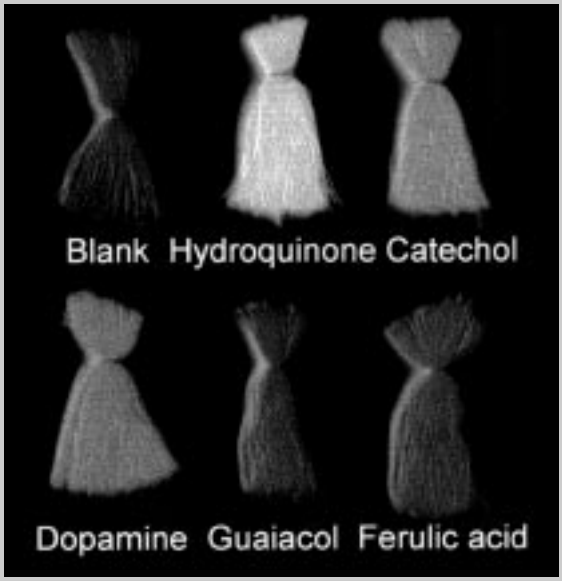
Communication: Phenolic compounds such as hydroquinone, catechol and ferulic acid can polymerise and therefore develop deep colours when treated with peroxidase and laccase enzymes. We have attempted to apply this phenomenon for wool dyeing. Wool was padded with phenolic compounds and deep colours were developed by enzymatic treatment in buffer solutions. Mordant with chromium compounds or heating under the condition of acid catalysis was available for fixation of these coloured compounds on the wool fibres.



Enzymatically dyed wool samples.

"In Situ" Enzymatically Prepared Polymers for Wool Coloration

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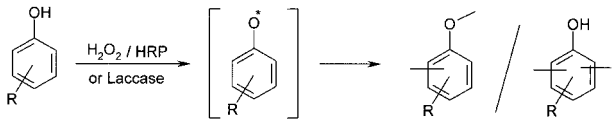
Introduction

The textile industry is looking for new coloration methods for dyeing and enzyme technology can represent an alternative for this approach. Oxydoreductase enzymes such as laccases (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) and horseradish peroxidase (donor: hydrogen peroxide oxidoreductase, HRP, EC 1.11.1.7) can provide novel ways for wool coloration in the face of actual state of the art of these enzymes.

HRP has been reported as a very useful enzyme for the synthesis of phenolic polymers.[1] A free radical at the hydroxyl position in the phenolic compounds is formed by HRP enzymatic oxidation through activation with hydrogen peroxide. This free radical moves along the aromatic ring and contributes to the coupling between monomeric units.[2] Scheme 1 shows a simplified scheme of the mechanism of the enzymatic polymerisation reaction. A similar enzymatic reaction proceeds for the system using enzyme laccase.[3,4]



Scheme 1. Enzymatic polymerisation of phenol compounds.

Many enzymatically polymerised phenolic compounds tend to have a characteristic colour because polyphenol forms a big conjugated structure along the main chain. Hydroquinone is also one of the phenolic compounds that show a colour change when it is catalysed with oxidoreductases. Enzymatic application for human hair dyeing has been carried out with hydroquinone under activation with laccase.[5] This investigation aims to reach a safe hair dyeing without using hydrogen peroxide. Laccase catalyses the oxidation of hydroquinone, producing a delocalized free radical at the oxidised intermediate. Colouring products of dimer, oligomer and polymer result by a radical coupling reaction between the intermediates.

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This phenomenon is interesting because the colour can be obtained easily from the enzymatic reaction and therefore can be available for textile dyeing.

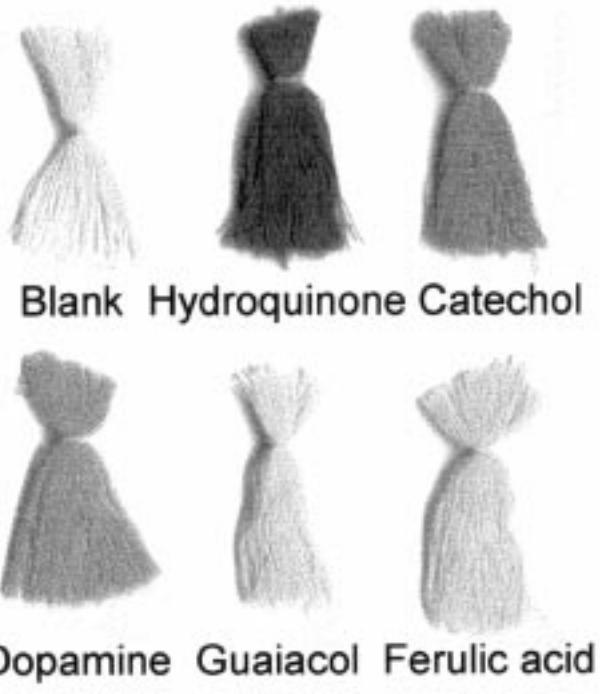
In this study, we try to dye wool fabric including mordant processing in an enzymatic method. We investigate the effects of dyeing conditions such as hydroquinone concentration, enzyme concentration and hydrogen peroxide concentration on the enzymatic dyeing towards wool fabric. We also discuss the enzymatic dyeing of wool with ferulic acid through curing processing.

Results and Discussion

Application of Hydroquinone

Colour in wool can be developed using several phenolic compounds such as hydroquinone (brown-blacks), catechol (greys), dopamine (greys), guaiacol (colourless) and ferulic acid (yellows) after treatment with HRP. Wool samples were rinsed with running water without any further treatment after the enzymatic treatment. They show that each of these samples have different characteristic colours which depend on the different phenolic compounds chosen for the enzymatic dyeing. This result indicates that different kinds of coloration can be performed by selection of phenolic compounds. Hydroquinone and ferulic acid will be mostly employed as phenolic compounds in this study.

The wool fabric used for enzymatic treatment has previously been padded with hydroquinone. The colour of the wool fabric changed to brown during enzymatic treatment as shown in Scheme 2. This colour change may be explained by the fact that hydroquinone on the fabric is oxidised by enzymatic oxidation, then forming dimer, oligomer and polymer. Evidence for enzymatic polymerisation using similar conditions was described before. Laccase was also employed for enzymatic dyeing of wool. Wool fabric that was previously padded with hydroquinone was also coloured to brown during enzymatic treatment with laccase. This is the same effect as when HRP is used for enzymatic dyeing, indicating that the enzymatic reaction using laccase may be similar to the enzymatic reaction using HRP, as shown in Scheme 1.



Scheme 2. Enzymatically dyed wool samples.

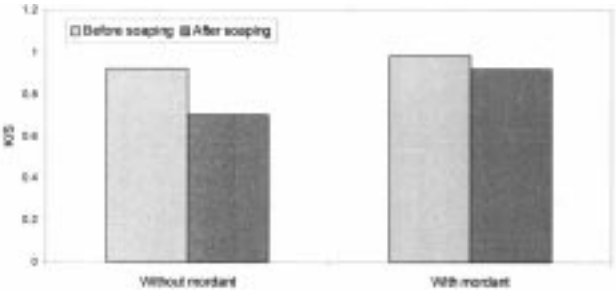


Figure 1. Washing test of enzymatically dyed wool fabric that was previously padded with hydroquinone.

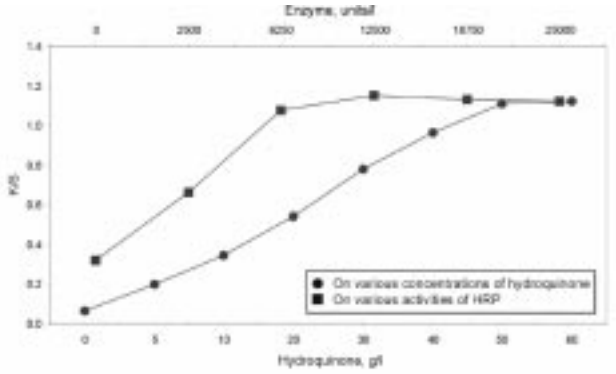


Figure 2. Colour depths of enzymatically dyed wool fabric at various concentrations of hydroquinone and on various activities of HRP.

Washing tests were carried out on dyed wool fabrics as described in the *Experimental Part*. Concentration of the hydroquinone solution was 40 g/l. The results are shown in Figure 1. The *K*/*S* value was employed to evaluate the colour depth for the wool fabrics. The reflectance values at 700 nm are employed because of the biggest difference between the values at this wavelength. One of them was treated with potassium dichromate solution, while the other was not treated. Colour depth of the dyed fabric decreased after the washing test. However, the decreasing colour depth could be reduced by mordant treatment. This indicates that the chromium combines with quinonoid oxygen and hydroxyl group of the enzymatically treated hydroquinone in wool fabric.

The diagram in Figure 2 shows the colour depths of wool fabrics that were padded previously with hydroqui-

none solutions in several concentrations and treated with HRP solution with a concentration of 25000 units/l. The concentration of hydroquinone solution is in the range of saturation, i.e., 60 g/l. This Figure shows that the colour depth of wool fabric increases with increasing concentration of hydroquinone in the padding solution, indicating that the colour depth of the fabric can be controlled by the concentration of hydroquinone padded on the wool fabric.

The colour depth of wool fabric against enzyme concentration is also shown in Figure 2. Wool fabric has been padded previously with hydroquinone solution (concentration 60 g/l). Colour depth increases remarkably with increasing enzyme activity to 6250 units/l, then the plot is nearly getting flat in the subsequent activity. This indicates that 6250 units/l is sufficient activity to oxidise almost all hydroquinone padded on the fabric, for a 1 h dyeing process. The *K*/*S* value of the untreated fabric is about 0.4. This value is higher than the value of the untreated fabric shown in the other plot, which is about 0.1, because this untreated fabric has previously been padded with hydroquinone.

In Figure 3, the colour depth of wool fabric is shown against concentration of added hydrogen peroxide in enzyme solution. The concentration of hydroquinone solution used for padding the wool fabric was 40 g/l. Colour depth of wool fabric increases with increasing concentration of hydrogen peroxide until 5 ml/l. The colour depth, however, starts to decrease in the subsequent concentration of hydrogen peroxide. This indicates that a high amount of hydrogen peroxide apparently inhibits the HRP reaction, although hydrogen peroxide is necessary for the HRP catalytic reaction. In this case, it seems that the enzyme loses its activity because hydrogen peroxide may oxidise and may inhibit the protein enzyme. A suitable concentration of hydrogen peroxide should be applied in order to increase the colour depth obtained in the enzymatic reaction without damage of the protein enzyme.

Application of Ferulic Acid

Ferulic acid was also used as phenolic compound to dye wool fabric using the enzymatic method. The diagram in Figure 4 shows the reflectance value of wool fabric that was padded previously with ferulic acid and treated with HRP. This fabric has been coloured by enzymatic treatment. The Figure shows, however, that the wool fabric after soaping presents a lighter colour than the untreated wool fabric. This indicates that the coloured ferulic acid on the wool fabric has been removed throughout soaping because the ferulic acid was not strongly fixed on the fabric. On the other hand, the wool fabric treated under acidic conditions after enzymatic treatment had a yellow colour even after soaping. This indicates that the coloured ferulic acid has been fixed on the wool fabric during boiling under acid condition. Ferulic acid contains useful reactive sites such as double bond and carboxyl group, except for hydroxyl group. These sites can be expected to be responsible for fixation of ferulic acid on the wool even without mordant. The fixation mechanism, however, is not clear at the present.

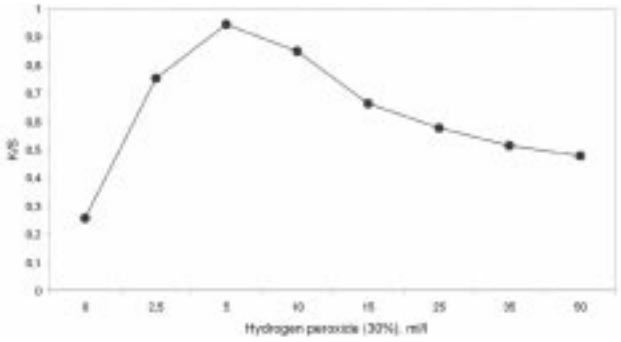


Figure 3. Colour depths of enzymatically dyed wool fabrics at various concentrations of hydrogen peroxide.

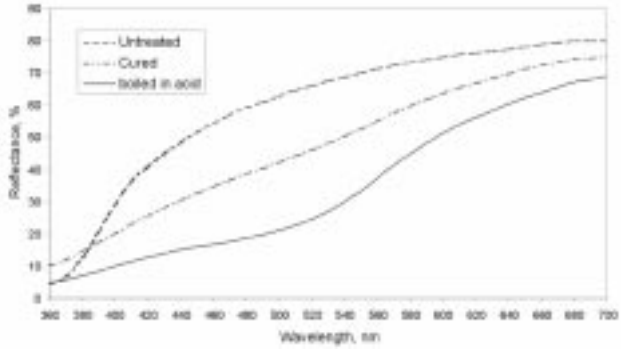


Figure 4. Colour of wool fabric that was padded with ferulic acid and then treated with HRP.

Conclusion

We have attempted to obtain new dyeing methods using an enzymatic reaction that contributes to the coloration for wool. It is known that particular phenolic compounds such as hydroquinone, catechol, dopamine, guaiacol and ferulic acid can be used for the enzymatic dyeing of wool. It was expected to obtain various characteristic colours of wool fabric by selection of the phenolic compounds.

The colour depth of enzymatically dyed wool fabrics can be changed depending on the controllable amount of hydroquinone on the fabric. Mordant processing with chromium compound was available for fixation of this coloured hydroquinone derivative on the wool fabrics. The hydroquinone on the fabric should be oxidised into a radical form by enzymatic oxidation, then forming dimmer, oligomer and polymer. This enzymatically polymerised hydroquinone, which has a big conjugated structure along the main chain, should cause the coloration of

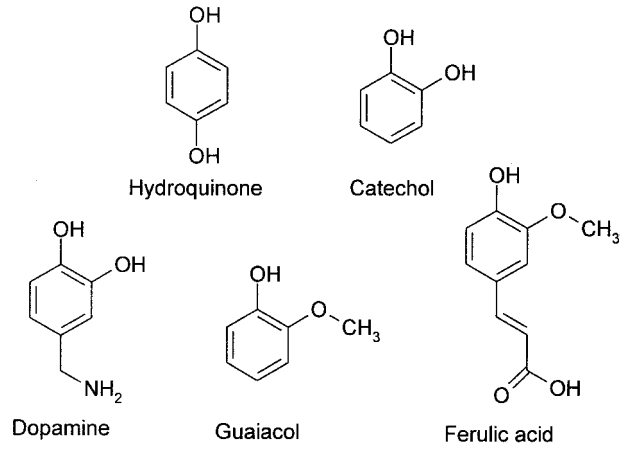
the wool fabric. To obtain a higher colour depth, appropriate dyeing conditions such as concentration of enzyme and concentration of hydrogen peroxide should be used.

Ferulic acid can be also used for the enzymatic dyeing of wool. In this case, coloured ferulic acid can be fixed rather strongly on the wool fabric through boiling treatment under acidic conditions instead of using chromium as mordant.

Experimental Part

Materials

Raw wool fabric was obtained from Albano Antunes Morgado, Lda and was used in this study without any further treatment. Hydroquinone, catechol, dopamine, guaiacol and ferulic acid from Sigma were used as phenolic compounds. The chemical structures of these compounds are represented in Scheme 3. Peroxidase from horseradish (Type VI-A) (HRP, EC 1.11.1.7), supplied in powder form, and laccase (EC 1.10.3.2), supplied in solution form, were purchased



Scheme 3. Chemical structures of phenolic compounds.

from Sigma. These enzymes are classified as oxidoreductases. 30% hydrogen peroxide solution was used as oxidising agent. The buffer systems used in this study were sodium phosphate – disodium hydrogen phosphate (0.1 M) for pH 7.0 and acetic acid – sodium acetate (0.1 M) for pH 5.0. HRP was diluted in the buffer solution (pH 7.0) and kept in a refrigerator as stock solution. This stock solution contains 5000 units/ml (Sigma Units). A 29.4 g/l potassium dichromate solution was prepared for mordant processing. Household surfactant (5 g/l) was used for washing wool fabrics.

Enzymatic Dyeing

1 g of hydroquinone was dissolved in 20 ml of water for 1 g of a wool fabric (8 cm × 8 cm). The fabric was immersed in a hydroquinone solution for 1 h at room temperature and padded in 100% pick up ratio. Two enzyme solutions were prepared using HRP and laccase, respectively. One was prepared with 0.1 ml of the HRP stock solution, 0.1 ml of the hydrogen peroxide solution and 20 ml of the buffer solution (pH 7.0). The other one was prepared with 0.1 ml of the laccase stock solution, however without using hydrogen peroxide, in a buffer solution in the same conditions with HRP solution. The wool fabrics were dipped in the enzyme solution without stirring for 1 h at room temperature. After the enzymatic reaction, the fabrics were slightly rinsed with water to remove residual compounds on the fabrics. 20 ml of the potassium dichromate solutions were employed to mordant the fabrics for 1 h at room temperature. The coloured fabrics were thoroughly rinsed with water at 85 °C for 15 min to remove residual compounds.

On the other hand, ethanol (50%) was used as solvent to prepare the ferulic acid solution (25 g/l) because this compound has a very low solubility in water. The wool fabric was padded with this ferulic acid solution under the same conditions as mentioned before. After drying, each wool fabric was treated with 20 ml of enzyme solution containing 0.1 ml of the HRP stock solution, 0.1 ml of the hydrogen peroxide solution and 20 ml of the buffer solution (pH 5.0 or 7.0), at room temperature during 1 h. After the enzymatic treatment, the temperature of the enzyme solutions was raised up to 100 °C for 40 min and kept at that temperature for 1 h. Each fabric was washed with 100 ml of the surfactant solution at 71 °C for 30 min to remove residual compounds.

Colour Measurement

Colour depth was evaluated in terms of *K*/*S* values and these were calculated using Kubelca-Munk's equation. For each fabric sample, five reflectance measurements were made and the arithmetic mean of these determinations was used for *K*/*S*. The reflectance values were measured with a Spectraflash SF600+CT of Datacolor International with illuminating D65 at 10° observer.

$$\text{Kubelca-Munk's equation}: K/S = \frac{(1-R)^2}{2R}$$

with R = reflectance.

Washing Test

The wool fabric was put into 300 ml of a pot containing 100 ml of 0.5% commercial surfactant (commercial name "xau") solution. The test was carried out for 30 min at 71 °C under 20 rpm.

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