A novel bio-technique using laccase enzyme in textile printing to fix natural dyes

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Laccase enzyme has been used as a substitute of harmful mordants in fixation of three different types of flavonoids (natural dyes), namely rutin, morin and quercetin, in printing of fabrics and the optimum conditions to achieve the best results are determined. The effects of enzyme concentration, \( \text{pH} \) of printing paste, treatment time and temperature as well as the nature of the printed substrate on the properties of printed goods expressed as \( K/S \) and overall fastness properties have been thoroughly investigated. The results show that the optimum conditions for using rutin in printing cotton fabrics are enzyme concentration 60g/kg printing paste, \( \text{pH} \) 4.5, treatment time 60 min and temperature 60\( ^\circ \)C. Laccase undergoes enzymatic oxidative polymerization of flavonoids giving the coloured pigment. It is also observed that with this technique, the above three kinds of flavonoids could be used for printing all kinds of fabrics, as in case of pigments, with very good to excellent colour fastness. However, the colour strength of the printed goods depends on the concentration of enzyme, \( \text{pH} \) of printing paste, treatment time, temperature of treatment, fixation conditions, nature of flavanoids used, and nature of printed fabric.

**Keywords**: Antibacterial activity, Blended fabrics, Cotton, Flavonoids, Laccase, Natural dye, Screen printing

**1 Introduction**

In the recent years, the subject of natural colours has become the matter of topical interest due to their environment friendly and non-toxic nature, although these dyes do not perform well with artificial fibres. Flavonoids present in most of the natural dyes obtained from fruits and vegetables are found to be important in producing colour in textile dyeing and printing.

The flavonoids consist of a large group of low-molecular weight polyphenolic substances, and are an integral part of the human diet\textsuperscript{1,2}. They are usually present almost exclusively in the form of \( \beta \)-glucosides and can be divided into four main groups, namely flavones, flavonols, flavonones and isoflavones, on the basis of their molecular structure\textsuperscript{3,4}. The flavonoids have been the object of extensive studies due to their biological, pharmacological and chemical properties\textsuperscript{5}. Flavonoid polyphenols can be oxidized by some oxido-reductive enzymes, e.g. laccases, peroxidases and tyrosinases. Laccases (EC 1.10.3.2) are multicopper polyphenol oxidases capable of oxidizing phenols by hydrogen atom abstraction, resulting in the formation of corresponding phenoxy radicals\textsuperscript{6,7}. These reactive radicals can perform non-enzymatic reactions in further steps. By this way laccase can oxidize flavonoids forming \( o \)-quinones that can perform further polymerization\textsuperscript{8}, indicating its dye fixing ability on to textile fabric.

Since natural dyes alone has no affinity to the fibres, they require chemicals in the form of metal salts (mordants) to produce an affinity between the fibre and the pigments for developing colour on the fibre. The present work is therefore aimed at investigating the suitability of using laccase enzyme formula as a substitute of hazardous mordants to fix three different flavanoids (rutin, morin and quercetin) onto the fibre surface under a variety of conditions. The effect of enzyme concentration, \( \text{pH} \) of the printing paste, treatment time and temperature as well as the nature of the fabric used on the properties of printed goods has also been studied.

**2 Materials and Methods**

**2.1 Materials**

Commercial laccase formulation (Denilite IIS; 120 U/g) from genetically modified Aspergillus was kindly supplied by NovoNordisk Company, Denmark.

Three different types of flavonoids, namely rutin, morin and quercetin were purchased from Aldrich and

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used as supplied. There chemical structures are shown in Fig.1.

High viscosity sodium alginate from brown algae, manufactured by Fluka Chemical Company; mill desized, bleached and mercerized cotton fabrics (130 g/m$^2$); mill scoured wool fabric (100%); nylon-6 fabric (96 g/m$^2$) produced by El-Shourbagy Co.Cairo, Egypt; and various kinds of blended fabrics, such as viscose/ polyester (80/20), wool/ polyester (70/30) and cotton / polyester (60/40) were also used.

2.2 Methods

2.2.1 Preparation of Printing Paste

The printing paste was prepared using the following recipe:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural colour</td>
<td>30 g</td>
</tr>
<tr>
<td>(rutin, morin or quercetin)</td>
<td></td>
</tr>
<tr>
<td>Laccase enzyme</td>
<td>X g</td>
</tr>
<tr>
<td>Sodium alginate</td>
<td>30 g</td>
</tr>
<tr>
<td>Water</td>
<td>Y g</td>
</tr>
<tr>
<td>Total</td>
<td>1000 g</td>
</tr>
</tbody>
</table>

Sodium alginate was soaked in small amount of water overnight at room temperature before preparing the printing paste. The enzyme and natural colour were then added on the thickener suspension. The printing pastes were adjusted at the required pH using sodium carbonate or dilute acetic acid. Finally, the total weight of the whole paste was adjusted to one kilogram with the addition of necessary amount of water. All the printing pastes were applied to fabrics according to the conventional screen printing technique. After printing , the samples were kept in an oven for different intervals of time at different temperatures. The printed samples were subjected to thermofixation at 140°C for 5min. After fixation, the printed goods were subjected to washing in three steps, first washing with cold running water, soaping (using 2 g/L non-ionic detergent, Espycon 1030) at 45°C for 15 min followed by rinsing with cold water. Finally, the printed fabrics were dried and assessed for colour strength ($K/S$) and overall fastness properties.

2.2.2 Test Methods


The antibacterial activity was assayed in the Micro Analytical Centre of Cairo University using Kirby-bauer disc diffusion method [Standard M38-A. NCCLS, Wayne, PA, USA (2002)].

3 Results and Discussion

The current work aims to investigate the ability of using laccase enzyme to enhance the affinity of the used flavanoids towards different fabrics and improve its fastness properties.

3.1 Effect of Enzyme Concentration

To study the effect of amount of commercial laccase formula concentrate, a series of printing pastes containing the thickening agent , rutin and different enzyme concentrations ranging from 20 g/kg to 80 g/kg was prepared as per the recipe mentioned at pH 4.5. Cotton fabric samples were then printed with these pastes using screen printing technique. Another sample was printed in the absence of enzyme for the sake of comparison. The samples were kept in a laboratory oven at 40°C for two and half hour followed by thermofixation at 140°C for 5 min. Finally, the samples were washed as per the procedure mentioned earlier followed by drying at ambient conditions and assessed for $K/S$ measurements. The laccase plays a dominate role in the fixation of natural dye (rutin), where the $K/S$ increases from 0.63 to 3.11 as the amount of laccase enzyme in the printing paste increases from 20g/kg to 60g/kg of the printing paste. Further increase in laccase more than 60g is accompanied by a decrease in $K/S$ of the printed goods. This reveals that the affinity of cotton fabrics towards natural dye (rutin) is very low, but the addition of laccase enzyme highly
improves the affinity of cotton towards rutin. The improvement in the affinity of cotton towards rutin may be due to the formation of high molecular weight compounds with the action of laccase enzyme (Fig. 2).

The laccase reactions presumably proceed by formation of a radical cation, with subsequent deprotonation of the hydroxyl group to give a radical. The radical may then undergo formation of a quinonoid derivative as shown in Fig. 2. The quinones are highly reactive compounds and can polymerize spontaneously to form high molecular weight compounds or brown pigments (melanins). The latter is precipitated on the fabrics surface to form a film like structure as obtained with binders. This finding is in complete confirmation with the previous works.

The formed brown pigment can be precipitated on any fabric surface, viz. wool, polyester, cotton, viscose or nylon. Furthermore, this enzymatic oxidative polymerization of flavonols has been previously studied by several researchers using HPLC analysis and UV–vis spectroscopic studies.

The data show that by increasing the laccase concentration the colour strength gradually increases due to the increase in the oxidative polymerization reaction, producing the coloured pigment. The highest colour strength is achieved at laccase concentration of 60g/kg and then it decreases. The slight decrease in the reaction rate as the concentration of enzyme increases more than the maximum may be due to the decrease in mobility of the enzymes because of the huge number of enzyme molecules or the less rutin molecules.

3.2 Effect of pH

To study the effect of pH of the printing paste on oxidation polymerization reaction of rutin, the pH values of the pastes have been adjusted at 2.5, 3.5, 4.5, 5.5 and 6.5. The prepared pastes are then applied onto the cotton samples separately via screen printing technique. After printing, the printed goods are left in oven for two and a half hour. Finally, the samples were fixed via thermofixation, washed according to the procedure mentioned earlier followed by drying at ambient conditions and assessed for K/S values. The results confirm that laccase enzyme has its optimum activity (maximum K/S) at pH 4.5 as already observed in previous studies.

3.3 Effect of Temperature

The effect of drying temperature on colour fixation of rutin was investigated using cotton fabrics printed with a paste containing 30g of rutin and 60g of laccase/kg of printing paste and subjected to drying at different temperatures between 30 ºC and 70ºC. It is observed that as the drying temperature increases from 30ºC to 60ºC, the K/S value increases regularly from 2.8 to 4.18. The increase in K/S may be due to the increase in the enzyme activity. Further increase in the drying temperature up to 70ºC shows a decrease in the K/S.

3.4 Effect of Enzymatic Treatment Time

Figure 3 represent the effect of enzymatic treatment time on the colour strength of printed cotton fabrics. At the initial period of time the amount of substrate which has been transformed is directly proportional to the length of treatment time. After 60 min, the rate of reaction is almost constant and then start decreasing. The amount of reaction is no longer directly proportional to the treatment time. The explanation of this phenomenon is the progressive loss of enzyme activity after a period of time. This may due to the effect of heat on the tertiary structure of the enzyme.

3.5 Effect of Temperature and Time of Thermofixation

Figure 4 shows the effect of temperature and time of thermofixation on the K/S value of the printed samples. The data reveal that by increasing the temperature of thermofixation from 140 ºC to 160 ºC and time from 4min to 8 min, the K/S value increases showing

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**Fig. 2**—Formation of quinonoid derivative

**Fig. 3**—Effect of treatment time on the K/S of the printing cotton samples
darker brown colour, while the K/S decreases at higher temperature (170 °C), irrespective of the time. It seems that the higher temperature (170 °C) may affect the formed polymer. It can be concluded that the best conditions for thermofixation is 8 min at 150°C.

3.6 Effect of Nature of Fabrics
From the above study, it is clear that laccase enzyme could be used successfully in printing of cotton fabrics with natural dye namely rutin via formation of high molecular weight compounds or brown pigments. Hence, a trial was made to investigate the suitability of this system to print other substrate, viz wool, nylon and polyester blends with cotton, viscose or wool using the optimum conditions. The results obtained are given in Table 1.

It is clear from Table 1 that the rutin with this technique could print all kinds of fabrics under investigation with very good to excellent colour fastness to rubbing, washing or perspiration even for light fastness. However, the K/S follows the order viscose/ polyester blend > cotton / polyester blend > cotton > nylon > wool > wool/ polyester blend. This may due to the variation in the physico-chemical properties of these fabrics, and hence the affinity between the natural dye and the printed substrate.

3.7 Printing with Two Other Classes of Flavonoids
Other two classes of flavonoids namely morin (which can be used on leather, silk, wool and nylon when mordanted with chrome) and quercetin (found in citrus fruit, buckwheat and onions) were chosen and used in preparing printing pastes which have been applied to different fabrics under the optimum conditions as obtained in case of using rutin. After printing, drying, fixation and washing, the printed fabrics were assessed for K/S and overall colour fastness properties of different fabrics printed with rutin and morin. The results are given in Tables 1 and 2 respectively.

![Fig. 4 — Effect of fixation temperature and time on the K/S of the goods printed](#)

| Table 1 — K/S as well as the overall colour fastness properties of different fabrics printed with rutin |
|---|---|---|---|---|---|
| Fabric | K/S | Rubbing | Washing | Perspiration | Light fastness |
| | | Dry | Wet | St. | Alt. | Alkali | Acidic | St. | Alt. | St. | Alt. | Light |
| Cotton (100%) | 5.66 | 4-5 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 5 |
| Wool (100%) | 4.67 | 4 | 4 | 4 | 4 | 3 | 3 | 3 | 3 | 3 | 3 |
| Nylon (100%) | 5.56 | 3-4 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3-4 |
| Viscose/ polyester (80/20) | 7.56 | 4-5 | 4-5 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Wool/ polyester (70/30) | 4.45 | 4-5 | 4-5 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Cotton / polyester (60/40) | 3.72 | 4-5 | 4-5 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |

Wool has been fixed by steaming at 102 °C for 15 min.

| Table 2 — K/S as well as overall colour fastness properties of the fabrics printed with morin |
|---|---|---|---|---|---|
| Fabric | K/S | Rubbing | Washing | Perspiration | Light fastness |
| | | Dry | Wet | St. | Alt. | Alkali | Acidic | St. | Alt. | St. | Alt. | Light |
| Cotton (100%) | 2.08 | 4-5 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 5 |
| Wool (100%) | 1.72 | 4 | 4 | 4 | 4 | 3 | 3 | 3 | 3 | 3 | 3 |
| Nylon (100%) | 2.01 | 3-4 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3-4 |
| Viscose/ polyester (80/20) | 4.2 | 4-5 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Wool/ polyester (70/30) | 1.1 | 4-5 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Cotton / polyester (60/40) | 1.39 | 4-5 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |

Wool has been fixed by steaming at 102 °C for 15 min.
colour fastness properties. The data obtained are given in Tables 2 and 3 for morin and querectin respectively.

Both morin and querectin could also be used successfully in printing all kinds of fabrics using this technique. It is observed that irrespective of the fabrics used, prints acquire very good to excellent colour fastness properties even for light fastness. In both cases, the difference in the K/S could be attributed to the difference in their chemical structures and on the number and position of OH groups.

3.8 Antibacterial Evaluation

It is worthy to mention that rutin has an antibacterial activity to different positive and negative bacteria as it is clear from the data of Table 4.

4 Conclusion

4.1 The laccase enzyme has been successfully used under variety of conditions as a substitute of harmful mordant, in fixation of three different types of flavonoids namely rutin, morin and querectin on different fabrics.

4.2 The optimum conditions for using Rutin, as a natural colour, in printing cotton fabrics are found to be enzyme concentration 60 g/kg printing paste, pH 4.5, treatment time 60 min and temperature 60 °C.

4.3 The K/S of the printed fabrics is found to depend on the nature of flavonoids, nature of printed fabric, concentration of enzyme, and pH of printing paste.

4.4 The colour fastness to washing, rubbing, perspiration and light for cotton, wool, viscose/polyester and cotton/polyester blend shows good to excellent results, confirming the feasibility of this new and promising colouration technique.

4.5 It is found that rutin acquires antibacterial activity to different positive and negative bacteria.

References