Mapping Tear Strength and Collagen Fibril Orientation in Bovine, Ovine and Cervine Hides and Skins

by

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Abstract

Leather is a natural and variable material. The variation in strength has previously been shown to be due in part to the collagen fibril orientation. However, the extent of variation in strength and orientation over a skin/hide is not well established. Synchrotron small angle X-ray scattering is used to measure collagen fibril orientation (O) and orientation index (OI). Tear strength is measured in two orthogonal directions across ovine and cervine skins and bovine hides. Average normalized strengths varied between species with cervine leathers having the greatest overall strength followed by bovine then ovine leathers. Ovine had the greatest variability in strength across the skin. There were no obvious regions of generally stronger or weaker skin within individual skins or hides. The predominant collagen fibril direction was perpendicular to the backbone of all species, with the greatest perpendicular alignment in ovine followed by cervine. Collagen fibril orientation in leathers made from of ovine, bovine and cervine skins have been analyzed quantitatively and in detail. Findings suggest an unpredictable variability in collagen arrangements within each species but a notable difference in strength between species.

Introduction

Collagen is a major constituent of skin and is responsible for many of its functional properties such as strength and flexibility.[1] It accounts for 75% of the dry weight of skin, the mechanical properties of which are mostly dependent on the structural arrangements of the fibers that make up the collagen.[2,3] The structural arrangement of the fibers in collagen most influences its mechanical properties. The collagen fibers are anisotropic and they are strongest in the direction of their component fibres[4,5] and weakest in the direction perpendicular to the fibres.[6,7] Slight variations in the collagen fiber arrangement can alter the mechanical properties of skin significantly, for example increasing its flexibility over joints to facilitate movement and increasing its strength in areas of high friction, such as the soles of the feet, for protection.[8] The orientation of collagen fibers in the skin varies from area to area to provide a local architecture to create the required mobility, flexibility and rigidity[9] as required by the forces operating at these sites.[10,11] This variability in skin's properties around the body was recognized in the late 1800's, when studies were performed on people to determine the optimal direction for surgical incisions. Langer lines,[12] Kraissl lines[13] and Borges lines[14] were developed to show the natural collagen orientation and maximum skin tension in human skin as a topological map of the body that are still used by surgeons today.[15,16] This idea has been extended further to dogs where collagen orientation mapping has also been performed, resulting in a set of canine tension lines to assist with surgical incisions.[17]

A series of studies have been performed to determine the mechanical properties of skin[2,18,19] and more specifically the application of biaxial loading where its effects on collagen orientation were observed.[18,20-22] In unloaded skin the collagen fibers are largely unaligned; when tensile loading is applied the fibers become more aligned. The classical theory behind this change in alignment suggests that the overall strength of the collagen becomes dependent on the mechanical properties of the fibers themselves,[20-23] with fibers initially orienting in the direction of the applied strain. Then, once aligned, the individual fibers themselves bear the strain load. However, another theory suggests that a more plastic response is observed due to inner sliding within each fiber, contributing a given stress to the global response. The mechanical response, on the other hand, is primarily due to the structural effect of the fibers network.[24]

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Information on the collagen fiber alignment and its influence on the mechanical properties of animal skins could have important implications for the tanning and leather industries. In particular, information is needed on how the natural collagen arrangement varies in skin around the body and how this influences the mechanical properties of the skin from different areas. To date there are only limited studies in animals. The collagen fibril orientation has been measured over a bovine hide, using the angular dependence of transmitted microwave intensity. Findings alluded to a correlation between the fibril orientation and the forces that would have occurred in the live animal.[25-27] A small angle x-ray scattering (SAXS) and tear testing study on ovine hides demonstrated that tear strength was distributed with decreasing strength when moving from the backbone down to the flanks.[28] The relationship between tear strength and collagen alignment in a selection of mammals including horse, goat, sheep, water buffalo and cattle[29] showed a significant correlation between the edge-on collagen fibril alignment and tear strength, where a trend of greater collagen alignment resulted in greater tear strength.

In this work the variation in and relationship between collagen orientation, orientation index and tear strength is mapped using tear testing and SAXS imaging, measured normal to the grain surface, for standard beamhouse tanned ovine and cervine skins and bovine hides. This has only previously been studied at the official sampling position (OSP) of selected mammals[29] and at two other locations (back and belly) of ovine leathers.[28]

Methods

Leathers

The leathers used in the study had been processed to crust according to standard beamhouse methods. Bovine leathers were obtained from a co-operating leather tannery. The leather was made using conventional processes which included: lime-sulfide liming, sulfuric acid pickling and chrome tanning. Cervine leathers were also obtained from a co-operating deer skin tannery using conventional processes which included: painting with lime-sulfide paint, depilation, liming, pickling with sulfuric acid and conventional chrome tanning. In addition, ovine leathers were produced in-house for this study from Romney cross animals of the same age according to the following procedure. After rehydrating the skins, the adhering fat and flesh were removed mechanically with a conventional fast acting (<4 hours) lime sulfide paint (200g/L commercial flake sodium sulfide, 45g/L sodium hydroxide, 50g/L hydrated lime and 23g/L pre-gelled starch thickener) applied to the flesh (inner) side of the raw skins at a rate of 400g/m^2. The painted skins were incubated at 10-15°C for 3 hours. The wool was manually removed and the skins washed to remove the lime. Ammonium sulfite (2% (w/v)) was added to lower the pH to 8 followed by the addition of a commercial bate enzyme (Tanzyme (0.1% w/v)). After incubation at 35°C for 75 min the treated skins were washed and pickled in 20% w/v sodium chloride and 2% w/v sulfuric acid. The pickled pelts were degreased in a drum with degreasing solution (10% common salt, 8% non-ionic surfactant and 4% Oxazolidine A (Zoldine ZA-78 from Angus)) for 30 minutes at 35°C. Sodium bicarbonate was added to neutralize the skins to be tanned (4.5% (based on pickled weight) of 33% basicity, 25% chromium content chromium sulfate powder (Chromosal B from Lanxess, Germany)). The wet blue pelts were then neutralized for 1 hour in 1% sodium formate and 0.15% sodium bicarbonate solution followed by water washes then retannage (2% synthetic retanning agent (Tanicor PW, Clarient, Germany), 3% vegetan (mimosa, Tanac, Brazil)). Finally, fatliquors were added (a total of 6% mixed fatliquors) and the temperature rose to 50°C for 45 minutes. The addition of 0.5% formic acid fixed the crust leathers for 30 minutes was followed by draining. The tanned pelts were then washed with water before being dried, conditioned and staked.

Test Samples

Samples for testing were taken from a grid (Figure 1) placed on one half of two ovine skins, two bovine hides processed to upholstery grade and two cervine skins; all were halved from neck to tail. The sampling grid was adjusted to accommodate the species differences in hide size with the grids for the ovine skins being 80 x 80 mm, the smallest size enabling two tear tests and SAXS measurements (see below); the grids for the bovine hides and cervine skins were 200 x 200 mm to limit the total number of samples (Table I). From each sampling grid square, two samples (35 x 20 mm) were removed for tear tests and one (8 x 8 mm) for SAXS measurements (solid gray

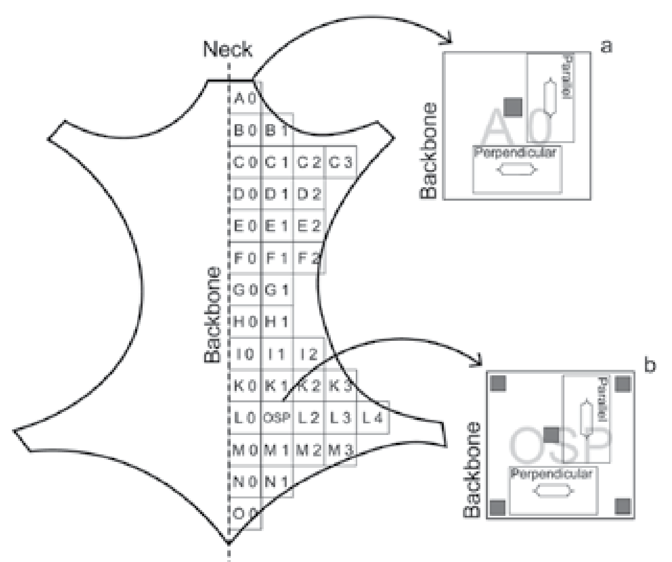

Figure 1. Grid pattern used for sampling hides showing the locations of the samples taken for tear testing and SAXS measurements (solid grey squares) at (a) general locations and at (b) the official sampling position (OSP).

Table I

List of skins and hides with size and number of samples taken from each for analysis using industry standard tear testing (ISO 3377-2:2002) and small angle x-ray scattering (SAXS) measurements.

Sample	Size of halved hide (m^2)	Sample spacing (m)	Number of samples for tear strength measurements	Number of samples for SAXS measurements*
Ovine skin halved (neck to tail) – skin I	0.20	0.08 0.04 at OSP	31 parallel; 31 perpendicular	35
Ovine skin halved (neck to tail) – skin II	0.22	0.08 0.04 at OSP	35 parallel; 35 perpendicular	39
Bovine hide halved (neck to tail) – skin I	1.52	0.2 0.1 at OSP	38 parallel; 38 perpendicular	44
Bovine hide halved (neck to tail) – skin II	1.64	0.2 0.1 at OSP	41 parallel; 41 perpendicular	45
Cervine skin halved (neck to tail) – skin I	1.80	0.2 0.1 at OSP	45 parallel; 45 perpendicular	49
Cervine skin halved (neck to tail) – skin II	2.08	0.2 0.1 at OSP	52 parallel; 52 perpendicular	56

*Five areas were analyzed in the OSP samples and one area in each of the other samples. Nine SAXS measurements (3 x 3 grid in the center of the sample with 0.5 mm spacing between measurement points) were made on each sample and used to calculate the orientation (O) and orientation index (OI) which were the averaged.

square in Figure 1(a)). To establish if SAXS measurements taken in the central 8 x 8 mm sample for each grid square was representative of the skin or hide in the rest of the grid square, extra samples were taken from the official sampling position (OSP). 8 x 8 mm samples were taken from the center as well as the corners of the grid square at the OSP for comparison. These additional samples were 40 mm apart in the ovine skin samples and 100 mm apart in the bovine and cervine skin samples.

Tear Strength

Measurements of tear strength were performed according to standard methods (ISO 3377-2:2002) and normalized for thickness so strengths are comparable between samples. Two samples (at right-angles or parallel to the backbone) were cut from each sample grid position and conditioned at a constant temperature (20°C) and relative humidity (65%) for 24 hours before tear testing with an Instron 4467.

Small Angle X-ray Scattering (SAXS)

The Australian Synchrotron SAXS/WAXS beamline was used to establish the SAXS diffraction patterns in each sample. SAXS has proven useful in analyzing collagen structures in a range of materials, such as cornea,[30] bones[31] and leather.[32] A total of nine SAXS measurements were taken from the center of each sample in a 3 x 3 pattern with 0.5 mm spacing between the measurement points (Figure 1). To determine the representativeness of a single SAXS measurement in the middle of the sample, at the Official Sampling Position (OSP), additional SAXS measurements were taken in the corners of the sample (Figure 1(b)).

For each of the above measurements a high-intensity undulator source was used with an energy resolution of 10^{-4} from a cryo-cooled Si(111) double-crystal monochromator. The beam size (FWHM focused at the sample) was 250 x 80 µm with a total photon flux of about 2 x 10^{12} photons s^{-1}. All diffraction patterns were recorded with an X-ray energy of 8 keV using a Pilatus 1M detector with an active area of 170 x 170 mm and a sample to detector distance of 3371 mm. The exposure time for diffraction patterns was in the range of 1- 5 s, and data processing was carried out using the Scatterbrain software.[33] Intensities were displayed as absolute detector counts.

Orientation and Orientation Index.

The Orientation (O) and the Orientation Index (OI) were calculated from each of the nine SAXS measurements taken for each sample and the average value taken to represent the O and the OI for the grid square. The OI was defined as (90°–OA)/90°, where OA is the minimal azimuthal angle range, centered at 180°, that contains 50% of the micro-fibrils.[32,34] The OI varies between 0 and 1 with 0 describing an isotropic, random arrangement and 1 describing fibrils all oriented in the same direction. The OI was calculated from the spread in the azimuthal angle of the D-spacing peak at 0.059 – 0.060 Å^{-1}. The O was taken as the center of the Gaussian curve fitted to azimuthal angle – intensity plot. The O is determined as the point on the x-axis corresponding to the greatest measured intensity where the x-axis has a horizontal angle measured clockwise from 0° at the graph origin (Figure 5).

Table II

Average tear strength, collagen fibril orientation (O) and orientation index (OI) of the ovine and cervine skins and bovine hides studied. An O angle of 0° is along the backbone.

Species	Perpendicular Tear Strength (N/mm)	Parallel Tear Strength (N/mm)	O (°)	OI
Ovine	20	22	79	0.44
Bovine	42	43	66	0.38
Cervine	54	51	44	0.24

Samples

The selection of samples used in this study is listed in Table I. The bovine hides and cervine skins are much larger than the ovine, so the sampling points are more widely spaced.

Results

Tear Strength

The average tear strength normalized for thickness varied between species with ovine skins having the least strength which was around 50% that of the bovine hides and cervine skins (Table II). The cervine skins had the greatest strength which was about 20% higher than those of the bovine hides.

When tear strengths in individual animals were compared (Table III), there was a similar trend with the individual ovine skins having lower average tear strengths than the individual

Table III

Average tear strengths , collagen fibril Orientation (°) and Orientation Index (OI) of the individual ovine, bovine and cervine half hides studied. An orientation angle of 0° is parallel to the backbone. P-values are for a t-test of whether the means are different where bold values are those that were statistically significant (P < 0.05).

Sample	Perpendicular Tear Strength (N/mm) Average (St.Dev)	Parallel Tear Strength (N/mm) Average (St.Dev)	O (°) Average (St.Dev)	OI Average (St.Dev)
Ovine 1	12 (8)	13 (8)	84 (33)	0.47 (0.15)
Ovine 2	27 (11)	30 (8)	73 (28)	0.40 (0.11)
P-value Ovine 1 and Ovine 2	0.121	0.156	0.054	**0.030**
Bovine 1	33 (11)	39 (17)	65 (25)	0.29 (0.17)
Bovine 2	50 (14)	47 (12)	66 (27)	0.25 (0.11)
P-value Bovine 1 and Bovine 2	**<0.001**	**<0.001**	**<0.001**	**0.031**
Cervine 1	50 (13)	45 (12)	44 (28)	0.22 (0.15)
Cervine 2	58 (10)	56 (10)	43 (26)	0.26 (0.11)
P-value Cervine 1 and Cervine 2	**0.002**	**<0.001**	**0.007**	**0.039**

bovine hides; these in turn had lower strengths than the individual cervine skins. There were significant differences between the individuals in each species, in particular in ovine, where Ovine 1 had tear strength values more than double that of Ovine 2.The difference between the individual bovine hides was not as marked, and the difference between the individual cervine skins was the least. Ovine skins are known in the industry to be more variable than bovine hides or cervine skins, even within the same breed and age.

There were only small differences between the tear strengths measured parallel and perpendicular to the backbone at each individual sample point (data not shown) and for each species (Table II). However, the average perpendicular tear strength varied considerably between individuals and between species which was also the case for parallel tear strengths (Table III). The tear strengths at the different measuring points varied between individuals of each species and between the species.

There was no apparent pattern to the distribution of perpendicular or parallel tear strengths at the various measuring points in the different individuals and species (Figure 2-4), rather the tear strengths at each position varied widely between individuals of a species and between the species. Although there were considerable differences between individuals within a species, there were only insignificant differences between the average tear strengths measured perpendicular and parallel to the backbone between species (Table II).

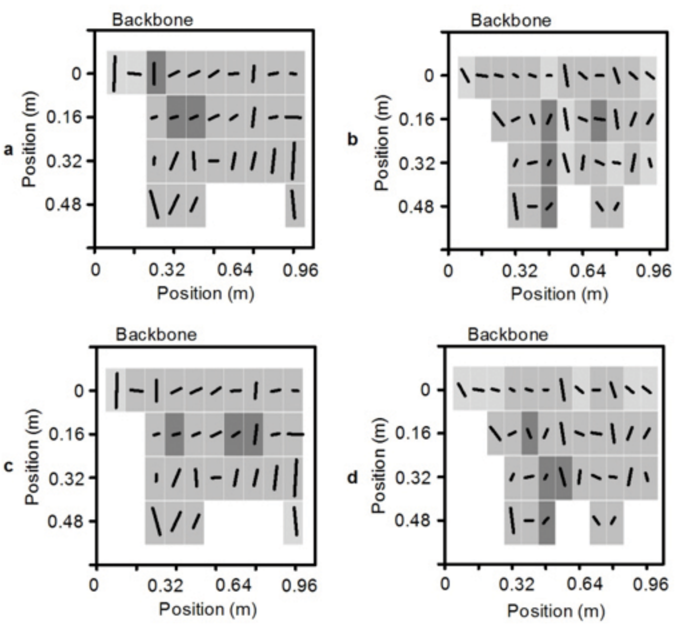

Figure	Key		
a	< 4 N/mm	4 N/mm – 19 N/mm	> 19 N/mm
b	< 4 N/mm	4 N/mm – 21 N/mm	> 21 N/mm
c	< 16 N/mm	16 N/mm – 38 N/mm	> 38 N/mm
d	< 22 N/mm	22 N/mm – 39 N/mm	> 39 N/mm

Figure 2. Ovine skin collagen orientation, orientation index and directional tear strength map. Data is for two ovine halved skins from two animals (a, c) and (b, d) with neck on the left. (a, b) display the perpendicular tear strength. (c, d) display the parallel tear strength relative to the backbone. Vector direction indicates the collagen fibril orientation; vector magnitude indicates the OI; shading indicates tear strength relative to the backbone as detailed in the key: middle column (grey) average for the half hide +/- one standard deviation; left column (light grey) average – one standard deviation; right column (dark grey) average + one standard deviation.

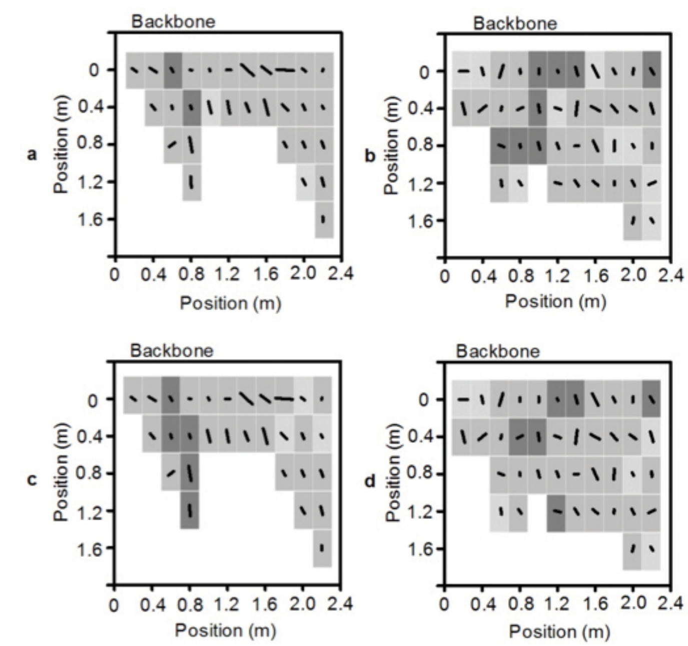

Figure	Key		
a	< 21 N/mm	21 N/mm – 44 N/mm	> 44 N/mm
b	< 37 N/mm	37 N/mm – 65 N/mm	> 65 N/mm
c	< 13 N/mm	13 N/mm – 48 N/mm	> 48 N/mm
d	< 36 N/mm	36 N/mm – 60 N/mm	> 60 N/mm

Figure 3. Bovine hide collagen orientation, orientation index and directional tear strength map. Data is for two bovine halved hides from two animals (a, c) and (b, d) with neck at the left. (a, b) display the perpendicular tear strength. (c, d) display the parallel tear strength relative to the backbone. Vector direction indicates the collagen fibril orientation; vector magnitude indicates the OI; shading indicates tear strength relative to the backbone as detailed in the key: middle column (grey) average for the half hide +/- one standard deviation; left column (light grey) average – one standard deviation; right column (dark grey) average + one standard deviation.

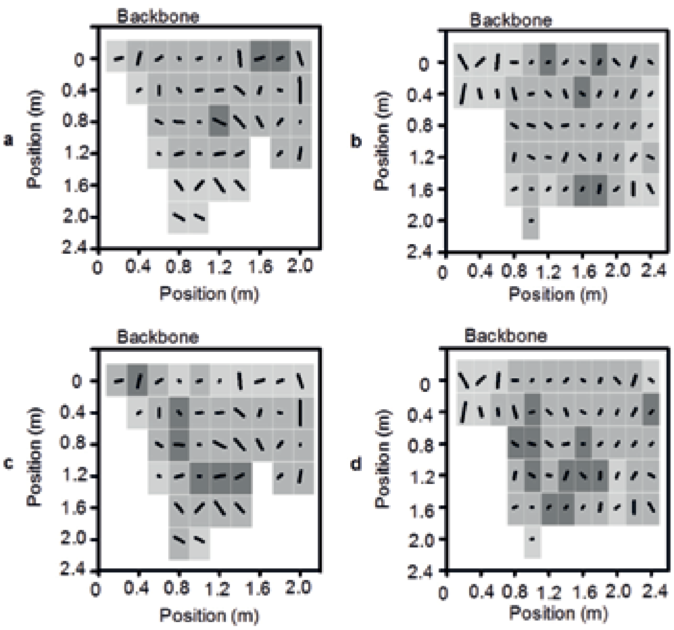

Figure	Key		
a	< 35 N/mm	35 N/mm – 57 N/mm	> 57 N/mm
b	< 50 N/mm	50 N/mm – 66 N/mm	> 66 N/mm
c	< 38 N/mm	38 N/mm – 66 N/mm	> 66 N/mm
d	< 53 N/mm	53 N/mm – 69 N/mm	> 69 N/mm

Figure 4. Cervine hide collagen orientation, orientation index and directional tear strength map. Data is for two cervine halved hides from two animals (a, c) and (b, d) with neck at the left. (a, b) display the perpendicular tear strength. (c, d) display the parallel tear strength relative to the backbone. Vector direction indicates the collagen fibril orientation; vector magnitude indicates the OI; shading indicates tear strength relative to the backbone as detailed in the key: middle column (grey) average for the half hide +/- one standard deviation; left column (light grey) average – one standard deviation; right column (dark grey) average + one standard deviation.

SAXS Measurements

Small angle X-ray scattering produced clear scattering patterns with pronounced diffraction rings due to the collagen D-spacing (Figure 5). From these the azimuthal angle intensity variation was plotted and the well-defined distributions were used to obtain the average fibril orientation and orientation index at the 6th order diffraction peak. Most of the samples showed a well-defined unimodal fibril orientation.

Variation of O in the OSP Grid Square Sample

There was little variation between the O values calculated for each of the nine measuring points separated by 0.5 mm in the 3 x 3 grids studied (Figure 6 (b, d, f)). There was also little variation between SAXS measurements taken in the center of the OSP sample as compared to those taken in the corners of the OSP samples (Figure 6 a, c, e) indicating central sampling for O gave representative data for the entire grid sample area on the skins and hides in this study. The OSP is known to be the most consistent in regards to physical testing (such as tear), and hence so it was important to confirm the idea in the context of these SAXS measurements.

Orientation Index

The average OI varied between species with cervine skins having the least aligned collagen fibers and ovine hides having the most aligned fibers (Table II).

Orientation

The average collagen alignment was predominately perpendicular to the backbone in all species (average = 63°; SD ± 27°) (Table II). Despite this trend the O varied considerably between measurement points (Figure 2-4) and between species (Table IV).

Table IV

A comparison of averaged tear strength parallel and perpendicular to the backbone, orientation (O) and orientation index (OI) between species. P-values are for a t-test of whether the means are different (where P < 0.05 indicates the means are different).

Comparison	Parallel Tear Strength (N/mm)	Perpendicular Tear Strength (N/mm)	Orientation (°)	Orientation Index
P-value Ovine and Bovine	0.314	0.192	0.143	0.115
P-value Ovine and Cervine	0.103	0.056	0.172	0.140
P-value Bovine and Cervine	0.036	0.032	0.095	<0.001

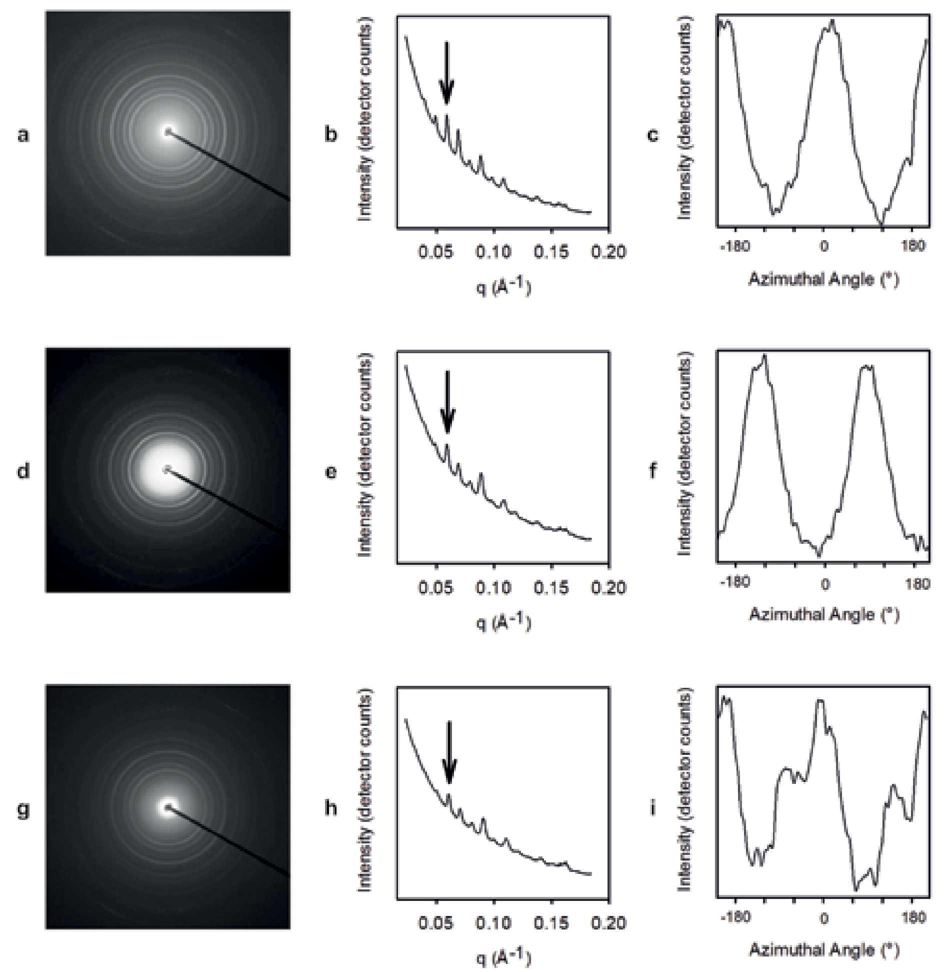

Figure 5. SAXS of leathers from the OSP of hides halved from neck to tail. (a, d and g) SAXS scattering patterns for (a) ovine; (d) bovine and (g) cervine species; (b, e and h) equatorial intensity profile where the selected 6th order D-period diffraction peak is indicated by the arrow; (c, f and i) azimuthal intensity profile of the selected 6th order D-period diffraction peak.

Correlation between O and Tear Strength

When the O and tear strength at each measurement site were compared (Figure 7), there was no significant correlation between the direction of the collagen fibers and the tear strength. Tear strength perpendicular to the backbone was not correlated with a low orientation angle, and the tear strength parallel to the backbone was not correlated with a high orientation angle.

Difference between Belly and Back

The individual values of OI and tear strength for each of two regions, the back area and the belly, of the skins and hides have been selected as a basis for comparison between species (Figure 8). These locations are likely to have similar structure-property relationships and may provide better measurements for comparison between species than averages for whole skins/hides. With this comparison we see the correlation between OI and tear strength in both tear directions is stronger in the backbone region of the bovine hides and cervine skins. In ovine skins the correlation is weak at both locations and tear directions (Table V). These finding are consistent with what has been tentatively proposed previously.[29]

Discussion

This study has shown that tear strength varies considerably over the skins of ovine, and hides of bovine and cervine leathers, and that there is no predictable pattern in an individual. However, there are limitations in this study with the sample size of just two animals per species.

Overall it was found that cervine and bovine hides had the greatest strength and ovine hides the least; in none of the species could a part of the hide be identified that had consistently higher strength than another. The variation in leather strength in different species has been observed before,[29] where it is primarily

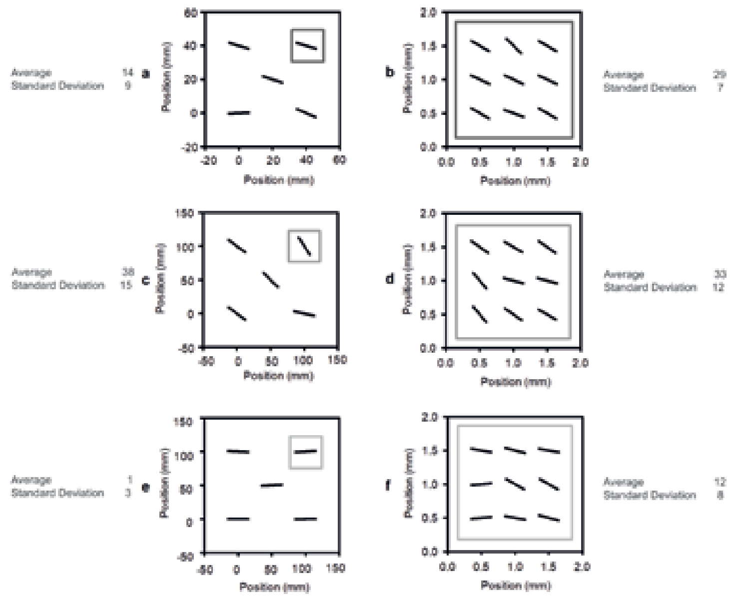

Figure 6. Collagen orientation (O) represented by the vector direction at different small angle x-ray scattering (SAXS) measuring positions in samples from the Official Sampling Position (OSP) of (a, b) an ovine skin; (c, d) a bovine hide; and (e, f) a cervine hide. For (a, c, e) the values are for the 5 SAXS measuring points made at the OSP sample grid square. For (b, d, f) the values are for the 3 x 3 sampling grid for the grey box, highlighted on the left figures, where the average O across the nine points creates this vector. 0° represents alignment from head to tail. Averages and standard deviations are calculated across all the vectors shown on the graph.

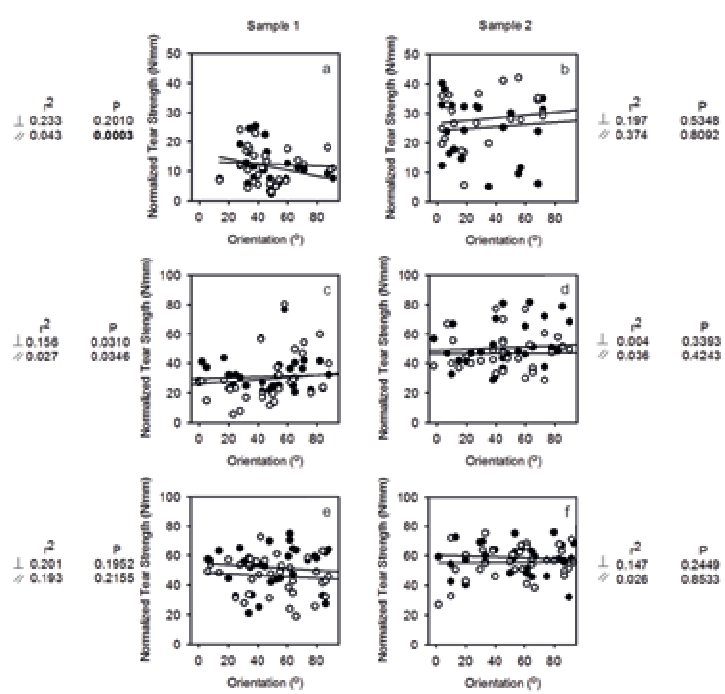

Figure 7. Relationship between orientation and tear strength measured perpendicular (filled circles) and parallel (hollow circles) to the backbone for (a,b) ovine (c,d) bovine and (e,f) cervine half hides at all measurement sites. r^2 and P values reflect best linear fit parameters where ⊥ represents perpendicular to the backbone measurements and // represents parallel to the backbone measurements. An orientation of 0° represents alignment from head to tail while 90° is perfectly perpendicular to the backbone.

Table V

Linear interpolation r^2 values for directional tear strength and Orientation Index (OI) in Figure 8.

Species	Trend line color	r^2 backbone (perpendicular)	r^2 backbone (parallel)	r^2 belly (perpendicular)	r^2 belly (parallel)
Ovine	Blue	0.12	0.25	0.33	0.33
Bovine	Green	0.54	0.42	0.05	0.30
Cervine	Black	0.55	0.74	0.15	0.15

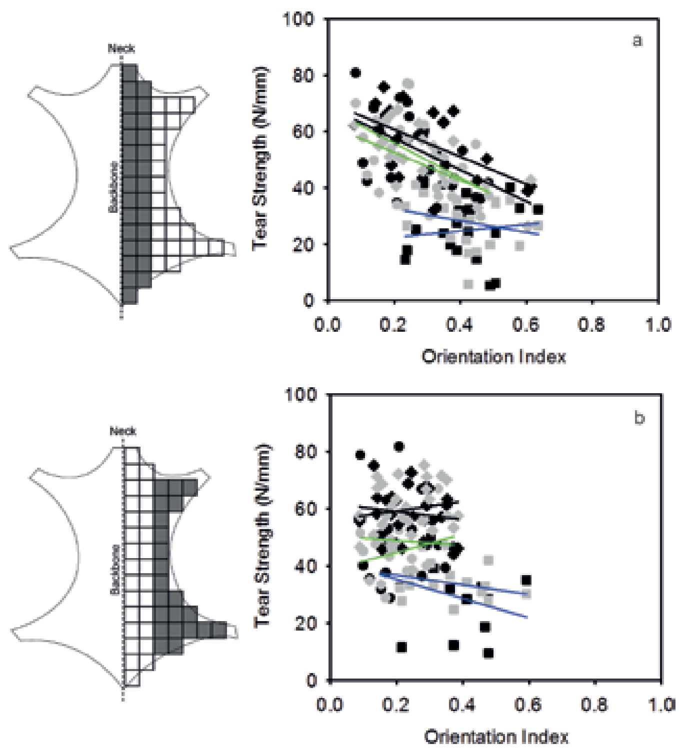

Figure 8. Relationship between tear strength measured perpendicular (black) and parallel (gray) with the orientation index (OI) along the (a) backbone and (b) belly for ovine (square), bovine (circle) and cervine (diamond). Linear interpolation r^2 values are shown in Table V.

thought to be due to the collagen alignment, viewed edge-on, with stronger leathers having a greater in plane alignment with little cross over between layers[35] or high angle of weave. We see different trends here with SAXS measurements made normal to the grain surface showing us the collagen alignment viewed flat-on. Fibril diameters have also been found to weakly correlate with leather strength in bovine leather, but not in ovine leather.[36]

Previous work on a calf hide and ovine skin found a preferred collagen alignment parallel to the backbone and limbs.[25,28] In contrast, the averaged results presented here found the preferred arrangement was perpendicular to the backbone across all species which may be attributed to the larger number of sampling points taken at the limbs, where the alignment tended to be more perpendicular to the backbone. Alignment parallel to the backbone is consistent with the idea that the collagen lies perpendicular to the maximum stretch direction needed for animal movement. A variety of studies have been performed on humans to determine surface movement errors in biomechanics resulting from markers placed on the skin when trying to determine bone movement during exercise.[37,38] Significant surface movement difference relative to bone movement is observed over joints,[39] suggesting skin has significant movement during motion. This supports the idea that forces applied to the skin resulting from animal movement could influence collagen alignment and that, this movement could result in collagen aligning in the direction of strain.[3,21] In cases where aligned collagen fibre experience repetitive strain, below their failure limit, the collagens helix substructure has been show to recover when the load is removed.[40] However, these forces are not acting in isolation. The downward force acting on skin from gravity is constant and can become larger when the weight is increased due to dense hair coverage. In the case of the ovine skins an additional 4-9 kg/animal of clean fleece weight[41] (with an additional 15-80% when grease and dirt is present[42]) is loaded onto the skin. If this persistent force is contributing to the strain applied on the skin this could provide an explanation for why the ovine skins were observed here to have the collagen fibrils most aligned in the direction of gravity for a standing animal.

Conclusions

Normalized tear strength measured across ovine and cervine skins and bovine hides showed cervine leathers had the greatest overall strength followed by bovine then ovine leathers. Ovine had the greatest variability in strength across the skin. There were no obvious regions of generally stronger or weaker skin within individual skins or hides. The predominant collagen fibril direction was perpendicular to the backbone of all species, with the greatest alignment found in ovine followed by cervine then bovine. These findings have important implications for manufacturers of leather products. They show there is no relationship between flat-on orientation measurements over the whole skin and with tear strength. The orientation maps presented here suggest it is the selection of the species that should be considered first if strength is a priority rather than the location within the hide.

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