Collagen Alignment and Leather Strength

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Abstract

Leather strength is believed to be largely due to the fibrous collagen which makes up a major proportion of the material. However, the strength of leather is not proportional to the amount of collagen which it contains. The structure of collagen in the leather produced from a range of animals is investigated using synchrotron based small angle x-ray scattering. It is shown that the tear strength of leather depends upon the alignment of the collagen fibrils. Tear-resistant material has the fibrils contained within parallel planes with little cross-over between the top and bottom surfaces. For tear strengths in the range 20–110 N/mm^2 the orientation index ranges from 0.420–0.633 with a direct relationship between orientation index and strength. Greater alignment within the plane of the tissue results in stronger material. This study provides a valuable insight into the structural basis of strength in leather and the inherent differences between animal skins.
Keywords: Synchrotron, small angle X-ray scattering, collagen, orientation, strength

Introduction

Collagen is the main structural component of leather, skin (Fratzl 2008) and other materials such as medical scaffolds (Floden et al. 2010). The strength of these collagen materials is of crucial importance for each application. In a medical context, strength is a necessity for collagen-based extracellular matrix materials whilst a primary requirement for leather applications, such as shoes and upholstery, is strength. Here we investigated the strength of collagen fibrils in leather and whether this corresponded with any physical properties of the material.

Leather, a material obtained through processing, is made up of two parallel planes that are mostly comprised of fibrous collagen. As a strong, flexible, water-resistant material, leather is used in a wide variety of manufacturing applications including shoes, bags, furniture coverings and car upholstery. However the leather used for these products is mostly bovine leather as it has a far greater strength than that of ovine leather. If the nanostructure of ovine leather was understood then the ability to manipulate processes in order to achieve ovine leather of higher strength may be realized.

In this experiment we analyse how the orientation of the collagen fibrils affects the strength of the leather. The amount of collagen present, the molecular structure of the collagen (D-spacing, collagen type), the nature of the cross-linking between collagen (Chan et al. 2009)

and collagen bundle size are all factors previously considered as possibly contributing to the strength of collagen materials.

Synchrotron based technique small angle X-ray scattering (SAXS) provides an ideal platform for nanostructure analysis of the fibrous collagen. The small angle scattering pattern acquired provides information regarding the structure and alignment of the collagen fibrils. This synchrotron technique allows us to determine why leather has the properties it does and how the fibril structure relates to the desirable attributes present. Leather manufactured from different animals is able to be looked at in depth through the use of synchrotron techniques. The leather produced from the skin of different animals has varying strength values. Thus it is possible to compare the strength of each sample with the orientation of the collagen fibrils using SAXS data results.

A statistically significant relationship was found between tear strength and edge-on orientation in our recent study of ovine and bovine leathers of differing strengths (Basil-Jones et al. 2011). We speculated that this trend may be of a more general nature among leather from various animal species. Thus to see if this relationship is more widely applicable among other animals, we have now measured fibril orientation in seven species of mammals. We used SAXS at a modern synchrotron facility as it allows analysis of a small area (250 × 80 μm). Therefore measurements of fibril orientation edge-on in tissues that are of limited thickness were able to be obtained.

Material and Methods

Leathers were generated using conventional techniques. Specifically fat and flesh was mechanically removed from the skin. Lime sulfide paint comprising of 140 g/L sodium sulfide, 50 g/L hydrated lime, and 23 g/L pre-gelled starch thickener was applied to the flesh side of the skin. The keratinaceous material was then removed after incubation of the skin at 20 °C for 16 hours. After this treatment, the skin was washed and the pH was lowered to 8 with ammonium sulphate followed by the addition of Tanzyme, a commercial bate enzyme. The treated skin was then washed followed by pickling in 20% sodium chloride and 2% sulfuric acid. The pickled pelt was degreased using a non-ionic surfactant, neutralised using 8% NaCl, 1% disodium phthalate solution and 1% formic acid, and tanned using chromium sulfate. The resulting wet-blue pelt was neutralised in 1% sodium formate and 0.15% sodium bicarbonate for 1 hour and then washed. The pelt was retanned using Tanicor, a synthetic retanning agent, and Mimosa, a vegetable extract. Fat liquors were added prior to drying and mechanical softening.

Tear strengths were measured for all samples using standard methods (Williams 2000a). In brief, samples were cut from the official sampling position (OSP) (Williams 2000b). The samples were then conditioned at a constant temperature and humidity (20 °C and 65% relative humidity) for 24 h after which time they were then tested on an Instron strength-testing device.

Samples were prepared for SAXS analysis by cutting strips of leather of 1 × 30 mm from the OSP. Each sample was mounted without tension in the X-ray beam in two directions; edge on so measurements could be taken through the thickness of the leather and flat on or normal to the surface of leather. Note that the flat samples were physically split into two layers, grain

and corium, to produce two samples from each piece of leather, before diffraction patterns were recorded. For the edge-on samples measurements were made every 0.25 mm with the measurements moving from the corium to the grain. The flat-on samples were mounted with the uncut face of the leather directed toward the X-ray beam. Four measurements were made per sample in a rectangular grid. Diffraction patterns were recorded on the Australian Synchrotron SAXS/WAXS beamline, using a high-intensity undulator source. Energy resolution of 10^{-4} was obtained from a cryo-cooled Si (111) double-crystal monochromator. The beam size [full width at half maximum (fwhm) focused at the sample was 250 × 80 μm, with a total photon flux of about 2×10^{12} photons s^{-1}. Diffraction patterns were recorded with an X-ray energy of 8 keV using a Pilatus 1M detector with an active area of 170 × 170 mm and a sample-to-detector distance of 3371 mm. Exposure time for the diffraction patterns was 1 s.

Data processing was carried out using SAXS15ID software (Cookson et al. 2006). Orientation index (OI) is defined as (90° − OA)/90°, where OA is the minimum azimuthal angle range, centered at 180°, that contains 50% of the microfibrils (Basil-Jones et al. 2010; Sacks et al. 1997). OI provides a measure of the spread of microfibril orientation. An OI approaching 1 indicates that the microfibrils are parallel to each other, whereas an OI of 0 indicates the microfibrils are randomly oriented. The OI is calculated from the spread in azimuthal angle of the most intense d-spacing peak (at around 0.059−0.060 $Å^{-1}$) (Basil-Jones et al. 2011). Each OI value presented here represents the average of 14−36 measurements of one sample. For edge-on mounted samples these measurements were taken at 0.25 mm intervals moving from the top of the corium to the bottom of the grain so that the whole thickness of the sample was covered. For flat-on analyses, measurements were taken at a number of points in a grid pattern. For the sheep and cattle samples the averages are derived from 228, 249, and 167 measurements from 15, 14,and 10 samples respectively and have been reported previously (Basil-Jones et al. 2011). The D-spacing was determined for each pattern by taking the central position of several of the collagen peaks, dividing these by the peak order (usually from n = 5 to n = 10), and averaging the resulting values.

Results and Discussion

Synchrotron based SAXS technique has become a crucial part of the project. The repeating fibril structure of collagen in the leather samples is represented by the SAXS pattern obtained on the beamline (Figure 1a). Each ring represents a collagen peak. Following integration, the collagen peaks become clearly distinguishable and the D spacing can be determined (Figure 1b).

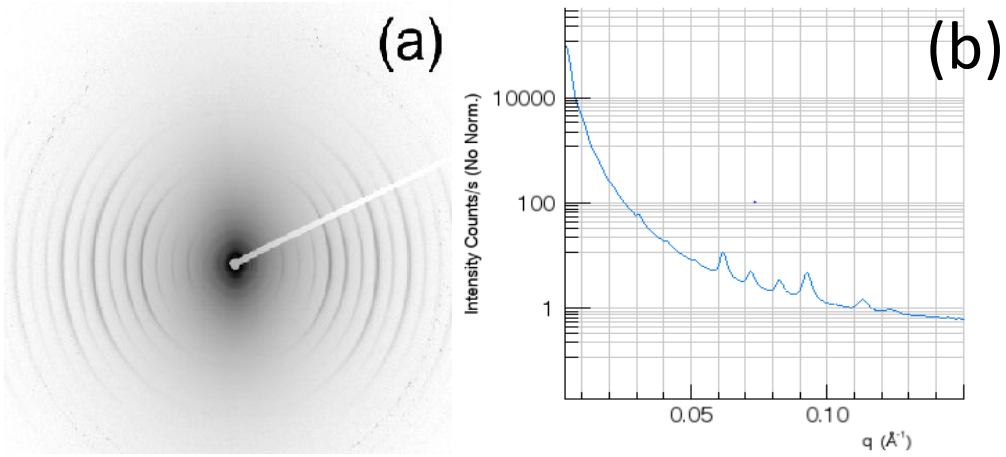

Figure 1. Leather analysis using SAXS (a) raw SAXS pattern. Reproduced from *J. Agric. Food Chem.* (2013) **61**, 887-892 © American Chemical Society; (b) integration of SAXS pattern.

Through a series of processing steps the orientation index (OI) of the collagen fibrils can be obtained. Both the D spacing and the OI illustrate the fibrous collagen structure within the two layers of the leather and the cross over between them. Upon the application of stress the fibrils may become more aligned and the individual fibres may stretch. We are able to determine how the fibrils react to the tension by looking at the D spacing and orientation index values.

The D spacing for the samples analysed ranged from 62.8 to 65.3 nm. This variation was found across a large range of strength (Figure 2). We do not find any significant correlation between d spacing and strength.

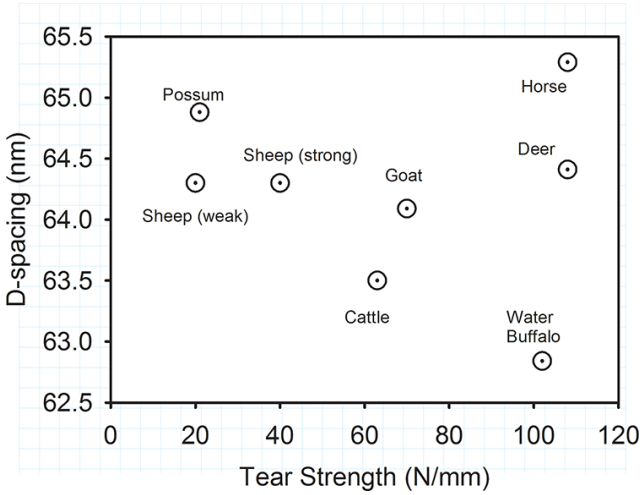

Figure 2. D spacing and tear strength of collagen fibrils in leather for selected mammals. Reproduced from *J. Agric. Food Chem.* (2013) **61**, 887-892 © American Chemical Society.

Leather samples analysed at the Australian Synchrotron were cut from leather skins in specific directions. With a full range of samples measured from all angles, a clear three dimensional understanding of the collagen fibrils was obtained. Figure 3 refers to the direction in which measurements were taken. "Backbone" indicates the direction of the animal backbone on the leather pelt. The edge on measurements taken span through the full thickness of the leather thus enabling the corium and grain layers and the crossover of fibrils

between the layers to be analysed. The OI for these measurements conveys the degree to which the collagen fibrils are aligned within planes of the leather. The measurements taken normal to the surface portray the fibrils on the surface of the leather.

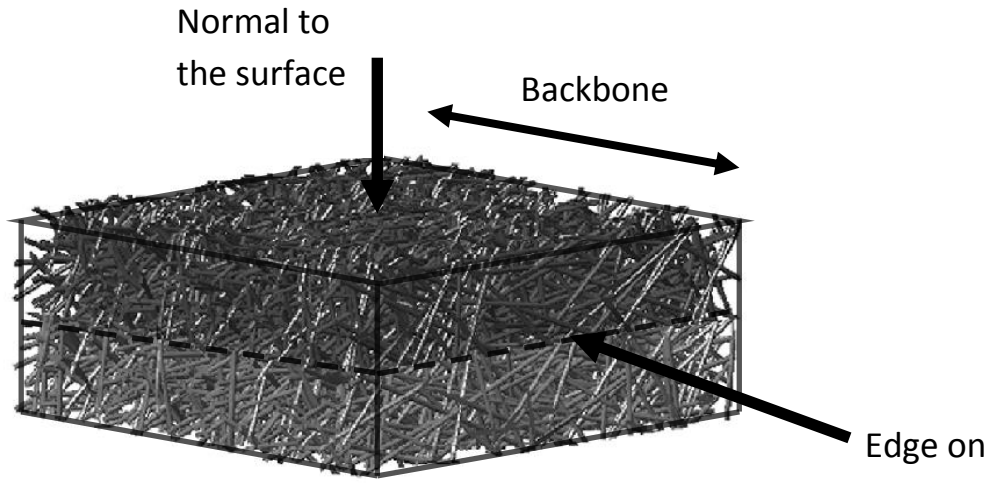

Figure 3. Measurement directions of leather samples used during analysis on the SAXS beamline at the Australian Synchrotron.

There is a large difference in OI between the measurements taken normal to the surface and measurements taken edge on. The OI numbers for the measurements normal to the surface are in the range of 0.18−0.35, with the exception of horse leather (Figure 4a). The edge-on measurements displayed OI values significantly higher (0.41−0.63) indicating the major component of fibril alignment is within the planes of the leather (Figure 4b) (Sizeland et al. 2013).

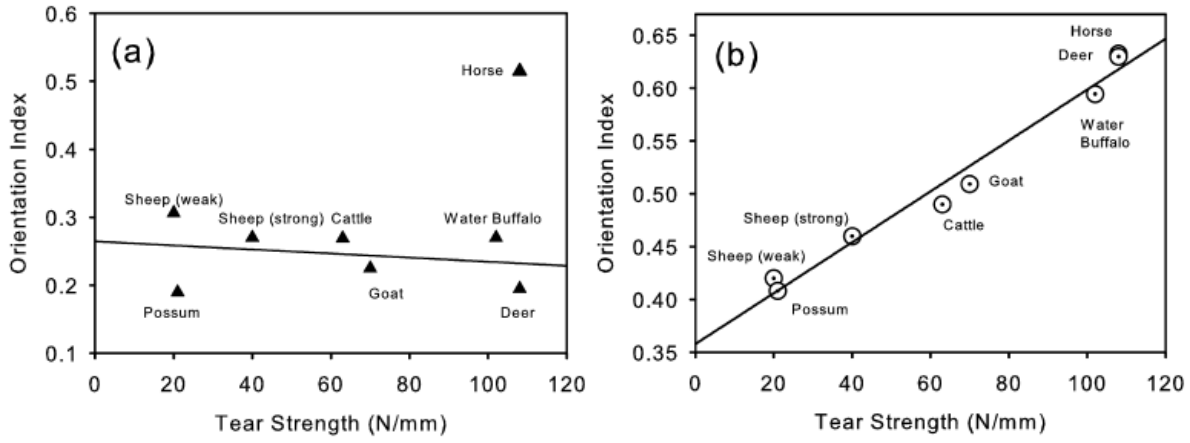

Figure 4. Orientation index and tear strength of collagen fibrils in leather for selected mammals (a) measured normal to the surface, (b) measured edge on. Reproduced from *J. Agric. Food Chem.* (2013) **61**, 887-892 © American Chemical Society.

We find a strong correlation between OI and tear strength for the edge on measurements. For the measurements taken normal to the surface of leather, we find little correlation if horse leather is excluded as an outlier. Therefore the tear strength of leather is relative to the planar alignment of the collagen fibrils.

When fibrils are not aligned within the planes but rather are aligned perpendicular to the planes, the fibres will not put up much resistance as they will be separated by any force applied (Figure 5a). This occurs in Hereford cattle (Amos 1958; Kronick and Sacks 1991) and is known as vertical fibre defect. No samples with this defect were included in this study. When there is a high degree of alignment of the fibres in the planes, maximum strength is obtained (Figure 5c). When fibres are found to be anisotropic, strength will be greater than samples with vertical fibre defect as some of the fibrils will be more in line with the parallel planes of the leather.

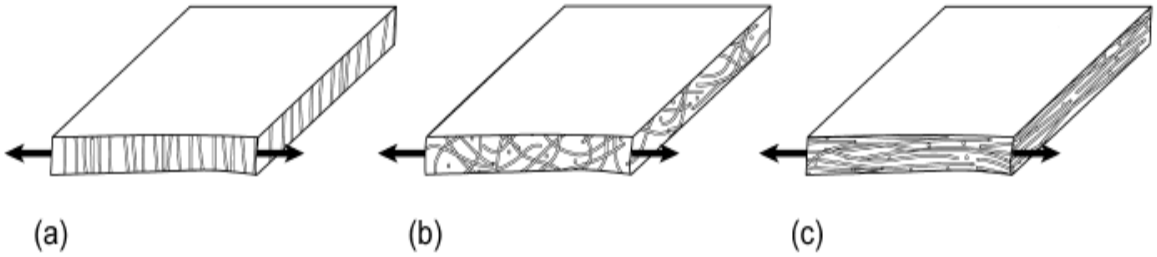

Figure 5. Relationship between OI of collagen fibrils and strength of skin. OI measured edge-on with orientation that results in leather that is (a) very weak (vertical fibre defect), (b) medium strength (low OI), or (c) strong (high OI). Arrow indicates direction of applied stress in tear measurements. Reproduced from *J. Agric. Food Chem.* (2013) **61**, 887-892 © American Chemical Society.

Tearing is used as the industry standard for leather strength. Tearing occurs at the two ends of a linear cut hole when looking normal to the surface of the leather (Figure 6). High OI values may indicate fibres running both parallel to (Figure 6a) and perpendicular to (Figure 6b) the hole. Strength will be low for samples with these structures as fibrils can be pulled apart along the shear lines with minimal force. Consequently it is expected that maximum strength will be associated with low OI indicating anisotropically arranged fibrils (Figure 6c).

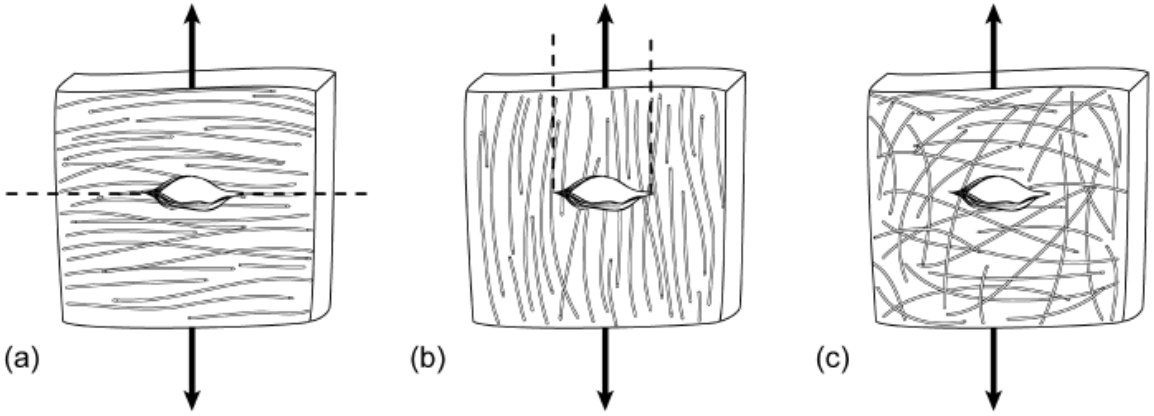

Figure 6. Relationship between OI of collagen fibrils and strength of skin. OI measured normal to the surface with orientation that results in leather that is (a) weak (high OI), (b) fairly weak (high OI), (c) strong in all directions. Arrow indicates direction of applied stress in tear measurements. Dashed lines represent probable lines of failure. Reproduced from *J. Agric. Food Chem.* (2013) **61**, 887-892 © American Chemical Society.

Conclusion

We investigated leather samples from a range of different mammals to develop an understanding of the structure-strength relationship. The correlation between tear strength and orientation of fibrils for edge on samples is remarkably quantitative. The strength range across which this relationship holds is much greater than has previously been demonstrated. This insight into collagen fibril structure may extend to tissues other than those studied here.

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