ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

ADDIS ABABA INSTITUTE OF TECHNOLOGY

SCHOOL OF CHEMICAL AND BIO-ENGINEERING

Transesterfication of Neem Seed Oil Into Fatty Acid Methyl Ester Using Na_2O/CaO Catalyst

By: Dagmawi Abebe Zewude

January, 2015
Addis Ababa, Ethiopia

ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

ADDIS ABABA INSTITUTE OF TECHNOLOGY

SCHOOL OF CHEMICAL AND BIO-ENGINEERING

Transesterfication of Neem Seed Oil Into Fatty Acid Methyl Ester Using Na_2O/CaO Catalyst

A thesis submitted to the Research and Graduate School of Addis Ababa University, Addis Ababa Institute of Technology, school of Chemical and Bio Engineering in partial fulfillment of the requirements for the attainment of Masters of Science in Environmental Engineering.

By: Dagmawi Abebe Zewude

Advisor: Dr. Beteley Tekola

ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

ADDIS ABABA INSTITUTE OF TECHNOLOGY

SCHOOL OF CHEMICAL AND BIO-ENGINEERING

Transesterfication of Neem Seed Oil Into Fatty Acid Methyl Ester Using Na_2O/CaO Catalyst

A thesis submitted to the Research and Graduate School of Addis Ababa University, Addis Ababa Institute of Technology, school of Chemical and Bio Engineering in partial fulfillment of the requirements for the attainment of Masters of Science in Environmental Engineering.

By: Dagmawi Abebe Zewude

Approved by the Examining Board:

.....	.....	.....
Chair man, Department's Graduate Committee	Signature	Date
.....	.....	.....
Advisor	Signature	Date
.....	.....	.....
Internal Examiner	Signature	Date
.....	.....	.....
External Examiner	Signature	Date

Acknowledgement

I have no words to praise my mother Ayelech Hunde for giving me reason, passion, and purpose in life which derives me to highness with great wisdom. I would like to thank my thesis advisor Dr. Beteley Tekola for providing me guidance, encouragement, and for sharing his knowledge and experience.

Moreover, I would like to express my heartfelt appreciation and thanks to School of Chemical and Bio-Engineering laboratory technicians, Addis Mojo Edible Oil Share Company quality control laboratory members, Ethiopian Petroleum Supply Enterprise laboratory technicians, and Bahir Dar University Industrial Chemistry Department staff members for their great contribution while I was doing my thesis.

Table of contents

List of tables

List of figures

Acronyms

ANOVA	Analysis of Variance
ASTM	American Standard Testing Materials
AV	Acid Value
CN	Cetane Number
CP	Cloud Point
DG	Diglycerides
EN	European committee for standardization
FAME	Fatty Acid Methyl Ester
FFA	Free Fatty Acid
FP	Flash Point
HHV	Higher Heating Value
IV	Iodine Value
NOME	Neem oil methyl ester
PLC	Private Limited Company
rpm	Rotation per minute
RSM	Response Surface Methodology
SV	Saponification Value
SG	Specific Gravity
TG	Triglycerides
THF	Tetrahydrofuran

Abstract

Environmental deterioration is affecting the way of living on earth. A lot of efforts are being made to reduce impact on the environment. Generating better sources for transportation fuels is part of the effort, since it reduce higher level fossil fuel consumption. Biodiesel is an alternative energy fuel to petroleum diesel with clean, renewable, biodegradability, and less exhaust emission advantage.

Ethiopia's economy is swifting, parallelly the energy demand is increasing. However, the country is completely dependent on imported petroleum fuel. The price of petroleum fuel is increasing from time to time. Therefore, there is a need to secure energy that can substitute the import and support the economic development as well.

Biodiesel can be produced from plenty of oil containing feedstock. Non edible oil seed bearing plants are the major choice for Ethiopia. Neem tree that can grow on degraded soil and/or waste land with very low input requirement is considered as a potential biodiesel feedstock based on its oil content and biodiesel yield. In addition, the tree can provide a lot of socioeconomic importance for the community around the plantation site.

In this study, oil content of Neem seed was found to be 42% with 15% FFA. Neem oil free fatty acid reduction screening was conducted via acid treatment. Successfully, FFA was reduced to 0.375% by 0.60% v/v sulfuric acid to oil ratio and 60ml CH_3OH.

Heterogeneous catalyst (Na_2O/CaO) loading screening based on fatty acid methyl ester yield was performed; higher yield (84.73 %) was obtained at 40% wt/wt Na_2O/CaO loading. Acid treated Neem oil was subjected to heterogeneous catalyzed transesterfication reaction.

Transesterfication reaction input process variables: reaction temperature, catalyst to oil weight ratio, and methanol to oil molar ratio optimum point were determined to provide maximum fatty acid methyl ester yield with central composite design, which end up in agreement with the real process data. Thus, maximum yield of 92.80% was obtained at 58^0C temperature, 11.71% methanol to oil molar ratio, and 8.50% catalyst to oil weight

ratio. Physico-chemical characteristics of fatty acid methyl ester was determined and found to be within the standard limit.

1. Introduction

1.1. Background

The depletion of fossil fuel, the resulting high price, and its adverse effects on the environment have encouraged governments and researchers to consider alternative renewable sources of fuels. Renewable and clean energy fuel can be produced from different bio based raw material as a substitute for petro based products. Production of biofuels from renewable feedstock's can improve energy security and reduce greenhouse emissions.

Ethiopia heavily depends on the imported fuel to fulfill the domestic energy demand for transportation, and other industrial activities. As the country's agricultural, infrastructural and industrial development increases, so does its demand for fuel. As a result, Ethiopia, in its green development strategy has outlined the importance of bio-fuel (Bioethanol and biodiesel) production and utilization to substitute fossil fuel import.

Currently, biodiesel and bioethanol are the major liquid biofuel produced in different part of the world for transportation sector. In Ethiopia as well bioethanol is being produced in sugar industries from molasses for transportation. However, the development of biodiesel is at its initial stage.

Biofuels in comparison to fossil fuels should be relatively cheap and rich in energy, locally produced (saving foreign currency), have environmental benefits (low greenhouse gas emission) and be producible in large quantities without impacting on food supplies. A lot of research was being carried out to achieve this characteristics of biofuel.

Biodiesel, which is fatty acid methyl ester, can be produced starting from non-edible agricultural seeds with high oil content such as Jatropha, Castor etc and alcohol in presence of a catalyst.

As alternative to Jatropha and Castor, Neem seed can be a potential and promising feedstock for production of biodiesel (Radha and Manikandan, 2011) with extra environmental and socioeconomic importance in Ethiopia. Neem tree is available in different part of Ethiopia: Amhara, Afar, Tigray, Gambella, Jigjiga, and in some part of

Oromia. Currently the tree doesn't have any significant use, mostly used for shade, except rarely used for its medicinal purpose (Workneh,W., 2011).

Neem tree is fast growing tree, easily cultivable, drought resistant, grow on poor soil condition, less annual rain fall requirement (not more than 200 mm), and other farming can also be carried out together. In addition, the by-product like the seed cake can be used for soil conditioning; leaf extract can be used for waste water treatment and for production of pesticide and insecticide. This and other significant multi benefit makes the tree attractive for biodiesel production.

Conventional biodiesel production via homogeneous catalysts (acid or bases) is associated with several drawbacks. In homogeneously catalyzed transesterification reaction catalyst recovery is difficult, results huge amount of catalyst loss, generates large amount of waste water from washing of biodiesel product and byproduct (glycerol), and by product (glycerol) has less purity due to the presence of residual catalyst. Moreover, most homogenous acid or alkali catalyst leads to corrosion of the equipment. As a result, in order to minimize the above adverse effects of homogenous catalysts, researches are continuously engaged in finding active and stable heterogeneous solid catalyst such as CaO, MgO, etc.

Therefore, this research has focused on developing a Na promoted CaO catalysts (Na_2O/CaO catalyst), with different Na loading, for transesterfication of Neem oil (Triglycerides) with Methanol into biodiesel (FAME). The undesired free fatty acid contained in the crude Neem oil was previously acid treated in the presence of methanol and small amount of acid (H_2SO_4). Optimal reaction operating conditions (Temperature, Methanol/oil ratio and Catalyst/Oil weight ratio) were investigated in presence of best performing Na_2O/CaO catalyst using response surface methodology.

1.2. Problem Statement

Ethiopia's economy is moving forward and striving to change from agricultural led economy to industrial led economy. Hence, has achieved rapid economic growth. However, this development requires huge amount of fuel energy source. Currently, the country is completely dependent on imported oil and large sum of country's earnings are used to pay for the imported petroleum fuel.

To meet the growing energy demand , support and stabilize the economy locally produced cheap, renewable, clean, and efficient source of energy is necessary.

Renewable and clean energy fuel can be produced from different bio based raw material as a substitute for petro based products. Production of bio fuels from renewable feedstock can improve energy security and reduce greenhouse emissions.

Biodiesel, fatty acid methyl ester, can be produced from non-edible, locally available Neem seed oil via trasesterification reaction using alcohol in the presence of catalyst. FAME yield is greatly affected by type and amount of alcohol, catalyst, oil purity, and reaction temperature. Industrial biodiesel production is being carried out via homogenous catalyst (Acids and Bases) which is blamed to have a lot of drawbacks. Despite the relatively high catalytic activity of homogeneous catalysts, their uses are still uneconomical since the process suffers from severe corrosion, costly separation and neutralization of waste acids. Recently, to overcome the aforementioned drawbacks of homogeneous catalysts, a lot of research is being conducted on developing novel heterogeneous catalysts such as nano MgO, SO_4^{2-}/ZrO_2, Mg-Co-Al-La, $Na/NaOH/Al_2O_3$, K_2CO_3/MgO, $LiNO_3/CaO$, KNO_3/Al_2O_3 etc that has superior activity and stability. Heterogeneous catalysts offer numerous advantages over homogeneous catalysts as it can easily be separated from reaction mixture and can be reused, possess high thermal stability, have limited corrosion effect, and have the potential to be easily applied to a continuous flow fixed-bed reactor (scale up).

One of the characteristics of non-edible seed derived oils is the presence of significant amount of free fatty acid. The presence of higher amount of free fatty acid in Neem oil, reduces the efficiency of base catalyst and hence lowers the biodiesel yield. However,

conventionally FFA can be removed by neutralization reaction (forming soap) with the alkali before the transesterfication reaction was carried out. As a result, FAME production leads to 15% FFA loss. However, this can be saved by acid pretreatment.

1.3. Objectives

1.3.1. General objective

The general objective of this research is to develop Na_2O/CaO heterogeneous catalyst for transesterification of Neem seed oil-triglyceride (acid treated) into biodiesel (FAME); and find optimal operating conditions (temperature, catalyst/oil weight ratio, and methanol/oil molar ratio) using response surface method.

1.3.2. Specific objectives

The specific objectives of this thesis are;

- To efficiently extract crude Neem seed oil from locally available Neem seeds using sohxlet extraction technique
- To convert traces of free fatty acid in crude oil via acid treatment with methanol in the presence of small amount of acid (H_2SO_4)
- To evaluate physicochemical properties of acid treated Neem seed oil.
- To prepare Na_2O/CaO heterogeneous catalysts with Na_2O/CaO weight ratio of 30%, 40% & 50% and find the optimal catalyst with high FAME yield.
- To determine the optimal reaction conditions (temperature, methanol/oil molar ratio, and catalyst/oil weight ratio) to obtain maximum FAME yield
- To determine the physico-chemical characteristics of biodiesel (FAME) fuel

1.4. Significance of the study

The government of Ethiopia is considering jatropha, castor, and palm as alternative source of biodiesel feedstock. This thesis considers Neem seed as additional alternative source with extra socioeconomic and environmental importance for farmers and community around.

Moreover, this thesis provides environmentally safe and closed loop industrial production system from high free fatty acid feedstock through the use of acid treatment and heterogeneous catalyzed process to enhance the yield of FAME. Generally, Small scale and large scale biodiesel production companies can use the material as reference.

2. Literature review

2.1. Introduction

The global energy demand is increasing from time to time. This energy demand is being quenched by conventional fossil fuel which is threatening the environment. Currently, the reserve of this conventional fossil fuel is at risk and its cost is also increasing. Thus, there is a growing interest to replace fossil fuel and meet the demand by clean and renewable energy source. Biodiesel and bioethanol are the major environmental friendly energy for transportation sector that the world is working on at this time.

Biodiesel is defined as monoalky ester of long chain fatty acid. It is produced through the reaction of oil or animal fat with alcohol in the presence of catalyst. Most commonly used alcohol in the production process is methanol and ethanol, especially methanol, because of its physical and chemical advantages (polar and shortest chain alcohol). Based on the alcohol type in the reaction, methy/lethyl ester is produced with glycerol by product. The stoichometry of reaction is 3 mol of alcohol to 1 mol of triglyceride to give 3 mol of fatty acid methyl/ethyl ester and 1 mol of glycerol. This reaction, is consists of three consecutive reversible reactions with intermediate formation of diglycerides and monoglycerides.

2.2. Biodiesel as a renewable fuel

Biodiesel is a renewable fuel made from plant oils that can be used directly in any existing unmodified conventional diesel engine. It is environmental friendly alternative to petro diesel which is produced from biological renewable source. It can be used alone or mixed in any amount with regular diesel fuel and it has a lot of benefits.

Advantage of biodiesel as a fuel over petro diesel:

- ✓ Produced from renewable resources
- ✓ Less greenhouse gas emissions
- ✓ Grown, produced and distributed locally
- ✓ Cleaner biofuel refineries
- ✓ Biodegradable and non-toxic
- ✓ Positive economic impact and smaller trade deficit
- ✓ Reduced foreign oil dependence

2.3. Biodiesel fuel characteristics

There are a wide variety of biodiesel feedstock with their different physical and chemical property, which can influence the fuel energy content and property. Fuel efficiency was dependent on the composition, structure, chain length, and molecular weight of its component. Thus, determining physical and chemical property of biodiesel is important. There are a plenty of fuel characteristics which is used to compare with the biodiesel standards.

2.3.1. Cetane number

One of the most influential properties of the diesel fuel is the dimensionless cetane number (CN), which represents the ignitability of the fuel, particularly it is critical during cold starting conditions (Giakoumis, 2013). Cetane number is commonly used as indicator for the determination of diesel fuel ignition quality and measures the readiness of the fuel to auto ignite when fuel is injected to the engine, and also it is proportionate to the fuel ignition delay time in CI engine (Sivaramakrishnan and Ravikumar, 2012). Higher value of cetane number indicates that the fuel ignites faster and often leads to lower NO_X emission (Oliveira and Da Silva, 2013). Conversely, lower cetane number indicates long ignition delay (long time between fuel injection and start of combustion). Generally, it is dependent on the composition of the fuel and can impact the engine storability, noise level, and exhaust emission.

The cetane number of biodiesel is usually higher than that of the conventional diesel fuel. European specifications dictate a cetane number of biodiesel fuel of at least 51, which means that some of the investigated feedstocks, namely castor, croton and rubber seed lie outside the acceptable limits in pure form. On the contrary, in the US minimum CN limit is 47 a fact that practically renders all analyzed feedstocks acceptable, except for B100 castor (Oliveira and Da Silva, 2013).

There is also interdependency between cetane number and degree of unsaturation. Cetane number decreases as the number of double bond increases, highly saturated ester such as those derived from coconut, palm, and tallow exhibit the highest value CN. In addition, CN increase as chain length increases and/or branching decreases (Giakoumis, 2013).

2.3.2. Kinematic viscosity

Kinematic viscosity which defined as a resistance to flow of a fluid under gravity of 40^0C. In a diesel engine, higher viscosity leads to less accurate operation of the fuel injectors, and to poorer atomization of the fuel spray, increase in the Sauter mean diameter of the fuel droplets and of the jet break-up time; these inefficiencies are exaggerated during cold starting (Giakoumis, 2013). Viscosity affects the atomization of a fuel upon injection into the combustion chamber and thereby ultimately the formation of engine deposits. It also plays a dominant role in the fuel spray, mixture formation, and combustion process (Çanakçı, 2008).

On the contrary, significant reduction in viscosity can result: loss of lubricity which causes excessive wear, higher mechanical friction which causes high heat generation and/or high energy consumption, reduction of oil film which cause friction between moving parts in the system which in turn leads to particle contamination (Gutti et al., 2012).

Vegetable oil has higher viscosity than the acceptable limit for diesel fuel. This makes difficulty of using as it was, specially to use them in compression ignition engine, as a result mechanism of reducing viscosity was devised: heating, transesterification, and blending are some of the mechanism. European specification (EN 14214) dictates an acceptable biodiesel viscosity range between 3.5 and 5 mm^2 /s and US standard (ASTM D 6751) is between 1.9 to 6 mm^2/s.

It was observed that there is interdependence between average number of double bond, chain length, and Kinematic viscosity; double bond reduces kinematic viscosity in both fatty compounds and aliphatic hydrocarbon (Knothe and Steidley, 2005). Larger proportions of saturated fatty acids with longer carbon chains cause kinematics viscosity to increase. It was also observed that as the chain length increases, viscosity will also increase (Giakoumis, 2013). In addition to double bond number, kinematic viscosity of unsaturated fatty acid depends on the place of double bond, double bonds at the end of straight chain hydrocarbons have a minimum reducing effect on the viscosity (Knothe Gerhard, 2005c and Knothe and Steidley, 2005). Viscosity was also noted to increase with molecular weight.

2.3.3. Specific gravity

Specific gravity of a substance is the ratio of density of the substance to that of density of water, which is unit less. It helps in determine the purity and/or relative comparison with the required standard. Specific gravity fuel is correlated with fuel property such as heating value, viscosity, and cetane number.

Fuel is supplied in to injection system by volume without knowing the mass, if denser biodiesel with greater mass was injected into combustion chamber it will affect the stocheometric ratio of air to fuel which in turn negatively affect the combustion process. Hence, specific gravity helps to notice this property.

Specific gravity of biodiesel depends on molecular mass and chemical structure of the fuel, free fatty acid content, water content, and temperature. Generally, it was observed that most of oil and their methyl ester specific gravity is higher than that of diesel fuel.

2.3.4. Iodine value

Iodine value of biodiesel indicates degree of unsaturation of the fuel, the higher the number of double bond, the higher will be the iodine value. High iodine value indicates a higher potential for biodiesel degradation either through thermal oxidation or free radical attack, and which leads to higher potential of being oxidized (Reda et al., 2007). Iodine value determines the chemical stability of biodiesel fuels. Higher iodine value indicates less stability of biodiesel (Sokoto et al., 2013)

Iodine value is of real significance in the examination of fatty oils since most fatty oils have their own characteristic value (Sayyed Siraj et al.,

2.3.5. Acid value

Acid value determine the level of free fatty acid or processing acid that may be present in biodiesel and is an important indicator of oil and biodiesel quality (Sokoto et al., 2013). Mainly, the cause for higher total acid value is free fatty acid. It is an indication of degree of oxidation and hydrolysis (Wang et al., 2008). Biodiesel with high acid value causes increase in fueling system deposit and increases the likelihood of corrosion (Sokoto et al., 2013), consequently, it will damage fuel pumps and fuel filters (Tyson, 2001).

Acid number is expressed as the amount of KOH (in mg) required to neutralize free fatty acid contained in 1g of sample. It measures the amount of unreacted acids, which remained in the fuel.

2.3.6. Flash point

Flash point is ignition temperature when it is exposed to the flame. It can also be defined as the lowest temperature at which fuel emits enough vapors to ignite (Sanford et al., 2009). Its greater importance is in defining flammability and combustibility of a material. Flashpoint of biodiesel (150°C) is much higher than petro diesel (55-66°C) (Knothe et al., 2005). This property makes biodiesel safe for storage and transportation over petro diesel.

The flash point determination was carried out by heating a sample of the fuel in a stirred container and passing a flame over the surface of the liquid; If the temperature is at or above the flash point, the vapor will be ignited and an easily detectable flash would be observed (Kaisan et al., 2013).

2.3.7. Cloud Point

Cloud point is the temperature of a liquid specimen in which the smallest observable cluster of hydrocarbon crystals (milky cloud) appear first upon cooling under prescribed conditions**.** As a matter of fact, the crystal start at lower circumferential wall of the test jar where the temperature is lowest (ASTM D 2500-05). As the temperature keeps on decreasing below, more amount of material solidifies and the compound approaches the pour point **(**no flow). This crystal, if it appears in the biodiesel, can clog fuel lines.

Generally, it was observed that CP and PP of biodiesel is higher than that of petroleum diesel however can be decreased by blending with petroleum diesel (specially CP) (Rajagopal et al., 2012).

The Cloud Point of biodiesel depends on chain length of saturated FAME, degree of unsaturation, and orientation of double bonds.

2.3.8. Pour point

Pour point is the lowest temperature (below the cloud point) at which the oil specimen can still be moved in the presence of large amount of crystal within the fuel. Hence, it is a measure of fuel gelling point (Dwivedi and sharma, 2013). Biodiesel fuels derived from fats or oils with significant amounts of saturated fatty compounds will display higher CPs and PPs (Knothe, 2005). Blending biodiesel with diesel fuel improves the cold flow properties of the biodiesel blend (Pradhan, 2007).

2.3.9. Cold filter plugging point and low temperature flow test

Cold filter plugging point (CFPP) is the lowest temperature (expressed in 1°C) at which a given volume of diesel type of fuel still passes through a standardized filtration device in a specified time when cooled under certain conditions (Dwivedi and sharma, 2013). American Society for testing Material (ASTM, 1999) define it as temperature at which a given volume of fuel fails to pass through a standardized filtration device in a specified time when cooling proceed under the prescribed conditions. This gives an estimate for the lowest temperature that a fuel will give trouble free flow in certain fuel systems (Dwivedi and sharma, 2013).

Low temperature flow test reports the lowest temperature at which 180mL of a sample can pass through the filter in 60 seconds or less, while the CFPP reports the lowest temperature at which 20mL of sample can pass through the filter in 60 seconds or less. Although both the tests are identical, the LTFT is a better approach because it takes into account the rigorous conditions within the engine (Edith et al., 2012).

2.3.10. Cold flow property

One of the major problems associated with the use of biodiesel is there poor flow properties at low temperature (Knothe, 2005). Higher-melting point components in the fuel at low temperatures start to nucleate and form solid crystals due to the presence of high amount of saturated fatty acid (Dwivedi and sharma, 2013). Prolonged exposure of fuel at a temperature below cloud point cause growth of crystal and it will affect their flow ability and other related property. Cold flow properties are expressed by cloud point, pour point, low temperature filterability test and cold filter plugging point (Dwivedi and sharma, 2013). Biodiesel cold flow properties depends on many factors including impurities, oil feedstock type, alcohol types, amount of free fatty acid, bound glycerin, moisture content, and amount of fatty acid ester (Pradhan, 2007), and also directly depend on its fatty acid methyl ester composition (Rajagopa et al., 2012).

2.4. Biodiesel production methods

There are different method of biodiesel production such as pyrolysis, micro–emulsification, dilution, and transesterfication. However, biodiesel produced by

transesterfication reaction was found to be a choice because of its relative simplicity and less costy in production process and efficient fuel property on use.

2.4.1. Pyrolysis

Pyrolysis is a method of conversion of material by a means of heat or with the aid of catalyst in the absence of Oxygen. Variety of reaction path are available, this makes the product obtained from the reaction to be plenty in there type. The pyrolyzed material can be vegetable oils, animal fats, natural fatty acids and methyl esters of fatty acids (Ma and Hanna, 1999).

The liquid fraction obtained from the decomposition of vegetable oil is likely to be approached diesel fuel. The first pyrolysis of vegetable oil was conducted in an attempt to synthesize petroleum from vegetable oil. Since World War I different investigators have been investigating pyrolysis of vegetable oil to obtained product suitable for fuel. The fuel obtained by pyrolysis cetane number and viscosity are high; the concentration of sulphur, water, and sediment for the resulting product were acceptable, and ash & carbon residue are very higher than the expected value for fuel (Ma and Hanna, 1999).

2.4.2. Micro–emulsification

Micro-emulsion is a colloidal equilibrium dispersion of optically isotropic fluid which has a micro structure with the dimension generally in the 1± 150 nm range formed spontaneously from two normally immiscible liquid and one or more ionic or non ionic amphiphiles liquid (Schwab et al., 1987). This method solves the problem associated with high viscosity of vegetable oil (Ma and Hanna, 1999). Micro emulsion can improve spray properties by explosive vaporization of the low boiling constituents in the micelles and also it was observed reduction in viscosity, increase in cetane number, and good spray characters in the biodiesel (Patel and Krishnamurthy R., 2013).

As it was reported by (Ma and Hanna, 1999), prepared nonionic emulsion of 53% (vol) alkali-refined and winterized sunflower oil, 13.3% (vol) 190-proof ethanol and 33.4% (vol) 1-butanol was found to have viscosity of 6.31 cSt at 40°C, a cetane number of 25 and an ash content of less than 0.01%. There lower viscosities and better spray patterns were observed to increase with an increasing of 1-butanol.

2.4.3. Dilution

Vegetable oil can be used directly or blended with diesel fuel without any change to the engine. Even, the very first engine was tasted using vegetable oil. The primary concern with vegetable oil is its high viscosity, which makes atomization difficult. Low viscosity was good for better performance of engine; it decreases with increasing the percentage of diesel fuel (Singh, S.P. and Singh,D., 2009 , Ma and Hanna, 1999).

Advantage of vegetable oil as diesel fuel includes: its high heat content, renewability, readily availability, portability and liquid nature. Conversely, the problem associated with includes higher viscosity, lower volatility, reactivity of unsaturated hydrocarbon, carbon deposit, oil ring sticking, and thickening and gelling (Ma and Hanna, 1999, Vivek and Gupta, 2004). In addition, the two severe problems associated with the use of vegetable oil as a fuel were oil deterioration and incomplete combustion. Polyunsaturated fatty acids were very susceptible to polymerization and gum formation caused by oxidation during storage or by complex oxidative and thermal polymerization at the higher temperature and pressure of combustion (Ma and Hanna, 1999).

Mixtures of degummed soybean oil and No. 2 diesel fuel in the ratios of 1:2 and 1:1 were tested for engine performance and crankcase lubricant viscosity in a John Deere 6-cylinder, 6.6 L displacement, direct-injection, turbocharged engine for a total of 600 h. The lubricating oil thickening and potential gel-ling doesn't existed with 1:2 blend, this indicated that 1:2 blend should be suitable as a fuel for agricultural equipment as it was reported on (Ma and Hanna, 1999). Caterpillar in Brazil, in 1980 has also found successful result by mixing 20% vegetable oil and 80% diesel fuel without any adjustment to the engine.

Winter rape seed was studied as diesel fuel the result obtained shows that the rate of gum formation is five times slower than that of high linoleic oil. A blend of 70/30 winter rapeseed oil and No. 1 diesel was used successfully to power a small single-cylinder diesel engine for 850 h as indicated by (Peterson et al., 1983). Viscosity was dependent on temperature, so are flow rate; flow rate of canola oil drops to zero at -4 ^{0}C. However, viscosity can be lowered by blending with ethanol, at 37°C the viscosity of canola oil with 10% ethanol was 21.15 cSt, while that of straight canola oil was 37.82 cSt (Ma and Hanna, 1999).

2.4.4. Transesterification reaction

Biodiesel is commonly produced by transesterification process. Oil or fat are used as raw material with an alcohol, which is excess, in the presence of catalyst to produce ester and glycerol. Transesterfication process consists of three consecutive reversible reaction, in the first step triglyceride react with alcohol to produce diglyceride and ester, then diglyceride, which is obtained from the first step, react with alcohol to form monoglyceride and ester, and lastly, monoglyceride, which is obtained from the previous stage, react with alcohol to produce glycerol and ester, as it was shown in the following reaction mechanism (Ma and Hanna, 1999).

Triglyceride (TG) + R'OH $\longleftrightarrow$ Diglyceride (DG) + $R'COOR_1$

Diglyceride (DG) + R'OH $\longleftrightarrow$ Monoglyceride (MG) + $R'COOR_2$

Monoglyceride (MG) + R'OH $\longleftrightarrow$ Glycerin (GL) + $R'COOR_2$

The stecheometric relation between alcohol and the oil is 3 to 1. Despite the reaction is reversible, excess alcohol is used to derive the forward reaction (Marchetti et al., 2007). Most commonly used alcohol are methanol, ethanol, propanol, butanol, and amyl alcohol, specially, methanol is the most common, due to its low cost, and chemical and physical property advantage over the others. On the process, catalysts are used to make the rate of reaction fast. Acid, base, and enzyme can be used as a catalyst. Sulfuric acid, sulfonic acid, hydrochloric acid are the most commonly used acid catalyst. For commercial production of biodiesel alkali catalyst is preferred, which is sodiun hydroxide (NaOH), Potasium hydroxide (KOH), and corresponding sodium and potassium alkoxide, sodium propoxide, and sodium butoxide.

2.5. Process variables affecting transesterification reaction

Biodiesel production process is affected by its input variables, which are alcohol, catalyst, reaction temperature and time, and oil purity. In addition, different research has showed that addition of co-solvent to the reaction mixture improves the yield and rate of reaction.

2.5.1. Effect of alcohol molar ratio

Molar ratio is important variable in the production process of biodiesel. Furthermore, it has determinant effect on the yield of biodiesel. Theoretically, it was expected to be in stoichiometric ratio of 3:1 mole of alcohol to triglyceride to produce three mole of fatty acid ester and one mole of glycerol. In practice, molar ratio should be higher than the stoichiometric ratio because the reaction of biodiesel production is reversible in its nature, as a result higher amount is important to shift the reaction equilibrium forward and for complete conversion of the reactant at higher rate. Unlikely, with very high molar ratio, separation of glycerol is difficult. This leads to increase in separation cost. It was found that optimum molar ratios depend upon type & quality of oil (Shikha and Rita, 2012, Maa and Hanna, 1999).

According to research done by (Maa and Hanna, 1999) molar ratio is associated with the type of catalyst used, acid catalyzed reaction requires 30:1 ratio of BuOH to soya bean oil, while alkali catalyzed reaction requires only 6:1 ratio to achieve the same ester yield for a given reaction time. The study conducted by varying methanol to oil molar ratio (0.35:1%, 0.40:1%,0.45:1%,0.50:1%, and 0.55:1%) and keeping other variables constant showed that the yield has increased up to 0.45:1% methanol to oil molar ratio then start to decrease (Sathya and Manivannan, 2013).

2.5.2. Effect of reaction time and reaction temperature

Temperature is another variable with strong influence on the rate of biodiesel production. Depending on the type of oil used transesterfication can proceed at different temperature (Maa and Hanna, 1999). At lower temperature the conversion efficiency is very low, as it increases toward the boiling point of alcohol its conversion rate increases significantly. Around and above melting point of alcohol, bubble formation will start takes place, which have strong affect on the reaction so that the rate conversion will be lower (Sathya and Manivannan, 2013).

However, at the room temperature even if the reaction rate is low, it will come to completion after longer reaction time at atmospheric pressure, in the case of alkali catalyst. At very lower temperature, the observed problem is biodiesel recovery (Shikha and Rita, 2012). The importance of higher temperature is, it will reach maximum

conversion within short reaction time (Refaat, 2010). Even if, the yield was observed to increase with reaction time, but extended reaction time does not increase the yield, instead backward reaction (hydrolysis of ester) starts to take place (Refaat, 2010).

2.5.3. Effect of catalyst concentration

Concentration of catalyst is important to derive the reaction forward. As the amount of catalyst increases, a chance of getting more triglyceride increase, which leads higher conversion. However, when its concentration gets higher than the optimum value, its yield start to reduces. The reason behind is: at higher catalyst concentration, the catalyst starts to get a chance to react with triglyceride, which leads to formation of soap.

2.5.4. Effect of moisture and free fatty acid

Free fatty acids and moisture adversely affect the biodiesel yield. In the case of alkali catalyzed condition, tolerable total FFA and water content (0.5% and 0.1%-0.3%, respectively) are required (Xiaohu Fan, 2008). In the presence of high amount of FFA, it requires more alkali to neutralize it and carry the transesterfication reaction. The resulting higher amount of soap leads to increase in viscosity and formation of gel, which in turn has strong negative effect on the biodiesel yield. Feedstock that contains higher amount of FFA, esterfication is required before transesterfication was carried out. The presence of significant amount of water also causes formation soap, which consumes the catalyst, reduced catalyst efficiency, and makes difficulty of separation of ester, glycerol, and washing water as of FFA. It was recommended for biodiesel feedstock containing FFA and water beyond acceptable level acid catalyzed condition is preferred (Maa and Hanna, 1999).

2.5.5. Effect of co-solvent

Alcohol and oil are immiscible which exist in two different phase. In the case of homogenous catalyzed transesterfication reaction due to the presence of more than one phase mass transfer resistance was observed which has negative effect on the biodiesel yield. Consequently, throughout the reaction continuous agitation was required to improve mass transfer process else the reaction will be carried out at the contact surface of two phase system only.

Different research has shown that co-solvent increases the rate of reaction and improves the yield. Basically, co-solvent is introduced to bring immiscible oil and alcohol to one

phase. Cyclic ethers are most effective co-solvents because they form hydrogen bonds with polar compounds such alcohols, and contain sufficient non-polar hydrocarbon groups to dissolve the high molecular weight of oils and fats. This reduces the viscosity and reduces mass transfer resistance. Cyclic ethers were increased the reaction rate by 15 times (Ji-Yeon Park et al., 2009).

In the case of heterogeneous catalyzed biodiesel production process catalyst, oil, and alcohol exist in three different phase. Co-solvent reduces one phase because the co-solvent has affinity for both alcohol and oil which leads to solublise one in the other. Co-solvent currently being investigated are: hexane, dimethylether, diethylether, tert-butyl methylether, and THF. After completion of the reaction, those co-solvents are easily separated out, since they are chemically inert.

2.6. Transesterification reaction catalyst type

Transesterfication reaction can be catalyzed by homogenous catalyst, heterogeneous catalyst, and biocatalyst. Most industry in the world are using homogenous catalyst, however a lot of research were being conducted on heterogeneous and biocatalyst to replace it because it has a lot of drawback such as environmental, economical, and technological.

2.6.1. Homogeneous catalysts

Currently, in the world majority of industry are producing biodiesel by using homogenous catalyst since it has cost effectiveness over the other. Furthermore, it also provides easily attainable reaction condition (25 ^{0}C –130 ^{0}C, atmospheric pressure), high yields with low amount of catalyst per batch, and high activity (Yan, 2010).

2.6.1.1. Homogeneous alkali catalysts

Commonly used homogeneous alkali catalyst is sodium hydroxide, potassium hydroxide, sodium methoxide, and potassium methoxide (Xiaohu Fan, 2008). Sodium hydroxide and potassium hydroxide flakes are widely preferred by small scale producer, but for large continuous flow process alky oxide solution of sodium methoxide or potassium methoxide are preferred (Refaat, *2010)*. Sodium hydroxide is also used widely in large-scale processing due to its cheaper price (Fangrui Ma, 1999).

Homogenous alkali catalyzed condition has a lot of advantage over acid catalyzed reaction condition as it was indicated on (Xiaohu Fan, 2008).

- ✓ The transesterfication reaction is very fast
- ✓ Consumption of alcohol is less
- ✓ The catalyst is less corrosive
- ✓ Methanol to oil molar ratio and catalyst concentration requirement is low as compared to acid catalyzed process.

However, homogenous alkali catalyzed transesterfication was disturbed and/or affected by the presence of moisture and high level of free fatty acid. The tolerable total FFA and water content are 0.5% and 0.1%-0.3%, respectively (Xiaohu Fan, 2008). The presence of water beyond the acceptable limit leads to saponification reaction (formation of soap), this in turn will leads to lower ester yield, reduced catalyst efficiency, increase in viscosity and formation of gel due to the presence of soap as by product in the reaction mixture (Shrestha et al., 2013). Furthermore, separation of ester, glycerol, and water washing becomes difficult. Therefore, in the presence higher amount of water and FFA, acid catalyzed condition is preferred (Ma, and Hanna, 1999).

According to (Xiaohu Fan, 2008) biodiesel yields after separation and purification steps are higher for methoxide catalysts ($NaOCH_3$, $KOCH_3$) than for hydroxide catalysts (NaOH, KOH) when methanolysis of sunflower oil was conducted. This phenomenon has lead the researcher to conclude that the yield lose of hydroxide catalysts was due to more triglyceride saponification and methyl ester dissolution in glycerol.

2.6.1.2. Homogeneous acid catalysts

Homogenous acid catalysts are not affected by the presence of free fatty acids and water, but the reaction rate is 4 000 times slower than in base catalysis (Ibrahim, H 2013). These catalysts give very high yields in alkyl esters, but the reactions are slow, requiring temperatures above 100 °C and more than 3 hr reaction time for complete conversion (Freedman et al., 1984).

Most commonly used acid in the transesterfication reaction of high free fatty acid and moisture containing feedstock are sulfuric acid, sulfonic acid, and hydrochloric acid (Fangrui Ma, 1999). Study done on single step acid catalyzed transesterification of soybean oil using sulfuric acid, hydrochloric acid, formic acid, acetic acid, nitric acid at 0.1 and 1 wt.% loadings and temperatures of 100 and 120°C in sealed ampules indicated that only sulfuric acid was found to be effective (Xiaohu Fan, 2008).

2.6.1.3. Stepwise acid and base catalyzed transesterfication reaction

Free fatty acid and moisture content are key parameters for determining the viability of the vegetable oil transesterification process (Refaat et al., 2008). Feedstocks with high free fatty acid (> 1%) are not easily converted by alkali catalysed transesterification, because of concurrent soap formation, which has a potential of reducing catalyst efficiency, increase viscosity, and difficulty in separation of glycerol (Shrestha et al., 2013). To avoid this all, free fatty acids must be reduced with a base or preprocessed with acid (Gerpen, 2005). Base pretreatment which is added to neutralize the free fatty acid cause significant loss, especially if the oil contains more than 5% FFA. Unlike to base, acid pretreatment is thought to be the best route, because it converts FFA into esters, as a result it reduces the losses which could have produced from base pretreatment (Gerpen, 2005). In the acidification stage, there is no bond cleavage instead the reaction was occurring directly between alcohol and free fatty acid; consequently no glycerol was generated as it was shown on the following reaction. After acid pretreatment, base catalyzed transesterfication continues as explained in homogeneous alkali catalyzed section.

$$RCOOH + CH_3OH \xleftrightarrow{H_2SO_4} RCOOCH_3 + H_2O$$

2.6.2. Heterogeneous catalysts

Currently, majority of industrial biodiesel production was proceeding by homogenous catalyst. But, some problems are associated with it, like formation of soap in the presence of high FFA and moisture in the feedstock, difficulty of separation of catalyst after completion of the reaction, difficulty of reusability of catalyst, corrosivity, and time consuming & costy process (Yan et al., 2010). Furthermore, huge amount of waste water is generated while biodiesel washing was carried out to separate residual homogeneous catalyst from the product.

Heterogeneous catalyst is an optional with multi environmental and economic benefit. It can proceed either with batch or continuous mode of operation. In heterogeneous catalyzed process, contaminated waste water generation, washing and neutralization are reduced (Yan et al.2010). In addition, higher yield of methyl ester which is close to the theoretical value could be obtained; and also the generated by product (glycerol) has high purity (Di Serio et al., 2008).

Heterogeneous catalyst categorized as solid acid and solid base catalyst. Solid base catalysts include a wide group of compounds in the category of alkaline earth metal oxides, hydrotalcites/layered double hydroxides, alumina loaded with various compounds, zeolites, and various other compounds showing high basicity coupled with active basic sites, pore size, and other parameters (Ibrahim, 2013).

The activity of the solid catalyst is dependent on the active sites on the surface of catalyst. This, surface of catalyst, especially in the case of metal oxides, can easily be poisoned by absorption of carbon dioxide and water from the air to form carbonates and hydroxides, respectively. Consequently, it reduces the activity of the catalyst so that the yield (Thanh et al., 2012).

Research don by (Huaping et al., 2006) showed that the transesterification of Jatropha curcas oil with methanol catalyzed by calcium oxide (solid base catalyst) has provided FAME yield higher than 93% at 1.5 wt.% catalyst amount, 70 °C temperature 9:1molar ratio, and 3.5 hr reaction time. The other type (solid acid catalyst) was not widely applicable due to low reaction rate and adverse side reaction. Those, which are used in the process of esterification and transesterfication reaction studies in the past include Zeolite with varous acidic and textural property, tungsten oxides, sulphonated zirconia (SZ), sulphonated saccharides, Nafion1 resins, and organosulphonic functionalized mesoporous silicas (Chopade et al., 2012). Sulfonic acid ion-exchange resins have been reported to show excellent catalytic activity in esterification reaction as a pretreatment step for oils containing a high amount of FFA (Russbueldt et al., 2009).

Transesterification of refined and crude vegetable oils with a sulfonic acid-modified mesostructured catalyst has provided a promising result, oil conversion close to 100% with 95 wt.% purity was obtained at 180 °C temperature, 10:1 methanol to oil molar ratio, and 6 wt.% catalyst to oil weight ratio (Melero et al.,2009). Kinetics of esterification of a mixture of triglyceride and oleic acid with initial acidity in the range of 47.1–58.3 wt.% was studied by (Santacesaria et al., 2005). It was obtained that esterification with the conversion of oleic acid to methyl oleate reachs more than 80% within 2 hr reaction time at 85 °C using Sulfonic acid resin (2 wt. %) with methanol.

2.6.3. Biocatalysts

Biocatalysis refers to catalysis of a reaction by highly complex proteins called enzymes (Güzel, 2012). Enzymes have enormous potential for reducing energy requirements and environmental problems, they can handle large variation in raw material quality, they have ability to esterify both FFA and triglyceride in one step, simplify the separation of products, produce a high quality glycerol, and allows for the reuse of the catalyst (Ghaly et al., 2010). In biocatalysis transesterfication reaction, all side and by products are minimized, hence higher yield will be obtained.

Furthermore, the current worldwide industrial biodiesel production is proceeding with help of conventional chemical catalytic process, even if it was suffering with a lot of drawback such as:

- Higher energy consumption,
- Formation of soap in the presence of significant amount of FFA, specially in alkali catalyzed condition, this will lead as to reduction in yield of ester
- Homogenous catalyst can be removed with glycerol layer, cannot be reused
- Purification and separation of glycerol is difficult
- Generation of huge amount of waste and toxic chemicals, which are not environmentally friendly.

Enzymatic process has a potential of solving the above drawbacks (Shah et al., 2003).

Biocatalysts are getting more attention, nowadays and have the potential to outperform in the future over the chemical catalysts for biodiesel production (Vasudevan and Briggs, 2008). Because, unlike to the current biodiesel feedstock, biocatalysis can efficiently use waste cooking oil, industrial waste oil, and non edible oil with high free fatty acid and moisture content as feedstock, in which the conventional chemical means of biodiesel production fail in its economic feasibility.

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) constitute a diverse and ubiquitous family of enzymes which are produced by animals, plants and microorganisms (Ghaly et al., 2010). It has a growing potential in industrial process since 1980s, they have been exploited as a catalyst for a number of transformation of fats and oils. Their main application has been in modifying the fatty acid composition of triglycerides by

interesterfication, the hydrolysis of triglyceride, and direct synthesis of ester (esterfication and transesterfication). Industrially lipase is produced by controlled fermentation of Aspergillus niger var., Aspergillus oryzae var., Candida rugosa.

However, enzymatic transesterification reaction and yield are affected by several factors including selection of alcohol, use of solvents, lipase pretreatments, alcohol to oil molar ratio, and reaction temperature (Ghaly et al., 2010). And also some draw back are associated with, for industrial production application, such as high cost of enzymes, low activity of enzyme (low rate of reaction), and high concentration of enzyme requirement (Ghaly et al., 2010) and low worldwide availability and/or non optimal operational features of naturally available enzymes (Güzel, 2012). In addition, short chain alcohol like methanol and ethanol are known to inactivate enzymes, even they can also be inhibited by glycerol (Shah et al., 2003).

2.7. Biodiesel production process technology

A lot of technology was being introduced to the biodiesel production process which is microwave heating, ultrasonic, and supercritical assisted biodiesel production. In addition, currently, Biox process was developed by Professor David Boocock. This process uses inert co-solvent, like tetra-hydrofuran and methyl tert-butyl ether that dissolves both alcohol, oil and generates an oil rich one phase system.

2.7.1. Microwave assisted biodiesel production

Nowadays microwave radiation is being researched to produce biodiesel from different raw materials due to many advantages that are associated with this technology as compared to traditional transesterification process (Cancela et al. 2012).

In the case of conventional heating, heat energy is transferred to the reaction through convection, conduction, and radiation from the surfaces of the reactor, which requires large amount of heat energy and longer reaction time. In the contrary, microwave radiation delivers energy directly to the reactants and heat is generated within the material (Zare et al. 2013). Hence, reduce reaction time and heat energy requirement, additionally it will improve product yields under atmospheric conditions (Cancela et al. 2012). Clean reaction products, and reduced time of separation-purification are also the other benefit of microwave assisted production technique as compared with conventional technique (Gude et al., 2013).

Microwaves activate a small degree of variance in polar molecules and ions, such as alcohol, with the continuously altering magnetic field. When molecular dipoles and charged ions interact, as they are trying to alien, with the altering electrical field, end up in a rapid rotation; intermolecular friction is generated this in turn causes the medium to heat (Nezihe and Aysegul, 2007). It was observed that due to the presence of continuous interaction process, there is localized overheating for short period of time. Basically, microwave technology was dependent on the use of electromagnetic wave. The electromagnetic spectrum for microwave is between infrared radiation and radiofrequency of 0.3 GHz to 300GHz, respectively, corresponding to wavelength of 1cm to 1m (Gude et al., 2013). This radiation can influence molecular movement, which is ion migration or dipole reaction, but could not change the molecular structure. In addition, this energy much lower than ionization energy of biological compounds, covalent bond, hydrogen bonds, van der Waals intermolecular interactions (Gude et al., 2013).

Research done by (Lin et al., 2012) showed that 99% biodiesel yield was obtained from soybean oil under following reaction conditions: 0.75 wt% CH_3ONa catalyst, a methanol-to-oil molar ratio of 6%, a reaction time of 3 min, and a microwave power of 750 W. Similarly, (Lertsathapornsuk et al., 2008) found that the continuous conversion of waste frying palm oil to ethyl ester was over 97% with an ethanol-to-oil molar ratio 12:1, 3.0% NaOH (in ethanol), a 30-s residence time, and a microwave power of 800 W. According to (Sherbiny et al., 2010) a conversion of 97.4% was obtained by applying microwave irradiation for two minutes, 1 wt% KOH, and 1:7.5 oil to methanol ratio compared to 98% that was obtained after one hour reaction time using the conventional transesterification method.

2.7.2. Ultrasonic assisted biodiesel production

Ultrasound refers to a sound pitch and/or sound wave that are above human hearing ability (i.e. usually above 20 kHz). Ultrasound wave travels like any other sound wave by a successive series of rarefaction and compression cycles vibrating the molecules of the carrier media. As it passes through, the attraction forces between the liquid molecules became less than the negative pressure of the cyclic rarefaction, a gap will be generated and is filled with vapor from the liquid, and end up in formation of cavitation bubble (Badday et al., 2012). Huge number of these bubbles can be generated in the liquid with

smaller size at the beginning but increase with time. Some of them are stable and others undergo collapse after reaching certain critical size. The strong collapses generate local pressure in the order of 2000 atm and the temperatures could reach up to 5000 K (Stavarache et al., 2005); this enhance the reactivity and mixing, and also reduce reaction time (Badday et al., 2012). Despite formation of micro jets and localized temperature increase, production of biodiesel by ultrasonic application doesn't require heating or agitation (Worapun et al., 2010). Moreover, the yields of biodiesel in the ultrasonic process were higher than those from traditional methods (Somnuk et al., 2012).

(Somnuk et al., 2012) has compared FAME conversion in the presence and absence of ultrasonic irradiation using 23% by volume of methanol, 7g NaOH per liter of oil, and reaction temperature of 45^0C. The result has showed that 90% conversions by weight were achieved with the help of ultrasonic and 6 min residence time; but in the absence of ultrasonic, the conversion is 65% by weight even after the residence time of 12 min. (Stavarache C. et al., 2005) has researched the effect of alcohols on the transesterfication of neat vegetable oil under ultrasound with the frequency of 28 KHz and 40 KHz, and mechanical stirring. The result exhibited that methanol, ethanol, n-propanol, and n-butanol provide a yield of 88%-98% by weight of ester within 10-20 min retention time with 40KHz ultrasonic frequency. (Worapun et al., 2010) has investigated stepwise transesterfication crude jatropha curcus L. oil using ultrasonic irradiation, Accordingly, 96% conversion was obtained, but the result is 83% for conventional stirring methods under similar condition. It has also enhanced equilibrium conversion from 91% in 60 minute to 96% in 40 minute of reaction time.

2.7.3. Supercritical biodiesel production

A substance can existed in three different phase, which is solid, liquid, and vapor or gas; and is characterized by both uniform physical structure and uniform chemical composition. Varying the temperature and pressure, will cause change in state, and change in important properties.

Super critical condition is defined as the temperature and pressure above the critical point of the reaction mixture. Critical point of a fluid marks the terminus of the vapor-liquid coexistence curve. Super critical fluid is characterized by physical and thermal properties that are in between pure liquid and gas. At temperatures above the critical temperature, a

fluid cannot undergo a transition to a liquid phase, regardless of the pressure applied (Savage et al., 1995).

Basically, supercritical treatment is based on the relationship between pressure and temperature upon the thermophysical properties of the solvent, such as dielectric constant, viscosity, specific weight and polarity (Kusdiana, 2001).

Biodiesel can be produced by a non catalytic, supercritical process using supercritical methanol. Supercritical methanol has very low dielectric constant; as a result there is formation of a single phase than the formation of two phase alcohol and oil mixture. The transesterfication of triglycerides by supercritical methanol, ethanol, propanol and butanol has proved to be the most promising process (Demirbas, 2006). Supercritical transesterfication has many important benefits such as less expensive, environmental friendly, less production time requirement (probably 3 to 5 minute), less energy consumption, and there nature can be adjusted by varying temperature and pressure (Krishna et al, 2013). In addition, study has shown that the process is not sensitive to free fatty acids and water contents as well (Nascimento et al., 2013). Supercritical transesterification has better production efficiency than the conventional catalytic method because it requires a smaller number of processing steps. And the feedstock quality is far less influential under supercritical conditions than with the heterogeneous catalytic method (Ngamprasertsith and Sawangkeaw, 2011).

In supercritical methanol transesterfication method using vegetable oil as feedstock, the yield of conversion rises 95% in 10 min (Demirbas, 2005). Research don by (Nascimento et al., 2013) on soybean oil with reaction time 15 minutes, 39:1 initial alcohol to oil molar ratio, temperature range of 260 to 300 °C and pressure over 100 bar showed significant conversion of oil into biodiesel at a temperature of 300 °C and reduction in conversion was observed as the temperature drops, at 260 °C the conversion was negligible. Research don by (Ezeanayanso et al., 2011) under supercritical condition in a tubular reactor at 250°C with 200 rpm by varying the pressure in the range of 1 to 100 bars showed that non-catalytic supercritical methanol technology required 3, 4, 5 min reaction time to produce 98, 97.17 and 87.1% biodiesel from J. curcas, A. indica and H. brasiliensis seed oil, respectively; compared to conventional catalytic methods, it required at least 1 hr reaction time to obtain similar yield.

However, the supercritical method requires higher temperatures and pressures, and large amounts of methanol (Ebtisam K., 2013). This will reduce its viability for industrial process. In some research addition of supercritical CO_2 as a co-solvent to increase the mutual solubility of ethanol and vegetable oil at low reaction temperatures showed reduction in expected high operating cost (Han et al., 2005).

2.7.4. Biox process

This process was developed by Professor David Boocock from the University of Torronto. Biox process uses inert co-solvent, like tetra hydrofuran and methyl tert-butyl ether that dissolves both alcohol and oil to overcome the initial slow reaction rate caused by alcohol-oil immiscibility and will generates an oil rich one phase system. This reaction is over 99% complete in seconds at ambient temperatures, compared to previous processes that required several hours (Demirbas, 2009). After the reaction is complete, the excess methanol and co-solvent is recovered in a single step (Gerpen, 2004). The process is not feedstock specific and no catalyst residues appear in either the ester or the glycerol phase (Krishna et al, 2013)

The initial mixture in traditional biodiesel production processes consists of two phases. This makes the reaction slow by creating a mass transfer problem (Mamilla et al., 2012). Biox process brings the two phases into one homogeneous phase, and hence reduces the need for extensive agitation, multiple batch reactors and extensive reaction time requirement, and improves the yield and product quality. It can also handle high free fatty acid containing feed stock, by stepwise esterfication within 30 second and transesterfication within a couple of seconds at ambient temperature and pressure in a single phase and continuous process (Mamilla et al., 2012, Demirbas, 2009). In addition, the co-solvent is recycled and reused continuously; this makes the process environmentally friendly.

2.8. Alternative biodiesel feedstock

Different country uses different feedstock for biodiesel production. As a matter of fact selection was dependent on the availability of raw material, economic level of the country, and environmental impact generated by feedstock. United State of America uses soybean, most European country uses rapeseed and sunflower oil, Canada uses canola oil (Gonsalves, 2006).

Even if the biodiesel is environmentally friendly product, but there is greater concern on the raw material and the process of getting it. Currently, in greater part of the world biodiesel was being produced from edible oil that is raising a competition between food fuel prices. Thus, the cost of biodiesel is not able to compete with petro diesel. Even the technology of processing was being advanced; still it was difficult to make its price competitive, since around 80% of its cost goes to feedstock (In tech, 2011).

In addition, oil seed production for biodiesel is using the land intensively, fertilizer and water input is also higher to generate higher yield. On the process, the available land for food cultivation was being reduced which indirectly leads to increase food price (especially in the developing country). As a matter of fact, increased demand for food can lead to slowdown in biodiesel production due to reduced raw material availability. Therefore, it was important to produce it from crops providing high productivity with low input requirements, or from low-cost feedstock, and lower land coverage (In tech, 2011).

Currently, Ethiopian government focused on production of biodiesel from jatropha, cator, and palm oil. Even if they have non-edibility importance, but there socioeconomic importance was minimum. In the country with food security problem those source of biofuel generates another burdon on the available land. It will deprive them from their daily needs because Jatropha leaves do not serve as fodder and it does not yield enough wood as well. Some research paper has shown that planting Jatropha as a promising biofuel crop is not a feasible strategy both economically and ecologically. Important feed stock for biodiesel is those without affecting the community way of living and ecology that can provide support for the society. Neem & Karanja are found to be promising tree (khandelwal and Chauhan, 2013) because they have a lot of socioeconomic and environmental advantage.

2.8.1. Brassica carinata seed oil

It is commonly known as Ethiopian mustard, which is locally called “Gomenzer”, is among the oldest crops cultivated in Ethiopia. Different study has suggested that the crop is evolved from the highlands of Ethiopia and adjoining portion of East Africa and Mediterranean coast. The B. carinata cultivars Dodolla (yellow seeded) and S-67 (brown seeded) which are presently commercially grown in Ethiopia, has provide a yield up to 3 t /ha of seed with an oil content of up to 420 g/kg (Getinet, et al.,1996). Currently, the seed

is being evaluated as an option to the traditional canola /mustard cultivation, especially for low rainfall areas of the world (Sheikh et al., 2010).

The oil from the seed is characterized by a high level of erucic acid (35 to 44%) (Getinet, et al., 1996) and excessive amount of linolenic acid, this makes it unsuitable for human consumption.

Its greater importance is it has higher resistance to biotic, abiotic stress, and rapeseed pests, additionally; it has high adaptability to hard pedo-climatic conditions. This and its higher oil yield make B.carinata the most promising oilseed for biodiesel purpose in temperate zones (InTech, 2011). Additionally, the crop has a potential to be used as feedstock for oleochemichals and bio-fumigant industries.

2.8.2. Castor seed oil

The castor bean plant (*Ricinus communis L.*) belonging to the family of Euphorbiaceae family, most research consider it as native to tropical Africa, particularly Ethiopia, but now it is indigenous to the southeastern Mediterranean basin, Eastern Africa, India, and South Asia (Gana et al., 2013). Currently, it is widely grown as a commercial crop in Ethiopia for its oil, which is a medicinal commodity (Chakrabarti and Ahmed, 2008).

Castor bean plant is fast growing suckering perennial shrub which can reach a height of 12 m and under warm climate it reaches a height of 2-3 m. Most importantly, it is drought resistant and can stand moderate arid/saline environments. The gestation period of harvesting the plant for oil is 4-6 months only (A. Goswami, 2011).

The seeds contain between 40 and 60% oil that is rich in triglycerides that is 35 to 55% of the weight of the seeds (Oplinger et al., 1990) and 85 to 90% of the seed is ricinoleic acid which is responsible for its solubility at room temperature in alcohol ether, glacial acetic acid, chloroform, carbon sulfide, and benzene.

It is non-edible as it contains ricin, which is a poisonous protein substance (Efeovbokhan et al., 2012). The oil has versatile utility such as cosmetics, lubricants, brake fluids, softener in tanning, solar cell, textile company, small components of PC, mobile phones, boots and shoe manufacturing (Goswami, 2011).

Castor oil is an alternative feedstock for biodiesel production. The castor biodiesel has very interesting properties (specially, very low cloud and pour points) which show that this fuel is very suitable for use in extreme low temperatures (Efeovbokhan et al., 2012).

2.8.3. Jatropha curcas L. seed oil

Jatropha tree belongs to the Euphorbiaceae family. It can grow in tropical areas all around the world. Normally the plant reaches a height of three to five meters but can reach up to eight to ten meters when grown under favorable conditions. It has a life span of 50 years and reaches maturity within four to five years (Axelsson and Franzen, 2010).

Jatropha is drought-resistant plant, can easily adapt to semi-arid and arid conditions, can grow on gravelly, sandy, saline, even on poorest stony soil. The required average annual rainfall for seed production is between 1000-1500 mm, but at least 600 mm is needed for flowering and fruit yield, and the most favorable temperature are 20-28 ^{0}C. The preferred pH for maximum yield is in the range of 6.0 and 8.5. On Average, have annual seed yield of 5 tons per hectare (Aderibigbe et al., 1997, Axelsson and Franzen, 2010).

Oil content of the seeds range from 32 to 40 %, and it was found that the oil cannot be used for human consumption and the cake as well cannot be used as cattle feed or for any edible purpose (Baroi et al., 2009).

Jatropha seed oil has low acidity and good oxidation stability as compared to soybean oil, low viscosity as compared to castor oil and better cold properties than palm oil that provides it a tremendous potential for biodiesel production (Tapanes et al., 2008).

2.8.4. Algae

Algae are a large and diverse group of simple plant like organisms, ranging from unicellar to multicellar forms. There are nine major groups of algae which are cyanobacteria (Cyanophyceae), green algae (Chlorophyceae),diatoms (Bacillariophyceae), yellow-green algae (Xantophyceae), golden algae (Chrysophyceae), red algae(Rhodophyceae), brown algae (Phaeophyceae), dinoflagellates (Dinophyceae) and 'pico-plankton' (Prasinophyceae and Eustigmatophyceae). Green algae are the largest taxonomic group of all. These cells have the ability to convert carbon dioxide to biomass that can further be processed to produce biodiesel, fertilizer and other useful products (Pokoo-Aikins et al., 2010).

Algae require primarily three components to grow: sunlight, carbon-dioxide and water. For better growing condition it requires temperature in the range of 20 to 30 ^{0}C and other nutrients like phosphorus and nitrogen. Algae have much faster growth-rates than

terrestrial crops, greatly minimize or avoid use of land, have easy adaptability to growth conditions, and can be harvested more than once a year (Pokoo-Aikins et al., 2010).

Algae can have 20-80% oil by weight of dry mass. The per unit area yield of oil from algae is estimated to be from 20,000 to 80,000 L per acre per year; this is 7–31 times greater than the next best crop (palm oil) (Demirbas & Demirbas, 2011). Oil production per unit area of land far exceeds other oil crops such as corn, soybean, and coconut by as much as 2–3 orders of magnitude (Yesuf, 2007). Hence, algae were considered as promising feedstock sources for biodiesel production.

2.8.5. Neem seed oil

The neem tree whose common botanical name Azadirachta indica believed to originates from Myanmar and India. It belongs to Meliaceae family. Currently, it is widely grown and naturalized in the dry climatic zones of different part of the world. Neem is fast growing and tropical evergreen tree that can reach height of 15 - 32 m. The tree normally starts fruiting after 3-5 years. From the tenth year onwards it can produce up to 50 Kg of fruits per tree annually (Kumar, et al., 2002).

The tree has good adaptability to a wide range of climatic, topographic, and edaphic factors; can grow in areas where the rainfall is as low as 150 to 250 mm, naturally it grows in the area of rainfall range 450 to 1200 mm and with altitudes up to 1500 m. Its deep tap root system makes free of competition with annual crops on scarce soil moisture. The tree can grow on clay, saline and alkaline soil in the temperature range of 0^0C to 49^0C and pH range of 4 to 10, but cannot withstand water-logged areas and poorly drained soils (Girish and Shankara, 2008).

Neem plant possesses a maximum noon wood product such as leaves, bark, flower, fruit, seeds, gum, oil, and neem cake than any other tree species. Biologically, it has numerous bioactive ingredients with diverse application. Most of the plant part contains compound with proven antiseptic, antiviral, antipyretic, anti-inflammatory, anti-ulcer, and antifungal use. Generally, it has a greater potential in pest management, environmental protection, and medicinal application (Ogbuewu et al., 2011).

The seeds have 45% oil which has high potential for the production of biodiesel. It comprises mainly of triglycerides and large amounts of triterpenoid compounds, which are responsible for the bitter taste. Neem oil also contains steroids (campesterol, beta-

sitosterol, stigma sterol) and a plethora of triterpenoids of which Azadirachtin is the most widely studied (Radha and Manikandan, 2011).

Fig.2.1. Neem tree plantation with 216 trees in a square plantation of 7x7 m.
(Source: Sergio et al., 2007)

2.8.5.1. Socioeconomic importance of Neem plant

Some part of Ethiopia is suffering with drought, soil erosion, and other related environmental deterioration. Greater part of the population is farmers, in which there life has close interaction with the environment. As a result environmental rehabilitation and protection has to be paid great attention. The forest need to be recovered, especially, arid, saline, and less fertile soil, which can't be used for farming, has to be given priority.

Neem tree has great advantage in environmental protection like stabilizing the local climate, rehabilitation of local biodiversity, and conservation of soil fertility and water quality. It can also provide to local farmers and pastoralist financial support. In addition, as a matter of fact Ethiopia's farmers and pastoralists life was dependent on cattle's and domestic animals, this tree can provide them grass and shrub in the dry time because the tree is long enough and spaced for the growth of other at the ground as shown in the Fig 2.1.

The seed is the major part of income generator. when the tree arrives at the maturity level start to generate more than 50 kg per tree per year with 45% oil content, depending on the climate, growth condition, and seed type (Radha and Manikandan, 2011).

All parts of the tree have been used medicinally for centuries in countries like India. A neem fruit, seeds, oil, leaves, bark and roots have such uses as general antiseptics, antimicrobials, antifungal activity, and are found to be active against both gram-positive and gram-negative bacteria. In agriculture as well it used to control various insects and nematodes (parasitic worms). Triterpenes with the most effective compounds, limonoids, of these Limonoids, Azadirachitin which is abundant in its oil is responsible for the effectiveness. The leaves by itself are also used as an insect repellent (Mugnai, E., 2009).

The cake which is by product from oil extraction process contains nutrient like nitrogen and carbon that can inhibits denitrifying microorganisms which makes it important soil conditioning. In addition, it contains pesticide property hence it can protect the farm land from pest. Those, soil conditioning and agricultural pest controlling chemicals can easily produced even by farmers. This helps specially small scale and house hold farmers.

Most importantly, the neem plantation land can be used for other farming activity which requires shading and/or doesn't affect the growing condition.

2.9.Treatment of oil impurities

The oil obtained by mechanical expelling or solvent extraction is termed as a crude oil, since it contains a number of impurities. Fats and oils are primarily composed of triglyceride. However, they contains minor fraction of non-triacylglyceride components, which are mono- and diglycerides, phosphatides, cerebrosides, sterols, terpenes, fatty acids, fat-soluble vitamins, and other substances as a minor components. The amount of those non triglyceride fraction of crude oils vary depending on oil source, extraction process, season and geographical origins.

Phospholipids pose many problems and are removed from oil during refining process. There are two types of phospholipids: hydratable (HPL) and nonhydratable (NHPL); degumming process is used to remove both type of Phospholipids. Generally, there are a lot of degumming methods, including water degumming, superdegumming, TOP degumming, acid treatment, which is currently being used worldwide as removal means of phospholipids (Zufarov et al., 2008).

Hydratable phosphatides can be removed by adding the proper quantity of water, mixing thoroughly, allowing time for the hydration to occur, and separating by centrifugation, which separates hydrated phosphatides (gums) from the oil stream. The other, nonhydratable type of phospholipids (NHPL), are not hydratable with water; cannot swell and precipitate out from oil. Hence, removing of NHPL requires more complex process, such as increased temperature and acids like phosphoric acid, citric acid etc (Zufarov et al., 2008).

Furthermore, colouring material and other non-triglyceride part of the oil can be removed by a process known as bleaching. It is a mass transfer process. Thus, non-triglyceride components, which are solubilised in a liquid state, are changed to a solid state by adsorption on to the surface of the solid bleaching earth particles, which are added to the oil stream (Karasulu, H., 2011).

The adsorption process is carried out by adding the proper amount of clay to the refined oil stream. Typical bleaching condition includes a minimum of 20-25 minutes contact time between the clay and the oil, a temperature range 104 to 116 °C and a vacuum of 50 mmHg absolute. Subsequently, the spent clay is filtered out of the bleached oil (Karasulu, H., 2011).

3. Material and method

3.1. Material and reagents

Dry neem seed was collected from Metehara city. All reagents used in the experiment are analytical grade: hexane (96%), methanol (98.9%), absolute ethanol (99.8%), sulfuric acid (98%), phenolphthalein indicator, hydrochloric acid, potassium hydroxide, anhydrous sodium carbonate, and calcium oxide. Those chemicals are purchased from Neway Chemical P.L.C.

Equipments used during the experimental works are soxhlet extraction apparatus, water bath, micropipette, hot plate magnetic stirrer, agitator, three and two neck round bottom flask, condenser, thermometer, desiccators, crucibles, furnace, mortar and pestle, mill, viscometer, hydrometer, and oven.

Laboratory work was conducted in School of Chemical and Bio-Engineering Laboratory, JIJE Analytical Testing Laboratory, and Ethiopian Petroleum Supply Enterprise.

The first part of experiment was pretreatment of the collected seed. Then, consecutively oil extraction and free fatty acid reduction was conducted prior to heterogeneous catalyzed transestrfication reaction. The catalyst, which is required for transesterfication reaction, was prepared and screened. Finally, optimal reaction condition was determined as it was shown on framework of the experiment in Fig.3.1.

3.2. Neem seed oil extraction

3.2.1. Neem seed collection and pretreatment

Neem seed (18 kg), which is composed of kernel and pulp, was brought to School of Chemical and Bio-Engineering laboratory.

Prior to processing, impurities and damaged seed was picked out by hand then soaked in water for five hour and washed repeatedly in order to separate the pulp from the kernel.

Depulped seed was sun dried, decorticated using disc mill, and sifted to separate the kernel. The Neem kernel was further dried in an oven at 50 ^{0}C for 48 hours and milled to obtained about two inch particle size.

3.2.2. Oil extraction

Neem seed oil was extracted using Sohxlet apparatus found in Addis Mojo Edible Oil Share Company laboratory using hexane solvent. In the process, Neem seed powder (50 g) was packed into thimble and placed in Sohxlet extractor. Round bottom flask containing hexane to its mark was attached to the lower part of the extractor, which in turn attached to condenser.

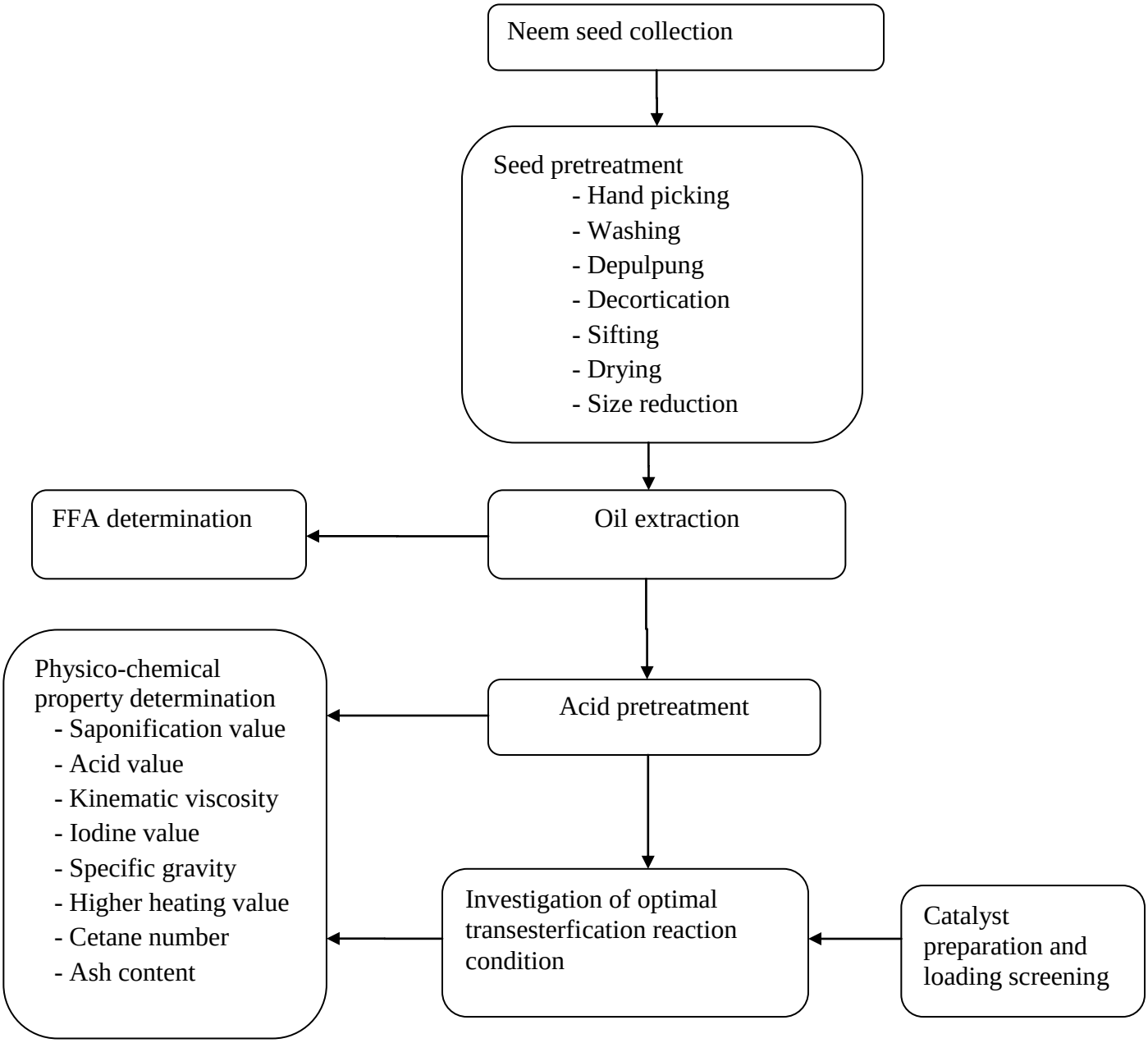


Fig.3.1.Framework of the experiment

The extraction continued for 4 hr at the boiling point of hexane. After completion of extraction, hexane and oil was separated using distillation process with the same apparatus. Finally the oil yield % was calculated as follow:

$$\% \, Oil \, content = \frac{Mass \, of \, extracted \, oil}{Mass \, of \, powder \, used} \, x \, 100 \quad \text{.....3.1}$$

3.2.3. Esterification of FFA using sulfuric acid catalyst

The reaction between FFA found in neem oil and methanol was carried out using sulfuric acid catalyst in order to reduce its amount.

For each reaction experiment, 100 ml Neem seed oil, which was heated at 60 ^{0}C for 30 min, was poured in to 500 ml round bottom flask and cooled to 50 ^{0}C. Throughout esterification reaction, sulfuric acid/oil volume ratio (0.60 v/v %), reaction time (90 min.), and reaction temperature (50 ^{0}C) were kept constant. However, varying amount of methanol/oil volume ratio of 30 %, 40%, 50%, 60%, 70%, and 80% was investigated to obtain lower FFA content. In the process, methanol was added in to the heated oil and then sulfuric acid was added while stirring. The reaction was continued in the 500 ml round bottom flask equipped with agitator and condenser. After completion of the reaction, it was transferred to separator funnel, washed with distilled water (50 ^{0}C warm) four times.

Then, it was allowed to stand for 2 hr till oil and converted ester was clearly separated from water, unreacted methanol, and catalyst. Based on the experimental data, less free fatty acid containing result was chosen prior to production of biodiesel.

3.3. Preparation of Na_2O/CaO catalyst

Three catalyst (Na_2O/CaO) containing 30, 40 and 50 wt/wt % of Na_2O were prepared starting from CaO and Na_2CO_3 solutions.

In the process, an appropriate amount of CaO was first soaked in 50 ml distilled water, placed in round bottom flask. Appropriate amount containing 50 ml Na_2CO_3 solution, was then poured into prepared CaO/H_2O mixture and vigorously stirred for 2 hr, aged for 10 min., and dried overnight at 120 ^{0}C, consecutively.

This dried Na_2CO_3/CaO flakes was then milled into powder and calcined at 730 ^{0}C for 3 hr. The prepared Na_2O/CaO catalyst was placed in a desiccators and then packed with

plastic bag to avoid any adsorption of moisture and carbon dioxide on the surface. In this way, N_2O/CaO-1, Na_2O/CaO-2, and Na_2O/CaO-3 catalyst with 30 wt/wt%, 40 wt/wt% and 50 wt/wt% of Na_2O/CaO ratio were prepared, respectively.

3.3.1 Na_2O/CaO catalyst screening

The performance of N_2O/CaO-1, Na_2O/CaO-2, and Na_2O/CaO-3 catalysts was primarily tested under constant operating conditions. In order to select catalyst with better loading, transesterification reaction was performed under constant input operating variable conditions: Esterified oil (100 ml), methanol to oil molar ratio of 10, catalyst to oil weight ratio of 6.5, temperature of 64 ^{0}C, and 3 hr reaction time. Best performing catalyst that provide high fatty acid methyl ester yield was then selected for future experiment.

3.4. Transesterification reaction setup

Transesterification reaction between acid treated oil (100 ml) and methanol was conducted in 500 ml three neck round bottom flask, which is equipped with water condenser, thermometer, and mechanical agitator at atmospheric pressure.

Appropriate amount of methanol and catalyst was primarily added to the flask and mixed vigorously. Then, 100 ml of acid treated oil was added into the mixture. When the water bath reaches at the desired temperature, mixture containing and equipped flask was placed on. The reaction continued for 3 hour with continuous and constant stirring.

After completion of reaction, the mixture is cooled to the room temperature; the solid catalyst was removed by centrifugation at 6000 rpm for 30 minute. The product then was transferred to separator funnel, washed with warm distilled water (50 ^{0}C) five times to remove residual impurities such as soap, residual methanol, and unreacted tri, di, and monoglyceride.

Fatty acid methyl ester was separated from glycerol and remaining catalyst by centrifugation at 6000 rpm for 50 minute.

3.4.1. Investigation of optimal reaction condition by employing central composite design

Best performing catalyst from previous section was selected and used in order to find the optimal reaction conditions for production of FAME. In this experiment, reaction temperature, catalyst to oil weight ratio, and methanol to oil molar ratio were selected as

independent input process variables as shown in Table 3.1. Time of reaction was maintained constant at 3 hours.

Table 3.1: Independent input variables levels for central composite design.

Independent variables	Symbol	Unit	Levels				
			1.68	-1	0	1	1.68
Temperature	T	^{0} C	53.91	58.00	64.00	70.00	74.09
Methanol to oil molar ratio	M	Mol%	5.80	7.50	10.00	12.50	14.20
Catalyst to oil weight ratio	C	Wt%	3.14	4.50	6.50	8.50	9.86

The relationship that exist between input variables and their influence on the output response variable was studied using Response Surface Method central composite design 3-factor-5-level, which requires a total of 20 experiment that is composed of 8 factorial point, 6 axial point, and 6 center point using Design Expert 7.0.0 software as shown in Table 3.2. In both Table 3.1 and 3.2, -1 and 1 represents low and high level of the factors, 0 represents center point, and -1.68 and 1.68 represents high and low level of axial point. The design involves 2^3 factorial point (2-level for 3 factor) that will provide a total of 8 run. The factorial points are efficient for estimation in the region of first order polynomial. However, if the region of interest involves curvature or second order polynomial, it needs addition of 2 x 3 axial points, which is 6 run in this experimental case. Central composite design repeats center point, in this case 6 times, to determine pure error. All the factorial, axial, and center point are shown on Figure 3.1. The distance from center to lower and higher point was unity, but for axial point, it was 1.68 for all 3 factors. The axial point value was selected to make the variance constant at the same distance from the design center that gives rotatability property to the design.

For temperature: $\text{Coded factor} = \frac{X-64}{6}$

For methanol to oil molar ratio: $\text{Coded factor} = \frac{X-10}{2.5}$

For catalyst to oil weight ratio: $\text{Coded factor} = \frac{X-6.5}{2}$

Based on the data entered, the software generates a model that represents the relation and interaction between dependent and independent variables. Thereafter, the model adequacy and graphical analysis was carried out.

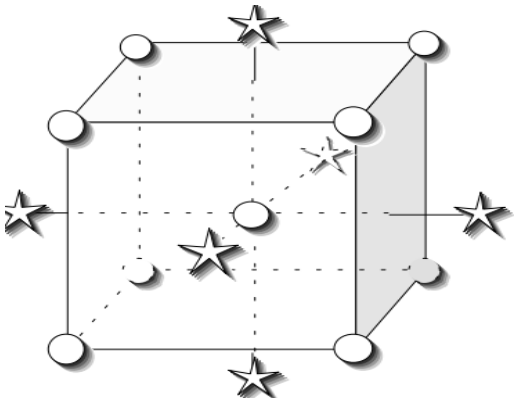


Fig.3.2 Central composite design for 3 factor.(Source: RSM tutorial)

3.5. Characterization of seed, oil, and biodiesel

3.5.1. Determination of moisture content

Neem seed powder (30 g) was taken into weight measured, clean, and dry crucible. The sample was placed in an oven at 105 ^{0}C; taken out every 2 hr, placed in the desiccators, and its weight measured till constant weight was obtained. Moisture content was calculated using the following formula:

$$\text{Moisture content} = \frac{Wi - Wf}{Wi - Wc} \text{x}100 \quad \text{.....3.2}$$

where: Wi - initial weight of sample with crucible

Wf - final weight of sample with crucible

Wc - weight of crucible

Table 3.2: Experimental design matrix

Run No	Coded factor			Actual factor		
	T	M	C	Temperature	Methanol to oil ratio	Catalyst to oil weight ratio
1	-1	-1	-1	58	7.5	4.5
2	-1	-1	1	58	7.5	8.5
3	-1	1	-1	58	12.5	4.5
4	1	-1	-1	70	7.5	4.5
5	-1	1	1	58	12.5	8.5
6	1	-1	1	70	7.5	8.5
7	1	1	-1	70	12.5	4.5
8	1	1	1	70	12.5	8.5
9	-1.682	0	0	53.91	10	6.5
10	1.682	0	0	74.09	10	6.5
11	0	-1.682	0	64	5.8	6.5
12	0	1.682	0	64	14.20	6.5
13	0	0	-1.682	64	10	3.14
14	0	0	1.682	64	10	9.86
15	0	0	0	64	10	6.5
16	0	0	0	64	10	6.5
17	0	0	0	64	10	6.5
18	0	0	0	64	10	6.5
19	0	0	0	64	10	6.5
20	0	0	0	64	10	6.5

3.5.2. Determination of saponification value

An esterified oil weighing 2 g was added to 250 ml conical flask and 0.5 M ethanolic KOH (25 ml), which is prepared by dissolving KOH pellet in 95% ethanol, was added to the oil. Similarly, blank solution (all chemicals except oil) was prepared in another conical flask. Reflux condenser was attached to both sample and refluxed for an hour while stirring. After completion of refluxing, 5 drops of phenolphthalein indicator was

added, while it was hot, titrated against 0.5 M HCl to the end point. S.N was calculated using the following formula:

$$S.V. = \frac{56.1\ xNx\ (Vo - V1)}{W} \quad \text{.....3.3}$$

where: N - concentration of Hydrochloric acid

V_0 -volume of HCl used for the blank

V_1 -volume of HCl used for the test

W- weight in gram of sample taken

3.5.3. Determination of acid value

A known amount of esterified oil (2 g) was added to 250 ml round bottom flask and heated to 70 ^{0}C for 3 minute. 25 ml of 1:1 ratio of absolute ethanol and diethylether solution was added to the oil under continuous stirring. Phenolphthalein indicator (5 drops) was added to the prepared solution and titrated against 0.1 M KOH to the end point, which is appearance of faint pink color. Similarly, blank solution was prepared and titrated against 0.1 M KOH to the end point. Acid value was calculated using the following formula:

$$A.V. = \frac{56.1\ xNx\ (V1 - V0)}{W} \quad \text{.....3.4}$$

where: N - concentration of KOH

V_0 - volume KOH used for the blank

V_1 - volume of KOH used for sample

W - weight of sample taken

3.5.4. Determination of kinematic viscosity

Viscosity was measured using Vibro viscometer. The sample and its holder brought to equilibrium temperature of 40 ^{0}C. The tip of the vibro viscometer was inserted in to the sample; viscosity was then seen from the controller. Kinematic viscosity was calculated using the following formula:

$$\mu = \frac{\upsilon}{\rho} \quad \text{.....3.5}$$

where: υ - dynamic viscosity, mm^2/s

ρ - density, Kg/m^3

μ - kinematic viscosity

3.5.5. Determination of iodine value

A known amount of the esterfiedoil (0.5 g) with 20 ml carbon tetrachloride was dissolved in 250 ml conical flask. 25 ml of 0.2 N Wijs reagents, which is prepared by dissolving 9 g iodine trichloride in a mixture of 700 ml glacial acetic acid and 300 ml of carbon tetrachloride, are added and mixed after it was plugged with stopper. Then, the mixture was placed in dark at room temperature for 1 hr and 20 ml of 100 g/l potassium iodine solution and 100 ml distilled water was added. 4 drops of 1% starch indicator was added and titrated against 0.1 M sodium thiosulfate solution till the disappearance of color.

$$Iodinevalue = \frac{12.69\ xNx(Vo - V1)}{W} \quad \text{.....3.6}$$

where: N - concentration of sodium thiosulfate

V_o-volume of sodium thiosufate used for blank

V_1 - volume of sodium thiosufate used for sample

W - mass of the sample

3.5.6. Determination of ash content

An oil weighing 20 g was added into weight measured crucible then, placed in the furnace at 500 ^{0}C for an hour. After all organic substance burned out, placed in the desiccators till it attain room temperature. The weight of residual inorganic substance was measured. Ash content was calculated using the following formula:

$$\%\ Ashmass = \frac{Wa}{Ws}\ x\ 100 \quad \text{.....3.7}$$

where: Wa - mass of ash

Ws - mass of sample

3.5.7. Determination of specific gravity

Specific gravity was measured using Hydrometer. The sample was poured into graduated cylinder and then hydrometer was dropped into measure specific gravity. This reading was used to calculate density

3.5.8. Determination of higher heating value

Higher heating value (energy content per unit quantity) of acid treated oil and fatty acid methyl ester was estimated from their Saponification value and iodine value using the following correlation:

$$HHV = 49.43 - [0.041(S.V.) + 0.015(I.V.)] \quad \text{.....3.8}$$

3.5.9. Determination of cetane number

Cetane number, which is the ignition delay after injection of the fuel, of neem oil and Fatty acid methyl ester was estimated based on saponification value and iodine value using the following correlation.

$$C.N. = 46.3 + \frac{5458}{S.V.} - 0.225(I.V.) \quad \text{.....3.9}$$

4. Result and discussion

Neem seed oil content was found to be 42%; out of it, 15% was FFA. This free fatty acid was converted to ester using H_2SO_4, prior to solid catalyzed transesterfication reaction.

The catalyst loading screening based on FAME yield was shown that 40% Na_2O/CaO loading has better performance. In this thesis, maximum of 92.80% FAME yield was obtained at optimum point of input process variables (temperature, catalyst and methanol).

4.1. Extraction of oil from Neem seed

The primary raw material used during the production of biodiesel (Fatty acid methyl ester) via transesterification reaction is oil (Triglycerides). In this study, crude oil was extracted from Neem seeds using hexane solvent in Soxhlet unit. After completion of extraction and removal of hexane, the total yield of crude oil obtained was found to be 42%.

4.2. Moisture and oil content of Neem seed

Table 4.1: Yield and characterization result of Neem seed

Parameter	Value (%)
Oil content	42
Moisture content	6.13
FFA(oil)	15

The crude extract oil primarily composed of oil (triglycerides) and portions of FFA. Previously, it has been sited that lower free fatty acid and moisture content promote the transesterfication reaction. Hence, the crude oil was further characterized for its FFA content; it was found to be 15%. This result showed that crude oil obtained from Neem seeds possess higher amount of free fatty acid. The crude Neem seed oil content without including FFA was found to be 35.7%. It is important to note that dehydrated and FFA reduced crude Neem seed oil can be regarded as the best feedstock for the production of biodiesel via acid treatment and transesterification reaction.

4.3. Esterification of FFA using sulfuric acid

Free fatty acid and moisture content are key parameters for determining the viability of the vegetable oil transesterification process. Feedstocks with high free fatty acid (> 1%) are not easily converted by base catalyzed transesterification, because of concurrent soap formation, which has a potential of reducing catalyst efficiency, increase viscosity, and glycerol separation difficulty. To avoid the above problem, the free fatty acids were preprocessed via sulfuric acid treatment into esters with methanol as shown in Scheme 4.1.

$$RCOOH + CH_3OH \xrightleftharpoons{H_2SO_4} RCOOCH_3 + H_2O$$

Scheme 4.1: Esterification of FFA using methanol in presence of sulfuric acid.

In this study, constant amount of H_2SO_4 (0.6 v/v%), temperature, and reaction time was employed. The optimal amount of methanol required to have high conversion of FFA into esters are investigated and showed in Table 4.2.

As the amount of methanol increases from 30 ml to 60 ml, the free fatty acid content decreased from 2.51% to 0.38%. Thereafter, with increasing methanol to 80 ml, FFA content increase to 1.67%. The observed higher FFA conversion as volume of methanol increases to 60 ml was due to optimum mixing between methanol and sulfuric acid but afterwards methanol start to inhibit the process and resulted lower FFA conversion. This is probably due to the dilution effect of methanol to that of sulfuric acid; excess methanol start to stabilize the acid (excess methanol act as a nucleophile) and also it reduce the availability of FFA for protonation (adding H^+ to FFA) by sulfuric acid.

Table 4.2: Acid value for different amount of methanol and 0.60% v/v sulfuric acid to oil ratio.

Volume of methanol (ml)	Acid value (mgKOH/g)	Free fatty acid (%)	FFA conversion (%)
30	5.020	2.51	83.26
40	3.801	1.90	87.33
50	2.523	0.84	94.40
60	0.7508	0.38	97.46
70	2.816	1.41	90.60
80	3.340	1.67	88.86

Table 4.2 shows that higher FFA conversion is obtained when 60 ml of methanol in presence of small amount of H_2SO_4 (0.6 v/v%) is used. Based on this optimum result the Neem crude oil acid pretreatment was carried out prior to heterogeneous catalyzed transesterification reaction.

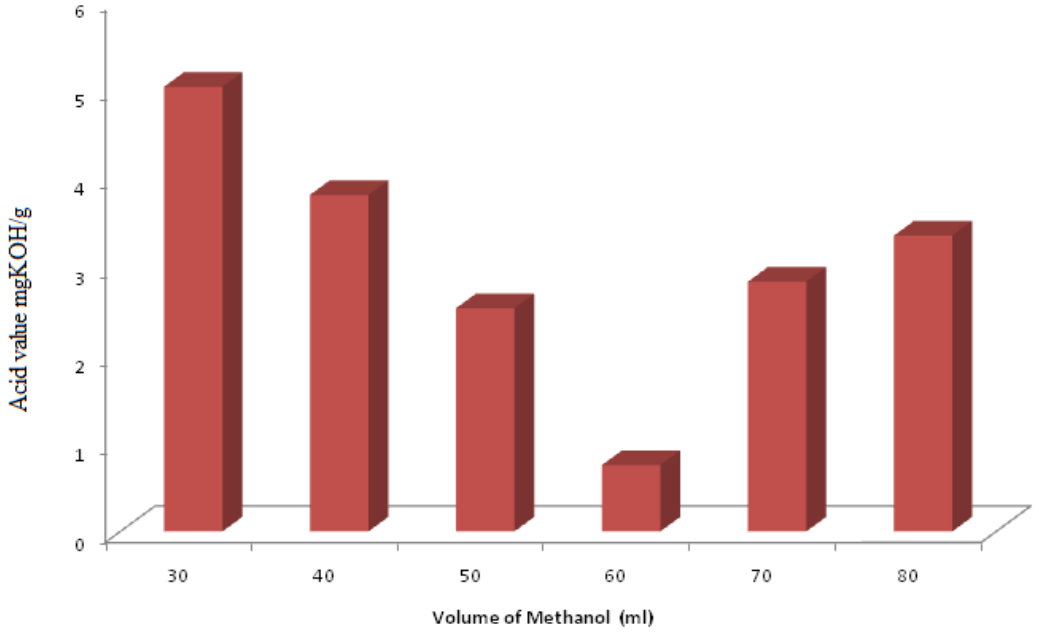


Fig. 4.1: Effect of methanol on acid value reduction at constant 0.60% v/v sulfuric acid to oil ratio.

4.4. Effect of Na_2O loading on the catalytic performance of Na_2O/CaO catalyst

Na_2O/CaO catalysts, with appropriate Na_2O/CaO loading were prepared by impregnation of appropriate amount of Na_2CO_3 on CaO followed by drying and calcination (730 ^{0}C). Effect of Na_2O/CaO loading on FAME yield with reaction condition of 10% methanol to oil molar ratio, 64 ^{0}C reaction temperature, and 6.5% catalyst to oil weight ratio was investigated.

During Na_2CO_3 impregnation, uniform and longer mixing can result high dispersion of Na. Drying and calcination forces the water to leaves the hydrated mixture with the disruption of crystalline structure. This is important in increasing the surface area and porosity of Na_2O/CaO catalysts. Higher temperature treatment (730 ^{0}C) decomposes Na_2CO_3 to Na_2O and from the surface of CaO, the adsorbed CO_2 and moisture that exist in the form of $CaCO_3$ and $Ca(OH)_2$ decomposes to CaO as it was shown in the following

reaction. Thus, increases the strength of the active site and surface area of the catalyst. In addition, it can also increase the availability of efficient active site for the reaction with triglyceride.

$$Na_2CO_3 \xrightarrow{Heat} Na_2O + CO_2$$

$$CaCO_3 \xrightarrow{Heat} CaO + CO_2$$

$$Ca(OH)_2 \xrightarrow{Heat} CaO + H_2O$$

Scheme 4.2: Surface phenomena occurring during calcination process.

The N_2O/CaO-1, Na_2O/CaO-2, and Na_2O/CaO-3 catalysts have produced 78.6 %, 84.73 %, and 63.2 % FAME yield, respectively, as shown in Figure 4.2. As Na_2O loading increases from 30% (N_2O/CaO-1) to 40% (Na_2O/CaO-2) the FAME yield was also increased from 78.6% to 84.73%. The 30% loading in Na_2O/CaO-1 catalyst was not sufficient for complete transesterfication reaction.

Furthermore, as the Na_2O loading increases to 50%, the FAME yield declines to 63.2%. The reason behind this decrement can be explained by looking into the intrinsic behavior of the Na_2O/CaO catalysts. It is known that surface area, porosity, and number and strength of the active site are important parameter for the efficiency of heterogeneous catalyst. Any activity with negative effect on those parameter leads to reduction in catalytic activity and hence the yield.

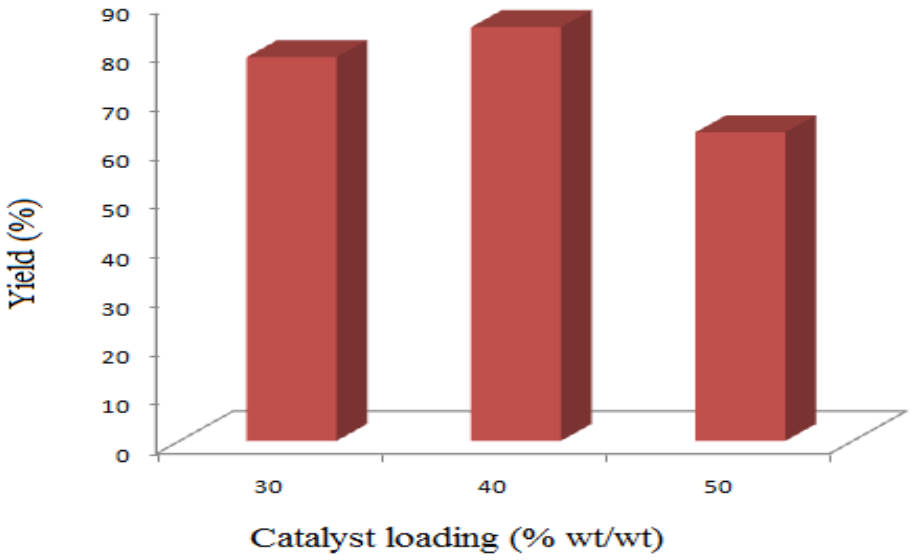


Fig.4.2: Effect of Na_2O/CaO loading on FAME yield with reaction condition: 10% methanol to oil molar ratio, 64 ^{0}C reaction temperature, and 6.5% catalyst to oil weight ratio.

Catalyst with pore diameter lower than the critical diameter of triglyceride, defined as the diameter of the smaller cylinder through which that molecule can pass without distortion, inhibits the diffusion through the pore. Thus, mainly external active catalytic surface takes part in the reaction, which leads to the reduction in catalytic activity. In this experiment as well, probably increasing of Na_2O beyond 40% might lead to reduction in pore diameter which results diffusion resistance through the pore. In addition, at higher loading, the active site of the catalyst has probability of agglomerating (covering of active site) which reduces the available active surface area for the reaction. Consequently, incomplete reaction might occur which is responsible for reduction in yield in the latter case.

4.5. Transesterfication reaction process variables optimization.

The effect of process variables (reaction temperature, methanol to oil molar ratio, and catalyst to oil weight ratio) was investigated by response surface methodology. Both main and interaction effects of the variables towards FAME yield was thoroughly studied as shown on Table 4.3. In addition, optimization of FAME yield was carried out by varying the input variables to predict the model and it was analyzed with surface and contour plot. The adequacy of fitted equation was also checked.

4.5.1. Effect of process variables on transesterfication reaction

Temperature, methanol to oil molar ratio, and catalyst to oil weight ratio was considered as input process variables. Analysis of variance on Table 4.4 has shown that main and interaction effect are significant in this model and variation of those process variables affects the product greatly.

4.5.2. Main effect of process variables on FAME yield

- ✓ **Effect of temperature on FAME yield**

Heterogeneously catalyzed biodiesel production is strongly influenced by temperature. Increasing temperature leads to decrease in viscosity of oil (i.e. increases solubility of methanol in oil), increase in mass transfer between solid catalyst and liquid reaction mixture, and increases rate of penetration of the oil into the pore of the catalyst. Thus, temperature is important parameter for higher reaction rate and higher yield.

Table 4.3: Experimental data

Run	Temperature (^{0}C)	Methanol to oil molar ratio	Catalyst to oil weight ratio	Actual FAME yield (%)	Predicted FAME yield (%)	Residual
1	58	7.5	4.5	73.3	73.36	-0.061
2	58	7.5	8.5	84.16	83.43	0.730
3	58	12.5	4.5	77.1	76.26	0.840
4	70	7.5	4.5	73.97	74.12	-0.150
5	58	12.5	8.5	93.55	92.62	0.930
6	70	7.5	8.5	65.01	65.07	-0.059
7	70	12.5	4.5	81.78	81.73	0.050
8	70	12.5	8.5	79.81	78.97	0.840
9	53.91	10	6.5	79.74	80.81	-1.070
10	74.09	10	6.5	69.94	69.97	-0.030
11	64	5.8	6.5	68.51	68.41	0.100
12	64	14.20	6.5	81.33	82.53	-1.200
13	64	10	3.14	81.79	81.82	-0.028
14	64	10	9.86	86.89	87.96	-1.070
15	64	10	6.5	86	85.00	1.000
16	64	10	6.5	85.23	85.00	0.230
17	64	10	6.5	85.33	85.00	0.330
18	64	10	6.5	84	85.00	-1.000
19	64	10	6.5	84.24	85.00	-0.760
20	64	10	6.5	85.39	85.00	0.390

The effect of temperature at constant methanol to oil molar ratio (10%) and catalyst to oil weight ratio (6.5%) was studied. Figure 4.3 shows that as the temperature increases from 58 ^{0}C to 61 ^{0}C, very small amount of increase in FAME yield is observed. However, the yield has started to decline after 61 ^{0}C. This reduction was significant after 64 ^{0}C. The reason behind is as the temperature approaches the boiling point of methanol, methanol start to appear in the vapor phase at the expense of available methanol for the reaction. In addition, as the temperature gets beyond the boiling point, significant methanol bubble formation was appeared, which inhibits the mass transfer process. Those are summed up to reduce the yield near and above boiling point of methanol.

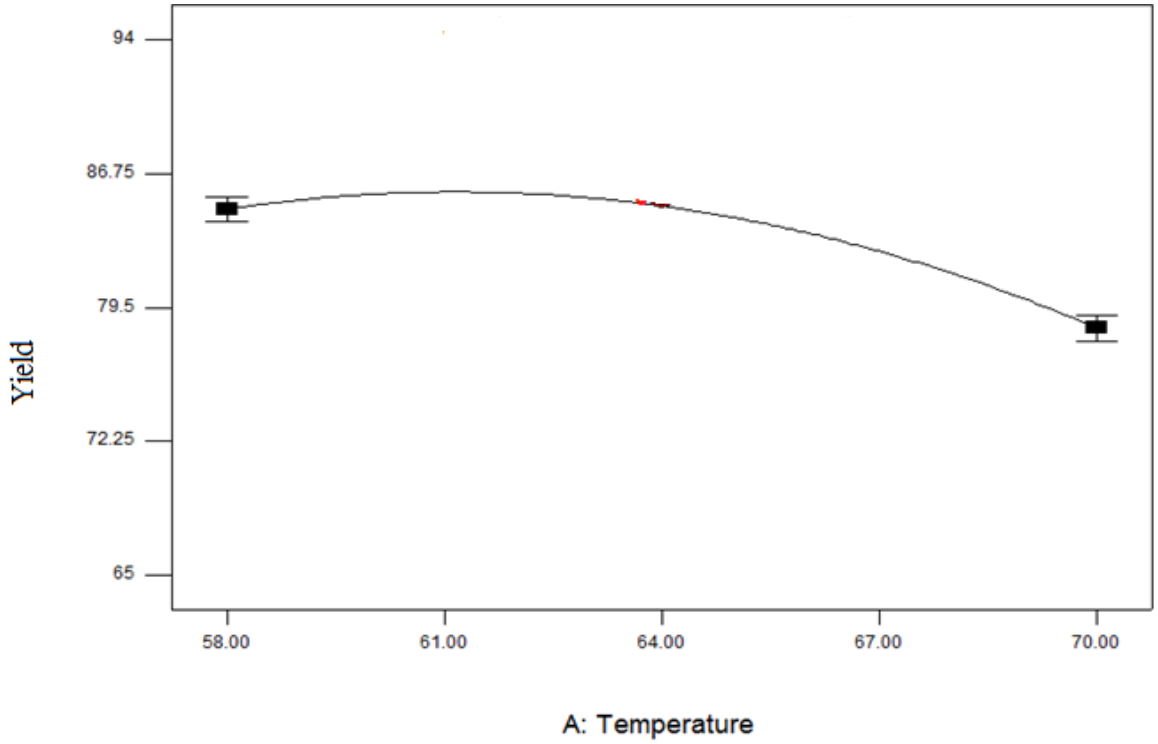


Fig.4.3: The effect of temperature on yield at methanol to oil molar ratio 10% and catalyst to oil weight ratio 6.5%.

✓ **Effect of methanol to oil molar ratio on FAME yield**

Theoretically one mole of triglyceride requires three mole of methanol, but in practice higher amount is required to shift the reaction equilibrium forward and maximize the yield. Effect of methanol on the FAME yield was studied at constant temperature and catalyst to oil weight ratio, 64 ^{0}C and 6.5%, respectively, as shown in Figure 4.4. It indicates that increasing of methanol to oil molar ratio from 7.5 to 10, increases FAME

yield from 77.43% to 85.33%. However, further increase doesn't have significant impact on the yield. Methanol to oil molar ratio of 12.5 results 85.83% FAME yield which is almost similar to methanol to oil molar ratio of 10. This is because, as the amount of methanol increases, it will shift the reaction equilibrium forward (i.e. since transesterfication reaction is reversible) and at the same time it will result increase in rate of reaction as well. However, further increase in methanol leads to dilution of the catalyst.

In addition, most probably at higher amount of methanol, the reverse reaction which is recombination of glycerol and ester may had started to takes place, this in turn can leads to formation of di and monoglyceride, which ends up with incomplete conversion.

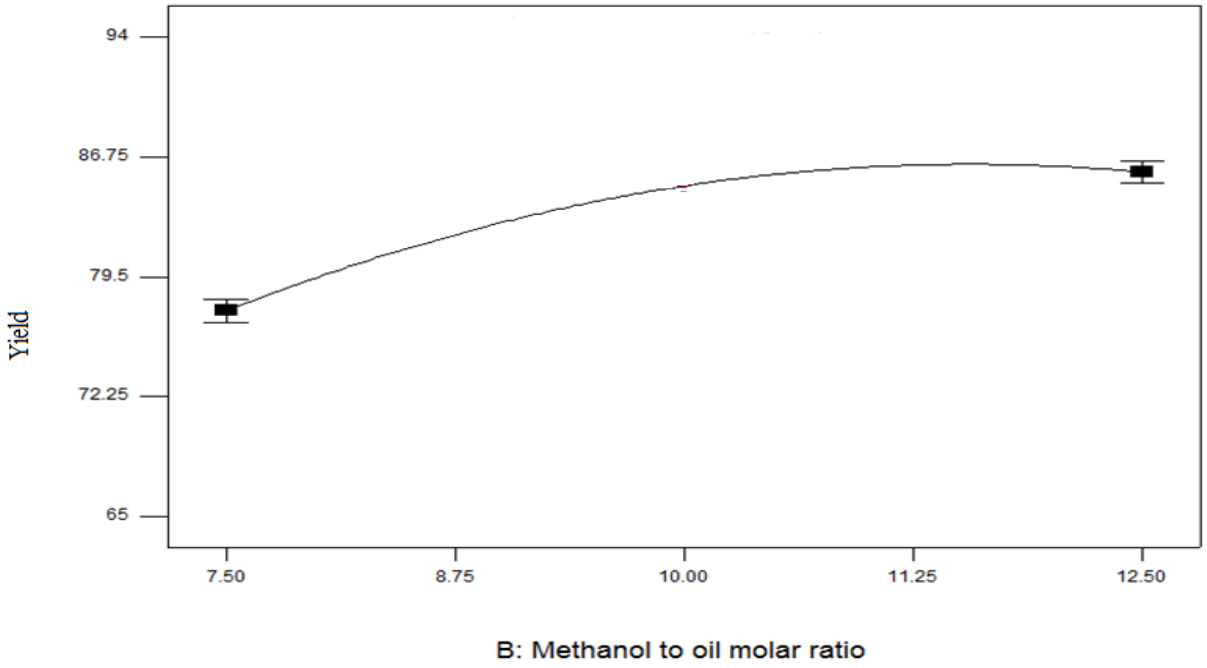


Fig.4.4:.Effect of methanol on the yield at 64 ^{0}C and catalyst to oil weight ratio 6.5%

- ✓ **Effect of catalyst to oil weight ratio on FAME yield**

The amount of catalyst is important process variables for production of biodiesel. In this experiment, catalyst to oil weight ratio was varied from 4.5% to 8.5% at constant temperature and methanol to oil weight ratio. Figure 4.5 shows that increasing of the catalyst amount has significant positive effect on FAME yield. It was observed that when the catalyst dose is low, incomplete transesterfication was occurred. However, as the amount increases, more triglyceride is changed to ester. The reason behind is

increasing of catalyst provides more active sites for the reaction of available triglyceride and methanol. In addition, the amount of available catalytic active surface is one important parameter to shift the reaction equilibrium forward in addition to the amount of methanol in the reaction mixture.

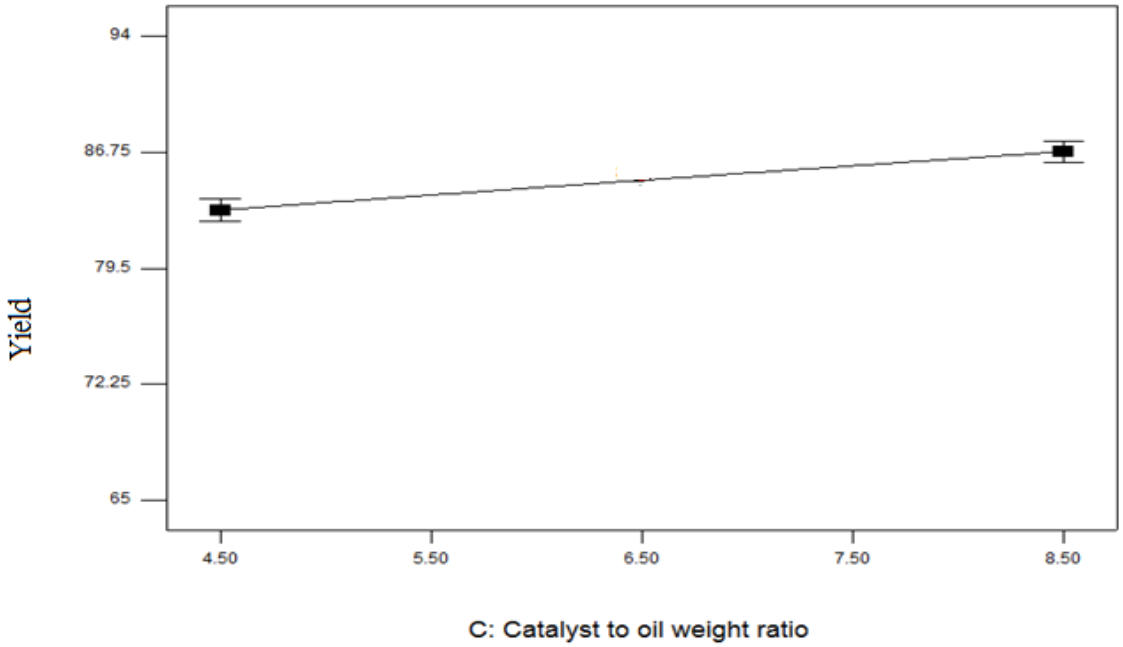


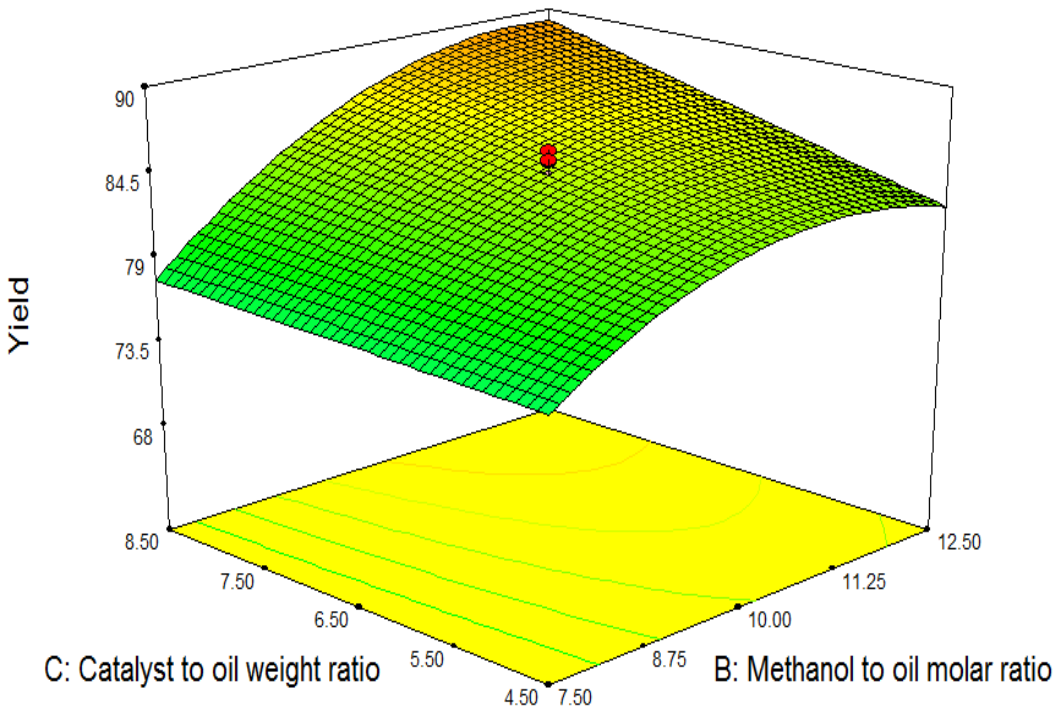
Fig.4.5:Effect of catalyst on the yield at 64^{0}C and methanol to oil molar ratio 10

4.5.3. Interaction effect of process variables on FAME yield

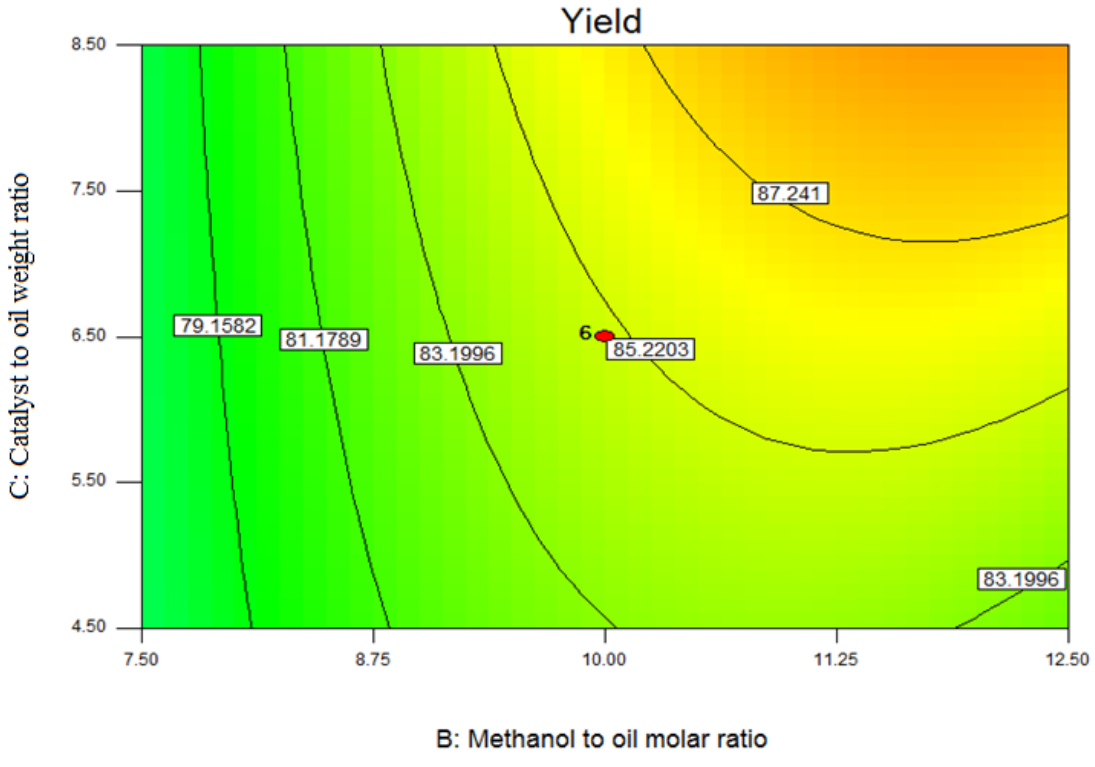
The interaction effect of reaction temperature, methanol to oil molar ratio, and catalyst to oil weight ratio on the FAME yield was studied. Based on the generated data, response surface and their contour are plotted.

- ✓ **Interaction effect of methanol to oil molar ratio and catalyst to oil weight ratio on FAME yield**

Interaction effect of methanol and catalyst was studied by holding temperature constant (64 ^{0}C). From Figure 4.6, it is observed that increasing of catalyst and methanol together, has increased the yield; and keeping one at higher and increasing the other has also generated similar effect on the yield. However, holding one at lower and increasing the other, provided lower improvement on FAME yield.



a)



b)

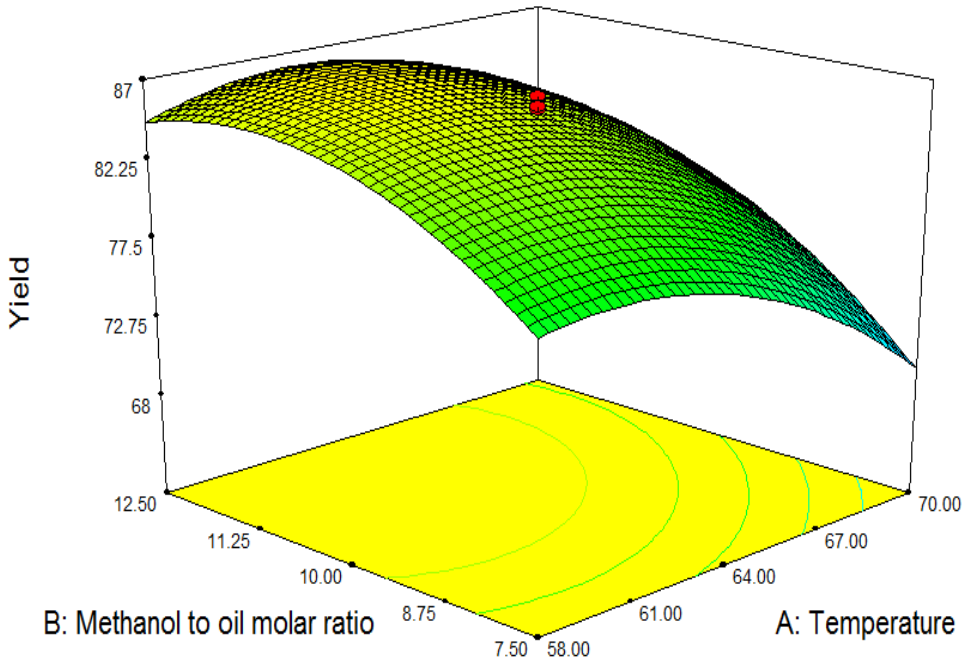
Fig.4.6: Interaction effect of methanol to oil molar ratio and catalyst to oil weight ratio at 64 ^{0}C: a) 3D response surface plot. b) Contour plot.

Main effect study shows that catalyst and methanol has positive effect on the yield. This observation is clearly shown on interaction effect plot of Figure 4.6, which is proportional addition of catalyst and methanol resulted higher yield. However, the FAME yield was reduced when the addition was disproportional (one excess than the other). Because, excess methanol leads to dilution of the catalyst and excess catalyst leads to formation of soap.

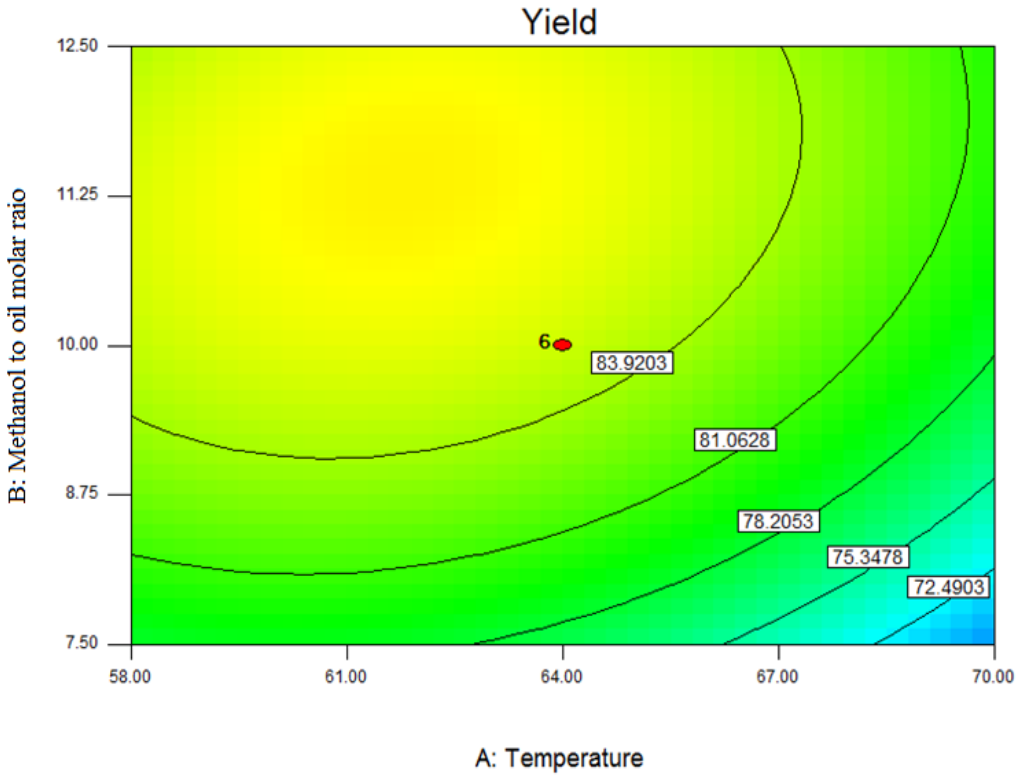
- ✓ **Interaction effect of temperature and methanol to oil molar ratio on FAME yield**

Interaction effect of temperature and methanol was studied by holding catalyst to oil weight ratio constant at 6.5%. As it was shown on Figure 4.7, increasing of methanol at any temperature level provide relatively better yield except above methanol to oil molar ratio of 11.25%, which starts to lie down slightly. In the other way round, increasing temperature up to 61 ^{0}C, at any level of methanol has resulted insignificant increment in FAME yield. Thereafter, temperature has shown negative effect on the yield. This negative effect of temperature was exaggerated at lower methanol amount.

Temperature has two opposing effect on the reaction. As the temperature increase, it will increase rate of reaction and reduce mass transfer resistance, which affects the yield positively which was shown on Figure 4.7 up to 61 ^{0}C. But, at higher temperature (near and beyond boiling point of methanol), it reduces the availability of methanol in the reaction mixture and mass transfer process, which has negative effect on the yield. This negative effect has been shown on Figure 4.7 to be solved by adding excess methanol that can compensate the lost in the vapor form. However, when the methanol gets excess, it turned the yield down, because the methanol to catalyst ratio is getting higher, this effect was explained in effect of methanol to oil molar ratio section.



a)

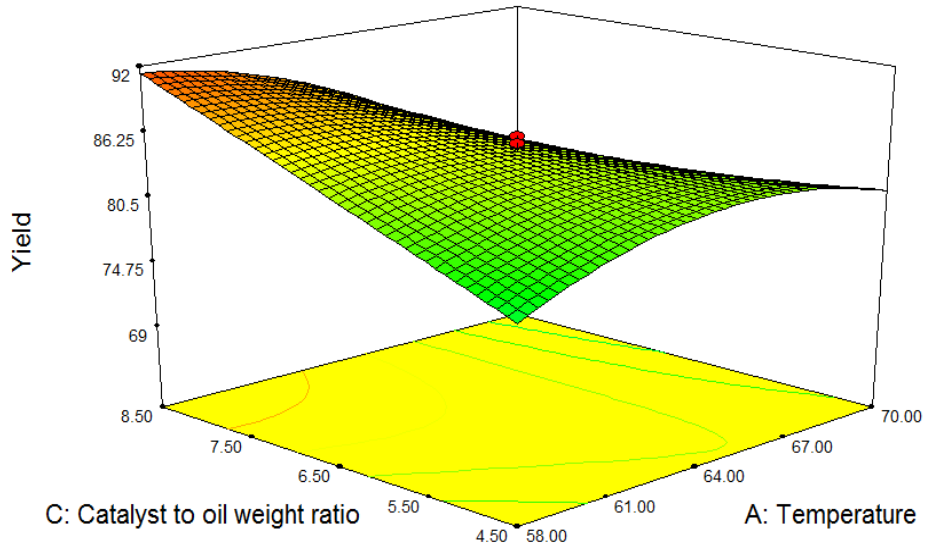


b)

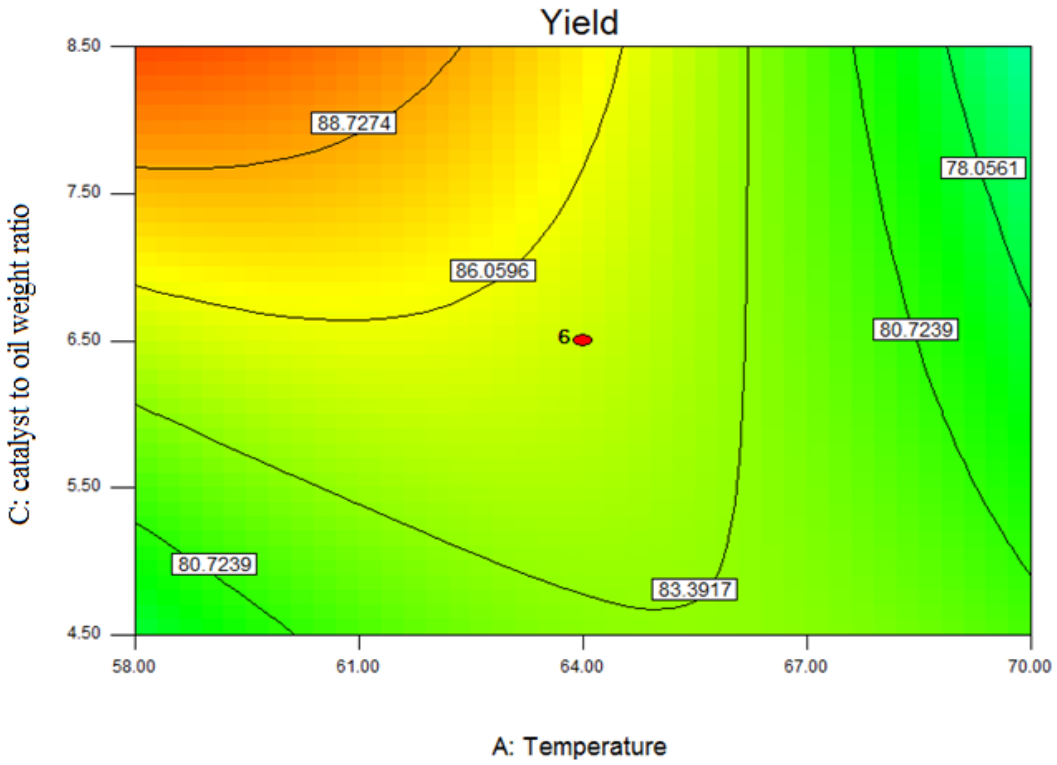
Fig.4.7: Interaction effect of temperature and methanol to oil molar ratio at catalyst to oil weight ratio of 6.5: a) 3D response surface plot. b) Contour plot

✓ **Interaction effect of temperature and catalyst to oil weight ratio on FAME yield**

Interaction effect of temperature and catalyst was studied by holding methanol to oil molar constant at 10%. Figure 4.8 has shown that increasing of temperature at higher catalyst loading (beyond 6.5%) has negative effect on the yield, however this effect was negligible at lower catalyst to oil weight ratio. This is because maximum yield was observed at higher catalyst, methanol amount; any action disrupting this combination has negative effect on the FAME yield. Increasing of temperature and catalyst at the same time leads to lower amount of methanol in the reaction mixture, which was responsible for the observed lower yield as it was shown on Figure 4.8. However, lowering the catalyst has improved the yield, because the methanol amount was increased and starts to keep its proportion. Bringing down the reaction temperature to 58 ^{0}C and increasing catalyst to higher level (8.5%) has shown to increase the yield to 91.3% because all the methanol was available with reaction mixture (i.e. there is no methanol in vapor form).



a)



b)

Fig.4.8: Interaction effect of temperature and catalyst to oil weight ratio at methanol to oil molar ratio of 10%: a) 3D response surface plot. b) Contour plot.

4.5.4. Optimization of response parameters

It was noted from main and interaction effect that temperature, methanol amount, and catalyst amount have strong effect on the yield. In order to maximize FAME yield, optimization of those input process variables were necessary. Optimization was performed by using Design-Expert 7.0.0 software. The model has predicted 92.962% yield at the following operating condition: 58 ^{0}C temperature, 11.71% methanol to oil molar ratio, and 8.50% catalyst to oil weight ratio. Experiment was conducted at optimum parameter to verify the predicted value; 92.80% yield was obtianed, which is in a good agrement with predicted value.

4.6. Statistical analysis of FAME yield affecting factors

Statistical relationship that exists between the input variables (methanol to oil molar ratio, catalyst to oil weight ratio, and reaction temperature) and output variable (FAME yield) led to empirical model construction by central composite design method. The experimental data was analyzed to fit to the quadratic polynomial equation and then regression analysis, analysis of variance, and model adequacy checking was carried out.

The Fatty acid methyl ester yield was calculated with the following formula:

The predicted model for Fatty acid methyl ester is given below in terms of actual factors:

$$Yield = -439.22928 + 13.34622T + 5.39265M + 23.38732C + 0.078500TM - 0.39833TC + 0.31450MC - 0.094369T^2 - 0.53904M^2 - 9.63555E\text{-}003C^2 \quad .....4.1$$

where: T - temperature

M - methanol to oil molar ratio

C - catalyst to oil weight ratio

4.6.1. Model adequacy checking

The effectiveness of the model in approximating to the true value was measured using regression coefficient (R^2). Always its value is between [0 1], however, a value close to 1 indicates well fitted model, otherwise indicates failure of approximation. In this case, R^2 0.9901 was obtained, which is close to 1 and the value of Adj-R^2 was 0.9811 that is in agood agreement with R^2.

Figure 4.9 indicates the plot of actual verses predicted value of biodiesel yield upon unit slope line. This graph indicates that all the points are very close to the line; thereby it validates the reliability of the model.

The validity of the model was further analyzed with the analysis of variance as shown on Table 4.4. Central composite design has generated the Model F-value of 110.81 and "Prob > F" less than 0.0500 which indicates that the model term are significant. In this case, main effect of T (temprature), M (methanol to oil molar ratio), C (catalyst to oil weight ratio), interaction effect of TM (temprature with methanol to oil molar ratio), TC (temprature with catalyst to oil weight ratio), MC (methanol to oil molar ratio with catalyst to oil weight ratio), and the quadratic effect of T^2 (temprature), M^2 (methanol to oil molar ratio) are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

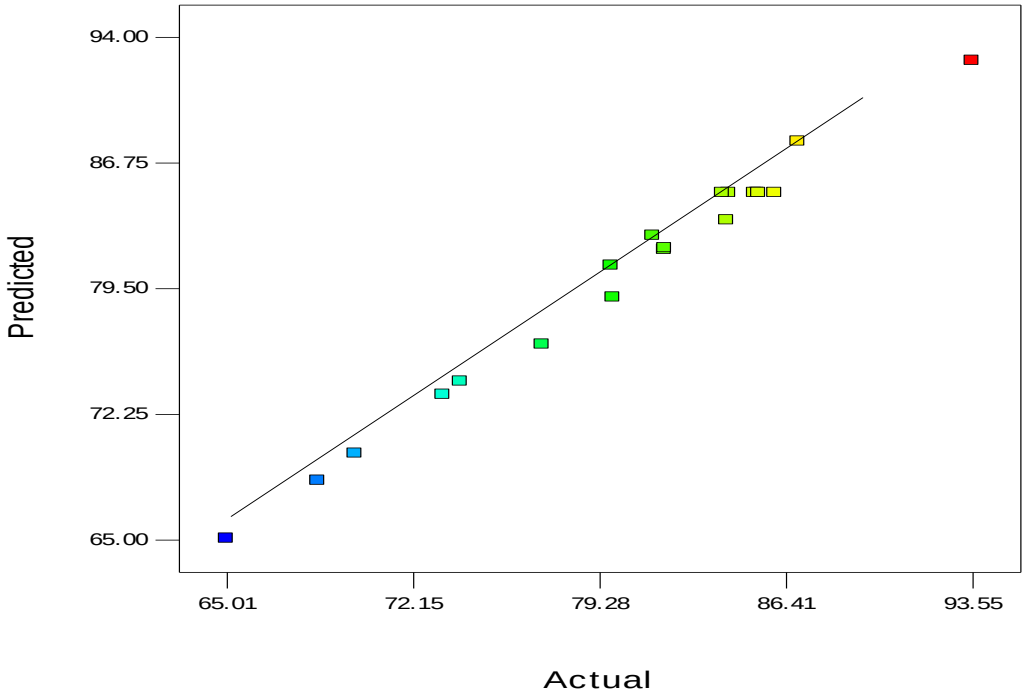


Fig.4.9: Predicted verses actual fatty acid methyl ester yield

Furthermore, lack of fit is important parameter in describing the varaition of data around the model. Lack of fit result was shown on Table 4.4. The analysis had provided lack of fit F-value of 2.29, which implies that it is not significant relative to the pure error. As a result, it was possible to conclude that the data are adquetly fitted to the model.

Table 4.4:ANOVA for Response Surface Quadratic Model

Source	Sum of square	df	Mean square	F value	P value prob > F
Model	947.01	9	105.22	110.81	< 0.0001
T: Temprature	*141.90*	1	*141.90*	*149.44*	< 0.0001
M: methanol/oil molar ratio	*240.92*	1	*240.92*	*253.72*	< 0.0001
C: catalyst/oil weight ratio	*45.61*	1	*45.61*	*48.03*	< 0.0001
TM	*11.09*	1	*11.09*	*11.68*	*0.0066*
TC	*182.79*	1	*182.79*	*192.50*	*< 0.0001*
MC	*19.78*	1	*19.78*	*20.83*	*0.0010*
T^2	*166.33*	1	*166.33*	*175.17*	*< 0.0001*
M^2	*163.57*	1	*163.57*	*172.26*	*< 0.0001*
C^2	*0.021*	1	*0.021*	*0.023*	*0.8836*
Residual	9.50	1	0.95		
Lack of fit	*6.61*	*5*	*1.32*	*2.29*	*0.1921*

4.7. Physico-chemical characteristics of acid treated oil and FAME

Physical, chemical, and fuel property of acid treated oil and FAME are presented along with ASTM and EN Standard in Table 4.5.Generally, it was shown that FAME properties are within the described standards.

Density is one important biodiesel fuel property. In this experiment, transesterfication reaction has reduced the density of acid treated oil by 2.2%, but it is still higher than diesel oil. Most probably, the observed higher density than diesel was due to the presence of higher molecular weight of triglyceride molecule.

Viscosity affects the atomization of a fuel upon injection into the combustion chamber and thereby ultimately results the formation of engine deposit. Transesterification reaction of this experiment was able to substantially reduce viscosity by 82.9%.

Iodine value indicates degree of unsaturation of the biodiesel component. In this experiment transesterfication reaction has reduced the iodine value by 15.21%, but still it was higher than diesel by 95.46%, which indicates that FAME from Neem oil was more susceptible to formation of polymerized methylester gum inside the engine, as it was compared with diesel.

Acid value was significantly reduced on acid pretreatment stage, since it affects FAME yield. ASTM and EN has limited the amount of acid value to maximum of 0.8 and 0.5 mg KOH/g in the biodiesel, respectively. Because, it will cause fuel aging due to hydrolytic cleavage of ester bond, increase fueling system deposit, and increases the likelihood of corrosion. In this experiment, the biodiesel acid value was 0.31 mgKOH/g, which is lower than specified standard. Transestrfication reaction of acid treated neem oil has reduced the saponification number by 33.12%.

Fuel with less ash content are preferred for better engine operation and maintenance. Acid treated oil was found to have no ash, but after tranesterfication reaction 0.01% ash was obtained. ASTM D 6751 - 02 has specified the maximum of 0.02% ash for biodiesel, hence, the result was lower than the standard.

Cloud point is the temperature at which the biodiesel starts to form small observable solid crystal. ASTM D 6751 - 02 has prescribed the range to be between -3 ^{0}C and 12 ^{0}C. FAME cloud point was found to be +3 ^{0}C, which is inside the limit.

Flash point was found to be 180 ^{0}C, which is much higher than petro diesel. Based on the ASTM and EN standard, there minimum value is 120 ^{0}C and 130 ^{0}C, respectively. Thus, the obtained flash point result makes FAME of Neem oil safe for storage and transportation.

Cetane number is known as a measurement of the combustion quality of diesel fuel. Better ignition quality is associated with higher cetane number. Solid catalyzed transesterfication reaction of this experiment was able to increase CN from 44.98 to 61.08, which was higher than the minimum standard of both ASTM and EN.

Heating value of biodiesel is dependent on the composition of the fuel. High heat content results from higher hydrogen content, higher carbon content, and lower carbon to hydrogen ratio (unsaturation reduces energy content). However, increasing of oxygen results reduction in energy content. The experimental value of higher heating value of

acid treated oil and fatty acid methyl ester was 38.59 MJ/Kg and 41.87 MJ/Kg, respectively. It was observed that its energy content was increased by 7.83%. This is probably due to the presence of relatively higher number of hydrogen in fatty acid methyl ester than acid treated oil.

Table 4.5: Physico-chemical characteristics of acid treated oil and fatty acid methyl ester along with American and European Standards.

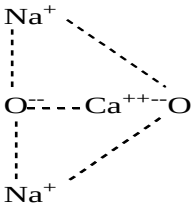
Property	Acid treated oil	FAME	ASTM standard, D6751	EN standard, 14214
Specific gravity	0.910	0.89	-	-
Density, Kg/m^3	910	890	870 - 900	860 - 900
Kinematic Viscosity (mm^2/s), 40 ^{0}C	28.6	4.89	1.9 - 6.0	3.5 - 5.0
Acid value (mg KOH/g)	0.7508	0.31	≤ 0.8	≤ 0.5
Free Fatty Acid	0.375	0.155	-	-
Saponification value (mgKOH/g)	222.2962	148.66	-	-
Iodine Value (g/ 100g)	115	97.50	-	≤ 120
Higher heating value (MJ/Kg)	38.59	41.87	-	-
Flash point (^{0}C)	-	180	≥ 120	≥ 130
Ash content (%)	0.00	0.01	-	-
Cloud point (^{0}C)	-	+3	-	-
Cetane number	44.98	61.08	≥ 47	≥ 51

4.8. Discussion on the transesterfication reaction

In this Na_2O/CaO catalyzed transesterfication reaction, the reaction was carried out on the surface of the Na_2O/CaO. Initially, one end of Na^+ adsorbs CH_3O^-, at the same time O^- abstract H^+ from CH_3OH, similarly the other end of Na^+ and O^- adsorbs tryglyceride as it was shown in Scheme 4.3. Then, the adsorbed species on the two end of the catalyst react to produce fatty acid methyl ester and diglyceride. Similarly, diglyeceride and then monoglyceride react to produce three mole of FAME and one mole of glycerol. Most probably, in this reaction active site surface provider is Na_2O, because the reaction carried out at 58 ^{0}C, 12.5% methanol to oil molar ratio, and 8.5% catalyst to oil weight

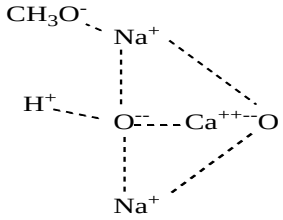
ratio without Na_2O has generated very small amount of yield, which couldn't be measured due to the presence of large amount of solidified product.

The solid catalyst has preserved its stability to stay together under normal condition. Most probably, this is due to the presence of ionic interaction between the two end of Na^+ and O^{--}.



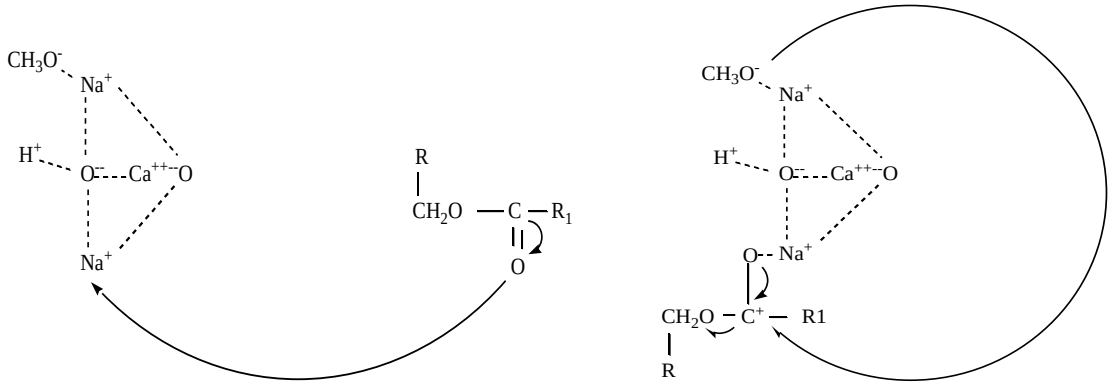
a)

The above catalyst in the presence of methanol (CH_3OH) changes to the following structure.



b)

And the other end of the catalyst start to interact with the triglyceride molecule



c) d)

Scheme 4.3: a) Structure of the catalyst; b) Structure of the catalyst in the presence of methanol; c) Interaction of methanol-catalyst with triglyceride; d) Intra-interaction of methanol-catalyst-triglyceride.

Finally, RCH_2O^- in the presence of H^+ goes to RCH_2OH and this will lead to the final product R_1COOCH_3 and RCH_2OH

5. Conclusion and recommendation

5.1. Conclusion

Ethiopia is completely dependent on imported fossil fuel to fulfill increasing domestic energy demand. Huge amount of the countries earning is being invested on the imported fuel. As a result, the government has started to look for clean and renewable energy source from the inside. Thus, jatropha, castor, and palm is seen as alternative source of biodiesel.

Neem seed was found to contain 42% oil; out of it 15% was FFA. This high amount of FFA has negative effect on the process efficiency and yield as well. Esterfication reaction was conducted using sulfuric acid catalyst to reduce FFA content to 0.38%. This esterfication process was shown to preserve loss as compared to neutralization.

Na_2O/CaO catalyst was prepared from Na_2CO_3 and CaO by wet impregnation method. Based on FAME yield, catalyst with 40% loading (Na_2O/CaO-2) was selected as high potential of catalyzing low free fatty acid feedstock.

Reaction temperature, methanol to oil molar ratio, and solid catalyst to oil weight ratio was seen to have strong main and interaction effect on the fatty acid methyl ester yield. At optimum operating condition: 58^0C temperature, 11.71% methanol to oil molar ratio, and 8.50% catalyst to oil weight ratio, 92.8% FAME was produced.

Physicochemical analysis and fuel property comparison with American and European Standard has proved that sulfuric acid treated and then (Na_2O/CaO-2) catalyzed FAME production from Neem oil is within the standard limit.

In this thesis, Neem seed was shown to be a potential and promising feedstock for biodiesel production based on its oil content and biodiesel yield.

5.2. Recommendation

- ✓ In this experiment transesterfication reaction was carried out with acid pretreated FFA (low FFA) feedstock. However, the catalyzing efficiency of high FFA containing feedstock need to be assessed in order to diversify the utilization of the catalyst (Na_2O/CaO) to other non edible oil.
- ✓ One of the important advantages of heterogeneous catalyst is it's reusability. Hence, further investigation need to be carried out on the catalyst (Na_2O/CaO) reusability and/or regeneration capacity.
- ✓ Full characterization of the catalyst need to be carried out to identify the major players in the transesterfication reaction.
- ✓ The catalyst (Na_2O/CaO) performance with ethanol need to investigated because ethanol is being produced in Ethiopia (it will reduce cost of production).
- ✓ Performance and emission characteristics of FAME and there blend need to be further investigated.

Reference

Aderibigbe A.O.; Johnson C.O.L.O.; Makkar H.P.S.; Becker K.; Foidl N., 1997. Chemical composition and effect of heat on organic matter and nitrogen degradability and some antinutrional components of jatropha oil. *Animal Feed Science Technology*; 67:223–243.

Axelsson, L. and Franzen, M., 2010. Performance of Jatropha biodiesel production and its environmental and socio-economic impacts, A case study in Southern India. Thesis in the Master Degree Programme Industrial Ecology, Chalmers University of Technology.

Badday, A. S.; Abdullah, A. Z.; Lee, K. T.; Khayoon, M. S., 2012. Intensification of biodiesel production via ultrasonic-assisted process: A critical review on fundamentals and recent development. *Renewable and Sustainable Energy Reviews;* 16:4574–4587.

Balaubramanian, R.K., 2010. Heterogeneous catalysis of plant derived oils to biodiesel, National university of Singapore , Division of environmental science and engineering, Dessertation.

Baroi, C.; Yanful, E.K.; Bergougnou, M.A., *2009.* Biodiesel Production from Jatropha curca Oil Using Potassium Carbonate as an Unsupported Catalyst. *International Journal of chemical and reactor Engineering;* 7: A72.

Chopade, S.G.; Kulkarni, K. S.; Kulkarni, A. D.; Topare, N.S., *2012*. *Acta chemica and pharmaceotica indica*; 2(1):8-14.

Çanakçı, M.Ş., 2008. Biodiesel production from various feedstocks and their effects on the fuel properties. *J Ind Microbiol Biotechnol;* 35:431– 441.

Cancela, A.; Maceiras, R.; Urrejola, S.; Sanchez, A., 2012. Microwave-Assisted Transesterification of Macroalgae. *Energies;* 5:862-871

Chakrabarti, M.H. and Ahmed, R., 2008. Transesterification studies on castor oil as a first step towards its use in biodiesel production. *Pakistan journal of botany;* 40(3):1153-1157.

Chisti,Y., 2007. Biodiesel from microalgae. *Biotechnol Advance*; 25:294-306.

Demirbas, A., 2005. Biodiesel production from vegetable oil by supercritical methanol. *Journal of scientific and industrial Research;* 64:858 - 865.

Demirbas, A., 2006. Biodiesel production via non-catalytic SCF method and biodiesel fuel characteristics. *Energy Conversion and Management;* 47:2271–2282.

Demirbas, A., 2009. Progress and recent trends in biodiesel fuels. *Energy Conversion and Management;*50:14–34.

Demirbas, A. and Demirbas, M.F., 2011. Importance of algae oil as a source of biodiesel. *Energy Conver. and Manag;*52:163-170.

Dwivedi, G. and Sharma, M.P., 2013. Cold flow behavior of biodiesel-A review. *International Journal of Renewable Energy*; 3(4):15-20.

Ebtisam, K.; Heikal, S.; Khalil, A.; Ismaeil K.A., 2013. Jatropha biodiesel Production technologies. *International Journal of Bioscience, Biochemistry and Bioinformatics*; 3(3):288 - 292.

Edith, O.; Janius, R. B.; Robiah Y., 2012. Factors affecting the cold flow behavior of biodiesel and methods for improvement – A Review, *Pertanika J. Sci. & Technol*; 20(1):1–14.

Efeovbokhan; Vincent E.; Ayoola A.; Anawe P.; Apeye L; Oteri O., 2012. The effects of trans-esterification of castor seed oil using ethanol, methanol and their Blends on the Properties and yields of Biodiesel. *International Journal of Engineering and Technology*; 2(10):1734-1742.

Ezeanayanso, C.S.; Okonkwo, E. M.; Agbaji, E. B.; Ajibola, V.I.; Okunola, O. J., 2011. Evaluation of homogeneous catalysis and supercritical methodology for biodiesel production from Jatropha curcas, Azadirachta indica and Hevea brasiliensis oil seeds. *Journal of Petroleum Technology and Alternative Fuels;*2(10):190-199.

Fernandez, M.B.; Tonetto, G.M.; Crapiste, G.; Damiani, D.E., 2007; Int. J. Chem. React. Eng. 5:A10.

Freedman, B.; Pryde, E.H.; Mounts, T.L., 1984. *J. Am. Oil Chem. Soc.;* 61:1638.

Fitamo, T. M., 2011. Investigation of biodiesel production using heterogeneous catalyst, AAIT, chemical engineering department, thesis.

Gana, A. K.; Yusuf, A. F; Apuyor, B., 2013. castor oil plant and its potentials in transformation and industrialization of under developing nations in the world. *Advanced Journal of Agricultural Research;*1(005):072-079.

Gerhard K., 2005. Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. *Fuel Processing Technology*; 86:1059–1070.

Gerhard, K., 2005c. Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. *Fuel Process Technol*; 86:1059–70.

Gerhard K.; Gerpen, V.J.; Krahl, J., 2005. The Biodiesel Handbook. Champaign, IL: AOCS Press.

Gerhard, K. and Kevin, S.R., 2005. Kinematic viscosity of biodiesel fuel components and related compounds. Influence of compound structure and comparison to petro diesel fuel components. *Fuel;* 84:1059–1065.

Gerpen, J.V.; Shanks, B.; Pruszko, R.; Clements, D.; Knothe, G., 2004. Biodiesel Analytical Methods. *National Renewable Energy Laboratory*; NREL/SR-510-36240.

Gerpen, V.J., 2005. Biodiesel processing and production. *Fuel Processing Technology;* 86:1097-1107.

Getinet, A.; Rakow, G.; Raney, J.P.; Downey, R. K., 1996. The inheritance of erucic acid content in Ethiopian mustard, *Canadian journal of plant science*, Contribution No.1192:33-41.

Ghaly, A.E.; Dave, D.; Brooks M.S.; Budge, S., 2010. Production of Biodiesel by Enzymatic Transesterification: Review. *American Journal of Biochemistry and Biotechnology;* 6 (2):54-76.

Giakoumis, E.G., 2013. A statistical investigation of biodiesel physical and chemical properties, and their correlation with the degree of unsaturation. *Renewable Energy*; 50:858-878.

Girish K. and Shankara B.S., 2008. Neem – A Green Treasure. *Electronic Journal of Biology*; 4(3):102-111.

Gonsalves, J.B., 2006. An Assessment of the Biofuels Industry in India. United Nations Conference on Trade and Development.

Goswami, A., 2011. An Alternative Eco-Friendly Avenue for Castor Oil Biodiesel: Use of Solid Supported Acidic Salt Catalyst, Biodiesel: Feedstocks and Processing Technologies, Dr. Margarita Stoytcheva (Ed.). *InTech,* ISBN: 978-953-307-713-0.

Gude, V. G.; Patil, P.; Martinez-Guerra; Deng, E. S.; Nirmalakhandan, N., 2013. *sustainable chemical process*;1:5.

Gutti, B.; Bamidele, S.S.; Bugaje, I.M., 2012. Biodiesel Kinematic viscosity Analysis of Balanite aegyptiaca Seed Oil. (*Asian Research Publishing Network) Journal of Engineering and Applied Sciences*; 7(4).

Güzel, G., 2012. Sustainable biocatalytic biodeseil production: thermodynamic analysis. Aarhus University, Department of Engineering, PhD dissertation.

Han, H.; Cao, W.; Zhang J., 2005. Preparation of biodiesel from soybean oil using supercritical methanol and CO_2 as co-solvent. *Process Biochemistry;*40:3148-3151.

Huaping, Z.; Zongbin, W.; Yuanxiao, C.; Ping, Z.; Shije, D.; Xiaohua, L.; Zongqian, M., 2006. Preparation of biodiesel catalyzed by solid super base of calcium hydroxide and its refining process. Chin. J. Catal.; 27:391–396.

Ibrahim, H., 2013. Advancement in heterogeneous catalysis of triglycerides for biodiesel production, *International Journal Of Engineering And Computer Science;*2:1426-1433.

Kaisan, M. U.; Pam, G. Y.; Kulla, D. M., 2013. Physico-chemical properties of biodiesel from wild grape seeds oil and Petrodiesel blends. *American Journal of Engineering Research*; 02(10):291-297.

Karasulu, H., 2011. Soybean oil: production process, benefits and uses in pharmaceutical dosage form, University of Ege, Department of Pharmaceutical Technology, Faculty of Pharmacy, Izmir, Turkey. InTech, No-13.

Kasim, F.H., 2012. In Situ Transesterification of Jatropha Curcas for Biodiesel Production newcastle university, thesis.

Krishna, M.T.P.; Monisha, J.; Harish, A.; Sushma, R.; Blessy, B.M.; Ananda S.; 2013. Biodiesel: A Review, *Journal of Engineering Research and Applications*; 3(6):902-912.

Kumar, R.V. and Gupta, V.K., 2002. Thrust on neem is need of today. In: Employment news, New Delhi, India.

Kusdiana, D, and Saka, S., 2001. Kinetics of transesterification in rapeseed oil to biodiesel fuels as treated in supercritical methanol. *Fuel*;80:693–8.

Lertsathapornsuk, V.; Pairintra, R.; Aryusuk, K.; Krisnangkura, K., 2008. Microwave assisted in continuous biodiesel production from waste frying palm oil and its performance in a 100 kW diesel generator. *Fuel Process. Technol*.; 89(12):1330-1336.

Lin, Yuan-Chung; Lin, Jia-Fang; Hsiao, Yi-Hsing; and Hsu, Kuo-Hsiang, 2012, Soybean oil for biodiesel production assisted by a microwave system and sodium methoxide catalyst, *Sustain. Environ. Res.;* 22(4):247-254 .

Ma, F. and Hanna. A., 1999. Biodiesel production: a Review. *Bioresource Technology;* 70:1-15.

Mamilla, V.R.; Mallikarjun M.V.; Rao, G.N., 2012. Biodiesel: The Use of Vegetable Oils and Methyl Esters as Alternative Diesel Fuels. *Indian Streams Research Journal;*2(7).

Marchetti, J.M.; Miguel, V.U.; Errazu, A.F., 2007. Possible methods for biodiesel production, *Renewable and Sustainable Energy Reviews*, 11:1300–1311.

Melero, J.A.; Bautista, L.F.; Morales, G.; Iglesias, J.; Briones, D., 2009. Biodiesel production with heterogeneous sulfonic acid-functionalized mesostructured catalysts. *Energy Fuels*;23:539–547.

Mugnai, E.**,** 2009. Azadirachta indica: Neem tree, the "village pharmacy". *Associazione Scienze Agrarie Tropicali;*1:1-5.

Nascimento, F.P.; Oliveira, A.R.G.; Paredes, M. L. L.; Costa, A. L. H.; Pessoa, F. L. P., 2013. Biodiesel production from supercritical ethanolysis of soybean oil. *Chemical engineering transaction*; 32:829 - 834.

Nezihe, A. and Aysegul, D. 2007. Alkali catalyzed transesterification of cottonseed oil by microwave irradiation. *Fuel*; 86:2639-2644.

Ngamprasertsith, S. and Sawangkeaw, R., 2011. Transesterification in Supercritical Conditions, Biodiesel – Feedstocks and Processing Technologies, Dr. Margarita Stoytcheva (Ed.). *InTech*; 12:247 - 268.

Ogbuewu, I.P.; odoemenam, V.U,; obikaonu, H.O.; Opara, M.N.; Emenalom, O.O.; uchegbu, I.C.; Okoli, M.C.; Esonu, B.O.; Iloeje., M.U., 2011. Growing importance of neem (Azadirachita indica A.Juss) in Agriculture, industry, medicine, and environment: Review. *Research journal of medicinal plant* 5(3):230-245.

Oliveira L.E.and Da Silva M.L.C., 2013. Relationship between cetane number and calorific value of biodiesel from Tilapia visceral oil blends with mineral diesel. *Renewable energy and power quality journal;* No.11, ISSN 2172-038 X.

Oplinger, E.S.; Oelke E.A.; Kaminski A.R.; Combs S.M.; Doll J.D.; Schuler R.T., 1990. Castor beans: Alternative Field Crops Manual, Wisconsin and Minnesota Universities, USA.

Park, J.; Kim, D.; Wang, Z.; Lee, J.,2009. Fast Biodiesel Production with One-Phase Reaction, *Appl Biochem Biotechnol*;154:246–252.

Patel Z. and Krishnamurthy R.,2013. Biodiesel: source material and future prospect, *International Journal of Geology, Earth and Environmental Sciences*; 3(2):10-20.

Peterson, C.L.;Auld, D.L.; Korus, R.A., 1983. Winter rape oil fuel for diesel engines: Recovery and utilization; *JAOCS.* 60:1579-1587.

Pokoo-Aikins, G.; Nadim, A.; El-Halwagi, M.M.; Mahalec, V.D., 2010. Analysis of biodiesel production from algae grown through carbon sequestration. *Clean Techn Environ Policy*; 12:239–254.

Pradhan, A., 2007. Impact of Some Common Impurities on Biodiesel Cloud Point. American society of Agricultural and Biological Engineering meeting presentation, paper number 076090.

Radha, K.V. and Manikandan, G., 2011. Novel production of biofuels from Neem oil. *Bioenergy technology*, world renewable energy congress:471 - 478.

Rajagopal , K.; Bindu, Ch.; Prasad, R.B.N.; Ahmada , A., 2012. Cloud point of biodiesel and blends. *Journal of Chemical, Biological and Physical Sciences, An International Peer Review E-3 Journal of Sciences;* 2(4):1998-2003.

Reda, S.Y.; Costa, B.; Freitas, R. J. S., 2007. Determination of iodine value in ethylic biodiesel samples by ^{1}H-NMR. *Ann. Magn. Reson;* 6(3):69-75.

Refaat, A. A.; Attia, N. K.; Sibak, H. A.; El Sheltawy, S. T.; ElDiwani, G. I., 2008. Production optimization and quality assessment of biodiesel from waste vegetable oil, *Int. J. Environ. Sci. Tech.;* 5 (1):75-82.

Refaat, A.A., *2010.* Different techniques for the production of biodiesel from waste vegetable oil, Review paper. *International Journal of science and technology;*7 (1):183-213

Russbueldt, B.M.E. and Hoelderich, W.F., 2009. New sulfonic ion exchange resins for preesterification of different oils and fats with high content of free fatty acid. *Appl. Catal. A;* 362:47–57.

Sanford, S.D.; White, J.M.; Shah, P.S.; Wee, C.; Valverde, M.A.; Meier, G.R., 2009. Feedstock and Biodiesel characteristics report. Renewable Energy Group.

Sathya, T. and Manivannan, A., 2013. Biodiesel production from neem oil using two step transesterification, *International Journal of Engineering Research and Applications;*3:488-492.

Savage, P.E.; Gopalan, S.; Mizan, T.I.; Martino, C.J.; Brock, E.E., 1995. Reactions at Supercritical Conditions: Applications and Fundamentals. *AIChE Journal*; 41(7):1723 - 1778.

Schwab, A.W.; Bagby, M.O.; Freedman, B., 1987. Preparation and properties of diesel fuels from vegetable oils. *Fuel*; 66:1372-1378.

Sergio M., Alberto A. I., Luis M. R., Humberto V., and Jesús B., 2007. Neem tree morphology and oil content, *new crops and new uses,* 126-128.

Singh, S.P. and Singh, D., 2010. Biodiesel production through the use of different sources and characterization of oils and their esters as the substitute of diesel: *A review*, *Renewable and Sustainable Energy Reviews;*14:200–216.

Shah, S.; sharma, S.; Gupta, M.N., 2003. Enzymatic transesterfication for biodiesel production. minireview. *Indean Journal of biochemistry and biophysics;* 40:392-399.

Sherbiny, S.A.E.; Refaat, A.A.; sheltawy, S.T.E., 2010. Production of biodiesel using microwave technique. *Journal of advanced research*; 1:309-314.

Sheikh, F. A.; Najeeb, S.; Rather, A.G.; Banga, S., 2010. Resynthesis of Ethiopian mustard (Brassica carinata L.) from related digenomic species: An unexplored possibility. *Journal of Agricultural Biotechnology and Sustainable Development;* 2(2):030-034.

Shikha, K. and Rita, C.Y., 2012. Biodiesel production from non edible-oils. *A Review, Journal of Chemical and Pharmaceutical Research*, 4(9):4219-4230.

Shikha K., and Rita Y.C., 2013. life cycle assessment of neem and karanja biodiesel an overview. *International Journal of ChemTech Research*; 5(2):659-665.

Shrestha, K.; Ghimire, J.R.; Gewali M.B., 2013. Biodiesel production from jatropha curcas (L.) oil of Nepal by transesterfication process, *International journal of pharmaceutical, chemical and biological science;* 3(3), 603-609.

Siraj S.R; Uttarwar, L.; Pagey S.; Suryawanshi R.; Effect of Acid &Iodine Value of Karanja oil Methyl Easter & Its statistical correlation with Gross Calorific Value, *IJRET: International Journal of Research in Engineering and Technology* ,eISSN: 2319-1163 | pISSN: 2321-7308.

Sivaramakrishnan, K. and Ravikumar, P., *2012.* Determination of cetane number of biodiesel and its influence on physical properties. *ARPN Journal of Engineering and Applied Sciences; 7(2) ISSN 1819-6608.*

Somnuk, K. ; Smithmaitrie, P.; Prateepchaikul, G., 2012. Feasibility of Using Ultrasound-Assisted Biodiesel Production from Degummed-Deacidified Mixed Crude Palm Oil Using Small-Scale Circulation. *Kasetsart J. (Nat. Sci.);*46:662-669.

Stavarache C.; Vinatoru M.; Nishimura R; Maeda Y., 2005. Fatty acids methyl estersfrom vegetable oil by means of ultrasonic energy. *Ultrasonics Sonochemistry;*12:367–372.

Stoytcheva, M. and Montero, G., 2011. Biodiesel- feed stock and processing technology. ISBN 978-953-307-713-0.

Tapanes, N.C.O.; Gomez Aranda D.A.; Carneiro de Mesquita J.W.; Antunes Ceva O.A., 2008. Transesterification of Jatropha curcas oil glycerides: Theoretical and experimental studies of biodiesel reaction. *fuel*; 87:2286–2295.

Tesser, R.; Serio, M.D.; Guida, M.; Nastasi, M.; Santacesaria, E., 2005. Kinetics of oleic acid esterification with methanol in the presence of triglycerides. *Ind. Eng. Chem. Res;*44:7978–7982.

Thanh, L. T.; Okitsu, K.; Boi, L. V.; Maeda, Y.; 2012, Catalytic technologies for biodiesel fuel production and utilization of glycerol: A Review, *Catalysts;* 2:191-222.

Tyson, S.K., 2001. Biodiesel Handling and Use Guidelines. National Renewable Energy Laboratory. Report, Golden, Colorado.

Vasudevan, P. T. and Briggs, M., 2008. Biodiesel production—current state of the art and challenges. *J Ind Microbiol Biotechnol*; 35:421–430 .

Vivek and Gupta, A K, 2004. biodiesel production from Karanja oil. *Journal of scientific and industrial research* .63:39-47.

Wang, H.; Tang, H.; Wilson, J.; Salley, S.O.; Simon Ng, K.Y., 2008. Total Acid Number Determination of Biodiesel and Biodiesel Blends. *J Am Oil Chem Soc*;85:1083–1086.

Worapun, I.; Pianthong, K.; Thaiyasuit, P., 2010. Synthesis of biodiesel by two-step transesterification from crude jatropha curcus L.oil using ultrasonic irradiation assisted. *KKU Engineering Journal*; 37(3):169-179.

Workneh,W., 2011, Extraction and characterization of essential oil from margosa, School of chemical and bioengineering, Addis Ababa institute of technology. Thesis.

Yan, S.; DiMaggio, C.; Mohan, S.; Kim, M.; Salley, S.O.; Simon Ng, K. Y., 2010. Advancement in heterogeneous catalysis for Biodeseil synthesis. *Springer Science+Business Media*; 53:721–736.

Zare, M.; Ghobadian, B.; Fayyazi, E.; Najafi, G.; Hosseinzadeh, B., *2013.* Microwave-assisted Biodiesel Fuel Production from Waste Cooking Oil. *International Journal of Agriculture and Crop Sciences;* 5(12):1314-1317.

Zufarov, O.; Schmidt, S.; Sekretár, S., 2008, Degumming of rapeseed and sunflower oils. *Acta Chimica Slovaca;* 1(1):321–328.

Appendix

Appendix A: Standard specification for biodiesel

Table A 1: EN 14214 (European biodiesel Standard)

Properties	Biodiesel	Diesel
Density (288K)	0.86 - 0.90	0.82 - 0.86
Viscosity (313K)	3.5 - 5	2.0 - 4.5
Flash point (K)	> 130	> 377
Sulfur (% mass)	< 0.01	< 0.20
Sulphated ash (% mass)	< 0.02	< 0.01
Water (mg/kg)	< 500	< 200
Carbon residue (% weight)	< 0.03	< 0.30
Cetane number	> 51	> 45
Acid value (mgkKOH/g)	< 0.80	-
Methanol (% mass)	< 0.20	-
M.G. content	< 0.80	-
D.g. content	< 0.20	-
T.G. content	< 0.40	-
Free glycerin (% mass)	< 0.02	-
Total glycerin	< 0.25	-
Iodine number	< 120	-
Cloud point	Report to customer	-
Pour point	Report to customer	-
Phosphorus (mg/kg)	<10	-

Source:(Fitamo, T. M., 2011)

+

Table A 2: American and European biodiesel standard

Properties	ASTM D 6751	EN 14214
Density (Kg/m^3) at 15^0C	-	860 - 900
Kinematic viscosity (mm^2/s), at 40^0C	1.9 - 6.0	3.5 - 5.0
Flash point (^0C)	≥ 120	≥ 130
Cloud point (^0C)	-	-
Sulfur content at 100%	≤ 0.05	≤ 0.01
Sulphated ash (wt%)	≤ 0.02	≤ 0.02
Water content (mg/kg)	-	≤ 500
Total contamination (mg/kg)	-	≤ 24
Water and sediment (%V)	≤ 0.05	-
Corrosion (Cu) at 15^0C	≤ No.3	Class 1
Cetane number	≥ 47	≥ 51
Acid number (mg KOH/g)	≤ 0.80	≤ 0.50
Oxidation stability (hr) at 110^0C	-	≥ 6
Methanol content (wt%)	-	≤ 0.2
Ester content (wt%)	-	≥ 96.5
Carbon residue (wt%) at 100%	< 0.05	-
Triglyceride (wt%)	-	≤ 0.20
Diglyceride (wt%)	-	≤ 0.80
Monoglyceride (wt%)	-	-
Free glycerol (wt%)	≤ 0.02	≤ 0.02
Total glycerol (wt%)	≤ 0.24	≤ 0.25
Iodine value ($gI_2/100g$)	-	≤ 120
Phosphorus (mg/kg)	≤ 10	≤ 10

Source:(Fitamo, T. M., 2011)

Table A 3: Homogeneous acid and base catalyzed reaction condition in transesterfication reaction

	Base catalyzed reaction	Acid catalyzed reaction
Feedstock	Low FFA (<0.5%) containing oil	High FFA (> 4%) containing oil
Methanol/oil (molar ratio)	6:1	50:1
Temprature	60 - 65 ^{0}C	80 ^{0}C
Pressure	1.4 -4.1 bar	4 bar
Catalyst	NaOH (0.5 - 2 wt %)	H_2SO_4(10 wt %)
Conversion	> 95% in 1 hr	> 95% in 4 hr

Source: (Balaubramanian, R.K., 2010)

Table A 4: Neem oil and biodiesel characteristics literature value

Property	Biodiesel standard ASTM 6751-02	Neem oil	Neem biodiesel	High speed diesel
Density (kg/m^3), 25 ^{0}C	870 - 900	910	870	822
Viscosity (mm^2/s), 40 ^{0}C	1.9 - 6.0	55.33	5.38	2.56
Flash point (^{0}C)	≥ 130	210	175	70
Pour point (^{0}C)	-15 - 10	11	10	-15
Calorific value (mJ/kg)	-	36.04	38.4	42.5
Ash content	-	0.98	0.01	0.01
Iodine value	-	66.25	53.29	4.42

Source: (Karmakar, A., 2012)

Appendix B: Experimental Result and Method of Calculation

Table B 1: Experimental result based at CCD Design point

Run	Temperature	Methanol/oil mol%	Catalyst/oil wt%	1st result	2nd result	Average value
1	58	7.5	4.5	74	72.6	73.3
2	58	7.5	8.5	84.82	83.5	84.16
3	58	12.5	4.5	77.6	76.6	77.1
4	70	7.5	4.5	73.5	74.44	73.97
5	58	12.5	8.5	94	93.1	93.55
6	70	7.5	8.5	64.42	65.6	65.01
7	70	12.5	4.5	81.21	82.35	81.78
8	70	12.5	8.5	80.47	79.15	79.81
9	53.91	10	6.5	79.1	80.38	79.74
10	74.09	10	6.5	70.58	69.3	69.94
11	64	5.8	6.5	69.02	68.0	68.51
12	64	14.20	6.5	81.76	80.90	81.33
13	64	10	3.14	82.08	81.5	81.79
14	64	10	9.86	86	87.78	86.89
15	64	10	6.5			86
16	64	10	6.5			85.23
17	64	10	6.5			85.33
18	64	10	6.5			84
19	64	10	6.5			84.24
20	64	10	6.5			85.39

Table B 2: AV and SV of Neem oil and fatty acid methyl ester

Property	1st Result	2nd Result	Average
Acid value of FAME (mg KOH/g)	0.22	0.40	0.31
Saponification value of FAME (mgKOH/g)	151.22	146.1	148.66
Saponification value oil (mgKOH/g)	223.3824	221.21	222.2962

Table B 3: Acid value for different amount of methanol and 0.60% v/v Sufuric acid/oil ratio.

Volume of methanol (ml)	1st AV (mgKOH/g)	2nd AV (mgKOH/g)	Average AV (mgKOH/g)
30	5.14	4.90	5.020
40	4.15	3.45	3.801
50	2.926	2.12	2.523
60	0.8716	0.63	0.7508
70	3.222	2.41	2.816
80	3.58	3.1	3.340

Table B 4: Screening of catalyst loading

Catalyst loading	1st result	2nd result	Average
N_2O/CaO-1	80.1	77.1	78.6 %,
Na_2O/CaO-2	84.36	85.1	84.73 %,
Na_2O/CaO-3	64.3	62.1	63.2 %

Experimental Method Calculations

Feed material requirement

Methanol to oil molar ratio and catalyst to oil weight ratio need to be specified in equivalent term as of the volume of oil.

- Calculation of Molecular weight of Oil

Molecular weight of acid treated oil was determined from its acid value and saponification value using the following formula:

$$M = \frac{56.1\ X\ 1000\ X\ 3}{S.V - A.V.}$$

Where: S.V - Saponification Value

A.V - Acid Value

- Methanol to oil molar ratio calculation:

The following basic formula works for all cases:

$$Methanol\ to\ oil\ molar\ ratio = \frac{Number\ of\ mole\ of\ methanol}{Number\ of\ mole\ of\ oil}$$

$$Number\ of mole = \frac{Given\ mass}{Molecular\ mass}$$

$$Density(\ \rho\ ) = \frac{Mass\ (m)}{Volume(V)}$$

Substitute Number of mole and Density in to methanol to oil molar ratio equation:

$$Methanol\ to\ oil\ molar\ ratio = \frac{\left(\frac{Density\ x\ Volume}{Molecular\ mass}\right)\ of\ methanol}{\left(\frac{Density\ x\ Volume}{Molecular\ mass}\right)\ of\ oil}$$

Then, solve for Volume of methanol:

$$Volume\ of\ methanol = (Methanol\ oil\ ratio)X\frac{\left(\frac{Density\ x\ Volume}{Molecular\ mass}\right)of\ oil}{\left(\frac{Density}{Molecular\ mass}\right)of\ methanol}$$

- Catalyst to oil weight ratio calculation

As it was cited before, from Density and Volume of oil, mass of Oil can easily be calculated. Mass of catalyst was calculated as follow:

$$Catalyst\ weight\ \% = \frac{Mass\ of\ catalyst}{Mass\ of\ oil}$$

Then:

$$Mass\ of\ Catalyst = Catalyst\ weight\ \%\ x\ Mass\ of\ oil$$

- Molecular weight of acid treated neem oil calculation:

$$M = \frac{56.1\ X\ 1000\ X\ 3}{S.V - A.V.}$$

$$M = \frac{56.1\ X\ 1000\ X\ 3}{222.2962 - 0.7508}$$

$$= 759.6637$$

Declaration

I declare that this M.Sc. thesis is my original work and has not previously been submitted for degree at this or any other university and that all resources of materials used for this thesis have been duly acknowledged.

Name: Dagmawi Abebe Zewude

Signature: .....

Date of Submission: .....

This thesis has been submitted for examination with my approval as a university advisor.

Name: Dr. Beteley Tekola

Signature: .....

Date: .....