Peroxidase-catalysed coloration of wool fabrics

Nalinee Netithammakorn, Edward Smith, Claire Lerpiniere and Jinsong Shen*

Textile Engineering and Materials (TEAM) Research Group, School of Fashion and Textiles, De Montfort University, Leicester, LE1 9BH, UK

Abstract

An enzyme-based textile coloration process using peroxidase (EC1.11.1.7) was investigated for its potential as an alternative to conventional textile dyeing processes, with the benefits of being low in energy use and non-damaging to fibres. The current study presents a process for the coloration of wool fabric using peroxidase oxidation of a range of different aromatic compounds in the presence of hydrogen peroxide. The results revealed that wool can be successfully dyed by peroxidase-catalysed coloration at temperatures as low as 30°C. By controlling the pH values and buffer systems during processing, a diverse colour palette was produced, depending on the small molecular aromatic compound used as the precursor. Colour fastness testing found that fastness to washing, rubbing and light properties achieved good to excellent ratings, with further improvement to wash fastness provided by a post-soaping wash. No fibre damage occurred due to peroxidase-catalysed coloration. This enzyme coloration process is a promising alternative to conventional wool dyeing processes with the advantage of effective dyeing at low temperatures, therefore having the potential of reducing energy consumption and preventing fibre damage.

Introduction

Textile products usually undergo a coloration stage during processing to enhance their appearance and consumer desirability. Conventional coloration methods used in the textiles industry require the use of synthetic dyes, chemical auxiliaries and large quantities of water heated to elevated temperatures which may have a negative impact on the environment due to the creation of polluting effluent and high energy use. There is an increasing demand for more sustainable coloration and wet processing methods for textiles to reduce the impact on the environment.

Enzyme-based wet processing methods are regarded as sustainable alternative methods for textile processing. Textile processing using enzymes can reduce the consumption of chemicals, energy and water, as well as generating less effluent, as enzymes are highly specific biocatalysts that can operate under mild temperature and neutral pH conditions. The residue of enzymes from enzymatic processing is reusable or biodegradable. Various enzyme-based textile wet processing methods have become well

established within the industry, such as desizing, bio-polishing, bio-stonewashing and bleach clean-up.[1] In terms of textile coloration, no enzyme-based method has yet been commercialised, although initial research studies have been carried out. Previous studies have found that enzymes belonging to the oxidoreductases, a class of enzymes which catalyse oxidation/reduction reactions, can catalyse the polymerization of certain simple aromatic compounds to form colour due to the formation of a conjugated structure. Two types of oxidoreductases have been used in previous studies to form colour, namely laccases and peroxidases.[2] A study by Shin et al[3] found that in-situ coloration of wool could be achieved if wool fabric was pretreated with phenolic compounds such as hydroquinone or ferulic acid then subsequently treated with either laccase or peroxidase. Since the study conducted by Shin et al, the majority of studies investigating enzyme coloration of textiles have focussed on the use of laccase rather than peroxidase.[2,4-7] Recently published studies have explored the application of laccase on the in-situ coloration of wool and cotton fabrics.[5,7-10] Peroxidase-oxidation has also been suggested for the dyeing of human hair.[11-13] The reaction of peroxidase is generally to catalyse the oxidation or reduction of a variety of substrates or precursors in the presence of hydrogen peroxide as an electron provider to generate intermediate radicals for further coupling or polymerisation.[2, 14-17]

The aim of this research was to investigate and develop an enzyme-catalysed in-situ dyeing process of wool using peroxidase. The potential for the formation of polymeric colourants, with a diverse colour palette, by the peroxidase-catalysed oxidation of a range of aromatic compound precursors in the presence of hydrogen peroxide, was explored. In addition, processing parameters such as processing time, temperature, buffer and pH conditions, precursor concentration, and their effects on colour, were investigated. Each sample was tested according to commercial standards in terms of tensile strength and colour fastness properties.

Experimental

Materials

Undyed plain-woven 100% wool fabric with a dry weight at 189 g/m^2, 50 ends per inch, 45 picks per inch, and a mean fibre diameter of 23 μm was supplied by Drummond Parkland (Huddersfield, UK).

Peroxidase (*Coprinus cinereus* peroxidase, CiP) in liquid form was supplied from Novozymes (Bagsvaerd, Denmark); 35 wt. % hydrogen peroxide (H_2O_2) solution, which was used as an oxidising agent, was purchased from Sigma-Aldrich (Dorset, UK). The dye precursors used for enzyme catalysis to form the colourants were also purchased from Sigma-Aldrich; their chemical structures are listed in Table 1.

Different buffer systems were prepared for a range of different pH values. Citric acid monohydrate and trisodium citrate were used to prepare 0.1 mol/L citrate buffers at pH 3 - 8 (Acros Organics). Monobasic sodium phosphate (Fisher Scientific) and dibasic sodium phosphate (Acros Organics) were used for preparing a 0.1 mol/L phosphate buffer at pH 9. Sodium bicarbonate and sodium carbonate anhydrous (Fisher Scientific) were used for preparing 0.1 mol/L bicarbonate/carbonate buffers at pH 10 and 11. Ultravon PL (UPL), a synergetic preparation based on nonionic surfactants, was purchased from Town End and used as a wetting agent and for post-treatment soaping. All other chemicals used were of specified laboratory reagent grade.

[Table 1 about here]

Enzymatic dyeing of wool fabrics

Two grams untreated woven wool fabric samples were dyed at a liquor to goods ratio of 20:1 in 0.1 mol/L of buffer solution containing 0.05% w/v of a specific precursors, 0.50% w/v of hydrogen peroxide and 0.50% v/v of peroxidase at various pH values. pH was controlled by citrate buffer for pH 3 - 8, phosphate buffer for pH 9 and bicarbonate/carbonate buffer for pH 10 - 11. Enzymatic dyeing was carried out in a Datacolor Ahiba Nuance Top Speed II–IR dye machine, which was set with an agitation speed of 40 rpm. The temperature was raised at 2.5°C/minute and maintained at 30, 40, 50, 60, or 70°C for 1, 2, or 4 hours, respectively. When the coloration process was completed, the samples were immediately rinsed repeatedly with cold tap water to remove excess dye then left to air-dry at room temperature.

Soaping wash for enzymatically dyed wool fabrics

A soaping wash of enzymatically dyed wool samples was followed to eliminate any excess, unfixed surface colour. Dyed wool samples were washed at a liquor to goods ratio of 50:1 in soaping wash solution containing 2 g/L UPL. The soaping wash process was performed in a Datacolor Ahiba Nuance Top Speed II–IR dye machine under agitation at the speed of 40 rpm. The temperature was raised at 2.5°C/minute then maintained at 60°C for 30 minutes. Once the process was finished, the wool samples were rinsed thoroughly with cold tap water and left to air-dry at room temperature.

Colour measurement

The CIE *L*a*b** colorimetric values and colour strength (*K/S*) of dyed fabric samples were measured using a Datacolor SF6000 Plus CT reflectance spectrophotometer. All values were measured and calculated under controlled conditions using Color-Tools QC software, with the illuminant and observer conditions of D_{65} and 10°, respectively. The instrument was set using a small aperture plate (9 mm), with the specular included, and the ultraviolet (UV) filter off for the assessment. Each dyed fabric

sample was folded into four and measured four times. The colour measurement value of each sample was obtained from the average of four measurements of each dyed fabric sample.

The *K/S* value, used to analyse the relationship between absorption and scattering coefficients (K and S) between the wavelengths of 400-700 nm, is directly proportional to the concentration of colourant in the textile fabric. The *K/S* values of the coloured fabric samples were determined.

Scanning electron microscopy analysis

The surface morphology of dyed fabric samples was examined using scanning electron microscopy (SEM). Fabric samples were prepared by attaching a double-sided adhesive carbon conductive tab to an aluminium pin stub and then placing a small piece of the fabric sample on the sticky surface of the stub. The samples were then placed into a Quorum Q150RS rotary-powered sputter coater for 4 minutes of sputter cycle under argon to coat with insulating gold to a thickness of 15 nm in order to have more conductive electrons. After this stage, the gold-coated samples were examined using Carl Zeiss EVO HD15 scanning electron microscope operating with a working distance of 9.5 or 10 mm with an accelerating voltage of 10 kV. The magnifications used were at 250x to gain an overview structure on the surface of the dyed fabric sample and at 2,500x to examine the microstructure of the wool fibre scales of a particular sample.

Tensile strength

A tensile strength test was performed to determine the change in mechanical properties of enzymatically dyed wool fabrics using an Instron 3345 with Bluehill 3 software following test method ISO 13934-2: 2014, determination of maximum force and elongation at maximum force using the grab method. Test samples were prepared at the width of 85 mm and length of 100 mm. The jaws used for grabbing the samples were 25 mm-wide for the front jaw and 50 mm-wide for the back jaw. The gauge length was adjusted to assess the samples at 50 mm with a width of 75 mm. The rate of traverse was set at 100 mm/minute. Each sample was assessed 10 times.

The tensile strength of the fabrics at maximum load was determined. The extension percentage or elongation was calculated from the increase in length of sample from its starting length according to Equation 1.

$$Extension\ (\%) = \frac{Elongation}{Initial\ length} \times 100 \qquad \textbf{Equation 1}$$

Colour fastness of enzymatically dyed fabrics

The colour fastness to wash of dyed wool fabric samples was tested to determine the resistance of the colour on the dyed fabrics according to British Standard test method as BS EN ISO 105-C10:2007 (A1) (Part C10: colour fastness to washing with soap or soap and soda). Wool fabrics were dyed by peroxidase catalysis of selected precursors in different buffer systems and pH values. After peroxidase-catalysed coloration, wool fabric samples were either just rinsed or soap-washed prior to the testing of colour fastness to washing. The dyed fabric sample was attached to an SDC multifibre strip fabric containing six different types of fibres (wool, acrylic, polyester, nylon 6.6, bleached cotton and cellulose acetate). The dyed fabric samples were washed in a washing solution containing 5 g/L of soap flakes at a liquor to goods ratio 50:1. The samples were incubated and agitated in a Roaches RO2008 Washtec wash wheel set at a temperature of 40°C for 30 minutes. After washing, the samples were rinsed with cold water and left to air-dry at room temperature.

Colour fastness to rubbing of enzymatically dyed fabric samples was determined in the accordance with British Standard test method BS EN ISO 105-X12:2002 (Part X12: colour fastness to rubbing) using a digitally recorded hand-operated crockmeter, James Heal Crockmaster Model 670HD. Colour change of the dyed samples and staining on cotton lawn rubbing cloths under dry and wet conditions were graded.

Colour changes due to washing and rubbing were assessed and graded under a VeriVide lightbox with standard artificial daylight following test method BS EN 20105-A02:1995, ISO 105:A02:1993, determining changes in colours using grey scale. Colour loss and staining results were used for assessing the colour differences corresponding to grey scale grading numbers from 5, 4/5, 4, 3/4, 3, 2/3, 2, 1/2, and 1, representing no colour loss/stain to complete colour loss/stain respectively.

The light fastness properties of wool fabric samples from enzymatic coloration was tested according to the British Standard test method BS EN ISO 105-B02:2014 (Part B02: colour fastness of textiles to light: artificial light: Xenon arc fading lamp test). Fabric samples were attached on a card and placed in specimen holder, which was placed in the test chamber of a James Heal Apollo light and weather fastness tester. The samples were exposed to light for 6 hours and 12 hours. A set of five blue wool references grades (1 - 5) were used during the test as known controls to obtain a numerical rating for the light fastness of each tested sample.

Results and discussion

The application of the enzyme peroxidase on the coloration of wool fabrics was explored. During the enzymatic process, various parameters, including the presence of hydrogen peroxide, precursor, processing temperature, processing time and pH condition, can inhibit or accelerate the enzymatic

coloration. The optimum level can enhance the performance of peroxidase-catalysed coloration of wool fabrics.

Effect of peroxidase and hydrogen peroxide on the enzymatic coloration of wool fabrics

The enzyme peroxidase requires the presence of peroxides, such as hydrogen peroxide, to act as a co-enzyme to catalyse the oxidation of substrates.[2,18-21] The potential for the generation of colourants through peroxidase-catalysed oxidation of the substrate in the presence of hydrogen peroxide was explored. The precursor 1,4-dihydroxybenzene (1,4DHB) was used as the substrate in the enzyme catalysis at pH 5 and 30°C for an hour for coloration of wool. The results are shown in Table 2.

[Table 2 about here]

It was found that formation of the colourant cannot be achieved in the absence of hydrogen peroxide, peroxidase or precursor. When these three components were combined together in the process, enzymatic coupling or polymerisation was achieved to produce colourant on wool fabrics. Peroxidase catalysation in the presence of hydrogen peroxide occurs through a process whereby hydrogen peroxide binds to an active site of peroxidase, which converts hydrogen peroxide to water through two electron reduction and peroxidase to its oxidised state.[22,23] The relocation of electrons in a redox reaction oxidises the precursor while the peroxidase returns to its original form.[24] Therefore, the precursor can be oxidised to form active intermediate radicals, resulting in their further coupling or polymerisation to produce a conjugated colourant,[3,22,25] as illustrated in Figure 1. The results confirmed that peroxidase-catalysed oxidation requires hydrogen peroxide and a precursor to achieve wool coloration.

[Figure 1 about here]

Effect of processing time and temperature on the enzymatic coloration of wool fabrics

The processing time and temperature are vital control parameters in the enzymatic coloration process. Therefore, the effect of processing time and temperature in peroxidase-catalysed coloration was investigated. The processing time was set for 1, 2 and 4 hours at different processing temperatures between 30 and 70°C.

[Table 3 about here]

Table 3 shows the influence of processing time and temperature on peroxidase-catalysed coloration of wool fabrics. The highest depth in colour shade was achieved from a 1- or 2-hour treatment. Therefore, the optimum duration was 1- 2 hours for peroxidase-catalysed coloration of wool fabrics because a longer dyeing time of 4 hours was unable to achieve a higher depth of colour and may also risk fibre damage.

It has been suggested from previous studies that peroxidase reacts at a wide range of temperatures from 10 to 100°C[26,27] with different reactions. The results in the current study found that peroxidase-catalysed coloration occurred at temperatures of 30 or 40°C and achieved a higher depth of colour on the wool fabrics compared with when fabrics were dyed at 50, 60 or 70°C, at which point colours gradually fade due to the decrease in peroxidase catalysis. These observations on the effect of temperature on peroxidase catalysis in the current study appear to be consistent with previous studies using this enzyme.[28-30] This is because increasing the temperature causes denaturation of the enzyme protein, resulting in the loss of enzyme activity, and may result in damage to the wool fibres and shrinkage of the wool fabric.

The *K/S* value was used to assess the colour strength of wool fabrics enzymatically coloured at different processing times of 15 - 240 minutes. Figure 2 shows that the *K/S* value of wool fabrics were increased with increasing treatment time. After 15 minutes, the *K/S* value reached 12.6. Then, *K/S* gradually increased to 26.9 at 60 minutes and reached a plateau of coloration of 32.1 at 120 minutes, without any further increase at 240 minutes. The results indicate that peroxidase was powerful enough to react and polymerise a colour on wool fabrics and generate colour within 15 - 60 minutes, thereby achieving time-savings in comparison with conventional coloration methods. However, when considering colour achieved on fabrics, dyeing for 60 minutes would be more appropriate, so that the peroxidase can allow the colourants to penetrate deep into the wool fibre to produce the strongest shade and hue. These results confirm that peroxidase-catalysed coloration conditions of 30°C for 60 minutes can produce optimal colour strength without causing damage on wool fibres.

[Figure 2 about here]

Because the enzymatic coloration process was undertaken through the peroxidase-catalysed oxidation in the presence of a low concentration of hydrogen peroxide, the effect of enzymatic dyeing processes on the mechanical properties of wool fabrics were investigated. Wool fabrics dyed enzymatically at 30 - 70°C for 1 and 2 hours were tested for their tensile strength at maximum load, and their results are presented in Table 4. There was no fibre damage to the wool fabrics during the peroxidase-catalysed coloration process. After comparing the average tensile strength and strain at the maximum load for treated and untreated wool sample, it was concluded that there was no significant difference. Thus, the overall tensile strength of wool fabrics was not affected during enzyme-catalysed processing lasting either 1 or 2 hours at 30 or 40°C. These parameters may preferably be used for the peroxidase-catalysed coloration of wool fabrics.

[Table 4 about here]

SEM analysis was carried out to assess whether the surface of the dyed wool fibre underwent any damage attributable to peroxidase-catalysed coloration. Dyed samples that had been treated for 1 or 2 hours at 30, 50 or 70°C were selected to observe any changes in the surface morphology of wool using SEM analysis (Figure 3). For fabrics dyed for 1 hour at 30°C (see Figure 3b) or 40°C (not shown), no damage on the fibre surface was observed, and there was no substantial difference when compared with the untreated sample (Figure 3a). After dyeing the wool fabric for 1 hour at either 50 or 70°C, it was observed that there was a slight but insignificant change to the wool scales. However, dyeing the wool for 2 hours at either 50 or 70°C caused the wool scales to break down. With increasing temperature, the wool scales were gradually opened, cracked and peeled. It was concluded that peroxidase-catalysed coloration for 1 hour at 30 and 40°C would not cause any damage to wool fibres and therefore would not make any significant difference in colour shade and depth in comparison with other dyeing conditions. Thus, further experiments for enzymatic coloration were undertaken at 30°C.

[Figure 3 about here]

Effect of pH values on the enzymatic coloration of wool fabrics

pH is an essential parameter for controlling enzyme activity. Enzymes are usually active under mild or neutral conditions[30] or within a restricted range of pH values.[27] In the case of peroxidase catalysation, it was found that the coloration under alkaline or acidic conditions could produce different results in terms of colour hue and depth of shade. Different pH values were employed for analysing the peroxidase-catalysed coloration of wool fabrics by using citrate buffer for pH 3 - 8, phosphate buffer for pH 9, or bicarbonate buffer for pH 10 - 11.

Table 5 shows that the peroxidase catalysis of the precursor 1,4DHB generated a strong brown colour on wool fabrics at pH 5, which then became a lighter yellow when the pH of enzyme treatment increased from 5 to 11. These results indicate that colour formation by peroxidase catalysis of 1,4DHB can achieve various shades of a particular colour corresponding to pH conditions. It appears that pH could be the influence on the colour hue and shade from peroxidase-catalysed coloration. The change in colour hue and shade caused by pH could be due to different structures of polymeric colourants formed during the enzymatic synthesis or ionisation of hydroquinone to hinder the enzymatic catalysis.[30] In order to confirm this effect, more precursors were tested for enzyme catalysis at different pH values and are described in the following section.

[Table 5 about here]

Effect of different precursors on the peroxidase-catalysed coloration of wool fabrics

Enzymatic coloration of wool fabrics was further developed by peroxidase catalysis of different precursors at different pH values to extend the range of colour hues and shades. The simple aromatic compounds, including 1,2-dihydroxybenzene (1,2DHB), 2,7-dihydroxynaphthalene (2,7DHN), catechin hydrate (CAT), 2,5-diaminobenzenesulfonic acid (2,5DABS), 3-amino-4-hydroxybenzenesulfonic acid (AHSB), p-phenylenediamine (PPD), 2-aminophenol (2-AP), and 4-aminophenol (4-AP), were used as precursors for peroxidase catalysation.

Table 6 shows that a range of colour on wool fabrics was achieved by peroxidase-catalysed coloration with the selected precursors at pH values ranging from 3 to 11. The results show that colour varies corresponding to the use of different pH values in the peroxidase-catalysed coloration. It was confirmed that peroxidase can actively catalyse the reaction across a range of acidic to alkaline pH values, but this was dependent on the individual substrate. By controlling pH, various colour hues and shades on wool fabric can be achieved, even through peroxidase catalysis of the same precursor.

[Table 6 about here]

It was observed when using the precursor 2,7DHN under peroxidase catalysis that the strongest shades of grey and blue occurred under neutral or milder alkaline conditions using either citrate buffer or phosphate buffer. Under acidic processing conditions ranging from pH 3 to 6, colour shades and hues varied from yellow to light orange, but a green and brown colour was produced on wool under stronger alkaline dyeing conditions with peroxidase at pH 10 - 11.

With the precursor PPD under peroxidase catalysis, the darkest colour shade can be produced across a wide range of pH from 4 to 11. At pH 7 - 9, wool fabric can be dyed almost black with an L^* value of 28.9 to 24.9 through peroxidase catalysis of precursor PPD. By increasing pH to 10 and 11, a strong brown colour can be achieved on wool. The mechanism of colourant synthesis under enzymatic oxidation of precursors may be different to that under chemical oxidation, especially at different pH values. Further research is needed to understand the conjugated structures of the different colours formed under peroxidase catalysis at different pH values.

The use of the precursors AHSB and 2-AP under peroxidase catalysis achieved strong red and orange colour on wool when buffered at pH 4 - 6 during the enzymatic process. By increasing the pH in the dyeing process, the colour on wool shifted to yellow.

Interestingly, there were significant changes in the colour obtained on wool fabrics when the dyeing condition were changed from weak alkaline to stronger alkaline conditions of pH 10 and 11 using a bicarbonate/carbonate buffer. Colours shifted from greenish blue to brown in the presence of the precursor 2,7DHN, pink to yellow in the presence of the precursor 2,5DABS, black to brown in the presence of the precursor PPD, and orange to yellow in the presence of the precursors 2-AP and 4-AP.

This is because strong alkaline conditions either dramatically affect the peroxidase-catalysed reaction, resulting in the formation of different coupling or polymeric structures of the conjugated system, or affect the activity of peroxidase to achieve strong colour. Enzyme-catalysed oxidation is very specific to the oxidation of precursors, so that wool fibres might suffer physical damage caused by the strong alkaline conditions, rather than oxidation by the trace amount of peroxide. To limit the fibre damage in the process, the enzymatic wool coloration could be carried out under mild alkaline conditions through rapid enzyme catalysis at low temperatures.

The effect of different precursors on the peroxidase-catalysed coloration of wool fabrics indicates that the difference in colour could be attributable to the different conjugated structures of colourants, due to the organism of the precursor and its chemical structure containing phenolic, amino or carboxylic groups. Therefore, a wide colour spectrum can be achieved on wool via selection of precursors and controlling pH for peroxidase catalysation.

Effect of precursor concentration on the enzymatic coloration of wool fabrics

When the concentration of precursor in peroxidase-catalysed coloration was increased, the colour on wool achieved was assessed to discover whether the depth of colour shade intensifies or if the colour hue shifts. Treatment using the pH conditions that gave the strongest colour for six of the precursors were selected. The six precursors selected for their strong colour were 2,7DHN (pH 9), 2,5DABS (pH 4), AHSB (pH 4), CAT (pH 5), PPD (pH 9), and 4-AP (pH 4) at their respective concentrations (w/v) of 0.05%, 0.12%, 0.25%, and 0.50%.

[Table 7 about here]

When increasing the precursor concentration in the peroxidase-catalysed coloration process, significant differences in colour and shade occurred with the precursor 2,7DHN at pH 9, 2,5DABS at pH 4 and AHSB at pH 4. Table 7 shows that the colour shades slightly intensified after increasing the precursor concentration 10-fold from 0.05% w/v to 0.50% w/v. By contrast, it was interesting to note that, when dyeing with 2,7DHN, a colour hue shift occurred with an increase in its concentration. Blue and greenish blue colours can be produced by coloration with precursor 2.7DHN at the concentration of 0.05% w/v and 0.12% w/v, shifting to a dark greyish green colour at 0.25% w/v and 0.50% w/v.

A positive correlation between the concentration of precursor and depth of colour was found when dyeing with the precursor 2,5DABS at pH 4 and AHSB at pH 4. The depth of colours on the wool fabric increased slightly without any shifting of colour shade from peroxidase-catalysed coloration while increasing the precursor concentration from 0.05% to 0.50% w/v .

Colour fastness assessments

The colour fastness to washing of wool fabrics dyed by peroxidase-catalysed coloration was tested to observe colour loss, colour changes, colour fading of dyed test samples and colour staining onto multifibre strip fabric during wash fastness testing. The selected colours of wool fabrics from peroxidase catalysation of six precursors at different pH values were prepared for testing. After peroxidase-catalysed coloration, wool fabric samples were either soap-washed or simply rinsed in running tap water without soap-washing prior to testing their colour fastness to washing.

[Tables 8 and 9 about here]

Table 8 shows the colour fastness to washing properties of wool samples dyed by peroxidase catalysation without post-soaping wash. Colour loss occurred on all selected coloured samples. A fair colour fastness to washing for wool samples dyed by peroxidase catalysis of 2,7DHN, CAT, 2,5DABS, AHSB, PPD, and 4-AP was achieved with grades of 2, 3, 3/4, 3/4, 2, and 4 respectively without colour shifting. After evaluating colour staining on a multifibre strip fabric, it was found that all the selected colours achieved excellent to very good grades on adjacent fabrics. The dyed sample with PPD and 4-AP had an exceptionally poor grade on nylon. On cellulose acetate, 4-AP achieved a rating at 3/4 while PPD achieved an unacceptable grade. Therefore, without a soaping wash after peroxidase-catalysed coloration, there were unfixed colourants absorbed on the surface of the fabric through Van der Waals forces and hydrogen bonds.

Table 9 shows colour fastness to washing properties of wool samples dyed by peroxidase catalysation with a post-soaping wash. The colour fastness of dyed samples after a post-soaping wash was better than that of the dyed samples without a post-soaping wash. There was no visible change in dyed colour with CAT. A minor change occurred on dyed sample with 4-AP achieving a rating of 4/5 while 2,7DHN and PPD each achieved a colour fastness rating of 4. However, 2,5DABS and AHSB both achieved a rating of 3/4. Therefore, a soaping wash after peroxidase-catalysed coloration is required to remove any unfixed surface colour to help achieve better colour fastness to wash.

[Table 10 about here]

Colour fastness to rubbing under dry and wet conditions was assessed to examine any colour changes of wool samples dyed by peroxidase-catalysed coloration. The results in Table 10 indicate that wool fabrics dyed by peroxidase-catalysed coloration had excellent rubbing fastness. There were no colour losses or changes on any dyed samples although there were slight colour staining on cotton lawn rubbing cloths under dry conditions especially for the samples dyed by peroxidase catalysis of PPD. However, these were still at an acceptable rating of 3/4. This could be attributed to the high depth of the black colour shade of the wool fabric produced by the peroxidase-catalysed coloration with PPD. Under wet

conditions, the results showed a similar trend. The rubbing fastness of the dyed wool fabrics under dry conditions was slightly better than that under wet conditions.

According to light fastness results, after the exposure of the dyed wool samples to UV light for 6 and 12 hours, dyed colour on wool fabrics by peroxidase-catalysis of CAT, 2,5DABS, PPD and 4-AP achieved a blue wool grade of 3-4. However, 2,7DHN and AHSB had a high degree of colour change after 6 and 12 hours of exposure. The enzyme-synthesised colour from 2,7DHN actually changed from a blue to a brownish shade with a reduction of colour brightness, which is why the rate of colour fastness to light was rated low, due to the colour shift responding to UV light. The difference in colour fastness to light could be attributed to the different structure of enzymatically synthesised colourants.

Peroxidase-catalysed coloration using binary precursors

The potential of peroxidase-catalysed coloration was further investigated in the use of binary precursors in the enzymatic process for wool coloration. The combination of two precursors with different chemical structures was used in peroxidase-catalysed coloration of wool fabrics. Precursor 2,7DHN was combined selectively with 2,5DABS at pH 7 and pH 9, AHSB at pH 8, CAT at pH 9, 2-AP at pH 7, pH 9 and pH 10, respectively. Two precursor concentrations at 0.05% w/v and 0.025% w/v were used. The resulting colour shade and hue from the enzymatic coloration between two precursors were assessed, as shown in Table 11.

[Table 11 about here]

It was found that the combination of two different precursors for peroxidase catalysis in the enzymatic dyeing process of wool fabrics did not achieve an exact overlay of the individual colour from the peroxidase catalysis of corresponding precursors. This could be due to the competition between precursors for peroxidase, or the coupling or polymerisation between different precursors rather than individual precursors. Although more colour shades on wool fabrics can be achieved by the peroxidase catalysis of the combined precursors, it might be difficult to predict the resulting colour based on the colour from the individual catalysed coloration.

By combining 2,7DHN and 2-AP in peroxidase-catalysed coloration at pH 7, a strong orange colour was produced on the wool fabric. This colour could be a mixture of the light greenish colour from the 2,7DHN polymerised colourant and the red colour from the 2-AP polymerised colourant. The *K/S* values of dyed fabrics over the whole visible spectrum of wavelengths from 400 to 700 nm (Figure 4) confirm that the colour from the combination was overlapping from the individual coloration. However, when 2,7DHN was combined with 2-AP in enzymatic synthesis at pH 9 or 10, it appears that the colour was mainly contributed by 2-AP. This could be attributed to self-coupling of 2-AP being more

preferential than the reaction between 2-AP and 2,7-DHN, therefore occupying most of the enzyme activity during enzyme catalysis.

When 2,7DHN was combined with other precursors in the enzymatic synthesis at pH 9, the strong blue colour from the peroxidase catalysis of 2,7DHN does not appear to develop. This blue colour was not fully shown in the resulting colour from the combination. The *K/S* spectrum between 400 and 700 nm (Figures 5 and 6) confirmed that colour development via enzyme catalysis of 2,7DHN by itself might not occur in binary combinations. This could be because the coupling or polymerising reaction between 2,7DHN and precursors CAT or 2,5DABS is more rapid that of 2,7DHN by itself, which occupied the enzyme activity during enzyme catalysis. This could be the reason for the lack of any contribution from 2,7DHN by itself to coloration. It is proposed that two precursors were oxidised by peroxidase and that both of them were coupled or polymerised together and incorporated in the same conjugated chain. Therefore, a different colour could be formed rather than the combination colour from the two conjugated structures of different colours.

[Figures 4, 5 and 6 about here]

Conclusions

Over recent years, there has been growing concern about the environmental impact of the textile industry, particularly in relation to coloration and finishing processes. To address this problem, sustainable textile bio-processing has gained attention due to its reusability and/or biodegradability. The current research study contributes to an understanding of the use of enzyme peroxidase for textile coloration of wool fabrics as an alternative coloration method to conventional textile coloration. The pocessing parameters in this enzymatic coloration process, namely, pH, temperature and processing duration, along with various precursors and their concentrations, were investigated. The results revealed that wool fabrics can be successfully dyed by peroxidase-catalysed coloration producing a broad range of colours at 30°C for 60 minutes rather than using the time and energy required in conventional high-temperature coloration of wool. It was found that peroxidase can be active over a broad range of pH values to catalyse the synthesis of colourants, resulting in successful coloration of wool fabrics. Under different pH conditions, the colourants of wool fabrics were produced with different shade of particular colours via the enzyme catalysis of nine different precursors. From tensile and colour fastness tests, it was found that there was no significant damage to wool fibre during peroxidase-catalysed coloration. The bioprocessing method developed using peroxidase catalysis for coloration of wool fabrics has potential not only as an alternative coloration process, but also saving energy and preventing wool fibre damage.

Acknowledgement

The authors would like to thank Novozymes for supplying the enzyme used in this study.

References

1. Shen J, Smith E. Enzymatic treatments for sustainable textile processing. In: Blackburn RS, ed. *Sustainable Apparel - Production, Processing and Recycling*. Cambridge: Woodhead Publishing; 2015:119–133.

2. Fu J, Nyanhongo GS, Gübitz GM, Cavaco-Paulo A, Kim S. Enzymatic colouration with laccase and peroxidases: recent progress. *Biocatal. Biotransform.* 2012;30(1):125–140.

3. Shin H, Guebitz G, Cavaco-Paulo A. "In Situ" enzymatically prepared polymers for wool coloration. *Macromol. Mater. Eng.* 2001;286:691–694.

4. Jeon J, Kim E, Murugesan K, Park H, Kim Y, Kwon J, Kim W, Lee J, Chang Y. Laccase-catalysed polymeric dye synthesis from plant-derived phenols for potential application in hair dyeing: Enzymatic colourations driven by homo- or hetero-polymer synthesis. *Microb. Biotechnol.* 2010;3:324–335.

5. Bai R, Yu Y, Wang Q, Yuan J, Fan X, Shen J. Laccase-catalyzed in-situ dyeing of wool fabric. *J. Text. Inst*. 2016;107(8):995–1003.

6. Sun S, Xing T, Tang R. Enzymatic dyeing and functional finishing of textile fibres with ferulic acid. *Indian J. Fibre & Text. Res.* 2015;40:62–69.

7. Prajapati CD, Smith E, Kane F, Shen J. Laccase-catalysed coloration of wool and nylon. *Color. Technol*. 2018;134:423–439.

8. Zhang T, Bai R, Shen J, Wang Q, Wang P, Yuan J, Fan X. Laccase-catalyzed polymerization of diaminobenzenesulfonic acid for pH-responsive color-changing and conductive wool fabrics. *Text. Res. J*. 2018;88(19):2258–2266.

9. Bai R, Yu Y, Wang Q, Fan X, Wang P, Yuan J, Shen J. Laccase-catalyzed poly(ethylene glycol)-templated 'zip' polymerization of caffeic acid for functionalization of wool fabrics. *J. Clean. Prod.* 2018;191:48–56.

10. Bai R, Yu Y, Wang Q, Shen J, Yuan J, Fan X. Chitosan-templated bio-coloration of cotton fabrics via laccase-catalyzed polymerization of hydroquinone. *Eng. Life Sci.* 2019;19:643–654.

11. Kaiser ET, inventor; The Procter & Gamble Company, assignee. Hair dyeing premixes containing peroxidase enzymes stabilized with heme complexing agents. US Patent 3,893,803. 8 July 1975.

12. Zeffren E, Sullivan JF, inventors; The Procter & Gamble Company, assignee. Enzyme-activated oxidative process for coloring hair. US Patent 3,957,424. 18 May 1976.

13. Wever R. Applications for (Halo) peroxidases. Paper presented at: Proceedings of the colloquium: Machanisms of biohalogenation and dehalogenation; 16–18 September 1996; Amsterdam.

14. Hamid M, Rehman K. Potential application of peroxidases. *J. Food Chem.* 2009;115:117–1186**.**

15. Bansal N, Kanwar SS. Peroxidase(s) in Environment Protection. *Sci.World J*. 2013:ID714639. doi:10.1155/2013/714639.

16. Nakayama T, Amachi T. Fungal peroxidase: its structure, function, and application. *J. Mol. Catal. B Enzym.* 1999;6(3):185–198.

17. Banci L. Structural properties of peroxidases. *J.Biotechnol.* 1997;53(2-3):253–263.

18. Reihmann M, Ritter H. Synthesis of phenol polymers using peroxidases. In: Kobayashi S, Ritter H, Kaplan D, eds. *Enzyme-Catalyzed Synthesis of Polymers*. Leipzig: LE-TeX Jelonek, Schmidt Vöckler GbR; 2006;194:1–49.

19. Uyama H, Kobayashi S. Enzyme-catalyzed polymerization to functional polymers. *J. Mol. Catal. B: Enzym.* 2002;19-20:117–127.

20. Freddi G. Functionalisation of wool and silk fibres using non-proteolytic enzymes. In: Nierstrasz VA, Cavaco-Paulo A, eds. *Advances in Textile Biotechnology*. Cambridge: Woodhead Publishing; 2010:193–234.

21. Hollmann F, Arends IWCE. Enzyme Initiated Radical Polymerizations. *Polymers.* 2012;4(1):759–793. doi:10.3390/polym4010759.

22. Won K, Kim YH, An ES, Lee YS, Song BK. Horseradish Peroxidase-Catalyzed Polymerization of Cardanol in the Presence of Redox Mediators. *Biomacromolecules.* 2004;5(1):1–4.

23. Nyanhongo GS, Prasetyo EN, Kudanga T, Guebitz G. Grafting of functional molecules: Insights into peroxidase-derived materials. In: Torres E, Ayala M, eds. *Biocatalysis Based On Heme Peroxidases: Peroxidases as Potential Industrial Biocatalysts*. Heidelberg: Springer-Verlag Berlin Heidelberg; 2010:155–177.

24. Renneberg R, Berkling V. Enzymes: Molecular supercatalysts for use at home and in industry. In: Demain AL, ed. *Biotechnology for Beginners.*2nd ed. London: Elsevier; 2017:36– 63.

25. Veitch NC. Horseradish peroxidase: a modern view of a classic enzyme. *Phytochemistry.* 2004; 65(3):249–259.

26. Dawson TL. pH and its importance in textile coloration. *JSDC.* 1981;97:115–125.

27. Scopes RK. Enzyme activity and assays. *ELS*. 2002:1–6. doi:10.1038/npg.els.0000712.

28. Saeidian S, Ghasemifar E. Effect of temperature on guaiacol peroxidase of pyrus communis. *ILNS.* 2013;5:46–51. doi:10.18052/www.scipress.com/ILNS.5.46.

29. Mizobutsi GP, Finger FL, Ribeiro RA, Puschmann R, Neves LLM, Mota WF. Effect of pH and temperature on peroxidase and polyphenoloxidase activities of litchi pericarp. *Sci. Agric.* 2010;67(2):213–217. doi:10.1590/S0103-90162010000200013.

30. Cavaco-Paulo A, Gübitz G. Catalysis and processing. In: Cavaco-Paulo A, Gübitz G, eds. *Textile Processing with Enzymes.* Cambridge: Woodhead Publishing; 2000:86–119.

Table 1 Chemical structure of precursors

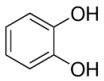
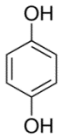
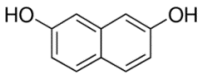
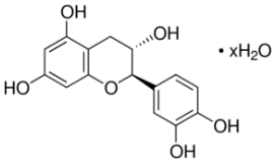
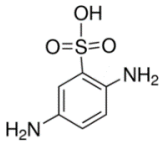
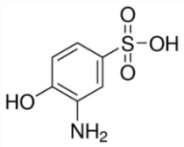
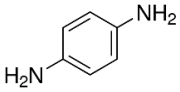
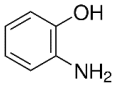
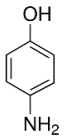
Precursors	Acronym	Chemical structure
1,2-dihydroxybenzene	1,2DHB	
1,4-dihydroxybenzene	1,4DHB	
2,7-dihydroxynaphthalene	2,7DHN	
catechin hydrate	CAT	
2,5-diaminobenzenesulfonic acid	2,5DABS	
3-amino-4-hydroxybenzenesulfonic acid	AHSB	
p-phenylenediamine	PPD	
2-aminophenol	2-AP	
4-aminophenol	4-AP	

Table 2 Effect of hydrogen peroxide, peroxidase and precursors on their own or combination on the coloration of wool fabrics

Chemical combination			Colour obtained	CIE $L^*a^*b^*$values		
Hydrogen peroxide	Enzyme peroxidase	Precursor (1,4DHB)		L^*	a^*	b^*
-	-	-		86.0	-0.5	10.0
✓	-	-		87.3	-0.6	10.0
✓	✓	-		80.5	-0.6	9.9
✓	-	✓		85.9	-0.1	10.0
-	✓	-		85.3	-0.6	11.0
-	✓	✓		85.9	-0.5	11.2
-	-	✓		87.3	-0.7	10.8
✓	✓	✓		33.4	13.1	15.2

Table 3 Colour achieved from peroxidase catalysation at different processing time and temperature with precursor 1,4DHB at pH 5

Temperature (°C)	Colour obtained and CIE *L*a*b** values		
	1 hour	2 hours	4 hours
30	 *L** 54.6 *a** 6.1 *b** 15.6	 *L** 51.2 *a** 5.2 *b** 15.9	 *L** 51.5 *a** 4.7 *b** 16.2
40	 *L** 51.3 *a** 5.9 *b** 16.5	 *L** 48.9 *a** 5.8 *b** 17.2	 *L** 52.9 *a** 7.0 *b** 19.5
50	 *L** 52.3 *a** 7.7 *b** 20.1	 *L** 51.3 *a** 7.6 *b** 19.9	 *L** 55.2 *a** 8.0 *b** 20.8
60	 *L** 52.3 *a** 8.3 *b** 20.6	 *L** 56.4 *a** 8.2 *b** 20.5	 *L** 57.1 *a** 9.0 *b** 22.1
70	 *L** 54.7 *a** 8.7 *b** 20.8	 *L** 56.8 *a** 9.1 *b** 21.9	 *L** 59.5 *a** 9.1 *b** 22.3

Table 4 Tensile properties (warp direction) of wool fabrics dyed by peroxidase catalysis of precursor 1,4DHB at pH 5 and different processing temperature for 1 or 2 hours

Temperature (°C)	Processing time (hr)	Maximum load (N)	Tensile strain at maximum load (%)
Untreated	-	393	43.4
30	1	414	46.9
40		415	46.6
50		412	46.8
60		411	47.1
70		401	47.0
30	2	420	49.4
40		418	48.7
50		404	47.9
60		413	48.6
70		400	49.9

Table 5 Colour achieved from peroxidase catalysation at different pH values from pH 3 to pH 11 with precursor 1,4-DHB

pH value																		
3		4		5		6		7		8		9		10		11		
*L**	69.5	*L**	59.3	*L**	54.6	*L**	65.5	*L**	74.1	*L**	78.5	*L**	84.4	*L**	86.0	*L**	86.3
*a**	6.7	*a**	6.3	*a**	6.1	*a**	6.6	*a**	4.7	*a**	4.5	*a**	1.3	*a**	0.6	*a**	0.0
*b**	17.8	*b**	15.2	*b**	15.6	*b**	17.8	*b**	14.9	*b**	11.8	*b**	11.6	*b**	13.8	*b**	13.4

Table 6 Colour shades of wool fabrics achieved from peroxidase-catalysed coloration with the selected precursors at different pH values from pH 3 to pH 11

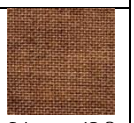
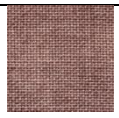
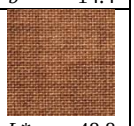
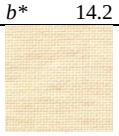
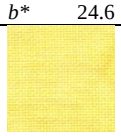
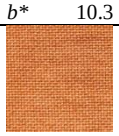
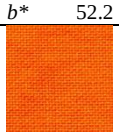
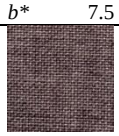
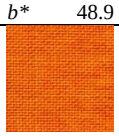
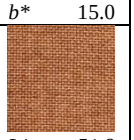
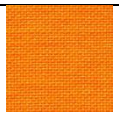
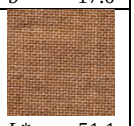
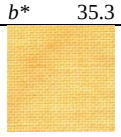
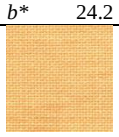
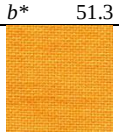
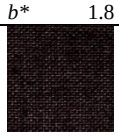
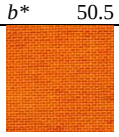
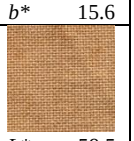
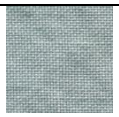
pH value	Precursor							
	1,2DHB	2,7DHN	CAT	2,5DABS	AHSB	PPD	2-AP	4-AP
3	-	*L** 85.3 *a** 1.2 *b** 11.0	*L** 85.2 *a** 1.3 *b** 12.9	 *L** 49.7 *a** 10.4 *b** 5.2	*L** 81.3 *a** 2.6 *b** 24.4	 *L** 66.6 *a** 2.6 *b** 4.1	 *L** 64.0 *a** 17.9 *b** 46.2	 *L** 42.3 *a** 8.1 *b** 14.4
4	 *L** 55.6 *a** 8.2 *b** 13.9	*L** 82.0 *a** 1.1 *b** 14.2	*L** 83.3 *a** 0.1 *b** 24.6	 *L** 43.5 *a** 13.3 *b** 10.3	 *L** 49.5 *a** 35.4 *b** 52.2	 *L** 49.5 *a** 6.0 *b** 7.5	 *L** 54.7 *a** 23.1 *b** 48.9	 *L** 48.8 *a** 7.9 *b** 15.0
5	 *L** 62.6 *a** 6.8 *b** 14.0	 *L** 77.8 *a** 2.2 *b** 12.5	 *L** 79.8 *a** 2.7 *b** 32.2	 *L** 54.2 *a** 13.0 *b** 18.0	 *L** 54.0 *a** 33.2 *b** 53.4	 *L** 46.0 *a** 4.2 *b** 3.3	 *L** 54.4 *a** 25.6 *b** 51.2	 *L** 51.9 *a** 8.6 *b** 17.0
6	 *L** 74.6 *a** 3.8 *b** 13.5	 *L** 80.3 *a** 0.3 *b** 11.7	 *L** 77.7 *a** 7.8 *b** 35.3	 *L** 64.3 *a** 15.1 *b** 24.2	 *L** 60.0 *a** 27.1 *b** 51.3	 *L** 39.2 *a** 1.7 *b** 1.8	 *L** 54.8 *a** 24.1 *b** 50.5	 *L** 51.1 *a** 6.2 *b** 15.6
7	 *L** 78.1 *a** 2.8 *b** 12.6	 *L** 69.4 *a** 2.8 *b** 7.3	 *L** 74.7 *a** 10.6 *b** 29.6	 *L** 72.3 *a** 11.6 *b** 27.4	 *L** 65.2 *a** 16.2 *b** 43.2	 *L** 28.9 *a** 3.9 *b** 2.1	 *L** 54.7 *a** 23.4 *b** 50.6	 *L** 59.5 *a** 5.7 *b** 17.6
8	-	 *L** 56.4 *a** -5.8 *b** -7.1	*L** 78.1 *a** 7.3 *b** 26.0	*L** 73.6 *a** 8.8 *b** 24.0	*L** 75.0 *a** 7.2 *b** 32.8	*L** 25.1 *a** 6.4 *b** 2.7	*L** 57.1 *a** 17.8 *b** 50.2	*L** 58.1 *a** 6.7 *b** 19.1
9	-	*L** 52.4 *a** -6.1 *b** -6.4	*L** 81.6 *a** 3.4 *b** 21.2	*L** 72.8 *a** 7.4 *b** 15.3	*L** 82.4 *a** -0.7 *b** 12.7	*L** 24.9 *a** 9.4 *b** 4.0	*L** 56.4 *a** 10.2 *b** 45.2	*L** 66.8 *a** 5.8 *b** 19.0
10	-	*L** 55.1 *a** -4.9 *b** -4.8	*L** 84.0 *a** 5.6 *b** 16.9	*L** 81.9 *a** 2.7 *b** 14.4	*L** 84.4 *a** 0.8 *b** 12.0	*L** 34.2 *a** 11.5 *b** 9.5	*L** 67.7 *a** 1.7 *b** 29.3	*L** 71.4 *a** 4.7 *b** 18.9
11	-	*L** 64.6 *a** 4.8 *b** 13.9	*L** 81.0 *a** 4.3 *b** 13.5	*L** 83.3 *a** 1.8 *b** 14.3	*L** 84.1 *a** 0.0 *b** 10.7	*L** 35.1 *a** 11.8 *b** 8.2	*L** 69.1 *a** -1.1 *b** 17.3	*L** 78.4 *a** 2.9 *b** 18.4

Table 7 Colour achieved from peroxidase catalysation at different precursors, precursor concentration, and pH values

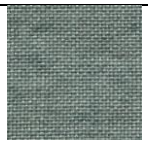
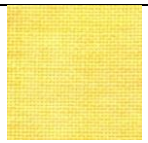
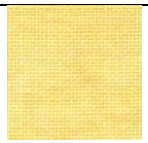
Precursor	pH value	Precursor concentration (%w/v)			
		0.05	0.12	0.25	0.50
2,7DHN	9	 *L** 52.4 *a** -6.1 *b** -6.4	 *L** 52.1 *a** -7.9 *b** -7.4	 *L** 51.3 *a** -7.9 *b** 5.0	 *L** 57.1 *a** -9.7 *b** 10.4
CAT	5	 *L** 79.8 *a** 2.7 *b** 32.2	 *L** 79.1 *a** 4.7 *b** 37.3	 *L** 79.0 *a** 3.7 *b** 34.7	 *L** 78.9 *a** 3.4 *b** 32.2
2,5DABS	4	 *L** 43.5 *a** 13.3 *b** 10.3	 *L** 42.8 *a** 11.1 *b** 10.0	 *L** 37.7 *a** 7.2 *b** 5.1	 *L** 32.1 *a** 3.7 *b** -0.5
AHSB	4	 *L** 49.5 *a** 35.4 *b** 52.2	 *L** 42.8 *a** 36.0 *b** 45.5	 *L** 36.7 *a** 35.6 *b** 36.8	 *L** 31.4 *a** 34.4 *b** 30.4
PPD	9	 *L** 24.9 *a** 9.4 *b** 4.0	 *L** 25.9 *a** 8.8 *b** 7.2	 *L** 24.4 *a** 8.0 *b** 7.6	 *L** 22.2 *a** 7.3 *b** 6.2
4-AP	4	 *L** 48.8 *a** 7.9 *b** 15.0	 *L** 48.9 *a** 8.0 *b** 13.7	 *L** 46.7 *a** 8.2 *b** 15.6	 *L** 46.5 *a** 8.0 *b** 17.1

Table 8 Colour fastness to washing property of peroxidase-catalysed coloration on wool fabrics with selective precursors and pH values in the absence of post-soaping wash

Precursor	pH value	Colour change	Colour staining					
			Cellulose acetate	Cotton	Nylon	Polyester	Acrylic	Wool
2,7DHN	9	2	5	4	4/5	5	5	5
CAT	5	3	5	4/5	4/5	5	5	5
2,5DABS	4	3/4	4/5	5	5	5	5	4/5
AHSB	4	3/4	4/5	4	4/5	4/5	4/5	4/5
PPD	9	2	1	4	1	4/5	4/5	4
4-AP	4	4	3/4	4	1	4/5	4/5	4

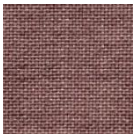
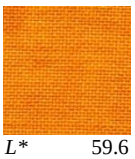
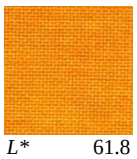
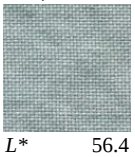
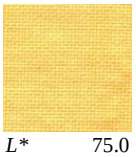
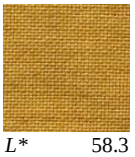
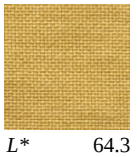
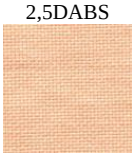
Table 9 Colour fastness to washing properties of post-soaping washed peroxidase-catalysed coloration on wool fabrics with selective precursors and pH values in the presence of post-soaping wash

Precursor	pH value	Colour change	Colour staining					
			Cellulose acetate	Cotton	Nylon	Polyester	Acrylic	Wool
2,7DHN	9	4	5	5	5	5	5	5
CAT	5	5	5	5	5	5	5	5
2,5DABS	4	3/4	4/5	5	5	5	5	4/5
AHSB	4	3/4	4/5	4	4	4/5	4	4
PPD	9	4	4	4/5	4	5	5	5
4-AP	4	4/5	4/5	5	4/5	5	5	4/5

Table 10 Colour fastness to rubbing and light properties of peroxidase-catalysed coloration on wool fabrics with selective precursors and pH values

Precursor	pH value	Colour fastness to rubbing			Colour fastness to light	
		Colour change	Rubbing conditions		Exposure period (hrs)	
			Dry	Wet	6	12
2,7DHN	9	5	4/5	4/5	1	1
CAT	5	5	4/5	4	3	3
2,5DABS	4	5	5	4/5	4	4
AHSB	4	5	4/5	4	2	1
PPD	9	5	3/4	3	3	3
4-AP	4	5	4	4	3	3

Table 11 Colour achieved on wool fabrics from peroxidase-catalysed coloration with the binary precursor system

pH value	Precursor/Colour obtained and CIE *L*a*b** values		Colour obtained / CIE *L*a*b** values	
	Precursor A 0.05% w/v	Precursor B 0.05% w/v	Precursor A+B at 0.05% w/v each	Precursor A+B at 0.025% w/v each
7	2,7DHN  *L** 69.4 *a** 2.8 *b** 7.3	2,5DABS  *L** 72.3 *a** 11.6 *b** 27.4	 *L** 50.3 *a** 6.1 *b** 6.8	 *L** 62.8 *a** 7.2 *b** 13.6
	2,7DHN  *L** 69.4 *a** 2.8 *b** 7.3	2-AP  *L** 54.7 *a** 23.4 *b** 50.6	 *L** 59.6 *a** 21.1 *b** 50.5	 *L** 61.8 *a** 19.4 *b** 50.0
8	2,7DHN  *L** 56.4 *a** -5.8 *b** -7.1	AHSB  *L** 75.0 *a** 7.2 *b** 32.8	 *L** 58.3 *a** 5.1 *b** 29.5	 *L** 64.3 *a** 3.1 *b** 25.1
9	2,7DHN  *L** 52.4 *a** -6.1 *b** -6.4	CAT *L** 81.6 *a** 3.4 *b** 21.2	 *L** 75.9 *a** 3.5 *b** 25.8	 *L** 70.4 *a** -4.8 *b** 7.0
	2,7DHN  *L** 52.4 *a** -6.1 *b** -6.4	2,5DABS  *L** 72.8 *a** 7.4 *b** 15.3	 *L** 69.2 *a** 0.0 *b** 10.3	 *L** 75.8 *a** 0.9 *b** 10.5
	2,7DHN  *L** 52.4 *a** -6.1 *b** -6.4	2-AP  *L** 56.4 *a** 10.2 *b** 45.2	 *L** 57.5 *a** 8.5 *b** 44.1	 *L** 61.8 *a** 6.0 *b** 44.2
10	2,7DHN  *L** 55.1 *a** -4.9 *b** -4.8	2-AP  *L** 67.7 *a** 1.7 *b** 29.3	 *L** 61.5 *a** 0.7 *b** 25.0	 *L** 66.2 *a** 0.2 *b** 14.9

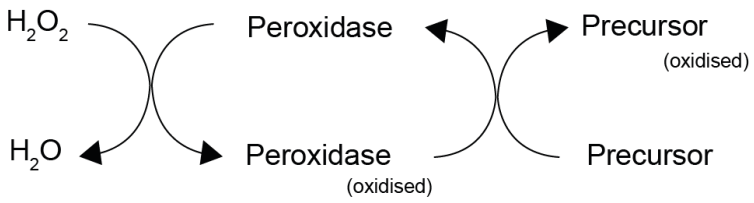


Figure 1 Mechanism of peroxidase-catalysed oxidation of a precursor acting as the enzyme's substrate

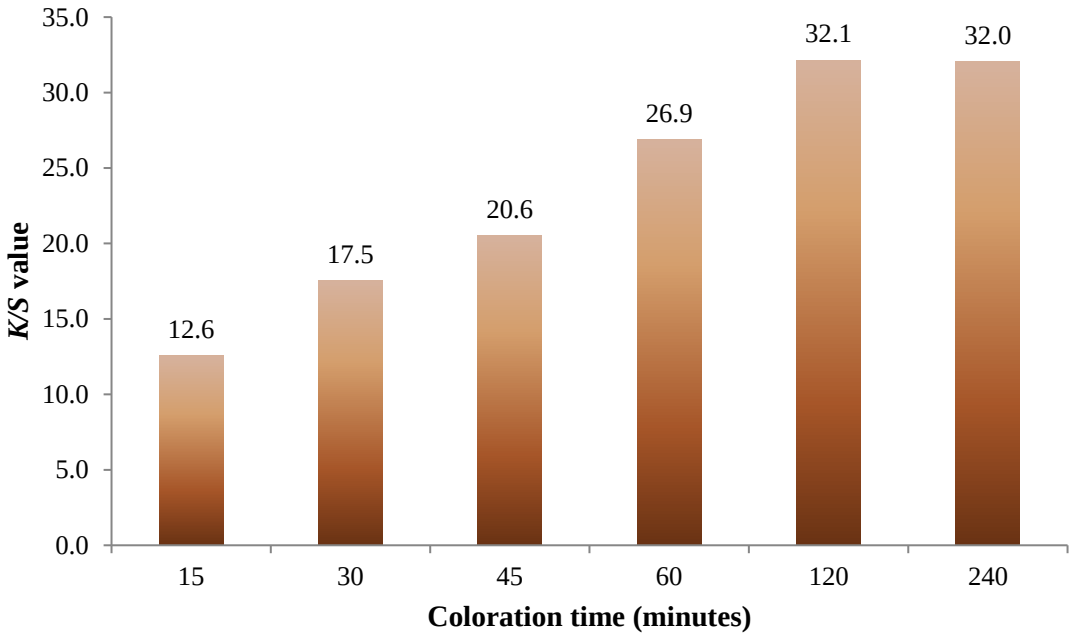


Figure 2 Colour absorption (700 nm) in different treatment times (minutes) of peroxidase-catalysed coloration

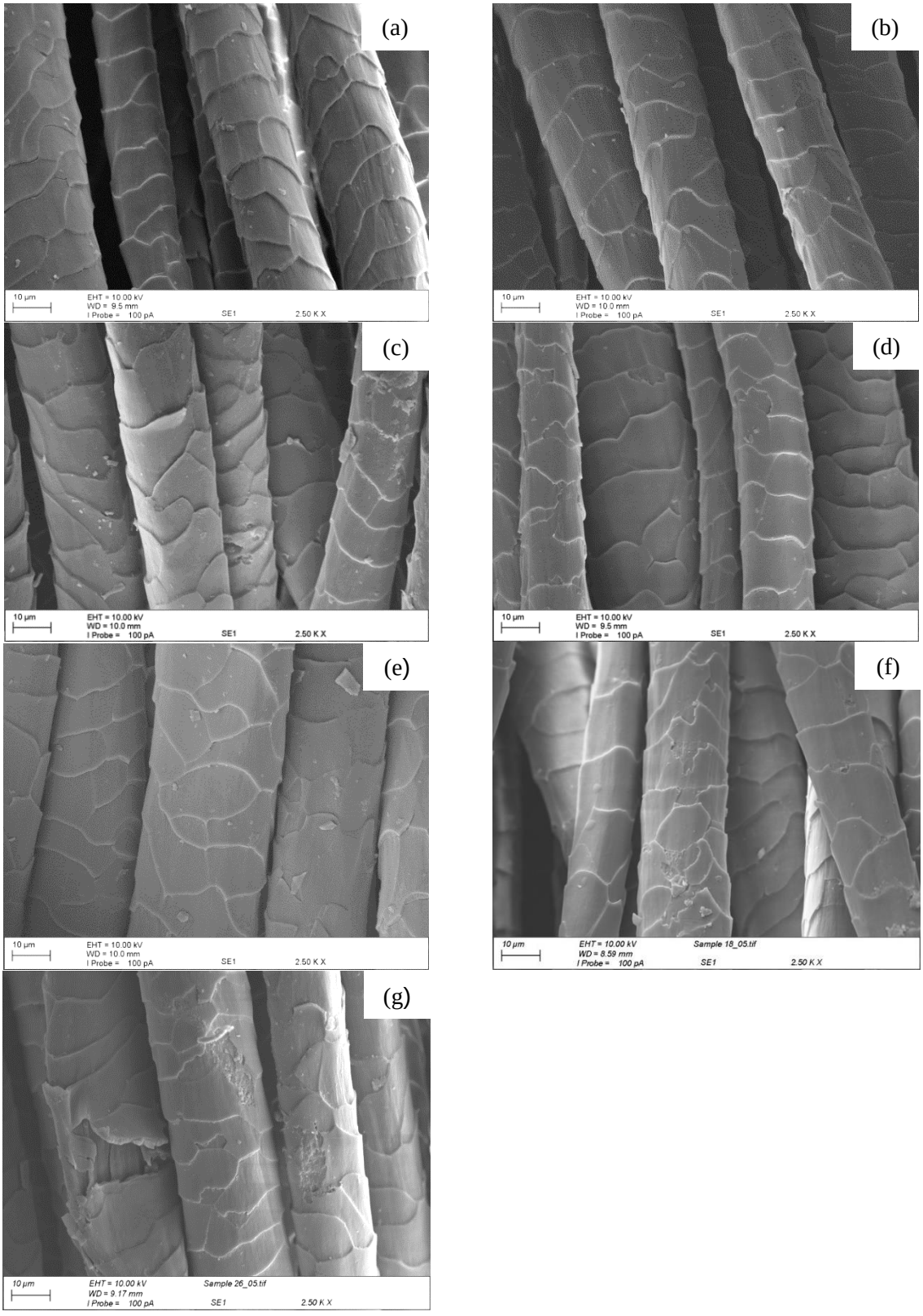


Figure 3 SEM images of wool fibre from fabric samples dyed using different processing time and temperature conditions: (a) untreated wool fabric; (b) wool fabric dyed at 30°C for 1 hour; (c) wool fabric dyed at 50°C for 1 hour; (d) wool fabric dyed at 70°C for 1 hour; (e) wool fabric dyed at 30°C for 2 hours; (f) wool fabric dyed at 50°C for 2 hours; (g) wool fabric dyed at 70°C for 2 hour

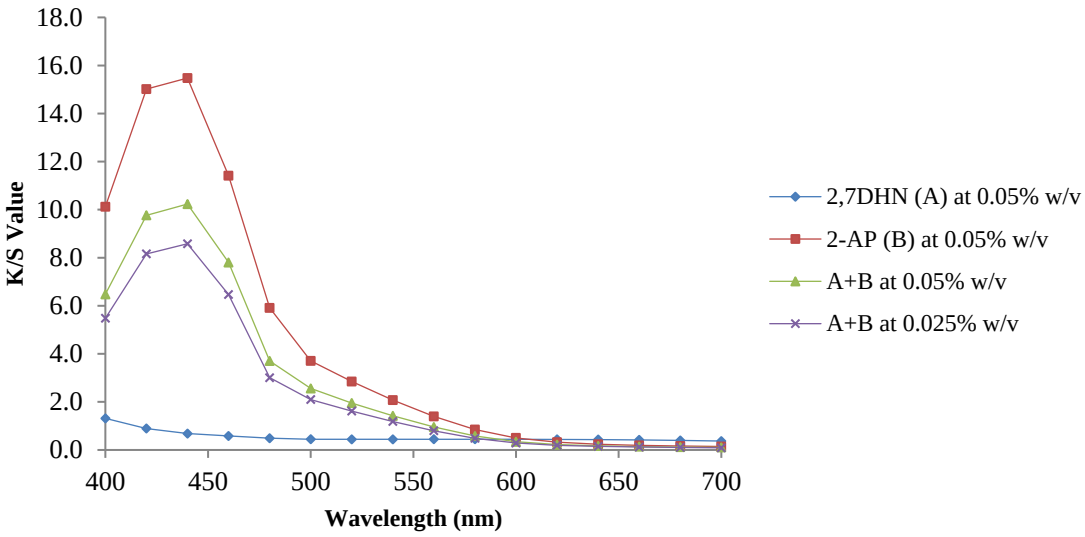


Figure 4 Peroxidase-catalysed coloration between precursor 2,7DHN (A) and 2-AP (B), and their combination at pH 7 on wool fabrics

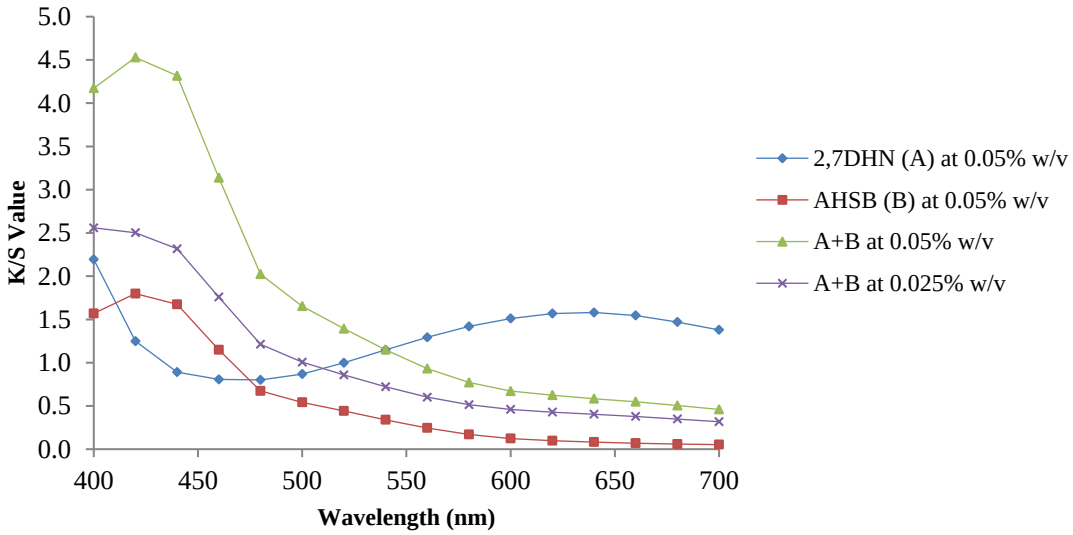


Figure 5 Peroxidase-catalysed coloration between precursor 2,7DHN (A) and AHSB (B), and their combination at pH 8 on wool fabrics

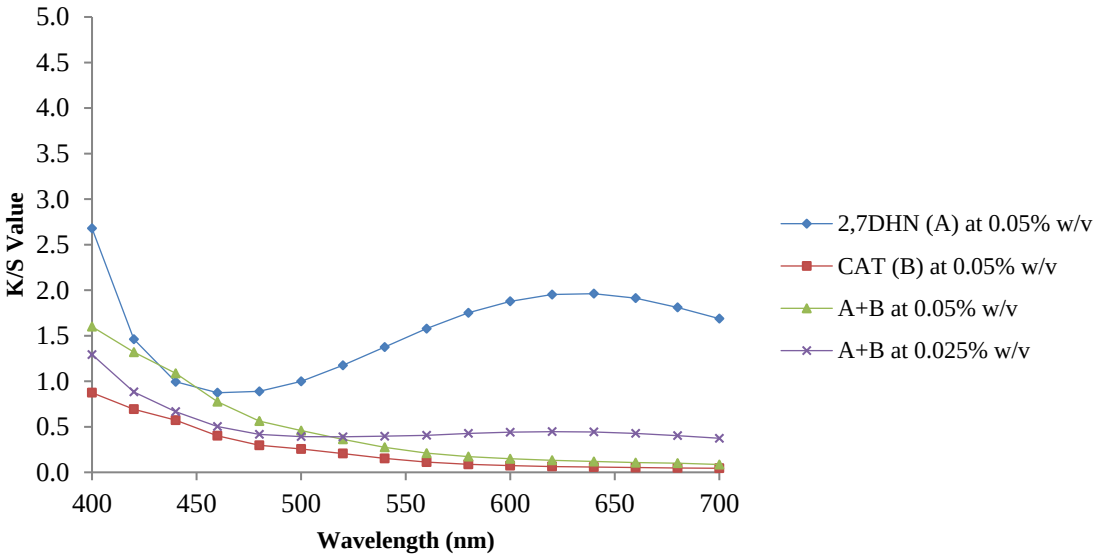


Figure 6 Peroxidase-catalysed coloration between precursor 2,7DHN (A) and CAT (B), and their combination at pH 9 on wool fabrics