New Automated Industrial Technologies for Improving Chemical Penetration of Bovine Pieces in the Raw Material Processing and Conditioning Areas of Gelatine Manufacture

A Thesis Submitted in Partial Fulfilment for the

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William John Wittich

Department of Chemical and Process Engineering

University of Canterbury

Christchurch, New Zealand

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Abstract

The production of gelatine at Gelita N.Z. Ltd. is a time consuming process. The time limiting step in the process is the pre-treatment of the collagen tissue of the raw material in a lime/sodium sulfide solution. The liming solution breaks down the collagen in the tissue to gelatine. This is a necessary step prior to the extraction of gelatine from the hide pieces. The current liming process takes nearly 50 days to complete.

Methods were investigated to increase the rate of penetration of the chemicals into the bovine hide raw material. An increase in the penetration of the liming solutions would lead to shorter processing times for this step in the process. The methods that were investigated were temperature controlled mixing, fluidization of the hide pieces and the use of ultrasound. Of all the methods tested, the fluidization of the hide pieces gave the best results. The pretreatment time of the hide pieces was reduced 9 days with this technique.

Methods were also investigated to monitor the levels of conditioning in the raw material An accurate technique to measure hide conditioning was important to pilot plant trials. This helped determine how well any of the trail methods increased the penetration of chemicals into the hide pieces. The use of an ultraviolet dye proved an effective method of measuring conditioning for all the pilot plant trials. The level of chemical penetration was monitored by assessing the penetration of the UV dye. The penetration of the UV dye could be quantified by using imaging software. A possible method of monitoring conditioning in full-scale production was tested. It was determined that the glycosaminoglycans and soluble collagen released into the liming solution could be accurately measured, and related to the overall conditioning of the raw material.

Chapter 1: Introduction

Gelatine is a hydrolyzed protein derived from collagen, which has a broad range of capabilities and uses in a variety of industries. Gelatine can be used as either an ingredient or a processing aid. There are four main categories of gelatine: edible, pharmaceutical, technical, photographic and hydrolyzed. The majority of gelatine products are edible and pharmaceutical. Gelatine is used in food production, as stabilizers/emulsifiers, production of film and for a vast variety of pharmaceutical applications. Final gelatine products contain approximately 84-90 % protein, 1-2 % mineral salts and the rest is water (Ward and Courts 1977). Gelatine dissolves in aqueous solutions at temperatures 37ºC. Gelation of gelatine solutions occur at different temperatures ranging from 5-30 ºC, and depend on concentration and setting time.

1.1. History of Gelatine

The history of gelatine use goes back to Ancient Egypt, though it didn't get its current name until the 1700s. Gelatine got its first patent in 1754 in England for the manufacture of glue. Early research on gelatine and its properties was investigated during the early 20th century by the photography industry, mainly by Eastman Kodak. The majority of technical advancements occurred in the 1950s when progress was made towards achieving high standards in gelatine production and its products (Ward and Courts 1977). It was in this period that important characteristics of gelatine were defined.

1.2. Makeup and Structure of Collagen

Gelatine is a protein which is derived from a larger protein, collagen. Collagen is a fibrous protein molecule which occurs in all multicellular animals and is often one of the most abundant proteins in an organism. There are many sources of collagen-containing tissues, such as mammalian skin, fish skin, bone, tendon and cartilage. A molecule of collagen has the physical characteristics of a molecular mass of ~285 kD, width of 15 Å and a length of 3,000 Å (Voet and Voet 1995). Like all proteins, collagen consists of a large number of amino acids grouped together to form polypeptide chains. Mature collagen is made up of three α-chains, each consisting of approximately 1000 amino acid residues. Collagen has a number of

unique characteristics. There are large percentages of glycine (Gly), 33%, and proline and hydroxyproline, approximately 11% of each (Voet and Voet 1995). Collagen also contains the rare amino acid hyroxylysine (Johnston-Banks 1990). It is common for the amino acid sequence, particularly in bovine collagen, to have repeating triplets of Gly-X-Y, where a high proportion of "X" and "Y" are proline and hydroxyproline (Fig. 1.1) (Voet and Voet 1995).

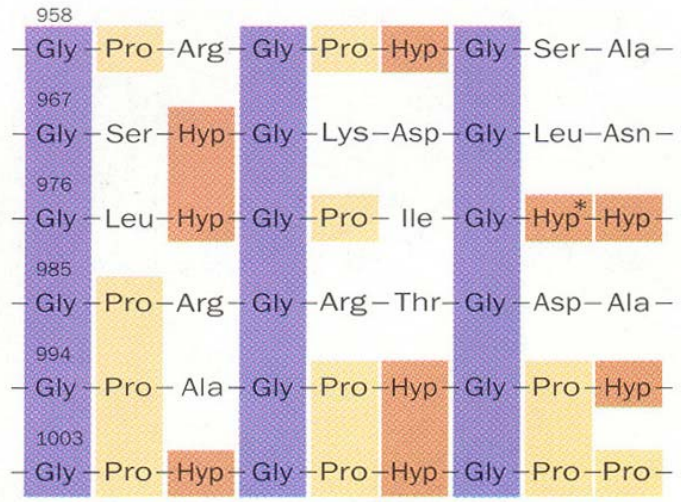


Figure 1.1: Section of amino acid sequence of bovine α_1(I) collagen showing repeating Gly-X-Y triplets (Voet and Voet 1995).

The repeating Gly-X-Y triplets form the individual α-chains. The α-chains making up the collagen are not always identical. There are subtle differences in the composition of the α-chains that create several different types of collagen. Collagen found in mammals consists at least of 30 different α-chains which make up 16 different variations of the collagen protein (Voet and Voet 1995). The most abundant types of these collagen variations are listed in Table 1.1. The different types of collagen are readily found in different tissue sources.

Table 1.1: Common Types of Collagen (Woodhead-Galloway 1980)

Type	*Molecular Form*	*Tissue*
I	$[\alpha_1(I)_2]_2\ \alpha_2$	Bone, dermis, tendon, cornea, dentine
II	$[\alpha_1(II)]_3$	Cartilages
III	$[\alpha_1(III)]_3$	Fetal dermis, cardiovascular system
IV	$[\alpha_1(IV)]_3$	Basement membranes

Three α-chains then form the stable twisted triple helical structure that gives collagen its high tensile strength.

1.2.1. Collagen Cross-linking

Collagen molecules are arranged into groups of four or five to create fibrils. These fibrils are further joined with other fibrils to form groups with a diameter of 100 to 2000 Å depending on the type of collagen and tissue sources (Voet and Voet 1995). The collagen fibrils are stabilized by the formation of intermolecular cross-links between each other. This produces bonds between the different amino acids, mainly the lysine and/or hydroxylysine residues bond with adjacent lysine/hyroxylysine and histidine residues in similar regions (Fig. 1.2).

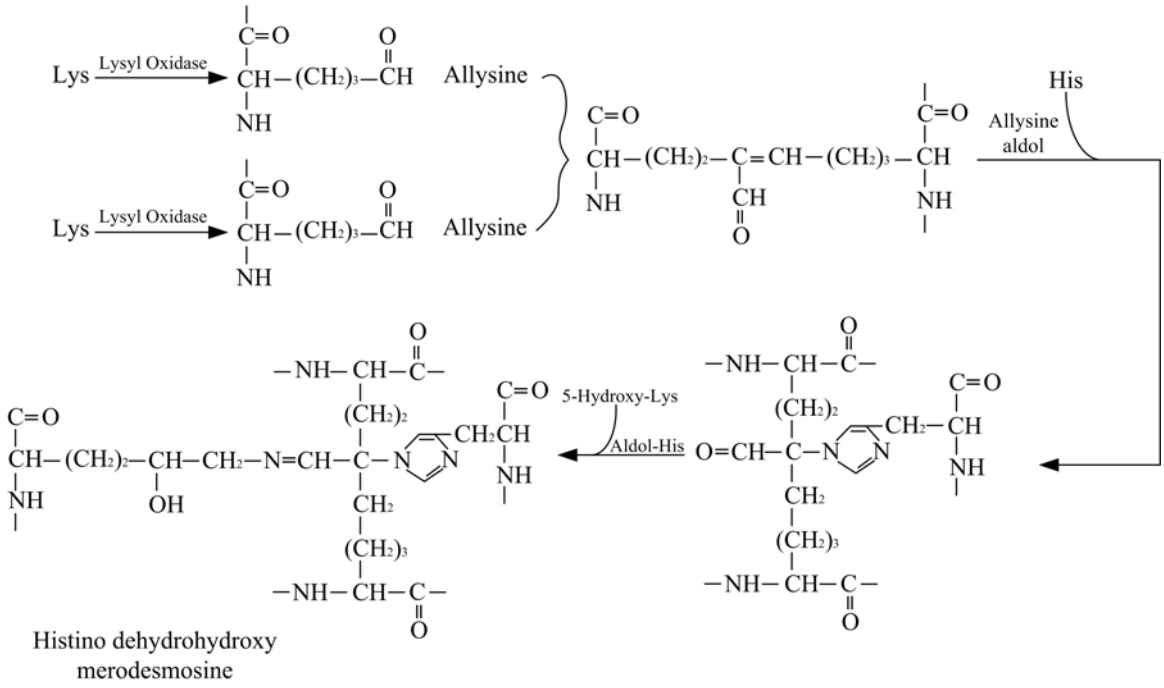


Figure 1.2: The biosynthetic pathway for cross-linking lysine with histidine side chains in collagen (Voet and Voet 1995).

These bonds produce stable covalent cross-links which ensure that collagen is insoluble in water and cannot be attacked by most enzymes (Johnston-Banks 1990). There are various different bond structures which have been proposed for the cross-link bonds between collagen fibrils. These include a head to tail, which is an overlapping of the α-chains, as well as side to side bonding structures (Fig. 1.3).

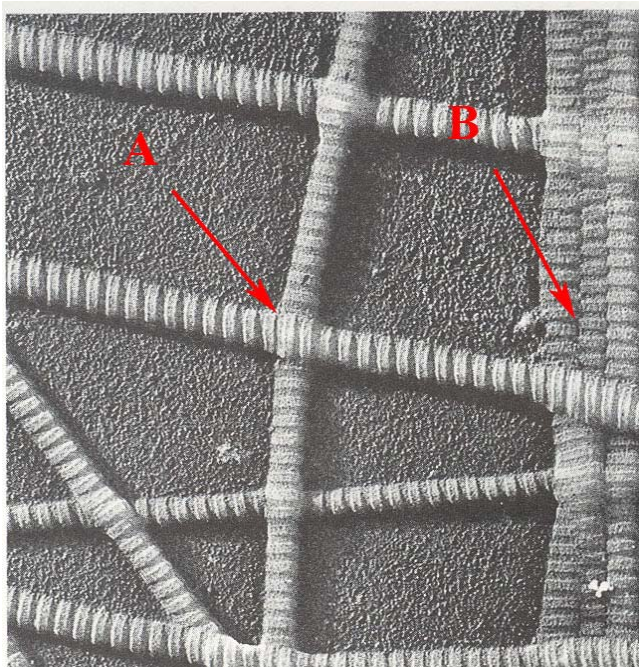


Figure 1.3: Collagen skin fibrils showing overlapping (A) and side to side (B) cross-linking (Voet and Voet 1995).

The arrangement of the bonds between fibrils varies with different tissue sources. As the animal ages the degree of cross-linking in the tissue increases and the proportion of procollagen, a soluble predecessor in collagen development, reduces significantly as the collagen matures. There is still some dispute over the exact nature of the cross-linking in tissue as it ages (Johnston-Banks 1990).

1.3. Structure of Gelatine

The structure of gelatine is similar to the collagen protein from which it is derived except that it is soluble in water. A molecule of gelatine has a width of ~14 Å and length of 3,000 Å, which is nearly identical to that of collagen. The molecular weight of gelatine varies from 20,000 to 250,000 (Ward and Courts 1977), which makes it difficult to relate the physical properties. Gelatine is not a completely polydisperse colloid, but it does has a definite molecular weight distribution. This distribution corresponds to the α-chain and its oligomers that are from cleavage of the collagen cross-links. One to eight oligomers are may be seen in solution (Table 1.2).

Table 1.2: The major molecular fractions found in gelatine (Johnston-Banks 1990)

Molecular fraction	*Description*
Q	Very high molecular weights
1-4	Oligomers of α-chains, levels of five to eight
X	Oligomers of four α-chains
γ	285,000 Daltons, i.e. 3 x α-chain
β	190,000 Daltons, i.e. 2 x α-chain
α	95,000 Daltons
A-peptide	86,000 Daltons

Doublets, β-chains, are formed from both α_1- and α_2-chains. It is possible to have three α-chains remain intact as triple helices, and chains greater than four α-chains. The majority of the oligomers in gelatine are typically single α-chains and β-chains (Table 1.3)

Table 1.3: Molecular fractions found in gelatine (Johnston-Banks 1990)

Source	Fractions (%)								
	<A	A	α	β-pept.	β	γ-pept.	γ-X	1-4	Q
Limed ossien	20	9	28	5	11	4	12	6	5
Limed hide	28	4	30	6	13	3	8	4	3
Acid pigskin	35	4	10	12	11	6	11	7	4

1.3.1. Raw Material Sources

Gelatine is produced from a variety of sources of collagens that must be available in large quantity and at a low cost. This is because a typical yield of gelatine product usually ranges from one tonne of gelatine per 12-20 tonne of raw material. The sources of raw material that are available commercially are pigskin, fish skin, bovine skin, and cattle bone (ossien). Bovine skin is the raw material used at Gelita N.Z. Ltd., which are trimmings not suitable for leather production.

1.3.2. Types of Gelatine

There are two types of gelatine that are produced commercially, Type “A” and “B”. Type A gelatine is produced from pigskin, fish skin or calf skin and is pretreated with acid prior to extraction. Type B gelatine is composed of mature bovine hide or bones which goes through an aggressive alkali pretreatment and/or acid process

(Johnston-Banks 1990). There are differences in the gelatine structure which result in the different molecular fractions (Table 1.3). These differences are due to the raw material source of collagen and the difference in pretreatment of the raw material.

1.3.3. Structural Changes from Collagen to Gelatine

One of the most noticeable changes in the conversion of collagen to gelatine is the hydrolysis of amide groups of asparagine and glutamine (Fig. 1.4) (Radhika and Sehgal 1997).

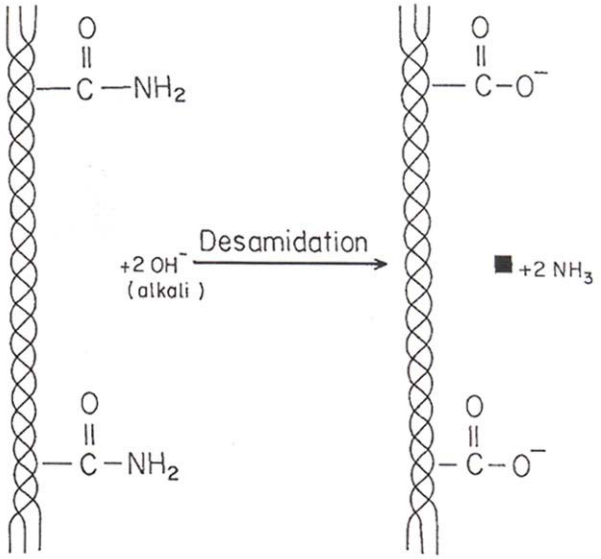


Figure 1.4: The desamidation of collagen during conversion to gelatine. (Radhika and Sehgal 1997)

This conversion leads to an increase of aspartic and glutamic acid residues in gelatine. On average, there is an approximately a 1.5% increase of aspartic acid and a 2-3% increase in glutamic acid. This does vary considerably between the types A and B gelatine. Pigskin is pretreated in a mild acid and is a relatively short process as the collagen from pigskins and fish skins have not developed a high degree of cross-linking. There are fewer amide groups removed and this leads to a marginal increase in aspartic and glutamic acid. Type B gelatine from bovine skin requires a much more aggressive alkali pretreatment and pretreatment takes a considerably longer time (Johnston-Banks 1990). This is because the raw material comes primarily from mature cattle whose tissue has developed a higher degree of cross-linking. Throughout the course of this process there will be almost a complete hydrolysis of the amide groups of asparagine and glutamine which releases ammonia.

The pretreatment in an alkaline solution also causes the alkaline cleavage of the complex carbohydrates (glycosaminoglycans or GAGs) associated with the collagen extra-cellular matrix. The removal of GAGs from the skin results in the loosening of the collagen fibrils (Gelita Project #107102).

The alkali pretreatment also converts of arginine to ornithine, which occurs when urea groups are removed from arginine. This is a slow reaction; gelatine produced from a pretreatment period of 8-10 weeks has only a 3% conversion of arginine. Where gelatine is produced from a longer pretreatment period, 4-8 months, it has a higher conversion of about 34%. There are other subtle changes in amino acid composition in gelatine. Cysteine, tyrosine, isoleucine, and serine residues are usually lower than found in collagen. This is due to the removal of some telopeptides during the cleavage of collagen cross-links, and is lost into the pretreatment solution (Johnston-Banks 1990).

1.4. Gelatine Characteristics and Definitions

There are several different characteristics that are used by gelatine manufacturers to describe the unique properties of gelatine. These properties are always reported by the manufacturer in commercially produced gelatine.

1.4.1. Gel Strength/Bloom

The most important property of gelatine is the gel strength or "bloom", which is a function of the molecular weight of the gelatine. The gel strength properties are related to the α- and β-chain components in the gelatine. The bloom strength, which is also related to viscosity, is an important property in the food industry as it is a good guide to the behavior of the gel. The bloom strength usually determines the grade of the gelatine. The gelatine must be tested under strict guidelines to be referred to as gel strength/bloom, which is defined as, "the weight required to push a cylindrical plunger, of 13mm diameter, 4mm into a previously prepared gel of 6 ⅔ % w/w concentration matured at 10ºC for 16-18h." (Johnson-Banks 1990). The gel strengths typically range from 50-300. Different gel strengths are used for different applications. For example, Type B gelatine with gel strengths from 125-250 is commonly used for confectionary product. Type A gelatine with low gel strength (70-90) can be used for the fining of wine and juice.

1.4.2. Viscosity

Viscosity is another important property in gelatine production and has been studied extensively. When quoted by a manufacturer the viscosity is determined from 6 ⅔ % solution at 60 ± 0.1 ºC and is measured in millipoise. Viscosity is close related to the gel strength of the gelatine. Table 1.4 shows the different viscosity ranges and gel strengths produced for Types A and B gelatines (Johnston-Banks 1990).

Table 1.4: Viscosity and bloom ranges in commercially produced gelatine.

High Bloom (250-300)	30-70 mpoise (limed hide/ossien)
	30-60 mpoise (acid pigskin/ossien)
	70-130 mpoise (specialized hide/ossien)
Medium Bloom (150-200)	20-80 mpoise (limed hide/ossien)
	20-40 mpoise (acid pigskin/ossien)
Low Bloom (50-100)	15-30 mpoise (limed hide/ossien)
	15-30 mpoise (acid pigskin/ossien)

1.4.3. Isoelectric Point

The isoelectric pH values vary with the different types of gelatine, which are dependent upon how the raw material was pretreated. Type A gelatine typically has isoelectric points in the range of 6.5-9.0, with acid-treated ossien being at the low range and acid pigskin gelatine being at the high end. Type B gelatines, produced from an alkaline process, are typically lower ranging from approximately 4.8-5.4 (Johnston-Banks 1990). The alkaline produced gelatine is lower because of the different structural changes that occur in the conversion of collagen during pretreatment. In Type B gelatine the hydrolysis of amide groups from asparagines and glutamine is much more extensive than in Type A, which increases the amount of free carboxyl groups and lowers the isoelectric point (Radhika and Sengal 1997). Some of the physical properties of gelatine have a min/max at the isoelectric point.

1.4.4. Other Properties/ Definitions

There are several other properties of gelatine usually measured in gelatine production.

pH The final pH of a 6 ⅔ % gelatine solution

Color The color of the sample, usually measured by comparison to a standard.

Clarity The clearness of a sample, often measured by optical transmission techniques.

Moisture The moisture remaining after 1 milliliter of water is added to 1 gram of gelatine and placed in an oven at 105 ºC for 18 hours.

There are other properties reported (varying from company to company) including sulfur dioxide content, melting/setting points, odor, ash and other determinations of non-gelatine components.

1.5. Gelatine Production

The initial steps of gelatine production are similar to the leather industry's process. The gelatine process is simply broken down in to two steps, pretreatment and extraction. The purpose of pretreatment is to convert insoluble collagen in the raw material into soluble gelatine, by means of either acid or alkaline processing. Often there are dual soak processes which go through an extensive alkaline process, followed by a shorter acid process. The solublized gelatine is then extracted through five basic steps: washing, extraction, purification, concentration and drying (Johnston-Banks). The process is optimized to obtain a high yield combined with the desired physical properties, which primarily consist of bloom, pH, and viscosity.

Some defined quantities used in gelatine production are shown in Eq. 1.1-1.3.

$$Yield(\%) = \frac{Mass\ of\ Gelatine\ Extracted}{Mass\ of\ Intial\ Raw\ Material} \qquad \text{Eq. 1.1}$$

$$Extractability(\%) = \frac{Mass\ of\ Gelatine\ After\ First\ Extract(50°C)}{Total\ Mass\ of\ Gelatine\ Extracted} \qquad \text{Eq. 1.2}$$

$$Scutch(\%) = \frac{Raw\ Material\ Remaing\ after\ Extraction}{Mass\ of\ Initial\ Raw\ Material} \qquad \text{Eq. 1.3}$$

1.5.1. Gelita N.Z. Ltd. Production Process

The Gelita N.Z. Ltd. facility produces gelatine from bovine hide trimmings that are not suitable for leather production. The majority of the raw material is salted for preservation prior to gelatine production. The hide pieces go through a lengthy alkaline pretreatment process and produces Type B edible gelatine, which is distributed world-wide. Fig. 1.5 shows a flow diagram of the Gelita production process.

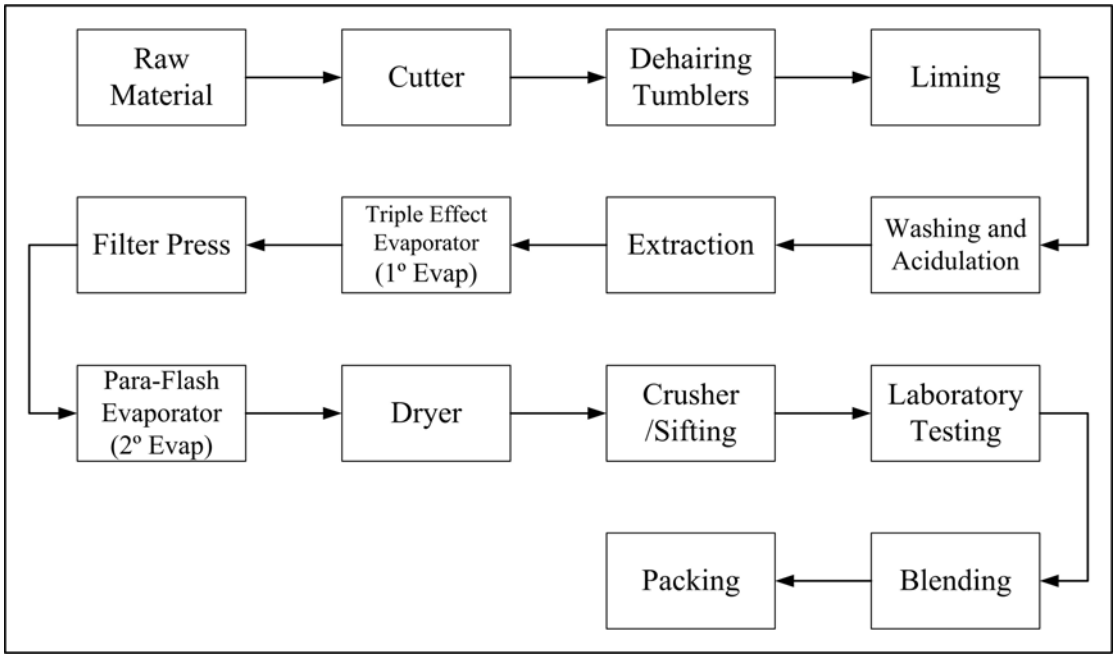


Figure 1.5: Summary of Gelita N.Z. Ltd Gelatine Production Process

1.5.1.1. Dehairing/Liming

Pieces of hide, the majority of which are fresh and salted pieces, are first cut into sizes suitable for processing, and then sent into washing/dehairing (Fig. 1.6). The hide is initially washed to remove salt and other extraneous material. It is dehaired by tumbling overnight in a solution of calcium hydroxide (hydrated lime) and sodium sulfide at 40 g/L and 15 g/L, respectively (Fig. 1.7). The lime loosens the epidermal layer from which the hair can be easily removed. Hair, which is primarily made up of the fibrous protein α-keratin, is broken down by the sodium sulfide (Voet and Voet 1995).

Figure 1.6: Cutting of the hide raw material in preparation for dehairing.

Figure 1.7: Tumblers where hide pieces are washed and dehaired prior to liming.

The same solution of sodium sulfide and hydrated lime makes up the liming solution. The hide remains in the liming solution for an extensive period in order to break the collagen cross-links. During this liming process the hide swells up to several times its original size. At this point the gelatine processing differs from leather processing. Leather processing uses methods to control swelling while gelatine processing promotes swelling. The liming solution is kept at a minimum pH of 12.0 because anything below this pH value tends to allow bacterial growth. The process takes place at ambient temperatures ranging from 8-16°C. The ideal temperature for the manufacture of gelatine is 16-20 ºC. Prolonged temperatures above 20ºC increases the soluble collagen lost into the liming solution, decreasing the final gelatine yield. Liming times typically range from 40-50 days and pits are periodically mixed manually using air spears (poled) (Fig. 1.8). This is the most time consuming step in the Gelita process. The progress of the liming process is monitored by empirically monitoring the appearance of the hide. Optimum alkaline conditioning is needed to maximize yields and produce high quality gelatine. Currently there is no method being employed which quantifies how well the raw material has been conditioned.

Figure 1.8: Pits where hide pieces are limed in a lime/Na_2S solution and periodically poled.

1.5.1.2. Acidulation

After liming, the material is transferred to the acidulation tumblers (Fig. 1.9). The acidulation step further promotes the breaking of the collagen cross-links prior to extraction. The hide pieces go through a series of washing and conditioning with sulfuric and sulfurous acid of 0.1 M concentration and a pH of 1.8. The acidulation procedure takes approximately 2 ½ days. This is the final pretreatment of the material and prepares the material for gelatine extraction.

Figure 1.9: Acidulation is the final pretreatment step prior to extraction

1.5.1.3. Extraction –Evaporation – Filtration

The conditioned collagen in the hide pieces can then be converted into solublized

collagen, or gelatine. The extraction rate is dependent upon the desired properties of the gelatine product, which decreases as the bloom, viscosity and pH increases (Johnston-Banks 1990). Gelatine is extracted over a 16-20 hour period starting at 40 ºC and increasing to 80 ºC over this time (Fig.1.10). The extraction pH and level of conditioning influence the extraction rate, which results in different bloom strength and viscosity.

Figure 1.10: Extraction of gelatine from pretreated hide pieces.

This typically gives a solution containing 3-5% gelatine. The gelatine solution is then concentrated to approximately 22-33% by using a triple-effect evaporator to remove excess water (Fig. 1.11) The temperature decrease from 90 ºC to 77 ºC between the first and second effect of the evaporator then to 56 ºC for the final effect.

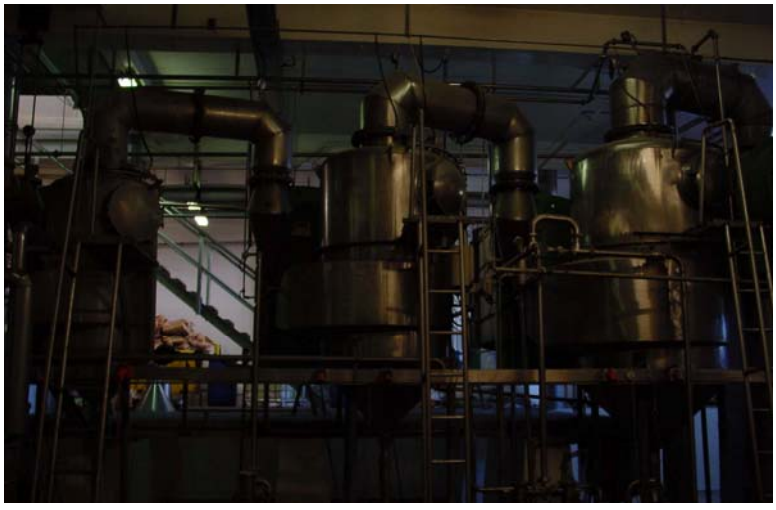


Figure 1.11: A triple-effect evaporation unit is used to initially concentrate the gelatine solution.

After the extraction, there are left over materials consisting of skin, fats and other

unextracted collagen fibers, which are referred to as “scutch” and is left in the extractors. The gelatine solution is then filtered to remove small fibers and insoluble particles (Fig. 1.12).

Figure 1.12: Filtration of gelatine solution to remove impurities.

The gelatine solution is then further concentrated by a secondary evaporation stage. A para-flash evaporator unit is used for the secondary evaporation stage (Fig. 1.13).

Figure 1.13: A flash evaporation unit is used to further concentrate the gelatine solution.

This gives a 30-38% concentrated gelatine solution and is treated to give a pH of 5.0 in the final product.

1.5.1.4. Drying - Crushing/Screening - Testing/Blending

The concentrated gelatine solution is cooled to 21ºC which solidifies the material. It is then extruded into a noodle cake (Fig. 1.14) and sent to an updraft drier increasing from 30 ºC to 50 ºC over eight zones (Fig. 1.15). The maximum water content of the

final dried gelatine is 12%.

Figure 1.14: Extrusion of gelatine into a noodle cake.

Figure 1.15: Drying of the final gelatine product.

The gelatine cake goes through a crushing/screening process after which the pieces are sorted by size. Laboratory testing is done on all gelatine products to test for the final gel strength, viscosity and pH. Upon completion, the different grades of gelatine are blended to meet the specifications of the customers.

Chapter 2: Experimental Background/Literature

The production of gelatine at Gelita is time consuming compared to other gelatine processes. The limitation is the liming process, which can take as long as 50 days to properly condition the raw material. Other gelatine liming process can take as little as seven days depending on the raw material. The reason for the long processing time is because a traditional technique is being used of soaking the hide pieces in the liming solution which does not promote a high degree of mass transfer. Other processes involving mixing have shown a decrease in liming times. The slower the rate of chemical penetration, the longer it takes to break the collagen cross-links in the hide pieces prior to extraction. Time spent on this step could be dramatically reduced if the rate of penetration of the liming chemicals could be increased. There is not much literature directly pertaining to gelatine production. Therefore, literature from the related leather industry and general literature pertaining to techniques for enhancing mass transfer was reviewed.

2.1. Enhancing Mass Transfer with Mixing and Controlled Temperature

The rate of mass transfer rate in a system can be significantly enhanced through mixing. One key to obtaining higher mass transfer rates in a mixed solid-liquid systems such as hide/lime solution comes with the suspension of the solids in the liquid. In general, the solid-liquid reactor is most efficient in a fully suspended condition. Suspension levels increase with an increase in mixer power levels. With a fully suspended solid-liquid system there is an increase in the effective interfacial area, and this corresponds to a large increase in the overall mass transfer coefficients. The turbulence of the mixer in the solution increases the concentrations at the boundary layer between the solution and the hide.

There are several physical properties that affect mass transfer in solid-liquid systems, which include: solid density, liquid density, density difference, viscosity and diffusivity. Geometrical parameters such as particle size and shape, tank size and shape, impeller type and liquid depth, also affect mass transfer (Harnby et al 1992). Particle size is particularly important in gelatine production. Due to current processing limitations hide pieces must be a minimum size when exiting the liming process. Bovine hide is quite a rigid material and would require high mixer power

levels in order to reach a fully suspended solid-liquid system. Mixer power levels would be dependent on hide size. Impeller type is also important because some impellers types could cut the hide pieces. This would result in a decrease in the amount of material that reaches extraction and thus overall decrease in yield.

Mixing trials have been conducted at Gelita N.Z. with less than desirable results. The major problem encountered is that the mixing action accelerates the conversion of collagen to gelatine on the surface of the skin faster than it accelerates the penetration of the liming chemical. The result is the over-conditioning of the surface of the hide that produces a gelled layer on this surface of the hide pieces. The gelled layer slows down any further penetration of the liming chemicals. It is possible to avoid the surface reaction and the creation of a gelled layer by controlling the temperature of the liming solution. The conversion of collagen to gelatine increases with temperature with the ideal temperature of the liming solution being approximately 15-18°C. High temperatures can lead to degradation of hide and a loss of collagen to solution. Decreasing the temperature of the liming solution decreases the rate of reaction. This is seen in the seasonal variation in gelatine production at Gelita N.Z. The reaction rate could be significantly decreased by dropping the temperature of the liming solution to 0°C. This would potentially slow the surface reaction so a gel-layer would not form on the surface of the hide pieces mixed. The mixing action would enhance the penetration of the liming chemicals without being hindered by the formation of a surface gel. Upon full penetration of the liming chemicals, the temperature could be raised to ambient temperatures for the conversion of collagen to gelatine.

2.1.1. Solubility of Calcium Hydroxide (Lime)

The lower temperature could also help increase the mass transfer in relation to the solubility of the lime. Calcium hydroxide is part a relatively small groups of compounds which do not follow typical solubility rules of increasing with temperature. Lime is generally considered only slightly soluble in water, with a solubility product K_{sp} of 5.02×10^{-6}. The solubility of calcium hydroxide decreases with temperature increase. The highest solubility in water occurs at 0°C dissolving 0.185 g/100 ml H_2O. At a temperature of 100°C lime is partially soluble, dissolving .077g/100 ml H_2O (Lide 2002). A higher concentrated lime solution will

increase the mass transfer of chemicals into the pieces of hide.

Decreasing the temperature of the solution, increases the solubility, thereby increasing the amount of hydroxide ions available (Eq. 2.1), though the equilibrium still lies far to the left.

$$Ca(OH)_2 \leftrightarrow Ca^{2+} + 2(OH^-) \quad \text{Eq. 2.1}$$

The liming solution also contains of sodium sulfide, which is slightly more soluble in water (Eq. 2.2). Sodium hydroxide is produced from the dissociated sodium and hydroxide ions (Eq. 2.3)

$$NaS \leftrightarrow Na^+ + S^- \quad \text{Eq. 2.2}$$

$$Na^+ + OH^- \leftrightarrow NaOH \quad \text{Eq. 2.3}$$

Sodium hydroxide solutions have been used as an aggressive form of alkali pretreatment (Ward and Courts 1977). The amount of hydroxide ions produced is greater a lower temperatures, which can accelerate the cleavage of the collagen cross-links.

The combination of increasing the solubility of calcium hydroxide and increasing rates of chemical penetration is more effective at lower temperature. This would optimize the mixing actions to increase the rate of chemical penetration into the hide pieces. Once the chemicals have been fully penetrated by more efficient mixing, raising the temperature would promote the breaking of collagen cross-links, resulting in more evenly conditioned hide pieces.

2.2. Ultrasound

Ultrasound is used to enhance mass transfer and reaction rates in several different systems. The ultrasound creates the implosive collapse of microbubbles formed during a negative pressure period of sound waves, which forms highly reactive free radicals (Hagenson and Doraiswamy 1997). The implosive collapse of microbubbles is referred to as cavitation and has mechanical effects on a system as well. There are two effects thought to increase the mass transfer of a system. The first is microstreaming or symmetric cavitation, which increases mass transfer by causing microscopic turbulence and thinning of the solid-liquid film. Asymmetric cavitation also occurs and increases mass transfer by forming microjets of solvent,

with velocities up to 10^4 cm s^{-1}, which bombard the surface of the solid.

2.2.1. Ultrasound in Leather Processing

The potential of using ultrasound technology in the leather industry was initially investigated in the 1950s and 1960s. Due the immaturity and cost of this technology it was never integrated into the industry. From the mid-90s there has been renewed interest in investigating ultrasound technology and its incorporation into leather processing. This is due to significant developments in the theory and practice of ultrasound. The commercial availability of ultrasound equipment has increased, significantly reducing the cost. Increasing environmental concerns has also been a driving force in the development of more efficient technologies in the leather industry (Ding et al. 1999).

The steps in leather processing include: soaking, liming/dehairing, pickling, degreasing, dyeing, and tanning. Leather processing involves diffusion of these various different chemicals through the pores of the skin, usually accomplished by a drumming or paddling action (Sivakumar and Roa 2003). The pore diameter of bovine skin typically ranges from 3 x 10^{-8} to 1.5 x 10^{-2} cm. The goals of implementing ultrasound technology into leather processing include: minimizing excess use of chemicals for the particular unit operation, reducing the processing time by enhancing rate of chemical penetration through the pores of the hide, and decreasing chemicals discharged into wastewater streams.

The use of ultrasound in leather processing has been investigated for all the above mentioned steps (Ding et al. 1999, Sivakumar and Roa 2001) .The most relevant work to gelatine production is the effect of ultrasound on the soaking and liming/dehairing processes. The leather processing liming ranges between 8-20 hours for wet salted skins and 24-48 hours for dried skin. Early trials with ultrasound technologies demonstrated a considerable reduction in soaking times. In addition, there was less consumption of water and chemicals. Recent results of ultrasound in soaking have given promising results reducing soaking times to around one hour, with a 10-15% increase in water uptake when compared to the control process (Sivakumar et al. 2005).

Soaking enhanced with ultrasound can remove non-collagenous materials present in the hide, such as, salt, dirt blood, feces and globular proteins, with little destructive effect on the skin material and the collagen fiber structure. Tests conducted by Sivakumar and Roa suggest that the tanned leather fiber structure was not adversely affected by ultrasound treatment. This was confirmed by comparing physical test characteristics against control leathers. Therefore the ultrasound at low ultrasonic intensity levels (~0.5 W cm^{-2}) had little to no destructive effect on the overall structure of the hide (Sivakumar et al 2005).

In 1995 an Italian company, Alpa S.p.A. patented a process for dehairing hide using ultrasound. The main emphasis of this process was not time reduction rather it was to reduce chemical-oxygen-demand (COD) of the effluent liming/dehairing liquor. Conventional leather processes have typical COD levels between 36,000 and 65,000 mg/L. These levels were significantly reduced with the application of ultrasound in which COD levels were reduced to 17,200-23,700 mg/L. The ultrasound action itself also helps loosening of hair from the hair bulb reducing the use of conventional chemicals and processing time. The hides were treated with ultrasound transducers placed inside a conventional rotating drum (Fig. 3).

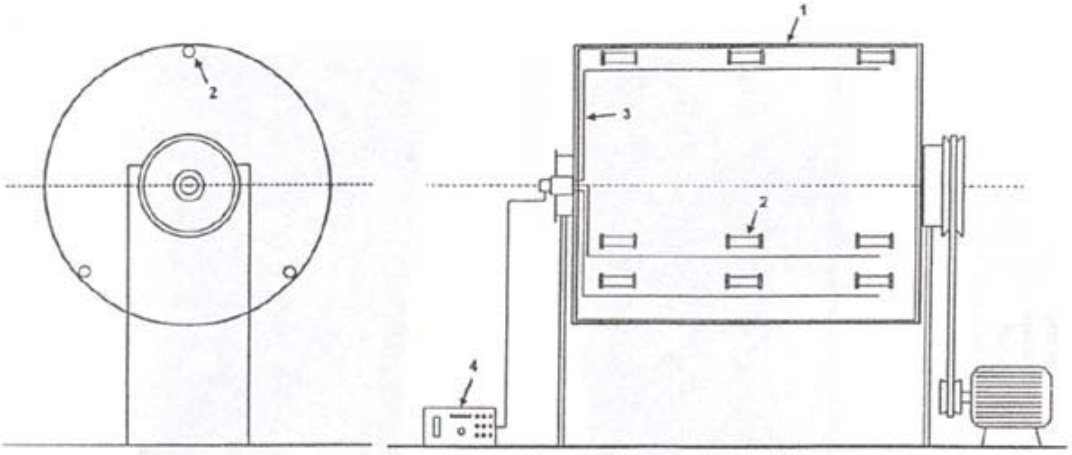


Figure 2.1: Industrial Drum Equipped with ultrasonic transducers, developed by APLA S.p.A.

The hides were treated with ultrasound for a three hour period followed by soaking overnight in the liming/dehairing solution. The resulting hides from this process had the quality of those of the 36 hours conventional process rather that the 18 hours process (Ding et al 1999). Ultrasound has also been used as a method to treat the effluent sulfide levels from liming solutions in which sulfide is oxidized to sulfate. Sulfide levels have been reported to drop from 200 mg/L to less than 1 mg/L (Ding et

al 1999).

Ultrasound has the potential to increase chemical penetration in the liming/dehairing steps of gelatine production. The addition of ultrasound gives a more thorough removal of hair from the skin allowing for better penetration through the hair shaft. Mass transfer rate can be increased as the ultrasound creates cavitation near the hide surface and generates microjets and shock waves. The liquid phase surrounding the microbubbles during cavitation creates micromixing and increases the diffusion of chemicals into the pores of the skin (Sivakumar and Roa 2001). There is also the potential that ultrasound could reduce the COD and sulfide levels of the liming effluent, reducing wastewater treatment costs.

2.3. Fluidization/Single Vessel Processing

Mass transfer of a solid-liquid system can also be enhanced by fluidization. This is achieved by pumping a fluid, gas or liquid, through a bed of solid particles with velocity high enough to counteract the weight of the solid particles in the bed. When solid particles become fluidized there is an increase in the surface area of the solids which promotes mass transfer of the system. The concentrations of the liming solution increase at the boundary layer of the hide during the fluidization, increasing the mass transfer of the system. One important definition of fluidization is the minimum fluidization velocity, which is defined as the velocity in which the upward drag force of the fluid is equal to the weight of the solid particle bed (Kunii and Levenspiel 1991). This point is where the solid bed starts to expand. The expansion of the solid particle bed is often measured in relation to the fluidization velocity and is shown at the minimum fluidization velocity in Fig. 2.2.

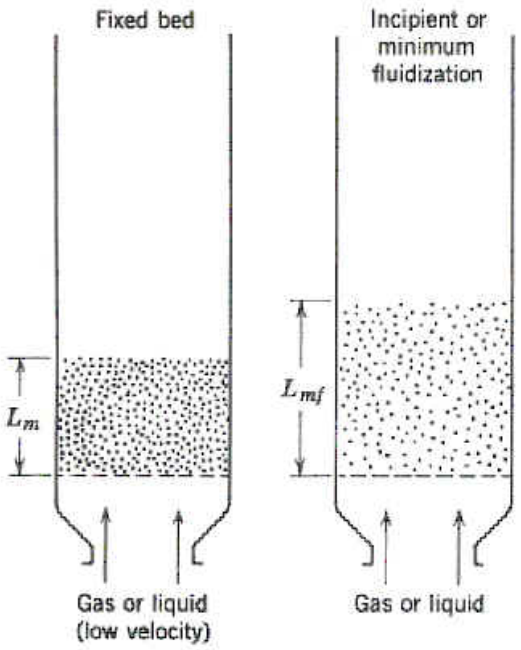


Figure 2.2: Bed expansion at the minimum fluidization velocity (Kunii and Levenspiel 1991).

The minimum fluidization velocity is described as a function of the following parameters: diameter of the column and solid particles, densities of the fluid and solid, and viscosity of the fluid. In practical applications the solids are often not spherical and the diameter of the solid can be corrected by using the volume of the solid. The effective diameter (d_p) is the diameter of a sphere having the equivalent volume of the solid (Eq. 2.4).

$$d_p^3 = \frac{6a^3}{\pi} \qquad \text{Eq. 2.4}$$

The Archimedes number is defined as:

$$Ar = \frac{d_p^3 \rho_f (\rho_s - \rho_f) g}{\mu_f^2} \qquad \text{Eq. 2.5}$$

The Reynolds number of the minimum fluidization velocity can be calculated from the Archimedes number (Eq. 2.5).

$$\mathrm{Re}_{mf} = \sqrt{1135.7 + 0.0408 Ar} - 33.7 \qquad \text{Eq. 2.6}$$

The minimum fluidization velocity corrected for a non-spherical particle can then be calculated using Eq. 2.7.

$$u_{mf} = \frac{\mathrm{Re}_{mf} \mu_f}{d_p \rho_f} \qquad \text{Eq. 2.7}$$

Chapter 3: Experimental Procedure/ Methods

The overall project goal was determining a method to increase the speed of chemical penetration into the bovine hide pieces of the pretreatment processing steps of gelatine production, leading to a decrease in processing time. This was accomplished by carrying out trials using different technologies and techniques to increase mass transfer. Each trial carried out was run simultaneously with a control hide samples pretreated using the same technique as Gelita N.Z. full scaled gelatine production. Control runs were reduced to pilot plant scale and were based upon Gelita N.Z. pilot plant liming and/or acidulation standard operating procedures (SOP's).

3.1 Hide Preparation/Dehairing

All the raw material for the trials, both experimental and standard, followed the same procedure for hide preparation and dehairing. This is a necessary step in preparing the hide for the pretreatment/conditioning process. The process uses different sources of raw material for gelatine production, these include: salted mature hide, fresh mature hide, salted calf hide, fresh calf hide, and splits. The majority of the raw material is bovine face pieces and trimmings that are not suitable for leather production. The raw material that was used for all the trials was salted mature hide as it is the predominant raw material used at the Gelita N.Z. facility.

During the preparation and dehairing step hide pieces were "twinned" and marked. Twinning is a procedure used at Gelita NZ in which the raw material is cut from the same hide piece of the same animal and run through different processes. This helps reduce variability in the procedure as each hide piece is different due to factors such as fatty tissue remaining on the hide, hide thickness and the age of the animal.

The hide pieces are salted for preservation and can remain in hide storage for up to several months. The hide pieces require a thorough washing to remove any extraneous materials before processing. The hides were cut into approximately 15cm x 15cm pieces and between one and six kilograms of hide were treated depending on the trial conducted. The hide pieces were loaded into tumblers and washed for 15 minutes with water to remove salt and other extraneous material then drained. The hide was then treated with a 0.1 M sodium hydroxide solution in

tumblers for one hour, which serves to loosen the hair from the skin.

The hide pieces were then treated in a lime/sodium sulfide (Na_2S) solution with concentrations of lime and sodium sulfide at 40 g/L and 15.0 g/L, respectively. This gave a final sulfide concentration of approximately 2.5 g/L based on previous dehairing trials, which is the typical lime pits sodium sulfide concentration. The hide pieces were then placed in a tumbler in the lime/Na_2S solution for three hours. After the dehairing period the hide pieces were checked to ensure all hair was removed from the skin. If there was hair still on the hide, the pieces remained in the tumbler for another hour or until all the hair was removed from the hide.

3.2. Standard Pilot Plant Liming

The pilot plant SOP for liming starts with placing the hide pieces into a bucket filled with the lime/ Na_2S solution from the dehairing step. The hair removed from the hide pieces remained in the solution and was not filtered. Hair remains in the solution in full-scale production. The solution was then diluted with water by adding 100% the initial volume of the lime/Na_2S solution. The pH of the solution and the initial Na_2S concentrations were measured. The pH of the solution should be 12.0 or greater. If the pH of the solution drops below 12.0 during the liming process, additional lime must be added to prevent bacterial growth, or “pit rotting”. The Na_2S concentration was determined by the LIRI method (Sec. 3.3), if the Na_2S concentration was below 2.0 g/L at the beginning of the process, additional sodium sulfide was added.

The hide pieces remained in the lime/ Na_2S solution for approximately three weeks at ambient temperature. This approximates the same level of conditioning that is obtained in the full scale liming pits. The solution was stirred every few days as the undissolved lime begins to settle. The solution temperature and pH were measured every few days as well as monitoring the level of conditioning of the hide pieces. Currently, there is no standard method to quantitatively determine the level of conditioning of the hide pieces, and it relies on experience and comparison to other samples. Several methods of determining level of conditioning were developed and tested throughout the course of the project. The methods developed will be discussed in detail later.

3.3. Sodium Sulfide Determination

Sodium sulfide levels were monitored throughout the liming process. The concentration of sodium sulfide was determined by the LIRI method. In this method the sulfide solution was titrated with a potassium ferricyanide solution standard with a ferrous dimethylglyoxime ammonia complex present. The sulfide in the solution was oxidized to sulfate which precipitated with barium chloride (Booth 1956).

Table 3.1: Reagents required for LIRI method of determination of sulfide.

Reagent	Description
Potassium ferricyanide	0.1 N solution must be kept in dark
Buffer	1 g/ml solution of NH_4Cl in ammonia (s.g. 0.880)
Barium Chloride	12.5 g/L solution of $BaCl_2$ in water
Indicator	10 ml 0.6% $FeSO_4$ 50 ml 1.0% dimethylglyoxime in ethanol 0.5 ml concentrated H_2SO_4

A sample of liming solution was filtered to remove any extraneous material. 20 ml $BaCl_2$ solution, 20 ml buffer and 1 ml of indicator are added to a suitable sample of liming solution. The solution was left for ten minutes to precipitate the sulfite. The solution was titrated with 0.1 N potassium ferricyanide until the pink color is destroyed for a period of one minute. The sulfide concentration was then determined from Eq. 3.1.

$$Na_2S\ Conc.\ (g/L) = \frac{Titre\ (ml)\ x\ N\ (Fe(CN)_6\ )\ x\ 0.0390\ x\ 1000}{Sample\ Volume\ (ml)} \qquad \text{Eq. 3.1}$$

3.4. Standard Acidulation Procedure

When hide pieces have completed the liming procedure they go through the washing/acidulation step. The hide pieces were thoroughly washed to remove as much residual liming solution as possible. Several stages of washing were required to drop the washing solution to a pH of below 10.0. Twelve kilograms of hide was added to 4.5 liters of water and placed into tumblers. The hide was washed in the tumblers for periods of two, three, six, and nine hours with the solution drained and replaced with fresh water after each stage. If the pH of the solution was not below 10.0, an additional stage was required.

The hide pieces were then treated with a series of sulfuric and sulfurous acid solutions. The first stage consisted of turning the hides in a 0.1 M sulfuric acid solution for a period of five hours. The hide was further treated with 0.1 M sulfurous acid in tumblers for three additional periods of five, seven and eight hours with the solution drained and replaced with fresh sulfurous acid after each stage. The hide was then left standing in a final sulfurous acid solution overnight. A visual assessment of the hide was made after this step to determine if another static stage was required.

3.5. Temperature Controlled Mixing

Trials were conducted to see how well the penetration of liming chemicals could be accelerated by means of temperature controlled mixing. The trial was set up to compare mixing at low temperature mixing versus room temperature mixing.

Table 3.2: Description of the set-up for the temperature controlled mixing trial.

Trial Abbreviation	*Description*
LTM	Continuous mixing of hide pieces with temperature maintained at 1°C.
RTM	Continuous mixing of hide pieces at ambient temperature (control).

The hides were mixed for an initial period of time then mixing was discontinued and both were left at ambient conditions. Sodium sulfide concentrations were monitored throughout this trial as mixing increases the reaction of sodium sulfide with keratin (hair).

In this trial each face piece was twinned and cut into equal parts of 15 cm x 15 cm and marked. A basis of three kilograms of hide was used for each of the trials. The LTM set-up consisted of temperature controlled mixed hides in liming solution. The temperature was kept at approximately 1 ºC by keeping the solution in an insulated container immersed in ice. The hide was continuously mixed with a Waitron Type R2R2-64 100 watt mixer. The mixing impeller was a radial-flow impeller with three flat panels as to minimize damage to the hide pieces during mixing. Ice was replenished each day to ensure the temperature was kept at approximately 1 ºC. Temperature and pH values were recorded each day, as well as sodium sulfide concentrations every third day. An identical set-up was made up for mixing under

ambient conditions (RTM).

3.6. Ultrasound

Trials using ultrasound as to enhance chemical penetration were conducted. It is particularly important to control temperature in these trials, as hides degrade in highly basic solutions above 20 ºC for excess periods of time. The ultrasound apparatus consisted of a 1 L glass beaker inside an ultrasonic cleaner (Transsonic TS 540) filled with water (Fig. 1.1). The glass beaker was lifted off the bottom of the ultrasound reservoir and positioned in the center of the tank (Fig. 1.2). Below the tank surface, in the center, is an ultrasonic transducer which generates ultrasound of 300 W at a frequency of 35 kHz. This gives an ultrasonic intensity of 0.71 W cm^{-2}. The bath temperature was controlled by running cooling water through a copper coil positioned inside the reservoir.

Figure 3.1: Set-up of equipment for ultrasound trials.

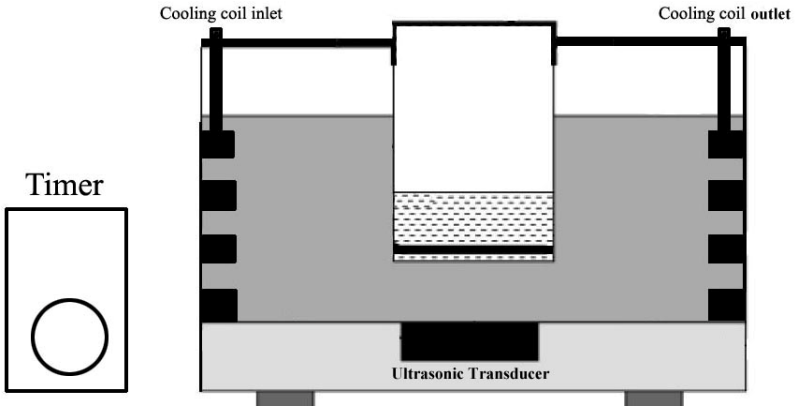


Figure 3.2: Schematic of the ultrasound set-up.

Hides were twinned for this trial. Approximately 500 g of hide was treated with

ultrasound. Temperature, pH and sodium sulfide concentrations were measure throughout the trial on both the ultrasound and standard hide pieces.

3.7. Fluidized Column Combining Liming and Acidulation

A fluidized column was investigated as a technique to reduce raw material pretreatment times. The fluidized column was set up to incorporate both liming and acidulation steps and treated in a single vessel. The combination of both steps would decrease the amount of processing vessels as well as the handling of the material. A fluidized column would provide good mixing of the fluid and is gentler on the material than conventional mixing.

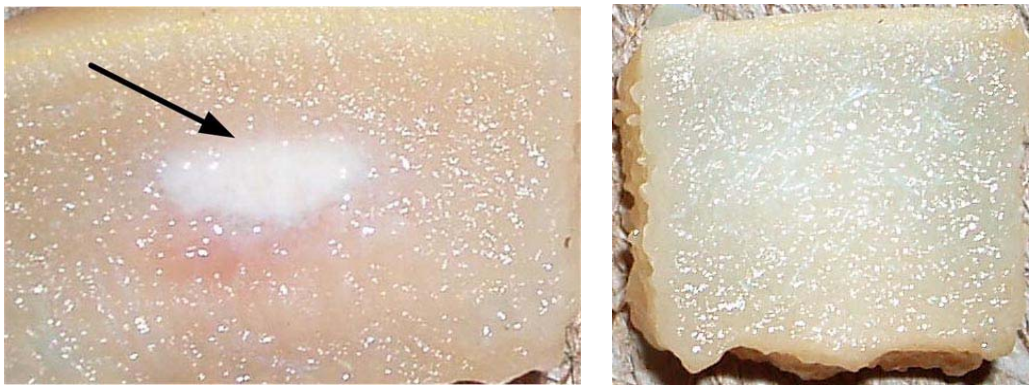
A graduated cylinder with a diameter of 53 mm was used as the fluidized column. The bottom of the column was modified with an inlet for recirculation of the chemical solutions. Hide pieces were cut sizes of 30 mm x 30 mm. Hide pieces that have gone through the dehairing process had a density of 1.22 g/L. The density of the liming solution was a similar density to water. Using the column diameter, the size of the samples and the determined hide density, a minimum fluidization velocity with a correction for non-spherical particles was determined (Eq. 2.5-2.7). The minimum fluidization velocity was determined to be 0.037 m s^{-1}, which corresponds to a minimum fluidization flow rate of 4.9 L min^{-1}. The initial bed height of the hide pieces was 225 mm. A Sandpiper ½" diaphragm pump was used at a flow rate of approximately 6.0 L min^{-1}. The Sandpiper pump was made of polypropylene which is resistant to highly basic and highly acidic solutions. The chemical solutions were drawn from the top of the column and recirculated continuously through the column. The control trial for comparison consisted of twinned hide cut to the same size as used in the fluidized column of 30 mm x 30 mm and also to the standard size of 15 cm x 15 cm. Temperature, pH and sodium sulfide concentrations of both the experimental and standard process were monitored throughout the trial. Fig. 3.3 shows the experimental set- up for the fluidization of hide.

Figure 3.3: The set-up of a fluidized column

Following the completion of the liming step the hide pieces went through the washing/acidulation procedure in the same vessel. The hide pieces were fluidized in the sulfuric and sulfurous acids stages then left static for the final sulfurous acid stage as in the acidulation SOP. The control trial was treated with the Gelita N.Z. acidulation SOP.

Chapter 4: Measurement of Conditioning

Conditioning is a term used in the gelatine industry to describe how well the hide pieces have been pretreated in the liming process. This relates to the extent of the breakdown of collagen cross-links. The conditioning degree of the hide pieces can be seen after hide pieces go through the acidulation process.



(a) **(b)**

Fig 4.1: Examples of under conditioned hide (a), shown by the dense white section in the middle and fully conditioned hide (b)

Fig. 4.1 shows examples of both under conditioned and fully conditioned hide. The white area in the center is where the liming chemicals have not fully penetrated and thus the collagen cross-links have not been broken.

Currently at Gelita N.Z. there is no quantitative method of determining how well the hide pieces have been conditioned in the liming pretreatment. In the current method transferred to the next process after assessing the visual appearance of the hide and relies on production experience and comparison to previous samples. In some cases raw material is moved to downstream processes based upon the average liming time of approximately fifty days, even though the raw material might be under conditioned. The lack of accurate determination of conditioning leads to variability in further processing of the material and ultimately final gelatine product yield and quality. A reliable method for determining the level conditioning of the raw material could potentially improve overall processing time and product yield.

The lack of a reliable means to determine conditioning affects the experimental trials. It is difficult to determine the effectiveness of the different methods of enhancing chemical penetration without a quantitative measurement of conditioning. Therefore,

it is necessary to develop a method to determine the level of conditioning during the pretreatment process of the hide pieces. Development of such a method would be beneficial the pilot-plant scale trials as well as the full scale processing.

4.1. Ultraviolet Dye Penetration

Dyes have been tested in pilot plant trials, in which the penetration of the dye approximates the penetration of the chemicals and hence the conditioning of the hide piece. The problem encountered with this method is that the lime/Na_2S solution ranges from a dark green to a black color and the dye is not clearly visible. The basic idea of using dyes in the liming solution is promising. It could make monitoring the chemical penetration possible, if the dye could be easily detected in a dark solution.

Dyes that are visible under ultraviolet light would be easily detectable in a dark liming solution. A sample of Leucophor PAT liquid was provided by Chemcolour N.Z. Ltd. Leucophor is a fluorescent brightener for polyamide fibers, wool and silk and their blends. One important property of the Leucophor PAT liquid is the stability of the dye in solutions. Leucophor is very stable in acids to pH 1.0 and in alkalis to pH 13.0. This dye could be used in the liming and acidulation solutions without breaking down.

Most of the applications of the Leucophor PAT liquid in the clothing industry use the dye at a 1% (v/v) concentration. A sample of the dye was tested at this concentration in the liming solution. The Leucohpor PAT was clearly visible in the liming solution at this concentration when viewed under ultraviolet light. This method of tracking chemical penetration with ultraviolet light was tested with the standard pilot plant liming process, using a 1% (v/v) concentration in the liming solution. Samples were taken from the liming process every few days in order to monitor the dye penetration and were photographed using the set up shown in Fig. 4.2. The samples were illuminated from the UV lamp at an offset angle, inside a black box, so the samples could be photographed.

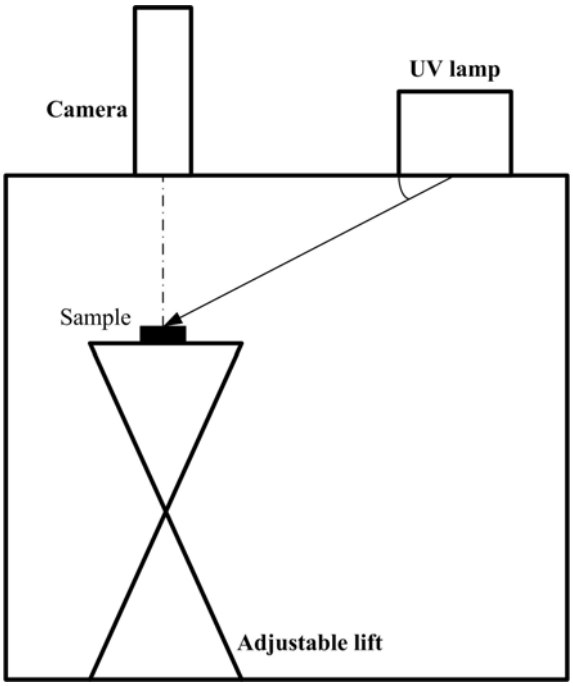


Figure 4.2: Photograph set-up for hide samples.

The hide samples were then frozen so the material could be easily sliced. The samples were cut to approximately 1mm thick using a razor, and viewed under ultraviolet light. Fig 4.3 shows the penetration of dye after an initial three day liming period.

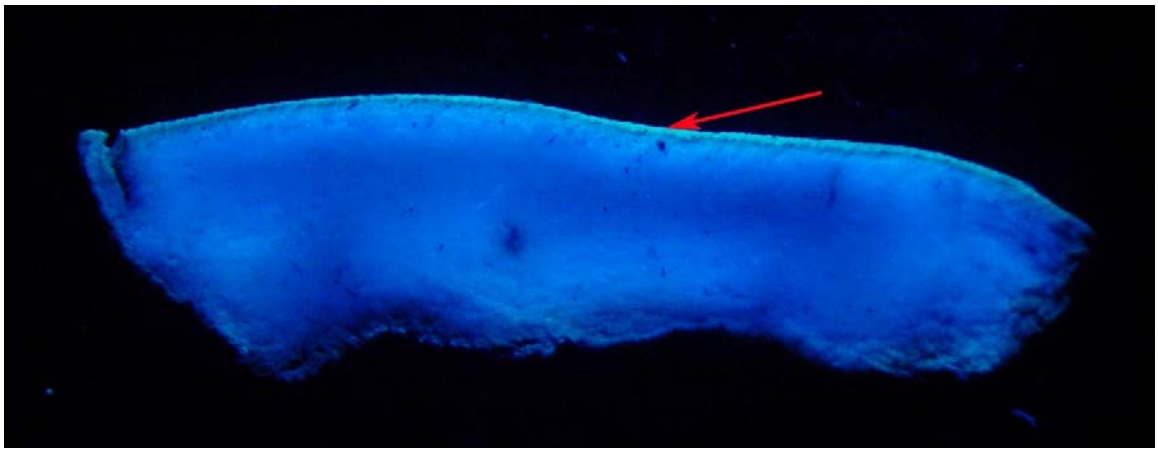


Figure 4.3: A hide sample after three day liming period with Leucophor dye.

The color of the skin shown in Fig. 4.3 closely represents the color of the dye in the liming solution. Areas with this color are sections where the dye has fully penetrated. The darker colors are where there has been little to no penetration of the dye. Fig. 4.4 shows a hide sample after completing the pilot plant liming procedure of twenty-three days. It shows a more even penetration of the dye throughout the entire hide piece.

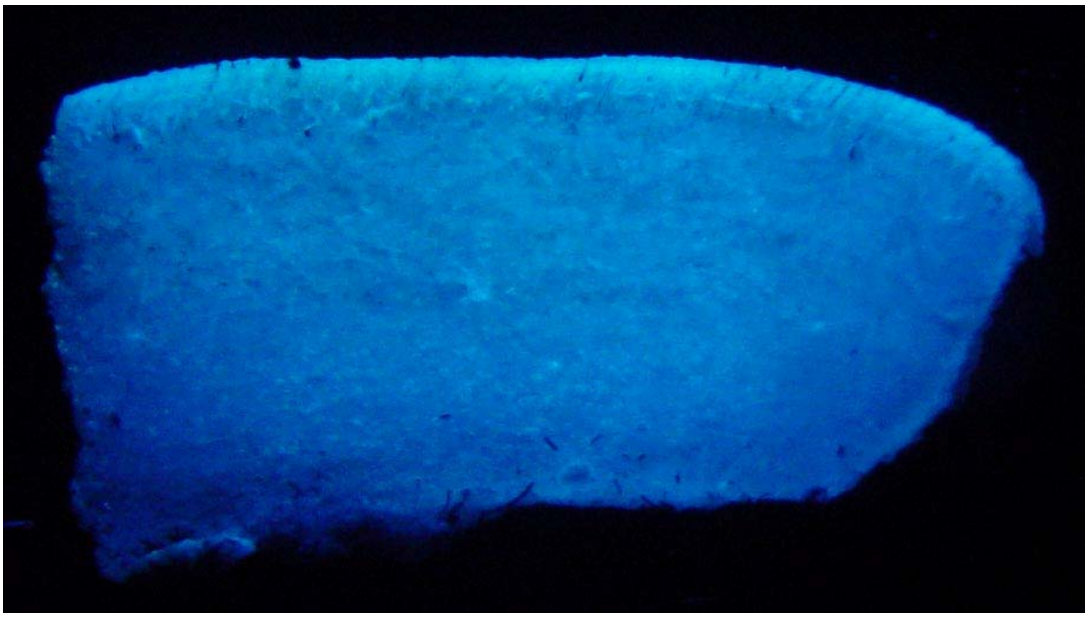


Figure 4.4: A hide sample after 23 days in liming solution with Leucophor dye.

After completion of the trial using Leucophor dye, it was determined that the chemical penetration of the dye could track the penetration of the liming chemicals, which at completion is shown by seeing an even and full strength penetration of the dye into the hide pieces. The time required to obtain the level of penetration shown in Fig. 4.4 was approximately three weeks, the same time required for standard pilot plant liming process. The use of an ultraviolet dye seemed a plausible method of monitoring the level of conditioning in pilot plant processes. This method cannot be used in the full scale production because it appears that the dye remained in the material throughout downstream processing, and would not be acceptable for edible gelatine production.

4.1.1. Sigma Scan Color Intensity Scan

The use of the dyed hide pieces illuminated with ultraviolet light produces a variety of different color intensities, with the highest color intensity being the color of the dye in solution, and a black color is an indication that dye has not penetrated the hide at all. It is difficult to accurately distinguish the difference of the color intensities visually. Image measurement software can detect the small differences in overall color intensities in the hide pieces. The intensity scan creates a set of data points representing the intensity at many points throughout the thickness of the hide sample. Therefore, using the software to display an array of color intensities indicates how well the dye has penetrated into the sample. This is a much more quantitative

method of determining dye penetration into the sample and ultimately how well the hide piece is conditioned.

An analysis method was developed using Sigma Scan Pro 5.0. Since the quality of the photograph of the hide piece affects the analysis, when using the software, the hide samples were photographed using a Sony Cybershot DSC-F707 5.0 megapixel digital camera equipped with a Cannon 2x magnifying lens. The sample was then photographed using the setup shown in Fig. 4.2, giving a high quality close up photograph. A line color intensity scan was then run, using Sigma Scan Pro 5.0 image measurement software.

An example of the analysis method is shown on a piece of hide that has been soaked in liming/Na_2S solution with 1% Leucophor PAT liquid for 23 days (Fig 4.3). After photographing the sample an intensity scan was run through the center thickness, as shown in Fig. 4.5.

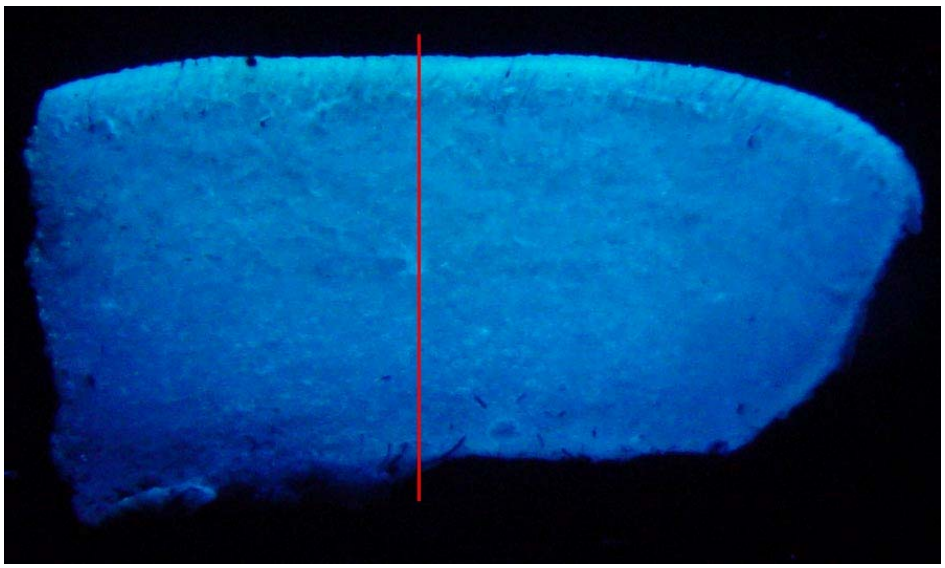


Figure 4.5: Line intensity scan configuration of hide sample.

The intensity scan in Sigma Scan is calibrated to indicate where the black background represents a color intensity of zero and the brightest color the highest intensity. The intensity data points can then be converted to a percent intensity, based upon the highest color intensity, which is on the surface of the skin. The hide thickness was normalized to account for the difference in hide thicknesses of the samples. This is done to easily compare different sample of hide. The x-axis is equal to zero at the skin

of the hide and one is the fatty tissue on inside of the hide piece. Graphs plotted percent intensity versus the normalized thickness of the hide sample (Fig. 4.6).

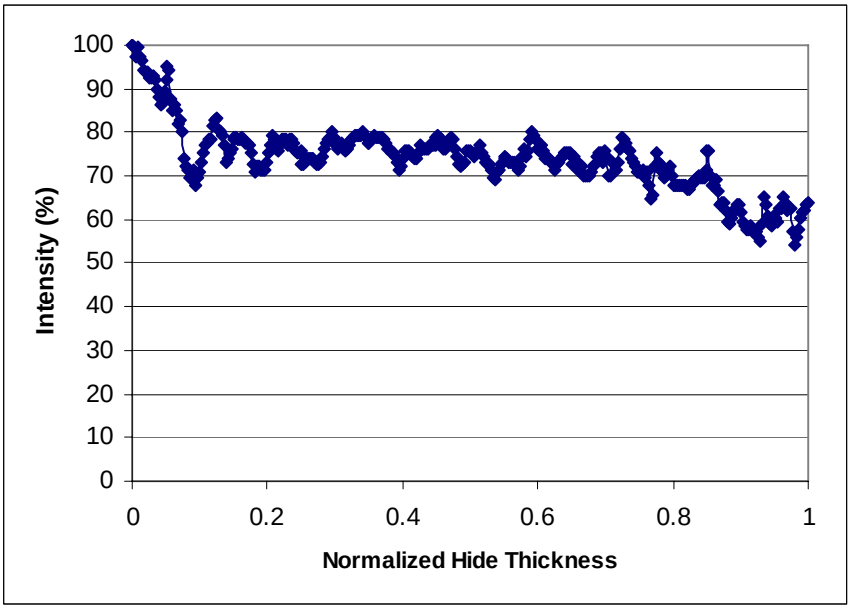


Figure 4.6: Plot of percent intensity versus hide thickness for a hide sample after 23 days of liming.

The plot of intensity percent versus hide thickness shows a generally even penetration of the dye throughout the sample. There is a trend of the color intensity decreasing while going through the thickness of the hide. This is not due to uneven penetration of the dye, but is a result of the placement of the UV lamp (Fig 4.2). The UV light illuminates the sample from an angle which results in higher color intensity on the skin side and decreases toward the fatty side of the hide.

Even though placement of the UV lamp gives the appearance that the color intensity is slightly higher at the skin surface, the analysis technique gives a method for accurate comparison of chemical penetration between the experimental and standard samples.

4.1.2. Microtome Techniques

In the analysis technique described the samples were lit from above with ultraviolet light and at an offset angle, so they could be photographed. The pieces were cut to approximately 1 mm in thickness. A microtome could cut much thinner samples. If the samples were cut into thin slices, on the scale of microns, the samples could then be lit with ultraviolet light from directly underneath and not at an angle. This

would give a more accurate measurement of color intensity.

A sample of hide from liming solution with dye was cut into thin sections using a microtome. A freezing microtome was used since the thinly sliced samples tend to break when cut at ambient temperature. Tissue-Tek OCT compound was used as the embedding compound. The OCT compound is a formulation of water-soluble glycol and resins, which is a supportive medium that binds tissue together at temperatures below -10 ºC. Upon freezing, the OCT compound stopped any tearing of the tissue sample during cutting (Graeme Bull, personal communication, School of Biosciences, UoC May 2005).

The OCT compound was allowed to infiltrate the hide samples overnight. The sample was then placed on a metal cutting chuck and surrounded with OCT compound, then placed in a freezing chamber. Upon freezing, at -10 ºC, the OCT compound binds the sample to the metal cutting chuck. Once frozen, the sample was ready to cut.

A Brights Cryostat was used at -20 ºC and set to cut the hide samples to 24 μm. Excess OCT compound was removed until a full cross-section of the hide sample was visible. The hide section was then placed on slide and dried for several hours. Once the sample has dried it is covered with a cover slip. The cover slip was mounted on the sample using glycerine jelly. The hide section was lit from underneath using the UV lamp at the Gelita N.Z facility.

The microtome technique was ineffective. Once the sample was placed under the UV the whole slide was illuminated because glycerine jelly, used to mount the cover slip, was slightly fluorescent. Therefore, it was difficult to determine how well the Leucophor dye had penetrated into the hide section. It is possible that this technique is still a viable option if a non-fluorescent mounting agent were used. Another possibility is just allowing the sample to air dry, and then analyzing the sample. The only drawback being the slide would not be preserved for any further analysis. Further investigation is required to improve upon the technique.

4.2. pH Indicator

Measurement of conditioning could also be determined by monitoring the pH of the hide pieces. The pH of native bovine hide is about 9.0 (Ward and Courts 1977), while the pH of the liming solution is typically around 12.8. Attempts have been made at Gelita N.Z. to monitor the pH of the hide throughout the liming process using a universal pH indicator. This was unsuccessful because the color change was difficult to detect as the hide pieces turn into a dark green from the liming solution. Additionally, the universal indicator covers a broad pH range from 1.0 -12.0 while the pH change in the hide is between 9.0-13.0.

A different pH indicator was chosen with an indicator range that was closer to the range of the pH change in the hide. Alkali Blue 6B indicator properties have a visual transition interval of pH 9.4 (blue) to pH 14.0 (red) (Green 1990). The Alkali Blue 6B indicator solution was prepared with 1.0% (w/w) in 95 % ethanol. This indicator range was closer to the range of the pH change in the hide during the liming process.

4.2.1. Microtome Techniques with pH Indicator

Difficulties were also encountered when trying to see a color change on a limed piece of hide. A thin section of hide was cut. The indicator was then applied to the sample and any changes in color were observed. . The preparation of the hide sample and sectioning was the same as described above to obtain an even section. Before the cover slip was mounted on the slide a few drops of the Alkali Blue 6B indicator solution was applied. Fig. 4.7 shows the hide section after the indicator solution had been applied and the cover slip mounted.

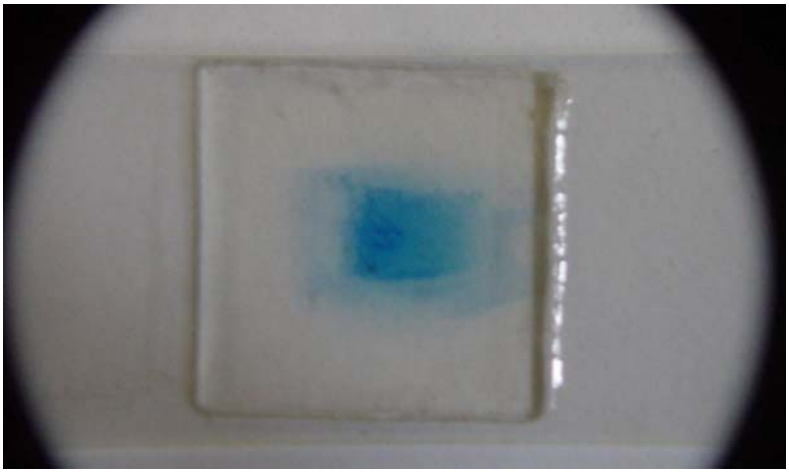


Figure 4.7: A section of hide with pH indicator solution.

As shown in Fig. 4.7, the piece of hide section was stained completely blue by the indicator solution and no color change could be seen. A possible explanation for this was that Alkali Blue 6B is also a biological stain and the indicator solution only stained the tissue rather than showing any color change. Therefore, Alkali Blue 6B was not a reliable method for monitoring the pH change in hide pieces during the liming process.

4.3. Determination of Glycosaminoglycans (GAGs) and Collagen Released into Liming Solution

While the use of fluorescent dye can be used in pilot plant level to monitor levels of conditioning, this method cannot be used in full-scale production at Gelita N.Z. A series of chemical assays for monitoring lime conditioning was developed by Gelita Australia. Their project was based on the theory that the release of hide components into the liming solution during conditioning reflects the quality of the raw material. The chemical assay measures the soluble products of the chemical reactions that occur in the hide pieces during the liming process. The products concentrations could be measured versus time to determine the progress of the liming process.

One particular chemical reaction that occurs during the liming process is the alkaline cleavage of the glycosaminoglycans (GAGs) associated with the collagen extra-cellular matrix. The removal of GAGs from the hide during conditioning resulted in the opening up of the fiber structure of the hide. The fibril organization of the hide and its diameter also relates to the GAGs content of the skin. Increased

removal of GAGs from the collagen in the hide resulted in an easier extraction of collagen from the hide. This can result in some collagen being solubilized and lost in the liming solution. The increase in the levels of soluble collagen lost in the liming solution could be a chemical marker to indicate the level of conditioning. The measurement of GAGs and collagen in the liming solution could potentially be used to measure the level of conditioning.

The Gelita Aus. liming process is not exactly the same as the process used at the Gelita N.Z. facility. The Gelita Aus. liming process usually takes seven days, and the assays were developed to detect the chemical changes in the hide for this period of time. The GAGs and soluble collagen concentrations were measured throughout the liming process using chemical assay methods that were developed. Fig. 4.8 shows how the GAGs and soluble collagen levels in the liming solution change with respect to conditioning time.

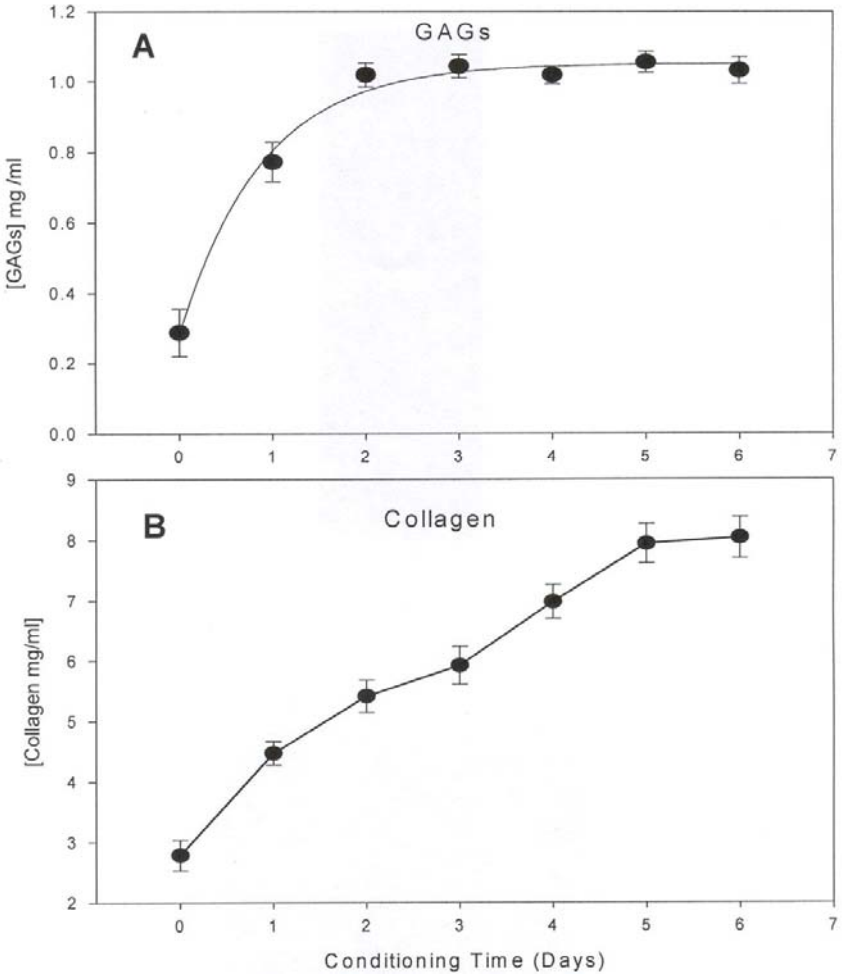


Figure 4.8: GAGs and soluble collagen concentration from Gelita Aus. liming process.

Since the Gelita N.Z. liming process varies from the Gelita Aus. process, the assay method needed to be tested at the Gelita N.Z. facility to determine if the release of GAGs and soluble collagen into the liming solution follows the same trend as the Australia liming process. This was done by following a pit at Gelita N.Z for the entirety of the liming process using the chemical assay for GAGs and soluble collagen released into solution.

4.3.1. Methods for Determination of GAGs and Collagen in Liming Solution ("Investigation of Chemical Assays for Lime Conditioning in the Production of Gelatine" Gelita Project # 107102)

The chemical assay used the procedure developed by Gelita Aus. A 250 ml liming liquor samples was taken from the pit being followed and filtered through glass wool to remove any extraneous materials. The pH of the liquor was then adjusted to a pH

of 5.0 using 5 M sulfuric acid, to precipitate interfering protein matter. A 2 ml sample of the solution was used for the GAGs assay. The remaining solution was then adjusted to a pH of 4.0 using 5 M sulfuric acid. A 2 ml sample of the pH 4.0 liming solution is taken for the soluble collagen assay. The two samples were then spun in a Heraeus Christ GmbH 60 W centrifuge at 7000 rpm for five to ten minutes.

The GAGs assay used dimethylmethylene blue (DMB) as the color reagent. The DMB powder was dissolved in 95 ml of 0.1 M hydrochloric acid and 3.04 g of glycine and 2.37 g of sodium chloride were added. The solution was diluted with distilled water to obtain pH of 3.0. The supernatant of the centrifuged pH 5.0 liquor sample was diluted five times with distilled water. The color reaction was obtained by mixing 100 µl of the diluted pH 5.0 supernatant with 2.5 ml of the DMB color reagent. The absorbance of the solution was measured immediately at 525 nm. A standard GAGs solution was made up using chondroitin sulfate A (Sigma No. C-8529) at a concentration of 0.3 mg/ml. A standard GAGs absorbance was created by mixing 100 µl of the standard with 2.5 ml of the DMB color reagent and the absorbance was measured immediately at 525 m. The concentration of GAGs in the sample was calculated using Eq. 4.1

$$GAGs\,(mg/ml) = \frac{Abs\ 525\ of\ Sample}{Abs\ 525\ of\ Standard}\ x\,1.5 \qquad \text{Eq. 4.1}$$

The determination of soluble collagen was done using an assay method with Coomassie Brilliant Blue G-250 dye combined with 50ml ethanol and 100 ml of ortho-phosphoric acid. This color reagent is known as the Bradford reagent. For the assay of soluble collagen 350 µl of 10 % sodium lauryl sulfate was added to the Bradford reagent. The supernatant of the centrifuged pH 4.0 liquor sample was diluted two times with distilled water. The color reaction was obtained by mixing 20 µl of the diluted pH 4.0 solution with 2 ml of the Bradford reagent. The absorbance was measured at 595 nm after a few minutes. A standard collagen solution was created by using Type B gelatine dissolve in water at concentration of 2 mg/ml. A standard collagen absorbance was created by mixing 20 µl of the collagen standard with 2 ml of the Bradford reagent. The absorbance was measured at 595 nm after a few minutes. The concentration of soluble collagen in the sample was calculated using Eq. 4.2

$$Soluble\ Collagen\ (mg/ml) = \frac{Abs\ 595\ of\ \ Sample}{Abs\ 595\ of\ \ Standard}\ x\ 4 \qquad \text{Eq. 4.2}$$

Chapter 5: Results and Discussion

The results are discussed from the different trials: temperature controlled mixing, ultrasound, and fluidization, used as different methods for enhancing chemical penetration. The level of conditioning was monitored by measuring the penetration of the Leucohpor PAT dye into the liming hide. The dye intensity as a function of the hide thickness was measured for both experimental and standard samples. This was the basis on which determinations were made as to how well experimental methods enhanced the penetration of the liming chemicals, thus decreasing the conditioning times.

5.1. Low Temperature Controlled Mixing

The effect of mixing hide pieces at low temperatures on chemical penetration of the lime/Na_2S solution was investigated. This trial was based upon the idea that the decrease in temperature would slow any surface reaction allowing for the mixing effect to enhance the penetration of chemicals. Initially the standard liming procedure was used to try to develop a chemical penetration standard as a function of time. It was quickly determined there was too much variability in the raw material to develop a simple penetration standard. The raw material differences affected the level of penetration, such as the amount of fatty material on the hide pieces, potentially as much as the method. Therefore, the experimental method was modified with controls run in parallel in each experiment (Table 5.1).

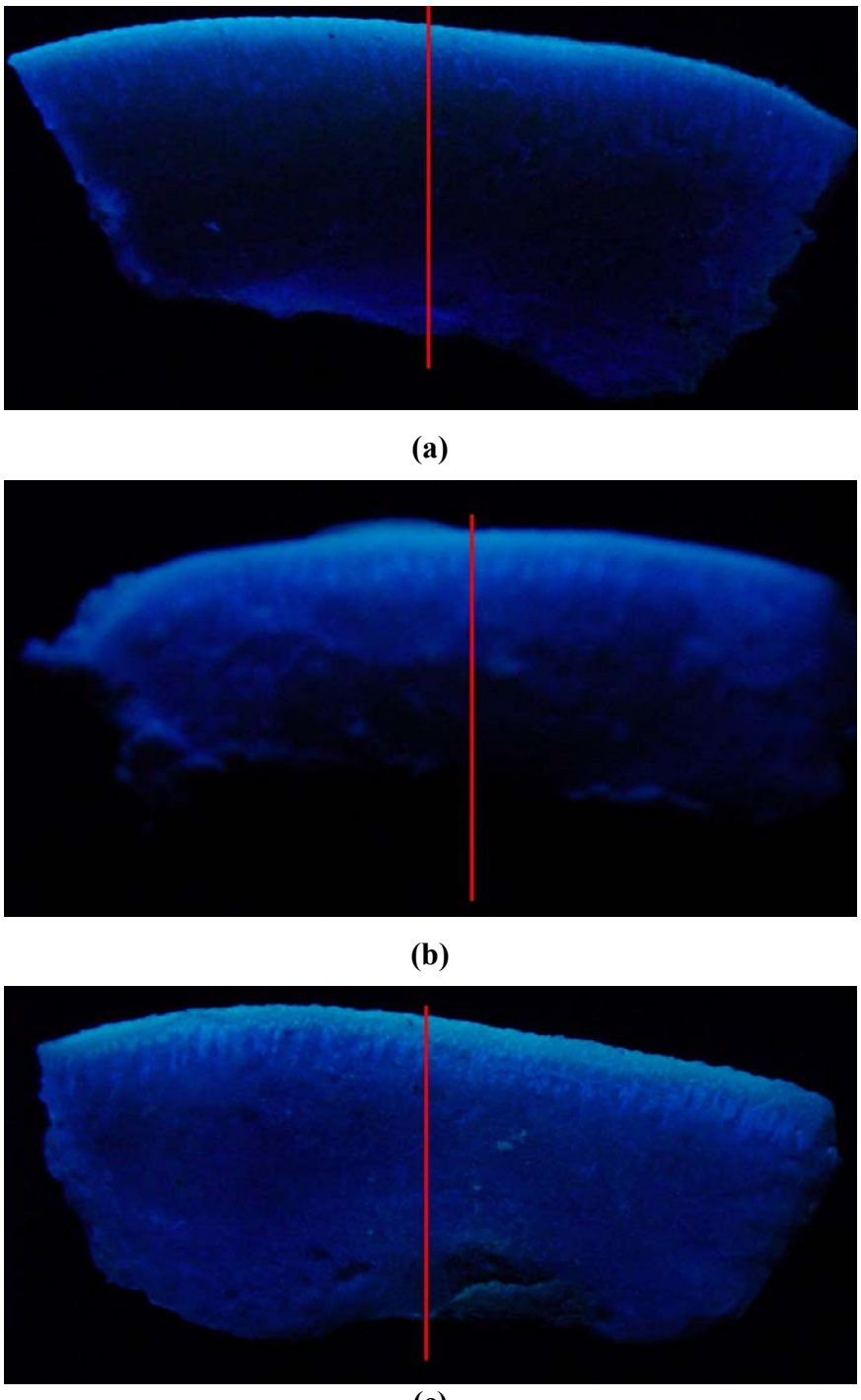
Table 5.1: Description of modified trial set up with standard for temperature controlled mixing (TCM) trial.

Trial Abbreviation	*Description*
LTM	Continuous mixing of hide pieces with temperature maintained at 1°C.
RTM	Continuous mixing of hide pieces at ambient temperature used as a control to determine the effect of mixing at different temperatures.
STD	Gelita N.Z. standard liming procedure used as a control to see the effects of mixing versus non-mixing.

Each of the three trials contained sections of hide from the same animal. Samples were taken from these hide sections and analyzed using the UV dye method. This system reduces variability due to differences in raw materials.

5.1.1. Dye Penetration

The experiment was conducted running the three trials in parallel. Samples were taken on days 3, 10 and 20 and the dye penetration was determined. Visually the samples did not appear dramatically different after day 3 (Fig. 5.1).



(a)

(b)

(c)

Figure 5.1: Sample of LTM (a), RTM (b) and STD (c) at day three of the liming process. The red line indicates where the color intensity scan was performed.

However, the dye intensity scan showed the LTM sample to have the slowest

penetration of the dye, while the RTM and the STD samples showed nearly at the same level of penetration (Fig 5.2).

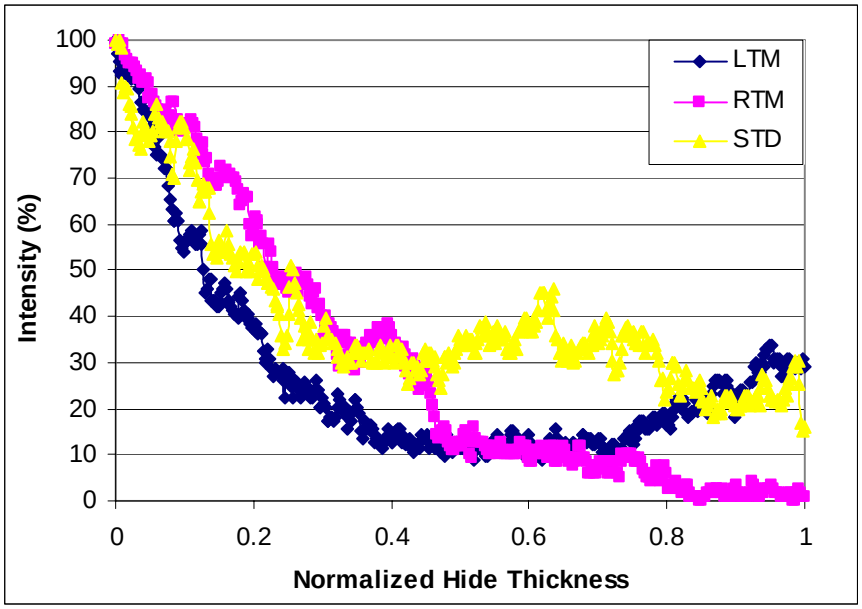


Figure 5.2: Comparison of the dye penetration after three days in TCM trial.

The RTM sample showed the best penetration from the skin but the poorest penetration from the inside. This could have been due to a large fatty deposit the inside on the sample. The STD sample had the most even penetration after three days of processing. After ten days of processing it was apparent that the decrease in temperature severely slowed and inhibited the level of chemical penetration (Fig 5.3).

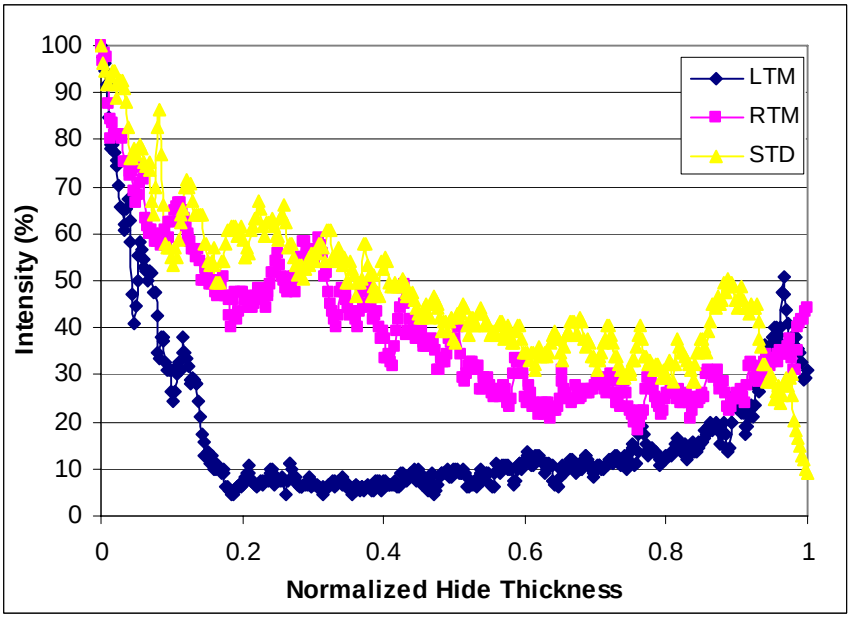


Figure 5.3: Comparison of the dye penetration after ten days in TCM trial.

The STD sample started to show better penetration from both sides. The slower penetration on the RTM sample could have been due to the acceleration of the

reaction on the surface of the skin and the beginning of the formation a gelled layer. The experiment concluded showing the STD trial having the best levels of chemical penetration of the three trials conducted (Fig. 5.4).

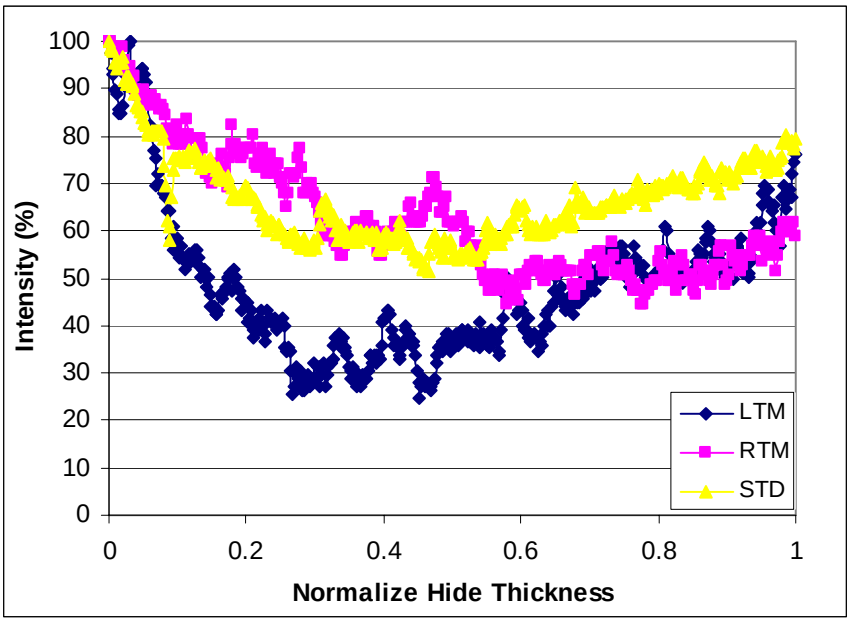


Figure 5.4: Comparison of the dye penetration after 20 days in TCM trial.

The RTM trial, which started out showing a higher level of penetration through the skin, but eventually slowed and showed similar results to the STD sample. The level of penetration from the fatty side was considerably slower in the RTM sample than either of the other two samples. While it is possible that this was caused as an effect of continuous mixing it is more probable some fatty deposits on the sample decreased the level of penetration. It is likely that the hide pieces the samples were taken from for the RTM trial had fatty deposits on the inside and thus decreased penetration levels. In the LTM sample, the decreased temperature likely slowed the level of penetration and the continuous mixing did not counteract this effect. The decreased temperature slowed down the surface reaction on the skin and did not have a gelled layer, which did occur in the RTM sample. The formation of a gel-layer can be easily felt when assessing the hide, though the difference in texture can be seen after the hide pieces went through the first step of acidulation (Fig 5.5 and 5.6).

Figure 5.5: LTM sample after first step of acidulation.

Figure 5.6: RTM sample after first step of acidulation.

While surface reaction in the LTM trial was slowed down. The formation of a gelled layer slows the penetration of chemical. This is often observed in full-scale production during summer months, when ambient temperatures are higher, and the hide pieces have a gel-like texture after acidulation. During winter months of full-scale production, with ambient temperatures of approximately 8 ºC, the hide pieces have a less gel-like texture after acidulation. The decreased temperature decreased the levels of penetration and the hide was generally under-conditioned after 20 days of liming. This resulted in a white section of collagen in the middle of the hide. The RTM sample initially showed higher levels of penetration although as time progressed the STD sample showed similar levels of conditioning. The RTM sample likely slowed down as a gelled layer was formed during the process, which is easily seen after the first step of acidulation. The STD trial showed the best overall results in levels of conditioning.

5.1.2. Sodium Sulfide Dissolution

The sodium sulfide concentrations were monitored throughout the experiment for all

three trials (Fig 5.7).

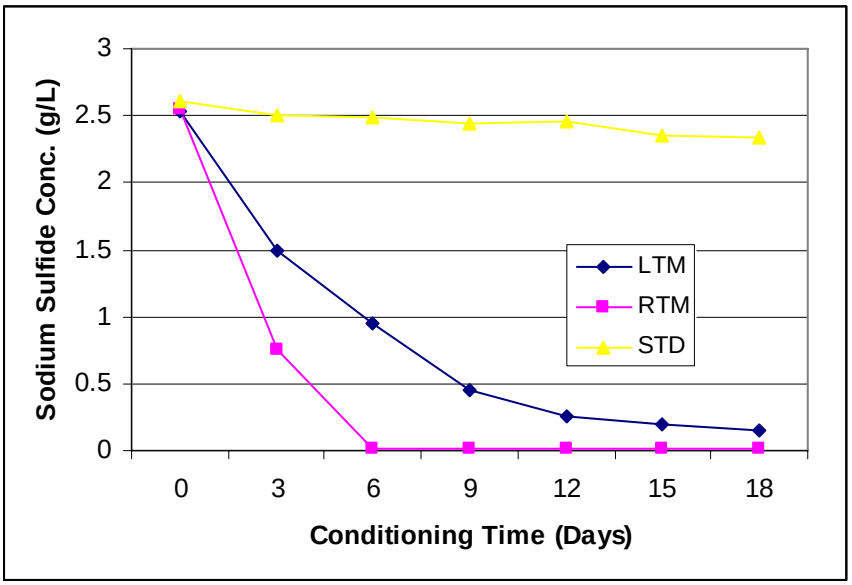


Figure 5.7: Sodium sulfide concentrations levels throughout all TCM trials as determined by the LIRI method.

Both of the trials with continuous mixing showed sodium sulfide concentrations drop rapidly in the first few days. The rapid drop of sodium sulfide concentrations was a result of the mixing. The oxidation of the sulfide during mixing could be related to the LTM and RTM being less conditioned that the STD. In addition, the mixing increased the sulfide reaction with the hair in the liming solution by attacking the keratin-S-S-keratin disulfide linkages (Gelita report "Hair Dissolution in Lime Pits", 27/03/00). This resulted in the hair being dissolved in solution which gives a dramatic change in the liming solution color (Fig 5.8). A trial would need to be conducted mixing just a lime/Na_2S solution with no hide to determine the decrease of sulfide levels due to oxidation.

Figure 5.8: The change in liming solution color caused by hair dissolution.

It is advantageous to Gelita N.Z to consume all the sodium sulfide within the liming process as the waste solution must be treated to lower sulfide concentrations in order to reach acceptable levels. Neither of the mixing processes enhanced the level of chemical penetration enough for this to be a viable option.

5.2. Ultrasound Trials

Several ultrasound trials were carried out in order to determine if ultrasound would enhance the penetration of the liming chemicals into the hide pieces. The most important factor in this experiment, discovered after several trials had failed, was maintaining the temperature of the liming solution. The ultrasound transducer gave off a large quantity of energy and raised the temperature of the liming solution above 30 ºC and degraded the hide (Fig. 5.9). When the hide degraded, collagen was solublized from the high temperatures and lost into the liming solution.

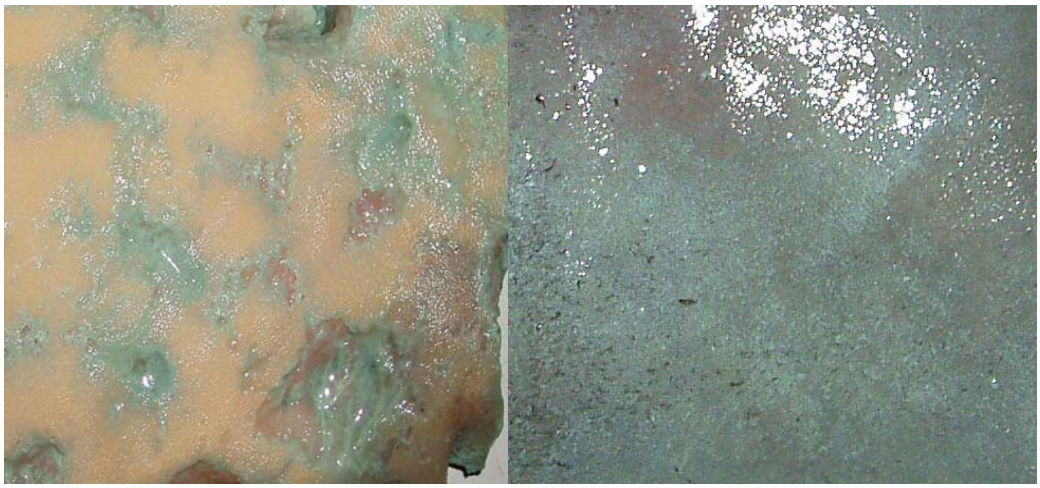
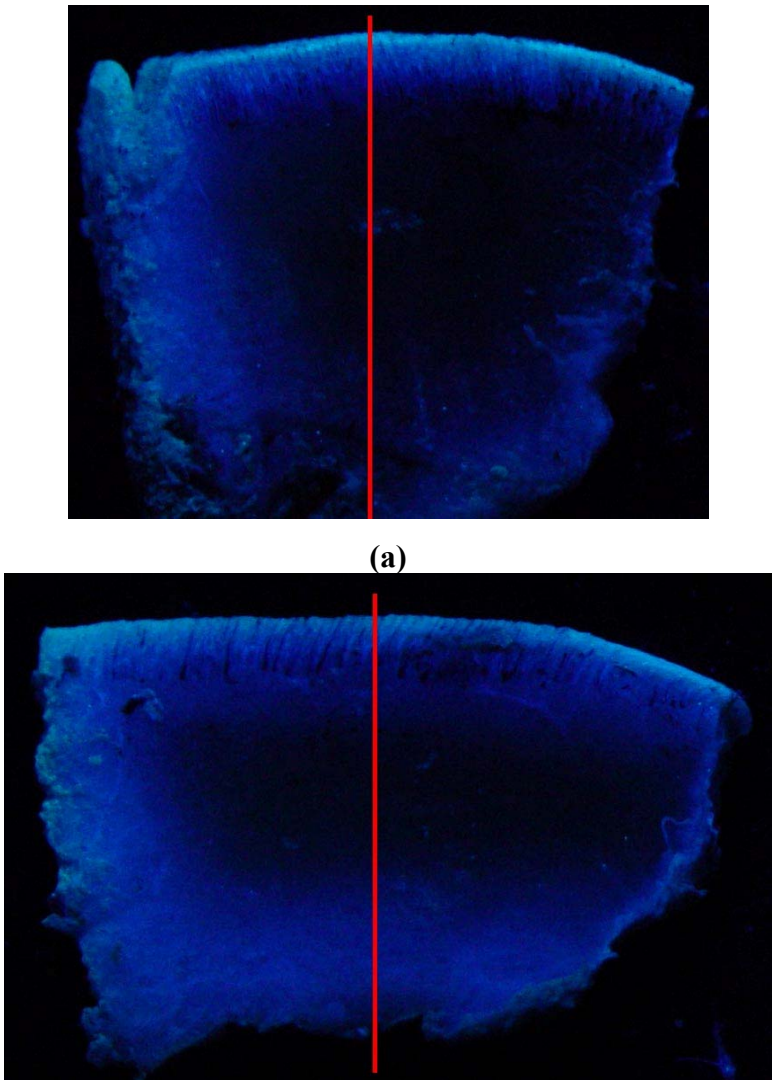


Figure 5.9: Comparison of hide degraded from high temperatures during liming process (left) and normal limed hide (right).

5.2.1. Liming with Periodic Ultrasound and Controlled Temperature

Ultrasound trials were conducted with the temperature ranging from 18-23 ºC with an ultrasound irradiation period of ten minutes per hour. The standard samples were at an ambient temperature of 18 ºC. Initial samples were taken after three days and the images were analyzed to determine the dye penetration (Fig 5.10).



(a)

(b)

Figure 5.10: Standard (a) and ultrasound (b) sample after three days of liming with color intensity scan.

The ultrasound sample showed better penetration of dye, particularly through the fatty side of the hide (Fig 5.11).

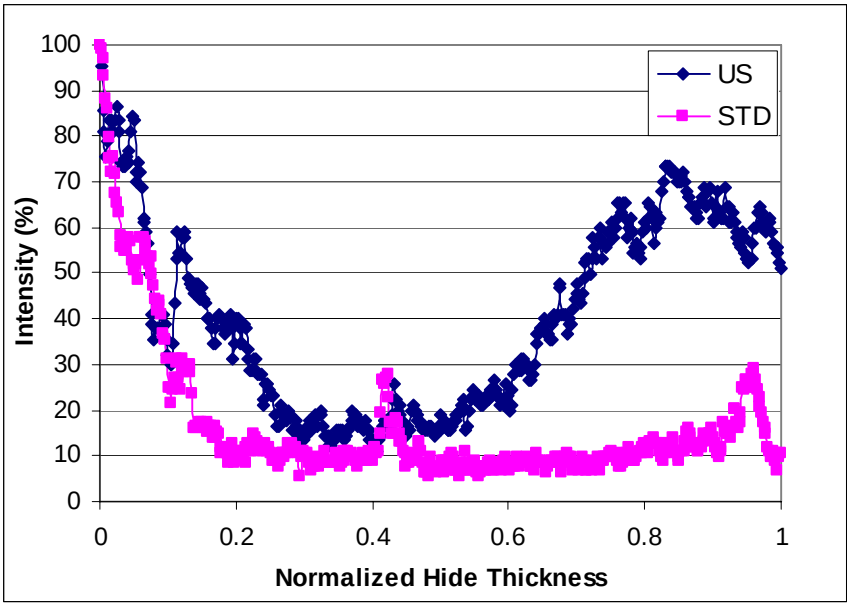


Figure 5.11: Comparison of dye penetration in ultrasound (US) and the control liming process (STD) trial after three days.

The penetration of the dye through the skin side of the hide was only slightly enhanced. There was a large degree of enhancement of chemical penetration through the fatty side. This is likely caused by the ultrasound, which has the capability to breakdown the fatty tissue remaining on hide pieces and other non-collagenous materials, as claimed by Sivakumar et. al. (2005). Fatty residues were found in the liming solution, indicating the breakdown of fatty tissue into the solution. The removal of excess fatty tissue from the hide allowed for better penetration of the liming chemicals into the hide pieces.

As the ultrasound trial progressed the hide began to degrade in the liming solution even with the temperature controlled to approximately 22 ºC. After six days with the hide pieces being periodically irradiated with ultrasound, the hide pieces started to show signs of degradation (Fig 5.12).

(a)

(b)

Figure 5.12: The ultrasound treated hide (a) which shows degradation after six days of process when compared to the standard (b).

The degree of hide degradation continued to progress. After nine days the hide showed extensive degradation, in which a significant amount of the hide had dissolved into the liming solution (Fig 5.13).

Figure 5.13: Ultrasound treated hide after nine days showing extensive degradation.

Trials were revised altering the ultrasound irradiation periods to seven minutes per hour and three minutes per hour. All hide treated by ultrasound, with an ultrasonic intensity of 0.71 Wcm^{-2}, showed signs of degradation after several days. Despite claims by Sivakumar et. al. (2004) that ultrasound used at low intensity levels of approximately 0.5 Wcm^{-2} had no destructive effect on skin material and collagen fiber structure. It is more likely that ultrasound used at low intensity levels has no destructive effect on the hide when only used for several hours. Their trials only used ultrasound for irradiation periods of 3 x 3 minutes at 22 ºC for a maximum of three hours. Therefore when ultrasound combined with a highly basic solution used on a scale of days rather than hours, was considerably destructive on the skin material and collagen fiber structure of the hide pieces.

The use of ultrasound enhanced the penetration of chemicals into the hide pieces, particularly on the fatty side due to the removal of excess fatty material and non-collagenous material. However, the destructive effect of the ultrasound after several days of processing degraded the hide to a point where the collagen material needed for gelatine production was lost into the liming solution. It is possible the lowering the concentration of the lime/Na_2S in the solution might not lead to such extensive degradation.

5.2.2. Sodium Sulfide

The sodium sulfide concentrations were measured throughout the ultrasound trials to determine any effect from the ultrasound (Fig 5.14).

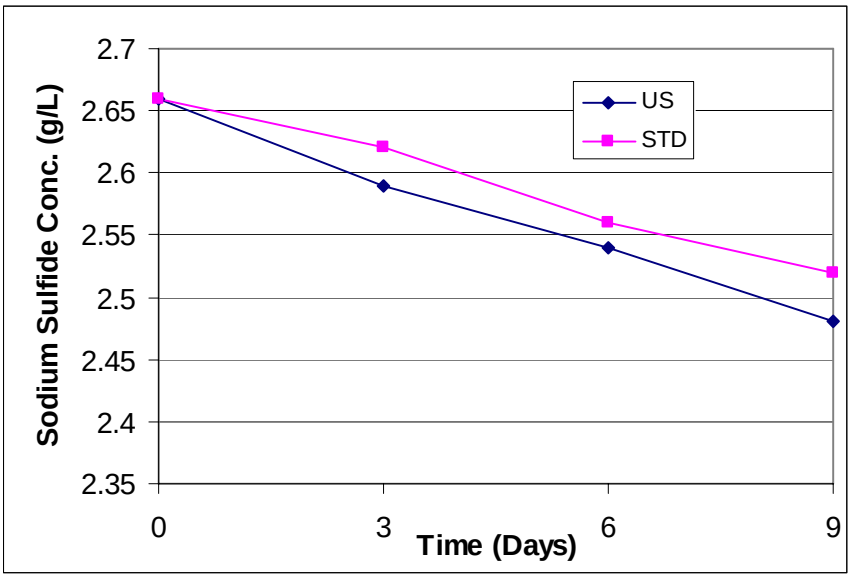


Figure 5.14: The changes in sodium sulfide concentration in ultrasound (US) and the control liming (STD) throughout the trial.

It was been suggested that the effect of ultrasound lowers the concentration of sodium sulfide in liming solutions (Ding et. al. 1999). Fig 5.14 shows that the concentration of sodium sulfide in the liming solution treated with ultrasound was only slightly lower than that of the standard liming process. The ultrasound trials were cut short due to the degradation of the hide, and the full effect of the ultrasound on the sodium sulfide concentrations was not determined.

5.3. Fluidized Column Combining Liming and Acidulation

A trial was conducted in which the liming and acidulation processes were carried out in a fluidized column. The fluidization of provided a method to increase chemical penetration by gently mixing the material. Both the liming and acidulation processes were carried out in the same vessel to reduce handling of the raw material.

The temperature throughout this trial was approximately 23 ºC. The liming and acidulation chemicals were recirculated through the column with a minimum fluidization velocity of 0.037 m s^{-1} calculated using Eq. 2.5- 2.7. The average size of the hide pieces were 30mm x 30 mm. This gave a bed expansion of 70 mm (L_{mf}) from an initial bed height of 225 mm (L_m) (Fig 5.15).

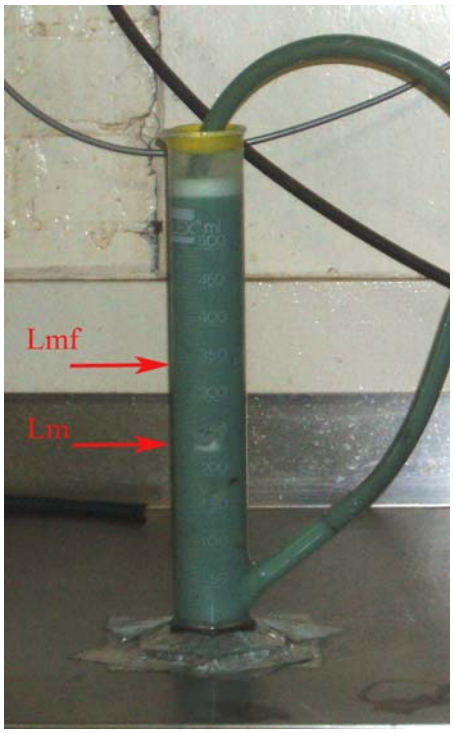
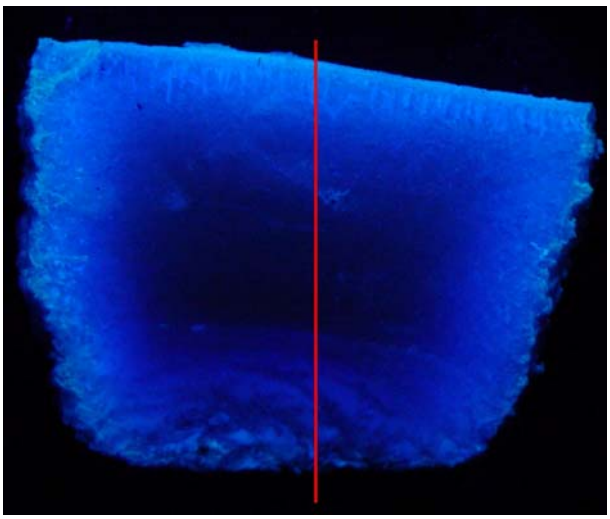


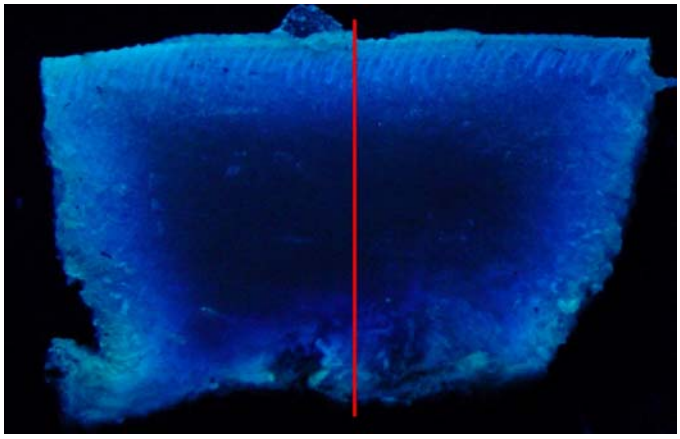
Figure 5.15: The bed expansion of fluidization of hide pieces.

5.3.1. Liming in Fluidized Column

Samples were analyzed for dye penetration from the fluidized column and the standard process to determine the effects of the fluidized column on the penetration of liming chemicals (Fig 5.16).



(a)



(b)

Figure 5.16: The penetration of dye into hide pieces for fluidized hide (a) and the standard process (b) after three days.

The initial penetration of the dye into the hide pieces after three days was slightly better for the fluidized hide as compared with the standard process. This can be seen in the comparison of image color intensity versus hide thickness (Fig 5.17), where the fluidized hide shows slightly better penetration in the center of the hide pieces.

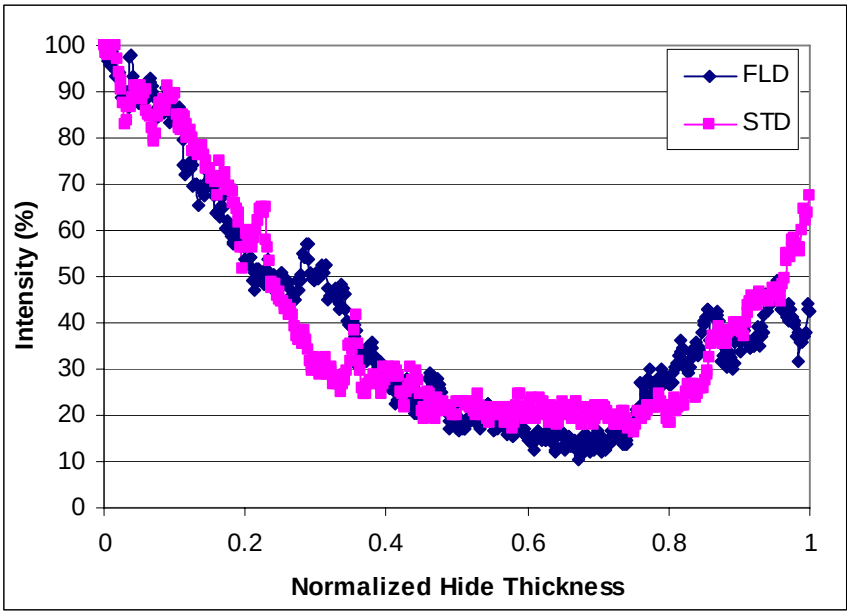
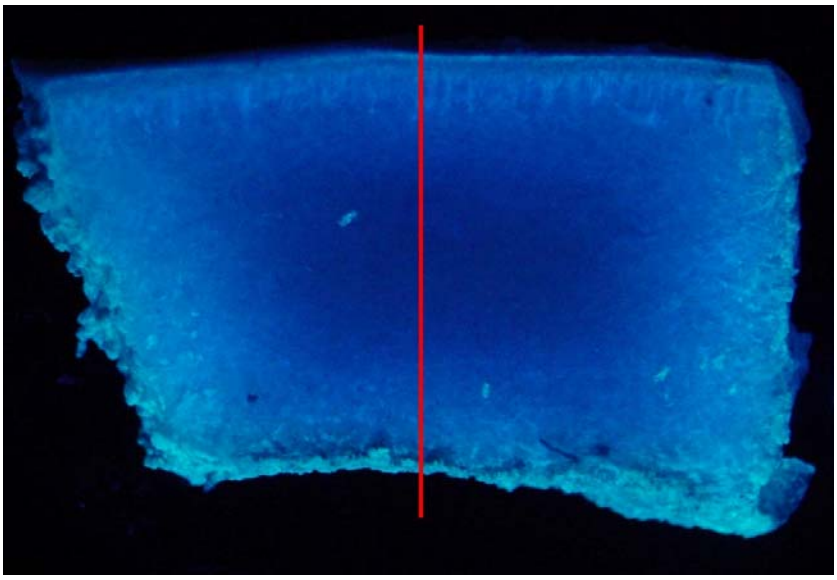
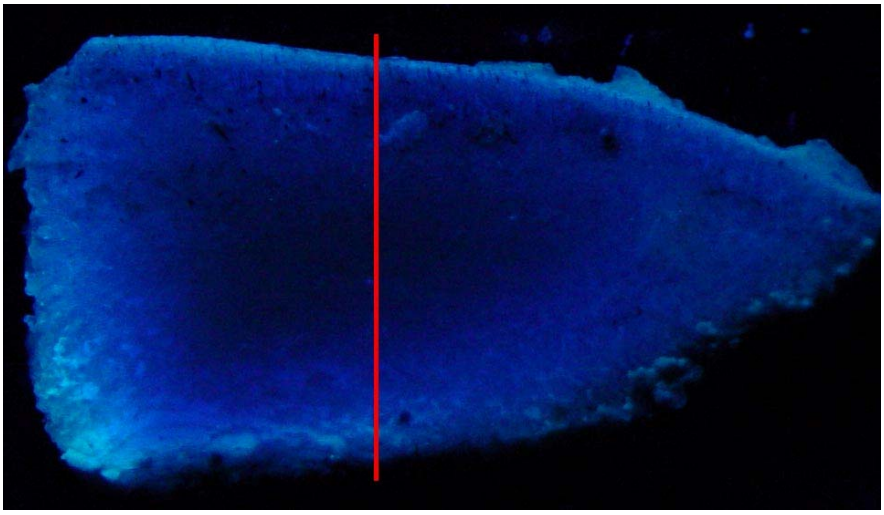


Figure 5.17: Comparison of dye intensity for fluidized column (FLD) versus the control liming process (STD) after three days.

The next samples were taken after six days of processing. The fluidized hide showed considerably better penetration of the dye throughout the entire hide pieces (Fig 5.18), while the standard process showed little penetration of the dye to the center of the hide pieces.



(a)



(b)

Figure 5.18: The penetration of dye into hide pieces for fluidized hide (a) and the standard process (b) after six days.

The comparison of the image color intensity versus the hide thickness showed the fluidized hide to have a more even penetration of the dye throughout the hide pieces (Fig 5.19). The standard process showed little penetration of the dye into the center of the hide pieces, though the penetration of the dye was better from the fatty side of the hide.

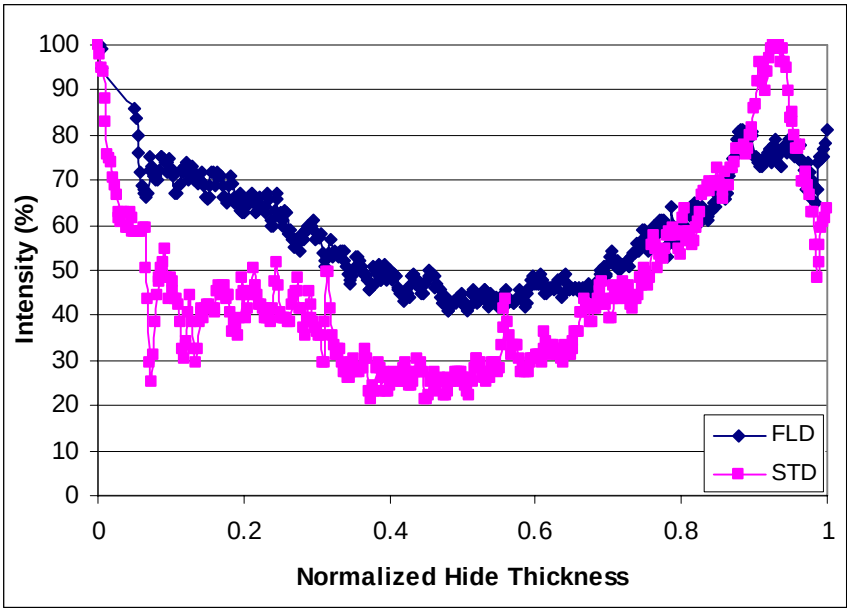
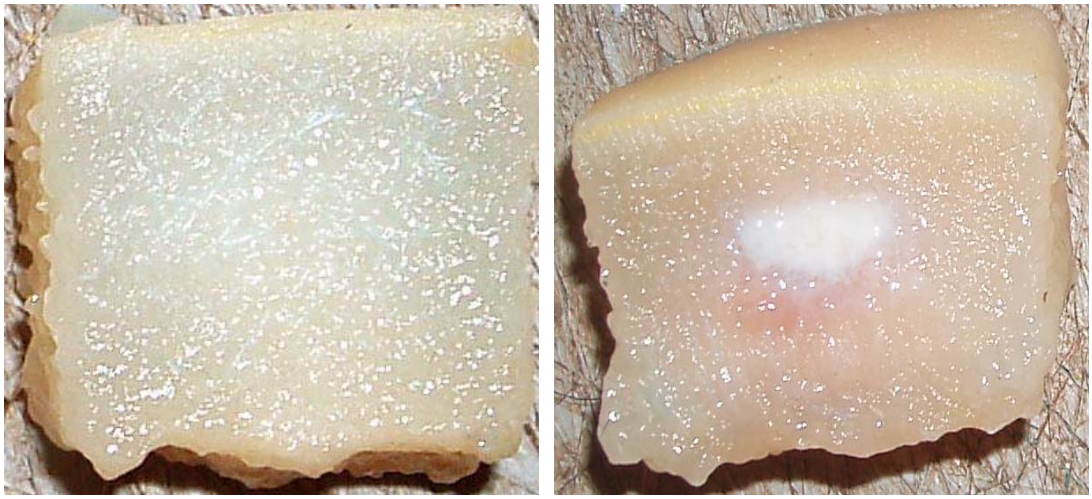


Figure 5.19: Comparison of dye intensity for fluidized column (FLD) versus the control liming process (STD) after three days.

Based on the image color intensity scan it was determined that the fluidized hide, after six days, was sufficiently conditioned to move to the acidulation part of the process.

5.3.2. Acidulation in Fluidized Column

After completion of the liming process, both the fluidized and standard hide pieces went through acidulation. The chemicals were changed and the hides were treated in the same column. The fluidized hide went through the same washing and acid treatment periods as in the standard process (Sec 3.4). The fluidization velocity, of 0.037 m s^{-1}, was kept the same giving the same bed expansion as in the liming process. The quality of the hide pieces was checked after the acidulation process had been completed for both the fluidized and standard hides (Fig 5.20). Sodium sulfide concentrations could not be accurately monitored as there was a loss of solution from the column when samples were taken.



(a) **(b)**

Figure 5.20: Sample of fluidized hide (a) and standard hide (b) after the completion of the acidulation process.

The hide from the fluidized column showed better overall conditioning after the acidulation period than the standard hide. This can be seen in Fig 5.20 where the standard hide had a white section in the center of the hide which indicated it was severely under-conditioned. This was a result of liming chemicals not fully penetrating into the hide and the collagen cross-links have not being broken down. The fluidized hide showed no sign of being under-conditioned. The standard hide pieces also showed a slight gelled layer on the outside of the hide. This is a common occurrence in Gelita N.Z full scale processing when the ambient temperature is higher during the summer months, which typically leads to lower yields. The fluidized hide showed no signs of gelling on the outside even though the temperature for both trials was approximately 23 ºC.

Treating hide pieces in a fluidized column reduced the pretreatment to only 9 days of processing including acidulation. The standard process in Gelita N.Z takes approximately 50 days. There are several reasons why the processing time was reduced in the fluidized column. The first was the reduction of size of the hide piece to 3 cm x 3 cm rather than the larger size of 15 cm x 15 cm in the standard process. The smaller sized hide pieces took less time for the chemicals to fully penetrate. Hide pieces are not cut to this smaller size in the standard Gelita N.Z full-scale process because the small pieces would be lost in the transfer from liming to acidulation. In the fluidized column the hide pieces were treated in the same vessel and the different liming and acidulation solutions were recirculated through the

column. The transfer of hides to different processing vessels was not required. The second reason for reduced processing time was the fluidization of the hide. When the hide was kept in a fluidized state, the surface area in which the chemicals could penetrate the hide was increased. The higher the surface area available for penetration led to a shorter time period required for the liming chemicals to fully penetrate the hide pieces.

This trial also confirmed that the UV dye method of measurement of conditioning was a reliable analysis technique. The image analysis of the fluidized hide showed a more even penetration of the dye into the hide, while the standard hide showed little penetration of dye into the center of the hide. This was confirmed after complete acidulation when the standard hide shows an area of under-conditioning in the center of the hide pieces.

5.4. Results of GAG and Soluble Collagen in Liming Solution

A single liming pit was followed for the duration of the liming period starting after dehairing until transfer to acidulation. The GAGs concentration (Fig. 5.21) and soluble collagen concentration (Fig. 5.22) were measured each day of the process.

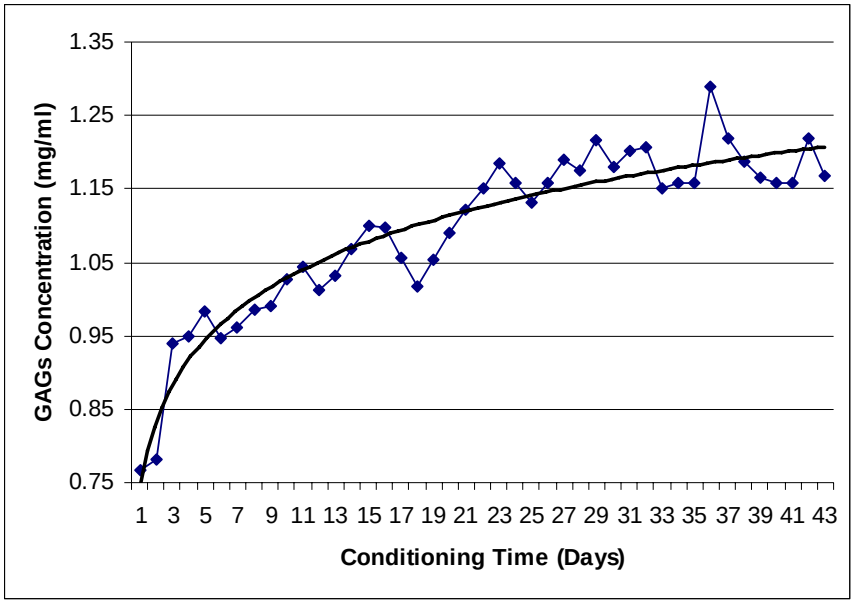


Figure 5.21: The changes in GAGs concentration throughout the liming process.

The initial GAGs concentration was 2.5 times greater than the Gelita Aus. trial (Fig. 4.8). The trend that follows is similar with a sharp increase during the initial conditioning period and leveling off as the process continues. There is considerable

variability in the day to day GAGs concentrations. There are two explanations for this trend. The first is Gelita Aus. liming pits are continuously mixed where the Gelita N.Z. pits are only poled every four days. This lead to less consistent results since the liming solution is not evenly mixed when samples are taken. Secondly with the longer process the Gelita N.Z pits often required to be refilled with liming solution. This would account for the sharp drops of GAGs concentrations in the liming solution.

The effect of these differences in the two liming processes can also be seen in the measurement of soluble collagen concentrations (Fig. 5.22).

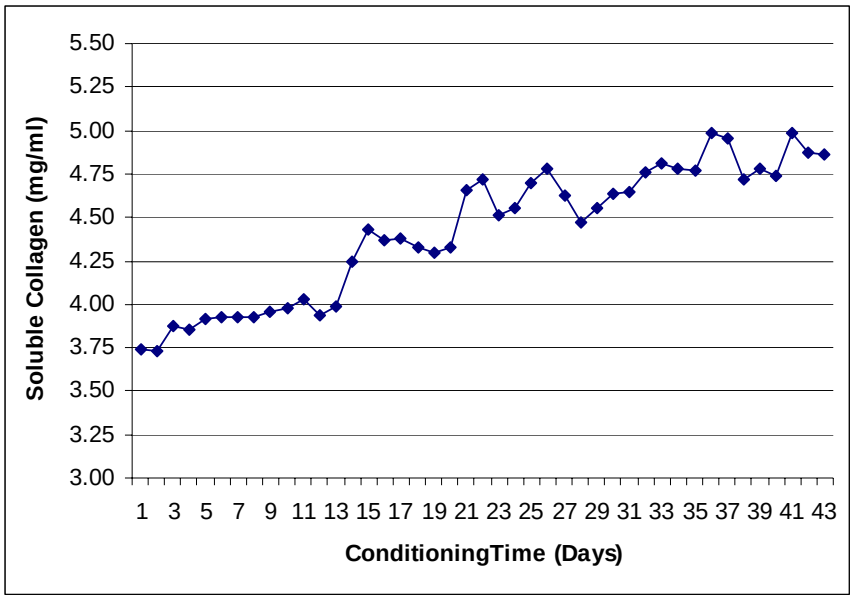


Figure 5.22: The changes in soluble collagen concentration in the liming process.

Some differences are seen in the soluble collagen concentrations as the liming process progresses, though the trend is similar to Gelita Aus. The initial collagen concentration is slightly higher, which is most likely due to the different hide preparation and dehairing methods. The variability in the raw material could account for these differences.

The procedure for monitoring the GAGs and soluble collagen concentrations is applicable to the Gelita N.Z. liming process. It is impractical to measure the concentration levels in the pits everyday. The most reliable concentration measurements would be after the pits have been poled and the liming solution has been thoroughly mixed. This would make it possible to accurately follow the

changes in GAGs and soluble collagen concentrations in the pits, though there will still be some variability when the pits are refilled with liming solution.

5.5. Specialized Hide Mixer with Temperature Control

A trail was conducted by Gelita Aus., in co-operation with a leather processing facility, in which the liming processing time was decreased using the combination of a specialized hide mixer and manipulation of the processing temperature. In this process the hide was soaked for a period of four hours. The hide was then dehaired in the same lime/Na_2S solution for 6.4 hours. The hide was then limed using only a 3.5 % (w/w) liming solution for 10.3 hours starting with an initial temperature of 28 ºC. This high temperature accelerated the liming process without loss of collagen into solution because the time period was short. The liming time was reduced to four days for this process. The use of the specialized hide mixer allowed for gentle mixing of the hide which is much less aggressive than conventional paddle mixers (Fig 5.23).

Figure 5.23: Specialized hide mixer showing the gentle mixing action on the hide.

The less aggressive mixing did not cause any over-conditioning of the outside of the hide and allows the chemicals to evenly penetrate into the hide pieces.

It would be necessary to conduct a full-scale trial with the specialized hide mixer to determine any feasibility of utilizing the process at Gelita N.Z. While it was not possible to conduct this trial a review of the required equipment was conducted. While several models of the type of hide mixers were available the mixer produced by Canbar was most applicable. The Canbar mixers are made of fiberglass which would be resistant to most chemicals. In addition, the mixer can be drained through the fins while in motion which would allow for recirculation of chemicals during the process. It would also make it possible to conduct both the liming and acidulation process in the same vessel, which showed positive results in the fluidization trials. A tour of the Canbar equipment was arranged at Graeme Lowe Tanneries to view the mixers and to arrange future full scale trials to asses the feasibility of the process.

Chapter 6: Cost Analysis

A capital cost analysis was conducted on the proposed liming process modifications. The fixed capital investment (FCI) was estimated using percentages of the delivered equipment costs (Peters et. al. 2003). This FCI method used the delivered cost estimates for the required equipment. The additional direct and indirect costs associated with equipment installation were estimated using a percentage of the delivered equipments costs.

The equipment costs were obtained from two sources. The first and most accurate equipment cost estimates were obtained from quotes of potential suppliers. When direct quotes from suppliers were not available, cost estimates were obtained from purchase cost estimation curves (Peters et. al. 2003), which gives purchased equipment cost estimates for a variety of processing equipment. These costs were corrected for to inflation and different country location. A New Zealand country location factor of 1.3 was used (Perry and Green, 1997). Purchased equipment costs (C_p) were corrected by using the ratio of the capital goods prices indices (CGPI) for New Zealand (Statistics New Zealand) from the old year (l_r) to the current year (l_s), in New Zealand dollars (Eq. 6.1).

$$C_{p,s} = C_{p,r}\left(\frac{l_s}{l_r}\right) \qquad \text{Eq. 6.1}$$

The CGPI from the quarter ending March 2005 of 1166 was used for the current CGPI.

6.1. Ultrasound Capital Cost

The addition of ultrasound to the liming process would be quite simple. The pits would be irradiated with ultrasound for a given period of time from an ultrasound transducer probe. The only equipment required would be industrial scale ultrasonic transducer probe. The most powerful transducer commercially available for this type of process would be a Hielscher 16 kW ultrasonic transducer probe with an adjustable pulse. This would give an ultrasonic intensity per pit of 0.23 W cm^{-2}. It is possible that at this ultrasonic intensity the hides would not degrade as they did with the experimental trials of 0.71 W cm^{-2}. The most beneficial aspect of using ultrasound in the liming process would be the removal of the fatty tissue on the inside

of the hide allowing for better chemical penetration. Little process modification would be required to implement the use of ultrasound. The liming pits would remain the same and the ultrasound transducer probe could be applied to each pit for a given irradiation period. The purchased equipment cost was obtained from a quote from Hielcher GmbH.

Table 6.1: The breakdown of FCI costs for the installation of ultrasound into the current liming pretreatment process.

Direct Costs		**Indirect Costs**	
Purchased Equipment Delivered	$224,500	Engineering and Supervision	$74,080
Purchased Equipment Installation	$101,025	Construction Expenses	$87,555
Instrumentation and controls	$20,205	Legal Expenses	$8,980
Piping	$0.00	Contractor's Fee	$38,165
Electrical Systems	$22,450	Contingency	$78,575
Buildings	$0.00		
Yard Improvements	$0.00		
Service Facilities	$22,450		
Total Direct Plant Cost	$390,630	**Total Indirect Plant Cost**	$287,360
		Fixed Capital Investment	$678,000

Some direct costs were omitted from the cost analysis. This was because some items, such as piping, would not be applicable to implementing ultrasound into the liming process. There would also be no need to change the buildings or infrastructure due to the installation of ultrasound into the liming process.

The initial equipment cost for the ultrasonic transducer would be high. It would have to be determined whether ultrasound would be most efficient means of removing the fatty tissue from the hides without any degradation. It is recommended that pilot plant trials be conducted with ultrasound transducer probes rather than with ultrasonic cleaning baths. A pilot plant trial could be conducted using ultrasonic transducer probes with a lower power rating of 400 W. A 400 W ultrasonic processor would be available from Hielscher GmbH at a cost of $9,000.

6.2. Fluidized Column Capital Cost

The fluidized column trials produced considerable improvement in the liming process. The liming times for the hide pieces were reduced from fifty days, in the current Gelita N.Z liming process alone, to approximately ten days in the trial runs to complete both the liming and acidulation processes. The liming pits and acidulation tumblers would be replaced by a fluidization column in which both liming and acidulation processes would take place.

The capital cost required to implement the fluidization process at Gelita N.Z was calculated. The basis for the calculations was the required number vessels to process 35 tonne of hide each day. The main pieces of equipment that would be required for the fluidization of the hide pieces are a vertical processing column and a fluidization pump. One specialized food pump manufactured by Svaertek would be required for the transfer of the small hide pieces after pre-treatment to extraction. The size of the fluidization pump would be based on the size of the hide pieces as well as the column diameter. The cost estimates for the column was from purchased cost estimation curves (Peters et. al. 2003) and was adjusted to current prices.

The hide loading capacity was based upon a 50% float. It was determined how many vessels were required to process 35 tonne of hide (Table 6.2).

Table 6.2: The required number of vessels required to process 35 tonne of hide for different column diameters.

Column Diameter (m)	Volume Column (m^3)	Tonne of Hide per Column (50% Float)	Total Columns for 35 tonne
0.5	1.96	1.31	27
1	7.85	5.24	7
2	31.42	20.95	2

The fluidization process takes approximately ten days to complete. Therefore, ten times the number of columns would be required so 35 tonne of hide would have completed pretreatment each day. The cost of the required number of vessels to produce 35 tonne of pretreated hide each day was then calculated (Table 6.3).

Table 6.3: Purchased equipment costs for vertical processing column based on column diameter.

Column Diameter (m)	Current Cost per Column	Total Columns (35 tonne/day)	Total Cost of Columns (35tonne/day)
0.5	$63,787	270	$17,222,598
1	$80,574	70	$5,640,149
2	$134,289	20	$2,685,785

While the cost of the column would increase with diameter so would the amount of hide that could be processed in the column. Therefore, the overall number of column required was lower for a larger column diameter. This made the 2 m diameter column the cheapest option to process 35 tonne of hide a day.

The pumping requirement would also change with the column diameter as well as the hide size. The minimum fluidization flow rates were calculated using Eq. 2.5-2.7 (Sec 2.3) based upon different hide sizes. This was used to determine the pump size required for fluidization of the hide pieces. Flow rates for fluidization would range between 4600 and 13000 L min^{-1}. For large flow rates such as these, centrifuge pumps would be the best option (Fig 6.1).

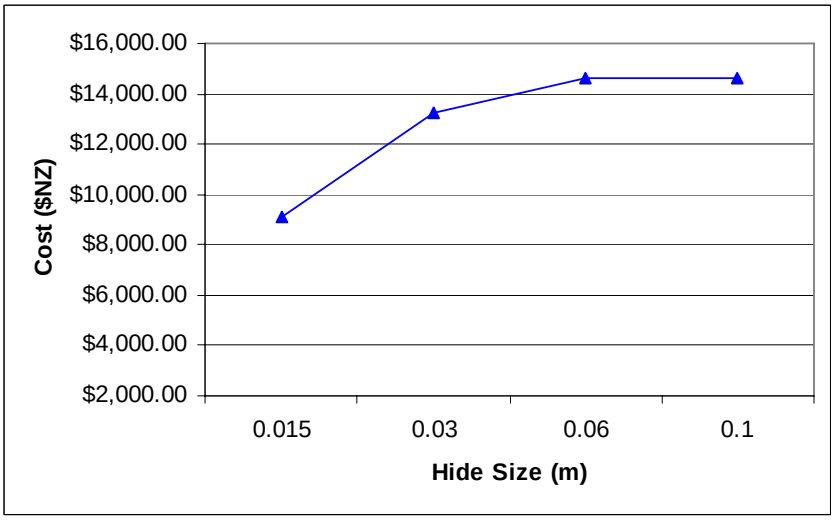


Figure 6.1: Purchased equipment costs for centrifuge pumps based on hide size and a column diameter of 2 m.

The smaller hide pieces require lower fluidization flow rates and would be the best option for fluidization since the material would not be transferred between the liming and acidulation steps. Smaller hide pieces would also require less time to complete the pretreatment process. The combination of hide pieces of 15 mm x 15 mm with a

column diameter of two meter would require lower processing times and pumping requirement.

Table 6.4: Breakdown of purchased equipment delivered costs for fluidization.

Equipment	Delivered Cost
Svaertek Food Pump	$90,000
20 316 SS Vertical Process Columns (2m Diameter)	$2,685,785
Centrifuge Pumps	$182,633
Purchased Equipment Delivered Cost	$2,958,419

A capital cost analysis was determined using the fluidization purchased equipment delivered cost (Table 6.5).

Table 6.5: The breakdown of FCI costs for the installation of a fluidized column for the pretreatment of gelatine raw material.

Direct Costs		Indirect Costs	
Purchased Equipment Delivered	$2,958,419	Engineering and Supervision	976,278
Purchased Equipment Installation	$1,331,288	Construction Expenses	1,153,783
Instrumentation and controls	$532,515	Legal Expenses	118,337
Piping	$473,347	Contractor's Fee	165,967
Electrical Systems	$295,842	Contingency	1,035,447
Buildings	$591,684		
Yard Improvements	$443,763		
Service Facilities	$295,842		
Total Direct Plant Cost	$6,922,700	**Total Indirect Plant Cost**	3,449,812
		Total Capital Investment	10,400,000

Experimental trials showed the use of a fluidization column to decrease the liming time considerably. However, the FCI associated with installing would be a considerable investment. This process could be installed over several years by integrating a few columns into the process each year. Further investigation of the fluidization of hide pieces could reduce the processing time and the number of vessels

required to produce 35 tonne of hide per day. This would make the FCI much lower and a more feasible process.

6.3. Specialized Hide Mixer Capital Cost

The final option available would the use of specialized hide mixers. In this process, the liming pits and acidulation tumblers would be replaced with specialized hide mixers. The initial temperature of the liming solution would be increased to 28 ºC bye means of a heat exchanger. While the hides were being mixed, the solutions would be recirculated through the mixer throughout the process. A capital cost analysis was conducted in which the equipment required was the hide mixer, heat exchanger and recirculation pump. . The basis for the calculations was the required number of mixers to process 35 tonne of hide each day. Of the specialized mixers available the one manufactured by Canbar Inc. would be the most appropriate (Fig 6.2).

Figure 6.2: Specialized hide mixer manufactured by Canbar Inc. (Graeme Lowe Tanneries Ltd).

According to the specifications of the mixer the hide loading capacity for 17,500 L mixer was 11,600 kg of hide at 50% float. This is one of largest capacities of all the mixers manufactured of this type. The fiberglass construction would also be longer lasting, in both the liming and acidulation solution processes, than the stainless steel

mixers supplied by other manufacturers

A quote for used Canbar mixers was obtained from Leather Technology Ltd. A quote for the Fybroc centrifuge pump was obtained from Wallace Pumps Ltd. The cost for the heat exchanger was estimated from cost estimation curves (Peters et. al. 2003) and was adjusted to current prices. For hide loading capacity of 11,600 kg at a 50% float, four mixers were required to process 35 tonne of hide. The process would take four days to complete and therefore 16 mixers would be required to produce 35 tonne of pretreated hide per day.

The capital cost analysis was based on the purchased equipment delivered cost for 16 used Canbar mixers, a heat exchanger with a large enough surface area to heat the solution to 28 ºC, and centrifuge pumps to recirculated the solution in the mixers.

Table 6.6: Breakdown of equipment cost for specialized mixing.

Equipment	Delivered Cost
16 Used Canbar Mixers (50% Float)	$1,138,463
Centrifuge Pumps Required	$227,047
Heat Exchangers Required	$18,465
Purchased Equipment Delivered Cost	$1,383,974

The FCI for the installation of hide mixers to produce 35 tonne of hide a day was calculated using the purchased equipment delivered costs (Table 6.6).

Table 6.7: The breakdown of FCI costs for the installation of the specialized hide mixer process for pretreatment of gelatine raw material.

Direct Costs		Indirect Costs	
Purchased Equipment Delivered	$1,400,000	Engineering and Supervision	462,000
Purchased Equipment Installation	$630,000	Construction Expenses	546,000
Instrumentation and controls	$126,000	Legal Expenses	56,000
Piping	$224,000	Contractor's Fee	238,000
Electrical Systems	$140,000	Contingency	490,000
Buildings	$280,000		
Yard Improvements	$210,000		
Service Facilities	$140,000		
Total Direct Plant Cost	$3,150,000	**Total Indirect Plant Cost**	1,792,000
		Fixed Capital Investment	$4,942,000

In the capital cost analysis, modifications to the buildings were included. This would include the cost for removal of the current lime pits and the installation of new equipment. This option was considerable cheaper as the process only took approximately four days to complete. Therefore, the number of mixers required to produce 35 tonne of hide a day was less. The FCI associated with this process would be a considerable investment. It would be possible to gradually integrate the mixers into the process over several years. It would also be advantageous to run a process, similar to the one presently in operation at Gelita Aus, which is currently being implemented as a pretreatment method.

Chapter 7: Conclusions and Recommendations

The purpose of this project was to test a variety of methods for enhancing the penetration of liming chemicals into the bovine hide raw material for pretreatment (hide conditioning) in the production of gelatine. Various methods, based upon techniques for enhancing mass transfer in a system while minimizing excess degradation of the material, which included temperature controlled mixing and fluidization. Research in the leather industry, which has similar pretreatment steps as gelatine production, suggested ultrasound as a means to enhance chemical penetration into the hide pieces.

During the course of the project it was decided that quantitative measurement of the chemical penetration/hide condition was required to determine the effectiveness of the different processing methods. Several methods were tested including monitoring: ultraviolet dye penetration, the pH profile in the hide pieces, and the GAGs /soluble collagen concentration released into solution. The UV dye is applicable to pilot plant trials but not production scale due to food-grade purity requirements. Dye penetration can be visually assessed, or quantified using imaging software. Further work could enhance this method with microtome techniques to improve imaging.

The concentration of GAGs/ soluble collagen released into solution was determined using the chemical assay techniques. The GAGs concentration was 0.77 mg/ml at the beginning of the liming process and increased to 1.15 mg/ml after liming was completed. The final concentration of soluble collagen in the liming solution was 3.86 mg/ml. Further trials are needed in order to fully understand how these concentrations relate to the conditioning level of the hide pieces. Furthermore, it would not be efficient to monitor the levels in 72 different lime pits as it took approximately one hour to complete the assay for one pit. A method would need to be developed in which this assay could be used to monitor all the lime pits.

The UV dye technique was used to monitor the levels of conditioning in all of the experimental trials. This technique was first tested in the temperature controlled mixing trials. In this trial the decrease in temperature slowed the penetration of

chemicals which continuous mixing could not counteract. However, the mixing at lower temperatures did prevent the formation of a gelled layer on the outside of the hide. Gelling did occur in room temperature mixing which slowed the penetration of chemicals into the hide pieces. In the temperature controlled mixing trials the standard liming process gave the best results.

The use of ultrasound had beneficial effects on the system. With the ultrasound there was a slight increase of the chemicals through the skin side of the hide pieces. The ultrasound also dissolved fatty tissue on the inside of the hide pieces. The absence of fatty tissue allowed for a large increase in penetration of the liming chemicals through the inside of the hide pieces. The ultrasound also had destructive effects on the hide when a temperature of 22 ºC was maintained, despite what was suggested in literature. The hide pieces irradiated ultrasound started to degrade after several days of processing.

It is possible that the ultrasound could be used in the liming process to increase the penetration of the liming chemical. However, further work would be required to overcome the problems with hide degradation. It is recommended that trails be conducted using an ultrasound probe rather than an ultrasonic cleaner. This would be the same type of equipment that would be implemented in large scale processing. Ultrasonic intensities could be easily changed then by varying the volume of the liming solution to see the effect on the degradation of the hide. This was not possible in the past trials as the volume of an ultrasonic cleaner is constant.

Of all the methods tested to enhance the penetration of chemicals into the hide pieces, the fluidization of the hide pieces was the most effective. The processing time for completing both the liming and acidulation processes was reduced to 9 days from the 50 days of the standard process. This was due to the decrease in size of the hide pieces, to 3 cm x 3 cm, and the increase of mass transfer due to fluidization. Hide pieces of the same size were under-conditioned when processed for the same amount of time using the standard liming procedure.

A capital cost analysis was conducted to determine the feasibility of implanting these processing methods into the production of gelatine at Gelita N.Z. The FCI required

to implement each process with a basis of 35 tonne of hide was calculated (Table 7.1).

Table 7.1: The comparison of FCI for different processing methods.

Process	Fixed Capital Investment
Ultrasound	$678,000
Specialized Hide Mixer	$4,942,000
Fluidization of Hide Pieces	$10,400,000

While experimental results showed that fluidization of the hide pieces decreased the pretreatment time considerably, the FCI required for this process was the most expensive with an expenditure of $10.5 million. The FCI required for the ultrasound transducer probe and was approximately $700,000. This was the cheapest option because none of the liming pits would have to be removed and replaced with different processing vessel. However, further research would be required with ultrasound to develop a method in which the benefits of the ultrasound could be used without causing the hide to degrade into the liming solution. The hide mixer would require a FCI of approximately $5.0 million and would completely replace the current 72 lime pits with 16 hide mixers.

Currently the best option would be the use of the specialized hide mixer process as developed by Gelita Aus. While this process was expensive it could be integrated into the process over several years. This process combines a gentle mixing and temperature manipulation by heating the initial liming solution to then allowing it to cool to room temperature as the process progresses. The gentle mixing of the specialized hide mixer increased chemical penetration without the formation of a gelled layer on the outside of the hide. This process decreased the liming time to approximately four days at Gelita Aus, though a full-scale trial would be required at Gelita N.Z. to see if the similar results could be obtained. It would also be advantageous for Gelita N.Z to be running a similar process as Gelita Aus.

7.1 Recommendations

The following are recommendations for further research or future projects base on the results previously discussed

- The enhancement of UV technique by improving the microtome sections so

they could be properly photographed under ultraviolet light. This would give allow for the samples to be photographed from underneath and would results in a better profile of the UV dye.

- Investigation of a UV dye that would be safe for food production. This would make it possible to use the UV technique for full-scale production.
- Research relating the GAGs/soluble collagen concentration to overall hide conditioning. Development of a method in which the chemical assay for these concentrations could be practically applied to monitor each pit at Gelita N.Z
- Conducting trials with more advanced ultrasound equipment, a Hielscher 400 W ultrasound probe. This would allow for varying the ultrasonic intensities to where the effect of ultrasound removes excess fat from the hide and increases chemical penetration. By varying the ultrasonic intensity it might be possible to accomplish this with out degrading the hide.
- Conducting a large scale fluidization trial and implementing the temperature profile of the specialized hide mixer process. This could potentially increased chemical penetration due to both the effect of fluidization and temperature.
- Running a large-scale specialized hide mixer trial. Pretreatment could be done at Graeme Lowe Tanneries, and then brought to Gelita N.Z. for gelatine extraction.

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Appendix

Nomenclature:

d_h^3 - effective diameter of a sphere having the equivalent volume of the hide pieces

ρ_f - the density of the liming solution which was approximated as the density of water

ρ_h - the density of the hide pieces which was experimentally determined to be 1.12 g/ml

μ_f - the viscosity of the liming solution which was approximated as the viscosity of water

Ar - the Archimedes number

Re_{mf} - the Reynolds number at minimum fluidization velocity

u_{mf} - the minimum fluidization velocity corrected for non-spherical partials

Corrected Minimum Fluidization Velocity

→ Corrected bide diamter for size of 30 x 30mm with column diameter of 2.0 m

$$d_B^3 = \frac{6a^3}{\pi} = \frac{6(0.03m)^3}{6}$$

$$d_B^3 = 5.2 \times 10^{-5} m^3$$

$$Ar = \frac{d_B^3 \rho_f (\rho_B - \rho_f) g}{\mu_f^2} \Rightarrow \frac{(5.2\times10^{-5}m^3)(1000\frac{Kg}{m^3})(1120\frac{Kg}{m^3} - 1000\frac{Kg}{m^3})(9.81\frac{m}{s^2})}{(0.001\frac{Kg}{m3})^2}$$

$$\Rightarrow \boxed{Ar = 31780000}$$

$$Re_{mf} = \sqrt{1135.7 + (0.0408)Ar} - 33.7 = \sqrt{1135.7 + (0.0408)(31780000)} - 33.$$

$$\Rightarrow \boxed{Re_{mf} = 1106}$$

$$u_{mf} = \frac{Re_{mf}\,\mu_f}{d_p\,\rho_f} = \frac{(1106)(0.001\frac{Kg}{ms})}{(0.03m)(1000\frac{Kg}{m^3})}$$

$$\boxed{u_{mf} = 0.037\frac{m}{s}}$$

$$F_{mf}(\frac{m^3}{s}) = u_{mf} A_c = (0.037\frac{m}{s})(\pi(\frac{2}{1})^2)$$

$$\Rightarrow F_{mf} = 0.116\frac{m^3}{s}$$

Determination of Vessels required to process 35 tonne of Hi
in a fluidized column

Column diameter = 2.0 m Column Height = 10. m

Volume of Column = $(\pi r^2)h = (3.14(1m)^2)(10.0m)$

$$V_c = 31.4\ m^3$$

Density of hide with 50% float = 667 $\frac{Kg\ hide}{m^3}$

Tonne of hide per Column

$= V_c(\rho_{50\%}) = (31.4\ m^3)(667\ \frac{Kg\ hide}{m^3}) = 20950\ Kg = 20.95$ tonne

Columns required for 35 tonne of hide

$$= \frac{35\ tonne}{20.95\ \frac{tonne}{Colun}} = 1.7$$

$$\Rightarrow 2$$

For a column diameter of 2.0 m would require 2 columns
to process 35 tonne of hide.

Breakdown of Indirect and Direct Costs from Purchased Equipment Costs

Direct Costs

Purchased Equipment Installation - 45% of the delivered purchased equipment costs (DPEC)

Instrumentation and Controls - 8% of the DPEC

Piping – 16% of the DPEC

Electrical Systems- 10% of the DPEC

Buildings- 20 of the DPEC

Yard Improvements- 15 of the DPEC

Service Facilities- 40 of the DPEC

Direct Costs

Engineering and Supervision- 33% of the DPEC

Construction Expenses- 39% of the DPEC

Legal Expenses- 4% of the DPEC

Contractors Fee- 17% of the DPEC

Contingency- 35% of the DPEC

These percentages were slightly modified when there would be no application to implementing the new process at Gelita N.Z as noted in the text.