Enzymatic dyeing of wool

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Introduction

This study reports for the dyeing of wool using an enzymatic system comprising laccase (EC 1.10.3.2), dye precursor - 2,5-diaminobenzenesulfonic acid and dye modifiers – cathehol and resorcinol. The effect of the process variables - reaction time, enzyme and modifiers concentration on fabrics colour was studied, according to an appropriate experimental design. Different hues and depth of shades could be achieved varying the concentration of the modifiers and the time of laccase treatment. The duration of the enzymatic reaction appeared to be the most important factor in the dyeing process. Thus the dyeing process, performed at low temperature and mild pH, was advantageous in terms of reduced enzyme and chemicals dosage. The ability of the laccases to generate colour "in situ" from originally non-coloured, low-molecular substances, appears as an alternative to the conventional dyeing processes. The dye precursors can be used alone or in combination with suitable modifier (coupler) – another phenolic compound, which together with the dye precursor will enlarge the colour pallet achieved in the enzymatic dyeing.

Enzyme dyeing

The textile material used in the experiments was scoured and washed 100 % wool fabric. The dyeing was carried out in 0.1 M acetate buffer pH 5, at 50°C with 2.5 – 10 ml/l *Trametes Villosa* laccase from Novo Nordisk (6.8 g prot./l), 0.1 M dye precursor - 2,5-diaminobenzene sulfonic acid, 5 – 50 mM dye modifiers – cathehol or resolcinol, for 1 - 9 hours. All reagents were from analytical grade, provided by Sigma. After the dyeing the fabrics were thoroughly washed at boil with non-ionic detergent Lutensol ON 30 (BASF) until no more dye was released in the washing bath. Transmission optic microscope (Olympus BH2) with magnification 40 × was used to observe the dye distribution across the fibres.

Experimental design

The influence of the dyeing process variables on the colour of the fabrics was studied using a 2^3 full factorial design with three coded levels leading to eleven sets of experiments. The data were analyzed using "Design Expert" software (version 5.0). The responses analyzed were the colour characteristics: K/S, L*, a*, b*.

Table 1 Factor levels according to the adopted 2^3 full factorial design

Independent Variables	Symbol	Range and Levels		
		-1	0	+1
Modifier concentration (mM)	A	5	27.5	50
Laccase amount (mL/L)	B	2.5	6.25	10
Dyeing time (h)	C	1	5	9

Results

Screening experiments were conducted to identify the factors that influence the colour (in terms of K/S, L*, a* and b*) of the dyed fabrics. The effects of different experimental variables (modifier concentration, laccase amount and dyeing time) on the dyeing results were simultaneously investigated, applying a full factorial design experiment.
The highest K/S value for cathehol was achieved at the uppermost levels of the three factors. Interestingly, for resorcinol the highest K/S value was attained when the modifier was applied in the lowest concentration, while the amount of enzyme and the time of treatment were at their highest levels. Independently on the other variables, increasing the dyeing time from 1 to 9 hours drastically increased K/S and decreased L* values. This high time-dependent increase of K/S suggested that deeper colour could be achieved simply prolonging the contact time between textile material, enzyme, dye precursor and modifier. From another point of view it means that the enzymatically initiated reaction of colour generation proceeds continuously and was not inhibited by the products of the reaction.

Table 2 Experimental design and dyeing results with modifiers cathecol and resorcinol, according to the 23 factorial design.

Runs	Variables			Cathecol				Resorcinol			
	A	B	C	K/S	L*	a*	b*	K/S	L*	a*	b*
1	-1	-1	-1	2.61	48.89	6.58	7.17	2.17	52.57	8.90	8.22
2	+1	-1	-1	5.14	39.04	5.91	7.46	2.40	52.64	8.40	11.91
3	-1	+1	-1	3.43	45.01	6.54	7.41	2.49	51.52	8.84	10.26
4	+1	+1	-1	3.34	45.14	6.66	7.13	2.21	53.76	8.24	11.81
5	-1	-1	+1	14.16	26.42	6.45	7.85	15.22	23.99	11.29	3.16
6	+1	-1	+1	14.94	25.72	5.72	7.93	9.04	33.55	10.76	10.18
7	-1	+1	+1	19.49	22.47	6.11	7.30	18.35	22.78	11.17	5.50
8	+1	+1	+1	23.91	18.30	10.00	3.01	13.97	27.05	10.65	8.05
9	0	0	0	11.50	28.72	6.31	7.56	7.95	33.75	10.04	7.25
10	0	0	0	12.86	27.24	6.17	7.42	8.89	32.47	10.29	7.49
11	0	0	0	14.36	25.77	6.10	7.16	9.60	31.11	10.01	6.80

Cathehol and resorcinol are respectively ortho- and meta-substituted diphenols. The position of the second OH group in the molecule of the modifier was responsible for the hue change. The statistical analysis for each of the response variables is summarized in Tables 4 and 5. According to the Student's test, the factor with most significant effect (99 % confidence level in most cases) on all responses was the time of dyeing.

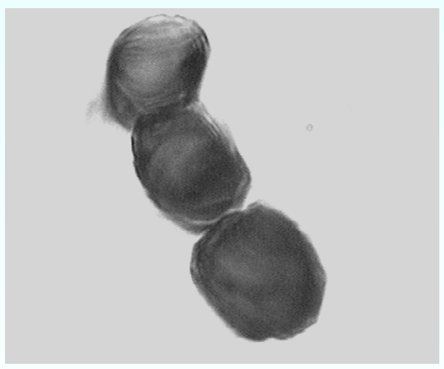

Figure 1 The cross-section image of the enzymatically dyed fibres showed penetration of the dye into the interior of the keratin fibre. This image suggests that the small molecules of the dye-precursor and modifiers could penetrate beyond the wool cuticles and some portion of the colour was formed in the fibre itself.

Tables 3 Statistical analysis for each of the response variables is summarized in. According to the Student's test.

Factors	K/S (± 0.51)		L* (± 0.52)		a* (± 0.04)		b* (± 0.07)	
	Effect	t value	Effect	T value	Effect	t value	Effect	t value
CATHECOL								
Mean	10.88	---	33.87	---	6.75	---	6.91	---
A	0.96	1.89	-1.82	-3.50 [c]	0.33	8.63 [b]	-0.52	-7.32 [b]
B	1.66	3.29 [c]	-1.14	-2.19	0.58	15.38 [a]	-0.69	-9.68 [a]
C	7.25	14.33 [a]	-10.65	-20.41 [a]	0.32	8.56 [b]	-0.39	-5.36 [b]
AB	0.13	0.25	0.81	1.56	0.68	17.89 [a]	-0.62	-8.60 [b]
AC	0.34	0.68	0.61	1.16	0.46	12.27 [a]	-0.53	-7.35 [b]
BC	1.91	3.78 [c]	-1.70	-3.26 [c]	0.40	10.68 [a]	-0.67	-9.37 [b]
ABC	0.78	1.55	-1.68	-3.22 [c]	0.48	12.66 [a]	-0.48	-6.62 [b]
RESORCINOL								
Mean	8.23	---	39.73	---	9.780	---	8.64	---
A	-1.33	-4.53 [b]	2.02	4.32 [b]	-0.270	-4.94 [b]	1.85	14.95 [a]
B	1.02	3.50 [c]	-0.96	-2.05	-0.056	-1.03	0.27	2.17
C	5.91	20.21 [a]	-12.89	-27.62 [a]	1.190	21.83 [a]	-1.91	-15.45 [a]
AB	0.16	0.55	-0.39	-0.84	-0.011	-0.21	-0.83	-6.67 [b]
AC	-1.31	-4.49 [b]	1.44	3.09 [c]	0.006	0.11	0.54	4.37 [b]
BC	0.99	3.39 [c]	-0.97	-2.08	-0.001	-0.023	-0.22	-1.75
ABC	0.29	0.99	-0.93	-2.00	0.014	0.25	-0.29	-2.35

The statistical analysis for each of the response variables is summarized in Tables 3. According to the Student's test, the factor with most significant effect (99 % confidence level in most cases) on all responses was the time of dyeing.

Table 4 Attained models for the responses studied

	Cathecol		Resorcinol	
	Model	ANOVA	Model	ANOVA
K/S	$\hat{y}_1 = 10.88 + 1.67\times B + 7.25\times C + 1.91\times BC$	$p<0.0001$; $R^2= 0.96$	$\hat{y}_3 = 8.23 - 1.33\times A + 1.02\times B + 5.91\times C - 1.31\times AC + 0.99\times BC$	$p=0.0002$; $R^2=0.99$
L*	$\hat{y}_2 = 33.87 - 1.82\times A - 10.65\times C + 0.61\times AC$	$p=0.0006$; $R^2=0.93$	$\hat{y}_4 = 39.73 + 2.02\times A - 12.89\times C + 1.44\times AC$	$p<0.0001$; $R^2 = 0.98$

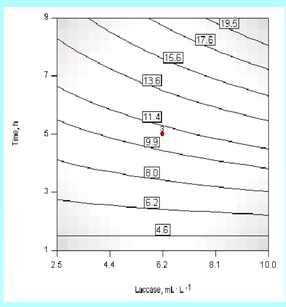

Figure 2 Contour plot of K/S as a function of laccase concentration and reaction time. The factor modifier was kept constant (27.5 mM cathehol).

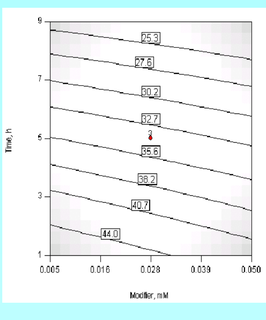

Figure 3 Contour plot of L* as a function of modifier (cathehol) concentration and reaction time. The factor laccase was kept constant (6.2 mL·L-1).

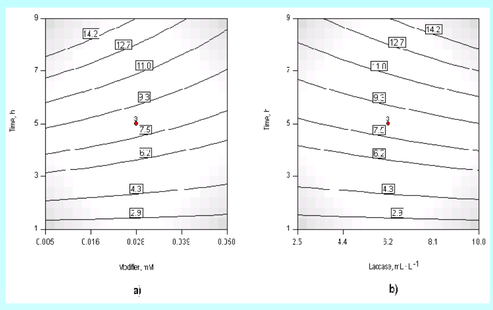

Figure 4 Contour plots of K/S as a function of modifier resorcinol (a) or laccase (b) concentration and time of reaction. The other factor was kept at the zero level.

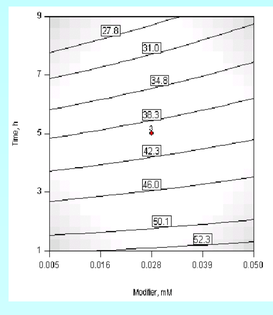

Figure 5 Contour plot of L* as a function of modifier (resorcinol) concentration and reaction time. The factor laccase was kept constant (6.2 mL·L-1).

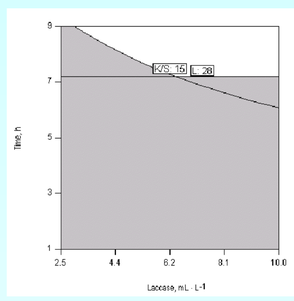

Figure 6 The optimum region defined by the overlaid plots of the two responses K/S and L* evaluated for modifier resorcinol as a function of laccase concentration and reaction time. The other factor was kept in the zero level

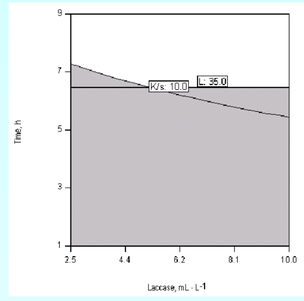

Figure 7 The optimum region defined by the overlaid plots of the two responses K/S and L* evaluated for modifier cathehol as a function of laccase concentration and reaction time. Modifier was kept constant (27.5 mM).

Conclusions

This study was an attempt to optimize the process variables of a novel laccase assisted dyeing process of wool. The adopted statistical techniques demonstrated their usefulness in finding the optimal modifier and enzyme concentration, and duration of the process to achieve different depth of colour and hue on the fabrics. Increasing the reaction time, and minimizing the enzyme and modifiers loading could obtain darker colouration of the samples. This renders the laccase dyeing an economically attractive alternative to the conventional high water, dyes, auxiliaries and energy consuming acid dyeing of wool. Additionally the enzymatic reaction was carried out at safe to the textile material pH and temperature. The dyeing experiments with two modifiers having the same molecular weight, but with different position of the substitutes revealed the potential of the enzymatic approach for achieving large diversity of colours and hues on the fabrics, varying the starting compounds.The statistical analysis showed that resorcinol should be used in low concentration to attain deep shade dyeing. Microscopic observation of the cross-section of the enzymatically dyed wool demonstrated penetration of the colorant into the mass of the fibres.