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THERMOANALYTICAL CHARACTERISATION OF MODIFIED KERATIN FIBRES

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Abstract

In the wool textile industry, several processes serve to improve the commercial properties of the fibres such as fineness, softness, length, strength and lustrous. For example, wool is chemically treated with reductive agents then stretched and set. This leads to modifications of the original protein structure causing changes in thermal behaviour, dyeing, colouristic and wet resistance properties. A multidisciplinary approach was used to investigate treated and untreated wools, with the aim of exploiting the nature of the structural changes. SEM and TEM revealed changes on the cuticle and cortical cell morphology; structure modification were studied by FT-IR and DSC.

Keywords: DSC, electron microscopy, keratin, melting, thermal denaturation, wool

Introduction

The composite structure of keratin fibres, such as sheep's wool and other animal hairs, although subjected to species-specific differences, is characterised by the presence of three main morphological components, namely the cuticle, the cell membrane complex and the cortex. The cuticle consists of a thin layer of flat overlapping 'cuticle cells' surrounding the cortex, which is made up of spindle shaped 'cortical cells' arranged in the direction of the fibre axis. The cell membrane complex, sometimes referred to as intercellular cement, performs the function of cementing cortical and cuticle cells together [1]. Within each cortical cell, microfibrils are embedded in a matrix containing two non-filamentous protein types, one cystine rich (high-sulphur proteins) and the other rich in glycine and tyrosine [2]; microfibrils, are composed of multiple α-helical, closely packed, low sulphur subunit, referred to as intermediate filaments (IFs), arising from the interaction of Type I and Type II keratins, a subclass of the larger group of structural proteins called cytokeratins which make up the cytoplasm of mammalian cells. The molecular ordered packing of α-helices into the microfibrils is identified as the crystalline fraction of the fibre [3–5]; all keratin from mammals contains α-form crystallites, whereas both α- and β-forms occur in

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keratin from birds and reptiles. Stretching fibres under certain conditions leads to the unfolding of the α-helices to make a β-pleated secondary sheet structure [6].

Differences in the cystine (sulphur) content lead to the identification of two types of cortical cells: *ortho*-cortical cells, containing a lower proportion of high sulphur matrix material, and *para*-cortical cells, with a higher concentration of disulphide linkages, belonging to two distinctive domains called respectively the *ortho*-cortex, more hydrophilic and heavily stained by some dyes, and the *para*-cortex, more readily stained by silver salts [1].

Arrangement and proportion of *ortho*- and *para*-cortex in fibre cross sections vary from one fibre to another: i.e. fine Merino wool shows a bilateral arrangement, Lincoln wool shows a cylindrical arrangement, Mohair consists of predominantly *ortho*-cortex [7]. Moreover, while microfibrils do not appear to differ from one keratin to another, important differences in composition between keratin fibres seems to be mainly due to differences in the amount and type of high-sulphur proteins from the matrix, which should not be regarded as completely amorphous, but must have a certain structure [3].

Some modern industrial textile processes serve to improve the commercial characteristics of wools, by means of chemical and mechanical treatments that reduce diameter and increase the length, strength and lustrous of the fibres. Thus, cheap sheep's wools became as fine as the more expensive Cashmere, as lustrous as Mohair (the hair of Angora goats) or Cashgora (the crossbreed of Cashmere or feral goat with Angora goat) and as 'silky' as silk. Although process parameters are not revealed due to patent rights, most industrial treatments consist of chemical reduction of disulfide links by sodium bisulphite, steaming and mechanical stretching involving cleavage of hydrogen bonds, followed by quick cooling in order to set the modification imparted to the fibres. This implies strong modifications of the original protein structure that largely determine the mechanical and other physical performances of the fibres, causing changes in thermal behaviour, dyeing and colouristic properties and wet resistance due to modified water-fibre interactions.

Thermal analysis represents a powerful tool to investigate the fine structure of complex materials such as wool and hairs. Fundamental relationships were found between thermal behaviour of the main morphological components, fibre properties and associated performances. Moreover, differential scanning calorimetric (DSC) traces of virgin wool and hairs, show thermal events typically related to the histological structure of the original fibres. This is sometimes useful for fibre-species identification purposes [8]. In this work, untreated and industrially treated wool samples were characterised by a multidisciplinary approach using DSC, Fourier Transform Infra-Red Spectroscopy (FTIR) with Attenuated Total Reflectance technique (ATR), Transmission and Scanning Electron Microscopy (TEM and SEM) and Energy Dispersive X-ray Microanalysis (EDXS), in the attempt of investigating type and amount of structural changes occurred in treated wools and how treated and untreated wools can be easily identified by means of thermal analysis.

Experimental

Material and methods

Merino wool tops before (19.3 μm) and after (16.9 μm) an industrial chemical reductive treatment with aqueous solution of sodium bisulphite, combined with mechanical twisting and stretching, were kindly supplied by Woolmark Italy s.r.l.; process parameters are covered by patent rights.

Mohair and Silk were withdrawn from the Scientific Archives of the Italian National Research Council. Fibre samples were cleaned with petroleum-ether for 2 h in a Sohxlet, then dried and conditioned in standard atmosphere at 20°C, 65% R.H. for 24 h.

Microscopic investigation, SEM investigation was performed with a LEO (Leica Electron Optics) 135 VP SEM, at an acceleration voltage of 15 kV and a 30 mm working distance. The fibres were mounted in aluminium specimen stubs with double-sided adhesive tape and sputter-coated with a 20 nm thick gold layer in rarefied argon, using an Emitech K 550 Sputter Coater, with a current of 20 mA for 180 s.

For TEM investigation, fibres were cut into smaller pieces (≈1 mm), washed three times in 0.1 M Cacodylate buffer (pH 7.2) for 1 h, fixed in 6% glutaraldehyde in 0.1 M Cacodylate buffer (pH 7.2) for 24 h at 4°C, rinsed three times for 20 min each in a cold Cacodylate buffer and then soaked for five days in dark ammoniacal silver nitrate solution 0.1 M (pH 10), dehydrated and infiltrated with Poly/Bed 812 resin (Polysciences, Inc., Warrington, PA) for 5 h at room temperature by using a rotator, embedding overnight at 48°C and for 48 h at 60°C. The ultra-thin sections (≈90 nm) were obtained by Leica AG Ultracut ultramicrotome with a diamond blade. The sections were picked up on a 3.05 mm 300 mesh nickel grid and examined in a Zeiss LEO 900 TEM operating at 80 kV.

For EDXS, after embedding wool samples in Poly/Bed 812 resin, according to the method explained above, the plastic blocks were trimmed with a steel razor blade into a four-sided pyramids with a flat top and shadowed with carbon in an Edwards 306 Vacuum Coater equipped with a twin hearth electron beam source. Sulphur levels were quantified by focusing the electron beam onto selected regions using an EDXS Oxford INCA 300 Microprobe, with an acceleration tension of 10 kV, working distance of 15 mm and analysis live time of 100 s.

Physical properties were determined in conditioned standard atmosphere at 20°C, 65% R.H. The measurement of the fibre fineness was performed with a BSC Optical Fibre Diameter Analyser (OFDA) on about 10,000 fibres per sample according to IWTO TM 47-00 [9].

Tensile properties were measured on 100 fibres per sample, with an Instron AMTS Series IX Dinamometer, according to EN-ISO 5079 [10].

FT-IR spectra with attenuated total reflection technique (ATR), were obtained by using a NICOLET NEXUS Infra-Red Spectrometer. Spectra of untreated and treated wool, dried at 105°C for 1 h, were obtained from 4000 to 650 cm^{-1}, using 100 scans at a resolution of 4 cm^{-1}. The spectra were baseline corrected, smoothed by the Savitsky-Golay method (9 points) and fitted with Gaussian band shapes by an iterative curve fitting procedure.

Thermal analysis, DSC was performed with a Mettler Toledo DSC 821, flushing the calorimeter cell with 150 mL min^{-1} of nitrogen. The temperature program was set in the range from 30 to 300°C, at a heating rate of 10°C min^{-1}, and the instrument was calibrated with indium as a standard. The data were collected on a computer using the Mettler Toledo Star System. About 3 mg of fibres were used in each test, cut into a few thousand snippets of 1 mm length by a hand microtome to optimise the heat transmission to the crucible. All tests were repeated three times, and the repeatability of the DSC traces was even confirmed.

DSC curves were differentiated to the second order derivative in order to resolve the peaks. The peaks areas, evaluated for Mohair, untreated and treated wool, were determined in the second order derivative traces using the ORIGIN 7.0 software.

Results and discussion

The surface morphology of sheep's wool, untreated and treated as described in the experimental part, is shown in the SEM micrographs Figs 1 and 2.

Stretching in plastic condition causes a strong modification of the cuticle cells shape that became thinner and elongated in the direction of the fibre axis, somewhat similar to those of mohair as shown in Fig. 3, improving the surface smoothness, thus the lustrous of the fibres.

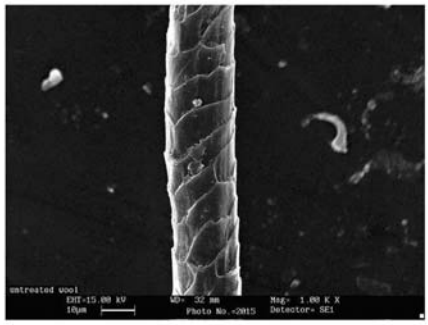


Fig. 1 Untreated wool, longitudinal view (SEM 1000×)

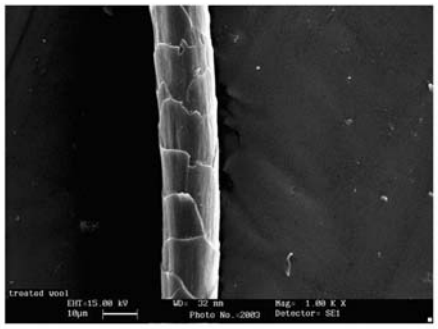


Fig. 2 Treated wool longitudinal view (SEM 1000×)

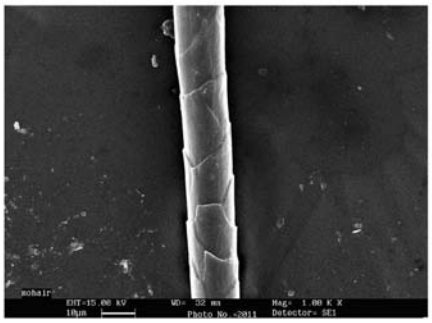


Fig. 3 Mohair longitudinal view (SEM 1000×)

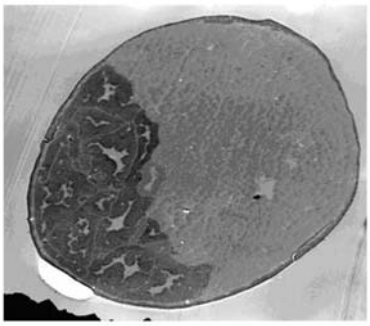
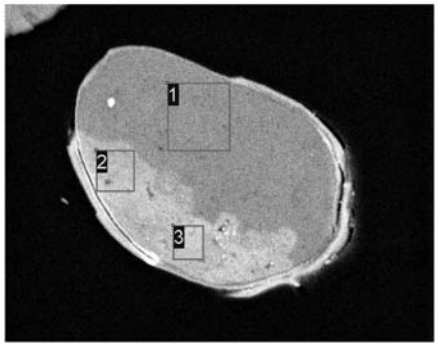


Fig. 4 Treated wool, cross section (TEM 5000×)

Also the fibre cross section change, from circular-elliptical of untreated wool to polygonal; however, the *ortho*- and *para*-cortex domains reveal the unchanged bilateral structure, with *para*-cortical cells more intensely stained by silver nitrate (TEM micrograph Fig. 4) due to the higher sulphur concentration, that was measured by EDXS analysis and reported in Fig. 5 (note that the image of Fig. 5 was made in back scattered electrons thus the lighter the area the higher the silver and the sulphur



Processing option: All elements analyzed (Normalised)

Spectrum	C	O	S	Ag	Total
1	68.52	27.09	1.29	3.10	100.00
2	67.11	26.63	1.91	4.34	100.00
3	65.29	28.16	2.05	4.50	100.00
Max.	68.52	28.16	2.05	4.50	
Min.	65.29	26.63	1.29	3.10	

All results in Atomic Percent

Fig. 5 Treated wool cross section (SEM-Backscattered 3300×)

concentrations). The fibre diameter decreases from an average value of 19.3 μm (standard deviation: 4.1 μm) to 16.9 μm (st. dev. 3.8 μm), while the shape of the fibre diameter distribution does not change as a consequence of the treatment.

Tensile properties are modified by the action of reductive agents, stretching and setting, as shown in Fig. 6: the reduction of load at break, elongation at break, increase of the tenacity peak and initial elastic modulus, are reflected in a small general loss of fibre resilience and softness.

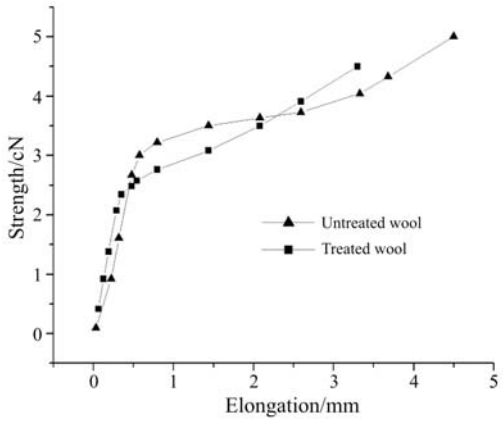


Fig. 6 Tensile behaviour of treated and untreated wools

Infrared spectroscopy is a technique widely employed to determine protein secondary structure [11, 12]. While many vibrational bands give information on protein structure, the amide I absorption (1700–1600 cm^{-1}) is widely used in studies of protein secondary structure. This vibrational mode, that originates primarily from the C=O stretching vibration, consists of several overlapping bands. The Gaussian curve-fitting procedure allows the identification of overlapped bands and their assignment to α-helical, β-sheet, disorder and turn structure in proteins.

The manipulated spectra of untreated and treated wool were curve fitted in the 1720–1580 cm^{-1} region using the method of Marquardt [13–15].The absorption bands that comprise amide I band are shown in Figs 7 and 8 and described in Table 1.

In the untreated wool spectrum, the absorption band at 1651 cm^{-1} is characteristic of α-helical structure, the bands at 1690, 1680, 1668 cm^{-1} are assigned to various types of β-turns and disorder structures. The two bands at 1631 and 1615 cm^{-1} are in the typical range of β-sheet structures. The large intensity of the band at 1631 cm^{-1} is due to the greater molar absorptivity of β strands, which is 1.6 times larger than that of α-helices [16].

When the wool sample is stretched, the fitting of amide I absorption shows bands primarily related to β-sheet structures. The α helix band at 1651 cm^{-1} disappears and the major absorption band at 1645 cm^{-1} is from parallel β-sheet structures.

Substantial modifications involve the microfibril-matrix structure, as shown in Fig. 9, where the DSC trace in the dry state of the treated wool is compared with those of the untreated one.

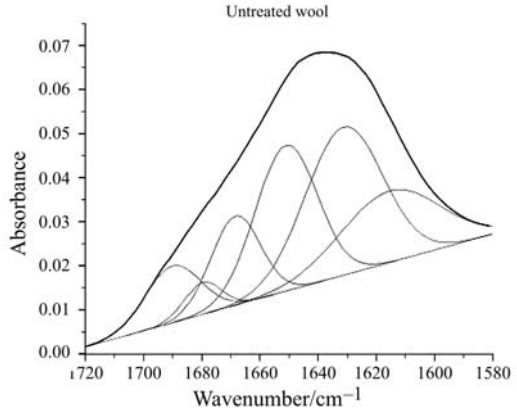


Fig. 7 Curve-fitted amide I region of untreated wool

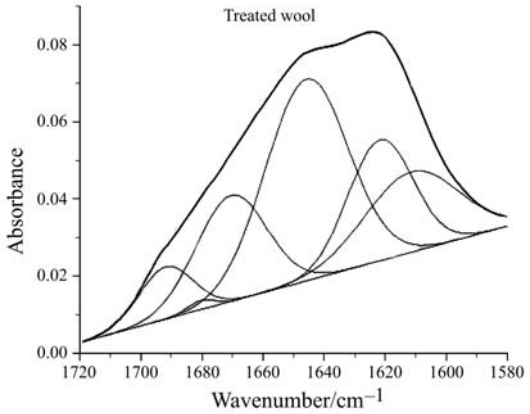


Fig. 8 Curve-fitted amide I region of treated wool

Table 1 Amide I absorption bands of untreated and treated wool

Band/cm^{-1}	Band assignment	Band/cm^{-1}	Band assignment
untreated		Treated	
1690	β-turn	1692	β-turn
1680	β-turn/Disorder	1680	β-turn/Disorder
1668	β-turn/Disorder	1670	β-turn/Disorder
1651	α-helical	1645	β-sheet parallel
1631	β-sheet	1622	β-sheet parallel
1615	β-sheet	1611	β-sheet

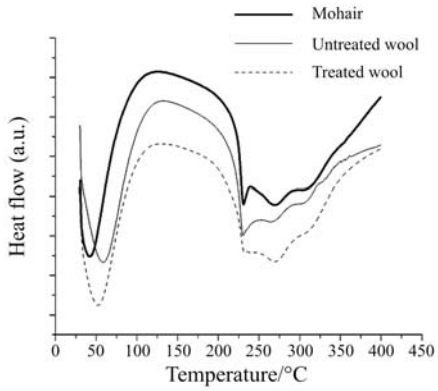


Fig. 9 DSC of mohair, untreated wool and treated wool

Apart for the large endotherm due to water evaporation, with temperature peak at around 60°C, a characteristic thermal events takes place in the temperature range 230–255°C, with a bimodal endothermic peak (the presence of a shoulder in the higher temperature side of the trace). This behaviour has been mainly explained by two principal theories: one attributes the doublet to differences in the thermal denaturation of the α-form crystallites with respect to the degradation of other histological components, namely the matrix [17–21]; the other attributes the bimodal trace to differences in the transition enthalpy of the α-helical material in the domains of *ortho*- and *para*-cortical cells [22, 23].

Melting or denaturation of other histological components are observed around 270°C for both untreated and treated wool.

Nevertheless, even though the bimodal trace in the temperature range 230–255°C is no longer univocally explained, the area under the first peak, that represents a measure of the relative α-helix content (only in the *ortho*-cortex by the *ortho-para* hypothesis), can be used as a measure of the extent of the structural modification caused by the industrial treatment

The DSC trace of the treated wool sample shows a decrease of the area under the first peak, due to the transition of the α-form crystallites in β-pleated sheet structures, similar the those found in silk fibroin and in feather keratins. The peak areas of mohair, untreated and treated wool, calculated in the second order derivative and normalized, were compared assuming mohair (Fig. 10), which has the highest α-helix content among the animal fibres and contains prevalently *ortho*-cortex, as a reference for 100% α-helix [24]; the result shows a residual α-helix content of 44%, meaning that the fibres were subjected to treatment conditions (temperature, time, elongation) not strong enough to cause the complete α-β structural change, as referred to in the technical literature [25].

Explaining the structural modification on the basis of the *ortho-para* hypothesis [22, 23], the treatment should be effective to a limited degree to the *ortho*-cortex, while microfibrils from the *para*-cortex, that are less densely packed into a higher

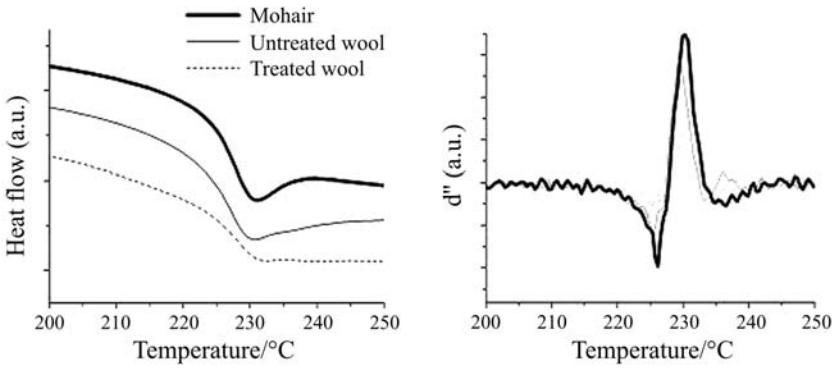


Fig. 10 DSC (A) and second order derivative (B)

amount of high sulphur matrix, seem to be more thermally stable, thus practically unaffected by the industrial process.

Conclusions

Chemical reductive treatments combined with mechanical stretching, carried out during industrial textile processing with the aim of improving the commercial characteristics of wools, cause substantial modification of the morphological and mechanical properties of the fibres. A multidisciplinary approach revealed elongation of the surface cuticle cell, changes of the fibre cross section and keratin structure transitions, mainly reflecting a general loss of softness related elastic properties. As a general result, fibres become finer, more lustrous and silky with respect to untreated wools, but a little more droopy. DSC analysis enabled the identification of treated wool as well as the evaluation of the severity of the structural changes that occur in the process under investigation.

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