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Preparation of leather Fat liquor cum filler from fleshing waste for Retanning process in leather manufacture

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DECLARATION

I, the undersigned, declare that this thesis is my original work and that all sources of materials used for the thesis have been dully acknowledged.

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List of Abbreviations

SGF = Sheep green fleshing

PDI= Polydispersity index

SLF = Sheep lime fleshing

GLF = Goat lime fleshing

HLF = Hide lime fleshing

SHL = Sheep hydrolysates limed

GHL= Goat hydrolysates limed

HHL = Hide hydrolysates limed

SHoL =Sheep homogenization limed

GHoL = Goat homogenization limed

HHoL = Hide homogenization limed

mV= millivolts

TAI= Total active ingredient

TA= Total alkalinity

HLB= Hydrophilic lipophilic balance

DCM= Dichloromethane

SDS= Sodiumdedico sulphate

ANOVA= Analysis of variance

DLS= Dynamic Light Scattering

PSC= photon correlation spectroscopy

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Abstract

Skin/Hide fleshing wastes are the day to day activities to remove the flesh from the pelt, from tanneries are generated during leather processing when flesh of the soaked or limed hides skin/hides is removed with the fleshing machine. These skin/hide fleshing wastes from tanneries contain significant quantity of protein and fat content and are currently being wasted into dumping sites or in open areas, consequently creating the fleshing waste disposal major environmental problem of the tanning industries throughout the world. Approximately, 9-14 tonnes of fleshing waste are being generated from Ethiopian tanneries per day.

The objective of this work is to elaborate a method/technique to develop fatliquor cum retanning agent for use in leather industry from the waste (fleshing) generated by the tanning industry.

Skin/hide fleshing is source of fat/oil and protein matter. The extracted fleshing fat was first emulsified by adding emulsifier. Then the fleshing hydrolysate, containing the protein fractions was prepared by alkaline hydrolysis in the presence of sodium hydroxide at a pH of 5.6. The hydrolysate and the extracted oil were further characterized to find the suitability of production of fatliquor cum filler for retanning process. The fatliquor cum filling product was prepared and used in leather processing during retannage stage. Physical characterization of the leathers were carried out and compared with that of control leather processed with commercial fatliquors and retanning agents.

Moisture, protein and fat content of the fleshing from cow hide were found to be 63.30, 12.82 and 4.84%, respectively. Similarly, the fleshing of sheep and goat skins have also been characterized and reported in the results and discussion section. The fat extracted from the fleshing was characterized for iodine, saponification and acid value. The fleshing hydrolysate was characterized for degree of hydrolysis, solid content, molecular weight, and particle size and zeta potential measurement. For making upper leathers, 8% of the fatliquor cum retanning agent per weight of skins/hides was employed, which gave better results. Similarly, for making nappa leathers, 16% of the prepared fatliquor cum retanning agent per weight of skins/hides was employed. Data from physical testing was quantified with statistical analysis and it was found significant. The leathers were not found greasy, indicating that the product penetrated into the leather matrix. The particle size of the prepared product was also determined and was found in the range of the commercially available retanning agents.

Organoleptic properties such as colour uniformity and intensity, roundness, softness and fullness properties of the two leather articles prepared using the developed product were evaluated by experts and found to be on par with that of the control leathers.

This study represents that the waste protein and fat from skin/hide fleshing wastes can be utilized as a beneficial product after some chemical modifications. Waste skin/hide fleshing is a good alternative source for preparation of fatliquor cum retanning agent. This project also answered the disposal problem associated with the solid wasted (fleshing) generated form tanning industries. The proposed methodology could be easily adopted by all leather industries.

Key words: Hydrolysis, Fleshing wastes, Fatliquor cum filler, Extraction, homogenization,

CHAPTER ONE

1. Introduction

The leather industry generally uses hides and skins as raw materials, which are the by-products of meat and meat products processing industry. In this respect, the leather industry could have easily been distinguished as an environmentally friendly industry, since it processes waste products from meat production [1]. However, the leather industry has commonly been regarded as a polluting industry due to the bad smell, organic and inorganic wastes and high water consumption caused during traditional manufacturing processes [2]. Leather is animal skin that has been chemically modified to produce a strong, flexible material that resists decay. Almost all the world output of leather is produced from cattle hides and calfskins, goatskins and kidskins, and sheepskins and lambskins. Other hides and skins used include those of the horse, pig, kangaroo, deer, reptile, seal, and walrus.

The leather can generally be processed in three steps. The first step is removal of the unwanted components, hair, adipose tissue, fats, etc., leaving a network of fibers of hide protein. The second step involves reacting of this network with tanning materials to produce a stabilized fiber structure. The third step is to build onto the tanned fibers characteristics of fullness, colour, softness and lubrication and finish the fibers surface to produce a useful product. The transformation of hide or skin into leather utilizes many chemical and mechanical processes.

Beamhouse operations

Cleaning and conditioning hides and skins by beamhouse operations like soaking, liming, unhairing, deliming and bating represent the biggest part of the effluent load of tanneries.

Soaking

During this process, hides and skins are rehydrated and at the same time, salts, bacteria and soluble proteins are washed out as well as dirts, blood and dung.

Liming and unhairing

Liming and unhairing are usually performed in one single operation. The treatment of hides in an acid or alkali solution with the addition of reducing agents is attained to destroy or to

remove the hair, unwanted proteins...etc. The removed substances can be separated from effluent in a solid form.

Deliming and bating

The purpose of this process is the neutralization of the alkaline hides and removal of lime as well as the further loosening and peptizing of the skin fiber texture to prepare them for the acid tanning process.

Pickling

It is the last beamhouse operation, at which adjustment pH of hides before chrome tannage occurs and thus reducing the astringency of the chrome tanning agents by addition of acid liquor and salts. Pickling is also used for preserving.

Degreasing

Degreasing process is necessary to attain good leather quality. Degreasing is normally performed together with or in addition to, soaking, pickling or after tanning, depending on the applied mixture, processed raw stock and on the desired quality.
The degreasing agents commonly in use are organic solvents. Emulsifiers are used as degreasing agents, instead of solvents. Enzymes and hot water may be applied as auxiliary agents in some mixtures.

Tanning

The purpose of tanning is to bring about irreversible of the skin substance that is prone to putrefaction. The object of converting pelt into leather by tanning is to:

- Stabilize it against enzymatic degradation and increase its resistance to chemicals
- Raise its shrinking temperature and increases its resistance to hot water.
- Reduce or eliminate its ability to swell.
- Enhance its strength properties.
- Lower its density by isolating the fibers.
- Reduce its deformability.
- Reduce its shrinking in volume, area and thickness. 8- Enhance the porosity of its fiber texture.

These effects are achieved by cross-linking the collagen chains with various tanning agents.

Wet finishing

This process includes, retanning, neutralization, dyeing and fat liquoring operations. The wet finishing processes are sometimes performed in a one single float. Retanning process aims at filling up of the meatuses of the already tanned leather through the incorporation of vegetable or synthetic tannins or resins with the aim of giving more fullness to the final product. Neutralization conditions the pH values of the chrome tanned leather for the dyeing and fat liquoring treatment.

Dyeing is to impregnate the material with the desired colour. Fat liquoring of the derma fibers improves the leather physical properties such as extensibility, tensile strength, wetting properties, water proofness and permeability to air and water vapour.

Finishing

The mechanical and coating treatment steps during finishing enhance the appearance of the leather and give special properties to the grain and flesh surfaces, i.e., to improve the use properties of the leather.

Mechanical treatment

The production of leather by transforming raw hides into finished leather requires several mechanical treatment steps. Most of them is to separate connective and adipose tissues, to remove useless parts of the surface of hides and leathers, to reduce the thickness of the pieces and to remove damaged grain, i.e., mechanical changes of the superficial appearance and of the rheological features (plasticity and elasticity) of the article "anchorage" of a polymerical aesthetical covering film to the derma. Other mechanical processes like samming, buffing and trimming can be also applied [3].

1.1 Ethiopian Leather Industry

Ethiopia is well gifted with livestock resources being among the ten top in the world. The leather industry is one of the country's most vibrant and important industries for Ethiopian economy as it generates the largest export revenue in industrial sector. Modern tanning in Ethiopia has started 70 years ago [4]. Annual production is only 2 million pieces of hides and 13.6 million pieces of skins which amounts to only 21% of the country's livestock population [5]. The fact that production of hides and skins heavily relies on the demand for meat makes the rate of expansion of the leather sector dependent on the rate of growth of meat industry.

Currently there are 30 tanneries in operation and most of them are located in vicinity of Addis Ababa. Annually, all leather industries put together use 2.3 million pieces of hides and 44.3 million pieces of skins as an input for processing at full capacity operations [6]. According to the Central State Agency of Ethiopia, in 2012/13[7] Ethiopia has a livestock population consisting of 53.99 million cattle, 25.5 million sheep, 24.06 million goats, 1.91 million horse and 0.92 million camels. The existing daily soaking capacity of tanning industries is 141,500pieces of skins and 9,050pieces of hides (see Annex 1). About one-third of these tanneries are found in Addis Ababa and it is surrounding [8].

1.2 Problem Statement

The sustainability of the leather industry crucially depends on how well it manages the liquid and solid wastes. While end-of-pipe treatment systems are in place to comply with discharge standards for liquids, solid waste management is becoming critical. With fleshing representing approximately 50% of all solid wastes generated in the leather industry, a proper management plan is essential. Value addition to leather such as providing customer specified aesthetic features are provided through use of host of proprietary products classified as fat liquors and syntans. These products are imported into the country leading to huge loss of national revenue. There is ample scope for adopting a multifunctional strategy such as use of fleshing for preparation of retanning and fatliquoring agents that can not only provide for replacing proprietary products but also tackle the vast solid waste menace.

Therefore, this thesis helps to realize a clean, new, renewable leather chemical option from locally available and environment polluting tannery solid waste as an alternative solution to current leather lubrication and filling chemicals, the rise in price of which has had an adverse effect on the economy of the country. The emerging industrial zones in Ethiopia will create a great opportunity to establish an industry that produce fatliquor cum filler in an industrial scale whereby the country can conserve its environment. In addition to this, the industry will enhance its public image and also reduce the influence of the environmental regulating agency on the tanning sector.

1.3 General and Specific Objectives

1.3.1 General Objective

The primary objective of this research study is the development of a fatliquor – retanning agent for use in leather industry from the wastes generated by the industry itself, so as to find an effective utilization for the large quantities of solid wastes generated.

1.3.2 Specific Objectives

The specific objectives of this study are:

- To characterize and quantify the water, fat and protein content in fleshing wastes generated from the processing of cattle, sheep and goat skins
- Further characterize the isolated protein for its extent of denaturation/ alkylation.
- Identify the specific fleshing waste which would enable maximum extraction of oil
- Based on the above information, identify and adopt suitable procedures for emulsification of the oil, so as to obtain a fatliquoring cum retanning agent
- Optimize the process parameters for fatliquor cum retanning agent production such as temperature required for hydrolysis of fleshing, reaction time required for complete hydrolysis, oil to emulsifier ratio.

1.4 Framework of the Study

The framework of this study is categorized in to two main groups. The first one is the assessment on the existing fleshing waste hydrolysed and homogenised that provides information on the physical composition of fleshing wastes, their further characterization and hydrolysis methods and secondly, the characterization of the fleshing wastes for selected and homogenised physicochemical parameters that are used to for preparation of fatliquor cum retannage agent for applicable of leather process itself and compare the leather after applied the product of Fatliquor-filler agent. The general conceptual framework of this study is shown the Figure 1.1 as below.

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Framework of the Thesis

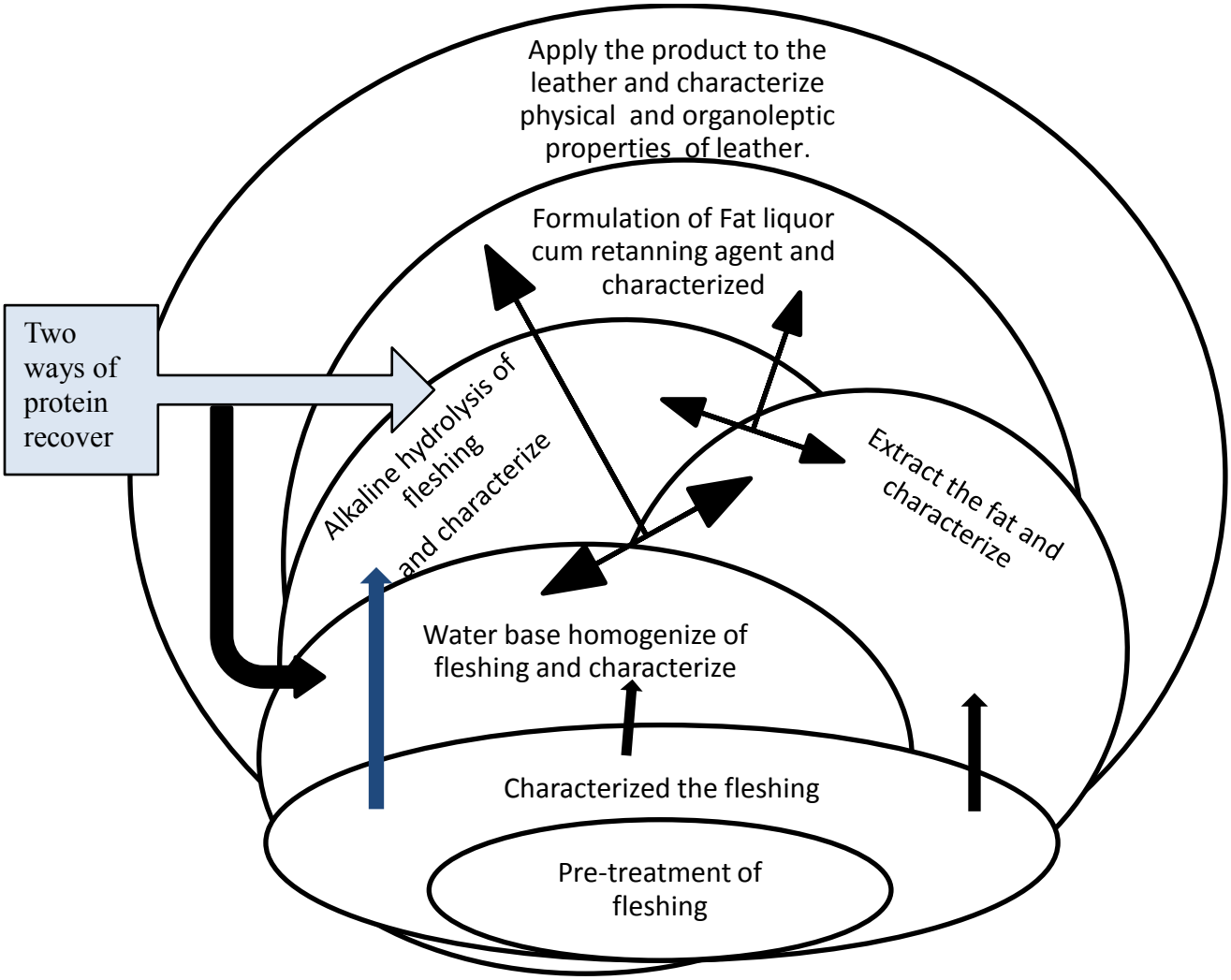


Figure 1.1: Framework of the Study

1.5 Significance of the research

It is predicted that solid wastes generated in the leather industry, unless properly management could hamper the sustainability of the industry itself. Fleshing is a major component of the solid wastes and the proposal provides for a viable option to use this waste as a fatliquoring cum retanning agent in the leather industry itself. In doing so, the product developed would provide reduction in cost of procurement of specialty chemicals and also on over dependence on imports for leather processing. The product is also expected to reduce the time for leather processing by

way of replacing two steps retanning and fatliquoring with one, leading to reduction in energy costs associated with leather processing. From the societal point of view, this process provides for a cleaner environment around the tanneries as well.

1.6 Scope and Limitation of the Study

The scope for the present study is to identify and develop a viable methodology for the utilization of fleshing wastes generated in leather industry for applications within the industry itself, leading to cost saving in terms of waste management as well as avoidance of use of proprietary products in processing. The scope is limited to fleshing wastes alone and does not cover other wastes such as hair or tanned wastes.

The present study is aimed at development, characterization and optimization the hydrolysate of fleshing waste and preparation of fatliquor cum retannage product for application in leather retanning stage.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Histology of animal skins/hides

The natural structure of animal skin or hide is very important in the ultimate leather which can be made from the structure [9]. Therefore, the histology of fresh animal skin is very interested to the tanner and the leather chemist [10]. More recently, however, the use of the microscope and cross-sectional pictures of the skin or hide after various stages in the tannery process have given the tanner and the leather chemist a much better insight into the changes which occur during the chemical conversion from the raw stock to the finished leather [11&12]. The natural structure of animal skin was described by O' Flaherty et al as follows:

I. Flesh

The flesh is composed of varying amounts of fatty adipose tissue, blood, vessels, nerves, and voluntary muscles. The amount present and its character influence the salt curing of hide because the fatty tissue as well as the voluntary muscle will serve as a barrier to salt penetration. The flesh layer forms 15% of total thickness of raw skin and it is removed in mechanical beam house operations.

II. Derma (epidermal area)

Derma or the epidermal area is that portion of the fresh skin or hides which contains the hair, the hair follicles, the epidermis, grain layer, corium and glands as sebaceous and the sudoriferous glands, which are surrounded and supported by a collagenous fiber bundle structure. Also, throughout this structure of collagenous fiber bundles are dispersed a network of elastic tissue fibers, erector pile muscle, blood vessel, and nerves.

III. Hair and hair follicles

The hair is composed of the cuticle, the cortex, and the medulla, going from the outside to the centre of the hair .The cuticle is composed of the same material as the horny layer of the epidermis. The surface epidermis dips down into the derma and forms a hair pocket, which it lines.

IV. Epidermis

The epidermis is known as stratified epithelium, because it is composed of four strata going from top to bottom and these strata are:

a) Stratum corneum,

b) Stratum lucidium,

c) Stratum granulosum,

d) Stratum germinativum.

The stratum germinativum is the lowest stratum and is in direct contact with the derma. When this layer is completely removed from the skin the so-called basement membrane underneath is left behind to form the grain surface of leather. The stratum germinativum is composed of similar cells that are at all times reproducing during the life of the animal.

V. Sebaceous and sudoriferous glands

The sebaceous glands are associated with hair and the excretory duct of the gland opens in the hair follicle on the shaft. The sebaceous glands are commonly referred to as the oil glands because of their functional characteristics of oiling or lubricating the hair cells and the corneum stratum of the epidermis. The contractions of the erector pile muscles regulate the discharge of oily material into the hair follicle [13 & 14].

VI. Epidermal area-fibrous tissues

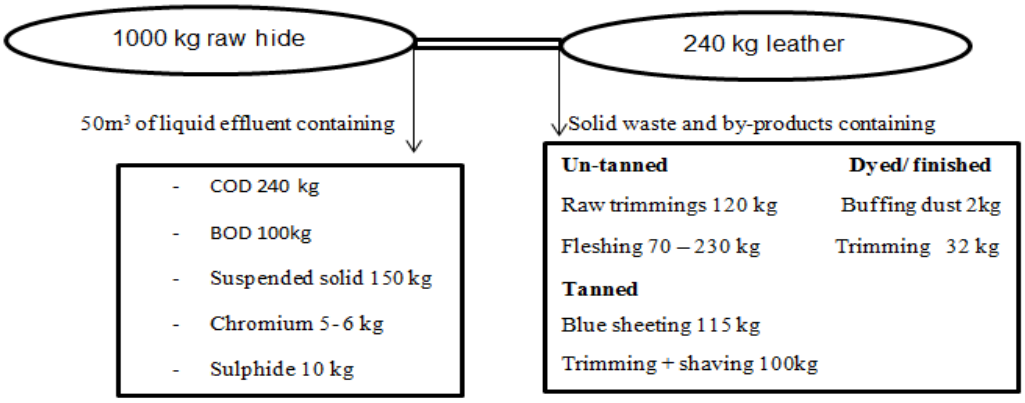
The fibrous tissue of the epidermal area is composed of collagenous fiber bundles, elastic tissue fibers, reticular tissue and nerve tissue. The fiber bundles of collagenous tissue in the epidermal area are small in comparison to the pronounced bundles in the corium. The collagen fiber structure of the epidermal or grain area has a different coefficient of swell than the corium area. The elastic tissue was found as a network of branching fibers that surround each hair follicle. The fibers making up this network are found along the entire length of the hair follicle, being most numerous at the level of the sebaceous gland.

2.2 Introduction to Fleshing Waste in Leather Manufacturing Process

Leather industry has been categorized as one of highly polluting industries and it has adverse impact on environment because of the generation of liquid, solid and gaseous wastes [15 and 16]. Fleshing wastes generated from tanning industries contain different chemicals which are used during leather manufacturing process. These fleshing wastes have different characteristics as different chemicals and mechanical processes are applied to the raw hides/skins. If these fleshing waste generated during after soaking, after liming operations [17] are not properly utilized or disposed they are likely to cause a number of problems on the environment.

Liquid and solid wastes are produced through leather processing where waste water is the most significant source of pollution, followed by solid wastes and by-products generated in nearly each one of the numerous production steps. One tonne of raw hides produce an average of 240 kg leather, up to 600 kg solid wastes (shaving, trimming, fleshing and dust) and approximately 20,000 litters of liquid effluents. Fig2.1 gives an overview [18]. Chrome shavings, fleshing and trimming are the principal solid wastes produced in leather industries and these are partly consumed by different industry.

Figure 2.1: Environment input and output of leather processing



Discharging hair waste and lime sludge wastes along with the effluents causes choking of drains. Raw and green fleshing, limed fleshing, splits (splitting waste) and trimmings putrefy easily and give rise to venomous smells. In many tanneries, it is the foul odour which starts from some of these putrescible solid wastes which accounts for much of the

smell traditionally associated with tannery wastes.

Solid waste (fleshing) disposal problem can be reduced by converting them to some value added product and finding a suitable use for the product. This is possible by adopting suitable technologies and suitable techniques and the prepared product can find application in leather processing. Thus would help in achieving the general and specific objectives of this research project.

2.2.1. Fleshing From Tanning Industry

The flesh is composed of varying amounts of fatty adipose tissue, blood, vessels, nerves, and voluntary muscles. The amount present and its character influence the salt curing of hide because the fatty tissue as well as the voluntary muscle will serve as a barrier to salt penetration. The flesh layer forms 15% of total thickness of raw skin and it is removed in mechanical beamhouse operations.

Leather processing is a highly water intensive process, with a very poor input to output ratio. The variety and quantity of solid wastes depends on animal species, breeding conditions, slaughterhouse practices, conservation conditions, leather process stages, mechanical operations, qualification of the personnel, and chemicals used in processes. Out of 1000 kg of raw hide, nearly 850 kg is generated as solid wastes in leather processing. Only 150 Kg of the raw material is converted in to leather. Percentage compositions of solid wastes generated in tanneries are [19]:

Table 2.1: Percentage compositions of solid wastes generated in tanneries are

Type of solid waste	Discharge percentage (%)
Fleshing	56-60
Chrome shaving, chrome splits and buffing dust	35-40
Skin trimming	5-7
Hair	2-5

During leather processing, high amount of solid waste are generated. Fleshing waste is one of them. Substantial quantities of fleshing waste in the form of green fleshing, lime fleshing, lime splitting and trimmings are generated from the tanning industry. For instance, every 150 tonne per day of processed leather generates 200-300 kg as wet limed

fleshing (LFs) in addition to other wastes. Due to putrescible nature of fleshing's it cannot be stored inside the tannin premises [20]. It is possible to recycle these products and even use them as raw materials for different industries [21]. Fleshing's are generally used in the manufacture of glue, adhesives, and gelatine [22 & 23]. Both de-limed fleshing's and residual hair have been found to be important sources of protein with several applications as biological fertilizers in agriculture or horticulture [24].

Over the past several years, researchers have developed processes for recovering collagen, protein and fat fraction from this material [25, 26 and 27]. It has been reported that the fleshing contains 4-18% fat, 5-7% proteins, 1-2% sodium chloride and 2-4% sodium sulphide in addition to 62-80% water [28 and 20].

2.2.2. Fleshing Waste Generated in Cow hides, Sheep skins and Goat skins processing

Skin/hide fleshing wastes from tanneries are generated in the pre-tanning process when flesh in the limed hide or skin is get rid of by means of sharp knives. These skins and hides fleshing wastes from tanneries contain the highest protein content and currently being wasted in the open areas, thus creating the solid waste disposal problem in tanneries [29&30]. The quantification of fleshing generated during leather processing has been carried out in some tanneries in Ethiopia (M/s Batu Tannery PLC, M/s Dire tannery and LIDI model tannery). The amount of fleshing generated per kilogram of raw hide or skin processed at different stage of leather manufacturing process have been quantified and provided in the Tables 2.2 and 2.3.

Fresh/green and salt preserved cattle hides/skins were used to produce shoe upper leather and the fleshing wastes generated from green fleshing and lime fleshing operation were determined. In order to determine the weight of a single piece of green and wet salted hide two batches of wet salted hides/skins were taken randomly from different batches prepared for soaking operation and then each sample batch containing different number of pieces of hides ranging from 1-5 pieces were weighed. Based on this calculation the average weight of a single piece of green and wet salted cow hide was found to be 31.16 and 10.74 kg, respectively (Table 2.2). When

compared the fleshing from green cattle hides are heavier due to the presence of water content in the green hides.

2.2.2.1 Fleshing Waste Generated in Cattle Hides Process

Fleshing are a solid waste generated during a mechanical process called fleshing that aims to remove the flesh or fats from the inner part of the hide or skin. Trimmings are unwanted parts removed by cutting the edge of hides just after fleshing operation is completed. To determine the fleshing and trimmings waste generated per kilogram of raw hide processed five pieces of hides that have already passed through soaking and liming processes were randomly taken from two pieces different batches and different source of raw material (i.e. fresh and salted) and weighed before and after fleshing and trimming operations, then rough calculate of the generated fleshing & trimming wastes in tannery. The data obtained from the assessing are presented in the Table 2.2.

From the assessment performed it was observed that 0.26 kg of fleshing and trimming waste per kg of wet salted hides and 0.25 kg of fleshing per kg of green hides are generated during leather processing. This indicates that approximately 250-260 kg of fleshing is generated per tonne of wet salted and fresh hides, respectively.

Table 2.2: Fleshing wastes generated per kilogram of green and wet salted hides.

Ref.No. Green	Weight of wet salted hide (kg)	Weight hide after fleshing (kg)	Weight of a fleshing waste generated (kg/kg of raw hide
1	32.00	27	0.28
2	29.00	26	0.24
3	30.5	27	0.25
4	33.7	30	0.23
5	30.6	25.8	0.29
Average	31.16	27.36	0.26
Salted			
6	10.90	12.50	0.22
7	11.30	12.48	0.25
8	11.20	12.62	0.23
9	8.90	10.59	0.26
10	1140	12.09	0.29
Average (kg)	10.74	12.06	0.25

2.2.2.2 Fleshing Waste Generated in Sheep and Goat skins Processing

In order to determine the fleshing waste generated per kilogram of wet slated sheep skin processed, a sample of five pieces of goat skin and un-haired sheep skins were taken randomly from five different batches of un-haired sheep skins prepared for fleshing process and weighed before and after fleshing process then calculate roughly the fleshing waste of sheep and goat almost the same, so due to this case the result can be generalized as skin fleshing waste and the results are presented in Table 2.3.

Table 2.3: Fleshing wastes generated per kilogram of green and wet salted skins processed.

Ref.No. Green	Weight of wet salted skin (kg)	Weight skin after fleshing (kg)	Weight of a fleshing waste generated (kg/kg of raw skin
1	1.22	1.77	0.016
2	1.23	1.76	0.081
3	1.25	1.75	0.024
4	1.26	1.73	0.048
5	1.35	1.79	0.022
Average	1.26	1.76	0.038
Salted			
6	1.23	1.76	0.016
7	1.20	1.75	0.017
8	1.22	1.76	0.016
9	1.20	1.73	0.017
10	1.21	1.75	0.008
Average (kg)	1.21	1.75	0.015

From the above Table 2.3 it can be seen that 0.015kg of fleshing waste generated from a kilogram of wet salted sheep skin and 0.038 kg of fleshing from fresh skin leather processing. This indicates that 15.00kg from wet salted sheep/goat skin and 38kg from fresh skins the 26.5 kg of fleshing waste average are generated from a tannery having a daily soaking capacity of 1000.00kg of wet salted and green sheep/goat skin.

2.2.3 Types of Fleshing

Green fleshing: In some countries, the raw hides undergo fleshing operation yielding

green fleshing and fatty tissues. This operation is carried out before washing and soaking. The yield of green fleshing is estimated to be 10% on the weight of the raw hide [31]. These fleshing are not contaminated with chemicals.

Limed fleshing: This is obtained while scraping out the limed hides and skins either by hand or by machines. The fleshing is protein rich comprising of cutaneous muscle layers and sub cutaneous adhering tissues, which are undesirable in the subsequent operations of leather manufacture. The limed fleshing are currently collected by the glue manufacturers and also by animal feed manufacturers.

Table 2.4: The composition of the limed fleshing in percentage [32]

Average composition of the fleshing		
Fat	11.0	(4-18) %
Proteins	6.0	(5-7) %
Not nitrogenous	1.5	(1-2) %
Sodium chloride	3.0	(2-4)%
Lime	4.0	(2-6)%
Sodium sulphide	3.0	(2-4)%
Water	71.5	(62-80)%

The fleshing obtained by employing machines in tanneries is potentially thermal denatured. The utilization of the same fleshing for glue manufacture is not economically viable. Similarly, fleshing obtained from hides treated with a high percentage of sodium sulfide is found to be unfit for the production of glue. They are at best disposed through landfill. Disposal of such fleshing is currently a serious problem. Limed fleshing can also be used to produce tallow by pressure boiling to separate the fats from the other components. Then, the process to obtain tallow continues in the same way as for green fleshing.

Figure 2.2: Lime fleshing waste of leather industry.

Limed trimmings: After the fleshing operation, the hides and skins are trimmed to remove the rugged and torn edges. These trimmings are also protein rich in nature and found to be good raw material for glue, technical gelatine, and animal feed [33]. The limed trimmings are 2–7% on the wet weight of the hides [34]. Un-tanned trimmings can be a good source for production of collagen [35]. Extraction of high-molecular-weight protein and enzymic hydrolysis of the residue from pig and fish skin has been reported. The extract had been found to have properties for use as a cosmetic material due to its high water retention capacity, ability to repair rough skin, lack of any odour problem, and absence of harmful effects on skin [36].

2.2.4 Characterization of Fleshing

The fleshing consists of water, fat, proteins (containing impurities due to the treatment of the leather with lime, sulphides and other products in lesser quantity: in fact a standard analysis states that it consists of 10% about fat, 7% of proteins, 2% of not nitrogenous substances, 8% of mineral salts, the rest of water. The present mineral salts are, mainly, calcium salt and sodium, the first one added as an oxide in the liming operation, the second one coming from the salting of the leather. This composition represents anyhow an average indication as the various origins of the hides and the different pre-treatments make very variable the fleshing produced, especially with regard to the contents of fat and the residual chemical agents used.

Fleshing wastes from tanneries are characterized by a very high water and protein content [37]. Hydrolysis with alkaline proteinase results in 4-12% fat, 5-10% collagen hydrolysate and 1-3% protein concentrate. Purification of the collagen hydrolysate fraction into edible

gelatine has been reported [38].

Alkaline hydrolysates of fleshing have been used as fillers and syntans, with good exhaustion and leather properties [39]. Enzymatically modified leather waste has also been used as fillers in leather production [38]. These polymerized potential filler products were shown to be evenly distributed throughout the hide and, more importantly, were not removed during the washing steps [40]. Small amounts of gelatine, a by-product of the leather industry, could be effectively used as filling agents for both shoe upper and upholstery leather [41].

2.3 Fat liquor

2.3.1 Introduction

Fatliquors have changed considerably over the years, particularly after the introduction of so-called synthetic fatliquors. It has gone through several periods of development from currying and stuffing to soap type fatliquors, and to sulphated, sulphited oils and evolved in recent years into a multiplicity of synthetic lubricants which bear very little resemblance to the soap type fatliquors and stuffing compounds of the past [42&43].

Fat liquor normally consists of an emulsifier and a water insoluble oil or fatty matter. The oil or fatty matter serves two main purposes. First, it has to separate the leather fibers to some degree to prevent them sticking together during drying and secondly, it has to act as a friction reducer enabling the fiber to pass against each other without too much resistance or squeak. A further requirement should be to impart the desired feel and gloss on suede or aniline leather, or to prepare the surface for finishing.

A useful leather oil should be water insoluble, of reasonably high molecular weight, non-volatile, fairly linear in structure (Heavily branched molecules are unsuitable) and a reasonably viscous fluid or paste.

These oils need to be emulsified to reach inside the leather in water medium. For these purpose, there are a wide variety of emulsifier available in the market. They are a means of dispersing the lubricants very finely in water to enable it to penetrate into the leather. Such emulsifier should have the desired property of allowing the emulsion to break at a point predemined by the tanner to give him the required penetration and complete uptake

of the applied lubricate. In order to do this, they must be the anionic or cationic. Although non- ionic emulsifiers are used, it is primarily as an adjunct to other systems.

The emulsifiers are responsible for:

- The electric charge
- The stability
- The degree of dispersion of the emulsion
- The binding power of the respective fatliquors to leather
- The reaction towards the influence of water.

The simplest way to make a lubricant dispensable is to mix this with some surface active agents. The charge of the resultant emulsion would depend on the nature of surfactant used, namely anionic, cationic or non-ionic. This should be remembered that a fat molecule consists of two chemical components with different chemical characteristics as shown below: [42]

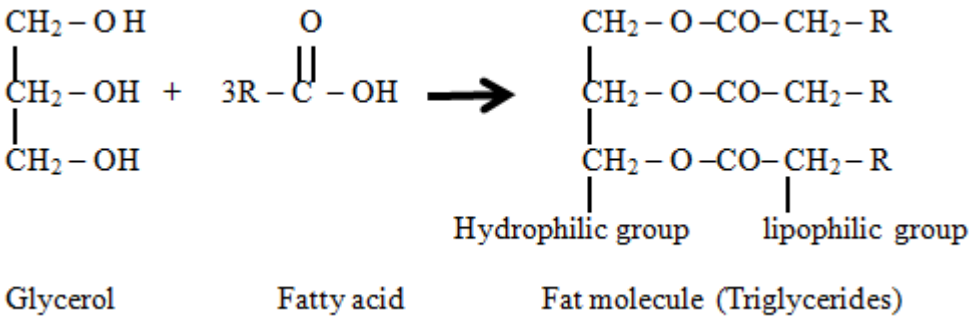
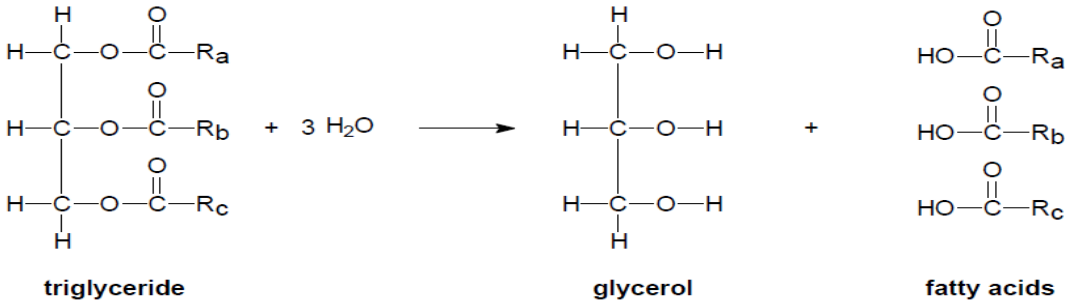


Figure 2.3: Triglycerides Structure

Fat and oils are esters of the tri–alcohol, (or glycerine). Therefore, fats and oils are commonly called triglycerides, although a more accurate name is triacyglycerols. One of the reactions of triglycerides is hydrolysis of the ester groups.



Triglyceride molecules contain mostly carbon and hydrogen atoms, with only six oxygen atoms per molecule. This means that fats and oils are highly reduced (that is, un-oxidized). Fatty acids contain an even number of carbon atoms, from 4 to 36, bonded in an un-branched chain. Most of the bonds between carbon atoms are single bonds. If all of these bonds are single bonds, the fatty acid is said to be saturated, because the number of atoms attached to each carbon is the maximum of four. Of some of the bonds between carbons atoms are double bonds, then the fatty acid is unsaturated. [44]

Sulphated oils are used frequently because they give good, fine-oil dispersions and are less sensitive to acid than soap fat liquors. This results in deeper penetration of the oil into the leather before they are deposited. Sulphated oil is prepared by treating animal or vegetable oils with concentrated sulphuric acid at a temperature of 10 to 20^{o}C. The resultant product is washed with a strong brine solution to remove excess acid. The salt is necessary to prevent the sulphated oil from emulsifying with the water. Soda ash is then added to form the sodium salt of the sulphated oil and to neutralize the last traces of the acid. The more the oil is sulphated, which is to say, the more sulphuric acid that has been fixed, the greater will be its stability to acid and the more thorough its penetration into the leather. Conversely, the more acid in the leather are less penetration. However, increasing the amount of sulfation or water miscibility decreases the "Oiliness" of the oil and therefore its lubricating powers [42 & 43]. Properly controlled sulphation process, the properties of sulphated products depends on the following factors:

- Strength of the sulphuric acid
- Proportion of the sulphuric acid to oil used
- Rate of addition of the sulphuric acid
- Degree of mixing or agitation.

The reactions take places during sulphation of oil are as follows: [42]

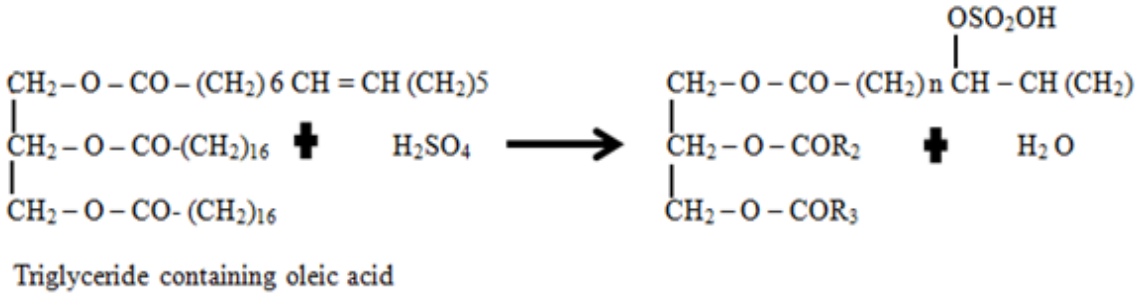


Figure 2.4: Triglyceride Containing Oleic Acid Sulphation of Oil Reaction.

The double bonds of a triglyceride are containing any unsaturated fatty acid like, oleic acid as one of the component of the fatty acids. This is simple sulphate because the sulphur atom is combined with the carbon atoms of the fatty acid via the oxygen atom.

Sulfonated oils are prepared by a similar process, usually at a higher temperature; the fatliquor contains the sulfonic group which gives greater stability and emulsions which penetrate deeper into the leather under acid conditions. Hydrolysis of the triglycerides takes place during the sulphation with the formation of sulphated mono glycerides. The hydrolysis does not take place during the sulphonation process because of excess sulphuric acid removed at washing stage. [42]

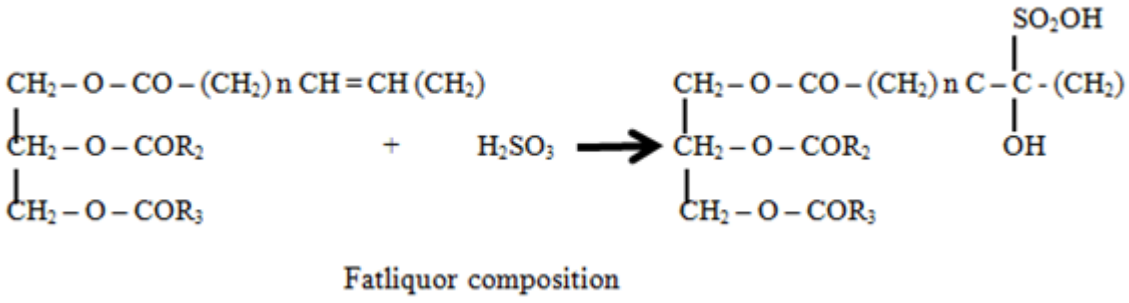


Figure 2.5: Triglyceride Containing Oleic acid Sulphonation of Oil Reaction

This is done by treating the oil with concentrated acid at room temperature. During sulphation if the temperature of the product goes up or if any other drastic method of

sulphatation is followed the $–SO_3H$ group is directly linked up with the carbon atom $R–SO_3H$ and thus a sulphonated product results.

The fat liquor consists of aqueous liquid containing oil in a state of fine dispersion. It is a three-phase system: [42]

a) The dispersed i.e. the oil which lubricates the leather

b) The water which is dispersion medium is the carrier of oil droplets into the leather.

c) The emulsifier which is the disperses the oil and stabilises the oil-in-water emulsion

2.3.2 Fat liquor Manufacturing

In the fatliquoring process, oils/fats are employed as oil in water emulsion known as fatliquor. Oil and water are immiscible because oil particles 'are held up together by its natural' cohesive force' (express surface tension), which are higher than the force of attraction (adhesive force) they can exert towards water particles. When the cohesive forces are stronger than the adhesive forces, the interfacial tension is higher as in the case between oil and water. The lower interfacial tension are required an Emulsifier (surface- active) material [42]. Fatliquor may be anionic, cationic or non-ionic. Anionic fat liquors are commonly employed for fat binding with chrome-tanned leather, which is cationically charged. Anionic fatliquors are commonly prepared by sulphation, sulphonation or bi-sulphitation of oils/ fats. Depending upon the source of the oils/fats used, the fatliquor can be classified as vegetable, synthetic and semi synthetic. Generally, castor oil is used as a source for vegetable based fatliquors. The synthetic fatliquors are usually obtained by sulphochlorination of C_{10}-C_{20} fractions obtained through the Fischer-Tropsch method of paraffin synthesis or from the petroleum industry [45]. Semi-synthetic fatliquors are prepared from both the vegetable and synthetic sources. Characterization and possible use of oil extracted from Seal Hides as leather fatliquor has also been studied [46]. Typical fatliquor raw materials are presented in Table 2.5.

Table 2.5: Typical Fatliquor Raw Materials

From natural source	**Synthetic source**
Fish oils	Paraffin
Animal oils	Mineral oils
Vegetable oils	Synthetic alcohol silicone oil
Fatty alcohol	Solvent
Lecithin	Oxo-oil
Wool grease	
Waxes	

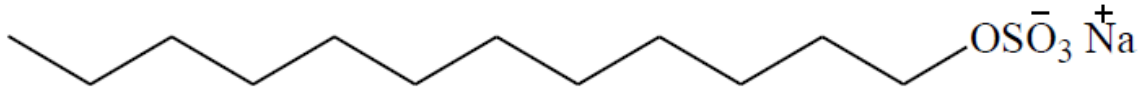
By far the largest amount of surfactant in leather industry is used in the preparation of fatliqours. Fatliquors have changed considerably over the years, particularly after the introduction of synthetic fatliquors. It has gone through several periods of evolution from carrying and stuffing to soap type fatliquors, and to sulphated, sulphited oils and evolved in recent years into multiplicity of synthetic lubricating solution which bear little resemblance to the soap type fatliquors and stuffing compounds of the past. In the preparation of commercial fatliquors, considerable amount of substances, this may be natural or synthetic.

The usual sulfating agent reacts both with the double bonds and the hydroxyl groups. Not all oils or fat products are made emulsifiable under identical conditions, because of their varying nature; also, the nature of oil has its own influence on the mode of addition of sulfuric acid for sulfation. Sulfated liquors are fairly stable at lower pH values. The degree of sulfation is an important factor for the stability of fat liquor and for the fixation of fatty matter while fat-liquoring. Sulfated oils are by far the largest category of fat-liquoring agents used by the tanning industry. These fat liquors are held in the leather by electrostatic forces between the protonated amino acids of the collagen molecules or the cationic groups of the chromium complexes and the negatively charged sulphate groups on the fat-liquor molecule. The sulfating agent has reacted mainly with the double bonds, which is shown by a decrease of the iodine value.

2.3.3 Choice of Emulsifier for Fatliquor Preparation

An emulsion is a fine dispersion of one liquid in another liquid with which it is immiscible. An emulsion is a two-phase system. The principal components are an oil phase and an aqueous (water) phase. The aqueous phase is water plus any combination of materials, which are polar and dissolve, at least to some extent, in water. The oil phase comprises one or more oily materials, or other ingredients, which are non-polar and exhibit at least some solubility in oily materials. Depending on how an emulsion is prepared, they are classified as Oil-in-water (o/w) (oil droplets dispersed in water; the oil is referred to as the internal or dispersed phase and the water as the external or continuous phase) or Water-in-oil emulsion (w/o) (water droplets are dispersed in oil; the water is the internal or dispersed phase and the oil the external or continuous phase) [47].Emulsifiers must therefore have both a strong hydrophilic and a strong hydrophobic group [48].

Surface-active or other agents that are added to an emulsion to increase its stability by interfacial action is known as emulsifiers or emulsifying agents. Emulsions are a fundamental product form for many lubricant categories and these are made possible by careful selection of the optimum emulsion. Emulsifiers are employed in fatliquor preparation to get water miscible emulsions. Efficacy of emulsifiers depends on their ability to reduce surface tension, form complex films on the surface of emulsified droplets, and create a repulsive barrier on emulsified droplets to prevent their coalescence. [49 & 50] Emulsions are suspensions of droplets (greater than 0.1μm) of one immiscible fluid dispersed in another fluid. Their kinetic stability is a consequence of small droplet size and the presence of an interfacial film around oil droplets.[51] An emulsifying agent must be present to form stable oil-in-water emulsions Chemically emulsifiers or surfactants in general can be classified as anionic (e.g. sodium dodecyl sulphate, fatty acid soaps, alkyl sulphates, alkoxylated carboxylic acids, phosphorous compounds etc.); cationic (e.g. Quaternary compounds); amphoteric (e.g. betaines) non-ionic (e.g. sorbitan esters and ethoxylated esters Glucosides; Transesters and Glyceryl esters) and others.

a) 

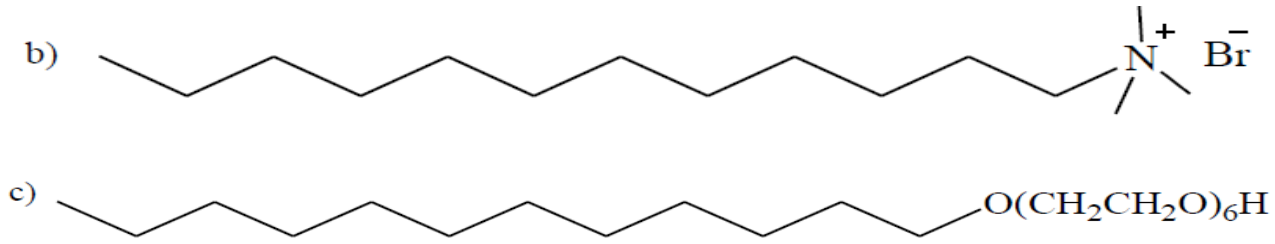


Fig.2.6: a) anionic surfactant sodium dodecyl sulphate {CH_3 $(CH_2)_{10}$ CH_2 OSO_3^- Na^+ } (SDS), b) cationic surfactant dodecyl trimethyl ammonium (DTAB) and c) non-ionic surfactant hexaethylene glycol monododecyl ether (C12E6).

Anionic surface active agents: - Anionic surface active agents account for about 65% of worldwide surfactants used. Generally, they are high foaming and sensitive and sensitive to hard water and thus required additional substance to complex calcium and magnesium ions. They also are more effective than others in particulate soil removal, especially for natural fabrics and gives softer handle in leather [42].

a. How to Make Water-in-Oil (W/O) Emulsions

Formulating water-in-oil (W/O) emulsions is inherently more difficult than oil-in-water (O/W) emulsions. But the difference between the W/O and O/W emulsions, oil is dispersed in water, it is an oil-in-water emulsion and vice versa, when water droplets are dispersed in oil, the resulting emulsion is called a water-in-oil emulsion. In W/O emulsions water droplets are dispersed in oil. This process is dependent on the type of emulsifier, and not the water-to-oil ratio. [52 & 53]

b. Manufacturing of W/O Emulsions

When making an O/W emulsions you can easily add the oil phase to the water phase (or vice versa), or dump everything into one container and heat to 66 - 80^{o}C. This will not work for W/O emulsions. For making W/O emulsions you have to add the water very slowly to the oil phase (which must contain the emulsifier), making sure that the water does not pool up excessively on the surface. Hence, you have to keep stirring continuously while adding the water.

c. Use the Right Emulsifiers

Often, O/W emulsions are made with more than one emulsifier. By combining emulsifiers, the oil-water interface is strengthened resulting in a more stable product. This is not necessarily the case for W/O emulsions. Often adding a second emulsifier may stabilize the system. For W/O emulsions only a limited number of emulsifiers are available since the entire range of ionic emulsifiers (with their typically high HLB) value will not work. For W/O emulsions a very low HLB (hydrophilic/lipophilic balance) of 3 - 6 is required. The important W/O emulsifiers [53] examples Sorbitan Stearate (HLB 4.7), Polyglyceryl Oleate (HLB 5.0), Lecithin (HLB approx. 4.0)

The concept of hydrophile –lipophile balance (HLB) is one of the most important properties of emulsifiers. The HLB is an expression of the relative simultaneous attraction of an emulsifier for water and oil or for the two phases, this mean the HLB of an emulsifier determines the emulsion type that tends to be formed [54].

Hydrolysates are surface active materials and promote oil-in –water emulsions because they are water soluble and contain hydrophilic and hydrophobic functional groups [55].

2.3.4 Fatliquoring Process in Leather Industry

Leather processing involves many chemical reactions and mechanical processes in converting the raw hide or skin, a highly putrescible material into leather. From the protein point of view, keeping oil around the collagen protein bonds is necessary to protect them and give longer life to the leather. If the collagen protein bonds dry out entirely they will shrink, become stiff and break. The broken bonds weaken the leather permanently and damage the product in general. Introducing oil back into the leather at this point is useless, as the damage is already done, and irreversible.

Chemistry of fatliquoring: To allow a small amount of oil to be spread uniformly over the large surface area of the leather, it is necessary first to dilute the oil. In an emulsion with water, the oil is dispersed in microscopically small droplets. It is important that the drops of oil in the water remain as an emulsion until they penetrate the leather, and not separate

out as large drops or as a layer of oil, which could not penetrate the leather fiber and result in merely a greasy surface layer

Tanned leather, which has not been treated with oils, fats, or greases in emulsion form will dry out hard and stick during the drying process. To avoid this problem and to produce soft leathers, fatliquoring is added as a step after the tanning process [56]. This process introducing oil into leather by treatment with Oil in water emulsion is known as Fatliquoring [57]. It prevents before the leather is dried. Fatliquor improves physical characteristics of leather such as tensile strength, tear strength, flexible endurance, wetting properties, waterproofness, and permeability to water vapour and air [58].

The fatliqouring agents are roughly classified in as emulsion type and non-emulsion type. The emulsion type fatliquoring agents include anionic, cationic, amphoteric and non-ionic ones. The non-emulsion type fatliquoring agents include natural oils such as fish oil, beef tallow oil, vegetable oil (olive oil), animal oil (beef tallow, lard and mutton tallow), wool grease, mineral oil, wax, paraffin wax and the like.

The self-emulsifying oils exist in two main categories: sulphated and sulfited. The sulphated are chemically treated with sulfuric acid, what increases the affinity with the tanned fibers. The sulfited oils have smaller particles and higher capacity of bonding. There are also chemicals elaborated with emulsifiers. Crude oils can also be added to the fatliquoring bath. They are water insoluble, but they are emulsified by the sulfated and sulfited parts. Both sulfated and sulfited oils are anionic and they bond to the amino groups of collagen. According to [59] the effect of a fatliquor may depend on several interacting factors: solution pH and substrate charge, the nature of the neutral oil and its reactive counterpart which combine to determine the emulsion properties and the flow characteristics of the oil. The sulfated oils were developed near 1950 and they are largely used, due to their capacity of improving the pleasant feel and softness. In 1980 new synthetic products have emerged. The sulfosuccinic compounds provide high softness with no risk of migrating substances, in addition of giving the leather hydrophobic character. [60]

Besides correct choice of added chemicals, it should be considered: keeping bath temperature constant, for example at 50ºC and making a previous mix of components,

adding them slowly to the hot water, to build the emulsion, and just then add this mixture to the drum.

Fatliquoring plays an important role on hydrophobisation of leather. [61] Made a correlation between hydrophobisation and production parameters. According to the author, the fatliquoring is the main step for the production of hydrophobic leather. The deeper the fatliquoring, the better will be the results for hydrophobisation. For production of hydrophobic leather, the oils used must be non-polar (crude). The emulsifying oil has surfactant structure, what does not allow the leather to become hydrophobic.

Sulphated, sulphonated, sulphited and phosphated fat as well as mixtures of neutral fat with different emulsifiers are used to obtain fatliquoring emulsions to lubricate leather [62 & 63]. Fatliquoring processes are important to have comforted leather products as well as these processes effect the physical properties of leathers such as tensile strength, stitch tear etc. Skin/hide varies greatly in its physical structure not only from one to another, but within a single one [64]. The aim of this research is to find out value added of natural fat /fleshing waste for application of leather chemical.

Penetration of the Oil: - Fatliquors which penetrate deeply into leathers have a tendency to give loose leather. Applying increased amount of oil of increasing penetration power, improvement of the quality of leather was obtained up to a maximum and then loose leather was produced [65].

The optimum softness is achieved by a penetrating as well as surface fatliquor. Application of fatliquor over several process steps leads ultimately to a maximum softness. It should be mentioned that excessive softness may give looseness. Softness is influenced by viscosity and interfacial tension of oils, and depends upon the ratio of emulsifier fraction to neutral fraction of the oil. The particle size distribution and emulsion stability of an emulsion are variables which influence the stability and penetration of the oil. Sulphonation imparts greater stability than sulphation [66].

Fullness of leather:- The quality and kind of raw material used in the manufacture of the fatliquors considerably influence the fullness, tightness of the grain and handle of the

leather. Fullness is commonly judged by subjective assessment. In some cases, however, the increase in thickness is used as a measure of fullness. The difference in filling action is more noticeable for thin leather up to 1.2mm. It is possible to reduce the amount of ratanning agent offered, particularly vegetable tanning agent and some replacement syntans to limit the increase the thickness of such leather. According to Wyss, the following order of the filling action of emulsified component of fatliquor could be used [67].

2.4 Retanning Agents

Conventional chrome tanned leathers are empty as the chromium salt forms coordinate covalent linkages with the protein without any filling. To add value to the leather through properties such as feel, various types of fillers such as vegetable tannins, aromatic condensed products, resin based products etc. are employed. In recent years, there has been tremendous interest in the use of protein or protein hydrolysates as fillers for chrome tanned leathers.

Preparation of protein fillers for making leather using animal fleshing as starting material is one of the objectives of reusing fleshing generated in the leather industry. In a recent patent, the preparation of protein fillers involved pre-treating with inorganic acid, hydrolysing the pre-treated fleshing in the presence of alkaline compounds, neutralizing the same to obtain the protein filler.

2.4.1 Leather Filler Agents

The filler assist in obtaining the desired fullness, plumpness, temper and roundness of leather. Since the filling material penetrate deep into the leather and are deposited within the voids or between the looser fibre structures, they prevent the voided areas from collapsing. Filler therefore not only produce uniformity in substance, roundness in feel and an improved aesthetic touch to the leathers but also facilitate uniform buffing, finishing and plating operations [68]. Filler may be composed of either inert or polar substances or a mixture of both. Use of leather fillers, in some stage or other during leather manufacture, deserves special attention for quality products.

2.4.3 Protein Filler

During the preliminary stages of the leather manufacture, dehairing, liming, bating and degreasing, most of the natural oils and protein from the skins/hides are removed, and at the time of completion of tanning, the leather does not contain sufficient amounts of lubricants and fullness to prevent it from drying into a hard material. Proper lubrication or fat-liquoring and fullness are necessary to obtain leathers with requisite characteristics. The term fatliquoring is used for the incorporation of oils and fats into leather in the form of an emulsion mixing with the protein parts. It safeguards the leather against cracking because adhesion of the fibers is prevented during drying by this operation. The main characteristics of fat-liquored leathers are softness, feel, and a certain degree of water repellence and protein use as filler to give for leather fullness. The physical characteristic such as break, stitch tear resistance, elongation and tensile strength, as well as comfort properties (particularly for clothing) of leathers, depends on fat-liquoring [69]

The fat-liquoring operation is designed to introduce oils and fats into the leather matrix in finely dispersed form in a water medium. This is usually achieved by emulsification processes through the introduction of sulfate, sulfonate, and sulfite groups into the structure of oils and fats [70] or by addition of surfactants to the composition of fat liquors. The ability of emulsifiable products to impart different characteristics to leather is governed by various factors, such as fatty acid composition of the fat products and the extent of unsaturation in the emulsifiable oil. The softness of fat-liquored leather increases with unsaturation of the oil, but the increase in softness is at the cost of fullness (firmness).

A great quantity of oil and protein are recovered when fleshing wastes of skins/hides the use in leather processing itself is interesting. After characterization of these fats, we studied the more appropriate formulation of fat liquors cum retanning product and assessed their retanning ability by practical tests these product.

2.4.3.1 Chemical nature of the proteins

All living matter contains protein, they occur in nature in a number of different physical forms and with a wide variety of chemical characteristics. All proteins are composed of polypeptide chains, which are polymeric structures of indefinite size formed by the elimination of water between the carboxyl and amino groups of different α-amino acid molecules. The polypeptide chains (proteins) may be represented as [52]:

Where the chains are extends indefinitely on either side, presumably terminating in free amino and carboxyl groups on the respective ends. The groups -CO-NH- within these chains are called the peptide groups. An alternative generalized formula can be represented as follows:-

$$(\text{-NH-CH-}\underset{\displaystyle R}{\overset{\displaystyle |}{\text{CO-}}})_n$$

Where the radical R characterizes the amino acids, and the n-is the number of residues in the chain, Fig.3 illustrates the structure of a polypeptide chain of amino acids; the following represents the formation of peptide group through the combination of two amino acids:

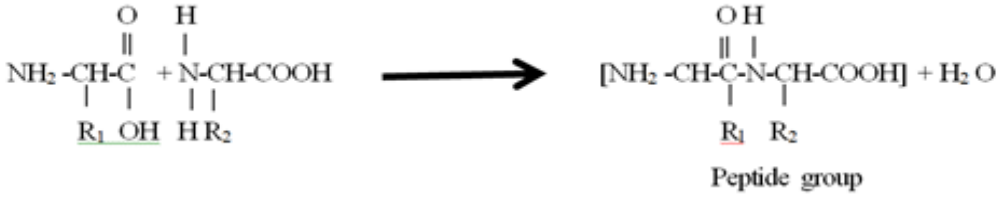


Figure 2.7: The Formation of Peptide Group through the Combination of Two Amino Acids

Protein or polypeptide chain also shows that the chain has different terminals- namely, $\alpha -$ amino (N- terminal) and the $\alpha -$carboxyl (C-terminal) [71]. The sequence of the amino acids in a polypeptide chain is normally expressed, conventionally, by starting with the N-terminal.

The polypeptide concept in the formation of protein is supported by the following facts:

- Amino acid are contains the amino and carboxyl groups, which produce abundant when protein molecules are hydrolzed.
- Lower molecular weights of hydrolysate protein have been isolated from the hydrolysed products.

During the hydrolysed of protein the breakdown of polypeptide in to peptide with different charges it can be anionic (-) or cationic (+) charges.

2.5 Hydrolysis

Alkaline hydrolysis of protein: Alkaline hydrolysis is a fairly simple and straightforward process; first the protein is solubility by heating followed by the addition of alkaline agents like calcium, sodium or potassium hydroxide and maintaining the temperature to a desired set point (typical range 55- 90 ^{o}C. The hydrolysis will be continued for several hours until it reaches the desired degree of hydrolysis and then the product is evaporated, pasteurized and spray dried [72].

Alkaline hydrolysis leads to the random breaking of nearly 40% of all peptide bonds in proteins, the major solid constituent of animal cells and tissues. The vast majority of the products of the hydrolysis are single amino acids or small peptides in the 2-5 residues range (nearly 98% of the hydrolyzate). [73]

Alkaline hydrolysis is a simple, natural process by which complex molecules are broken down into their constituent building blocks by the insertion of ions of water (H_2O), H^+, and OH^- between the atoms of the bonds that held those building blocks together.

CHAPTER THREE

3. MATERIALS AND METHODS

This chapter presents the raw material, chemical and reagent required for these research and the experimental procedures followed, the instruments used for extraction, instrument used for hydrolysis, characterized and analysis, and illustration depicting the experimental setup.

3.1 Raw materials, Chemicals and Reagents

3.1.1 Raw Materials

Fleshing waste: Fleshing coming from the fleshing operation may be produced after the soaking or after the liming. It is the basic raw material. Normally the fleshing waste contains very less amount of oil and protein. Fleshing from sheep, goat and cow hide were utilized in this study.

Sodium dodecyl sulphate: It is one of the anionic emulsifier used as such. It is reported to have good emulsifying properties. It has an HLB value above 10 and hence can be effectively used in making oil in water emulsion [74]. M7 emulsifier is an anionic emulsifier. Hence it is used in making oil in water emulsions.

Goat skins: - The developed product was used as a retanning cum fatliquoring agent in processing of goat skins in to upper and garment leathers.

3.1.2 Chemicals, Reagents and Solvents

All the chemicals used like, sodium dodecyl sulphate, M-7, Sodium hydroxide, Sulphuric acid, Castor oil, Petroleum ether, Dichloromethane (DCM), chemicals for characterization of extracted fats, nitrogen content determination, determination and characterization hydrolysed product were of LR grade and the leather chemicals used for processing were of commercial grade.

3.1.3 Apparatus used in Laboratory instruments

- ✓ 10 ml, 250 ml, 500 ml glass beaker, Standard Measuring Flask, Round bottom flask, Burettes, Micropipettes, Heating mantle, Dropper, Scissor.

- ✓ Refrigerator, Hot air Oven, Magnetic stirrer, Analytical weighing balance, Funnels, Crucible, aluminium foil and filter paper and Grinder
- ✓ Automatic setting of Oil Extractor (Soxhlet), Autoclave (for degraded of protein), Zeta potential apparatus, viscometer, GPC and Drums for leather test processing

3.1.4 Leather processing equipment's and apparatus

Testing drums, Fleshing machine, Shaving machine, Sam- setting machine, vacuum dryer, staking machine and roto-press machine

3.1.5 Laboratory Apparatus used for physico-chemical analysis of the leather

Tensile strength, tear strength and elongation at break tester, lasto-meter (Burst ball), flexo-meter and colour fastness tester (rub fastness tester)

3.2 Experimental Setup

3.2.1 General Process Descriptions:

Calculated quantities of fleshing from each raw material (cow hides, sheep and goat) are taken. The fleshing waste sample was brought from LIDI Model tannery and M/s Batu Tannery PLC. The fleshing material was washed, de-limed and was cut in to small pieces.

The alkaline hydrolysis of fleshing was carried out and the various parameters like temperature, pH and time were optimized. The fleshing hydrolysate prepared were characterize and used for making the product by mixing together using different combination of emulsifier to improve the stability of the final product.

3.2.2 Sample Collection and Sample Pre-treatment

Enough amounts of goat, sheep and hide fleshing waste were collected from M/s Batu Tannery PLC and LIDI Model tannery. Two types of samples were collected; green fleshing and limed fleshing. Green fleshing was washed with copious amount of water and taken for hydrolysis. The limed fleshing has lime and sodium sulphide. Hence, the limed fleshing was washed with water and de-limed with ammonium chloride and taken for further processing. The pH of the fleshing before hydrolysis was 7.5 - 8 and stored at 4 ºC in refrigerator (Annex 2).

3.3 Methods

3.3.1 Characterization of Fleshing Wastes

The first step in the sample preparation was de-limed and washing then to reduce the size by cutting, the wet sampled.

a) pH: The sample's pH was checked using pH paper.

b) Moisture Content: - Moisture content in skin and hide fleshing wastes were carried out according to SLC 3 (IUC 5), the amount of moisture present in the fleshing's were measured using a gravimetric difference. A set of five samples from each raw material fleshing waste weighing approximately five grams were placed in a china crucible (weight of the crucible already noted) and dried at 102 ^{o}C in an electric oven for 5 hours. Then the crucibles were cooled in desiccators and weighed [75]. The entire samples used in the experiment were used on a wet weight basis [76]. The procedure of moisture content determination explained under (Annex 3).

Calculate the moisture content of the sample is calculated using the following equation:

$$W\% = \frac{A-B \text{ x } 100}{B}$$

Where: %W = Percentage of moisture in the sample,

A = Weight of wet sample (grams),

B = Weight of dry sample (grams)

c) Nitrogen content

To determine the total nitrogen content of the sample a Kjeldhal method was used [77]. It involves three steps digestion, distillation and titration.

Digestion

Ten grams of the fleshing was weighed into a 500ml kjeldhal flask moistened with distil water. Selenium powder and sodium sulphate was added as a catalyst and 30ml of concentrated sulphuric acid was also added and then digested for 2 hours using the Bunsen burner flame. The solution was then cooled and decanted into a 100ml volumetric flask and made up to the mark.

Distillation and Titration

An aliquot of 10ml of the digested sample was taken into a distillation unit and 20ml of 40% NaOH was taken and 10ml of 4% boric acid was added to it resulting in a pink colour. The distillate was then collected over NaOH solution and boric acid for about 5 minutes. The presence of nitrogen gave a blue colour. The solution was titrated with 0.49N H_2SO_4 until the blue colour changed to pink signifying the end point. Using the recorded titre value and the relation below the % of nitrogen was then calculated.

$$\%\text{Total Nitrogen} = \frac{14 * (A - B) * N * 100}{1000 * 1}$$

Where: A= is the volume of standard HCL used in the sample titration.

B= is the volume of the standard solution used in the blank titration.

N = is the normality of standard H_2SO_4

d) Carbon Hydrogen Nitrogen Ratio (CHNS)

The carbon to hydrogen to nitrogen ration of the sample fleshing wastes was determined by dividing the percentage of carbon content to the percentage of hydrogen to the percentage of nitrogen content to percentage of sulphur content of the samples. The samples were then analysed for the content of N_2 (expressed as % N_2). The technique involves combustion of test sample in an oxygen rich environment. The products of combustion in a CHNS analysis (CO_2, H_2O, N_2 and SO_2) are carried through the system by helium carrier gas. The combustion products are measured quantitatively by means of a non-dispersive IR absorption detection system, except for the N_2 which is determined via a thermal conductivity detector (TCD).

3.3.2 Extraction of Fat

Soxhlet method was employed for the extraction of fat from the fleshing. The fleshing was de-limed, washed and dried in an oven. Measured quantity of the dried fleshing sample was placed in a thimble or bag filter paper inside soxhlet glass chamber of the extractor. The extraction was carried out using the electrical manual setup Soxhlet apparatus was arranged as show in Figure 3.1. The extractor was connected to 6 pieces of soxhlet beakers and condenser. The solvent was poured on the top of the condenser. The

sample was dipped in the solvent. Then the extraction program was set as given in Table 3.1. For this experiment taken 10 and 40 grams of dried powder and wet fleshing samples, respectively were taken and dichloromethane was used as the solvent. The entire extraction process was carried out for approximately 5 hr. After the completion of extraction, the excess solvent present in the beaker was separated from the fat and reuse for the next batch process. The fat extract was characterized for fat content, pH, acid value, saponification value, un-saponifiables, iodine value, density and viscosity.

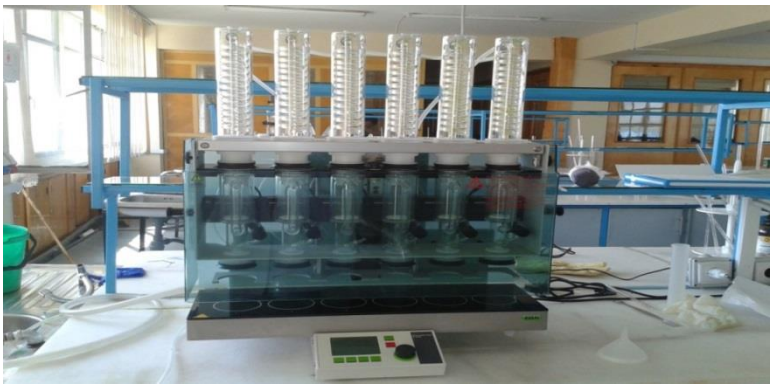


Figure 3.1: Fleshing waste oil extraction manual electrical setup apparatus

Table 3.1: Manual setup of electrical Soxhlet apparatus programme setting condition

Programme types	*Value setting*
Position occupied by extractor beaker	*6*
Extraction cycle	*30*
Rinsing time	*30min*
Temperature of extraction for DCM	*85 ^{o}C*
Rinsing Temperature	*85 ^{o}C with 20 min.*
Drying Temperature	*80 ^{o}C-90^{o}C at 3 ^{o}C/min.*
Drying Time	*40 min.*

3.3.2.1 Chemical Characterization of Extracted Fleshing Fat/Oil

a. Determination of fat extracted

Extract fat/oil content was determined from gravimetric analysis. The samples were weighted in digital weighting balance apparatus. The method followed for analysis was: fat was obtained according to SLC 319 (ISO 2/14) from the skins/hides fleshing wastes.

- ❖ The empty beaker were weight and this weight was recorded,
- ❖ The beaker with the extract was weighted and recorded, and
- ❖ The difference in weight between the first measurement and the last gives the weight of the extract oil.

The maximum yield of fleshing wastes is determined under this formula. Its help for calculation of fat amount present in each types of fleshing wastes. [78]

$$\text{Fat yeild } \%\text{ by wt} = \frac{W2-W1}{W} * 100$$

Where: W_1= weight of the extraction flask (g)

W_2 = weight of the extraction flask plus the dried crude fat (g)

W = weight of sample (g)

b. Acid value (Acid Number)

The acid value (*AV) is* the number that expresses, in milligrams the quantity of potassium hydroxide required to neutralize the free acids present in 1 g of the substance. The acid value may be overestimated if other acid components are present in the system, *e.g.* amino acids. The acid value is often a good measure of the breakdown of the triacylglycerol into free fatty acids, which has an adverse effect on the quality of many lipids. The acid value of the extracted oil was obtained according [79] method.

$$RCOOH + KOH \longrightarrow KOH + KOH$$

Figure3.2: Reaction of extracted oil with KOH for determination of acid value.

Significance:-Acid value is the measure of hydrolytic rancidity. [Annex 4a] containing the procedure of acid value of fleshing waste.

$$AV = \frac{ml\ of\ KOH\ x\ Nx\ 56.1}{Weight\ of\ sample} = mg\ of\ KOH$$

N = Normality of KOH

% Free Fatty Acid (FFA) = AV x 0.503

Calculate the acid value (AV) and free fatty acid (%FFA) using above laws.

c. Saponification Number according to [80] method.

The saponification value is the number of mg of potassium hydroxide required to neutralize the free acids and to saponify the esters in 1 g of the substance. The saponification number is a measure of the average molecular weight of the triacylglycerols in a sample. Saponification is the process of breaking down a neutral fat into glycerol and fatty acids by treatment with alkali. The smaller the saponification numbers the larger the average molecular weight of the triacylglycerols. Saponification value is inversely proportional to the mean molecular weight of fatty acids (or chain length) the procedure explained on [annex- 4b].

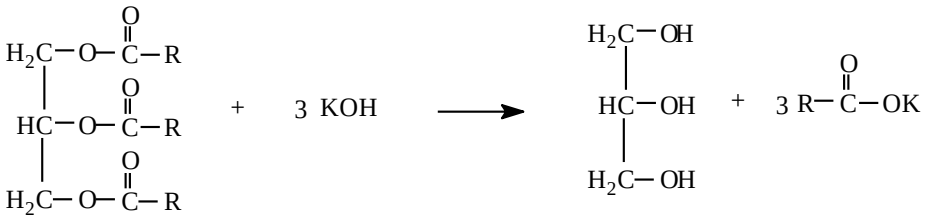


Figure 3.3: Reaction of extracted fat with KOH for determination of saponification value.

Fats (triglycerides) upon alkaline hydrolysis (either with KOH or NaOH) yield glycerol and potassium or sodium salts of fatty acids (soap)

Calculate the saponification number by using the following law:

$$SP\# = \frac{56.1\ (B-S)x\ N\ of\ HCl}{gram\ of\ sample}$$

Where: - B = ml of HCl required by Blank.

S = ml of HCl required by Sample.

Ester Value: - The ester value is defined as the mg of KOH required to react with glycerin (glycerol / or glycerin) after saponify one gram of fat. It is calculated from the saponification Value *(SV)* and the acid Value (AV):

Ester Value (EV) = Saponification Value (SV) – Acid Value (AV)

d. Iodine Value (I.V) according to [81] method.

The iodine value (*IV*) gives a measure of the average *degree of unsaturation* of a lipid: the higher the iodine value, the greater the number of C=C double bonds. By definition the iodine value is expressed as the grams of iodine absorbed per 100g of lipid. Iodine value (I.V.) is directly proportional to the degree of unsaturation (No of double bonds.) and inversely proportional to the melting point (M.P.) of lipid. An increase in I.V. indicates high susceptibility of lipid to oxidative rancidity due to high degree of unsaturation. The procedure explained on the [annex 4c]

Calculate the iodine number by using the following law:

$$\text{Iodine Value} = \frac{(B-S)\ x\ N\ of \text{Na2S2O3 x 0.127 g/meq}}{Weight\ of\ sample\ (g)} \text{X } 100$$

Where: - B: V ml of $Na_2S_2O_3$ volume for blank

S: V ml of $Na_2S_2O_3$ volume for sample

e. Un-saponification value: - Determined the amount of un-saponification present in the fat liquor determined according to the [82] method. This method determines the materials in oil which cannot be saponified and which are not easily volatile. The procedure explain [annex-4d]

$$\text{Un-saponification matter (\%)} = 100\ X\ \frac{w1}{w}$$

Where W_1 = weight of residual

W= weight of sample taken

3.3.3 Preparation of Protein Hydrolysate

Alkaline Hydrolysis of fleshing waste

In this research alkaline hydrolysis method by sodium hydroxide in the autoclave apparatus shown in Figure 3.4 was employed. Firstly, fleshing of sheep, goat and cow hide were cut in to small pieces and weighted. 100 g of fleshing were put in 500 ml of beaker and 150 ml of 5% sodium hydroxide was added to the weighed samples. The solution was sealed and put in to autoclave apparatus for hydrolysis of the fleshing with the set temperature (75 and 85 ^{o}C), time (2 and 4hr), and pH (10 and 12) for different samples. The hydrolysate solution was cooled and vacuum filtered for separation of the residue and the supernatant from impurities. The fluid consists of amino acids, small peptides, soap, oil and possibly some minor lime residues. Solid content of the fleshing hydrolysate was determined. Solid content determination was useful to calculate the active matter and degree of hydrolysis. The fleshing hydrolysate was neutralized carefully with 1N sulphuric acid. Water based hydrolysis of the fleshing was also carried out with the same time and temperature and used as control. The fleshing hydrolysates were taken for various analyses and for leather application.

Figure 3.4: Autoclave apparatus for fleshed waste hydrolysis

Homogenization of fleshing waste

The fleshing from different raw materials were chopped, dried and ground as powder form. The dried samples were then put in measured quantity of water and then homozenized in the homozenizer, shown in Figure 3.5. This homogenized sample was then analysed for various parameters as given below.

Figure 3.5: Powdered fleshing waste Homogenized mechanisms

3.3.3.1 Characterization of Fleshing Hydrolysate

Solid Content of the fleshing Hydrolysate

About 5 grams of filtered supernatant was taken in a porcelain crucible and heated in an electrical oven till it gets dry. The crucible was then transferred into the hot air oven and dried for 3 hours at 105 ^{o}C, until getting constant weight. The weight of the dried sample was taken after cooling the crucible in a desiccator. Same procedure was carried out to find the solid content of the residual part of fleshing hydrolysate.

$$\text{Total solids} = \frac{(A-B)x\ 100}{Sample\ volume\ (ml)}$$

Where A = weight of (dish residue + dish) (mg)

B = Weight of the dish (mg)

Measurement of Particle size distribution: - Particle size distributions of the samples were measured by using the integrated of dynamic Light Scattering (DLS) Instrument (Nanosizer, Malvern particle size Analyser). DLS, also known as photon correlation spectroscopy (PCS) uses time-depended intensity fluctuations of scattered laser light caused by Brownian movement of particles in the nanometre size range to derive via an autocorrelation function a diffusion coefficient of the particles. Via the Einstein-Stokes relationship the hydrodynamic particle diameter is calculated. DLS normally calculates also a polydispersity index (PDI) as a measure for the width of the particle size distribution. The smaller the PDI the more homogeneous the particles are distributed in

the sample [83, 84]. The chamber sample dilution was in to 1:1000 (sample / water) and the process carried out at 25^{o}C. Relative refractive index and absorption were set to be 1.330 and 0.000, respectively. Toluene was used as a blank. The average particle size reported by intensity (volume surface weighted diameter) and volume percentage were recorded.

Measurement of zeta potential: - Zeta potential is a scientific term for electrokinetic potential. The zeta potential is a key indicator of the stability of colloidal dispersions. The magnitude of the zeta potential indicates the degree of electrostatic repulsion between adjacent, similarly charged particles in dispersion. The zeta potential was measured using Nanosizer, Malvern particle size Analyser. [83, 84]

Table 3.2: The particle size and Zeta potential apparatus setting condition

Properties	*Value*
Material RI	*1.30*
Absorption	*0.00*
Dispersant	*Water*
Dispersant RI	*1.33*
Viscosity(cP)	*0.8872*
Temperature(^{O}C)	*25*
Measurement position (mm)	
Cell description	*Glass cuvette with square*
Cell description for Zeta	*Zeta dip cell*

Determination of Molecular weight [85]:- The count rate and solvent refractive index calculated from a toluene reference standard as well as the sample concentrations and time averaged scattering intensities are entered into the Molecular Weight Calculator.

Table 3.3: The Molecular weight setting condition

Properties	*Value*
For Toluene RI(25^{O}C)	*1.4960*
Standard Raleigh ratio Rθ (cm^{-1})	*1.35e-5*
Standard interest (kcp)	*726*
Solvent RI(25^{O}C)	*1.33*
Solvent interest (kcp)	*79*

dn/dc	*0.0100*
Temperature	*25*
Correlation coefficient(R^2)	*0.803*
Shape correction	*0.00*
Cell description	*Glass cuvette with square*

Viscosity: - The viscosity is measure of the fluid resistance to shear or flow and is a measure of the adhesive/cohesive or frictional fluid property. The resistance is caused by intermolecular friction exerted when layers of fluids attempt to slide by one another [86]. There are two related measures of fluid viscosity - known as dynamic (or absolute) and kinematic viscosity.

$$\eta = \mu / \rho$$

Where η = kinematic viscosity

μ = absolute or dynamic viscosity

ρ = density

Pour some sample in a beaker. Insert one of the spindles in the Brookfield and put the end of the sample in the liquid. The rpm, spindles number was used a reading mid-scale at room temperature 100, L1 and 25^{O}C respectively. The particular spindle (with its specific geometry) must be noted.

Determination of degree of hydrolysis: The degree of hydrolysis was estimated by taking 10 grams of fleshing hydrolysate and 10 grams of residue after hydrolysis of fleshing. Weighed china crucible dish put the two samples in two different dishes kept in oven for drying at 102^{o}C for 3 hr. and until to get constant weight.

$$\% DH = AM\% - RC\%$$

Where: - AM% = active matter present in hydrolysate liquor.

RC% = residual content in hydrolysate

3.3.4 Preparation of Fatliquor cum Filler Agent

The optimized process parameters were used to extract fat and the protein hydrolysate from the fleshing. The fat and the hydrolysate from the optimized process were used for

the preparation of fatliquor cum filing agent. There are so many factors, which needs to be considered for the preparation of fatliquor, they are

- Order of addition of the raw material
- Ratio of emulsifier: extracted fat: fleshing hydrolysate
- Selection of emulsifiers
- Type of fleshing hydrolysate: Alkali hydrolysis and homogenized
- Temperature and reaction time

Two types of product based on alkali hydrolysed fleshing and homogenized fleshing was prepared.

3.3.4.1 Fatliquor from Alkali hydrolysed Fleshing Wastes

Synthesis of FH_{SDS} (A): - 30, 40, 50 grams of extracted fat was taken in the beaker and it was melted by heating it to a temperature of 60- 70^{o}C. The temperature was controlled by means of a thermostat. Once the fat completely melts and the flow characteristics improve, the stirring is started. Then, different volumes (15, 30 and 40 ml) of 5% solution of SDS (anionic emulsifier) were added to the molten extracted fat slowly till a homogeneous mass is obtained. Then the calculated quantity (35, 100 and 108 ml) of the fleshing hydrolysate was added to the emulsion and stirring was continued for further 15 minutes at a temperature 70 ^{o}C and pH was adjusted to 5-6. This product after cooling was used as a retanning agent during leather process. The resultant emulsion was very fine and homogeneous when dispersed in water.

Synthesis of FH_{M7} (B): - 30, 40, 50 grams of extracted fat was taken in the beaker and it was melted by heating it to a temperature of 60- 70^{o}C. The temperature was controlled by means of a thermostat. Once the fat completely melts and the flow characteristics improve, the stirring is started. Then, different volumes (15, 30 and 40 ml) of M7 (anionic emulsifier) were added to the molten extracted fat slowly till a homogeneous mass is obtained. Then the calculated quantity (35, 100 and 108 ml) of the fleshing hydrolysate was added to the emulsion and stirring was continued for further 15 minutes at a temperature 70 ^{o}C and pH was adjusted to 5-6. This product after cooling was used as a

retanning agent during leather process. The resultant emulsion was very fine and homogeneous when dispersed in water.

Synthesis of $FH_{SDS\ with\ M7}$ (C) : - 30, 40, 50 grams of extracted fat was taken in the beaker and it was melted by heating it to a temperature of 60- 70^{o}C. The temperature was controlled by means of a thermostat. Once the fat completely melts and the flow characteristics improve, the stirring is started. Then, different volumes of 5% solution of SDS and M7 (anionic emulsifier) were added to the molten extracted fat slowly till a homogeneous mass is obtained. Then the calculated quantity (35, 100 and 108 ml) of the fleshing hydrolysate was added to the emulsion and stirring was continued for further 15 minutes at a temperature 70 ^{o}C and pH was adjusted to 5-6. This product after cooling was used as a retanning agent during leather process. The resultant emulsion was very fine and homogeneous when dispersed in water.

3.3.4.2 Fatliquor from Water Based Homogenized Fleshing

The similar procedure as mentioned in Section 3.4.1 was followed for preparing the product. The only difference is instead of alkaline hydrolysed fleshing, water based homogenized fleshing was employed.

3.4.5 Characterization of Fatliquor cum Filler

3.4.5.1 Stability of Fat liquor cum Filler

The stability of the emulsion is one major thing to be taken into account. The emulsions are always thermodynamically unstable and the stability is maintained by the equilibrium between the opposite forces. Some of the forms of instability observed in the emulsions are

Breaking: - Breaking is the spontaneous joining of small droplets in the emulsion to form larger ones, leading ultimately to two separate liquid layers

Flocculation:- Flocculation is the sticking together of individual droplets in the forms of three dimensional clusters without the coalescence of the individual droplets, whereas creaming is bound to occur in any dilute emulsion where the phases are not equal in density and is the trivial form of instability [87 & 88].

The product was tested for its stability towards temperature, acids and alkalies. In addition to that the stability was checked by adding small drop of the product in to water and checked for any variation in colour.

3.4.5.2 Determination the Active Ingredient of the Final Product

Total Fatty Matter (TFM) is one of the most important characteristics describing the quality of fat liquor cum filler products and it is always specified in commercial transactions [89]. The procedure explained on annex 5.

$$\text{Free oil, in percentage (A)} = \frac{F}{W} \text{ X } 100$$

$$\text{Emulsifier in percentage (B)} = \frac{E}{W} \text{ X } 100$$

Where: - F= weight of petroleum ether soluble in g,

E= weight of alcohol soluble in g, and

W = weight of the fat liquor sample in g.

Total active ingredient in present = A + B

3.4.5.3 Determination of Total Alkalinity of Fatliquor cum Filler

The requirement apparatus for carried out this properties conical flask and burette graduated. The alkalinities results can be calculated using the formula below: [90]

$$\text{Total alkalinity in ml 0.1N per grams of oil} = \frac{t \ x \ 5}{w} = \text{A}$$

Where: t = ml 0.5 N sulphuric acid require

w = weight of sample taken

The detailed procedure for total alkalinity determination is provided in Annex 6.

3.4.6 Application of Fatliquor cum Filler in Leather Retanning Process

Wet blue goat leathers were taken and treated with the prepared fat liquor cum retanning product for preparing upper (0.9±0.1 mm) and garment (0.6±0.1 mm) leather. The process was carried in LIDI Model tannery. Two wet blue leathers processed using conventional retanning and fatliquoring agent and were used as control leathers (T_1) for upper and

nappa leather. The process recipe is provided in Annex 8 and 13. Two wet blue leathers were processed in to upper and nappa leather using the developed product (T_2) (Annex 9 &12), respectively. Two wet blue goat leathers were processed in to upper leathers using the developed product in admixture with the commercial fatliquor and retanning agents (T_3) (Annex 10).

3.4.7 Measure the Exhaustion of Fatliquor cum Filler

The percentage of materials given recipe in Annex 8-10 were based on the shaved weight of wet blue goat leathers. The spent liquor was collected for determining of exhaustion of the products. It was done based on visual assessment.

3.4.8 Physical Test of Leather

After the leathers are produced, it is necessary to test them to assess whether they will serve the ultimate purpose. As the properties of leather are affected by atmospheric temperature and varying humidity and as in the same place in different seasons of the year and the hour of the day, it is essential to conditions the leather, prior to testing, in a room under controlled conditions. The condition specified by the Indian Standard Specification are 20±2 ^{o}C and 60% R.H.±2 (R.H. = relative humidity) over a period of 48hrs. For leather this conditioning procedure is defined in ISO 2419 test method [91].

The conditioned samples are tested for various properties. The analyses for resistance were: tensile strength, elongation at break and tear load, in parallel (//) and perpendicular directions (⊥), the samples were analysed parallel and perpendicular to the dorsal line. The principles involved in testing various properties following with Official Standards methods are given below.

3.4.8.1 Tensile Strength

It is the load per unit area of cross-section required to pull apart or break a strip of leather. A dump- bell shaped sample is punched out using a steel die of standard and dimension of 110 mm total length with 25 diameter but the machine used for measuring of tensile strength test only 50 mm between the two edged dump shape: this particular shape aids in concentrating the stresses in the narrow portion forcing sample to break there or otherwise the sample may break near the jaw of the machine and this will give a wrong value. The thickness of the specimen is measured at three equidistant points on the straight portion of the specimen using a thickness

gauge and the average calculated. The width is measured using a graduated scale correct to 0.02 inch, at the corresponding positions, and these values are used in calculating tensile strength of the sample, cutting the crust leather 50 mm. [92]

Tensile strength (N/ mm^2) or (Kg/cm^2) = $\frac{Breaking\ load\ in\ (kg)}{Thickness(cm)\ X\ width(cm)}$ or

$$= \frac{Force(N)}{Area\ (\text{width in mm X Thickenss in mm})}$$

3.4.8.2 Elongation

The percentage elongation of leather is also a useful index of the stretching quality in many cases. The elongation is measured simultaneously with the measurement of tensile strength. Two reference marks are made in the narrow portion of the specimen before testing and the distance between these points is measured. The elongation also measure simultaneously with the tensile test of sample. The extension can be expressed as the percentage elongation at that load. [92]

$$\%\ \text{Elongation at break} = \frac{L2-Lo\ X100}{Lo}$$

Where: -L_2 = Initial distance between the jaws in mm

L_o= final distance between the jaws in mm

3.4.8.3 Tearing Strength

This is the load required to continue a tear in leather, once started. Tear strength is also an important bulk property test. This test is the most preferable test for leather than tensile strength by many of the customers including BS EN ISO 20345 Safety shoe standards. There are two types of tear strength tests for leather material, for this research used Double edge tears strength - Baumann Tear strength. The leather cutting 70mm x 40mm size with central cut parallel to the longer edge to 50 ml length to form two legs [93].

Record the maximum force. Continue the test for remaining test specimen.

Tear strength = Maximum tear force (N)

Thickness (mm)

3.4.8.4 Flexing Endurance

Test pieces were cut from the leather 45X 70mm and placed on a flexometer (based on ISO

5402:2002). Choosing at least three samples of different parts of the leather and placing static test specimen on the temperature at 20 ℃ and Relative humidity of 60% can be measured when time is over 48hr.Two ends of the leather specimen were folded and gripped on one end, and then subjected to 25000 flexes, after which any sign of cracking or peeling was observed. [94]

3.4.8.5 Lastometer (Bursting Ball Test)

Aim of this test method is intended to determine the grain crack force and distension of leather when used for shoe upper. These can be defined in [95]. Shoe upper leather often shows slight crack in the toe area at the time of lasting operation in spite of the leather has good tensile and tear strength properties. This is due to weak grain surface characteristics of leather by more filling and loading of tanning and retanning materials in the grain side. To determine the grain crack distension and load when a circular leather test specimen is secured in between two circular rings of 25 mm diameter and stretched with the help of a spherical head. Unto grain crack appears on the grain surface of leather.

3.4.8.6 Colour Rub Fastness Dry Base

Test pieces were cut from the crust leather 75mm$^{2.}$ Small damp cotton swabs were placed on a die which was fitted to the rub fastness tester [96]. The leather specimen was gripped on the lower platform and subjected to 50,100,150 rub run. The specimens of colour degree subjected to the rub fastness test before and after staining on the white pelt were done using ISO grey scale for colour change and staining.

3.4.8.7 Fat liquor cum Filler Effect on Organoleptic properties of leathers

Organoleptic feelings of colour-fixed leathers were determined by experienced technician/experts on the leather and the fixing capacities were determined through the colour intensity. The softness, fullness, round ness, feel, colour uniformity and colour intensity of fat liquor cum filler leather might change

The leather handle and Fullness is made use of contrast-scoring, scores from 1 ~ 10 minutes by touched the crust leather in every side. Control samples and test samples were evaluated by this method. For the above all physical testing sample cutting was carried out by (BS-3144 IUP-1/EN ISO2419: 2006), but for tensile strength, elongation and tear strength tests were required the measuring of thickness by [97].

3.4 Statistical Analysis

Result of fleshing waste hydrolysate analyses were compared with each fleshing types and the results of all physical testing were statistically evaluated using ANOVA. All experiments were carried out in triplicates. All data were stated as mean $\pm$ standard deviation Analysis of variance (ANOVA) at 5% level of significance was used to compare the hydrolysate of each fleshing among all factors affecting the hydrolysate process. The result were analysed using statistical software SAS.

CHAPTER FOUR

4. Results and Discussion

4.1 Characteristics of Fleshing Waste

I. Moisture content

The pH of the limed fleshing used for the experiments was found to be 12.0. Determinations of moisture content of the fleshing wastes for all species has been determined and tabulated in Table 4.1. The average moisture content of fleshing from cow hide, goat and sheep was found to be 64.88, 56.64 and 63.3% respectively. Fleshing obtained from sheep skins showed higher moisture content.

Table 4.1: Moisture content determination for sheep and goat and cow hide lime fleshing

Sample code	weight of LF (Lime fleshing) gram	Moisture content(MC)	Moisture content(MC) %
SLF_1	10.09	5.7109	76.7
SLF_2	10.02	6.0213	59.9
SLF_3	10.00	5.7689	73.34
SLF_4	10.02	6.2389	60.61
SLF_5	10.03	6.5201	53.83
average	10.04		**64.88**
GLF_1	10.02	6.6423	50.85
GLF_2	10.01	6.1200	63.56
GLF_3	10.09	6.4880	55.5
average			**56.64**
HLF_1	10.00	6.5023	53.79
HLF_2	10.01	6.2398	60.42
HLF_3	10.01	5.6975	75.69
average			**63.3**

II. Nitrogen content

The nitrogen content of the fleshing sample for each species (dry based) were determined and shown in Table 4.2. The hide fleshing had the maximum nitrogen content of 12.59% when compared with other fleshing types.

Table 4.2 Nitrogen content of fleshing wastes

Sample code	Weight of sample (grams)	Nitrogen content (%)
$SLF_{@\ 70\ ^oC}$	2.0048	8.3006
$SLF_{@\ 70\ ^oC}$	2.0046	8.0262
$SLF_{@\ 102\ ^oC}$	2.0019	8.9866
$SLF_{@\ 102\ ^oC}$	2.0050	9.3296
average	2.0041	**8.6608**
$GLF_{@\ 70\ ^oC}$	2.0020	10.2900
$GLF_{@\ 70\ ^oC}$	2.0020	10.5644
$GLF_{@\ 102\ ^oC}$	2.0012	10.7016
$GLF_{@\ 102\ ^oC}$	2.0082	11.0208
Average	2.0034	**10.6442**
$HLF_{@\ 70\ ^oC}$	2.0085	12.4166
$HLF_{@\ 70\ ^oC}$	2.0086	12.0736
$HLF_{@\ 102\ ^oC}$	2.0016	13.0340
$HLF_{@\ 102\ ^oC}$	2.0010	12.8282
average	2.0049	**12.5881**

The % nitrogen content was used to calculate the protein content present in fleshing. From the results obtained, the nitrogen content of the fleshing from different raw material source such as cow hide, goat and sheep skin was calculated to be around 64.96, 54.90 and 44.69%, respectively. Hence, it could be observed that the fleshing from cow hides have more protein content.

III. Carbon to Hydrogen to Nitrogen to Sulphur ratio (CHNS)

The carbon to hydrogen to nitrogen ratio (CHN) is very important factor to prepare value added product from fleshing waste by mixing with other modified chemicals for leather industry itself. The obtained laboratory result was shown in Table 4.3, for all species of fleshing types. For sheep fleshing waste (dry base) was found the C: N ratio higher than when compared to other fleshing waste types. The reason was due to high carbon present in sheep fleshing, if it is the lower ratio of C: N had high nitrogen content it's was refers the hide fleshing wastes. The same results seen in the ratio of C: H.

Table 4.3: CHNS content of fleshing wastes

Sample code	Weight (mg)	N%	C%	H%	S%	C/N ratio	C/H ratio
SLF	4.75	2.4533	42.5405	6.309	0.0830	17.3398	7.0537
GLF	4.75	3.0512	30.8965	5.230	0.0792	10.1260	5.9076
HLF	4.75	4.1051	22.9567	4.589	0.0785	5.5922	5.0025

From the above table a basic understanding on the C: N could be obtained. Higher C:N ratio means the protein content is high. Hence, from the Table it could be observed that the cow hide fleshing had the higher protein content and sheep skin fleshing had the lowest protein content. Thus cow hide fleshing would be the most suitable product to prepare the fatliquor cum retanning agent.

4.2 Identifying Maximum Yield of Oil Extraction from Specific Fleshing Wastes

4.2.1 Determination of Fat content and yield

The fat content of various green and limed fleshing from different raw materials are determined and tabulated in Table 4.4. Dichloromethane was used as the solvent for fat extraction. It could be observed that the green fleshing from sheepskin had the maximum fat content of 23.88%. Other limed fleshing had lower fat content. It is obvious that during liming process, the fat gets saponified and hence the lowering of fat content in the limed fleshing. However, limed sheep fleshing had the higher fat content of around 12.29%. The goat and cow hide limed fleshing had fat content of 5.09 and 4.83%, respectively.

Table 4.4: The amount fat content in percentage of fleshing waste of each species

Sample code	Fleshing weight(grams)	Oil weight in grams	Fat content (%)
SGF_1	10.0421	3.9876	39.70
SGF_2	10.0119	4.1065	41.02
SGF_3	10.6048	3.2793	30.93
Average	10.2196	3.4578	**23.88**
LSF_1	20.00	2.6820	13.41
LSF_2	20.00	2.9093	14.55
LSF_3	20.00	1.7852	8.93
Average	20.00	2.4588	**12.29**
LGF_1	20.00	1.0359	5.18

LGF_2	20.00	0.9257	4.63
LGF_3	20.00	1.0901	5.45
Average		1.0172	**5.09**
LHF_1	20.00	0.9871	4.94
LHF_2	20.00	1.0023	5.02
LHF_3	20.00	0.9079	4.54
Average	20.00	0.9658	**4.83**

The consolidated results obtained from the % fat estimation are depicted in Figure 4.1.

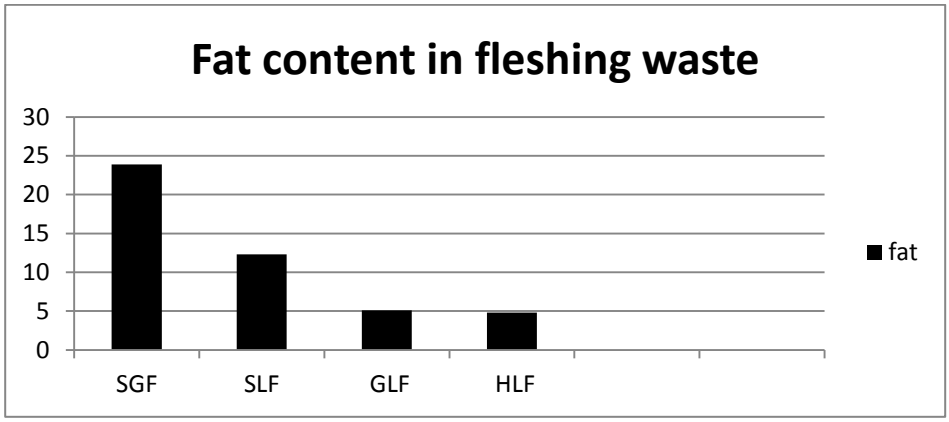


Figure 4.1: The %fat yield present in different fleshing waste.

It could be observed that green fleshing, which is devoid of any chemicals would be a suitable material for preparation of the product of higher quality. However, most of the tanneries carry out only limed fleshing. Hence, it becomes necessary to clean the limed fleshing devoid of chemicals, so that a quality product can be obtained. Hence, in the present work, the limed fleshing was washed, de-limed and used for oil and protein hydrolysate extraction. The colour of the products obtained from Green and limed fleshing are shown in Fig. 4.2. It could be observed that the both the products showed similar colour properties. Hence, limed fleshing was used for further experiments.

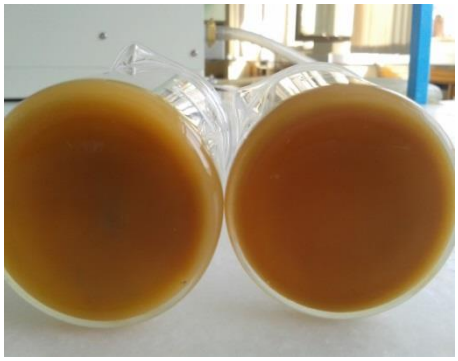


Figure4.2: The colour of the extracted Oil from fleshing waste of two types of source.

4.2.2 Extraction and Characterization of Fleshing Wastes Oil

a. Saponification Value

The main characteristics of oil from fleshing wastes is required for preparing the fat liquor cum filler agents, the saponification value of oil is a measure of the average molecular weight of the triacylglycerols in a sample. The smaller the saponification numbers the larger the average molecular weight of the triacylglycerols presents i.e. Saponification value is inversely proportional to the mean molecular weight of fatty acids was lower.

Saponification value of green and limed fat extracted fleshing for all species have been determined and tabulated in Table 4.5. The saponification value of sheep green fleshing ($SGF_{average}$) was 198.98, sheep limed fleshing ($SLF_{Average}$) was 193.07, for goat lime fleshing ($GLF_{Average}$) 214.185 and for hide limed fleshing ($HLF_{average}$) 228.890. From the above result shows the Hide Oil fleshing has shown higher molecular weight than the other type of fat fleshing. All results were falls under the standard value that is 180 -233. [98]

Table 4.5: Saponification value of each fleshing waste fat extracted

Sample code	Weight of sample (g)	Saponification value (ml.N/meq)	Sample code	Weight of sample (grams)	Saponification value (ml.N/meq)
SLF	2.0329	186.27	HLF	2.0370	229.960
SLF	2.0349	199.870	HLF	2.0315	227.82
	2.0339	**193.07**		**2.0343**	**228.890**
GLF	2.0627	217.580	SGF	2.0372	205.17
GLF	2.0626	210.790	SGF	2.0369	192.79
	2.0627	**214.185**	**SGF**	**2.0904**	**198.98**

Oils having saponification value "between" 180-233 have smaller molecules and so their penetration powers into the leather should be more, this in turn will improve the softness property of the final leather.

b. Acid Value

Acid value of the green and limed fleshing from different source of raw material was determined and the values are tabulated in Table 4.6.

Table 4.6: Acid value of limed fleshing the extract and water base hydrolysis Oil

Sample code	**Weight of sample (grams)**	**Acid value(AV_1)(mg)**
SGF_1	2.0069	8.65
SGF_2	2.0069	8.11
Average	**2.0069**	**8.38**
SLF_1	2.0070	8.82
SLF_2	2.0281	7.19
Average	**2.0176**	**8.01**
GLF_1	2.0039	6.99
GLF_2	2.0118	8.02
Average	**2.0079**	**7.51**
HLF_1	2.7347	4.35
HLF_2	2.5028	4.71
Average	**2.6188**	**4.53**

Acid value is a measure of rancidity. If the values are high, the fat or oil will become more rancid and vice versa. Fat or oil to be used for the preparation of fatliquor, it should be below 8.6 mg/g of oil. It is observed from the table that the average acid value for the fat extracted from green fleshing from sheep skin is 8.38 mg and that of limed sheep fleshing is 8.31 mg/g of oil. The acid value of goat limed fleshing and the cow hide limed fleshing is 7.51 and 4.53 mg/g of oil, respectively. From the obtained result, it is very clear that acid value of all the extracted fat/ oil is less than 8.6 mg/g of oil.

The % free fatty acid can be calculated based on acid value. The results are provided in Table 4.7, this can be calculated under the following formula Acid (FFA) = AV x 0.503

Table 4.7: the free fatty acid of extracted fat from fleshing wastes

Sample code	Average AV	% FFA_1
SGF	8.38	4.22
SLF	8.01	4.03
GLF	7.51	3.78
HLF	4.53	2.28

c. Iodine value

Table 4.8 shows the determined iodine value of fleshing fat. A low iodine number shows that the fat has a low quantity of unsaturated fatty acid [100]. All the samples showed lower iodine value indicating that the fat/oil had lower level of unsaturation and is suitable for making fatliquor and would give better lubrication. [101]

Table 4.8: Iodine value determination green and lime fat extracted fleshing wastes

Sample code	Weight of sample(g)	Iodine value (IV)
SGF	0.4060	63.81
SGF	0.4094	60.18
Average	**0.4079**	**61.995**
SLF	0.4091	59.60
SLF	0.4067	60.27
Average	**0.4079**	**59.94**
GLF	0.2502	56.85
GLF	0.2503	57.84
Average	**0.2503**	**57.35**
HLF	0.2514	58.09
HLF	0.2507	55.72
Average	**0.2511**	**56.91**

A low iodine number also indicates a high melting point and soft lubricating value of the fleshing fat. The iodine value is used to determine the degree of unsaturation of the fatty acids. The standard requirement for Fat/Oil is explained in Annex 7. Fats and oils are usually classified, on the basis of their iodine value as drying oil (125 - 181), semi-drying oil (85-128) and non-drying (8- 129) [55]. All results are falling in the range of 58.6 – 61.4. Hence, based on the iodine values of extracted fleshing fat, they can be called non- drying oils and all types of fat can be used for preparation of fat liquor cum filler. The chemical properties of the extracted fleshing fat are summarized in Table 4.9.

Table 4.9: Physico-chemical properties of extracted fat from fleshing

S.No.	Characteristics	Source of Oil			
		Sheep		Goat	Hide
		Green	Limed		
1	Colour	Light yellow	Light yellow	Light yellow	Light yellow
2	Acid value mg KOH/gm.	8.35	8.01	7.51	4.53
3	Iodine value	61.995	59.94	57.35	56.91
4	Saponification value	193.07	214.185	228.890	198.98
5	Free fatty acid %	4.22	4.03	3.78	2.28

4.3 Appearance and Factors of fleshing hydrolysates

During the hydrolysis, the fleshing wastes were rapidly converted from solid pieces of fleshing into a free flow liquid Fig.4.3 shows the fleshing hydrolysate after hydrolysis. The hydrolysis was carried out at different time, temperature and pH and the effect of those factors obtained are tabulated in Table 4.10.

Table 4.10: Characteristics of the hydrolysates involving various parameters

Sample code	Weight gram	5%NaOH Soln. (ml)	Hydrolysis time (hr.)	Hydrolysis temperature (^{o}C)	Colour	Observation after Hydrolysis
SLF_1	100.04	150	2	75/85	Grey brownish	Three layer formation (top oil/fat part, middle protein hydrolysate and bottom some unhydrolysed protein and unwanted lime sludge
SLF_2	100.03	150	4	75/85	Grey brownish	In this case have been shown less residual formation. The liquor more viscous when compared to 2hr Hydrolysates
GLF_1	100.06	150	2	75/85	Grey brownish	Three layer formation (top oil/fat part, middle protein hydrolysate and bottom some unhydrolysed protein and unwanted lime sludge
GLF_2	100.02	175	4	75/85	Grey brownish	In this case have been shown less residual formation. The liquor more viscous when compared to 2hr Hydrolysates
CLF_1	100.05	150	2	75/85	Grey brownish	Three layer formation (top oil/fat part, middle protein hydrolysate and bottom some unhydrolysed protein and unwanted lime sludge
CLF_2	100.61	150	4	75/85	Grey brownish	In this case have been shown less residual formation. The liquor more viscous when compared to 2hr Hydrolysates

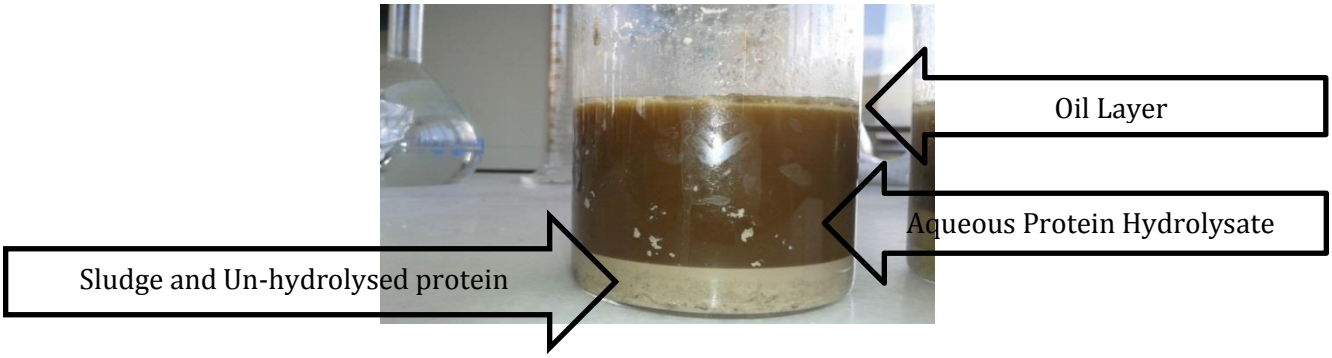


Fig. 4.3: Three layer of fleshing hydrolysates before filtration

After completion of hydrolysis the approximate estimation of the hydrolysed protein part, fat and the unhydrolysed residue were calculated and tabulated in Table 4.11. When 100 grams of fleshing waste was hydrolysed, 18.29 grams from $SLF_{2hr.}$, and 17.09 from $SLF4_{hr}$ of the fat was obtained. For 100g of goat limed fleshing, 9.65 and 8.43 g of fat was obtained after 2 and 4 hrs of treatment, respectively. In the case of cow hide fleshing, 2.36 g of fat was obtained after 4 hrs of treatment.

Table 4.11: After hydrolyses separated fat part on the top of the flesh hydrolysate

Sample	Filtered hydrolysate part	Top part/oil/fat(gram)	Sludge part (gram)
$SLF_{2hr.}$	121 ml	18.29	27.37
$SLF_{4hr.}$	160 ml	17.09	25.56
GLF_{2hr}	133 ml	9.65	35.69
$GLF_{4hr.}$	143 ml	8.42	33.23
$HLF_{2hr.}$	145ml	3.25	25.36
$HLF_{4hr.}$	160ml	2.36	22.69

4.3.1 Effect of Temperature on Hydrolysis

The effect of temperature on the hydrolysis of fleshing was determined by maintaining the temperature at 75 and 85^{o}C. The effect of temperature was not very significant as there was a slight difference in the yield of hydrolysate and the fat obtained at these two temperatures. Hence, the hydrolysis temperature of 75^{o}C was fixed as optimum temperature.

4.3.2 Effect of Time

The time of hydrolysis plays a vital role for hydrolysis of fleshing samples. The Figure 4.4 provides the samples of fleshing hydrolysate from different raw materials at 2 different time intervals. It could be observed that the sample after 2 hours of hydrolysis was viscous and was darker in colour. On the other hand, the fleshing sample was less viscous and lighter in colour after 4 hrs. Of hydrolysis. This could be attributed to the fact that with increased time of hydrolysis, more amount of protein got hydrolysed and might result in low molecular weight peptide and fatty acids.

A B C

Figure 4.4: Effect of time on the hydrolysis of fleshing waste by different temperature and pH of alkaline reagents a. SLF at 2 hr. and 4hr; b. GLF at 2hr and 4hr; c. HLF at 2hr and 4 hr.

4.3.3 Effect of pH

The pH of the solution during alkaline hydrolysis was maintained at 10 and 12. The results obtained at these 2 different pH's are depicted in Figure 4.5. It is clearly seen the product obtained at a pH of 12 was clearer than that obtained at a pH of 10. This might be due to enhanced hydrolysis at higher pH. As per the literature review [99], the hydrolysis of protein was higher at higher pH, which has been substantiated in this study.

Figure 4.5: The Image of fleshing hydrolysate at different pH value.

Annex 14 as shown full information about the hydrolysate effect parameter.

4.4 Characterization of fleshing hydrolysates

The effect of degree of hydrolysis (DH) on the physicochemical properties of fleshing hydrolysates was determined. Fleshing hydrolysates liquid samples were analysed for their active matter and other physicochemical properties.

4.4.1 Solid Content/ Active Matter

The active matter of the hydrolysed fleshing sample was calculated and tabulated in Table 4.12. Degree of hydrolysis is defined as the percentage of the total number of peptide bonds in a protein which have been cleaved during hydrolysis [100]. Hence, it could be observed that the increased duration of hydrolysis results in increased degree of hydrolysis.

Table 4.12: Active matter/ solid content present in the hydrolysed fleshing after different times of hydrolysis from different raw material sources.

No.	Sample code	Weight of sample (grams)	Solid content	Active matter for 100ml of hydrolysed fleshing wastes
1	$SLF_{2hr.}$	5.061	0.444	8.872
2	SLF_{4hr}	5.056	0.643	12.867
3	$GLF_{2hr.}$	6.350	0.515	10.300
4	GLF_{4hr}	6.332	0.632	12.637
5	$HLF_{2hr.}$	5.07	0.538	10.755
6	HLF_{4hr}	5.07	0.861	17.225

4.4.2 The Viscosity of fleshing hydrolysate

The viscosity of fleshing hydrolysate liquor of each fleshing types have different value for different time of hydrolysis. The determined viscosity has been tabulated in Table 4.13.

Table 4.13: Viscosity of the fleshing hydrolysate for different duration of hydrolysis

Sample code	Dynamic viscosity μ) cP or mPa.s	kinematic viscosity (η) (cSt)
SLF_{2hr}	3.85	34.9444
SLF_{4hr}	3.35	23.4958
GLF_{2hr}	4.27	37.6923
GLF_{4hr}	4.01	23.1658
HLF_{2hr}	4.57	29.9672
HLF_{4hr}	4.32	17.8660

It could be clearly seen from the Table that the, irrespective of the samples used, both dynamic and kinematic viscosity decreased with increase in hydrolysis duration. This might be due to the increased degree of hydrolysis with increased time of hydrolysis.

4.4.3 Particle Size Distribution and Zeta Potential of the Fleshing Hydrolysates

4.4.3.1 Determination of Particle Size Distribution

Particle size distribution (PSD) of the material is an important factor influencing the efficiency of value-added processing and a valuable indicator of quality and performance [101]. For this and many reasons, it is important to measure and control the particle size distribution of many products. In this experiment the particle size distribution result values was given based on intensity. The basic information for this experiment result was shown Table 4.14. Z-average size (also known as the "cumulants mean") only gives two values, a mean value for the size, and a width parameter known as the Polydispersity, or the Polydispersity Index (PDI). Peak means displays the size and percentage by intensity, for this research was obtained one and two peak value.

Table 4.14: Result of each fleshing wastes hydrolysed particle size distribution.

Particle Size Distribution Report by Intensity							
Sample detail	z-average (d.nm)	PDI	intercept	Peak	Size	% intensity	Standard deviation(d.n)
$HHL_{2hr.}$	789.2	0.500	0.879	1	780.2	78.9	154.2
$HHL_{4hr.}$	603.3	0.250	0.972	2	592.6 549.2	98.9 1.1	174.3 216.1
$GHL_{2hr.}$	1825	0.789	1.08	1	766.5	100.0	85.60
$GHL_{2hr.}$	1877	0.825	1.07	1	686.5	100.0	64.40
$GHL_{4hr.}$	2528	1.000	1.20	2	68.49 2.488	85.0 15.0	2.111 0.1824
SHL_{2hr}	2503	0.635	1.02	1	962.4	100.0	91.96
$SHL_{4hr.}$	1315	0.718	0.971	1	910.6	100.0	172.6
SHG_{2hr}	2512	0.589	0.956	1	792.3	75.3	160.3
$SHG_{4hr.}$	2756	1.000	0.913	2	75.73 5.075	71.4 28.6	4.948 0.4530

The Polydispersity Index is dimensionless and scaled such that values smaller than 0.05 are rarely seen other than with highly monodisperse standards. A value is greater than 0.7 indicate that the sample has a very broad size distribution. The various size distribution algorithms work with data that falls between these two extremes [88]. In this study PDI value of sample SHG_{2hr}, SHL_{2hr}.,SHL_{4hr}, $GHL_{2hr.}$, HHL_{4hr} and HHL_{2hr} were failed between the standard values, the rest samples were greater than 0.7

The y-intercept can be used to evaluate the signal-to-noise ratio from a measured sample and thus is often used to judge data quality. It is usually scaled such that an ideal signal will give a value of 1, and a good system will give intercepts in excess of 0.6, and greater than 0.9 for the best systems [101]. The intercept of these experiment samples was shown greater than 0.9 values so it's indicated the system was best.

4.4.3.2 Interpretation of Particle Size Distribution

The PSD results were also shown in graphical form Fig. 4.6.The graph below has been arranged using the dialogue to display a size distribution by Intensity graph as a histogram peak.

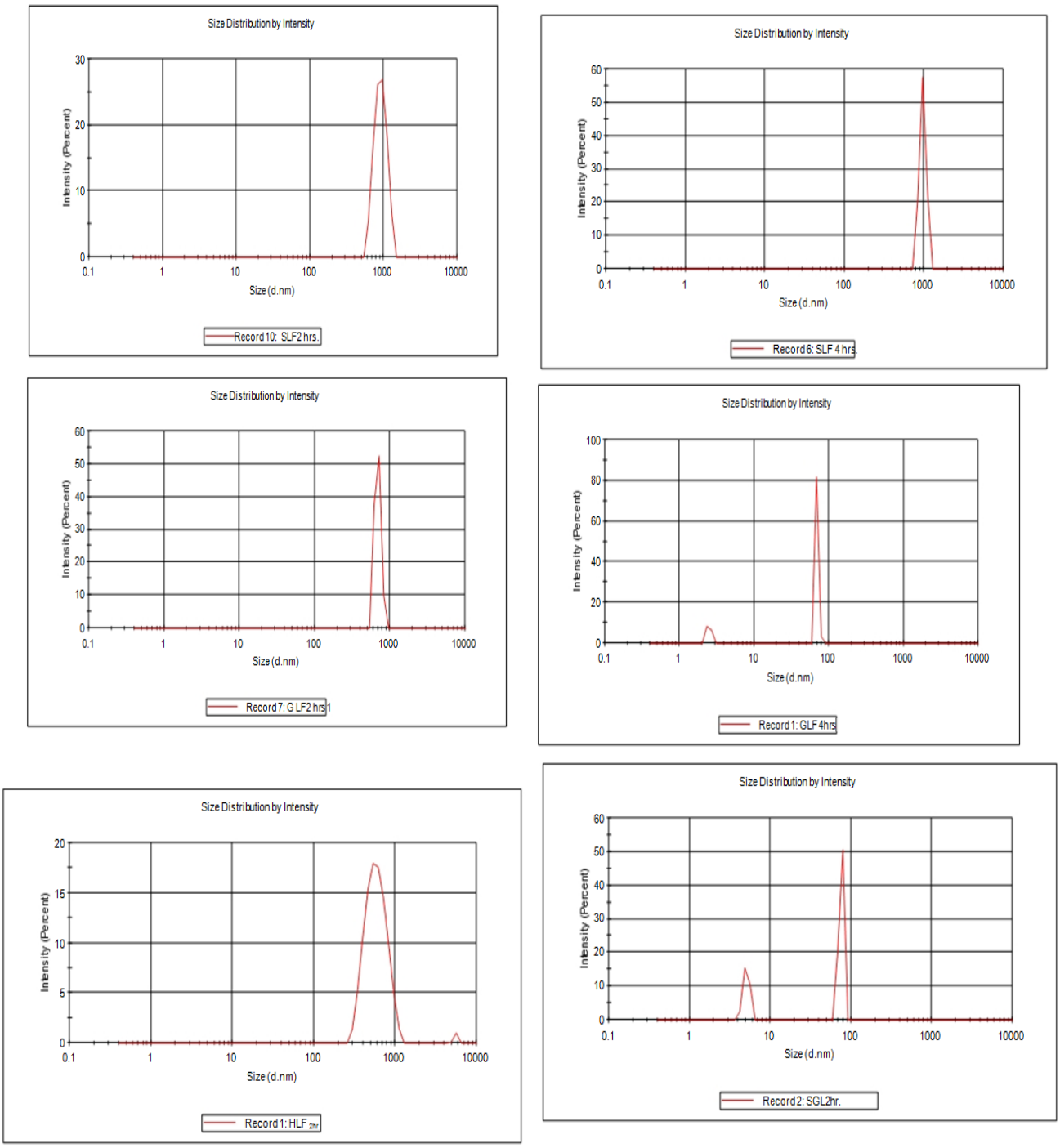


Figure 4.6: Graphical expression of each types of fleshing hydrolysates PSD

The recorded parameter include count rate, duration used, Z-average, PID, Interception, Peak number in graph, Size, % intensity and standard deviation. Moreover, the size in microns that splits the distribution with half above and half below as this diameter. The median has also been defined above as the diameter where half of the population lies below this value. The results of particle size distribution for each types of fleshing hydrolysates were found in Table 4.14 according to Figure 4.6. The two peaks of particle size distribution were found for hydrolysate obtained after hydrolysate of 4hr. The particle size distribution concentrated was shown in the regions of high peak, for SLF 962 to 5.075nm, GLF 766.5 to 2.488 nm and HLF 5492 to 592.6 nm . The 4hr hydrolysate particle size was lower than the 2 hr. hydrolysate. In the case of sheep, goat and hide shown different particle size at the same hydrolysate times, and Sheep and goat has lower particle size than the hide sample. The value of 2 hr. hydrolysate particle size also decreased significantly ($p< 0.05$) compared with those of the hide 4hr. hydrolysates sample. The hydrolysates of fleshing at 4hr had two peaks in the range of 68.49 - 2.488 for goat samples and 75.73- 5.075 for sheep green fleshing and for hide sample 592.6-5492. One peak of particle size distribution was fall in the range of 962.4n m – 686.3nm. The curve of particle size distribution gradually migrated to smaller particle sizes. It means that the particle size of fleshing waste hydrolyses decrease during alkali hydrolysis.

4.4.3.3 Interpretation of Zeta Potential Graph

Zeta potential is therefore a function of the surface charge of the particle and the nature and composition of the surrounding medium in which the particle is dispersed. Zeta potential is an important and very useful parameter in the study of the properties and performance of hydrolysate fleshing solution and colloidal particles in general. Zeta potential has values that typically range from + 25mV to – 25mV, when the zeta value above +25 and below -25 values will get higher degree of stability. The magnitude of the zeta potential is predictive of the colloidal stability. Zeta potential is an important tool for understanding the state of the nanoparticle surface and predicting long term stability of the nanoparticle [102].

Table 4.15: zeta potential of result of each fleshing hydrolysates at different times variation

Sample code	Measurement position(mm)	Zeta Potential (mV)	Conductivity (mS/cm)	Peak	Mean (mV)	Area (%)	Standard deviation (mV)
$HHL_{2hr.}$	4.65	-40.0	4.88	3.00	-37.9 -75.7 11.7	84.3 11.3 4.4	17.5 6.59 5.19
$HHL_{4hr.}$	5.03	-35.43	2.03	1	-33.9	86.2	18.6
$GHL_{2hr.}$	2.00	-563	603	0.00	0.00	0.00	0.00
$GHL_{4hr.}$	4.50	-39.8	9.78	0.00	0.00	0.00	0.00
SHL_{2hr}	4.50	-48.99	0.2988	1.00	-48.9	74.5	7.123
$SHL_{4hr.}$	4.50	-44.5	9.85	0.00	0.00	0.00	0.00
SHG_{2hr}	4.50	-41.2	10.3	0.00	0.00	0.00	0.00

Zeta potential is therefore a function of the surface charge of the particle (it is not, as is all too often erroneously stated, itself the surface charge) and the nature and composition of the surrounding medium in which the particle is dispersed. It is usually, but not necessarily, of the same sign as the potential actually at the particle surface [102, 103] but, unlike the surface potential, the zeta potential is readily accessible by experiment. Zeta potential has recognized to be extremely relevant to the practical study and control of colloidal processes and properties. In this study results shown Table 4.15 the zeta potential value was obtained negative charge that means the anionic charge more dominated in the system of the samples. In all samples case had been shows the zeta potential was demonstrated below -25mV, it was indicated the higher degree of stability. The conductivity value were indicated the ability of a samples to conduct electrical currents sample $GHL_{2hr.}$ have a higher conductivity to compared to other samples. This gave information of higher salt concentration present in sample $GHL_{2hr.}$ The zeta potential results were also shown in graphical form.

The hydrolysates fleshing was more dominated with anionic peptide charge, as mentioned in chapter two the protein part have anionic and cationic due to breakdown in to peptide during hydrolysis and the molecular size became to lower.

4.4.4 Molecular Weight of the Hydrolysate Fleshing [104-106]

Dn/dc**:** This is the differential refractive index increment; the change in refractive index as a function of the change in concentration. Second Virial Coefficient **(**SVC): A property is describing the interaction strength between the molecule and the solvent. Fit error: This is an indication of the quality of the measurement. The lower value of the fit error has the better the measurement.

Table 4.16: Molecular Weight DLS software for all hydrolysed fleshing wastes

Sample	MW (kDa)	A_2 (SVC) (mL mol/g^2)
$SHF_{2hr.}$	2.57e2+05	-6.75e-05
$SHF_{4hr.}$	15.4e+32	$2.41e \pm 6.90e-6$
$GHF_{2hr.}$	2.68e2+4	4.47
$GHF_{4hr.}$	16.0e+06	5.32
$HHF_{2hr.}$	18.5+45	4.62e+06
$HHF_{4hr.}$	6.19e+05	-5.64e+05

Using these values, a Debye plot is created as shown in Figure 4.7, with molecular weight (M_w) and 2nd Virial Coefficient (A_2) highlighted in red (Figure 4.8).

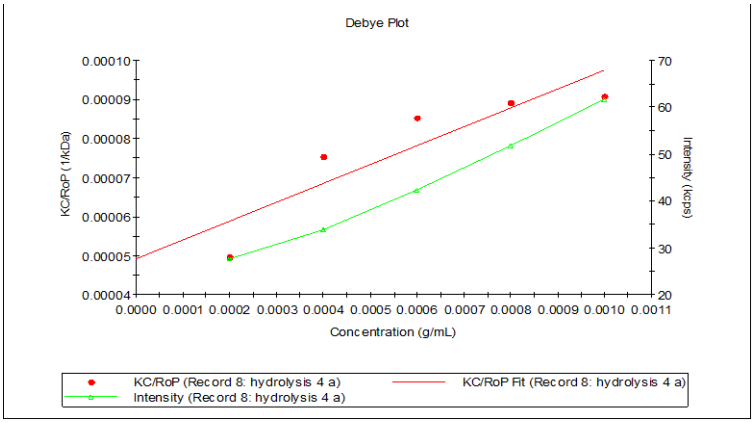


Figure 4.7: Debye plots of protein of fleshing hydrolysates solutions and a toluene reference

Molecular weight has been thought to be an important feature of fleshing hydrolysates. It was generally assumed that lower molecular weight polypeptides were more substantive to damage fleshing wastes. The study reported here shown Table 4.16 that peptides in the range of molecular weight2.68e2+4 to 6.19e+05 kDa are more substantive than the very high molecular weight polypeptides. Molecular weight of the system was smaller and can be suitable for making of the fat liquor cum filler products and it can be penetrated through the goat fibre porosity due to having the lower particle diameter when it compared with the goat fibre porosity.

4.5 Synthesis, Optimization and Characterization of Fat liquor cum Filler Agents

The product was prepared by combination of the hydrolysed protein, fat and emulsifiers. SDS and M7 are the two emulsifiers used in the study. The emulsifier concentration was varied to study the influence of emulsifiers on the product quality. It is observed from Table 4.17 that with increase in emulsifier concentration the stability of the product developed was better. M7 was seen to be a better emulsifier that SDS. The next experiment was use of SDS and M7 in combination. When both the emulsifiers were used, the products developed were more stable even with lower concentrations of emulsifiers. The pH of the final product was maintained at 5.5 to 6.0 by neutralizing with required amount of 1N sulphuric acid.

As shown in Table 4.17, sample A the formulation with 10 parts of oil, 25 parts of protein hydrolysate and 7 parts of 5% SDS solution as emulsifier gave a stable product, which was less viscous. Similarly, Sample B with the following formulation 10 parts of oil, 25 parts of protein hydrolysate and 5parts of M7 was more stable than sample A. In formulation of sample C, which was prepared using 15 parts of oil, 25 parts of protein hydrolysate and 3 parts of M7 and 5% SDS solution each gave a product showing the better stability when compared to the sample A and B formulation. And it has advantage based on economical by using small amount of emulsifier. The combination of the two emulsifiers has better emulsion. They give a very fine and uniform range of particle size. And also gave low viscosity.

Table 4.17: Synthesized and optimized the formation of fat liquor cum filler agent with ratio of Oil to Emulsifier to Hydrolysates liquor.

Sample code	Extracted oil in grams	Type of emulsifier in ml	4hr hydrolysate solution in ml	1N of Sulphuric acid(H_2SO_4)	pH	Appearance
Control	10	6	-	-	-	Stable and light brownish.
A	5	2, SDS	10	5	4.50	Viscous whitish in colour but not much stable. Separation of phases seen after 15 min.
	5	3, SDS	15	10	5.60	Whitish viscous and stable till 20 min
	10	5, SDS	20	15	5.50	High viscous and whitish with greater stability
	10	7, SDS	25	20	5.00	Less viscous
B	5	2, M7	10	5	5.00	Viscous whitish in colour and stable till 30 min. When checked the stability with small water added drop of water obtained blue colour
	5	3, M7	15	10	5.00	Stable product obtained
	10	4, M7	20	15	5.50	More viscose and more stable
	10	5, M7	25	20	6.00	Better product
C	5	2.SDS & 2,M7	10	5	5.00	The combination of emulsifier gave better products with improved stability and also was homogeneous.
	5	2SDS & 3,M7	10	5	5.5	
	10	3SDS &2,M7	10	5	6.00	
	15	3SDS &3,M7	25	5	5.30	

4.5.1 Stability of Products

The acid stability of the products A, B and C was measured and the results are provided in Table 4.18. The stability of sample A in acid media was for 3 hr without separation of oil. In the case of sample B stability was for 3:30 hr. without separation of oil and in samples C the stability was for more than 6 hours and better than products A and B. Hence, it could be concluded that the combination of 2 emulsifiers during the preparation of the products is required to get maximum stability. The pictorial representation of the stable products is provided in Fig 4.8 All the emulsion shows considerable and improved stability comparable to control sample.

Table 4.18: Stability test in acids

Sample	Stability in hours
A (emulsifier 6 %)	3
B (emulsifier 8 %)	3:30
C (emulsifier 10 %)	6
Control synthetic fatliquors	7

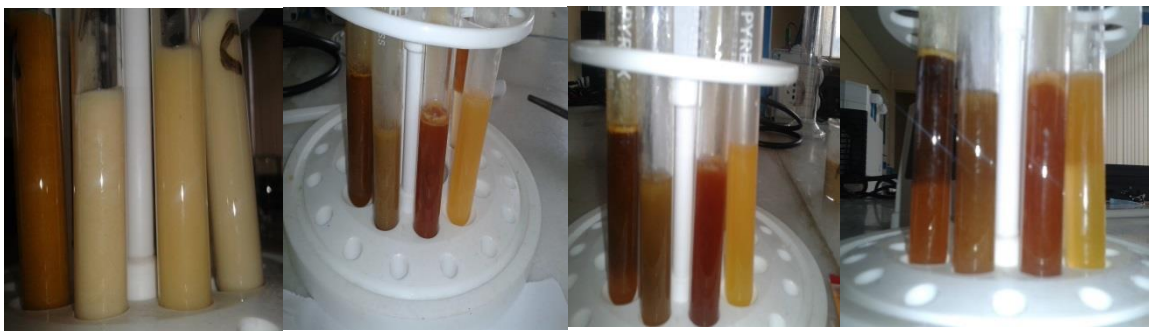


Figure 4.8: The sustanability of of Fatliquor cum filler agent in Acdi condition stability Image with different time. a. after 2 hr; b. after 3hr; c. after 4hr; d. after 6hr

From the figure, it could be observed that the seperation of layers was not observed till 4 hrs. However, after 6 hours the the separation of layers were observed.

4.5.2 Determination of total active ingredient and total alkalinity

To determine the amount of the active ingredient in the prepared fat liquor cum filler

products the data obtained during experiment is presented in Table 4.19 as below.

Table 4.19: The active Ingredient of each prepared sample of fatliquor cum filler

Sample	Weight of sample (g)	Weight of the residue (g)	% Free oil	% Emulsifier	% active matter
A	5.0733	0.756	18.04	11.80	29.84
B	5.0148	1.312	34.89	17.45	52.34
C	5.0510	1.338	34.72	18.28	53.00

It could be observed from the table that the % free oil presnt in sample A was 18.04%, whereas the % free oil present in sample B and C was 34.89 and 34.72%, respectively. However, the prodcut B and C had around 53% active ingredient. Hence, product B and C are more suitable for using in the leather processing tna product A, owing to its low active ingredient. The total alkalinity of each prepared sample was determined to be around 4.44 ml/g. This is on par with the Indian standard value for sulphited fatliquor [107]. The unsaponifiable matter in the developed product was found to be around 0.5%, which is very low compared to the values for commercially available fatliquors.

4.6 Application of developed products in leather processing

Developed fatliqour cum filling agent and the protein hydrolysate were used in the retanning stage. Four trials were carried out 1) control process using commercial fatliquor and retanning agent (T_1); 2) the developed alkaline hydrolysed product was used alone (T_2); 3) the developed alkaline hydrolysed products were used in combination with the commercial syntan and fatliquors (T_3) and 4) homogenized product used alone (T_4). Both upper and garment leathers were developed using the process recipe provided in (Annex 8-13).

From all the experiments, the leathers obtained had similar properties. Especially, the leathers did not show any oiliness on the surface. Hence, it could be inferred that the prepared fatliquor cum filling agent was stable during leather processing and had penetrated in to the leather matrix without any problem. The dye exhaustion of the experimental leathers were better ta the control leathers. This could be due to the presence of protein ingredient of the prepared sample. Owing to the presence of the protein

fractions, additional reactive sites are available for fixation of dyes. Similarly, the leather processing involving the homogenized fat liquor cum filler products without any commercial product show better exhaustion and better colour intensity on par with that of the leathers retanned with alkaline hydrolysed products. However, the leathers developed slight grain roughness when compared to other experiments. Various physical analyses of the leathers were carried out as per standard procedures.

4.7 Physical Testing Results of Leather Samples

The crust leathers, both upper and nappa leather were analysed for tensile strength, % elongation at break, teat strength and bursting strength. The determined values are provided in Table 4.20.

Table 4.20: Physical characteristics of control and experimental leathers

Sample	Thickness	Tensile strength (N/mm^2)	% Elongation	Thickness (mm)	Tear strength (N/mm)	Lastometer test	
						Distension (mm)	Load (Kgf)
Upper Leathers							
Control (T_1)	1.142	15.6	66.55	1.0988	41.57	10.24	33.05
HT(T_2)	1.219	20.9	56.1	1.1129	47.55	11.7	51.56
HT_C (T_3)	1.235	15	76.90	1.0206	50.22	13.9	45.47
Ho (T_4)	1.319	23.95	66.85	1.0813	48.72	13.9	34.46
Nappa leather							
Control (T_1)	0.892	15.330	64.535	1.085	44.38	13.4	27.09
HT (T_2)	1.133	15.706	57.83	0.9150	42.33	11.9	25.69

4.7.1 Tensile Strength

A good tensile strength value is desired in all leather types and this characteristic is an important indicator about leather quality [108]. From the Table 4.20, it could be observed that tensile strength values of control upper and control nappa leathers were 15.6 and 15.330 N/mm^2, respectively. The leathers retanned with the homogenized sample showed better tensile strength of 23.95 $N/mm^{2.}$ This could be attributed to the fact that, it contains more unhydrolysed oil fractions. Also, it could be observed that the leathers retanned with the alkaline hydrolysed product showed better tensile strength higher than the control leathers. Tensile strength of leathers made using the products was higher

than the standard value of 15 N/mm^2 [109]. Fatliquor cum filler products for making leather have been developed and it gives sufficient strength and fullness properties to the leathers.

4.7.2 Elongation at Break

The elongation at break of the leather provides an idea about elastic behaviour of the leathers. The results are tabulated in Table 4.20. In the present study, leathers retanned with the alkaline hydrolysed product in combination with commercial syntan and fatliquor gave highest value of 76.9%. Whereas, the leather obtained from experiments T2 and T3 gave relatively lesser values. This might be due to the fact that the leathers are fuller due to more protein fractions. However, all the values are on par with the stipulated minimum value of 40% [110].

4.7.3 Tear Strength

The tear strength of the leather provides an idea about strength of the leathers. The results are tabulated in Table 4.20. In the present study, leathers retanned with the alkaline hydrolysed product in combination with commercial syntan and fatliquor gave highest value of 50.22%. Whereas, the leather obtained from experiments T2 and T3 gave relatively lesser values. This might be due to the fact that the leathers are fuller due to more protein fractions. However, all the values are on par with the stipulated minimum value of 40 N/mm [109]. Tear strength of nappa was found to be 42.33N/mm. As per UNIDO norms, the minimum tear strength value reburied for chrome tanned nappa leathers is 35 N/mm [111]. Hence, it could be inferred the strength properties are not affected by the use of alkaline hydrolysed and homogenized product in leather processing.

4.7.4 Ball Burst (Distension and Load Crack)

Shoe upper leather often shows slight crack in the toe area at the time of lasting operation in spite of the leather has good tensile and tear strength properties. This is due to weak grain surface characteristics of leather due to more filling and loading of tanning and retanning materials in the grain side. The result of grain crack distension and load shown in Table 4.20. It could be observed from the table that all the leathers had higher distension and the load at break/burst than the stipulated minimum values of 20 Kgf and 7 mm, respectively.

Thus it could be inferred that fat liquor cum filler agent enhanced the ball burst test property of leather in retanning process.

4.7.5 Colour Rubs Fastness (Dry Based)

The results of the dry rub fatness test are shown in Table 4.21. It could be observed from the table that the leathers with stood the dry rubbing (150 cycles), proving that the dye was well fixed in the leathers and not migrated to the felt.

Table 4.21: Results of fastness by dry rubbing (150 cycles)

Samples	Grey scale reading of leather	Grey scale reading of leather
Upper Leathers		
Control (T_1)	3/4	3/4
HT(T_2)	3/4	3/4
HT_C (T_3)	3/4	3/4
Ho (T_4)	3/4	3/4
Nappa leather		
Control (T_1)	3/4	3/4
HT (T_2)	3/4	3/4

4.7.6 Flexing Endurance

Flexing endurance of the upper leathers retanned with the prepared product was tested for flexing endurance for 250,000 flexes. The leathers did not develop any damage after this test. Hence, it could be inferred that the products does not affect the flexing endurance of the leathers.

4.7.7 Organoleptic properties of Upper and Nappa leathers

Goat Crust Upper Softness, rub resistance, and organoleptic Feeling

It is essential to study the influence of fat liquor cum filler agent on the organoleptic properties of leather. The various organoleptic properties of the upper leather such as uniformity of colour, intensity of colour, roundness, fullness, feel and softness were evaluated by tanners and the values are provided in Table 4.22. Three experienced tanners rated the

leathers on a scale of 0-10 points score for each functional property, where higher values indicated better property of leathers. It could be observed that all the leathers had similar organoleptic properties. However, the intensity of colour was better in experimental leather, due to its higher protein fractions.

Table4.22: Organoleptic properties of goat upper chrome crust leathers

Parameter	Control (T_1)	HT (T_2)	HT_C(T_3)	HO (T_4)
Uniformity of colour	7.5	9	9	9
Intensity of colour	7.5	8.5	9	8.5
Roundness	8	9	8.5	8
Fullness	8	8.5	8.5	8.5
Feel	8.5	8	8	8
Softness	8.5	8	8	8.5

Similarly, various organoleptic properties such as uniformity of colour, intensity of colour, feel, softness and drape were evaluated by experts and the values are provided in Table 4.23. It could be observed that the leathers had similar organoleptic properties. However, the feel, softness and drape were better than the control leather.

Table4.23: Organoleptic properties of goat nappa chrome crust leathers

Parameter	Control (T_1)	HT (T_2)
Uniformity of colour	8	8
Intensity of colour	8	8
Feel	7.5	8
Softness	7.5	9
Drape	Better	More better

4.8 Statistical Analysis Data Result

The ANOVA results can be seen at Annex 15, for hydrolysates fleshing wastes and annex 16 (Table 1 – 4) results shown the physical test of the leather articles when applied to the fatliquor cum filler agents in ratanning process.

The physical effect of the leather can be significance of the fleshing hydrolysates expressed by ANOVA result on Annex 15. The model F-value of 10.36 implies that the model is significant. There is only 0.01% chance that a “Model F-value” this large could occur due to noise in the experiments. Values of “prob. > F” less than 0.05 indicates that

the model is significant. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C, BC are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The R^2 value of the model was 0.8381, which further indicates that the model was suitable for adequately representing the relationships among the variables. The "Pred R-Squared" (Predicted correlation coefficient) value of 0.5243 not as close to the "Adj R-Squared" (Adjusted determination coefficient) value of 0.7572. This may indicate a large block (types of fleshing waste hydrolysates i.e. Sheep, Goat and Hide) effect or a possible problem with your modeland/or data. Things to consider are model reduction, response tranformation, outliers, etc. The value of R^2 indicates that there is a good agreement between the experimental values and predicted values obtained from the model. In general, the P-value determines the significance of each coefficient in the model. However, in order to minimize error, all of the coefficients were considered in the design. The model proved suitable for the adequate representation of the real relationship among the selected factors.

The statistical results of tensile strength, elongation, tear load and distension and load at grain crack of the upper and nappa goat leathers produced are presented respectively in Annex 16. Table21 shows that the average data for the three samples were different from each other. The values were 18.5, 20.4 and 21.9 N/mm2 for the samples $Econtrol_1$, EHT_2 and $EHTc_3$, respectively. Tensile strength for both parallel and perpendicular to the backbone test samples showed with ANOVA decreased in // and increased in ⊥, with respect to the corresponding control. The analysis of variance for the tensile strength test the data was shown at Table 1. The model F-value of 7.40 implies that the model is significant. There is only a 0.58% chance that a "Model F-Value" this large could occur due to noise.Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A are significant model terms.The R^2 value of the model was 0.4968, which further indicates that the model was suitable for adequately representing the relationships among the variables. The "Pred R-Squared" (Predicted correlation coefficient) value of 0.2753 is in reasonable agreement with the "Adj R-Squared" (Adjusted determination coefficient) value of 0.4297. The elongation data show annex

13 Table 2 that the amounts use of fat liquor cum filler utilization of test had no influence on elasticity of the produced leather. The stretching values for upper 66.55, 76.9, 89.00 and 66.85 % obtained are not recommended for making upper leather but 56.1% can be used it for making upper article. The model F-value of 10.85 implies that the model is significant. There is only 0.01% chance that a "Model F-Value" this large could occur due to noise.Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.The R^2 value of the model was 0.5913, which further indicates that the model was suitable for adequately representing the relationships among the variables. The "Pred R-Squared" (Predicted correlation coefficient) value of 0.4115 is in reasonable agreement with the "Adj R-Squared" (Adjusted determination coefficient) value of 0.5368. According to Annex13 Table3, was indicating that the percentage of fatliquor cum filler used in ratanning influenced in progressive tear load. It is observed that as the fatliquor cum filler % in fatliquoring increases, the resistance to progressive tearing also increases. The values obtained for the three samples are higher than that of minimum recommended (40N) for goat leather for shoes. The combination of products with commercial test was provided better touch, filling and resistance. The model F-value of 27.27 implies that the model is significant. There is only 0.01% chance that a "Model F-Value" this large could occur due to noise.Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.The R^2 value of the model was 0.7843, which further indicates that the model was suitable for adequately representing the relationships among the variables. The "Pred R-Squared" (Predicted correlation coefficient) value of 0.6894 is in reasonable agreement with the "Adj R-Squared" (Adjusted determination coefficient) value of 0.7555.

Annex 13(Table 4a-4b). The data shows that the leather grain distension at crack differences between the samples of leather and Load at crack respectively. The retanning process carried on the three samples and thereafter fatliquoring with fatliquor cum filler provided in two sample tests of leather products has given excellent characteristics such as the grain distension and the minimum recommended value of 7.00 mm. The model F-value of 10.93 implies that the model is significant. There is onlya 0.01% chance that a

"Model F-Value" this large could occur due to noise.Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.The R^2 value of the model was 0.7847, which further indicates that the model was suitable for adequately representing the relationships among the variables. The "Pred R-Squared" (Predicted correlation coefficient) value of 0.5156 is in reasonable agreement with the "Adj R-Squared" (Adjusted determination coefficient) value of 0.7555.The model F-value of 10.59 implies that the model is significant. There is onlya 0.01% chance that a "Model F-Value" this large could occur due to noise.Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.The R^2 value of the model was 0.7057, which further indicates that the model was suitable for adequately representing the relationships among the variables. The "Pred R-Squared" (Predicted correlation coefficient) value of 0.5033 is in reasonable agreement with the "Adj R-Squared" (Adjusted determination coefficient) value of 0.7057.

In the two samples test was seen that the use of fatliquor cum filler in retanning caused improved the physic-mechanical aspects of its collagen structure and principally the aspect of fullness and softness of the grain.

CHAPTER FIVE

5. Conclusion and Recommendation

This study focuses on the reutilization of fleshing wastes (i.e. sheep, goat and hide) as beneficial product in leather processing after some chemical and thermal modification. Fleshing wastes are one of the most important by-products from leather industry. Fleshing have two part of fraction i.e. protein and fat components. The fleshing hydrolysate was obtained by alkaline hydrolysis or the hydrolysate was obtained by water base homogenises of fleshing and the fat was extracted from fleshing obtained by dichloromethane solvent. The hydrolysate and the extracted oil from fleshing was used for preparation of fatliquor cum filler agents and used in retannage of upper and nappa leathers.

The oil and the protein fractions were characterized and then used in the development of the product. Various parameters, such as time, temperature and pH of hydrolysis have been optimized. The optimized product was used in the preparation of fatliquor cum filling agent. During the preparation, two types of surfactants SDS and M7 were used and the offer of these emulsifiers was optimized. The optimized product was used in the retanning of upper and garment leathers.

The total substitution of the fatliquor cum filler for upper and nappa leather products 8% of fatliquor cum filling agent per weight of skins/ hides and 16% of fatliquor cum filling agent per weight of skins/hides, respectively. The physical properties of goat shoe upper and nappa leather were on par with that of the control leathers. The colour intensity of the experimental leathers is better than the control leathers.

It is recommended that the tanner can consider converting the fleshing waste in to a usable product, as given in this project report. Even the Chemical manufacturers in Ethiopia could avail the opportunity and try to produce indigenous chemicals, which can be used by the Ethiopian tanners. By doing this the tanner can reduce the cost of elimination of fleshing waste dump and create jobs opportunity, minimize the load of environmental regulation agent and reduce the cost of fatliquor-filler importation, thereby making savings in foreign exchange.

The tanning sector can sell the solid waste and get extra profit and save the money invested to dispose the solid waste. This project could allow the tanneries to solve or at least to simplify their management of the fleshing; on the other side to favor new employments for the fleshing, decreasing its environmental impact and improving its positive features.

Future research will be done on feasibility study for this technology. Previously there was no effort to implement this new technology in Ethiopian. Can done further work to change the Investor attitude on waste generate reusing material and Tanneries should be wisely to hand this wastes by apply this techniques.

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Annex

Annex 1: Daily soaking capacity of Ethiopian Leather Industries

Ref. No.	Name of Tannery	Soaking Capacity (pieces/day)		Soaking Capacity (tonne/day)	
		Skin	Hide	Skin	Hide
1	Addis Ababa Tannery	2500	900	3.25	18.9
2	Bahir Dar Tannery Plc.	2000	300	2.60	6.3
3	Bale Tannery Plc.	3000	300	3.9	6.3
4	Batu Tannery Plc.	2500	1000	3.25	21
5	Blue Nile Tannery(under investment)	0	0	0	0
6	China Africa Tannery	12,000	0	15.6	0
7	Colba Tannery Plc.	6000	600	7.8	12.6
8	Crystal Tannery	0	0	0	0
9	Debrebrehan Tannery	5000	0	6.5	0
10	Dire Tannery	6000	600	7.8	12.6
11	DX industrial	0	0	0	0
12	East Africa tannery	7000	0	9.1	0
13	ELICO	13,000	1000	16.9	21
14	Ethiopia Tannery Share	12,000	12,00	15.6	25.2
15	Farida Tannery	7000	0	9.1	0
16	Friendship Tannery	10,000	1000	13	21
17	Gellan Tannery Plc.	3000	0	3.9	0
18	Habesha Tannery	3000	0	3.9	0
19	Hafde Tannery Plc.	6000	250	7.8	5.25
20	Hora Tannery Plc.	3000	0	3.9	0

21	Kombolcha Tannery	6000	0	7.8	0
22	Mersa Tannery Plc.	6000	300	7.8	6.3
23	Mesaco Global Tannery	2500	0	3.25	0
24	Modjo Tannery Share Company	7000	500	9.1	10.5
25	New wing leather finishing unit	0	0	0	0
26	Sheba Tannery	6000	600	7.8	12.6
27	Sun industrial Tannery	3000	0	3.9	0
28	United vasn Tannery	3000	0	3.9	0
29	Wallia Tannery Plc.	5000	500	6.5	10.5
30	Xiang xin xang	0	0	0	0
Total		141,500	9,050	183.95	190.05

Source: Leather industry development institute and tanneries 2012/13 current 30 tannery soaking capacity performance.

Annex 2

Delimed of fleshing wastes - performed in the liming drum

Material	Fleshing wastes	Sheep		Goat	Hide
	Weight kg	20		20	20
Process	Chemical	%	Temperature	Time	pH
Washing I	Water	200	30	30’	
W/D/W					
Washing II	Water	200	30	30’	
W/D/W					
Deliming	water	200	30		
	Ammonium sulphate $(NH_4)_2SO_4$	3		60’	
		3		60’	
		3		60’	7-8
Washing	Water	250	30	30’	
D/W/D after this kept in laboratory refrigerator until to start the work					

Annex 3

Determination of Moisture content

Weigh moisture sample immediately and record as "wet weight of each samples" Dry the wet samples at a temperature not exceeding 215.6 º F (102 º C) using the suitable drying equipment usually for 3hr. after that cool and measured and placed again inside the hot air oven for 1hr until to get a constant weight . Allow the sample to cool. Weigh the cooled sample again, and record as the "dry weight of samples" The moisture content of the samples is calculated using the following equation:

$$W\% = \frac{A-B \text{ x } 100}{B}$$

Where: %W = Percentage of moisture in the sample,

A = Weight of wet sample (grams),

B = Weight of dry sample (grams)

Annex 4

Characterization of fat/oil properties procedure

a. Determination of acid value

Place 5.0 g of fat or oil in a dried conical flask. Add 25 ml of absolute ethanol alcohol and add (2-3) drops of phenolphthalein. Heat with shaking in water bath (65%) for 10 minutes ,then cool Titrate the solution against 0.1 N KOH until pink colour appears (end point). Record your observations and calculated the acid value of fat/oil based on the following equation:

$$AV = \frac{\text{ml of KOH x N x 56}}{\text{Weight of Sample}} = \text{mg of KOH}$$

N = Normality of KOH

% Free Fatty Acid (FFA) = AV x 0.503

Calculate the acid value (AV) and free fatty acid (%FFA) using above laws

b. Determination of saponification value

Weigh approximately 2 g of the fleshing fat or oil into a 250 mL conical flask. Add 25 mL of alcoholic potassium hydroxide solution (0.5 N).Attach a reflux condenser and heat the flask contents on a boiling water bath for 1 hour until formation of some foam. While the solution is still hot, add 3 drops of phenolphthalein indicator and titrate the excess potassium hydroxide with the 0.5 N hydrochloric acid (Vml of hydrochloric acid at end point represents S).Do same above procedure but without sample (Vml of hydrochloric acid at end point represents B).

Calculate the saponification number by using the following law:

$$SP\# = \frac{56.1(B - S) \times N \text{ of } HCl}{\text{Gram of Sample}}$$

Where: - B = ml of HCl required by Blank.

S = ml of HCl required by Sample.

c. Determination of Iodine value

Weigh approximately 0.25 g of the fat or oil into a 250 mL conical flask. Add 10 ml of chloroform. Add 30 ml of Hanus solution and close the flask completely by Para film, then leave the solution for 30 minutes in dark place. Add 10 ml of 15% potassium iodide solution and then shake. Add 100 ml of distilled water (DW).Titrate the iodine solution against 0.1 N Sodium thiosulfate solution till yellow colour formed, then add 2-3 drops of starch solution where blue solution formed and then continue with titration till the blue colour is disappeared (Volume (ml) of $Na_2S_2O_3$ at end point represents S) Do same above procedure but without sample (Volume (ml) of $Na_2S_2O_3$ at end point represents B).

Calculate the iodine number by using the following law:

$$\text{Iodine Value} = \frac{(B - S) \times N \text{ of } Na_2S_2O_3 \times 0.127 g/meq}{\text{Weight of Sample (g)}} \times 100$$

B: V ml of $Na_2S_2O_3$ volume for blank

S: V ml of $Na_2S_2O_3$ volume for sample

Annex 5

DETERMINATION OF TOTAL ACTIVE INGREDIENT

Weigh about 5.0733 g, 5.0148g and 5.0510g of the prepared Fatliquor cum filler agents A,B and C respectively in a 250 ml flask and add 25 ml of 50 % ethyl alcohol and 25 ml of petroleum ether. Transfer the contents to a separating funnel. Shake the contents vigorously and allow the layers to separate. Transfer the lower alcohol layer to another separating funnel and extract it three or four times with petroleum ether. Extract upper layer, namely, the petroleum ether layer, with 75 % alcohol and allow the layers to separate and extract further with 90 % alcohol and absolute alcohol and allow the layers to separate.

Collect the petroleum ether layer and alcohol layer in two tarred flasks. Evaporate the solvents, dry the residues to constant weight in the oven cool and weigh.

Alcohol layer contains the emulsifier and the petroleum ether layer contains the neutral oil.

$$\text{Free oil, in \% (A)} = \frac{F}{W} \text{ x } 100$$

$$\text{Emulsifier in \% (B)} = \frac{E}{W} \text{ X } 100$$

Where

F= weight of petroleum ether soluble in g,

E= weight of alcohol soluble in g, and

W = weight of the fatliquor sample in g.

Total active ingredient in % = A + B

Annex 6

Determination of total alkalinity

For determined of TA of fatliquor cum filler agent carried out at the condition of temperature 21.2^{O}C and relative humidity 47.1. After this the samples were measured 10.1203g from each sample types of fatliquor cum filler products (i.e. A, B and C) put into conical flask and diluted with 100ml of distilled water until to get homogenised solution by given heating for one hour, then cool and added 30g of sodium chloride (NaCl), 25 g of diethyl ether and 5 drops of methyl orange indicator, the titrated with 0.5N sulphuric acid until the aqueous layer is orange, shaking the flask frequently to ensure a permanent end- point.

The Total Alkalinity of the samples is calculated using the following equation:

Total alkalinity in ml 0.1N per grams of oil $= \frac{t \ x \ 5}{w}$ = A

Where: t = ml 0.5 N sulphuric acid require

w = weight of sample taken

Annex 7: Standard requirements for characteristic of Oil/Fat

Characterized	Standard
Iodine value in ml.N/meq	58.6 – 61.4
Acid value in mg of KOH	8.6
Saponification value ml.N/g	180 -233
Un- saponification value	0.37

Annex 8: Application of the formulated fatliquor cum filler on leather process Recipe

Article Goat Upper					
Weight = 2 kg				Pcs = 4	Remark
Process	%	Chemical	Temp.(^{O}C)	Time	pH
Wetting back	400	Water	25		
	0.5	Wetting agent			
	0.25	Oxalic acid		30’	
D/W/D					
Re-chroming	150	Water	35		
	0.3	Formic acid		10’	Check,2.8-3
	4	Basic chromium (III) sulfate (33% Cr_2O_3		30’	
	4	Chrome syntan		40’	
Basification	1.5	Sodium format		10’	
	1	Sodium bicarbonate		2x15’+1hr	dilute 1:10 pH= 3.8 - 4
D/W/D					
Neutralization	150	water			
	0.5	Sodium format		10’	
	1	Sodium bicarbonate		3x10’+30’	5 – 5.3
Separate the goat skin for control and for test in to two pcs					
For control					
Weight = 1kg		Pcs = 2			Remark
Process	%	Chemical	Temp.(^{O}C)	Time	pH
Re-tanning and Dyeing	100	Water	35		
	2	Novaltan MAP		20’	
	2	Fosfol LP (vegetable fatliquor)		20’	
	3	Nerfill powder (protein filler)		30’	
	3	Retinal LSF 100			
	4	Mimosa powder			
	3	Black dye		30’	
	4	Ratanal MD 80		60’	Check cross section
Fatliquoring	3	Synthetic fatliquor			
	3	Synthetic fatliquor			
	2	Castor oil based fatliquor		60’	
Fixation	3	Formic acid		3x10’+30’	pH = 3.8 - 4 check exhaustion
D/W/D, Pile, Sum-setting, Vacuum, overhead drier, staking					

Annex 9: Application of Fatliquor cum Filler Agent Alone

For Hydrolysate fleshing and extracted oil fleshing test					
Weight= 1kg		Pcs= 2			Remark
Process	%	Chemical	Temp.(^{O}C)	Time	pH
Retanning and Dyeing	100	Water	35		
	2	Novaltan MAP		20’	
	2	Fatliquor cum filler		20’	
	6	Mixing of Hydrolysate fleshing		30’	
	4	Mimosa powder			
	3	Dark Brown		30’	
	4	Ratanal MD 80		60’	Check cross section
Fatliquoring	3	Fatliquor cum filler product(A)			
	3	Fatliquor cum filler product(B)			
	2	Fatliquor cum filler product(C)			
Fixation	3	Formic acid		3x10’+30’	
D/W/D, Horse up overnight, set out, wet-toggle, Vacuum, , stake					

Annex 10: Mixing of the prepared product with the commercial fatliquor and protein filler

For hydrolysed fleshing and extracted oil fleshing test					
Weight = 1 kg		Pcs = 2			Remark
Process	%	Chemical	Temp.(^{O}C)	Time	pH
Re-tanning and Dyeing	100	Water	35		
	2	Novaltan MAP		20’	
	2	Fatliquor cum filler		20’	
	1	Nerfill powder		30’	
	3	Mixing of Hydrolysate fleshing			
	3	Retinal LSF 100			
	4	Mimosa powder			
	3	Black dye		30’	
	4	Ratanal MD 80		60’	Check cross section
Fatliquoring	3	Fatliquor cum filler			
	3	Synthetic fat liquor			
	1	Fatliquor cum filler		60’	
Fixation	3	Formic acid		3x10’+30’	
D/W/D, Horse up overnight, set out, wet-toggle, Vacuum, , stake					

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Annex 11: For homogenized the powdered fleshing with water

Homogenized the powdered fleshing with water and extracted oil fleshing test					
Weight = 1 kg		Pcs = 2			Remark
Process	%	Chemical	Temp.(^{O}C)	Time	pH
Retanning and Dyeing	100	Water	35		
	2	Novaltan MAP		20’	
	2	Fat liquor cum filler		20’	
	4	Mixing of Hydrolysate fleshing		30’	
	3	Retinal LSF 100			
	4	Mimosa powder			
	3	Black dye		30’	
	4	Ratanal MD 80		60’	Check cross section
Fatliquoring	3	Fatliquor cum filler product(A)			
	3	Fatliquor cum filler product(B)			
	1	Fat liquor cum filler		60’	
Fixation	3	Formic acid		3x10’+30’	
D/W/D, Horse up overnight, set out, wet-toggle, Vacuum, , stake					

Annex 12: For Nappa leather with application of commercial

Article		Goat Nappa			
Weight = 1kg				Pcs = 4	Remark
Process	%	Chemical	Temp.(^{O}C)	Time	pH
Neutralization	150	water			
	0.5	Sodium format		10’	
	1.5	Sodium bicarbonate		3x10’+30’	5.4-6
Separate the goat skin for control and for test in to two pcs					
For control					
Weight = 500 g		Pcs = 2			Remark
Process	%	Chemical	Temp.(^{O}C)	Time	pH
Retanning and Dyeing	100	Water	35		
	2	Novaltan MAP		20’	
	2	Fosfol LP (vegetable fatliquor)		20’	
	3	Nerfill powder (protein filler)		30’	
	2	Retinal LSF 100			
	3	Mimosa powder			
	3.5	Grave		60’	Check cross section
Fatliquoring	16	Synthetic fatliquor		60’	
Fixation	3	Formic acid		3x10’+30’	pH = 3.8 - 4 check exhaustion
Top dye	50	Water			
	0.5	Grave		20’	
	0.5	Grave		20’	
Fixation	3	Formic acid		30’	
D/W/D					
	50	Water			
	1	Synthetic fatliquor		30,	
D/W/D, Pile, Sum-setting, overhead drier, staking					

Annex 13 The Prepared products alone for nappa leather

For hydrolysis fleshing and extracted oil fleshing test					
Weight = 250g		Pcs = 2			Remark
Process	%	Chemical	Temp. (^{O}C)	Time	pH
Retanning and Dyeing	100	Water	35		
	2	Novaltan MAP		20’	
	2	Fatliquor cum filler agents		20’	
	3	Mixing of Hydrolysate fleshing		30’	
	1	Retinal LSF 100			
	3	Mimosa powder			
	3	Grave		60’	
					Check cross section
Fatliquoring	4	Fatliquor cum filler product(A)			
	4	Fatliquor cum retanning agent (B)			
	8	Fatliquor cum filler (C)		60’	
Fixation	3	Formic acid		3x10’+30’	
D/W/D					
Top dye	50	Water			
	0.5	Grave		20’	
	0.5	Grave		20’	
Fixation	3	Formic acid		30’	
D/W/D					
	50	Water			
	1	Prepared fatliquor		30,	
D/W/D, Horse up overnight, set out, wet-toggle, Stake					

Annex 14: Effect of time on the hydrolysis of fleshing waste by different temperature and pH of alkaline reagents.

S.No.	Hydrolysis time	NaOH of pH	Temperature ^{O}C	Degree of hydrolysis
1	$SLF_{2hr.\ -}$	10 -	75 -	Slightly hydrolyzed
2	$SLF_{4hr.+}$	10 -	75 -	Completely hydrolyzed
3	$SLF_{2hr.-}$	12 +	75 -	Partially hydrolyzed
4	$SLF_{4hr.+}$	12 +	75 -	Completely hydrolyzed
5	$SLF_{2hr.-}$	10 -	85 +	Slightly hydrolyzed
6	$SLF_{4hr.+}$	10 -	85 +	Completely hydrolyzed
7	$SLF_{2hr.-}$	12 +	85 +	Completely hydrolyzed
8	$SLF_{4hr.+}$	12 +	85 +	Completely hydrolyzed
9	$GLF_{2hr.-}$	10 -	75 -	Slightly hydrolyzed
10	$GLF_{4hr.+}$	10 -	75 -	Completely hydrolyzed
11	$GLF_{2hr.-}$	12 +	75 -	Partially hydrolyzed
12	$GLF_{4hr.+}$	12 +	75 -	Completely hydrolyzed
13	$GLF_{2hr.-}$	10 -	85 +	Partially hydrolyzed
14	$GLF_{4hr.+}$	10 -	85 +	Completely hydrolyzed
15	$GLF_{2hr.-}$	12 +	85 +	Completely hydrolyzed
16	$GLF_{4hr.+}$	12 +	85 +	Completely hydrolyzed
17	$HLF_{2hr.-}$	10 -	75 -	Slightly hydrolyzed
18	$HLF_{4hr.+}$	10 -	75 -	Completely hydrolyzed
19	$HLF_{2hr.-}$	12 +	75 -	Partially hydrolyzed
20	$HLF_{4hr.+}$	12 +	75 -	Completely hydrolyzed
21	$HLF_{2hr.-}$	10 -	85 +	Partially hydrolyzed
22	$HLF_{4hr.+}$	10 -	85 +	Completely hydrolyzed
23	$HLF_{2hr.-}$	12 +	85 +	Completely hydrolyzed
24	$HLF_{4hr.}+$	12 +	85 +	Completely hydrolyzed

Annex 15: ANOVA FOR HYDROLYSATES OF FLESHING WASTES

Degree of Hydrolysates

Source	Sum of Squares	DF	Mean Square	F-value	P-Value Prob. > F	
Block	0.11	2	0.055			
Model	0.51	7	0.074	10.36	0.0001	Significant
A- Time	0.27	1	0.27	38.48	<0.0001	
B- Temperature	0.083	1	0.083	11.65	0.0042	
C- pH	0.083	1	0.083	11.71	0.0041	
AB	0.013	1	0.013	1.87	0.1933	
AC	0.017	1	0.017	2.43	0.1412	
BC	0.042	1	0.042	5.96	0.0286	
ABC	2.814E-003	1	2.814E-003	0.40	0.5391	
Residual	0.099	14	7.098E-003			
Cor. Total	0.72	23				

Annex 16: ANOVA for Physical Test for leather

Analysis of variance of Tensile Strength Table - 1

Source	Sum of squares	DF	Mean square	F value	p-value Pro.> F
Model	57.69	2	28.85	7.40	0.0058
A-concentration	*57.69*	*2*		*7.40*	*0.0058*
Pure Error	58.45	15	*28.85*		
Cor Total	116.14	17	3.90		

Analysis of variance of Elongation Table - 2

Source	Sum of squares	DF	Mean square	F value	p-value Pro.> F
Model	1464.59	2	732.30	10.85	0.0012
A-concentration	1464.59	2	732.30	10.85	0.0012
Pure Error	1012.26	15	67.48		
Cor Total	2476.86	17			

Analysis of variance of Tear Strength Table - 3

Source	Sum of squares	DF	Mean square	F value	p-value Pro.> F
Model	884.58	2	442.29	27.27	< 0.0001
A-concentration	*884.58*	*2*	*442.29*	*27.27*	
Pure Error	243.29	15	16.22		
Cor Total	1127.87	17			

Analysis of variance of Destation of Crack Table – 4a

Source	Sum of squares	DF	Mean square	F value	p-value Pro.> F
Model	23.50	2	11.75	10.93	0.0100
A-concentration	23.50	2	11.75	10.93	0.0100
Pure Error	6.45	6	1.07		
Cor Total	29.94	8			

Analysis of variance of Load at Crack Table - 4b

Source	Sum of squares	DF	Mean square	F value	p-value Pro.> F
Model	41020.67	2	20510.33	10.59	0.0108
A-concentration	41020.67	2	20510.33	10.59	0.0108
Pure Error	11621.33	6	1936.89		
Cor Total	52642.00	8			