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Comparative Study of dyeing Properties of Two Yellow Natural Pigments—Effect of Enzymes and Proteins

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ABSTRACT

*The crude water extract of saffron stigmas (*Crocus sativus *L.) as well as crocin, the yellow pigment isolated from* Crocus sativus *L. were used for dyeing cotton and wool fibres after enzymatic treatment. The dyeing results were compared with those obtained with another yellow natural pigment, curcumin. Wash and light fastness of all dyed samples were studied. The enzymes used were α-amylase, amyloglycosidase and trypsin for the cotton and wool samples respectively. In addition, casein was used in the case of curcumin as a pretreating agent and its effect on the dyeing properties of the pigment was studied.* © *1998 Elsevier Science Ltd. All rights reserved*

Keywords: crocin, curcumin, dyeing, enzymes, proteins.

INTRODUCTION

In previous studies [1, 2] we reported the effect of enzymatic treatment on the dyeing of cotton and wool fibres with natural pigments, with respect to pigment adsorption and wash and light fastness properties. The aim of this present work was to extend the study on the dyeing properties of two yellow natural pigments, viz, curcumin and crocin, under enzymatic or protein treatment at various conditions of pH and temperature.

Crocin 1 (Fig. 1) (C.I. Natural Yellow 6), is the main colorant species among the water-soluble carotenoids isolated from the dried stigmas of the plant *Crocus sativus* L.

Curcumin 2 (Fig. 1) (C.I. Natural Yellow 3) is extracted from the rhizomes of various *curcuma* species. Commercially obtained curcumin consists of a

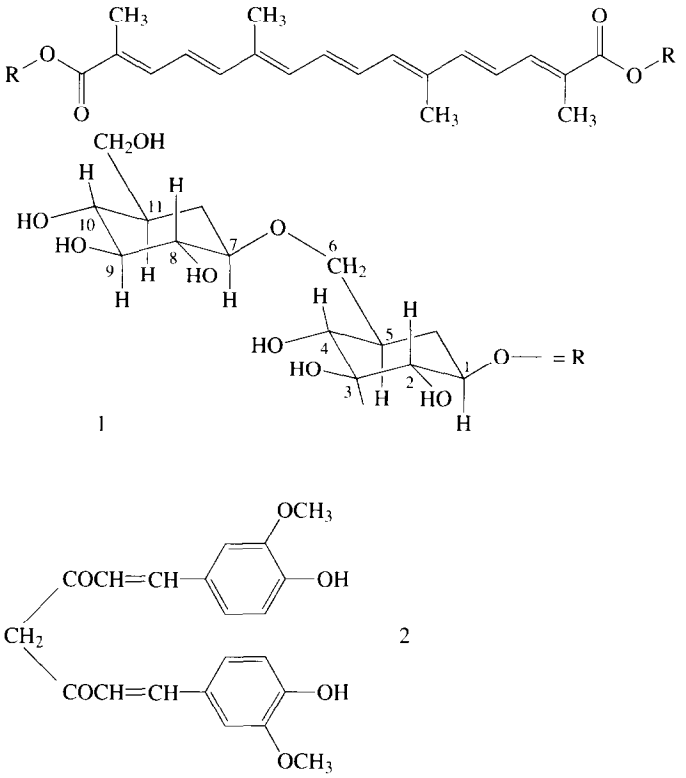

Fig. 1. Pigments used: **1** crocin, **2** curcumin.

mixture of the three naturally occurring curcuminoids, viz, curcumin, dimethoxy- and bis-dimethoxy-curcumin, with the first as the main constituent [3].

Dyebath exhaustion, and the wash and light fastness of cotton and wool samples dyed with the two pigments after treatment with α-amylase, amyloglycosidase and trypsin and the protein casein, were studied.

EXPERIMENTAL

Materials

Saffron stigmas were supplied by the Cooperative of Saffron, Krokos, Kozani, Greece. Curcumin was purchased from Riedel de Häen. All other reagents and solvents used were from Sigma Chem. Co as were α-amylase, amyloglycosidase, trypsin and casein. Commercial bleached cotton and wool fibres were used for the dyeings.

Pretreatment with enzymes and casein

Treatment conditions 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). Spectrophotometric measurements were recorded in a Shimadzu UV-2101 spectrophotometer. An air-cooled Hanau Suntest apparatus (Heraeus) with a xenon lamp was used for the light fastness tests. Colour change during the light fastness tests was evaluated in a Verivide colour assessment cabinet (Leslie Hublle) with an artificial daylight (D65) lamp.

Preparation of saffron extract and isolation of saffron pigments

The preparation of water extract of saffron stigmas was carried out as follows: 2 g of saffron stigmas were added in cold water. The mixture was shaken for 30 min, heated to 60°C for 30 min and allowed to stand in the dark for 24 h. The clear supernatant liquid obtained before filtration was used for the dyeings [4].

The isolation of the saffron pigment was effected as previously described [2].

Dyeing

The fabrics were dyed with 0.5, 1 or 2% owf at a liquor ratio of 20:1. The temperature was raised to 90°C over 30 min and maintained at this level for 1 h. In the case of the aqueous extract of saffron stigmas, portions of the above extract prepared as described were used directly as dyeing liquor.

TABLE 1
Pretreatment Conditions for the Enzymes Used

Pigment	*Pretreating agent*	*pH*	*Temperature °C*
Saffron extract	α-amylase	7	25
	trypsin	8	25
Crocin	α-amylase	7	25
	trypsin	8	25
Curcumin	α-amylase	7	25,40,60
	amyloglycosidase	7.5	25,40,60
	trypsin	8	30,60
	casein	7	25,40,60

Determination of dye adsorbed on the fibre

This was done spectrophotometrically by extracting the saffron pigment of the dyed fabrics with a pyridine–water mixture (25% v/v) and measuring the absorbance of the extracts at 444.8 nm (λ_{max} of crocin in the above solvent).

In the case of curcumin, quantitative pigment extraction of the dyed fabric was not possible by the use of several solvents, and determination of the pigment was therefore made indirectly by measuring the absorbance of the remaining pigment in the dyeing liquor after dyeing in a mixture of ethyl alcohol/water 1:1 at $\lambda = 430$ nm (λ_{max} of curcumin in the above solvent).

Fastness determination

Wash fastness tests were carried out according to BS 1006: 1990 CO2 with a soap solution (5 g litre^{-1} 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 blue standards.

RESULTS AND DISCUSSION

The dyeing results, i.e. percentage pigment exhaustion, wash and light fastness values for the dyed crocin samples with and without enzymatic pretreatment are given in Table 2.

The same results for the pretreated, and dyed with curcumin, cotton and wool samples, using several pretreatment conditions and various depths of dyeing, are given in Tables 3 and 4, respectively.

The dyeing results for the cotton and wool samples pretreated with casein and dyed with curcumin are given in Table 5.

From Tables 2–5 the following conclusions can be made:

TABLE 2
Adsorbed Amount of Crocin, % Exhaustion, Wash and Light Fastness of Cotton and Wool Samples Dyed with and without Enzymatic Pretreatment (depth of dyeing 1% owf)

Fibre	*Enzyme*	*g pigment per 100 g fibre (% exhaustion)*	*Wash fastness (colour change)*	*Light fastness*
Cotton	—	0.41 (41.0)	4	3
Cotton	α-amylase	0.60 (60.0)	4	3
Wool	—	0.40 (40.0)	4	4
Wool	trypsin	0.61 (61.0)	4	4

TABLE 3

Adsorbed Amount of Curcumin (g pigment per 100 g fibre, % exhaustion) and Wash and Light Fastness of Cotton Samples Dyed with Curcumin after Treatment with Enzymes at Different pH, Concentration and Temperature Conditions

Pretreatment conditions					*g pigment per 100 g fibre (% exhaustion)*	*Fastness*	
Enzyme	*Enzyme concentration (units g fibre^{-1})*	*T(°C)*	*pH*	*Depth of dyeing (% owf)*		*Wash (colour change)*	*Light*
—	—		—	0.5	0.235 (47.2)	3–4	2–3
Amylase	1500	25	7	0.5	0.446 (89.7)	4	2–3
	3000	25	7	0.5	0.444 (89.2)	4	2–3
	1500	40	7	0.5	0.476 (95.7)	3–4	3
	3000	40	7	0.5	0.469 (94.5)	3–4	3
	1500	60	7	0.5	0.489 (98.4)	3–4	2–3
	3000	60	7	0.5	0.485 (97.6)	3–4	2–3
	—		7	2.0	0.580 (30.0)	2–3	2–3
Amylase	1500	25	7	2.0	0.983 (49.1)	3	2–3
	3000	25	7	2.0	0.788 (39.5)	3–4	2–3
	1500	40	7	2.0	0.707 (35.4	3	2–3
	3000	40	7	2.0	0.635 (31.7)	3	2–3
	1500	60	7	2.0	0.705 (35.2)	3	2–3
	3000	60	7	2.0	0.738 (36.9)	2–3	2–3
Amyloglycosidase	1500	25	5	0.5	0.444 (89.5)	3	3
	3000	25	5	0.5	0.418 (84.3)	3	3
	1500	40	5	0.5	0.489 (98.4)	3	2–3
	3000	40	5	0.5	0.464 (93.3)	3–4	2–3
	1500	60	5	0.5	0.408 (82.0)	3	3–4
	3000	60	5	0.5	0.455 (90.4)	3–4	3–4
	1500	25	7	0.5	0.470 (94.5)	4–5	2–3
	3000	25	7	0.5	0.479 (96.3)	4	2–3
	1500	40	7	0.5	0.464 (93.3)	3–4	2–3
	3000	40	7	0.5	0.471 (94.9)	3–4	2–3
	1500	60	7	0.5	0.457 (91.9)	3–4	3
	3000	60	7	0.5	0.449 (90.4)	3	3
	1500	25	5	2.0	0.591 (29.6)	3	3–4
	3000	25	5	2.0	0.702 (35.2)	2–3	3
	1500	40	5	2.0	1.484 (74.2)	2–3	3
	3000	40	5	2.0	0.836 (41.8)	2–3	3
	1500	60	5	2.0	0.853 (42.7)	2–3	2–3
	3000	60	5	2.0	0.728 (36.4)	2–3	3
	1500	25	7	2.0	0.703 (35.1)	3–4	2–3
	3000	25	7	2.0	0.767 (38.4)	3–4	2–3
	1500	40	7	2.0	1.368 (68.4)	3	2–3
	3000	40	7	2.0	0.780 (39.0)	2–3	3
	1500	60	7	2.0	0.673 (33.6)	3	3
	3000	60	7	2.0	0.842 (42.1)	2–3	3

Pretreatment with enzymes results, in all cases, in an improvement in pigment adsorption on the fabric. This is in good agreement with previous results related to crocin and other natural pigments [1, 2].

TABLE 4
Adsorbed Amount of Curcumin (g pigment per 100 g fibre, % exhaustion) and Wash and Light Fastness of Pretreated with Trypsin and Dyed Wool Samples

Pretreatment conditions		*Depth of dyeing (% owf)*	*g pigment per 100 g fibre (% exhaustion)*	*Fastness*	
Enzyme concentration (g l^{-1})	*T (°C)*			*Wash (colour change)*	*Light*
—		0.5	0.430 (86.1)	2(R)[a]	2–3(R)
1	30	0.5	0.404 (80.8)	3(R)	3(R)
2	30	0.5	0.409 (81.9)	2–3(R)	3(R)
1	60	0.5	0.485 (96.9)	2(R)	3(R)
2	60	0.5	0.489 (97.8)	2(R)	2–3(R)
—		2.0	1.894 (94.7)	2–3(R)	2–3(R)
1	30	2.0	1.906 (95.3)	2–3(R)	3(R)
2	30	2.0	1.911 (95.6)	2–3(R)	3(R)
1	60	2.0	1.892 (94.6)	2–3(R)	3(R)
2	60	2.0	1.891 (94.5)	2–3(R)	2–3(R)

[a]Redder.

TABLE 5
Adsorbed amount of Curcumin (g pigment per 100 g fibre, % exhaustion) and Wash and Light Fastness of Cotton and Wool Fabrics Dyed with and without Pretreatment with Casein (pretreatment pH = 7)

Pretreatment conditions		*Fibre*	*Depth of dyeing (% owf)*	*g pigment per 100 g fibre (% exhaustion)*	*Fastness*	
Enzyme concentration (g l^{-1})	*T (°C)*				*Wash (colour change)*	*Light*
—		Cotton	0.5	0.235 (47.2)	3–4	2–3
2	25		0.5	0.470 (94.6)	3	3
4	25		0.5	0.475 (95.7)	3–4	3
2	40		0.5	0.477 (96.0)	3	3
4	40		0.5	0.541 (97.0)	3–4	3
2	60		0.5	0.471 (94.4)	4	2-3
4	60		0.5	0.471 (94.4)	3–4	3
—			2.0	0.58 (30.0)	2–3	2–3
2	25		2.0	0.617 (30.9)	3	2–3
4	25		2.0	0.703 (35.2)	3	2–3
2	40		2.0	0.539 (26.9)	3	2–3
4	40		2.0	0.675 (33.7)	2–3	2–3
2	60		2.0	0.913 (45.6)	3–4	2–3
4	60		2.0	0.854 (42.7)	3	3
—		Wool	0.5	0.430 (86.1)	2(R)[a]	2–3(R)
2	25		0.5	0.436 (87.2)	2–3(R)	2–3(R)
4	25		0.5	0.441 (88.3)	3(R)	2–3(R)
2	60		0.5	0.430 (86.0)	2(R)	2–3(R)
4	60		0.5	0.421 (84.2)	2(R)	2–3(R)
—			2.0	1.894 (94.7)	2–3(R)	2(R)
2	25		2.0	1.908 (95.4)	2–3(R)	2–3(R)
4	25		2.0	1.892 (94.6)	2–3(R)	2–3(R)
2	60		2.0	1.887 (94.3)	3(R)	2–3(R)
4	60		2.0	1.887 (94.3)	3(R)	2–3(R)

[a]Redder.

Comparison between the % adsorption values of the two pigments shows that it is almost the same for the reference cotton samples. Enzymatic treatment with α-amylase gives better results in the case of curcumin than crocin, especially at 0.5% depth of dyeing, while at 2.0% depth of dyeing it does not. Furthermore, variation of the enzymatic pretreatment conditions (pH, temperature and enzyme concentration) seems to have little effect on the curcumin adsorption or it does not affect it at all. The use of another amyloglycosidase as a pretreating agent for cotton did not result in a significant change in the curcumin adsorption values compared to the corresponding values obtained after pretreatment with amylase (Table 3).

Regarding the wool samples, it can be concluded from Table 2 that the use of trypsin as a pretreating agent results in an improvement in crocin adsorption of the pigment on the untreated reference sample. In contrast, this improvement was not significant in the case of curcumin, where the pigment adsorption was high, even on the untreated reference samples.

In an attempt to examine the effect of proteins in addition to that of enzymes, casein was used as a pretreating agent in the dyeing of both fibres with curcumin. The reason for using casein is that proteins such as albumin have been used from early times as a mordant for printing cotton cloth to produce 'calico' [5].

Regarding the fastness properties of the two pigments, it is concluded from Tables 2–5 that crocin has medium light fastness, in all cases higher than curcumin, which does not change for the dyeings after enzymatic pretreatment of the samples. Wash fastness values for cotton samples dyed with curcumin are comparable to those dyed with crocin, while the wool samples dyed with crocin show higher value than those with curcumin.

The fastness properties above were also estimated for cotton and wool samples dyed with curcumin with the same depths of dyeing (0.5 and 2.0% omf) after treatment with aluminium sulphate, zinc chloride and sodium potassium tartrate, which have been traditionally used as mordants for natural pigments (Table 6).

Comparison of these values with the corresponding values obtained after enzymatic pretreatment shows that there is no advantage for either of the two procedures, except for in the case of the wash fastness of the mordanted wool samples, which is significantly higher than that of the enzymatically pretreated ones.

Finally, and in addition to previous work [6] on the dyeing properties of the water extract of saffron stigmas, dyeing of cotton and wool fabrics was carried out directly and after enzymatic treatment with α-amylase and trypsin, respectively, under the dyeing conditions mentioned above. As was visually apparent, the adsorption of the saffron extract obtained under ISO 3632 conditions [4] on the fabrics, with and without enzymatic treatment,

TABLE 6
Wash and Light Fastness of Cotton and Wool Fabrics Dyed with Curcumin after Pretreatment with Mordants

Sample	*Depth of dyeing (% owf)*	*Mordant*	*Wash fastness (colour change)*	*Light fastness*
Cotton	0.5	$Al_2(SO_4)_3$	3	3
		$ZnCl_2$	3	3
		$C_4H_4KNaO_6$	3–4	3
	2.0	$Al_2(SO_4)_3$	3–4	2–3
		$ZnCl_2$	3–4	2–3
		$C_4H_4KNaO_6$	4	2–3
Wool	0.5	$Al_2(SO_4)_3$	4–5(R)[a]	2–3(R)
		$ZnCl_2$	4–5(R)	2–3(R)
		$C_4H_4KNaO_6$	4–5(R)	3(R)
	2.0	$Al_2(SO_4)_3$	4–5(R)	2–3(R)
		$ZnCl_2$	4(R)	2–3(R)
		$C_4H_4KNaO_6$	4(R)	3(R)

[a]Redder.

was satisfactory. Quantitative determination of the pigment was not possible, mainly due to the large amounts of pigments and other constituents contained in the extract. Extraction with a mixture of pyridine and water (25:75) of the two wool samples (one reference and one dyed after treatment with trypsin) resulted in two extracts which differed in their absorbances at $\lambda = 440$ nm, that corresponding to the pretreated sample having almost 35% higher absorbance than the other. That implies that enzymatic treatment has, on the dyeing with the saffron water extract, a similar effect to that on dyeing with crocin. Wash and light fastness values also were similar to those obtained with crocin. This study will be further extended to other natural dyes with the use of additional enzymes and proteins.

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