ELSEVIER

Dyes and Pigments, Vol. 36, No. 3, pp. 215–221, 1998

PII: S0143-7208(97)00013-2 0143-7208/97 $17.00+0.00

Dyeing of Cotton and Wool Fibres with Pigments from *Crocus Sativus*—Effect of Enzymatic Treatment

M. Liakopoulou-Kyriakides,[a] E. Tsatsaroni,[b] P. Laderos[a] & K. Georgiadou[b]

[a]Department of Chemical Engineering, Aristotle University of Thessaloniki, Greece, 54006
[b]Faculty of Chemistry, Aristotle University of Thessaloniki, Greece, 54006

(Received 17 December 1996; accepted 31 January 1997)

ABSTRACT

Natural pigments from Crocus sativus *stigmas were used for the dyeing of cotton and wool fibres after treatment with the enzymes α-amylase and trypsin, respectively. The separation of various compounds and pigment constituents of the stigmas was effected by fractionation of the methanolic extract on a silica gel column, and the use of these fractions for dyeing is described. The dyeing properties of the fractions were compared with those of commercial trans-β-carotene. Wash and light fastness of the dyed samples after the enzymatic pretreatment were also studied.* © *1997 Elsevier Science Ltd*

INTRODUCTION

Commercial saffron consists of the stigmas of the flowers of *Crocus sativus L* and it is widely used as a condiment because of its aroma and colour. In addition, some applications of saffron and the bulbs in medicine [1–3] and textile dyeing [4] have been reported.

A large number of carotenoid compounds have been isolated by different methods from saffron [5–9]. Extraction of saffron with various solvents such as chloroform, acetone, ether, hexane and methanol has been reported, and the latter seems to be more advantageous. Methanol extraction gives a dark red syrup with the highest yield in crocins.

We have reported previously the dyeing of cotton and wool fibres with some natural dyes after pretreatment with enzymes [10]. In an attempt to

extend this study to vegetable dyes, we used fractions containing crocins (Fig. 1a), obtained from separation of the crude methanolic extract from *Crocus sativus* and compared their dyeing properties with those of commercial trans-β-carotene (Fig. 1b). Thus, we report here fractionation of the methanolic extract of saffron by column chromatography on silica gel, and the use of the main and characteristic fractions for dyeing of cotton and wool fibres after pretreatment with enzymes. Enzymatic treatment has been reported to minimize wool shrinkage and to improve its dyeing properties [11, 12].

EXPERIMENTAL

Plant material and reagents

Stigmas of pure red Greek saffron were obtained from the Cooperative of Saffron, Krokos Kozanis, Greece. Trans-β-carotene was purchased from Aldrich Chemical Co. All other reagents and solvents used in this work were from Sigma Chem. Co (Germany).

Commercial bleached cotton and wool fibres, trans-β-carotene, crocin and fractions A, B, C, D and E from *Crocus sativus* stigmas (obtained as outlined in the experimental section) were used for the dyeings. Before dyeing, cotton and wool fabrics were treated with amylase and trypsin, respectively, as in previous studies.

Enzymatic treatment

Treatment conditions for the enzymes used are given in Table 1. After treatment, all samples were rinsed and squeezed.

Apparatus

Dyeings and wash fastness tests were carried out in a Rotadyer apparatus (John Jeffreys Ltd, Rochdale and Banbury, U.K.). Spectrophotometric measurements were recorded in a Shimadzu UV-2101 PC spectrophotometer.

TABLE 1
Enzymatic Treatment Conditions (T = Room Temperature)

	α − Amylase	*Trypsin*
g/lt	2	2
pH	7	8
t(min)	10	30

An air-cooled Hanau Suntest apparatus (Heraeus) with xenon lamp was used for light fastness tests.

Colour change during the light fastness tests was evaluated in a Verivide colour assessment cabinet (Leslie Hubble) with an artificial daylight (D65) lamp.

Fractionation of components and pigments from saffron

Dried stigmas (30 g) were Soxhlet extracted with methanol for 10 h. The methanolic solution was then evaporated to dryness *in vacuo*. This extract, called henceforth, crude methanolic extract, was further fractionated on a silica gel column using chloroform or chloroform-methanol (from 1 to 50%). Two column volumes from each solvent system were used, except for the last one where more than three column volumes were used for the elution of crocins. All fractions obtained were checked by thin layer chromatography and by UV- spectrum for their content. Fractions corresponding to the same class of compounds were combined and used for dyeing wool and cotton fibres. Fractions A,B, C, D and E were obtained and their UV-Vis spectra were recorded on a Shimadzu UV 2002 spectrophotometer.

Dyeing

The fabrics were dyed with 1 or 2% o.w.f. (on the weight of the fibre) depth of dyeing at a liquor ratio 20:1. The temperature was raised to 90°C over 30 min and maintained at this level for 1 h. Sodium chloride 10% o.w.f. or a few drops of 40% acetic acid solution were added in the dyeing liquors of cotton or wool fabrics, respectively.

Determination of dye adsorbed on the fibre

This was done spectrophotometrically by extracting the pigment of the dyed fabrics with pyridine (25% v/v in water) and measuring the absorbance of the extracts at 434.8 nm (λ_{max} of crocin in this solvent).

Fastness determination

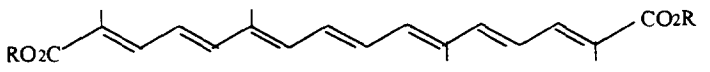
Wash fastness tests were carried out according to BS 1006:1990 CO2, with a soap solution 5 g/l (liquor ratio 50:1) for 45 min at 50 ± 2°C. The samples were assessed against the standard grey scale for colour change.

Light fastness tests were carried out according to BS 1006: 1990 BO2. Colour change of the samples was assessed against the grey scale and the blue wool standards.

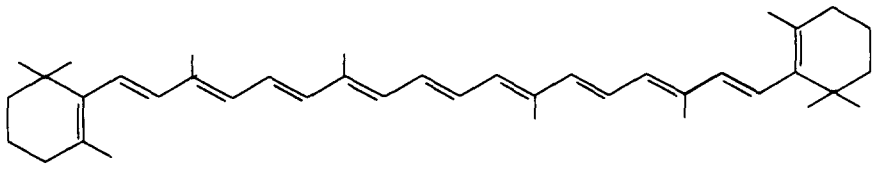
RESULTS AND DISCUSSION

Fractionation of the crude methanolic extract by column chromatography on silica gel resulted in five main fractions: A, B, C, D and E. The first three were eluted with a relatively non-polar solvent system (chloroform, or chloroform-methanol, 95/5). These fractions contained picrocrocins and derivatives, as well as flavonoids, as could be concluded from mass spectra and UV measurements. Each fraction contained more than four compounds (or isomers), as shown by GC or HPLC. From these fractions, picrocrocin and safranal were detected by mass spectroscopy in fractions A and B. Picrocrocin [4-(β-D-glucopyranosyloxy)-2,6,6- trimethyl-l-cyclohexen-l-carboxaldeyde] is the bitter agent in saffron. The UV-Vis spectrum of picrocrocins exhibits a characteristic broad absorption band at 250 nm due to the α, β-unsaturated cycloaldehyde system in the molecule. Polyphenols (including flavonoids) were also detected in fractions B and to a lesser extent in fraction C. Polyphenols are the most widespread constituents in all plants.

Dyeing with fractions A, B, and C proved unsuccessful under the conditions used. Fractions D and E, eluted with the more polar solvent system chloroform-methanol 50/50 and methanol, respectively, were found to dye cotton and wool after enzymatic pretreatment. Crocins were detected in those two polar fractions, and mainly in fraction E. Crocins and their derivatives show the characteristic UV-Vis spectra of the carotenoid moiety in the molecule. Crocins are glucosyl esters of crocetin (Fig. 1a), the central unit consisting of seven conjugated double bonds and four side chain methyl groups. The end groups are esterified with one, two and three glucose units. Carotenoids have a characteristic spectra in the visible region with double peaks between 400 and 500 nm. The UV-Vis spectra of fractions D and E were identical (Fig. 2). Crocin 2 was isolated as pure material from fraction



1a



1b

Fig. 1.

TABLE 2
Percentage Exhaustion and Wash and Light Fastness of Cotton and Wool Samples Dyed with Crocins with and without Enzymatic Treatment (Depth of Dyeing 1% o.w.f)

Sample	*Enzyme*	*Percentage pigment exhaustion*	*Wash fastness (colour change)*	*Light fastness*
Cotton	—	41	2	4–5
Cotton	α-Amylase	60	3	4–5
Wool	—	40	4	4
Wool	Trypsin	62	4	4

TABLE 3
Wash and Light Fastness of Wool Samples Dyed with trans-β-carotene (Depth of Dyeing 2% o.w.f)

Enzyme	*Wash fastness*			*Light fastness*
		Staining		
	Colour change	*Acrylic*	*Acetate*	
—	2	5	4–5	4
Trypsin	3	4–5	4–5	4–5

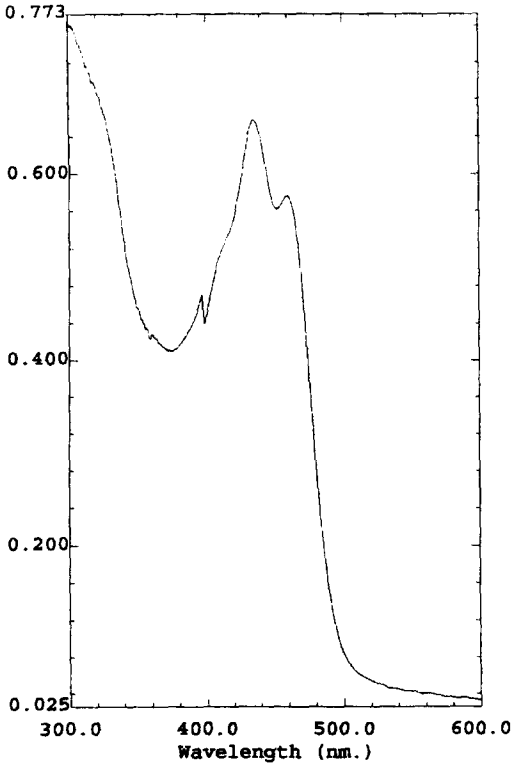


Fig. 2. UV-Visible spectrum of fraction with crocins.

E. It was crystallized from methanol and obtained as dark red crystals, m.p. 208°C. This pure material was also used for the dyeing of wool and cotton fibres.

Thus, the fractionation of the crude methanolic extract on a silica gel column resulted in separation of the crocins from the other constituents of saffron such as picrocrocins, flavanoids, etc. The dyeings with fractions D and E were further compared with dyeings using commercial trans-β-carotene. The dyeing results, percentage pigment exhaustion, wash and light fastness values for the dyed samples with enzymatic treatment of all the compounds used are shown in Tables 2 and 3. It was found that while, for D and E, dyeing and colour fastness were comparable, giving the possibility to use these fractions instead of the pure pigments, carotene adsorption was not satisfactory, especially for cotton dyeing, as was visually confirmed. Thus, cotton samples dyed with carotene were not tested further for their fastness properties. It is worth mentioning that even the commercial trans-β-carotene showed the presence of other minor peaks (most probably isomers), when analyzed by HPLC.

ACKNOWLEDGEMENTS

The authors are grateful to the Cooperative of Saffron, Krokos Kozanis, for providing the stigmas of saffron and Mr A. Karakatsanis for technical assistance. The work was supported by the General Secretariat of Research and Technology, Ministry of Industry, Research and Technology.

REFERENCES

1. Hojima, Y., Pierce, J. V. and Pisano, J. J., *Thrombosis Research*, 1980, **20**, 163.
2. Liakopoulou-Kyriakides, M., Sinakos, Z. and Kyriakides, D. A., *Plant Science*, 1985, **40**, 117.
3. Liakopoulou-Kyriakides, M. and Skubas, A. I., *Biochemistry International*, 1990, **22**, 103.
4. Tsatsaroni, E. and Elefteriadis, I., *Journal of the Society of Dyers and Colourists*, 1994, **110**, 313.
5. Dhingra, V. K., Seshadri, T. R. and Mukerjee S. K., *Indian Journal of Chemistry*, 1975, **13**, 339.
6. Pfander, H. and Rychener, M., *Journal of Chromatography*, **234** (1982) 443.
7. Sujata, V., Ravishankar, G. A., Venkataraman, L. V., *Journal of Chromatography*, 1992, **624**, 497.
8. Tarantilis, P., Polissiou, M. and Manfait, M., *Journal of Chromatography A*, 1994, **664**, 55.
9. Tarantilis, P., Polissiou, M. and Tsoupras, G., *Journal of Chromatography A*, 1995, **699**, 107.

10. Tsatsaroni, E and Liakopoulou-Kyriakides, M., *Dyes and Pigments*, 1995, **29**, 203.
11. Riva, A., Cegarra Sanchez, J. and Prieto, R., *Melliand Textilberichte*, 1991, **11**, 934.
12. Riva, A., Cegarra Sanchez, J. and Prieto, R., *Journal of the Society of Dyers and Colourists*, 1993, **109**, 210