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Comparison of Surface Modification of Wool Fibres Using Pronase, Trypsin, Papain and Pepsin

Abstract
Nowadays the uses of enzymes in textile industries are being developed because of their harmless effluents and good effectiveness. One of these uses is the shrink proofing of wool fabrics using proteolytic enzymes. In this research, some proteolytic enzymes, such as pronase, trypsin, papain and pepsin were used to treat wool fibers in optimum conditions for 30, 60 and 120 minutes. Afterwards, the effectiveness of these enzymes on the surface of wool was studied by scanning microscopy (SEM). Comparison of resulting micrographs showed that papain is more proteolytically efficient for wool fiber morphology.

Key words: *proteolytic enzymes, SEM, wool, morphology.*

Introduction

Wool is a complex natural fiber composed mainly of proteins (97%) and lipids (1%), with a heterogeneous morphological structure [1]. It can be regarded as a composite material mainly made up of keratinous proteins. Wool fibers have a shape similar to that of elliptical cylinders, with an average diameter ranging from 15 μm to 50 μm and length determined by the rate of growth of the wool and the frequency of sheering [2]. Wool and other keratin fibers consist of two major morphological parts: the cuticle layer (usually referred as the scale layer of wool), which is composed of overlapping cells that surround the cortex (the inner part of the fiber). Cuticle and cortical cells are linked to one another by the cell membrane complex (CMC). The cortex is comprised of spindle-shaped cortex cells that are separated from each other by a cell-membrane complex, which consists of non-keratinous proteins and lipids [2 - 6].

The cuticle cells are located on the outermost part of the fiber surrounding the cortical cells forming a layer of flat scales (about 1 μm thick) overlapping one another [6 - 8]. They comprise 10% of the total weight of the wool fibre [9]. Cuticle cells are composed of three distinct layers, as shown in **Figure 1** [4]. The outermost layer is the outer resistant surface membrane (epicuticle); the next layer from the surface of the cells is called the exocuticle, which is subdivided into two main layers (A and B layer) that differ mainly in the cysteine content. Finally, the end cuticle is the cuticular layer nearest the cortex [1, 3, 4, 8].

The substructure of the cuticle is directly relevant to felting, friction and shrink proofing processes. The epicuticle consists of an outermost monolayer (F-layer) (N25% by mass) of fatty acid and a protein matrix of 75% by mass. The fat is covalently bound to proteins by means of ester or three-ester bonds, the 18-methyleicosanoic acid (18-MEA) being the main component (~65% by mass). Fatty acids are oriented away from the fiber to produce a Polyethylen-like layer on the fiber surface, thus making the epicuticle hydrophobic and resistant to the attack of different agents [10].

This is why wool is known to develop hydrophobic grease during aqueous scouring or solvent extraction. This layer of fatty acid can by removed by treatment with alcoholic or chlorine solutions in order to enhance many textile properties, such as wetting ability, uptake and polymer adhesion [6].

One of the intrinsic properties of wool, which is peculiar to wool only, is its tendency to felt and shrink. 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 [2]. Hence, the friction of a wool fiber in the scale direction is lower than the friction against the scale direction. The hydrophobic character and scaly structure of the wool surface are the main factors causing the differential frictional effect (DFE), resulting in all fibers moving to their root end when a mechanical action (such as moister, heat, and pressure) is applied in a wet state [2].

The shrinkage behavior of wool can be regulated to a greater or smaller degree by various chemical means, but there are a number of drawbacks which make the

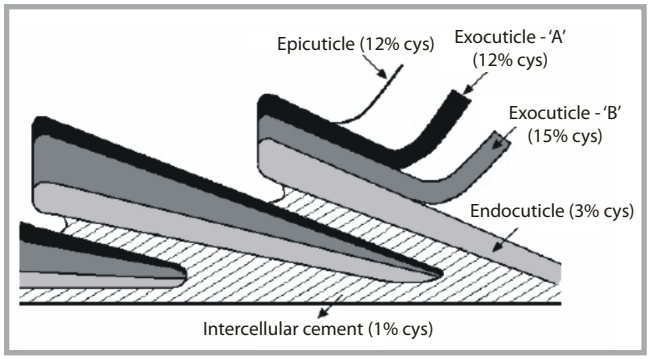

***Figure 1.** The cortex structure.*

search for an ecologically clean alternative worthwhile: limited durability, poor handling, the yellowing of wool, difficulties in dyeing, and the most important today, the environmental impact (Like the release of absorbable organic halogens-AOX into the effluents)[11].

The development of clean technologies, such as enzymatic finishing processes, is a priority. Biotechnology applied in the textile industry with the use of enzymes has already contributed to a reduction in energy costs and also a reduction in pollutant emissions into the environment [12].

Today the most promising method of modifying a wool surface are low – temperature plasma (LTP) and enzymatic treatments, which are considered environmentally acceptable and produce considerable energy savings, compared with those of conventional finishing methods. Nowadays, the use of enzymes to achieve wool shrink resistance, better whiteness and improved handle are also successfully employed in wool bleaching [13]. Enzymes are employed as auxiliary agents in wool dyeing [13, 14], and as modifiers of wool handle by reducing wool fiber stiffness and prickle [15].

Proteolytic enzymes are capable of attacking natural keratin, hydrolying some peptide linkages. In this study various proteolytic enzymes (papain, pepsin, trypsin and pronase) were applied to wool fibers, and their effects on wool morphology were studied by electron microscopy.

Materials

Merino wool top with an average fibre diameter of 26 μm. Enzymes: papain (roche), Trypsin (Sigma), Pepsin (Sigma) Pronase (sigma).

Methods

Prewashing

Before enzyme treatment the wool fibers were subjected to pre-washing under the following conditions: liquor composition – SDS (anionic detergent, sigma): 1 gl^{-1}; 25% ammonia 0.7 gl^{-1}.

The pre-washing was performed for 25 min within 15 min of heating the composition to 90 °C. Afterwards, the wool was rinsed and cooled simultaneously for another 30 min.

Prepration of buffers

Since each of the enzymes acts/reacts in certain conditions, the buffer systems presented in ***Table 1*** were prepared for optimum pH.

Enzyme treatment

100 mg of wool top was washed as before and put on a flask which contained 10 cm^3 buffer and 5mg enzyme.

Table 1. *Bufferic systems.*

Enzyme	Buffer	pH
Papain	Citrate/phosphate	6
Trypsin	Phosphate	8
Pepsin	HCl/KCl	2
Pronase	Citrate/phosphate	7

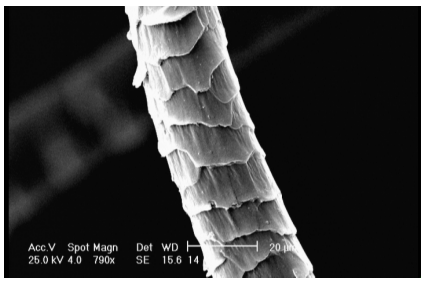

Figure 2. *SEM photograph of an untreated wool fibre.*

At the optimum temperature for each enzyme, the flasks were shacked at 60 r.p.m. in a water-bath shaker for 30, 60 and 120 minutes.

After washing

After enzyme treatment for denaturing and deactivation of enzymes, samples were sunk in boiling water for 10 minutes and then rinsed with tap water and dried in air.

Micrographs

Treated and untreated wool fibers were coated with gold and micrographs prepared by SEM.

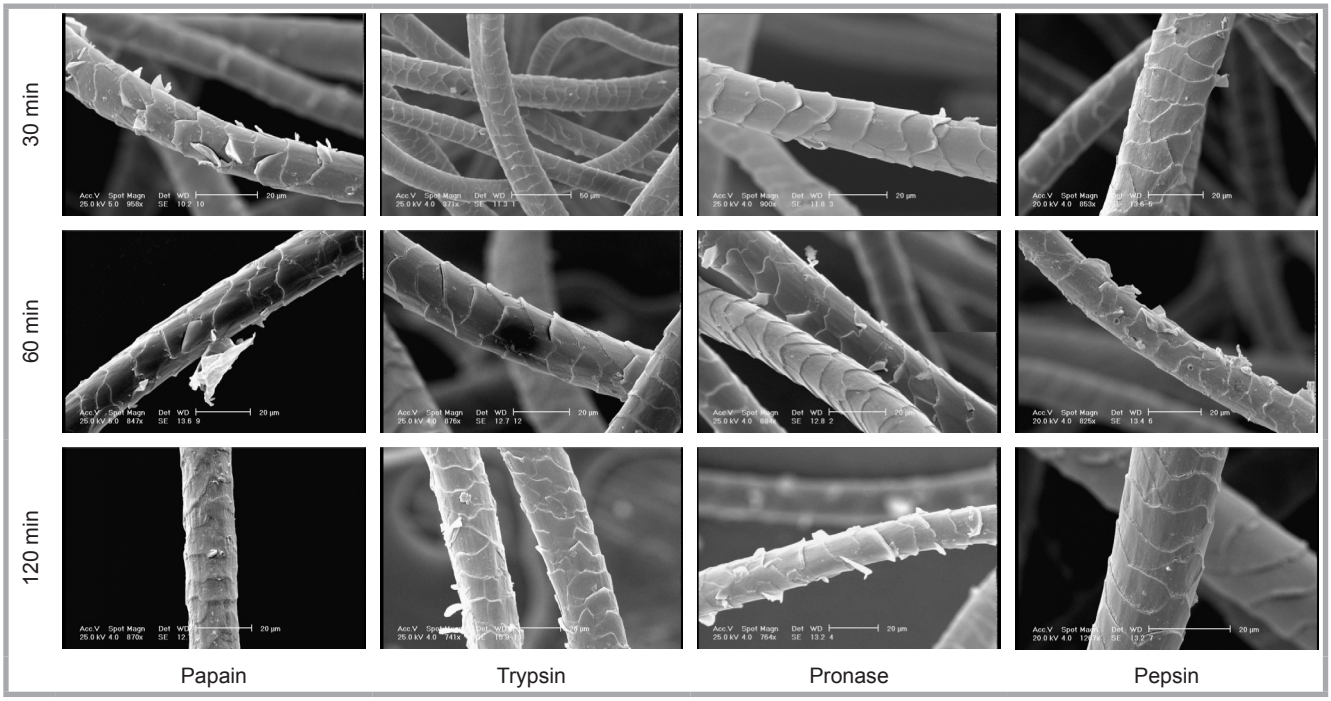

Figure 3. *SEM micrographs of the enzyme (Papain, Trypsin, Pronase and Pepsin) treatment wool fibres over different time (30, 60 and 120 min).*

Results and discussion

The surface appearance of an untreated wool fiber is presented as an SEM photograph (***Figure 2***, see page 91). It can be observed that the untreated fiber has flat scales with well-defined scale edges characteristic of wool. The boundaries which separate neighboring auricular cells are clearly resolved.

Figure 3 (see page 91) shows micrographs for the enzyme treated wool fibers. The micrographs show that the edges of the first layer of the overlapping scales of the papain treated fibres rose within half an hour. After one hour of treatment, the first layer was completely destroyed, and the next layer of scales were attacked by the enzyme.

In two hours the scale layers were completely destroyed and impart a smooth surface to fiber without any scales. After half an hour and one hour treatments of the wool fibers with trypsin, there were not any clear observations. However, for longer periods (more than two hours) the effects of the trypsin attacks on the scales are presented. Apparently, mechanical shaking during enzyme treatment causes the destroyed scales to depart from the wool fibers.

The effect of pronase started after half an hour and in two hours the scales had risen from the surface of the fiber. However, the scale removal was not complete, and more time was required.

In the case of fibers treated with pepsin, attacking had begun after half an hour, and after two hours the scale layers had risen. Shaking during treatment removed scales and made a smooth surface on the fibers.

With respect to enzyme activity, it can be concluded that papain has a stronger effect on wool surface.

Conclusions

This work compared the effect of a number of proteolytic enzymes on wool fiber morphology; however, it should be continued by to identify more effective enzymes. Examination of other proteases, optimisation of enzymatic reaction media, and screening for microorganisms which are able to produce potent proteases could be useful.

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