

Technological Evaluation of Laccase Enzyme in Discharge Printing Using Natural Colours

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Abstract: *The suitability of using eco-friendly laccase enzyme instead of the harmful reducing agents to obtain discharge printing on natural fabrics (cotton, wool and silk) coloured with different natural dyes (Henna, corcoma or leucaena) was investigated under a variety of conditions. The latter comprise, the effect of enzyme concentration, steaming time and temperature. The results obtained indicate that, increasing the concentration of laccase enzyme formula from 50 to 200 g/Kg pastes accompanied by an increase in the % decrease in the colour strength (K/S) of the printed natural fabrics. The optimum conditions to obtain colour discharge on using laccase enzyme were achieved. Samples of different attractive colour half-tone could be also achieved via changing enzyme's concentration or in admixture with conventional reducing agent. Harmful reducing agents which are used in discharge printing viz. Redoxal-Z could be substituted partially or completely by laccase enzyme in order to reduce the price and environmental pollution.*

Keywords: Textile, Printing, laccase, natural dyes and discharge.

1. Introduction

There are three basic styles of printing a colour on fabric namely: direct, discharge and resist (Abd El-Thalouth, et al., 2012). In printing discharge style, which is considered as the most technically complete art of printing, both science and art combine to give a great aesthetic look to the printed fabric. Hence it is also the most difficult to produce, but if the science behind this is well understood, the results will be easier to obtain (Layer, 2001).

Discharging refers to the standard way of making small white motifs on a solid dark background (Brito, 2004) in discharge printing, the fabric must first be dyed with dyes that can be destroyed by selected discharging agents. The discharge paste is printed onto the dyed fabric and, usually during subsequent steaming, the dye in the pattern areas is discharged, to produce white discharge. It is also possible to add a discharge resist (illuminating) dye to the discharge print paste, to produce a colour discharge (Milles, 1994).

The success of discharge printing depends on the proper selection of the ground colours (dyes), illuminating dyes and the discharging agent as well as proper printing, fixation and washing conditions (Wang et al., 2007).

Recently, the environmental and industrial safety conditions increased the potential for use of enzymes in textile processing to ensure eco-friendly production. The use of enzymes in textile processing is well-known and for some technologies is well established, e.g. in denim bleaching (El-Hennawi and Abd El- Thalouth, 2010).

Laccase is the newest enzyme class to be introduced into the denim finishing area. The first commercial product was introduced in 1996 (Kierulff, 1997; Vollmond, 1997). This product exhibited good performance, but handling characteristics were not ideal. A fully- formulated solid Laccase has been commercially available for the denim market since 1999 (Muller and Shi, 2001). The commercial

Laccase formula contains Laccase, an enzyme mediator, buffer and a non-ionic surfactant (Novo Nordisk, 1998; Soares et al., 2002).

The first Laccase studied was originated from *Rhus vernicifera* tree and many other Laccase are being discovered and studied from various plant and microbial sources (Mayer, 1987; Reinhammar & Malmstron, 1981; Yaropolov et al., 1994).

The ability of Laccase to catalyse the oxidation of phenolic and non-phenolic compounds has gained much attention over the years in many industrial and environmental fields; particular commercial interest is the potential use of these enzymes to decolourize a wide range of synthetic dyes (Abadulla et al., 2002). The range of dyes, which can be decolourized by using Laccase, may be expanded by additionally applying defined mediators. Mediators are low molecular weight compounds that are easily oxidized by Laccases producing very reactive radicals which attack more complex substrates before return to its original state (Guebitz, 2004; Zille et al., 2004; Almansa et al., 2004).

The present work was, therefore, aimed at using an eco-friendly Laccase enzyme instead of the harmful reducing agents to obtain discharge printing on natural fabrics coloured with different natural dyes.

2. Experimental

2.1. Materials

Fabrics: Wool fabrics: Mill scoured 100% wool fabric supplied by Misr Co. for spinning and weaving (Mehalla El-Kubra) 210 g/m² were used.

Silk fabrics: Mill scoured natural Silk fabric of plain weave 140 g/m² supplied by El-Khateib Co. Souhag, Upper Egypt.

Cotton fabrics: Mill desized, bleached and mercerized cotton fabrics 165 g/m² produced by Misr/Helwan for Spinning and Weaving Company, were used.

Thickener: High viscosity sodium alginate from brown algae, manufactured by Fluka Chemical Co., Denmark was used as a thickening agent.

2- British Gum, supplied by Misr Company for starch and Glucose, Cairo, Egypt.

2.1.3. Dyestuffs (Natural Colouring matter):

Natural Colouring substances used in this work were extracted from turmeric (Curcuma) henna, and leucaena seeds (which have been purchased from local market) according to the procedure described latter.

Enzyme: Denilite ITS which is a commercial product based on Laccase enzyme, supplied by NovoNordisk Company, Denmark.

Other chemicals:

Acetic Acid and sodium carbonate are of the Laboratory grade chemicals, Redoxal Z commercial reducing agent supplied by Daico for chemical Industry, Cairo, Egypt.

2.2 Methods

2.2.1. Extracting of natural colouring matter

From Henna: The colouring matter was extracted from Henna using different solvents via water, Sodium carbonate solution or commercial ethyl alcohol as follows:

On using water: The colouring matter was extracted using 100 g. Henna in 1000 ml water at the boil under reflux for 30 min. The solution was left to cool and then filtrated off. The filtrated was concentrated using a laboratory Rotavapour.

On using Sodium Carbonate: 100 g. of Henna was extracted in 1000 ml sodium carbonate solution 5% at the boil for 30 min under reflux for 30 min. The solution was left to cool and then filtrated off. The filtrated was concentrated using a laboratory Rotavapour.

On using Alcohol: The colouring matter was extracted using 100 g. Henna in 750 ml Ethyl alcohol and 250 ml water at room temperature for 24 hours followed by filtration and the obtained solution was concentrated using a laboratory evaporator Rotavapour.

From Curcuma and Leucaena seeds:

N.B.: Using the aforementioned procedure the colour matter was extracted from Curcuma and Leucaena seeds using water only.

2.2.2. Dyeing Method

The fabrics (wool, silk and cotton) were dyed using natural colouring matter extracted from three natural colours [henna, leucaena, and turmeric (curcuma)] in the presence of sodium sulphate (1-20 g). The pH was adjusted at 6.5 using acetic acid at L.R. (1:50).

The samples were added to the dyeing bath at 30°C then the temperature was raised to 80 °C during 10 minute. The samples were dyed for one hour then rinsed with cold water and finally were air dried.

2.2.3. Discharge printing:

2.2.3.1 Printing fabrics with discharge printing:

The dyed fabrics (Wool, Silk and Cotton) were printed with a printing paste containing the thickening agent and the enzyme only (i.e. uncoloured paste). The recipe of the uncoloured printing paste was as follows:

Enzyme -----	X g
Sodium alginate -----	30 g
Water -----	Y g
<hr/>	
Total	1000 g

While (X was 50, 100, 120, 140, 160, 180, or 200 g/Kg).

The enzyme was then poured on the thickener suspension and the whole paste was adjusted to one kilogram with addition of necessary amount of water. The printed fabrics were allowed to dry at ambient conditions.

Also the dyed fabrics were printed with printing paste containing both of reducing agents [conventional and ecofriendly products (enzyme)] with deferent amount as follow:

Enzyme -----	X g
Reducing agents (Redoxal Z) ----	Y g
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Total	100 g

While (X was 25, 50, 75 g).

After printing the samples were lifted in an oven for different intervals of times and also at different degrees of temperature. Finally the printed goods we steamed at 102 °C for five minutes, followed by washing.

2.3 Measurement

2.3.1. colour strength (K/S) & fastness:

Colour strength (Judd & Wyszecski, 1975), expressed as (K/S) and the colour fastness properties: The washing, perspiration and rubbing fastness were assessed according to standard methods (Society of Dyers and Colourists, 1955).

3. Results and Discussion

As previously mentioned the main aim of the present work is to investigate the suitability of laccase enzyme instead of conventional reducing agents viz- Redoxal-Z (Which is harmful to the environment) in discharge printing using natural colours. Hence, 3 different natural colours namely henna, curcuma and leucaena were selected and the colour from which were extracted via 3 different techniques as mentioned in the experimental section.

Samples wool, silk or cotton were subjected to dyeing using the aforementioned concentrated three natural colours according to the produce described in the experimental part. The dyed fabrics were assessed for K/S and overall colour fastness properties, after which subjected to bio-discharge

printing using laccase enzyme under a variety of conditions. The effect of enzyme concentration, time and temperature of steaming on colour discharge performance has been studied.

Given below the results obtained in each case along with the appropriate discussion.

3.1. Effect of Enzyme Concentration on K/S:

To investigate the effect of commercial Laccase formula concentration, a series of white printing pastes containing only the thickening agent and different concentrations of laccase (50,100,120,140,160,180,or 200 g/Kg printing paste), maintained the pH at 4.5 were prepared according to the recipe mentioned in the experimental section. Samples of wool, silk and cotton which dyed with natural dyes (henna, curcuma and leucaena) were printed with discharge printing paste using screen printing technique. After printing the goods were left in a laboratory oven at 70°C for 1 hour followed by fixation via steaming at 102 °C for five minutes. At the end, the goods were washed, followed by drying at ambient conditions and assessed for K/S. The results obtained are given in Figures (1-5).

It's clear from Figures (1,2 and3) as the concentration of Laccase enzyme increases of the K/S decreases then either levels off or slightly increases.

This phenomenon holds true regardless of the technique of extraction of natural dye or the nature of fabric used. For examples on wool fabric as the concentration of the Laccase increases from 50 to 200 g/Kg, the K/S decreases from 12.29% to 61.42%, from 9.52% to 42.94%, and from 11.28% to 45.13% for Wool fabrics dyed using henna extracted with water, sodium carbonate or ethyl alcohol respectively Figure (1).

The same trend was also obtained in case of silk and cotton Figures (2 and 3).

Figures (4,5) represent the effect of laccase concentration or the three mentioned fabrics upon using leucaena and curcuma extract respectively.

It is clear from the data that increasing the concentration of Laccase enzyme from 50 to 200 g/Kg printing paste is accompanied by increasing in the percent decrease in the colour strength of the printed natural fabrics. This holds true regardless of the natural dye used.

For example as the concentration of the Laccase increases from 50 to 200 g/Kg paste, the percent decrease in colour strength increases from 12.58% to 61.12%, from 9.03% to 51.44%, and from 17.14% to 67.01% for Wool, Silk and Cotton fabrics respectively.

3.2 Effect of Enzyme concentration in comparative with conventional agents:

Since enzyme could not be able to remove all the natural colours to obtain a white discharge printing. A trial was made to mix it with commercial reducing agent namely Redoxal Z.

To achieve this goal two deferent printing pastes were prepared one contain laccase enzyme (120 g/Kg paste) and the other contain Redoxal Z. Other three mixed pastes were also prepared containing 25:75, 50:50, 75:25 Enzyme : Redoxal Z respectively. Samples of dyed Wool, Silk and Cotton fabrics were printed with these pastes. After printing, fixation and washing off the samples were assessed for K/S. The results obtained are given in Figures (6-8).

It is clear from the data of Figure (6) that as the amount of Redoxal Z in the printing paste increases, the percent decrease of colour removal also increases. For example in case of the fabrics dyed with Henna, as the amount of Redoxal increases from 25% to 50% to 75% the percent decrease in K/S increases from 45.08% to 61.06% to 68.96% , and from 27.12% to 46.15% to 54.37% and from 21.43% to 41.33% to 52.04% for wool, silk and cotton respectively.

This is expected since Redoxal Z is more powerful than Laccase enzyme as a reducing agent for the natural colour. The same trend was obtained on using curcuma and leucaena Figures (7-8). On comparing laccase enzyme with commercial reducing agent namely Redoxal Z., It was found that, samples of different attractive colours halftone could be obtained on using Laccase enzyme.

3.3. Effect of steaming temperature on K/S of the printed samples

It has been reported that the enzyme exhibit maximum activity at specific range of temperature (Shukia et al., 2000). Hence, it is a great interest to investigate the effect of steaming temperature on natural colour dye namely Henna, discharged by Laccase enzyme. To achieve this goal white paste containing Laccase enzyme (120g/Kg) printing paste thickened with sodium alginate was prepared and adjusted at pH 4.5 and used for printing of samples.

After printing the printed goods were subjected to drying for 1 hour followed by streaming at different temperatures (98, 100, 102, 104, or 106) at the end, the goods were washed according to the procedures previously motioned in the experimental section. The data of K/S obtained and their % decrease are reported and presented in Figure (9).

It is clear from the data that as the temperature of steaming increases from 98 : 102°C the percent decrease in K/S increases while on further increases in the steaming degree from 102 to 106°C the opposite holds true.

This phenomenon holds true for all the three fabrics under investigation i.e. for wool, silk, or cotton. For example in case of wool fabric the decrease in K/S increases from 23.55% to 32.75% to 35.17% as steaming temperature increases from 98 to 100 to 102°C by increasing the steaming temperature to 104 and 106°C the percent decrease in K/S decreases to 28.79 % and 26.45% respectively.

The decrease in K/S by increasing the drying temperature from 98 °C to 102°C may be due to the increase in the activity of enzyme because of the increased possibilities of

its molecule coming closer to substrate molecule. Increasing the temperature beyond 102°C may affect reversibly the activity of the enzyme.

From the previous investigation it's clear that the optimum steaming temperature to reach to the highest colour discharge is 102°C irrespective of the nature of the fabric used.

3.4. Effect of steaming time on K/S of the printed samples

It has been reported (Hardalov, 1992) that the reaction takes place during the steaming fixation step and the quality of the product depends to great extent, on the temperature and moisture content of the fixation room; any error will cause destruction of the cellulose material.

In the initial period of time the amount of substrate which has been transformed is directly proportional to the length of treatment time. After this initial period, the rate of reaction starts decreasing and the amount of reaction is no longer directly proportional to the treatment time. Provided the substrate is excess, the explanation of this phenomenon is the progressive loss of enzyme activity after a period of time. This may be due to the effect of heat on the tertiary structure of the enzyme or to the formation of some product or side product of the reaction which inhibits the enzyme (Vignon, 1924).

Therefore, to obtain the optimum steaming time at which the highest percent decrease in K/S was achieved. White discharge printing paste containing laccase enzyme thickened with sodium alginate adjusted at pH 4.5 was prepared and used in printing of wool, silk or cotton dyed with either curcuma or leucaena. After dyeing and printing the discharge printed goods were subjected to steaming at 102°C for different intervals of time viz. (5, 10, 15 and 20 minutes). Figures (10, 11) represent the percent decrease in K/S.

It's clear from the data that the percent decrease in K/S depends on (a) The nature of the printed fabric, (b) The nature of colour used and (c) On the time of thermo-fixation.

The highest % decrease in K/S was attained on conducting the steaming for 5 minutes in all three mentioned fabrics, this phenomenon holds true irrespective of the nature of the natural colour used i.e. for the fabrics dyed with either curcuma or leucaena.

The colour fastness properties of the discharge printed fabrics previously dyed with henna, curcuma or leucaena and printed with white discharge printing paste containing different amounts of enzyme were measured. Tables (I to III) represent the data for colour fastness to washing, to rubbing, and to perspiration for different fabrics (Wool – Silk – Cotton) dyed with (Henna, Leucaena, or Curcuma) respectively.

Table (I): shows that washing properties ranging from good to very good whereas the rubbing and perspiration range from very good to excellent.

Table (II): shows that washing properties ranging from good to very good whereas the rubbing and perspiration range from very good to excellent.

Table (III): shows that fastness properties of wool and cotton range from good to excellent whereas silk fabric ranges from good to very good

4. Conclusion

Innovative Technology of using laccase formulation has been done successfully in discharge printing of natural fabrics (wool, silk or cotton) dyed with different natural colours of dyes (henna, leucaena, and curcuma).

Factors affecting the discharge printing activity of the laccase enzyme were thoroughly investigated. It was found that:

Increasing the concentration of laccase enzyme formula from 50 to 200 g/Kg paste is accompanied with increase in the percent decrease in the colour strength of the printed natural fabrics then either levels on or slightly decreases. This holds true regardless of the technique of extraction of natural dye or the nature of fabric used.

Irrespective of the nature of fabric and/or the natural colour used the overall fastness properties, i.e. colour fastness to washing, to rubbing, or to perspiration for the printed goods ranges between good and very good.

The optimum temperature of steaming to achieve colour discharge on using either Redoxal Z or laccase enzyme was 102°C irrespective of the nature of fabric used, for 5 minutes.

The discharge printed areas using laccase enzyme have K/S different than that of the ground shade. Hence different colour tones could be obtained which reflect attractive and beauty feature.

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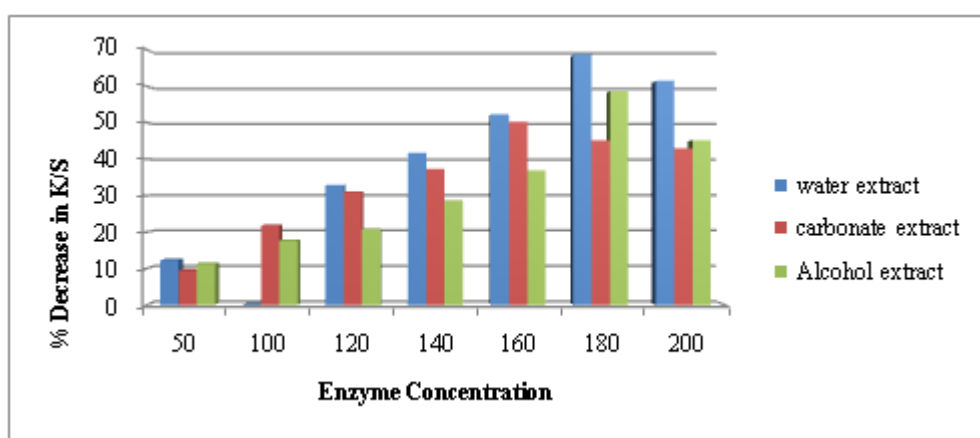


Figure 1: Effect of enzyme concentration on the colour discharge of Wool fabric dyed with Henna:

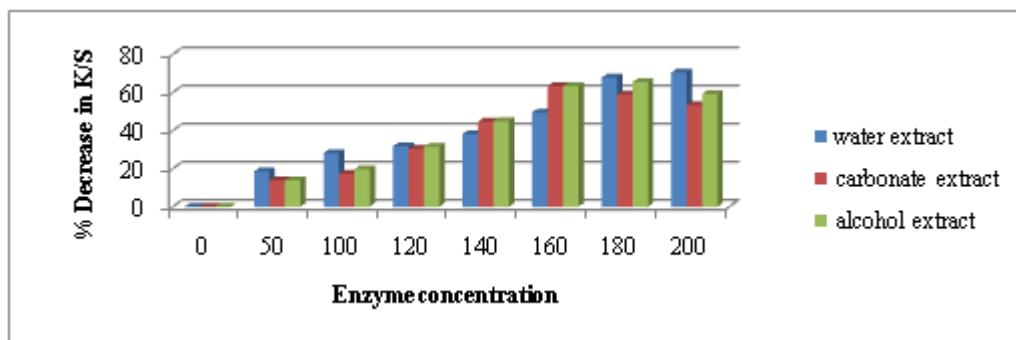


Figure 2: Effect of enzyme concentration on the colour discharge of Silk fabric dyed with Henna:

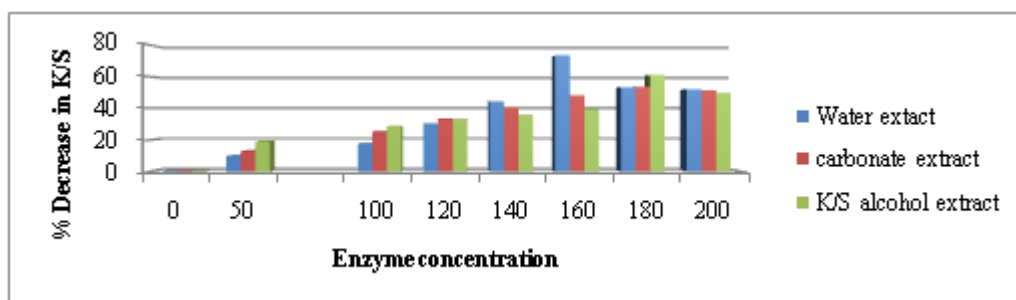


Figure 3: Effect of enzyme concentration on the colour discharge of Cotton fabric dyed with Henna:

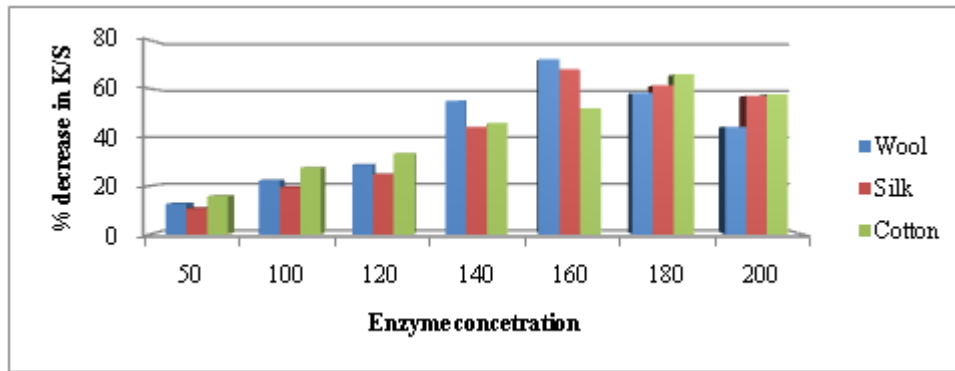


Figure 4: Effect of enzyme concentration on the colour discharge of the fabrics dyed with Leucaena:

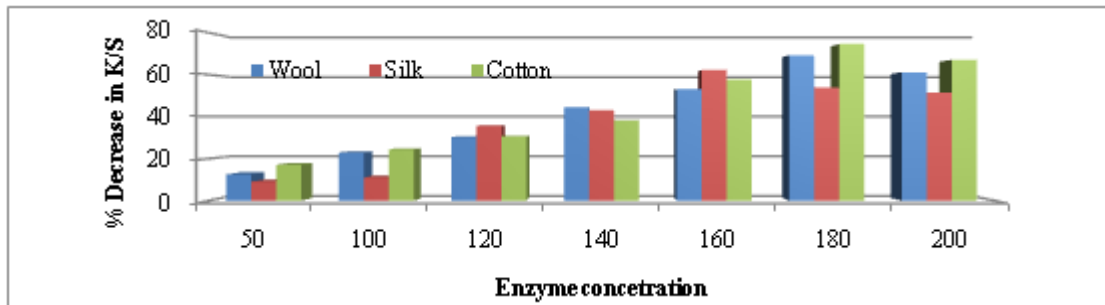


Figure 5: Effect of enzyme concentration on the colour discharge of the fabrics dyed with turmeric (Curcuma):

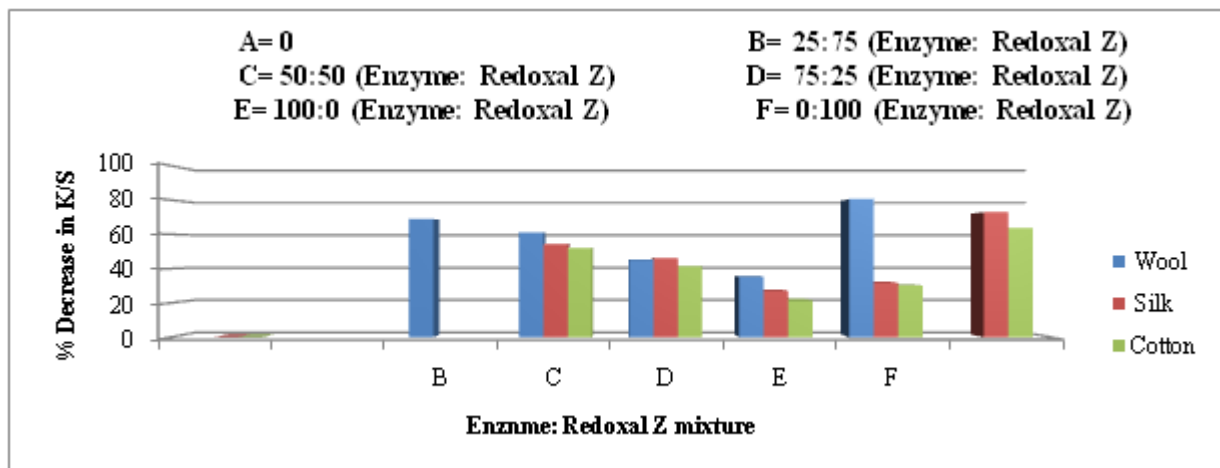


Figure 6: Effect of mixed enzyme concentration on the colour discharge of the fabrics dyed with Henna:

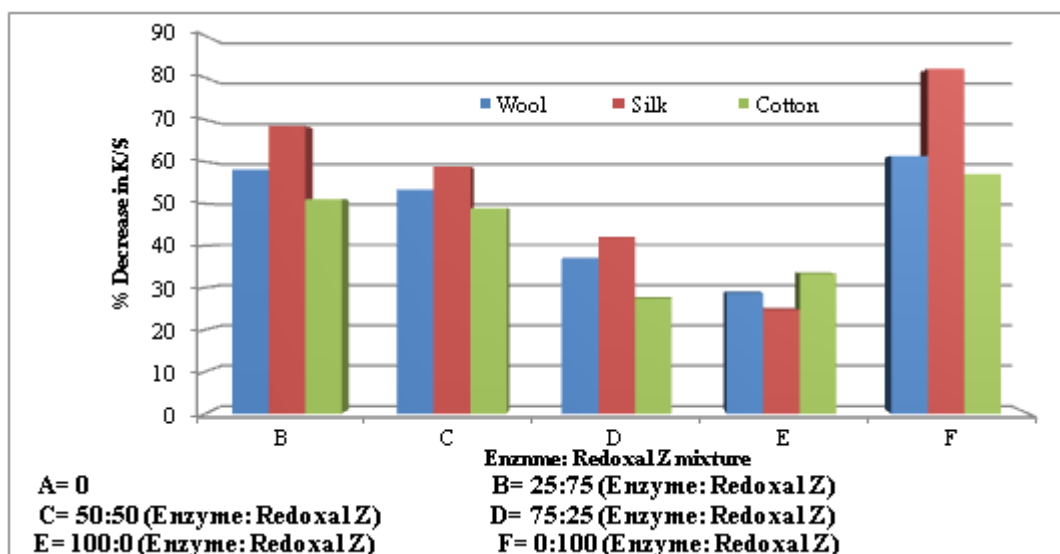


Figure 7: Effect of mixed enzyme concentration on the colour discharge of the fabrics dyed with Leucaena:

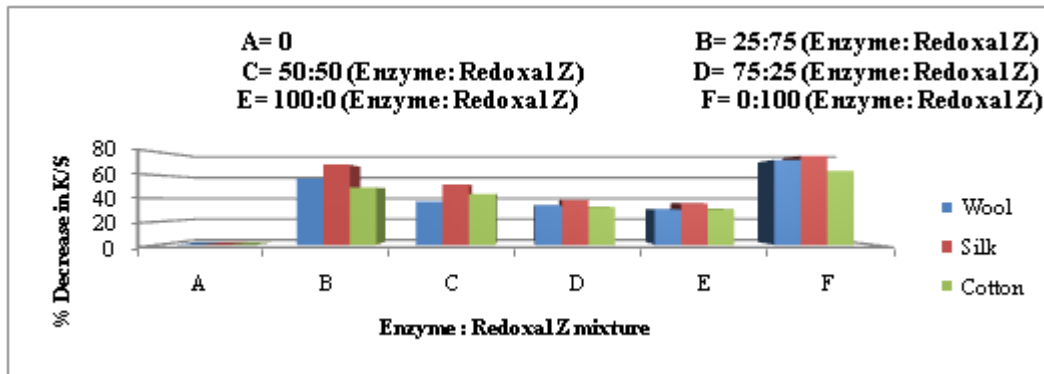


Figure 8: Effect of mixed enzyme concentration on the colour discharge of the fabrics dyed with Curcuma:

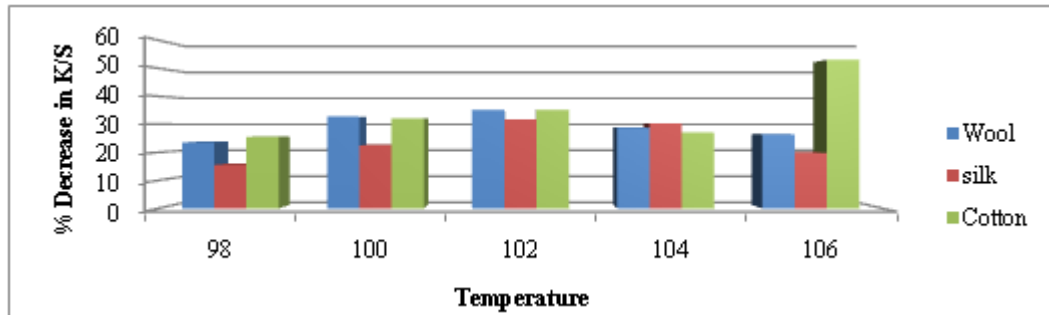


Figure 9: Effect of steaming temperature on K/S of the printed samples:

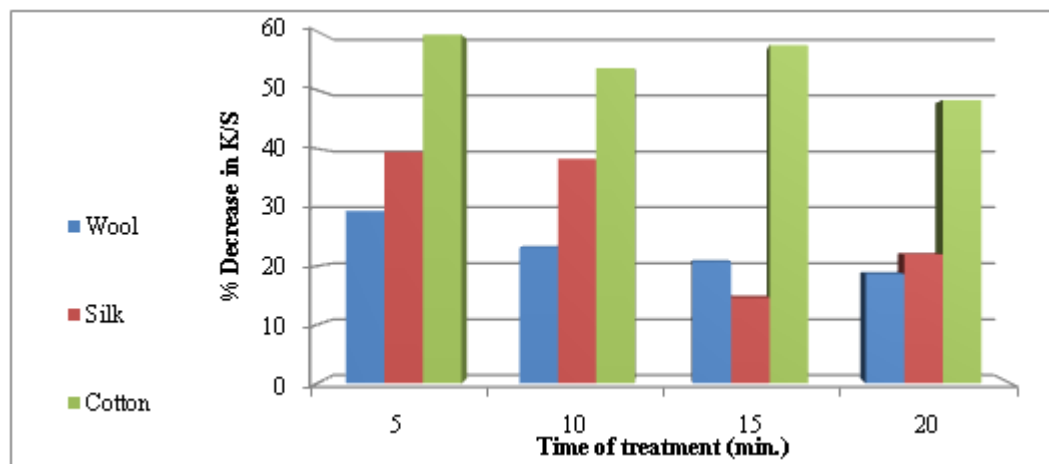


Figure 10: Effect of steaming time on K/S of discharge printed samples previously dyed with Curcuma:

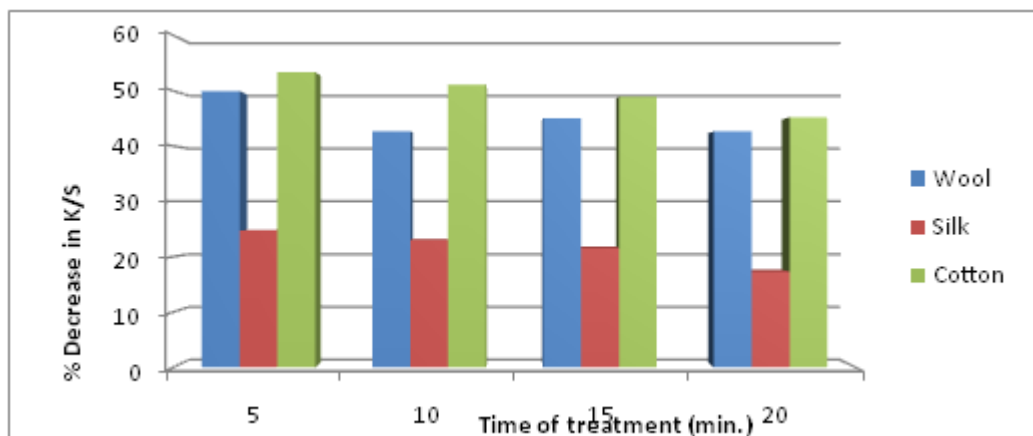


Figure 11: Effect of steaming time on K/S of discharge printed samples previously dyed with Leucaena:

Table 1: Fastness properties of natural fabric samples coloured with Henna and discharged by Enzyme

Sample		Washing fastness		Rubbing fastness		Perspiration			
Printed fabric	Concentration of bower's yeast g/Kg paste					Acidic		Alkaline	
		Alt.	St.	Dry	Wet	Alt.	St.	Alt.	St.
Wool	50	4 - 5	3 - 4	4 - 5	4	4 - 5	4 - 5	4 - 5	4
	100	4	4	4 - 5	4	4	3 - 4	4 - 5	4 - 5
	120	4	3	4 - 5	4	4	4	4 - 5	4 - 5
	140	4 - 5	3	4 - 5	4	4	3 - 4	4 - 5	4 - 5
	160	4 - 5	3 - 4	4 - 5	4	4 - 5	4 - 5	4 - 5	4 - 5
	180	4	3 - 4	4 - 5	4 - 5	4	4	4 - 5	4
	200	4 - 5	3 - 4	4 - 5	4 - 5	4	4	4	4
Silk	50	4	3 - 4	4 - 5	4 - 5	4	4	4 - 5	4
	100	4	3 - 4	4 - 5	4 - 5	4 - 5	4	4 - 5	4 - 5
	120	4	4	4 - 5	4	4	4	4 - 5	4 - 5
	140	4	4	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5
	160	4	3 - 4	4 - 5	4	4 - 5	4	4 - 5	4
	180	4	4	4 - 5	4	4 - 5	4 - 5	4 - 5	4
	200	4	4	4 - 5	4 - 5	4	4	4 - 5	4 - 5
Cotton	50	4 - 5	4	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5
	100	4 - 5	4	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5
	120	4 - 5	4	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5
	140	4 - 5	4	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5
	160	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5
	180	4 - 5	4	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5
	200	4 - 5	4	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5

Table 2: Fastness properties of natural fabric samples coloured with Leucaena and discharged by Enzyme.

Sample		Washing fastness		Rubbing fastness		Perspiration			
Printed fabric	Concentration of bower's yeast g/Kg paste					Acidic		Alkaline	
		Alt.	St.	Dry	Wet	Alt.	St.	Alt.	St.
Wool	50	4	3 - 4	4 - 5	4 - 5	4	4	4 - 5	4 - 5
	100	4	3 - 4	4 - 5	4 - 5	4	3 - 4	4 - 5	4 - 5
	120	4	3 - 4	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5
	140	4	3 - 4	4 - 5	4 - 5	4	4	4 - 5	4 - 5
	160	4	3 - 4	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5
	180	4	3 - 4	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5
	200	4	3 - 4	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5	4
Silk	50	4	4	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5
	100	3 - 4	4	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5
	120	4	4	4 - 5	4 - 5	4 - 5	4 - 5	4	4
	140	4	4	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5
	160	4	4	4 - 5	4	4 - 5	4 - 5	4 - 5	4 - 5
	180	4	4	4 - 5	4 - 5	4 - 5	4 - 5	4	4
	200	4	4	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5
Cotton	50	4	4	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5
	100	4	4	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5
	120	4	4	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5
	140	4	4	4 - 5	4 - 5	4	4	4 - 5	4 - 5
	160	4	4	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5
	180	4	4	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5
	200	4	4	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5	4 - 5

Table 3: Fastness properties of natural fabric samples coloured with Curcuma and discharged by Enzyme.

Sample		Washing fastness		Rubbing fastness		Perspiration			
Printed fabric	Concentration of bower's yeast g/Kg paste	Alt.	Sta.	Dry	Wet	Acidic		Alkaline	
						Alt.	St.	Alt.	St.
Wool	50	4-5	4	4-5	4	3-4	3	4	3-4
	100	4-5	4	4-5	4	3	3	4	3-4
	120	4-5	4	4-5	4	4	2-3	4-5	4
	140	4-5	3-4	4-5	4-5	3-4	3	4-5	4
	160	4-5	3-4	4-5	4	4	3-4	4-5	3-4
	180	4	3	4-5	4	3-4	3	4	4
	200	4	4	4	4	4	3	4	3
Silk	50	4	2-3	4-5	4	4	3	4	3
	100	4	3	4-5	4	3-4	2-3	4	2-3
	120	4	2-3	4-5	4	3	2-3	4	2-3
	140	4	2-3	4-5	4-5	4	3	3-4	2-3
	160	4	3	4-5	4-5	3	3	4	3
	180	4	3	4-5	4-5	3	3	4	3-4
	200	4	3-4	4-5	4-5	3-4	3-4	4	3
Cotton	50	4-5	3-4	4-5	4-5	4-5	4-5	4-5	4
	100	4-5	3-4	4-5	4-5	4	4	4	4
	120	4-5	3-4	4-5	4-5	4	4	4-5	4
	140	4-5	4	4-5	4	4	4	4-5	4
	160	4-5	4	4-5	4-5	4	3-4	4-5	4-5
	180	4-5	4	4-5	4-5	4	4	4	4
	200	4-5	2-3	4-5	4-5	4-5	4-5	4-5	4