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DIFFERENTIATING ALPACA FIBRES BY SCANNING ELECTRON MICROSCOPY AND ENERGY DISPERSIVE SPECTROMETRY

S. Shim and K. A. Jakes[1]

[1]Department of Consumer Sciences, The Ohio State University, Columbus, Ohio 43210, U.S.A.

ABSTRACT

The research reported describes a method proposed to distinguish the fibre from two breeds of alpaca, the Huacaya and the Suri, using scanning electron microscopic examination of cross-sections etched with cold plasma. Sulphur distribution in the cross-sections, obtained by energy dispersive spectroscopy, support the distinctions made between the fibre types. Cell size, appearance, and fibre shrinkage indicate that Huacaya is composed of two cell types, while Suri is composed of one in the sample fibres examined. Sulphur is unevenly distributed in Huacaya while it is more evenly distributed in Suri fibres. Applying these methods to a larger number of fibre may resolve controversies concerning the difference in fibre structure between the two breeds.

Key words: Alpaca, fibres huacaya, orthocortical cell, paracortical cell, scanning electron microscopy, suri

Two breeds of alpaca, the Huacaya and Suri, provide specialty hair fibre used in the textile industry. One readily apparent difference between these alpaca fibre types is in the crimp properties; the Huacaya exhibits a high amount of crimp and the Suri is more straight. Using the fine structure of sheep's wool (Birbeckand Mercer, 1957; Fraser *et al*, 1972; Swift, 1977; Jones *et al*, 1998) as a model for this research, the physical and chemical composition of the alpaca fibre cortical cells were examined.

Wool is a bicomponent fibre with bilaterally distributed ortho and paracortical cells; the orthocortex has a lower sulphur content and is stained darkly with dye while the paracortex has a higher sulphur content and exhibits lower dye take-up. The bicortical structure is linked to the fibre crimp because orthocortical cells are always found outside the curve of the fibre crimp (Fraser *et al*, 1972; Horio and Kondo, 1953; Mercer, 1953; Mercer, 1954). A third cell type, labeled the mesocortex, that is intermediate between the para- and orthocortex has been reported as well (Whiteley and Kaplin, 1977).

Each cortical cell is closely packed with macrofibrils (0.05-0.2 μm diameter) and these macrofibrils are aggregates of intermacrofibrillar matrix and microfibrils (7-8 nm diameter). The intermacrofibrillar matrix consists of high-sulphur, high-tyrosine proteinaceous material. The paracortex contains more densely packed macrofibrils, has a more parallel arrangement of microfibrils, and contains a larger matrix content compared to the orthocortex (Filshie and Rogers, 1961). Each cortical cell and cuticle cell is surrounded by a cell membrane complex (CMC), composed of a lipid membrane (β-layer) and proteinaceous intercellular material (δ-layer).

Without treatment the cortical cells in wool fibres are not readily visible using either light or electron microscopy. Common methods employed to aid in observation of the distinctions between the cells include dyeing with methylene blue (Horio and Kondo, 1953; Mercer, 1953; McCloghry and Uphill, 1997) for light microscopy or staining with osmium tetroxide, silver nitrate, silver methanamine or potassium permanganate for scanning electron microscopy (SEM) and transmission electron microscopy (TEM) (Jones *et al*, 1998; Swift, 1977; Kulkarni *et al*, 1971). These methods are limited by the possible alteration of the fibre structure in the dyeing process and the subjective judgment employed to define ortho- and para-cortical cells according to the relative amounts of stain take-up. In other approaches, cortical cells are separated through the destruction of the CMC using enzymes (Mercer, 1953; Kulkarni *et al*, 1971) or acid hydrolysis (Leach *et al*, 1964; Leeder and Rippon, 1982) before they are examined.

Cold plasma etching of embedded fibre cross-sections (Jakes and Mitchell, 1996; Swift, 1980) is an ideal method for examining the cells *in situ* without

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dyeing or alteration of the cells. High temperatures are not required thereby preventing thermal degradation of the fibre. In sheep's wool, components with lower sulphur content, such as the CMC and intermacrofibrillar matrix, are etched more rapidly than those with higher sulphur content, such as the macrofibrils (Swift, 1980), thus providing a controlled degradation of the boundaries between the cortical cells. Etching must be conducted with care, however, since continued plasma treatment will destroy the cell structures as well. In plasma-etched Merino wool fibres, orthocortical cells show macrofibrils more clearly than do paracortical cells. Paracortical cells are larger, and differ in general appearance from orthocortical cells (Swift, 1980).

Ortho and para cortical cells can also be differentiated by sulphur content. For example, Jones *et al* (1990) used TEM with a microprobe and a proton beam accelerator for sulphur X-ray mapping of cortical cells in sheep's wool. In this study, an energy dispersive spectrometer (EDS) attached to an SEM was used to create an elemental map along line scans of the cross-section of individual fibres thereby providing a semi-quantitative means to discern ortho-and para-cortical cells based on relative differences in sulphur content.

The literature is contradictory in reporting whether alpaca has a bilateral distinction between cortical cells (Jones *et al*, 1998; Hoffman and Fowler, 1997) or is primarily comprised of a single type of cell (Jones *et al*, 1998; Kidd, 1977). Dyeing fibres from a selected group of fleeces, Villaroel (1959) concluded that Huacaya is bicomponent and that distinction between the two cell types could be seen more clearly in finer and more crimped fibres. Suri did not dye well, and the cell structures were difficult to study. Holt (1996) agrees that Huacaya is bilateral while "suri types" are not. In this work, then, a pilot study was conducted to evaluate whether examination of etched cross sections reveals any differences in Huacaya and Suri and whether any differences can be noted in sulphur composition across the regions of the cross sections.

Meterials and Methods

White fleeces from 9 Huacaya and 8 Suri alpacas were obtained from 2-year old healthy males raised in two farms in Ohio. Locks of fibre were randomly selected from each fleece and washed with ethanol. Embedments of the cleaned fibres from each alpaca were made by arranging the fibres in a parallel manner, embedding in Epofix® resin (Struers), cutting with a diamond saw, polishing with grits of 2500 and 4000, and drying in a vacuum desiccator overnight. The samples were etched with an SPI Plasma Prep II in a cold oxygen plasma for 30 minutes with radio frequency power of 13.56 MHz. Mounted on carbon planchettes with carbon tape, the etched samples were sputter-coated with gold using a Pella Pelco sputter coater. For the EDS elemental analysis, fibres were not plasma etched but were sputter-coated with carbon using a Denton Vacuum Desk II coater. Although the samples for both coatings were obtained from the same lock of hairs and the same set of fibre embedments, the SEM observation and EDS elemental analysis were not conducted on the exact same fibre.

Coated samples were observed using a JEOL JSM 820 scanning electron microscope, equipped with an Oxford Instruments Analytical Group INCA energy dispersive spectrometer, a Pentafet light element detector, and INCA microanalysis software suite. An accelerating voltage of 15kv was used for elemental analysis and of 7kv for collecting images.

In order to minimise fibre surface destruction as well as to reduce overall time for data collection, sulphur elemental analyses were conducted on two line scans bisecting the cross-sections and arranged at approximate 45° angles from each other, rather than on a scan of the entire fibre cross-sectional area. Since the trend of relative sulphur contents was small and there was some data fluctuation, data were smoothed using the Savitsky-Golay second order algorithm for 25 data points before they were plotted. To better illustrate the sulphur content, Igor Pro software, version 4.07 (WaveMetrics, Inc.) was used, allowing the viewer to rotate the spectra from the two line scans in three dimensions.

Results

In etched Huacaya fibres (Fig 1), it is possible to discriminate cortical cells by the relative size and shape of the cells (Fraser *et al*, 1972; Swift, 1980) although the difference is judged subjectively. It is not feasible to set a numeric threshold of the size separating the types of cortical cells because the differences observed are relative and the cell sizes change continuously rather than dichotomously along a distinct boundary. Moreover small cells are sometimes present among larger cells. In contrast, Suri fibres (Fig 2) do not show localisation of the cell sizes but rather display cell sizes that are more uniform and randomly mixed throughout the entire cross section.

As a result of the etching process, fibres shrank, resulting in a void area surrounding the fibres and separating them from the resin embedment. The void is seen as a dark ring encircling the fibre (Fig

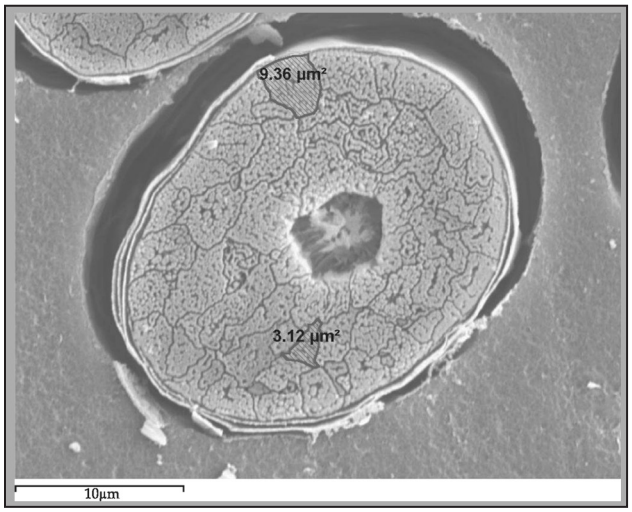

Fig 1. SEM image of a plasma-etched Huacaya fibre.

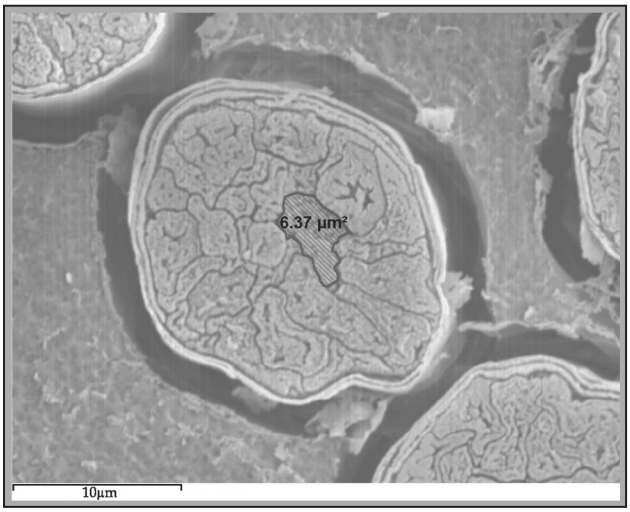

Fig 2. SEM image of a plasma-etched Suri fibre.

1,2). While the void areas appear only on one side of Huacaya fibres, those of the Suri fibres surround the fibres equally. Furthermore, it can been seen that in Huacaya fibres the larger cells abut the crescent-shaped void and smaller cells are clustered on the opposite side of the fibre in a region which is not as separated from the resin embedment, indicating that when exposed to the plasma these fibres preferentially shrink on one side.

In spite of the fluctuations seen in the sulphur spectra along the line scans of the fibres (Fig 3), larger quantities of sulphur can be seen near one side of the cross-section of Huacaya fibres. In contrast, most Suri fibres display sulphur that is evenly distributed across the entire fibre cross-section. These results, however, are relative and it is difficult to identify the boundaries between ortho- and para-cortical cells by the sulphur content alone. The regions of the line scans near the fibre centres with low sulphur content are due to the presence of the sulphur-free medulla. Also, sudden increases in sulphur at the edges of the fibres are sometimes observed because the scanning passed over the sulphur-rich cuticle cells.

The overall sulphur distribution can be seen more easily by the rotation of spectral data by Igor Pro software (Fig 4). Limitations of the printed word do not alow the same visual impact noted when the fibre image is rotated smoothly in all directions from the centre of the spectral image. An attempt to show some aspects of the consequences of rotating the scan image is presented in Fig 4; although limited, it is presented to show the value of the technique. By increasing the number of scans this technique has the potential to describe the elemental mapping in a more rapid and simple manner compared to the time-consuming area scan.

Discussion

Demarcation between cortical cells of alpaca fibres became visible through careful plasma etching. The distribution of cortical cells can be used for the discrimination between Huacaya and Suri alpaca fibres since the Huacaya fibres display a distinguishable trend of cortical cell sizes while Suri fibres display randomly mixed cells in more uniform sizes. The difference in cell sizes is likely to be related to the type of cortical cell present. Assessing size and appearance alone, Huacaya fibres display two types of cells, while Suri fibres display only one.

Huacaya fibres shrink differentially in regions of the cross-section, reflecting its composition of two types of cells that they are unequally distributed throughout the cross-section. Suri fibres display uniform shrinkage and a more uniform cell size distribution supporting the concept that they are composed of one type of cortical cell or, at least, of a random distribution of two cell types.

Differential shrinkage of the two phases in Huacaya may be related to the fact that the macrofibrils of a paracortical cell in a wool fibre are more closely packed than those of an orthocortical cell (Swift, 1977) so it does not shrink as much. The orthocortex, which has a lower sulphur content, would be etched at a greater rate, and would shrink more in comparison to the paracortex, resulting in the bending of the fibre toward the void area and closing the gap between the fibre and the surrounding resin. This corresponds to the explanation of the crimping mechanism in the literature (Horio and Kondo, 1953; Mercer, 1953; Mercer, 1954; Kulkarni *et al*, 1971). Cells that shrank more would contract the fibre and therefore be located on the inside of the bending curve

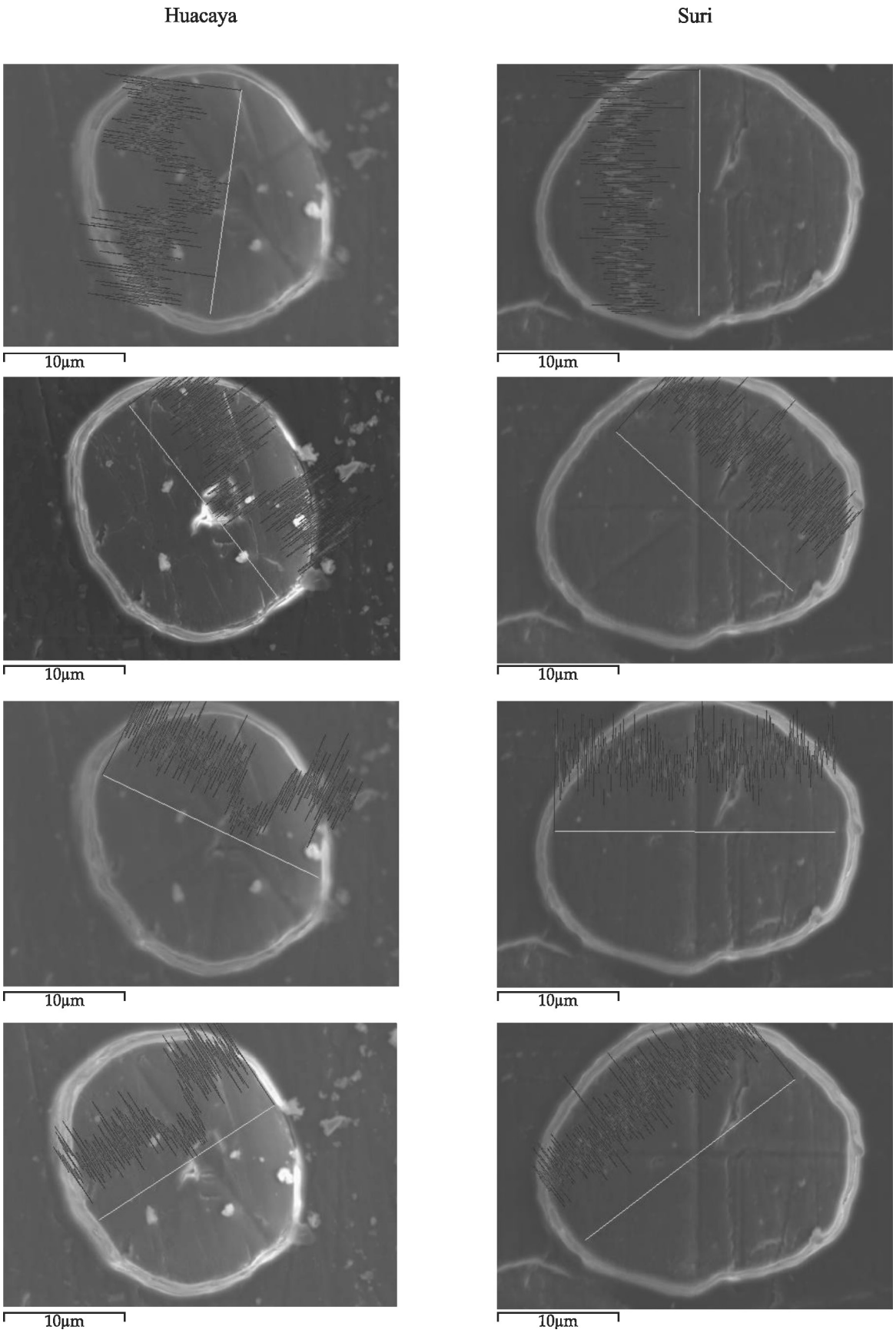

Fig 3. Sulphur distribution in Huacaya and Suri alpaca fibres.

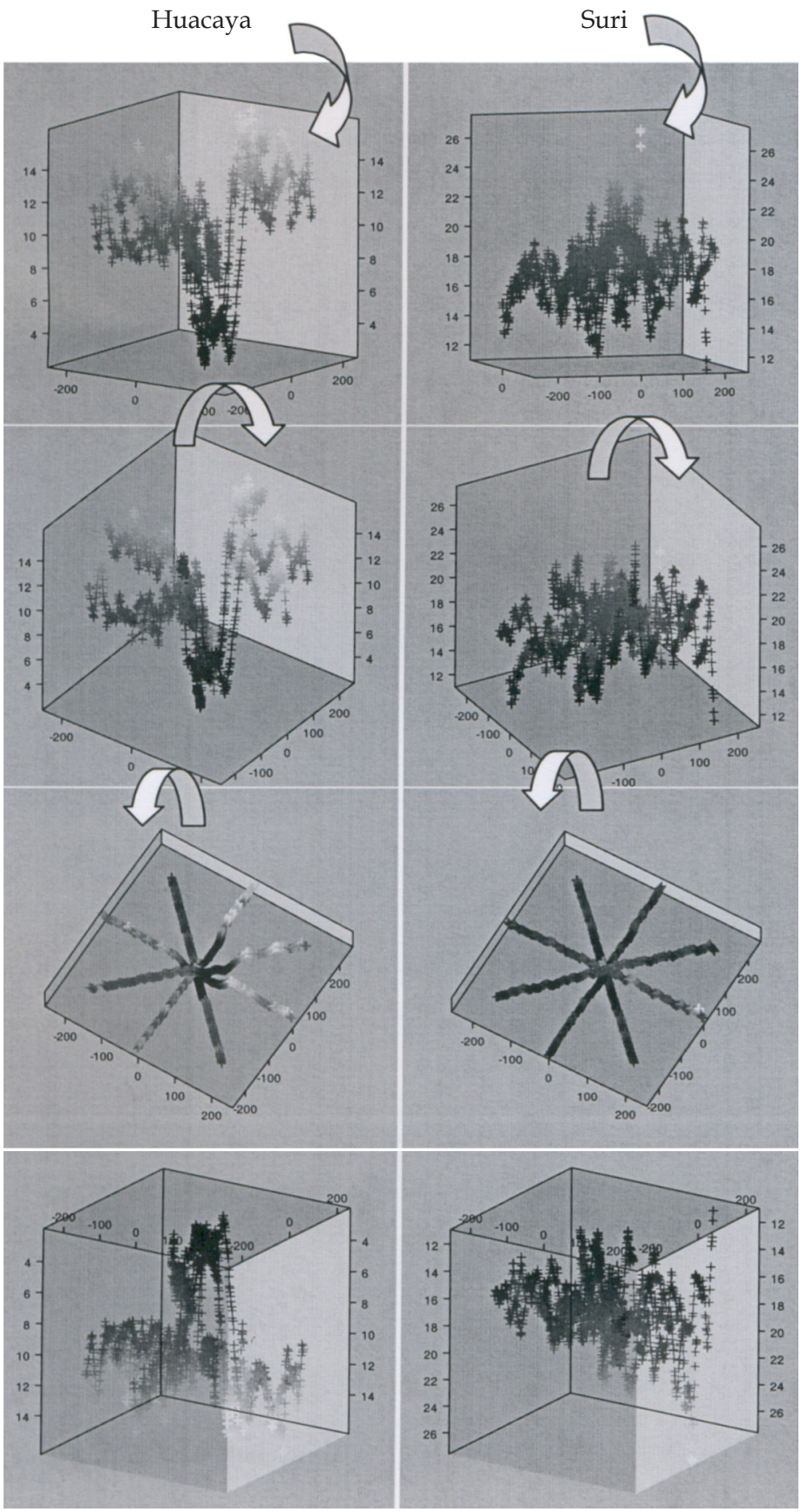

Fig 4. Three-dimensional plot of sulphur distribution in Huacaya and Suri alpaca fibres.

thus bringing the fibre closer to the surrounding resin and closing the void area that was created as the fibre shrank in the plasma.

While it may be concluded from observation of the cross-sections alone that Suri fibres do not display a bicomponent structure with preferential distribution of the cells as is displayed in crimped Huacaya fibres or in sheep's wool, the uniform shrinkage in Suri fibres does not indicate which type of cortical cells is contained in these fibres. These cells could be all ortho, all para, or a random distribution of both types throughout the fibre. In other research on the same fibres, evidence was provided for the presence of paracortical cells in Suri fibre. Differential scanning calorimetric thermograms of Huacaya fibre displayed two decomposition peaks, while those obtained of Suri fibre displayed only one. The peak temperature for the endotherm in Suri corresponds to that of paracortical cells (Shim, 2003).

The sulphur analysis does not provide evidence of difference in cell structures between Huacaya and Suri fibres that is as definitive as that obtained from the observation of etched cortical cells. Due to noise and the intricacy of the internal structure of each fibre, the spectra fluctuate, thus hindering data interpretation. The interaction volume of the electron beam is not a small spot, but penetrates some distance into the material, thus the Sulphur content acquired by EDS reflects a volumetric space with an axial depth, and not just a cross-sectional surface (Goldstein *et al*, 2003; Slayter & Slayter, 1992) and this, too, can contribute to spectral fluctuation. Although the distinction between fibre types is not readily apparent, however, the observations made in this research suggest that the Huacaya and Suri fibres show some differences in the overall trend of sulphur distribution. Further examination in this regard in warranted.

Conclusions

The plasma etching technique improved the visibility of internal structures of Huacaya and Suri alpaca fibres. Huacaya fibres displayed a perceptible trend of localised cortical cell sizes from large to small while the cortical cells in Suri fibres are similar size and are randomly mixed throughout the cross-section. The different shrinkage rates of ortho- and para-cortical cells may explain the Huacaya fibre's asymmetric arrangement in the void area surrounding etched embedments. Further clarification of the cortical cell sizes and types contained in the fibres could be made by study of separated ortho- and para-cortical cells.

The difference in sulphur content distributions between Huacaya and Suri alpaca fibres was not as clear as desired but the data do suggest a more uniform distribution throughout the Suri fibres compared to Huacaya fibres. For the further investigation of sulphur content, the use of an electron microprobe analyser with wavelength dispersive spectrometry (WDS) would be suggested for a better resolution.

One of the limitations of this study is that the images of the etched fibre surfaces were not collected from the exact same fibre from which the EDS results were obtained. A study linking the visual investigation of cortical cells and the sulphur distribution mapping of the exact same cells would be ideal. Further investigation is needed to develop a method in which elemental analysis can be conducted on the same plasma-etched surface used to collect cortical cell images.

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