JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

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Collagen Fibril Orientation and Tear Strength across Ovine Skins

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ABSTRACT: Variability of physical properties across hides and skins requires careful consideration when manufacturing goods from leather. Therefore, an understanding of the extent of this variation and its nanostructural basis is useful. Tear strength tests were performed on ovine leather from a grid of 81 positions on skins. Synchrotron small-angle X-ray scattering measurements were made from three positions on the skin, from 26 skins. The X-ray structural measurements are compared with tear strengths of the samples. It is found that the thickness normalized tear strength does not vary greatly between different positions on the skin, in contrast to bovine hides. There is more variation between different skins than within the same skin. The collagen fibril orientation and orientation index, which has previously been shown to be correlated with tear strength, do not vary significantly between the different sampling positions in ovine skins. The collagen fibril orientation varies through the thickness of the skin in a consistent way. The consistency of collagen orientation in ovine leather between different positions on the skin is in marked contrast to bovine hides and informs the use of ovine leather for manufacturing applications.

KEYWORDS: *collagen, leather, small-angle X-ray scattering, location, orientation*

INTRODUCTION

Leather possesses a variety of desirable properties that make it suitable for use in footwear, upholstery, and clothing. Currently no synthetic material has been designed that can match the combination of useful physical and aesthetic properties that leather possesses.[1] However, as a natural product, leather may be subject to significant variation between different skins and between different regions of a skin.

It is has been shown that there is a significant variation in the physical properties of leather across cattle hides,[2−7] kangaroo skins,[8] and mink and fox skins.[9] These studies found significant variation in tear strength and elongation according to sample location and direction of measurement for these animals. For bovine leather tear strength was found to be greatest in the belly region,[4] whereas for kangaroo the rump region had the greatest tear strength.[8]

Tear strength is an important physical property when considering the manufacturing applications of leather. It is particularly important for ovine leather, since ovine leather is generally weaker than bovine leather. This limits the use of ovine leather in some applications, particularly high value applications such as shoe manufacture. Leather has a complex structure where the primary component responsible for strength is collagen. Collagen fibrils may exist in different arrangements in leather, and it has been shown that the orientation of the collagen fibrils affects tear strength in ovine and other leather.[10−13]

Collagen orientation can be measured by different methods, and one of these methods is small-angle X-ray scattering (SAXS), which provides a quantitative measure of fibril orientation.[10] SAXS can also be used to generate detailed structural information regarding the *D*-spacing and amount of fibrillar collagen in leather.[10] SAXS has previously been used to investigate collagen structure in a number of other tissues including tendons,[14] ligaments,[15] mitral valve leaflets,[16] pericardium,[17] and materials for tissue regeneration scaffolds.[18]

Significant variation in collagen fiber orientation across bovine skins has been observed. When measured parallel to the skin, collagen fibers were found to be oriented parallel to the backbone in the torso and parallel to the limbs in the extremities with greater alignment of the fibers in limb and abdomen samples than those taken near the backbone.[19]

Here we measure the variation in tear strength across different regions of ovine leather. We then choose three of these regions to do a detailed investigation of the variation in the nanostructure of the leather and between these regions.

METHOD

Preparation of Leather. Skin processing has been detailed previously.[10] Briefly, leather was produced from 5-month-old "black face" lamb skins. The pelts were treated with conventional lime sulfide depilatory paint containing 160−200g/L sodium sulfide and a 50 g/L hydrated lime. After depilation any remaining wool was removed by processing the skins in a 0.8−2.4% sodium sulfide solution at 16−24 °C for 8−16 h. The skins were treated in a 0.025−0.1% solution of proteolytic enzyme, either pancreatic (Tanzyme bate, Tryptec Biochemicals Ltd.) or bacterial (Rohapon ANZ bate, Shamrock Ltd.). Skins were then pickled in a 10% sodium chloride and 2% sulfuric acid solution. The skins were pretanned using oxazolidine, then degreased with an aqueous surfactant before being tanned using chromium sulfate and retanned with a mimosa vegetable extract. The skins were then fatliquored in a 5% solution of mixed fatliquors and

Received: August 29, 2013
Revised: November 24, 2013
Accepted: November 25, 2013
Published: November 25, 2013

   dx.doi.org/10.1021/jf4038375 | J. Agric. Food Chem. 2013, 61, 12327−12332

dyed before being dried and mechanically softened. Mechanical softening was carefully controlled and with a specific humidity conditioning process.

Measurement of Tear Strength. Tear strengths were determined using standard methods for double edge tear testing.[20,21] Samples were cut from the appropriate position on the leather skin, in an orientation such that the direction of pull would be parallel to the backbone. These samples were stored at 20 °C and 65% relative humidity for 24 h before the tear strength was measured using an Instron device. Thickness was measured with calipers using a uniform pressure.

Two sets of samples were prepared: one set to cover the entire ovine skin for just tear strength testing and another set at three positions, the belly, neck, and official sampling position (OSP),[22] for tear strength testing and SAXS measurements. For the complete coverage, 81 sites were selected (a grid of 9 × 9) on each of five skins and samples were cut parallel to the backbone. For the tensile testing for SAXS, samples from 29 ovine skins were cut parallel to the backbone from three different positions: the belly, neck, and official sampling position (OSP).[22] The locations of these positions on the skin for SAXS are shown in Figure 1.

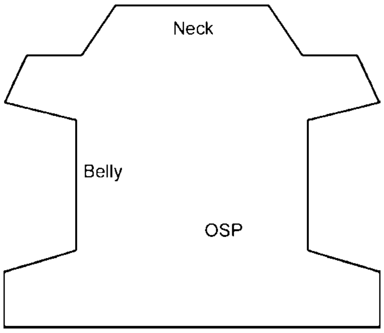

Figure 1. Diagram of sample locations on skin.

Small Angle X-ray Scattering. Diffraction patterns were recorded on the Australian Synchrotron SAXS/WAXS beamline. The beamline has an undulator source and a cryocooled Si (III) double-crystal monochromator. It has an energy resolution of 10^{-4}, a beam size at sample, full width half-maximum (fwhm), of 250 μm × 80 μm, and a photon flux of approximately 2×10^{12} ph s^{-1}. Patterns were recorded with an X-ray energy of 11 keV and an exposure time of 1 s on a Pilatus 1M detector with an area of 981 × 1043 pixels. A sample to detector distance of 3371 mm and an active area of 170 mm × 170 mm was used.The energy is calibrated using the zinc K-edge from zinc foil at 9.659 keV and is maintained with 2 eV of the nominal energy.

Each sample was mounted, without tension, on a remotely controlled sample plate that was placed in the path of the X-ray beam. Samples were analyzed from grain to corium with spectra recorded at 0.25 mm increments. Beam damage was tested by comparing successive diffraction patterns taken on the same region, but no changes were observed under the conditions of these measurements. All samples were stored under the same humidity conditions. Data were processed using SAXS15ID software.[23]

The *D*-spacing was determined using Bragg's Law, with the value from several collagen peaks (usually $n = 5$ to $n = 10$) being averaged for each spectrum.

Orientation index (OI) is used to measure the spread of orientation of the collagen fibrils. It is calculated from the azimuthal angle spread of the most intense Bragg's peak at around 0.058−0.060 $Å^{-1}$. OI is defined as $(90° - \text{OA})/90°$ where OA is the minimum azimuthal angle range, centered at maximum peak intensity, that contains 50% of the fibril scattering intensity. An OI value of 1 indicates that the fibrils are completely parallel to each other, while a value of 0 indicates orientation of the fibrils is completely random.

The orientation was estimated using the most intense Bragg's peak at around 0.058−0.060 $Å^{-1}$. It was defined as the azimuthal angle corresponding with the location of the maximum peak intensity above the baseline. The amount of fibrillar collagen I was estimated by dividing the area above the baseline of the sixth-order collagen Bragg's peak by the intensity of the baseline under the peak. Only collagen I that has formed into fibrils will contribute to this peak, but as we expect all the collagen I to do this, this should provide a measure of the total amount of collagen I.

RESULTS

Variation of Tear Strength with Sampling Position. The tear strengths determined for the detailed coverage of the skins show that the absolute tear strength is variable across the skin. A strip down the middle of the skin had the highest value with the flanks having lower tear strength (Figure 2a). However, the thickness also varies in a similar manner (Figure 2c) so that when the tear strength is normalized for thickness (Figure 2b), much of this difference is accounted for.

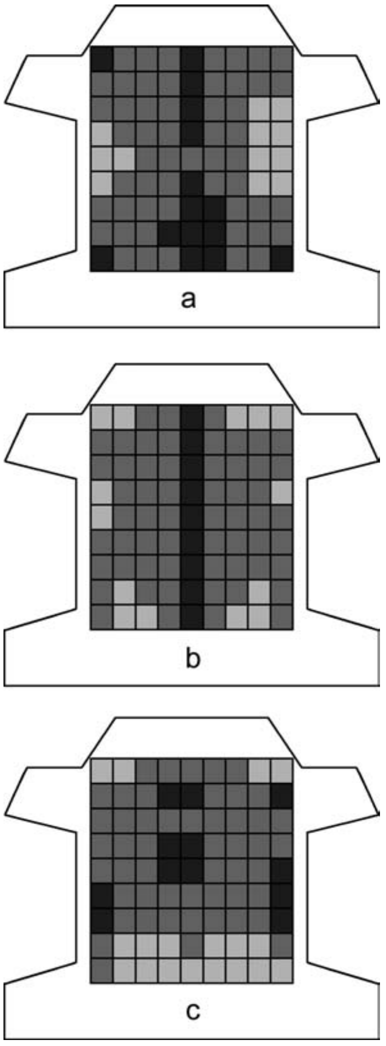

Figure 2. (a) Thickness (mm), (b) absolute strength (N), (c) normalized strength (N/mm) for 81 positions on the average of 5 ovine skins. The scale is the following. Thickness: dark gray, >1.28 mm; medium gray, 1.28−1.03 mm; light gray, <1.03 mm. Absolute strength: dark gray, >36.4 N; medium gray, 36.4−28.0 N; light gray, <28.0 N. Normalized strength: dark gray, >31.9 N/mm; medium gray, 31.9−24.6 N/mm; light gray, <24.6 N/mm. The strength for light gray and dark gray is set at greater than and less than 1 standard deviation away from the mean strength, respectively.

Tear Strengths at the Three Samples Positions Used for SAXS Analysis. The normalized tear strength for the three sample positions of 29 ovine skins (which were also used for the SAXS analysis) gives average values (standard deviation) of 28.5 (6.0) N/mm for the neck, 28.2 (7.7) N/mm for the OSP, and 33.6 (9.7) N/mm for the belly position. A one way analysis of variance finds that the difference between these three is not enough to exclude the possibility that the differences are due to random sampling variability ($P = 0.6$). In other words there is no significant difference in the tear strengths between the three positions.

In contrast, when individual values of normalized tear strength at each of the three positions are plotted against the average normalized tear strength of a skin, there is a strong correlation between the strength at any position with the strength of the whole skin (Figure 3). Therefore, it is clear that the variation from one skin to another is greater than the variation within a single skin.

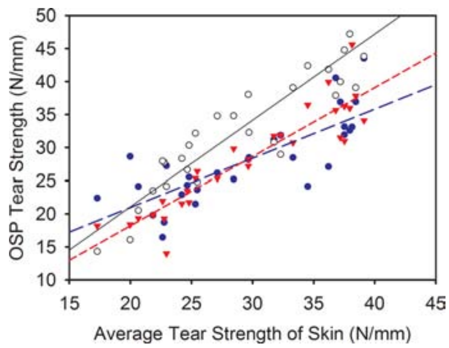

Figure 3. Normalized tear strength of position versus the average normalized tear strength across the entire skin for the (a) neck (closed circles, blue, long dashed line), $r^2 = 0.62$, $t = 6.7$, $P < 0.0001$; (b) belly (open circles, black, solid line), $r^2 = 0.83$, $t = 11.7$, $P < 0.0001$; and (c) OSP (triangles, red, short dashed line) $r^2 = 0.84$, $t = 12.0$, $P < 0.0001$.

Small Angle X-ray Scattering Analysis. The ovine skins gave scattering patterns from which the OI could be clearly measured. Examples of a scattering pattern, an integrated intensity plot, and an intensity variation with azimuthal angle from which the OI is calculated are shown in Figure 4.

Orientation of Collagen Fibrils. The average OI measured edge-on for each of the three positions, neck, belly, and OSP (averaged for all the skins measured), as shown in Figure 5a, was found not to be statistically different. Alignment of collagen fibrils in ovine leather measured edge-on is therefore similar across the animal skin, showing a consistency in the arrangement of the fibrils in different regions of the skin.

The preferred direction of alignment for collagen fibrils is also found to be unaffected by sampling position, with no statistical difference between the average values for each position (measured edge-on). However, the OI has a weak but statistically significant correlation with tear strength for the three sample positions studied (Figure 5b).

Measurements of OI taken normal to the surface, where the leather was split into the grain layer and the corium, found no significant difference in OI between the different positions measured across the skin (not shown). Although the average OI is generally lower in the grain than in the corium, there is no significant difference between the OI of the grain and the corium for any of the positions studied.

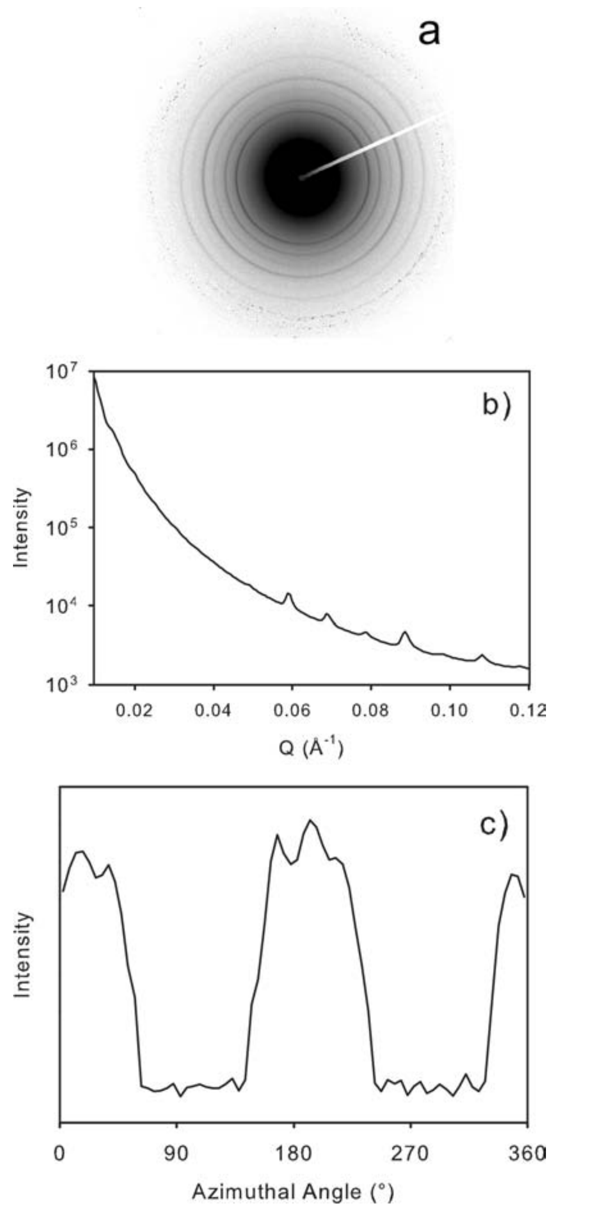

Figure 4. Examples of (a) a scattering pattern (logarithmic intensity scale), (b) an integrated intensity plot, and (c) a plot of the intensity variation with azimuthal angle at the sixth collagen diffraction peak (using the q range 0.058–0.060 Å^{-1}).

There is also no significant difference in the preferred direction of alignment between positions in both corium and grain samples measured normal to the surface. The average peak location for corium measured normal to the surface is between 0° and 5° from parallel to the direction of the backbone for each of the three positions measured, and much of this variation from 0° may be due to misalignment of the sample during measurement.

These findings are similar to those previously reported for calf skin, where collagen fibers were found to align with the backbone in the OSP and neck and belly positions.[19]

Variation in Structure through the Thickness. Three aspects of the structure, OI, amount of collagen, and D-spacing, were measured through the thickness of samples from each of the three positions. Each of these aspects of the structure changes through the thickness of the skin.

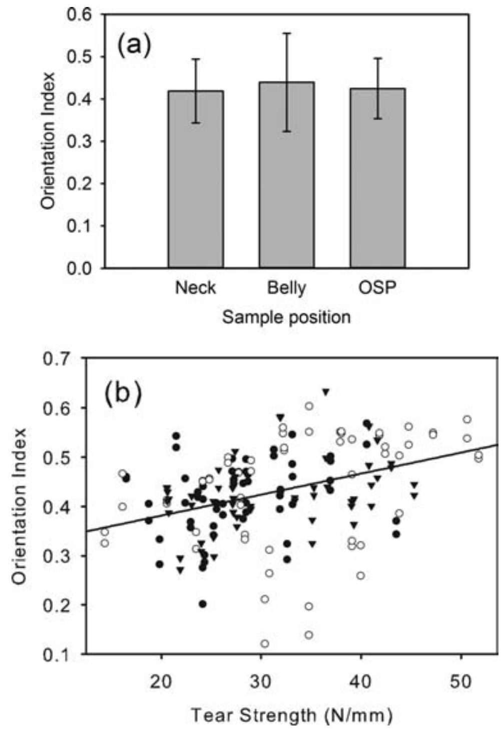

Figure 5. (a) Average OI for each position and (b) OI versus tear strength for ovine leather samples cut parallel to the backbone from the neck (closed circle), belly (open circle), and OSP (triangle). For individual regressions the statistics are the following: belly, $r^2 = 0.13$, $t = 2.8$, $P = 0.006$ (linear regression line shown, others similar); neck, $r^2 = 0.07$, $t = 2.0$, $P = 0.05$; OSP, $r^2 = 0.16$, $t = 3.2$, $P = 0.003$.

The variation of the OI through the sample thickness follows a similar profile for all three positions (Figure 6a). The greatest fibril alignment (highest OI) occurs in the corium and the least alignment at the corium−grain boundary.

The amount of fibrillar collagen I also varies through the cross-section of ovine leather with a similar variation for each of the three positions sampled (Figure 6b). There is a higher concentration of fibrillar collagen I in the corium region compared with the grain. There is no relationship between the amount of fibrillar collagen I and tear strength taken across all the samples (not shown). This is consistent with a previously reported analysis of samples from the OSP position.[11]

The *D*-spacing also varies through the cross-sction (Figure 6c) with a dip in *D*-spacing near the middle of the cross-section. Each of the three positions sampled displays this profile but with varying magnitudes of the dip in the corium near the center of the skin. The neck had the greatest variation through the thickness and the belly the least. A statistical analysis finds that there is a small but significant difference between the *D*-spacing at the three sample positions. The average *D*-spacing for the neck is 64.15 (0.25) nm, for the OSP 64.30 (0.22), and for the belly 64.44 (0.20). A pairwise multiple comparison using the Tukey test finds that there is a difference between all pairs (belly vs neck, $q = 8.8$, $P < 0.05$; belly vs OSP, $q = 4.5$, $P < 0.05$; OSP vs neck, $q = 4.4$, $P < 0.05$).

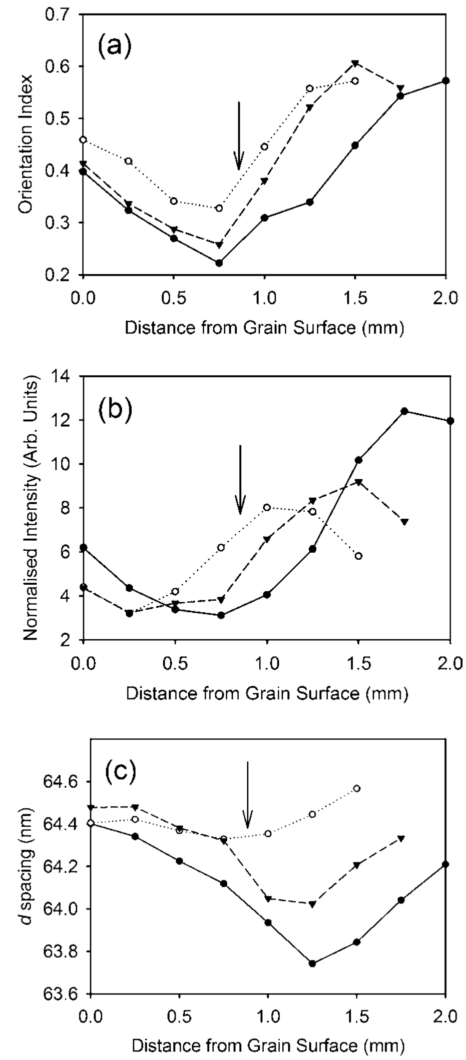

Figure 6. Profiles across samples cut parallel to the backbone: (a) orientation index; (b) amount of fibrillar collagen I; (c) *D*-spacing; neck, closed circle; belly, open circle; OSP, triangle. The profiles start at the grain, and the arrow indicates the approximate position of the grain−corium boundary.

■ DISCUSSION

Measurements of the tear strength variability across ovine skins have not been reported previously. It is therefore revealing to learn that there is little variation in strength with position on the skin, in contrast to bovine leather where it is well-known that the strength varies in different position.[24] That there is little difference in fibrillar collagen OI across different positions in ovine skin is surprising given the variation in stresses that would be experienced by the skin in the living animal. While strength is only one property of leather, and other properties are also important depending on the manufacturing application, strength is a fundamental and critical property for many of the high value applications. The ovine leather we measured spanned a range of absolute strength from 23 to 49 N (or 13−48 N/mm with variable thickness). For comparison the leather for men's shoes needs to meet ISO 20344:2011(E) with a double edge tear test of 120 N and for women's fashion shoes 40 N. For apparel leather such high strength is not needed and the leather should meet the standard BS EN ISO 14931 with a single edge tear of 20 N. So the range of leather strengths we display in Figure 2 would have strength sufficient for apparel and women's fashion shoes but not for men's shoes, with the higher strength samples plotted being about two-thirds of the required minimum strength.

With bovine leather it is necessary to select the region of the hide to suit the in service requirements of the leather, whereas from the uniformity of the strength measurements in ovine hides it is apparent that strength does not vary to the same extent so that selecting a specific region on the skin is less important. Of course, ovine skins are smaller than bovine hides, so this is a rather fortunate situation. What we can see is that the variation from one ovine skin to another is much greater than the variation within a skin. Therefore, of more importance is selecting skins that have the required strength for the manufacturing application rather than selecting specific regions on skins. This is likely to become possible with the introduction of stock traceability, which is being implemented in New Zealand through NAIT (National Animal Identification and Tracing) with similar systems likely to become legally mandated throughout the world. Full traceability from farm to processing plant would assist breed selection by the leather processor.

The alignment of fibrils in the ovine skins, which may affect the directional strength and the stretch of the leather, is parallel to the backbone throughout the useable areas of the processed skin. This is useful knowledge when laying out cutting patterns on skins.

The collagen fibril structural studies reported here provide evidence for the consistency of strength across different regions in the skin. It has previously been shown that a primary determiner of strength in leather is the alignment of collagen fibrils, with greater alignment (more in parallel planes) leading to stronger leather.[11,13] Collagen I forms fibrils and exhibits a great deal of strength along the direction of the fibrils but little strength across the direction of the fibrils. Therefore, the strength is a property of the number of fibrils aligned in the direction of stress. This relationship has been observed previously at the OSP for ovine and bovine leather[11] and for a range of other animals.[13] Therefore, the lack of variation in OI we observe here between the three different sampling positions would be expected to lead to a lack of variation of tear strength, and that is indeed what we find. In contrast, we find a large variation of OI between different skins, and this explains some of the variation in tear strength between skins. Therefore, an important goal for the ovine leather industry is to reduce this variability in fibril orientation and produce skins with consistent highly aligned fibrils. There may also be some scope to use selective breeding to improve the skin, and therefore, leather quality and this work could be used to inform breed selection. However, as the skin currently represents only a small portion of the total value of the animal, it is unlikely that skin quality will become a priority for breeding. Despite this lack of difference in average OI with different positions in the skins, we do find some differences in the cross-sectional structure of ovine leather from different parts of the skin. These do not appear to have a marked effect on tear strength, but perhaps they impart different properties to the leather that we have not tested in this study or perhaps they do not have a large bearing on physical properties. The OI varies in a consistent way through the thickness and in a similar way to bovine leather.[11] It is well-known that the corium provides most of the strength to leather and this contains a greater amount of diffracting collagen and is in general more highly aligned, both of which would be expected to lead to greater strength. The *D*-spacing varies through the thickness in the neck and the OSP positions but less so in the belly. We have previously not found any correlation of *D*-spacing with strength and neither do we here, since despite the marked variation in *D*-spacing between positions, the tear strength does not change. We do not have a good explanation of the reason for this variation in *D*-spacing.

In summary, we found that ovine leather has similar thickness normalized tear strength across the entire skin. We found that the reason for this is that the collagen fibril alignment does not significantly vary from one region of the skin to another. The structural features common across the skin include an increased concentration of fibrillar collagen I in the corium and a greater alignment of collagen fibrils in the corium. A relationship between tear strength and average orientation index in the edge-on direction is maintained across the skin. The variation in tear strength and structure was found to be greater between different skins than between regions on one skin. That there is little difference in fibrillar collagen structure across different positions in ovine skin is surprising given the variation in stresses that would be experienced by the skin in the living animal. It is also in contrast to the variations that have been observed in bovine hides between different positions on the hide. This work suggests that when selecting regions of ovine skin for manufacturing on the basis of tear strength, the choice of skin is more important than the location on the skin chosen in contrast to bovine hides and some other skins. This knowledge is important in informing selection processes in leather manufacture and cutting design in finished good manufacture.

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Funding

The NZ Synchrotron Group Ltd is acknowledged for travel funding. This work was supported by the Ministry of Innovation, Business and Employment grant number LSRX0801.

Notes

The authors declare the following competing financial interest(s): This work was supported by the Ministry of Innovation, Business and Employment Grant Number LSRX0801.

■ ACKNOWLEDGMENTS

This research was undertaken on the SAXS/WAXS beamline at the Australian Synchrotron, Victoria, Australia.

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