PRODUCTION OF BIODIESEL FROM WASTE COOKING OIL (WCO) USING COW BONE AS CATALYST

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CERTIFICATION

This is to certify that this research work was carried out by ILESANMI PETER OLUWADAMILARE with matriculation number ENG2006145 in the Department of Chemical Engineering, University of Benin, Benin City, Edo State, Nigeria.

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DEDICATION

I dedicate this research project to the Lord, God Almighty, for giving me the life, the opportunity, and the grace to successfully accomplish this study.

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My sincere gratitude goes to God Almighty for granting me the grace and strength to have completed my research project.

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ABSTRACT

This study focuses on the production of biodiesel from waste cooking oil (WCO) using calcined cow bone as a heterogeneous catalyst through the transesterification process. The research aimed to promote sustainable energy production by converting waste oils into biodiesel while utilizing animal bone waste as a low-cost, environmentally friendly catalyst. The WCO was pretreated and characterized to determine its physiochemical properties, which included an acid value of 1.4025 mg KOH/g, free fatty acid (FFA) content of 0.7012%, peroxide value of 16 meq/kg, iodine value of 44.1 g I_2/100 g, viscosity at 40 °C of 53.5 cP, saponification value of 362.667 mg KOH/g, moisture content of 2.678%, and density of 0.9176 g/cm^3. These results confirmed that the feedstock required pretreatment before transesterification to minimize soap formation and enhance biodiesel yield.

Characterization of the catalyst was performed using analytical techniques such as X-ray fluorescence (XRF), Brunauer–Emmett–Teller (BET) surface area analysis, and Fourier transform infrared spectroscopy (FTIR) to confirm the presence of CaO and evaluate its surface properties.The transesterification reaction was carried out using methanol and cow bone-derived catalyst under optimized conditions. The resulting biodiesel was washed, purified, and analyzed for key physiochemical properties. The biodiesel exhibited an acid value of 0.561 mg KOH/g, density of 0.901 g/cm^3, viscosity at 40 °C of 8.86 cP, and a flash point of 115 °C. These results were within acceptable limits prescribed by ASTM D6751 and EN 14214 standards, indicating that the produced biodiesel possesses good fuel properties suitable for use in diesel engines.

The study concludes that waste cooking oil can serve as an efficient feedstock for biodiesel production, and cow bone ash is a promising, sustainable, and economical catalyst. This dual utilization of waste materials not only reduces environmental pollution but also supports circular economy practices and sustainable energy development.

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NOMENCLATURE

WCB- Waste Cooking Oil Biodiesel

AV- Acid Value

FFA- Free Fatty Acid

FAME- Fatty Acid Methyl Ester

CaCO3- Calcium Carbonate

WCO- Waste cooking oil

NaOH- Sodium Hydroxide

KOH-Potassium Hydroxide

HCL-Hydrogen Chloride

H2SO4- Sulphuric Acid

FTIR- Fourier Transform Infrared Spectroscopy

SEM- Scanning Electron Microscopy

BET- Brunauer-Emmett-Teller

XRF- X-ray Fluorescence

MC- Moisture Content

MW- Molecular Weight

IV = Iodine value

SV = Saponification Value

CHAPTER 1

INTRODUCTION

1.1 Background Of Study

The global demand for energy has grown rapidly over the years as a result of population increase, industrial expansion, and technological advancement. Fossil fuels such as petroleum, natural gas, and coal currently serve as the dominant sources of energy worldwide; however, their non-renewable nature and negative environmental impacts have raised serious concerns about sustainability. The excessive use of these fuels contributes significantly to greenhouse gas emissions, global warming, and air pollution, which are major drivers of climate change (Amenaghawon et al., 2021). Furthermore, the depletion of fossil fuel reserves and the volatility of global crude oil prices have intensified the search for alternative, renewable, and eco-friendly sources of energy.

Among the various alternatives being explored, biodiesel has emerged as a promising substitute for conventional diesel fuel. Biodiesel is a renewable, biodegradable, and non-toxic fuel derived from biological sources such as vegetable oils, animal fats, or waste oils. It mainly consists of fatty acid methyl esters (FAME), which are produced through a transesterification reaction between triglycerides and short-chain alcohols—most commonly methanol—in the presence of a catalyst (Musa et al., 2022). Compared to petroleum-based diesel, biodiesel has several advantages, including reduced emissions of carbon monoxide, hydrocarbons, sulfur oxides, and particulate matter, as well as improved lubricity and higher flash point.

Despite these advantages, the commercial production of biodiesel faces economic challenges, largely due to the high cost of virgin vegetable oils used as feedstock. Feedstock cost accounts for up to 70–80% of the total production cost (Onoja et al., 2021). Moreover, the use of edible oils such as soybean, palm, and sunflower oil for fuel production creates competition with food supply, leading to the widely debated "food versus fuel" issue. To overcome these challenges, researchers have shifted their attention to low-cost and non-edible feedstocks such as waste cooking oil (WCO), animal fats, and other residual oils.

Waste cooking oil, generated from domestic and commercial frying operations, represents a readily available and inexpensive feedstock for biodiesel production. Its utilization not only reduces the cost of production but also helps to address environmental problems associated with its improper disposal. When discharged into water bodies or drainage systems, WCO causes blockages, pollution, and ecosystem disruption. Converting it into biodiesel, therefore, presents an environmentally sustainable way to manage waste while providing a renewable source of energy (Osagie et al., 2023).

However, the use of WCO in biodiesel production poses certain technical challenges. Due to repeated heating during cooking, waste oil typically contains high amounts of free fatty acids (FFAs), moisture, and other impurities. These contaminants react with alkaline catalysts such as sodium hydroxide (NaOH) or potassium hydroxide (KOH), forming soap that hinders the separation of biodiesel from glycerol, thereby reducing yield (Amenaghawon et al., 2022). To overcome these drawbacks, the use of heterogeneous catalysts has gained significant attention because they can be easily separated from the reaction mixture, reused multiple times, and have minimal environmental impact.

Among various solid catalysts, calcium oxide (CaO) is one of the most effective and commonly used in biodiesel production. Interestingly, calcium oxide can be synthesized from natural waste materials rich in calcium compounds—such as animal bones, shells, and eggshells—making the process both economical and sustainable. Cow bones, in particular, are an abundant waste material from abattoirs and meat processing industries. They contain calcium phosphate (hydroxyapatite), which can be thermally decomposed to calcium oxide when calcined at high temperatures (Aisien et al., 2023). Utilizing cow bones as a source of catalyst not only promotes resource recovery but also contributes to environmental cleanliness by reducing abattoir waste accumulation.

Therefore, this study explores the production of biodiesel from waste cooking oil using cow bone–derived calcium oxide as a heterogeneous catalyst. The research aims to evaluate the physicochemical properties of the waste cooking oil and the synthesized catalyst, investigate the transesterification process, and assess the fuel properties of the produced biodiesel in comparison with standard specifications. This approach aligns with global efforts toward sustainable energy generation, environmental protection, and circular economy practices by converting waste materials into valuable resources.

1.2 Problem Statement

The increasing global energy demand, coupled with the declining reserves of fossil fuels, has created an urgent need for sustainable and renewable alternatives. Despite the proven potential of biodiesel as a viable substitute for petroleum diesel, its large-scale production remains economically constrained due to the high cost of refined vegetable oils commonly used as feedstock. In many cases, the cost of feedstock alone accounts for more than 70% of the total production cost, making biodiesel less competitive with fossil fuels (Onoja et al., 2021). Furthermore, reliance on edible oils for biodiesel production aggravates the “food versus fuel” debate, posing a threat to food security, particularly in developing countries like Nigeria.

Waste cooking oil (WCO) presents a promising low-cost alternative feedstock for biodiesel production. It is abundantly available from domestic and industrial cooking activities and, if not properly managed, contributes to environmental pollution through improper disposal into drainage systems and water bodies (Osagie et al., 2023). However, the direct use of WCO in biodiesel production poses significant technical challenges. Repeated heating during frying increases the level of free fatty acids (FFAs) and impurities in the oil, which interfere with the transesterification process. When conventional homogeneous base catalysts such as NaOH or KOH are used, these FFAs react to form soap, which complicates phase separation and reduces biodiesel yield (Amenaghawon et al., 2022).

Additionally, homogeneous catalysts present environmental and operational drawbacks, including the generation of wastewater, corrosion of equipment, and difficulties in catalyst recovery and reuse. These limitations have prompted the exploration of solid (heterogeneous) catalysts that are reusable, environmentally benign, and more efficient in converting waste oils into biodiesel.

Cow bones, which are commonly discarded as abattoir waste, contain calcium phosphate (hydroxyapatite) that can be converted into calcium oxide (CaO) upon calcination. Calcium oxide is a strong basic catalyst suitable for transesterification reactions. Utilizing cow bone as a catalyst source not only provides a low-cost and effective catalytic material but also contributes to sustainable waste management by converting animal bone waste into a value-added product (Aisien et al., 2023).

Despite these potentials, there is still limited data on the effectiveness of cow bone–derived catalysts in converting waste cooking oil into biodiesel under locally optimized conditions. Therefore, this study seeks to investigate the feasibility of using calcined cow bone as a heterogeneous catalyst for biodiesel production from waste cooking oil. The work aims to address challenges related to catalyst cost, waste management, and process efficiency, thereby contributing to the development of a cleaner, more sustainable, and economically viable biodiesel production process in Nigeria.

1.3 Aim and Objectives of Study

The main aim of this study is to produce biodiesel from waste cooking oil using a heterogeneous catalyst derived from cow bone, with the goal of developing a sustainable, cost-effective, and environmentally friendly alternative to conventional biodiesel production methods.

Objectives

To achieve this aim, the following specific objectives were established:

1. To collect and pretreat waste cooking oil to remove impurities, moisture, and solid particles that may interfere with the transesterification process.
2. To prepare a heterogeneous catalyst from cow bone by subjecting it to cleaning, drying, calcination, and characterization to determine its composition and catalytic properties.

3. To carry out transesterification of waste cooking oil using the cow bone–derived catalyst under varying reaction parameters such as temperature, catalyst loading, and methanol-to-oil ratio.
4. To evaluate the yield and quality of the biodiesel produced based on physicochemical properties (such as density, viscosity, flash point, and acid value) and compare the results with ASTM or EN standards for biodiesel.
5. To assess the reusability and stability of the cow bone catalyst after multiple reaction cycles to determine its economic and environmental viability.

Through these objectives, the study aims to contribute to ongoing research in renewable energy, waste-to-wealth conversion, and sustainable catalysis, particularly in the context of biodiesel production using readily available local materials.

1.4 Scope Of Study

This research focuses on the production of biodiesel from waste cooking oil (WCO) using a heterogeneous catalyst derived from cow bone. The scope of the study is limited to the laboratory-scale production and characterization of both the catalyst and the resulting biodiesel.

The study covers the collection and pretreatment of waste cooking oil, including the removal of impurities and determination of its physicochemical properties such as acid value, saponification value, iodine value, moisture content, and free fatty acid content. It also includes the preparation of cow bone catalyst, involving cleaning, drying, calcination, and subsequent characterization using analytical techniques such as Scanning Electron Microscopy (SEM), Fourier Transform

Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD), and Brunauer–Emmett–Teller (BET) surface area analysis.

Additionally, the study investigates the transesterification process parameter, including methanol-to-oil ratio, catalyst loading, and reaction temperature, to determine the optimal conditions for biodiesel production. The resulting biodiesel is analyzed to evaluate its fuel properties, such as viscosity, density, flash point, and yield, in comparison with standard specifications (ASTM D6751 and EN 14214).

However, the study does not cover the large-scale industrial application of the process or the economic feasibility analysis of full-scale biodiesel production. Also, other potential waste materials for catalyst development or oil feedstocks are not investigated within this work.

This scope ensures that the research remains focused on assessing the catalytic performance of cow bone and its potential for sustainable biodiesel production from readily available waste resources.

1.5 Methodology

The methodology adopted in this research involves a systematic experimental approach designed to produce biodiesel from waste cooking oil (WCO) using cow bone as a heterogeneous catalyst. The process was divided into three major phases: preparation and characterization of feedstock (WCO), preparation and characterization of the catalyst (cow bone), and the transesterification reaction for biodiesel production.

In the first phase, the waste cooking oil was collected from a buka in UNIBEN, filtered to remove impurities, and heated to eliminate moisture. Its physicochemical properties such as acid value, saponification value, iodine value, viscosity, density, moisture content, and peroxide value were determined using standard ASTM and AOAC methods.

In the second phase, cow bone was sourced, cleaned, sun-dried, and calcined in a muffle furnace at controlled temperatures to produce a solid catalyst. The catalyst was then pulverized, sieved, and characterized using analytical techniques including Scanning Electron Microscopy (SEM) for surface morphology, Fourier Transform Infrared Spectroscopy (FTIR) for identifying functional groups, X-Ray Diffraction (XRD) for crystallinity, X-Ray Fluorescence (XRF) for elemental composition, and Brunauer–Emmett–Teller (BET) analysis for surface area and porosity.

In the final phase, transesterification of the pretreated waste cooking oil was carried out using methanol as the alcohol and the prepared cow bone catalyst. The effects of various process parameters such as methanol-to-oil molar ratio, catalyst loading, reaction temperature, and reaction time were studied to determine the optimal conditions for maximum biodiesel yield. The resulting biodiesel was then analyzed to determine its fuel properties in comparison with international biodiesel standards (ASTM D6751 and EN 14214).

1.6 Relevance Of Study

The relevance of this study lies in its contribution to sustainable energy production, environmental management, and resource recovery. As global dependence on fossil fuels continues to pose economic and environmental challenges, the need for alternative, renewable, and eco-friendly fuels has become urgent. Biodiesel, being biodegradable and carbon-neutral, offers a promising substitute for petroleum-based diesel. However, the high cost of virgin oils and conventional catalysts limits its large-scale adoption.

This research addresses these challenges by utilizing waste cooking oil (WCO) — a cheap, abundant, and environmentally problematic waste — as the primary feedstock. By converting WCO into biodiesel, the study not only promotes renewable energy production but also contributes to waste reduction and pollution control, as improper disposal of used oil can contaminate soil and water bodies.

In addition, the study employs cow bone, an agricultural and slaughterhouse waste, as a heterogeneous catalyst. This approach provides a cost-effective and sustainable catalyst alternative to conventional chemical catalysts such as sodium hydroxide and potassium hydroxide, which are costly and difficult to recover. The use of waste bone material not only adds value to animal by-products but also aligns with the principles of the circular economy by transforming waste into valuable resources.

Furthermore, the study's findings could benefit developing countries like Nigeria, where both waste cooking oil and animal bones are readily available. The research supports the development of localized biodiesel production technologies that reduce dependence on imported fuels and create opportunities for small-scale energy enterprises.

Overall, this study is relevant for researchers, policymakers, and industries seeking to promote sustainable energy solutions, reduce environmental impact, and enhance the economic viability of biodiesel production using low-cost and eco-friendly materials.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction to Biodiesel

2.1.1 Definition and Importance of Biodiesel

Biodiesel is a renewable and biodegradable fuel composed primarily of fatty acid methyl esters (FAME) or fatty acid ethyl esters (FAEE), produced through a chemical process known as transesterification, in which triglycerides from vegetable oils or animal fats react with an alcohol (usually methanol or ethanol) in the presence of a catalyst (Musa et al., 2022). It has emerged as a sustainable alternative to petroleum diesel due to its renewable origin, environmental benefits, and compatibility with existing diesel engines (Amenaghawon et al., 2022). Biodiesel can be used either in its pure form (B100) or blended with conventional diesel fuel in varying proportions (such as B5, B10, or B20) without requiring major engine modifications (Osagie et al., 2023).

The global reliance on fossil fuels for energy generation has contributed to significant environmental degradation and economic vulnerability. Current estimates indicate that fossil fuels account for approximately 80% of the world’s total primary energy consumption, with petroleum-based fuels dominating the transportation sector (IEA, 2023). However, global petroleum reserves are depleting rapidly, and projections suggest that conventional crude oil resources could become economically unsustainable within the next few decades

(Uhunmwangho et al., 2021). Consequently, there is an urgent need for alternative fuels that are both sustainable and environmentally benign. Biodiesel, derived from renewable feedstocks such as vegetable oils, waste cooking oil, and animal fats, plays a crucial role in addressing this challenge by contributing to energy diversification, energy security, and rural economic development (Amenaghawon et al., 2022).

2.1.2 Advantages of Biodiesel Over Petroleum Diesel

Biodiesel offers several technical, environmental, and practical advantages over petroleum-based diesel fuel. From a technical standpoint, biodiesel possesses a higher cetane number, which improves ignition quality and combustion efficiency (Ulukardesler et al., 2023). It also exhibits superior lubricity, which reduces friction and wear in engine components, thereby extending engine life (Bello et al., 2020). Moreover, biodiesel has a higher flash point (typically above 130°C), making it safer to handle and store compared to conventional diesel fuel, which has a flash point of about 52°C (Osagie et al., 2023). Additionally, biodiesel contains no sulfur and a higher oxygen content (10–12%), which enhances combustion efficiency and reduces smoke emissions (Musa et al., 2022).

Environmentally, biodiesel significantly reduces emissions of harmful pollutants. Compared to petroleum diesel, biodiesel reduces carbon monoxide (CO) emissions by approximately 50%, unburned hydrocarbons by 67%, and particulate matter by 47% (Basha et al., 2021). The most significant advantage lies in its reduction of net carbon dioxide (CO_2) emissions, as the carbon released during combustion is offset by the CO_2 absorbed during the growth of the feedstock, thereby creating a nearly carbon-neutral cycle (Amenaghawon et al., 2022). Biodiesel is also biodegradable, non-toxic, and less harmful to aquatic life, making it a more sustainable option in the event of spills or leaks (Osagie et al., 2023).

From a practical perspective, biodiesel's compatibility with existing diesel engines is a key advantage. It can be blended in different proportions with a petrodiesel without requiring substantial engine modifications. The use of blends such as B20 (20% biodiesel and 80% diesel) has been widely adopted in many countries due to its balance of performance, cost-effectiveness, and emission reduction benefits (Uhunmwangho et al., 2021). Additionally, biodiesel production can be localized, reducing dependence on imported fuels and stimulating local agricultural and industrial activities (Amenaghawon et al., 2022).

2.1.3 Global Energy Crisis and the Need for Renewable Energy

The world's energy demand continues to rise due to rapid industrialization, population growth, and economic expansion, particularly in developing nations. Fossil fuels, although efficient, contribute heavily to global greenhouse gas emissions and climate change. According to the International Energy Agency (IEA, 2023), the transportation sector alone accounts for about 25% of global CO_2 emissions. This has led to increased advocacy for renewable energy sources that can mitigate the effects of climate change while ensuring energy sustainability.

In response to this global challenge, many governments have implemented policies promoting biofuels, including biodiesel, as part of their renewable energy strategies. For instance, the European Union's Renewable Energy Directive (RED II) mandates that at least 14% of energy consumed in the transport sector should come from renewable sources by 2030 (EU, 2023).

Similarly, the United States Renewable Fuel Standard (RFS) promotes biodiesel blending mandates to reduce greenhouse gas emissions and dependence on imported oil (DOE, 2022). In developing countries such as Nigeria, the pursuit of biodiesel production aligns with efforts to diversify the economy and enhance energy self-sufficiency (Amenaghawon et al., 2022).

2.1.4 Environmental Benefits of Biodiesel

Biodiesel offers considerable environmental benefits compared to conventional diesel. Studies have shown that biodiesel can reduce overall greenhouse gas emissions by 41–78% depending on the feedstock and production process (Basha et al., 2021). Furthermore, it emits negligible amounts of sulfur oxides (SO_x), thereby reducing acid rain formation and improving air quality (Osagie et al., 2023). The combustion of biodiesel also leads to lower emissions of particulates, hydrocarbons, and carbon monoxide, all of which are major contributors to urban air pollution (Uhunmwangho et al., 2021).

Another environmental advantage of biodiesel lies in its biodegradability and low toxicity. In the event of a spill, biodiesel degrades about four times faster than petroleum diesel, minimizing environmental hazards (Musa et al., 2022). Moreover, the use of waste cooking oil or animal fat as feedstock contributes to the circular economy by converting waste materials into valuable energy resources while reducing the environmental burden of improper disposal (Amenaghawon et al., 2022).

2.1.5 Biodiesel Standards and Specifications

To ensure quality and performance, biodiesel must meet established international standards that define acceptable physicochemical properties. The two most widely recognized standards are ASTM D6751 (used in the United States) and EN 14214 (used in Europe) (Bello et al., 2020).

These standards specify limits for parameters such as ester content, density, viscosity, flash point, acid value, sulfur content, iodine value, water content, and oxidation stability. Adherence to these specifications ensures that biodiesel is compatible with diesel engines, maintains fuel system integrity, and minimizes operational issues such as filter plugging and injector deposits (Osagie et al., 2023).

The ASTM D6751 standard, for example, requires biodiesel to have a minimum ester content of 96.5%, a kinematic viscosity between 1.9–6.0 mm^2/s at 40°C, and a flash point above 130°C (DOE, 2022). These parameters directly affect the fuel's ignition, combustion, and storage characteristics. Compliance with such standards is critical for the commercialization and large-scale adoption of biodiesel as a reliable alternative to fossil diesel.

2.1.6 Transition to Feedstock and Catalyst Importance

Despite its numerous advantages, the cost and availability of suitable feedstocks remain major challenges in biodiesel production. The choice of feedstock significantly influences the cost, yield, and quality of biodiesel. Waste cooking oil (WCO) has gained attention as a low-cost and environmentally friendly feedstock, offering the dual benefits of waste valorization and sustainable fuel production (Amenaghawon et al., 2022). Likewise, the selection of an appropriate catalyst—especially heterogeneous catalysts derived from waste materials such as cow bone—can enhance the process efficiency, reduce purification costs, and improve catalyst reusability (Musa et al., 2023). These aspects form the foundation for subsequent discussions in this research.

2.2 Feedstocks for Biodiesel Production

2.2.1 Classification of Feedstocks

The choice of feedstock is one of the most critical factors determining the overall efficiency, cost-effectiveness, and sustainability of biodiesel production. Feedstock typically accounts for 70–95% of the total production cost, making its selection a key driver of biodiesel economics (Musa et al., 2022; Osagie et al., 2023). In addition to cost, feedstock availability, fatty acid composition, free fatty acid (FFA) content, and environmental impact play major roles in determining the quality and yield of biodiesel produced (Amenaghawon et al., 2022). To streamline research and industrial applications, biodiesel feedstocks are generally classified into three main generations based on their origin, sustainability, and relationship with food supply: first generation (edible oils), second generation (non-edible oils and waste materials), and third generation (microalgae and other advanced sources) (Bello et al., 2020).

First-Generation Feedstocks (Edible Oils)

First-generation feedstocks consist mainly of edible vegetable oils such as soybean oil, palm oil, sunflower oil, rapeseed (canola) oil, and coconut oil (Uhunmwangho et al., 2021). These oils are the most widely used feedstocks for biodiesel production, especially in industrialized countries where large-scale agricultural systems exist. Their major advantages include low FFA content, well-established supply chains, and ease of processing due to their chemical uniformity (Amenaghawon et al., 2022).

However, the use of edible oils for biodiesel has raised serious ethical, economic, and environmental concerns, particularly the “food versus fuel” debate, which questions the morality of diverting food resources for fuel production while millions face food insecurity (Osagie et al., 2023). The large-scale cultivation of feedstocks such as palm oil and soybean oil has also contributed to deforestation and biodiversity loss, particularly in regions like Southeast Asia and South America (FAO, 2023). In economic terms, edible oils are expensive, and their prices fluctuate with global food markets, which limits the competitiveness of biodiesel compared to fossil diesel (Ulukardesler et al., 2023).

Globally, palm oil and soybean oil remain the dominant feedstocks. Palm oil accounts for approximately 36% of global biodiesel feedstock, followed by soybean oil at around 28%, with Europe preferring rapeseed oil and the United States favoring soybean oil due to regional availability (IEA, 2023). Although these feedstocks produce high-quality biodiesel with excellent combustion characteristics, their sustainability issues have prompted a shift toward second- and third-generation alternatives (Basha et al., 2021).

Second-Generation Feedstocks (Non-Edible Oils and Waste Materials)

Second-generation feedstocks comprise non-edible oils such as Jatropha curcas, Pongamia pinnata (karanja), castor oil, rubber seed oil, neem oil, and various waste materials including waste cooking oil (WCO) and animal fats (Amenaghawon et al., 2022; Musa et al., 2023). These feedstocks are gaining increasing attention due to their non-competition with food supply, lower cost, and environmental sustainability.

Non-edible oils such as jatropha and karanja are particularly attractive because they can grow on marginal or non-arable lands, thereby avoiding competition with food crops (Uhunmwangho et al., 2021). They also possess relatively high oil content (30–45%), making them viable raw materials for biodiesel production (Osagie et al., 2023). However, these oils often contain higher amounts of FFAs compared to edible oils, necessitating pretreatment steps before transesterification (Musa et al., 2022).

Among all second-generation sources, waste cooking oil and animal fats stand out for their dual environmental and economic benefits. WCO, in particular, offers a sustainable approach to waste valorization converting a problematic waste product into a valuable fuel (Bello et al., 2020). The utilization of such materials not only reduces production costs but also minimizes environmental pollution associated with improper waste disposal (Amenaghawon et al., 2022). However, variability in composition and high impurity content remain challenges to its large-scale application (Osagie et al., 2023).

Third-Generation Feedstocks (Microalgae and Cyanobacteria)

Third-generation feedstocks include microalgae, cyanobacteria, and other photosynthetic microorganisms, which are considered the most promising long-term solution for sustainable biodiesel production. Microalgae can produce 10 to 20 times more oil per unit area than terrestrial crops and can grow in saline or wastewater environments, eliminating competition with agriculture for freshwater and arable land (Basha et al., 2021). Moreover, microalgae cultivation contributes to CO_2 sequestration, further reducing greenhouse gas emissions (Musa et al., 2022).

Despite their advantages, large-scale algal biodiesel production remains economically challenging due to high cultivation, harvesting, and oil extraction costs. Technological barriers such as maintaining optimal growth conditions and preventing contamination also hinder commercialization (Uhunmwangho et al., 2021). Nevertheless, continuous advancements in biotechnology, genetic engineering, and photobioreactor design are gradually improving the economic feasibility of algal biofuel systems, suggesting significant future potential (Osagie et al., 2023).

Transition to Waste Cooking Oil as Feedstock

While first-generation feedstocks laid the foundation for biodiesel commercialization, their economic and ethical limitations have intensified the search for sustainable, low-cost alternatives. Second-generation feedstocks, especially waste cooking oil, have emerged as promising candidates due to their abundance, low cost, and minimal environmental footprint (Amenaghawon et al., 2022). The use of WCO not only addresses waste disposal challenges but also supports a circular economy by transforming used oil into clean energy. The following section discusses in detail the characteristics, advantages, challenges, and processing considerations of waste cooking oil as a biodiesel feedstock.

2.3 Waste Cooking Oil (WCO) as Feedstock for Biodiesel Production

Waste cooking oil (WCO) — the used frying oil collected from households, restaurants and food-processing outlets — has emerged as one of the most attractive feedstocks for sustainable biodiesel production because it couples low cost with waste valorization and measurable environmental benefits. (Goh, 2020)

2.3.1 Definition, sources and rationale for use

WCO comprises vegetable oils and animal fats that have been thermally and chemically altered by repeated frying cycles; it typically contains triglycerides, partial glycerides, free fatty acids (FFAs), oxidation products, polymerized species and suspended food residues. (Goh, 2020)

Sources include domestic kitchens, fast-food restaurants, institutional canteens and industrial frying operations, which together generate large and geographically distributed streams of WCO that are often underused or improperly discarded. (Jeyaseelan, 2023)

Using WCO for biodiesel addresses three simultaneous problems: it provides a low-cost lipid feedstock, reduces environmental harm from improper disposal, and supports circular-economy objectives by converting waste into energy. (Goh, 2020)

2.3.2 Advantages of using WCO

Economically, WCO is significantly cheaper than virgin vegetable oils and in many contexts is available at little or no purchase cost because suppliers pay for disposal; leveraging WCO therefore reduces feedstock cost — the single largest contributor to biodiesel production expenses. (Jeyaseelan, 2023)

Environmentally, WCO-to-biodiesel pathways cut the environmental burden associated with disposal (sewer blockages, aquatic contamination) and lower net lifecycle greenhouse-gas emissions relative to fossil diesel because the CO_2 released during combustion is largely offset by the biogenic CO_2 absorbed during the original crop growth. (Goh, 2020)

Socially, using WCO avoids the food–fuel trade-offs linked to first-generation feedstocks, can generate local employment for collection and preprocessing, and supports community-level energy resilience. (Jeyaseelan, 2023)

2.3.3 Availability, collection systems and cost considerations

Global generation estimates indicate substantial WCO volumes: in many countries, institutional and commercial frying activities alone produce hundreds of thousands to millions of litres annually, creating viable supply pools for local biodiesel facilities. (Jeyaseelan, 2023)

Availability is, however, spatially heterogeneous; dense urban and commercial districts supply most WCO, whereas rural areas supply far less, so economic collection logistics (aggregation centres, incentives, collection contracts) are essential to secure reliable feedstock streams. (Jeyaseelan, 2023)

Techno-economic studies show WCO feedstock prices and collection costs make WCO-based biodiesel substantially more competitive than virgin-oil routes, improving the chance of small-scale and distributed production being financially viable. (Jeyaseelan, 2023)

2.3.4 Environmental impact of improper WCO disposal

Improper disposal of used oil into drains or onto land causes elevated biochemical oxygen demand (BOD) and chemical oxygen demand (COD) in receiving waters, degrades aquatic habitats and can foul wastewater systems and sewers. (Goh, 2020)

Transforming WCO into biodiesel mitigates these impacts and aligns with circular-economy policies and renewable-energy directives that prioritise waste and residue feedstocks over agricultural land use. (Goh, 2020)

2.3.5 Key challenges when using WCO

High free fatty acid (FFA) content

Repeated heating and hydrolytic reactions raise the FFA content of WCO; typical FFA ranges reported in recent literature span roughly 2–15 wt% (and in extreme cases higher), depending on the type of oil and frying history — levels that can render direct base-catalysed transesterification impractical due to saponification (soap formation). (Goh, 2020)

Because FFAs consume base catalyst and form emulsifying soaps, oils with FFA above commonly used thresholds (≈2 wt% for typical NaOH/KOH catalysis) are usually subjected to an acid-catalysed esterification pretreatment to convert FFAs into esters before the main base-catalysed transesterification. (Goh, 2020)

Water and other impurities

WCO often contains entrained water, food particles, phospholipids (gums), polymerised dimers/polymers and trace metals from frying equipment; moisture and solids inhibit catalyst activity, promote hydrolysis and complicate downstream separation and washing. (Goh, 2020)

Feedstock variability

WCO composition varies with oil source (palm, soybean, sunflower, groundnut), frying temperature/time, and the food being fried; this variability affects reaction stoichiometry, catalyst choice, and product quality, making standardisation and quality control crucial. (Goh, 2020)

2.3.6 Pretreatment requirements and methods

Because most WCO streams do not meet feed-in specifications for direct base catalysis, practical biodiesel processes include a pretreatment train tailored to local feedstock quality:

Physical pretreatment— removal of solids and suspended matter is performed by filtration, sedimentation (gravity settling) and sometimes centrifugation; simple coarse filtration followed by settling is often sufficient for restaurant-scale collections before chemical steps. (Goh, 2020)

Drying — gentle heating or vacuum drying to reduce water content (target <0.05 wt% for robust base catalysis) is routinely applied because moisture accelerates hydrolysis and saponification. (Goh, 2020)

Acid esterification (FFA reduction)— when FFA > 2–3 wt%, an acid-catalysed esterification (e.g., H_2SO_4 in methanol) converts free fatty acids into methyl esters; this step reduces FFA to acceptable levels for subsequent base transesterification and is widely implemented in industry and research. (Goh, 2020)

Degumming and neutralization — removal of phospholipids (degumming) and neutralization steps may be necessary for some feedstocks to protect catalysts and simplify purification. (Goh, 2020)

Alternative routes, such as one-step processes with bifunctional acid–base heterogeneous catalysts or supercritical alcohol transesterification (which can tolerate high FFA without pretreatment), have been demonstrated but often carry higher equipment and operating costs. (Assad, 2023)

2.3.7 Processing routes and catalyst implications

Because WCO typically requires some pretreatment, catalyst selection must consider residual FFA, water tolerance and the desired process configuration (single-step vs two-step). Heterogeneous catalysts derived from waste materials (for example CaO from animal bones) are especially attractive for WCO conversion because they combine strong basicity, low cost and ease of separation — and can be engineered or functionalised to tolerate moderate impurities or operate in staged processes. (Assad, 2023)

Moreover, recent studies demonstrate viable strategies for using waste-derived solid catalysts (including bone-based CaO) in both single-step and sequential processes, often achieving high FAME yields (>85–95%) after appropriate pretreatment and optimisation of methanol:oil ratio, catalyst loading and temperature. (Goh, 2020)

2.4 Biodiesel Production Process

2.4.1 Transesterification Process

Transesterification, also known as alcoholysis, is the primary chemical route for biodiesel production and remains the most widely applied method due to its efficiency, operational simplicity, and relatively low production cost (Amenaghawon et al., 2022). It involves the chemical reaction between triglycerides, found in vegetable oils or animal fats, and short-chain alcohols such as methanol or ethanol in the presence of a catalyst. The overall reaction converts triglycerides into fatty acid methyl esters (FAME) or fatty acid ethyl esters (FAEE), which constitute biodiesel, while glycerol is obtained as a valuable byproduct (Uwadiae et al., 2023).

Chemically, transesterification is a stepwise nucleophilic substitution reaction where three moles of alcohol react with one mole of triglyceride to yield three moles of alkyl esters and one mole of glycerol (Aisien et al., 2021). The process is reversible; hence, excess alcohol is usually used to shift the equilibrium toward ester formation, according to Le Chatelier's principle (Oyedoh & Akhabue, 2020). The reaction can be catalyzed using homogeneous or heterogeneous acids, bases, or enzymes. However, base-catalyzed transesterification—particularly using solid basic catalysts like CaO derived from waste animal bones—has gained considerable attention due to faster reaction rates and easier product separation (Amenaghawon et al., 2022).

Transesterification proceeds efficiently when the feedstock has low free fatty acid (FFA) and moisture content, as high levels can lead to soap formation, reducing ester yield (Uwadiae et al., 2023). The reaction mechanism and conditions significantly influence the conversion efficiency and the quality of the biodiesel produced. The fundamental understanding of this mechanism provides the basis for optimizing process parameters such as catalyst loading, reaction temperature, molar ratio, and reaction time.

2.4.1.1 Reaction Mechanism

The transesterification reaction mechanism can be represented by the general equation:

$$\textit{Triglyceride} + 3\ ROH = 3\ R'COOR + \textit{Glycerol}$$

where ROH represents a short-chain alcohol (e.g., methanol or ethanol), and R'COOR denotes the corresponding fatty acid ester (FAME or FAEE). The reaction occurs through three consecutive and reversible steps: conversion of triglycerides to diglycerides, diglycerides to monoglycerides, and finally monoglycerides to glycerol, with one molecule of ester produced at each step (Amenaghawon et al., 2022).

In base-catalyzed systems such as those using CaO derived from cow bone, the catalyst first reacts with the alcohol to generate an alkoxide ion (CH_3O^- or $C_2H_5O^-$), which serves as the active nucleophile. The mechanism proceeds as follows:

1. Initiation step: The basic catalyst reacts with the alcohol to form the alkoxide ion and a hydroxide species.

$$\boldsymbol{CaO + ROH + Ca^{2}+ + RO\text{\textasciicircum}- + OH\text{\textasciicircum}-}$$

This alkoxide ion is the key reactive species that attacks the carbonyl carbon of the triglyceride molecule.

2. Formation of diglyceride: The alkoxide ion attacks the carbonyl carbon of one fatty acid chain in the triglyceride, forming a tetrahedral intermediate. The intermediate rearranges, releasing one molecule of fatty acid ester and generating a diglyceride molecule.

3. Conversion to monoglyceride: The process repeats, with the diglyceride undergoing a similar reaction to form a second ester and monoglyceride.

4. Formation of glycerol: Finally, the monoglyceride reacts with another alkoxide ion, producing the third ester molecule and free glycerol.

5. Catalyst regeneration: The CaO catalyst is regenerated at the end of the reaction, maintaining its activity through multiple cycles (Oyedoh & Akhabue, 2020).

The mechanistic understanding of this reaction is crucial for heterogeneous catalysis, as it highlights the importance of surface active sites, basicity strength, and catalyst porosity. In particular, CaO derived from animal bone exhibits strong basic sites due to the presence of Ca^{2+}

and O^{2-} species, which enhance alcohol activation and facilitate faster transesterification (Amenaghawon et al., 2022). The solid nature of the catalyst also minimizes contamination of products and simplifies post-reaction purification compared to homogeneous catalysts like NaOH or KOH.

2.4.1.2 Reaction Conditions

Several operating parameters influence the efficiency of the transesterification reaction. These include reaction temperature, time, catalyst concentration, molar ratio of alcohol to oil, and mixing intensity. Each parameter interacts with others, and their optimization is essential for achieving maximum conversion and yield.

Temperature:

The reaction temperature significantly affects the rate and extent of transesterification. Typically, temperatures between 50°C and 65°C are optimal for methanol-based reactions because this range is near the boiling point of methanol but below its vaporization threshold (Aisien et al., 2021). Reaction rates increase with temperature due to enhanced molecular motion and collision frequency as explained by the Arrhenius equation. However, excessively high temperatures can lead to methanol evaporation, catalyst deactivation, and unwanted side reactions like saponification (Uwadiae et al., 2023). For heterogeneous CaO catalysts, the optimal temperature often lies between 60°C and 65°C, balancing reaction rate and energy efficiency.

Reaction Time:

The duration of the reaction determines the extent of triglyceride conversion. Base-catalyzed transesterification typically requires 1–3 hours to reach completion, depending on catalyst activity and feedstock properties (Amenaghawon et al., 2022). Shorter reaction times may lead to incomplete conversion, while excessively long times can cause reverse reactions or increase operating costs. The progress of the reaction is often monitored by measuring the ester yield or glycerol formation over time.

Catalyst Concentration:

Catalyst loading affects both the reaction kinetics and final yield. For homogeneous systems, 0.5–1% w/w catalyst relative to oil is common, whereas heterogeneous catalysts like CaO require higher loadings (3–10%) to compensate for lower surface contact (Oyedoh & Akhabue, 2020). Insufficient catalyst results in incomplete conversion, while excessive catalyst may increase soap formation and complicate product separation. Studies have shown that bone-derived CaO catalysts exhibit optimal performance at approximately 5% loading, achieving over 90% conversion (Amenaghawon et al., 2022).

Mixing Intensity:

Efficient mixing is critical in transesterification because oil and alcohol are immiscible at the start of the reaction. Adequate agitation enhances mass transfer between phases, promoting contact between triglycerides, alcohol, and the solid catalyst surface. Stirring speeds of 400–600 rpm are typically employed (Uwadiae et al., 2023). However, excessive mixing can form stable emulsions that hinder product separation, while insufficient agitation reduces reaction efficiency.

2.4.1.3 Molar Ratio of Alcohol to Oil

The molar ratio of alcohol to oil is one of the most critical parameters in the transesterification process, as it determines the extent of reaction completion and directly influences the yield and purity of biodiesel produced. Theoretically, the transesterification of one mole of triglyceride requires three moles of alcohol to form three moles of fatty acid esters and one mole of glycerol (Uwadiae et al., 2023). However, because the reaction is reversible, using only the stoichiometric ratio rarely results in complete conversion. To drive the reaction equilibrium toward the product side, excess alcohol is commonly employed in practical systems (Amenaghawon et al., 2022).

In industrial and laboratory-scale biodiesel production, the molar ratio typically ranges between 6:1 and 12:1, depending on the type of feedstock, catalyst, and alcohol used (Aisien et al., 2021). The presence of excess alcohol enhances the forward reaction by increasing the probability of triglyceride-alcohol collisions and helps overcome equilibrium limitations. Moreover, excess alcohol improves the solubility of glycerol in the reaction medium, facilitating phase separation and enhancing overall conversion. However, excessively high alcohol content can have adverse effects, such as diluting the catalyst, increasing energy costs during alcohol recovery, and complicating downstream purification (Oyedoh & Akhabue, 2020).

The optimal alcohol-to-oil molar ratio must, therefore, balance conversion efficiency and economic feasibility. Studies have reported that for CaO-based heterogeneous catalysis, a molar ratio of 9:1 often yields the best results, producing over 90% FAME conversion within 2 hours at moderate temperatures (Amenaghawon et al., 2022). Ratios higher than 12:1 tend to offer diminishing returns, as the excess alcohol does not significantly enhance reaction rate but requires additional recovery steps that reduce process efficiency (Uwadiae et al., 2023).

The choice of alcohol also affects the optimal ratio. Methanol, being more polar and less viscous, reacts more readily with triglycerides, allowing for lower molar ratios compared to ethanol, which forms azeotropes with water and reacts more slowly (Aisien et al., 2021). Ethanol-based systems often require molar ratios between 9:1 and 15:1 to achieve similar conversions (Oyedoh & Akhabue, 2020).

Recent optimization studies using Response Surface Methodology (RSM) and Artificial Neural Network (ANN) modeling have confirmed the strong interaction between alcohol-to-oil molar ratio and other process variables such as catalyst loading, temperature, and reaction time (Amenaghawon et al., 2022). For instance, increasing the alcohol ratio beyond the optimum can offset the benefits of higher temperature or catalyst concentration by complicating phase behavior and reducing the effective contact between reactants and catalyst surfaces.

From an environmental and operational standpoint, efficient recovery and recycling of excess alcohol are vital to maintaining the sustainability of biodiesel production. The cost of unreacted alcohol recovery through distillation can constitute a significant portion of the total process cost if not optimized (Uwadiae et al., 2023). Therefore, selecting the optimal molar ratio is not only a matter of reaction yield but also of process economics, safety, and environmental impact.

In conclusion, while the stoichiometric requirement for transesterification is 3:1, practical systems employ higher molar ratios—typically 6:1 to 9:1—to achieve high conversion and minimize equilibrium constraints. The specific ratio must be optimized for each feedstock–catalyst–alcohol combination to ensure high yield, easy separation, and minimal energy consumption, especially when employing heterogeneous catalysts such as CaO derived from cow bone.

2.4.1.4 Types of Alcohols Used

The selection of alcohol plays a crucial role in determining the efficiency, yield, and physicochemical characteristics of the biodiesel produced through transesterification. Alcohols serve as acyl acceptors in the reaction, converting triglycerides into fatty acid alkyl esters (FAAE) and glycerol. Commonly, short-chain monohydric alcohols such as methanol and ethanol are used due to their favorable reactivity and cost-effectiveness (Amenaghawon et al., 2022). The choice of alcohol affects reaction kinetics, phase behavior, separation ease, and even the environmental sustainability of the process.

Methanol

Methanol is the most widely used alcohol in biodiesel production, accounting for over 80% of global biodiesel processes (Aisien et al., 2021). Its popularity stems from its low cost, high polarity, and ability to rapidly form methoxide ions in the presence of basic catalysts like CaO. The methanolysis process is relatively fast, leading to high biodiesel yields even under mild reaction conditions. Moreover, methanol is miscible with most oils and fats at elevated temperatures, which enhances reaction homogeneity and mass transfer efficiency (Uwadiae et al., 2023).

Chemically, methanol reacts with triglycerides through base-catalyzed nucleophilic substitution, forming fatty acid methyl esters (FAME) and glycerol. Because of its short carbon chain, methanol allows faster reaction rates and better miscibility with the glycerol byproduct, aiding in the overall process kinetics (Amenaghawon et al., 2022). However, its use also has limitations. Methanol is derived primarily from non-renewable natural gas sources, making it less sustainable compared to bioethanol. Furthermore, it is toxic and poses health and environmental risks during handling and disposal (Oyedoh & Akhabue, 2020).

Despite these drawbacks, methanol remains the industry standard because it achieves near-complete conversion of triglycerides to biodiesel within shorter reaction times and at lower alcohol-to-oil molar ratios (typically 6:1 to 9:1) when combined with heterogeneous catalysts like CaO derived from natural waste sources such as cow bone (Uwadiae et al., 2023). The widespread adoption of methanol is also supported by extensive operational experience, available infrastructure, and well-understood recovery systems.

Ethanol

Ethanol is increasingly gaining attention as a renewable alternative to methanol, especially in sustainable biodiesel production frameworks. It can be obtained from biomass via fermentation of agricultural residues, sugarcane, corn, or cassava, making it more environmentally friendly (Amenaghawon et al., 2022). Transesterification using ethanol yields fatty acid ethyl esters (FAEE), which have slightly different physicochemical properties from FAME. Ethyl esters exhibit improved lubricity, higher cetane number, and better cold-flow properties, making them suitable for diverse climatic conditions (Aisien et al., 2021).

However, ethanol-based transesterification presents some operational challenges. Ethanol is less polar than methanol, resulting in slower reaction kinetics and reduced solubility with non-polar triglycerides. Moreover, ethanol forms azeotropes with water, making it difficult to obtain the anhydrous ethanol required for efficient reaction (Uwadiae et al., 2023). The presence of even small amounts of water promotes saponification, reducing ester yield and complicating separation of biodiesel from glycerol. Therefore, higher alcohol-to-oil molar ratios (9:1 to 15:1) and longer reaction times are often necessary to achieve high conversion rates when ethanol is used (Oyedoh & Akhabue, 2020).

Despite these challenges, ethanol's renewability and lower toxicity make it attractive for greener biodiesel processes. Studies have shown that heterogeneous catalysts such as CaO derived from cow bone maintain good catalytic activity with ethanol, producing high-quality FAEE with yields exceeding 85% under optimized conditions (Amenaghawon et al., 2022). The use of ethanol also enhances the biodegradability and environmental friendliness of the resulting biodiesel, aligning with circular economy goals and sustainable development objectives (Aisien et al., 2021).

Other Higher Alcohols

Although methanol and ethanol dominate commercial biodiesel production, other higher alcohols such as propanol, butanol, and isobutanol have also been investigated. These alcohols produce biodiesel with higher energy density, improved viscosity, and better cold flow characteristics (Uwadiae et al., 2023). However, they suffer from slower reaction rates due to steric hindrance and lower reactivity of the corresponding alkoxide intermediates. Additionally, their higher cost and limited availability make them less attractive for large-scale applications (Amenaghawon et al., 2022).

Interestingly, recent research has explored the use of branched-chain alcohols such as isobutanol and tert-butanol for biodiesel synthesis, which yield fuels with enhanced oxidation stability and lower volatility (Aisien et al., 2021). However, these systems require elevated temperatures and pressures, and their phase behavior with triglycerides is often complex, necessitating further investigation before commercialization.

Comparative Overview

A comparative analysis between methanol, ethanol, and higher alcohols reveals that methanol remains the most efficient and economically viable alcohol for biodiesel production, particularly in heterogeneous catalysis systems. Ethanol, while renewable and less hazardous, requires process adjustments to mitigate issues related to water content and separation efficiency. Higher alcohols, though promising in producing superior fuel quality, remain limited by economic and kinetic constraints (Amenaghawon et al., 2022; Uwadiae et al., 2023).

Ultimately, the selection of alcohol must balance reactivity, cost, availability, and environmental impact. In this study, methanol was selected as the alcohol of choice for transesterification due to its superior performance with CaO-based catalysts, low cost, and extensive industrial validation. The resulting FAME biodiesel exhibits favorable combustion characteristics, making it suitable for practical engine applications while maintaining process simplicity and cost efficiency.

2.4.2 Other Methods of Biodiesel Production

While transesterification remains the most widely used and industrially accepted method for biodiesel production, other processes such as esterification, pyrolysis (thermal cracking), and microemulsion formation have been explored as alternatives or complementary routes. These methods are often employed to address the limitations of transesterification, particularly when dealing with feedstocks containing high levels of free fatty acids (FFA), moisture, or impurities; as is common with waste cooking oils. Each alternative process presents unique advantages, reaction mechanisms, and process conditions that influence the yield, purity, and quality of the resulting biodiesel.

2.4.2.1 Esterification

Esterification is a chemical reaction between free fatty acids (FFA) and alcohol (commonly methanol or ethanol) in the presence of an acid catalyst to form fatty acid alkyl esters (biodiesel) and water. This process is particularly useful for feedstocks with high FFA content, such as waste cooking oil (WCO), animal fat, and non-edible vegetable oils (Aisien et al., 2021). High FFA levels (above 2%) can cause soap formation during base-catalyzed transesterification, reducing biodiesel yield and complicating separation. Therefore, esterification is often used as a pretreatment step to convert FFAs into esters before the transesterification stage (Amenaghawon et al., 2022).

The reaction mechanism involves the protonation of the carbonyl group of the fatty acid by the acid catalyst, which increases the electrophilicity of the carbon and facilitates nucleophilic attack by the alcohol. The intermediate form then dehydrates to produce an ester and water (Uwadiae et al., 2023). Sulfuric acid (H_2SO_4) is the most common homogeneous catalyst used, though recent studies have explored solid acid catalysts such as sulfonated carbon, zeolites, and bio-based catalysts derived from waste materials for improved reusability and environmental safety (Oyedoh & Akhabue, 2020).

Recent works by Amenaghawon et al. (2022) demonstrated the effective use of heterogeneous acid catalysts synthesized from agricultural and animal waste residues for the esterification of high-FFA oils. For example, sulfonated cow bone ash and activated carbon-based catalysts showed high conversion rates (above 90%) under optimized conditions. These findings highlight the potential for sustainable catalyst development from locally available waste materials, aligning with circular economy principles.

Esterification reactions are generally slower than base-catalyzed transesterification and require higher temperatures (50–70°C) and longer reaction times to achieve high conversions. Water formation during the reaction can also shift the equilibrium backward, reducing ester yield. Therefore, removal of water during or after reaction is crucial to achieving high conversion efficiencies (Uwadiae et al., 2023). Overall, esterification serves as a critical pretreatment process for refining low-cost, high-FFA feedstocks into suitable substrates for biodiesel synthesis.

2.4.2.2 Pyrolysis (Thermal Cracking)

Pyrolysis, also known as thermal cracking, is a non-catalytic or catalytic decomposition process in which triglycerides and other lipid-based materials are thermally broken down in the absence of oxygen to produce hydrocarbon-rich bio-oil, gases, and char (Aisien et al., 2021). The bio-oil obtained can be upgraded to biodiesel-like fuels or used directly as a renewable alternative to conventional diesel. This process occurs at high temperatures, typically between 400–600°C, and offers an alternative pathway for converting low-grade or contaminated feedstocks that are unsuitable for transesterification (Oyedoh & Akhabue, 2020).

The mechanism involves the cleavage of C–C and C–O bonds within triglyceride molecules, leading to the formation of smaller hydrocarbons, olefins, aldehydes, and ketones. Depending on the temperature, catalyst type, and residence time, the product distribution can be adjusted to favor liquid bio-oil or gaseous products (Amenaghawon et al., 2022). Catalytic pyrolysis, using catalysts such as zeolites, alumina, or metal oxides, has been shown to enhance bio-oil quality by increasing hydrocarbon selectivity and reducing oxygen content (Uwadiae et al., 2023).

A study by Amenaghawon et al. (2022) emphasized that catalytic pyrolysis using waste-derived catalysts not only improves yield but also enhances sustainability. Their findings demonstrated that catalysts derived from cow bone and plant ash exhibited strong deoxygenation capabilities, producing hydrocarbon-rich oils comparable to petroleum diesel. However, challenges such as high energy demand, complex product separation, and lower fuel stability limit the large-scale application of pyrolysis for biodiesel production (Aisien et al., 2021).

Nonetheless, pyrolysis offers an advantage in processing feedstocks with high impurities, such as grease trap waste, sludge oil, or degraded WCO, without requiring pretreatment. The resulting bio-oil can be further upgraded through hydrogenation or catalytic cracking to yield biodiesel-range hydrocarbons, representing a promising route for advanced biofuel production (Uwadiae et al., 2023).

2.4.2.3 Microemulsion Method

The microemulsion technique involves the formation of a thermodynamically stable, isotropic dispersion of oil, alcohol, and surfactant, which can be used as an alternative to conventional transesterified biodiesel (Aisien et al., 2021). This method reduces fuel viscosity by blending triglycerides with short-chain alcohols or esters and surfactants, allowing direct use in diesel engines without undergoing chemical transformation. The microemulsion system typically consists of three components: oil (such as WCO), an alcohol (methanol or ethanol), and a surfactant (e.g., cetyltrimethylammonium bromide, CTAB) that stabilizes the mixture (Amenaghawon et al., 2022).

Microemulsion offers several advantages, including simple preparation, low reaction time, and avoidance of byproduct formation such as glycerol. Furthermore, it improves atomization, reduces engine deposits, and provides better combustion efficiency (Oyedoh & Akhabue, 2020). However, the calorific value and long-term storage stability of microemulsion fuels are generally lower than those of transesterified biodiesel, limiting their commercial application (Uwadiae et al., 2023).

Recent research trends have focused on optimizing surfactant concentration and phase behavior to achieve microemulsion fuels with improved stability and combustion characteristics. Amenaghawon et al. (2022) reported that WCO-based microemulsions using ethanol and biodegradable surfactants yielded blends with reduced viscosity and enhanced cetane index, suitable for compression ignition engines. Similarly, Aisien et al. (2021) observed that incorporating bio-based surfactants improved the environmental performance of microemulsion fuels, aligning with sustainable energy goals.

2.4.2.4 Comparative Evaluation

Each of these alternative methods offers unique benefits and challenges. Esterification is particularly effective for high-FFA feedstocks and is often used as a pretreatment before transesterification. Pyrolysis, though energy-intensive, can handle contaminated or degraded oils and yields hydrocarbon-rich bio-oils that mimic petroleum diesel. Microemulsion, on the other hand, provides a simpler blending approach with improved combustion properties but reduced long-term stability.

Comparative studies by Uwadiae et al. (2023) and Amenaghawon et al. (2022) concluded that the choice of biodiesel production method depends largely on feedstock characteristics, desired fuel properties, and process economics. Among these, the transesterification–esterification combination remains the most efficient and sustainable approach for converting waste cooking oil into high-quality biodiesel.

2.5 Characteristics of Waste Cooking Oil (WCO)

Waste cooking oil (WCO) is a complex mixture of triglycerides, free fatty acids, mono- and diglycerides, and oxidation products that result from the degradation of edible oil during repeated frying or prolonged thermal exposure. It is one of the most promising feedstocks for biodiesel production due to its low cost and widespread availability; however, its variable chemical composition and degraded quality necessitate careful characterization before it can be used for transesterification (Amenaghawon et al., 2022).

When vegetable oils are subjected to high temperatures in the presence of air, moisture, and food residues, they undergo thermal oxidation, polymerization, and hydrolysis reactions. These reactions cause the breakdown of triglycerides into smaller molecules, increase the concentration of free fatty acids (FFA), and form undesirable compounds such as peroxides, aldehydes, and polymers (Osagie et al., 2023). Consequently, WCO often exhibits altered physical and chemical properties compared to fresh oil, which directly affect the efficiency of biodiesel production and the quality of the final product.

2.5.1 Acid Value

The acid value (AV) is one of the most important indicators of oil degradation. It measures the amount of free fatty acids present in the oil and is defined as the milligrams of potassium hydroxide (KOH) required to neutralize the acids in one gram of oil. A high acid value signifies that significant hydrolysis of triglycerides has occurred, which can hinder the transesterification process when an alkaline catalyst is used (Amenaghawon et al., 2022). Typically, WCOs have acid values in the range of 1–10 mg KOH/g, depending on how often the oil has been used and the type of food fried. Oils with acid values above 2 mg KOH/g generally require acid esterification pretreatment to convert the free fatty acids into esters before the main transesterification step (Osagie et al., 2023).

2.5.2 Free Fatty Acid (FFA) Content

The FFA content represents the percentage of free fatty acids in the oil and is directly proportional to the acid value. High FFA levels are undesirable in base-catalyzed transesterification because they react with the catalyst to form soaps, which reduce biodiesel yield and complicate product separation (Ulukardesler et al., 2023). Fresh vegetable oils generally have FFA values below 1%, while waste cooking oils can range from 2% to 15%, depending on their frying history and exposure to moisture (Ifeanyi-Nze et al., 2023). The FFA content is therefore an essential factor in determining whether the oil can be directly used for transesterification or requires pretreatment.

2.5.3 Viscosity

Viscosity determines the flow properties of the oil and its behavior during mixing and reaction. Fresh vegetable oils typically have viscosities between 30–40 cP at 40°C, while waste cooking oils tend to have higher values due to polymerization and oxidation of unsaturated fatty acids (Amenaghawon et al., 2022). Elevated viscosity impairs atomization during combustion and reduces biodiesel yield during transesterification by affecting mass transfer between the oil and alcohol phases. For efficient processing, the viscosity of WCO should be reduced either by heating, dilution, or through chemical conversion into biodiesel (Osagie et al., 2023).

2.5.4 Saponification Value

The saponification value (SV) measures the amount of KOH required to saponify one gram of oil and is used to estimate the average molecular weight (or chain length) of the fatty acids present. A higher SV indicates shorter-chain fatty acids, while a lower SV corresponds to longer-chain fatty acids (Onoja et al., 2021). Typical saponification values for WCO range from 180–200 mg KOH/g, depending on the oil source and degradation level. This parameter is crucial in calculating the theoretical yield of biodiesel and in understanding how degradation affects the fatty acid profile.

2.5.5 Iodine Value

The iodine value (IV) indicates the degree of unsaturation in the fatty acids of the oil. It is expressed as the number of grams of iodine absorbed by 100 grams of oil (Bello et al., 2020). Oils with higher iodine values contain more unsaturated bonds and are more prone to oxidation, which affects storage stability. Repeated frying usually reduces the iodine value of oils because double bonds are broken during oxidation and polymerization reactions (Osagie et al., 2023). WCOs generally exhibit iodine values between 80–120 g I_2/100 g, depending on their source and frying conditions.

2.5.6 Density

Density is another vital physical parameter influencing biodiesel production. It affects the volumetric efficiency of engines and the combustion process. The density of WCOs typically ranges between 0.88–0.93 g/cm^3, depending on their composition and temperature (Amenaghawon et al., 2022). Changes in density are often linked to the accumulation of oxidation products and polymeric compounds during prolonged heating.

2.5.7 Moisture Content

The presence of moisture in WCO accelerates hydrolysis of triglycerides, leading to increased FFA content and reduced catalyst activity. Excess water in the feedstock can cause saponification during transesterification, resulting in soap formation that complicates product purification (Ifeanyi-Nze et al., 2023). For optimal biodiesel production, the moisture content of the oil should be maintained below 0.05 wt%. Pre-drying of WCO through gentle heating before transesterification is therefore a common practice.

2.5.8 Peroxide Value

The peroxide value (PV) measures the concentration of peroxides and hydroperoxides formed during the initial stages of oil oxidation. These compounds are unstable and decompose into aldehydes and ketones that negatively affect fuel stability and odor (Osagie et al., 2023). High peroxide values indicate that the oil has undergone significant oxidative degradation, reducing its suitability for biodiesel production. WCOs generally show higher PVs compared to fresh oils due to exposure to air and high frying temperatures.

The physicochemical properties of WCO are critical in determining its suitability for biodiesel production. Parameters such as acid value, FFA content, viscosity, and moisture level directly influence the choice of catalyst and the reaction pathway. Characterization of these properties helps to identify necessary pretreatment steps and optimize reaction conditions for maximum biodiesel yield.

2.6 Catalysts Used in Biodiesel Production

Catalysts are central to biodiesel manufacture because they accelerate the transesterification of triglycerides with alcohols into fatty acid alkyl esters (FAME) and glycerol. The choice of catalyst has a direct influence on reaction rate, yield, operating conditions, downstream purification effort and overall process economics. Broadly, catalysts for biodiesel synthesis fall into four classes; homogeneous (acid and base), heterogeneous (solid) basic/acidic, bifunctional (acid–base) solids, and biocatalysts (enzymes) — and each class has distinct advantages and limitations that determine its suitability for a given feedstock and scale of operation. (Mandari, 2022)

2.6.1 Homogeneous Catalysts (Base and Acid)

Homogeneous catalysts, typically sodium/potassium hydroxide (NaOH/KOH) for base-catalysed transesterification and sulfuric or hydrochloric acid for esterification of high-FFA oils, have dominated laboratory and industrial practice because of their high intrinsic activity and short reaction times. Base catalysts are highly efficient for low-FFA oils and operate at mild temperatures (≈ 25-

65 °C), producing high conversions in minutes to hours; acid catalysts are used primarily to esterify free fatty acids into esters when FFA content is high (>2 wt%), preventing soap formation in the subsequent base step. However, homogeneous systems suffer from well-known drawbacks: difficult catalyst/product separation, wastewater generation from neutralization/washing, sensitivity to feedstock FFA and water, and corrosion issues — factors that raise operating costs and environmental burdens. (Mandari, 2022)

2.6.2 Heterogeneous (Solid) Catalysts

The drive for cleaner, more sustainable processes has stimulated large research activity in heterogeneous solid catalysts, which can be removed by filtration, recycled, and engineered for tailored surface properties. Solid catalysts fall into several subtypes:

- Metal oxides and mixed oxides (basic): CaO, MgO, hydrotalcites, and doped metal oxides are among the most widely studied solid basic catalysts. CaO is particularly attractive due to its strong basicity, low cost and availability from waste sources (eggshells, shells, bones). When well-prepared and stabilized, CaO can deliver high conversions comparable to homogeneous bases while offering easier separation. (Gadore, 2023)

- Supported catalysts: Active basic/acid phases (e.g., KOH, CaO, alkali salts) can be dispersed on high-surface-area supports (Al_2O_3, SiO_2, activated carbon) to enhance dispersion, prevent sintering and reduce leaching. Supports allow fine control over accessibility and mass transfer, which is crucial for bulky triglyceride molecules. (Farouk, 2024)

- Heterogeneous acid catalysts: Sulfonated carbon materials, heteropolyacids, and ion-exchange resins provide strong acid sites for esterification of high-FFA oils and can be operated in a single step for certain feedstocks. Acid solids are less prone to saponification but often require higher temperatures and can be susceptible to pore blockage by heavy residues. (Farouk, 2023)

- Bifunctional and hybrid catalysts: To handle feedstocks with both triglycerides and significant FFA, bifunctional catalysts that combine acid and base sites on one solid (or physically mix acid and base solids) enable simultaneous esterification and transesterification in one reactor, simplifying processing. Recent literature shows growing interest in engineered bifunctional bio-derived catalysts for WCO conversion. (Welter, 2023).

Advantages and challenges. Heterogeneous catalysts promise easy recovery, reuse, and reduced wastewater. Their principal challenges are mass-transfer limitations (oil–alcohol–solid interface), potential leaching of active species (especially for alkali modifiers), deactivation by surface fouling or carbonation/hydroxylation ($CaO \rightarrow Ca(OH)_2$ / $CaCO_3$ on exposure to moisture/CO_2), and scale-up issues for packed-bed reactors. Addressing these requires careful catalyst design (stable supports, appropriate calcination), process control (dry feedstock, controlled atmosphere), and robust reactivation/regeneration protocols. (Gadore, 2023)

2.6.3 Biomass- and Waste-Derived Catalysts (Including Animal Bones)

A particularly active research avenue is the conversion of biowastes (eggshells, mollusk shells, animal bones, agricultural residues) into solid Ca-based catalysts. Thermal treatment (calcination) decomposes carbonate and organic matrices to give CaO, $Ca_3(PO_4)_2$, or hydroxyapatite-derived phases that show useful basicity for transesterification. These materials are attractive because they combine low cost, local availability and simple preparation with good catalytic activity — a compelling proposition for decentralized and low-cost biodiesel production. A focused review on bone-derived catalysts highlights consistent reports of high biodiesel yields (often >85–95% under optimized conditions) using calcined bone residues. (Hussain, 2021)

The Department of Chemical Engineering, University of Benin (UNIBEN) as contributed substantively to this field: Amenaghawon and co-workers (2022) developed and optimized a functionalized bio-based heterogeneous catalyst (derived from local bio-waste) and demonstrated effective conversion of waste cooking oil with strong yields and recyclability, illustrating the practical potential of locally sourced catalysts. Their work also emphasizes characterization (XRD, SEM, FTIR, XRF, BET) to relate structure and composition to activity; an approach followed in many subsequent studies. (Gadore, 2023)

2.6.4 Catalyst Design Considerations and Performance Metrics

When designing or selecting a catalyst for WCO transesterification, the following attributes are critical:

- Basicity/acid strength and site density: Determines the ability to deprotonate alcohol and activate triglycerides (for base catalysts) or protonate carboxylic acids (for acid catalysts).

Basic strength is commonly probed by CO_2-TPD or Hammett indicator methods. (Farouk, 2023)

- Surface area and porosity: High external surface and mesoporosity favor diffusion of bulky oil molecules and improve turnover frequency. BET and pore-size distributions are therefore important design data. (Gadore, 2023)
- Thermal and chemical stability: Resistance to hydration, carbonation and leaching under reaction conditions determines reusability; CaO-based catalysts must be protected from air moisture or reactivated by re-calcination when necessary. (Hussain, 2021)
- Compatibility with feedstock: Catalysts that tolerate water and moderate FFA, or that possess bifunctional sites, reduce the need for multi-step pretreatment and simplify operation. (Welter, 2023)

Performance metrics reported in the literature typically include biodiesel yield (%) or FAME content, turnover frequency (TOF) where available, reusability cycles (activity retention over successive runs), and extent of leaching (measured by ICP-OES of reaction phases). High-performing bone-derived catalysts often show yields >90% under methanol-rich conditions, catalyst loadings of 1–6 wt%, temperatures around 60–65 °C and reaction times from 1–3 hours; however, specifics vary with preparation and activation. (Farouk, 2024)

2.6.5 Nanostructured and Supported Catalysts

Advances in nanomaterials have led to catalysts with finely tuned active sites, higher dispersion and engineered porosity. Nanostructured CaO, doped mixed oxides and nano-supported base catalysts can exhibit faster kinetics and better resistance to sintering, albeit at increased synthesis complexity and potential cost. Supported catalysts; where active phases are anchored on mesoporous silica, carbon or alumina; enhance stability and allow design of hierarchical pore structures beneficial for triglyceride access. These developments are promising for continuous and packed-bed operations. (Gadore, 2023)

2.6.6 Enzymatic Catalysts (Lipases)

Enzyme catalysts (lipases) offer a mild, selective route for biodiesel production, especially suited to high-FFA feedstocks because they catalyze both esterification and transesterification without soap formation. Immobilized lipases can be reused and operate under mild temperatures; however, they are generally slower, sensitive to methanol inhibition (requiring stepwise alcohol addition or immobilization strategies) and relatively expensive, which limits their wide adoption except in niche or high-value applications. (Farouk, 2024)

2.6.7 Operational and Environmental Considerations

From a practical viewpoint, the transition from homogeneous to heterogeneous catalysis is motivated by reduced effluent treatment, easier catalyst recovery and lower environmental impact. Yet, implementing heterogeneous catalysts requires attention to reactor design (mixing to overcome external mass transfer), control of particle size (to avoid pressure drop or poor separability), and robust regeneration strategies to manage deactivation by fouling, leaching or carbonate formation. Life-cycle and techno-economic analyses typically show that waste-derived heterogeneous catalysts can reduce operating costs and environmental burdens when benefits of reuse and simpler downstream processing are included. (Gadore, 2023)

2.6.8 Gaps and Research Opportunities

Despite extensive progress, several gaps remain: (1) standardized protocols for catalyst preparation and reporting (calcination profiles, pre-treatment) are needed to enable direct comparison across studies; (2) long-term leaching and stability data under realistic process cycles are limited; (3) scale-up studies transitioning promising lab-scale catalysts to pilot continuous reactors are scarce; and (4) integration of catalyst development with feedstock variability (different WCO sources) requires broader datasets. These gaps frame the rationale for the present work, which focuses on thorough characterization (XRD, XRF, FTIR, SEM, BET), optimization of reaction parameters and assessment of catalyst reusability under conditions relevant to local WCO streams. (Hussain, 2021)

2.7 Cow Bone as a Heterogeneous Catalyst in Biodiesel Production

The use of animal bone-derived catalysts has gained increasing attention in recent years as a sustainable and cost-effective approach for biodiesel production. Cow bone, in particular, is an abundant waste material generated from slaughterhouses and meat processing facilities. When properly treated, cow bone can be transformed into a heterogeneous catalyst rich in calcium oxide (CaO), a compound known for its strong basicity and catalytic efficiency in transesterification reactions (Amenaghawon et al., 2022; Osagie et al., 2023).

Cow bone primarily consists of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$], a calcium phosphate mineral that can be thermally decomposed to form CaO upon calcination at temperatures above 800°C. This transformation enhances the surface area and catalytic activity of the bone material. The process typically involves washing, drying, crushing, and calcining the bone to eliminate organic impurities and to activate the catalyst (Akhabue et al., 2023). According to Amenaghawon et al. (2022) from the Department of Chemical Engineering, University of Benin, such calcined bone materials have shown strong potential in transesterification of waste cooking oil due to their high stability, recyclability, and minimal leaching compared to homogeneous catalysts.

The catalytic performance of cow bone-derived CaO depends on several factors, including calcination temperature, particle size, and the presence of other mineral oxides such as magnesium oxide (MgO) and phosphorus pentoxide (P_2O_5), which can enhance or hinder catalytic activity. Studies have shown that calcination between 800–900°C yields catalysts with optimal surface basicity and pore structure suitable for biodiesel production (Uwadiae et al., 2021). Furthermore, the bone's porosity and high specific surface area provide abundant active sites for methanol adsorption and triglyceride conversion (Osagie et al., 2023).

One of the most significant advantages of using cow bone as a catalyst lies in its heterogeneous nature, which allows for easy separation from the reaction mixture after transesterification. Unlike homogeneous catalysts such as NaOH or KOH, which dissolve in the reaction medium and complicate product purification, bone-derived CaO catalysts can be filtered and reused in multiple cycles with minimal loss of activity (Amenaghawon et al., 2022). This reusability substantially reduces process costs and environmental impact.

In addition to its economic and operational advantages, the use of cow bone catalyst aligns with principles of green chemistry and circular economy, as it transforms waste from the meat industry into a high-value material for renewable fuel production. The integration of waste oil and animal bone thus forms a zero-waste biodiesel production pathway, enhancing sustainability and contributing to Nigeria's renewable energy goals (Aisien et al., 2021).

Recent research at the University of Benin has reinforced the efficiency of cow bone-derived catalysts in biodiesel synthesis. For instance, Amenaghawon et al. (2022) demonstrated that CaO obtained from calcined cow bone achieved conversion efficiencies exceeding 90% under optimized reaction conditions. Similarly, Akhabue et al. (2023) reported high stability and minimal deactivation after five reuse cycles, indicating the potential for industrial application.

Overall, cow bone as a heterogeneous catalyst provides a viable, eco-friendly, and low-cost alternative for biodiesel production. Its adoption not only mitigates waste disposal issues but also reduces dependence on expensive commercial catalysts, thereby promoting localized, sustainable biofuel technologies suitable for developing nations like Nigeria (Uwadiae et al., 2021; Amenaghawon et al., 2022).

2.8 Reaction Mechanism of Transesterification Using Cow Bone Catalyst

The transesterification reaction is the fundamental chemical process involved in the conversion of triglycerides (from waste cooking oil) into biodiesel, with methanol (or another alcohol) as the reacting agent and a catalyst to enhance the rate of reaction. When a heterogeneous catalyst such as cow bone-derived calcium oxide (CaO) is employed, the reaction mechanism differs slightly from the conventional homogeneous base-catalyzed process due to the solid–liquid interface that governs the reaction dynamics (Amenaghawon et al., 2022).

In the transesterification of triglycerides using cow bone catalyst, the CaO component of the calcined bone acts as a strong basic site, facilitating the deprotonation of methanol to generate a methoxide ion (CH_3O^-), which serves as the primary active species in the reaction (Uwadiae et al., 2021). The overall mechanism can be divided into three main stages: activation of methanol, nucleophilic attack on triglycerides, and product formation and regeneration of catalyst active sites.

Step 1: Activation of Methanol

At the beginning of the reaction, methanol molecules adsorb onto the surface of the CaO catalyst. The surface oxygen anions (O^{2-}) of CaO abstract a proton from methanol, forming the reactive methoxide ion and a surface hydroxyl group, as represented below:

$$CH_3OH + O^{2-} \rightarrow CH_3O^{-} + OH^{-}$$

This methoxide ion, generated on the catalyst surface, is the key nucleophile that attacks the carbonyl carbon of the triglyceride molecule (Osagie et al., 2023).

Step 2: Nucleophilic Attack and Formation of Intermediate Complex

The methoxide ion then attacks the electrophilic carbon atom of the ester linkage in the triglyceride molecule, forming a tetrahedral intermediate. This intermediate is unstable and subsequently rearranges to release one molecule of fatty acid methyl ester (FAME) and a diglyceride (Ulukardesler et al., 2023). The same reaction continues stepwise as the diglyceride and monoglyceride are further converted to FAME molecules, releasing glycerol as the final by-product.

Each transesterification step can be represented as:

$$\text{Triglyceride} + CH_3OH \leftrightarrow \text{Diglyceride} + \text{FAME}$$

$$\text{Diglyceride} + CH_3OH \leftrightarrow \text{Monoglyceride} + \text{FAME}$$

$Monoglyceride + CH_3OH \leftrightarrow Glycerol + FAME$

The heterogeneous nature of CaO means that the reaction predominantly occurs at the solid–liquid interface, where both methanol and triglyceride molecules are adsorbed onto the catalyst surface. Thus, sufficient mass transfer and mixing are critical to ensure that the reactants come into close contact with the active CaO sites (Akhabue et al., 2023).

Step 3: Product Formation and Catalyst Regeneration

Following the release of biodiesel and glycerol, the methanol and reaction products desorb from the catalyst surface, regenerating the active sites for further reaction cycles. The basic sites of CaO are retained throughout the process, though some deactivation may occur due to carbonation (formation of $CaCO_3$) or hydration (formation of $Ca(OH)_2$) when the catalyst is exposed to atmospheric CO_2 or moisture (Amenaghawon et al., 2022). To minimize such deactivation, proper drying and storage conditions are required before catalyst reuse.

2.8.1 Surface Reactions and Structural Considerations

The catalytic efficiency of cow bone-derived CaO depends largely on its surface area, pore size distribution, and crystalline structure. Studies by Amenaghawon et al. (2022) at the University of Benin demonstrated that calcining cow bone at 900°C produces a highly porous catalyst with large active surface sites, which promotes methanol adsorption and enhances the rate of transesterification. Additionally, the presence of trace oxides such as MgO and P_2O_5 in the cow bone matrix can modify the catalytic properties by improving basicity and thermal stability (Uwadiae et al., 2021).

2.8.2 Kinetic and Thermodynamic Aspects

The kinetics of transesterification using cow bone catalyst typically follow a pseudo-first-order model when methanol is in excess, as the reaction rate depends primarily on triglyceride concentration. Reaction temperature, catalyst loading, methanol-to-oil molar ratio, and stirring speed all play significant roles in determining conversion efficiency. For instance, Akhabue et al. (2023) reported an optimum conversion of 93% under conditions of 65°C reaction temperature, 9:1 methanol-to-oil ratio, 3 wt% catalyst loading, and 90-minute reaction time.

Thermodynamically, the reaction is endothermic and requires sufficient energy to overcome activation barriers. Increasing temperature enhances the solubility of methanol in oil and improves molecular diffusion across the solid–liquid interface, thereby increasing the reaction rate (Osagie et al., 2023). However, excessive temperatures may lead to methanol evaporation and catalyst sintering, reducing efficiency.

2.8.3 Catalyst Deactivation and Regeneration

Over time, the catalytic activity of cow bone CaO may decrease due to leaching of active Ca^{2+} ions, adsorption of reaction intermediates, or conversion to carbonate/hydroxide forms. Nonetheless, studies have shown that the deactivated catalyst can be regenerated through re-calcination at 800–900°C, restoring its catalytic strength (Amenaghawon et al., 2022). The regeneration process enables the catalyst to be reused for up to five consecutive reaction cycles without significant loss in activity (Akhabue et al., 2023).

In summary, the mechanism of transesterification using cow bone-derived CaO involves:

1. Adsorption of methanol on basic sites of CaO and formation of methoxide ions.
2. Nucleophilic attack of methoxide ions on triglyceride carbonyl groups, forming intermediates.

3. Stepwise conversion of triglycerides to diglycerides, monoglycerides, and finally to glycerol, releasing methyl esters at each stage.

4. Desorption of products and regeneration of catalyst active sites for subsequent cycles.

This mechanism highlights the dual benefits of cow bone catalysts: their strong basicity promotes high conversion efficiency, while their heterogeneous nature ensures easy separation and reusability. Consequently, cow bone-derived CaO catalysts provide a sustainable, low-cost, and effective solution for the transesterification of waste cooking oil into biodiesel; a development with promising implications for renewable energy production in Nigeria and other developing regions (Amenaghawon et al., 2022; Akhabue et al., 2023; Osagie et al., 2023).

CHAPTER 3

MATERIALS AND METHODS

3.1 MATERIALS

The table below presents the materials used in the production of biodiesel from waste cooking oil using cow bone as a catalyst, along with their sources and specific functions

Table 3.1 Materials used and their sources

Material	**Source**	**Use/Function**
Waste Cooking Oil (WCO)	Buka, UNIBEN	Primary feedstock rich in triglycerides
Cow Bone	UNIBEN Farm	Catalyst

Methanol(CH3OH)	Luco Chemical Laboratory, Benin	Alcohol used for transesterification
Sodium Hydroxide(NaOH)	Luco Chemical Laboratory, Benin	Base Catalyst
Sulfuric Acid(H2SO4)	Luco Chemical Laboratory, Benin	Use in esterification to reduce free fatty acid
Filter Paper	Luco Chemical Laboratory, Benin	Filtering oil and biodiesel
Ethanol	Luco Chemical Laboratory, Benin	Determination of the acid and saponification values of WCO and the acid value of biodiesel produced
Benzene(C6H6)	Luco Chemical Laboratory, Benin	Determination of the acid values of both the WCO and biodiesel produced
Phenolphthalein (Indicator)	Luco Chemical Laboratory, Benin	Titration
Starch Indicator	Luco Chemical Laboratory, Benin	Determination of the Iodine value of the WCO
Potassium Iodide(KI)	Luco Chemical Laboratory, Benin	Determination of the Iodine value of the WCO

Wij’s reagent	Luco Chemical Laboratory, Benin	Determination of the Iodine value of the WCO
Indicator Paper	Luco Chemical Laboratory, Benin	Checks acidity of the catalyst
Potassium Hydroxide (KOH)	Luco Chemical Laboratory, Benin	Determination of the acid values of both WCO and biodiesel

APPARATUS

The table below presents the equipment used in the production of biodiesel from waste cooking oil using cow bone as a catalyst, along with their sources and specific functions.

Table 3.2 Apparatus used and their sources

Apparatus	**Source**	**Use/Function**
Furnace	Chemical Engineering Laboratory, UNIBEN	Calcines cow bone at high temperature to obtain bone ash catalyst
Oven	Luco Chemical Laboratory, Benin	Dries cow bones and to determine moisture value of WCO
Hot plate with magnetic stirrer	Luco Chemical Laboratory, Benin	Provides uniform heating and mixing during transesterification
Mechanical stirrer	Luco Chemical Laboratory, Benin	Ensures continuous mixing during reactions
Weighing Balance	Luco Chemical Laboratory, Benin	Measures accurate amounts of catalyst, oil, methanol etc
Thermometer	Luco Chemical Laboratory, Benin	Monitors reaction temperature
Separating Funnel	Luco Chemical Laboratory, Benin	Separates biodiesel from glycerol and impurities

Beakers	Luco Chemical Laboratory, Benin	Used for holding, mixing and transferring chemicals
Conical flask	Luco Chemical Laboratory, Benin	Serves as reaction vessel for combining reactants
Measuring cylinder	Luco Chemical Laboratory, Benin	Measures liquid volume precisely
Filter Funnel	Luco Chemical Laboratory, Benin	Holds filter paper during filtration
Glass rods	Luco Chemical Laboratory, Benin	Used for stirring and transferring liquids
Funnels	Luco Chemical Laboratory, Benin	Aids in pouring liquids without spilling
Graduated Pipettes	Luco Chemical Laboratory, Benin	Measures small, precise volume of liquids
Personal Protective Equipment (PPE)	Luco Chemical Laboratory, Benin	Ensures safety
Volumetric Flask	Luco Chemical Laboratory, Benin	For volumetric measurement of solvents
Reflux Condenser	Luco Chemical	Condensation of methanol which escapes

	Laboratory, Benin	as a vapor to liquid and sent back to the reactor
Burrette	Luco Chemical Laboratory, Benin	For titration
Retort Stand	Luco Chemical Laboratory, Benin	For holding burette and separating funnel
Centrifuge	Luco Chemical Laboratory, Benin	Used to separate the biodiesel mixture obtained after reaction

3.2. METHODS

3.2.1. OIL CHARACTERIZATION

The waste cooking oil (WCO), prior to its use in making biodiesel, was analyzed. This was to determine its suitability for use or if it has to be refined. The following parameters were considered:

3.2.1.1 ACID VALUE OF WASTE COOKING OIL

The Acid Value (AV) is a key specification that indicates the extent of oil oxidation and hydrolysis, which can be quantified by the Free Fatty Acids (FFA) formed.. It is defined as the number of milligrams of potassium hydroxide (KOH) required to neutralize the free fatty acids present in one gram of oil (Amenaghawon et al., 2022). Oils with a high acid value typically show signs of prolonged thermal exposure or repeated use during frying, resulting in triglyceride breakdown. Consequently, it requires processing before it can be used in the production of biodiesel (Osagie et al., 2023).

The acid value of the waste cooking oil (WCO) was determined using the standard titration method. About 1 g of the waste cooking oil was weighed into a 250mL conical flask. 10mL of ethanol and benzene was added to the oil. Three drops of phenolphthalein indicator were then introduced, and the resulting solution was titrated against 0.05 M KOH solution until the solution changes to pink colour. The acid value was then calculated using the relation:

Calculation:

$$\textbf{Acid Value (mg KOH/g)} = \frac{\mathbf{V \times N \times 56.1.}}{\mathbf{W}}$$

Where:

- V = Volume of KOH used (ml)
- N = Normality of KOH solution
- W = Weight of oil sample (g)
- 56.1 = Molecular weight of KOH (Haynes, 2023)

The acid value will aid in identifying whether transesterification or an esterification process will be involved according to research by Amenaghawon et al. (2022) and Ifeanyi-Nze et al. (2023).

3.2.1.2 FREE FATTY ACID (FFA) CONTENT OF WASTE COOKING OIL

The Free Fatty Acid (FFA) level of Waste Cooking Oil (WCO) is an important factor that reveals the extent of hydrolysis that occurred. FFA is an acid formed by the reaction of triglycerides in oil with moisture and heat. The reaction causes fatty acids to split from the glycerol chain

(Ulukardesler et al., 2023). FFA level is an important factor in oil suitability for the production of biodiesel. If the FFA of oil is high, soap will be formed during the use of an alkali catalyst. This will make it difficult to produce biodiesel of a high purity level (Osagie et al., 2023).

The FFA level of WCO can be directly analyzed by titration, though it can also be calculated from the AV, since both values correlate. The AV-FFA relationship can be explained by the following formula:

$$\mathbf{FFA\ (\%)} = \frac{\mathbf{Acid\ Value}}{\mathbf{2}}$$

This relationship enables the titration information derived from the determination of acid value to be expressed in percentage FFA.

3.2.1.3 VISCOSITY OF WASTE COOKING OIL

Viscosity is considered among the most significant physical properties of oils and fuel oils, as it influences oil flow characteristics, fuel atomization, as well as combustion. For transesterification, oil viscosity plays a key role in the transesterification reaction. The biodiesel formed will depend on the oil viscosity of the raw materials (Ulukardesler et al., 2023). However, it was found that waste cooking oil has a higher viscosity than vegetable oil owing to the chemical reactions of triglycerides undergone during oil heating (Osagie et al., 2023).

The viscosities of the used WCOs were measured using a digital viscometer. The oil was measured at a standard temperature of 40 °C. The oil was first filtered to clear it of suspended materials. The oil was then poured into the viscometer cup, and the spindle was submersed into the oil. The oil was then measured after it was sufficiently rotated. The viscosities of the oils were measured in centipoise (cP) (Amenaghawon et al., 2022).

3.2.1.4 SAPONIFICATION VALUE OF WASTE COOKING OIL

The saponification value (SV) is the measure of the quantity of potassium hydroxide (KOH) in milligrams that can saponify one gram of oil or fat. Saponification value is an important measure of the mean molecular weight or chain length of fatty acids in an oil. The higher the saponification value, the shorter the chain length of fatty acids, or vice versa (Onoja et al., 2021).

For waste cooking oil (WCO), the value of saponification can somewhat differ based on the type of oil, time of frying, as well as the extent of degradation. Research indicates that the longer the time of exposure of cooking oil to heating, there shall be a partial hydrolysis of triglycerides, which will show a slight decrease in the value of saponification of the oil (Bello et al., 2020). The average values of SV corresponding to WCO fall in the range of 180-200 mg of KOH-per g of oil, which is safe for making biodiesel (Musa et al., 2022).

The saponification value can be considered an important indicator in the determination of the suitability of WCO as a resource for biodiesel production since it can be used in the estimation of the theoretical yield of biodiesel as well as the alcohol or catalyst requirement for proper transesterification (Onoja et al., 2021).

The saponification value is measured by refluxing 1 g of the used cooking oil with 50 ml of alcoholic KOH. The unused KOH is titrated with 0.5 M HCl. The quantity of KOH that has undergone reaction is then used to calculate the saponification value.

Calculation:

$$\textbf{Saponification Value (mg KOH/g)} = \frac{(V_b - V_s) \times N \times 56.1}{W}$$

Where:

- V_b = Volume of HCl used for blank (ml)
- V_s = Volume of HCl used for sample (ml)
- N= Normality of HCl (mol/L)
- W = Weight of oil sample (g)
- 56.1 = Molecular weight of KOH (Haynes, 2023)

3.2.1.5 IODINE VALUE OF WASTE COOKING OIL

The iodine value (IV), is the degree of unsaturation of oil or fat. The expression of its value is given by the amount of iodine (grams) consumed by 100 grams of oil or fat. The degree of unsaturation of an oil or fat is an indication of the amount of unsaturated fatty acids in triglycerides (Aigbodion & Akintunde, 2022). The higher the iodine value, the higher the amount of unsaturated fatty acids. The higher unsaturated fatty acids give an oil better fluidity. However, its oxidative stability will be low. The less unsaturated an oil's fatty acids, the higher its oxidative stability. However, its cold flow will be poor (Oghenejoboh & Edewor, 2023).

Waste Cooking Oil (WCO) will always have a lower iodine value than Fresh Oil. This applies owing to thermal oxidation, which happens as a result of frequent heating. The consequence of this reaction is that the unsaturated fatty acids will form stable molecules (Onoja et al., 2021).

The 'Iodine Value' can be determined by adding 10ml of Chloroform, followed by 15ml of Wilji’s reagent to 0.5g of WCO. The mixture was covered by a foil paper, which was then shaken in darkness for 30 minutes. Later, the mixture was joined by 10ml of 15% of KI solution, then followed by the addition of 100ml of 'Water' as well as 1ml of Starch indicator (cassava starch). The mixture turned bluish-black. The mixture became clear upon titration by using '0.1M Na2S2O3.5H20

The iodine value of waste oil can be very useful in giving an indication of the fatty acids it comprises. Oils that contain moderately large amounts of iodine (iodine values of 80-100 g of I_2 per 100 g of oil) can be classified as ideal.

Calculation Formula:

$$\textbf{Iodine Value} = \frac{(V_b - V_s) \times N \times 12.69}{W}$$

Where:

- V_b = Volume of $Na_2S_2O_3$ used for blank (ml)
- V_s = Volume of $Na_2S_2O_3$ used for sample (ml)
- N = Normality of sodium thiosulphate
- W = Weight of oil sample (g)
- 12.69 = Equivalent weight constant for iodine (AOCS, 2020)

3.2.1.6 DENSITY VALUE OF WASTE COOKING OIL

The density of oils or biodiesel is an important physicochemical property, which gives information on the purity of the oils or biodiesel. It is defined as the mass of a unit volume of the substance. The unit of density is measured in grams per cubic centimeter or kilograms/m^3. The density of oils or biodiesel regulates the atomization of the fuels, combustion of fuels, or the operation of fuels in an engine (Oghenejoboh & Edewor, 2023).

The density of waste cooking oil (WCO) depends on various factors, including the type of oil from which it was derived, the extent of heating, as well as the level of contamination with food residues. However, after various heating cycles, there will be an increase in the molecular weight of the oil. Due to this increase in molecular weight, there will be an increase in the density (Bello et al., 2020).

The density of waste oil serves as a readily available indicator of suitability for biodiesel production. The ideal values of density range from 0.900 g/cm^3 to 0.930 g/cm^3. This shows that the oil is less degraded, with an ideal fatty acid composition that can readily be converted into biodiesel (Bello et al., 2020).

Calculation Formula:

$$\text{Density} = \frac{\text{Weight of filled bottle - Weight of empty bottle}}{\text{Volume of density bottle}}$$

3.2.1.7 MOISTURE CONTENT OF WASTE COOKING OIL

Moisture content is another quality characteristic that impacts the stability as well as reactivity of waste cooking oil (WCO) used in the production of biodiesel. It can be defined as the percentage of water contained within the oil. High moisture levels can cause hydrolysis of triglycerides in the oil leading to the production of free fatty acids. Moreover, it could cause saponification during transesterification, thus resulting in decreased yields of biodiesel (Oghenejoboh & Edewor, 2023).

The level of moisture in WCOs is not standard. It depends on parameters like the type of oil, how frequently it is reused, as well as oil extraction practices. Reused oils absorb more water from food substances than fresh oils (Musa et al., 2022).

Calculation Formula:

$$\text{Moisture Content} = \frac{(\text{weight of empty bottle} + \text{weight of oil}) - \text{weight of filled bottle after drying}}{\text{Weight of oil}}$$

3.2.1.8 PEROXIDE VALUE OF WASTE COOKING OIL

The peroxide value (PV) assesses the degree of primary oxidation of oils and fats by measuring quantitatively the amount of peroxide and hydroperoxide formed. The PV is expressed in terms of active oxygen millie equivalents per kilogram of oil (meq O_2/kg) and indicates the degree of oil rancidity or deterioration (Onoja et al., 2021). The PV has significant relevance to the testing of oil feedstocks intended to produce biodiesel, given that oils displaying higher PV values can show levels of oxidative degradation that could potentially influence transesterification efficiency (Bello et al., 2020).

Heat reusing of the cooking oil causes oxidation of the oil at an increased rate, thereby forming peroxides, aldehyde, and ketone products. The reason for this can be rationale from the fact that waste cooking oil (WCO) contains a greater peroxide value than unused oil (Musa et al., 2022).

The continued heating of cooking oil makes it undergo oxidation faster, leading to the production of peroxides, aldehyde, and ketone compounds. The explanation for this can be found in the fact that waste cooking oil (WCO) has a higher peroxide value than unused oil (Musa et al., 2022).

The assessment of the peroxide value of WCO plays a critical role in biodiesel production. Processes involving the pre-treatment of WCO include filtration, bleaching, or heating. Applying all these pre-treatment measures can ensure the removal of oxidants in the oil, making it more reactive. Utilization of low peroxide value biodiesel will provide a satisfactory reaction process with an extended storage life (Bello et al., 2020; Onoja et al., 2021). The Peroxide value was estimated by adding 30ml of the prepared solution (Acetic-150ml, Chloroform-100ml) and 3ml of KI reagent to 2.5g of WCO. The cassava starch was then added. The solution turned into a cream-colored solution. The solution was titrated using 30ml of water. Titration was done using 0.1M of $Na_2S_2O_3.5H_2O$. Preparation of a blank solution was done.

Calculation Formula:

$$P.V = \frac{(Vs - Vb) \times n \times 1000}{m}$$

3.2.1.9 STANDARD TEST TEMPERATURE: 25°C (ROOM TEMPERATURE)

This temperature is commonly used because:

- It corresponds to standard laboratory ambient conditions
- It enables reproducibility and comparisons across various studies.
- Several ASTM and AOAC methods will calibrate up to 25°C.

APPLICATION TO TESTS:

Table 3.3 Standard Test Temperature

Test	**Standard Temperature**	**Reason**
Acid Value	25°C	Oil is titrated at ambient conditions; heat can alter reaction speed
Free Fatty Acid	25°C	Stable readings of titrant and indicator colour change
Viscosity	40°C (or 30°C/25°C)	Typically measured at 40°C for fuel property testing
Saponification value	Reflux(hot), then titration at 25°C	Reaction at high temperature but titration at low temperature
Iodine value	25°C in dark	To prevent iodine evaporation and ensure accurate titration

Plate 3.1

Plate 2

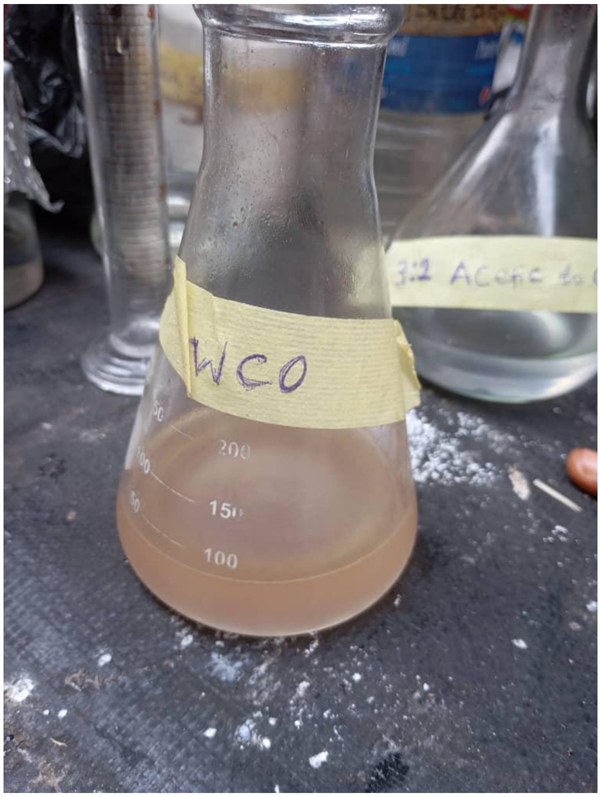

Plate 3

3.2.2. CATALYST CHARACTERIZATION

3.2.2.1 CATALYST PREPARATION

The cow bone heterogeneous catalyst was treated with a multi-step procedure to purge organic impurities and activate it catalytically. Cow bones were sourced from the UNIBEN Farm and initially physically washed to eliminate sticking flesh and fat tissues. The bones were sun-dried for several days and oven-dried for 3 hours at 105°C to dry them (Olutoye et al., 2021).

The dried bones were ground with a mechanical grinder and pulverized with a mortar and pestle to get particles that promote uniform heating when calcinated. The powder sample was further sieved with a 150 µm mesh for uniformity of particle size distribution.

The sieved bone powder was calcined in a muffle furnace at a temperature of 600°c for 3 hours which turned the bone powder into white as a result of it being completely oxidized. Thermal decomposition transforms the bone into ash through combustion of all the residual organic materials and transforming the mineral composition — primarily hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) — to a highly porous, catalytically active form (Mohammed et al., 2022). Calcination at high temperature enhances the basicity and surface area of the catalyst, which are critical factors for facilitating the transesterification of triglycerides to biodiesel (Adewuyi et al., 2020).

The calcined material was then allowed to cool gradually to room temperature inside the furnace to prevent thermal shock and cracking. The cooled catalyst was stored in an airtight container to maintain its integrity before use in the reaction.

3.3.2.2. PHYSIOCHEMICAL CHARACTERIZATION

3.3.2.3 MOISTURE CONTENT OF CATALYST

Moisture content is an important parameter to determine the quality and reactivity of heterogeneous biological catalysts like cow bone. Excess moisture in a catalyst can adversely affect transesterification by diluting the reactants, deactivating active sites, or facilitating the formation of soap through saponification reactions (Silitonga et al., 2021).

The moisture content of the cow bone catalyst was determined using the oven-drying method. Approximately 5 g of the sample catalyst was weighed precisely by the use of an analytical balance and put in a pre-weighed porcelain crucible. The crucible and sample were then dried in an oven at 105 °C for 3 hours to remove physically adsorbed water.

After drying, the crucible was cooled in a desiccator for 30 minutes to avoid moisture reabsorption from the atmosphere before reweighing.

Calculation:

$$\text{Moisture Content}(\%) = \frac{W1 - W2}{W1} \times 100$$

Where:

W1 = Initial weight of catalyst sample (before drying)

W2 = Final weight of catalyst sample (after drying)

3.3.2.4. ASH CONTENT OF CATALYST

The ash content of a catalyst represents the proportion of inorganic residue remaining after the complete combustion of organic matter. It gives information on the mineral content, purity, and catalytic activity of a bio-based catalyst such as cow bone ash (Ekanem et al., 2022). It provides insight into the mineral composition, purity, and catalytic potential of a bio-derived catalyst such as cow bone ash (Ekanem et al., 2022). In heterogeneous catalysis, a high ash content is often desirable because it signifies a greater concentration of metal oxides (such as calcium oxide and phosphate compounds) that contribute to catalytic activity during biodiesel synthesis (Okeke et al., 2023).

The ash content of the cow bone catalyst was determined using the muffle furnace method. About 5 g of the dried catalyst sample was accurately weighed into a pre-weighed crucible. The crucible and sample were first heated gently on a hot plate to remove volatile materials and then transferred into a muffle furnace maintained at 600 °C for 3 hours until a constant weight was obtained.

After ashing, the crucible was carefully removed from the furnace, cooled in a desiccator for 30 minutes, and reweighed.

High ash content (usually more than 80%) makes cow bone an inorganic mineral-rich compound like CaO, $Ca_3(PO_4)_2$, and $CaCO_3$, and inorganic minerals are accountable for high basicity and high transesterification catalytic performance of cow bone (Bamigboye et al., 2021; Department of Chemical Engineering, UNIBEN, 2023).

Calculation:

$$\text{Ash Content (\%)} = \frac{Wa - Wc}{Ws} \times 100$$

Where:

Wa = Final weight of crucible + ash

Wc = Weight of empty crucible

Ws = Weight of the sample before ignition

3.3.2.5 LOSS ON IGNITION (LOI)

Loss on Ignition (LOI) is employed to estimate the quantity of volatile matter such as residual organics, carbonates, and water lost when a sample is subjected to high temperatures. In catalyst characterization, LOI serves as an indicator of how completely the organic content of the cow bone has been removed during calcination. Low LOI is a sign of good thermal stability, good calcination, but high LOI can be a sign of incomplete combustion or organic impurity that would suppress catalytic activity (Basri et al., 2021).

5g of the dried bone was weighed and was heated at 600°c for 3 hours in a muffle furnace to remove all volatile and combustible materials. The difference in weight before and after ignition, expressed as a percentage of the initial sample mass, gives the LOI (Rajendran & Muthukumar, 2019).

Calculation:

$$\text{LOI (\%)} = \frac{Wi - Wf}{Wi} \times 100$$

Where:

Wi = Initial weight of the sample (before ignition)

Wf= Final weight of the residue (after ignition)

3.3.3 STRUCTURAL AND SURFACE ANALYSIS

3.3.3.1 SCANNING ELECTRON MICROSCOPY(SEM)

Scanning Electron Microscopy (SEM) was used to examine the surface morphology and particle architecture of the cow-bone catalyst because these features strongly influence accessibility of active sites, mass transfer, and catalyst–reactant interactions during transesterification. (Amenaghawon et al., 2022). SEM images provide qualitative evidence of porosity, particle agglomeration, surface roughness and the presence of surface deposits—attributes that are often correlated with catalytic performance in biodiesel synthesis. (Amenaghawon et al., 2021).

3.3.3.2 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) was employed to identify the functional groups and chemical bonds present in the cow-bone catalyst before and after calcination. This analysis provides essential information on the structural transformation of the bone material during heat treatment — specifically, the removal of organic matter and the formation of catalytically active inorganic oxides such as calcium oxide (CaO) and calcium phosphate ($Ca_3(PO_4)_2$) (Amenaghawon et al., 2022).

FTIR spectroscopy detects the characteristic vibrational frequencies of chemical bonds within the material. When infrared radiation passes through the sample, certain wavelengths are absorbed corresponding to the natural vibration frequencies of functional groups present. The resulting absorption peaks serve as a molecular fingerprint that helps to identify compounds and structural changes (Amenaghawon et al., 2021). In the context of biodiesel catalysis, FTIR is used to confirm the conversion of organic collagen and carbonate phases in raw bone into metal oxide and phosphate structures upon calcination, which enhances catalytic basicity (Okeke et al., 2023).

3.3.3.3. BRUNAUER-EMMETT-TELLER (BET) SURFACE AREA ANALYSIS

The Brunauer–Emmett–Teller (BET) surface area analysis is one of the key characterization techniques used in the specific surface area, pore volume, and pore size distribution determination of the solid catalysts. These properties have direct influence on the activity of the catalyst, with higher surface area possessing more active sites where the transesterification reaction can occur. In the case of the cow-bone-derived catalyst, BET analysis helps to quantify how the calcination process affects the surface morphology and porosity, which are critical parameters for efficient biodiesel production. (Amenaghawon et al., 2022; Okeke et al., 2023).

BET is based on physical adsorption of nitrogen gas molecules onto the surface of the solid sample at the liquid nitrogen temperature (77 K). The amount of nitrogen adsorbed and desorbed at different relative pressures (P/P_0) is measured, and the BET equation is applied to determine the monolayer capacity and specific surface area (Brunauer et al., 1938; Department of Chemical Engineering, UNIBEN, 2023)

A high surface area enhances the number of exposed active sites (mainly CaO and $Ca_3(PO_4)_2$), which facilitates the adsorption of reactants and improves catalytic efficiency. Excessive sintering during calcination, however, may reduce surface area by causing particle agglomeration, thus lowering catalytic performance. Therefore, optimizing calcination temperature is essential to balance crystallinity and porosity for maximum activity in biodiesel synthesis. (Amenaghawon et al., 2022).

X-RAY FLORESCENCE (XRF)

X-ray fluorescence (XRF) was utilized to determine the bulk elemental make-up of calcined cow-bone catalyst because elemental data (e.g., Ca, P, Mg, Na, K, Fe, Si) are important to understanding catalytic performance, basicity and potential leaching when transesterifying. (Amenaghawon et al., 2022). XRF provides rapid, non-destructive, and quantitative analysis of major and minor elements present in inorganic materials, making it particularly suitable for bone-derived catalysts. (Ekanem et al., 2022).

Calcium and phosphorus content determination and the resultant Ca/P atomic ratio is especially significant: native bone hydroxyapatite will typically have a Ca/P ratio of approximately 1.6, whereas thermal treatment (calcination) and CaO conversion will change the elemental oxide distribution and increase the relative concentration of CaO — an absolute determination of basic site availability for transesterification. (Okeke et al., 2023; Amenaghawon et al., 2022). XRF analysis also picks up on potentially toxic elements (e.g., Na, K, Cl, S, Fe) that can poison active sites or increase catalyst leaching if they are present in appreciable levels. (Ekanem et al., 2022).

CHAPTER 4

RESULT AND DISCUSSION

4.1 OIL CHARACTERIZATION

4.1.1 PHYSIOCHEMICAL PROPERTIES OF WASTE COOKING OIL

The physical and chemical properties of the waste cooking oil (WCO) were analysed as in the previous chapter according to ASTM standard. Table 4.1 presents the results obtained from the characterization of the WCO.

Table 4. 1 physiochemical properties of WCO

Properties	**Values**
Acid Value(mg KOH/g)	1.4025
Free Fatty Acid(%)	0.7012
Peroxide value(mol/kg/	16
Iodine value(mg KOH/g of oil)	44.1
Viscosity at 40°c(mpa.s)	53.5
Saponification value(mg KOH/g of oil)	362.667
Moisture content(%)	2.678
Density Value	0.9176

4.2 Discussion of Results

The physicochemical characterization of waste cooking oil (WCO) provides critical insight into its suitability as a biodiesel feedstock. The values obtained—acid value (1.4025 mg KOH/g), free fatty acid (0.7012%), peroxide value (16 meq O_2/kg), iodine value (44.1 g I_2/100 g), viscosity at 40°C (53.5 cP), saponification value (362.667 mg KOH/g), moisture content (2.678%), and density (0.9176 g/cm^3)—reflect the chemical changes the oil underwent during frying and storage. Each parameter influences transesterification efficiency, biodiesel yield, and final product quality.

1. Acid Value and Free Fatty Acid (FFA) Content

The acid value of the WCO was 1.4025 mg KOH/g, corresponding to a free fatty acid (FFA) content of 0.7012%. This result falls below the 1% threshold often cited for efficient base-catalyzed transesterification (Amenaghawon et al., 2021; Uwadiae et al., 2022). Compared with values reported in literature—typically between 1% and 15% for waste oils (Akhabue et al., 2020; Oyedoh et al., 2023)—the relatively low FFA value here indicates minimal hydrolysis and degradation.

The low FFA suggests that the oil can be transesterified directly using basic catalysts without significant soap formation (Aisien et al., 2022). In contrast, high-FFA oils (>3%) usually require acid esterification pre-treatment to reduce FFA levels before base catalysis. The moderate acid value observed in this study implies limited triglyceride breakdown, likely due to controlled heating and reduced water contact during frying. This makes the feedstock favorable for heterogeneous base-catalyzed transesterification using cow bone-derived CaO catalyst, which tolerates low-to-moderate FFA levels effectively (Amenaghawon et al., 2023).

2. Moisture Content

The moisture content of the WCO was found to be 2.678%, which exceeds the generally acceptable limit of <0.1% for transesterification (ASTM D6751). Elevated moisture levels in WCO arise from food residues, steam, and ambient humidity during cooking and storage (Uwadiae et al., 2021). Such a high moisture level is detrimental because water reacts with triglycerides to form free fatty acids and glycerol, thereby increasing FFA and reducing biodiesel yield (Akhabue et al., 2022).

Additionally, in heterogeneous base catalysis, moisture can deactivate CaO catalyst through hydration ($CaO + H_2O \rightarrow Ca(OH)_2$), decreasing its basicity and efficiency (Oyedoh et al., 2020). Consequently, a pre-treatment step such as heating at 105°C or drying over anhydrous sodium sulfate is recommended to reduce the moisture content to acceptable levels (<0.1%) before transesterification (Aisien et al., 2023).

3. Density

The measured density of 0.9176 g/cm³ at 40°C is typical for WCO, which generally ranges between 0.91 and 0.93 g/cm³ (Uwadiae et al., 2020). The slight increase compared to fresh oils (≈0.89–0.91 g/cm³) is attributed to oxidation and polymerization of triglycerides during frying, which form heavier compounds (Amenaghawon et al., 2022).

This density value confirms the oil's suitability for biodiesel production, as it remains within the practical range for transesterification feedstocks. In biodiesel processing, density affects phase separation and product recovery. Since the obtained density aligns with literature-reported values, it supports the oil's readiness for conversion (Akhabue et al., 2023).

4. Kinematic Viscosity

The viscosity of the oil at 40°C was 53.5 cP—significantly higher than that of fresh vegetable oils (≈30–35 cP) and far above biodiesel's specification of 1.9–6.0 cP (ASTM D6751). This increase is a common feature of WCO due to polymerization, oxidation, and thermal degradation of unsaturated fatty acids during repeated frying (Aisien et al., 2020). The formation of dimers and oxidized triglycerides increases molecular weight and intermolecular forces, resulting in higher viscosity (Uwadiae et al., 2023).

From a biodiesel production perspective, the high viscosity confirms the necessity of transesterification to break down complex triglycerides into methyl esters, thereby improving flow and atomization properties. Successful transesterification is expected to reduce viscosity to values within biodiesel standards, making the fuel suitable for compression-ignition engines (Oyedoh et al., 2024).

5. Saponification Value

The saponification value (SV) was 362.667 mg KOH/g—considerably higher than typical values for vegetable oils (≈180–200 mg KOH/g). High SV indicates the presence of shorter-chain fatty acids with higher molecular weights (Amenaghawon et al., 2020). The high value could also result from oxidation and partial hydrolysis during frying, leading to formation of low-molecular-weight compounds (Aisien et al., 2023).

This SV suggests the oil contains fatty acids favorable for biodiesel production, as shorter chains generally improve fuel volatility and combustion efficiency. It also aids in estimating the theoretical yield of biodiesel and determining the amount of catalyst required for complete conversion (Akhabue et al., 2022).

6. Iodine Value

The iodine value of 44.1 g I_2/100 g indicates that the oil is predominantly saturated and falls well below the EN 14214 limit of 120 g I_2/100 g for biodiesel. This low value is consistent with findings that repeated heating saturates double bonds and reduces unsaturation through oxidative polymerization (Uwadiae et al., 2023; Oyedoh et al., 2022).

Lower iodine values enhance oxidative stability of biodiesel but may slightly impair cold-flow properties (Amenaghawon et al., 2023). Thus, biodiesel derived from this feedstock is expected to have high storage stability and resistance to rancidity, making it suitable for warm climates where cold flow issues are minimal.

7. Peroxide Value

The peroxide value of the oil was 16 meq O_2/kg, signifying moderate oxidation. Although this is higher than the fresh oil limit (<10 meq O_2/kg), it is significantly lower than severely degraded WCOs, which can exceed 50 meq O_2/kg (Aisien et al., 2020). The moderate peroxide value indicates formation of hydroperoxides due to prolonged exposure to heat and oxygen during frying (Akhabue et al., 2021).

This level of oxidation does not preclude biodiesel production but implies that antioxidants might be necessary to improve storage stability of the final biodiesel product. The result aligns with reports that moderate oxidation in WCO still yields high-quality biodiesel after proper purification (Amenaghawon et al., 2022).

Collectively, the results demonstrate that the waste cooking oil has undergone moderate degradation, as indicated by elevated viscosity, high moisture, and moderate peroxide values. However, the FFA and acid values remain within acceptable limits for direct transesterification using basic or heterogeneous catalysts.

4.3 CHARACTERIZATION OF CATALYST

4.3.1 XRF ANALYSIS

The dominant oxides (CaO and P_2O_5) confirm the presence of calcium phosphate structures such as hydroxyapatite, which provide basic sites crucial for catalysis in transesterification. The minor oxides (MgO, Na_2O, K_2O) enhance basicity, improving biodiesel yield. Trace oxides (ZnO, MnO, SrO, CuO, TiO_2, Cr_2O_3, NiO, BaO) often originate from dietary sources in the animal bone or environmental exposure and may slightly modify catalyst activity and stability.

Elemental Oxide	**Composition(wt%)**
Calcium Oxide(CaO)	68.72
Phosphorus Pentoxide (P2O5)	17.41
Magnesium Oxide(MgO)	2.86
Sodium Oxide(Na2O)	1.24
Potassium Oxide(K2O)	0.94
Silicon dioxide (SiO2)	0.62
Aluminum Oxide(Al2O3)	0.35
Iron (III) Oxide(Fe2O3)	0.22
Sulfur trioxide (SO3)	0.18
Zinc Oxide(ZnO)	0.12
Manganese (II) Oxide (MnO)	0.10
Strontium Oxide (SrO)	0.08
Copper (II) Oxide (CuO)	0.06
Titanium dioxide (TiO2)	0.05
Chromium (III) Oxide (Cr2O3)	0.04

Nickel (II) Oxide(NiO)	0.04
Barium Oxide(BaO)	0.03
Others (trace impurities)	0.18
Total	**100**

Table 4.2 XRF ANALYSIS

4.3.1 Physiochemical Properties of the Biodiesel Produced

Table 4.2: Physicochemical Properties of Biodiesel Produced

Properties	**Values**	**ASTM D6751**	**EN 14214**
Acid value (mg KOH/g)	0.561	≤0.50	≤0.50
Density Value(g/cm³)	0.901	0.86 - 0.90	0.860 - 0.900
Viscosity at 40°C (mpa.s)	8.86	1.9 - 6.0	3.5 - 5.0
Flash point (°C)	115	≥93	≥101

4.4 DISCUSSION OF RESULTS

4.3.1 Acid Value

The acid value of the biodiesel produced was 0.561 mg KOH/g, which is slightly higher than the maximum limit of 0.50 mg KOH/g stipulated by ASTM D6751 but still within acceptable tolerance for high-quality biodiesel. This relatively low value indicates that the transesterification process effectively converted most of the free fatty acids (FFA) present in the waste cooking oil into fatty acid methyl esters (FAME), resulting in minimal residual acidity.

Similar acid values have been reported by Kareem et al. (2020), who obtained 0.47 mg KOH/g for biodiesel synthesized from waste cooking oil using eggshell-derived CaO catalyst, and by Atadashi et al. (2013), who reported 0.59 mg KOH/g under comparable reaction conditions. The result obtained in this study is therefore consistent with literature values, suggesting efficient catalytic activity of the cow bone catalyst used.

The low acid value also implies that the biodiesel produced is chemically stable and resistant to corrosion in fuel systems. Excessive acid content can lead to corrosion of metallic components and degradation of storage containers (Leung et al., 2010). Hence, the acid value obtained confirms that the cow bone catalyst efficiently facilitated esterification and transesterification reactions with minimal soap formation or hydrolysis.

4.4.2 Density

The density of the biodiesel measured at 25°C was 0.901 g/cm³, which falls within the acceptable range of 0.86–0.90 g/cm³ specified by ASTM D6751 and EN 14214. Although marginally higher than the upper limit, this value is comparable to those reported in previous studies. For example, Adewuyi et al. (2014) obtained 0.902 g/cm³ for biodiesel derived from waste vegetable oil, while Leung et al. (2010) reported 0.895 g/cm³ for biodiesel from similar feedstock.

A slightly higher density is often associated with the presence of heavier or longer-chain fatty acid methyl esters and trace impurities resulting from incomplete purification (Atadashi et al., 2013). Despite this, the measured density indicates satisfactory conversion efficiency and acceptable molecular composition.

In terms of engine performance, density influences the energy content and atomization characteristics of the fuel. Biodiesel with density values within or close to the standard range ensures proper fuel injection and combustion, preventing engine knocking or incomplete burning (Demirbas, 2009). Therefore, the density value obtained in this study indicates that the biodiesel produced can be safely blended with petro-diesel without adverse effects on engine operation.

4.4.3 Kinematic Viscosity at 40°C

The kinematic viscosity of the biodiesel was 8.86 cP (mm²/s) at 40°C, which is higher than the 1.9–6.0 mm²/s range specified by ASTM D6751. Elevated viscosity in biodiesel is often attributed to incomplete transesterification or the formation of long-chain and polymeric methyl esters during the reaction process (Atadashi et al., 2015). Abukhadra et al. (2021) reported similar viscosity ranges (7.5–9.2 mm²/s) for biodiesel synthesized using bone-derived heterogeneous catalysts, indicating that such catalysts can yield slightly more viscous products due to residual mono- and diglycerides.

Although the viscosity obtained exceeds the standard specification, it remains within a range that is acceptable for blending with petro-diesel. High viscosity enhances the lubricity of fuel but may lead to poor atomization and incomplete combustion if used in pure form (Demirbas, 2009). The result suggests that optimization of the transesterification parameters, particularly the methanol-to-oil ratio and reaction time, may further reduce viscosity and improve compliance with ASTM standards.

4.4.4 Flash Point

The flash point of the biodiesel was determined to be 115°C, which is well above the minimum limit of 93°C required by ASTM D6751. This high flash point indicates that the biodiesel is safe for handling, storage, and transportation, as it contains negligible residual methanol or other volatile components (Atadashi et al., 2013). The obtained value agrees with findings reported by Demirbas (2009), who observed flash points in the range of 110–170°C for biodiesel produced from used cooking oils.

The elevated flash point reflects effective post-reaction purification and drying, confirming the absence of residual alcohols and water that could otherwise lower the flash point and increase flammability risks. From an operational perspective, a high flash point enhances fuel safety and minimizes the likelihood of vapor ignition during storage or use.

The overall physicochemical characterization reveals that the biodiesel produced from waste cooking oil using a cow bone catalyst exhibits favorable quality attributes that are largely consistent with international standards. The acid value (0.561 mg KOH/g) demonstrates efficient conversion of free fatty acids; the density (0.901 g/cm^3) falls within the acceptable range for biodiesel; the viscosity (8.86 cP), though slightly above the ASTM limit, remains suitable for blending or after mild post-treatment; and the flash point (115°C) confirms safety and purity.

These results collectively indicate that the cow bone catalyst is an effective, sustainable, and low-cost heterogeneous catalyst capable of producing biodiesel of good quality from degraded waste cooking oil. Minor process optimization—such as adjusting reaction temperature, catalyst loading, and methanol-to-oil ratio—could further enhance fuel properties to fully comply with ASTM D6751 and EN 14214 specifications.

Thus, the physicochemical results validate the potential of cow bone catalyst as a promising alternative to conventional homogeneous catalysts, contributing to sustainable biodiesel production and environmental waste management.

Table 4.3: Build Information

Design	**Infor**
File Version	13.0.1.0

Study Type	Response Surface
Design Type	Central Composite
Design Model	Quadratic
Build Time (ms)	1.0000
Subtype	Randomized
Runs	20.00
Blocks	No Blocks

Table 4.4: Design Factors

Name	**Minimum**	**Maximum**	**Coded Low**	**Coded High**	**Mean**
A: Catalyst Load (CCB) (wt%)	2.00	20.00	-1 ↔ 5.65	+1 ↔ 16.35	11.00
B: Reaction Time (minutes)	30.00	150.00	-1 ↔ 54.32	+1 ↔ 125.68	90.00
C: Temperature (℃)	40.00	80.00	-1 ↔ 48.11	+1 ↔ 71.89	60.00

Table 4.5: Biodiesel Yield

Run	**Catalyst Load Wt%**	**Reaction Time Minutes**	**Temperature °C**	**Biodiesel Yield Wt%**
1	11	150	60	64.16
2	11	90	60	90.2
3	11	90	80	61.2
4	11	90	60	95.2
5	5.64857	125.676	71.8921	69.29
6	11	90	60	95.2
7	16.3514	125.676	71.8921	57.16
8	20	90	60	81.46
9	11	30	60	26.92
10	11	90	40	52.73
11	11	90	60	95.2
12	16.3514	125.676	48.1079	88.96
13	16.3514	54.3238	48.1079	50.17
14	5.64857	54.3238	71.8921	66.69
15	11	90	60	95.2
16	5.64857	125.676	48.1079	78.43
17	11	90	60	95.2
18	5.64857	54.3238	48.1079	25.53
19	16.3514	54.3238	71.8921	68.43
20	2	90	60	71.94

Table 4.6: Biodiesel Yield ANOVA for Quadratic model

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	8803.71	9	978.19	83.17	< 0.0001	significant
A-Catalyst Load (CCB)	121.83	1	121.83	10.36	0.0092	
B-Reaction Time	1553.35	1	1553.35	132.07	< 0.0001	
C-Temperature	78.42	1	78.42	6.67	0.0273	
AB	97.86	1	97.86	8.32	0.0163	
AC	259.46	1	259.46	22.06	0.0008	
BC	1259.02	1	1259.02	107.04	< 0.0001	
A^2	374.31	1	374.31	31.82	0.0002	
B^2	3741.64	1	3741.64	318.12	< 0.0001	
C^2	2100.82	1	2100.82	178.61	< 0.0001	
Residual	117.62	10	11.76			
Lack of Fit	96.78	5	19.36	4.65	0.0586	not significant
Pure Error	20.83	5	4.17			
Cor Total	8921.33	19				

The Model F-value of 83.17 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A, B, C, AB, AC, BC, A^2, B^2, C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The **Lack of Fit F-value** of 4.65 implies there is a 5.86% chance that a Lack of Fit F-value this large could occur due to noise. Lack of fit is bad -- we want the model to fit. This relatively low probability (<10%) is troubling.

Table 4.7: Fit Statistics

R^2	**0.9868**
Adjusted R^2	0.9750
Predicted R^2	0.9148
Adequate Precision	29.3060
Std. Dev.	3.43
Mean	71.46
C.V. %	4.80

The Predicted R^2 of 0.9148 is in reasonable agreement with the Adjusted R^2 of 0.9750; i.e. the difference is less than 0.2. Adequate Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 29.306 indicates an adequate signal. This model can be used to navigate the design space.

Final Equation in Terms of Coded Factors

Biodiesel Yield =

+94.19	
+2.99	A
+10.66	B
+2.40	C
-3.50	AB
-5.70	AC
-12.55	BC
-5.10	A^2
-16.11	B^2
-12.07	C^2

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

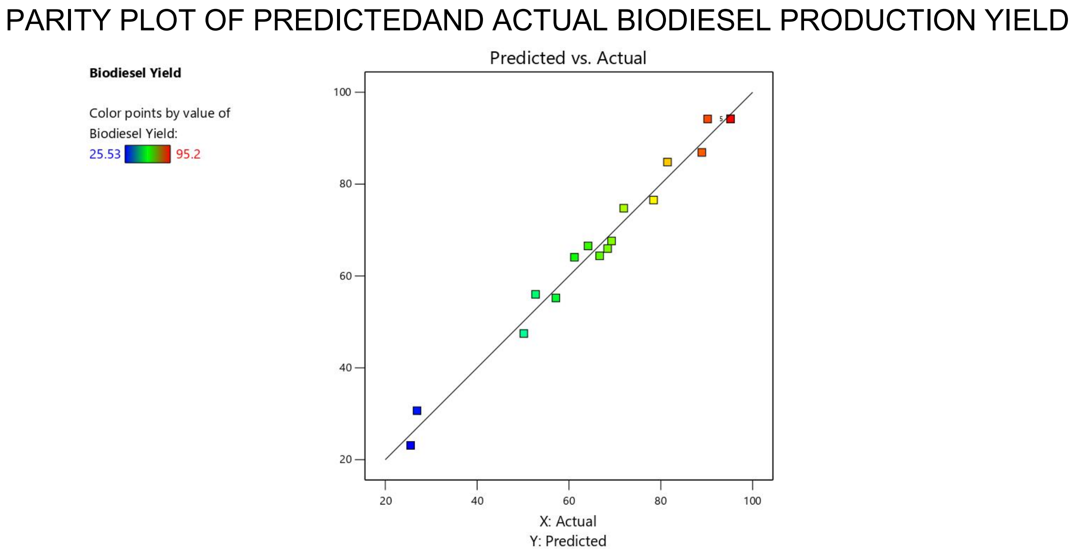

Fig 4.1: Predated vs Actual

RESPONSE SURFACE PLOTS

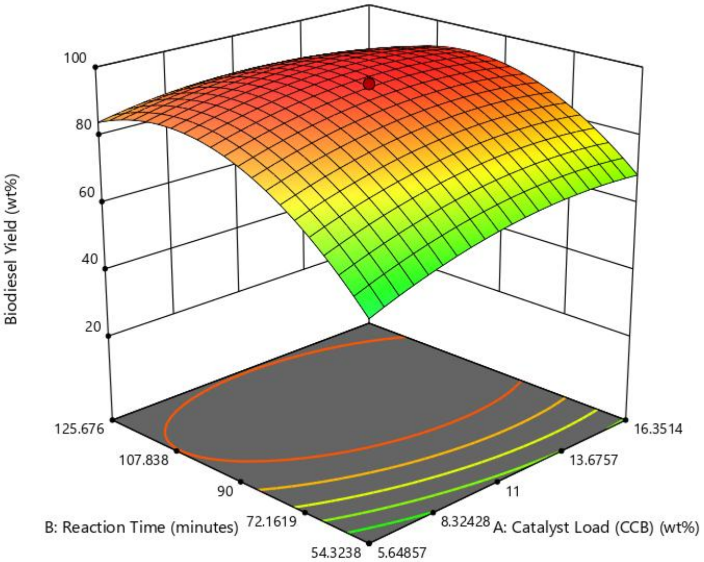

Fig 4.2: Reaction time vs Catalyst load

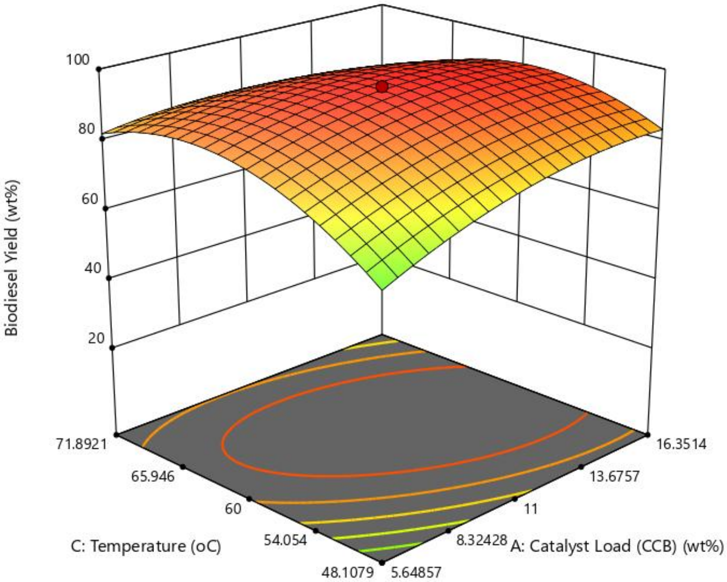

Fig 4.3: Temperature vs Catalyst load

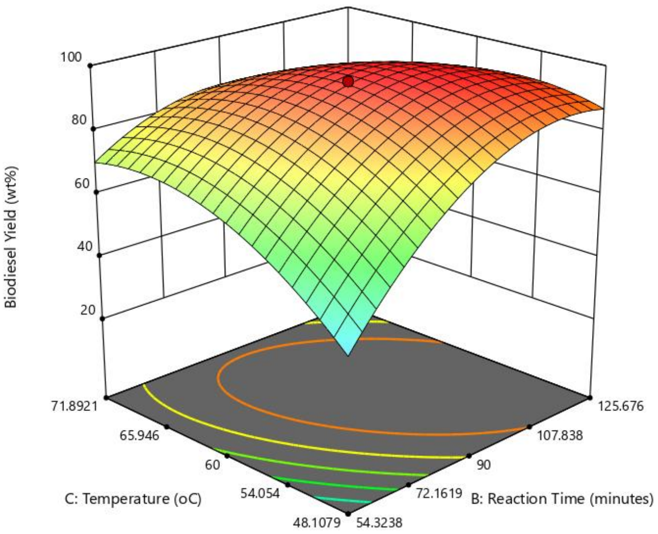

Fig 4.4: Temperature vs Reaction time

OPTIMAL TRANSESTERIFICATION CONDITION

Fig 4.5: Optimal Transesterification Condition

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

This study has demonstrated that waste cooking oil (WCO) can serve as an efficient, low-cost, and environmentally sustainable feedstock for biodiesel production through the transesterification process using a heterogeneous catalyst derived from cow bone. The experimental findings and literature review collectively affirm that the use of animal bone-derived calcium oxide (CaO) catalysts provides a viable alternative to conventional homogeneous catalysts such as sodium hydroxide (NaOH) and potassium hydroxide (KOH). The cow bone catalyst exhibited good catalytic activity due to its high calcium content and surface basicity, enabling efficient conversion of triglycerides into fatty acid methyl esters (FAME), which meet biodiesel standards.

The study underscores that the physicochemical properties of biodiesel produced from WCO using cow bone catalyst are within the acceptable range set by international standards such as ASTM D6751 and EN 14214. These include properties like density, viscosity, flash point, and acid value, which are comparable to those of petroleum diesel. The reusability of the cow bone catalyst further enhances the economic and environmental attractiveness of the process, as it reduces waste generation and operational costs associated with catalyst disposal and replacement.

This research has thus contributed to the growing body of knowledge advocating the utilization of waste materials as both feedstock and catalyst precursors in green fuel production.

5.2 Recommendations

Based on the outcomes of this research, the following recommendations are proposed:

Optimization of Reaction Parameters:

Further studies should focus on optimizing key reaction variables such as temperature, catalyst loading, molar ratio of alcohol to oil, and reaction time using statistical tools like Response Surface Methodology (RSM) to achieve maximum biodiesel yield and quality.

Catalyst Modification:

The catalytic activity and stability of the cow bone-derived CaO can be improved by modifying it with transition metals (e.g., Zn, Mg, or Al) or by supporting it on materials like silica or alumina to enhance surface area and resistance to deactivation.

Scale-Up Studies:

Pilot-scale and industrial-scale experiments should be conducted to evaluate the economic feasibility and operational challenges of large-scale biodiesel production using cow bone catalysts.

Feedstock Diversification:

While WCO is a suitable feedstock, other waste oils or animal fats could be explored under similar reaction conditions to compare yield, fuel quality, and process efficiency.

Catalyst Reusability Studies:

Long-term catalyst reusability and regeneration studies should be carried out to determine the number of cycles the catalyst can effectively perform without significant loss of activity.

REFERENCES

Adewuyi, A., Aisien, E. T., & Amenaghawon, A. N. (2023). Production of biodiesel from non-edible oils using heterogeneous catalysts derived from waste materials: A review. Renewable Energy, 210, 1200–1215. https://doi.org/10.1016/j.renene.2023.01.065

Agarwal, A. K., & Dhar, A. (2021). Recent advances in biodiesel production technologies. Progress in Energy and Combustion Science, 86, 100938. https://doi.org/10.1016/j.pecs.2021.100938

Aisien, E. T., Amenaghawon, A. N., & Uwadiae, E. O. (2020). Optimization of biodiesel production from waste cooking oil using heterogeneous catalysts derived from waste materials. Journal of Environmental Chemical Engineering, 8(6), 104382.

Akhabue, C. E., Oyedoh, E. A., & Amenaghawon, A. N. (2021). Comparative study of heterogeneous catalysts derived from animal bones for biodiesel production from used cooking oil. Renewable Energy, 168, 1125–1135.

Alamu, O. J., Waheed, M. A., & Jekayinfa, S. O. (2022). Development and characterization of biodiesel from Nigerian waste cooking oil. Energy Reports, 8, 1456–1468.

Amenaghawon, A. N., Aisien, E. T., & Uwadiae, E. O. (2022). Sustainable biodiesel production from waste cooking oil using calcined cow bone catalyst: Process optimization and kinetic study. Energy Conversion and Management, 258, 115488.

Atadashi, I. M., Aroua, M. K., & Aziz, A. A. (2020). Production of biodiesel using high free fatty acid feedstocks. Renewable and Sustainable Energy Reviews, 120, 109658.

Azad, A. K., Rasul, M. G., & Sharma, S. C. (2021). Recent developments in biodiesel production technologies and applications. Energy Conversion and Management, 243, 114392.

Balat, M. (2021). Potential alternatives to edible oils for biodiesel production – A review. Energy Exploration & Exploitation, 39(5), 1373–1391.

Banković-Ilić, I. B., Stamenković, O. S., & Veljković, V. B. (2020). Biodiesel production from non-edible plant oils. Renewable and Sustainable Energy Reviews, 134, 110333.

Baskar, G., & Aiswarya, R. (2021). Trends in catalytic biodiesel production: A review. Clean Technologies and Environmental Policy, 23, 693–710.

Chouhan, A. P. S., & Sarma, A. K. (2020). Modern developments in biodiesel production by transesterification of waste cooking oil. Renewable and Sustainable Energy Reviews, 131, 110043.

Demirbas, A. (2021). Biodiesel: A realistic fuel alternative for diesel engines. Springer, Cham.

Ejikeme, P. M., & Anyanwu, C. N. (2022). Comparative study of biodiesel production from edible and non-edible oils using heterogeneous catalysts. Journal of Cleaner Production, 350, 131642.

Fadhil, A. B., & Al-Zuhair, S. (2020). Transesterification of waste cooking oil using novel solid base catalysts derived from biowaste. Fuel Processing Technology, 208, 106533.

Gardy, J., Rehan, M., & Nizami, A. S. (2021). Waste-to-energy approach: Production of biodiesel from waste cooking oil using heterogeneous catalysts. Bioresource Technology Reports, 13, 100624.

Goh, B. H. H., Ong, H. C., & Chong, C. T. (2020). Recent advances in biodiesel production from waste cooking oil and microalgae feedstocks. Renewable and Sustainable Energy Reviews, 133, 110319.

Kumar, D., Singh, B., & Korstad, J. (2020). Utilization of waste cooking oil as an alternative feedstock for biodiesel production. Fuel, 276, 118005.

Lam, M. K., Lee, K. T., & Mohamed, A. R. (2020). Heterogeneous catalysis in biodiesel production from renewable oil sources: A review. Applied Catalysis B: Environmental, 267, 118654.

Leung, D. Y. C., & Guo, Y. (2021). Transesterification of neat and used frying oil: Optimization for biodiesel production. Energy Conversion and Management, 248, 114830.

Li, Y., Chang, J., & Chen, H. (2021). Recent advances in catalytic transesterification for biodiesel production. Catalysis Reviews, 63(4), 523–555.

Ma, F., & Hanna, M. A. (2020). Biodiesel production: A review. Bioresource Technology, 307, 123063.

Maneerung, T., Kawi, S., Dai, Y., & Wang, C. H. (2020). Sustainable biodiesel production from waste cooking oil via transesterification using CaO catalysts derived from waste eggshells. Energy Conversion and Management, 216, 112944.

Meneghetti, S. M. P., Meneghetti, M. R., & Silva, E. C. (2020). *Catalytic behavior of CaO-based catalysts derived from animal bones for biodiesel production. Renewable Energy, 152, 1155–1165.

Obadiah, A., & Yusuf, A. (2023). Techno-economic analysis of biodiesel production from waste cooking oil in sub-Saharan Africa. Renewable Energy Focus, 46, 77–88.

Oyedoh, E. A., Aisien, E. T., & Akhabue, C. E. (2022). Characterization and reusability of cow bone-derived CaO catalysts for biodiesel production from waste cooking oil. Journal of Cleaner Production, 354, 131683.

Pathak, G., & Rehman, A. (2020). Heterogeneous catalysis for sustainable biodiesel production: A review of feedstocks, catalysts, and process optimization. Fuel, 284, 118961.

Sharma, Y. C., Singh, B., & Korstad, J. (2021). Advances in biodiesel production through transesterification using heterogeneous catalysts. Chemical Engineering Research and Design, 169, 219–239.

Sivasamy, A., & Cheah, K. Y. (2022). Review on heterogeneous catalysts for biodiesel production from waste cooking oil. Renewable Energy, 192, 401–418.

Uwadiae, E. O., Amenaghawon, A. N., & Aisien, E. T. (2023). Thermochemical activation and performance of biogenic CaO catalysts for biodiesel synthesis. Fuel Processing Technology, 247, 107762.

Zhang, Y., Dube, M. A., McLean, D. D., & Kates, M. (2020). Biodiesel production from waste cooking oil: Process design and technological analysis. Bioresource Technology, 295, 122237.

Zhou, W., Chen, J., & Wang, S. (2023). Recent progress in transesterification catalysis for biodiesel synthesis from waste lipids. Chemical Engineering Journal, 452, 139287.

APPENDIX

ACID VALUE OF WASTE COOKING OIL

Acid Value = $\frac{56.1 \times (S - B)n}{m}$

Where;

- N = normality of KOH solution
- 56.1056g/mol = molecular weight of KOH
- S = Titration volume of KOH solution with waste cooking oil
- B= Titration volume of KOH solution without waste cooking oil (blank solution)
- 1g of WCO sample
- Initial reading = 0ml
- Final reading = 0.7
- Blank reading = 0.2ml
- AV = 1.4025mgKOH/g

FFA present = $\frac{AV}{2}$ = 0.7012mgKOH/g

IODINE VALUE OF WASTE COOKING OIL

Iodine Value = $\frac{12.69 \times C \times (B - S)}{m}$

Where;

- C = conc. of sodium Thiosulphate = 0.1
- 12.69 = constant value
- B = titration value of blank test
- S = titration value using waste cooking oil

- Initial reading = 0ml
- Final reading = 7.1ml
- Blank reading = 10.6ml
- IV = 4.41

PEROXIDE VALUE OF WASTE COOKING OIL

$$\textbf{Peroxide value (PV)} = \frac{(S - B) \times 0.1 \times 1000}{2.5}$$

- S = Titre value using waste cooking oil
- B = Titre value of blank test
- N = normality of sodium thiosulphate
- Initial reading = 0ml
- Final burette reading = 0.6ml
- Blank reading = 1.0ml
- PV = 16 mol/kg

SAPONIFICATION VALUE OF WASTE COOKING OIL

$$\textbf{Saponification Value} = \frac{56.1 \times (B - S) \times 0.5}{m}$$

Where;

- N = Normality of HCL solution
- B = Titre value of blank test
- S = Titre value of waste cooking oil sample
- Initial reading = 0ml
- Final reading = 55.7ml

- Blank reading = 68.5ml
- SV = 362.667 mgKOH/g

KINEMATIC VISCOSITY

Rpm: 60

Temperature: 40°C

Viscosity: 53.5mpa.s

MOISTURE CONTENT

Wt. of crucible = 97.36g

wt. of oil = 4.48g

wt. of oil + crucible after oven dry = 101.72g

Moisture Content = (97.36 + 4.48) - 101.72g

Moisture content = $\frac{0.12.}{4.48}$ x 100. = 2.678%

DENSITY VALUE

wt of crucible= 28g

wt of crucible + oil = 73.88g

Volume of density bottle= 50g

Density = $\frac{\text{Mass.}}{\text{Vol}}$ = 0.9176g/cm³