Extraction of DNA from Leather and applications to the supply chain.

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Abstract.

Many articles that are sold as 'genuine' chamois leathers do not meet the requirements of this definition. Many people use chamois leather for its unique ability to absorb large amounts of moisture.

There is a British Standard that details what can be described as genuine chamois (along with specifications in other countries such as the USA). This limits the type of material that can be used for chamois production and also the type of tannage.

The authenticity testing of chamois leather is complicated. The most technically challenging element has been proving species of origin of chamois. This is because chamois leather does not have the grain layer present which would facilitate species diagnosis by microscopy.

A technique has been developed that allows the extraction and amplification of DNA from leather samples to provide unequivocal species identification. This is an exciting development for the leather industry, using state of the art genetic testing procedures and could offer scope with other authenticity issues including trading standards and protected species.

The definition of chamois leather also relates to the type of tannage (which must be of an oil type). Aldehyde tanning followed by marine oil fatliquoring does not meet the British Standard classification of chamois leather (In the USA the leathers must be solely oil tanned). It is known that the mechanism of oil tanning is through cross-linking of acrolein and peroxides liberated during the auto oxidation of the marine oil, with the lysine residues on the collagen. This cross-link is very stable to acid digestion.

Research has determined that it is possible to determine whether leather has been subjected to a full oil tannage through analysis of the concentration of lysine released after acid hydrolysis. Genuine chamois leather exhibits lower levels of free lysines. This analysis along with quantification of the level of formaldehyde provides a suite of tests that can be used to authenticate chamois.

Introduction

Chamois leather is an important commodity product internationally. The bulk of chamois leather is used as wash leather for cleaning windows, car bodies and similar surfaces where its particular speciality is softness, high water absorbance and 'wringability'. Chamois leather is produced using a specific tanning process which involves damp flesh splits being impregnated with cod oil. The skins are then heated to effect drying and oxidation of the oil.

Historically the skin of the chamois mountain goat was used for the material. It had a relatively poor grain and so was usually tanned with the grain removed in order to give a fine 'suede' surface. Today chamois is made from the flesh splits of sheepskins. The grain layer is removed by splitting, leaving a flesh split with a loose and soft, fibrous texture. This is significant in obtaining the properties associated with chamois leather. Few other skins are so suitable.

A particular requirement of a wash-leather for window or automotive cleaning is that the material should be sufficiently soft so as not to scratch the surface during cleaning. Any grit encountered should be enmeshed in the fibrous structure, thus minimising the scratching potential. In addition chamois leather should absorb large amounts of water (typically 600% based on dry weight). A key property of chamois leather is the ability to retain these characteristic properties after subsequent drying and rewetting.

Specifications

Whilst many people are aware of the unique properties of chamois leather, what is not perhaps known is that there is a British Standard that details what can be described as genuine chamois leather. There is also a current work item in the CEN committee for leather TC 289, which is looking to develop a Europe wide specification for this material.

The British Standard BS 6715:1991 describes chamois as:

A) Leather made from the flesh split of sheepskin or lambskin, or from sheepskin or lambskin from which the grain has been removed by frizzing, and tanned by processes involving the oxidation of marine oils in the skin, using either solely such oils (full oil tannage) or first an aldehyde and then such oils (combination chamois).
B) Leather made from the skin of the mountain antelope or chamois.

(Note in the USA the term ‘chamois’ without any qualification is restricted to the flesh split of sheep or lambskin tanned solely with oils – US Federal Standard CS99-1971)

Under this definition, leathers manufactured from any substrate other than sheep or lamb cannot be marketed as genuine chamois leather. Also any leather that is first tanned with a non-oil system and then subsequently fatliquored with marine oils can not be classed as chamois leather. The key problem faced by the industry is the difficulty in proving that cheaper inferior quality products are actually made using inappropriate substrates or are not oil tanned.

The problem

Authenticity testing of chamois leather is complicated. The most technically challenging element has been proving the species of origin of the leather. Typically analysis of leather under the microscope can provide an indication of the animal type. In the case of chamois leather this is not possible as the grain layer is not present.

A solution to this problem would be the development of a technique to identify the animal type and also the type of tannage.

Solution

Within this research the two aspects of the problem were tackled and these are detailed below.

Determination of Species

All animal species are distinguishable by characteristic DNA profiles, with no two species sharing exact copies of DNA. The ability to extract and identify DNA from leather would provide an opportunity to unequivocally identify its species of origin (sheep, goat or pig for example).

DNA (Deoxyribonucleic acid) is the fundamental genetic material of life. It is as unique as a human fingerprint and can be used to identify species and more importantly individuals within a given species. For example, DNA is different for every human, with the exception of identical twins.

Each DNA molecule is tightly folded into structures called chromosomes which can be viewed as a subset of the total DNA compliment of an individual. Chromosomes are further split into functional units, called genes, each of which guides the production of a protein, the building blocks of life. A set of human chromosomes contains one copy of each of the roughly 30,000 genes in the human "genome" – the term used to refer to the complete genetic instructions for an organism.

Even though each animal species shares effectively identical numbers of genes, the instructions contained within the genes are not always exact copies. Each person or animal is unique at the DNA level. However, people have different hair colour, facial structures, and other traits. These differences between individuals can result from very minor differences in their DNA sequences.

The DNA molecule is comprised of a long string of chemical building blocks known as "nucleotides." There are four different nucleotides, which are labelled as follows:

- Adenine (A)
- Thymine (T)
- Guanine (G)
- Cytosine (C)

These strands of A's, T's, G's and C's are paired with complimentary DNA strands (also comprised of A's, T's, G's and C's) to form a double helix.

Within the field of forensic science the ability the ability to unequivocally identify individuals through their DNA code has been exploited and substances such as bodily fluids or hair can be used for individual identification purposes. DNA profiling or fingerprinting was pioneered in the UK in 1985 by Professor Alec Jeffreys. The technique developed by Jeffreys targets fragments of DNA called mini-satellites which produced patterns on electrophoretic gels unique to the individual and consequently could be used for individual identification purposes.

Since this time DNA profiling has received other applications including testing for the presence of genetically modified organisms (GMO's) and food authenticity.

In working with forensic specimens where frequently only small amounts of DNA are available, a technique known as the polymerase chain reaction (PCR) is employed. Using this process, small amounts of DNA are copied through a specific reaction to produce millions of copies of the original target fragment of DNA. This can be achieved in a matter of hours through a thermo-cycling process. This PCR technique has revolutionised the identification of DNA which previously was not possible due to low concentrations and/or poor condition of the molecules extracted from biological samples.

Following this amplification process the extracted DNA is cleaned and screened using electrophoresis which separates the DNA within an agarose gel inside an electric field. The resulting profile (which can be visualised using an UV indicator) can be compared to standard DNA markers (along with appropriate controls) to determine absolute identity.

Experimentation

The application of DNA profiling techniques to the leather industry is novel. To evaluate the potential for DNA profiling of leather, samples of different leather types were prepared for genetic testing to determine the species of origin of the leather sample. This technique has not previously been applied to a leather substrate and the method required extensive development and modification.

DNA is a very robust molecule and can survive extreme environmental conditions. The extensive pH changes associated with leather processing (in particular pickling) does cause damage to DNA which needs to be considered within the analytical process. The samples typically require an extended incubation process to sufficiently solubilise the leather and recover the DNA to facilitate PCR.

A range of leather types were tested (including sheep, goat and pig) in addition to three chamois samples (one of which was known to be authentic). After extensive trials, the results obtained show that it is possible to profile leathers to prove their origin. Table 1 illustrates the results obtained in these trials. These results indicate that chamois leathers 1 and 2 are produced from goat rather than sheepskin whilst the genuine chamois was definitely not produced from goat. Subsequent experiments have proven a positive identification for this product. Of the four leathers tested only one was indicative of being a genuine chamois.

Table 1: Showing the DNA identification results for a range of leather samples

Sample Description	Goat DNA Detectable	Pig DNA detectable
Sheep leather	Negative	N / A
Goat leather	Positive	N / A
Hair sheep	Negative	N / A
Pig leather	Negative	Positive
Chamois 1	Positive	N / A
Chamois 2	Positive	N / A
Genuine Chamois	Negative	N / A

Determination of tannage

During the production of chamois leather, marine oil is used as a tanning agent with oxidation of the oil being essential for the tanning action to occur. Auto-oxidation is induced during processing in a stage known as 'heating off' of the oil. During this oxidation process, peroxides are generated and further smaller quantities of acrolein, aliphatic keytones and other volatile compounds are formed. These thermal decomposition products combine with the skin, imparting the yellow colour characteristic of chamois leather. The ceasing of heat generation indicates the completion of tanning. The oil tanning mechanism unique to chamois leather is thought to involve the lysine groups of the collagen reacting with peroxides and acrolein liberated during the oil tannage process.

In true chamois leather the cross-link formed between the lysine and acrolein should be very stable to acid digestion. Therefore any leathers that have been fatliquored in an attempt to make them appear chamois-like should have larger quantities of free lysine present following acid hydrolysis.

Initial trials were carried out to determine whether any variation in the lysine content of leather samples can be determined throughout chamois processing.

Leather was analysed at varying points in a typical chamois process. Analysis was by gas chromatography to determine the level of lysine solublised following acid hydrolysis. The results are illustrated in Figure 1.

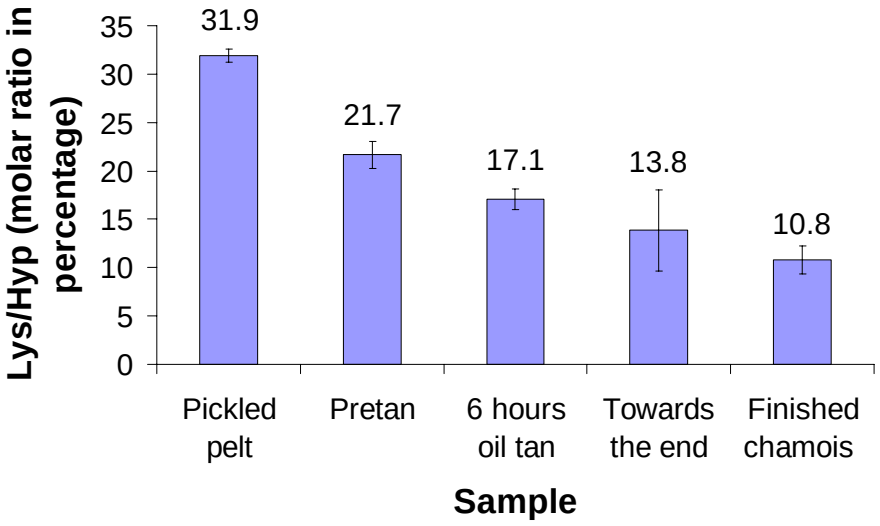

Figure 1 Quantification of lysine in acid hydrolysate using gas chromatography

The results illustrated in Figure 1 show clearly that the amount of released lysine decreases during chamois tanning. This is indicative of the formation of acid stable bonds.

Subsequent samples were analysed to determine if the suitability of the technique for the identification of a true oil tannage. The results are shown in Figure 2.

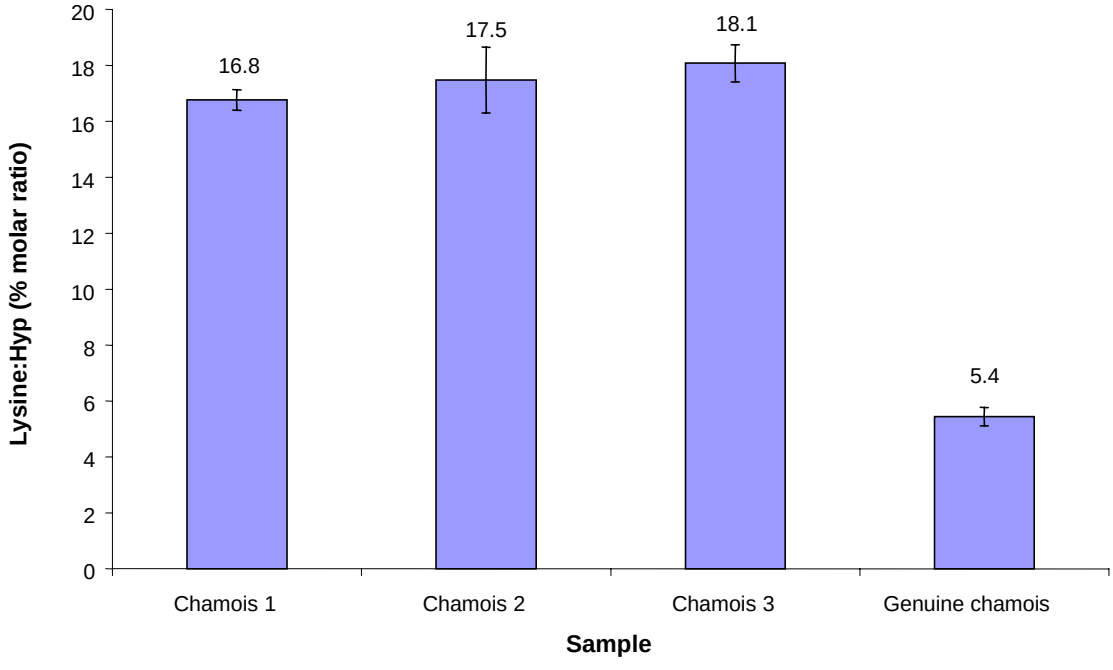

Figure 2 Variation observed in the free lysine detected in the samples analysed

Figure 2 provides evidence that the chamois leather known to be genuine has undergone a different tanning mechanism to the leathers manufactured by competitors. Whereas the lysine content in chamois samples 1, 2 and 3 is around 17% this decreases to around 5% for the genuine sample. This indicates that the leather has been oil tanned.

Leathers that have undergone an aldehyde tannage followed by fatliquoring would be expected to exhibit higher levels of free formaldehyde than true chamois leather. The formaldehyde content of chamois samples 1, 2 and 3 was found to be much higher than seen in the genuine chamois leather which suggests that the competitors' product contains formaldehyde in the finished product. The levels of formaldehyde detected in the genuine sample are very low and can be considered as background levels suggesting that formaldehyde is unlikely to have been used in the tanning process. Consequently, this analytical technique also allows for differentiation between genuine chamois leather and leathers which are produced by aldehyde tanning and subsequent fatliquoring.

Conclusions

This is an exciting development and a scientific first for the leather industry, using state of the art genetic testing procedures. This study has demonstrated how advanced techniques such as these can be applied to the leather industry to help resolve trade issues such as chamois authentication.

The potential for a robust and reliable system of species identification within the leather industry is significant and has the potential to act as a significant deterrent to malpractice. Certain trade issues such as those described with chamois leather could take advantage of this highly specific test. In the future definitive species testing for the control of protected species may also be a possibility and the identification of sub-species of animals, for example Zebu. Another potential application is the identification of historical leathers and ultimately traceability of leather through the supply chain using DNA technology.

References

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