Journal of Biotechnology 156 (2011) 134–140

Contents lists available at SciVerse ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Surface modification of wool with protease extracted polypeptides

Edward Smith, Jinsong Shen*

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

ARTICLE INFO

Article history:
Received 24 June 2011
Received in revised form 4 August 2011
Accepted 11 August 2011
Available online 19 August 2011

Keywords:
Polypeptides
Wool
Enzyme
Protease
Shrink-resistance

ABSTRACT

Polypeptides were extracted from wool protein fibres using the serine type protease Esperase 8.0L (EC 3.4.21.62), a subtilisin from *Bacillus* sp., in a reducing solution. The extracted polypeptides, in aqueous liquor, were then applied to modify the fibre surface of wool fabric with or without additional protease. The treated wool fabric was subsequently treated with the cross-linking agent, glycerol diglycidyl ether, and then underwent a curing process to affix the polypeptide to the fibre. The resulting knitted fabric showed a very high level of shrink-resistance to machine washing, without excessive fibre damage. Shrinkage of 1–2% could be achieved after 5 times 5A washes with minimal (<1%) weight loss due to washing and a burst strength of 317 kPa.

1. Introduction

Felting shrinkage is a typical property of wool when washed and must be controlled to achieve a washable wool product. Due to the configuration of the cuticle scales on the surface of wool fibre, the mechanical action of aqueous washing causes the progressive entanglement of wool fibres leading to irreversible shrinkage of wool fabric. Smoothing or eroding the cuticle scales lowers the friction between the fibres and therefore can prevent shrinkage. Shrink-resist finishing processes often consist of an oxidation/reduction step to degrade the cuticle scales and/or an additive polymer process to mask the scales. The conventional chemical process to achieve shrink-resistant wool, which consists of a chlorination step followed by polymer deposition (Lewis, 1977, 1978), has major drawbacks with respect to chlorination causing severe ecological problems due to contamination of wastewater effluent with absorbable organic halogens (AOX) (Müller, 1992). Recent European Union legislation has imposed restrictions on AOX releases to water (Environment Agency, 2011). If the chlorination step is omitted to avoid the effluent problem, increased polymer deposition then becomes necessary, resulting in a product with a harsh handle that feels more like a synthetic fabric and less like wool. This necessitates the use of alternative, environmentally acceptable shrink-proofing processes. Extensive research has been undertaken to develop an enzyme-based shrink-resistant finishing treatment of wool (Heine and Höcker, 1995; Shen, 2009). Protease can promote the hydrolysis of protein compounds and would appear ideal for degrading the cuticle scales on the wool fibre surface leading to shrink proofing of the wool fibre. However proteolytic attack is not limited to the fibre surface and will penetrate into the fibre causing significant damage in terms of loss of weight and tensile strength (Nolte et al., 1996; Shen et al., 1999; Heine et al., 2000). If the molecular size of the protease is enlarged by covalent coupling with an enteric polymer the proteolytic attack is limited to the cuticle scales thus controlling the damage to the wool (Cavaco-Paulo and Silva, 2003; Silva et al., 2005, 2006a, 2006b; Shen et al., 2007; Smith et al., 2008, 2010a, 2010b). An improvement in shrink-resistance was observed, however there is extra costing involved in the modification of enzymes and commercial standards for machine washability were difficult to meet especially for knitted wool fabrics.

In the current work it was considered whether commercial anti-shrinkage standards could be met by the attachment of a protein resin to the surface of pre-treated wool fabrics and fibres. The protein resin could be a soft protein polypeptide extracted and separated from low quality wool fibre. It was considered that treatment with a protein resin would give the treated wool fibre a softer handle than using a synthetic polymer resin. Proteins such as casein (Needles, 1970), collagen (Needles, 1970; Hesse et al., 1995) and silk sericin (Cortez et al., 2007) have been used as polymer deposition treatments in previous studies in an attempt to achieve shrink resistant wool.

Several different techniques have been reported for fixing proteins on to wool using cross-linking agents. Needles (1970) used a number of different commercially available difunctional epoxides to graft commercially available proteins onto wool and found

* Corresponding author. Tel.: +44 116 2577558; fax: +44 116 2577582.
E-mail address: jshen@dmu.ac.uk (J. Shen).

0168-1656/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jbiotec.2011.08.012

that the only epoxide to give a durable protein graft onto wool fabric was glycerol diglycidyl ether. Hesse et al. (1995) used the trifunctional epoxide Araldite PT 810 (1,3,5-triglycidyl isocyanurate) to covalently fix collagen onto the fibre surface of plasma (glow discharge) pre-treated wool fabric or top and reported almost complete shrink-resistance of the treated wool could be obtained. Cortez et al. (2007) used the enzyme transglutaminase to graft silk sericin onto wool by forming cross-links with the amino acids glutamine and lysine. An improvement in the shrink-resistance, strength and perceived softness of wool was observed. However, the extent of enzymatic reaction is highly dependent on the accessibility of the target amino acids within the wool fibre proteins (Fatarella et al., 2010).

In the current work, sodium sulphite was used to break down the disulphide bonds in combination with a protease to catalyse the hydrolytic cleavage of the protein molecule into smaller peptide chains. Studies show that as long as cystine disulphide bond remains intact the rate of enzyme attack on wool is relatively slow, but once some of these cross-links are broken the rate of reaction is greatly increased (Moncrieff, 1953). The extracted wool polypeptide was separated by centrifugation and the resulting supernatant liquor layer was used to treat pre-scoured knitted wool fabric. The properties of the treated knitted wool fabric were assessed.

2. Materials and methods

2.1. Materials

2.1.1. Enzyme and reagents

The enzyme used was a serine type protease, Esperase 8.0L (EC 3.4.21.62), which is a subtilisin from *Bacillus* sp., supplied by Novozymes A/S (Bagsvaerd, Denmark). The reducing agent sodium sulphite was purchased from Fisher Scientific (Loughborough, UK). Ultravon PL, a synergetic preparation based on non-ionic surfactants, was supplied by Ciba Speciality Chemicals (Cheshire, UK). The cross-linking agent glycerol diglycidyl ether (GDE) was purchased from Sigma–Aldrich (Dorset, UK). IEC reference detergent B and sodium perborate were purchased form SDC Enterprises Ltd (Bradford, UK). The reactive dye, Lanasol Red CE used to test the dyeability of treated wool fabric was supplied by Ciba. All other chemicals used were of specified laboratory reagent grade.

2.1.2. Wool material

The wool fibre used in the extraction process was clean wool top with a mean fibre diameter of 23 μm and was supplied by Drummond Parkland (Huddersfield, UK). The knitted wool fabric used was supplied by Lokateks (Skofja Loka, Slovenia) and was a fine rib 1:1 knit with a mean fibre diameter of 21.3 μm.

2.2. Preparation of wool polypeptide extract

Clean wool top was cut into snippets and placed in a 0.02 M phosphate buffer (pH 8) containing up to 12 g/L sodium sulphite, with a liquor to goods ratio of 20:1 and treated at 60 °C for 30 min using a Datacolor Ahiba Nuance Top Speed II infrared dyer with the agitation set at 40 rpm. 18 activity units of the protease, Esperase 8.0L, per gram of wool fibre (u/g) was added to the mixture and mixed for a further 2 h at 65 °C with an agitation of 40 rpm. The enzyme present in the mixture may be deactivated by raising the temperature to 80 °C for 10 min and maintaining the agitation at 40 rpm. The resulting suspension was separated by centrifugation at 4500 rpm for 5 min using a Hettich Rotina 420 bench centrifuge with a swing out rotor. The supernatant liquid layer was collected for use in the treatment of wool.

2.3. Pre-treatment of wool fabric by alkali scour

Fine rib 1:1 knitted wool fabric was pre-treated in an alkali scour solution containing 2 g/L of the non-ionic surfactant Ultravon PL and 1.6 g/L sodium carbonate at a liquor to goods ratio of 50:1 for 30 min at 60 °C using a Datacolor Ahiba Nuance Top Speed II infrared dyer with the agitation set at 5 rpm. The fabric was then rinsed in deionised water with a liquor to goods ratio of 50:1 for 10 min at 60 °C with 5 rpm agitation. After treatment, the wool sample was washed thoroughly with water, hydro-extracted at 2800 rpm and then left to air-dry.

2.4. Treatment of wool fabric using polypeptide extract

The alkali scoured wool fabric was treated in the neat supernatant wool extract with or without additional Esperase at a liquor to goods ratio of 12:1 for up to 2 h at 60 °C using a Datacolor Ahiba Nuance Top Speed II infrared dyer with the agitation set at 5 rpm. The fabric could then be transferred into a new bath set at pH 7.3 using 0.02 M phosphate buffer containing 10 g/L of the cross-linking agent glycerol diglycidyl ether (GDE) with a liquor to goods ratio of 12:1 for 30 min at 60 °C with 5 rpm agitation. After the wet treatment steps, the fabric was hydro-extracted at 2800 rpm to remove excess wetness. The fabric was then cured at 140 °C for 10 min in a fan assisted oven. The treated wool fabric samples were then conditioned for 24 h at 20 °C and 65% relative humidity prior to property and performance testing.

2.5. Weight loss

The weight loss of the wool fabric after extracted polypeptide treatment was expressed as a percentage, W_L and was calculated using Eq. (1):

$$\%W_L = \frac{100 \times (W_1 - W_2)}{W_1} \quad (1)$$

where W_1 is the weight of conditioned wool fabric prior to extracted polypeptide treatment and W_2 is the weight of conditioned wool fabric after extracted polypeptide treatment.

2.6. Bursting strength

The strength of the knitted wool fabric after extracted polypeptide treatment was measured using bursting strength. A James H Heal TruBurst 610 Bursting Strength Tester was used according to ISO 13938-2:1999. A test area of 10 cm^2 (35.7 mm diameter) was used and the pressure rate was set at 21 kPa/s. The mean bursting pressure and mean height (distension) at burst were recorded.

2.7. Shrinkage

The measurement of shrinkage due to washing of the treated knitted wool fabric was tested according to Woolmark Test Method TM31: Washing of Wool Textile Products. The samples were subjected to a 7A wash cycle for relaxation shrinkage and 5A wash cycles up to 5 times for felting shrinkage using a Miele Novotronic W980 computer controlled washing machine. Between each washing cycle the samples were flat-dried in a 50 °C oven for 4 h then conditioned at 20 °C and 65% relative humidity for 24 h and then weighed. Weight loss due to washing was determined and expressed as a percentage, W_{LW}, which was calculated using Eq. (2):

$$\%W_{LW} = \frac{100 \times (W_3 - W_4)}{W_3} \quad (2)$$