UNIVERSITY OF NAIROBI

ENZYMATIC HYDROLYSIS OF CHROMIUM TANNED LEATHER WASTES, AMINO ACID COMPOSITION ANALYSIS AND CHEMICAL CHARACTERIZATION OF HYDROLYSATE

BY

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DECLARATION

I declare that this thesis is my original work and has not been submitted elsewhere for examination, award of degree or publication. Where other people's work or my own work has been used it has been acknowledged and referenced in accordance with the university of Nairobi requirements.

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DEDICATION

This work is dedicated to my family for supporting me and advising me. I also dedicate this to my supervisors for continuous guidance and support during the period of my research.

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ABSTRACT

The disposal of leather wastes poses many challenges and is a major threat to the environment in many countries across the world. Large amounts of wastes from the tanneries including: shavings, trimmings, sludge and untreated waste waters are disposed to the environment and pose a threat to the environment and humans. Solid wastes in particular are non-biodegradable and therefore remain in the environment for a long period of time. The major tanning agent is chromium, a clear indication that the solid wastes and even the liquid waste expose the environment to significant amount of chromium.

In this study enzymes extracted from Lake Bogoria, an extremophile environment were used to hydrolyze leather trimmings to generate valuable products and consequently reduce the effect of solid waste disposal and chromium discharged to the environment. Different parameters such as pH, temperature and time were optimized for enzymatic hydrolysis. Characterization of the hydrolysate was done using FTIR and HPLC and the heavy metals were analyzed using Inductively Coupled Plasma mass spectroscopy (ICP-MS).

The enzyme was able to hydrolyze the trimmings under optimum conditions. The optimum conditions for hydrolysis were pH of 12 and a temperature of 75oC. FTIR analysis of the samples showed peaks at 3290.56 cm -1 and 3334.92 cm -1 indicating the presence of amide bands. The HPLC analysis indicated the presence of 16 and 15 amino acids in the freeze dried and dry filtrate respectively. Further, glycine was the most abundant amino acid comprising 37.83% and 43.36% mole fraction in the freeze dried and dry filtrate samples respectively. Glycine also indicated large percentage by mass in both samples, 25.17% in freeze dried sample and 29.23% by weight in the dry filtrate. Both Histidine and Tryptophan were absent in both samples. The element content of the samples was analyzed using Inductively Coupled Plasma mass spectroscopy (ICP-MS) and it indicated the presence of Cr and S, in addition to several other elements. The dry filtrate showed higher element content filtrate than in the freeze dried samples. This study shows that gelatin can be obtained from the leather wastes and the enzyme hydrolysis process can be achieved within 21 hours, under the optimum conditions established.

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrometry
ATR	Average True Range
CTLSs	Chrome-tanned leather shavings
DHHS	Department of Health and Human Services
DEFRA	Department for Environment, Food and Rural Affairs
FTIR	Fourier Transform Infra-Red Spectroscopy
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
NEMA	National Environment Management Authority
pH	Potential Hydrogen
PAHS	Polycyclic Aromatic Hydrocarbons
UV	Ultra Violet radiation
UNIDO	United Nations Industrial Development Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Leather trimmings are pieces of leather wastes that are obtained when various items are made from leather such as wallets, belts, boots and shoes, hand bags etc. Due to the various shapes and sizes required from tanned leather, the chopping results in generation of a huge volume of waste trimmings in the leather industries.

Leather has been the main economic sector providing employment to many people across the world. In leather based industries in Pakistan for example, the leather industry is amongst the greatest vital commercial sectors employing about 1 million people and sales above 1 billion dollars and is ranked the second in the exporting sector (Saif, 2012). In Kenya, only about 140 million leather products are made in a year which is approximately 0.14% of the world's total export. The leather sector in Kenya employs about 14000 people and out of these 10,000 works in the informal sector (Hansen et al., 2015). Therefore, many countries rely on the leather industries as a source of income for economic development (Hansen et al., 2015).

Enormous volumes of solid wastes are released from the tanning industries each year. The report from world bank specified that the solid waste constitute about 70% of the total original weight (Singh et al., 2011). Chromium based wastes formed in leather production comprises of Chrome-tanned leather shavings (CTLSs), chromium sludge and chrome skin clippings. The resulting wastes cause a serious risk to the environment. It has been predicted that about 0.02 million tons of CTLSs are produced in India annually and nearly (0.8 million tons) of chromium tanned leather shavings are produced worldwide every year (Gupta, 2016). Most of these wastes are disposed physically through landfills, chemically through chemical hydrolysis or incineration (Sundar et al., 2011). However, reutilization of leather wastes is desirable since it is environmental friendly and saves on financial loses.

Kanpur town in India (formerly known as Leather City of World) contains more than 10,000 tanneries and disposes over 22 tons of noxious waste into River Ganges daily (Gupta, 2016). In Brazil, there are about 700 tanneries processing 7.6 million hides and 37.2 million skins (Aftab et al., 2006). The industrial leather process produces plentiful amounts of solid (mainly inorganic solids), liquids and gaseous wastes. This is an increasing threat since most companies rely on chromium tanning technology and release a lot of chromium tanned solid and liquid wastes to the environment (Sundar et al., 2011). When one metric

ton of raw skins is processed it produces about 200 kilograms of tanned leather, about 190 to 350 kilograms of untanned waste 200 to 250 kilograms of leather waste (tanned) and fifty thousand kilograms of waste waters (Sundar et al., 2011). In the leather manufacturing industry, only 20% of the saturated brackish rawhides/skins are transformed into marketable leather, whilst massive fraction turns out to be trimmings, leather shaving (Sundar et al., 2011).

Leather disposal technologies have evolved from small-scale to large scale industrial activities. Connected with leather processing, there are serious environmental challenges which transpire from the form and volume of waste released in every dispensation industry. These wastes in addition to resolvable proteins or fats with solid contaminants are disposed in wastelands. The structure of newly ready hides has 65% water, 33% protein and fat substances (2-6 % cattle/ calf, 5-30 % sheep and 2-10 % goat) with slight amounts of carbohydrates, lipid, globulin and albumins. Besides the 33% protein content, values of 96.5% fibrous proteins, 3- 5% is globular protein where there are 1% keratin 1% elastin and 98% collagen (Counts et al., 1996). It is clearly evident that the leather contains a reasonable amount of important materials that can be used commercially through recycling process of leather wastes (Counts et al., 1996).

Chromium leather wastes are not biodegradable naturally and this is the main problem when the wastes are dumped in solid form to the environment. The major browning agent used in the leather industry is chromium and now accounts for about 85% of leather produced internationally. Chromium tanned leather has better comfort and strength properties compared to other tanning systems (Kanagaraj et al., 2008; Rao et al., 2002). This poses a threat when these wastes from chromium tanned leather are released to the environment (Cabeza et al., 1998).

In Kenya, there are 14 tanneries producing about 40 tons of wet blue leather which accounts for the large percentage of chromium tanned solid wastes (shavings and trimmings). Chromium leather wastes forms the largest percentage (63%) of the total solid wastes. Such wastes do not disintegrate when disposed to the environment hence they pose a threat to the environment and human health (Mwondu et al., 2020).

The common mode of disposal in Kenya and the world is land fill and open dumping which is not a recommended form of waste management (DEFRA, 2011) because it eventually contaminates underground water through chromium leaching. Whereas some companies have adopted the use of chemical hydrolysis, is expensive and the treatment process still leaks the chromium to the environment. Further, others have adopted incineration techniques; however, it releases noxious gases to the environment such as SO2 and Nitrogenous gases which is required to be removed by air pollution control devices hence increasing the cost of production. The ash also contains Cr, hydrogenated organic compounds and poly aromatic hydrocarbons (PAH'S). The National Environment Management

Authority (NEMA, 2014) emphasize on reusing and recycling of solid wastes as the environmental friendly way of dealing with solid wastes. (Mwondu et al., 2020).

Therefore, with all these problems posed by the leather industry, it's important to develop a suitable and sustainable way of dealing with leather trimmings and other types of solid wastes from the tanneries. Various technologies adopted in leather waste disposal have side effects to the environment and are also not economically viable. Significant research has been done in recycling the leather wastes. In this study the use of enzymatic technology in hydrolysis of the chromium tanned leather trimmings, will be investigated as an environmentally friendly and sustainable method. The advantage of the enzymatic technology is that enzymes can be obtained locally and they can also be recycled. Most enzymes like Bacillus Sp works well under optimum affordable conditions that can easily attained in the laboratories (Reddy et al., 2008).

1.2 Statement of the problem

The world volume of leather processing is about 15 million tons of rawhide and skins annually and it releases about 6 million tons of solid wastes per annum (Niculescu et al., 2012). The present research highlights the possibility of extracting the protein component from chrome leather waste in the leather industry through various techniques and using it in various agricultural and industrial applications.

The average waste water is over 15,000 million liters per day. Also, there is a large disposal of sludge, roughly 4.5 million tons annually. All these waste released to the environment are the major source of environmental contaminations (Bajza and Vrcek, 2001). Disposal methods such as land fill, open dumping, incineration and chemical hydrolysis have been used for decades in trying to deal with solid wastes from the leather industry. These methods are not effective because they increase production cost, exposure of noxious gases to the environment (such as SO2) and they leach chromium to the underground waters (Mwondu et al., 2020).

Therefore, there is need for environmentally friendly disposal methods to help in controlling the amount of wastes released to the environment from the leather industries. Of particular interest are chromium tanned solid wastes. Such methods involve enzymatic hydrolysis which may be applied in hydrolysis of the leather shaving and dehairing of the leather skin (Janacova et al., 2006). The use of such technologies can control and promote recycling of the chromium from the hydrolyzed solution before freeze drying and conversion to other products.

1.3 Objectives

1.3.1 General objective

To analyze the efficacy of locally isolated enzyme in hydrolysis of tanned leather wastes.

1.3.2 Specific objectives

1. To investigate the optimum conditions for enzymatic hydrolysis of tanned Leather Trimmings.
2. To determine the Effluent physicochemical properties such as pH, conductivity and chromium levels.
3. To study the properties of the hydrolysate obtained using FTIR.
4. To determine the amino acid composition of hydrolysis products.

1.4 Justification

Several efforts have been attempted to tackle the problem posed by wastes generated by the leather industry. An overwhelming majority of current technologies used involve use of chemicals which are toxic as well as expensive and most leather industries face a serious challenge in their waste management practices to comply with environmental waste management regulations. Some other disposal methods such as incineration, introduce noxious gases to the environment and also the ashes contain chromium and polyhydrocarbons which are difficult to control. Enzymatic technology has been proposed for both de-haring of the skins and hydrolysis of the shavings.

However, inadequate research has focused on leather waste trimmings, yet huge volumes of waste are generated annually. This study explored the use of enzymes in treatment of leather wastes trimmings (chromium tanned leather trimmings). This is because enzymes can be produced locally and can be recycled unlike chemicals which are imported and not reused. This technique is able to break down the trimmings to a viscous liquid with tinny insoluble particles. The freeze-dried value addition products can be used in various applications in industries. This is also advantageous since the leaching of chromium into the environment can subsequently be controlled through adsorption or other possible techniques.

It is anticipated that the proposed technology will help in reducing the amount of the solid wastes discharged to the environment and cleaning up of the environment. Most of the wastes released to the environment contain chromium because it is used as a tuning agent and therefore control of chromium is important to avoid seepage to the environment, sewer lines and the ground water. This study will help to assess levels of chromium in tannery trimmings effluents and conversion of solid wastes into useful recyclable products. The optimum conditions for enzyme hydrolysis will be investigated and the efficiency and sustainability of the process investigated.

CHAPTER TWO

2. 0 LITERATURE REVIEW

2.1 Leather

Leather remains strong and stretchy material formed by tanning animal's raw hide and skins. The most common type of leather is cattle hides and it has been processed from the artisan to current manufacturing scales. However, there are a number of other animals whose skins can used commercially for leather products. Leather is obtained from animals in the raw form then it is tanned to obtain its stability, color and strength. The tanned leather is the one that is shaped in different sizes for different applications. Most tanning companies in Kenya export leather as wet blue or raw hides. Tanning process converts leather in the different strengths and stabilities. However leather has come to criticism by animal right groups such as People for the Ethical Treatment of Animal (PETA) in 20th and 21st century and they claim that it is unethical because it involves killing of animals (Regan, 2004a).

Leather can make a variety of products such as: automobiles, seats, foot ware (shoes and boots), bindings, clothing, book accessories, bags, auto upholstery, wallets, leather belts, home furnishings and can be fashioned in many forms, styles and adorned by a large scope of practices. Skins and artifacts usage started during 2200 BC to date (Gosden, 2008).

Most of these commodities are produced for local use and commercial purposes. Most leather products are associated with stability and strength. They last longer and they are not affected by the physical stress and environmental challenges. Peripatetic tools and foot ware from the leather are durable and trusted by many tourist and local markets in African markets especially in Kenya (Mwondu et al., 2020). The sort of skin shaped in a particular zone is determined principally with the primary supply existing. In USA, they essentially use cattle hide/skin upgraded with goat, sheep, ostrich, yak, bison and deer (Cabeza et al., 1998). A great deal of the intriguing cow hides is likewise getting more considered currently. The skin of stingray also can be converted to leather and commonly found particularly in Thailand because they are in plenty (Karthikeya et al., 2011; Regan, 2004b). This skin is rottenly tinted dark and secured with round knocks in the typical example of the back edge of an animal. These knocks were constantly being shaded white to feature the adornment. Stingray leather is additionally utilized as hangs on Japanese Katanas, Scottish crate hilted swords and Chinese swords. Stingray leather is additionally used in high scraped spot regions in bike hustling leathers (Regan, 2004b).

In recent times, most leather is made of cow's skins which include sixty-five percent of the total leather products in the world (Shapiro, 2018). Different animals likewise are utilized which include: goats (11 %), sheep (13 %), and pigs which about 10% (van Driel-Murray, 2008). Hides of the Horses are utilized primarily as strong leather. Shell cordovan is a horse skin made from an under coat originating uniquely in equestrian species known as the shell and it is well recognized from its mirror-like completion and against wrinkling assets (van Driel-Murray, 2008).

Deerskin is comprehensively utilized in work gloves and inside shoes. Deer and Lambskin are also utilized for tolerant leather in progressively extravagant wear. Reptilian skins for example gator, crocodile and snakes are famous for their disparate improvements that echo the scales of their group. Consequently, this has prompted farming of these species (Blaine, 2002). Kangaroo skin is utilized to create substances that are strong, durable and flexible. This material is the one normally used in Bullwhips. A few motorcyclists recommend kangaroo skin for bike leather covers since it is light in weight and resist scratches (Lee et al., 2011). Kangaroo skin is also utilized in punching rate packs and soccer footwear (Blaine, 2002). In spite of the fact that at first brought for their plumes during the nineteenth century, many ostriches are currently progressively pervasive for meat and skin. Ostrich skin consists of a distinctive appearance in light of the enormous hair follicle where the plumes developed. Various methods produce various completions for some applications such as: adornments, car, items upholstery, footwear and apparel (Blaine, 2002).

2.2 Importance of leather products

There is an increasing demand for leather products worldwide. There is 23 billion square feet of leather that are delivered every year with an estimated value of $77 billion (Morelli et al., 2015). Skin foot ware is among the significant outlet for the material prized at $47 billion which is 60 percent of the worldwide business. This includes leather products like ornaments estimated at $12.3 billion and constitute 15.9% of the overall global trade (Hansen et al., 2015). Therefore, leather manufacturing industries and leather products are the main contributor of financial growth internationally by providing employment and luxury leather products (Martin, 2010). The massive volumes of leather manufacturing process yields to large piles of chromium leather wastes leading to environmental pollution (Sarker et al., 2013).

Despite Kenya being the third largest livestock holder in Africa, it still has relatively low levels of employment in the leather industry. During peak seasons it employs about 14,000 employees in the leather sector (Krishnan et al., 2019), with the informal sectors accounting for 10,000 people. The slow growth of the leather sector is due to the high cost of equipment, leather inputs, high cost of labor, electricity and the high cost of domestically produced leather compared to imported products. The informal sector in Kariokor in Nairobi is very competitive and it accounts for the largest number of foot ware in the region

and it provides a tangible employment in the leather industry. Few companies in Kenya produce finished products like Alpharama which is located in Machakos and it's the largest leather industry in Kenya (Hansen et al., 2015). Despite creating employment and improving the economy, the leather sector in Kenya also release a lot of solid wastes to the environment containing chromium hence increasing the threat to the environment.

2.3 Leather waste management in Kenya

A study done by Mwondu et al., (2020) in six leading selected tanneries investigated the volume of chromium wastes released by various industries in the Kenya. These tanneries include: Alpharama, Bata, Nakuru Tanneries, East Africa tanneries, Aziz Tanneries and Redamac. Alpharama is the largest and it processes about 40 tons of leather and 2000 pieces of skin (goat and sheep) per day to finished leather (Mwondu et al., 2020).

The total solid wastes from these industries were classified as; Chrome spilt trimmings (36.2%), Chrome shavings (32.1%) and vegetable split trimmings (14.9%) etc. of the total waste produced. This finding gives a clear indication that a large volume of chromium solid wastes is discharged by these industries per month (Mwondu et al., 2020).

Table 1 shows the summary of the leather processed and the solid wastes released in a month.

Table 1: The volume of solid wastes released in selected tanneries in Kenya.

Industry Name		Quantity processed (Kg/month)	Quantity of waste generated (Kg/month)
1.	Alpharama	2,128,420	675,060
2.	Bata	2,077,992	663,000
3.	Nakuru tanneries	1,027,230	325,000
4.	East Africa tanneries	811,414	254,500
5.	Aziz tanneries	314,289	101,000
6.	Redamac	283,180	89,000
	Total	6,642,525	2,112,560

(Source: Mwondu et al., 2020)

2.3.1 Modes of leather waste disposal in Kenya

The mode of leather wastes disposal in industries includes land filling, open dumping, incineration and pyrolysis. There is limited information on recycling, re-use or other methods of disposal. Also options for solid leather waste disposal in Kenya are limited. Consequently, the greatest challenge faced by tannery operators is disposal of chromium tanned solid wastes to the environment to comply with effluent discharge standards. Chrome is the major tanning agent used for many years because its products are strong and produce durable leather products (Mwondu et al., 2020).

Waste disposal techniques such as land fill and open dumping comes with a number of challenges including inadequate land, challenges to access dumping sites due to poor roads and leaching of the chromium to the underground waters. There is need therefore for the recycling and reuse method of the solid wastes particularly leather wastes (DEFRA, 2011).

Incineration has been adopted as a method of waste disposal but in return it releases noxious gases to the environment such as SO2 and nitrogen related gases which need to be removed using air pollution control devices hence increasing the cost of production. Besides, there are also other sources of pollutants in the ash like chromium, hydrogenated organic compounds and poly aromatic hydrocarbons (PAHs). However, there are no appropriate methods of dealing with the listed pollutants from the ash hence becoming a threat again (Mwondu et al., 2020). Chromium tanned leather wastes accounts for about 68.3% (largest percentage) and it persists in the environment hence posing a challenge to the eco system (Paliwal et al., 2014).

2.4 The Process of Tanning

The procedure of tanning leather remains basically the one intended to wrinkle a hide and mitigate the subsequent material with the end goal that it won't rot or reinforced into a clumsy structure. The process includes scratching the hide to remove the meat, fat and hair, spread to destroy lime glues, decolorizing and safeguarding the skin. The variation between treated leather and skin depends on their reaction to warmth and wet environment. Tanned leather remains elastic in heat and does not decompose when moistened. Rawhide on the other hand can strengthen when heated and when rewetted it putrefies (Garone et al., 2015). There are various available assorted tanning methodologies, dependent upon the desired products characteristics and applications. Several methods such as Chromium tanning, vegetable tanning, synthetic tanning, tanning with aluminium and aldehyde tanning have been adopted over the years. However, chromium is the most dominant because it results to strong leather products and non-degradable materials of leather which can last longer to the environment (Garone et al., 2015).

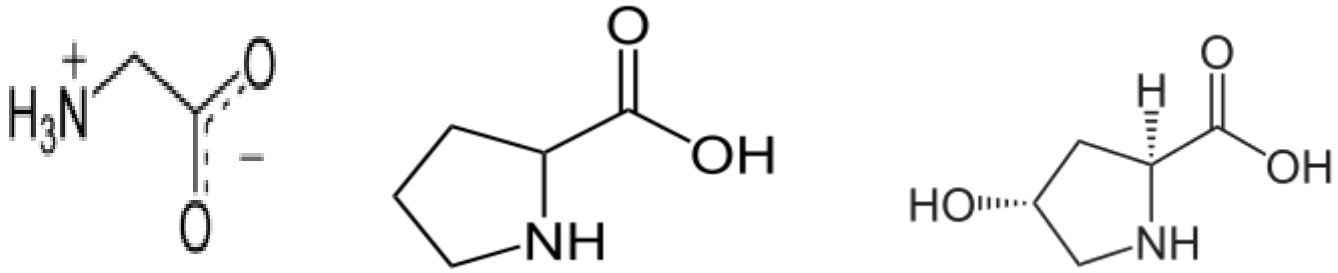
2.4.1 The chemistry of using chromium in tanning hide/skins

Chromium is a chemical element with atomic number 24 and it is steel grey and lustrous, hard and brittle transition metal. Chromium metal and Cr3+ ions are not toxic and they are considered essential nutrients in human for insulin sugar and lipids metabolism (Rowland et al., 2019). However, Cr6+ is both toxic and carcinogenic; and this is the reason why chromium should not be disposed to the environment and its essentially important to clean areas where chromium has been predisposed before like damping sites for leather wastes and in tanneries where chromium is used as tanning agent (Kaiser, 2000). Most of Cr3+ compounds such as Chromium III nitrate ([Cr(H2O)6](NO3)3•3H2O) are used in dyeing industries and in laboratories (Sauter et al., 1990). Chromium III acetate [Cr3O(O2CCH3)6(OH2)3]Cl(H2O)6 exists as a cation ([Cr3O(O2CCH3)6(OH2)3]+), and the common chromium III oxide which is used as a pigment Cr2O3 (Walker, 2018). Cr+6 occurs as CrO4 2- ions or dichromate ions Cr2O7 2- state which exist at equilibrium determined by pH.

$$2\ [CrO_4]^{2-} + 2\ H^+ \rightleftharpoons [Cr_2O_7]^{2-} + H_2O$$

The two commercially available chromate compounds are sodium chromate and potassium chromate which are used as oxidizing agents (Holleman et al., 1985). Chromium IV compounds also can be used to preserve wood (the chromate copper acetate) (CCA) (Hingston et al., 2001). The tanning process in the leather industry is important because it increases the crosslinking between the collagen chains from 10 to 12 Å. The pH must be very acidic when Cr is introduced to ensure chromium complexes are small enough to fit in between the fibers and residue of the collagen. The pH is raised once the desired level of penetration of Cr to the skin is attained to enhance the "Basification" process. In the raw state the chrome tanned skin they appear greyish in color and they are called 'wet blue'. The bath of chromium is also treated with sodium bicarbonate at a pH of 4 to 4.3 which induces cross linking between the chromium and the collagen, the pH is increased normally accompanied by increase in temperature up to 40˚C.

Chromium III sulphate has been the most effective tanning agent ([Cr (H2O) 6]2(SO4)3 over the years (Rao et al., 2002). During tanning process chromium three sulphate dissolve to give hexaaquachromiuim III cation [Cr (H2O) 6]3+; which undergoes olation process to give polychromium III compounds that are active in tanning being the cross linking of tanning substitutes. The chemistry of [Cr (H2O) 6]3+ is more complex in the tanning bath due to presence of a variety ligands such as sulphate ions, collagens carboxylic groups and amine groups which form side chains amino acids and masking agents such as acetic acids that suppress the formation of chromium III chains (Priyadharshini, 2019). Collagen is characterized by high content of glycine, proline and hydroproline compounds (figure1) in the repeat of –gly-pro-hypro-gly- gives collagen high helical structure, collagens high content of hydroproline allows for significant cross linking by hydrogen bonding (Priyadharshini, 2019).



Glycine proline hydroxyproline

Figure 1: Some of the collagen compounds.

Chromium tanning is the predominant strategy for making leather nowadays and essentially it depends upon the toxic slop of chromium salts and browning liquor to yield a flexible and frequently bluish hued product. The ready skins are protected in a holder with chromium until when the pH drops to 2.8 - 3.2; from there it's moved to an auxiliary compartment filled by tanning alcohol that infiltrates the skin. When the alcohol has been added, it makes the pH in the compartment to increase to about 3.8 and 4.2 (Garone et al., 2015).

2.5 leather wastes

There are different forms of wastes produced in leather industries which includes, solid wastes (shavings and trimmings), liquid wastes, sludge and gaseous wastes during incineration processes. All these forms of wastes are produced and released to the environment hence becoming a threat to human and marine life. Solid wastes acquired throughout tanning process consist of leather trimmings and shavings that comprise of a high protein content and collagen (Rao et al., 2002). Solid wastes are produced in large amounts and become a risk to the environment due in adequate disposal options. Invariably they are disposed in landfills or open dumpings which positions a threat to the environment (Ludvik & Buljan, 2000).

Waste water discharge is mostly an outcome of the essential beginning stage of tanning process, wherein bits of substance like hair, crap, and other animals side products are blended into wash-down water and push off. Small measures of chromium are required in nature for different plants and animals in controlling their metabolic capacities. Most of these discharges from these processes contain chromium that is released to the environment and may pose toxic effect if they exceed the maximum permissible level. Of particular concern, disposal of large amounts of waste bound with chromium into nearby water bodies, is harmful to fish gills, provoke breathing challenges, and can lead to sterility, and birth defects. It can

likewise trigger various genuine malignant growths in creatures through the evolved way of life. Other forms of industrial wastes include gaseous wastes and sludge (Shrivastava, 2012; Ludvik & Buljan, 2000).

2.5.1 Projection of leather wastes increase in Kenya

The rapid growth of Kenyan population and urbanization has directly influenced the consumption of high valuable products such as meat, milk and eggs. Higher earning Kenyans use a lot of money on dairy, meat and eggs which can be ascribed to equally high grade luxurious products and high quantities. It is estimated that, eating of beef and milk will increase by over 170% between 2010 and 2050 that is approximately 0.81 and 8.5 million tons correspondingly (Pardey et al., 2014).

The main livestock species in Kenya include cattle (18 million), sheep (18 million), goats (28 million), camels (3 million) pigs (334,689) and poultry (31 million). Presently, about 60% of entire households keep livestock (Bouwman et al., 2013; Smith et al., 2013). In response to the growing demand for animal source foods, livestock custody households as well as secluded commercial livestock enterprises, will expand their livestock possessions and adopt productivity-enhancing rehearses. Consequently, more leather will be processed and a lot of wastes will be discharged to the environment over the years hence becoming a threat to the environment (Turk, 2016).

2.6 Dangers of chrome tanned leather waste to the environment.

Most chromium tanned leather wastes are disposed to the environment through land fill and open dumping. Fibbi et al,, (2012) observed that when one ton of raw hides is tanned, it produces about 20 - 80 M3 of wastewater containing chromium fixations (250 mg/L) and sulfide (500 mg/L) that are discharged to the environment. More than 70 % of unrefined skin is disposed as solid waste (Kolomazník et al., 2007).

However, there are various technologies available to reduce these impacts. The United Nations Industrial Development Organization has reported chrome management techniques for use in industries, such as direct recouping chromium submersion method in the underlying tanning and succeeding re-tanning steps, which reduces chromium fixation in wastewater by 21%. Similarly, by recouping chromium, at times by quickly hastening it from the corrosive shower with NaOH or Na2CO3 or gradually coaxing it out with MgO, one can recover in any event 25% to 30% of the shower's chrome content. An investigation done in 2000 in more than 540 Indian tanneries suggested that a blend of 70% new chrome and 30% recovered chromium delivers almost similar outcomes as utilizing 100 % new chrome (Rogers et al., 2002).

2.6.1 Pollution due to chromium tanned leather wastes in the environment

Chromium is found in either trivalent, Cr (III), or hexavalent, Cr (VI) states. However, the Chromium (VI) is a strong oxidizing agent, and poses a high risk and adverse impacts to humans and animals and id known

to have carcinogenetic properties. Cr (VI) is marked as a human toxic substance by the EPA, the US Department of Health and Human Services (DHHS), the IARC, and the WHO. Therefore, it has turned out to be solidly controlled through skirting or on supreme forbidding (He, 2002).

Therefore, when chromium tanned wastes are released to the environment they pollute the environment and cause adverse health effects to the humans. When taken in, chromium can cause lung and the upper respiratory problems, and increases the probabilities of rising nasal, lung, or sinus malignant growth (Famielec & Wieczorek-Ciurowa, 2011). Chromium can also cause intensified phases of asthma, bronchitis, polyps of the upper respiratory tract, pharyngitis, and the development of the hilar locale and lymph hubs (He, 2002).

2.7 Application of enzymatic technology in leather industry

The unique properties of enzymes have made it attractive for applications in leather manufacturing. The attractive properties of enzymes includes, is its non-toxicity, non-pollutant and their ability to catalyze chemical reactions at advanced rates under mild conditions. Enzymes can be employed with advanced quality leather production with a lesser amount of pollution impact (Choudhary et al., 2004a).

In the leather industrial process there are a number of stages that are involved. These includes: curing, soaking, pickling, dehairing, bating, degreasing, liming and tanning. Specific appropriate enzymes can be employed in each of these stages with a reduced amount of environmental pollution. The enzyme technology in the leather industry originated in early 20th century; the initial trial was done by Rohm in the year 1910 where enzymes were used in bating, which later took over 70 years to be useful in industrial scale for dehairing method and it was also applied in treating the industrial waste waters (Souza & Gutterres, 2012). All the stages of leather development cause quite a lot of health complications. Enormous quantities of industrial effluent contain sulphides and chromium which are the main contaminants to the environment. The first large scale production of enzyme originated about 1874 with the primary industrial batch of chymosin. In leather manufacturing the application of enzymes was used since 1917 and for the starch industry in 1950. Most of the industrial enzymes are produced from plant and animal systems and by cultivating microorganisms in the laboratories (Choudhary et al., 2004a).

About 12 categories of enzymes are applied in industrial purposes and some are shown in table 2.

Table 2: Categories of enzymes used in industrial scale in the world.

	Enzyme	Tons
1.	Bacillus protease	550

2.	Aspergillus amyloghucosidase	350
3.	Bacillus amylase	350
4.	Glucose isomerase	60
5	Microbial rennet	25
6.	Fungal amylase	20
7.	Pectinase	20
8.	Fungal protease	15

(Source: Choudhary et al., 2004a)

The major enzyme manufacturing countries in the world are shown in table 3 below

Table 3: Top leading enzyme manufacturing countries in the world

	Country	Amount (Tons)
1.	Denmark	249
2.	Netherlands	100
3.	USA	64
4.	Japan	42
5.	Germany	32
6.	France	16
7.	UK	11
8.	Switzerland	11
9.	Others	5

(Source: Choudhary et al., 2004a)

Enzymes have proved to be the best in dealing with chrome shavings (Cabeza and Taylor, 2001). Enzymes are widely used in skin industry, farming, food, cloth and medicinal industries (Taylor et al., 2001). Enzyme play a significant role in treating industrial waste, petroleum sludge dilapidation, water treatment, crude oil spills treatment, fly ash dump reclamations, eco-renovation of mine dumps and tarnished eco

system (Choudhary et al., 2004b). The present technology enables the separation, cleansing, even immobilizing (bind to offer support) the exact enzyme required for a definite purpose.

2.7.1 Enzymatic hydrolysis application

Historically, trimmings, shavings and splits from the chrome tanning's and coatings have been disposed through land fill or open dumping. Recently strict environmental regulations have triggered the tanning industries to find alternative techniques for management of Leather trimmings. The enzymatic technology is greatly applied in the leather shavings hydrolysis. However, limited work has been done on the trimmings and that this has formed the basis for this research. Enzyme hydrolysis of the chrome trimmings will be done and the amino acids obtained we are analyzed in cake powder obtained and evaluate the properties of polypeptide products obtained. The protein obtained has a potential as animal feed additive and as a fertilizer additives (Rao et al., 2002).

2.7.2 Enzymatic digestion of chrome shavings

The technology of enzymatic breakdown was adopted in United States of America in the Department of Agriculture in Philadelphia which was applied in TANEX Company by Hradek and Nisou and applied greatly in Czech Republic in early 21st century (Kolomaznik et al., 2001). This plant had the ability to process 3 tons of waste daily and give three outcome products which are gelatable protein, filter cakes and protein hydrolysate. This technology was established by Prof Karel Kolomazník of BRNO University by UNIDO between May 1999 and September 2000. The process was conducted in two phases, denaturation and enzymatic hydrolysis. With denaturation the chrome shavings were processed at a pH of 9.0 and a temperature of 70˚C for 3- 5 hours. After digestion the blend was filtered to obtain the gelatable protein and the filter cakes (Kolomaznik et al., 2001).

2.8 Leather waste processing

Fleshing's are the main solid waste from the leather manufacturing industries that the enzyme technology has been applied broadly in hydrolysis. Being the case the fleshing's are treated and hydrolyzed with pancreatic enzyme within aim of accepting a simple technique of solid waste management (Kumaraguru, et al, 1998). The pH for the enzyme was set at 8.0 and the complete hydrolysis happened to full liquefaction of the fleshing's. Bajza and Marcovic (2001) studied the effect of alkaline protease on untanned leather waste; trimmings obtained at pH of 10 and a temperature of 55˚C and demonstrated that these conditions favored the enzyme (bacillus species) hydrolysis process (Bajza & Vrček, 2001).

It was shown that the leather solubility increases with increase in the enzyme concentration from 500 - 1500 unit per 1g of leather. The leather waste breakdown produces a water-soluble hydrolysate which

could be concentrated on vacuum evaporator and then dried to form a fine flour to be analyzed (Janacova et al., 2006; Choudhary et al., 2004b).

Chrome -tanned wastes ⟶ gelatin/ protein hydrolysate + chrome sludge.

Chrome sludge was found suitable for glassmaking, heat resistant bricks, and alkaline chromate; it can similarly be processed and used tanned salt to be produced. Enzymatic hydrolysis has a number of advantages since it works under reasonable reaction conditions. This reaction can continue under the temperature of up to 80 ˚C and the pH between 8 and 9 and under normal atmospheric conditions. Also, the resulting product has a molecular weight that is dependent on the reaction composition and by adjusting the amount of enzyme, different products with different specifications that meet the client requirements can be prepared (Janacova et al., 2006).

In India, tanneries yield about 150,000 tons of solid wastes (raw hides / skin trimmings, lime flashings, hide splits and chrome shavings) and these solids are ultimately dumped to the environment and contaminates the environment. To address this concern, various methods for de-liming of limed tannery fleshing's (TF) using enzymatic hydrolysis and lactic acid fermentation have been explored. A bacitracin making native lactic acid bacteria (enterococcus faecium HAB01) has been studied (Rai et al., 2009). Acid ensilaging is broadly used in translating wastes from livestock/fish and fruits or vegetables. This includes the use of mineral acids like sulphuric acid / hydrochloric acid and a neutralization stage before application of silage. Milder organic acids are similarly used (propanoic acid and formic acid) as substitutions for the dangerous and durable mineral acids. Acid hydrolysis of de-limed TF is one of the low cost techniques that was used to utilize the solid wastes from tanneries and the acid hydrolysate shown to be a good source of arginine, leucine and lysine in livestock aquaculture feeds (Rai et al., 2009).

2.9 Analytical methods used to characterize heavy metal

Most of industrial effluents and agricultural practices release a lot of wastes to the environment that contain heavy metals, some of which are toxic to the humans and ecosystem when they exceed the normal concentrations (Lokhande et al., 2011). When these metals are discharged to the environment they contribute greatly to the pollution of water bodies and soil. However, some of them are essential at certain permissible levels, but they become toxic when they surpass the normal concentrations (Florea & Büsselberg, 2006; Plum et al., 2010).

The heavy metals can interact with proteins and DNA in humans causing oxidative deformation of biological molecules. Due to the toxic nature of metals, different techniques have been adopted to analyze heavy metals from industrial effluents over the years (Beck & Sneddon, 2000; Sneddon, 2002). The most predominant techniques used to analyze the heavy metals include: Atomic absorption spectrometry

(AAS), Atomic emission/fluorescence spectrometry (AES/AFS), inductive coupled plasma optical emission spectroscopy (ICP-OES), Neutron activation analysis (NAAS), X-Rays fluorescence and anodic stripping voltammetry (Helaluddin et al., 2016).

AAS technique can detect up to 70 elements and it measures the concentration of element by passing light of specific wavelength emitted by radiation source to a particular element. Atoms receives and absorb this light from energy source known as hollow cathode lamp and the light is directed to a monochromator which scatter light of other wavelength before the photomultiplier detects the desired signals (Alemnesh, 2019). The flame is atomized by flame or electro thermal (graphite tube) atomizers. The flame operates at a temperature of about 2300˚C (Koirtyohann, 1991). Then the atoms are irradiated then passed through the monochromator in order to separate the element. Atomic absorption/emission spectroscopy techniques provide not only analysis of wide range of heavy metals but also ensure immense reliability by exhibiting precise and accurate trace metal levels (Helaluddin et al., 2016).

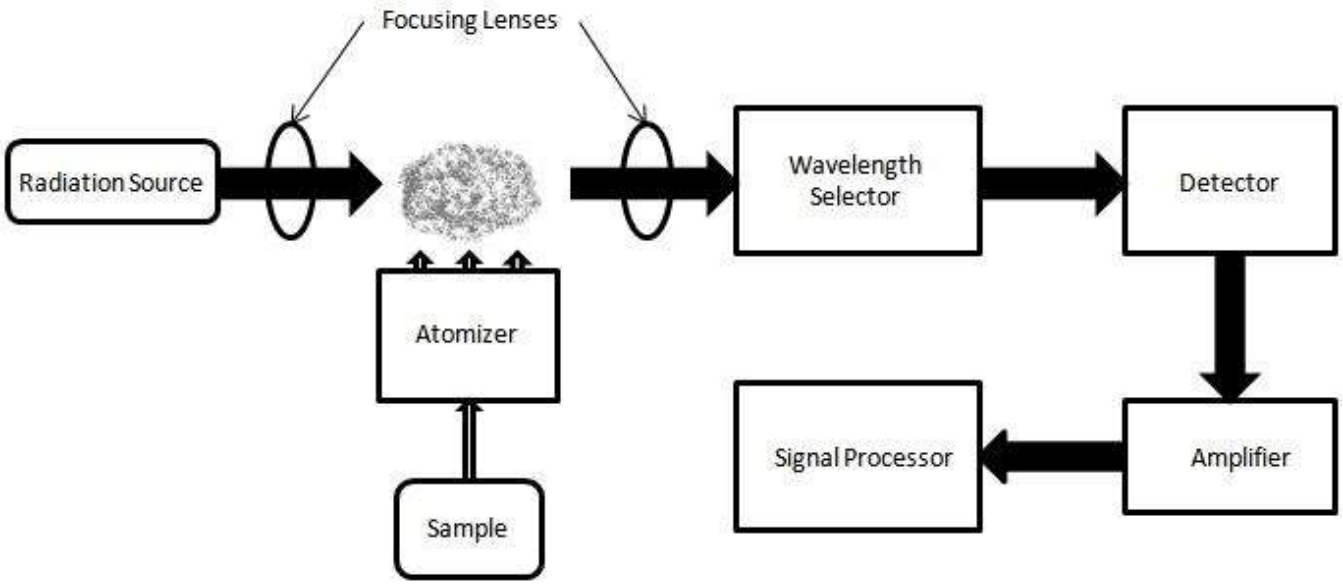


Figure 2: Parts of Atomic Absorption Spectrometer.

FAAS is also another efficient technique in analyzing samples in parts per billion (ppb) with good precision for many elements, the analysis is fast to about 10-15 seconds per sample (Lajunen, 2007).

ICP-MS, (Inductively coupled plasma mass spectroscopy) is a quantitative multi element measuring system that offer wide detection of elements. It uses Argon plasma source to dissolve the samples into its basic atoms or ions. The ions are released from the plasma and handed to the mass spectrometer where they are isolated according to the mass to charge ratios by quadrupole or magnetic sector analyzer, in this state metal ions are detected instead of the light they emit (Brown & Milton, 2005). The sample are

injected in gaseous form or aerosol, while liquid samples needs a nebulization before injection (Brown & Milton, 2005).

ICP-MS, was used in selection of trace elements in society traditional herbal medicine and various element were detected in different concentration such as Zn, Mn, Se, and Cd. This technique also was used to analyze lipsticks and the concentration of lead was found to be 1.07 ppm (Hepp et al., 2010). The type of sample and concentration level determines the techniques to be used.

ICP MS consists of the ion source, sampling surface, ion lens, mass spectrometer and a detector. Ion source is used to ionize the samples and it can ionize up to 90% efficient for many elements. The ions produced are led though sampling interface to the mass analyzing unit where the samples are analyzed and then detected. A multi element standard is ran to obtain a standard calibration curve for the analysis.

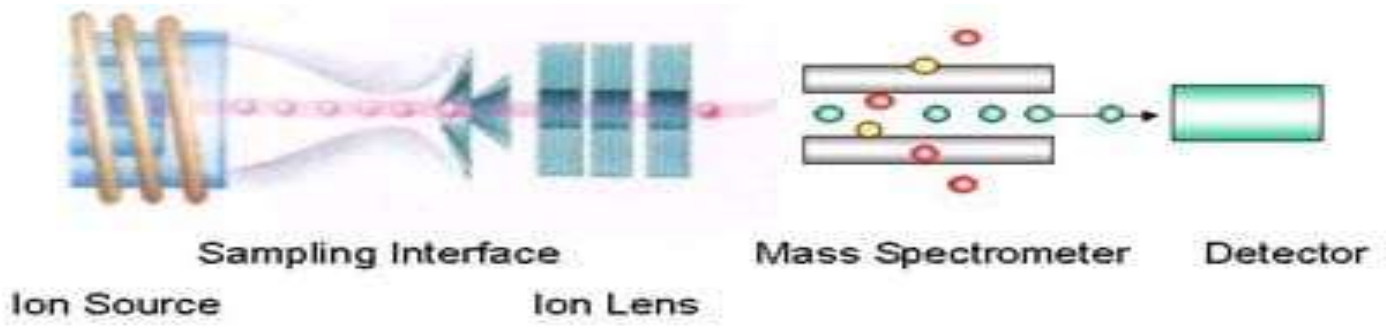


Figure 3: Parts of the ICP-MS

2.10 FTIR Analytical methods or techniques

The use of FTIR spectroscopy for analysis is broadly used in various fields including analyzing biological, water and food samples among others. This is because the molecular bonds contains dipole moments that can change by atomic displacement owing to natural vibration that are IR active; and these vibrations are quantitatively measured by IR spectroscopy. FTIR is used to obtain infrared spectrum of absorption or emission of solids with high resolution data over a wide range (Baker et al., 2014; Griffiths & De Haseth, 2007).

In UV-Vis spectroscopy technique, it uses a monochromatic light beam to samples and ensure how much the light is absorbed and repeat this at different wavelength. The absorption or reflection in the visible range directly affects the perceived color of the chemical used and how atoms and molecules undergo electronic transitions (Skoog et al., 2017).

In FTIR, the machine shines beams containing many frequencies of light at once and measure how much of the beam is absorbed by samples, next the beam is modified to contain different combinations of frequencies giving a second data point. The process is rapidly repeated many times over a short time span,

afterwards the computer takes all this data and acts afterwards to infer the absorption at each wavelength. The computer turns, the raw data, i.e., light absorbed for each mirror positions into desired results (light absorbed for each wavelength). The process required turns out to be a common algorithm called Fourier transform which converts the displacement of the mirror in cm into inverse domain cm-1 (wavenumber)(Griffiths & De Haseth, 2007).

By investigating biological materials, the most important spectral region that is measured are normally the finger print region which is around (600-1450 cm-1); the amide I and amide II region that appears at (1500-1700cm-1) region. We also have a higher wavenumber region at (2550-3500cm-1) which is associated with the stretching vibration such as S-H, C-H, N-H and O-H. Whereas the lower wavenumber regions are associated with bending and carbon skeleton fingerprint vibration. (Baker et al., 2014; Walsh et al., 2008)

2.11 Analytical methods used to characterize amino acid

HPLC also referred to as High Pressure Liquid Chromatography, is a technique used to identify, separate, and quantify each component in a mixture. It relies on the pumps to pass the pressurized liquid sample (solvent) through a column filled with solid adsorbent material. HPLC is used broadly in manufacturing (during production processes), in pharmaceuticals and biological products, used in legal process in performance enhanced in drugs and urine, in research to separate complex compounds among others (Gerber et al., 2004).

The sample mixture to be analyzed is introduced in discrete amounts into the mobile phase in the column. The sample moves through the column at different velocity which is consequence of different interaction with the adsorbent. The velocity of each component is dependent on its chemical nature and the nature of the stationary phase and the mobile phase. Different samples elutes the column at different times called retention times. Different columns exist but the smaller the particles in the column the higher the resolution. The aqueous composition of the mobile phase may contain some acids such as formic acids and phosphoric acids that helps in the separation of the sample (Gerber et al., 2004).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection of the trimmings

The leather trimmings were collected manually from one of the Leather Industry in Kenya (LIK) situated in Thika as shown in Figure 4.

Figure 4: The Location of Thika Tanneries.

(Source: Google map; Scale: 1:100)

Figure 5: Chromium tanned trimmings obtained from tanned leather.

The materials were screened to remove all the unwanted components, and further separated to distinguish large particles from the fine or tiny trimmings ranging from 1gram to 50 grams. The isolated materials

were stored in a cool dry place in the laboratory waiting for the hydrolysis, Figure 5 shows the images of leather obtained from the trimmings. The large particles are ground to small pieces for quick hydrolysis as shown below.

3.2 Tyrosine standard curve

The standard calibration curve was obtained in order to determine the level of tyrosine concentration during the hydrolysis process. To obtain the standard curve the following procedure was followed. Various solutions containing 0.4 M sodium carbonate, 1.1mM of the PC reagent and 1.1µM tyrosine was prepared.

The various proportions of the above reagents were measured as summarized in Table 4 to obtain the number of moles of tyrosine for various solution mixtures.

Table 4: Reagents for preparing Tyrosine standard curve.

Reagents	Standards							
	Blank	1	2	3	4	5	6	7
Tyrosine standard	0.0 µl	5.0 µl	10.0 µl	15.0 µl	20.0 µl	25.0 µl	30.0 µl	35.0 µl
Water	250 µl	250 µl	250 µl	250 µl	250 µl	250 µl	250 µl	250 µl
Sodium Carbonate	625 µl	625 µl	625 µl	625 µl	625 µl	625 µl	625 µl	625 µl
Dilute F–C Reagent	125 µl	125 µl	125 µl	125 µl	125 µl	125 µl	125 µl	125 µl

The volumes were obtained using micropipettes and transferred into the falcon tubes, in each ratio as shown in Table 4 and the color allowed to form. Thereafter, the eight falcon tubes with various ratios solution were shaken to ensure complete mixing before adding the FC reagent. The solutions were then incubated at 35˚C for 20 minutes then centrifuged at a rate of 1200 rpm for 5 minutes. Each sample was then transferred into the clean falcon tubes and incubated at 35˚C for 30 minutes. Different absorbance was recorded using UV-Vis spectrometer at a wavelength of 660nm and calibration curve plotted.

3.3 Enzyme preparation

To produce the enzyme, the bacteria species (bacillus cereus stain wwcp1) was obtained from Lake Bogoria in Kenya. The enzyme was obtained using the following procedure; 200ml portion of 0.5% of casein and 0.25% glucose culture medium was prepared in 500ml conical flask. Each sample was autoclaved in a well-covered flask at 121˚C for 15 min. The mixture was allowed to cool and then inoculated with a 5% seed bacteria and left overnight. The mixture was then transferred into a rotary

shaker incubator (140rpm) for 72 hours at 45˚C. The cultured medium was centrifuged at 5000 rpm for 15 minutes and transferred into the media (solution) for the growth of the enzyme (Wanyonyi et al, 2014). The bacteria growth was done using (R'ALF plus Duet fermenter, 3.7L) bioreactor, where the media was prepared and placed in together with the bacteria for 72 hours. The medium was autoclaved using Tuttnauer steam sterilizer at 121˚C for 30 min to destroy all the living organisms and to ensure only the targeted enzyme is growing. Crude alkaline protease was generated using a modified laboratory procedure (Rao et al., 2002).

To prepare the media, 4.5 g of potassium hydrogen sulphate was weighed into a mixing vessel and 3.75g of potassium dihydrogen sulphate, 0.9g of magnesium sulphate, 0.9g of calcium chloride and urea were added respectively. In addition, 1.5g of yeast, 15g of casein and 7.5g of glucose were also added. The mixture was diluted with 3 liters of distilled water. This is the media used to grow the Bacillus bacteria (Lakshmi et al., 2014). The mixture was stirred and the pH adjusted to 11.5 before inoculation by autoclaving for 25 minutes and subsequently transferred into the bioreactor. The bacillus enzyme was cultured overnight. The fermentation process was set up as shown in figure 6.

Figure 6: Bioreactor and the fermentation process.

The incubation was done in the Bioreactor at 45˚C for three days with the enzyme solution, the medium was stirred continuously to ensure uniform growth of the enzyme. The enzyme was subsequently harvested for further use, before use it was centrifuged using a serval ST16R centrifuge at 12000 rpm for 15 minutes. The 100% enzyme solution obtained was used for hydrolysis of the leather trimmings (Khatoon, 2017).

3.4 Optimization of hydrolysis conditions

3.4.1 Optimizing the pH

Enzymes have different activities when exposed to different conditions, such as pH and temperature. The optimization of the pH was done by weighing a specific mass (2g) of the leather trimmings and put in the same volume of the enzyme concentration at varying pH conditions. The pH was varied from 1 to 14 to

obtain the optimum condition for the hydrolysis. The tyrosine concentrations were measured using the UV spectrometer at varying time intervals.

This experiment was done using fresh enzymes from the bioreactor by weighing 100mL into twelve 100ml conical flasks (for pH 2 to 13). The pH was adjusted using 0.1M sodium hydroxide and 0.1M hydrochloric acid. Approximately 2g of the leather trimmings was added to each solution in the flasks. The absorbances of these solutions were recorded before any hydrolysis take place. The hydrolysis was conducted at a temperature of 50˚C, because most enzymes perform well at this temperature and monitored closely to see the changes in color. Absorbances were obtained at an interval of 6 hours, 12 hours and 24 hours for the start (first day) at a wavelength of 660nm.

A specific volume of the enzyme solution obtained from each flask was mixed with other reagents and absorbance read at 660nm. In this case, 25 µl of the enzyme solution was measured then few drops of TCA reagent, 125µg of FC reagent and 625µg sodium carbonate solution were added. The solution was incubated for 20 minutes to ensure the reaction is complete then centrifuged at a rate of 1200rpm for 30 minutes again before reading the absorbencies. This was done concurrently and repeatedly at a time interval of 12 to 24 hours until all trimmings were hydrolyzed at desired pH. The pH was adjusted to maintain the set pH using the base (0.1 M NaOH) and an acid (0.1M HCl) every day. The pH was monitored closely at a range of 6-12 hours until the hydrolysis process was complete.

3.4.2 Optimizing the Temperature.

A fixed mass (2g) of the trimmings was hydrolyzed at a fixed optimum pH of 12 and a varying temperature to obtain the optimum temperature for the hydrolysis. In this study a fixed mass of leather trimmings was hydrolyzed at varying temperature from 25˚C to 75˚C to determine the variability of the hydrolysis. The level of tyrosine concentration was monitored closely at a fixed time interval using a similar procedure used in reading absorbance in the optimizing the pH conditions. That is, measuring a specific volume of the enzyme solution is obtained from each flask mixed with other reagents and absorbance read at 660nm. In this case, 25 µl of the enzyme solution was measured then few drops of TCA reagent, 125µg of FC reagent and 625µg sodium carbonate solution were added. The solution was incubated for 20 minutes to ensure the reaction was complete, then centrifuged at a rate of 1200rpm for 30 minutes again before reading the absorbance values. The absorbance values were recorded and a plot drawn to show the effect of temperature in the hydrolysis.

3.5 Gelatin extraction

500g of the chromium tanned leather trimmings were weighed separately and placed in a 1-liter conical flask and 500mLs of the enzyme was added (at pH 12). This was covered by aluminium foil and cotton

wool, this pH was used because it was the optimum pH for this experiment. The experiment was set at a temperature of 65˚C and 50˚C (the optimum temperatures obtained experimentally) separately to investigate the effect of temperature on large volumes of trimmings. The pH was adjusted to 12, every day and the temperature maintained.

The filtered solution was frozen at -4 ˚C for 48 hours to obtain the solid ice. Thereafter the freeze drier was set at a temperature of 30 ˚C, then the sample was loaded to the freeze drier for 60 hours. The dried sample was stored in an air tight container to avoid moisture filtration.

Figure 7: Freeze drying of gelatin obtained from hydrolysis of leather trimmings

The residue was transferred to a piece of aluminium foil then placed in an oven at a temperature of 50˚C for a period of 24 hours until the whole sample is completely dried. The freeze dried sample and the residue are stored in tight containers for further analysis using FTIR and HPLC. The freeze drying technique was adopted because it does not distort the chemical and physical properties of the products.

3.5.1 Heavy metal analysis

Solid samples

About 0.1 ± 0.0001 grams of finely ground solid samples were weighed into digestion specimens' tubes. The samples were converted to ashes in a muffle furnace for 41/2 hours at 450˚C and allowed to cool down. The samples were digested using 1:1 HCl, 1:1 HNO3 and 20 % H2O2 mixtures. The mixture was

reacted with the acid mixture and heated on the hot plate till the sample mixture had completely evaporated. The samples were re - dissolved using 0.05N of HCl, the specimen tube was corked and allowed to stand for at least 5 hours to re – extract (Desorption).

Liquid samples

About 1 ml solution of the sample was measured and diluted with 5 mls of distilled water. The dilution is normally determined by how viscous the sample is and this can be varied depending on the instrument response. If the readings are high, further dilutions was done, but if the instrumental readings were low dilution was reduced. This dilution factor was taken into consideration during the final calculations. The calibration curve of the standards were ran followed by the sample for quantification of the analyte of interest. After all that was done, the samples are injected to the ICP-MS for analysis.

The standards preparation involved premixed standards of mineral stock solution of 1000ppm separately containing sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), manganese (Mn), zinc (Zn), copper (Cu) and iron (Fe), cadmium (Cd), cobalt (Co), chromium(Cr), Silver(Ag), Sodium(Na), was used to prepare 0ppm, 1ppm, 3ppm, 5ppm,10ppm, 15ppm and 20ppm of each element standard solutions for equipment calibration. Blank solution of 0.05NHCL used during the mineral extraction is normally used to calibrate the instrument including the standards. The samples were loaded onto the ICP--MS auto-sampler and automatically analyzed. Each element is detected based on its specific wavelength emission spectrum. The results were read from the monitor connected to the ICP-MS and expressed in ppm, ppb and percentage.

3.5.2 Determination of effluents pH and electrical conductivity

This was done to the hydrolyzed solution of the leather waste trimmings. The solution was filtered to remove the segments. Then 5mls of the solution was measured into a beaker and 100 ml of distilled water added to it. The solution was stirred continuously to obtain a homogeneous mixture. The solution was allowed to settle for 10 minutes. There after the pH and the Electrical conductivity values were done in triplicate using the multipara-meter (Hanna Model) equipment. The machine was cleansed by distilled water thoroughly before the readings were made.

3.6 FTIR Analysis

This was done for the pure gelatin obtained from the hydrolysis of the chromium tanned leather trimmings and its residue using Fourier transformed infrared spectrometer equipment (IR- Affinity IS Shimadzu Model). The number of scans were run and after completion the spectra were drawn in the range of 500-4500cm-1.

The sample holders were cleansed using acetone and maximum care was taken to ensure that acetone is not splashed on the instrument. The sample name were logged in and the range scanned (the range limit is 650-4500 cm-1) then the back ground button was clicked to collect back group information of the sample holder as recommended by the manufacturer.

For the liquid samples, few drops around 25µl was placed in the sample holder. The machine was run by pressing 'Apply' then data acquisition started to collect the spectrum. While for the solid samples, small mass of 2µg was placed in the sample holder, then the monitor button clicked on the Scan and Instrument Setup dialog, and then the pressure arm lowered down through the monitor dialog to monitor the total pressure applied to the sample. The 'Force Gauge' was set to be around 80 in the instrument, then data acquisition started to collect the spectrum.

3.7. Determining amino acid composition

The amino acid analysis was done using Hitachi 8800 (Sigma A-9906) analyzer and standards were verified using National Institutes of Standards and Technology (NIST) guidelines. The experimental procedure included turning on both power strips to apply power to the pump, column heater and detector. The detector power switch was turned on 15 – 20 minutes before running samples and the flow rate on the pump was set to 00.

The system set-up was checked and the buffer/mobile phase reservoir (minimum of 540 ml) and the inject switch was verified before loading the sample to the machine. The reference cell was flushed by filling syringe with mobile phase – use large gas type syringe with metal fitting on the end - with 5 ml buffer/mobile phase. The buffer/ mobile phase solution was injected into reference inlet.

Setting up of the column procedure included verifying buffer/mobile phase column was exiting and the flow rate was set to around 3 mL/min. The column heater at plug strip was switched on since it has no power switch of its own and the temperature was set to 40˚C. The column was allowed to achieve steady state temperature (about 30 minutes for temperature stabilization and flushing of system). The start button was clicked and the system verified to 'Waiting for Injection' status.

For the sample injection, the flow rate was set at around 3ml/min. and the Hamilton 10 micro litter syringe selected. The injection port plug was released by turning the bottom lever down. The round silver port plug was removed and placed in the hole in the valve handle and 10 micro liters of sample was drawn into the Hamilton syringe. The syringe was fully inserted into the sample port and the sample injected to the column. The round silver port plug was replaced into port then the bottom lever turned up until the lever was tight. The upper switch was turned to 'Inject' position, data acquisition automatically started, after

waiting for 30 seconds the upper switch was turned to ‘Load’ position. The data acquisition started and the display changed from ‘Waiting for Injection’ to ‘Running’.

The known dry masses (50µg) of the samples were transferred to the mass hydrolysis tube and then the liquid phase hydrolysis (6N HCL, 1%Phenol at 110˚C) done in vacuum for 24 hours. The mass was cooled and unsealed, then dissolved in the solution buffer (sodium Diluent) and in Pickering then 400nmol/Ml NorLeucine was added. The machine was run and different spectrum recorded for the freeze dried sample and the dry filtrate samples and the composition of the different amino acids in the samples analyzed.

CHAPTER FOUR

4.0 RESULTS AND DISCUSION

4.1 Preparation of tyrosine standard curve

The solution was made by mixing different concentration of the tyrosine reagent and the sodium carbonate, FC reagents and incubated to form the blue colour. The final solution was read using the UV/VIS calorimetric at 660 nm. Various absorbencies were noted and the curve was plotted as shown in figure 8.

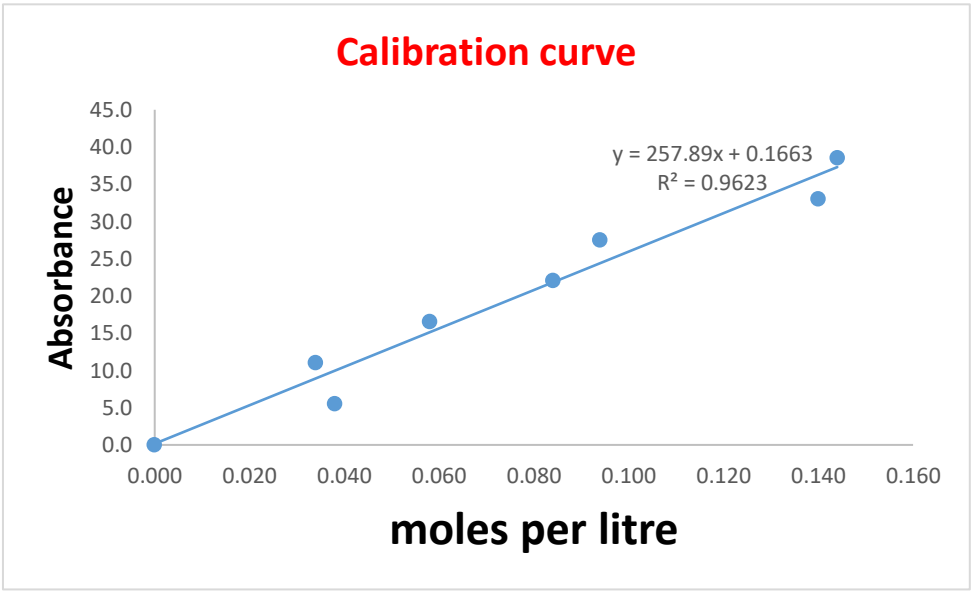


Figure 8: Standard calibration curve of tyrosine

A standard curve was used to detect the level of tyrosine obtained during hydrolysis process. It also helps in knowing the rate of the hydrolysis of the enzymes in different conditions, R2 is the correlation coefficient which is 0.9623 and y is the absorbance of the tyrosine in nanometres. Beers lambert law was used to calculate the Absorbance,

$A=\varepsilon lc$,

Where: A – Absorbance; ε- Absorptivity; l- Optical path length (cm) and c- Concentration

4.2 Investigating optimum hydrolysis conditions for the trimmings

4.2.1 Effects of pH on the hydrolysis of the trimmings

Experimentally, 100 ml of crude protease was measured into different conical flasks and its pH varied from 1-13. Then a fixed mass of the trimmings were hydrolysed at a temperature of 50oC. The figure 9 shows the effect of different pH on the hydrolysis of the trimmings. The results obtained showed that the enzyme hydrolysis were different at different in pH and it was fast at high pH values as reported by Janacova et al., (2006).

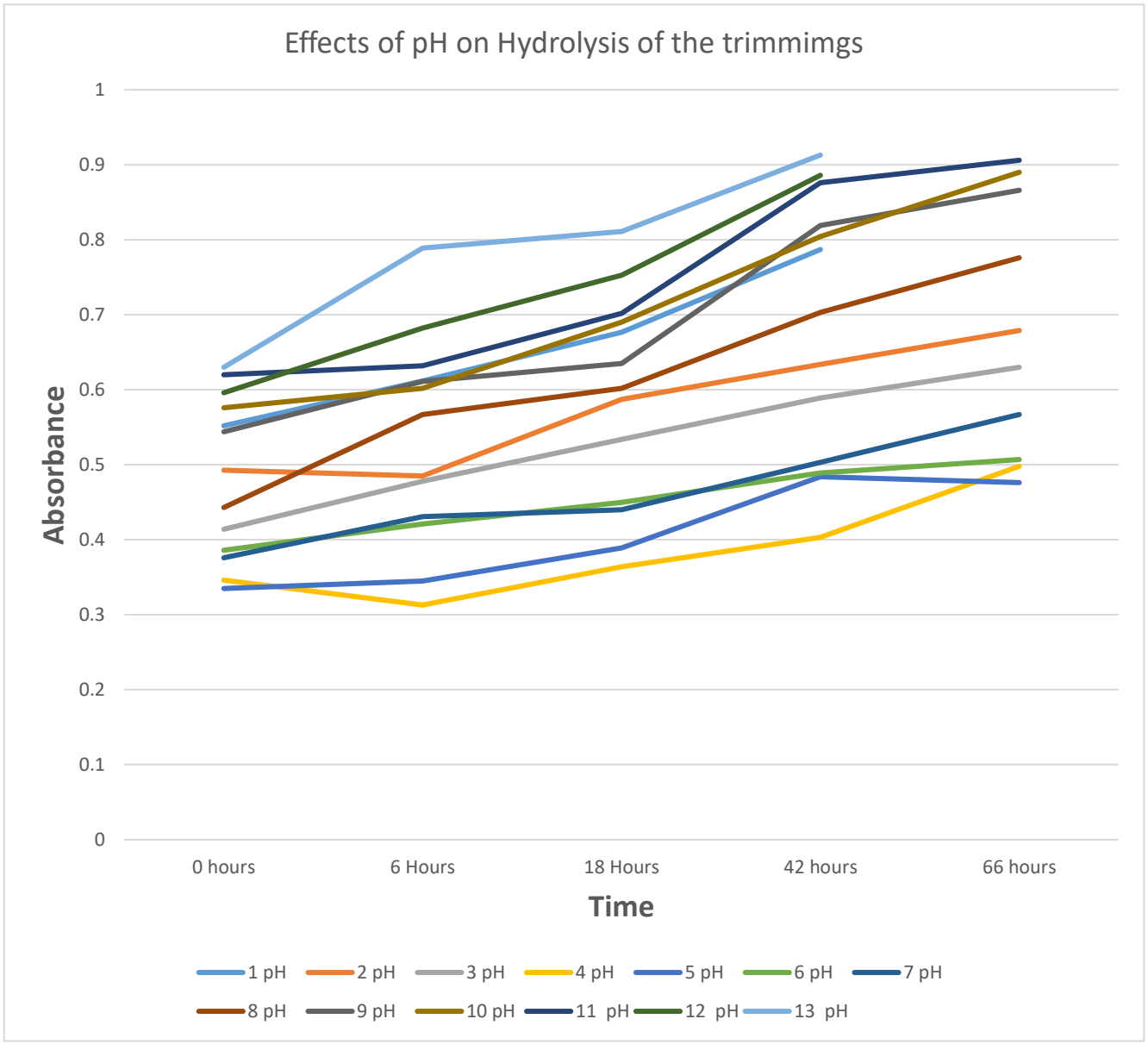


Figure 9: Effects of pH on hydrolysis the leather trimmings.

As shown in figure 9 the reactions were very fast at high pH. This is because the enzymes perform well in the basic environment since they were obtained from Lake Bogoria which has high pH. The tyrosine

concentration was high in the pH 12 and pH 13 and it took shorter time to complete the hydrolysis yielding these PH (Janacova et al., 2006; Wanyonyi et al., 2014). From the literature review done, the performance of various enzymes varies with the different pH conditions. However for hydrolysis of raw hides and skin most enzymes perform better at a pH of 7 to 10 (Niculescu et al., 2012; Wanyonyi et al., 2014).

The higher pH above 8 showed a high concentration level of tyrosine above 0.5nm, the reactions rate were fast and the complete hydrolysis took short times than at other pH concentrations. At pH 4 to 7, the reaction were slower and the rate of hydrolysis were very slow, the absorbance was below 0.5nm. The acidic hydrolysis also took a short period of time and it indicated high level of tyrosine concentration released (0.4-0.8nm). It is clearly observed that the protease concentration increases with increase in time of hydrolysis, but it was extreme in the high pH values. Though various enzymes such as neutrase and papain works very well in the mid pH and they yield the desired products in hydrolysis of raw hides, an indication that different enzymes works well at different conditions (Niculescu et al., 2012; Damrongsakkul et al., 2008).

The optimum pH is 12 because of the steady hydrolysis and the duration that it took for complete hydrolysis. Muyonga et al., (2004) showed that the protease enzyme works well at high pH. Bajza and Marcovic (2001) studied the effect of alkaline protease on untanned leather waste; trimmings obtained at pH of 10 and a temperature of 55˚C and demonstrated that these conditions favored the enzyme (bacillus species) hydrolysis process (Bajza & Vrček, 2001).

4.2.2 Effect of temperature on the hydrolysis of the trimmings

Figure 10 shows the effect of temperature on hydrolysis. It is shown that the hydrolysis occurs faster at high temperatures compared to low temperatures. The protease enzyme showed a higher activity at high temperatures (observed at 75˚C), whereby it took less time to complete the hydrolysis. This confirms the thermophilicity of the enzyme based on where it was isolated from (Janacova et al., 2006). At the optimum temperature of 75˚C, the activity was maximum and a short time was taken for complete hydrolysis and less evaporation of the enzyme medium occurred (Wanyonyi et al., 2014).

At temperatures above 75˚C, the triple helix bonds of the amino acid begin to break due to thermal hydrolysis. Most protease like bacillus, papain and neutrase enzymes performs well at the temperature of above 50 ˚C up to 70 ˚C producing maximum yields in the hydrolysis of the raw hides and leather wastes (Damrongsakkul et al., 2008).

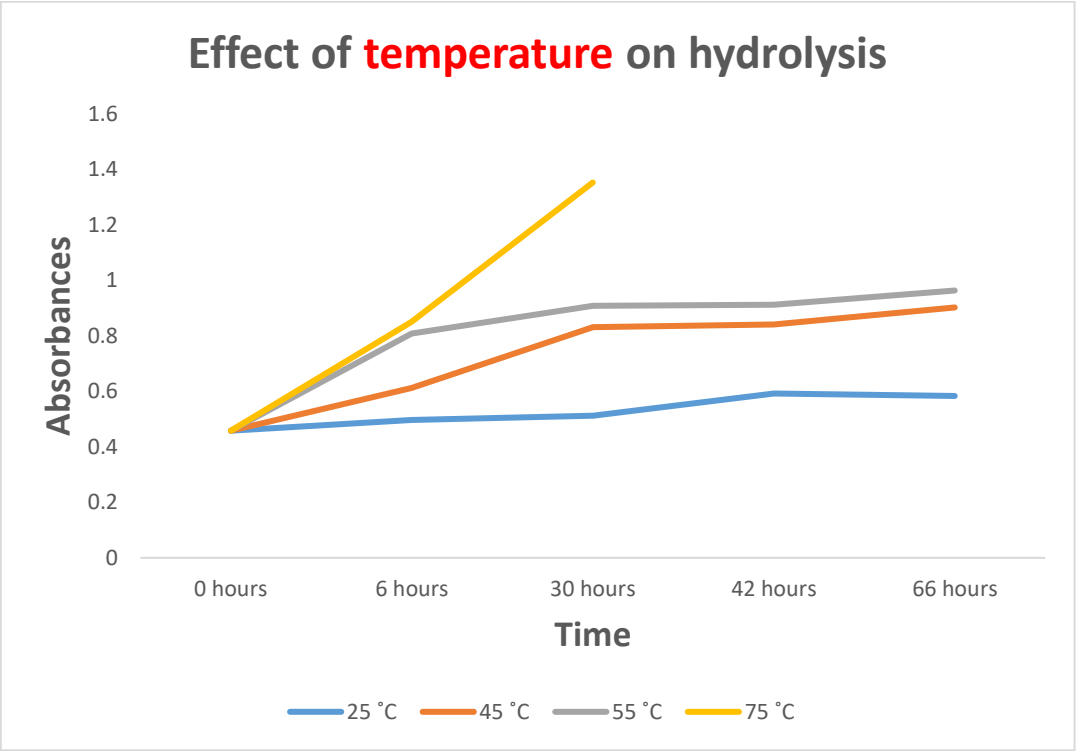


Figure 10: The effect of temperature on hydrolysis of the trimmings

4.3 Gelatin yield

Freeze drying which is also known as lyophilisation or cryosiccation was done to the hydrolyzed solution. During freeze drying stage, the material is cooled below triple point, which ensures that sublimation rather than melting takes place, this occurs in the following steps. To obtain large crystals, the products should be frozen slowly or can be cycled up and down in temperature in a process called annealing.

Figure 11: Freeze dried powder obtained from the gelatin solution

After complete hydrolysis, the solution containing gelatin was separated from the insoluble particles and taken to a freeze drier to obtain a powder as shown in figure 7. The freezing phase is the most critical in the whole freeze drying process because it impacts on the speed of reconstitution (Rey, 2016).

After freeze drying, a brownish powder which is gelatin was obtained from the solution as shown in Fig 11. The gelatin obtained from the freeze drier has potential application in fertilizer and animal feeds since the leather skin contain about 80% protein content; an indication that the gelatin has a lot of proteins (Aftab et al., 2006). Lyophilisation is a low temperature dehydration process (Ratti, 2001). It involves freezing the product, lowering pressure and then removing the ice by sublimation (Garcia Campos, 2019). Freeze drying result in a high quality product because of the low temperature used in processing. The original shape of the product is retained and quality of the hydrolyzed product is expected (Ratti, 2001).

The filtrate was also dried and crushed into powder form. The filtrate also contains similar properties as the gelatin, since it is obtained from the same process of hydrolysis. The gelatin obtained is rich in proteins and amino acids and it can also have a diverse application in fertilizer and animal feeds industry. However, it is usually rich in Cr and sulphur than the freeze dried gelatin.

The filtrate was taken to the oven and dried for 24 hours at a temperature of 50 ˚C to constant weight. The powder was then crushed into small particles as shown in the figure 12.

Figure 12: The filtrate powder obtained from the hydrolysate.

4.4 Heavy Metal Analysis

The analysis of heavy metals was done to determine the number and concentration level of different elements including chromium (Cr), sodium (Na) and Sulphur (S) that are utilized in the tanning process. The concentration of several metals in the hydrolyzed samples were determined using ICP-MS and the results are presented in Table 5. The metals of interest which is chromium and sulphur were detected in both the filtrate powder and the freeze dried powder at different concentrations as shown in table 5.

Table 5: The concentration of heavy metals in the hydrolyzed samples.

Elements	Concentration in mg/L		
	Freeze dried sample	Dry filtrate	Hydrolyzed solution
B	0.00857	0.0103	0.0433
Ba	0.019	0.0322	0.0000434
Ca	2.87	18.8	0.00382
Cd	0.00183	0.046	0.000206
Co	0.00231	0	0.0000051
Cr	1.9	68.7	0.0639
Cu	0.00893	0.0107	0.000248
K	10.8	7.57	1.56
Mg	0.507	0.784	0.000429
Mn	0.00522	0.0494	0
Mo	0.0235	0.115	0.00077
Na	196	152	10.2
Ni	0	0	0
P	2.49	8.39	1.02
Pb	0.00654	0	0
S	15.1	11.7	0.975
Se	0.32	16.2	0.0109
Sr	0.0204	0.0473	0
Zn	0.0123	0.0384	0.000193
Fe	218	1090	1.18

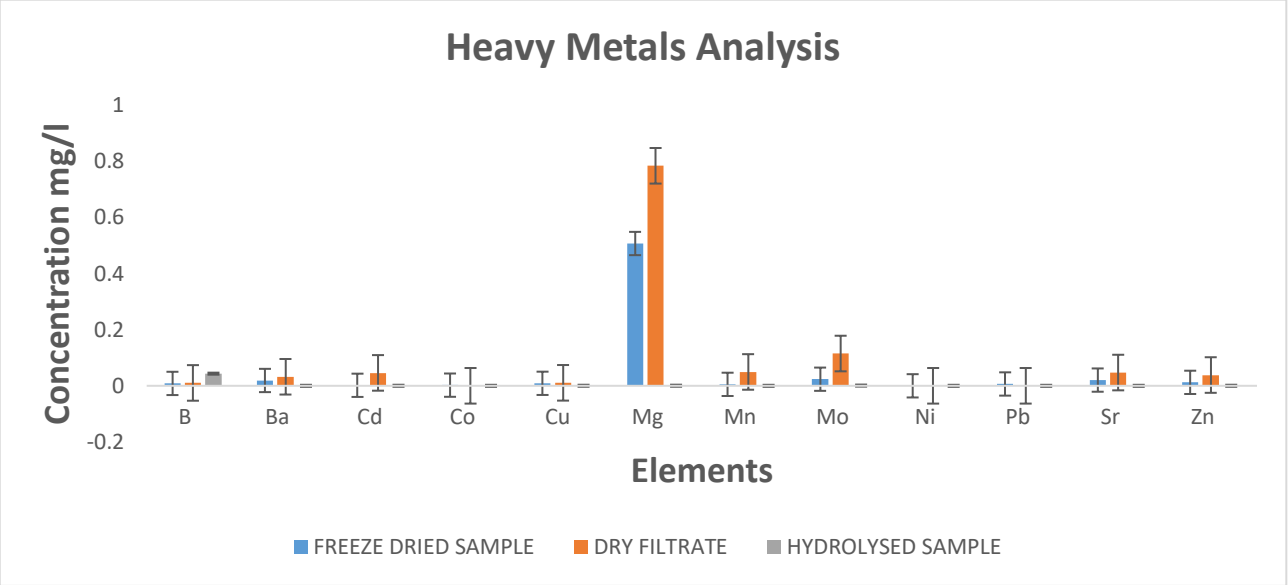


Figure 13a Heavy metal analysis of the freeze dried sample dry filtrate.

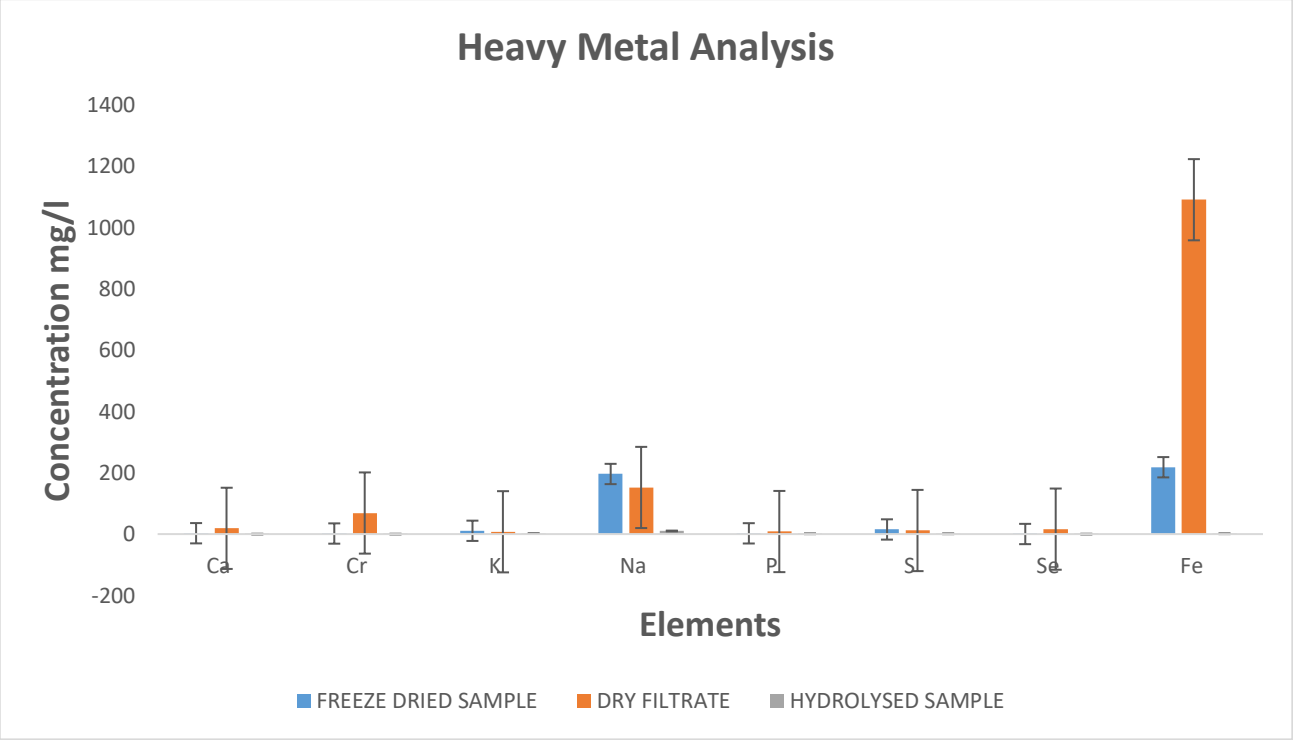


Figure 13b Heavy metal analysis of the hydrolyzed solution..

Figure 13a and 13b shows the results of the Heavy metal analysis of the freeze dried sample dry filtrate and the hydrolyzed solutions respectively.

Graphical presentation of the heavy metal content of the hydrolysate products are shown in figure 13. From the freeze dried sample both Cr and S were detected in trace amounts of about 1.9 mg/l and 15.1 mg/l respectively. Notably, the filtrate showed higher levels of Cr 68.7 mg/l and sulphur value of 11.7 mg/l. As shown in Table 5, about 20 elements were detected in the samples, however, the concentration

values obtained are relatively low. In the dry filtrate, different elements were detected and chromium and sulphur being one of the predominant elements as shown in figure 13a and figure 13b. High concentrations of Fe, above 1,000 mg/L in the dry filtrate and above 200 mg /l on the freeze dried samples were obtained. Other elements observed in the filtrates were Na, Al, Ca, K, Mg, P, S, and Se. The relatively high levels of Chromium 68, 7 mg/l in dry filtrate samples demonstrate the need for removal and recovery of the metal during the treatment process, Chromium is used in tannery sector as the tanning agent. Chromium concentration values exceeding 9.7 mg/l , have been reported in the blood and urine samples of the workers in the leather industries, an indication of the high levels of chromium in leather industries (Basaran et al., 2006; Junaid et al., 2017).

United States environmental agencies have identified Cr+6 as one of the 17 most noxious chemicals posing a threat to humans (Chauhan et al., 2015). Among the well-known contaminants include metals such as Cd, Cr, Cu, Hg, Pb and Ni which are toxic when taken in excess amounts. The presence of heavy metals in biological systems is undesirable due to their toxicity and oxidative nature (Chauhan et al., 2015). The significance of enzymatic hydrolysis is that most of the noxious elements like Cr and sulphur can be controlled from the hydrolysate, hence releasing products and wastes that are environmentally friendly from the leather industries. The hydrolysate solution also showed similar properties of heavy metals as the freeze dried samples (da Trindade Alfaro et al., 2015).

4.4.1 Analysis of pH and electrical conductivity

This was done to detect the hydrolysates pH and electrical conductivity. The sample was analysed in three replicates and the results indicated in the table below

Table 6: The pH and Electrical conductivity of the hydrolysate

	1st reading	2nd reading	3rd reading	Average
pH	9.14	9.18	9.21	9.18
Electrical conductivity(sm-1)	1.551	1.611	1.407	1.523

The electrical conductivity of the hydrolysate was found to be 1.523 sm-1 and an average pH of 9.18 obtained. Literature data shows that enzymes are used in leather processing technology at pH values varying from 3 to 11. This indicates that various enzymes performs differently under different pH conditions. At higher pH above 8, the activity of most enzymes such as Bacillus species increases (Choudhary et al., 2004c).

4.5 FTIR Analysis

This analysis was done to detect the possible functional groups in samples. The FTIR spectrometry for the gelatin obtained from the chromium tanned trimmings for the filtrate and the freeze dried powder showed similar properties as shown in figures 14 and 15.

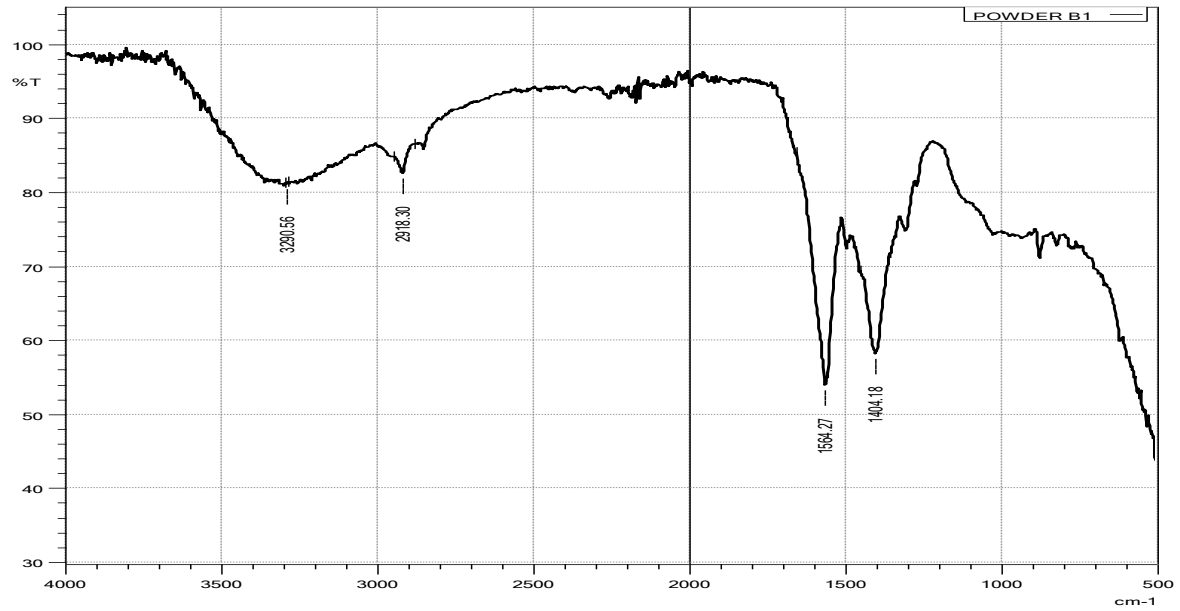


Figure 14: FTIR spectra for the dry filtrate from the hydrolysate.

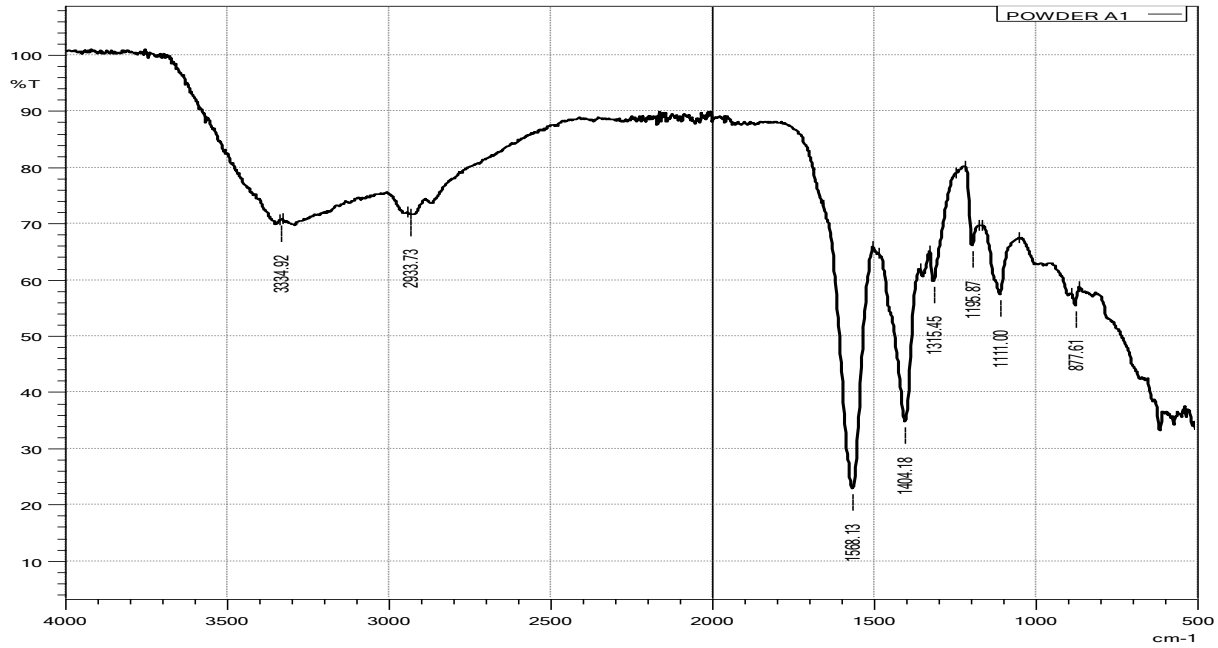


Figure 15: FTIR spectra for the freeze dried powder of gelatin solution

The amides bands were seen at 1564.27 cm -1 and 1568.13 cm -1 respectively. The bands at 3334.92 cm -1 and 3290.56 cm -1 this band also indicates the presence of partially free asymmetrical –OH groups (OH

stretching vibration. From the literature the OH bands ranges from the 3000 -3700 cm -1 (Hinterstoisser & Salmén, 1999). A clear indication of a good characteristics of the OH bands at these regions of the spectra as shown in figure 15 and 16 above. The small peaks at 2000- 2500 cm -1 indicates the presence of X=Y=Z groups where (XYZ maybe C, N, O, S) and also representing N-H stretching vibration. The bands at 2933.73 cm -1 and 2918.30 cm -1 indicated C-H stretching bands. The peaks at 2918.30 cm -1 and 2933.73 cm -1 indicated the C-N and N-H in plane bending. The hydrolysate products shows similar properties with the analysis done in raw skins and animal bones (Aftab et al., 2006). The FTIR spectra for these two products indicated the presence of the Amides bands which is an indication of possible amino acids in the products and that the products are suitable for analysis for suitable applications (Famielec & Wieczorek-Ciurowa, 2011).

4.6 Amino Acid composition

The animal skin contains proteins and other mineral elements that exist as gelatin. Therefore the aim for analysing this product was to obtain the number of possible amino acids present in the gelatin. For the analysis, 11.5 mg of each samples were used. The hydrolysis products, sample A (freeze dried sample) and sample B (dry filtrate) were analysed in triplicate. The results are summarized in Table 7 and 8.

Table 7: Amino acid composition of the freeze dried sample.

Amino acid	nm/inj	nm/50µl	µg/50ul	Mole %	Weight%	µm/mg	%(w/w)
Asx	2.765	2.80	0.32	6.26	8.40	0.2	2.26
The	0.28	0.28	0.03	0.63	0.75	0.02	0.2
Ser	0.937	0.95	0.08	2.12	2.15	0.07	0.58
Glx	4.701	4.76	0.61	10.65	16.02	0.33	4.31
Pro	6.586	6.67	0.65	14.92	16.89	0.47	4.54
Gly	16.695	16.90	0.96	37.83	25.17	1.19	6.77
Ala	6.385	6.46	0.46	14.47	11.99	0.45	3.22
Val	1.26	1.28	0.13	2.85	3.30	0.09	0.89
Ile	0.470	0.48	0.05	1.06	1.4	0.03	0.38
Leu	1.493	1.51	0.17	3.38	4.46	0.11	1.20
Try	0.237	0.24	0.04	0.54	1.02	0.02	0.27
Phe	1.024	1.04	0.15	2.32	3.98	0.07	1.07
His	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lys	1.232	1.25	0.16	2.79	4.17	0.09	1.12
Arg	0.071	0.07	0.01	0.16	0.29	0.01	0.08
Cysteic acid	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Met sulfone	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Trp	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total		44.7	3.83				
Total mg			3.07				
%protein			26.9				

Table 8: Amino acid composition of the dry filtrate.

Amino acid	nm/inj	nm/50µl	µg/50ul	Mole %	Weight%	µm/mg	%(w/w)
Asx	1.483	1.49	0.17	5.52	7.5	0.1	1.19
The	0.043	0.04	0.00	0.16	0.19	0.00	0.03
Ser	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glx	4.061	4.07	0.53	15.12	23.04	0.28	3.65
Pro	3.635	3.64	0.35	13.53	15.51	0.25	2.46
Gly	11.648	11.67	0.67	43.36	29.23	0.81	4.64
Ala	3.109	3.12	0.22	11.57	9.71	0.22	1.54
Val	0.876	0.88	0.09	3.26	3.81	0.06	0.61
Ile	0.332	0.33	0.04	1.24	1.65	0.02	0.26
Leu	0.937	0.94	0.11	3.49	4.66	0.07	0.74
Try	0.064	0.06	0.01	0.24	0.46	0.0	0.07
Phe	0.503	0.50	0.07	1.87	3.25	0.04	0.52
His	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lys	0.173	0.17	0.02	0.64	0.97	0.01	0.15
Arg	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cysteic acid	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Met sulfone	0.0	0.0	0.0	0.0	0.0	0.00	0.0
Trp	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total		26.92	2.28				
Total mg			1.82				
%protein			15.9				

As shown in Table 7, the most abundant amino acid (mol %) obtained for the freeze dried sample was glycine (37.83 %) and 43.36% in the dry filtrate (Table 8). On the weight % basis, the most abundant

amino acid was glycine 25.17% (sample A) and 29.23% (sample B). The Tables 6 and 7 shows the percentage composition of all the amino acids present in the samples.

About 16 amino acids were detected excluding tryptophan and cysteine which were destroyed during hydrolysis. The amino acid that were observed were: Glycine, Alanine, Proline, Glutamic acid, Aspartic acid, Arginine, Leucine, Lysine, Valine, Serine, Methionine, Phenylalanine, Isoleucine, Threonine Tyrosine Histidine, cysteine. Figure 16 shows the structures of the amino acids detected from the analysis.

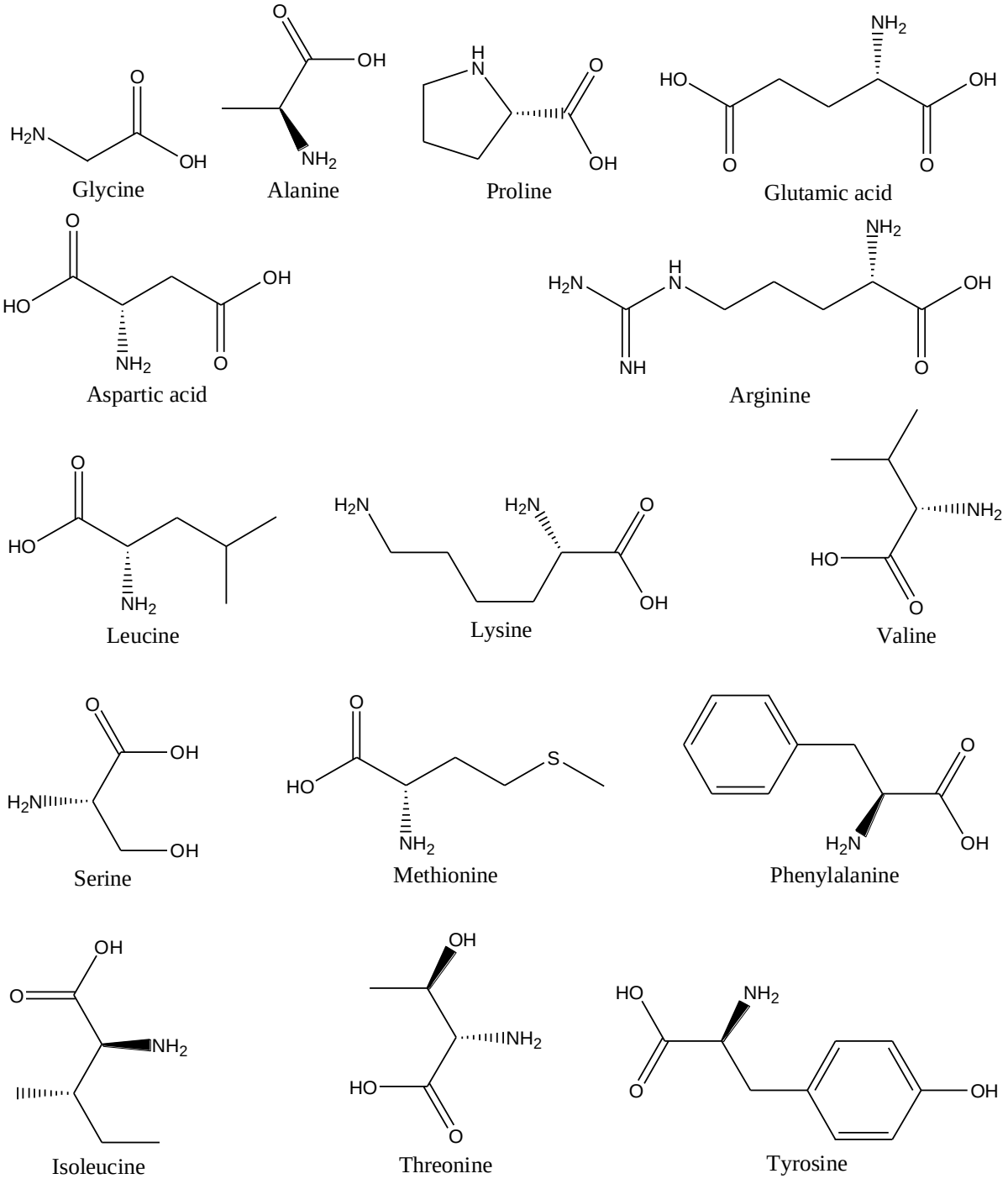


Figure 16: Amino Acids detected in the samples.

Glycine is the predominant amino acid with a composition of about 33% then proline and hydroxyproline which is 20% and alanine which is 11%. Gelatin from mammals have about 30% of this amino acids while the gelatin of fish from warm water is 22-25% and that from cold waters is 17%. Other types of gelatins, (glycine, serine, threonine, methionine and histidine) show similar properties in mammalian and fish gelatin (da Trindade Alfaro et al., 2015; Jiang, 2013).

Glycine is the most abundant amino acid due to the repeating sequence of glycine bonds (Muyonga et al., 2004; Lakshmi et al., 2014) . The non-polar region of collagen accounts for high percentage of alanine, together with proline and hydroproline which is responsible for viscoelasticity of gelatin (Khatoon, 2017).

Both gelatin and the filtrate show the similar types of protein except in the filtrate. arginine, serine and histidine were not detected. The absence of hydroproline indicates the low process of hydroxylation (Muyonga et al., 2004). The percentage yield of the proteins content obtained was 18.96% indicating high content of amino acids in gelatin as reported by Khatoon, (2017). Tyrosine showed a low percentage in abundance which is an inherent property of all Gelatin and Histidine was absent in both samples. Tryptophan was absent in both samples of gelatin studied, This finding is in agreement with results obtained by Sundar et al., (2011). The abundance of amino acids and protein content indicates that gelatin obtained from leather trimmings could potentially be used in the fertilizer, animal feeds or any other possible formulations in the industry as suggested by (Aftab et al., n.d.).

The HPLC spectra for amino acid analysis are shown below in figure 17 and 18.

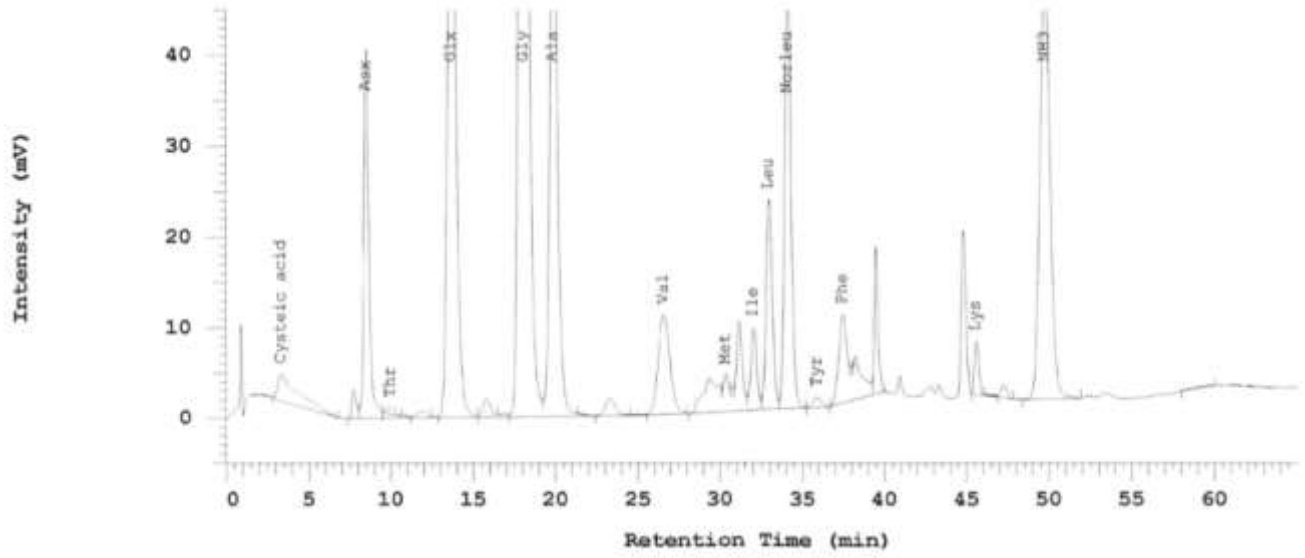


Figure 17: HPLC chromatogram for the gelatin from the leather trimmings.

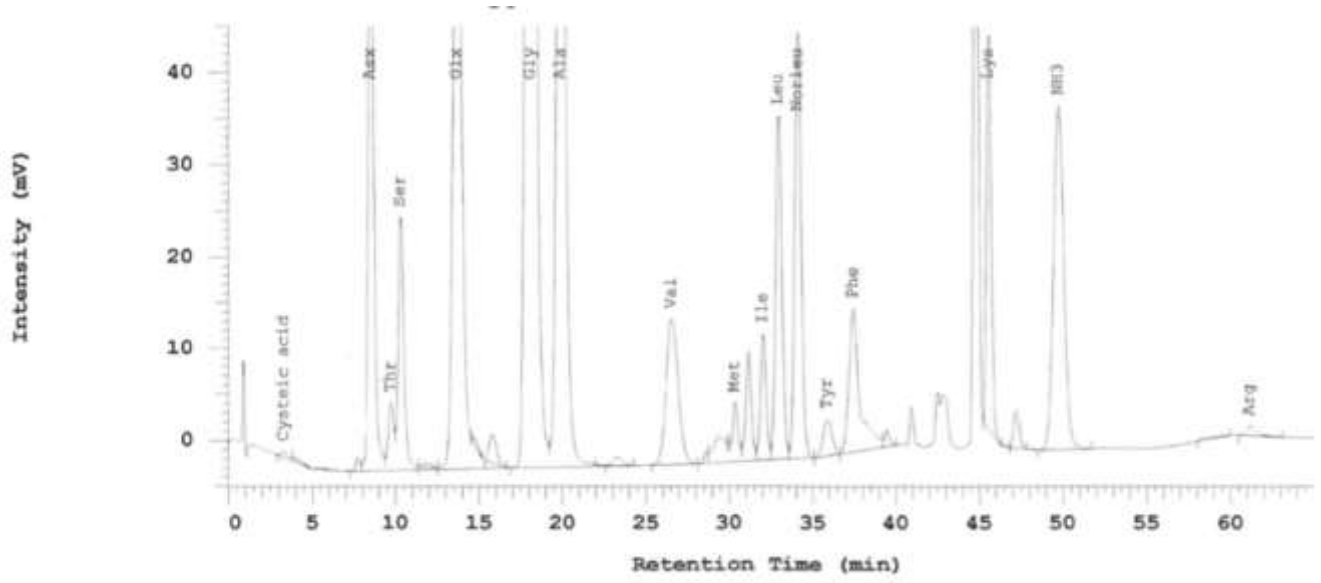


Figure 18: HPLC chromatogram for the dried filtrate of the hydrolysate.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENTATION

5.1 Conclusions

The aim of the study was to develop an enzymatic process for hydrolysis of leather trimmings wastes to address the solid waste disposal problem. The optimum condition for enzyme hydrolysis were investigated and it is shown that the process is efficient and the hydrolysis product obtained (gelatin) contain several important amino acids. The optimum conditions for enzyme hydrolysis was at pH 12 and a temperature of 75˚C. It took 21 hours for complete hydrolysis of the leather trimmings. The proposed enzyme technology is more environmentally friendly compared to the conventional acid and base hydrolysis which are expensive and have more adverse effects to the environment.

The gelatin shows low levels of chromium which can be extracted and make it safe for human use in various applications. The filtrate contains higher chromium levels and further studies are therefore recommended to renove Cr before discarding to the environment or reused for other applications. The FTIR results indicated the presence of the amino acids and the characteristic absorption bands were observed.

Overall it is shown in this study that about 18.9656 % of gelatin was obtained from 1kg of the hydrolyzed trimmings, an indication that the trimmings are a potential sources of gelatin. The HPLC results demonstrated the presence of several amino acids in both the freeze dried sample and dry filtrate. The gelatin obtained showed the presence of 16 Amino acids. Further, the filtrate. yielded 15 Amino Acids (Glycine, Alanine, Proline, Glutamic acid, Aspartic acid, Arginine, Leucine, Lysine, Valine, Serine, Methionine, Phenylalanine, Isoleucine, Threonine, Tyrosine Histidine, cysteine). It can therefore be concluded that leather trimmings that currently pose a major disposal problem for the tannery industries, can be used to produce value addition gelatin products that have commercial value.

5.2 Recommendations

1. It is recommended that a study to be done on the trimmings that were tanned by other means like vegetable tanned trimmings and aldehyde turned trimmings among others.
2. A further study to be done on the extraction of chromium from the gelatin and the filtrates.
3. Practical experiments to be done on the application of the gelatin obtained from the hydrolyzed trimmings

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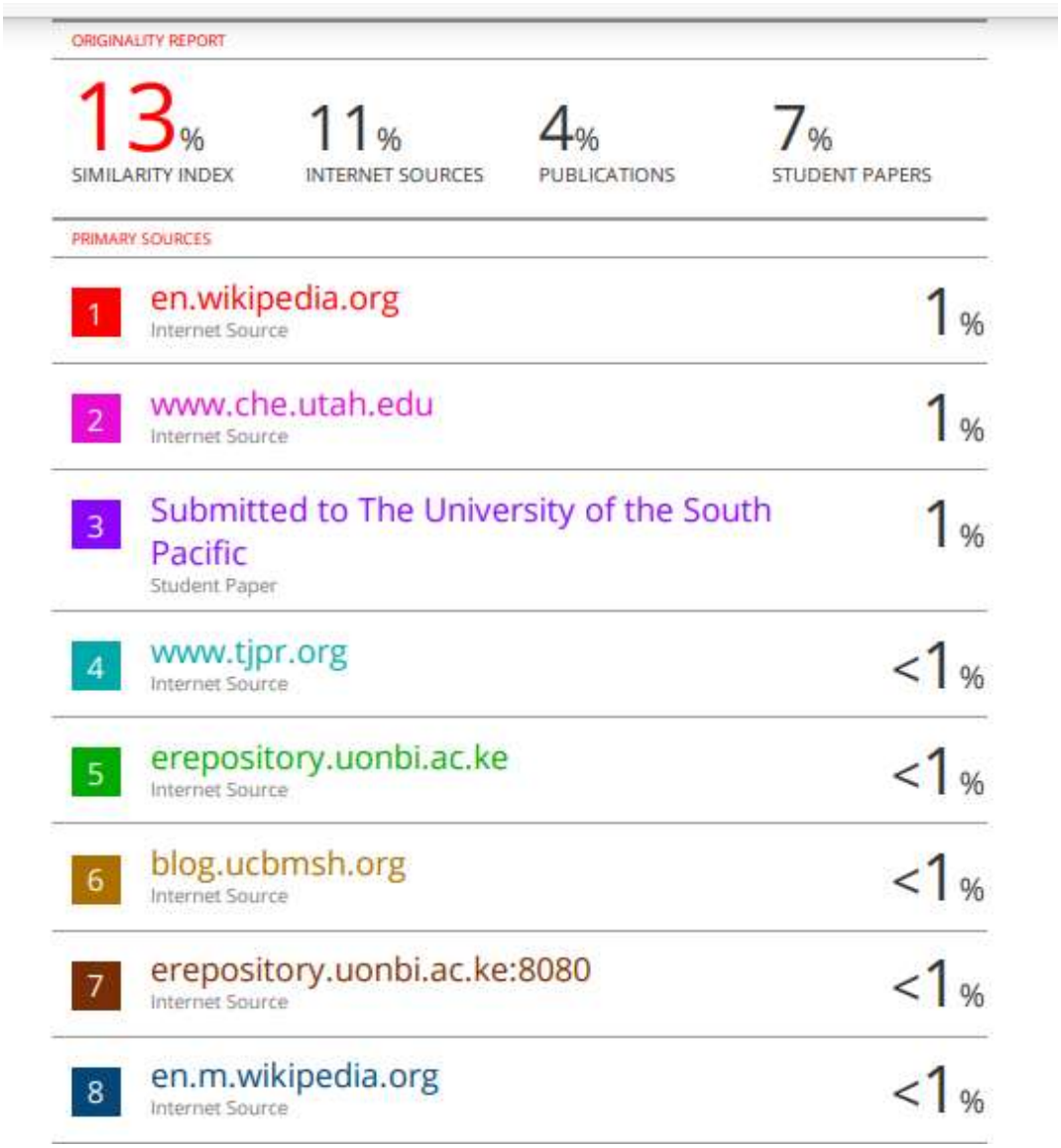

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ENZYMATIC HYDROLYSIS OF CHROMIUM TANNED LEATHER WASTES, AMINO ACID COMPOSITION ANALYSIS AND CHEMICAL CHARACTARIZATION OF HYDROLYSATE

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