Addis Ababa University
Addis Ababa Institute of Technology
School of Chemical and Bioengineering

VALUE ADDED PROTEIN PRODUCTS FROM CHROME SHAVINGS

By

Hiwot Getahun

Advisor

Dr. R.Karthikeyan

A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the Degree of Master of Science in Environmental Engineering

October 2017

Addis Ababa, Ethiopia

ADDIS ABABA UNIVERSITY

ADDIS ABABA INSTITUTE OF TECHNOLOGY

SCHOOL OF CHEMICAL AND BIO ENGINEERING

This is to certify that the thesis prepared by Hiwot Getahun entitled: ***Value Added Protein Products From Chrome Shavings*** and submitted in partial fulfillment of the requirements for the degree of Master of Sciences in Biochemical Engineering complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

By

Hiwot Getahun

Approved by Board of Examiners

_____	_____	_____
Dean, Department	Signature	Date
_____	_____	_____
Advisor	Signature	Date
_____	_____	_____
Internal Examiner	Signature	Date
_____	_____	_____
External Examiner	Signature	Date

Declaration

I, the undersigned, declare that this thesis entitled "***Value Added Protein Products From Chrome Shavings*****"** is my original work under School of Chemical and Bio Engineering, AAiT, and has not been presented by any other person for an award of a degree in this or any other University, and that all resources of materials used for this thesis have been duly acknowledged.

Name: Hiwot Getahun _____

Signature

This thesis has been submitted to the Addis Ababa University with my approval as the University

Advisor: Dr.R. Karthikeyan (Asst. Prof.**)** _____

Signature

Acknowledgments

- Firstly I would like to thank the God and his Mother St. Merry; it is because of their help and courage this study is possible.

- I wish to express my deep gratitude to Dr. R. Karthikeyan, Assistant Professor of Leather Technology, School of Chemical and Bioengineering for his guidance and advice throughout this research.

- I would also like to say thanks Mr. Zerihun Abate for his encouragement and support and Mr. Hintsaselassie Seifu, for his help during my laboratory work.

- I am grateful to all the staff members of School of Chemical and Bioengineering, AAiT and Leather Industry Development Institute (LIDI), Ethiopia.

- I take this opportunity to thank my dear brother Alemseged Taddese, and to my lovely Husband and to my parents and friends.

Table of Contents Page

List of Tables

List of Figures

Acronyms

CH	Collagen Hydrolysate
CH-Si	Collagen hydrolysate and sodium silicate complex
Cr	Chromium
DSC	Differential Scanning Calorimetry
FTIR	Fourier Transform Infrared Spectroscopy
LIDI	Leather Industry Development Institute
pH	A measurement of acidity or basicity
SEM	Scanning Electron Microscopy
TGA	Thermo gravimetric analysis
UNIDO	United Nations Industrial Development Organization

Abstract

Attention is invited to the fact that leather industry is the most polluting of all chemical industries, which in turn are more polluting than others. The generation of huge quantity of chromium containing solid wastes is the beginning the real threat. With respect to solid waste potential, over 25% of the waste contains chromium. Implementation of cleaner technologies, waste minimization, recovery and reuse of proteins and chemicals in the tanneries are very important for suitable growth in leather industry, in the present context of stringent environmental regulations on one hand and increased public awareness on the other. Environmental protection aims at preventing pollution of soil, water and air. These require evolving efficient solid waste management and no effluent technologies.

Hence, in the present investigation an attempt has been made to isolate the chromium and collagen from chrome shaving waste thereby, converting the waste into valuable protein products. Initially, the raw material and the isolated collagen hydrolysate were characterized for ash, fat and chrome content and the results are 9.2%, 0.85%, 4.2mg/l and 0.9%, 0.9%, 2.3mg/l respectively. Later the collagen was crosslinked with silica for application in retanning as protein filler. The retanning study has been carried out on wetblue sheep skins and the resultant leathers are subjected to scanning electron microscopic analysis and physical testing and the results are comparable with those of conventional ones. Finally, the isolated collagen is converted into glue for industrial applications. The colour of the prepared glue was compared with the commercially available glue (prepared from limed fleshing) and the overall colour difference between local glue and the glue from chrome shavings is more ($\Delta E=10$).

1 Introduction

1.1 Background of Study

At present, Ethiopian leather industry is in the forefront of the leather sector development within the Eastern and Southern Africa region. Ethiopia has one of the largest livestock populations in the world providing a strong raw material base for the leather industry. Ethiopia's livestock population is estimated at 45 million cattle, 23 million sheep and 23 million goats (CSA, 2004). The total number of tanning industries in Ethiopia under good running condition is twenty six. All of them are of considerable size with the smallest having a soaking capacity of 3000 skins per day. According to the Ethiopian Leather Industry Development Institute (LIDI), Ethiopia exports finished leather to 40 countries.

During the process of manufacturing, substantial amount of solid and liquid wastes are generated. It is known that 1 ton of wet salted hide yields only 200kg leather while 600kg comes out as solid wastes. The various solid wastes generating in tanneries are hair, wools, raw trimming and fleshings, wet blue shavings and splits, crust trimmings, buffing dust and sludge. Some of the huge quantities of solid wastes produced from various operations of tanneries are put to practical use, but the majority of the wastes is dumped without any effective usage and becomes a source of pollution. So the disposal of these wastes becomes a serious problem for the world tanning industry (Rae et al 2002).

The leather industry has gained a negative image in the society not only because of pollution causing potency but also of its dirty nature due to the generation of huge amount of solid waste. Solid wastes generated from tanning industries contain different chemicals which are used during leather manufacturing process. These tannery solid wastes have different characteristics mainly these wastes constitute protein (collagen) as the main component. If these protein and other chemicals, which are present in the chemical treated protein (e.g. Cr leather waste), are not utilized properly it will pose hazardous pollution problem to the environment (Kanagaraj, 2006).

Historically, shavings, trimmings and splits from the chrome tanning of hides and skins have been disposed off in landfills. Recently, tighter local restrictions have caused the tanning industry to seek out alternatives to dumping. Utilization of these waste products has been utilized in preparation of building materials and composites with polymers have been molded into sheets. Acidic and basic hydrolysis has yielded animal feed and fertilizer. Collagen proteins have application for making gelatin, additive component for cosmetics, biomaterial for medical products (Sastry et al 2005).

Therefore, it is important to regenerate the collagen from these wastes so as to reduce the pollution and to have better value addition to these wastes. Thus in the present investigation, an attempt has been made to isolate the collagen from chrome shavings and the isolated collagen is further crosslinked with Silica for use as a protein filler. Further, the separated collagen is converted into glue for industrial applications.

1.2 Statement of problem

During leather processing, after the completion of chrome tanning process, the leathers are leveled with splitting and shaving methods. By this operation large quantities of chrome containing solid wastes are generated in the tanneries. The disposal of solid waste from leather manufacture is a significant issue in the tannery- environment relationship. With reference to the solid balance in the conversion of hides and skins into leather, out of every 1,000 kilos of salted bovine hides only 260kg are finally converted into leather. Among the remaining solids, 230kg are in the wet blue state, comprising 100 kg shavings, 110 kg unusable splits, and 20 kg trimmings. In terms of collagen, the yield as leather is 50%, with approximately 34% distributed among wet blue solid wastes (Kanagaraj, 2006).

Hence, when the huge quantity of chromium containing solid wastes are dumped into the environment without any treatment that will leads serious environmental and health issues since Cr(VI) is declared as highly carcinogenic. As a consequence, the leather industry world over is coming under pressure from environmental authorities to comply with the pollution and the

pressure is so much that it has become a common occurrence that the tanneries are forced to close down not only in developed countries but also in developing countries. Thus it is the need of the time to develop alternative technologies for the utilization of chromium containing solid wastes.

1.3 Objective of the study

1.3.1 General objective

The general objective of the present work was to develop protein based value added products from chrome shavings by isolating the collagen from chromium.

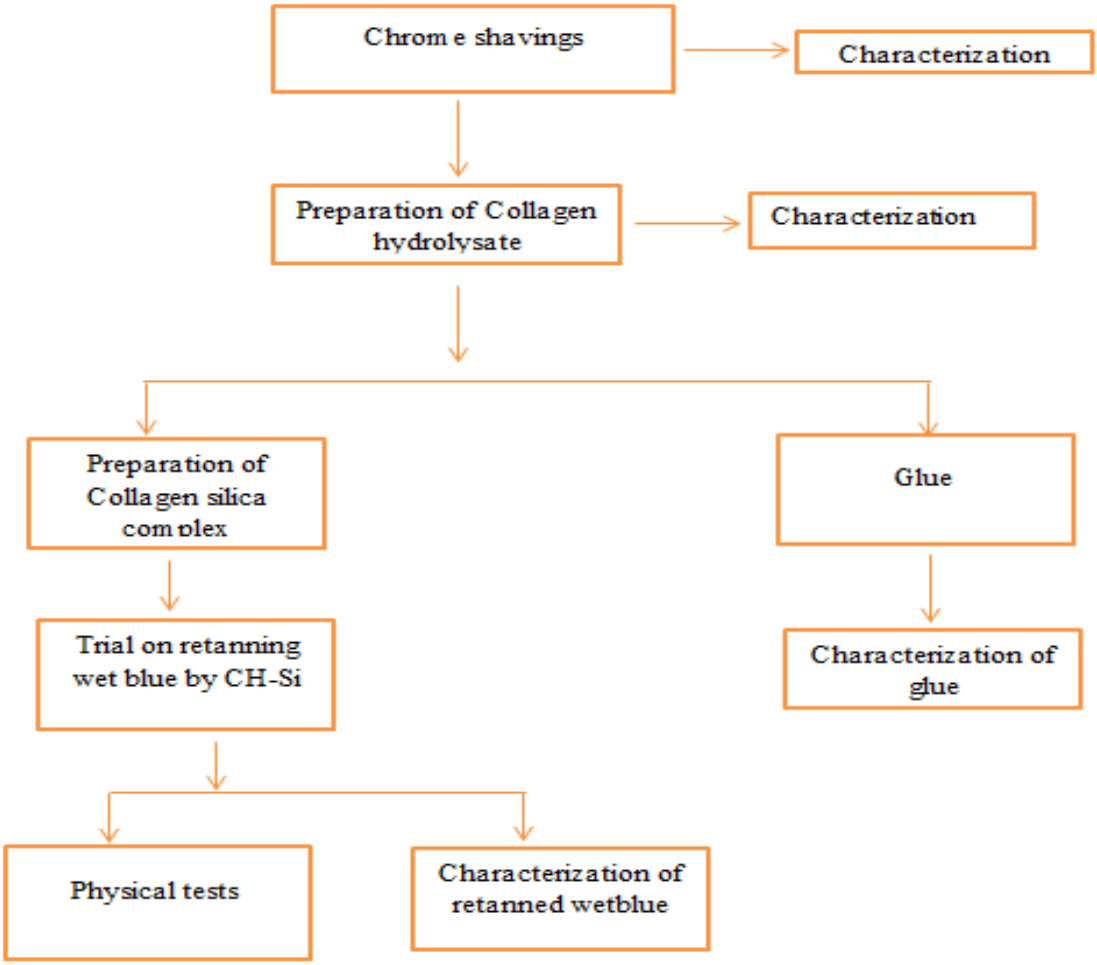
1.3.2 Specific objectives

The specific objectives of this study:-

- ✓ To characterize the chrome shavings
- ✓ To characterize the collagen hydrolysate.
- ✓ To study the effect of CH-Si on retanning of wet blues
- ✓ To characterize glue made from collagen hydrolysate

1.4 Framework of the study

The Framework of the study is presented as follows



1.5 Scope of the study

The scope for the present research is to develop value added protein products from chrome shavings by isolating the chromium from collagen. The present study aimed at the characterization of chrome shavings and development and characterization of hydrolysate from chrome shavings and preparation of valuable protein filler in leather retanning and glue for industrial applications. The scope is limited to chrome shavings but not other chromium containing solid wastes such as trimmings, buffing dust, finished trimmings and splits. The isolation of chromium and collagen is also Alkaline and Acid hydrolysis.

1.6 Significance of study

Cleanliness is considered next only to Godliness. This has been one of the avowed objectives of environmental engineering from time immemorial. The leather industry has gained a negative image in the society not only because of pollution causing potency but also of its dirty nature due to the generation of huge amount of solid waste. During the process of manufacturing, substantial amount of solid and liquid wastes are generated. It is known that 1 ton of wet salted hide yields only 200-250kg leather while 750kg comes out as solid wastes. The various solid wastes generating in tanneries are hair, wools, raw trimming and fleshings, wet blue shavings and splits, crust trimmings, buffing dust and sludge (Sharphouse, 1995). Some of the huge quantities of solid wastes produced from various operations of tanneries are put to practical use, but the majority of the wastes is dumped without any effective usage and becomes a source of pollution. Chrome shavings accounts for about 10-15% of the weight of raw material processed. So the disposal of these wastes becomes a serious problem for the world tanning industry. So the developments of protein based products from waste chrome shavings have both economic and environmental benefits.

2 Literature Review

1.1 Introduction

Tanning of leather is a very old industry since the advent of civilization of mankind. In the tanning process, putrescible skins and hides of the animals are processed to a stable non-putrescible form which can be utilized for making apparels, footwear, leather goods etc. In the process of converting raw hides and skins to finished leather, the leather industry produces all three categories of waste: liquid, solid waste and air emissions. One ton of wet salted hides yield only 200 kg of leather but over 600 kg of solid waste, or by-product if a market can be found (Cabeza, 1998).

❖ Leather processing

Skin is the largest organ of the body and comprises of 3-5% of an animal's weight. The skin performs many functions protection against physical injury, a barrier to microorganisms, protection from the element and against drying out; it even helps to regulate body temperature. The skin is composed of two major divisions the epidermis or grain layer and the corium layer and the flesh layer, the aesthetic value of leather derived from the grain layer; the corium layer gives leather strength and resiliency. (Dutta, 1999)

Hides and skins as they are obtained after flaying contain water up to 60-70% of their weight. The presence of such high quantity of moisture makes them liable to bacterial attack and mold growth, which in turn decompose the skins and hides. To prevent this decomposition cleaning and protective treatment against bacteria and mold must be given which is curing.

- Curing: is a temporary treatment of hide so that microorganisms cannot break it down for food and damage it the reason it is designed to be temporary is that various end products can be manufactured from the raw hide or skin. The purpose of curing a hide or skin is to temporarily preserve starting from the time it is removed from the animal until it can be processed into final product.

The leather making process is batch process and it is classified in to four main categories: - Beamhouse operation, Tanning operation, Post tanning operation and Finishing. Operations carried out in the beamhouse, tanning and post tanning operations are wet processes because they are performed in a vessels filled with water; after post tanning the leather is dried and operations are referred to as dry processing.

The skeleton of the leather processing is as follows: -

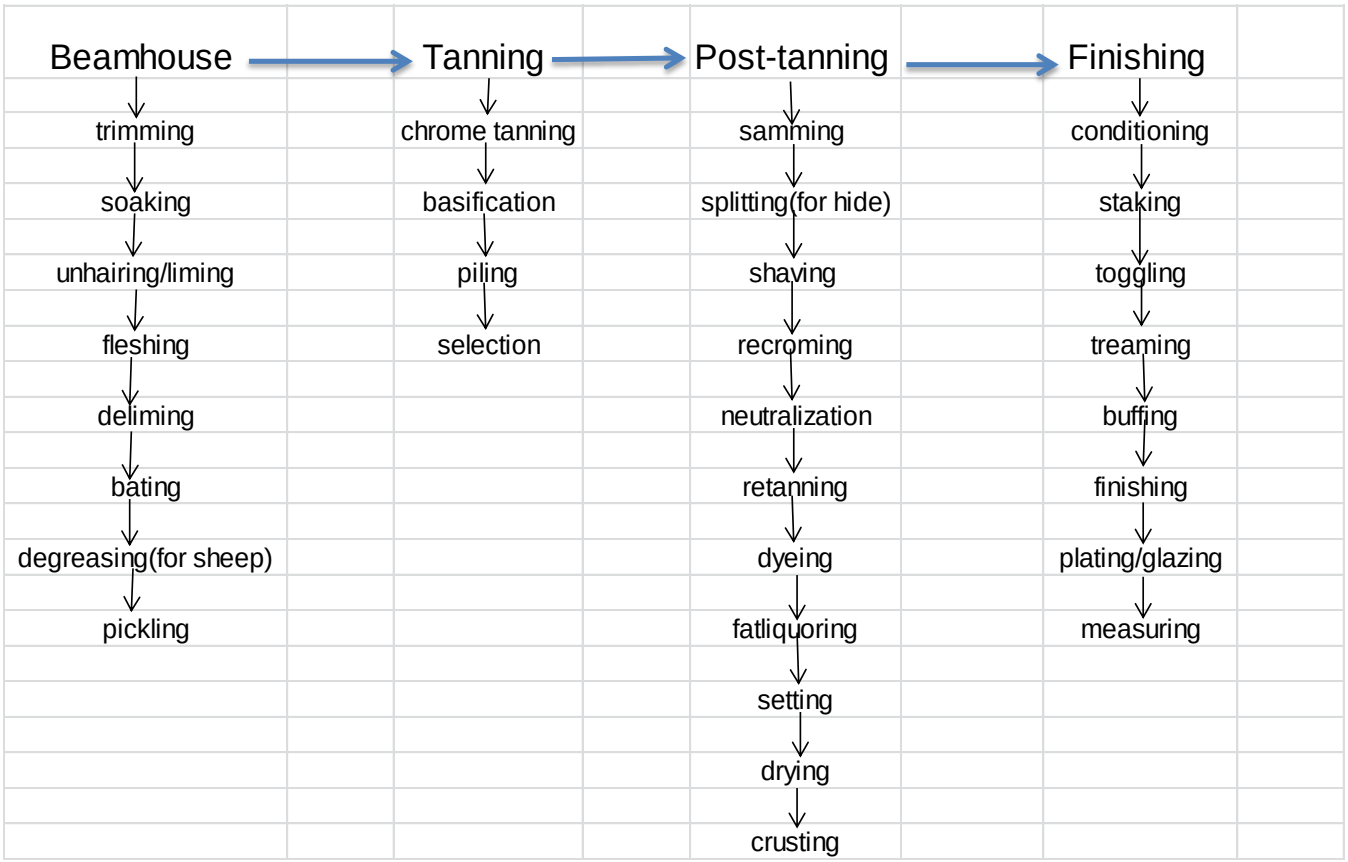


Figure 2.1: Process flow of leather making

❖ Beamhouse operation

This process in the tannery is between the removal of hide/skin from the storage and there preparation for tanning. Beamhouse operations have a tremendous importance on the ultimate quality of leather and it includes operations such as trimming, soaking, pickling, liming/unhairing, fleshing, deliming, bating, degreasing and pickling.

- Trimming: The hides and skins are trimmed to remove long shanks and other perimeter areas which do not go into making of leather goods it helps in preventing unnecessary wastage of chemicals. The trimmed hides or skins are sorted for size and weight to form in to batches then undergoes the next treatment.

- Soaking: The hides and skins loss there natural moisture due to curing therefore the main objective will be to restore the lost moisture so that the chemical treatments that follow will achieve optimum results. The method and duration of soaking vary according to the available equipment in the tannery (pit, drum or paddle) and the condition of the raw stock.
- Liming: The process of liming will uniformly open up the fiber structure and remove the hair and epidermis. The requirement for flat tight and mellow leathers with maximum area yield will be fulfilled if correct liming process is done. The liming process opens up the collagen fibers by the alkaline swelling of the structure and further pulping of the hair or epidermal protein (keratin) occurs by reduction of the disulphide bridges under the influence of alkali. After liming the hides or skins are called PELTS.
- Fleshing: is a mechanical operation that is carried out after liming and it helps to remove the unwanted flesh part on the hide or skin of the animal.
- Deliming: is the removal of alkali and the adjustment of pH for bating. After fleshing the pelts are in plumping conditions and they are full of lime and the pH is high (above 12) so before they are taken for tanning it is necessary to free them from lime , reduce the plump and lower the ph nearer to the tanning range.
- Bating: This process is used to remove scud i.e. hair roots, hair pigment and other protein substances left from liming thus avoid the precipitation of this matter on to the grain during the subsequent pickling and tanning processes.
- Pickling: is a process of acidification of pelt by using salt and dilute acid (organic or inorganic) in order to preserve or make ready for tanning. The salts used in this process helps in avoiding acid swelling by reducing concentration gradient between the pickle liquor and the pelt.

❖ **Tanning operation**

Tanning is a process by which the putrescible raw skin and hides are rendered stable by treating them with suitable chemicals. The purpose of tanning for hides or skins is: - to stabilize against enzymatic degrading and increase its resistance to chemicals, to raise the shrinkage temperature and increase its resistance to hot water, enhance its strength property, reduce its shrinkage in volume area and thickness.

Tanning is a process of converting putrescible outer coverings of animal to non-putrescible leathers with definite physical, chemical and biological properties so that they can be used in our daily life (Dutta, 1999).

Tanning agents are classified in to two:

1. Mineral tanning agent
    - Chrome tanning
    - Aluminum, Zirconium and other tannin
2. Organic tanning agent
    - Vegetable tanning
    - Syntans
    - Aldehydes
    - Oil tanning

- Chrome tannage

Chrome tanning is the most stable and the most widely used process throughout the world. It is done by using basic chromium salts and chrome tanned leather is called wetblue. Some of the reasons why chrome tannage widely used are because:

- The method is simple
- A wide range of leather types can be made
- Good hydrothermal stability can be achieved
- It is a convenient holding stage - hides and skins are commonly shipped around the world in this condition
- Special properties can be introduced by incorporating polymers, syntans and fatliquors.

- Basification: Is an addition of sodium bicarbonate to maintain the ph of the leather between 3.8- 4. As the pH of the tanning is increased the sulphate associated with chromium becomes displaced by the hydroxyl groups become shared by Cr atoms through Basification – Olation.

- Piling: Up on drying, the tannage becomes more stable as the complex gives up hydrogen ions (H+) and Oxolation results.

At low pH conditions, the concentration of OH- in the solution is low, the basicity of chromic salt is also low and the sulphate will be present in the complex. The chrome complex reacts with the protein carboxyl group and the cross linking of chrome – collagen occur.

- Selection: After the hides or skins have been tanned and conditioned to compose lots that are more homogeneous especially as regards grain quality. The selection of the hides/skins that are to go toward making a particular finished product must be essentially basic on the following two criteria grain quality and hide/skin thickness.

❖ Post Tanning operation/ Crust preparation

After tanning and conditioning the hide undergoes a production cycle that directly modified the finished products characteristics. While shaving and splitting (for hide) establish the final thickness, the final drumming operations give the hides their basic color, flexibility and desired feel. Steps required for bringing wetblue to crust state are:

- Samming: is a mechanical operation that helps to decrease the moisture content of the wetblue so that there won't be a problem when the next operation (shaving) is performed.
- **Shaving**: evens the skins thickness and for hides also permits greater precision than is possible by splitting. The shaving machine the wetblue will be advanced by the still roller at the bottom with the grain against the roller, as the wetblue is shaved by another roller at the top which is fitted with helical shaving blades. The rollers have a converging motion. The bottom roller has low speed which is equivalent to the feed speed and the top roller has higher rotational speed. The blades on the top roller will remove the excess wetblue material from hides/ skins by means of a scraping action that produces shavings.
- Rechroming: is a useful process because after selection and shaving in tanneries different batch wetblue will come together for the retanning operation and this wet blues will not have the same tannage so doing this rechroming operation will help in bringing all the wetblue to same characteristics because once they are rechromed the rest operation will be done in one drum.

- Neutralization: To assure stability in heated conditions and resistance to boiling the amount of chrome left in the hide or skins must not be excessive. This also holds as regards avoiding the aggregation of tanning salts. In dyeing for example, this could cause non uniform coloring due to the negative effects on the absorption of the chemical products.

Therefore the free acids present in the leather must be removed and neutralized. These acids lower the ph and this could favor the release of chromium. Neutralization depends on the required characteristics of the article to be produced.

- Retanning: is done to fill the interfibrillary spaces with different types of substances to give the finished product greater firmness or modify its physical characteristics in some way. Retanning is a process of further imparting different properties to the skin matrix and it is an important unit operation and the purpose of retanning is to produce a further stabilization of the collagen network.
- Fatliquring: is a process of introducing oil in to the leather so that the individual fibers are uniformly coated. To bring stability the emulsifying components in the fatliquor such as acid esters and sulfonic acid groups will link to basic collagen amino acids by ionic bond. Fatliquors are oils, fats and waxes, synthetic or natural, plus associated products prepared in some way so that they become emulsifiable in water and suitable for use in leather in a water float.
- Dyeing: The dyeing of leather into a wide variety of colors plays an important part in meeting fashion requirements. Some leathers are only surface dyed, while others need completely penetrated dyeing, as is the case with suede leathers. Almost all leather is dyed. With few exceptions, such as vegetable tanned leathers with the natural look, leather is artificially colored and this visual aspect is an essential part of its aesthetic properties.
- Drying: The leather is dried to various moisture levels (commonly 14-25%). Leather is normally dried to 10-20% water content. This can be achieved in a number of ways and each method has a different effect on the finished leather.

❖ **Finishing**

Finishing consists of placing a series of coatings on the surface of the leather. These coatings are designed to protect the leather and produce surface effects pleasing to the eye and hand. Various mechanical operations are necessary to obtain the desired effect. Hydraulic presses, printing,

embossing machines, automatic spray applicators and vacuum driers are a few of the machines used in the finishing process. The aims of finishing are to level the colour, cover grain defects, control the gloss and provide a protective surface with good resistance to water, chemical attack and abrasion.

2.2 Tannery solid waste

The tanning process, with the exception of finishing, produces 35 m^3 wastewater on the average of per meter ton of raw hide or skin. According to UNIDO while processing tones of hide or skin 380kg of chemical waste and 637kg of solid waste mainly from the raw hides are generated. Others estimate show that during the pre-tanning leather processing stage, out of tons of raw hide or skin 510kg or 60% is solid waste and 30 m^3 of wastewater is generated in the process. Currently the tanneries in Ethiopia produce 234 metric tons of solid waste and 11,312 m^3 wastewater daily which are disposed to the surrounding without treatment (Legesse, 2011).

The production of chromium-containing solid waste including chrome shavings and tanned splits in tannery has been recognized as a problem for many years, but recent years, pressure from environmental authorities has given the problem increasing urgency. I n past decades, significant efforts have been made to decrease the amount of chrome shavings, but with more than 90% of tanneries adopting chrome tanning, chrome shavings from leather industry are unavoidable. (Deqiang Su, Preparation of Protein Retanning Agent by Grafting Modification of Collagen Hydrolysate Extracted from Chrome Shavings)

Solid wastes generated in leather industries contribute mainly skin trimmings, Keratin wastes, fleshing wastes, chrome shaving wastes and buffing wastes. It constitutes protein as the main component. If these protein and other chemicals, which are present in the chemical treated protein, are not utilized properly it will pose hazardous pollution problem to the environment (Kanagaraj, 2006).

2.2.1 Chrome shavings

After chrome tanning the chrome tanned leather will be shaved. Shaving evens the skins thickness and for hides also permits greater precision than is possible by splitting. This savings

and splits will contribute highly to the total solid waste generation of the tannery. Chrome shavings are small, thin pieces of chrome tanned fibrous matrix of collagen formed during the levelling operation.

Chrome shavings primarily consist of chromium and protein, which could be treated to give the potential resources of collagen protein and chromium. These wastes can be utilized with or without the presence of chromium. Attempts have also been made to reduce potassium dichromate using chrome shavings directly to give a chrome tanning agent product, usable in the tanning or retanning processing of leather industry. Prior research has demonstrated that it's an effective way to acid hydrolyze chrome shavings into a chromium-containing protein hydrolysate which can also be reused in retanning processing. It's found that it's cleaner and more economical to separate the protein-bound chromium by the treatment of alkali or enzyme and use the protein and chrome cake or chrome sludge for several applications (Deqiang Su, Preparation of protein Retanning agent by Grafting modification of collagen hydrolysate extracted from chrome shavings)

Land application and disposal of solid, chromium-containing tannery wastes has been widely practiced during most of the twentieth century. This is a rather expensive and environmentally inappropriate way of handling a waste material that has the potential for reutilization. In addition, the costs of disposal will continue to increase as fewer landfill sites can be found and the cost of transportation increases. About 75% of the chromium containing solid waste is produced when the tanned hide is shaved to a uniform thickness. These chrome shavings are small particles, in a variety of shapes, mainly consisting of collagen cross-linked with Cr (III) complexes. (Cabeza, 1998)

Chrome shavings hydrolyzed using magnesium oxide alone or in combination with calcium hydroxide, sodium hydroxide or sodium carbonate increases efficiency of the Solubilization and at the same time reduce the amount of enzyme needed and thus making the treatment more cost effective (Kanagaraj, 2006).

In general Leather industry is one of the polluting industries because of generation of huge amount of liquid and solid wastes, also emits obnoxious smell because of degradation of

proteinous material of skin and generation of gases such as NH_3, H_2S and CO_2. Solid wastes are raw trimmings, fleshings, chrome shavings, buffing dusts and keratin wastes. Accumulation of these wastes lead to sludge problem and choking of treatment pipes and finally results in reduction in efficiency of treatment plant. Treatment of solid wastes also is not cost effective, posing economic burden to the tanners. (Kanagaraj, 2006).

Hides come to the tanner as a by-product of the meat industry. The tanning process, in turn, generates even greater quantities of by-products and wastes than of finished leather. One metric ton of wet salted hides yield 200 kg of leather, along with about 250 kg of tanned solid waste and about 350 kg of non-tanned waste; 100 kg is lost in wastewater (Cabeza, 1998).

2.3 Collagen

Collagen /ˈkɒlədʒɪn/ is the main structural protein in the extracellular space in the various connective tissues in animal bodies. As the main component of connective tissue, it is the most abundant protein in mammals, making up from 25% to 35% of the whole-body protein content. Depending upon the degree of mineralization, collagen tissues may be rigid (bone), compliant (tendon), or have a gradient from rigid to compliant (cartilage). Collagen, in the form of elongated fibrils, is mostly found in fibrous tissues such as tendons, ligaments and skin. It is also abundant in corneas, cartilage, bones, blood vessels, the gut, intervertebral discs and the dentin in teeth. In muscle tissue, it serves as a major component of the endomysium.

Collagen constitutes one to two percent of muscle tissue, and accounts for 6% of the weight of strong, tendinous muscles. The fibroblast is the most common cell that creates collagen. Gelatin, which is used in food and industry, is collagen that has been irreversibly hydrolyzed. Collagen also has many medical uses in treating complications of the bones and skin (https://en.wikipedia.org/wiki/Collagen).

The term collagen is used as a generic term for proteins forming a characteristics triple helix of three polypeptide chains and all members of the collagen family from these supera molecular structure in the extracellular matrix although there size, function and tissue distribution. Collagens are centrally involved in the formation of fibrillar and microfibrillar networks of the

extracellular matrix. Despite the rather high structural diversity among the different collagen type, all members of the collagen family have one characteristic feature: a right-handed triple helix composed of three α-chains (Gelse.K, 2003).

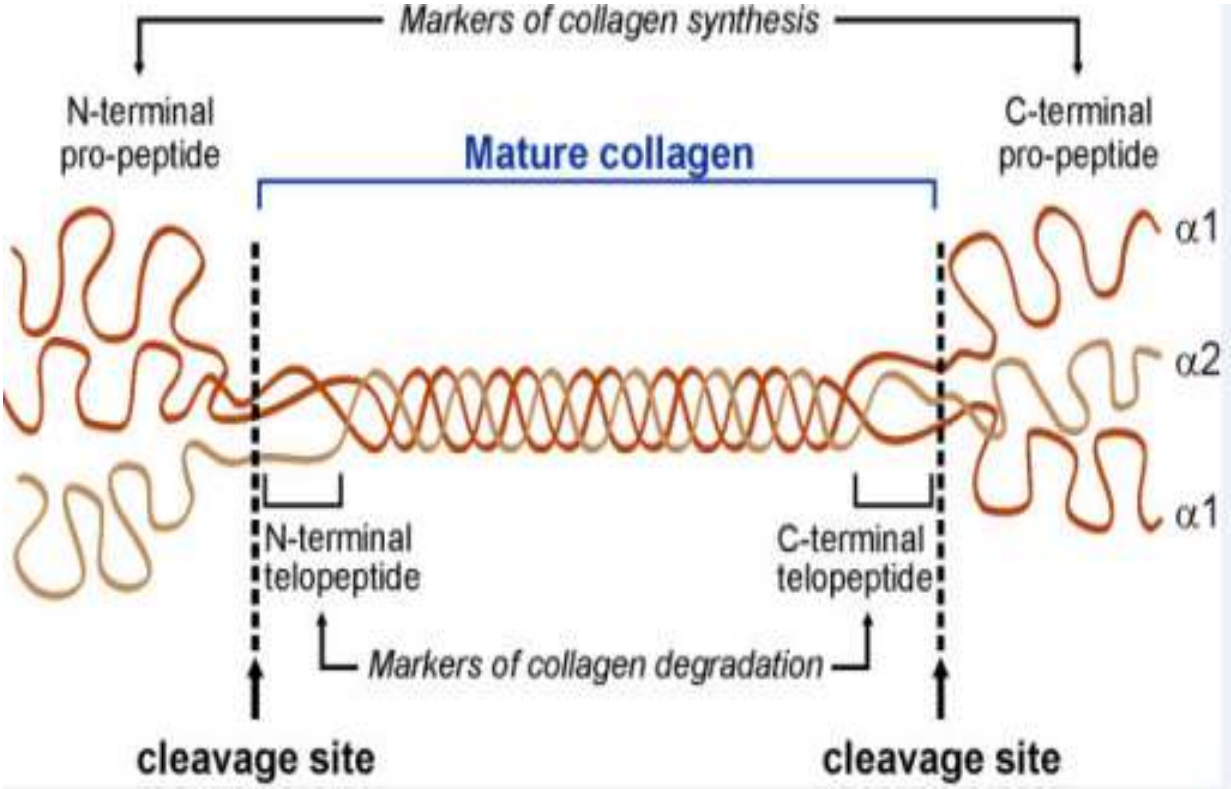


Figure 2.2: Molecular structure of fibrillar collagens with the various subdomains as well as the cleavage sites for N- and C-propeptides

The notation of the triple helix structure of collagen was first proposed by Ramachandran. In type one collagen, the monomeric molecule, protocollagen, contains three chains, designated αI (2) and αI (2): there are two αI (1) and αI (2) chain, which differ only in the details of the amino acid sequence. These three chains twist about each other in right-handed or clockwise triple helix: this is only possible because of the high glycine content, which has the simplest α-carbon side chain, a hydrogen atom, so that glycine is always situated in the center of the triple helix. Each α-chain is about 1050 amino acid long so the triple helix takes the form of a road about 300nm long with a diameter of 1.5nm: this is the monomeric unit from which the polymeric fibrous structure is created (Convington, 2009).

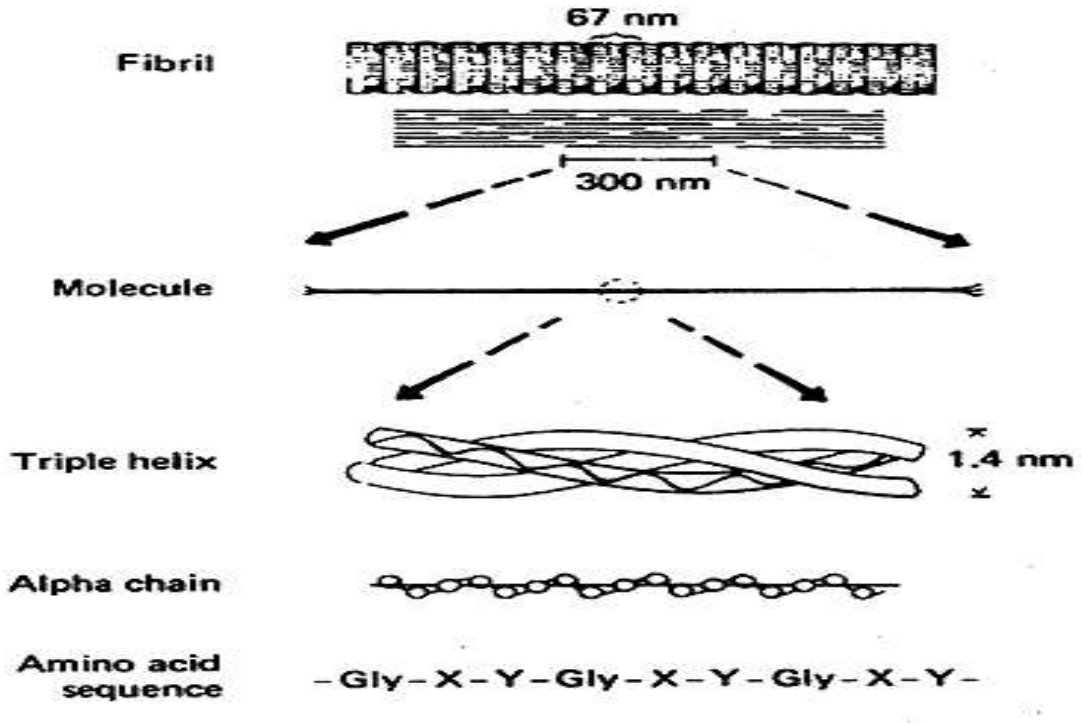


Figure 2.3: Triple helix collagen structure

2.3.1 Collagen Hydrolysate

Hydrolyzed collagen is produced from collagen found in the bones, skin, and connective tissue of animals. The process of hydrolysis involves breaking down the molecular bonds between individual collagen strands and peptides using combinations of physical, chemical or biological means. Typically, with skin-sourced collagen (Type-I collagens), hides are put in a lime slurry pit for up to 3 months, loosening collagen bonds; the hides are then washed to remove lime, and the collagen extracted in boiling water. The extracted collagen is evaporator concentrated, desiccated with drum driers and pulverized (https://en.wikipedia.org/wiki/Hydrolized_collagen).

Several researchers have detanned the chrome product for gelatin preparation and isolation of collagen fibers. Chromium containing leather waste can be treated with enzymes, but only after pretreatment to denature the collagen. Gelable and hydrolysed protein products from chromium containing leather waste have been obtained using magnesium oxide (MgO), carbonates and hydroxides. Carbonates and hydroxides have a detrimental effect whereas MgO has the most significant effect.

The use of Hydrolysate from chrome-tanned wastes for producing biodegradable plastic particularly applicable in agriculture for the breakdown of synthetic polymers has been studied. Protein Hydrolysate from chrome shavings markedly increases biodegradation of material and also exerts a positive influence on mechanical properties (Kanagaraj, 2006).

M.M Taylor etal (Taylor M.M, 1993) treated chrome shavings using alkaline agent without and with enzyme showing the effect of various alkali treatments on total solids and total ash content of the final product and detailed characterization of the gelable protein and hydrolyzed protein products and effects of processing parameters on chrome content of protein.

L.F Cabeza etal (Cabeza, 1998) have done isolation of protein products and chrome cake from chrome shavings using an alkali in the first step and with alkaline proteinase in the second to isolate two different protein products: gelatin and hydrolysate. They also try to demonstrate that very different range of molecular weight distribution of collagen degradation products.

Eleanor M etal (Eleanor M. Brown)`used Magnesium oxide with alkaline hydroxide, for the hydrolysis of chrome shavings. Their aim was to develop a more recent two-step process that treats the chrome shavings first under mild alkaline condition to produce a high molecular weight glable protein fraction for value added production of gels, adhesives and films then treat the remaining sludge with enzyme.

E. Langmaler etal (Langmailer.E)treated chrome tanned leather waste by magnesium oxide and a commercial proteolytic preparation (i.e. Alkalase Novonordisk) was added and enzymatic hydrolysis was conducted together they have conducted Electrophoresis on tricine 10-20% polyacryloamide gel at a pH of 8.45 in a mini-cell (X Cell II^{TM}, Innovex, Vienna) using a programmable Electric current intensity of 80mA at the beginning and 40mA at the end.

M. Catalina etal (Catalina.M) study the effect of crosslinking agents on the production of isolated gelatin from dechromed shaving by thermal and chemical degradation of collagenous materials obtained from shavings. They evaluated Thermal Analysis, Swelling and Mechanical property by testing tensile test and molecular weight distribution using SDS-PAGE analysis.

Research over several years in our laboratory has been designed to fully utilize chrome shavings, and a complete set of technological alternative based on separating the protein-bound chromium by the treatment of sodium hydroxide for reutilization of these waste has developed, including modifying collagen hydrolysate to give a retanning agent and using chrome cake or chrome sludge as a reductant for potassium dichromate to give a chrome tanning agent. (Deqiang Su, Preparation of Protein Retanning Agent by Grafting Modification of Collagen Hydrolysate Extracted from Chrome Shavings)

2.4 Retanning syntans

Chrome tanned and shaved hides are retanned to give them the required uniform fullness and ability to retain their consistency after the drying process tends to flatten the hides and reduce their thickness. Retanning is done on hides which already have a base tanning employing either one or combination of mineral tanning agents or vegetable syntans, aldehydes or raw oils (e.g. fish, cod or sulpho-chlorinated oils) (K.T, 1996).

Retanning is a process of further imparting different properties to the skin matrix and it is an important unit operation and the purpose of retanning is to produce a further stabilization of the collagen network. This involves further processing of the stabilized collagen network and may comprise a further tannage (e.g. with combinations of chrome, vegetable, glutaraldehyde or syntan agent) when special characteristics such as perspiration resistance are required. Conditioning, softening, dyeing or bleaching may also be carried out.

Fiber structure of hide or skin is not uniform throughout the entire area and it is most common to fill the empty nature of chrome tanned leathers by retanning to improve the required properties of leathers, which are intended for making foot wear, garments, gloves, furniture and automotive upholstery etc. (Karthikeyan, 2007).

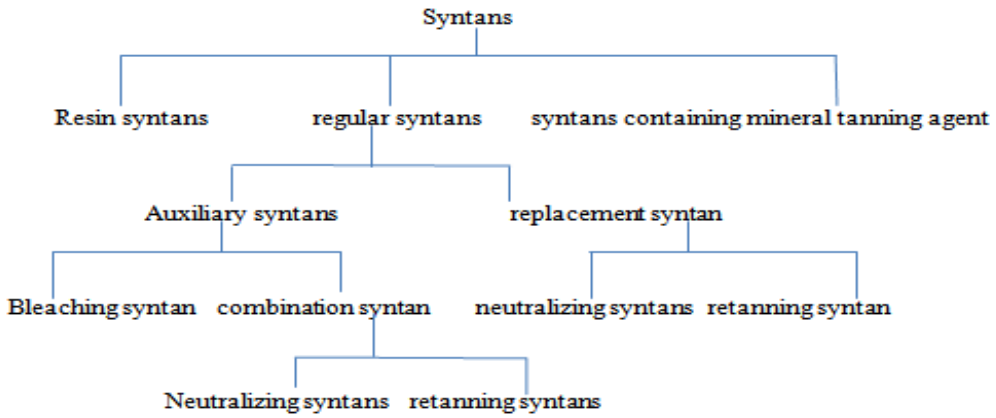
The following properties can be influenced by the retannage: Fullness, grain tightness softness, fat distribution, leather color, degree of whiteness, dyeing properties, levelness of the dyeing, light fastness, grain fineness, smoothness, dry drumming properties, embossing properties, water proofness, physical and chemical properties etc. The amounts of product required for the

retannage vary considerably depending on the type of leather, the nature of chrome leather and other criteria, and range from about 2% to 30%, calculated on the shaved weight of the leather.

Retanning agents like vegetable extracts and retanning syntans preferentially go to the looser regions such as bellies due to more opened up structure and produce a filling effect, bringing about uniformity over the entire area of the leather. These retanning materials are mostly anionic in nature and hence have high affinity for chrome tanned leather, particularly vegetable tanning and replacement type of syntans. Syntans are synthetic tanning agents which are high molecular organic compounds and they are used in retanning in the production of chrome tanned leathers to give them new properties distinguished it from conventional chrome leather (K.T, 1996).

Today several developments are taking place in the field of retanning such as phenol formaldehyde and naphthalene formaldehyde condensates, melamine, dicyandiamide and carbodiamide based syntans, polymers of various types, such as acrylates, urethanes and melamine resins. Most of these retanning agents are still suspected in their application due to release of high COD, TDS, free phenol and free formaldehyde. Proteins and protein hydrolysate are finding increasing amounts of applications as fillers in retanning operations. Protein based retanning agents offer better prospects as they fill loose areas such as belly, flanks and poor substance materials without contributing much load to tannery effluent (KarthiKeyan, 2011).

Classification of syntans:-



Leather must have the necessary body, thickness, tightness of grain, improved cutting value and minimum or no looseness. The alternative is to fill the voids between the corium major and the corium minor with some foreign materials like natural or synthetic tannins and different types of resins. The syntans used in the process are explained below.

2.4.1 Resin syntans:

Resin syntans are the condensation products of urea, Dicyanamide melamine with formaldehyde. Resin syntans particularly have no tanning property and so penetrate more to the empty regions of the leather, bellies and shanks. These syntans thus bring uniformity in thickness and substance between the butt, bellies and shanks by filling up the voids of the looser parts of the leather (K.T, 1996).

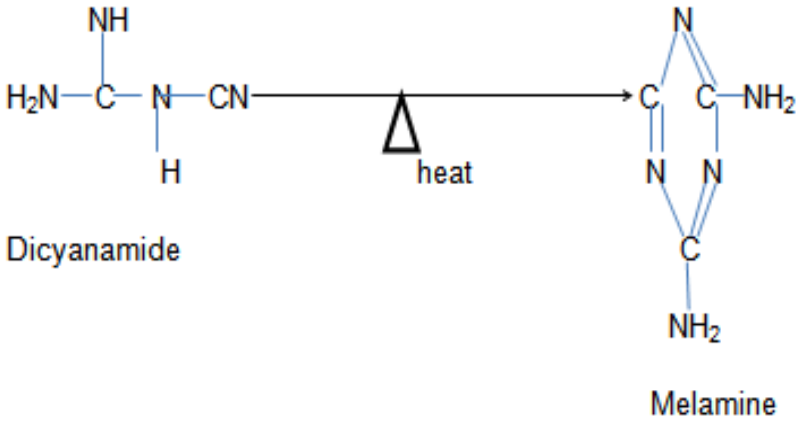
Resin syntans may be anionic or cationic in nature, for fixation the cationic leathers (chrome tanned) are treated with anionic resins and anionic leathers (vegetable tanned) are treated with cationic resin syntans. Resin syntans are classified into: -

- urea formaldehyde resin syntan
- Dicyanamide resin syntan
- melamine resin syntan
- acrylic resin syntan

Acrylic syntans are leather chemicals which are polymeric in nature. They are homo-polymers or co-polymers of acrylic acid, methacrylic acid and acrylonitrile in aqueous solutions.

- Acrylic syntans are useful in retanning of chrome leather for making suede leather.
- Acrylic syntans help in achieving round full firm handle & good grain characteristics with softness.
- Acrylic syntans are anionic in nature and can be used in combination with other anionic syntans.
- Acrylic tanning agents are light fast in nature.

Melamine syntans are cyclic compounds formed by heating Dicyanamide.



Dicyanamide

Melamine

Property of melamine syntan: -

- Good filling property
- Good bleaching effect
- Give shrinkage temperature up to 92 - 93^0c
- Not suitable for treating vegetable tanned leathers but suitable for alum tanned leathers.

2.4.2 Replacement syntans:

Replacement syntans are leather chemicals used as synthetic substitutes of vegetable tanning agents for retanning of chrome tanned wet blue leather. Replacement syntans are based on the condensation products of phenol and naphthalene sulfonic acids. Replacements are less soluble in water but have better tanning properties than combination syntans 60-65%. The solution of this syntans shows less acidity high ph value, low bleaching and sludge solubilizing capacity (K.T, 1996).

- Replacement syntans are favored for retanning chrome leather to improve fullness and level dyeing thru uniform uptake and penetration of fatliquors and dyes which leads to improvement in buffing properties.
- Replacement syntans reduce cationic charge of chrome leather.

- Replacement syntans are used to produce white leathers.
- Replacement syntans are also amphoteric with respect to their ionic charge and these can be used at different pH conditions exhibiting pH dependent tanning properties.

2.4.3 Vegetable tannins

Vegetable tannins are natural organic astringent materials obtained from plants. They are derivatives of phenol (with several OH groups). Phenols are more acidic than alcohols (pKa~10), but are weak acids therefore form salts only with strong bases. Solubility of phenol is ~7% in cold water. But the sodium salt is soluble.

Vegetable tannins react with atmospheric oxygen, particularly at high pH values to form quinones (two oxygen atoms attached to opposite points of benzene ring) for OH groups that are ortho-para to one another. Vegetable tanning liquors are very complex and continually changing physically, chemically and biologically. They are partly colloidal but easily aggregate and will then sediment. Yeasts, molds and bacteria can grow in the liquors, the main consequence being the fermentation of sugars to acids (K.T, 1996).

All the syntan do not have the same power of tanning. The factors that tanning power of the syntan depends on are: -

- Phenolic hydroxyl groups
- Position of hydroxyl groups: - compounds with hydroxyl group is the ortho and para positions have tanning power but compounds with –OH groups in the meta positions have no tanning properties.
- Distance of hydroxyl groups from strong acid groups: - syntans with sufficient phenolic hydroxyl groups in proper positions may not have tanning power if strong acid groups are present very near to –OH groups.
- Syntans with no hydroxyl groups
- Nature of connecting bridge
- Molecular size

Research over several years in our laboratory has been designed to fully utilize chrome shavings, and a complete set of technological alternative based on separating the protein-bound chromium

by the treatment of sodium hydroxide for reutilization of these waste has developed, including modifying collagen hydrolysate to give a retanning agent and using chrome cake or chrome sludge as a reductant for potassium dichromate to give a chrome tanning agent (Deqiang Su, Preparation of protein Retanning agent by Grafting modification of collagen hydrolysate extracted from chrome shavings).

In order to understand the possible interactions between silicon species and collagen, the effects of sodium silicate, molecular complexes of silicon and silica nanoparticles on the collagen self-assembly process have been extensively studied at a range of concentrations from ca. 8 × 10−5 to 1 × 10−2 M. The mode of interaction between collagen and 'silicon' appears species dependent. Depending on its concentration, silicate solutions either promote or hinder collagen fibrillogenesis. Low concentrations of a silicate solution promote fibril formation as does the addition of a silicon catecholato complex. The presence of silica nanoparticles and concentrated silicate solutions hinders fibril formation. The data obtained suggest that there may be direct interaction between the various 'Si' containing species and the collagen triple helices as initially formed (David Eglin).

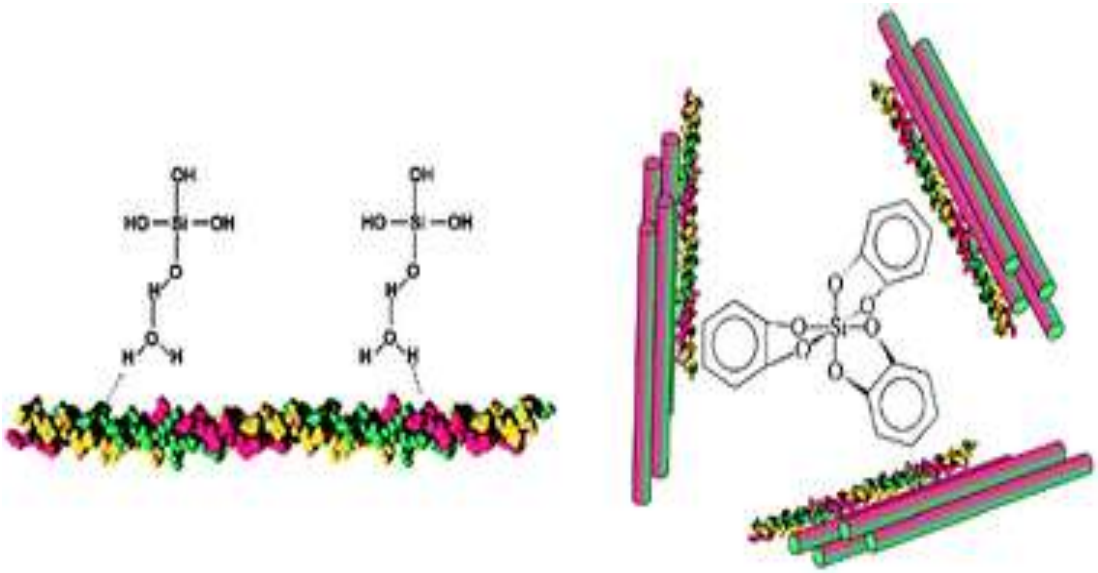


Figure 2.4: The effect of collagen self-assembly on 'silicon' speciation/condensation

Keratinous wastes are converted into keratin hydrolysate by hydrolysis with sodium hydroxide to get a soluble hydrolysate suitable for leather processing, particularly in retanning process.

Conventionally, keratin hydrolysate (KH) prepared by alkali (e.g. NaOH) and acid (e.g. HCl) hydrolysis requires neutralization to ensure that it is suitable for applications in animal feed, cosmetic and leather processing industries. Besides, the existing keratin hydrolysate prepared by alkali hydrolysis and microbial degradation for retanning of wet blue leathers imparts color to the leather, which is not desirable and moreover, the product does not have antimicrobial and tanning potency. This limitation of these products eventually affects the economy of application adversely. The advantages of using silicates to convert keratin into water soluble keratin hydrolysate for application in retanning are many fold. Conventionally, silicates have been used for the preparation of various industrial products exhibiting antimicrobial property. Silicate containing sheet with antimicrobial and antifouling properties and a fiber structure comprising a zeolite layer and an alkyl silicate layer suitable for textiles are some of the examples. Another advantage of using silicate is that it would enhance tanning action (shrinkage temperature) of the keratin hydrolysate; as the use of alkali metal silicates for tanning of animal skins is well known. Alkali silicates have also been used to improve the exhaustion of added auxiliaries, especially of chrome tannins in leather processing. Silicates could also be used for the preservation of hides and skins. Hence, there is a scope to develop keratin-silica based retanning agent that overcomes the existing problems in the conventional keratin hydrolysate used as a retanning agent (KarthiKeyan, 2011).

2.5 Animal glue

Animal glue is an adhesive that is created by prolonged boiling of animal connective tissue. These protein colloid glues are formed through hydrolysis of the collagen from skins, bones, tendons, and other tissues, similar to gelatin. The word "collagen" itself derives from Greek κόλλα kolla, glue. These proteins form a molecular bond with the glued object. Today, animal glues are sparsely industrialized, but still used for making and restoring objects, paintings, illuminated parchment manuscripts, and other artifacts Retanning syntans. Gelatin, a form of animal glue, is found in many contemporary products, such as gelatin desserts, marshmallows, and pharmaceutical capsules, and is used to reinforce sinew wrappings, wood, leather, bark, and paper. Chrome tanned and shaved hides are retanned to give them the required uniform fullness and ability to retain their consistency after the drying process tends to flatten the hides and

reduce their thickness. Retanning is done on hides which already have a base tanning employing either one or combination of mineral tanning agents or vegetable syntans, aldehydes or raw oils (e.g. fish, cod or sulpho-chlorinated oils) bath for the container of glue. Most animal glues are soluble in water, useful for joints which may at some time need to be separated. Alcohol is sometimes applied to such joints to dehydrate the glue, making it more brittle and easier to crack apart. Specific types include hide glue, bone glue, fish glue, and rabbit skin glue (https:// en.wikipedia.org/Animal_glue).

Most common production of animal glue is Animal hides will be soaked in water to produce "stock." The stock is then treated with lime to break down the hides. The hides are then rinsed to remove the lime, any residue being neutralized with a weak acid solution. The hides are heated, in water, to a carefully controlled temperature around 70 degrees Celsius. The 'glue liquor' is then drawn off, more water added, and the process repeated at increasing temperatures. The glue liquor is then dried and chipped into pellets (https:// en.wikipedia.org/Animal_glue).

The source of raw stock for manufacturing of glue and gelatin are animal connective tissues comprising protein collagen. They are mostly present in hides and skins by-products. Limed fleshings and trimmings are invariably good stock for production of glue and gelatin. In every extraction cycle the float stands at least 5 to 8 cm above the level of the stock at the start of the cycle. The stock to float ratio may be about 1:2 to 1:2.5. The extraction of the glue is carried out in 5 to 6 extractions at approximate temperatures of 60^0C, 70^0C, 80^0C, 85^0C and 90^0C in an extraction tank and finally by boiling and the extraction period varies. (Technology of Animal and Tannery by-product Utilization.).

The main objectivities of this work is to prepare a retanning agent by modifying CH with sodium silicate and to evaluate the behavior of protein retanning agent that is modified using sodium silicate and also to prepare glue from the collagen Hydrolysate.

3 Materials and Methods

3.2 Materials

The materials used for this study was Hide wet blue shavings, Sodium hydroxide, Sulphuric acid, Collagen hydrolysate, Sodium silicate and chemicals for leather processing (wetting agent, sodium formate, sodium bicarbonate, protein containing syntan (Nelfill powder), synthetic fatliquor (FosFol BPC), formic acid).

3.3 Equipment

Conical flask, heating mantle, hot plate, pH paper, filter pad, beaker, hot air oven, refrigerator, small testing drum, shaving machine, setting out machine, overhead drier, staking machine, analytical instruments (FTIR, DSC/TGA, SEM, CIE color measurement) and physical testing equipments.

3.4 Methods

3.4.1 Raw material collection and pre treatment

The chrome shavings were collected from local tannery in Addis Ababa. The raw weight of these shavings kept at room temperature was determined and placed in to a container. 500% water and 0.3% surfactant was added based on the raw weight and mixed well then left overnight to continue the wet back process. Next day, the soaked shavings was washed thoroughly with water and the color of shavings will turned into light blue, which means some of free (unreacted surface) chromium was removed.

3.4.2 Characterization of chrome shavings

Chrome shavings were kept at room temperature after collection. The raw material was characterized for the moisture content, ash content, fat content, and FT-IR analysis (Fourier Transform Infrared Spectroscopy).

3.4.2.1 **Moisture content**

Moisture content of the chrome shavings was determined according to ISO 4684:2005 or SLC 3 (IUC 3; BS 1309:3). For moisture determination, the samples were weighed into dry, tared porcelain dishes. The samples were dried for 17hrs at 105^{o}C. The samples were cooled in a desiccator, weighed and the percent moisture determined.

3.4.2.2 **Ash content**

Total ash content of the chrome shavings was determined according to ISO 4047:1977 or SLC 6 (IUC 7; BS 1309:6). For ash determination, the dried samples were ashed at 750^{o}C for two hours. The samples were cooled in a desiccator and weighed to determine the ash content. The detailed procedure is attached as Annex 1.

3.4.2.3 **Fat content**

Fat content of chrome shaving was determined according to ISO 4048:2008 or SLC 4 (IUC 4; BS 1309:4). The procedure is attached as Annex 2.

3.4.2.4 **Chromium content**

Chromium content of chrome shavings was determined according to ISO 5398:2007 SLC 8 (IUC 8; BS 1309:8). The chromium content in the chrome shavings was estimated through perchloric acid digestion method. A known quantity of sample was accurately weighed and digested by using an acid mixture containing 11.5 mL of perchloric acid, 3.5 mL of sulfuric acid and 5 mL of concentrated nitric acid. The trivalent chromium was converted into hexavalent chromium during digestion which could be seen by change of colour from green to orange. The flask was immediately cooled by dipping in through cold water with constant stirring. 50 mL of distilled water was added with a few porous tiles. The content of the flask were boiled once again for 5-10 min to expel chlorine. It was then cooled and titrated against standard N/10 sodium thiosulphate using 10% KI and starch indicator. End point was the disappearance of blue colour.

The chromium content as percentage by mass on the original material has been calculated using the factor

1 mL of 0.1 N titrant = 0.00173 g Cr = 0.00253 g Cr_2O_3.

3.4.2.5 **Fourier Transform Infrared Spectroscopy (FT-IR)**

The FT-IR spectra of chrome shaving sample were measured by using spectrum 65 FT-IR (PerkinElmer) in the range 4000-400 cm-1 using KBr pellets.

3.4.3 Isolation of Protein (Collagen) and Chromium from chrome shavings

The wet backed shavings were tumbled in tap water for one hour with repeated washings to remove the chromium on the surface and latter with distilled water. The washed chrome shavings were treated initially with 5% sodium hydroxide solution (100g chrome shavings: 100ml 5% NaOH solution) for two hours and washed again with running water (to bring the pH 7 and the pH was measured using pH paper) and later with distilled water and finally dechromed with concentrated Sulphuric acid (100g chrome shavings: 10ml Conc. H_2SO_4) for 30 minutes. The chrome shavings were thoroughly washed in running water till they are completely dechromed. Chrome liquor was collected in each steps of washings from this chromium can be recovered.

3.4.4 Characterization of Collagen Hydrolysate

Then the collagen hydrolysate prepared was characterized for moisture, ash, chrome, fat content and FT-IR (Fourier Transform Infrared Spectroscopy), DSC (Differential Scanning Calorimetry) and TGA (Thermo gravimetric analysis).

3.4.4.1 **Moisture content**

Moisture content of the collagen hydrolyaste was determined according the method mentioned in section 3.4.2.1.

3.4.4.2 **Ash content**

Total ash content of the collagen hydrolysate was determined according to 3.4.2.2.

3.4.4.3 **Fat content**

Fat content of collagen hydrolysate was determined according to 3.4.2.3.

3.4.4.4 **Chromium content**

Chromium content of collagen hydrolysate was determined using methodology in 3.4.2.4

3.4.4.5 **Fourier Transform Infrared Spectroscopy (FT-IR)**

The collagen hydrolysate sample was measured on spectrum 65 FT-IR (PerkinElmer) in the mid-infrared range 4000 to 400 cm-1 using KBr pellets.

3.4.4.6 **Differential Scanning Calorimetry and Thermo Gravimetric Analysis (DSC- TGA)**

The thermal stability of the collagen hydrolysate was determined with a thermo gravimetric analyzer SDT Q V20.9 Build 20 instrument with a temperature range of 0-1000^{0}C.

3.4.4.7 **Scanning Electron Microscopy - SEM**

The scanning electron microscopic analysis was carried out on the collagen hydrolysate using instrument JSM-IT300 scanning electron microscope.

3.4.5 Preparation of Collagen Silica Complex

Protein and protein hydrolysates have found applications in leather processing during retanning very long time before. Instead of using collagen hydrolysate alone for retanning/tanning, if the silicates are incorporated into the collagen matrix the advantages are manifold. Conventionally, silicates have been used for the preparation of various industrial products exhibiting antimicrobial property (Sugizaki et al 2003). Another advantage of using silicate is that it would enhance tanning action (shrinkage temperature) of the collagen hydrolysate and the use of alkali metal silicates for tanning of animal skins is well known (Fernald and Iler 1946). Silicates could also be used for the preservation of hides and skins (Munz 2007). Hence there is a scope to develop collagen-silica based retanning agent that overcomes the existing problems in the conventional collagen hydrolysate used as a retanning agent.

Collagen hydrolysate isolated from chrome shavings were kept under refrigeration. The collagen hydrolysate weighing 1kg was treated with 250gms of sodium silicate in a glass vessel. The temperature was maintained at 90-100ºC for a period of 2 hrs, the collagen hydrolysate starts to dissolve in the sodium silicate solution when the temperature starts rising. The product contains smaller peptides linked with silica species and it was denoted as CH-Si solution. The yield was stored in the liquid form for application in retanning.

Figure 3.1: collagen silica combination at 90^0C

3.4.5.1 **Application of CH-Si in Post tanning**

The CH–Si sample rich in protein content was used as protein filler in the retanning of chrome tanned leathers. Shaved wet blue sheep leathers having 0.9-1mm thickness were used as raw material for retanning trials. The wet blues were cut into sides on the backbone and marked as 1L, 1R, 2L, 2R … The leathers were washed and neutralized to pH 5.2 and washed twice. The process details for making sheep upper crust using CH-Si as a retanning syntan (Protein filler) is presented in the Table 3.1.

Table3.1: Retanning Process recipe for the experiment and control

Process	Chemicals	Percent	Time	Remark
Wet back	Water	200		
	Wetting agent	0.1	20 min	
	Formic acid	0.7	20 min	pH 3.2
Neutralization	Water	150		
	Sodium formate	1.5	25 min	
	Sodium bicarbonate	0.75	60 min	pH= 4.8-5.2
				Drain/ wash /drain
Retanning	Water 50^0c	50		
	Collagen hydrolysate (experimental)	15	45 min	
	Protein containing syntan (control)	15		
				Check exhaustion
Fatliquoring	Water 60^0c	100	50 min	
	Synthetic fatliquor	10		
				Check exhaustion
Fixation	Formic acid	0.5	20 min	
	Formic acid	0.5	20 min	pH 3.4-3.6
				Drain/ wash /drain
	Pile and leave over night the next day Sam setting and hung overhead			

3.4.5.2 **Scanning Electron Microscopy Analysis**

The scanning electron microscopic analysis was carried out on the collagen-silica treated crust leathers and the results were compared with commercial protein product. The samples measuring 5mm x 2mm were cut from the crust leathers using fresh stainless steel blades. The samples were mounted both vertically and horizontally on aluminum stubs using an adhesive. These were then coated with gold. The stubs were introduced into the specimen chamber of a JSM-IT300 scanning electron microscope. The stubs mounted on the stage could be tilted, rotated and moved to the desired position and orientation. The micrographs for the cross-section were obtained by operating the microscope at higher voltage.

3.4.5.3 **Physical Testing and Visual Assessment**

The samples for physical testing were cut from the CH-Si treated and control sheep crust leathers according to the official sampling position (IUP2 2000) from each trial run. The samples were conditioned at 20^0C ±2 and 65±4% R.H. for 48 h. The tensile and tear strengths were measured as per the IULTCS method (IUP6 2000, IUP8 2000). Experienced technologists assessed the organoleptic properties such as fullness, feel, grain tightness and general appearance. The leathers were rated on a scale of 0-10 points for each functional property, where higher points indicate better property.

3.4.6 Preparation of Glue from Collagen Hydrolysate

In some of the earlier studies glue was made from limed fleshings and trimmings by boiling them in the presence of sulphuric acid at a temperature between 60 and 90^0C. Chrome leather wastes, splits, shavings etc. could also used as raw stock for the extraction of glue. In such wastes, collagen is cross linked with tanning agents, namely basic chromium sulphate. To prepare glue, the tanning agent, namely chromium sulphate, should be removed or detanned from the waste (See Section 3.3.3) and subsequently hydrolysis should be carried out to yield glue.

Here in our study, the collagen hydrolysate isolated from chrome shavings was used. The extraction of glue was carried out in a glass beaker and the temperature was maintained around 90^0C for a period of 2-3h. The stock (collagen hydrolysate) to float ratio may be about 1:4 to 1:5.

Figure 3.2: Preparation of glue: Collagen Hydrolysate, CH and water being boiled and final glue solution respectively

3.4.7 Characterization of glue

3.4.7.1 Moisture content

Moisture content of the collagen hydrolysate was determined using methodology in 3.4.2.1.

3.4.7.2 Ash content

Total ash content of the collagen hydrolysate was determined according to 3.4.2.2.

3.4.7.3 CIE color measurement

The color characteristics of the glue prepared from collagen hydrolysate in terms of CIE color coordinates L, a, and b were studied using a computer controlled Gretagmacbeth spectrolino instrument and the results were compared with the locally prepared commercial glue made from fleshing wastes. Where L represents the difference between light (where L=100) and dark (where L=0), a represents the difference between green (-a) and red (+a), and b represents the difference between yellow (+b) and blue (-b).

The colour difference between glue prepared from chrome shavings and the glue from limed fleshings was calculated in terms of ΔE, the overall color difference using standard equation (Randall 1994, Tremlett 2003, Malathy et al 2004).

- $\Delta L = L_{Sample} - L_{Local}$ (if $+\Delta$ L, Sample is lighter than Local)
- $\Delta a = a_{Sample} - a_{Local}$ (if $+\Delta$ a, Sample is redder than Local)
- $\Delta b = b_{Sample} - b_{Local}$ (if $+\Delta$ b, Sample is yellower than Local)

4 Results and Discussion

4.1 Characterization of chrome shavings

The chrome shavings used as raw material to run experiments for the preparation of value added proteins were obtained from a local tannery at Addis Ababa. They were analyzed for moisture, ash, fat and chromium dry weight basis and the results are presented in Table 4.1. These values were typical for chrome shavings.

Table 4.1: Studies of Chrome Shavings

Parameter	Result
Moisture	51.5%
Ash	9.2%
Fat	0.85%
Total Chromium	4.2%

4.1.1 Fourier Transform Infrared Spectroscopy (FT-IR)

The infrared spectra of chrome shaving sample are shown in the Figure 4.1. The absorption peak at 1638.46 cm-1 represents amide I of chrome shavings. Amide II and III absorption peaks normally appear in the wavelengths ranges from 1500-1550 and 1220-1280 respectively. But in the FT-IR spectra of chrome shavings, these peaks are not present. This may be due to the reaction of chromium with collagen protein. A broad strong absorption at 3428.85 cm^{-1} region results from superimposed O-H and NH_3^+ stretching bands.

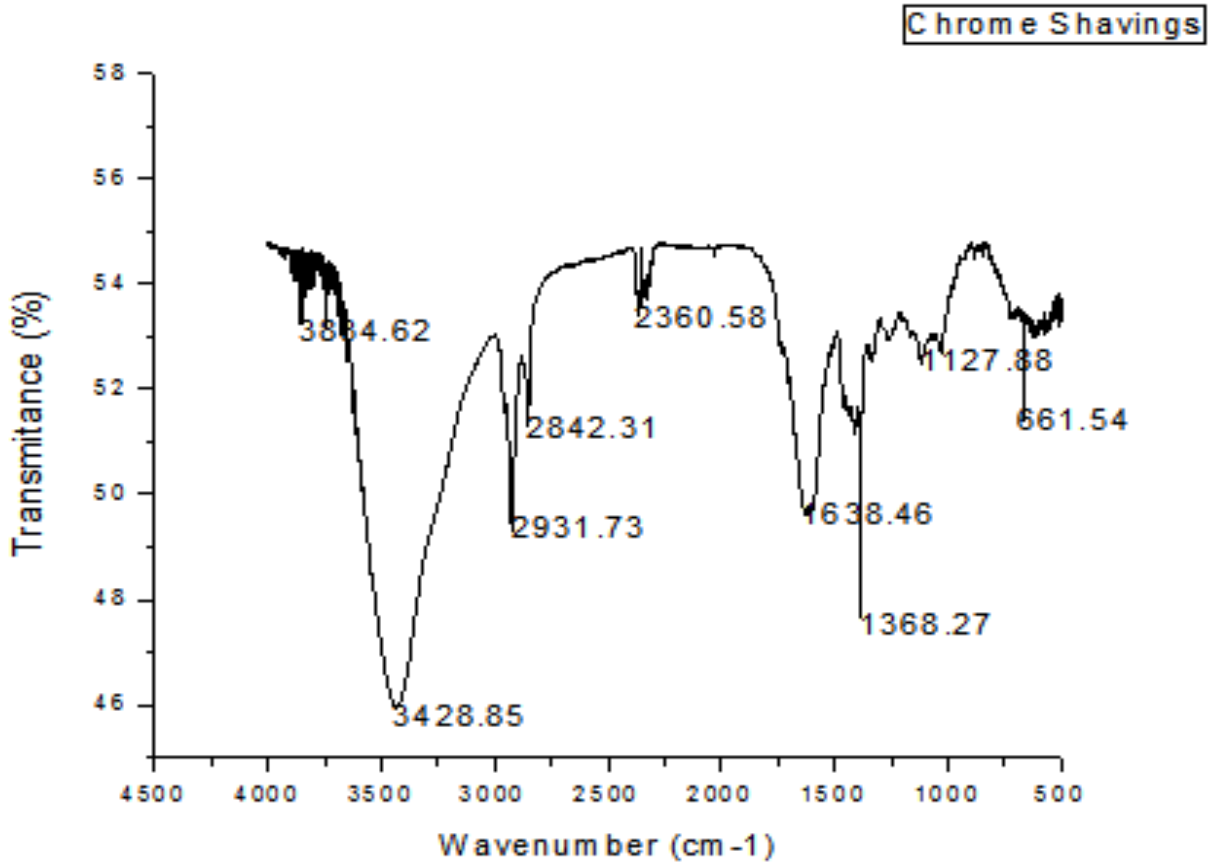


Figure 4.1: FT-IR Spectra of chrome shavings

4.2 Characterization of Collagen Hydrolysate

- ✓ **Moisture content**

The moisture content based on the procedure mentioned in section 3.3.1.1 and according to SLC 3 (IUC 3; BS 1309:3) was determined. It was found that the collagen hydrolysate contains 46.9% moisture.

- ✓ **Ash content**

Total ash content of the collagen hydrolysate was determined according to SLC 6 (IUC 7; BS 1309:6) and it is found that the collagen hydrolysate contains 0.9% ash based on dry collagen hydrolysate which lets us know that chromium is removed.

- ✓ **Fat content**

Fat content of collagen hydrolysate was determined according to SLC 4 (IUC 4; BS 1309:4). Accordingly the fat content of the collagen hydrolysate is 0.9% based on dry collagen hydrolysate weight.

- ✓ **Chromium content**

Chromium content of collagen hydrolysate was determined according to SLC 8 (IUC 8; BS 1309:8), and it was found the chrome shaving contains 2.3 mg/L. This result shows that the removal of chromium from the chrome shavings was successful but still a negligible quantity of chrome is available in the collagen hydrolysate.

4.2.1 Scanning Electron Microscopy

The SEM picture of isolated collagen hydrolysate sample is shown in Figure 4.2. This figure show loosely knit fibrous matrix and interspaces are clearly seen due to the treatment of alkali and acid. Well organized cohesion between the fibers is not seen as like in crust leathers.

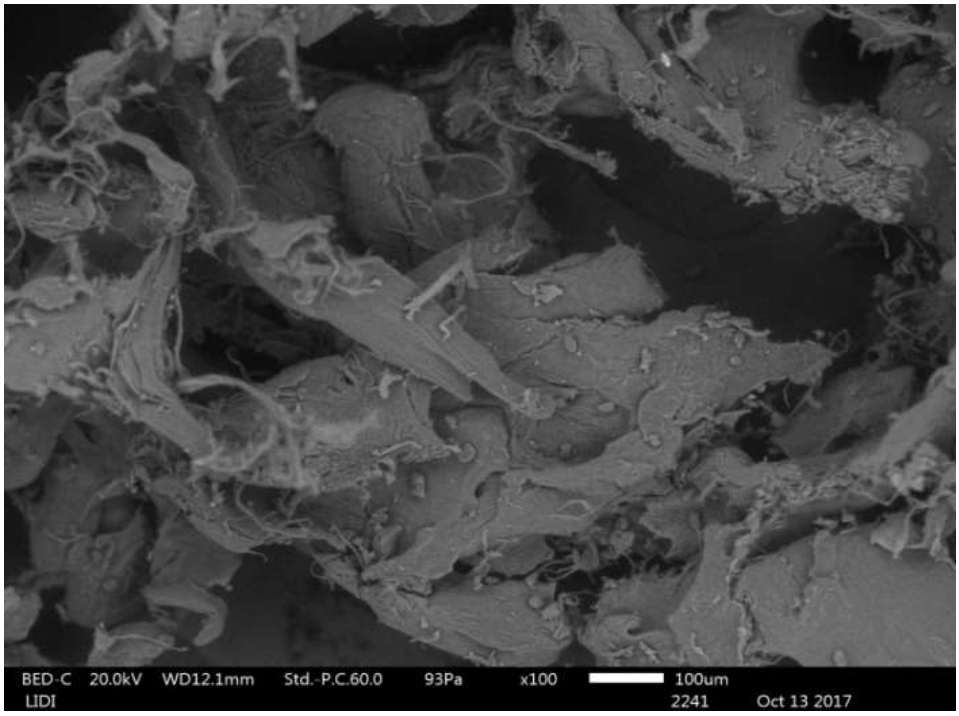


Figure 4.2: Scanning Electron Micrograph of Collagen Hydrolysate

4.2.2 Differential Scanning Calorimetry and Thermo Gravimetric Analysis

The thermal stability of collagen hydrolysate sample was measured by using DSC and TGA and the results are presented in Figure 4.3. The DSC graph indicates that the collagen hydrolysate shows lower thermal stability (70^oC). This low temperature is due breakage of the crosslinks between chromium and collagen during isolation. The thermogravimetric profile of collagen hydrolysate indicates the weight percent of residual composite at different temperature. Generally, the TGA curve shows a gradual weight loss due to absorbed moisture upon initial heating up to around 100^oC, followed by a slow weight loss until around 380^oC and the final degradation of the peptides occurs from around 380 to 500^oC.

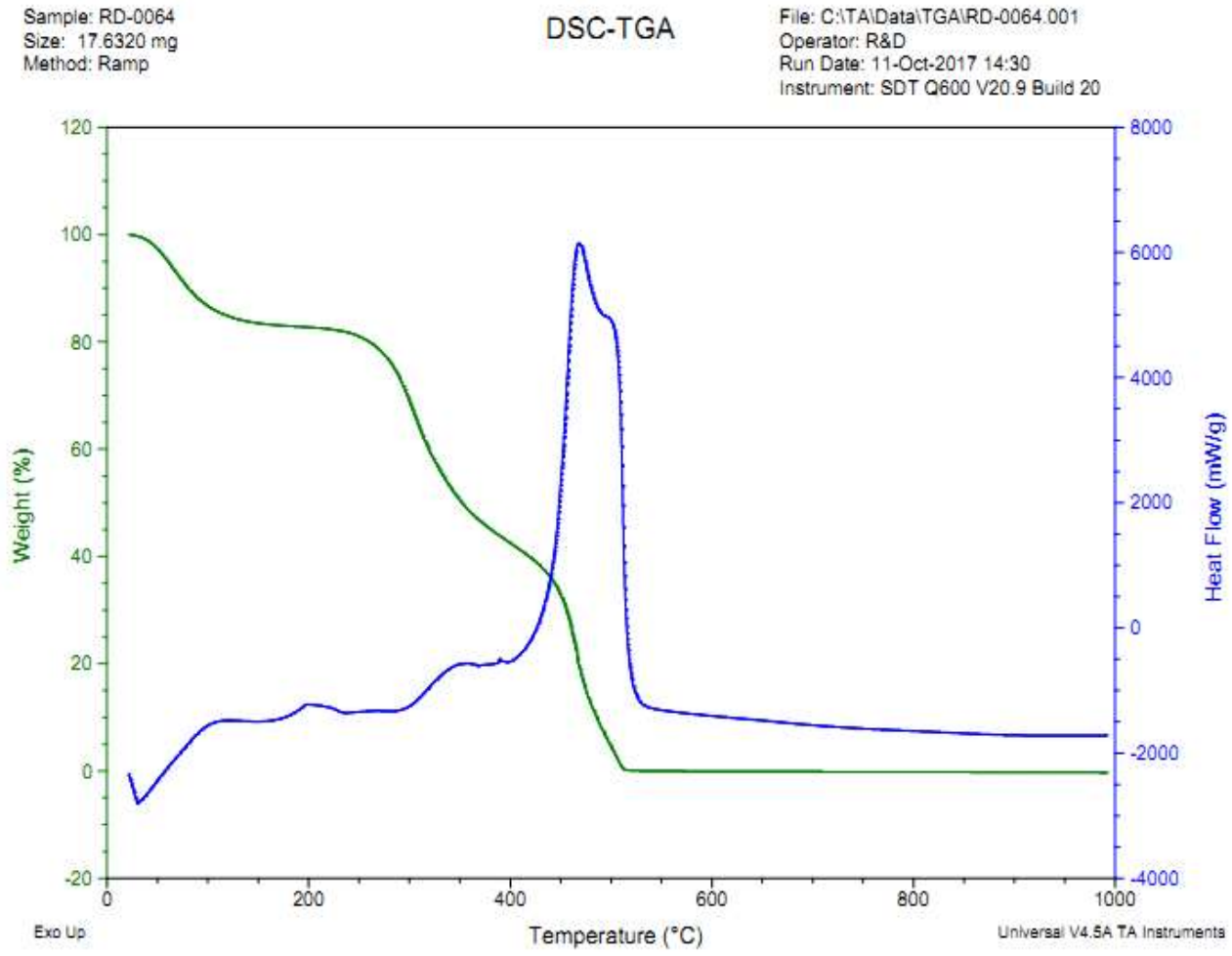


Figure 4.3: DSC-TGA curve for collagen hydrolysate

Where:-

Green graph denotes DSC curve

Blue graph denotes TGA curve

4.2.3 Fourier Transform Infrared Spectroscopy (FT-IR)

Infrared absorption a spectrum of collagen hydrolysate (Figure 4.4) shows characteristic absorption bands assigned mainly to the peptide bonds (–CONH–). The amide I band is connected mainly with the C=O stretching vibration and it occurs in the range of 1700–1600 cm^{-1}. The amide I of collagen hydrolysate peaks at 1684cm^{-1}. The amide I peak of collagen hydrolysate shifted to higher wave number was an indicative of more disordering structure due to treatment alkali hydrolysis during isolation. The absorption near at 1400 cm^{-1} is the characteristic absorption band of cis-peptide bond. The amide III band occurs in the range of 1220–1300cm^{-1} and it results from the in-phase combination of C–N stretching and N–H in-plane bending, with some contribution from C–C stretching and C=O bending vibrations. The amide III of collagen hydrolysate peaks is 1238.5cm^{-1}.

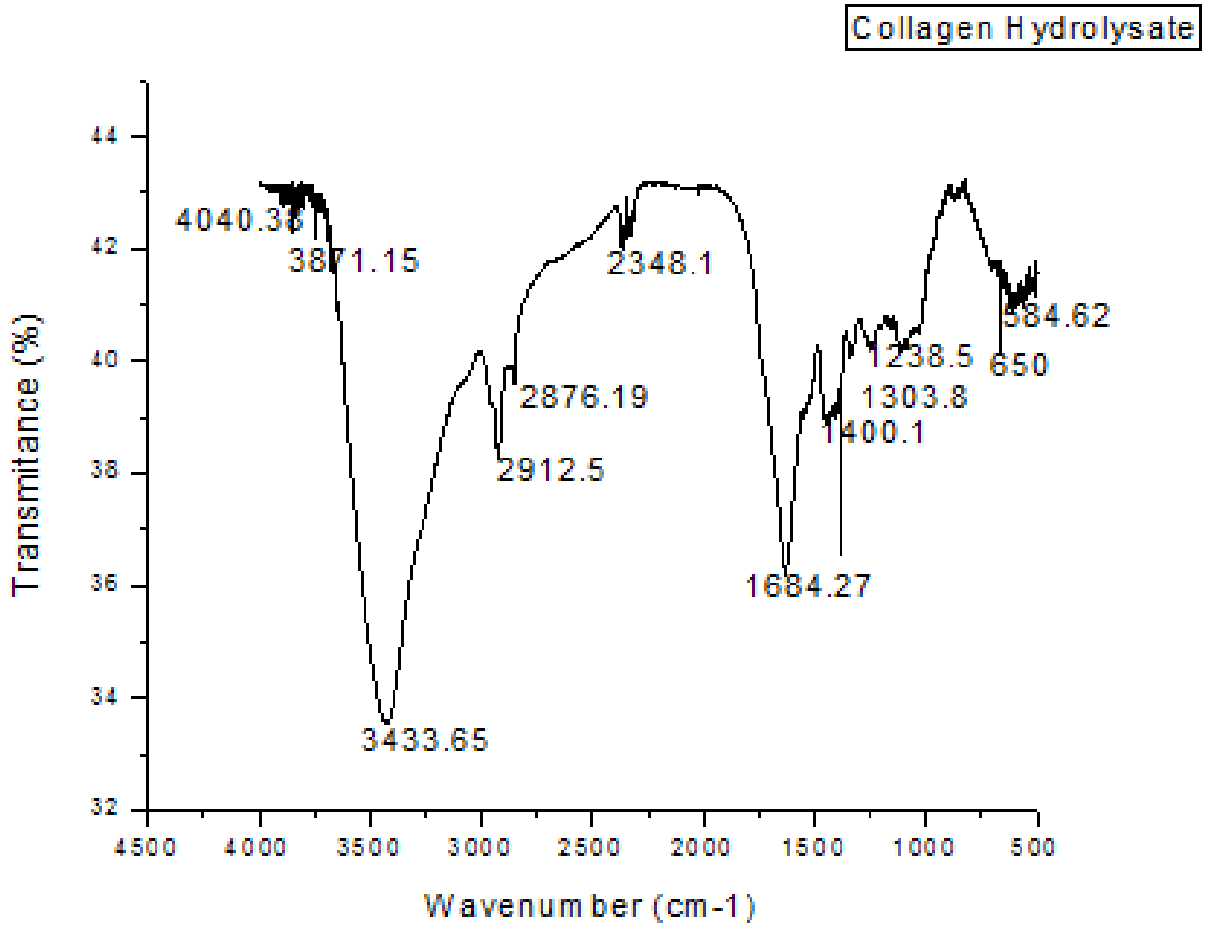


Figure 4.4: FT-IR spectra of Collagen Hydrolysate

4.3 Effect of CH–Si on the Retanning of Wet Blues

Fiber structure of hide or skin is not uniform throughout the entire area and it is most common to fill the empty nature of chrome tanned leathers by retanning to improve the required properties of leathers (Bienkiewicz 1983, Dix 1998), which are intended for making footwear, furniture and automotive upholstery etc. Protein based retanning agents offer better prospects as they fill loose areas such as belly, flanks and poor substance materials without contributing much load to tannery effluent.

From the retanning studies, it is clear that CH–Si have been successfully employed as protein filler in the retanning of wet blue sheep leathers. This is because collagen preparations (CH–Si) have low molecular weight peptides which penetrate through the pores, deep into the layers and fills the available gap present in the looser portions of the wet blue sheep leathers. The general assessment and physical properties of the crust leathers retanned with CH–Si shows encouraging results which are presented in Table 4.2 and in the Figure 4.5 and 4.6. But comparing with control, the physical properties such as thickness tear strength, grain crack strength and organoleptic properties such as fullness, grain tightness, and general appearance of the crust leathers retanned with CH–Si show marginally better values as compared to control. The use of CH–Si in retanning process also influences lubricating effect that enhances the grain smoothness and softness characteristics of the leathers.

Table 4.2: Organoleptic evaluations of the control and experiment

Properties	Control	Experiment
Fullness	5	6
Softness	7	7.5
Grain smoothness	8	8
Roundness	7	7
Overall Appearance	6.5	7

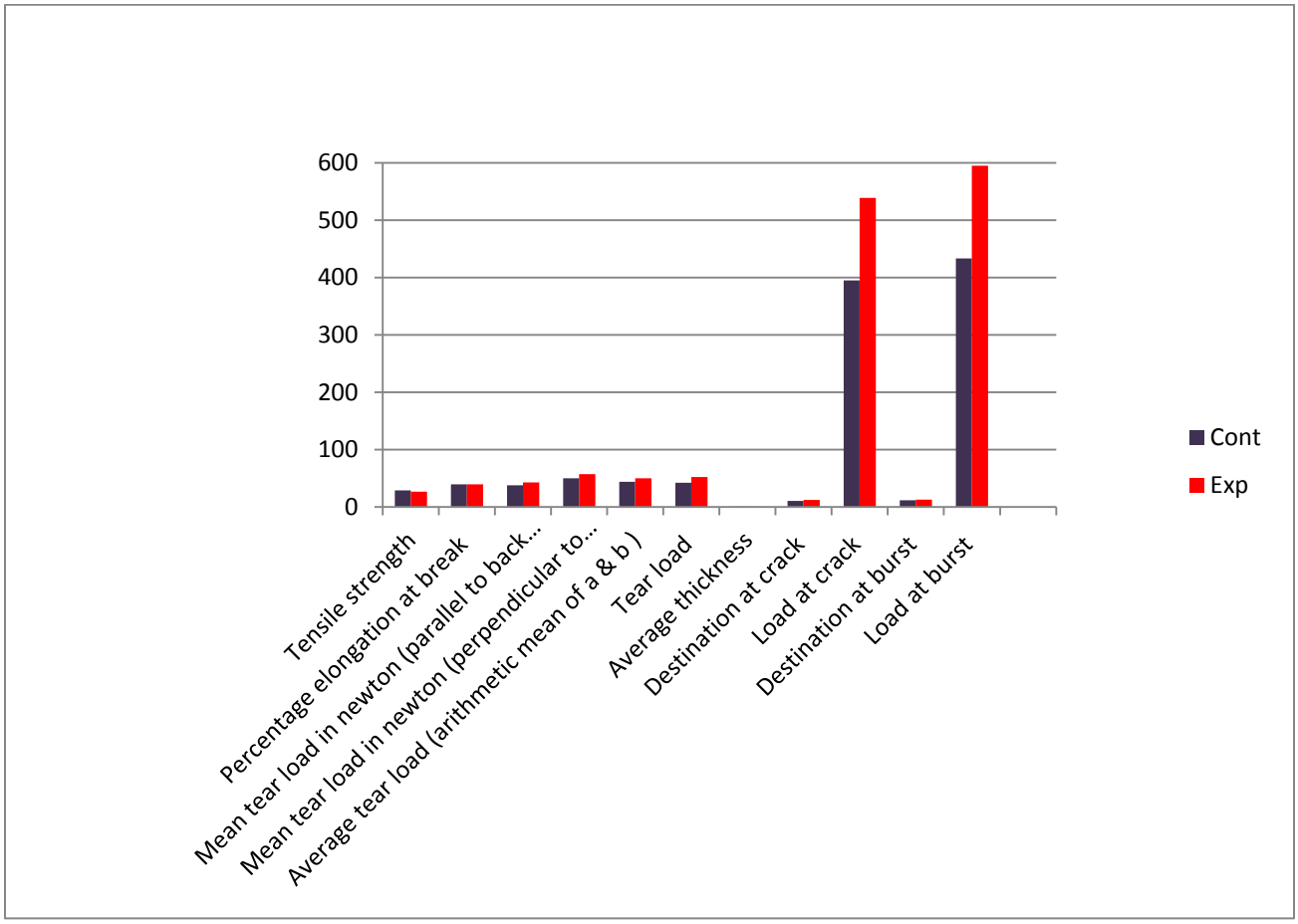


Figure 4.5: Strength characteristics of the CH-Si treated and control leather

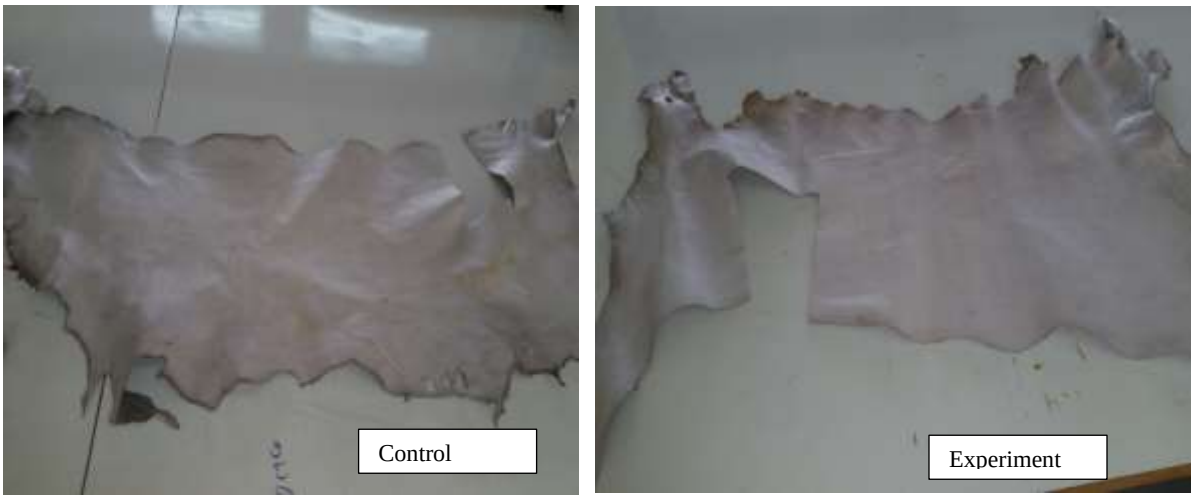


Figure 4.6: Control (Nelfill powder) and Experimental (CH-Si) treated Leather

4.3.1 Effect of CH–Si on Collagen Fibers by SEM

The scanning electron microphotographs of leathers obtained by the use of commercial filler (control) (Right hand side) and CH–Si(Left hand side) in retanning process showing their cross section at a magnification of 300x and 1000x are given in Figures 4.7 & 4.8. From the Figures it is evident that the fibre structure of control and CH–Si retanned crust leathers do not show any adverse physical change. From the micrograph pictures it is observed that most of the interspaces are filled up with protein preparations, control and CH–Si but the filling effect is better for leathers retanned with CH–Si. Fibre compactness is an indirect measure of fullness which is clearly evident from the visual assessment data of CH–Si retanned leather.

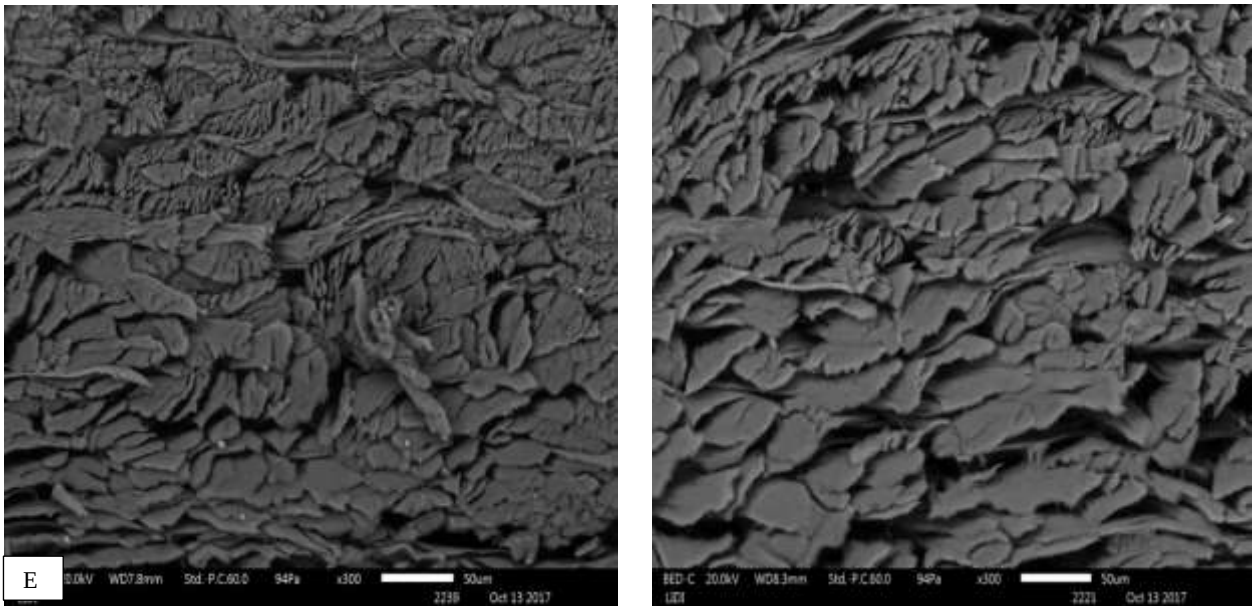


Figure 4.7: Scanning electron microphotographs at 300 × magnification

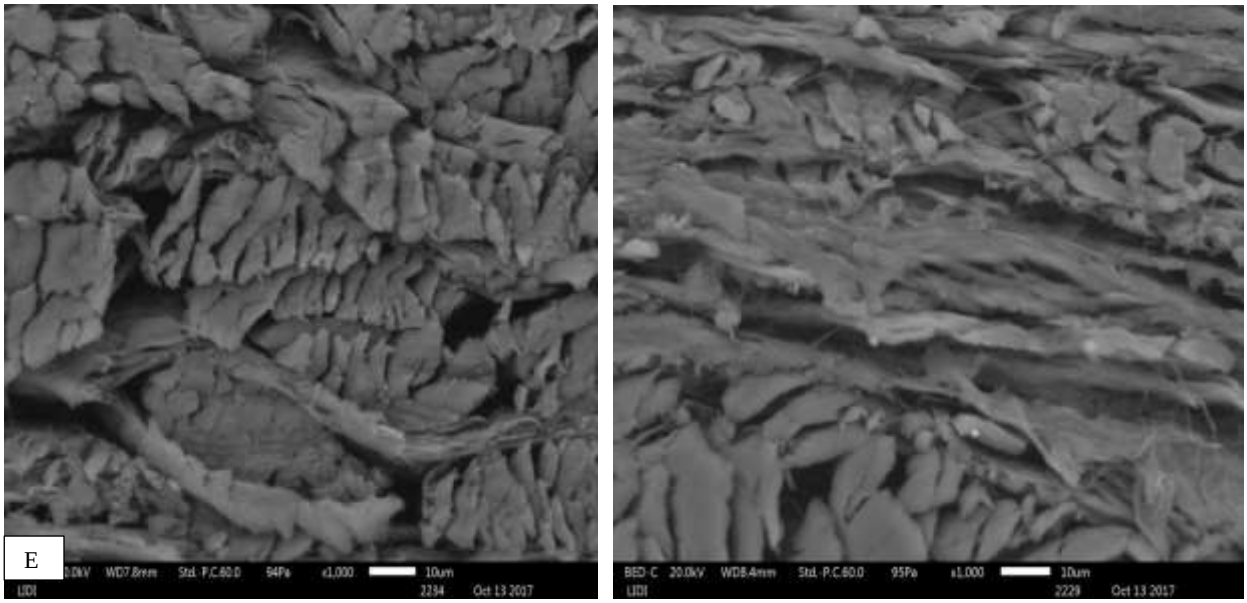


Figure 4.8: Scanning electron microphotographs at 1000× magnification

4.4 Characterization of Glue from Chrome Shavings

The Glue prepared in the present study is free from sulphide/ lime and other toxic substances. The colour of the glue is also lighter in colour free from bad odour in contrast to the glue prepared from limed fleshings. The use of the mixture aluminium sulphate and dicalcium phosphate in 1% concentration is employed for clarification which results in lighter colour. They are dissolved in water and slowly added to the hot glue liquor with continuous stirring. 0.1% sodium penta chlorphenate is also added to prevent bacterial action.

Figure 4.9: Glue from Chrome Shavings

- ✓ **Moisture content**

The moisture content based on the procedure mentioned in section 3.3.5.1 was determined. It was found that the glue contains 24.4% moisture.

- ✓ **Ash content**

Total ash content of the collagen hydrolysate was determined according to procedure mentioned in section 3.3.5.2 and it is found that the collagen hydrolysate contains 13.0% ash.

- ✓ **CIE Color measurement Data**

The CIE colour coordinates L, a and b for the glue sample produced from collagen hydrolysate and local glue sample was measured and the variables of L, a, and b represented as ΔL, Δa and Δb in addition with overall color difference ΔE is presented in Table 4.3 and Figure 4.10. From the table and figure it is clear that the overall colour difference between local glue and the glue

from chrome shavings is more (ΔE=10) indicating that the glue from chrome shavings is lighter compared to the glue from limed fleshings.

Table 4.3: CIE color values of glue samples compared to local Glue

Sample	ΔL	Δa	Δb	ΔE
1	1.58	-0.88	9.84	10.00

Where: - **Δ**L - over all difference between light and dark

Δ a - over all difference between green and red

Δb - over all difference between yellow and blue

ΔE- over all color difference

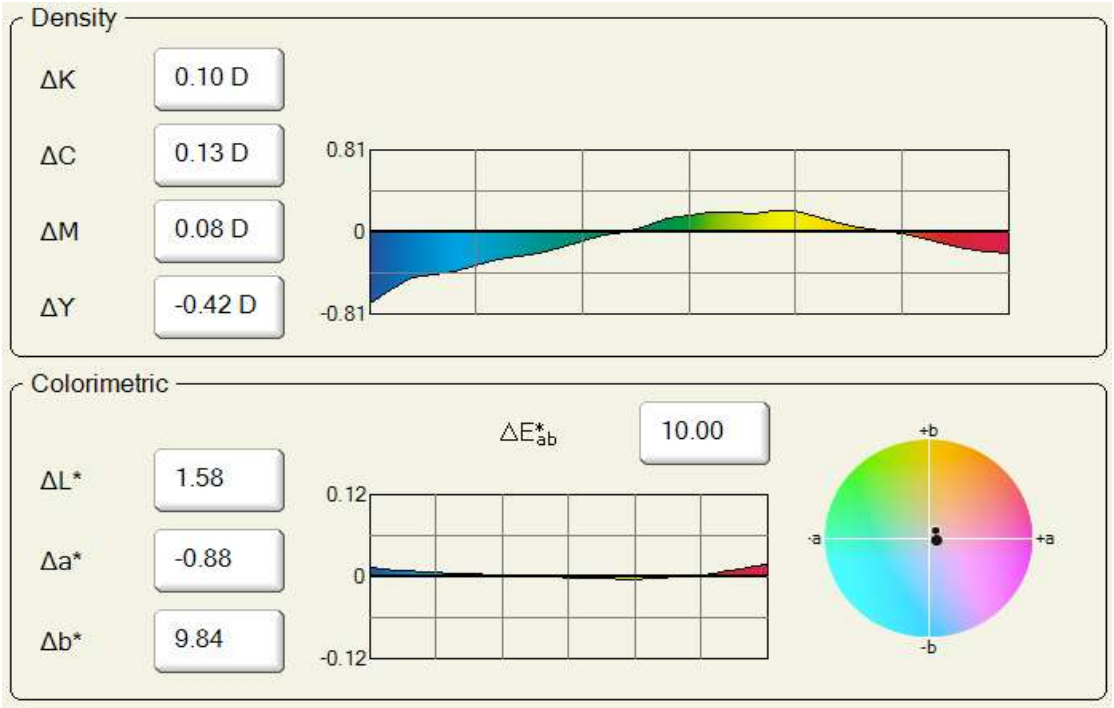


Figure 4.10: Color value of Glue Sample made from CH compared to Local glue sample

5 Conclusions and Recommendation

5.2 Conclusions

The objective of the present investigation was to develop value added protein products from chrome shavings generated during chrome tanning. Leather processing is one such industrial activity that generates chromium-bearing waste in different forms, one of them is chrome shavings. Chrome shavings are the prominent solid waste in tanning industry since chromium is known for its toxicity and hence the disposal of chrome shavings has been identified as a serious problem from the environmental point of view. Hence, in the present an attempt has been made to prepare value added proteins from shavings.

- Retanning studies confirmed that CH–Si have been successfully employed as protein filler in the retanning of wet blue sheep leathers. The collagen preparations have low molecular weight peptides penetrates through the pores, deep into the layers and fills the available gap present in the looser portions of the wet blue sheep leathers. The retanning efficiency of CH-Si was comparatively better as evidenced by scanning electron microscope studies, physical testing and visual assessment data.

- The glue prepared from chrome shavings results in lighter in colour ($\Delta E=10$) compared to glue produced from limed fleshings and also free from toxic substances lime, sulphide etc.

5.3 Recommendation

Any research and development should meet the actual needs of commercial / industrial people. The efficient value added products developed in the present investigation, viz. collagen-silica complex would be useful in retanning processes in commercial tanneries and the glue from shavings would have many industrial applications. But it needs further standardization, characterization and additional trials. We plan to upscale the production of collagen-silica complex and glue and conduct extensive tannery trials for the commercial exploitation of the valuable products.

References

Bienkiewicz K. (1983), 'Physical chemistry of Leather Making', Robert E. (ed.), Krieger Publishing Company, Florida, pp.413-416.

Cabeza L.F, T. M. (1998). Processing of leather waste: pilot scale studies on chrome shavings. Waste Management.

Catalina.M, A. J. Mechanical and Chemical properties of modified Gelatin obtained through thermal hydroloysis of chrome shavings.

Convington, T. (2009). Tanning Chemistry: The Science of Leather.

David Eglin, K. L. Comparative study of infulence of several silica precursors on collagen self-assembly and of collagen on 'Si' separation and condensation. (43, 2006).

Deqiang Su, K. W Preparation of protein Retanning agent by Grafting modification of collagen hydrolysate extracted from chrome shavings.

Dix J.P. (1998), 'Characteristics and mode of action of modern polymer in post tanning treatment process', J. Am. Leather Chem. Assoc., Vol.93, pp. 283-294.

Dutta, S. (1999). An Introduction to the Principle of Leather Manufacture. Culcatta: Indian Leather Technologists Association.

Eleanor M. Brown, M. M. Production and potential Uses of Co-Products from Solid Tannery Waste.

Fernald H.B. and Iler R.K. (1946), 'Tanning compositions and their manufacture', United States Patent, US2395472.

Gelse.K, P. A. (2003). Collagen- Structure, Function and Biosynthesis.

https://en.wikipedia.org/wiki/Animal_glue.

https://en.m.wikipedia.org/wiki/Hydrolized_collagen.

https://en.wikipedia.org/wiki/Collagen.

K.T, Sarkat. (1996). Retanning Dyeing and Finishing of Leather.

Kanagaraj. J, V. K. (April 2006). Solid wastes generation in the leather industry and its utilization for cleaner environment. Jornal of Scientific & Industrial Research.

Karthikeyan R, B. S. (2007). Industrial application of keratins. Vol 66.

KarthiKeyan R, C. B. (2011). Keratin-Silica Matrix – a new Protein Filler from Chicken Feathers for Retanning. Vol 105.

Langmailer.E, K. S. (n.d.). Products of inzymatic decomposition of chrome tanned leather waste. 83.

Legesse, S. (2011). Carbon Neutral Development in the Tanning Industry. Addis Ababa, Ethiopia.

Munz K.H. (2003), 'Soluble silicates in leather products', J. Am. Leather Chem. Assoc., Vol. 98, pp. 159-167.

Rao, J.R., Thanikaivelan, P., Sreeram, K.J., Nair, B.U., Green route for the utilization of chrome shavings (chromium-containing solid waste) in tanning industry. *Environ Sci Technol*. 36 (6), 1372-6, (2002).

Sastry, T.P., Sehgal, P.K and Ramasami,T., Value Added Eco-friendly products from Tannery Solid Wastes. *Journal of Environ. Science & Engg.* 47, (4), 250-255, (2005).

Sharphouse J.H. (1979), 'Leather Technician's hand book', Leather Producers

Sugizaki T., Node S., Kageyama T. and Moriya O. (2003), 'Silicate-containing sheet', United States Patent, US 6544641.

Taylor M.M, D. E. (1993). Effects of Processing Variables on Ash Content of Gelable and Hydrolized Protein Products Isolated from Treatment of Chromium Leather Waste. VOL 88.

Technology of Animal and Tannery by-product Utilization. . (n.d.).

Malathy J., Venba R., Jyothi G., Jinandra Doss M. and Chandra Babu N.K. (2004) 'Instrumental assessment of levelness of dyed materials', J. Am. Leather Chem. Ass., Vol. 99, p. 233-241.

Randall D.L. (1994), 'Color measurement and control in leather education committee invited lecture', J. Am. Leather Chem. Assoc; Vol.89, pp.309-319.

Tremlett R. (2003), 'Colour matching and measurement', World Leather, Vol.16, pp.29-31.

IUP: 2 (2000), 'Sampling', J. Soc. Leather Technol. Chem., Vol.84, pp.303-309.

IUP: 6 (2000), 'Measurement of tensile strength and percentage elongation', J. Soc. Leather Technol. Chem., Vol. 84, pp.317-321.

IUP: 8 (2000), 'Measurement of tear load –Double edge tear', J. Soc. Leather Technol. Chem., Vol.84, pp.327-329.

Annexes

Annex 1

Determination of Sulphated Total Ash (SLC 6)

1. Scope

This method is applicable to all types of leather. The method may be inaccurate by the extent to which the leather contains silicone or Organo-metallic compounds.

The amount of mineral substances found by ashing can differ from the actual content owing to decomposition, reduction, or the escape by volatilization of certain salts. By treating the ash with sulphuric acid the salts and oxides are converted into sulphates, but some salts will again be transformed into oxides at the selected temperature of ignition.

To determine the total mineral content, e.g., within the framework of a complete leather analysis, the water-soluble and water-insoluble inorganic substances can be ascertained by calculation or determined separately.

Ammonium salts are not determined by this method (compare with SLC 5), but can be determined as described in SLC 8.

2. Definition

For purpose of this method the following definitions apply.

Sulphated total ash: - Residue obtained from burning leather in an open crucible after sulphating, as described in this method.

Sulphated water insoluble ash: - Residue obtained when leather, previously extracted with water as described in SLC 5, is burnt in an open crucible after sulphating, as described in this method.

3. Reagents

The following reagents are required.

Sulphuric acid reagent solution, approximately 2N

4. Apparatus

Usual laboratory apparatus is required and, in particular, the following

(a) **Crucibles and dishes**, of glazed porcelain, platinum, or quartz.
(b) **Muffle furnace**, capable of being maintained at temperature close to, but not exceeding, 750^{o}C.

5. Procedure

Sample and grind in accordance with SLC 1 and 2. Weigh 2.5 g of the sample to the nearest 0.001 g, and carefully carbonize it over a low flame in a crucible which has been previously heated to 750^{0}C, cooled, and weighed, so that the leather burns with a small flame. Carbonize fatliquored leather particularly carefully so that the grease burns very slowly. Then thoroughly moisten with the sulphuric acid solution and heat over a low flame until sulphur trioxide fumes are no longer visible. Heat more vigorously, and then ignite in the furnace at 750^{0}C until completely ashed. Cool in the desiccators and weigh.

Repeat the addition of acid, heating, cooling and weighing until the mass of the residue is constant.

6. Notes on the procedure
   a. It is advisable to extract silicone-impregnated leather with dichloromethane before determining the sulphated total ash.
   b. For the determination of the sulphated total ash, the dry leather obtained from the determination of volatile matter, in accordance with SLC 3, can be used in all cases.
   c. If a carbon-free residue cannot be obtained in spite of heating at 750^{0}C, it should be moistened with a little ammonium nitrate solution and the heating repeated until the ash is free from carbon.
   d. At temperature above 750^{0}C some loss of mass from the residue is possible due to volatilization of certain inorganic salts. For this reason, close control is essential to prevent the maximum furnace temperature from exceeding 750^{0}C.
7. Expression of results

Calculate the following percentages.

$$sulphated\ total\ ash, percentage\ by\ mass = \frac{100\mathrm{M}_1}{\mathrm{M}_0}$$

Where $\mathbf{M_1}$ is the mass of sulphated total ash

And $\mathbf{M_0}$ is the mass of the original sample of leather

Annex 2

Determination of Substances (Fats and Other Solubles) Soluble in Dichloromethane SLC 4 (IUC 4; BS 1309:4)

1. Scope

This method is applicable to all types of leather.

Not all fatty and similar substances can be extracted from leather with organic solvents; they may be in part soluble and partly bound to the leather. On the other hand, the solvent can dissolve non-fatty substances, e.g. sulphur and impregnants, both of which cause difficulty in the determination of the acid value and saponification value of the fat.

As the extraction is frequently done in conjunction with determination of free fatty acid content of the leather, a suitable procedure for determination of the free fatty acids extracted by this method si included.

NOTE: - The apparatus and technique described in this method are also suitable for the extraction of leather by solvents other than dichloromethane. If, for any purpose, other solvents are used, the solvent or solvents used should be stated in the test report.

2. Definition

For the purposes of this method the following definition applies.

Extractable substances: - Fats and other soluble matter which can be extracted from leather with dichloromethane.

3. Principle

The prepared leather is extracted continuously with dichloromethane. Solvent is evaporated from the extract which is then dried at 102^0C.

4. Reagents

The following reagent is required

Dichloromethane, boiling point 38^0C to 40^0C, freshly distilled and kept in a dark flask over calcium oxide.

NOTE 1:- Dichloromethane that has stood for a long time should be tested for the presence of any hydrochloric acid which may have formed, as follows.

Shake 10 ml of dichloromethane with 1 ml of 0.1N silver nitrate solution. If the silver nitrate solution becomes turbid the dichloromethane should be redistilled and kept in a dark flask over calcium oxide.

NOTE 2:- Dichloromethane which has been used for this method can be recovered and re-used after distillation.

NOTE 3:- Dichloromethane has toxic properties and should be used with caution.

5. Apparatus

Usual laboratory apparatus is required and, in particular, the following

a) Soxhlet apparatus

b) **Filter paper thimbles** of suitable size and manufacture, or suitable glass filter bells. Schleicher and Schull Thimbles No 603 or Whatman Extraction Thimbles 33 mm x 80 mm are known to be satisfactory.

c) **Oven**, capable of being maintained at 102 ± 2^0C, complying with the requirements of BS 2648.

6. Procedure

Sample and grind in accordance with SLC 1 and 2. Weigh 10 ± 0.1 g of the prepared sample and press evenly into the filter paper thimble, or into the filter paper thimble, or into the glass bell. Cover the leather with a thin layer of cotton wool. Dry the extraction flask with two glass beads in it by heating for half an hour at 102 ± 2^0C. Weigh after cooling in a desiccator.

Begin the continuous extraction with dichloromethane, then, after at least 30 changes of solvent, distil the dichloromethane from the flask containing the extract. Dry the extract in the oven for four hours at 102 ± 2^0C (if drops of water are visible before drying, add 1-2 ml of ethanol). Weigh after cooling for 30 min in the desiccator. Repeat the drying, cooling and weighing, at least twice more, but with drying periods of 1 hour until either the further loss in mass does not exceed 10 mg, or the total drying time equals 8 hours.

7. Notes on the procedure

a) Dichloromethane can also dissolve non-fatty materials from the leather, e.g. sulphur (the presence of sulphur is recognizable by yellow precipitate in the flask). As sulphur causes difficulty it can be removed in the following way. Dissolve the extract in the smallest possible quantity of diethyl ether and filter through a little cotton wool into a previously weighed flask. After thoroughly washing out the cotton wool filter with ether, remove the ether from the extract in the flask by distillation over a bath of hot water from which any flame has previously been

removed. If sulphur is again precipitated, repeat the procedure. After the diethyl ether has been distilled off, dry the flask and residue and weigh.

b) The extract can be used for analysis, e.g. to determine acid and saponification values of the fats, or to determine the free fatty acid content of the leather.

c) After removal of the solvent the extracted leather may be required for determination of water soluble matter in accordance with SLC 5.

8. Expression of results

Calculate the following percentage

$$extractable\ substannce, percentage\ by\ mass = \frac{100}{M_0}$$

Where M_1 is the mass of the extract

And M_0 is the mass of sample used

Annex 3

LIDI	LEATHER INDUSTRY DEVELOPMENT INISTITUTE TESTING & RESAERCH LABORATORY DIRECTORATE	
Title	TEST REPORT	Page : 1of 1

Test date(s): 21/09/17 - 06/10/17
Lab. Design. Code: C-11504
Type of Sample: Leather (Chrome shaving)
Sample Identification: _____
Sampling Condition: _____
Environmental test condition: Temp.22.7 (^{0}C) & RH 49.9 (%)

Report No: C-11504/17
Test Order No: SCBE-687/2009
Sampling date & place: _____
Sampling location: _____
Sample receiving date: 24/08/17
Sampled by: Customer
Report date: 09/10/2017

Name of Customer: Addis Ababa Institute of Technology (Hiwot Getahun)
School of Chemical & Bio Engineering
Address of customer: Tell: 011-123 24 35
Fax: 011-123 94 80, P.O.Box 384
Email: info@aau.edu.et
Addis Ababa, Ethiopia

ORIGINAL

Customer code: (CHS1)

S/No	Type of Test	Test Result	Unit	Test Method	Uncertainty	Standard Required	Remark
1.	Sulphated Total Ash	0.9	%	SLC 6	-		
2.	Moisture Content	46.9	%	SLC 3	-		
3.	Chromium	2.3	mg/L	SLC 8	±0.3		
4.	Fat Content	0.9	%	SLC 4	-		On dry base

Note: The test results relates only to the item tested
This test report is for technical information of the client only. Not for the advisement, promotion, publicity litigation or legal purpose.

Tested By:Abebaw D. Jun. Chem/Insl Researcher Signature
Checked By: Meron M. Senior chem/ Insl Researcher (Team Leader) Signature
Authorized By: Signature
Director, Research & Testing Laboratory Directorate

☎ +251-11-439 1700, +251-11-439 4846 24 692(1000) Fax: +251-11-439 2259 E-mail: berhanunegus@gmail.com
Mobile +251-911 252713 elidilab@gmail.com
AKAKI -KALITY KEFLE KETEMA, ADDIS ABABA

Annex 4

LIDI	LEATHER INDUSTRY DEVELOPMENT INISTITUTE TESTING & RESAERCH LABORATORY DIRECTORATE	
Title	TEST REPORT	Page : 1of 1

Test date(s): 21/09/17 - 28/09/17
Lab. Design. Code: C-11503
Type of Sample: Leather
Sample Identification: _____
Sampling Condition: _____

Environmental test condition: Temp.22.5 (°C) & RH 50.5 (%)

Report No: C-11503/17
Test Order No: SCBE-687/2009
Sampling date & place: _____
Sampling location: _____
Sample receiving date: 24/08/17
Sampled by: Customer
Report date: 05/10/2017

Name of Customer: Addis Ababa Institute of Technology_ (Hiwot Getahun)
School of Chemical & Bio Engineering
Address of customer: Tell: 011-123 24 35
Fax: 011-123 94 80, P.O.Box 384
Email: info@aau.edu.et
Addis Ababa, Ethiopia

ORIGINAL

Customer code: (GS1)

S/No	Type of Test	Test Result	Unit	Test Method	Uncertainty	Standard Required	Remark
1.	Sulphated Total Ash	13.0	%	SLC 6	-		
2.	Moisture Content	24.4	%	SLC 3	-		

Note: The test results relates only to the item tested
This test report is for technical information of the client only. Not for the advisement, promotion, publicity litigation or legal purpose.

Tested By:Maereg H. Senior Chem/Insl Researcher Signature
Checked By: Meron M. Senior chem/ Insl Researcher (Team Leader) Signature
Authorized By: Signature

Berhanu Negus Baraki
Director,Research & Testing
Laboratory Directorate

☎ +251-11-439 1700, +251-11-439 4846 ✉ 24 692(1000) Fax: +251-11-439 2259 E-mail: berhanunegus@gmail.com
Mobile +251-911 252713 elidilab@gmail.com
AKAKI -KALITY KEFLE KETEMA, ADDIS ABABA

Annex 5

LEATHER INDUSTRY DEVELOPMENT INISTITUTE
TESTING & RESAERCH LABORATORY
DIRECTORATE

Title: TEST REPORT — Page : 1 of 1

Test date(s) : 21/09/2017
Lab. Desg .Code No : P-11505
Type of Sample: Leather
Sample Identification: White
Sampling: ISO 2418:2008
Conditioning: Tem=20°C±2 & RH=65%±5
Equipment used: UTM, Thickness gauge, Lastometer,

Report No: P-11505/17
Test order No: SCBE-687/2009
Sampling date & place: Customer
Sampling location : ISO 2418:2008 (Fig 1)
Sample receiving date: 24/08/2017
Sampled by : Customer
Report date: 29/09/2017

Name of Customer: Addis Ababa Institute of Technology (Hiwot Getahun)
School of Chemical & Bio Engineering
Address of customer: Tell : 011-123 24 35
Fax: 011-123 94 80, P.O.Box 384
Email info@aau.edu.et
Addis Ababa (Ethiopia)

ORIGINAL

Customer code: CHATRI

/No	Type of test	Unit	Test Result	Uncertainty	Test method	Standard Requ.	Remark
1.	**Tensile strength & percentage extension**						
1.1	Tensile strength	N/mm²	29.1	-	ISO 3376: 2011		
1.2	Percentage Elongation at break	%	39.7	-			
2.	**Tear Load (Double edge tear)**						
2.1	Mean Tear Load in Newton(Parallel to the back bone)	N	38.0	-	ISO 3377-2 :2011		
2.2	Mean Tear Load in Newton(perpendicular to the backbone)	N	50.0	-			
2.3	Average Tear Load (Arithmetic mean of 2.1 & 2.2)	N	44.0	-			
2.4	Tear Load	N/mm	42.2	-			
3.	**Distention & strength of grain Ball burst**						
3.1	Average Thickness	mm	0.7	-	ISO 3379:2015		
3.2	Distention at crack	mm	10.7	-			
3.3	Load at crack	N	395.0	-			
3.4	Distention at burst	mm	11.6	-			
3.5	Load at burst	N	433.3	-			

Note: _____

Tested By: H/Mariam Tesfay — Jun Phy/Mech. Testing Researcher — Signature
Checked/Verified by: Maereg Haile. — Ass.Phy/Mech. Testing Researcher — Signature
Authorized By: Berhanu Negus (Berga) — Director, Research & Testing Laboratory Directorate

1. This test report is for technical information of the client only. Not for advertisement, promotion, publicity litigation or legal purpose.
2. The test result relates only to the item tested.
3. uncertainty calculated with expanding factor K=2 with confidence limit=95%
4. The test report must not be reproduced without approval of LIDI.
5. LIDI lab shall be indemnified against any dispute arising out of issue of this report

☎ +251-11-439 1700, +251-11-439 4846 ✉ 24692 Code 1000 Fax: +251-11-439 2239 E-mail:berhanunegus@gmail.com
Mobile +251-911 252713 elidilab@gmail.com
AKAKI -KALITY SUB CITY, ADDIS ABABA, ETHIOPIA

Annex 6

LEATHER INDUSTRY DEVELOPMENT INISTITUTE
TESTING & RESAERCH LABORATORY
DIRECTORATE

Title: TEST REPORT — Page : 1 of 1

Test date(s) : 21/09/2017
Lab. Desg ./Code No : P-11506
Type of Sample: Leather
Sample Identification: White
Sampling: ISO 2418:2008
Conditioning: Tem=20°C±2 & RH=65%±5
Equipment used: UTM, Thickness gauge, Lastometer.

Report No: P-11506/17
Test order No: SCBE-687/2009
Sampling date & place: Customer
Sampling location : ISO 2418:2008 (Fig 1)
Sample receiving date: 24/08/2017
Sampled by : Customer
Report date: 29/09/2017

Name of Customer: Addis Ababa Institute of Technology (Hiwot Getahun)
School of Chemical & Bio Engineering
Address of customer: Tell : 011-123 24 35
Fax: 011-123 94 80, P.O.Box 384
Email info@aau.edu.et
Addis Ababa (Ethiopia)

ORIGINAL

Customer code: EXPERIMENT

S/No	Type of test	Unit	Test Result	Uncertainty	Test method	Standard Requ.	Remark
1.	**Tensile strength & percentage extension**				ISO 3376: 2011		
1.1	Tensile strength	N/mm²	26.9	-			
1.2	Percentage Elongation at break	%	39.7	-			
2.	**Tear Load (Double edge tear)**				ISO 3377-2 :2011		
2.1	Mean Tear Load in Newton(Parallel to the back bone)	N	42.6	-			
2.2	Mean Tear Load in Newton(perpendicular to the backbone)	N	57.1	-			
2.3	Average Tear Load (Arithmetic mean of 2.1 & 2.2)	N	49.8	-			
2.4	Tear Load	N/mm	52.4	-			
3.	**Distention & strength of grain Ball burst**				ISO 3379:2015		
3.1	Average Thickness	mm	0.8	-			
3.2	Distention at crack	mm	12.1	-			
3.3	Load at crack	N	538.7	-			
3.4	Distention at burst	mm	12.9	-			
3.5	Load at burst	N	595.3	-			

Note: _____

Tested By: H/Mariam Tesfay Signature — Jun.Phy/Mech. Testing Researcher
Checked/Verified by: Maereg Haile. Signature — Ass.Phy/Mech. Testing Researcher
Authorized by: Berhanu Negus Bayeh, Director, Research & Testing Laboratory Directorate

1. This test report is for technical information of the client only. Not for advertisement, promotion, publicity litigation or legal purpose.
2. The test result relates only to the item tested.
3. uncertainty calculated with expanding factor K=2 with confidence limit=95%
4. The test report must not be reproduced without approval of LIDI.
5. LIDI lab shall be indemnified against any dispute arising out of issue of this report

☎ +251-11-439 1700, +251-11-439 4846 ✉ 24692 Code 1000 Fax: +251-11-439 2259 E-mail:berhanunegus@gmail.com
Mobile +251-911 252713 elidilab@gmail.com
AKAKI–KALITY, SUB CITY, ADDIS ABABA, ETHIOPIA