Synchrotron Studies of Leather Structure

Richard G. Haverkamp[1], Melissa M. Basil-Jones[1], Katie H. Sizeland[1], Gillian E. Norris[2], Richard L. Edmonds[3].

[1]School of Engineering and Advanced Technology, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand.

[2]Institute of Fundamental Sciences, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand.

[3]Leather and Shoe Research Association, PO Box 8094, Hokowhitu, Palmerston North 4446, New Zealand.
r.haverkamp@massey.ac.nz

Abstract

The arrangement of collagen fibrils in leather is complex. Synchrotron based small angle X-ray scattering enables detailed structural information to be obtained. The variation in fibril orientation through cross sections of leather, and structural responses to dynamic loads between strong and weak leather were studied. Under tension, fibrils reorient at low strain then individual fibrils stretch at higher strain. In strong leather the load is taken up more uniformly across the thickness of leather compared with weak leather. This study provides an insight into the structural basis of strength in leather and the response of leather to stress.

Keywords: Synchrotron, Small angle x-ray scattering, orientation, strength

1. Introduction

The physical properties of leather depend upon the composition (proteins, fat, other components), the structural arrangement of these components and the chemical interactions between these components. Collagen is the main structural component of leather, responsible for its strength. Other molecular components, even at minor concentrations, can interact with collagen to affect the physical characteristics of leather. In particular, chemical cross-linking agents such as chromium salts or tannins link collagen fibrils and alter their mechanical properties and oils may act as lubricants of the collagen matrix and alter the strength and suppleness of the leather. These components are fundamental to leather making.

Therefore, to understand leather, knowledge of the collagen fibril structure and arrangement is important. Measurement and an understanding of the variation of this structure with different processing methods, from different skins and hides, in good leather and defective leather, and during tension can contribute to the control of mechanical properties and to the art of leather manufacture.

The arrangement of collagen fibril structure in biological tissues has long been a subject of interest and has been investigated by a variety of methods. These methods include reflection

anisotropy (Schofield et al. 2011), atomic force microscopy (Friedrichs et al. 2007), small angle light scattering (Billiar and Sacks 1997), confocal laser scattering (Jor et al. 2011), raman polarization microscopy (Falgayrac et al. 2010), anisotropic raman scattering (Janko et al. 2010), multiphoton microscopy (Lilledahl et al. 2011) and small angle X-ray scattering (SAXS) (Basil-Jones et al. 2010; Basil-Jones et al. 2011; Basil-Jones et al. 2012a; Boote et al. 2002; Kronick and Buechler 1986; Sizeland et al. 2013b).

It is recognized that defects in the collagen fibre arrangement can lead to poor quality leather with vertical fibre defect, a well know issue with some Hereford leather (Duivestein et al. 2000; Kronick and Sacks 1991).

X-ray diffraction is is a powerful technique for investigating material structures, giving information on long range structural order, with repeat distances in the order of 0.1 to 10 nm. It can be used for protein assemblages, such as collagen. For protein structures, small diffraction angles, θ, are measured, typically less than 10 degrees so that this X-ray diffraction technique is referred to as small angle X-ray scattering (SAXS). In SAXS the scattering momentum q is generally used rather than θ ($q = 4\pi sin\theta/\lambda$ where λ is the X-ray wavelength used) as it allows direct comparison of SAXS measured at different incident X-ray wavelengths. SAXS can provide information on macromolecules either in solution or in solid materials (Bernado et al. 2007; Tsutakawa et al. 2007) and has been used to investigate the structure of collagen (Cameron et al. 2002) and collagenous materials such as tendon (Sasaki and Odajima 1996a; Sasaki and Odajima 1996b); bone (Burger et al. 2008; Cedola et al. 2006) and human articular cartilage (Mollenhauer et al. 2003). SAXS provides a quantitative measurement of both fibril orientation and the average d-spacing of collagen within the irradiated volume of sample.

SAXS can be performed on laboratory based instruments or on more synchrotron based instruments. The SAXS beam line at the Australian Synchrotron has several advantages over laboratory sources for characterization of leather. These are a focused beam (typically 250 x 80 μm) enabling detailed cross sectional analysis of leather, very rapid sample collection, and very good signal to noise ratio due to the very high X-ray flux and very sensitive two dimensional detector. In addition the facility is staffed by scientists who are expert in their field and who maintain the facility at optimum performance and provide support to users. Access to the SAXS beamline is by competitive application. The facility operates 24 hours per day and experiments normally must be completed over a limited time, typically one or two days, so that very careful preparation is necessary prior to each experiment.

Here we describe the use of SAXS to understand the structure and arrangement of collagen fibrils in ovine and bovine leather and to derive the relationship between collagen structure and the physical characteristics, particularly strength, of leather.

2. Material and Methods

The methods have been described previously (Basil-Jones et al. 2012a).

Ovine pelts were from 5-month-old, early season "black faced" lambs, of breeds which may include Suffolk, South Suffolk and Dorset Down. The bovine hides were from 2–3 year old cattle of a variety of breeds.

Leathers were generated with various properties by using a range of processing parameters both during the conventional beamhouse process and then during the conventional tanning of the pelts. Specifically, the pelts were depilated using a caustic treatment comprising sodium sulfide (ranging from a slow-acting paint containing 160 g/L flake sodium sulfide to a quick-acting paint containing 200 g/L sodium sulfide) and a saturated solution of calcium hydroxide. Depilated slats were then processed to remove the residual wool in a solution of sodium sulfide ranging in concentration from 0.8 to 2.4% for 8–16 hours at temperatures ranging from 16 °C to 24 °C. After this treatment, the pelts were washed and treated with a proteolytic enzyme – either a bacterial enzyme (Tanzyme, Tryptec Biochemicals Ltd) or a pancreatic one (Rohapon ANZ, Shamrock Ltd) – at concentrations ranging from 0.025 % to 0.1 %, followed by pickling in a 2% sulfuric acid, 10% sodium chloride solution. The pickled pelts were then pretanned using oxazolidine, degreased with an aqueous surfactant and before being tanned using chromium sulfate. The resulting “wet blue” was then retanned using a mimosa vegetable extract and impregnated with lubricating oil prior to drying and mechanical softening.

The tear strength of the crust leathers was tested using standard methods (Williams 2000a).Samples (strips 1 x 50 mm) were cut from the leather at the official sampling positions (OSP) (Williams 2000b), parallel to the backbone. The bovine leather was shaved to produce samples approximately 1.3 mm thick consisting, on average, of 34% grain and 66% corium. All samples were then conditioned by storage at a constant temperature and humidity (20°C and 65% relative humidity, respectively) for 24 hours, after which time they were tested using an Instron strength-testing device.

Leather samples were tested either statically or under load. Static samples were mounted on a plate containing up to 24 samples with sample changing taking place remotely (Figure 1, left).

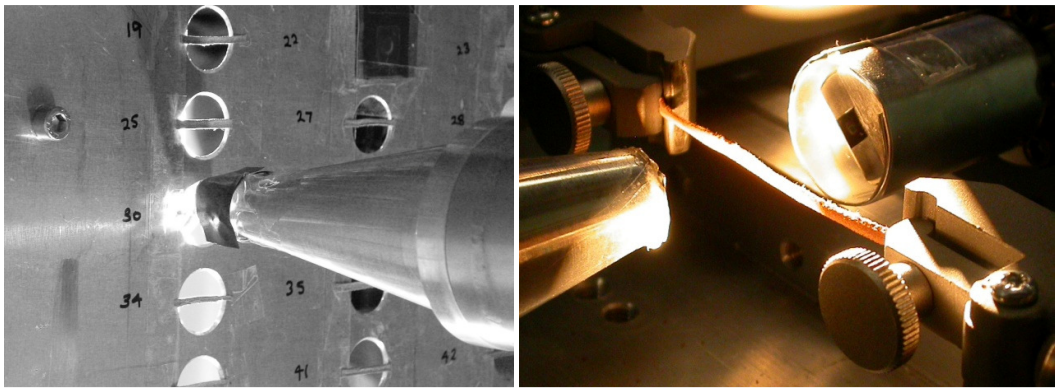

Figure 1. Sample mounting for SAXS measurements for left) static measurements. Reproduced from *J. Agric. Food Chem.* (2010) **58**, 5286-5291 © American Chemical Society; right) measurement during stretching. Reproduced from *J. Agric. Food Chem.* (2012) **60**, 1201−1208 © American Chemical Society.

For the samples under load, a stretching apparatus was built (Figure 1, right). A linear motor, Linmot PS01 – 48x240/30x180-C (NTI AG, Switzerland), was mounted on a purpose-built frame with a custom-made clamp fitted to the end of the slider. The clamp was designed so that the leather would not be subjected to a sharp point load. A L6D Aluminum Alloy OIML single-point loadcell (Hangzhou Wanto Precision Technology Co., Zhejiang, China) was attached to second clamp that held the other end of the sample and was attached to the frame. Each leather sample was mounted horizontally between the clamps without tension and then moved into the X-ray beam. The sample was stretched in 1 mm increments until a force was registered by the loadcell. The slider was then moved back 1 mm to reduce the tension on the sample and diffraction patterns were recorded edge-on and parallel to the backbone. Measurements were made, depending on the mounted orientation of the leather, either normal to the leather surface (flat) in a 0.5 mm grid of four points or edge-on at 0.10 to 0.20 mm intervals across the sample from the grain to the corium. The sample was stretched 1 mm, and maintained at this extension for 1 minute to stabilize, before a SAXS pattern was recorded. The extension and the force information were recorded. This process was repeated until the sample failed. Note that the flat samples were physically split into two layers, grain and corium, resulting in the production of two samples from each piece of leather, before diffraction patterns were recorded.

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 to produce a beam size (FWHM focused at the sample) of 250 x 80 µm, with a total photon flux of about 2 x 10^{12} ph.s^{-1}. All diffraction patterns were recorded with an X-ray energy of 11 keV (or 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 absorption edge of zinc at 9.659 keV was used to calibrate the energy and to set the zero angle of the monochromator. This resulted in energy calibration across the energy range used being better than 5 eV and typically better than 2 eV. A diffraction peak of silver behenate was used to scale the camera length. The correct value of q is then calculated by trigonometry for each pixel in each diffraction image. Exposure time for diffraction patterns was 1 s and data processing was carried out using the SAXS15ID software (Cookson *et al.* 2006). No normalization was performed for changes in beam intensity.

3. Results and Discussion

The SAXS patterns recorded on leather show clearly the d-spacing and the orientation of the fibrils (Figure 2). The d-spacing is visible as a series of partial (or full) rings. The rings are of increasing diffraction order, so that the d-spacing can be calculated from any ring by dividing the space between rings by the order. In practice, the d-spacing is determined by averaging the central values of several collagen peaks (usually from n = 5 to n = 10). The orientation is apparent in the extent to which the collagen d-spacing diffraction rings extend around the full circle. This is quantified this by defining an orientation index (OI), which is defined as (90° – OA)/90°. The OA is the minimum azimuthal angle range that contains 50% of the microfibrils centered at 180°. The OI is used to give a measure of the spread of microfibril orientation (an OI of 1 indicates the microfibrils are completely parallel to each other; an OI of 0 indicates

the microfibrils are completely randomly oriented). The OI is calculated from the spread in azimuthal angle of one of the most intense d-spacing peaks (at around 0.059–0.060 $Å^{-1}$).

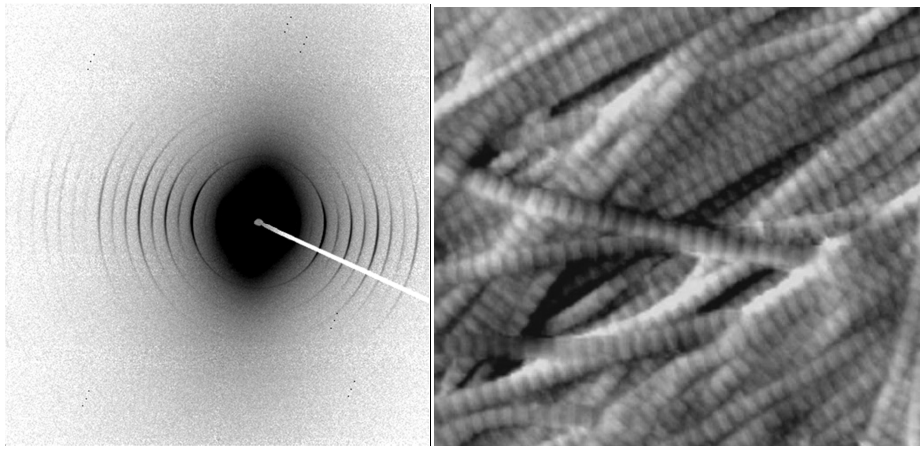

Figure 2. Left: SAXS pattern of leather, showing the rings due to the d-banding of collagen. The orientation is apparent by the rings not extending around 360°. Right: An atomic force microscope image of bovine intestine, for an illustration of the structure of collagen fibrils to aid in understanding the SAXS pattern.

Selective information can be extracted from the SAXS pattern and represented in different forms. For example a radially integrated pattern can be plotted (losing the information on azimuthal variation) as in Figure 3 (left). The sharp peaks at higher *q* are due to the d-period, whereas the broader pattern at low *q* is due to the thickness of the collagen fibrils and the bundling of these fibrils. The variation in intensity with azimuthal angle can also be plotted. For example the variation in intensity at just one radial angle (corresponding to a d band) can be plotted as in Figure 3 (right) showing fibrils well aligned in the horizontal axis (0° – 180°). An orientation profile can be obtained by taking just this portion of data for a series of measurements through the thickness of a piece of leather from grain to corium (Figure 4).

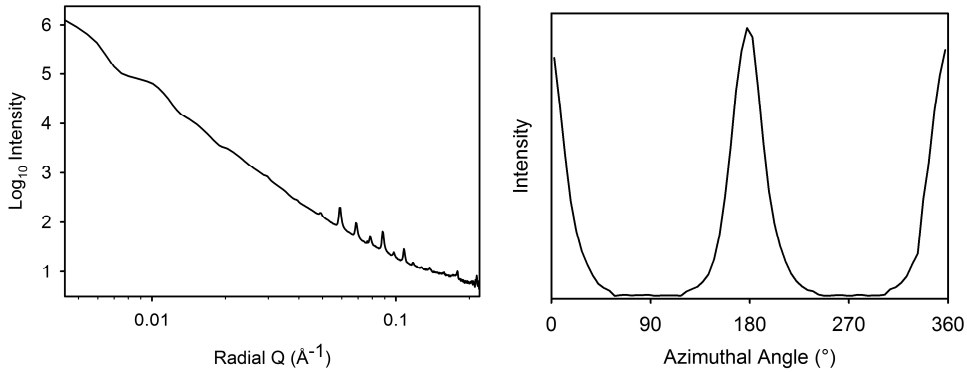

Figure 3. Left: Radially integrated SAXS pattern with the sharp peaks due to the d-period visible; Right: Azimuthally integrated SAXS pattern at a radial angle corresponding with a d-period indicating fibril orientation.

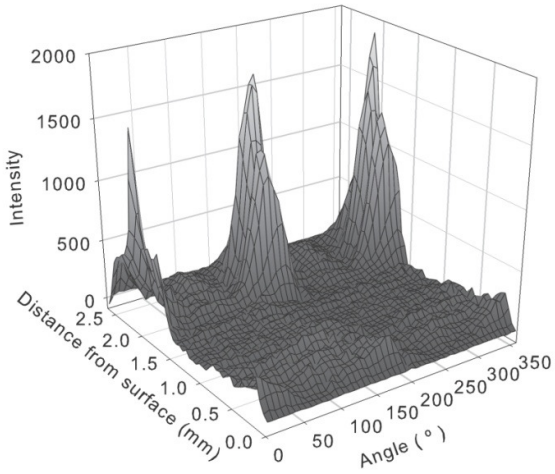

Figure 4. The Azimuthally integrated SAXS pattern through the thickness of ovine leather at the collagen d-spacing of around 0.059-0.06 $Å^{-1}$. Reproduced from *J. Agric. Food Chem.* (2010) **58**, 5286-5291 © American Chemical Society.

With these techniques it is possible to investigate the variation in d-spacing and fibril orientation through the thickness of leather, or at different positions in a piece of leather. For example, scanning through ovine and bovine leather a variation in d-spacing is observed with a lower d-period in the central region (Figure 5, left). The OI also varies through the leather, with a lower OI in the grain and a higher OI in the corium. This difference in orientation is known from optical imaging, however, SAXS enables a more reliable quantification of the average fibril orientation.

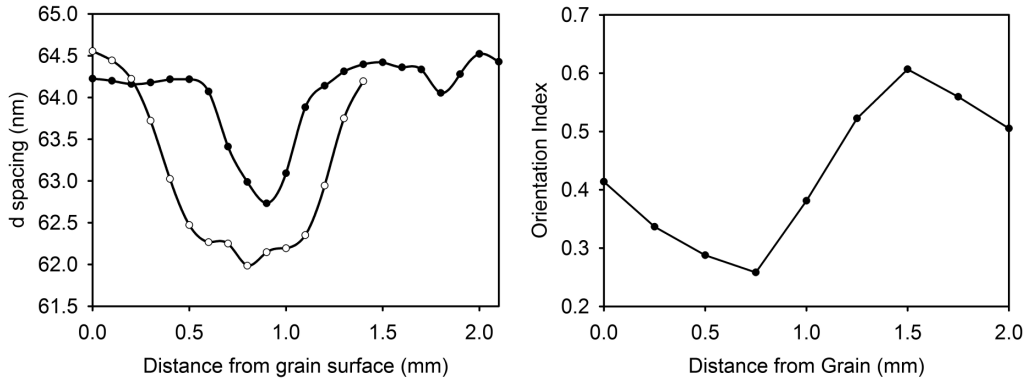

Figure 5. Left: Variation in d-spacing through representative samples of (●) ovine and (○) bovine leathers. Right: Average orientation index across the thickness of ovine leather measured parallel to the backbone. An average of 24 leather samples (1 measurement per sample). Adapted from *J. Agric. Food Chem.* (2011) **59**, 9972–9979 © American Chemical Society.

Variations in d-period and OI for leathers of differing strength have been compared. There is not a strong correlation of d-period with strength (Basil-Jones et al. 2011; Sizeland et al. 2013b). There is however a good correlation of OI with strength (Basil-Jones et al. 2011; Sizeland et al. 2013a; Sizeland et al. 2013b). A comparison of ovine leather grouped into weak and strong samples, and bovine leather which is stronger than the ovine leather, shows a significant correlation between average OI and strength (Figure 6). A regression line fitted to the averages of the three groups of data for OI and tear strength (low strength ovine: 15 samples, 228 analysis points; higher strength ovine: 14 samples, 249 analysis points; and bovine: 10 samples, 167 analysis points) has a slope of 1.708×10^{-3} mm/N, $r^2 = 0.20$, $p = 4.45 \times 10^{-5}$. This relationship applies within a species (i.e. sheep) and is maintained between species (sheep and cattle). These measurements have recently been extended to hides from other mammals (Sizeland et al. 2013a; Sizeland et al. 2013b). The results obtained suggest that the relationship between fibril orientation and strength is a universal property of leather: strength is determined by fibril orientation, such that stronger leather has the fibrils arranged mostly parallel to the plane of the leather surface (low angle of weave), while weaker leather has more out-of-plane fibrils (higher angle of weave).

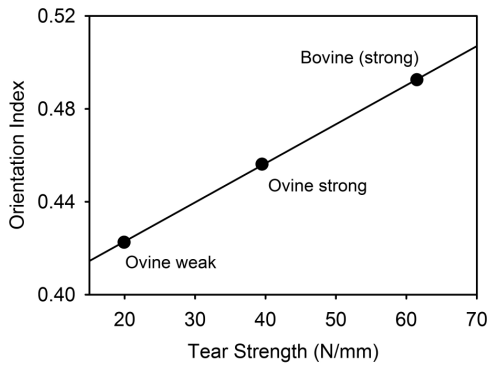

Figure 6. Orientation versus tear strength for the averages of each of the leather types measured through the edge parallel to the backbone. Adapted from *J. Agric. Food Chem.* (2011) **59**, 9972–9979 © American Chemical Society.

Further information on the mechanical performance of leather can be obtained by stretching during SAXS measurements. It is observed that, under applied stress, the collagen fibrils in leather first rearrange to become more aligned (OI increases) before the fibrils stretch and the d-period increases (Figure 7). The change in d-period can be used as an internal strain gauge, giving a measure of the strain experienced by individual fibrils.

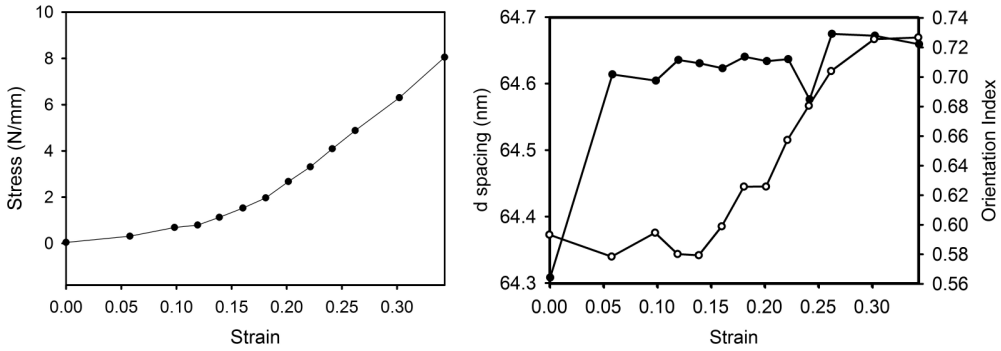

Figure 7. d-spacing and orientation versus strain measured edge-on, parallel to the backbone: (○) d spacing and (●) OI (parallel, OSP) for stronger ovine, 39 N/mm tear strength. Reproduced from *J. Agric. Food Chem.* (2012) **60**, 1201−1208 © American Chemical Society.

It is also possible to monitor the variation in fibril orientation and d-spacing through the thickness of leather during strain. The stress that the collagen fibrils experience in different parts of the leather differs, as shown in Figure 8 where the fibrils in the centre of the corium and the surface of the grain of weak ovine leather undergo significantly more deformation that in the central region of the leather. There are differences in the deformation behaviour of weak and strong leather with strong leather tending to take up the stress over the full width of the leather and weak leather tending to have an uneven distribution of stress (Basil-Jones et al. 2012b).

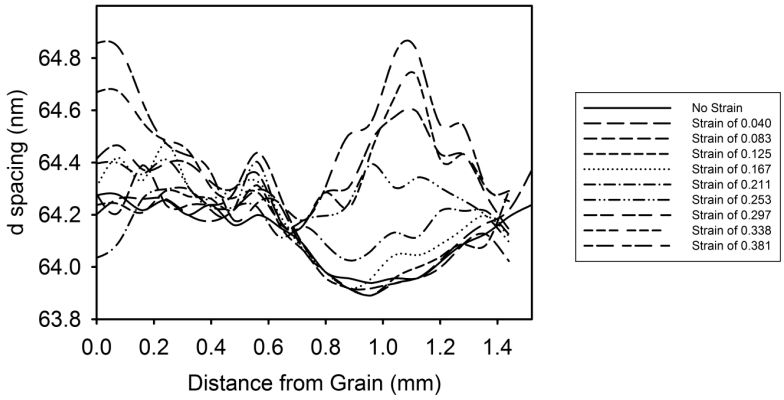

Figure 8. d spacing through the thickness of the leather and change in d spacing as a consequence of increasing strain, measured edge-on parallel to the backbone for weak ovine, 19 N/mm tear strength. Adapted from *J. Agric. Food Chem.* (2012) **60**, 1201−1208 © American Chemical Society.

4. Conclusion

Synchrotron based small angle X-ray scattering has been shown to be a powerful technique for understanding the structure of leather. It provides information on fibril orientation and structure. Changes taking place during strain of the leather have been observed. These techniques have been used to study strength–structure relationships. It has been possible to

show a correlation between strength and collagen fibril orientation, where leather that has fibrils more aligned in the plane of the leather is stronger. During tension, collagen fibrils first realign and then stretch. These methods can be extended to study the structure of leather during the stages of processing, with different tanning techniques and can enable the properties of leather obtained by different tanning procedures to be understood in terms of microstructure.

5. Acknowledgements

This research was undertaken on the SAXS/WAXS beamline at the Australian Synchrotron, Victoria, Australia. Nigel Kirby, Adrian Hawley and Stephen Mudie of the Australian Synchrotron are acknowledged for their contribution to this work. The NZ Synchrotron Group Ltd is acknowledged for travel funding. This work was supported by the Foundation for Research Science and Technology grant number LSRX0801.

6. References

Basil-Jones, M.M., Edmonds, R.L., Allsop, T.F., Cooper, S.M., Holmes, G., Norris, G.E., Cookson, D.J., Kirby, N.Haverkamp, R.G., 2010, Leather structure determination by small angle X-ray scattering (SAXS): cross sections of ovine and bovine leather, J. Agric. Food Chem., 58(9) 5286-5291p.

Basil-Jones, M.M., Edmonds, R.L., Cooper, S.M.Haverkamp, R.G., 2011, Collagen fibril orientation in ovine and bovine leather affects strength: A small angle X-ray scattering (SAXS) study, J. Agric. Food Chem., 59(18) 9972-9979p.

Basil-Jones, M.M., Edmonds, R.L., Norris, G.E.Haverkamp, R.G., 2012a, Collagen fibril alignment and deformation during tensile strain of leather: A SAXS study, J. Agric. Food Chem., 60(5) 1201-1208p.

Basil-Jones, M.M., Edmonds, R.L., Norris, G.E.Haverkamp, R.G., 2012b, Collagen Fibril Alignment and Deformation during Tensile Strain of Leather: A Small-Angle X-ray Scattering Study, J. Agric. Food Chem., 60(5) 1201-1208p.

Bernado, P., Mylonas, E., Petoukhov, M.V., Blackledge, M.Svergun, D.I., 2007, Structural characterization of flexible peoteins using small-angle X-ray scattering, J. Am. Chem. Soc., 129(17) 5656-5664p.

Billiar, K.L.Sacks, M.S., 1997, A method to quantify the fiber kinematics of planar tissues under biaxial stretch, J. Biomech., 30(7) 753-756p.

Boote, C., Sturrock, E.J., Attenburrow, G.E.Meek, K.M., 2002, Psuedo-affine behaviour of collagen fibres during the uniaxial deformation of leather, J. Mat. Sci., 37 3651-3656p.

Burger, C., Zhou, H.W., Sics, I., Hsiao, B.S., Chu, B., Graham, L.Glimcher, M.J., 2008, Small-angle X-ray scattering study of intramuscular fish bone: collagen fibril superstructure determined from equidistant meridional reflections, J. Appl. Crystallogr., 41 252-261p.

Cameron, G.J., Alberts, I.L., Laing, J.H.Wess, T.J., 2002, Structure of type I and type III heterotypic collagen fibrils: an x-ray diffaction study, J. Struct. Biol., 137 15-22p.

Cedola, A., Mastrogiacomo, M., Burghammer, M., Komlev, V., Giannoni, P., Favia, A., Cancedda, R., Rustichelli, F.Lagomarsino, S., 2006, Engineered bone from bone marrow stromal cells: A structural study by an advanced x-ray microdiffraction technique, Phys. Med. Biol., 51(6) N109-N116p.

Cookson, D., Kirby, N., Knott, R., Lee, M.Schultz, D., 2006, Strategies for data collection and calibration with a pinhole-geometry SAXS instrument on a synchrotron beamline, J. Synchrotron Radiat., 13 440-444p.

Duivestein, J., Pitchford, W., Bottema, C., Fassbender, R., Cusack, D., Meldrum, R.Leather, M., 2000, Detection of vertical fiber hide defect (VFHD) in Hereford cattle hides by biopsy, J. Am. Leather Chem. As., 95(3) 92-101p.

Falgayrac, G., Facq, S., Leroy, G., Cortet, B.Penel, G., 2010, New method for Raman investigation of the orientation of collagen fibrils and crystallites in the haversian system of bone, Appl. Spectrosc., 64(7) 775-780p.

Friedrichs, J., Taubenberger, A., Franz, C.M.Muller, D.J., 2007, Cellular remodelling of individual collagen fibrils visualized by time-lapse AFM, J. Mol. Biol., 372(3) 594-607p.

Janko, M., Davydovskaya, P., Bauer, M., Zink, A.Stark, R.W., 2010, Anisotropic Raman scattering in collagen bundles, Opt. Lett., 35(16) 2765-2767p.

Jor, J.W.Y., Nielsen, P.M.F., Nash, M.P.Hunter, P.J., 2011, Modelling collagen fibre orientation in porcine skin based upon confocal laser scanning microscopy, Skin Res. Technol., 17(2) 149-159p.

Kronick, P.L.Buechler, P.R., 1986, Fiber orientation in calfskin by laser-light scattering or X-ray-diffraction and quantitative relation to mechanical-properties, J. Am. Leather Chem. As., 81(7) 221-230p.

Kronick, P.L.Sacks, M.S., 1991, Quantification of vertical-fiber defect in cattle hide by small-angle light-scattering, Connect. Tissue Res., 27(1) 1-13p.

Lilledahl, M.B., Pierce, D.M., Ricken, T., Holzapfel, G.A.Davies, C.D., 2011, Structural analysis of articular cartilage using multiphoton microscopy: input for biomechanical modeling, IEEE T. Med. Imaging, 30(9) 1635-1648p.

Mollenhauer, J., Aurich, M., Muehleman, C., Khelashvilli, G.Irving, T.C., 2003, X-Ray Diffraction of the Molecular Substructure of Human Articular Cartilage, Connect. Tiss. Res., 44(5) 201-207p.

Sasaki, N.Odajima, S., 1996a, Elongation mechanisms of collagen fibils and force-strain relations of tendon at each level of structural hierarchy, J. Biomech., 29(9) 1131-1136p.

Sasaki, N.Odajima, S., 1996b, Stress-strain curve and youngs modulus of a collagen molecule as determined by the x-ray diffaction technique, J. Biomech., 29(5) 655-658p.

Schofield, A.L., Smith, C.I., Kearns, V.R., Martin, D.S., Farrell, T., Weightman, P.Williams, R.L., 2011, The use of reflection anisotropy spectroscopy to assess the alignment of collagen, J. Phys. D Appl. Phys., 44(33).

Sizeland, K.H., Basil-Jones, M.M., Edmonds, R.L., Cooper, S.M.Haverkamp, R.G., 2013a, Collagen Alignment and Leather Strength, IULTCS 2013, Istanbul, Turkey,

Sizeland, K.H., Haverkamp, R.G., Basil-Jones, M.M., Edmonds, R.L., Cooper, S.M., Kirby, N.Hawley, A., 2013b, Collagen Alignment and Leather Strength for Selected Mammals, J. Agric. Food Chem., 61(4) 887-892p.

Tsutakawa, S.E., Hura, G.L., Frankel, K.A., Cooper, P.K.Tainer, J.A., 2007, Structural analysis of flexible proteins in solution by small angle X-ray scattering combined with crystallography, J. Struct. Biol., 158(2) 214-223p.

Williams, J.M.V., 2000a, IULTCS (IUP) test methods - Measurement of leather softness, J. Soc. Leather Tech. Ch., 84(7) 377-379p.

Williams, J.M.V., 2000b, IULTCS (IUP) test methods - Measurement of tensile strength and percentage elongation, J. Soc. Leather Tech. Ch., 84(7) 317-321p.