ADDIS ABABA UNIVERISITY

INSTITUTE OF TECHNOLOGY

SCHOOL OF GRADUATE STUDIES

CHARACTERIZATION AND ENZYMATIC TRANSESTERIFICATION OF PORK LARD FOR THE SYNTHESIS OF LEATHER FATLIQUOR AND BIODIESEL

BY

TEKLU TSEGA

A RESEARCH THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES OF ADDIS ABABA UNIVERISITY IN THE PARTIAL FULLFILMENT OF THE REQUIREMENT FOR THE MASTERS DEGREE IN SCHOOL OF CHEMICAL AND BIOENGINEERING, STREAM OF LEATHER TECHNOLOGY

ADVISOR

DR. GEETHA BASKAR

SEPTEMBER, 2014

ADDIS ABABA, ETHIOPIA

ADDIS ABABA UNIVERISITY

INSTITITUTE OF TECHNOLOGY

SCHOOL OF GRADUATE STUDIES

CHARACTERIZATION AND ENZYMATIC TRANSESTERIFICATION OF PORK LARD FOR THE SYNTHESIS OF LEATHER FATLIQUOR AND BIODIESEL

BY

TEKLU TSEGA

A RESEARCH THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES OF ADDIS ABABA UNIVERISITY IN THE PARTIAL FULLFILMENT OF THE REQUIREMENT FOR THE MASTERS DEGREE IN SCHOOL OF CHEMICAL AND BIOENGINEERING, STREAM OF LEATHER TECHNOLOGY

Approved by Board of Examiners

Chairman, Department Graduation Committee	**Signature**	**Date**
Advisor		
Dr Geetha Baskar	**Signature**	**Date**
External Examiner	**Signature**	**Date**
Internal Examiner	**Signature**	**Date**

Acknowledgement

First and above all, I praise God for providing me this opportunity and granting me strength and all the capability to proceed successfully. Next to my God I would like to express my sincere gratitude to my advisor Dr. Geetha Baskar for her continuous support of my study with full of patience, motivation, enthusiasm, and immense knowledge that helped me in all the time of research work; and Dr. Kamini for her guide and allowing me to use the biotechnology lab facilities, reagents and gas chromatogram instrument for FAME analysis in CLRI.

My sincere thanks also goes to Dr Kalarical Janardhanan Sreeram for his support in doing DSC analysis and Dr.R.Aravindhan facilitating to get the pork skin and lard used as a raw material and for their valuable advice for my thesis. I would like to thank Aarthy Mayil (PhD student in biotechnology lab), S.Raja and Ms. Ramania in Excel laboratory at Central Leather Research Institute (CLRI) of India for helping me in laboratory process.

I am so grateful for the scholarship opportunity provided by Leather Industry Development Institute (LIDI) of Ethiopia under twinning program conceded by CLRI and LIDI for making me to study my MSc both in India and Ethiopia. I give deep and heartily thanks to Mr. Wondu Legesse, director general of LIDI for his quality leadership and he has been committed and played a great role for this twinning program to be happened and completed successfully. Thanks to Mr. Taye Tibebu, leather manufacturing technology department director he has been supportive and for his valuable contribution by providing all the necessary facilities required for the successful completion of the research work. This thesis appears in its current form due to the assistance and guidance of all these and several people.

Last but not the least; I would like to thank my family: my parents for giving birth to me at the first place and supporting me financially, psychologically and spiritually throughout my life.

Table of Contents

List of Figures

List of Tables

Acronyms or symbols

A: Molar Ratio of oil to methanol

AB: Interaction effect of Molar Ratio of oil to methanol &Temperature

AC: Interaction effect of Molar Ratio of oil to methanol &Time

ANOVA: Analysis of Variance

ASTM: American Standards Tests and Materials

AV: Acid Value

B: Temperature

BC: Temperature

C: Time

CCD: Central Composite Design

CLRI: Central Leather Research Institute of India

DSC: Differential Scanning Calorimetry

EN: European Normalization (European Standards)

FAAE: Fatty Acid Alkyl Esters

FAME: Fatty acid methyl esters

FFA: Free Fatty Acid

FTIR: Fourier transform infrared spectroscopy

GC: Gas Chromatography

LIDI: Leather Industry Development Institute

ml : Millilitre

N : Normality

PPL: Porcine Pancreatic Lipase

Rpm: Revolution per minute

SV: Saponification Value

TER: Transesterification Reaction

TG: Triglyceride

UFA: unsaturated fatty acid

°C: Degree Celsius

µl: Microliter

cP: Centipoise

Abstract

Pork lard (PL) is a class of lipids and oils organic compounds consisting of both saturated and unsaturated fatty acids that can be modified into biodiesel and leather fatliquor for industrial applications. The fat of the pork was collected from the back attached below the skin and around the belly and kidney of the Caracas. After the oils and fats were extracted and purified, characterization for its physicochemical properties, pattern of triglycerides and fatty acid compositions was done by analytical analysis and using Gas Chromatography (GC), Gas Chromatography Mass Spectroscopy (GC-MS) and Fourier transform infrared spectroscopy (FTIR) instrumental tools. The aim of present research work was the synthesis of biodiesel or Fatty Acid Methyl Ester (FAME) by enzyme catalysed transesterification process using Porcine Pancreatic Lipase (PPL) as a catalyst and the production of leather fatliquor by sulfitation and external emulsification processing methods. The effect of three parameters including Pork Lard to methanol molar ratio, temperature and reaction time on the transesterification reaction (TER) was investigated by keeping 5% enzyme concentration (based on oil weight) and 200 revolutions per minute (rpm) stirring rate constant. The maximum yield of biodiesel was 81 % at 1:4 molar ratio of oil to methanol and 40 ^{o}c temperatures after 36 hours reaction time. PL consists of 44% oleic acid and 11%linoleic acid which are suitable to add polar functional group by sulfitation reaction. In this study a promising fatliquor was synthesized from PL by sulfitation and external emulsification method after complete characterization of the fats and lipids. The synthesized sulphited fatliquor gives the leather better multifunctional properties such as softness, handle, colour uniformity, roundness, and better tensile and tear strength properties. This new fatliquor also has unique waxy characteristics which give an attractive burnishable effect in finishing especially for shoe upper leather that most of the conventional commercial fatliquors cannot give.

Chapter one

1. Introduction

1.1. Background

The main component of oils and fats is triglycerides which composed of glycerol and free fatty acids. Different types of oils and fats are exploited for industrial applications both for food and oleo-chemical products. Since the most reactive sites of any oils and fats are carboxyl and unsaturated double bond group; the industrial products are synthesized by chemical modification of both these groups present in fatty acids. Therefore identification of the chemical composition and functional groups of these oils is necessary for further analysis of transesterification, emulsification and chemical modification of the oil for their intended use as in the production of leather fatliquor. Oils from vegetable, marine and land animal sources are being used for the production of biodiesel and lubricants in a variety of industrial applications including metalworking and leather making [1].

PL is triglycerides composed of fatty acids and the distribution of these fatty acids varies from oil to oil. Lard is being used traditionally as an ingredient by many people for making laundry soap, cosmetics, shortening and heat transfer medium in cooking. Using of lard for industrial application is advantages because it is cheap, easily obtainable, and easy for modification. This fat can be combined with vegetable oils for different applications.

Animal fat including pig fat is recovered from slaughterhouse usually by applying heat treatment which melts it allowing gravity separation from non-fat materials. This treatment may be applied in heated vats or by means of continuous cooker-extruder type technology. One of the main industrial uses of oils and fats is the production of biodiesel which is the mono alkyl ester of long chain fatty acids obtained by the transesterification of biological resources such as vegetable and animal fats and oils. Transeterification is an important class of organic reactions where an ester is transformed into another through interchange of the alkoxy moiety, it used to get new esters with special properties and chemical compositions based on the selected ester or alcohol reactants. Transesterification reaction is an equilibrium reaction which is accelerated by the presence of catalysts such as strong acid, Alkalis or biological enzymes [1]. Using biodiesel as alternative sources of fossil and petroleum fuels helps to reduce the

environmental pollution from emissions of CO_2, SO_2, particulate matter and poly-aromatic hydrocarbons during the combustion of fossil fuel. It also helps to minimize the cost spent for importing the petroleum based fuel especially for the importer countries like Ethiopia. Biodiesel is a renewable non-toxic biodegradable fuel, with a high heating value and high energy oxygen content [2].

Leather fatliquor is another important industrial products obtained from oils and fats. Fatliquoring products are formulated from a variety of different raw materials, such as different vegetable oils, alcohol and fatty acids, fish oil, paraffin, lanolin, lecithin, and others. Fatliquors are prepared by sulphation, sulfitation, sulphochlorination and external emulsification process in order to add different polar groups that make them emulsifiable in water and to enable them to properly penetrate the leather matrix for better lubrication. Nowadays many types of fat-liquoring products with different chemical natures are being synthesized which give certain specific functional and desirable properties to the leather. Fat liquors have an important role in the leather processing which gives most of the desired leather physical and organoleptic properties. All types of leather need to be treated with fatliquor to a greater or lesser degree. It affects the physical properties of leather and creates better softness, flexibility, touch and fluffiness. Most of the developing countries like Ethiopia are importing most of the fat-liquors from all over the world especially from china and Europe for the fulfilment of the requirement and need of the leather industries.

For this particular research porcine pancreatic lipase (PPL) enzyme is used as a reaction catalyst for the transesterification of pork lard. This enzyme has been widely applied in organic synthesis because of it is readily available in commercially, easy to handle, do not require any coenzymes, reasonably stable, and often tolerate organic solvents. PPL is a class of lipase enzyme that hydrolyse fatty acids from lipid species triacylglycerol or phospholipids. A number of lipases, mainly of bacterial origin, are now available immobilized onto a solid support for use as industrial scale catalysts. The significant differences between lipase and chemically catalysed reactions include, lipase catalysed reactions take place at a lower temperature and has few side reactions that gives clean products and it is an environmentally friendly in comparison to acid and alkali catalysed transesterification. The other advantages of enzyme catalysed reactions are more selective than chemical catalyst [1]

1.2. Statement of The Problem

Insufficient raw material availability, poor chemical quality and environmental effects are the major problems recently have been growing and affecting the chemical and leather industries. In connection to leather fatliquors one of the most challenges affecting the leather industry is the shortage of the common oils such as castor oil, linseed oil, fish oil, cod oil, sperm oil and greases used as leather lubricants. As a result of this the price of leather chemicals and petroleum products are increasing from time to time which highly affects the competitiveness of the tanning industries and transportation in the developing countries like Ethiopia where there is no imminent technological solution for the forthcoming oil shortage. In those countries most of the petroleum products and leather chemicals including fatliquors are being imported from foreign countries. Researches and technologies to substitute these imported chemicals by producing them locally from local raw materials like PL is the best option to solve the problems. This thesis work is aim to introduce scientific and technological option for the production of biodiesel and leather fatliquors from local PL based on eco-friendly manufacturing process using lipase enzyme as a catalyst.

1.3. Objective

1.3.1. General Objective

The general objective of this study is to produce biodiesel by transesterification reaction using PPL enzyme as a catalyst and to synthesize leather fatliquor from PL and its FAME.

1.3.2. Specific Objectives

- To characterize the fatty acid composition and physicochemical properties of PL.
- To optimize the process variables such as temperature, molar ratio of oil to alcohol and reaction time for the transesterification reaction to get the highest possible yield of biodiesel.
- To produce biodiesels and leather fatliquors and to study the physical and chemical properties of the product against the standard value.
- To evaluate the fatliquor performance on the leather by producing sheep garment leather.

1.4. Significance of The Study

The production of fatliquor and biodiesel from PL by enzymatic transesterification reaction has economic and environmental advantages for the country and the tanning sector.

Some of the significances of this thesis study include:

- New source of fatliquor and biodiesel was identified from local PL.
- The fatliquor can be used for the leather making process directly or by blending with other existing commercial fatliquors that enables the country to substitute the imported leather lubricants that save foreign currency and the same is true for biodiesel.
- Substituting fossil and petroleum based fuel by biodiesel helps to minimize environmental impact by reducing the emission of greenhouse gases from these mineral oils.
- Transesterification of the PL by enzymatic method has many advantages such as: reactions take place at a lower temperature, fewer side reactions are formed; cleaner products are obtained, more selective and an environmentally friendly alternative than the existing chemical catalyzed transesterification reaction.
- The result of this research work used as a base line for other researchers who further study on this area beyond the scope of this thesis work, this is because only few leather chemicals such as salt, lime and sulphuric acid are produced locally from available indigenous materials in Ethiopian and most of the other basic and speciality chemicals like syntans, dyes, fat liquors, special auxiliaries and finishes for leather industries need to be imported.

1.5. Scope of The Study

The present research work covers the scientific study of the nature and composition of PL, standardization of lipase catalysed transesterification reaction, production of fatliquor and biodiesel. It also covers the development of finished leathers from this fatliquor and comparing the quality of leather produced from experimental work with comparable commercial fatliquors.

Chapter Two

2. Literature Review

2.1. History And Description of Pig

Pigs are believed to have been domesticated from wild boar as early as 9000 years ago. They were originally native to Europe and parts of Asia. Later on they have been introduced to many parts of the world over the centuries. Most pigs live as livestock, but some have become feral, having escaped from farms or been deliberately introduced into the wild for hunting [3].

Around 1.3 billion pigs are slaughtered annually for meat worldwide. The majority of these are in East Asia, particularly China, which rears around half of the world's pigs. This is followed by the EU, North America, Vietnam and Brazil. The majority of pigs are reared for meat and a smaller number are kept for breeding [4].

Figure 2.1 Ethiopian pig

2.2. History And Description of PL

Pig fat is commonly known as lard which is fat in both its rendered and unrendered forms. Pig fat is triglycerides which are composed of three fatty acids like tallow composition and the distribution of fatty acids varies from oil to oil. Pigs that have been fed different diets will have lard with a significantly different fatty acid composition and

different chemical properties such as free fatty acid value, iodine value, soap value and ester value. Lard or pig fat can easily melt when it exposed to heat. Lard contains high levels of cholesterol and has no natural antioxidants. Hence, the level of oxidation should be maintained by adding natural or synthetic antioxidants to the oil (Akoh and Moussata, 1998; Gunstone, 1999; Yang et al., 2003).

Lard was commonly used for soap making and as a cooking fat or shortening, or as a spread similar to butter. The qualities of lard vary depending on the part of the pig from which the fat was taken and how the lard was processed.

2.3. Status of Pig Population in Ethiopia

The livestock population of Ethiopian pig is small compared to cattle, sheep, goat and camel. The overall livestock population is believed to be the largest in Africa , and the 10^{th} largest in the world which accounts 35 million cattle and buffalo, 40 million sheep and goat and 25 000 pig. Annually 2.7 million hides, 8.1 million sheepskins and 7.5 million goatskins are produced. (Kiruthu 2002). Based on the data 2003, the annual growth rate of pig population reached 2.3 % which is the largest comparing to the population growth of cattle & buffalo (1.9%) and sheep & goat (0.3%) during the period 1993-2003. This indicates that the meat industry of pig has been growing vastly as a result of the change in attitudes of people to eat the pig meat previously which believed as a prohibited and unclean in some religious and cultural belief. According to FAO 2005 report the pig population in Ethiopia was estimated to be 29,000. In many rural parts of Ethiopia, pig production was characterized by extensive production system whereby pigs are allowed to scavenge at backyard and municipal garbage dumping sites [10].

2.4. Transesterification of Oil

Transesterification is the important class of organic reactions where an ester is transformed into another through interchange of the alkoxy moiety. This reaction process is carried out by mixing the original ester or triglycerides with alcohols or with other esters. The fatty acid and alcohol groups can be exchanged by reaction with an excess of other fatty acids (acidolysis), alcohols (alcoholysis), or other esters (interesterification).

The most commonly used alcohol in transesterification reaction is methanol, ethanol, iso-propanol, and butanol. The main factor for the selection of alcohol is the water content. Triglycerol is the starting point for this reaction and by which the composition and properties of oils and fats can be modified.

Both Acidolysis and alcoholysis reaction can be catalysed by acid or alkali or enzyme and used to modify triacylglycerol composition through the reaction process. Methanolysis of triglycerols is termed as transesterification which is used to prepare fatty acid methyl esters by which fatty acid analysed.

As reported in several literature biodiesel and other lubricating chemicals are produced on the industrial scale by methanolysis of different animal and vegetable oils or waste fat, particularly using frying oils [1].

Bio diesel is a chemical compound made by the transesterification of vegetable oil. The triglycerides, or fat, in the oil are converted into useable fuel through the process of reaction. The natural oil is nothing but triglycerides that is an ester. Since one triglyceride contain 3 reactive carboxylic group, the stoichiometric reaction requires 1mol of a triglyceride and 3mol of the alcohol. Adding excess alcohol is used to increase the yields of the new alkyl or methyl esters and to allow its phase separation from the glycerol formed.

Biodiesel has many advantages over petroleum diesel. Some of them include better lubricity, low toxicity, renewable, domestic feedstock, superior flash point and biodegradability, negligible sulphur content, and lower exhaust emissions. It has also some disadvantages like high feedstock cost, inferior storage and oxidative stability, lower volumetric energy content, inferior low-temperature operability, and in some cases, higher NOx exhaust emissions [5]

2.5. Chemical Reaction in Biodiesel Production

Vegetable oil or animal fat feedstock and alcohols are the reactants in biodiesel production and they requires selective catalysis and controlled process conditions to get quality products that meet the standard specification to satisfy the customer. The purity of the feedstock is an important factor for the reaction. In the TER catalyst speeds up the

exchange of glycerol with methanol creating methyl esters of fatty acids [9]. The chemical reaction is given in fig 2.2

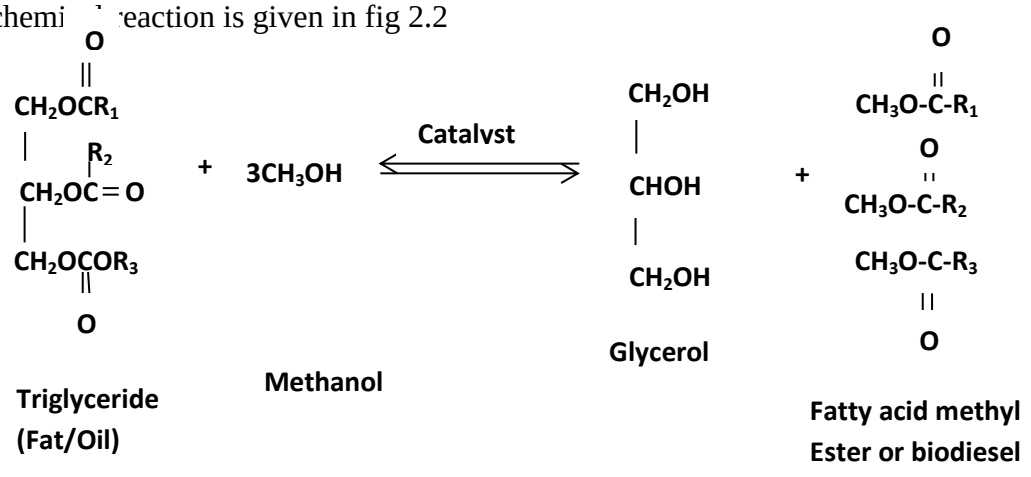


Figure 2.2: Transesterification chemical reaction Process

Where:

- R_1, R_2 and R_3 are the fatty acid group of triglyceride components which can be:
  - Palmitic acid = $CH_3(CH_2)_5CH=CH(CH_2)_7COOH$
  - Myristoleic acid = CH3(CH2)3CH=CH(CH2)7COOH
  - Oleic acid = $CH_3(CH_2)_7CH=CH(CH_2)_7COOH$
  - Linoleic acid = $CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$
  - Linolienic acid = $CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$ and etc…

In biochemistry, a fatty acid is a carboxylic acid with a long aliphatic tail, which is either saturated or unsaturated. Most naturally occurring fatty acids have a chain of an even number of carbon atoms, from 4 to 28. Fatty acids are usually derived from triglycerides or phospholipids. When they are not attached to other molecules, they are known as free fatty acids (FFA) [6].

2.6. The Process of Transesterification Reaction

The manufacturing process of biodiesel is based on the conversion of triglycerides and alcohols to fatty acid methyl esters and glycerol using acid, alkali or enzyme as a catalyst [7]. The process of biodiesel production can be either batch or continuous. Most of the time conventionally batch reaction process is being used to transesterified Vegetable oils to FAME in the presence of an excess amount of alcohol, and catalyst. Adding excess of

alcohol helps to increase the solubility of triglyceride, to make the viscosity of Fatty Acid Methyl Este (FAME) low and to shifting the chemical equilibrium. After the reaction is completed the excess ethanol can be recovered for the next batch process [8].

Transesterification reaction in continuous is preferable for the bulk production of biodiesel. Two reactors are needed for this; in the first reactor, esterification of free fatty acids with ethanol is takes place and then the transesterification reaction follows in the second reactor. This process working at low pressure that is capable of processing a feedstock with a larger amount of free fatty acids, such as unrefined non edible vegetable oils, tallow fat and used cooking oil [8].

"A modern transesterification plant is continuous instead of batch. A continuous plant leads to better heat economization, better product purity from phase separation by removing only the portion of the layer furthest from the interface, better recovery of excess methanol in order to save on methanol cost and regulatory issues, minimal operator interference in adjusting plant parameters, and lower capital costs per unit of biodiesel produced." [9].The synthesis of biodiesel has the following major unit operations. It contains oil and catalyst mixer, main reactor, methyl ester/glycerol separator, methanol flash unit (for continuous recycling of feedstock methanol), water wash for methyl esters, glycerol/water separator, and a water pH balance unit. All pumps and motors are included, along with the necessary process controls. A glycerin/methanol distillation module can be added to the system to remove methanol from crude glycerin. The following figure shows the general process flowsheet for TER [11].

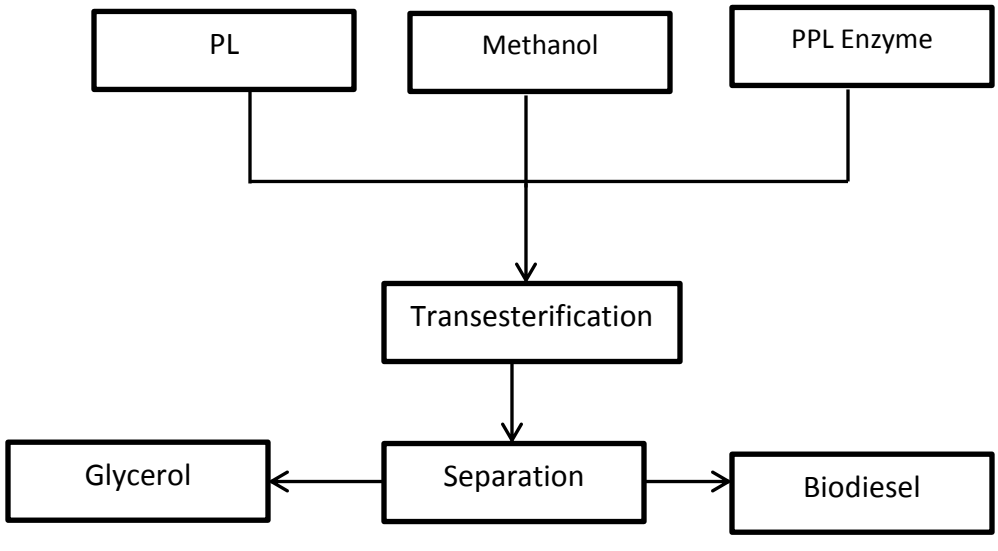


Figure 2.3: Process flow sheet diagram of transesterification reaction

In general, transesterification reaction process consists of five sections that include:

- Two-stage reaction unit
- Separation unit
- Water washes unit
- Methanol and water recovery unit
- Methanol and water purification unit by distillation for reuse.
- Each separate unit operations are highly dependent on one another [9].

2.7. Mechanism of Transesterification Reaction and its Kinetics

Transesterification process is a process of converting fatty acid and free fatty acid into a single fatty acid alkyl ester with an alcohol in two-step mechanism; release of alcohol by hydrolysis of triglycerides followed by esterification with the second substrate.

A Ping-Pong bi-bi one mechanism is a widely accepted mechanism for TER of triglycerides of oils and fats. Addition of the substrates between the reactions is important after each product is being released. The kinetics of the conversion is regulated based on the concept of simplified Michaelis - Menten kinetics.

2.8. Application of Transeterification Reaction

Several relevant industrial processes use this reaction to produce different types of compounds. The production of polyethylene terephthalate, which involves a step where dimethyl terephthalate is transesterified with ethylene glycol in the presence of zinc acetate as catalyst is an example. Furthermore, a large number of acrylic acid derivatives are produced by transesterification of methyl acrylate with different alcohols, in the presence of acid catalysts [11].

The most widely used application of TER is the alcoholysis of vegetable oils and animal fats to produce Fatty Acid Alkyl Ester (FAAE) which is an excellent substitute for diesel fuel. Although, conventional chemical technology using alkaline catalysts has been applied to alkyl ester production, there are several drawbacks to this approach including difficulties in the recovery of glycerol, the need for removal of catalyst and the energy intensive nature of the process. The utilization of lipase in alcoholysis is considered as an effective means of circumventing these problems. Several reports describe enzymatic

alcoholysis of vegetable oils. When ethanol, isopropanol, butanol and long chain alcohols were used as substrates, the oils were efficiently converted to their fatty acid esters [13].

2.9. Factors Affecting Transesterification of Oil

2.9.1. The Effect of Molar Ratio of Oil To Alcohol

Molar ratio of alcohol to oil is one the important factors affecting the yield of the reaction. According to the stoichiometric ratio of transesterification reaction 3 moles of alcohol per mole of triglyceride are required which produce 3 moles of ester and 1 mole of glycerol. The molar ratio to achieve the desired yield is correlated with the type of catalyst used. Transesterification is an equilibrium controlled reaction in which excess of alcohol can be used to get complete conversion and alcohol is easily recoverable. Further, the conversion efficiency is remains the same, but to decrease the energy increment required for the recovery of alcohol, we should avoid increasing molar ratio of alcohol to oil. Excessive amount of alcohol makes the recovery of the glycerol more difficult by hindering the decantation by gravity that decreases the yield of ester since part of the glycerol remains in the biodiesel phase. The molar ratio of alcohol to oil is associated with the type of catalyst used such as enzyme, alkali and acid and it should be optimized by experiment. The molar ratio of alcohol to oil has no effect on acid value, saponification values and iodine values of esters.

2.9.2. The Effect of Purity (Moisture and Free Fatty Acids Content) of Triglycerides and Alcohols

The quality of reactants (oil and alcohol) in the enzyme catalysed transesterification process has a greater effect on reaction rate and yield of a new ester product. Less acid content of triglycerides and less moisture content gives better conversion as explained in many literatures. High acid content will consume more catalysts and higher moisture content will increase the formation of soap especially for alkali catalysed reaction which results in the decrease in catalyst activity or efficiency. The soaps formed cause an

increased product viscosity, the formation of colloidal solids and the difficulty in glycerol separation.

2.9.3. The Effect of Catalyst

As it has been already noted earlier the transesterification process catalysts are categorized either as alkaline, acidic or enzymes.

Alkali and acid catalysed transesterification

Commercially, the most common method of biodiesel production is alkali-catalyzed transesterification (Freedman et al, 1984, 1986). In this process triglyceride of an oil and fat is reacted with alcohol in the presence of an alkali catalyst to yield biodiesel and glycerol. Alkali catalyzed transesterification is faster than the acid catalyzed reaction. Acid catalyzed transesterification reaction is more suitable to prevent the formation of soap in the case of the oil or fat when it has a high free fatty acid and water content. 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 process

The following chemicals have been used as a catalyst for this reaction as reported in different papers.

- Alkalis
- Sodium hydroxide (NaOH)
- Potassium hydroxide (KOH)

  Acids
- Sulphuric acid (H_2SO_4)
- Phosphoric acid (H_3PO_4)
- Hydrochloric acid (HCl)
- Sulfonic acid (RSO_3H)

  Other salts
- Sodium methoxide (CH_3ONa)
- Sodium amide ($NaNH_2$)

- Potassium amide (KNH_2)
- Sodium and Potassium hydrides

Enzymes

- Different lipase enzymes

[Source: F. Ma, et al (1999)]

2.9.4. The Effect of Reaction Temperature

The transesterification can take place at different temperatures depending on the types of enzyme catalyst, oil and alcohol used. Immobilized enzyme is more stable for temperature as compared to free lipase. The heat of reaction for transesterification is generally small and the equilibrium conversion is expected to be not influenced in the temperature range for production of biodiesel (20–70^{o}c) [17].

The effect of temperature on enzymatic transesterification of oil was summarized until now as follows [18]:

- The initial rate of reaction increases with reaction temperature
- Immobilized lipases show more temperature resistance than free ones
- Some enzymes are partially deactivated at 60 ^{o}c by methanol or ethanol within the first 24 hours, while others are practically not affected during such a short reaction time.

2.9.5. The Effect of Stirring Rate

Transesterification reaction occurs in the interfacial region between the oil and alcohol and the reaction rate is a moderately slow since oil and alcohol is not completely miscible. So to increase the reaction rate for dynamic mixing or agitation is required to increase the area of contact between the two immiscible phases. Since the reaction is carried out between heterogeneous mixtures, the mass transfer of biodiesel from the oil phase towards the alcohol-oil interface is a vital step that limits rate of alcoholysis reaction. Therefore the degree of stirring can varies the kinetics of TER, as the stirring rate is faster, the reaction will be accelerated [19].

2.10. Lipase-Catalysed Transesterification Reaction

Lipase is an enzyme that hydrolyzes lipids by breaking down the ester bonds in triglycerides to form fatty acid and glycerol to get new alcoholic ester from triglyceride and alcohol through esterification or TER. Hydrolytic enzymes have been widely applied in organic synthesis because of they are ready available, easy to handle, do not require any coenzymes, are reasonably stable, and often tolerate organic solvents. Their potential for regioselective and especially for enantioselective synthesis makes them valuable tools.

Although the enzyme-catalyzed transesterification processes are not yet commercially developed, more recent studies have been reported in recent articles and patents shown that biodiesel can be enzymatically produced by lipase-catalyzed transesterification process. The advantage of using lipases as a biocatalyst for transesterification of oil over chemical methods using alkaline or acid catalysts includes [20].

- The high efficiency and selectivity,
- Easy to separate glycerol from biodiesel and easy purification [21-23]
- Toleration of water into the oil and raw material impurities
- Low energy consumption (reaction requires mild conditions) and
- Low waste amounts (more environmentally friendly)
- Esterification of free acids also takes place
- Soap formation is avoided,
- Both reactions producing in the same step and thus the washing step is eliminated.
- Can produce biodiesel in a lower number of steps using less energy and with drastically reduced amount of wastewater
- Disadvantage of enzymes as catalysts for transesterification over chemical methods using alkaline or acid catalysts includes [14]:
- Low reaction rate
- Very high cost for industrial scale use:
- Loss of activity within about 100 days of operation

The common aspects of these studies consist in optimizing the reaction conditions (solvent, temperature, pH, type of microorganism which generates the enzyme, etc.) in

order to establish suitable characteristics for an industrial application. The reaction media highly affects the enzyme activity for the transesterification reaction. Water and solvent are the two common reaction media as reported in many literatures in the production of biodiesel from different oil sources. The enzyme activities for oil hydrolysis can be estimated by enzyme assay using P-NPL as a substrate by spectrophotometer or titration. The assay to estimate the enzyme activity for transesterification reaction also done by following standard method by using butyric acid as a substrate [13].

2.10.1. Choice of Enzymes

Nonspecific lipase should be chosen in order to transform glycerides (mono, di and tri) and free fatty into alkyl esters. A large variety of lipases has been used for transesterification reaction [15]. The origin and formulation of lipase enzyme highly affects the use and performance on hydrolysis and esterification of lipids and oils.

The lipases enzymes are able to perform effectively and give conversions over 90% at temperatures ranging between 30 and 50 ^{o}c and reaction times from 8 hours for immobilized enzymes to 90 hours for the same free enzymes, depending of the oil and the alcohol used.

The maximum biodiesel yield in general depends on the origin of lipase, enzyme formulation (immobilized or not), alcohol type used, alcohol to oil molar ratio, optimal activity of water, reaction temperature, reaction time, enzyme concentration, presence and types of solvent and enzyme life time [15, 16].

PPL is triacylglycerol lipase (EC 3.1.1.3) which is a biomolecule that has been known and used for several years. PPL is used mainly for hydrolysis of all types of ricemic esters and for transesterification. It is easy accessible enzyme on the international market, has high degree of stability, don't require cofactor and cheaper than any other enzymes [38].

2.10.2. Enzyme Recovery and Reuse

The goal of enzyme recovery is to extract, concentrate, and purify as much enzyme as possible after the completion of reaction to reduce the cost of enzymes. To recover the

enzymes in the production of biodiesel the reaction mixture two different stages of settling is required for purification. “Initial settling results in a heavy phase that includes glycerol, methanol, water and enzymes, as well as a light phase of crude biodiesel. At this point, the crude biodiesel can be withdrawn from the reactor and refined, while the heavy phase requires further separation. The second stage of settling results in a transparent glycerin phase, which falls to the bottom of the reaction vessel, and the enzymes, which migrate to the interface between crude biodiesel and glycerin. This second separation can be expedited by the use of flocculants or mechanically separated with membranes. A portion of the glycerin phase is withdrawn and refined, while the remaining glycerin and the layer of active enzymes are retained in the reactor and reused to catalyze the next reaction. Combining proper feedstock, pre-treatment and optimized reaction conditions allows running a series with a minimum of eight to ten enzyme reuses, with a 10 percent addition of fresh enzymes to each batch.” [39].

2.11. Physicochemical Properties and Quality Parameters of PL and FAMEs

The physicochemical and quality standards and specifications of FAMEs / biodiesel/ is determined based on the standard test methods. Currently there are two specifications that determine the market and commercial character of biodiesel production. These are the ASTM (D6751) and the CEN (pr EN14214) that require the purity levels demanded by engine manufacturers and petroleum suppliers. “Product specifications are driven by consumers with limited input by producers, unlike petroleum fuel markets. In most cases, the distribution chain from manufacturing to consumption involves a product quality assurance program based upon ISO (BQ9000). The U.S. based National Biodiesel Board (NBB) supported the ASTM specification development” [9].

The ASTM and EU biodiesel property specifications with the recommended test methods are given in Table 2.1.

Table 2.1: Standard Specifications of Biodiesel: USA and European

Property	Unit	USA	EU	Recommended
		ASTM D 6751	EN 14214	Test method
Density at 15^0C	Kg/m^3	-	860 – 900	ASTM D 4052
Kinematic viscosity at 40^oC	mm^2/s	1.9 -6.0	3.5 - 5.0	ASTM D 445
Flash point	^{o}C	≥ 120	≥ 130	ASTM D 93
Cloud point	^{o}C	-	-	ASTM D 2500
Sulphur content,100%	w%	≤ 0.05	≤ 0.01	ASTM D 5453
Sulphated Ash	wt%	≤ 0.02	≤ 0.02	ASTM D 874
Water content	mg/Kg	-	≤ 500	EN ISO 12937
Total contamination	mg/Kg	-	≤ 24	EN 12662
Water and sediment	% vol.	≤ 0.05		ASTM D 2709
Cetane number	-	≥ 47	≥ 51	ASTM D 613
Acid number	Mg KOH/g	≤ 0.8	≤ 0.5	ASTM D 664
Oxidation Stability ,110^oC	Hours		≥ 6	EN14112
Ethanol content	wt%		≤ 0.2	EN 14110
Ester content	wt%		≥ 96.5	EN 14103
Carbon Residue,100%	wt%	0.05 max		ASTM D 4530
Triglycerides	wt%		≤ 0.20	EN 14105
Diglycerides	wt%		≤ 0.80	EN 14105
Monoglycerides	wt%			EN 14105
Free glycerol	wt%	≤ 0.02	≤ 0.02	ASTM D 6584
Total glycerol	wt%	≤ 0.24	≤ 0.25	ASTM D 6584
Iodine value	$gI_2/100g$		≤ 120	EN 14111
Phosphorus	mg/Kg	≤ 10	≤ 10	ASTM D 4951

Source: Adopted from Biodiesel industries, Australia 2003.

The definition of some physicochemical properties of biodiesel was explained as follows.

Density: - is explained as mass per unit volume of a substance at a specific temperature. The higher density of biofuel indicates presence of large mass in a specific volume. The higher the density means the biofuel is heavy [29].

Cold Flow Properties - These properties describe the fluidity of biodiesel at low temperature. Compared to petro-diesel, biodiesel has higher cloud point/pour point. Pure biodiesel begins to cloud at 55°F and become gel at a temperature of 32°F [24]. Biodiesel produced from vegetable oils such as rapeseed oil has better fluidity in cold clime than beef tallow from animal fats [25].

Viscosity - The viscosity of a fluid is a measure of its resistance to gradual deformation by shear stress or tensile stress. For liquids, it corresponds to the informal notion of "thickness" [26]. A fluid with large viscosity resists motion because its molecular makeup gives it a lot of internal friction. A fluid with low viscosity flows easily because its molecular makeup results in very little friction when it is in motion.

Kinematic Viscosity - is the resistance to flow of a fluid under gravity. The kinematic viscosity is the ratio of the dynamic viscosity μ to the density of the fluid ρ. It is usually denoted by the Greek letter nu 'ν'.

Flash Point- is the lowest temperature at a volatile material can be vaporized to form an ignitable mixture in air. Measuring a flash point requires an ignition source. At the flash point, the vapour may cease to burn when the source of ignition is removed. The flash point is often used as a descriptive characteristic of liquid fuel, and it is also used to help characterize the fire hazards of liquids. "Flash point" refers to both flammable liquids and combustible liquids. There are various standards for defining each term [27].

The Acid Number or Acid Value (AV) - In chemistry, acid value or acid number is the mass of potassium hydroxide (KOH) in milligrams that is required to neutralize one gram of oil. It is an important quality measurement of crude oil [28].
The acid value may increase with time as the fuel degrades due to contact with air or water.

Ash Content - is the residue remaining after a fuel sample has been burned. For biodiesel, this test is an indicator of the quantity of residual materials in the fuel that came from the catalyst used in the transesterification reaction and raw material used. Ash is the general term used to describe the inorganic matter in a fuel.

Cetane Number (CN) - is a measurement of the combustion quality of diesel fuel during compression ignition. CN is the approximate equivalent of octane rating for gasoline. It is an important factor in determining the quality of diesel fuel [30].

Iodine Value (IV) - is a measure for the number of double bonds in a sample. It is the amount of iodine in g/100 g sample that can be added to the sample under the given conditions. The determination of the iodine number in fatty acids or biodiesel is covered by European standard EN 14111. According to ASTM, the iodine number helps to indicate the oxidation stability of the biodiesel. The higher iodine number represents the lower oxidation stability [31].

2.12. Leather Fatliquor

Fatliquoring of leather is performed to impart softness, flexibility, feel, drape, run and strength. This is done by coating the individual fibres or fibre bundles with the oil that reduces the internal friction and the fibre can slide over one another to give required softness and flexibility. The medium used in leather processing is water, so the oil cannot be used as such but in the form of emulsion to ensure ease of application and to get the required diffusion. The oil in water emulsion is called fatliquors. Emulsification is done in two ways; external emulsification and internal emulsification. External emulsification of oil is done by mixing the oil with emulsifiers in appropriate proportion. Internal emulsification is done by building the emulsifying groups in the oil molecules to fix the fat to the fibre structure. Most commonly fatliquoring is done by oils-with inbuilt emulsifying agent.

The traditional raw material used for fatliquor for many decades has been fish oil. Fish oil contains high amount of long unsaturated carbon chains which gives superior softness to the leather. The presence of these unsaturated groups helps for sulfitation or Sulphation

reaction to takes place so that fish oil can easily be processed into the anionic, lubricating components, which effectively prevent the leather fibres from sticking together.

Regarding the use of fish oil, nowadays nearly 90% of the yearly outputs are used for aqua feed, and only 7% for industrial applications, including also the production of leather fatliquors. In comparison to 1 million ton of fish oil, almost 60 million tons of rape seed oil or 30 million tons of soy bean oil are produced annually. Both oils are potential precursors for leather fatliquors. Main application of these vegetable oils include again animal feed, direct human consumption, and of course, with increasing importance, the production of bio-fuel [37].

2.13. Classification of Leather Fatliquor

There are many classifications of fatliquors. They are classified based on three main categories. These are:

- Origin of the oil
- Preparation method
- Charge characteristics

2.13.1. Based on Origin of the Oil

- Animal based fatliquor which commonly includes fish / cod fish/ sardine, sperm oil, neats foot oil and frog leg oil.
- Vegetable based fatliquor: these oils have comparatively less unsaturated fatty acid than animal's oil. Castor oil, cotton seed oil and linseed oil are the common source of vegetable based fatliquor.
- Synthetic fatliquors: these are mineral based fatliquor or paraffin oil with no unsaturation and have high emulsifying power and good fastness. Synthetic fatliquors are made into emulsion by sulphochlorination. The most probable fatty acids present in natural oil are lauric acid, oleic acid, linoleic acid, linoleinic acid. The choice of oils for fatliquor preparation is donning based on molecular size, viscosity, drying characteristics, degree of unsaturation, free fatty acid content and pour point or solidification point in the case of paraffin oil.

2.13.2. Based on Preparation Method

Fatliquors are classified into sulphated, sulphited and sulphochlorinated based on their preparation method. In sulphated fatliquors the sulphur is directly attached to the hydro

carbon by oxygen. Sulphited fatliquors are a mixture of sulphated and sulphonated fatliquors where sulphur atom is directly attached to the hydrocarbon. The third preparation method is sulphochlorination which is the combined reaction of sulphur dioxide and chlorine with saturated aliphatic hydro carbons under the action of UV- light.

Sulphated Fatliquor

Sulphated fatliquors are obtained from the reaction of animal or vegetable oil with sulphuric acid or gaseous SO_3 at low temperatures so as to add polar hydrophilic functional groups into the triglycerides to make it water soluble. The degree of emulsion formed in water depends on the degree of sulphation process. As the sulphation is higher than condensation it will have greater stability to acids, higher emulsifying power, greater penetration power inside the skin or hide collagen matrix and the lower the lubricating power. Since the lubricating power comes from only the neutral oil the fatliquor should have optimum oil to sulphate ratio to get better softness property to the leather.

Sulphated fatliquor was important to impart softness, filling, grain tightness, elasticity and strength. Most of the sulphated fatliquors exhibits anionic properties and the pH is adjusted by adding solution of NaOH which neutralize traces of sulphuric acid.

The Chemistry of the Preparation of Sulphated Fatliquor

Three main steps are carried out to synthesize sulphated fatliquors. These are preparation, brine wash and neutralization [40].

Preparation

10-20 % of concentrated sulphuric acid was on the weight of the oil is added slowly to the oil with constant stirring by maintaining the temperature less than 28 ^{o}c. This exothermic reaction must be controlled to prevent the drastic increase in temperature that causing the oil to be charred and darkening. Overheating may cause darkening of the oil due to oxidation or polymerization as well as hydrolysis of triglycerides may occur that can release free fatty acids causing fat spew problems observed on the finished leather due to migration of carboxylic acid from internal structure of the skin to the grain surface.

CH_3-$(CH_2)_n$=CH-OH-CH_2-CH=CHOH-$(CH_2)_n$-COOH + H_2SO_4

$\longrightarrow$$CH_3$-$(CH_2)_n$=CH-OH-$CH_2$-$CH_2$-$CH_2$-$OSO_2OH$-$(CH_2)_n$-COOH (sulfonic acid) [40].

Brine Wash

This is the process of removing excess free acid by washing the partially sulphate with brine. Washing also separates the oil part from the aqueous part. Brine also used to avoid creating an emulsion when water alone was to be used [40].

Neutralization

This is the last step done to neutralize the free acid group with alkali mainly using sodium hydroxide [40].The degree of sulphation affects the properties of fatliquor during application on the leather; as the degree of sulphation is less it will have high oiling effect on the surface of the leather but less penetration power to the leather matrix whereas the greater the sulphation the greater the emulsion penetration into the wet leather, reducing the oiliness of the grain and flesh surface. The degree of sulphation can be expressed in terms of SO_3 percentage combined with the oil or in terms of percentage of neutral oil present.

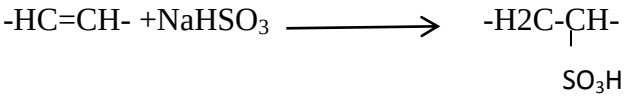
Sulphited Fatliquor

This is another class of fatliquor that prepared by oxidation method using sodium Meta bisulfite as a reagent. The preparation has two major processing steps; sulfitation/ oxidation followed by brine washing at a temperature of 60 – 80^{o}c. For oxidation process air/ oxygen or hydrogen peroxide can be used. Washing with brine is used to remove excess sodium bisulfite. Sulfitation of oil has the following advantages over sulphated fatliquor.

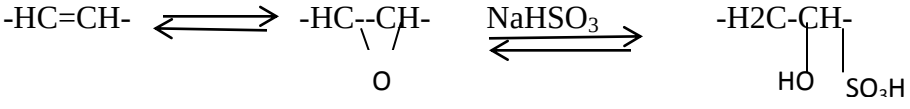
- No darkening or charring at high temperature
- Has high emulsion stability to acid, hard water salts and metal ions [40].

Reaction in sulfited fatliquor preparation

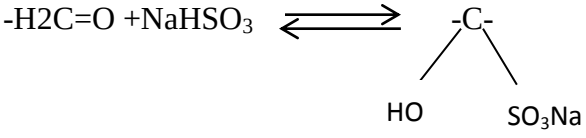
- Bisulfite reacted with the double bond of oil



- Oxidation of double bonds, then reaction with bisulphite



- Reaction between carbonyl group and bisulphite



2.13.3. Based on Charge Characteristics

Fatliquors are classified as anionic, cationic, non-ionic and multi charged or amphoteric fatliquors.

Chapter Three

3. Materials And Methods

3.1. Materials

3.1.1. Raw Materials

- Pig skin
- Pig tallow
- Sheep skin
- Castor oil
- Emulsifiers

3.1.2. Chemicals

porcine pancreatic lipase, methyl alcohol, ethyl pentanoll, methoxy mono ethylene glycol, n-hexane, Standard fatty acids (lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, linolineic acid and their corresponding fatty acid methyl ester, Internal standard), Porcine pancreatic lipase enzyme and different laboratory reagents such as n-hexane analytical GC standard, acetone, methanol, potassium hydroxide (reagent grade, 90%, flakes), Sodium hydroxide (reagent grade, 97%, flakes),absolute ethanol alcohol, phenolphthalein, chloroform, hanus solution, potassium iodide, starch, Sodium thiosulfate, synthetic fatliquor and fish oil based fatliquor

3.1.3. Laboratory Equipment

- Soxhlet Apparatus,
- Shaker,
- Flasks
- Beakers
- Thermometer
- Measuring cylinder
- Separating Funnel
- Protective Equipment
- Mixer
- Centrifuge

3.1.4. Scientific Instruments

- GC- MS
- GC
- FTIR
- DSC

3.2. Methodology

3.2.1. Raw Material Sample Preparation and Moisture Content Analysis

After the pig flesh or tallow is collected, its impurities like stones, dusts and other insoluble solid matters were removed mechanically.

Extraction and purification of the oil

The oil of PL was extracted by using Soxhlet apparatus and by heating at 80 ^{o}c. Soxhlet apparatus is the most commonly used method to determine the amount of oils and fats. The extraction was conducted by petroleum ether solvent as the fat is soluble in this solvent. For this experiment soxhlet apparatus was used to determine the oil content of the tallow but for easy separation of oils and fats from other insoluble matters and proteins during the bulk extraction heating at the melting point of the pork lard can be useful.

Procedure

- Petroleum ether solvent was poured into round bottom flask.
- 50 g of the sample was put in the thimble and inserted in the centre of the extractor.
- The Soxhlet was heated to a boiling temperature of the solvent.
- The extract was Collected and all the solvent evaporate was evaporated
- The experiment was repeated by placing the same amount of the sample into the thimble again.
- The weight of the oil was determined for each run hours.

3.2.2. Characterization of PL for Its Physicochemical Properties

Acid and free fatty acid value, iodine value, soap and unsaponifiable matter, density and viscosity of PL were determined by following the standard procedure according to ASTM method.

3.2.2.1. Determination of Moisture Content

Moisture content of the oils and fats is the loss of the sample on heating at 105 ^{o}c under operating conditions specified. The moisture content of the fat was determined by taking the weight difference after the sample has been dried in an oven at 105°C. Apparatus
Crucible dishes with 7-8 cm diameter and 2-3 cm deep provided with tight fitting slip on covers.
Procedure

Thirty gram of oil was measured in a dried and crucible and heated in an oven at 105 oc for an hour. The dish was removed from the oven and cooled in a desiccator and weighed again. The sample was heated again in the oven for further period of 1 hour, cooled and weighed. The same process was repeated until change in weight between two successive observations does not exceed 1mg. the determination was carried out in triplicate.
Finally the moisture content was detrmined by equation 4.1 specified on page 44 on the discussion part.

3.2.2.2. Determination of Density of PL

The melted sample was filtered through a filter paper to remove any impurities and the last trace of moisture. The sample was cooled to 30 ^{o}c which is ambient temperature desired for density determination.
The apparatus used for density and specific gravity determination is Pycnometer fitted with a calibrated thermometer. Balance and water bath maintained at 30 ^{o}c was also used for the experiment.

To know the mass of the oil, take the mass of the Pycnometer + stopper + PL was taken and the mass of the Pycnometer + stopper was subtracted.

3.2.2.3. Determination of Viscosity

Viscosity can be defined as measurement of fluid internal resistance to flow at a specified temperature. There are two ways to measure a fluid's viscosity, namely Dynamic (Absolute) Viscosity and Kinematic Viscosity.

Dynamic (Absolute) Viscosity

Dynamic Viscosity is defined as a fluid's resistance to flow, or the fluid's resistance to deform when subjected to a force. The dynamic viscosity of PL was measured by rotary viscometer at 30 ^{o}c, 100 rpm using spindle L_2. The oil sample was kept in the water thermostat bath until it reaches the equilibrium temperature of 30^{o}C. After maintaining the equilibrium temperature, the vibro-viscometer tip was inserted to the sample and the reading was taken from the digital board controller.

Kinematic Viscosity of PL

The kinematic viscosity is defined as a fluid's resistance to flow which is found as the ratio of dynamic viscosity to density of the oil. It is traditionally measured by noting the time taken for a fluid sample to travel through an orifice in a capillary under the force of gravity. Vibro-viscometer was used to determine the dynamic viscosity as it explained above.

3.2.2.4. Determination of Ash Content of PL

Ash content of oil was determined using a furnace. A 15 g of oil was added in a burning cup. Then, the sample was placed in a furnace. A furnace was heated at a temperature of 500^{o}C for an hour and after burning the residue sample was weighted and ash content was calculated as shown on the result and discussion part on page 48.

3.2.2.5. Acid Value (AV)

The (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. AV is often a good measure of the breakdown of the triacylglycrols into free fatty acids, which has an adverse effect on the quality of many lipids.

AV is the measure of hydrolytic rancidity. In general, it gives an indication about edibility of the lipid and applicability of the oil for leather fatliquoring.

Experimental Procedure

The AV of the PL was determined by titration with 0.1N of KOH. Two gram of oil was placed to 250ml conical flask 20ml of anhydrous methanol (99.5%w/w) were added and heated at 70^{o}C for 10 minutes with shaking in water bath. Then transfer the solution to the titration 3 drops of phenolphthalein as an indicator was added into the titration beaker with sample oil. Then the mixture was titrated against 0.1N of KOH until pink color appeared (end point). Finally after the colour change was observed, the volume (ml) of 0.1 N KOH consumed by the titration was recorded and titration was stopped. The titration volume recorded (ml) was used to calculate the acid value by using equation 4.3 on page 45

3.2.2.6. Saponification Value (SV)

The SV is the number of mg of potassium hydroxide required to neutralize the free acids and to saponify the esters in 1 g of the substance. The saponification number is a measure of the average molecular weight of the triacylglycerols in a sample. Saponification is the process of breaking down a neutral fat into glycerol and fatty acids by treatment with alkali. The smaller the saponification number the larger the average molecular weight of the triacylglycerols present i.e. SV is inversely proportional to the mean molecular weight of fatty acids (or chain length).

Experimental procedure

Approximately 2 g of the oil was placed into a 250 mL conical flask 25 mL of alcoholic potassium hydroxide solution (0.5 N) was added. By attaching a reflux condenser, the flask contents were heated on a boiling water bath for 1 hour with occasional shaking.

While the solution is still hot, 3 drops of phenolphthalein indicator was added and the excess potassium hydroxide was titrated against 0.5N hydrochloric acid. (The volume of hydrochloric acid at consumed at the end point is recorded and represented as S). The above procedure was repeated but without sample (volume of hydrochloric acid consumed at the end point is represented B). Finally the SV was calculated the by using equation 4.5 as specified on page 47.

3.2.2.7. Ester Value (EV)

The EV is defined as the mg of KOH required to react with glycerin (glycerol / or glycerin) after saponify one gram of fat. EV and % glycerin were calculated from the SV and the AV of the PL by equation 4.6 and 4.7 respectively as represented on page46 and 47.

3.2.2.8. Iodine Value (IV)

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

Experimental Procedure

Approximately 0.25 g of the fat or oil was placed into a 250 mL conical flask and then 10 ml of chloroform and 20 ml of Hanus solution was added. Then the flask was completely closed by Para film, and the solution was left for 30 minutes with shaking continuously. After that 20 ml of 15% potassium iodide solution and100 ml of distilled water were added by shaking.

Finally the iodine solution was titrated against 0.1 N Sodium thiosulfate solution till yellow color formed , then 3 drops of starch solution was added where blue solution formed and then continue with titration till the blue color is disappeared (Volume (ml) of $Na_2S_2O_3$ at end point represented as S). The same procedure was done but without sample

(Volume (ml) of $Na_2S_2O_3$ at end point represented as B). The IV was calculated by using equation 4.8 as written on page 47.

3.2.3. Characterization of PL For Its Fatty Acid Composition

The determination of fatty acid present in the oil was mainly done by gas chromatography. Other scientific instruments such as GC-MS, FTIR, NMR and DSC also used to get supportive idea in the characterization of oil and yield of fatty acid methyl ester.

3.2.3.1. Analysis of PL by GC-MS Chromatography

Gas chromatography is an important instrument for analysing lipids and oils. Typically GC is suitable for analysing organic and non-ionic compounds that are vaporisable at 400 ^{0}c or less (22). For the analysis of PL derivetization or transeterification of the lipids into their more volatile derivatives of ester is done prior to GC analysis. The objective of using GC analysis is to determine the fatty acid composition present in the oil and to know the composition of ester formed after TER.

The primary reasons to analyse fatty acids as fatty acid methyl esters include:

Fatty acids without derivetization may be difficult to analyze because they are highly polar compounds tend to form hydrogen bonds, leading to adsorption issues. Reducing their polarity may make them more amenable for analysis.

To distinguish between the very slight differences exhibited by unsaturated fatty acids, the polar carboxyl functional groups must first be neutralized. This then allows column chemistry to perform separations by boiling point elution, and also by degree of unsaturation, position of unsaturation, and even the cis vs. trans configuration of unsaturation.

The esterification of fatty acids to fatty acid methyl esters is performed using an alkylation derivetization reagent. Methyl esters offer excellent stability, and provide quick and quantitative samples for GC analysis.

Procedure for Sample Preparation for GC Analysis / Derivetization of oil/

There are several methods to prepare fatty acid methyl ester from crude oil based on derivetization. For this experiment DB-wax company sample preparation method was applied by using oil and 2 N KOH as a reactant and hexane as a solvent.

The derivetized FAME was prepared by following the procedure; 100 mg of oil sample was weighed in a 20 ml reaction vial and dissolved in 10 ml hexane. Then 100 µl of 2N KOH in methanol (11.2gm in 100ml) was added in the vial. After closing the reaction vial it was mixed by vortex for 30 Second and centrifuged. Then the sample was dehydrated by anhydrous sodium sulphate and then the clear supernatant was transferred into a 2 ml auto sampler vial for injection into GC.

GC Conditions

- Agilent technology of GC fitted with DB-5 capillary column
- Injection volume - 1µl
- Oven temperature – 50 ^{o}c hold for 2 minutes, ramp10 ^{o}c to 280 ^{o}c, hold 5 minute, total 30 minute.,
- Detector temperature – source 230 ^{o}c, quadruple 250 ^{o}c.,
- Carrier gas – helium

3.2.3.2. FTIR Analysis

An infrared spectrum of a compound can reveal information about molecular structure as the existence of specific groups of atoms may be confirmed from the presence of their characteristic absorptions. FTIR analysis helps mainly to identify the presence of double bond due to unsaturated fatty acids which are necessary for sulfitation reaction in the process of fatliquor production. It also gives idea whether the changes happened during TE reaction by comparing against the spectrum of the raw oil.

3.2.4. Optimization of Transesterification

The experiment of enzymatic transesterification reaction was carried out in a 25-ml flask; Stoppard with a plug. Experiments were done to determine the yield of transesterified ester by optimizing mainly the molar ratio of methanol to oil, reaction time and temperature. The selection of these factors was based on previous research works and practical considerations of the enzyme technology on biodiesel production. Aqueous environment is unsuitable for lipase catalyzed ester synthesis because water has an effect on the lipase behaviour either by affecting the hydration of the enzyme or by changing the nature of the reaction media of the support material. The water content of the catalyst is more important in dictating the catalytic activity than the total water content in the system. Therefore the transesterification reaction was carried out under solvent condition.

3.2.4.1. Effect of Molar Ratio of Methanol to Oil

Three different molar ratio of methanol to oil (3:1, 4:1 and 5:1) were investigated by keeping the other parameters constant.

The amount of methanol required for each molar ratio of methanol to oil ratio was calculated based on the molecular weight of oil which was estimated from its soap value and from molecular weight of methanol. The amount of methanol required when the molar ratio of ethanol to oil ratio 3:1, 4:1 and 5:1 were calculated as follows.

For 3:1 Molar Ratio of Methanol to Oil;

$$\frac{\text{mole of methanol}}{\text{mole of PL}} = 3 \quad \text{……. (3.1)}$$

$$\frac{\text{Given mass of methanol} / \text{Molecular mass of methanol}}{\text{Given mass of PL} / \text{Molecular mass of PL}} = 3$$

Where:

Molecular mass of methanol is= 32g/mol.

Average molecular mass of pork lard = 857 g/mol. Average molecular mass of pork lard was estimated from its soap value or from the percentage composition of fatty acid present in the PL which was determined in the oil characterization part. For this experiment 50ml of Pork oil is going to be used, and density of oil is 893kg/m3. Therefore given mass of oil is;

$Given\ mass\ of\ PL = density\ of\ oil * volume\ of\ oil$ ……. (3.2)

$$Given\ mass\ of\ PL = \frac{893kg}{m3} * 50 * 10^{-6}m = \ 44.65\ \text{g}$$

For 1:3 Molar Ratio of Methanol to Oil:-

$$\frac{\text{Given mass of methanol}/_{32\text{g/mol}}}{44.65\text{g}/_{859\text{g/mol}}} \ = \ 3$$

So the given mass of methanol = 4.99g

To get the volume of methanol used for 50ml of oil;

$given\ mass\ of\ methanol = density\ of\ methanol\ x\ volume\ of\ methanol$

Volume of ethanol= 4.99 g / 0.791 *g/mL* at 25ºC = 6.31

Volume of methanol taken for 1:6 molar ratio of methanol to oil was 6.31ml.

For 1:4 molar ratio of methanol to oil:-

$$\frac{\text{Given mass of methanol}/_{32\text{g/mol}}}{44.65\text{g}/_{857\text{g/mol}}} \ = \ 4$$

So the given mass of methanol = 6.67g

To get the volume of methanol used for 50ml of oil;

$Given\ mass\ of\ methanol = density\ of\ methanol\ x\ volume\ of\ methanol$

Volume of methanol = 6.67 g / 0.791 *g/mL* at 25ºC

Volume of methanol taken for 1:4 molar ratio of methanol to oil was 8.43ml.

For 1:5 Molar Ratio of Methanol to Oil:-

$$\frac{\text{Given mass of methanol}/_{32\text{g/mol}}}{44.65\text{g}/_{859\text{g/mol}}} = 5$$

So the given mass of methanol = 8.31g

$$given\ mass\ of\ methanol = density\ of\ methanol\ x\ volume\ of\ methanol$$

Volume of methanol= 8.31 g / 0.791 *g/mL* at 25ºC =10.51ml

Volume of methanol taken for 1:5 molar ratio of methanol to oil was 10.81ml.

$$Mass\ of\ PPL\ catalyst = \frac{Mass\ of\ catalyst}{Mass\ of\ PL} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.3)$$

3.2.4.2. Effect of Temperature

The effect of temperature was studied at 30, 40 and 50 ^{o}c by keeping 1: 4 molar ratio of oil to methanol constant after 36 hours.

3.2.4.3. Effect of Time

The effect of time was studied for 12, 24 and 36 hours. 1:4 molar ratio and 50 ^{o}c temperature were used for the investigation of reaction time.

3.2.5. Separation of FAME from Glycerol

For the separation of biodiesel, glycerine and other impurities; separatory funnel and centrifuge are used. Separation is done by settling the mixture under gravitational force.

3.2.6. Biodiesel Yield Analysis

The yield of enzymatic transesterified FAME product was analysed by gas chromatography in a similar manner that have been used for the analysis of crude oil based on derivetization as specified on page 32. The quantification of individual fatty acids was also determined by internal standard method by using the peak area data from the chromatogram based on internal response factor. The entire standard pure FAMEs that have been used for the oil analysis also used in the analysis of biodiesel.

The standard FAMEs were prepared for GC analysis by diluting into 4 ml of n-hexane.

The sample preparation was done by talking 200 µl aliquots samples from the reaction mixture at selected time intervals (12, 24 and 32hours) in triplicates and centrifuged to obtain the upper layer.

After the centrifugation, 100 µl of the upper layer and 60 µl of methyl heptadecanoate were mixed in a 15 ml bottle, to which some amount of anhydrous sodium sulphate was added as a dehydrating agent and 3.0 ml of hexane was added.

After centrifugation and taking the upper layer, 2µl of the sample was injected into a gas chromatography for the determination of yield of FAME in each experiment.

The programing of the GC and its analytical condition is the same as that has been used in the analysis of derivetized oil which was written on section 3.2.3.1 on page 32.

Amount of total fatty acid is the sum of the individual fatty acid being converted in the reaction. Therefore the general conversion of oil to fatty acid methyl ester (biodiesel) was estimated by using equation 3.4 and 3.5. Equation 5 is used to calculate the yield of FAME if the analysis is done by GC.

$$\%\, Total\ Methyl\ Ester\ = \frac{Amount\ of\ ester}{Amount\ of\ cruid\ oil\ taken\ for\ the\ reaction} x\ 100 \quad \text{..... (3.4)}$$

From the chromatogram data the conversion yield of each FAME was also calculated based on equation 3.5.

$$\text{Conversion yield of Peak area A (wt\%)}\ = \frac{Peak\ area\ A\ x100}{\sum Peak\ Area\ A + peak\ Area\ B + \cdots + peak\ Area\ N} \quad \text{..... (3.5)}$$

Where: A, B…and N are individual FAME

3.2.7. Experimental Design and Statistical Analysis

Experimental design for TER was done by the Design Expert 7.0.0 software application based on Central Composite Design (CCD). The molar ratio, temperature and reaction time was optimized to get maximum conversion of triglyceride to FAME or biodiesel at 200 pH 8.0 and 5% enzyme based on PL weight. Biodiesel yield are set as a response in the experimental design.

Three factors at three levels; a total of 20 experiments were done in the optimization study and statistical analysis was made.

Table 3.1: Complete experimental design matrix of CCD

Variables	Factor coding	Unit	Levels		
			-1	0	+1
Methanol to Oil ratio	A	-	1:3	1:4	1:5
Reaction Temperature	B	^{o}c	30	40	50
Reaction time	C	Hours	12	24	36

The summary of central composite design arrangement of main and interaction effect and the responses / yield of biodiesel / is presented on table 3.2.

Table3.2: Summary of central composite design arrangement of main and interaction effect.

Run	PL to Methanol molar ratio	Temperature	Time (hours)	Biodiesel (% w/w)
1	1:4	50.00	24.00	
2	1:5	50.00	12.00	
3	1:5	50.00	36.00	
4	1:3	50.00	12.00	
5	1:4	30.00	36.00	
6	1:4	40.00	24.00	
7	1:4	40.00	36.00	
8	1:5	30.00	12.00	
9	1:3	40.00	24.00	
10	1:3	30.00	36.00	
11	1:4	40.00	24.00	
12	1:4	30.00	24.00	
13	1:4	40.00	24.00	
14	1:4	40.00	24.00	
15	1:3	50.00	36.00	
16	1:4	40.00	24.00	
17	1:3	30.00	12.00	
18	1:4	40.00	24.00	
19	1:5	40.00	24.00	
20	1:4	40.00	24.00	

3.2.8. FAME Quality Analysis

The major physicochemical properties that determine the quality of biodiesel for its application were analysed. These biodiesel parameters include:

- Density
- Kinematic viscosity
- Flash point
- Moisture content
- Cetane number

The density, viscosity, acid value, saponification value, moisture content, ash content and Cetane number was determined for the biodiesel with similar procedure used for the determination of raw oil.

Flash Point (°C)

The flash point of the biodiesel was determined using pensky Marten (open cup) method. The method determines the temperature at which the sample will flash when a test flame is applied under the conditions specified for the test. Before analysis the sample was dehydrated with Calcium chloride. The cup was filled with the biodiesel (about 75 ml) and the cup was heated by a Bunsen burner. A small open flame was maintained from an external supply of LPG. Periodically, the flame was passed over the surface of the oil. When the flash temperature was reached the surface of the oil catch the flame. Therefore the temperature at which the surface of the oil catches the flame was noted and reported as flash point temperature.

Procedure of the Experiment

All parts of the cup and its accessories were cleaned and dried thoroughly before starting the test. The cup was filled with biodiesel to be tested up to the level indicated by the filling mark. Then the lid was placed on the cup and properly the heating devices were engaged. The thermometer was inserted and the test flame was lighted and adjusted to 4 mm in diameter. By heating the sample the temperature was increased to 5 to 6 ^{o}c per minute. The flame was applied when the temperature of the sample is a whole number

not higher than 17 ^{o}c below the flash point. At every 5 ^{o}c raise the temperature, discontinue stirring and apply the test flame and by opening the device which control the shutters and lowers the test flame into the shutter opening.as soon as the test flame has been returned to the raised position, and the string was stopped. The flash point is the temperature indicated by the thermometer at the time of the flame application that causes a distinct flash in the interior of the cup [33].

3.2.9. Fat liquor Preparation

After the determination of physicochemical properties and fatty acid characterization of the oil, the fatliquors were prepared by external emulsification and chemical modification (Sulfitation) method. Four different commercial emulsifiers such as A B, C & D and HNP as a dispersing agent were used for fatliquor production. The optimization of formulation was performed based on the emulsion stability of the fatliquor.

3.2.10. Physicochemical Properties of Fatliquor

The physicochemical properties of the fatliquor synthesized from crude oil and transesterified ester by means of both external emulsification and sulfitation process were estimated. These parameters have a direct relationship to the quality of leather produced from this fatliquor and the degree of lubrication. These parameters include:

Physical Condition

- Pourablility
- Emulsion Characteristics or emulsion stability
- Emulsion particle size
- Odour

3.2.11. Determination of Chemical Requirements of Fatliquor

PH of emulsion (1:10 dilution in distilled water)

Ten percent emulsion was prepared by dispersing 10 g of fatliquor in 90 ml water and the pH was measured by using electrode type *p*H meter.

Total active Ingredient, Percent by Mass

About 5 g of the sulfited oil fatliquor was weighed accurately in a 250 ml flask and 25 ml of 50 percent ethyl alcohol and 25 ml of petroleum ether was added. The contents were transferred to a separating funnel and stirred the contents vigorously and allowed the layers to separate. The lower alcohol layer was transferred to another separating funnel and it was extracted three times with petroleum ether. The upper petroleum ether layer was extracted with 75 percent alcohol and allowed the layers to separate and extracted further with 90 percent alcohol and absolute alcohol and again allowed the layers to separate.

Then the petroleum ether layer and alcohol layer were collected in two flasks. The solvents were evaporated and the residues are dried to constant weight in the oven, cooled and weighed.

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

Determination of Total Alkalinity

Ten gram of the sample was dissolved in 100 ml of water in a conical flask and heat to obtain uniform solution. After cooling, 30 g of sodium chloride, 25 ml of ether and 5 drops of 0.1 percent methyl orange indicator was added and titrated with 0.5 N sulphuric acid until the aqueous layer is orange. Stopper the flask and the content was shaken frequently during the titration. The calculation result was explained and discussed on the result and discussion part on page 76.

Determination of Total Ash (Referee Method)

Three gram of sample was weighed in a platinum crucible and heated gently until a charred residue remains. The residue was extract with hot water to remove soluble salts, filtered through a low ash filter paper and washed thoroughly. The filter paper was gotten ash and charred residue, and it was moistened with ash-free hydrogen peroxide to

minimize the difficulty. Aqueous extract and evaporate was added to a cooled dish on a steam bath. Finally the ashing was completed at as low a temperature as possible. Then the dish was cooled and weighed.

3.2.12. Evaluation of PL Performance on the Leather

Effect of the new synthesized fatliquor was analyzed on the leather by producing garment leather from sheep skin. The physical and chemical properties of the leather were analyzed comparatively with the commercial fatliquors.

Organoleptic tests, Physical resistance tests and Chemical tests were conducted for both controlled and test sample garment leather.

3.2.12.1. Organoleptic Test

Touch, colour, firmness factor and softness were assessed under organoleptic test. Softness was measured by softness tester but the other properties were assessed subjectively.

3.2.12.2. Physical Resistance Tests

These are the major functional and performance properties of the leather that determine its acceptance for the intended purpose. The physical resistance tests involve:

- Thickness (mm)
- Tensile strength (N/mm2)
- Elongation at break (%
- Tear strength(N)

Tensile Strength and Elongation at Break

Tensile strength is the ultimate strength of the leather that includes grain, corium and flesh layers. In this test elongation property of the leather can also be measured.

Tensile strength is defined as strength of material in terms of force per unit area of cross section while applying force in linear direction. The tensile strength was measured by tensile strength testing Machine (Dynamometer) according to the procedure specified on ISO 3376/iup 6 test method.

Tear Strength

Tear strength is also another important bulk property test. This test is the most preferable test for leather than tensile strength by many of the customers including BS EN ISO 20345 Safety shoe standards. There are two types of tear strength tests are followed for leather material.

- Double edge tear strength – Baumann Tear strength
- Single edge tear strength - Tongue/trouser tear strength

Table 3.3: Standard physical test methods of leather

Physical tests /parameter	Standard test methods
Thickness(mm)	ISO 2589:2002(IULTCS/IUP 4) Determination of thickness
Tensile strength (N/mm2)	ISO 3376:2002 (IULTCS/IUP Determination of tensile strength and percentage extension
Elongation at break (%	ISO 3376:2002 (IULTCS/IUP 6) Determination of tensile strength and percentage extension
Tear strength(N)	ISO 3377-2:2002 (IULTCS/IUP 8) Determination of tear load -- Part 2: Double edge tear
Grain burst (mm)	ISO 3379:1976 (IULTCS/IUP 9) Determination of distension and strength of grain -- Ball burst test

CHAPTER FOUR

4. Result and Discussion

4.1. Extraction and Processing of Pork Lard

Waste fat from pork carcasses and pork skin were removed and then made into oil using a rendering process. Rendering consists of grinding the animal by-products to a fine consistency and heating them until the liquid fat separates and pathogens are destroyed. The cooking process also removes water, which makes the fat and solid material stable against rancidity. The physical appearance of PL is semisolid at room temperature. Since it contains both saturated and unsaturated fatty acids, some part is liquid and the rest are solid. So by mixing the PL gently a soft enough semisolid oil was obtained and taken for its physicochemical analysis and for the synthesis of biodiesel and leather fatliquor. The fats taken from the skin part is mainly liquid whereas the fat taken from other Carcasses is mainly solid at room temperature.

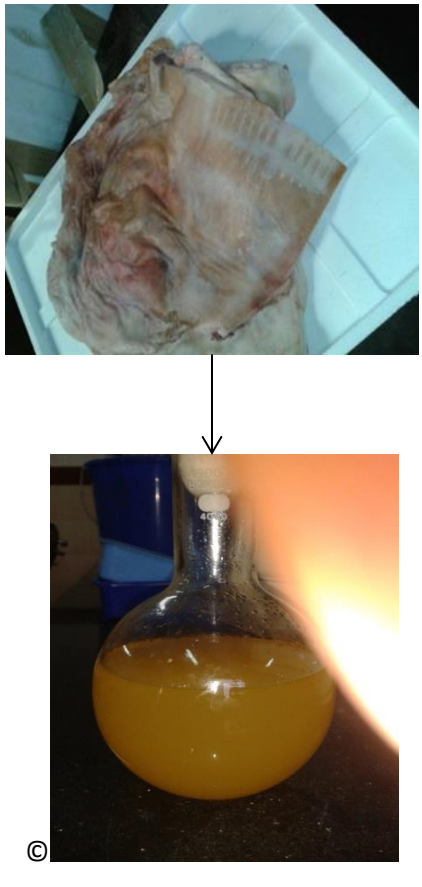


Figure 4.1 (A) Fat under the skin of Pig (B) Melted Pork lard

4.2. Characterization of PL for its Physicochemical Properties

The physicochemical parameters tested in the oil have a relation to the purpose for which the analysis is required. For each experimental analysis a triplicate result was taken. Most of the physicochemical tests were conducted by analytical and gravimetric method of analysis. All the tested parameters are discussed in detail in the following sections.

4.2.1. Moisture Content of PL

The moisture content of PL was estimated by an air-oven method by heating the sample at 105 ^{o}c under operating conditions as specified on page 26.

The moisture content of PL was obtained to be 0.34% by calculating using equation 4.1.

$$\text{Moisture content and volatile matter \% by weight} = \frac{W-W_1}{W} * 100 \quad \ldots\ldots\ldots\ldots\ldots (4.1)$$

W = Average Initial mass of oil taken= 30 g

W_1 = loss in gram of the oil on drying = 29.898 g

Inserting the above values in equation

$$\text{Moisture content\%}\left(\frac{w}{w}\right) = \frac{30g - 29.898g}{30g} * 100\% = 0.34\%$$

Thus, the moisture content of oil was 0.34% (w/w). The low moisture content of the oil helps to prevent the formation of soap during transesterification reaction which causes difficulty of glycerol separation and reduction of biodiesel yield [52].

4.2.2. Density of PL

The density of PL was determined by Pycnometer fitted with a thermometer as specified on page 26.

Therfore the density of the oil was determined by dividing the mass to the volume of oil after being determined by the following method.

Pycnometer + stopper + PL=94.4891

Pycnometer + stopper = 49.8391

Mass of oil = Pycnometer + stopper + PL - Pycnometer + stopper + PL=44.65

The volume of the Pycnometer is 50 ml, which is the same as the volume of sample inside the Pycnometer.

Then you have the volume of the Pycnometer and the mass of your unknown liquid to find the density of that liquid just divide the liquid's mass by the volume of the Pycnometer.

$\rho = m/v = 44.65g/50\ ml = 0.893g/ml$

So the density of the PL was found to be 0.893g/ml. The value was taken from triplicate experiment data result.

4.2.3. Viscosity of PL

Dynamic (Absolute) Viscosity of PL

The dynamic viscosity of PL was measured by rotary viscometer under specified conditions at 30 ^{o}c, 100 rpm using spindle L_2. The viscosity of the oil was measured and is about 56.2 Centipoise (cP) or mPa.s.

Kinematic viscosity of oil of PL

Viscosity can be reported in both dynamic and kinematic viscosity which is interchangeable by the following formula.

$$Kinematic\ Viscosity\ of\ oil = \frac{Dynamic\ viscosity\ of\ oil}{Density\ of\ oil}, \quad \nu = \frac{\mu}{\rho} \text{ ---------- (4.2)}$$

Where:

ν =kinematic viscosity, mm^2/s

μ = dynamic viscosity, mPa.sec and ρ = density, kg/m^3

By inserting 56.2 cP dynamic viscosity and 893 kg/m3, the kinematic viscosity is obtained.

Kinematic Viscosity of pork Lard = $56.2x\ 10^{-3}$kgm.s x 893kg/m3 =

Thus, the kinematic viscosity of oil was $50.2mm^2/s$. Most laboratories worldwide follow kinematic viscosity which is defined by ASTM D445

4.2.4. Acid value of PL

The AV of the PL was estimated by the experimental procedure explained on page 27 and the results of titration are illustrated on table 4.1.

Table 4.1: Volume of 0.1NKOH consumed in titration for Acid value analysis

Trial	Volume of 0.1N KOH consumed	Color change
1	1.0	Brown to pink
2	1.1	Brown to pink
3	1.0	Brown to pink
Average	1.067	

Insert the value in equation 4.3 and the result was:-

$$AV = \frac{\text{ml of KOH x N x 56}}{\text{Weight of Sample}} = \text{mg of KOH} \quad \text{---------- (4.3)}$$

Where N = Normality of KOH

$$\text{Acid value} = \frac{\frac{0.1mol}{l} * \frac{56.1g}{mol} * 1.067 * 10^{-3} l}{2g} = 2.991 \text{mg KOH/g of oil}$$

Free Fatty Acid (FFA) = AV x 0.503 ---------- (4.4)

Free Fatty Acid (FFA) = 2.991 x 0.503= 1.5 mg KOH/g of oil.

Therefore the AV and FFAV of PL were found to be 2.991 mg KOH/g of oil and 1.5 mg KOH/g of oil by calculating using equation 4.3 and 4.4 respectively. FFA value gives a good idea of how degraded the oil is. The high value of FFA in the feed stock can lead to soap formation in alkali catalysed TER but not a problem for enzymatic catalysed TER.

4.2.5. Saponification Value (SV) of PL

The experimental procedure of SV was explained on the methodology part on page 29. The blank titration was consumed 24.5 ml of 0.5 M HCL and the volume of HCL consumed by the sample titration was tabulated on table 4.2.

Table 4.2: Volume of HCL consumed in titration for saponification value analysis

Trial	Volume of HCL consumed	Color change
	Sample	
1	10	Brown to pink
2	10.5	Brown to pink
3	11	Brown to pink
Average	10.5	

By inserting the above value in equation 4.5 the SV was calculated as:

$$SP\# = \frac{56.1(B - S) \times N \text{ of HCl}}{\text{Gram of Sample}} \quad \text{---------- (4.5)}$$

Where B: ml of HCl required by Blank.

S: ml of HCl required by Sample.

$$SV = \frac{\frac{56.1g}{mol} * \frac{0.5mol}{l}(24.5 - 10.5) * 10^{-3}l}{2g}$$

Therefore the SV obtained is about196.35 mgKOH/gOil.

4.2.6. Ester Value (EV) and % Glycerin

EV and % glycerin were calculated from the SV and the AV of the PL by using equation 4.6 and 4.7 respectively. For this the average value results were taken.

Ester Value (EV) = Saponification Value (SV) – Acid Value (AV) ---------- (4.6)

% glycerin =Ester Value x 0.054664 ---------- (4.7)

By inserting the values in the above equation The EV and % glycerin were found to be 193.31 mg KOH/g Oil.and 11.15% respectively.

4.2.7. Iodine Value (IV) of PL

The IV of the lard was done by the experimental procedure explained on page 29 on the methodology chapter. In the blank titration 19.66 ml (B) of $Na_2S_2O_3$ was recorded from the triplicate experiment and the volume of sodium thiosulphate consumed in the sample titration was recorded on table 4.3 below [43].

Table 4.3: Volume of $Na_2S_2O_3$ consumed in titration for iodine value analysis

Trial	Volume of $Na_2S_2O_3$ consumed (S), ml
1	7.5
2	8
3	7.5
Average	

The IV of the PL was calculated by using equation 4.8 which is found to be59.28 g KOH/ 100g Oil.

$$IV = \frac{\text{Equivalent weight of iodine} * \text{Volume of sodium thiosulphate used} * \text{N of Na2S2O3} \times 100\text{x}10^{-3}}{\text{Weight of oil Sample (g)}}..$$

…… (4.8)

Where:

Volume of Sodium thiosulphate used = [Blank- Test] ml

Equivalent Weight of Iodine = 127

Normality of sodium thiosulphate ($Na_2S_20_3$) = 0.1

Inserting the value in equation __

$$IV = \frac{(19.5 - 7.83)\text{ml} \times 0.1\text{mol/l} \times 0.127\text{g/meq} \times 100}{\text{Weight of Sample (g)}} = 59.28\text{g per g of oil}$$

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

S: V ml of $Na_2S_2O_3$ volume for sample

Therefore the IV of the PL is about 59.28g per g of oil

4.2.8. Ash Content of PL

For the determination of ash content furnace was used at a temperature of 500^{o}C. A 15 g of oil was added in a burning cup and placed in a furnace. A furnace was heated to a temperature of 500^{o}C for 1 hour and after burning the residue sample was weighted and ash content was calculated. The experiment was done in triplicate and by using equation 4.9 as follows the ash content was calculated.

$$Ash\ content\%\ (w/w)\ =\ \frac{Final\ mass\ of\ oil\ after\ burning*100\%}{Initial\ mass\ of\ sample}$$ ---------- (4.9)

Initial mass of sample=15g

Final mass of sample=0.1185g

Inserting values in equation gives the ash content of oil.

$Ash\ content\ \%(^{w}/_{w}) = \frac{0.1185g*100\%}{15g} = 0.79\%$, The ash content of the oil is relatively lower that makes the oil suitable for biodiesel synthesis.

Table 4.4: summary of physicochemical properties of PL

Physicochemical properties of PL	Values	Units
Moisture content	0.34	% (w/w)
Density @ 20^{o}C	893	kg/m^{3}
Kinematics viscosity @ 40^{o}C	50.2	mm^{2}/s
Acid value	2.991	mgKOH/g
Free fatty acid	1.5	mg/g
Saponification value	196.35	mgKOH/g
Ester value	11.15	mgKOH/g
Iodine value	59.28	gKOH/100g oil
Ash content	0.79	% (w/w)

4.3. Characterization of PL for its Fatty Acid Composition

GC, GC-MS, and FTIR instruments were used for characterization and the determination of the type and amount of fatty acid present in the PL.

4.3.1. Gas Chromatography

To analyse the fatty acid composition of PL, derivetization of oil into its more volatile fatty acid methyl ester (FAME) was performed prior to GC analysis. This is because of GC can be used to analyse fatty acids either as free fatty acids or as fatty acid methyl esters. This analysis was done in central leather research institute of India and leather industry development institute of Ethiopia by using gas chromatography (Agilent technology) equipped with flame ionization detector and DB-5 capillary column. The derivetization of oil and GC analytical conditions were explained on page 31 and 32 on the methodology section.

Individual fatty acids of the oil were identified by comparing the retention time (the time at which the peak is observed) and chromatographic profiles of the sample to the retention times and of chromatograms profiles of standard oils. Standards for the determination of free fatty acids were obtained from central leather research institute, Chennai, India which was bought from Sigma-Aldrich chemical company. Methyl palmitate, methyl stearate methyl oleate, methyl linoleate, and methyl myristate were used as a standard solution to prepare the standard curve and methyl pentadecanoate was served as internal standard for GC analysis.

The main fatty acid present in the PL from the chromatogram result include palmitic acid, oleic acid, stearic acid, linoleic acid and myristic acid.

The chromatogram of FAME formed in the transesterification of PL is illustrated in fig 4.2.

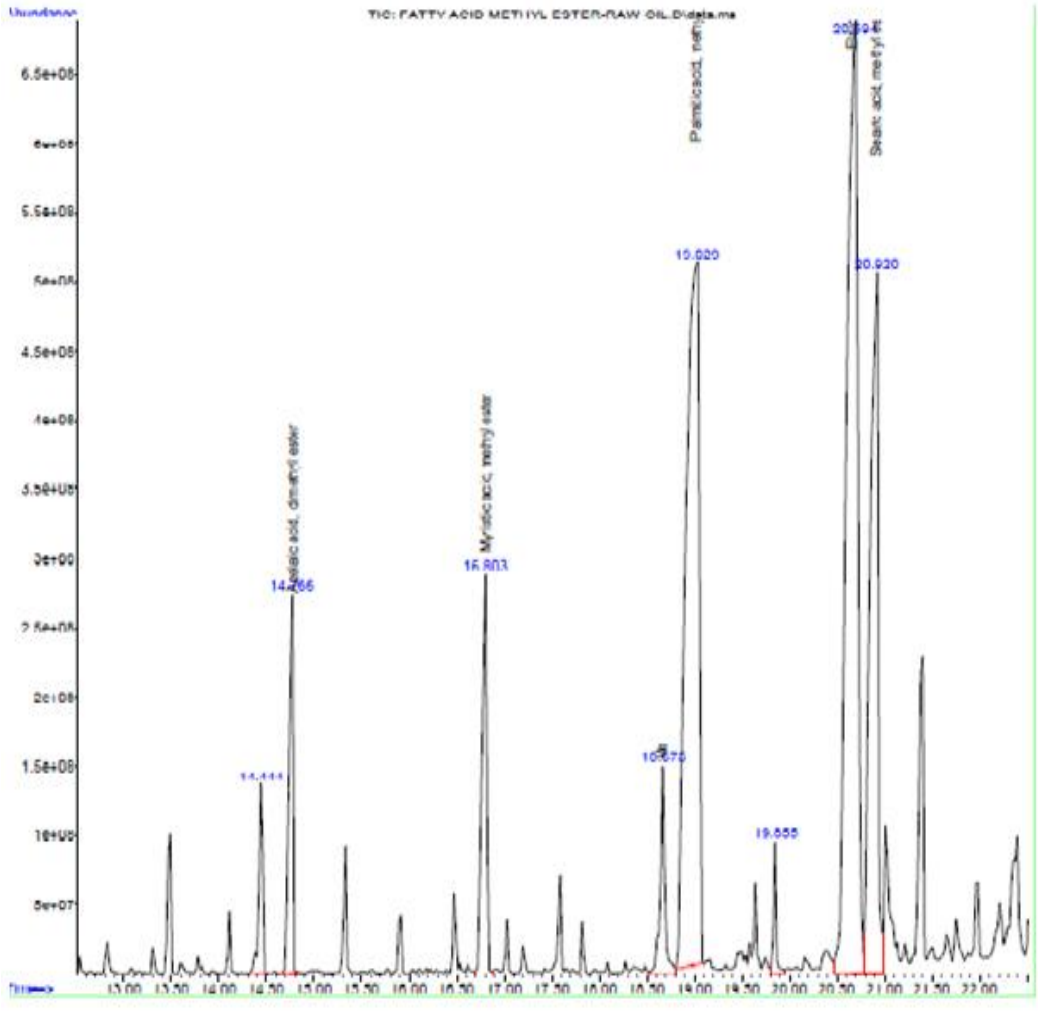


Figure 4.2: FAME Gas Chromatogram

4.3.1.1. Quantification of Fatty Acid in PL

There are several types of quantification methods commonly used for the determination of individual fatty acid components present in the crude pork oil. This is based on the chromatographic data either in the form of peak height or peak area which is obtained from an integrated chromatogram. For this particular experiment single Point Internal Standard method of quantification was used to determine the percent quantity of individual fatty acid present in the oil.

The internal standard method accounts for any variances in gas chromatograph performance. The analyte chosen for the internal standard has a predictable retention time and area, allowing it to be used to determine if abnormalities have occurred.

The Single Point Internal Standard method requires at least two analyses. The first analysis contains a known amount of internal standard and the compounds of interest the oil sample. Then the response factor was calculated using Equation 4.11 [42]. All the data were gathered very accurately to minimize error.

Quantification of individual fatty acid was determined based on an area of the chromatogram. This method is more simplified method of calculation recently developed for FAME estimation. The areas under the peaks on the GC traces are within limits linearly proportional to the amount (by weight) of material eluting from the columns [41]. The content of individual fatty acids was expressed as a percentage of the total content of all acids in the sample. The amount of each fatty acid present in the Pl was calculated based on equation 4.10 [42].

$$\text{Amount of specific compound} = \frac{\text{AmountIS x area SC x IRFSC}}{\text{area IS}} \text{ ---------- (4.10)}$$

Where

IS = Internal Standard

SC = Specific Compound of Interest

IRF = Internal Response Factor

$$\text{Internal Response Factor (IRF)} = \frac{\text{area IS x amount SC}}{\text{amount IS x area SC}} \text{ ---------- (4.11)}$$

Where

IS = Internal Standard

SC = Specific Compound of Interest

Then 100 µl of the internal standard was added to the sample containing analyte of unknown concentrations.

4.3.1.2. Standard FAME Sample Preparation for GC Analysis

Based on the density of individual fatty acid methyl ester, 20 µg of specific compound of interest (fatty acid methyl ester including methyl oleate, methyl myristate methyl

palmitate, methyl stearate and methyl linolate) and internal standard (methyl pentadecanoate) was prepared.

Amount of liquid FAME such as methyl oleate, methyl myristate and methyl linolate for the mixture was calculated from density.

Based on the calculation the quantity of fatty acid present in the PL was presented on figure 4.3 as follows.

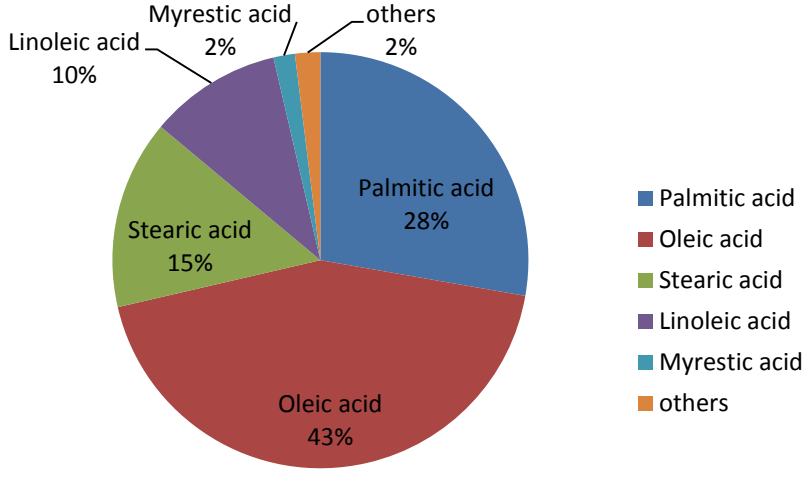


Figure 4.3: Chart for fatty acid composition of PL

4.3.2. FTIR

The infrared spectrum was recorded by using a Fourier infrared spectrometer, Perkin Elmer spectrophotometer model RX1 in central leather research institute. A film of small amount of pork oil was put between two KBr disks. Then the spectra were recorded in the wavelength range 500- 4000 cm-1. From the FTIR spectra the main functional groups were identified that determine the properties of oil for Oxidation**,** Sulphation**,** and sulphonation which are the important parameters during the fatliquor synthesis.

The spectrum of the crude oil and transesterified fatty acid methyl ester was shown in the following figures.

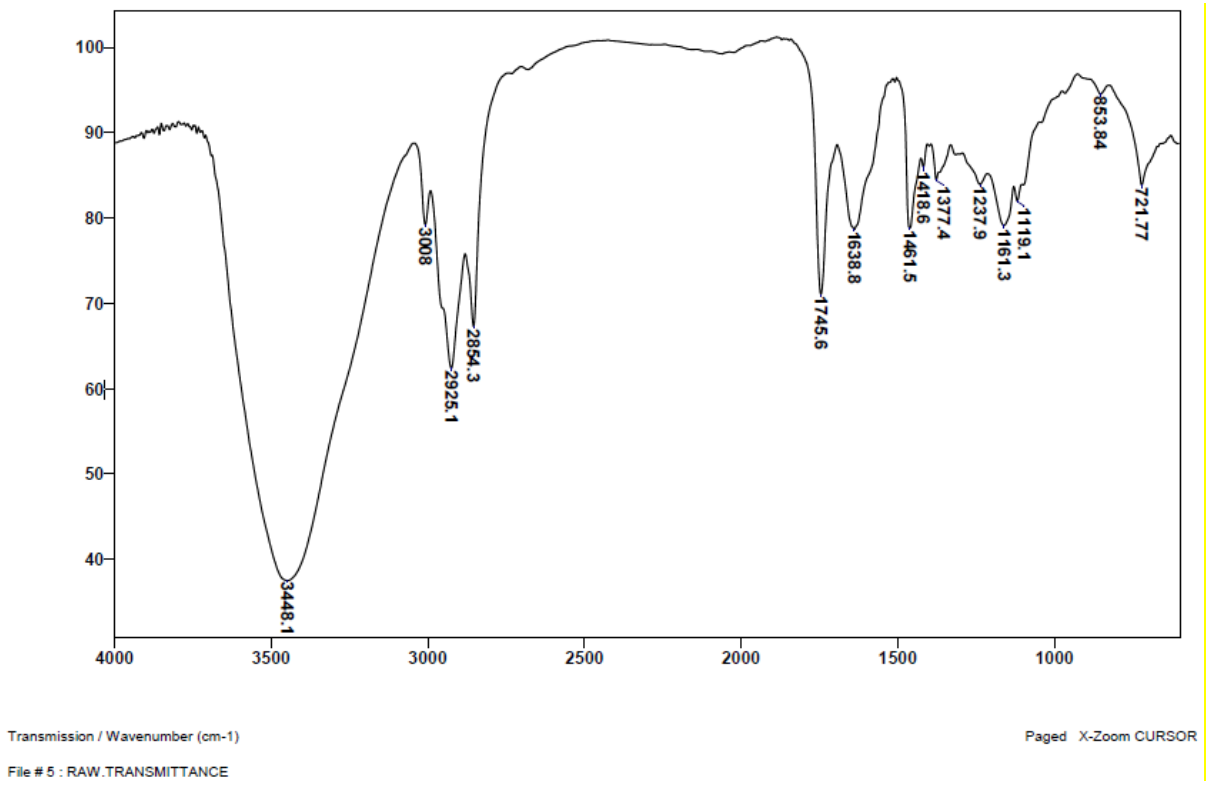


Figure 4.4: IR spectrum for raw oil

Table 4.5: Summary of IR spectrum for PL

Wave number	Functional group	Mode of vibration	Intensity of band
3008	Trans =C–H	Stretching	Weak
2925	C-H (–CH2)	Asymmetrical stretching	Medium
2854	C-H (–CH2)	Symmetrical stretching	Medium
1745	–C=O (ester)	Stretching	Medium
1638	Cis –C=C	stretching	Short
1462	C-H	Bending (scissoring)	Medium
1377	–CH3	Bending	Weak
1161	$–CH_2$, C-O	Bending, Stretching	Weak
722	cis –CH=CH–	bending out of plane	Medium

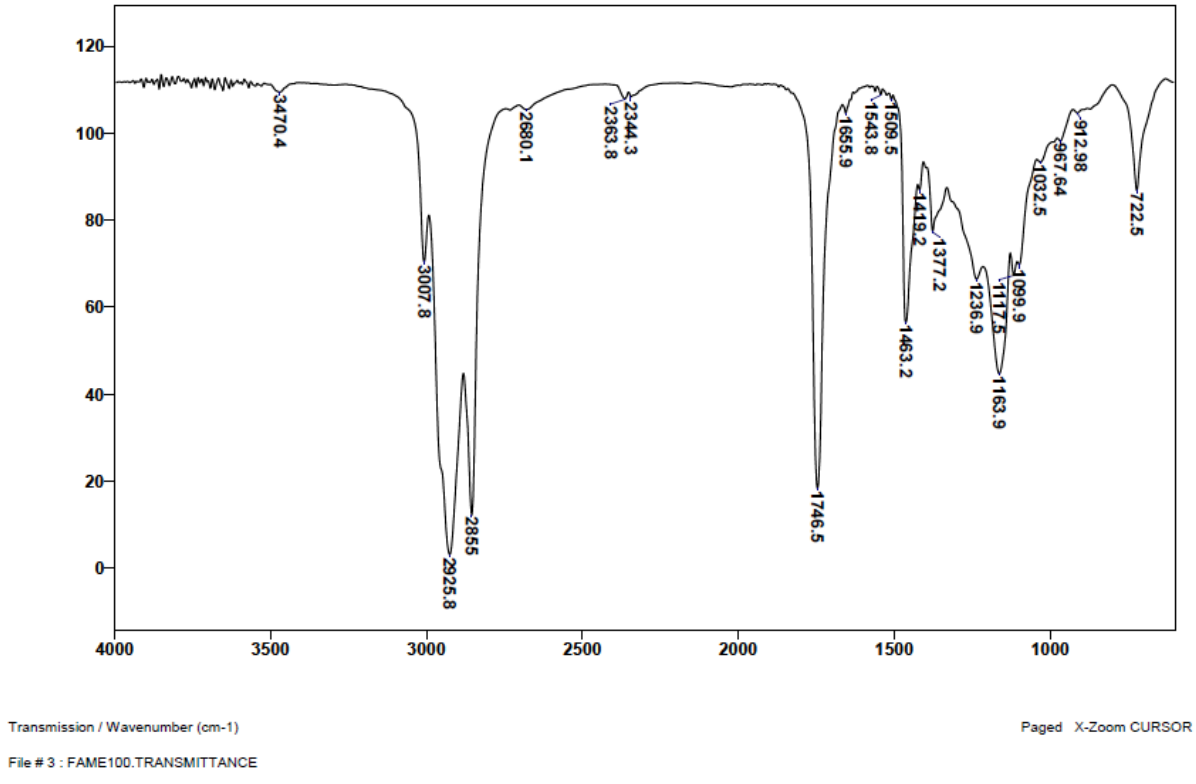


Figure 4.5: IR spectrum for transesterified PL (FAME)

Table 4.6: Summary of IR spectrum for FAME from PL

Wave number	Functional group	Mode of vibration	Intensity of band
3008	Trans =C–H	Stretching	Weak
2925	C-H ($–CH_2$)	Asymmetrical stretching	Strong
2855	C-H ($–CH_2$)	Symmetrical stretching	Strong
1746	–C=O (ester)	Stretching	Strong
1463	C-H	Bending (scissoring)	Medium
1377	$–CH_3$	Bending	Weak
1163	$–CH_2$, C-O	Bending, Stretching	Weak
1099	–C–O	Stretching	Weak
722	cis –CH=CH–	bending out of plane	Medium

Figure 4.4 & 4.5 above shows the FTIR spectra of crude pork lard and fatty acid methyl ester formed after transesterification reaction. From the spectra it can be revealed some difference between crude oil and FAME. FTIR spectroscopy is important in the identification of molecular structures based on the information content obtained and the possibility to assign certain absorption bands related to its functional groups. An intense band observed at 3448 on the spectrum of raw PL and it was vanished from the spectrum of FAME shows the conversation of alcohols to esters.

The dominant peaks in the spectra observed at represent triglyceride functional group which is the major component of the PL. These peaks was observed around 2925 cm^{-1} (C–H stretching (asymmetry)), 2855 cm^{-1} (C–H stretching (symmetry)), 1747 Cm^{-1} (C=O stretching), 1463 cm^{-1}(C–H bending (scissoring)), 1164 cm^{-1}(C–O stretching and C–H bending), and 722 cm^{-1}(C–H bending (rocking)).

4.4. Transesterification Reaction of PL for FAME Production and Yield Analysis

The transesterification reaction was carried out by optimizing molar ratio of oil to alcohol, temperature and time using 5% PPL enzyme at 200 rpm and pH 8.0.

4.4.1. Effect of Molar Ratio of PL to Methanol

Effect of molar ratio was studied at 1:3, 1:4 and 1:5 molar ratio of oil to alcohols at 40 ^{o}c and specified conditions above after 36 hours and the yield of biodiesel recoded was 74.4%, 81% and 76.5% respectively. Oil to methanol molar ratio of 1:3 is the stoichiometric molar ratio needed for the reaction but the maximum yield was obtained at molar ratio 1:4; and this is because of as the concentration of methanol decreases the reversible hydrolysis reaction will favours and that yield of ester decreases. Figure 4.6 shows the graphical presentation of effect of molar ratio on biodiesel yield.

Table 4.7: Effect of Molar ratio of PL to methanol

Run	Molar ratio of PL to Methanol	Biodiesel Yield%(w/w)
1	1:3	74.4
2	1:4	81
3	1:5	76.5

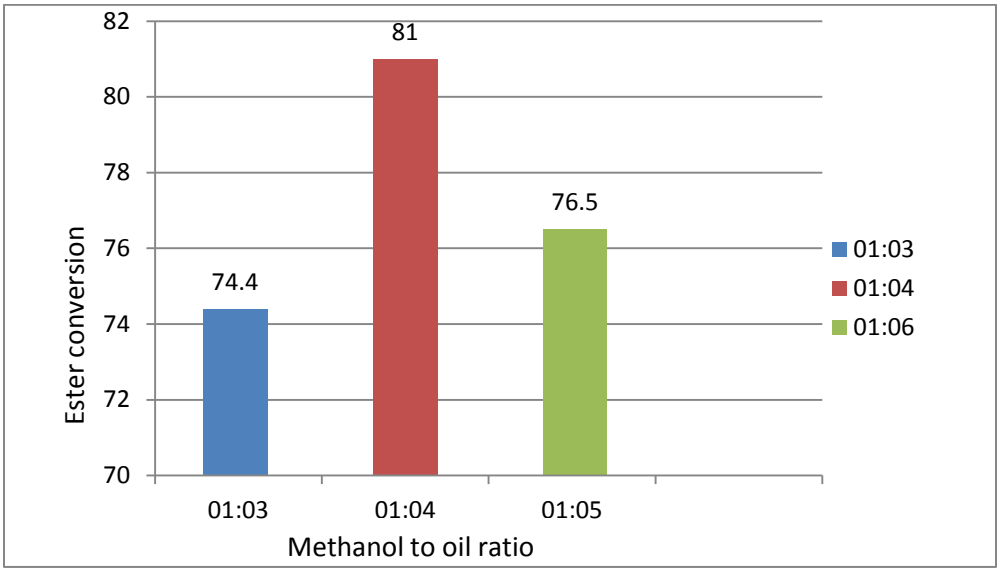


Figure 4.6: Chart for effect of Molar ratio of PL to methanol

4.4.2. Effect of Reaction Temperature on Biodiesel Yield

The effect of temperature on the transesterification reaction catalysed by PPL was studied at 30^{o}C, 40^{o}C and 50^{o}C by keeping the other parameters constant: (4:1 molar ratio of alcohol to oil and other specified conditions above after 36 hrs. The yield of ester was found to be 68%, 81% and 74% at temperature 30^{o}C, 40^{o}C and 50^{o}C respectively and the maximum yield was 81% at 40^{o}C .The results are illustrated in graph 4.7 as shown below.

Table4.8: Effect of temperature on biodiesel yield

Run	Temperature(^{O}C)	Biodiesel Yield%(w/w)
1	30	68
2	40	81
3	50	74

Table4. 4: Effect of temperature on biodiesel yield

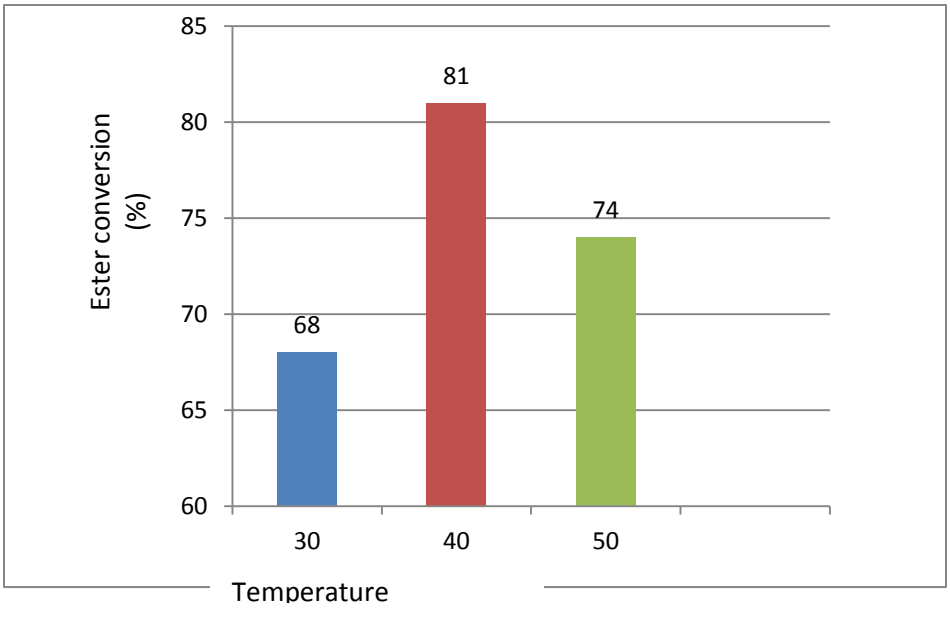


Figure 4.7 Chart for effect of temperature on transesterification

4.4.3. Effect of Time

The optimization of time to get the highest possible yield was investigated for 12, 24 and 36 hours at 40^{o}c temperature and other specified conditions above after 36 hours reaction time. The yield of transesterified product increased as time increase from 12 to 36 hours as the result is shown in table 4.8 below.

Table 4.9: Effect of time on biodiesel yield

Time	FAME yield
12	61.3
24	79
36	81

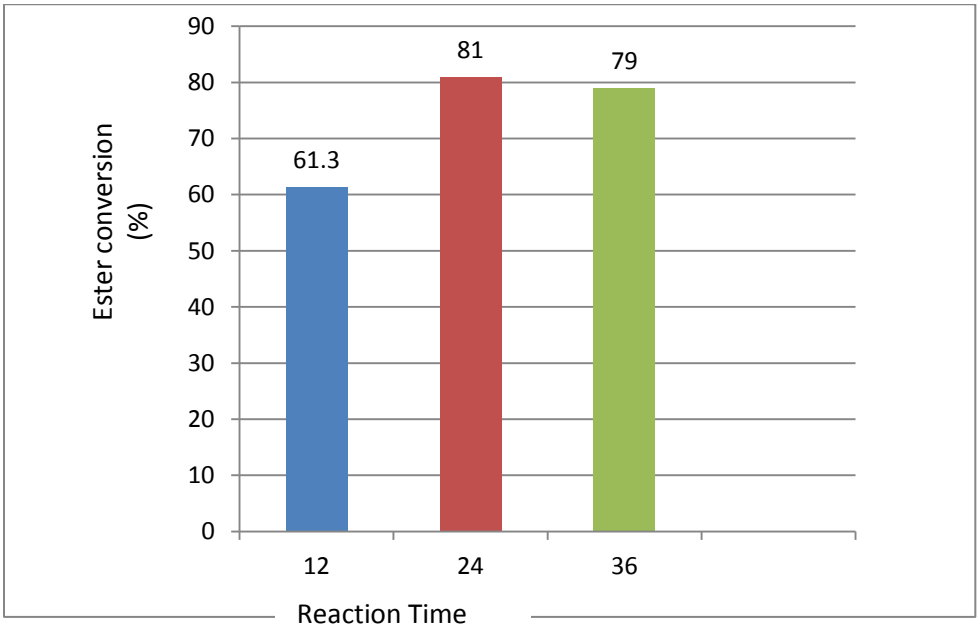


Figure 4.8 Chart for effect of reaction time on transesterification

4.5. Separation of FAME from Glycerol

The FAME /biodiesel/ and glycerine were separated by settling the mixture under gravity for 24 hours in the separatory funnel after the reaction was completed. After the mixture was allowed to settle, the glycerol was settled at the bottom of the settling vessel and FAME /biodiesel/ was floated at the top. Centrifuge was also used to separate the two materials faster by screening both phases when the separation by settling takes time.

Both upper and lower layer consisted of excess methanol, catalyst, and other impurities. After separation both the glycerine and biodiesel were further purified from these impurities by evaporation process or by distillation process to remove the excess alcohol and by washing gently with hot distilled water with 1:1 ratio of FAME product to water to prevent the possible loss of biodiesel due to emulsion formation. The washed biodiesel was properly dried to remove trace impurities.

4.6. Statistical Analysis on Factors Optimization on the Yield of Biodiesel

The optimization study was conducted based on an experimental design using Design Expert 7.0.0 software application. Three process parameters or factors such as oil to methanol molar ratio, temperature and time were investigated in three levels by keeping stirring speed and enzyme concentration fixed at 200 rpm and 5% respectively in the presence of n-hexane solvent as a media. Central Composite Design (CCD) was selected for the statistical analysis of the study and a total of twenty experiments were run. The yield of biodiesel or fatty acid methyl esters (FAME) was measured as a response. The surface plots were generated from the Regression analysis and analysis of variance (ANOVA).

The central composite design arrangements and results of the experiment and the results of statistical analysis of the ANOVA are given in tables 4.9 and 4.10 respectively. The actual yield of biodiesel produced at different process parameters are calculated using equation 4.10.

As it was shown from the table the maximum yield of biodiesel was 81% (w/w) and the minimum yield was 47 % (w/w) obtained from experimental run number 7 and 8 respectively. The maximum biodiesel yield was obtained at 1:4 molar ratio of PL to methanol and 40 ^{o}c temperatures after 36 hours reaction time. The minimum yield was obtained at 1:5 molar ratio of PL to methanol and 30 ^{o}c temperatures after 12 hours reaction time.

Table4.10: Experimental design arrangement by CCD and yield of biodiesel

Run	PL to Methanol molar ratio	Temperature	Time (hours)	Biodiesel (% w/w)
1	1:4	50.00	24.00	72.4
2	1:5	50.00	12.00	53.8
3	1:5	50.00	36.00	61
4	1:3	50.00	12.00	56.1
5	1:4	30.00	36.00	55.5
6	1:4	40.00	24.00	77
7	1:4	40.00	36.00	81
8	1:5	30.00	12.00	46.9
9	1:3	40.00	24.00	62.2
10	1:3	30.00	36.00	64
11	1:4	40.00	24.00	76
12	1:4	30.00	24.00	69
13	1:4	40.00	24.00	77.8
14	1:4	40.00	24.00	79
15	1:3	50.00	36.00	58.9
16	1:4	40.00	24.00	75.4
17	1:3	30.00	12.00	47
18	1:4	40.00	24.00	77.7
19	1:5	40.00	24.00	66.3
20	1:4	40.00	24.00	78

4.6.1. Regression Model Equation

The regression model equation that correlates the biodiesel yield and factors affecting transesterification reaction are obtained based on Response Surface Regression Model which is a hybrid type of design with characteristics of both polynomial regression designs and fractional factorial regression designs.

The quadratic regression model equation in terms of codded factor and actual value factor was given in equation 4.12 and 4.13respectively.

Final Equation in Terms of Coded Factors:

$$\text{Biodiesel yield} = 77.43 - 0.47 * A + 1.98 * B + 4.52 * C + 1.05 * A * B - 0.50 * A * C - 1.95 * B * C - 13.46 * A^2 - 7.01 * B^2 - 1.50 * C^2 \quad \ldots\ldots\ldots\ldots\ldots (4.12)$$

Final Equation in Terms of Actual Factors:

$$\text{Biodiesel yield} = 273.81089 + 103.97051 * \text{Molar Ratio of oil to methanol} + 5.77205 * \text{Temperature} + 1.69325 * \text{Time} + 0.10500 * \text{Molar Ratio of oil to methanol} * \text{Temperature} * \text{Time} - 0.016250 * \text{Temperature} * \text{Time} - 13.45506 * \text{Molar Ratio of oil to methanol}^2 - 0.070051 * \text{Temperature}^2 - 0.010417 * \text{Time}^2 \quad \ldots\ldots\ldots\ldots\ldots (4.13)$$

Table 4.10 shows the ANOVA for Response Surface Quadratic Model, Analysis of variance table [Partial sum of squares - Type III]

The Model F-value of 54.68 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 0.0500 indicate model terms are significant. In this case B, C, BC, A^2, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

Table 4.11: ANOVA analysis of experimental result

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	2292.46	9	254.72	54.68	< 0.0001
A-Molar Ratio of oil to methanol	2.21	1	2.21	0.47	0.5067
B-Temperature	39.20	1	39.20	8.42	0.0158
C-Time	170.94	1	170.94	36.69	0.0001
AB	8.82	1	8.82	1.89	0.1989
AC	2.00	1	2.00	0.43	0.5271
BC	30.42	1	30.42	6.53	0.0286
A^2	426.93	1	426.93	91.64	< 0.0001
B^2	115.72	1	115.72	24.84	0.0006
C^2	4.00	1	4.00	0.86	0.3759
Residual	46.59	10	4.66		
Lack of Fit	37.41	4	9.35	6.12	0.0260
Pure Error	9.17	6	1.53		
Cor Total	2339.05	19			

4.6.2. Interaction Effect of Reaction

The interaction effect between molar ratio of PL to methanol, temperature and time was analysed by ANOVA. The process variables have significant interaction effect as it was shown on figure 4.9 to 4.14 below which are the Contour plot and surface plot of the interaction effect of temperature and molar ratio of PL to methanol versus biodiesel yield, interaction effect of time and molar ratio of PL to methanol versus biodiesel yield and interaction effect of temperature and time versus biodiesel yield respectively. An increase in reaction time from 12 to 36 hours increases the yield of biodiesel. The highest biodiesel yield was obtained at a temperature of 4o oc, 1:4 molar ratio of PL to methanol after 36 hours. At 1: 5 molar ratio of PL to methanol the yield of biodiesel slightly reduced and this might be the inhibition effect of enzyme by an excess methanol.

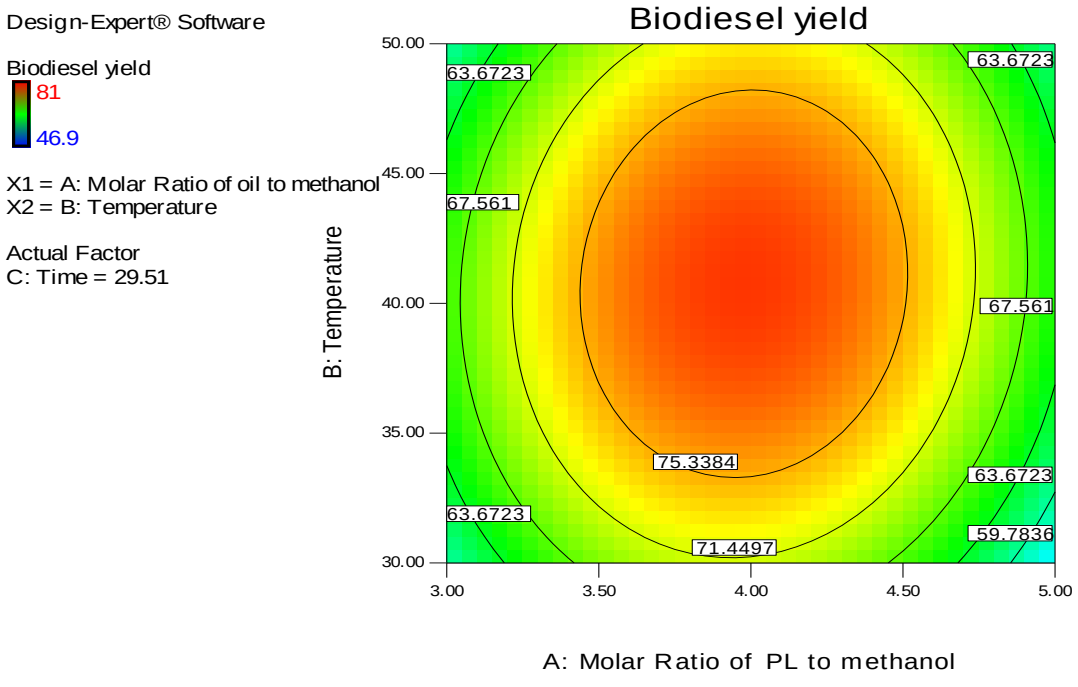


Figure4. 9: Contour plot of the interaction effect of temperature and molar ratio of PL to methanol versus yield

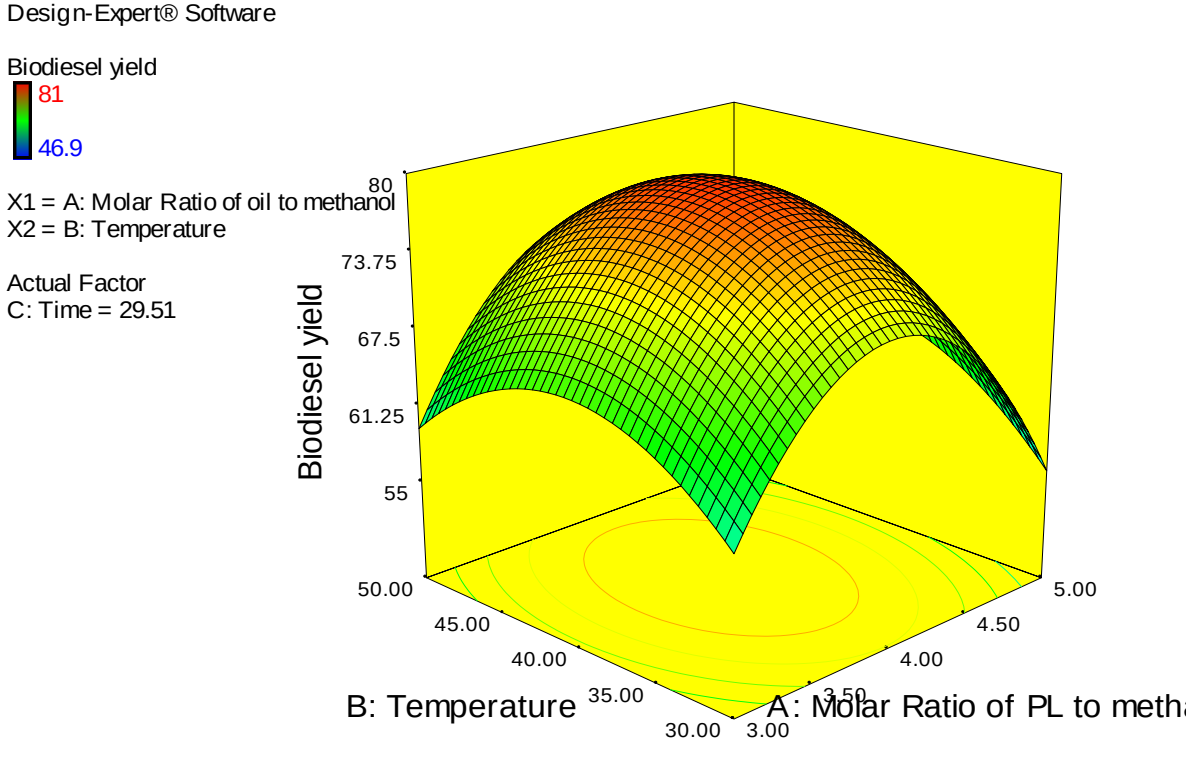


Figure4. 10: Surface plot of the interaction effect of molar ratio and temperature versus biodiesel yield

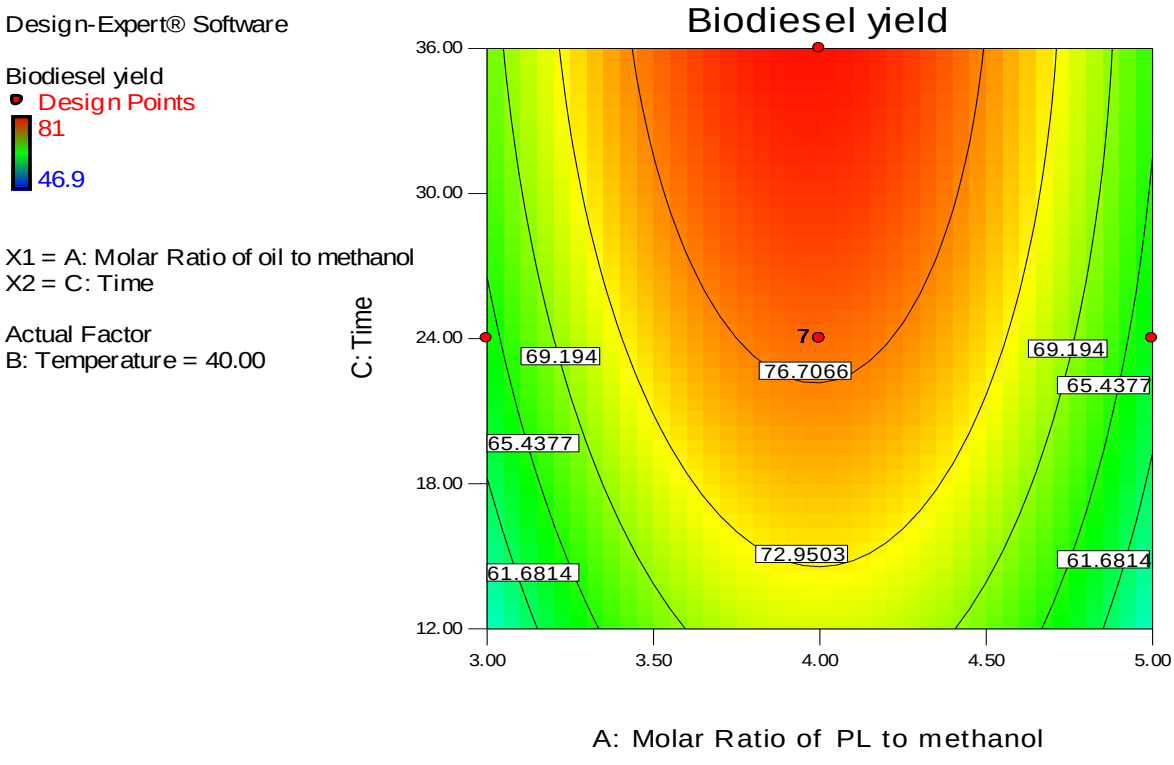


Figure4. 11: Contour plot of the interaction effect of time and molar ratio of PL to methanol versus yield

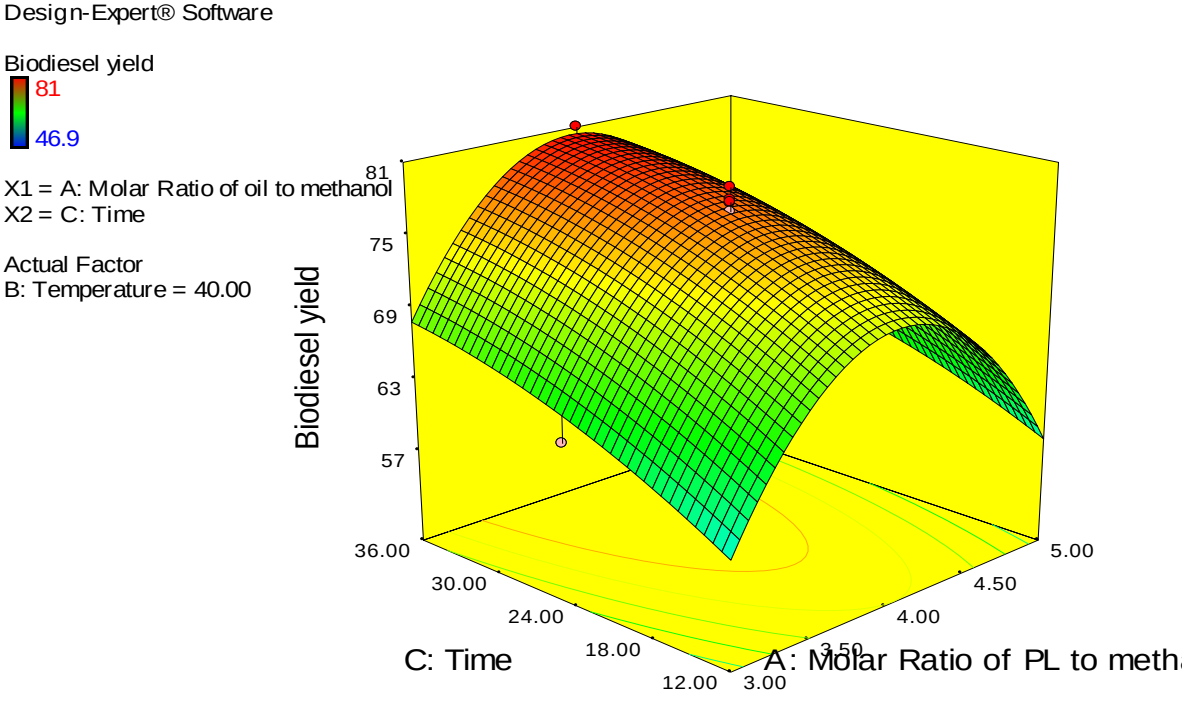


Figure4. 12: Surface plot of the interaction effect of molar ratio and time versus biodiesel yield

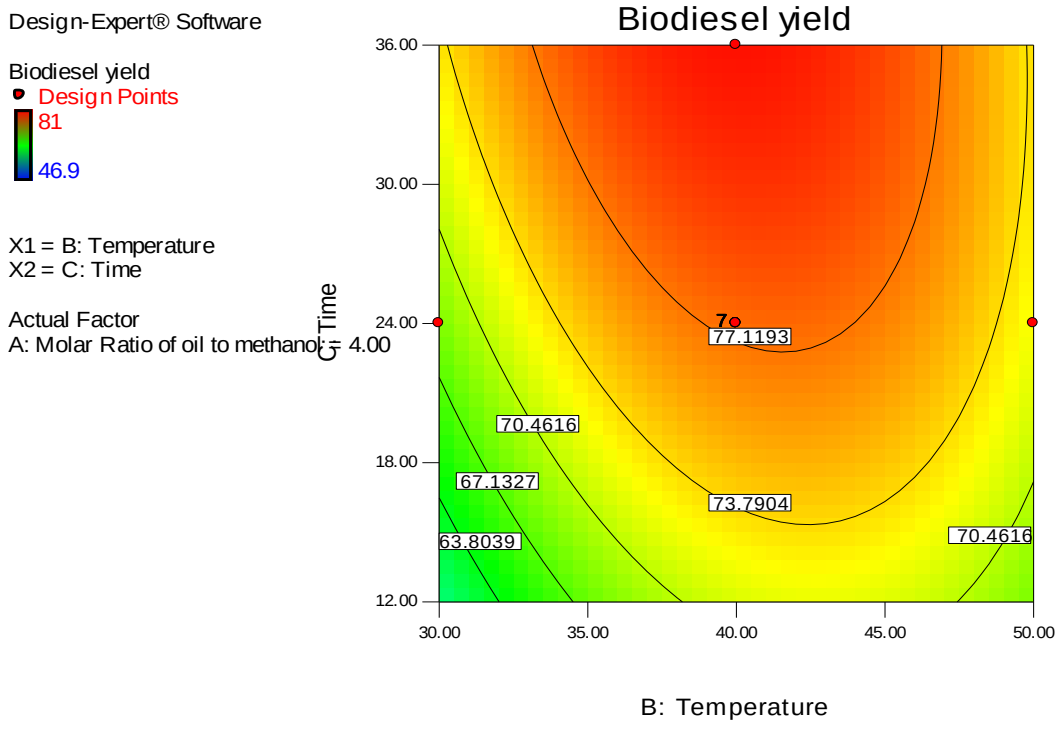


Figure4. 13: Contour plot of the interaction effect of temperature and time versus yield

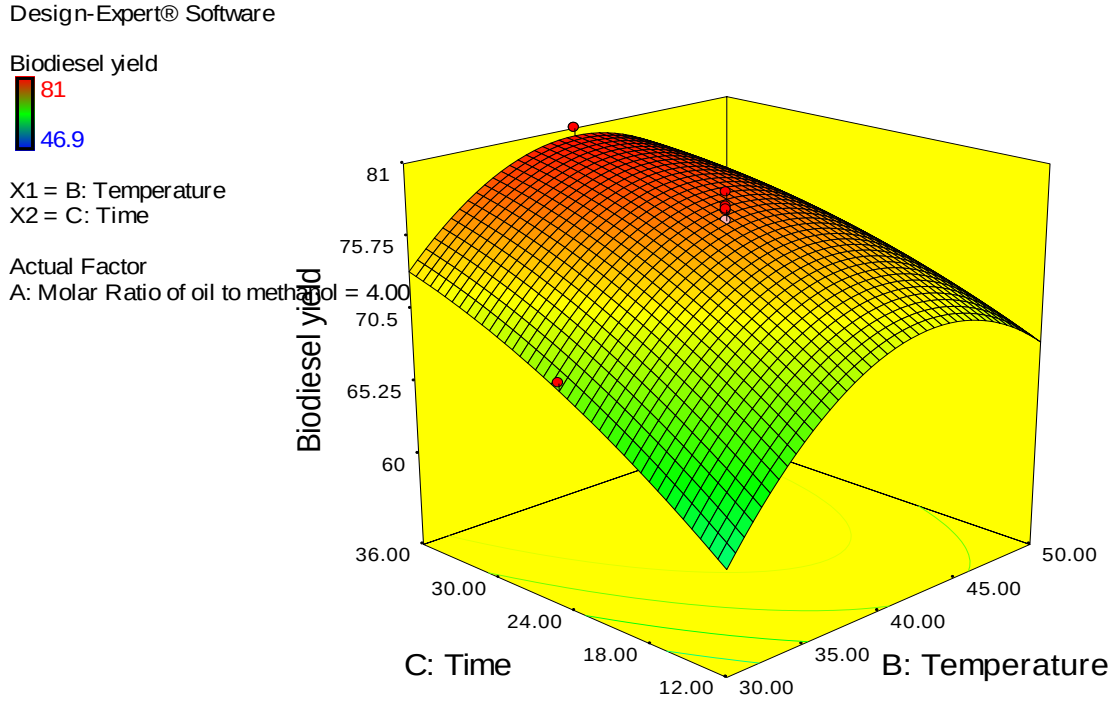


Figure4. 14: Surface plot of the interaction effect of time and temperature versus biodiesel yield

4.7. FAME Quality Analysis

The quality of FAME was analysed for its physicochemical properties are density, kinematic viscosity, flash point, moisture content and Cetane number by using the standard method. All the tests are measured based on the standard method of ASTM and EN as presented on table 2.1.

4.7.1. Density

The density and specific gravity of biodiesel was done based on the procedure described on methodology section on page 26 by which the density and specific gravity of oil was measured.

So the density of FAME from PL was calculated and found to be 867 kg/m^3. The standard value of biodiesel density is 860 – 900 kg/m3according to EU EN 14214. The lower density fuel burns quickly and consumed immediately while higher density fuel burns for longer time.

4.7.2. Kinematic Viscosity

The kinematic viscosity of the biodiesel was measured based on procedure written for raw oil and the value was 4.68mm^2/s. This value is within the ASTM and EN 14214 standard value of kinematic viscosity which are 1.9-6.0 and 3.5-5.0 mm^2/s.

4.7.3. Flash Point (°C)

The flash point of the biodiesel was determined using pensky Marten (open cup) method as the procedure was explained on the methodology part. The flash point of biodiesel from PL obtained from the experiment was 144^{o}c which is above the ASTM and EN 14214 standard values 130 ^{o}c and 120 ^{o}c respectively. The fuel having lower flash point is more favour for spontaneous ignition during transportation and storage for longer time but fuel with higher flash point can resist spontaneous combustion [34].Therefore the

flash point indicates the biodiesel from PL can resist spontaneous combustion during storage.

4.7.4. Moisture Content

The moisture content of biodiesel from PL was done in similar manner with that of raw oil by heating at 105 ^{o}c in oven. It was calculated based on the weight difference method as explained on equation 4.1 and it was found 246 mg/kg of biodiesel. This value is less compared to the US ASTM standard value which is ≤ 500 according to EN ISO 12937. The water content of the biodiesel can be come from wash water during purification and poor drying.

4.7.5. Ash Contents of Biodiesel

Ash content of biodiesel was determined by using furnace at a temperature of 500^{o}C in a similar way done for the determination of raw oil. A 15 g of FAME was added in a burning cup and placed in a furnace. A furnace was heated to a temperature of 500^{o}C for 1 hour and after burning the residue sample was weighted and ash content was calculated. The value of ash content of biodiesel was found to be 0.023% (w/w) which is slightly higher than the limits of ASTM standard (<0.02).

Ash content indicates the contents of inorganic contaminants, such as abrasive solids and catalyst residues, and the concentration of soluble metal soaps contained in a fuel sample [35].

4.7.6. **Iodine Value**

This test was done by the procedure explained on the methodology part on page 29. The value of iodine value of biodiesel obtained from the test was 58.1 mgKOH/ gram of sample which is within the limit of EN 14214 standards (<120). The value is almost

similar to the iodine value of raw PL. Iodine value is the measure of unsaturation of the biofuel.

Table 4.12: Summary of physicochemical properties of biodiesel from PL

Biodiesel properties	Measured values	ASTM Standard	EN 14214 Standard
Density @ 20^{o}C (kg/m^{3})	867	875-900	860-900
Kinematic viscosity @40^{o}C (mm^{2}/s)	4.68	1.9-6.0	3.5-5.0
Flash point (^{o}C)	144	$\geq$130	$\geq$120
Moisture content (mg/kg)	246	< 500	-
Iodine value (Mg KOH/g)	58.1	<120	-
Ash content% (w/w)	0.023	< 0.02	

4.8. Fatliquor preparation

The leather fatliquors were prepared by Sulphation and an external emulsification of PL.

4.8.1. Fatliquor Preparation by External Emulsification

This fatliquor was prepared by blending the crude PL and FAME with four different emulsifiers such as A, B, C, D and HNP as a dispersing agent with different ratio. Heavy Normal Paraffin (HNP) has carbon chain length C_{14}-C_{17} and average Molecular weight 195 g/mol is used as a dispersing agent with different formulation ratio based on the emulsion stability.

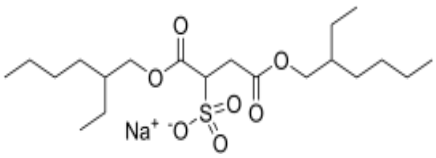
Where

A- Stearyl amine ethylene oxide, commonly known as stearyl amine:- Chemical formula : R N (CH_2CH_2O)x H, R : stearyl. X = ~ 9.10,

B- Steary poly(oxyethylene) (POE) ether:- commonly known as Poly (oxyethylene)
Chemical formula: $C_{18}H_{35}O(CH_2CH_2O)_n H$, n= ~10.

C- Tallow amine ethylene oxide: - Chemical formula: $R\,N\,(CH_2CH_2O)_x H$, R = Tallow fatty acids mixtures, x: 8.40

D- Sulfosuccinate dispersion in water: IPA/PPG mixtures.
Active matter: 60%.



Sulfosucciantes: 2 ethyl hexyl ester (diester) of maleic anhydride, converted into sulfosuccinate, through addition of sodium bisulphite at the maleyl unsaturation site. This is also popularly known as Aerosol OT.

The optimization study was done based on the emulsion stability of the fatliquor for 40 minutes. After several trials 40:20: 20: 20 mass ratio of oil: FAME: HNP: Emulsifiers respectively were optimized. The 20 percentage emulsifier consists of four different emulsifiers A, B, C, and D at each percentage of, 6, 6, 5 and 3% respectively and presented on table 4.12.

Table 4.13: composition of externally emulsified fatliquor

Components	**Percentage**
Crude PL	40
PL FAME	20
HNP	20
A	6
B	6
C	5
D	3

4.8.2. Fatliquor Preparation by Sulphitation Process

The sulphited fatliquor was prepared by mixing of pork lard, sodium meta bisulfite and stearyl amine emulsifier. The reaction between oil and PL contains 44% oleic acid and 11 % Linolic acid which is unsaturated fatty acid (UFA) as it was seen in the characterization of part. The presence of these UFA makes the oil suitable by providing reactive site (double bond) for the sulphitation reaction to takes place.

The reaction was takes place in a reactor with Ms-jacket fitted with agitator. 400 gram of PL and 100 gram of FAME was charged into the reactor. The mixture was heated to 70-80^0c by passing steam through the jacket. Then 2.5 gram of hydrogen peroxide was added as a catalyst to speed up the reaction and sparking air into the reactor for half and an hour. 25 gram ionic emulsifiers (Stearyl amine ethylene oxide) was added to the mixture and stirred for 15 minutes. Emulsifier is used to bind the insoluble $NaHSO_3$ and immiscible oil with water. Then a paste solution of $NaHSO_3$ was added in 6 feed/ instalment. After each instalment the reaction was allowed to go for 30 minutes. After the completion of all instalments the reaction continues for 4 hours to completely consume the unreacted $NaHSO_3$.

4.9. Physicochemical properties of Fatliquor

4.9.1. Physical Condition

4.9.1.1. Fatliquor Emulsion Stability Characteristics

The stability of the prepared fatliquor emulsion was studied by observing phase separation (as oil and water) if any takes place with respect to time.

The fatliquor was formed a stable emulsion in hot water (55^oC-65°C) when diluted in 1:10 ratio and the water emulsion containing 3 to 4 percent total fatty matter was remain stable for at least 40 minutes at room temperature 27 ± 2°C without creaming and oil separation.

The fatliquor was also tested for 10 percent emulsion stability in three different salts such as sodium chloride, calcium chloride, and 5 percent basic chromium sulphate solution in

separate containers without creaming and oil separation. This test helps to know the degree of emulsion break during the fixation step in the leather fatliquoring process.

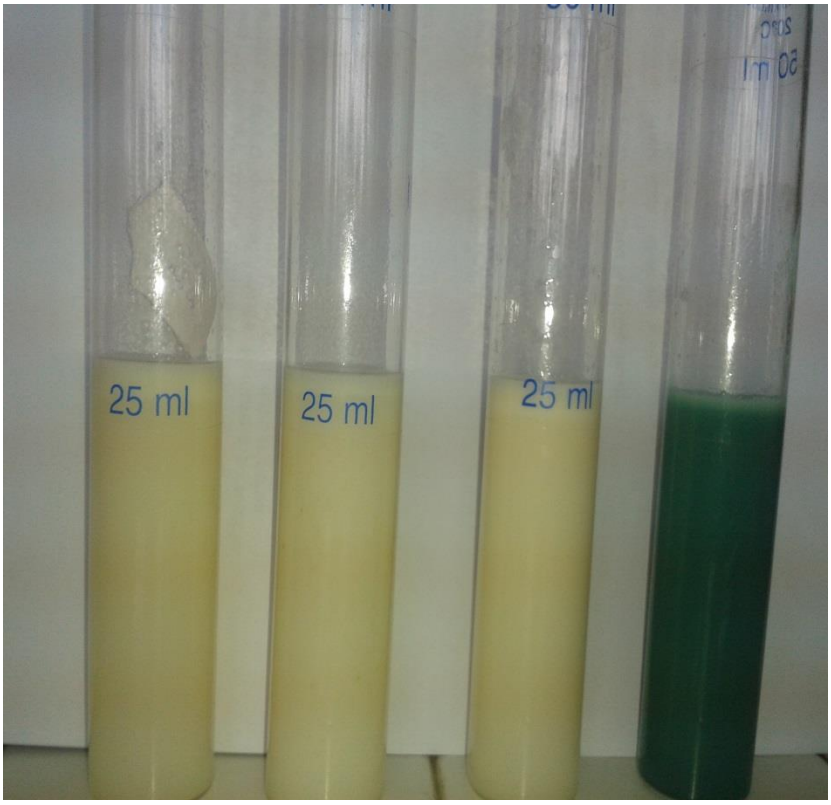


Figure 4.15: Emulsion of sulfited fatliquor in hot water, 5% brine solution, 5%calcium chloride solution and 5% chrome sulphate solution from left to right respectively.

4.9.1.2. Emulsion Particle Size

Emulsion particle size is an important parameter which influences properties of an emulsion. The particle size will be determined using Laser Diffraction Particle Size Analyzer for both commercial fatliquor and new fatliquor comparably.

Measurements of zeta potential, along with particle size, can be used to predict the stability of fat emulsions.

Control of the zeta potential and size enables to control the characteristics of colloidal particles, in production a high zeta potential prevents aggregation of the emulsion, increases stability to give a shelf life greater than 2 years. The data output from the Zeta-sizer is shown in graph as follows for both the controlled and sample fatliquors.

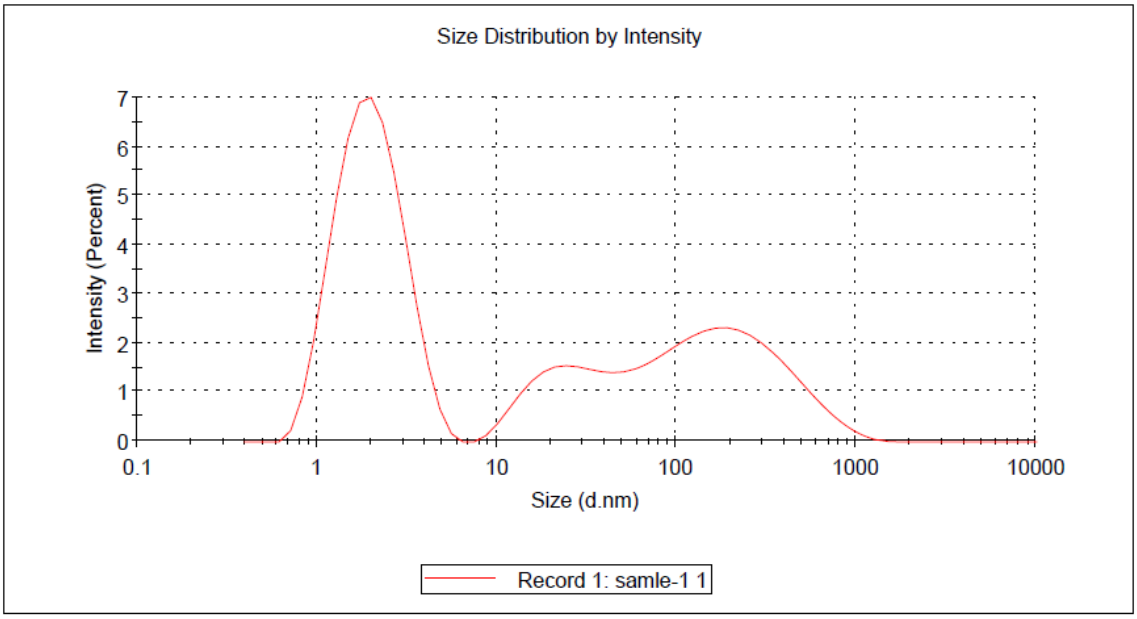


Figure 4.16: Size distribution of raw oil using heptane as a dispersant

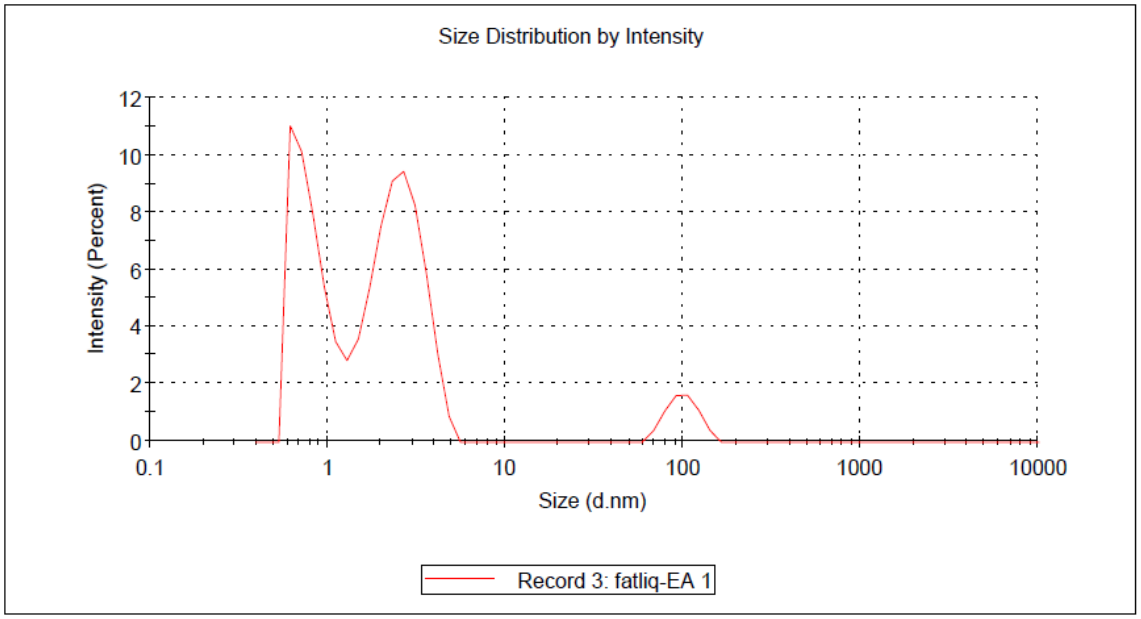


Figure 4.17: Size distribution of externally emulsified fatliquor from PL

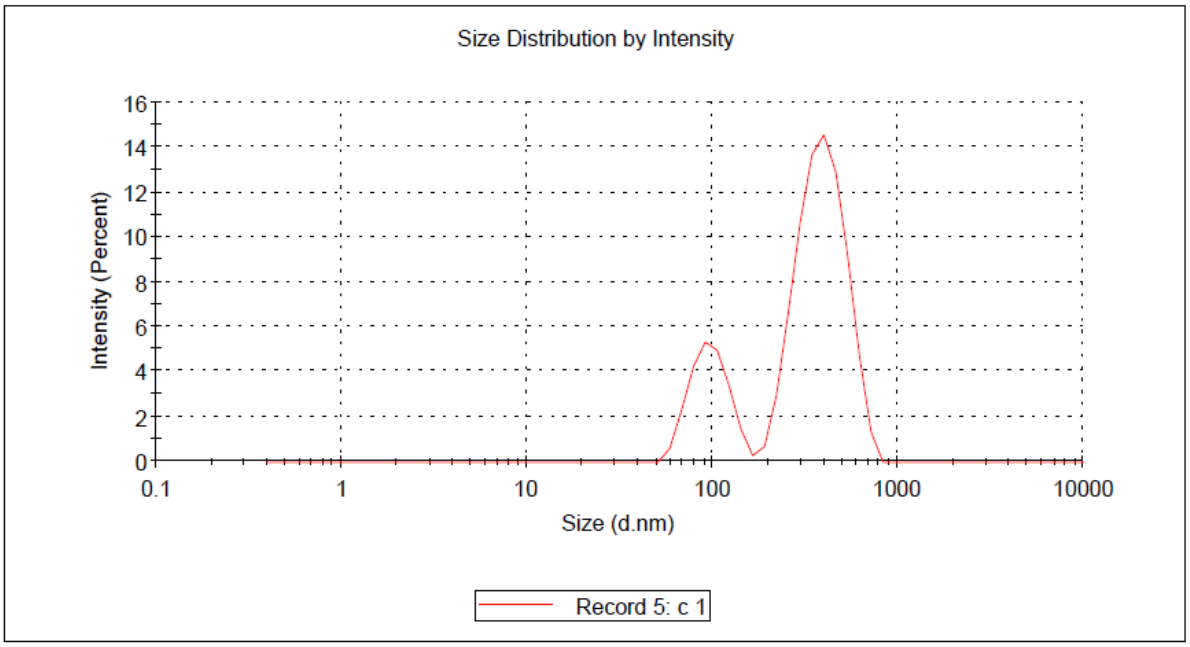


Figure 4.18: Size distribution of sulphited fatliquor from PL

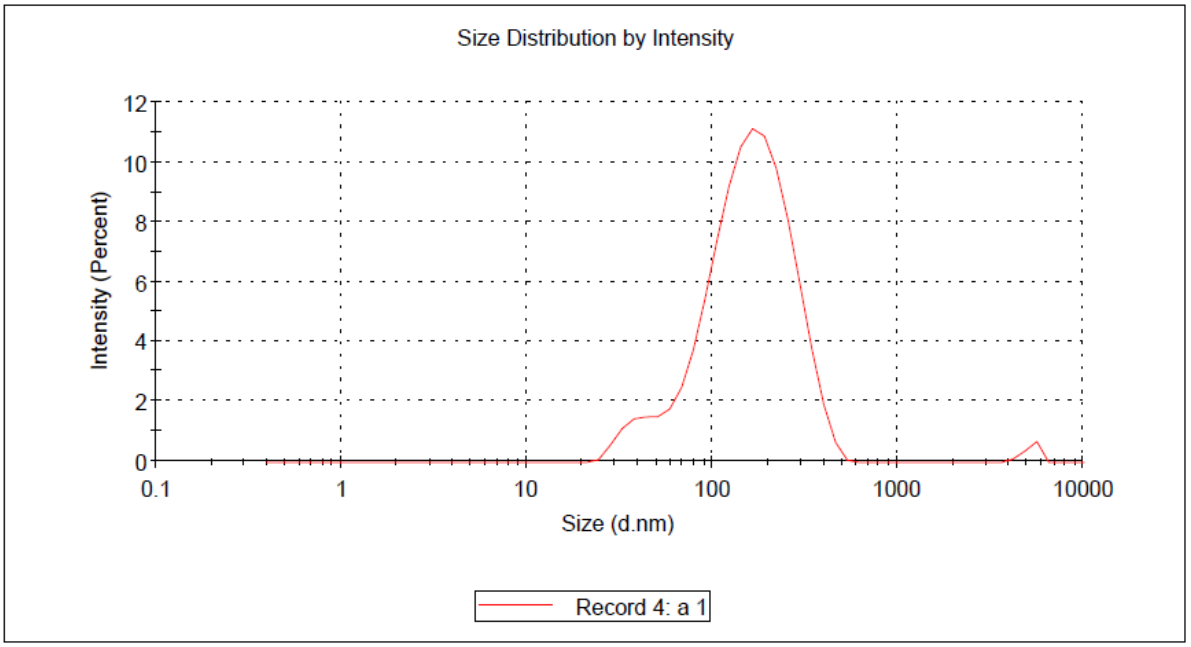


Figure 4.19: size distribution of conventional sulfited fatliquor from fish oil

The size distribution of raw oil was done by using heptane as a dispersant and all the rest new and conventional fatliquors were analysed by using water as a dispersing agent. As it was shown from the figure the size distribution of raw oil, sulfited fatliquor from PL and conventional sulphited fish oil is ranging from 0.7-1000 nm, 25-500 nm and 50 – 800nm. Externally emulsified fatliquor from PL has a size distribution of 0.5 -6nm and 60 – 200

nm. This shows it can penetrate the collagen structure of the skin easily. The sulfited fatliquor ha a comparable size distribution with conventional sulfited fish oil fatliquor. According to literature review, the average pore radius of chromium crosslinked collagen matrix of chrome tanned leather from goat is around 2–30 nm and that of sheep is 2–20 nm which was measured by thermoporometry. Nitrogen adsorption result shows, the average pore diameter of goat and sheep is 289 nm and sheep 385 nm respectively [52]. Therefore the new fatliquor is suitable to penetrate and cover the internal fibre structure of the skin to give the desired property to the leather.

4.9.2. Chemical Requirements of Fatliquor

4.9.2.1. pH of emulsion (1:10 Dilution in Distilled Water)

The pH of ten percent emulsion was measured by using electrode type *p*H meter. The pH of each fatliquor was illustrated on the table 4.13 below.

Table 4.14: pH value of fatliquors

Fatliquor type	Emulsion dilution (fatliquor to oil)	pH of emulsion
Sulfited fatliquor from PL	1:10	6.5-7.0
Externally emulsified fatliquor from PL	1:10	6-7

The pH range of both sulfited and externally emulsified fatliquors was within the anionic pH range which is suitable for leather fatliquoring process comes after retanning.

4.9.2.2. Total Active Ingredient, Percent by Mass

The experiment for the determination of total active ingredient, percent by mass of sulfited fatliquor was performed by following the procedure specified on page 40 on the methodology part.

The free oil and emulsifier content in percent was calculated based on equation 4.14.

$$\text{Free oil, in percent (A)} = \frac{F}{W} \text{ x } 100 \text{ ---------- (4.14)}$$

Where

F= weight of extract soluble in petroleum ether in g = 3.7g

W = weight of the fatliquor sample in g = 5 gram

Based on the calculation the total active ingredient of the sulfited fatliquor prepared from PL was found to be 74% which is within the standard requirement according to Indian standards. Since externally emulsified leather fatliquor was prepared by simple formulation of 80% of purified oil and 20% of emulsifiers no need of determining the active oil content by titration method. 80 % is the active matter for the fatliquor.

4.9.2.3. Determination of Total Alkalinity

The titration volume of 0.5 N sulphuric acid in the triplicate experiments after the end point were recorded on table 4.14. The total alkalinity of the fatliquor was estimated by using equation 4.15.

Table 4.15: volume of 0.5 N sulphuric acid consumed in total alkalinity test

Trial	volume of 0.5 N sulphuric acid consumed
1	6ml
2	5.9ml
3	6ml
Average	5.97

Calculation

$$\text{Alkalinity} = \frac{10 \text{ x } V \text{ x } N}{W} \text{ ---------- (4.15)}$$

Where

A = total alkalinity in milli equivalent per 10g of oil,

V = volume of sulphuric acid in ml,

N = normality of sulphuric acid, and

W = weight of the sample in g.

Based on the calculation the alkalinity of the fatliquor was 2.98 milli equivalent per 10 gram which is within the Indian standard alkalinity value (<5 milli equivalent per 10 gram sample). NOTE - This method is not applicable in case sodium acetate or other compounds which are not accurately titratable to methyl orange in aqueous solution is present in the fatliquor.

4.9.2.4. Determination of Total Ash (Referee Method)

The total ash content of the fatliquor was calculated by using equation 4.16 as follows.

Calculation

The ash content was calculated by using equation 4.16.

$$\text{Ash content in percent} = \frac{W2}{W1} X\ 100 \text{ ---------- (4.16)}$$

Where

W1 = weight of the sample before ashing, in g = 3gram

W2 = weight of the residue after ashing in g = 0.11gram

The ash content of sulfited fatliquor was 3.67 percent by mass of fatliquor which is slightly lower than the standard ash content value (4-5) of Indian standard.

4.10. Application of Fatliquor on Leather

Ten sheep skins were processed for the research from soaking to tanning in one batch. Each wet blue was divided into two sides along the backbone. Then three trials were conducted in three different drums as control, test for sulfited fatliquor and test for externally emulsified fatliquors. Three sides of skin wet blue were taken for each trial (total of nine wet blue) for the post tanning process. For each trial only one fatliquor has been used to study their effects on the leather comparatively. Since the new fatliquor was prepared from animal based PL, commonly used commercial sulfited fish oil was used to

process the controlled leather. The leather processing was done in the testing drums provided with the automatic control of speed and temperature in Leather Industry Development Institute (LIDI) of Ethiopia model tannery.

Table 4.16: Process recipe for Retanning and dyeing for sheep garment leather (% based on wet blue shaved weight)

Operation& chemicals	T ^{o}c	%	Kg/lt	Time/min	Remark
Water Formic acid Selasol DLA	35	200 0.2 0.2		 20	Drain /wash/drain
Water SalChrom-26 /Chromotel XGS	38	150 4		 40	
Gene tan LD Gene tan neutro A2 Sodium formate		3 1 1		45 60	
Leave O/N					3min.run, 27min. stop
Next day				15	Run, drain
Water Sodium formate Sodium bicarbonate	35	150 1 1.5		 60	 Check PH (5.5-6.0)- Drain
Water	40	300		10	Drain
Water Genecryl QS	40	75 3		 20	
Retanal SLF 200		3		45	
Retanal MD 80 Nerphil powder Retanal SLF 200		3 3 2		 30	
Dyestuff(Incoflore black GTN)		4		60	
Water	60	100			
Sulfited fish oil		**12**		**0**	
Formic acid		1		15	
Formic acid		1		25	Check PH,3.7-3.8,drain
Water	60	100			
Dyestuff		2			1:10@60^{0}c
Formic acid		1		20	Drain
Water	60	100			

For Sulfited PL and externally emulsified PL the same recipe was used by replacing only the Sulfited fish oil by new synthesized fatliquors.

4.11. Physico-Chemical Tests of Leather

The fatliquored leather was characterized from three points of consideration such as: Organoleptic tests, Physical resistance tests and Chemical tests.

4.11.1. Organoleptic Tests

Under organoleptic tests the parameters considered are: touch, colour, and firmness factors. The touch of the leathers was tested and graded on the level of softness. These properties are evaluated physically by Dr. Madhan, Dr. Nishad and Mr. suril who have best experiences on leather processing and leather products. The results are shown in table 4.16.

Table 4.17: Results of Organoleptic tests

Parameters	Control-1	Control-2	Sulfited fatliquor	Externally emulsified fatliquor
Softness	7.5	8	8	8.5
Fullness	9	9	8.5	8.5
Roundness	7.5	7	8	8
Smoothness of grain	7.5	7.5	7.5	8
Uniformity of color	8	8	8	8
Overall appearance	8	8	9	8.5

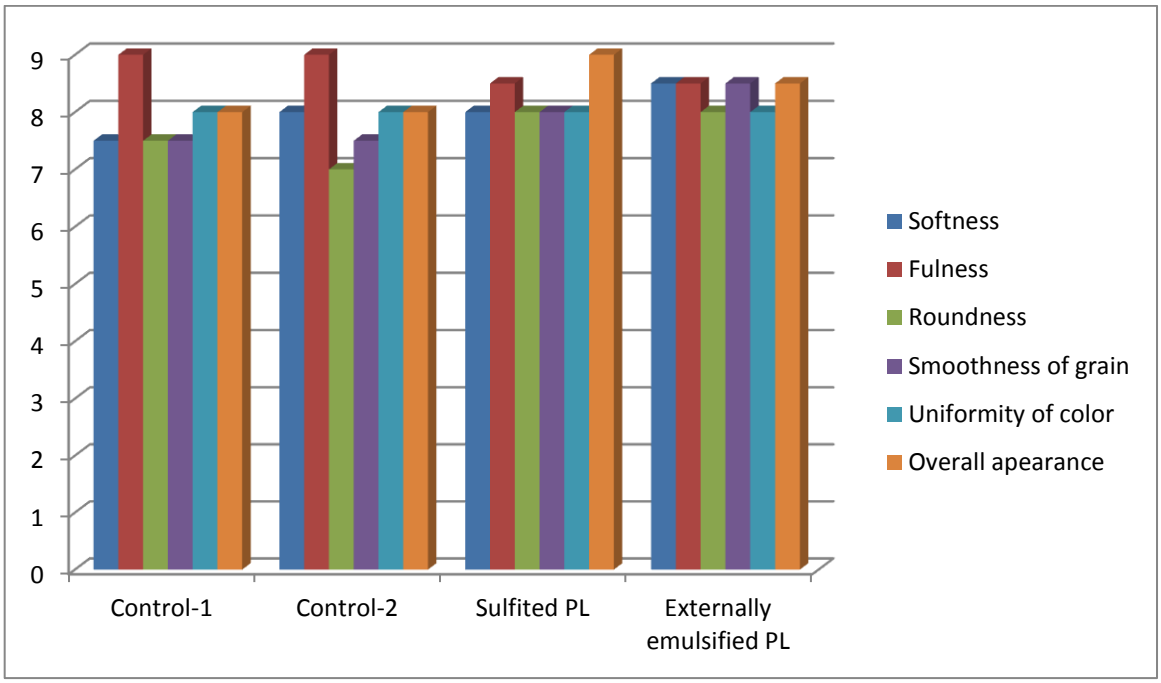


Figure 4.20: Chart for organoleptic properties of leather

From the observation of Organoleptic Properties the new sulfited and externally emulsified fatliquors showed improved softness, roundness and overall appearance than the control fatliquors, all the rest properties are almost comparable. Grading above 5 is considered as a pass for organoleptic properties.

4.11.2. Strength Properties

The physical tests of the sample leathers produced by the new fatliquors and commercial reference fatliquors were assessed in accordance with accepted ISO standard test methods. For the current work garment leather was produced and its physical or strength properties such as thickness, tensile strength, and tear strength and percentage elongation of the leather were conducted in LIDI research and testing laboratories. The test results of leather physical strength are shown in table 4.18 respectively as shown below.

4.11.2.1. Tensile Strength and Elongation at Break

The tensile strength was measured by tensile strength testing Machine (Dynamometer) according to the procedure specified on ISO 3376/iup 6 test method and the value of tensile strength of the garment leather was calculated by equation 4.17.

$$\text{Tensile strength, N/mm}^2 = \frac{\text{Force (N)}}{\text{Area (Width in mm x Thickness in mm)}} \text{-------- (4.17)}$$

Percentage Elongation at Break

The elongation measurement was made at a designated load and is expressed as a percentage of the original distance between the jaws or between bench marks of the unstretched specimen which is calculated by equation (4.18).

$$\%\ Elongation\ at\ break = \frac{(b-a)\ x\ 100}{a} \text{---------- (4.18)}$$

Where a = Initial distance between the jaws in mm

b = final distance between the jaws in mm

4.11.2.2. Tear Strength

The tear strength test of the leather was measured by double edge tear strength – Baumann tear strength test methods.

$$\text{Tear strength} = \frac{\text{Maximum tear force (N)}}{\text{Thickness (mm)}} \text{---------- (4.19)}$$

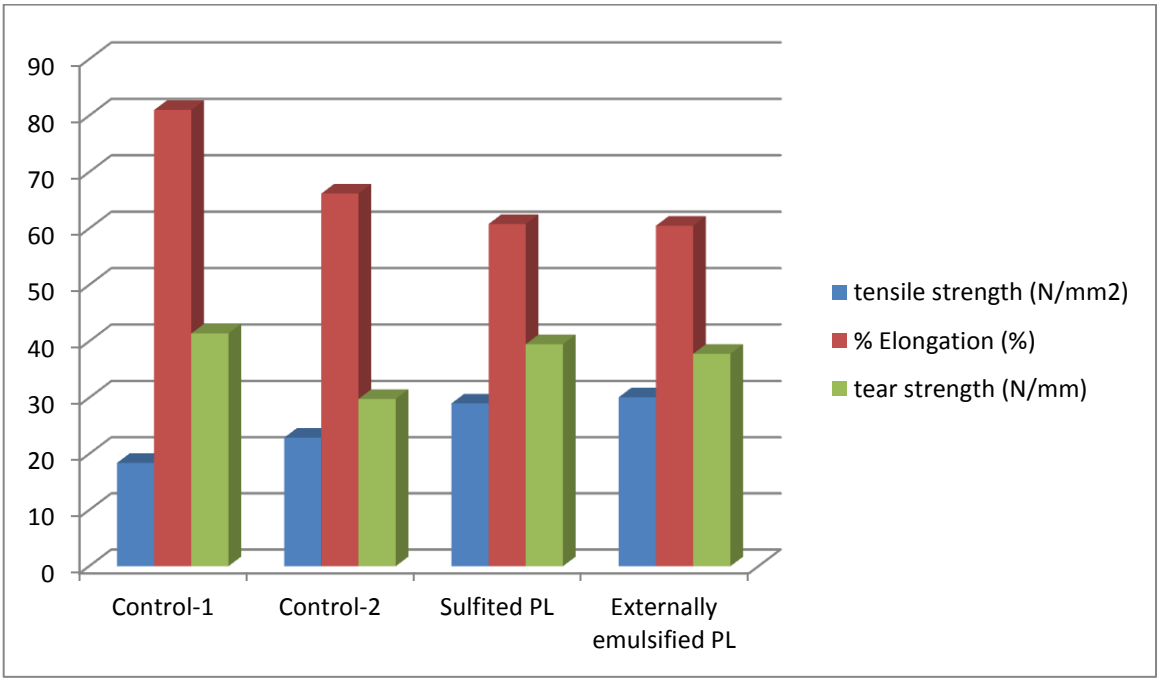


Figure 4.21: Chart for strength properties of leather

The test for this experiment was done by double edge tear strength (Bauman tear strength method) using dynamometer according to the procedure specified on this iso 3377/iup 8 test method. The value was calculated based on equation 4.19.

Table4.18: Summary of results and standard values of strength properties of garment leather

Leather tested property	Test of direction	Fatliquors Tested			
		Controlled-1	Controlled-2	Sulfited PL	Emulsified raw oil
Extractable fatty matter	-				
Tensile strength (N/mm)	-	18.4	22.9	29	30.1
% Elongation	-	81	66.2	60.8	60.5
Tearstrength (N/mm)	Parallel	36.5	30.4	33.5	32.4
	Perpendicular	46.3	29.1	45.6	43.2
Average tear strength (N/mm)	-	41.4	29.8	39.5	37.8

Controlled – 1: Leather treated with Commercial sulfited fish oil fatliquor which is a control for sulphated PL.

Controlled – 2: Leather treated with Commercial sulfited fish oil fatliquor control for externally emulsified PL.

From table 4.18, leather treated with sulfited and emulsified pork lard has better tensile strength than the controlled leather treated with sulfited fish oil. The tear and percentage elongation of both tested and controlled leathers is comparable.

Outcomes

- Leathers treated with fatliquors prepared from sulfited and externally emulsified PL exhibits good softness, fullness, fine grain with improved strength properties suitable for light upper and garment leathers.

- Mixing FAME to crude PL in the preparation of fatliquor improves the quality of the fatliquor by reducing its viscosity and increasing emulsion stability against hard water and salt solution.

5. Economic Potential Evaluation

5.1. Flow Sheet Synthesis and Economic Evaluation of Biodiesel Production

Once the process has been described, the flow sheet of transesterification and fatliquor production was synthesized which give an overview of the whole process. For synthesizing the flow sheet for transesterification and fatliquor production various points should be considered before decisions are made at different level. These different levels decisions include 'Level -1 to Level- 5' decisions [51].

- Batch v/s Continuous Process
- Input-Output Structure of Flow sheet
- Recycle Structure of Flow sheet
- Separation System for Flow sheet
- Heat Exchanger Network for Flow sheet

Batch v/s Continuous Process

To decide the process to be either batch or continuous process four important guidelines such as Production Rates, Market Forces and Operational Problems should be analysed.

Table 5.1: shows comparison between batch and continuous operation.

Process characteristics	**Batch process**	**Continuous process**
Production Rate and economy of scale	• if more than one product is planned (Multiple plants) • less than 50,000 TPA	• Only one product is planned (Multiple plants) • less than 50,000 TPA
Typical plant size	• Smaller	• Greater
Market Forces Basis	• Products having short life span • Product is seasonal	• Products having long life span • Product is not seasonal
Scale-up / Operational basis	• Very long reaction times (very slow processes) • Handling slurries at low flow rates • Rapidly fouling materials	• Shorter reaction times (rapid processes) • Handling slurries at high flow rates • Slowly fouling materials
Feed stock flexibilities	• Greater flexibility	• Less flexibility

From the two batch and continuous process alternatives given in 5.1 a continuous process is selected for the present biodiesel production by considering the advantage listed in the table for the better production capacity and to able to use the recycled unreacted methanol and catalyst.

Flow Sheet for TER Process

Process flow sheet design in biodiesel production involves the design of each unit operations processes for desired physical and chemical transformation of triglycerides to fatty acid methyl ester. This process includes many unit operations such as reactor in which reaction takes place and separation unit operations. Determining the flowsheet, equipment needs, and implementation requirements for a particular process are the activities in designing the process. [44]

Since enzyme catalysed transesterification does not form soap from free fatty acid, pre-treatment reactor is not needed before the main reactor to reduce free fatty acids entering to the main reactor. In alkali catalysed TER the pre-treatment is used to avoid the formation of soap from the FFA of the oil. In a conventional biodiesel process, an excess methanol is recovered by using a distillation column and a by-product glycerol is separated from biodiesel by a decanter. The product biodiesel is first purified by using a distillation with partial condenser.

A flash column is chosen to recover the excess methanol in crude biodiesel product. The light key components were sent to a distillation column in order to separate methanol and water and then the recovered methanol from the column is recycled to the biodiesel production process. The heavy key product from the flash separation consists of mostly biodiesel, glycerol and unreacted mixed triglyceride. Then the biodiesel was further clarified by washing and evaporation. The input output and recycle stracture are shown in figure 5.1 and 5.2 respectively.

The component and their destination for the TER process are as follows:

Component	**Destination**
PL	Feed to the reactor
Methanol	Feed to the reactor
PPL Enzyme	Feed to the reactor
Glycerol	By-products
Biodiesel	Main –products

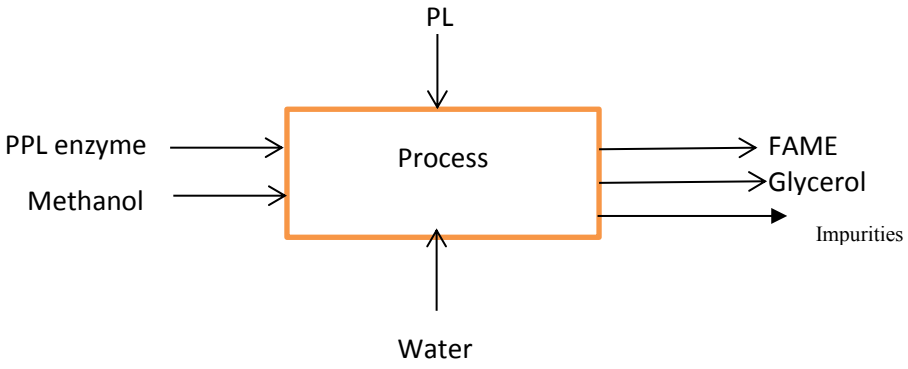


Figure 5.1: Input output structure of transesterification process.

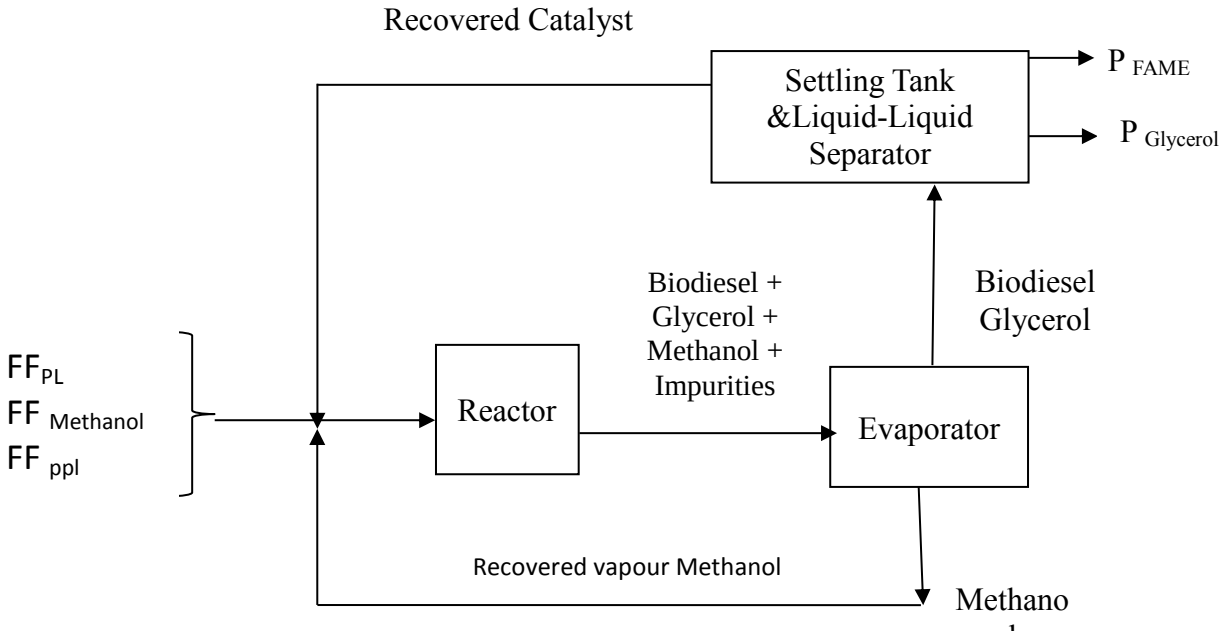


Figure 5.2: recycle flow sheet structure of biodiesel production

5.1.1. Mass Balance in the Production of Biodiesel on Major Unit Operations

Overall Material Balance

The overall balance encloses the entire process. The system defined by this boundary has input feed streams PL, methanol, PPL, and water for washing or purification and product streams contains FAME /biodiesel, glycerol, and washing water. The general mass balance equation can be written as:

Accumulation / Depletion = Input + Generation – Output – Consumption

Transesterification reaction

1 mole TG + 3 mole Methanol ⟶ 3 mole biodiesel + 1 mole Glycerin

The chemical reaction of transesterification

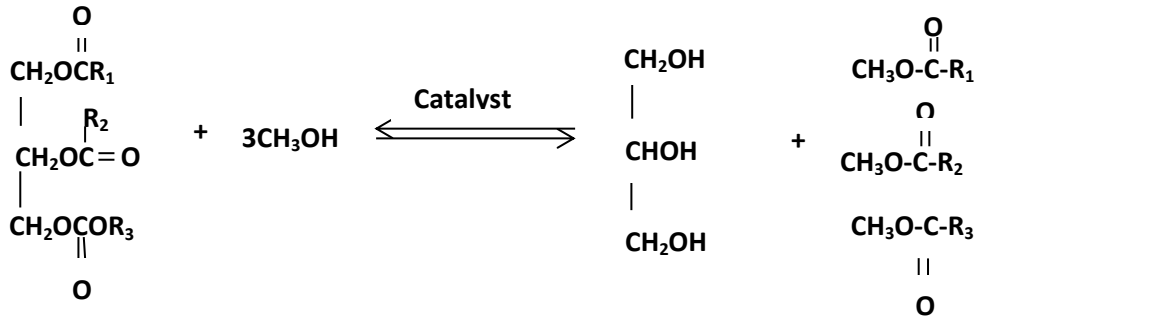


Figure 5.3: The chemical reaction of transesterification

From the stoichiometric reaction the biodiesel was produced in 1:3 molar ratio of oil to methanol. According to the optimization study 1:4 molar ratio of PL to methanol gives better biodiesel yield which was 81 % conversion of fat to biodiesel. Adding excess amount of methanol is advisable to ensure the completion of reaction to convert all fats and oils to biodiesel.

For the current study the TER was carried out with 1:4 molar ratio of oil to alcohol and the volume and mass ratio was calculated based on the densities and molecular weight of the feed stocks. For this molar ratio the volume of PL was calculated on the methodology part on page 33. 44.65 gram (50 ml) of oil and 6.67 gram (8.43 ml) of methanol was taken which is approximately 5:1 volumetric ratio or 6.7: 1 mass ratio of PL to methanol. But stoichiometrically 4.99 gram of methanol was enough to complete the reaction the excess is used to prevent the reverse reaction or hydrolysis. 2.01 gram of PPL enzyme catalyst was being used for this reaction which is 5% based on the PL weight. From these input materials 40.2 Kg of biodiesel was produced which are 81% reactants were converted to FAME. The remaining 6.43 gram (16 %) is converted to Glycerol. The complete material balance of the TER process was done based on this information by taking 1000 kg of biodiesel production per day as a basis. Table 5.2 shows the mole, mass and volume of each component in the input and output streams.

Basis = 1000 kg biodiesel production per day

From the optimization study 81% the oil was converted to biodiesel and the remaining 16% and 3% was converted to glycerol and impurities respectively. The amount of oil and methanol was calculated based on the optimization study of 1:4 molar ratio of oil to methanol. At this molar ratio the amount of PL, methanol and PPL used to produce 1000kg of biodiesel per day are 1064 kg, 185 kg and 55 kg respectively. These values are calculated as follows. 5% of PPL based on the oil weight was feed into the reactor process.

Selectivity and reaction Stoichiometry

Selectivity (S) is defined as the fraction of PL converted in the reactor that corresponds to the biodiesel flow at the reactor outlet. All the biodiesel should be removed and recovered.

$$\text{Selectivity} = \frac{\text{moles of desired product}}{\text{Moles of reactant converted}}$$

$$\text{Selectivity} = \frac{\text{moles of biodiesel}}{\text{Moles of PL converted}} = \frac{P_{FAME}}{FF_{PL}} = S$$

S =1161/1280 =0.91

Therefore the selectivity is equal to 0.91.

- **Pork Lard in the feed (FF_{PL})**

Since the conversion of PL to FAME is 81 % (w/w), 1064 Kg or 1280 mol of purified pork lard is needed in the feed. Or it can be calculated from selectivity as:

$$FF\ PL\ = \quad \frac{PFAME}{S} \quad = \frac{1161mol}{0.91} = 1280 \text{ mol}$$

Methanol in the Feed (FF CH_3OH)

The amount of methanol required for the reaction in the feed is the sum of methanol required for the reaction and the excess methanol which will recycle to reactor after being separated from FAME, glycerol and impurities. From the stoichiometric 1:3 molar ratio, 138 kg (429 mol) of methanol is needed but in this case 1:4 molar ratios has been used, hence additional 47kg of methanol was taken and total amount was 185kg.

PPL Lipase in the feed (FF_{ppl})

- 54.8 kg of PPL is needed which is 5% w/w of oil.

FAME in product stream (P_{FAME})

- 1000 kg/day (1161 mol) biodiesel production was taken as a basis which is 81% conversion of oil and fat.

Glycerol in Product stream:

- 170 kg/ day will be produced which is 16 % of oil conversion.

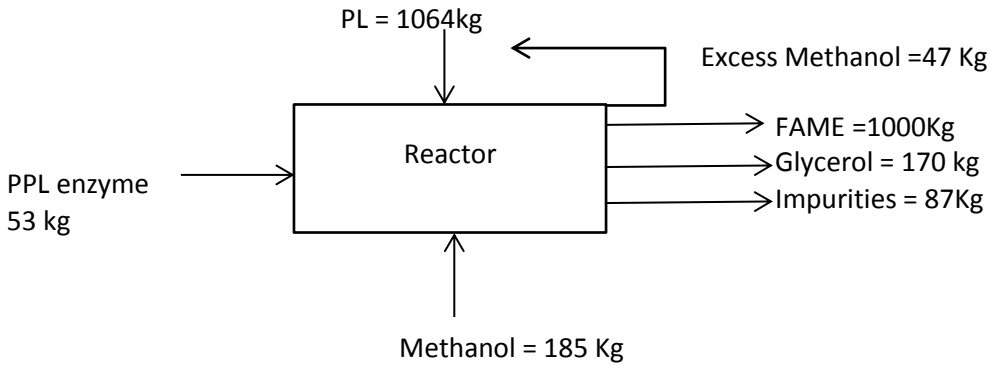
Other Impurities:

- 87Kg which is around 3% impurities produced from the feedstock and enzyme residue

Table 5.2: Summary input output streams for over all process

Input component	**Molecular weight (g/mol)**	**Density (g/ml)**	**Total mass (Kg)**	**Total volume (L)**
PL	857	0.893	1064	1191
Methanol	32	0.791	185	234
PPL enzyme	-	-	55	-
Output components				
Biodiesel	860.9	0.867	1000	1153
Glycerol	92.1	1.26	105	83.3
Impurities	-	-	32	-

Mass balance on Reactor



Over all material balance on the reactor

Input = output

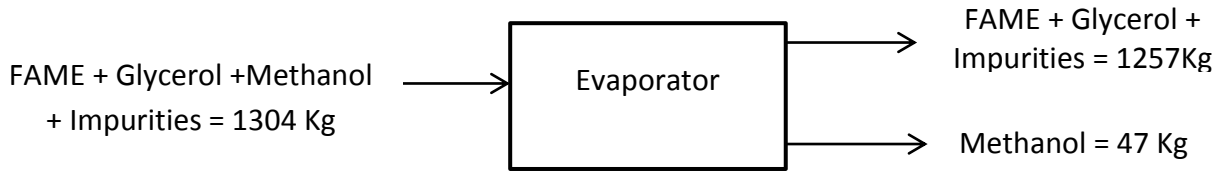
FFPL + FF Methanol +FF PPL = P FAME + P Glycerol + P Impurities + Excess Methanol

1064 Kg + 185 Kg + 55 Kg = 1000 Kg + 170 Kg + 87 Kg + 47 KG

1304 Kg = 1304 Kg, it is balanced

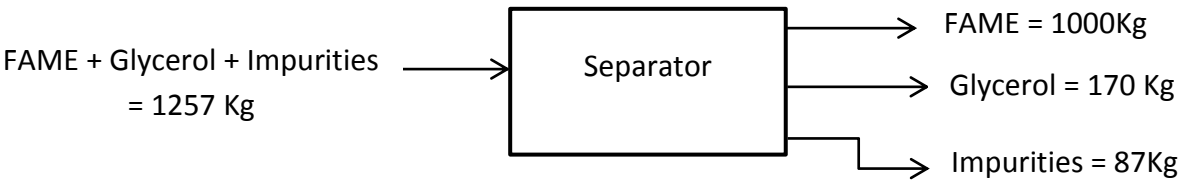
Evaporator

The mixture of FAME, glycerol, excess methanol and impurities are entered to the evaporator. Since methanol has lower boiling point than three other components it separates in this unit and recycle to reactor and used for the next transesterification reaction.



Settling Tank &Liquid-Liquid Separator

In this separator a mixture of biodiesel, glycerol and impurities are separated by removing a layer of liquid glycerol and biodiesel from settled impurities. In the settling tank the glycerol and sludge of catalyst and other impurities should be removed. Then biodiesel and glycerol can be separated by gravitational and centrifugal technology which has been widely used for separation of glycerol from biodiesel.



5.1.2. Economic Potentials and cost Analysis of Biodiesel production

The economic potential and costs of Production is primarily influenced by:

- Feedstock (PL, Methanol and PPL)
- Capital for investment and operating costs
- Main product (Biodiesel)
- Glycerol by-product

- The yields and quality of the biodiesel and glycerol

The economic potential analysis shows the base line to determine the profitability of the project and which is calculated as: [51].

Economic potentials (EP) =product value + by-product value- raw material cost

For TER, EP= (Biodiesel value + Glycerol Value) - (PL cost + Methanol cost + PPL cost)

Cost data for TER process

- Value of PL feed= $0.55/kg
- Value of Methanol feed = $0.7/kg
- Value of PPL feed = $1.2/kg
- Value of biodiesel (main product) = $1/kg
- Value of glycerol (by-product) =$0.9/kg

Therefore the feedstock, main product and by-product cost to produce 1000kg of biodiesel per day is presented on table 5.3.

Table cost data for input output components for 1000 Kg biodiesel production

Components	Value /Price/ ($)	Remark
PL	550	Feedstock
Methanol	96	Feedstock
PPL	66	Feedstock
Biodiesel	1000	Main product
Glycerol	153	By-product

EP= (Biodiesel value + Glycerol Value) - (PL cost + Methanol cost + PPL cost)

EP = (1000 + 153) – (550 +96 +66)

EP = 440

This indicates 62% value addition can be gain from the production of biodiesel from PL by using enzyme as a catalyst. In addition to this $25-30 per ton of CO_2 carbon tax credit can be gained as revenue by reducing the CO_2 emission to the environment as a pollutant.

5.2. Process Flow Sheet and Economic Evaluation for Fatliquor Production

In the present work the leather fatliquor was produced by sulfitation and externally emulsification process as it was optimized earlier. The fatliquor prepared by emulsifying the oil using external emulsification is simply done by blending the oil, four emulsifiers and dispersing agent. Therefore the flow sheet process was done for sulfitation process only that helps to control the reaction parameters and easily to perform the material balance of the process. The flow diagram for the preparation of sulfited fatliquor was given below on figure 5.3.

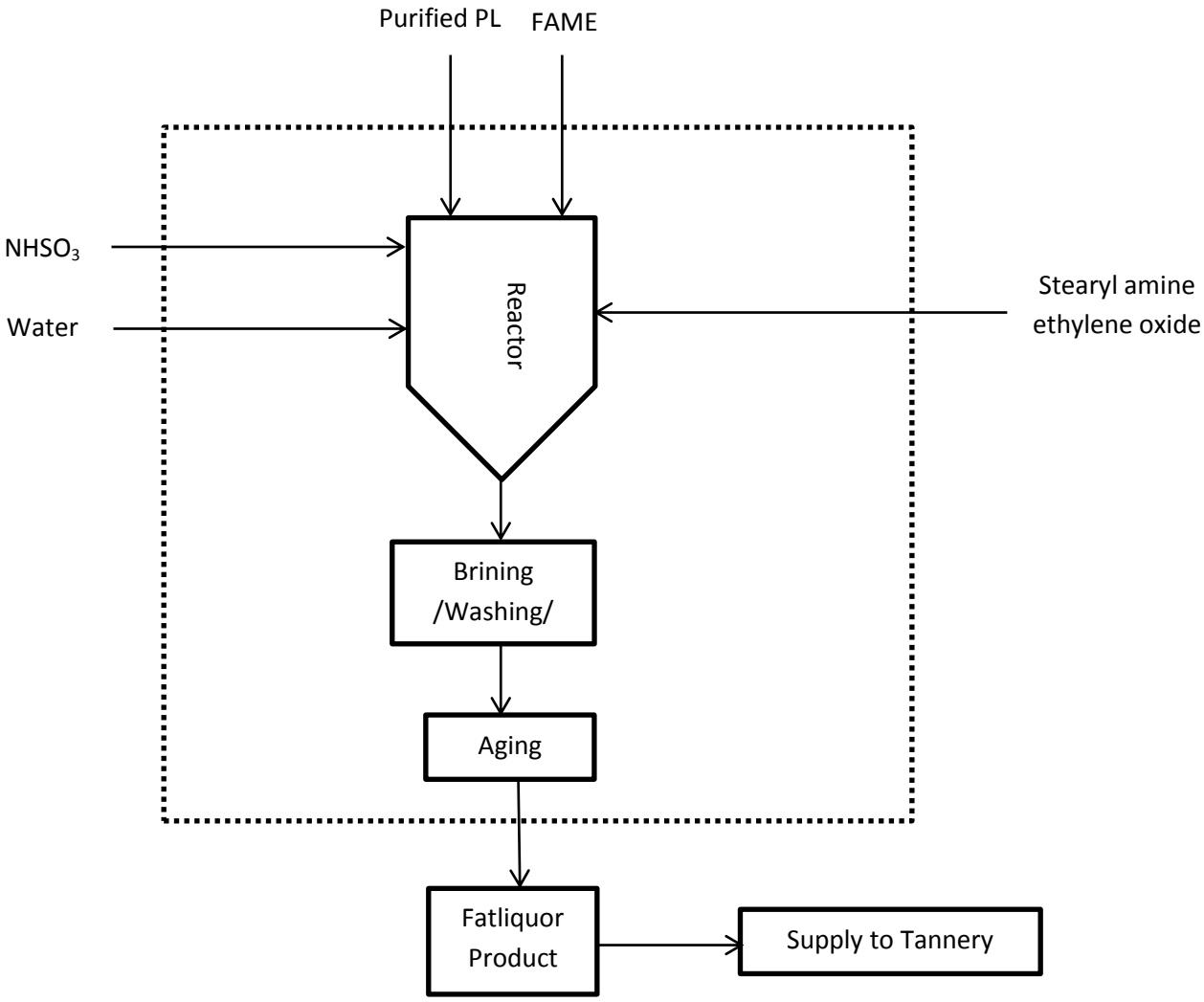


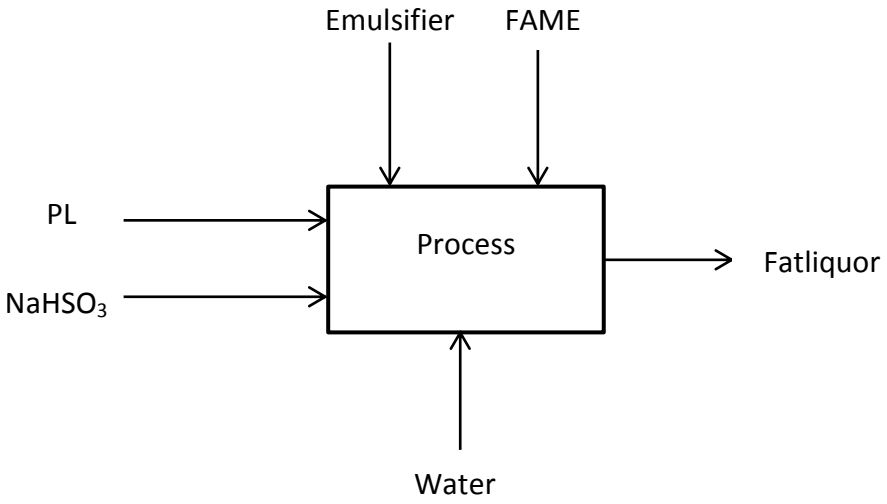
Figure 5.3: process diagram for fatliquor production by sulfitation

5.2.1. Material Balance for Fatliquor Production on Major Unit Operation

The mass balance of fatliquor manufacturing by sulfitation method is an important part in the analysis of physical system. 1000 litre production of fatliquor per day was taken as a basis.

Mass Balance on Boundary -1 (Over all mass balance)

On the overall process 15 % of $NaHSO_3$, 4% emulsifiers, 0.5% catalyst and 1% water, 15.5% FAME of PL and 63% (w/w) crude PL were feed to the system. 1% water is used to prepare the past or slurry solution of $NaHSO_3$. Assume 1000 Kg of fatliquor will be produced per day as a basis and all the $NaHSO_3$ is being reacted with the oil.



Mass of PL, $NaHSO_3$, Emulsifier, water and catalyst needed for the production of 1000 kg of fatliquor are:

PL = 0.63 x 1000 Kg = 630 Kg

FAME = 0.155 x 1000 Kg = 155 Kg

$NaHSO_3$ = 0.15 x 10000 kg = 150 Kg

Emulsifier = 0.05 x 1000 kg = 50 Kg

Water = 0.01 x 1000 Kg = 10 Kg

Catalyst = 0.005x 1000 Kg =5 kg

Therefore the overall system material balance is:

Mass In = Mass Out

Mass of PL + Mass of FAME + Mass of $NaHSO_3$ + Mass of Emulsifiers + Mass of water = mass of fatliquor

(630 + 155 + 150 + 50 + 10 +5) Kg = 1000

1000 Kg = 1000 kg

After the completion of reaction all the input materials are goes to output stream in the form of sulfited fatliquor.

5.2.2. Economic Potential Analysis of Fatliquor Production

The economic potential of fatliquor production from PL by sulfitation process was estimated in a similar manner done for the production of biodiesel as explained above on page 92.

EP = Value of main product + value of by-product – Cost of raw material

EP = Value of sulfited fatliquor – Cost of PL- Cost of NaHSO3 –Cost of Emulsifier.

The addition of Emulsifier is optional which is used to enhance the miscibility of the oil with the paste solution of sodium metabisulfite and increase the rate of reaction and it also improves the emulsion characteristics of the fatliquor. In most literature the sulfitation reaction was done in the absence of emulsifier.

Table 5.3: Cost data of materials used to produce 1000 kg of fatliquor

Materials	Total price ($)	Remark
PL	347	Input material
$NaHSO_3$	97	"
FAME	155	"
Emulsifier	75	"
Catalyst (H_2O_2)	5	"
Fatliquor	1500	Output /Product?

EP = Value of sulfited fatliquor – Cost of PL- Cost of NaHSO3 – Cost of FAME –Cost of Emulsifier.

EP = 1500 - (345 + 97 + 155 + 75 +80)

EP = 748

The value indicates more than 100% value addition can be gained by processing PL to leather fatliquor. The prices of all these chemicals are taken based on Ethiopian current market price of. This pre-feasibility study can be used as the basis for investment decisions of the tanners itself, chemical companies or other interpreters.

6. Conclusions and Recommendations

6.1. Conclusions

Pork lard was investigated for its physicochemical properties and fatty acid compositions for the production of biodiesel and leather fatliquor. The biodiesel from PL was synthesized using methanol and PPL enzyme as a catalyst. The effect of molar ratio of oil to alcohol, temperature and reaction time were determined by optimization study designed by design expert 7.0.0 software to see the main and interaction effects of parameters on the TER. The biodiesel yield was 81 % after 36 hours at 3:1 molar ratio of oil to alcohol, 50 ^{o}c temperature 180 rpm and pH 8.0. The conversion of fats and oils into biodiesel is very important not only to replace non-renewable fossil fuels by renewable source of energy for transportation and industrial application but also it has significant environmental benefits by mitigating the emission of pollutants from the petroleum fuel. The physicochemical properties of biodiesel produced from PL showed its suitability to replace the commercial fossil fuel; hence PL can be a cheap alternative feed stock for the production of biodiesel.

Another important industrial chemicals synthesized in the present research from PL feedstock was leather fatliquor. The fatliquor was produced by sulfitation and external emulsification process which are the common manufacturing methods of leather lubricating and retanning chemicals. The physico-chemical properties of the fatliquor including its emulsion stability, pH, total alkalinity, and molecular size showed its suitability in leather processing. The performance of pork lard fatliquor was tested on sheep skin garment leather and gives better and comparable softness and strength properties to the leather as compared to the leather treated with conventional fatliquoring process.

6.2. Recommendations

Pork lard easily extracted and rendered by heating which is cheaper preparation method to use as a substituent feedstock material in the synthesis of biodiesel to replace diesel fuel. Enzyme catalysed transesterification is expensive than alkali or acid catalysed transesterification process but preferable for environmental issue. Chemical catalysed process can be taken as an option to be more economical by saving the cost of enzyme.

The contaminants and insoluble materials of the lard should be removed before feed to the reactor and the fuel should be free from these impurities to use in an engine.PL contains both saturated and unsaturated fatty acid and biodiesel produced from PL will solidify at lower even room temperature saturated, which means that the fat solidifies at a relatively high temperature. Therefore, the biodiesel from PL is ideal to use in warm climate area by blending with petroleum diesel.

The fat liquor has been synthesized from PL can be used as a potential substituent of the imported fatliquor for the leather industries of developing country like Ethiopia. The new fatliquor can be used by blending with other vegetable, animal or synthetic based fatliquor to get better effect on the leather. The fatliquor can also be synthesized by blending with other oils. Since pork lard will solidify at lower temperature sulfitation process is suitable that carries out at high temperature 60 -80 ^{o}c. Addition of FAME helps to reduce the viscosity of the fatliquor and will give better emulsion characterstics.

Reference

1. Charlie Scrimgeour, Chemistry of Fatty Acids, , Scottish Crop Research Institute, Dundee, Scotland, Published by: Neeraj Bhusari on Aug 20, 2013
2. Ram B. Gupta, Ayhan Demirbas, Gasoline, Diesel and Ethanol Biofuels from Grasses and Plants, Cambridge University Press, Apr 19, 2010 - Technology & Engineering
3. E.Giuffra, J. M. H. Kijas, V. Amarger, Ö. Carlborg, J.-T. Jeon and L. Andersson, The Origin of the Domestic Pig: Independent Domestication and Subsequent Introgression, Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala Biomedical Centre, S-751 24 Uppsala, Sweden.
4. Global Pig Numbers - World Hog Population, FAO 1990-1998 - January 2003.
5. Bryan R. Moser, Biodiesel production, properties and feedstocks, The Society for In Vitro Biology 2009.
6. IUPAC Compendium of Chemical Terminology (2nd ed.). International Union of Pure and Applied Chemistry. 1997. ISBN 0-521-51150-X. Retrieved 2007-10-31.
7. D, C G. El Diwani, N. K. Attia, S. I. Hawash, Development and evaluation of biodiesel fuel and by products from jatropha oil, chemical Engineering and Pilot Plant Department, National Research Center, Dokki, Egypt Spring 2009; pp.219-224.
8. Ayhan Demirbas, Biodiesel, a Realistic Fuel Alternative for Diesel Engines, , Energy Technology Sila Science and Energy Trabzon Turkey, Springer, 2008.
9. Dan Anderson, Derek Masterson, Bill McDonald and Larry Sullivan, Industrial Biodiesel Plant Design and Engineering, , Minneapolis, Minnesota 55440, USA, 2003.
10. Theodros Tekle, Abreha Tesfay and Tsegabirhan Kifleyohannes, Smallholder pig production and its constraints in Mekelle and southern zone of Tigray region, north Ethiopia, Mekelle University, College of Veterinary Medicine, Livestock Research for Rural Development 2013.
11. Gerard Hillion, Bruno Delfort, Dominique le Pennec, Laurent Bournay , Jean-Alain Chodorge, Biodiesel production by a continuous process using a heterogeneous catalyst, , Institut Français, Rueil-Malmaison Cedex – France.

12. Wilhelm Riemenschneider and Hermann M. Bolt, "Esters, Organic" Ullmann's Encyclopedia of Industrial Chemistry, 2005.
13. N.R. Kamini, H. Iefuji, Lipase catalyzed methanolysis of vegetable oils in aqueous medium by Cryptococcus spp. S-2, National Research Institute of Brewing, Kagamiyama, Higashi-Hiroshima 739 -0046, Japan, 2001.
14. Minodora Leca, Luminiţa Tcacenco, Marin Micutz, Teodora Staicu, Optimization of biodiesel production by transesterification of vegetable oils using lipases, Romanian Biotechnological Letters, University of Bucharest Vol. 15, No.5, 2010
15. Cynthia Lahey, Novalina Lingga, ph.D, Analysis of vegetable oils using gas chromatography, customer support center, Shimadzu (Asia pacific), Pte. Ltd., application news GC, January 2004.
16. Antolín G1, Tinaut FV, Briceño Y, Castaño V, Pérez C, Ramírez AI, Optimization of Sunflower Oil Transesterification Process Using Sodium Methoxide, US National Library of Medicine National Institutes of Health, technol., (2002).
17. V.M. Balcao, A.L. Paiva, F.X. Malcata, Bioreactors with immobilized lipases: state of the art, US National Library of Medicine National Institutes of Health, Enzyme Microb. Technol., 18, 392–416 (1996.).
18. Cynthia Lahey, Novalina Lingga, ph.D, Analysis of vegetable oils using gas chromatography, customer support center, Shimadzu (Asia pacific), Pte. Ltd., application news GC, January 2004.
19. Enkuahone Abebe Alamineh , Biodiesel production from vernonia galamensis oil using ethanol with alkali catalys, Addis Ababa, Ethiopia, November, 2012
20. Minodora Leca, Luminiţa Tcacenco, Marin Micutz, Teodora Staicu, Optimization of biodiesel production by transesterification of vegetable oils using lipases, Romanian Biotechnological Letters, University of Bucharest Vol. 15, No.5, 2010
21. , Zhang y., Dube M.A, Mclean D.D., Kates M., Bioresour. Biodiesel production from waste cooking oil: Process design and technological assessment, US National Library of Medicine National Institutes of Health, 2003.
22. Miller C., Austin H., Posorske L.H., Gonzlez J., J. Am., A review of the current state of biodiesel production using enzymatic transesterification, Oil Chem. Soc.,1988.

23. Fjerbaek l., Christensen K.V., Norddahl B., Development of Process Technology for Two Stage Enzymatic FAEE biodiesel Production, Biotechnol. Bioeng., 102, 1298-1315 (2009).
24. Petroleum Diesel Fuel and Biodiesel Technical Cold Weather Issues, Ralph Groschen, (2009). Report to the Legislature. Minnesota department of agriculture. Retrieved June 12, 2013.
25. Nwadike Isioma, Yahaya Muhammad, O'Donnell Sylvester, Demshemino Innocent, Okoro Linus, Cold Flow Properties and Kinematic Viscosity of Biodiesel, Universal Journal of Chemistry 1(4): 135-141, 2013.
26. Mechanics (Third ed.), Symon, Keith (1971). Addison-Wesley. ISBN 0-201-07392-7.
27. Flammable and Combustible Liquids Code, NFPA 30, 2012 Edition Retrieved January 4, 2014.
28. Total Acid number test, Lab Services, CPI Engineering. Retrieved 2 June 2014.
29. The National Aeronautic and Atmospheric Administration's Glenn Research Centre. "Gas Density Glenn research Centre". grc.nasa.gov.
30. Werner Dabelstein, Arno Reglitzky, Andrea Schütze and Klaus Reders, Automotive Fuels, Ullmann's Encyclopedia of Industrial Chemistry, 2007.
31. Iodine Value of Animal and Vegetable Fats and Oils, EN ISO 3961 (1999) & ISO 3961 1996).
32. Quantitation Methods in Gas Chromatography, Copyright 1998 Alltech Associates, Inc.
33. IS 1448 – 1970 Method of test for petroleum and its product (p:21 flash point (closed) by pensky Martin apparatus.
34. J. Vangerpen, B.Shanks, R. Pruszko, D. Clements, G. Knothe, Biodiesel production technology, National Renewable Energy Laboratory 2004; pp.22-28.
35. Umer rashid, Farooq anwar, Amer Jamil and Haq nawaz Bhatti. Jatropha Curcas Seed oil as a viable source for biodiesel, Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad-38040, Pakistan, Pak. J. Bot., 2010.
36. Gerhard John, possible defect in leather production,

37. Ivo Reetz, Francina Izquierdo, Christine Fischer, Ramon Segura, Peter Mähner, Josef Drexler, Fatliquoring from a Viewpoint of Sustainability, Pulcra Chemicals GmbH, Geretsried, German, journal of aqeic, Vol:63 N4 2012.
38. H.L Bergstron and H. Brockman, Elsevier, Lipases eds. Amsterdam, 1984.
39. Rebecca Hobden, Chemical Engineer, Commercializing Enzymatic Biodiesel Production Viesel Fuel, January 03, 2014.
40. Tony Covington, Cambridge, Tanning chemistry: the science of leather, UK: Royal Society of Chemistry, ©2009.
41. Timothy Ruppel, Timon Huybrighs, PerkinElmer,Application note Gas Chromatography, Inc. Shelton, CT USA.
42. Quantitation Methods in Gas Chromatography, Copyright 1998 Alltech Associates, Inc.
43. Iodine Value of Animal and Vegetable Fats and Oils (EN ISO 3961 (1999) & ISO 3961 1996))
44. L. Simasatitkula, R. Ganib a*, A. Arpornwichanopa, Optimal design of biodiesel production process from waste cooking palm oil, a Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University, Bangkok 10330, Thailand.
45. Enkuahone Abebe Alamineh, Biodiesel production from vernonia galamensis oil using ethanol with alkali catalys, ,Addis Ababa, Ethiopia, November, 2012.
46. Fatty Acid / FAME Application Guide, Analysis of Foods for Nutritional Needs, Supelco analytical.
47. Flowsheet Synthesis, Prasad Jayavant Parulekar, 2010
48. Alfred Thomas, Fats and Fatty Oils. "Ullmann's Encyclopedia of Industrial Chemistry". Ullmann's Encyclopedia of Industrial Chemistry, (2002).
49. Charlie Scrimgeour, Chemistry of Fatty Acids, Scottish Crop Research Institute, Dundee, Scotland, Published by: Neeraj Bhusari, 2013.

50. Ram B. Gupta, Ayhan Demirbas, Gasoline, Diesel and Ethanol Biofuels from Grasses and Plants, Cambridge University Press, - Technology & Engineering, Apr 19, 2010

51. James M. douglas, Conceptual design of chemical processes, Univrisity of Massachusetts, International edition, 1988.

52. Nishter Nishad Fathima, Analysis of variations in porosity of metal crosslinked collagen matrix, Journal of Applied Polymer Science, 2014.