Research Article

Chitosan-Templated Bio-coloration of Cotton Fabrics via Laccase-Catalyzed Polymerization of Hydroquinone

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Abbreviations: **FT-IR,** fourier-transform infrared spectroscopy ; **PHQ,** polymerized hydroquinone; **SEM,** scanning electron microscopy; **XPS,** x-ray photoelectron spectroscopy; **HQ,** hydroquinone; **TGA,** Thermogravimetric analysis; **2, 5-DABSA,** 2, 5-diaminobenzene sulfonic acid; **ABTS,** 2, 2′-azino-bis(3-ethylthiazoline-6-sulfonate); **UPF,** ultraviolet protection factor; **UVA,** ultraviolet radiation a; **UVB,** ultraviolet radiation b

Practical application

This study presents a novel bio-coloration method of cellulose materials using laccase catalysis of hydroquinone. Cotton fabric was pre-treated with chitosan to form the template and then followed by laccase-catalyzed in-situ dyeing with hydroquinone. Chitosan was used as template for the polymerization of hydroquinone, which endows high colorfastness and antibacterial activity simultaneously for dyed cotton fabric. This provides a new approach for preparing textiles with multiple functions.

Abstract

There is an increasing interest in the development of enzymatic coloration of textile fabrics as an alternative to conventional textile dyeing processes, which is successful for dyeing of protein fibers. However, unmodified cotton fabrics are difficult to be dyed through enzyme catalysis due to the lack of affinity of biosynthesized dyes to cotton fibers. In order to improve the enzyme-catalyzed dyeability of cotton fibers, chitosan was used to coat cotton fabrics as template. A novel and facile bio-coloration technique using laccase catalysis of hydroquinone were developed to dye chitosan-templated cotton fabrics. The polymerization of hydroquinone with the template of chitosan under the laccase catalysis was monitored by UV-vis spectrophotometer on the absorbance of reaction solution. A significant peak of UV-vis spectrum at 246 nm corresponding to large conjugated structures appeared and increased with increasing the duration of enzymatic catalysis. The effect of different treatment conditions on the laccase-catalyzed dyeing of cotton fabric was investigated to determine their optimal parameters of laccase-catalyzed coloration. FT-IR spectra demonstrated the formation of H-bond and Schiff base reaction between chitosan and polymerized hydroquinone (PHQ). Scanning electron microscopy (SEM) indicated that the surface of dyed cotton fiber was much rougher than that of the control sample. Moreover, X-ray photoelectron spectroscopy also revealed the existence of the chitosan/PHQ complex and polymerized hydroquinone on the dyed cotton fibers. This chitosan-templated approach offers possibility for biological dyeing coloration of cotton fabrics and other cellulosic materials.

1 Introduction

As an alternative to conventional dyeing method, enzymatic coloration of textile fabrics using oxidoreductases has been investigated for many years [1-4]. A wide range of simple aromatic substrates including phenols and phenol derivatives could be catalyzed by oxidoreductases and polymerized to colorant products (polymeric colorants) through oxidative coupling reactions [5-7]. Most of these polymeric colorants and enzyme-catalyzed coloration methods are suitable for dyeing of protein fibers, since protein fibers have abundant amino and phenolic hydroxyl groups which could form covalent bonds with the colorant products during enzyme catalysis [8-13]. Enzyme bioprocesses are generally carried out under milder conditions in aqueous media, resulting in the reduction of energy consumption in dyeing processes of textile fabrics and the prevention of fibers damage.

By contrast, cellulose fibers, especially cotton fiber, do not contain any amino groups. Therefore cellulose fibers have difficulty to be dyed with enzymatic bio-coloration process due to the low substantivity of enzymatically-generated colorants towards fibers. To date, there is still no feasible way or clear mechanism for *in-situ* enzymatic dyeing of cellulose fibres [14]. Hadzhiyska *et al*. found that satisfactory dye fixation on cotton fabrics could be achieved by increasing the concentration of the phenol component in laccase catalysis and transforming them into less soluble colorant products upon laccase oxidation [15]. Calafell *et al*. further studied the mechanism of laccase-catalyzed cross-coupling catechol and 2, 5-diaminobenzene sulfonic acid (2, 5-DABSA) for the coloration of cotton fabrics. High fixation levels were achieved only when catechol was applied at least fourfold excess [16]. However, wet rubbing fastness of the dyed cotton fabrics cannot meet the requirement due to poor affinity between dyes and cellulose. To overcome the deficiency, the pre-functionalization of cotton fibers tends to be an effective method to improve dyes fixation. It was reported by Kim *et al*. that amination of cellulose could be an approach for the subsequent "*in-situ*" coating by laccase-catalyzed poly(catechol) [17]. Moreover, Blanco *et al*. investigated a novel quantitatively controlled method for the amination of cellulose to improve the dyeing depth on cotton fabrics. A two-step procedure: cotton

tosylation and nucleophilic attack with 2, 5-DABSA was used for the amination of cellulose fibres [18].

Chitosan, consisting of β-(1-4)-linked Nacetyl-D-glucosamine and D-glucosamine, was widely applied as non-toxic and biodegradable polymers for improving color depth of cotton fabrics due to its water solubility, fiber compatibility and good affinity [19-21]. Cotton fabric was pre-treated with chitosan to improve its dyeing depth when using natural dyes, direct dyes and reactive dyes [22-25]. However, this method still cannot reduce the environmental impact which was caused by conventional dyeing methods.

In this paper, chitosan-templated bio-coloration was investigated and found to be more attractive as an alternative method for dyeing of cotton fabrics due to the milder processing conditions used and improved colorfastness to wash. Chitosan was applied on the surface of cotton fabrics to form the template and then followed by laccase-catalyzed *in-situ* dyeing with hydroquinone. The laccase catalysis of hydroquinone in the presence of chitosan at different stages of the process was monitored by UV-vis spectrophotometry. Effects of different processing conditions (concentration of chitosan, treatment temperature, treatment duration, dosage of hydroquinone) on the enzymatic dyeing of cotton fabrics were investigated. The color properties of the dyed cotton fabrics were examined by a Color-Eye 7000 A Spectrophotometer. Surface structures of the dyed cotton fabrics were studied by FTIR and XPS analysis.

2 Materials and Methods

2.1 Materials

Laccase (EC 1.10.3.2) from *Trametes versicolor,* hydroquinone (HQ, $C_6H_4H_2$) and chitosan (with a viscosity of 20-300 mPa·s and approximate 75-85% degree of deacetylation) were all purchased from Sigma-Aldrich (Shanghai, China). Scoured and bleached plain woven cotton fabric with a

fabric weight of 150 g/m^2 were supplied by Wuxi No. 1 Cotton Textile Company (Wuxi, China). All other chemicals used were analytical grade from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and used without further purification.

2.2 Determination of laccase activity

Laccase activity was determined by assessing the enzymatic oxidation of 2, 2′-azino-bis(3-ethylthiazoline-6-sulfonate) (ABTS; ε_{420} = 36,000 $M^{-1}\times cm^{-1}$) in 50 mM acetate buffer (pH 5.0) as described by Niku-Paavola *et al*. with some modifications [26]. 3 mL mixture of 0.1 mL laccase (5 g/L) and 2.9 mL ABTS (0.5 mM) in 50 mM acetate buffer (pH 5) was incubated over a period of 30 min. The absorbance due to enzymatic oxidation of ABTS was monitored at 420 nm using a Spectrometric UV-1800 to determine the time taken for 0.05 unit increment in the absorbance. One unit of laccase (U) was defined as the amount of enzyme required to oxidize 1 μmol of ABTS per minute.

2.3 Laccase-catalyzed coloration of chitosan-templated cotton fabrics with hydroquinone

2.3.1 Pretreatment of cotton fabrics with chitosan

5 g/L chitosan solutions were prepared in 0.1 M acetic acid solution with constant stirring for 1 hour. Cotton fabrics (0.5 g, 5cm×5cm) were immersed in 100 mL of chitosan solution for 24 hours under room temperature. Then the cotton fabrics were padded and cured in the curing chamber at 120°C for 5 min [27].

2.3.2 Enzymatic dyeing process of pre-treated cotton fabrics

Hydroquinone (1.1g) and 8 mg laccase (approximately with the activity of 0.6 U/mg) were dissolved in 100 ml of 0.2 M sodium acetate buffer (pH 5.0). The chitosan-pretreated cotton fabric samples were immersed in the reaction solution of hydroquinone (0.1M) and laccase (0.08 mg/mL) at a liquor-to-fabric ratio of 200:1 for enzymatic incubation at 55°C (optimal temperature

of laccase activity) for 6 hours with an agitation of 40 rpm. Enzymatically dyed cotton fabrics were washed thoroughly with cold tap water and dried at room temperature.

In order to optimize the reaction conditions, experiments were performed at different dosage of hydroquinone, temperature, reaction time and concentration of chitosan.

2.4 UV-Vis spectrum analysis

UV-Vis spectra of hydroquinone catalyzed by laccase were recorded on a UV-1800 spectrophotometer (Shimadzu, Japan), in the range of 200-400 nm. The polymerization kinetics was monitored by changes in absorption of samples at 246 nm [28].

2.5 Evaluation of color depth of dyed fabrics

After completing the enzymatic dyeing process, cotton fabric samples were thoroughly washed several times with a solution of 1:3 (v/v) acetones: deionized water in order to remove any residual laccase or non-reacted hydroquinone as well as any unfixed dye from the fabrics [29]. The fabric samples were then dried overnight at room temperature prior to their color measurement.

The CIELab color coordinates (L*, a*, b*, c*, h°) and color depth of the dyed cotton fabrics were evaluated using a Gretag Macbeth Color-Eye 7000 A Spectrophotometer (Datacolor, America) with the standard illuminant D65 using a 10° observer. The K/S values were calculated based on the Kubelka-Munk equation (1) below:

$$K/S = (1 - R)^2 / 2R \qquad (1)$$

Where K is the absorbance coefficient, S is the scattering coefficient, and R is the reflectance ratio.

2.6 Colorfastness tests

The colorfastness tests of dyed cotton samples were evaluated using the standard test procedures. The wash-fastness of dyed wool samples was measured according to ISO 105-C06:2010. The dry and wet rubbing fastness of dyed wool fabrics were tested based upon ISO 105-X12:2001 by

mounting the fabrics on a panel and applying ten strokes for both dry and wet rubbing fastness tests [30].

2.7 FTIR spectroscopy

FTIR spectra of the untreated and dyed cotton fabrics were recorded using a Nicolet IS10 infrared spectrophotometer (Thermo Nicolet, USA) with an ATR attachment. Absorbance measurements were performed in the range of wavelength 800-3750 cm^{-1} at a resolution of 8 cm^{-1} and 16 scans per sample.

2.8 X-ray photoelectron spectroscopy (XPS) analyses

XPS analyses of the untreated and dyed cotton fabrics were investigated using a RBD upgraded PHI-5000C ESCA system (Perkin Elmer) with Mg $K\alpha$ radiation ($h\gamma$ = 1253.6 eV). The entire spectra in the range 0–1100 eV and the narrow spectra of all the elements with a high resolution were recorded using a RBD 147 interface (RBD Enterprises, USA) with Auger Scan 3.21 software [31].

2.9 Thermogravimetric analysis (TGA) measurements

TGA tests of the cotton samples were performed using a TGA/SDTA 851e thermogravimetric analyzer (Mettler Toledo, Switzerland). The cotton fabric samples were cut into a fine powder to eliminate the effects of fabric thickness and 5 ± 0.1 mg of sample was used for each test. The untreated and dyed cotton samples were heated from 50°C to 600°C with a heating of 10°C min^{-1} under nitrogen atmosphere.

2.10 Scanning electron microscopy

The surface morphology of the dyed cotton samples was observed using a SU-1510 scanning electron microscope (Hitachi, Japan) with a 2k× and 4k× magnification. The surface of the cotton samples was sputtered with a thin layer of gold to avoid electrical charging.

2.11 Antioxidant activity test

The antioxidant activity of cotton fabrics was evaluated according to its ABTS radical scavenging ability [32]. ABTS (7 mM) was prepared and added into the 2.45 mM potassium persulfate solution (final concentration), then ABTS radical cation ($ABTS^{\cdot+}$) was produced after keeping the mixture at room temperature for 12-16 hours. The $ABTS^{\cdot+}$ solution was diluted with ethanol to reach an absorbance of 0.70 (±0.02) at 734 nm. Cotton fabrics with different treatment were immersed in the diluted $ABTS^{\cdot+}$ solution for 30 min, respectively. $ABTS^{\cdot+}$ radical scavenging activity was calculated based on the equation (2):

$$\text{Inhibition percentage (\%)} = (1 - Abs_{sample}/Abs_{control}) \times 100 \qquad (2)$$

2.12 Anti-ultraviolet properties measurements

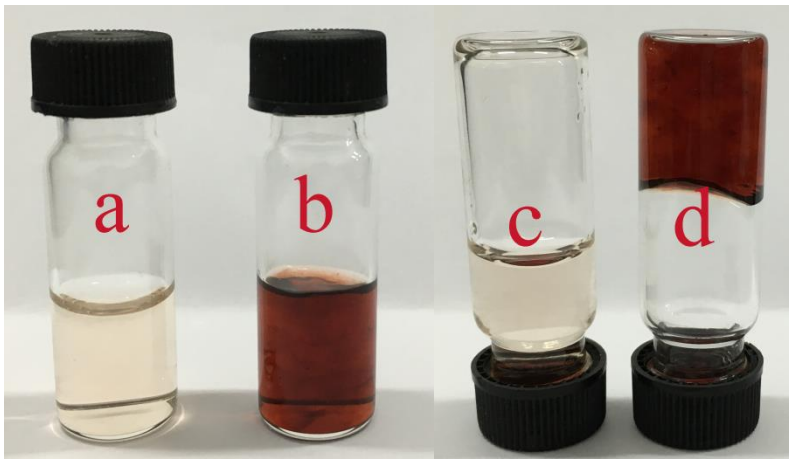
The anti-ultraviolet ability of untreated and treated cotton fabrics was determined by a UV transmittance analyzer YG(B)912E (Darong, China) [33]. The detective wavelength ranged from 280 nm to 400 nm. Each sample was measured three times and the results were averaged.

3 Results and Discussion

3.1 Oxidative polymerization of HQ under the catalysis of laccase in the presence of chitosan.

In the current research, chitosan was prepared and used as a template to improve dyeability of cotton fabric by laccase catalysis of hydroquinone because of the strong affinity between chitosan and cotton fibers.

Before the cotton was dyed by laccase, the effect of chitosan in the laccase catalyzed polymerization of hydroquinone was investigated. It was found that when the concentration of chitosan increased above to 5 g/L in the solution containing laccase and hydroquinone (HQ), the solution of chitosan/HQ under the laccase catalysis clearly became more and more viscous with the time of catalysis. Finally an *in-situ* laccase-catalyzed hydrogel with dark brown color was formed as shown in Figure 1.



chitosan/HQ chitosan/HQ+laccase chitosan/HQ chitosan/HQ+laccase

Figure 1 Photographs of chitosan / hydroquinone (HQ) solution without laccase (a and c) and with laccase (b and d) in 0.2 M sodium acetate buffer (pH 5.0) incubated at 50°C for 5h.

3.2 UV–Vis spectrophotometry analysis of chitosan/hydroquinone

To investigate the polymerization of hydroquinone via laccase catalysis in the presence of chitosan as template, the reaction for polymerization at a low concentration of laccase (5 mg/L) and HQ (10 mM) over different durations of incubation was monitored by UV–vis spectrophotometry. Figure 2 showed the absorption of the reaction mixture at the ultraviolet region from 220 to 320 nm. A significant peak was observed at 290 nm prior to the reaction. With prolonging duration of laccase catalysis, a broad peak appeared at 246 nm and trended to increase in absorbance. The appearance of the new absorption peak at 246 nm in the ultraviolet region

indicates the generation of large conjugated structures, while the decrease of the adsorption peak at 290 nm could be due to the consumption of hydroquinone. Moreover, the color of hydroquinone solution in the presence of laccase changed from colorless to dark brown during enzymatic oxidation. The inset of Figure 3 plotted the absorbance at 246 nm versus the treatment time. A good linear relationship was found at the rate of 0.15 unit increment in the absorbance per hour for the first two hours. This indicated that the color formation by laccase catalysis was increased steadily. For the further four hours of the reaction, the absorbance increased slightly with increasing time. Therefore, reaction rate of enzyme catalysis was faster at the first 2 hours and then tended to level off with the time. That is because most of monomers (hydroquinone) in the solution had been oxidized to form colored polymers during the first two hour reaction.

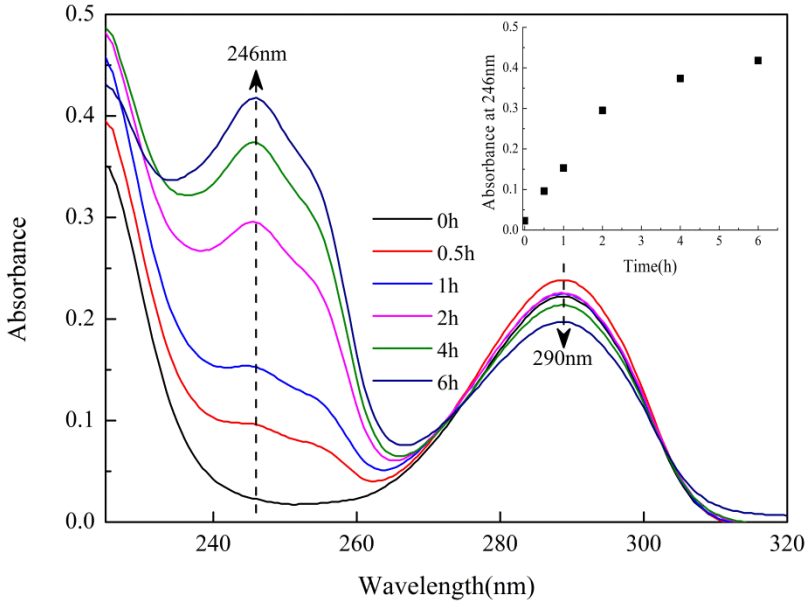


Figure 2 UV-vis spectra of chitosan/HQ mixture in 0.2 M NaAc-HAc buffer in the presence of laccase. The spectra were measured at 0, 0.5, 1, 2, 4 and 6h, respectively. Inset: plot of absorbance at 246 nm versus time.

It is well known that hydroquinone could be polymerized under the catalysis of laccase and the polymerized hydroquinone (PHQ) has higher capability to form hydrogen bonds with chitosan. Importantly, quinone groups of PHQ can react with amino groups of chitosan through Schiff bases or Michael-type mechanism, leading to the currently proposed template-polymerization

mechanism. Therefore, this mechanism could explain the main reason of the higher color depth of cotton fabric to be achieved through laccase-catalyzed polymerization of hydroquinone with the assistance of chitosan as the template as shown in Figure 3.

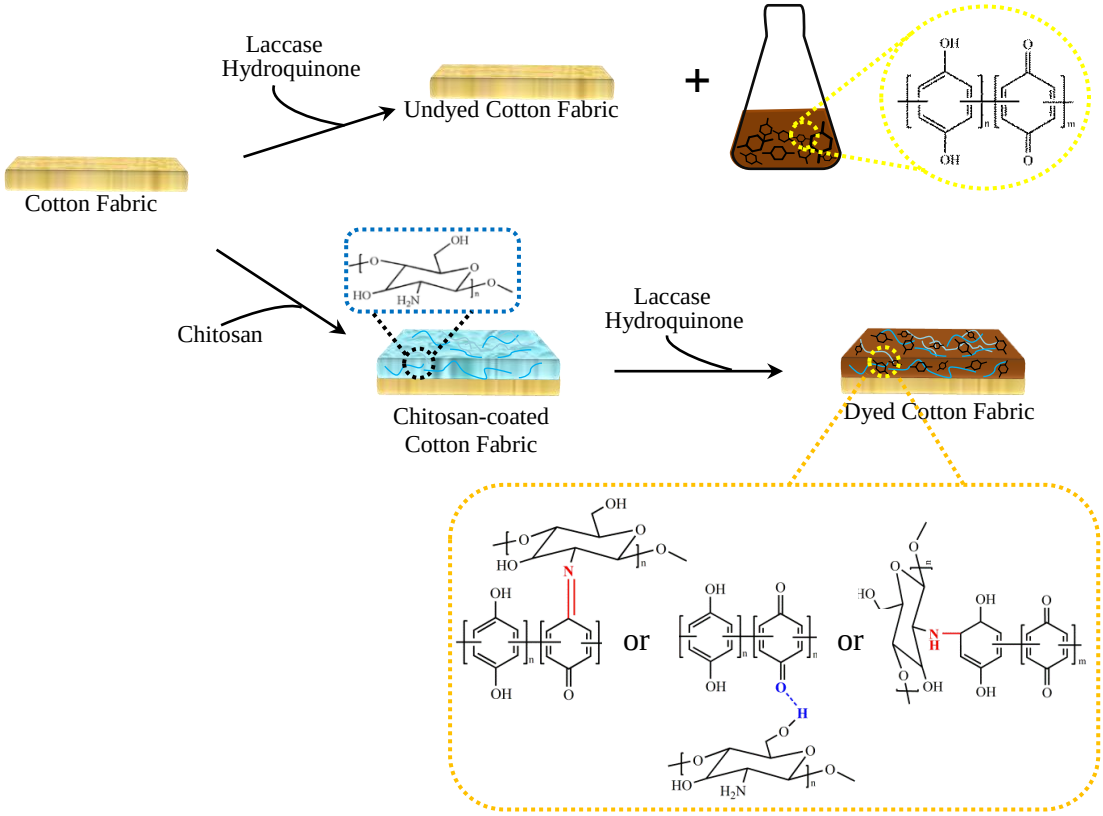


Figure 3 Chitosan-templated dyeing process of cotton fabric with hydroquinone/ laccase

3.3 Effects of different conditions on dyeing of cotton fabrics

The depth of color through enzyme-catalyzed coloration is highly dependent on the treatment conditions in term of concentration of chitosan, temperature, reaction time and dosage of hydroquinone. The results of effect of various treatment conditions of enzyme-catalyzed coloration process on the color depth were presented in Figure 4.

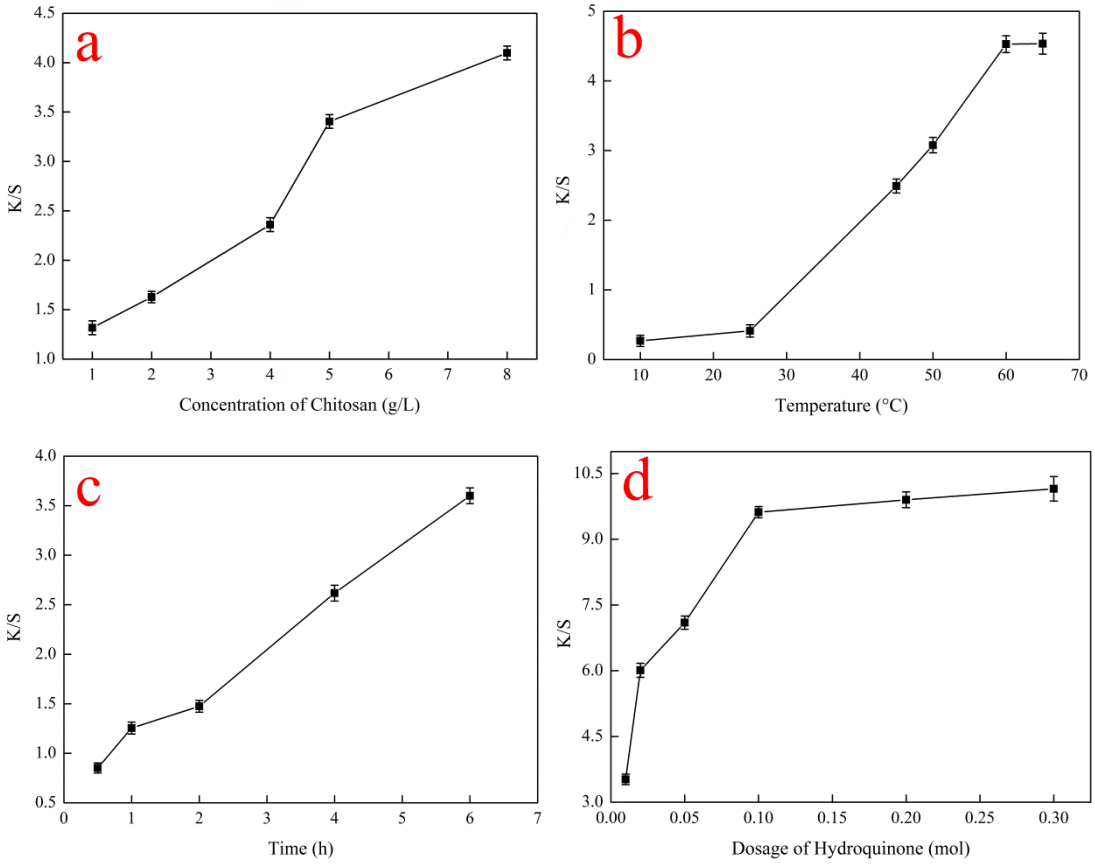


Figure 4 K/S values of the cotton fabrics treated with chitosan/hydroquinone in the presence of laccase at various conditions of enzyme-catalyzed coloration process.

Chitosan was pre-adsorbed onto cotton fibers through hydrogen bonds to provide a template for enzymatic polymerization of hydroquinone in the subsequent laccase catalysis. The concentration of chitosan could be one of the most important factors affecting the coloration of cotton fabric. The influences of different concentrations of chitosan on the dyeing depth of dyed cotton fabric were shown in Figure 4a. It can be seen that K/S value of dyed cotton fabric corresponding to the depth of color shade increased with increasing the dosage of chitosan. The color depth of dyed cotton fabric might still not reach the highest level even at the highest concentration of chitosan (8 g/L) used in the treatment. However, low concentration of hydroquinone was the main reason for the low dyeing depth of cotton fabric at high dosage of chitosan.

Figure 4b shows the effects of reaction temperature on the color depth of dyed cotton fabric. It is known that temperature is a significant factor for enzymatic reactions because activities of enzymes vary with the temperature. Above the enzyme application temperature, the rate of enzyme-catalyzed reaction could be reduced dramatically due to denature or deactivation of enzyme proteins at elevated temperature. The results showed that the K/S values of dyed cotton fabric increased with treatment temperature. From Figure 4b, the K/S value of cotton fabrics dyed at 60°C looks the same as that at 65ºC. Brown color can be successfully achieved from the reaction solution through oxidation of hydroquinone under laccase catalysis. Dyeing of cotton fabric needs temperature around 60-70°C because higher temperature could increase the rate of cellulose fiber swelling and the kinetic energy of the system.

Figure 4c shows the effect of reaction time on the color depth of cotton fabric dyed with PHQ. The results indicated a trend similar to that of the data under different temperatures. Deeper depth of color shade on chitosan-templated cotton fabric samples dyed through laccase-catalyzed polymerization of hydroquinone could be achieved at longer reaction time. The K/S value of dyed cotton fabric at 6 hours reached 3.7 which was almost double that of precedent 4 hours treatment.

Hydroquinone is one of the phenolic compounds capable to form colorants by the catalysis of laccase for dyeing of textile fabrics. Figure 4d shows the effect of different concentrations of hydroquinone used in the laccase catalysis on the K/S values of dyed cotton fabric. The dyeing depth of cotton fabric increased rapidly with the initial increase in the concentration of monomer hydroquinone. It was easy to be detected by a simple visual inspection. K/S value of the dyed cotton fabrics increased to reach 10 at 0.1 mol of the hydroquinone concentration and then almost levelled off when the concentration of hydroquinone was increased further.

3.4 FTIR spectra of dyed cotton fabrics

The surface structural characteristics of the untreated cotton fabric and the chitosan-templated cotton fabric dyed by laccase catalysis of hydroquinone were investigated by FTIR (Figure 5). As shown in Figure 5(a), both of the spectra showed stronger peaks around 3000 - 3700 cm^{-1}, corresponding to the O-H and N-H stretching vibrations. This could confirm that a number of hydrogen bonds existed. For the enzymatically dyed cotton fabric, the absorption peaks at about 3350 cm^{-1}still remained. A prominent peak at 1507 cm^{-1} appeared in the FTIR spectrum (Figure 5(b)). This peak could be ascribed to C=N stretching vibration, indicating that Schiff base reaction occurred between amino groups of chitosan and quinone groups of hydroquinone. Moreover, the absorption peak of Schiff base (C=N) at 1640 cm^{-1} both appeared in the FTIR spectra of untreated and dyed cotton fabrics.

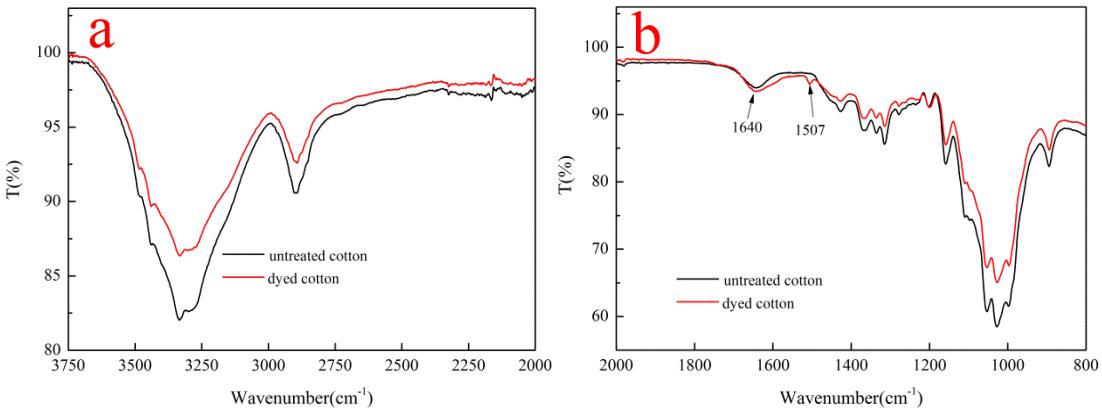
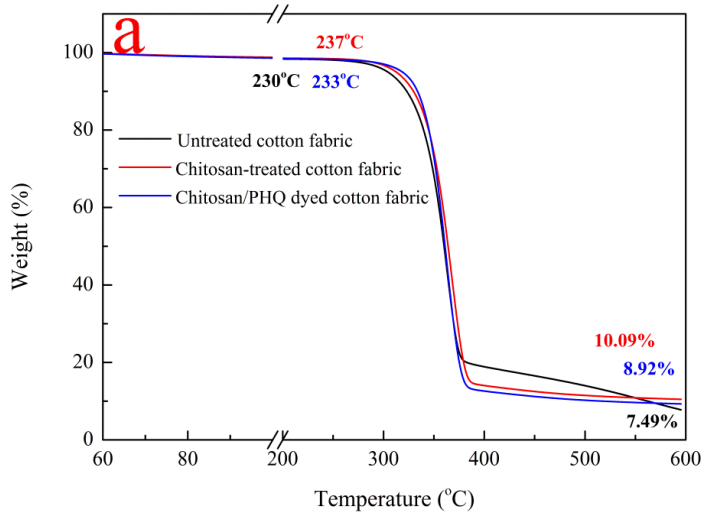


Figure 5 ATR-FTIR spectra of untreated cotton and dyed cotton at 2000-3750 cm^{-1} (a) and at 800–2000 cm^{-1} (b).

3.5 Thermal analysis of dyed cotton fabrics

The thermal stabilities of untreated and dyed cotton fabrics were investigated by non-isothermal thermogravimetric analysis. Figure 6 shows the TGA and DTG curves of untreated and dyed cotton fabrics. It can be seen from the TG curves that the overall weight loss can be generally attributed to thermal degradation. The initial thermal degradation temperatures of untreated cotton fabric, chitosan-treated and chitosan/PHQ-dyed cotton fabrics were 230ºC, 237ºC and 233ºC, respectively. The major thermal degradation temperature of untreated cotton fabric, chitosan-treated cotton fabric and dyed cotton fabric were 362ºC, 367ºC and 363ºC, respectively. It can be

concluded that the thermal degradation temperature of chitosan-treated cotton fabric was slightly higher than that of dyed cotton fabric. Up to 600°C, about 7.49% and 8.92% mass of untreated cotton fabric and dyed cotton fabric remained, but 10.09% mass of chitosan treated cotton fabric still remained. In theory, the intermolecular forces between polymer chains were the main factor that affects the thermal stability. There are high affinity between chitosan and cellulose due to their similar chemical structure. The possible intermolecular interactions between them are intermolecular H-bonds and Van der Waals forces which were physically cross-linked. The intermolecular forces predominate at higher temperature, causing an increase in thermal stability [34-36]. However, partial amino groups of chitosan were consumed in Schiff base reaction between PHQ and chitosan, which result in the lack of bonding between chitosan and cotton. Consequently, the cotton fabrics dyed with chitosan/PHQ had lower thermal stability than those treated with chitosan.



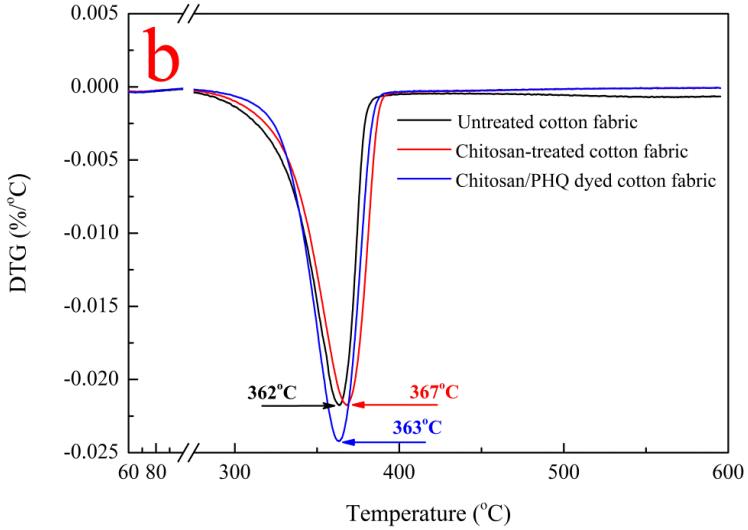


Figure 6 TGA (a) and DTG curves (b) for untreated cotton fabric and treated cotton

3.6 XPS analysis

Table 1 Surface elemental composition of cotton fibers before and after treatments.

Sample	N (%)	C (%)	O (%)
Untreated cotton	0.60	65.12	34.28
Chitosan-treated cotton	0.89	69.43	29.68
Chitosan/PHQ dyed cotton	1.94	73.23	24.84

XPS analysis is one of the most effective methods to investigate the surface structure and elements of the dyed cotton fabrics, as the dyeing reaction mainly occurs on the surfaces of cotton fibers. XPS can reach to the limited depth (~10 nm deep) on the surface of the sample. Table 1 shows the surface elemental compositions of the untreated, chitosan treated and chitosan/PHQ-dyed cotton fabrics examined by the XPS analysis. The results show an increase in the nitrogen content of the cotton fibers after chitosan treatment, indicating the coating of chitosan on the surface of cotton fibers. There was a further increase in the nitrogen content of samples from 0.89% (chitosan treated cotton fabrics) to 1.94% (the dyed cotton fabrics). As oxygen elements of poly(hydroquinone) on the chitosan/PHQ-dyed cotton fabrics were consumed during reaction

with chitosan through Schiff base reaction, the oxygen contents of chitosan treated cotton fabric decreased. Relatively speaking, the contents of carbon and nitrogen increased. Most of chitosan/PHQ complex possibly attach on the surfaces of cotton fabrics, because chitosan is a macromolecule that might not be capable to diffuse into the interior of cotton fibers.

3.7 SEM analysis

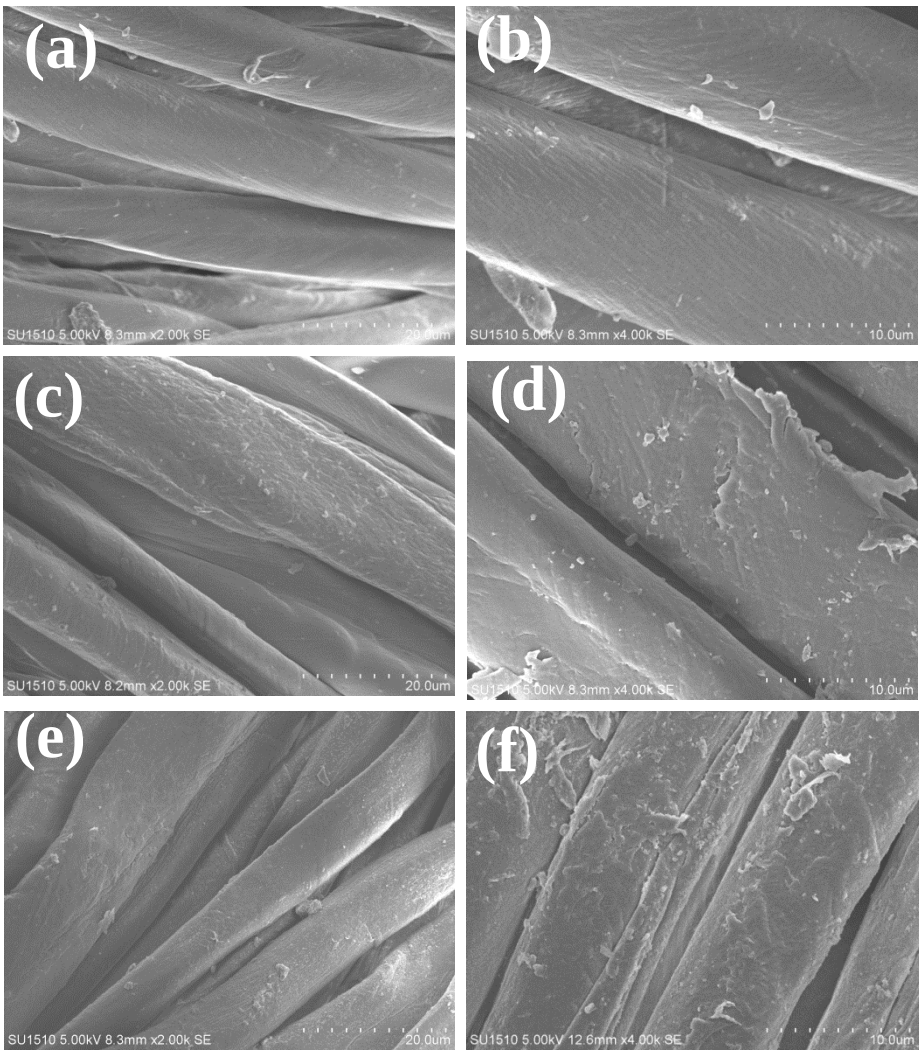


Figure 7 Surface morphology of untreated cotton fibers (a: 2k, b: 4k), chitosan-treated cotton fibers (c: 2k, d: 4k) and cotton fibers dyed with chitosan/PHQ (e: 2k, f: 4k)

As shown in Figure 7, the morphology of untreated cotton fibers, chitosan treated cotton fibers and dyed cotton fibers was investigated by means of scanning electron microscope (SEM) analysis. The surface of untreated cotton fibers was relatively smooth as shown in Figure 7(a and b) as expected. SEM images of cotton fibers treated with chitosan and chitosan/PHQ in Figure 7(c, d, e and f) clearly showed the coating and particles on the surface of cotton fibers due to the attachment of chitosan films. There were significant differences on the surface between untreated cotton fibers and cotton fibers dyed with chitosan/PHQ. After treatment by laccase/chitosan/hydroquinone, the surface of the dyed cotton fibers became rougher than that of the untreated cotton, and some granules presented on its surface. These granules observed on the surface of dyed cotton fibers were chitosan/PHQ complex catalyzed by laccase, resulted in color depth of dyed cotton fabrics. The photos of the contrast of the cotton fabrics before and after dyeing at regular size were shown on Table 2.

Table 2 Images of untreated cotton fabrics and treated cotton fabrics

Untreated cotton fabric	Chitosan-treated cotton fabric	Chitosan/PHQ dyed cotton fabric
		

3.8 Colorfastness test

Colorfastness of the cotton fabrics dyed with chitosan/hydroquinone/laccase was shown in Table 3. Washing fastness, scrubbing fastness and rubbing fastness tests were carried out to study the durability of the colored polymers attached to cotton fabrics. The dyed cotton fabrics treated with chitosan/hydroquinone/laccase achieved better colorfastness (4-5 grade), with the exception of the fading of washing fastness. The staining fastness to adjacent cotton fabrics was higher than that to adjacent wool fabrics, because hydroquinone has low affinity to cotton fabrics. The results

demonstrated that the hydroquinone/laccase dyed cotton fabric pre-coated with chitosan possessed favorable color fastness, which was acceptable for textile coloration. The colorfastness of the chitosan/PHQ dyed cotton fabrics was slightly higher than that of dyed cotton fabrics using conventional dyeing method. Moreover, the chitosan-templated bio-coloration was carried out under milder conditions and no dye fixing agent added.

Table 3 Colorfastness of the dyed cotton fabrics

Sample	Washing fastness			Scrubbing fastness	Rubbing fastness				
					Dry			Wet	
Dyed cotton	Fading	Staining		Fading	Fading	Staining		Fading	Staining	
		wool	cotton			wool	cotton		wool	cotton
	3	4-5	5	5	5	4-5	4-5	4-5	4-5	4-5

3.9 Anti-oxidant activity of cotton fabrics

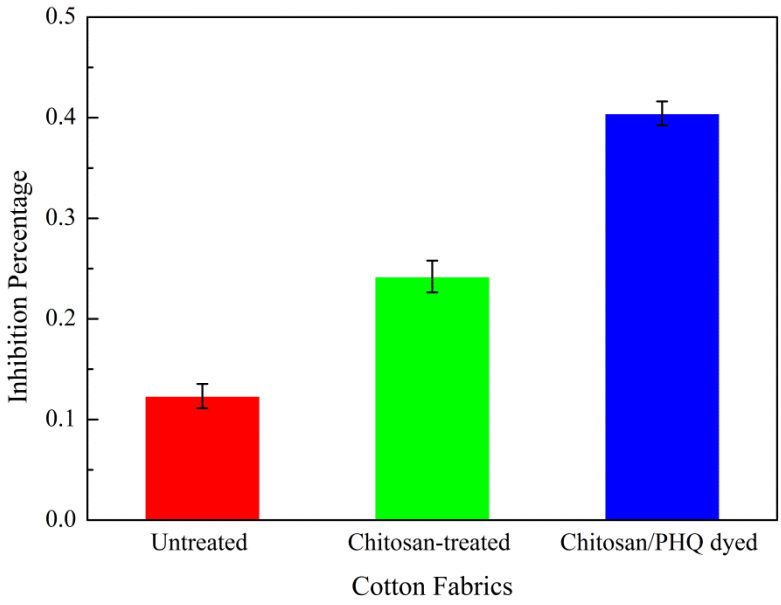


Figure 8 Antioxidant activity of untreated and treated cotton fabrics

The chitosan/PHQ dyed cotton fabric might acquire free radical scavenging ability due to anti-oxidant activity of chitosan and poly(hydroquinone) , The free radical scavenging activity of cotton samples were assessed by the ABTS radical cation method. It is a widely used method for

evaluating the antioxidant activity of samples. Figure 8 showed the ABTS radical scavenging activity of untreated and the treated cotton fabrics. All the samples exhibited different level of oxidation resistance. The untreated cotton fabric had poor ABTS radical scavenging capacity. By contrast, chitosan/PHQ dyed cotton fabrics exhibited much higher ABTS radical scavenging activity than that of untreated and chitosan-treated cotton fabrics. The improvement of free radical scavenging capacity of cotton fabrics was mainly attributed to the aromatic structure of PHQ.

3.10 Anti-ultraviolet activity of cotton fabrics

Table 4 Anti-ultraviolet activity of untreated cotton fabrics and treated cotton fabrics

Cotton fabrics	UPF	UVA%	UVB%
Untreated	7.36	11.35	11.85
Chitosan-treated	10.11	11.42	7.63
Chitosan/PHQ dyed	101.06	9.25	7.09

The anti-ultraviolet ability of the untreated and treated cotton fabrics was investigated by ultraviolet protection factor (UPF), transmittance of UVA and UVB. As shown in Table 4, both untreated cotton fabrics and chitosan-treated cotton fabrics showed poor anti-ultraviolet ability. However, the chitosan/PHQ dyed cotton fabric exhibited excellent anti-ultraviolet ability according to the high UPF value of 101.06. The UPF value of chitosan/PHQ dyed cotton fabrics higher than that of chitosan-treated cotton fabrics, this is because the aromatic structure of PHQ coated on the cotton fabrics can absorb high-energy ultraviolet rays, effectively reducing the transmittance of UVA and UVB.

4 Concluding remarks

Chitosan was pre-adsorbed onto cotton fabrics to provide a template for the enzymatic-catalyzed coloration of cotton fabrics. Precursor hydroquinone was polymerized by laccase catalysis to

produce polymeric colorants. Polymerized hydroquinone (PHQ) was used for the dyeing of the chitosan-pretreated cotton fabric. Dyed cotton fabrics by laccase catalysis showed dark brown color and higher color fastness, which revealed that chitosan/PHQ complex existed on the surface of cotton fibers. This was also confirmed by SEM and XPS analysis of the dyed cotton fabrics. FTIR analysis demonstrated that there are Schiff base reactions between amino groups of chitosan and quinone groups of hydroquinone. The mechanism of the reaction between hydroquinone and chitosan will be further studied. Although cellulose fibre materials, like cotton fibers are difficult to be dyed with enzyme-catalyzed polymeric dyes due to their poor affinity to polyphenol, this study presents a novel chitosan-templated bio-coloration technique of cellulose materials via laccase-catalyzed polymerization of hydroquinone. The cotton fabrics treated with chitosan/PHQ not only possess high dyeing depth and good colorfastness, but also impart anti-oxidation and anti-ultraviolet abilities due to the aromatic structure of PHQ. In addition, chitosan used as template could endow the dyed fabrics with antibacterial function. Therefore, this study could lead to the successful development of biologically dyed textiles with multiple functions.

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Conflicts of interest

The authors have declared no conflicts of interest.

5 References

1. Shin, H., Georg, M. G., and Cavaco-Paulo, A., “In situ” enzymatically prepared polymers for wool coloration. *Macromol. Mater. Eng.* 2001, *286*, 691-694.

2. Kim, S., Lopez, C., Güebitz, G. and Cavaco‐Paulo, A., Biological coloration of flax fabrics with flavonoids using laccase from Trametes hirsuta. *Eng. Life Sci.* 2008, *8*, 324-330.

3. Guimarães, C., Kim, S., Silva, C. and Cavaco‐Paulo, A., In situ laccase–assisted overdyeing of denim using flavonoids. *Biotechnol. J.* 2011, *6*, 1272-1279.

4. Su, J., Noro, J., Fu, J., Wang, Q. et al., Enzymatic polymerization of catechol under high-pressure homogenization for the green coloration of textiles. *J. Clean. Prod.* 2018, *202*, 792-798.

5. Fu, J., Nyanhongo, G. S., Gübitz, G. M., Cavaco-Paulo, A. and Kim, S., Enzymatic colouration with laccase and peroxidases: Recent progress. *Biocatal. Biotransfor.* 2012, *30*, 125-140.

6. Sun, X., Bai, R., Zhang, Y., Wang, Q. et al., Laccase-catalyzed oxidative polymerization of phenolic compounds. *Appl. Biochem. Biotech.* 2013, *171*, 1673-1680.

7. Zhang, L., Zhao, W. S., Ma, Z. L., Nie, G. G. and Cui, Y. C., Enzymatic polymerization of phenol catalyzed by horseradish peroxidase in aqueous micelle system. *Eur. Polym. J.* 2012, *48*, 580-585.

8. Yuan, M., Wang, Q., Shen, J., Smith, E. et al., Enzymatic coloration and finishing of wool with laccase and polyethylenimine. *Text. Res. J.* 2017, 0040517517712096.

9. Jeon, J. R., Kim, E. J., Murugesan, K., Park, H. K. et al., 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.

10. Sun, S., Xing, T. and Tang. R., Simultaneous coloration and functionalization of wool, silk, and nylon with the tyrosinase-catalyzed oxidation products of caffeic acid. *Ind. Eng. Chem. Res.* 2013, *52*, 8953-8961.

11. Bai, R., Yu, Y., Wang, Q., Yuan, J. and Fan, X., Laccase-mediated in situ polymerization of pyrrole for simultaneous coloration and conduction of wool fabric. *Text. Res. J.* 2016, *88*, 27-35.

12. Zhang, T., Bai, R., Shen, J., Wang, Q. et al., Laccase-catalyzed polymerization of diaminobenzenesulfonic acid for pH-responsive color-changing and conductive wool fabrics. *Text. Res. J.* 2017, 0040517517720497.

13. Bai, R., Yu, Y., Wang, Q., Yuan, J. et al., Laccase-catalyzed in-situ dyeing of wool fabric. *J. Text. I.* 2016, *107*, 995-1003.

14. Barfoed, M., Kirk, O., Salmon, S., Enzymatic method for textile dyeing. US Patent 6805718, 2004.

15. Hadzhiyska, H., Calafell, M., Gibert, J. M., Dagà, J. M. et al., Laccase-assisted dyeing of cotton. *Biotechnol. Lett.* 2006, *28*, 755-759.

16. Calafell, M., Díaz, C., Hadzhiyska, H., Gibert, J. M. et al., Bio-catalyzed coloration of cellulose fibers. *Biocatal. Biotransfor.* 2007, *25*, 336-340.

17. Kim, S. Y., Zille, A., Murkovic, M., Güebitz, G. and Cavaco-Paulo, A., Enzymatic polymerization on the surface of functionalized cellulose fibers. *Enzyme Microb. Tech.* 2007, *40*, 1782-1787.

18. Blanco, C. D., González, M. D., Monmany, J. M. D. and Tzanov, T., Dyeing properties, synthesis, isolation and characterization of an in situ generated phenolic pigment, covalently bound to cotton. *Enzyme Microb. Tech.* 2009, *44*, 380-385.

19. Sun, T., Zhu, Y., Xie, J. and Yin, X., Antioxidant activity of N-acyl chitosan oligosaccharide with same substituting degree. *Bioorg. Med. Chem. Lett.* 2011, *21*, 798-800.

20. Zhao, D., Wang, J., Tan, L., Sun, C. and Dong, J., Synthesis of N-furoyl chitosan and chito-oligosaccharides and evaluation of their antioxidant activity in vitro. *Int. J. Boil. Macromol.* 2013, *59*, 391-395.

21. Zhou, Y., Yang, H., Liu, X., Mao, J. et al., Electrospinning of carboxyethyl chitosan/poly(vinyl alcohol)/silk fibroin nanoparticles for wound dressings. *Int. J. Biol. Macromol.* 2013, *53*, 88-92.

22. He, X. X., Liu, S. and Li, Z. J., The effects of chitosan on dyeing abilities of cotton fabric dyed with reactive dyes. *Appl. Mech. Mater.*2013, *310*, 3.

23. He, X., Tao, R., Zhou, T., Wang, C. and Xie, K., Structure and properties of cotton fabrics treated with functionalized dialdehyde chitosan. *Carbohyd. Polym.* 2014, *103*, 558-565.

24. Ren, Q., Zhao, T., Pan, H. and Wang, J., The application of water-soluble and reactive chitosan on antimicrobial activity and salt-free dyeing of reactive dyes. *Res. J. Text. Apparel*, 2009, *13*, 9-18.

25. Morakotjinda, P. and Nitayaphat, W., Dyeing properties and color fastness of chitosan treated cotton fabrics with thian king leaves extract. *Appl. Mech. Mater.* 2015, *749*, 89-93.

26. Niku-Paavola, M. L., Karhunen, E., Salola, P. and Raunio, V., Ligninolytic enzymes of the white-rot fungus Phlebia radiata. *Biochem. J.* 1988, *254*, 877-884.

27. Dev, V. G., Venugopal, J., Sudha, S., Deepika, G. and Ramakrishna, S., Dyeing and antimicrobial characteristics of chitosan treated wool fabrics with henna dye. *Carbohyd. Polym.* 2009, *75*, 646-650.

28. Zhang, Y., Dong, A., Fan, X., Wang, Q. et al., Laccase-catalyzed synthesis of conducting polyaniline-lignosulfonate composite. *J. Appl. Polym. Sci.* 2016, *133*, 42941.

29. Montazer, M., Dadashian, F., Hemmatinejad, N. and Farhoudi, K., Treatment of wool with laccase and dyeing with madder. *Appl. Biochem. Biotech.* 2009, *158*, 685-693.

30. Shahid, M., Ahmad, A., Yusuf, M., Khan, M. I. et al., Dyeing, fastness and antimicrobial properties of woolen yarns dyed with gallnut (Quercus infectoria Oliv.) extract. *Dyes Pigments*, 2012, *95*, 53-61.

31. Yu, Y., Wang, Q., Yuan, J., Fan, X. et al., Hydrophobic modification of cotton fabric with octadecylamine via laccase/TEMPO mediated grafting. *Carbohyd. Polym.* 2016, *137*, 549-555.

32.Yu, S. H., Hsieh, H. Y., Pang, J. C., Tang, D. W. et al., Active films from water-soluble chitosan/cellulose composites incorporating releasable caffeic acid for inhibition of lipid oxidation in fish oil emulsions. *Food Hydrocolloid.* 2013, *32*, 9-19.

33. Cao, J., and Wang, C., Multifunctional surface modification of silk fabric via graphene oxide repeatedly coating and chemical reduction method. *Appl. Surf. Sci.* 2017,*405*, 380-388.

34. Neto, C. G.T., Giacometti, J. A., Job, A. E., Ferreira, F. C. et al., Thermal analysis of chitosan based networks. *Carbohyd. Polym.* 2005, *62*, 97-103.

35. Mocanu, G., Nichifor, M., Mihai, D. and Oproiu, L. C., Bioactive cotton fabrics containing chitosan and biologically active substances extracted from plants. *Mat. Sci. Eng. C-Mater*, 2013, *33*, 72-77.

36. Hu, Z., Chan, W. L. and Szeto, Y. S., Nanocomposite of chitosan and silver oxide and its antibacterial property. *J. Appl. Polym. Sci.* 2008, *108*, 52-56.