Measurement of Fat-Liquor Distribution in Ovine Leather

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Abstract

Fat-liquor is an important component in the processing of leather. The impact of processing changes that result in changes in the fat-liquor content have previously been shown to impact on the organoleptic and physical properties of the final leather. Fundamental research is therefore being carried out within a research consortium funded by the New Zealand Ministry of Science and Innovation to understand the impact of fat-liquor in the rheological properties of the resulting leather.

Analytical techniques have been previously applied to examine the relationship between total fat-liquor content and ultimate leather properties. These techniques do not however take into account the differences in distribution of fat-liquor during processing.

An objective image analysis technique was therefore developed for this work to examine differences in fat-liquor distribution during processing and its resulting impact on the final leather.

In this work we describe an easy to implement method of measuring and quantifying fat-liquor distribution and show that the distribution of fat-liquor is an important factor in the final properties of leather.

Introduction

Techniques for determining the distribution of fatliquor have been previously described (Haines et al. 1969). However, the aim of this work was to develop a staining method or methods for fats both in raw skins and in leathers; One which could then be quantitatively analysed through image analysis of cross sections. The method/s had to differentiate the fats by colour from other skin components and also be suitable for the computer-assisted deconvolution technique already described for the analysis of elastin (Edmonds and Cooper 2011). Briefly, this technique involves staining the sections with a stain for the component of interest, counterstaining the other components with a different-coloured stain, photographing the section (where necessary making a mosaic of several photomicrographs to get a whole

cross-section in one photomicrograph), separating the photomicrograph into the colours of the different stains, and analysing the distribution of the stain of interest.

Materials and methods

Initial work concentrated on raw skin.

Requirements of stain
1 Must stain fat
2 Must stain only fat, differentiating it from other components
3 Must be suitable for the colour separation technique of quantifying

Three fat stains were tried:-

Nile blue
This stained fat either pink or blue, depending on the fat (Figure 1(a)). There was also some background staining. This stain was rejected on the grounds that two colours for fat and the blue non-specific staining was too complicated for computer analysis to deal with.

Sudan Black
This stains fat black (Figure 1 (b)), and was rejected because there was some non-specific staining even when counter-stained, and also black is not a good colour for either deconvoluting or for quantifying by colour density.

Sudan III & IV
This stains fat red (Figure 1 (c)). There is some non-specificity when it is used alone but counterstaining removes this. Accordingly it was decided to use this stain.

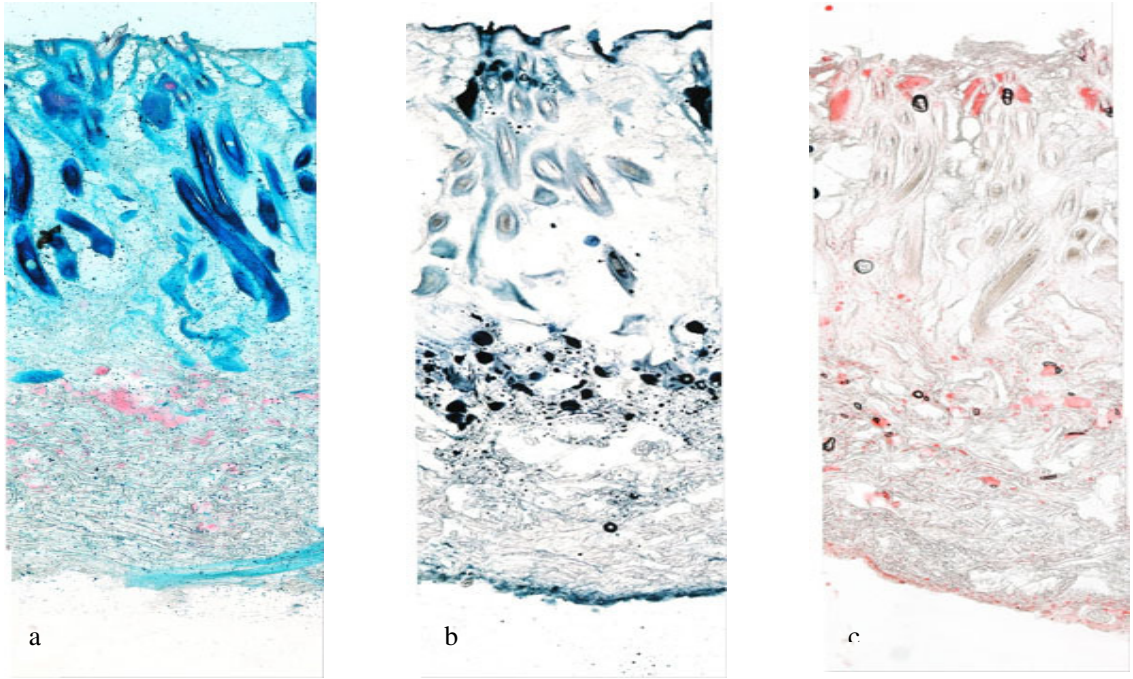

Figure 1: Photomicrographs of cross-sections of raw skin stained for fat with Nile Blue (a), Sudan Black (b), and Sudan III & IV (c)

Counter stains that were tried were Azur A and Malachite Green (both of which gave problems with colour leaching into the mounting medium), Celestine Blue (which went purple, which was too difficult to differentiate from red because the red component of the purple was also extracted) and Alcian Blue (which was suitable).

The Sudan III & IV plus Alcian Blue stain coloured fat pink/red and other components blue. The third component of colour (not shown) is essentially the material not fully stained with either of the stains used and in a sence represents the error of the staining process, this stain was very faint (not visible on the printed page) and is not shown. The pink/red component could be quantified. The end result is shown in Figure 32.

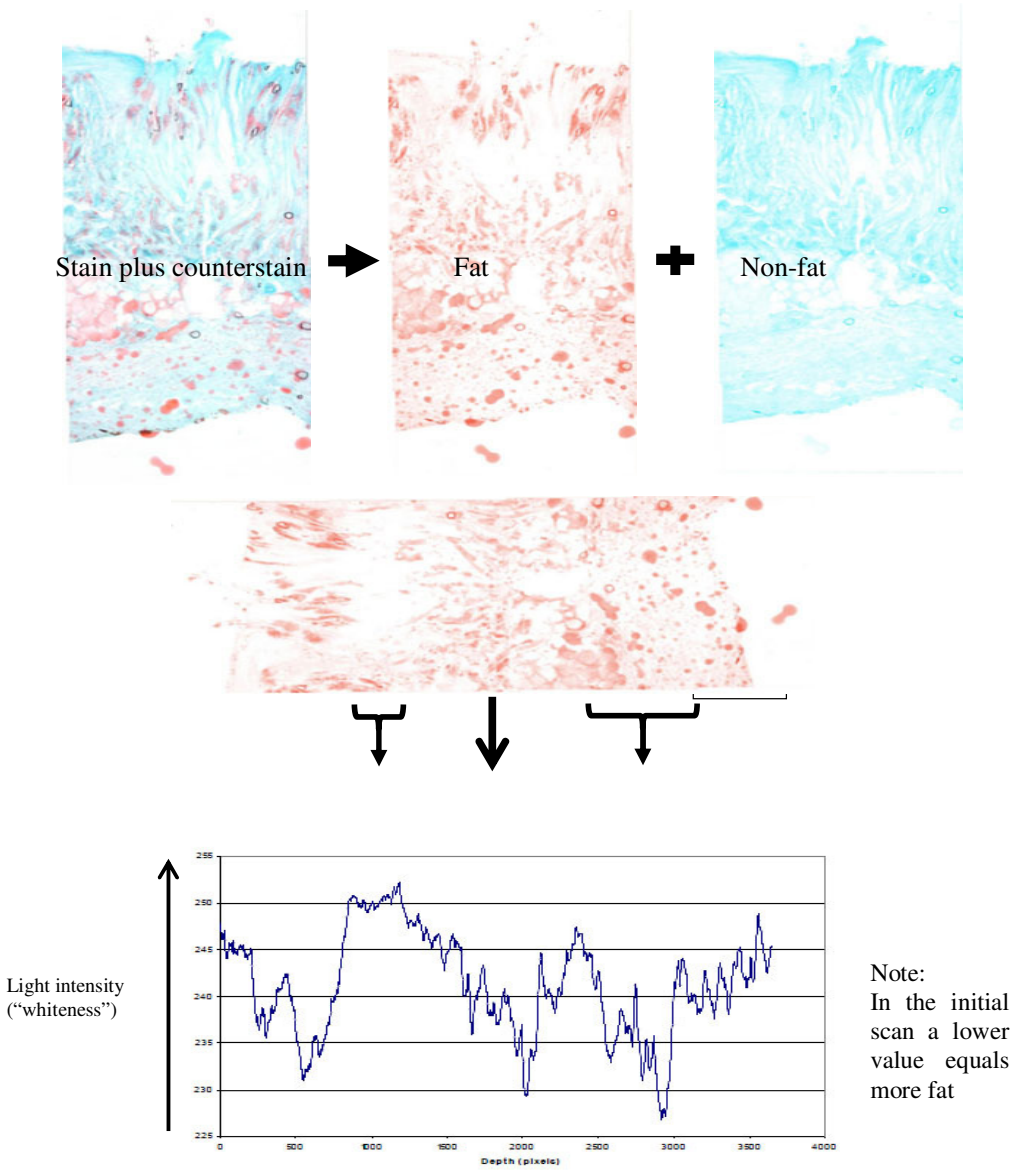

Figure 2: Shows how the raw skin section stained with Sudan III & IV and Alcian blue is deconvoluted into fat and the other components (top), then the section with red fat staining alone (centre) is scanned to quantify the amount of fat present throughout the section (bottom)

The next step was to extend the technique to crust leather, which is more demanding to stain than raw skin.

One of the problems with staining crust leather is that it has usually been dyed and the colour of the dye interferes with the stain. Four methods of de-dyeing the leather were trialled – a hypochlorite bleach, pyridine, acetic acid plus sodium dithionite, and potassium permanganate plus sodium dithionite. The first three all removed some colour, but only the potassium permanganate/sodium dithionite combination removed all of it.

The same stains and counter-stains were trialled as for the raw skin, because crust leather may respond to staining differently to raw skin. Again the Sudan III & IV/Alcian Blue combination was found to be the most successful. However, if the same concentrations were used as in raw skin the counter-staining was far too dark and most of the red Sudan staining was obscured (Figure 3).

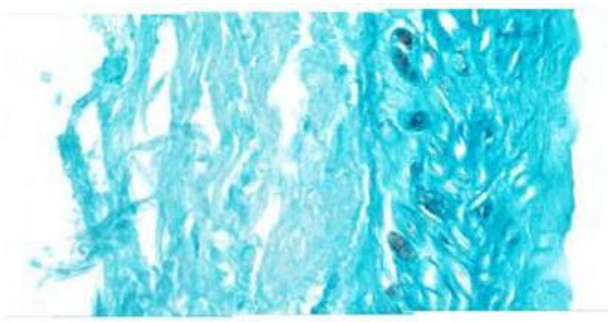

Figure 3: Photomicrograph of de-dyed crust leather stained with Sudan III & IV and counter-stained with 1% Alcian Blue.

It was found necessary to reduce the Alcian Blue concentration to approximately one fiftieth of that used for raw skin. On leather, Sudan III & IV plus Alcian Blue at the lower concentration gave pink/red fat and blue other components.

Validation and calibration

In order ensure that the analysis method for the chosen stain was measuring only fat, and to produce an absolute estimate of the amount of fat present, a validation and calibration step was required. By taking samples of differently fatliquored leathers and analysing them using the optical microscope image analysis technique and then measuring the fat content of the samples using the standard analytical technique, we were able to correlate the fat content measured by the two methods. The results for differently treated skins using two fatliquors are shown in Figures 4 and 5.

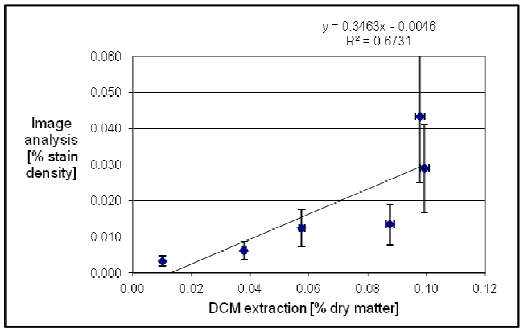

Figure 4: The correlation for Lipsol between grease measured by DCM extraction and grease measured using the optical technique

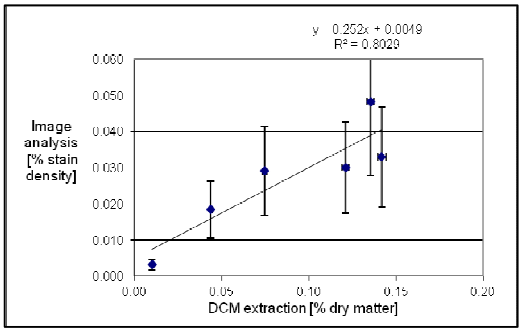

Figure 5: The correlation for Derminol between grease measured by DCM extraction and grease measured using the optical technique

From these two graphs, the correlation between the Image analysis technique and the DCM extractible matter is clear. The size of the error bars generated results from the fact that only two sections were assessed for the calibration curves. This is enough to provide differential or semi quantifiable measures of fat content. If an absolute quantifiable measure of fat content is needed a larger number of samples would be required to reduce the experimental error.

Application of the technique results and discussion

The first application of the technique was to investigate the previously described empirical trial of fatliquor mixtures. Leather samples of extreme strength and extreme looseness were examined. The fat distribution scans are shown in Figures 6 and 7.

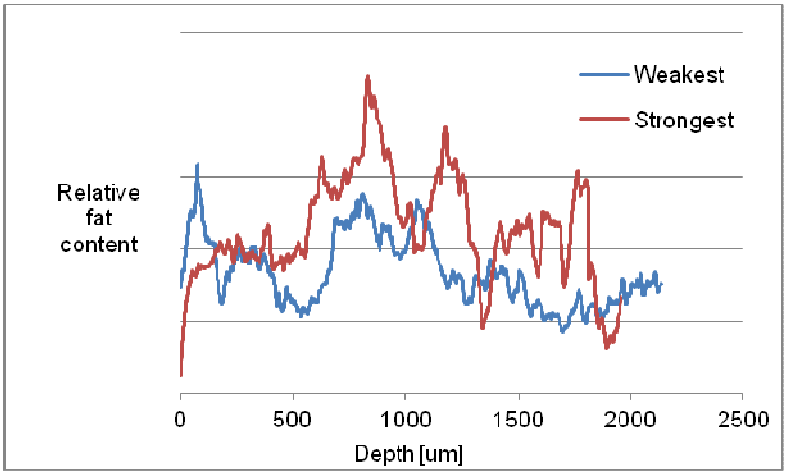

Figure 6: Fat distribution scans for leather with high and low tear strength

Figure 6 shows that greater fat present through the centre of the leather and into the corium corresponded to higher strength whereas a slightly greater fat concentration at the grain surface was found in the weaker leather. This was as expected, since the strength of leather is usually associated with the corium, and it illustrates the importance

of this technique in identifying the distribution of fat through the thickness of the leather rather than simply the overall concentration of the fat.

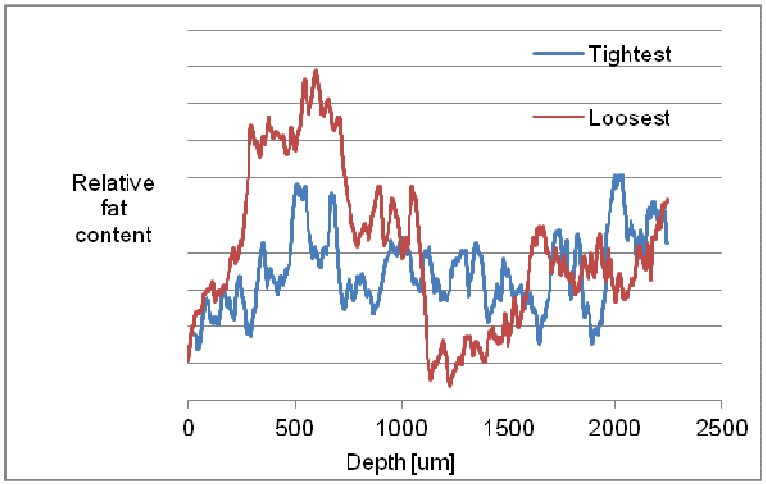

Figure 7: Fat distribution scans for leather with high and low looseness scores

Figure 7 shows that the looser leather had a greater concentration of fat in the grain layer. If the presence of fatliquor allows fibres a greater ability to move in the structure then these results are to be expected. A greater ability of fibres to move through the structure will allow more fibres to take up the strain during tearing thereby increasing the tear strength but making the leather more loose.

The technique was also used to compare leather which had not been neutralised prior to fatliquoring with leather thoroughly neutralised before fatliquoring, to see if the expected effects (shown in Table 2) actually occurred.

Table 1: The expected effects of not neutralising/neutralising leather prior to fatliquoring

Not neutralised	Neutralised overnight
Surface pH remains low	Surface pH is higher
Fatliquor emulsion destabilises at the surface	Fatliquor emulsion destabilises within the leather when it is acidified
Fat is deposited at the leather surface	Fat is deposited within the leather
Leather is weaker	Leather is stronger
Leather is less soft	Leather is softer

The results of the fat scans of the two leathers are shown in Figure 8.

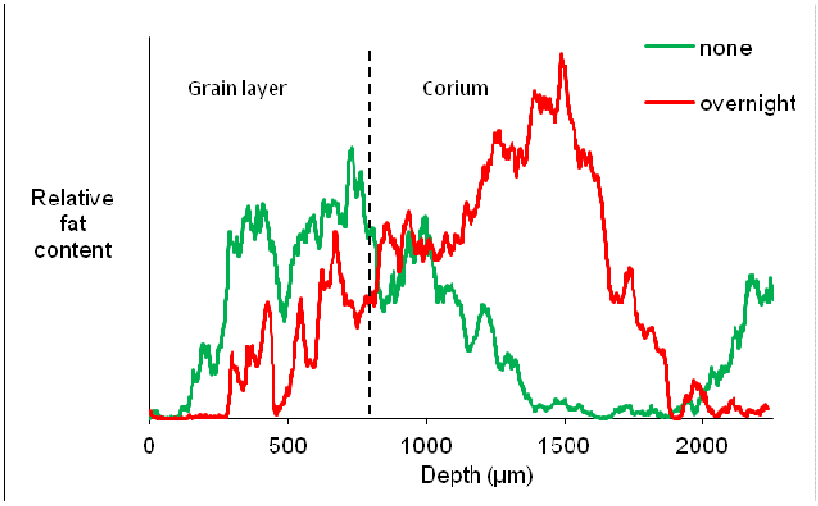

Figure 8: Fat scans of leathers not neutralized and neutralised overnight

For the leather which had not been neutralised prior to fatliquoring (green line), most of the fat was either in the grain layer (possibly within follicles) or at the flesh surface, as expected. The leather which had been neutralised overnight prior to fatliquoring (red line) had much more fat within the corium, also as expected.

The strength of the two leathers is shown in Figure 9(left) and the softness in Figure 9(right).

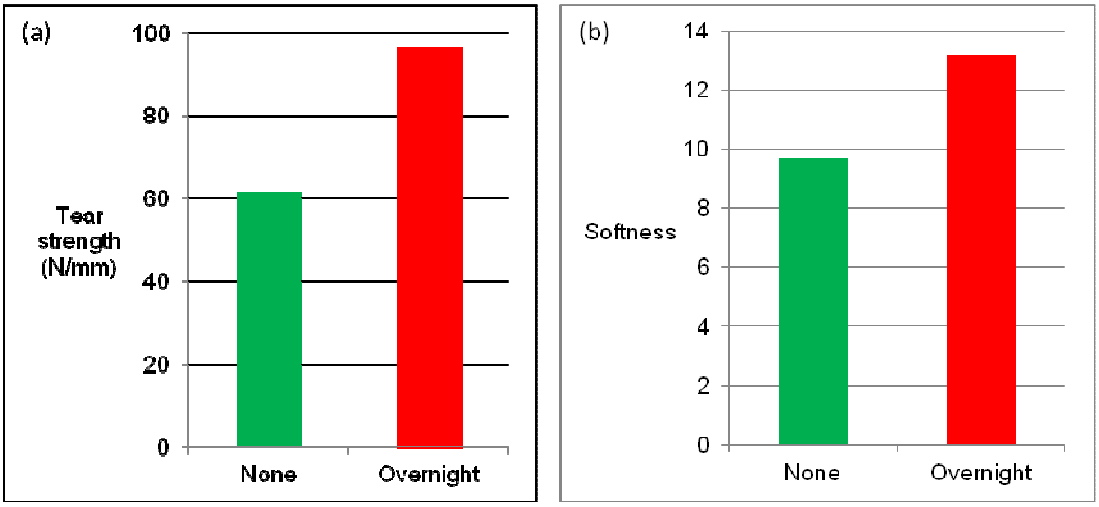

Figure 9: Tear strength and softness of leathers not neutralized and neutralised overnight

Figure 9 shows that the leather which had been neutralised overnight prior to fatliquoring (red bars) was both stronger and softer than the leather which had not been neutralised prior to fatliquoring (green bars) as would be expected due to the greater quantity of lubricating fat within the corium.

The next experiment compared the distribution of four different types of fatliquor within leather, and demonstrated the effects of the different fatliquors on both tear

strength and grain strength. The properties of the fatliquors are shown in Table 2. Each was used alone on bovine leather.

Table 2: Description of the fatliquors used

Fatliquor A	sulphited lanolin derivative
Fatliquor B	emulsified synthetic oil
Fatliquor C	sulphited fish oil
Fatliquor D	sulphated natural oil

Fat scans for the four fatliquors are shown in Figures 10 -13.

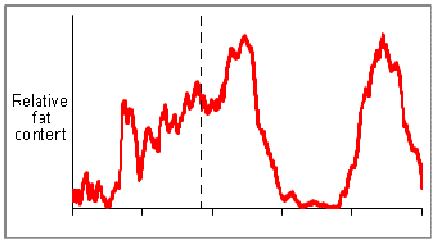

Figure 10: Fat scan for Fatliquor A

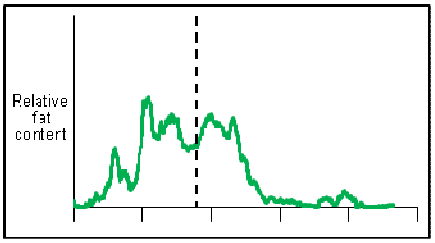

Figure 11: Fat scan for Fatliquor B

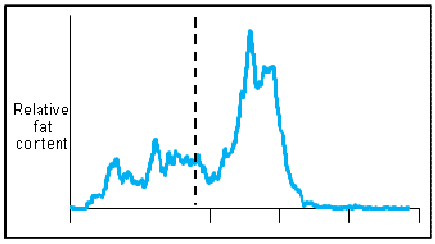

Figure 12: Fat scan for Fatliquor C

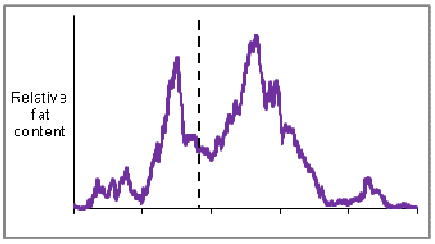

Figure 13: Fat scan for Fatliquor D

The distribution of the four fatliquors is summarised in Table 3.

Table 3: Distribution of the fatliquors

Fatliquor A	Reasonable amount in grain layer. A peak in the upper corium and at the flesh surface
Fatliquor B	Most in the lower grain and upper corium. Almost none at the flesh surface
Fatliquor C	A peak in midcorium. Little in the grain, none at the flesh surface
Fatliquor D	A peak in the grain and at midcorium. Little at the flesh surface

The tear strength and grain strength of the leathers prepared with the four fatliquors are shown in Figures 14 and 15.

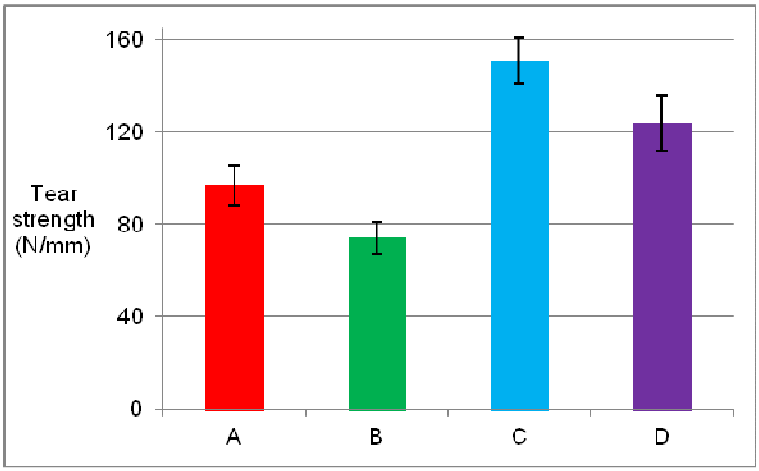

Figure 14: Tear strength of leathers prepared with fatliquors A, B, C and D
Note the wide variation in strength, with the strongest (C) being twice as strong as the weakest (B). The two strongest leathers (C and D) are those with a fatliquor peak in mid-corium.

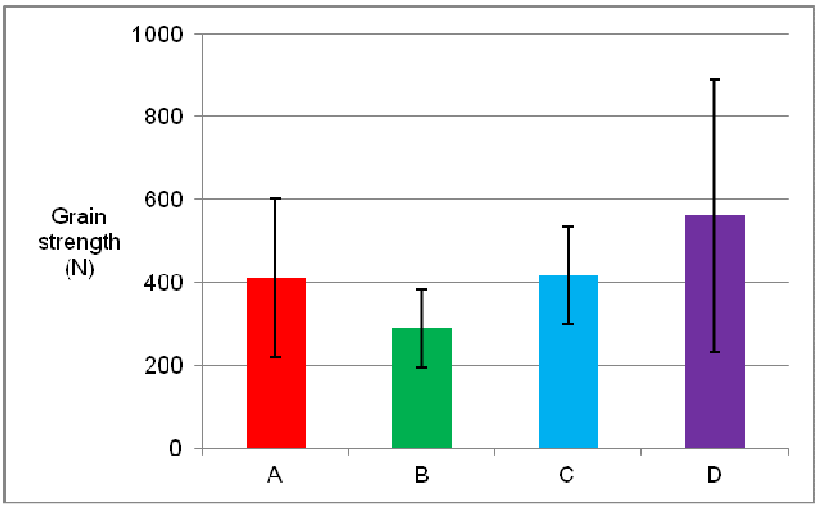

Figure 15: Grain strength of leathers prepared with fatliquors A, B, C and D

Note that the relativities have changed somewhat. Leather B still has the lowest value, but D has a higher grain strength than C, the opposite of what was seen for tear strength. Leathers C and D had a similar distribution of fatliquor in the corium, but D had an additional peak in the grain layer which may have given it the somewhat higher grain strength.

Conclusions

A new technique has been described; to monitor the distribution of fatliquor within leather. This technique has application in:-

- Research
- Quality control
- Development of new fatliquoring systems
- Adjusting recipes
- Controlling process times

Acknowledgements

We would like to thank Geoff Holmes for his work on fatliquor mixtures and LASRA staff members William Aitkenhead and Emma Crawford for their technical assistance. This work was funded by MSI, Meat and Wool NZ and MIRINZ (Inc).

Software

The software use was wholly based on open source packages available to all for free. The specific packaged used was ImageJ with add-on plugins incorporated to achieve the image corrections and image analysis required. Links for each are given below.
Instructions for installation of imageJ and incorporation of the add-on plugins can be found on the imageJ site and readily found on the internet.

ImageJ:
http://rsb.info.nih.gov/ij/
OrientationJ:
http://bigwww.epfl.ch/demo/orientation/OrientationJ_.jar
MosaicJ:
http://bigwww.epfl.ch/thevenaz/mosaicj/MosaicJ.zip
Deconvolution:
http://www.dentistry.bham.ac.uk/landinig/software/cdeconv/cdeconv.html
Brightfield correction:
http://imagejdocu.tudor.lu/doku.php?id=howto:working:how_to_correct_background_illumination_in_brightfield_microscopy
Distortion correction:
http://www.imatest.com/docs/distortion.html
For the distortion correction the author developed a custom distortion module based on the equations describe in the above link and is available at the following link.
https://raw.github.com/mrpurplenz/NZ-LASRA/master/ImageJ/Microscope_Correction.java
Subsequently to the development of this work alternative distortion correction modules have become available at the following link but are untried by this author.
http://rsb.info.nih.gov/ij/plugins/polar-transformer/index.html

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