EISSN: 2707-0425

East African Journal of Science, Technology and Innovation, Vol. 4 (Special Issue 2): September 2023

Assessment of the change in the conformation and content of collagen of the goat skin due to different unhairing process

[1]CHONGERI A M*

[1]Dar es salaam Institute of Technology (DIT), Mwanza Campus.

***Corresponding Author**: azariamikas@gmail.com

Abstract

Recently, more attention has paid to the reduction of environmental pollution due to leather processing, the beamhouse step especially unhairing part has been named as the one which contribute high pollutants in the total industrial waste. Therefore, different types of unhairing have been studied intending to reduce waste produced by unhairing step, however, no more findings on the influence of those alternatives to the collagen of the skin which determine the features of the leather. This study explores the impacts of those unhairing alternatives to the collagen of the skin. The shrinkage temperature values were lower as compared to that of raw skin sample, where oxidative unhaired skin, shows high difference (50.5^0C) from raw skin sample which was 64.9^0C. There were no significant differences in the hydroxyproline concentration between unhairing, this was due to triple helix structure of the collagen which make it more stable and not easily destroyed by unhairing chemicals. FT-IR results shows the differences in intensity (percentage) for side chain of collagen, where raw sample was 99.67%, oxidative was 90.83% while for conventional, hair saves and painting were 97.38%, 97.93% and 96.02% respectively. The stretching of N-H bond and bending for C-N bond for amide II, displays vibrations with wavenumber of 1519.20cm^{-1} for oxidative unhaired wet blue, as compared to other unhaired wet blue and raw skin sample which reads at 1539.20cm^{-1}. The small differences in strength of the leather produced were associated with the change in the side chain intensity and conformation of collagen. The strength properties of the leather do not depend only on the concentration of the collagen but also on the proper packing of the collagen structures. The disturbance occurs to the hierarchical network of the collagen was reflected in the physical properties of the leather.

Keywords: *Collagen, Unhairing, hydroxyproline, Triple helix structure*

Cite as: *Chongeri, (2023)* Assessment of the change in the conformation and content of collagen of the goat skin due to different unhairing process. *East African Journal of Science, Technology and Innovation 4(special issue 2).*

Received: 27/06/23
Accepted: 06/07/23
Published: 14/09/23

Introduction

Unhairing is the removal of hair from the skin or hide during leather processing. It takes place in alkaline condition because it induce swelling which facilitate an effective unhairing.

The removing of hair is the primary purpose of unhairing; however, it also involves the removal of non-structural proteins such as fats, blood vessels, proteoglycans, sweat glands, sebaceous glands, and epidermis to open up the fibre

structure. These help to open-up of the fibre structure and allow better penetration of different materials such as tanning agents, fatliquors, retanning agents, and dyes for proper leather processing.

The hairs, non-structural proteins and collagen are all proteins in nature, then the chemical reactions which occur to non-structural and hair proteins during their removal can occur simultaneously to collagen because they basically have the same chemical structure (bond) even if collagen are stronger due to its triple helix structure (Covington, 2009). Therefore, the study aims to investigate how collagen can maintain their structure and strength in spite of different chemicals that are applied during unhairing process.

Collagen is the main structural protein found in the skin and other musculoskeletal tissues. It makes the skin cells strong and resilient thus responsible for tensile strength of the skin. The primary structure of collagen is composed of repeating triplets of (Glycine-X-Y)n, where X and Y are often proline and hydroxyproline. The repeating sequence is responsible for the helical structure and the inherent and predictable mechanical strength of collagen. The glycine content accounts for the flexibility of the collagen chain and increased glycine gives rise to more flexibility. Collagens contain three chains which twisted about each other in a clockwise triple helix, this is only possible because of the high glycine content, situated in the centre of the triple helix. At each end of triple helix there are region called telopeptide regions, the importance of this region in leather processing lies in their role in bonding, to hold the collagen macromolecule together. (Covington, 2009). The triple helix also held together by electrostatic bonds formed by electrostatic reaction between acidic and basic sidechains of the protein, which together determine the isoelectric point of collagen. Therefore, if unhairing process is not well controlled, can cause the degradation of the collagens and affect the final leather because the tanning involves the conversion of collagen from a putrescible organic matter into a non-putrescible and stable materials and lead to leather, thus why, any procedure example unhairing which is done before the tanning step should not destroy/degrade collagen in anyway (Naffa *et al.,* 2019).

The hair burn, hair saves, painting and oxidative unhairing has been used to compare the impact which are caused by those approach to the conformation and content of collagen of the skin.

This part gives out different concepts regarding the findings on unhairing process. The more attention was on the types of unhairing to be studied in this research work, it shows views from different literatures, on how the process can be done, and at the end to bring the required results.

This is also known as hair burn unhairing because it involves the hair burning. It is unhairing process in which hairs burns/dissolves in the mixture solution of lime and sulfide compounds, the burning process starts at the tip of the hair down to the entire hair (Covington, 2009).
The unhairing chemicals normally attack only soft keratin of hairs, and this will cause the breaking down (peels off) of scales attached to it. The medulla cortex of hair is not attacked by unhairing chemicals, but it is broken down due to hydrolytic effect, which is caused by high pH condition. The high pH is due to lime added as one of unhairing chemicals, these results into the decomposition of entire hair (Buljan and Ivan, 2019).

The lime also creates the buffer system, which favor the formation of more sulphide ions (S^{2-}) than hydrogen sulfide ions (HS^{-}), because is the only sulphide ions which can attack bisulphide bond and not hydrosulphide (HS^{-}) (Covington, 2009).

The mechanism is accomplished by sulfide in the presence of lime which create conducive condition (pH) for the process.
The sulphide added will ionize in water to form nucleophilic sulphide ion, S^{2-}. This has reducing power to attack the sulfide-to-sulfide bond of keratin (cystine), $Na_2S \rightarrow Na^{+}+S^{2-}$

When bisulphide is attacked by sulfide ions and broken, it creates a cystine bisulphide ion and

cysteine ion, R-S-S-R + S^{2-} ⟶ R-S-S^{-} +RS^{-} (Covington, 2009), where R is any alkyl group.

Cystine bisulfide (R-S-S^{-}) is then attacked by sulphide ion (S^{2-}), to form cysteine ion by reduction process, while sulphide ion is changed to multi-sulphide (S_2^{2-}),
R-S-S^{-} + S^{2-} ⟶ RS^{-} +S_2^{2-} .This mark the burning (destructions) of the hairs (Buljan and Ivan, 2012).

The burning start taking place at the soft part of hair, where the scales fragment off from the damaged cortex. This takes place in high alkaline condition as it help to weaken medulla due to hydrolytic impact. The lime is added to maintain pH above 12 which favor production of sulphide ions (S^{2-}), this attack disulfide bond of keratin of hair. The pH below 12 and above 6 favor formation of hydrosulphide ions (HS^{-}), this does not make unhairing (Wise *et al,* 2019), as it can't attack the disulphide bond of keratin.

If the pH drops more to less than 6, can facilitate the formation of hydrogen sulfide (H_2S). This is very poisonous gas to the human being (Covington, 2009). Thus why, the pH must be maintained at 12 to avoid formation of H_2S.
This mechanism has currently challenged by Wise *et al*, 2019, with argument that, unhairing mechanism can't be in that way, because it has been proved with concrete evidence by current research that, sulfide ions (S^{2-}) which attack the disulphide bond of keratin (cystine) in hair, as per previous mechanism, cannot exist in an aqueous solution, therefore, mechanism might proceed in other way around, and he propose different hypothetical thought of how mechanism can be, however, the postulates are not yet proved. This can confirm that, the mechanism of hair burns unhairing still need more research, to find what exactly happen in disulphide bond breaking of keratin (cystine).

This type of unhairing is practiced by most of the tanneries, this is due to its advantage over the other types of unhairing. This includes the following

It is a very short time-consuming type of unhairing. This is because unhairing is done simultaneously with liming process, as the lime creates and maintain pH for unhairing sulfide to work. It also induces swelling, hence liming of the skins (Buljan and Ivan, 2019).

Simple and inexpensive drums can be used to accomplish the process. In contrast to other type of unhairing, for example, oxidative unhairing needs drums made up of steel iron or polyethylene (polyethene) only, this is due to corrosion impact caused by hydrogen peroxide used in the process, also hair saves unhairing needs complicated technological drums to facilitate filtering of the hair during the process, but hair burn uses a very simple drum in terms of technology and their materials (Buljan and Ivan, 2019).

With advantages of this type of unhairing, but it has some of disadvantages, which made different leather scientists and technologists to think out, on how to have other alternative process to overcome the negative impact of hair burn unhairing. These includes the following;
It is a highly polluting type of unhairing. This produces high nitrogen due to hair decomposition, hence eutrophication impact. This may cause the death of aquatic organisms such as fish and disturb an ecosystem. (Buljan and Ivan, 2019)

It may leave some of the hairs which reduce the quality of the leather produced. This is due to hair staples which remains in the follicles, and at the end affect the dyeing of the leather (Covington, 2009).

It is very expensive. This is due to the high cost which leather industry incur in the wastewater treatment, because the use of sulfide and lime chemicals lead to high Chemical oxygen demand (COD), thus why high cost in the tannery effluent treatment (Bronco *et al.,* 2005)

The process does not involve the dissolving of hairs, as in conventional unhairing, however, it uses the same chemicals as in conventional unhairing. The chemicals are applied in the way that; the whole hair is removed without destruction. This help to reduce organic pollution, because hairs will not be the part of effluent, as in conventional unhairing (Buljan and Ivan, 2019).
The hairs will be filtered from an effluent, and can be used in various uses, such as making

blanket and others depending on the nature of the hairs.

The process starts by applying/adding lime which react with bisulphide bond of hairs. It takes place in alkalinity condition of pH 11 or above to form Lathionine, which is made up of thioether bond (Covington, 2009).

This bond is resistant to sulphide attack therefore, it will cause hair to be resistant to sulphide, while the hair root in its normal state is easy to be attacked by sulphide compound,
R-S-S-R' (Keratin) + $Ca(OH)_2$ ⇌ R-S-R' (Lathionine)

After thioether bond formation, then sulphide is added to attack the hair root, and make it easy to remove the whole hair from the hide/skin. The sulfide applied at this point will ionize to form the sulfide ions (S^{2-}), which attack the bisulphide bond of hair root as in conventional unhairing (Buljan and Ivan, 2019).

When hair root is attacked by sulfide, it loosens the hairs and make it easy to remove just by rubbing, therefore, the mechanical action will be enough to peels off the hairs from the skin/hide.

This unhairing process has the following importance over the conventional unhairing.
There is high reduction of chemical oxygen demand (COD). Because there is no decomposition of hairs, therefore, it is termed as the cleaner unhairing process (Buljan and Ivan, 2019).
It produces the clear grain surface. This improves the quality of the leather produced, and simplify other proceeding process such as dyeing, due to clear grain pelt surface.
Hairs can be used for other purposes. For example, wools may be used to make clothes, carpet, upholstery, and blankets (Buljan and Ivan, 2019).

It requires a very complicated and sensitive technology. For example, drums used need special technological modifications to allow hair filtering, before its decomposition in the mixture of lime and sulfide (Buljan and Ivan, 2019).
The act of unhairing is separated from liming process. This makes the process to use more time (time-consuming), when compared to conventional unhairing (Covington, 2009)

Painting is an old-style hair-save unhairing for young animal or animal with thin skins; it is used for thin skin that can allow better penetration of unhairing chemicals (paste), from the fresh side to hairs (root) follicles. The paste is applied on the flesh side of the skin, and penetrate to hair root, where keratin reduction will take place and make easily remove of the hair (Buljan and Ivan, 2019).
It is necessary when there is a need of using those hairs for making winter clothes, blankets, carpets, and others (Yahia *et al.*, 2019).

The paste is made by the mixture of lime, sulfide, and optimum amount of water. Then after skin soaking and fleshing is painted at the flesh side using hand or machine. Thereafter, the skins are piled flesh-to-flesh side and the chemicals penetrates to hair roots.
The sulfide will cause the reduction of keratin of the hair root in the presence of lime, this reduction cause destruction of the hair root, and results into loosing of the hair and easily removed by scudding off or simply by pulling off (Buljan and Ivan, 2019)

The reserved hairs can be used in other ways, such as making carpets, insulations, winter clothes and other more.
The small quantity of water is used in the process, therefore, there is gradual swelling of collagen which decrease the possibility of collagen destructions (Naffa *et al.*, 2019)

The amount of unhairing chemicals used are reduced as the only one side of the skin is painted, therefore, low cost with small BOD/COD output (Buljan and Ivan, 2019).

The process is restricted to only skin with small (thin) cross section, at which chemicals can penetrate through. It is suitable for skins from young animals, such as calf, goat, and sheep. It cannot be used for thicker hides, as chemicals cannot penetrate through the cross section completely.
The process needs large floor surface to keep the piled skins, and it becomes a problem when large production is required.

It separates unhairing and liming process; therefore, it is the time-consuming process (Yahia *et al.,* 2019).

This unhairing use oxidizing agent to reduce the bisulphide link of keratin of hair. One of the common oxidizing agents which are currently used is hydrogen peroxide. It acts in the presence strong alkaline conditions, such as sodium hydroxide (Yahia *et al.,* 2010).

The process starts by adding sodium hydroxide to create alkaline condition. The pH about 12.5-13 is suitable for hydrogen peroxide to work (Marsal *et al.,* 2011).

Then hydrogen peroxide (H_2O_2) is added to the system. This decomposes to form peroxy anion (HOO^-) in alkaline medium, $H_2O_2 \leftrightharpoons HOO^- + H^+$ (take place in alkaline medium) (Bronco *et al.,* 2005)

The formed peroxy anion attack bisulfide bond (-S-S-) of keratin and cause the destructions of hair root. The mechanism on how peroxy anion attack the disulfide bond of keratin is not well known, therefore, there is a need of further research on this important part of the oxidative unhairing mechanism (Zengin *et al.,* 2010).

It is necessary to use sodium hydroxide to create alkaline condition, because hydrogen peroxide work best at high pH (12.5-13). This can be created by only strong alkali such as sodium hydroxide. It does not cause immunization of hair as calcium hydroxide ($Ca(OH)_2$). This would prevent breaking of bisulfide bond by hydrogen peroxide (Zengin *et al.,* 2010)

It produces less pollutants as compared to conventional unhairing. Both takes place in alkaline medium, but oxidative uses H_2O_2 which decomposes to water during unhairing (Yahia *et al.,* 2010).
It brings the solution for sulfides and lime. One of the challenges of the leather industry is the use of the sulfides and lime, which contribute in the large extent to the environmental pollution, hence high COD/BOD. The process does not need the use of those chemicals, hence low cost in the wastewater treatment (Buljan and Ivan, 2019). Other unhairing types use calcium hydroxide ($Ca(OH)_2$), because apart from creating an alkaline medium for the reaction to take place, also Ca^{2+} has ability to cross-link the site of carboxylic side of collagen, this led to excellent packed collagen structure. Unlike to sodium hydroxide (NaOH) which causes the excess swelling due to greater osmotic impact of Na^+ as compared to Ca^{2+}, thus why the use of sodium hydroxide (NaOH) can even affect the physical strength of the leather (Bronco *et al.*, 2005)

It produces more nitrogen which can cause eutrophication. The situation whereby, nitrogen favors the development of more plants and algae. These use more oxygen for aerobic respiration, deplete the oxygen content in the water for aquatic animals, and can cause the death of aquatic animals such as fish (Daniels and Landmann, 2013).

Materials and methods

Raw materials and chemicals used
Three (3) wet salted goat skins obtained from Latco Ltd (UK) were used. They were cut into four small pieces to ensuring that the official sampling position was present in each piece under BS EN ISO 2418:2017 (SLP2/IUP2) method (SLTC, 1996). This was done to ensure that for each unhairing the test sample will be obtained from the official sampling position for the same skin in order to maintain similarity of the sample source for each unhairing alternative for easily and proper comparisons. Goat skins were used in the study because they are suitable for all types of unhairing to be explored in this study. For example, the cow hide has large cross section area, therefore cannot be suitable for painting unhairing as it works better for hide/skin with small cross section area to allow better penetration of the paint from the flesh side to hair roots.

Unhairing chemicals
The chemicals used were Hydrogen peroxide (H_2O_2) sourced from VWR International, Sodium Hydroxide (NaOH) from Fisher Scientific chemicals, Calcium Hydroxide ($Ca(OH)_2$), Sodium Sulfide (NaS) and Sodium Hydrogen Sulfide (NaHS) of the commercial grade were used in the process because the process does not necessarily need the use of analytical grade which are very expensive.

Reagents used for chemical tests.

The chemicals used for the collagen content determination in leather (hydroxyproline) in leather were of analytical grade as it involves the quantitave analysis thus why necessary to use this type of chemical grade in order to obtain the required and correct results as the chemical of this grade are free from impurities which might interfere the obtained results.

Equipment and apparatus used in the study.

Different equipment and apparatus used in the study for leather processing, testing and other related activities were from Teaching and Research Tannery, Chemical testing Laboratory, Analytical Laboratory and SATRA Physical Testing Laboratory from ICLT at University of Northampton United Kingdom.

Experimental design

A complete block flow design of an experimental work was created. This provide the fully details in a simple way showing what to be done from the initial point (raw materials), chemicals requirement at each point and other accessories which were necessary to accomplish the experimental work.

This design was important as it create a sense of preparation whereby all necessary requirements were easily identified and collected before the commencement of an experimental work. The work starts with several trials following the same design which also create opportunity of making design correction before the experimental work. The block flow is presented in the Figure 1.

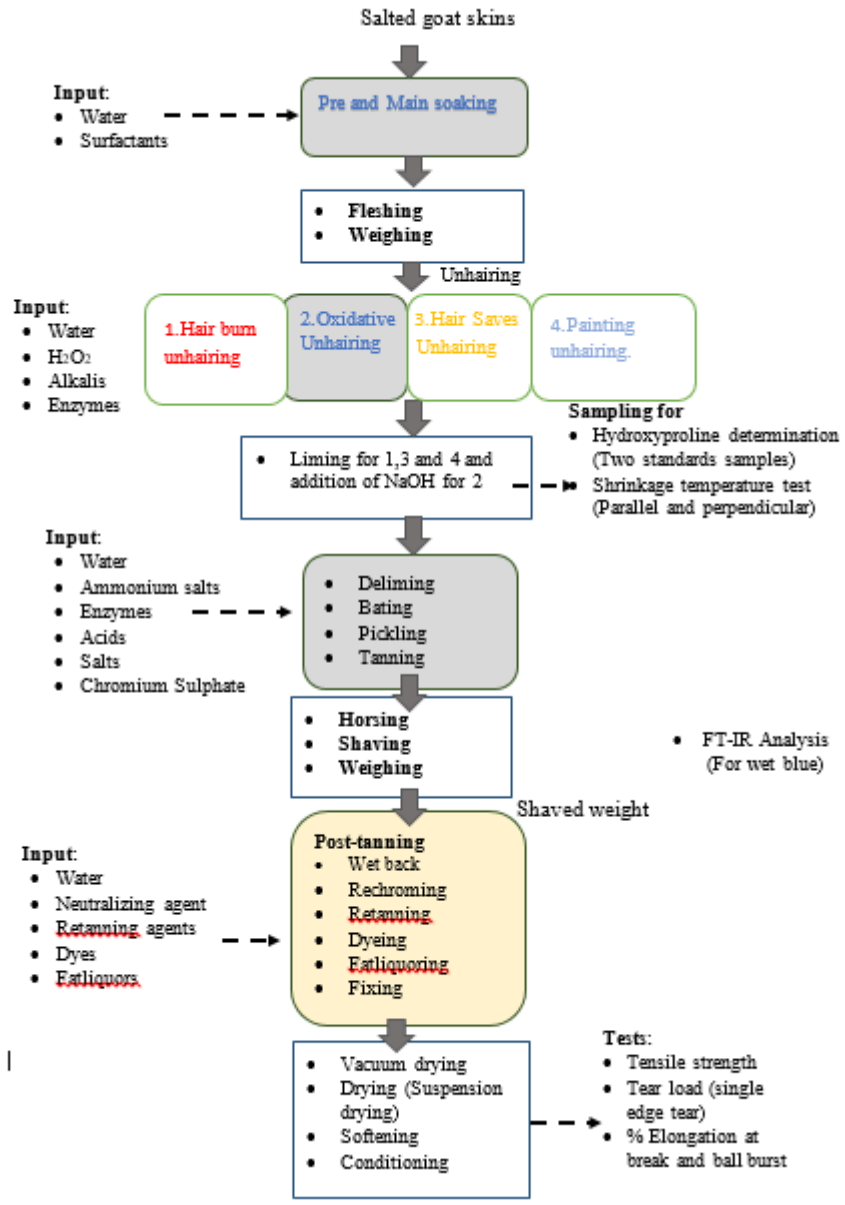

Figure 1. Flow Chart showing the Framework (workflow) of the study

Soaking

Three goat skins were soaked as per standard procedures (Covington, 2009). Thereafter, were fleshed and divided into four sides as per Figure 2. They were divided in the way that each side was having an official sampling position (OSP), each approach of unhairing takes one piece from each skin and make a total of three pieces of skin per each unhairing approach. The trial work expresses an expected result; therefore, the same approach and recipe were done for experimental work.

Figure 2. Cutting the skin into four pieces and distributed in each unhairing approach (One piece to each unhairing approach, the process was done for three goat skins)

The Unhairing Process

Conventional unhairing

Conventional unhairing and liming were done and controlled following the required procedures and the process control (Covington, 2009). The trial results meet expectations therefore, the same recipe was used for experimental work. At the end of the process, shrinkage temperature was measured as per SLP 18 method (SLTC, 1996). The skin samples for hydroxyproline determination were collected and placed in the oven for drying at 40^0C for 24hours, then transferred to desiccator for cooling and storage ready for analysis.

Hair saves unhairing

Hair saves unhairing and liming were done and controlled as conventional unhairing chemicals with different modality in order to save hair (Covington, 2009). At the end of the process, shrinkage temperature was measured as per SLP 18 method. The skin samples for hydroxyproline determination were collected and placed in the oven for drying at 40^0C for 24hours, then transferred to desiccator for cooling and storage ready for analysis (SLTC, 1996).

Painting unhairing

Painting unhairing and liming were done and controlled as per standard procedures (Buljan and Ivan, 2019). At the end of the process, shrinkage temperature was measured as per SLP 18 method. The samples for hydroxyproline determination were collected and placed in the oven for drying at 40^0C for 24hours, then transferred to desiccator for cooling and storage ready for analysis (SLTC, 1996).

Oxidative unhairing

Trials were conducted using 4.5%, 6%, 7.5%, 9% and 12% of hydrogen peroxide (H_2O_2) simultaneously where in all 6% of NaOH were added slowly to create a constant alkaline condition. An assessment shows that 7.5% of hydrogen peroxide pelt was clean (all hairs were removed) with no any destruction on the grain surface as compared to other pelt produced. Therefore, 7.5% H_2O_2 with 6% NaOH were used during an experimental work. The process was conducted in the stainless-steel tanning drum (Bronco *et al.,* 2005). At the end of the process shrinkage temperature were measured as per SLP 18. The samples for hydroxyproline determination were collected and placed in the oven for drying at 40^0C for 24hours, then

transferred to desiccator for cooling and storage ready for analysis (SLTC, 1996).

Pickling and Tanning

Pickling was done to lower the pH to 2.8 which was suitable for tanning agent (chrome salt) to penetrate the pelt during the tanning (Covington, 2009). The cross-section check, pH and shrinkage temperature test were done to confirm the successful tanning. After fixation, the wet blue sample for FT-IR spectrometer analysis, from each unhairing approach were collected, and placed in the oven for drying at 40^0C for 24hours, then transferred to desiccator for cooling and storage ready for analysis (SLTC, 1996). The wet blue leathers were horsed for three days before post tanning.

Shrinkage temperature of skins (SLP 18)

The shrinkage temperature was determined using IULTCS (International Union of Leather Technologists and Chemists Societies), standard method of testing the shrinkage temperature up to 100^0C (SLP 18), for raw skin sample and after the liming and tanning stage. The sample was extracted from the official sampling position, it cut in rectangular form with the measurement of 50mm ± 2mm × 3.0mm ± 0.2mm, four test sample were done in each type of unhairing, where by two samples were in perpendicular to backbone and other two were parallel to backbone. The samples were tested using standard shrinkage temperature tester. In each unhairing the average shrinkage temperature recorded, was presented as the result for the shrinkage temperature obtained for specific unhairing type (SLTC, 1996).

FT-IR spectrometer analysis

The skin samples after liming process, were well dried and for raw sample was well shaved to remove all hairs and dried as well, then the sample were subjected to FT-IR spectrometer, where grain side face the detector part of the plate. The infra-red radiations were allowed to pass through a sample, the generated information was well recorded for analysis (Naschekina *et al.,* 2021). IRTracer-100 FT-IR Spectrometer supplied by Shimadzu company was used for analysis.

Hydroxyproline determination (SLTC 21 (IUC17))

About 0.4g of raw and delimed dried sample was put into the digestion tube followed by 10ml of 50% hydrochloric acid and closed immediately. The digestion tube was placed in the test tube rack then in the oven for 16 hours at 100^0C for digestion process. The sample solution was cooled and transferred to 100ml volumetric flask, then water was further added to the mark of the volumetric flask. Thereafter, 2ml of the solution was diluted to 100ml with distilled water which was used as a sample solution to determine the concentration of hydroxyproline.

The standard solutions were prepared from 100mg/L stock solution of hydroxyproline by transferring different aliquots into 100ml volumetric flask and then diluted by filling the flask with distilled water to the mark as shown in Table 1.

Table 1.

Preparation of Standards; Aliquot showing the volume from the stock solution (100mg/L) which diluted to 100ml to make the respective standard concentration

Standard concentration	Aliquot from the stock solution
0mg/L	0ml
2.5mg/L	2.5ml
5.0mg/L	5.0ml
10mg/L	10ml
15mg/L	15ml
20mg/L	20ml

The sample and the standard solution were prepared for reading in the spectrophotometer, taking 0.55ml of standards and samples into different test tubes in the metal test tube rack, and

then in each test tube 1.27ml of distilled water were added, followed by 0.88ml of Chloramine T reagent and shaken well to mix thoroughly and left at room temperature for 5 minutes. In each test tubes 2.30ml of Ehrlichs reagent were added, shaken well to mix, and then incubated in the water bath at 70^{0}C for 10 minutes. It was left for cooling to room temperature, then shaken well to mix and transferred to cuvette at the require mark, then placed in the Genesys 10S UV-VIS spectrophotometer at 555nm for absorbance reading, starting with standard solutions then the sample solutions (SLTC, 1996).

Physical strength test of the crust

This was done to assess the suitability of the leather (crust) for shoe upper in regarding to the different unhairing. The sample was cut from the crust in the official sampling position (OSP) as per IUC 2/IUP 2 sampling method by IULTCS using standard shape and size of the test sample per each test. Then the sample was conditioned for 24 hours at standard atmospheric temperature of 23.0 ± 2.0^{0}C and 50.0± 5.0% relative humidity as specified in IUP 3 (SLP3) conditioning method of IULTCS (SLTC, 1996).

The number of the test samples and the way samples were cut (test sample shapes) were specified in each physical parameter tested (SLTC, 1996).

Thereafter, the tensile strength, percentage elongation at break, single edge tear strength and distension at the grain crack (ball and burst test) were tested as per standard procedures shown below.

Tensile strength and percentage elongation (IUP 6 (SLP 6))

The three parallel and three perpendiculars to the backbone sample for each unhairing type were cut using a dumbbell shape as per BS EN ISO 2418 and the test were done as per IUP 6 (SLP 6) method by IULTCS (SLTC, 1996).

Single edge tear strength (IUP 40 (SLP 40))

The three parallel and three perpendiculars to the backbone sample for each unhairing type were cut in accord with BS EN ISO 2418 and tested as per IUP 40 (SLP 40) method by IULTCS (SLTC, 1996).

Distension and strength of grain by the ball burst Test (SLP9 (IUP9))

Six test samples were cut from official sampling position as per BS EN ISO 2418 and tested as per BS EN ISO 3379:2015 and SLP 9 (IUP 9) testing method for each type of unhaired crust sample (SLTC, 1996).

Results

Shrinkage Temperature

The oxidative unhairing shows low average shrinkage temperature (50.50^{0}C) as compared to raw skin sample which was 64.9^{0}C, the readings reported the shrinkage temperature of the raw skin sample as 65^{0}C (Covington, 2019). Other unhairing types express almost the same average shrinkage temperature which were 55.5^{0}C, 54^{0}C and 52.5^{0}C for conventional, hair saves and painting unhairing respectively as per Figure 3. All unhairing types shows the low shrinkage temperature of limed skin (pelt) as compared to raw skin sample.

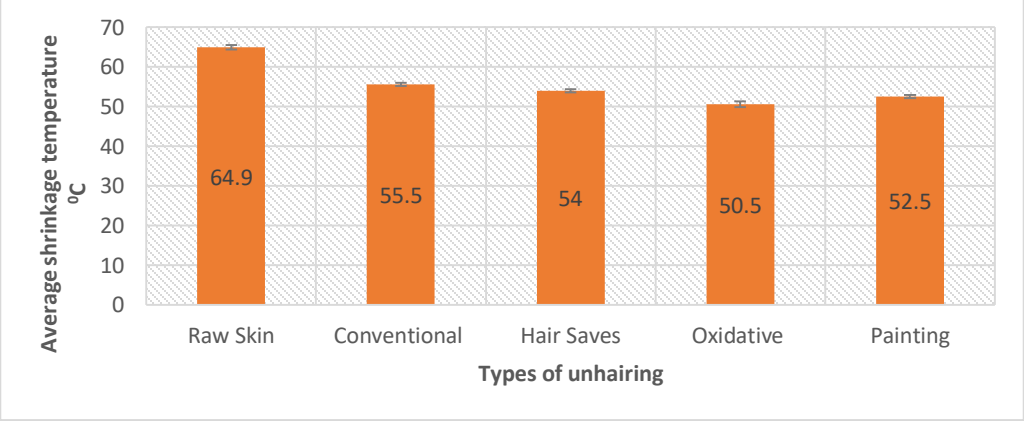

Figure 3. The shrinkage Temperature of Pelts/skin

Note: For *Raw skin; shrinkage temperature measured after soaking and shaving hairs using sharp scissors; For conventional, hair saves, and painting unhairing shrinkage temperature were measured after unhairing and the action of liming (swelling); while for Oxidative shrinkage temperature was measured after unhairing and the action of sodium hydroxide (swelling).*

FT-IR spectrometer analysis

The amide II of oxidative unhaired wet blue shows the wavenumber of 1519.91cm^{-1}, while for other unhaired wet blue with the raw skin sample their wavenumber reads as 1539.20cm^{-1}, which is like that of the normal absorption band of amide II whose absorption band is 1539cm^{-1} (Ganesan *et al.*, 2018 and Yao *et al.*, 2019). The amide I and amide II express the wavenumber of 1639cm^{-1} and 1234cm^{-1} respectively, which are the same absorption band amide I and III (Ji *et al.*, 2020)

The changes were noted to the intensity of the side chains of collagen with wavenumber range of 1005-1100cm^{-1} which correspond to ν(C-O-C) bond (Nashchekina *et al* 2021). The intensity for raw skin sample was 99.67% and for conventional, hair saves, painting and oxidative unhaired wet blue were 97.38%, 97.98%, 96.02% and 90.83% respectively (Figure 4).

.

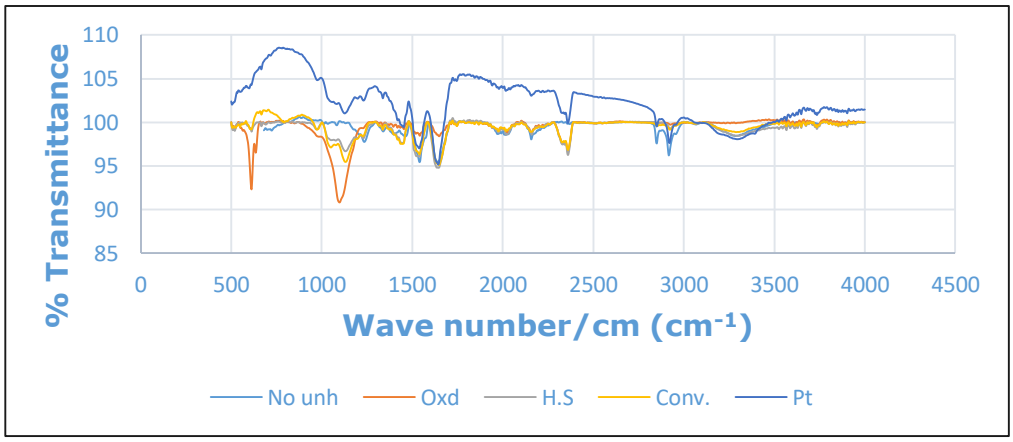

Figure 4*:* FT-IR spectral lines of fibril collagen for skin/wet blue*: "No unh" this was haired skin (Raw skin) after soaking and shaved with sharp scissors, "Oxd" this was Oxidative unhaired wet blue leather, "H.S" -Hair saved unhaired wet blue, "Conv"- Conventional unhaired wet blue and "Pt"- Painting unhaired wet blue*

The Hydroxyproline Reading results for the experimental samples.

The absorbances were read after standard calibration of the spectrophotometer at 555nm. The reading help to obtain the percentage of collagen per skin sample, which was calculated using the following formula equation (Heidemann, 2000)

Collage

$= Weight\ of\ hydroyproline/dry\ weight\ of\ the\ skin \times (100/12.5) \times 100\%$

The values obtained were recorded in the Table 2

Table 2

The collagen percentage for the skins after different unhairing and raw skin sample.

s/n	*Name of the sample*	*percentage of collagen*
1	Raw skin	42.25±0.55
2	Conventional unhaired skin	43.66±0.58
3	Hair saved unhaired skin	43.75±0.53
4	Painting unhaired skin	45.21±0.37
5	Oxidative unhaired skin	46.64±0.39

Note: The sample for Raw skin was taken after soaking and shaving all hairs using sharp scissors, for conventional, hair saves and painting unhaired skin sample was taken after liming while for oxidative unhairing was taken after the action of sodium hydroxide. (Total number of three (3) replicates and percentage are based on dry weight).

Physical Test Results

The physical test results for the crust leathers for all types of unhairing studied are given in Table 3. The values were not the same however, most of the value meet the required value as per international organization for standardization, except for tearing strength and percentage elongation which shows the degree of deviation from the required values for oxidative unhairing.

Table 3

Physical tests result of the crust leathers for respective unhairing

Types of unhairing	Tensile strength (Mpa)	Tear strength N	Percentage elongation %	Ball Burst %
Conventional	30.30± 17.80	40.10±9.60	60.50±28.50	12.60±0.08
Oxidative	21.80±6.50	34.10±12.10	39.60±10.10	9.60±0.62
Hair saves	32.10±21.50	46.40±11.30	64.20±22.90	13.30±1.16
Painting	30.30±8.10	38.60±9.40	41.50±17.10	11.00±1.07

***Note**: Six samples for each test parameter (Samples were obtained from OSP (official sampling position): 3 parallel and 3 perpendiculars to backbone) and conditioned at 23.0 ± 2.0^{0}C and 50.0± 5.0% relative humidity for 24 hours*

Discussion

Shrinkage temperature

The results show that Oxidative unhairing cause high stress to the collagen as the shrinkage temperature of the pelt after the action of sodium hydroxide (swelling) was observed to be lower as compared to raw skin and other unhairing alternatives. This was due to use of sodium hydroxide which is strong alkali with high solubility 1110g/L at 20^{0}C (Zengin *et al*., 2010).

The high solubility causes rapid change in isoelectric point (swelling) and causing damage to the collagen and its side chains (Covington, 2009). Also, the use of sodium hydroxide (NaOH) cause swelling which is not reversible completely as compared to weak alkali such as calcium hydroxide whose solubility is 1.3g/L at 20^{0}C (Beghetto *et al*., 2013). At the end, there was high change in the conformation of the collagen fibers which affect the stability of the collagen and lowering its shrinkage temperature (Nashchekina *et al*., 2021)

The use of hydrogen peroxide causes the destruction of the hierarchical package of the collagen by breaking the (-C-O-C-) bond of galactose and fructose attached to hydroxylysine of collagen (Figure 5). This side chains attached to the collagen has great contribution to the stability of the collagen including the shrinkage temperature (Baghetto *et al*., 2013 and Nashchekina *et al*., 2021)

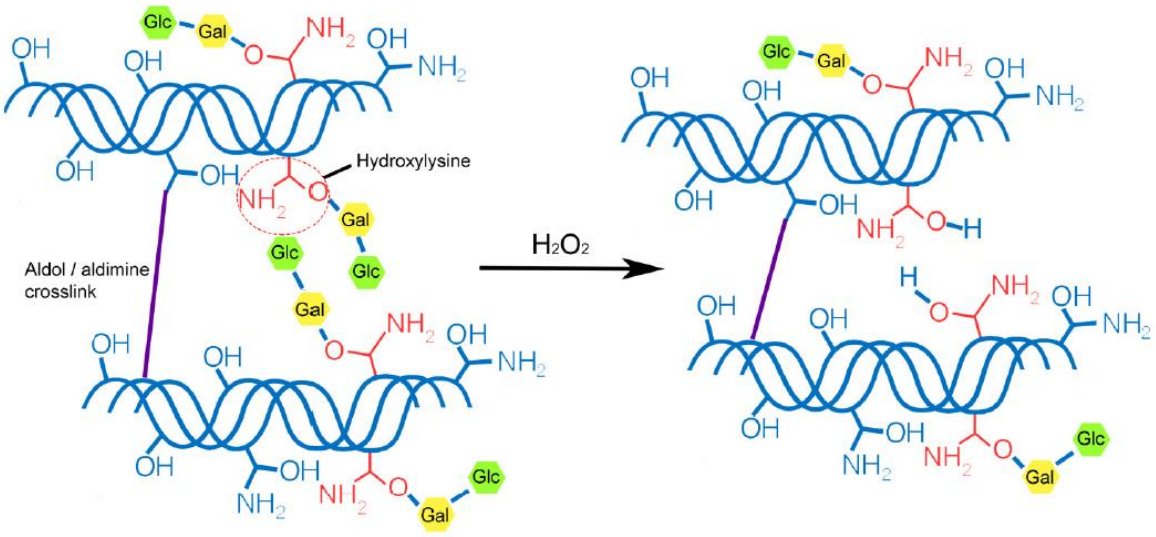

Figure 5. *The action of H_2O_2 to the Hierarchical structure of Collagen (Naschekina, et al., 2021), where Glc represent Glucose and Gal represent Galactose.*

Note: *The breaking down of the -C-O-C- bond of the collagen disturb its structural network which contribute to its stability, thus why this breaking down lower its stability thus why lower the shrinkage temperature as compared to raw skin sample* (Baghetto *et al.,* 2013*).*

FT-IR spectrometer analysis

The amide II associated with absorption band of 1539 cm^{-1} which is associated with vibrations bending of N-H amide bond and vibrations which stretch C-N bond (Ganesan *et al*., 2018), the FT-IR reading revealed that there was small wavenumber reading for oxidative unhaired wet blue which was 1519.91cm^{-1} which signify the formation of the weak isomer of the collagen structure (conformation) (Naffa *et al*., 2019), while for other unhaired wet blue with the raw skin sample their wavenumber reads 1539.20cm^{-1} (Figure 4), which identify that the collagen was able to maintain its original strong isomer (Yao *et al.,* 2019 and Ji *et al.,* 2020).

The similar wavenumber for amide I and amide II shows that, there were no C=O stretching vibrations for protein amide for amide I and stretching vibrations of C-N and in-plane bending of N-H for protein amide III

The significant change of the side chains intensity for oxidative unhaired wet blue 90.83% as compared to raw skin sample 99.67% means that, there was significant destructions of glycoproteins and proteoglycans which form complex network with collagen (Nashchekina *et al.,* 2021), and form hierarchical structure which determine the stability of the collagen, therefore, its destruction affect the physical features of the leather which are associated to the stability of collagen such as tensile strength and tearing strength (Beghetto *et al.*, 2013)

Hydroxyproline determination

The results show that there were no significant differences in the percentage of the collagen content determined. This indicate that unhairing chemicals do not cause the breakdown of collagen, however, it might cause only change in conformation of the collagen and breakdown of the side chain of the collagen (Nashchekina *et al.,* 2021).

The small difference obtained between unhairing types was due to mass differences, as there was the removal of non-structural proteins (non-collagenous proteins) which takes place during unhairing and liming (Buljan and Ivan, 2019).

This makes raw skin sample to contain more mass of non-collagenous content than any unhaired skin which lose some of non-collagenous content, thus why, the results shows that it has low collagen content than all unhaired

skins. Also, oxidative unhaired skin shows high collagen content, because it loses more non-nitrogenous content thus the most of its total mass was due to collagen (Bronco *et al.,* 2005).

Physical Test

It was very important to assess the influence of the types of unhairing to the strength properties of the leather because any changes happen to the collagen should be reflected in the strength of the leather because the strength of the leather is determined by the properties of the collagen of the leather such as crosslinking, and concentration of the collagen (Covington, 2009).

The tensile strength measures the resistance of the leather to breaking under tension. Its value should be a minimum of $15N/mm^2$ as per international organization for standardization (ISO) (Andualem, 2014).

The value obtained for crust leather from all types of unhairing meet the minimum specification required, this means that with the different effects observed to the collagen of the leather still the crust obtained was able to meet the standard as per ISO, however, there were small differences in the magnitude of the value obtained, this was associated with the differences observed to the collagen structure due to different unhairing process.

Percentage elongation at break shows how much crust leather can be stretched before it breaks, its value should not be less than 40% for shoe upper as per ISO (Andualem, 2014). The value obtained for both unhairing meets the required minimum specifications.

Tear strength express the extent to which leather materials can overcome the ripping effect its value should not be less than 35N for shoe upper as per ISO (Soebijarso, 1994). The value obtained for conventional, hair saves, and painting unhairing meet the required results, however there were differences in the magnitude of the value obtained, but the oxidative unhairing results was below the required standards. The oxidative unhaired leather failed due to high pH change as the result of isoelectric point changes and the more breaking of the collagen side chain as compared to other types of unhairing (Nashchekina *et al*., 2021).

The ball and burst test intended to test the suitability of crust leather for shoe upper. This is due to high force which is applied to grain of the leather during the shoe lasting, if the grain is not strong enough there will be the possibility of grain cracking especially at the toe part of the shoe as is the part which expected to experience more force during the lasting.

The crust to be able to sustain the force applied during the lasting, it should not show any grain crack at 7.0mm of leather extension as per ISO (SLTC, 1996).

The experimental results (table 5) for both unhaired crust leathers were even more than the minimum acceptable standard (≥7.0mm) as specified by ISO, however there were some of the differences among them especially oxidative which show the low value among them, and the difference was associated with the collagen side chain breaking and the swelling which was observed to be high in the oxidative unhairing.

The average result at the end, shows that the crust leather was able to sustain the force applied on it without grain cracking especially during the lasting process (Soebijarso, 1994).

Conclusion

It has been revealed that there is no significant change in the collagen content in the skin due to unhairing, however, there was the differences in the degree of collagen helix conformation and breakdown of the collagen side chains which at the end affect the strength properties of the leather.

The strength properties of the leather do not depend only on the concentration of the collagen but also on the proper packing of the collagen structures. The disturbance occurs to the hierarchical network of the collagen was reflected in the physical properties of the leather.

Oxidative unhairing was possible at the industrial scale (large scale) using polyethylene drums, and the leather produced meet the required standard as per ISO with some of suggested improvement. This shows that it can be possible to eliminate or reduce the use of sulfide and lime in the beamhouse process if further study is done as per recommendations.

This will lead to excellent environmental improvement in the leather industry and make it more economical as the wastewater treatment cost much the leather industry due sulfide and lime used in the leather processing.

Further study is required on how to minimize the concentration of hydrogen peroxide with proper unhairing results. This will reduce the extent of change of collagen conformation and destruction of the collagen side chain, which lower the strength of the leather (Nashchekina *et al.,* 2021).

To make a study on the effective collagen fiber packing, together with crosslinking density of the leather fibers. The difference in the physical test has been connected to the swelling, and the change in the side chain percentage expressed by FT-IR spectrometer. This study will show how swelling and the change in the side chain can affect the proper packing of collagen fibers and the cross-linking density of the collagen fibers (Marsal *et al.,* 2011).

More findings are required on the mechanism of unhairing as regarding the current research on the unhairing mechanisms, there is no any clear agreed mechanism of unhairing, however there are different postulates on the mechanism which need scientific research to prove those thoughts. Wise *et al*, 2019, had propose postulates on the unhairing mechanism which use sulfide in alkaline ($Ca(OH)_2$) medium in its operations. Also, Shi *et al.,* 2003, suggest making a further study on the mechanisms on the hydrogen peroxide in unhairing as it is not clear of how peroxy anion from H_2O_2 attack the (-S-S-) sulfide bond. The clear mechanism will help to express the impact of the chemicals to the fibers of the leather which have impact on the properties of the leather.

References

Andualem G (2014), Combination Tanning based on Tara and Sodium Metasilicate a new Chrome free Tanning system (MSc-thesis), Addis Ababa University, Ethiopia.

Ali. F, Kamal. M & Islam S. (2020) Comparative study on the physical properties of different types of leather in Bangladesh, Journal of Engineering Research and Applications, 10 (2) PP 55-63.

Beghetto. V, Zancanaro. A, Scrivanti. A, Matteoli. U, & Pozza. G (2013), The Leather Industry: A Chemistry Insight Part I: An Overview of the Industrial Process, sciences at ca' Foscari, pp 12-22.

Biskauskaite. R, Valeikiene. V, & Valeika. V (2021), Enzymes for Leather Processing: Effects on Pickling and Chromium, Materials, Vol 14, 1480 pp 1-11.

Bronco S, Castiello D, D'Elia M, Salvdori M, Seggiani M, & Vitolo S (2005), Oxidative unhairing with Hydrogen Peroxide: Development of an industrial scale process for high quality upper leather, Journal American Leather Chemistry Association 100(2) 45-53.

Buljan J & Ivan K (2019), The Framework for sustainable leather manufacture 2nd Edition, UNIDO, Viena.

Covington. D (2009), Tanning Chemistry; The Science of Leather, RSC Publishing, Cambridge.

Dai. H, Zhang. Z, Fan. S & Zhang. X (2004), Analysis of Hydroxyproline in Collagen of Pig Skin Tissue by Low Pressure Ion Chromatography Separation and Conductivity Detection, Journal of the Society of Leather Technologists and Chemists, Vol. 89 pp.145-148.

Daniels R & Landmann W (2013), The Framework for Leather Manufacture, UNIDO, World Trades Publishing Limited, Liverpool.

Ding, Z & Zhang, M (2000), Linear Relationship Between Collagen Degradation and degree of Swelling in Beamhouse, Journal of the Society of Leather Technologists Chemists, Vol. 85, pp 164-166.

Galarza, B. C., Cavello, I., Greco, C. A., Hours, R., Schuldt, M. M., & Cantera, C. S. (2009), Alternative Technologies for adding value to bovine hair waste, Journal of Soc. Leather Technol. Chem., 94, 26-32.

Ganesan, Dhanasekaran, Thangavel, & Dhathathreyan (2018), Elastic compliance of fibrillar assemblies in type I collagen, Biophysical Chemistry, Vol. 240, pp 15-24.

Haidemann. E (2000), The Determination of Hydroxyproline in Materials Containing Collagen, Journal of the Society of Leather Technologists and chemists, Vol. 64, p. 57-59.

Haiming. C, Min. C & Zhiqiang (2009), Properties of Collagen Fibre in Alkali and Neutral Salt Solutions, Journal of the Society of Leather Technologists Chemists, Vol. 94, pp 65-69.

ICLT (2019), Chemical Testing of Leather, 6th Edition, University of Northampton, Northampton

Ji Y, Yang X, Ji Z, Zhu L, Ma N, Chen D, Jian X, Tang J, & Cao Y (2020), DFT- Calculated IR spectrum Amide I, II, and III Band Contributions of N-Methylacetamide Fine Components, ACS Omega, Vol. 5, pp 8572-8578. https://dx.doi.org/10.1021/acsomega.9b0421.

Marsal. A, Cot. J, Bartoli. E, Bosch. T and Manich. A (2011), Oxidising Unhairing Process with Hair Recovery, Journal of the Society of Leather Technologists and Chemists, Vol. 86, pp 30-33.

Naffa R, Maidment C, Ahn M, Ingham B, Hinkley S, & Norri G (2019), Molecular and structural insights into skin collagen reveals several factors that influence its architecture, International Journal of Biological Macromolecules, 128: 509–520.

Nashchekina. Y, Nikonov. P, Mikhailova. N, and Nashchekin. A (2021), Collagen Scaffolds Treated by Hydrogen Peroxide for Cell Cultivation, Polymers, 13, 4134 https://doi.org/10.3390/polym13234134

Priya, S., Rajaram, A., Rajaram, R., & Ramasami, T. (2008), Depilation of skins by pure enzymes. Journal of the Society of Leather Technologists and Chemists, 92, 214-221.

Puyana. V, Wieringa. P, Guerrero. A, Romero. A, & Moroni. L (2021), (Macro) Molecular Imprinting of Proteins on PCL Electrospun Scaffolds, ACS Applied Materials interfaces, pp 1-10, https://doi.org/10.1021/acsami.1c0022

Rabotyagova, O.S., Cebe, P., & Kaplan, D.L. (2008) Collagen structural hierarchy and susceptibility to degradation by ultraviolet radiation, Mater. Sci. Eng, 28, 1420–1429.

Senthilvelan T, Kanagaraj J, & Mandal A (2012), Application of enzymes for dehairing of skins: cleaner leather processing, Clean Techn Environ Policy, 14:889–897.

Shi, B.; Lu, X.; Sun, D.; (2003) Further investigations of oxidative unhairing using hydrogen peroxide. JALCA, 98, 185-192.

Shoulders M, & Raines. R (2010) Collagen Structure and Stability, Annu Rev Biochem , 78: 929-958; doi:10.1146/annurev.biochem.77.032207.120833

SLTC (1996), Official methods of analysis, Charlesworth Group, Huddersfield.

Soebijarso K (1994), Leather specification according to IULTCS II, Majalah Barang Kulit, Karet dan Plastik, Vol X, No 19, pp 16-27.

Wise W, Ballantyne A, & Covington A (2019), Sulfide unhairing: Rethinking the received wisdom, XXXV IULTCS Congress, Dresden.

Yahia M, Musa A, Gasmelseed G, Faki E, Ibrahim H, Haythem O, Manal M, & Haythem S (2019), Painting unhairing for manufacture of upper Leather,

International Journal of Engineering and Applied Science (IJEAS), Volume 6, Issue 5, 75-79

Yao Q, Chen H, & Chen Y, Zhan L (2021), Improvement of hydrothermal stability and chrome uptake of leather: Amino-terminal hyper-branched as high exhausted tanning auxiliary, Journal of the Indian Chemical Society 98, 100-175

Yorgancioglu A, Onem E, Yilmaz O, & Karavana A (2022), Interaction Between Collagen and Alternative Leather Tanning Systems to Chromium Salts by Comparative Thermal Analysis Methods, Jonson Matthey Technology Review, 66 (2), 215-226.

Zengin G, Colak S, & Ozgunay H, (2010) Usage of hydrogen Peroxide in Leather manufacturing process and its effects on Leather Characteristics, Testily ve Konfeksiyon, p.p 235-240.

Zhang. Y, Liu. H, Wang. F, & Tang. K (2020), KCl-assisted Enzyme in Unhairing and Collagen Fibre Bundle Opening for Cleaner Leather Making, Journal of the Society of Leather Technologists and chemists, Vol. 104, pp. 235-239.

Zhi-Hua, S., Qing, L. S., Jian-Wei, L., & Ollis, D. L. (2009), Lime-free removal of non-collagen proteins from sheepskins: quantitative analysis by gel electrophoresis. Journal of the Society of Leather Technol. and Chem., 93, 229-23