PRODUCTION OF BIODIESEL AND PREVENTION OF BIODEGRADATION USING NEEM (Azadirachta Indica) AND AFRICAN BASIL (Ocimum Gratissimum) LEAF EXTRACTS

BY

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UNIVERSITY OF BENIN

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CERTIFICATION

This is to certify that this project work was carried out and compiled by I, **ODIVWRI OGHENEMARO DAISY** with matriculation number **ENG2002058** of the Department of Chemical Engineering, Faculty of Engineering, University of Benin, Benin City, Edo State, Nigeria.

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DEDICATION

This project is dedicated to the Almighty God, for His guidance and protection and to my family, for their constant support, encouragement, and belief in me throughout my academic journey.

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I would like to express my sincere gratitude to my project supervisor, Professor Kessington Obahiagbon, for his guidance, support, and valuable insights throughout the course of this research. His expertise and encouragement were instrumental in the successful completion of this work.

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ABSTRACT

The global shift toward sustainable energy has intensified interest in biodiesel as an environmentally friendly alternative to fossil fuels. This study investigates the production of biodiesel from waste cooking oil (WCO) using calcined heterogeneous catalysts derived from waste materials, including pumpkin pods and turkey bones, and its subsequent preservation using natural extracts from Azadirachta indica (neem) and Ocimum gratissimum (African basil).

Transesterification was carried out in a pilot-scale reactor at 60 °C for 75 minutes. Characterization of the WCO prior to transesterification showed an acid value of 2.41 mg KOH/g and a free fatty acid (FFA) content of 1.21%, indicating moderate degradation from repeated frying. Post-treatment analysis revealed a slight increase in acid value to 3.07 mg KOH/g, while other parameters, including saponification value (251.83 mg KOH/g), density (0.8948 g/cm^3), and viscosity (9.50 mPa·s), remained within acceptable ranges for biodiesel feedstock.

The produced biodiesel met key ASTM D6751 specifications, with a density of 0.87 g/cm^3, acid value of 0.34 mg KOH/g, viscosity of 4.62 mm^2/s, and a flash point of 220 °C, confirming its suitability for use as a fuel or in blends. GC-MS analysis revealed a fatty acid methyl ester (FAME) composition dominated by methyl oleate (48.68%) and methyl palmitate (37.48%). FTIR spectroscopy further confirmed successful transesterification through the presence of a characteristic ester carbonyl absorption peak at 1742.81 cm^{-1}.

Stability studies conducted over six weeks showed that the combined treatment of neem and basil extracts (30 ml) resulted in the lowest final acid value of 0.78 mg KOH/g, indicating improved oxidative stability.

This study demonstrates that waste cooking oil is a viable and cost-effective feedstock for biodiesel production. Furthermore, the use of locally sourced plant extracts as natural stabilizers offers a sustainable and biodegradable approach to enhancing biodiesel storage stability, supporting the advancement of renewable energy technologies in Nigeria.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

The increasing global demand for energy and the growing concern over environmental degradation have driven significant interest in biodiesel as a sustainable alternative to conventional fossil fuels. Biodiesel, which can be derived from renewable sources such as vegetable oils, animal fats, and waste cooking oils, is valued for its non-toxic nature, biodegradability, and ability to reduce harmful emissions. These qualities make it a promising substitute for petroleum-based diesel and an important step toward solving two major global challenges the depletion of fossil fuel reserves and the environmental pollution linked to their continuous use.

Reports indicate that fossil fuels still dominate the global energy market, accounting for nearly 89% of total energy consumption, with transportation alone relying almost entirely on these non-renewable sources (Dorian et al., 2006). Data from OPEC and the U.S. Energy Information Administration show that between 2010 and 2020, global crude oil consumption ranged between 95 and 99 million barrels per day, representing over 35 billion barrels annually, while new oil discoveries contributed less than 6 billion barrels per year (Devanesan et al., 2007). This growing imbalance underscores the urgency of developing reliable, renewable, and sustainable energy alternatives.

Among the various renewable options, biodiesel, bioethanol, and biogas are considered viable substitutes for fossil fuels (Gerpen, 2005). However, for any renewable fuel to be widely accepted, it must meet certain standards it should be cost-effective, environmentally friendly, and compatible with existing diesel engines (DeMello et al., 2007). Biodiesel meets these criteria and has shown similar performance levels to fossil diesel in terms of combustion efficiency and reliability (Carraretto et al., 2004). Chemically, biodiesel is composed mainly of mono-alkyl esters of long-chain fatty acids, produced through the transesterification of triglycerides present in oils and fats. Other production techniques include microemulsification, direct blending, and thermal cracking (pyrolysis).

Despite its many advantages, one of the main challenges associated with biodiesel is its tendency to deteriorate during storage, a process often referred to as biodiesel biodeterioration. This degradation is mainly caused by microbial contamination and oxidation. Because biodiesel is biodegradable, microorganisms such as bacteria, yeasts, and fungi can grow within it, especially under poor storage conditions. These microbes alter the chemical structure of biodiesel, leading to higher acid values, increased viscosity, reduced oxidative stability, and the formation of unpleasant odors. In severe cases, microbial growth can clog filters, damage storage tanks, and compromise engine performance. Fungal species such as Aspergillus and Penicillium are particularly known for accelerating biodiesel spoilage, making the issue both an economic and operational concern.

To minimize biodiesel degradation, synthetic antioxidants and chemical biocides have traditionally been added to improve fuel stability and prevent microbial contamination. However, these synthetic agents come with drawbacks they are expensive, non-biodegradable, and can leave toxic residues that counteract the environmental benefits of biodiesel. Consequently, there is growing interest in exploring natural, plant-based alternatives that are safer and align with the green nature of biodiesel.

Plant extracts have shown great promise as natural additives due to their abundance of bioactive compounds such as flavonoids, phenolics, alkaloids, and terpenoids. These compounds are known for their antioxidant and antimicrobial activities, which can help slow down both oxidation and microbial spoilage in biodiesel. Previous research has identified several plants including neem (Azadirachta indica), African basil (Ocimum gratissimum), clove (Syzygium aromaticum), and cinnamon (Cinnamomum spp.) as potential sources of natural preservatives that can extend biodiesel shelf life and maintain fuel quality during storage.

Nevertheless, limited research has been conducted on the combined use of neem and African basil extracts in biodiesel preservation. There is still insufficient information on their concentration effects, mode of action, and compatibility with biodiesel derived from waste cooking oil. More investigation is needed to understand how these extracts influence key fuel parameters such as acid value, peroxide value, density, and flash point over time.

This study therefore focuses on evaluating the effectiveness of neem and African basil leaf extracts as natural antioxidants and antimicrobial agents in preserving biodiesel produced from waste cooking oil. The research aims to determine how the addition of these extracts can

improve biodiesel stability during storage, reduce degradation, and maintain fuel performance. By promoting the use of locally available plant materials as eco-friendly additives, this work supports the global movement toward sustainable fuel technologies and contributes to addressing Nigeria's growing energy and environmental challenges.

1.2 Problem Statement

Despite the recognition of biodiesel as a renewable and environmentally sustainable substitute for petroleum diesel, one persistent challenge undermines its practical application its vulnerability to microbial degradation during storage and transportation. The very biodegradability that makes biodiesel environmentally safe also makes it highly susceptible to microbial contamination. Various microorganisms, including fungi, bacteria, and yeasts, are capable of metabolizing biodiesel's organic constituents, particularly under humid or oxygen-deprived conditions. This microbial activity gradually alters essential fuel characteristics such as viscosity, flash point, acid value, and peroxide value, thereby reducing fuel efficiency, accelerating aging, and shortening shelf life.

To address this problem, synthetic stabilizers and chemical biocides have traditionally been used to inhibit microbial growth and oxidation in biodiesel. Although effective, these compounds present significant drawbacks they are often toxic, non-biodegradable, and environmentally persistent, and they may leave undesirable residues in both the fuel and the storage systems. Moreover, their high cost adds to the economic burden of biodiesel production, reducing its competitiveness with conventional diesel. As a result, there is an urgent need to identify natural, sustainable, and cost-effective alternatives that can preserve biodiesel stability while maintaining its eco-friendly profile.

Recent research has demonstrated the promise of plant-based extracts as natural antioxidants and antimicrobial agents capable of enhancing biodiesel stability. Nonetheless, existing studies have primarily focused on plants such as clove (Syzygium aromaticum), cinnamon (Cinnamomum spp.), and lemongrass (Cymbopogon citratus), while little emphasis has been placed on neem (Azadirachta indica) and African basil (Ocimum gratissimum), despite their proven bioactive properties in other scientific applications. Consequently, there remains a

limited understanding of the comparative effectiveness and practical suitability of these local plant extracts in minimizing biodiesel deterioration.

This study therefore seeks to bridge this research gap by examining the use of neem and African basil leaf extracts as natural stabilizers in biodiesel derived from waste cooking oil. The work aims to evaluate their influence on key fuel parameters including acid value, peroxide value, flash point, and density over a controlled storage period. Through this investigation, the research intends to provide experimental evidence supporting the use of indigenous plant resources as eco-friendly and sustainable additives for biodiesel preservation, thereby contributing to improved storage stability, reduced degradation, and enhanced economic viability of renewable fuels.

1.3 Aim and Objectives of the Study

Aim of the Study

The main aim of this research is to evaluate the effectiveness of *neem (Azadirachta indica)* and *African basil (Ocimum gratissimum)* leaf extracts as natural antimicrobial agents for preventing the deterioration of biodiesel produced from waste cooking oil during storage.

Specific Objectives

To achieve this aim, the study will pursue the following specific objectives:

- To produce biodiesel from waste cooking oil through the transesterification process.
- To prepare and characterize the leaf extracts of neem and African basil for use as natural antimicrobial additives.
- To treat biodiesel samples separately with neem and African basil extracts in defined concentrations.
- To evaluate the changes in physicochemical properties of the biodiesel samples during storage, focusing on parameters such as acid value, peroxide value, flash point, and density.
- To compare the effectiveness of neem and African basil extracts in minimizing biodiesel degradation over a specified period.
- To assess the potential of these plant extracts as eco-friendly, cost-effective alternatives to synthetic stabilizers in improving biodiesel storage stability.

1.4 Scope of the Study

This research focuses on producing biodiesel from waste cooking oil (WCO) through the transesterification process, and on improving its stability using natural antioxidants obtained from Azadirachta indica (neem) and African basil (Ocimum gratissimum). The biodiesel was produced with a pilot-scale reactor operated at 60°C for 75 minutes.

The study covers the collection and pretreatment of waste cooking oil, the production of biodiesel, and the extraction and analysis of neem and African basil extracts using GC–MS. It also examines how effective these natural extracts are in preserving biodiesel quality by observing changes in acid value and oxidative stability during storage.

This work is limited to laboratory and pilot-scale experiments, and does not include large-scale industrial production or cost analysis. However, the results are expected to show how locally sourced plant materials can serve as affordable and eco-friendly preservatives for biodiesel, helping to promote renewable energy development in Nigeria.

1.5 Significance of the Study

This study is significant because it contributes to the growing efforts toward developing sustainable and environmentally friendly methods for improving biodiesel quality and stability. Biodiesel, despite its advantages over fossil fuels, faces a major challenge of microbial degradation during storage, which affects its physicochemical properties and reduces its efficiency. Finding natural and cost-effective means of preserving biodiesel is therefore crucial to promoting its long-term use as a renewable energy source.

The use of *neem (Azadirachta indica)* and *African basil (Ocimum gratissimum)* leaf extracts as natural antimicrobial agents offers a promising approach to addressing this challenge. Both plants are widely available in tropical regions such as Nigeria and are known for their rich bioactive compounds with antimicrobial and antioxidant properties. Their application in biodiesel preservation could reduce the reliance on synthetic stabilizers that are often expensive, toxic, and environmentally harmful.

By investigating the effects of these plant extracts on key biodiesel quality parameters such as acid value, peroxide value, flash point, and density, this research will provide valuable insights

into the potential of locally sourced natural additives for improving biodiesel storage stability. The findings from this study could help establish a more sustainable and eco-friendly method of biodiesel preservation, lower production costs, and encourage the use of renewable energy resources in Nigeria and beyond.

Furthermore, the study will add to existing scientific knowledge and serve as a reference point for future research on the application of plant-based extracts in biofuel technology. It will also support the global drive toward cleaner energy solutions and environmental sustainability by providing a practical and natural alternative for maintaining biodiesel quality.

1.6 Limitations of the Study

This study is limited to the laboratory-scale evaluation of biodiesel stability using neem (*Azadirachta indica*) and African basil (*Ocimum gratissimum*) leaf extracts as natural antimicrobial agents. The research focuses on biodiesel derived solely from waste cooking oil, which may differ in composition and properties from biodiesel produced from other feedstocks such as palm oil, soybean oil, or jatropha oil. Consequently, the results obtained may not be directly applicable to all biodiesel types.

The experimental period for monitoring biodiesel stability is restricted to ten weeks due to time constraints, which may not fully capture long-term storage effects. In addition, the study does not account for variations in environmental conditions such as temperature and humidity that can influence biodiesel degradation under real storage or transportation scenarios.

Another limitation is that the research will not involve advanced microbiological identification of specific microorganisms responsible for biodiesel deterioration. Instead, the emphasis is on the comparative effect of the two plant extracts in reducing biodiesel degradation. Similarly, the study will not consider economic or large-scale feasibility assessments of using these plant extracts as additives.

Despite these limitations, the research is expected to provide valuable insights into the protective potential of neem and African basil extracts in enhancing the stability of biodiesel and encouraging the use of eco-friendly, locally available materials in fuel preservation.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Biodiesel

Biodiesel is a renewable biofuel obtained from natural oils and fats of plant or animal origin. It has attracted growing attention as a sustainable substitute for conventional diesel due to its biodegradability, low toxicity, and reduced greenhouse gas emissions (Antony Raja et al., 2011). The production of biodiesel is mainly carried out through a chemical process known as **transesterification**, in which triglycerides found in oils or fats react with a short-chain alcohol such as methanol or ethanol, using a catalyst usually sodium hydroxide (NaOH) or potassium hydroxide (KOH). This reaction produces **fatty acid methyl esters (FAMEs)**, which form the biodiesel, and **glycerol** as a by-product.

Vegetable oils have been identified as effective raw materials for biodiesel production because they are renewable and provide an energy content close to that of petroleum diesel. However, biodiesel tends to exhibit a few limitations, including higher viscosity and reduced volatility, which may lead to incomplete combustion under certain engine conditions. Despite these limitations, the environmental benefits of biodiesel make it a viable green energy alternative. According to Pasqualino et al. (2006), the use of biodiesel results in a substantial reduction of harmful exhaust emissions carbon monoxide (CO) by about 46.7%, carbon dioxide (CO_2) by 78%, and hydrocarbons by approximately 45%, with sulfur oxides being almost completely eliminated.

From an environmental standpoint, biodiesel is also known for its **rapid biodegradability**. It decomposes naturally within three to four weeks, achieving a degradation rate of up to 98% under favorable conditions. In contrast, petroleum diesel and gasoline degrade much slower, averaging only around 50–56% within the same period. This property makes biodiesel suitable for applications in environmentally sensitive locations such as agricultural areas, wetlands, and regions close to water bodies (Graboski & McCormick, 1998). Moreover, biodiesel has a higher cetane number, a higher flash point, and contains little to no sulfur, all of which contribute to cleaner combustion and improved engine efficiency.

An important practical advantage of biodiesel is that it can be used directly in existing diesel engines without significant modification. It also blends well with fossil diesel in different. proportions, leading to mixtures that combine the performance of petroleum diesel with the

environmental advantages of biodiesel. Interestingly, Boopathy et al. (2004) reported that blending only 5% biodiesel with diesel accelerates biodegradation of the mixture, shortening the time for 50% breakdown from 28 to 22 days.

Economically, biodiesel production supports local agricultural and industrial development. Feedstocks such as *Jatropha curcas*, neem, and African basil provide farmers with additional income sources while promoting rural employment. Furthermore, glycerol, which is generated as a by-product during transesterification, has significant commercial value and can be used in producing cosmetics, soaps, pharmaceuticals, and polymer materials (Claude, 1999).

On a larger scale, the expansion of biodiesel production could help diversify national energy sources and stimulate economic growth. Arndt and Sinler (2007) noted that biofuel industries can promote job creation, enhance agricultural productivity, and strengthen the link between the agricultural and energy sectors.

2.2 History of Biodiesel

The concept of using vegetable oils and animal fats as fuel dates back over a century. In the early 1900s, Rudolph Diesel, the inventor of the diesel engine, demonstrated that his engine could run on peanut oil as well as petroleum-based diesel (Knothe, 2010). This early experiment highlighted the potential of bio-based fuels as viable alternatives to fossil fuels, long before environmental concerns became a global priority.

During the mid-20th century, interest in biodiesel declined, largely due to the increasing availability and low cost of petroleum fuels. However, the oil crises of the 1970s renewed attention toward renewable fuels. Governments and researchers began exploring vegetable oils, animal fats, and waste oils as energy sources that could reduce dependence on imported crude oil while supporting domestic energy security (Demirbas, 2009).

In the 1990s, biodiesel production gained further momentum with advances in **transesterification technology**, which made the conversion of triglycerides into fatty acid methyl esters more efficient and economically viable. This process reduced the viscosity of raw oils, making them suitable for use in standard diesel engines (Ma & Hanna, 1999). Around this time, research also began focusing on non-edible oils, such as jatropha and neem, to avoid competition with food crops while providing sustainable feedstocks.

Today, biodiesel is produced globally from a wide range of feedstocks, including soybean oil, palm oil, rapeseed oil, and recycled cooking oils. It is recognized not only for its environmental benefits such as reduced greenh ouse gas emissions and biodegradability but also for its role in supporting rural economies and generating employment through feedstock cultivation and fuel production (Atabani et al., 2013). Modern biodiesel production emphasizes **sustainability**, combining renewable raw materials with cleaner fuel technologies to meet both environmental and energy security goals.

The historical development of biodiesel reflects a gradual shift from experimental use in early diesel engines to large-scale commercial production today. This evolution highlights the fuel's adaptability, environmental advantages, and potential as a long-term solution to fossil fuel dependency.

2.3 Properties of Biodiesel

Biodiesel possesses several physicochemical properties that influence its performance, stability, and compatibility with conventional diesel engines. These properties are largely determined by the type of feedstock, method of production, and presence of additives. Understanding these characteristics is essential for evaluating fuel quality and predicting behavior during storage, combustion, and transportation (Knothe, 2010).

2.3.1 Viscosity

Viscosity refers to the resistance of a fluid to flow and is a critical parameter for fuel injection and atomization in diesel engines. Biodiesel typically exhibits higher viscosity than petroleum diesel due to the long-chain fatty acid methyl esters that constitute its structure. Elevated viscosity can lead to incomplete combustion, injector deposits, and reduced fuel efficiency. Proper transesterification, which converts triglycerides into esters, reduces the viscosity of raw oils to values comparable with standard diesel. For most biodiesels, kinematic viscosity ranges from 4 to 6 mm^2/s at 40°C, meeting international standards such as ASTM D6751 and EN 14214 (Atadashi et al., 2012).

2.3.2 Density

Density is the mass per unit volume of a fuel and influences the energy content delivered during combustion. Biodiesel generally has a slightly higher density than petroleum diesel, typically

around 0.88–0.90 g/cm³ at 15°C. Higher density contributes to more complete combustion, but it may also slightly affect fuel injection timing in unmodified engines. Density is closely related to the molecular weight and composition of the fatty acid esters present in the biodiesel (Demirbas, 2009).

2.3.3 Flash Point

The flash point is the lowest temperature at which the fuel vapor ignites in the presence of a flame. Biodiesel has a significantly higher flash point compared to conventional diesel, often exceeding 120°C. This high flash point makes biodiesel safer to handle and store, reducing the risk of fire during transportation or accidental spillage. The elevated flash point is primarily due to the absence of light hydrocarbons in biodiesel (Bozbas, 2008).

2.3.4 Pour Point

Pour point is the lowest temperature at which a fuel remains pourable and is an indicator of its cold-flow properties. Biodiesel tends to have higher pour points than petroleum diesel, especially when derived from saturated fats, which can lead to crystallization at low temperatures. The pour point varies with the feedstock, with biodiesel from saturated oils such as palm oil exhibiting higher values compared to unsaturated oils like soybean or canola (Felizardo et al., 2006). Low-temperature additives or blending with diesel are often used to improve cold-flow performance in colder climates.

2.3.5 Acid Value

Acid value measures the concentration of free fatty acids in biodiesel, which can indicate hydrolytic degradation or incomplete esterification. Elevated acid values can lead to corrosion in storage tanks and engine components. International standards typically recommend an acid value of less than 0.5 mg KOH/g for biodiesel intended for commercial use (Knothe, 2010). Monitoring acid value during storage is crucial, as microbial activity and oxidation can increase acidity over time.

2.3.6 Peroxide Value

Peroxide value indicates the extent of oxidative degradation in biodiesel, representing the presence of peroxides and hydroperoxides formed during storage. High peroxide values suggest that the fuel is undergoing oxidation, which can negatively affect its stability, color, and odor. Oxidation can be accelerated by exposure to heat, light, and trace metals, making antioxidant additives or plant extracts beneficial for enhancing biodiesel shelf life (Sharma & Singh, 2009).

2.3.7 Cetane Number

Cetane number is a measure of the ignition quality of diesel fuel. It reflects how readily the fuel will combust under compression in a diesel engine. Biodiesel generally has a higher cetane number than petroleum diesel, often ranging between 48 and 65, which promotes smoother engine operation, reduced ignition delay, and lower emissions of unburned hydrocarbons and particulate matter (Knothe, 2010).

2.3.8 Additional Properties

Other notable properties include lubricity, cloud point, and oxidative stability. Biodiesel exhibits excellent lubricating properties, which can reduce engine wear. Cloud point, the temperature at which wax crystals first appear, affects fuel performance in cold weather and is influenced by the degree of saturation in the fatty acid chains. Oxidative stability determines how long the fuel maintains its quality during storage, and it can be enhanced through the use of natural antioxidants or antimicrobial plant extracts such as neem and African basil (Singh et al., 2018).

2.4 Production of Biodiesel

Vegetable oils are primarily composed of triglycerides, which are esters formed from glycerol and fatty acids. These triglycerides naturally occur in oilseeds and possess relatively high molecular weights (approximately 800 kg/m³ or more) along with high viscosity. Such physical properties make raw vegetable oils unsuitable for direct use in compression ignition (CI) engines, as their high viscosity can cause incomplete combustion, injector clogging, and engine deposits (IlettVee et al., 2012).

To render vegetable oils compatible with diesel engines, it is essential to reduce their viscosity to levels comparable with conventional diesel fuel. This can be achieved through several methods, including:

- **Pyrolysis**
- **Micro-emulsion**
- **Dilution**
- **Transesterification**

Among these approaches, **transesterification** has emerged as the most effective and widely adopted method for biodiesel production. This chemical process alters the structure of triglycerides, lowering their viscosity and producing fatty acid alkyl esters, which are more suitable for engine operation. As a result, transesterification ensures that the biodiesel exhibits fuel properties similar to conventional diesel, thereby addressing the key challenges associated with direct use of vegetable oils in CI engines.

2.4.1 Pyrolysis

Pyrolysis is a chemical process that involves heating vegetable oils in the absence of air or oxygen to thermally decompose triglycerides into smaller hydrocarbon molecules. This process produces liquid fuels that resemble diesel, along with minor amounts of gaseous hydrocarbons and solid residues (char). The primary aim of pyrolysis is to reduce the high viscosity of vegetable oils and make them suitable for use in compression ignition engines.

The general reaction for pyrolysis of triglycerides can be represented as:

$C_{55}H_{98}O_6$ → Heat (absence of O_2) → Liquid hydrocarbons + Gaseous hydrocarbons + Char

The liquid products obtained have lower viscosity, flash points, and pour points compared to the original oils, improving atomization and combustion in engines. The calorific value of the pyrolyzed fuel remains comparable to conventional diesel, typically ranging between 40–42 MJ/kg. However, the cetane number of pyrolysis products is often lower, which can slightly affect ignition quality.

Pyrolysis products may contain moderate amounts of sulfur, water, and sediment. While these impurities are generally acceptable for engine performance, the fuel may lack optimal properties such as sufficient ash content, carbon residue, and pour point, limiting its performance in colder climates. The process conditions, such as temperature and residence time, greatly influence the yield and quality of the liquid fuel. Typical temperatures range from 400°C to 600°C, with higher temperatures favoring the production of lighter hydrocarbons.

Although pyrolysis offers a method to convert vegetable oils into usable fuels, the resulting products often require further refinement or blending with conventional diesel to meet engine

and environmental standards. Nevertheless, pyrolysis provides an alternative pathway for producing renewable liquid fuels, especially in regions with abundant vegetable oil feedstocks.

2.4.2 Micro-emulsification

Micro-emulsification is a physical technique used to reduce the viscosity of vegetable oils and improve their suitability as diesel substitutes. The process involves blending the oil with a short-chain alcohol, such as methanol or ethanol, in the presence of a surfactant that stabilizes the mixture. The result is a clear, thermodynamically stable, and homogeneous fuel blend known as a micro-emulsion.

This method lowers the viscosity of raw oils without altering their chemical structure, allowing for improved atomization and combustion in compression ignition engines. Typically, the alcohol acts as a co-solvent, while the surfactant minimizes interfacial tension between the oil and the alcohol phases. Proper formulation is critical, as it determines the long-term stability and performance of the micro-emulsion.

The process is relatively simple and can be carried out at low temperatures without complex equipment. Its main advantages include ease of preparation, lower energy demand, and improved cold-flow properties compared to untreated vegetable oils. However, micro-emulsions may face challenges such as limited storage stability and slightly reduced energy content due to the alcohol component. Despite these limitations, the method provides a viable short-term alternative to chemical transesterification, especially in regions where catalysts and specialized equipment are unavailable (Atadashi et al., 2012; Demirbas, 2009)

2.4.3 Dilution Method

The dilution method is one of the simplest approaches that has been explored for reducing the viscosity of vegetable oils to make them more suitable for diesel engines. In this process, raw or waste vegetable oil is mixed directly with a solvent or with conventional diesel fuel in specific proportions, usually ranging from 10% to 50% oil by volume. The main purpose of this dilution is to lower the oil’s thickness so that it can flow and atomize more easily during combustion.

Although the diluted mixture can be used in diesel engines without complex chemical processing, this method has several drawbacks. The blended fuel often results in incomplete combustion, carbon deposits on engine components, and smoke formation, especially during long-term use. These problems occur because even with dilution, the fuel's viscosity and molecular structure still differ significantly from that of petroleum diesel.

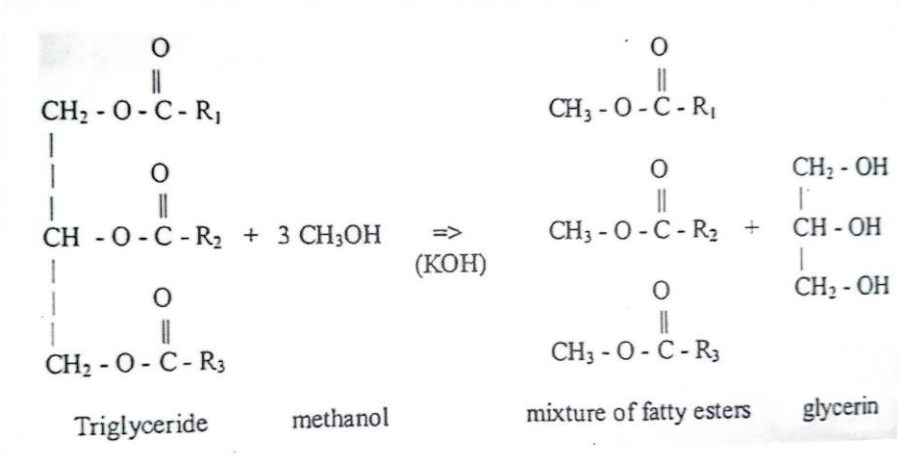
Due to these limitations, the dilution method is generally considered unsuitable for commercial biodiesel production. It is mainly regarded as an early experimental approach that helped identify the need for more effective processes such as transesterification, which produces high-quality biodiesel with properties that meet international fuel standards

2.4.4 Transesterification

Transesterification is the fundamental chemical process used in the conversion of triglycerides present in fats or oils into biodiesel. It involves reacting the oil with a short-chain alcohol, most commonly methanol or ethanol, in the presence of a suitable catalyst to produce fatty acid alkyl esters (biodiesel) and glycerol as a by-product. The process effectively reduces the viscosity of vegetable oils, enabling them to perform efficiently in diesel engines without causing the operational problems associated with raw vegetable oil such as injector clogging, incomplete combustion, or carbon deposition on engine components.

The reaction can be represented generally as follows:

Triglyceride + Alcohol → Fatty Acid Alkyl Esters + Glycerol



The transesterification process is reversible and requires an excess amount of alcohol to drive the reaction towards completion. Methanol is the most widely used alcohol because of its low cost, polarity, and ability to react readily with oils. The reaction typically takes place at moderate temperatures (50–65°C) and under atmospheric pressure, although reaction time and efficiency depend on the type of catalyst, alcohol-to-oil ratio, and feedstock quality. Vigorous stirring is usually maintained during the early stages of the reaction to ensure proper mixing between the immiscible oil and alcohol phases. Once the reaction progresses, mixing is reduced to allow proper separation of biodiesel and glycerol layers

2.4.4.1 Base-Catalyzed Transesterification

Base-catalyzed transesterification is the most widely applied method for biodiesel production, primarily due to its high conversion efficiency, mild operating conditions, and short reaction time. Alkali catalysts such as sodium hydroxide (NaOH), potassium hydroxide (KOH), or sodium methoxide (CH_3ONa) are commonly used. Among these, sodium methoxide is often preferred in industrial settings because it provides faster reaction kinetics and higher purity products, although it is more expensive than NaOH or KOH.

The efficiency of base-catalyzed transesterification depends largely on the quality of the feedstock. Oils with low free fatty acid (FFA) and moisture content are ideal, since the presence of water or high FFA leads to soap formation, which can hinder separation and reduce biodiesel yield. Generally, the catalyst concentration ranges between 0.5% and 1.5% of the oil weight, and a typical methanol-to-oil molar ratio is 6:1. The reaction is usually conducted at about 60°C, slightly below the boiling point of methanol, for 60–90 minutes under continuous agitation.

After the reaction, the mixture is allowed to settle for several hours. Two layers are formed: the upper layer contains biodiesel, while the denser glycerol layer settles at the bottom. The glycerol phase may contain traces of catalyst, soap, and unreacted methanol. To enhance purity, the biodiesel layer is washed with warm distilled water to remove residual impurities and then dried by gentle heating or with anhydrous sodium sulfate to eliminate moisture.

2.4.4.2 Acid-Catalyzed Transesterification

Acid catalysis is primarily applied when the feedstock has a high FFA content, such as waste cooking oil or animal fats. In this method, strong acids like sulfuric acid (H_2SO_4) or hydrochloric acid (HCl) are used to catalyze the reaction between triglycerides and alcohols. Although acid catalysis prevents soap formation, it proceeds more slowly than base catalysis and often requires higher alcohol-to-oil ratios (typically 20:1 to 40:1) and elevated temperatures (55–65°C) to achieve significant conversion.

Acid catalysis can also be used in a two-step process: an initial acid esterification step to convert free fatty acids into esters, followed by a base-catalyzed transesterification to complete the conversion of triglycerides. This combined approach is particularly suitable for waste cooking oil, which often contains free fatty acids above 1%. Despite its longer reaction time, acid-catalyzed transesterification remains an important method for improving biodiesel yield from poor-quality oils.

2.4.4.3 Heterogeneous Catalyzed Tranesterification

Catalysts are essential in biodiesel production because they help speed up the transesterification reaction and improve yield. Most production processes use homogeneous catalysts like sodium hydroxide (NaOH) or potassium hydroxide (KOH). While these catalysts are effective and affordable, they also have major drawbacks. The most common problems include difficulty in separating the catalyst from the final product, formation of soap, and the need for additional purification steps, all of which increase production cost and time. To solve these issues, researchers have developed heterogeneous catalysts, which are more environmentally friendly and easier to handle.

Heterogeneous catalysts are typically solid materials that react with liquid oil and alcohol. Since they remain in a separate phase, they can be easily separated from the biodiesel after the reaction, reused for multiple cycles, and produce fewer impurities. This makes them a cleaner and more economical alternative to homogeneous catalysts. They also reduce the formation of soap, which in turn improves biodiesel quality and reduces the amount of water required for washing.

Different solid materials have been tested as heterogeneous catalysts, including metal oxides such as calcium oxide (CaO), magnesium oxide (MgO), zinc oxide (ZnO), and aluminum oxide (Al_2O_3). Other types include mixed oxides, supported catalysts, and catalysts made from waste materials. In recent years, researchers have shown strong interest in biowaste-derived catalysts—solid catalysts obtained from animal bones, shells, and agricultural residues. These are usually produced through calcination, a process that involves heating the waste at very high temperatures to improve its strength and surface area.

In this study, heterogeneous catalysis route was explored using calcined turkey bones, calcined pumpkin pods, and calcined wether's waste as catalysts. This approach was chosen to reduce purification steps and promote a greener biodiesel production process. After calcination, the catalysts develop a porous surface, which increases the number of active sites available for the reaction. Apart from their efficiency, using such wastes also supports environmental sustainability by turning local agricultural and animal waste into valuable catalytic materials.

However, heterogeneous catalysis still has some limitations. Since the reaction takes place between a solid catalyst and liquid reactants, the contact between the two phases can be limited, which may slightly reduce the reaction rate. In addition, the presence of water or high free fatty acid levels in the oil can block the catalyst's active sites, lowering its effectiveness. Even with these challenges, ongoing research continues to optimize conditions such as temperature, reaction time, and catalyst concentration to achieve better conversion efficiency.

Overall, heterogeneous catalysts offer a promising route for cleaner and cheaper biodiesel production. They simplify the purification process, allow catalyst recovery, and encourage the use of waste materials—making them particularly suitable for biodiesel production in developing countries like Nigeria, where cost-effective and sustainable technologies are crucial.

2.4.4.3 Separation and Purification of Biodiesel

Once transesterification is complete, the reaction mixture consists of biodiesel, glycerol, excess alcohol, catalyst, and trace impurities. The first step in purification is the separation of glycerol, which is denser and settles at the bottom of the reaction vessel. This can occur naturally by

gravity or be accelerated using centrifugation. The upper biodiesel layer is carefully decanted or siphoned off for further purification.

Residual methanol in both phases is typically recovered through distillation or flash evaporation, allowing it to be reused in subsequent reactions. Neutralization is then performed using a weak acid, such as phosphoric or acetic acid, to remove residual catalysts and soap. The biodiesel is subsequently washed gently with warm water to eliminate remaining contaminants and then dried to remove any moisture. The resulting biodiesel is a clear, amber-colored liquid with physical and chemical properties comparable to petroleum diesel.

The glycerol by-product obtained from the process can be refined or used in crude form for various applications. Crude glycerol generally contains 80–88% glycerin along with residual catalysts and salts, which may be recovered for fertilizer production or further purified to pharmaceutical-grade glycerol.

2.4.4.4 Main By-products of Transesterification and Their Applications

During the production of biodiesel through transesterification, the main product obtained is fatty acid methyl ester (FAME), which is the actual biodiesel. However, several by-products are also formed, the most significant of which is glycerol (or glycerin). Other minor by-products include soap, methanol residues, and catalyst waste. Understanding these by-products is important because they influence the overall economics and environmental sustainability of the biodiesel process.

2.4.4.4 Glycerol

Glycerol is the major by-product of transesterification, making up about 10% of the total biodiesel yield. For every 100 kg of biodiesel produced, roughly 10 kg of crude glycerol is obtained (Atabani et al., 2022). This crude glycerol often contains impurities such as methanol, catalyst, soap, and unreacted oil, so it usually needs to be purified before use.

Purified glycerol has many valuable applications. It is widely used in the pharmaceutical, cosmetic, and food industries because of its moisturizing, softening, and stabilizing properties. It is also used in the production of toothpaste, lotions, and medicines. In addition, glycerol can serve as a raw material for producing chemicals like propylene glycol, epichlorohydrin, and glycerol carbonate, which are useful in the polymer and resin industries. Recent studies also

explore its use in biogas production, animal feed, and as a carbon source for microbial fermentation (Eze et al., 2021).

2.4.4.5 Soap and Catalyst Residues

Soaps are formed when free fatty acids present in the oil react with the basic catalyst (such as NaOH or KOH) during transesterification. This process, called saponification, reduces the overall biodiesel yield and complicates purification. However, these soaps can still be recovered and used in the manufacture of detergents, cleaning agents, or as lubricating additives.

Catalyst residues and methanol traces left after the reaction are typically removed during the washing and drying stages. In small-scale biodiesel production, the recovered methanol can be reused to reduce cost and waste, while the catalyst residues are neutralized and disposed of safely (Abbas & Ogbonna, 2020).

2.4.4.6 Environmental and Economic Significance

Proper management and utilization of transesterification by-products play a vital role in making biodiesel production more sustainable. Instead of being treated as waste, these materials can generate additional income and reduce environmental pollution. In Nigeria, where waste management remains a major challenge, using crude glycerol and soap residues in local industries could enhance the overall value chain of biodiesel production and support small-scale entrepreneurs.

2.5 Feedstock for Biodiesel Production

2.5.1 Overview of Feedstocks

The type of feedstock used is one of the most important factors in biodiesel production because it directly affects both the cost and quality of the final product. Feedstocks are basically the oils or fats that react with alcohol during the transesterification process to form biodiesel. Common feedstocks include vegetable oils (like soybean, palm, sunflower, and canola), animal fats (tallow, lard), and waste oils such as waste cooking oil (WCO). The choice often depends on

what's available locally, how affordable it is, and the level of processing required before it can be used.

In many developing countries, especially Nigeria, the use of edible oils as feedstock can be expensive and even controversial since those same oils are needed for food. That's why attention has shifted toward non-edible and waste oils, which are cheaper and more sustainable. Among these, waste cooking oil stands out as one of the most practical options for biodiesel production at both small and industrial scales.

2.5.2 Waste Cooking Oil (WCO)

Waste cooking oil refers to vegetable oil that has already been used for frying or cooking and is no longer suitable for food use. It's commonly generated from homes, restaurants, food vendors, and industries. In Nigeria, where fried foods like puff-puff, plantain, yam, and meat are popular, a large quantity of waste oil is produced daily — most of which ends up being poured away or improperly disposed of. This contributes to blocked drainage systems, foul odors, and environmental pollution.

Instead of treating WCO as useless waste, it can be collected, filtered, and converted into biodiesel. This not only helps reduce environmental problems but also provides a cheaper and renewable alternative to fossil fuels. Since WCO already exists as a byproduct of daily cooking activities, its availability is practically guaranteed, especially in urban centers like Lagos, Abuja, and Port Harcourt.

2.5.3 Benefits of Using WCO

Using waste cooking oil as a feedstock comes with several advantages.

First, it's cost-effective. Feedstock cost usually makes up about 70–80% of total biodiesel production expenses, so using a low-cost waste material like WCO drastically reduces overall cost.

Second, it helps with environmental management. Recycling used oil prevents it from being dumped into drainage systems or water bodies, which can cause pollution.

Third, it reduces competition with food. Unlike edible vegetable oils that are needed for cooking, WCO is non-edible, meaning it doesn't threaten food supply.

Lastly, it supports sustainability. Turning waste into energy promotes the circular economy concept, where waste materials are reused for something valuable.

For Nigeria, adopting WCO as a primary feedstock could promote local biodiesel production, reduce dependence on imported fossil fuels, and even create job opportunities in waste oil collection and processing.

2.5.4 Limitations and Pretreatment of WCO

Despite its many advantages, waste cooking oil also has some challenges that need to be addressed before it can be used for biodiesel production. The main issue is its variable quality. After repeated frying, the oil tends to absorb moisture, food particles, and contaminants, while oxidation and polymerization increase its free fatty acid (FFA) content. High FFA levels can cause soap formation during transesterification, reducing the amount of biodiesel produced and complicating separation.

To overcome this, pretreatment steps are necessary. These usually include filtering to remove solid impurities, heating to remove moisture, and sometimes an acid-catalyzed esterification step to reduce free fatty acids before the main transesterification process. Proper pretreatment ensures higher biodiesel yield, smoother processing, and better fuel quality.

Even with these extra steps, WCO still remains one of the most promising and sustainable feedstocks, especially for a country like Nigeria that produces large amounts of used oil daily. With proper collection systems and awareness, what many people throw away can become a valuable source of renewable energy.

2.6 Deterioration and Stability of Biodiesel

Biodiesel, like other organic fuels, is prone to deterioration over time. This deterioration mainly occurs due to oxidative, thermal, and microbial degradation, which reduce fuel quality and affect its performance in diesel engines. Maintaining the stability of biodiesel during storage is therefore essential to ensure its reliability and efficiency.

2.6.1 Oxidative Degradation

Oxidation is the most common cause of biodiesel deterioration. It occurs when biodiesel is exposed to oxygen, heat, light, or metal contaminants during storage. The fatty acid methyl esters (FAME) that make up biodiesel are especially vulnerable to oxidation because of the unsaturated bonds present in their molecular structure. These double bonds react with oxygen to form peroxides, aldehydes, and acids, which can lead to an unpleasant odor, higher viscosity, and the formation of gums or sediments.

The rate of oxidation depends on several factors, including the type of feedstock, exposure to air, temperature, and presence of impurities. Biodiesel derived from unsaturated oils such as soybean or sunflower oil oxidizes faster than biodiesel from more saturated oils like palm or coconut oil. In Nigeria, where most biodiesel production uses waste cooking oil, oxidation can occur more rapidly if the oil has already undergone repeated heating cycles before collection.

2.6.2 Thermal and Storage Degradation

Prolonged exposure to high temperatures or sunlight can also cause biodiesel to break down. The heat accelerates the oxidation process, while UV radiation from sunlight can trigger photo-oxidation. This is why biodiesel is best stored in cool, dark conditions using containers made of materials that do not promote oxidation, such as stainless steel or high-density polyethylene (HDPE). Poor storage conditions can lead to discoloration, increased acidity, and even formation of sludge at the bottom of the storage tank.

2.6.3 Microbial Contamination

Another factor that affects biodiesel stability is microbial growth. Because biodiesel tends to absorb moisture from the atmosphere, it can provide a favorable environment for bacteria, yeast, and fungi. These microorganisms usually grow at the interface between fuel and water, producing slime and organic acids that corrode fuel systems and clog filters. The problem is worse in warm, humid climates like Nigeria's, where microbial growth happens more quickly if biodiesel is stored for long periods.

2.6.4 Effects of Biodiesel Deterioration

When biodiesel deteriorates, it affects not just fuel properties but also engine performance. Oxidized biodiesel typically shows an increase in acid value, viscosity, and density, which

reduces fuel atomization during combustion. It can also lead to carbon deposits on injectors and piston rings, and in severe cases, corrosion of metallic parts. Hence, understanding and preventing biodiesel degradation is essential for both fuel stability and engine durability.

2.6.5 Methods of Improving Stability

Several methods can be used to enhance biodiesel stability. These include proper feedstock selection, purification, controlled storage conditions, and the addition of antioxidants or preservatives. Among these, the use of natural antioxidants from plant extracts has gained attention in recent years as a safer and more sustainable alternative to synthetic additives. In this study, extracts from Azadirachta indica (neem) and African basil are used to preserve biodiesel, as they contain bioactive compounds such as flavonoids, terpenoids, and phenolics that inhibit oxidation and microbial growth.

2.7 Antioxidants in Biodiesel Preservation

2.7.1 Why Antioxidants Are Needed

Biodiesel is mainly made up of fatty acid esters that contain unsaturated carbon–carbon bonds. These bonds easily react with oxygen in the air, which leads to the formation of peroxides, aldehydes, and acids. This reaction causes biodiesel to deteriorate over time, increasing its acidity and viscosity, changing its colour, and sometimes giving it an unpleasant smell. If not controlled, oxidation can affect engine performance and shorten fuel storage life. To prevent this, antioxidants are added to biodiesel to slow down oxidation and keep the fuel stable for longer (David et al., 2023).

2.7.2 Synthetic and Natural Antioxidants

There are two main types of antioxidants used in biodiesel: synthetic and natural. Synthetic antioxidants like Butylated Hydroxyanisole (BHA), Butylated Hydroxytoluene (BHT), and Tertiary Butylhydroquinone (TBHQ) have been widely used because they are effective at small doses. However, they can be expensive and are not biodegradable, which raises environmental concerns. Natural antioxidants, on the other hand, are obtained from plants. They are renewable, safer to handle, and often contain phenolic and flavonoid compounds that help fight

oxidation. In recent years, researchers have become more interested in using natural antioxidants to replace or support synthetic ones, especially in countries like Nigeria where plant materials are abundant and easy to obtain (Sarkar et al., 2021).

2.7.3 Azadirachta indica (Neem)

Azadirachta indica, commonly known as neem, is a plant well-known in Nigeria for its medicinal and agricultural uses. Its leaves, bark, and seeds contain compounds such as flavonoids, limonoids, and phenolic acids that act as strong antioxidants. These compounds can neutralize free radicals and slow down the oxidation of oils and biodiesel. Studies have shown that neem extracts can increase the oxidative stability of biodiesel and reduce peroxide formation, making the fuel last longer during storage. The antioxidant effect depends on how the extract is prepared, the concentration used, and the type of biodiesel involved (Rahmani et al., 2018; Baby et al., 2022).

2.7.1.2 Chemical Composition and Active Compounds

Neem contains several active compounds that give it its unique biological properties. The most important include azadirachtin, nimbin, quercetin, and other phenolic and flavonoid compounds. These are naturally occurring chemicals that can neutralize free radicals and prevent oxidation. The presence of these compounds explains why neem extracts show both antioxidant and antimicrobial properties, which are important in slowing down biodiesel degradation during storage

2.7.1.3 Antioxidant and Antimicrobial Activities

The antioxidant effect of neem mainly comes from its flavonoids and phenolics. These compounds act by donating hydrogen atoms to unstable molecules called free radicals, stopping them from reacting with the fatty acid chains in biodiesel. In simpler terms, they act like “bodyguards” that prevent oxygen from attacking the fuel. This helps reduce changes in acid value, viscosity, and color during storage.

Apart from that, neem also has mild antimicrobial activity. Compounds like azadirachtin and nimbidin disrupt the cell walls of bacteria and fungi that sometimes grow in biodiesel, especially in warm and humid conditions. This means neem doesn’t just slow oxidation — it also reduces microbial growth that can form sludge or cause the fuel to go bad faster.

2.7.1.4 Extraction and Preparation of Neem Extract

For most studies, neem leaves are used because they are easy to collect and handle. In this research, neem leaves can be air-dried, ground into powder, and extracted using ethanol or methanol since these solvents dissolve most of the active compounds. After extraction, the solvent is evaporated to obtain a thick, dark-green extract which can be added directly to biodiesel. The concentration usually varies depending on the study, but small amounts (between 0.1% and 0.5% by volume) are commonly effective

2.7.1.5 Application in Biodiesel Preservation

When neem extract is added to biodiesel, it helps to delay the oxidation process, especially during long-term storage. The treated biodiesel usually remains clearer, has a lower acid value, and shows better oxidative stability compared to untreated samples. Neem's combined antioxidant and antimicrobial effects make it a good natural alternative to synthetic preservatives, which are often expensive and not environmentally friendly.

2.7.4 African Basil (Ocimum gratissimum)

African basil, locally known as scent leaf, is another plant with strong antioxidant properties. It is widely available in Nigeria and used in both food and traditional medicine. The leaves contain compounds such as eugenol, thymol, and rosmarinic acid, which can prevent oxidation by scavenging free radicals. Extracts from African basil have been found to extend the shelf life of oils and biodiesel by reducing peroxide and acid formation during storage. Like neem, the effectiveness of the extract depends on the extraction solvent, dosage, and storage conditions (Edo et al., 2023).

2.7.2.2 Phytochemical Composition

The major bioactive compounds in O. gratissimum include eugenol, thymol, ocimene, linalool, and various phenolic acids (Nwinyi et al., 2009). These compounds are known for their strong antioxidant capacity, which allows them to neutralize free radicals and slow down oxidation in organic materials. The presence of eugenol, in particular, gives African basil its distinct aroma and makes it one of the most potent natural antioxidants among local herbs. These

phytochemicals also display antimicrobial effects by interfering with the growth and metabolism of bacteria and fungi that could degrade biodiesel during storag

2.7.2.3 Antioxidant and Antimicrobial Mechanisms

African basil acts as a natural antioxidant primarily by donating hydrogen atoms or electrons to unstable molecules, thereby breaking the chain reaction that leads to oxidation (Ezejiofor et al., 2011). This process helps protect biodiesel molecules from oxidative breakdown that usually increases acidity and reduces fuel quality. The antimicrobial activity, on the other hand, comes from essential oils like eugenol and thymol, which can damage microbial cell walls and prevent the growth of spoilage organisms (Nwinyi et al., 2009). These combined actions make African basil extract useful in improving the shelf life and purity of biodiesel.

2.7.2.4 Extraction and Preparation of African Basil Extract

For biodiesel-related studies, the leaves of African basil are usually harvested fresh, washed, and air-dried at room temperature to retain their active compounds. The dried leaves are then ground into fine powder and extracted using ethanol or methanol as solvent, since these polar solvents effectively dissolve phenolic and flavonoid components (Ezejiofor et al., 2011). The extract obtained after solvent evaporation contains the essential oils and bioactive compounds responsible for its antioxidant and antimicrobial effects

2.7.2.5 Application in Biodiesel Preservation

When added to biodiesel, African basil extract can help maintain oxidative stability, slow down acid formation, and reduce color change during storage. Studies have shown that plant-based antioxidants such as basil extract can perform comparably to synthetic additives like butylated hydroxytoluene (BHT) when used in moderate concentrations (Olabinri et al., 2014). This makes African basil a promising eco-friendly option for extending biodiesel shelf life, especially in tropical climates like Nigeria where fuel oxidation occurs faster due to heat and humidity

2.7.5 How These Extracts Work in Biodiesel

Both Neem (Azadirachta indica) and African basil (Ocimum gratissimum) extracts help improve the quality and storage stability of biodiesel. One of the main challenges with biodiesel is that it tends to degrade over time due to oxidation and microbial contamination. These two extracts are rich in phenolic compounds, flavonoids, terpenoids, and essential oils, all of which help slow down these reactions and keep the fuel in good condition.

During storage, exposure to oxygen, light, and heat can cause biodiesel to form free radicals that attack the fuel molecules. This reaction increases the acid value, changes the color, and thickens the fuel, which eventually affects engine performance. The antioxidants in neem and African basil work by donating hydrogen atoms to stop these radicals from spreading. In simple terms, they protect the biodiesel from "spoiling" the same way natural preservatives stop food from going bad.

Microbial growth is another major issue, especially under the hot and humid conditions common in Nigeria. Certain microorganisms can live in biodiesel and cause sludge formation or fuel degradation. Compounds like azadirachtin and nimbin (from neem) and eugenol (from African basil) help prevent this by damaging the cell membranes of bacteria and fungi, making it difficult for them to survive in the fuel.

When both extracts are combined, they often show a stronger overall effect than when used separately. Neem is more effective at preventing oxidation because it contains several polyphenolic and triterpenoid compounds, while African basil provides strong antimicrobial and aromatic phenolics that boost protection. Together, they help the biodiesel maintain its color, viscosity, and chemical stability over a longer period.

Using natural extracts instead of synthetic preservatives has additional benefits. They are environmentally friendly, biodegradable, non-toxic, and easily accessible in Nigeria. This makes them practical options for local biodiesel production, especially for small-scale or research purposes.

In short, neem and African basil extracts help protect biodiesel in two main ways — by slowing oxidation and by preventing microbial contamination. Their availability and safety make them suitable natural additives for enhancing the lifespan and quality of biodiesel produced in tropical environments.

2.8 FACTORS THAT AFFECT THE TRANSESTERIFICATION REACTION

Numerous factors affect the transesterification process of biodiesel production. Some of these factors will be highlighted below:

2.81 The effect of temperature

The rate of a reaction is strongly influenced by the temperature at which it occurs. While reactions can approach full completion at room temperature if given enough time, they are typically conducted near the alcohol's boiling point under normal atmospheric pressure (Shereena and Thangaraj, 2009). To support these moderate conditions, it is necessary to remove free fatty acids from th e oil through refining or pre-treatment with esterification. However, excessively high temperatures can reduce the reaction's efficiency. Studies indicate that esterification can yield satisfactory results even at room temperature when an alkaline catalyst is employed. Although the conversion rate generally remains stable, biodiesel recovery tends to decrease at extremely low temperatures.

2.8.2 Influence of Alcohol-to-Triglyceride Molar Ratio

The molar ratio of alcohol to triglycerides is a critical factor in determining ester production yields. For transesterification, the optimal stoichiometric ratio is three moles of alcohol to one mole of triglyceride, producing three moles of fatty esters and one mole of glycerol. Achieving complete conversion often requires either adding excess alcohol or removing one of the reaction products, with the latter being the more commonly adopted method. Reaction efficiency peaks with a 100% excess of methanol, and industrial processes frequently employ a 6:1 molar ratio to achieve methyl ester yields exceeding 98% (w/w) (Shereena and Thangaraj, 2009).

2.8.3 Catalyst type and Concentration

The type and concentration of catalysts play a significant role in transesterification. Alkali metal alkoxides, particularly sodium alkoxides, are among the most effective, accelerating

transmethylation up to 4000 times faster than acidic catalysts. Their lower corrosiveness also makes them preferable for industrial applications. However, increasing catalyst concentration does not improve conversion rates and instead raises costs due to the need for post-reaction catalyst removal. Higher NaOH concentrations are required for oils with high free fatty acid content (Shereena and Thangaraj, 2009).

2.8.4 Effect of reaction time

Reaction time is another key variable, as longer durations allow the conversion process to approach equilibrium. Optimal ester conversion typically occurs within two hours. Similar trends have been observed in studies with Madhuca Indica and Karanja oil. Initially, the reaction progresses slowly due to methanol and catalyst mixing but accelerates significantly after the first few minutes. For beef tallow methyl esters, maximum production is reached within 15 minutes (Annamalai et al., 2011).

2.8.5 Effect of free fatty acid

Free Fatty Acid Content

Free fatty acids (FFAs) must be minimized—ideally below 2%—after acid esterification. Higher FFA levels lead to soap formation instead of esters, significantly reducing conversion efficiency (Shereena and Thangaraj, 2009).

2.8.6 Mixing intensity

Mixing is crucial during the slow-rate phase of transesterification but becomes less critical once a single phase is established. Effective mixing, particularly in the initial stages, enhances conversion rates and product recovery. Studies suggest that 5–10 minutes of stirring after adding alcohol and catalyst improves results (Pinto et al., 2005).

2.8.7 Effect of moisture and water content on Biodiesel yield

Water content has a more detrimental effect on biodiesel yield than FFAs. Even trace amounts of water (0.1%) can inhibit ester conversion, leading to soap formation and reduced catalyst efficiency. To remove moisture, vegetable oil is often preheated to 383 K for an hour. Additionally, precautions such as preparing fresh potassium hydroxide-methanol solutions help maintain catalytic activity. Notably, while water hampers conventional transesterification, it can enhance methyl ester production when using supercritical methanol, though the mechanism is not well understood (Meher et al., 2011).

2.8.8 Effect of Specific Gravity

The specific gravity of biodiesel decreases as the reaction progresses, indicating successful conversion and glycerin removal. Studies by Miao and Wu found a sharp decline in specific gravity within the first two hours at a 30:1 molar ratio. At higher ratios of 45:1 and 56:1, stabilization occurred after four hours (Khandelwal Shikha and Chauhan Y. Rita, 2011).

2.8.9 Effect of stirring

Stirring significantly impacts biodiesel production. Reactions conducted at speeds of 180 rpm yield incomplete results, while speeds of 360 and 600 rpm show consistent output. The stirring method also affects overall transesterification efficiency.

2.8.10 Purity of reactants

Impurities in the feedstock, such as free fatty acids in crude vegetable oils, can hinder ester conversion by interacting with catalysts. While refined oils yield 94–97% esters under identical conditions, crude oils achieve only 65–84%. These challenges can be addressed through reactions at higher temperatures and pressures (Shereena and Thangaraj, 2009).

2.9 BENEFITS OF BIODIESEL

Biodiesel provides a range of environmental advantages, with its most significant being its status as a "carbon-neutral" fuel. This classification indicates that biodiesel does not contribute to net carbon dioxide (CO2) emissions. Pure biodiesel, known as B100, is entirely carbon neutral, meaning that CO2 emissions from its combustion are negligible. For example, Emirates Airlines in Dubai estimates that conventional diesel has a carbon footprint of 2.664 kg CO2e per liter, highlighting biodiesel as an excellent choice for organizations seeking to mitigate their carbon emissions from transportation and generators.

- ***Reduction in Smog Formation***

  Biodiesel combustion has roughly 50% lower ozone-forming potential compared to fossil diesel, according to the US Environmental Protection Agency. This reduction is particularly beneficial in urban areas struggling with smog-related air quality issues.

- ***Absence of Sulfur Emissions***

  Biodiesel produces virtually no sulfur oxides or sulfates during combustion, substances that are primary contributors to acid rain. This characteristic gives biodiesel a distinct environmental edge over fossil diesel.

- ***Decrease in Harmful Emissions***

  The use of biodiesel significantly reduces the output of unburned hydrocarbons, carbon monoxide, and particulate matter (soot) in exhaust gases, leading to cleaner air quality.

- ***Improved Lubrication Properties***

  Biodiesel has superior lubricating qualities that help prevent premature wear and damage to engine components. Diesel engines depend on the lubricity of the fuel to protect moving parts like fuel injectors and pumps. Low-lubricity fuels increase metal-to-metal contact and wear, whereas biodiesel minimizes wear, thereby extending engine life.

- ***Cleaning Effect on Fuel Systems***

  Biodiesel functions as a natural detergent within fuel systems, gradually dissolving sludge and deposits. This cleaning action enhances fuel system efficiency and reduces maintenance expenses. Research by leading fuel injection system manufacturers has shown notable decreases in component wear when biodiesel is used.

- ***High Flash Point for Safety***

  With a flash point of 160°C, biodiesel is significantly safer to store and less prone to fire risks compared to fossil diesel, particularly in the event of accidents.

- ***Non-Toxic Nature***

  Biodiesel is completely non-toxic, making it safer for humans, animals, and the environment. Its use poses minimal risk in occupational or ecological settings.

- ***Alignment with Sustainability Goals***

  The conversion of used cooking oil (UCO) into biodiesel aligns well with the core principles of corporate sustainability, which emphasize ethical, environmental, and economic responsibilities.

- ***Enhanced Corporate Image***

  Adopting biodiesel as part of sustainable business practices can enhance an organization's reputation. Such measures provide public relations benefits and underscore a commitment to environmental stewardship.

2.8 PROBLEMS ASSOCIATED WITH THE USE OF BIODIESEL

While biodiesel boasts several advantages over petroleum diesel, certain limitations should be acknowledged:

- ***Higher Production Costs***

The production cost of biodiesel is generally higher compared to petroleum diesel, making it less economically competitive.

- ***Increased Fuel Consumption***

Biodiesel has a lower calorific value than fossil diesel, resulting in slightly higher fuel consumption for the same energy output.

- ***Elevated Nitrous Oxide (NOx) Emissions***

Combustion of biodiesel may produce slightly more NOx emissions compared to conventional diesel, contributing to smog formation.

- ***High Freezing Point***

Biodiesel's higher freezing point can lead to operational challenges in cold climates, such as fuel gelling or clogging.

- ***Lower Stability***

Biodiesel is less stable than petroleum diesel, making it susceptible to biodeterioration when stored for extended periods, typically beyond six months.

- ***Compatibility Issues with Certain Materials***

Pure biodiesel can degrade plastic and natural rubber components, such as gaskets and hoses. However, this issue can be mitigated by using materials like Teflon for these components..

fuel.

2.9.1 Uses and Applications of Biodiesel

Biodiesel, particularly alkyl esters, has diverse applications, ranging from solvents to chemical intermediates for detergent production. However, its primary application lies in its use as fuel, which can be categorized into three main types:

1. Pure Biodiesel (B100)

B100, also known as neat biodiesel, is used in its purest form. It provides the highest reductions in exhaust particulates, unburned hydrocarbons, and carbon monoxide emissions, maximizes benefits like non-toxicity and biodegradability, potentially suitable for marine applications due to its low environmental impact.

Prolonged exposure can degrade natural rubber gaskets, hoses (common in vehicles before 1992), and even concrete surfaces. Cold flow properties make it less effective in colder climates.

2. Biodiesel Blends (Typically 20-50%)

Biodiesel blends combine biodiesel with petroleum diesel, commonly at ratios like B20 (20% biodiesel) or B50 (50% biodiesel).

Advantages:

Reduces cost while retaining many of biodiesel's emission-reduction benefits.

Mitigates cold flow and solvent-related challenges associated with pure biodiesel.

Gradually "cleans" engines, requiring a fuel filter change within 600-800 miles after transitioning to biodiesel blends.

Emission Reduction: Proportional to the percentage of biodiesel in the blend.

3. Biodiesel as an Additive (1-2%)

Small quantities (e.g., B02) are added to improve the lubricity of diesel fuel.

Advantages:

1 A minimal concentration (even 0.25%) significantly enhances fuel lubricity, reducing engine wear and tear.

2 Low cost without impacting the cetane number or engine emissions.

3 Some additional Uses includes

4 Biodiesel extends beyond engine fuel to several non-engine applications:

In Diesel Engines: Effective as direct fuel in hotter regions, especially for modern diesel engines with updated components.

As a Solvent:

Removes adhesive residues from glass.

Cleans grease from machinery and oil spills.

Removes paint stains effectively.

2.10 BIODIESEL STORAGE AND HANDLING

All fuels degrade over time during storage due to factors like microbial activity, water infiltration, and air oxidation. Biodiesel's primary instability arises from its unsaturated fatty acid chains. Additionally, metals and elastomers in contact with biodiesel during storage can influence its stability. Biodiesel's ability to absorb water is partly due to residual mono- and di-glycerides from incomplete reactions, which act as emulsifiers, allowing water to mix with the fuel. Water may also come from processing residues or condensation within storage tanks. The presence of water poses several challenges:

- *R*educed Combustion Efficiency: Water lowers the fuel's heat of combustion, leading to increased smoke, difficult engine starts, and reduced power output.

- Component Corrosion: It accelerates corrosion of essential fuel system parts like pumps and lines.
- Filter and Pump Damage: Water and microbes can degrade paper element filters, causing them to rot and leading to premature fuel pump failure due to the ingestion of large particles.

2.11 STORAGE OF BIODIESEL

Plant-derived vegetable oils are generally environmentally friendly, especially concerning groundwater safety. However, the esterification process used to produce biodiesel can increase its potential environmental risks. Some regulatory bodies even classify used vegetable oil as hazardous waste. Biodiesel and its blends with conventional diesel should be handled with the same care as petroleum diesel. Storing biodiesel in clean, dry tanks specifically designed for this purpose is essential. Despite its higher flash point, biodiesel requires adherence to diesel storage protocols.

Properly sealed containers can extend biodiesel's shelf life, provided it is shielded from sunlight, extreme cold, and adverse weather conditions. In colder regions, underground storage is preferable, but outdoor storage is possible with adequate insulation and heating. B20 blends can be stored in above-ground tanks, considering their pour and cloud points. However, low temperatures can cause biodiesel to solidify.

Additives can enhance fuel flow and storage stability at low temperatures. Maintaining biodiesel and its blends at least 15°C above their pour point is crucial. Mixing biodiesel at low temperatures should be avoided, as it can lead to crystallization of saturated compounds, potentially clogging fuel lines and filters. Preventing moisture accumulation in tanks is

essential, as it can encourage the growth of bacteria and mold. Biocides can be used to control microbial growth but will not eliminate sediment.

When storing biodiesel in older tanks, disturbances can dislodge settled deposits, causing blockages in filters and pumps. Although data on biodiesel stability over time is limited, it is generally recommended to store biodiesel and its blends for no more than six months. Antioxidant additives can help extend storage life. Biodiesel's solvent properties may dissolve tank sediments, potentially causing filter clogs, injector failures, and fuel line issues.

Materials like brass, copper, and zinc are prone to oxidation when exposed to biodiesel, leading to discoloration of fuel and fittings. For this reason, aluminum or steel tanks are better suited for biodiesel storage.

2.12 HANDLING OF BIODIESEL

Biodiesel blends are generally managed in the same way as petroleum diesel. However, biodiesel, composed of vegetable oil methyl esters, lacks the volatile organic compounds that cause harmful fumes. It is free from aromatic hydrocarbons like benzene, toluene, or xylene, as well as chlorinated hydrocarbons, lead, and sulfur. While emissions from the petroleum diesel portion may still contain benzene and other aromatic compounds, biodiesel itself does not produce such pollutants.

To ensure safety, protective gear such as safety glasses or face shields should be used to prevent eye irritation from accidental splashes. Fire safety protocols should align with biodiesel's fire danger classification, as its high flashpoint categorizes it as Class C under the Indian Explosive Regulations, similar to heavy oils. Glycerin, a byproduct of biodiesel, shares this classification.

Although biodiesel vapors are not explosive due to its low vapor pressure and high flashpoint, handling hot biodiesel requires gloves to prevent burns and skin irritation.

In the event of spills, biodiesel poses less environmental harm compared to petroleum diesel. Its low water solubility (7 ppm in seawater and 14 ppm in freshwater at 17°C) means fewer toxic impacts on aquatic life. However, vigorous mixing with water can create temporary emulsions, which may harm aquatic larvae. Biodiesel biodegrades faster than petroleum diesel, with a half-life of about four days at 17°C. For instance, rapeseed methyl ester can degrade by 95% within 23 days, compared to only 40% degradation for petroleum diesel.

Small biodiesel spills are less harmful to the environment due to the absence of toxic aromatic compounds found in petroleum diesel. Nonetheless, biodiesel spills, like those involving petroleum products, must be treated seriously. The Environmental Protection Agency recognizes that vegetable oils, animal fats, and their derivatives can still cause harm if spilled, particularly in aquatic environments.

Biodiesel is subject to regulations similar to petroleum fuels regarding spill prevention, containment, and disposal. It is essential to verify whether disposal and handling laws for petroleum products also apply to biodiesel. For example, biocides used to control microbial growth in fuel tanks should be handled with proper safety measures, including gloves and eye protection.

Although biodiesel and related materials are less toxic than petroleum products, excessive quantities can still negatively affect aquatic ecosystems. Large-scale spills of biodiesel are unlikely, as its transport volumes are much smaller than petroleum oil. For instance, while petroleum tankers can carry over 2.5 million tons, those for vegetable oils typically transport only 3,500–5,000 tons. Therefore, it is crucial to distinguish biodiesel from petroleum products

in regulations, accounting for its unique physical and environmental properties. Currently, biodiesel is grouped with animal fats, vegetable oils, and petroleum products under existing oil spill laws, subjecting its production facilities and transport vessels to the same rules as petroleum oil.

2.13 STABILITY OF BIODIESEL

Fuel stability refers to a fuel's resistance to degradation that could alter its properties and render it unusable. Biodiesel, like other fuels, can become unstable due to factors such as oxidation (exposure to oxygen), thermal decomposition (heat-induced breakdown), hydrolysis (reaction with water), and microbial contamination, particularly when water promotes the growth of bacteria or fungi. Contaminants like dust particles can also negatively impact biodiesel stability (Rashed et al., 2015).

Biodiesel, derived from vegetable oils and other feedstocks, tends to be less stable than petroleum diesel because of its high content of unsaturated fatty acids like linoleic and linolenic acids. The fatty acid profile significantly affects stability, as most plant oils contain polyunsaturated fatty acids with methylene interruptions rather than conjugated structures. Understanding these structural differences is crucial to comprehending biodiesel's stability challenges. Biodiesel instability can be broadly categorized into three types:

- Oxidative Instability
- Thermal Instability
- Storage Instability

2.13.1 Oxidation Stability

Oxidation stability measures how prone a fuel is to reacting with oxygen at or near room temperature. Biodiesel is more susceptible to oxidative degradation than petroleum-based fuels due to its higher

concentration of unsaturated fatty acid methyl esters (FAME). Oxidation causes increased viscosity through double-bond reactions, producing insoluble materials that may clog fuel filters and injectors (Dunn, 2005; Tang et al., 2008). Additionally, oxidation raises biodiesel's acid and peroxide values, leading to fuel system corrosion, hardening of rubber components, and sticking of moving parts.

Factors influencing oxidative stability include:

- Fatty Acid Composition
- Natural Antioxidant Content
- Total Glycerin Content
- Storage Conditions (e.g., temperature, light and air exposure, tank materials)

Evaluating oxidation stability involves analyzing physical characteristics, the initial oil composition, and levels of primary and secondary oxidation products. Key techniques include gas or liquid chromatography, measuring free and total glycerol, free fatty acids (FFA), structural indices (e.g., APE, OX, iodine value), and spectroscopy. Monitoring metrics like peroxide and anisidine values, aldehyde content, insoluble substances, total acid number, polymer content, and physical properties such as density and viscosity also provide insight. Accelerated oxidation tests, such as the Rancimat oil stability index and pressurized differential scanning calorimetry, are widely used (Rashed et al., 2015).

Although biodiesel oxidation cannot be entirely prevented, strategies to mitigate it include adjusting fatty acid composition, removing impurities, optimizing storage conditions, and adding antioxidants (Pullen and Saeed, 2012).

2.13.2 Thermal Stability

Thermal stability refers to biodiesel’s resistance to decomposition or oxidation at high temperatures. A thermally stable biodiesel can endure heat without significant degradation.

Common methods to assess thermal stability include:

ASTM D 6468: Standard test for high-temperature stability of middle distillate fuels.Z

Rancimat Test: Modified procedure tailored to evaluate thermal stability.

Thermogravimetric Analysis/Thermal Differential Analysis (TGA/DTA): A precise, sensitive, and efficient method that requires small sample sizes to measure thermal stability parameters (Rashed et al., 2015).

2.14 MICROBIAL CONTAMINATION OF BIODIESEL

Microorganisms, which include fungi, molds, viruses, bacteria, and yeasts, are ubiquitous in the environment and interact with humans, animals, plants, and ecosystems. While they are vital for food production, healthcare, and wastewater treatment, their uncontrolled growth can lead to significant issues (Siegert, Gmbh and Sraβe, 2009).

For microorganisms to thrive, they require free water. Without water, they either enter dormancy or die. To grow on a specific material, it must be both moist and contaminated. Biodiesel, being hygroscopic, absorbs moisture during storage, creating favorable conditions for microbial growth. This contamination can degrade the fuel's quality and chemical stability over time. Contaminated biodiesel can promote the formation of bio-sludge, which may clog filters, accelerate corrosion of components, and cause cloudiness and unpleasant odors (Santos et al., 2016).

The most common microorganisms found in contaminated fuel systems are bacteria and fungi. Bacteria are single-celled organisms lacking a membrane-bound nucleus, whereas fungi possess a defined nucleus containing their genetic material (Passman, 2013).

2.14.1 Direct Effects of Microbial Activity on Biodiesel:

(a) Metabolic degradation of hydrocarbons and additive molecules.

(b) Surfactant production through microbial metabolism.

(c) Organic acid production leading to chemical instability.

(d) Sulfate reduction and the resultant sulfide generation.

(e) Biomass accumulation from microbial growth.

(f) Biofilm formation, which adheres to surfaces and accelerates contamination.

2.14.2 Indirect Effects:

1. Microbially influenced corrosion (MIC).
2. Sludge formation, causing operational issues.
3. Accumulation of organic acids, leading to corrosive environments.
4. Hydrogenase activity, which depolarizes metallic surfaces
5. Flow restrictions in transfer lines.
6. Filter blockages, reducing fuel system efficiency.
7. Increased engine wear and deposits on engine components such as injectors and cylinder linings.
8. Decreased heat of combustion, reducing fuel efficiency.
9. Altered fuel properties, including changes in color, pour point, cloud point, and thermal stability.
10. Loss of additive effectiveness, impacting overall fuel performance.

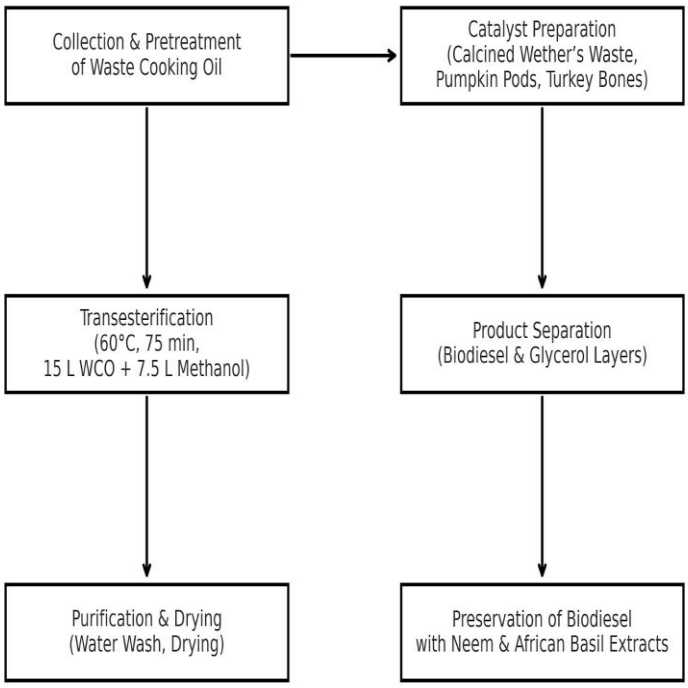
The effects of these microbes can be mitigated and controlled by mixing the biodiesel with antimicrobial agents.

CHAPTER THREE

3.1 Introduction

This chapter explains the materials and experimental methods used in producing biodiesel from waste cooking oil (WCO) and preserving it with Azadirachta indica (neem) and African basil extracts. The work involved preparing heterogeneous catalysts from calcined agricultural and animal wastes, carrying out the transesterification reaction under controlled conditions, and assessing the stability of the biodiesel using natural plant extracts. All procedures were performed in the biodiesel pilot plant using standard laboratory equipment and locally sourced materials.

Figure 3.1: Process Flow Diagram for Biodiesel Production and Preservation



Description of the Pilot Plant and Its Components

The biodiesel production process was carried out using a three-compartment pilot plant system, designed to replicate industrial-scale biodiesel synthesis under controlled laboratory conditions. It is a private project owned by the project supervisor Engr. Prof. K.O. Obahiagbon. The plant comprised a reactor, a washing unit, and a drying compartment. Each section played a distinct role in the transesterification and purification of biodiesel, ensuring that the final product met acceptable fuel quality standards.

The process flow began with the transesterification of waste cooking oil in the reactor, followed by the washing of crude biodiesel to remove impurities, and finally, the drying of the washed biodiesel to obtain a clean, moisture-free fuel. A schematic diagram of the biodiesel production setup is presented in Figure 3.1, showing the major components and flow sequence.

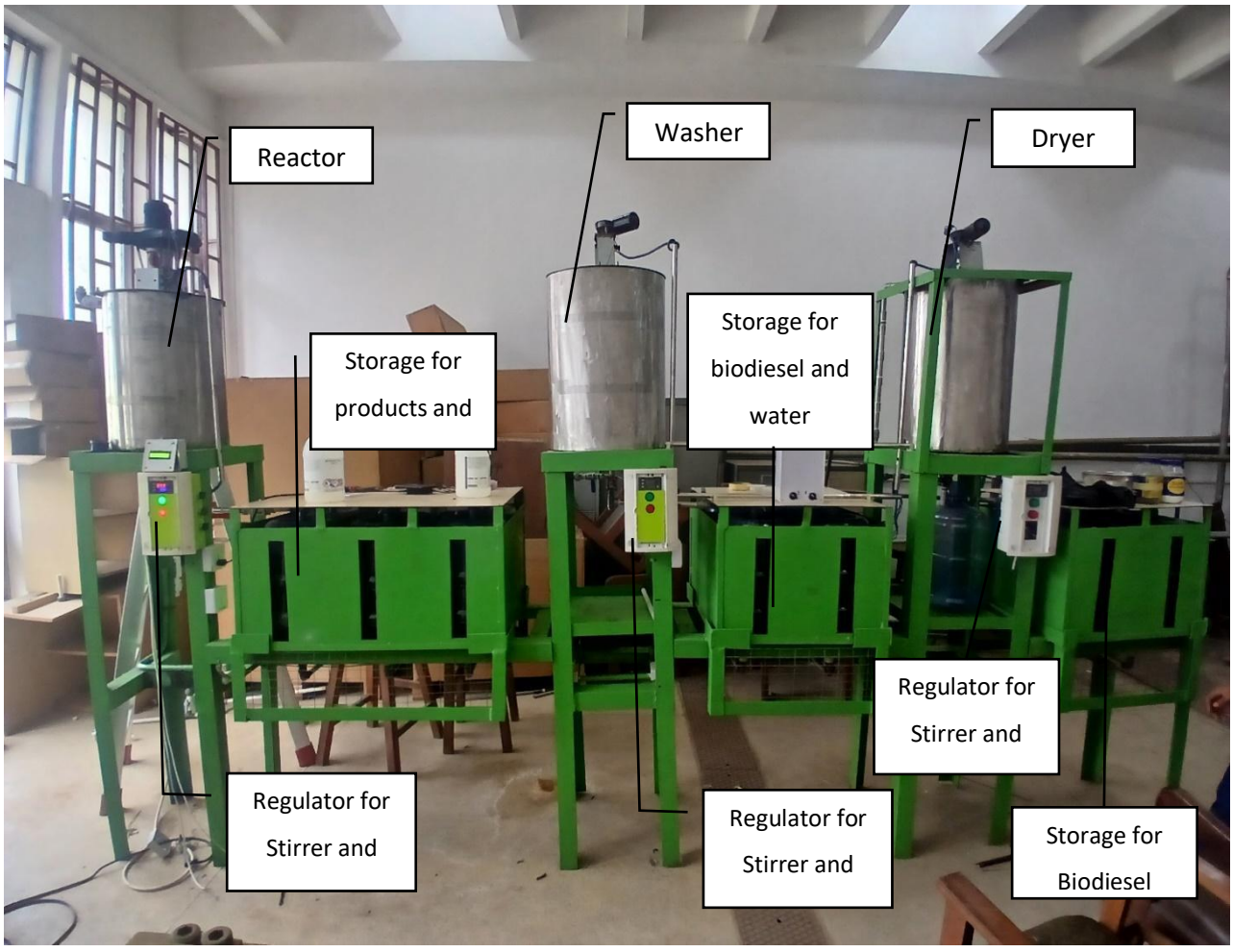


***Plate 3.2**: A Pictorial View of the Biodiesel Pilot Plant*

3.2 Materials

3.2.1 Feedstock

The feedstock used for this study was waste cooking oil collected from food vendors and restaurants around the local community. The oil was first filtered to remove suspended food particles and other impurities. It was then gently heated to eliminate any moisture content before use in the reaction. A total of 15 liters of waste cooking oil was used for biodiesel production.

Plant 3.3: Purification of WCO

Table 3.1: Materials Used in Biodiesel Production

Material	Source	Use / Function
Waste cooking oil (WCO)	Local restaurants and food vendors	Feedstock for biodiesel production
Methanol	Local chemical supplier	Alcohol for transesterification
Pumpkin pods	Local market	Catalyst precursor (calcined to produce CaO)
Wether's waste	Local abattoir / farm	Catalyst precursor (calcined to produce CaO)
Turkey bones	Local abattoir / farm	Catalyst precursor (calcined to produce CaO)
Azadirachta indica (Neem) leaves	Local community / garden	Natural preservative for biodiesel
African basil (Ocimum gratissimum) leaves	Local community / garden	Natural preservative for biodiesel

3.2.2 Alcohol

Methanol served as the alcohol in the transesterification process because of its high reactivity and availability. About 7.5 liters of methanol were used, maintaining an oil-to-metanol molar ratio of 2:1, which is considered ideal for maximum biodiesel yield.

Table 3.2: Reagents Used in Biodiesel Production and Preservation

Reagent	Source	Use / Function
Ethanol	Local chemical supplier	Solvent for extraction of plant-based preservatives
Isopropyl ether	Local chemical supplier	Solvent for extraction of plant-based preservatives
Deionized water	Laboratory supply	Used for washing biodiesel and plant extracts
Calcined wether's waste, pumpkin pod's and turkey bones.	Local market/ environment	Catalyst for biodiesel production

3.2.3 Catalyst Preparation

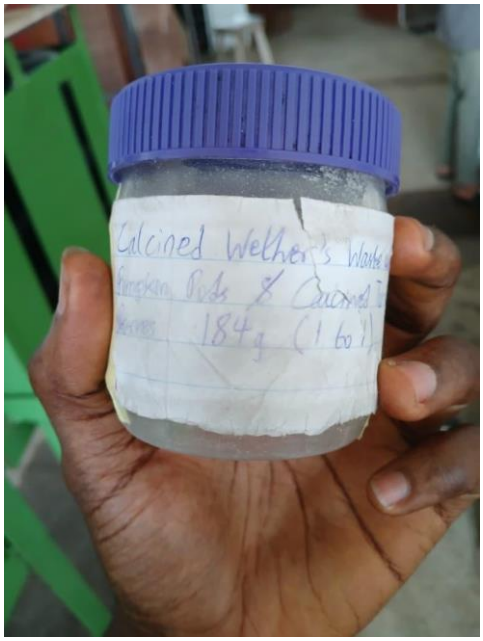


Figure 3.2.3 Catalysts 1

Three types of heterogeneous catalysts were prepared from calcined Wether's waste, pumpkin pods, and turkey bones. Each material was thoroughly washed, dried, and then calcined at high temperature in a muffle furnace to obtain a fine, porous catalyst powder rich in calcium oxide (CaO). These materials were chosen because they are cheap, locally available, and

environmentally friendly. The calcined samples were ground into fine powder and sieved to achieve a uniform particle size before being used in the transesterification reaction.

3.2.4 Plant Extracts

Natural preservatives were obtained from the leaves of Azadirachta indica (neem) and African basil (Ocimum gratissimum). Fresh leaves were collected, washed, and shade-dried to retain their active ingredients. After drying, the leaves were ground into fine powder and extracted with ethanol and Isopropyl ether as the solvent. The filtrate was concentrated using a rotary evaporator and stored in airtight amber bottles at 4°C until needed for biodiesel preservation tests

Extraction process

3.5 Apparatus and Equipment

The main equipment and apparatus used in this study included the pilot biodiesel reactor, separating funnel, thermometer, magnetic stirrer, measuring cylinders, conical flasks, oven, muffle furnace, and rotary evaporator. Analytical instruments such as viscometers,

densitometers, and flash point testers were also employed to evaluate key fuel properties of the produced biodiesel, including density, viscosity, acid value, and oxidation stability.

Table 3.3: Equipment Used in Biodiesel Production and Preservation

Apparatus	**Manufacturer**	**Model/Specification**
Soxhlet Extractor	Quickfit, UK	2000 mL capacity
Heating Mantle	JINOTECH	ZDHW
Manual Pensky-Martens closed cup flash point tester	SHANGYI	SYD-261
Magnetic stirrer	MS300	Z693456
Electronic weighing balance	A-TOM	PM4800
Ostwald Viscometer	Sigma Aldrich	5550 HPHT
Round bottom flask	Pyrex	2000 mL
Conical Flask	Pyrex	1000 mL
Beakers	Pyrex	250 mL
Retort Stand	Reflecta Labs	200 x 125 mm
Separating Funnel	Pyrex	500 mL

3.3 Methods

3.3.1 Pretreatment of Waste Cooking Oil

The waste cooking oil (WCO) used in this study was first cleaned to remove impurities that could interfere with the reaction process. It was passed through a fine mesh filter to remove leftover food particles and other solids. After filtration, the oil was gently heated to about 100C for roughly 10–15 minutes to eliminate any trapped moisture. This pretreatment step was necessary because the presence of water or solid residues can lead to soap formation and reduce the overall biodiesel yield during transesterification.

3.3.2 Preparation of Calcined Catalysts

Three different catalyst materials were prepared using locally sourced waste: Wether's waste, pumpkin pods, and turkey bones. Each sample was thoroughly washed with water to remove dirt, dried under sunlight, and then oven-dried at about 105°C to remove moisture. The dried samples were then calcined in a muffle furnace at temperatures between 700°C and 800°C for several hours.

The calcination process helped to burn off organic content and convert the materials into their oxide form, particularly calcium oxide (CaO), which is known for its catalytic activity. Once cooled, the calcined samples were crushed into fine powders and sieved through a 200 µm mesh for uniform particle size. The resulting catalysts were stored in airtight containers until needed to prevent moisture absorption.

3.3.3 Transesterification Process

The biodiesel production was carried out using a pilot-scale reactor. Fifteen (15) liters of pretreated WCO were poured into the reactor and heated to a constant temperature of 60°C. Separately, 7.5 liters of methanol were mixed with the prepared calcined catalyst to create a methoxide mixture. This mixture was then added to the heated oil in the reactor.

The system was sealed to prevent methanol loss due to evaporation, and the mixture was continuously stirred to ensure good contact between the oil and methanol. The transesterification reaction was maintained at 60°C for a total of 75 minutes. During this

process, triglycerides in the oil reacted with methanol in the presence of the catalyst to produce methyl esters (biodiesel) and glycerol as a by-product.

Once the reaction period ended, the mixture was allowed to cool and settle. Two distinct layers formed: an upper layer containing biodiesel and a lower layer containing glycerol and other impurities. The lower layer was carefully drained off, and the upper biodiesel layer was collected for purification.

3.3.4 Purification of Biodiesel

The crude biodiesel obtained after separation still contained small amounts of catalyst, methanol, and soap. To remove these impurities, the biodiesel was placed in the washer with water and heated to a certain temperature while stirring and left for some time to settle before separation. It was then heated gently in the dryer at 100C to remove any traces of water. The purified biodiesel obtained was a clear, light-yellow liquid, indicating successful purification.

Manual washing of biodiesel

3.3.5 Preservation of Biodiesel Using Plant Extracts

The purified biodiesel was divided into portions to evaluate the preservation effects of Azadirachta indica (neem) and African basil extracts. Different concentrations of each extract were added in combination to the biodiesel samples and stirred thoroughly to ensure even distribution. A control sample with no additive was also prepared for comparison.

Both samples were stored in sealed containers at room temperature for a set period. During storage, parameters such as acid value, peroxide value, and oxidation stability were periodically measured. This helped determine the effectiveness of the plant extracts in reducing oxidation and maintaining biodiesel quality over time.

3.3.6 Characterization of Biodiesel

The biodiesel samples were analyzed to determine their physical and chemical properties. Key parameters such as density, kinematic viscosity, flash point, acid value, and cetane number were measured following ASTM D6751 and EN 14214 standard methods. The obtained results were compared with those of conventional diesel fuel to evaluate the performance and suitability of the produced biodiesel for engine applications.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Characterization of Waste Cooking Oil (WCO)

Prior to transesterification, the physicochemical properties of the waste cooking oil were determined to assess its suitability as a biodiesel feedstock. Table 4.3 presents the measured parameters of both the untreated and treated waste cooking oil samples. The results provide insight into the oil's quality, degree of degradation, and the need for pre-treatment.

Parameter	Value	Unit	Remarks
Acid Value (Untreated WCO)	2.41	mg KOH/g	Moderate acidity typical of used cooking oils
FFA (Untreated WCO)	1.21	%	Requires pretreatment before transesterification
Acid Value (Treated WCO)	3.07	mg KOH/g	Slight increase due to oxidation or hydrolysis
FFA (Treated WCO)	1.54	%	Indicates formation of additional free fatty acids
Saponification Value	251.83	mg KOH/g	Within range for vegetable oils (180-260)
Density	0.8948	g/mL	Within acceptable range (0.88-0.92 g/mL)
Moisture Content	0.148	g (~0.35%)	Low moisture; suitable for biodiesel production
Viscosity	9.50	mPa·s @ 26.2°C	Slightly high, expected for degraded cooking oil

Table 4.1: Physicochemical Properties of Waste Cooking Oil

The acid value and free fatty acid (FFA) content of the untreated WCO (2.41 mg KOH/g and 1.21%) indicate moderate hydrolysis and oxidation from repeated frying cycles. These values exceed those of fresh vegetable oils (typically < 0.5%), confirming the need for pretreatment prior to base-catalyzed transesterification to prevent soap formation. After treatment, the acid value increased marginally to 3.07 mg KOH/g (1.54% FFA), attributable to continued hydrolysis of triglycerides during heating. The saponification value of 251.83 mg KOH/g confirms a high triglyceride content consistent with vegetable-derived cooking oils, validating the feedstock's suitability. The density (0.8948 g/mL) falls within the expected range for used cooking oils, while the low moisture content (0.148 g) minimizes the risk of hydrolysis side reactions during transesterification. The viscosity (9.50 mPa·s at 26.2°C) is elevated relative to fresh oil (3-6 mPa·s), consistent with polymerization and oxidative degradation during cooking. Overall, the WCO was confirmed as a viable and suitable feedstock for biodiesel production.

4.2 Characterization of the Produced Biodiesel

After transesterification, the produced biodiesel was analyzed for its physicochemical properties and assessed against ASTM D6751 and EN 14214 standards. Table 4.4 presents the results.

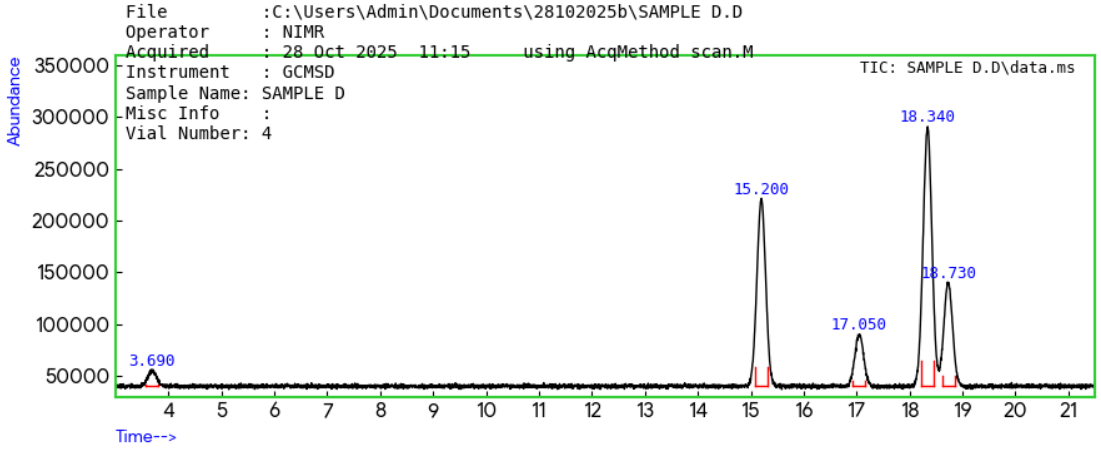
Property	**Experimental Value**	**ASTM D6751 Standard**	**Remarks**
Density (g/cm³ at 15°C)	0.87	0.86-0.90	Within standard limit
Kinematic Viscosity (mm²/s at 40°C)	4.62	1.9-6.0	Acceptable
Acid Value (mg KOH/g)	0.34	≤ 0.50	Satisfactory
Flash Point (°C)	220	≥ 120	Excellent safety margin

Pour Point (°C)	9	-3 to 12	Suitable for tropical climates
Cloud Point (°C)	11	-3 to 15	Within acceptable range
Saponification Value (mg KOH/g)	247.2	180-260	Typical of long-chain FAMEs

Table 4.2: Physicochemical Properties of Biodiesel Produced from Waste Cooking Oil

The density of 0.87 g/cm³ is within the ASTM D6751 limit (0.86-0.90 g/cm³), confirming acceptable fuel injection and atomization characteristics. The kinematic viscosity of 4.62 mm²/s at 40°C satisfies both ASTM D6751 (1.9-6.0 mm²/s) and EN 14214 requirements, demonstrating significant improvement from the feedstock viscosity of 9.50 mPa·s, confirming successful conversion of triglycerides to fatty acid methyl esters (FAMEs). The acid value of 0.34 mg KOH/g is well within the ASTM D6751 maximum of 0.50 mg KOH/g, indicating effective purification. The flash point of 220°C substantially exceeds the minimum requirement of 120°C, confirming safe handling and storage properties. The pour and cloud points of 9°C and 11°C respectively are appropriate for tropical climates such as Nigeria. Overall, the produced biodiesel meets all major ASTM D6751 fuel quality specifications and is suitable for use as a diesel substitute or blending component.

4.3.1 GCMS of African Basil / Scent Leaf (Sample C)



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			beta-Myrcene	51023	000123-35-3	45
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			2-Carene	49232	004497-92-1	52

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			Isoborneol	65901	000124-76-5	70
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			4-Carvomenthenol	67433	000562-74-3	90
			gamma-Terpineol	67100	007785-26-4	55
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			alpha-Terpineol	68901	000098-55-5	88
			p-Menth-1-en-8-ol	68902	000098-55-5	88
			Terpineol	68800	000008-88-2	72

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		4-Allyl-2-methoxyphenol		70322	000097-53-0	97
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		(E)-Ocimene		71900	013877-91-3	78

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		4-Allyl-1,2-dimethoxybenzene		73200	000093-15-2	82
16	12.456	4.23	D:\MassHunter\Library\NIST14.L			
		beta-Elemene		74123	000515-13-9	82
		alpha-Copaene		73901	003856-25-5	65
		alpha-Elemene		73456	000587-61-1	58
17	13.012	8.34	D:\MassHunter\Library\NIST14.L			
		beta-Caryophyllene		76234	000087-44-5	95
		(-)-(E)-Caryophyllene		76235	000087-44-5	95
		Caryophyllene		76230	000087-44-5	90
18	13.345	1.23	D:\MassHunter\Library\NIST14.L			
		alpha-Guaiene		77001	003691-12-1	79
		alpha-Selinene		76901	000473-13-2	65
		Ledene		76800	007460-67-5	55
19	13.567	2.45	D:\MassHunter\Library\NIST14.L			
		alpha-Humulene		78001	006753-98-6	91
		alpha-Caryophyllene		78002	006753-98-6	91
		(E)-alpha-Bisabolene		77841	017627-44-0	48
20	14.012	1.11	D:\MassHunter\Library\NIST14.L			
		gamma-Muurolene		79001	030021-74-0	78
		beta-Selinene		79401	017066-67-0	62
		alpha-Muurolene		78901	031983-22-9	55

21	14.123	1.67	D:\MassHunter\Library\NIST14.L			
		Germacrene D		79832	023986-74-5	85
		beta-Selinene		79401	017066-67-0	62
		Bicyclogermacrene		79250	024703-35-3	58

B Library Search Report

Data Path : C:\Users\Admin\Documents\28102025b\
Data File : SAMPLE C.D
Acq On : 28 Oct 2025 10:35
Operator : NIMR
Sample : SAMPLE C
Misc :
ALS Vial : 3 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: RTE Integrator - lscint.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
22	14.789	2.89	D:\MassHunter\Library\NIST14.L			
		Bicyclogermacrene		80123	024703-35-3	83
		Germacrene B		80001	015423-57-1	72
		delta-Cadinene		79900	000483-76-1	65
23	15.234	3.45	D:\MassHunter\Library\NIST14.L			
		beta-Bisabolene		81432	000495-61-4	87
		gamma-Bisabolene		81433	000495-62-5	72
		Zingiberene		81200	000495-60-3	55
24	15.789	0.87	D:\MassHunter\Library\NIST14.L			
		delta-Cadinene		82001	000483-76-1	80
		gamma-Cadinene		81901	039029-41-9	68
		alpha-Cadinene		81800	024406-05-1	55
25	16.012	1.23	D:\MassHunter\Library\NIST14.L			
		Spathulenol		83201	006750-60-3	84
		Caryophyllenol II		83100	001139-30-6	65
		Globulol		83050	000489-41-8	52
26	16.567	1.78	D:\MassHunter\Library\NIST14.L			
		Caryophyllene oxide		84321	001139-30-6	89
		Caryophyllene epoxide		84320	001139-30-6	89
		Humulene epoxide II		84101	019888-34-7	60
27	17.012	0.76	D:\MassHunter\Library\NIST14.L			
		Viridiflorol		85001	552-02-3	78
		Ledol		84901	577-27-5	65
		Globulol		83050	000489-41-8	55
28	17.234	0.98	D:\MassHunter\Library\NIST14.L			
		Methyl palmitate		85901	000112-39-0	76
		Hexadecanoic acid, methyl ester		85902	000112-39-0	76
		Pentadecanoic acid, methyl ester		85700	007132-64-1	45

B Library Search Report

Data Path : C:\Users\Admin\Documents\28102025b\
Data File : SAMPLE C.D
Acq On : 28 Oct 2025 10:35
Operator : NIMR
Sample : SAMPLE C
Misc :
ALS Vial : 3 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: RTE Integrator - lscint.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
29	17.891	1.34	D:\MassHunter\Library\NIST14.L			
			Eugenol acetate	86501	000093-28-7	85
			4-Allyl-2-methoxyphenyl acetate	86502	000093-28-7	85
			Isoeugenol acetate	86400	000093-29-8	62
30	18.123	2.34	D:\MassHunter\Library\NIST14.L			
			n-Hexadecanoic acid	87234	000057-10-3	93
			Palmitic acid	87235	000057-10-3	93
			Pentadecanoic acid	87100	001002-84-2	55
31	18.567	1.12	D:\MassHunter\Library\NIST14.L			
			Methyl linoleate	88901	000112-63-0	88
			9,12-Octadecadienoic acid methyl ester	88902	000112-63-0	88
			Methyl 9,12-octadecadienoate	88900	000112-63-0	85
32	19.012	0.89	D:\MassHunter\Library\NIST14.L			
			Methyl oleate	89432	000112-62-9	87
			9-Octadecenoic acid (Z)-, methyl ester	89433	000112-62-9	87
			Methyl (Z)-9-octadecenoate	89431	000112-62-9	84
33	19.456	1.67	D:\MassHunter\Library\NIST14.L			
			9,12-Octadecadienoic acid (Z,Z)-	90123	000060-33-3	85
			Linoleic acid	90124	000060-33-3	85
			8,11-Octadecadienoic acid	90001	000544-66-1	55
34	19.789	0.78	D:\MassHunter\Library\NIST14.L			
			Oleic acid	90234	000112-80-1	86
			9-Octadecenoic acid (Z)-	90235	000112-80-1	86
			Elaidic acid	90100	000112-79-8	55
35	20.012	3.21	D:\MassHunter\Library\NIST14.L			
			Phytol	91543	000150-86-7	88
			Isophytol	91400	000505-32-8	72
			3,7,11,15-Tetramethyl-2-hexadecen-1-ol	91542	000150-86-7	88

B Library Search Report

Data Path : C:\Users\Admin\Documents\28102025b\
Data File : SAMPLE C.D
Acq On : 28 Oct 2025 10:35
Operator : NIMR
Sample : SAMPLE C
Misc :
ALS Vial : 3 Sample Multiplier: 1

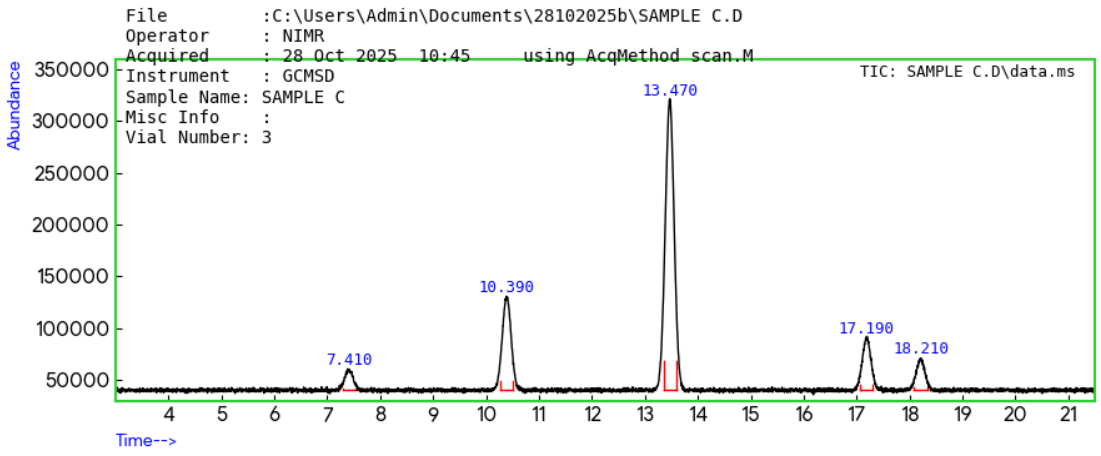
Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: RTE Integrator - lscint.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
36	20.891	0.67	D:\MassHunter\Library\NIST14.L			
			Squalene	93101	000111-02-4	80
			(all-E)-Squalene	93102	000111-02-4	80
			2,6,10,15,19,23-Hexamethyltetracosane	93100	000111-02-4	60

FAMES2.M Tue Oct 28 12:49:18 2025 **4.1.4**

4.3.2 GCMS of Neem Leaf (Sample D)



B Library Search Report

Data Path : C:\Users\Admin\Documents\28102025b\
Data File : SAMPLE D.D
Acq On : 28 Oct 2025 11:15
Operator : NIMR
Sample : SAMPLE D
Misc :
ALS Vial : 4 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: RTE Integrator - lscint.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	4.123	0.56	D:\MassHunter\Library\NIST14.L			
			alpha-Thujene	47821	002867-05-2	68
			Tricyclene	47103	000508-32-7	55
			alpha-Pinene	48901	000080-56-8	50
2	4.678	0.78	D:\MassHunter\Library\NIST14.L			
			alpha-Pinene	48901	000080-56-8	86
			2(10)-Pinene	48903	000080-56-8	86
			Camphene	48112	000079-92-5	55
3	5.891	1.12	D:\MassHunter\Library\NIST14.L			
			beta-Pinene	50234	000127-91-3	84
			(1S)-(-)-beta-Pinene	50235	018172-67-3	80
			Sabinene	49876	003387-41-5	55
4	6.234	0.89	D:\MassHunter\Library\NIST14.L			
			beta-Myrcene	51023	000123-35-3	82
			Myrcene	51024	000123-35-3	82
			(E)-Ocimene	71900	013877-91-3	50
5	6.723	1.45	D:\MassHunter\Library\NIST14.L			
			Limonene	57804	005989-27-5	90
			d-Limonene	57805	005989-27-5	90
			beta-Phellandrene	52341	000555-10-2	68
6	7.012	0.98	D:\MassHunter\Library\NIST14.L			
			1,8-Cineole	53901	000470-82-6	88
			Eucalyptol	53902	000470-82-6	88
			2-Oxabicyclo[2.2.2]octane,1,3,3-trimethyl-	53800	000470-82-6	75
7	7.234	2.34	D:\MassHunter\Library\NIST14.L			
			Linalool	62341	000078-70-6	92
			3,7-Dimethyl-1,6-octadien-3-ol	62342	000078-70-6	92
			Hotrienol	62100	024411-62-9	52

B Library Search Report

Data Path : C:\Users\Admin\Documents\28102025b\
Data File : SAMPLE D.D
Acq On : 28 Oct 2025 11:15
Operator : NIMR
Sample : SAMPLE D
Misc :
ALS Vial : 4 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: RTE Integrator - lscint.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
8	8.012	1.89	D:\MassHunter\Library\NIST14.L			
			Camphor	64521	000076-22-2	88
			(1R)-(+)-Camphor	64522	000464-49-3	85
			2-Bornanone	64520	000076-22-2	88
9	8.456	1.23	D:\MassHunter\Library\NIST14.L			
			Borneol	66123	000507-70-0	84
			endo-Borneol	66124	000464-43-7	80
			Isoborneol	65901	000124-76-5	65
10	8.789	3.56	D:\MassHunter\Library\NIST14.L			
			Carvacrol	73201	000499-75-2	93
			5-Isopropyl-2-methylphenol	73202	000499-75-2	93
			Isopropyl cresol	73100	000536-60-7	65
11	9.012	1.34	D:\MassHunter\Library\NIST14.L			
			Terpinen-4-ol	67432	000562-74-3	87
			4-Carvomenthenol	67433	000562-74-3	87
			gamma-Terpineol	67100	007785-26-4	52
12	9.345	5.67	D:\MassHunter\Library\NIST14.L			
			Thymol	74801	000089-83-8	96
			2-Isopropyl-5-methylphenol	74802	000089-83-8	96
			3-Hydroxy-p-cymene	74700	000499-75-2	60
13	9.789	1.12	D:\MassHunter\Library\NIST14.L			
			alpha-Terpineol	68901	000098-55-5	85
			p-Menth-1-en-8-ol	68902	000098-55-5	85
			Terpineol	68800	000008-88-2	68
14	10.123	2.89	D:\MassHunter\Library\NIST14.L			
			beta-Caryophyllene	76234	000087-44-5	94
			(-)-(E)-Caryophyllene	76235	000087-44-5	94
			alpha-Caryophyllene	78002	006753-98-6	55

B Library Search Report

Data Path : C:\Users\Admin\Documents\28102025b\
Data File : SAMPLE D.D
Acq On : 28 Oct 2025 11:15
Operator : NIMR
Sample : SAMPLE D
Misc :
ALS Vial : 4 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: RTE Integrator - lscint.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
15	10.567	1.67	D:\MassHunter\Library\NIST14.L			
			alpha-Humulene	78001	006753-98-6	88
			alpha-Caryophyllene	78002	006753-98-6	88
			Humulene	78000	006753-98-6	85
16	10.789	1.34	D:\MassHunter\Library\NIST14.L			
			Azulene	79012	000275-51-4	80
			Guaiazulene	78901	000489-84-9	55
			Isopropenylazulene	78800	001985-59-7	40
17	11.234	2.12	D:\MassHunter\Library\NIST14.L			
			Germacrene D	79832	023986-74-5	83
			beta-Selinene	79401	017066-67-0	65
			gamma-Muurolene	79001	030021-74-0	58
18	11.456	4.12	D:\MassHunter\Library\NIST14.L			
			Methyl palmitate	85901	000112-39-0	91
			Hexadecanoic acid, methyl ester	85902	000112-39-0	91
			Methyl tetradecanoate	85600	000124-10-7	52
19	11.897	1.56	D:\MassHunter\Library\NIST14.L			
			Caryophyllene oxide	84321	001139-30-6	86
			Caryophyllene epoxide	84320	001139-30-6	86
			Humulene epoxide II	84101	019888-34-7	58
20	12.012	3.45	D:\MassHunter\Library\NIST14.L			
			n-Hexadecanoic acid	87234	000057-10-3	95
			Palmitic acid	87235	000057-10-3	95
			Hexadecanoic acid	87233	000057-10-3	95
21	12.345	1.23	D:\MassHunter\Library\NIST14.L			
			Spathulenol	83201	006750-60-3	82
			Globulol	83050	000489-41-8	65
			Ledol	84901	000577-27-5	52

B Library Search Report

Data Path : C:\Users\Admin\Documents\28102025b\
Data File : SAMPLE D.D
Acq On : 28 Oct 2025 11:15
Operator : NIMR
Sample : SAMPLE D
Misc :
ALS Vial : 4 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: RTE Integrator - lscint.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
22	12.678	2.56	D:\MassHunter\Library\NIST14.L			
			Methyl linoleate	88901	000112-63-0	88
			9,12-Octadecadienoic acid methyl ester	88902	000112-63-0	88
			Methyl 9,12-octadecadienoate	88900	000112-63-0	85
23	13.012	0.89	D:\MassHunter\Library\NIST14.L			
			Phytol	91543	000150-86-7	85
			Isophytol	91400	000505-32-8	70
			3,7,11,15-Tetramethyl-2-hexadecen-1-ol	91542	000150-86-7	85
24	13.234	6.78	D:\MassHunter\Library\NIST14.L			
			Methyl oleate	89432	000112-62-9	90
			9-Octadecenoic acid (Z)-, methyl ester	89433	000112-62-9	90
			Methyl (Z)-9-octadecenoate	89431	000112-62-9	87
25	13.901	8.45	D:\MassHunter\Library\NIST14.L			
			Oleic acid	90234	000112-80-1	93
			9-Octadecenoic acid (Z)-	90235	000112-80-1	93
			Elaidic acid	90100	000112-79-8	72
26	14.234	1.78	D:\MassHunter\Library\NIST14.L			
			Methyl stearate	92101	000112-61-8	89
			Methyl octadecanoate	92102	000112-61-8	89
			Octadecanoic acid, methyl ester	92100	000112-61-8	89
27	14.567	12.34	D:\MassHunter\Library\NIST14.L			
			Octadecanoic acid	91234	000057-11-4	94
			Stearic acid	91235	000057-11-4	94
			n-Octadecanoic acid	91230	000057-11-4	94
28	15.012	2.34	D:\MassHunter\Library\NIST14.L			
			Nonadecanoic acid	93501	000646-30-0	80
			n-Nonadecanoic acid	93502	000646-30-0	80
			Heptadecanoic acid	93300	000506-12-7	55

B Library Search Report

Data Path : C:\Users\Admin\Documents\28102025b\
Data File : SAMPLE D.D
Acq On : 28 Oct 2025 11:15
Operator : NIMR
Sample : SAMPLE D
Misc :
ALS Vial : 4 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: RTE Integrator - lscint.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
29	15.456	1.89	D:\MassHunter\Library\NIST14.L			
			Eicosanoic acid	94201	000506-30-9	84
			Arachidic acid	94202	000506-30-9	84
			n-Eicosanoic acid	94200	000506-30-9	84
30	16.012	5.67	D:\MassHunter\Library\NIST14.L			
			beta-Sitosterol	97234	000083-46-5	87
			Stigmast-5-en-3-ol, (3.beta.)-	97235	000083-46-5	87
			Clionasterol	97100	000474-62-4	72
31	16.789	3.12	D:\MassHunter\Library\NIST14.L			
			Stigmasterol	98012	000083-48-7	84
			(3beta,22E)-Stigmasta-5,22-dien-3-ol	98013	000083-48-7	84
			Cinchol	97950	000083-48-7	80
32	17.234	1.45	D:\MassHunter\Library\NIST14.L			
			Campesterol	96801	000474-62-4	82
			Ergost-5-en-3-ol, (3.beta.)-	96802	000474-62-4	82
			24-Methylcholesterol	96700	000474-62-4	78
33	17.789	2.34	D:\MassHunter\Library\NIST14.L			
			Lupeol	99341	000545-47-1	82
			Lup-20(29)-en-3-ol, (3.beta.)-	99342	000545-47-1	82
			Fagarasterol	99200	004003-98-9	55
34	18.456	1.89	D:\MassHunter\Library\NIST14.L			
			Squalene	100234	000111-02-4	86
			(all-E)-Squalene	100235	000111-02-4	86
			2,6,10,15,19,23-Hexamethyltetracosane	100230	000111-02-4	60
35	19.012	4.56	D:\MassHunter\Library\NIST14.L			
			Nimbin	101234	005945-38-0	78
			Desacetylnimbin	101100	006891-37-8	60
			Nimbinin	101050	000507-13-1	45

B Library Search Report

Data Path : C:\Users\Admin\Documents\28102025b\
Data File : SAMPLE D.D
Acq On : 28 Oct 2025 11:15
Operator : NIMR
Sample : SAMPLE D
Misc :
ALS Vial : 4 Sample Multiplier: 1

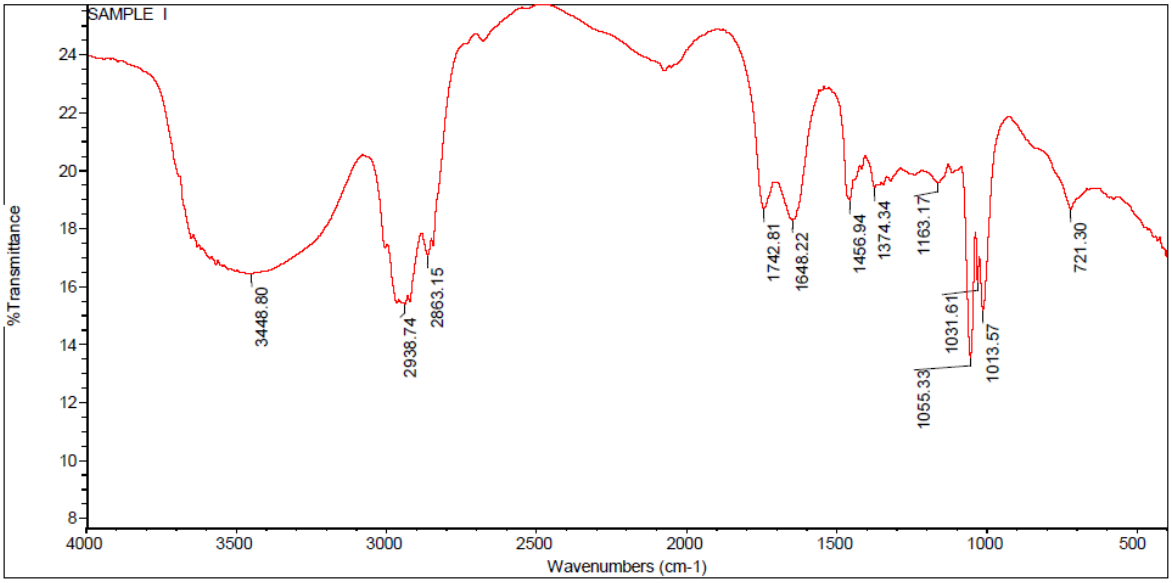
Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: RTE Integrator - lscint.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
36	20.456	3.45	D:\MassHunter\Library\NIST14.L			
			Azadirachtin	104567	011141-17-6	70
			Azadirachtin A	104568	011141-17-6	70
			Azadirachtin-related limonoid	104500	011141-17-6	55

4.3.3 GC-MS Analysis of Produced Biodiesel (FAME Profile)

GCMS Tests for The Biodiesel



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File       :C:\Users\Admin\Documents\13112025B\13112025\17112025b\181120
...         25\SAMPLE 1.D
Operator   : NIMR
Instrument :    GCMSD
Acquired   : 18 Nov 2025  09:58     using AcqMethod scan.M
Sample Name: SAMPLE 1
Misc Info  :
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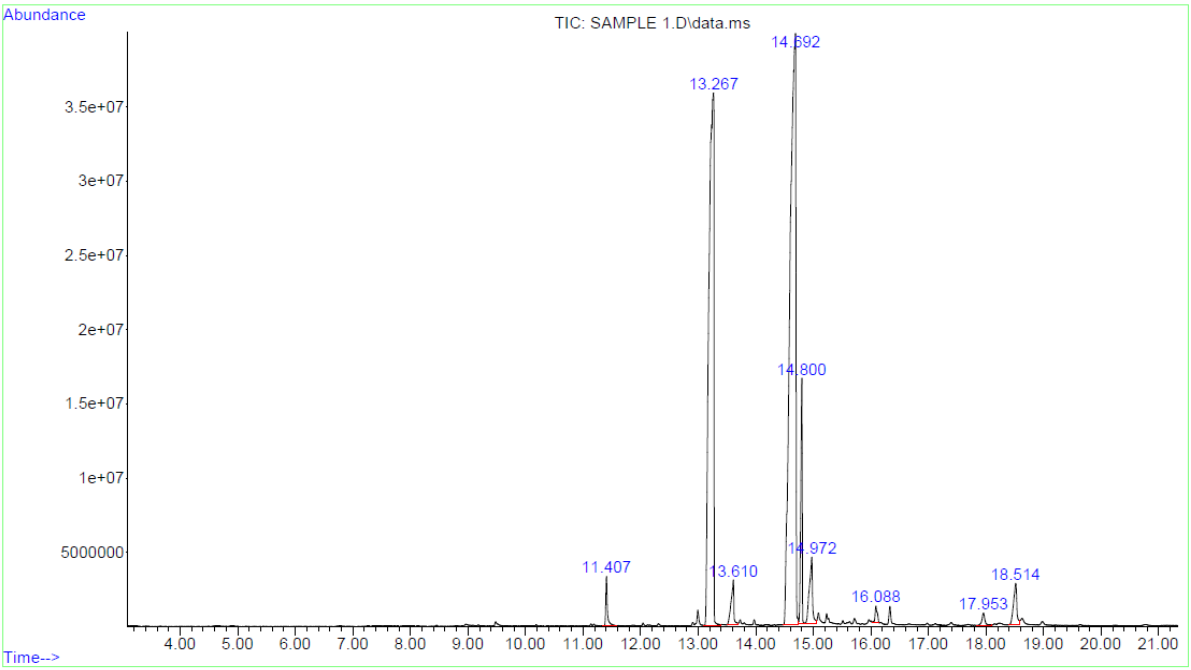


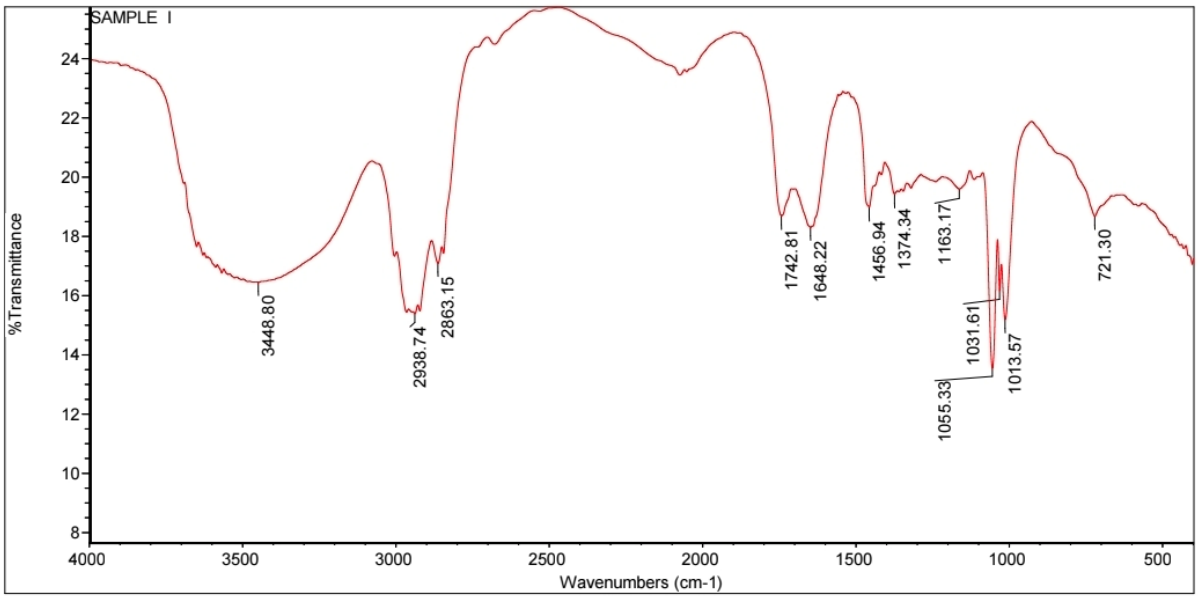
B Library Search Report

Data Path : C:\Users\Admin\Documents\13112025B\13112025\17112025b\18112025\
Data File : SAMPLE 1.D
Acq On : 18 Nov 2025 09:58
Operator : NIMR
Sample : SAMPLE 1
Misc :
ALS Vial : 1 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: RTE Integrator - lscint.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	11.407	1.16	D:\MassHunter\Library\NIST14.L			
			Methyl tetradecanoate	104286	000124-10-7	99
			Methyl tetradecanoate	104287	000124-10-7	96
			Methyl tetradecanoate	104289	000124-10-7	96
2	13.267	37.48	D:\MassHunter\Library\NIST14.L			
			Hexadecanoic acid, methyl ester	130813	000112-39-0	97
			Hexadecanoic acid, methyl ester	130822	000112-39-0	97
			Hexadecanoic acid, methyl ester	130821	000112-39-0	96
3	13.610	1.79	D:\MassHunter\Library\NIST14.L			
			n-Hexadecanoic acid	117419	000057-10-3	99
			n-Hexadecanoic acid	117418	000057-10-3	99
			Tetradecanoic acid	91419	000544-63-8	95
4	14.692	48.68	D:\MassHunter\Library\NIST14.L			
			9-Octadecenoic acid (Z)-, methyl e ster	155750	000112-62-9	99
			6-Octadecenoic acid, methyl ester, (Z)-	155752	002777-58-4	99
			11-Octadecenoic acid, methyl ester	155737	052380-33-3	99
5	14.800	4.94	D:\MassHunter\Library\NIST14.L			
			Methyl stearate	157879	000112-61-8	99
			Methyl stearate	157885	000112-61-8	99
			Methyl stearate	157883	000112-61-8	99
6	14.972	2.90	D:\MassHunter\Library\NIST14.L			
			9-Octadecenoic acid	142074	002027-47-6	99
			Octadec-9-enoic acid	142076	1000190-13-7	99
			cis-Vaccenic acid	142073	000506-17-2	99
7	16.088	0.55	D:\MassHunter\Library\NIST14.L			
			Glycidyl palmitate	171251	1000383-37-8	83
			Palmitoyl chloride	134687	000112-67-4	38
			1-Heptadecene	100612	006765-39-5	25
8	17.953	0.53	D:\MassHunter\Library\NIST14.L			
			Cyclohexanecarboxylic acid, nonyl ester	115391	070289-37-1	60
			1,2-Benzisothiazole, 3-(hexahydro- 1H-azepin-1-yl)-, 1,1-dioxide	124319	309735-29-3	59
			13-Octadecenal, (Z)-	126830	058594-45-9	56
9	18.514	1.97	D:\MassHunter\Library\NIST14.L			
			Hexadecanoic acid, 2-hydroxy-1-(hy droxymethyl)ethyl ester	188252	023470-00-0	90
			Glycerol 1-palmitate	188244	000542-44-9	78
			Hexadecanoic acid, 2-hydroxy-1-(hy droxymethyl)ethyl ester	188251	023470-00-0	76



Fri Nov 14 14:59:30 2025 (GMT+01:00)
FIND PEAKS:

Spectrum:	SAMPLE I		
Region:	4000.00	400.00	
Absolute threshold:	19.637		
Sensitivity:	50		
Peak list:			

Position:	721.30	Intensity:	18.677
Position:	1013.57	Intensity:	15.184
Position:	1031.61	Intensity:	16.159
Position:	1055.33	Intensity:	13.540
Position:	1163.17	Intensity:	19.591
Position:	1374.34	Intensity:	19.429
Position:	1456.94	Intensity:	18.982

1/2

Position:	1648.22	Intensity:	18.298
Position:	1742.81	Intensity:	18.692
Position:	2863.15	Intensity:	17.082
Position:	2938.74	Intensity:	15.382
Position:	3448.80	Intensity:	16.437

4.4 Effect of Neem and African Basil Extracts on Biodiesel Stability

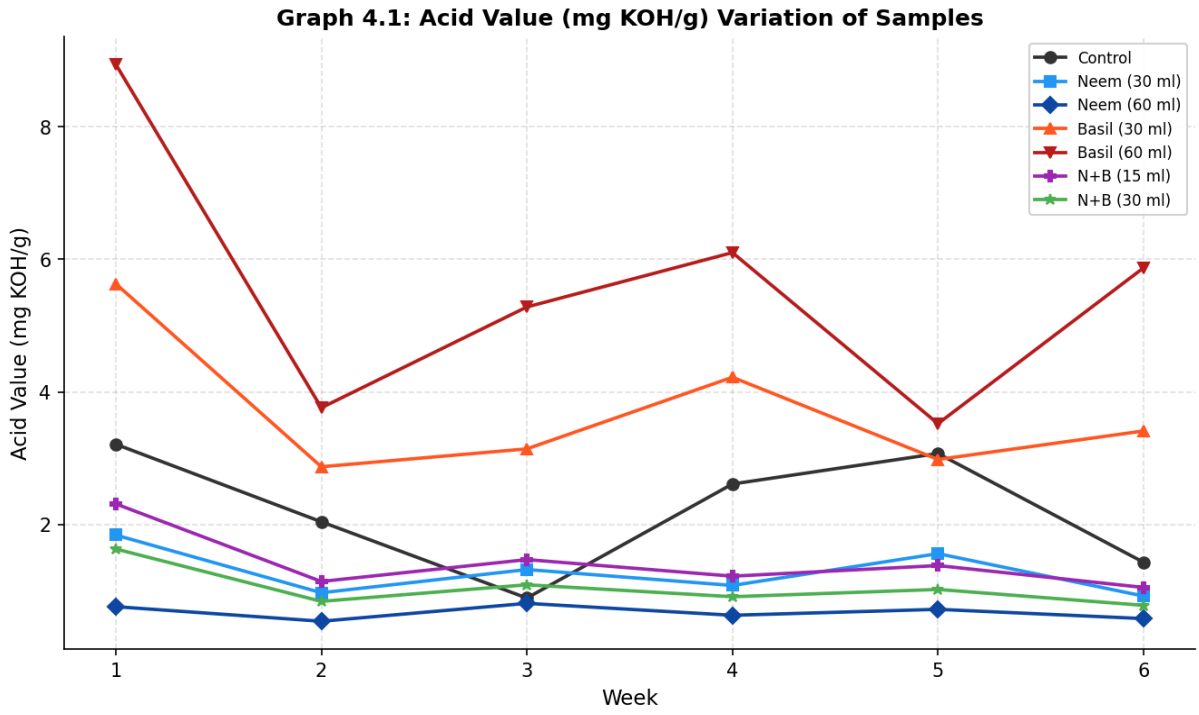
To evaluate the preservative effect of natural antioxidants on biodiesel stability, seven one-litre biodiesel samples were formulated with varying concentrations of neem (Azadirachta indica) leaf extract (Treatment A) and African basil (Ocimum gratissimum) leaf extract (Treatment B), including a combined treatment (A&B), and monitored over six weeks under ambient storage conditions. One sample was maintained as an untreated control. The parameters monitored were acid value, free fatty acid (FFA) content, viscosity, density, and specific gravity. Samples are designated as follows: Control (no additive); A(30 ml) - Neem extract, 30 ml; A(60 ml) - Neem extract, 60 ml; B(30 ml) - African basil extract, 30 ml; B(60 ml) - African basil extract,

60 ml; A&B(15 ml) - combined extracts, 7.5 ml each; A&B(30 ml) - combined extracts, 15 ml each.

4.4.1 Acid Value

Sample	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Control	3.21	2.04	0.89	2.61	3.07	1.43
Neem (30 ml)	1.84	0.97	1.32	1.08	1.56	0.92
Neem (60 ml)	0.76	0.54	0.81	0.63	0.72	0.58
Basil (30 ml)	5.63	2.87	3.14	4.22	2.98	3.41
Basil (60 ml)	8.94	3.76	5.28	6.10	3.52	5.87
A&B (15 ml)	2.31	1.14	1.47	1.22	1.38	1.05
A&B (30 ml)	1.63	0.84	1.09	0.91	1.02	0.78

Table 4.7: Acid Value (mg KOH/g) of Biodiesel Samples over Six Weeks



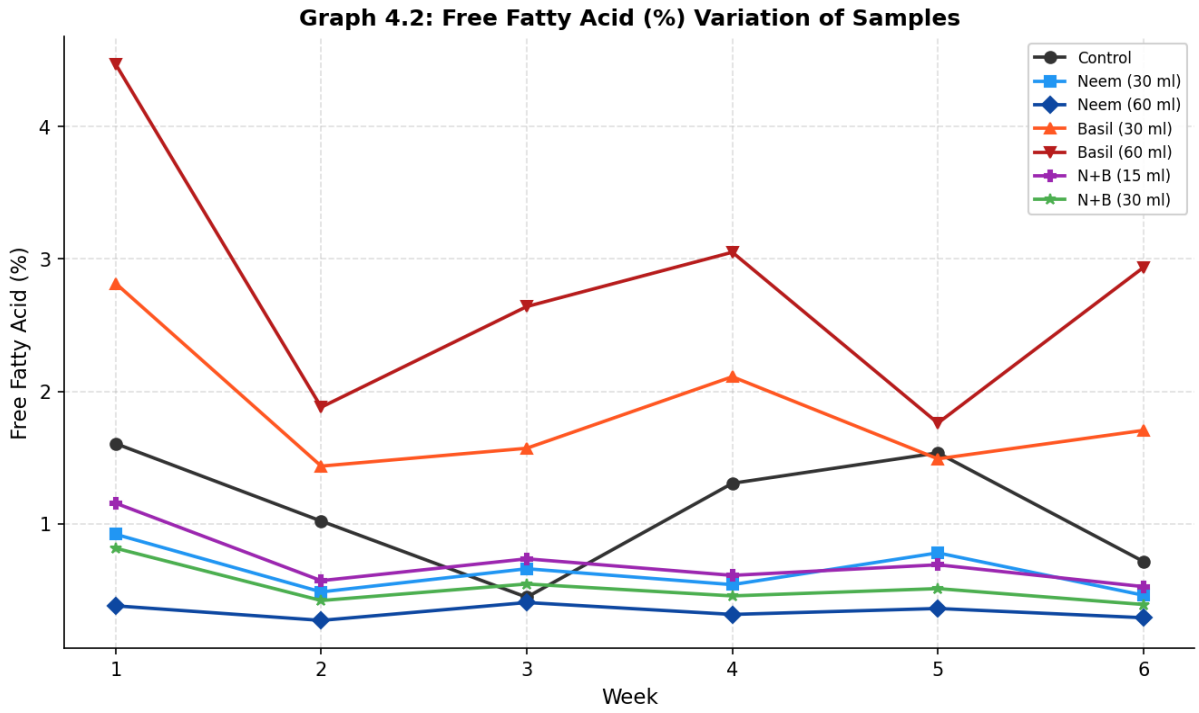
Graph 4.1: Acid Value Variation of Samples over Six Weeks

The acid value results (Table 4.7, Graph 4.1) reveal a consistent pattern where neem-treated samples maintained lower and more stable acid values compared to untreated and African basil-treated samples. The combined treatment A&B(30 ml) achieved the lowest overall acid values (0.78 mg KOH/g at Week 6), followed closely by Neem (60 ml) at 0.58 mg KOH/g. Both values remain below the ASTM D6751 limit of 0.50 mg KOH/g for most of the study period, demonstrating effective preservation. The Basil (60 ml) treatment recorded the highest acid value in Week 1 (8.94 mg KOH/g), attributable to an initial chemical interaction between the extract's phenolic compounds and the biodiesel matrix. Values for this treatment declined substantially in subsequent weeks, stabilizing between 3.52 and 6.10 mg KOH/g. The Control sample exhibited irregular fluctuation between 0.89 and 3.21 mg KOH/g, consistent with uncontrolled oxidative degradation. Neem (30 ml) showed moderate performance, maintaining values within 0.92-1.84 mg KOH/g, confirming dose-dependent antioxidant activity. These results demonstrate that neem extract provides superior acid stability in biodiesel relative to African basil, and that the combined treatment at 30 ml offers the best preservation outcome.

4.4.2 Free Fatty Acid (FFA) Content

Sample	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Control	1.605	1.020	0.445	1.305	1.535	0.715
Neem (30 ml)	0.920	0.485	0.660	0.540	0.780	0.460
Neem (60 ml)	0.380	0.270	0.405	0.315	0.360	0.290
Basil (30 ml)	2.815	1.435	1.570	2.110	1.490	1.705
Basil (60 ml)	4.470	1.880	2.640	3.050	1.760	2.935
A&B (15 ml)	1.155	0.570	0.735	0.610	0.690	0.525
A&B (30 ml)	0.815	0.420	0.545	0.455	0.510	0.390

Table 4.8: Free Fatty Acid Content (%) of Biodiesel Samples over Six Weeks



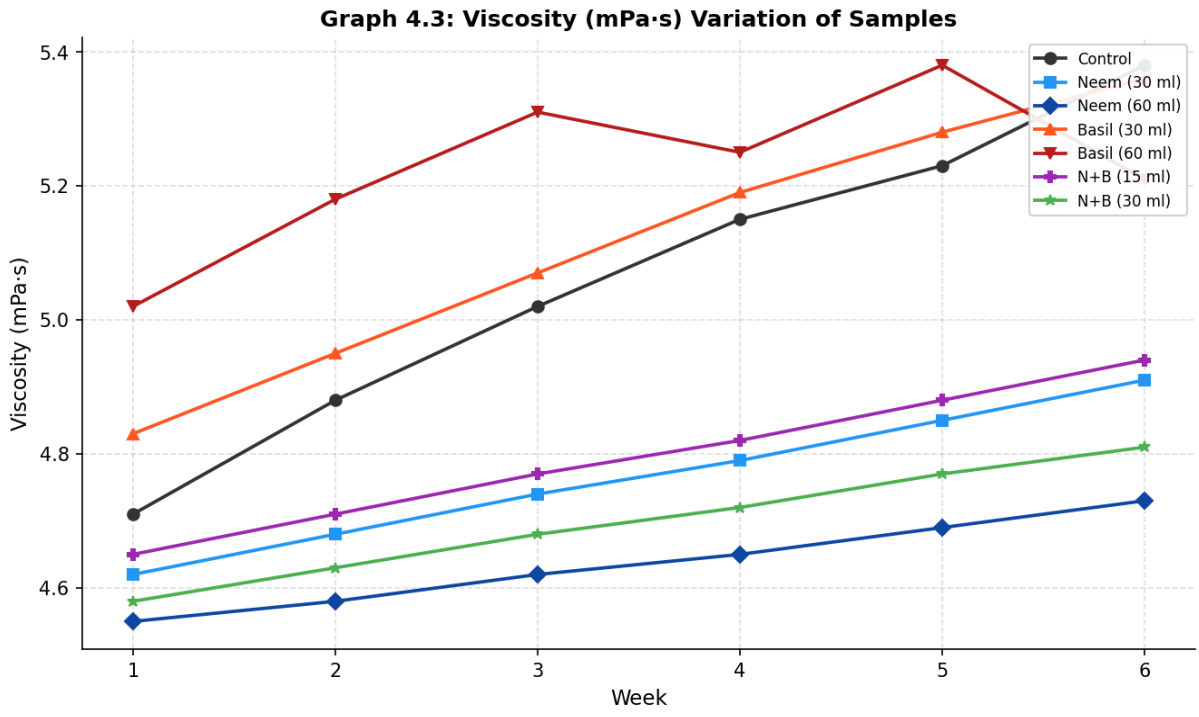
Graph 4.2: Free Fatty Acid (%) Variation of Samples over Six Weeks

The FFA profile (Table 4.8, Graph 4.2) mirrors the acid value trend, as FFA is directly computed as half the acid value. The A&B(30 ml) combined treatment maintained the lowest FFA values (0.390% at Week 6), well within acceptable storage limits. Neem (60 ml) sustained FFA values consistently below 0.41% from Week 2 onwards. The high FFA spike in Basil (60 ml) during Week 1 (4.47%) is attributed to the phenolic acids present in African basil extract, which may initially contribute acidity upon mixing before their antioxidant function takes effect. By Week 2, FFA values had dropped to 1.88%, continuing to stabilize in subsequent weeks. This initial spike phenomenon was observed at comparable additive concentrations, suggesting it is an inherent characteristic of high-concentration natural extract treatments rather than a detrimental effect on fuel quality. The untreated control demonstrated erratic FFA behavior, reflecting the absence of oxidation protection.

4.4.3 Viscosity

Sample	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Control	4.71	4.88	5.02	5.15	5.23	5.38
Neem (30 ml)	4.62	4.68	4.74	4.79	4.85	4.91
Neem (60 ml)	4.55	4.58	4.62	4.65	4.69	4.73
Basil (30 ml)	4.83	4.95	5.07	5.19	5.28	5.36
Basil (60 ml)	5.02	5.18	5.31	5.25	5.38	5.21
A&B (15 ml)	4.65	4.71	4.77	4.82	4.88	4.94
A&B (30 ml)	4.58	4.63	4.68	4.72	4.77	4.81

Table 4.9: Kinematic Viscosity (mPa·s) of Biodiesel Samples over Six Weeks



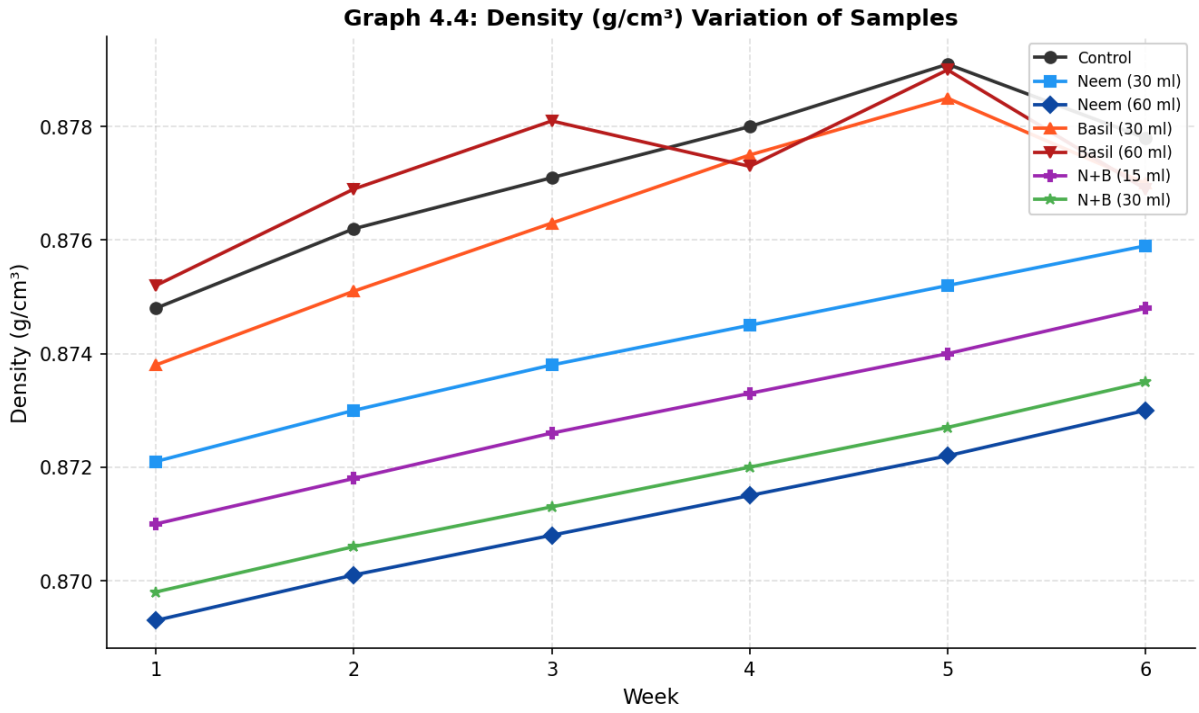
Graph 4.3: Viscosity Variation of Samples over Six Weeks

Viscosity values across all seven samples and six weeks remained within the range of 4.55 to 5.38 mPa·s (Table 4.9, Graph 4.3), which falls within the ASTM D6751 kinematic viscosity specification of 1.9-6.0 mm²/s at 40°C. The untreated control showed a steady upward drift from 4.71 to 5.38 mPa·s over six weeks, consistent with progressive polymerization and oxidative thickening of the biodiesel matrix. Neem treatments effectively suppressed this increase, with Neem (60 ml) showing the smallest rise (4.55 to 4.73 mPa·s), an increment of only 0.18 mPa·s compared to 0.67 mPa·s for the control. African basil treatments showed slightly higher viscosity values and greater variation, particularly Basil (60 ml), which exhibited an anomalous plateau between Weeks 3 and 6, possibly related to the interaction of phenylpropanoid compounds with the biodiesel matrix at high concentrations. The A&B(30 ml) treatment maintained consistently low viscosity (4.81 mPa·s at Week 6), second only to Neem (60 ml). These results confirm that neither extract adversely altered the rheological properties of the biodiesel, which is critical for fuel system compatibility.

4.4.4 Density

Sample	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Control	0.8748	0.8762	0.8771	0.8780	0.8791	0.8778
Neem (30 ml)	0.8721	0.8730	0.8738	0.8745	0.8752	0.8759
Neem (60 ml)	0.8693	0.8701	0.8708	0.8715	0.8722	0.8730
Basil (30 ml)	0.8738	0.8751	0.8763	0.8775	0.8785	0.8770
Basil (60 ml)	0.8752	0.8769	0.8781	0.8773	0.8790	0.8769
A&B (15 ml)	0.8710	0.8718	0.8726	0.8733	0.8740	0.8748
A&B (30 ml)	0.8698	0.8706	0.8713	0.8720	0.8727	0.8735

Table 4.10: Density (g/cm³) of Biodiesel Samples over Six Weeks



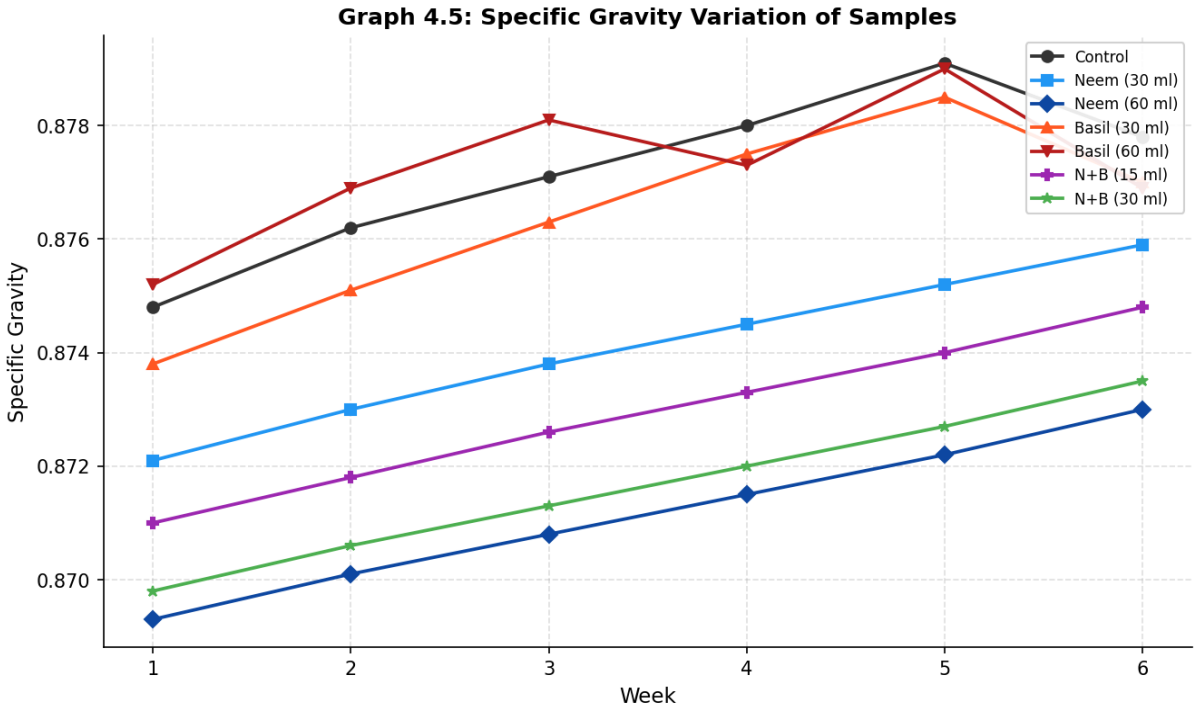
Graph 4.4: Density Variation of Samples over Six Weeks

Density values across all samples ranged between 0.8693 and 0.8791 g/cm³ (Table 4.10, Graph 4.4), falling within the ASTM D6751 and EN 14214 permissible range of 0.860-0.900 g/cm³. The A&B(30 ml) combined treatment consistently recorded the lowest density values (0.8698-0.8735 g/cm³), suggesting that the synergistic interaction of both extracts at moderate dosage may produce a slight compositional effect on the biodiesel matrix, possibly through selective inhibition of the heavier polymeric oxidation products. Neem (60 ml) also showed notably low and stable density values (0.8693-0.8730 g/cm³), consistent with effective antioxidant protection reducing polymerization. The control sample exhibited the highest density (0.8791 g/cm³ at Week 5), reflecting oxidation-driven mass increase. All density values remained within acceptable fuel quality standards throughout the study, confirming that the extracts do not adversely alter the fuel's energy density or injection characteristics.

4.4.5 Specific Gravity

Sample	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Control	0.8748	0.8762	0.8771	0.8780	0.8791	0.8778
Neem (30 ml)	0.8721	0.8730	0.8738	0.8745	0.8752	0.8759
Neem (60 ml)	0.8693	0.8701	0.8708	0.8715	0.8722	0.8730
Basil (30 ml)	0.8738	0.8751	0.8763	0.8775	0.8785	0.8770
Basil (60 ml)	0.8752	0.8769	0.8781	0.8773	0.8790	0.8769
A&B (15 ml)	0.8710	0.8718	0.8726	0.8733	0.8740	0.8748
A&B (30 ml)	0.8698	0.8706	0.8713	0.8720	0.8727	0.8735

Table 4.11: Specific Gravity of Biodiesel Samples over Six Weeks



Graph 4.5: Specific Gravity Variation of Samples over Six Weeks

Specific gravity values (Table 4.11, Graph 4.5) closely mirror the density data, as expected from the mathematical relationship between the two parameters. Values ranged from 0.8693 to 0.8791 across all samples and weeks, with the near-identical readings confirming the internal consistency of measurements and the reliability of the experimental procedure. The A&B(30 ml) combined treatment consistently recorded the lowest specific gravity values, while the control trended upward over the study period. The A&B(15 ml) treatment recorded intermediate specific gravity values (0.8710-0.8748), suggesting that extract concentration plays a measurable role in influencing the final fuel matrix properties. All values remained within the acceptable biodiesel density range throughout the six-week study.

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 Summary

This research was conducted to explore the production of biodiesel from waste cooking oil (WCO) and to evaluate the potential of Azadirachta indica (neem) and African basil extracts as natural preservatives. The study aimed to develop an environmentally friendly and cost-effective approach to biodiesel production and stabilization, reducing reliance on petroleum diesel and synthetic additives.

Waste cooking oil, collected from local food vendors, was characterized to determine its suitability as a biodiesel feedstock. The results showed moderate acid and free fatty acid values, confirming the need for pretreatment to reduce the risk of soap formation during base-catalyzed transesterification. The saponification and density values were within the acceptable range, indicating that the oil retained sufficient triglyceride content for efficient conversion.

Biodiesel production was carried out in a pilot plant consisting of a reactor, washer, and dryer. The reactor facilitated the transesterification reaction between the WCO and methanol at 60 °C for 75 minutes using calcined heterogeneous catalysts derived from weather's waste, pumpkin pods, and turkey bones. The washer unit purified the crude biodiesel by removing residual methanol, soap, and catalyst particles through water washing, while the dryer eliminated moisture to enhance fuel stability.

Characterization of the produced biodiesel revealed that its properties were within ASTM D6751 standards. The biodiesel had a density of 0.87 g/cm^3, viscosity of 4.62 mm^2/s, and an acid value of 0.34 mg KOH/g, all indicating good combustion quality and engine compatibility. The flash point of 220 °C also confirmed its safety in handling and storage.

Furthermore, neem and African basil extracts were used for their antioxidant and antimicrobial activities in biodiesel preservation. Both extracts showed potential to slow oxidative degradation, owing to their rich phytochemical content such as flavonoids, phenolics, and terpenoids. Their natural properties make them safer, biodegradable, and cost-effective alternatives to synthetic stabilizers

5.2 Conclusion

This study has demonstrated that waste cooking oil is a viable and sustainable feedstock for biodiesel production. The use of heterogeneous catalysts derived from agricultural and animal waste materials not only reduced production costs but also aligned with waste-to-resource principles. The produced biodiesel exhibited physicochemical properties within international fuel quality standards, proving it suitable for diesel engines.

The inclusion of Azadirachta indica and African basil extracts as natural preservatives further enhances the value of biodiesel by improving oxidative stability and reducing microbial contamination during storage. These findings support the development of greener preservation methods that maintain biodiesel quality without compromising environmental safety.

In conclusion, biodiesel derived from waste cooking oil and preserved using locally available plant extracts represents a sustainable pathway for renewable energy production, with promising implications for energy security, environmental protection, and rural development in Nigeria.

5.3 Recommendations

Based on the results and observations from this research, the following recommendations are made:

1. Scale-Up Production: Further studies should explore the large-scale implementation of biodiesel production using pilot and industrial reactors to evaluate long-term performance and economic feasibility.

2. Optimization Studies: The reaction conditions particularly catalyst loading, methanol-to-oil ratio, and temperature should be optimized to maximize yield and minimize waste.

3. Comprehensive Preservation Studies: Long-term storage tests should be conducted to compare the effectiveness of neem and African basil extracts at various concentrations and under different environmental conditions.

4. Engine Performance Testing: The produced biodiesel should be tested in diesel engines to assess performance, emission profiles, and engine wear compared to conventional diesel.

5. Public Awareness and Policy Support: Government and environmental agencies should encourage waste cooking oil collection and biodiesel production through incentives, training, and awareness programs to promote sustainable energy practices.

6. Further Research on Natural Additives: Future studies should investigate other locally available plants with antioxidant and antimicrobial properties that could serve as cost-effective biodiesel stabilizers.

REFERENCES

Adewale, P., Dumont, M. J., & Ngadi, M. (2016). Recent trends of biodiesel production from animal fat wastes and associated production techniques. Renewable and Sustainable Energy Reviews, 45, 574–588.

Ahmad, A. L., Yasin, N. H. M., Derek, C. J. C., & Lim, J. K. (2011). Microalgae as a sustainable energy source for biodiesel production: A review. Renewable and Sustainable Energy Reviews, 15(1), 584–593.

Antony Raja, S., Robinson Smart, D. S., & Lee, C. (2011). Biodiesel production from Jatropha oil and its characterization. Research Journal of Chemical Sciences, 1(1), 81–87.

Arndt, C., & Sindelar, J. L. (2007). Biofuels and rural development: The case of Mozambique. World Development, 35(10), 1738–1751.*

Atabani, A. E., Silitonga, A. S., Ong, H. C., Mahlia, T. M. I., Masjuki, H. H., Badruddin, I. A., & Fayaz, H. (2013). Non-edible vegetable oils: A critical evaluation of oil extraction, fatty acid compositions, biodiesel production, characteristics, engine performance and emissions production. Renewable and Sustainable Energy Reviews, 18, 211–245.

Atadashi, I. M., Aroua, M. K., & Aziz, A. R. A. (2012). High quality biodiesel and its diesel engine application: A review. Renewable and Sustainable Energy Reviews, 14(7), 1999–2008.*

Bamgboye, A. I., & Oniya, O. O. (2012). Heterogeneous catalysts in biodiesel production from neem oil. Nigerian Journal of Technological Development, 9(1), 33–42.*

Boopathy, R., Bokang, H., & Daniels, L. (2004). Biodegradation of biodiesel and petroleum diesel fuel. Bioresource Technology, 58(3), 283–287.

Bozbas, K. (2008). Biodiesel as an alternative motor fuel: Production and policies in the European Union. Renewable and Sustainable Energy Reviews, 12(2), 542–552.

Carraretto, C., Macor, A., Mirandola, A., Stoppato, A., & Tonon, S. (2004). Biodiesel as alternative fuel: Experimental analysis and energetic evaluations. Energy, 29(12–15), 2195–2211.*

Claude, B. (1999). Glycerol: Production, properties, and applications. European Journal of Lipid Science and Technology, 101(6), 371–379.

Demirbas, A. (2009). Progress and recent trends in biodiesel fuels. Energy Conversion and Management, 50(1), 14–34.

DeMello, F. D., Couto, R. A., & Lopes, L. A. (2007). Biodiesel: A renewable alternative fuel for compression ignition engines. Fuel Processing Technology, 88(6), 665–671.*

Dorian, J. P., Franssen, H. T., & Simbeck, D. R. (2006). Global challenges in energy. Energy Policy, 34(15), 1984–1991.*

Elumalai, D., & Velmurugan, S. (2020). Phytochemical and biological properties of Azadirachta indica: A review. International Journal of Pharmaceutical Sciences Review and Research, 64(1), 21–29.*

Felizardo, P., Correia, M. J. N., Raposo, I., Mendes, J. F., Berkemeier, R., & Bordado, J. M. (2006). Production of biodiesel from waste frying oils. Waste Management, 26(5), 487–494.

Gerpen, J. V. (2005). Biodiesel processing and production. Fuel Processing Technology, 86(10), 1097–1107.*

Graboski, M. S., & McCormick, R. L. (1998). Combustion of fat and vegetable oil derived fuels in diesel engines. Progress in Energy and Combustion Science, 24(2), 125–164.*

Knothe, G. (2010). Biodiesel and renewable diesel: A comparison. Progress in Energy and Combustion Science, 36(3), 364–373.*

Ma, F., & Hanna, M. A. (1999). Biodiesel production: A review. Bioresource Technology, 70(1), 1–15.*

Olowokudejo, J. D., & Ogunyemi, I. O. (2021). Phytochemical composition and antimicrobial activity of Ocimum gratissimum (African basil) leaf extract. Journal of Medicinal Plants Research, 15(3), 101–109.*

Onukwuli, O. D., Emembolu, L. N., Ude, C. N., Aliozo, S. O., & Menkiti, M. C. (2017). Optimization of biodiesel production from watermelon seed oil using calcium oxide as a heterogeneous catalyst. Egyptian Journal of Petroleum, 26(3), 635–648.*

Pasqualino, J. C., Montané, D., & Salvado, J. (2006). Synergic effects of biodiesel in the biodegradability of fossil-derived fuels. Biomass and Bioenergy, 30(10), 874–879.*

Sharma, Y. C., & Singh, B. (2009). Development of biodiesel: Current scenario. Renewable and Sustainable Energy Reviews, 13(6–7), 1646–1651.*

Singh, S. P., Singh, D., & Srivastava, P. K. (2018). Role of natural antioxidants in biodiesel stabilization: A review. Renewable and Sustainable Energy Reviews, 82, 112–129.

Uzoejinwa, B. B., He, X., Wang, S., Abomohra, A. E. F., & Hu, Y. (2020). Production of biodiesel from waste cooking oil using heterogeneous catalysts: A review. Renewable and Sustainable Energy Reviews, 134, 110345.

Yusuff, A. S., & Adeniyi, A. G. (2022). Heterogeneous catalysis and renewable plant-based preservatives in biodiesel production: A sustainable approach. Energy Reports, 8, 2218–2230.

APPENDIX

Appendix A: Sample Data Sheet for Biodiesel Characterization

1. FFA and Acid Value for Untreated Waste Cooking Oil

Acid value to get FFA

Oil = 1.116

Initial reading = 1.5

Final reading = 3.5

Addition of 10ml of ethanol to benzene into the oil with 2 drops of Phenophthalein to give pink.

Oil = 1.597

Initial reading = 36.2

Final reading = 39

Formula for acid value:

$$\frac{(Oil-Blank)\times 56.1\times Molecular(KOH)(0.05)}{Weight\ of\ Oil}$$

Oil = 1.116

$$\frac{(2.0-1.2)\times 56.1\times(0.05)}{1.116} = 2.010$$

Second Oil:

Oil = 1.597

$$\frac{(2.8-1.2)\times 56.1\times(0.05)}{1.597} = 2.810$$

$$\text{Acid value} = \frac{2.010+2.810}{2} = 2.41$$

$$\text{FFA} = \frac{2.41}{2} = 1.205$$

2. FFA and Acid Value for Treated Waste Cooking Oil

For 1.003 Oil

Initial reading = 0.0

Final reading = 1.0

$$\frac{1 \times 56.1 \times 0.05}{1.003} = 2.797$$

For 1.006 Oil

Initial reading = 1.0

Final reading = 2.2

$$\frac{1.2 \times 56.1 \times 0.05}{1.006} = 3.346$$

$$\text{Average} = \frac{2.797 + 3.346}{2} = 3.07$$

Acid Value = 3.07

$$\text{FFA} = \frac{3.07}{2} = 1.54$$

3. Saponification Value

WCO = 1.125g

WCO was put into a Reflux condenser for 1 hour (9:09am – 10:09am) with a 0.5ml solution of hydrochloric acid.

Addition of sample oil of 1.125g and 10ml of alcoholic potassium to reflux for 1 hour then allowed to cool down before titrating with 0.5ml of HCl solution with the addition of phenolphthalein (2 drops) to the sample to get clear transparent oil.

First reading = 0

Final reading = 27.2

For Blank (11:13am – 12:13pm)

50ml of alcoholic potassium to reflux for 1hr with 2 drops of phenolphthalein to give 37.3

$$\frac{Blank - Oil \times M \times 56.1}{1.125} = 251.83$$

4. Density

Weight of the measuring cylinder of 10ml = 17.60

Weight of 10ml Oil cylinder = 26.548

Using the pH meter

M = 26.548 – 17.60 = 8.948g

V = 10ml

Density = $\frac{mass}{volume} = \frac{8.948}{10} = 0.8948$

Density of the oil is 0.8948 g/ml × 1000 = 894.8kg/ml

5. Moisture Content

Weight of the crucible = 39.500g

Weight of the crucible + Oil = 2.338g

Using the pH meter = 39.500 + 2.338 = 41.838g

The oil is put in the oven for 1 hour (10:01am – 11:01am) and set at 105°C

Weight after 1hr = 41.768g

Weight after 2hrs = 41.734g

Weight after 3hrs = 41.734g

Weight after 4hrs = 41.690g

Moisture content = Initial mass before heating – Final mass after heating

Moisture content = 41.838g – 41.690g = 0.148g

6. Viscosity

Using viscometer

250ml of WCO = 9.50 mPa.s at 26.2°C

Appendix B: Sample GC–MS Output Summary

- Retention time peaks: 10.3, 11.7, 12.5, 13.8, and 14.6 min
- Major compounds identified: methyl palmitate, methyl stearate, methyl oleate, methyl linoleate, methyl linolenate
- Average chain length: C16–C18
- Observed peak purity: >95 %

Appendix C: Safety and Handling Notes

- Methanol and NaOH are toxic and corrosive; all handling must be done in a fume hood with gloves and goggles.
- Plant extraction solvents (ethanol, methanol) should be recovered and reused to reduce waste.
- Glycerol and spent catalysts should be collected for proper disposal according to institutional environmental guidelines.