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Hell Bent For Leather!

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Contents

Preface

The purpose of this document is to bring together in one place the exercises carried out in class during the course “An Introduction to Tanning in English” for fourth year students at the technical school for tannery in Arzignano, Vi, Italy.

The exercises are based on a text currently in preparation which will form the basis of the course.

Some of the materials are not original (references are given where appropriate), the exercises are.

The materials will be subject to modification.

General structure of skin

Skin is the largest organ in the body of a mammal. It has a complex layered structure which allows it to carry out its excretory and protective functions. Each layer is (1) _____different types of cells and organs with different functions. A typical bovine skin is made up of 64% of water, 33% of proteins, 2% of fats, 0. 5% of minerals and other substances.

The skins of mammals are essentially the same across species, histologically we can distinguish three main layers: (2) _____, dermis and subcutaneous layer.

The epidermis is the (3) _____part of the skin. It is made up of a thin film of cells which is easily removed from the underlying layer during processing. There are no nerves or blood vessels in the epidermis. Under the microscope it can be seen to be made up of five layers of superimposed cells.

In cowhide the epidermis represents about 1% of the thickness of the skin and it is (4) _____in the liming step along with the hair follicles, sebaceous and sweat glands. The roots of the hairs and the hairs themselves are (5) _____in the **grain layer** which is part of the dermal layer (**dermis**).

Both the epidermis and the hair are made up of (6) _____, a protein characterized by the presence of cysteine residues and then disulphide bonds between adjacent protein chains which make it very resistant to proteolytic and chemical attack by dilute acids and bases, and vulnerable to attack by reducing agents.

The dermis is the main part of the (7) _____and is off-white in colour. It protects the underlying flesh from mechanical attack and provides the epidermis with nutrients via a network of fine capillaries. It is the skin layer that is transformed into leather during the tanning process

The dermis is mainly composed of **connective tissue.** The dermis also (8) _____other proteins such as **elastin** and non-structural proteins with a cementing action, such as **albumins** and **globulins**.

The dermis contains two layers with different structures. The upper layer, which reaches down to the level of the hair roots and is a mixture of glands and hair bulbs. It has a compact fine-grained structure made of small cells and is called (9) _____by the tanner. It is made up of a dense (10) _____of fine collagen fibres oriented perpendicular to the surface of the skin. Elastin fibres, which give elasticity to the skin, are present between the collagenic fibres.

The innermost layer of the dermis is in contact with the subcutaneous tissue. It contains thick bundles of collagen fibres that show variable (11) _____and which become parallel to the surface of the skin in the deeper layers of the dermis. The more varied orientation of these fibres is responsible for the physical strength of leather.

Underneath the dermis lies the **subcutaneous layer** which is rich in fat and collagen fibres arranged parallel to the skin surface with a more relaxed structure. This is easy to cut, allowing the dermis to be obtained intact from a careful operation of skinning. In a cowhide, it (12) _____about 14% of the thickness of the skin and fixes the skin to the underlying tissues.

Questions

1) Complete the cloze exercise by filling in the gaps with the correct word

represents	composed of
eliminated	epidermis
located	external
keratin	network
orientation	contains
hide	grain

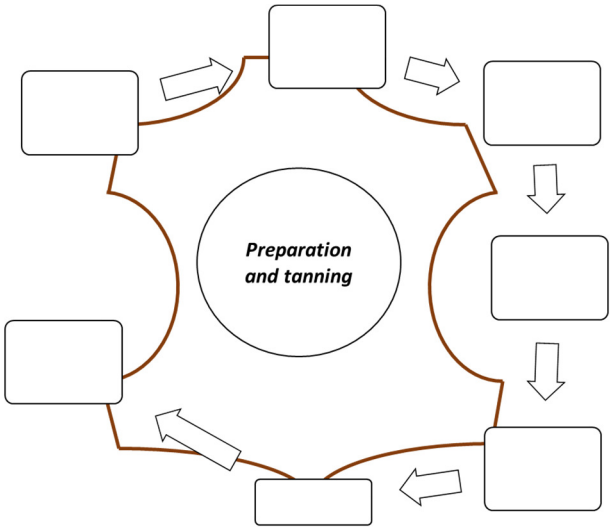
Vocabulary

- Underline any words that you don't know in the text – find their meaning.

2) Answer the following questions about the text.

a) What are the different layers of the skin called?
b) In which layers can you find collagen?
c) What percentage of the thickness of the skin is represented by the epidermis?
d) What is the biological function of skin?
e) Which layer is used by the tanner?
f) What does the subcutaneous layer do?
g) What is elastin?

3) The tanning process exercise – map



a	Tanning		1	Acidify pelts to prepare them for chrome tannage
b	Soaking		2	Introduce chemical cross-links to irreversibly stabilise collagen
c	Reception		3	Loosen collagen fibres with enzymes
d	Pickling		4	Receive hides from suppliers
e	Liming		5	Remove deposited and chemically bound lime
f	Deliming		6	Remove hair, wool and epidermis; loosen collagen fibre and partially saponify natural grease
g	Bating		7	Restore hide to natural state; remove dirt, curing agents, soluble proteins

Match the letter of the step to the number of the description then fill in the diagram.

Exercise: Amino acids and proteins

The chemistry of leather is essentially the chemistry of collagen. To understand how to make leather, we need to understand what collagen is made of and how this affects its properties. Collagen is a protein and as all proteins, it is made up of amino acids.

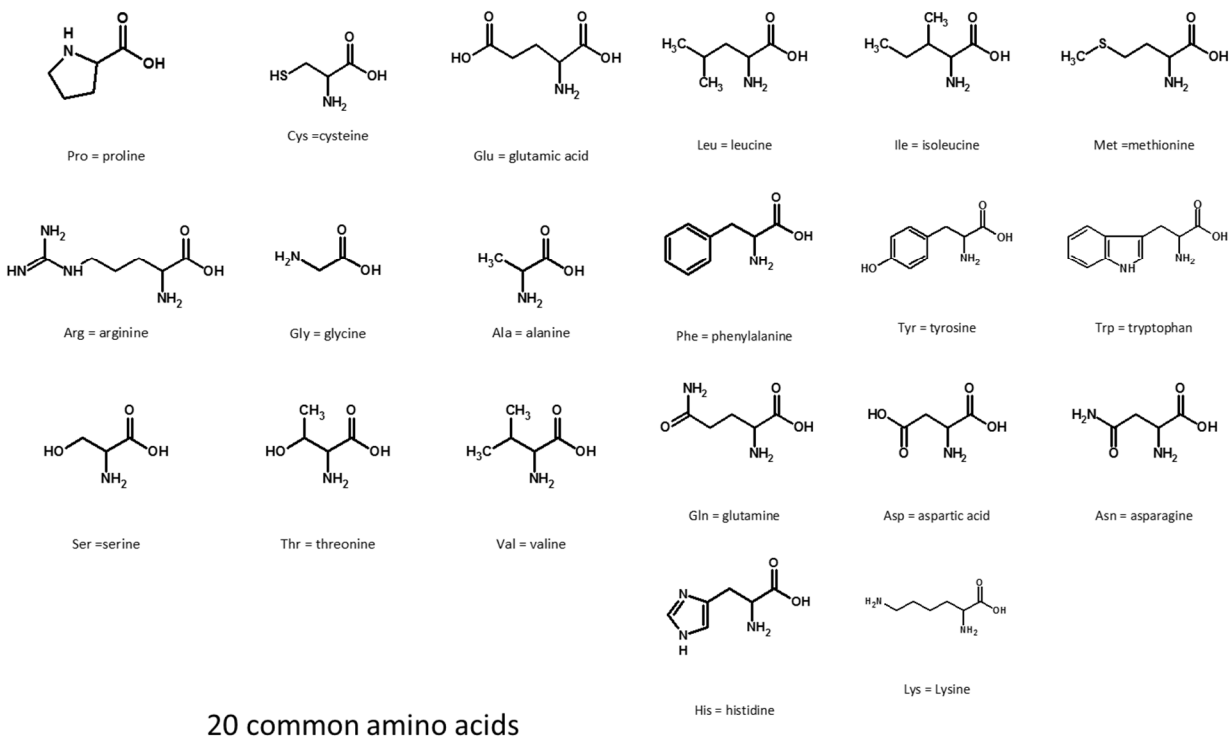
There are 20 essential amino acids needed for healthy growth in humans although there are many more amino acids in nature. Amino acids are the Lego™ blocks of proteins and all proteins in the human body are composed of combinations of these amino acids.

These amino acids all have an amine group and a carboxylic acid group attached to a carbon atom. This is called the alpha carbon. There is usually a side chain attached to the alpha carbon (except in glycine, the simplest amino acid). These side chains are important because they can have many different structures and give particular physical and chemical properties to the protein.

Look at the amino acids below. Mark them according to whether their side chains contain:

- a non-polar (hydrophobic) group,
- a nucleophilic group,
- an acidic group (e.g. a carboxylic acid),
- a basic group (e.g. an amine group),
- contain an aromatic ring.

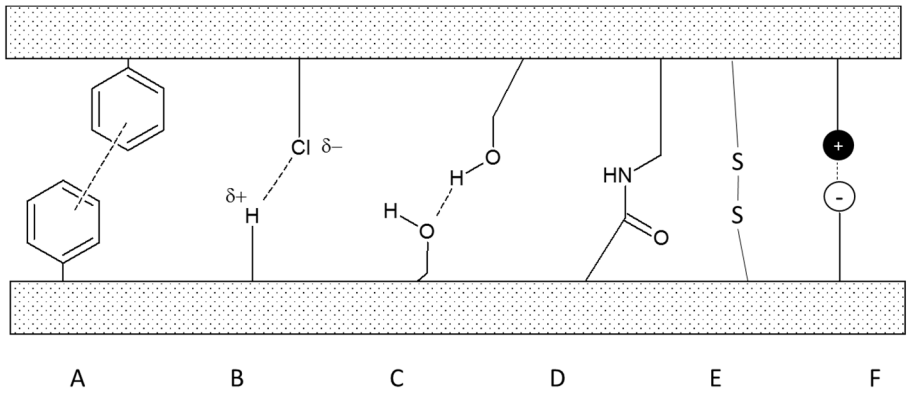
Remember, they ***all*** contain an amino group and a carboxylic acid group attached to the alpha carbon, this exercise is only interested in the side chain groups.



20 common amino acids

Exercise: Bonding and structure in proteins

What types of bonding can we find in proteins? Match the bonding to the name.



	Which amino acids might make this type of bonding?
Covalent bond	
Disulfide bridge	
Hydrogen bond	
Ionic bond	
London (dispersion) forces	
Permanent dipole	

These bonds are important in stabilising how the protein folds: the secondary, tertiary and quaternary structures of the peptide chain.

Polypeptides (proteins) are made up of amino acids joined together by amide bonds (=peptide bonds). When two amino acids come together the amine on one can react with the acid on the other via an enzyme catalysed reaction to give a dipeptide.

Choose any two amino acids from the amino acid table and draw the dipeptide formed:

The isoelectric point

The **isoelectric point** (pI) is the pH at which an amino acid does not migrate in an electric field. In a similar way, the isoelectric point of a protein is the pH at which the net charge on the protein (ie the sum of all of the positive and negative charges) is zero. Under these conditions, the protein will not move in an electric field.

- At this pH the amino acid or protein is neutral. For an amino acid, the zwitterionic form is dominant.
- For a protein, the pI is given by the average of the pKas that involve the zwitterion

We need to consider the following equilibrium: $HA = H^+ + A^-$

$$K_a = \frac{[H+][A-]}{[HA]}$$

And $$pKa = pH + \log\frac{[HA]}{[A-]}$$ (Henderson-Hasselbach)

Neutral side chains

These amino acids are characterised by two pK_as : pK_a1 and pK_a2 for the carboxylic acid and the amine respectively.
The isoelectronic point will be halfway between, or the average of, these two pK_as, *i.e.* **$pI = 1/2 (pK_{a1} + pKa_2)$**.

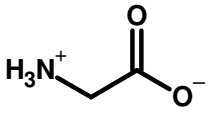
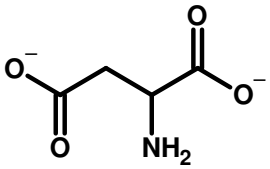
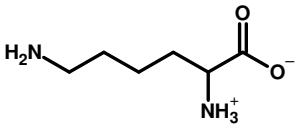
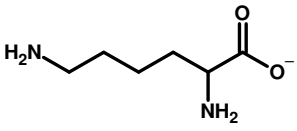
Acidic side chains

The pI will be at a lower pH because the acidic side chain introduces an "extra" negative charge. So the neutral form exists under more **acidic** conditions when the extra -ve has been neutralised. For example, for **aspartic acid**, the neutral form is dominant between pH 1.88 and 3.65, pI is halfway between these two values, *i.e.* **$pI = 1/2 (pK_{a1} + pK_{a3})$**, so pI = 2.77.

Basic side chains

The pI will be at a higher pH because the basic side chain introduces an "extra" positive charge. So the neutral form exists under more **basic** conditions when the extra +ve has been neutralised. For example, for histidine, the neutral form is dominant between pH 6.00 and 9.17, pI is halfway between these two values, *i.e.* **$pI = 1/2 (pK_{a2} + pK_{a3})$**, so pI = 7.59.

Draw the forms that you would expect to find in these solutions:

Amino acid	*Form in acidic solution (low pH)*	*Form at the **isoelectric** point*	*Form in basic solution (high pH)*
Neutral e.g. glycine			
Acidic e.g. aspartic acid			
Basic e.g. lysine			

The calculation of the isoelectric point in proteins is complicated by the fact that there are many different side chains which contribute. It can be measured experimentally and calculated using sophisticated algorithms (see the QR references).

Why is pI important for the chemistry of leather making?

Understanding the pI and how it changes during the process of the preparation of the hide is important because changes in the pH of the internal environment of the hide affects how it reacts to the presence of salts and water in the surrounding solution. The pI of a hide does not remain constant as the side chains of the amino acids are chemically modified during the various steps of the tanning process. The presence of a charged or neutral environment in the hide affects how it will react towards liming and tanning agents as well as causing physical effects such as swelling or contraction caused by lyotropic and osmotic effects. Collagen has a pI of about 4.7 under normal conditions.

Reading: Collagen

From: Collagen: molecule of the month: https://pdb101.rcsb.org/motm/4

Your Most Plentiful Protein

About one quarter of all of the protein in your body is collagen. Collagen is a major structural protein, forming molecular cables that strengthen the tendons and resilient sheets that support the skin and internal organs. Bones and teeth are made by adding mineral crystals to collagen. Collagen provides structure to our bodies, protecting and supporting the softer tissues and connecting them with the skeleton. But, in spite of its critical function in the body, collagen is a relatively simple protein.

The Collagen Triple Helix

Collagen is composed of three chains, wound together in a tight triple helix. The illustration included here shows only a small segment of the entire molecule--each chain is over 1400 amino acids long and only about 20 are shown here. A repeated sequence of three amino acids forms this sturdy structure. Every third amino acid is glycine, a small amino acid that fits perfectly inside the helix. Many of the remaining positions in the chain are filled by two unexpected amino acids: proline and a modified version of proline, hydroxyproline. We wouldn't expect proline to be this common, because it forms a kink in the polypeptide chain that is difficult to accommodate in typical globular proteins. But, as you can see in the examples below, it seems to be just the right shape for this structural protein.

Vitamin C

Hydroxyproline, which is critical for collagen stability, is created by modifying normal proline amino acids after the collagen chain is built. The reaction requires vitamin C to assist in the addition of oxygen. Unfortunately, we cannot make vitamin C within our bodies, and if we don't get enough in our diet, the results can be disastrous. Vitamin C deficiency slows the production of hydroxyproline and stops the construction of new collagen, ultimately causing scurvy. The symptoms of scurvy--loss of teeth and easy bruising--are caused by the lack of collagen to repair the wear-and-tear caused by everyday activities.

Collagen on the Grocery Shelf

Collagen from livestock animals is a familiar ingredient for cooking. Like most proteins, when collagen is heated, it loses all of its structure. The triple helix unwinds and the chains separate. Then, when this denatured mass of tangled chains cools down, it soaks up all of the surrounding water like a sponge, forming gelatin.

Ropes and Ladders

We make many different kinds of collagen, which form long ropes and tough sheets that are used for structural support in mature animals and as pathways for cellular movement during development. All contain a long stretch of triple helix connected to different types of ends. The simplest is merely a long triple helix, with blunt ends. These "type I" collagen molecules associate side-by-side, like fibers in a rope, to form tough fibrils. These fibrils crisscross the space between nearly every one of our cells.

This illustration depicts a basement membrane, which forms a tough surface that supports the skin and many organs. A different collagen-"type IV"-forms the structural basis of this membrane. Type IV collagen has a globular head at one end and an extra tail at the other. The heads bind strongly together, head-to-head, and four collagen molecules associate together through their tails, forming an X-shaped complex. Using these two types of interactions, type IV collagen forms an extended network, shown here in light blue. Two other molecules-cross-shaped laminin and long, snaky proteoglycans -fill in the spaces, forming a dense sheet.

Some references:

Collagen reading:	
Isolelectric point calculations for proteins http://isoelectric.org/index.html;	

The process of soaking: "Right first time"

The text has been divided into sections with each section organised as an exercise.

A:

WORDLIST: (some may be used more than once!)

In	*along*	*between*	*by*	*during*	*for*
in	*of*	*out*	*to*	*up*	*with*

__ the process __ soaking, the skin reacquires the water which is lost _____ storage. The fundamental properties __ the leather are determined __ what happens __ this stage and it is impossible __ fully correct any defects introduced _____ the activities __ this stage.

This is a fundamental operation because all __ the processes performed afterwards exploit the exchange __ solutes _____ the solution and the skin. The more water present __ the substrate, the faster the exchange __ products will be because diffusion __ and ___ __ the skin will be facilitated.

Soaking cleans the skin and opens __ the structure. The non-collagenic components such as hyaluronic acid, glycosylamines and non-structural globular proteins are removed _____ ____ the salt used __ preservation. Soaking causes the fibril bundles __ the collagen __ separate and prepares them ___ tanning.

B: Soaking and subsequent steps are carried out in rotating drums of various dimensions although the initial steps may be carried out in open vessels in which the hides are moved with paddles. Brusque movements must be avoided at the start because the fibres are rigid and mechanical bending can break them.

C:

WORDLIST: *amount* *concentration* *content pH presence* *process* *time temperature*

Several parameters are important during the soaking stage, including:

1. _____ of preservation
2. water _____ of the hide
3. salt _____ in the bath
4. _____ of water used (float to pelt ratio)
5. _____ in the bath
6. _____ or absence of added biocides
7. _____ of the bath
8. _____ of the bath

D:

During preservation of the hide with salt, water is removed from the pelt causing a collapse of the collagen fibres that renders the hide stiff and which obstructs the movement of the solution within the hide. During soaking, the fibre structure of the collagen is rehydrated to reach an equilibrium such that dissolved chemical agents can move through the hide. If rehydration is not complete, subsequent chemical processing will be non-uniform leading to variation in the final product.

E: *(match the heading to the description)*

Three main approaches are used in soaking:

1. Under running water		a. short first soak followed by longer subsequent soaks. Effectiveness depends on partitioning of salt between the pelt and the aqueous solution. The amount of water used depends on the number of washes used.
2. Washing in batches		b. reduces the amount of water used. Salt and dirt are accumulated in the small volume of water used.
3. Counter current washing		c. this maximises the rate of transfer of the salt out of the pelt and water into the pelt, however, it uses a large volume of water.

F: Before starting, the excess salt on the surface of the pelt may be mechanically separated and recovered in a practice called “whipping”.
Since the concentration of the salt in solution determines the rate of salt removal, the soaking water (=float) should be changed several times.

G:
MISSING NUMBERS: 25 45 65 30 30 25

The temperature must be kept below __ _°C since the contraction temperature of the raw pelt is just __ _°C and gelatinization occurs at __ _°C. If the temperature is too low, the erector muscles of the pili contract increasing the coarseness of the grain. In addition, bacteria grow exponentially around __ _°C, therefore if the bath temperature exceeds __ _°C, the addition of an antibacterial agent is required.

Working at __ _°C, without an antibacterial, a slight bacterial action contributes to the elimination of globular proteins.

H:
WORDLIST: Detergents Removal This are by collagen detergents effective of post-tanning removed

_____ accelerate rehydration by wetting the fibres. _____ of fatty components is not particularly _____ at these temperatures since the lipids ___ not particularly mobile. Triglycerides are best _____ by alkyl poly (ethylene oxide) non-ionic _____ whereas sebaceous grease is best removed __ anionic detergents. The latter bind to _____ and may be carried through to _____-.

The major risk at this stage __ processing a hide is bacterial activity. ____ can be controlled by the use __ a variety of biocidal components.

I:

The pH is typically kept around 7, although it may be increased to 10 with sodium carbonate to improve wetting. Auxiliaries to help soaking can be acids, bases or acidic or basic salts. Changing the pH causes collagen swelling in both acid and basic conditions although alkaline swelling is preferred because with an isoelectric point of 4-5, globular proteins precipitate in the bath if it is too acidic.
The most widely used salts are bicarbonates, carbonates or sodium sulphide, paying attention not to exceed the pH of the bath.

Preservation

Preservation – part 1

Salting

By far the most widespread method is based on treating the hide with common salt (NaCl) either in mineral form or as sea salt. The salt is sprinkled on the meat part. The salting must be complete to avoid any bacterial growth with approximately 40 - 50% weight of salt used in relation to the weight of the skin.

Salting in piles or in presses

Heavy bovine hides such as pelts from oxen, cows, and sometimes calf skins are usually salted in piles or in presses. The flesh side is uniformly sprinkled with salt, the skin is folded with the flesh against the meat following the dorsal midline, with the tail inside. The pile is placed on an inclined floor so that the brine (=salt water exudate) does not stagnate near the pile.

The skins remain on the stack until they are shipped or for a period of 1 to 2 months. The pelts lose weight during stacking because of the water lost in the brine. This weight loss is typically around 12-14% but if the skin is very thick, it may reach 20%.

Brining

Calf skins are usually treated in a tank with the meat side up and sprinkled abundantly with salt (45-50% by weight).

Brine which forms spontaneously as water is removed from the skin, submerges the skins thus increasing the effectiveness of the salting process as the skins are no longer in direct contact with the air. The high salt concentration of the solution and the lack of exposure to oxygen reduce the risk of bacterial growth. In addition, the room in which this is carried out must be kept cool and well-ventilated (this also helps reduce the risk of putrefaction) and the skins must not be exposed to direct light.

When the skins are to be sold, they are removed from the bath, drained, salted and then packaged.

Problems with using salt

All of the salt used to preserve the skin must be removed in the soaking step before the hide can be processed further. While sodium chloride itself is relatively cheap and easily available, the large amount used in the process of preservation causes problems in waste management since the effluent water of the soaking process will have a very high salinity and must be desalinated before the water can be released into the water system.

This has led to a lot of efforts to reduce the environmental impact of salt in the creation of green leather processes.

(428 words)

Vocabulary.

Identify any words you do not know – ask your colleagues, ask the teacher

LANGUAGE EXERCISE: Underline the verbs in the text with a coloured pen or pencil – to be discussed as a class

Questions

1. What is putrefaction and what causes it?
2. Why is it important to avoid putrefaction when preparing a hide?
3. How are the skins folded during salting on a pile?
4. Where does the brine come from?
5. What is a green leather process?

Preservation – part 2

Drying

Drying is the oldest form of preservation of hides. It is a very simple process relying on the availability of a source of heat (the sun) and a flow of air to remove the moisture from the skin and so is a practice that is typical of warmer countries. Fresh skins must not be exposed to direct sunlight (direct sunlight can chemically alter them), and drying must not be carried out too quickly to avoid the transformation of the skin into a horn-like substance which is difficult to recover. Skins are dried in a shady place such as a well-ventilated room in which they are suspended and dried slowly in a stream of air that constantly removes the moisture to avoid putrefaction.

Small skins are typically only suspended while larger skins are usually staked out to keep them under tension. They may be impregnated with antibacterial and repellent solutions to discourage attack by insects.

The dried skin is quite stiff and must not get wet for example by exposure to even light rain which can cause the putrefaction process to start again in the areas where the skin is wet.

Cooling and Refrigeration

By far the simplest way of preserving raw hides is to keep them cool through the use of refrigerators though without reaching the temperature of ice formation which causes the breakdown of the collagen fibres. Fresh hides are kept cool in special cooling rooms. The big advantage of this method is that it avoids the use of large amounts of salt usually used for conservation although it does require a lot of energy to run the refrigerators. Another advantage is that storage time is essentially unlimited and when thawed, the skins can be processed immediately as if they were fresh.

Newer methods of conservation

Other alternatives to salt have been tested recently including mixtures with lower amounts of salts containing antibacterial substances (some natural) and alternative conserving agents such as sodium chlorite ($NaClO_2$). This is a strong oxidizing agent which has been successfully used although it must be used with certain safety precautions such as avoiding contact with combustible materials or reducing agents.

(word count 357)

LANGUAGE: identify the **adjectives** in the text. Underline them.

Questions.

1. Why are larger skins staked out during drying?
2. What happens if skins are exposed to sunlight?
3. Why do we need a flow of air when drying the skin?

Group work then presenting

- What are the advantages and disadvantages of each method of preservation of the hide?
- Complete the table using full sentences.

	Advantages	Disadvantages
Drying		
Salting		
Brining		
Oxidising agents		
Refrigeration		

1. Which form of preservation does not involve the removal of water?
2. Which form of preservation produces most salt water effluent (waste)?
3. Which form of preservation needs more energy?
4. In the processes which remove water, where does this water come from and what happens to the structures and molecules in the skin?

Preservation – tanning crossword exercise

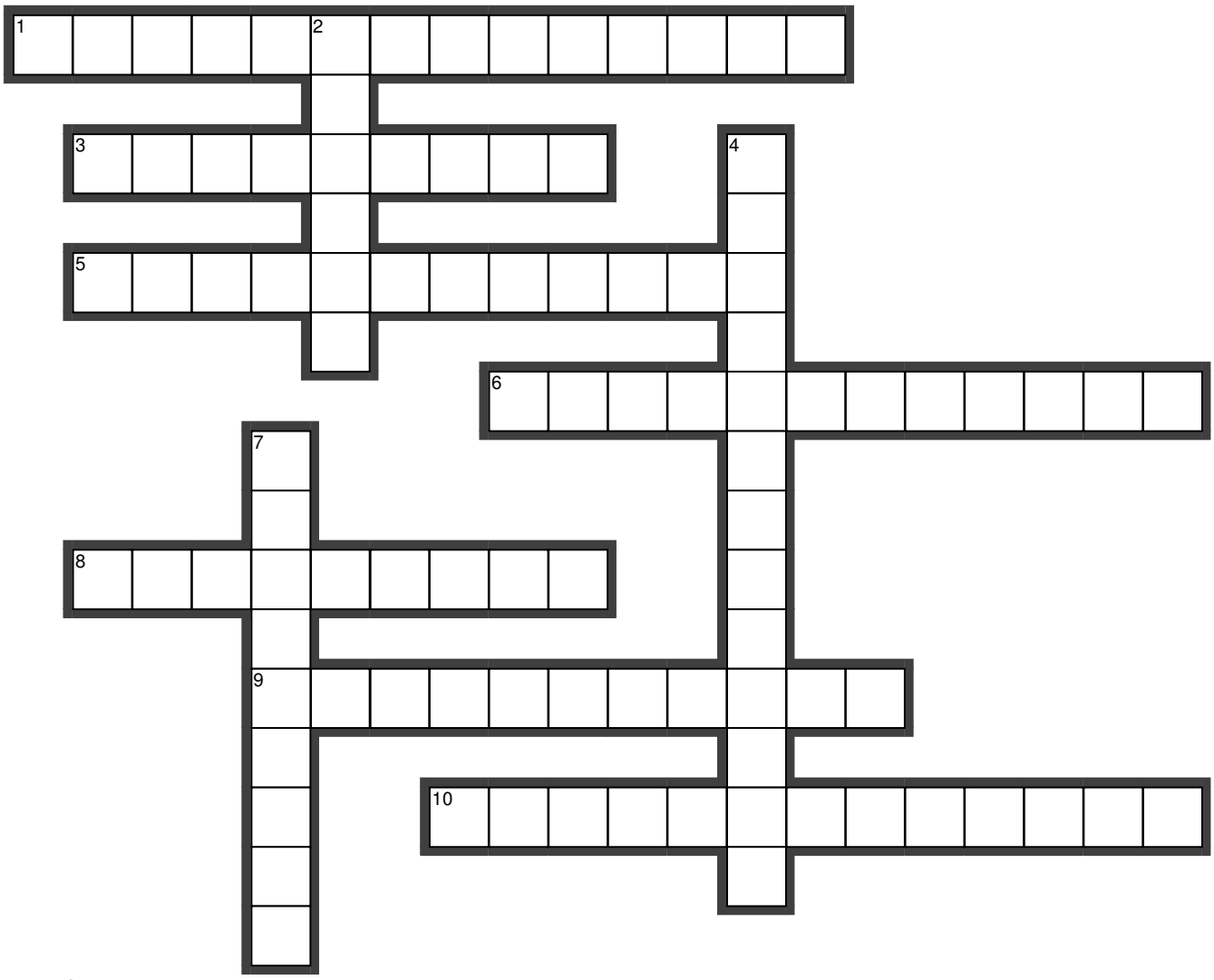
REFER TO THE TEXTS ABOVE

Across

1. T... the process of changing one thing into another (14)
3. R... it keeps unwanted things away from something that you wish to protect (9)
5. P... the process of stopping the degradation of the hide (12)
6. P... the process of decompostion by bacteria (12)
8. S... small pieces of a solid which are spread over a surface (9)
9. I... something which absorbs a liquid or a substance (11)
10. E... when we design a process, we must think of the e... impact that the waste has (13)

Down

2. F... something which has been f... down the middle is half the size (6)
4. C... in this context it refers to how much salt is dissolved in a quantity of water (13)
7. O... a type of substance which can be used to substitute for common salt (9)



EclipseCrossword.com

The process of unhairing and liming

Exercise: alternative words: The process of unhairing and liming

Note: this is the text of the listening exercise used in class.

At the end of soaking, the skin is ready for the processes of liming and unhairing which can be conducted individually, although they are usually carried out simultaneously.

These processes are used to

- Remove keratinous parts such as hair, nails etc.;
- Remove soluble proteins (mucins) and solubilize the residual globular proteins;
- Hydrolyse part of the polypeptide chains in order to allow a relaxation of the protein structure causing the fibres to swell and split. This introduces spaces between the fibres that will be exploited for the penetration of the tanning agent;
- Remove a certain amount of grease and fat from the skin through saponification reactions to facilitate their extraction;

Hair is removed in two ways: removal intact to allow its use in other economically important activities or through simple destruction.

Hair is made of keratin, a protein which has a different composition compared to other proteins found in the hide. It is particularly rich in the amino acid cysteine.

The hair shaft is composed of an outer cuticle which is protected by scales. Inside , the shaft is composed of bundles of protofibrils arranged within a microfibril. The microfibrils are made of protofibrils composed of helical keratin chains which are linked to each other with strong sulfur-sulfur bonds together with weaker salt bridges and hydrogen bonds. It is the sulfur-sulfur bonds which dominate the chemistry of keratin.
In the hair fibril, four of these chains are held together by hydrogen bonding, salt bridges and above all very stable covalent disulphide bridges.

The disulphide bridges are the product of an oxidation reaction between the sulfhydryl side chains of two cysteine residues on two neighbouring protein chains.

The reversibility of this reaction is exploited during the unhairing reaction with sodium sulphide. In this reaction, the hide is treated with a solution of sodium sulphide, a good nucleophile, which readily attacks the disulphide bridge to break the S-S bond holding the chains together.

Write answer below

Below are some words which can be used instead of the words underlined in the text. Match the alternative to the original word.

A. Used	B. make easier	C. carried out
D. reacted with	E. made up of	F. are important in
G. opening up	H. joined	I. remaining

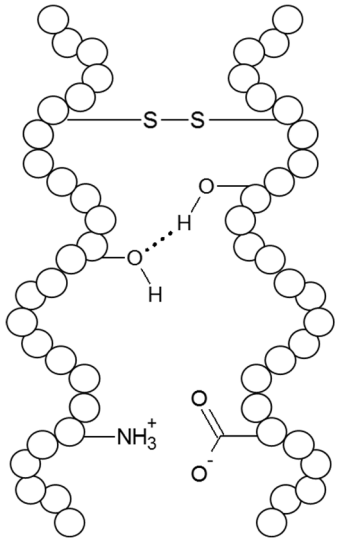
Questions on the text.

What is removed from the skin during unhairing?

1.
2.
3.
4.

Why might you want to not destroy the hair?

Which types of bonds are present in keratin? (Note them on the diagram)



Describe the structure of a hair?

Why is the hide treated with a solution of sodium sulfide?

Why might you use enzymes instead of sodium sulfide to unhair a hide? What are the problems?

Short writing task: comparing protein structures

Consider the amino acid compositions of these three proteins found in the skin. In what ways are these proteins similar to or different from each other. Write four or five phrases using *some of the comparison words* below to help you.

Amino Acid	**α-keratin**	**Collagen**	**elastin**
Gly	8.1	32.7	32.3
Ala	5.0	12.0	23.0
Ser	10.2	3.4	1.3
Glu/Gln	12.1	7.7	2.1
Cys	11.2	0	0
Pro	7.5	22.1	10.7
Tyr	4.2	0.4	1.7

- both
- same
- also
- lower
- like
- similar
- whereas
- higher
- each
- just as
- in the same way
- equal

Listening – part 2

The unhairing reaction solubilizes the keratin by chemical attack to facilitate the detachment of the hair from the skin and takes advantage of the reaction of sodium sulphide with the disulphide bridges of the keratin, however, it must be carried out under alkaline conditions. This is traditionally done with lime (calcium hydroxide) although more recently, enzymes have been studied as greener alternatives since the process avoids the use of potentially toxic reagents and the production of unpleasant and toxic waste.

The keratin in hair bulbs is weaker than that of the shaft and the collagen of the skin and this means that it is rapidly hydrolysed by enzymes. Since the action of enzymes on the skin is not specific, part of the collagen will also be hydrolysed with the risk of obtaining a finished product with poor mechanical properties. If the hides are well preserved, the fibre will weaken, but if the hides are badly preserved, the enzymes cause further degradation and local solubilization of the fibres.

There are three equilibria involved in the unhairing reaction:

$$Na_2S \rightleftharpoons 2Na^+ + S^{2-}$$

$$S^{2-} + H_2O \rightleftharpoons HS^- + OH^-$$

$$HS^- + H_2O \rightleftharpoons H_2S + OH^-$$

The positions of these equilibria depend on the pH of the solution which must be kept strongly basic to avoid the formation of hydrogen sulphide which is a highly toxic gas. Lime (calcium hydroxide) is used to buffer the reaction, keep the pH high and control the equilibrium between hydrosulfide and sulphide in favour of the latter. Hydrosulfide (HS-) does not attack the disulfide bonds.

The highest concentrations of sulfide anion (S^{2-}) are present at pH values above 12.

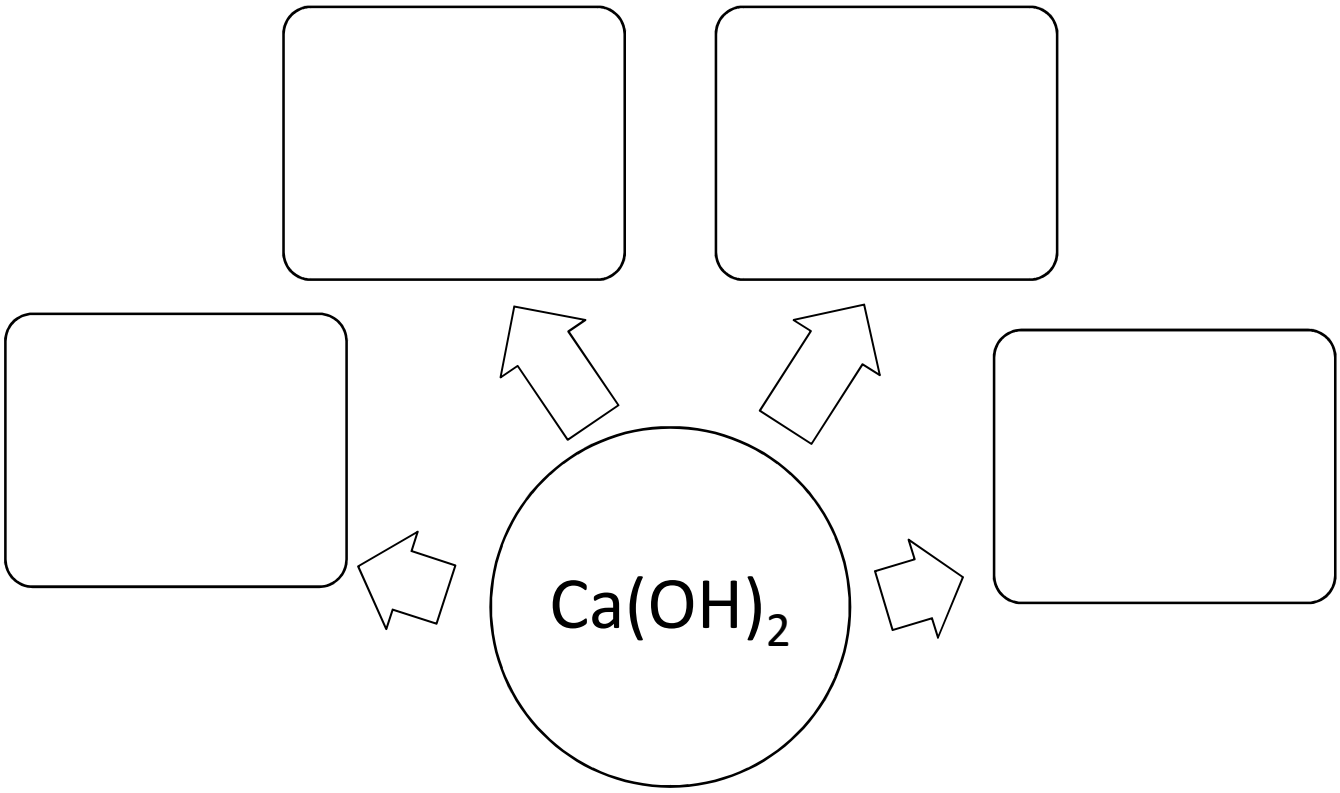
In this process, about 3-5% calcium hydroxide is added by weight of raw skin. This corresponds to an excess which serves as a reserve, as part of the hydroxide in the solution will be consumed by the saponification reaction of fats and part by the hydrolysis of proteins present as well as some which will react with carbon dioxide in the atmosphere. The hydroxide consumed is therefore continuously replenished by the solid lime present thanks to the equilibrium.

An excess of solid lime also acts as a cushion between the skin and the inner wall of the drum, so that during the rotation the hides are not damaged.

True or false

1. All of the lime dissolves during this reaction
2. The amount of lime added is exactly the amount needed to remove the keratin
3. The lime reacts with carbon dioxide in the atmosphere
4. The lime is used to suppress the formation of hydrogen sulfide which is a dangerous gas by keeping the pH low
5. All of the sulfur species in solution can be used to attack the keratin

What is the role of the Ca(OH)2 in the liming reaction?



Exercise: asking questions.

- Make three questions about any aspects of the unhairing and liming process.

What…

How…

Which…

Why…

The process of deliming

At the end of liming and unhairing, the skin 1) _____ a mixture of unreacted calcium hydroxide as particles 2) _____ to it as well as calcium ions in the skin itself both as free cations and cations 3) _____ to the carboxylic acid groups of the collagen.

The process of deliming does not simply 4) _____ the excess lime from the hide: it 5) _____ the pH of the skin to around 7.5-8.5 in preparation for bating, it removes the calcium hydroxide still present on the fibres of the skin after liming as well as the calcium ions present in the limed pelt and it partially 6) _____ the swelling observed during liming. During the reversal of swelling (depletion), water molecules 7) _____ from the internal part of the pelt to the surrounding solution.

In the skin from the liming process, the calcium is present in a bound form (as calcium collagenate) as well as in an unbound form as the 8) _____ Ca^{2+} ion. There is also a small amount of 9) _____ lime present on the skin.

For the tanning process to be successful, all of the calcium must be removed because it readily forms insoluble salts with many anions and is able to 10) _____ some dyes used in colouring as well as forming partially soluble greases by the saponification of lipids during liming. These can 11) _____ stains on the surface of the skin as well as causing rigidity of the hide by 12) _____ the internal sliding movement of the fibres.

As the pH is lowered from the strongly alkaline conditions used in unhairing to a more weakly alkaline value, the swelling of the hide is 13) _____ as the skin releases the water previously absorbed.

attached	hindering	move
complexed	hydrated	precipitate
contains	lowers	reduced
create	reverses	remove
	undissociated	

Questions. *Answer In your own words,*

1. In a limed pelt, where is the calcium?

2. Beyond simply removing the excess lime, what else happens in the deliming process?

3. Explain why it is important to completely remove calcium from the hide.

READING: Deliming agents

A large variety of strong and weak acids, acidic salts and even carbon dioxide and water can be used.

The simplest reagent to use is water. It reduces the pH by dilution of the hydroxyl anions present in the skin. If the water contains dissolved carbon dioxide (like normal water does), some of the hydroxide anion will react with the bicarbonate anion (HCO_3^-) present in the water in a neutralisation reaction.
The pelt acts like a semipermeable membrane but the exchange is slow and large amounts of water are required. There is a risk of over-swelling the grain.

Strong acids

Strong acids such as hydrochloric acid or sulfuric acid remove calcium and lower the pH of the hide. Hydrochloric acid reacts to form calcium chloride which is a *lyotropic agent*, causing swelling of the fibre (lyotropic agents = decrease the affinity of a macromolecule for water altering its structure).

Sulfuric acid, on the other hand, neutralizes lime to form calcium sulfate which is not very soluble and settles on the fibres making them heavier and impeding the sliding between fibres.

Strong acids are cheap since only small quantities are needed to cause a rapid lowering of the pH. However, they must be accurately weighed and diluted during addition to the drum because if the pH is lowered too rapidly and goes below 5.5 even only locally, the pH difference between the surface of the hide and the centre which will still be at pH 12 stops reagents penetrating and causes uneven swelling.

Weak acids

Formic, acetic or boric acids are weaker and are easier to use during additions and require less accuracy in weighing. They are more expensive because we need to use more of them and we cannot be completely certain that the skin is totally delimed.

Boric acid, the weakest acid, will remove the free calcium cations from the collagen but not those complexed to the collagen. To do this we need to use a stronger acid.

Recently, organic dicarboxylic acids such as maleic, succinic, glutaric and adipic acid have been used. These are usually used as mixtures.

Acid salts

Salts which dissociate in water to give acidic solutions can also be used for deliming. Among these, ammonium chloride (NH_4Cl) and ammonium sulfate ($(NH_4)_2SO_4$) are easy to handle, give good results and bring the pH of the skin to an optimal value for bating.

The formation of highly soluble ammonium sulfate instead of almost insoluble calcium sulfate is an advantage and the combination of price and quality of result make ammonium sulfate the best deliming agent.

Disadvantages of ammonium salts are the liberation of ammonia gas during the process, which is uncomfortable for operators and increased nitrogen present in the effluent which is an important environmental contaminant.

Use of deliming agents

Deliming agents are normally prepared by mixing different salts which are measured according to three values: the deliming index (I_d), the buffer index (I_b) and the lime solubility index (I_{sol}).

The deliming index (I_d) = grams of deliming agent needed to neutralize one gram of calcium hydroxide.

The buffer index (I_b) = volume of 1M NaOH needed to bring the pH of a solution containing I_d grams of deliming agent in 100ml of aqueous solution from 8.5 to 10.5.

In practice, it is necessary to draw a titration curve; the longer the curve the greater the margin of error we can have in the weighing of the product. We are looking for systems that are buffered to pH 7.5-8.5.

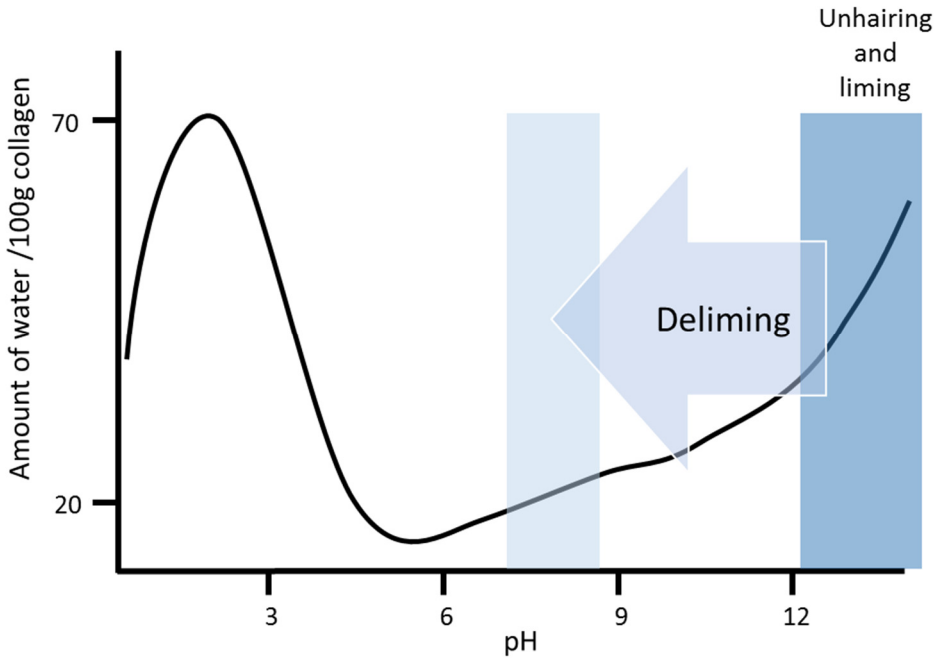
The ammonium ion present determines the buffer index.

The safest deliming operation is obtained with agents that have a buffer index greater than 20.

The solubility index (I_{sol}) depends on the ability of the deliming agent to form soluble salts, avoiding chalking and weighing down the skin. I_{sol} is expressed as a percentage of solubilized calcium as a function of the neutralized calcium.

The best deliming agent is one that has a low deliming index while the other two indices are high. Ammonium sulfate is the best because of its low cost, high buffer index and rapid action (less than an hour).

1. Writing exercisethe graph below which shows the effect of pH on the amount of water contained in the collagen (Adapted from Covington p155).



- Compare the amount of water absorbed at high, neutral and low pH.
- What happens to the collagen during the deliming step?

Some of these words may help you:

- *both*
- *same*
- *also*
- *lower*
- *like*
- *similar*
- *whereas*
- *higher*
- *each*
- *just as*
- *in the same way*
- *equal*

2. Write a balanced equation for the reaction between calcium hydroxide and hydrochloric acid and a balanced equation for the reaction with sulfuric acid.
   - Name the products and say what type of reactions they are.
   - How can you tell when the reaction is finished?

3. The K_a is a measure of the equilibrium. The further the equilibrium is to the right, the stronger the acid.

$$HA = H^+ + A^-$$

$$Ka = \frac{[H+][A-]}{[HA]}$$

   - The following are typical acids that are used in the deliming step. Put these acids in order, from the strongest to the weakest.

	Name	K_a
	succinic acid	6.4×10^{-5}
	maleic acid	1.4×10^{-3}
	oxalic acid	5.4×10^{-2}
	glutaric acid	4.5×10^{-5}
	formic acid	1.8×10^{-4}
	adipic acid	3.7×10^{-5}
	acetic acid	1.8×10^{-5}

4. Calcium ions bind well to the carboxylate groups on collagen. Give two examples of typical carboxylic acids found in collagen and find their K_a values. Would boric acid be able to remove this calcium? If not, why not?

5. What happens to the hide if the pH is changed too quickly during the deliming step? Why?

6. How does water work as a deliming agent?

7. In the deliming step, we can use a titration curve to help us determine the amount of reagent to add. What is a titration curve? Why should it be long?

8. Ammonium salts are widely used in deliming. Why are ammonium chloride and ammonium sulfate acidic when dissolved in water? Write an equation for what happens

9. In solutions of these two salts, what acts as an acid and what acts as a base?

10. List the advantages and disadvantages of different deliming agents.

The process of bating

Fill in the spaces with the best sentence.

Bating is a general term for the use of enzymes at an early stage in the tanning process. In particular, it is applied to the step after the completion of deliming in which the skin is 1)…

epidermis and hair as well as more resistant molecules that could interfere with the tanning process.

Enzyme are proteins which act as biochemical catalysts, increasing the rate of a specific chemical reaction. They are sensitive to the reaction conditions and typically work best in defined in ranges of pH and temperature. At higher temperatures (above 40^{o}C) 2) …

leading to a rapid loss of activity. Below 20^{o}C, the enzyme has little effect on the rate.

The pH profile of the enzyme depends on the environment in which it usually works and the chemical mechanism by which it acts. Trypsin and 3)

which has an alkaline pH. These enzymes work best in basic conditions and are used to solubilise the elastic fibres by hydrolysis. Sometimes enzymes from bacterial or fungal cultures are also used.

Enzymes used in bating are always as mixtures in diluted form because of their high activities. This allows for possible effects of error in weighing and eventual effects on consistency. The 4) …

needed in the fibres of the leather.

Bating itself is carried out directly in the deliming bath, if its pH and temperature are suitable for pepsin and trypsin.

There are no physico-chemical tests for the end of bating, only manual testing can be used for example seeing if the imprint left on the skin by a pressure exerted by a finger remains. Another is performed by forming a bag with the skin; if the bag is compressed, the air flows outwards through the skin, which is considered to be macerated.

The enzymes used may be endo or 5)

peptide (amide) bonds along the protein chain. Exo proteases work on the telopeptide zones of the collagen which are located at the ends of the molecule. The hydrolysis of the chains in this region leads to a relaxation of the collagen fibres, improving softness, elasticity and fineness of the grain layer.

The presence of hydroxyproline in the bating bath indicates hydrolysis of the main collagen chains while tyrosine indicates the hydrolysis of the telopeptide zone. The 6)

of bating which under normal conditions, must be limited and in any case lower than 1% even after four hours.

Bacterial proteases cause a greater loosening of the collagenous structure compared to pancreatic enzymes.

Bating agents are able to degrade the protein part of proteoglycans which are distributed over the skin as well 7)

causing the release of fats.

Collagen, is more easily dissolved by lyotropic agents after enzymatic treatment therefore, bating must be carefully controlled to avoid excessive solubilization of the collagen.

Skins bated with 8)

, but are less full and less thick. Moreover, since these enzymes also degrade elastin and collagen, the grain layer on the finished is rougher and tends to swell, especially on the sides. Hides bated with pancreatic agents have a firmer grain layer, are much fuller. The fullness is maintained even after a prolonged bating.

Insert the best phrase for the text.

a) bacterial proteases are distinguished by their high softness
b) pepsin which are typically used in bating are secreted into the small intestine
c) release of hydroxyproline is proportional to the activity and duration
d) exo proteases and they work by catalysing the hydrolysis of the
e) the enzyme will be denatured as the 3D structure of the protein is disrupted
f) as affecting the walls of the sebaceous glands and the cytoplasm of the fat cells
g) cleaned of unwanted components such as degradation of products of proteins
h) amount of enzyme and time required are directly proportional to the amount of relaxation

Questions

How do you know when the bating step is complete?

What happens to the collagen if the bating goes on for too long?

Why do bacterial enzymes give softer skins?

The process of pickling

READING

The pickling step chemically prepares the collagen for the reactions of the tanning step. During this step, acid is added to reduce the activity of the collagen. Pickling also opens up the pelt by the slow hydrolysis of the peptide bonds.

Pickling makes the collagen unreactive towards the tanning agent by using enough acid to bring the pH below the isoelectric point. Under these conditions the carboxylic groups are not charged and therefore there is no possibility of interaction with the cations in the tanning agent.

At pHs lower than 3, there is a significant osmotic swelling which can be avoided by increasing the osmotic pressure of the solution by adding common salt.

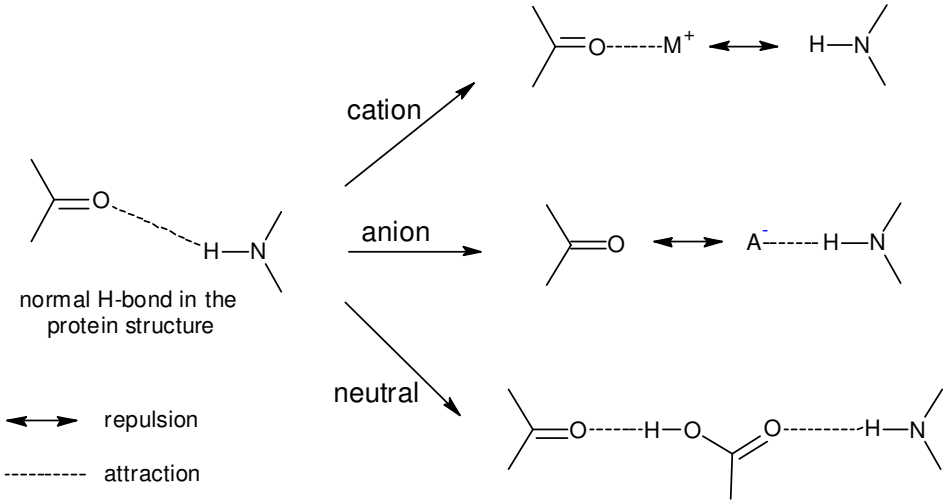
A typical pickling bath contains water (also called float), acid and salt. The most commonly used acid is sulfuric acid, but mixtures of acids such as formic and sulfuric acid are also used. Common salt (NaCl), which is cheap and unreactive towards tanning agents, is added to limit the amount of swelling caused by the acid. The density of the pickling solution is measured to monitor swelling.

Acidic swelling is most evident at low densities. Swelling gives spongy products with poor mechanical characteristics. If too much salt is used, the skins dehydrate and are excessively flat.

The sodium ions influence the equilibrium of how much water passes into or out of the collagen.

The collagen presents two surfaces to the solution and the pelt surface works like a semi-permeable membrane. This means that the diffusion of reagents (water, salt cations, protons and counter-anions…) into and out of the collagen is controlled by the relative chemical potentials of the species involved, as well as the potentials of those groups on the collagen which physically cannot move.

The lyotropic effect contributes to swelling and is caused by the species present in the pickling solution inserting themselves in the hydrogen bonding network of the collagen. This disrupts the network and causes the tertiary structure of the protein to relax. The effect depends on whether the species is neutral or carries a positive or negative charge.



Sulfuric acid should always be used by diluting the concentrated acid and allowing time for the acid to cool before addition to avoid gelatinization of the collagen. Any scalding from sulphuric acid is visible in the tanned product as dark spots and gives a very fragile grain layer.

Questions

1. What is the lyotropic effect and why is it important in pickling?

2. How does an anion exert its lyotropic effect?

3. What is a semi-permeable membrane?

4. How can swelling be monitored during pickling?

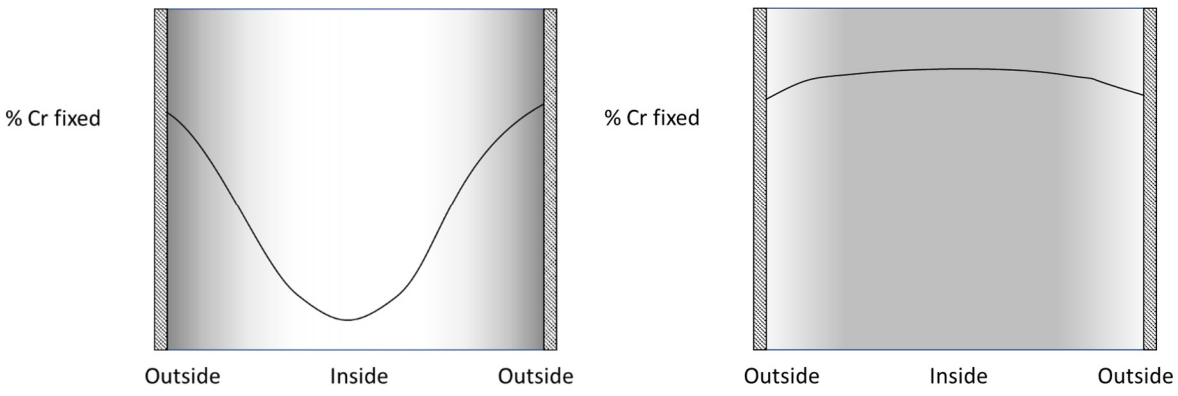
5. Why does the hide swell during pickling?

6. How can you control swelling at low pH values?

7. How does pickling make the hide unreactive towards tanning agents?

8. Why is it important to dilute the sulfuric acid and cool it before adding it to the pickling bath?

"Equilibrium and non-equilibrium" pickle



The result of tanning depends on the pickle: the distribution of the chromium in the layers of the pelt: left: equilibrium pickle; right: non-equilibrium pickle (Based on Berto p67)

Exercise: match the phrase to the equilibrium or non-equilibrium pickle

1. the pH is higher in the middle than at the surface
2. At the end of the process, the pH is uniform across the skin section.
3. The difference in pH between the central and peripheral sections can be monitored with the use of an indicator such as phenolphthalein
4. the central zone tends to fix Cr very quickly during tanning
5. When the pH of the innermost layers begins to increase during basification, additional Cr cannot pass into the centre of the pelt because it is obstructed by the Cr already fixed on the surface.
6. This pickle can last over 8 hours.
7. At the end of tanning, there is more Cr on the surface than in the middle of the pelt
8. the time of pickle is kept relatively short (between 2 and 4 hours)
9. is the most common method and to achieve an equilibrium more rapidly, the pelt is treated with a small amount of formic acid before the addition of the sulfuric acid which penetrates the pelt very easily.
10. there is more Cr on the inside after tanning.

EXERCISE: compare and contrast the two graphs

References

Glossary: from the International School of Tanning (SA)