CHAPTER 4

REDUCTION OF DISULPHIDE LINKAGES IN
KERATIN PROTEINS AND THEIR APPLICATION IN
LEATHER PROCESSING

4.1 INTRODUCTION

Reduction of the disulphide bonds of Keratin has been studied much as it leads to the formation of soluble derivatives. Early work on the reduction of keratin suggested that complete reduction of cystine could only be obtained under conditions where the keratin was solubilised at high pH.

The reduction of the disulphide bond occurs in two stages:

(i) hydrolysis of strained disulphide bond.

\[
R - S - S - R' + H_2O \rightarrow R - S - OH + SH - R'
\]

(ii) Linkage rebuilding to give new cross links (see Chapter II for more details)

The cleavage of disulphide bonds by aqueous cupric sulphite is extremely rapid and appear to be very highly specific (Swan 1957).

\[
RSSR + 2Cu^{++} + 2SO_3^{−} \rightarrow 2RSSO_3^{−} + 2Cu^{+}
\]

\[
RSH + 2Cu^{++} + SO_3^{−} \rightarrow RSSO_3^{−} + H^{+}
\]
The reaction shown above, has been made the basis of a method for dissolving keratin and may be employed generally for the modification of SH and SS groups.

Similarly the reaction of disulphides with silver and mercury salts (Kolthoff et al 1950 and Stricks et al 1954) result in dissolution of keratin.

\[
\begin{align*}
\text{RSSR} + \text{Ag}^+ + \text{SO}_3^- & \rightarrow \text{RSSO}_3^- + \text{RSG} \\
2\text{RSSR} + \text{Hg}^2+ + 2\text{SO}_3^- & \rightarrow 2\text{RSSO}_3^- + \text{(RS)}_2 \text{Hg} + 2\text{Cl}^- 
\end{align*}
\]

(i) (ii)

Degradation of wool keratin by iron (III) is recently described (Fukatsu 1990). The activity of Fe(III) complex is lower than that of similar copper (II) complex (Fukatsu et al 1993).

In leather processing unhairing is generally done using lime and sodium sulphide (Proctor 1922). The depilation procedure used in tanneries all over the world is based on treatment either in a solution or in a slurry of 1% - 5% sodium sulphide (or sodium hydrogen sulphide) and 3% - 10% calcium hydroxide. Some times, dimethylamine, sodium hydroxide, sodium chloride or calcium chloride is added to the mixture. The epidermal keratin, the hair roots and hair root sheaths are dissolved or degraded to such an extent that the hair is removed in the rotating drum or by the mechanical pressure given during the unhairing with the blunt knife. The collagen fibre containing tissue swells strongly but nevertheless the surface area of the hide diminishes as a result of hide shrinkage. The Ca\textsuperscript{2+} present in the lime bath is strongly bound by the carboxyl groups of the collagen molecule though it may be released from the hide by the action of weak organic acids or by enzyme treatment (Hormann and Schubert 1958) after neutralization. Lime is the preferred alkaline substance for opening up the collagen fibre network. Although the swelling is greater with either potassium hydroxide or sodium hydroxide the grain structure of the leather seems to be protected.
by reaction with divalent Ca\(^{2+}\) ions. Leather limed with calcium hydroxide as a bright grain surface though the soluble part of the hide (which includes fat, sebaceous substances, blood, proteoglycans, cellular materials and incompletely cross-linked collagen) is removed leaving voids in the fibre structure. The opening-up of the structure increases the accessibility of the reactive protein sites allowing better uptake of fat, dyes and tannin molecules, thus resulting in a softer leather.

During liming the disulphide linkages in keratins of the epidermis are hydrolysed. Lime and other alkalis not only hydrolyse the epithelial cells but also disintegrate and loosen the entire epidermis, so that it can be removed. The hydrolysed proteins get partly dissolved in the lime and form a culture medium for bacteria. Subsequently the keratins of the epidermis are completely hydrolysed and the hair roots are loosened. Liming generates large quantities of solid and liquid effluents and also suffers a disadvantage as bacteria may attack the collagen fibers (Sarkar 1981) and the quality of leather may be affected (Wilson 1941). Due to regulations from the environmental health authorities, tanneries all over the world are facing problems with the toxic sulphide containing beam house effluent. Further sodium sulphide can generate H\(_2\)S during subsequent operations after unhairing viz deliming, pickling etc. which is a health hazard to the worker handling. To overcome this, various alternatives are suggested.

Use of serine protease (Microdep-C from M/s Textan Chemical (P) Ltd., Chennai India) formulations of unhairing has been reported (Anselm De'souza and Vedaranjan R 1997). This enzymatic unhairing in the sulphide poor system resulted in pelts which had a bright and uniform finished surface. Reduction in sulphide used resulted in a less wrinkled pelt and consequent area increase. From the economic standpoint enzymatic unhairing costs Rs.0.25 per sq. ft which is costlier than the conventional method. However, this increase is more than offset from the realisation gained through additional area obtained.
Thomas Feigel (1994) has also discussed the use of proteolytic enzyme in low sulphide liming system.

CLRI (Jayaraman 1991) has been working on this particular aspect of unhairing enzyme for the past couple of years on the development of a cost effective method of production of an enzyme depilant of microbial origin. A new strain by *Aspergillus flavus* has been isolated which can produce large amounts of extracellular protease. It is widely recognised that solid substrate fermentation has distinct economic advantages over submerged fermentation wherever it is feasible to apply this system. It has also been found that this particular species is non-toxicogenic. The properties of this enzyme have been characterised and it has been found to be active over a broad pH range of 6 to 10 and the activity also ranges over a temperature of 25 to 50°C with an optimum at 45°C. It has been found that it has very little activity on collagen, elastin or keratin, so that during the application of the enzyme it is expected that the solubilization of collagen might not have taken place and the hair will come out without loosing its strength.

Working on similar aspects were are reporting in this chapter for the first time, an alternative process of unhairing which will lead to a sulphide free non-enzymatic system in leather processing. Some recent studies on the action of metallic salts on the disulphide linkages in keratin led to this study and paved the way for the development of some unhairing formulations consisting a metallic compound which acts on the disulphide linkages in keratin, at ambient temperature resulting into complete depilation.

Among various metallic salts described here it is observed that nickel salts are very effective in reducing the disulphide linkages at room temperature. Nickel carbonate appears to have an edge over others in the process of unhairing (Sehgal *et al* 1991 and 1996). The details of the investigations are reported here.
4.2 MATERIAL AND METHODS

4.2.1 Materials

Fresh goat skins (22 Nos) were purchased from the local slaughter house and kept in the freezer before use. The skins were directly taken for unhairing. All the chemicals used were of commercial grade available locally.

4.3 METHODS

4.3.1 Unhairing process

22 goat skins were cut into halves and numbered as 1L, 2L, 3L ... 22L, 1R, 2R, 3R ... 22R. 1L to 8L and 11L to 20R (18 sides) were treated with a paste consisting of Nickel carbonate (1%), sodium hydroxide (1%), lime (5%) Kaolin (5%) china clay (2%) and water Q.S. 1R - 8R and 11L - 20L were unhaired by the conventional procedure using sodium sulphide-3%, lime 10% and water Q.S. and were kept as a control batch at ambient temperature (30°C). Both experimental and control lots were kept for 16 hours. The experimental skins after unhairing were delimed whereas control skins were first re-limed and were then delimed. Subsequent processes such as pickling, chrome tanning, rechroming, neutralization, prefatliquoring, retanning, fat liquoring and dyeing for both the experimental and control batches were performed in a single drum as described below. Finally they were converted into crust leather.

4.3.2 Conversion of skins upto crest leather

Pickling

<p>| | | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Water</td>
<td>-</td>
<td>60%</td>
</tr>
<tr>
<td>Salt</td>
<td>-</td>
<td>7%</td>
</tr>
</tbody>
</table>
The drum was run for 10 minutes. Then 1% sulphuric acid dissolved in 10% water was added in 5 feeds at 15 minutes interval. The drum was run for 30 minutes after the last feed. The goods were left overnight in the bath. Next day the drum was run for 20 minutes and checked for pickling and pH of the bath. The pH of the cut section was found to be 2.8 - 3.0. The pickle bath was drained.

**Washing**

<table>
<thead>
<tr>
<th>Water</th>
<th>-</th>
<th>150%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid</td>
<td>-</td>
<td>0.2%</td>
</tr>
<tr>
<td>Run 15 mins.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drained</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Rechroming**

| Basic chromium sulphate | - | 5%  |
| Dry drum for 40 mins    |   |     |
| Added 300% water (40°C) |   |     |
| Cationic fat liquor     | - | 2%  |
| (catalix GSIN)          |   |     |
| Run 45 mins.            |   |     |
| Sod. bicarbonate        | - | 1%  |
| Water                   | - | 10% |
| Added in 4 feeds at 15 mins. intervals |   | |
| Check pH                | - | 4.0 |
| Run 1 hour              |   |     |
| Drained and piled       |   |     |
**Neturalisation**

Sod. formate - 2%
Sod. bicarb - 1%
Water - 300%
Run 80 mins.
pH - 6 (Cross Section)
(pH Checked with Bromocresol purple)
Drained

**Washing**

Water - 100%
Run 10 mins - Drain
Water - 100%
Run 10 mins
Drained

**Dyeing, Retanning & Fat liquoring**

Water - 100% (55°C)
Veg. based fat liquor - 2%
(vernal liq. PN)
Water - 20%
Run 20 mins
Added dye levelling agent - 1%
(TAMOL-NNO)
Water - 10%
Run 10 mins.
Added suitable dye - 1%
(acid or direct dye)
Hot water - 10%
Run 30 mins
Added replacement syntan - 2.5%
(Vernatan OS)
Run 30 mins
Added Wattle (G.S.) - 2.5%
Run 30 mins.
Added
Vernal liq. SP - 6%
Tannit LSW - 2%
Vernal. Liq. ASN - 1%
Run 60 mins.
Formic acid - 1.25%
Water - 10%

Added in four feed at 5 min. intervals. Finally run 20 mins. exhaustion was checked. Finally rinsed and piled overnight.

Next day set, dried, reset, dried completely, saw dusted, staked, toggled, trimmed, buffed on flesh side with 240 emery paper - Dusted off, to get the crust leather.

4.4 EFFECT OF TEMPERATURE

Skins 9 and 10 were cut transversely and longitudinally into twelve symmetrical samples. The six left side samples of the skins (9 and 10) were treated with the paste of Nickel carbonate as described above and kept at temperatures between 10°C to 40°C. The corresponding right side samples of 9 and 10 skins were kept at the same temperature under identical conditions as above using the conventional lime-sulphide unhairing system. Both batches of the skins were covered to minimise moisture loss. The above processes were repeated for skins 21 and 22. The end point of unhairing was
determined by visual examination of skins at ½ hourly intervals. The pelt was scraped with the thumb nail to check unhairing.

4.5 VISUAL ASSESSMENT

The subjective assessment of the crust leathers were made by six qualified leather technologists drawn from CLRI, Chennai.

4.6 PHYSICAL PARAMETERS OF CRUST LEATHER

(Please refer to Chapter II for tensile strength, elongation at break).

4.6.1 Tongue tear strength

The load (Kg) required to tear the leather beyond the cut made perpendicular to its surface, expressed per unit thickness (cm) of the test specimen is the tongue tear strength.

Two third of the length of test specimen (75 x 25 mm) is cut and the mean thickness of the remaining one-third portion is determined and the sampling is done parallel as well as perpendicular to the back bone. The tearing load is determined using the dynamometer.

\[
\text{Tongue tear strength, } \frac{\text{Tearing load (Kg)}}{\text{Thickness (cm)}} = \frac{\text{kg/cm thickness}}{}
\]

4.6.2 Double hole stitch tear strength

The load (kg) required to tear the sample of leather between two holes of 2mm diameter each of which centres are 6mm apart, expressed on its unit thickness (cm) is the stitch tear strength.
The sampling is carried out in both parallel and perpendicular
directions to the back bone and the mean thickness is determined at the
area of the leather of 6mm length throughout its 25mm width. A soft steel
wire (of 1mm diameter) of about 10mm length bent into U-shape is
introduced into the holes of the test piece from the grain side in such a way
that the two ends of the wire project at the flesh side. The wire is wrapped
and then clamped to the upper jaws of the dynamometer, the test specimen
is fixed to the lower jaws. The tearing load is determined.

\[
\text{Double hole Stitch tear strength (kg/cm thickness)} = \frac{\text{Tearing load (Kg)}}{\text{Thickness (Cm)}}
\]

4.6.3 Dynamic water absorption

The leather piece is being subjected to the conditions of wear using
penetrometer to obtain an indication of water proofness.

The test piece is of the size 74 x 60 mm. The size of the absorbent
cloth (Terry towel) is 120 x 40mm. Water absorption is measured as the
difference in the weight of leather cylinder assembly at an hourly interval
from the beginning of the test and is expressed as the percentage on the
original weight of leather. The amount of water transmitted at the given
time interval is measured as the difference in the weights of the absorbed
cloth at an hourly interval from the beginning of the first hour after the
occurrence of penetration is expressed as gms.

4.6.4 Grain Crack Strength

The pressure required to cause first signs of crackiness on the grain
is the grain crack strength.
The pressure is accomplished either by hydraulic means (glycerine or any non-compressible liquid) through a rubber diaphragm as in the Muller apparatus or by mechanical means through a spherical head as in lastometer. A circular test specimen is used and the thickness is read at the middle of the specimen before starting the test.

4.6.5 Bursting strength

Bursting strength is the pressure required to burst the leather. The bursting strength is determined by continuing the pressure beyond the grain crackness till the leather bursts.

4.7 RESULT

Visual assessments of the unhaired skins by a panel of Leather Technologists (drawn from CLRI tennery) showed that unhairing with nickel carbonate appears to give an improved appearance over the leathers unhaired with lime and sodium sulphide. The experimental unhaired skins were soft and free from stains whereas the control lot though soft had green stains over the skins due to the sodium sulphide.

Table 4.7.1 shows the effect of temperature on unhairing for both lots. From this table it is apparent that the duration of unhairing at a higher temperature is reduced to almost half that at the lower temperature. Much improvement of unhairing is not observed at nickel carbonate concentration below 1% at the higher temperature. At temperature lower than 20°C the unhairing was incomplete for the control skins.
Table 4.7.1

Effect of temperature on unhairing

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>Experimental 1% NiCO₃</th>
<th>Control 3% Na₂S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Duration of unhairing (hr)</td>
<td>Duration of unhairing (hr)</td>
</tr>
<tr>
<td>10</td>
<td>19</td>
<td>21.5</td>
</tr>
<tr>
<td>18</td>
<td>15</td>
<td>18.5</td>
</tr>
<tr>
<td>24</td>
<td>13</td>
<td>13.0</td>
</tr>
<tr>
<td>30</td>
<td>12</td>
<td>13.0</td>
</tr>
<tr>
<td>36</td>
<td>9</td>
<td>12.5</td>
</tr>
<tr>
<td>40</td>
<td>8</td>
<td>12.5</td>
</tr>
</tbody>
</table>

* Mean of 2 experiments
Table 4.7.2 shows the subjective assessment of the crust leathers prepared by nickel carbonate unhairing (experimental) and sodium sulphide unhairing (control). In this table the sample of the crust leathers were given separately to six Qualified leather technologists to offer their visual assessment of the two batches (experimental and control). Each assessment was separately done and later all the six assessments were pooled together to get the mean values of the properties of the crust leather like general appearance, surface smoothness, fullness, feel and strength. In all the properties assessed by the technologists, the experimental crust leathers were found to be either good or better than the control crust leathers. In this assessment no tools were used by the technologists to give their assessment. This type of assessment commands very high merit in the Leather Institute (CLRI) and the trade.

To get the actual picture about the properties of the crust leathers the physical parameters were measured as shown in the table 4.7.3. Here properties like Tensile strength, Elongation at break, Tongue tear strength, were measured using standard equipments available in our tannery. From the results obtained as shown in the table it is clearly indicated that the physical parameters of experimental crust leathers are comparable with the control. This shows that unhairing with Nickel carbonate does not have any adverse effect on the physical parameters of the leather.

The test report 4.7.4 is the copy of the test report given by the committee of three technologists of CLRI who were asked by CLRI Director to see the demonstration of unhairing of the goat skins using nickel carbonate formulation (CLARINE : Name given to NiCO$_3$ formulation).
Table 4.7.2
Subjective Assessment of Crust Leather*

<table>
<thead>
<tr>
<th>Property</th>
<th>Experimental</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D.</td>
</tr>
<tr>
<td>General appearance</td>
<td>7.8</td>
<td>1.4</td>
</tr>
<tr>
<td>Surface smoothness</td>
<td>8.3</td>
<td>0.9</td>
</tr>
<tr>
<td>Fullness</td>
<td>7.8</td>
<td>1.4</td>
</tr>
<tr>
<td>Feel</td>
<td>8.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Strength</td>
<td>7.4</td>
<td>0.6</td>
</tr>
</tbody>
</table>

* Mean and S.D. were calculated from 6 assessments by leather technologists.
**Table 4.7.3**

**Physical Parameters of Crust Leather**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Property</th>
<th>Experimental*</th>
<th>Control*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D.</td>
<td>Mean</td>
</tr>
<tr>
<td>1.</td>
<td>Tensile strength Kg/Cm²</td>
<td>202.4</td>
<td>23.6</td>
</tr>
<tr>
<td>2.</td>
<td>Elongation at break %</td>
<td>55.4</td>
<td>5.8</td>
</tr>
<tr>
<td>3.</td>
<td>Tongue tear resistance Kg/Cm thickness</td>
<td>16.3</td>
<td>2.6</td>
</tr>
<tr>
<td>4.</td>
<td>Double hole stitch tear strength Kg/cm thickness</td>
<td>82.5</td>
<td>16.4</td>
</tr>
<tr>
<td>5.</td>
<td>Dynamic water absorption %</td>
<td>109.6</td>
<td>10.5</td>
</tr>
<tr>
<td>6.</td>
<td>Bursting strength Kg/Cm thickness</td>
<td>399.4</td>
<td>36.7</td>
</tr>
<tr>
<td>7.</td>
<td>Grain crack strength Kg/Cm</td>
<td>225.6</td>
<td>36.8</td>
</tr>
</tbody>
</table>

* Mean values of 6 samples.
As per the communication from TIL dated 4.11.91, the team (Dr. P. K. Sehgal and Mr. G. Ramamurthy) demonstrated the product to the Committee. The report of the Committee is given below.

The inventor team prepared the product and applied the same to two nos. of goat skins on the flesh side on 19.11.91 at 2.30 P.M. After applying the formulation, the skins were packed in polythene bag for air tightness and kept overnight for 20 hours and hair is removed from the samples at 10.00 hrs on 20.11.91. The unhairing operation, application and the cleaned skin were closely observed by the Committee.

The Committee has the following observations:

a) The product is working for unhairing of goat skins and idea of the new formulation is effective.

b) The product need to be standardized as regards to commercial application.

c) A new project is to be proposed to study the process in detail and develop the same for commercialisation.

(Dr. B. U. Nair) (Dr. P. G. Rao) (Mr. J. K. Khanna)
4.8 DISCUSSION

Unhairing with nickel carbonate appears to be promising and needs evaluation on a larger scale (Sehgal et al 1991 & 1996).

The pelts treated with nickel carbonate were white and had no stains of sodium sulphide. Both these properties ultimately helped to get uniform and smooth crust leathers in sodium sulphide and lime unhairing the hairs are completely burnt. This not only increases BOD/COD levels in effluent but also interferes with the bacterial oxidation process itself.

So, it is very important to adopt hair saving product to reduce BOD/COD level in the effluent. Hair saving method can be followed by employing dehairing using nickel carbonate formulations. This gives the following advantages over the sodium sulphide and lime process.

1. Hair gets removed with epidermal layer.
2. Less pollution due to non use of sodium sulphide and lime.
3. Free from stains and cleaner pelt.
4. Strength properties are better.
5. Hair are better, cleaner and not contaminated with sulphide.
6. No odour during unhairing.

7. Better scope for assortment as the pelts are cleaner.

Another advantage in this new unhairing process using nickel carbonate after unhairing we observed that, the skins were not required to be relimed with fresh lime and water.

This helps to save 5-10% lime. It also helps in reduction in labour and time. In the conventional process, the skins are required to be relimed after unhairing.

Data has been collected on toxicity of nickel and its compounds (Othmer 1981). Toxicological information and epidemiological data on nickel were examined (Irving Saks 1975).

This included (Toxicological profile 1988) toxicological properties, routes of exposure, government recommendations, health effects (inhalation, oral and dermal exposure) environmental levels as indicators of human exposure potential.

The above available information indicates that occupational exposure to inorganic nickel is widespread but air borne concentrations vary widely. Extensive monitoring data in manufacture of nickel alloy shows average air born nickel concentration of about 0.4 - 1.0 mg/m³ (Santodonato et al 1985).
In tannery operations we are using nickel carbonate in a paste with other ingredients.

The risk is less but has to be evaluated on a larger scale and for a longer period to assess the toxicity. Inorganic nickel compounds including nickel carbonates are absorbed to a low level from the gastro intestinal tract (Lars Friberg et al 1979). Absorbed nickel will accumulate in kidneys, liver and lungs.

Excretion is rapid chiefly via urine. Dermatitis due to nickel is common in nickel workers. (NAS Nickel Nation Research Council 1975) Wearers of shoes with residual nickel may develop an itching or burning popular erythema, in chronic stages it occurs more as a popular or papulocasicular dermatitis.

Precautions must be taken to bring down the residual level of nickel to a bare minimum to eliminate such possibilities. Even then shoes made which may contain nickel compounds must be evaluated by conducting trials on its long term effects.

The residual paste containing nickel should be deposited in concrete tanks for drying, its disposal or recovery of nickel compounds will need to be investigated.
Regarding the cost of nickel carbonate, it is seen that unhairing using nickel formulations will be more expensive than the lime-sulphide process. But attempts could be made to convert available nickel waste into unhairing compositions. Such nickel wastes are available in very large quantities in the sludge generated in electroplating and vegetable oil hydrogenation plants where nickel is used extensively. These aspects are being looked into for preparation of a composition from these waste materials. Particularly the sludge from vegetable oil hydrogenation plants contains very high percentage of nickel along with fuller's earth and oil. Recovery of nickel and converting into nickel carbonate from this sludge is possible.