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Addis Ababa Institute of Technology (AAiT)

School of Chemical and Bio-Engineering

Production and Characterization of Biodiesel by Hybridization of *Moringa stenopetala* and *Azadirachta indica* Seed Oil

By: Degnechew Genene Demisu

Advisor: Dr.-Ing. Shimelis Kebede Kassahun (Asst. Professor)

Thesis submitted to School of Chemical and Bioengineering, Addis Ababa Institute of Technology, in partial fulfillment for the requirements of Degree of Master of Science (MSc) in Chemical Engineering (Environmental Engineering)

July, 2021
Addis Ababa, Ethiopia

DECLARATION

I declare that this Thesis is the result of my original work and it is submitted to Addis Ababa University, Addis Ababa Institute of Technology, in partial fulfillment of the requirements of Degree of Master of Science (MSc) in Chemical Engineering (Environmental Engineering). The Thesis has not been submitted to this University or any other Institutions, anywhere, for the award of any academic Degree, Diploma or Certificate. I have followed all ethical and technical principles of scholarship in the preparation, data collection, data analysis and interpretation and compilation of this Thesis. Any scholarly material included in the Thesis has been given recognition through citation and all sources have been duly acknowledged. The Thesis is deposited in the Addis Ababa University Library and is made available at the University's Library under the rules of the Library.

Signature: ____________________

Degnechew Genene Demisu

Date: 27/07/21

This is to certify that the above declaration made by the candidate is correct and the Thesis has been submitted for examination with my approval as the University advisor.

Advisor: Dr.-Ing. Shimelis Kebede Kassahun

Signature: ____________________

Date: 29/07/21

DEDICATION

This Thesis is dedicated to my beloved family and all my best regards.

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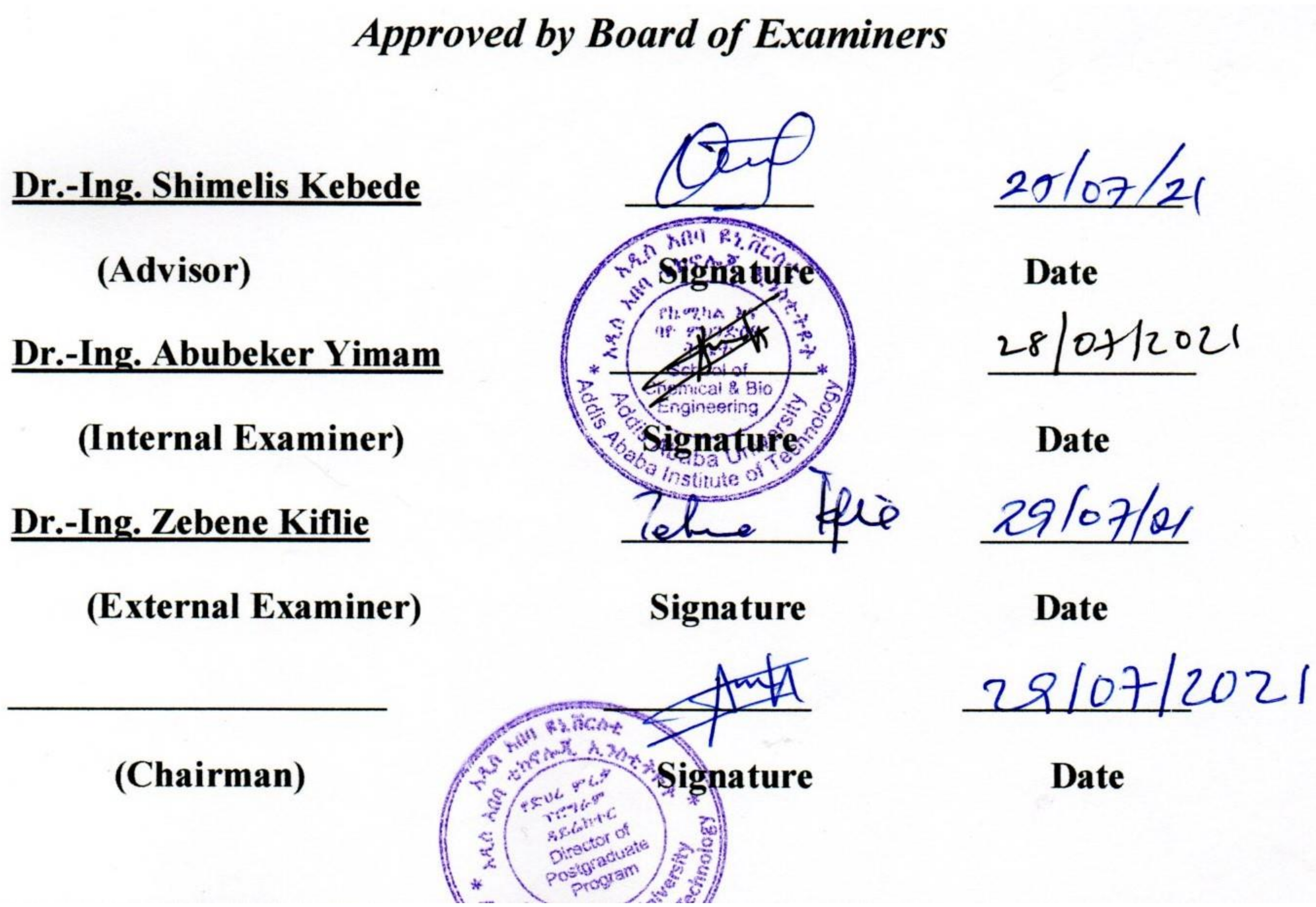

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ABBREVIATIONS AND SYMBOLS

$(C_2H_5)_2O_3$ – Diethyl ether

AAiT – Addis Ababa Institute of Technology

AAU – Addis Ababa University

Adj- R^2 – Adjusted coefficient of determination

ADO – Automotive Diesel Oil

ANOVA – Analysis of Variance

AOAC – Association of Official Analytical Chemists

ASTM – American Society for Testing Materials

BBD – Box-Behnken Design

BMN – Biodiesel of hybridized *M. stenopetala* and *A. indica* seed Oil

$C_{16}H_{34}$ – Hexadecane

$C_{20}H_{14}O_4$ - Phenolphthalein indicator

C_2H_5OH – Ethanol

C_6H_{14} – n- Hexane

CCl_4 – Carbon tetrachloride

CDM – Clean Development Mechanism

CH_3OH – Methanol

CO_2 – Carbon dioxide

CRGE – Climate Resilient Green Economy

CV – Coefficient of Variation

EIAR – Ethiopian Institute of Agricultural Research

EPSE – Ethiopian Petroleum Supply Enterprise

EU – European Union

FAME – Fatty Acid Methyl Ester

FAO – Food and Agricultural Organization

FDRE – Federal Democratic Republic of Ethiopia

FFA – Free Fatty Acid

FTIR – Fourier- Transform Infrared Radiation

GHG – Greenhouse Gas

H_2SO_4 – Sulfuric acid

H_3PO_4 – Phosphoric acid

HCl – Hydrochloric acid

HDPE – High Density Poly Ethylene

ICRAF – International Center of Research Agroforestry

ISO – International Standard Organization

IV – Iodine value

$KOCH_3$ – Potassium methoxide

KOH – Potassium hydroxide

LDPE – Low Density Poly Ethylene

LSD – Least Significance Difference

MAE – Micro-wave Assisted Extraction

MGR – Motor Gas Regular

MN – Hybridized Oil of M. stenopetala and A. indica Seed oil

$Na_2S_2O_3$ – Sodium thiosulfate

NaCl – Sodium chloride

$NaOCH_3$ – Sodium methoxide

NaOH – Sodium Hydroxide

NH_4Cl – Ammonium chloride

NH_4OH – Ammonium hydroxide

NRC – National Research Council

OPEC – Organizations of Petroleum Exporting Countries

PAH – Poly Aromatic Hydrocarbons

PFD – Process Flow Diagram

PPE – Personnel Protective Equipment

Pred- R^2 – Predicted coefficient of determination

R^2 - Coefficient of determination

RI – Refractive Index

RoW – Rest-of-the-World

RSM – Response Surface Methodology

Symbols and units:

°C – Degree Celsius

C_p – Central replication point

g – Gram

g/ml – Gram per milliliter

GHz – Giga Hertz

Kg – Kilogram

Kg/m^3 – Kilogram per cubic meter

Km- Kilometer

L – Liter

mg/Kg – milligram per Kilogram

MHz – Mega Hertz

MJ/Kg – Mega Joule per kilogram

MJ/Kg – Mega Joule per kilogram

ml – Milliliter

mm – Millimeter

mm^2/s - millimeter squared per second

mPa.s – milliPascal-second

ppm – parts per million

rpm – revolution per minute

ρ – Density

ABSTRACT

Biodiesel (Fatty acid alkyl ester) has been regarded as cleaner, alternative, renewable, biodegradable and environmentally benign energy source. It can replace petrol-diesel and solve challenges accompanied with environmental pollution, climate change, socio-economic, energy crisis and global warming. The aim of this study was production and characterization of biodiesel by hybridization of *M. stenopetala* and *A. indica* seed oil to substitute conventional diesel fuel with greener, alternative and eco-friendly energy source. The statistical software package Design-Expert® Response Surface Method (RSM), Box-Behnken Design (BBD) method, was employed in experimental design and result analysis. The considered factors of the study were: Oil mixing composition, Catalyst dose and Reaction time with three levels for each factor, low (-1), medium (0) and high (+1); and the other operational parameters, alcohol to oil molar ratio, temperature and mixing speed, were kept constant. The interaction effects of factors and their influences on response were studied. The considered oil hybrid compositions subjected to the transesterification reaction were: $M_{25}N_{75}$ (25%v/v *M. stenopetala* seed oil : 75%v/v *A. indica* seed oil), $M_{50}N_{50}$ (50%v/v *M. stenopetala* seed oil : 50%v/v *A. indica* seed oil) and $M_{75}N_{25}$ (75%v/v *M. stenopetala* seed oil : 25%v/v *A. indica* seed oil) with their corresponding biodiesel, $BM_{25}N_{75}$, $BM_{50}N_{50}$, $BM_{75}N_{25}$; catalyst dose of 1 %w/v, 1.5 %w/v and 2 %w/v; and reaction time of 20 min, 40 min and 60 min. A total of 15 number of runs were conducted with 3 levels for each numeric factors. The quadratic model was developed for conducted experiment with statistical significance, P - $value < 0.0001$. The analysis of variance (ANOVA) and coefficients of determinations were used to evaluate the quality of the model, where the main comparison was conducted at 5% LSD. The model adequacy was expressed using, $R^2 = 0.9933$, Adj- $R^2 = 0.9812$, $Pred$- $R^2 = 0.8993$ and lack of fit test with P - $value = 0.1071$ (statistically not significant). The value of $Pred$- $R^2 = 0.8993$ was adequately in agreement with the value of $R^2 = 0.9933$, showed that the developed quadratic model equation can be used to navigate the design space. The optimum, 91.72% biodiesel yield was obtained at oil mixing composition (57.17 %v/v of *M. stenopetala* and 42.83 %v/v of *A. indica* seed oil), catalyst dose (1.88 %w/v KOH) and reaction time (60 min). The evaluation of FTIR and physicochemical characteristics were conducted for the optimized product according to ASTM and EN 14214 standard test methods; and the obtained results were in agreement with the standard limit of biodiesel quality.

Keywords: *Azadirachta indica*, Biodiesel, Catalyst, Hybridization, Methanol, *Moringa stenopetala*, n- Hexane, Transesterification

CHAPTER ONE

1. INTRODUCTION

1.1. Background Information and Justification

Energy is an indispensable commodity and the principal driver of socio-economic growth and development of all nations across the globe. Any aspects of human activity is associated with energy. The operation of heavy duty machines, power generating plants, transport vehicles, mechanized agriculture induces the production and utilization of large amount of energy (OPEC Bulletin, 1994). The geometric increase in number of world population has caused a subsequent increase in the demand of energy which in turn lead to inadequate supply of energy (Yahya A.B., 2013). The consequence of inadequate energy supply can also be detrimental to the economy of the globe (Ayoola A. *et al*., 2017).

Fossil fuels (i.e. Crude oil, coal and natural gas) have been a principal source of energy supply for some years now (Carraretto C. *et al.*, 2004). Nevertheless, these fossil fuels are non-renewable sources of energy, limited in their supply, poses several environmental and health problems, unevenly distributed across the world (i.e. more concentrated in some countries than others). Hence, countries which do not have such fossil fuel sources are imposed to import crude oil while encountering a number of challenges. For instances, high cost of procurement and foreign exchange crisis accompanied by importing petroleum fuels (Singh S.P. and Singh D., 2010). Moreover, the depletion of crude oils, global energy crisis, unstable energy prices, harmful gaseous emission and environmental pollution are the main problems induced by utilization of conventional fossil fuels (Barnwal and Sharma, 2005; Amish *et al.*, 2009). The consumption of about 89 million barrel of petroleum was anticipated per day worldwide in 2012 (Balat M., 2009). At this consumption rate, the crude oil resources are estimated to run out within the next 50 years (Arifn Y. *et al.*, 2014). Therefore, with geometric population growth and coping with energy demand, several countries are striving to search for alternative, cleaner, renewable, environmentally friendly and greener energy sources (Balat M., 2009; Kirtay E., 2011). To this end, production and utilization of biomass energy or biofuels (i.e. biodiesel, bio-ethanol and bio-oil) have been regarded as alternative and eco-friendly energy sources, thereby substituting crude oil (Hossain *et al.*, 2008).

Furthermore, Ethiopia, as one of the developing country, has had vision for greening its economy or Climate Resilient Green Economy (CRGE) strategy based on its national Growth and Transformation Plan (GTP), 2010, in which the country seeks to reach middle-income status by 2025 (Federal Democratic Republic of Ethiopia [FDRE], 2010). The CRGE aims to strengthen the improvement of agriculture, sustainable management of natural resources and poverty reduction. Thus, the ambitious CRGE strategy rests on the four major pillars: First, Improving agricultural practices to enhance food security; Second, Protecting and re-establishing forests for ecosystem services, including enhanced carbon stocks and for direct economic benefits; Third, Expanding electricity generation from renewable sources; and Fourth, Leapfrogging inefficient technologies and instead implementing modern, energy-efficient technologies in transport, industry and infrastructure. Incorporated in the fourth pillar of the CRGE strategy, the country is striving toward decarbonizing transport fuel via production and utilization of biofuels (i.e. biodiesel and ethanol). As per planned strategy, the implementation of 5% biodiesel and 15% ethanol blends by 2030 would substitute 0.28 billion liters of diesel and 0.09 billion liters of gasoline. During the second Growth and Transformation Plan (2015/16-2019/20), for development of biodiesel in Ethiopia, it was planned to identify about 23 million hectares of land for Biofuel production. Hence, about 16.6 million hectares of land is partially planted and being planted with non-edible Biodiesel production feedstocks, such as Jatropha, Castor, Moringa and Neem (*Azadirachta indica*) tree.

Therefore, in compliance with the CRGE of Ethiopia, the rationale of the present study was biodiesel production by hybridization of oils from locally available feedstocks (i.e. *Moringa stenopetala* and *Azadirachta indica* seeds) as cleaner, alternative, biodegradable, domestic fuel, renewable and eco-friendly energy source with improved quality. In this study, synthesizing biodiesel by blending the considered feedstocks paves a way to new approach towards biodiesel production and utilization. Biodiesel production by mixing *M. stenopetala* and *A. indica* seed oil at various mixing composition has not been tried so far; and hence, it can be regarded as alternative way of exploiting biodiesel (fatty acid methyl ester) as alternative and renewable energy source from locally available feedstocks.

1.2. Statement of problem

Challenges associated with the hastened global warming, energy insecurity, the rise in crude-oil prices, environmental pollution, climate change mitigation and scarcity of fossil fuels have provoked the growing interests in the development and utilization of alternative, sustainable and renewable energy sources, such as bioenergy or biofuels world-wide. In a developing countries like, Ethiopia, the increased in demand of energy, reliance on imported petroleum fuel for transport, increasing the price of crude-oil (i.e. immense amount of foreign currencies are spent to import the diesel fuel), crude-oil scarcity, environmental air pollution due to emissions of harmful gaseous into atmosphere are major problems uprising due to utilization of conventional fossil fuel as energy source. These problems are also becoming the critical issue in energy security, growth and development of Ethiopia.

Diesel fuel is one of the most important commercial freight public transport fuel in Ethiopia. Ethiopia is importing petroleum products like, Motor Gas Regular (MGR), Kerosene, Automotive Diesel Oil (ADO), Gas oil, Light fuel oil and Heavy fuel oil to meet the growing demand of transport fuel. The amount of imported Petroleum has been rising over the past two decades and reached 3,788,961 metric ton in 2019/20. According to the importation data of Ethiopian Petroleum Supply Enterprise (EPSE), in 1999/2000, 1,022,377 metric ton of refined petroleum was imported with importation bill of $1.869*10^9$ Birr; In 2009/10, 2,034,193 metric ton of Petroleum was imported with importation bill of $18.729*10^9$ Birr; and in 2019/20, 3,788,961 metric ton of petroleum was imported with the importation bill of $85.336*10^9$ Birr. The rapid growth in demand of diesel fuel and the rise in importation bill contributes to the imbalance between imports and exports; and thereby affect the national economy of the country.

Therefore, substitution of imported petroleum oil by clean, renewable, locally available, alternative, and sustainable biofuel (i.e. biodiesel) as energy source will make valuable contribution to overcome or diminish the aforementioned environmental, energy and socio-economic challenges associated with conventional fuel utilization. Thus, this study aimed at developing clean, environmentally friendly, renewable biofuel energy source, specifically, Production and Characterization of Biodiesel by Hybridization of *M. stenopetala* and *A. indica* seed oil, as alternative solution to conventional diesel fuel, in response to the increase in oil price and transport fuel scarcity, while improving the energy quality and the economy of the country.

1.3. Objectives of the Study

1.3.1. General objective

The general objective of this study was Production and Characterization of Biodiesel by Hybridization of *Moringa stenopetala* and *Azadirachta indica* seed oil for substitution of fossil fuel with clean, alternative, renewable, locally available and environmentally benign energy source.

1.3.2. Specific Objectives

The specific objectives of this study were:

- To extract, prepare and purify oil from *M. stenopetala* and *A. indica* seeds for biodiesel productions
- To produce biodiesel by base-catalyzed transesterification from hybridized oils of *M. stenopetala* and *A. indica* seeds
- To optimize statistically and observe the effect of operation parameters (i.e. Oil mixing composition, catalyst dose and reaction time) on the yield of product.
- To characterize the Fatty Acid Methyl Ester (Biodiesel) produced from the considered biomass feedstocks

1.4. Scope of the Study

The scope of the intended study was raw materials collection, sample preparation, extraction of oils from the considered feedstocks, purification of oils, characterization of the extracted oils, and production of biodiesel by base-catalyzed transesterification process and characterization of the resulting fatty acid methyl ester (biodiesel) product.

1.5. Significance of the Study

In this study, the development and utilization of renewable, cleaner, alternative and eco-friendly energy sources such as, biofuel (i.e. biodiesel) produced from *M. stenopetala* and *A. indica* seed oil will make significant contributions in achieving Clean Development Mechanism (CDM) across regions of the country (i.e. Ethiopia). Moreover, the production of biodiesel from locally available biomass feedstocks and substitution of conventional diesel fuel will also enhance development of potential agriculture-based business industry, employment generation via biomass feedstock utilization value chains, reducing the amount of petroleum import and increasing the export, contribute to water and soil conservation, reducing emission of pollutants including Greenhouse gases; and hence, offer local and global environmental welfare. Furthermore, biofuel (i.e. biodiesel) energy source is helpful in securing fuel supplies, meeting the growing energy demand, reduction of petroleum import bill via the development of domestic fuel production. When we substitute and utilize biodiesel as transportation fuel, agricultural machineries running fuel, household energy and electricity generation fuel, instead of diesel fuel, a large amount of foreign currencies will also be saved.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Overview of Biofuels

Biofuel is a fuel produced from biomass feedstocks through biological, biochemical or Physico-chemical processes (Vera I. and Langlois L., 2007). It comprises solid fuels (i.e. wood fuel or fire wood, agricultural residues, pellets and briquettes), liquid fuels (i.e. biodiesel, bioethanol, bio-oil and bio-butanol) and gaseous fuels (i.e. biogas and syngas) of which liquid and gaseous fuels are predominantly utilized for transportation (Balat M., 2009). Liquid fuels are considered as relatively cleaner, renewable and alternative energy sources to fossil fuels (Demirbas A., 2008; Balat M., 2009). They are vitally employed in fueling vehicle and engines (Domínguez Y.D. *et al.*, 2019) and they are domestic fuel, economical and sustainable energy source (Eevera T. *et al.*, 2011).

The potential for development and utilization of liquid biofuels (i.e. biodiesel and bioethanol) from non-edible energy crops is extensively disseminated across the globe, whereas the reservations of world’s petroleum oil are centralized in some countries. Thus, it is a great opportunity for poor countries importing petroleum products to produce and use biofuels (Fraiture de *et al*., 2008); and thereby saving the reserves of their foreign currencies while gaining the domestic economic benefits. Besides, the liquid biofuels possess the potential to substitute and leverage the limitation of fossil fuel supply as it is a renewable fuel source (Core J., 2002). The utilization of biofuels also offers a number of benefits. For instances, biofuel utilization results in minimization of unburned hydrocarbons and other particulate matters; and hence, it diminishes greenhouse gas emissions (Core J., 2002; Bultzen S., 2006) and reduces the associated risks of public health as biofuel exhibits good fuel quality (i.e. high lubricity of fuel and high attributes of fuel performance like enhanced Cetane rating)(Bultzen S., 2006); and it provide job opportunity and employment generation for rural livelihoods (Dufey, 2006).

Furthermore, the integration of biofuels (i.e. biodiesel and bioethanol) in the transport sectors (Hammond *et al.,* 2008) has been taken into account by European Union to meet targets of climate change mitigation; thereby enhancing the capacity of regional energy security. Biodiesel, which is considered as a second generation biofuels, has been proven as alternative fuel to fossil diesel fuel; and it is non-toxic, eco-friendly and biodegradable fuel produced from locally available biomass feedstocks (i.e. non-edible oilseeds) (Demirbas, 2008). Biofuels can be categorized into three major categories, including first generation biofuels, second generation biofuels and third generation biofuels.

2.1.1. First Generation Biofuels

First generation biofuels (i.e. ethanol) can be produced from food crops, such as corn, barley, wheat and sugar beet, etc.; and biodiesel production from edible oils. These biofuels have some positive attributes (i.e. relatively simple, low unit investment for production, simple production technology and fungibility with petroleum-derived fuels); however, first generation biofuels pose challenges and threats on food price and animal feeds, for instances, direct competition with food production, relatively high production costs in most cases due to the competition for feedstocks with food and the use of feedstocks optimized for food production are some of threats accompanied with first generation biofuels.

2.1.2. Second Generation Biofuels

The second generation biofuel can be produced from locally available non-edible biomass feedstocks, for instances, biodiesel produced from non-edible oilseeds, and bio-ethanol produced from lignocellulosic materials (Gomez *et al*., 2008; Zabaniotou *et al*., 2008). These biofuels are helpful to overcome challenges accompanied with direct food and fuel competition; and can be produced from considered feedstocks by biochemical, thermochemical and physico-mechanical processes.

2.1.3. Third Generation Biofuels

The third generation biofuels can be developed from engineered crops like algae as a source of energy. The algal species can be grown, harvested, extracted and their corresponding triglyceride oil can be refined and converted into other fuels substituting petrol-based fuels. Production and utilization of biodiesel from algae has been widely considered as one of the effective and efficient way of generating biofuels and has great potential to substitute a fossil-fuel based in transportation (Pabbi *et al*., 2011). The algal biofuels are very attractive due to its ease of production by employing waste water, salt water and fresh water (Potts *et al*., 2012); and the oil extracted from algae is biodegradable and hence, environmentally friendly. In comparison with non-edible oilseeds and lignocellulosic biomasses, algae can be grown at faster rate and require small land surface (Lü *et al.*, 2011). Per area of harvest, the third generation biofuels are more energy dense (i.e. high energy) than the first and the second generation biofuels.

2.2. Biodiesel as Renewable Energy Source

Biodiesel is a liquid biofuel produced from vegetable oils and animal fats by transesterification process (Upadhyay and Sharma, 2013); and it can be employed either alone or blended with diesel oil in diesel engines (Van Gerpen J. *et al*., 2005; Romano S.D. *et al*., 2006). According to the definition of ASTM- D6751, biodiesel is a mixture of long-chain of fatty acid mono-alkylic esters obtained from renewable resources (i.e. triglycerides of fatty acids - vegetable oil) in the presence of alcohol and suitable catalyst with glycerol as by-product (Knothe, 2009; Domínguez Y. *et al*., 2019). In twenty-first century, biodiesel has received attention as renewable, biodegradable, cleaner and environmentally safe fuel source (Liaquat *et al*., 2010). Sustainable utilization of biodiesel will diminish dependence on fossil fuel (Rashid U. *et al.*, 2008) and pollutants contributing to environmental pollution and global warming (Kafuku G. *et al.*, 2010). Moreover, Biodiesel utilization do possess a number of advantages like its substitution for conventional diesel fuel (Romano S. *et al*., 2006), lower emission of contaminants such as poly aromatic hydrocarbons (PAH), aldehydes, carbon monoxide and hence, low toxicity in comparison with conventional diesel fuel, degrades more rapidly and its excellent lubricant properties (Van Gerpen J. *et al*., 2005).

2.3. Global Biodiesel Production

The increasing cost of energy, socio-economic challenges and concerns of the greenhouse gas emission impacts necessitated the production and utilization of biofuels, partially substituting about 1,200 billion liters of annual consumption of petroleum fuel, across the globe (Fraiture de *et al*., 2008). In this regard, the approximated capacity of 35 billion liters of biofuel (i.e. biodiesel and bio-ethanol) was produced and utilized worldwide thereby making substantial contribution for the modern supply of energy. Moreover, the efforts of extended research and development of liquid biofuels for the replacement of petroleum products emphasized on production of biodiesel and bio-ethanol predominantly. The world's largest liquid biofuel producers include, Austria (biodiesel), Germany (biodiesel), United States of America (bioethanol) and Brazil (bioethanol) (Garba M.U. *et al.*, 2006). Since the 2007/2008 crisis of transport fuel-price, the production and utilization of the first and second generation liquid biofuels (i.e. biodiesel and bioethanol) have been encouraged as a solution for energy insecurity and challenges of climate change mitigation coupled with global warming (Food and Agricultural Organization [FAO], 2008). In addition, the adopted European Union Renewable Energy Directive (i.e. the Directive 2009/28/EC) necessitated the member states of European Union to meet at the minimum ten percent of their transport fuel demand from renewable sources of energy by 2020. The production of Biofuel in European Union (EU) is centralized in the main markets of transport fuel hosting countries, such as France, Spain, Germany, Sweden, Italy, Denmark and Poland (Eikeland P.O., 2006). In particular, the four largest global biodiesel producers offer approximately 78.63% out of the whole production capacity (Figure 1 below). Among these, the estimated 31% of biodiesel (14,600 million liters) was produced by European Union (EU), about 19% of biodiesel (i.e. 9031 million liters) was produced by United States (USA), 5% of Biodiesel was produced by Argentina, 16 % of biodiesel (i.e. 7380 million liters) was produced by Indonesian and 13% share of biodiesel (i.e. 5800 million liters) was produced by Brazil, whereas, the Rest-of-the-World (RoW) produced a 16 % share of biodiesel (Mizik T. and Gyarmati G., 2021). As a whole, the European Union is the principal biodiesel producer across the globe; and this implies the preponderating share of biodiesel cars in Europe that was about 41.9% vehicles among which approximately 35.9% share of modern biodiesel cars in 2018. Nonetheless, this share widely differs among the member states of European Union, for instances, about 32.2% was in Germany, 44.4% in Italy, 51.9% in France and 60.0% in Spain (European Automobile Manufacturers' Association [ACEA], 2020).

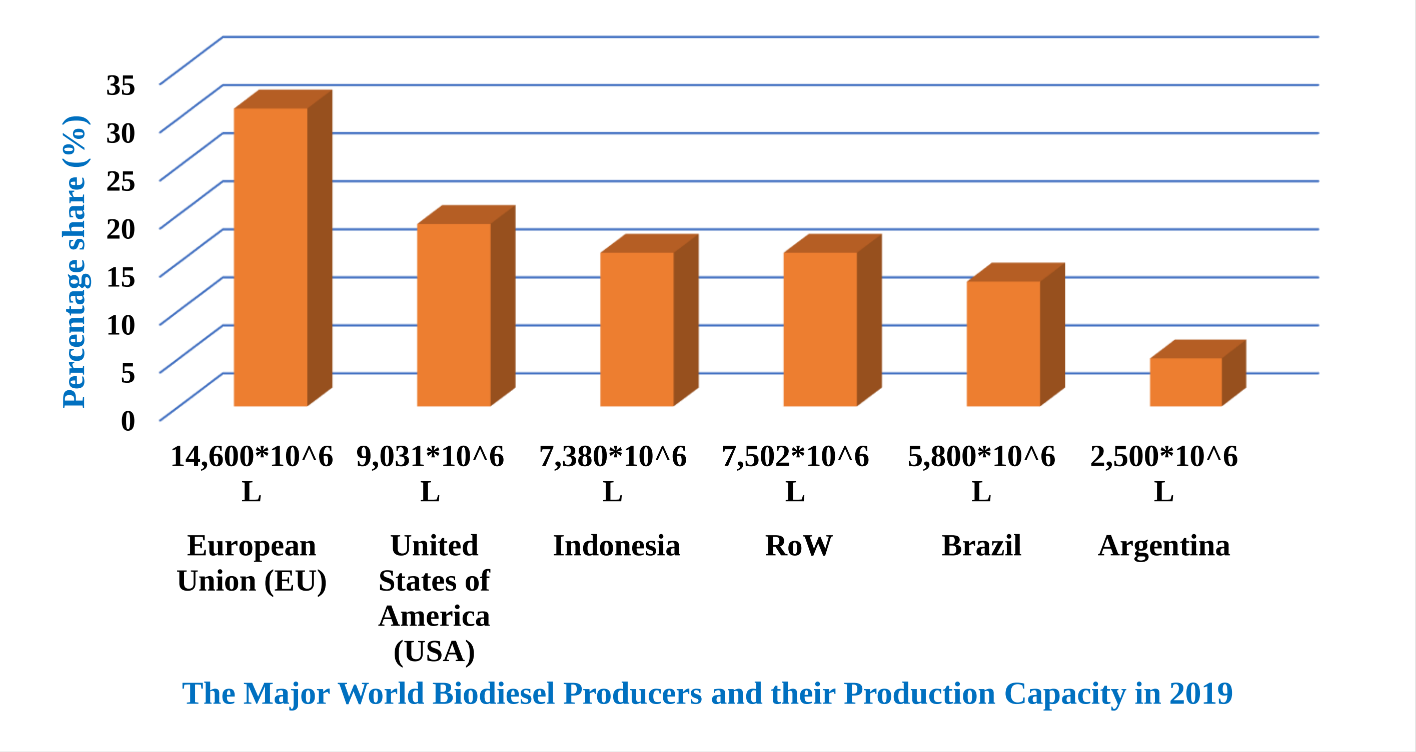


Figure 1: The composition of the major global biodiesel producers in 2019

Source: (*Mizik, T. and Gyarmati, G., 2021*)

In addition to vehicle fleets, biodiesel can be employed as blended fuel with petroleum products. Its blending ratio may vary in the range of B1 (1%) to B100 (100%). The mandates of biodiesel blending with petroleum fuel boost the demand and production of biodiesel though it is not uniform across the globe. For instances, the blending objective of European Union was B7 as of 2020; Argentina has introduced B10 even though the country consumes less biodiesel than its exports; Indonesia has introduced the biodiesel-petroleum blending mandate of B20 recently; and Brazil has a blending mandate of B10. The biodiesel production and utilization in India and China is insignificant, whereas in United States of America (USA) there is no biodiesel mandates at the state level (Lane J., 2019).

2.4. Biodiesel Production in Ethiopia and its Prospects

Ethiopia has been considered as one of the African countries, with total area of landmass of 1.2 million square kilometer, suitable for exploitation of domestic renewable energy like biofuels (i.e. biodiesel and bioethanol) due to its location. Approximately, the land area of 25 million hectares was identified as auspicious potential for the growth and development of biodiesel feedstocks (Gebremeskel and Tesfaye, 2008). Following the increase of price of petroleum products across the world, the country has been focusing on the development of biofuel industries for the past two decades; and thereby increasing its domestic energy potential (Gebreegziabher and Mekonnen, 2011). In addition, the Ethiopian Government's Strategy of Biofuel Development, aiming at diminishing the reliance on petroleum, promotes the domestic production and utilization of biofuels (Ministry of Mines and Energy [MoME], 2007). With this regard, there are a number of biofuel production investments in various regions of the country emphasizing on the synthesis and utilization of biodiesel and bioethanol. According to the planned strategy of Climate Resilient Green Economy (CRGE) of Ethiopia, implementation of biofuel production and its blends (i.e. about 5 % biodiesel and 15% bioethanol) with petroleum will substitute the estimated amount of 0.09 billion liters of gasoline and 0.28 billion liters of diesel by 2030 (FDRE, 2010).

2.5. Feedstocks for Production of Biodiesel

The utilization of non-edible vegetable oils for production of biodiesel is important because of the great need of edible oil for food. The non-edible oils, like Moringa seed, *A. indica* seed, Jatropha seed, Mahua seed, castor seed, linseed, cotton seed, rubber seed, and oils from microalgae, are locally available and accessible in developing countries and are economical compared to edible oil

(Balaji G. and Cheralathan M., 2013). They are considered to be highly promising reliable feedstock possessing seed with high oil content. The prospects of utilizing oils of these non-edible seeds as alternative fuel source has vided scope and very good understanding of biodiesel production from non-edible seeds will make significant contribution while supporting the countries' economy by diminishing the import of crude oil and commercializing the biodiesel product (Patel N.K. *et al.*, 2013). The *M. stenopetala* seed (i.e. indigenous species to Ethiopia) and *A. indica* have been considered as a reliable raw materials and locally available oilseeds for the intended study.

2.5.1. Moringa stenopetala

2.5.1.1.The ecological distribution and botanical description of *M. stenopetala*

Moringa is a tropical plant that originates and grows all around the tropics; and belongs to *Moringaceae* family. The genus Moringa comprises fourteen species that covers the sub-tropics and tropical parts of the Earth's surface (Beyene, 2005; Jiru *et al.*, 2006; NRC, 2006; Gebregiorgis *et al.*, 2011), out of which the five Moringa species including, *Moringa stenopetala*, *Moringa rivae*, *Moringa oleifera*, *Moringa ruspoliana* and *Moringa longituba* exist in the Northeast tropic of Africa (Jiru *et al.*, 2006). Among these species, *M. stenopetala* is predominantly found in southern part of Ethiopia and Northern part of Kenya; and hence, considered as African Moringa tree. It has also been domesticated in the lowlands of east Africa, specifically, it is known as indigenous species to Ethiopia among the *Moringaceae* family (Jiru *et al.*, 2006; Gebregiorgis *et al.*, 2011; Addis G. *et al*., 2014). It covers wide range of south-western part of Ethiopia, for instances, Konso, Gamo, Burji and Gofa people cultivated it as food crop and consume its leaves as a vegetable (Jahn, 1991; Demeulenaere, 2001; Abuye *et al*., 2003). Its English name is known as 'Cabbage tree', 'Ben oil tree', 'Africa Moringa tree' and 'Horse-radish tree'. In Ethiopia, *M. stenopetala* is known by various vernacular names, such as 'Haleko' in Wolaita and Gofa Zones, 'Shiferaw' in Amharic and 'Shelagda' in Konso areas (Demeulenaere *et al.*, 2001; Teketay, 2010; Yisehak 2011; Addis G. *et al.*, 2014).

M. stenopetala is a drought tolerant, evergreen perennial plant, well-adapted to semi-arid, arid and poor soil areas with a 500-1,400 mm annual rainfall (Steinmüller *et al*., 2002). It grows naturally in riverine; and can also be cultivated in gardens and terraced fields due to its ease of propagation, multiple usage and adaptability to degraded and harsh environments (Jahn S., 1991; Addis G. *et al*., 2014). Nonetheless, it does not grow in waterlogged or in swampy soils; and it grows best in well-drained soils, altitude varying from 400 - 2,100 m above sea level and annual temperature varying from 24-30 °C (Steinmüller *et al*., 2002; International Center of Research Agroforestry [ICRAF], 2006).

Moreover, *M. stenopetala* is a deciduous plant (i.e. shedding foliage at the end of its growing season) with average height of 6-12 m and estimated diameter of 60 cm at the breast height. It is referred as a soft wood and its crown (i.e. upper part of the tree) is highly branched with a number of trunks (Edwards *et al.,* 2000). The *M. stenopetala* trunk is substantially thicker at the base and it provides larger foliage and seeds (Educational Concerns for Hunger Organization [ECHO], 2009). Furthermore, *M. stenopetala* pods are reddish, elongated with grayish blooms and twisted when the fruit is fresh (ICRAF, 2006). Its seeds are triangular in shape and covered with a thick, spongy and yellowish seed coat. The seed kernel has oval shape and a whitish-grey color. *M. stenopetala* tree can produce up to 4,500 - 10,000 seeds that weigh 2.3-5 Kg from about 500-1,000 pods (Ethiopian Institute of Agricultural Research [EIAR], 2003). The *M. stenopetala* seed is crucial source of oil (i.e. 45% w/w oil yields) that can be used for either cooking or various industrial applications, for instances, it is a potential feedstock for production and utilization of biodiesel (Andinet E. *et al.*, 2010).

c

Figure 2: a) Moringa stenopetala Tree, b) Un-dehulled Moringa stenopetala Seeds, c) Dehulled Moringa stenopetala Kernels

The oil extracted from *M. stenopetala* seed powder comprises various components of fatty acids at various percentage compositions. These components of fatty acid includes, 6.10% palmitic acid ($C_{16}H_{32}O_2$), 7.50% Stearic acid ($C_{18}H_{34}O_2$), 76.00% Oleic acid ($C_{18}H_{32}O_2$), 3.80% Arachidic acid ($C_{20}H_{40}O_2$), 1.70% Arachideic acid ($C_{20}H_{38}O_2$), 4.40% Behenic acid ($C_{22}H_{44}O_2$) and 0.50% other components; and the corresponding molar mass of extracted *M. stenopetala* seed oil is 285 g/mol. (Andinet E. *et al.*, 2010). *M. stenopetala* seed oil contains 76.00% $C_{18}H_{32}O_2$ and hence, the oil was classified as oleic family.

2.5.1.2. The use of *Moringa stenopetala* and its economic contributions

The *Moringa* species has been considered as a 'miracle tree', a fast growing and it is one of the most world's valuable plants (Fuglie, 2003; Yisehak *et al.*, 2011; Accra *et al.*, 2012). The *Moringa* plant is a multipurpose tree possessing a number of economic importance, as it has essential industrial, medicinal and nutritional values (Jahn, 1991; NRC, 2006). Almost every part of the Moringa tree (i.e. the leaf, stem, bark, roots and seed) is useful. Among the broad range of its applications, *Moringa stenopetala* tree offers the benefit as fuel wood, soil and water conversation, livestock forage, medicine, water purification, green manure, dye and it generates income for farmers and *Moringa* growing enterprises (Demeulenaere, 2001; Palada and chang, 2003, Jiru *et al.*, 2006; ECHO, 2009; Melesse *et al.*, 2011).

2.5.2. Azadirachta indica

2.5.2.1. The ecological distribution and botanical description of *A. indica*

The neem tree, *Azadirachta indica*, belongs to a family of *Meliaceae (Mahogany)*. It is native to regions of Indian subcontinent and Burma (Vietmeyer, 1992). It is evergreen perennial tree species found in tropical and subtropical areas of South Asia, Africa, Australia and America (Schmutterer, 1990). *A. indica* tree has a broad range of adaptability potential to various topographic and climatic conditions. It is a drought tolerant and grows under humid and semi-arid conditions. The neem tree thrives well on poor soil fertility (i.e. calcareous, dry and stony soils); and improves the soil conditions (Anonymous, 2006). Currently, the tree has been cultivated and grown in several countries across the globe (i.e. in Australia, Asia and Africa, Central, Southern and Northern part of America). In Ethiopia, *A. indica* is planted and grown widely in the dry, moist 'Kolla' and 'Weyna Dega' agro-climatic regions, such as Gambella, Afar, Humara, Metema, Gonder, Kefa, Arsi, Hararge, Illubabor, Shoa and other regions. It grows in the altitude ranges from 400 - 2,000 m above sea level. It is also a medium sized and fast-growing tree which can reach over 20-25 m in height with an oval-shape canopy and a dense leaf. The neem tree begins fruiting in 3-5 years and in about 10 years, it become fully productive. Based on the climatic conditions and genotype of the plant, a single *A. indica* tree can produce about 30 - 100 Kg of fruit annually (Saxena, 1989). The neem seed comprises about 40-45% oil and the remaining part is the matrix of the seed. The neem oil is non-edible, brownish yellow with unpleasant odor (Johnson and Morgan, 1997). Thus, the neem oil, which is extracted from neem seeds, can be employed for production of biodiesel, cosmetics, soap and medicinal products in pharmaceutical industries.

a

b

Figure 3: a) Azadirachta indica Tree, b) Azadirachta indica Seeds

The oil extracted from *A. indica* seed powder composed of various portions of fatty acids at various percentage compositions. These components of fatty acid includes, 0.44 % Myristic acid ($C_{14}H_{28}O_2$), 26.00 % palmitic acid ($C_{16}H_{32}O_2$), 9.50 % Stearic acid ($C_{18}H_{34}O_2$), 51.00 % Oleic acid ($C_{18}H_{32}O_2$), 13.00% Linoleic acid ($C_{18}H_{32}O_2$) and 0.06 % Linolenic acid ($C_{18}H_{30}O_2$) with the corresponding molar mass of extracted crude oil 275.76 g/mol. (Adepoju T. and Olawale O., 2015).

2.5.2.2. The use of *Azadirachta indica* and its economic contributions

The *A. indica* (Neem) is a multipurpose tree species that provides various industrial benefits (i.e. to produce cosmetics, biofuels and to synthesize medicines - Antiseptic, Antituberculosis, Antiviral, Antitumour (Biswas *et al.,* 2002), environmental (i.e. it serves as fixation of dune, reclamation of soil in salinity areas, and organic manure or organic fertilizer from non-edible Oil cake) (Orwa C. *et al*., 2009; Ramachandran *et al.,* 2007); and socio-economic benefits such as employment generation, income generation (Orwa C. *et al.,* 2009). Every parts of the *A. indica* tree (i.e. roots, stem, leaf, seed, twigs and bark) are useful and hence, it can be utilized in many different way. The seeds, leaves and bark of *A. indica* tree have been employed in ethno-veterinary medicine and ethno-medicine for several years (Biswas *et al.,* 2002; Subapriya *et al.*, 2005) as their respective extracts comprise, essential compounds like, a limonoid and azadirachtin; and hence, it can serve as insect repellent (Boeke *et al.,* 2004; Orwa *et al.,* 2009; Jindal *et al.,* 2012). Moreover, the extracts of neem plant can be used for the treatment of various diseases including, allergies, diabetes, herpes, ulcers and cancer (Imam *et al.,* 2012).

2.6. Biodiesel Production Technologies

There are a number of problems that hinder direct utilization of vegetable oil for replacement of petrol-diesel. For instances, very high viscosity of vegetable oils due to its complex chemical structure and its higher molecular weight than conventional diesel fuel and lower heating value of vegetable oil than diesel fuel due to the presence of oxygen in the vegetable oil. Moreover, higher viscosity of vegetable oil leads to inefficient mixing of oil with air induces incomplete combustion, pumping and spray (i.e. penetration and atomization) problems. Therefore, vegetable oils must be modified chemically in order to overcome accompanied challenges in engine while making appropriate and compatible fuel for the existing engines. Such modification is predominantly targeted at diminishing the viscosity of the oil, thereby overcoming the fuel combustion and flow related problems. The modification of vegetable oils can be carried out by employing methods, such as Transesterification, Dilution and Pyrolysis (thermal cracking) and Micro-emulsion (Atabani A. E. *et al*., 2013).

2.6.1. Transesterification process

Transesterification is one of the most effective and promising processes employed in the conversion of triglycerides of vegetable oils into biodiesel. It can be conducted in the presence of alcohol (i.e. either methanol or ethanol) in homogeneous or heterogenous system using base, acid or enzyme as catalysts (i.e. commonly used homogeneous base catalysts are KOH, NaOH, $KOCH_3$ and $NaOCH_3$) (Leung D. Y. C. and Guo Y., 2006) to form biodiesel and glycerol (Bello E. I. *et al*., 2013). The transesterification process eliminates the glycerin in the triglycerides and substitutes it with the alcohol utilized in the process of conversion (Van Gerpen J.H. *et al.*, 1997; Raheman H. *et al*., 2013). This process helps to reduce the viscosity of the resulting product while it maintains the heating value and the Cetane number.

In the presence of liquid base catalysts, the vegetable oil with high free fatty acid (FFA) tends to form soap; and hence, reduces the biodiesel yield. For high FFA containing vegetable oils, like neem seed oil, a two-step transesterification process (i.e. liquid acid and base catalyst respectively) will be employed, thereby improving the yield of biodiesel (Wang Y. *et al*., 2010).

The mechanism of the base-catalyzed transesterification of vegetable oils involves: First, the reaction of base catalyst with alcohol producing an alkoxide and the protonated catalyst. Second, the nucleophilic attack of the alkoxide at the carbonyl group of the triglyceride generating a tetrahedral intermediate (Guthrie J.P., 1991; Meher L.C. *et al*., 2006). Thirdly, the formation of the alkyl ester and the corresponding anion of diglyceride. Finally, deprotonating the catalyst, thereby regenerating the active species, which will able to react with a second molecule of the alcohol, starting another catalytic cycle. Diglycerides and mono-glycerides are converted by the same mechanism to a mixture of alkyl esters and glycerol. The process of transesterification reaction in the presence of catalyst can be given as follow (Sanli H. and Canakci M., 2008):

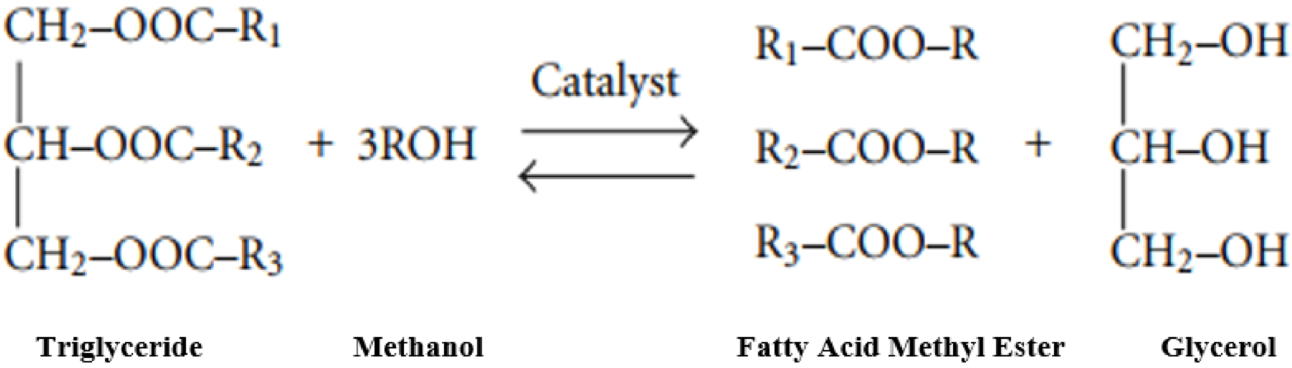


Figure 4: The chemical reaction of triglyceride with methanol in the presence of catalyst to form fatty acid methyl ester (FAME) and glycerol

Source: *(Sanli H. and Canakci M., 2008)*

2.6.2. Dilution

In dilution process, the refined vegetable oil is diluted with petroleum diesel to run the engine. The main problem with oil dilution in diesel fuel is its higher viscosity that induces gelling of lubricating oil, carbon deposits and formation of coking and trumpet in the engine part. Besides, blending of 80% diesel fuel and 20% vegetable oil is somehow possible; however, substitution of diesel fuel by 100% vegetable oil is not practical (Shikha K. and Chauhan Y.R., 2012).

2.6.3. Pyrolysis (Thermal Cracking)

Pyrolysis, in the absence of oxygen, is the process of heating organic matter in combination with catalyst to break oilseeds or vegetable oils into its smaller constituents, for instances, bio-oil with maximum conversion temperature 400-450 °C. The flashpoint, pour point, viscosity and calorific value of the resulting bio-oil is lower than that of diesel fuel. The product of pyrolysis comprises a heterogeneous molecules, like particulate matter, alkanes, sulfur, carboxylic acids and water (Sani Y. M. *et al.*, 2013). Moreover, pieces of equipment for pyrolysis process are expensive for production of biodiesel. Pyrolysis also requires a separate distillation equipment to various fractions of the product (Gashaw A. *et al.*, 2015).

2.6.4. Micro-emulsion

The micro-emulsion method is a colloidal equilibrium dispersion of optically isotropous fluid micro-structures with dimensions in the range of 1-150 mm, spontaneously formed from two immiscible liquid mixtures of water, oil and surfactants (i.e. the interfacial tension between two liquids or compounds that lower the surface tension of a liquid). This method is helpful to overcome challenges associated with viscosity of oil and some other atomization properties of vegetable oil (Rajalingam A. *et al*., 2016). The biodiesel micro-emulsion comprises, vegetable oil, alcohol, surfactants, diesel fuel and Cetane improver in appropriate proportions. Alcohols like ethanol and methanol are used to lower viscosity of oil; and higher alcohols are used as surfactants, whereas, alkyl nitrates are used to improve Cetane number. Moreover, the micro-emulsion method can be employed to get better spry property when injected into the diesel engine by the nozzle. Nonetheless, a continuous use of micro-emulsified diesel in engine induces some problems such as, nozzle failure, carbon deposit and incomplete combustion (Gebremariam S.N. and Marchetti J.M., 2017; May Y.K. and Tinia I.M., 2011).

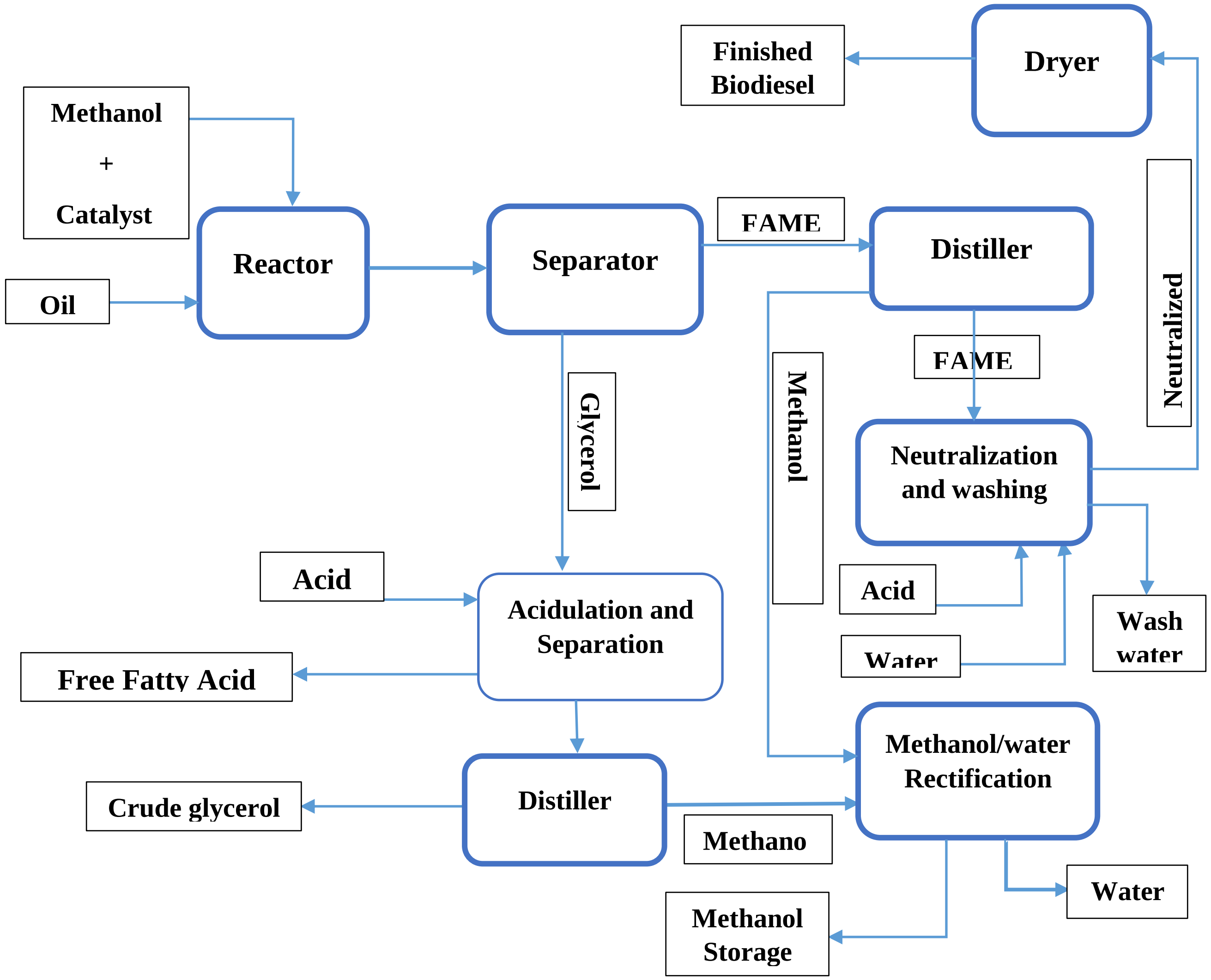


Figure 5: Biodiesel Production Process Flow Diagram (PFD)

2.7. Extraction and Purification of the Non-edible Vegetable Oils

Extraction of oil is a process of obtaining or separating triglycerides from oil bearing vegetables or animal tissues. This process can be carried out via a number of extraction techniques or methods while diminishing the alteration of the desired product quality and maximizing the yield of the product. The extraction of oil from non-edible vegetable oils predominantly depends on the part of the plant, for instances, the seed kernel, the pulp or foliage (Baskar G. *et al.,* 2019). Firstly, oil extraction involves a solubility controlled phase which is a fast physical process helps to separate oil from the outer surface of the considered particle; and secondly, oil extraction involves the diffusion-controlled phase which is relatively slower process enable to obtain oil from the internal part of the subjected particle (Mustapa A. N. *et al.,* 2009). Moreover, a process of oil extraction from oleaginous seed material is affected by a number of operational variables such as moisture content of the oilseed, particle size, quality of the solvent used, time allowed for the extraction, the amount of solvent employed and extraction temperature (Chaiklahana R. *et al*., 2008; Siddiquee M. and Rohani S., 2011) that in turn affects the quality and the yield of the desired oil (Olaniyan, 2010). Regardless of the method of extraction to be employed, pretreatment of the non-edible oilseeds is an indispensable step prior to extraction. The oilseed pretreatment step comprises, cleaning, removal of pod or seed coat (de-hulling), winnowing, sorting and the particle size reduction or milling (Ogunniyi, 2006; Yusuf *et al.,* 2015). The removal of moisture content or drying of oilseeds by either oven drying or micro-wave assisted heating or open air heating is necessitated before grinding proceeds (Patel *et al.,* 2016). Size reduction or grinding of the oilseeds ruptures or breaks the cell embedded in the structure of fiber to increase the surface area of the oil-bearing minute cells for and ensure the release of the desired oil by leaching (Akpan *et al.,* 2006; Tayde *et al.,* 2011). Several extraction methods or technologies, such as, Mechanical extraction method (Arisanu, 2013), Chemical or solvent extraction method (Buenrostro and Lopez-Munguia, 1986), Supercritical liquid extraction method (Huang Z. *et al.,* 2012) and Micro-wave assisted extraction method can be employed accordingly to obtain the desired oil from the oil-bearing materials.

2.7.1. Mechanical Extraction Method

The mechanical extraction method is the technique that involves the applied pressure (i.e. via either screw or hydraulic pressing) to expel oil from the oilseeds (Arisanu, 2013). This extraction technique is usually applied to extract oil from vegetable oilseeds with high oil content. Increasing the exerted mechanical pressure on the considered oil-bearing material increases the yield of the desired oil and vice-versa. The mechanical extraction technique provides the advantage of producing good quality oil with relatively lower free fatty acids content and lower operation cost (Carr, 1976). Nonetheless, the yield of extracted oil by mechanical method is relatively lower than the other extraction technologies; and hence, it is inefficient method as it usually lefts large percentage of oil content in the seed cake (Buenrostro and Lopez-Munguia, 1986; Anderson, 1996), for instances, mechanical pressing technique applied on the castor seeds removes approximately 45% of crude castor oil while the remaining portion of oil in the cake was obtained by the solvent extraction technique (Ogunniyi, 2006). Moreover, this method of extraction is labor intensive and time consuming (Bhuiya *et al.*, 2015). Therefore, the mechanical pressing method is not suitable for extraction of the whole oil in the low oil comprising oilseeds. In order to overcome challenges associated with mechanical extraction method, chemical or solvent extraction techniques have been formulated and it is being employed, even, to separate the remaining oil in the seed cake resulted from the mechanical extraction method (Kessler N., 1985).

2.7.2. Chemical or Solvent Extraction Method

The solvent or chemical extraction method is a conventional technique of extraction that implemented to oleaginous materials comprising low percentage of oil. This extraction technique is regarded as the most efficient method (i.e. with less residual oil in the cake) of oil extraction from oil-bearing materials (Buenrostro and Lopez-Munguia, 1986; Tayde *et al.*, 2011). The Soxhlet extraction has been considered as the most preponderantly employed technique of extraction for obtaining oil from the solid matrix (Abdelaziz *et al.*, 2014). Moreover, the chemical extraction method requires the utilization of suitable organic solvent(s) during the process; and the choice of appropriate solvent for the extraction process depends upon availability and cost, extraction capacity, the relative volatility of solvent, relative polarity or solubility of solvent with oil, high solvent-to-solute ratio (Muzenda *et al.*, 2012; Takadas and Doker, 2017) and the highest possible leaching nature of the required solute substrate (Dutta *et al.*, 2015). The main solvents employed in the chemical extraction method are n-Hexane, ethanol, methanol, petroleum ether and diethyl ether. Among these solvents, n-Hexane is predominantly used solvent for extraction of oil due to its low corrosiveness, high stability and solubility with oil, relatively low boiling point and low greasy residual effects (ISO 659-1988). After the completion of the oil extraction process, the mixture of organic solvent and oil is subjected to either rotary evaporator or distillation column to separate the desired crude oil and recover the organic solvent. The obtained crude oil is then subjected to oil refining process for oil purification. The Soxhlet extraction techniques is relatively effective and efficient method of obtaining oil from the solid matrix and it enables to recover and re-use of the organic solvent. However, the Soxhlet extraction method possess a certain drawbacks like the dilution of sample in the solvent (Rassem *et al.,* 2016), energy and time requirement, and high requirement of the extraction solvent (Takadas and Doker, 2017).

2.7.3. Supercritical Liquid Extraction Method

The supercritical extraction method is a technique that helps to overcome the aforementioned challenges associated with the above described methods of extraction. It is eco-friendly as it does not use organic solvents; and it is also considered as supercritical carbon extraction technique (Nuttawan Y., 2013). Several solvents (i.e. compressed gaseous like dinitrogen oxide, ethylene, ethane, carbon dioxide, propane) can be employed in this extraction technique. Among these, the most frequently used solvent in the supercritical extraction technique is liquid carbon dioxide (CO_2) which is of low viscosity, non-toxic, non-polar, non-flammable, high diffusivity, with good solvent power, available at low price, and it can be easily removed from desired oil (Bravi M. *et al.,* 2002; Huang Z. *et al.,* 2012; Katayoun M. *et al.,* 2016). In addition, other solvents often considered as co-solvents (i.e. acetone, ethanol, isopropanol and methanol) can also be employed with a supercritical carbon dioxide. These solvents help to enhance the solubility power of CO_2 with the desired analyte while boosting the relative polarity of the super critical CO_2 (Wejnerowska G. *et al.,* 2018).

2.7.4. Micro-wave assisted extraction method

The micro-wave assisted extraction (MAE) technique is one of the modern and advanced technique used for separating the desired oils from oil-bearing seeds (Rassem *et al.,* 2016). In MAE, the initial sample pretreatment is carried out in the microwave oven that uses the radio waves to transfer energy; and thereby converting to heat with frequency ranging from 300 MHz to 300 GHz (Singh and Heldman, 2001). Moreover, the application of microwave radiation breaks or ruptures the cell membrane of the considered oilseeds to enhance the coefficients of mass transfer and to increase the yield of the desired product (Azadmard D. *et al.,* 2011). The microwave assisted extraction method provides several advantages like the direct capability of extraction, reduced the consumption of solvent, improved quality and yield of oil extraction, faster extraction time and the consumption of low energy during the process of oil extraction (Azadmard D. *et al.,* 2011). However, a certain drawbacks are associated with the MAE. For instances, the degradation of oil quality during its operation at high temperature (Amarni and Kadi, 2009) and it may not be appropriate for plants at all as the higher energy of microwave disrupts the tissue of the plant (Uquiche *et al.*, 2008).

2.8. Variables Affecting Synthesis of Biodiesel by Transesterification process

The biodiesel synthesis from non-edible vegetable oil via the transesterification process (Meher L.C. *et al*., 2006; Encinar J. M. *et al*., 1999) is affected by several operational variables including, the oil moisture content (Leung D. Y. and Guo Y., 2006), the amount of free fatty acid (FFA) presented in the oil (Kulkarni M. G., and Dalai A., 2006), reaction time (Refaat A. A. *et al*., 2008a), reaction temperature (Colucci J. *et al.*, 2005), types of alcohol used (Encinar J. M. *et al.*, 2007), alcohol to oil molar ratio (Tomasevic A. *et al.*, 2003), types and dosage of catalyst employed (Freedman B. *et al.*, 1984), Mixing intensity (Canakci M. and Van Gerpen, J., 2003) and purity of feedstocks (Sergejus L. and Andrius V., 2006).

2.8.1. Moisture Content of the Oil

The base-catalyzed transesterification reaction of the non-edible vegetable oil is mainly affected by the presence of moisture in the oil. The oil water content causes and stimulates the hydrolysis of the ester with the accompaniment saponification reaction (Leung D. and Guo Y., 2006; Arun N. *et al*., 2011); and thereby reduces the amount of triglyceride ester in the non-edible vegetable oil (Arun N. *et al*., 2011). During the free fatty acid conversion into triglyceride ester (i.e. acid-catalyzed esterification reaction), water was also formed as side product (Canakci M., 2007). Thus, the amount of water content in the oil was removed or evaporated by heating the oil at 120 °C (Ahmad M. *et al.*, 2009); and then, the oil was allowed to cool at the temperature of 60 °C (Chen K. *et al.*, 2012).

2.8.2. Amount of Free Fatty Acid Presented in the Oil

The free fatty acid contents of the non-edible vegetable oil inhibits the occurrence of the base-catalyzed transesterification reaction (Ahmad M. *et al.*, 2010). When the free fatty acid (FFA) in the oil is greater than 3%, the reaction will not take place, rather it induces the saponification reaction (i.e. soap and water formation). The saponification reaction on turn reduces the catalyst efficiency and effectiveness leading to the gel formation; and hence, it rises the viscosity of FAME (fatty acid methyl ester) or biodiesel and makes the separation of glycerol difficult. A two-step transesterification was used to overcome challenges with transesterification of high free fatty acid oil, of which the first step was the esterification of FFA (i.e. a homogeneous acid pretreatment of the non-edible oil) until amount of FFA in the oil was less than 0.5% (Van Gerpen J., 2005; El Sherbiny S. A. *et al.*, 2010). In the second step, homogeneous base catalyst was employed in order to complete the chemical reaction (Muthu H. *et al.*, 2010; Mathiyazhagan M. *et al*., 2011).

2.8.3. Reaction Time

The optimum reaction time should be used to synthesize the fatty acid methyl ester (biodiesel) through base-catalyzed transesterification of non-edible vegetable oil while maximizing the yield of the desired product and minimizing the accompanied cost of production. Adequate reaction time is required for conversion of reactants (i.e. the non-edible vegetable oil and alcohol) and completion of the transesterification reaction. In this regard, it has been found that the best yield of biodiesel product has taken place at the optimum reaction time of 60 minutes (Tomasevic A. V. *et al.*, 2003; Refaat A. *et al*., 2008a). It has also been shown that, when the base-catalyzed transesterification reaction is carried out for longer time, approximately 99% yield of the biodiesel can be obtained. Nonetheless, beyond the optimum time required for the reaction, extra reaction time favors the reverse reaction (i.e. hydrolysis of fatty acid methyl ester or biodiesel), and hence, reduces the yield of the desired product (Leung D. Y., and Guo Y., 2006; Refaat A., 2010).

2.8.4. Reaction Temperature

The reaction temperature is another operational variable that affects the base-catalyzed transesterification reaction (Darnoko D. and Cheryan M., 2000). A higher reaction temperature induces higher rate of conversion of reactants and diminishes the required time to complete the reaction while increasing the yield of biodiesel (Colucci J. *et al*., 2005; Highina *et al*., 2011). However, the reaction temperature should not exceed the boiling point of the alcohol used in the transesterification reaction because increasing the temperature of reaction beyond the alcohol stimulates formation of bubble due to vaporization of the alcohol inhibiting the reaction process (Meng X. *et al.*, 2008). In contrast, keeping the reaction temperature far below the alcohol boiling point increases the viscosity of biodiesel (Kapilakarn K., and Peugtong A., 2007). Hence, to obtain the maximum possible yield of fatty acid alky ester (biodiesel) the base-catalyzed transesterification reaction should be conducted at the optimum operation temperature of 55-68 °C (Van Gerpen J., 2005).

2.8.5. The Molar Ratio of Alcohol to Vegetable Oil

The alcohol that is employed in the base-catalyzed transesterification process is selected based on the availability, relative boiling point of alcohol, the cost, operational performance and the ease of its recovery in the downstream process (Encinar J. M. *et al*., 2007). Thus, Methanol, ethanol and butanol are frequently employed alcohols in the transesterification reaction, out of which methanol (CH_3OH) is a predominantly used alcohol in the chemical reaction (Refaat A. A. *et al.*, 2008a) due to its lower boiling point, relatively lower price, its higher effectiveness during the reaction process and its availability (Zhou W. and Boocock D., 2006). With the presence of base catalyst in the transesterification reaction, 1 mole of fatty acid triglyceride reacts with 3 mole of alcohol (i.e. CH_3OH) to produce 3 mole of fatty acid methyl ester (FAME) and 1 mole of glycerol (Figure 4 above). From the Le Chatelier's principle, the rate formation of the desired product is enhanced by increasing the concentration of the reacting species. Thus, the concentration of fatty acid methyl ester (biodiesel) will increase as the concentration of the employed alcohol (methanol) is increased until the equilibrium point (Knothe G., and Steidley K., 2009). The highest conversion of the triglyceride of the non-edible vegetable oil occur at the oil to alcohol molar ratio of 1:6 (Tomasevic A. and Siler-Marinkovic S., 2003; Encinar J. M., *et al*., 2005).

2.8.6. Types and dosage of Catalyst

Catalysts are either biological or chemical agents that used to increase the rate of biological or chemical reaction while decreasing the required activation energy in the transesterification reaction. Several chemical catalysts can be applied in the transesterification of alcohol and the vegetable oil and production of the fatty acid methyl ester (Vicente G. *et al*., 2004). For instances, the flakes of homogeneous catalyst like potassium hydroxide (KOH), Sodium hydroxide (NaOH) and their respective methoxide solutions are mainly employed in biodiesel producing industries as they are easy for handling, safe for transportation, available and low in price (Encinar J. M. *et al.*, 2005; Encinar J. M. *et al.*, 2007). Moreover, the strong and concentrated acids such as hydrochloric acid, sulfuric and phosphoric acids can be used in the esterification step of the free fatty acid in the vegetable oil followed by the base catalyst in the transesterification step in order to maximize the yield of biodiesel (Freedman B. *et al.*, 1984; Refaat A. A. *et al.*, 2008a). About 1 % wt optimum dosage of catalyst is applied in transesterification of vegetable oil to increase the forward rate of conversion of reactants while maximizing the yield of the desired product (Highina B. K. *et al.*, 2011).

2.8.7. Mixing Intensity

During the base catalyzed transesterification, mixing is required to increase the surface area of contact between alcohol and the non-vegetable oil thereby avoiding the occurrence of reaction between the interfacial surfaces of reactants while enhancing the rate of reaction (Singh A. and Fernando S., 2006). The higher the agitation intensity, the higher the forward rate of reactants (i.e. shorter time for reaction) and providing the maximum yield of the desired product (Adeyemi N. *et al.*, 2011). Thus, optimum agitator or mixer speed in the range of 100 - 500 rpm should be employed for various biodiesel feedstocks considering their corresponding physical and chemical properties (Kumari A. *et al.*, 2009).

2.9. Biodiesel Specification and Quality Parameters

2.9.1. Cetane number

The Cetane number (CN) a fuel's quality parameter that measure or indicate the combustion or ignition quality of the fuel in the engine. It is derived from unbranched or straight chain of saturated hydrocarbon, hexadecane ($C_{16}H_{34}$) (Knothe G., 2014). The Cetane number of biodiesel is relatively larger than that of petroleum fuel as the biodiesel comprises the straight and long-chain of hydrocarbons. The degree of unsaturation of fatty acid methyl ester or biodiesel induces the decrease in the Cetane number of the fuel (Ramirez-Verduzco L. *et al.*, 2012).

2.9.2. Kinematic viscosity

Fuel viscosity is the measure of resistance or internal friction of the flowing fuel opposite to the change in fluid dynamics. Increasing the temperature of the flowing fluid decreases the viscosity of the flowing fluid and vice-versa. The kinematic viscosity of the given fuel can be obtained from the ratio of the coefficient of viscosity of the fuel by the corresponding density of the fuel; and it affects the injection characteristics of the fuel (Allen *et al.*, 1999). Higher viscosity of fuel causes poor atomization of the fuel as it induces poor vaporization of fuel, relatively larger droplet sizes and narrower angle of injection spray thereby resulting the higher emissions and combustion problems in the engine. The viscosity of fatty acid methyl ester is relatively higher than that of petroleum (Su Y. and Liu Y., 2011). The fuel kinematic viscosity can be determined using the standard method of (American Society for testing materials [ASTM D445], 1970).

2.9.3. Specific gravity

The specific gravity (relative density) of fatty acid methyl ester (biodiesel) is the ratio of the fuel density to the density of distilled water at the same temperature. The fuel specific gravity influences the performance characteristics of engine (i.e. effectiveness and efficiency) as it affects the mass of air-to-fuel ratio during injection of the fuel into the combustion chamber. The specific gravity of biodiesel can be determined using the standard test method, ASTM D1298.

2.9.4. Flash point

The flash point is the measure of the flammability of the given fuel under controlled conditions. Its fundamental objective is to determine the flammability of hazardous liquids, i.e. liquid biofuels (ASTM D92-05, 2005). For instances, the flash point of biofuel is helpful to decide and avoid the contamination due to the presence of impure volatile materials (i.e. residual solvents like methanol) post-stripping of the desired product like biodiesel (Hoekmana S. *et al.,* 2012). The biodiesel flash point can be determined using standard method, ASTM D92-05.

2.9.5. Pour point

The pour point of the biodiesel is the flowing property of the fuel inside the engine at various temperature. It is the temperature at which the fuel loses its nature of flowing thereby inducing the failure of the engine operation system; and hence, helps to determine and decide the suitable geographical and environmental conditions to utilize the biodiesel (Alptekin and Canakci, 2011). The pour point of the given fuel can be determined according to the standard test method provided in ASTM D5853.

2.9.6. Cloud point

The cloud point is the temperature at which the formation of cloudy appearance occur in the fatty acid methyl ester. It is the index of the minimum possible temperature of biodiesel and petroleum utilization (ASTM D2500-02, 2002). If the temperature of the operating system is far below the cloud point of the fuel, then the fuel will be solidified and formation of crystals will take place which in turn affects the part of engine thereby varying the performance efficiency of the engine (Chiu *et al.*, 2004). The procedure provided in the standard test method of ASTM D2500 was used to determine the cloud point of the biodiesel.

2.9.7. Moisture content

The moisture content in the fatty acid methyl ester is the critical quality parameter that influences the combustion behavior in the engine as it induces formation of corrosion, spray and foaming problems in the engine (ASTM D6304-2007). By its nature, fatty acid methyl ester exhibits a hygroscopic traits; and hence, it absorbs the humidity from the atmosphere. The moisture in the biodiesel predominantly resulted from washing the final product (i.e. wet washing) after transesterification process is completed. The moisture content of biodiesel was carried out using the standard test method of, ASTM D6304-07.

2.9.8. Higher Heating Value (HHV)

The higher heating value (HHV) is a fuel quality specification parameter that measures the amount of thermal energy liberated from unit quantity of fuel provided that the fuel is subjected to complete combustion. It is helpful quality parameter to determine the suitability of fuel for combustion and the fuel energy content. The HHV of biodiesel (fatty acid methyl ester) can be determined using bomb calorimeter following the standard test method of ASTM D240.

2.9.9. Acid number

The acid value or acid number is a quality parameter that shows the amount of fatty acids content in the fatty acid methyl ester (biodiesel) (Wang H. *et al.*, 2008). It is an indispensable parameter that helps to evaluate and monitor the formation of corrosion and degradation of FAME throughout storage (Bouaid *et al.*, 2009). The acid number of the given fuel or the fatty acid methyl ester can be determined by employed the standard test method of ASTM D664.

2.9.10. Oxidation stability

The oxidation stability of fatty acid methyl ester is the propensity of the fuel to undergo oxidation at ambient temperature and the tendency of the fuel to resist the degradation (James P. and Khizer S., 2012). The Oxidation stability of the fuel indicates the comparative susceptibility of the FAME to degradation (Frankel E., 1998). The biodiesel oxidation stability can be determined according to the standard method of testing, EN 14112.

CHAPTER THREE

3. MATERIALS AND METHODS

This chapter comprised, materials and methods employed in the laboratory during experiment, experimental design and experimental procedures for all laboratory activities including, raw material collection, sample preparation, oil extraction and characterization, fatty acid methyl ester (biodiesel) production from considered feedstocks and characterization of the final product.

3.1. Materials and Chemicals Used

Materials used:

Moisture analyzer, Analytical balance, Oven drier, Milling machine, Sieves, Vibrated sieve shaker, Soxhlet apparatus, Thimble paper, Boiling water bath, Rotary evaporator, Chiller, Desiccator, Measuring cylinders, Round bottom flasks, Volumetric flask, Conical flask, Erlenmeyer flask, glass stoppered bottle, Reflux condenser, Retort stand and clamp, Centrifuge, Plastic bags, Parafilm, Filter paper, Beakers, Funnel, Aluminum foil, Spatula, Scissors, SV-10 Vibro-viscometer (capacity 0.3 - 10,000 mPa.s), Dropper, pH meter, Motor stirrer with a capacity of 2000 rpm, Three neck glass reactor, Burette graduated in 0.1 ml, Thermometer, Pycnometer, Separatory funnels, Stop watch, Low Density Poly Ethylene (LDPE), High Density Poly Ethylene (HDPE) and Personnel Protective Equipment (PPE).

Chemicals/Reagents Used:

Methanol (CH_3OH), n- Hexane (C_6H_{14}), sulfuric acid (H_2SO_4), Phosphoric acid (H_3PO_4), Potassium hydroxide (KOH), Hydrochloric acid (HCl), Sodium hydroxide (NaOH), Sodium chloride (NaCl), Ethanol (99.5 %), Diethyl ether $(C_2H_5)_2O$, Standard Sodium thiosulfate ($Na_2S_2O_3$), Distilled water, Phenolphthalein indicator ($C_{20}H_{14}O_4$), Soap, Carbon tetrachloride (CCl_4), Iodine (I_2), Glacial acetic acid, Chloroform, Starch solution indicator, Ammonium hydroxide (NH_4OH) and Ammonium chloride (NH_4Cl).

3.2. Methods

3.2.1. Sample Collection, Preparation, Oil Extraction and Purification

Sample collection and Preparation:

The sample of *M. stenopetala* seed was collected from Sidama Region Agricultural Center, 273 Km from Addis Ababa, with geographical location of latitude 7° 3' North (N), Longitude 38° 28 East (E) and elevation of 1,708 m above sea levels. The sample of *A. indica* seed was collected from Afar Region, Awash Arba (Amibera woreda), which is 242 Km far from Addis Ababa in Northeastern part of Ethiopia. The collected samples were transported to Addis Ababa Institute of Technology (AAiT), School of Chemical and Bioengineering Laboratory to conduct the intended study. Then, the samples were cleaned and free from foreign materials such as, weed seed, molds, stones and other contaminants. In order to increase the efficiency of oil extraction, the collected seeds were first de-hulled (i.e. decorticated). Following the decortication, the outer husk was separated from the kernels by winnowing. Then, samples of seed kernels were dried in oven drier at 60 °C for 24 hours to remove its moisture content. The sample drying was continued until the moisture content was below 5% using standards of American Society of Testing Materials (ASTM, 2008).

$$\textit{Moisture content} = \frac{W0 - W1}{W0} * 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(1)}$$

Where, W_0 - initial weight of the sample before drying

W_1 - final weight of the sample after drying

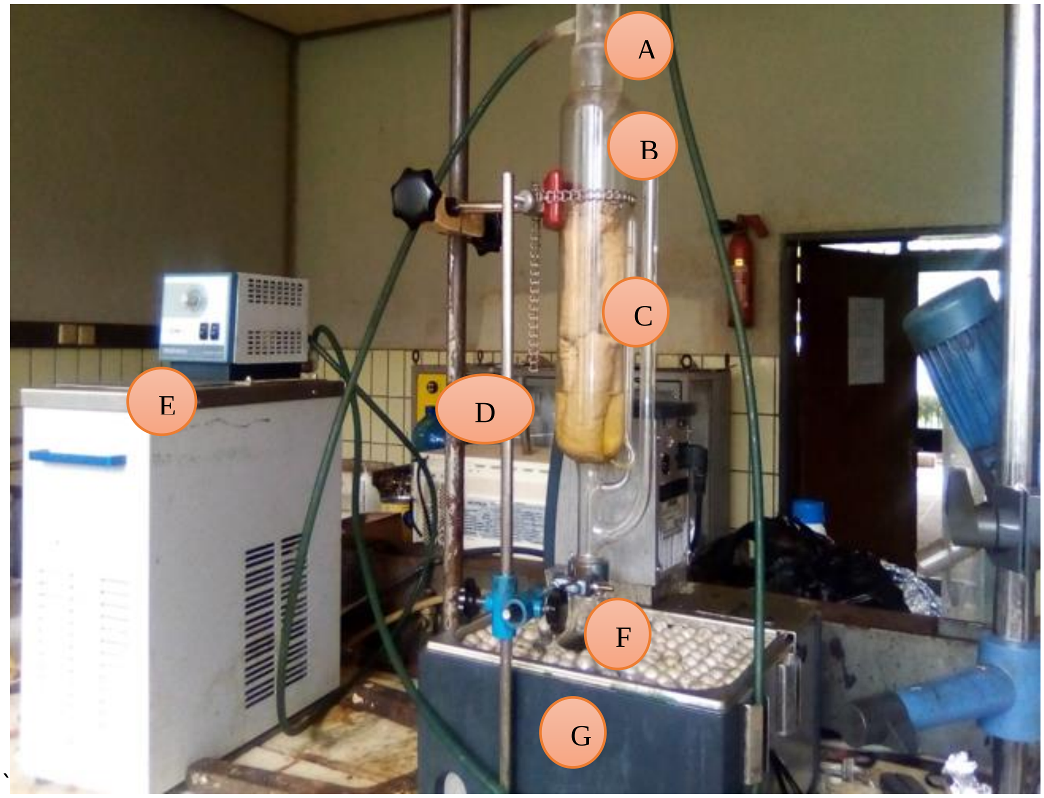
The dried samples of *M. stenopetala* and *A. indica* seed kernels were grinded into the paste at particle size in the range of 0.25 - 0.425 mm, 0.425 - 0.60 mm, 0.60 - 0.85 mm turn-by-turn using grinder to provide a higher surface area of particles for the ease of extraction. The range of desired particle size was obtained through sieving using the Vibrated sieve shaker. The milling operation ruptured the cell wall and released the solute for direct contact with the solvent during oil extraction process (i.e. by Soxhlet Extraction Method). The grinded samples were put into a Low Density Poly Ethylene (LDPE), labelled and stored until dispatch.

Oil Extraction and Purification:

The Soxhlet extraction method, with *n*-Hexane as a solvent, was employed for the extraction of oil from the considered species. A 120 g of grinded sample was weighed and placed in thimble paper. Then, the thimble paper containing the sample was inserted into the Soxhlet apparatus. Using a measuring cylinder, 600 mL of the solvent (n- Hexane) was measured and poured into a 1000 mL round bottom flask; and adjusted in the set-up of the extraction vessel. The extraction temperature was adjusted at 72 °C and extraction process was continued for 5 hours to obtain the desired crude oil. As the extraction temperature increased and process of heating continued, the solvent commenced to evaporate and condensed back to the thimble containing the sample. The extracted crude oil containing the solvent was recycled and refluxed back to the round bottom flask and this process was continued until the extraction hour was reached. Then, to recover the solvent from the crude oil - solvent mixture, a rotary evaporator was used at the temperature of 72 °C. This extraction process was continued until a reasonable amount of crude oil was obtained (Aliyu *et al.*, 2013). Finally, the amount of the extracted crude *M. stenopetala and A. indica* oil were recorded at the end of each steps of extraction process and the percentage of extracted oil was determined as follow:

$$\textbf{Oil yield (\%)} = \frac{\boldsymbol{Mass\ of\ crude\ extracted\ oil}}{\boldsymbol{Total\ Mass\ of\ Seed\ kernel}} * \mathbf{100} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

3.2.2. The Experimental Set-up for Oil Extraction Process



Description: A – Condenser, B – Soxhlet, C – Thimble containing sample, D – Stand with clamp, E – Chiller, F – Round bottom flask, G – Boiling water bath

Figure 6: The experimental set-up for oil extraction process using Soxhlet Apparatus

The extracted crude oil of each species were subjected to filtration and the removal of potentially unwanted particles was carried out using the centrifuge. For further purification or removal of trace solvent, the oil and solvent mixture was placed in the boiling water bath until the trace n-hexane was liberated completely. Then, the extracted crude oils (*M. stenopetala* and *A. indica* Seed Oil) were subjected to the determination of physicochemical properties of oil.

3.3. Determination of Physicochemical Properties of Extracted Oil

The Physico-chemical properties of the extracted oil was determined following the method of American Standards for Testing Materials (ASTM) and Association of Official Analytical Chemists (AOAC). These includes: Determination of Acid value and free fatty acid content, Saponification value, Specific gravity, Viscosity, pH value, Iodine value and the refractive index.

3.3.1. Determination of Acid value and free fatty acid

The amount of acid number (AN) or acid value and free fatty acid (FFA) are used to show the edibility and rancidity of vegetable oils (Amos *et al.*, 2013). The acid number (AN) indicates the quantity of milligram of potassium hydroxide (mg KOH) required in order to neutralize about 1g of FFA in the given vegetable oil; and the free fatty acid (i.e. usually half of the acid value) is the weight percent of the determined fatty acid like percentage of Oleic acid in the non-edible vegetable oils (Othman and Ngaasapa, 2010). The acid value and free fatty acid determination of *M. stenopetala* and *A. indica* Seed Oil were conducted according to the standard test method (Association of Official Analytical Chemists [AOAC], 2000). The experimental procedure was also provided in Annex (Annex A- 1).

3.3.2. Determination of Saponification Value

The saponification value of the non-edible vegetable oil is used to indicate the quality, size and characteristics of chains of fatty acids that can be esterified into glycerol. It also provides the magnitude of the mean length of fatty acid chain that comprises fat (Garrett R. *et al*., 2012). In amalgamation with acid number, the saponification value is helpful in offering information such as, the average weight, the amount and the type of glycerides presented in the provided sample of the non-edible vegetable oils (Fazal *et al*., 2015; Mohammed and Ali, 2015). The determination of saponification value of both samples of crude Oils (i.e. *M. stenopetala* and *A. indica* Oil) has been carried out following the AOAC standard test method (AOAC - 920.160, 2000). The experiment was conducted following the procedure provided in Annex (Annex A- 2).

3.3.3. Determination of Iodine value

The iodine number or iodine value (IV) indicates the mass of iodine (g) that is added to a 100 g of the considered sample while measuring the unsaturation levels of the subjected organic compound. The iodine value also shows the number of double bonds that exist in the test samples; and the higher the iodine value presented in the sample, the higher the number of double bonds exist and vice-versa. The iodine value of samples of *M. stenopetala* and *A. indica* Seed Oil has been determined according to the standard test method provided in AOAC, (AOAC -920.159, 2000). The experiment was carried out according to the procedure given in Annex A- 3.

3.3.4. Determination of Peroxide value

The peroxide value is a measure of the extent at which the vegetable oils undergo oxidation during the storage of oils and it is helpful in predicting the stability and nature of oils (Nangbes *et al.*, 2013). A high degree of oil unsaturation induces higher peroxide value; and the peroxide value increases with increasing duration of oil contact with atmospheric oxygen, storage temperature and oil storage time (Mohammed and Ali, 2015) which in turn causes the oxidative rancidity of oil (Annonymous, 1978). The determination of peroxide value for each samples (i.e. *M. stenopetala* and *A. indica* Oil) has been conducted using the standard test method (AOAC - 965.33, 2000) (Annex A- 4).

3.3.5. Determination of Kinematic Viscosity

Viscosity is a measure of opposition or resistance of flowing fluid (i.e. liquid or gaseous) to a deformation at a provided rate (Rao M.A., 1999). The viscosity of the non-edible vegetable oil can be expressed in two ways based on the dynamic viscosity ($\boldsymbol{\mu}$) and kinematic viscosity ($\boldsymbol{\upsilon}$). The dynamic viscosity of vegetable oil is its shear force or resistance to flow because of the internal friction force in the oil molecule. On the other hand, the kinematic viscosity (mm^2/s) of the oil is its shear force or opposition to flow due to gravity; and this can be determined by dividing the dynamic viscosity of the non-edible vegetable oil by its corresponding density ($\boldsymbol{\rho}$). The dynamic viscosity of each oil sample (i.e. *M. stenopetala* and *A. indica* Oil) was determined using the SV-10 Vibro-viscometer following the standard test method (ASTM D445). The volume of oil sample was kept uniform throughout measurement and the corresponding dynamic viscosity of each oil sample was recorded at various temperature (i.e. at 22 °C, 40 °C and 60 °C) respectively. The required temperature was adjusted by hot water bath. Then, the kinematic viscosity of oil sample was computed as follow:

$$\textbf{Kinematic viscosity } (\upsilon) = \frac{\textbf{Dynamic viscosity of Oil } (\mu)}{\textbf{Density of Oil } (\rho)} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3)$$

Where, $\boldsymbol{\upsilon}$ (mm^2/s), $\boldsymbol{\mu}$ (mPa.s), $\boldsymbol{\rho}$ (Kg/m^3)

3.3.6. Determination of pH value

The pH value of each samples of *M. stenopetala* and *A. indica* seed Oil were determined using the standardized Digital pH meter. Three grams of each oil samples were weighed and added to the dry and clean beaker of 25 ml. Then, hot distilled water of 15 ml was added into beaker containing sample and gently stirred; and using the cold water bath, the sample was cooled. A mixture of NH_4OH and NH_4Cl buffer solution was used to standardize the pH electrode. Then, the electrode was immersed into the beaker containing sample and the corresponding pH value of each Oil sample was recorded (Akpan U. *et al.*, 2006).

3.3.7. Determination of Specific gravity

The specific gravity of the non-edible vegetable oils (i.e. *M. stenopetala* and *A. indica* seed oil) were determined by pycnometer according to the standard test method (AOAC- 920.212, 2000) (Annex A- 5).

3.3.8. Determination of Refractive Index (RI)

The refractive index of oil, usually known as the index of refraction, is ratio of velocity of light in vacuum to the velocity of light in the given non-edible vegetable oil (Jack *et al*., 2013). The longer fatty acid chain in the oil results the higher refractive index of oil and vice-versa. The refractive index of extracted *M. stenopetala* and *A. indica* seed oil were determined using Digital Refractometer as per the AOAC standard test method (AOAC- 921.08, 2000).

3.4. The Extracted Oil Refining Process

3.4.1. Oil Degumming Process

In degumming step, the extracted and purified non-edible vegetable oils (i.e. *M. stenopetala* and *A. indica* Seed Oil) were subjected to the removal of gums or phospholipids. To get rid of phospholipids from the crude oils, boiling water bath was used for heating oils at 65 °C turn by turn. Then, distilled water of 2% (v/v of oil) was added into the crude oil and stirred for 5 minutes. The mixture of crude oil and distilled water was added with 1.3 % v/v of concentrated H_3PO_4 and the mixture was agitated in a hot water bath at mixing intensity of 200 rpm for 60 minutes at 65 °C; and finally, it was subjected to separation.

3.4.2. Acid- Pretreatment (Esterification) of *M. stenopetala* and *A. indica* Seed Oil

In the esterification reaction or acid pretreatment step, the *M. stenopetala* and *A. indica* seed oil were heated turn by turn at 60°C using boiling water bath for 10 mins and mixed with methanol (45%v/v of oil) and stirred for 5 mins. The mixture was added with 1.2% v/v of concentrated H_2SO_4 and the resulting mixture was stirred on a hot water bath for another 45 mins at 60°C, before it was poured into a separatory funnel and allowed to settle for 4 hrs. The bottom layer of the oil was separated from the methanol-water phase at the top and stored in an air tight water proof container in a cool dry place. Once the oil was refined (i.e. degummed, purified and esterified), it was packaged into High Density Polyethylene (HDPE) to prevent oxidation, appropriately labeled; and stored in a cool place until dispatch.

3.4.3. Neutralization of the refined oil:

In the removal of excess FFA (i.e. neutralization) of esterified *M. stenopetala* and *A. indica* Seed Oil, a solution of 0.1 N of NaOH was added into each flasks containing the esterified oils and the mixture was heated at 60°C for 30 minutes with uniform speed of agitation at 300 rpm. Then, to enhance the rate of soap settling, 5% w/v of NaCl was added into the mixture. Finally the mixture of neutralized oil was subjected to separation and the neutralized oil was obtained.

3.5. Determination of the Amount of Alcohol Required for Stoichiometric Reaction of Transesterification Process

Basis of Computation: Batch size = 60 ml of hybridized *M. stenopetala* and *A. indica* Seed Oil was fed to the reactor in each experimental runs.

To determine the amount of alcohol (i.e. Methanol - CH_3OH) required for the transesterification reaction, the molecular mass of each samples of oil (i.e. *M. stenopetala* seed oil and *A. indica* seed oil) were employed. Theoretically, it has been assumed that, complete conversion of triglycerides occurred when one mole of triglyceride reacts with three mole of methanol to provide three mole of fatty acid methyl ester (FAME) and one mole of glycerol (Figure 4 above). Then, from the relationship of mole-mole stoichiometric reaction, for the alcohol to oil molar ratio of 6:1, the required amount of methanol (CH_3OH) was computed for each oil mixing compositions (i.e. $M_{75}N_{25}$, $M_{50}N_{50}$, $M_{25}N_{75}$):

Case 1: For 3:1 Oil mixing composition ($M_{75}N_{25}$, 75 %v/v *M. stenopetala* seed oil and 25%v/v *A. indica* seed oil):

Batch size = 60 ml of hybridized oil was employed

V_1 = 45 ml (Volume of *M. stenopetala* seed oil), ρ_1 = 0.89 g/ml (Density of *M. stenopetala* seed oil)

V_2 = 15 ml (Volume of *A. indica* seed oil), ρ_2 = 0.92 g/ml (Density of *A. indica* seed oil)

M_1 = 285 g/mol. (Molar mass of *M. stenopetala* seed oil); and M_2 = 275.76 g/mol. (Molar mas of *A. indica* seed oil)

Then, the amount of Methanol required for the transesterification reaction was computed as follow:

$$m_1 = V_1 * \rho_1 = 45\ \text{ml} * 0.89\ \text{g/ml} = \underline{\underline{40.05\ \text{g}}}\ \text{(Mass of } \textit{M. stenopetala} \text{ seed oil)}$$

$$m_2 = V_2 * \rho_2 = 15\ \text{ml} * 0.92\ \text{g/ml} = \underline{\underline{13.80\ \text{g}}}\ \text{(Mass of } \textit{A. indica} \text{ seed oil)}$$

$$\textbf{Density of mixture} = \frac{\boldsymbol{total\ mass\ of\ mixture}}{\boldsymbol{total\ volume\ of\ mixture}} = \frac{\boldsymbol{m1 + m2}}{\boldsymbol{V1 + V2}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.. (4)$$

$$\rho_{\ Oil\ mixture} = \frac{m1 + m2}{V1 + V2} = \frac{40.05\ g + 13.80\ g}{45\ ml + 15\ ml} = \underline{\underline{0.8975\ \text{g/ml}}}$$

$$\text{Mass of mixed oil} = \rho_{\ Oil\ mixture} * \text{Volume of mixture} = 0.8975\ \text{g/ml} * 60\ \text{ml} = \underline{\underline{53.85\ \text{g}}}$$

$$\text{Mole of } \textit{M. stenopetala} \text{ seed oil, } n_1 = \frac{\text{m1}}{\text{M1}} = \frac{40.05\ g}{285\ \frac{g}{mol}} = \underline{\underline{0.1405\ \text{mol.}}}$$

$$\text{Mole of } \textit{A. indica} \text{ seed oil, } n_2 = \frac{\text{m2}}{\text{M2}} = \frac{13.80\ g}{275.76\ \frac{g}{mol}} = \underline{\underline{0.0500\ \text{mol.}}}$$

Then, the mole fractions of each oil in the hybridized oil of *M. stenopetala* and *A. indica* seed oil was computed as follow:

$$\text{Mole fraction of } \textit{M. stenopetala} \text{ seed oil, } X_1 = \frac{mol.\ of\ M.\ stenopetala\ seed\ oil}{mol.\ of\ M.\ stenopetala\ seed\ oil + nol.of\ A.\ indica\ seed\ oil}$$

$$\text{Mole fraction of } \textit{M. stenopetala} \text{ seed oil, } X_1 = \frac{n1}{n1 + n2} = \frac{0.1405\ mol.}{0.1405\ mol. + 0.0500\ mol.} = \underline{\underline{0.737}}$$

Mole fraction of *A. indica* seed oil, $X_2 = \frac{n2}{n1 + n2} = \frac{0.0500\ mol.}{0.1405\ mol. + 0.0500\ mol.} = \underline{\underline{0.263}}$

The average molar mass of hybridized *M. stenopetala* and *A. indica* seed oil was computed as:

Average molar mass of hybridized oil:

$$M_{Average} = \sum_{i=1}^{2}(Xi * Mi), = X_1M_1 + X_2M_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

$$M_{Average} = 0.737*285\ g/mol. + 0.263*275.76\ g/mol. = \underline{\underline{282.57\ g/mol}}.$$

Then, Mole of hybridized oil, $n_{mixture} = \frac{Mass\ of\ mixed\ oil}{Average\ molar\ mass\ of\ mixture} = \frac{53.85\ g}{282.57\ \frac{g}{mol}.} = \underline{\underline{0.1905\ mol.}}$

Considering the 6:1 alcohol to oil molar ratio, the amount of alcohol (methanol) required for the base-catalyzed transesterification reaction was determined as follow:

Mole of Methanol (CH_3OH) = 6 * (Mole of hybridized oil) = 6*0.1905 mol. = $\underline{\underline{1.143\ mol}}$.

Mass of methanol = (mole of methanol) * (Molar mass of methanol)

= (1.143 mol.) * (32.4 g/mol.) = $\underline{\underline{37.033\ g}}$

The required volume of methanol was computed as,

$$\text{Volume of methanol } (CH_3OH) = \frac{Mass\ of\ methanol}{Density\ of\ methanol},\ \rho\ (CH_3OH) = 0.79\ g/ml$$

$$\text{Volume of methanol } (CH_3OH) = \frac{37.033\ g}{0.79\ g/ml} = \underline{\underline{46.87\ ml}}$$

By adding 5 % excess methanol for the completion of the transesterification reaction,

Total amount of methanol employed, V = 46.87 ml + (0.05*46.87 ml) = $\underline{\underline{49.21\ ml}}$

Case 2: For 1:1 Oil mixing composition ($M_{50}N_{50}$, 50 %v/v *M. stenopetala* seed oil and 50 %v/v *A. indica* seed oil):

Batch size = 60 ml of hybridized oil was employed

V_1 = 30 ml (Volume of *M. stenopetala* seed oil), ρ_1 = 0.89 g/ml (Density of *M. stenopetala* seed oil)

V_2 = 30 ml (Volume of *A. indica* seed oil), ρ_2 = 0.92 g/ml (Density of *A. indica* seed oil)

M_1 = 285 g/mol. (Molar mass of *M. stenopetala* seed oil); and M_2 = 275.76 g/mol. (Molar mas of *A. indica* seed oil)

Then, the amount of Methanol required for the transesterification reaction was computed as follow:

$$m_1 = V_1 * \rho_1 = 30\ \text{ml} * 0.89\ \text{g/ml} = \underline{\underline{26.70\ \text{g}}}$$ (Mass of *M. stenopetala* seed oil)

$$m_2 = V_2 * \rho_2 = 30\ \text{ml} * 0.92\ \text{g/ml} = \underline{\underline{27.60\ \text{g}}}$$ (Mass of *A. indica* seed oil)

$$\textbf{Density of mixture} = \frac{\boldsymbol{total\ mass\ of\ mixture}}{\boldsymbol{total\ volume\ of\ mixture}} = \frac{\boldsymbol{m1 + m2}}{\boldsymbol{V1 + V2}}$$

$$\rho_{\ Oil\ mixture} = \frac{m1 + m2}{V1 + V2} = \frac{26.70\ g + 27.60\ g}{30\ ml + 30\ ml} = \underline{\underline{0.905\ \text{g/ml}}}$$

$$\text{Mass of mixed oil} = \rho_{\ Oil\ mixture} * \text{Volume of mixture} = 0.905\ \text{g/ml} * 60\ \text{ml} = \underline{\underline{54.30\ \text{g}}}$$

$$\text{Mole of } \textit{M. stenopetala} \text{ seed oil, } n_1 = \frac{\text{m1}}{\text{M1}} = \frac{26.70\ g}{285\ \frac{g}{mol}} = \underline{\underline{0.0936\ \text{mol.}}}$$

$$\text{Mole of } \textit{A. indica} \text{ seed oil, } n_2 = \frac{\text{m2}}{\text{M2}} = \frac{27.60\ g}{275.76\ \frac{g}{mol}} = \underline{\underline{0.1000\ \text{mol.}}}$$

Then, the mole fractions of each oil in the hybridized oil of *M. stenopetala* and *A. indica* seed oil was computed as follow:

$$\text{Mole fraction of } \textit{M. stenopetala} \text{ seed oil, } X_1 = \frac{mol.\ of\ M.\ stenopetala\ seed\ oil}{mol.\ of\ M.\ stenopetala\ seed\ oil + nol.of\ A.\ indica\ seed\ oil}$$

Mole fraction of *M. stenopetala* seed oil, $X_1 = \frac{n1}{n1 + n2} = \frac{0.0.0936\ mol.}{0.0936\ mol. + 0.1000\ mol.} = \underline{\underline{0.483}}$

Mole fraction of *A. indica* seed oil, $X_2 = \frac{n2}{n1 + n2} = \frac{0.1000\ mol.}{0.0936\ mol. + 0.1000\ mol.} = \underline{\underline{0.517}}$

The average molar mass of hybridized *M. stenopetala* and *A. indica* seed oil was computed as:

Average molar mass of hybridized oil:

$$M_{Average} = \sum_{i=1}^{2}(Xi * Mi), = X_1M_1 + X_2M_2$$

$$M_{Average} = 0.483*285\ g/mol. + 0.517*275.76\ g/mol. = \underline{\underline{280.22\ g/mol}}.$$

Then, Mole of hybridized oil, $n_{mixture} = \frac{Mass\ of\ mixed\ oil}{Average\ molar\ mass\ of\ mixture} = \frac{54.30\ g}{280.22\frac{g}{mol}.} = \underline{\underline{0.1937\ mol.}}$

Considering the 6:1 alcohol to oil molar ratio, the amount of alcohol (methanol) required for the base-catalyzed transesterification reaction was determined as follow:

Mole of Methanol (CH_3OH) = 6 * (Mole of hybridized oil) = 6*0.1937 mol. = $\underline{\underline{1.1622\ mol}}$.

Mass of methanol = (mole of methanol) * (Molar mass of methanol)

= (1.1622 mol.) * (32.4 g/mol.) = $\underline{\underline{37.655\ g}}$

The required volume of methanol was computed as,

$$\text{Volume of methanol } (CH_3OH) = \frac{Mass\ of\ methanol}{Density\ of\ methanol},\ \rho\ (CH_3OH) = 0.79\ g/ml$$

$$\text{Volume of methanol } (CH_3OH) = \frac{37.655\ g}{0.79\ g/ml} = \underline{\underline{47.66\ ml}}$$

By adding 5 % excess methanol for the completion of the transesterification reaction,

Total amount of methanol employed, V = 47.66 ml + (0.05*47.66 ml) = $\underline{\underline{50.00\ ml}}$

Case 3: For 1:3 Oil mixing composition ($M_{25}N_{75}$, 25%v/v *M. stenopetala* seed oil and 75 %v/v *A. indica* seed oil):

Batch size = 60 ml of hybridized oil was employed

V_1 = 15 ml (Volume of *M. stenopetala* seed oil), ρ_1 = 0.89 g/ml (Density of *M. stenopetala* seed oil)

V_2 = 45 ml (Volume of *A. indica* seed oil), ρ_2 = 0.92 g/ml (Density of *A. indica* seed oil)

M_1 = 285 g/mol. (Molar mass of *M. stenopetala* seed oil); and M_2 = 275.76 g/mol. (Molar mas of *A. indica* seed oil)

Then, the amount of Methanol required for the transesterification reaction was computed as follow:

$$m_1 = V_1 * \rho_1 = 15\text{ ml} * 0.89\text{ g/ml} = \underline{\underline{13.35\text{ g}}} \text{ (Mass of } M.\ stenopetala \text{ seed oil)}$$

$$m_2 = V_2 * \rho_2 = 45\text{ ml} * 0.92\text{ g/ml} = \underline{\underline{41.40\text{ g}}} \text{ (Mass of } A.\ indica \text{ seed oil)}$$

$$\textbf{Density of mixture} = \frac{\boldsymbol{total\ mass\ of\ mixture}}{\boldsymbol{total\ volume\ of\ mixture}} = \frac{\boldsymbol{m1 + m2}}{\boldsymbol{V1 + V2}}$$

$$\rho_{\ Oil\ mixture} = \frac{m1 + m2}{V1 + V2} = \frac{13.35\ g + 41.40\ g}{15\ ml + 45\ ml} = \underline{\underline{0.9125\text{ g/ml}}}$$

$$\text{Mass of mixed oil} = \rho_{\ Oil\ mixture} * \text{Volume of mixture} = 0.9125\text{ g/ml} * 60\text{ ml} = \underline{\underline{54.75\text{ g}}}$$

$$\text{Mole of } M.\ stenopetala \text{ seed oil, } n_1 = \frac{\text{m1}}{\text{M1}} = \frac{13.35\ g}{285\ \frac{g}{mol}} = \underline{\underline{0.0468\text{ mol.}}}$$

$$\text{Mole of } A.\ indica \text{ seed oil, } n_2 = \frac{\text{m2}}{\text{M2}} = \frac{41.40\ g}{275.76\ \frac{g}{mol}} = \underline{\underline{0.1501\text{ mol.}}}$$

Then, the mole fractions of each oil in the hybridized oil of *M. stenopetala* and *A. indica* seed oil was computed as follow:

$$\text{Mole fraction of } M.\ stenopetala \text{ seed oil, } X_1 = \frac{mol.\ of\ M.\ stenopetala\ seed\ oil}{mol.\ of\ M.\ stenopetala\ seed\ oil + nol.of\ A.\ indica\ seed\ oil}$$

$$\text{Mole fraction of } M.\ stenopetala \text{ seed oil, } X_1 = \frac{n1}{n1 + n2} = \frac{0.0468\ mol.}{0.0468\ mol. + 0.1501\ mol.} = \underline{\underline{0.237}}$$

Mole fraction of *A. indica* seed oil, $X_2 = \frac{n2}{n1 + n2} = \frac{0.1501\, mol.}{0.0468\, mol. + 0.1501\, mol.} = \underline{\underline{0.763}}$

The average molar mass of hybridized *M. stenopetala* and *A. indica* seed oil was computed as:

Average molar mass of hybridized oil:

$$M_{Average} = \sum_{i=1}^{2}(Xi * Mi), = X_1M_1 + X_2M_2$$

$$M_{Average} = 0.237*285 \text{ g/mol.} + 0.763*275.76 \text{ g/mol.} = \underline{\underline{277.95 \text{ g/mol}}}.$$

Then, Mole of hybridized oil, $n_{mixture} = \frac{Mass\ of\ mixed\ oil}{Average\ molar\ mass\ of\ mixture} = \frac{54.75\, g}{277.95 \frac{g}{mol}}. = \underline{\underline{0.1969 \text{ mol.}}}$

Considering the 6:1 alcohol to oil molar ratio, the amount of alcohol (methanol) required for the base-catalyzed transesterification reaction was determined as follow:

Mole of Methanol (CH_3OH) = 6 * (Mole of hybridized oil) = 6*0.1969 mol. = $\underline{\underline{1.1814 \text{ mol}}}$.

Mass of methanol = (mole of methanol) * (Molar mass of methanol)

= (1.1814 mol.) * (32.4 g/mol.) = $\underline{\underline{38.27 \text{ g}}}$

The required volume of methanol was computed as,

$$\text{Volume of methanol } (CH_3OH) = \frac{Mass\ of\ methanol}{Density\ of\ methanol}, \rho\ (CH_3OH) = 0.79 \text{ g/ml}$$

$$\text{Volume of methanol } (CH_3OH) = \frac{38.27 \text{ g}}{0.79 \text{ g/ml}} = \underline{\underline{48.44 \text{ ml}}}$$

By adding 5 % excess methanol for the completion of the transesterification reaction,

Total amount of methanol employed, V = 48.44 ml + (0.05*48.44 ml) = $\underline{\underline{50.86 \text{ ml}}}$

3.6. Production of Biodiesel by Base-Catalyzed Transesterification Process

3.6.1. Experimental Design

In this study, the experiment was designed according to Response Surface Methods - the Box-Behnken Method (BBD) with selected variables of study or factors (i.e. Oil mixing composition, Catalyst dose and Reaction time) and three levels for each factors, Low (-1), Medium (0) and High (+1). The interaction effects of factors and their influences on response was studied while optimizing the required response (i.e. Biodiesel yield). The oil mixing composition, catalyst dose and reaction time were symbolized as A, B and C respectively. Factors and levels were provided below (in Table 1) and total number of experiment was computed according to BBD (Aslan N. and Cebeci Y. 2007*)*:

$$\mathbf{N = k^2 + k + C_p} \qquad \text{………………………………………………..…...……….} \quad (6)$$

Where, N – number of experiment; k – number of factors; and C_p - a central replication point

Table 1: The experimental levels of considered variables of study (i.e. Oil mixing composition, catalyst dose and reaction time) for BBD

Symbols	**Levels**				
	Variables	**Units**	**-1 (Low)**	**0 (Medium)**	**+1 (High)**
A	Oil mixing composition	% v/v	25	50	75
B	Catalyst dose	% w/v	1.0	1.5	2.0
C	Reaction time	Min	20	40	60

The mixture of *M. stenopetala* and *A. indica (*Neem*)* seed oils were subjected to the base-catalyst transesterification reaction at various operation parameters, including, Oil hybridization ratio (i.e. Oil mixing composition), catalyst dose and reaction time. The considered oil hybrid compositions were: $M_{25}N_{75}$ (25% *M. stenopetala* seed oil : 75% *A. indica* seed oil) , $M_{50}N_{50}$ (50% *M. stenopetala* seed oil : 50% *A. indica* seed oil) and $M_{75}N_{25}$ (75% *M. stenopetala* seed oil : 25% *A. indica* seed oil) with their corresponding Biodiesel products: $BM_{25}N_{75}$, $BM_{50}N_{50}$, $BM_{75}N_{25}$; catalyst dose of 1%, 1.5% and 2%; and reaction time of 20 min, 40 min and 60 min; whereas, the other operation parameters (Alcohol to oil molar ratio, temperature and mixing speed) were kept constant as per provided below (Table 2). According to BBD, a total of 15 number of runs were conducted with 3 levels for each numeric factors.

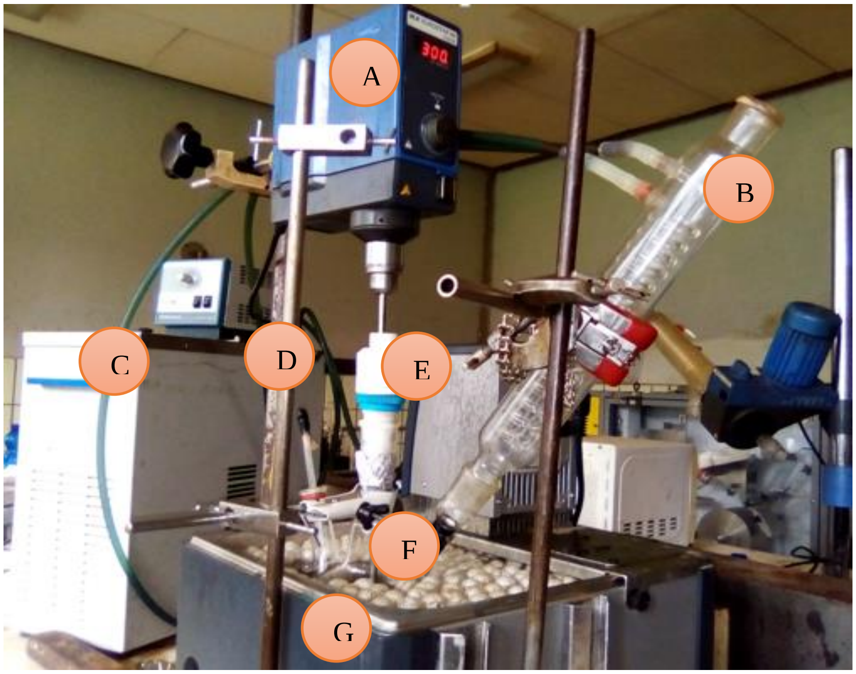
Table 2: The effect of operation variables (i.e. Oil mixing composition, catalyst dose and reaction time) on the yield of Biodiesel

Run	Factor 1	Factor 2	Factor 3	Alcohol to Oil Molar ratio	Temperature (°C)	Speed of stirrer (rpm)	Response
	A: Oil mixing Composition (M : N) (%)	B: Catalyst dose (KOH) (%)	C: Reaction time (min)				Biodiesel yield (BMN) (%)
1	50.00 (0)	2.00 (+1)	60.00 (+1)	6:1	60	300	X
2	50.00 (0)	2.00 (+1)	20.00 (-1)	6:1	60	300	X
3	50.00 (0)	1.50 (0)	40.00 (0)	6:1	60	300	X
4	50.00 (0)	1.00 (-1)	60.00 (+1)	6:1	60	300	X
5	50.00 (0)	1.00 (-1)	20.00 (-1)	6:1	60	300	X
6	75.00 (+1)	1.00 (-1)	40.00 (0)	6:1	60	300	X
7	25.00 (-1)	1.50 (0)	20.00 (-1)	6:1	60	300	X
8	25.00 (-1)	1.50 (0)	60.00 (+1)	6:1	60	300	X
9	50.00 (0)	1.50 (0)	40.00 (0)	6:1	60	300	X
10	50.00 (0)	1.50 (0)	40.00 (0)	6:1	60	300	X
11	75.00 (+1)	2.00 (+1)	40.00 (0)	6:1	60	300	X
12	25.00 (-1)	2.00 (+1)	40.00 (0)	6:1	60	300	X
13	25.00 (-1)	1.00 (-1)	40.00 (0)	6:1	60	300	X
14	75.00 (+1)	1.50 (0)	20.00 (-1)	6:1	60	300	X
15	75.00 (+1)	1.50 (0)	60.00 (+1)	6:1	60	300	X

M- M. stenopetala oil; N- A. indica Oil; BMN- Biodiesel of mixed M and N; X – the yield of biodiesel obtained from each run

$$\textit{Yield of Fatty Acid Methyl Ester (Biodiesel)} = \frac{\textit{Mass of obtained biodiesel}}{\textit{Mass of refined oil input}} * 100 \quad \ldots\ldots\ldots\ldots\ldots (7)$$

3.6.2. The Experimental Set-up for Biodiesel Production



Description: A – Motor stirrer, B- Condenser, C- Chiller, D- Stand with clamp, E- Fittings, F- Glass reactor, G- Boiling water bath

Figure 7: The Experimental Set-up for Biodiesel Production Process

3.6.3. Biodiesel Production Procedure

The refined oil was trans-esterified to mono-esters of fatty acids using methanol (CH_3OH) and potassium hydroxide (KOH) as catalyst. The base-catalyzed transesterification was carried out using the three necked round bottom flask (i.e. Glass reactor) containing 60 mL of Hybridized oils of *M. stenopetala* and *A. indica*. The KOH was dissolved in CH_3OH forming mixture of potassium methoxide. Then, the methoxide solution was added to the Glass reactor containing oil sample at 6:1 (alcohol to oil molar ratio). The reaction was allowed to take place at 60 °C and 300 rpm speed of stirrer for each trials as per specified in the experimental design (Table 2 above). After the reaction takes place, 3 drops of aqueous hydrochloric acid (HCl) was added into the solution to quench the reaction in order to stop further reaction from occurring. Then, the solution was transferred to a separating funnel for glycerol removal.

The lower glycerol layer was drained off from the bottom of the separatory funnel and the remaining upper layer (i.e. biodiesel or mono-methyl ester) was obtained. For further purification, the biodiesel was added into separatory funnel and allowed to settle for 12 hours to remove traces of KOH, glycerol and unreacted methanol.

The remaining trace materials and unreacted methanol induces corrosion in engine components; and trace glycerol in the fatty acid methyl ester (biodiesel) diminishes lubricity of the fuel, causes injector coking and safety risks (Hanumanth M. *et al*., 2012). The resulted hybridized biodiesel product was washed (4 times) with distilled water (1:2 biodiesel to water ratio) at 40-50 °C by spraying hot water over the biodiesel in order to remove these impurities. Finally, the pure biodiesel was heated to 110 °C to get rid of the trapped traces of water; and the Biodiesel was subjected to characterization and physicochemical determinations.

3.6.4. Characterization of the Produced Fatty Acid Methyl Ester (Biodiesel)

Determination of Physicochemical Properties of the produced biodiesel follows ASTM 6751-09 and European’s EN14214 standards including specific gravity, flash point, calorific value, kinematic viscosity, Cetane number, Acid number, pour points, free glycerin and total glycerin (Tariq *et al*., 2015). Moreover, the following parameters of the desired biodiesel such as, moisture content, carbon residue, water and sediments, oxidation stability, methanol content, sulfur content, phosphorus content, calcium and magnesium content were determined using the standard test methods.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1. Extraction and Characterization of Oil from *M. stenopetala* and *A. indica* Seeds

4.1.1. Determination of Moisture Content of the collected Seeds

Determination of the moisture content was conducted for both *M. stenopetala* and *A. indica* Seeds based on the dry biomass. The initial moisture content was carried out using the Moisture Analyzer and the corresponding results were triplicated and the average percentage of moisture contents were provided as follow (Table 3):

Table 3: Percentage of Moisture content of M. stenopetala and A. indica Seeds based on Dry Biomass

S/N	**Collected Samples**	**Initial Moisture content (%) before drying (**Mean ± SD**)**	**Final Moisture content (%) after drying (**Mean ± SD**)**
1.	*M. stenopetala* seeds	6.48 ± 0.32	3.12 ± 0.38
2.	*A. indica* Seeds	11.64 ± 0.54	4.34 ± 0.42

SD – Standard deviation

The obtained initial average moisture content of *M. stenopetala* seed (6.48 ± 0.32%) was comparable with 5.70 % value of moisture content obtained for *M. oleifera* (Anwar and Bhanger, 2003). To enhance the effectiveness of oil extraction and avoid challenges of oil water content in the downstream process (i.e. diminishing efficiency of catalyst and formation of soap), the collected samples were dried and pretreated accordingly. Thus, using the oven drier, the collected samples were dried continuously until the moisture content was below 5% (ASTM, 2008). After drying, average moisture contents of *M. stenopetala* and *A. indica* Seeds were triplicated and obtained as 3.12 ± 0.38% and 4.34 ± 0.42% respectively.

4.1.2. The Yield of *Moringa stenopetala* and *Azadirachta indica* Seed Oil

Extraction of oil from *M. stenopetala* seed and *A. indica* seed was conducted at various range of particle sizes (i.e. 0.250 – 0.425 mm, 0.425 – 0.600 mm and 0.600 – 0.850 mm); and the corresponding average values of oil yield (%) were triplicated and recorded as per the result provided below, average ± SD (Table 4).

Table 4: The Yield of Oil Extracted from M. stenopetala and A. indica seed powder using Solvent Extraction Method

S/N	Feedstocks for biodiesel production	Particle size in range (mm)	Average volume of extracted oil (ml)	Average mass of extracted oil (g)	Average yield of oil (%) (mean ± SD)
1	*M. stenopetala* seeds	0.250 – 0.425	53.67	46.1533	38.46 ± 0.45
		0.425 – 0.600	62.33	53.6067	44.67 ± 0.33
		0.600 – 0.850	59.00	50.7400	42.28 ± 0.21
2	*A. indica* seeds	0.250 – 0.425	52.00	46.8000	39.86 ± 0.18
		0.425 – 0.600	58.67	52.8000	44.98 ± 0.54
		0.600 – 0.850	56.33	50.7000	43.19 ± 0.37

SD – Standard deviation

In this study, the maximum value of average oil yield (44.67 ± 0.33 %) was obtained from *M. stenopetala* seed powder with particle sizes in the range of 0.425 – 0.600 mm. This value was comparable with the value obtained in the previous study, 44.90% of oil yield from *M. stenopetala* seed powder (Andinet E. *et al.*, 2010). The obtained value was also in agreement with the reported value *M. stenopetala* seed oil, which ranges from 40.50 - 44.90 % (Lalas S. *et al*., 2003). In addition, the minimum value of oil yield (38.46 ± 0.45%) was recorded and obtained from the *M. stenopetala* seed powder of particle size in the range of 0.250 – 0.425 mm. This indicated that the particle size highly affects the yield of oil extracted from the considered species while the rest extraction variables (i.e. Temperature, solid-solvent ratio and time) were kept constant at their respective optimum operation points.

Furthermore, the maximum value of average oil yield (44.98 ± 0.54%) was obtained from *A. indica* seed powder in the range of 0.425 – 0.600 mm particle sizes. The recorded value was in the range of the previous reported result, which was 41.1 - 47.4% (Elteraifi I. and Hassanali A., 2011). The minimum oil yield (39.86 ± 0.18%) was obtained from *A. indica* seed powder at the particle sizes in the range of 0.250 – 0.425 mm.

4.1.3. The Physico-chemical Properties of Extracted *M. stenopetala* and *A. indica* Seed Oil

The physicochemical properties of *M. stenopetala* and *A. indica* Seed Oil were conducted according to the standard test methods. The results were triplicated and their corresponding mean values with standard deviation (SD) were provided below (in Table 5). The main physicochemical characteristic of *M. stenopetala* and *A. indica* seed oil were: Acid value, free fatty acid, Saponification value, Iodine value, Peroxide value, Kinematic viscosity, Specific gravity, pH value and Refractive index.

Table 5: The Physico-chemical properties of extracted M. stenopetala and A. indica seed Oil

S/N	**Physico-chemical properties of the extracted oil**		**Measurement units**	**Experimental Values** (*mean* ± *SD* **)**		**Standard Method Used**
				***M. stenopetala* Oil**	***A. indica* Oil**	
1	Acid value		mg KOH/g	5.68 ± 0.08	18.60 ± 0.64	AOAC, 2000
2	Free fatty acid (FFA)		mg KOH/g	2.84 ± 0.04	9.30 ± 0.32	AOAC, 2000
3	Saponification value		mg KOH/g	188.42 ± 0.46	194.36 ± 0.54	AOAC - 920.160, 2000
4	Iodine value		g I_2/100 g	68.20 ± 0.35	72.78 ± 0.36	AOAC -920.159, 2000
5	Peroxide value		meq/Kg	17.12 ± 0.15	16.24 ± 0.74	AOAC - 965.33, 2000
6	Kinematic Viscosity	at 22 °C	mm^2/s	47.73 ± 0.42	49.84 ± 0.33	ASTM D445
		at 40 °C	mm^2/s	26.50 ± 0.30	28.32 ± 0.42	
		at 60 °C	mm^2/s	12.30 ± 0.20	14.12 ± 0.12	
7	Specific gravity		-	0.89 ± 0.01	0.92 ± 0.02	AOAC - 920.212, 2000
8	pH value		-	7.35 ± 0.05	8.26 ± 0.35	-
9	Refractive index		-	1.4132 ± 0.03	1.4628 ± 0.02	AOAC - 921.08, 2000

In this study it has been found that the acid number of *M. stenopetala* seed oil (5.68 ± 0.08 mg KOH/g oil) was relatively lower than that of *A. indica* seed oil (18.60 ± 0.64 mg KOH/g oil). These value showed that the extracted oil from the two species (i.e. *M. stenopetala* and *A. indica* seeds) could not be subjected to the transesterification process directly. Thus, they necessitated acid pretreatment steps (i.e. esterification reaction) to convert the free fatty acid in the considered raw oils until their respective FFA values were less than 0.5% while enhancing the yield of biodiesel (FAME).

The obtained saponification values of *M. stenopetala* seed oil (188.42 ± 0.46 mg KOH/g oil) and *A. indica* seed oil (194.36 ± 0.54 mg KOH/g oil) were of high value signifying that high free fatty acid (FFA) was presented in each extracted crude oils. In this study, the obtained saponification value of *M. stenopetala* seed oil was in agreement with reported values of previous work, 186 mg KOH/g saponification value of oil was reported by (Anwar *et al*., 2006) and 178.23 mg KOH/g by (Andinet E. *et al.,* 2010), Whereas, the obtained saponification value of *A. indica* seed oil (194.36 ± 0.54 mg KOH/g oil) was in comparable with the previous value, 199.810 ± 1.584 mg KOH/g oil, reported by (Bakari H. *et al.,* 2020) for the sample of *A. indica* seed oil collected from *Zidim*, Cameroon.

The iodine number indicates the degree of unsaturation of oils. The lower iodine value of the oil signifies the lower degree of unsaturation and vice versa. In this study, the iodine number of *M. stenopetala* seed oil was 68.20 ± 0.35 g I_2/100 g oil (Table 5 above). The obtained value of iodine number was relatively lower and hence, it showed that *M. stenopetala* seed oil was less likely susceptible to oil rancidity. The result was also in agreement with the report of the previous study, 69 g I_2/100 g oil by (Andinet *et al.,* 2010) and 65.8 g I_2/100 g oil by (Lalas *et al.,* 2003). Moreover, the obtained value of iodine number of *A. indica* seed oil was 72.78 ± 0.36 g I_2/100 g oil. This value was in line with the previous reported result, 74.448 ± 0.564 g I_2/100 g oil and 73.814 ± 0.366 g I_2/100 g oil in *A. indica* seed oil collected from the districts of *Maroua* and *Zidim*, Cameroon, respectively (Bakari H. *et al.,* 2020).

The peroxide number determines the amount of hydro-peroxides presented in the crude extracted vegetable oil (Kapoor *et al.,* 2009). Crude oil with lower number of peroxide is highly resistant to oxidation reaction. The peroxide value of *M. stenopetala* seed oil obtained in this study was 17.12 ± 0.15 meq/Kg; and this value was in comparable with previous reported peroxide value of 17.60 meq/Kg for *M. stenopetala* seed oil collected from *Konso*, Ethiopia (Meseret H. *et al.,* 2019). The peroxide value of *A. indica* seed oil (16.24 ± 0.74 meq/Kg) was less than that of *M. stenopetala* seed oil signified that *A. indica* seed oil was more resistant to oxidation than *M. stenopetala* seed oil.

The kinematic viscosities obtained in this study were recorded at various temperature (i.e. at 22 °C, 40 °C and 60 °C) and the corresponding values at each specified temperature were recorded, 47.73 ± 0.42 mm^2/s, 26.50 ± 0.30 mm^2/s, 12.30 ± 0.20 mm^2/s, respectively for *M. stenopetala* seed oil; and for *A. indica* seed oil the kinematic viscosities, 49.84 ± 0.33 mm^2/s, 28.32 ± 0.42 mm^2/s, 14.12

± 0.12 mm^2/s were recorded respectively (Table 5 above). For both crude oil samples, the result showed that the kinematic viscosities were decreased with increasing temperature of oil and vice versa. The obtained physicochemical properties for *M. stenopetala* seed oil and *A. indica* seed oil (i.e. kinematic viscosity, specific gravity and refractive index) were in agreement with the reported values of previous work. The specific gravity of *M. stenopetala* and *A. indica* seed oil were 0.89 and 0.92 respectively; and these values were in comparable with the previous reported value (Adegbe *et al.*, 2016). Whereas, the recorded values of refractive index, 1.4132 ± 0.03 and 1.4628 ± 0.02, for *M. stenopetala* and *A. indica* seed oil respectively; and these values were in line with the result showed in the previous study (Anwar *et al.*, 2006).

4.2. Production and Characterization of Biodiesel from Hybridized *M. stenopetala* and *A. indica* Seed Oil

4.2.1. Experimental Design

In this study, the experiment was designed according to Response Surface Method - the Box-Behnken Design (BBD) Method with selected variables of study or factors (i.e. Oil mixing composition, Catalyst dose and Reaction time) and three levels for each factor (i.e. Low, Medium and High). The interaction effects of factors and their influences on response was studied while optimizing the required response (i.e. Biodiesel yield). The oil mixing composition, catalyst dose and reaction time were symbolized as A, B and C respectively. A total of 15 experiments were conducted according to BBD as per provided below (Table 6).

The statistical software package Design-Expert® was employed for analysis of variance (ANOVA), analysis of regression and estimation of coefficients of the model equation of fatty acid methyl ester (Biodiesel) synthesized by hybridization of *M. stenopetala* and *A. indica* seed Oil. The analysis of variance (ANOVA) was also employed to evaluate the adequacy of the model equation. The statistical significance and the quality of fit of the model equation was expressed using prediction coefficients of determination (Pred- R^2), coefficient of determination (R^2), and adjusted coefficients of determination (adj- R^2), F- test, and coefficient of variation (CV), where the main comparison was conducted at 5% levels of the Least Significance Difference (LSD).

Table 6: The effect of operation variables (i.e. Oil mixing composition, catalyst dose and reaction time) on the yield of fatty acid methyl ester (Biodiesel)

Run	Factor 1	Factor 2	Factor 3	Alcohol to Oil Molar ratio	Temperature (°C)	Speed of stirrer (rpm)	Response	
	A: Oil mixing Composition (M : N) (%v/v)	**B: Catalyst dose (KOH) (%w/v)**	**C: Reaction time (minute)**				**Biodiesel yield (%)**	
							Actual value	**Predicted value**
1	50.00	2.00	60.00	6:1	60	300	89.00	90.00
2	50.00	2.00	20.00	6:1	60	300	72.00	71.00
3	50.00	1.50	40.00	6:1	60	300	88.00	87.67
4	50.00	1.00	60.00	6:1	60	300	73.00	74.00
5	50.00	1.00	20.00	6:1	60	300	60.00	59.00
6	75.00	1.00	40.00	6:1	60	300	74.00	73.75
7	25.00	1.50	20.00	6:1	60	300	70.00	70.75
8	25.00	1.50	60.00	6:1	60	300	86.00	84.75
9	50.00	1.50	40.00	6:1	60	300	87.00	87.67
10	50.00	1.50	40.00	6:1	60	300	88.00	87.67
11	75.00	2.00	40.00	6:1	60	300	90.00	89.75
12	25.00	2.00	40.00	6:1	60	300	84.00	84.25
13	25.00	1.00	40.00	6:1	60	300	72.00	72.25
14	75.00	1.50	20.00	6:1	60	300	70.00	71.25
15	75.00	1.50	60.00	6:1	60	300	92.00	91.25

M- M. stenopetala oil; N- A. indica Oil; BMN- Biodiesel of mixed M and N

The resulting actual yield of fatty acid methyl ester (biodiesel) synthesized by hybridization of *M. stenopetala* and *A. indica* Seed Oil at various operational variables (i.e. Oil mixing composition, catalyst dose and reaction time) was computed according to equation 7 above. The results provided above (Table 6) showed that the maximum yield of biodiesel (92 %) was obtained from Experiment number 15 at Oil mixing composition of $M_{75}N_{25}$ (i.e. 75 %v/v *M. stenopetala* seed oil and 25 %v/v *A. indica* seed oil), catalyst dose of 1.5%w/v and reaction time of 60 min. Whereas, the minimum yield of biodiesel (60%) was obtained from Experiment number 5 at Oil mixing composition of $M_{50}N_{50}$ (i.e. 50 %v/v *M. stenopetala* seed oil and 50 %v/v *A. indica* seed oil), catalyst dose of 1 %w/v and reaction time of 20 min.

4.2.2. Statistical Analysis of the Fatty Acid Methyl Ester (Biodiesel) Using Response Surface Methodology (RSM)

A polynomial equation of second-order (quadratic equation) was employed to find the relationship between the independent variables or factors (i.e. A- oil mixing composition, B- catalyst dose and C- reaction time) and the response (i.e. Biodiesel yield). For the three selected factors, the response was provided as follow:

$$Y = \beta_{11}A^2 + \beta_{22}B^2 + \beta_{33}C^2 + \beta_{12}AB + \beta_{13}AC + \beta_{23}BC + \beta_1 A + \beta_2 B + \beta_3 C + \beta_0 + \varepsilon \ldots\ldots\ldots (8)$$

Where,

Y- the response (yield); A, B, and C are the independent variables, $\boldsymbol{\beta_i}$ - Coefficients of linear interaction effect, $\boldsymbol{\beta_0}$ – a constant, $\boldsymbol{\beta_{ii}}$ – coefficients for cross-product interaction effect, $\boldsymbol{\beta_{ij}}$ – coefficients for quadratic interaction effect, and $\boldsymbol{\varepsilon}$ –is the random error. The estimation of coefficients and the regression analysis was carried out with a statistical software package Design-Expert®. The analysis of variance (ANOVA) was employed to evaluate the adequacy of the model equation. The statistical significance and the quality of fit of the model equations was expressed using prediction coefficients of determination (Pred- R^2), coefficient of determination (R^2), and adjusted coefficients of determination (adj-R), F- test, and coefficient of variation (CV), where the main comparison was conducted at 5% levels of the Least Significance Difference (LSD).

The response surface methodology (i.e. Box-Behnken Design method) was employed for the analysis of variance (ANOVA) of the produced fatty acid methyl ester; and thereby providing the corresponding model equation for the desired product.

Table 7: Analysis of variance (ANOVA) of the synthesized biodiesel at various operational variables

Response 1	**Biodiesel (FAME) Yield**					
ANOVA for Response Surface Quadratic Model [Partial Sum of Squares –Type III]						
Source	**Sum of Squares**	**Degree of freedom**	**Mean square**	**F value**	**P value Prob > F**	
Model	*1356.17*	*9*	*150.69*	*82.19*	*< 0.0001*	*significant*
A- Oil mixing composition	*24.50*	*1*	*24.50*	*13.36*	*0.0147*	
B- Catalyst dose	*392.00*	*1*	*392.00*	*213.82*	*< 0.0001*	
C- Reaction time	*578.00*	*1*	*578.00*	*315.27*	*< 0.0001*	
AB	*4.00*	*1*	*4.00*	*2.18*	*0.1997*	
AC	*9.00*	*1*	*9.00*	*4.91*	*0.0776*	
BC	*4.00*	*1*	*4.00*	*2.18*	*0.1997*	
A^2	*2.56*	*1*	*2.56*	*1.40*	*0.2901*	
B^2	*172.41*	*1*	*172.41*	*94.04*	*0.0002*	
C^2	*198.56*	*1*	*198.56*	*108.31*	*0.0001*	
Residual	*9.17*	*5*	*1.83*			
Lack of Fit	*8.50*	*3*	*2.83*	*8.50*	*0.1071*	*not significant*
Pure Error	*0.67*	*2*	*0.33*			
Cor Total	*1365.33*	*14*				

The Model F-value of 82.19 indicated that the model is significant (*P- value < 0.0001*). There is only a 0.01% chance that a "Model F-Value" is large, which could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C, B^2, C^2 are significant terms of the developed model. Values greater than 0.1000 shows that the model terms are not significant statistically. Thus, AB, AC, BC and A^2 are insignificant terms of the model. If there are many insignificant model terms (i.e. not counting those required to support hierarchy), model diminution would meliorate the model. The "Lack of Fit F-value" of 8.50 indicates the Lack of Fit is not significant (*P- value = 0.1071*) relative to the pure error. There is a 10.71% chance that a "Lack of Fit F-value" is large, occur due to noise.

4.2.3. Fatty Acid Methyl Ester (Biodiesel) Modelling and Analysis of the Model

Table 8: The suggested model for the produced biodiesel from hybridized M. stenopetala and A. indica seed oil at various operational parameters

Response 1	**Biodiesel (FAME) Yield**					
Sequential Model Sum of Squares [Type I]						
Source	**Sum of Squares**	**Degree of freedom**	**Mean square**	**F value**	**P - value Prob > F**	
Mean vs Total	*95201.67*	*1*	*95201.67*			
Linear vs Mean	*994.50*	*3*	*331.50*	*9.83*	*0.0019*	
2FI vs Linear	*17.00*	*3*	*5.67*	*0.13*	*0.9407*	
Quadratic vs 2FI	*344.67*	*3*	*114.89*	*62.67*	*0.0002*	***Suggested***
Cubic vs Quadratic	*8.50*	*3*	*2.83*	*8.50*	*0.1071*	*Aliased*
Residual	*0.67*	*2*	*0.33*			
Total	*96567.00*	*15*	*6437.80*			

After evaluation of a model fit summary of the obtained experimental data, polynomial expression of the highest degree (i.e. second order) with significant terms of the model was selected while insuring the model is not aliased. Therefore, a quadratic model was suggested as statistically significant model for the required response of biodiesel (fatty acid methyl ester) yield.

Table 9: Lack of fit Testes for the Suggested Quadratic Model of the Produced Biodiesel (FAME)

Response 1	**Biodiesel (FAME) Yield**					
Lack of Fit Tests						
Source	**Sum of Squares**	**Degree of freedom**	**Mean Square**	**F value**	**P - Value Prob > F**	
Linear	*370.17*	*9*	*41.13*	*123.39*	*0.0081*	
2FI	*353.17*	*6*	*58.86*	*176.58*	*0.0056*	
Quadratic	*8.50*	*3*	*2.83*	*8.50*	*0.1071*	***Suggested***
Cubic	*0.000*					*Aliased*
Pure Error	*0.67*	*2*	*0.33*			

The "Lack of Fit F-value" of 8.50 implies the Lack of Fit is not significant relative to the pure error. There is a 10.71% chance that a "Lack of Fit F-value" this large could occur due to noise.

Table 10: Measures of Adequacy of the Produced Biodiesel (FAME) Model

Std. Dev.	1.35	R^2	0.9933
Mean	79.67	Adj - R^2	0.9812
C.V. %	1.70	Pred - R^2	0.8993
PRESS	137.50	Adeq Precision	29.171

Std. Dev. – standard deviation, C.V. - coefficient of variation, R^2 *– Coefficient of determination*

The " Pred - R^2" of 0.8993 was in reasonable agreement with the "Adj - R^2" of 0.9812. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Thus, the ratio of 29.171 indicated an adequate signal for the developed model. Hence, the developed model can be used to navigate the design space. The quality or strength of produced biodiesel (FAME) Model can be determined by taking coefficient of correlation into consideration. The coefficient of determination (R^2) value for the developed model was 0.9933. This indicated that 99.33% of the overall variation in fatty acid methyl ester (biodiesel) yield was associated with operational variables of the intended study. In addition, the lower value of coeffficient of variation (1.70 %) obtained from ANOVA showed the reliability and precision of the conducted experiment. This coefficient of variation (i.e. the ratio of standard error of estimate to average value of obtained biodiesel yield) measured the reproducible tendency of the selected model (i.e. quadratic model).

Table 11: The lower and higher coefficients of regression at confidence interval (CI) of 95%

Factor	**Coefficient of Estimate**	**Degree of freedom (df)**	**Standard Error**	**95% CI**	**95% CI**
Intercept	87.67	1	0.78	85.66	89.68
A-Oil mixing composition	1.75	1	0.48	0.52	2.98
B-Catalyst dose	7.00	1	0.48	5.77	8.23
C-Reaction time	8.50	1	0.48	7.27	9.73
AB	1.00	1	0.68	-0.74	2.74
AC	1.50	1	0.68	-0.24	3.24
BC	1.00	1	0.68	-0.74	2.74
A^2	-0.83	1	0.70	-2.64	0.98
B^2	-6.83	1	0.70	-8.64	-5.02
C^2	-7.33	1	0.70	-9.14	-5.52

Final equation of the produced Biodiesel (FAME) Yield in terms of Coded factors:

$$\begin{aligned}\text{Biodiesel (FAME) Yield, } Y = \; & +87.67 \\ & +1.75 * A \\ & +7.00 * B \\ & +8.50 * C \\ & +1.00 * A * B \\ & +1.50 * A * C \\ & +1.00 * B * C \\ & -0.83 * A^2 \\ & -6.83 * B^2 \\ & -7.33 * C^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(9)}\end{aligned}$$

The developed quadratic model equation indicated the relationship between operational variables (i.e. factors) and the response (i.e. yield of biodiesel). The model equation was expressed in terms of both significant and insignificant terms of the model. By avoiding the insignificant terms of the model (i.e. terms with P - *value* > 0.05) (Table 7 above), the model equation can be simplified and expressed in terms of significant model terms (i.e. terms with P - *value* < 0.05):

$$\begin{aligned}\text{Biodiesel (FAME) Yield, } Y = \; & +87.67 \\ & +1.75 * A \\ & +7.00 * B \\ & +8.50 * C \\ & -6.83 * B^2 \\ & -7.33 * C^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{(10)}\end{aligned}$$

Final Equation of Biodiesel (FAME) Yield in Terms of Actual Factors:

Biodiesel (FAME) Yield, Y =

$$
\begin{aligned}
&-30.00000\\
&-0.036667 \ * (\text{Oil mixing composition})\\
&+88.00000 \ * (\text{Catalyst dose})\\
&+1.59167 \ * (\text{Reaction time})\\
&+0.080000 \ * (\text{Oil mixing composition} * \text{Catalyst dose})\\
&+3.00000\text{E-}003 \ * (\text{Oil mixing composition} * \text{Reaction time})\\
&+0.10000 \ * (\text{Catalyst dose} * \text{Reaction time})\\
&-1.33333\text{E-}003 * (\text{Oil mixing composition})^2\\
&-27.33333 \ * (\text{Catalyst dose})^2\\
&-0.018333 * (\text{Reaction time})^2 \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots \ (11)
\end{aligned}
$$

Therefore, from the developed quadratic model equation, it has been observed that significant terms affecting the response (i.e. yield of biodiesel) were: Oil mixing composition (A), Catalyst dose (B), Reaction time (C), the square of catalyst dose (B^2) and the square of reaction time (C^2). Whereas, the insignificant terms of the model were: the square of oil mixing composition (A^2), the interaction effects of oil mixing compositon with catalyst dose (AB), the interaction effects of oil mixing composition with reaction time (AC) and the interaction effects of catalyst dose with reaction time (BC).

Furthermore, to evaluate the model property of statistical distribution, the Box-Behenken Design (BBD) normal probability versus residual plot was employed (Figure 8 below). The plot showed that residual errors were distributed normally in linear pattern. Thus, the data points in the plot were estimated to be in straight line and there was no abnormality sign in the developed model.

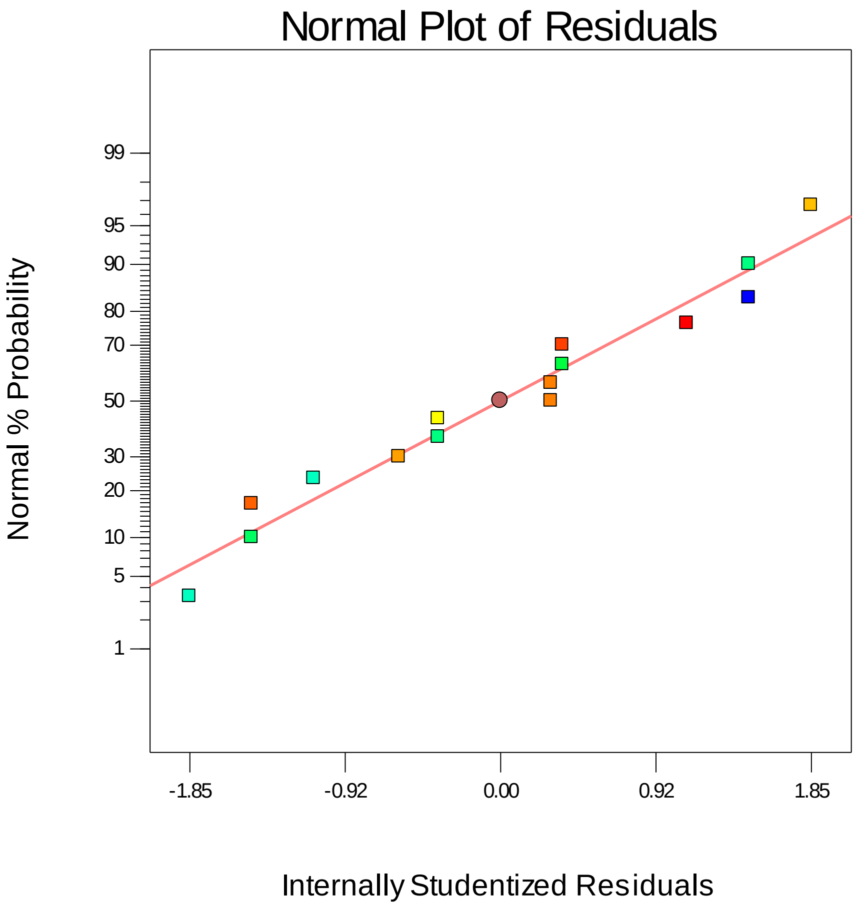


Figure 8: The plot of normal % probability versus internally Studentized residuals

The relationship between actual and predicted value of biodiesel yield was observed from the high correlation coefficient, $R^2 = 0.9933$, which was close to unity. Hence, the predicted values of the biodiesel yield were in reasonable agreement with actual obtained values (Table 12 below) and the experimental data fit with the developed model providing the precise estimate of the required response for the transesterification reaction process in the considered range of operational variables.

Table 12: The Diagnostics Case Statistics of Biodiesel (FAME) yield

Response 1	**Biodiesel (FAME) yield**					
Standard order	**Actual value**	**Predicted value**	**Residual**	**Leverage**	**Internally Studentized residual**	**Externally Studentized residual**
1	72.00	72.25	-0.25	0.750	-0.369	-0.335
2	74.00	73.75	0.25	0.750	0.369	0.335
3	84.00	84.25	-0.25	0.750	-0.369	-0.335
4	90.00	89.75	0.25	0.750	0.369	0.335
5	70.00	70.75	-0.75	0.750	-1.108	-1.141
6	70.00	71.25	-1.25	0.750	-1.846	-2.928
7	86.00	84.75	1.25	0.750	1.846	2.928
8	92.00	91.25	0.75	0.750	1.108	1.141
9	60.00	59.00	1.00	0.750	1.477	1.760
10	72.00	71.00	1.00	0.750	1.477	1.760
11	73.00	74.00	-1.00	0.750	-1.477	-1.760
12	89.00	90.00	-1.00	0.750	-1.477	-1.760
13	88.00	87.67	0.33	0.333	0.302	0.272
14	88.00	87.67	0.33	0.333	0.302	0.272
15	87.00	87.67	-0.67	0.333	-0.603	-0.560

Moreover, the relationship between actual values and predicted values of biodiesel (FAME) yield was indicated by the plot provided below (Figure 9) using the data provided above (in Table 12).

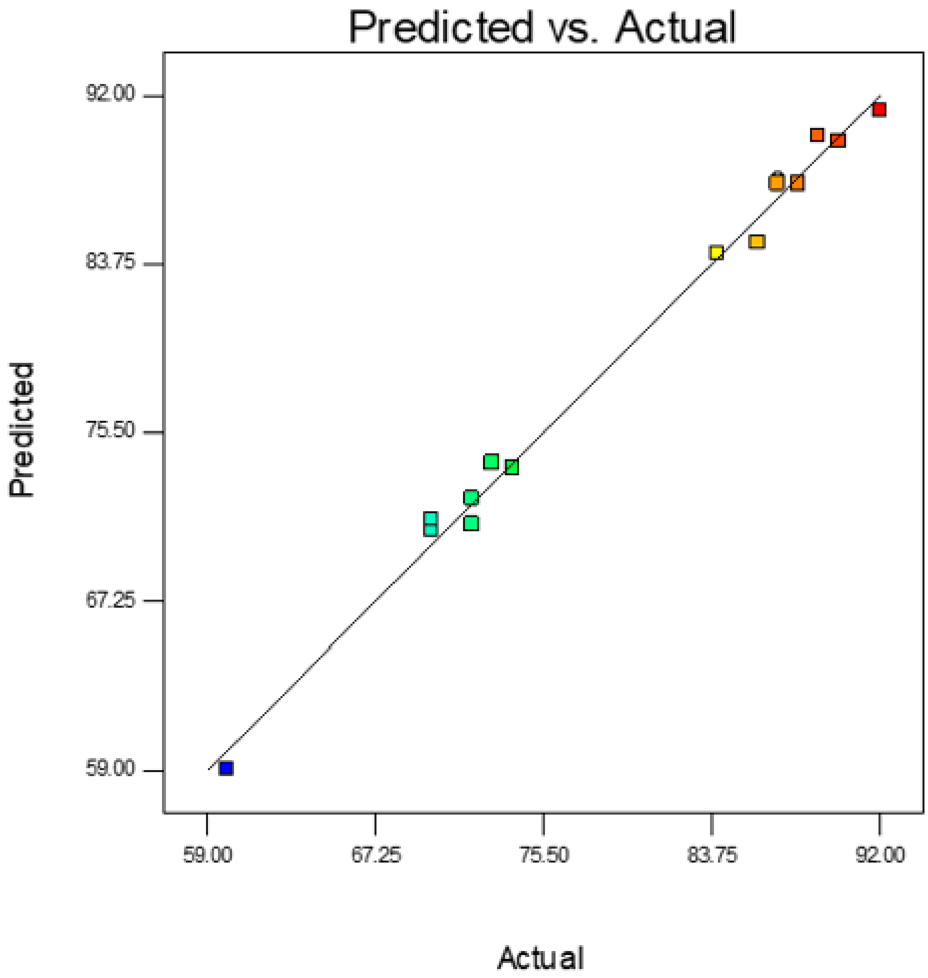


Figure 9: Actual Values versus Predicted Values of Biodiesel (FAME) Yield

4.2.4. The Effect of Operational Variables on the Yield of Fatty Acid Methyl Ester (Biodiesel)

The effect of considered operational variables on the yield of biodiesel (FAME) was evaluated based on values obtained from 15 experimental runs. The effect of oil mixing composition, catalyst dose and reaction time on the response (yield of biodiesel) were presented with their corresponding 3D plot in the Box-Behnken Design (BBD).

4.2.4.1. The Effect of Oil Mixing Composition and Catalyst dose on the Yield of Biodiesel (FAME)

The result of ANOVA showed that oil mixing composition (A) and catalyst dose (B) affect the yield of fatty acid methyl ester (Biodiesel) significantly as they had *P- value* < 0.05 (Table 7 above). The reaction time (C) was fixed at 40.00 Min. The maximum value of fatty acid methyl ester (Biodiesel) percentage yield (90 %) was achieved when the oil mixing composition was $M_{75}N_{25}$ (i.e. 75 %v/v *M. stenopetal*a seed oil and 25 %v/v *A. indica* seed oil) and the catalyst dose of 2 %w/v (Figure 10 below). The 3D plot of Box-Behnken Design (BBD) indicated that for the considered range of these operational variables, increasing the oil mixing composition increases the yield of FAME and as the catalyst dose increased (i.e. from 1 - 2 %w/v), the yield of FAME was also increased and vice-versa. Adequate catalyst dose during the transesterification reaction enhanced the forward rate thereby achieving completion of the reaction and enhancing the yield of FAME.

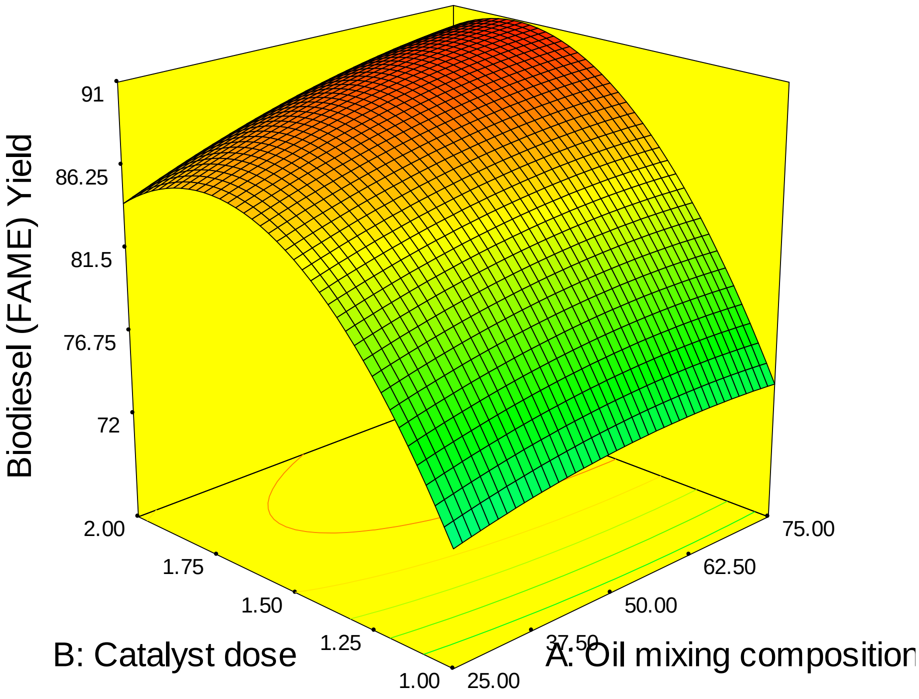


Figure 10: The effect of Oil mixing composition (A) and Catalyst dose (B) on the yield of Biodiesel (FAME)

4.2.4.2. The Effect of Oil Mixing Composition and Reaction Time on the Yield of Biodiesel (FAME)

For the data obtained from 15 experimental runs, the result of ANOVA indicated that oil mixing composition (A) and reaction time (C) were significant operational variables because they had *P-value* < 0.05 and hence, they had significant effect on the yield of biodiesel. The catalyst dose (B) was kept constant at 1.5 %w/v. From the provided 3D plot of Box-Behnken Design (BBD), it has been observed that percentage yield of Biodiesel was increased with increased oil mixing composition (i.e. from 25 - 75 %v/v) and reaction time (i.e. from 20 - 60 min). The maximum yield of biodiesel (92%) was obtained when the oil mixing composition was $M_{75}N_{25}$ (i.e. 75 %v/v *M. stenopetal*a seed oil and 25 %v/v *A. indica* seed oil) and the reaction time of 60 min (Figure 11 below). The optimum and sufficient reaction time enhanced the conversion of reactants and completion of the transesterification reaction while maximizing the yield of biodiesel. However, longer reaction time beyond the optimum induces the reverse reaction (i.e. hydrolysis of FAME into triglycerides); and thereby diminishing the yield of the FAME.

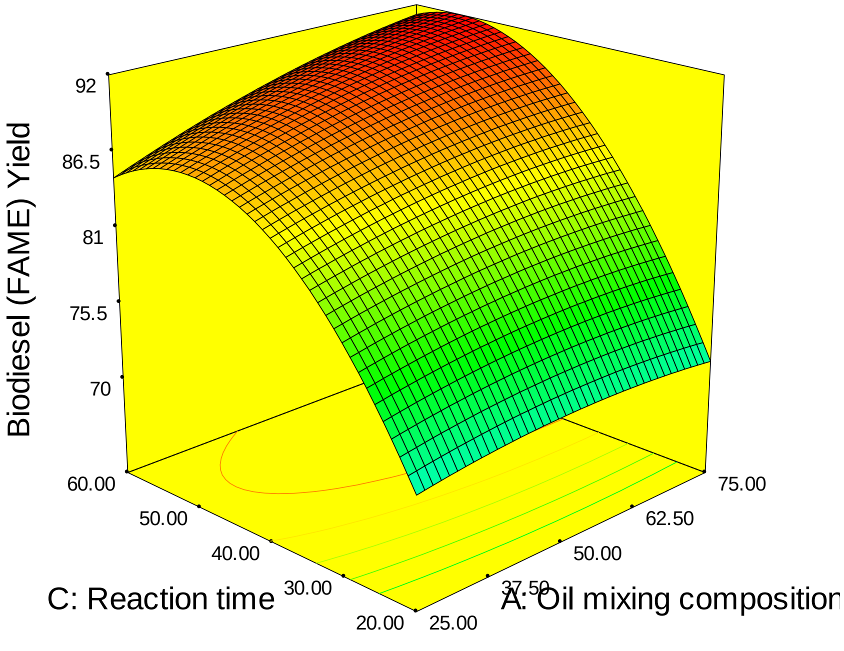


Figure 11: The effect of oil mixing composition and reaction time on the yield of biodiesel

4.2.4.3. The Effect of Catalyst dose and Reaction Time on the Yield of Biodiesel (FAME)

To observe the effect of catalyst dose (B) and reaction time (C) on the yield of biodiesel, the oil mixing composition (A) was kept constant at 50.00 %v/v (i.e. 50 %v/v *M. stenopetala* seed oil and 50 %v/v *A. indica* seed oil). From the result of ANOVA, both operational variables (i.e. Catalyst dose and reaction time) had *P- value < 0.0001* (Table 7 above); and hence, these variables had significant effect on the yield of fatty acid methyl ester. From the 3D plot of Box-Behnken Design (BBD) provided below (Figure 12), it has been indicated that the yield of biodiesel increased significantly with increased catalyst dose (%w/v) and reaction time (min). The maximum value of 89% biodiesel yield was obtained at the maximum catalyst dose of 2 %w/v and maximum reaction time of 60 minutes. Whereas, the minimum value of 60 % biodiesel yield was obtained at the minimum catalyst dose of 1%w/v and reaction time of 20 minutes. Thus, during the base-catalyzed transesterification reaction, optimum catalyst dose and reaction time promoted the forward rate of reaction and derived the reaction system to reach the equilibrium while maximizing the yield of the desired product (FAME).

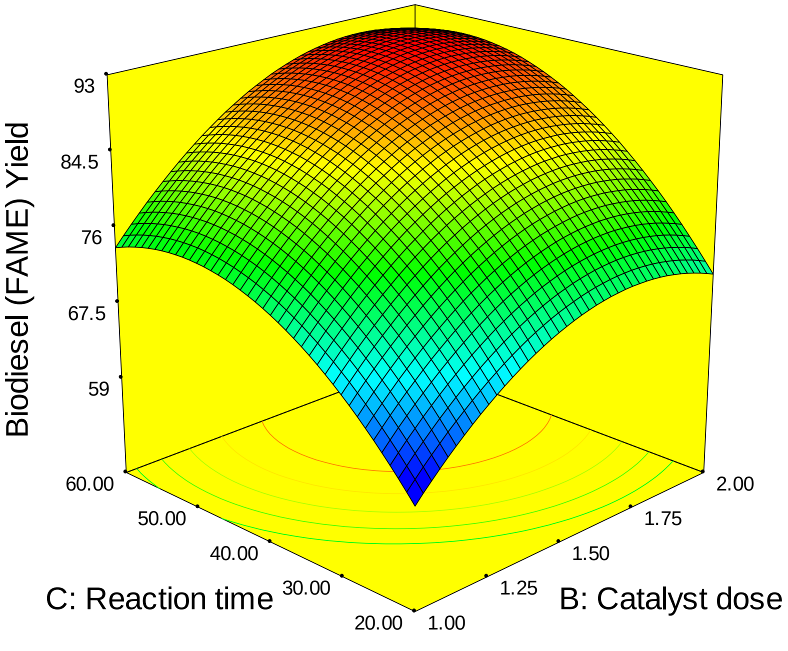


Figure 12: The effect of catalyst dose and reaction time on the yield of biodiesel

4.2.5. Optimization of the Biodiesel Production Process Using Response Surface Methodology

The optimum conditions for operational variables (i.e. oil mixing composition, catalyst dose and reaction time) were determined by Response Surface Methodology (i.e. by Box-Behnken Design Method). The Design expert software was employed to evaluate combinations of operational variables achieving the required criteria set for the response (i.e. Maximizing the yield of biodiesel). In the process of optimization, the operating conditions (i.e. Lower limit, Upper limit and Ultimate goal) were determined for each variables of study and the response or biodiesel (FAME) yield (Table 13). To maximize the yield of biodiesel (FAME), the value of oil mixing composition and catalyst dose were set in ranges with their corresponding Lower and Upper limits, whereas, the value of reaction time was set to be 60 min (i.e. the sufficient time required for completion of the reaction).

Table 13: Criteria for Numerical Optimization of fatty acid methyl ester (Biodiesel) Yield

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
Oil Mixing Composition	is in range	25	75	1	1	3
Catalyst dose	is in range	1	2	1	1	3
Reaction time	maximize	20	60	1	1	3
Biodiesel (FAME) Yield	maximize	60	92	1	1	3

Then, using the Box-Behnken Design desirability, the determined operating conditions were evaluated in order to observe degree of satisfaction (i.e. it ranges from 0 - 1) of the proposed solutions achieving the ultimate goal of maximizing the product (Table 14).

Table 14: Solutions for Numerical Optimization of fatty acid methyl ester (Biodiesel) Yield

Number	**Oil mixing composition**	**Catalyst dose**	**Reaction time**	**Biodiesel (FAME) Yield**	**Desirability**	
1	57.17	1.88	60.00	92.0261	1.000	**Selected**
2	74.37	1.61	60.00	92.8265	1.000	
3	58.77	1.84	60.00	92.3924	1.000	
4	59.81	1.80	60.00	92.5554	1.000	
5	68.99	1.61	60.00	92.4693	1.000	
6	68.52	1.89	60.00	93.4440	1.000	
7	61.63	1.77	60.00	92.7376	1.000	
8	69.00	1.60	60.00	92.2652	1.000	
9	60.63	1.80	60.00	92.6617	1.000	
10	65.77	1.97	60.00	92.6050	1.000	
11	71.64	1.82	60.00	93.8972	1.000	
12	74.84	1.60	60.00	92.7646	1.000	
13	63.99	1.84	60.00	93.0476	1.000	

From the proposed numerical solutions provided above (in Table 14), it has been observed that the optimum operating conditionss for variables of study were: 57.17 %v/v of oil mixing composition, 1.88 %w/v of catalyst dose, 60 min of reaction time and the predicted value of 92.026 % biodiesel (FAME) yield with its correspondiing maximum desirability of 1.000. However, the actual or experimental value of the biodiesel (FAME) yield was 91.72%, with minor deviation 0.30 % of predicted value from the actual value of biodiesel yield. Therefore, the developed model equation was valid for the optimized variables of study.

4.2.6. Evaluation of the Physico-chemical Properties of the Optimized Yield of Biodiesel

4.2.6.1. Results of Fourier Transform Infrared (FTIR) Spectrometer

The Fourier Transform Infrared Radiation (FTIR) Spectroscopy was employed to examine the functional groups of *M. stenopetala* seed oil, *A. indica* seed oil and biodiesel produced by hybridization of *M. stenopetala* and *A. indica* seed oil. The analysis of FTIR was conducted using 5 ml of each samples (i.e. *M. stenopetala* seed oil, *A. indica* seed oil and Biodiesel synthesized from hybridized oil) at the resolution of 1 cm^{-1} for the wave number in the range of 400.0000 - 4000.0000 cm^{-1} (Figure 13).

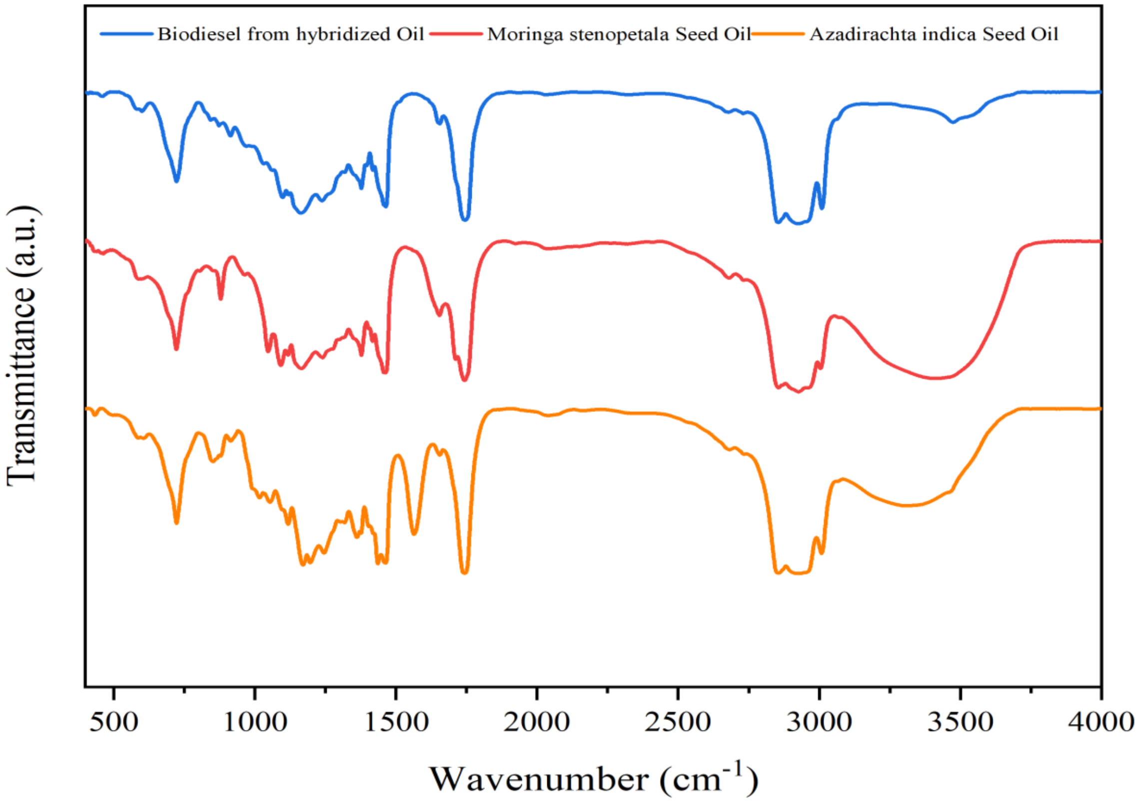


Figure 13: The FTIR spectrum of M. stenopetala seed oil, A. indica seed oil and the biodiesel produced from hybridized oil

The analysis of peak points of infrared spectrum for each sample indicated that there was significance difference between the spectrum of feedstocks and biodiesel produced in the considered range of wave number which on turn signified the existence of different functional groups in each sample. This variation in infrared spectrum occurred due to the transition of triglyceride esters in the non-edible oils into fatty acid methyl ester (biodiesel) via transesterification reaction. The peak of infrared spectrum in the range of 400 - 750 cm^{-1} wave number indicated that the existence of functional groups of C-H – aromatic bending vibration in the feedstocks (*M. stenopetala* and *A. indica* seed oil) and the presence of alkyl (methyl) – functional group in the fatty acid methyl ester (FAME) (Donnell S. *et al*., 2013). The infrared spectra peak in the range of 750-1470 cm^{-1} wave number, showed the presence of C - H bending vibration of alkane (- CH_2 -) in the feedstocks and in biodiesel. This results were in agreement with the results obtained by Banik S. K. *et al*. (Banik S. K. *et al*., 2018). Moreover, the peak of spectrum in the range of wave number from 1500 - 1740 cm^{-1} indicated the existence of functional group of C-H stretching vibration of alkenes (= CH_2) in fatty acid methyl ester (biodiesel) and feedstocks (Dube, M. A. *et al*., 2004). The transmission band of feedstocks and fatty acid methyl ester were similar in the range of wave number 1750 - 2850 cm^{-1}. The functional group of C = O stretching vibration of esters (i.e. carboxylic acid group) occurred in *M. stenopetala* seed oil, *A. indica* seed oil and biodiesel at the peak of spectrum in the region of 1750 - 2850 cm^{-1} wave number. This indicated that fatty acid methyl ester (biodiesel) can be prepared from the considered feedstock successfully via transesterification reaction (Oyerinde A. Y. and Bello E. I., 2016). The range of infrared spectrum peak with wave number of 3000 - 4000 cm^{-1} showed that the presence of water and – OH group in *M. stenopetala* and *A. indica* seed oil; and from the spectra of biodiesel, it has been observed that there was no presence of moisture or water content in the biodiesel (Figure 13 above) signifying that the synthesized biodiesel can be utilized in the diesel engine as alternative fuel or energy source.

4.2.6.2. Results of Physicochemical Determination of the Biodiesel

Table 15: Results of The Physicochemical Properties of Biodiesel Produced from Hybridized M. stenopetala and A. indica Seed Oil

Properties		Unit of measurement	Standard Method Used	Experimental Value	Standard Values	
					EN 14214 Standard	ASTM Standard
Specific gravity		-	ASTM D1298	0.87 ± 0.01	0.86 - 0.90	-
Flash point		°C	ASTM D93	163 ± 1.54	> 130	≥ 120
Pour point			ASTM D5853	4.36 ± 0.50	*	*
Cloud point		°C	ASTM D2500	8.68 ± 1.12	*	*
Higher heating value		MJ/Kg	ASTM D240	41.64 ± 0.33	-	40 - 42
Kinematic viscosity	at 22 °C	mm^2/s	ASTM D445	5.64 ± 0.35	3.5 - 5.0	1.9 - 6.0
	at 40 °C			3.35 ± 0.15		
	at 60 °C			2.42 ± 0.56		
Cetane number		-	ASTM D613	53 ± 0.58	> 51	≥ 47
Acid number		mg KOH/g	ASTM D664	0.134 ± 0.12	≤ 0.5	≤ 0.5
Water and sediment		% Vol.	ASTM D2709	0.067 ± 0.06	-	≤ 0.05
Carbon residue		% mass	ASTM D4530	0.03 ± 0.01	-	< 0.05
Methanol content		%	EN 14110	0.01 ± 0.005	< 0.2	-
Free glycerin		% mass	ASTM D6584	0.013 ± 0.005	< 0.02	< 0.02
Total glycerin		% mass	ASTM D6584	0.015 ± 0.01	< 0.25	< 0.24
Oxidation stability		Hours	EN 14112	7.20 ± 0.50	8 hours	3 hours
Phosphorous		mg/Kg	ASTM D4951	0.03 ± 0.01	< 4.0	< 0.001
Sulfur content		mg/Kg	ASTM D5453	0.06 ± 0.03	< 10.0	< 0.05
Calcium and Magnesium		mg/Kg	EN 14538	3.13 ± 0.04	< 5	≤ 5

* - *Not determined (i.e. depends on season and location)*

The evaluation of physicochemical characteristics of the resulting product was carried out for the optimized biodiesel (FAME) yield that was synthesized at optimum operating variables. The resulting 91.72 % of biodiesel yield was obtained by hybridization of 57.17 %v/v of *M. stenopetala* seed oil and 42.83 %v/v of *A. indica* seed oil with the presence of 1.88 %w/v catalyst dose (KOH) for 60 min reaction time (Table 14 above). The physicochemical characteristics of the optimized fatty acid methyl ester or biodiesel (i.e. specific gravity, flash point, pour point, cloud point, higher heating value, kinematic viscosity, Cetane number, acid number, water and sediment, carbon residues, methanol content, free glycerin, total glycerin, oxidation stability, phosphorus and sulfur content, calcium and magnesium content) were evaluated according to the procedures provided in their respective standard test method, ASTM and EN 14214 standards (Table 15 above). The obtained results were in agreement with the standard limit of biodiesel quality specification parameters; and hence, the synthesized FAME from hybridized *M. stenopetala* and *A. indica* seed oil was suitable for application in the diesel engine. In this study, biodiesel production by hybridization of considered feedstocks involves mixing of *M. stenopetala* and *A. indica* seed oil at various percentage of mixing composition (%v/v); and thereby obtaining the final product with improved quality. Moreover, the presence of high percentage of mono-unsaturated fatty acids (i.e. predominantly, oleic acid) in each feedstocks (i.e. *M stenopetala* and *A. indica* seed oil) induces the improvement of biodiesel quality parameters. Thus, the presence of 76.00 % of oleic acid ($C_{18}H_{32}O_2$) in *M. stenopetala* seed oil (Andinet E. *et al.*, 2010) and 51.00 % oleic acid ($C_{18}H_{32}O_2$) in *A. indica* seed oil (Adepoju T. and Olawale O., 2015) enhances and improves the flash point, the higher heating value, Cetane number and other properties of the produced biodiesel (FAME).

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

A fatty acid alkyl ester (Biodiesel) - is a long-chain of fatty acid mono-alkylic esters synthesized by transesterification reaction of triglycerides of vegetable oils and animal with alcohol in the presence of suitable catalyst. It has been taken into consideration as cleaner, alternative, renewable, biodegradable and environmentally benign energy source. Upon its sustainable utilization, biodiesel can replace conventional diesel fuel and solve challenges accompanied with environmental pollution due to emissions of harmful gaseous and particulate matters from conventional diesel fuel, climate change, socio-economic, energy crisis and global warming. The main objective of this study was production and characterization of biodiesel by hybridization of *M. stenopetala* and *A. indica* seed oil to substitute conventional diesel fuel with greener, alternative and eco-friendly energy source. The considered samples (i.e. *M. stenopetala* and *A. indica* seeds) were collected from Sidama Region Agricultural Center and Afar Region, respectively. Then, the samples were transported to Addis Ababa Institute of Technology (AAiT), School of chemical and Bioengineering Laboratory, cleaned and freed from foreign materials such as, weed seed, molds, stones and other contaminants. The collected seeds were first de-hulled (i.e. decorticated). Following the decortication, the outer husk was separated from the kernels by winnowing. The dried samples of *M. stenopetala* and *A. indica* seed kernels were grinded into the paste at particle size in the range of 0.25 – 0.425 mm, 0.425 – 0.60 mm, 0.60 – 0.85 mm turn-by-turn using grinder to provide a higher surface area of particles for the ease of oil extraction and thereby determining the range of particle size to obtain better oil yield. The Soxhlet extraction method, with *n*-Hexane as a solvent, was employed for the extraction of oil from the considered species. The Physico-chemical properties of the refined oil was determined following the method of American Society for Testing Materials (ASTM) and Association of Official Analytical Chemists (AOAC). These properties include, determination of Acid value and free fatty acid content, Saponification value, Specific gravity, Viscosity, pH value, Iodine value and the refractive index. Then, the crude oil extracted from the considered feedstocks were, degummed, acid pre-treated prior to biodiesel production.

In biodiesel production process, the statistical software package Design-Expert$^{®,}$ Response Surface Methods (RSM), Box-Behnken Design (BBD) method, was employed in experimental design and result analysis. The considered factors of the study were: Oil mixing composition, Catalyst dose and Reaction time with three levels for each factor, low (-1), medium (0) and high (+1); and the other operational parameters, alcohol to oil molar ratio, temperature and mixing speed, were kept constant. The interaction effects of factors and their influences on response (biodiesel yield) were studied. The considered oil hybrid compositions subjected to the transesterification reaction were: $M_{25}N_{75}$ (25%v/v *M. stenopetala* seed oil : 75%v/v *A. indica* seed oil), $M_{50}N_{50}$ (50%v/v *M. stenopetala* seed oil : 50%v/v *A. indica* seed oil) and $M_{75}N_{25}$ (75%v/v *M. stenopetala* seed oil : 25%v/v *A. indica* seed oil) with their corresponding biodiesel, $BM_{25}N_{75}$, $BM_{50}N_{50}$, $BM_{75}N_{25}$; catalyst dose of 1 %w/v, 1.5 %w/v and 2 %w/v; and reaction time of 20 min, 40 min and 60 min. A total of 15 number of runs were conducted with 3 levels for each numeric factors. The quadratic model was developed for conducted experiment with statistical significance, P - $value < 0.0001$. The analysis of variance (ANOVA) and coefficients of determinations were used to evaluate the quality of the model, where the main comparison was conducted at 5% LSD. The model adequacy was expressed using, $R^2 = 0.9933$, $Adj\text{-}\ R^2 = 0.9812$, $Pred\text{-}\ R^2 = 0.8993$ and lack of fit test with P - $value = 0.1071$ (i.e. statistically not significant). The value of $Pred\text{-}\ R^2 = 0.8993$ was adequately in agreement with the value of $R^2 = 0.9933$, signified that the developed quadratic model equation can be used to navigate the design space. The optimum, 91.72% biodiesel yield was obtained at optimum operating variables, oil mixing composition (57.17 %v/v of *M. stenopetala* and 42.83 %v/v of *A. indica* seed oil), catalyst dose (1.88 %w/v KOH) and reaction time (60 min) with desirability 1.000. The physicochemical characteristics of the optimized fatty acid methyl ester or biodiesel (i.e. specific gravity, flash point, pour point, cloud point, higher heating value, kinematic viscosity, Cetane number, acid number, water and sediment, carbon residues, methanol content, free glycerin, total glycerin, oxidation stability, phosphorus and sulfur content, calcium and magnesium content) were evaluated according to the procedures provided in their respective standard test methods, ASTM and EN 14214 standards; and the obtained results were in agreement with the standard limit of biodiesel quality.

5.2. Recommendations

Biodiesel (fatty acid methyl ester) production by hybridization of multi-feedstocks (i.e. predominantly, oil-bearing seeds) that are locally available is a new approach in developing the product with improved quality. Thus, it is recommended that proper characterization of feedstocks and understanding of the Physico-chemical nature and compositions of the considered raw materials is an indispensable step prior to mixing or hybridization of feedstocks to synthesize the biodiesel. In addition, the developed biodiesel product can be blended with the petrol-diesel at various blending ratios and its corresponding diesel-engine emission characteristics should be investigated. To scale-up the developed biodiesel (FAME) product obtained via transesterification reaction (i.e. biodiesel from hybridized *M. stenopetala* and *A. indica* seed oil), it has also been recommended to carry out process simulation, process development, feasibility study at large scale, preliminary pilot plant design and operation of the plant. Furthermore, it is important to work in collaboration with relevant multi-stakeholders across regions of Ethiopia, including, Governmental and Non-governmental biofuel processing industries, Higher Learning Institutions (i.e. University-Industry Linkage), Transportation sectors, Agricultural and plantation sectors, Agro-forestry sectors, Environmental policy makers, Environment and Forest Research Institute, Environmental Protection Agency, Biodiversity Institute and Ministry of Water, Irrigation and Electricity, Petroleum Supply Enterprises and Ministry of mine, Petroleum and Biofuel to ensure the sustainable production and utilization of biodiesel as a renewable energy source contributing to Environmental protection, solve energy crisis, climate change mitigation and contributing to the national economy of the country. Moreover, the production of biodiesel from locally available biomass feedstocks and substitution of conventional diesel fuel will also enhance development of potential agriculture-based business industry, employment generation via biomass feedstock utilization value chains, reducing the amount of petroleum import and increasing the export, contribute to water and soil conservation, reducing emission of pollutants including Greenhouse gases (GHG); and hence, offer local and global environmental welfare.

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ANNEXES:

Annex A: Determination of Physicochemical Properties of Extracted *M. stenopetala* and *A. indica* Seed Oil

Annex A- 1: Determination of Acid Value and Free Fatty Acid:

In 1000 mL conical flask 0.1 N ethanolic KOH (99.5% ethanol) was prepared, filtered and stored for 24 hours in dark bottle (i.e. glass stoppered bottle). About 2.5 g oil was dissolved in a mixture of 95% ethanol (25 mL) and diethyl ether (25 mL) solution. The solution was titrated with 0.1 N ethanolic KOH solution in the presence of pipetted 5 drops of phenolphthalein till colorless to faint pink color that persisted for ≥ 30 seconds. The Free fatty acid (FFA) was determined from acid value.

$$\textbf{Acid value (\%)} = \frac{56.1*(Vs-Vb)*N}{w}$$

Where, *Vs* is volume for sample (mL) and *Vb* is volume for blank of 0.1 N ethanolic KOH solution (mL); *N* is concentration of ethanolic KOH; *w* is weight of Oil (g).

$$\textbf{Free Fatty Acid (\%)} = \frac{AV}{2}$$

Annex A- 2: Determination of Saponification Value:

Two grams sample of each species (i.e. *M. stenopetala* and *A. indica* Seed Oil) were weighed using analytical balance; and added into 250 ml Erlenmeyer flasks respectively. Then, the solution of alcoholic potassium hydroxide (25 ml) was added into flasks. The determination of the blank was carried out along with the determination for the sample. The blank flask and flasks with samples were connected with an air condenser turn by turn and gradually boiled in the water bath at constant rate for 30 minutes until the completion of saponification reaction occurred (i.e. shown by the appearance of clear solution and disappearance of the oily matter). The condenser and flasks were subjected to cooling; and then, the inside part of condenser was washed with 10 ml of ethanol (C_2H_5OH). Finally, the phenolphthalein indicator of 1 ml was added into the solution. The extra amount of KOH was titrated with concentrated HCl of 0.5 N until the formation of cloudy solution occurred. Then, the saponification value has been computed as:

$$\textbf{Saponification Value (SV):} \frac{(Vb-Va)*N*56.1}{w} \text{ mgKOH/g}$$

Where, *Vb* is volume of HCl required for the blank (mL); *Va* is volume of HCl required for the sample (mL); *N* is Normality of the standard HCl; *w* is weight of oil sample (g) and 56.1 is molecular weight of KOH (g).

Annex A – 3: Determination of Iodine Value:

Using analytical balance, 5 g sample of oil for each species (i.e. *M. stenopetala* and *A. indica* Oil) was weighed and added into a 250 ml conical flasks. Then, 25 ml of carbon tetrachloride (CCl_4) was measured and added into the flasks containing sample of Oil; and entirely, the content of the mixture was mixed properly. Then, 25 ml of Hanus reagent (i.e. a mixture of 18.2 g of Iodine in 1L of glacial acetic acid and 3 ml of bromine water) was added into each respective flasks containing oil sample. Then, the mixture was mixed gently and allowed to be in the dark place for about 30 minutes. The potassium iodide solution of 15 ml was added to each mixture in the flasks and 100 ml of distilled water was added into each sample mixture. About 1 ml of starch solution was added into the solution of samples. Finally, the liberated iodine was titrated with sodium thiosulfate ($Na_2S_2O_3$) solution of normality, 0.01N until a blue color formed. The iodine determination for the blank (i.e. without sample of oil) was also conducted following the same procedure. Then, the iodine value was calculated using the following formula:

$$\textbf{Iodine value } (\mathbf{I_2 g/100g}) = \frac{(Vb - Vs) * N * 1.269}{w} * 100$$

Where, V_b is volume of standard thiosulfate required for the blank (mL); V_s is volume of standard thiosulfate required for the sample (mL); *N* is Normality of the standard thiosulfate solution; *w* is mass of oil sample (g)

Annex A- 4: Determination of Peroxide Value:

Five grams of samples of *M. stenopetala* and *A. indica* seed oil were weighed and added into a conical flasks of 250 ml separately. Then, 30 ml of mixture of chloroform and acetic acid solvent (i.e. at the mixture ratio of 2 : 3) was added into each flasks containing oil samples and dissolved gently. Then, the solution of potassium iodide (1 ml) was added into each samples. The solution was allowed to be in the dark place for about 1 minute with periodic shaking. Then, distilled water of 30 ml was added into the solution. The solution was titrated with 0.01 N of sodium thiosulfate ($Na_2S_2O_3$) until yellow color was disappeared. Then, the starch solution indicator of 1 ml was added and titration was continued until all iodine (I_2) was liberated and the blue color was disappeared.

$$\textbf{Peroxide value} = \frac{V * N * 100}{w}$$

Where, V is volume of sodium thiosulfate; N is normality used for sodium thiosulfate; and w -is mass of Oil sample (g)

Annex A- 5: Determination of Specific Gravity:

A pycnometer of 50 ml capacity was cleaned and dried. Then, the mass of dried pycnometer (**m_1**) was measured using analytical balance. The pycnometer was filled with sample of oil and its corresponding mass (**m_2**) was obtained. Then, the pycnometer was washed and dried; and filled with distilled water. The mass of pycnometer containing the distilled water (**m_3**) was recorded. Then, the specific gravity of each sample of oil (i.e. *M. stenopetala* and *A. indica* seed oil) was computed as follow:

$$\textbf{Specific gravity} = \frac{Mass\ of\ Oil}{Mass\ of\ distilled\ water\ of\ equal\ volume} = \frac{m2 - m1}{m3 - m1}$$

Where,

m_1 – mass of empty pycnometer (g); m_2 – mass of pycnometer containing oil sample (g); m_3 – mass of pycnometer containing distilled water (g)

APPENDICES:

Appendix A: Raw Materials Preparation and Oil Extraction from *M. stenopetala* and *A. indica* Seeds

Appendix B: The Physicochemical Determination of Extracted oil and Oil Refining Process

Appendix C: Fatty Acid Methyl Ester (Biodiesel) Production by Hybridization of *M. stenopetala* and *A. indica* Seed Oil

Appendix D: Results of Oil Extraction Using Solvent Extraction Method

Table D - 1: Results of Oil Extraction from both Species (i.e. *M. stenopetala* and *A. indica* seed powder) using Solvent Extraction Method

S/N	Feedstocks for Biodiesel Production	Particle Size (mm)	Solid mass to solvent volume ratio	Temperature of extraction (°C)	Time of extraction (hr.)	Volume of Extracted Oil (ml)		Mass of Extracted Oil (g)		Yield of Extracted Oil (%)	
						Value	**Mean**	**Value**	**Mean**	**Value**	**Mean**
1	*M. stenopetala* seed	0.250 – 0.425	1:5	72.00	5:00	52	53.67	44.7200	46.1533	38.13	38.46
			1:5	72.00	5:00	56		48.1600		40.13	
			1:5	72.00	5:00	53		45.5800		37.98	
		0.425 – 0.600	1:5	72.00	5:00	62	62.33	53.3200	53.6067	44.43	44.67
			1:5	72.00	5:00	63		54.1800		45.15	
			1:5	72.00	5:00	62		53.3200		44.43	
		0.600 – 0.850	1:5	72.00	5:00	60	59	51.6000	50.7400	43.00	42.28
			1:5	72.00	5:00	58		49.8800		41.57	
			1:5	72.00	5:00	59		50.7400		42.28	
2	*A. indica* seed	0.250 – 0.425	1:5	72.00	5:00	53	52	47.7000	46.8000	40.63	39.86
			1:5	72.00	5:00	52		46.8000		39.87	
			1:5	72.00	5:00	51		45.9000		39.10	
		0.425 – 0.600	1:5	72.00	5:00	59	58.67	53.1000	52.8000	45.23	44.98
			1:5	72.00	5:00	57		51.3000		43.70	
			1:5	72.00	5:00	60		54.0000		46.00	
		0.600 – 0.850	1:5	72.00	5:00	55	56.33	49.5000	50.7000	42.17	43.19
			1:5	72.00	5:00	57		51.3000		43.70	
			1:5	72.00	5:00	57		51.3000		43.70	

$$\textbf{Yield of Extracted Oil (\%)} = \frac{\boldsymbol{Mass\ of\ extracted\ Oil\ (g)}}{\boldsymbol{Mass\ of\ sample\ of\ Seed\ Powder (g)}} * \mathbf{100}$$

Appendix E: Results of Biodiesel Production by Hybridization of *M. stenopetala* and *A. indica* Seed Oil and Effect of Operational Variables on the Yield

Run	Factor 1	Factor 2	Factor 3	Batch size (ml)	Amount of Refined Oil				Mass of hybridized Oil (g)	Alcohol to Oil Molar ratio	Volume of alcohol employed (ml)	Reaction Temperature (°C)	Speed of stirrer (rpm)	Mass of conical flask (g)	Mass of biodiesel and conical flask (g)	Mass of Biodiesel (g)	Response
	A: Oil mixing composition (M : N) (%)	B: Catalyst dose (KOH) (%)	C: Reaction time (min)		*M. stenopetala* Oil (ml)	Mass of *M. stenopetala* Oil (g)	*A. indica* Oil (ml)	Mass of *A. indica* Oil (g)									Biodiesel yield (BMN) (%)
1	50	2.0	60	60	30	26.70	30	27.60	54.30	6:1	50.00	60	300	41.9294	90.2564	48.3270	89.00
2	50	2.0	20	60	30	26.70	30	27.60	54.30	6:1	50.00	60	300	46.8495	85.9455	39.0960	72.00
3	50	1.5	40	60	30	26.70	30	27.60	54.30	6:1	50.00	60	300	47.2083	94.9923	47.7840	88.00
4	50	1.0	60	60	30	26.70	30	27.60	54.30	6:1	50.00	60	300	47.9982	87.6372	39.6390	73.00
5	50	1.0	20	60	30	26.70	30	27.60	54.30	6:1	50.00	60	300	48.7438	81.3238	32.5800	60.00
6	75	1.0	40	60	45	40.05	15	13.80	53.85	6:1	49.21	60	300	34.7321	74.5811	39.8490	74.00
7	25	1.5	20	60	15	13.35	45	41.40	54.75	6:1	50.86	60	300	48.9534	87.2784	38.3250	70.00
8	25	1.5	60	60	15	13.35	45	41.40	54.75	6:1	50.86	60	300	44.2540	91.339	47.0850	86.00
9	50	1.5	40	60	30	26.70	30	27.60	54.00	6:1	50.00	60	300	47.2068	94.1868	46.9800	87.00
10	50	1.5	40	60	30	26.70	30	27.60	54.60	6:1	50.00	60	300	49.6403	97.6883	48.0480	88.00
11	75	2.0	40	60	45	40.05	15	13.80	53.85	6:1	49.21	60	300	46.5253	94.9903	48.4650	90.00
12	25	2.0	40	60	15	13.35	45	41.40	54.75	6:1	50.86	60	300	35.3071	81.2971	45.9900	84.00
13	25	1.0	40	60	15	13.35	45	41.40	54.75	6:1	50.86	60	300	49.3484	88.7684	39.4200	72.00
14	75	1.5	20	60	45	40.05	15	13.80	53.85	6:1	49.21	60	300	44.0946	81.7896	37.6950	70.00
15	75	1.5	60	60	45	40.05	15	13.80	53.85	6:1	49.21	60	300	35.1835	84.7255	49.5420	92.00

M – *Moringa stenopetala* Seed Oil; N- Neem/*Azadirachta indica* Seed Oil

$$\textbf{Biodiesel (FAME) yield (\%)} = \frac{\boldsymbol{Mass\ of\ Biodiesel\ (g)}}{\boldsymbol{Mass\ of\ Refined\ Oil\ (g)}} * \mathbf{100}$$