Biodiesel Preparation and Characterization from *Maesa Lanceolata* Seed Oil using Sodium Hydroxide Catalytic Transesterification Method.

Ephrem Debissa Yadessa

A Thesis Submitted to the Department of Applied Chemistry, School of Applied Natural Science

Presented in Partial fulfillment of the Requirement for the Degree of Masters in Applied Chemistry (Specialization in Industrial Chemistry).

Office of Graduate Studies
Adama Science and Technology University

June, 2023
Adama, Ethiopia

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Advisor: Enyew Amare Zereffa (PhD)

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Declaration

I hereby declare that this Master Thesis entitled "Biodiesel Preparation and Characterization from *Maesa Lanceolata* Seed Oil using Sodium Hydroxide Catalytic Transesterification Method" is my original work. That is, it has not been submitted for the award of any academic degree, diploma or certificate in any other university. All sources of materials that are used for this thesis have been duly acknowledged through citation.

Ephrem Debissa	_____	07/07/23
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Recommendation

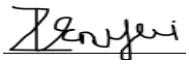
I/we, the advisor(s) of this thesis, hereby certify that I/we have read the revised version of the thesis entitled "Biodiesel Preparation and Characterization from *Maesa Lanceolata* Seed Oil using Sodium Hydroxide Catalytic Transesterification Method" prepared under my/our guidance by Ephrem Debissa submitted in partial fulfillment of the requirements for the degree of Master's of Science in Applied chemistry (Specialization in Industrial Chemistry).

Therefore, I/we recommend the submission of revised version of the thesis to the department following the applicable procedures.

Dr. Enyew Amare		07/07/23
Major Advisor	Signature	Date

Approval

I/we, the advisors of the thesis entitled "Biodiesel Preparation and Characterization from *Maesa Lanceolata* Seed Oil using Sodium Hydroxide Catalytic Transesterification Method" and developed by Ephrem Debissa , hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

Dr. Enyew Amare		07/07/23
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We, the undersigned, members of the Board of Examiners of the thesis by Ephrem Debissa have read and evaluated the thesis entitled "Biodiesel Preparation and Characterization from *Maesa Lanceolata* Seed Oil using Sodium Hydroxide Catalytic Transesterification Method" and examined the candidate during open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Applied chemistry (Specialization in Industrial Chemistry).

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ACKNOWLEDGEMENTS

Above all and utmost I would like my thanks to goes to our Almighty God which let all this extraordinary things happens and allows us to explore different kinds of dreams and vision comes to manifest with fair willingness which indeed further endeavors to be carried out.

Based on their concrete and extraordinary lecture style plus being the reason behind my insight to study such kind of research topics my Advisors Dr. Enyew Amare and Dr. Eshetu Bekele deserves a lot acknowledgments and whole hearted appreciations.

Some institution that supported with laboratory analyses and tests like Ethiopian Petroleum supply agency, Geological survey laboratory and Addis Ababa Science and Technology University I would like to extend my gratitude for their strong support and for the tests results.

Dr. Tegene Desalegn, Mr. Hadish Teklehaimanot and Mr. Guta Amanu and those who directly and indirect participate and suggest arable comments and citations my gratitude and thanks needs to be submitted to them without any reservation.

Last but not the least, my best and loyal friend Mr. Tilahun I would like to extend my thanks for his constructive comments and suggestions.

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Lists of Acronyms and Abbreviations

AOCS…… American Oil Chemists Society

ASTM …….. American Society for Testing and materials

atm……. Atmosphere

CN……..Cetane Number

CP……Cloud point

EN…….. European Standards

EPA…….Environmental Protection Agency

EPSE……..Ethiopian Petroleum Supply Enterprise

EU……..European Union

FAME…… Fatty Acid Methyl Ester

FFA…….Free Fatty Acid Content

g/ml…….gram per millileter

HV……..Heating Value

IEA……. International Energy Agency

ISO…….International Organization for Standards

NSFA…… Non Saturated Fatty Acid

PJ…… Peta Joules

PP……Pour Point

ppm…… Parts per millions

POV…….. Peroxide Value

RSM …….. Response surface method

SCF…….Super Critical Fluids

SFA…….Saturated Fatty Acid

SFE…… Supercritical Fluid Extraction

TG…….. Triglycerides

USA……United State of America

USD……United State Dollar

Abstract

Biodiesel is an eco-friendly and a sustainable Biofuel with fossil fuel–like properties. Fossil fuels are the major sources of energy across the world. These energy sources are nonrenewable and highly concentrated in specific part of the world. The depletion rate of these fossil fuels is also much faster than their regeneration rate and is the major sources of greenhouse gases, air pollution and global warming. This study aimed to produce biodiesel from a Nobel plant Maesa Lanceolata seed oil using sodium hydroxide catalytic transesterification process. Soxhlet extraction method was employed for oil extraction process by applying petroleum ether solvents. ANOVA and Box Behnken standard design for optimization of process parameters was done by response surface method (RSM) and tested for the significance. A maximum oil yield of 36.96% were obtained at the temperature of 70^{o}C, 6 hours of reaction time, and plant seed powder to solvent ratio of 1: 5 at constant particle size of 0.25mm. The physical and chemical characteristics of the oil; moisture content of 3%, density 0.917g/cm^{3}, acid value of 1.39 mg KOH/g oil and free fatty acid of 0.696% were determined and most of the results agreed with the American society testing for materials as feedstocks for biodiesel synthesis. The Maximum Biodiesel yield of 98.67% were found at 4:1 of oil to Sodium methoxide ratio, 25 minutes of reaction time and 65^{o}C of reaction temperature. The biodiesel characteristics; water and sediment content of 0.025%, density of 0.885 g/cm^{3}, Acid value of 0.64 mg KOH/g of biodiesel, Saponification value of 180.27 mg KOH/g of biodiesel, Iodine value of 111.83gI_2/100g, Cetane number of 51.41, Flash point of 135°C and Sulfated ash of 0.016% mass results were within ASTM standard limits except the kinematic viscosity of 6.7 mm^{2}/s value that was little higher and could overcome by blending and further purification process. The Fourier transform infrared spectroscopy analysis showed that the oil was efficiently transesterified to Biodiesel. In general, the major findings of the study showed that Maesa Lanceolata seed oil is among the non-edible oil sources which have a great potential for biodiesel synthesis due to its feedstock characteristics and being as alternative to other sources.

Keywords: *Biodiesel, Maesa Lanceolata, transesterification, catalyst, optimization*

CHAPTER ONE

1. INTRODUCTION

1.1. Background of the Study

The rapid growth of the world energy demand is noticeable to the shoot up growth in world population, industrialization and technology. Global energy demand is expected to grow by 50% in the year 2025. Currently, the globe is dependent heavily on coal, petroleum and natural gas as sources of energy. These sources, also known as fossil or nonrenewable resources, release life threatening emissions into the environment as a result of their usage. Hence, enormous and intensive efforts must ceaselessly be made to ensure that there is steady exponential growth in the production rate of renewable and sustainable forms of energy at the global level (Niju et al., 2019).

Most developing countries including Ethiopia mainly depends upon fossil fuels for energy requirements for different activities like transportation, industrialization and other business activities that were exported from outside and demanding huge foreign currencies. Lack of abundant foreign currency exchange demand and rapid population growth aggravates the situation so badly. The depletion rate of these fossil fuels is also much faster than their regeneration rate and is the major sources of greenhouse gases, air pollution and global warming (Ahmad et al., 2011). These factors have stimulated scientist and researchers to search a renewable energy source such as biodiesel, hydropower, biomass, wind, solar, geothermal, hydrogen and nuclear is of vital importance (Demirbas, 2005). Thus, production and utilization of biodiesel can solve the problems related to utilization of fossil fuel such as lack of foreign currency, and environmental impacts(Tsegay, 2019)

Biodiesel is a liquid biofuel obtained by chemical processes from vegetable oils or animal fats and an alcohol that can be used in diesel engines, alone or blended with diesel oil. ASTM International (originally known as the American Society for Testing and Materials) defines biodiesel as a mixture of long-chain monoalkylic esters from fatty acids obtained from renewable resources, to be used in diesel engines. Biodiesel is a promising biofuel which is renewable, biodegradable and nontoxic. Now it is gaining as one of the most important clean energy source. There are many advantages of using biodiesel as an alternative form of energy.

It can be used as such in the diesel engine without any engine modification indicating that it has similar physical and chemical properties as conventional diesel fuel (Knothe et al., 2018).

The second and third generation of biodiesel is produced from non-edible oil crops (vegetable and algal oil) and oil waste, respectively. Also, the feasibility of using a wide range of feedstocks, process optimization, and cost reduction for biodiesel production has been challenging for over 20 years. This has led to the search for low-cost alternatives, such as non-edible resources and animal- based feedstocks. Hence, the use of non edible oils is increasing for industrial-scale biodiesel production, which makes the entire production process more sustainable (Rehan et al., 2018).

The most common way to produce biodiesel is by transesterification, which refers to a catalyzed chemical reaction involving vegetable oil and an alcohol to yield fatty acid alkyl esters (i.e., biodiesel) and glycerol. Now day, industrial scale biodiesel production use base catalysis was widely used (Ali & Tay, 2013). The basic catalysts commonly used are NaOH and KOH since they are relatively cheap and widely available. It was reported that the rate of base catalyzed transesterification is 4000 times faster than that of the acid-catalyzed one. These two base catalysts are commonly used for the reasons: they are able to catalyze at lower reaction temperature and atmospheric pressure, high conversion (high biodiesel yield) can be obtained in a minimal time. Alcohols that can be used in biodiesel production are those with short chains, including methanol, ethanol, butanol, and amylic alcohol. The most widely used alcohols are methanol (CH_3OH) and ethanol (C_2H_5OH) because of their low cost and properties. Methanol is often preferred to ethanol in spite of its high toxicity because its use in biodiesel production requires simpler technology; excess alcohol may be recovered at a low cost and higher reaction speeds are reached. (Romano & Sorichetti, 2010).

1.2. Statement of the Problem

Now a day, fossil fuels are the major sources of energy across the world. However, these energy sources are nonrenewable and highly concentrated in specific part of the world**.** Moreover, this energy sources demands huge amount of foreign currencies that might impose a burden upon the developing countries economy for the country like Ethiopia. These factors have stimulated some scientist and researchers to search a renewable energy source such as biodiesel, hydropower, biomass, wind, solar, geothermal, hydrogen and nuclear is of vital importance (Demirbas, 2005).

Ethiopia is a rich country in biodiversity of oil bearing plants specially those of non edible ones are important aspects for biodiesel production. As one of the developing countries having no petroleum reserves and being affected by lack of foreign currency, searching alternative renewable source of energy such as biodiesel is necessary to overcome these fossil fuel related problems. Ethiopia is highly endowed with wide variety of non edible oils that can be used as a feed stock for biodiesel production but not well exploited (Tsegay, 2019). Hence, *Maesa Lanceolata* is non-edible oil plant, a shrub or small tree that belongs to the genus family of *Maesa* with 150 species. It is growing in many African countries native to including Central Africa, Ethiopia, and Kenya, where it has been used as an ethno-medicine for a long time (Shin et al., 2014). It grows well in moist and Wet Weyna Dega and Dega agroclimatic zones in nearly all regions, 1,500 - 3,000 m. Some findings identified that the plant seeds contain a maximum oil yield of 35.3% was obtained at the optimum parametric values. The chemical compositions and functional group of the extracted oils were determined and the result shows that the abundant fatty acid was monounsaturated oleic acid C18:1 (53.49%) and poly-unsaturated linoleic acid C18:2 (31.48%), that related with the major feedstock oils for biodiesel productions (Bayisa & Bultum, 2022).Thus, production of biodiesel from *Maesa Lanceolata* seed oil can be a potential source and overcome fossil fuel dependency.

1.3. Objectives of the study

1.3.1. General Objective

The general objective of the study was to produce and characterize biodiesel made from *Maesa Lanceolata* plant seed oil using sodium hydroxide catalytic transesterification process.

1.3.2. Specific Objectives

The Specific Objectives of this study were:

- To extract and purify oil from *Maesa Lanceolata* seed by solvent extraction method
- To determine the physical and chemical properties *of Maesa Lanceolata* seed oil.
- To characterize the physical and chemical properties of the biodiesel.
- To optimize the process parameters of oil to sodium methoxide ratio, reaction time and reaction temperature

- To evaluate the effect of ratio of oil to sodium methoxide, reaction time and temperature on biodiesel production yield from *Maesa Lanceolata* seed oil using sodium hydroxide catalyst via transesterification process.

1.4. Significance of the study

In this study, *Maesa Lanceolata* seed oil which can be produced in large quantity on lands which cannot be used for food crop cultivation can be used as a feed stock for biodiesel production. Thus, it will be significant in terms of motivating local farmers to produce the plant in large quantity which can help them improve their income. It can also be significant in terms of shifting the use of *Maesa Lanceolata* seed oil from its traditional use like medicinal use of antifungal and antibacterial to biodiesel production which can help minimize the economic losses of the oil and partially substitute fossil fuel. This study has a great significance in terms of assuring the production of clean alternative energy source from locally available feed stock.
This study also introduced the new potential feed stock (*Maesa Lanceolata* seed oil) that did not discover in the previous and extend the choice for alternative energy source. Moreover, this study provided eco-friend, underutilized tree seed, oil rich, fast growing and higher yield feed stock for biodiesel production. The study finding could be used as a benchmark for further studies in the area. Hence, the production of biodiesel from indigenous *Maesa Lanceolata* seed oil has a great significance in energy sector as well as for environmental protection.

1.5. Scope and limitation of the study

The performance of analysis of blended biodiesel with petrol diesel in compression ignition engine did not done due to lack of laboratory set up and equipment required to measure these parameters. This study did not compare biodiesel with conventional fuels by using diesel engines. Moreover, it did not characterize the blend of biodiesel with other petroleum fuels and the study focused on biodiesel produced from *Maesa Lanceolata* seed feedstock unlike other vegetable based feedstocks. Some properties like carbon residue, cold soak filterability, Copper Strip Corrosion, sulfur content, oxidation stability analysis, combined metals and phosphorus were not carried out due to resource limitations and unavailability of instrumentation.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Uses and Properties of *Maesa Lanceolata* Plants

Maesa Lanceolata is a shrub or small tree that belongs to the genus family of *Maesa* with 150 species. It is growing in many African countries including Central Africa, Ethiopia, and Kenya, where it has been used as an ethno medicine for a long time (Shin et al., 2014). It grows well in moist and Wet Weyna Dega and Dega agroclimatic zones in nearly all regions, 1,500 - 3,000 m. The Tree with its rounded crown is usually up to 5m but may reach 10m+ high in forests. It usually has a single trunk that is up to 20cm in diameter. It may also be a multi-stemmed shrub. The Bark is smooth to roughish grey, to red-brown. Young stems are smooth and may have reddish hairs. Branches develop either near ground level or near the top of the tree. Here there are often visible raised Lenticels (usually raised corky oval or elongated area on the plant that allows the uncontrolled interchange of gases with the environment).The fruit is very small, round, white and fleshy, topped by the flower remains. Small black seeds inside. (Shin et al., 2014). The white or pinkish, thinly fleshy, small – up to 6mm wide, almost spherical Fruit **i**s a Drupe (a fleshy, 1-seeded indehiscent fruit with the seed are enclosed in a stony endocarp; stone fruit e.g. peach). The fruit may be crowded in dense hanging sprays and occurs in bunches up to 10cm wide. Here each fruit is up to 6mm in diameter. Each drupe ends with the remains of both the persistent Calyx lobes and an inner point – the style**.** Many fruit are usually crowded in leaf axils in dense sprays. Within the collective fruit are many black Seeds containing endosperm (the starch and oil-containing tissue of many seeds; often referred to as the albumen) (Bayisa & Bultum, 2022).

Its medicinal value is evident from its use for the treatment of pain against various diseases including infectious hepatitis, bacillary dysentery, impetigo, bacterial infections in the small intestine, viral infections in the liver and throat, as well as for treatment of some types of dermatoses and neuropathies. The presence of bioactive components such as polyphenols, tannins, and alkaloids in this plant makes it highly wanted due to their beneficial effects on the human body (Uoonlue & Muangrat, 2019).The maximum oil yield of 35.3% was obtained at the optimum parametric values; the plant is rich in oil composition that can be used as alternative feed stock for biodiesel production (Bayisa & Bultum, 2022).

The Seeds are used as verminfuge and the oil extract from the seeds is used for greasing the baking plate of Injera and Bread. Some findings identified that the plant seeds maximum oil yield of 35.3% was obtained at the optimum parametric values. The chemical compositions and functional group of the extracted oils were determined and the result shows that the abundant fatty acid was monounsaturated oleic acid C18:1 (53.49%) and poly-unsaturated linoleic acid C18:2 (31.48%) (Ambat et al., 2018). Also the US Department of Energy indicates that a perfect biodiesel should only comprise mono-unsaturated fatty acids (Handling, 2004).

Figure 2. 1 Pictorial representation of *Maesa Lanceolata* plant.

2.2. Fatty Acid Composition of *Maesa Lanceolata* Seed Oil

Maesa Lanceolata plant seed contain the oil maximum yield of 35.3% was obtained at the optimum parametric values reported by (Bayisa & Bultum, 2022).The oil contains different fatty acid components that shown in table 2.1 as follows:

Table 2. 1 A fatty acid profile of *Maesa Lanceolata* oil.

Fatty Acid	Carbon Structure	Saturation level	% Total Balance
palmitic	18:0	saturated	5.14
heptadecanoic acid	17:0	Saturated	1.1
pentadecanoic acid	15:0	Saturated	1.03
oleic	18:1n-9	Monounsaturated	53.49
linoleic	18; 2n-6	Polyunsaturated	31.38
linolenic	18:3n-3	Polyunsaturated	3.87
stearic	16:0	Monosaturated	4.93

The obtained *Maesa lanceolata* oil compose seven main components from these three saturated fatty acids, two monounsaturated fatty acids, and two polyunsaturated fatty acids were identified. Among them, the most abundant fatty acid was monounsaturated oleic acid C18:1 (53.49%), poly-unsaturated linoleic acid C18:2 (31.48%), linolenic acid (3.87%), saturated palmitic acid C16:0 (5.14%), stearic acid C18:0 (3.9%), heptadecanoic acid (1.1%) and pentadecanoic acid (1.03%). It is, however, important to note that the high percentage of mono-unsaturated oleic acid provides a high degree of oiliness. Acid's degree of unsaturated fatty acid leads to solidification at low temperature or cloud formation. Oils rich in unsaturated free fatty (e.g. oleic acid and linoleic acid) are generally more stable to oxidative rancidity and stable as deep-frying oils. Oils and fats, known as lipids, are hydrophobic substances insoluble in water and are of animal or vegetal origin. They differ in their physical states at room temperature. From a chemical viewpoint, lipids are fatty glycerol esters known as triglycerides. Fatty acids may be saturated fatty acids (SFA) or non-saturated fatty acids (NSFA). In the former, there are only single covalent bonds in the molecules. The composition of vegetable oils influences their properties. For instance, the pour point and cloud point temperatures, cetane number and the iodine index depend on the number of unsaturations and the length of the fatty acid chains. A higher content of double-covalent bonds gives a lower solidification point and a higher iodine index (Pryde, 1981).

Jatropha oil, Moringa Olifera oil and Castor oil are well known feedstocks for biodiesel productions. Different scholars' and researches approved their biodiesel properties after production processes as shown below in table 2.2. In line with this *Maesa Lanceolata* oil can fulfill the properties as compared to these oils. From the above table 2: all the three oil seeds composed of high percentages of oleic acid like, *Maesa Lanceolata* (53.49%), Jatropha oil (44.61) and Moringa Oleifera oil (67.3%) whereas Castor oil contains the highest percentages of Ricinoleic acid (88.8%). Oleic acids are monounsaturated fatty acids (Bayisa & Bultum, 2022; Omonhinmin et al., 2020; Sáez-Bastante et al., 2015).

The total percentage of unsaturated fatty acid in this study of extracted oil of *m. lanceolata* oil was 88.83% which was lower than sunflower oil, but higher than the total percent of unsaturated fatty acid value of Soybean oil, Mustard oil, Palm oil, and Coconut oil for values 81.14, 86.18, 53.30, and 7.12% respectively. This indicates that the extracted *m. lanceolata* oil has the potential to produce alternative healthy oil since it contains high polyunsaturated fatty acid percentages.

Table 2. 2 A fatty acid profile comparison between different plants oil.

Fatty Acid	Chemical formula	MaesaLanceolata oil (%)	Jatropha oil (%)	Moringa oil (%)	Castor oil (%)
Palmitic	$C_{16}H_{32}O_2$	5.14	22.55	7.9	1.1
Heptadecanoic acid	$C_{17}H_{34}O_2$	1.1	1.85	-	
Pentadecanoic acid	$C_{15}H_{30}O_2$	1.03	-	-	
Oleic	$C_{18}H_{34}O_2$	53.49	44.61	67.3	3.6
Linoleic	$C_{18}H_{32}O_2$	31.48	13.64	-	4.4
Linolenic	$C_{18}H_{30}O_2$	3.87	-	1.1	0.7
Stearic	$C_{18}H_{36}O_2$	4.93	8.64	4.5	1.3
Ricinoleic	$C_{18}H_{34}O_3$	-	-	-	88.8

The AOCS standards list the required properties of oils. Anyway, the properties required by the oils are finally determined by the biodiesel industry in each country. For instance, in Argentina the oils for biodiesel production usually have: Acidity level < 0.1 mg KOH/g, Humidity < 500 ppm, Peroxide index < 10 meq/kg and Non-saponificable substances < 1% (Firestone, 2013). The US Department of Energy indicates that a perfect biodiesel should only comprise mono-unsaturated fatty acids (Handling, 2004). Flax seeds have nutritional value for human consumption since they are a source of polyunsaturated fatty acids necessary for human health, Linolenic acid being from 40 to 68% of the total. *Maesa Linceolata* plant seed oil also contains high amounts of polyunsaturated fatty acids which can be comparable to Avocado with content of saturated fatty acids in the pulp of the fruit and in the oil is low; on the contrary, it is very high in mono-unsaturated fatty acids (about 96%being oleic acid).The abundant fatty acid was monounsaturated oleic acid C18:1 (53.49%) and poly-unsaturated linoleic acid C18:2 (31.48%). (Bayisa & Bultum, 2022; Romano & Sorichetti, 2010)

Table 2. 3 Approximate content (in weight) of saturated and non-saturated fatty acids in some vegetable oils and animal fats.

Oil/fat	SFA (≈ % w/w)	NSFA (≈ % w/w)
Coconut	90	10
Corn	13	87

Cottonseed	26	74
Olive	14	86
Palm	49	51
Peanut	17	83
Rapeseed	6	94
Soybean	14	86
Sunflower	11	89
Safflower	9	91
Castor	2	98
Yellow grease	33	67
Lard	41	59
Beef tallow	48	52

From the above table 2. 3 we can see that the well-known biodiesel producing oil plants like Jatropha, corn, cottonseed, olive, peanut, rapeseed, soya bean, sunflower and safflower high content of non-saturated fatty acids content (NSFA) of; 75.3%,87%, 74%, 86%, 83%, 94%, 86%, 89%, 91% and 98% respectively also matches with *Maesa Lanceolata* plant seed oils NSFA content of 92.73%.The specific gravity of the extracted oil was 0.92±0.14 which was lighter than the pure water (Jain & Sharma, 2010; Romano & Sorichetti, 2010).

2.3. Raw materials used for Biodiesel production

The raw materials for biodiesel production are vegetable oils, animal fats and short chain alcohols. The oils most used for worldwide biodiesel production are rapeseed (mainly in the European Union countries), soybean (Argentina and the United States of America), palm (Asian and Central American countries) and sunflower, although other oils are also used, including peanut, linseed, safflower, used vegetable oils, and also animal fats. As shown below in table 2.4. Methanol is the most frequently used alcohol although ethanol can also be used (Romano & Sorichetti, 2010).

There are various feed stocks for biodiesel production. Oil crops are the common pillar raw materials for biodiesel production. It is very important to select the suitable feedstock for biodiesel production since feed-stock alone takes 75% of biodiesel production cost. Currently, there are about 350 oil-bearing crops that have been identified for biodiesel production

globally. They are categorized as edible and non-edible oil sources. The main edible oil sources are: peanut oil, soybean oil, sunflower oil, corn oil, rice bran oil, palm oil, coconut oil, olive oil, rapeseed oil, castor oil, milkweed seed oil and linseed oil. Non-edible oil sources are: Jatropha curcas, Pongami, Madhuca indica, Salvadora oleoid, cotton seed oil, Tobacco, *Calophyll uminophyllum, Eruca Sativa Gars, Terebinth*, rubber seed oil, desert date, fish oil, Jojoba, Neem oil, apricot seed, *Pistacia Chinensis*, Bunge Seed, *Moringa Olifera* (Moringa Stenopetala native to Ethiopia) and croton mega Locarpus. Besides to this oil crops, microalgae, Terpenes, waste cooking oil and animal fats are also alternative feed stocks for biodiesel production (Tsegay, 2019).

Generally edible feed stocks such as sunflower, coconut oil and palm oil which are used for biodiesel production are called first generation feed stocks while non-edible vegetable oils are second generation. Feed stock alone takes the highest of overall production cost of biodiesel. Hence, it is important to employ inexpensive feed stocks to be competitive with petroleum derived fuels.

Table 2. 4 Alternative feed stocks for biodiesel production

Edible oils	No-edible oils	Animal fats	Other sources
Barley	Camelina sativa	Beef tallow	Cyanobacteria
Canola	Cotton seed	Chicken fat	Bacteria
Coconut	Croton megalo carpus	Fish oil	Cooking oil
Soybeans	Jatropha curcas	Pork lard	Fungi
Ground nut	Jajoba	Poultry fat	Latexes
Sorghum	Karanja (pongamiapinnata)	Waste salmon	Microalgae(Chlorella vulgaris)
Peanut, wheat	Mahua (madhucaindica)		Miscanthus
Pumpkin seed	Moringa seed		Pomace oil
Rape seed	Neem (Azadirachtaindica)		Poplar
Rice brain oil	Passion seed (Passifloraadulis)		Soap stocks
sunflower sesame, corn	Rubber tree seed (Heveabra siliensis), Tobaco seed		Switch grass, Tall oil,

			Tarpenes
sesame, palm			

The yield of biodiesel production highly depends on the oil content of the selected feed stocks. The higher the oil content of the feeds stock, the higher yield of biodiesel produced from the corresponding feed stock.

2.4. Oil Extraction and Characterization

There are three main methods that have been identified for the extraction of the oil/fats/lipids:

(i) Mechanical Extraction,

(ii) Chemical Extraction (Solvent Extraction) and

(iii) Enzymatic oil extraction.

Most commonly used commercialized methods of oil extraction are solvent extraction and mechanical extraction. Sufficient drying of sources before oil extraction eases the process and improves the extraction efficiency. Oil extraction sources are to be processed depending on the method of extraction. For instance, whole seeds or kernels can be extracted using mechanical expellers or presses whereas; solvent extraction is possible with kernels. On the other side, biological extraction is possible only with the finely crushed sources (Atabani et al., 2013).

2.4.1. Mechanical Extraction

The most traditional method of oil extraction is the use of mechanical presses/expellers. Mechanical expression is the forceful extraction of oil from the oleaginous material under applied pressure (hydraulic or screw presses) (Atabani et al., 2013).

Oil seed is fed to mechanical press machine which exerts high pressure. When high pressure is applied on the required amount of seed, the solid cake is removed at one side while oil and some impurities are collected on another side. Finally, oil and impurities are separated using gravity settling. Mechanical oil extraction method is most applicable in industrial scale. These techniques are used for particular feed stocks which has high oil yield (40% -70%). Oil extracted by mechanical technique has large amount of gum, and wastes and needs extra treatments of purification and degumming. The problem of conventional mechanical press is it is designed for particular seeds and not suitable for other seeds as it affects the yield. The

advantageous of mechanical extraction technique are: can be done by simple equipment and low investment, low operating cost and oil need not undergo separation process (Tsegay, 2019).

2.4.2. Chemical extraction

Chemical extraction methods mostly involve organic solvents. Hence, chemical extraction is also termed as solvent extraction. Solvent extraction is mostly widely used and commercial method for the separation of oil and fats (lipids). To overcome the disadvantages associated with mechanical methods, solvent methods have been developed. Even, solvent extraction is extensively being used to extract residual oil present in the seed cake from mechanical extraction. Solvent extraction is the isolation of specific or desired components from solid or liquids by using a solvent. There are different types of solvent extractions; most common are liquid – liquid and solid-liquid extractions (Kessler et al., 1985).

Chemical properties of the extractant as well as the analyte influence the phenomena of extraction. Mostly, solubility, hydrophobicity or hydrophilicity, vapor pressure, molecular weight and acid dissociation are the fundamental properties of an analyte that decide the selection of solvent for extraction. Particle size (reduced particle size limits the barrier between two phases), agitation speed and temperature of extraction influences the efficiency of the solvent extraction (Wells, 2003).

2.4.2.1. Soxhlet Extraction

Soxhlet extraction is a popular method and is being considered as a reference for several existing modern extraction techniques.

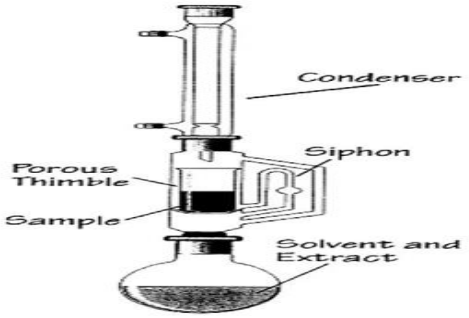

Figure 2. 2 Soxhlet extraction apparatus set up.

Operation is very simple and at first, the Soxhlet thimble is loaded with a cellulosic pack of oleaginous material. Extraction solvents are used as individual or mixture of solvents; hexane, ethanol, chloroform and methanol, hexane and methanol, terpene, 2-propanol, chloroform,

toluene. Most commonly hexane is used for oil extraction, added to the flask and the flask is heated slowly to the boiling point of the solvent so that the solvent vaporizes. Soxhlet extraction is based on the principle that, the vaporized solvent passes through the side arm, condenses at the condenser and floods in to the thimble. When the moderately hot solvent interacts with the oil source in the thimble, extraction of analyte (oil, lipid, fat or phytochemicals) occurs and the solvent with analyte drains again in to the flask through the siphon device. Repetitions of the cycle will result in complete extraction of the analyte. Analyte gets accumulated at the bottom of the flask, as it has higher boiling point than the extraction solvent. Therefore, selection of a solvent for oil extraction is a most critical step for Soxhlet extraction. Hexane, an extensively used solvent for oil extraction has peculiar characters such as excellent oil miscibility, distinguishable boiling point range (63-69°C) and easily recoverable (Bhargavi, Nageswara Rao, & Renganathan, 2018).

2.4.2.2. Supercritical Fluid Extraction (SFE)

In general, solvent extractions (Soxhlet) are associated with the limitations of longer durations, large quantity of solvent requirement and incomplete expulsion of hexane from the oil. Hence, finding alternate innovative technologies that could overcome these limitations is imperative (Maran & Priya, 2015).Gases such as CO_2, propane; toluene, ethane and water are used as solvents in SFE at their supercritical conditions. A gas at supercritical conditions possesses the properties such as density and solubility as a liquid whereas, viscosity, diffusivity and surface tensions similar to that of a gas. These changes in the properties improve the selectivity, flow rates and mass transfer than the normal solvents. In addition, the use of these gases as solvents in SFE makes the extraction more efficient and environmentally benign when compared to conventional solvent extractions (Rudzinski & Aminabhavi, 2000).

Carbon dioxide (CO_2) is the most commonly used oil/lipid extraction solvent in SFE owing to its acceptable critical conditions (temperature 304°K and pressure 73atm), low cost, non flammability and non toxicity. The properties of supercritical CO_2 for the extraction of oils and fats are summarized as,

- High solubility of non polar compounds,
- High dissolution of low molecular weight components,
- High affinity towards oxygen containing organic substances,
- Less solubility of water at <100°C,

- Less affinity towards proteins, polysaccharides and other salts and
- Compound of interest can be extracted by adjusting temperature and pressure (Sahena et al., 2009).

2.4.3. Enzymatic Oil Extraction

Enzymatic oil extraction technique is a promising oil extraction method that uses suitable enzymes to extract oil from crushed seeds. The main advantages associated with this method are: it is environmentally friend and does not produce volatile organic compounds. Some disadvantages of this technique are long extraction time and operation cost (Atabani et al., 2013).

Enzymes breakdown the cell structure of the plant material parts that present within the plant. The mass of plants is made explicitly out of pectic materials, cellulose, hemicelluloses, lignin and protein, while lipid bodies are encompassed in a lipoprotein layer. Enzymes like cellulase, hemicellulase and pectinase crush down the cell, while proteases permeabilize the liposome layer and encourage oil discharges from the oil outline. Aqueous enzymatic oil extraction is essentially founded on concurrent confinement of oil and protein from the oil seed by utilizing scattering finely ground seed in water and isolating the scattering by methods for centrifugation into oil, steady and watery stages. Protein consortia containing different compounds including cellulase, pectinase, protease and so on encourages in hydrolyzing the versatile divider polysaccharides of oil seeds, involving predominantly of cellulose and pectines, accordingly improving of oil from the seeds (Prajapati et al., 2020).

2.4.4. Characteristics of Oils and Fats Used in Biodiesel Production

Oils and fats, known as lipids, are hydrophobic substances insoluble in water and are of animal or vegetal origin. They differ in their physical states at room temperature. From a chemical viewpoint, lipids are fatty glycerol esters known as triglycerides. The three hydrocarbon chains may be of equal or different lengths, depending on the type of oil; they may also differ on the number of double-covalent bonds in each chain. Fatty acids may be saturated fatty acids (SFA) or non-saturated fatty acids (NSFA) referred in table table 2.5. In the former, there are only single covalent bonds in the molecules (Romano & Sorichetti, 2010).

Table 2. 5 chemical formulas of the main fatty acids which present in vegetable oils.

Fatty acid	Chemical formula
Lauric (12:0)	$CH_3(CH_2)_{10}COOH$
Palmitic (16:0)	$CH_3(CH_2)_{14}COOH$
Estearic (18:0)	$CH_3(CH_2)_{16}COOH$
Oleic (18:1)	$CH_3(CH_2)_7$ $CH{=}CH(CH_2)_7COOH$
Linoleic (18:2)	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$
Linolenic (18:3)	$CH_3CH_2(CH{=}CHCH_2)_3(CH_2)_6COOH$
Erucic (22:1)	$CH3(CH_2)_7(CH{=}CH(CH_2)_{11}COOH$
Ricinoleic (18:1)	$CH_3(CH_2)_5CHOHCH_2CH{=}CH(CH_2)_7COOH$

2.4.4.1. Saponification Value

The Saponification value represents milligrams of potassium hydroxide required to saponify one gram of fat or oil. Esters had higher Saponification values than the corresponding oils. Knowing that a triglyceride has 3 fatty acid chains associated and each triglyceride will give 3 methyl esters, stoichiometrically it may be expected that the same amount of fatty acid carbon chain in neat feedstock oil and the biodiesel will react with the same amount of KOH giving the soaps, i.e. their saponification values will be the same (Shalaby, 2013).

2.4.4.2. Acid Value

The acid value measures the content of free acids in the sample, which have influence on fuel aging. It is measured in terms of the quantity of KOH required to neutralize sample. The base catalyzed reaction is reported to be very sensitive to the content of free fatty acids, which should not exceed a certain limit recommended to avoid deactivation of catalyst, formation of soaps and emulsion. The acid value and peroxide value of the oil were investigated respectively. Acid values less when compared with 0.5mg KOH/g specified as the maximum value according to JUS EN14214 [JUS EN 14214:2004] (Shalaby, 2013).

2.4.4.3. Iodine value

The iodine value (IV) of oils or fats is very important parameter since it is used to quantify the amount of double bond present in that oil. It is also used to evaluate its stability to oxidation and higher iodine value indicates more sensitive to oxidation process (Tsegay, 2019).

2.5. Biodiesel Production

Biodiesel production is dominated by European Union (EU) countries followed by USA, Argentina and Brazil. Globally, ethanol consists of 80 % of liquid biofuels produced while 20 % is biodiesel. EU is the world major biodiesel manufacturer with a share of 57 % of total world production in 2009. Currently, the production capacity of European biodiesel has reached approximately 22 million tons. Within the EU, the first four largest biodiesel-producing countries share two-thirds of total production are Germany (33 % of total European production), France (18 %), Spain (7 %), and Italy (5.6 %). Currently, majority of the world countries produce biodiesel from edible oil resources which affects the food security by increasing price of food and land. For these reasons the world is looking for biodiesel oil resources which are non-edible, biodegradable and environmentally friend. In Australia, 90 % of biodiesel are produced from non-edible oil resources and 10 % from edible oil resources. Australia started producing biodiesel in 2004 and the potential production capacity reached 9100 barrels per day in 2011 (Azad et al., 2015).

Biodiesel production increased rapidly starting from 2002 to 2006 with 40% increase annually. According to the United States Environmental Protection Agency (EPA), the total diesel demand in US and EU was 147 billion gallons. The following table 2.6 shows summary of biodiesel production in USA. As it can be seen from the table, production rates are increasing every year and the target of EPA for 2020 was to ramp up the production to 12 billion gallons in USA. (Bidir et al., 2021).

Table 2. 6 Biodiesel production summary in 2009-2011 in USA (million gallons)

Year	2009	2010	2011
Consumption	326	263	878
Production	516	343	967
Gross imports	77	23	36
Gross exports	266	105	73
Consumption (% of distillate fuel by volume)	0.6	0.5	1.5

IEA, *Monthly Energy Review,* August 2012. Biodiesel Production and Consumption:

Ethiopia's fuel import expense has intensified the strain of the country's equilibrium of payments significantly. For instance, the Ethiopian Petroleum Supply Enterprise (EPSE) purchased 3.3 million tons of oil at an expense of US$1.37 billion in 2017 (Ahmad et al., 2011). The imported volume surpasses the sum from the previous year by 10% and the expense is 248 million USD higher than that of 2016, and it keeps increasing as shown in Figure 2.3. This is affecting the balance of expenses negatively as it is increasing with time. In reaction to the price upswing of oil, and partially in reaction to climate fluctuations, Ethiopia has been looking into its energy strategies to move from imported oil fuels to domestically produced biofuels (Bidir et al., 2021).

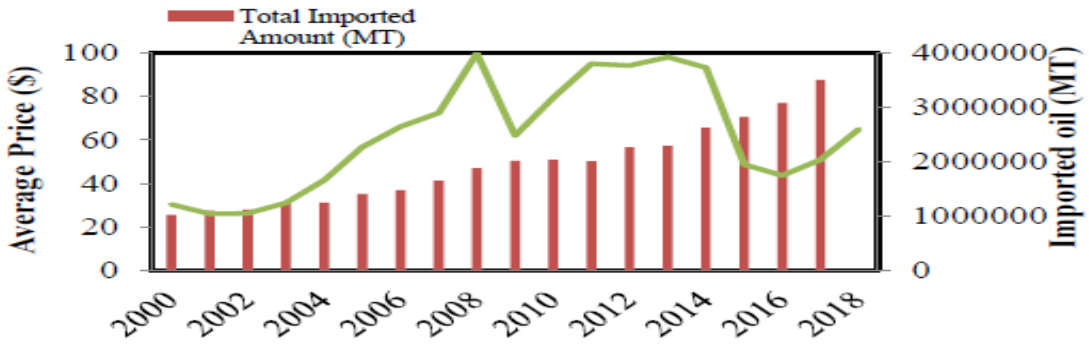

Figure 2. 3 Net energy imports of Ethiopia up to 2016.

Ethiopia is estimated to have 23.3 m ha of land suitable for biodiesel. A joint report by a team of experts from the Ministry of Agriculture and the Ministry of Energy and Water Resources indicated investment licenses were offered to 83 investors in Ethiopia to produce biodiesel feedstock in various regions (Shete & Rutten, 2014).

Ethiopia has huge biomass potential and many non-edible and edible oil crops, which can be used for the production of biofuel. Furthermore, it has all types of topographies and climatic conditions that can accommodate the growth of various potential biofuel feed stocks. Ethiopia could deliver up to 60 Peta-joules (PJ) of energy from sugarcane, which is comparable to 1.7 billion liters of bio-jet fuels. It has roughly about 30% of its land distinguished as feasibly accessible for purposes other than food-based plant growth and ecological preventions, about 25 *x* 106-hectare accessible land and favorable natural conditions for biofuel development. There is about 7 *x* 105 hectare recognized suitable land only for sugar cane cultivation. Furthermore, different oil-bearing seeds and potential biomasses including castor bean,

Jatropha, cassava, croton, palm, Pongamia, and Moringa grows in Ethiopia readily (Bidir et al., 2021).

Fats and oils are primarily water-insoluble, hydrophobic substances in the plant and animal kingdom that are made up of one mole of glycerol and three moles of fatty acids and are commonly referred as triglycerides.[39]Biodiesel is a liquid biofuel obtained by chemical processes from vegetable oils or animal fats and an alcohol that can be used in diesel engines, alone or blended with diesel oil. ASTM International (originally known as the American Society for Testing and Materials) defines biodiesel as a mixture of long-chain monoalkylic esters from fatty acids obtained from renewable resources, to be used in diesel engines (Knothe, Dunn, & Bagby, 1997)

Biodiesel is made in a chemical process called transesterification, where organically derived oils (vegetable oils, animal fats and recycled restaurant greases) are combined with alcohol (usually methanol) and chemically altered to form fatty esters such as methyl ester. Chemically, most Biodiesel consists of alkyl (usually methyl) esters instead of the alkanes and aromatic hydrocarbons of petroleum derived diesel (Knothe et al., 1997).

Oil, ester and diesel have different number of carbon and hydrogen compound. Diesel has no oxygen compound. It is a good quality of fuel. On the other hand, in the case of vegetable oils Oxidation resistance is markedly affected by the fatty acid composition. The large size of vegetable oil molecules (typically three or more times larger than hydrocarbon fuel molecules) and the presence of oxygen in the molecules suggest that some fuel properties of vegetable oil would differ markedly from those of hydrocarbon fuels (Knothe et al., 1997).

Petroleum based diesel fuels have different chemical structures from vegetable oils and esters. The former contain only carbon and hydrogen atoms, which are arranged in normal (straight chain) or branched chain structures, as well as aromatics configuration. The normal structures preferred for better ignition quality. Diesel fuel can contain both saturated and unsaturated hydrocarbons, but the latter are not present in large enough amounts to make fuel oxidation problem. Petroleum-derived diesel is composed of about75% saturated hydrocarbons (primarily paraffins including n, iso, and cycloparaffins), and 25% aromatic hydrocarbons (including naphthalenes and alkylbenzenes). The average chemical formula for common diesel fuel is $C_{12}H_{23}$, ranging from approximately$C_{10}H_{20}$ to $C_{15}H_{28}$. Vegetable oils contain fatty acid, free fatty acids (generally 1–5%), phospholipids, phosphatides, carotenes, tocopherols, sulfur

compound and traces of water. The fatty acids, which are commonly found in vegetable oils, are stearic, palmitic, oleic, linoleic and linolenic (Knothe et al., 1997).

2.5.1. Biodiesel Production Methods

Biodiesel can be produced using different production processes. The production process to adopt depends on the availability of the raw materials (feedstocks) and ease of the application of the available production technology. Some of the biodiesel production methods are examined below (Ayoola et al., 2021).

2.5.1.1. Pyrolysis process

Pyrolysis can be simply defined as the decomposition or disintegration of organic compounds at very high temperatures, aided either by the presence of a suitable catalyst or absence of air. It is an irreversible reaction involving the simultaneous change in both the physical and chemical and physical behaviors of the materials used to produce other useful material(s) due to the application of heat (Schobert, 2013).

The organic materials that can be paralyzed include animal fats, vegetable oils, and natural triglycerides. The liquid components of the paralyzed fats and triglycerides include biodiesel which function in the same manner as petroleum diesel in diesel engine. These liquid products have cetane number, flashpoint, pour point, heating values and viscosity properties that are similar to that of petroleum diesel (Masjuki et al., 2013).

So the fuel treated by this process which could be use directly in diesel engine without any modification. Generally alumina, zeolite and red mud are used as a catalyst in thermal cracking process for biodiesel production. The thermal cracking process will happens by the temperature between $250^{O}C$ and $350^{O}C$. The oil or animal fat need to be converting into the biodiesel is placed inside the reactor, and then heat is applied to the reactor. Now the oil or animal fat get vaporized and reach the condenser through pipe. The condenser cools the vapor in the liquid then the liquid is collected in the beaker which is called as biodiesel (Rajalingam et al., 2016).

The pyrolysis of any material is generally considered not to be environmentally friendly due to the release of high carbon residue, green gases and ash content that exceed the quantities obtained from the fossil fuels. Also, the technology involved is an expensive one. These hinder its wide acceptance as a major process of biofuel production (Schobert, 2013).

2.5.1.2. Transesterification process

The transesterification is the most convenient process due to its low cost and simplicity while the selection of feedstock is critical as it accounts about 70% of the total production cost from edible and non edible sources. The transesterification process involves the chemical conversion of different types of oils into fatty acid methyl esters (FAME) which depicted in figure 2.4. In the presence of a catalyst, the oil is reacted with short-chain alcohol. Biodiesel or mono-alkyl esters of long-chain fatty acids, such as FAME, are produced by a transesterification reaction with TGs and alcohol. Alcohols, such as methanol and ethanol (acyl acceptor), can be catalyzed by the alkoxy group of an ester to convert TGs to FAME and glycerol. Then, a lipid reacts with a mono-alcohol in the presence of alkaline, acidic, or enzymatic catalysts to produce FAME and glycerol (Rezania et al., 2019).

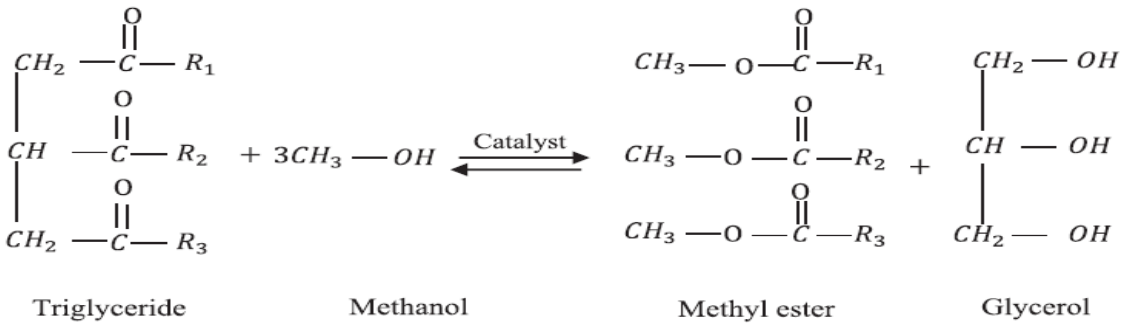

Figure 2. 4 Conversion of TGs to glycerol through transesterification.

Several factors, such as catalyst type, reactor type, raw material characteristics, agitation speed, temperature, reaction time, solvent type, and solvent-to-oil ratio, have been shown to be important to obtain the optimum yield of biodiesel via the transesterification process (Verma & Sharma, 2016). Catalysts are divided into two main categories:

- homogeneous catalysts, such as sodium hydroxide (NaOH) and potassium hydroxide (KOH) and
- heterogeneous catalysts (like solid acid/base, polymer, and zeolites)

2.5.1.3. Microalgae biodiesel production process

This approach of biodiesel production, chemical or mechanical process is engaged in the extraction of oil or lipid from the microalgae biomass. The main challenge associated with this

technique is the low yield of the extracted algal oil or lipids from microalgae cells, and this ultimately resulted into low yield of biodiesel economical. The extraction of lipid can be carried out through various methods such as mechanical press, solvent extraction, and supercritical fluid extraction. The extracted lipid can then be further processed into biodiesel production via different techniques such as transesterification. The low yield of the extracted oil and the high cost of auto bioreactor equipment required for biodiesel production make this technique not to be embraced as a common practiced (Omonhinmin et al., 2020).

2.5.2. Catalysts for transesterification process and their role on yield of Biodiesel

Catalyst plays a significant role to fasten the rate of chemical reaction in transesterification process. Catalysts break the bond of triglyceride which is reacted with alcohol to separate fatty acid methyl ester from glycerol. Catalysts increase the yield of biodiesel by fastening the rate of the transesterification reaction. The main catalysts used for biodiesel production are categorized as chemical and biological catalysts. Chemical catalysts comprise of homogeneous catalyst, heterogeneous catalyst and super critical fluids (SCFs).

2.5.2.1. Chemical catalysts

2.5.2.1.1. Homogeneous catalysts

Homogeneous catalysts include basic and acidic catalysts. The basic catalysts commonly used are NaOH and KOH since they are relatively cheap and widely available. It was reported that the rate of base catalyzed transesterification is 4000 times faster than that of the acid-catalyzed one. Currently, most biodiesel production is carried out using homogenous base catalysts of sodium hydroxide (NaOH) or potassium hydroxide (KOH). These two base catalysts are commonly used for the reasons: they are able to catalyze at lower reaction temperature and atmospheric pressure, high conversion (high biodiesel yield) can be obtained in a minimal time, cheap and available. Now day, industrial scale biodiesel production use base catalysis was widely used (Ali & Tay, 2013).

Transesterification can be conducted under homogeneous or heterogeneous catalysis. In homogeneous catalysis, the chemical reaction is faster and requires lower catalyst loadings than in the heterogeneous one. However, the used catalyst cannot be reused or recycled. Therefore, it needs several washings for product separation. Transesterification reactions are

homogeneously catalyzed for biodiesel production results with high yields in a relatively short time. However, biodiesel does not compete favorably with fossil fuels because of the catalyst cannot be reused (Di Serio et al., 2008).

2.5.2.1.2. Heterogeneous catalysts

In heterogeneous catalysis, the catalyst and the reaction are in different phases. This has advantage of easily removal of the catalyst and its reuse without intense washing. However, leaching of the active phase may be a problem. Therefore, the synthesis of active reusable heterogeneous catalysts is a great challenge in biodiesel production. They are still not currently viable for wide industrial scale production, as most of these catalysts are expensive, lower activity and lower yield when compared to homogeneous catalysts (Di Serio et al., 2008).

Environmental issue has led to search for solid heterogeneous catalyst since they are eco-friend and effective. The chemical process to produce biodiesel using solid heterogeneous catalyst in catalyzing the reaction has advantages of reducing time and cost of the process through the reuse of the catalyst, decreasing the level of impurities in the reaction products and conducting the operation in a continuous fixed bed (Colombo, Ender, & Barros, 2017).

Metal oxides have important applications in the preparation of biodiesel, such as CaO, MgO, and La_2O_3. However, their reusability is poor and their active sites are easily lost. Based on this situation, more complex metal oxide materials have emerged, among which zinc oxide has more complex metal oxide. This is due to the stable nature of zinc oxide, which can better disperse strong alkaline active sites, improve catalytic activity, and prevent leaching of active sites. The application of CaO-ZnO, MgO-ZnO, and La_2O_3-ZnOcatalysts in biodiesel synthesis was introduced. ZnO and its composite nanomaterials are usually prepared by co-precipitation, sol–gel method, solvothermal method template method and impregnation method. Ultimately, the ideal nanocatalysts need to be obtained by calcinations at a specific temperature (Wang et al., 2021).

2.5.2.2. Biological catalysts (Enzymes)

Enzymes have received increased attention because of their high selectivity, biocompatibility, biodegradability, environmental acceptability and forming few or no by-products. Enzymatic transesterification has environmental advantage over other catalysts. However, enzymes are not widely used in commercial biodiesel production processes as they are very expensive and show

longer reaction time (Di Serio et al., 2008). Free lipase, traditional immobilized lipase, lipase immobilized on magnetic nanoparticles is commonly used biological catalysts. They have their own merit and demerit during their application. Among biological catalysts immobilized lipase are more preferable than others (Colombo et al., 2017).

2.5.3. Characteristics of Alcohols Used in Biodiesel Production

Alcohols that can be used in biodiesel production are those with short chains, including methanol, ethanol, butanol, and amylic alcohol. The most widely used alcohols are methanol (CH_3OH) and ethanol (C_2H_5OH) because of their low cost and properties. Methanol is often preferred to ethanol in spite of its high toxicity because its use in biodiesel production requires simpler technology; excess alcohol may be recovered at a low cost and higher reaction speeds are reached.

Methanol is the most widely used for biodiesel synthesis, in spite of its toxicity. It is also a substance of petrochemical origin. Ethanol is less used and requires more complex production technology and the reaction speeds are lower. It can be produced from biomass.

It must be remembered that in order for biodiesel to be a fully renewable fuel, it should be obtained from vegetable oils and animal fats, together with an alcohol that is produced from biomass, such as bioethanol, instead of being a petrochemical product. Several countries are carrying out research towards this objective, such as Spain and Brazil (Romano & Sorichetti, 2010).

2.5.4. Characterization of Biodiesel

2.5.4.1. Density

Density is defined as the mass of unit volume, measured in a vacuum. It is one of the quality parameters in biodiesel standardization. It strongly affects the cetane number, heating value and viscosity of a biodiesel. The density also affects atomization and combustion qualities. Density of biodiesel is dependent on the molar mass, the free fatty acid content, the water content and temperature. The density of biodiesel is typically higher than that of diesel fuel and is dependent on fatty acid composition. The densities of biodiesels are generally higher than those of fossil diesel fuel. The values depend on their fatty acid composition as well as on their purity. Density increases with decreasing chain length and increasing number of double bonds,

or can be decreased by the presence of low-density contaminants such as methanol (Sarin, 2012).

2.5.4.2. Kinematic Viscosity

Viscosity which is measure of resistance to flow is one of the most important biodiesel quality indicators. Because it affects engine performance like lubrication and fuel atomization. Biodiesels with high viscosity tend to form larger droplets on injection, which can cause poor combustion, increased exhaust smoke and emissions. This leads to an increase in engine deposits and energy requirement for fuel pumping. Similarly, too low viscosity results in very fine spray which leads to insufficient penetration and the formation of black smoke. It also affects the lubricity of the fuel as some elements of the fuel system can only be lubricated by the fuel. Biodiesels are 10 times less viscous than the oils from which they are made. Thus, direct use of vegetables oils as fuel in diesel engines results in engine deposits. However, the viscosity of biodiesel is higher than that of the conventional diesel fuel.(Knothe & Steidley, 2005).

2.5.4.3. Cloud Point (CP) and Pour Point (PP)

The CP is the temperature at which a sample of the fuel starts to appear cloudy, indicating that wax crystals have begun to form which can clog the fuel lines and filters in a vehicle's fuel system. The PP is defined as the temperature at which the fuel ceases to flow. The cessation of flow results from an increase in viscosity or from the crystallization of wax from oil (Sarin, 2012)

2.5.4.4. Flash Point

The flash point is defined as the lowest temperature at which a fuel gives off sufficient vapors, which when mixed with air will ignites momentarily. The flash point for biodiesel is used as the mechanism to limit the level of un-reacted alcohol remaining in the finished fuel. The flash point is also of importance in connection with legal requirements and for the safety precautions involved in fuel handling and storage, and are normally specified to meet insurance and fire regulations. The flash point of pure biodiesel is considerably higher than the prescribed limits, but can decrease rapidly with increasing residual alcohol. As these two aspects are strictly correlated, the flash point can be used as an indicator of the presence of methanol in the

biodiesel. Flash point is used as a regulation for categorizing the transport and storage of fuels, with different thresholds from region to region, so aligning the standards would possibly require a corresponding alignment of regulations (Sarin, 2012).

2.5.4.5. Cetane Number

Cetane number (CN) is widely used as a diesel fuel quality parameter related to the ignition delay time and combustion quality. The higher the CN is the better the ignition properties of the fuel. It is measured by matching against the blend's two reference fuels namely *n*-cetane and a-methylnaphthalene. High CNs helps ensure good cold-start properties and minimize the formation of white smoke. Thus, a high CN is associated with rapid engine starting and smooth combustion. A low CN causes deterioration in this behavior and higher exhaust gas emissions (hydrocarbons and particulates). In general, biodiesel has slightly higher CNs than fossil diesel. The CN increases with increasing length of both fatty acid chains and ester groups, while it is inversely related to the number of double bonds (Sarin, 2012).

2.5.4.6. Acid Number

Acid value is the measure of free fatty acids (FFA) present in the extracted oil. Acid value is expressed in terms of milligram of base (KOH) per gram of oil to neutralize free fatty acids. Higher acid value reduces the quality of the oil since it because corrosion to oil. Acid number or neutralization number is a measure of the amount of free fatty acids contained in a fresh fuel sample and of free fatty acids and acids from degradation in aged samples. This test is used to determine the acidic constituents in the biodiesel. If mineral acids are used in the production process, their presence as acids in the finished fuel is also measured with the acid number. It is expressed in mg KOH required to neutralize 1 g of FAME. The sample is dissolved in a mixture of toluene and propan-2-ol containing a small amount of water and titrated potentiometrically with alcoholic KOH. The end points are noted and readings of the volumes of titrating solution are taken and used in a formula to calculate the total acid number of the biodiesel sample. The parameter characterizes the degree of fuel ageing during storage, as it increases gradually due to degradation of the biodiesel. High fuel acidity has been discussed in the context of corrosion and the formation of deposits within the engine which is why it is limited in biodiesel specifications (Tsegay, 2019).

2.5.4.7. Iodine Value

The iodine value (IV) is another biodiesel quality parameter which has been introduced to evaluate the stability to oxidation. It is a measure of the total unsaturation of fatty acids measured in g iodine/100 g of biodiesel sample, when formally adding iodine to the double bonds. Biodiesel with higher iodine number is sensitive to oxidation and is highly dependent on the nature and composition of the feedstocks used in biodiesel production. Biodiesel with high (IV) tends to polymerize and form deposits on injector nozzles and piston rings. This tendency is directly related to the degree of unsaturation of the fatty acids (Barabás & Todoruț, 2011; Sarin, 2012).

2.5.4.8. Heating Value (Calorie value) (HV)

The heat of combustion or hot worth of a fuel is a very important measurable parameter that shows the quantity of heat liberated by the fuel at intervals the engine that allows the engines to try and do the work. The heat content (Calorific value) of biodiesel ranges from is 32-42 MJ/kg (Knothe & Steidley, 2005)

The biodiesel physical and chemical characteristics based on the EN and ASTM standards and specifications were discussed below in the table 2.7.

Table 2. 7 Specification and Test Methods of Biodiesel as per ASTM D6751 & EN 14214 Standards.

Property	units	Limits		Test methods	
		ASTM D6157	EN 14214	ASTM D6751	EN 14214
Flash points	^oC	130 min	101 min	D93	ISOCD 3679e
Kinematic viscosity	mm^2/s	1.9 – 6	3.5 – 5	D445	EN ISO 3104
Cetane number	-	47 min	51 min	D613	EN ISO 5165
copper strip corrosion	-	no. 3 max	class 1	D130	EN ISO 2160
Acid value	mg KOH/g	0.80 max	0.5 max	D664	EN 14104
Free glycerol	% (m/m)	0.020 max	-	D6584	EN 14106
Total glycerol	% (m/m)	0.240 max	0.25 max	D6584	EN 141101

Carbon residue	% (m/m)	0.05 max	0.3 max	D4530	ENISO 10370
cloud point	^{o}C			D6950	
density at $15^{0}C$	Kg/m^{3}	-	860 – 900	-	EN ISO 3675
Sulfur content	mg/Kg	-	10 max	-	-
water content	mg/Kg	-	500 max	-	ENISO 12937
Iodine value	-	-	120 max	-	EN14111

2.5.5. Factor that affect Biodiesel Production

Biodiesel is produced from alcohol and triglyceride by transesterification reaction. During this reaction, there are different factors that affect this process such as alcohol to oil ratio, catalyst weight, and reaction temperature, mixing intensity (stirrer speed), fatty acid content and time of reaction. These parameters affect the fuel property, quality and yield of biodiesel product (Raj & Bhandari, 2017).

2.5.5.1. Alcohol to oil ratio

The most effective variable that affects biodiesel production during transesterification reaction is molar ratio of alcohol to oil .Since transesterification is an equilibrium reaction, large and excess amount of alcohol is required for the reaction to move forward and avoid the reversible reaction. From the stoichiometry of transesterification reaction, 3 moles of alcohol reacts with 1 mole of triglycerides to yield 3 moles of fatty ester and 1 mole of glycerol. Since transesterification is an equilibrium reaction, large excess alcohol is required to accelerate the reaction. Lower molar ratio decreases reaction rate and longer time is needed to complete the reaction. However, excess molar ratio beyond the optimum level makes separation of glycerol difficult (Kafuku & Mbarawa, 2010a).

2.5.5.2. Catalyst load (catalyst weight)

The most common catalysts used for transesterification reaction are the alkali catalysts such as sodium hydroxide and potassium hydroxides since they both react with the triglycerides of oil to break them apart so that methanol can bond with the fatty acids to produce fatty acid methyl ester (biodiesel) (Kafuku & Mbarawa, 2010a).

2.5.5.3. Reaction temperature

Reaction temperature affects the yield biodiesel produced via transesterification process. Higher reaction temperature increases reaction rate by reducing viscosity of oils. This shortens the transesterification reaction and higher biodiesel yield is obtained. Lower reaction temperature decreases the rate of reaction and decreases the yield of desired product (biodiesel). However, the increase of reaction temperature beyond the optimum accelerates the Saponification of triglycerides of oils which causes methanol to vaporize and results in decreasing the yield of biodiesel. To prevent the evaporation of alcohol (methanol), transesterification reaction temperature should be maintained below the boiling point of alcohol (Romano & Sorichetti, 2010).

2.5.5.4. Stirrer Speed

Mixing or agitating of reactants is very important to achieve completion of transesterification reaction as it increases the yield of product. Reactants can be well mixed through agitation and the role of agitation is to increase the collision between the particles and diffusion of one reactant into another, thorough mixing of catalyst with reactants and rate of reaction. Increase in stirrer speed will shorten the reaction time and increase the conversion. However, too much higher agitation speed would not result in significant increment in the yield of biodiesel (ester) (Gnanaprakasam et al, 2013)

2.5.5.5. Free fatty acid content (FFA)

In the conventional transesterification of fats and vegetable oils for biodiesel production, FFAs always produce negative effects. The presence of high FFAs in the vegetable oils or animal fats lead to soap formation, consumes the catalyst, reduces its catalytic effectiveness which results a low conversion in biodiesel production. Because the FFAs react with the alkaline catalyst to produce soaps that inhibit the separation of the ester, glycerin. The resulting soaps leads to increasing in viscosity, formation of gels and foams, and made the separation of glycerol difficult (Demirbas, 2005).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Materials and chemicals

The chemicals and equipment that was used in this study are mortar and pestle (miller machine), Oven, Soxhlet extractor, measuring cylinder, Vibrio-viscometer, Pensky Martens apparatus, furnace, refrigerator, digital balance, rotary evaporator, heating mantle, rotary evaporator, beaker, flask, reflex condenser, water bath, stirrer, pH meter, Pycnometer, Bomb calorimeter, burette, separator funnel, FT-IR, and thermometer. Also the chemicals that were used for the study are like Carbon tetra chloride (Alpha cheimka), Sodium sulfate, hydrochloric acid, sulfuric acid, Potassium hydroxide, sodium hydroxide (LABKEMICAL®), potassium iodide, toluene rectified (for synthesis, 99%, LOBA Chemie PVT. LTD), methanol (99.8%, ar/acs, LOBA Chemie PVT. LTD), ethanol (99.9% AR), petroleum ether (40-60°C, AR), and sodium thiosulfate (RELBMED, India), starch, phenolphthalein indicator.

3.2. Experimental methods

3.2.1. Raw material collection and preparation

The seeds of *Maesa Lanceolata* plant were collected at the harvesting time (from November, 2022 to January, 2023) from Bonga town which is located in south western Regional state. After the collection of seeds, the samples were transported to Adama Science and Technology University, where the study was conducted. The impurities that present with the sample seeds were separated and removed before take to any further activities. The seed was air dried in open dark place at ambient room temperature. The dried sample was grinded by milling machine. Size reduction is used to create high surface area for the reaction. After size reduction the sample was sieved to appropriate mesh size (0.25 mm). The prepared sample was stored by packing in plastic bag for the next analysis (Bayisa & Bultum, 2022)

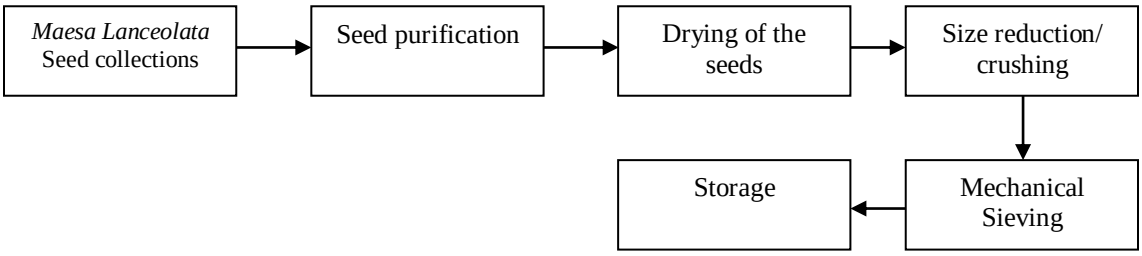

Figure 3. 1 Preparation of Feedstock for Biodiesel production.

3.2.2. Raw material proximate analysis

Proximate analysis is characterization that consists of determining physical and chemical properties which includes moisture, ash, volatile matter and fixed carbon content based on ASTM standards.

3.2.2.1. Determination of moisture content

The moisture content of the sample was determined according to EN 14774-1. The sample was conditioned by putting it in desiccators for 24 hours to attain its equilibrium condition. 2g of the sample was oven dried for 2 hours at 105°C. After 2 hours drying, the sample was cooled in desiccators for 30 minutes and weighed. This was continued until the constant weight was registered (Niju et al., 2019).

The percentage weight was calculated as:

$$Wt\%\ moisture = \frac{W1-W2}{W1} * 100 \quad \text{.....(1)}$$

Where; W1 is the original weight of the sample before oven drying and W2 is the weight of the sample after oven drying

3.2.2.2. Determination of ash content

The ash content was determined according to EN 14775. 2 g of sample was weighed in crucible and burned at 650 °C for 2 hours in muffle furnace (Niju et al., 2019).

$$\text{Ash } \% = \frac{W2}{W1} * 100 \quad \text{.....(2)}$$

Where, W1 is weight of sample initially taken and W2 is weight of Ash cooling after heating

3.2.2.3. Determination of volatile matter

Volatile matter was determined using high temperature furnace. 2 g of the sample was weighed in crucible and heated at temperature of 925 °C in a closed crucible for 8 minutes.

$$Volatile\ matter, \% = \frac{W1-W2}{W1} * 100 \quad \text{.....(3)}$$

Where, w1 is weight of sample before drying and W2 is weight of sample after drying

3.2.2.4. Determination of fixed carbon

Carbon content of fuels was obtained by subtracting the percentage composition of ash, moisture and volatile matter from 100 (Sarin, 2012).

Fixed carbon content = 100 – (ash, % + M.C, % + V.M, %)..... (4)

Where, ash, % = ash content, Mc, % = Moisture content and V.M, % = Volatile matter content

3.2.3. Extraction of *Maesa Lanceolata* seed oil

To extract the *Maesa Lanceolata* seed oil the Soxhlet apparatus was used. A grinded sample was placed in a thimble and loaded into the Soxhlet extraction unit. A solid: solvent (petroleum ether) of 1:5 and 1:6, extraction time from 5 to 6 h., temperature of 70^0C to 80^0 C, and particle size of 0.25 mm as constant was employed (Bayisa & Bultum, 2022). Finally, the solvent and oil was separated using a rotary evaporator (RotaVapor) and the solvent was recovered using a condenser.

Table 3. 1 determination of the optimum oil extraction from Maesa Lanceolata seed

Experimental run	Powder to solvent ratio	Temperature (^{0}C)	Extraction time (hour)	Yield (%)
1	1:5	70	5	
2	1:5	70	6	
3	1:5	80	5	
4	1:5	80	6	
5	1:6	70	5	
6	1:6	70	6	
7	1:6	80	5	
8	1:6	80	6	

The extracted oil contains undesired substances like: petroleum ether, gum, moisture, wax substance and phospholipids. These unwanted substances decreases the quality of the oil as they increases formation of soap during transesterification process. The extracted oil was purified by the methods likes: evaporation, degumming and drying.

Evaporation process is used to separate petroleum ether from oil. The oil and solvent are separated by evaporating at 60°C using rotary evaporator. The mixture of extracted oil and petroleum ether will be separated based on their boiling point differences. The boiling point of petroleum ether varies from 40°C – 60°C while that of oil is greater than 200°C. Petroleum ether solvent is evaporated above 60 °C.

Degumming is used to separate the unwanted mixtures of gum and wax from extracted crude oil. There are two types of degumming methods like acid degumming and water.

Water Degumming is also employed for Low FFA Crude Oils where degumming is followed by Neutralization and Water Washing. Crude oils were degummed by heating the oils to 80 °C, Mixed with water (to 5% vol.) and stirred for 15 min by magnetic stirrer. Then, the mixture was centrifuged for 20 minutes.

For removal of Non-hydratable gums, water degumming is not suitable and the oil is conditioned by acid media such as phosphoric acid. The content of phosphoric acid addition depends on the amount of gums present as well as the type of oil being refined. Acid Degumming is perfectly suitable for High FFA Crude Oils where acid washing is followed by Water Washing and ensures total degumming. Due to the variable content of phospholipids in crude oils, analysis of phosphorus prior to acid treatment is necessary to ensure that the acid dosage is correct.

Crude oils were heated to 80 °C and water solution of citric acid (30 %) was then added in amount of 2 % (by volume of the oil). The mixture was stirred for 20 minutes, The oil/acid mixture was kept at 80 °C up to 15 min, cooled down to 25 °C, Mixed with water (1 %) and transferred to a holding vessel. After settling for 60 min the mixture was centrifuged for 20 min to separate acid degummed oil from its by-product (Zufarov, Schmidt, & Sekretár, 2008).

After degumming process water may present in the oil then drying process using Na_2SO_4 is important in order to remove the water content from the oil.

The percentage oil yield will be calculated using:

% Yield of oil = Mass of oil obtained/ mass of seed used * 100% …….. (5)

3.2.4. Characterization of the oil

A. Moisture content determination

Moisture content of the sample was determined according to EN 14774-1 standard. Empty dish was used and weighed. 2 g of oil was taken on crucible and weighed. Then, the oil sample was oven dried for 105^0C at 2 hours until constant weight is obtained. Moisture content of sample was determined using equation: (Tsegay, 2019).

$$\text{Moisture content, \%} = \frac{W1-W2}{W1} * 100 \quad \text{…….……..} \quad (6)$$

Where: W1 = original mass before oven drying W2 = weight after oven drying

B. Determination of Acid value (AV)

5 g of oil sample was dissolved in 250 mm conical flask with 25 mm ethanol. After adding few drops of phenolphthalein (2 drops) and boiled for few minutes (5 minutes), and titrated with 0.1 N of KOH until the end point of colorless to pink was recognized.

The acid value was calculated using the following equation (Sarin, 2012).

$A.V = (V*N*56.1)/W$ ………………………………………………………….. (7)

Where, V is volume expressed in milliliter of 0.1 N ethanol KOH solutions, N is concentration of ethanol KOH solution, W is mass of oil taken in gram

C. Determination of specific gravity

A stopper density bottle (pycnometer) was filled with distilled water. The weight was taken after losing away any water drops on the bottle. After drying, the bottle was filled with the extracted oil and weighed (Sarin, 2012).

The specific gravity was calculated as follows:

Specific gravity = a –c/b-c ..……………………………………………………… (8)

Where, c is weight of empty density bottle or pycnometer, a is weight of bottle with oil, b is weight of bottle with water

D. Determination of density

Density of oil was determined from the following relation:(Sarin, 2012).

$Density of oil\ (\rho) = (specific gravity * density of water)$ ………….…..……... (9)

E. Determination of Kinematic viscosity

Kinematic viscosity of oil was determined by vibrio - viscometer. The sample was put in the water thermostat until it reaches the equilibrium temperature of 20 °C. The tip of the vibrio-viscometer was inserted in the sample and measures the viscosity of the oil. Viscosity decreases as temperature increases. Hence, kinematic viscosity is the ratio of dynamic viscosity to the density of oil (Tsegay, 2019).

$\mu = \upsilon/\rho$……………………………………………………………………….(10)

Where μ=kinematic viscosity in mm^2/s, υ =dynamic viscosity of oil, mPas (measured by using vibrio-viscometer) and ρ=density of oil ing/cm^3

F. Determination of Saponification value

Saponification value is the number of milligrams of base required to saponify 1 g of fat or oil. 3 g of the sample was weighed into a conical flask. 25 ml of 0.5 M KOH was added from burette by allowing it to drain for a definite period of time. Then a reflux condenser was connected to the flask and boiled gently for 30 minutes. The flask and condenser were allowed to cool and rinsed with little distilled water and the condenser will be removed. 1ml of indicator (5 drops) was added and tittered with 0.5M HCl until the pink color is disappeared. Finally volume of titrant (V1) was recorded(Tsegay, 2019).

The blank was determined with the same quantity of KOH solution under the same conditions. The final result was calculated using the following equation:

$$S.V = 56.1 * \mathrm{N} * (\mathrm{V2} - \mathrm{V1})/\mathrm{W} \quad \text{.....(11)}$$

Where, W = Weight of oil taken, N = Normality of HCL solution, V1 = volume of HCL solution used in the test in milliliter, V2 = volume of HCL solution used in the blank in milliliter and S.V = Saponification value

G. Determination of pH

pH value is the measurement of acidity or basicity of oil. Standard pH meter was used to determine the pH of the oil.

H. Determination of free fatty acid (FFA)

The content of free fatty acid of the oil was determined using the following equation:

$$\%\mathrm{FFA} = \mathrm{A.V}/2 \quad \text{..... (12)}$$

Where, A.V is acidic value of oil

I. Determination of molecular weight

Molecular weight of oil was determined according from the following equation:(Mundhe, 2017).

$$\mathrm{Mwt} = \frac{168300}{\mathrm{SV}-\mathrm{A.V}} \quad \text{.....(13)}$$

Where, S.V = Saponification value of oil and A.V = Acid value of oil

J. Iodine Value

Iodine value of the oil was determined according to EN 14111standard. 0.3 g of oil was dissolved with 20 ml of Carbon tetra chloride (CCl_4) in 250 ml conical flask. 20 ml of 15 % KI and 100 ml distilled water was added after one hour. Then titrated against 0.1 M Sodium thiosulfate by adding 2ml indicator until yellow color was disappeared. Iodine value was calculated using the following equation(Sarin, 2012).

$$I.V = ((B - S) * 0.1 * 12.69))/m \quad \text{(14)}$$

Where, B = Blank titration, S = Sample titration and m = molecular weight of Iodine

K. Caloric Value

Heating value or calorific value (CV) is determined by burning the oil in bomb calorimeter according to ASTM D240 protocol. Benzoic acid is used to standardize the calorimeter. 1 gram of sample was measured and taken in a crucible and made into a pellet, after taking the initial weight, it was placed in the bomb, which was pressurized to 18atm (18.2385 bar) of oxygen. Then, the bomb was placed in a vessel containing 2000 gram of water. The ignition circuit was connected and the temperature of water taken. Ignition temperature rise was taken every minute until a constant temperature attained. The pressure was released and finally, the length of unburned fuse wire was measured (Bello & Otu, 2015).

3.2.5. Biodiesel production process and Experimental design

The most common way to produce biodiesel is transesterification process. Transesterification process is a chemical reaction between one mole triglycerides and three mole alcohols to separate fatty acid from glycerol to produce Fatty acid methyl ester (FAME) and glycerin in the presence or no catalyst. Methanol becomes the first choice for transesterification reaction as its low cost (Tsegay, 2019).

3.2.5.1. Experimental set up and procedures of activity

(i) The experimental set up was adjusted

(ii) Sodium hydroxide catalyst and methanol (20g of NaOH : 1 liter of methanol, 0.5M) was mixed and prepared in the beaker on water bath until complete solution observed.

(iii) Based on the experiment design ratio and level of factors Sodium methoxide solution was added to hot *Maesa Lanceolata* oil and let to settle the solution in order to separate

the biodiesel from glycerol. The reaction was done by using a constant mixing regime of 500RPM.

(iv) The mixture obtained from the reaction was settled for 24 hours in separating funnel

(v) The upper layer (biodiesel) was separated from glycerol (lower part), soap and other residuals and the quantity of biodiesel obtained was measured. The procedure was repeated for all experimental runs by varying oil to sodium methoxide ratio, reaction time and reaction temperature. (Kafuku & Mbarawa, 2010b)

Table 3. 2 Independent variables and level of main factors that affect transesterification reaction

Factor	**Unit**	**Level**	
		Low	**High**
Oil : Sodium methoxide ratio	-	3	5
Reaction Time	minute	20	30
Reaction Temperature	^{0}C	60	70

Table 3. 3 Box Benhken design and optimization parameters.

Std	Run	Factor 1	Factor 2	Factor 3	Response 1
		A:Oil : Sodium methoxide ratio	B:Reaction Time (minutes)	C:Reaction Temperature (^{0}C)	Yield %
10	1	4	30	60	
3	2	3	30	65	
15	3	4	25	65	
8	4	5	25	70	
9	5	4	20	60	
11	6	4	20	70	
16	7	4	25	65	
17	8	4	25	65	
13	9	4	25	65	
12	10	4	30	70	
2	11	5	20	65	

14	12	4	25	65	
6	13	5	25	60	
1	14	3	20	65	
7	15	3	25	70	
5	16	3	25	60	
4	17	5	30	65	

3.2.5.2. Separation (purification) process of biodiesel

After the transesterification reaction was completed, the product formed was the mixture of biodiesel, glycerin, alcohol, catalyst, water and other unreacted residuals. To separate biodiesel from this mixture, different separation techniques were used. When the reaction reached the preset reaction time, heating and stirring was stopped. All the products of the experiments were allowed to settle overnight for 24 hours to enhance separation of biodiesel and unwanted substances. Two distinct liquid phases was formed during separation, with the crude ester phase at the top and the glycerol phase at the bottom. The bottom glycerol phase was removed and the crude methyl esters layer at the top then washed with 1/3 of warm deionized (distilled) water repeatedly until the water becomes clear (Kafuku & Mbarawa, 2010b).

Percentage yield = volume of biodiesel produced/volume of oil used *100

= pure biodiesel/oil used/* 100 ……...…..…….. (15)

3.2.5.3. Characterization of the biodiesel

A. Water and sediment

Water content and sediment was measured according to ASTM D-2709 and limited to 0.050% by volume. Equal volumes of biodiesel and water-saturated toluene were placed into a cone-shaped centrifuge tube. Each of the two centrifuge tubes, 50-mLof biodiesel was added directly from the sample container. By using a pipet 50 ± 0.05 mL of toluene was added, which has been water saturated at 60°C. The tubes in the trunnion cups place on opposite sides of the centrifuge to establish a balanced condition and pinned for 10 min at a minimum relative centrifugal force of 600. After centrifugation, the volume of the higher density water and sediment layer at the bottom of the tube was read (Sarin, 2012).

B. Specific gravity

Specific gravity of biodiesel was determined according to EN 14214 standard. An improvised specific gravity bottle was washed and dried in the oven. The bottle was cooled at room temperature in a desiccators and the weight of the empty bottle was taken. Then, the bottle was filled with water and its weight is taken. Water was poured out and rinsed with acetone and oven dried. Then, it was filled with biodiesel and weighed (Sarin, 2012).

$$S.G = \frac{W3 - W2}{W1}$$

Where: W_3 is weight of bottle and biodiesel, W_2 is weight of empty bottle and W_1 is weight of equivalent volume of water (weight of water)

C. Density

Density of biodiesel was determined according to EN 14214 standard. Density of biodiesel influences the efficiency of fuel combustion. High density fuel has higher energy content (calorific value). Density of biodiesel was determined from the following equation:

Density = specific gravity * density of water.

D. Kinematic Viscosity

Dynamic viscosity of oil (biodiesel) was determined by viscometer according to ASTM D445 standard. Kinematic viscosity of biodiesel was determined using the following equation.

$$\mu = \upsilon/\rho$$

Where, μ is dynamic viscosity, υ is kinematic viscosity (mpa.sec), ρ is density (kg/m^3)

E. Saponification value

3g of biodiesel sample was placed in a 250ml conical flask and 25ml of 0.5M ethanol potassium hydroxide solution was added. A reflux condenser was attached and the flask content refluxed for 30 min on a water bath with continuous swirling until it simmered. The excess potassium hydroxide was titrated with 0.5 hydrochloric acid adding phenolphthalein indicator. A blank determination was carried out under the same condition and the saponification value was calculated from the following equation (Tsegay, 2019).

$$S.V = (B-R)*Mwt.C)/m$$

Where, B = blank titration Mwt = molecular weight of KOH, R = real titration of oil (biodiesel) C = Concentration of KOH solution and m = weight of biodiesel sample

F. Acid value (acid number)

Acid value was determined according to EN14104 standard. 5 gram of biodiesel was added to the mixture of 25 ml of ethanol and 1ml (5 drops) of phenolphthalein in 250 ml conical flask. The mixture was heated for about 7 minutes. The mixture titrated with 0.1 M of KOH with consistent shaking until dark pink color is observed (colorless to pink). Acid value was determined using the following equation according to ASTM D664 standard.

$$A.V = (V*C*Mwt)/m$$

Where, V = volume of titrated solution Mwt = molecular weight of KOH, C = concentration of KOH and m = mass of biodiesel taken

G. Free fatty acid value (FFA)

Free fatty acid of biodiesel was determined from acid value from the following equation.

$$\%\ FFA = (acidvalue)/2$$

H. Calorific value (High Heating Value)

Heating value or calorific value (CV) was determined by burning biodiesel in bomb calorimeter according to ASTM D240 protocol. Benzoic acid was used to standardize the calorimeter. 1 gram of sample was measured and taken in a crucible and made into a pellet, after taking the initial weight, it was placed in the bomb, which was pressurized to 18atm (18.2385 bar) of oxygen. Then, the bomb was placed in a vessel containing 2000 gram of water. The ignition circuit was connected and the temperature of water taken. Ignition temperature rise had taken every minute until a constant temperature was attained. The pressure was released and finally, the length of unburned fuse wire was measured. (Bello & Otu, 2015).

I. Flash point

Flash point of biodiesel was determined according to ASTM D93 standard using Penisky Martens apparatus. 70 ml of the biodiesel was poured into an open metal cup and put on heat source of closed system. Automatic temperature reader machine was attached and read the temperature, recorded. A test flame applicator used to pass flame across it with continuous smooth motion and the temperature at which the sample ignited was recorded as the flash point.

J. Determination of fire point

Fire point is the temperature at which the fuel starts to create continuous burning fire. Also the Fire point of biodiesel was determined using the same procedure with that of flash point determination.

K. Determination of Iodine value

Iodine value was determined according to EN 14111standard. 0.3 g biodiesel was dissolved with 20 ml of Carbon tetra chloride (CCl_4) in 250 ml conical flask. 20 ml of 15 % KI and 100 ml distilled water was added after one hour. Then, titrated against 0.1 M Sodium thiosulfate by adding 2ml indicator until yellow color was disappeared. Iodine value was calculated using the following equation (Sarin, 2012).

$$I.V = ((B - S) * 0.1 * 12.69))/m$$

Where, B = Blank titration, S = Sample titration and m = molecular weight of Iodine

L. Determination of Cetane number

Cetane number of biodiesel was determined according to ASTM D6751 using the following empirical formula.

C.N = 46.3 + 5458/S.V – 0.225 * I.V…….. (16)

Where, S.V = saponification value, I.V= Iodine value and C.N = Cetane number

M. Determination of cloud and pour point

Cloud point was determined according to ASTM D6950. Pour point is the temperature at which the liquid form of sample starts to flow or stop to flow. Pour point was determined according to ASTM D5771.

N. Sulfated ash

Sulfated ash content of the biodiesel sample was determined according to ASTM D4057. 10g of the biodiesel sample was taken and placed on crucibles. The sample is ignited and burned until only ash and carbon remained. After cooling, the residue was treated with sulfuric acid and heated at 775^0C until oxidation of carbon is complete. The ash was cooled and retreated with sulfuric acid and again heated at 775^0C to constant weight for 30 minutes. The sulfated ash was determined by calculating:

Sulfated ash, mass % = $\frac{W1}{W2} * 100$ ……………………………………………(17)

Where: W1 – grams of sulfated ash and W2- grams of biodiesel sample used

O. FT-IR Analysis

FTIR analysis was used to determine the functional groups present in *Maesa Lanceolata* oil and biodiesel. The effectiveness of the transesterification process and change of functional groups were determined using FTIR instrumentation.

3.3. Statistical analysis of Experimental results

The transesterification process parameters were optimized using Response Surface Method (RSM) and the significance of the experimental result was determined from analysis of variance (ANOVA).

The individual effect of process parameters on yield of biodiesel was analyzed both numerically and graphically using Box Behinken design method. The interaction effect of the process parameters on the yield of biodiesel; effect oil to sodium methoxide ratio at constant temperature and time, effect of temperature at constant oil to sodium methoxide ratio and time and effect of reaction time at constant oil to sodium methoxide ratio and temperature was discussed using design expert software.

CHAPTER FOUR

4. RESULT AND DISCUSSION

4.1. Plant material preparation and proximate analysis

The moisture content of the plant seed (*Maesa Lanceolata*) from the table 4.1 as shown below, was found to be 2.67 ± 0.35 % which was lower than 5.02 ± 0.13% compared to the study reported by (Bayisa & Bultum, 2022) studied on the same plant *Maesa Lanceolata* Seeds. In addition, the moisture content of a given plant seeds determines the combustibility, maturity and quality of the seed. High moisture content decreases the heating (calorific) value of the product. Moreover, it forms glycerol and decreases yields of biodiesel during transesterification process. According to (Niju et al., 2019) the appropriate range of moisture content of feed stock is 0.5 to 10.The Ash content, volatile matter and fixed carbon were determined accordingly and the results were found as 4.47 ± 0.37 %, 79.88 ± 0.03, and 12.98 %, respectively.

The ash content value of the studied plant is similar to (Manju, et al, 2018) done on *Moringa Oliefera* plant seed with result of 4.45%. Ash is the remained impurity after the burned fuel. High ash content affects the combustion efficiency of fuels by causing slagging and clinkering. Average ash content of the fuel is 3 % - 10 %. The result of the ash content agreed with the range limit specified.

The Volatile matter of plants seed affects the ignitability of the biodiesel, the result found agreed with (Jenkins et al., 1998) range of volatile matter of biomass which is 70 -80 %.

Fixed carbon (FC) determines the estimate of heating value of fuels. Fuels that have high carbon content have higher flame length and easy to ignite. In addition the fixed carbon value indicated that the feed stock has good fuel properties and is promising resource for biofuel production.

Table 4. 1 Proximate analysis of *Maesa Lanceolata* plant seed

s.no	Parameters	Result (%)
1	Moisture content	2.67 ± 0.35 %
2	Ash content	4.47 ± 0.37 %
3	Volatile matter	79.88 ± 0.03
4	Fixed carbon	12.98 %

4.2. Oil yield determination of *Maesa Lanceolata* Seed

Table 4. 2 Extracted and oil yield of *Maesa Lanceolata* seed

Exp run	Powder to solvent ratio	Temperature (^{0}C)	Extraction time (hour)	Yield (%)
1	1:5	70	5	33.34
2	1:5	70	6	36.96
3	1:5	80	5	32.67
4	1:5	80	6	32.67
5	1:6	70	5	28.87
6	1:6	70	6	29.12
7	1:6	80	5	31.11
8	1:6	80	6	31.20

The maximum oil yields of 36.96% were obtained at the temperature of 70^0C, 6 hours of reaction time, pore size of 0.25mm, and powder to solvent ratio of 1: 5 as shown in above table 4.2. The result agreed with the previous work done by (Bayisa & Bultum, 2022). However, the maximum oil yield was found by this study to some extent more than the past findings done. The result also showed that solvent to solid ratio, extraction time, temperature and particle size had influence on the yield of oil. Increasing the temperature will increasing the rate of diffusivity through the cell walls of the seed, decreasing solvent density, and increasing mass transfer and vapor pressure of the solutes. Due to this, the effect of temperature difference was observed having impact upon the oil extraction processes. As the time of the extraction process increased, the rate of diffusion will be decreased as the concentration of the oil increased in the solvent. Eventually, the extraction process is said to have reached its saturation capacity when the highest oil yield is obtained and remained unchanged. Moreover the particle size have effect on the yield as the smaller the size, the greater is the interfacial area between the solid and liquid, and therefore the higher is the rate of transfer of material and the smaller is the distance the solute must diffuse within the solid as already indicated (Bayisa & Bultum, 2022).

4.3. The Physico-chemical Characteristics of *Maesa Lanceolata* seed Oil

The physical and chemical characteristics of the extracted oil were summarized below in the table 4.3:

Table 4. 3 The physical and chemical characteristics of Maesa Lanceolata seed oil.

s.no	Parameters	Measurement units	Experimental values	ASTM standard
1	pH		7.36 ± 0.05	----
2	Moisture content	%	3 ± 1	----
3	Specific gravity	-	0.917 ± 0.0014	----
4	Density	g/cm^3	0.917 ± 0.0014	0.957-0.968
5	Acid value	mg KOH/g oil	1.39 ± 0.179	0.4 - 4
6	% Free fatty acids	--	0.696 ± 0.089	0.2-2
7	Saponification value	mg KOH/g oil	190.68 ± 3.631	175-187
8	Kinematic viscosity	mm^2/sec	10.90	6.3-8.8
9	Molecular weight	g/mol	888.68	----
10	Iodine value	$mgI_2/100g$ oil	118.38	82-88
11	Caloric Value	MJ/Kg	38.7934	

We can observe that the pH of the extracted *Maesa Lanceolata* seed oil was 7.36 ± 0.05around the neutral zone that could show us that the oil was free from acidity or basicity properties i.e, acidic conditions creates corrosion and higher pH value makes the oil more basic and this makes soap during transesterification reaction (Patil et al., 2009).

The moisture content of the extracted oil was found to be 3 ± 1% which was less than as compared to other non edible oils like Moringa, Castor and Jatropha oil studies carried out by (Balat, 2011; Bayisa & Bultum, 2022). Indeed high water content of the oil affects the quality of biodiesel by forming more soap and difficulty in separation process of the FAME from the glycerol.

The specific gravity of the oil was 0.917 ± 0.0014 which was lighter than pure water and agreed with the result found by (Bayisa & Bultum, 2022) studied on the same plant material.

Additionally the density measurement of the oil was within the limits of ASTM requirements which were 0.917 ± 0.0014 g/cm^3.

The acid value of oil is the measure of number of carboxylic acid groups such as free fatty acid. The acid value measures the content of free acids in the sample, which have influence on fuel aging (Shalaby, 2013). The acid value of the study showed that 1.39 ± 0.179 mg KOH/g oil which was good for transesterification process to proceed with one step without pretreatment processes that efficiently matches with the ASTM standard. Also the FFA has significant effect on the transesterification of glycerides with alcohol using catalyst. The high FFA content (>1%w/w) will cause soap formation and the separation of products will be exceedingly difficult, and as a result, low yield of biodiesel product would be obtained(Ahmad et al., 2011). The result of the study was 0.696 ± 0.089 which was within the ASTM standard limit for biodiesel feedstock.

Saponification is used to determine the total acid content, both free and combined, of fatty acids (acid number only measure the free fatty acids) of fat. The combined acids are primarily esters formed by reaction with the neutral components present in the original fat. The SV is therefore a measure of fatty acid quantity. The Saponification value of the extracted oil was found to be 190.68 ± 3.631 mg KOH/g oil, which was higher than the previous finding (Bayisa & Bultum, 2022) and the ASTM standard.

The kinematic viscosity of the purified oil was found as10.90 mm^2/sec (10mpas); and out of the ASTM standard limit for oil products which was viscous and needed extra purification process. Viscosity of oil affects the storage and handling of fuel oil. As reported by the works of (Knothe et al., 2018) highly viscous oil is difficult for pumping and to operate in engine.

The iodine value of the oil extracted from *Maesa Lanceolata* was obtained to be 118.38 mgI_2/100g oil. This result is an indication for the presence of high amount of unsaturated fatty acids (double bonds) in the oil.

Furthermore, the caloric value of *Maesa Lanceolata* seed oil was found 38.7934 MJ/Kg which was high and a promising value for energy uses.

4.4. Preparation and Characterization of the Biodiesel

Table 4. 4 the physical and chemical characteristics of Biodiesel made from Maesa Lanceolata seed oil comparison against standard requirements.

Property	Units	Experimental results	Biodiesel Standards	
			ASTM 6751	EN 14214
Water and sediment	% volume	0.025	0.05 max	0.05 max
Density at 15^0C	Kg/m^3	885.7	-	860 - 900
Specific gravity	-	885.7	-	-
Acid value	mgKOH/g	0.64	0.80 max	0.5 max
free fatty acid	% mgKOH/g	0.32	-	-
Kinematic viscosity at 40^0C	mm^2/s	6.7	1.9 - 6	3.5 - 5
Saponification value	mgKOH/g	180.27	370 max	-
Iodine value	g I_2 /100 g	111.83	-	120 max
Cetane number	-	51.41	47 min	51 min
Flash point	^{0}C	135	130 min	101 min
Fire point	^{0}C	146.	-	120 min
Cloud point	^{0}C	8.6	Report	Report
Pour point	^{0}C	0.8	Report	Report
Caloric value	MJ/Kg	44.6635	-	-
Sulfated ash	% mass	0.016	0.02 max	0.02ax

4.4.1. Water and sediment

The water and sediment value of the studied biodiesel was 0.025% that agreed with the EN14214 standards of maximum limit of 0.05% by volume. As biodiesel is hygroscopic, it can absorb water in concentrations up to 1000 ppm during storage. Appreciable amounts of water and sediment in a fuel oil tend to cause fouling of the fuel-handling facilities and cause trouble for the fuel system of a burner or engine. An accumulation of sediment in storage tanks and on filter screens can obstruct the flow of oil from the tank to the combustor that supported by (Sarin, 2012).

4.4.2. Specific gravity and Density

The specific gravity and Density at 15^0C was obtained 0.8857 g/cm^3 which was within the standard limit of EN 14214 (860 – 900 Kg/m^3 or 0.86 – 0.9 g/cm^3). Density of the biodiesel influences the efficiency of fuel combustion and also affects fuel quality and fuel injection system (Sarin, 2012)

4.4.3. Acid value and free fatty acid

By using EN14104 standard method of the synthesized biodiesel acid vale were found around 0.64 mg KOH/g oil, the free fatty acid value was around 0.32 both results were agreed with ASTM D6157 maximum limit for acid value and free fatty acid value respectively. The parameter characterizes the degree of fuel ageing during storage, as it increases gradually due to degradation of the biodiesel. High fuel acidity has been discussed in the context of corrosion and the formation of deposits within the engine which is why it is limited in biodiesel specifications (Sarin, 2012; Tsegay, 2019)

4.4.4. Kinematic viscosity

Viscosity is a measure of resistance to flow of a liquid due to internal friction caused by one part of a fluid moving over another. This is a critical property because it affects the behavior of fuel injection. The kinematic viscosity value of *Maesa Lanceolata* was found as 6.7 mm^2⁄s which was slight higher than both ASTM (1.9 - 6 mm^2⁄s) and EN14214 (3.5 - 5 mm^2⁄s) standard specification of Biodiesel fuels. The deviation of the results can be overcome by blending with diesel for usages. A higher viscosity leads to poorer fuel atomization, can cause larger droplet sizes, poorer vaporization, a narrower injection spray angle, and greater in-cylinder penetration of the fuel spray. A low viscosity can result in an excessive wear in injection pumps and power loss due to pump leakage whereas high viscosity may result in excessive pump resistance, filter blockage, high pressure, coarse atomization and low fuel delivery rates (Sarin, 2012).

4.4.5. Saponification value

Saponification is used to measure the total mixture of combined and free fatty acids and which are primarily esters formed by reaction with the neutral components present in the original fat. The saponification value being found as 180.27 ± 1.23 mg KOH/g oil that showed reduction from the oil saponification value of 190.68 ± 3.63 mg KOH/g oil due to transesterification

process. Also the saponification value of the determined biodiesel agreed with the ASTM standard maximum limit of specification of 370 mgKOH/g.

4.4.6. Iodine value

The Iodine value of the study was about 111.83 g I_2 /100 g comfortably agreed with the standard of EN14214 (120 max).The Iodine value is simply a measure of total unsaturation, while oxidative stability is more strongly affected by the number of FAME molecules having multiple double bonds. The iodine number is an index of the number of double bonds in biodiesel which determines the unsaturation degree of the biodiesel. This property can greatly influence the oxidation stability and polymerization of glycerides. This can lead to the formation of deposits in diesel engines injectors. Iodine value is directly correlated to biodiesel viscosity, Cetane number and cold flow characteristics (cold filter plugging point).The iodine number is set to a maximum value of 120mgI2/g according to EN 14111 (Atabani et al., 2013)

4.4.7. Cetane number

Cetane number (CN) is widely used as a diesel fuel quality parameter related to the ignition delay time and combustion quality. CN is a measure of the ignition quality of diesel fuel during combustion ignition (Colombo et al., 2017). Based on the formula of C.N = 46.3 + 5458/S.V – 0.225 * I.V the calculated value of the Cetane number was found 51.41 which satisfied both the ASTM and EN14214 standard minimum requirements for biodiesel fuels.

4.4.8. Flash and Fire (ignition) point

Flash point of a fuel is the temperature at which it will ignite when exposed to a flame or a spark. The flash point is the lowest temperature at which fuel emits enough vapors to ignite. Fire or Ignition Point the temperature at which an oil sample will continue to burn on its own without the application of additional external heat—expressed in degree Celsius (°C) (Firestone, 2013). The flash point of study was found to be 135^0C, and the fire point was around 146.5^0C. The studied value agreed with ASTM D6157 and EN14214 standards limits of 130 and 101 ^{0}C respectively. Flash point varies inversely with the fuel's volatility. Biodiesel has a high flash point which is usually more than150^0C, while generally conventional diesel fuel has a flash point of 55–66 ^{0}C (Atabani et al., 2013).

4.4.9. Cloud and pour point

The cloud point of biodiesels starts at -1.11°C to 0.55°C for most of the vegetable oils that are made up primarily of mono- or poly-unsaturated fatty acid chains and can go as high as 26.67°C or higher for animal fats or frying oils that are highly saturated. The cloud point of *Maesa Lanceolata* biodiesel was determined and found as 8.6°C and the pour point was 0.8 °C. Crystals formed in biodiesel or diesel fuel can drift to the bottom of the tank and begin to build up a gel layer. Slow agitation can prevent crystals from building up on the tank bottom or, once present in the fuel, agitation can help to dissolve crystals back into solution (Handling, 2004).

4.4.10. Caloric value

Calorific value is an important parameter in the selection of a fuel. The heat of combustion or hot worth of a fuel is a very important measurable parameter that shows the quantity of heat liberated by the fuel at intervals the engine that allows the engines to try and do the work. The caloric value of the study was found around 44.6635 MJ ⁄ Kg, however, the heat content (Calorific value) of biodiesel ranges from is 32-42 MJ/kg, which is significantly above the heat content of crude oil diesel in MJ/kg (Atabani et al., 2013; Knothe & Steidley, 2005).

4.4.11. Sulfated ash

The sulfated ash content of the biodiesel was done and found to be 0.016%. The ASTM standard and maximum limit is 0.02% and our study results agreed with the standard. The Ash content describes the amount of inorganic contaminants such as abrasive solids and catalyst residues, and the concentration of soluble metal soaps contained in the fuel. These compounds are oxidized during the combustion process to form ash, which can contribute to injector, fuel pump, piston and ring wear, and also to engine deposits (Sarin, 2012).

4.4.12. Fourier Transform Infrared Ray (FTIR) analysis

In figure 6 and 7 as shown below, the result of FTIR analysis showed that both the oil and biodiesel formation of the *Maesa Lanceolata* seed had similar results. However, the carbonyl (C=O) group of the oil parts was 1742.86 cm^{-1} and changed to 1741.80cm^{-1} in the case of biodiesel formation. Furthermore, the ester group (C-O) in the oil was found peak value of 1158.64cm^{-1} and changed to 1680.00cm^{-1} in biodiesel formation. This changes depicted us that

the oil was efficiently undergoes transesterification and process changed to Biodiesel. The result also supported by (Tsegaye, 2019) in the study of biodiesel production from castor oil.

Within the wave number range of 1650 -1540cm^{-1}, there is no any peaks observed, thus shows us that no soap was formed in the biodiesel sample. Moreover, no broad peaks were observed in the region of 2500-3300 cm^{-1} indicating that the low concentration of hydrogen impurities such as water, methanol, free glycerol and fatty acids. The result also supported by (Neju et al) study done on

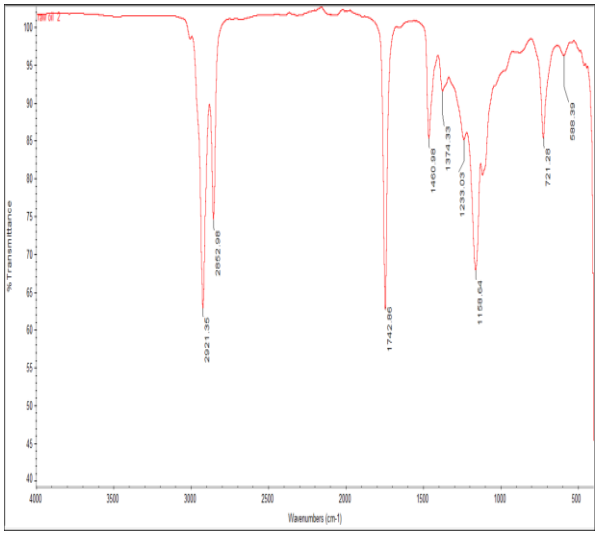

Figure 4. 1 FTIR spectrum of *Maesa Lanceolata* seed oil

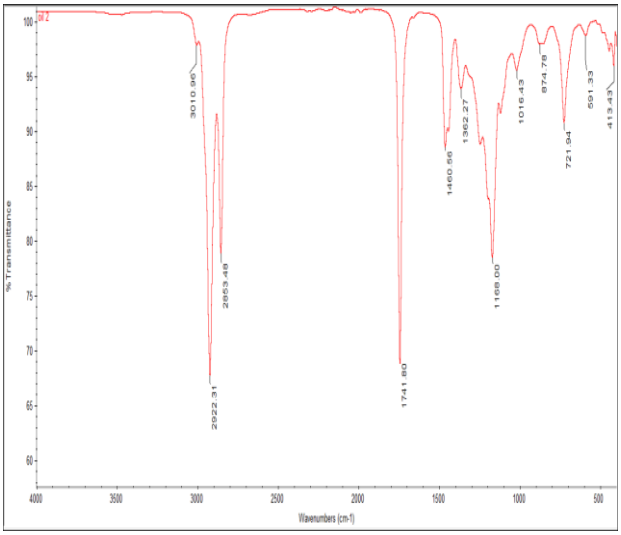

Figure 4. 2 FTIR spectrum of Biodiesel made from *Maesa Lanceolata* seed oil

4.5. Statistical Analysis of Study variables

4.5.1. ANOVA for Quadratic model

Table 4. 5 Analysis of variance (ANOVA) for quadratic model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1,222.26	9	135.81	214.20	< 0.0001	significant
A-Oil : Sodium methoxide ratio	60.50	1	60.50	95.42	< 0.0001	
B-Reaction Time	3.55	1	3.55	5.60	0.0499	
C-Reaction Temperature	9.40	1	9.40	14.82	0.0063	
AB	0.1122	1	0.1122	0.1770	0.6866	

AC	0.0000	1	0.0000	0.0000	0.9952	
BC	1.0000	1	1.0000	1.58	0.2495	
A^2	943.14	1	943.14	1,487.55	< 0.0001	
B^2	32.99	1	32.99	52.03	0.0002	
C^2	103.96	1	103.96	163.97	< 0.0001	
Residual	4.44	7	0.6340			
Lack of Fit	3.00	3	1.00	2.79	0.1737	not significant
Pure Error	1.44	4	0.3591			
Cor Total	1,226.70	16				

Factor coding is coded.

Sum of squares is Type III - Partial

The Model F-value of 214.20 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B, C, A^2, B^2, C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve the model.

The Lack of Fit F-value of 2.79 implies the Lack of Fit is not significant relative to the pure error. There is a 17.37% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good -- the model is fit.

4.5.2. Fit Statistics

Table 4. 6 Model adequacy measures

Std. Dev.	0.7963	R^2	0.9964
Mean	87.57	Adjusted R^2	0.9917
C.V. %	0.9093	Predicted R^2	0.9590
		Adeq Precision	38.9254

As shown above in table 4.6 the Predicted R^2 of 0.9590 is in reasonable agreement with the Adjusted R^2 of 0.9917; i.e. the difference is less than 0.2.Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Our ratio of 38.925 indicates an adequate signal. This model can be used to navigate the design space.

4.5.3. Coefficients in Terms of Coded Factors

Table 4. 7 Regression coefficients and significance of response surface quadratic model

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	98.27	1	0.3561	97.43	99.11	
A-Oil : Sodium methoxide ratio	-2.75	1	0.2815	-3.42	-2.08	1.0000
B-Reaction Time	0.6663	1	0.2815	0.0006	1.33	1.0000
C-Reaction Temperature	1.08	1	0.2815	0.4181	1.75	1.0000
AB	-0.1675	1	0.3981	-1.11	0.7739	1.0000
AC	0.0025	1	0.3981	-0.9389	0.9439	1.0000
BC	-0.5000	1	0.3981	-1.44	0.4414	1.0000
A^2	-14.97	1	0.3880	-15.88	-14.05	1.01
B^2	-2.80	1	0.3880	-3.72	-1.88	1.01
C^2	-4.97	1	0.3880	-5.89	-4.05	1.01

The coefficient estimate depicted in table 4.7 represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1; VIFs greater than 1 indicate multi-colinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable.

4.5.4. Final Equation in Terms of Coded Factors

$$\text{Yield (\%)} = +98.27 - 2.75A + 0.6663B + 1.08C - 0.1675AB + 0.0025AC - 0.5BC - 14.97A^2 - 2.8B^2 - 4.97C^2$$

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

4.5.5. Final Equation in Terms of Actual Factors

$$\text{Yield (\%)} = -1{,}093.072 + 117.787A + 7.16525B + 26.55355C - 0.0335AB + 0.0005AC - 0.02BC - 14.9665\,A^2 - 0.11196B^2 - 0.19876C^2$$

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space.

4.5.6. Diagnosis of Model adequacy test

The normal probability plot is a graphical method used to check whether the error occurred during the experimental analysis is normally distributed throughout the experiment. The normality assumption of analysis of variance (ANOVA) is valid if all the data points lie along the straight line of the normality plot of residuals. As shown below the normal probability distribution in Figure 4.3, most of the points of the studied variables results were on the graph are fitted to the straight line. This feature shows us that the quadratic model satisfies the assumption of analysis of variance. The distribution of the error is approximately normal.

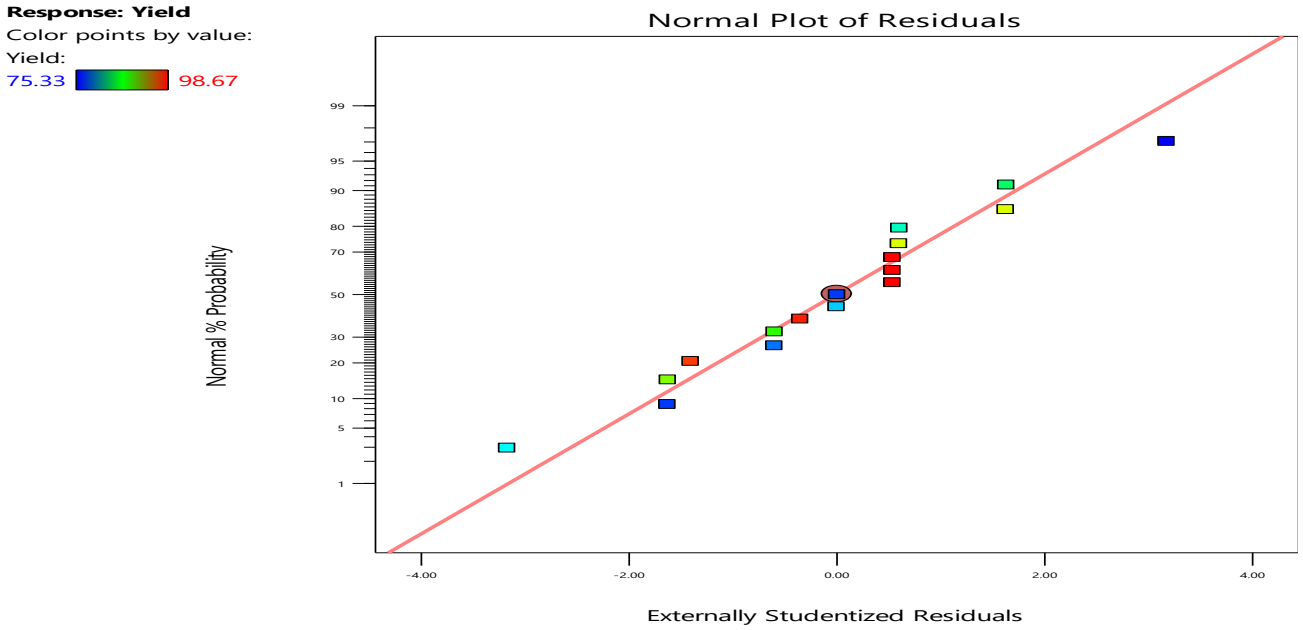

Figure 4. 3 Normal probability plot of residuals

A model is considered to be perfect if the slope of the line is unit (all data points are perfectly along the line) which corresponds to zero error. The graph of Predicted versus actual plot figure 4.4 is the correlation between actual experimental data and predicted value. The line on the plot has a line of perfect fit. The points on the line correspond to zero error between actual and predicted value. Therefore, the regression model equation approved accurate description of experimental data.

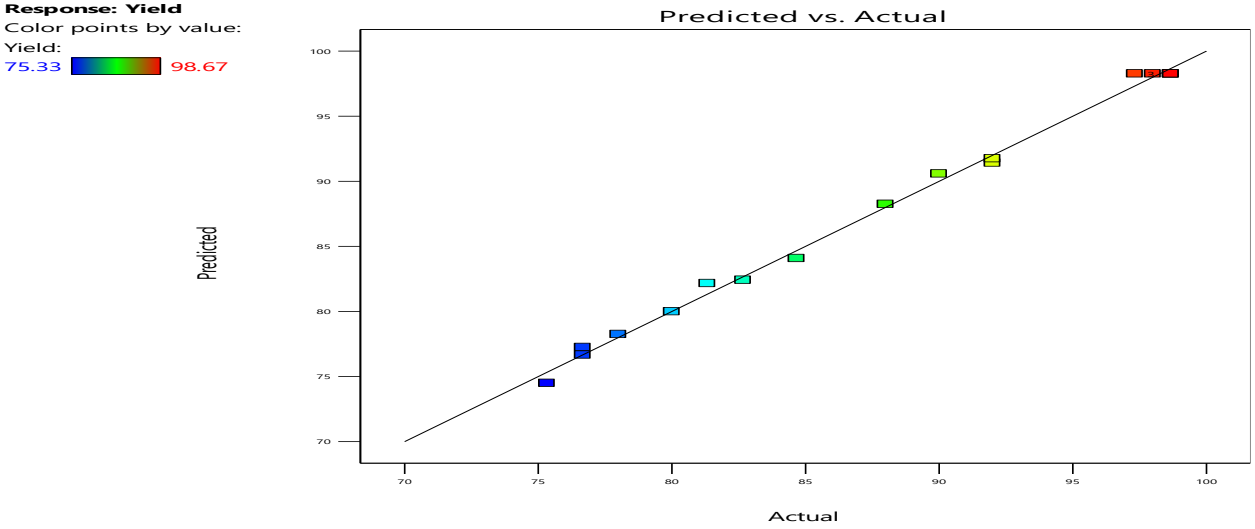

Figure 4. 4 Predicted versus actual value plot

The plot of the residual versus predicted value is also another graphical technique used to assess the validity of the model. The model developed to describe the process is considered to be perfect if the residuals are structure less. Residuals versus predicted value plot shows that the residuals are less structured and they are unrelated to any of other variables including the predicted value. Hence, figure 4.5 showed a random scatter distribution of points and justifying that no need for alteration of minimizing personal error.

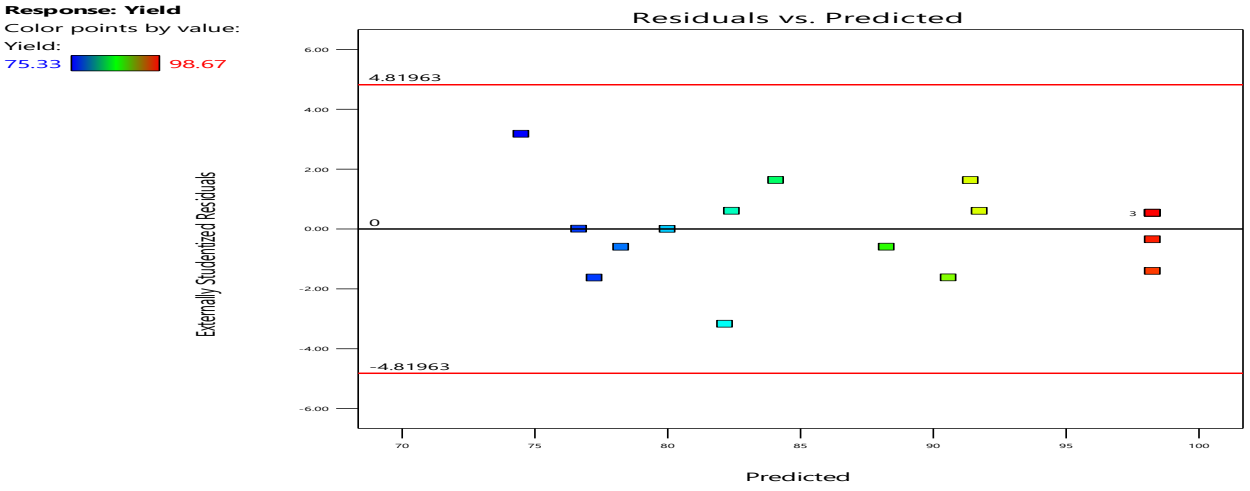

Figure 4. 5 Residual versus predicted value plot

4.6. Optimization of *Maesa Lanceolata* Biodiesel yield

Response surface method found 100 solutions and selected the optimum values to minimize the economic cost of temperature, sodium methoxide solution, and reaction time required for transesterification reaction and to maximize the yield of biodiesel. The selected optimum yield of biodiesel was observed at oil to sodium methoxide solution of 4.983, Temperature of 61.181°C and reaction time of 21.266 minutes shown below in table 4.8 and 4.9.

Table 4. 8 Optimization goal and limit of process variables

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A:Oil : Sodium methoxide	is in range	3	5	1	1	3
B:Reaction time	is in range	20	30	1	1	3
C:Temperature	is in range	60	70	1	1	3
Yield	none	75.33	98.67	1	1	3

Table 4. 9 Optimum numerical solutions by RSM

Number	Oil : Sodium methoxide	Reaction time	Temperature	Yield	Desirability	
1	**4.983**	**21.266**	**61.181**	**75.150**	**1.000**	**Selected**
2	3.000	25.000	70.000	82.164	1.000	
3	5.000	25.000	60.000	74.496	1.000	
4	4.000	25.000	65.000	98.268	1.000	
5	5.000	25.000	70.000	76.669	1.000	
6	4.000	20.000	60.000	88.250	1.000	
7	4.000	20.000	70.000	91.418	1.000	
8	4.000	30.000	70.000	91.750	1.000	
9	5.000	30.000	65.000	78.251	1.000	
10	4.000	30.000	60.000	90.582	1.000	
11	3.000	30.000	65.000	84.086	1.000	
12	3.000	25.000	60.000	80.001	1.000	

13	5.000	20.000	65.000	77.254	1.000	
14	4.988	25.220	61.376	77.576	1.000	
15	4.106	21.438	68.258	94.756	1.000	
16	3.300	24.995	61.551	89.739	1.000	
17	3.699	29.494	62.542	94.611	1.000	

4.7. The effect of different factors on Biodiesel Yield

4.7.1. The effect of Oil to Sodium methoxide ratio on Biodiesel yield

Oil to sodium methoxide ratio is one of the main factors that affect transesterification reaction. Figure 4.6 shows the effect of oil to sodium methoxide ratio on biodiesel yields at constant temperature and reaction time. It indicated that the yield of biodiesel increases as oil to sodium methoxide ratio increases, however, increasing oil to sodium methoxide ratio beyond optimum value results in excess methanol and catalyst which decreases the yield of biodiesel and increase the cost of post -production treatment. Furthermore, oil to sodium methoxide ratio makes the separation process difficult and causes excessive soap formation in the biodiesel products. The maximum yield of biodiesel (98.67 %) was obtained at oil to sodium methoxide ratio of 4: 1.Therefore, this ratio was taken as an optimum value of oil to sodium methoxide ratio. On the other hand, decreasing oil to sodium methoxide ratio below the optimum value (3:1) decreased the conversion efficiency of the transesterification reaction and resulted in lower biodiesel yield. Similar reports were observed in (Atabani et al., 2013) findings approving that the effects of molar ratio variation (oil to methanol) on the biodiesel yield percentages.

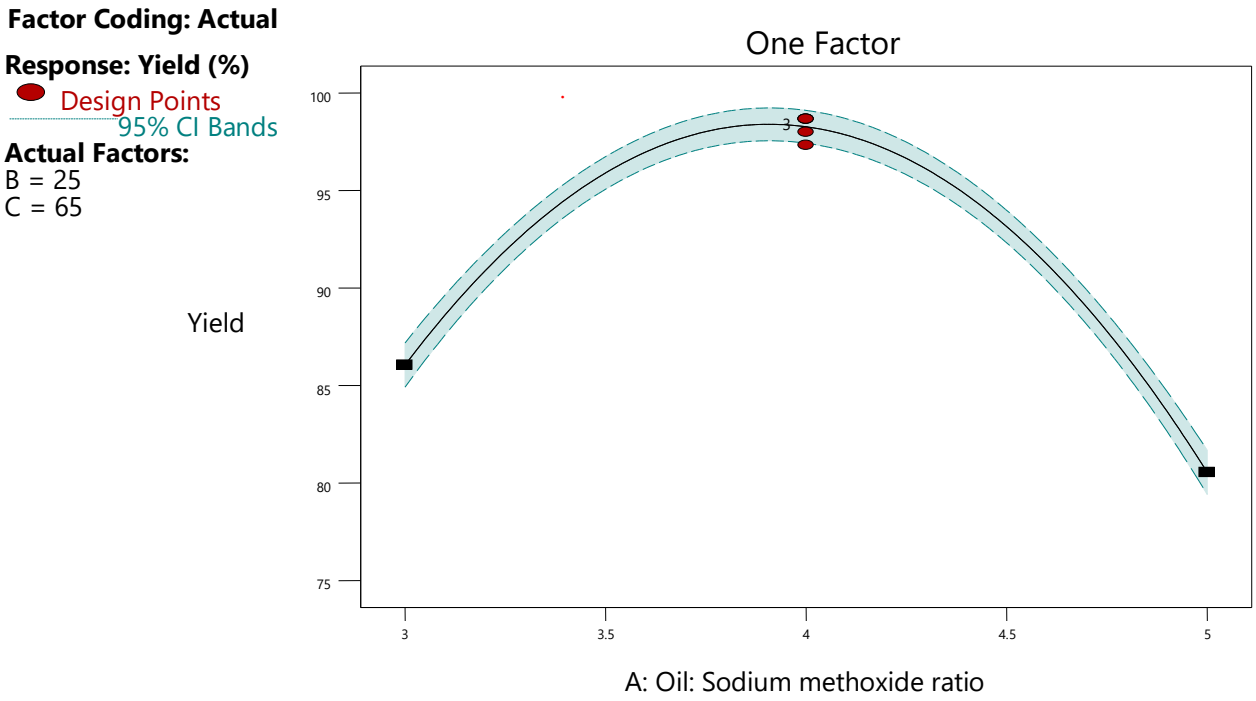

Figure 4. 6 the effect of oil to sodium methoxide ration on the biodiesel yield.

4.7.2. The effect of reaction time on Biodiesel yield

The effect of reaction time at constant oil to sodium methoxide ration and temperature on the biodiesel yield of Maesa Lanceolata was briefly shown in figure 4.7. It showed that as the reaction time increased from 20 to 25 minutes the biodiesel yield increases. The maximum yield of biodiesel (98.67 %) was obtained reaction time of 25 minutes. However, increasing the reaction time beyond the optimum range them yield value declined steadily because of more mixing disturb the formation of the biodiesel. As reported on the finding of (Demirbas, 2005) reaction time had influences on the Biodiesel yield (ester conversion) that supported this findings too.

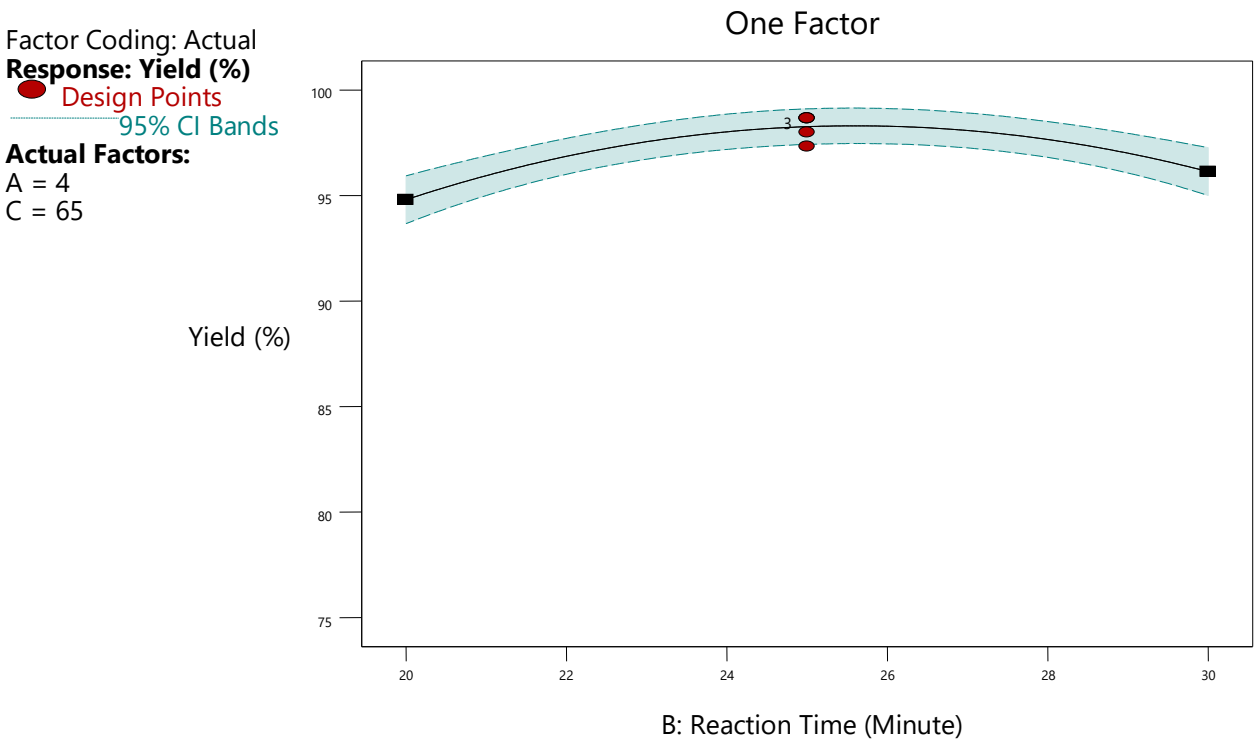

Figure 4. 7 the effect of reaction on biodiesel yield.

4.7.3. The effect of Reaction Temperature on Biodiesel yield

The effect of reaction temperature was observed in the study as shown below in Figure 4.8 on yield of biodiesel at constant oil to sodium methoxide ratio and reaction time. The rate of transesterification reaction is highly influenced by reaction temperature. The rate of the reaction and the yield increased as temperature increased from 60 °C to 65 °C. Maximum yield (98.67 %) was obtained at 65 °C and the yield started to decrease slightly from 65 °C to 70 °C due to the vaporization of methanol results in soap formation. The boiling point of methanol is 64.7 °C. Increasing reaction temperature above this point leads to methanol loss by evaporation and decreases the conversion efficiency of reaction. A research carried out by (Patil et al., 2009) showed that the biodiesel yield was increased with increasing reaction temperature up to a

certain level and then decreased, also the findings agreed with this result.

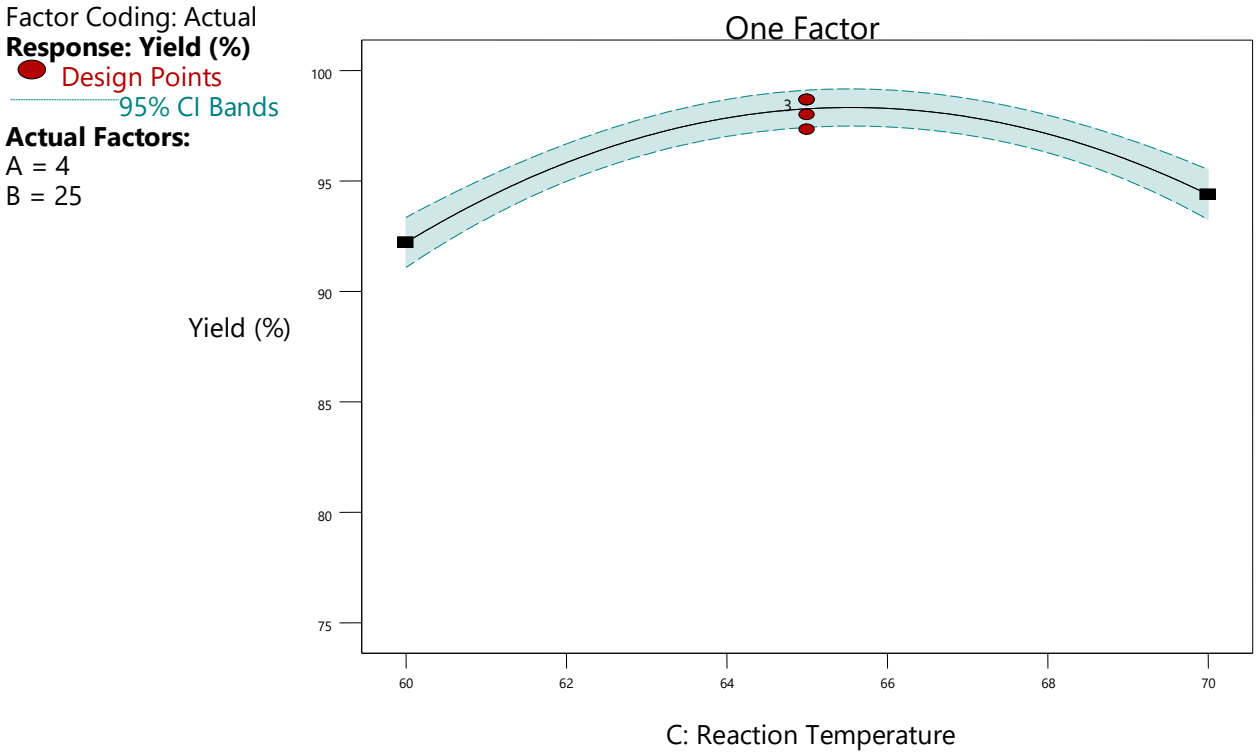

Figure 4. 8 the effect of reaction temperature on biodiesel yield.

4.8. The effect of Interaction between process variables

The analysis of variance (ANOVA) as given in table 4.5 shows that the yield of biodiesel was highly affected by the interaction between oil to sodium methoxide ration, reaction temperature and reaction time.

4.8.1. The effect of interaction between oil to sodium methoxide solution ratio and Reaction time

The effect of interaction between oil to sodium methoxide solution ratio and reaction time observed below in figure 4.9 at constant temperature of 65^0C. The response surface is curved because the model contains quadratic terms that are statistically significant. The highest value of yield of the *Maesa Lanaceolata* biodiesel found in the middle of upper plot, which corresponds with the middle value of reaction time and oil to sodium methoxide solution ratio. The lowest values of yield for biodiesel are in the lower right corner of the plot, which corresponds with the low value of reaction time and high value of oil to sodium methoxide

solution ratio. The 3D surface plot indicates us that as the yield increases from 76.67% to 98.67%, oil to sodium methoxide ratio decreased from 5 to 4 (reversely increasing the methanol volume) as the reaction time increased from 20 minutes to 25 minutes. This implies that the effect of interaction between reaction time on the yield of biodiesel was positive. This result is also conveyed in the analysis of variance (ANOVA). According to stoichiometry, excess methanol is generally preferred to drive the reaction forward and increases the conversion. On the other hand, excess methanol present in the reaction mixture for a longer time would solubilize the glycerol and favors glycerolysis which decreases the biodiesel conversion (Niju et al., 2019).

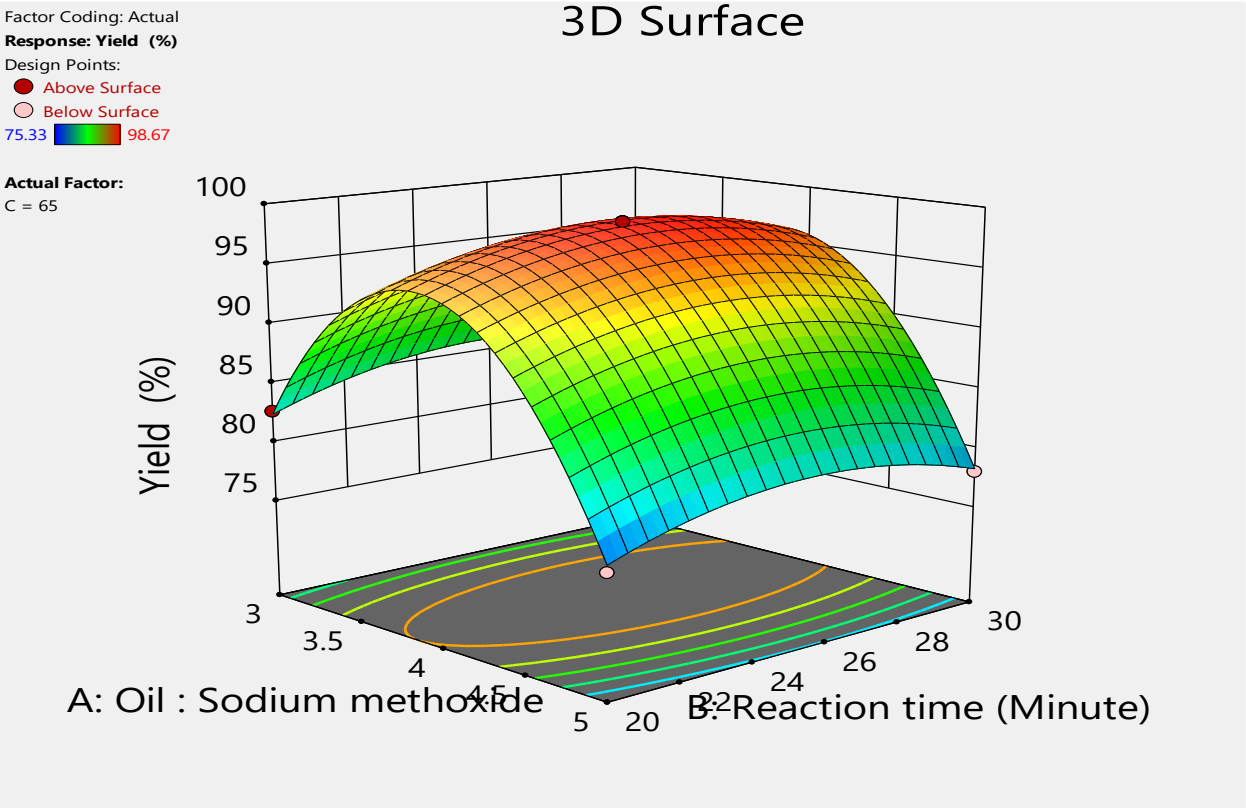

Figure 4. 9 Effect of interaction between oil to sodium methoxide solution ratio and Reaction time

4.8.2. The effect of interaction between oil to sodium methoxide solution ratio and Reaction temperature

The effect of interaction between oil to sodium methoxide solution ratio and reaction temperature observed below in figure 4.10 at constant time of 25minutes. The response surface

is curved because the model contains quadratic terms that are statistically significant. The highest value of yield of the *Maesa Lanaceolata* biodiesel found in the middle of upper plot, which corresponds with the middle value of reaction temperature and oil to sodium methoxide solution ratio. The lowest values of yield for biodiesel are in the lower right corner of the plot, which corresponds with the low value of reaction temperature and high value of oil to sodium methoxide solution ratio. The 3D surface plot indicates us that as the yield increases from 75.33% to 98.67%, oil to sodium methoxide ratio decreased from 5 to 4 (reversely increasing the methanol volume) as the reaction temperature increased from 60^0C to 65^0C. This implies that the effect of interaction between reaction temperatures on the yield of biodiesel was positive, however, the interaction between yield and oil to sodium methoxide ratio is negative (inversely positive with methanol ratio). This result is also conveyed in the analysis of variance (ANOVA).As reported by (Niju et al., 2019) that at higher catalyst concentration and insufficient reaction time results in lower yield. However, increasing the reaction time influences the biodiesel yield.

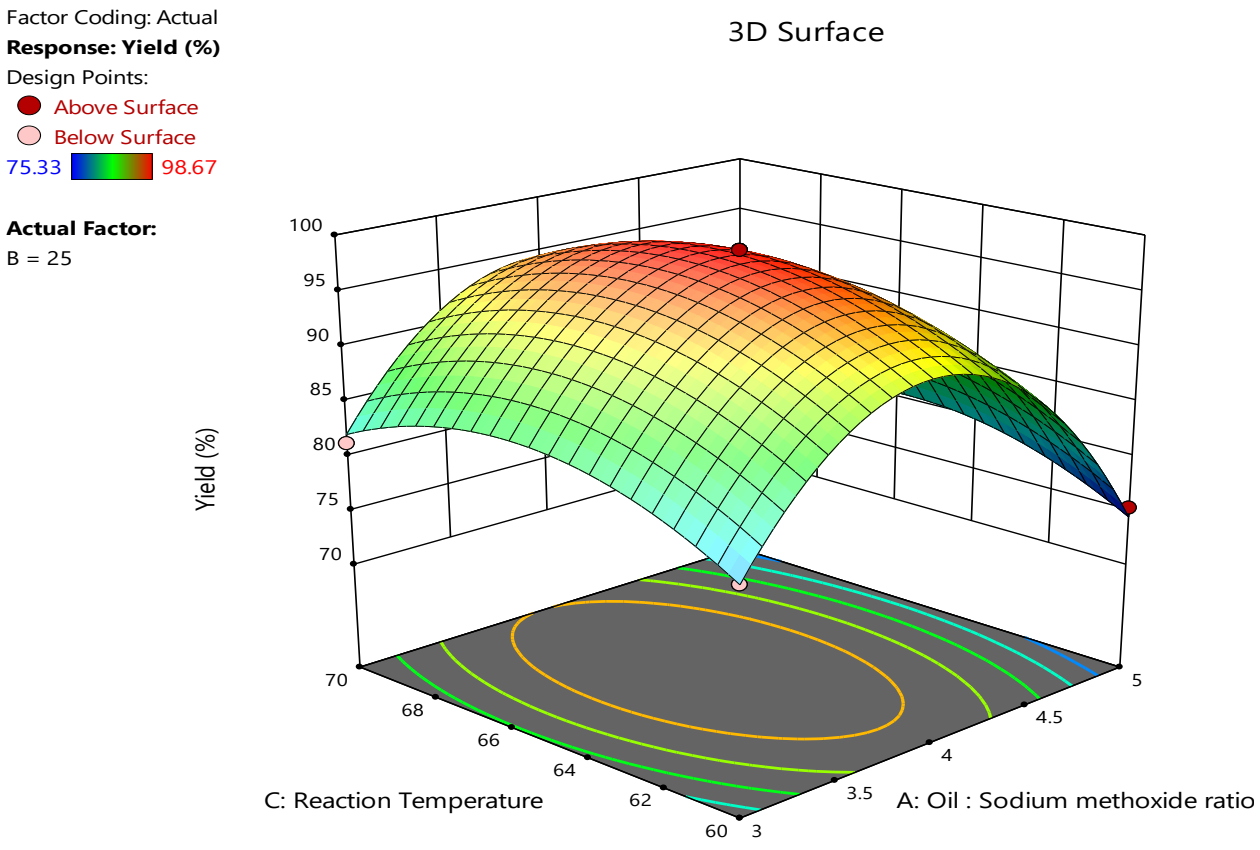

Figure 4. 10 Effect of interaction between oil to sodium methoxide solution ratio and Reaction temperature

4.8.3. The effect of interaction between Reaction time and Reaction temperature

The effect of interaction between reaction temperature and reaction time observed below in figure 4.11 at constant oil to sodium methoxide solution ratio of 4. The response surface is curved because the model contains quadratic terms that are statistically significant. The highest value of yield of the *Maesa Lanaceolata* biodiesel found in the middle of upper plot, which corresponds with the middle value of reaction time and reaction temperature. The lowest values of yield for biodiesel are in the lower middle of the plot, which corresponds with the low value of both reaction time and temperature. The 3D surface plot indicates us that as the yield increases from 88% to 98.67%, reaction temperature increases from 60^0C to 65^0C as the reaction time increased from 20 minutes to 25 minutes. This implies that the effect of interaction between reaction time and reaction temperature on the yield of biodiesel was positive. This result is also conveyed in the analysis of variance (ANOVA).

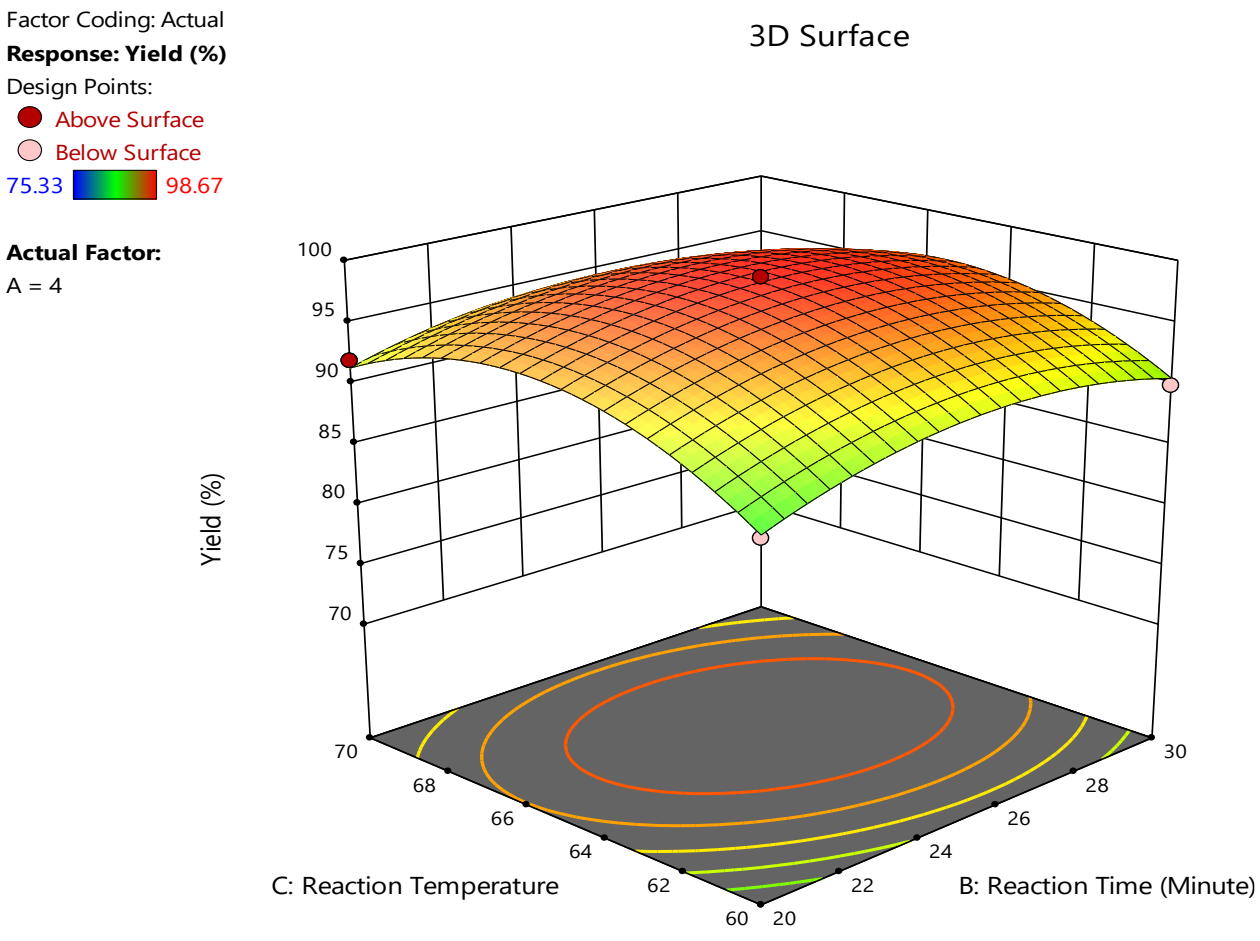

Figure 4. 11 Effect of interaction between Reaction time and Reaction temperature

CONCLUSSION AND RECOMMENDATION

Conclusion

Maesa Lanceolata seed oil is among the non edible oil which has a great potential for biodiesel synthesis due to its fatty acid profile that found in the oil which was comparable to the well known oil feedstocks for biodiesel synthesis. The plant is indigenous to Ethiopia and for many other African countries which was used for many different purposes like medicinal plants. Thus, using of the plant seed oil as a feedstock for biodiesel production can be economically feasible, alternative sore to fossil fuel and economical source income for the local peoples.

In this study, oil was extracted from *Maesa Lanceolata* seed using solvent extraction method like soxhlet extraction by varying different study parameters like pore size, solvent to powder ratio, reaction temperature and time to obtain the maximum yield of 36.96%. The physical and chemical characterization was done on the oil sample and the results were promising for biodiesel production.

The extracted oil was converted to biodiesel using catalytic transesterfification process using methanol and catalyst sodium hydroxide solutions. An optimum biodiesel yield of 98.67% was obtained by varying different parameters like oil to sodium methoxide solution ratio, reaction temperature and time and also there effects were determined. For significance of study, ANOVA and Box Behnken standard design for optimization of process parameters was done by response surface method (RSM) and tested for the significance. Most of the characteristics of the biodiesel synthesized meet the standard limit of ASTM and EN requirements. However, kinematic viscosity of the biodiesel deviates from acceptable requirements which could overcome by blending with the diesel for end users.

Recommendation

This study which was done on the preparation and characterization of biodiesel made from *Maesa Lanceolata* seed oil is a new investigation study and needs further analysis by varying different study variables. The promising oil content of the plant material, the fatty acid profile content and widespread forestation of the plant in the country make it astonishing study area for further investigation to be done ahead.

Thus, to make the production of biodiesel from *Maesa Lanceolata* seed oil technically, economically and Environmentally feasible, the researcher believes that the following improvements shall be under take:-

- Different investigation should be under taken on how to improve the yield of the plant and oil, expansion of the cultivation of the plant in other area of the country.
- Comparative study on mechanical and solvent extraction methods.
- The effect of mixing speed (intensity) on the yield of biodiesel
- Further study should be done on engine test to determine fuel consumption efficiency, brake thermal efficiency and exhaust gas emissions
- Government and concerned body should give training on plantation, utilization and multipurpose use of *Maesa Lanceolata* plants.

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APPENDICES

Appendices A. Figures for raw material preparations

Figure A, Maesa Lanceolata seed before drying

Figure B, Maesa Lanceolata seed after drying

Figure C, powder form of*Maesa Lanceolata* seed

Figure D, crude oil in the presence of solvent-petroleum ether

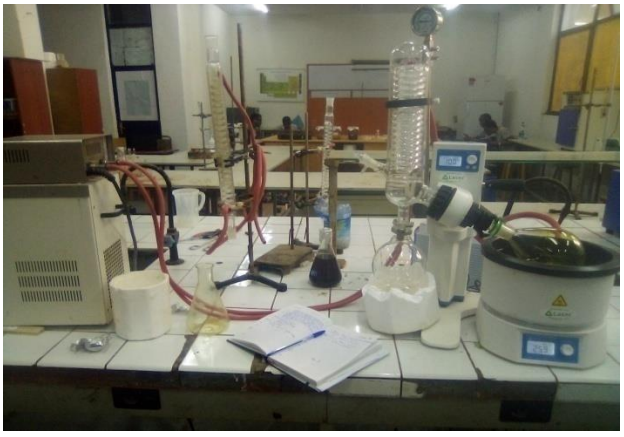

Figure E, Rotary Evaporator machine

Figure F, Maesa Lanceolata seed oil before purification

Figure G, Maesa Lanceolata seed oil after purification

Figure H, Titration for acid value, iodine value,

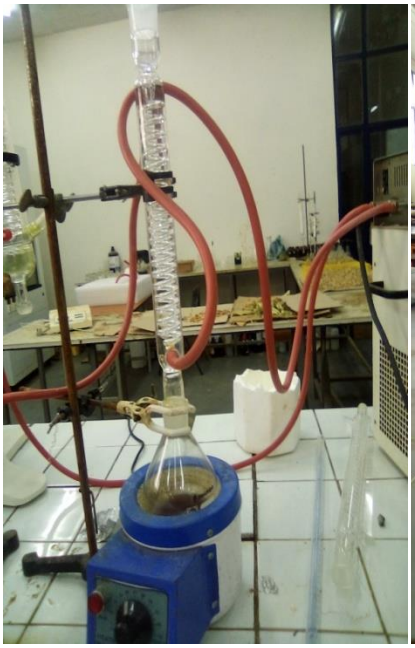

Figure I, Saponification,

Figure J, Pycnometer,

(k) Transesterification process (L) Biodiesel before separation (M) pure biodiesel

(O) Flash point, fire point. Pour point and cloud point determination

(P) Optimization of biodiesel sample (Q) Vibro viscometer (R) FTIR

Appendices B. *Maesa Lanceolata* seed oil characterization and calculation

Parameters	Formula	Replicates		
		1st	2nd	3rd
Moisture content	Moisture content, % = $\frac{W1 - W2}{W1} * 100$. Where, W1= original mass before oven drying, W2 = weight after oven drying	2%	4%	3%
pH		7.4	7.4	7.3
Specific gravity	Specific gravity = a –c/b-c Where, c is weight of empty density bottle or pycnometer a is weight of bottle with oil b is weight of bottle with water	0.9185	0.9157	0.9168
Density	Density of oil (ρ) = (specific gravity ∗ density of water)	0.9185	0.9157	0.9168
Molecular weight	$\text{Mwt} = \frac{168300}{\text{SV} - \text{A.V}}$	888.68		
Acid value	$A.V=(V*N*56.1)/W$ Where, V is volume expressed in milliliter of 0.1 N ethanol KOH solutions N is concentration of ethanol KOH solution W is mass of oil taken in gram	1.3	1.6	1.28
Saponification value	$S.V = 56.1 * N * (V2 - V1)/W$ W = Weight of oil taken N = Normality of HCL solution V1 = volume of HCL solution used in the test in milliliter V2 = volume of HCL solution used in the blank in milliliter S.V = Saponification value	191.8	186.62	193.62
Kinematic viscosity	$\mu = \upsilon/\rho$ μ = kinematic viscosity, mm^2/s υ = dynamic viscosity of oil, mPa.s ρ = density of oil, g/cm^3	11.80	11.00	9.90
Iodine value	$I.V= ((B-S) *0.1*12.69))/m$ Where, B = Blank titration S = Sample titration and m = molecular weight of Iodine	120.12	118.46	116.56
free fatty acid	$\%\text{FFA} = \text{A.V}/2$ A.V is acidic value of oil	0.65	0.80	0.64

- X (mean) = $\frac{\sum xi}{n}$
- Variance (δ^2) = $\frac{\sum(Xi-X)2}{N-1}$
- Standard deviation = $\sqrt{\delta^2}$

Appendices C. *Maesa Lanceolata* Biodiesel characterization and calculation

Parameters	Formula	Replicates		
		1st	2nd	3rd
Water and sediment	Reading the graduated cylinder to the nearest point.	0.025%	0.025%	0.025%
Specific gravity	**Specific gravity = a –c/b-c** Where, c is weight of empty density bottle or pycnometer a is weight of bottle with oil b is weight of bottle with water	0.88	0.8739	0.8739
Density	Density of oil (ρ) = (specific gravity ∗ density of water)	0.88	0.8684	0.8684
Acid value	$A.V=(V*N*56.1)/W$ Where, V is volume expressed in milliliter of 0.1 N ethanol KOH solutions N is concentration of ethanol KOH solution W is mass of oil taken in gram	0.64	0.64	0.64
free fatty acid	$\%\mathbf{FFA} = \mathbf{A.V/2}$ A.V is acidic value of oil	0.32	0.32	0.32
Saponification value	$S.V = 56.1 * N * (V2 - V1)/W$ W = Weight of oil taken N = Normality of HCL solution V1 = volume of HCL solution used in the test in milliliter V2 = volume of HCL solution used in the blank in milliliter S.V = Saponification value	178.87	181.18	180.78
Kinematic viscosity	$\mu = \upsilon/\rho$ μ = kinematic viscosity, mm^2/s υ = dynamic viscosity of oil, mPa.s ρ = density of oil, g/cm^3	6.7	6,7	6,7
Iodine value	$I.V= ((B-S)*0.1*12.69))/m$ Where, B = Blank titration S = Sample titration and m = molecular weight of Iodine	109.12	114.54	111.83
Flash point	The lowest temperature to form a flash (^{0}C)	136	134	135
Fire point	the lowest temperature burn continuously for 5 seconds(^{0}c)	148	145	146.5
Cloud point		8.6	8.6	8.6
Cetane number	**C.N = 46.3 + 5458/S.V – 0.225 * I.V** Where, S.V = saponification value I.V= Iodine value C.N = Cetane number	51.41		
Pour point		0.9	0.8	0.8
Caloric value	MJ/Kg	44.6635	44.4548	
Sulfated ash	Sulfated ash, mass % = $\frac{W1}{W2} * 100$ Where: W1 – grams of sulfated ash and W2- grams of biodiesel sample used	0.01%	0.02	0.02

- X (mean) = $\frac{\sum xi}{n}$
- Variance (δ^2) = $\frac{\sum(Xi-X)2}{N-1}$
- Standard deviation = $\sqrt{\delta^2}$

Appendices D. Biodiesel optimization and calculation

Sequence no.	Oil: sodium methoxide solution	Reaction time (minutes)	Temperature (^{0}C)	Volume of oil used (ml)	Volume of FAME produced (ml)	% yield
1	3	20	65	150	124	82.67%
2	5	20	65	150	115	76.67%
3	3	30	65	150	127	84.67%
4	5	30	65	150	117	78%
5	3	25	60	150	120	80%
6	5	25	60	150	113	75.33%
7	3	25	70	150	122	81.33%
8	5	25	70	150	115	76.67%
9	4	20	60	150	132	88%
10	4	30	60	150	135	90%
11	4	20	70	150	138	92%
12	4	30	70	150	138	92%
13	4	25	65	150	146	97.33%
14	4	25	65	150	147	98%
15	4	25	65	150	148	98.67%
16	4	25	65	150	148	98.67%
17	4	25	65	150	148	98.67%

% yield = $\frac{\text{volume of FAME produced}}{\text{Volume of oil used}}$ * 100

Appendices E. Outsourced tests and results

Company Name: ETHIOPIAN PETROLEUM SUPPLY ENTERPRISE QUALITY ASSURANCE SERVICE	Document No: OF/EPSE/QAS/033	
	Page 1 of 1	
TITLE: CERTIFICATE OF TEST REPORT		
Issue No.: 2	Date of Issue: August,2020	
Prepared by: QT	Approved by: Tadesse H/Mariam	

Sample Originator: ADAMA SCIENCE AND TECHNOLOGY UINIVERSITY	**Request No**: 7/2-2-1/7m80/9087/15
Sample Type: BIODISEL	**Analytical Method**: ASTM /IP/ISO.
Sample Number: 01	**Analytical Result**: Interpreted with stds.
Lab. Number: 2023- ADO-EXT-13	**Report No**: QA/0015/2023
Sample Submitted By: CUSTOMER	**Test date**: 24/04/2023
Date Submitted: 19/04/2023	**Issued Date**: 24/04/2023
Customer Address: ADAMA	**Telephone**. +251-931-020748

S.N	Property	Test method ASTM	Limits	Test results of the BIODIESEL sample	
				Maesa Lanceolata Seed Oil	MU
1	Density@15°C, g/mL	D4052	Report	0.8857	0.0002
	Density@20°C, g/mL	D4052	Report	0.8817	0.0002
2	Kinematic viscosity @ 40 °c	D 445		6.761	
3	Calorific value, calc, BTU/LB	Calculated		19,198	

Liability:- Ethiopian Petroleum supply Enterprise, its Laboratory, its Servants or agents Shall not be held liable for any damages, loss, claims or expenses direct or indirect howsoever caused, arising in connection with the laboratory test carried out on BIODIESEL sample submitted to it for analysis whether in tort or in contract, due to any act, omission or error of whatever nature, whether or not negligence and howsoever caused. Furthermore all expenses and implied warranties are specifically disclaimed. This test report shall not be reproduced or given to a third party without written approval of the Petroleum Quality Assurance Service.

Lab. Expert
Name--
Signature--

Senior expert
Name--
Signature--

Quality Control Head
Name--
Signature--

PLEAS MAKE SURE THAT THIS IS THE CORRECT ISSUE BEFORE USE

	GEOLOGICAL INSTITUTE OF ETHIOPIA	Doc. Number: GLD/F5.10.2	Version No: 1
	Geochemical Laboratory Desk		Page 1of 1
Document Title:-	**Hydrocarbon Analysis Report**	**Effective date:**	**Nov. 2022**

Customer Name:- Ephrem Debissa Yadessa
Sample type: -Liquid
Sample Preparation:- 60 Mesh
Date Submitted:-19/04/2023
Elements to be determined:-(Calorie),
Method of analysis:- Adiabatic Calorie Metter

Issue Date:- 04/05/2023
Request No:-GLD/RN/1165/23
Report No:- GLD/TR/17452/23
Number of Sample: -Two (02)

Collectors' Code	Calorific Value Cal/gm.	Weight of Sample
Biodesel	9,925.55	
Oil	9,265.66	

Note: - This result represent only for the sample submitted to the laboratory.

Analysts
Bethelhem Tefera
Shashe Haile

Approved By
Alemnesh Abate

Quality Control
Negash Worku