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Research Article

Casein and transglutaminase-mediated modification of wool surface

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In this study, casein, a protein found in milk, was used as a surface-coating material for improving wool fabric properties mediated by a microbial protein cross-linking enzyme: transglutaminase (TGase). For the wool fabric used in this study, 4% casein (owf) treatment for 1 h combined with 20 U/g fabric of microbial TGase reduced the area shrinkage of the KMnO₄-pretreated wool fabric from 11.32 ± 0.26 to $4.57 \pm 0.15\%$. The tensile strength increased from 275 ± 5.0 to 315 ± 2.1 N. The casein cross-linked by TGase on wool exhibited better stability than that with the inactivated enzyme in warm water, Na₂CO₃ and SDS solutions. Scanning electron micrographs demonstrated that the casein and TGase treatment smoothened the wool fiber surface by coating or filling the raised scales of the wool.

Keywords: Casein / Modification / Shrink-resist finishing / Transglutaminase / Wool

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1 Introduction

In the textile industry, wool is one of the most important natural protein fibers. But wool fabrics must be subjected to a variety of chemical treatments in order to provide them with desired properties, such as anti-shrinking or anti-pilling ability. The typical commercial shrink-resist finishing used for decades is the chlorine-Hercosett process. This effective process, widely used even today, consists of a strong acid chlorine treatment with a subsequent neutralization and an application of polymer resin [1, 2]. A big disadvantage of the process is the problem of environmental pollution, for example, the disposal of adsorbable organic chlorides. Different other kinds of chemical pretreatment methods involving oxidation or reduction treatments, such as: hydrogen peroxide (H₂O₂), dichlorodicyanuric acid, potassium permanganate (KMnO₄) and sodium sulfite (Na₂SO₃), have been employed to enhance the anti-felting ability of wool fabrics [3–5].

Enzymes can be used in the textile industry for developing environmental-friendly alternative processes. Especially, proteases and lipases have been investigated for wool fiber modification. Current enzymatic processes, especially those using proteases, are difficult to control and not sufficiently predictable and reproducible in the industrial scale. During

such treatments, proteases can easily penetrate into the fiber cortex, causing severe degradation at macrofibril level and unacceptable tensile strength loss. The application of proteases alone is therefore not yet widely used industrially [6–8]. Transglutaminase (TGase; protein-glutamine-glutamyltransferase, EC 2.3.2.13) is an enzyme capable of catalyzing acyl transfer reactions by introducing covalent cross-links between proteins as well as peptides and various primary amines, as shown in Fig. 1 [9], which can lead to enhanced protein stability. At present, TGase is frequently used to improve functional properties of food proteins [10, 11]. In the recent years, it has been found that TGases can remediate the damage caused by the action of chemicals and proteases, prevent strength loss and maintain color during wool processing [5, 7].

Casein, a protein that is found in milk and makes up 80% of milk protein, has a number of interesting properties, including high solution viscosity, high heat stability and the ability to melt that make it to be used widely in food ingredients, pharmaceutical products and as a binding agent in industrial products such as glue, paint and paper. In addition, casein has also been processed into silk-like fibers with the properties of warmth, soft handle and resilience [12]. At the same time, as a widely employed biomacromolecule, casein is a good substrate for TGase because it has a relatively open structure that allows the enzyme to reach appropriate side chains. The aim of this work is to study the effect of TGase-mediated cross-linking of casein with wool on the dimensional

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Abbreviations: SEM, scanning electron microscopy; TGase, transglutaminase

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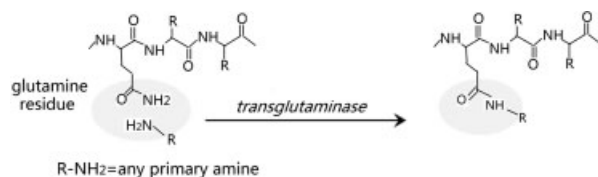


Figure 1. The cross-linking reaction mechanism catalyzed by TGase.

stability and mechanical properties of the fabric. The surface morphologies of the wool samples were analyzed by means of scanning electron microscope (SEM).

2 Materials and methods

2.1 Reagent and wool fabric

TGase was separated from the culture broth of *Streptomyces hygroscopicus* according to the method reported previously [11]. The measured activity of the TGase preparation was 100 U/g. The casein used was of chemical grade and the other chemicals were of analytical grade. Except where otherwise stated, reagents were purchased from Sinopharm Chemical Reagent, China.

Worsted wool fabric (33 tex $\times$ 33 tex, 325 g/m²) was supplied by Wuxi Xiexin Group (China) and used in all the experiments. Before using, all the fabrics were washed with a solution containing 1 g/L non-ionic detergent, at 40°C for 1 h, thoroughly rinsed with water and air dried. Then, the wool fabrics were relaxed in a Y(B)089A washing machine (Wenzhou Darong Textile Instrument, China) using the ISO 6330 7A program as described in the IWS Test Method 31.

2.2 Tensile strength of fabrics

The fabrics were tested on a YG (B) 026D-250 fabric tensile strength tester (Wenzhou Darong Textile Instrument) to measure the tensile strength in accordance with the method of ISO5081. The samples were conditioned at 25°C and a relative humidity of 60% for 24 h before testing. The sample was in a dimension of 50 mm $\times$ 250 mm after drawing out the yarns of 5 mm at both sides. Tensile strength of wool fabrics was only tested in the warp direction and the results were given as the arithmetic means of at least three different samples.

2.3 Shrinkage analysis

Wash area shrinkage of the fabrics was determined according to the IWS Test Method 31 in a Y(B)089A washing machine. Each fabric was washed with ISO 6330 program 5A for six times. The percentage of area shrinkage of wool fabric was calculated and the results were the arithmetic means of three different samples. The dimensions of the testing samples were 200 mm $\times$ 220 mm.

2.4 Treatments of wool fabric

The wool samples were pretreated with 3% (owf) KMnO₄ at pH 4–5 for 30 min at 40°C and subsequently rinsed in water and dried at room temperature. To investigate the effect of casein alone on area shrinkage and tensile strength of wool fabric, the samples without KMnO₄ pretreatment and the KMnO₄-pretreated samples were treated with 0, 1, 2, 4, 6% (owf) casein in 20 mmol/L phosphate buffer (pH 7.0) for 1 h at 40°C and a liquor-to-fabric ratio of 20:1, respectively.

To study the effect of casein with TGase on area shrinkage and tensile strength of wool fabric, the KMnO₄-pretreated samples were treated with 4% (owf) casein in the presence of TGase activity (10, 20 and 30 U/g fabric) in 20 mmol/L phosphate buffer (pH 7.0) for 1 h at 40°C and a liquor-to-fabric ratio of 20:1, respectively.

All the treated samples were tested for shrinkage analysis and fabric tensile strength as described above. The sample without any treatments was used as the control.

2.5 Durability of casein on the wool fabric

The durability of casein on wool fabric was determined according to Cortez et al. method of measuring the effect of TGase on the resistance of dyes on wool fiber to detergent damage, with some modifications [7].

The KMnO₄-pretreated samples were treated using 4% (owf) casein combined with 20 U/g fabric TGase and the same amount of inactivated TGase in 20 mmol/L phosphate buffer (pH 7.0) for 1 h at 40°C and a liquor-to-fabric ratio of 20:1, respectively, washed with water and air dried. TGase was inactivated by boiling the enzyme solution for 10 min. Then, the fabric samples were washed in water, 0.5 mmol/L sodium carbonate (Na₂CO₃) of pH 10 and 0.5 g/L of SDS with a liquor ratio of 30:1 respectively in beakers placed in a shaker at 100 rpm during 30 min at 50°C for three times. The durability of casein on wool fabric was expressed as the amount of casein released from the fabric into each washing bath. The amount of casein was determined by the Lowry method with casein as the standard [13].

2.6 Fiber surface analysis

The surface morphologies of the wool samples were visualized using an S-3000N/H scanning electron microscope (Hitachi, Japan), operating at a typical accelerating voltage of 10 kV. The samples were sputter-coated with gold for 40 s at 15 mA prior to the observation.

3 Results and discussion

3.1 Effect of casein alone on area shrinkage and tensile strength of wool fabric

The effect of casein on the properties of wool fabric was shown in Fig. 2. The area shrinkage level and tensile strength of the

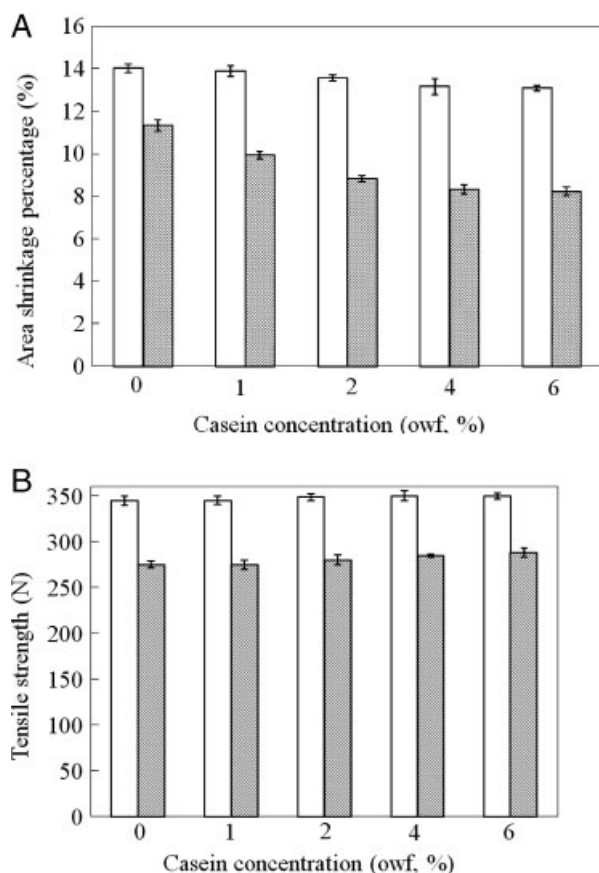


Figure 2. Effect of casein concentration without TGase on the (A) area shrinkage and (B) tensile strength of wool fabrics. Without KMnO₄ pretreatment; KMnO₄ pretreatment.

fabric without KMnO₄ pretreatment were not obviously affected with the increasing casein concentration. Because wool fiber surface is covered by a covalently bound fatty layer, which is responsible for the strong hydrophobicity of the fiber. This fatty layer diminishes the affinity of the hydrophilic casein to wool fiber. Chemical or protease treatments are often used to remove some of the hydrophobic substances to alter the surface properties of the fiber [14].

In our previous study on the effect of TGase on the wool properties, among the different chemical pretreatments chosen: KMnO₄, H₂O₂, Na₂SO₃ and hypochlorite (NaClO), KMnO₄ pretreatment played a more efficient role in the following TGase treatment to wool fiber than the other three pretreatments [5]. KMnO₄ is a strong oxidant. It diffuses right through wool fibers and readily oxidizes all the accessible cystine to increase the wool hydrophilicity [15]. Accordingly, the hydrophobic nature of the fiber is reduced and the adhesion of hydrophilic compounds to wool is improved. So, KMnO₄ was selected as the pretreatment method in this study. The results in Fig. 2 indicated that KMnO₄ pretreatment decreased the percentage of area shrinkage (from 14.06 ± 0.13 to $11.32 \pm 0.26\%$) but simultaneously resulted in serious damage to the wool fiber surface and consequently the loss of fabric tensile strength (from

345 ± 3.6 to 275 ± 5.0 N). And a pretreatment of the fabric with KMnO₄ followed with a treatment with 4% (owf) casein reduced the area shrinkage of the fabric to $8.52 \pm 0.26\%$ and increased the tensile strength to 284 ± 2.1 N. It was suggested that the oxidant pretreatment of wool fabric enhanced the casein–wool affinity and the efficiency of following casein treatment. Increasing the casein concentration above 4% (owf) did not change the properties of wool fabric significantly. Therefore, the below experiments were performed at a casein concentration of 4% (owf).

3.2 Effect of casein with TGase on area shrinkage and tensile strength of wool fabric after Kmno₄ pretreatment

It is known that the conventional process chlorine-Hercosett can almost confer the complete unshrinkability upon wool [16]. At the same time this method causes very serious environmental problems. Recently, many different types of adsorbable organic chloride-free wool anti-shrinkage processes have been reported and different reduction levels of wool shrinkage were obtained. Cardamone *et al.* applied alkaline hydrogen peroxide (H₂O₂) systems, including dicyandiamide, gluconic acid and Triton X surfactants, followed by enzymatic treatment, 1.16% area shrinkage was achieved [17]. Gaffar *et al.* developed a bioprocess combining the advantages of both protease and TGase in a simultaneous enzymatic treatment and the felting tendency of woven wool fabrics was reduced by 9% at the expense of only 2% weight and tensile strength loss [18]. Wang *et al.* used a mild oxidation and cutinase/lipase pretreatment process and the area shrinkage of worsted wool fabric decreased to 3.1% [19]. In Cortez *et al.* study, after permonosulphuric acid pretreatment, the knitted wool fabric was then treated with either tissue or microbial TGase from *S. mobaraense* and the area shrinkage level was reduced from 16 to 6% [20]. The effect of TGase dosage on the area shrinkage and tensile strength of KMnO₄-pretreated wool fabric combined with 4% (owf) of casein was shown in Fig. 3. The percentage of area shrinkage of the fabric further reduced notably and the tensile strength recovered to some degrees after casein treatment with increasing TGase dosage. The percentage of area shrinkage of the fabric continually reduced to $4.57 \pm 0.15\%$ and the tensile strength increased to 315 ± 2.1 N after treatment with 4% (owf) casein combined with 20 U/g fabric of TGase at 40°C for 1 h. It was implied that chemical pretreatment might make wool keratin more accessible to TGase and cause the cross-linking of wool keratin and casein easier at the same time. By comparing with the results of the previous reports, casein and TGase-mediated process after KMnO₄ pretreatment provided a new possibility to make wool garments more competitive in the global market.

3.3 Stability of casein on the wool fabric

During the process of wear, wool fabrics are often submitted to washing in the presence of heat, alkaline and detergent.

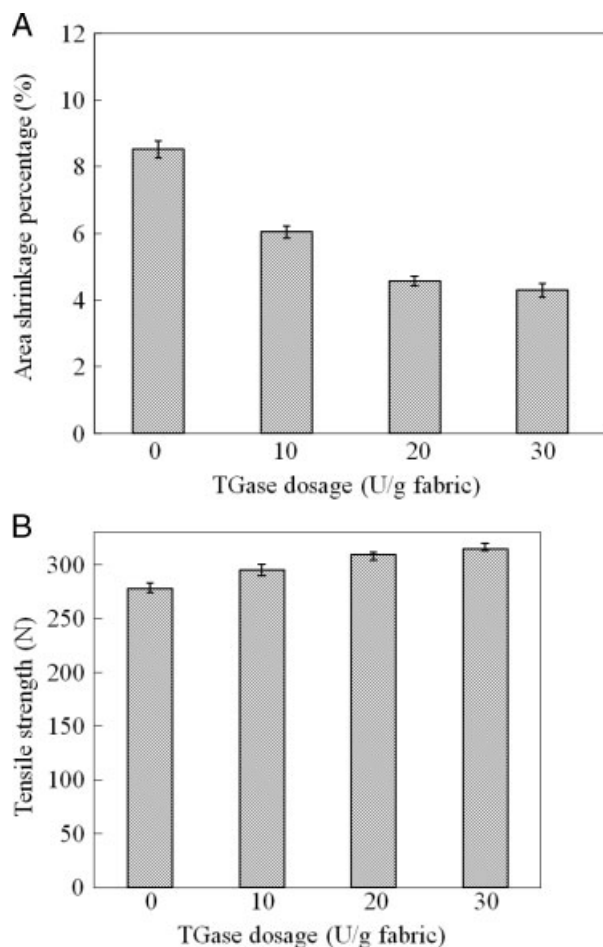


Figure 3. Effect of TGase dosage and 4% (owf) casein on the (A) area shrinkage and (B) tensile strength of KMnO_4 -pretreated wool fabrics.

Durability of casein on the wool fabric to washing is one of the important factors to consider for the anti-felting finishing process of wool. Figure 4 depicted the amount of casein released from different treated wool fabrics into water, Na_2CO_3 and SDS solution at 50°C .

For the fabric treated with KMnO_4 and casein combined with inactivated TGase, casein proteins were mainly absorbed on wool fibers from the solution *via* hydrogen bonds and/or hydrophobic interactions. After the first washing cycle, 0.048 ± 0.002 , 0.116 ± 0.001 , 0.083 ± 0.002 mg/mL of casein were released from the fabric into warm water, Na_2CO_3 and SDS solutions respectively. Even after three washing cycles, there was still a small quantity of casein released from the fabrics into the solutions (Fig. 4a). On the contrary, the casein cross-linked with TGase on KMnO_4 -pretreated wool had a better washing stability. As shown in Fig. 4b, after the first washing cycle, only 0.015 ± 0.0028 , 0.049 ± 0.001 , 0.040 ± 0.001 mg/mL of casein was soluble in warm water, Na_2CO_3 and SDS solutions. After the second washing cycle, there was hardly any casein dissolved in the solutions. It is because that the casein attached to the fiber without

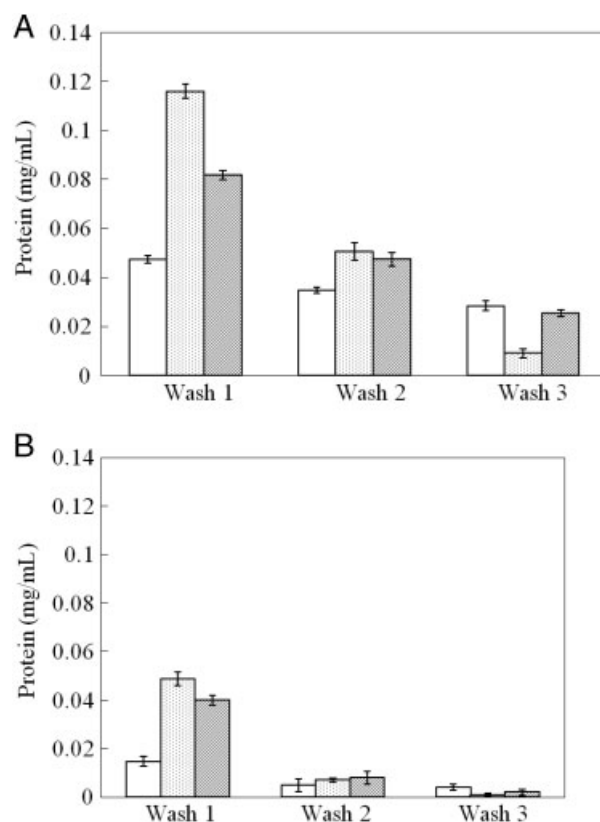


Figure 4. Durability of casein with inactivated TGase (A) and with TGase (B) on the KMnO_4 -pretreated wool fabrics. Water; Na_2CO_3 solution; SDS solution.

covalent cross-linking owing to enzyme inactivation tends to be washed out into water, alkaline solution and detergent solution. The covalent cross-linking between the casein proteins or between casein and wool keratin catalyzed by TGase impart a better stability of casein on the fiber than that without TGase activity.

3.4 Surface analysis of the treated wool fibers

The SEM images of different treated fabrics are shown in Fig. 5. The cuticle scale on the control fabric without any treatments appeared intact as demonstrated in Fig. 5a, which is considered as the primary cause of felting in wool during an aqueous washing process. The control sample exhibited an average area shrinkage of $14.06 \pm 0.13\%$ and tensile strength of 345 ± 3.6 N. KMnO_4 pretreatment resulted in rugosities on the fiber surface and the observed damage appeared as partial lifting of the scale edges as depicted in Fig. 5b, which caused fiber damage. Thus, the tensile strength of KMnO_4 -pretreated fabric reduced to 275 ± 5.0 N and the area shrinkage decreased to $11.32 \pm 0.26\%$. Figures 5c and d revealed how the combined system of casein and TGase provided anti-felting property to KMnO_4 -pretreated wool fabrics. No

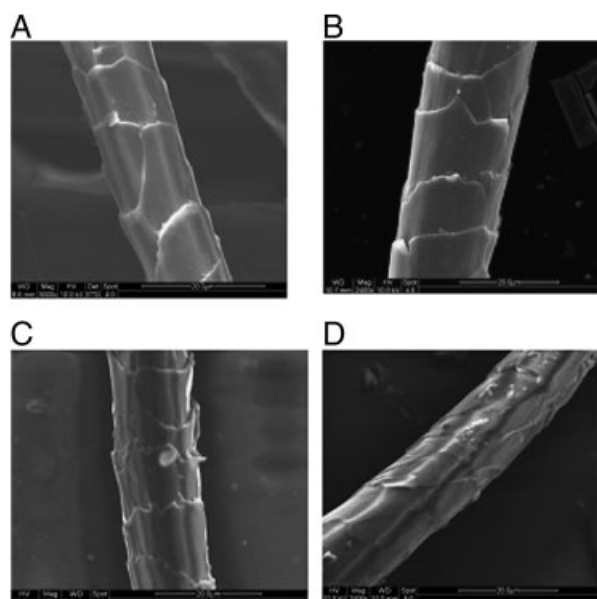


Figure 5. SEM of surface structure of wool fabrics after different treatments ((A): Control; (B): KMnO_4 pretreatment; (C): KMnO_4 and casein treatment without TGase; (D): KMnO_4 and casein treatment with TGase).

morphological changes appeared on KMnO_4 - and casein-treated wool fibers and the scales still could be clearly distinguished (Fig. 5c). This treatment method continually reduced the area shrinkage of the fabric to $8.52 \pm 0.26\%$ and increased the tensile strength to $284 \pm 2.1 \text{ N}$. KMnO_4 and casein combined with TGase treatment completely smoothened the fiber surface by filling or coating the protuberant scales (Fig. 5d), which removed the directional friction effect of wool and helped to prevent interlocking of the scales induced by washing. So the wool fabric had better shrinkage resistance. The area shrinkage was only $4.57 \pm 0.15\%$ and the tensile strength increased to $315 \pm 2.1 \text{ N}$ evidently.

4 Concluding remarks

Casein was used to modify the surface properties of wool fiber combined with TGase. KMnO_4 pretreatment could enhance the casein–wool affinity and the efficiency of following casein and TGase treatment, which was concluded from the results of area shrinkage and tensile strength of wool fabrics. The results also showed that casein cross-linked on wool surface catalyzed by TGase exhibited a better stability in warm water, Na_2CO_3 and SDS solutions than that with inactivated TGase. SEM provided evidence that the casein was coupled to the wool surface using TGase to smoothen the wool fiber surface by coating or filling in the scales, which imparted the anti-felt property to wool fiber. It is suggested that casein combined with TGase treatment could provide an option as an environmental-friendly method to functionalize wool fiber.

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