Studies on Isolated Enzyme on Wool

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The free enzyme penetrated into wool fiber cortex while the modified enzyme, with a bigger size, was retained at the surface, in the cuticle layer. It was also confirmed that the diffusion of pro teases was facilitated by the hydrolytic action. Scanning electron microphotographs were also used to observe the intensity of the proteolytic attack. In this paper we mainly discussed about the different types of scale removing techniques and advantages of proteolytic enzymes for this process.

The tendency of wool to felt and shrink is mainly due to its scaly structure. The Chlorine-Hercosett is the most widespread process used to modify the scales of wool fibers with the purpose of providing resistance to felting and shrinkage. There have been many attempts to replace this chlorine process by an environmental friendly enzymatic process that would similarly degrade the scales. However; although pro teases are large molecules, their attack is not only limited to the scales; they penetrate inside the fiber causing unacceptable weight and strength loss. It is believed that if the proteases are chemically modified in order to increase their molecular weight, then they will act just on the surface of the fibers, thus providing wool with anti-shrinking behavior. The free enzyme penetrated into wool fiber cortex while the modified enzyme, with a bigger size, was retained at the surface, in the cuticle layer. It was, also confirmed that the diffusion of proteases was' facilitated by the hydrolytic action. Scanning electron microphotographs were also used to observe the intensity of the proteolytic attack. In this paper we mainly discussed about the different types of scale removing techniques and advantages of proteolytic enzymes for this process.

Wool is a complex natural fiber composed mainly of proteins (97%) and lipids (1 %), consisting of two major morphological parts: the cuticle and the cortex. The former is composed of overlapping cells (scales) that surround the latter. This scaly structure of wool is responsible, to a great extent, for the tendency of wool to felt and shrink (Heine and Hocker 1995; Feughelman 1997).

Chlorination is a commonly used process to modify the scales of wool fibers with the purpose of providing resistance to felting and shrinkage. There have been many attempts to replace this chlorine process by an environmental friendly enzymatic process that would similarly degrade the scales (Nolte et al. 1996; Heine and Hocker 1995; Silva and Cavaco-Paulo 2003; Cortez et al. 2004).

The utilization of enzymes in the textile industry has been known and applied commercially for many years, principally in cellulosic fibers, like the use of amylases for desizing of cotton and cellulases for indigo abrasion on denim (Cavaco-Paulo and Almeida 1994; Cava coPaulo et al. 1996; Ericksson and Cavaco-Paulo 1998). However, for protein fibers, there are other possibilities for enzyme application, including the use of proteases for wool and silk processing (Nolte et al. 1996; Heine and Hocker 1995; Riva et al. 1999) and for the surface modification of cashmere fibers (Hughes et al. 2001).

Properties of Wool

Wool, one of the oldest textile fibers known, has survived the test of time because of its unique natural properties. The basic characteristics that wool possessed in the Stone Age

are still the fundamental qualities that make wool unique in this century. Today there are many other textile fibers, but science as yet to produce another fiber containing all the natural properties of wool (Fact Sheet 2005; FAO 1995). Hence, wool is a remarkable renewable resource with exceptional properties - cool in summer, warm in winter and in a *variety* of weights suitable for both apparel and interior fiber applications. Wool has excellent flame-resistant properties. This factor is of importance in industrial safety garments and in institutions. Legislation on children's night clothes has been a reminder of its value in domestic clothing also. The natural flame resistance of wool is inherent - it will not wash out or decrease in effectiveness with age. Consumers' perception of the benefits of using natural products has stimulated interest in industrial uses of wool (Glaser 1996). *However,* the laundry and durability performance of wool is inferior to synthetics; a factor which is a key selling point for synthetic fiber manufacturers and the cause of much research and development work in the wool industry.

Morphological structure of wool

A merino wool fiber, *viewed* under the scanning electron microscope is shown in Figure 1. Raw wool contains 25-70% by mass of impurities. These consist of wool grease, perspiration products (suint), dirt and vegetable matter such as burrs and seeds (Rippon 1992; Garner 1967; Pearson et al. 2004; Glaser 1996). The s e impurities are removed by specific processes (scouring and/or carbonizing) that will be further explained ahead in section 1.2 (Pearson et al. 2004; Lewis 1992). The wool discussed in this chapter is the fibrous material from which the surface contaminants, described above, have been removed.
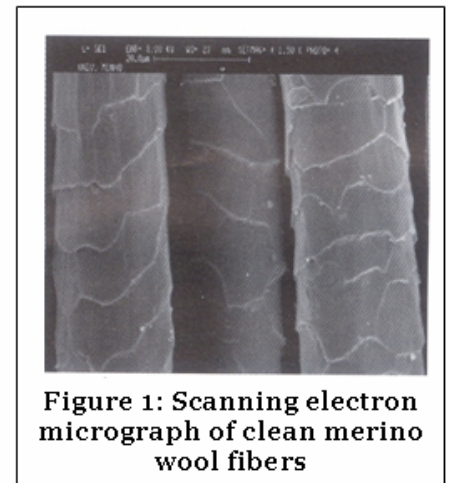

Figure 1: Scanning electron micrograph of clean merino wool fibers

Wool is an extremely complex protein, *evolved over* millions of years for the protection of warm-blooded animals in a great *variety* of climates and conditions. Wool is produced in the fiber follicle in the skin of the sheep. Because of the multitude of variations possible in, for example, the diet, breed and health of the sheep, as well as the climate, wool fibers *vary* greatly both in their physical properties, such as diameter, length and crimp, as in their chemical composition Wool consists principally of one member of a group of proteins called keratins (Hughes et al. 2001; Fiadeiro et al. 2000; Hogg et al. 1994). Keratin fibers are not chem ically homogeneous; they consist of a complex mixture of widely different polypeptides (Rippon 1992). Despite the classification of wool as a keratin, clean wool in fact contains only approximately 82% of the keratinous proteins, which are characterized by a high concentration of cystine. Approximately 17% of wool is composed of proteins which *have* been termed nonkeratinous, because of their relatively low cystine content (Rippon 1992). The wool fiber also contains approximately 1 % by mass of non proteinaceous material; this consists mainly of waxy lipids
plus a small amount of polysaccharide material. The nonkeratinous proteins and lipids are not uniformly distributed throughout the fiber but are concentrated in specific regions of the structure (Rippon 1992). Their location and their importance in determining the behaviour of wool are discussed later in this section.
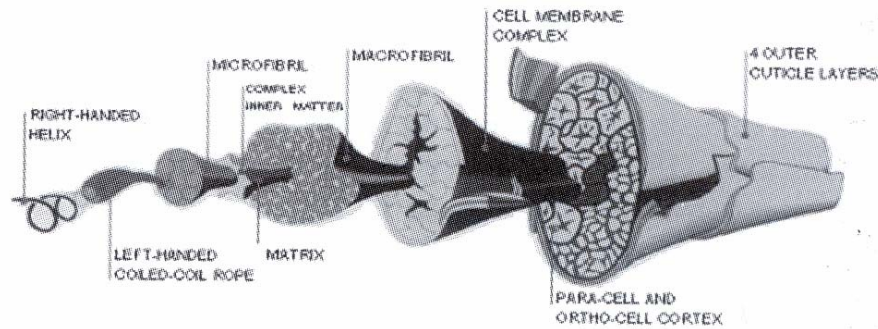
Figure 2 : Cross-section diagram of a merino wool fiber showing the structure at progressive magnifications

The substructure of the cuticle cells is directly relevant to felting, friction and shrink proofing processes. The epicuticle, which constitutes about 0.25 percent of the total mass of the fiber, is *very* inert chemically, being resistant to acids, oxidising and reducing agents, enzymes, and alkalis (Makinson 1979; Negri et al. 1993). This membrane does not form a continuous sheet *over* the whole fiber, but *covers* the outer surface of each cuticle cell (Naik 1994). The epicuticle membrane is raised in the form of characteristic bubbles or sacs (Allworden bubbles) when the fiber is immersed in aqueous chlorine solutions (Makinson 1979; Rippon 1992).

The epicuticle is known for its hydrophobicity, probably due to the lipid component bound to the membrane (Negri et al. 1993). The resistance of the surface membrane is thought to be due to the naturally occurring covalent isopeptide crosslinks as well as ro *covalent* attached lipid, predominantly 18-methyleicosanoic acid (Naik 1994; Negri et al. 1993; Brack et al. 1999; Swift and Smith 2001; Heine and Hocker 1995). This fatty acid is *covalently* boLt'd to the protein matrix *via* cysteine residues, forming a layer that can be *removed* by treatment with alcoholic alkaline or chlorine solutions in order to enhance many textile properties such as wetability, dye uptake and polymer adhesion (Negri et al. 1993; Brack et al. 1999).
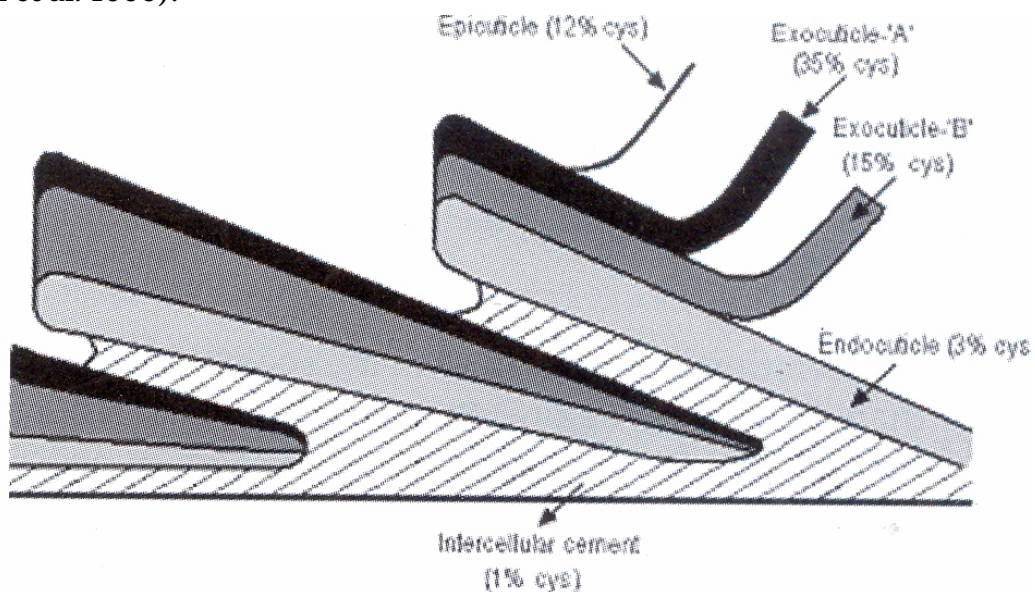

Figure 3: **Schematic scale structure of the cuticle showing the major components**

Thirty-five percent of the exocuticle A-layer is made by cystine residues, and in addition to normal peptide bonds, the cuticle is crosslinked by isodipeptide bonds (E-(Y-glutamyl)lysine) (Naik 1994; Rippon 1992; Heitle and Hocker 1995). The A and Blayers are both resistantto boiling in diluted hydrochloric acid and to trypsin digestion; however they can be solubilised by trypsin treatment after oxidation or reduction. The endocuticle is preferentially attacked by proteolytic enzymes, and readily degraded in diluted boiling hydrochloric acid (Naik 1994; Sawada and Ueda 2001).

Therefore, as pointed out by several authors, wool cuticle forms a diffusion barrier to chemicals and other treatment agents (Naik 1994; Schafer 1994; Nolte et al. 1996). This diffusion barrier (to dye molecules, for example) is mostly due to the hydrophobic character of the exocuticle A-layer, caused by the large amount of disulphide crosslinks and the bound lipid material. Consequently, the fiber pre-treatment processes modify mainly the composition and morphology of the wool surface (Brack et al. 1999; Millington 1998; Pascual and Julia 2001).

Felting and Shrinkage

One of the intrinsic properties of wool, that is peculiar to wool only, is its tendency to felting and shrinkage. Under certain conditions, such as moisture, heat and mechanical agitation, wool shrinks, basically due to its morphological and scale structure. There are two kinds of shrinkage: relaxation shrinkage and felting shrinkage. Relaxation shrinkage describes the shrinkage which appears during production when fabrics are subjected to more or less strong mechanical tensions in warp or in weft direction. In finished garments these tensions can still be present either completely or partially. If such a garment gets wet either during wearing or washing, these tensions are loosened and the garment shrinks.

The anti-felt finishing does not remove relaxation shrinkage. Therefore, to obtain a lower relaxation shrinkage the garment must be processed with as less tension as possible or it must be relaxed by corresponding finishing processes. Felting shrinkage describes the shrinkage of garments due to the felting of wool fibers. Legends state that the felting of wool was discovered by a mediaeval saint who packed wool or fur into his shoes to ease his blistered feet and subsequently found that it had matted into a fabric. Only keratin fibers, grown on animals from their skin, can be induced to felt. This is because a directional surface structure is provided by the scales (the cuticle scales are arranged towards the fiber tip) which occur on all animal fibers but are not present on vegetable or man-made fibers (Makinson 1979).

Hence, the friction of a wool fiber in the scale direction is lower than the friction against the scale direction. There are different theories concerning the origin of wool felting (Heine 2002). The hydrophobic character and the scaly structure of the wool surface are the main factors causing the differential frictional effect (D F E) resulting a II fibers to move to their root end when mechanical action (such as moisture, heat, and pressure) is applied in the wet state (Makinson 1979; Chi-wai et al. 2004; Hocker 2002). The felting changes not only the garment dimensions but its look as well. The woven or knitted structure becomes less visible, and the garment becomes thicker and less elastic.

Conventional Finishing Processes for Wool Fiber

A variety of processes are available to improve the appearance, handle, performance and durability of the wool fabrics. Before the more specialized finishing processes are applied,

fabrics usually require cleaning (scouring) to remove warp sizing, oils, other additives and dirt. Processes such as bleaching and dyeing are known as wet-finishing processes, since the fabric is exposed to bleaches or dyes in aqueous solutions. Treatment of fabric with particular resin systems can improve crease and shape resistance.

These are examples of chemical finishing processes, which depend upon the ultimate use of the textile. (Glaser 1996). Some of the finishing processes in" which enzymes can be employed will be described later (section 1.4). In common with almost all other manufacturing activities, wool processing has the potential to cause environmental damage that should be eliminated or minimized for wool to maintain its naturalness. Pollution is a concern in several areas of wool finishing.

In a study performed by the International Wool Secretariat (lWS), four high priority areas were identified (Shaw 1996)

- Pesticide residues in wastewater from chemicals applied to sheep.
- Discharge of mothproofing agents from wool carpet manufacture.
- Emissions of halo-organics from wool shrink proofing.
- Chromium releases from chrome dyeing operations.

The finishing processes that may be carried out on wool prior to commercialization are discussed bellow. Such processes include scouring, carbonizing, bleaching, dyeing, antimicrobial finishing and shrink proofing.

Enzymes

Protein-degrading enzymes dominate the market, accounting for approximately 40% of all enzyme sales (Godfrey and West 1996; Sharma et al. 2001; Gupta et al. 2002). Proteases have found new applications but their use in detergents is still the major market.

Enzymes are categorized according to the compounds they act upon. As in the development of organic chemistry, many enzymes were given "trivial" names before any attempt was made to create a system of nomenclature. Some of the most common include: proteases which break down proteins, cellulases which break down cellulose, lipases which split fats (lipids) into glycerol and fatty acids, and amylases which break down starch into simple sugars. The present-day accepted nomenclature of enzymes is that recommended by the Enzyme Commission (EC), which was set up in 1955 (lUB 1992; Price and Stevens 1999; Fornelli 1995). Proteolytic Enzymes

Proteases have been used in food processing for centuries, like rennet obtained from calves' stomachs used traditionally in the production of cheese and papain from the leaves and unripe fruits of the pawpaw used to tenderize meats (Chaplin and Bucke 1990). Proteolytic enzymes catalyse the hydrolysis of certain peptide bonds in protein molecules, as already mentioned in the previous section.

The classical antifelting treatments for wool assume a chlorine treatment with polymer deposition, which has many ecological drawbacks, as well as some handling and durability disadvantages. Although many attempts have been made to replace this process by an environmental friendly one, the unacceptable weightloss caused by proteolytic attack eliminates the potentiality of these enzymatic finishing methods.

The aim of the present work is to develop an enzyme-based antifelting treatment for wool. The specific objectives intend to increase the molecular size of proteases in order to reduce their diffusitivity on wool, thus controlling the proteolytic attack. The methods used for that purpose include increasing molecular size of proteases with normal crosslinking agents of proteins agents like glutaraldehyde and covalent attachment to soluble-insoluble polymers of high molecular mass. This strategy represents a new approach for the study of the proteolytic finishing of wool, allowing overcoming some of the most important drawbacks that the previous attempts were facing, such as the penetration of protease inside the wool fiber and the controllability of the enzyme.

Conclusion

Enzymes can often replace chemicals or processes that present safety or environmental issues. Surface modification of wool is currently attracting considerable attention. Such modifications seek to obtain the optimum level of beneficial effects by the modifying only the fiber surface. Among the most promising treatments for surface modification of wool is an enzymatic treatment. The application of proteases for surface modification of wool fibers is a challenging task and has not yet been accomplished in a fully satisfying manner. The major problem concerning this enzyme/substrate system is that the action of these enzymes so far could not be limited to the cuticle of the wool fiber. Full penetration and fiber damage has been the consequence.

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