ASSESSMENT OF POLLUTION REDUCTION IN THE TANNERY PRE-TANNING PROCESSES THROUGH THE USE OF ENZYMES

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DECLARATION

This thesis is my original work and has not been presented for a degree award in any other University

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DEDICATION

This thesis is dedicated to my dear wife Peninnah Kaimenyi, father Peter Kuria, mother Joyce Nyambura, my sister and my brothers for their prayers and continued support.

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LIST OF ABBREVIATIONS AND ACRONYMS

BOD	Biochemical oxygen demand
COD	Chemical oxygen demand
DS	Dissolved solids
EIPPCB	European Integrated Pollution Prevention and Control Bureau
SLTC	Society of Leather Technologist and Chemist
SS	Suspended solids
TDS	Total dissolved solids
TS	Total solids
WBGETG	World Bank Group, Economic and Transformation Group
GAGs	Glycosaminoglycan
SI	Isoelectric point

DEFINITION OF TERMS

Bating: The process carried out after deliming where proteolytic enzymes are used

Degreasing: The process of removing fat from hides and skins

Deliming : The step after liming when the pH is reduced (8-9) in readiness for enzymatic bating

Hide: Outer covering of a large animal eg cattle

Leather: Tanned hide or skin

Liming : Alkaline treatment to the pelt to cause swelling and hydrolysis

Pelt : Partially processed hide or skin before tanning

Pickling: Acidification of the pelt in brine for preparation for tanning

Pre-tanning: The early stages of processing hides and skins for tanning

Skin: Outer covering of small animal eg sheep

Soaking: First stage of processing, primarily to rehydrate preserved pelt

Unhairing: The process of removing the hair

Wet-blue leather: Chrome tanned pelt

ABSTRACT

The process of transforming hides/skins into leather has been categorized as one of the most environmentally detrimental industries globally. Because of elevated pollution levels linked to the leather manufacturing sector, there has been a concerted search for clean technology options that could replace or reduce the hazardous chemicals used in tanneries. Application of enzymes has been suggested but has not been fully adopted in Kenya and there have been complaints about the poor performance of enzymes available in the market. In order to address these problems, a study was conducted to assess the potential of enzymes in reduction of pollution in the tannery pre-tanning processes. Commercial beamhouse enzymes in Kenyan market and special formulations of enzymes prepared by mixing different enzymes (lipase, amylase, keratinase and protease) were assessed for their effectiveness in different pre-tanning processes. Their potential in reducing pollution and improving leather quality was also evaluated and compared with the conventional method.

To determine the potential of enzymes on pollution reduction, several parameters were measured on the tannery effluent: biochemical and chemical oxygen demand, total and suspended solids. The quality of produced leathers was also determined by organoleptic tests, tearing strength, tensile strength, shrinkage temperature and flexing endurance. SPSS Statistical Software was used for data analysis and T-test and ANOVA analysis were performed to identify the means that were statistically significant ($p \leq 0.05$).

Microbate, micro enzyme-P, microbate elbate and microenzyme elbate enzymes were found to be the only commercial enzymes in the Kenyan market used by the tanners specifically in the bating process. Assessment of protease activity of these bating enzymes from five local tanneries showed that Microbate elbate -AHITI had 11,341.2 ± 68.05 (U/g), Microbate- Ewaso Nyiro had

21,321.33± 54.64 (U/g), Microenzyme elbate- LIK had 23,883.6 ± 97.10 (U/g), Microbate-Yetu leather had 24,137.4 ± 65.25 (U/g) and Micro enzyme P-Sagana had 24,717.6 ± 109.84 (U/g). The majority of the other evaluated variables (fat content, protein, suspended and total solids) to compare the efficiency of these enzymes in various pre-tanning techniques revealed no significant difference ($p > 0.05$). The commercial enzymes were only effective in the bating process only.

In the case of various combinations, the utilization of a blend of keratinase and lipase enzymes (KL) was chosen for the soaking process, leading to a clean pelt with a weight gain of 68.3% and a residual fat content of 8.40%. The most effective combination for unhairing process was found to be a formulation containing keratinase, protease, and lipase enzymes (KPL), resulting in a completely unhaired pelt with a residual fat content of 6.4%. Additionally, the lipase enzyme was employed for degreasing purposes.

Goatskins and cattle hides were processed using the selected formulations. In most of the physio-chemical parameters analysed on the effluent from processing leather, the enzymatic method had lower values compared to the conventional method. The enzymatic method was highly effective in pollution reduction in the unhairing process with a percentage reduction of total solids (48.89%), suspended solids (66.73%), biochemical oxygen demand (52.1%), chemical oxygen demand (80.96%) and sulphide (100%) for goatskins and total solids (74.26), suspended solids (73.52), biochemical oxygen demand (49.79%), chemical oxygen demand (80.01%) and sulphide (100%) for cattle hides.

On the assessment of the organoleptic properties, both conventional and enzymatic tanned leather had a rating of more than 8. Elongation, shrinkage temperature, tearing and tensile strength of enzymatic processed goat leather was found to be 67.24 %, >100^{o}C, 17.12 N and 30.17 mpa

respectively while that of conventional process was found to be 66.5%, >100 ^{o}C, 29.25 N and 26.7 mpa. A comparison of these properties in cattle leather also revealed the same results where enzymatic method had higher values. For enzymatic method of cattle leather the shrinkage temperature was > 100 ^{0}C, tearing strength (79.54 N), tensile strength (23.91 mpa) and elongation (68.62 %) while that of the conventional method was shrinkage temperature > 100 0C, tearing strength (81.44 N), tensile strength (16.2 mpa) and elongation (66.29 %). In addition, both cattle and goat leather processed by enzymatic and conventional methods met most of the minimum requirement for shoe uppers such as tearing strength 50 N, tensile strength 15 mpa, percentage elongation > 30%, shrinkage temperature > 100 0C and flexing endurance > 50, 000 flexes. In conclusion, use of enzymes can replace some of chemicals used in the tannery, reduce pollution and produce quality leathers that meet the requirement for making different leather products. Therefore, it was recommended that the special formulations should be adopted for use in the tanneries to significantly reduce the pollution.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Leather sector which utilizes hides/skins as raw materials, is one of the flagship projects that has been identified by the government to make Kenya a middle-income industrialized country by 2030 (WBGETG, 2015). Since only 88% of raw materials are processed to wet blues and 2% to dyed and pigmented leathers, the government is encouraging local manufacturers to process up to finished products to create jobs for thousands of unemployed youths and women. Unfortunately, the leather sector has been classified among the industries which releases a lot of pollution to the ecosystem (Azom *et al.*, 2012). Because of high use of chemicals, the waste from tanneries must be treated before disposal, which increases the production costs. In addition, some of the chemicals used in the tanneries are known to cause undesirable health effects and even deaths (Azom *et al*., 2012).

In the tannery, excessive amount of chemicals and water are used to process wet-salted hides/skins into leather. This produces highly concentrated waste water that pollutes the environment (Saxena *et al.*, 2016). Chemicals commonly used in beamhouse stages include sodium salts (sulphide, sulphate bicarbonate, carbonate and chloride) calcium hydroxide, bactericide, sulphuric acid, ammonium salts, formic acid etc. (Azom *et al.*, 2012). According to Mondal *et al.* (2005), chrome and sulphide are found in large quantities in waste water from the tanneries and when discharged into the environment they permanently pollute the ground water and make it unsuitable for drinking, crop cultivation and for domestic use.

Application of enzymes in pre-tanning processes (soaking, unhairing, and degreasing) can substitute harmful chemicals and auxiliaries used in the tannery. Although there have been some

commercial enzymes in Kenyan market, there have been complaints from various tanneries in Kenya that the enzymes are ineffective leading to low adoption of their use. In this study, enzymes were applied in the soaking, unhairing, bating and degreasing processes to investigate their potential in the reduction of the amount of sulphide, COD, surfactants, BOD, total solids (TS) and suspended solids of tannery effluent. The application of enzymes in soaking and unhairing process would eliminate the need for bating process which significantly reduces the amount of tannery effluent. The use of inorganic degreasing agents causes health hazards to aquatic animals and the use of enzymes could also help eliminate this problem.

1.2 Statement of the problem

Pre-tanning stages releases effluents into the environment which has high concentration of sodium sulphides, ammonium sulphate/chloride, sodium chloride, calcium hydroxide and protein. This produces waste water with lower pH but with high amount of COD, BOD, SS, total dissolved solids (TDS) and others. Over 70% of COD, BOD and TDS in the tannery effluent are generated in pretanning processes (Islam *et al.,* 2014). Out of this 45% of COD and 50% of BOD comes from unhairing and liming processes. The pretanning process also contributes 90% of the tannery total SS with liming/unhairing contributing to about 60% of the beamhouse processes. Research in India has shown that most of the tanners suffer from digestive tract diseases, respiratory diseases and skin infections and ninety percent (90%) die before the age of fifty years (Azom *et al*., 2012). Mondal *et al.* (2005), established that one tannery has a potential to pollute ground water around a radius of seven to eight kilometers, causing the total amount of dissolved solids of ground water to increase up to 17,000 mg/l and therefore unsuitable for growing crops and for other uses.

Although some studies have been conducted in search of appropriate enzymes for use in different stages of leather processing, there is need for more intensive studies to investigate the best

formulations of enzyme that can substitute the most polluting chemicals and auxiliaries used in the tannery. Since hides and skins have many components that can be degraded by specific enzymes, there was a need to formulate an enzyme for each specific stage. The aim of using enzymes was to reduce or eliminate the polluting chemicals which could lead to the reduction of overall beamhouse pollution. The types of enzymes used must maintain or improve the quality of leathers as compared to the conventional method.

1.3 Objectives

1.3.1 General objective

To assess the potential of enzymes in the reduction of pollution in the tannery pre-tanning processes.

1.3.2 Specific objectives

1. To determine the effectiveness of commercial beamhouse enzymes in different pre-tanning processes
2. To formulate and to assess the potential of new enzyme combinations for use in pre-tanning processes
3. To assess the potential of the new enzyme's formulations in the reduction of beamhouse pollution
4. To compare the quality of leathers processed by use of enzymes with the leather processed by conventional pre-tanning processes

1.4 Justification of the study

The high levels of pollution caused by use of harmful chemicals in the tannery has prompted worldwide research to develop cleaner technologies such as use of enzymes to reduce or eliminate

hazardous chemicals in the tannery. At the same time, the type of enzyme used must maintain or improve the quality of leather. Clean technology also emphasizes on pollution prevention measures rather than treatment of the final tannery waste.

Most documented past studies have investigated the application of single enzymes in specific pre-tanning processes. In addition, only few have determined the extent of pollution reduction. In Kenya today, there is also no documentation on the quality of enzymes used by the tanners. The enzymes are marketed by trade names such as Batezyme AC, Debazyme DK-03, Degreasezyme, Dermilize SK30, Novozyme etc. Since hides and skins have different components that can be removed by different specific enzymes, it was believed that a different approach to enzyme formulation should be carried out in order to replace the hazardous chemicals used in the pretanning processes. If successful, application of enzymes in different pre-tanning processes should eliminate or significantly reduce some of the chemical pollutants and improve leather quality. In addition, pollution reduction in the tannery reduces the cost of effluent treatment and some of the diseases and death associated with some chemicals and emissions such as hydrogen sulphide gas. The quality of leather was also expected to be improved and this means more income for tanners since high quality leather will be sold at a higher price.

CHAPTER TWO: LITERATURE REVIEW

Leather industry is not only among the most profitable industries in the world, but also classified among the most contaminating industries (Saikia *et al.*, 2017). This is because of the release of various harmful materials used in leather processing into the environment (Arunachalam and Saritha, 2009). Leather is a product obtained by processing hides/skins through a sequence of mechanical and chemical steps such that it is not easily affected by microorganism, moisture and stress (Fúquene *et al*., 2018). Each processing steps is very important and if omitted the resultant quality of the leather is affected. To produce quality leather, the tanners must have adequate knowledge of the physical and chemical composition of hides and skins. In addition, most of the chemicals used are known to cause a lot of pollution and many studies are being conducted to find eco-friendly products that can replace the hazardous chemicals (Dixit *et al*., 2015). This chapter reviews physical and chemical structure of hides/skins, associated pollution in the conventional method, enzymes classification and their application in leather sector and physical properties of leather that are important for quality assessment.

2.1 Physical Structure of skins/hides

For consistent processing of high-quality leather, there is need to understand the physical and chemical structure of hides (Basil *et al*., 2013, Lischuk *et al*., 2006). This will enable removal of all the undesirable non-collagenous substances without affecting the collagen protein. Hides/skins are main raw materials used in leather production (Sundar *et al*., 2011). In a living animal, they perform vital functions in the body, such as protection of the underlying tissues from physical abrasion, invading pathogenic macro and microorganisms, dehydration and ultraviolet (UV) light (Rosso and Levin 2011). They also contribute to homeostasis and sensation (Alsodany *et al*., 2019, Moyo *et al*., 2018). Hides and skins are a multi-layered structure that can be categorized into three

main layers: epidermal layer, dermal layer and subcutaneous/flesh layer (Maxwell, 2007). The dermal/corium layer is the main leather-forming material while the epidermis and subcutaneous layers are removed in pre-tanning processes Fig 2-1.

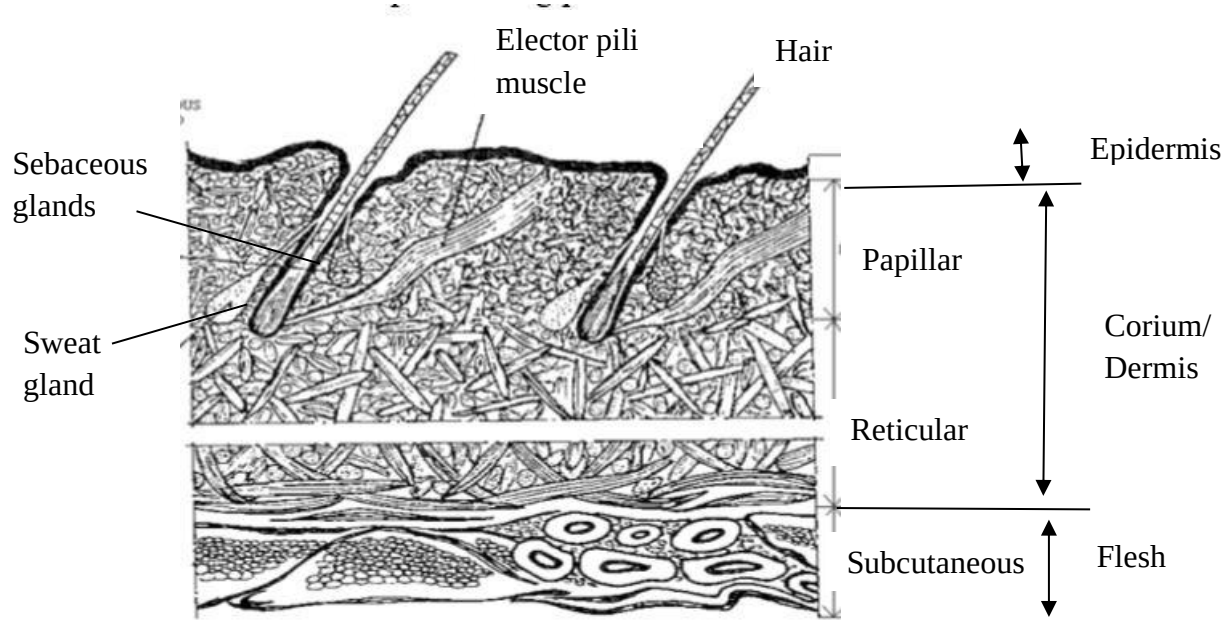

Figure 2-1: Structure of hide/ skin (ADZET 2010)

The skin's outer layer, known as the epidermis, consists of four primary layers: the stratum corneum, stratum granulosum, stratum spinosum, and stratum basale (Hole *et al*., 2008, Razv *et al*., 2015, Rohankar *et al*., 2018). Stratum lucidum is absent in hides and only found in areas such as the palm and sole (Koruga *et al*., 2012, Yousef *et al*., 2022). Basement membrane which consists of a thin layer (50-100 nm) between the epidermal layer and the grain layer of the corium have been reported by some researchers (Frendrup and Buljan, 2000). This membrane controls passage of substance between the epidermis and the grain layers and gives additional protection against invaders and prevents loss of water and other vital substances (Ali *et al*., 2015). It consists of a

special type of collagen as well as proteoglycans and glycoproteins that are interlinked in a close network. (Frendrup and Buljan, 2000, Khalilgharibi and Mao, 2021).

The epidermis mostly consists of Keratinocytes cells which are later transformed into corneocytes as new cells are produced (Wicket, and Visscher, 2006). The epidermis also consists of other important cells such as Langerhans and melanocytes cells that play important roles in the skins. Melanocytes make melanosomes containing melanin at the base of the skins. The production of melanin is continuous as the skin grows but may be facilitated by exposure to UV (Wicket and Visscher ,2006). The reactivity of all these cells with the unhairing chemicals is different and the tanner's knowledge about them is very important to enable them to unhair during leather processing.

The middle layer is known as dermis/corium and the layer consists of grain/corium minor and the corium major (Sujitha *et al*., 2018). This layer is the main leather forming material that is mostly used by the tanners (Sangeetha *et al*., 2016). This layer has fairly distinct sections that include the papillary/grain layer and the corium major. The thickness of the papillary layers varies between animals and it ranges between 40-65 percent in goatskins, 25-35 percent in cattle hides and 50-70 percent in sheepskins (Yu, 1999). Corium major is known to be a fibrous structure that changes across the section of the hide or skin. From the grain layer, the diameter of fibers structures increases in sizes towards the center of the corium and then decreasing toward the flesh layer (Covington, 2011). The physiochemical properties of leather are affected by genetic traits, fibers alignment within the corium and the degree of tanning process (Gbolagunte, 2016). The subcutaneous is the innermost layer of skin adjacent to the flesh (Wanyoike *et al*., 2018).

The hides and skins also contain other components such as hair/wool, hair follicle, erector pili muscle, sweat glands, fat glands, veins and arteries (Covington, 2011, Naffa *et al*., 2019). Most of these components are removed from the skin in pre-tanning processes.

2.2 Chemical constituents of skins/hides

The proteins, fats, carbohydrates, mineral matter and water are the main chemical constituents of skins/hides (Garg *et al*., 2019). The relative proportions of different constituents vary depending upon the type, race, feeding habit, sex, age and species of the animals (Ulugmuratov *et al*., 2022). The constituents of raw hide/skins consist of 33% protein, 65% moisture, 0.5% mineral matters and 0.5 -2-6 % fatty matter (Vijayalakshmi *et al*., 2009).

The largest proportion of rawhides and skin is water, varying from 60-70 per cent (Santos *et al*., 2005, Sarker *et al*., 2018). The fat content is relatively low in many animals but present in higher content in pigskin and sheepskin (Tsanov *et al*., 2012). The availability of fat prevents the penetration of chemicals to the hides and skins and therefore they have to be removed (Santos and Gutterres 2007). Also, the amount of fat in hides differs depending on the age and feeding program of the animal. Raw hides and skins also contain a small percentage of mineral salts such as sodium, potassium, carbonates, phosphates, chlorides sulphates, magnesium, calcium and potassium (Mwinyihija, 2006).

Proteins are the chief constituents of hides and skins and may be classified into two major groups: non-fibrous proteins (albumins, globulins and various protein mucins) and fibrous proteins (keratin, elastin, collagen and reticulin) (Kayalvizhi *et al*., 2008). Collagen protein is found in higher proportion in hides/skins and can be altered chemically by the tanner to make desirable product from an unappealing raw hides and skins (Covington, 2011). Collagen protein is the main

leather making material but it has other uses such as food, cosmetics, drugs and biomedical industries (Iskandar and Rizal, 2018, Lee, 2001).

A collagen structure is a heteropolymer composed of three polypeptide chains arranged to form a triple helix chain Fig 2-2 (Silvipriya *et al*., 2015). Several polypeptide chains form a complex collagen structure (Fig 2-3) that has the ability to retain many times its mass in water, as well as the ability to disperse water and these unique properties allow it to be used for various applications (Mastauskas and Maffia 2012).

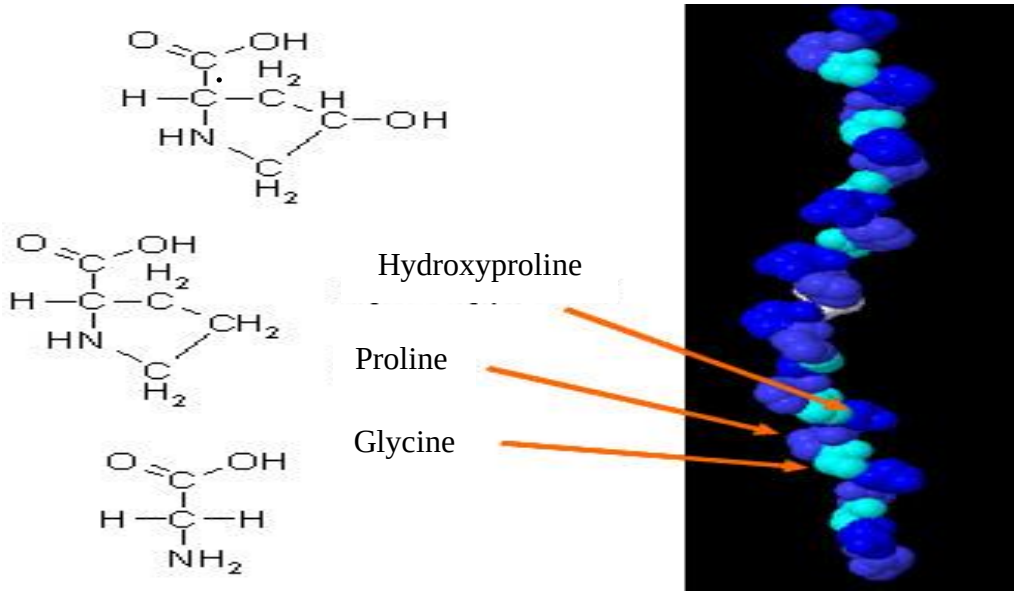

Figure 2-2: Collagen triple helix structure (Silvipriya *et al*., 2015)

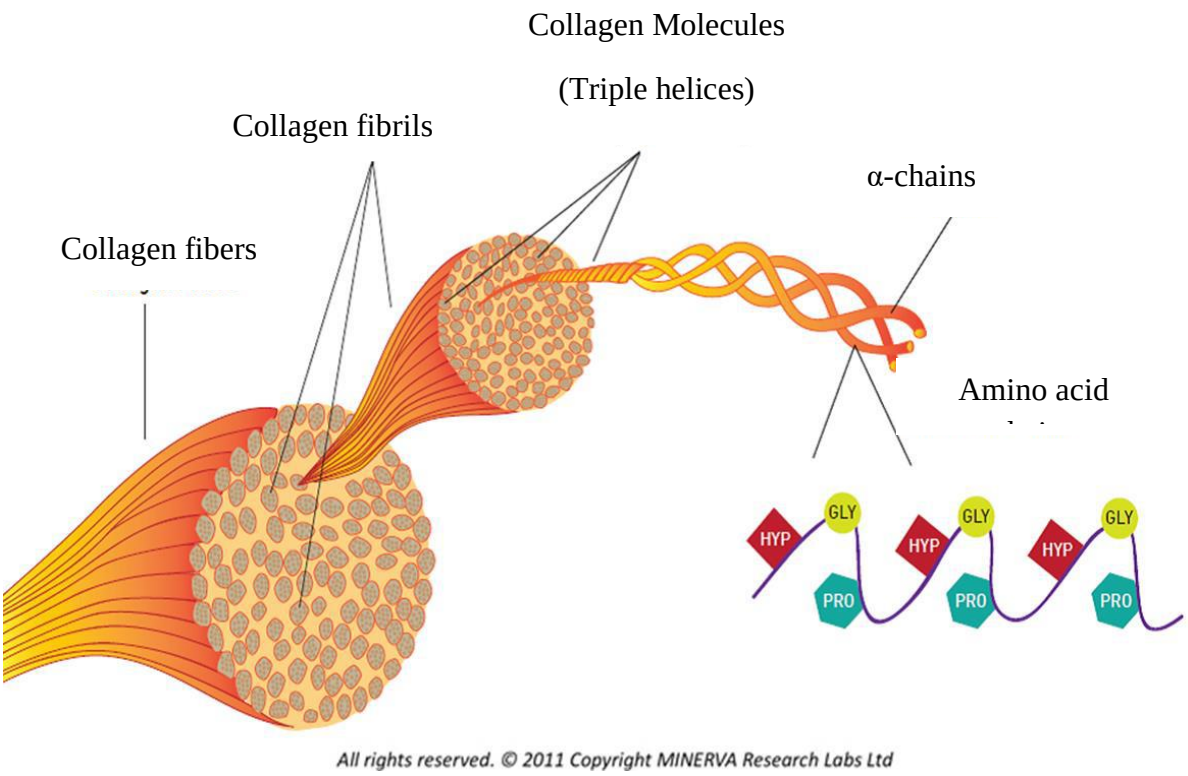

Figure 2-3: Structure of collagen fibre (Sibilla *et al*., 2015)

The chains are left-handed polyproline helix which consist of a sequence of repeating amino acids (glycine, proline and hydroxyproline) and the three chains interact to form a triple helix structure (Paul and Bailey, 2003). Collagen triple helix molecules are stabilized by several forces such as hydrophobic, hydrogen, van dar Waals and electrostatic forces (Adzet, 2010). The stability of these bonds can be altered by addition of chemicals during tanning operations (Adzet, 2010).

Collagen is known to have higher heat stability/shrinkage temperature and several researchers have given their views. According to Naffa *et al*., (2019), hydroxyproline is responsible for hydrothermal stability of the collagen by mediating through hydrogen bonding and the formation of a cage of water molecules around and within tropocallagen. According to covington (2011), the ring structure of proline and hydroxyproline restrict steric conformation wich contribute to higher

stability.The presence of hydroxylysines in the collagen is essential for the formation of collagen crosslinks (Naffa *et al*., 2019).

The elastin protein has unique amino acids isodesmosine and desmosine which are present only in elastin protein and are involved in skin elasticity (Choudhury *et al*., 2006). It also consists of phenylalanine, leucine, valine and isoleucine amino acids (Covington, 2011).

Reticulin is a structural protein of the skin also known as collagen type III and it is secreted by reticular cells (Ushiki, 2002). Albumin and globulin are nonstructural proteins found in the hides and skins. Albumin dissolves in water while globulin dissolves in dilute salt solution (Hřivna *et al*., 2013). These properties enable them to be removed during the soaking process (Mohamed, 2003). Albumin is one of the soluble proteins that is highly ionized over a large pH range. All the interfibrillar substances must be removed completely from hides and skins to hinder the fibres structure from re-sticking together during drying (Maina *et al*., 2019).

Keratin which has a high content of tyrosine and cysteine is the main protein of the epidermis. It also characterized by low content of glycine and proline than in collagen and no hydroxylysine (Covington, 2011). The stability of keratin is caused by the presence of cystine amino acid that contains a disulphide bonds. Another important non-collagenous substance is the glycosaminoglycan's (GAGs) which can affect the usability of hides and skins if not removed in the beamhouse processes. Several classes of glycosaminoglycan's have been reported which include Hyaluronic acid, chondroitin sulphate, chondroitin, dermatan, keratin sulfates and heparin (Casale and Crane, 2022). Hyaluronic acids are polyelectrolyte and can react with chromium (III) salts during tanning to produce stiff leather. The presence of proteoglycans may affect the physiochemical properties of leather by acting as an adhesive on the collagen fibre structure and

therefore their removal is important (Choudhury *et al.*, 2006). The content of chondroitin sulfate GAGs is present in high quantities in young animals but as the animal matures, the dermatan sulfate becomes most abundant due to conversion of chondroitin sulfate to dermatan sulfate (Maxwell, 2007)

Fat is also another important component of hides and skins and swine skins and sheep skins have a higher content of fatty substances than those of cattle (Mocanu, *et al*., 2018). Most of the fats in the skin are found in the cutaneous layer and in the grain-corium. The structure is in the form of large lipocyte, closely associated in a mass and act as a barrier to the penetration of aqueous reagents (Covington, 2011).

2.3 Conventional pre-tanning processes

Since environmental pollution has become a global concern, tanners all over the world are facing a lot of challenges in order to meet the strict discharge limits for effluents disposal. Chemicals used in the tanneries are not completely absorbed by the leather leaving huge amounts in wastewater (Kanaguaraj *et al*., 2015). The tanners also tend to use a huge amount of chemicals to reduce the processing time. According to the Fick's Law, the diffusion of substances is affected by its concentration. To increase the rate of diffusion of chemicals and to shorten the processing period, tanners normally use high concentration of chemicals. The high concentration of chemicals discharged in the effluents has caused the sector to be rated among highly polluting industries (Chowdhury *et al*., 2015, Ilou, *et al*., 2014, Sah, 2013).

If hides/skins are not processed immediately after the flaying process, they must be preserved/cured since putrefaction may begin to occur four minutes after the death of the animal. The fats and non-structural protein such as albumins, globulin, mucoprotein are less resistance to

putrefaction while fibrous protein such as collagen, elastin and hair keratin are more resistance (Ahmed *at al*., 2015).

There are various pre-tanning processes that are carried out in the tannery which are determined by conditions of hides/skins and product to be produced (EIPPCB, 2013). The pre-tanning (beamhouse) processes that are carried out in the tannery include soaking, dehairing, liming, fleshing, deliming, bating, pickling and degreasing which varies depending on the conditions of the skin/hide (Choudhary *et al*., 2004). Pre-tanning and tanning stages are the most contaminating processes in the tannery and they cause serious health and environmental risks (Choudhary *et al*., 2004). Each processing step has its own uniqueness which contributes to the production of effluent with different characteristics in terms of DS, SS, BOD, COD, sulphide and other organic matters (Kanagaraj *et al*., 2020). These processes and the type of pollutants associated with them are described below:

2.3.1 Soaking

It is the first wet process in the tannery which aims at cleaning and rehydrating dry/wet salted hides and skins (Covington, 2009). During this step, curing salts, dirts, blood, hyaluronic acid and some of the interfibrillar protein are also removed (Sundar *et al*., 2011, Covington, 2011). Failure to remove the dirt and the blood encourages bacterial growth which degrades the raw stock. Proper rehydration is very important as it allow the chemicals to penetrate to the level of the fibril (Covington, 2011). The nonstructural protein albumin and globulin are removed during this process. Albumin is soluble in water and globulin is soluble in dilute salt solution (Mohamed 2003)

. These properties enable them to be easily removed and mostly when the raw material is wet-salted. The hyaluronic acid is also removed by dilute salt solution in the soaking process. Since it

is not complexed to the skin, the presence of salt electrolyte causes the structure to collapse and can now be easily removed (Siddique *et al*., 2015). Hyaluronic acid reacts with both lime and chrome during leather processing (Covington, 2011). It is advisable to remove it before the liming stage because it's precipitated by lime and this hinders its removal. It is then reactivated during pickling process as a free carboxylate which then react with chrome to form stiffer leather (Covington, 2011).

The soaking methods adopted by various tanneries differ depending on the condition of hide/skin and it is mostly carried out in two steps in order to clean and to rehydrate them. There are many chemicals and auxiliaries that are used in the soaking process. Soaking auxiliaries such as enzymes and surfactants may be utilized (EIPPCB, 2013). Biocide, sodium bicarbonate and detergent are some of the commonly used chemicals. The tanners have several parameters to consider when choosing the ingredients used in the soaking process such as water content of hides and skins, nature of the protein solubilized in the soak, pH and the temperature of the float, float length, soaking time and character of soaking water (Covington, 2011). The pollutants associated with this processing step include: BOD, COD, TDS, SS and salts (Islam *et al.*, 2014).

2.3.2 Unhairing and liming

Sodium sulphide unhairing method is the main method used in the unhairing process because of its efficiency (Andrioli *et al*., 2015). Unfortunately, this method is known to contribute heavily to pollution. Other methods that are also used for unhairing include hair saving, enzyme-assisted chemical unhairing, chemical-assisted enzyme unhairing, enzyme unhairing, painting, hair burning, oxidative unhairing and reductive unhairing (Obiero, 2016). Keratin protein is the main target by conventional lime sulphide unhairing.

Keratin protein which is characterized by the presence of disulphide bonds (-S-S-) is the main protein of nails, hairs and epidermis. Highly keratinized and fully developed keratin is highly resistant to biological and chemical attack but can easily be degraded by sulphide (Qiu *et al*., 2020). Immature keratin is less resistant to chemicals and is easily degraded. The resistance of keratin to chemical attack can be increased by immunizing the hair by treating it with an alkali without the sulphide (Quadery *et al*., 2014). The mechanism has been utilized in hair-save processes, where mature keratin becomes more resistance to chemical attack and the hair root is attacked by chemicals hence loosening the whole epidermis.

Unhairing process is aimed at removing the hair and non-fibrous proteins from the hides and skins and preparing the pelt for tanning process (Covington, 2009). The unhairing process is normally performed by use of sodium sulphide, sodium hydrosulphide, thioles or by sodium thioglycolate although some tanners normally add some enzymes (EIPPCB, 2013).

The huge amount of water and toxic sulphide used in this process lead to the generation of a lot of waste that requires treatment prior to disposal (Dettere *et al*., 2011). Sulphides used in unhairing process produces wastewater with a COD of about 60,000 mg/l and constitute the polluting aspect of leather industry. The pollutants in this effluent include: BOD, COD, SS, TDS, alkalinity, sulphide, ammonia nitrogen, hair and lime (Islam *et al*., 2014). Sodium sulphide has also been associated with the toxicity of hydrogen sulphide gas and can corrode the concrete in the sewer and may also cause poor settling of sludge (Valeika *et al*., 2012). Inhalation of Hydrogen sulphide gas leads to a loss of consciousness and in large quantities may affect olfactory nerve and cause death (Omor *et al*., 2017).

2.3.3 Fleshing operation

Although fleshing is normally performed after liming, some clean technology techniques recommend fleshing to be carried out before or after soaking. It is normally performed to remove the adhering fat and flesh on the subcutaneous layer of hides/skins (EIPPCB, 2013). This operation may be carried out by hand or by fleshing machines and produces waste water containing flesh and fatty suspension (EIPPCB, 2013). The pollutants associated with this process include: BOD, COD, SS, TDS, sulphide and fat containing organic matter (Islam *et al.*, 2014).

2.3.4 Deliming

Deliming is an important process that is normally done to prepare the pelt for bating. Sometimes it is normally done together with the bating process as a compact process. Deliming has three main objectives which are: lowering pH, depleting the pelt and partially reducing the swelling of the pelt by removing lime (Sivakumar *et al*., 2015). Water and various chemicals are used in this process. Water does not only act as a carrier for deliming chemical but it can also act as a deliming agent through two mechanisms. It dilutes the hydroxyl content and neutralizes the hydroxyl with the bicarbonate content in the water (Covington, 2011). Other deliming agents include strong and weak acids and ammonium salts (Chowdhury *et al*., 2018, Sivakumar *et al*., 2015).

Ammonium salts are mostly used as they buffer at a pH 8-9 (Zeng *et al*., 2018). It is easier to monitor the process as the deliming agent can be added as a whole offer at the start of the process without causing sudden drop of pH. Application of ammonium salts in deliming process releases ammonium gas into the working environment, which causes discomfort to workers and it is also poisonous to fish and aquatic life when disposed to the environment (Covington, 2011).

The duration of deliming process depends on the requirements of leather that the tanner intends to produce. Thorough deliming process produces softer leather while incomplete deliming of the pelt gives leather that is very firm. Hydrogen sulphide gas may be produced at this stage due to acidification of the liquor that contains sulphide (Qiang *et al*., 2011). The production of hydrogen sulphide gas during deliming can be reduced by the oxidation of the sulphide's ions in the effluent by use of sodium hydrogen sulfite or hydrogen peroxide but this increases the salt content in the tannery effluent (EIPPCB, 2013). Carbon dioxide deliming has shown a significant reduction of ammonia in the effluent (Deng *et al*., 2015). The pollutants associated with this process include: BOD, COD, and TDS, ammonia nitrogen, ammonium and hydrogen sulphide gas.

2.3.5 Bating

Bating process utilizes commercial proteolytic enzymes to remove the remnant of hair roots and hair pigments that remained during unhairing (Covington, 2009). Enzymes are biological catalyst that can accelerate a reaction to equilibrium, but not chemically changed in the process, although it may be physically changed (Covington, 2011). Enzymes reactions are influenced by pH, temperature, time of reaction, enzyme and substrate concentration (Covington, 2011). The presence of remnant of ammonium sulphate in this process maintains the proteolysis activity of trypsin (Wang *et al*., 2013). This process also removes the remains of the non-fibrous protein of the skin and makes the pelt to be soft. It is sometimes conducted as a compact process and its pollutants in the effluent include BOD, COD, DS and Ammonium salts. (Ludvik and Buljan, 2000)

2.3.6 Degreasing

Degreasing process is performed to eliminate fat from skins/hides before application of tanning agent (Kirubanandan, and Babu, 2012). There are different methods of degreasing such as use of lipase, detergents or solvents such as trichloroethylene, methylene chloride, kerosene and ultrasound-assisted aqueous degreasing (Kirubanandan, and Babu, 2012, Sivakumar *et al.*, 2012). The availability of fat prevents penetration of tanning chemicals and dyes and their removal leads to soft and pliable leather (Mohammed, 2012). Application of lipase in degreasing have been documented to contribute to more uniform color, cleaner appearance and prevent dryness in the leather (Khambhaty, 2020, Moujehed *et al*., 2022). This is normally carried out when processing sheep skins but may also be carried out on other hides and skins that have a lot of fat. The amounts of fat in the hides and skins are affected by age, breed, sex and feeding program of the animal (Salehi *et al.*, 2013). It also varies from one region of the hide/skin to the other and it is approximated that thirty to forty percent fat is found in the neck regions, twenty to thirty percent in the back, five to ten percent at the sides and one to five percent at the flank region (Afsar and Cetinkaya, 2008). Excessive fat prevents penetration of tanning agents and dyes causing difficulty in post-tanning (finishing) processes (EIPPCB, 2013). In addition, excess fat affects finished leather by causing defects such as fatty spew, stained appearance and poor bounding of pigments (Afsar and Cetinkaya, 2008). Degreasing may be accomplished by use of solvent medium and aqueous media with a non-ionic surfactant. Afsar and Cetinkaya (2008), reported that most of the solvents and emulsifiers used in degreasing process have harmful effects to the environment. During leather processing, natural oils and grease or fat liquoring oils may be released to the environment. This fat and oils may cause blockage of effluent system, covers water and reduces

transfer of oxygen from the atmosphere (Bosnic *et al*., 2000). Organic degreasing agents may also inhibit biological degradations of wastewater which also contribute to pollution (Sah, 2013).

2.3.7 Pickling

Pickling is normally performed to lower pH of delimed pelt (pH 2-3) for chrome tanning process (Sundar and Muralidharan, 2006). This pH is determined by many factors, first the Pka values (dissociation constant) for aspartic and glutamic acid which are pH 3.8 and 4.2 respectively (Ballantyne, and Davis, 2019). The side chains of these two main proteins are responsible for chrome tanning (Ballantyne, and Davis, 2019). This means that the collagen carboxyl groups are deprotonated at a pH higher than 3.8. Deprotonated carboxyl groups are charged and can easily form complex with chrome causing surface tannage (Prokein *et al*., 2020). Chromium salts are also stable in the pH range of 2-4 but are precipitated at a higher pH. Therefore, lower pH than three stabilizes the chrome complex and protonate the carboxyl side chains groups allowing penetration of chrome salts to the center of the pelt without fixation.

There are many types of acid that can be used in the pickling process which include: sulfuric, formic, hydrochloric, sulfonic and other non-swelling acids (Zhang *et al*., 2016). Sulfuric acid is the main acid used by tanners because it is cheap and readily available and has no harmful effects on leather quality. The presence of salts is very important in the pickling process to avoid acid swelling of the pelt (Jia *et al*., 2020). In chrome tanning, pickling allows the chrome tanning agent to penetrate through the whole cross-section of the pelt without fixation. Delimed and bated pelts are treated with a concentrated solution of salt and acid. The pollutants associated with this processing step are BOD, COD, SS and salts (Islam *et al.*, 2014).

2.4 Enzyme classifications

Enzymes are proteinous substances obtained from living organisms and speed up chemical reactions in a living organism (Gurung *et al*., 2013). They are formed by several amino acids joined together by peptide bonds and they have special ability of speeding up a biochemical reaction without being changed (Christy and Kavitha 2014). Although they are able to perform these roles, they are not alive and they cannot reproduce (Christy and Kavitha, 2014).

Most enzymes have common/trivial names which relate to reaction they catalyze, with the suffix *-ase (*Robinson, 2015*).* Example of these enzymes includes, protease, amylase, lipase and oxidase. Some proteolytic enzymes have the suffix -in for example chymotrypsin and papain. Some of the common names do not give detailed information about the reactions involved (eg diastase and invertase) and due to this complexity, Enzyme Commission has classified enzymes into seven major categories according to their nature and chemical reaction they catalyze which include: transferases, hydrolases, oxidoreductases, isomerases, lyases, ligases and Translocases (Okpara, 2022, Robinson, 2015).

Although there are very many types of known enzymes, only a few are commercially available in in detergent, feed, laundry, food, tanning, cosmetics, textiles and pharmaceuticals industries (Tiwari *et al*., 2015). More than eighty percent of enzymes in the market are used in industries to make various products (Miguel *et al*., 2013). The class hydrolases consist of over 75% of all commercial enzymes (Shukla *et al*., 2022) where protease constitute about 40- 60 % of all enzymes sales (Zhou *et al*., 2018). Lipases, proteases and carbohydrase's, enzymes contribute to over 70% of enzyme sold globally and their sales is predicted to increase significantly by the year 2024 (Ramnath *et al.*, 2017). Enzymes can be used to perform specific roles such a production of

washing powder, modification of antibiotics, sweetening powders and in analytical devices (Robinson, 2015).

Due to environmentally friendly nature of enzymes, they are substituting many chemicals used in industrial processes (Okpara, 2022). There are different sources of enzymes which include fungal, bacterial, plant and animals. The most popular enzyme production sources are microbial ones because they are readily available, grow faster, and can be genetically altered to produce enzymes that work well under a variety of industrial production conditions (Okpara, 2022). There are various industries that use microbial enzymes such as pharmaceutical, leather, photography, textiles, biorefineries, detergent, agriculture and food industries (Okpara, 2022).

Some of the factors that hinder the use and adoption of the use of enzymes in the industries include narrow range of substrates, high cost, low productivity and stability (Fernandes, 2010). Protease, amylase, lipase and keratinase are some of the common enzymes that may be used by tanners in large quantities.

2.4.1 Protease

Proteases enzymes breakdown the bonds in proteins to produce amino acids (Okpara, 2022). They are mostly applied in pharmaceutical, food and detergent manufacturing industries. There are different ways of classifying protease enzymes which include: exopeptidases and endopeptidases according to cleavage site. They may also be categorized into alkaline, neutral, and acidic enzymes based on their optimal pH (Zhu, 2011). Alkaline proteases are very active under high pH value conditions (Poonam, 2013). Alkaline protease normally loosens the hair root making unhairing process easier (Andrio and Gutterres, 2014).

2.4.2 Amylase

This enzyme speedup the breakdown of starch in plants and glycogen in animals to fermentable sugars, primarily maltose (Sweta *et al*., 2019). Amylase enzymes represent approximately thirty percent of commercial enzyme (Vaidya *et al*., 2015). The three sub-classes of amylase enzymes include (α- β- γ-) classified according to their cleaving site (Yasmeen 2021, Paul, 2016). Their ideal pH range is between pH 2 -12 (Christopher and Kumbalwar 2015, Paul, 2016). According to Paul (2016), β -amylase break starch to maltose during ripening of fruits (Yasmeen, 2021). Gamma-amylase speedup the breakdown of amylopectin and amylose to produce glucose (Yasmeen 2021, Paul 2016). Amylase enzymes may also be classified into endo-and exo- amylases according to their mode of action (Sweta *et al*., 2019). Amylase is mostly used in textiles, animal feed, baking, starch, ethanol, food, paper industries, bread-making and digestive aid (Verma and Verma, 2018).

2.4.3 Lipase

Lipases enzymes catalyze breakdown of triglycerols (Christopher and Kumbalwar 2015). They can also be used in leather, detergent/laundry, paper textile, and pulp processing industries (Okpara, 2022). Lipase enzymes have also been reported to help in the cleaning of the environment by catalyzing lipid degradation (Ashok *et al*., 2022). Presence of lipids in the soil reduces the ability of soil to absorb water. In addition, oil layer on the water surface affects the aquatic ecosystem (Ashok *et al.*, 2022). Lipase can be obtained from several sources but the suitable sources are fungi, bacteria and yeast. These microorganisms can produce lipase of high quality in a shorter time, lowering production costs (Padmapriya, 2011). Approximately thirty two percent of total lipase sales are used in detergent industry. These types of enzymes are thermal stable and remain active in alkaline environment (Sharma *et al*., 2001). They can work effectively at a pH 7

or pH 4.0 - 8.0 without a cofactor but also divalent cation of calcium can stimulate its activity (Chandra *et al*., 2020).

2.4.4 Keratinase

Keratinases are serine and metallo proteases that degrade keratin by splitting the peptide bonds (Rani *et al*., 2013). According to Li (2021), most keratin degradation reactions require keratinase to break the disulphide bond but they need other protease for peptide degradation. Keratinases perform well across a wide pH and temperature range, with optimal activity occurring between 40 ^{0}C and 70^{o}C at neutral and alkaline pH 7-8.5. These enzymes have been reported to be effective in leather, detergent, textile and in biodegradation of keratin to feed and fertilizer (Avdiyuk and Varbanets 2012). Although several studies have indicated several microorganisms that can produce keratinase, keratinase from B. *Licheniformis* is the only keratinase that is produced commercially (Rani *et al*., 2013). Due to non-collagenolytic activity in keratinase enzymes, they can be used selectively to breakdown the keratin tissue in hair follicle, making it easier to remove the hair without damaging the leather (Rani *et al*., 2013).

2.5 Application of enzymes in leather industry

The leather industry is facing a lot of challenges in an attempt to meet environmental regulations and ensuring that finished goods are of the required quality. Due to elevated levels of pollution linked to the use of various chemicals in tannery, most tanners are exploiting the potential of enzymes in different pre-tanning stages. Use of enzyme is considered as a clean technology since it is focused on pollution prevention approach rather than treatment of pollution once it has been released from the tannery (Dandira *et al.*, 2012). The cost of enzymes and strict process control for most enzymes has affected the adoption of the use of enzymes by the tanners (Arunachalam and Saritha 2009).

Among the most used enzymes in the tannery are protease, amylase and lipase (Choudhary *et al.*, 2004). According to Gutterres *et al.,* (2009), most enzymes used by the tanners do not have sufficient specificity and when used in the soaking process, they reduce the soaking time, solubilize and remove the fibrous proteins, fats and carbohydrate and this causes better opening up of the skin fibres. The soaking time of dry-salted hides and skins can be greatly reduced when enzymes are used in this process. Application of protease in combination with surfactants, lipase in combination with surfactants and protease in combination with lipase can rehydrate the wet-salted skins within five hours as compared with the conventional method that utilizes soda ash in combination with surfactants which took nine hours. Therefore, use of enzymes in soaking of hides/skins can reduce the quantity of sodium carbonate utilized in soaking stage and consequently the COD of the effluent (Kanagaraj *et al.*, 2020). Enzymes in the soaking liquor facilitates the rehydration of hides and skin by dissolving non-fibrous proteins (albumins and globulins) that cement the collagen fibres together and break up fats (lipids) present on the skin (Jiaan, 2014). The shorter time taken for soaking after the adoption of use of enzymes also eliminates the need of adding bactericide in soaking process.

In the unhairing process, use of enzymes has been investigated and proteolytic enzymes have shown to be more efficient than amylolytic enzymes (Shivasharana and Naik, 2012). Any unhairing protease should not damage the skins but most of them are known to damage the skin during practical unhairing process. Arunachalam and Saritha (2009), indicated that application of enzymes in unhairing process is very important because it can significantly reduce or eliminate sodium sulphide, and create a conducive atmosphere for tanners.

Another study by Haggran (2014), working on the digestion/breakdown of feathers by use of keratinase enzyme from *Bacillus* species concluded that keratinase enzyme can be utilized

successively in the degradation of feather which is normally composed of keratin. The epidermis and hair are made up of keratin and therefore they can be removed by this enzyme in unhairing process. The strength and elongation of leather are also improved due to use of alkaline protease in unhairing stage (Gutterres *et al.*, 2009). The main target by the unhairing enzymes is the hair bulb and malpighia layer. Digestions of cells of this layer lead to the loosening of hair root and entire epidermis which can be removed by mechanical scrapping (Shivasharana and Naik, 2012). Enzyme protease from *Bacillus* isolate has been reported to unhair within 18-24 hours (Choudhary *et al.*, 2004). Although this was achieved by use of 2% of the crude enzyme the researcher recommended 3% of enzyme because higher concentration was able to completely remove tougher hair at the neck region (Choudhary *et al.*, 2004).

Other researchers have reported that α-amylase can unhair the hides and skins but others have rejected this opinion by suggesting that commercial amylase preparations may be a mixture of protease because of the cost of making a pure sample (Zeng *et al*., 2013). In leather production, amylases can be used to open up the fibrous structure of hides (Dettmer *et al*., 2013).

According to Valeika *et al*., (2012), application of enzymes enables the removal of an intact hair, causing better opening up of collagen structure and faster removal of dermatan sulphate. In addition, it also offers quick penetration of tanning agents which lead to production of soft leather with clean grain. The disadvantage of this process is that some chemicals must be added to remove some remnants of hair, scuds and epidermis (Valeika, *et al*., 2012).

Bating is another important pre-tanning process that has been using enzymes for many years and cannot be replaced with chemical process. Bating softens the leather and makes its surface smooth and clean (Jiaan, 2014).

Fats and oils in hides and skins can be removed by use of lipase enzyme that has no effect on the collagen structure in a process called degreasing. According to Jiaan (2014), the use of lipase leads to production of leather with a clean surface and uniform color. From all these studies, there is a clear indication that the adoption of enzyme in pre-tanning process can be effective in replacing most of the polluting chemicals and auxiliaries and also improve the leather quality as compared to the conventional methods.

2.6 Tanning process

Tanning is not a beamhouse process, but all the pre-tanning processes carried out in the tannery are aimed at ensuring that the tanning process will be effective. The selection of tanning materials mostly depends on cost of tanning agents and leather quality required by the customers. Tanning agents are broadly classified into mineral, vegetable and aldehyde tannages (Sreeram, and Ramasami, 2003). According to Saxena *et al.* (2016), the developing countries are tanning using chrome because it is cost effective and less time consuming while the developed countries are using vegetable tanning method because it can be performed immediately after bating and this significantly reduces the content of sodium chloride and sulfate in effluent hence eco-friendly.

During tanning, the tanned leather becomes resistant to high temperature, reduces the swelling of the fibres and makes the pelt resistance to decay (Maraz 2021). More than ninety percent of the leather sold globally is tanned using chrome (Maraz, 2021). There are many theories that have been suggested to explain the mechanism that occur during tanning. Some of these methods include: reduction of number of reactive groups in a collagen structure, reduction of moisture content of the fibres, separation of fibres and intra- and inter-molecular crosslinking that occurs during tanning (Sreeram, and Ramasami, 2003).

Chrome tanning method is known to produce leather with desirable qualities and it is regarded as the best tanning method (Kanagaraj *et al.,* 2022, Jian-xun *et al*., 2019). Other mineral tanning agents include aluminium, iron and Zirconium (Covington, 2011). Other alternative tanning agents include polyphenols, oxazolidine, formaldehyde, polymers, carbohydrates etc. Aluminum tanning agent is the most promising replacement for chrome in the leather industry as it is readily available, cheap and it produces leather with a competitive performance (Haroun *et al*., 2008).

Vegetable tannins are the second mostly used tanning agents in Kenya. In these methods, tannins which are water soluble plant polyphenolics molecules with a molecular weight between 500-3000 D are used (Ali *et al.,* 2013). Vegetable tanned leathers are mostly used in production of heavy leathers such as shoes uppers, furniture and sole due to their excellent fullness, wear resistance, solidness, air permeability and moldering properties (Koloka and Moreki, 2021).

Zirconium oxide can also be used in a tanning process giving leather with a shrinkage temperature of 95-98 ^{o}C but it must be applied at a high percentage (10%) and produce leather with a fuller substance (Lampard, 2000). Titanium (IV) tanning agent give less versatile leather that is overfilled and large quantities must be applied to obtain a shrinkage temperature above 95^{o}C. Ti (IV) tanning agent is less effective when used alone in a tanning process and large quantities are needed to attain a shrinkage temperature above 95°C. This causes titanium tanned leather to become less flexible (Lampard, 2000). Another important tanning agent is the use of synthetic tanning agents (syntan) (Suresh *et al*., 2001). Different types of syntans are available in market and most of them are used in the re-tanning processes.

2.7 Post-tanning

There are three main post tanning operations in the leather sector. These include: re-tanning, dyeing, and fatliquoring (Fathima *et al*., 2010). They are carried out before the finishing operation. Re-tanning processes are normally carried out to improve the embossing property, cutting value, perspiration resistance, buffing property, fastness to washing and handle of finished leather. The final properties of leather are affected by the level at which the tanning agents penetrate and be fixed into the leather. According to Musa *et al*., (2019), penetration and fixation are affected by the charge of the re-tanning agent and collagen fibers. To ensure proper penetration without fixation, the knowledge of isoelectric point (PI) of materials used is very important. It is known that the PI of raw collagen ranges from pH 7-7.8. The processes that occur during the beamhouse processes and the tanning processes are known to alter these conditions. For vegetable tanned leather the PI is pH 4.5, aldehyde, quinone and oil tanned leather is about pH 4.5 while chrome tanned collagen is pH 6.5 (Fathima *et al*., 2010). Therefore, it is crucial to completely comprehend the surface charges of retanning, dyeing, and fatliquoling agents and how they affect their penetration and uptake in leather throughout the processes in order to optimize their performance scientifically (Musa *et al*., 2019). There are many commercial re-tanning agents such as, vegetable tannins, polymers, Syntans, mineral re-tanning agents and resins and they are used depending on the properties required for the final leathers.

The reaction of collagen fibres toward dye is affected by the action of tanning agents used (Lawal and Nwokocha 2014). There are different types of dyes but anionic dyes are widely utilized in leather industry and comprises of 70% of all the dyestuff (Özgünay *et al*., 2009). They are known to have good penetrating, dyeing properties and also give a brilliant shade. Unfortunately, these dyes are not good in vegetable tanned leathers since they do not fix into the leather and also has

poor fastness properties (Özgünay *et al*., 2009). Therefore, the types of the dyes used must be compatible with the tanning and re-tanning agents.

Fat liquoring is usually the last wet post-tanning processes prior to drying and finishing (Nkwor *et al*., 2021a). It is done by applying warm dilute oil emulsion to leather (Nkwor *et al*., 2021b, Sivakumar *et al*., 2008). The aim of this process is lubrication of collagen fibres, adjustment of the physical properties, waterproofing, filling and protection of leather (Santos and Gutterres, 2007). During fatliquoring stage, the leather and the emulsion must have the same charge to allow good penetration. Conversely when leather or any layer of the leather has an opposite charge to that of an emulsion, then rapid fixation occurs and the deposition of fat occurs. After the penetration of the oil, the pH value is reduced by addition of formic acid to break the oil emulsion to allow fixation and deposition of oil to the fibre structure of collagen (Fathima *et al.,* 2010).

2.8 Assessment of quality of leather

Chemical, organoleptic and physical tests are very important parameters that help to define the usefulness of the leather. Proper sampling is very important for the accuracy of the results. Correct sampling for physical testing is very important in order to get results that are representative. Leather is known to be a heterogeneity material and therefore the physical and chemical properties vary from one part of skin to another. Since most of the physical tests are destructive, accuracy and reproducibility must be maintained but simultaneously avoiding excessive destruction of leather (UNIDO, 1996)

Apart from sampling, changes that occurs in the beamhouse may also affect the quality of leather (Choudhury *et al*., 2006). For example, decorin which is an example of proteoglycans may cause sticking together of collagen molecules leading to production of hard leather if not removed

(Choudhury *et al*., 2006). Collagen, keratin and elastin are the commonest structural proteins of hides/skins. Uncontrolled elimination of fibrous proteins from hide/skin in leather processing leads to unattractive crease formation that are not easy to detect at beamhouse stages and only becomes visible on the finished leathers (Mukhopadhyay *et al*., 2008).

There are many physical tests that can be carried out on the leather to assess their quality. Some of the common tests include: Tensile and tearing strength, percentage elongation, color rub fastness, grain crack load, stitch tearing strength and flexing endurance (Ali *et al*., 2020). The characteristics of leather are determined by physical structure, chemical content and mechanical operations during the leather manufacturing operation. Re-tanning hides and skins causes a decrease in percentage elongation but also improves shrinkage temperature, ball burst, tear and tensile strength of leather (Nalyanya *et al*., 2018). Therefore, different processes in leather processing can change the quality of finished leather and proper control is needed.

CHAPTER THREE: DETERMINATION OF EFFECTIVENESS OF COMMERCIAL BEAMHOUSE ENZYMES IN DIFFERENT STAGES

3.1 Abstract

The study objective was to evaluate the beamhouse enzymes available in the Kenyan market for prospective usage in various pre-tanning procedures in an effort to employ less dangerous chemicals. The study involved five tanneries (Sagana, LIK, AHITI, Ewasonyiro and Yetu leathers) where one hundred and fifty grams of enzymes were gathered from each of them. Moisture content, activity of enzyme and solubility were assessed on the collected enzymes. Protein content, TS and SS of the processing effluent was also determined to assess the effectiveness of enzymes in different pretanning stages. In addition, fat content and organoleptic tests were also determined on the pelt. SPSS statistical software was employed to examine the data and an ANOVA test was conducted to determine whether the difference was statistically significance between the parameters analyzed. Protease activities of the sampled enzymes were found to be: Micro enzyme P-Sagana 24,717.6 (U/g), Microenzyme elbate LIK 23,883.6 (U/g), Microbate Ewaso Nyiro 21,321.33 (U/g), Microbate elbate -AHITI- 11,341.2 (U/g and Microbate-Yetu leather 24,137.4 (U/g). Determinations of fat, protein, suspended and total solid showed no significance difference ($p > 0.05$) in the unhairing and soaking processes and did not unhair. The bated pelt's organoleptic testing yielded a range of 4-5 on a scale of 1–5 (poor–very good), which is a strong indicator that enzymes were appropriate in bating stage. Since these enzymes did not unhair and showed no significant difference in TS, SS, protein content of the of the wastewater and fat content of the pelt, they were regarded as ineffective in soaking, unhairing and degreasing process. In summary, commercial enzymes were particularly efficient only in bating process. Soaking, unhairing, and degreasing steps required the employment of particular formulations.

3.2 Introduction

Trade in leather and leather goods is on the rise worldwide (Mwinyihija, 2014 a) and their demand will continue to grow as human population increases. Africa has a lot of hides and skins which are underutilized (Jabbar *et al*., 2002). Due to high number of animals that are reared in Kenya and other east African countries, leather sector is one of the mushrooming agro-based industries (Mwinyihija, 2014 b) and it has employed thousands of people in rural areas. The availability of hides/skins for use by these tanneries is mostly determined by the number of animals slaughtered and are mostly regarded as a by-product (Tesfaye *et al*., 2015). To produce leather which is a non-putrescible and hydrothermal stable product, the raw materials go through a series of chemical treatments. These chemicals processes are aimed at removing most of the non-collagenous substance before tanning process (Li *et al*., 2010).

It is known that the hides and skins have many components that have to be removed before the tanning process (Zambare *et al*., 2013). Collagen protein is tanned into leather and all other components are completely or partially removed depending on type of leather being made (Beghetto *et al*., 2013). It is in the beamhouse stages where most of these non-collagen substances are removed (Hasan *et al*., 2022). The increasing demand of leather and leather products with different characteristics has constantly pushed the tanners to change and improve the processing techniques.

Tanneries produces inevitable solid and liquid wastes that emanate from various pre-tanning processes and can cause a serious pollution threat if not treated in some ways before discharge (Oruko *et al*., 2014). This is because of the use of concentrated chemicals and this has also led to classification of leather sector among the top hazardous industries in the world (Hasan *et al*., 2022). This solid and liquid waste contain hazardous chemicals that are not biodegradable and toxic

chemical becomes mobile after synergies in the environment thereby posing pollution threat to several ecosystems where they are disposed (Oruko *et al*., 2014).

A lot of chemicals used in the conventional processing methods produces effluent with high content of BOD, COD, DS, SS and Sulphide. Tanneries in Kenya are still facing a lot of challenges and many have been closed due to lack of proper effluent treatment plant. This has called for a worldwide study on the search for a clean technology that can replace use of hazardous chemicals. Use of enzymes has proved to be an area of interest to tanners since they are not toxic and they are biodegradable (Khambhaty, 2020). Despite the fact that tanners have traditionally used enzymes, they have not replaced the use of chemicals (Khambhaty, 2020). The main reasons for this poor adoption are that enzymes work well at optimum temperature, pH, concentration and poor control of these parameters may solubilize the hides and skins (Jayakumar *et al*., 2019).

Since enzymes are very specific, their use has become a popular option for environmentally friendly processing in the leather industry (Hasan *et al*., 2022). Numerous studies are being conducted in an effort to replace dangerous chemicals but complete substitution has not yet been achieved. There are several enzymes that have been proposed for use in the tannery. Amylase, lipase and protease can work effectively in beamhouse stages (Jayakumar *et al.*, 2019). Lipase aid in the removal of fat, protease help in the removal of nonfibrous protein while amylase aid in the removal of proteoglycans (Jayakumar *et al*., 2019).

Enzymatic soaking accelerates the removal of non-collagenous substances, reduces wrinkles and also reduces the time required in a soaking process (Thanikaivelan *et al.,* 2004). Zambare *et al*. (2013), reported an increase in moisture content of the skin due to break down of non-fibrous protein and also reported a complete softening of the hide after 16-18hrs. This is very important

since water content of the skin acts as a carrier for chemicals and other reagents used in the tanneries.

Application of unhairing enzymes helps to eliminate the problem of waste disposal as hair is removed while intact and also reduces the use of sulphide (Andrioli, 2015). The unhairing enzymes act on the soft keratins of the hair root and of the epidermis, loosening the whole epidermis and making them to be easily removed by scrapping. This reduces the BOD, COD and nitrogen level of the wastewater. In addition, leather with cleaner grain layer is produced (Andrioli, 2015). Bating is the only stage in beamhouse where enzymes cannot be replaced by use of chemicals. They have been used successfully for many years in the removal of the remnant of albumins, globulins mucoid and scuds (Mhya, and Mankilik, 2015). Bacterial protease is still used although the common one is protease from bovine and pigs (Mhya, and Mankilik, 2015). Lipase enzyme is very effective enzyme that is mostly used in the degreasing process (Thanikaivelan *et al*., 2004). Enzymatic degreasing helps to prevents defects such as waxy patches, fatty spues, uneven dyeing and finishing (Mhya, and Mankilik, 2015).

From these studies, it is evident that enzymes can play a significant role in leather processing but their adoption has been very low in most of the developing countries. Most of the tanneries are still using the conventional methods which are known to pollute the environment. Kenya being one of the developing countries is also expected to be slow in the adoption of clean technologies. This study was undertaken to determine the commercial enzymes, their quality, and effectiveness in various pre-tanning stages.

3.3 Materials and methods

3.3.1 Study area

There were 12 operating tanneries during the time of study in different parts of the country. Letters were sent to all the tanneries requesting for their involvement in the study. Only five tanneries approved the request and they were all selected for the study. They included Ewaso Nyiro, Yetu leather, Sagana, AHITI and LIK (Fig 3-1).

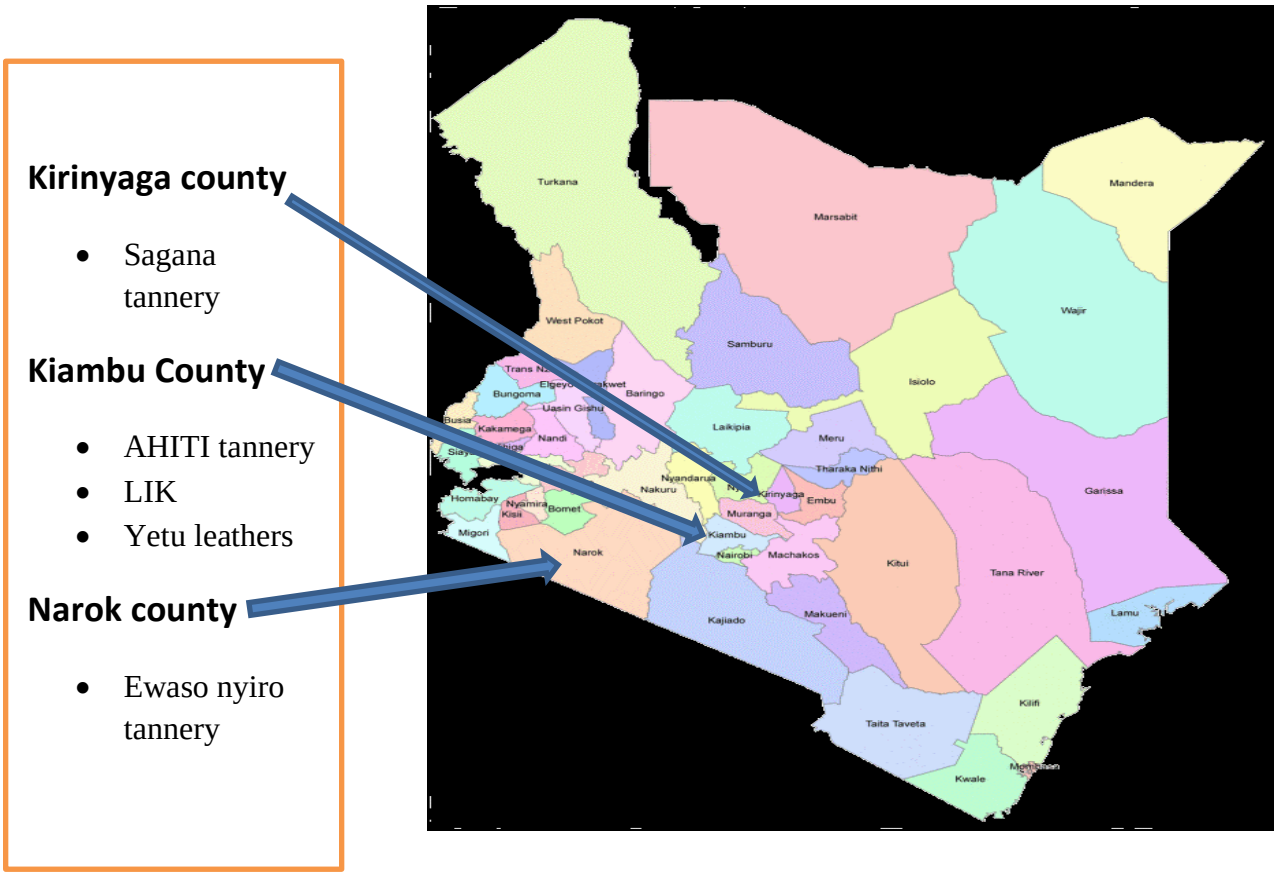

Figure 3-1: Location of the study areas

3.3.2 Sample collections

A structured questionnaire was used to collect more information about the enzymes, types of raw materials processed and challenges they were facing (appendix 1). 150 g of enzymes were gathered from each of the tanneries for further analysis.

3.3.3 Determination of protease activity

It was determined in accordance with Bureau of Indian Standards (BIS) (1991). One hundred milliliters of distilled water was added into a beaker containing one gram of enzyme. The beaker's contents were held at 37°C for two hours while being occasionally stirred, and after filtering, the enzyme extract was obtained. To the previously prepared 10 ml of casein solution, distilled water and enzyme extracts (2.5 mL each) were added. The preparation was placed in Erlenmeyer flask and incubated for 30 minutes at 45 °C. 30 ml of a 5 percent trichloroacetic acid solution was added after the time interval to stop the reaction. Boiling water bath was then used to heat the mixture for three minutes then filtered and cooled to room temperature. A solution of filtrate (0.5 ml), sodium hydroxide (5.0 ml), distilled water (2 ml) and diluted Folin phenol reagent (1.5 ml) were combined. After shaking, the color produced (blue) was determined at 660 nm using a spectrophotometer. The identical procedures were used to carry out a control, but the enzyme solution was introduced after addition of trichloroacetic acid and before placing the samples in an incubator. For the calculation of the enzyme unit, the control values were subtracted from the experimental results.

3.3.4 Determination of moisture content of enzymes

It was assessed in accordance with Bureau of Indian Standards (1991). Bating enzymes weighing five grams were placed in a porcelain basin and dried for six hours at 105°C. The porcelain basin

with dried enzymes was cooled inside a desiccator and its weight taken. The final weight was recorded after subsequent drying with no weight change noticed.

$$\text{Moisture content} = \frac{(\text{M} - \text{m})}{M} \times 100$$

M= mass (g) of dried enzyme

M= Mass (g) of undried sample

3.3.5 Determination of insoluble matter

It was determined as described by BIS, (1991) specifications. In a 500-ml beaker, 10 grams of enzymes were carefully weighed before being dissolved into four hundred milliliters of distilled water. It was dissolved and stirred before filtering through Whatman filter paper (No. 4), which was then repeatedly rinsed using distilled water. The filter paper was subjected to a uniform drying process in a hot oven set at 105°C. Afterward, it was cooled in a desiccator and its weight was measured.

$$\text{Insoluble matter} = \frac{\text{m}}{\text{M}} \times 100$$

m=mass of residue (g)

M= mass of sample (g)

3.3.6 Effectiveness of enzymes in different pretanning stages

Eight goatskins were utilized to evaluate efficiency of the commercial beamhouse enzymes in various pre-tanning procedures. Samples from the butt region weighing about ten grams were taken in accordance with the Society of Leather Technologist and Chemists' (2001) recommendation. Five percent (5%) of the enzymes were utilized for the soaking and unhairing

processes for 5 and 24 hours, respectively. Percentage weight gain and the amount of fat in the pelts were evaluated. The liquor's protein level, TS, and SS were assessed as described by Clesceri and the American Public Health Association (1989). To assess the effectiveness of enzymes in a bating stage, sampled pelts were processed by commonly used unhairing method (Table 3-1) and the delimed skins bated by use of two percent of enzymes.

Table 3-1: Processing of delimed pelt by conventional method

PROCESS	**%**	**PRODUCT**	**RUN**	**REMARKS**
Dirt soak	400	water@	30min	Drain
Main soak	400	water@		
	0.5	Wetting agent		
	0.5	Biocide		Leave it overnight (18hrs
Unhairing and liming	200	Water		
	0.85	Na_2S	15min	
	1.30	Na_2S	15min	
	0.85	Na_2S		
	0.7	Lime	20min	
	1.0	Lime	10min	
	1.3	Lime	10min	Leave it for 18 hours Check for Unhairing, swelling/plumping
Fleshing with machine				
Deliming	100	Water		
	2	Ammonium sulphate	1hr	Phenolthalein indicator check for lime free- clear colour change.
Bating	100	water@ 35^oC		

	2	Bating enzymes	1hr	Thumb imprint test

The following parameters grain firmness, appearance, softness, flexibility and thumb imprint were rated on a range of 1-5 (very Poor- very good).

3.3.7 Total solids of the effluents

The total solids was determined according to Clesceri and American Public Health Association, 1989). An oven was employed to dry a clean dish at 105^{o}C for one hour before placing it in a desiccator to cool until usage. After weighing the dish, 25 ml of well-mixed liquid samples were introduced. To prevent splattering, an oven's temperature was decreased below the boiling point (by 2 ^{0}C). They were then evaporated for one hour in an oven at 103-105^{o}C. They were cooled inside a desiccator and their weight was measured. The cycle of drying and cooling was repeated until the weight loss change was < 0.5 mg.

$$\text{TS (mg /L)} = \frac{(A-B)\times 1000}{\text{Volume of sample (ml)}}$$

B= weight of dried residue (mg)

A=weight of dried residue + dish, (mg)

3.3.8 Total suspended solids

The TS was determined according to Clesceri and American Public Health Association, 1989).The filter and the filtering apparatus were assembled. Distilled water (small amount) was used to wet the filter to seat it. Glass fiber filter paper was used to filter twenty-five millimeters of the samples. It was then cleaned three times in succession with distilled water (10 mL), allowing for full drainage between washes and three minutes of continuous suction following the completion of

filtration. The filter was taken out of the filtering device and placed on an aluminum disc. The leftover materials were heated at 103^{o}C and cooled inside a desiccator. The dying and cooling process were repeated until a fixed weight loss of < 0.5 milligrams was achieved.

$$SS\ (mg\ /L) = \frac{(A-B)\times 1000}{Sample(ml)}$$

B= weight of filter paper (mg).

A= weight of filter paper + dried residue (mg),

3.3.9 Determination of fat

It was carried out as described by International Union of Leather Technologist and Chemist societies (2001) IUC/4. Wet samples of skins were dried at 50^{0}C in an oven and then conditioned to a relative humidity and temperature of 65 % and of 20 ^{0}C respectively for 24 hours prior to grinding. 10 g of ground samples were weighed and placed in filter paper thimble. Cotton wool was used to cover the materials in the thimble. The flask used for extraction was dried at 102 ±2^{0}C for 30 minutes Afterward, it was cooled in a desiccator and its weight was recorded. The continuous extraction using dichloromethane was carried out for a minimum of 30 solvent replacements. The extracts were dried at 102 ± 2 ^{0}C for four hours after distillation of the dichloromethane. The dried extracts were cooled inside a desiccator for 30 minutes and its weight taken. The process repeated until weight loss was <10 mg.

$$\% \text{ fat} = 100\ M_1/M_*$$

Where: M_1= mass of extracts

M_*= mass of the sample used

3.4 Data analysis

It was done by use of Social Science Statistics Package (SPSS). Results were presented using means, standard deviation and graphs. Significance levels were tested using ANOVA and post hoc analyses were conducted, employing Duncan's multiple comparison test to identify statistically distinct means among the parameters ($p \leq 0.05$).

3.5 Results and discussion

All identified tanneries were involved in the study and during the sample collection, it was noted that all the tannery were using cattle hide, sheep and goatskins as a raw material. In addition to this, AHITI tannery was also processing fish and ostrich skin but on order basis. All the tanneries were only using bating enzyme in the bating stages of leather processing which was sourced from the local suppliers. Forty percent (40%) of the assessed tanneries indicated that the bating enzymes were too costly whereas 60% indicated that the bating enzymes were affordable. The study also found that 40% of the tanneries wanted to know whether there is a place where they could get quality enzymes at affordable prices. From the samples collected it was found that the tanneries were using microbate, microbate elbate, microenzyme p and microenzyme elbate enzymes and all of them were sold in Kenyan market. Their effectiveness in soaking, unhairing, bating and degreasing were assessed and they were different. These enzymes had different solubility, protease activity and moisture as presented in table 3-2.

Table 3-2: Solubility, Moisture content, and Enzyme activity of commercial bating enzymes

Enzyme	Solubility (%)	% Moisture	Enzyme activity (U/g)
Microbate-Ewaso Nyiro	60.53 ±2.42	3.08 ± 0.88^{a}	$21{,}321.33 \pm 54.64^{d}$
Microbate-Yetu leather	65.65 ± 2.16	0.28 ± 0.08^{d}	$24{,}137.4 \pm 65.25^{b}$

Microenzyme P-Sagana	64.06 ± 1.95	0.91 ± 0.07[c]	24,717.6 ± 109.84[a]
Microbate elbate -AHITI	53.81 ± 7.28	0.20 ± 0.07[d]	11,341.2 ± 68.05[e]
Microenzyme elbate- LIK	67.74 ± 1.9	1.86 ± 0.23[b]	23,883.6 ± 97.10[c]
p- Values	0.140	< 0.001	< 0.001

When assessing the quality of bating enzymes, parameters such as moisture, solubility and enzyme activity are very important. There was a high significant difference in moisture content and protease activity of enzymes (table 3-2). Micro enzyme-p had the highest protease activity (24,717.6±109.84 U/g) while Microbate elbate enzyme-AHITI had the lowest protease activity (11,341.2 ± 68.05 U/g). Duncan multiple comparison tests showed a significant difference in all the enzyme activities. The minimum required enzymes activity of bating enzymes is 10, 000 U/g and all the assessed enzymes had higher values than this (Table 3-2). The composition of the formulations of most of the enzymes used in the leather sector has remained a secret to the manufacturers and most of the leading enzyme companies do not indicate the activity of the enzymes in their package and tanners have known the correct amount to add based on their experience. The use pancreatic proteolytic enzyme with an activity of 250,000 U/g in a bating process has been documented (Song *et al*., 2019). A study by Ding *et al*., (2021), also documented use of TM enzyme with an activity of 100,0000 U/g, and active at a pH of 8-11. From this comparison it is evident that different enzymes in the market have different activity. The difference in solubility of enzymes was not statistically significant (p= 0.140) and all failed to meet the minimum requirement of 90%. Microbate elbate -AHITI had the lowest solubility (53.81 ± 7.28 %) while Micro enzyme elbate- LIK had the highest solubility (67.74 ± 1.9%) as indicated in Fig 3-2. Moisture composition of all tested enzymes was within the required range <5 %.s

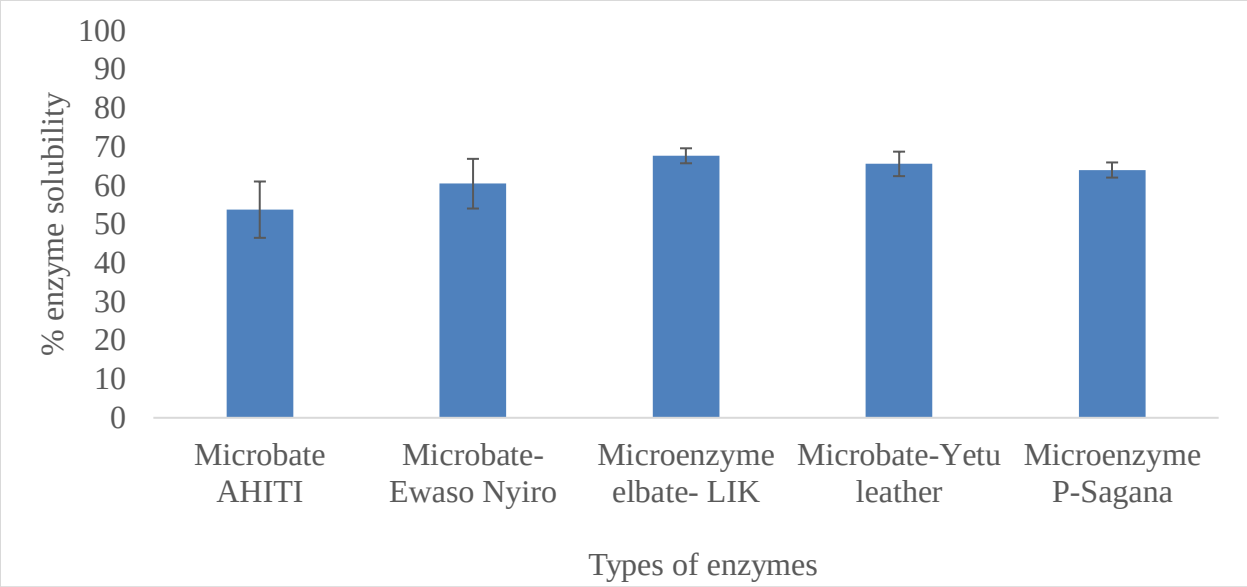

Figure 3-2: Solubility of commercial bating enzymes

The determined protein content, TS, SS and fat content to investigate the performance of enzymes in soaking stage indicated no significant difference ($p > 0.05$) as can be seen in Table 3-3.

Table 3-3: Weight gain, total solids, suspended solids, protein content and fat content assessed in a soaking process

	%Weight gain (g)- (5 Hrs)	**TS (mg/l)**	**SS (mg/l)**	**Protein (%)**	**Fat content (%)**
Microenzyme P- Sagana	57.17 ± 0.82	89.07 ± 28.76	11.38 ± 0.90	0.47 ± 0.14	4.80 ±2.4
Microbate-Yetu leather	66.29 ± 5.06	115.58 ± 6.26	10.25 ± 8.10	0.48 ± 0.16	3.98 ±1.22

Microbate elbate-AHITI	63.69 ± 8.77	71.79 ± 1.51	11.73 ± 0.12	0.42 ± 0.11	7.88 ±3.39
Microbate-Ewaso nyiro	57.34 ± 4.94	99.22 ± 42.28	10.35 ± 2.19	0.49 ± 0.18	5.96 ±2.28
Micro enzyme elbate-LIK	75.01 ± 5.56	93.68 ± 39.25	8.14 ± 3.76	0.56 ± 0.18	7.47 ±1.28
Blank	56.86 ± 3.13	73.57 ± 5.44	7.67 ± 1.80	0.41 ±0.30	7.82 ±1.483
P-values	0.02	0.183	0.4229	0.682	0.423

Soaking process is very important as absorbed water in the pelt acts as a carrier and facilitates penetration of chemicals into the pelt. The amount of absorbed water can be assessed by assessing the weight gain of the pelt. A mixture of enzyme with soda ash and surfactant has been reported to enhance weight gain of cured hide by 45 % (Stockman *et al*., 2008). In this study application of enzymes increased protein content of soaking liquors. Although the blank sample had lower protein content as compared to enzymatic samples the difference wasn't significant (P= 0.68). The protein content mostly represents nonfibrous protein and mostly albumins and globulins removed from the hides and skins. Microbate-Yetu leather was the most effective in the removal of fats. The samples processed by these enzymes had a residue fat content of (3.98 ±1.22%) and the difference wasn't statistically significant (P= 0.42) from the others. According to Afsar and Cetinkay, (2008), use of enzymes protease and lipase in unhairing and liming reduced the fat to below 4%. From this study microbate enzyme from Yetu leather was the only enzyme that produced a pelt with a fat content lower than 4% (3.98 ±1.22). The difference in total solids was

not statistically significant (P= 0.18) for all the samples tested. Total soluble includes all the dirt's, dung, and the soluble components of hides and skins.

Table 3-4: Measured parameters in the unhairing process

	% Weight gain (g)-(24Hrs)	Proteins (%)	SS (Mg/l)	TS (Mg/l)	Fat content (%)	Unhairing
Micro enzyme P-Sagana	57.17 ± 0.82	0.75 ± 0.19	11.32 ± 0.95	79.29 ± 11.41	8.65 ± 1.43	No
Microbate-Yetu leather	66.29 ± 5.06	0.70 ± 0.13	12.44 ± 3.21	81.37 ± 0.79	7.04 ± 0.01	No
Microbate elbate- AHITI	54.86 ± 2.57	0.66 ± 0.09	14.88 ± 3.44	57.35 ± 23.35	7.97 ± 0.65	No
Microbate-Ewaso Nyiro	57.38 ± 0.79	0.79 ± 0.28	10.39 ± 2.48	79.81 ± 14.82	8.15 ± 1.42	No
Micro enzyme elbate LIK	72.88 ± 4.58	0.67 ± 0.08	15.69 ± 2.12	85.96 ± 18.72	7.42 ± 1.16	No
Blank	58.21±0.749	0.61 ± 0.14	11.10 ± 3.43	64.95 ± 4.39	9.21 ±0.62	No
P – Values	p< 0.001	0.967	0.273	0.684	0.971	

Of all the unhairing parameters only percentage weight gain within 24 hours had a significant difference p< 0.001. All the test samples absorbed a lot of water within the first hour as indicated on Fig 3-3

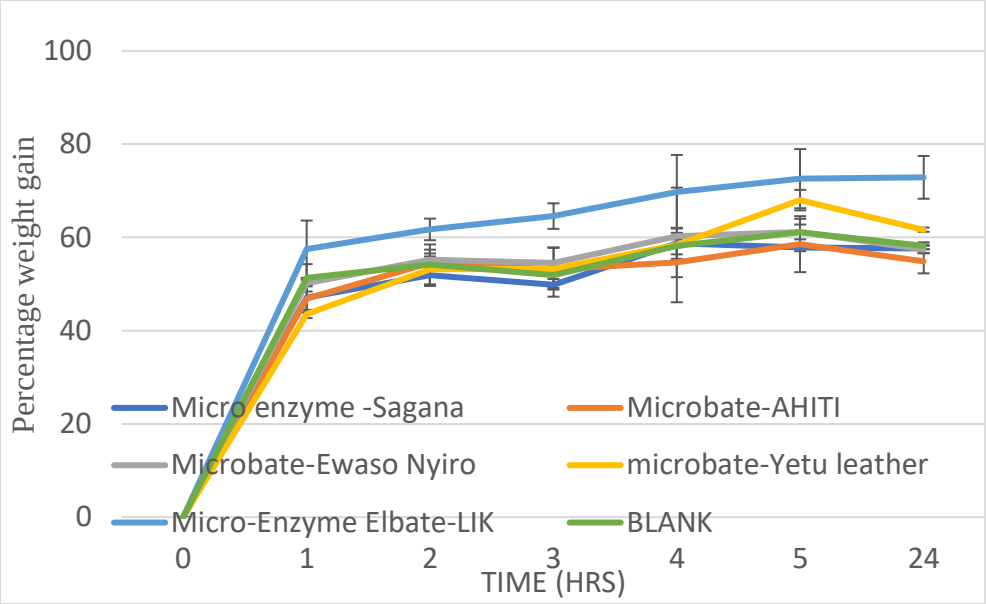

Figure 3-3: Percentage weight gain of the pelt during 24 hours

Time and the type of enzymes used in the process had an effect on percentage weight gain of the skin (P< 0.001). Multiple comparison tests showed no significant differences in percentage weight gain when Microbate-yetu leather, microbate elbate-AHITI, Microbate-Ewasonyiro, microenzyme p-sagana and the blank were used in the process but the difference in percentage weight gain when microenzyme elbate-LIK was used showed a significant different from all the other enzymes. Soaking time also had an effect on weight gain. The skin experienced an increase in weight gain during the initial hours after soaking (Figure 3-3). This study showed a lot of improvement as the percentage weight gain ranged from 54.86% - 72.88 % as compared to Ângela *et al.*, (2018), that reported a weight of 41.1% when wet-salted pelts were soaked for a period of 24 hours

Organoleptic properties of bated skins (appearance, flexibility, thumb imprint, grain firmness and softness) were determined at a range 1-5 (poor - very good). In all the evaluated tests, they had a range of 4-5 which is good evidence that enzymes were appropriate for this processing stage

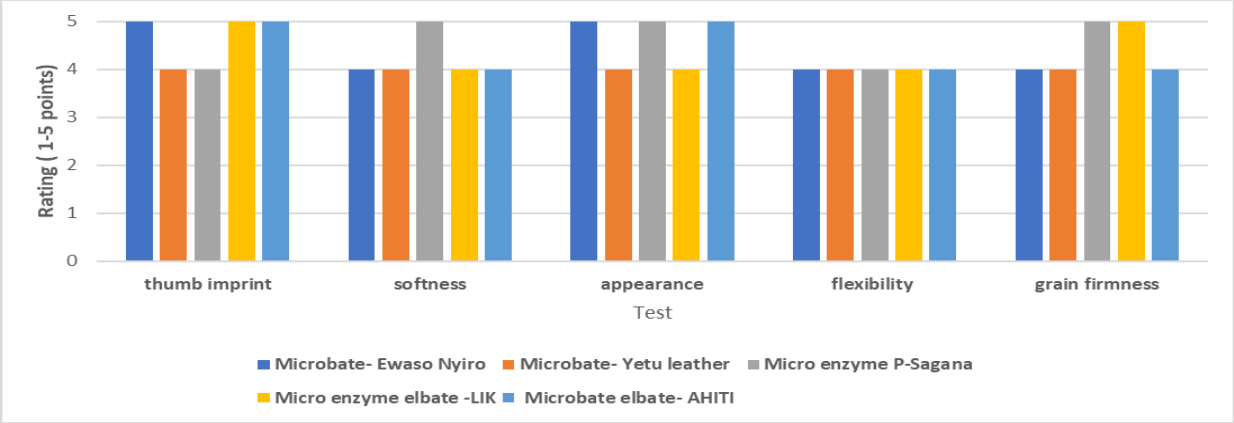

Figure 3-4: Organoleptic test of bated pelts

Application of enzymes during the bating processes aids in the removal of remnants of hairs, epidermis and non-fibrous proteins. Complete removal of these substances causes the skin to be clean, soft, flexible and ability to retain a thumb imprint. Since the rating was high for all the tested organoleptic properties, it is evident that the enzymes were successive in opening up of the skin collagen structure (Zambare *et al*., 2010). Higher efficiency of bating enzymes in the removal of non-collagenous substances without affecting the collagen protein produces leather with smooth grain that is soft and flexible (Hameed *et al*.,1996).

Conclusion

Bating enzymes in Kenyan market are ineffective in soaking, unhairing and degreasing processes hence new enzymes formulations are required for use in these processes.

CHAPTER FOUR: ASSESSMENT OF THE EFFECTIVENESS OF DIFFERENT ENZYME COMBINATIONS FOR USE IN DIFFERENT PRETANNING PROCESSES

4.1 Abstract

High pollution resulting from the use of hazardous chemicals in tanneries necessitates the exploration of alternatives. This research study aimed to evaluate the efficiency of various enzyme combinations for application in pre-tanning stages. The study utilized the enzymes keratinase, amylase, lipase, and protease. Various enzyme formulations, comprising single, dual, triple, and quadruple enzyme combinations, were prepared for the soaking, unhairing, and degreasing stages. The study also involved assessing the protein content, as well as the TS and SS in the resulting solutions to assess the effectiveness of the enzymes. Furthermore, organoleptic tests, weight gain, and the remaining fat on the pelt were examined. The analyses, both qualitative and quantitative, were conducted using SPSS software. A formulation combining keratinase and lipase (KL) was chosen for the soaking process, resulting in a clean pelt with a weight gain of 68.3% and a residual fat content of 8.40%. For complete unhairing of the pelt with a residual fat content of 6.4%, the best formulation was found to be one combining keratinase, protease, and lipase (KPL). Lastly, the application of lipase enzyme was recommended for the degreasing process. Notably, the organoleptic tests of the pelts processed with these selected enzyme formulations received ratings between 8 and 10, indicating that the use of enzymes can serve as a viable and environmentally friendly alternative to hazardous chemicals while still producing high-quality leather. In conclusion and formulation of KL and KPL performed well as compared to other formulations and they were recommended for use in soaking and unhairing process respectively while lipase enzymes were recommended for degreasing process.

4.2 Introduction

Hides/skins, byproducts of the meat industry, serve as the primary raw materials in tanneries. These skins consist of three main layers: epidermis, corium, and hypodermis (Naffa *et al*., 2019). When processing leather, the hypodermis and epidermis layers are typically removed, leaving the corium as the primary layer utilized by tanners. The chemical makeup of hides and skins can vary due to a range of factors, such as the animal's breed, age, gender, dietary habits, and other farming techniques (Salehi *et al*., 2013). For intance, cattle hide contain pigments (0.5 %), proteins (33%), inorganic components (0.5%) fats (2%), and water (64%) (Beghetto *et al*., 2013). Collagen, the main structural protein, constitutes approximately 29%, while elastin and keratin make up around 0.3% and 2% respectively (Beghetto *et al*., 2013). Tanners make deliberate choices to either eliminate or keep these elements, depending on the specific kind of leather they intend to produce, with collagen being the fundamental protein for leather manufacturing.

Hides/skins pasess through subsequent beamhouse stages aimed at cleaning the collagen stracture by removing non-collagenous materials (Mella *et al*., 2016). Throughout these stages, a significant volume of water and chemicals is employed, which has resulted in categorizing the leather sector among ecologically harmful sectors on a global scale (Yorgancioglu *et al*., 2020). In the course of these activities, a substantial amount of wastewater is generated, characterized by elevated levels of chromium, sulfide, organic nitrogen, TS, SS, COD and BOD. The significant pollutants discharged by these industries present a hazard to both the natural world and the well-being of people (Mella *et al*., 2016, Zhihua, 2017). As per a 2015 report from China's environmental protection agency, more than 300 million individuals lack immediate access to clean drinking water, and an additional 60 million are exposed to subpar water quality because of the pollution

resulting from the discharge of chromium and other metallic substances by tanneries. (Han *et al*., 2021).

Given that the pre-tanning phase produce over 70% of overall pollution in tanneries (Zhihua *et al.,* 2017), it is imperative to promptly embrace eco-friendly technologies that can significantly reduce or entirely substitute the hazardous chemicals within the tanning stage. The use of enzymes has been proposed as the most favorable substitute. Various classes of enzymes can be employed during different pre-tanning phases in leather processing to remove various noncollagenous constituents of skins. Lipases, amylases. proteases, chondroitinases, amidases and phospholipases can be utilized in the soaking stage to expedite the elimination of non-fibrous proteins, fats, and enhance the rehydration process (Biškauskaitė *et al*., 2021, Valeika *et al*., 2019).

Protease and keratinase enzymes can be employed in the unhairing stage. Reports have indicated that they can achieve thorough unhairing without harming the grain, resulting in high-quality leather, while also reducing the discharge of pollutants in both wastewater and emissions. The utilization of enzymes for unhairing eliminates the need for sulphides and hydrosulphides as dehairing agents. The use of sulphides during hair removal raises the COD levels and the expenses associated with wastewater treatment (Spinosi *et al*., 2018).

Protease and keratinase enzymes can be employed in unhairing stages, and studies has proved that they effectively remove hair without damaging the grain and result in high-quality leather. Additionally, their use contributes to a reduction in pollution, particularly in terms of emissions and wastewater (Spinosi *et al*., 2018. By employing enzymes for unhairing, the need for hydrosulphide and sulphides as unhairing agents is eliminated. The use of sulphides in unhairing process not only raises the COD but also raises the expenses associated with effluent treatment

The traditional degreasing process typically involves the utilization of emulsifying degreasing and solvent degreasing techniques. However, these methods require significant initial expenses and are marked by safety concerns (Yu *et al*., 2021). Furthermore, their overuse results in the release of degreasing agents into wastewater, thereby contributing to environmental contamination (Yu *et al.*, 2021). Enzymes can be a good substitute to most of these hazardous chemicals. The majority of previous research has focused on individual processing stages involving the use of one or more enzymes. This study, however, seeks to evaluate the efficiency of various enzyme combinations across various pre-tanning stages, with the objective of reducing the use of harmful chemicals within the beamhouse procedures.

4.3 Materials and Methods

4.3.1 Sample collection

Four distinct enzymes with well-documented activity levels were acquired from Jinan Grace Industry Co. Ltd in China and employed in this investigation. These enzymes include lipase (100,000 U/g), keratinase (200,000 U/g, protease (200,000 U/g) and amylase (100,000 U/g). The wet-salted goat skins utilized in this research were sourced from the curing facilities located adjacent to Dagoretti slaughterhouse.

4.3.2 Assessment of the optimum pH and Temperature for the enzyme's activity

The pre-tanning stages (soaking, unhairing/liming, deliming, bating and degreasing) are normally carried out at a pH of 7-12 and a temperature between 25-35 ^{0}C, the optimum conditions were assessed within this range. The optimum conditions for enzymes were assessed using different substrate by use of qualitative and quantitative test. The assessed enzymes include keratinase, amylase, lipase and protease.

4.3.3 Effects of Temperature on lipase activity

It was determined as described by Rose (2010). Five test tubes were labeled with the following temperatures: 25, 28, 30, 33, and 35 °C. Each tube received five drops of indicator (phenolphthalein). Each tube received additional 5 ml of milk and 7 ml of a solution of sodium carbonate. The mixture took on a pink hue. A thermometer was inserted into the test tube, which was then submerged in a water bath and allowed to stand until the required water bath's temperature was attained. The thermometer was taken out and replaced with a glass rod. The test-tube was filled with one milliliter of lipase enzymes equilibrated to temperature under investigation and a stopwatch started. The content of the test-tube was stirred until the pink color of solution was lost (plate: 4-1). The time noted in a suitable result table

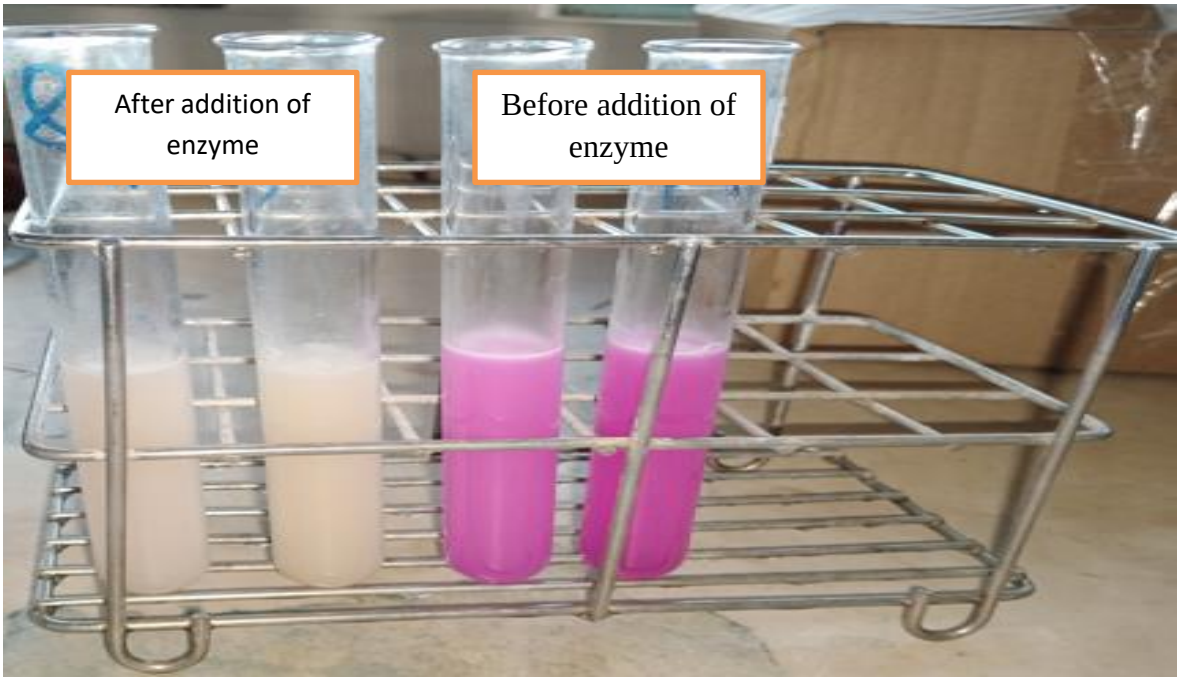

Plate 4-1: Observed color change due to addition of lipase enzyme in milk samples

4.3.4 Effects of pH on lipase activity

To assess the effects of pH on activity of lipase, a whole bated pelt from goat skins was used. The pelt was processed using the conventional method. Samples 2 cm X 2 cm were cut from the butt area. Since sodium carbonate is mostly used to raise pH (8 -9) during processing of hides and skins, it was used in this experiment to create different pH. The assessed pH includes pH 7,8,9 and 10. Two percent of enzymes based on pelt weight were added at a temperature of 30 ^{0}C and run for one hour. The samples were then dried and the fat content determined according to official method (SLTC, 1989).

4.3.5 Effects of temperature on amylase enzyme

Different temperatures were set on water bath: 25, 28, 30, 33 and 35 °C. One drop of solution of iodine was put inside the well of a spotting tile. Five test-tubes with two milliliters of 1% starch solution and five test-tubes containing two milliliters of one percent amylose solution were prepared. Each pair of the test tube was labelled and placed in the appropriate water bath for ten minutes to equilibrate. Two milliliters of a solution of starch were added into 1% amylase solution and put in a water bath at 25^{0}C and timer started. A drop of solution mixture was removed and placed into iodine in its first well and color change observed. It was repeated after 30 seconds until no color change was noticed (the iodine remains yellow-brown). The steps were repeated for the other temperature. The method was repeated to obtain three repeats for each temperature (Rose, 2010).

4.3.6 Effect of pH on amylase enzyme activity

Four test tubes were labeled pH 7, 8, 9, and 10. Four milliliters of appropriate buffers with corresponding pH was added. Four milliliters of amylase solution were added to each test tube. Another additional seven test tubes were obtained and 4 ml of one percent starch solution added

to each tube. All fourteen tubes were immersed in a water bath for a duration of five minutes to reach a consistent temperature. The contents of each test tube were transferred into a separate test tube containing a distinct amylase buffer. After stirring, they were subsequently positioned in a water bath set at 37°C. After fifteen minutes, four drops from each reaction were taken using a dropper to a spot plate. Iodine solution (one drop) was placed to each spot plate and color change observed and recorded. A graph of enzyme activity level vs pH was then recorded.

4.3.7 Effects of temperature and pH on protease and keratinase enzymes

Since both keratinase and alkaline protease enzymes are known to unhair hides and skins although through different mechanism, the methods of determining optimum temperature and pH conditions were the same. These optimum conditions were performed as described by Nyakundi *et al*., (2021). The effect of pH and temperature were determined by immersing pieces of goatskin into the enzyme solution and checking the extent of hair removal regularly. Pieces weighing 10 grams were cut from the butt area of goatskins. They were washed thoroughly with water and submerged in water for two hours. The pieces were dipped in a flask containing 100% of water and 5% enzyme at 28 ^{o}C and a pH range of 7-13. A blank test was also run with 100% of water but without enzymes. The assessment of unhairing percentage was based on the ratio of the area from which hair had been removed to the total area of the test piece. A complete unhairing was treated as 100% while no unhairing was zero percent.

To evaluate the effect of temperature in unhairing process, pieces of goatskin were immersed in the flasks containing 2% enzyme and 100 % water at pH 12 and temperature range of 25 - 35°C. An additional control experiment was conducted within the same temperature range, in which a solution consisting of 100% water without the presence of enzymes at a pH of 12 was used. The

flasks were closed and shaken at intervals of 10 minutes. Unhairing was assessed after every one hour.

4.3.8 Formulation of enzymes

The assessment of enzyme effectiveness involved the use of individual enzymes, pairs of enzymes, sets of three enzymes, and all four enzymes combined. The details of these enzyme combination trials can be found in Table 4-1. The formulations were prepared by mixing equal proportions of enzymes for each formulation.

Table 4-1: All formulations of enzyme

Formulation type	**Possible Combinations**
Single enzymes	Keratinase (k)
	Amylase (A)
	Lipase (L)
	Protease (P)
Two enzyme combinations	Keratinase + protease (KP)
	Lipase + amylase (LA)
	Amylase + keratinase (MK)
	Lipase + keratinase (LK)
	Lipase + protease (LP)
	Amylase + protease (AP)
Three enzyme combinations	Amylase + lipase + keratinase (ALK)
	Keratinase +protease+ amylase (KPA)

	Keratinase + protease + lipase (KPL)
	Protease + amylase + lipase (PAL)
Four enzymes' combinations	Keratinase + amylase + protease+ lipase (KAPL)

4.3.9 Assessment of the effectiveness of enzymes in different pre-tanning processes

A total of sixteen goat skins were employed. Skin samples weighing 10 grams were extracted from the buttock area, following the guidelines outlined by the Society of Leather Technologies and Chemists (1996). During the soaking and unhairing stages, a mixture consisting of five percent of the enzyme formulations and an amount of water equivalent to two hundred percent based on skin's weight was applied. These processes were carried out for durations of 5 and 24 hours, respectively. The samples were positioned within 1-liter beakers and gently agitated using a horizontal laboratory shaker (as shown in Plate 4-2)

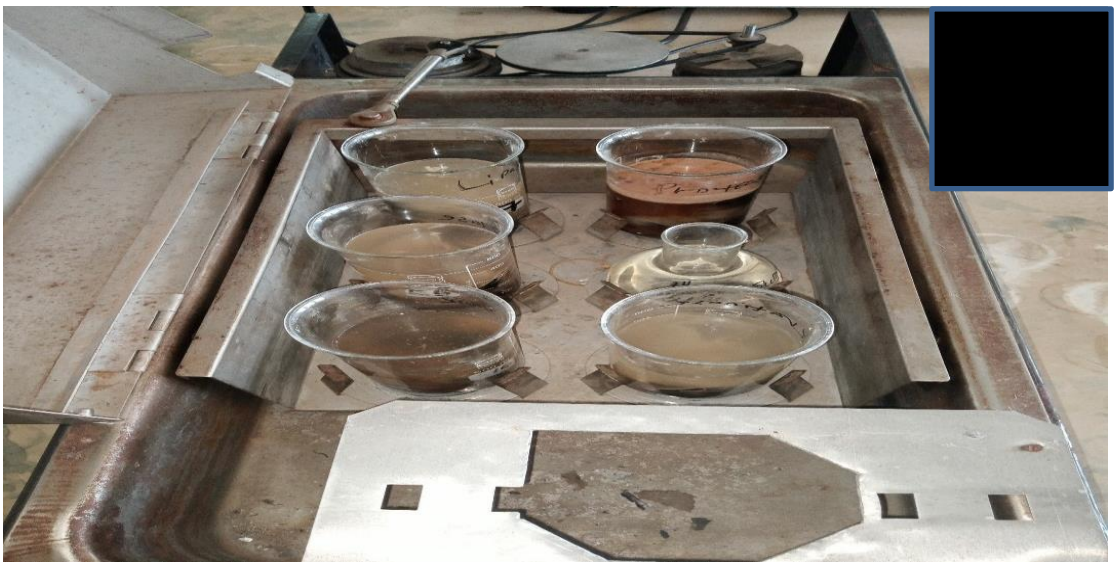

Plate 4-2: Laboratory shaker used to soak the skins

Regular observations were made on all segments of goat skins to monitor the extent of hair detachment and any changes in weight. The evaluation of formulations' performance in degreasing step was evaluated on the remaining fat content following the soaking of hides/skins for durations of 5 and 24 hours. Various factors were assessed, including fat content and pelt weight gain. Total and suspended solids of the processing liquors were also determined according to Clesceri and the American Public Health Association (1989). In every set-up, a control sample without enzymes was employed to serve as a point of reference for comparison.

4.3.10 Total solids and suspended solids

TS and SS were determined as described by Clesceri and the American Public Health Association (1989).

4.3.11 Protein content

Protein content was determined both in soaking and unhairing liquors as described by Association of Official Analytical Chemists (1995).

4.3.12 Determination of fat

It was determined according to SLC 4 (IUC4) (1996).

4.3.13 Assessment of the Organoleptic tests of unhaired and degreased pelts

The most efficient enzyme combinations were chosen for the soaking, unhairing, and degreasing stages. Ten-gram skin samples underwent soaking and unhairing using these enzymes. Subsequently, they were subjected to liming with a 5% calcium hydroxide solution and then delimed with a 2% ammonium sulfate solution. To this, 5% lipase enzyme based on the weight of the pelt was added and left for one hour. Various parameters, including cleanliness, firmness, appearance, flexibility, softness and thumb imprint were evaluated for both enzyme-treated and

sodium sulfide-treated unhaired pelts (the conventional method). Ratings were assigned on a scale from 1 to 10, ranging from very poor to very good, as per the method outlined by Unango *et al*. (2019)

4.4 Data analysis

SPSS was used to analyse the data. The results were presented using descriptive statistics, which included measures such as averages, standard deviations, and graphical presentations. ANOVA analysis was performed to determine the significance level and Duncan multiple comparison tests were also carried out to identifies means that were statistically significant ($p \leq 0.05$).

4.5 Results and discussion

From results obtained, the optimum pH for amylase, lipase and keratinase was found to be pH of 8. Alkaline protease was highly effective at a pH 8-10 although the optimal pH was found to be 10. The optimum conditions for these enzymes were recorded (appendix 6) and these variations can be seen clearly in Fig 4-1, Fig 4-2, Fig 4-3, Fig 4-4.

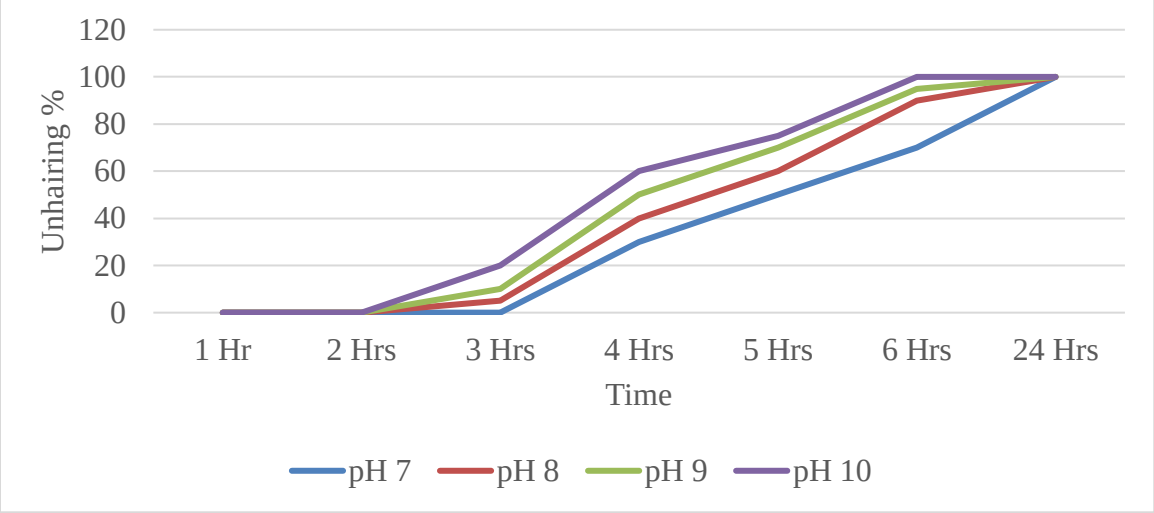

Figure 4-1: Effect of pH and time on unhairing process by use of protease enzymes

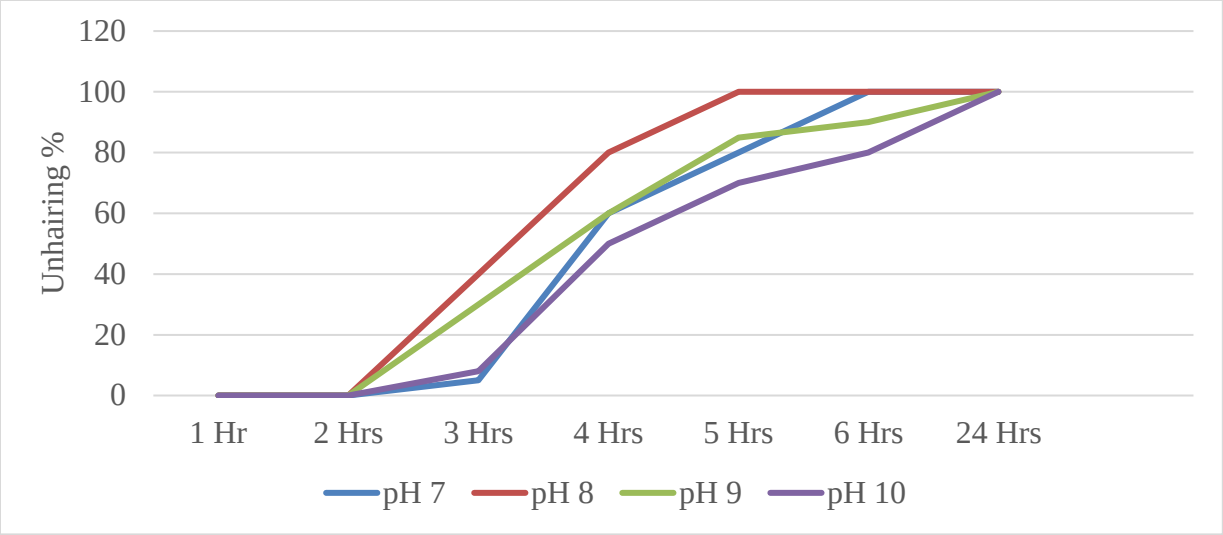

Figure 4-2: Effects of pH and time on the unhairing process by use of keratinase enzyme

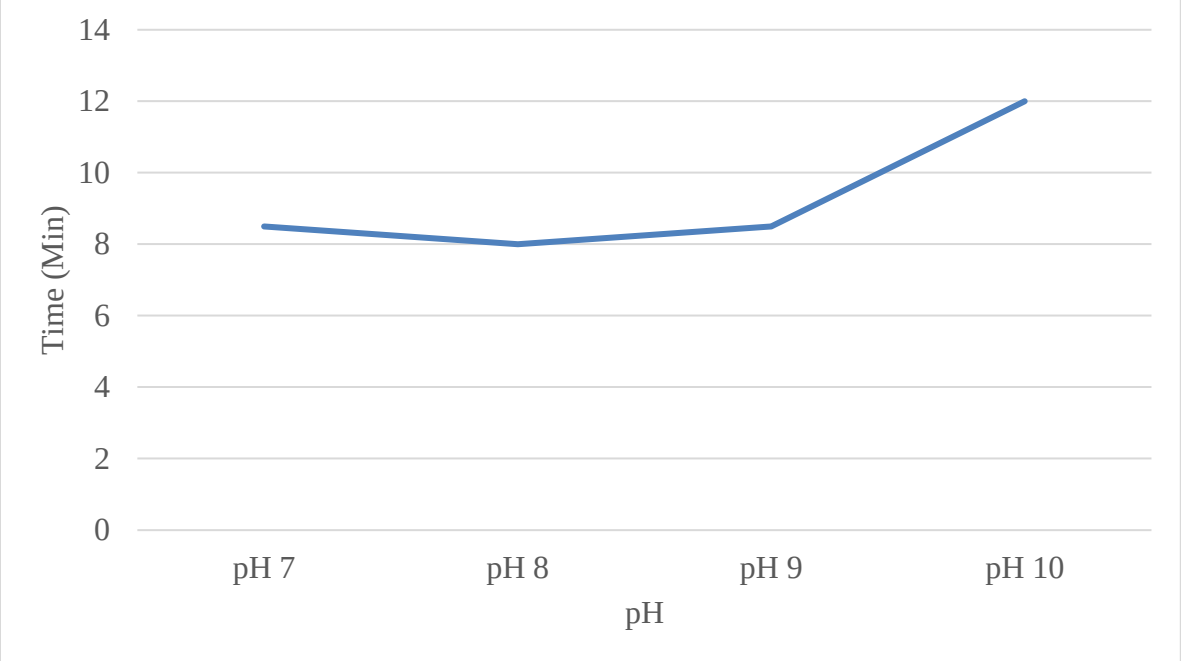

Figure 4-3: Time taken to change the blue-black color after addition of amylase enzyme

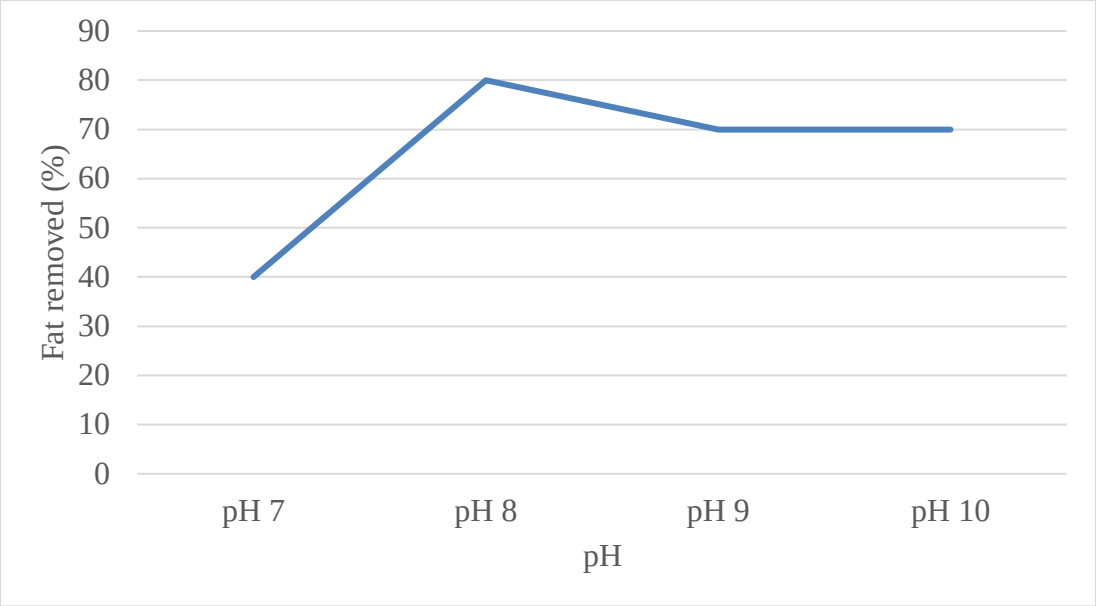

Figure 4-4: Percentage fat removed from the pelt by application of lipase enzymes

Under different pH conditions, the blue-black color of iodine solution was reduced after 8 min which was considered optimum. Under pH 7,9 and 10 the process took 8.5, 8.5 and 12 minutes respectively. For lipase enzymes there was a total reduction of fat content by 80% at pH 8 and this pH was also considered as the optimal pH. At pH 7, 9 and 10 the effectiveness of fat removal was found to be 40%, 70% and 70% respectively.

All the test enzymes were effective at a higher temperature (fig 4-5, fig 4-6 and fig 4-7). The activity of enzymes increased with rise in temperature. The highest activity was found to be 35 ^{0}C

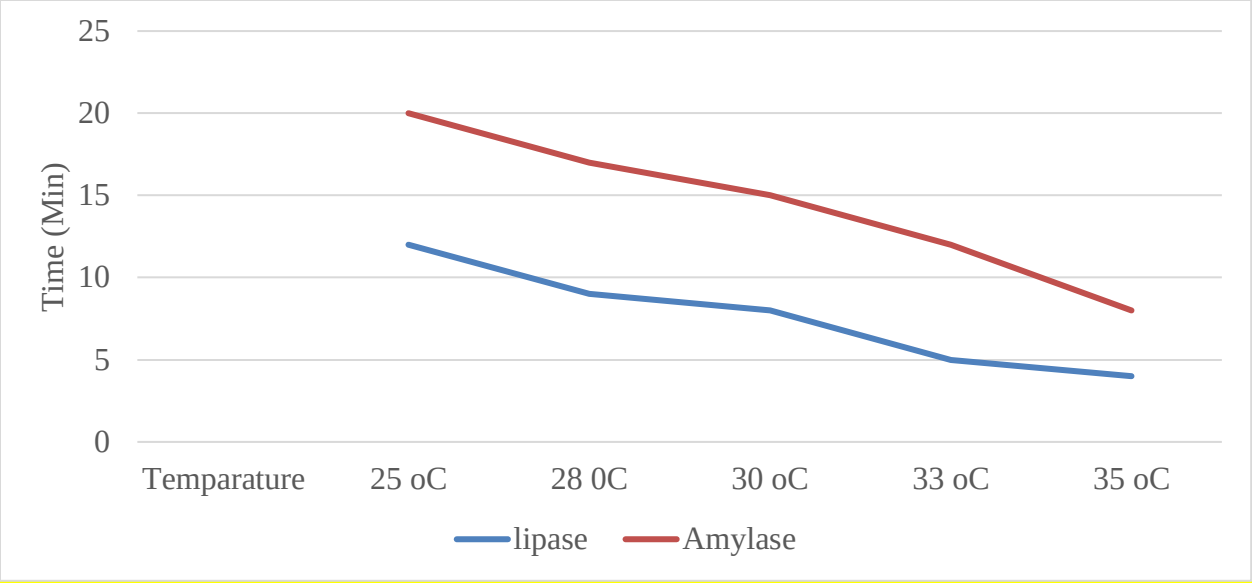

Figure 4-5: Time taken to change the color of the test solutions (amylase and lipase)

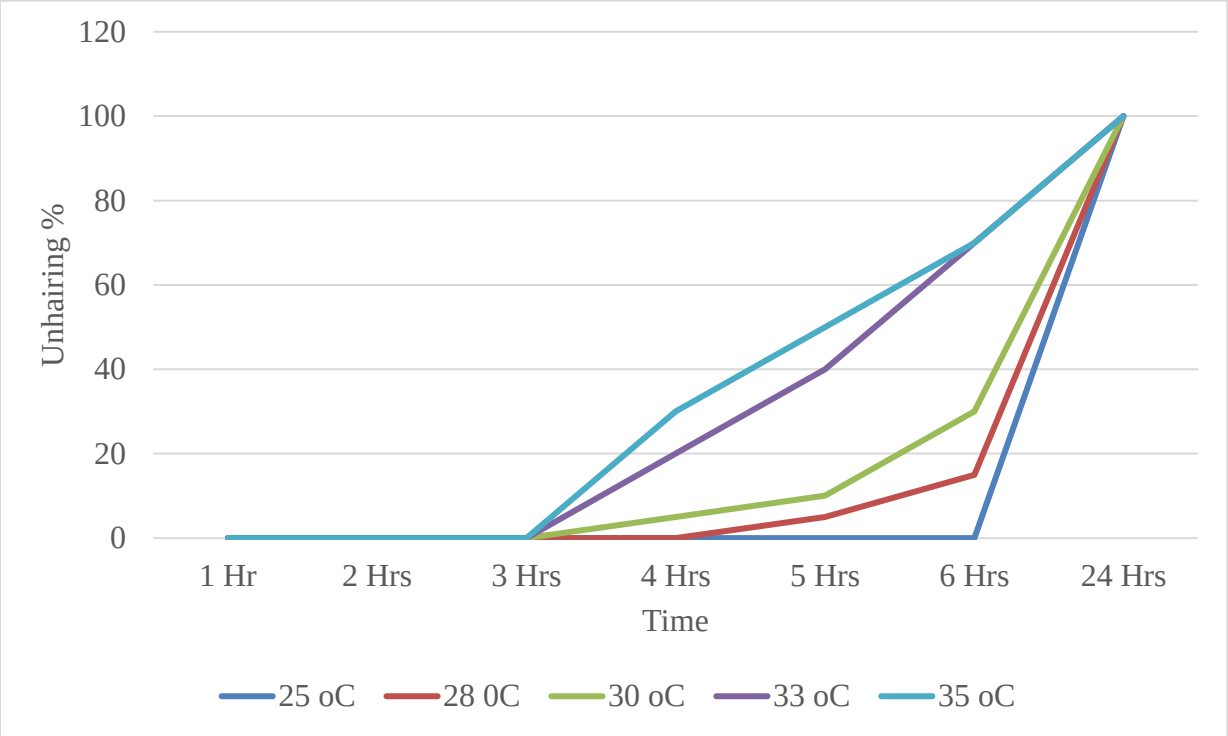

Figure 4-6: Effect of temperature on unhairing of pelt by use of protease enzyme

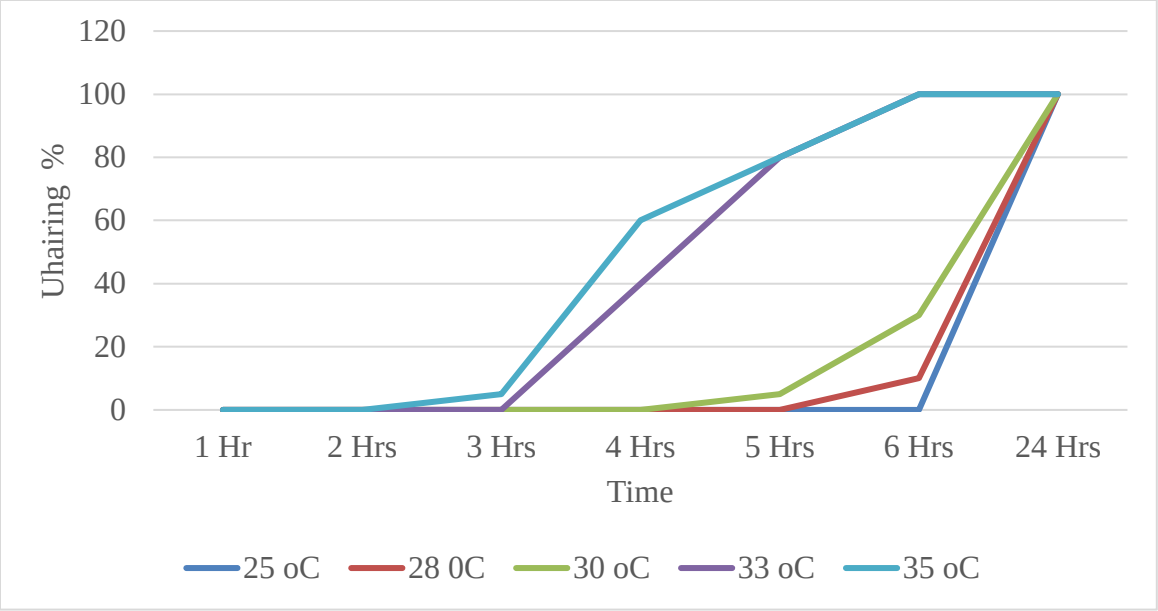

Figure 4-7: Effect of temperature on unhairing of pelt by use of keratinase enzyme

Several studies have reported different optimum temperature and pH for different enzymes. Ma *et al*., (2021), have reported a lipase enzyme that performed optimally at 25 - 35 °C and a pH 6.0 - 8.0. Another study by Sirisha *et al*., (2011), reported a lipase enzyme performing optimally at 37°C and pH 7.0. It can be seen from this study that the pH was within this reported range. Amylase enzymes have been found to be active within a pH range of 7.7 to 10.5 and at a temperature of 80 °C (Amoozegar *et al*., 2003 and Burhan *et al*., 2003). A stable keratinase enzyme at 30-40 0C and pH 6-8 has been documented (Vigneshwaran *et al*., 2010). In addition, Cai *et al*., (2008), reported another keratinase enzyme active at pH and temperature of 8.5 and 55 °C respectively. Sanghvi *et al*., (2016), also reported a purified enzyme of keratinase to be active within a pH 8.0-12.0. For the proteolytic enzymes, the optimal pH (8) and temperature (60°C) has been documented Bayoudh *et al*., (2000). A similar study by Kumar *et al*., (2011), have documented a protease enzyme highly active at pH 8.5- 12.5 and temperature of 45 ^{0}C.

Except protease all the test enzymes had an optimum pH of 8. Since protease enzyme was highly effective at a pH 8-10, pH 8 was selected since it was also suitable for other enzymes. All the test enzymes seemed to have an optimal pH 8 and temperature above 35 ^{0}C. Although the performance of the enzymes from other studies was higher at higher temperature, the use of the higher temperature in leather processing was limited by the shrinkage temperature of pelt. The highest best possible temperature for processing of skins is 28 ^{0}C. Therefore, a temperature of 28 ^{0}C and a pH of 8 were considered as the best parameters for the working enzyme formulations.

Various formulations (comprising one enzyme, two enzymes, three enzymes, and four enzymes) produced varying outcomes for the measured parameters during the soaking and unhairing process, as illustrated in Table 4-2 and Table 4-3 respectively

Table 4-2: Parameters measured in the soaking process

Enzymes combinations	**Parameters**				
Single enzyme	**% Weight gain (5 Hours)**	**SS (mg/l)**	**TS (mg/l)**	**Fat content**	**Protein content**
Lipase	49.45 ±13	34.99 ± 5	86.40 ± 8	5.84 ± 0.7	0.5227 ± 0.25
Amylase	42.78 ±5	29.75 ± 5	93.14 ±2	11.09 ± 0.9	0.55 ± 0.05
Protease	45.39 ± 14	33.04 ± 4	94.34 ± 0.8	8.78 ± 1	0.62 ± 0.18
Keratinase	50.21± 13	32.70± 6	72.36 ± 10	10.78 ± 0.5	0.37 ± 0.2
Blank	45.63 ± 9	16.94 ± 4	59.37 ± 2	11.70 ± 0.03	0.52 ± 0.15
P values	0.31	0.13	< 0.001	< 0.05	0.443
AP	57.42 ± 12	18.58 ± 4	64.21 ± 2	11.16 ± 1	0.51 ± 0.04
LA	54.33 ± 16	18.58 ± 1	62.22± 1	11.95 + 0.7	0.34 ± 0.02
LP	62.57 ± 13	16.04 ± 3	62.17 ± 1	12.87 ± 3	0.56 ± 0.03
AK	51.97 ±4	15.13 ± 2	61.57 ± 3	13.19 ± 2	0.63 ± 0.3
KP	54 65 ± 8	20.66 ± 3	63.8 ± 1	12.96 ± 1	0.52 ± 0.3
LK	62.83 ± 6	16.57 ± 0.2	62.02 ± 2	8.40 ± 2	0.4393 ± 0.3
Blank	50.08 ± 5	18.17 ± 1	53.56 ± 1	13.65 ± 2	0.23 ± 0.1
P= values	0.02	0.295	P ≤ 0.05	0.132	0.59
KPL	55.76 ± 5	7.66 ± 0.76	75.93 ± 9	13.99 ± 3	0.46 ± 0.04
ALK	59.10 ± 1	11.30 ± 0.2	70.83 ± 9	16.79 ± 0.6	0.3 ± 0.07
KPA	53.73 ± 10	9.54 ± 0.8	76.80 ± 12	16.31 ± 1	0.43 ± 0.05
PAL	51.35 ± 5	9.39 ± 1	70.43 ± 9	15.08 ± 0.5	0.58 ± 0.1
Blank	46.37 ± 7	12.45 ± 1	53.70 ± 3	9.30 ± 0.6	0.24 ± 0.01
P Values	0.02	0.001	0.075	0.005	0.002
Blank	65.65 ± 4	11.04 ± 0.7	65.23 ± 4	14.67 ± 0.68	0.4 ± 0.03
KLPA	68.95 ± 1	12.51 ± 0.2	67.16 ± 3	10.64 ± 0.7	0.50 ± 0.06
P Values	0.001	0.03	0.606	0.964	0.078

Key AK=Amylase + Keratinase

LK= Lipase + Keratinase

LA= Lipase +Amylase

AP= Amylase + Protease

LP= Lipase + Protease

KP Keratinase + Protease

KPA= Keratinase + Protease + Amylase

KPL= Keratinase + Protease + Lipase

PAL= Protease +Amylase + Lipase

ALK= Amylase + Lipase + Keratinase

KLPA= Keratinase + lipase + protease + amylase

Using a single enzyme in the soaking stage revealed that the keratinase enzyme was particularly successful in enhancing pelt weight, with a significant increase of 50.21% ± 13%. This is a critical parameter because weight gain results from the absorption of water, which acts as a carrier for all the chemicals used in the tannery. Skins that underwent soaking with the lipase enzyme exhibited the second-highest increase in weight (49.45%), while those treated with the protease enzyme followed closely. However, the distinctions between these results were not found to be statistically significant (p=0.31). Zambare *et al*. (2013) reported a substantial increase in weight gain (84.37%) when 0.55% of protease enzymes were employed to soak a buffalo hide. In a separate investigation conducted by Ramamoorthi *et al*. (2020), it was documented that the skins exhibited an elevated weight gain of 10.5% ± 1.5% when subjected to a soaking process involving the use of 0.75% and 1% of alpha-amylase with non-aqueous solvents. Notably, alpha-amylase demonstrated greater efficacy in non-aqueous solvents when compared to water (Ramamoorthi *et al*., 2020)

In the case of formulations involving two enzymes, the combination of lipase and keratinase yielded the highest weight gain at 62.83% ± 6%, closely followed by the combination of lipase and protease at 62.57% ± 13%. The use of enzymes had a noticeable impact since all the

formulations resulted in greater weight gain in comparison to the blank (the control sample without enzymes). It's worth noting that the application of amylase and protease enzymes has been reported to reduce the soaking time required for hides and skins and also lead to the production of cleaner pelts (Zambare *et al*., 2013).

A formulation involving three enzymes was also successful, resulting in an increase in pelt weight when compared to the control sample without enzymes. Among the three-enzyme combinations, the mixture of amylase, lipase, and keratinase (ALK) proved to be the most effective, with a weight gain of 59.10% ± 1%. However, when all the enzymes were combined, there was only a marginal increase in percentage weight gain compared to the control sample.

The presence of fat within hides/skins can impede the permeation of water and other chemicals, making it a significant factor in leather processing. Across all formulations, the utilization of lipase enzymes on their own demonstrated the most effective reduction in fat, resulting in a pelt with a residual fat content of 5.84% ± 0.7%. Formulations involving lipase and keratinase (LK) and keratinase, protease, and lipase (KPL) enzymes also appeared to be effective, yielding residual fat contents in the pelt of 8.40% ± 2% and 7.66% ± 0.76%, respectively.

The TS, SS, and protein levels in the soaking water are also significant parameters that provide insights for tanners to evaluate the effectiveness of enzymes. Total solids serve as an indicator of the enzymes' capacity to eliminate impurities such as salts, dung, dirt and non-fibrous proteins from the skins (Zambare *et al*., 2013). In the case of single enzymes, protease enzymes were found to yield a liquor with higher total solids content (94.34 ± 0.8 mg/l) and protein content (0.62 ± 0.18%) compared to other enzymes. This effect can be attributed to the protease enzymes

accelerating the removal of globulin and albumin from hides/skins, likely contributing to the higher protein content in the liquors in comparison to other enzymes.

A mixture of amylase and protease enzymes exhibited increased levels of TS (64.21 ± 2 mg/l) and SS (18.58 ± 4 mg/l) in comparison to the other enzyme combinations. The findings suggest that there is no notable distinction ($p > 0.05$) in SS and protein content between these two enzyme combinations. Past research has indicated that a three-hour application of α-amylase enzyme is adequate for effectively eliminating interfibrillar substances, particularly proteoglycans (Madhan et al., 2010). The research also indicated that the protein content in the liquors increased by 2.34 ± 0.10 mg/l and 2.95 ± 0.14 mg/l when the enzyme concentration was adjusted from 1% to 3%. In a separate small-scale unhairing trial using the Erhavit Mc enzyme, the SS in the liquors were recorded as 1.5-1.82 mg/l (blank) and 1.6-2.32 mg/l (enzyme), as reported by Crispim and Mota in 2003

Among the combinations of enzymes, the one consisting of keratinase, protease, and lipase enzymes displayed a greater concentration of total solids (75.93 ± 9 mg/l) in this category, albeit it had lower levels of protein and suspended solids of the liquors. In contrast, the combination of protease, amylase, and lipase enzymes exhibited the highest levels of suspended solids (15.08 ± 0.5 mg/l) and protein content (0.58 ± 0.1%)

All the enzymes did not unhair after 5 hours as can be seen in plate 4-3 and therefore more time was needed to evaluate the ability of enzymes the unhairing process

Plate 4-3: Condition of the skin after soaking with different enzymes for 5 hours

For unhairing stage, several parameters were determined on the pelt and on the liquor after processing to assess the effectiveness of different formulations after 24 hours as shown in table 4-3.

Table 4-3: Assessed parameters in the unhairing process for 24 hours

Enzymes formulations	**Test**					
Single enzymes	**% Weight gain (24 Hours)**	**Fat content %**	**Total solids (mg/l)**	**Suspended solids (mg/l)**	**Protein content (%)**	**Unhairing conditions**
Lipase	55.65 ± 2	11.30 ± 2	75.24 ± 0.98	20.49 ± 5	0.44 ± 0.02	No unhairing
Keratinase	58.55 ± 2	15.84 ± 0.3	67.43 ± 5	12.29 ± 2	0.48 ± 0.04	Complete unhairing
Protease	55.91 ± 4	14.89 ± 4	73.81 ± 5	22.7 ± 3	0.8486 ± 0.02	Hair looseness
Amylase	46.68 ± 3	16.8 ± 2	66.1 ± 1	12.2 ± 1	0.28 ± 0.002	
Blank	53.45 ± 5	17.14 ± 2	64.08 ± 2	11.04 ± 1	0.3 ± 0.01	No unhairing
P values	0.05	p< 0.0001	0.21	0.02	p< 0.0001	
Two enzymes' combinations						
AK	60.34 ± 6	14.32 ± 1	70.68 ± 3	8.11 ± 2	0.5 ± 0.08	Incomplete
LK	65 .65 ± 8.5	9.78 ± 0.7	74.69 ± 6	8.5 ± 4	0.65 ± 0.11	Complete
LA	55.59 ± 1	10.95 ± 1	88.89 ± 9	7.3 ± 3	0.35 ± 0.04	No unhairing
AP	71.08 ± 7	14.41 ± 0.7	113 ± 3	9.8 ± 2	0.73 ± 0.1	Incomplete
LP	77.49 ± 3	10.5 ± 1	97.08 ± 16	11.05 ± 3	0.64 ± 0.07	Incomplete
KP	73.89 ± 5	14.82 ± 0.75	86.00 ± 17	9.3 ± 1	0.74 ± 0.1	Complete
Blank	51.61 ±7	15.42 ± 5	80.48 ±7.3	6.05	0.29 ± 0.01	No unhairing
P values	< 0.0001	< 0.0001	< 0.0001	0.54	< 0.0001	
Three enzymes' combinations						
KPA	61.82 ± 5	10.58 ± 4	68.34 ± 6.4	9.34 ± 6	0.918 ± 0.1	Complete
KPL	72 ± 2	6.4 ± 1	70.28 ± 5	10.28 ± 5	0.92 ± 0.2	Complete
PAL	71.9 ± 3	11.56 ± 2	67.90 ± 0.46	9.73 ± 0.7	1.03 ± 0.23	Incomplete

ALK	72. 61 ± 11	9.5 ± 0.5	65.71 ± 0.7	8.04 ± 0.4	0.8144 ± 0.06	Incomplete
Blank	55.52 ± 5	11.54 ± 2	60.30 ± 3	8.01 ± 3	0.793 ± 0.3	No unhairing
P Values	< 0.0001	0.001	0.1	0.9	0.74	
Four enzymes' combinations						
KLPA	74.67 ± 5	10.64 ± 0.7	112.82 ± 16	18.38 ± 2	1.705 ± 0.7	Incomplete unhairing
Blank	74.47 ± 3	14.61 ± 0.6	79 ± 15	11.90 ± 0.8	0.688 ± 0.03	No unhairing
P Values	0.42	0.264	0.538	0.104	0.08	

Key

AK=Amylase + Keratinase

LK= Lipase + Keratinase

LA= Lipase +Amylase

AP= Amylase + Protease

LP= Lipase + Protease

KP Keratinase + Protease

KPA= Keratinase + Protease + Amylase

KPL= Keratinase + Protease + Lipase

PAL= Protease + Amylase + Lipase

ALK= Amylase + Lipase + Keratinase

KLPA= Keratinase+ lipase+ Protease+ Amylase

When evaluating the percentage weight gain in single enzyme formulations, it was found that keratinase enzymes produced the greatest weight gain at 58.55% ± 2%, followed by protease at 55.91% ± 4%. In the case of two-enzyme formulations, such as (lipase + protease) and (amylase + protease), the highest weight gains recorded were 77.49% ± 3 and 71.08% ± 7, respectively. The difference in percentage weight gain was highly significant ($p < 0.001$) for formulations involving three enzyme combinations, with the combination of amylase, lipase, and keratinase (ALK) and keratinase, protease, and lipase (KPL) demonstrating the highest weight gains of 72.61% ± 11% and 72% ± 2%, respectively. A comparison between the control group (blank) and formulations

containing all four enzymes did not reveal a significant difference in weight gain ($p > 0.05$). It has been documented that the utilization of enzyme combinations or enzymes in combination with surfactants can yield beneficial outcomes when applied in soaking process. Choudhary *et al*. (2004) documented a complete soaking time of 5 hours when combinations of surfactant and protease, surfactants and lipase and lipase and protease were used, resulting in a 45% reduction in soaking time. In another investigation conducted by Queirós *et al*., (2018), which focused on optimizing the soaking process for cattle hides, it was determined that a combination of a wetting agent and a conventional degreaser resulted in a weight gain percentage of 41.1%. Similarly, a blend of protease and lipase enzymes produced a weight gain of 41.6% (Choudhary *et al*., 2004).

The assessment of TS, proteins and SS in the liquors after 24 hours was also conducted. In single enzyme applications, it was observed that protease and lipase had the highest levels of total solids, with protease leading in total SS at 22.7 ± 3 mg/l and protein at 0.8486 ± 0.02. Significantly, the keratinase enzyme was the second enzyme to produce solutions with an elevated protein content, measuring at 0.48 ± 0.04. The reason behind these results is that protease and keratinase enzymes target different proteins, which explains the higher protein levels in their respective liquors. The extent of removal of interfibrillar substances is directly linked to the opening of the fiber structure. The availability of these substances impedes the passage of various agents during the leathermaking (George *et al*., 2014).

In the case of two-enzyme combinations, protease and lipase exhibited higher levels of TS (97.08 ± 16 mg/l) and SS (11.05 ± 3 mg/l). Meanwhile, a mixture of protease and keratinase produced the highest protein content. In formulations involving three enzymes, KPL had the highest levels of total solids (10.28 ± 5 mg/l) and suspended solids (0.92 ± 0.2 mg/l), although these values were lower in comparison to traditional methods. A study by Quandary *et al*. (2014), which compared

hair-saving methods and sulfide methods of unhairing, reported suspended solids levels of 4.7 and 6.2 mg/l, respectively. It's important to note that variations in these variables can occur between researchers, depending on factors such as the experimental conditions, the type of hides/skins used, and their quantity.

A combination of PAL enzymes recorded the highest protein content at 1.03 ± 0.23, although it did not exhibit a statistically significant difference from the other combinations ($p = 0.74$). Furthermore, employing a blend of all the enzymes did not yield a notable disparity ($p = 0.08$) in the protein content of the solutions.

The appearance and percentage unhairing of pelt were assessed by scrapping the epidermis (plate 4-4). Five formulations of the enzymes gave a complete unhairing and. Plate 4-5 indicate the samples that were completely unhaired.

Plate 4-4: Assessment of the unhairing and appearance of the pelt by scrapping the epidermis

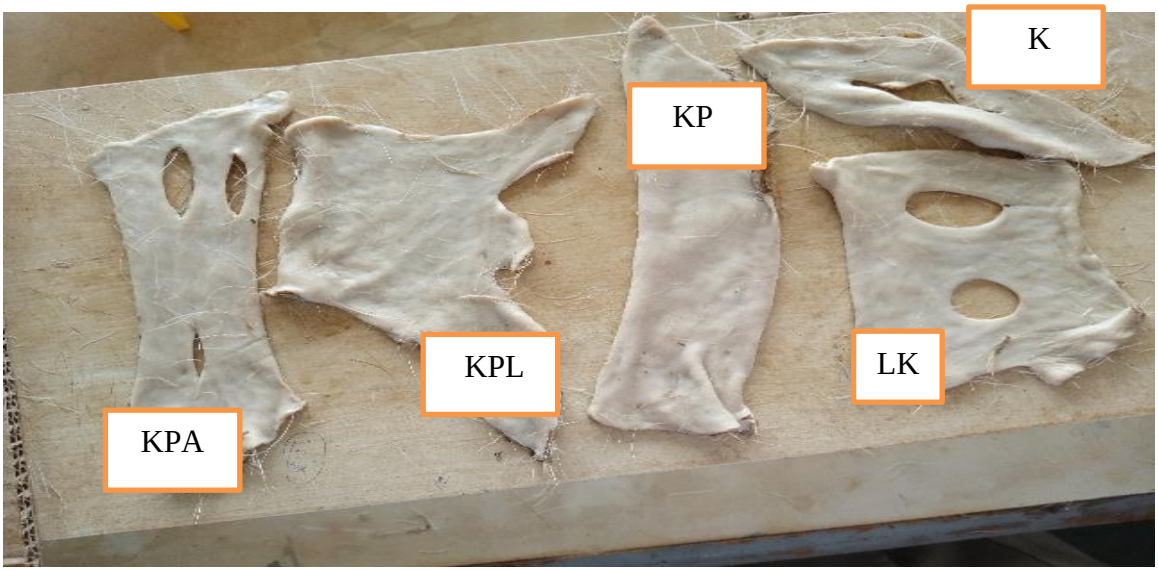

Plate 4-5: Pelts from samples that were completely unhaired by different enzymes

In a comparable investigation conducted by Mamun *et al*. (2015), it was observed that a combination of 2.5% protease and 2.5% keratinase enzymes effectively unhair 85% of pelt area within a 26-hour duration. The introduction of calcium oxide alongside the protease and keratinase enzymes enhanced their performance, leading to complete unhairing of the entire pelt area within a 24-hour period (Mamun *et al*., 2015). Several formulations gave a complete unhairing (table 4-4). This study showed a lot of improvement as compared to the reported studies as samples were completely unhaired within 24 hours without addition of other chemicals.

4.5.1 Assessment of the Organoleptic properties of completely unhaired pelt

A comparison of the organoleptic tests of the samples that were completely unhaired by use of enzymes was done and presented in Fig 4-8.

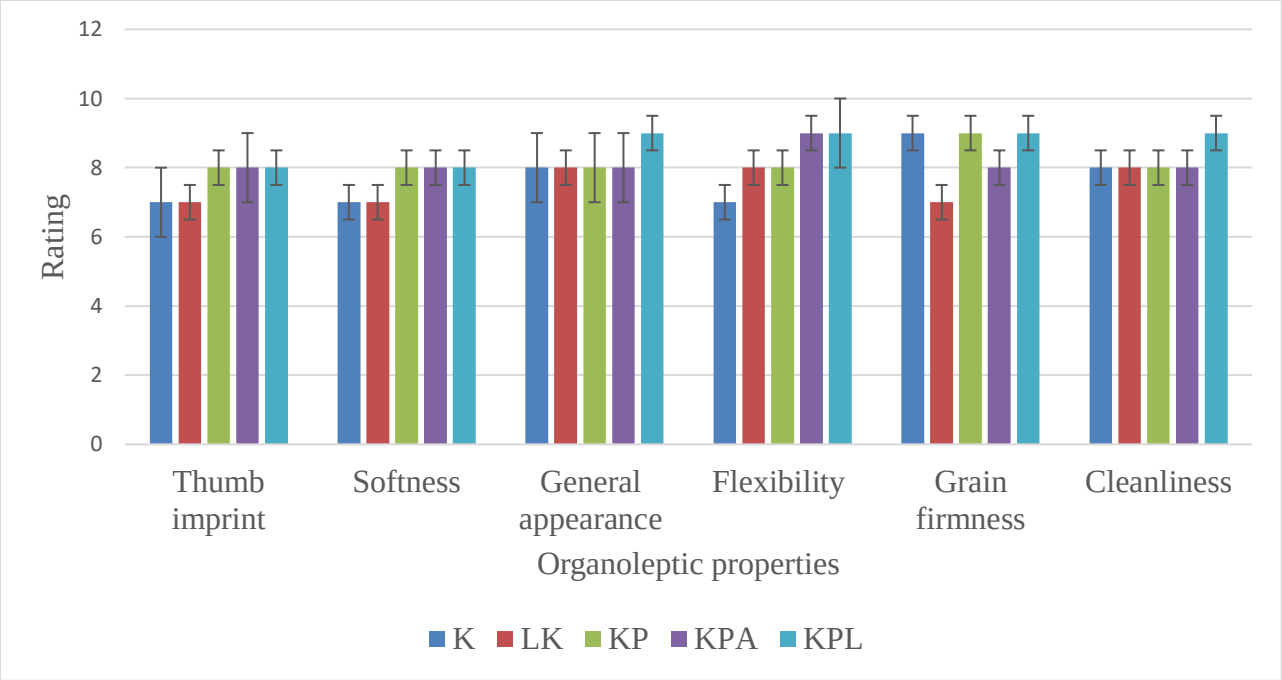

Figure 4-8: Organoleptic properties of completely unhaired pelt

Of all the properties tested all the completely unhaired pelts had a score of 8-10 and this serves as a compelling example of the potential for using enzymes in beamhouse processes to yield pelts with characteristics akin to conventionally processed ones.

4.6 Conclusion

While multiple formulations demonstrated successful unhairing of the skin, a combination of keratinase, protease, and lipase (KPL) enzymes was identified as the most effective for unhairing purposes. A formulation comprising both keratinase and lipase (KL) enzymes was chosen for the soaking process, and a single formulation of lipase enzyme was utilized for degreasing. When assessing the organoleptic properties of the pelts processed using enzymes for soaking, unhairing, and degreasing, the results were comparable to those processed using conventional methods. This suggests that enzymes can be employed sequentially in the early stages of leather processing to replace the use of harmful chemicals.

CHAPTER FIVE: ASSESSMENT OF POLLUTION REDUCTION IN TANNERY PRE-TANNING PROCESSES BY USE OF ENZYMES

5.1 Abstract

Because of high pollution load of wastewater released from tanneries, a study was done to assess the performance of enzymes in reducing this pollution. Twenty-one goatskins and twenty-one cattle hides were used in the study, where they were cut along the backbone and randomly assigned to conventional and enzymatic processing methods. For the enzymatic method, a combination of keratinase and lipase (KL) enzyme was used in soaking process, keratinase, protease, and lipase (KPL) enzyme was used for the unhairing process and lipase was used for the degreasing process. BOD, COD), TS, SS and sulphide were assessed on the effluent. SPSS statistical software was applied to performed inferential and descriptive statistics. The means of quantitative parameters were compared by t-test and significant level indicated as $p \leq 0.05$. The enzymatic method was highly effective in pollution reduction in the unhairing stage with a percentage reduction of TS (48.89%), SS (66.73%), BOD (52.1%), COD (80.96%) and sulphide (100%) in goat skins and TS (74.26), SS (73.52), BOD (49.79%), COD (80.01%) and sulphide (100%) for cattle hide. In all organoleptic tests, both enzymatic and conventionally processed pelts had a rating of 8-10. Therefore, use of enzymes can replace chemicals used in the tanneries, to reduce pollution and produce leather with comparable properties with the conventional method.

5.2 Introduction

Environmental pollution is the primary factor to many diseases and premature deaths globally (Landrigan *et al*., 2018). More people die from pollution every year than from AIDS, tuberculosis and malaria combined (Landrigan *et al*., 2018). Many nations are presently raising public consciousness regarding the impacts of pollution and enacting stringent measures to decrease it.

Despite worldwide involvement, the long-term effects of environmental contamination are still felt today due to release of contaminated wastewater (Ukaogo *et al*., 2020). Although leather industries play an important role, it has been categorized among the harmful industries in the world (Kanagaraj *et al*., 2015). Huge amount of chemicals are used in the tanneries and most of them are not fully absorbed by the hides and skin and are released into the effluents. The chemicals used are unique to specific pre-tanning processes.

During operations of leather processing, various chemicals are used which include: sodium sulphide, sodium chloride, bactericides, lime, ammonium sulphate, chrome salts, tannins etc. (Kanagaraj *et al*., 2015, Selman *et al*., 2019). These chemicals help to remove the non-collagenous substances and to convert collagen to non-putrescible and hydrothermal stable products called leather. In this process a lot of waste in forms of liquid, solid and gases are produced and they contribute to high COD, BOD, TS, TDS, sulphates, chlorides and other heavy metals (Hashmi *et al*., 2017).

Chemicals including sulphide, chromium, and lime are used in traditional leather manufacturing and are responsible for 80–90% of pollution. Hydrogen sulphide may be released at pH levels lower than 10, and it is harmful to sewage and tannery workers (Saran *et al*., 2013). According to Hussein (2021), sixty percent of Na_2S and forty percent of lime based on weight of hides/skins, remains in wastewater and this residue together with solubilized hair increases the COD and BOD of the effluents (Saran *et al*., 2013).

In additional, most of the heavy metals are not degraded by microorganism and hence persist in the environment for extended periods, causing serious health hazards to human and animals (Laxmi and Kaushik 2020). It is reported that most workers in the tanneries suffers from skin and

stomach diseases that are associated to tanneries and most of them (90%) die before they reach the age of 50 years (Dobson 2001). Other effects of tannery chemicals include headache, dizziness, eye irritation, poisoning of liver, nervous system and the kidney (Tadesse *et al*., 2017). The presence of a lot of organic matters in wastewater causes the reduction of dissolved oxygen in the water bodies due to bacterial decomposition and this affects animals that live in water (Tadesse *et al*., 2017).

Many studies have suggested several methods that can be used to reduce the effect of tannery pollution which include: reduction, reuse, recycle and recovery of the tannery effluents (Hu *et al*., 2011). Another important method is the elimination of the chemicals causing the pollution. Extensive studies are currently underway in pursuit of ecofriendly chemicals to fully replace or drastically reduce the harmful chemicals used in the tanneries. Application of enzymes may serve as the best available options in the leather sector if fully adopted. Enzymes have the potential to reduce or completely substitute a substantial portion of the harmful chemicals used in the tanning industry. The substitution of chemicals with enzymes can result in reduction of pollution in the tannery wastewater.

5.3 Materials and methods

5.3.1 Selection of raw materials

Wet-salted goatskins and cattle hide utilized in this study were sourced from curing premises adjacent to Dagoretti slaughterhouse. In this study, 21 wet-salted goatskins and 21 cattle hides were used.

5.3.2 Hides/skins preparation

Full hides/skins were cut along the backbone (Plate 5-1) into two sides. One side was randomly selected for enzymatic method while the other side was selected for the conventional methods. Each category was processed in three batches of seven sides. The weight of hides and skins used per batch was taken using a weighing balance (plate 5-2).

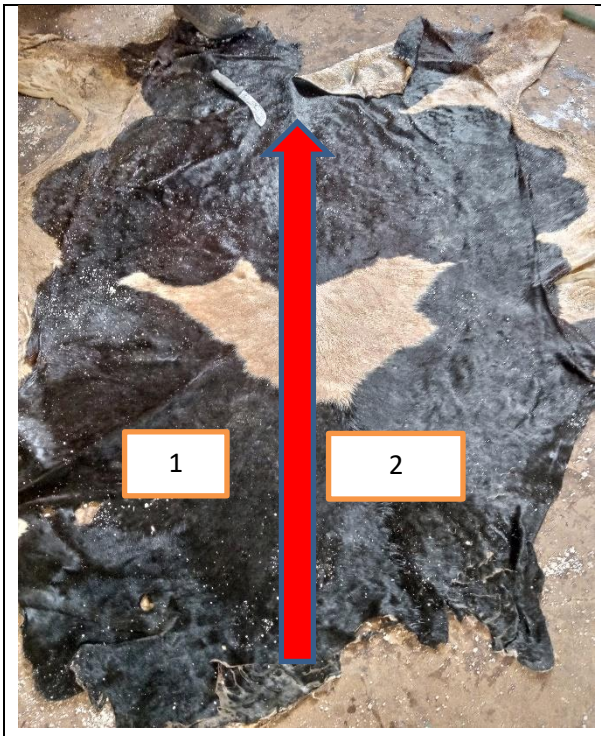

Plate 5-1: Cutting the hides along the backbone

Plate 5-2: Weighing of goatskins

5.3.3 Processing of hides and skins by conventional method

The following recipe was used to process hides and skins by conventional method. The amount of chemicals used was depending on the weight of hides/skins.

Table 5-1: Processing recipe for hides and skins

PROCESS	%	PRODUCT	RUN	REMARKS
Dirt soak	400	Water	30min	Drain
Main soak	400	Water		
	0.5	Wetting agent		
	0.5	Biocide		Leave it overnight (18hrs
Unhairing and liming	200	Water		
	0.85	Na_2S	15min	
	1.30	Na_2S	15min	
	0.85	Na_2S		
	0.7	$Ca(OH)_2$	20min	
	1.0	$Ca(OH)_2$	10min	
	1.3	$Ca(OH)_2$	10min	Leave it for 18 hours Check for unhairing, swelling/plumping
Fleshing with machine				
Deliming	100	Water		
	2	Ammonium sulphate	1hr	Phenolthalein indicator check for lime free- clear colour change.
Bating	100	Water@ 35^oC		
	1	Microbate	1hr	Thumb imprint
Degreasing	1	Degreasing agent	1 hr	
Pickling	100	Water		
	7	NaCl	20min	
	0.5	Formic acid (1:5)	45min	
	1	Sulphuric acid (1:10)	1.5hr	Leave it overnight
Tanning	50	Pickle liquor		
	2	Basic chrome sulphate	1hr	
	2	Aluminium chrome	30min	
	3	Basic chrome sulphate	2hrs	Check penetration of chrome /thickness of the hide
Basification	0.5	Sodium formate	30min	
	1	Magnesium oxide (1:5)	4 portions at interval of 15 min Run for 1 hr	Pile on the floor.
Retannage	50	Water		45 ^oC

	4	Mimosa extract		
	4	Melanin syntan MD	1 Hr	
	2	Mimosa syntan MF	1 Hr	
Dyeing	1	Ammonium liquid	30 Min	
	1.25	Black dye	2 Hrs	Checked penetration
Fatliquoring	50	Water		50 ^{o}C
	3	Fosfal M1		
	2	Fosfal CL	30 Min	
	2	Replan	30 Min	
Fixation	1	Formic acid	3x10+60min	pH 4 Wash and drain

5.3.4 Processing of skins by the enzymatic method

Five percent of enzyme formulation (based on hides and skins) made by mixing an equal proportion of KL (keratinase and lipase) enzyme was us for soaking process for a period of 6 hours. Another 5% of equal proportion of keratinase, protease, and lipase (KPL) enzyme was used for unhairing process for a duration of 18 hours, and 5% of lipase was used for the degreasing process for a duration of 1 hour. The processed pelt did not require bating as compared to the conventional method. Liming, deliming, pickling, tanning, and all the post tanning process were the same for the two processing methods.

5.3.5 Determination of physiochemical parameters of wastewater

To determine the quality of wastewater produced by both processes, TS, SS, COD and BOD were assessed by use of standard methods.

5.3.6 Analysis of total solids and suspended solids

The TS and SS solids were determined as described by Clesceri and the American Public Health Association (1989).

5.3.7 Chemical Oxygen Demand

Fifty milliliters of diluted effluent were put in a five hundred milliliters refluxing flask. One gram of $HgSO_4$, 5.0 ml of sulfuric reagent and glass beads were added and mixed to dissolve. During mixing, they were cooled to prevent the volatile materials from being lost. Twenty-five millimeters of a solution 0.0417 M K_2CrO_7 was added. The flask and the condenser were connected and water was turned on to allow cooling. Seventy millimeters of the left sulfuric acid solution was added. Addition of sulfuric acid reagent was continued while swirling and mixing. A small beaker was used to cover the open end of the condenser to prevent materials from entering to the refluxing mixture and refluxed for two hours. Distilled water was used to cool the condenser. It was then disconnected and the mixture diluted two times with distilled water. After cooling, FAS was used to titrate the excess K_2CrO_7 using ferroin as an indicator. The first sharp change in color was taken as the endpoint. In the same manner a blank sample was also refluxed and titrated. The COD was calculated using the following formula (Clesceri and American Public Health Association, 1989).

$$\text{COD (mg/l)} = \frac{(\text{A}-\text{B}) \times M \times 8000}{\text{mL sample used}}$$

A- ml FAS (for blank)

B- ml FAS (for sample)

M- Molarity of FAS

5.3.8 BOD determination method

The dilution water was aerated and 1mL each of $MgSO_4$, phosphate buffer, $FeCl_3$ and $CaCl_2$ solution per liter of water added. The dilution water was shaken for one minute. It was then stored at a temperature of 20°C until the start of the analysis. The effluent samples were neutralized to a

pH 6.5-7.5 with sulphuric acid and sodium hydroxide solutions and sample container placed at 20^{o}C to start warming. The sample was aerated for about 15 minutes while still warming. The sample effluent was diluted with dilution water and 300 mL put in a BOD bottle. Another blank sample containing only dilution water was set for quality control purposes. The initial dissolved oxygen (D1) of sample and dilution water was determined after saturating the samples and after preparing the dilutions. BOD bottle was closed tightly ensuring no water bubbles were left enclosed in the bottles. The sealed BOD samples were placed in an incubator at 20°C for five days. The glass stopper was removed after five days and the final dissolved oxygen concentration (D2) measured. The BOD results were accepted when the BOD of water of dilution was found to be < 0.2 mg/l and the following formula used to calculate the BOD of the samples (Clesceri and American Public Health Association, 1989).

$$\text{BOD} = \frac{D1 - D2}{P}$$

D_1- initial sample dissolved-oxygen (DO) concentration (mg/l)

P- decimal volumetric fraction of sample used

D_2- sample DO (mg/l) after 5 days

5.4 Data analysis

Analysis of the data was carried out using SPSS Statistical software version 21. Descriptive statistic such as parameters mean and standard deviation were calculated and results presented in tables and graphs. Inferential statistics was carried out by t- test to identify whether there was a significant difference on the means of parameters measured between enzymatic and conventional method ($p \leq 0.5$).

5.5 Results and discussion

Enzymes can perform a crucial role in reduction of most chemicals used in the tannery. This may result in the reduction of most of the physiochemical properties in the tannery effluent as shown in table 5-2 and table 5-3.

Table 5-2: Physiochemical parameters of effluent from goatskins processed by enzymatic and conventional method.

Parameters assessed	**Conventional method**	**Enzymatic method**	**P- Values**	**% Reduction**
Soaking process				
Total Solids (mg/L)	211.66 ± 2.16	120.5 ± 3.62	< 0.001	43.06
Suspended solids (mg/L)	34.67 ± 1.86	46.17 ± 2.48	< 0.001	-
BOD (mg/L)	1286.66 ± 2.8	994.67 ± 3.32	< 0.001	22.69
COD (mg/L)	3226 ± 5.55	993 ± 3.6	< 0.001	69.21
Unhairing and liming				
Total solids (mg/L)	465.33 ± 3.67	237.83± 2.4	< 0.001	48.89
Suspended solids (mg/L)	286.5 + 4.09	95.33 ± 3.78	< 0.001	66.73
BOD (mg/L)	3579.33 ± 7.09	1714.66± 8.48	< 0.001	52.1
COD (mg/L)	17892 ± 7.52	3405.83 ± 11.27	< 0.001	80.96
Sulphide (mg/L)	11± 2			100
Deliming				
Total solids (mg/L)	97.17 ± 4.71	99.67 ± 5.31	0.409	-
Suspended solids (mg/L)	27.33 ±1.75	32.33 ± 2.06	0.001	-
BOD (mg/L)	583 ± 5.55	581.5 ± 2.07	0.549	0.26
COD (mg/L)	1465.67 ±3.50	1455.83 ± 3.43	0.001	0.67
Bating				
Total solids (mg/L)	80	-		100
Suspended solids (mg/L)	29	-		100
BOD (mg/L)	225	-		100
COD (mg/L)	500	-		100
Degreasing				
Total solids (m g/L)	13 ± 0.71	12.23 ± 0.77	0.102	5.92
Suspended solids (mg/L)	3.8 ± 0.69	4.15 +0.5	0.34	-
BOD (mg/L)	202.83 ± 4.9	186.5 ± 3.62	< 0.001	8.05

COD (mg/L)	599.167 ± 5.78	351.33 ± 3.7	< 0.001	41.36

The effluent from conventional and enzymatic processes showed a lot of variation in most of the pre-tanning processes assessed: soaking, unhairing and liming, deliming, bating and degreasing. The variations in the physiochemical parameters for goatskins can be seen clearly in Figure 5-1, Figure 5-2, Figure 5-3, and Figure 5-4.

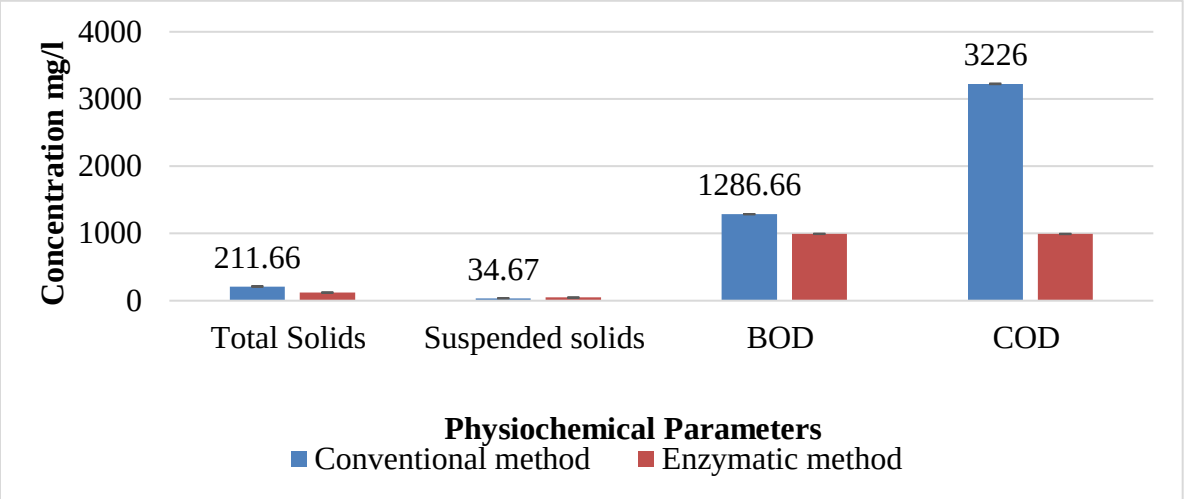

Figure 5-1: Physiochemical parameter for soaking process

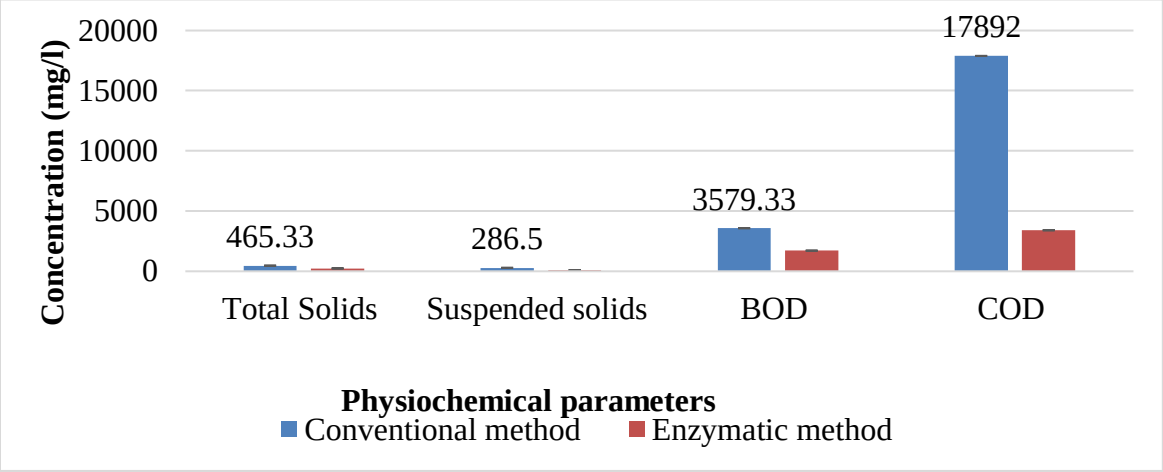

Figure 5-2: Physiochemical parameter for unhairing process

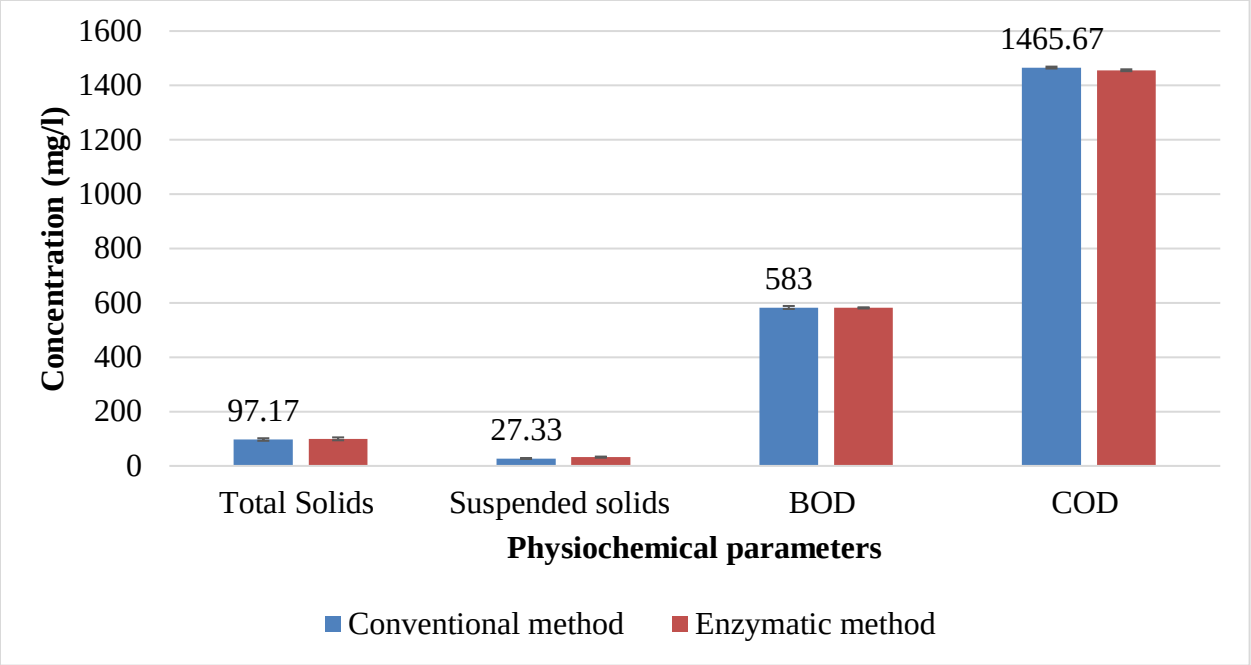

Figure 5-3: Physiochemical parameter for the deliming process

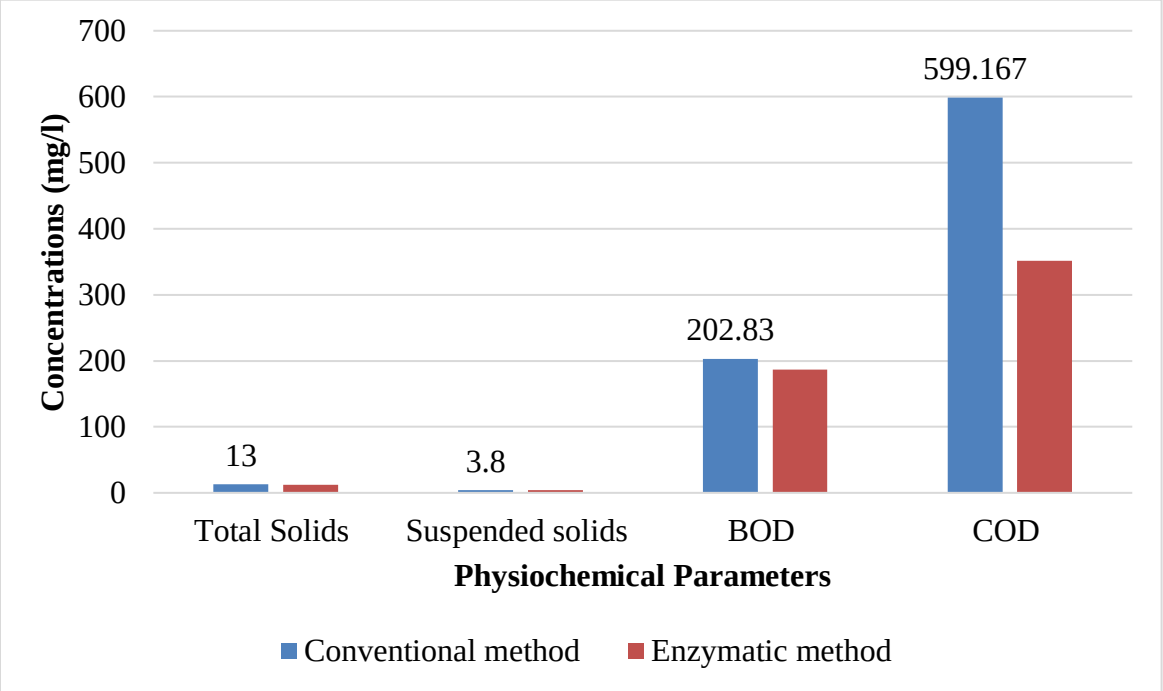

Figure 5-4: Physiochemical parameters for the degreasing process

In the soaking process of the goatskins, the enzymatic method had a lower total solid, BOD and COD as compared to the effluent from conventionally processed skins with a percentage reduction of 43.06%, 22.69% and 69.21% respectively. Due to the enzymes' ability to dislodge dirt and hair in hides and skins, the enzymatic approach had higher suspended solids (46.17 mg/l) than the conventional method (34.67 mg/l). The state of the preserved skins and the variations in the soaking process may affect the physio-chemical properties of the effluent. COD and BOD levels of 2987 mg/l and 586 mg/l, respectively, have been reported by Hashem *et al*. (2022). This was close to the findings of this study that found a BOD to be 1286 mg/l) and a COD of 3226 mg/l. When assessing and describing the tannery wastewater, Islam *et al*., (2014) reported BOD and COD values of 1850 mg/l and 4600 mg/l. Ma *et al*., (2014) investigating the impact of two distinct enzymes during soaking process also reported the SS, BOD, and COD ranging from 748-967 mg/l, 907- 956.5 mg/l, 1010.5- 1089.5 mg/l respectively.

For all the physio-chemical properties analyzed in the unhairing process, they were higher in the conventional method as compared to the enzymatic method as indicated on fig 5-2. This led to a reduction of TS, SS, BOD and COD by 48.89%, 66.73%, 52.1% and 80.96% respectively. A statistical analysis of the tested variables in the unhairing process indicated a highly significant difference $p < 0.001$ between the enzymatic and conventional method in all physio-chemical analyses as indicated in table 5-2.

The deliming process was carried out the same way for both conventional and enzymatic method. Although the chemicals used were the same, physio-chemical parameters were different. The enzymatic method had slightly higher total solids as compared to the conventional method (table 5-2). The BOD and COD for the enzymatic method had lower values as compared to conventional

method by 0.26% and 0.67% respectively (Table 5-2). These variations in the physio-chemical parameters are due to the residual chemicals used in the unhairing and liming processes.

Since the pelts processed by the conventional method were adequately soft, there was no need for bating process. The pelts processed by the conventional method were bated to soften them. This means that there was a total reduction of all the physio-chemical parameters on enzymatic method by a 100%.

A variation of physio-chemical parameters was also observed in the degreasing process as indicated on fig 5-4. Degreasing is a crucial step in the leather-making process where extra fat is removed from the raw hides and skins. In the conventional method organic chemicals that have negative environmental effects, such as emissions of volatile organic compounds and an increase in BOD and COD are used. A comparison of BOD and COD between the conventional and enzymatic methods was highly significant ($p < 0.001$) and the conventional method had higher values as indicated in table 5-2.

A comparison of the physiochemical parameters of cattle hides processing for the conventional and enzymatic methods also indicated a significant reduction of pollution in most of the pre-tanning processes (Table 5-3).

Table 5-3: A comparison of physiochemical parameters of cattle hides processing for the conventional and enzymatic methods

Parameters assessed	**Conventional method**	**Enzymatic method**	**P values**	**Pollution reduction (%)**
Soaking				
Total solids (mg/l)	216.5 ± 1.87	176.17 ± 5.63	< 0.001	18.63%
Suspended solids (mg/l)	45 ± 2.82	93.33 ± 7.3	< 0.001	-
BOD (mg/l)	1563 ± 4.60	1166.83 ± 5.34	< 0.001	25.35
COD (mg/l)	3894.16 ± 7.30	2338.83 ± 3.66	< 0.001	39.94
Unhairing and liming				
TS (mg/l)	566 ± 10.29	145.67 ± 2.80	< 0.001	74.26
SS (mg/l)	395.83 ± 4.02	104.83 ± 4.02	< 0.001	73.52
BOD (mg/l)	3722.166 ± 7.67	1869 ± 8.12	< 0.001	49.79
COD (mg/l)	18620.5 ± 7.42	3722.5 ± 5.99	< 0.001	80.01
Sulphide mg/l	7			100
Deliming				
Total solids (mg/l)	167.5 ± 2.73	110.83± 3.18	< 0.001	33.83
Suspended solids (mg/l)	28.33 ±3.26	34.5 ± 2.42	0.004	
BOD (mg/l)	627.33± 2.16	622.83 ± 1.72	0.003	0.72
COD (mg/l)	1567.166 ± 4.62	1559 ± 1.55	0.002	0.52
Bating				
Total solids (mg/l)	114			100
Suspended solids (mg/l)	22			100
BOD (mg/l)	225			100
COD (mg/l)	510			100
Degreasing				
Total solids (mg/l)	11.75 ± 1.08	13.167 ± 1.47	0.87	-
Suspended solids (mg/l)	2.53 ± 0.45	4.0 ± 0.89	0.005	-
BOD (mg/l)	74.33 ± 3.01	59.66± 8.80	0.003	19.73
COD (mg/l)	506 ± 5.78	305.16 ± 8.58	< 0.001	39.69

Analysis of BOD, COD, TS and SS revealed highly significant difference P< 0.001 in all the assessed parameters in the soaking process of cattle hides. In addition, the enzymatic method showed a reduction of TS, BOD and COD by 18.63%, 23.35% and 39.95% respectively. The suspended solids are due to loosened hair particles by enzymes. Fig 5-5, Fig 5-6, Fig 5-7 and Fig 5-8 shows the graphical presentations for the variations in the physical properties analyzed for different pre-tanning processes.

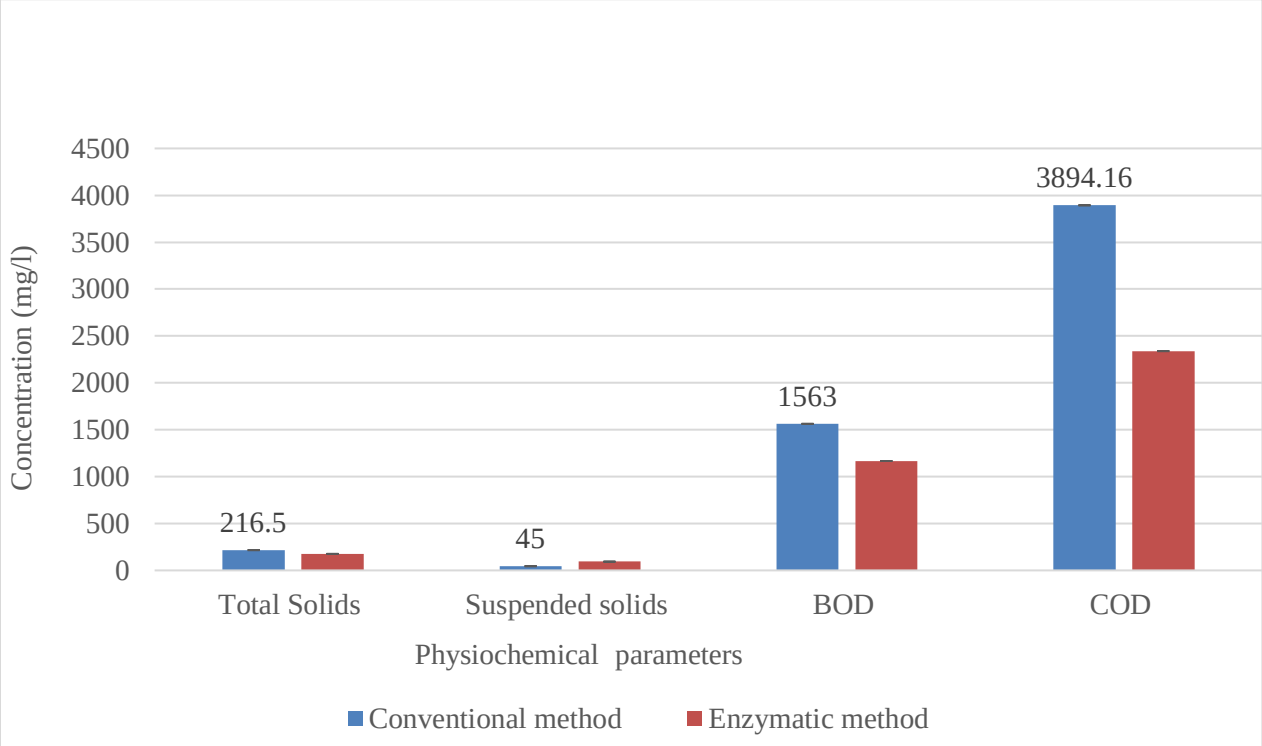

Figure 5-5: Physiochemical parameters for the soaking process of cattle hide

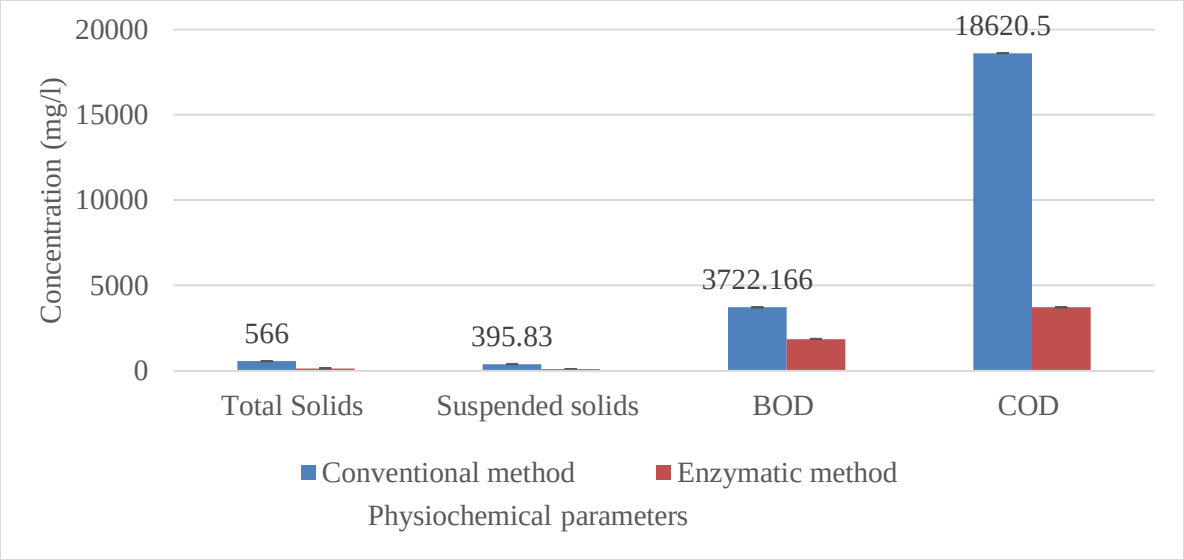

Figure 5-6: Physiochemical parameters for unhairing and liming process of cattle hide

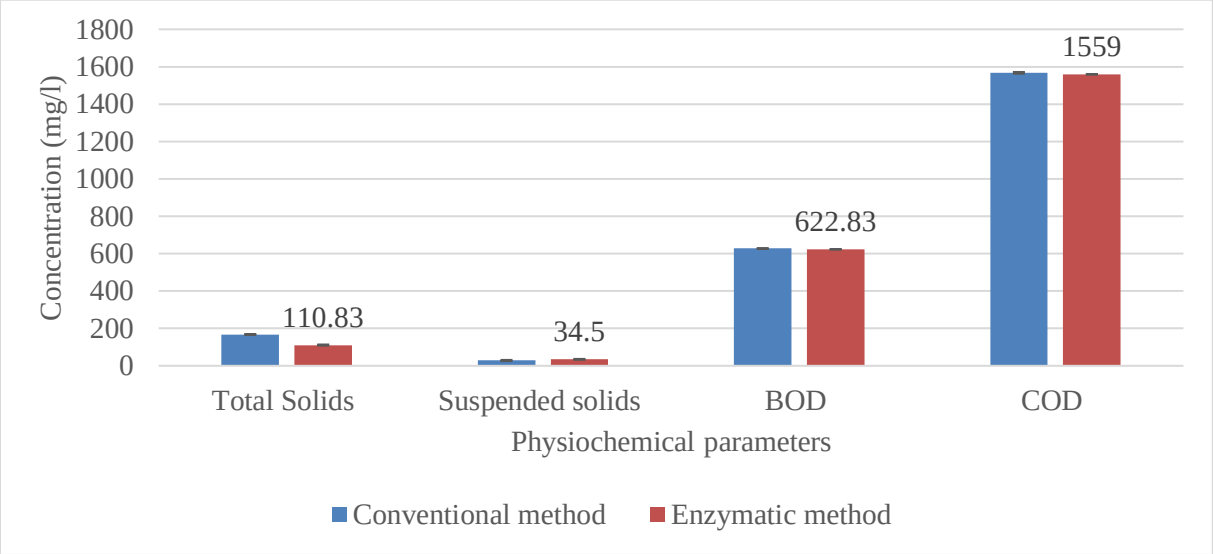

Figure 5-7: Physiochemical parameters of deliming process of cattle hide

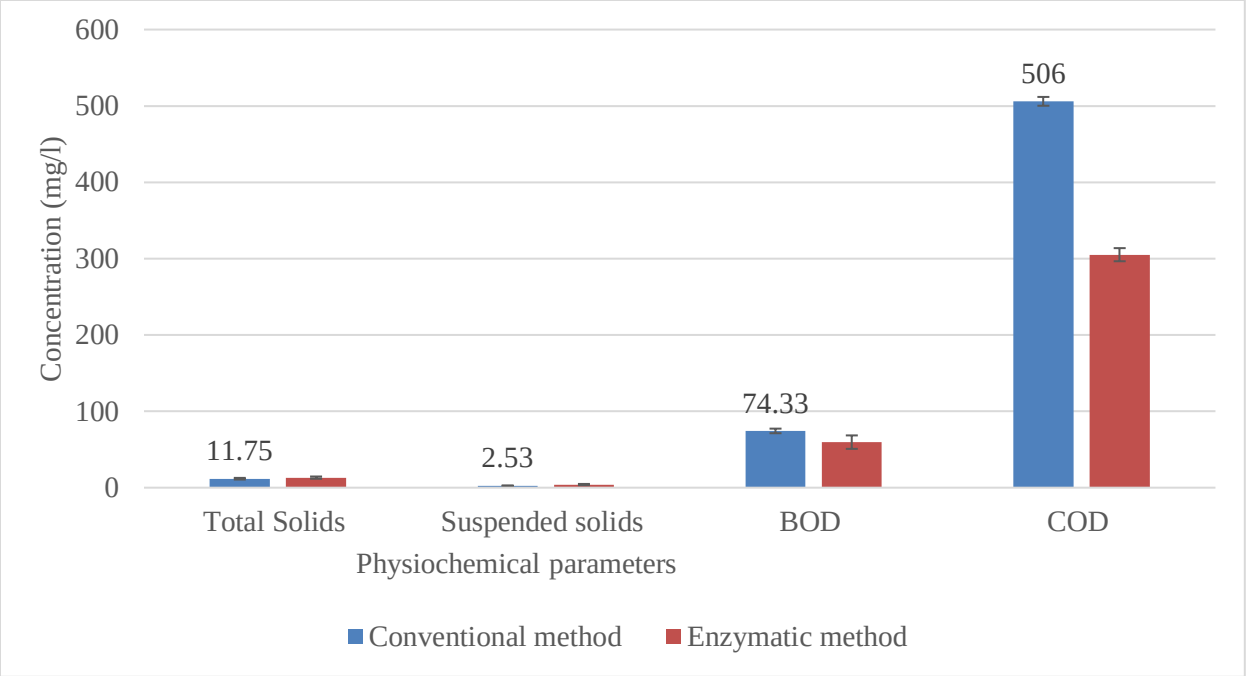

Figure 5-8: Physiochemical parameters of the degreasing process of cattle hide

For the unhairing and liming process, the conventional method showed very positive results in the reduction of physiochemical parameters as shown in fig 5-6. TS, SS, COD, BOD and sulphide were reduced by 74.26%, 73.52%, and 49.79%, 80.01% and 100 % respectively. The application of sodium sulfide in unhairing process is a major contributor to these higher physio-chemical parameters and its substitution with other chemicals is of great advantage. Less value of BOD indicated in the enzymatic method in both cattle hides and goatskins processing represent high-quality water as compared to the conventional method. It can be seen from this study that the wastewater quality from enzymatic method was better compared to the conventional method. Unhairing effluent of cattle hide having a SS, BOD, COD and sulphide of 813 mg/l, 580 mg/l, 8200 mg/l and 788 mg/l respectively have been reported in Sudanese tanneries by Ali *et al*., (2018). A related work by Dettmer *et al*., (2013) has reported a reduction of BOD in unhairing, deliming and bating by 39.78%, 100% and 81.73% respectively when enzyme was used in unhairing

process. The same study reported a reduction of COD in unhairing, deliming and bating by 42.11%, 100% and 82.29% respectively. In another wok by Saran *et al*., (2013), the BOD and COD load from the effluent was reduced by 75% and 80%, respectively when enzymes were used to unhair goat skins. A reduction of 82% and 85% of BOD and COD respectively has also been reported for enzymatic processing of buffalo hides (Saran *et al.*, 2013).

Although the deliming process was the same for both conventional and enzymatic method, the physiochemical parameters were different as indicated in fig 5-7. The conventional method had higher values than the enzymatic method except in suspended solids. The reduction in suspeded solids in the conventional method is due to the removal of hair and other keratinous substance during the liming and unhairing process. A study by Nugraha *et al.,* (2020), comparing effluent of the deliming process by conventional ammonium and tartaric acid, reported a BOD, COD, TSS and TDS of 647 mg/l, 1605 mg/l, 3810 mg/l, and 3810 mg/l respectively for conventional deliming process. Other studies have also reported BOD and COD in the range of 1625 mg/l- 2500 mg/l and 6300 mg/l- 74851 mg/l respectively (Hassen and Woldeamanuale, 2017, Islam *et al.,* 2014).

In the degreasing process, only the BOD and COD showed a reduction by 19.73% and 39.69% respectively. Lipase degreasing can play a major role in pollution reduction if fully adopted by the tanneries. A study by Kilic, (2013), assessing the effect of different degreasing agents and different concentrations reported a COD in the range of 15- 55 mg/l . Degreasing is an important process that helps to reduce fat from skins/ hides. Fats in animal skins/hides are enclosed in a fat cell which consists of protoplasmic envelope invested in reticular tissue. Tanners' faces a lot of difficulty when degreasing as the degreasing agent penetrates to the fat site, emulsify the fat and remove the emulsified fat (Sivakumar *et al*., 2009). Use of lipase helps in reductions of pollution as illustrated from other studies. For example, Moujehed *et al*., (2022), reported a reduction of BOD and COD

by 78% and 84% respectively when degreasing skins with enzymes as compared to the conventional method.

It can be concluded from this study that processing of hides and skins by application of enzymes can play a crucial role in pollution reduction. It is also observed from this study that the conventional processes produce an effluent with higher BOD and COD in all the pre-tanning processes. The COD analysis is the most used method for predicting oxygen amount needed by organic matter in a sample. A higher BOD in the samples causes dissolved oxygen to be depleted in the water bodies and may have a negative effect on an aquatic living organism (Sugasini and Rajagopal, 2015).

Total suspended solid is also a very important parameter as very high suspended solids may hinder the penetration of light to the water bodies and affects the photosynthesis of the algae. This means that the aquatic animals will lack adequate food and also the oxygen released during the photosynthesis process will be reduced. Total dissolved solid is mostly related to hardness, alkalinity, and salinity of wastewater and it increases due to dirt and chemicals used in the tannery. The high content of dissolved solids will also affect aquatic life. It is therefore important that effluent produced in the tanneries should have lower contents of all the parameters assessed.

Despite the use of enzymes in leather processing being expensive as compared to the conventional method, it has been proved from this study that it significantly reduces the pollution. Pollution has been reported as a major cause of death worldwide. Since the use of enzymes will reduces the numbers of death and diseases associated with pollution, the relative high cost of enzymes should not be a hinderance for their adoption in leather sector.

5.6 Conclusion

In processing of hides and skins the enzymatic method had lower BOD, COD, SS and TS values in comparison to the conventional method. It's evident from this study that application of enzymes can reduce or substitute the chemicals used in the tanneries and therefore should be adopted.

CHAPTER SIX: COMPARISON OF THE QUALITY OF LEATHER PROCESSED BY USE OF ENZYMES WITH THE LEATHER PROCESSED BY CONVENTIONAL PRE-TANNING PROCESSES

6.1 Abstract

Consistent production of quality leather products that meet and exceed customer expectation is very important. To achieve this, visual and physical evaluation of leather has become part of quality control in leather sector. Organoleptic tests, tearing and tensile strength, percentage elongation, shrinkage temperature and flexing endurance tests were determined on enzymatic and conventional processed leather using standard methods to assess whether they met the required standards. SPSS was used for data analysis. Both descriptive and inferential statistics were performed. T-test analysis was done to identify the parameters means that were statistically different between the conventional and enzymatic processed goat and cattle leather. Tensile strength, tearing strength and percentage elongation for enzymatic method was 28.5 Mpa, 17.5 N, 58.3% while that of the conventional method was 26.4 Mpa, 29.3 N, and 80% respectively for goat leather. For the cattle leather the enzymatic method had higher values in all the test properties except tearing strength where the conventional method had a higher value of (90.85 N) as compared to the enzymatic method (74.85 N). For organoleptic tests both enzymatic and conventionally processed crust leathers had a rating of 8-10. Both conventional and enzymatic method produced leathers that passed the minimum requirement for shoe uppers in most of the assessed parameters: tearing strength 50 N. tensile strength 15 Mpa, percentage elongation 30%, shrinkage temperature > 100 ^{o}C, and flexing endurance 50,000 flexes. It was recommended that the use of enzymes be adopted in soaking, unhairing and degreasing processes.

6.2 Introduction

The leather and leather products industries are experiencing rapid growth due to high-demand of products such as sandals, wallets, shoes, jackets, gloves, bags, and belts (Winiarti *et al*., 2018). For this trend to continue, production of high-quality leather has to be sustained through testing. The usefulness of leather is mainly affected by location and defect sizes which have an adverse effect on physical characteristics of leather (Winiarti *et al*., 2018).

Physical properties of leather are very crucial as they determine the performance required by different leather products (Nalyanya *et al*., 2018). These properties are modified by sequences of mechanical and chemical operations which are carried out during leather processing (Nalyanya *et al*., 2018). Beamhouse process is performed to open-up the collagen structure through elimination of non-collagenous substances and splitting the fibre bundles to the level of fibrils (Islam *et al*., 2014, Das *et al*., 2022). Tanning is an important process that changes putrescible and non-hydrothermal stable hide/skins to hydrothermal and non-decaying stable product (Mohammed, 2014, Maina *et al*., 2019). Post-tanning processes are performed to modify the characteristics of leather (Jian *et al*., 2012, Nalyanya *et al*., 2018).

Several studies have reported leather with different chemical and physical properties due to variations of chemicals and mechanical operations carried out during leather processing (Nalyanya *et al*., 2018). Since tanners are exploring clean technologies options that can replace the hazardous chemicals, the new methods must produce leather with equal or better physical and chemical properties than the conventional method. In addition, there has been a growing consumer awareness in the last 20 years towards the demand of quality leather goods and footwear that meets occupational and safety standards (Nalyanya *et al*., 2021). Most of the developed countries have strict rules that prohibit process that are a threat to human and environment (Saxena *et al*., 2017).

The best options for tanners are to produce quality leathers by use of environmentally friendly methods in order to secure markets even in developed countries (Nalyanya *et al*., 2021). Some of the proposed methods include use of enzymes, carbon dioxide deliming and vegetable tanning.

There are many qualities aspect that the customers look for when buying leather products which include the aesthetic values, physical, hydro- thermal, chemical and fastness, properties (Nalyanya *et al*., 2021). Use of enzymes can give acceptable leather if the processes are well controlled. Some of the physical parameters that are of important to most of the leather products include: flexing endurance, ball burst test, tearing and tensile strength. The organoleptic properties include: fullness, softness, flexibility, grain firmness. The tensile test is normally done to test whether the leather have structural resistance and mostly determined by the moisture and fat content of the leather (Hossain *et al*., 2021). Lastometer test is normally done to assess whether the leather will withstand operation of shoes making (Hossain *et al*., 2021). Flexing endurance is also an important parameter as this value indicates ability of leather to resist folding and breaking. The requirement standard for leather goods varies from one product to another and therefore leather testing help to classify the test leather into different use.

6.3 Materials and methods

Quality of chrome tanned leather produced in chapter 5 was assessed by organoleptic and physical tests. All the organoleptic and physical properties were assessed after retanning, dyeing and fatliquoring. The parameters assessed include: shrinkage temperature, flexing endurance, elongation, tearing and tensile strength

6.3.1 Organoleptic properties of the processed pelt

Organoleptic characteristics of treated leather were evaluated in both enzymatic and conventional methods. Parameters like grain firmness, appearance, softness, flexibility, and cleanliness were rated on a scale of 1 to 10 (from very poor to very good), as per the methodology outlined in (Unango *et al.*, 2019).

6.3.2 Sampling

All the samples used were obtained from butt area according to IUP2 by applying a press knife to the grain surface. Only one sample in each direction was taken from each test pieces.

6.3.3 Tensile strength

Instron 1026 was used to determine the tensile strength according to International Union of Leather Technologist and Chemist Societies (IULTC) (2001) IUP/6. The leather samples were cut across and along the backbone using a dumbbell shaped press knife. The samples were clamped into the jaw of the tensile machine after setting the jaw at 50 mm apart and the jaws lied along the midline. The machine was run to break the test piece. The testing apparatus also recorded the percentage elongation automatically

6.3.4 Shrinkage temperature determination

SATRA STD 114 test apparatus was used in this test as described by IULTC, (2001) IUP/16. The leather samples (50 mm ×2 mm) were cut across and along the backbone line. Small holes were made at the ends of the leather samples and a small weight connected to the lower end. The samples were then held vertically in the test chamber that was filled with water. An adjustable marker outside the tube was used to indicate the position of the lower end. The device was then shut, and the external heat source was applied to the boiler parts to heat the water at a constant temperature

of about 4 ^{o}C. The shrinkage temperature was recorded to be the temperature at which the leather began to shrink.

6.3.5 Measurement of tear strength

Instron 1026 was used to determine the tear strength of the leather samples according to IULTC, (2001) IUP/8. A rectangular leather samples (50 × 25 mm) were cut across and along the backbone line. The samples were clamped into the jaw of the Instron. The process was started until the sample broke and the maximum force obtained at break recorded as the breaking load.

6.3.6 Flexing endurance

It was determined by a bally flexometer as described by IULTC, (2001) IUP/20. Leather samples of dimension 70 mm were folded and attached to the jaws of the instrument with the grain side facing outside. The machine was started when one clamp remained fixed and the other moved to and fro causing folds in the specimen to run along it. The samples were flexed 100,000 times and the grain surface checked for cracks

6.4 Data analysis

SPSS statistical package was used for data analysis. Both descriptive and inferential statistics were carried out. Means and standard deviation were calculated and the results presented in tables and in graphs. T-test was performed to check whether there was a significant difference between the enzymatically processed leather and the conventional method. The significant level was set at $p \leq 0.05$.

6.5 Results and discussion

Quality of the resulting crust leathers: plate 6-1, Plate 6-2, Plate 6-3, and Plate 6-4 was determined visually and by carrying out the physical tests.

Plate 6-1: Goat leather (Enzymatic method)

Plate 6-2: Goat leather (Conventional method)

Plate 6-3: Cattle leather (Enzymatic method)

Plate 6-4: Cattle leather (Conventional Method)

The organoleptic test was determined according to ratio of 1-10 and higher points indicated a superior property. Of all the properties tested, both enzymatic and conventionally processed crust leathers had a rating of 8-10 (Fig 6-1 and Fig 6-2)

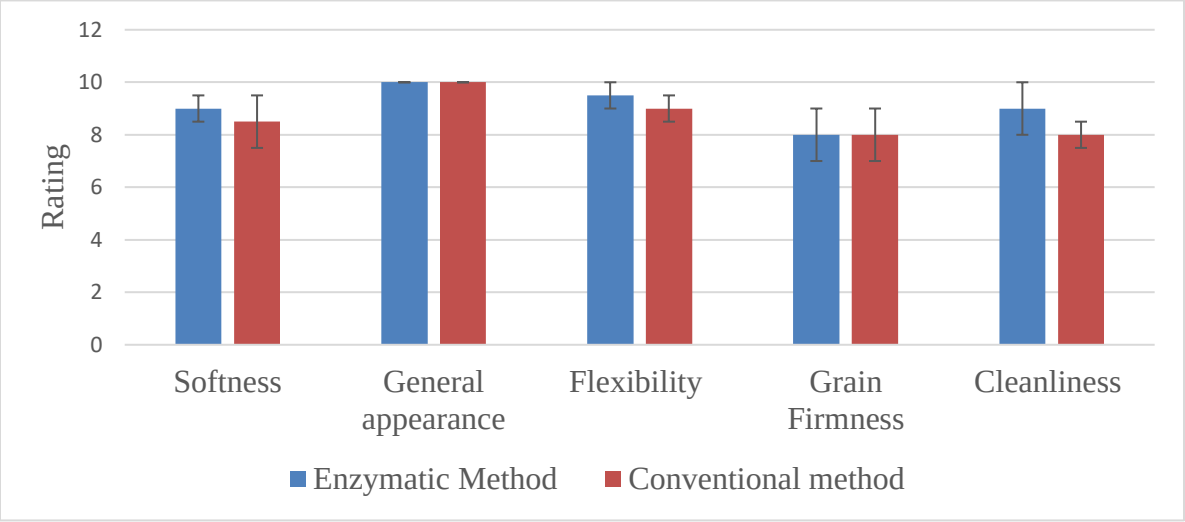

Figure 6-1: Comparison of the organoleptic properties of chrome-tanned goat leather processed by enzymatic and conventional methods in pre-tanning processes

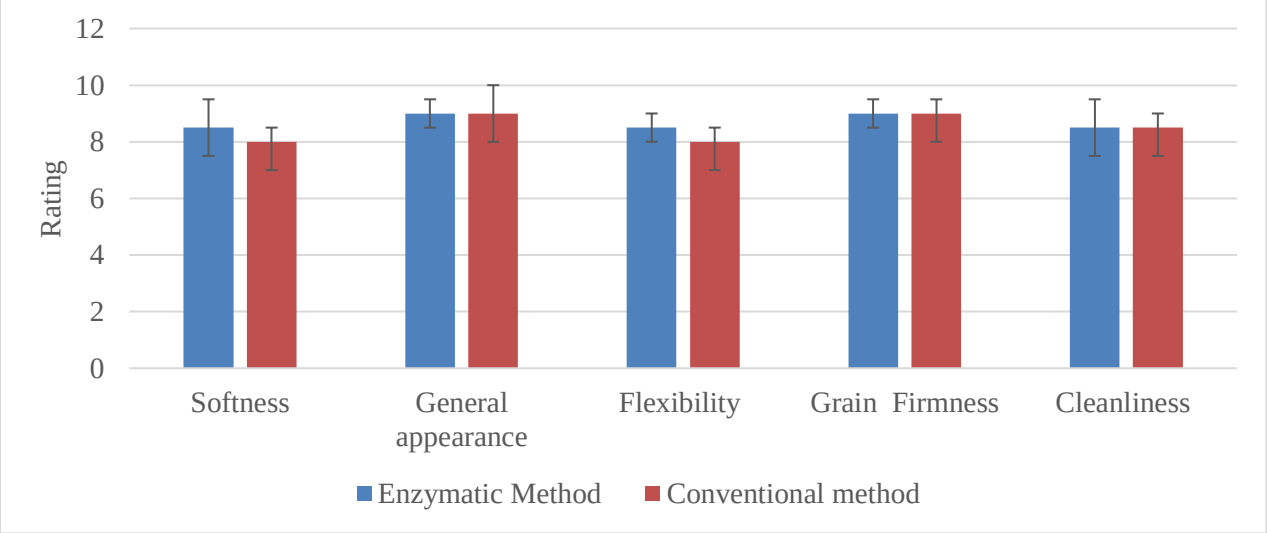

Figure 6-2: Comparison of the organoleptic properties of chrome-tanned cattle leather processed by enzymatic and conventional methods in pre-tanning processes

This is a good indication that enzymes can be used in earlier stages of leather production to produce leather with similar or superior properties to conventionally processed pelts. In addition, the enzymatic method had a superior characteristic in properties such as general appearance, flexibility and softness. The leathers produced by enzymatic method were softer than the conventionally processed leather and this is due to action of enzymes on non-structural protein and this is also associated with fibre opening up (Nyakundi *et al*., 2021). A similar study by Saravanabhavan *et al*., (2004), also reported leather having properties such as grain, fullness, grain tightness, smoothness and softness in the range of 8-9. Nyakundi *et al*., (2021), carrying out the visual assessment of enzymatic unhaired leather and sulphide unhaired leather reported them having a rate of 8.5-9.6. The good rating of enzymatic method is attributed to enhanced uptake of processing chemicals following the sufficient opening up of the collagen fibre matrix by use of enzymes.

Assessment of the physical properties of leather also showed some variations between the conventional and enzymatic method for both bovine and goat leather as can be seen in Tables 6-1 and 6-2

Table 6-1: Comparison of the physical properties of goat leather processed by conventional and enzymatic method

Goatskin leather				
Type of test	**Enzymatic method**	**Conventional method**	**P values**	**Min standards**
Tensile strength	30.17+3	26.7+ 4	0.32	15 mpa
Tearing strength	17.12+4	29.25 + 7	0.08	50 N
Shrinkage temperature	> 100 ^{o}C	> 100 ^{o}C		> 100 ^{o}C
Percentage elongation	67.24+6	66.55 + 7	0.37	30-80%

Flexing endurance	No damage @ 100,000 flexes	No damage @ 100,000 flexes		No damage @ 100,000 flexes

Table 6-2: Comparison of the physical properties of cattle leathers processed by enzymatic and conventional method

Bovine leather				
Type of test	**Enzymatic method**	**Conventional method**	**P values**	**KEBS standard* minimum**
Tensile strength (Mpa)	23.91 + 7	16.2+3	0.04	15 mpa
Tearing strength (N)	79.54+16	81.44+17	0.84	50 N
Shrinkage temperature	> 100 ^{o}C	> 100 ^{o}C		> 100 ^{o}C
Percentage elongation	68.62+ 9	66.29+ 16	0.7	30-80%
Flexing endurance	No damage @ 100,000 flexes	No damage @ 100,000 flexes		No damage @ 50,000 flexes

Tensile and tearing strength tests were performed across and along the backbone line. Mean of these parameters corresponding to across and along the backbone was determined for each leather (Aravindhan *et al*., 2007). The enzymatic processed goatskin leather had a higher tensile strength (30.17 ± 3) than the conventional method (26.7 ± 4) (Fig 6-7 and Fig 6-8) although the difference was not statistically significant (p= 0.32). Tensile strength represents the maximum tensile stress that the leather can hold without breaking (Nalyanya *et al*., 2021). The required minimum standard of tensile strength for shoe upper leather, lining, furniture and leather goods is 15, 15, 10 and 10 N/mm2, respectively (Nalyanya *et al*., 2021).

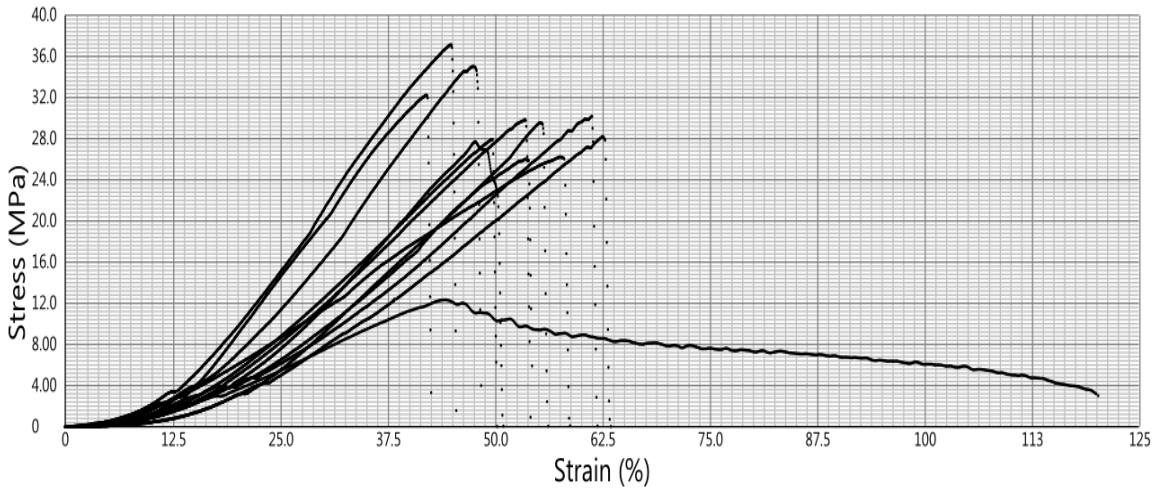

Figure 6-3: Tensile strength of goat leather processed by enzymatic method

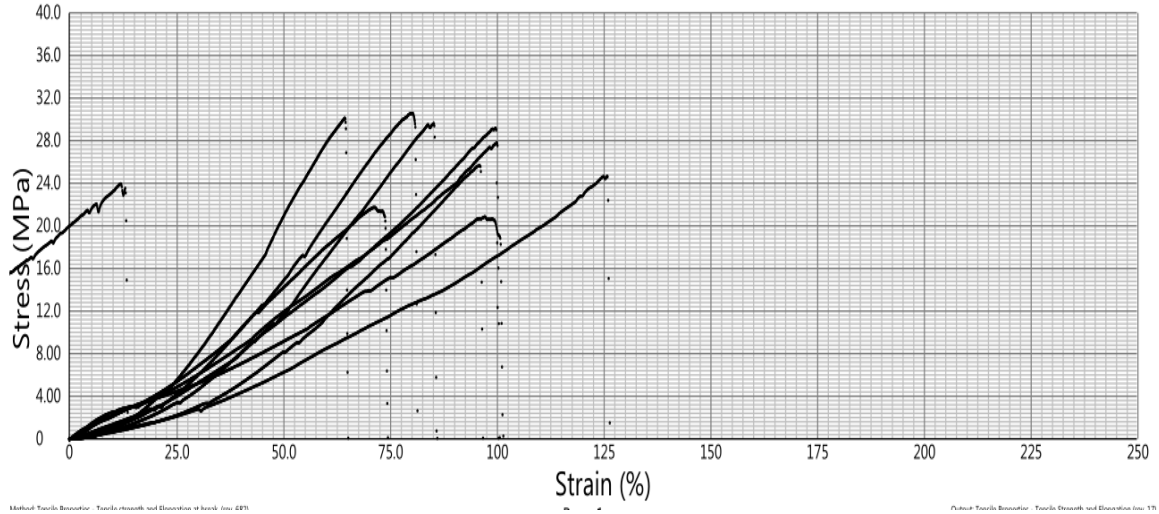

Figure 6-4: Tensile strength of goat leather processed by conventional method

The leather processed by conventional method had a higher tearing strength (29.25 ± 7 N) as compared to the enzymatic method (17.12 ± 4 N) and the difference was not statistically significant (P=0.08). The conventional method had a lower percentage elongation than the enzymatic method as can be seen in table 6-1. Percentage elongation is a crucial parameter and mostly in garment leather making because leathers with low elongation values are easily torn while leathers with

excessive elongation values cause leather goods to deform fast or even lose their usability (Nalyanya *et al*. 2018). All samples passed the shrinkage and flexing test as indicated in table 6-2. Although there seemed to be a difference on the indicated values, all the leather samples passed the minimum requirement for making a shoe upper as required by KEBS.

For bovine leather, the enzymatic method performed better in most of the properties except tearing strength where the conventional method had a higher value (81.44 ± 17 N) and the difference was not statistically significant (p=0.84). Tearing strength measures material resistance to the expansion of cuts when under tension and indicates how well leather will withstand the effects of tearing (Nalyanya *et al*., 2018). All the test samples passed the KEBS specification for shoe uppers as indicated in table 6-2. Several studies have reported different values for physical testing although this is expected due to change of processing recipe, sex, type of water, breed of the animals and chemical modification (Mesa *et al*., 2019). High percentage elongation allows the leather to withstand elongation stresses during lasting process of the shoes and also gives the user comfort by extending the material as they move their feet (Mutlu *et al.*, 2016). Maximum flexibility prevents cracking and tear of the leather during uses (Mesa *et al*., 2019). A similar study investigating lime-sulphide dehairing and enzymatic dehairing of goatskin and sheepskins reported the conventional method having an elongation of 90% (goat), 80% (sheep) while the enzymatic method had 60% (goat) 66% (sheep) (Choudhary *et al*., 2014). In addition, the enzymatic method seemed to have higher tensile strength and tearing strength as compared to the conventional method (Choudhary *et al*., 2014).

Leather samples also passed the flexing endurance and shrinkage temperature tests. The shrinkage temperature is the temperature at which the leather starts to shrink when put in a heated water and it related to the amount of tanning agents absorbed by the pelts (Griyanitasari *et al*., 2018). Higher

degree of penetration and fixation of tannins with the collagen structure raises the shrinkage temperature (Nalyanya *et al*., 2013). Presence of non-collagenous substance hinders the penetration of tanning agents. Since shrinkage temperature of all the leather was above 100 ^{0}C, it is a clear indication that enzymatic method was also efficient in the opening up of the collagen structure to allow fixation of chrome tanning agents. This test is very important in shoe making process where the upper leather is heated to stick into the midsole material (Griyanitasari *et al*., 2018).

Flexing endurance measures leather resistance to cracking and creasing when bent repeatedly, simulating actual use of the shoe (Nalyanya *et al*., 2018, Ali *et al*., 2020). All the leather samples were flexed 100, 000 times without fail and they surpassed the minimum requirement of 50,000 flexes.

6.6 Conclusion

Crust leather processed using enzymatic method showed similar physical and organoleptic properties as observed for conventional processed leather. Therefore, use of enzymes should be adopted in soaking, unhairing and degreasing process to reduce pollution in tannery wastewater and in the production of quality leather.

CHAPTER SEVEN: GENERAL CONCLUSIONS AND RECOMMENDATIONS

7.1 General conclusions

- Four types of bating enzymes (Microbate, micro-enzyme P, Microbate elbate and microenzyme elbate) were commercially available in Kenyan market and the assessment of their effectiveness in different pretanning processes revealed that these enzymes were only effective in one processing step (bating). Further analysis of their protease activity, the study found these enzymes having different activities: Microbate-Yetu leather 24,137.4 (U/g), Microbate elbate -AHITI- 11,341.2 (U/g), Micro enzyme P-Sagana 24,717.6 (U/g), Microbate- Ewaso Nyiro 21,321.33 (U/g and Microenzyme elbate- LIK 23,883.6 (U/g). Although all the enzymes failed to meet the minimum requirement of 90% solubility, they were effective in bating process but ineffective in soaking, unhairing and degreasing process.
- A combination of keratinase and lipase (KL) was selected for the soaking process. A formulation of keratinase, protease and lipase (KPL) enzymes was selected for unhairing stage while lipase enzyme was selected for application in a degreasing process.
- A comparison of enzymatic and conventional methods in pollution reduction showed the enzymatic method to be highly effective in pollution reduction in the unhairing process with a percentage reduction of TS (48.89%), SS (66.73%), BOD (52.1%), COD (80.96%) and sulphide (100%) for goatskins and TS (74.26), SS (73.52), BOD (49.79%), COD (80.01%) and sulphide (100%) for cattle hides. In most of the parameters assessed in other pretanning stages such as soaking, deliming bating and degreasing, enzymatic method had lower values as compared to the conventional method and therefore effective on pollution reduction.

- On the assessment of the organoleptic properties, both conventional and enzymatic tanned leather had a rating of more than 8. Both cattle and goat leather processed by enzymatic and conventional methods met most of the minimum requirement for shoe uppers such as tearing strength 50 N, tensile strength 15 mpa, percentage elongation > 30%, shrinkage temperature > 100 ^{0}C and flexing endurance 50, 000 flexes

7.2 Recommendations

- Commercial enzymes met most of the requirement for bating enzymes and they were recommended for use only in the bating process since they were not effective in other processes. These enzymes failed the solubility test and the manufacturers should ensure that they meet the required specification. KEBS should also develop specific standards for enzymes used in leather sector.
- A formulation of keratinase and lipase (LK), Keratinase protease and lipase (KPL), and single use of lipase were recommended for use in soaking unhairing and degreasing processes respectively since they were highly effective in those pre-tanning processes
- The enzymatic method seemed to be highly effective on pollution reduction as compared to the conventional method. It is high time that the use of enzyme be adopted to reduce pollution associated with leather sector.
- Enzymatic method had a higher rating in most of the organoleptic properties tested and even higher physical properties as compared to the conventional method. The use of these formulations was recommended for adoption in the tannery pretanning processes
- Since all the enzymes in Kenyan market are imported, there is an urgent need to have local manufacturers that can make cheap and readily available enzymes for use in leather sector

- The government of Kenyan should put more research funds in the area of leather to ensure that the processing techniques are eco-friendly.

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APPENDECES

9.1 A questionnaire for the identification of the type of enzymes used by the tanners

Introduction

Processing of hides and skins is a lucrative business that has created a lot of jobs for thousands of Kenyans. Different enzymes and chemicals are normally used in the process of leather manufacture and the amount used varies from one tannery to another. This study is meant to identify the types of enzymes and the stages they are used in the tanneries with the aim of analyzing their effectiveness and this will enable us to advice the tanners accordingly. All the information that you will provide will be confidential and I will highly appreciate for your cooperation.

Name of the company …….

Contact person name……..

Date ……year…….....

Required information

Which type of hide/skin do you process?

Sheep ☐ Goat ☐ Cattle ☐ Others……

Do you use enzymes in your company?

Yes ☐ No ☐

If yes, which types of enzymes?

Protease ☐ lipase ☐ keratinase ☐ amylase ☐ others……

In which stage of leather processing do you use enzymes?

Soaking ☐ Unhairing ☐ Liming ☐ Deliming

Bating ☐ Pickling ☐ others……

Where do you buy your enzymes……

How costly are the enzymes?

Cheap ☐ affordable ☐ costly ☐

What problems are you experiencing for using enzymes in your company

……

……

……

……

Apart from these questions that you have answered, do you have any other information about enzymes that you would like me to know? Yes ☐ NO ☐

If yes, which one?

……

……

……

……

……

END, THANK YOU

9.2 Time taken to discolor the test solutions

Name of the enzyme	Temperature	25 ^{o}C	28 ^{0}C	30 ^{o}C	33 ^{o}C	35 ^{o}C
lipase		12	9	8	5	4
Amylase		20	17	15	12	8

9.3 Optimum temperature for protease and Keratinase enzymes

Keratinase					
Hours	25 ^{o}C	28 ^{0}C	30 ^{o}C	33 ^{o}C	35 ^{o}C
1 Hr	0	0	0	0	0
2 Hrs	0	0	0	0	0
3 Hrs	0	0	0	0	5
4 Hrs	0	0	0	40	60
5 Hrs	0	0	5	80	80
6 Hrs	0	10	30	100	100
24 Hrs	100	100	100	100	100
Protease					
Hours	25 ^{o}C	28 ^{0}C	30 ^{o}C	33 ^{o}C	35 ^{o}C
1 Hr	0	0	0	0	0
2 Hrs	0	0	0	0	0
3 Hrs	0	0	0	0	0
4 Hrs	0	0	5	20	30
5 Hrs	0	5	10	40	50

6 Hrs	0	15	30	70	70
24 Hrs	100	100	100	100	100

9.4 Optimum pH for protease and keratinase enzymes

Keratinase pH				
Hours	pH 7	pH 8	pH 9	pH 10
1 Hr	0	0	0	0
2 Hrs	0	0	0	0
3 Hrs	5	40	30	8
4 Hrs	60	80	60	50
5 Hrs	80	100	85	70
6 Hrs	100	100	90	80
24 Hrs	100	100	100	100
protease				
Hours	pH 7	pH 8	pH 9	pH 10
1 Hr	0	0	0	0
2 Hrs	0	0	0	0
3 Hrs	0	5	10	20
4 Hrs	30	40	50	60
5 Hrs	50	60	70	75
6 Hrs	70	90	95	100
24 Hrs	100	100	100	100