between 2 and 4/5. The highest indigo amounts were determined spectrophotometrically as 4.19 mg/g and 2.53 mg/g in I. tinctoria and I. candolleana, respectively. Results also showed that harvesting season was important for indigo production and the highest indigo amount was observed in mid-June.

Key words: *Isatis* spp., woad, indigo, natural dye, fermentation, fastness, spectrophotometry

INTRODUCTION

Most of the dyes used in textile industries are synthetic (Gilbert and Cook 2001) but there has been an increasing interest about natural dyes in fabric coloration. This is because of the stringent environmental standards imposed by many countries in a response to the toxic and allergic reactions associated with synthetic dyes (Kamel et al. 2005). Among the naturals dyes, indigo has been obtained from a variety of plant sources such as *Indigofera tinctoria*, *Polygonum tinctorium*, *Nereum tinctorium* and *Isatis tinctoria* (Gilbert and Cook 2001; Tozzi et al. 2005). However, once its chemical synthesis from aniline was established, it has been largely produced by chemical industry and almost superseded natural indigo (Bechtold et al. 2002; Puchalska et al. 2004). Natural indigo is produced by fermenting the leaves of indigo bearing plants. During the fermentation, indican in the leaves is hydrolized to form indoxyl and glucose by the action of endogenous β-glucosidase and subsequently oxidized to form indigo in contact with air (Song et al 2010). Although indigo could be produced from the plants in large quantities, the decrease in trade of the natural indigo has been mainly due to the lack of knowledge with regard to simple production of natural indigo from plant material and high production cost (Gilbert and Cook 2001; Bechtold et al. 2002). Beyond the technical approach of dyeing to achieve the displacement of synthetic with natural dyes, the requirement of modern dyehouse and dye manufacturers should be considered (Bechtold et al. 2003).

[*]Author for correspondence: noktem @ksu.edu.tr

Isatis tinctoria L. (woad, Brassicaceae), which is an ancient European dye and medicinal plant, has a long and well-documented history (Brattström et al. 2010; Han et al. 2011). Successful cultivation of *I. tinctoria* in mountain and marginal areas offers an alternative to traditionally grown crops (Spataro et al. 2007); therefore, *I. tinctoria* could be an important indigo source for natural dyeing applications. There are several reports on the extraction of indigo from *I. tinctoria* leaves (Balfour-Paul 1998; Stoker et al. 1998; Gilbert et al. 2004; Tozzi et al. 2005; Sales et al. 2006; Angelini et al. 2007; Rocha et al. 2011). Simple extraction method is required for an effective and ecological production of natural indigo (Bechtold et al. 2002). Detailed information is needed for a basic optimized and standardised extraction of indigo precursors and dye formation. The dye yield is usually low with traditional fermentation method.

The aim of this study was to develop an efficient and eco-friendly method for coloring textiles by using natural indigo and to determine the amount of indigo potentials in the plants. Hence, four different species of *Isatis* genus were cultivated and rosette leaves of the plants used as raw materials for extraction. Natural dyestuff extractions and seasonal indigo production from culture and native species of Isatis were tested and dyeing potentials were compared.

MATERIALS AND METHODS

Material

Fresh leaves of four *Isatis* species (culture form of *I. tinctoria* and wild forms of *I. buschiana, I. candolleana* (endemic) and *I. tinctoria* subsp. *corymbosa*) were used for indigo production. The seeds of *I. tinctoria* were provided from IPK-Institute for Plant Genetics and Crop Plant Research, Gatersleben, Germany and were grown in Kahramanmaras/Turkey. *I. candolleana, I. buschiana*, and *I. tinctoria* subsp. *corymbosa* were biennial, while *I. tinctoria* was annual in trial field. The other wild species were collected from South-East Mediterranean Region of Turkey. All of the specimens were identified according to the Flora of Turkey (Davis 1965). Cotton yarn was used as cellulose material for the dyeing experiments. Raw cotton yarn was soaked in 500 mL tap water for 1 h before the dyeing treatments.

Extraction of Indigo

Rosette leaves were harvested from the cultivated plants by cutting 10 cm from the ground they reached about 30 cm in the end of May and early June. Leaves (100 g) were washed and cut into small pieces of 1 cm^2. The dyestuff was then extracted by using 1000 mL of hot water (60, 70 or 80ºC) or fermentation (24 h). Two different pH (9.0 and 11.0) and four different reducer ($Na_2S_2O_4$, Sigma) concentrations (1, 5, 10 and 20 g l^{-1}) were tested in extraction experiments. All the experiments were repeated three times and mean values were used.

Fermentation Application

Plant materials (100 g) were fermented in 1000 mL water for 24 h in the dark and placed at the room temperature. The extraction liquor was divided into two equal volumes and the pH adjusted to 9.0 and 11.0 using 2 N NaOH (Merck) solution. Extraction liquor was aerated for 1 h by using a compressor to enable complete oxidation of the indigo precursors (Stoker et al. 1998; Chanayath et al. 2002).

Hot Water Application

The extraction was carried out at temperatures of 60, 70 or 80ºC for 10 min. The leaves were removed and the sample was rapidly cooled to 50ºC. Extraction medium was divided into two equal volumes and the pH adjusted to 9.0 and 11.0 with 2 N NaOH solutions. The extract was aerated by using a magnetic stirrer until the blue color development (Stoker et al. 1998; Balfour-Paul 1998).

Reduction Step

The pH adjusted dye solutions were equally divided into 100 mL volumes and then $Na_2S_2O_4$ (1, 5, 10 and 20 g L^{-1}) was added for reduction of indigo. After that the cotton yarns (1 g yarn/100 mL dye solution) were soaked in it and incubated for 60 min at 50ºC in water bath. During the dyeing process, stirring and aeration were performed manually every 15 min for 1 min. Finally, dyed cotton yarns were rinsed with tap water (approx. 500 mL) three times for the removal of excess unbound dyes.

Fastness Tests

Color fastness tests on dyed samples were carried out using ISO standard methods. The light fastness of the yarns was examined by using the

ISO/FDIS 105-B02. Then, color fading was evaluated by comparing each sample with standard blue scale. The washing fastness of the samples was also tested on the basis of ISO 105-C06:2010. Dry and wet rubbing fastness of the samples were tested using ISO 105-X12:2001and ISO 105-X16:2001 methods. Shade changes in washing and rubbing fastness were determined using a grey scale (Marks 1–5, 1 = poor, 5 = excellent).

Indigo Extraction and Sample Preparation for Spectrophotometry

Indigo content of leaves was extracted following Stoker et al. (1998) and Sales et al. (2006) with some modifications. The chopped leaves (approx. 1 cm^2 in size) were weighed (1.0 g) and placed in tubes containing 10 mL of distilled water. Then the tubes were immersed in a boiling water bath for 10 min and cooled rapidly in ice bath. The leaf pieces were removed and NaOH solution was added to adjust the pH at 11.0. Samples were then aerated for 30 sec and allowed to stand at room temperature (20-25^{o}C) for 1 h before acidification to pH 1.0-2.0 with HCl (Merck). After 30 min, 5.0 mL of ethyl acetate (Merck) was added to 1.0 mL aliquot of sample and the absorbances were measured at 600 nm. Finally, indigo concentrations were calculated by using the indigo calibration curve. Calibration curve was prepared by dissolving indigo standard (Sigma) in ethyl acetate (Sales et al. 2006). Experiments were repeated three times and mean values were used.

RESULTS AND DISCUSSION

The extraction of dye components from the plant materials was performed with hot water and fermentation without the addition of chemicals or solvents. The results on the effects of pH, fermentation and temperature levels on dyeing potentials of *Isatis* species by using 1 g l^{-1} reducing agent are shown in Table 1. The main colors obtained from *I. tinctoria*, *I. candolleana* and *I. tinctoria* subsp. *corymbosa* were dark blue, blue and gray-blue, respectively (Table 1).

Table 1 - Effects of temperature and pH on dyeing performances of *Isatis* species.

	I. tinctoria		***I. candolleana***		***I. tinctoria subsp. corymbosa***	
	pH 9	**pH 11**	**pH 9**	**pH 11**	**pH 9**	**pH 11**
60 ^{o}C						
70 ^{o}C						
80 ^{o}C						
Fermentation						

No blue color was obtained from *I. buschiana*, therefore this plant was not studied in further experiments. The desired blue colors were obtained from *I. tinctoria* in all hot water extraction treatments at pH 11.0; however, extraction with fermentation failed to give blue color. The success in hot water extraction could possibly be due to leaf epidermal wax melting. Blue colors were also observed in *I. candolleana* extraction at pH 11.0 using both hot water and fermentation. The extraction carried out at the 70 and 80^{o}C give similar blue colors with *I. tinctoria* and *I. candolleana*; therefore, the extractions at 70^{o}C could be favorable for energy saving. Blue colors obtained from *I. tinctoria* subsp. *corymbosa* were lighter than that of *I. tinctoria*. This

difference between these closely related species could be due to their genetical makeup. The production of indigo precursors are affected by genetical differences (Angelini et al. 2007), hence, indigo producing capacity varies among the plants (Spataro et al. 2007). High dyestuff containing plants and easy extraction with water are the desired factors for the selection of dye plant (Bechtold et al. 2003).

The effects of reducer concentrations in dyeing were studied at 70°C and pH 11.0. Difference in color obtained by different reducer concentrations was insignificant (Table 2). Color improvement was not also observed with increasing concentration of reducer in *I. tinctoria* subsp. *corymbosa*.

Table 2 - Effects of reducer concentrations on dyeing performances of *Isatis* species.

pH 11	**1 g L $^{-1}$**	**5 g L $^{-1}$**	**10 g L $^{-1}$**	**20 g L $^{-1}$**
I. tinctoria **70 °C Hot Water Extraction**				
I. candolleana **70 °C Hot Water Extraction**				
I. tinctoria subsp. *corymbosa* **Fermentation Extraction**				

Various shades of blue color were obtained in all the tested *Isatis* species, except *I. buschiana*. The shades of light blue were much more than yellow-greenish and were obtained in *I. tinctoria* subsp. *corymbosa* while the most desired blue colors were obtained from *I. tinctoria* and *I. candolleana*. In caustic-hydrosulphite method, which is widely used to reduce indigo, dyeing vat is generally prepared in 80 g l^{-1} concentration. An excess of reducing agent results in irregular dyeing and increase of ecological damage (Kumbasar et al. 2006). For this reason, the control of pH and reducing agent concentrations are very important and this study represents an environmental friendly process with a small amount of hydrosulphite resulting succesfull dyeings. Hot water extraction method was reported as the most suitable choice to fulfill the extraction immediately after the harvest (Bechtold et al. 2002). It was simple and the yield could be obtained in the same day; however, this method required higher energy consumption. Energy consumption is not necessary for extraction with fermentation, but bad odor and overflows are the main problems and extra time is required for better results. However, simple and rapid dyeing process, no intermediate drying steps and one bath dying are the requirements of a technical dyehouse (Bechtold et al. 2003). Both the techniques studied here met these requirements.

Fastness Properties

The requirements for natural colorants in textile industry are the quality of fastness properties as well as color appearance in terms of reproducibility (Kumbasar et al. 2006). Washing fastness of the samples ranged in 2 to 4/5 for *I. tinctoria;* 2/3 to 4/5 for *I. candolleana*; 3 to 4/5 for *I. tinctoria* subsp. *corymbosa* (Table 3). Poor or insufficient washing fastness could be identified as a limitation for the use of natural dyes extracted from the different representative sources. Sufficient dyestuff fixation also can be seen as a good mark for washing fastness bleeding (Bechtold et al. 2006). The dry rubbing fastness ranged in 3/4 to 4 for *I. tinctoria* and *I. candolleana*; 4/5 for *I. tinctoria* subsp. *corymbosa.* The wet rubbing fastness ranged in 3 to 3/4 for *I. tinctoria* and *I. candolleana,* 3/4 for *I. tinctoria* subsp. *corymbosa.* The light fastness grade 2 or 3 was required as the lowest limit for positive selection; however, values of light fastness could be increased by further investigations to exceed the limit of grade 2-3 (Bechtold et al. 2006). Our results showed that light fastness grade were 3 and 3/4 for *I. tinctoria* and 3 for *I. candolleana while* 2/3 for *I. tinctoria* subsp. *corymbosa*. All the

results presented were at an acceptable light fastness level. The light fastness is also influenced by various factors such as chemical and physical properties of dye, dye concentration, nature of fibers (Cristea and Vilarem 2006; Guinot et al. 2006). Padfield and Landi (1996) also stated that indigo was much more light resistant on wool than that of cotton. In this study, the impurities in dyebath, which was released from raw cotton yarn, could reduce the affinity of dye particules to cotton yarn, therefore low fastness results could be caused by these impurities.

In the market, the main features of indigo-dyed denim fabrics demand has to meet the low washing fastness, average light fastness and low dry-rubbing resistance (Kumbasar et al. 2006). In this study, acceptable values for light, rubbing and washing fastness were obtained for denim fabrics (Table 3).

Table 3 - Fastness properties of dyed cotton yarn.

			I. tinctoria		***I. candolleana***		***I. tinctoria* subsp. *corymbosa***
		Mat blue	**Dark blue**	**Light blue**	**Light blue**	**Dark blue**	**Light blue**
Light Fastness		3*	3/4	3	3	3	2/3
Washing Fastness	Type of the reference fabric: acetate	2/3	2/3	3	3/4	3	3/4
	cotton	3	3	4	3/4	3/4	4
	nylon	2	2	2/3	3	2/3	3
	polyester	4	4	4	4	4	4
	acrylic	4	4	4/5	4/5	4/5	4/5
	wool	4	4	4	4	4	4/5
Rubbing Fastness	Dry	4	3/4	4	4	3/4	4/5
	Wet	3	2/3	3/4	3/4	3	3/4

*Scores for fastness tests: 1- Poor, 2-Weak, 3- Average, 4-Good, 5-Excellent

The Amount of Indigo

Seasonal variations of indigo were determined in Turkish native *Isatis* species and compared with *I. tinctoria*. Significant differences in indigo production were found between the species in growing season. The seasonal change in the amount of indigo production was determined and indigo extraction was performed between April 1 and August 7 from plant leaves with two-week interval. Seasonal course of indigo is shown in Figure 1. Indigo content generally increased from beginning of April until the mid of June, and began to decrease with temperature increase until beginning of July, then remained stable until the middle of August. Rosette plants completed their vegetative development in August. The highest indigo value was recorded at mid June in *I. tinctoria.*

The results of indigo content in *Isatis* spp. are given in Table 4. Indigo content were higher in *I. tinctoria* and *I .candolleana* than *I. tinctoria* subsp. *corymbosa* while indigo could not be detected in *I. buschiana.* Mean indigo content measured in fresh leaves of *I. tinctoria* and *I. candolleana* ranged from 0.34 to 4.19 mg g^{-1} and 0.01 to 2.53 mg g^{-1}, respectively. *I. tinctoria* subsp. *corymbosa* produced indigo in a range from 0.3 to 0.78 mg g^{-1}, which was 80% lower than that of *I. tinctoria.* These results were also supported with the dyeing experiments (Table 1). Blue colors obtained from *I. candolleana* were close to the colors from *I. tinctoria*. *I. tinctoria* subsp. *corymbosa* produced lighter blue colors; however, no blue colors were obtained from *I. buschiana*. The analysis showed that indigo reached to its maximum level in mid June, therefore mid-June could be the best time to harvest.

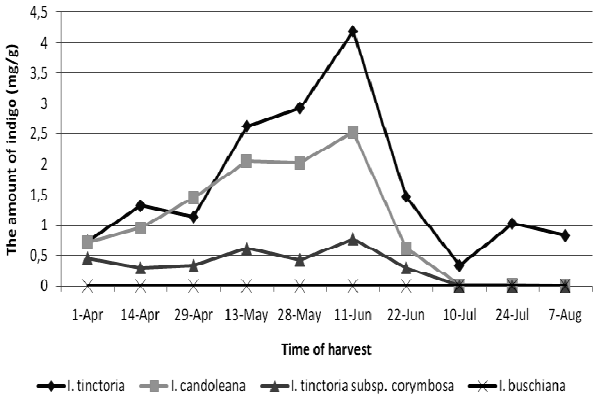

Figure 1 - Seasonal variation of indigo content in *Isatis* spp. leaves.

Table 4 - Seasonal variation of indigo content in *Isatis* spp (mg g^{-1}). The values represent the mean ± standard deviation.

	***Isatis* species**			
Harvest Dates of Leaves	***I. tinctoria***	***I. candolleana***	***I. tinctoria* subsp. *corymbosa***	***I. buschiana***
1st Apr	0.74 ± 0.07	0.72 ± 0.09	0.46 ± 0.05	ND
14thApr	1.32 ± 0.07	0.95 ± 0.11	0.30 ± 0.04	ND
29thApr	1.14 ± 0.12	1.45 ± 0.11	0.34 ± 0.02	ND
13thMay	2.63 ± 0.26	2.05 ± 0.12	0.62 ± 0.13	ND
28thMay	2.93 ± 0.24	2.02 ± 0.28	0.43 ± 0.10	ND
11thJun	4.19 ± 0.06	2.53 ± 0.23	0.78 ± 0.03	ND
22ndJun	1.47 ± 0.16	0.62 ± 0.07	0.30 ± 0.05	ND
10thJul	0.34 ± 0.04	0.01 ± 0.02	ND	ND
24thJul	1.03 ± 0.16	0.02 ± 0.06	ND	ND
7thAug	0.83 ± 0.31	ND	ND	ND

ND: Not detected

This study presented an indigo content between 0.34 to 4.19 mg g^{-1}, which was confirmed by Gilbert et al. (2004) and Angelini et al. (2007)'s previous observations. Angelini et al. (2007) reported that the harvest time had significant effect on leaf yield and indigo amount. They also stated that the genetical factor was another important criterion that affected the production of indigo precursors. Therefore, in this study, different *Isatis* species produced different indigo yield as a result of their genetic makeup. High temperature, light intensity and inadequate rainfall could limit the plant growth and indigo amount (Stoker et al. 1998; Sales et al. 2006). Therefore, the decrease in indigo in late-June could be due to the climatic changes.

CONCLUSIONS

Following cultivation of the plant material, a simple procedure for the extraction of indigo precursors was investigated in terms of crop and dye quality, aiming to bring some contribution to the knowledge of Turkish native *Isatis* species as a new potential dyeing plant. The study focused on the determination of indigo production potentials and effect of harvest times for indigo contents in *Isatis* spp. Results confirmed that indigo yield and quantity depended on the genetic factors, harvest time and extraction conditions. Extracted dye from *I. candolleana* was comparable to *I. tinctoria*. Thus, *I. candolleana* could be a promising plant for indigo dyeing. However, *I. tinctoria* subsp. *corymbosa* and *I. buschiana* were not suitable for this purpose.

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