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Variation and storage stability of juices extracted from potatoes and their influence on *in-situ* coloration of wool

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In this study, juices from four different varieties of potatoes are extracted and then stored at 4˚C. Thereafter enzymes have been assayed at different periods and the juices are applied for the coloration of wool. Results reveal that potato juices demonstrate good storage stability. Though there are variations in the quantity of enzymes present in potatoes, the same is not reflected in the *K/S* values of the wool. The polymerization of catechol with potato juice results in coloured quinone derivatives which further react to form dimers, oligomers and polymers as confirmed by UV-Vis spectroscopy studies.

Keywords: Polyphenol oxidases, Potato juices, Storage stability, Wool fibres

1 Introduction

Conventional dyeing of wool involves the use of synthetic dyes, such as acid dyes, reactive dyes, metal complex dyes with chemical auxiliaries through long dyeing application processes at elevated temperatures. This includes not only high energy consumption but also high waste water generation. These difficult circumstances create the need for finding out new types of ecofriendly dyes with potential benefits in reducing or eliminating additional chemical usage, saving time and energy and reduced effluent load. In the recent years, the adoption of an alternative dyeing approach using oxidative enzymes as ecofriendly biocatalysts has been well recognized[1].

Polyphenol oxidases (PPO) (EC 1.14.18.1) monophenol monooxygenases are a group of copper containing enzymes found in plants, animals, fungi and bacteria[2]. PPO's generally include tyrosinase, laccases, monophenol oxidase, cresolase and catechol oxidases. PPO's oxidize phenolic compounds to phenoxyl radicals, which next undergo nonenzymatic reactions to form colored dimeric, oligomeric and polymeric products. These coloured oxidation products are capable of being adsorbed onto or reacting with numerous textile fibres for fibre coloration[3]. This enzymatic approach has been used by several researchers for dyeing both cellulose and protein fibres[4-6]. Bai *et al*[7]. combined the laccases with catechol along with mediators such as 2,2 azino-bis-(3-ethylthiazoline-6-sulfonate) (ABTS), 2,6-dimethoxyphenol (DMP), 2-methoxy phenol to produce colours on wool fabric. In a recent paper, Netithammakorn *et al*[8]. investigated the use of peroxidases to catalyse different aromatic compounds in the presence of hydrogen peroxide to produce diverse colour palette on wool fabrics.

Several above-mentioned studies reporting the application of enzyme catalyzed coloration of wool fibres, have used the pure enzymes. However, the production of pure enzymes involves process, such as isolation and purification, making them expensive for application on large scale dyeing of textiles. Nevertheless, if these expensive enzymes are substituted by cheaper, safer and more abundant & easier to use options, the possibility of enzymatic dyeing process accepted at large scale can be enhanced. Vegetable juices are safe and abundant source of enzymes. Potato (*Solanum tuberosom*) is one of the important common vegetables consumed worldwide. It is rich in carbohydrates with significant amount of amino acids, vitamins, minerals, fats, sugars, proteins, phenolic compounds and PPO enzymes[9]. When potato encounters any damage, like cut, bruise or crush and exposed to air, it becomes brown, due to the PPO enzyme catalysed browning reactions. During the industrial processing of potatoes for the starch production, the process leads to generation of large amount of potato juice as waste

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effluent[10]. Approximately 3.5 tons of potato juice is generated as by product for producing a ton of potato starch[11]. These waste juices have a great scope as a source of PPO to be utilized in coloration of wool. In the previous work, the fresh potato juice extracted from potatoes without purification was combined with diphenolic compound catechol (a known substrate for PPO) to develop series of brown shades on wool fabric[12].

There are many varieties of potatoes available in the market and the result obtained in each case could be different. Also, for industrial application, large amount of juice would have to be extracted and stored before use. Therefore, juices should demonstrate good storage stability in order to realize their full potential as catalysts. Both these factors, viz variety of potato and time lapse between juice extraction and its use, can be possible sources of variation in colour. It is critical to ensure the consistency of the raw material in order to obtain consistent results. The current work is therefore undertaken to study the effect of these two factors on the colour obtained. To establish the statistical significance of results obtained, *t*-test analysis is used. UV-Vis spectra are employed to investigate the oxidation mechanism of catechol with potato juice. Colour coordinates, colour depth, colour evenness and colour fastness of the wool fabrics have been evaluated and the results are discussed.

2 Materials and Methods

2.1 Materials

Catechol (benzene-1,2-diol) (Sigma Aldrich, USA) having molecular weight of 110.11g/mol was used. Lissapol N (non-ionic surfactant) was purchased locally. Fresh potato tubers (*Solanum tuberosom)* of LSG Packed, Chipsona, Nilgiri and Haldwani varieties were procured from the local market. The potato varieties used are shown in the Fig. 1. DI water was used throughout for all experiments.

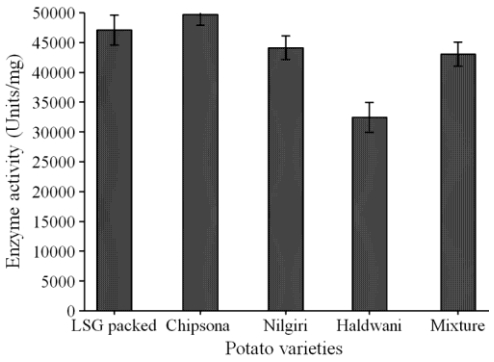

Fig. 1 — Enzyme activity determined for different varieties of potato

2.2 Wool Fabric

Hundred per cent wool fabric (matt weave, 30 µm fibre diameter, areal density 86.3 g/m^2, warp/weft count 23.5/18.02 tex and ends/picks per cm 22/22) was used. RFD fabric was obtained from Shingora Textiles, Ludhiana, Punjab and used without further preparation.

2.3 Characterization of Potato Juice

2.3.1 Comparison of Potato Varieties and Storage Stability of Potato Juice

All four varieties of potatoes were separately washed, padded dry, grated and squeezed to extract the juice. One hundred fifty grams of each variety of potato yield about 100 mL of juice. The fifth sample was prepared by mixing equal volumes of juice of all four varieties. All 5 juices were allowed to stand for 20 min at 35°C in a beaker to allow the starch to settle down. The clear juice at the top was decanted and then stored at 4˚C for 120 h. Thereafter, 20 mL aliquots of juice were removed after every 24 h. Out of this, 5 mL was used for estimating the quantity of PPO enzyme present in potato juice as per spectrophotometric procedure proposed by Sigma Aldrich[13] and the rest was used for colouration of wool. The fifth juice sample was used for the UV-visible spectra and colour fastness tests.

2.3.2 UV- visible Spectra Analysis

An aqueous solution containing catechol (0.05% w/vol) and potato juice (25% vol/vol) was incubated to study the UV-visible spectral analysis. The solutions were incubated at 30˚C for 0, 1, 2, and 4 h to study the change in colour with time. Solutions were diluted 10 times and UV -vis spectra scan was recorded in the region 200 - 700 nm. The catechol and potato juice solution was incubated independently as a reference. All studies of spectrophotometric evaluations were carried out using UV-Visible spectrophotometer (Model: D-2750, Shimadzu, Singapore).

2.4 Colouration of Wool

The *p*H of all the 5 juices were adjusted to 4.5 with acetic acid. To prevent any enzymatic oxidation, the juices were used for coloration within half an hour of preparation. Wool fabric was treated in a separate bath containing 1.75% precursor catechol, 25% potato juice (all five juices) at *p*H 4.5 and 70°C for 60 min in HT-HP beaker dyeing machine STARLET DLS – 7000, (DaeLim Starlet co Ltd, Korea) equipped with six medium wave infrared heating tubes. The beakers

were mounted on a mandrel that rotates alternately in the clockwise and anticlockwise direction. Treated samples were soaped with Lissapol D at 80°C for 3 min to remove the unreacted components. This was followed by thorough rinsing with cold water and drying. The samples were conditioned at 65% RH and 20°C for 24h. DI water was used for all experiments.

2.4.1 Colour Measurement

Computer colour matching system (Gretag Macbeth, USA) with 10° observer and D_{65} illuminant was used for recording the colour coordinates (L^*, a^*, b^*), colour strength (*K/S*) and colour difference (ΔE) of the dyed samples. The Kubelka Munk equation was used for determining colour strength.

Colour evenness (ΔE) between samples dyed with fresh extracted juices at 0h and stored juices at 120h are represented as ΔE and calculated using the following equation:

$$\Delta E = [(\Delta L^*) + (\Delta a^*) + (\Delta b^*)]^{1/2} \quad \ldots (1)$$

where ΔL*, Δa*, and Δb* represent the differences between the corresponding units of the samples. Colour measurements were recorded at 4 different positions on each sample and the average value was recorded. To establish the significance of results obtained, *t*-test analysis was used.

2.4.2 Colour Fastness Tests

Colour fastness in terms of washing and staining was tested using Launderometer according to ISO 2 (3361-1984) test method. Rubbing fastness under dry & wet conditions was performed using Crockmeter according to ISO 105-X12 test method. Xenon arc lamp tester (Atlas Xenotest Alpha LM, USA) was used to test the colour fastness of samples to light, according to the procedure ISO 105 B02. Samples of size 2×6 cm were exposed and rated against the Standard Blue Wool samples of Grade 1-8. Assessment was carried out with the help of Gray scales.

3 Results and Discussion

3.1 Comparison of Enzyme Activity of Different Varieties of Potato

Several varieties of potato are available in the local market. Also, in a processing house, it is not possible to extract juice and use it immediately. It would have to be stored for some time before processing. The variety of potato used, as well as the duration of storage of juice can influence enzyme activity and consequently the colour obtained. Thus, some amount of standardization is required.

In order to study these aspects, four local varieties of potato have been procured, their juices are extracted and compared in terms of enzyme activity as well as storage stability. A fifth sample was prepared by mixing the juice of all four varieties to study the effect of mixed variety. All samples are assayed for enzyme activity and used for colouration of wool as per the standard recipe. Results of enzyme assay are shown in Fig. 1. The amount of PPO enzyme detected in various varieties ranges from 32497 units/mg (Haldwani) to 49759 units/mg (Chipsona). The *t*-test for significance ($\alpha = 0.05$) has been performed between every two varieties to see whether the enzyme activity in the five test solutions is statistically similar. The *p*-values are given in Table 1. It can be seen that enzyme activity of Haldwani variety is significantly different from all other varieties of potato studied. It is interesting to note that while Chipsona and Nilgiri are significantly different from each other, they both are similar to LSG variety.

Figure 2 shows that the *K/S* values of samples treated with various varieties of potato juice is 8 ± 0.5 except for Haldwani variety which is lower (6.63). The mixture has a *K/S* value equal to the mean (7.84) of all varieties. A direct correlation between *K/S* value of coloured fabric and the concentration of enzyme in potato juice could be established (Fig. 3). The statistical significance of *K/S* values on enzyme activity of potato varieties by *t*-test was performed (Table 2). Here, the significance test ($\alpha = 0.05$) has been carried out by comparing *K/S* values of every two potato varieties. From the *t* test, it is observed that, there is no significant difference in mean *K/S*

Table 1 — Comparison of enzyme activity in various varieties of potato (*t*-test)

Potato varieties	*p*-values				
	LSG packed	Chipsona	Nilgiri	Haldwani	Mixture
LSG packed	-	0.216	0.187	0.002	0.0958
Chipsona	0.216	-	0.023	0.0006	0.013
Nilgiri	0.1871	0.0233	-	0.0032	0.5472
Haldwani	0.002	0.0006	0.003	-	0.0045
Mixture	0.0958	0.013	0.547	0.0045	-

values of combinations of potato varieties other than Haldwani. Although there is significant difference in amount of enzymes between some of the potato varieties, the same is not reflected in the *K/S* values.

3.2 Storage Stability of Juice Extracted from Various Varieties of Potato

Juices of 4 varieties of potato have been extracted and their stability is tested for up to 120 h of storage at 4°C. An aliquot is removed from every sample after every 24 h. It is assayed for enzyme activity and also used for colouring of wool. The enzyme activity is compared with that of freshly extracted juice and represented as % residual activity. *K/S* of wool dyed with fresh juice was compared with that of wool dyed with stored samples (Fig. 4). It can be seen from Fig. 5 that enzyme activity reduces by 30 - 47% in various varieties of potato after 24 h of storage. After 120 h of storage, the activity is reduced by 70-90%. Chipsona variety is most stable as it retains nearly 44% activity after 120 h of storage. It can thus be seen that different varieties vary significantly in terms of storage stability. It can thus be understood that storage of potato juice leads to severe loss of enzyme activity. However, due to the nature of reactions involved in enzyme catalyzed colouration of wool, loss of enzyme activity does not affect the colour obtained on wool (Fig. 4). Juice can be stored for up to 120h in a refrigerator after extraction, without any effect on the colour characteristics of wool. Also, the final shade obtained from various test varieties is not significantly different.

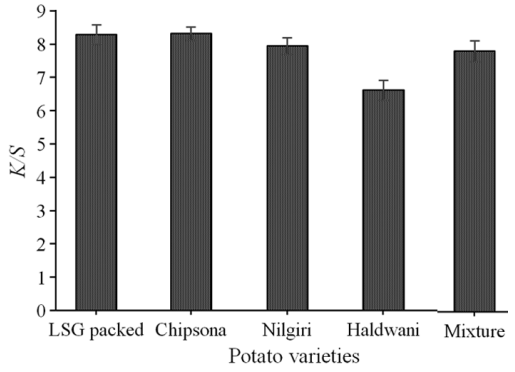

Fig. 2 — Colour value obtained with different varieties of potato

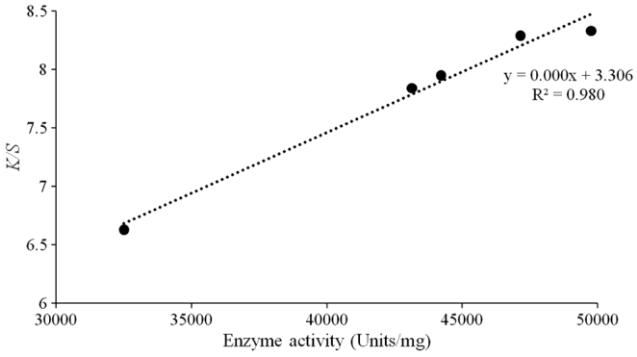

Fig. 3 — Correlation between enzyme activity and *K/S* value

These results provide indisputable evidence that the process of colouration of wool with potato juice occurs by both enzymatic and non-enzymatic pathways. When the juice is fresh, the enzyme is active and the enzymatic pathway is initiated. This pathway proceeds through enzyme mediated oxidations and leads to formation of intermediate products, such as *o*-quinones. During juice storage, the natural process of browning proceeds in the juice, leading to exhaustion of enzyme and formation of intermediate products. These intermediate products combine with catechol via non-enzymatic pathway, leading to formation of deep coloured compounds. Wool fibres contain a greater number of amino acids such as tyrosine and nucleophilic groups such as sulfhydryl and amino groups. The tyrosine residues present in wool gets oxidized and the electrophilic *o*-quinones condense and react with nucleophilic groups such as sulfhydryl (Thiol) and amino groups on wool. This finally leads to the synthesis of stable, covalent bonds of deeply coloured pigments called melanins of indeterminate structure bound to wool proteins. The mechanism of colour formation on wool is depicted in Fig. 6. This mechanism was further supported and confirmed by UV-Vis spectroscopy studies.

3.3 *UV-Vis Spectra*

UV-visible spectra were employed to monitor the oxidation reaction of catechol catalyzed by potato

Table 2 — Statistical significance of *K/S* values on enzyme activity of potato varieties by *t*-test

Potato varieties	*p*-values				
	LSG packed	Chipsona	Nilgiri	Haldwani	Mixture
LSG packed	-	0.8798	0.171	0.0018	0.1442
Chipsona	0.8798	-	0.201	0.0031	0.1616
Nilgiri	0.1711	0.2007	-	0.0042	0.6807
Haldwani	0.0018	0.0031	0.004	-	0.0104
Mixture	0.1442	0.1616	0.681	0.0104	-

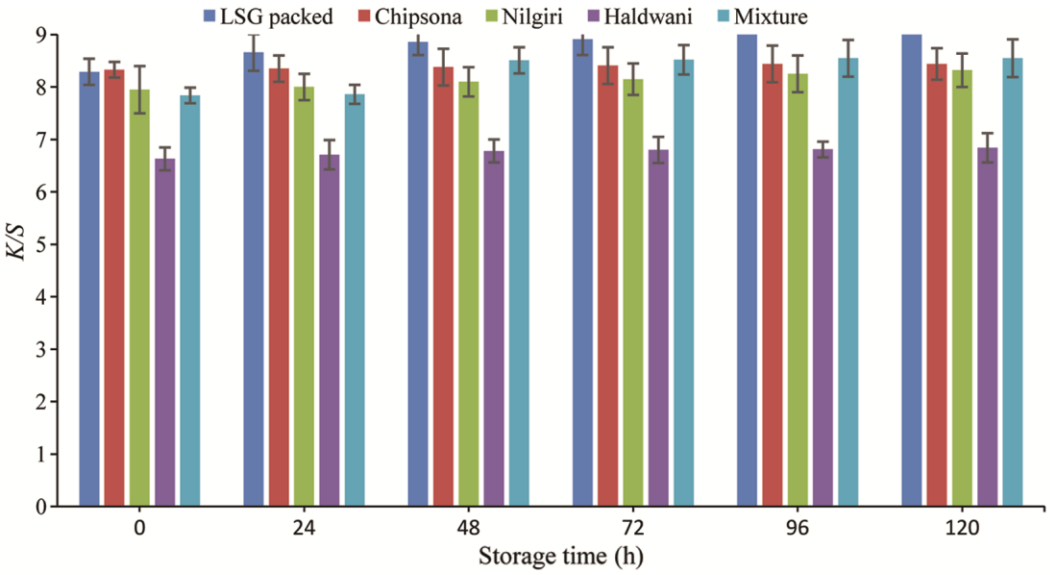

Fig. 4 — Effect of length of storage time of potato juice on *K/S* value

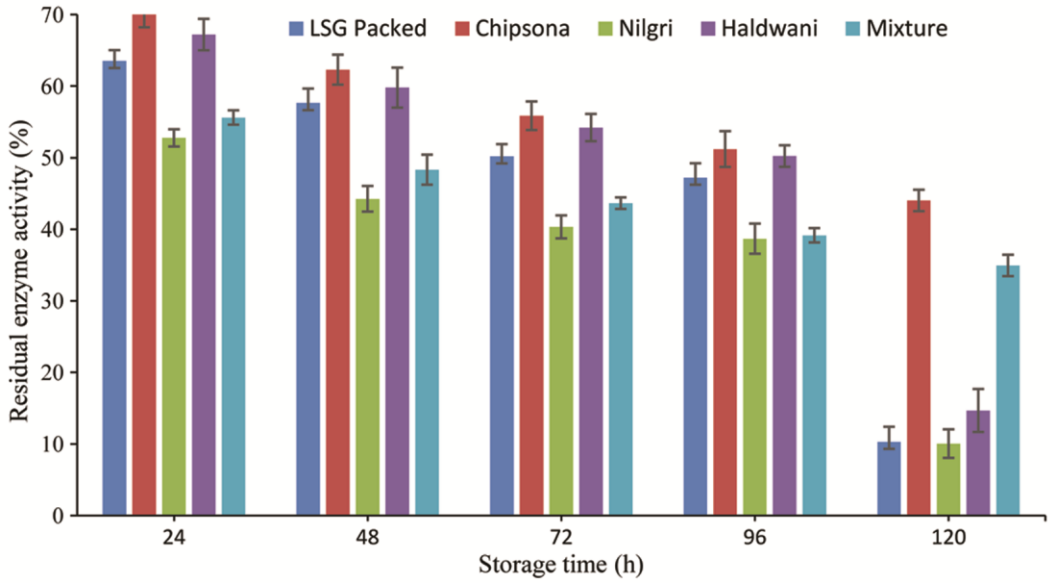

Fig. 5 — Effect of storage time of potato varieties on enzyme activity

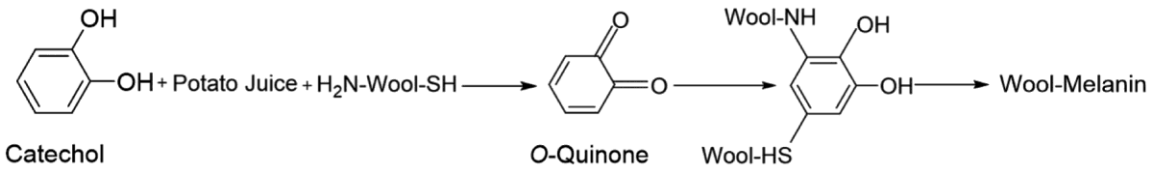

Fig. 6 — Mechanism of colour formation on wool

juice in aqueous solution. A mixture of potato juice and catechol incubated for various time duration are shown in Fig. 7. An absorption peak is observed at 280 nm with a peak intensity of 0.35 in case of freshly formed mixture. With increase in time of incubation, the intensity of the peak increases and at the same time it becomes broader. Hence, the change in peak intensity and curve broadening could be taken as a measure of the formation of melanin from the catechol and potato juice mixtures[14].

Potato juice consists of phenolic compounds, polyphenol oxidase enzyme, vitamins, minerals, proteins and sugars. Of these compounds, polyphenol oxidase enzyme could undergo reaction with itself and other phenolic compounds to form coloured compounds. These reactions might be accelerated by the addition of catechol to potato juice, leading to the formation phenoxy radical which is further turned into *o* – benzoquinone intermediates[14]. These quinones are very reactive and undergo non-enzymatic radical coupling reaction with the intermediates resulting in the formation of dimers, oligomers and polymers[4]. The reason for the disappearance of the sharp peak after 2 h may be due to the consumption of active intermediates during further reaction[14].

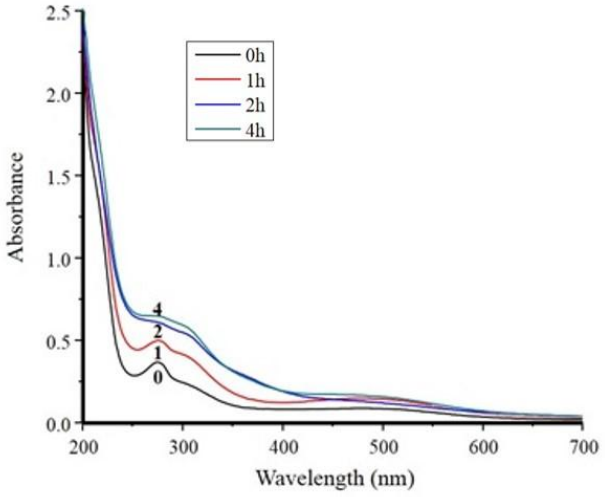

Fig. 7 — UV- vis spectra of mixture of catechol and potato juice after varying duration of incubation

3.4 *Colour Coordinates and Evenness*

Results of the colour measurements obtained for wool fabrics dyed with fresh potato juices (0 h) and those stored after 120 h is shown in Table 3. It can be observed that the results of *K/S* values are not consistent with those obtained for enzyme activity of stored juices (Fig. 5). Except the LSG variety, the samples dyed with other potato varieties, such as Chipsona, Nilgiri, Haldwani and mixed juice show only a slight increase in *K/S* values, and L^* values (the lower L^* value, darker the colour) are slightly decreased, indicating that the samples remain lighter. Interestingly, in case of LSG juice treated samples, *K/S* values increase from 8.29 to 10.31, and the L^* value decreases from 37.7 to 35.1, indicating that the samples become darker. Visual observation of the samples also show that LSG packed variety appears darker when compared with other varieties. However, the a^* (positive = redder; negative = greener) and b^* (positive = yellower; negative = bluer) values of all the samples remain unchanged in the positive side, displaying the redder and yellower tone of the samples.

Evenness of dyed sample is considered as an essential criterion that controls the success of dyeing in obtaining the reproducibility of shade. The

Table 3 — Colour measurements of wool fabrics treated with various potato varieties stored at different duration (h)

Potato varieties	L^*		a^*		b^*		*K/S*		Shade		ΔE
	0h	120h	0h	120h	0h	120h	0h	120h	0h	120h	
LSG Packed	37.7	35.1	8.1	7.9	12.5	12.2	8.29	10.31			2.6
Chipsona	38.2	37.9	5.7	5.5	11.7	11.6	8.33	8.44			0.4
Nilgiri	41.7	40.5	5.9	5.7	12.6	12.4	7.95	8.32			1.2
Haldwani	38.7	37.7	5.6	5.4	11.6	11.3	6.63	6.84			1.1
Mixture	38.4	37.1	6.1	5.8	11.6	11.8	7.84	8.55			1.4

Table 4 — Colour fastness properties of wool fabrics treated with juice stored at different durations

Duration h	Colour change	Wash fastness (Colour staining)						Rub fastness		Light fastness
		Acetate	Cotton	Nylon	Polyester	Acrylic	Wool	Dry	Wet	
0	4	5	5	3-4	5	5	3-4	4	4	5
120	4	5	5	3-4	5	5	3-4	4	4	5

evenness of the dyed fabrics is assessed using the ΔE values calculated between the samples dyed with fresh juices at 0h and stored juices at 120 h. It can be seen from Table 3 that the ΔE values are highest for LSG packed variety (2.6) followed by mixture (1.4), Nilgiri (1.2), Haldwani (1.1) and lowest for the Chipsona (0.4). According to the regulation of the National Bureau of Standards, it is acceptable for industrial application when this ΔE value is <2.0[15]. Therefore, it can be inferred that, except the LSG packed variety ΔE values of all the samples are in the acceptable range.

3.5 *Colour Fastness*

The colour fastness of the wool fabrics treated with mixed juice stored at different duration has been evaluated and results are presented in Table 4. It can be observed that both wash and rub fastness are found to be good (4). Staining on nylon (3-4) is observed for both the samples. This is attributed to the structural similarities between wool and nylon fibres. However, the colour fastness of the samples remains unaffected even after using the potato juice stored up to 120 h. Light fastness is found to be good with the rating of 5. The reason may be due to the presence of the covalently bound melanin pigment on wool, which confers photo protection from the artificial light source.

4 Conclusion

This study is an attempt to investigate the effect of variation and storage stability of different varieties of potato juices and its influence on catalyzing the oxidative polymerization reaction of catechol to dye wool. Results of the study show that storage stability of juices is good and colour is not affected by the variety of potato or the length of storage used. The mechanism of colour formation inside wool is complex and based on a mixture of enzymatic and non-enzymatic pathways due to the presence of a large number of compounds in the juice. The phenolic compounds formed (*o*-quinone) intermediates in the first stage of oxidation, which further undergo reactions to link up with covalent bonds to form deep coloured compounds. The dyed fabrics display light to deep brown shades with acceptable fastness properties.

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