

# Effect of Tanning Agents on Collagen Structure and Response to Strain in Leather

by

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## Abstract

A variety of tanning agents are used to produce leather of different mechanical performances and properties. How different tanning agents affect the structure of leather, and consequently the mechanism underlying the performance of the differently tanned leathers, is not well understood. We used small-angle X-ray scattering to investigate the structure of ovine leather tanned with chromium, zirconium, mimosa, oxazolidine or tetrakis hydroxymethyl phosphonium sulfate (THPS), or selected pairs of these agents. Leather tanned using THPS and THPS in combination with mimosa retained the most oriented collagen fibril structure. Treatment with chromium, zirconium or zirconium and mimosa resulted in the least oriented fibril structures. The effect of oxazolidine with mimosa was intermediate between these. A high natural collagen fibril orientation in a skin is normally associated with high strength while a high degree of crosslinking normally results in a lower fibril orientation. We suggest that the extent of crosslinking in the samples is reflected in their fibril orientation, with more crosslinking producing stronger leathers. This interpretation is supported by the differences in shrinkage temperatures: those leathers for which orientation index (a measure of collagen fibril alignment) changed most had a lower shrinkage temperature (which is normally interpreted as more “tanning”). This provides a greater understanding of the mechanism of action of tanning agents in the development of the properties of leather.

## Introduction

Tanning is an important part of leather manufacture as it converts a raw hide or skin into a stable and robust material. Tanning agents effect this change by binding to the hide's collagen and establishing crosslinks between its fibril, causing

the hide to become less hydrophilic and more resistant to bacterial attack. This process, in combination with exposure to fatliquors, also causes the final product to be more flexible.

Tanning agents fall into three main categories: chrome tans, vegetable tans and synthetic tans. Chrome tanning, using chromium (III) sulfate, is the most commonly used tanning method in the leather industry. It produces a very stable, fibrous network resistant to both bacterial attack and to high temperatures. Owing to increasing concerns about the perceived negative environmental effects of chromium there is substantial interest in pursuing alternatives to chrome tanning. Efforts are being made to reduce chromium in tannery waste liquors or to produce chrome-free leather.<sup>1-4</sup>

Vegetable tanning uses “tannins”, which are a group of complex phenolic compounds extracted from plant parts, including from the twigs, bark, nuts, leaves, and wood. Vegetable tanning imparts organoleptic “handle” qualities and resilience to the leather and is used for applications such as upholstery and saddlery. However, the specific properties imparted by vegetable tanned leather may render it less desirable compared to chrome-tanned leather for some final uses.<sup>5,6</sup>

Synthetic tanning agents like formaldehyde, glutaraldehyde, and phenols are highly effective and are often used to obtain specific properties, such as lighter colors and thinner leathers for the final product.

Combining tanning agents is common practice in the leather industry. Such combination tanning can improve the physical or mechanical properties of the final leather and can reduce the amount of chrome used. To counteract the aforementioned disadvantages of using just chromium as a tanning agent, normally a second tanning stage follows chromium tanning, using natural vegetable or synthetic tannins, to make the final

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leather feel softer with an improved handle or to modify the leather's strength, weather resistance or dye penetration. Tetrakis hydroxymethyl phosphonium sulfate (THPS) is used with aluminum or glutaraldehyde (retanning agents) to produce white leather.<sup>7-10</sup> Oxazolidine with chrome enhances chrome uptake, resulting in less chrome in the waste liquor.<sup>11</sup> while combining oxazolidine with zirconium produces a high quality leather.<sup>12</sup>

In addition to their effects on collagen, tanning agents may also alter the collagen fibril structure of leather. Investigations using small-angle X-ray scattering (SAXS) have provided detailed structural information about the fibril orientation, fibril diameter, and the d-spacing of collagen in leather and other tissues.<sup>13-22</sup> The orientation of leather collagen measured edge-on (alignment in-plane) has been shown to be correlated with strength across a range of mammal species.<sup>23-25</sup>

Structural changes to the arrangement of collagen fibrils during mechanical strain have been investigated<sup>26-28</sup> and other research has shed light on the changes in collagen fibril orientation and d-spacing that occur throughout the manufacture process.<sup>29-31</sup> However, it is not understood how different tanning agents affect the structure, and consequently the performance, of the leather.

Here, we use small-angle X-ray scattering to investigate the structure of ovine leather tanned with chromium, zirconium, THPS, mimosa or oxazolidine. The structural arrangements of collagen in the tanned leathers were compared with the tear strengths.

## Methods

Conventional beamhouse and tanning processes were used to generate the leather. Selected skins were cooled and all adhering fat and flesh was mechanically removed. The pelts were depilated using a fast-acting lime sulfide paint and then were washed, treated with carbon dioxide and a bate enzyme, Tanzym (Trypsect Biochemical), washed again, and pickled using sulfuric acid.

The pickled pelts were then tanned using one of the following processes:

**Chromium (standard):** Pickled pelts were first neutralized using sodium formate and sodium bicarbonate, and then washed. Next, they were degreased using a solution of 10% common salt, 8% non-ionic surfactant and 4% oxazolidine A. The neutralized, degreased pelts were then tanned using 4.5% (pickled pelt weight) chromium sulfate (33% basic) and were allowed to fix by raising the temperature to 40°C and left overnight. The tanned pelts were washed and retanned using 4% mimosa extract Clarotan (Tanac), and 5% fatliquor consisting of 1.5% sulfited fish oil and 3.5% Coripol UFB-W (TFL), and given a final wash.

**Zirconium:** Leathers were tanned following the chromium process but 5% zirconium sulfate (Blancerol ZB, Lanxess) was substituted for the 4.5% chromium sulfate at the tanning stage and, at the retanning stage, 3% Tanicor PW (Clariant; a syntan comprising polymers of naphthalene and sulfonic acid, which is typical of many syntans used in the industry) instead of the 4% Tanac.

**THPS:** Pickled pelts were degreased and neutralized following the chromium process but after washing, the pelts were tanned using 2% THPS (Albright AD, Albright and Wilson) and the fatliquor of the chromium process. Retanning then followed using 2% Tanicor PW (Clariant), instead of Tanac.

**Oxazolidine + Mimosa:** Pickled pelts were degreased and neutralized following the chromium process, but after washing, the pelts were tanned using 2% Tanicor PW (Clariant), 6% Tanac, and 2% oxazolidine A. After the addition of a further 8% Tanac, the tanned pelts were exposed to the standard fatliquor and then underwent a final wash.

**Zirconium + Mimosa:** Leathers were tanned following the chromium process but 5% zirconium sulfate was substituted for the 4.5% chrome sulfate at the tanning stage and, at the retanning stage, 3% Tanicor PW was used in addition to 8% mimosa extract.

**THPS + Mimosa:** Pickled pelts were degreased and neutralized following the chromium process but, after washing the pelts were tanned using 2% THPS, 2% Tanicor PW, and 6% mimosa extract. After the addition of a further 8% mimosa extract, the pelts were exposed to the standard fatliquor and then underwent a final wash.

The tanned pelts were then evaluated using the following methods:

**Mechanical Testing:** Twenty leather samples tanned by each agent or agent combination were cut from the hides according to standard sampling methods.<sup>32</sup> Likewise, standard methods<sup>33,34</sup> were followed for tests of tear and tensile strength on an Instron 4467.

**Shrinkage temperature:** Two leather samples tanned by each agent or agent combination were cut from the hides according to standard sampling methods.<sup>32</sup> Similarly, the standard method<sup>35</sup> was followed for shrinkage testing, with the modification that a glycerol solution was used to allow for leather samples with shrinkage temperatures greater than 100°C.

**SAXS:** Strips of leather 1 x 30 mm were cut from the "official sampling position" and mounted with the beam edge-on or parallel to the surface of the leather. Diffraction patterns were recorded for the leather samples on the SAXS/WAXS beamline at the Australian Synchrotron. This uses a high-intensity

undulator source and has an energy resolution of  $10^{-4}$  from a cryo-cooled Si (111) double-crystal monochromator. The beam size (FWHM focused at the sample) was  $250 \times 80 \mu\text{m}$  with a total photon flux of about  $2 \times 10^{12} \text{ ph}\cdot\text{s}^{-1}$ . All diffraction patterns were recorded with an X-ray energy of 12 keV using a Pilatus 1M detector with an active area of  $170 \times 170 \text{ mm}$  and a sample-to-detector distance of 3371 mm. Exposure time for diffraction patterns was 1 s. Data were processed using the scatterBrainAnalysis software.<sup>36</sup>

For the *in-situ* SAXS measurements, a stretching apparatus was custom built as described elsewhere<sup>37</sup>. Samples were mounted horizontally without tension and without slack between the jaws of the stretching machine. Force and extension information was recorded and SAXS patterns were taken through the full thickness of the sample. The leather sample was then stretched by 1 mm, maintained at this extension for a 1 minute interval, and then diffraction patterns were again recorded through the full thickness of the sample. This process was repeated with the sample stretched a further 1 mm each time until the sample failed.

Orientation index (OI) is defined as  $(90^\circ - \text{OA})/90^\circ$ , where OA is the azimuthal angle range that contains 50% of the fibrils centered at  $180^\circ$ . The OI provides a measure of the spread in orientation of the fibrils where an OI of 1 indicates the fibrils are completely parallel to each other and an OI of 0 indicates the fibrils are completely randomly oriented. The OI is calculated at the most intense d-spacing peak which, in this case, was the 6<sup>th</sup> peak around  $0.059\text{--}0.060 \text{ \AA}^{-1}$ .<sup>23</sup> The d-spacing was determined from Bragg's Law by taking the center of a Gaussian curve fitted to the 6<sup>th</sup> order diffraction peak of an integrated intensity plot for each spectrum.

## Results

**Tear strength:** The samples processed with chromium or zirconium tanning agents had higher tear strengths while the samples tanned with oxazolidine or THPS had lower tear strengths (Table I).

**Tensile strength:** Similar to the tear strength results, the tensile strengths of the samples from chromium- or zirconium-tanned leather were higher than those of the oxazolidine- or THPS-tanned leathers.

**Stress-strain:** Stress-strain curves were measured at the synchrotron while SAXS data were being recorded (Figure 1). Note that the size of each of the samples analyzed was small and the samples were not of uniform width; therefore, these data may not accurately represent the physical properties of the tanned leather as a whole. However, most samples yielded similar slopes (Figure 1) with the exceptions of leathers tanned with zirconium plus mimosa (a steeper slope) and with chromium (a shallower slope).

**SAXS:** Well defined SAXS patterns were obtained for all the leather samples. Diffraction rings that occur due to the regular spacing of the internal structure of collagen fibrils were clearly visible (Figure 2a). When the integrated intensity (of scattering angle over all azimuthal angles) of the pattern is plotted (Figure 2b) the d-spacing is easily measured using the central position of a peak divided by the peak's order. The orientation of the fibrils is calculated from the varying scattering intensity with the azimuthal angle usually at one scattering angle (around a ring) (Figure 2c).

**Table I**

**Tear strengths and tensile strength of leathers tanned using various agents, from strongest to weakest. Each value represents the average of 20 samples that were tested.**

Tanning Agent(s)	Tear Strength (N/mm)	Tear Strength Error at 95% confidence (N/mm)	Tensile Strength (N/mm <sup>2</sup> )	Tensile Strength Error at 95% confidence (N/mm <sup>2</sup> )
Zirconium + Mimosa	42.5	4.2	13.6	1.3
Zirconium	41.6	3.0	12.7	0.9
Chromium	30.9	1.8	12.5	0.8
Oxazolidine + Mimosa	26.0	1.5	11.6	0.8
THPS	24.9	1.4	10.8	0.7
THPS + Mimosa	22.3	1.3	9.8	1.0

**OI:** The orientation index of the samples differed among the tanning agents used. Samples tanned using THPS or oxazolidine had higher OIs while those tanned with chromium or zirconium had lower OIs (Figure 3). This corresponds with the pattern observed in the tensile strength and tear strength results.

**OI and strain:** Samples tanned with THPS, THPS in combination with mimosa, and oxazolidine in combination with mimosa developed the most oriented collagen fibril structures under tension (Figure 4). Leather tanned with chromium, zirconium, or zirconium in conjunction with mimosa resulted in the least oriented fibril structure.

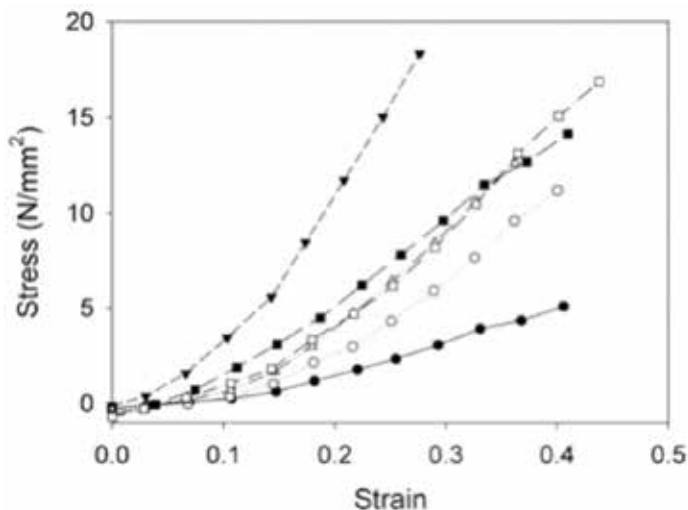


Figure 1. Stress versus strain for leathers tanned with the following agents: (▼, - - -) Zirconium + Mimosa; (■, — — —) Zirconium; (●, — — —) Chromium; (Δ, - · - ·) Oxazolidine + Mimosa; (□, - · - ·) THPS; (○, ·····) THPS + Mimosa.

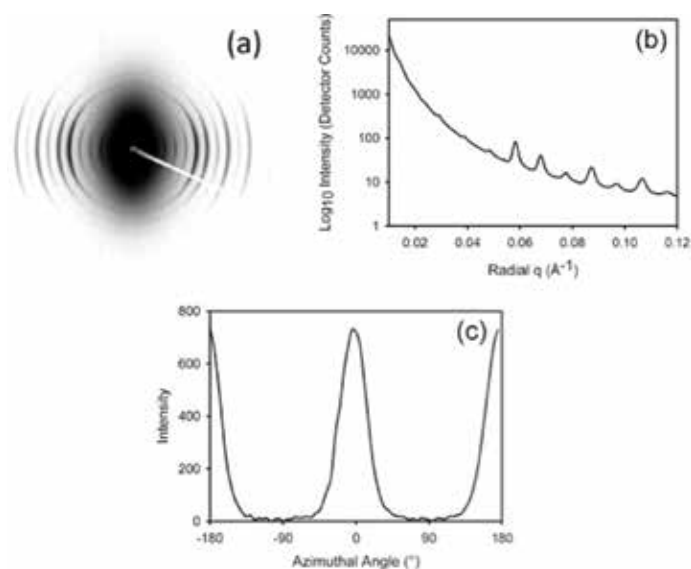


Figure 2. Example of SAXS of collagen in leather: (a) SAXS pattern; (b) integrated intensity plot; (c) intensity with azimuthal angle for the 6<sup>th</sup> order collagen diffraction peak at  $0.058 \text{ \AA}^{-1}$ .

**d-spacing and stress:** The d-spacing for each sample did not change initially when tension was applied, although the OI did increase during this initial stress. With further stress, however, the d-spacing of each sample increased. The change in d-spacing was relatively similar among samples, with increases being 0.28–0.50 nm (Figure 5).

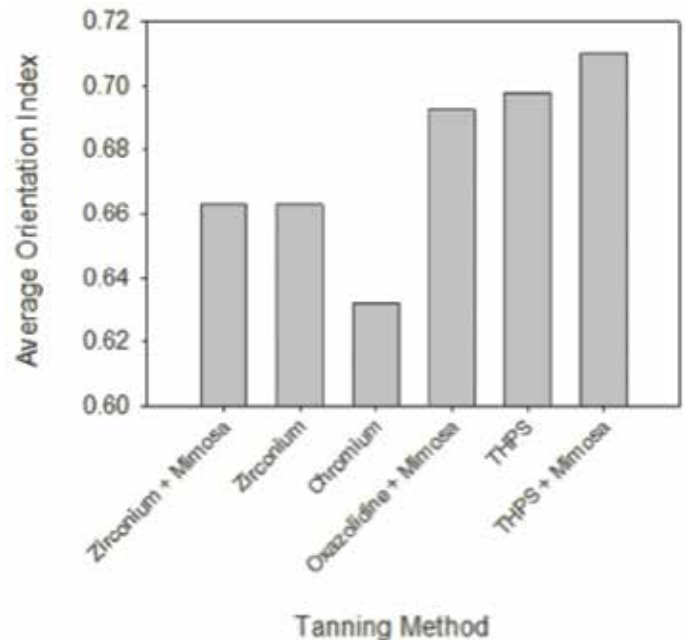


Figure 3. Average orientation index for collagen in unstrained leathers processed using different tanning agents. Each orientation index value is the average of 20 separate measurements taken at different positions on each piece of tanned leather ( $n = 20$ ).

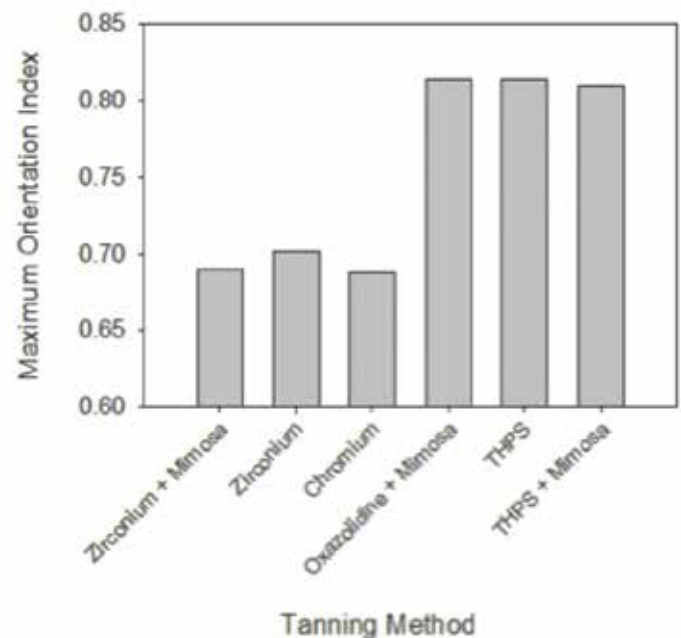


Figure 4. Maximum orientation index ( $n = 20$ ) for collagen in leathers after strain is applied for the different tanning methods.

**Shrinkage temperature:** The shrinkage temperatures for the different leathers (Table II) fall generally into two groups: higher temperatures, found for the chromium- and zirconium-tanned leathers, and lower temperatures, for the other leathers. The group with the shrinkage temperatures below 94°C is the same as the group with high OI, while the high shrinkage temperature samples (>98°C) is the group with low OI. No correlation is seen between the degree of tannage measured by the shrinkage and the amount of change in d-spacing.

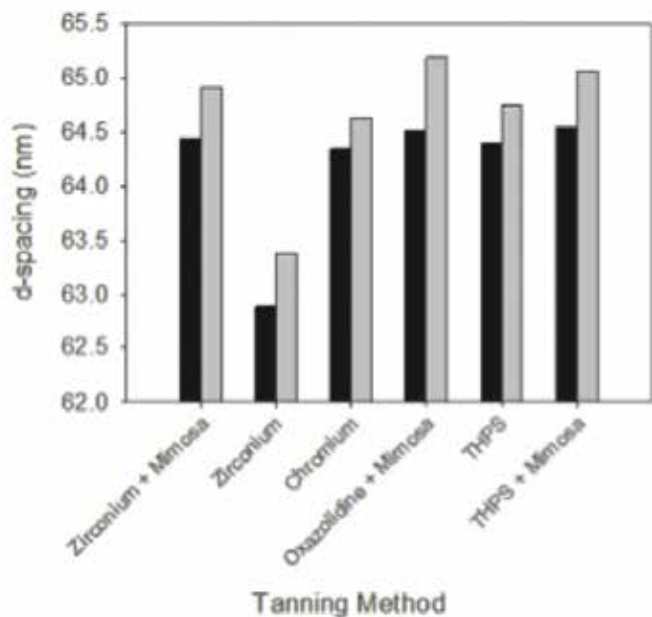


Figure 5. Initial d-spacing (black) and maximum d-spacing (grey) of leather samples when tension is applied for leathers tanned using different agents.

**Table II**  
Shrinkage temperatures of leathers tanned using various agents.

Tanning Agent	Shrinkage Temperature (°C)	
	(two measurements)	
Zirconium + Mimosa	108.3	112.3
Zirconium	98.4	98.2
Chromium	116.5	118.7
Oxazolidine + Mimosa	93.7	94.6
THPS	86.1	84.8
THPS + Mimosa	92.7	92.3

## Discussion

Results from both the tear and tensile strength tests are in accordance, indicating that the samples processed with chromium or zirconium have higher tear and tensile strengths compared to the samples processed with oxazolidine or THPS (Table I).

As stated in the Introduction, correlation between the OI of a leather's fibrils and its strength, where a higher OI means a stronger material, has been found<sup>23</sup>. However, here the stronger materials with higher tear and tensile strengths (chromium- or zirconium-tanned leathers) had lower OIs. These lower OIs of the stronger materials may reflect the greater extent of the crosslinking between the collagen fibrils that the specific tanning agent(s) produced. This has been documented already, where more crosslinked collagen produced a stronger material, with a lower OI.<sup>21,38</sup> Therefore, because the stronger materials here have lower OIs, it can be inferred that these stronger materials contain more crosslinks with the tanning agents. An alternative interpretation could be that the tanning agents dehydrate the collagen<sup>39</sup> and that this dehydration leads to stiffening of the collagen fibrils.

When tension is applied to a leather, the structure changes in two stages: first, the fibrils become more oriented relative to one another, then the fibrils themselves extend.<sup>37</sup> This is reflected in an initial increase in OI while the d-spacing remains relatively unchanged, and then the d-spacing increases. This pattern of structural change was observed here. The leathers that are able to develop the highest OIs are weaker and this may be due to less crosslinking in these leathers, whereas a lower OI is retained under strain for strongly crosslinked materials.

A high natural collagen OI in skin is normally associated with high strength, while a high degree of crosslinking normally results in a lower OI. In the leathers made with different tanning agents presented here, we suggest that the extent of crosslinking is reflected in the fibril OI, which in turn is reflected in the strength of the leathers. This interpretation of the SAXS data is supported by the observed differences in shrinkage temperatures, where those leathers that showed a greater decrease in OI (which we interpret as indicating more crosslinking) have a higher shrinkage temperature (which is normally interpreted as more "tanning"). There was no correlation between the degree of tannage measured by the shrinkage and the amount of change in d-spacing, whereas such a relationship might have been expected.<sup>39</sup>

## Conclusions

Leathers tanned with chromium and zirconium tanning agents have higher tear and tensile strengths but lower OIs, while THPS- and oxazolidine-tanned leathers have lower tensile and tear strengths but higher OIs. The stronger material with lower OI indicates that there is a higher level of crosslinking in the chromium- and zirconium-tanned leathers, which leads to their increased strength and decreased OI. This knowledge provides an understanding of how the different tanning agents result in different leather properties and may assist the tanner to optimize tanning formulations.

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