A novel cotton bio-scouring using pectinase in combination with Fenton system

Laipeng Luo  
Jiangnan University

Chenkun Ding  
Jiangnan University

Qinting Zhu  
Jiangnan University

Lei Liu  
Jiangnan University

Ping Wang  
Jiangnan University

Qiang Wang  
Jiangnan University

Yuanyuan Yu (✉ jnyyy@jiangnan.edu.cn)  
Jiangnan University

Research Article

Keywords: cotton fabric, scouring, pectinase, Fenton system, textiles

Posted Date: January 30th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-3896010/v1

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Additional Declarations: No competing interests reported.
Abstract

Untreated cotton fabrics are hydrophobic due to the high content of pectin and waxes, and the fabrics need to improve the wettability by scouring for the demands of the subsequent processing such as dyeing and finishing. In this study, a new scouring method for cotton fabrics was developed by pectin hydrolase combined with Fenton system (Fe$^{2+}$/H$_2$O$_2$). The mechanism of pectin degradation by pectinase combined with Fe$^{2+}$/H$_2$O$_2$ was investigated. The results showed that α-1,4-glycosidic bonds of water-soluble pectin could be degraded more efficiently by pectinase hydrolysis and Fe$^{2+}$/H$_2$O$_2$ oxidation. The fabrics scoured with pectinase→Fe$^{2+}$/H$_2$O$_2$ had less wetting time (4.25 s) and higher vertical wicking height (9.80 cm) compared to the fabrics treated with pectinase alone (>120 s, 5.15 cm). The results of bleaching and dyeing experiments indicated that the whiteness and dyeing properties of the fabrics were not negatively affected by pectinase→Fe$^{2+}$/H$_2$O$_2$ treatment, and the strength loss of the fabrics (11.79%) was lower than that of the fabrics with conventional NaOH treatment (20.16%). The results of the experiments in the pilot-scale machine showed that various performance indicators of the fabrics scoured by pectinase→Fe$^{2+}$/H$_2$O$_2$ reached the level of the conventional NaOH treatment, which demonstrated the possibility for practical application of pectinase→Fe$^{2+}$/H$_2$O$_2$ scouring. The cotton fabric scouring using pectinase→Fe$^{2+}$/H$_2$O$_2$ is an effective and feasible new method with advantages such as environmental friendliness, mild treatment conditions, and good treatment effect.

Introduction

Cotton is one of the most widely used cellulosic fibers in the textile industry for its natural, comfortable to wear, good moisture absorption, soft and skin-friendly (Cheng et al. 2021). Nevertheless, untreated natural cotton fibers are not 100% composed of cellulose, but also of non-cellulose impurities, including waxes, pectin, ash and proteins present in the cuticle and other parts of the cotton fiber (Aggarwal et al. 2020; Panda et al. 2023). Raw cotton fabrics are hydrophobic due to the presence of these impurities (Xia et al. 2021). Because of this hydrophobic barrier, dyes and auxiliaries are difficult to be adsorbed and diffused into the fibers, and wet processing of textiles such as dyeing is difficult. In textile processing, cotton fabrics need to be pre-treatment before dyeing, including scouring and bleaching processes (Hebeish et al. 2009).

Scouring is the key to improving the wettability of cotton fabrics. Conventional cotton scouring is carried out using sodium hydroxide solution under boiling conditions (Hoque et al. 2023; Zhou et al. 2019). This process generates a lot of wastewater, consumes high energy, and does not meet the requirements of green and sustainable development in the textile industry. Finding green and environmentally friendly methods for cotton scouring has always been the focus of research in the textile processing field (Raafi et al. 2023).

Enzyme scouring is considered as an alternative technology to traditional NaOH scouring because of its many advantages, such as mild processing conditions, less water pollution, less damage to the cellulose
structure, and good softness of the treated fabric (Kalantzi et al. 2019). Several different enzymes have been used in cotton fabric scouring, such as pectinase, hemicellulase, cellulase and cutinase (Colombi et al. 2021). Among these enzymes, pectinase has proved to be the most effective in removing impurities from cotton. Because waxes, other impurities and cellulose are bonded to each other by pectin, and waxes and other impurities removed when pectin is degraded by pectinase (Wang et al. 2007; Kumar et al. 2023). However, in practical applications, the effectiveness of pectinase in removing impurities is still not as good as that of NaOH scouring. This may be because the epidermal structure of cotton fibers is dense, lacking enzyme action sites and diffusion channels, resulting in some pectin deep in the epidermis of cotton being unable to be degraded. For this reason, pectinase is often used in combination with surfactant, ultrasound, plasma or other enzymes, etc. For example, Wang et al. obtained cotton fabrics with good wetting properties by treatment with pectinase and β-cyclodextrin. However, good wetting properties may be caused by residual β-cyclodextrin on fabrics (Wang et al. 2008). The limitations of the chemical method have led more researchers to consider mechanical action. A "wedge device" was used in the enzymatic scouring of cotton fabrics, as studied by Agrawal et al. The pectin removal rate had increased by 9% using this method, but the waxes were not effectively removed (Agrawal et al. 2008). Some novel physical methods have also been employed. Ultrasonic was used to assist pectinase for cotton scouring by Eren and Erismis et al. (Eren and Erismis 2013). But enzyme activity might be decreased in the presence of ultrasonic, which had been demonstrated by Ma et al. (Kan and Lam 2018; Ma et al. 2016). Wang et al. used a combination of plasma and enzyme treatments for pre-treatment of cotton fabrics (Wang et al. 2009). However, the disadvantages of these physical methods limited their application in textile industry, such as complex and expensive equipment, low processing efficiency and high energy consumption (Chen et al. 2022).

The Fenton system can catalyze the degradation of heparin, citrus pectin, and okra pectin according to recent studies (Li et al. 2019; Yeung et al. 2021; Zhi et al. 2019). Because the Fenton system can produce strong oxidizing radicals (mainly •OH) through the reaction of Fe$^{2+}$ and H$_2$O$_2$. The researchers proved the mechanism of polysaccharide degradation by Fe$^{2+}$/H$_2$O$_2$ that the α-1,4-glycosidic bond in the pectin polysaccharide molecule was broken in the presence of hydroxyl radicals (•OH) because the free radicals could extract H atom on glycosidic bonds. (Chen et al. 2021; Zhi et al. 2017). The reaction between Fe$^{2+}$ and H$_2$O$_2$ is usually considered to be harmless. The H$_2$O$_2$ decomposes into H$_2$O and O$_2$, which are not polluting to the environment. Bokare and Choi demonstrated that a trace amount of Fe$^{2+}$ could trigger the Fenton reaction that generated free radicals with good pectin degradation (Bokare and Choi 2014). Fe$^{2+}$/H$_2$O$_2$ can be reacted at temperatures close to the ambient temperature, and the reaction is fast and easy to carry out. Fe$^{2+}$/H$_2$O$_2$ has been widely used in the treatment of pulp and dyeing effluents (Gayathri et al. 2023; Su et al. 2019). To the best of our knowledge, the use of Fe$^{2+}$/H$_2$O$_2$ in the scouring of cotton textiles has not been reported.

In this study, a new green and environmentally friendly cotton scouring method has been proposed using the combination of pectin-hydrolases and Fenton system (Fe$^{2+}$/H$_2$O$_2$) (Fig. 1). The pectin on cotton fibers
was degraded more efficiently when combining the enzyme catalysis and Fe$^{2+}$/H$_2$O$_2$ oxidation. The effectiveness of the scouring was evaluated by the wetting properties and strength loss of the fabrics. Changes in the chemical structure of water-soluble pectin were investigated before and after the treatments of Fe$^{2+}$/H$_2$O$_2$ and pectin-hydrolases. The possible mechanisms of pectin degradation and removal of impurities from fabrics by pectinase→Fe$^{2+}$/H$_2$O$_2$ system were proposed.

**Experiment Section**

**Materials**

The 100% cotton knitwear fabric (230 g/m$^2$) was purchased from Meixinda Group Ltd. (Zhejiang, China). Pectin-hydrolase (83 U/mL) was kindly provided by the School of Bioengineering, Jiangnan University. Pectin (98%) was obtained from Weng Jiang Reagent Co (Guangdong, China). Ferrous sulfate (FeSO$_4$$\cdot$7H$_2$O), hydrogen peroxide (30% W/V), and sulfuric acid (GR) were purchased from Sinopharm Reagent Co., Ltd. (Shanghai, China). Deoxyribose, thiobarbituric acid, and trichloroacetic acid were purchased from Aladdin (Shanghai, China). TF-1871A, an additive to remove waxes, was purchased from Transfar Group Limited (Hangzhou, China). DM-1042A, a stabilizer for hydrogen peroxide, was purchased from Dymatic Fine Chemical Group Co. (Guangdong, China). All other chemicals used in this research were of analytical grade.

**Methods**

**Depolymerization of water-soluble pectin by pectinase and Fe$^{2+}$/H$_2$O$_2$**

The depolymerized pectin polysaccharides were prepared by pectinase and Fe$^{2+}$/H$_2$O$_2$ according to methods described by Yeung et al. with some modifications (Yeung et al. 2021). Briefly, the initial pH value of the deionized water was adjusted to 6 using CH$_3$COOH. Under continuous stirring, 0.25 g water-soluble pectin was dissolved in 100 mL of the prepared acetate solution. Then pectinase/Fe$^{2+}$/H$_2$O$_2$ (pectinase 1 mL, the concentration of Fe$^{2+}$ 1 mM, and the dosage of H$_2$O$_2$ 1 mL/L) was added. Moreover, pectinase (1 mL) or Fe$^{2+}$/H$_2$O$_2$ (the concentration of Fe$^{2+}$ 1 mM, and the dosage of H$_2$O$_2$ 1 mL/L) were added separately as controls. All the experiments were operated at 55°C for 30 min. For the reaction to be terminated and Fe$^{2+}$ to be isolated, the pH of the reaction mixture was adjusted to 8.5 by adding 5 M NaOH solution dropwise. Residual hydrogen peroxide was removed by heating at 100°C for 10 min, while the pectinase was inactivated. The reaction solutions were centrifuged at 3,500 rpm for 10 min. Finally, the solutions were concentrated and dialyzed for 72 h using 1000 Da molecular-weight dialysis bags. The degraded water-soluble pectin in solutions by pectinase and Fe$^{2+}$/H$_2$O$_2$ was obtained.

**Degradation of pectin on cotton fabrics by Fe$^{2+}$/H$_2$O$_2$**
Untreated cotton fabrics (5.0 g per sample) were placed in 250 mL beakers respectively, and acetic acid solutions of pH 6 were added with a weight ratio of liquid to the fabric of 15:1. Subsequently different ratios of Fe$^{2+}$ and H$_2$O$_2$ were added to initiate the reaction. The reaction was carried out at 55°C for 60 min. At the end of the reaction, the treated fabrics were washed twice in boiling water, two minutes at a time. Finally, the cotton fabrics were rinsed several times with deionized water and dried at 60°C. The control samples were treated similarly but without adding Fe$^{2+}$ and H$_2$O$_2$. Reaction conditions, such as temperature, pH, and reaction time, were also explored after determining the optimal concentrations of Fe$^{2+}$ and H$_2$O$_2$. All experiments were performed in triplicate (Reyhani et al. 2019).

**Scouring combined by pectinase and Fe$^{2+}$/H$_2$O$_2$ treatments**

The pH values of treatment baths were adjusted to 6 with 0.02 M CH$_3$COOH buffer solution. TF-1871A (0.5 g/L) was added to the treatment baths. Subsequently, untreated cotton fabrics were put into the solution with a fabric-to-solution mass ratio of 1:15. Pectinase and Fe$^{2+}$/H$_2$O$_2$ were added to the solutions at the beginning of the reaction and 1h later respectively, and the total reaction time was 1.5 h. The control samples were treated with pectinase or Fe$^{2+}$/H$_2$O$_2$ alone. At the end of the reaction, EDTA (0.5 g/L) was added to remove residual metal ions on the fabrics. The fabrics were taken out after 5 min, then washed with hot water (100°C) twice (two minutes at a time) to remove impurities, enzymes, etc. followed by washing with deionized water several times. Finally, the fabrics were placed in an oven at 60°C until dry. All experiments were performed in triplicate.

**Bleaching of cotton fabric**

The fabrics treated with pectinase, Fe$^{2+}$/H$_2$O$_2$, or pectinase→Fe$^{2+}$/H$_2$O$_2$ were bleached. The solutions for bleaching containing H$_2$O$_2$ (10 mL/L), NaOH (1 g/L), and DM-1402A (3 mL/L, hydrogen peroxide stabilizer) were used at 98°C for 60 min. After bleaching, the fabrics were washed with hot water (100°C) twice (two minutes at a time), followed by washing with deionized water several times and then dried at 60°C (Aggarwal et al. 2020).

**Dyeing of fabrics**

The fabrics after the treatments of pectinase→Fe$^{2+}$/H$_2$O$_2$ scouring and H$_2$O$_2$ bleaching were dyed with a fabric-to-liquor ratio of 1:50 at 60°C using the different reactive dyes (X-3b Red, Eo-3g Yellow, Kn-p Blue). The dyeing solutions contained the reactive dye of 1.5 g/L and NaCl of 30 g/L. The fabrics were immersed in the dye solutions for 30 min. Following the addition of Na$_2$CO$_3$ (10 g/L) for the fixation reaction between dyes and fibers, and this process was carried out at 90°C for 30 min. The dyed fabrics were washed with deionized water until no residual color in the effluent (Kalantzi et al. 2009). In addition, the dyed fabrics were boiled in a mixture of sodium carbonate and soap flakes to wash away the unfixed dyes on the fibers. Finally, these dyed fabrics were placed in an oven to dry.

**Estimation of hydroxyl radicals in Fe$^{2+}$/H$_2$O$_2$ system**
The method based on the reaction of deoxyribose with •OH was used for the study of the time course of production of •OH radicals by Fe$^{2+}$/$H_2O_2$ and pectinase/Fe$^{2+}$/H$_2$O$_2$ system. Aliquots of the reaction mixture (450 µL) were taken at 10-minute intervals and supplemented with 50 µL deoxyribose (28 mM). The reaction was stopped by the addition of 500 µl thiobarbituric acid (1% W/V in 50 mM NaOH) and 500 µL of trichloroacetic acid (2.8% W/V) after 5 min of incubation. The deoxyribose degradation product reacted with thiobarbituric acid during subsequent 30-minute incubation at 80°C, with the resulting formation of a pink compound. The product of the reaction was quantified by spectrophotometry (λ = 532 nm) after dilution with an equal amount of water. A reaction system containing only pectinase, Fe$^{2+}$, or $H_2O_2$ at the same concentration were used as a control. The relative amount of •OH was expressed in absorbance units (Verma et al. 2003).

**Characterization of untreated and degraded water-soluble pectin**

The FTIR spectra of pectins by pectinase, Fe$^{2+}$/H$_2$O$_2$, or pectinase/Fe$^{2+}$/H$_2$O$_2$ treatments were carried out by a Fourier transform infrared (FTIR) spectrophotometer (IRAffinity-1S, Shimadzu, Japan). The samples (~ 1 mg) were ground together with 200 mg KBr and pressed into pellets for IR scanning from 4000 cm$^{-1}$ to 400 cm$^{-1}$ with 64 scans. The XPS survey spectra of pectins were recorded by X-ray photoelectron spectroscopy (XPS, Thermo Scientific K-Alpha, America).

The average molecular weight of the pectin samples was measured by a Waters 1525EF system (Waters, Milford, USA) according to the approach of Conglei Ma, with some modifications. The pectin samples were dissolved in ultrapure water (3.0 mg/L) and injected separately into the GPC system after filtration through a 0.22 µm microporous membrane. The column temperature was maintained at 40°C, and 0.1 M NaNO$_3$ was used as the mobile phase (Ma et al. 2021).

**Pectin content on cotton fabric**

The method based on the reaction of sulfuric acid–carbazole with polysaccharide was used for the study of the pectin content on fabric treated by the pectinase, Fe$^{2+}$/H$_2$O$_2$, or pectinase→Fe$^{2+}$/H$_2$O$_2$. The fabric samples (1.5 g) were immersed in ammonium oxalate solution (5 g/L) in a sealed glass beaker, and the pectin on the fabrics was hydrolyzed at 98 °C for 2h. The hydrolysate (1 mL) was further hydrolyzed by $H_2SO_4$ (10 mL) and subsequently heated at 100 °C for 10 min. After being cooled, anhydrous ethanol-carbazole solution (0.5 mL) was added, and the absorbance values at 535 nm of the purplish-red solutions were detected at 30 min. Finally, the pectin content on the fabric was calculated by comparison with a standard sample (Liu et al. 2012).

**Fabric properties**

The wettability of the fabric was evaluated using the AATCC Test Method 39-1980 (Evaluation of Wettability) (AATCC Technical Manual, 1980). The vertical wicking height of the prepared fabric was
measured based on the standard AATCC 197–2013. The hunter whiteness was measured on the WSD-Automatic Whiteness Meter according to the AATCC 110–2005 standard.

The color strength [light absorption coefficient (K)/light scattering coefficient (S)] of fabrics was measured at the wavelength of the maximum absorbance (Degirmenci and Çoruh 2017). The bursting strength of the fabrics was tested according to ASTM D5035-11. The crystallinity of the fabrics was determined by an X-ray diffractometer (D2 PHASER A26-X1-A2E0B2A0, Bruker-Axs). The morphology of the fabrics was observed using scanning electron microscopy (SEM, SU8100, Japan) (Liu et al. 2023).

Results and discussion

Degradation of water-soluble pectin by the pectinase and Fe$^{2+}$/H$_2$O$_2$

As shown in Fig. 2a and Fig. 2b, the free radicals were generated in the reduction process of H$_2$O$_2$ by Fe$^{2+}$. A large amount of hydroxyl radicals (•OH) were produced in the Fe$^{2+}$/H$_2$O$_2$ system, and the concentration of radicals in the Fe$^{2+}$/H$_2$O$_2$ system was significantly higher than that in H$_2$O$_2$ alone system. However, the hydroxyl radical concentration decreased in the Fe$^{2+}$/H$_2$O$_2$ system when pectinase was added. The increase in •OH concentration could be attributed to the generation of •OH promoted by the reaction of Fe$^{2+}$ with H$_2$O$_2$. The •OH has strong oxidation due to the presence of an unpaired electron. The α-1,4-glycosidic bonds in polysaccharide molecules could be attacked and broken by the oxidation of •OH, therefore pectin molecules could be degraded by •OH. Hydroxyl radicals can react with most amino acids through a series of chemical reactions such as addition and electron transfer. This is the reason for the decrease in free radical concentration after the addition of pectinase in the Fe$^{2+}$/H$_2$O$_2$ system (Reyhani et al. 2019; Ma et al. 2020).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Detailed data on the distribution of molecular weights after different treatments</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Mn (Da)</td>
</tr>
<tr>
<td>Untreated pectin</td>
<td>157566</td>
</tr>
<tr>
<td>Pectin by different treatment</td>
<td>Pectinase</td>
</tr>
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<td></td>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe$^{2+}$/H$_2$O$_2$</td>
<td>3113</td>
</tr>
<tr>
<td>Pectinase/Fe$^{2+}$/H$_2$O$_2$</td>
<td>1886</td>
</tr>
</tbody>
</table>
The elution profiles of the pectin samples in GPC are shown in Fig. 2c. Untreated pectin had the highest average molecular weight (Mw) with 1969.2 kDa, while pectins treated by pectinase or Fe^{2+}/H_{2}O_{2} showed a decrease in Mw (15.9 kDa, 11.6 kDa). The pectin with the lowest molecular weight (9.0 kDa) was obtained by the pectinase/Fe^{2+}/H_{2}O_{2} treatment. All pectins showed a wide Mw distribution with polydispersity indexes > 1.10 (Yu et al. 2010; Olawuyi et al. 2022), and a broader distribution was observed in the pectin treated with pectinase/Fe^{2+}/H_{2}O_{2}. Some lower molecular weight signals were detected in pectin treated with pectinase, presumably due to a small amount of degradation of the RG-region in pectin. D-galacturonic acid and rhamnose were linked to constitute the backbone of the RG-I region. The D-galacturonic acid was linked by an α-1,4 glycosidic bond, which could be hydrolyzed by pectinase. However, the α-1,2 glycosidic bond between the rhamnose sugars could not be hydrolyzed by the pectinase that we used. Therefore, the signal might be attributed to the shorter lengths of rhamnose fragments (Noguchi et al. 2020). This signal did not appear in the pectin samples with Fe^{2+}/H_{2}O_{2} treatment, presumably because the small molecular weight polysaccharides were removed by dialysis.

In conclusion, water-soluble pectin was more efficiently degraded by pectinase/Fe^{2+}/H_{2}O_{2} treatment compared to the pectinase or Fe^{2+}/H_{2}O_{2} treatments alone (Noguchi et al. 2020; Yuan et al. 2023). A possible mechanism was proposed in Fig. 3, the more highly dispersed polysaccharide molecules were obtained after the treatment of Fe^{2+}/H_{2}O_{2} due to an unselective attack by •OH. These polysaccharides were further broken down into smaller units like D-galacturonic acid by the treatment of pectinase, so lower molecular weight polysaccharides were obtained after pectinase/Fe^{2+}/H_{2}O_{2} treatment (Wang et al. 2021; Xie et al. 2021).

As shown by the FT-IR spectra in Fig. 4a, both the untreated pectin and its depolymerized products showed similar spectral bands, because FT-IR was relatively insensitive to small structural changes in large polymer molecules. The broad absorption around 3425 cm\(^{-1}\) was attributed to the hydroxyl group stretching which indicated that polysaccharide chains had strong inter and intramolecular interactions. The peak at about 2932 cm\(^{-1}\) was the characteristic absorption of the C-H, including CH, CH\(_2\), and CH\(_3\) stretching and bending vibrations. Signals at 1640 cm\(^{-1}\) could be attributed to the stretching vibration of carboxyl groups (-COOH), and the relatively strong absorption peak at 1743 cm\(^{-1}\) was due to the stretching vibration of a carboxylic ester, suggesting the presence of esterified pectic polysaccharide. The absorption peaks between 950 cm\(^{-1}\) and 1150 cm\(^{-1}\) indicated that the presence of pyranose and the pyranose configuration of pectin did not change after the different treatments. The intensity of the carboxyl ester peak (-COOCH\(_3\)) in pectin was reduced after being treated by pectinase/Fe^{2+}/H_{2}O_{2}. Carboxyl esters might be destroyed by pectinase/Fe^{2+}/H_{2}O_{2} treatment as judged by the signal change at 1743 cm\(^{-1}\) (Ma et al. 2021; Fu et al. 2022).

In Fig. 4b, two peaks belonging to C1s and O1s could be observed in the spectra. The peak area of O1s was smaller than that of C1s in the untreated pectin spectrum, whereas the peak area of O1s was larger...
than that of C1s in the spectra of pectinase-treated or pectinase/Fe\(^{2+}/H_2O_2\)-treated pectins. This could be attributed to the generation of new C = O double bonds from the α-1,4-glycosidic bond was broken by the •OH. The amount of C-O bonds of the water-soluble pectin increased after pectinase treatment, as shown in Fig. 4d and 4g, and this result was attributed to the generation of C-OH from the hydrolysis of the α-1,4-glycosidic bond by pectinases. The increase in the content of C = O bonds of the water-soluble pectin was found after pectinase/Fe\(^{2+}/H_2O_2\) treatment, as shown in Fig. 4e, because the α-1,4-glycosidic bond was broken by the •OH attack, and the new C = O double bonds at the position of carbon atom one or carbon atom four of the polysaccharide ring was generated. As shown in Fig. 4h, the content of C-O/C = O bonds in pectin was increased after the treatment by pectinase/Fe\(^{2+}/H_2O_2\). This suggested that the α-1,4 glycosidic bond in pectin could be both hydrolyzed by pectinases and destroyed by •OH (Wang et al. 2021; Yan et al. 2021; Yu et al. 2022).

The increase in the C-O bond content was due to hydrolysis by pectinase, and the increase in the C = O bond content was attributed to dehydrogenation by •OH. Thus, a possible mechanism of pectin degradation by pectinase/Fe\(^{2+}/H_2O_2\) was proposed, as shown in Fig. 5. The α-1,4-glycosidic bond was broken in the presence of pectinase or Fe\(^{2+}/H_2O_2\), and the ester bond could also be broken attributed to the presence of Fe\(^{2+}/H_2O_2\).

**Degradation of pectin on cotton fabrics by Fe\(^{2+}/H_2O_2\) system**

As shown in Fig. 6a, the pectin on cotton fabrics was difficult to remove when the fabrics were treated with H\(_2O_2\) alone. The removal rate of fabric pectin was very low, even when the concentration of H\(_2O_2\) had been increased to very high. Because the low concentration of reactive oxygen species was produced by H\(_2O_2\) at low temperatures and near-neutral pH (Liu et al. 2021; Huang et al. 2023).

The concentration of Fe\(^{2+}\) or H\(_2O_2\) had a significant influence on the efficiency of the Fe\(^{2+}/H_2O_2\) reaction, and 1 mM was suggested to be the most suitable concentration for Fe\(^{2+}\) to promote the decomposition of 1 mL/L H\(_2O_2\). As shown in Fig. 6b, the pectin degradation rate increased with increasing H\(_2O_2\) concentration at lower H\(_2O_2\) dosages. It might be due to the increased chance of H\(_2O_2\) contact with Fe\(^{2+}\) as the H\(_2O_2\) concentration increased, resulting in more •OH being produced. However, when the concentration of H\(_2O_2\) reached a certain range, the degradation rate did not continue to increase, probably because too much H\(_2O_2\) could not be decomposed effectively. At the same time, the excess H\(_2O_2\) might trap the •OH in the reaction system, resulting in the production of H\(_2O\) and other by-products (Hu et al. 2018; Liu et al. 2021). Similarly, a similar trend was shown when changing the Fe\(^{2+}\) concentration in the reaction system.

As can be seen in Fig. 6c, the degradation rate of pectin increased with the addition of Fe\(^{2+}\) concentration, and the degradation rate decreased when Fe\(^{2+}\) concentration exceeded 1 mM. These excess Fe\(^{2+}\) were
involved in unexpected reactions, reducing the oxidizing capacity of the Fe\(^{2+}/\text{H}_2\text{O}_2\) system. Although the temperatures used in the Fe\(^{2+}/\text{H}_2\text{O}_2\) process were typically around 25 ~ 30°C, the influence of reaction temperature had been studied, because the higher reaction temperatures were thought to reduce reaction times. The experimental results indicated that reaction temperature had a significant effect on the removal rate of pectin from fabrics. It could be seen from Fig. 6d that increasing temperature from 20 to 50°C had a positive effect on the pectin removal. It was possible to speed up the Fe\(^{2+}/\text{H}_2\text{O}_2\) reaction by increasing temperature, thereby increasing the •OH generation rate. In contrast, only about 15% removal rate was reached at 65°C, and the pectin removal rate on fabrics was very low under high-temperature conditions because of the invalid decomposition of hydrogen peroxide into H\(_2\)O and O\(_2\) at these conditions (Watwe et al. 2021).

As shown in Fig. 6e, to clarify the effects of the reaction pH values on the pectin degradation, the experiments were carried out at pH 3 ~ 6. The removal rate of pectin was increased with the pH value of the solution decreased. This might be attributed to the pectin on the fabric being more easily released and dissolved at lower pH (Du et al. 2024). Moreover, more •OH was produced because Fe\(^{2+}\) was more stable under acidic conditions. On the contrary, Fe\(^{2+}\) was more easily oxidized to Fe(OH)\(_3\) which did not react with H\(_2\)O\(_2\) at near-neutral pH. However, the strength of cotton fabrics was reduced due to hydrolyzation of the β-glycosidic bonds in cellulose under acidic conditions (Ghasemi et al. 2017). Meanwhile, the most suitable pH for the pectinase we used in this study was 6. Consequently, the pH value for the Fe\(^{2+}/\text{H}_2\text{O}_2\) reaction was chosen to be 6 (Cui et al. 2021).

According to Fig. 6f, the effect of reaction time on the Fe\(^{2+}/\text{H}_2\text{O}_2\) reaction was not remarkable. The removal of pectin on cotton fabrics increased very slowly over more than 30 min. This was attributed to the reaction between Fe\(^{2+}\) and H\(_2\)O\(_2\) occurring very rapidly and the production of •OH decreased because of the consumption of Fe\(^{2+}\) and H\(_2\)O\(_2\) (Bae et al. 2013).

**Structure and properties of cotton fabrics after scouring by pectinase→Fe\(^{2+}/\text{H}_2\text{O}_2\)**

The wettability of the fabrics was improved after scouring by pectinase, Fe\(^{2+}/\text{H}_2\text{O}_2\), and pectinase→Fe\(^{2+}/\text{H}_2\text{O}_2\). The high removal rates of pectin or waxes on the fabrics after pectinase→Fe\(^{2+}/\text{H}_2\text{O}_2\) treatment were considered to be responsible for the satisfactory wettability. Wetting properties of the fabrics treated with pectinase or Fe\(^{2+}/\text{H}_2\text{O}_2\) were not as good as that of the fabric treated with pectinase→Fe\(^{2+}/\text{H}_2\text{O}_2\), because of the low removal rates of pectin or waxes (Fig. 7a). The wettability of the fabric treated with pectinase was the worst. Pectin and waxes removal rates from the fabrics increased after Fe\(^{2+}/\text{H}_2\text{O}_2\) were added into the treatment solution of pectinase because the hydroxyl radicals were easier to enter the interior of the fibers for pectin degradation. At the same time, loose waxes were taken away from the cotton fibers along with the pectin removed. The pectin could be
effectively removed from the fibers by pectinase→Fe$^{2+}$/H$_2$O$_2$ treatment. The α-1-4 glycosidic bond in pectin could be hydrolyzed by pectinase, and the methylated long chains of esterified pectin and rhamnogalacturonan might be degraded under hydroxyl radical attack. Therefore, the pectin could be more removed from the fibers under two degradation processes with pectinase→Fe$^{2+}$/H$_2$O$_2$. Waxes that coexist with pectin could be removed from the fibers along with pectin. In addition, waxes could also be degraded by the attack of hydroxyl radicals from Fe$^{2+}$/H$_2$O$_2$ reaction (Raafi et al. 2023; Colombi et al. 2021).

The wettability of the cotton fabric with pectinase→Fe$^{2+}$/H$_2$O$_2$ treatment was improved due to the effective removal of two major impurities, pectin and waxes. As shown in Fig. 7c, the fabrics had better wettability after the treatment with pectinase→Fe$^{2+}$/H$_2$O$_2$. The average time for complete wetting of the water droplets was 4.25 s on the fabric scoured by pectinase→Fe$^{2+}$/H$_2$O$_2$, and those of the fabric treated by pectinase or Fe$^{2+}$/H$_2$O$_2$ were greater than 120 s or 20.35 s, respectively. The results of the water contact angle of the fabrics were consistent with those of the wettability of the cotton fabrics. Moreover, the results of the vertical wicking height also indicated that the fabrics obtained better wettability after pectinase→Fe$^{2+}$/H$_2$O$_2$ treatment, as demonstrated in Fig. 7d.

Figure 7e showed the SEM micrographs of the untreated cotton fabric and the cotton fabrics scoured by pectinase, Fe$^{2+}$/H$_2$O$_2$ or pectinase→Fe$^{2+}$/H$_2$O$_2$. The untreated cotton fabrics had rough fiber surfaces due to the presence of a large amount of impurities. The smoother fiber surface was obtained after pectinase→Fe$^{2+}$/H$_2$O$_2$ treatment (Zhou et al. 2019).

Figure 7b showed the FT-IR spectra of the untreated cotton fabric and the cotton fabrics after the different scouring. The absorption band at 3200 ~ 3400 cm$^{-1}$ could be assigned to the OH stretching vibration and the intra and intermolecular hydrogen bond related to the chemical structure of cellulose. The narrower bands located at 2852 cm$^{-1}$ were attributed to the stretching vibration of CH$_2$ and CH groups. The bands at around 1732 ~ 1640 cm$^{-1}$ were characteristic of pectin and could be assigned to the COOH and COOCH$_3$ groups of polygalacturonic acid. However, it should be noted that the characterization of the carboxyl ion band around 1550 ~ 1700 cm$^{-1}$ by FT-IR was quite difficult (Ben Ticha et al. 2016; Zhou et al. 2020).

The change in the strength of the fabrics was demonstrated in Fig. 7f. The results indicated no significant loss of fabric strength after the scouring processes. Slight strength loss of the fabric by pectinase→Fe$^{2+}$/H$_2$O$_2$ scouring was mainly due to the weak damage of cellulose under the unselective attack of free radicals. This result was supported by the result of XRD (Fig. 7g). Comparison of crystallinity between the untreated and scoured cotton fabrics, the crystallinity of the cotton fabrics slightly decreased after the scouring.
Based on the above experimental results, better scouring results of the cotton fabric were obtained by pectinase→Fe^{2+}/H_2O_2 treatment as compared to pectinase or Fe^{2+}/H_2O_2 treatment alone.

**Properties of scoured fabrics after bleaching**

The scoured fabrics were further bleached to remove natural pigments and cottonseed hulls and obtain better wetting properties and whiteness for subsequent processing. The pectin removal rates of the fabrics reached about 90% after the bleaching treatment, as shown in Fig. 8a. The hydrophilicity of the fabrics after the bleaching had been further improved compared to the scoured fabrics because the hydrophobic impurities on the fiber surface were further removed.

As shown in Fig. 8c and 8d, almost the same whiteness was obtained after the scoured fabrics were bleached. The destruction of natural pigments on fabrics was associated with substitution or addition reactions by unstable •OOH and •OH attacks. Moreover, the strength loss difference between the fabrics scoured by pectinase→Fe^{2+}/H_2O_2 and those scoured by pectinase was only 2 ~ 2.5% after bleaching, suggesting that the fabrics scoured by pectinase→Fe^{2+}/H_2O_2 did not obtain more strength loss after bleaching (Bulut 2016; Tang and Sun 2017).

Based on the experimental results above, the bleaching effect of fabrics was barely affected after scouring with pectinase→Fe^{2+}/H_2O_2.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>The properties of the fabrics treated with NaOH scouring or pectinase→Fe^{2+}/H_2O_2 scouring, and bleaching. (NaOH scouring: temperature 98°C; NaOH 5g/L and time 60 min. Pectinase→Fe^{2+}/H_2O_2 scouring: pectinase, 1mL/L; H_2O_2 concentration, 1mL/L; Fe^{2+} concentration, 1mM; TF-1871A concentration, 0.5mL/L; temperature 55°C, pH 6, and time 90 min. The conditions for bleaching were the same: H_2O_2 concentration, 10mL/L; DM1402A concentration, 2 mL/L; temperature 98°C; pH 11.5; time 60 min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pectin removal rate (%)</td>
<td>95.67</td>
</tr>
<tr>
<td>Waxes removal rate (%)</td>
<td>97.76</td>
</tr>
<tr>
<td>Wettability (s)</td>
<td>&lt; 1.0</td>
</tr>
<tr>
<td>Vertical wicking height (cm)</td>
<td>10.35</td>
</tr>
<tr>
<td>Hunter whiteness (%)</td>
<td>89.79</td>
</tr>
<tr>
<td>Strength loss (%)</td>
<td>11.79</td>
</tr>
</tbody>
</table>
Table 3
The properties of the fabrics treated by pectinase→Fe$^{2+}$/H$_{2}$O$_{2}$ scouring and H$_{2}$O$_{2}$ bleaching using the pilot-scale dyeing machine. (Pectinase→Fe$^{2+}$/H$_{2}$O$_{2}$ scouring: Pectinase, 1mL/L; H$_{2}$O$_{2}$ concentration, 1mL/L; Fe$^{2+}$ concentration, 1mM; TF-1871A concentration, 1mL/L Temperature 55°C, pH 6, and time 90 min. Bleaching: H$_{2}$O$_{2}$ concentration, 10 mL/L; DM1402A concentration, 2 mL/L; temperature 98°C; pH 11.5; time 60 min.)

<table>
<thead>
<tr>
<th>Pectinase→Fe$^{2+}$/H$<em>{2}$O$</em>{2}$</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Pectin removal rate (%)</td>
<td>90.67</td>
</tr>
<tr>
<td>Waxes removal rate (%)</td>
<td>89.92</td>
</tr>
<tr>
<td>Wettability (s)</td>
<td>1.30</td>
</tr>
<tr>
<td>Vertical wicking height (cm)</td>
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<tr>
<td>Hunter whiteness (%)</td>
<td>89.66</td>
</tr>
<tr>
<td>Strength loss (%)</td>
<td>12.35</td>
</tr>
</tbody>
</table>

As shown in Table 2, pectin removal rate, waxes removal rate, wettability, vertical wicking height and whiteness of the fabric by pectinase→Fe$^{2+}$/H$_{2}$O$_{2}$ scouring were close to those of the fabric by NaOH scouring. This result indicated that the scouring method proposed in this study, pectinase→Fe$^{2+}$/H$_{2}$O$_{2}$ scouring, could achieve the same effect as the traditional chemical scouring method. However, the strength loss of the fabric treated by pectinase→Fe$^{2+}$/H$_{2}$O$_{2}$ was 11.79%, which was less than the strength loss of the fabric treated by traditional chemical scouring (20.16%). This result indicated that chemical scouring caused greater damage to the cotton fibers than pectinase→Fe$^{2+}$/H$_{2}$O$_{2}$ scouring. The dyeing performance of the different bleached fabrics was investigated by the color depth (K/S), as shown in Fig. 9a ~ c. It could be observed that there was almost no difference in the color depth of the dyed fabrics which were scoured by pectinase→Fe$^{2+}$/H$_{2}$O$_{2}$ or traditional chemical method (Li et al. 2023). This indicated that the new pectinase→Fe$^{2+}$/H$_{2}$O$_{2}$ method had no negative impact on the dyeing performance of the fabrics.

In summary, compared to the traditional chemical scouring, there were still many benefits of cotton fabrics with pectinase→Fe$^{2+}$/H$_{2}$O$_{2}$ scouring. The treatment temperature of pectinase→Fe$^{2+}$/H$_{2}$O$_{2}$ scouring was lower, and this was more in line with energy-saving requirements. The strength loss of the fabric by pectinase→Fe$^{2+}$/H$_{2}$O$_{2}$ scouring was lower than that by traditional chemical scouring, and the fabric had better application performance. Moreover, the fabric with enzyme scouring had a softer hand because there was still a certain amount of natural cotton waxes present.
Conclusion

In this study, the results showed that the pectin on cotton fabric could be efficiently removed by pectinase combined with Fenton treatment (Fe$^{2+}$/H$_2$O$_2$). The molecular weight and chemical structure of water-soluble pectin treated by pectinase→Fe$^{2+}$/H$_2$O$_2$ were evaluated, and lower molecular weight water-soluble pectin were obtained after the treatment. The α-1-4 glycosidic bonds of pectin were hydrolyzed by pectinase and broken by Fe$^{2+}$/H$_2$O$_2$, and the pectin was more effectively degraded by these two mechanisms. Furthermore, the ester bond in pectin might be broken by Fe$^{2+}$/H$_2$O$_2$ treatment.

The results of fabrics by scouring and bleaching showed that the fabrics treated with pectinase→Fe$^{2+}$/H$_2$O$_2$ obtained the better wetting properties than those treated with pectinase alone. The strength loss of fabric treated with pectinase→Fe$^{2+}$/H$_2$O$_2$ was less than that treated with the traditional chemical method (NaOH treatment). In addition, after dyeing the fabrics, it was found that the new pectinase→Fe$^{2+}$/H$_2$O$_2$ scouring method had no negative impact on the dyeing performance of the fabrics.

A pilot-scale machine was employed to investigate the effect of pectinase→Fe$^{2+}$/H$_2$O$_2$ scouring. The results showed that the fabrics the various properties of the fabric treated with pectinase→Fe$^{2+}$/H$_2$O$_2$ had reached the level of the traditional chemical method, which demonstrated the possibility of application in practice. This study combined pectinase with Fenton treatment for cotton scouring, improving the efficiency of enzyme scouring, and a new approach for using pectinase in cotton fabric scouring was provided.

Declarations

Acknowledgements

The National Key Research and Development Program of China (2021YFC2104000).

Author contributions

LP L: Conceptualization, Methodology, Investigation, Validation, Formal analysis, Writing-Original Draft, Visualization. CK D, QT Z and L L: Methodology, Investigation. P W and Q W: Methodology, Supervision, Writing-Review & Editing. YY Y: Resources, Writing-Review & Editing, Supervision, Project administration, Funding acquisition. All authors read and approved the final manuscript.

Data availability

The data and materials will be made available upon reasonable request.

Conflict of interest
The authors declare that they have no conflict of interest.

**Ethics approval**

This article does not involve any research on human participants or animals. Informed consent was obtained from all individual participants included in the study.

**References**


Figures
Figure 1

The research route and mechanism
Figure 2

Concentration of hydroxyl radicals in (a) deionized water and (b) pectin solution during incubation by H$_2$O$_2$ (1 mL/L), Fe$^{2+}$ (1 mM), and pectinase (1 mL/L) alone or mixed. (c) elution profiles of the pectin samples in the GPC system. (d) Conversion of pectin polysaccharides tested using DNS reagent.
Figure 3

Schematic representation of water-soluble pectin degradation by pectinase, Fe$^{2+}$/H$_2$O$_2$, or pectinase/Fe$^{2+}$/H$_2$O$_2$. 
Figure 4

(a) ATR–FTIR spectra of untreated water-soluble pectin and the pectins treated by pectinase, \(\text{Fe}^{2+}/\text{H}_2\text{O}_2\) or pectinase/\(\text{Fe}^{2+}/\text{H}_2\text{O}_2\), respectively. (b) XPS survey spectra and isolated C 1s, O 1s spectra of untreated water-soluble pectin (c and f), the pectins treated by pectinase (d and g) or pectinase/\(\text{Fe}^{2+}/\text{H}_2\text{O}_2\) (e and h).
Figure 5

Schematic representation of the changes in the structure of the water-soluble pectin molecule that may occur after treatment with pectinase, Fe$^{2+}$/H$_2$O$_2$, or pectinase/Fe$^{2+}$/H$_2$O$_2$. 
Figure 6

The effects of reaction conditions on removal rate of pectin on cotton fabric: (a) Concentration of \( \text{H}_2\text{O}_2 \) without \( \text{Fe}^{2+} \) (temperature 55°C, pH 6, and treatment time 60 min). (b~c) Concentration of \( \text{H}_2\text{O}_2 \) and \( \text{Fe}^{2+} \) (temperature 55°C, pH 6, and reaction time 60 min). (d~f) Reaction temperature, reaction time, and pH (\( \text{H}_2\text{O}_2 \) concentration 1mL/L; \( \text{Fe}^{2+} \) concentration 1mM).
Figure 7

(a) Removal rates of pectin or waxes on cotton fabrics. (b) ATR-FTIR spectra, (c) Surface morphology images, (d~e) Wetting time and vertical wicking height; Water contact angle was tested after 5s of water droplet contact, (f) Strength loss, (g) XRD spectra of fabrics scoured by pectinase, Fe^{2+}/H_2O_2, or pectinase→Fe^{2+}/H_2O_2 (Treatment conditions: pectinase, 1mL/L; H_2O_2 concentration, 1mL/L; Fe^{2+}
concentration, 1mM; TF-1871A concentration, 0.5mL/L; temperature 55°C, pH 6, and treatment time 90 min.)

Figure 8

Fabrics after bleaching: (a) Removal rate of pectin on cotton fabrics and Vertical wicking height, (b) Water contact angle of the bleached fabric scoured by pectinase→ Fe^{2+}/H_2O_2 and vertical wicking height of bleached fabrics, (c) Hunter whiteness, (d) Strength loss rate. (Bleaching conditions: temperature 98°C; pH 11.5; H_2O_2 concentration, 10 mL/L; DM1402A concentration, 2 mL/L; time 60 min.)
Figure 9
(a~c) The color depth of the fabrics after dyeing (delivered as K/S value).

Figure 10
(a~c) A pilot-scale dyeing machine and pictures of the front and side of the fabrics when they were bleached.