ADDIS ABABA UNIVERSITY

ADDIS ABABA INSTITUTE OF TECHNOLOGY

SCHOOL OF CHEMICAL AND BIO ENGINEERING

Development, Characterization and Optimization of sulphated Fatliquor from Neem (*Azadirachta Indica*) seed oil for crust leather process technology

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A Thesis submitted to School of Chemical and Bio-engineering, Addis Ababa institute of Technology, Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Chemical Engineering (Process Engineering)

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Declaration

I, the undersigned, declare that this thesis is my original work and has not been presented for a degree in any other university, and that all sources of materials used for the thesis have been duly acknowledged.

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A thesis Submitted to the Research and Graduate School of Addis Ababa University, Addis Ababa Institute of Technology, School of Chemical and Bio Engineering in partial fulfillment of the requirements for the attainment of the Degree of Masters of Science in Chemical Engineering under Process Engineering Stream.

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Acronyms and Abbreviations

ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
ASTM	American Standard Testing Methods
ATL	Average Tear Load
AV	Acid Value
BASF	Baden Anilline and Soda Factory
BCS	Basic Chromium Sulphate
$C_4H_{10}O$	Diethyl Ether
C_2H_5OH	Ethanol
CCD	Central Composite Design
CVLOFL	Commercial Vegetable Lecithin Oil Fatliquor
EB	Elongation at Break
EFA	Essential Fatty Acid
RSA	Response Surface Analysis
SNOSFL	Synthesized Neem Oil Sulphated Fatliquor
EV	Ester Value
FFA	Free Fatty Acid
G	Gram
H	Hour
H_2O	Water
ISO	International Standards Organization
IV	Iodine Value
LIDI	Leather Industry Development Institute
mg eq.	milligram equivalent
Min.	Minutes
Ml	Milliliters
$Na_2S_2O_3$	Sodium thiosulphate
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NSO	Neem Seed Oil
RH	Relative Humidity
RSM	Response Surface Methodology
Sg	Specific gravity
SV	Saponification Value
TS	Tear Strength
TT	Tensile Strength
UNIDO	United Nation Industrial Development Organization
UTM	Universal Tensile Testing Machine

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Abstract

The leather market demands quality and competitive cost for producers as well as users. Leather producers use effective substituent to make the process efficient in order to reduce the waste load and improve the quality of the final product. Fatliquoring is a fundamental stage during the production process of leather. The source of fatliquoring can be formulated from emulsification of raw materials such as vegetable or seed oils and fats, wax, fish oil and paraffin. These enable them to penetrate leather properly to enhance the major characteristics of leather such as softness, pleasant feel and strength. This study showed the process for synthesis of sulphated fatliquor from locally available raw material, neem seed oil (SNOSFL). The fatliquor production process involves preparation of seed, extraction of oil by solvent and mechanical screw press , purification of oil, sulphation of the neem oil with conc.H_2SO_4, washing and neutralize with 10% NaCl and 30% NaOH solutions respectively. The major sulphation reaction process parameters used for fatliquor production were reaction temperature, amount of conc.H_2SO_4, and reaction time. In the experimental analysis results, the optimal conditions that maximize the degree of sulphation were found to be reaction temperature at 23.98 $^{\circ}$C, amount of conc. H_2SO_4 at 16.76 Wt. % and reaction time 3.28 h that yield the degree of sulphation was 5.37%. The physicochemical characterization, spectroscopic analysis (FT-IR, GC-MS) of synthesized fatliquor and neem oil, and the organoleptic and physical test for the applied fatliquor on leather were done. The overall results confirm the sulphated group during sulphation process in SNOSFL and the synthesized product has a potential for the application garment leather.

Key word: Fatliquor, Sulphation, Neem seed oil, Leather

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1. INTRODUCTION

1.1 Background

The leather making operation consists of converting the raw hide or skin which is a highly putrescible material into leather, a stable material. The tough hides of bulls, oxen and cow make excellent shoe sole leather, while the softer skins of sheep are suitable for shoe upper leathers, coats and bookbinding. The whole process involves a sequence of complex chemical reactions and mechanical processes involve converting the raw hide or skin (a highly putrescible material) into leather (Covington, 2009).

Possession of oil around the collagen protein bonds is necessary to protect them and give longer life to the leather. If the collagen protein bonds dry out entirely they will shrink, become stiff and break then the broken bonds weaken the leather permanently and damage the product. After the damage is already done, introducing oil back into the leather is useless and irreversible. So to prevent drying in hard needs enough lubricant to soften the collagen protein bonds before the bonds dries out and breaks (Kite and Thomson, 2006).

Fatliquoring products can be formulated from raw materials of vegetable oil and fat, wax, fish oil and paraffin's. Emulsification of these raw materials enables them to properly penetrate leather. This process can be held with different polar groups that are added by emulsifying agents such as sulphation, sulphonation, phosphatisation and chlorination (Kumar, 2017). Fatliquoring has involved in lubricating the fibrils with fat layers and make the leather soft and pleasant feel. It also influences physical characteristics of leather, such as tensile strength, extensibility, wetting properties and permeability (Santos *et.al.*,2005).

Sulphation is one of the common methods followed to prepare fatliquor emulsion where sulphuric acid is used for maximum take up on chrome tanned leather. The use of sulphuric acid in fatliquor preparation reduces the neutral oil component in fatliquor, which is very essential for imparting good feel and better properties to leather. (Sivakumar *etal.*, 2008)

Neem is non –toxic and non-edible potentially novel industrial oilseed crop. Neem oil is vegetable oil obtained from the seeds of the neem plant *(Azadirachtaindica)* usually prepared from the seed kernels and is well known for its high insecticidal and medicinal value. The fruit flower and leaves are minor sources of neem essential oil. Neem kernels are about 45% oil, which is composed of essential saturated and unsaturated fatty acids (puri, 2006).

The neem tree has adaptability to a wide range of climatic and topographic factors. It thrives well in dry, stony shallow soils and even on soils having hard calcareous or clay pan, at a shallow depth. Neem tree requires little water and plenty of sunlight and rainfall in the range of 450 to 1200 mm with wide temperature range of 0 °C to 49 °C. However, it has been introduced successfully even in areas where the rainfall is as low as 150 to 250 mm (Balami *et.al*., 2014).

Like any other vegetable oils it contains triglycerides. Its major fatty acid is the unsaturated, with Systematic Name 9-octadecenoic acid, known familiarly as oleic acid. The fatty acid composition of a typical neem oil contains about (50%–60%), of oleic acid (Paul *etal.,* 2013).

1.2 Problem statement

Ethiopia has variety of vegetable oil sources treated for fatliquoring to process in leather production. The tanneries import fatliquor from abroad for higher expenses that brings an impact on utilization of resources, unemployment, and hard currency.

Currently Ethiopia has 32 leather industries, of which 28 tanneries are in operation owned by private sector that produce finished leather from cow hides, sheep and Goat skins. The majority of the tanneries in Ethiopia built relatively large capacity to process sheep skin. The annual production capacity and average fatliquor consumption from 2013-2018 G.C was 1000 metric tons for hide and 1235 metric tons for skin as shown in *Appendix G* (LIDI, 2018).

The total annual utilized production capacity in 2018G.C was 1,649,000 piece hide (8,245,000Kg) and 18,762,000 piece skin (9,381,000Kg) with 12% and 14% fatliquor consumption (production cost 70 birr/Kg) based on shaved weight of wet blue. This has high impact on the economic resources, availability and unemployment rate. So neem oil is vegetable oil easily available novel industrial oil seed for substituting the imported fatliquor to save the foreign currency.

1.3 Objective of the study

1.3.1 General objective

To study the effect of process variables, their interaction and characterization of synthesized fatliquor from neem seed oil using sulphation process.

1.3.2 Specific objectives

- Extraction, purification and characterization of the physicochemical properties of neem oil.
- Develop the model equation and the effect of sulphation reaction parameters to determine the optimal operating conditions that results maximum degree of sulphation.
- Characterize the physicochemical properties for produced sulfated fatliquor.
- Test the fatliquoring effect of synthesized fatliquor on leather at leather industry development institute.

1.4 Significance of the study

Neem seeds are non- toxic, non-edible and available novel industrial oil seed crop grown under large scales which have considerable economic significance due to a variety of commercial usages and a potential of producing neem oil. Since, Ethiopia has suitable weather for growth of Neem tree, it is necessary to exploit the potential of neem seed oil for fatliquor production that can substitute the imported product. Thus, these resources aggravate the availability of raw materials and characteristics for formation of the product. So it is useful to develop sulphated fat liquor production technology. This study has shown a significant advantage to provide a new potential industrial oilseed, neem seed oil (NSO), for production of sulphated fatliquor in Ethiopia. This can play a major role to substitute the imported fatliquor that conserve the utilization, and technological transformation of locally available resource. Also, it might contribute in saving expenditures in its economic value.

1.5 Scope of the study

Different experiments were performed in this study based on necessary equipment, raw materials, chemical and reagents and procedures such as preparation of neem seed, extraction, refining, characterization of neem oil, synthesis and characterization of sulphated fatliquor and test the performance of synthesized fatliquor on leather using protocol and working procedure of LIDI (2018).

2. LITREATURE RIEVIEW

Fatliquors are oil in water emulsion which is used for the lubrication of the tanned fibers of leather. For many centuries, the leather lubricants were incorporated into the leather by simply smearing wet leather with mixtures of greases and oils. The use of oil in water emulsion, now called fat liquors, originated during the twentieth century. The early fat liquors were prepared by emulsifying the oil in water through the use of common surface active agents such as soap, and/or the use of protective colloids such as egg albumen, casein or natural gums. Recently, the oils and fats are converted into emulsions by a process of sulphation, sulphonation, sulphitation or sulphochlorination (Lollar, 1978).

Fatliquoring of leathers are performed to impart as an adjustment of physical properties (softness, mellowness, fullness ,roundness; preserving grain break; regulating elasticity of the fiber and pleasing handle to leather) so as to improve its aesthetic value (Covington, 2009).

Different researcher studied that the amount and concentration acid, reaction temperature and time of reaction are some common variables used to produce sulphated fatliquor, however there is no report that the effect of those parameters on the degree sulphation from neem oil for the stability of fatliquor as well as its utilization in fatliquoring of leather. In order to optimize the degree of sulphation, Experiments under different amount of acid, reaction temperature, and reaction time was performed. Evaluation and its effectiveness of the prepared fatliquored chrome tanned leather on fixing light leather also compared against the imported fatliquor.

2.1 Neem seed oil

Neem oil is a vegetable oil that found from pressing the seed in the pulp of neem tree. Neem oil varies in color; it can be golden yellow, yellowish brown, reddish brown, dark brown, greenish brown or bright red. It has a rather strong odor that is said to combine the odors, of peanut and garlic. It composed mainly of triglycerides and contains many triterpenoid compounds, which are responsible for the bitter taste. Due to the hydrophobic nature of the oil it must be formulated with appropriate surfactants in order to emulsify in water for application purpose. *Azadirachtin* is the most well-known and studied triterpenoid in Neem oil (Elshierh & EldeenEdres, 2013).

i) Geographical distribution and description of neem tree

Neem, botanically known as *Azadirachta indica*, belongs to the Meliaceae (mahogany) family. This plant is now has wide distribution over the world and could be found in many countries due to its essentiality in medicinal properties. Even though the species of the plant are the same, many other factors such as the geographical origin, genetic makeup, environmental condition, climate cultivation and soil composition might result in differences of the characteristics of the plant (Puri, 2006).

Neem is indigenous to India and found in tropical and subtropical regions like Pakistan, Bangladesh, Sri Lanka, and Myanmar. The Science and Technology Panel of the International Development National Research Council has documented that around 60% of the total neem population of the world inhabits India. It is also grown and naturalized in Southeast Asian (Thailand, Indonesia, Peninsular Malaysia, the Philippines, and Singapore) and West African countries, as well as Australia and Saudi Arabia. More recently, it has been familiarized to the Caribbean and various zones of America (Paul *et.al.*,2013).

Indian immigrants introduced neem to Mauritius and may also have taken it to continental Africa. It is now widely cultivated in Mauritania, Senegal, The Gambia, Guinea, Ivory Coast, Ghana, Burkina Faso, Mali, Benin, Niger, Nigeria, Togo, Cameroon, Chad, Ethiopia, Sudan, Somalia, Kenya, Tanzania, and Mozambique (Tinghui *et.al.*,2001).

Ethiopia is located in the tropical region. As a result the weather condition, Kola and Weynadega, for agro climatic zones makes a suitable environment for the growth of plantation of neem tree and about 65-75% of the conditions in Ethiopia are appropriate for plantation of the tree. There are about 25 places that neem tree has observed in Ethiopia. It is widely planted in of Illubabor, Kefa, Wolega, Harerge, Shwoa, Gambella, Jijiga, and Umera. The people in Gambella do not have information about the high importance of the tree but some children eat the yellow fruit otherwise, they use to make the city green throughout the year. Neem is an evergreen, tall, fast-growing tree, with a height of 25m and 2.5m in girth which has an attractive crown of deep green foliage and honey scented flowers. It is the most versatile, multifarious tree of tropics, with immense potential possesses useful non-wood products (leaves, bark, flowers, fruits, seeds, gum, oil and neem cake) than any other tree species (Workneh, 2011).

(Tekie, 1999), reported that a single tree produces 30 to 100 kg of neem fruit annually depending on climatic conditions, edaphic factors, the ecotype or the genotype of the plant and According to (Balami *et.al.*, 2014) Fresh fruit yield per neem tree ranges between 37 and 50 kg per year. Forty kg fruit yields nearly 24 kg of dry fruit (60 %), which in turn gives 11.52 kg of pulp (48 %), 1.1 kg of seed coat (4.5 %), 6 kg of husk (25 %) and 5.5 kg of kernel (23 %). The kernel gives about 2.5 kg of neem oil (45 %) and 3.0 kg of neem cake (55 %). Similarly, (Paul *et.al*, 2013) shows that the fresh neem fruit produces greenish brown kernel 30%, other shell and pulp 70%. From the seed of neem 53.3% shell and47.7% kernel obtained. From this kernel the oil content is 46-48%. Other review (Paul *et.al.*, 2013) reported neem oil yield from seeds varies from 25% to 45%.

ii) Extraction of oil

There are broadly two classes of plant oils those are Essential Oils and Fixed Oils. Essential oils are volatile, and are usually derived from the non-seed parts of the plants different from oils produced from oilseeds. Most fixed oils are the so-called "fatty oils", and a majority of the fatty oils are derived from the seeds. Some of the fixed oils are derived from vegetables & nuts that are fatty, dense and non-volatile, such as olive, castor oil, coconut oil and sweet almond oil. This is in contrast to essential oils which are volatile in nature (Evbuomwan *et.al.*, 2015).

The methods to obtain Neem oil from the seeds are like mechanical pressing, supercritical fluid extraction, and solvent extraction (Liaw *et al*., 2008) reported that mechanical extraction is the most widely used method. The authors however, noted that the oil produced with mechanical extraction method usually have a low price, since it is turbid and contains a significant amount of water and metals contents. With extraction using supercritical fluid, the oil produced has very high purity but for the high operating and investment cost, not widely used. Extraction using solvents have a number of advantages which are higher yield and less turbid oil than mechanical extraction. It as well as relative low operating cost compared with supercritical fluid extraction.

Liaw *et al*., (2008) compared ethanol and n-hexane solvents as a medium of neem oil extraction. The maximum oil yields obtained were 41.11% and 44.29% with ethanol and n-hexane at a low extraction temperature (50°C), respectively. Hence, there is a possibility that more oil could still be extracted from the neem seed using ethanol at temperatures higher than 50°C, and possibly more oil than that extracted using hexane at 50°C. In another study, ethanol–hexane mixtures utilized for oil extraction by the Soxhlet extraction method in ratios of 60/40, 50/50 and 40/60 (v/v) furnished oil yields of 44%, 43%, and 41.2%, respectively. The ethanol–hexane mixtures gave a better yield than 100% hexane (40.25%) at 55°C over6 h duration (Ayoola *et.al.*, 2014).

iii) Chemistry of neem oil

The active ingredients in Neem oil (*Azadirachtin*) and clarified hydrophobic extract of Neem oil are derived from the natural oil found in seeds of the Neem tree. When the natural Neem oil is removed from the seeds and treated with alcohol, virtually all the Azadirachtin and related substances separate from the oil itself. The remaining oil without the Azadirachtin is called clarified hydrophobic extract of Neem oil (Elshierh & EldeenEdres, 2013).

The seed kernels of *A. indicia* contain an array of Azadirachtin of which seven isomers labeled as A to G are so far identified. They are potent growth inhibitors. Azadirachtin A is the main bioactive constituent in neem extracts which compose about 70 % and 99% together with Azadirachtin B. The molecular structure (Figure 1) and activity studies indicate that the complete carbon skeleton of the molecule is required for growth inhibition and anti-feedant activity (Tekie, 1999).

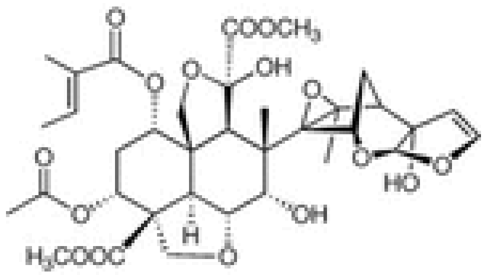


Figure 1: Structure of Azadirachtin
Source: (Paul *et.al.*, 2013)

Neem oil extract, which is rich in essential fatty acids (EFAs), triglyceride, vitamin D and calcium. Because of its EFAs and vitamin D, neem oil penetrates deep within the skin to heal the minute crack brought on by sever dryness. The physicochemical characteristics commonly analyzed for its quality by determining the saponification (SV), acid (AV), and iodine (IV) values, and so forth (Tawfig *et.al.*,2017). The major fatty acid composition and physicochemical characteristics of NSO are presented below in Table 1 and Table 2.

Table 1: Fatty acid composition of NSO

Source: (Aransiola *et al.*,2012)

Fatty acid	Formula	Systemic name	Structure	Wt. %
Palmitic	$C_{16}H_{32}O_2$	Hexadecanoic	16:0	18.1
Stearic	$C_{18}H_{36}O_2$	Octadecanoic	18:0	18.1
Oleic	$C_{18}H_{34}O_2$	cis-9-Octadecenoic	18:1	44.5
Linoleic	$C_{18}H_{32}O_2$	cis-9,cis-12-Octadecedianoic	18:2	18.3
Linolenic	$C_{18}H_{30}O_2$	cis-6,cis-9,cis-12-Octadecatrienoic	18:3	0.2
Arachidic	$C_{20}H_{40}O_2$	Eicosanoic	20:0	0.8
Total saturated fatty acid 37% Total mono-unsaturated fatty acids 44.5% Total poly-unsaturated fatty acids 18.5%				

Table 2: Physico-chemical properties of neem oil

Source: (Djibril *etal.*, 2015 and Leather Sectional Committee, 2001)

Characteristics	Requirement for Neem kernel oil	Depulped neem seed oil	Unit
Moisture and volatile content, max	0.30 %	0.50 %	-
Refractive index(25^{o}c)	1.46-1.47	1.46-1.47	-
Specific gravity(25^{o}c)	0.91-0.93	0.91-0.93	
Saponification value	180-205	175-200	mg KOH/g of oil
Iodine value	65-80	65-80	mg KOH/g of oil
Acid value, max	15.00	20.00	mg KOH/g of oil
Viscosity (40°C)	49.79	49.79	mm^2/s
pH	6.5-7.5	5.7-6.5	-

The poor quality of NSO can be confirmed by increased acid and peroxide values. A high acid value indicates the presence of a high amount of free fatty acids in oil due to the degradation caused by hydrolysis at high temperature with a result of rancidity that gives an unpleasant smell to the oil due to the degradation of glycerides or formation of aldehydes and ketones. Also, the specific gravity (SG) or density of NSO decreases with an increasing temperature of exposure, and results in reduced viscosity and increased flow of oil (Liaw *et.al.*, 2008).

The reactivity or the degree of unsaturation is determined by the amount of halogen with which 100 gram of fatty acid or oil can react and expressed as iodine value. An oil or fatty acid of high iodine value is naturally reactive and can easily oxidize, polymerize and react with different chemicals. Oils of low iodine value, on the other hand, are chemically inert. Oils of iodine value above 140 are thus very reactive and quickly dry up in atmosphere leaving a solid mass behind and so these oils are called drying oils. Similarly when iodine value of oils is between 95 to140, they are known as semidrying oils and non-drying oils should have iodine value below 95. The iodine value of neem oil makes the oil non-drying type. This makes the oil is suitable for synthesis of fatliquor. Higher saponification value means oils of smaller molecules gives more penetrating powers and softness to leather (Dutta, 1999). Characteristic average values of the main fatty substances are shown in Appendix A Table A-4.

iv) Industry application of neem tree and components

All parts of this tree are very useful in variety of biological activity. The most famous part of this tree is the oil obtained from the kernel of its seed (Adewoye & ogunleye, 2012).

In leather industry, Sodium chloride is commonly used for curing hides and skin. In a process developed by leather researches, neem oil has been used in place of salt (Puri, 2006). Moreover, neem oil is used to soften and supple skin. Also, it helps in reducing old scar (Tawfig *et.al.*,2017).

De pulped and dried neem seeds can be processed with an expeller oil-press to obtain the oil and neem cake .In addition to its use in pest control, the oil can be effectively used as a raw material in the soap industry. Neem cake is commonly used as a fertilizer in many places where neem trees are widely grown (Tekie, 1999).

Neem oils widely used in different region for medicinal and agricultural purpose and it has shown positive results as pesticide. In India, it has been demonstrated that Neem Oil is a potential new contraceptive for women (Adewoye and Ogunleye, 2012).

Every part of this fascinating tree has been used from ancient to modern times to treat hundreds of different maladies. While it is still revered in India for its superior healing properties, recent investigation has dramatically increased worldwide interest in Neem and many products are now manufactured using this miraculous herb (Paul *et.al.*, 2013).

Before synthetic detergents became popular in India, soap making, particularly for laundry, was a cottage industry and any readily available, cheap fatty oil was incorporated in soap formulations. Neem oil was one of the ingredients, but was not preferred because of the malodor and dark color of the soap so obtained. Conventional industrial methods failed to refine the oil because the bitter principles are mixed with the lipid constituents so intimately that it is difficult to separate the two. The soap industry takes various steps for refining neem oil. In due course of time, the soap manufacturer's study that neem oil could be incorporated up to 15 percent into the mixture of fatty oils along with cotton seed oil, coconut oil, etc. The cotton seed oil was found to mask the odor of neem (Puri, 2006).

Table 3: The industrial use of part of the neem tree
Source: (Workneh, 2011)

Parts of neem tree and its product	**Use in**			
	Pharmaceutical industry	**Cosmetic industry**	**Agriculture Industry**	**Oral care**
Neem leaf	inflammation, skin related diseases like acne, rashes	Face and body Cream	Natural pesticide, Insecticide	Manufacturing of tooth paste, mouse washes
Neem kernel		Manufacturing of skin products	pesticide, insecticide, crop and plant protection	
Neem seed cake			Pest repellant, organic fertilizer	
Neem flower	Manufacturing of drugs	As an astringent, facial cream	Honey	
Neem oil		Skin product, body lotion, beauty facial care	Pesticide, insecticide, Fungicide	
Neem seed	Drug and medicine: pimples, blemishes, skin infection, birth control	Skin creams, moisturizers, face packs	Pesticide, Insecticide	

2.2 Fatliquor Sources

The primary sources as a raw material for fat liquor production are fats, oils and waxes which have lubricating properties to promote the slipping of adjustment surfaces against each other or reduce the adhesion between them. In the absence of oil, the leather fiber structure is tightly woven, firmer and rather hard. However, small amount of oil spread over the fibers to reduce this hardness and make the leather remarkable for its softness and stretch (Sharphouse, 1983).

Oils in the leather industries are used in the production of fat liquor and oil tannage. Some of the oils used are; Sperm whale oil which is obtained from head cavities and blubber of the whale and is an ester of fatty alcohol with iodine value of 71 -93. It's very good oil for leather lubrication but became very scarce due to worldwide whale conservation program (Sarkar & Sorcar, 2005) Jojoba oil is a vegetable oil from a desert plant called Jojoba which is having 50 per cent oil yield by weight and is reported to have the same quality as whale (sperm) oil in fatliquor. Cod oil are commonly used for Chamoising (Leathers that are extremely soft mostly used for cleaning of glasses) and also in the production of fatliquor. Other oils that can be used in the leather industry are Linseed, cotton seed, neat foot, olive oils etc. (Habila *et al.*, 2016).

Fats and oils predominantly are trimesters of glycerol and aliphatic fatty acids containing up to 22 carbon atoms. Waxes are esters of long-chain fatty acids, usually containing 24-28 carbon atoms. Fats and oils are members of lipids, which has been classified by national research council in to non-polar lipids including easers of fatty acids that are virtually insoluble in water, but soluble in most organic solvents (Tawfig A *et.al.*, 2017).

The five fatty acids namely palmitic, stearic, oleic, linoleic and linolenic are the main fatty acids in oils and fats. The first two fatty acids are saturated and the rest three are unsaturated. Saturated compounds have single bonds (C-C) between the carbon atoms and the other bond is bound to hydrogen atoms. Unsaturated compounds have double bonds (C=C) between carbon atoms, reducing the number of places where hydrogen atoms can bond to carbon atoms(Evbuomwan *et.al.*,2015).The selection of oils and fats for fatliquoring depends upon the type and composition of fatty acids (Dutta, 1999).Some common fatty acids found in oils and fats are shown in Appendix A Table A-3.

2.3 Physicochemical properties of fatliquor

The physical properties of fat liquors are color, clarity, odor, viscosity and thawing point. These are the measurable properties that can describe the state of the matter. Color gives the physical appearance and can be measured using the Tintometer. Clarity and odor can be analyzed physically and organoleptically. Viscosity is the fluidity of the fatliquor and measured using Viscometer. Thawing point of a fatliquor is the temperature at which it is solidified and measured by gradual cooling of the fatliquor. The chemical properties of fat liquors are active matter, oil content, fatty matter, acid value, saponification value, iodine value of the free oil and salt composition (Leather Sectional Committee, 2001).

2.4 Fatliquoring substances category

i) Natural fat liquoring substances

Oils, fats and waxes are natural fatliquoring substances obtained from vegetables or animal sources. Oils and fats are triglycerides extract which could be vegetable oils and fats from plants; and animal sources as animal oil and fats. Vegetable oils are drying oils (linseed oil, hemp oil, poppy oil, nut oil, wood or Tung oil). Semi-drying oils (colza or rape oil, maize oil, sunflower oil, soya bean oil, cotton seed oil, rice oil).Non-drying oils are olive oil, castor oil, ground nut oil and fruit kernel oils (BASF).They are either hydrophobic or lipophilic in character. These oils from plant seed are produced either by mechanical extraction using oil mills which is termed as pressing or by chemical extraction using solvents. Vegetable fats are Coconut fat, palm kernel fat, palm oil fat, Japan tallow (Habila *etal.,* 2016).

Animal oils are oils of Marine animals like seal oil, whale oil, dolphin oil. Land animal oils (Neats foot oil and lard oil). Animal fats are Beef and mutton tallow, lard, and butter fat, bone fat, horse grease. The main drawbacks with animal fat is that it causes too much of spue formation on leather surfaces. To overcome this trouble fats are nowadays chlorinated in presence of suitable catalyst and ultraviolet rays. Chlorination converts solid fats in to liquid oil. The chlorinated fatty portions get fixed to the leather fibers and therefore cannot migrate to leather surface. Thus the spue formation is reduced considerably and also waxes like vegetable origin (carnauba wax and montan wax), animal origin (bees wax and wool grease) are the examples of this category (Dutta, 1999).

ii) Synthetic fatliquoring substances

It is an imitation of natural oils (Neats foot oil and sperm oil) and substituted natural oils. Chlorinated oils, silicones, polybutenes and paraffin waxes are belonging to this group (Tawfig A *et.al*, 2017).

iii) Semi synthetic fatliquoring substance

It is a mixture of synthetic and natural oils. To overcome dry, flat, show straw feel, poor roundness and resiliency nature, synthetic products are always used in combination with natural greasing agents. The blending not only improves the quality of the leather but also improves the stability of emulsion. The power of penetration into the hide of this type of emulsion is always more than the emulsions made either with natural or synthetic products alone (Sarkar & Sorcar, 2005).

2.5 Process technology for fatliquor production

Lubrication of leather is very essential during the manufacturing process. It increases the flexibility, pliability, softness and gives comfort to the user in whatever forms of finished goods the leather eventually end up. These properties face some challenges during application such as the electronic charges of the leathers, stability of the emulsions, uptake, types, colors and quantity of dyestuff and the method of drying (Covington, 2009). For these applications the demand for fatliquor increases and shortage in industrial demand became imminent. Therefore different technologies and sources need to be established with scientific proofs to fatliquor characteristic that meet the upcoming challenges (Habila *et al.,* 2016).

Fat liquor is processed from water emulsion of oils or fats. The presence of polar and non-polar groups in oil helps to determine the affinity or aversion of the oil toward water. A polar group (hydrophilic) has a natural ability to orient itself and dissolve in the water phase. Such groups include –COOH, -OH-, -SO_3, -Cl, etc. a non-polar group (lipophilic) dissolve in the oil phase and exemplified as–CH_3, -C_2H groups (Lollar, 1978).Fat liquors based on their charge characteristics are classified into anionic (soap –COOR where R is monovalent metal, sulfated –C-O-SO_3, and sulphonated C-C-SO_3) cationic (salts of tertiary amines or quaternary ammonium compounds) or non-ionic (oxide condensates, (-O-O-)). Anionic fatliquors are commonly employed for fat binding with chrome-tanned leather, which is cationically charged. Anionic fatliquors are commonly prepared by sulphation, saponification or sulphitation of oils/fats using different production technology (Sivakumar *et.al.,*2008).

In modern tanning practice the sulphonated or sulphated oils are being used presently the largest category of fatliquoring materials. This technology has the advantage of greater stability at low pH (Thorstensen, 1993).

i) Sulphation

Sulphated oil are produced by treating the oil with a given amount of sulfuric acid under controlled conditions of temperature, time, and agitation , followed by washing the acid and oil mixture with a salt solution to remove the excess acid. The acid salt solution is allowed to settle and is drained off. The sulphated oil is then generally neutralized with sodium or ammonium hydroxide until the desired PH is reached. Following this the moisture content, total alkalinity, etc. are adjusted to the desired levels (Lollar, 1978). The properties of sulphated product depend on strength and proportion of sulfuric acid to oil used, Rate of addition of sulfuric acid and degree of mixing or agitation (Tawfig *et.al.*, 2017).

Sulphated oil (often called sulphonated oils) are very commonly used because they give good, fine oil dispersions and are less sensitive to acid than soap fatliquors. Consequently the oil drops penetrate further in to the leather before they are deposited and give deeper lubrication to ensure soft and mellow handle. But the lubrication of the grain layer becomes rather poor. When surface lubrication is desired, highly sulphated oil should carry with it free or untreated oil (neutral oil) to provide deposition on the surface for grain lubrication (Sarkar, 1996).

The main reaction between oil (R-CH=CH-R) and sulfuric acid is exothermic and thus, acid is added very slowly with constant stirring to avoid any raise of temperature. The (-SO_3H) radical is thus linked up with a carbon atom through an oxygen atom (R-O-SO_3H) (Dutta, 1999). The further reaction of sulfated oil with caustic soda gives sulfated fat liquor. The mechanism of reaction for sulphation reaction is illustrated below in Scheme 1and Scheme 2 (El-Shahat *et.al.*,2011).

$$\mathrm{R-CH=CH-R+H_2SO_4 \rightarrow R-CH_2-\underset{\displaystyle OSO_3H}{\underset{|}{CH}}-R}$$

Scheme 1: Reaction for production of sulphated oil (sulphuric acid ester)

$$\mathrm{R-CH_2-\underset{\displaystyle OSO_3H}{\underset{|}{CH}}-R+NaOH \rightarrow R-CH_2-\underset{\displaystyle OSO_3Na}{\underset{|}{CH}}-R+H_2O}$$

Scheme 2: Reaction for production of sulphated Fatliquor

During sulphation if the temperature of the product goes up or if any other drastic method of sulphation is followed (-SO_3H) group. This is directly linked up with a carbon atom (R-SO_3H) and thus sulphonated product results as shown in Scheme 3

$$\mathrm{R-CH=CH-R+H_2}SO_4 \rightarrow \mathrm{R-\underset{\displaystyle OH}{\underset{|}{CH}}-\underset{\displaystyle SO_2OH}{\underset{|}{CH}}-R}$$

Scheme 3: The reaction for sulphonated oil (side reaction)

The degree of sulphation is expressed by the combined sulphate factor. The effect of increasing the combined sulphate is to increase the fineness of emulsion giving better penetration. The degree sulphation may range from 2% considered low level, to 3-4% considered medium level, to 6-8% considered to be high level (Covington, 2009).

- Low level of sulphation: Low stability of the emulsion to coagulating (cracking) by acids or metal salts used to lubricate the outer surfaces, particularly the grain.
- Medium level of sulphation: More stable to coagulation, therefore greater potential for penetration. Used for surface neutralized chrome leather.
- High level of sulphation: used for complete penetration, e.g. through neutralized chrome leather for gloving, clothing, and softie leathers.

As the level of sulphation increases,

⇒ Anionic charge increases, hence greater affinity for cationic leather

⇒ Lubricating effect decreases , due to the lower concentration of neutral oil

⇒ The oil functions more like wetting agent than lubricant, hence the leather becomes more hydrophilic

⇒ The leather becomes looser, in terms of break, possibly due to the damaging effect of the sulphate species on collagen

⇒ The likelihood of hydrolyzing the oil to create free fatty acids increases, thereby creating the possibility of chrome soaps, fatty acid spue, poor wetting back, uneven dyeing and poor finish adhesion (Covington, 2009).

The addition of sulphation or sulphonation group in to the oil modifies the emulsion characteristics in that the dispersive power of the sulphonated group is much greater than the carboxyl group of soap (Thorstensen, 1993).

ii) Sulphitation /Oxidation

In this oxidation process, the heated oil (60-80^{o}C) reacted with Sodium bi-sulphite ($NaHSO_3$) (40^{o}Be) following aeration for 8 to 20 h as shown in Scheme 4 and Scheme 5. The excess $NaHSO_3$ can be removed by washing with brine without necessitate the pH adjustment (Covington, 2009).

Sulphited oils possess superior penetrating, softening qualities and good stability in presence of acidity, alkalinity and hardness of water present in fatliquor bath.

$$R-CH=CH-R+O_2 \rightarrow R-\underset{\diagdown\,O\,\diagup}{CH-CH}-R$$

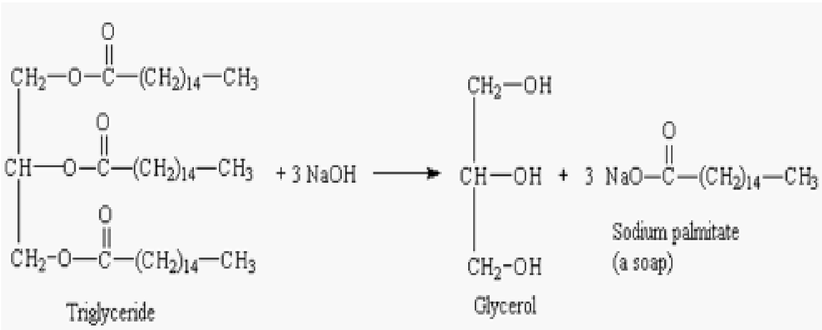
Scheme 4: Oxidation of oil to form epoxide oil (oxidized oil)

$$\mathbf{R-\underset{\diagdown\,O\,\diagup}{CH-CH}-R+NaHSO_3 \rightarrow R-\underset{OH}{CH}-\underset{SO_3Na}{CH}-R}$$

Scheme 5: Sulphitation reaction for sulphited fatliquor

iii) Saponification

Soap is a saponified fatliquors produced by saponification or basic hydrolysis reaction of fat or oil. The general overall hydrolysis reaction is given in (Betsy *et.al.*, 2013).



Scheme 6: Saponification of triglyceride

When saponification is complete the whole mass is solidified without separation of glycerin. The oil droplets in an oil-water emulsion formed by using soap as an emulsifying agent are generally coarse and also break very quickly with acid. Such emulsions cannot penetrate deep in to the leather. In such away, there is greater tendency to be deposited on the surface of the leather (Dutta, 1999).

A fatliquor emulsion of soap and raw oil is done by first dissolving soap in hot water to which the oil is slowly added. The soap oil mixture is then boiled or steamed when a coarse emulsion of oil-in-water is formed (Sarkar *et.al.*, 2005).

Soap fat liquor is limited due to the low emulsion stability. This is mainly applicable for shoe uppers (surface fatliquoring calf skin) and gloving leather that is formaldehyde tanned sheepskin (Covington, 2009).

2.6 Factors that affect sulphated fatliquor production

1) Amount and concentration of acid

The amount and concentration of sulfuric acid is very essential for imparting good feel and better properties to leather which reduces the neutral oil component in fatliquor (Sivakumar *et.al.*, 2008). The more the oil is sulphated or the more sulfuric acid has been fixed, the greater will be its stability to acid and the more penetration in to the leather. When the leather is more acidified, the penetration would be less. Thus, with chrome leather, better penetration is obtained by neutralizing the leather or using emulsifying agents of good acid stability in larger quantities. The amount of sulphation or water miscibility increases, the oiliness and its lubricating power become decreases (Sharphouse, 1983).

2) Mixing speed

The mixing speed in the reaction must be considered as constant to prevent variations and maintaining the temperature. The minimum speed may result in poor dispersion and increasing speed may cause certain emulsions to break and ability to raise the temperature (Lollar, 1978).

High micro mixing will increase the heat and mass transfer and even the diffusion of species inside the pores of the solid. The intense agitation and dispersion effect which is brought on by the effects of cavitation results in an increase in the number of collisions between the oil droplet and water and better emulsification of oil in water (Sivakumar *et.al.*, 2008).

3) Reaction temperature

As sulphation reaction is exothermic, it gives off heat which would char the oil unless the mixtures were kept cool. Hazards with this process are overheating may cause darkening of the color due to oxidation or polymerization and the triglyceride oil may be hydrolyses to release free fatty acids. This later effect can give rise to the problem of spue, when the longer chain carboxylic acids can migrate from the internal structure of the leather to the grain surface, visible as a white efflorescence (Tawfig *et.al.*, 2017).

4) pH

It is a factor for the stability of fatliquor and for fixation of fatty mater while fatliquoring. The stability of emulsion and penetration of the fatliquor into the leather depends on pH value of emulsion and leather to be fatliquored. Leather, particularly chrome tanned leather, is acid and has a PH of about 4 at the time of fatliquoring. The PH in the leather is lower than that of the emulsion to maintain the stability of the oil, and deposition of oil on to the leather. Higher acidity causes premature splitting up of fatliquor before it has a chance to penetrate into the leather. This results in undernourished leather of greasy surface (Thorstensen, 1993).

5) Purity of raw materials

Impurities present in vegetable oil also affect the sulphation reaction. The presence of high quantities of free fatty acids in the original raw oil will significantly change the degree of sulphation. This may have an effect on the dried leather as spue which results poor quality of fatliquored leather. Another trouble due to rancidity of the oil is that free fatty acid form compounds with chromium, alum or zirconium salts used in tanning, which may make the leather water repellent and difficult to wet back uniformly for dyeing and finishing purposes. So rancid oils and fats, especially if they contain hard fatty acids, should be avoided and the leather should be kept dry and free from mold. As a result purification of crude oil is necessary for production of desired quality sulphated fatliquor and fatliquored leather (Sharphouse, 1983).

6) Time of reaction

At low temperature the viscosity of neem oil increases considerably which results lower reaction rates and correspondingly higher reaction time for completion of the reaction.

A study by (Ariful, et al., 2015) shows that preparation of sulphated fatliquor from karanja requires 3hrs.similar work has been reported that 5hr is required to complete the reaction from oil for the production of sulphated fatliquor (Pervez, et al., 2015).

2.7 Properties and specification of sulphated fatliquor

Indian Standard IS.6357 was adopted for the specification of sulphated oil for leather fatliquoring with the recommended test method described below.

1. Physical Condition

The material shall be a free-flowing, clear, oily liquid, Appearance of any graininess or a slight physical separation like a liquid and semi-solid portion appearing especially in winter season or long storage shall not be considered as defect as long as the two portions individually or together form ready emulsions in water.

2. Emulsion Characteristics

For sulphated Fatliquor, the emulsion needs to satisfy the following requirements:

- The product shall form a ready emulsion in hot or cold water in any dilution.
- The sulphated oils shall be capable of being readily washed out with water without leaving any oily feeling to hand.
- The sulphated product when mixed with 25 percent raw oil(s) having an acid value not above 10, or other mineral oil shall forma ready emulsion when diluted with hot/cold water in all dilutions.
- Emulsion of 10% percent shall remain stable for 40 minutes when mixed with 5 percent solutions of sodium chloride, Magnesium sulphate and basic chromium sulphate in separate containers without creaming and oil separation.

3. Odor

The product shall be free from rancid or putrefactive odor of the oils.

4. Chemical Requirements

The product, besides meeting the other characteristics mentioned above, shall also meet the chemical requirements mentioned in Table 4

Table 4: Chemical requirement of sulphated fatliquor

Characteristics	Requirement	Method of test, Reference
pH of emulsion	6.5 to 8	IS.6357, Appendix A-3
Total active ingredients,(% by mass)	Min., 60	IS.6357, Appendix A-4
Unsaponifiable matter,(% by mass)	Max., 2	IS.6357, Appendix A-5
Total alkalinity in mg equivalent per 10g	Max., 3	IS.6357, Appendix A-6
Organically combine sulphate (SO_3) as sulphuric esters, % by mass	Min., 3	IS.6357, Appendix A-7
Total organically combine sulphate (SO_3)as sulphuric and sulphonic esters, % by mass	Min., 4.5	IS.6357, Appendix A-7 & A-8
Ash, % by mass	Max., 3	IS.6357, Appendix A-8

The pH of 10% fatliquor emulsion should be between 6.5 and 8 of sulphated fatliquors used for leathers lubrication for the stability and penetration in to the leather. The reaction depth and degree of conversion were determined by the amount of SO_3 fixed (degree of sulphation). The stability of the resulting emulsions also evaluated according to the content of SO_3 in the sulphated oil. (El-Shahat *et.al.*, 2011) states that the degree of conversion was estimated mostly as SO_3 content and should be noted that SO_3 content of the resulting constituents is of great significance.

Total alkalinity is the name given to the quantitative capacity of an aqueous solution to neutralize an acid. Measuring total alkalinity is important in determining the fatliquor ability to neutralize acidic media in fatliquoring process. Unsaponifiable mater is the fraction of substances in oil and fats which is not saponified by caustic alkali, but is soluble in ordinary fat solvent.

2.8 Fatliquoring of leather

Fatliquoring is the application of oils, greases and other materials having lubricating properties to the leather matrix in finely dispersed form of water medium (Cuq *et.al.*, 1998).In an emulsion with water, the oil is dispersed in microscopically small droplets, remain as an emulsion until they penetrate the leather and should not separate out as large drops or as a layer of oil and wouldn't give a greasy surface layer (Sharphouse, 1983).The ability of emulsifiable products to impart different characteristics to leather is governed by various factors, such as fatty acid composition of the fat products and the extent of unsaturation in the emulsifiable oil (Cuq *et.al.*, 1998).

A tanned leather which has not been treated with oils, fats or greases will dry out hard and bony. The fibers of the tanned leather must be lubricated with a coating of oils or fats to enable them to slide over one another as the material is flexed. Also treatment with oils and fats prevents the leather fibers from sticking together during dying. Proper treatment with oils and fats give the leather full and soft handle flexibility and additional strength (Sarkar & Sorcar, 2005). Fat liquors penetrate into the gaps of collagen fibers and their polar groups attract each other to form a lubricant film around the fibers (Zhengjun *et.al.*, 2014). One of the industrial processes which render the penetration of fat molecules is the formation of surface active compounds (El-Shahat *et.al.*, 2011).Unless the leather is fat liquored, it becomes hard on drying and the fibers cannot slide over one another and ultimately break when bent (Dutta, 1999).

Low grade and impure oil should not be put on chrome leather; as such oil undergoes a process of decomposition in the leather, imparts a bad odor to it, and spews out on the surface in the form of white scum, which is removed with difficulty. So it is advisable to use only the best grade for the purpose in view of the better quality of the finish (Covington, 2009).

Fatliquor type can affect the mechanical properties of skin immensely during fatliquoring process beyond other dependent structural and molecular factors for mechanical behavior of materials such as molecular weight, crosslinking and branching, crystal morphology, fillers, molecular orientation, phase separation, plasticization. Apart from these structural and molecular factors, there are many environmental or external factors like; temperature, time, rate of stressing, pressure, type of deformation, moisture content, stress and strain amplitude, thermal history etc. which affect mechanical behavior Among the mechanical tests, stress-strain tests are traditionally the most popular and are most widely used as these tests indicate the modulus, strength and toughness of the material. As per the prediction of kinetic theory, the modulus of the material increases as the degree of crosslinking increases .In this regard, the breaking stress should also be directly proportional to crosslink density (Kumar, 2017).

Physical-mechanical properties and sensory/organoleptic properties are two important parameters for evaluating the application performance of leather and leather products. Softness is especially one of the most important organoleptic properties to be taken into consideration when assessing the quality of light leathers (Zhengjun *et.al.*, 2014).The physical/strength properties of fat- liquored leather such as break, stitch tear resistance and tensile strength, as well as comfort properties (particularly for clothing) of leathers, depend on fat-liquoring (Cuq *et.al.*, 1998).

2.9 Leather and leather products

Leather is one of the most widely traded commodities in the world. The leather and leather products industry plays a prominent role in the world's economy, with an estimated global trade value of approximately US$100 billion per year (UNIDO, 2010). Leather is used in the production of a variety of different articles, for shoes, garments, upholstery and, increasingly, in the automotive sector. Leather is a very versatile material i.e. 90% of it used for various applications in which it is used make very varied demands on its performance.

Ethiopia, during 2007 and 2008 E.C. budget year started to develop its leather and derived products sector more effectively. A mix of export duties and incentives has led to the use of a larger proportion of domestic raw material for the manufacture of finished leather and finished leather products. In the 2006-07 E.C. budget year, Ethiopia exported leather and leather products worth to US$89.5 million. Italy was the leading recipient with 44%. The government expects the value of leather exports to increase to US$500 million within five years, and has imposed increasing duties on exports of semi-processed material to support this goal (UNIDO, 2010).

3. MATERIAL AND METHODS

3.1 Materials

3.1.1 Chemicals and reagents

In this study the following chemical and reagents were used:

Acetone (Ranchem, Turkey), Chloroform (Abron chemicals, India), Ethanol (Ranchem, Ethiopia), n-Hexane (Loba chemie, India), Glacial acetic acid (Abron chemicals, India), Diethyl ether (Loba chemie, India), Hydrochloric acid (Blulux, India), Potassium hydroxide (KI) (Abron chemicals, India), Potassium iodide (Alpha Chemika, India),$Na_2S_2O_3$(Abron chemicals, India), Starch ,H_2SO_4 (Loba chemie , India), Sodium Chloride (NaCl), Sodium hydroxide (NaOH).

3.1.2 Equipment and apparatus

The equipment and apparatus used in this experiment were:

Beakers diff. size, Burettes, Centrifuge (Pro-analytical C2004, UK), Chiller (Maalab, RC 3000G), Condenser, Density Meter (Aton Paar, DMA 4100 M), Electronic balance (+0.0001g, China), Erlenmeyer flasks diff. size, Mechanical screw oil press (CA59 G, Germany), stirrer (Euro-st 60c, Germany), Measuring cylinders, Muffle Furnace (MF106, Turkey), Crusher (Model-300, china), Oven, pH-Meter (AutoDeluxe , LT-10), Refractometer, Reactor glass, Rotary evaporator (Maalab , ML-EN2050), Separatory funnel, Soxhlet extraction unit (Bionics , India), Sieve (meshsize,1mm),Stirrer (Euro-st60c, Germany), Shaker, Sam-Setting machine, Shaving machine , Testing drum , Staking machine, Tear die double-edge, Tensile die, Universal tensile testing machine,(UTM ,UK) ,Viscometer (vibrioviscometer,0.3-10,000mpa.s), Water bath (Maalab , Great Britain).

3.1.3 Sampling and Raw material Preparation

Neem seed sample of 35 Kg was collected from Dire Dawa city, located at 532 Km to the eastern part of Ethiopia. The sample was cleaned from extraneous and washed manually. Then, it has been left for seven days to be sun dried. Then, the seed was decorticated and 15.86 kg of kernel was obtained. 60 g was used for Solvent extraction and the other 15.8 kg was used for mechanical screw oil press extractor. The solvent oil extraction, oil refining and characterization, synthesis of fatliquor and characterization were done at Addis Ababa Science and Technology University and Addis Ababa institute of Technology while the mechanical extraction using screw press was done at Bahirdar University. The Actual test of Synthesized Fatliquor was conducted in collaboration with Leather Industry Development Institute. The general scheme or overview of the experiment is shown below in Figure 2.

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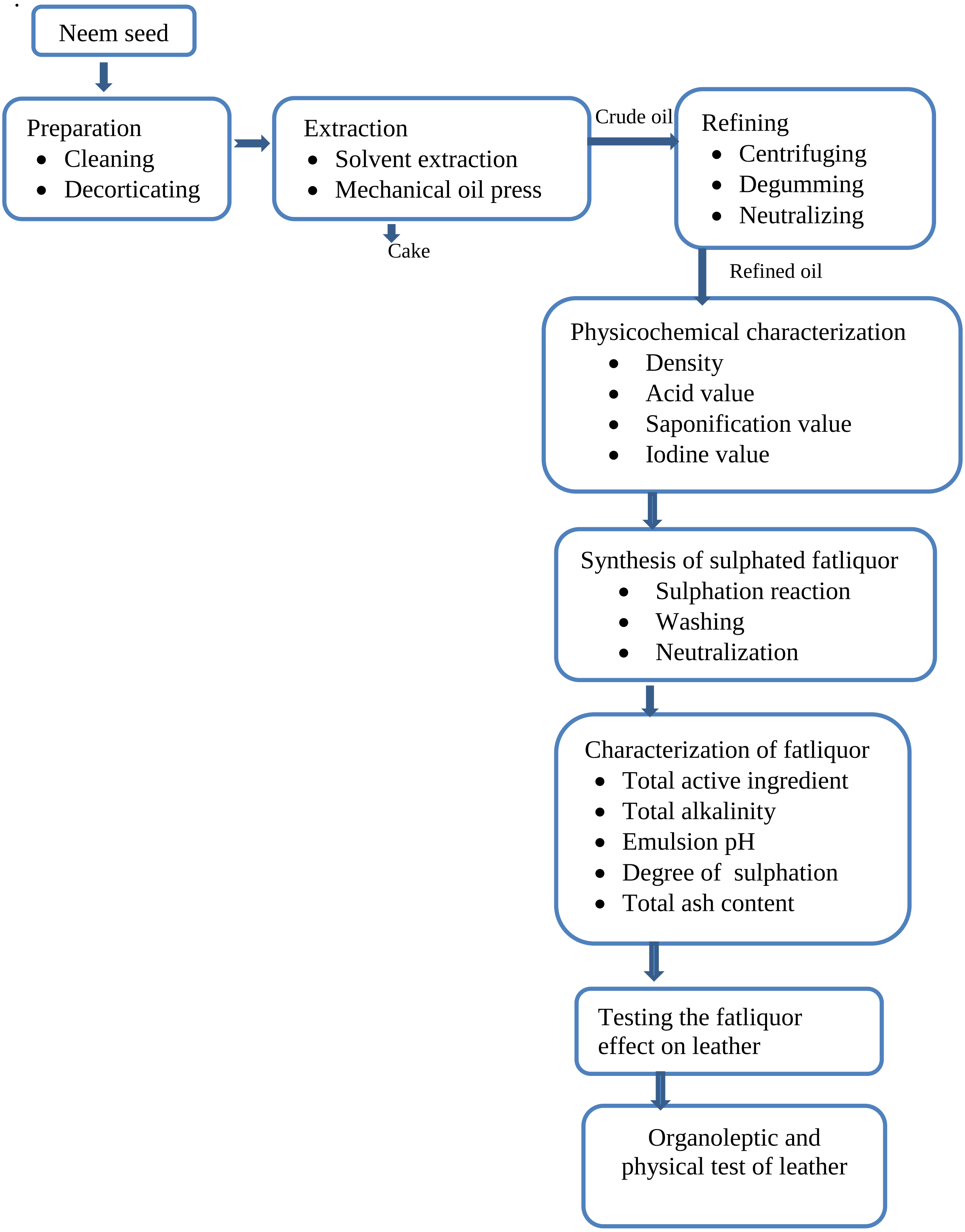


Figure 2: General overview for flow of the experiment

3.2 Methods

3.2.1 Neem seed kernel oil

3.2.1.1 Determination of moisture content of neem seed kernel

The moisture content was analyzed using the method by IS 3579. Sample of 5 g neem seed kernel was weighed and dried in an oven at 105°C for every 2 h. and the weight was measured until constant weight was obtained.

3.2.1.2 Extraction of neem oil

Mechanical and solvent extraction method was applied to extract neem oil. Mechanical extraction of Neem kernel performs using mechanical screw oil pressing machine. First, the pressing machine was cleaned and adjusted the speed to the desired level. Then the prepared kernel seeds feed continuously in to the screw pressing machine. Finally the crude neem oil was collected and the remaining cake removed. The mechanical screw press machine extracting neem oil is depicted in Figure 3 below.

Figure 3: Mechanical screw oil press

Solvent extraction method using hexane, ethanol and combinations of n-hexane and ethanol (60:40) were used to study the better yield performance of the solvent. Each extraction run was set up by measuring 20g of the seed powder into 120ml of the solvent in the soxhlet extraction unit. Then the solvent and crushed mixture was heated at constant temperature of 55^0C for 3 h to extract the oil. At the set time interval, the samples were taken and centrifuged to separate the solid fraction from the solution. Finally, the extracts were heated and evaporated using rotary evaporator apparatus to obtain solvent-free oils. The general process flow schematic diagram for neem oil extraction is shown in Figure 4. The yield of the extracted oil was determined from the proportionate mass of extracts to the sample as shown below.

$$\textbf{Yield}\ (\%) = \frac{\textbf{Mass of Extract}}{\textbf{Mass of Sample}} \times \textbf{100} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.1$$

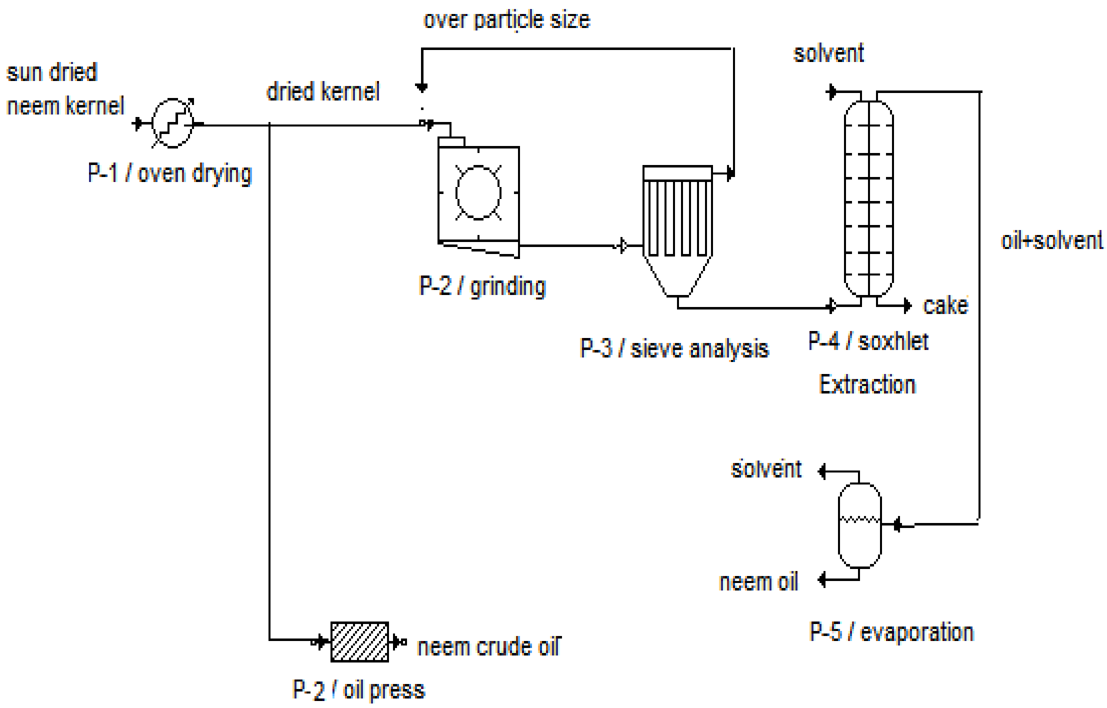


Figure 4: The process flow schematic diagram for neem oil extraction

3.2.1.3 Refining stage of crude neem oil

i) Centrifuging

The crude oil extracted had solid suspensions and impurities those impurities and solid suspensions were separated by using centrifuge at a speed of 3500 rpm for 30 minitues.

ii) Degumming

The undesirable complex compounds like phospholipids, gums and increment of free fatty acid during storage or rancidity were avoided by degumming. 3 % distilled water, by oil volume was brought in to contact with the crude at constant speed of 200 rpm with a temperature of 70 ^{0}C. Finally the oil separated from impurities and complex compounds using centrifuge at 3500rpm for 40min.

iii) Neutralization

The amount of sodium hydroxide needed to neutralize free fatty acid based on the free fatty acid content of crude neem oil was calculated by the given equation (Logan, 2005).

$$\text{Amount of lye (sodium hydroxide)} = \frac{A \times FFA \times 1000 \times K}{100 \times M \times N} \ldots\ldots\ldots\ldots 3.2$$

Where, A= Oil flow (kg/hr.); FFA= Content of free fatty acid (mgKOH/goil);

K =Factor of lye excess; M= molecular weight of fatty acid (Kg/mol);

N=normality of lye (mol/L)

The degummed oil was heated at 70 ^{0}C. Calculated amount of NaOH with concentration of 0.5 N and excess alkali (factor of lye excess) 15% was used to be mixed for 1 h. at a speed of 200 rpm. At this stage, the alkali reacted with FFAs and converts them into soap stock. The obtained soap has a higher specific gravity than the neutral oil and tends to settle at the bottom. Then the mixture transferred into a separating funnel and allowed to stand for more than one hour to separate the soap formed from the oil. Hot water was added again and again to the oil solution until the soap remaining in solution removed and completely separated from neutral oil.

3.2.1.4 Physicochemical analysis of neem oil

The moisture content, refractive index, pH value, acid value, saponification value, and iodine value of neem oil was determined using standard testing methods of IS 548 and IS 4765.

i) Determination of moisture and volatile matter content of neem oil

Sample of 10 g was weighed in glass vessel and kept in an oven at 105 ^{o}C, cooled in a desiccator and the sample was measured every 2 h until the difference between the two successive weighing does not exceed one milligram.

ii) Determination of specific gravity

The specific gravity was determined from density by using density meter. 5 ml sample was filled in to a tube of density meter and recorded the value at temperature of 20^{o}C.

iii) Determination of viscosity of the oil

Sample of 35m1 was poured into a beaker and the height of the sensor unit was adjusted to fix the sensor plates in the prescribed position then the viscosity measurement was started at room temperature and the viscosity was measured for approx. three minutes.

iv)Determination of refractive index

Refractive index was measured using refractometer. Few drop of the sample were transferred in to the glass slide of refractometer. Water at 25^{o}c was circulated round the glass slide to keep its temperature uniform. Through eye piece of the refractometer; the dark portion viewed was adjusted to be in line with the intersection of the cross. At no parallel error, the pointer on the scale pointed to the refractive index. This was repeated and the mean value noted and recorded as refractive index.

v) Determination of pH Value

Sample of 2g was poured into beaker and 13ml of hot distilled water added to the sample and stirred. It was then cooled in a cold-water bath to 25°C. The pH electrode was standardized or calibrated with buffer solution and the electrode immersed into the sample and the pH value was recorded.

vi)Determination of acid value

An equal volume proportionate (1:1) mixture of 98% C_2H_5OH and $C_4H_{10}O$ was prepared for total volume of 50ml. Then, 2g of oil was dissolved in a mixture and titrated with 0.1N KOH to the end point with consistent shaking for which a dark pink color was observed and the volume of 0.1N KOH (v) was noted. The Acid value was calculated as:

$$\text{Acid value} = \frac{V \times C \times 56.11}{M} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.3$$

Where, V = the volume in ml of 0.1N KOH; C = the conc. KOH; M = sample weight.

vii) Determination of saponification value

Sample of 2 g was added into 25 ml of 0.5 N Ethanolic NaOH solution and reflux condenser was placed on the flask containing the mixture. Then, a content which was constantly stirred was allowed to boil gently for 30 min. After the oil was completely dissolved, 5 drops of phenolphthalein indicator was added and the hot soap solution obtained was slowly titrated with 0.5 N HCl to the end point until the pink color of the indicator just disappeared. And the same procedure was used for blank. The final result was calculated using equation:

$$\text{Saponification value} = \frac{56.1 \times N \times (V0 - V1)}{m} \ldots\ldots\ldots\ldots 3.4$$

Where, N = normality of HCl Solution; V0 = volume of HCl solution used in blank; V1 = volume of HCl solution used in the test sample; m= mass of sample

viii) Determination of ester value

Easter value of neem oil was estimated from the relationship given below

$$EV = SV - AV \ldots\ldots 3.5$$

ix) Determination of iodine value

Iodine value and preparation of standard Hanus reagent was determined by the official method AOAC 920.158. The standard hanus reagent was prepared by dissolving 13.2 g of iodine in 1L of glacial acetic acid and 3 ml of bromine water in to brown glass bottle. The iodine number was determined by dissolvation of 0.25g oil in 10ml of chloroform and 25 ml standardized hanus reagent in 500 ml conical flask. Then, the solution was shaken continuously and allowed to stand for 60 minutes in dark. When the reaction was completed, 10 ml of 15% KI solution and 100 ml distilled water was added. Finally, the free iodine was titrated with 0.1N $Na_2S_2O_3$ using starch solution as indicator until the blue color disappears after vigorous shaking. Similarly a blank determination was done at the same length of time. The result was calculated using the following equation.

$$\text{Iodine value(IV)} = \frac{(B-S)\times N\times 12.69}{W} \ldots\ldots 3.6$$

Where, B=volume in ml of standard thiosulphate required for the blank;

S=volume in ml of standard thiosulphate required for the test sample;

N= normality of the standard thiosulphate solution;

W= weight in g of neem kernel oil taken for the test

3.2.2 Synthesis of fatliquor from neem oil

The synthesis was processed under sulphation process. Neem oil of 100 g was used for each run and exothermic reaction was carried out with conc. H_2SO_4 in a clamped burette poured in to weighted amount of oil at a different temperature of 15 ^oC, 20 ^oC and 25 ^oC with different amount of conc. H_2SO_4 (10, 15 and 20) percentages based on the weight of oil for about (2, 3.5, 5) h, to complete the time of reaction respectively. Then, the sulphated product was shaken by 200 ml of 10% NaCl solution and kept in a separating funnel overnight to separate the layer as shown in Figure 5. Finally the separated upper layer sulphated liquor was neutralized at pH of 5 by adding 30% NaOH solution then applied for leather processing.

Figure 5: Sulphated fatliquor washed with NaCl

3.2.2.1 Design of Experiment for sulphated fatliquor

To design the experiment and obtained suitable predictive model and optimum reaction conditions, design expert software, version 7.0.0 was used. The design expert selected for this study was three-level-three-factor face-centered central composite design (CCD) and the response variable measured was the degree of sulphation. RSM is a powerful tool for optimization of chemical reactions or industrial processes which includes factorial design and regression analysis. It helps in evaluating the effective factors and in building models to study interaction, and select optimum conditions of variables for a desired response. Response surface 3D plots can provide a good way for visualizing the parameters interactions.

Fatliquor production parameters of reaction temperature, reaction time and amount of sulphuric acid by experimental design and analysis of variance (ANOVA) used as statistical tools for determining the parameters effect. The three sulphation process parameters and the interaction among the parameters were analyzed to obtain the maximum degree of sulphation. In addition to achieve maximum degree of sulphation, rotational speed was set at 250 rpm. And to optimize the experimental design, a three-level-three-factors central composite design (CCD) generated 20 experimental runs.

The selection of levels for each factor was chosen by considering the operating limits of sulphation process conditions. The coded factor and levels of the three independent variables used are shown below in Table 5.

Table 5: The coded factor and levels of the process parameters used in experimental design

Parameter / factor	Factor coding	Unit	Coded levels		
			-1	**0**	**+1**
Reaction Temperature	A	°C	15	20	25
Amount of H_2SO_4	B	Wt %	10	15	20
Reaction Time	C	h.	2	3.5	5

3.2.2.2 Yield for sulphated fatliquor

The yield of sulphated fatliquor from neem oil was calculated using the given equation

$$\% \text{ Yield} = \frac{\text{Total mass out}}{\text{Total mass in}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.7$$

3.2.2.3 Physico-chemical analysis of sulphated fatliquor

For the synthesized of sulphated fat liquor ; pH of emulsion, stability of emulsion in Tanning salt solution ,total active ingredient , unsaponifiable matter, total alkalinity , total organically combined sulphate and total ash content was determined using Indian standard specification and testing method (IS:6357).

i) Determination of pH of water emulsion

Fatliquor emulsion (10%) was prepared by dispersing 10 ml of fatliquor in 90 mL of distilled H_2O. Then the pH of emulsion was determined by using an electrode pH meter.

ii) Stability of emulsion in tanning salt solution

Fatliquor emulsion (10%), 20ml was mixed with 5% solutions of NaCl, MgSO4 and 5% basic CrSO4 in separate 100 ml measuring cylinder and allowed to stand for 40 min. Then, phase separation was determined as a function of time.

iii) Determination of total active ingredient

5 g of the sulphated oil fatliquor was weighed in a 250 ml flask and 25 ml of 50% ethyl alcohol and 25 ml of petroleum ether was added. Then the contents were transferred to a separating funnel. The contents shaked vigorously and allowed the layers to separate. The lower alcohol layer was transferred to another separating funnel and it was extracted four times with petroleum ether. The upper layer was extracted, namely, the petroleum ether layer, with 75% alcohol and the layers was allowed to separate and further extracted with 90% alcohol and the layers was allowed to separate. The petroleum ether layer and alcohol layer were collected in two tarred flasks. The solvents was evaporated, the residues was dried to constant weight in oven; cooled and weighed.

Alcohol layer contains the emulsifier (fatliquor) and the petroleum ether layer contains the neutral oil. The calculation was done using equations given below.

$$\% \text{ free oil (A)} = \frac{F}{W} \times 100$$

$$\% \text{ emulsifier (B)} = \frac{E}{W} \times 100$$

$$\% \text{ Total active ingredient} = A+B \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.8$$

Where, F= weight of petroleum ether soluble in g;

E=weight of alcohol soluble in g

W= weight of the fatliquor sample in g.

iv)Determination of unsaponifiable matter

Sample of 5 g was weighed into the flask. 50 ml of alcoholic potassium hydroxide solution was added. Then the mixture was boiled gently but steadily under a reflux condenser for 1 hr. until the Saponification was complete. The condenser was washed with about 10 ml of ethanol. The mixture was cooled and transferred to a separating funnel. The transfer was completed by washing the flask first with some ethanol and then with cold water. Altogether 50 ml of water was added to the separating funnel followed by an addition of 50 ml of petroleum ether.

The stopper was inserted and shakes vigorously for one minute and it was allowed to settle until both the layers are clear. The lower layer was transferred containing the soap solution to another separating funnel, and the ether extraction was repeated six times more using 50 ml of petroleum ether for each extraction. All the ether extracts was collected in a separating funnel. The combined extracts were washed in the funnel three times with 25 ml portions of aqueous alcohol shaking vigorously and the alcohol-water layer after each washing was drawing off.

Again the ether layer was washed successively with 20 ml portions of water until the wash-water no longer turned pink on addition of a few drops of phenolphthalein indicator solution. Then ether layer was transferred to a tarred flask and evaporated to dryness on a water-bath.

To remove the last traces of ether, the flask was placed in an air-oven at 85^{o}C for about 1 h and last traces of moisture removed by adding 5 ml of acetone. Then kept in an oven at 105 ^{o}C for about 15 min. Cooled in a desiccator and weighed. The procedure was repeated until constant weight was obtained. After weighing, the residue was taken up in 50 ml of warm neutral C_2H_5OH, containing a few drops of phenolphthalein indicator solution and titrated with 0.1N NaOH solution to slightly pink color. Unsaponifiable matter can be calculated using equation12.

$$\text{Unsaponifiable matter, \% by weight} = 100 \times \frac{(A-B)}{W} \ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.9$$

Where: A= weight in g of the residue; W= weight in g of the material taken for the test

B=0.282*V*N

B= weight in g of the fatty acids in the extract;

V= volume in ml of standard sodium hydroxide solution;

N = normality of NaOH solution.

v) Determination of total alkalinity

The sample of 10 g was dissolved in 100 ml of water in a conical flask. Then 30 g of NaCl, 25 ml of ether and 5 drops of 0.1 % methyl orange indicator was added and titrated with 0.5 N H_2SO_4 until the aqueous layer is orange. The total alkalinity can be calculated from:

$$\mathbf{A} = \frac{\mathbf{10 \times V \times N}}{\mathbf{W}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.10$$

Where, A=total alkalinity in milligrams equivalent per 10 g of oil;

V=volume of sulfuric acid in ml;

N= normality of sulfuric acid; W= weight of the sample in g

vi)Determination of organically combined SO_3 as neutralized sulfuric ester

Sample of 10 g and 30 ml concentrated sulfuric acid from a burette was added into 250 ml conical flask with a few glass beads. Reflux condenser was attached and the mixture was boiled for one and half hour until oil and water layer cleared. Then the mixture was cooled and washed the condenser by 10 ml of water. Then, 50 ml H_2O, 30 g NaOH, 25 ml diethyl ether ($C_4H_{10}O$) and 5 drops methyl orange indicator was added and titrated with 0.5 N NaOH with shaking until the aqueous layer was changed to orange. The same procedure followed for blank solution. "Organically combined SO_3 as neutralized sulfuric esters G, % by mass"

$$G = (A + F)*0.8007 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.11$$

Where, A is total alkalinity in mg eq. per 10 g of oil;

F =increase in acidity in mg eq. per 10 g of oil;

$$F=\frac{(V1-V2)N}{W} \times 10$$

V1 = volume in ml of NaOH required in the titration of the blank;

V2 = volume in ml of NaOH required in the titration of the sample;

N = normality of NaOH solution, and

W = weight in g of sample taken.

vii) Determination of total organically combined SO_3 as sulfuric and sulfonic esters

The sample of 10 g and 25 ml of 0.1N NaOH was added into a platinum dish. The mixture was evaporated to dryness on the water bath and heated in an oven at 105°C for about 2hours, then ignited in to the muffle furnace at a temperature of 550^{o}c for 30 minute. The mixture was cooled and 5 ml of 30% H_2O_2 added to oxidize any bi sulphite formed and heated in a muffle furnace again at 350°C for 30 min. After it has been cooled, the sample was washed with hot distilled water I n to a beaker. 25 ml of 0.1N sulfuric acid was added, boiled the solution down to about 50 ml to expel carbon dioxide and allowed to cool. Finally the solution was titrated with 0.1N NaOH using five drops of 0.1 percent methyl orange as indicator until the color turns yellow. The organically combined sulphate (SO_3) existing as neutralized sulfonic ester I, % by mass was calculated as follows:

$$I = (T-F)*0.80066;$$

$$T = \frac{(X-Y)}{W} \times 10 \; ; \; X = (25*N_1) + (\text{titer value}*N_1) \; ; \; Y = (25*N_2) + (V*N_2)$$

Where, F = increase in acidity in mg eq. per 10 g of oil;

T = resultant acidity expressed as mg eq. NaOH per 10 g of oil;

N1 = normality of NaOH;

X = mg eq. alkali;

N2 = normality H2SO4;

Y = mg eq. acid used;

V = volume in ml of any additional H2SO4;

W = the weight of the sample

Total organically combined sulphate (SO_3) = I+G……………………….3.12

viii) Determination of total ash

Sample of 3 g was weighed in a measured crucible and heated on low oxidizing flame. Then the crucible was kept in muffle furnace at 800 ^{o}c for 3 hours and allowed to cool. Finally, the crucible containing the ash was weighed and calculated as follows;

$$\text{Ash content} = \frac{W_2 - W_0}{W_1 - W_0} \times 100 \ldots\ldots 3.13$$

Where: W0 = weight of dry crucible;

W1 = weight of crucible containing sample before ashing;

W2 = weight of crucible containing residue after ashing

3.2.3 Characterization of neem seed oil and synthesized sulfated fatliquor

3.2.3.1 GC-MS analysis of neem seed oil

The oil was characterized in terms of its fatty acid profile. Fatty acid composition was determined after converting the fatty acids in the oil to fatty acid methyl esters (FAME) by methylation process at 55^{o}c for 2h to increase the volatility of the oil.

3.2.3.2 FTIR analysis of neem seed oil and synthesized sulfated fatliquor

FT-IR spectroscopy (FTIR-65, Perkin-Elmer) was used to characterize structural functionalities in the oil. In this study FT-IR analysis was carried out in order to assess the functional groups present in purified neem seed oil and synthesized neem oil sulphated fatliquor for wavelength range of 500 cm-1 to 4000 cm-1. The resulting FT-IR spectral pattern was then analyzed and matched with the identified materials in the FT-IR library.

3.2.4 Application and test analysis of sulphated fatliquor on leather

3.2.4.1 Procedures for application of fatliquor on leather

Four sheep skin for garment with thickness (0.5$\pm$0.1mm) were used to compare the synthesized neem oil sulphated fatliquor (SNOSFL) with commercial vegetable lecithin oil fatliquor (CVLOFL) or named with Fosfol LP.

The wet blue was sammed by Sam setting machine and shaved to the appropriate thickness required for sheep garment leather. Then, the shaved wet blue weighed to calculate the quantity of the chemical required in the subsequent operation. In a testing drum, Washing was carried out with hot water and wetting agent for 30 minutes to remove contaminations and wetted back it properly to penetrate and disperse the chemicals used in the next operation of re-chroming, neutralization, retanning, dying, fatliquoring, fixation and top dye applied with the given percentages and time requirement given in the recipe of sheep garment which is provided in *Appendix E.*

After completion of neutralization stage, the wet blue leathers were washed and splited along the spinal cord in coding with EX1, EX2, EX3 and EX4 of the right sides for the synthesized fatliquor and CO1, CO2,CO3 and CO4 of the left sides for commercial fatliquor. EX stands for Experimental and CO for control value. Then two testing drums were prepared one for SNOSFL and the other was for CVLOFL and continued to the next operation.

In the fatliquoring stage, 14% fatliquor based on shaved weight was used as an emulsion with hot water at temperature of 60^{o}C and run the drum for 60 minutes. Then formic acid was added for fixation at a given amount and time interval to fix the retanning, dyeing and fat liquoring agents to the fibers. After that the leather pieces were washed and top dye was applied for adding aesthetic value and beauty. Finally the leather removed from the drum and piled overnight for proper distribution and fixation of the various treatments of chemicals of the retanning, dyeing and fat liquoring agents given. In the next day, sammed set out for removal of surplus moisture, crease, wrinkles and folds as well as for smoothing out coarse and drawn grain and left to dry in air through hanging up at room temperature. After the leather has been dried, staking was done to make the leather more flexible by massaging it to separate the fibers using staking machine and trim the unnecessary parts. At the end the leather pieces or crust were taken for investigation of organoleptic/sensory analysis and physical test parameters. The weight of wet blue and the amount of fatliquor used is shown in Table below;

Table 6**:** weight of wet blue and amount of fatliquor used

Testing drum		Weight of shaved wet blue(Kg)	%	Amount of fatliquor(g)
1	Synthesized fatliquor	0.7	14	98
2	Commercial fatliquor	0.7	14	98

3.2.4.2 Test analysis of sulphated fatliquor on leather

i) Organoleptic / sensory analysis test of leather

The organoleptic test was determined by visualizing the color uniformity or color intensity of crust leather and by touching the product whether it has smooth or rough surface. The organoleptic tests were performed for uniformity of color, softness, roundness, grain tightness and fullness of the fatliquored crust leather using panelists. The panelists, professionals, undertook the two samples, the synthesized (experimental) and the control (commercial), to be graded for ten points for each tests.

ii) Physical test of leather

After the leathers were produced, it was necessary to test them to assessed whether it was fit the ultimate purpose. For the test, both the samples were conditioned at 21.7^{o}c and 67.8 R.H for 48 h. For garment leather this conditioning procedure was defined in ISO 2418:2008 test method to support the specimen.

The standard testing methods (STM), ISO 3376 (2011), was used for sampling of materials for tensile strength test (TT) and percent elongation at break (EB) tests. Also, STM of ISO 3377-2 (2011) was used to tear load (TL) in test equipment of UTM maintaining a crosshead speed of 100 mm/min.

The Specimen used for physical testing of experimental and control crust leather were cut with special steel press knives known as double-edge tear die having a dimension of 50 mm length with 25 mm width and tensile die 110 mm length with 25 mm width.

The specimen for measurement of tensile strength and tear strength were cut parallel and perpendicular to the backbone and the thickness of each specimen was measured using a standard thickness gauge at three positions on the grain side and three positions on the flesh side, and then the mean of the six measurements was calculated. Each value for tensile and tear strength represents the value of four samples (2 values parallel to the backbone and 2 values perpendicular to the backbone). Then the area of cross section of each specimen was calculated by multiplying its width by its thickness. Finally the jaws of tensile machine set to 50 mm apart and clamped the specimen in the jaws then run the machine until the specimen breaks and the highest load reached was taken as the breaking load.

a) Measurement of tensile strength and elongation at break

The specimen was placed in the testing machine and fixed from its two sides between the two jaws. The machine set to 50 mm apart between the two edged dump shapes that aids in concentrating the stresses in the narrow portion and increased the force gradually with a speed of 100 ± 20 mm/min forcing sample to break (ASTM D-2211). So the tensile strength caused by the specified load and elongation at break was calculated using the following formula:

$$\text{Tensile strength, N/mm}^2 = \frac{\text{breaking load (N)}}{\text{thickness inmm} \times \text{widthin mm}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.3.14$$

$$\text{Elongation at break, \%} = \frac{\text{length at break in mm} - \text{initial length in mm}}{\text{initial length in mm}} \times 100 \quad \ldots3.15$$

b) Measurement of tear strength (double edge tear)

The tear strength was calculated from tear load required to tear the specimen from a steel rod passing through the hole of the specimen (ASTM D-2212).

$$\text{Tear strength, N/mm} = \frac{\text{tearload in N}}{\text{thickness of tear specimen in mm}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.3.16$$

4. RESULT AND DISCUSSION

4.1 Raw material preparation

From 35 kg neem seed taken about 520 gram (1.486%) waste was removed and 34.48 kg pure seed was obtained. From 34.48 kg of pure neem seeds 18.619 kg (54%) is shell and 15.86 kg (46%) is the kernel found inside the shell that contains maximum oil content used for the process of extraction of oil and production of fatliquor.

4.2 The moisture content of neem seed kernel

The moisture content was determined in triplicate for the analysis and the average value was taken. The laboratory result found in *Appendix C* Table C-1

4.3 Extraction of oil from neem seed kernel

i) Mechanical extraction method

From 15.86 kg of seed kernel, 15.8 kg was used to extract oil by screw press oil extractor and the rest 60g was used for solvent extraction. From 15.8kg of seed kernel 4.5 liter crude neem oil was extracted using mechanical screw pressing machine. The density of crude oil was found to be 0.9149g/ml using density meter. Therefore 4.117 kg (26.06%) crude neem oil was extracted and the rest 11.683kg (73.94%) was removed as a cake.

ii) Solvent extraction method

Extraction of neem oil using soxhlet extraction method for some selected organic solvents such as hexane, ethanol and ethanol-hexane mixture (60:40) was carried out to study the performance of the solvent towards better yield and the result is tabulated in the following Table 7.

Table 7: Performance of the solvent towards better yield

Solvent	Temperature (^{o}c)	Mass of sample (g)	Mass of extract (g)	Yield (%)
Hexane	55	20	8.32	41.60
Ethanol	55	20	8.20	41.00
Ethanol-hexane	55	20	8.62	43.10

The ability of the ethanol-hexane mixture performs better than ethanol or hexane. This result is agreed to the reported value (Ayoola *et.al.*, 2014).

Generally; the percentage yield found from mechanical extraction is lower than for solvent extraction however it has an advantage in minimum extraction cost and lower extraction time.

4.4 Purification of neem oil

From a total of 4.142 kg extracted by mechanical press and solvent extraction method 607g was removed during the purification process. Higher amount of suspended solid (526g) were removed by centrifuging and the rest 81 g removed as a lost by degumming and neutralization stages. Around 135ml of distilled water was used to degum the oil and 9.28ml of sodium hydroxide solution to neutralize the free fatty acids of crude oil in order to avoid rancidity and spue formation on fatliquored leather. The detail calculation is given in *Appendix D*. In general, the total of 3.535 kg of pure oil obtained after purification of crude oil to make it suitable for further fatliquor processing.

4.5 Physicochemical properties of neem oil

Density, refractive index, viscosity and pH were measured by appropriate devices three times each to increase the accuracy and the average value was taken.

The moisture content of neem oil was determined in triplicate for the analysis and the average value was taken. The acid value, saponification value, iodine value of neem oil was determined by doing the titration three times each and the average volume of titrant was taken. The laboratory results found and detailed calculations are given in *Appendix C and D.*

Comparison between density, refractive index, viscosity, pH, Sg, AV, FFA, IV and FFA of the refined neem oil agreed with the reference that is given in Table 8. The moisture content of refined oil (0.41%) observed minimal difference to that of the reported value of (0.3%). This difference could be considered as an experimental error due to measurement or reading.

Differences were observed between the value obtained for the viscosity of the crude and refined oil. The value of the viscosity of the crude neem oil was found to be outside the range of reported viscosity in literature. While the refined oil viscosity was quite within range. This may be attributed to the fact that some impurities and other components were removed during refining.

Table 8: Physicochemical properties for neem oil

Property	Experimental value		Literature value standards IS 4765	Unit
	Crude oil	Purified oil		
Moisture content	-	0.4100	0.30	Max, %
Density at 20^oc	-	0.9149	0.91 - 0.93	g/ml
Sg at 20^oc	-	0.9149	0.91 - 0.93	-
Viscosity at 23^oc	55.7	51.3	49.79±0.5	mm^2/s
Refractive index@25 ^oc	1.468	1.467	1.46-1.47	-
pH	6.47	6.96	5.70-6.50	-
AV	4.21	2.99	15	Max, mg KOH/g oil
FFA	2.104	1.403	7.5	Max, mgKOH/goil
SV	-	229.11	180-205	mgKOH/goil
EV	-	226.12	165-190	mgKOH/goil
IV	-	74.45	65-80	$gI_{2/}goil$

The pH value of the crude oil which was found to be 6.47 indicate that the oil is more acidic compared to 6.96 pH obtained for the refined oil. This may be as result of degumming and Neutralization carried out during the refining process. The acid value of crude and refined oil was 4.208mg KOH/g of oil and 2.993mg KOH/g of oil respectively. The acid value and corresponding FFA composition of the purified neem oil decreased after neutralization of crude neem oil. The value is higher in crude oil due to free fatty acid present; while it less for the refined oil as a result of the chosen strength 0.1M of KOH used in the treatment of the crude oil, which must have neutralized some of the free fatty acid present in it. Both values fall within the range specified in literature standards (IS 4765). The lower acid value of the oil is suitable for synthesis of fatliquor and increase the shelf life. The saponification value and ester value of purified neem oil was a little bit deviates from the standard value given in literature. However, this little difference could be considered being within an acceptable experimental error range that may be attributed to components of the mixture. According to (Dutta, 1999), higher saponification gives higher penetrating power to the leather and softness when it is used for fatliquoring and the iodine value which is below 95 is non-drying type and indicates a good lubricating value of the oil. Hence, neem oil is a non-drying type of oil which is suitable for the production of fatliquor and would give better lubrication.

4.6 Analysis of sulphated fatliquor production

4.6.1 Statistical analysis of factors that affect degree of sulphation

The experimental design selected for this study was the Central Composite Design (CCD) and the response variable measured was the degree of sulphation. Three-level-three-factor CCD applied the face-centered cubic design because it is very efficient for fitting a second order model. Having three levels instead of five was cited as desirable because it reduced the preparation time and lessened the potential for mistakes in preparing the test. Three-level-three-factor face-centered CCD with a complete 2^K factorial layout, 2K axial (star) points and 6 center points was employed in the optimization study, a total of 20 experiments were conducted, where K is three for three factors used in the analysis. The three sulphation process variables are reaction temperature, amount of sulfuric acid and reaction time.

Design-Expert, Software 7.0.0, was used for the regression ANOVA and to generate the model equation, interaction effects of the independent variables and surface plots using the fitted equation obtained from the regression analysis holding one of the independent variable constant. The actual and predicted degree of sulphation of fatliquor and the ANOVA are given below in Table 9 and Table 10 respectively.

Table 9: Experimental and predicted values for three level-three factor RSA

Run	Reaction Temperature (^{o}c)	Amount of H_2SO_4 (Wt %)	Reaction time (hr.)	Degree of sulphation	
				Experimental	Predicted
1	15	10	2.0	3.81	3.82
2	15	20	2.0	4.82	4.83
3	20	15	3.5	5.29	5.3
4	20	15	3.5	5.28	5.29
5	20	15	3.5	5.27	5.29
6	20	15	3.5	5.32	5.29
7	25	20	5.0	5.23	5.22
8	20	15	3.5	5.28	5.29
9	15	10	5.0	3.83	3.82
10	20	15	3.5	5.31	5.29
11	20	15	5.0	5.31	5.34
12	25	20	2.0	5.06	5.07
13	25	15	3.5	5.36	5.35
14	20	15	2.0	5.30	5.26
15	25	10	2.0	4.45	4.46
16	20	20	3.5	5.17	5.18
17	20	10	3.5	4.58	4.56
18	15	15	3.5	4.65	4.66
19	25	10	5.0	4.96	4.97
20	15	20	5.0	4.48	4.47

4.6.2 Analysis of variance (ANOVA)

From the ANOVA of response surface quadratic model for degree of sulphation of fatliquor, the model F-value of 886.36 and prob>F of < 0.0001 implied that the model was significant. There is only a 0.01% chance that a “Model F – Value” this large could occur due to personal error or disturbance. For the model terms Values of "Prob> F" less than 0.0500 indicate model terms are significant. In this case A, B, C, AB, AC, BC, A^2 and B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. This shows that the reaction temperature, amount of sulfuric acid, reaction time, interaction between reaction temperature and amount of sulfuric acid, interaction between reaction temperature and reaction time, interaction between amount of sulfuric acid and reaction time, square of the reaction temperature and square of amount of sulfuric acid affects the degree of sulphation significantly.

The variation between the model prediction and extra points is compared with pure error to test the lack of fit in statistical output; the lack of fit should not be significant. A small F- value and high p-value (greater than 0.1) are good in this test. In table it was observed that the lack of fit F-value of 2.01 and P-value of 0.2310 implies the Lack of Fit is not significant relative to the pure error. There is a 23.10% chance that a "Lack of Fit F – value" this large could occur due to noise. This indicates that the model desirably represent the actual relationships of reaction parameters, which are well within the selected ranges. Non-significant lack of fit is good because we want the model to fit.

Table 10**:** ANOVA for response surface model

Source	Sum of square	df	Mean square	F value	P value Prob>F	
Model	4.52	9	0.5	886.36	<0.0001	Significant
A-Reaction temperature	1.20	1	1.20	2124.30	<0.0001	
B-Amount of sulfuric acid	0.98	1	0.98	1728.40	<0.0001	
C-Time	0.014	1	0.014	24.15	0.0006	
AB	0.076	1	0.076	134.17	<0.0001	
AC	0.13	1	0.13	220.53	<0.0001	
BC	0.061	1	0.061	108.06	<0.0001	
A2	0.24	1	0.24	420.91	<0.0001	
B2	0.50	1	0.5	874.45	<0.0001	
C2	0.0001	1	0.0001	0.14	0.7119	
Residual	0.006	10	0.0006			
Lack of fit	0.004	5	0.0008	2.01	0.2310	Not significant
Pure error	0.002	5	0.0004			
Cor total	4.53	19				

4.6.3 Development of model equation

The model equation that correlates the response (degree of sulphation) to the process variables in terms of actual value after excluding the insignificant terms was given below. Design expert software has been suggested to select quadratic model in the regression analysis.

The predicted model for percentage of degree of sulphation in terms of the coded factors is given in equation

$$\text{Degree of sulphation} = +5.29+0.35*A+0.31*B+0.037*C-0.097*A*B+0.13*A*C$$
$$-0.087*B*C-0.29*A^2-0.42*B^2+0.0054*C^2$$

Where: A is the reaction temperature, B is the amount of sulfuric acid and C is the reaction time

Model adequacy check

The developed quadratic model has to be examined for its consistency and how it correlates the experimental variable to the response variables. Regression model was found to be significant with the correlation coefficients of determination of R^2, adjusted R^2 and predicted R^2 having a value of 0.9987, 0.9976 and 0.9930 respectively.

Table 11: Model adequacy measurement

Std. Dev.	0.024	R-squared	0.9987
Mean	4.94	Adj R-squared	0.9976
C.V.%	0.48	Pred R-squared	0.9930
PRESS	0.032	Adeq precision	90.532

The quality of the model developed could be evaluated from their coefficients of correlation. The value of R-squared for the developed correlation 0.9987 reveals that the model is in a good agreement with the experimental data and can be used to predict the response accurately.

The Predicted R^2 of 0.9930 is in reasonably agreed with the adjusted R^2 of 0.9976 that is less than 0.2 differences as one might expect. The adequate predict value measures the signal to ratio. A ratio greater than four is desirable. Ratio of 90.532 indicates an adequate signal and the model can be used to navigate the design space. The graph of the predicted values obtained using the developed correlation versus actual values is shown in Figure 6. It demonstrate that the regression model equation provided a very accurate description of the experimental data, in which all the points are very close to the line of perfect fit. This result indicates that it was successful in capturing the correlation between the three sulphation reaction process variables to the degree of sulphation.

As shown in Figure 7, the normal probability plot indicates the residuals following by the normal % probability distribution , in the case of this experimental data the points in the plots shows fitted to the straight line in the figure, this shows that the quadratic polynomial model satisfies the assumptions analysis of variance(ANOVA) i.e. the error distribution is approximately normal.

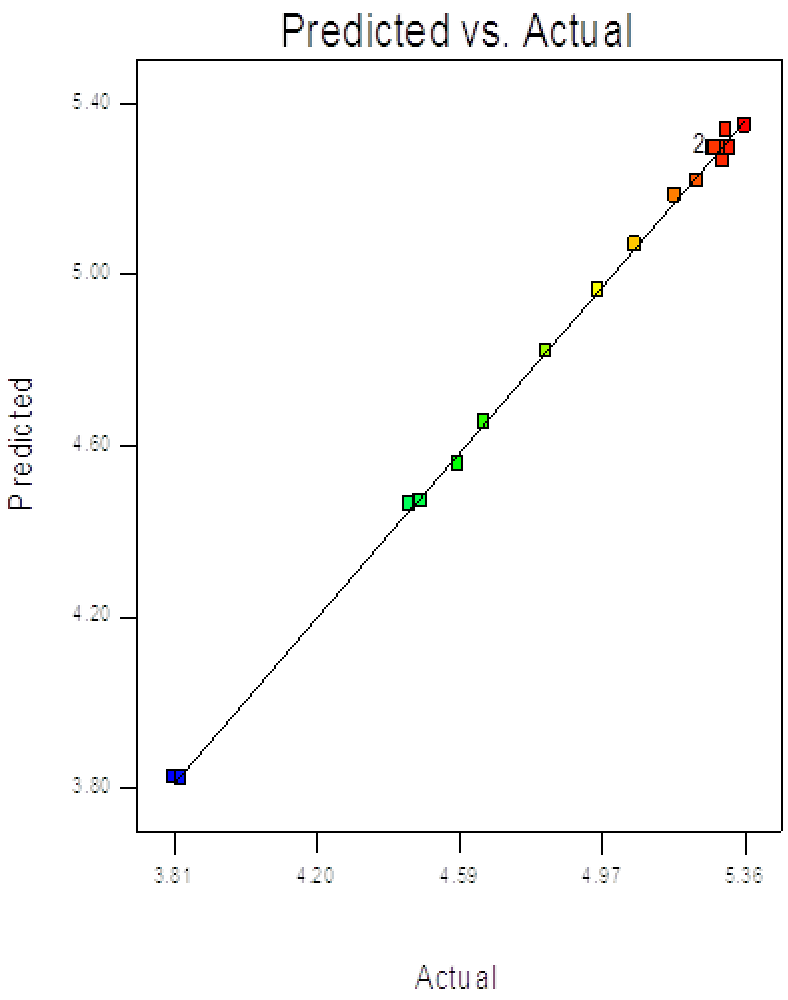


Figure 6: Comparison of predicted versus actual response value

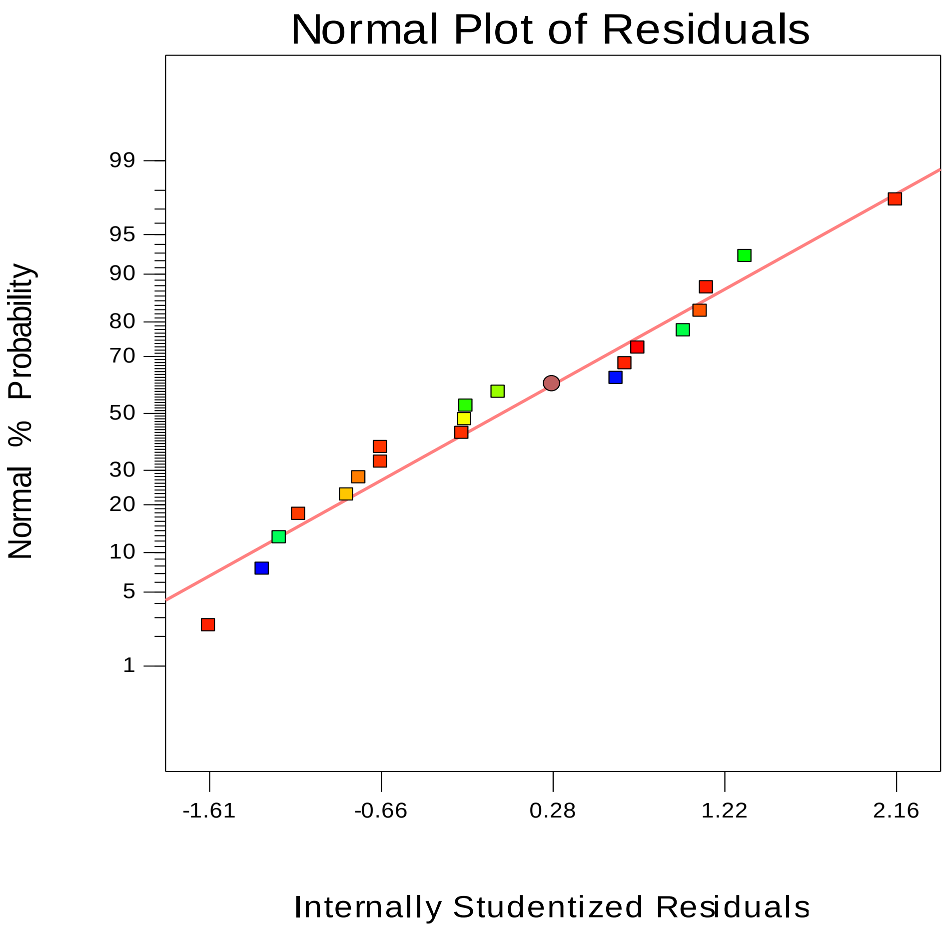


Figure 7: Normal probability of residuals

4.7 Effect of sulphation reaction process variables

4.7.1 Effect of individual process variables

The response optimized value for the production of sulphated fatliquor was based on the three process variables described on the response surface plot.

- **Effect of temperature**

As shown in Figure 8 below the degree of sulphation is significantly affected by reaction temperature. It can be seen from the figure that the degree of sulphation increases up to a maximum reaction temperature of 23.98 $^{\circ}$C. Further increase in temperature have a decrease in degree of sulphation may result hydrolysis of triglyceride causes the release of free fatty acids. Accordingly with the report on Tawfig *et al.*, (2017), the increase in the degree of sulphation at higher reaction temperature is due to higher rate of reaction. Is it well reported in the literature that the reaction rate of sulphation reaction is influenced by the reaction temperature. Higher reaction rate constant resulted from higher temperature not greater than 25 °C, if it would be greater to this level, the overheating of oil causes to oxidation and polymerization of oil.

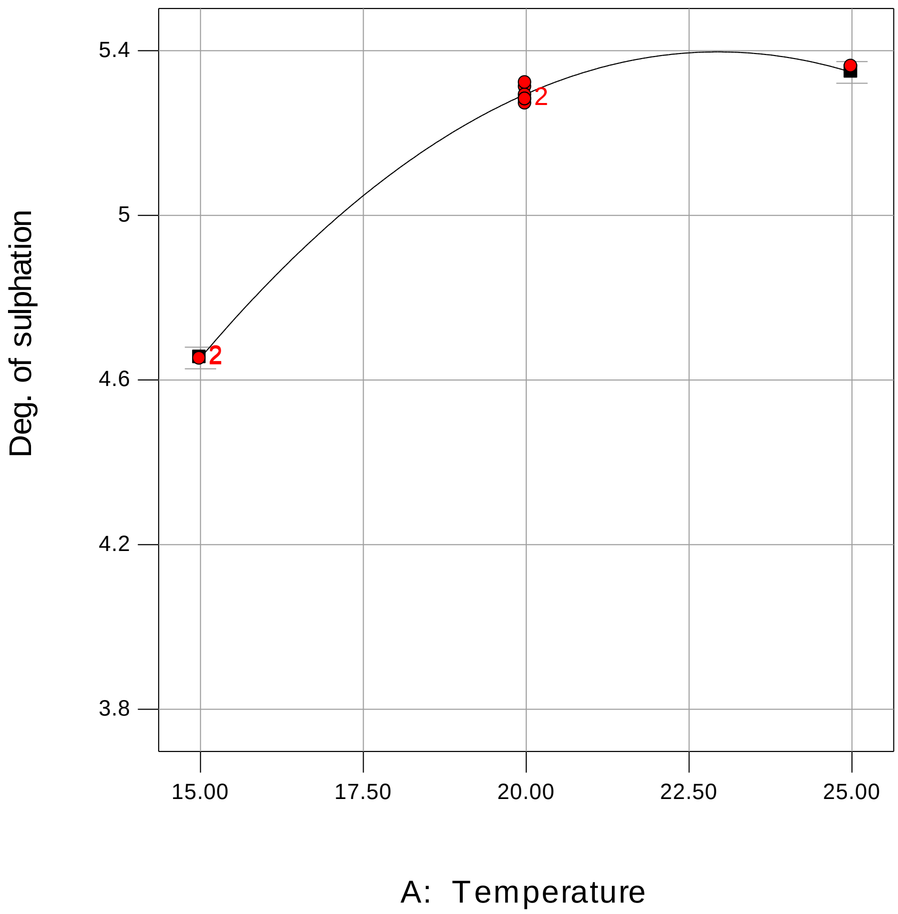


Figure 8: Effect of temperature on degree of sulphation

- **Effect of amount of sulfuric acid**

The amount of H_2SO_4 is one of the important factors that affect the degree of sulphation. As reported in literature the minimum standard value for degree of sulphation is 4.5 % by mass. Figure 9 shows that, the degree of sulphation was increased up to 16.76 Wt% of sulfuric acid and satisfied the standard value.

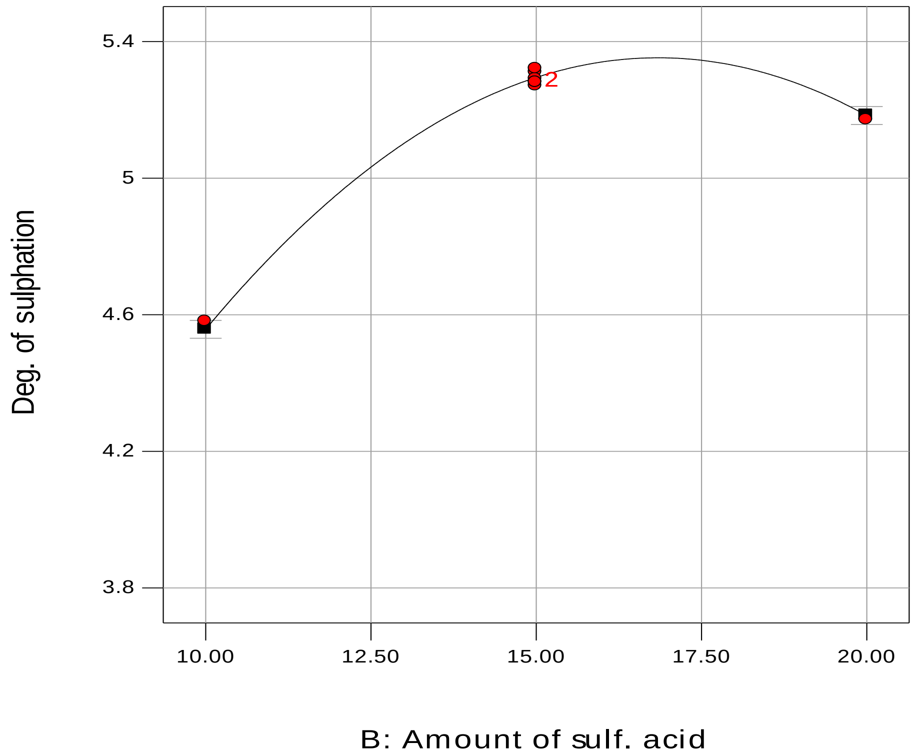


Figure 9**:** Effect of amount of sulphuric acid on degree of sulphation

- **Reaction time**

When the reaction time was raised from 2 to 5 h, the sulphation reaction gets enough time for formation of the sulphated group which increases the degree of fixation. The effect of reaction time on degree of sulphation in Figure 10 illustrate the reaction time is not highly significant compared to the reaction temperature and amount of sulfuric acid.

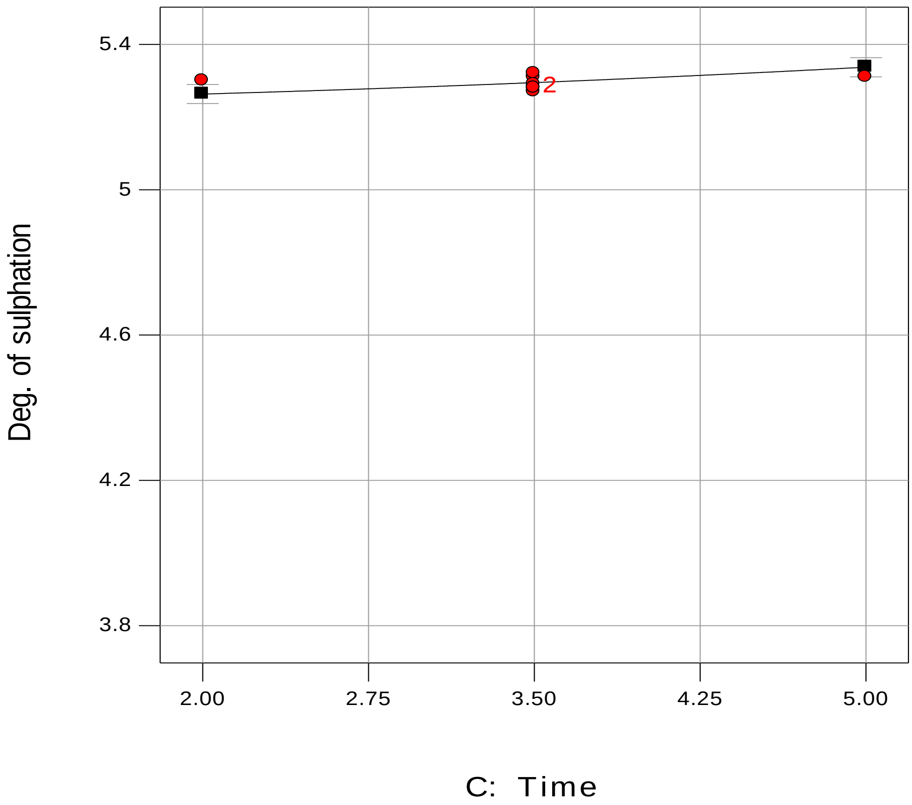
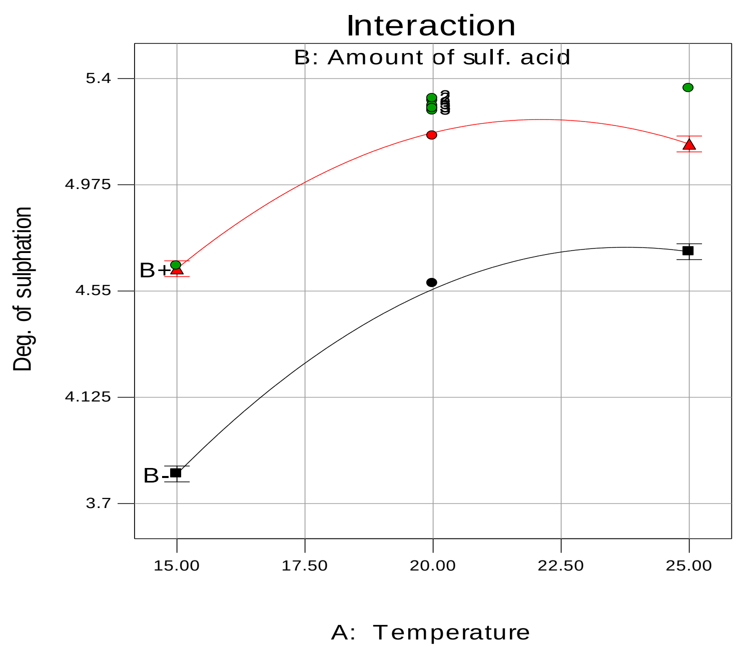


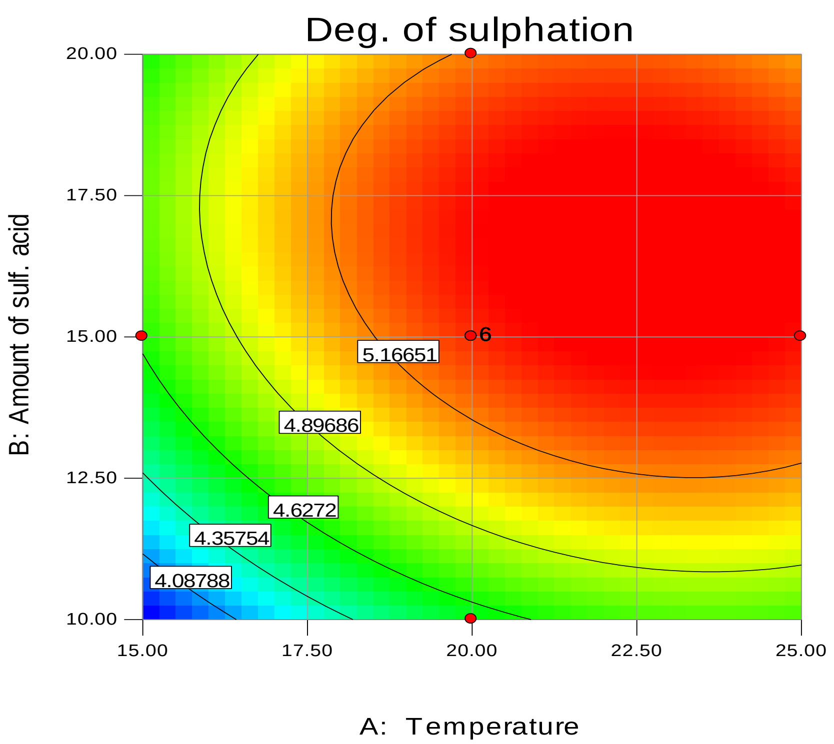
Figure 10**:** Effect of reaction time on degree of sulphation

4.7.2 Effect of interaction between process variables

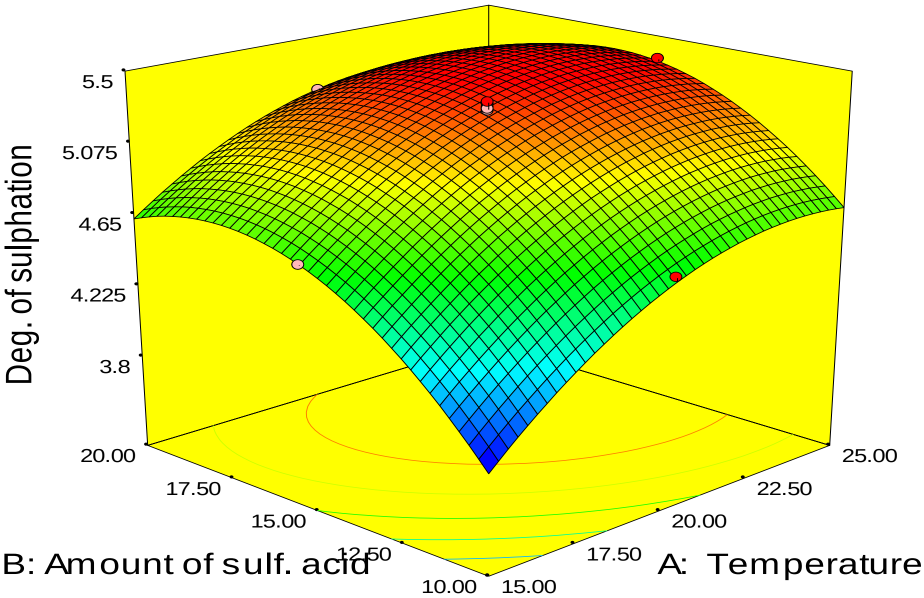
Interaction effects are effects that independent variable impose on one another. In order to absorb the interaction effect of process variables, 3D and 2D contour plots were obtained by plotting the response (degree of sulphation) on the z axis against any three variables while keeping the other variables constant. The process variables were found to have significant interaction effects. Figure 11, Figure 12, Figure 13 shows the interaction between reaction temperature and amount of sulfuric acid, reaction temperature and reaction time, and amount of sulfuric acid and reaction temperature on the degree of sulphation respectively.



a) The effect of reaction temperature and amount of sulfuric acid on the degree of sulphation



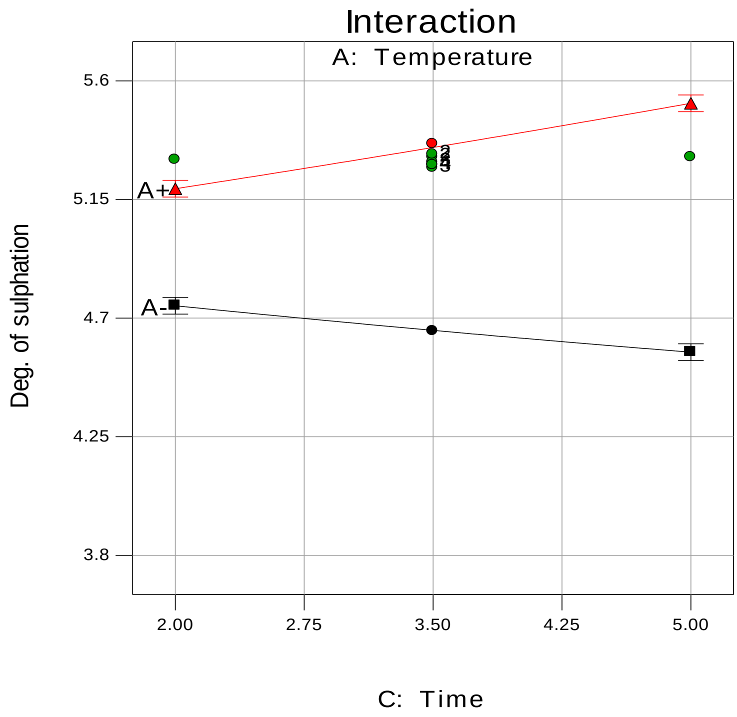
b) Contour plot of the effects of temperature and amount of sulfuric acid on the degree of sulphation of fatliquor



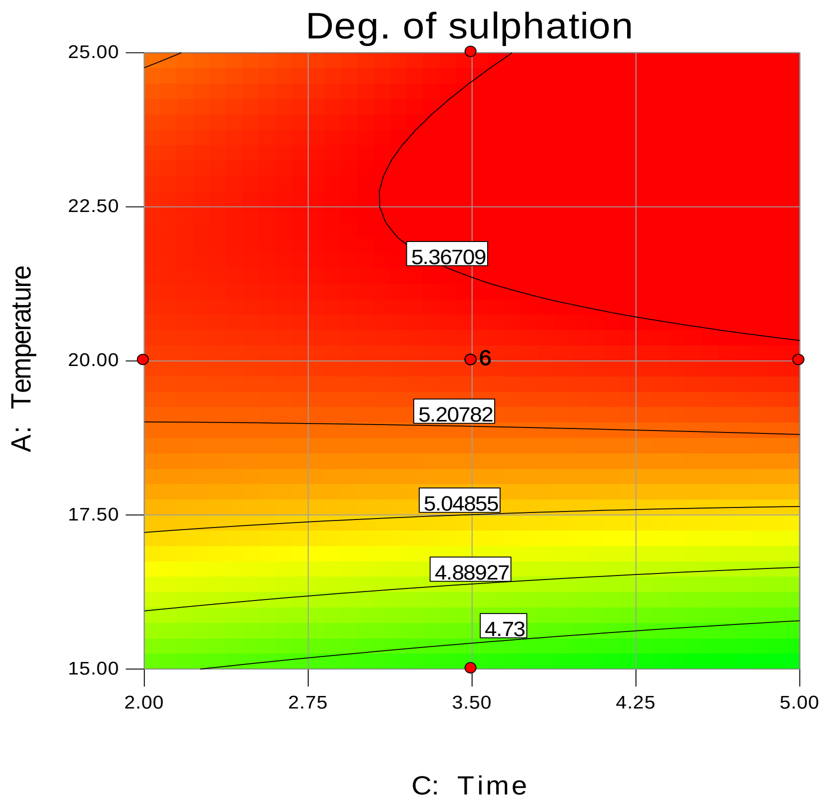
c) 3D - surface plot of the effects of temperature and amount of H_2SO_4 on the degree of sulphation of fatliquor

Figure 11: Interaction effect of temperature and amount of H_2SO_4 on degree of sulphation

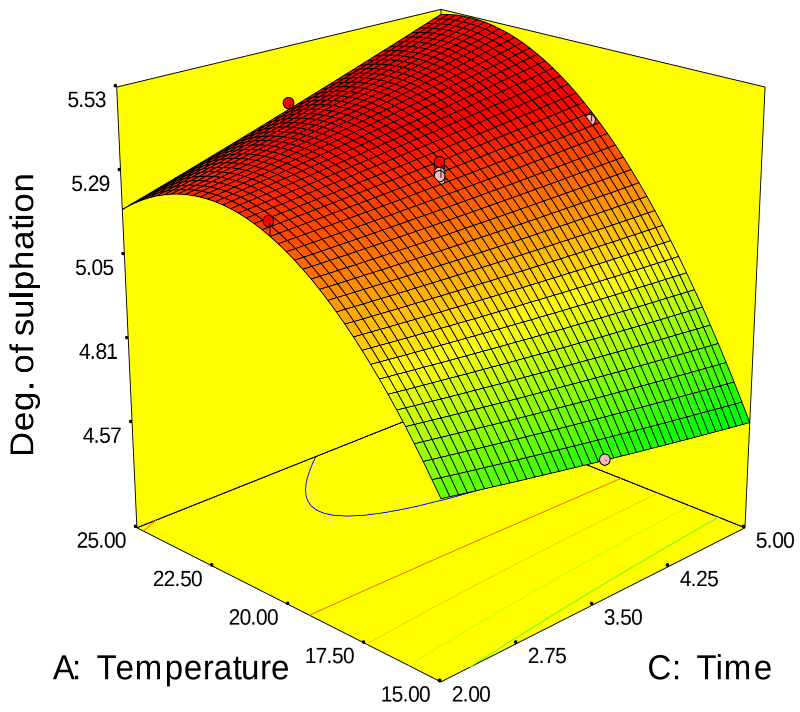
In the above Figure 11, the interaction effect of temperature and amount of H_2SO_4 on degree of sulphation was done at time being constant (center point). In (a), it was observed the effect at a lower and high amount of H_2SO_4. the degree of sulphation increases with increasing temperature up to optimum value because the oil is sulphated and more sulphuric acid has been fixed for the greater stability to acid and the more thorough penetration in to the leather .Hence both temperature and amount of H_2SO_4 have relationship to the degree of sulphation of fatliquor. The contour plot and 3D (b) and (c), represents the fatliquor response surface plot developed as a function of temperature and amount of H_2SO_4 while reaction time was kept constant at 3.5 h. as the amount of H_2SO_4 increases from 10 Wt% to 16.76 Wt%, the degree of sulphation increased and reached to peak level. However beyond 16.76 Wt% the degree of sulphation decreased due to losing the lubricating power and oiliness. As the temperature increases from 15^{o}c to 23.98^{o}c maximum pick was observed on the plot but further increase had a decrease the degree of sulphation due to overheating of oil which results oxidation and polymerization of oil.



a) The effect of reaction time and reaction temperature on the degree of sulphation,



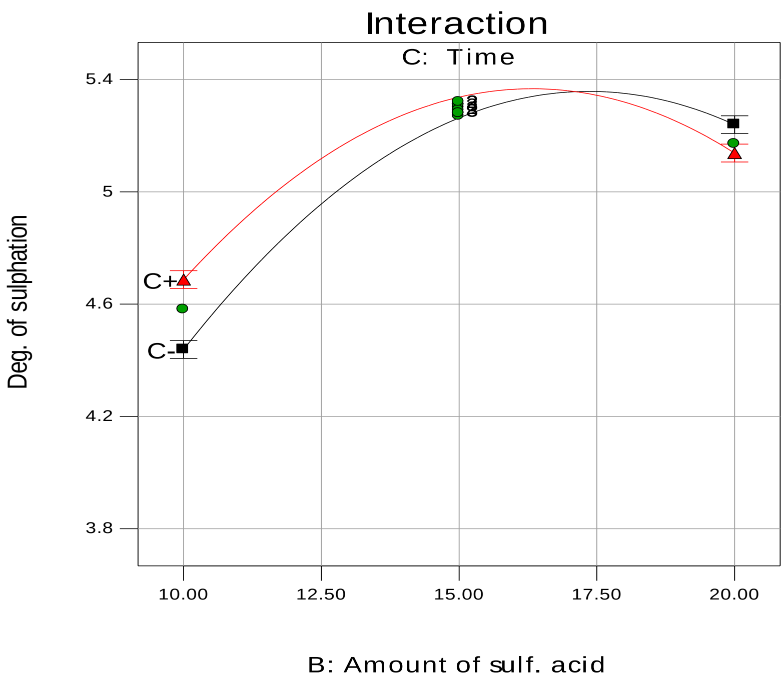
b) Contour plot for the effects of time and temperature on the degree of fatliquor sulphation



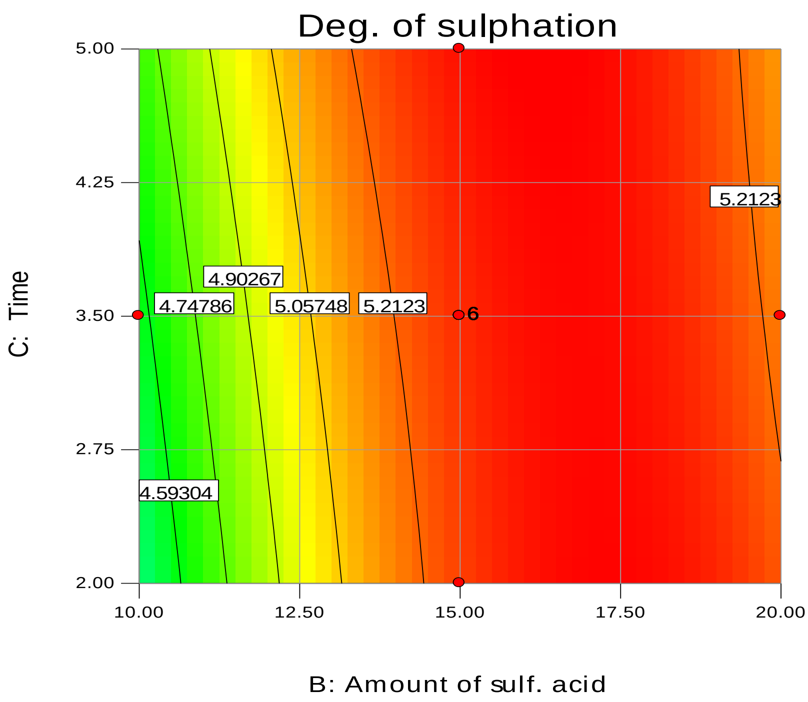
c) Surface plot of the effects of time and temperature on the degree of fatliquor sulphation

Figure 12: Interaction effect of time and temperature on degree of sulphation

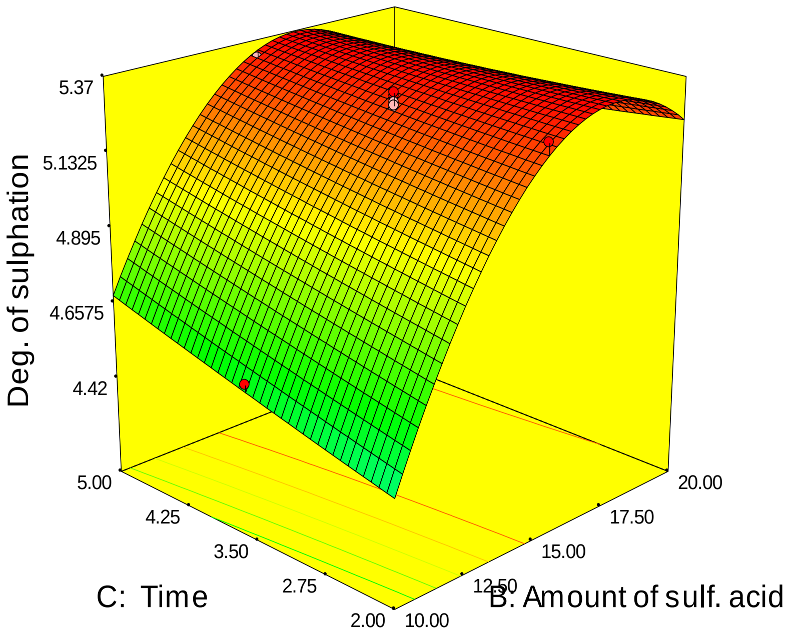
The above Figure 12, the interaction effect of time and temperature was observed for the amount of H_2SO_4 was at the center point. In (a) it is depicted that both the temperature and time have strong relationship to the degree of sulphation of fatliquor at amount of H_2SO_4 was constant. At high reaction temperature, the degree of sulphation was increased while at a lower temperature the degree of sulphation decreased with increasing time. This is because at a lower temperature the neutral oil component in fatliquor production could not be reduced or low capabilities of converting neural oil to sulphated oil which is essential for better properties to leather. The contour plot and 3D (b) and (c) represent the fatliquor response surface plot developed as a function of time and temperature while amount of H_2SO_4 was kept constant at 15 Wt%. As the temperature and time increases the degree of sulphation increased but the sulphation reaction needs more time for formation of the sulphated group which increases the degree of fixation.



a) The effect of amount of sulfuric acid and reaction time on the degree of sulphation, when the reaction temperature was at the center point



b) Contour plot of the effects of amount of sulfuric acid and reaction time on the degree of sulphation of fatliquor



c) Surface plot of the effects of reaction time and amount of sulfuric acid on the degree of sulphation of fatliquor

Figure 13: Interaction effect of reaction time and amount of H_2SO_4 on degree of sulphation

In the above Figure 13 a, both time and amount of sulfuric acid have strong relationship to the degree of sulphation of fatliquor at the constant temperature. At high and low reaction time, the degree of sulphation observed to parabolically decrease to a certain amount. At a minimum amount of H_2SO_4, the reaction needs more time for better degree of sulphation. Maximum amount of H_2SO_4 needs low reaction time for high degree of sulphation because the reaction requires the greater stability and fixation to acid at the specified time for penetration in to the leather.

The 3D and contour plot in Figure 13 b and c represents the fatliquor response surface plot developed as a function of reaction time and amount of sulfuric acid while reaction temperature was kept constant at 20 ^{o}C. As the amount of sulfuric acid increases from 10 Wt% to 16.76 Wt% , the degree of sulphation increased and reached to peak level then decreases due to losing the oiliness characteristics and lubricating effect during the sulphation reaction formation.

4.8 Optimization of process variables

The results above have shown that the three sulphation process variables and the interaction among the variables affect the degree of sulphation. Therefore, the next step is to optimize the process variables in order to obtain the highest degree of sulphation using the model Regression developed. The optimum conditions for the three factors, i.e. reaction temperature, amount of sulfuric acid and reaction time were determined using the numerical optimization feature of the design expert software. The software generates combination of parameters that satisfy the requirement for the response and each of the factors. Optimization of the process was conducted by setting the parameters and response with their high and low values was listed in Table 12 in order to identify the optimum condition.

Table 12: Optimization of process parameters

Name	Goal	Lower limit	Upper limit
Reaction temperature (RTe)	Is in range	15	25
Amount of sulfuric acid (AS)	Is in range	10	20
Reaction time (Rt)	Is in range	2	5
Degree of sulphation (DS)	Maximize	3.81	5.36

The optimum conditions obtained were then evaluated by the composite desirability, which has a value from 0 to 1, to determine the degree of satisfactory of the optimum conditions for the ultimate goal of response. Based on the predicted parameters as shown in Table 13, experiments were conducted and the results were computable with the prediction. It was found that the experimental value of 5.37% degree of sulphation which is well agreed with the predicted value. Therefore, this study shows that neem oil can definitely be used for synthesis of sulphated fatliquor and a higher DS can be obtained from the synthesis of neem oil using concentrated sulfuric acid by sulphation reaction.

Table 13: Optimization result

RTe (^{o}c)	AS (Wt %)	Rt (h.)	DS (%)		Desirability
			predicted	measured	
23.98	16.76	3.28	5.3995	5.3700	1.0

4.9 Yield of sulphated fatliquor

RTe, AS and Rt are the major parameters that affect the yield of sulphated fatliquor. It also observed in the experimental data analysis arises from the sulphation reaction**.**

Table 14: Yield of sulphated fatliquor

Run	RTe (^oc)	AS (Wt %)	Rt (h.)	Y (%)	DS (Wt %)
1	15	10	2.0	90.01	3.81
2	15	20	2.0	82.87	4.82
3	20	15	3.5	82.4	5.29
4	20	15	3.5	81.64	5.28
5	20	15	3.5	81.32	5.27
6	20	15	3.5	80.8	5.32
7	25	20	5.0	75.32	5.23
8	20	15	3.5	82.1	5.28
9	15	10	5.0	88.8	3.83
10	20	15	3.5	80.88	5.31
11	20	15	5.0	83.11	5.31
12	25	20	2.0	84.7	5.06
13	25	15	3.5	80.1	5.36
14	20	15	2.0	82.9	5.30
15	25	10	2.0	85.5	4.45
16	20	20	3.5	79.97	5.17
17	20	10	3.5	83.91	4.58
18	15	15	3.5	87.32	4.65
19	25	10	5.0	84.01	4.96
20	15	20	5.0	86.4	4.48

As indicated in the above Table 14, the yield was affected by the sulphation process parameters. The lower yield was found during increment of the reaction temp., amount of H_2SO_4 and reaction time that may be due to in favor of the backward reaction. The degree of sulphation at a minimal amount of H_2SO_4, lower reaction temperature and short residence time is lower that favor the reaction to be forwarded in order to increases the yield. At a lower temperature, the rate of reaction is slow and minimum degree of sulphation found. On the other hand at higher degree of sulphation and percent yield 80.1%, can be obtained at higher reaction temperature, optimal amount of sulfuric acid and optimal reaction time.

4.10 Physicochemical analysis of sulphated fatliquor

The physicochemical characterization of the synthesized sulphated fatliquor was done based on the Indian standard specifications and recommended methods, IS 6357. Stability of emulsion in tanning salt solution, Emulsion pH, total alkalinity and degree of sulphation was conducted for all the experiments and amount of total active ingredient, unsaponifiable matter, and total ash content was done for randomly selected three experiments and compared with the specifications. The results were calculated using equations given in the materials and methods chapter three.

i) Physical condition

To allow a small amount of oil to be spread uniformly over a very large surface area of the leather fibers, it is necessary to dilute the oil. For fatliquoring process the sulphated fatliquor is applied in the form of emulsion. The 10% emulsion formed using the sulphated fatliquor form a stable emulsion at room temperature.

The physical condition of sulphated fatliquor produced from neem oil appear as a brown liquid which is pourable at 23^0C, capable of being readily washed out with water without having any oily feeling on hand and does not have any rancid or putrefactive odor.

ii) Stability of emulsion in tanning salt solution

Emulsion stability in salt solution is a factor to determine the property of fatliquor. The prepared fatliquor was stable in tanning salt solution of 5% NaCl, 5% Na_2SO_4, and 5% basic CrSO4 for more than 40 min. The stability of the emulsion of the prepared sulphated fatliquor is due to the poly hydroxyl sulphate group which is resistance to hydrolysis.

iii) pH of emulsion

The pH of sulphated fatliquor emulsion is one of the most important properties for determining the property and performance of fatliquor.

Table 15: pH for 10% emulsion of sulphated fatliquor

Run	RTe (^{o}c)	AS (Wt %)	Rt (h.)	pH value
1	15	10	2.0	6.93
2	15	20	2.0	6.56
3	20	15	3.5	7.31
4	20	15	3.5	6.85
5	20	15	3.5	7.21
6	20	15	3.5	7.14
7	25	20	5.0	7.25
8	20	15	3.5	6.92
9	15	10	5.0	7.64
10	20	15	3.5	6.87
11	20	15	5.0	7.80
12	25	20	2.0	7.50
13	25	15	3.5	6.93
14	20	15	2.0	6.67
15	25	10	2.0	6.75
16	20	20	3.5	7.27
17	20	10	3.5	6.96
18	15	15	3.5	7.56
19	25	10	5.0	7.12
20	15	20	5.0	6.62

The pH value of emulsion should be neutral (6.5 – 8) to be used for fatliquoring. The pH of leather at the time of fatliquoring also plays an important role in the manner in which the oil is fixed and uniformly distributed. The principal result of neutralization of chrome tanned leather before fatliquoring process is reduction of the strong positive charges so that the negative fatliquor emulsion gets absorbed. The pH of 10% emulsion formed by the sulphated fatliquor was observed to be in the range from 6.56 to 7.8. Which is within the range given by IS 6357.

iv)Total active ingredient

Total active ingredient (TAI) shows the amount of fatty matter in sulphated fatliquor that give the desired characteristics to the fatliquor. High amount of TAI indicates high amount of oil is available for lubrication of the leather. The hydrophilic part makes this oil to bond with the protein to gain a stable effect.

The TAI of sulphated fatliquor produced was performed at different sulphation process parameters. The result of randomly selected three experiments was calculated and the value is shown in Table 16 below.

Table 16**:** Total active ingredient of sulphated fatliquor

Run No	RTe (°c)	AS (Wt %)	Rt (h)	W(g)	F(g)	E(g)	A (%)	B (%)	TAI (Wt %)
7	25	20	5.0	5	2.67	0.61	53.4	12.2	65.7
13	25	15	3.5	5	2.69	0.69	53.8	13.8	67.6
17	20	10	3.5	5	2.71	0.62	54.2	12.4	66.6

The total active ingredient of sulphated fatliquor produced was performed and observed Minimum of 65.7 Wt % of fatliquor used at specified sulphation process parameters. The experimental results is found agreeable with the standard IS 6357, which is minimum of 60 Wt%.

v) Unsaponifiable matter

The Unsaponifiable Matter of sulphated fatliquor produced was performed at different sulphation process parameters. The result of randomly selected three experiments was calculated and the value is shown in Table 17 below.

Table 17: Unsaponifiable matter of sulphated fatliquor

Run	RTe (°c)	AS (Wt %)	Rt (h.)	W (g)	A (g)	V (ml)	B (g)	Unsaponifiable matter (Wt %)
7	25	20	5.0	5	0.015	0.1	0.003	0.243
13	25	15	3.5	5	0.02	0.4	0.011	0.174
17	20	10	3.5	5	0.03	0.5	0.014	0.320

The unsaponifiable matter of sulphated fatliquor produced was found Maximal of 0.320Wt % of fatliquor used at specified sulphation process parameters. The experimental results is found agreeable with the standard IS 6357, which is a maximum of 2wt %

vi)Total ash content

The total ash content (TAC) of sulphated fatliquor produced was performed at different sulphation process parameters. The result of randomly selected three experiments was calculated and the value is shown in Table 18 below.

Table 18: Total ash content of sulphated fatliquor

Run	RTe (°C)	AS (Wt %)	Rt (h.)	W_0 (g)	W_1 (g)	W_2 (g)	TAC
7	25	20	5.0	23.341	26.361	23.416	2.470
13	25	15	3.5	23.012	26.014	23.070	1.932
17	20	10	3.5	23.514	26.515	23.589	2.517

The TAC of SNOSFL produced was found maximal of 2.517% at specified sulphation process parameters. The experimental results is found agreeable with the standard IS 6357, which is maximum of 3%.

vii) Total alkalinity

The total alkalinity (TA) of sulphated fatliquor produced was performed at different sulphation process parameters. The result of the experiment was calculated and the value is shown in Table 19 below.

Table 19: Total alkalinity of sulphated fatliquor

Run	RTe (^oC)	AS (Wt %)	Rt (h.)	W(g)	V(ml)	TA (mg/10g)
1	15	10	2.0	10	5.00	2.50
2	15	20	2.0	10	3.60	1.79
3	20	15	3.5	10	3.20	1.60
4	20	15	3.5	10	3.40	1.68
5	20	15	3.5	10	3.40	1.70
6	20	15	3.5	10	3.30	1.64
7	25	20	5.0	10	2.90	1.45
8	20	15	3.5	10	3.00	1.50
9	15	10	5.0	10	4.60	2.30
10	20	15	3.5	10	2.60	1.30
11	20	15	5.0	10	2.90	1.45
12	25	20	2.0	10	3.20	1.59
13	25	15	3.5	10	2.50	1.25
14	20	15	2.0	10	2.90	1.47
15	25	10	2.0	10	4.20	2.12
16	20	20	3.5	10	3.30	1.64
17	20	10	3.5	10	3.68	1.84
18	15	15	3.5	10	3.60	1.81
19	25	10	5.0	10	3.50	1.75
20	15	20	5.0	10	4.10	2.05

The TA of SNOSFL produced was found maximally 2.5 mg/10 g fatliquor at specified sulphation process parameters. The experimental results is agreeable with the standard IS 6357, which is maximum of 3 mg/ 10 g fatliquor.

viii) Determination of organically combined SO_3 as neutralized sulfuric ester

The sulphated product obtained were analyzed by Indian standard (IS 6357) method; taking into consideration that the degree of conversion was estimated mostly on combined SO_3 Content. The stability of the resulting emulsion also evaluated according to the content of SO_3 in the sulphated oil. In addition as stated by (El-Shahat *et.al.*, 2011) in the synthesis of Fatliquor it should be noted that SO_3 content of the resulting constituents is of great significance. (Sharphouse, 1983) States that the higher an oil is sulphated (the more polar groups put in), the more soluble it becomes and the greater the degree to which the oil is sulphated, the less will be its lubricating effect on the leather surface. This may be due to the consequent reduction in neutral oil whilst the greater the sulphation the greater the emulsion penetration in to the wet leather, reducing the oiliness of the grain and flash surface. The experimental results are shown in Table 20 and 21.

Table 20: Organically combined SO_3 as neutralized sulphuric ester

Run	RTe (^oc)	AS (Wt %)	Rt (hr.)	W (g)	V1 (ml)	V2 (ml)	F mg/10g	A mg/10g	G % by mass
1	15	10	2.0	10	23.5	12.3	1.12	2.50	2.896
2	15	20	2.0	10	43.1	13.4	2.97	1.79	3.808
3	20	15	3.5	10	48.9	13.3	3.56	1.60	4.128
4	20	15	3.5	10	46.3	11.7	3.46	1.68	4.112
5	20	15	3.5	10	45.9	11.6	3.43	1.70	4.104
6	20	15	3.5	10	49.0	13.3	3.57	1.64	4.168
7	25	20	5.0	10	56.2	19.5	3.67	1.45	4.096
8	20	15	3.5	10	57.3	20.6	3.67	1.50	4.136
9	15	10	5.0	10	24.5	11.1	1.34	2.30	2.912
10	20	15	3.5	10	57.9	18.5	3.94	1.30	4.192
11	20	15	5.0	10	57.5	19.7	3.78	1.45	4.184
12	25	20	2.0	10	44.6	11.1	3.35	1.59	3.952
13	25	15	3.5	10	58.6	18.6	4.00	1.25	4.192
14	20	15	2.0	10	56.2	18.9	3.73	1.47	4.160
15	25	10	2.0	10	38.8	18.5	2.03	2.12	3.320
16	20	20	3.5	10	45.0	11.5	3.35	1.64	3.992
17	20	10	3.5	10	42.9	17.5	2.54	1.84	3.504
18	15	15	3.5	10	43.0	15.8	2.72	1.81	3.624
19	25	10	5.0	10	44.2	13.6	3.06	1.75	3.848
20	15	20	5.0	10	40.4	27.2	2.22	2.05	3.416

ix)Determination of total organically combined SO_3 as sulfuric & sulphonic esters

The degree of sulphation of fatliquor produced or total organically combined SO_3 as sulphuric and sulphonic ester was performed and observed to be in the Range of 3.81 to 5.36 % by weight of fatliquor at different sulphation process parameters. When we compare the experimental results with the standard IS 6357, minimum percentage of 3.81% is a little bit lower than the standard minimum value which is 4.5%.

Table 21: Degree of sulphation of fatliquor

Run	RTe (^{o}c)	AS (W %)	Rt (h.)	Titer Vol. , ml	X (mg)	Y (mg)	T (mg/10g)	F (mg/10g)	I % by Mass	G % by Mass	(I+G) % by Mass
1	15	10	2	29.5	5.45	5	2.263	1.12	0.914	2.896	3.81
2	15	20	2	33.5	5.85	5	4.235	2.97	1.012	3.808	4.82
3	20	15	3.5	35.0	6.00	5	5.013	3.56	1.162	4.128	5.29
4	20	15	3.5	34.8	5.98	5	4.920	3.46	1.168	4.112	5.28
5	20	15	3.5	34.7	5.97	5	4.888	3.43	1.166	4.104	5.27
6	20	15	3.5	35.0	6.00	5	5.010	3.57	1.152	4.168	5.32
7	25	20	5	35.2	6.02	5	5.088	3.67	1.134	4.096	5.23
8	20	15	3.5	35.2	6.02	5	5.100	3.67	1.144	4.136	5.28
9	15	10	5	29.9	5.5	5	2.488	1.34	0.918	2.912	3.83
10	20	15	3.5	35.8	6.08	5	5.415	3.94	1.118	4.192	5.31
11	20	15	5	35.4	6.04	5	5.188	3.78	1.126	4.184	5.31
12	25	20	2	34.5	5.95	5	4.735	3.35	1.108	3.952	5.06
13	25	15	3.5	35.9	6.09	5	5.460	4.00	1.168	4.192	5.36
14	20	15	2	35.3	6.03	5	5.155	3.73	1.14	4.16	5.30
15	25	10	2	31.9	5.69	5	3.443	2.03	1.13	3.32	4.45
16	20	20	3.5	34.6	5.96	5	4.823	3.35	1.178	3.992	5.17
17	20	10	3.5	32.8	5.78	5	3.885	2.54	1.076	3.504	4.58
18	15	15	3.5	33	5.8	5	4.003	2.72	1.026	3.624	4.65
19	25	10	5	33.9	5.89	5	4.450	3.06	1.112	3.848	4.96
20	15	20	5	32.1	5.71	5	3.550	2.22	1.064	3.416	4.48

4.11 Spectroscopic Analysis

1) GC-MS Spectroscopy

GC-MS analyses were carried out to detect the components in the NSO. The chromatogram is presented in Figure 14 and Table 22. The major fatty acid component present in NSO was oleic acid 30.99 %. The saturated fatty acids 46.98% in the sample represented as Margaric acid 25.62%, Palmitic acid 19.44%, Lauric acid 1.92% .The unsaturated fatty acids 46.75% identified as oleic 30.99%, Linoleic acid15.76%, and other compounds 6.27%.

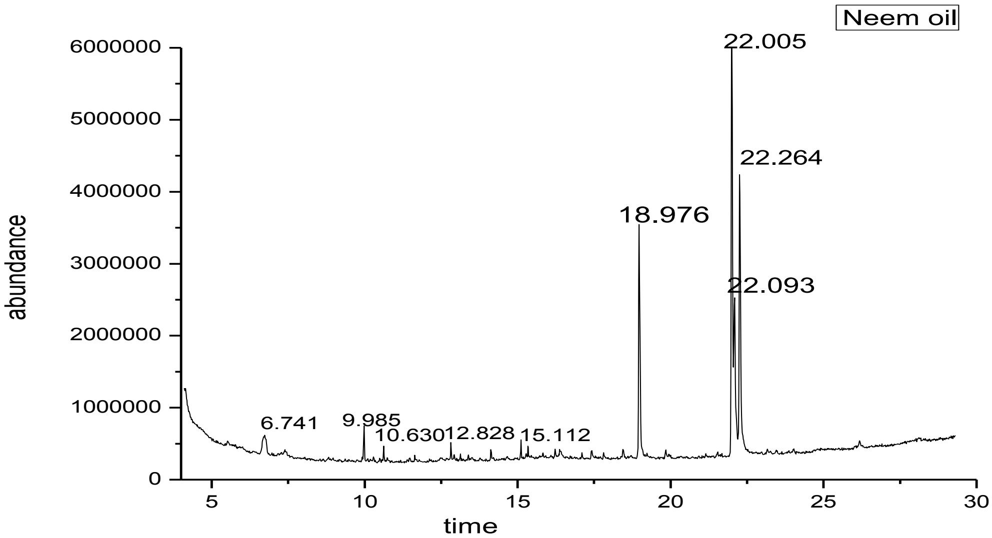


Figure 14: GC-MS spectroscopy for Neem seed oil

Table 22: GC-MS identification of major fatty acid Groups in NSO

S/N	Fatty acids		Area %	Retention time
	IUPAC/systemic name	Common name		
1	Dodecanoic acid	Lauric acid	1.92	9.984
2	Hexadecanoic acid, methyl ester	Palmitic acid	19.44	18.976
3	9-Octadecanoic acid, methyl ester	Oleic acid	30.99	22.005
4	10,13-Octadecanoic acid, methyl ester	Linoleic acid	15.76	22.093
5	Heptadecanoic acid, 16-methyl-ester	Margaric acid	25.62	22.264

The type, concentration and structural features influence the properties of sulphated fatliquor. As degree of unsaturation or reactivity of oil is more, creates a lot of troubles to a tanner because these oils undergo auto oxidation, rancification and polymerization. Therefore neem oil had of proportional value of degree of unsaturation to select for the production of sulphated fatliquor.

2) FT-IR Spectroscopy

FT-IR spectra for neem seed oil and Neem oil sulphated fatliquor are presented in Figure 15 and 16.The characteristics absorption band and functional groups are depicted in Table 23.

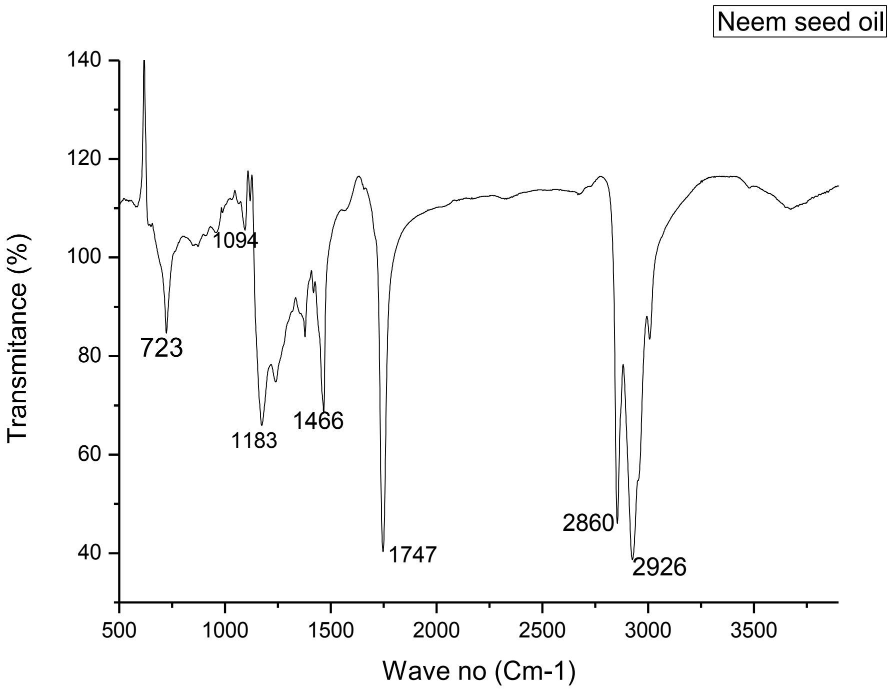


Figure 15: FT-IR spectroscopy for neem oil

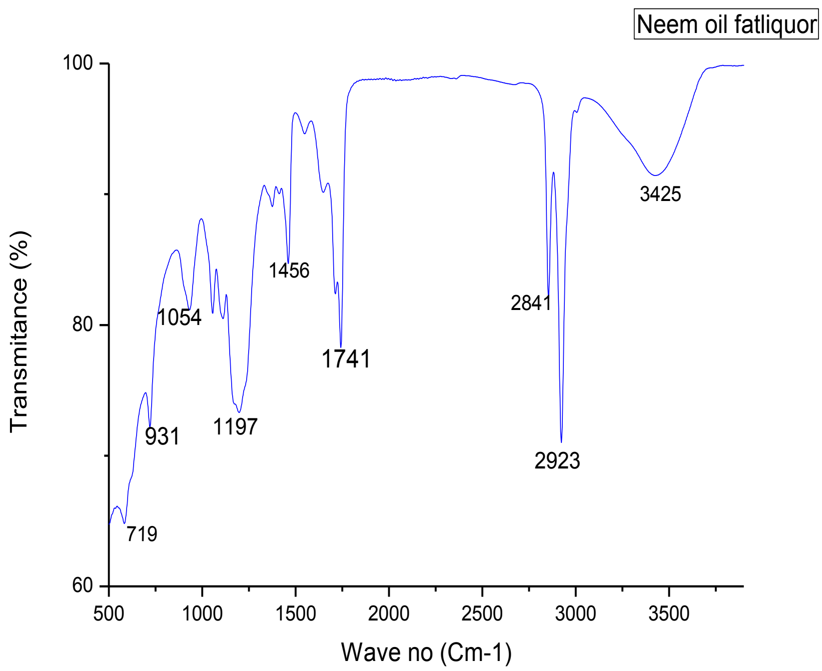


Figure 16**:** FT-IR spectroscopy for Neem oil fatliquor

Table 23: Absorption band, functional groups and vibration modes for Neem oil fatliquor Source: (Segneanu *et.al.*,2012).

Reference			Experimental	
Class	Group	wave no (Cm^{-1})	Wave no (Cm^{-1})	Transmittance (%)
Hydrocarbons				
Alkane	C-H	2800-3000	2841,2923	83.23,70.99
Alkane	-C-H	1350-1480	1466	68.46
Oxygen compounds				
Alcohol	O-H	3300-3600	3425	91.43
Carboxylic Acids	C-O	1100-1300	1197	68.36
Easter	C=O	1735-1750	1741	78.29
	C-O	1000-1300(2 band)	1054	80.89
Sulfur compounds				
Ester	S-OR	700-900	719	72.18
Thio carbonyl	C=S	1050-1200	1094	78.32
Sulphate	S=O	1350-1460	1456	85.39
Phosphorous compounds				
Esters	P-OR	900-1050	931	81.15

FT-IR analysis showed defined characteristic absorption bands at 2841, 2923, 1466, cm^{-1} to the presence of long linear aliphatic chain compounds of the hydrocarbon and thio carbonyl at 1094 cm^{-1}. The broad absorption band for the region of 3425 cm^{-1} corresponds to the resulting hydroxyl group. While C-O stretching due to carboxylic acid was observed at 1183cm^{-1}, sulfate was observed at 1456 cm^{-1}. The strong absorption peaks at around at 2,923–2,841 cm^{-1} were assigned to CH_3 and CH_2 asymmetric stretching vibration. Also, the spectra showed stretching absorption bands at 1741 cm^{-1} which corresponds to (C=O) and absorption bands at 1054 cm^{-1} corresponds to (C-O) due to ester group. The bands at 719 cm^{-1} and 931cm^{-1} resulting from the S-OR and P-OR stretching were representative of the presence of ester of sulfur and phosphorus compounds. The bands corresponds to stretching and bending absorption peaks at 3,007 and 723 cm^{-1} resulting from olefinic (=CH) group spectra of Neem oil.

4.12 Test analysis of sulphated fatliquor on leather

4.12.1 Organoleptic properties and outlook of garment leather

The various organoleptic properties of the leather such as uniformity of color, roundness, fullness, grain tightness and softness were evaluated by experienced professionals and rated the leathers on a scale of 0-10 points score for each functional property where higher values indicated better property of leathers. The average values are taken to be considered. The sample of crust leather is shown in below Figure 17.

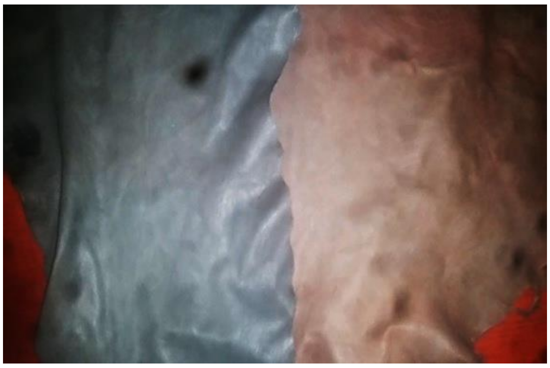


Figure 17: Fatliquored sheep garment crust

Leather especially that uses for clothes and footwear production, the properties with regard to the comfort to the wearer is a great demand. The designers and all leather industries always look first at the appearance of the leather. The suitability of leather fatliquoring is mainly shown by the organoleptic properties of the treated leather as shown below in Table 24.It also shown the synthesized (experimental) fatliquor has an approachable result to that of commercial (control) fatliquor but a little bit higher value of roundness, fullness, and grain tightness was recorded. In addition to the effect of fatliquor, the nature and quality of hides used has also an effect on the artistic beauty, appearance and performance of the leather.

Table 24: Organoleptic properties of sheep garment leather

Tests	Coded factors	
	Experimental (EF_1)	Control (CF_1)
Uniformity of color	9	9
roundness	7.5	7
fullness	8.5	7.5
Grain tightness	9	7.5
softness	8	8
Overall appearance	9	9

4.12.2 Physical test analysis of garment leather

The crust leathers, both experimental and control were analyzed for tensile strength, percent elongation at break and tear strength. The average value for each of the crust leather samples was taken in to consideration and the evaluated results identified by code are given in *Appendix F*.

The strength properties have been given the greatest consideration in evaluating fat-liquored leather because they give an indication of fiber lubricity. The mechanical properties/physical tests were evaluated according to ISO testing methods.

The experimental and control samples were treated with equal amount of fatliquor prepared from refined vegetable oils as fatliquoring agent. But Figure 19 shows that the tensile strength and elongation at break of treated leather via neem oil fat liquor were relatively higher than that of commercial fatliquor (Fosfol LP) commonly used in most of the tanneries. The high value of tensile strength indicates the high strength of collagen fibers. The elongation at break indicates the softness, flexibility, strength and toughness of leather. The enhancement in mechanical properties of treated leather is due to a good lubrication of fibers and penetration properties of prepared fatliquor. The sulfated portion of oil during liquoring of chrome tanned leather is chemically bound to the leather fibers, i.e. interacts with active centers in the collagen molecules of leather fibers, while the emulsified portion is mainly located between the fibers.

The result given in Appendix F shows that the average tear load of experimental and control value was 40.30 N and 40.10 N and the tear strength was 48.1N/mm and 48.6N/mm respectively. The value difference of tear load to that of tear strength of the samples might be due to the variation in thickness of samples caused by fullness of the leather.

Emulsion properties, particle size distribution of emulsion and the type of oil and contents have a great impact on the performance of the resultant leather. From the result shown, I conclude that the nature and property of neem oil give the fatliquored leather desired organoleptic and physical properties. This was agreeable with the reported study by Kumar (2017) that the mechanical properties of hide can be affected by type of fatliquor during fatliquoring process, structural and molecular factors, environmental and external factors such as temperature, time, pressure, moisture content.

The graphs shown in the Figure 18 and Figure 19 below indicated the average values of synthesized and commercial fatliquor effect. The synthesized sulphated fatliquor from neem oil was comparable to commercial one. This result shows that the potential of neem oil for substitution of the corresponding imported fatliquor. In general, the physical and organoleptic properties demonstrate the Suitability of the prepared sulphated fatliquor from neem oil for fatliquoring of chrome Tanned leather.

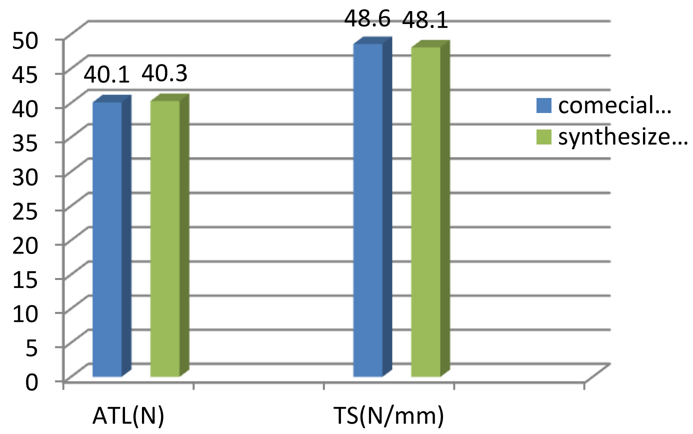


Figure 18: Comparison of average tear load (ATL) and tear streangth (TS) of commercial and synthesized fatliquor

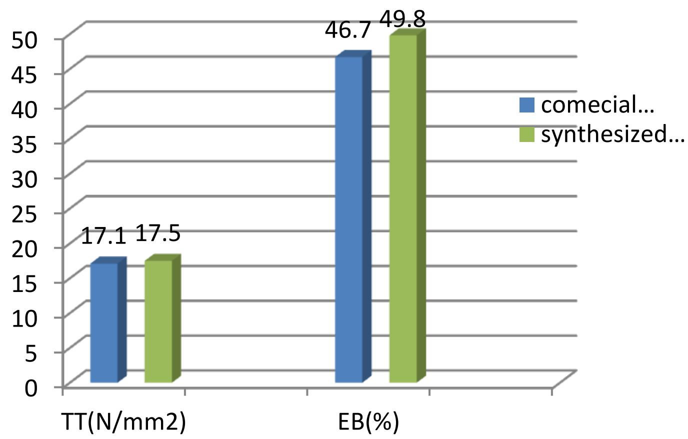


Figure 19**:** Comparison of tensile strength (TT) and elongation at break (EB) of commercial and synthesized fatliquor

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Sulphated Fatliquor was synthesized from locally available raw material Neem (*Azadirachata indica*) seed oil through sulphation reaction. The three sulphation reaction parameters affecting the degree of sulphation namely reaction temperature, amount of concentrated sulphuric acid and reaction time has been studied.

Based on the analysis of experimental results, it is found that all the three process variables exhibited significant interaction effect on the degree of sulphation. This shows that the capability of the design of experimental analysis in successfully capturing these effects. The optimal conditions that maximize the degree of sulphation were found to be reaction temperature at 23.98 °C, amount of conc. H_2SO_4 at 16.76 Wt. % and reaction time 3.28 h that yield the degree of sulphation was 5.37%. The fatliquoring effect of the synthesized fatliquor on leather has comparable organoleptic and physical properties to that of the imported vegetable oil based sulphated fatliquor.

Therefore, from experimental analysis we can conclude that sulphated fatliquor production using neem oil has a considerable potential application on leather processing industry as fatliquoring chemical. This is mainly because of its performance on leather for fatliquoring and the potential of exploiting local resources. Hence, it is conclude that neem (*Azadirachata indica*) seed oil sulphated fatliquor has a great potential for substitution of imported vegetable oil sulphated fatliquor in leather processing industry.

5.2 Recommendation

It is recommended to make leathers that can be fatliquored with sulphated neem oil that the characteristics of the leathers processed by the prepared neem oil fatliquor are comparable to that of leathers produced using imported fatliquor.

Industrially different types of fatliquor were used in blend for best outcome. Thereby, the following area can be recommended to further study

- ⇒ The effect of Neem oil sulphated fatliquor in combination with other fatliquors.
- ⇒ The principal parameters that affect fatliquoring process such as temperature, neutralization pH, which may increase the efficiency of fatliquor.
- ⇒ The determination of leather breathability to test the air and water vapor permeability and absorption.

Finally, it is recommended to investigate the potential of neem seed plantation for raw material supplement.

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APPENDICIES

Appendix A: Some common compositions and characteristics of fatty substances

Table A-1: Fatty acid composition of some common fats/animal origin

Product	Saturated			Unsaturated			Other
	Wt%	Name	Structure	Wt%	Name	Structure	
Beef fat	27 7 3	Palmitic Stearic myristic	16:0 18:0 14:0	48 11 2	Oleic Palmitoleic linoleic	18:1 16:1 18:2	2%
Butter	26 12 11 4 3 3 2	Palmitic Myristic Stearic Lauric Butyric Capric caproic	16:0 14:0 18:0 12:0 4:0 10:0 6:0	28 3 2	Oleic Palmitoleic linoleic	18:1 16:1 18:2	6%
Chicken/ turkey fat	22 6 1	Palmitic Stearic myristic	16:0 18:0 14:0	37 20 6 1 1	Oleic linoleic Palmitoleic gadoleic linolenic	18:1 18:2 16:1 20:1 18:3	6%
Lard (hog fat)	27 11 2	Palmitic Stearic myristic	16:0 18:0 14:0	44 11 4	Oleic Palmitoleic linoleic	18:1 16:1 18:2	1%

Source: (Gunstone, 1996)

Table A-2: Typical Fatty–Acid Compositions of Some Common Fats /plant origin

Product	Saturated			Unsaturated			Other
	Wt%	Name	structure	Wt%	Name	Structure	
Canola oil (rapeseed)	4 2	Palmitic Stearic	16:0 18:0	56 26 10	Oleic Linoleic linolenic	18:1 18:2 18:3	2%
Coconut oil	48 16 9 8 7 2	Lauric myristic palmitic caprylic capric stearic	12:0 14:0 16:0 8:0 10:0 18:0	7 2	Oleic Linoleic	18:1 18:2	1%
Corn oil	13 3	Palmitic Stearic	16:0 18:0	52 31 1	Linoleic Oleic linolenic	18:2 18:1 18:3	-
Cotton seed oil	23.4	palmitic	16:0	31.6 45	Oleic Linoleic	18:1 18:2	-
Olive oil	10 2	Palmitic stearic	16:0 18:0	78 7 1	Oleic Linoleic linolenic	18:1 18:2 18;3	2%
Palm oil	44 4	Palmitic stearic	16:0 18:0	40 10	Oleic linoleic	18;1 18:2	2%
Peanut oil	13 3	Palmitic stearic	16:0 18:0	41 38	Linoleic Oleic	18:2 18:1	5%

Source: (Gunstone, 1996)

Table A-3: some common fatty acids in oil and fats

	Common names	Systemic names	Formula and structure
Saturated fatty acids	Palmitic acid	Hexadecanoic	CH_3-$(CH_2)_{14}$-COOH; 16:0
	Stearic acid	Octadecanoic	CH_3-$(CH_2)_{16}$-COOH; 18:0
	Arachidic acid	Eicosanoic	CH_3-$(CH_2)_{18}$-COOH; 20:0
	Lauric acid	Dodecanoic	CH_3-$(CH_2)_{10}$-COOH; 12:0
	Capric acid	Decanoic	CH_3-$(CH_2)_2$-COOH; 4:0
	Caproic acid	Hexanoic	CH_3-$(CH_2)_4$-COOH; 6:0
	Caprylic acid	Octanoic	CH_3-$(CH_2)_6$-COOH; 8: 0
	Myristic acid	Tetradecanoic	CH_3-$(CH_2)_{12}$-COOH; 14:0
	Behenic acid	Docosanoic	CH_3-$(CH_2)_{20}$-COOH; 22:0
Unsaturated fatty acids	Oleic acid	9-octadecenoic	CH3-$(CH_2)_7$CH=CH$(CH_2)_{7}$-COOH; 18:1
	Ricinoleic acid	12-hydroxy-9-octadecenoic	CH_3-$(CH_2)_5$C(OH)HCH_2CH=CH$(CH_3)_7$-COOH; 18:1
	Linoleic acid	9,12-octadecadienoic	CH_3-$(CH_2)_4$CH=CHCH_2CH=CH$(CH_2)_7$-COOH; 18:2
	Linolenic acid	6,9,12-octadecatrienoic	CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CH$(CH_2)_7$-COOH; 18:3

Source: (Dutta, 1999)

Table A-4: Characteristic average values of the main fatty substance

Class	**Oil**	**Origin**	**Iodine value ($g\ I_2$ / 100 g oil)**	**Saponification value mgKOH/goil**	**Unsaponifiable matter (Wt %)**
Drying oil	Cod & other Fish oil	Marine	140-181	179-193	0.7-3
	Linseed oil	Vegetable	175-202	187-195	0.6-2
	Sunflower oil	Vegetable	125-136	188-194	0.3-1.5
Semi - drying oil	Corn oil	Vegetable	112-128	187-193	1.5-2.5
	Cotton seed oil	Vegetable	105-121	189-198	0.6-2
	Mustard oil	Vegetable	96-107	173-184	0.9-1.5
	Groundnut oil	Vegetable	85-107	188-197	0.3-1
	Sesame oil	Vegetable	103-116	187-195	0.5-1
Non- drying oil	Coconut oil	Vegetable	8-10	246-268	0.2-0.5
	Olive oil	Vegetable	79-88	190-195	0.5-1.4
	Neatsfoot oil	Animal	68-81	190-196	0.1-0.6
	Wool grease	Animal	15-129	77-130	39-50
	Egg yolk	Animal	64-82	184-198	0.2-4.2
	Beef tallow	Animal	32-47	190-200	0.1-0.3
	Bees wax	Animal	6-15	99-100	52-55
	Castor oil	Vegetable	81-89	176-191	0.3-0.4
	Sperm oil	Marine	71-93	125-149	35-44

(BASF and Thorstensen, 1993)

Appendix B: Standard Specification of Sulphated Fatliquor

Table B-1: Requirement of sulphated fatliquor based on IS 6357

Chemical Test Requirement	
Characteristics	**Requirement**
Moisture, % by mass	≤35
pH of emulsion	6.5 to 8
Total active ingredient,% by mass	≥60
Unsaponifiable matter, % by mass,	≤2
Acid value	≤10
Total alkalinity in mg equivalent per 10g	≤3
Ash content , % by mass	≤3
Organically combine sulphate(SO_3) as sulphuric esters, % by mass	≥3
Total organically combine sulphate (SO_3)as sulphuric and sulphonic esters, % by mass	≥4.5
Physical properties and test requirement	
Appearance	• The material shall be a free-flowing, clear, oily liquid and semi-solid portion appearing especially in winter season. Long storage shall not be considered as defect so long as the product forms a ready emulsion in water. • Sulphated fatliquor shall be capable of being readily washed out with water without leaving any oily feeling to hand.
Emulsion stability	• The product shall form a ready emulsion in hot or cold water in any dilution
Emulsion stability (1:10)ratio dilution	• Shall remain stable for 40 minutes when mixed with 5% solutions of NaCl, MgSO4 and 5% BCS in separate containers without creaming and oil separation.
Odor	Shall be free from rancid or putrefactive odor of oil

Appendix C: Experimental result

Table C-1: moisture content on neem seed kernel

	Drying time (hr.)							moisture content (%)	Average Moisture Content (%)
	0	2	4	6	8	10	12		
Weight Of sample(g)	5.021	4.873	4.820	4.790	4.762	4.711	4.710	6.194	6.68
	5.014	4.864	4.721	4.693	4.687	4.672	4.671	6.840	
	5.011	4.851	4.703	4.674	4.651	4.661	4.660	7.005	

Table C-2: moisture content of neem oil

	Drying time(hr.)					Moisture content (%)	Average moisture content (%)
	0	2	4	6	8		
Weight of purified neem oil(g)	10.010	9.985	9.984	9.961	9.960	0.40	0.41
	10.021	9.980	9.967	9.963	9.962	0.38	
	10.011	9.979	9.958	9.955	9.955	0.45	

Table C-3: Acid value of neem oil

Oil	Titration volume(ml)				Acid value
	V1	V2	V3	Average	
Crude	1.5	1.6	1.4	1.500	4.208
Refined	1.2	1	1	1.133	2.993

Table C-4: saponification value of neem oil

Oil	Titration volume (ml)										Saponi fication value
	Run 1			Run 2			Run 3			average Vo-V1	
	Vo	V1	Vo-V1	Vo	V1	Vo-V1	Vo	V1	Vo-V1		
Refined	31.5	12	19.5	30	15	15	28	13.5	14.5	16.333	229.11

Table C-5: iodine value of neem oil

Oil	Titration volume(ml)										Iodine value
	Run 1			Run 2			Run 3			Average B-S	
	B	S	B-S	B	S	B-S	B	S	B-S		
Refined	43	29	14	42	28	14	42	26	16	14.667	74.45

Appendix D: calculation of the experimental value

Note D-1: Purification of crude neem oil

The total amount of crude oil obtained was 4.53liter (4.142Kg) out of 15.86 kg of neem seed kernel

- **Degumming**

  3 % distilled water by oil volume of 4.53 L was used for degumming process of crude oil to remove phosphatides, gums and other complex compounds. Hence, 0.135L or 135ml amount of distilled water was required to proceed in degumming process.

- **Neutralization**

  The free fatty acids were neutralized by using appropriate amount of NaOH. The composition of free fatty acid (FFA) was determined from the acid value of the crude oil described in table C3. Hence,

  $$FFA=\frac{AV\ (ACID\ VALUE)}{2}=\frac{4.208}{2}=2.104\ \frac{mg\ KOH}{g\ of\ oil}$$

  The amount of alkaline solution (NaOH) required neutralizing the free fatty acid was calculated using the given in equation 3.2

  $$\text{Amount of lye (alkaline solution)} =\frac{A\times FFA\times 1000\times K}{100\times M\times N}$$

  A= oil flow (4.142Kg/hr); K = factor of lye excess (15%); M= molecular weight of fatty acid (282Kg/mol); N= normality of lye (0.5mol/l)

  Therefore,

  $$\text{Amount of lye (alkaline solution)} = \frac{4.142\times 2.104\times 1000\times 15}{100\times 282\times 0.5} = 9.28\text{ml of NaOH}$$

Note D-2: physicochemical properties of neem oil

- **Acid value**

  The acid value of both crude and purified neem oil was calculated using equation 3.3 and the average titration volume was taken as described in table

  $$\text{Acid value of crude oil} = \frac{V\times C\times 56.11}{M} = \frac{1.5\times 0.1\times 56.11}{2} = 4.208\text{mgKOH/goil}$$

  $$\text{Acid value of purified oil}= \frac{V\times C\times 56.11}{M} = \frac{1.133\times 0.1\times 56.11}{2} = 2.993\text{mgKOH/goil}$$

- **Saponification value**

The saponification value of both crude and purified neem oil was calculated using equation 3.4 and the required average titration volume difference of the blank and test sample (Vo-V1) was taken to be considered as described in table

$$\text{Saponification value of purified oil}=\frac{56.11\times N\times(V0-V1)}{m}=\frac{56.11\times0.5\times16.333}{2}$$

$$= 229.11\text{mgKOH/goil}$$

- **Iodine value**

The iodine value of both crude and purified neem oil was calculated using equation 3.6 and the required average titration volume difference of the blank and test sample (B-S) was taken to be considered as described in table

$$\text{Iodine value of purified oil} =\frac{(B-S)\times N\times12.69}{W}=\frac{14.667\times0.1\times12.69}{0.25}=74.45$$

Appendix E: Re-tanning, Dyeing and Fatliquoring sheep garment leather process sheet recipe

Date _________________

Sheep garment leather process recipe

Re-chroming, neutralization

Percent based on shaved weight

Thickness 0.5 mm,   Quantity 4 pcs

Wt 1.4 kg

Process	%	Chemicals	Weight	Time (min.)	pH, ^{o}Be
Wet back	150	Water, 40^{o}C	2 liter		
	0.3	Wetting agent	4.2 gram	30	Drain/ Wash
Add	150	Water, 40^{o}C	2 liter		
	0.5	Formic Acid	7 gram	20	pH=3.0-3.2
Re-chroming	3	BCS	42 gram	60	
	1	Bleaching Agent	14 gram	20	
	1	Cationic fatliquor	14 gram	10	
	1	Sodium Formate	14 gram	10	
	1	Sodium bicarbonate	14 gram	40'	pH=4.0, L/O/N, D/W/D
Neutralization	150	Water, 35^{o}C	2 liter		
	2	Neutralizing syntan (Clarimol AK)	28 gram	40	
	1	Sodium formate	14 gram	10	
	0.8	Sodium Bicarbonate	11.2 gram	60	pH 6.0/6.2, D/W/D

Re-tanning, dyeing and fat liquoring

Percent based on shaved weight

Wt 0.7 kg

	50	Water, 35^{o}C	0.35 liter		
Re- tanning	3	Magnopal RHN	21 gram		
	3	Pellitan CNS	21 gram	30	
Dyeing	4	dye	28 gram	60	Check penetration
Fatliquoring	100	Water, 60^{o}C	0.7 liter		
	14	Fatliquor	98 gram	60	
Fixation	1	Formic Acid	7 gram	15	
	1	Formic Acid	7 gram	15	
	2	Formic Acid	14 gram	30	Drain/Wash/Drain
	150	Water, 40	1 liter		
	0.4	Formic acid	2.8 gram	10	
Top dye	1	Black dye	7 gram	20	
	1	Formic acid	7 gram	30	Drain/Wash/Drain

Pile O/N Sam Setting, overhead drying, Stake, Trim, then for finishing.

Appendix F: Test result of control and experimental value of crust leather

LEATHER INDUSTRY DEVELOPMENT INSITITUTE
TESTING & RESEARCH LABORATORY
DIRECTORATE

Title | TEST REPORT | Page : 1 of 2

Test date(s) : 15/08/2019 - 16/08/2019
Lab. Desg .Code No : P-15416
Type of Sample: Sheep Garment
Sample Identification: Black
Sampling: ISO 2418:2008
Conditioning: Temp=21.7°C & RH=67.8%
Equipment used: Thickness gauge, UTM,

Report No: P-15416/19
Test order No: SCBE-688/2011
Sampling date & place: Customer
Sampling location : ISO 2418:2008 (Fig 1)
Sample receiving date: 12/08/2019
Sampled by : Customer
Report date: 19/08/2019

Name of Customer: ADDIS ABABA INSTITUTE OF TECHNOLOGY
School of Chemical & Bio Engineering
Address of Customer: Tell:011-123-24-17
Fax: 011-12394-80
P.O.BOX:-385
E-mail:-info@cheng.aait.edu.et

ORIGINAL

Customer code:- Control

S/No	Type of test	Unit	Test Result	Uncertainty	Test method	Standard Requ.	Remar
1.	Tensile strength & percentage extension				ISO 3376: 2011	-	
1.1	Tensile strength	N/mm²	17.1	-			
1.2	Percentage Elongation at break	%	46.7	-			
2.	Tear Load (Double edge tear)				ISO 3377-2 :2011	-	
2.1	Mean Tear Load in Newton(Parallel to the back bone)	N	36.2	-			
2.2	Mean Tear Load in Newton(perpendicular to the backbone)	N	43.9	-			
2.3	Average Tear Load (Arithmetic mean of 3.1 & 3.2)	N	40.1	-			
2.4	Tear Load	N/mm	48.6	-			

Note:

Tested By: Tigist W/Eyesus Signature Checked/Verified by: Aster Mekesha Signature Authorized by: Signature
Phy/Mech. Testing Expert III
Lead Phy/Mech. Testing Expert

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

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LEATHER INDUSTRY DEVELOPMENT INISTITUTE
TESTING & RESEARCH LABORATORY
DIRECTORATE

Title | TEST REPORT | Page : 1 of 2

Test date(s) : 15/08/2019 - 16/08/2019
Lab. Desg .Code No : P-15415
Type of Sample: Sheep Garment
Sample Identification: Brown
Sampling: ISO 2418:2008
Conditioning: Temp=21.7°C & RH=67.8%
Equipment used: Thickness gauge, UTM,

Report No: P-15415/19
Test order No: SCBE-688/2011
Sampling date & place: Customer
Sampling location : ISO 2418:2008 (Fig 1)
Sample receiving date: 12/08/2019
Sampled by : Customer
Report date: 19/08/2019

Name of Customer: ADDIS ABABA INSTITUTE OF TECHNOLOGY
School of Chemical & Bio Engineering
Address of Customer: Tell:011-123-24-17
Fax: 011-12394-80
P.O.BOX:-385
E-mail:-info@cheng.aait.edu.et

ORIGINAL

Customer code:- Experiment

S/No	Type of test	Unit	Test Result	Uncertainty	Test method	Standard Requ.	Remar
1.	Tensile strength & percentage extension				ISO 3376: 2011	-	
1.1	Tensile strength	N/mm²	17.5	-			
1.2	Percentage Elongation at break	%	49.8	-			
2.	Tear Load (Double edge tear)				ISO 3377-2 :2011	-	
2.1	Mean Tear Load in Newton(Parallel to the back bone)	N	38.2	-			
2.2	Mean Tear Load in Newton(perpendicular to the backbone)	N	42.5	-			
2.3	Average Tear Load (Arithmetic mean of 3.1 & 3.2)	N	40.3	-			
2.4	Tear Load	N/mm	48.1	-			

Note:

Tested By: Tigist W/Eyesus Signature Checked/Verified by: Aster Mekesha Signature Authorized by: Signature
Phy/Mech. Testing Expert III
Lead Phy/Mech. Testing Expert

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Appendix G: Production Capacity of Leather Factories

Table G-1: Annual installed soaking capacity and actual performance of Ethiopian tanneries

Description (E.C)		2005 ('000)	2006 ('000)	2007 ('000)	2008 ('000)	2009 ('000)	2010 ('000)	Average fatliquor Consumption(Kg) From 2005-2010
Hide	Installed(Pcs)	2,702	2,478	2,943	2,205	2,858	2,858	999,800
	Actual (Pcs)	1,391	1,487	2,026	1,837	1,608	1,649	
Actual production performance (%)		51.48	60.00	68.84	83.31	56.2	57.7	
Skin	Installed(Pcs)	44,352	34,100	45,466	34,100	46,586	46,586	1,235,068
	Actual(Pcs)	14,042	17,043	18,937	17,785	19,294	18,762	
Actual production performance (%)		31.66	49.98	41.65	52.16	41.42	40.27	

Source: (LIDI, 2018)

Appendix H: Laboratory equipment and experimental sample

Neem seed

Neem seed kernel

mechanical screw pressing neem Seed kernel

Soxhlet to extract oil

Neem seed oil

Rotary evaporator to remove solvents

Centrifuge to remove gum and phosphatides

Density meter to measure density of oil

Refractometer measures
Refractive index

synthesized fatliquor
and 10% emulsion

wet blue used to test the fatliquor
effect

Fatliquored sheep garment crust

Thickness tester measures
Thickness of crust sample

Universal testing machine to
test the tensile and tear strength