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Bio mimetic coloration of wool using plant juice

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The paper reports study on the optimisation of coloration of wool, through *in situ* reaction of diphenolic catechol with enzymes present in potato juice. Color formation takes place at low temperature and without any use of auxiliary chemicals. Response surface methodology has been used to study the effect of process variables, such as temperature and respective concentrations of catechol and plant juice, on the intensity of color obtained on fabric. A range of shades varying *K/S* from 2 to 10 is produced. Temperature is found to be the most significant factor affecting the color strength. Colour continues to become deeper with the increase in temperature of treatment. Optimised recipes have been proposed for producing a particular shade on wool. Spectroscopic analysis of coloured wool shows no specific peaks, pointing towards formation of colorants having a non specific molecular structure. Colours obtained on wool are found to be fast to light and washing.

Keywords: Bio mimetic coloration, Polyphenol oxidases, Potato juice, Wool

1 Introduction

Oxidative dyes based on colourless dye precursors have been used for permanent colouration of hair. These dyes represent a two-component system, namely a dye precursor and an oxidative agent. The oxidant initiates the coupling of the dye precursor and the formation of large molecular size coloured compounds, which remain fixed in the fibre structure[1]. Oxidative laccase enzymes have been used with small colourless aromatic compounds such as diamines, amino phenols, aminonaphthols and phenols, which undergo further non enzymatic reactions, to produce colour in textiles [2-8].

Vegetable juices are an abundant source of enzymes. However, no study has been carried out so far to study the process of coloration of fibres by enzyme rich plant juices. Potato starch industry produces large amounts of potato juice as waste[9]. This potato juice is rich in the enzyme polyphenol oxidase (PPO)[10]. PPO, also known as tyrosinase, is a bifunctional, copper containing oxidase having both catecholase and cresolase activity[11]. It is responsible for the browning reaction in fruits and plants. The browning phenomenon has been studied widely by biochemists and is attributed to the oxidation and dehydrogenation of colourless polyphenols by PPO. In nature, the initial reaction catalysed by PPO yields reddish brown products based on ortho quinones. (Fig. 1)[12]. These highly reactive species further undergo a series of non enzymatic reactions to yield insoluble black - brown melanin pigments[13-14]. Optimum *p*H of the enzyme activity is reported to be 6.6 and optimum temperature is 40°C.

Given that melanins are amongst the most stable and resistant biochemicals known, it is possible to exploit these natural reactions to produce deep and durable colour in textiles. With this understanding, fresh juice extracted from the tubers of common kitchen vegetable- potato (*Solanum tuberosum*), was combined with catechol to develop a series of brown shades on wool fabric. This paper reports the study on the optimization of process conditions for the oxidation reaction, with the aim of producing the required shade on wool fabric.

2 Materials and Methods

2.1 Materials

The textile material used was 100% pure merino wool fabric of 30 µm, having areal density of 86.3 g/m^2, warp/weft count of 23.5/18.02 tex and ends/picks per cm of 22/22. It was procured from Shingora Textiles, Ludhiana and used without any further preparation.

Catechol (benzene-1,2-diol), having molecular weight of 110.11g/mol, was procured from Sigma Aldrich Pvt Ltd. Fresh potatoes were procured from the local market, washed and peeled. They were

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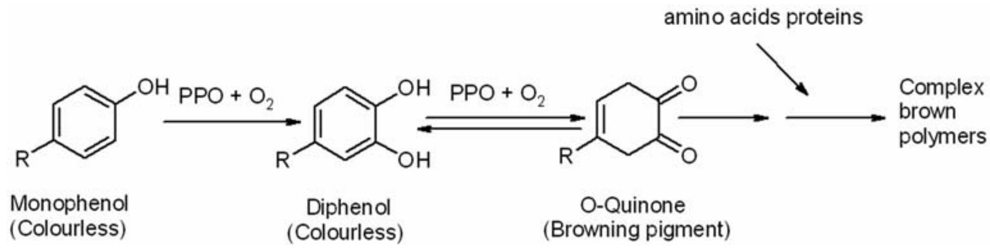

Fig. 1—Enzymatic browning mechanism in nature[12]

grated and the pulp was squeezed to extract the juice. Fresh juice was allowed to stand for 20 min so as to allow the starch to settle at the bottom. Clear juice was decanted and used for the study. *p*H of the extracted juice was 4.5. Concentration of protein in potato juice, as determined using Bradford protein assay method[15] using Bovine serum Albumin (BSA) as the standard was found to be 0.124 µg/µL.

2.2 Methods

Fabric was treated with an aqueous solution containing a mixture of catechol and potato juice in various proportions. Treatment was carried out at variable temperature (Table 1) in an Infra red dyeing machine DLS – 7000 (Daelim Starlet, Korea) equipped with six medium wave IR heating tubes. The materials-to-liquor ratio (MLR) was kept constant at 1:25.

2.2.1 UV- Vis Spectra

Ultraviolet - visible spectrum was recorded individually for aqueous solution of catechol and Potato Juice as well as that of the mixture. Catechol solution of 0.01% was prepared. One millilitre of freshly prepared juice was mixed with 99 mL of water to prepare 1% v/v solution. Two millilitre of this mixture was added to 10 mL of catechol solution to form the coloured complex. This mixture was then diluted 100 times and used to record the UV-vis spectra using a D-2750 UV-Vis spectrophotometer (Shimadzu, Singapore).

2.2.2 Colour Strength of Dyed Wool Fabric

Reflectance spectrum of dyed wool was recorded. Dyed samples were evaluated for colour strength using Kubelka Munk equation. *K/S* was recorded at 4 different positions on each sample and the average value was recorded. Computer colour matching system (Gretag Macbeth, USA) was used at 10° observer and D_{65} illuminant.

2.2.3 Light and Wash Fastness

Dyed samples were tested for colour fastness to light using Xenon arc lamp tester by Atlas Xenotest Alpha LM, as per ISO 105 B02. Samples of size 2×6 cm were exposed and rated against the standard blue wool samples of grade 1-8.

Table 1—Levels used in Box and Behnken design

Variable	Levels		
	Low(-1)	Medium(0)	High(+1)
Temperature, °C (X_1)	30	60	90
Concentration of catechol, g/L (X_2)	1	3	5
Concentration of potato juice, g/L (X_3)	5	15	25

Color fastness to washing and staining on adjacent fabric was tested in a launderometer at the speed of 40 rpm in accordance with the method prescribed in ISO 105 C06. Assessment was carried out with the help of Grey Scales.

2.2.4 X ray Diffraction (XRD)

XRD spectra were recorded for untreated and dyed wool to ascertain if there was any effect of *in situ* colour development on fibre crystallinity. Powdered samples were mounted onto sample stage to record the crystallinity index. Sample stage was mounted on horizontal axis and the diffracted beam optics and the detector were mounted on 2θ axis. The scanning rate was 0.5° /min at 2θ= 10° - 35° under acceleration voltage of 30 kV and 20 mA. X'pert PRO Xray diffractometer (PAN alytical, Netherlands) equipped with an X-ray tube producing monochromatic CuKα radiation was used for the purpose.

2.2.5 Experimental Design

The response surface methodology (RSM) is an empirical modeling technique used to evaluate a set of controlled experimental factors and observed results[16]. The factor levels in this study were evenly spaced and coded as –1 (low), 0 (central point) and +1 (high), as shown in Table 1. The influence of process variables [(X_1 -temperature (°C), X_2 –concentration of [catechol] (g/L) and X_3 –concentration of potato juice (g/L)] on the colour of wool was studied using a three level, three factorial Box and Behnken Design, leading to

15 experiments (Table 2). The responses analyzed were colour strength (*K/S*) and colour co-ordinates (L^*, a^*, b^*). Data was analyzed using Design Expert software. The module applied a quadratic polynomial equation to analyze the relation of each response with the independent variables as shown below:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_1X_2 + b_5X_2X_3 + b_6X_1X_3 + b_7X_1^2 + b_8X_2^2 + b_9X_3^2 + E \quad \ldots (1)$$

where b_0-b_9 are the regression coefficients; X_1, X_2 and X_3, the factors studied; E, an error term; and Y, the measured response (*K/S* value).

3 Results and Discussion

A range of *in situ* colours varying from light pinkish brown to deep reddish brown has been synthesized on wool by treating it with a mixture of catechol and fresh potato juice at different process conditions (Fig. 2). The process mimics the browning phenomenon witnessed in nature. Compounds present in potato juice have the property of catalysing the oxidation of phenolic compounds to produce delocalised free radical as the oxidised intermediate. They oxidize phenolic compounds and form coloured quinones with sulphhydryl and amino groups in wool[17].

Table 2 — Experiment conditions obtained by Box and Behnken surface response design

Sample No.	Temp. of bath, °C (X_1)	Concentration	
		Catechol (X_2), % w/v	Potato juice (X_3), % v/v
1	60	1	5
2	90	3	25
3	90	5	15
4	60	5	25
5	30	5	15
6	30	3	5
7	60	3	15
8	60	3	15
9	90	1	15
10	60	1	25
11	60	3	15
12	30	3	25
13	60	5	5
14	90	3	5
15	30	1	15

3.1 Spectroscopic Analysis

In the spectroscopic analysis, catechol shows a sharp peak at 284.5 nm while potato juice shows a broad peak, having λ_{max} value coinciding with that of catechol at 286 nm. This is because potato juice also contains phenolic compounds similar to catechol which are responsible for browning of the fruit. The coloured complex also shows the main peak at 286 nm, which is attributed to the presence of complex conjugated structures in the melanin molecule[18]. A second broad peak is observed between 280 nm and 600 nm. Reflectance spectra of the coloured fabric also shows a diffused spectrum with no sharp peak. Absence of a sharp peak in the visible region is a characteristic of several coloured compounds found in nature. In this case, colour is due to the synthesis of compounds belonging to the family of melanins. Melanins are heterogeneous copolymers having large complex conjugated aromatic structures with phenylene and oxyphenylene units resulting from the C-C and C-O coupling of phenols[19]. In Fig. 3[20], the curly red lines indicate the sites of attachment to wool protein. The exact structure of melanins is as yet unknown since standard analytical methods like UV and visible light analysis do not yield any meaningful data about this compound[21]. For a detail hypothesis on the chemical structure of melanin, the reader is referred to an interesting paper by Cheun[21].

3.2 XRD Studies

The X Ray diffraction spectra of dyed and undyed wool was studied. The 2θ peak for both samples is detected at 20.5, indicating that the colorant molecules are randomly distributed and do not

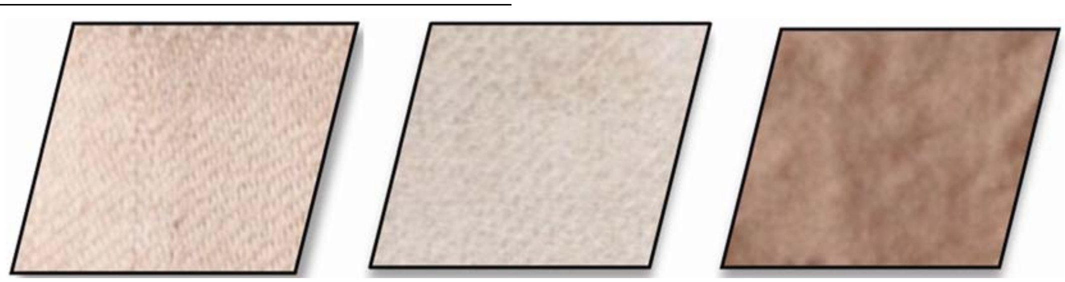

Fig. 2 — Range of colours produced in wool

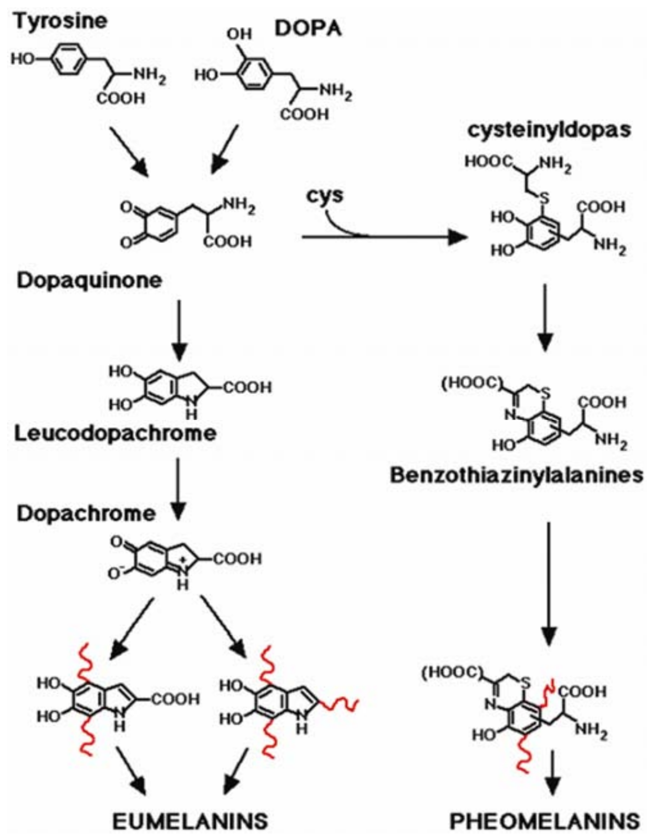

Fig. 3 — Synthesis of melanin pigment[20]

follow a regular pattern. No change is observed in the crystallinity of wool fibre due to *in situ* synthesis of colour.

3.3 Light and Wash Fastness

Since melanin is highly stable and insoluble compound, the fastness is expected to be high. Results of fastness tests indicate that wash fastness of these colours lies in the range of 4-5 (excellent) and light fastness grade is 4 on a scale of 1-8.

3.4 Design of Experiments

The colour developed in textiles is affected by several process parameters such as concentration of components, time and temperature of treatment. Optimisation is required to find the best possible combination of process variables with respect to conservation of energy as well as materials used. A statistical design of experiments is employed to determine the process parameters that influence the color synthesised by coupling of potato juice with catechol in wool (Table 3).

A wide range of colours can be obtained by changing the process variables. Although the *K/S* value for test samples varies widely from 1.94 to 10.65, there is not much change observed in the a* and b* values among samples, indicating that a tone on tone increase occurs in deeper coloured samples without any change in the hue or shade. Maximum depth of colour (*K/S* = 10.65) is obtained for Sample 2 where the concentration of potato juice as well as the temperature of treatment is at the highest level. The next highest K/S value of 9.04 is achieved for Sample 3, where the highest concentration of catechol is used at the highest temperature of treatment. Lower values of *K/S* (1.94 - 2.67) are obtained when treatment was carried out at the least temperature, irrespective of the concentration of catechol and potato juice used. This indicates that all parameters individually as well as collectively play a role in determining the final colour obtained on wool. Statistical analysis is carried out to study these interactions further.

Table 3 — Colour characteristics of test samples treated as per DOE

Sample No.	*A/B/C*	*K/S*	L*	a*	b*
Control	-	0.368	87.33	-1.57	11.25
1	0/-1/-1	4.52	47.14	9.85	12.64
2	+1/0/+1	10.65	33.25	7.67	10.03
3	+1/+1/0	9.04	35.94	8.74	10.43
4	0/+1/+1	3.25	50.31	8.06	9.82
5	-1/+1/0	2.19	57.17	9.38	11.31
6	+1/0/-1	1.94	60.07	9.63	12.37
7	0/0/0	3.49	48.62	7.44	9.89
8	0/0/0	3.68	48.81	8.24	11.14
9	+1/-1/0	7.38	39.82	7.07	11.82
10	0/-1/+1	5.13	49.22	8.34	9.37
11	0/0/0	3.49	48.87	7.85	9.42
12	-1/0/+1	2.67	52.83	9.31	12.17
13	0/+1/-1	3.14	64.36	9.83	11.38
14	+1/0/-1	5.32	45.09	8.92	12.06
15	-1/-1/0	3.02	52.75	8.72	12.71

A–Temperature, *B*–Concentration of catechol and *C*–Concentration of potato juice.

3.5 Statistical Analysis

Results obtained from ANOVA show that the model is highly significant for the treatment ($p < 0.0001$). Temperature of treatment has the maximum effect on colour; *K/S* increases with increase in temperature. At potato juice concentration of 15%, *K/S* increases from 1.94 to 8, while at potato juice concentration of 25%, *K/S* value is increased from 1.94 to 10.65 as the temperature is increased from 30˚C to 90˚C. The next significant parameter is concentration of potato juice, having the residuals distributed along a well randomized straight line. The

R^2 value, being the measure of the goodness of fit of the model, indicates that 93.69% of the total variation is explained by the model. The coefficients of variables included in the model for *K/S* response show high significance ($p < 0.05$). The coded form of equation obtained for *K/S* response is given by the following equation:

$$K/S_{\text{Coded}} = 3.66+2.82*A-0.30*B+0.85*C+0.62*A*B+1.15*A*C-0.13*B*C+1.44*A^2+0.30*B^2+0.042*C^2 \quad \text{... (2)}$$

where A is the temperature of the treatment; B , the concentration of catechol; and C , the concentration of potato juice. From the quadratic factor influence, only factor A has p value < 0.0500. Therefore, in this case, A&A^2 are found to be the most significant terms. Factor C having a p value of 0.0750 is less significant. The insignificant factors are eliminated from the previous equation and a simplified equation is obtained. The concentrations of [catechol] (1 - 5 %) and potato juice (5 - 25 %v/v) used in the study do not exhibit any effect on *K/S* values. The above simplified equation is given below:

$$Y_{\text{Coded}} = 3.66+2.82*A+0.85*C+1.15*A*C+1.44*A^2+0.042*C^2 \quad \text{... (3)}$$

The graph plotted between expected values and observed values of *K/S* shows an R^2 value of 0.905 indicating a good correlation between the two. Thus, it can be concluded that, within the design space of the factors studied, this model can be used to accurately predict the colour of wool when it is treated with a combination of catechol and potato juice.

3.6 Study of Contour Plots

Contour plots obtained from the Design Expert Software can be used to predict the recipe for obtaining a particular shade on wool. The plots are shown in Fig. 4. It can be seen from Option 1 in Table 4 that if the highest temperature level (90°C) is used, and the concentration of catechol is kept at the lowest, the *K/S* value increases with the increase in concentration of potato juice. However, the maximum value 10 of *K/S* can be obtained only with higher concentration (3.5%) of catechol. It is interesting to note from Option 2 that whether the concentration of potato juice is 25% or 15%, the *K/S* value remains same at 30°C.

Option 3 shows that the entire range of colours from light to deep (*K/S* 2-10) can be obtained if the highest concentration of potato juice (25%) is

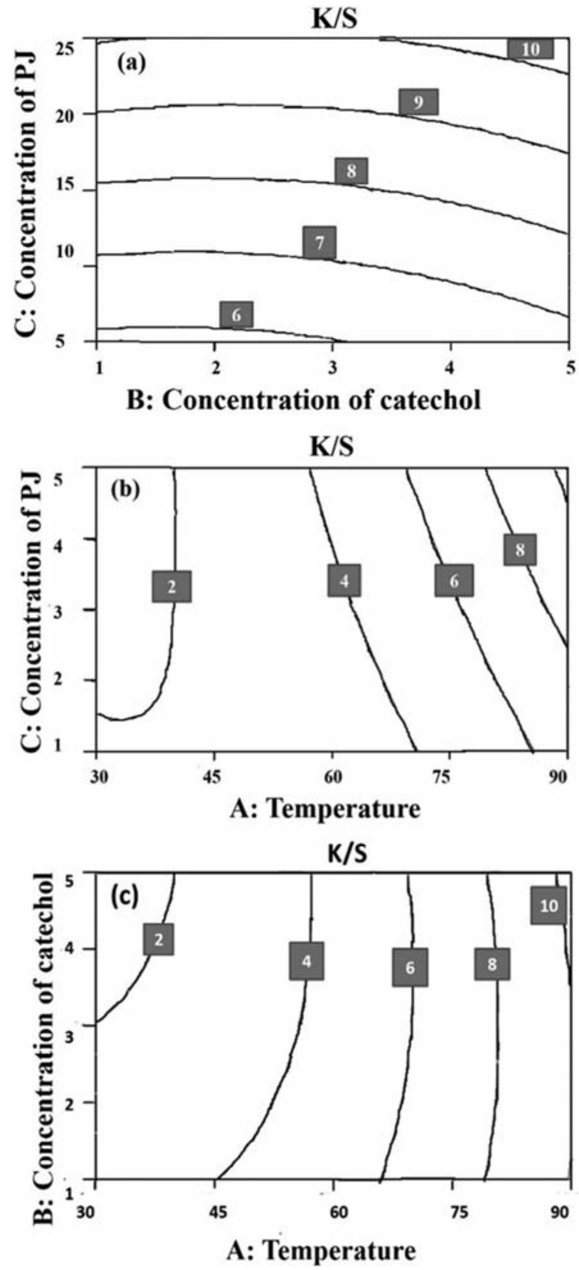

Fig. 4 — Selected contour plots obtained from RSM (a) Temperature -90°C, (b) Concentration of catechol – 5%, and (c) Concentration of potato juice - 25%.

combined with lowest concentration of catechol-1%. The depth of colour increases with increase in the temperature of treatment. Even the maximum concentration of the two reactants, when used at the lowest temperature (30°C), yields only a very light shade. This indicates that deep shades can only be obtained at higher temperatures, irrespective of the concentration of reactants used. Interestingly, a wide gamut of shades can be obtained simply by varying

Table 4 — Recipe options for obtaining a specific *K/S* value on wool

K/S	Option 1 (*A*-90°C)	Option 2 (*B*-5%)	Option 3 (*C*-25%)
2	–	A 30°C, C 1.5%	B 5%, A 30°C
4	–	A 58°C, C 1%	B 1%, A 45°C
6	B 1%,C 5%	A 70°C, C 1%	B 1%, A 66°C
7	B 1%,C 11%	–	–
8	B 1%,C 15%	A 80°C, C 2.5%	B 1%, A 80°C
9	B 1%,C 20%	–	–
10	B 3.5%,C 25%	–	B 1%, A 90°C

A–Temperature, B–Concentration of catechol and C–Concentration of potato juice

the temperature from 30°C to 90°C using different concentrations of catechol.

It can be concluded that the synthesis of colorant does not take place at the temperature of 30°C and whatever colour is observed may be due to the inherent colour of catechol itself. Therefore, 30°C should not be used as a treatment temperature and only >45°C should be used. Similarly the table can be used to pick the most optimum process parameters for getting a particular depth of colour on wool.

4 Conclusion

In this study, a process for *in situ* dyeing of wool in a range of shades has been proposed. The process of dyeing is based on a one step process of combining potato juice with polyphenolic compound catechol. The colorant formed inside wool is based on melanin, which is a heterogeneous polymer having an indeterminate chemical structure. Optimised recipes for obtaining various colour depths on wool have been proposed using the response surface methodology. The depth of colour produced is found to be significantly dependent on the temperature of treatment. No colour is observed when treatment is conducted at 30°C, while deep brown colour is obtained when treatment is carried out at 90°C. Concentration of the individual components used for colouring does not have a significant effect on the colour obtained.

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