ENZYME REACTIONS FOR REMOVING NON-CELLULOSIC CELL WALL COMPONENTS OF COTTON FIBERS

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Abstract

Enzymatic removal of the noncellulosics on cotton fiber surfaces has been extended from the use of pectinases to that of proteases. Ten proteolytic enzymes or proteases were employed on greige cotton fabrics which were pretreated in 100°C water for 2 seconds twice. Reactions with four of the proteases resulted in significantly improved wettability on cotton fabrics and the resulted wetting behavior is similar or superior to alkaline scoured cotton. Several other proteases also improved the wettability of cotton fabrics, but to lesser extents. The optimized reaction conditions for the most effective proteases afford a range of moderate scouring conditions including neutral to acid (pH 4) media, low temperatures (25ºC to 45ºC), and short reaction times (10 min. to 30 min.). The low temperatures of these protease reactions also lead to less change in fabric thickness and porosity than in the alkaline scoured cotton fabrics .

Introduction

As the nature's purest source of ?-cellulose, cotton fibers still contain approximately 10% of non-cellulosics, the hydrophobic nature of some impede uniform and efficient dyeing and finishing performed under aqueous conditions. Most noncellulosic materials on cotton fibers can be made soluble and removed with alkaline scouring, conventionally done with sodium hydroxide solutions at boil in the presence of wetting and sequestering agents. Alkaline scouring improves wettability and, at the same time, causes fabric shrinkage, i.e., reduction in fabric planar dimensions and an increase in fabric thickness [Hsieh *et. al*., 1996]. These changes in fabric geometry are expected to impact the physical properties and handle of the scoured fabrics.

Recent work has shown that cellulolases [Achwal, 1992; Bach and Schollmeyer, 1993; Li and Hardin, 1997; Rößner, 1993] and several other enzymes [Hartzell and Hsieh, 1998, Hartzell and Hsieh, *in press*; Li and Hardin, 1997], can produce similar effects to alkaline scouring on cotton fabrics. Our earlier work has evaluated several enzymes including cellulases, pectinases, proteases, and lipases for their effectiveness to improve water wettability and absorbency of cotton fabrics [Hartzell and Hsieh, 1998]. Among those studied, cellulases were the only enzymes that improved fabric wettability when used alone. Cellulases work by hydrolyzing the supporting cellulose, thus dislodging the non-cellulosics on the surface. This explains why cellulases and those pectinases which contain cellulase components cause greater weight loss than when either proteases or lipases were used alone. It was for this reason that strength reduction with cellulases was inevitable and often significant [Achwal, 1992; Bach and Schollmeyer, 1993; Rößner, 1993]. Greater effects of either cellulases or pectinases were observed if the cotton was pretreated with chloroform prior to the enzyme treatments [Achwal, 1992; Bach and Schollmeyer, 1993].

Among the noncellulosics, it is clear that waxes are present on the surface of the cotton fibers. We found that boiling water pretreatments prior to enzyme reactions enhanced the accessibility of enzymes to the other noncellulosics beneath the waxes thus improving enzymatic scouring effects on cotton [Hartzell and Hsieh, 1998, Hartzell and Hsieh, *in press*]. Depending on the length and repetition of such exposure, melting and partial removal and/or reorganization of the surface waxes facilitated greater pectinase access to the pectinacious substances on cotton fiber surfaces [Hartzell and Hsieh, *in press*]. Three pectinases from *Aspergillus niger* significantly improved surface wettability of cotton by achieving water wetting contact angles within the range of alkaline scoured fabrics. These results indicate that digestion of pectinacious matters can also dislodge the other noncellulosics and is effective in removing the hydrophobic compounds on the cotton fiber surfaces. These pectin-degrading enzymes have the advantage over cellulases in that they had no adverse effects on the fabric strength. Furthermore, these pectinase reactions function in completely aqueous systems without wetting agents nor organic solvents.

Considering that proteinacious contents are among the highest of the noncellulosics on cotton, the potential of proteolytic enzymes as scouring agents for raw cotton remains unverified. Proteolytic enzymes or proteases catalyze the hydrolysis of proteins and peptides. Depending upon the types of bonds on which they act, there are two types of proteases, i.e., the proteinases (endopeptidases) and peptidases (exopeptidases). Proteinases act on the interior peptide bonds of proteins and peptides. Proteases of this kinds include pepsin, trypsin, and chymotrypsin from animals and papaine from papaya. In this paper, ten commercially available proteolytic enzymes includinng subtilisin, trypsin, and chymotrypsin were investigated in terms of their potential to hydrolysis of surface proteins thus dislodging the hydrophobic compounds on cotton fiber surfaces.

Experimental

A 100% cotton fabric with a plain weave (400R, Test Fabrics, Inc.) and ten commercially available proteases including subtilisin, trypsin, and chymotrypsin were used in this study. The greige cotton fabrics were subjected to a

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two 2-s water pretreatment at 100°C prior to enzyme treatment. With the exception of one initial set of reactions conducted in phosphate buffer solutions, all enzyme reactions were performed in purified water. Reaction conditions included varied concentration, temperature, and time. The enzyme treatments were performed at constant temperatures in a water-bath at 30 revolutions per minute. Enzyme activity was ceased by immersion into a buffer solution whose pH was unstable for the enzyme, then rinsed in cold water several times until the rinse became neutral. The fabric was rinsed and centrifuged and dried at 65% relative humidity and 21°C until constant weight was obtained. Water wetting behavior was described by the cosine wetting contact angles (WCAs) calculated from the wetting force measured on a tensiometer apparatus described earlier [Hsieh and Yu, 1992]. A high cos WCA or low WCA indicate high water wettability whereas low cos WCA or a large WCA indicates low water wettability. This method also allow the derivation of liquid retention capacity and water absorption.).

Results and Discussion

The untreated cotton fabrics have a cosine water contact angle (cos WCA) of 0.0087 ($\pm$0.0003) or a water contact angle (WCA) of 89.5º ($\pm$2.9º). Initial comparisons among the proteases were performed at near neutral conditions under which these enzymes are most active. **?**Reactions with four N series proteases were conducted with a 5ml/L concentration in water (pH 7) at 45ºC for N1 and N2 and 60ºC for N3 and N4. Both proteases N2 and N4 produced significantly increased cos WCAs of 0.645 (WCA = 49.8º) and 0.531 (WCA = 57.9º), respectively. Proteases N1 and N3 were ineffective under the conditions employed. The reaction with protease N2 was futher optimized with respect to reaction time, enzyme concentration, and reaction temperature. Protease N2 appears to be effective in water at pH 7 with concentration as low as 1 ml/L for 30 minutes at 25ºC.

Two other protease series I (I1 and I2 from ICN) and S (S1, S2, S3, S4 from Sigma) were evaluated under similar conditions, i.e., at 5g/L concentration in buffer and in water (Table 2). The reactions were performed for 60 minutes at 45ºC for I1, I2, S2, S3, and S4 and at 25ºC for S1. All reactions conducted in the buffer in fact lowered the wetting properties of the cotton fabrics. Except for S2, all reactions conducted in water resulted in improved water wettability on the cotton fabrics when compared with those performed in the buffer. Further optimization of the reaction conditions for proteases I2 and S1 showed that the cos WCA increased with increasing concentrations, with most significant improvement of wettability observed up to 2 g/L concentrations. Both I2 and S1 were effective under acidic conditions, i.e., pH 5.5 and 4, respectively. Both required only a short reaction time of 10 minutes. Protease S1 was effective at 25ºC whereas a higher reaction temperature (45ºC) was necessary for protease I2.

Pectin-degrading enzymes have shown to reduce the weights of cotton fabrics by a total of 7.5 to 12.5% [Li and Hardin, 1997]. Since the pectin-degrading enzymes often contain cellulases, the higher weight losses resulted from reactions with some pectinases are due to the digestion of cellulose in addition to the pectins. In reactions with proteolytic enzymes, the lower weight reduction (than those with pectin-degrading enzymes) in combination with the same boiling water pretreatment indicates these proteases remove not only proteinacious substances but other hydrophobic materials thus imparting wetting behavior similar to alkaline scoured cotton. These lower removal levels of cotton cell wall materials appear to be sufficient in achieving comparable wettability to alkaline scouring.

Substantial increases in cotton fabric thickness are expected from hot alkaline scouring. The thickness increases observed ranged from 20 to 60% depending on the duration of scouring time. The effects of the 100ºC water pretreatment caused fabric thickness increase without changing overall pore volume in the fabric. However, none of the protease reactions caused additional changes in fabric thickness nor liquid capacity. Therefore, the increased thickness from the overall process involving proteolytic enzymes are caused by the pretreatment, not results of the proteolytic reactions, and are slightly less than those produced from alkaline scouring.

Although not all protease reactions were effective in improving the wetting behavior of the cotton fabrics, those proteases which improved the water wettability also increased the water absorbency of the cotton fabrics. In fact, when the cos WCA and the water absorption-to-capacity ratio (Cm/C_V) data from all the protease reactions, whether effective or not, are considered together, a positive linear relationship is observed (Figure 3). This relationship is expected as the improved water absorbency is a direct result of improved water wetting property when the overall fabric structure, i.e., thickness and porosity, of the treated fabrics remain unchanged. Under this assumption, the unity water absorption-to-capacity ratio (Cm/C_V) in any fabric indicates that all pores in the fabrics are completely filled with water. Calculations from the linear regression of this relation (Figure 3) give a cos WCA of 0.71 or a WCA of 44.7° as the minimum for water to completely fill all pores in the cotton fabrics. Therefore, the most effective proteases under the optimal conditions fulfill this requirement and produce completely water wettable and absorbent cotton fabrics. The water wetting contact angles produced by these proteases on cotton fabrics are much lower than those (55° to 65°) commercially alkaline scoured cotton fabrics but similar to those (~45°) scoured in the laboratory (4% NaOH at boil for 60 min.).

Conclusion

We have previously shown that pectin-degrading enzymes can effectively remove the pectins on the surface of cotton fibers thus improving the surface wetting properties of greige cotton fabrics [Li and Hardin, 1997]. This study further evaluated ten proteolytic enzymes for improving the water wettability and absorbency of cotton fabrics following a water pretreatment at 100°C. Four of the proteases produced superior wettability on cotton fabrics. Several other proteases also improved wettability of cotton fabrics, but to lesser extents. Overall, scouring cotton with proteolytic enzymes offers many advantages over alkaline scouring. With proteolytic enzymes, scouring requires only moderate temperature (25ºC-45ºC) and minimal lengths of time (10 min. to 30 min.); both much lower than those in alkaline scouring. Under the optimal reaction conditions, the resulted wetting behavior on the protease scoured cotton fabrics is similar or superior to that of the alkaline scoured cotton. These protease scoured fabrics exhibited slightly less increase in fabric thickness than that of the alkaline scoured fabrics. The thickness increase was a result of the pretreatment in water at 100ºC, but not from the protease reactions. Furthermore, scouring with proteolytic enzymes requires lower temperature and shorter reaction time than those employing pectin-degrading enzymes [Li and Hardin, 1997]. The mass loss from reactions in these proteolytic enzymes is less than those in pectin-degrading enzymes. These proteolytic enzyme reaction media also afford a greater range of pH options, from acidic (pH 4) to neutral (pH 7).

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Table 1. Proteases and their properties

	Protease (catalog number or name) EC number	Source	Form	Stability	
				pH	Temp.
S1	Chymotrypsin (C4129) 3.4.21.1	bovine pancreas	solid	7.0-9.0	25°C opt.
S2	Protease Type XXXI (P6670)	*Bacillus licheniformis*	liquid		
S3	Protease Type XXIII (P4032)	*Aspergillus oryzae*	solid		
S4	Protease Type XVI (P5459)/ 3.4.21.14a	*Bacillus subtilis*	solid	7.0-11.0	40-55°C
I1	Protease (101024)/ 3.4.22.2	papaya	solid	6.0-8.5	25-65°C
I2	Protease (150211)	*Streptomyces caespitosus*	solid	6-10	<55°C
N1	Subtilisin (Neutrase)	*Bacillus subtilis*	liquid	5.5-7.5	45-55°C
N2	Subtilisin (Durazyn)/ 3.4.21.14	*Bacillus*	liquid	8.0-12.0	25-50°C
N3	Subtilisin (Alcalase)	*Bacillus licheniformis*	liquid	6.0-9.0	50-65°C
N4	Subtilisin (Esperase)/ 3.4.21.14	*Bacillus*	liquid	6.0-11.0	40-60°C

Table 2. Cosine water contact angle (cos WCA) and retention characteristics of cotton fabrics treated[a] with I and S protease series

Protease[b]	Water		
	pH	cos WCA	Water/Capacity
I1	7	0.232 (0.021)	0.12
I2	5.5	0.588 (0.046)	0.74
S1	4	0.404 (0.056)	0.65
S2	7.5	-0.005 (0.0003)	0.07
S3	7	0.307 (0.031)	0.17
S4	5	0.182(0.033)	0.21

[a] All reactions were performed at 5 g/L concentration and 45ºC for 60 minutes except for the temperature for S1 was 25 ºC.

[b] I series proteases were from ICN Chemicals, Inc. and the S series proteases were from Sigma.

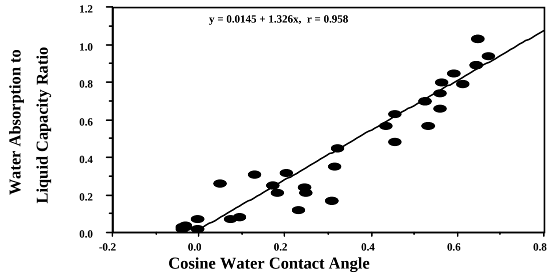

Figure 1. Relationship between water absorption-to-capacity ratio and cosine water contact angles of all protease treated cotton fabrics.