Addis Ababa University

Addis Ababa Institute of Technology

School of chemical and Bio-Engineering

Optimization and Characterization of Biodiesel Production from Leather Industry Fleshing Wastes

A thesis submitted to the school of graduate studies of Addis Ababa Institute of Technology in partial fulfillment for the Degree of Masters of Science in Environmental Engineering

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SCHOOL OF CHEMICAL AND BIOENGINEERING ENVIRONMENTAL ENGINEERING STREAM

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ABSTRACT

Leather industry fleshing wastes have been currently recognized as potential feed stocks for biodiesel production due to high fat content and less competition with food production. Moreover, utilization of industrial wastes for energy production has additional environmental protection purposes. This research work aims at optimization of process variables of biodiesel production from limed leather fleshing waste. Wet limed leather fleshing wastes were delimed using 1% boric acid, dried, chopped and subjected to Soxhlet extraction. The oil after concentrated was treated by 2% w/w orthophosphoric acid and 3% w/w distilled water to remove gums. The pretreated oil had acid value of 1.13 mg KOH /g of oil, FFA of 0.56 %. Since the FFA was below 2.5% direct homogenous base catalyzed transesterification was conducted. The conversion to biodiesel by potassium hydroxide-catalyzed transesterification was achieved above 96% under optimum conditions: a methanol-to-oil molar ratio of 6:1, catalyst amount of 1 % w/w, reaction temperature of 60 °C for an hour reaction time. The produced FAME was found to have viscosity of 5.99 mm^2/s density of 880 kg/m^3 , cetane number of 68.5, iodine value of 65.13g I_2 /100g and HHV of 42.38 MJ/kg. The results showed that majority of the fuel properties are within the ASTM and EN standards.

Keywords: Biodiesel, fleshing oil, homogenous catalysis, pretreatment, transesterification

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List of acronyms and abbreviations

ASTM	American standard for testing materials
CFPP	Cold filter plugging point
EGR	Exhaust gas recirculation
EN	European Norm
EPA	Environmental protection authority
FAAE	Fatty acid alkyl ester
FAME	Fatty acid methyl ester
FFA	Free fatty acid
GCC	Global climate change
GC-MS	Gas chromatograph- mass spectroscopy
GDP	Gross domestic productivity
LIDI	Leather industry development institute
OSI	Oxidative stability index
PUFA	Poly unsaturated fatty acid
SFA	Saturated fatty acid
TAG	Triacyl glycerol
USFA	Unsaturated fatty acid
WAF	Waste animal fat
WCO	Waste cooking oil

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1. INTRODUCTION

1.1. Background of the study

The human society, with its expansion and high technological development, is highly dependent on petroleum fuel for energy generation. However, fossil fuels are non-renewable resources, which take millions of years to form up and remained with limited reserves and high price. Around 60 % of world's oil consumption and one-fifth of global CO_2 emissions are related to transportation sector [1].

The demand for diesel fuel is constantly increasing, requiring its alternate that could **be** sustainable, technically feasible, price competitive and ecologically acceptable. Politico-environmental situations, emission of huge amounts of greenhouse gases, health problems, economic concerns, cost effectiveness as well as predicted shortage of conventional fuels forced in the exploration of alternative fuel to drive diesel engines.

The production and use of fossil fuel in engines with internal combustion causes environmental problems such as rising carbon dioxide levels in the atmosphere and in Global Climate Change (GCC).This decline of conventional fossil fuel reserve, growing emissions of combustion generated pollutants, and their increasing costs make biomass sources more attractive in the recent years to substitute the dependency on fossil fuel. Biofuel as a renewable an alternate and ecologically acceptable substitute for the conventional fuels, biodiesel has attracted concerned environmentalists and governments all over the world. Beside many advantages of biodiesel over diesel fuel, such as: renewability, ready availability, portability, lower sulfur and aromatic content, higher efficiency, higher cetane number, better emission profile and safer handling [2].

The high cost of biodiesel production is the main reason for its limited commercial application. The price of raw material consists of about 70–95% of the total biodiesel cost [2]. Since biodiesel production from food-grade oils is not ethically acceptable and economically competitive with petroleum-based diesel fuel. Therefore, it is necessary to use look for novel and low cost oil feed stocks for its production. The use of cheap waste cooking oils (WCOs), waste oil by-products from edible-oil refinery, non-edible oils and waste animal fats (WAFs) can be used as low cost source in the production of sustainable and ecologically acceptable product.

One of these biomass sources would be the leather industry fleshing waste. The leather fleshing waste generated from the tanneries always triggers secondary, and tertiary environmental impacts such as ground water contamination, greenhouse gas emission effects and odor problem.

The capacity of world leather process is around 15 million tons of hides and skins per year. Solid waste generation from tannery process is estimated over 6 million tons/year. The disposal of large quantity of sludge which is about 4.5 million tons from effluent treatment plants is one of the major environmental pollution issues [3].

Leather industry plays a major role in creating employment opportunity and contributing for national GDP in developing countries like Ethiopia. Ethiopia is relatively well endowed in its livestock base with a share of 12, 15 and 22 percent of the world cattle, sheep and goats population respectively. This makes the country one of the most promising leather producing economic center in Africa. Ethiopia's livestock population is estimated at 52 million Cattle ranking 1st in Africa, 27 million Sheep population which makes 3rd in Africa and 23 million Goat population which makes 3rd in Africa (LIDI, 2014).

The country owns around twenty-nine tanneries with varying processing capacity, the smallest having a soaking capacity of 3000 skins per day. According to the Ethiopian Leather Industry Development Institute (LIDI), the 29 tanneries are processing and producing about 46451520 square meters of finished leather. About 48% of the existing tanneries in Ethiopia are operating at Modjo area. Moreover, the government plan to setup the Modjo Leather City (MLC) at Modjo area makes a necessity to setup a glue and edible grade gelatin plant in order to reduce the impact of leather waste on the environment, since it generates huge amount solid waste.

The Ethiopian leather industries account for about 67% of the total manufactured exports as depicted in the Ethiopian Federal Environmental Protection Authority, Environmental Impact Assessment Guideline for Tanneries (EPA, 2007).

Although the leather industry contributes to the national economic growth, it has also negative environmental impacts by generating organic and inorganic pollutants. The wastes generated by these industries are solid, liquid and gaseous that poses a major challenge to the environment and the ecosystem. The solid wastes that are generated in the leather industries are originated

from the pre-tanning stages. These wastes are mainly originated from pre-fleshing, fleshing, shaving and trimming. These solid wastes have high amount of fat contents that can be used in the production of bio-energy such as biodiesel. Fleshing waste has considerable amounts of fatty acid composition as compared to the other vegetable oil fatty acid compositions. However, these wastes are not changed in to value added products. One way to recover the leather industry wastes is using them as feedstock in biodiesel production due to their rich fat content [4]. Consequently, the pollution coming from the leather industry wastes can be reduced dramatically and more value added products can be obtained by converting them to biodiesel.

Essentially, biodiesel is non-aromatic and sulfur-free as compared with petro diesel which contains 20 to 40 wt.% aromatic compounds and 500 ppm SO_2 [5]. Biodiesel release around 50% less smog smoke density decreased by 22.5 %, the release of CO to 17 % and CO_2 to 14 % reduced as compared to fossil fuel emission[6].

Currently, the production of oil directly from the agricultural industry has the greatest potential for the production of biodiesel. However, producing biodiesel from virgin oil is not commercially feasible to satisfy the need of the transportation due to the cost and dilemma of food or energy.

On the other side, there are potential sources in oil and leather industry wastes for the production of biodiesel. Producing biodiesel from these wastes has a dual purpose by reducing environmental pollution and value added products.

Different researchers have investigated the potential of animal fats and waste oils for biodiesel production [4, 7, 8]. Therefore, the purpose of the present study was to optimize parameters for biodiesel production from low cost goat fleshing oil feed stock through performing extensive experiments on the oil extraction, hydrolysis and trans esterification reactions throughout the production process.

1.2. Statement of the problem

Since the mid of 19th century when fossil fuel came into exploration for energy generation, the need for more energy and fossil reserve depletion in the 21st century forced to the search of new

energy sources. In 2012, the global petroleum consumption was estimated as 89 million barrels per day from which about a half was used for gasoline production. At this rate of consumption, the oil resources are predicted to run out within the next 50 years[9]. The price fluctuation and reserve depletion of fossil fuel, rejuvenate the use of biomass as energy source of material. These biomass resources are either generated from plant or animals.

Thermo-chemical-based biodiesel obtained from wastes can reduce carbon release by 90 % compared to the 75 % reduction offered by usual biodiesel [10].

Although tannery industry is built upon the waste from slaughter house and can be considered as one method of waste management and contribute to the national GDP but the solid, liquid and gaseous wastes produced from tannery are posing serous human health and environmental problems. These days waste is not something to be disposed off or kept out of sight. There is a search for use, reuse and recycling of wastes and changing waste to wealth via tireless research efforts. Production of biodiesel from leather industry fleshing wastes is therefore one of these attempts.

Ethiopia imports petroleum with foreign currency which is earned from agricultural products. The demand for petroleum fuel is rising rapidly due to the growing economy and increasing infrastructures. Import of fuel grows annually by 26.6 percent and reached USD 1.7 billion. The share of fuel import in total imports of goods was 16 percent and 20.1 percent in the years 2009/10 and 2010/11 respectively (Annual report of National Bank of Ethiopia, 2010/11). During the fiscal year 2012/13, a total of about 2.2 million metric tons of petroleum products worth Birr 38.7 billion were imported. The total value of imports was increased by 5.5 percent as compared to the performance in fiscal year 2011/12 (Annual report of National Bank of Ethiopia 2012/13).

Having high capacity of animal population and thereby huge potential of tannery work which will produce vast amount of fleshing waste which has high potential for biodiesel production, undertaking research to manage the waste and producing value added products is crucial.

Usage of expensive substrates for biodiesel production keeps the cost higher. Low-cost feedstock such as waste animal fat can make economically viable the production of biodiesel [11]. Although animal-derived fats have gained considerable attention across the world for the

production of biodiesel, sufficient studies have not been conducted in Ethiopia to attract leather sector towards biofuel generation. This study aims to provide valuable information concerning biodiesel production using low-cost, leather industry byproducts.

Management of industrial wastes is top priority agenda for developing countries like Ethiopia. The primary issue being waste minimization through conversion to valuable products, therefore, this research will focus on conversion of leather industries waste to biodiesel as an extra source of biodiesel for the country.

The conversion of low quality residual animal fats into commercial grade biodiesel could help to reduce environmental contamination besides contributing to overcome the fuel crisis [12].

Since many researchers have developed conceptual frame work on biodiesel production from waste animal fat, this research, aimed at optimization of parameters for biodiesel production from leather fleshing wastes specifically on goat fleshing oil.

1.3. Objectives

1.3.1. General objective

- Optimization of biodiesel production from leather industry fleshing wastes using homogenous base catalyzed method and characterization of the oil and biodiesel.

1.3.2. Specific objectives

i. To undertake physical characterization of the leather fleshing waste.
ii. To determine the crude oil yield from the leather industry fleshing wastes.
iii. To undertake physicochemical and compositional analysis for the extracted oil
iv. To define optimum conditions/ parameters / i.e. Alcohol to oil ratio, catalyst concentration and reaction temperature for maximum conversion using response surface composite central design.
v. To determine the fuel physicochemical properties such as acid value, saponification number, density, kinematic viscosity, cetane number, heating value, cloud point, iodine value of the biodiesel.

1.4. Significance of the study

There is strong scientific evidence that the average temperature of the earth's surface is rising, it is attributed to the increased concentration of carbon dioxide (CO_2), and other greenhouse gases (GHGs) in the atmosphere released by burning fossil fuels [13, 14]. This global warming will eventually lead to substantial changes in the world's climate, which will, in turn, have a major impact on human life and the environment. Reduction of GHG release to the atmosphere can be achieved by minimizing the energy demand, by rational energy use, use of efficient technologies, by recovering heat and the use of more green energies. This study is a step towards achieving this goal.

The growing environmental concerns due to increasing carbon dioxide emissions, global warming, declining petroleum reserves and rising crude oil prices have resulted in worldwide attention to biodiesel. With growing human population, more land is needed to produce food for human consumption, which poses a potential challenge to biodiesel production. Leather industry fleshing waste could way-out of feedstock for biodiesel production.

This study will enable to know the optimum operating conditions for biodiesel production from leather industry fleshing wastes and fill the gap in solid waste management with in leather production factories. The significance goes far beyond waste minimization to energy production. Furthermore the study will serve as a reference material for researchers, academicians, policy makers, leather industry owners and managers in the management of fleshing wastes. Since conversion of waste to energy technology is not common in developing countries including Ethiopia the study will contribute to fill the gap in this regard.

2. LITERATURE REVIEW

2.1. Introduction

Biodiesel, a fuel comprised of mono-alkyl esters of long chain fatty acids derived from vegetable oils, animal fat, and mixtures thereof, is produced by transesterification, with glycerol which has

many uses being produced as a byproduct. Due to the reserve depletion and increasing price of petroleum together with environment pollution caused by the combustion of fossil fuels, the search for alternative fuels has gained much attention [15]. Biodiesel (fatty acids methyl ester, FAME) can be derived from the trans esterification of triglycerides (the main component of vegetable oils or animal fats) with a short chain alcohol (mainly methanol). It has become popular as a possible alternative to fossil fuels. The main advantages of this fuel are renewability, cleanness and that its properties and performance are similar to conventional diesel fuels [16].

2.2. Leather processing

Producing different types of leather requires different types of processes and chemical usage. Consequently, leather wastes generated from each type of leather and process have different characteristics. For the utilization of these wastes in various fields, having more specific information about their characteristics has great importance. It is possible to divide leather processing into four main stages. It is known that processing 1 ton of wet salted hide yields only 200-250 kg leather and 25% of becomes chrome leather waste, and remainder becomes non tanned waste or is lost in waste water as fat soluble protein and solid suspended pollutants [17]. In order to reduce the environmental impact of leather processing, it is necessary to find a solution for the solid and liquid wastes generated from the industry. Raw hide Trimmings, Green Fleshings, Limed Fleshings and Trimmings are the most important non tanned wastes generated during pretanning operations. These wastes are the good source of raw material for the production of biodiesel.

2.2.1. Beam house processes:

The conserved hides are first subjected to a trimming process for removing the unwanted parts, and then they are soaked to restore the lost water and to remove substances like dirt, blood and conservation salt. After the wetted hides are fleshed they are treated with an intense alkali solution of lime (Ca (OH) $_2$) and sodium sulfide (Na_2S) to ensure hair and wool removal (unhairing process). Later the hides are swelled up in liming process by immersing them in a strong alkaline bath so as to open up the collagen structure. The hides may be treated with

a second fleshing process after liming in order to clean the flesh. At this stage, hides are treated with splitting Process and split into two or three layers.

Through bating process, hides are exposed to an enzymatic effect for both opening up the structures, and the removal of unwanted proteins from the hide. Following the bating process, a degreasing process is applied to hides for removing the excess natural fat in their structure and providing a homogeneous distribution of the fat in it.

Raw Trimmings

Trimming is one of the primary operations in a tannery, which involves removal of neck, leg, and tail pieces from hides/skins to provide a shape to the final leather. The quantities of trimmings are estimated to be around 8-10% (on raw weight) depending on the nature of the animal. The raw trimmings are the good source of raw material for the production of edible grade gelatin for human consumption.

Green fleshing

In some tanneries, the raw hides undergo fleshing operation yielding green fleshing and fatty tissues. This operation is carried out before washing and soaking. The yield of green fleshing is estimated to be 10% on the weight of the raw hide. Since these fleshing are not contaminated with chemicals they are suitable for the production of gelatin for human consumption.

Limed Fleshing

Limed fleshing is obtained while scraping out the limed hides and skins either by hand or by fleshing machines. The fleshing are proteinaceous in nature comprising cutaneous muscle layers and sub cutaneous adhering tissues which are not required in the subsequent operations of leather manufacture. The availability of limed fleshing is 35% on the wet weight of the raw hides (70% moisture). The limed fleshing can be utilized for the production of glue.

Limed Trimmings

After the fleshing operation, the hides, skins and sides are trimmed in order to remove the ragged and torn edges. These trimmings are proteinaceous in nature and found to be good raw material for glue. The limed trimmings are 2-7% on the wet weight of the hides.

2.2.2. Tanning Processes:

The hides at this stage are first treated with pickle process in a solution composed of salt and acids so as to obtain a homogeneous distribution of tanning materials through the cut. After the hides are conditioned as above, the tanning process is applied with various tanning materials (materials able to form stable bonds with collagen) in order to provide the leather with a stable form and high thermal stability. Tanning materials such as vegetable tannins, mineral tanning materials and syntans (synthetic organic tanning materials) are used in tannage. Among mineral tanning materials, chrome is the most widely used in leather production due to its unique features that it gives to the leather. Aluminum and vegetable tanning materials are also widely used in leather production. Before the leathers are treated with further processes, the setting out and samming process is applied, and shaving is done to obtain the desired thickness of the leather.

2.2.3. Post-Tanning Processes:

The next step for the leathers, which are tanned and standardized to a desired thickness, is re-tanning process with various retanning agents improving the requested characteristics of products. In this process, the structural differences within leathers are compensated to obtain uniform structure. The Fat liquoring process is applied by using a combination of various fat liquoring agents in order to allow the leather to be more supple and softer. In the dyeing process leathers are dyed to the desired color. After this stage, leathers are hanged and dried, and they are prepared for the finishing process through certain mechanical operations. The unwanted parts are trimmed and removed.

2.2.4. Finishing Processes:

After the leathers are fat liquored and dyed following the tanning process, they are processed with a series of coatings on the surface in order to improve their resistance and produce appealing and uniform surface effects. After this process, leathers are trimmed for a final form and sent to confection. Solid wastes generated by the leather industry in these stages of processes may be classified as follows.

i. wastes from untanned hides/skins (trimmings, fleshing wastes)
ii. Wastes from tanned leather (shaving wastes, buffing dust)

iii. Wastes from dyed and finished leather (trimmings from leather)

Data obtained from research reveals that 80% of solid wastes are generated during pre-tanning processes, while 20% of the wastes are caused by post-tanning processes[18].

2.3. Characterization of leather fleshing wastes

The variety and quantity of solid wastes depends on animal species, breeding conditions, slaughterhouse practices, conservation conditions, leather process stages, mechanical operations, qualification of the personnel, and chemicals used in processes. Yet this fact causes uncertainties in reusing the generated wastes. First of all, solid wastes should be characterized so that they can be reused.

Table 2.1.Mean values of some characteristics of limed leather fleshing wastes.

Type of limed fleshing waste	% Water content	pH	Fats & oils %	Nitrogen %	Salt %	SO_2 (ppm)	Calorific value Kcal/kg
Sheep	61.65	12.5	53	13.1	1.77	439	4852
Goat	83.72	10.06	25	25	0.87	243	4916
Hide lime	57.13	12.5	6.5	25	2.84	65	5450

(Adapted from, Ozgunay [19])

2.3.1. Water Content

Water content of solid wastes differs to a great extent in accordance with how and where they are collected, storage conditions, and climate. Due to the fact that fleshing wastes are generated during leather process steps called beam house processes, in which leathers are processed in watery environments where fleshing wastes have higher amounts of water.

2.3.2. pH

The pH of the fleshing wastes show similarities in parallel with the process stages disclosing the pH values of solid wastes, and the pH values at which these processes are carried out; and it was found that liming fleshing waste has the highest pH value, since the liming process is done

at around pH 11-12. In order to protect the current ecosystem, wastes should be subjected to a neutralization process before they are disposed to the environment.

2.3.3. Fat content

According to Bienkiewiczk [20] fat content varies between 0.5% and 4% in cattle and horse hides, 3% and 30% in sheep skin, 4% and 40% in pig skin, and 3% and 10% in goat skin over dry weights. Another fat source in solid wastes is the natural or synthetic fats used in the fat liquoring process in order to provide the leather with features like softness, elasticity and resistance, and to allow the leather fibers to slide easily over one another depending on the leather type and purpose of use. The fat content of leathers consists of the remaining natural fat after degreasing process, and the fat used in fat liquoring process. Due to this reason, different fat proportions may be detected in leather wastes. Pre-fleshing and lime fleshing wastes from the processes before degreasing include higher amounts of fat compared to other waste samples (40-70%). In addition, it should be kept in mind that wastes were generated at the end of the fleshing process that was applied to remove the hypodermis layer of the hide, which is the layer including the highest amount of fat. It should also be taken into consideration that flesh and fat layers left on hides may differ greatly, depending on slaughter and flaying techniques, which in turn directly affects the fat amount in waste.

The fat obtained from lime fleshing waste is generally regarded to be of low quality due to its dirty color and bad smell resulting from sulfide contact.

2.3.4. Thermal Values

Due to their high content of fat, fleshing wastes with an average of 5872 kcal/kg thermal value have higher thermal values than that of other wastes with an average of 4493 kcal/kg [19]. Thermal treatment of leather scrap must be justified not only by its high calorific value, but by taking into account as much as possible of the consequences of it, namely (i) pollutants and their levels in the released gasses; (ii) characteristics of the ashes and factors that influence changes in the chromium oxidation state during the burning process as well as in some of the further ash treatment options [21].

2.4. Biodiesel production

2.4.1. Pretreatment

The high FFA acid content of the waste animal fat renders it inadequate for the most traditional direct one-step base-catalyzed transesterification process to biodiesel due to soap formation. However, an indirect multistep process allows the use of these feed stocks with high FFA concentrations by first carrying out an acid-catalyzed esterification of the FFA before the base-catalyzed triglyceride trans esterification.

Moreover, the low quality of the animal fat used as feedstock as source of energy influenced with problems like higher nitrogen and sulfur contents of the animal fat indicate that still some protein and phosphoglycerides (usually called gums) remain in the feedstock; the phosphoglycerides are essential constituents of the animal cell membranes and they concentrate in the lipids fraction. For this reason, a degumming process to eliminate the phosphoglycerides has been proposed as the first step of this multistep process.

The esterification of the FFA was carried out at different temperatures, catalyst concentration and alcohol to oil molar ratio with stirring (750 rpm) for a period of one hour. Among the acids of choice to catalyze this esterification of the FFA, concentrated hydrochloric acid (ca.35 wt. %) would introduce an undesirable amount of water in the reaction.

2.4.2. Transesterification

There are usually three main steps for biodiesel production from waste oils including pretreatment, transesterification, and separation processes. Some types of oil usually needs pretreatments to reduce the viscosity and the content of water and free fatty acids (FFAs) which can influence trans esterification process negatively [22]. Even by carrying out a pretreatment on used oil, it is still more economical and can reduce the direct production costs in comparison with pure oils [23].

Transesterification is an equilibrium reaction which takes place in three consecutive steps. It is usually affected by variables such as molar ratio, temperature, catalyst type and catalyst amount, reaction time etc. [24]

Pretreatment is the first step in biodiesel production from waste oils in order to remove the impurities and solid particles or decrease the water content and acidity value [25]. The required

pretreatments in most cases are filtration and heating; however, pretreatments such as steam injection, neutralization, vacuum evaporation, degumming, esterification and vacuum filtration can be applied as well.

In the second step, transesterification reaction should be performed in which glycerides are converted into esters in the presence of a catalyst and an alcohol. However, in this step, using non-catalytic conversion techniques are also possible. In fact, each mole of lipid reacts with two or three moles alcohol to produces the respective alkyl esters (biodiesel) and a mole glycerol [26].There are three basic chemical processes used to produce biodiesel from oils and fats industrially; base catalyzed trans esterification of the oil with alcohol usually methanol, direct acid catalyzed esterification of the oil with methanol or conversion of the oil to fatty acids (acid pretreatment) and then to alkyl esters with acid catalysis.

Generally biodiesel is generated through transesterification of animal fats, which can be classified into catalyzed (using various chemical catalysts), enzymatic methods and non-catalyzed (supercritical alcohol conditions) reactions. The fats/oils are chemically esterified with an alcohol such as methanol or biochemically with an enzyme [27]. Based on the reaction state esterification process categorized into homogenous, heterogeneous, enzymatic and non-catalyzed systems [28]. One-step and two-step trans esterification reactions are applied to feedstock with low and high acid numbers, respectively [29].

Homogeneous reactions generally involve alcohol: fat molar ratio of 6:1, catalyst concentration of about 1% and reaction temperature of 60 °C for 1 h. Sulfuric acid constitutes the most commonly used acid catalyst whereas both KOH and NaOH are typically used as base catalysts for homogeneous transesterification[29]. Heterogeneous transesterification involves comparatively high catalyst concentration and reaction temperature with longer reaction time than its homogeneous equivalent.

Regardless of being a time-consuming procedure, enzymatic transesterification can be conveniently used for the processing of feedstock having high acid number without acid pre-treatment. Supercritical transesterification is considered as economically prejudicial due to involvement of extremely high temperature and pressure conditions. Although both homogeneous as well as heterogeneous transesterification methods are currently being applied

for the successful conversion of animal fats into biodiesel, introduction of novel techniques using ultrasonic irradiation, microwaves, enzymes and radiofrequency heating will further extend the global biodiesel production from animal-derived byproducts.

In general, depending on the presence of the catalyst in the process, there are two methods of transesterification of TAGs into biodiesel: catalyzed and non-catalyzed. According to the type of catalyst employed, the reactions can be classified into three groups: homogeneously-, heterogeneously- and enzymatically catalyzed. The transesterification reactions are conventionally performed using homogeneous base or acid catalysts. Different groups of heterogeneous catalysts such as: metallic, solid bases, solid acids and natural catalysts can be also applied for efficiently converting oil or fat to ester [30].

2.4.3. Catalysts for Biodiesel Production

Biodiesel is produced commercially using homogenous basic catalysts such as sodium or potassium hydroxide or methoxide because the trans esterification reaction is generally faster, less expensive, and more complete with these materials than with acid catalysts[31].

The biodiesel industry currently uses sodium methoxide, since methoxide cannot form water upon reaction with alcohol such as with hydroxides. Other alkoxides, such as calcium ethoxide, have also effectively catalyzed the biodiesel production, albeit with higher methanol and catalyst requirements [32]. Furthermore, base-catalyzed reactions are performed at generally lower temperatures, pressures, and reaction times and are less corrosive to industrial equipment than acid-catalyzed methods. Therefore, fewer capital and operating costs are incurred by biodiesel production facilities in the case of the base-catalyzed transesterification method [16].

However, the homogenous acid-catalyzed reaction holds an important advantage over the base-catalyzed method in that the performance of acid catalysts is not adversely influenced by the presence of FFA. A wide range of catalysts may be used for biodiesel production, such as homogenous and heterogeneous acids and bases, sugars, lipases, ion exchange resins, zeolites, and other heterogeneous materials.

In general, acids are more appropriate for feed stocks with high FFA content. Homogenously catalyzed reactions generally require less alcohol, shorter reaction times, and more complicated purification procedures than heterogeneously catalyzed transesterification reactions.

Heterogeneous lipases are generally not tolerant of methanol, so production of ethyl or higher esters is more common with enzymatic methods.

Feedstock quality dictates the catalyst media or process is needed to produce FAAE that satisfies relevant biodiesel fuel standards such as ASTM D6751 or EN14214. If the feedstock contains a significant percentage of FFA (>3 wt.%), typical homogenous base catalysts such as sodium or potassium hydroxide or methoxide will not be effective as a result of an unwanted side reaction in which the catalyst will react with FFA to form unreacted TAG, soap, DAG, MAG, biodiesel, glycerol, water, and/or methanol [33].

2.5. Leather Industry Fleshing Waste a challenge and opportunity

Solid wastes created from leather industry, constitutes about 56-60% of the pre-tanned solid wastes [34]. Disposal of leather industry fleshing wastes to the environment may harbor pathogenic microorganisms resulting in environmental pollution and health risks. Therefore, especial attention is given to these wastes on handling and converting to value added materials. Leather industry which plays a crucial role in the country's economy, contributes to environmental pollution by dumping the solid wastes into the land and the water bodies. Even though disposal methods such as landfill disposal, thermal incineration and anaerobic digestion are available, they again contribute to pollution and cause additional economic burden to the tanners.

One way to recover the leather industry wastes is using them as feedstock in biodiesel production due to their rich fat content [7]. Consequently, the pollution coming from the leather industry wastes can be reduced dramatically and more value added products can be obtained by converting them to biodiesel.

2.6. Correlation between chemical composition and physical properties of biodiesel

The fatty ester composition, along with the presence of contaminants and minor components, dictates the fuel properties of biodiesel fuel. Because each feedstock has a unique chemical composition; biodiesel produced from different feed stocks will in turn have different fuel properties. Important properties of biodiesel that are directly influenced by fatty ester

composition and the presence of contaminants and minor components include low-temperature operability, oxidative and storage stability, kinematic viscosity, exhaust emissions, cetane number, and energy content. In the context of biodiesel, minor components are defined as naturally occurring species found in vegetable oils and animal fats and may include tocopherols, phospholipids, steryl glucosides (also called sterol glucosides, steryl glycosides, sterol glycosides, or phytosterols), chlorophyll, fat soluble vitamins, and hydrocarbons (such as alkanes, squalene, carotenes, and polycyclic aromatic hydrocarbons). Contaminants are defined as incomplete or unwanted reaction products, such as free fatty acid, soaps, triacyl glycerol, diacyl glycerol, monoacyl glycerol, alcohol, catalyst, glycerol, metals, and water.

2.6.1. Specific gravity

Density is a key fuel property, which directly affects the fuel performance, as some of the engine properties, such as cetane number, heating value and viscosity of biodiesel. The density of the fuel also affects the quality of atomization and amount of fuel injected into the combustion chamber and thus, the air-fuel ratio. This is because fuel injection pumps meter fuel by volume not by mass and a denser fuel contains a greater mass in the same volume. Densities of biodiesel fuels are generally higher than those of petro diesel and are dependent on fatty acid composition and purity. Since density is strongly influenced by temperature, the quality standards state the determination of density at 15°C. Density limits in the European EN norm are in the range of 860-900 kg/m^3. Contamination of the biodiesel significantly affects its density; therefore, density can also be an indicator of contamination in the fuel. Experimental data showed that biodiesel density decreased with increasing unsaturation levels of FAMEs [35].

2.6.2. Low-temperature operability

Low-temperature operability is normally determined by three common parameters: cloud point, pour point, and cold filter plugging point (CFPP).

The cloud point (CP) is defined as the temperature at which crystal growth is large enough (diameter≥0.5μm) to be visible to naked eye. At temperatures below the CP, larger crystals fuse together and form agglomerations that eventually become extensive enough to prevent pouring of the fluid.

The lowest temperature at which the fluid will pour is defined as the pour point (PP). The CFPP is defined as the lowest temperature at which a given volume of biodiesel completely flows under vacuum through a wire mesh filter screen within 60 s. The CFPP is generally considered to be a more reliable indicator of low-temperature operability than CP or PP, since the fuel will contain solids of sufficient size to render the engine inoperable due to fuel filter plugging once the CFPP is reached. It should be noted that it is inappropriate to measure CP, PP, and CFPP of chemically pure compounds (pure methyl oleate, for instance). Instead, determination of melting point (MP) as a means to measure low-temperature operability is appropriate for chemically pure compounds. The CP, PP, CFPP, and LTFT attempt to quantify low-temperature behavior of complex mixtures (such as biodiesel) that are composed of constituents that generally exhibit a relatively wide range of melting point.

The low-temperature behavior of chemical compounds is dictated by molecular structure. Structural features such as chain length, degree of unsaturation, orientation of double bonds, and type of ester head group strongly influence MP of individual chemical constituents of biodiesel. As the chain length of saturated fatty acid alkyl ester (FAAE) increases, a corresponding increase in melting point is observed. With respect to unsaturation, compounds of similar chain length but increasing levels of unsaturation display lower melting point.

The orientation of the double bond(s) is another important factor influencing melting point. Nearly all naturally occurring unsaturated fatty acids contain cis-oriented double bond. However, trans isomers may be chemically introduced through catalytic partial hydrogenation. Constituents that contain trans double bonds generally exhibit higher melting point than the corresponding cis- isomers.

With respect to ester head group, the melting point of FAEE is generally lower with larger alkyl head groups up to about eight carbons.

2.6.3. Oxidative stability

Oxidative stability of biodiesel is determined through measurement of the oil stability index (OSI) by the Rancimat method (EN 14112). The Rancimat method indirectly measures oxidation by monitoring the gradual change in conductivity of a solution of water caused by volatile oxidative degradation products that have been transported via a stream of air (10 L/h) from the

vessel (at 110°C) containing the biodiesel sample. The OSI is mathematically determined as the inflection point of a computer-generated plot of conductivity (μS/cm) of distilled water versus time (h). The units for OSI are normally expressed in hours. Biodiesel fuels with longer OSI times are more stable to oxidation than samples with shorter values.

Oxidation initiates in the case of lipids at methylene carbons allylic to sites of unsaturation [36]. Autoxidation of lipids, including biodiesel, produces free radicals through hydrogen abstraction in the presence of various initiators such as light, heat, peroxides, hydro peroxides, and transition metals. These free radicals further react exothermically with molecular oxygen to produce peroxides, which react with un oxidized lipids to produce additional free radicals [36]. Generally, the rate-limiting step in the autoxidation of lipids is initial hydrogen abstraction. The rate of autoxidation is dependent on the number and location of methylene interrupted double bonds contained within FAAE. Materials that contain more methylene carbons allylic to sites of unsaturation (such as polyunsaturated esters) are particularly vulnerable to autoxidation.

Structural features such as degree of unsaturation, double bond orientation, chain length, and type of ester head group influence the OSI of FAAE. Esters that are otherwise similar but contain a greater number of methylene interrupted double bonds undergo oxidative degradation at progressively faster rates [37]. Consequently, superior oxidative stability is expected from biodiesel fuels prepared from feed stocks relatively high in saturated fatty acid content or relatively low in polyunsaturated fatty acid content.

Oxidative stability and low-temperature operability are normally inversely related: structural factors that improve oxidative stability adversely influence low-temperature operability and vice versa. Ester head group is the only exception to this relationship, as larger ester head groups tend to improve both low-temperature performance and oxidative stability.

2.6.4. Kinematic viscosity

Kinematic viscosity is the primary reason why biodiesel is used as an alternative fuel instead of neat vegetable oils or animal fats. The high kinematic viscosities of vegetable oils and animal fats ultimately lead to operational problems such as engine deposits when used directly as fuels. The kinematic viscosity of biodiesel is approximately an order of magnitude less than typical vegetable oils or animal fats and is slightly higher than petro diesel.

Kinematic viscosity is determined following ASTM D445 at 40°C. Several structural features influence the kinematic viscosities of FAAE, such as chain length, degree of unsaturation, double bond orientation, and type of ester head group. Factors such as longer chain length and larger ester head group result in increases in kinematic viscosity. Increasing the degree of unsaturation decreases kinematic viscosity. Kinematic viscosity at 40°C is the parameter required by biodiesel and petro diesel standards. In a diesel engine, the liquid fuel is sprayed into compressed air and atomized into small drops near to the nozzle exit. The liquid fuel, usually, forms a cone shaped spray at the nozzle exit and its viscosity affects the atomization quality, size of fuel drop and penetration. The viscosity of the fuel increases with chain length (number of carbon atoms). Fuels with high viscosity tend to form larger droplets on injection which can cause poor fuel atomization during the spray, increases the engine deposits, needs more energy to pump the fuel and wears fuel pump elements and injectors. Kinematic viscosity is the resistance to flow of a fluid under gravity. It is the time taken for a fixed volume of fuel to flow under gravity through the capillary tube viscometer immersed in a thermostatically controlled bath at 40ºC. It is the product of measured time flow and calibration constant of viscometer.

The kinematic viscosity is a basic design parameter for the fuel injectors used in diesel engines. Fuel viscosity has influence on fuel droplet size and spray characteristics. Viscosity is inversely proportional to temperature. Viscosity increases with chain length and degree of saturation. Fuel specification viscosity upper limit ensure that fuel will flow readily during cold starting. Higher viscosity leads to poor atomization, incomplete combustion and increases carbon deposits. More, higher viscosity fuel needs higher pumping power also. Fuels with lower viscosity leaks past plunger through the clearance between plunger and barrel during fuel compression [38]

2.6.5. Flash point

The flash point is the minimum temperature calculated to a barometric pressure of 101.3 kPa at which the fuel will ignite (flash) on application of an ignition source under specified conditions. Flash point helps to monitor the safe handling and storage of fuel. The flash point does not affect the combustion directly; flash point varies inversely with the fuel's volatility. For biodiesel the minimum flash point is 93°C in the United States, 100°C in Brazil and 120°C in Europe. The flash point indicates the residual alcohol present in biodiesel samples. For example, the presence of 0.5% methanol in biodiesel reduces biodiesel flash point from 170°C to 50°C.

2.6.6. Higher heating value (HHV)

The higher heating value (HHV) is an important property defining the energy content and thereby efficiency of fuels, such as vegetable oils and biodiesels. The higher heating value of a fuel increases with increasing carbon number in fuel molecules and also increases as the ratio of carbon and hydrogen to oxygen and nitrogen increases. Higher heating value (HHV) and composition of biomass are important properties which define the energy content and determine the clean and efficient use of these fuels.

The high heating value (HHV) is not specified in the EN 14214 and ASTM D6751 biodiesel standards. However, European standard for using biodiesel as heating oil (EN 14213) specifies a minimum HHV of 35MJ/kg. The HHV increases with increasing chain length and decreases with increasing unsaturation, and it is important for estimating the fuel consumption: the greater the HHV, the lower the fuel consumption.

Biodiesel fuels do not contain aromatics, but they contain methyl esters with different levels of saturation. Unsaturated esters have lower energy content on a weight basis, but due to their higher density, they have more energy per unit volume. The higher heating value (HHV) is an important property defining the energy content and thereby efficiency of fuels, such as oils and biodiesels. The HHV of biodiesel is approximately 10% less than that of petrodiesel. There exists a variety of correlations for predicting HHV from ultimate analysis of fuels. Energy content is an important indication of the suitability of a fuel.

2.7. Limitations in biodiesel production from leather industry fleshing wastes

The production of biodiesel from WAFs has certain limitations and concerns that have to be overcome, such as the presence of proteins, phosphoacylglycerols, water, FFAs, SFAs, pathogens etc. The major technical constraints associated with processing of waste animal fats include the presence of relatively high content of water, free fatty acids, saturated fatty acids, phosphoacylglycerols and pathogens. Desiccation, drying or gravitational settling can be used for the exclusion of excessive humidity. Two-step acid/base transesterification helps to cope with the relatively high concentration of free fatty acids [39]. Compared to vegetable oils, animal fats contain high amount of saturated fatty acids which diminish the efficacy of resultant biodiesel in cold weather. Nevertheless this problem can be overcome by suitable additives or winterization

process [40]. Phosphoacylglycerols can be eliminated from animal fats through degumming process using 60% orthophosphoric acid followed by centrifugation [41]. Pathogenic prions involved in the contamination of biodiesel thereby offering a risk towards the environment and public health could be effectively inactivated via esterification at high temperature catalyzed by sulfuric acid [42].

2.8. Factors affecting biodiesel production yield and quality

The main factors affecting transesterification are the molar ratio of glycerides to alcohol, reaction temperature and time, catalyst, acid pretreatment and mixing intensity. Methanol is the preferred alcohol for biodiesel production because of low cost and easy distinct layer formation between the biodiesel and the byproduct glycerol [43].

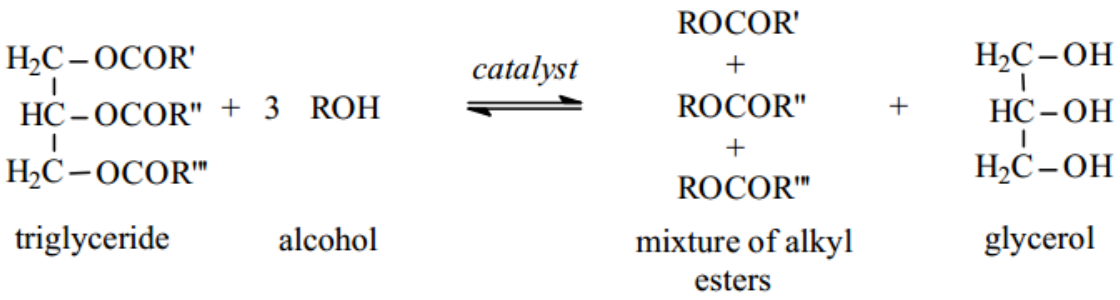


Fig. 2.1 Transesterification reaction

Animal fats are categorized into three distinct types: high quality fat has less than 2% of free fatty acids; medium and low-grade fats exhibit 3-5% and above 5% free fatty acids respectively and can be effectively used for biofuel production. Beef fat, lard, poultry fat and fish oil constitute the chief products of animal origin primarily used for biodiesel production [44]. Feather meal, duck fat, lamb fat and leather industry fleshing wastes can also be used as feedstock to generate biodiesel.

2.9. Advantages and Disadvantages of Biodiesel

Biodiesel has attracted considerable interest as an alternative fuel or extender for petrodiesel for combustion in compression–ignition (diesel) engines. Biodiesel is miscible with petrodiesel in any proportion and possesses several technical advantages over ultra-low sulfur diesel fuel

(ULSD, <15 ppm Sulfur), such as inherent lubricity, low toxicity, derivation from a renewable and domestic feedstock, superior flash point and biodegradability, negligible sulfur content, and lower overall exhaust emissions.

The major disadvantages of biodiesel include high feedstock cost, inferior storage and oxidative stability, lower volumetric energy content, inferior low-temperature operability versus petro diesel, and in some cases, higher NOx exhaust emissions [37]. Many of these deficiencies can be mitigated through cold flow improver and antioxidant additives, blending with petro diesel and/or reducing storage time [45].

Additional methods to enhance the low-temperature performance of biodiesel include crystallization fractionation [46] and transesterification with long or branched-chain alcohols. Strategies to improve the exhaust emissions of biodiesel include various engine or after-treatment technologies such as selective catalytic reduction (SCR), exhaust gas recirculation (EGR), diesel oxidation catalysts, and NOx or particulate traps.

2.10. Food versus fuel

By means of continuous efforts to produce biodiesel from non-food sources, it has been established that fats produced as a waste in leather industries offer a promising feedstock. The raw material of animal fat derived biodiesel is rejected for food, so its use for biodiesel production offers an added environmental perspective. It can avoid disposal problem in leather industries by minimizing the environmental impact associated with the accumulation of these residues. The economics of biodiesel production totally depend upon the cost of utilized feedstock. Therefore high vegetable oil prices are very unfavorable, whereas non-food, waste animal fats could solve the problem of the biodiesel industry by frequently providing inexpensive and abundant, high-quality feedstock.

3. MATERIALS AND METHODS

The leather fleshing wastes were collected from Addis Ababa Tannery S.C located in Addis Ababa, Ethiopia as main raw materials. Moisture content of the flesh was 65%. The flesh waste was washed with distilled water so as to remove the impurities. All chemicals used in this study were analytical grade. Sample preparation, extraction of oil, analysis and characterization of oil and its product were done at the Forest Products Research Center of the Ethiopian Environment and Forest Research Institute and at the Chemical and Bioengineering School of the Addis Ababa Institute of Technology.

a/ fleshing waste b/waste soaked in 1% wt. /wt. boric acid c/ chopped dried waste d/oil extraction using Soxhlet

Fig.3.1 Preparation of waste and extraction of oil

3.1. Experimental Setup and Descriptions

The GC-MS analysis was carried out at Ethiopian leather industry development institute (LIDI) Addis Ababa. The pH, high heat value, viscosity, density and ash content were done in school of Chemical and Bioengineering reaction laboratory. All the other analysis was done at Ethiopian forest product utilization research center, Addis Ababa Ethiopia. Soxhlet extraction was used to extract the oil from the flesh (fig.1. d). A weighed amount of flesh waste was charged into the Soxhlet. The heating mantle was heated to the desired temperature of boiling point of the solvent. Then, the extracted oil and solvent i.e. petroleum ether 60-80 ^{o}C was separated by rotator vapor (fig 3.2. b).

a/ oil and extractive b/ separation of oil and extractive using rota vapor

Fig 3.2. Separation of oil and extractive

The concentrated oil was subjected to degumming process to remove the phospholipids with centrifuge model TD_3, at 800 rpm to separate the oil from the mixture. Finally the fleshing oil was washed several times with distilled water and dried to keep it in moisture free container.

a/degumming b/ centrifuging c/gum and oil separation d/ storage of oil in moisture free container

Fig.3.3. removing gum from the crude oil

All the pretreatment and transesterification experiments were performed in a laboratory scale glass reactor. Hot plate magnetic stirrer was used to heat and agitate the reaction mixture. When the crude oil temperature is reached $70^{O}C$, a defined amount of orthophosphoric acid previously dissolved in distilled water was added to the heated oil. The mixture was stirred with magnetic stirrer at 200 rpm for 1h. After cooling the reaction mixture was centrifuged with centrifuge model TD3, 800 rpm for 20 minutes to separate the gum and oil mixture. The degummed fatty acid was further heated to $105^{O}C$ to remove any remaining water. The acid and FFA

values of the oil before and after degumming were determined and recorded. After obtaining the desired FFA level, the transesterification reaction experiment was performed in the same reactor. Vacuum Filter was used for separation of the oils and the biodiesel from impurities.

3.2. Physico-chemical characterization of raw material

3.2.1. pH measurement

The pH of the hide, goat and sheep fleshing waste samples were determined by shaking five gram of sample of solid wastes in 100ml of distilled water for 16 – 24 hours followed by direct measurement of the pH.

3.2.2. Moisture content of the delimed fleshing waste

Moisture content of solid waste is usually expressed as the mass of moisture per unit mass of wet or dry materials. In order to determine the moisture content of the solid wastes, samples of the solid waste was first weighed and put in an oven at 105 ^{0}C for 24 hours. It was kept in desiccators for about 30 minutes and then weighed and recorded. The wet-mass moisture content is expressed as follows.

$$\%\ \text{moisture} = \frac{\text{Weight of dried sample (g)}}{\text{Weight of sample taken (g)}} \times 100\% \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.1)$$

3.2.3. Ash content of limed fleshing waste and volatile organic content

About 1g of the oven dried LFW was taken in a silica crucible. The crucible containing the sample was ignited on the hot plate till the sample gets charred. Then the crucible along with the sample was kept in a muffle furnace and heated at 550-600°C for 4 h. Finally, it was allowed to cool and the ash formed was weighed. The percent of ash and volatile organic contents were calculated using the following formulae

$$\%\ \text{Ash} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample taken (g)}} \times 100\% \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.2)$$

$$\%\ \text{volatile organic content} = \frac{(\text{Weight of sample taken-Weight of ash})(\text{g})}{\text{Weight of sample taken (g)}} \times 100\% \ldots\ldots (3.3)$$

3.2.4. Total fat content of delimed fleshings

The Total Fat content of DFs was determined using A.O.A.C, 2005 method. About 1g of the DF sample was placed in the Soxhlet extraction apparatus and 150 ml of petroleum ether was added and the sample was extracted for 6 hours at the boiling point of the solvent. It was then allowed to cool and the ether was evaporated. The weight of the extraction flask along with the dried extract minus the weight of the empty extraction flask gives the weight of the total fat being extracted.

The % of total fat in de-limed fleshing wastes was calculated using the following formula-

$$\text{Total fat\%} = \frac{\text{weight of the fat (g)}}{\text{weight of sample (g)}} \text{ x } 100\% \ldots\ldots\ldots\ldots\ldots\ldots (3.4)$$

3.3. Physicochemical characterization of leather fleshing oil and biodiesel

The physiochemical properties of the oil before and after pretreatment and after biodiesel production were conducted to identify the effects of the different pretreatments i.e. degumming and acid catalyzed esterification. The physicochemical characterization of the biodiesel produced was performed to compare the results with the EN and ASTMD standards. The physicochemical parameters that have been investigated include specific gravity, kinematic viscosity, acid value, ester value, FFA content, iodine number, high heat value and saponification number.

3.3.1. Determination of specific gravity

The specific gravity of the oils extracted and biodiesel produced was determined by inserting hydrometer into a graduated cylinder containing 500ml samples heated to 25^{O}C.

3.3.2. Determination of kinematic viscosity

Digital sine-wave (SV-10, 2011, Australia) vibrio viscometer was used to determine the viscosity of the oil and the biodiesel. The viscosities were determined at different temperatures. Since the reading of the vibrio viscometer is dynamic viscosity the kinematic viscosity was determined using the following empirical formula.

$$\text{kinematic viscosity} = \frac{\text{dynamic viscosity}}{\text{density of oil}} \ldots\ldots\ldots\ldots\ldots\ldots (3.5)$$

3.3.3. Acid value (Acid Number) determination

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

For acid value determination of oil a titration solution of 0.1N KOH was prepared and stored in brown bottle for five days. Two grams of oil was added to a beaker and heated at 70 ^{O}C for three minutes. Next 20ml of anhydrous ethanol (99.5% W/W), 20 ml of diethyl ether and 5 drops of phenolphthalein were added to the titration beaker containing the 2 gram oil sample. The solution was mixed thoroughly and titrated with 0.1 N KOH by adding a drop at a time until pink color appeared. The acid value was determined by using the following correlation.

$$AV = \frac{M \times N \times V}{W} \quad \text{.....(3.6)}$$

Where N = Normality of KOH

V = volume of titrant i.e. KOH used for titration (ml)

M = Molecular weight of KOH

W = Weight of the oil sample

3.2.1. Free fatty acid (FFA) determination

The free fatty acid of the oil was determined using acid value previously determined using the following equation.

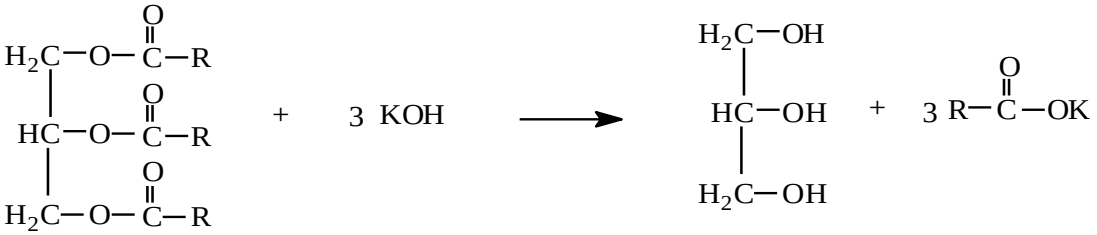
$$\% \text{ FFA} = \frac{AV}{2} \quad \text{.....(3.7)}$$

Where % FFA = percentage of free fatty acid, AV= acid value of the oil

3.3.4. Saponification value determination

The saponification value is the amount of mg of potassium hydroxide required to neutralize the free acids and to saponify the esters in 1 g of the substance. The saponification number is a

measure of the average molecular weight of the triacylglycerol in a sample. Saponification is the process of breaking down a neutral fat into glycerol and fatty acids by treatment with alkali. The smaller the saponification number the larger the average molecular weight of the triacylglycerol present i.e. Saponification value is inversely proportional to the mean molecular weight of fatty acids (or chain length).



Saponification reaction

Twenty-five milliliter of 0.5 ml ethanoic solution of KOH was added to 2 g of previously extracted oil of LFW. The mixture was heated to 70 ^{O}C for 30 minutes in reflex condenser. Then the heated mixture was cooled immediately and 5 drops of phenolphthalein was added for indicator subsequently the sample was titrated with 0.5 mol/l HCl solution. Titration was stopped when color change was observed. The same procedure was also performed using blank level test i.e. without the presence of oil sample in parallel to the above activity. The saponification was then determined by using the following equation.

$$SV = \frac{M \times N \times (Vb - Vs)}{W} \quad \text{.....(3.7)}$$

Where SV= Saponification value, mg KOH/g oil

M= Molecular weight of KOH

N= Normality of HCl solution

Vb =Volume of HCl solution used in blank

Vs = Volume of HCl solution used in the sample

W= Weight of the oil used

3.3.5. Determination of iodine value

The iodine value determination was done by measuring one gram of biodiesel/oil and dissolving it in 10ml of chloroform. 25ml of Hanus solution (which was prepared by dissolving 13.6g of iodine in 400ml glacial acetic acid by adding 6ml of bromine liquid) and allowed to stand in dark place for 30min. Ten milliliter of 15% KI (15g of KI dissolved in100 ml) added to the oil sample and titrated against 0.1N sodium thiosulfate until yellow solution turns in to almost colorless.

$$IV = \frac{MI \times (Vb - Vt) \times N}{Ma} \quad \text{.....(3.8)}$$

Where, IV = Iodine value (g I_2 / 100 g amostra), Vt = volume of sodium thiosulfate solution for the sample (mL), Vb = volume of sodium thiosulfate solution for the blank (ml), N = standard sodium thiosulfate solution concentration (N); Ma= mass of the biodiesel sample (g), MI= molar mass of iodine.

3.3.6. Determination of high heat value

Determination of HHV was conducted using empirical equation available. Therefore, for calculation of the HHVs (MJ/kg) of oils, the following equation was suggested [47].

$$HHV = 49.43 - \frac{(0.041 \times SV) + 0.015 \times IV}{Ma} \quad \text{.....(3.9)}$$

3.3.7. Determination of cetane number (CN)

Cetane number determination was done using empirical formula suggested [7]. The calculation was based on the results from saponification number (SN) and Iodine value (IV) of oils. The CN was calculated as below:

$$CN = 46.3 + \frac{5458}{SV} - 0.225IV \quad \text{.....(3.10)}$$

3.4. Quantification of leather fleshing oil fatty acids

Gas chromatography stands out among other methods of analysis of fatty acids because it allows their easy separation and quantification. However to analyze fatty acids by this technique, it is

necessary to increase the volatility of the fatty acids by converting them in to methyl ester derivatives[48]. The esterification procedure followed was the method described by Bannon et al [49]. Approximately 300 mg each from the sheep, goat and hide fleshing oil were weighed. About 10 ml of KOH in 0.5 mol/L methanol was added to each sample. The mixture was heated under reflux for 3 minutes. Next 10 mL BF_3 (14%) in methanol was added. Then the mixture was heated under reflux for another 3 minutes. After cooling 6 ml heptane and approximately 30 mL saturated NaCl were added and vigorously stirred for 15 seconds. After phase separation, the top phase was collected and 2.5 µL for each samples were injected into the gas chromatographer.

3.5. Experiments

3.5.1. Flesh Waste Treatment and Oil Extraction

One percent (Wt. / Wt.) boric acid was added with sufficient water in order to neutralize the pH. The flesh was soaked with the acid for an hour at atmospheric conditions. Then, the flesh was chopped into smaller sizes and the oil was extracted in a Soxhlet extraction apparatus a little above the boiling point of the extraction solvent. Finally, the oil and the solvent mixture was subjected to rotatory vapor for separation using the boiling point differences. The yield of the fleshing oil was calculated as:

$$\text{Yield}(\%) = \frac{\text{Total weihgt of dried oil}}{\text{Total weight of flesh waste sample}} \text{x} 100\% \quad \text{..... (3.11)}$$

3.5.2. Pretreatment of extracted oil

In this study degumming was used as options of oil pretreatment. Degumming is the pre-treatment of Feedstock for separation of gums, solid, water and free fatty acid. Technically, degumming is referred as an operation of purification of oil, which normally contains impurities in the colloidal state or dissolved in them. In more simple words, degumming is a process of removing the unwanted gums, which will interference the stability of the oil product (like Bio-diesel). In refining, physical and chemical processes are combined to remove undesirable natural as well as environmental-related components from the crude oil. These components comprise for example phosphatides, odors etc. The emulsifying action of phospholipids increases oil losses during alkali refining. Above all gums lead to brown discoloration of oil after heating during deodorization. They are also known causes of accelerated oxidative degredation of oil. Different

degumming processes are carried out to remove phosphatides. The four known categories of degumming techniques are water degumming, acid degumming, enzymatic degumming and membrane degumming. A large part of the phosphatides (gums) can be hydrated quickly and easily. If the extracted oil contains a considerable quantity of gums the oil is subjected to the water degumming process immediately following extraction. In this process, water is added to the oil. After a certain reaction period the hydrated phosphatides can be separated either by decantation (settling) or continuously by means of centrifuges. In this process step a large part of hydratable and even a small proportion of the non-hydratable phophatides are removed.

Dry acid degumming is particularly suit-able for processing oils with low gum contents such as palm oil, coconut oil, palm kernel oil or animal fats. Intensive mixing is implemented following addition of acid to the pre-heated crude oil. The benefits of the dry acid degumming process are high efficiency as a result of low energy consumption, low operation and maintenance costs (sturdy and reliable control system), long service life (the components are acid proof), low investment costs, environmental-friendly as no wastewater or soap stock occur.

The degumming technique employed in this study was chemical degumming using 2% v/v (orthophosphoric acid/oil) and 3% v/v (distilled water/oil) at 70 ^{o}C, 200 rpm mixing intensity for an hour. Then the gum was separated from the oil using a centrifuge at the speed of 800 rpm for 20 minutes. The acid value, FFA, pH and saponification values of the degummed and non-degummed oil were measured. The results obtained were tabulated for comparison.

3.5.3. Transesterification

After degumming pretreatment experimental work that lowered the FFA below 1% the next experimental stage in the production of biodiesel was transesterification. In this study alkali catalyzed transesterification was employed to convert the purified and pretreated oil. The catalyst (KOH) and the alcohol (CH_3OH) were mixed via vigorous stirring. Fifty milliliters of pretreated oil was charged to the experimental reactor. It was heated for 30 minutes at 100 ^{O}C to dehumidify the oil to avoid parallel saponification reaction. The alcohol-catalyst mixture was charged to the reactor containing dehumidified, pretreated oil. The alcohol-catalyst-oil mixture was agitated at 750 rpm using magnetic stirrer for an hour. The temperature of reaction, catalyst to oil weight basis ratio and alcohol to oil molar ratio were varied, to obtain a large range of methyl ester yields. Heating and stirring were then stopped, and the product was allowed to

settle, to allow the two phases to be separated. The top phase consists of biofuel, whereas the lower phase contained a mixture of impurities. The upper layer was purified using distilled water and then dried over anhydrous sodium sulfate (Na_2SO4). In this sense, 0.5 g of anhydrous Na_2SO4 was added for every 100 mL of fatty acid methyl ester, stirred for 15 min, and then was allowed to settle and be decanted. To remove solid traces from biofuel after the purification step, a filtration process was needed. This step was performed with the help of a vacuum pump and a 27-µm diameter filter paper. For each oil sample, three replications were performed. The optimum of each parameter involved in the process was determined while the rest of them remained constant. After each optimum was attained, this value was accepted and considered to be constant during the optimization of the next parameter. Ester yield results (given as percentages) were related to the weight of oil at the start (weight of ester/weight of oil). The biodiesel yield was determined as:

$$\text{Yield}(\%) = \frac{\text{Total weihgt of fatty acid methyl Ester}}{\text{Total weight of oil in the sample}} \text{ x } 100\% \quad \text{..... (3.12)}$$

a/separation of the glycerol and FAME

b/Purified FAME

Fig. 3.4. Separation of FAME from glycerol

Table 3.1: Independent variables used for CCD in transesterification process

Variable	symbol	Coded levels		
		-1	0	+1
Catalyst % (wt. /wt.)	A	0.5	1	1.5
Methanol to oil molar ratio	B	3:1	6:1	9:1
Temperature (^{o}C)	C	55	60	65

Table 3.2. CCD experimental design matrix of transesterification reaction

Std.		**Run**	**Catalyst (% wt. /wt.)**	**Methanol : oil**	**Temperature (^{o}C)**	**% Yield**
4		1	1.50	9.00	55.00	
5		2	0.50	3.00	65.00	
17		3	1.00	6.00	60.00	
13		4	1.00	6.00	55.00	
3		5	0.50	9.00	55.00	
14		6	1.00	6.00	65.00	
19		7	1.00	6.00	60.00	
1		8	0.50	3.00	55.00	
9		9	0.50	6.00	60.00	
7		10	0.50	9.00	65.00	
20		11	1.00	6.00	60.00	
12		12	1.00	9.00	60.00	
16		13	1.00	6.00	60.00	
15		14	1.00	6.00	60.00	
6		15	1.50	3.00	65.00	
11		16	1.00	3.00	60.00	
8		17	1.50	9.00	65.00	
2		18	1.50	3.00	55.00	
10		19	1.50	6.00	60.00	
18		20	1.00	6.00	60.00	

3.6. Experimental Design

Response surface methodology (RSM) is a useful statistical technique which has been applied in research into complex variable processes. It employs multiple regression and correlation analyses as tools to assess the effects of two or more independent factors on the dependent variables. Optimization of transesterification reaction was undertaken. Where the alcohol to oil molar ratio, catalyst amount and reaction temperature were investigated within one hour and 750 rpm mixing intensity. In order to optimize the central composite experimental design (CCD), a three-level-three-factor central composite design was employed in this study, which generates 20 experimental runs. The factors investigated in this study were catalyst concentration, temperature (°C), and oil/methanol ratio. Considering the treatments as in dependent variables, difference in means of each treatment were compared at a significance level of $p < 0.05$ was considered throughout the study.

4. RESULT AND DISCUSSION

4.1. Physical characterization of raw material

The raw material used in this study had unique characteristics, its color was greyish brown with a characteristic pungent odor. The odor decreases markedly after each consecutive water washing and deliming using boric acid. The pH of the hide and sheep fleshing wastes was found to be 10.6 whereas the goat flesh had originally a pH of 12.25. The pH values are attributed to the basicity values of soaking conditions. The tannery fleshing wastes with this high amount of limes should be first neutralized with boric acid. After washing with water to neutralize and wash out the alkalis about 1% (wt. /wt.) boric acid was added with sufficient water in order to neutralize the pH. The flesh was soaked with the acid for an hour at atmospheric condition. Then, the flesh was chopped into small size and dried with the atmospheric air before extraction was commenced. It was possible to reduce the pH of hide and sheep fleshing waste to 6.5 and that of goat to 8.6.

Table 4.1.Physical parameters of liming and Delimed Fleshing wastes

S.N^{o}.	Parameters	Limed fleshing waste			Delimed fleshing waste		
		sheep	hide	goat	goat	hide	sheep
1.	Color	Greyish brown	Greyish brown	Greyish brown	Pinkish brown	Pinkish brown	Pinkish brown
2.	Odor	pungent	pungent	pungent	normal	normal	normal
3.	Moisture Content	63%	58%	82%	61%	60%	80%
4.	Ash content	3.06%	3%	2.8%	2.05%	1.8%	1.6%
5.	Total fat content	NA	NA	NA	23.08%	12.05%	26.7%
6.	pH	10.6	10.6	12.5	6.5	6.5	8.6

Deliming made the fleshings vulnerable for fermentation with different species like probiotic bacterium *Lactobacillus plantarum.* Therefore, the delimed fleshing waste was kept in a refrigerator i.e. controlled environment that does not allow the growth of the bacterium.

4.2. Oil extraction and physicochemical characterization

About 2.5 liter i.e. 2.76 kg of goat fleshing oil was obtained from 10 kg delimed chopped and dried goat leather fleshing waste. The total fat content of DFs was determined using A.O.A.C, 2005 method. It was calculated based on equation 3.4 on page 28.

4.2.1. Physicochemical Characteristics of the Fleshing and Pretreated Oil

The density of the goat fleshing oil was reduced to 906 kg/m^3, viscosity to 57.8 mm^2/s, acid value to 1.13 mg KOH /g of oil, free fatty acid to 0.56 %, at the oil pretreatment stage. The experimental results of the physicochemical characteristics of fleshing oil and pretreated oil are shown in Table 4. In this study biodiesel was produced using goat fleshing oil because on other fleshing wastes two researchers have conducted studies at different times using different methods. The physicochemical parameters are performed to see the effect of pretreatment i.e. degumming for all the three types of leather fleshing wastes.

Table 4.2 Physicochemical characterization of untreated and treated fleshing oil

S.N^o.	Parameters	Unit	Fleshing oil before treatment			Fleshing oil after treatment		
			goat	sheep	hide	goat	sheep	hide
1.	Density @ 298.15 K	Kg/m^3	911	905	900	906	900	895
2.	Water content @ 378.15 K	% mass	0.4	0.3	0.15	0.32	0.24	0.04
3.	Kinematic viscosity @ 313.15 K	mm^2/s	70.8	40.2	30.9	57.8	27.65	21.23

4.	Acid value	mg KOH/g	6.1	3.5	3.25	1.13	2.8	2.7
5.	FFA	%	3.05	1.75	1.63	0.56	1.4	1.35
6.	Saponification value	mg KOH/g	165.6	198.6	191.57	148.1	170.5	171.3
7.	Higher heating value	MJ/Kg	42.2	40.2*	42.43**	42.38	40.86*	43.13**
8.	Iodine value	g/100g	66	74*	80**	65.13	65*	75**

* adapted from Abebe Reda [8]

**adapted from Eshetu Getahun and Nigus Gabiyye [7]

4.2.1.1 Density of the extracted oil

The densities of the untreated and treated fleshing oils were measured by hydrometer and the results of the experiment were recorded.

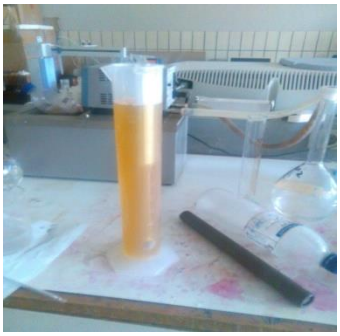


Fig 4.1 Oil density measurement using hydrometer

As depicted in table 3 degumming has reduced the density of the oil. The density of the oil is affected by contamination. It also shows the unsaturation level. Density values of oil samples depend on their fatty acid composition, as well as on their purity. On the one hand, density increases with decreasing chain length and increasing number of double bonds, explaining high values for fuels rich in unsaturated compounds, The higher the unsaturation the higher the

density of fuels. A widely used method for density prediction of vegetable oils was developed by Lund and discussed by Halvorsen et al [50]. The Lund relationship is: sg (298.15 K) = 0.8475 + 0.00030 SV + 0.00014 IV where sg is the specific gravity of oil at 298.15 K, SV is the saponification value, and IV is the iodine value of the oil. The results obtained experimentally are in close agreement to the values calculated by using the suggested correlation. The density of the oil is inversely related to the temperature. Higher density values may increase the required fuel/air mixture, which increases emission of pollutants, including hydrocarbons, carbon monoxide and particulate matter. Low values may favor the formation of a lean air/fuel ratio, leading to loss of engine power and consequent increased fuel consumption Experimental data showed that biodiesel density decreased with increasing unsaturation levels of FAME [35]. The results obtained from this experiment agree with this fact.

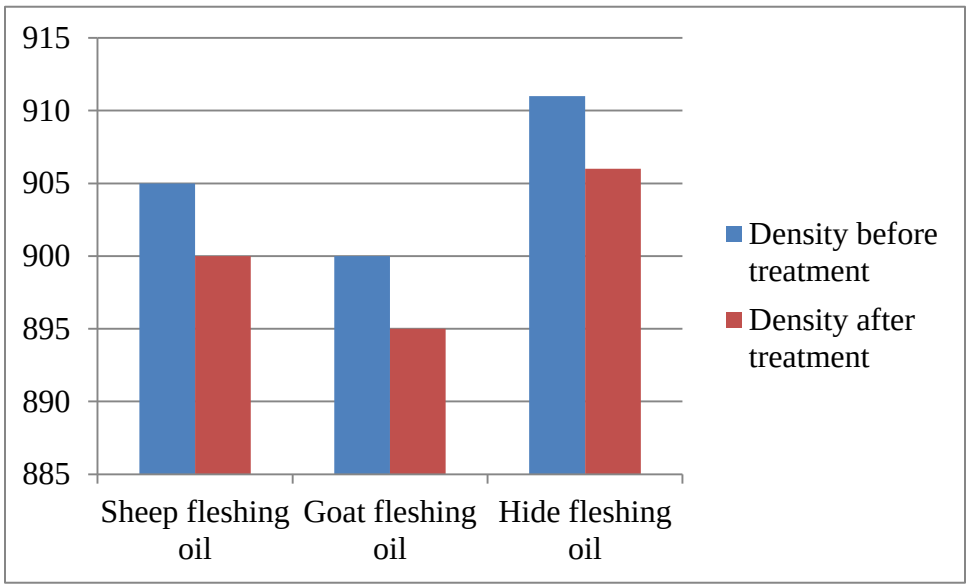


Fig 4.2 Density of the sheep, goat and hide oils before and after Degumming

4.2.1.2. Kinematic viscosity of oil and biodiesel

Viscosity is the measurement of a fluid's resistance to flow defined as the force in required to move a surface one square centimeter in area past a parallel surface at a speed of one centimeter per second, with the surfaces separated by a fluid film one centimeter thick. Laboratory measurements of viscosity normally use the force of gravity to produce flow through a capillary tube (viscometer) at a controlled temperature. This measurement is called kinematic

viscosity. Kinematic viscosity is absolute viscosity of a fluid divided by its density at the same temperature of measurement. It is the measure of a fluid's resistance to flow under gravity, as determined by test method ASTM D 445. To determine kinematic viscosity, a fixed volume of the test fluid is allowed to flow through a calibrated capillary tube (viscometer) that is held at a closely controlled temperature.

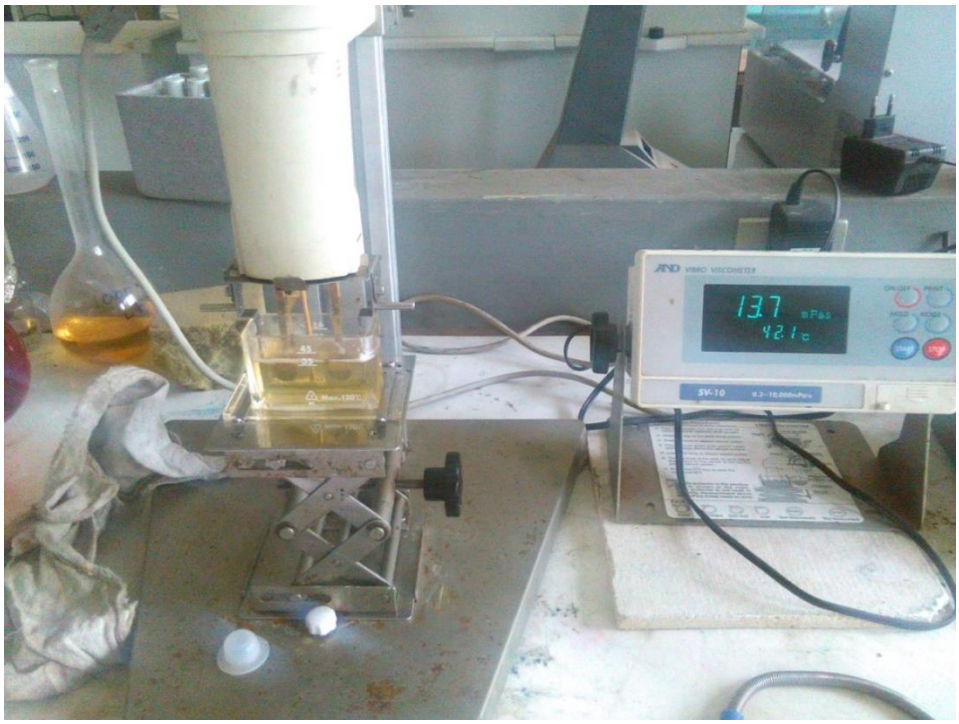


Fig 4.3.Determination of kinematic viscosity using viscometer

The dynamic viscosity of the treated oil and the biodiesel was measured at different temperatures. The kinematic viscosities were calculated using equation 3.5 under section 3.3.2 and the results are presented using graphs on figures 4.4 and 4.8.

Table 4.3. Kinematic viscosity of degummed goat leather fleshing oil at different temperature

Temperature (^{0}C)	30	35	40	45	50	55	60
Specific gravity	0.901	0.895	0.889	0.893	0.881	0.875	0.869
Dynamic viscosity (m. pa. s)	80.9	63.3	51.4	42.5	34.7	28.6	22.5
Kinematic viscosity (mm^2/S)	89.8	70.7	57.8	47.6	39.4	32.7	25.9

As clearly observed from figure 4.4 below temperature and kinematic viscosity are inversely related.

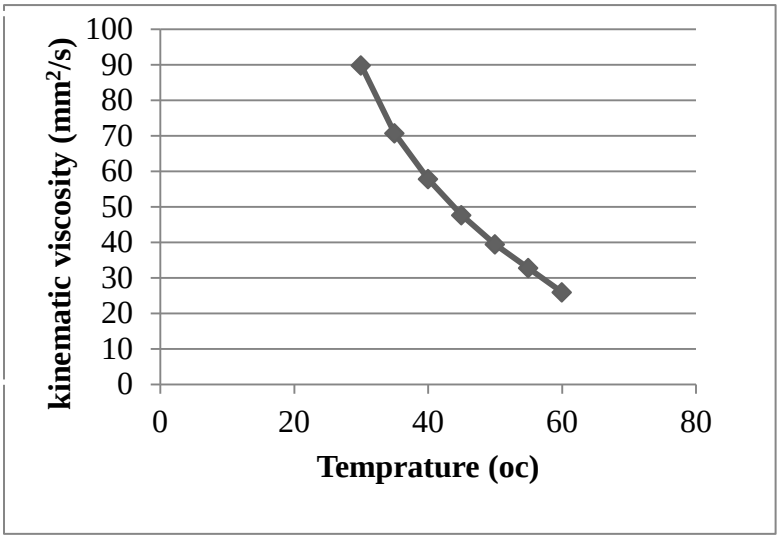


Fig.4.4 Effect of temperature on kinematic viscosity of degummed goat fleshing oil

Table 4.4 kinematic viscosity of goat fleshing biodiesel at different temperature

Temperature (^{0}C)	20	25	30	35	40	45	50	55	60
Specific gravity	0.880	0.875	0.872	0.868	0.865	0.862	0.859	0.850	0.848
Dynamic viscosity (m. pa. s)	8.32	7.06	6.90	5.85	5.18	4.24	3.80	3.70	3.30
Kinematic viscosity (mm^2/S)	9.45	8.06	7.91	6.74	5.99	4.90	4.42	4.35	3.91

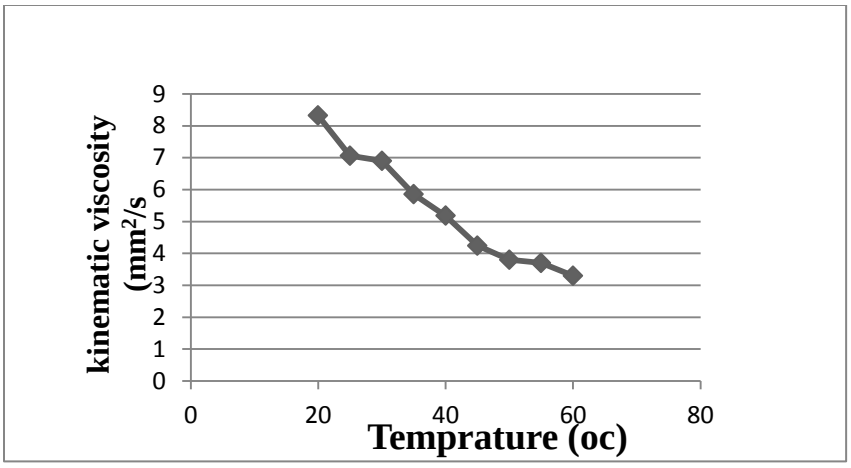


Fig. 4.5 effect of temperature on kinematic viscosity of goat fleshing biodiesel

4.2.1.3. Acid value of the oil

The acid values of the treated and the untreated the goat, sheep and hide fleshing oils were measured in triplicate for each and the average values were taken.

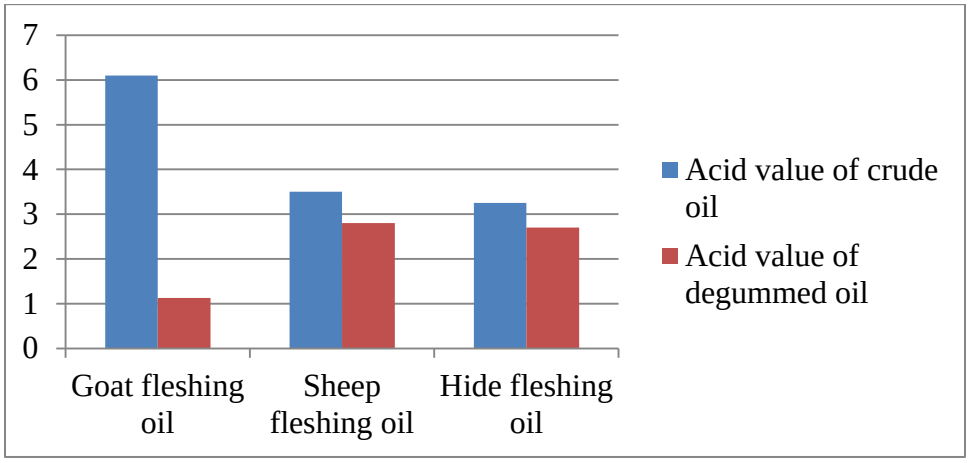


Fig 4.6. Acid values of oils before and after degumming treatment

The following consecutive tables show the acid values of oils of crude and degummed goat, sheep and hide fleshing wastes. It is possible to observe that degumming has reduced the acid values of the crude oils. The acid values and FFA are calculated based on section 3.3.3 using equations 3.6 and 3.7 respectively.

Table 4.5 Acid value of goat fleshing crude oil

Run number	Mass of oil (g)	Titrant (KOH) volume (ml)	Color change	Acid value (mg KOH/g oil)	FFA %
1.	2.044	2.200	Yellow to pink	6.040	3.020
2.	2.016	2.20	Yellow to pink	6.120	3.060
3.	2.050	2.250	Yellow to pink	6.160	3.080
Average	2.040	2.217	Yellow to pink	6.100	3.050

Table 4.6. Acid value of degummed goat fleshing oil

Run number	Mass of oil (g)	Titrant (KOH) volume (ml)	Color change	Acid value (mg KOH/g oil)	FFA %
1.	2.130	0.400	Yellow to pink	1.054	0.527
2.	2.050	0.500	Yellow to pink	1.368	0.684
3.	2.300	0.400	Yellow to pink	0.976	0.488
Average	2.160	0.433	Yellow to pink	1.133	0.566

Table 4.7. Acid value of sheep fleshing crude oil

Run number	Mass of oil (g)	Titrant (KOH) volume (ml)	Color change	Acid value (mg KOH/g oil)	FFA %
1.	2.010	1.300	Yellow to pink	3.628	1.814
2.	2.050	1.200	Yellow to pink	3.284	1.642
3.	2.000	1.280	Yellow to pink	3.588	1.794
Average	2.020	1.260	Yellow to pink	3.500	1.750

Table 4.8 Acid value of degummed sheep fleshing oil

Run number	Mass of oil (g)	Titrant (KOH) volume (ml)	Color change	Acid value (mg KOH/g oil)	FFA %
1.	2.174	1.090	Yellow to pink	2.813	1.406
2.	2.000	0.980	Yellow to pink	2.749	1.374
3.	1.980	1.000	Yellow to pink	2.833	1.417
Average	2.050	1.024	Yellow to pink	2.800	1.400

Table 4.9.Acid value of crude hide fleshing oil

Run number	Mass of oil (g)	Titrant (KOH) volume (ml)	Color change	Acid value (mg KOH/g oil)	FFA %
1.	1.998	1.150	Yellow to pink	3.229	1.614
2.	2.030	1.100	Yellow to pink	3.040	1.520
3.	1.999	1.250	Yellow to pink	3.508	1.754
Average	2.009	1.167	Yellow to pink	3.259	1.629

Table 4.10: acid value of degummed hide fleshing oil

Run number	Mass of oil (g)	Titrant (KOH) volume (ml)	Color change	Acid value (mg KOH/g oil)	FFA %
1.	2.050	0.9	Yellow to pink	2.463	1.232
2.	2.040	1.1	Yellow to pink	3.025	1.512
3.	2.140	1.0	Yellow to pink	2.621	1.310
Average	2.077	1.0	Yellow to pink	2.703	1.352

4.2.1.4. Saponification value of the oils

The procedure followed to calculate the saponification value of the crude and degummed oils is discussed in section 3.3.5. The results obtained are illustrated in the subsequent tables.

Table 4.11. Saponification value of crude goat fleshing oil

Run number	Mass of oil (g)	V_{bHCl} (ml)	Vs_{HCl} (ml)	V_{bHCl} - Vs_{HCl} (ml)	Saponification value (mg KOH/g oil)
1.	2.100	25.5	13.1	12.4	165.6
2.	2.200	25.5	12.7	12.8	169.4
3.	2.098	25.5	13.4	12.1	161.8
average	2.13	25.5	13.06	12.44	165.6

Table 4.12. Saponification value of degummed goat fleshing oil

Run number	Mass of oil (g)	V_{bHCl} (ml)	Vs_{HCl} (ml)	V_{bHCl} - Vs_{HCl} (ml)	Saponification value (mg KOH/g oil)
1.	2.50	25.5	12.3	13.2	148.1
2.	2.50	25.5	11.9	13.6	152.8
3.	2.54	25.5	12.5	13.0	143.4
average	2.47	25.5	12.23	13.27	148.1

Table 4.13. Saponification value of crude sheep fleshing oil

Run number	Mass of oil (g)	V_{bHCl} (ml)	Vs_{HCl} (ml)	V_{bHCl} - Vs_{HCl} (ml)	Saponification value (mg KOH/g oil)
1.	2.000	25.5	11.340	14.160	198.6
2.	1.964	25.5	11.500	14.000	200.0
3.	2.036	25.5	11.200	14.300	197.2
average	2.000	25.5	11.346	14.15	198.6

Table 4.14 Saponification value of degummed sheep fleshing oil

Run number	Mass of oil (g)	V_{bHCl} (ml)	Vs_{HCl} (ml)	V_{bHCl} - Vs_{HCl} (ml)	Saponification value (mg KOH/g oil)
1.	1.99	25.5	13.4	12.1	170.5
2.	2.00	25.5	13.5	12.0	168.3
3.	2.01	25.5	13.12	12.38	172.7
average	2.00	25.5	13.34	12.16	170.5

Table 4.15 Saponification value of crude hide fleshing oil

Run number	Mass of oil (g)	V_{bHCl} (ml)	Vs_{HCl} (ml)	V_{bHCl} - Vs_{HCl} (ml)	Saponification value (mg KOH/g oil)
1.	2.00	25.5	11.50	14.00	196.35
2.	1.95	25.5	12.45	13.04	187.50
3.	2.05	25.5	11.5	14.00	190.86
average	2.00	25.5	11.85	13.65	191.57

Table 4.16.Saponification value of degummed hide fleshing oil

Run number	Mass of oil (g)	V_{bHCl} (ml)	Vs_{HCl} (ml)	V_{bHCl} - Vs_{HCl} (ml)	Saponification value (mg KOH/g oil)
1.	2.02	25.5	13.42	12.08	167.7
2.	1.98	25.5	13.50	12.00	170.0
3.	2.00	25.5	13.00	12.50	175.3
average	2.00	25.5	13.30	12.20	171.0

The effect of degumming on saponification values of the oils can be easily observed using bar graph depicted below.

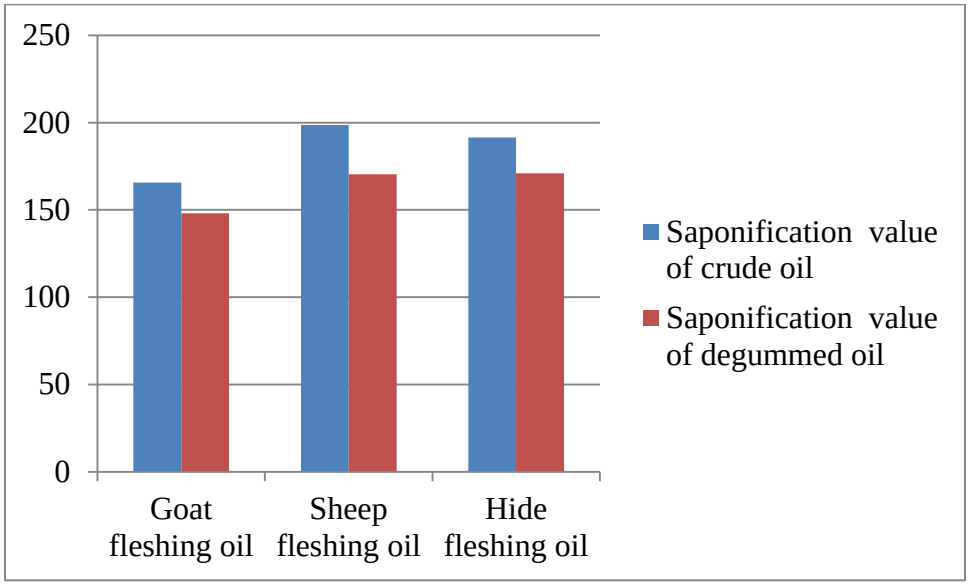


Fig. 4.7.Effect of degumming on saponification value of the oils

4.2.1.5. Iodine values of oil and biodiesel

The iodine values of the crude and degummed oil samples were measured using the method discussed on section 3.3.6 using equation 3.8. The average value of the triplicate result was recorded.

Iodine value is a measure of total unsaturation within a mixture of fatty acid. It is expressed in grams of iodine which react with 100 g of the respective sample when formally adding iodine to the double bonds. The iodine value of a vegetable oil or animal fat is almost identical to that of the corresponding methyl esters [51]. Iodine value is a measure of total unsaturation of fatty acids. The more unsaturation is present in the oil, the higher the iodine value [52]. The lower iodine value i.e. superior oxidative stability of the sheep fleshing oil dictates it to be more preferable for biodiesel production. All the fleshing oils have iodine value limits below the maximum value expressed by EN-norm. This result is similar with the result obtained by Eshetu Getahun and Nigus Gabiyye [7]. The low iodine index indicates less susceptibility to oxidation by oxygen. Decreasing the chain length and/or increasing the number of double bonds (i.e. higher iodine value (IV)) of FAAE results in an increase in NOx emissions [53].

4.2.1.6. HHV of the oils and biodiesel

The high heating value (HHV) is not specified in the EN 14214 and ASTM D6751 biodiesel standards. However, European standard for using biodiesel as heating oil (EN 14213) specifies a minimum HHV of 35MJ/kg. The HHV increases with increasing chain length and decreases with increasing unsaturation, and it is important for estimating the fuel consumption: the greater the HHV, the lower the fuel consumption. Factors that influence the energy content include the oxygen content and carbon to hydrogen ratio. Generally, as the oxygen content of FAAE is increased, a corresponding reduction in energy content is observed.

4.3. Biodiesel production and analysis of effects of parameters

4.3.1. Transesterification process

The transesterification process was carried out using the method described in section 3.6. In this study biodiesel was produced from goat leather fleshing oil using homogenous base catalysis. Since the oil extracted was of high quality grade i.e. significantly low acid value, direct base catalyzed transesterification after degumming was selected to produce the biodiesel. The acid values of the sheep and hide fleshing oils obtained in this study is significantly different from the values reported by Eshetu Getahun, Nigus Gabiyye [7] and Abebe Reda [8]. This variation might be due to the extraction method used. While Getahun and Gabiyye used extractive boiler, Reda used heating and decantation. In this study Soxhlet extraction was employed. Using Soxhlet extraction high quality oils were obtained. Only degumming was enough to reduce the acid values to go for direct transesterification reaction. Fifty milliliters of degummed goat fleshing oil was used for each run.

4.3.2. Statistical analysis

The Design Expert 7.0.0 software was used for the regression and graphical analysis of the data. The maximum values of percentage of yeild of biodesiel were taken as the response of the design experiment for transesterification process. The experimental data obtained by the above procedure was analyzed by the response surface regression using the following second-order polynomial equation.

$$y = B_0 + \sum_{i=1}^{k} B_i X_i + \sum_{i=1}^{k} B_{ii} X_i^2 + \sum_{i>j}^{k} \sum_{j}^{k} B_{ij} X_i X_j \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4.1)$$

Where; y is the response, i and j are the linear and quadratic coefficients respectively, x_i and x_j are the uncoded independent variables, β_0 is the regression coefficient, k is the number of factors studied and optimized in the experiment. Statistical analysis of the model was carried out to evaluate the analysis of variance (ANOVA). The equation was also validated by carrying out confirmatory experiments. The actual results obtained from average values of triplicate of the 20 runs were recorded in table 4.17 below.

Table 4.17: Actual and predicted value of biodiesel yield

Run	Factors			Biodiesel (ml)		% Biodiesel yield		Residuals
	Catalyst (wt. /wt. %)	Methanol to oil molar ratio	Temperature ^0C	Actual	Predicted	Actual	Predicted	
1	1.50	9.00	55.00	38.20	38.230	76.4	76.46	-0.061
2	0.50	3.00	65.00	36.00	35.970	72	71.94	0.059
3	**1.00**	**6.00**	**60.00**	**48.55**	**48.405**	**97.1**	**96.81**	**0.290**
4	1.00	6.00	55.00	45.00	45.015	90	90.03	-0.025
5	0.50	9.00	55.00	34.70	34.670	69.4	69.34	0.035
6	1.00	6.00	65.00	44.00	43.985	88	87.97	0.035
7	1.00	6.00	60.00	48.25	48.405	96.5	96.81	-0.310
8	0.50	3.00	55.00	35.50	35.525	71	71.05	-0.051
9	0.50	6.00	60.00	44.50	44.495	89	88.99	0.015
10	0.50	9.00	65.00	34.00	34.04	68	68.08	-0.081
11	1.00	6.00	60.00	48.40	48.405	96.8	96.81	-0.011
12	1.00	9.00	60.00	43.00	42.985	86	85.97	0.035
13	1.00	6.00	60.00	48.50	48.405	97	96.81	0.190
14	1.00	6.00	60.00	48.20	48.405	96.4	96.81	-0.410
15	*1.50*	*3.00*	*65.00*	34.40	34.430	68.8	68.86	-0.061
16	1.00	3.00	60.00	42.75	42.765	85.5	85.53	-0.025
17	1.50	9.00	65.00	35.75	35.725	71.5	71.45	0.049
18	1.50	3.00	55.00	35.9	35.86	71.8	71.72	0.079
19	1.50	6.00	60.00	45.5	45.505	91	91.01	0.000
20	1.00	6.00	60.00	48.52	48.405	97.05	96.81	0.240

From different models such as the linear, 2F1, quadratic and cubic, quadratic is selected on the bases of p-values, Lack of fit test and R-Square. A P-value ($P< 0.0001$) indicates the signifficance of quadratic model. In addition, absence of any lack of fit ($P> 0.05$) strengthened the reliability of the model.

The predicted model equation that relates the response to the parameters affecting the response interms of actual and coded factors is desplayed below.

Final equation in terms of coded factors**:**

$$\% \text{ biodiesel yeild} = 96.81+1.01\ A + 0.22\ B - 1.03\ C + 1.61\ AB - 0.94\ AC - 0.54\ BC - 6.82\ A^2 - 11.07\ B^2 - 7.82\ C^2$$

Where: A- Catalyst

B- methanol to oil molar ratio

C- temperature

Final equation in terms of actual factors:

% biodiesel yeild = 96.81+1.01 catalyst + 0.22 methanol to oil molar ratio - 1.03 temperature + 1.61 catalyst . methanol to oil molar ratio - 0.94 catalyst . temperature - 0.54 methanol to oil molar ratio. temperature - 6.82 catalyst^2 – 11.07 methanol to oil molar ratio^2 – 7.82 temperature^2

Table 4.18 Analysis of variance (ANOVA) table for response surface quadratic model

Source	Sum of Squares	df	Mean Square	F value	P-value Prob > F	Significance
Model	2492.56	9	276.95	5790.64	< 0.0001	Significant
A	10.2	1	10.2	213.29	< 0.0001	
B	0.48	1	0.48	10.12	0.0098	
C	10.61	1	10.61	221.82	< 0.0001	
AB	20.8	1	20.8	434.92	< 0.0001	
AC	7.03	1	7.03	147.01	< 0.0001	
BC	2.31	1	2.31	48.32	< 0.0001	
A^2	127.76	1	127.76	2671.19	< 0.0001	
B^2	336.75	1	336.75	7040.95	< 0.0001	
C^2	167.99	1	167.99	3512.5	< 0.0001	
Residual	0.48	10	0.048			
Lack of Fit	0.036	5	7.24E-03	0.082	0.9921	Not significant
Pure Error	0.44	5	0.088			
Cor Total	2493.03	19				

The Model F-value implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0100 indicate model terms are significant. In this case A, B, C, AB, AC, BC, A^2, B^2, C^2 are significant model terms. The "Lack of Fit F-value" of 0.08 implies the Lack of Fit is not significant relative to the pure error. There is a 99.21% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good; we want the model to fit. As it can be seen from p-values of the model coefficients, the value of temperature and catalyst mass in both linear and quadratic is much more less than 0.001. This indicated that both parameters are more significant than the third parameter i.e. methanol to oil molar ratio. The non-significant lack of from the ANOVA indicated that the model represent the actual relationships of reaction parameters.

Table 4.19 Adjusted versus predicted R-squared

Std. Dev.	0.22	R- squared	0.9998
Mean	83.96	Adjusted R- squared	0.9996
C.V. %	0.26	Predicted R- squared	0.9995
PRESS	1.34	Adequacy Precision	185.787

The predicted R- squared of 0.9995 is in reasonable agreement with the adjusted R- squared of 0.9996. A rule of thumb is that the adjusted and predicted R-squared values should be within 0.2 of each other. Here the values are within the order of 0.0001. Adequacy precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 185.787 indicates an adequate signal. This model can be used to navigate the design space. The plot between predicted vs. actual (fig. 4.8) is a good tool to study the significance of suggested model. The responses predicted from the empirical model are in agreement with the observed values in the range of the operating variables. Maximum points are on the line.

The normal probability plot is shown in(Fig. 4.9). The general impression from examining this display is that as the plot resembles a straight line so there is not much of a problem with the normality of the data. Outlier detection was done using index plots of externally studentized residuals (Fig. 4.10).

Linearity and quadratic effect of the independent variables and their interactions on the response variable were evaluated by analysis of variance (Table 4.18). The ANOVA of the regression

model showed that the model was highly significant due to a very low probability value (P< 0.0001).

The P-values were used as a tool for checking the significance of each coefficient, which in turn might indicate the interaction patterns between the variables. The smaller the P-value, the more significant was the corresponding coefficient.

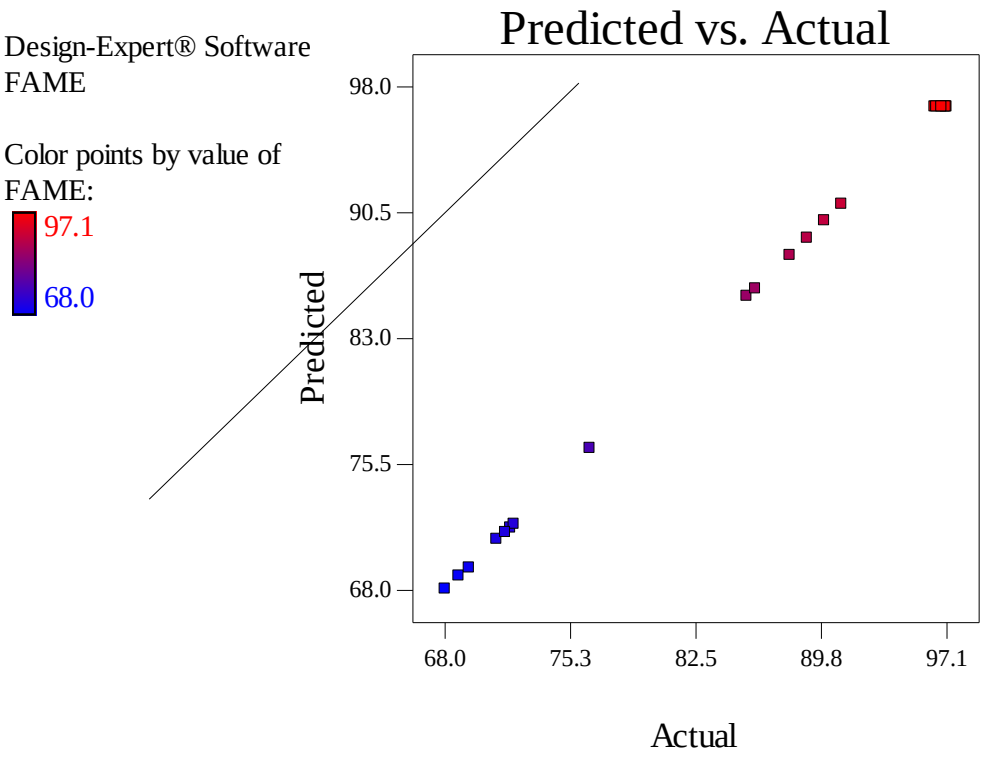


Fig.4.8 plot of actual versus predicted percentage yield of biodesiel

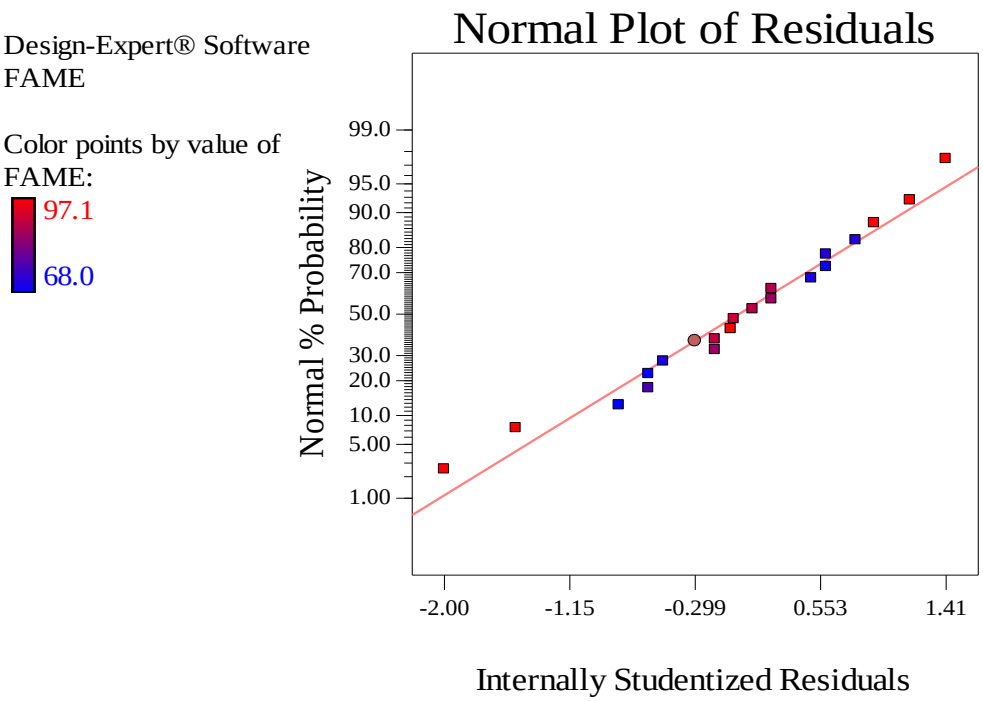


Fig.4.9

Normal probability plot of residuals.

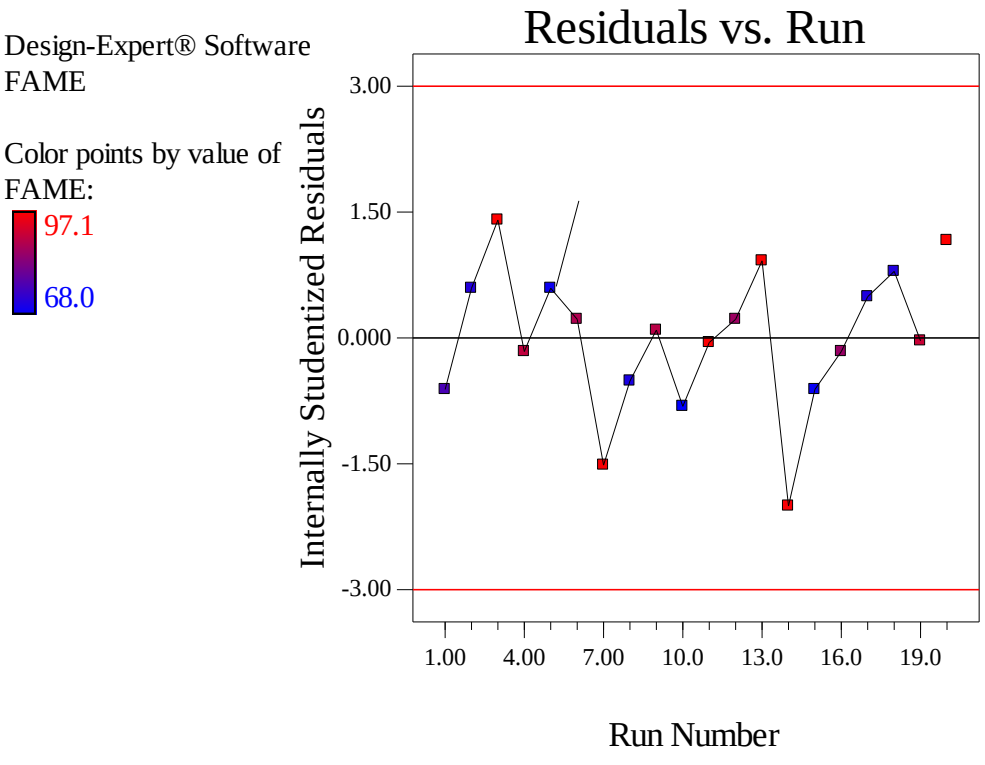


Fig4.10.Index plots of internally studentized reseduals

4.3.3. Optimum conditions

As the fitted model presented in equation 4.1 offered a good estimate to the experimental conditions, so the given model was employed to predict the best possible values of the transesterification process variables for obtaining a maximum yield of FAME. Optimum conditions are depicted by bold font in bold in table 4.17.

The optimal experimental conditions for biodiesel production using goat leather fleshing oil acquired using the model were as follows: catalyst concentration (%) on weight basis 1, reaction temperature (^{o}C) 60 ^{o}C, and molar ratio of methanol to oil 6:1 for 1 hour reaction time and 750 rpm mixing intensity. Under these optimal conditions, the model predicted a maximum response of 96.81 percentage yield of biodiesel from the oil.

4.3.4. Main factors affecting the yield of biodiesel

The effects of individual parameters and interaction factors are subsequently discussed in the proceeding subsections. The factors selected for this study are temperature, catalyst concentration and methanol to oil molar ratio.

4.3.4.1. Effects of individual process variables on biodiesel yield

4.3.4.1.1. Effect of methanol-to-oil molar ratio

Methanol-to-oil molar ratio is one of the important factors that affect the transesterification process. The stoichiometric ratio for transesterification requires three moles of alcohol and one mole of triglyceride to yield three moles of FAME and one mole of glycerol. However, transesterification is an equilibrium reaction in which a large excess of alcohol is required to drive the reaction to the right side of reaction products [54].

In the present work, the effect of a methanol-to-oil molar ratio was varied from 3:1 to 9:1 on conversion to biodiesel was studied. When the methanol-to-oil molar ratio was 3:1, the conversion to biodiesel was 85.5% (Figure 4.17). The maximum conversion to biodiesel was obtained at 6:1 and a further increment did not result in higher conversion to biodiesel since the excess of methanol deactivated the catalyst [55]. The excess methanol, with one polar hydroxyl group, could act as an emulsifier and thereby increase the solubility of glycerol in the ester phase, making the separation more difficult. The glycerol that remained in solution could drive the equilibrium back to the left, reducing the ester conversion. Consequently there will be

recombination of esters and glycerol to MAGs in the excess of methanol [56]. However, at industrial scale increasing the amount of methanol is not a problem in terms of yield, since methanol is recovered from ester and glycerol phases [57]. Even in industrial set up further increase in the amount beyond the optimal ratio will increase the cost of alcohol recovery. The use of excess methanol will shift the equilibrium to the right and improve FAME yield, but beyond a certain value (6:1 in this case) the excess methanol causes dilution.

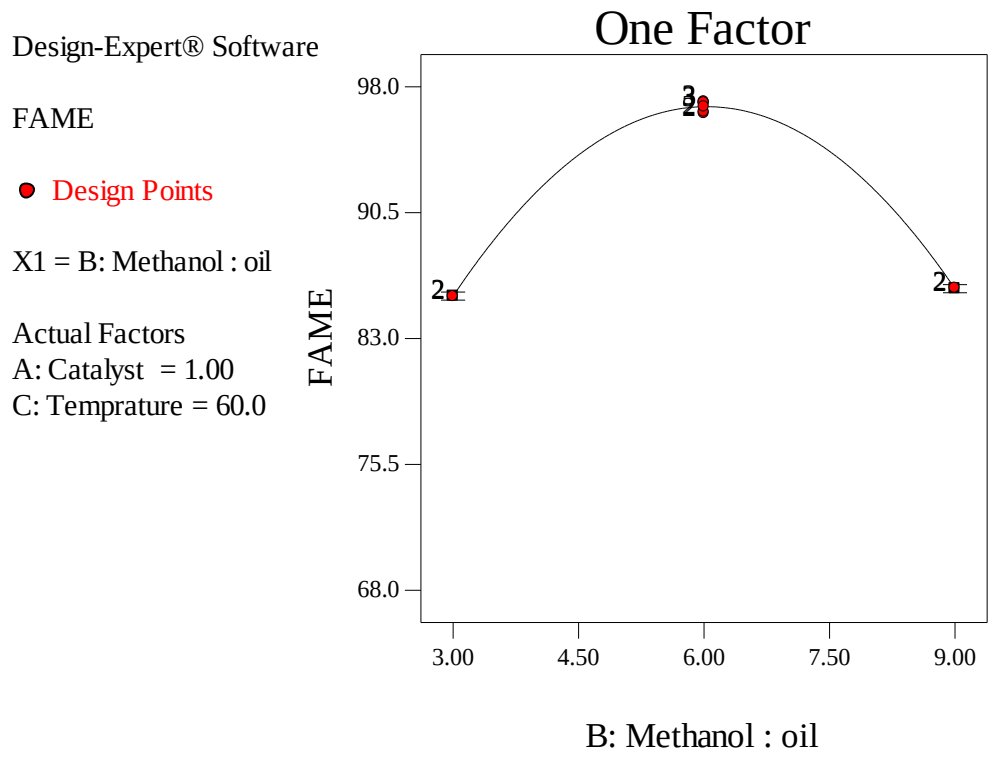


Fig. 4.11. Effect of methanol to oil molar ratio on percentage yield of biodiesel

4.3.4.1.2. Effect of reaction temperature

Temperature clearly influences the reaction and yield of the biodiesel product. The reaction temperature was varied at six different levels to determine the effect of reaction temperature on GLFO biodiesel production. As can be seen from table 4.17, when the reaction temperature was 60°C, the conversion to biodiesel reached 97.1%.

Initially increased biodiesel yield was observed as temperature increased due to the fact that viscosity of oils decreases at high temperature which results in an increased reaction rate. With

further increase in reaction temperature, there was little increase in conversion to biodiesel. The reaction temperature above the boiling point of methanol (65°C) should be avoided since at higher temperature, methanol tends to be lost and high temperature accelerates saponification of the glycerides by alkaline catalyst [58].

The results obtained in this study confirmed that higher a temperature of 65 °C was not beneficial for transesterification a decrease in conversion to biodiesel was observed.

The temperatures in the range from 62 to 70 ^{o}C caused evaporation of methanol and reduced the overall ester yield [56].

Although a reflux condenser was used to avoid methanol losses, the ester yield significantly decreased at temperatures of greater than 60 °C, probably because of a negative interaction between temperature and catalyst concentration, due to side reactions, such as soap formation completion of the alcoholysis.

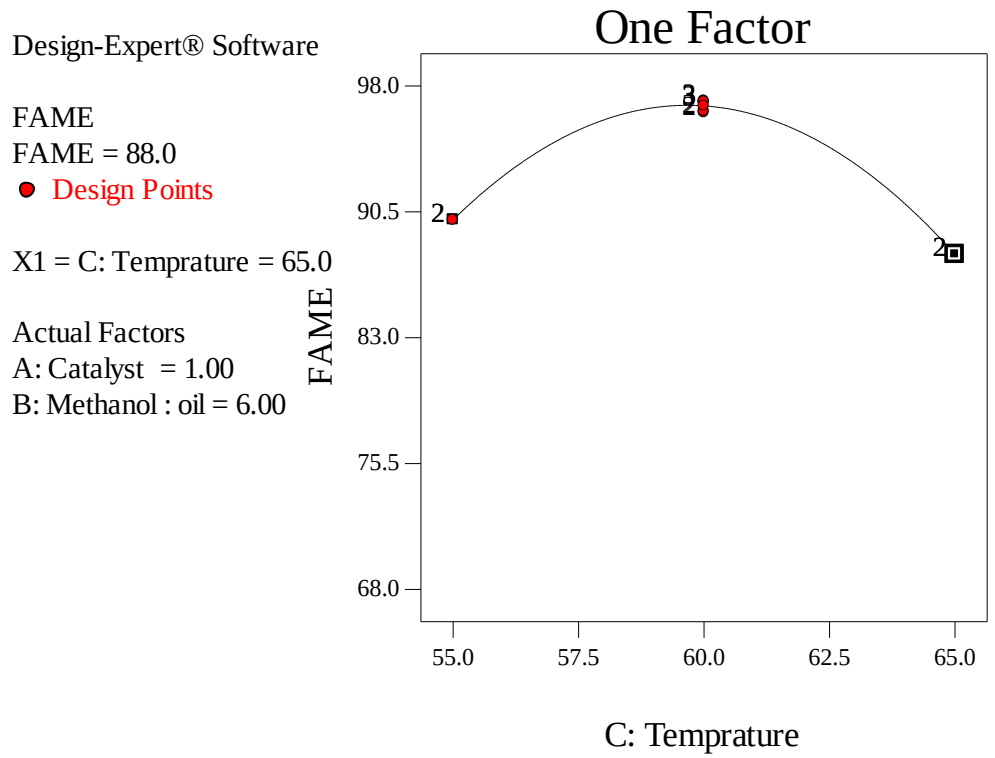


Fig. 4.12. Effects of temperature on percentage yield of biodiesel

4.3.4.1.3. Effect of catalyst mass/concentration

The biodiesel yield was increased as the catalyst concentration increased up to a certain level. Further increasing catalyst concentration beyond optimum values caused a decreased biodiesel yield. The addition of an excessive amount of catalyst, gives rise to the formation of an emulsion, which increases the viscosity and leads to the formation of gels. These hinder the glycerol separation and, hence, reduce the apparent ester yield. These results are in good agreements obtained by other investigators [59].

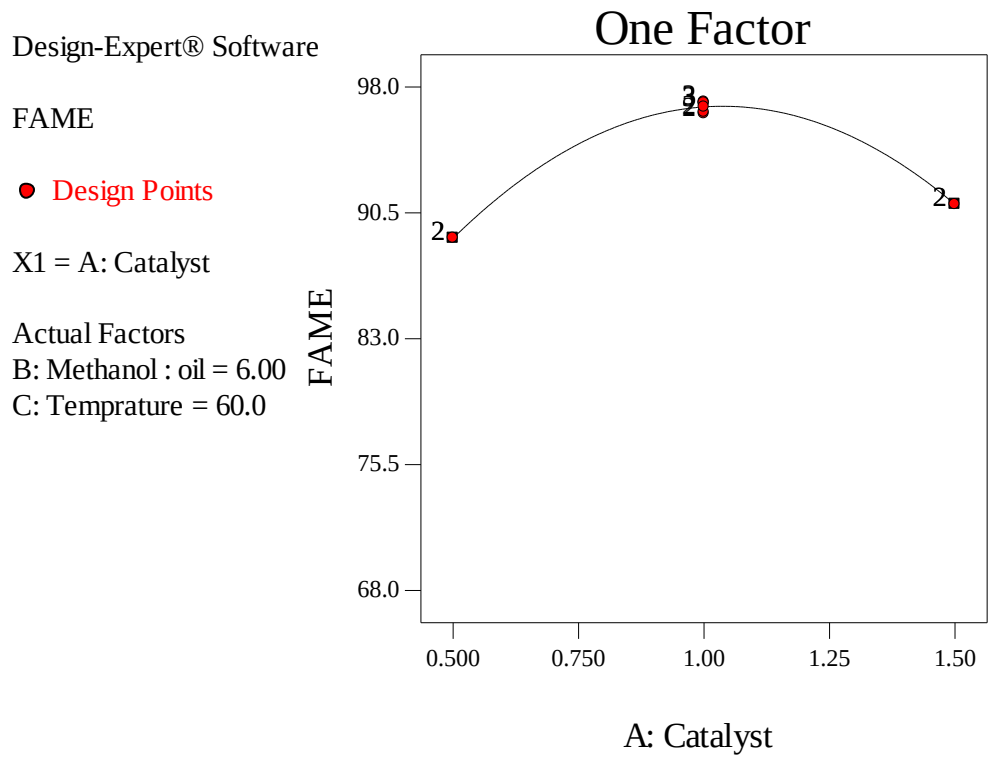


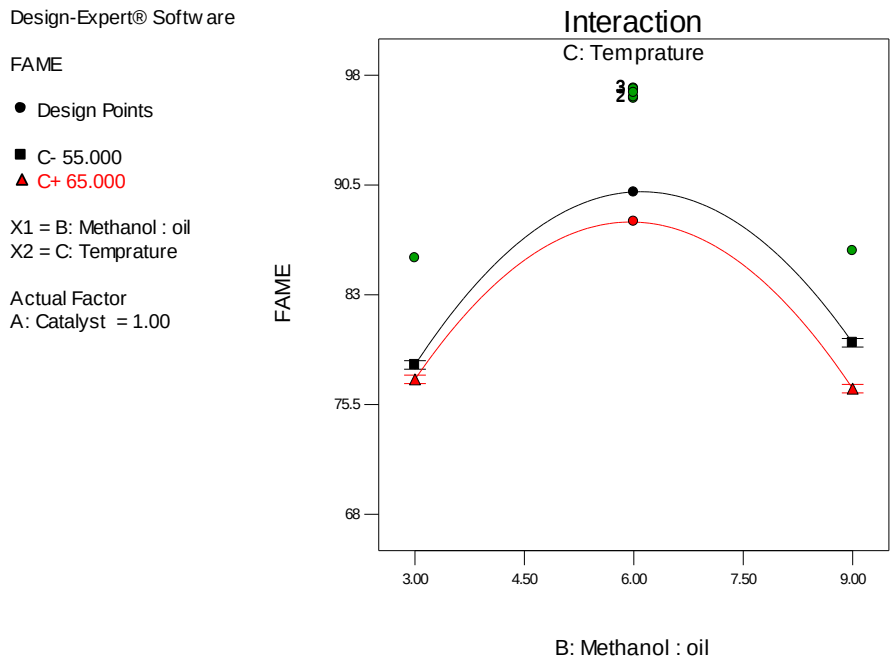
Fig.4.13 Effect of catalyst concentration on percentage yield of biodiesel

4.3.4.2. Effects of interaction of process variables on percent yield of biodiesel

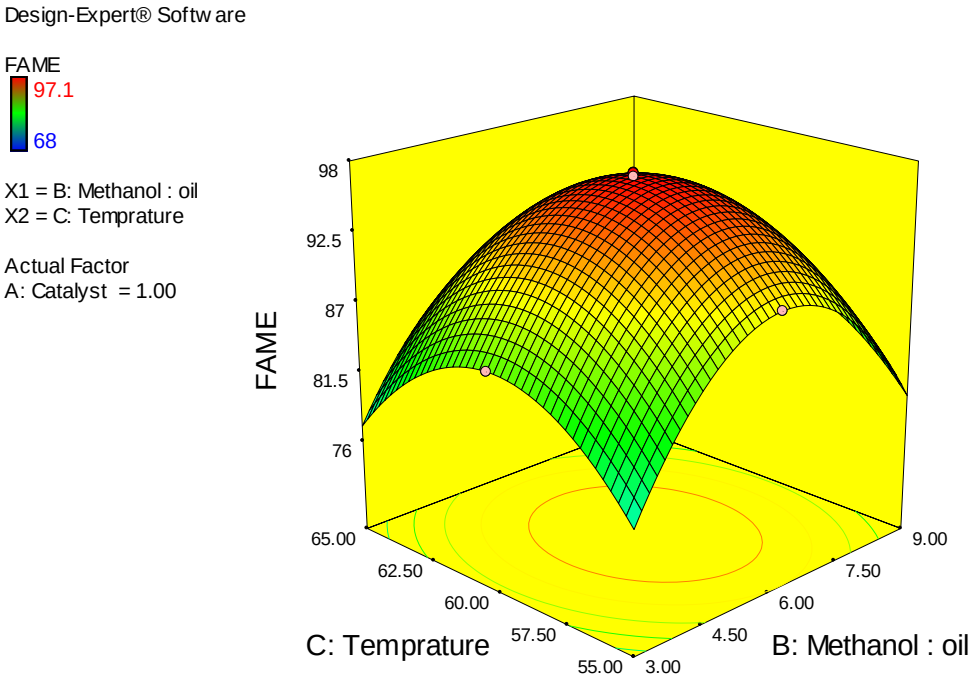
The 3D and contour plots of interaction effects of process variables over the FAME yield were drawn and subsequently descriptions are given. Each contour curve represents effects of two process variables while holding the third at constant level.

4.3.4.2.1. Effect of methanol to oil molar ratio and temperature on Biodiesel yield

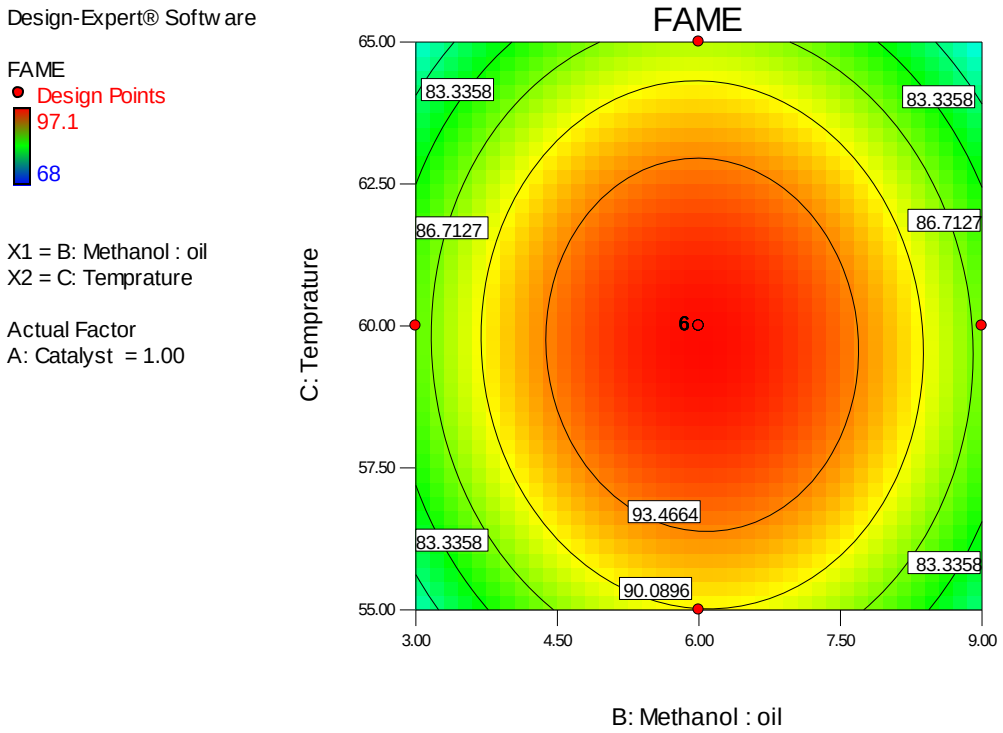
The following consecutive interaction, 3D and contour plots show the effect of varying temperature and methanol to oil molar ratio while the catalyst concentration i.e. weight by weight catalyst to oil is at 1. Initially the effect of low level of methanol to oil molar ratio and temperature produces low level of FAME percentage yield. Further increase of both process variables will enable optimum amount of yield where as an increase beyond some levels decreases the yield. The effect of increasing temperature beyond the boiling point of methanol will decrease the interaction of the oil with methanol which leads to a decreased yield.



A/ interaction plot


Design-Expert® Software
FAME
97.1
68
X1 = B: Methanol : oil
X2 = C: Temprature
Actual Factor
A: Catalyst = 1.00
FAME
98
92.5
87
81.5
76
65.00
62.50
60.00
57.50
55.00
C: Temprature
3.00
4.50
6.00
7.50
9.00
B: Methanol : oil

B/ 3D plot

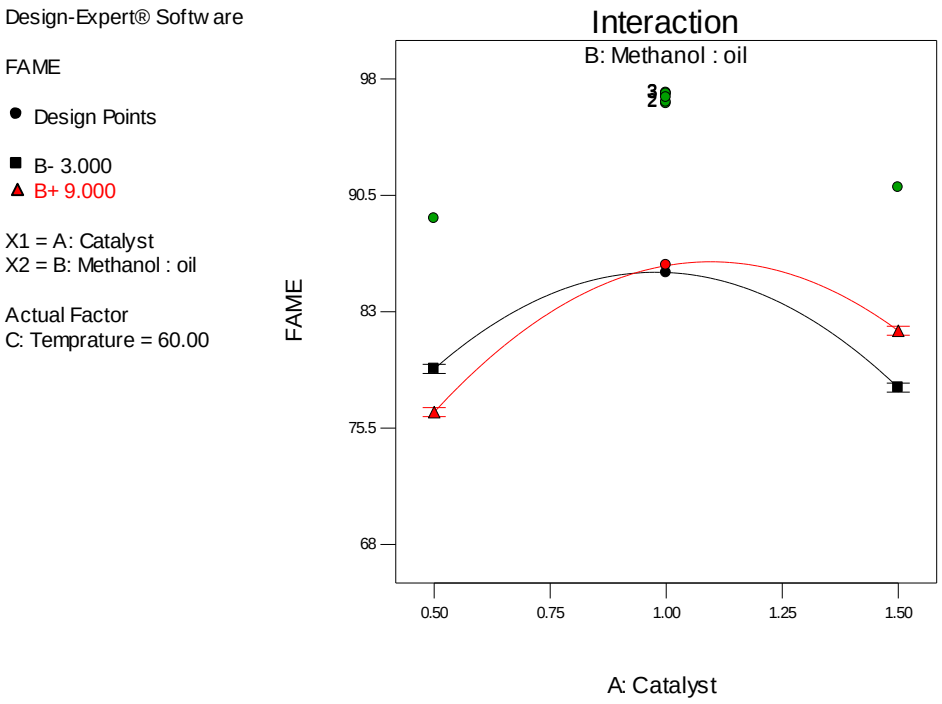

Design-Expert® Software
FAME
Design Points
97.1
68
X1 = B: Methanol : oil
X2 = C: Temprature
Actual Factor
A: Catalyst = 1.00
FAME
65.00
62.50
60.00
57.50
55.00
C: Temprature
83.3358
86.7127
93.4664
90.0896
3.00
4.50
6.00
7.50
9.00
B: Methanol : oil

C/ contour plot

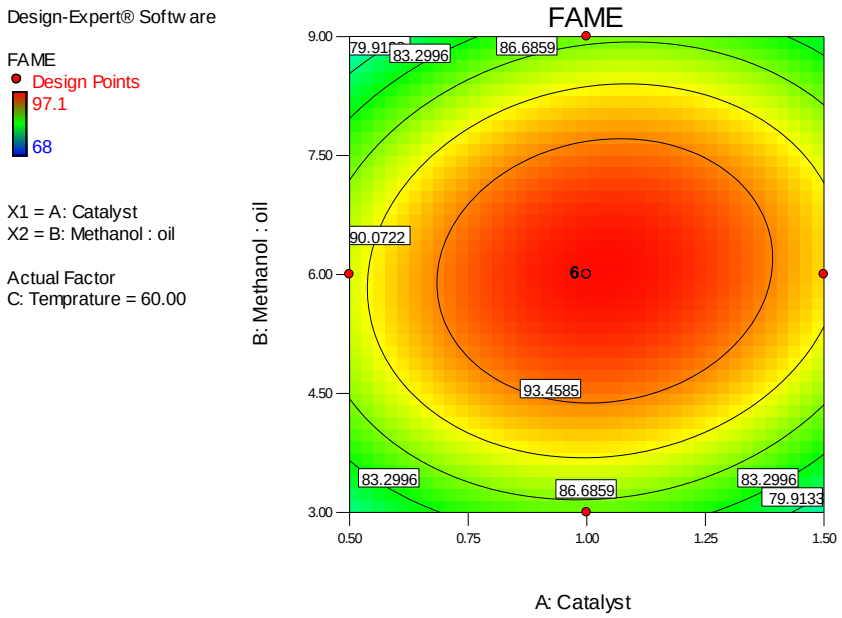
Fig.4.14 the effect of methanol to oil molar ratio and temperature on FAME percentage yield

4.3.4.2.2. Effect of methanol to oil molar ratio and catalyst on Biodiesel yield

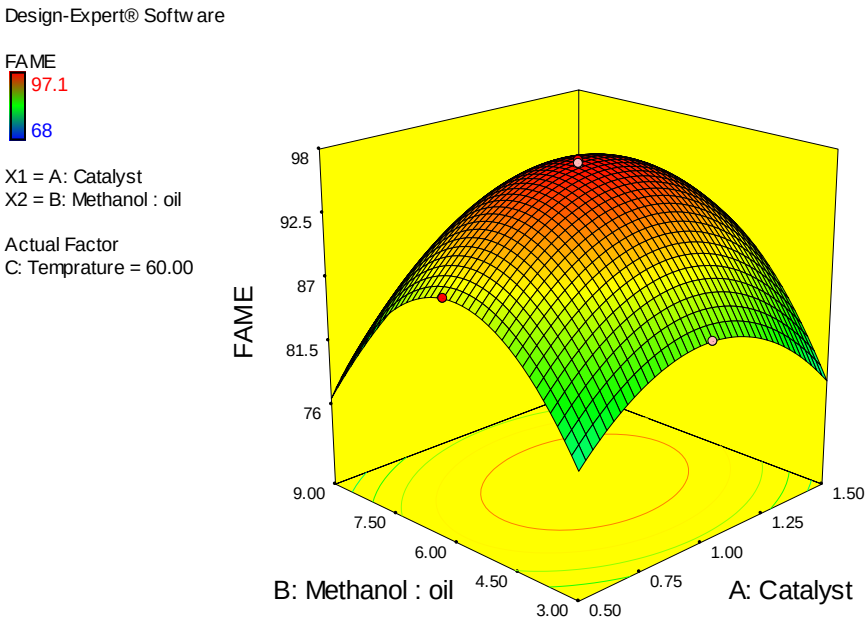
The individual effects of methanol to oil molar ratio and weight by weight catalyst ratio have been discussed in their respective sub sections. Here, the effect of the interaction of the two variables is depicted using contour, interaction and 3D plots. It is possible to see from the plots that initially percentage FAME yield increases with increasing catalyst and methanol to oil molar ratio. Further increase of both the methanol and the catalyst decreases the percent yield. This might be due to the fact that increasing catalyst mass favors formation of soap than the biodiesel and an increase in methanol beyond the limit will hinder the conversion of fatty acids to FAME. This might be due to the inactivation of the catalyst or emulsification of the glycerol by the alcohol.



A/interaction plot



B/contour plot



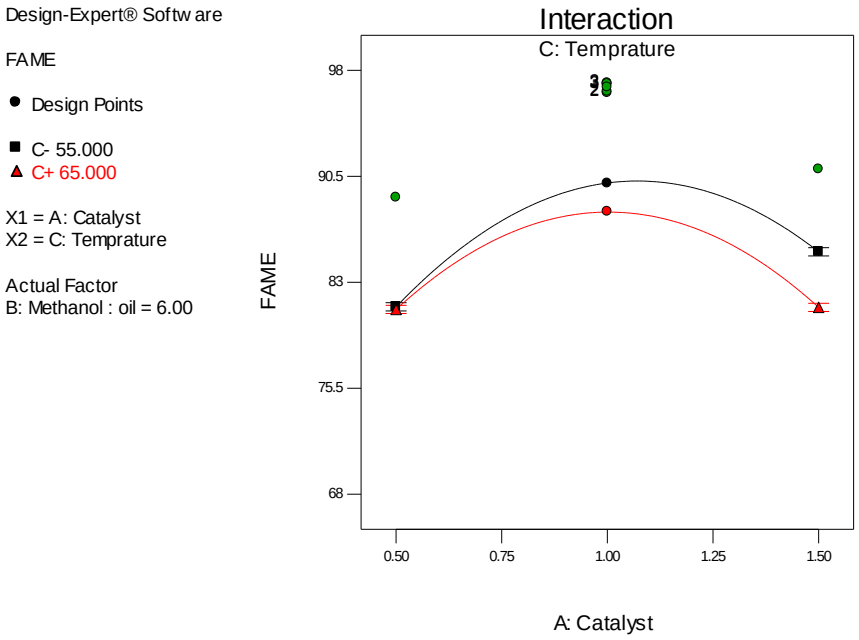
C/3D plot

Fig. 4.15 the effect of methanol to oil molar ratio and catalyst mass on FAME percentage yield

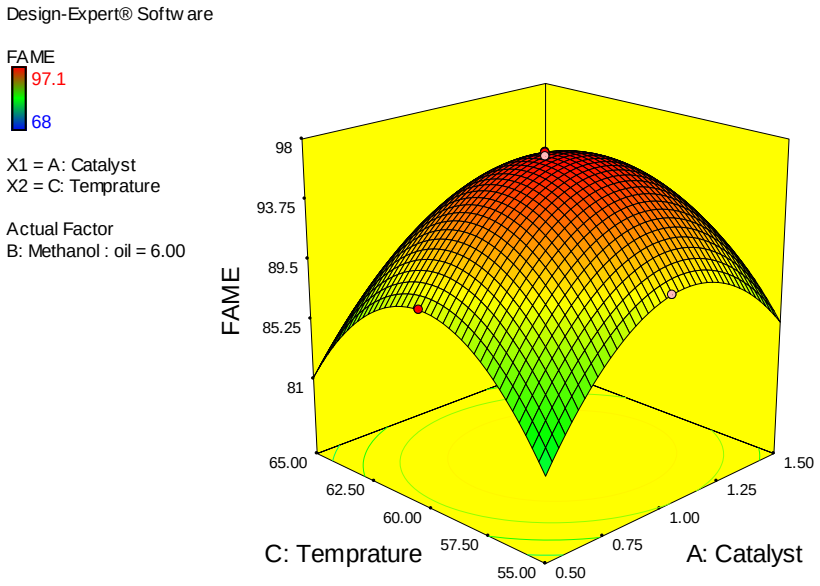
4.3.4.2.3. Effect of temperature and catalyst mass on Biodiesel yield

It can be observed from the following plots that initial increase in both the temperature and the catalyst increases the yield of FAME. As both the temperature and the catalyst increases the FAME percent yield starts to decline. The result might be due to the fact that an increase in temperature will lead to evaporation of the alcohol and an excessive amount of catalyst, gives rise to the formation of an emulsion, which increases the viscosity and leads to the formation of gels that hinder the glycerol separation and, hence, reduce the apparent ester yield.

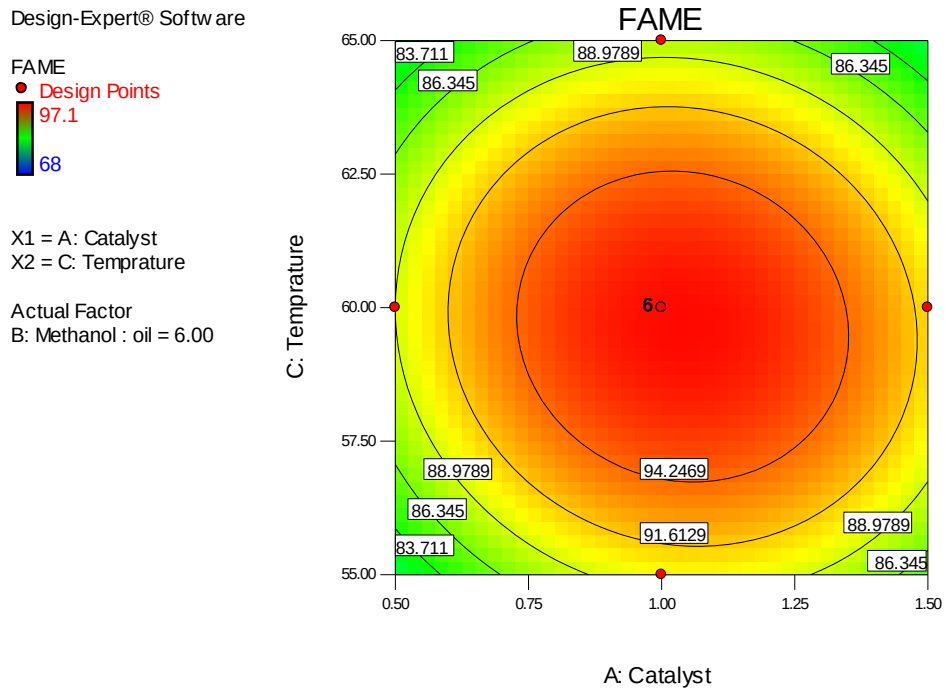
The contour, interaction and 3D plots of the interaction effect of catalyst and temperature is depicted in the subsequent figures below.



A/ interaction plot



B/ 3D plot



C/ Contour plot

Fig. 4.16. The effect of temperature and catalyst mass on FAME percentage yield

4.4. Optimization of process variables

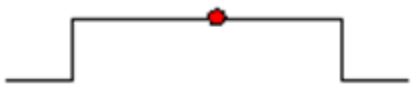
The optimization of process variables with in the selected ranges for maximum conversion of the oil to FAME was evaluated using Design Expert 7.0.0. The optimum result predicted by the model was found to be 96.891% FAME at catalyst weight by weight percent of 1.04, temperature of 59.64 ^{O}C and methanol to oil molar ratio of 6.05.

Table 4.19 optimization of process variables

Constraints Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
Catalyst	in range	0.5	1.5	1	1	3
Methanol to oil molar ratio	in range	3	9	1	1	3
Temperature	in range	55	65	1	1	3
FAME	maximize	68	97.1	1	1	5

Solution

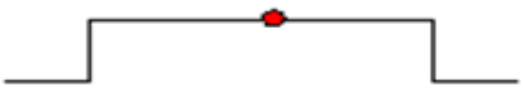
Solution number	Catalyst	Methanol to oil molar ratio	Temperature	% yield of FAME	Desirability	Remark
1	1.04	6.05	59.64	96.891	0.993	selected



0.5 Catalyst 1.04 1.5

55 Temperature 65

59.64^{o}c



0.5 Methanol to oil molar ratio 6.05 :1 1.5

68 FAME 97.1

96.891

Desirability = 0.993

Fig 4.17. Optimization of process variables for maximum conversion of oil to FAME

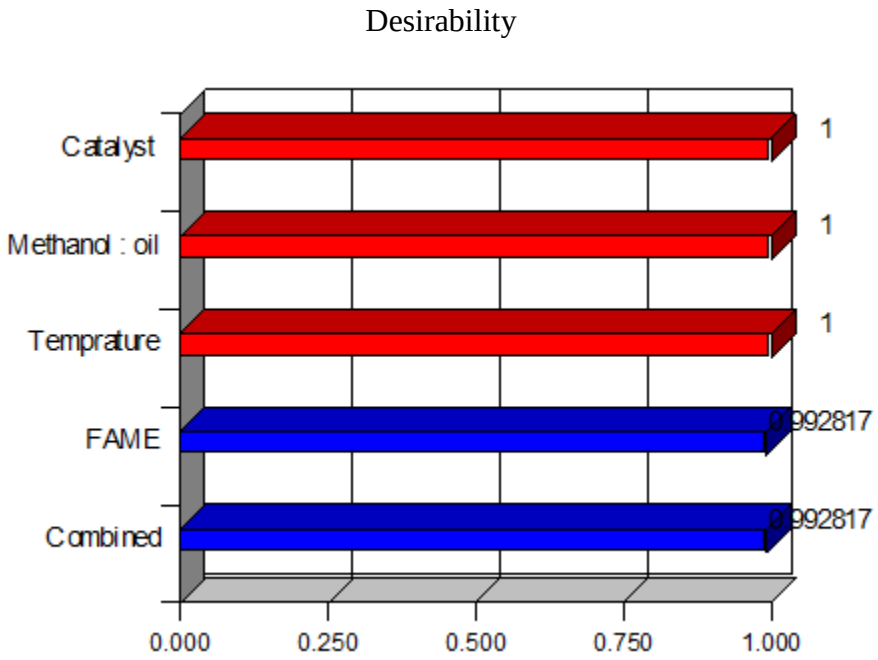


Fig 4.18. Desirability of the experimental process variables

4.5. Gas chromatograph-Mass spectroscopy analysis of the produced biodiesel

The samples for sheep, goat and hide fleshing FAMEs were prepared according to the method described in section 3.4. The method employed for identification of species was the internal standard. Individual fatty acids of the oil were identified by comparing retention time i.e. the time at which the peak is observed and the chromatographic profiles of the samples. The gas chromatograms of FAMEs of the sheep, goat and hide fleshing oils are presented subsequently in the following figures. The peak values of the corresponding FAMEs are presented in the annex.

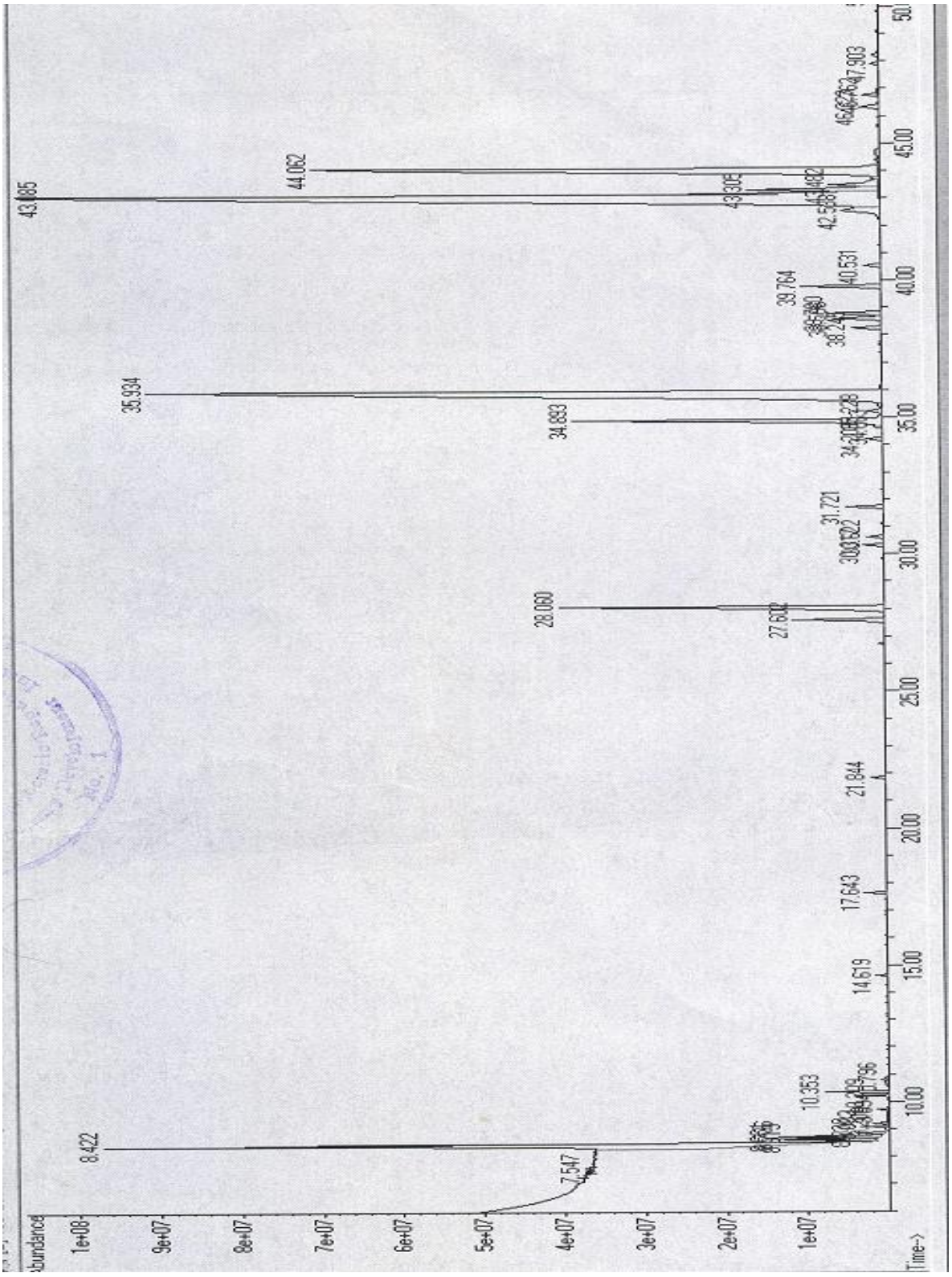


Fig.4.19. Goat fleshing FAME chromatogram

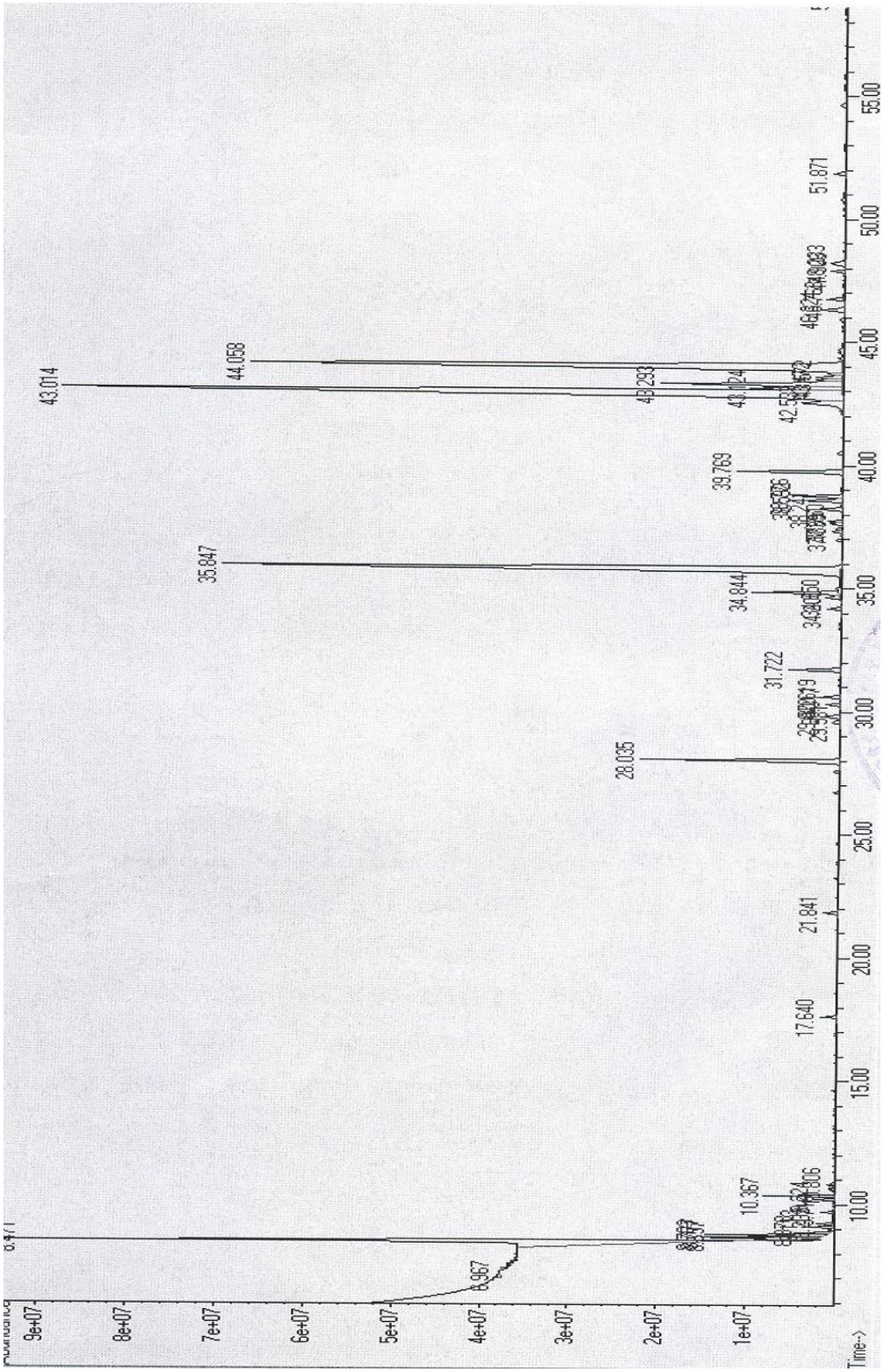


Fig 4.20. Hide fleshing FAME chromatogram

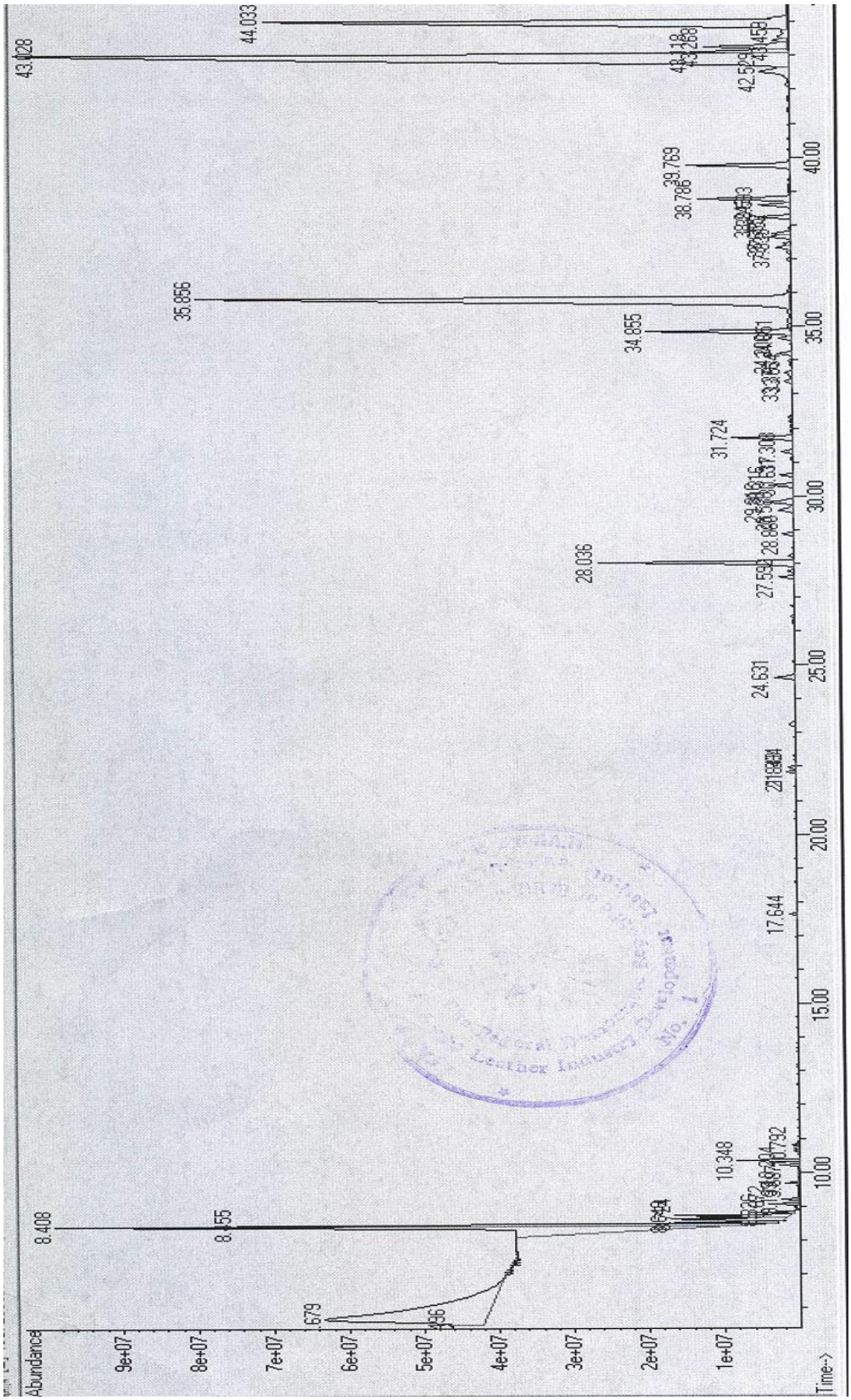


Fig 4.21. Sheep fleshing FAME chromatogram

4.6. Physicochemical properties of the FAME from goat fleshing oil

The physicochemical analysis of the biodiesel was conducted. The procedures followed were those discussed in the materials and methods section.

4.6.1. Density of the FAME of the goat fleshing oil

The density of the biodiesel was measured at different temperature using pycnometer. The result of the biodiesel density measurement at different temperatures is depicted by figure 4.22.

The density of biodiesel is a factor which influences the efficiency of atomization and depends on alkyl ester content and remained amount of alcohol. The density value of biodiesel at 25 ^{o}C is adopted to be between 860 and 900 kg/m^3 by EN14214 standard and between 875 and 900 kg/m^3 ASTM D 6751-02. The result obtained from the experiment i.e 880 kg/m^3 was found to be within the range.

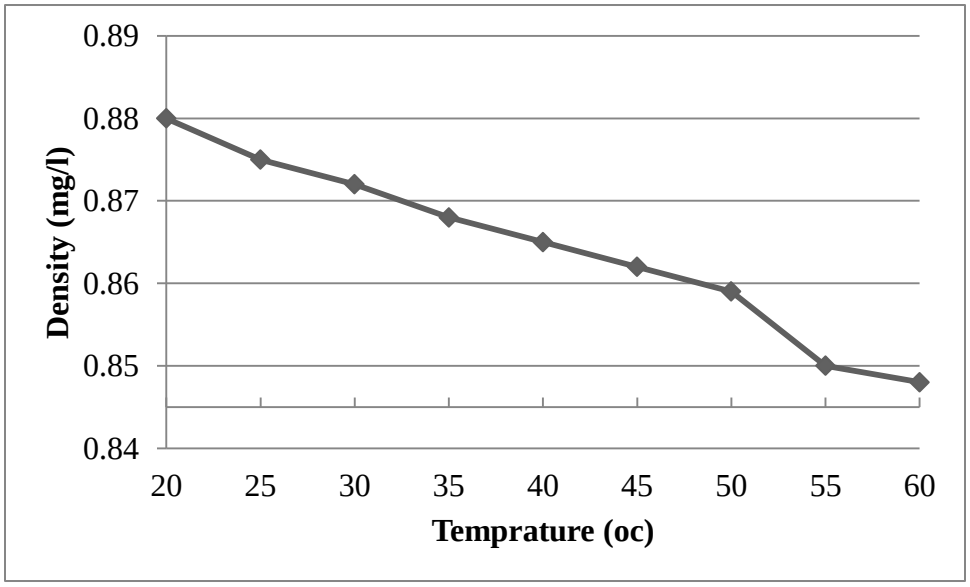


Fig. 4.22 Density of goat leather fleshing biodiesel at different temperature

4.6.2. Kinematic viscosity of the FAME of the goat fleshing oil

The viscosity of biodiesel is influenced primarily by the experimental conditions and the extent of transesterification reaction [60]. Therefore it is possible to find different viscosity values for esters originated from the same type of feedstock due to incomplete reaction i.e presence of acyl glycerol and inherent biodiesel purification. The kinematic viscosity of the treated oil and the

biodiesel was measured and the result was depicted under section 4.2.1.2. The method to calculate the kinematic viscosity was discussed under section 3.3.2.

The standard values for kinematic viscosity biodiesel at 40 ^{o}C are EN14214 (3.5-5.0 mm^2 /s) and the ASTM D6751 (1.9-6.0 mm ^{2}/s). The experimental result 5.9 mm^2/s was slightly higher than the limits dictated by EN.

4.6.3. Acid value of the FAME of the goat fleshing oil

The acid value of the biodiesel was determined employing the similar procedure stated under section 3.3.3. The result obtained was 0.48 mg KOH/g.

4.6.4. Moisture and ash contents of the FAME of the goat fleshing oil

The moisture and ash contents of the produced biodiesel were measured using procedures mentioned under sections 3.2.2 and 3.2.3. The moisture and ash contents were 0.023 and 0.021 respectively.

4.6.5. Iodine value of the FAME of the goat fleshing oil

The iodine value of the produced biodiesel was calculated using equation 3.8 that was discussed under section 3.3.6. Iodine value is a measure of total unsaturation within a mixture of fatty acid. It is expressed in grams of iodine which react with100 g of biodiesel when formally adding iodine to the double bonds. It is limited to 120 g I_2/100 g in the European biodiesel standard EN 14214. The iodine value of the biodiesel was found to be 65.13 g I_2/100g.

4.6.6. Cetane number of the FAME of the goat fleshing oil

The cetane number (CN) is a dimensionless number for diesel just as the octane number is for gasoline and is associated with ignition delay time, i.e., the time between fuel injection the beginning of ignition. The shorter the ignition time, the higher the cetane number.it is the calculated value based on equation 3.10 under section 3.3.8. Cetane number (CN) is determined in accordance with ASTM D 613 and is one of the primary indicators of diesel fuel quality. It is related to the ignition delay time a fuel experiences once it has been injected into a diesel engine's combustion chamber. Generally, shorter ignition delay times result in higher CN and vice versa. Hexadecane, also known as cetane (trivial name), which gives the cetane scale its name, is the high-quality reference standard with a short ignition delay time and an arbitrarily assigned CN of 100 [61]. The cetane number of the biodiesel was calculated to be 68.5.

4.6.7. Higher heat value of the FAME of the goat fleshing oil

The higher heat value of the biodiesel was calculated using equation 3.9 under section 3.3.7. The calculated value was 42.38 MJ/kg.

4.7. Comparison of the biodiesel properties with ASTM and EN Standards

The general comparison of the biodiesels produced in this experiment with international standards is illustrated in Table 4.20. As it can be observed in the table, majority of the physicochemical properties of the fleshing oil biodiesel were in agreement with the international standards.

Table 4.20 Comparison of experimental biodiesel with standards

Physicochemical properties	Unit	Experimental biodiesel	EN 14214	ASTM D 6751-10	Diesel	Test method
Density at 25 ^{o}C	kg/m^{3}	880	860-900	875-900	840-860	(EN ISO 3675 & ASTM D 5002)
Kinematic viscosity at 40^{o}C	mm^{2}/s	5.99	3.5-5.0	1.9-6.0	1.9 - 3.8	ASTM D 445
Acid value	mg KOH/g	0.48	≤ 0.5	≤ 0.8	-	ASTM D 664
HHV	MJ/kg	42.38	-	-	43.3-46.7	-
Iodine value	g I_2/100g	65.13	120 max	-	-	ASTMD 5558
Cetane number	-	68.5	-	46 - 70	47 - 55	ASTM D613
Moisture content	% w/w	0.023	-	< 0.03		
Ash content	% w/w	0.021	<0.02	<0.03		ASTM D482

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

The fat obtained from the leather industry fleshing wastes was used successfully for the production of acceptable quality biodiesel. It was proved that fleshing wastes from tanneries that storage and disposal are both troublesome and costly could be transformed to a fuel with low emission values and a performance close to diesel fuel.

The GC-MS analysis result showed that all the three types of wastes i.e goat, sheep and hide fleshing wastes can be acceptable candidates for producing biodiesel. In this research the experimental oil yield obtained from washed, delimed and dried goat fleshing waste was 23.08%. Degumming only was sufficient to reduce the FFA level to proceed with direct homogenous base catalyzed transesterification.

Three major factors and their interaction effects on transesterification were studied. The optimum conditions were evaluated using design expert 7.0.0 software. The transesterification reaction yield was 97% at 1% catalyst concentration on weight basis, reaction temperature of 60 ^{o}C, and methanol to oil molar ratio of 6 :1 for one hour reaction time and 750 rpm mixing intensity.

The produced biodiesel has density, kinematic viscosity, iodine value and HHV of 880 kg/m^3, 5.99 mm^2/s, 65.13 g I_2/100g and 42.38 MJ/kg respectively. The physicochemical analysis result shows that majority of the parameters fall within the range of values established by EN and ASTM. Therefore environmental polluting leather fleshing waste can be converted to useful energy generating product.

5.2. Recommendation

In this study of biodiesel production from leather industry fleshing wastes using Soxhlet extraction was employed, although it was possible to produce high quality oil, Soxhlet cannot be used for large scale industrial application. Further study on other ways of oil extraction is therefore essential.

Effect of blending the oils from goat, sheep and hide fleshing oils at different proportions and blending with other feed stocks should be studied to use the advantage of the qualities each of them have individually.

This study has focused on homogenous base catalyzed transesterification; a complimentary heterogeneous, super critical and biological catalysis should be tried for better environmental protection. Exhaust gas emission profile studies, engine performance studies and should be carried out.

The economic feasibility study on large scale production and commercialization should be undertaken. Since gum, glycerol and compostable waste residues are produced as side products during the production process additional studies on using these products for different applications shall be initiated.

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ANNEXES

Annex A: Qualitative GC-MS analysis test result for goat fleshing FAME

LEATHER INDUSTRY DEVELOPMENT INSTITUTE
RESEARCH AND TESTING DIRECTORATE
QUALITATIVELY GC-MS ANALYSIS TEST RESULT

Test date: 10/06/2015
Time: 2:00 pm
Test name: qualitative identification
Sample type: oil sample
Sample code: C-5108
Customer code: G
Method: Internal method
Room temperature: 20 degree celcius

ORIGINAL

Pk#	RT	Area%	Name of compounds	Ref#	CAS#	Qual(% Abundance)
15	14.617	0.04				
			Octanoic acid, methyl ester	30234	000111-11-5	94
			Octanoic acid, methyl ester	30223	000111-11-5	90
			Octanoic acid, methyl ester	30233	000111-11-5	80
16	17.641	0.12				
			Decanoic acid, methyl ester	50090	000110-42-9	97
			Decanoic acid, methyl ester	50098	000110-42-9	96
			Decanoic acid, methyl ester	50100	000110-42-9	95
17	21.845	0.11				
			Dodecanoic acid, methyl ester	72688	000111-82-0	98
			Undecanoic acid, 10-methyl-, methy l ester	72721	005129-56-6	91
			Dodecanoic acid, methyl ester	72687	000111-82-0	87
18	27.601	0.83				
			Methyl myristoleate	94118	056219-06-8	99
			Methyl Z-11-tetradecenoate	94132	1000130-82-8	91
			Methyl 9-tetradecenoate	94125	1000336-47-3	91
19	28.057	3.56				
			Methyl tetradecanoate	95859	000124-10-7	98
			Tridecanoic acid, 12-methyl-, meth yl ester	95899	005129-58-8	95
			Methyl tetradecanoate	95862	000124-10-7	95
20	30.316	0.17				
			Methyl 13-methyltetradecanoate	107583	1000336-31-4	98
			Methyl 9-methyltetradecanoate	107578	213617-69-7	97
			Pentadecanoic acid, methyl ester	107595	007132-64-1	95
21	30.621	0.15				
			Tetradecanoic acid, 12-methyl-, me thyl ester	107610	005129-66-8	90
			Tridecanoic acid, 12-methyl-, meth yl ester	95898	005129-58-8	83
			Pentadecanoic acid, methyl ester	107593	007132-64-1	81
22	31.721	0.34				
			Pentadecanoic acid, methyl ester	107593	007132-64-1	98
			Pentadecanoic acid, methyl ester	107595	007132-64-1	97
			Pentadecanoic acid, methyl ester	107596	007132-64-1	96
23	34.206	0.16				
			Pentadecanoic acid, 14-methyl-, me thyl ester	119423	005129-60-2	97
			Hexadecanoic acid, methyl ester	119405	000112-39-0	96
			Hexadecanoic acid, methyl ester	119407	000112-39-0	95
24	34.662	0.08				
			9-Hexadecenoic acid, methyl ester,	117507	001120-25-8	99

			(Z)-			
			7-Hexadecenoic acid, methyl ester,	117506	056875-67-3	99
			(Z)-			
			9-Hexadecenoic acid, methyl ester,	117513	001120-25-8	99
			(Z)-			
25	34.893	3.60				
			9-Hexadecenoic acid, methyl ester,	117507	001120-25-8	99
			(Z)-			
			Methyl hexadec-9-enoate	117464	010030-74-7	99
			9-Hexadecenoic acid, methyl ester,	117513	001120-25-8	99
			(Z)-			
26	35.228	0.18				
			Methyl hexadec-9-enoate	117464	010030-74-7	99
			9-Hexadecenoic acid, methyl ester,	117507	001120-25-8	99
			(Z)-			
			9-Hexadecenoic acid, methyl ester,	117513	001120-25-8	99
			(Z)-			
27	35.936	18.84				
			Hexadecanoic acid, methyl ester	119400	000112-39-0	97
			Hexadecanoic acid, methyl ester	119408	000112-39-0	95
			Pentadecanoic acid, 14-methyl-, me	119425	005129-60-2	94
			thyl ester			
28	38.242	0.28				
			Hexadecanoic acid, 15-methyl-, met	131321	006929-04-0	96
			hyl ester			
			Heptadecanoic acid, methyl ester	131301	001731-92-6	95
			Hexadecanoic acid, 14-methyl-, met	131316	002490-49-5	95
			hyl ester			
29	38.599	0.47				
			Hexadecanoic acid, 14-methyl-, met	131316	002490-49-5	97
			hyl ester			
			Hexadecanoic acid, 15-methyl-, met	131319	006929-04-0	96
			hyl ester			
			Hexadecanoic acid, 14-methyl-, met	131320	002490-49-5	95
			hyl ester			
30	38.782	0.52				
			cis-10-Heptadecenoic acid, methyl	129385	1000333-62-1	99
			ester			
			Cyclopropaneoctanoic acid, 2-hexyl	129411	010152-61-1	90
			-, methyl ester			
			Methyl myristoleate	94118	056219-06-8	89
31	39.763	0.79				
			Heptadecanoic acid, methyl ester	131301	001731-92-6	99
			Heptadecanoic acid, methyl ester	131299	001731-92-6	98
			Heptadecanoic acid, methyl ester	131300	001731-92-6	98
32	40.533	0.18				
			Hexadecanoic acid, 2-hydroxy-, met	132966	016742-51-1	97
			hyl ester			
			Hexadecanoic acid, 2-hydroxy-, met	132968	016742-51-1	93
			hyl ester			
			Hexadecanoic acid, 2-hydroxy-, met	132969	016742-51-1	80
			hyl ester			
33	42.536	0.81				
			9,12-Octadecadienoic acid (Z,Z)-,	139724	000112-63-0	99
			methyl ester			
			9,12-Octadecadienoic acid (Z,Z)-,	139725	000112-63-0	99
			methyl ester			
			10,13-Octadecadienoic acid, methyl	139716	056554-62-2	99
			ester			
34	43.086	28.13				
			9-Octadecenoic acid, methyl ester,	141306	001937-62-8	99
			(E)-			

			(Z)-			
			7-Hexadecenoic acid, methyl ester,	117506	056875-67-3	99
			(Z)-			
			9-Hexadecenoic acid, methyl ester,	117513	001120-25-8	99
			(Z)-			
25	34.893	3.60				
			9-Hexadecenoic acid, methyl ester,	117507	001120-25-8	99
			(Z)-			
			Methyl hexadec-9-enoate	117464	010030-74-7	99
			9-Hexadecenoic acid, methyl ester,	117513	001120-25-8	99
			(Z)-			
26	35.228	0.18				
			Methyl hexadec-9-enoate	117464	010030-74-7	99
			9-Hexadecenoic acid, methyl ester,	117507	001120-25-8	99
			(Z)-			
			9-Hexadecenoic acid, methyl ester,	117513	001120-25-8	99
			(Z)-			
27	35.936	18.84				
			Hexadecanoic acid, methyl ester	119400	000112-39-0	97
			Hexadecanoic acid, methyl ester	119408	000112-39-0	95
			Pentadecanoic acid, 14-methyl-, me	119425	005129-60-2	94
			thyl ester			
28	38.242	0.28				
			Hexadecanoic acid, 15-methyl-, met	131321	006929-04-0	96
			hyl ester			
			Heptadecanoic acid, methyl ester	131301	001731-92-6	95
			Hexadecanoic acid, 14-methyl-, met	131316	002490-49-5	95
			hyl ester			
29	38.599	0.47				
			Hexadecanoic acid, 14-methyl-, met	131316	002490-49-5	97
			hyl ester			
			Hexadecanoic acid, 15-methyl-, met	131319	006929-04-0	96
			hyl ester			
			Hexadecanoic acid, 14-methyl-, met	131320	002490-49-5	95
			hyl ester			
30	38.782	0.52				
			cis-10-Heptadecenoic acid, methyl	129385	1000333-62-1	99
			ester			
			Cyclopropaneoctanoic acid, 2-hexyl	129411	010152-61-1	90
			-, methyl ester			
			Methyl myristoleate	94118	056219-06-8	89
31	39.763	0.79				
			Heptadecanoic acid, methyl ester	131301	001731-92-6	99
			Heptadecanoic acid, methyl ester	131299	001731-92-6	98
			Heptadecanoic acid, methyl ester	131300	001731-92-6	98
32	40.533	0.18				
			Hexadecanoic acid, 2-hydroxy-, met	132966	016742-51-1	97
			hyl ester			
			Hexadecanoic acid, 2-hydroxy-, met	132968	016742-51-1	93
			hyl ester			
			Hexadecanoic acid, 2-hydroxy-, met	132969	016742-51-1	80
			hyl ester			
33	42.536	0.81				
			9,12-Octadecadienoic acid (Z,Z)-,	139724	000112-63-0	99
			methyl ester			
			9,12-Octadecadienoic acid (Z,Z)-,	139725	000112-63-0	99
			methyl ester			
			10,13-Octadecadienoic acid, methyl	139716	056554-62-2	99
			ester			
34	43.086	28.13				
			9-Octadecenoic acid, methyl ester,	141306	001937-62-8	99
			(E)-			

42	50.807	0.11	cis-13-Eicosenoic acid, methyl est er	164512	1000333-52-1	99
			cis-11-Eicosenoic acid, methyl est er	164513	1000333-63-8	99
			11-Eicosenoic acid, methyl ester	164508	003946-08-5	99
43	51.871	0.11	Methyl 18-methylnonadecanoate	166215	1000352-20-6	99
			Eicosanoic acid, methyl ester	166219	001120-28-1	98
			Eicosanoic acid, methyl ester	166218	001120-28-1	98

Note: The sample chromatogram is attached on the separated paper at the last page.

Analysis hour: 2 days
Remark:_____
Analysed by: Mulatu K. Signature_____ Checked by: Tigist K. Signature_____ Approved by:
(Chem./instrumentation researcher) (Chem./instrumentation team leader)

Tell. 0114391700
0114394846
P.O.Box: 24692(1000)
E-mail: llpti@ethionet.et
Fax: 0114392259

AKAKI KALITY KIFLE KETEMA, ADDIS ABABA

Annex B: Qualitative GC-MS analysis test result for hide fleshing FAME

LEATHER INDUSTRY DEVELOPMENT INSTITUTE
RESEARCH AND TESTING DIRECTORATE
QUALITATIVELY GC-MS ANALYSIS TEST RESULT

Test date: 10/06/2015
Time: 2:00 pm
Test name: qualitative identification
Sample type: oil sample
Sample code: C-5108
Customer code: H
Method: Internal method
Room temperature: 20 degree celcius

ORIGINAL

Pk#	RT	Area%	Name of compounds	Ref#	CAS#	Qual(% Abundance)
15	17.641	0.12				
			Decanoic acid, methyl ester	50090	000110-42-9	97
			Decanoic acid, methyl ester	50100	000110-42-9	95
			Decanoic acid, methyl ester	50098	000110-42-9	95
16	21.840	0.15				
			Dodecanoic acid, methyl ester	72688	000111-82-0	98
			Undecanoic acid, 10-methyl-, methy l ester	72721	005129-56-6	91
			Dodecanoic acid, methyl ester	72681	000111-82-0	90
17	28.036	2.52				
			Methyl tetradecanoate	95859	000124-10-7	99
			Methyl tetradecanoate	95862	000124-10-7	95
			Tridecanoic acid, 12-methyl-, meth yl ester	95899	005129-58-8	95
18	29.583	0.14				
			Pentadecanoic acid, methyl ester $ $ Methyl n-pentadecanoate	342264	007132-64-1	53
			Pentadecanoic acid, methyl ester $ $ Methyl n-pentadecanoate	342265	007132-64-1	53
			9-Octadecenoic acid, 12-(acetyloxy)-, methyl ester, [R-(Z)]- (CAS)	595919	000140-03-4	50
19	29.824	0.26				
			Tridecanoic acid, 4,8,12-trimethyl -, methyl ester	119428	010339-74-9	80
			1,3-Dioxolane, 2-methyl-2-(3-methy lbutyl)-	30323	014447-30-4	47
			1,3-Dioxolane-2-propanoic acid, 2- methyl-, methyl ester	41497	035351-33-8	47
20	30.317	0.16				
			Methyl 13-methyltetradecanoate	107583	1000336-31-4	98
			Methyl 9-methyltetradecanoate	107578	213617-69-7	97
			Pentadecanoic acid, methyl ester	107593	007132-64-1	94
21	30.621	0.23				
			Methyl 13-methyltetradecanoate	107583	1000336-31-4	94
			Tetradecanoic acid, 12-methyl-, me thyl ester	107610	005129-66-8	91
			Tridecanoic acid, 12-methyl-, meth yl ester	95899	005129-58-8	91
22	31.721	0.60				
			Pentadecanoic acid, methyl ester	107595	007132-64-1	98
			Pentadecanoic acid, methyl ester	107593	007132-64-1	98
			Pentadecanoic acid, methyl ester	107596	007132-64-1	96
23	34.201	0.16				
			Hexadecanoic acid, methyl ester	119406	000112-39-0	96

			Hexadecanoic acid, methyl ester	119408	000112-39-0	95
			Hexadecanoic acid, methyl ester	119405	000112-39-0	94
24	34.652	0.16				
			9-Hexadecenoic acid, methyl ester, (Z)-	117507	001120-25-8	99
			Methyl hexadec-9-enoate	117464	010030-74-7	99
			7-Hexadecenoic acid, methyl ester, (Z)-	117506	056875-67-3	99
25	34.846	0.99				
			Methyl hexadec-9-enoate	117464	010030-74-7	99
			9-Hexadecenoic acid, methyl ester, (Z)-	117507	001120-25-8	99
			9-Hexadecenoic acid, methyl ester, (Z)-	117513	001120-25-8	99
26	35.847	14.40				
			Pentadecanoic acid, 14-methyl-, methyl ester	119425	005129-60-2	97
			Hexadecanoic acid, methyl ester	119408	000112-39-0	97
			Hexadecanoic acid, methyl ester	119400	000112-39-0	97
27	37.341	0.13				
			Hexadecanoic acid, 14-methyl-, methyl ester $$ Methyl 14-methylhexadecanoate	422289	002490-49-5	58
			Cyclopentanetridecanoic acid, methyl ester (CAS)	455324	024828-61-3	58
			Tetradecanoic acid, 12-methyl-, methyl ester $$ Methyl 12-methyltetradecanoate	342298	005129-66-8	53
28	37.650	0.14				
			4,6-Di-O-methyl-.alpha.-d-galactose $$ 4,6-Di-O-methylhexopyranose #	202164	998202-16-4	50
			Methyl(methyl 4-O-methyl-.alpha.-d-mannopyranoside)uronate	281442	024916-47-0	50
			Hexadecanoic acid, 14-methyl-, methyl ester (CAS)	422296	002490-49-5	45
29	37.750	0.15				
			Tridecanoic acid, 12-methyl-, methyl ester	95899	005129-58-8	95
			Hexadecanoic acid, 14-methyl-, methyl ester	131318	002490-49-5	93
			Tridecanoic acid, 12-methyl-, methyl ester	95898	005129-58-8	91
30	38.242	0.34				
			Hexadecanoic acid, 14-methyl-, methyl ester	131316	002490-49-5	98
			Hexadecanoic acid, 15-methyl-, methyl ester	131321	006929-04-0	96
			Heptadecanoic acid, methyl ester	131301	001731-92-6	95
31	38.594	0.55				
			Hexadecanoic acid, 14-methyl-, methyl ester	131316	002490-49-5	97
			Hexadecanoic acid, 15-methyl-, methyl ester	131321	006929-04-0	95
			Hexadecanoic acid, 14-methyl-, methyl ester	131318	002490-49-5	94
32	38.777	0.58				
			cis-10-Heptadecenoic acid, methyl ester	129385	1000333-62-1	99
			Methyl 9-heptadecenoate or 9-17:1	129369	1000336-38-0	83
			9-Octadecenoic acid (Z)-, methyl ester	141302	000112-62-9	76

33	39.768	1.33				
			Heptadecanoic acid, methyl ester	131301	001731-92-6	99
			Heptadecanoic acid, methyl ester	131299	001731-92-6	98
			Heptadecanoic acid, methyl ester	131300	001731-92-6	98
34	42.536	1.02				
			9,12-Octadecadienoic acid (Z,Z)-, methyl ester	139724	000112-63-0	99
			9,12-Octadecadienoic acid (Z,Z)-, methyl ester	139725	000112-63-0	99
			10,13-Octadecadienoic acid, methyl ester	139716	056554-62-2	99
35	43.013	23.08				
			9-Octadecenoic acid, methyl ester, (E)-	141310	001937-62-8	99
			9-Octadecenoic acid, methyl ester, (E)-	141306	001937-62-8	99
			9-Octadecenoic acid (Z)-, methyl ester	141300	000112-62-9	99
36	43.123	1.27				
			9-Octadecenoic acid, methyl ester, (E)-	141310	001937-62-8	99
			9-Octadecenoic acid (Z)-, methyl ester	141300	000112-62-9	99
			9-Octadecenoic acid, methyl ester, (E)-	141306	001937-62-8	99
37	43.296	3.02				
			9-Octadecenoic acid, methyl ester, (E)-	141310	001937-62-8	99
			11-Octadecenoic acid, methyl ester	141290	052380-33-3	99
			9-Octadecenoic acid, methyl ester, (E)-	141306	001937-62-8	99
38	43.474	0.29				
			8-Octadecenoic acid, methyl ester	141273	002345-29-1	99
			9-Octadecenoic acid, methyl ester, (E)-	141306	001937-62-8	99
			11-Octadecenoic acid, methyl ester	141290	052380-33-3	99
39	43.573	0.35				
			9-Octadecenoic acid (Z)-, methyl ester	141300	000112-62-9	99
			11-Octadecenoic acid, methyl ester	141290	052380-33-3	99
			8-Octadecenoic acid, methyl ester	141273	002345-29-1	99
40	44.056	14.25				
			Methyl stearate	143131	000112-61-8	99
			Methyl stearate	143126	000112-61-8	99
			Methyl stearate	143130	000112-61-8	98
41	46.325	0.34				
			Methyl 9-cis,11-trans-octadecadienoate	139701	1000336-44-0	99
			Methyl 10-trans,12-cis-octadecadienoate	139709	1000336-44-2	97
			7,10-Octadecadienoic acid, methyl ester	139706	056554-24-6	93
42	46.761	0.20				
			cis-10-Nonadecenoic acid, methyl ester	153152	1000333-64-4	98
			10-Nonadecenoic acid, methyl ester	153149	056599-83-8	96
			Nonadecanoic acid, methyl ester	154941	001731-94-8	93
43	47.903	0.15				
			Nonadecanoic acid, methyl ester	154941	001731-94-8	99
			Nonadecanoic acid, methyl ester	154943	001731-94-8	98
			Nonadecanoic acid, methyl ester	154945	001731-94-8	98

44	48.234	0.28				
			9,10-Dihydro-10-oxo-12H-pyrrolo[2, 1-e][1,3,6]benzotriazocine	217797	998217-79-7	46
			Clauszoline-M	258171	998258-17-1	43
			Methyl (11R,12R,13S)-(Z)-12,13-epoxy-11-methoxy-9-octadecenoate	565555	088335-39-1	27
45	51.871	0.15				
			Methyl 18-methylnonadecanoate	166215	1000352-20-6	99
			Eicosanoic acid, methyl ester	166218	001120-28-1	98
			Eicosanoic acid, methyl ester	166219	001120-28-1	98
46	59.331	0.61				
			17-(1,5-Dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol	205911	1000210-38-4	99
			Cholesterol	205851	000057-88-5	99
			Cholesterol	205847	000057-88-5	99

Note: The sample chromatogram is attached on the separated paper at the last page.

Analysis hour: 2 days
Remark:_____
Analysed by: Mulatu K. Signature _____ Checked by: Tigist K. Signature _____ Approved by:
(Chem./instrumentation researcher) (Chem./instrumentation team leader)

Tell. 0114391700 P.O.Box: 24692(1000) Fax: 0114392259
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AKAKI KALITY KIFLE KETEMA, ADDIS ABABA

Annex B: Qualitative GC-MS analysis test result for sheep fleshing FAME

LEATHER INDUSTRY DEVELOPMENT INSTITUTE
RESEARCH AND TESTING DIRECTORATE
QUALITATIVELY GC-MS ANALYSIS TEST RESULT

Test date: 10/06/2015
Time: 2:00 pm
Test name: Qualitative identification
Sample type: Oil sample
Sample code: C-5107
Customer code: S
Method: Internal method
Room temperature: 20 degree celcius

ORIGINAL

Pk#	RT	Area%	Name of compounds	Ref#	CAS#	Qual(% ABUNDANCE
14	17.641	0.06				
			Decanoic acid, methyl ester	50090	000110-42-9	98
			Decanoic acid, methyl ester	50098	000110-42-9	96
			Decanoic acid, methyl ester	50100	000110-42-9	95
15	21.845	0.08				
			Dodecanoic acid, methyl ester	72688	000111-82-0	98
			Undecanoic acid, 10-methyl-, methy l ester	72721	005129-56-6	91
			Dodecanoic acid, methyl ester	72687	000111-82-0	87
16	21.982	0.08				
			Tridecanoic acid, 4,8,12-trimethyl -, methyl ester (CAS)	382675	010339-74-9	64
			Pentacosanoic acid, 4-methyl-, met hyl ester $$ Methyl 4-methylpentac osanoate #	690580	055281-93-1	59
			Methyl 4,10-Dimethyldodecanoate	302225	998302-22-5	53
17	24.629	0.24				
			Methyl 4,11-Dimethyldodecanoate	302226	998302-22-6	90
			Tridecanoic acid, 4,8,12-trimethyl -, methyl ester (CAS)	382675	010339-74-9	59
			Tridecanoic acid, 4,8,12-trimethyl -, methyl ester	382672	010339-74-9	50
18	27.591	0.17				
			Methyl myristoleate	94118	056219-06-8	99
			Methyl Z-11-tetradecenoate	94132	1000130-82-8	91
			Methyl 9-tetradecenoate	94125	1000336-47-3	91
19	28.036	2.40				
			Methyl tetradecanoate	95859	000124-10-7	98
			Methyl tetradecanoate	95862	000124-10-7	96
			Tridecanoic acid, 12-methyl-, meth yl ester	95899	005129-58-8	94
20	28.880	0.12				
			branched methyl pentadecan - oate, but NOT iso- or ante-isomultiple branching ?	342408	000000-00-0	72
			Tridecanoic acid, 4,8,12-trimethyl -, methyl ester	382672	010339-74-9	50
			1-Cyclopentyl-1-propanol	34577	019833-89-7	47
21	29.577	0.24				
			Heneicosanoic acid, methyl ester (CAS) $$ Methyl heneicosanoate	566309	006064-90-0	49
			Eicosanoic acid, methyl ester (CAS	533536	001120-28-1	49

			) $$ Arachidic acid methyl ester			
			Docosanoic acid, methyl ester (CAS) $$ Methyl behenate $$ Methyl doc osanoate	596558	000929-77-1	47
22	29.813	0.40				
			Dodecanoic acid, 4-methyl-, methyl ester $$ Methyl 4-methyldodecanoa te #	261770	055955-73-2	64
			Methyl 4,8-dimethylnonanoate	182345	013758-80-0	45
			Dodecanoic acid, 4-methyl-, methyl ester $$ Methyl 4-methyldodecanoa te #	261769	055955-73-2	43
23	30.316	0.28				
			Methyl 9-methyltetradecanoate	107578	213617-69-7	98
			Methyl 13-methyltetradecanoate	107583	1000336-31-4	96
			Pentadecanoic acid, methyl ester	107593	007132-64-1	95
24	30.615	0.15				
			Pentadecanoic acid, methyl ester	107595	007132-64-1	91
			Tetradecanoic acid, 12-methyl-, me thyl ester	107610	005129-66-8	91
			Pentadecanoic acid, methyl ester	107593	007132-64-1	91
25	31.307	0.14				
			Tridecanoic acid, 4,8,12-trimethyl -, methyl ester	382673	010339-74-9	70
			Methyl 4,13-Dimethyltetradecanoate	382759	998382-75-9	47
			Heptacosanoic acid, methyl ester (CAS) $$ Methyl heptacosanoate	708337	055682-91-2	43
26	31.721	0.76				
			Pentadecanoic acid, methyl ester	107593	007132-64-1	98
			Pentadecanoic acid, methyl ester	107595	007132-64-1	97
			Pentadecanoic acid, methyl ester	107596	007132-64-1	96
27	33.378	0.11				
			Hexadecanoic acid, methyl ester (C AS) $$ Methyl palmitate	382600	000112-39-0	64
			Hexadecanoic acid, methyl ester $$ Palmitic acid, methyl ester	382746	000112-39-0	64
			Triacontanoic acid, methyl ester $ $ Methyl melissate $$ Methyl melis sicate	747947	000629-83-4	64
28	33.635	0.13				
			2-Methyl-d-glucose $$ 2-O-Methylhe xose #	163779	004132-40-5	59
			2-Methyl-d-glucose $$ 2-O-Methylhe xose	163780	004132-40-5	59
			Tridecanoic acid, 4,8,12-trimethyl -, methyl ester	382674	010339-74-9	46
29	34.201	0.18				
			Hexadecanoic acid, methyl ester	119408	000112-39-0	95
			Hexadecanoic acid, methyl ester	119407	000112-39-0	95
			Hexadecanoic acid, methyl ester	119400	000112-39-0	95
30	34.652	0.17				
			9-Hexadecenoic acid, methyl ester, (Z)-	117507	001120-25-8	99
			7-Hexadecenoic acid, methyl ester, (Z)-	117506	056875-67-3	99
			Methyl hexadec-9-enoate	117464	010030-74-7	99
31	34.856	1.92				
			9-Hexadecenoic acid, methyl ester, (Z)-	117507	001120-25-8	99
			Methyl hexadec-9-enoate	117464	010030-74-7	99
			9-Hexadecenoic acid, methyl ester, (Z)-	117513	001120-25-8	99

32	35.857	15.22				
			Hexadecanoic acid, methyl ester	119406	000112-39-0	95
			Hexadecanoic acid, methyl ester	119400	000112-39-0	95
			Pentadecanoic acid, 14-methyl-, methyl ester	119425	005129-60-2	95
33	37.341	0.18				
			Cyclopentanetridecanoic acid, methyl ester (CAS)	455324	024828-61-3	58
			Methyl 8-methyl-decanoate	182445	998182-44-5	55
			Undecanoic acid, methyl ester (CAS) $$ Methyl undecanoate	182190	001731-86-8	53
34	37.655	0.27				
			Pentacosanoic acid, 4-methyl-, methyl ester $$ Methyl 4-methylpentacosanoate #	690580	055281-93-1	59
			4,6-Di-O-methyl-.alpha.-d-galactose $$ 4,6-Di-O-methylhexopyranose #	202164	998202-16-4	53
			D-Fructose, 3-O-methyl- (CAS) $$ 3-O-METHYL FRUCTOSE $$ 3-O-Methyl-D-fructose	163771	036256-85-6	47
35	37.755	0.22				
			Hexadecanoic acid, 14-methyl-, methyl ester	131318	002490-49-5	93
			Hexadecanoic acid, 14-methyl-, methyl ester	131316	002490-49-5	91
			Tridecanoic acid, 12-methyl-, methyl ester	95898	005129-58-8	87
36	38.242	0.42				
			Hexadecanoic acid, 14-methyl-, methyl ester	131316	002490-49-5	97
			Hexadecanoic acid, 15-methyl-, methyl ester	131321	006929-04-0	96
			Heptadecanoic acid, methyl ester	131301	001731-92-6	95
37	38.594	0.41				
			Hexadecanoic acid, 14-methyl-, methyl ester	131316	002490-49-5	97
			Hexadecanoic acid, 14-methyl-, methyl ester	131320	002490-49-5	96
			Hexadecanoic acid, 14-methyl-, methyl ester	131318	002490-49-5	95
38	38.787	1.21				
			cis-10-Heptadecenoic acid, methyl ester	129385	1000333-62-1	99
			9-Octadecenoic acid (Z)-, methyl ester	141302	000112-62-9	76
			Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester	129411	010152-61-1	76
39	39.768	1.43				
			Heptadecanoic acid, methyl ester	131301	001731-92-6	99
			Heptadecanoic acid, methyl ester	131299	001731-92-6	98
			Heptadecanoic acid, methyl ester	131300	001731-92-6	98
40	42.530	0.75				
			9,12-Octadecadienoic acid (Z,Z)-, methyl ester	139724	000112-63-0	99
			9,12-Octadecadienoic acid (Z,Z)-, methyl ester	139725	000112-63-0	99
			10,13-Octadecadienoic acid, methyl ester	139716	056554-62-2	99
41	43.028	25.77				
			9-Octadecenoic acid, methyl ester, (E)-	141310	001937-62-8	99
			9-Octadecenoic acid, methyl ester,	141306	001937-62-8	99

			(E)-9-Octadecenoic acid (Z)-, methyl ester	141300	000112-62-9	99
42	43.117	1.16				
			9-Octadecenoic acid, methyl ester, (E)-	141310	001937-62-8	99
			8-Octadecenoic acid, methyl ester	141273	002345-29-1	99
			9-Octadecenoic acid (Z)-, methyl ester	141300	000112-62-9	99
43	43.269	1.39				
			8-Octadecenoic acid, methyl ester	141273	002345-29-1	99
			9-Octadecenoic acid, methyl ester, (E)-	141310	001937-62-8	99
			9-Octadecenoic acid (Z)-, methyl ester	141300	000112-62-9	99
44	43.458	0.20				
			cis-13-Octadecenoic acid, methyl ester	141299	1000333-58-3	99
			11-Octadecenoic acid, methyl ester	141291	052380-33-3	99
			8-Octadecenoic acid, methyl ester	141273	002345-29-1	99
45	44.035	12.65				
			Methyl stearate	143131	000112-61-8	99
			Methyl stearate	143126	000112-61-8	98
			Methyl stearate	143130	000112-61-8	98
46	46.320	0.21				
			Methyl 10-trans,12-cis-octadecadienoate	139709	1000336-44-2	96
			7,10-Octadecadienoic acid, methyl ester	139706	056554-24-6	95
			Methyl 9-cis,11-trans-octadecadienoate	139701	1000336-44-0	95
47	46.755	0.14				
			cis-10-Nonadecenoic acid, methyl ester	153152	1000333-64-4	99
			Cyclopropaneoctanoic acid, 2-octyl-, methyl ester, cis-	153176	003971-54-8	96
			10-Nonadecenoic acid, methyl ester	153149	056599-83-8	91

Note: The sample chromatogram is attched on the separated last page

Analysis hour: 2 days
Remark_____
Analysed by: Mulatu K. Signature_____ Checked by: Tigist K. Signature:_____ Approved by: Ts
(chem./instrumentation researcher) (chem./instrumentation team leader)

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