EVALUATION OF THE BIODIESEL POTENTIALS OF SELECTED PLANT BIOMASSES IN IBADAN NORTH LOCAL GOVERNMENT AREA, NIGERIA

BY

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CERTIFICATION

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DEDICATION

This work is dedicated to Jehovah God Almighty, in whose mercy and grace I found favour to carry out this research work from start to finish; and for the gift of life.

Also dedicate this work to my Father (Cosmas Etim Udofia) and Mother (Eno Etim Udofia), both of whom have been my pillar of support and encouragement, without whom I might have even given up hope.

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ABSTRACT

There has been an increasing emphasis on renewable sources of energy following recurrent economic crises and environmental concerns associated with petrodiesel. In Nigeria, there is an abundance of oil-bearing inedible plant biomasses, which are underutilized. Research into biodiesel production from these renewable oil sources can provide a more sustainable alternative to petrodiesel. This study was designed to evaluate the biodiesel yielding potentials of selected locally available plant biomasses.

Four plant biomasses (*Moringa oleifera*, *Elaeis guineensis*, *Thevetia peruviana* and *Spirogyra africana*) were utilised. Oil extraction from the biomasses was carried out using Soxhlet and Cold-solvent extraction methods. Hexane-only (H-only) solvent was used in the Soxhlet extraction while two solvent systems were used in the Cold extraction [Hexane/Ether (H/E) mixture and H-only]. The extracted oils were processed to biodiesel via transesterification reaction using sodium hydroxide as catalyst, and two alcohol systems [Methanol/Ethanol (M/E) mixture and Methanol-only (M-only)]. Samples of biomasses were analysed for moisture content and levels of the elements-Phosphorus (P), Calcium (Ca), Sodium (Na) and Sulphur (S)]; and the oil samples for Kinematic Viscosity (KV), Free Fatty Acid (FFA) level and Saponification value. Samples of the biodiesels were also analysed for KV, Flash Point (FP), Acid Value (AV) and the levels of P, Ca, Na and S according to the methods described by the American Standard for Testing and Materials (ASTM D6751). Results of analyses were compared with ASTM D6751 guidelines. Data were analysed using descriptive statistics and t-test at 5% level of significance.

The oil yields from Soxhlet extraction, Cold extraction (H/E mixture) and Cold extraction (H-only) were: Moringa (45.0%, 27.7% and 18.0%), PK (38.4%, 33.2% and 25.4%), Thevetia (62.3%, 51.9% and 45.8%) and Spirogyra (22.3%, 11.5% and 6.4%) respectively. Similarly, biodiesel yield from the extracted oils in the M/E and M-only transesterification processes were: Moringa (61.2% and 65.5%), PK (72.4% and 75.3%), Thevetia (78.4% and 85.2%) and Spirogyra (19.1% and 26.2%) respectively. The M-only alcohol proved to be more effective than the M/E mixture as it gave better biodiesel yield. Moisture content of the seeds of Moringa, PK, Thevetia and Spirogyra were 9.4%, 8.3%, 6.6% and 39.7% respectively. The KV, FFA level and Saponification value of the oils were Moringa (44.5 mm^2/s, 3.0%, 192.5 mgKOH/g), PK (4.9

mm^2/s, 1.9%, 230.2 mgKOH/g), and Thevetia (21.5 mm^2/s, 0.6%, 120.1 mgKOH/g). Also, the KV, FP, and AV of the biodiesels were Moringa (5.0 mm^2/s, 176^oC and 0.7 mgKOH/g), PK (2.4 mm^2/s, 166^oC and 0.4 mgKOH/g), and Thevetia (4.7 mm^2/s, 130^oC and 0.4 mgKOH/g). Analyses of elemental composition of the biomasses and biodiesels revealed a significant decline in the percentage compositions of P, Ca, Na and S in the biomasses when compared to their respective biodiesel. Spirogyra oil and biodiesel were insufficient to undergo the physiochemical tests.

The seeds of Moringa, Palm kernel and Thevetia are good sources of oil for biodiesel production but Thevetia proved to be the highest oil- and biodiesel-yielding biomass. The quality parameters of the biodiesels were found to be within international acceptable standard.

Keywords: Biodiesel production, Plant biomasses, Cold-solvent extraction, Transesterification
Word count: 489

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GLOSSARY OF TECHNICAL TERMS AND ABBREVIATIONS

A.A.S	Atomic Absorption Spectrophotometer
A.O.A.C	Association of Official Analytical Chemists
ASAE	Division of American Society of Agricultural Engineers
ASTM	American Standard for Testing and Material
A.V	Acid Value
B20	Blend of biodiesel and petrodiesel (i.e. 20% biodiesel and 80% petrodiesel)
B50	Blend of biodiesel and petrodiesel (i.e. 50% biodiesel and 50% petrodiesel)
B100	Unblended biodiesel
B.Y	Biodiesel Yield
CBT	Computer-based Test
CI	Combustion Ignition
CIA	Central Intelligence Agency
CNG	Compressed Natural Gas
COPESCO	Conservation Policy, Education and Science Committee
C.P	Cloud Point
CRIN	Cocoa Research Institute of Nigeria
D.I	De-ionized
DME	Dimethyl ether
EBB	European Biodiesel Board
EEA	European Environment Agency
EIA	Energy Information Administration
EJ	Exajoule (1 exajoule = 10^{18} joules)
EPA	Environmental Protection Agency
FAME	Fatty Acid Methyl Esters
FAO	Food and Agriculture Organization
FAP	Fatty Acid Profile
FFA	Free Fatty Acid
F.P	Flash Point
FSU	Former Soviet Union
FTL	Fischer-Tropsch Liquid

GHG	Green House Gas
HC	Hydrocarbon
HDL-C	Cholesterol contained in High Density Lipoproteins
HFRR	High Frequency Reciprocating Rig
IAR&T	Institute of Agricultural Research and Training
ICIC	International Centre for underutilized Crops
IEA	International Energy Agency
IITA	International Institute of Tropical Agriculture
IPCC	Intergovernmental Panel on Climate Change
JIAS	Journal of the International AIDS Society
K.V	Kinematic viscosity
LDL-C	Cholesterol contained in Low Density Lipoproteins
LNG	Liquefied Natural Gas
LPG	Liquefied Petroleum Gas
mb/d	million barrels per day
MCRL	Multidisciplinary Central Research Laboratory
MDG	Millennium Development Goals
MMT	Million Metric Tonnes
MPH	Masters of Public Health
MSHA	Mining Safety Health Administration
MTBE	methyl tert-butyl ether
MTOE	Million Tonnes of Oil Equivalent
NEH	National Engineering Handbook
NFPA	National Fire Protection Association
NIFOR	Nigerian Institute for Oil Palm Research
NIHORT	National Institute for Horticultural Research and Training
NNPC	Nigeria National Petroleum Corporation
NISLT	Nigerian Institute of Science Laboratory Technology
OD	Optical Density
OMR	Oil Market Report
OPEC	Oil Producing and Exporting Countries

PK	Palm Kernel
PKO	Palm Kernel Oil
PM	Particulate Matter
PP	Pour Point
ppm	parts per million
R.D	Relative Density
SBSTTA	Subsidiary Body on Scientific, Technical and Technological Advice
SHS	Super Hybrid Sensor
TAG	Triacylglycerol
THF	Tetrahydrofuran
T.N	Total Nitrogen
T.O.C	Total Organic Carbon
T.P	Total Phosphorus
T.V	Titre Value
US	United States
USDA	United States Department of Agriculture

CHAPTER ONE

INTRODUCTION

1.1 Background Information

The relative availability of middle-distillate petroleum fuels over the years has provided little reason for man to experiment with alternative, renewable fuels for diesel engines. Global atmospheric concentrations of carbon dioxide, methane and nitrous oxide have also increased markedly as a result of human activities since 1750 and now far exceed pre-industrial values determined from ice cores spanning many thousands of years (Ding *et. al*., 2001).

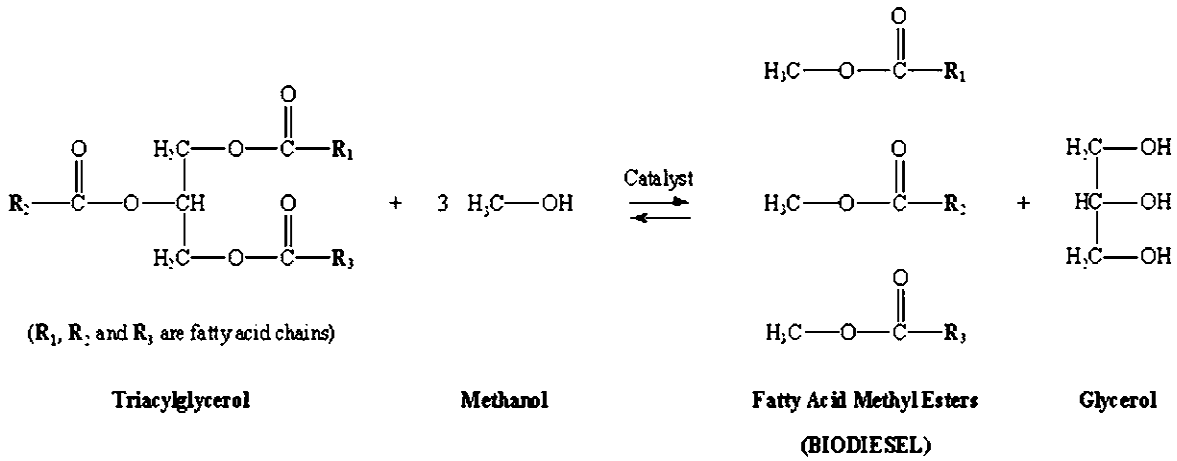
The global increases in carbon dioxide concentration are due primarily to fossil fuel use and land use change, while those of methane and nitrous oxide are primarily due to agriculture (Vaughn, 2011). However, since the oil crisis of the 1970s and considering the environmental impact of petroleum fuels as well as the decrease in world's reserve of petroleum, it becomes imperative to source for an alternative renewable energy. Hence, research interest has expanded in the area of alternative fuels (Oghenejoboh & Umukoro, 2011; Petchmata *et. al.*, 2008; Gupta *et. al.*, 2007; Math, 2007; Saravanan *et. al.*, 2007; Bobboi *et. al.*, 2006; Singh *et. al.*, 2006; Canakci, 2001).

Amongst the main renewable energy sources which include solar, wind, geothermal, hydro, tides, waves and biofuels, biodiesel (which is an example of a biofuel) holds much prospect as a viable alternative to conventional fossil-derived diesel. The concept of vegetable oils as fuel is not new. It was first proposed by the German Engineer, Rudolf Diesel (1858-1913) at the time of Second World War (1900) when he experimented with pea nut oil as a fuel in his compression ignition (diesel) engine (Leray, 2006) and Fujio Magao achieved operation with pine oil in 1948 (Thomas, 2003).

Biodiesel is defined as the mono alkyl esters of long chain fatty acids obtained from renewable feedstock such as vegetable oil or animal fats, for use in compression ignition engines; and it is much cleaner than conventional fossil-fuel diesel (Murugesan, *et. al.*, 2009). The most commonly used and most economical process is called the *base catalyzed transesterification of oil/fat with methanol*, sometimes referred to as "*the methyl ester process*".

Essentially, biodiesel production begins with pressing a crop (or using other oil extraction means), which yields a liquid oil fraction to be converted and a first by-product, oil cake, used as cattle feed. After filtering, transesterification provides a low-cost way to transform the large-branched molecule structure of the extracted oils into smaller, straight-chained molecules similar to the hydrocarbons in the diesel boiling range.

The process basically involves combining the oil/fat (made up of triglyceride molecules) with methanol and sodium or potassium hydroxide. This process creates three main products-methyl esters (biodiesel), glycerine and residual methanol; and the latter could be recycled back through the system (Franz et. al., 2005) (Formula 1.1).



Formula 1.1: Transesterification reaction used in conventional biodiesel production

Many proposals have been made regarding the availability and practicality of an environmentally sound fuel that could be domestically sourced. Methanol, ethanol, compressed natural gas (CNG), liquefied petroleum gas (LPG), liquefied natural gas (LNG), vegetable oils, reformulated gasoline, and reformulated diesel fuel have all been considered as alternative fuels. Of all alternative fuels, only ethanol and vegetable oils are non-fossil fuels. Many researchers have concluded that vegetable oils hold promise as alternative fuels for diesel engines (Oghenejoboh *et. al.*, 2010; Bajpai and Tyagi, 2006; Demirbas, 2003; Haque *et. al.*, 2009; Banerjee *et. al.*, 2009).

Biodiesel is highly favored as alternative to petroleum-based diesel because it is *renewable and environmentally friendly* (Zhang *et. al.* 2003). It has the advantages of being *non-toxic, highly biodegradable with non-flammable characteristics* (Bajpai and Tyagi, 2006). It is safer to handle (flash point above 110^{o}C), contains little or no sulfur or carcinogenic polyaromatic components, and decreases soot emission considerably, which is very advantageous in environmentally sensitive areas (Knothe *et. al.*, 2005).

Furthermore, biodiesel is a suitable outlet for the vegetable oil industry requiring little or no changes in current diesel engines when used in blends and also increases engine life due to its superior lubricity over petrodiesel (Knothe and Steidley, 2005a; Ramos and Wilhelm, 2005). Unlike ethanol, which is only two-thirds as efficient as gasoline (Al Gore, 2009), biodiesel is just as powerful as petroleum diesel while retaining its environmental advantages.

Biodiesel has been accepted as a possible substitute for conventional diesel fuel because of its certain desirable properties as stated earlier, but in spite of these properties as a diesel fuel substitute, biodiesel from food-grade oils is not economically competitive with petroleum-based diesel fuel. The major obstacle to this competitiveness is the cost of biodiesel. Approximately 70-90% of biodiesel cost arises from the cost of feed stocks (Zhang *et. al.*, 2003).

Cost of edible oils specifically is higher than petroleum-diesel and the use of edible oils for biodiesel production could lead to food oil crisis, hence it is rather impossible to justify the use of these oils for fuel purposes such as in biodiesel production. However, this could be justifiable if there is a massive commensurate increase in the production of the edible oil sources such that there would be surplus enough to guarantee food security as well as biofuel production.

1.2 Problem Statement

Fossil fuels such as petroleum, coal and natural gas, which have been used to meet the energy needs of man over the years, are associated with negative environmental impacts such as global warming (Saravanan *et. al.*, 2007; Munack *et. al.*, 2001).

The continuous emission of greenhouse gases (CH_4, CO_2, NO_x) into the atmosphere from burning of fossil fuels (mainly from petroleum) has also been identified as the major cause of

climate change, emergence of drought, spread of diseases and biodiversity loss (Oghenejoboh *et. al*., 2010; IPCC, 2007).

There are already certain projections that the supply of non-renewable fossil fuel sources are threatening to run out in a foreseeable future as not less than ten major oil fields from the 20 largest world oil producers are already experiencing decline in oil reserves (EIA, 2007; Alamu *et. al.*, 2007a). This is as a result of the spate of industrialization and "motorization" of the world, which has led to a steep rise in the demand of petroleum-based fuels. (Rambabu *et. al*., 2010 and Munack *et. al.*, 2001).

There is paucity of research work that have been carried out to specifically determine the biodiesel potential of locally available materials in Nigeria except for investigation such as Oghenejoboh and Umukoro, 2011; Agarry *et. al*., 2010; Alamu *et. al.*, 2008; Ibiyemi *et. al*., 2002; Abigor *et. al*., 2000).

1.3 Rationale for the study

Considering the *environmental impact of petroleum fuels* as well as the *dwindling World's reserve of petroleum* (Petchmata *et al*., 2008), it becomes imperative to source for alternative renewable fuels such as biodiesel, which has been found to be non-toxic, environmentally friendly, highly biodegradable with non-flammable characteristics.

In Nigerian perspective, the continuous reliance of the country on the oil and gas exploration and production sector since the discovery of commercial reserves in the Delta region in mid 1950s is *unsustainable*. This is especially so because of the fact that the current respective proven reserves of oil and gas, which are 36.20 billion barrels and 187 trillion cubic feet, as released by the Nigerian National Petroleum Corporation (NNPC) in 2007, could only last for the next 35 to 40 years. Hence, to reduce the country's dependence on imported petrol and to mitigate the country's total GHG emissions, certain types of biofuels must be targeted, such as biodiesel and cellulose-based ethanol (Galadima *et. al*., 2011; Forge, 2007).

Nigeria falls within the region of the world rated to have *high potential for biofuel production* based on three criteria: the level of water availability, level of available arable land and the state

of food insecurity (Von, 2007). Hence, in view of the failure of past policies of technology importation in the petroleum refining industries, there is need to emphasize the development of Nigerian indigenous technology to improve our vast biodiesel potentials from renewable biomasses. This would in turn ensure that the full benefits of the production of biodiesel are realized. However, the viability of the biodiesel production from these sources, amongst other factors, depends on the oil content of the oil-bearing biomasses as well as their product meeting basic fuel characteristics for diesel fuels (Oghenejoboh and Umukoro, 2011), which this work seeks to investigate.

Data on research works assessing the biodiesel potential of locally sourced substrates in Nigeria are very scanty; hence this work is intended to significantly contribute to the baseline data on this subject area.

1.4 Objectives of the study

1.4.1 Broad Objective

To determine the biodiesel yielding capacity of selected locally available oil-bearing substrates in Nigeria and their individual fuel characteristics.

1.4.2 Specific Objectives

1. Explore the sources of the oil-bearing substrates.
2. Quantitatively assess the oil-yield from the substrates.
3. Characterize certain physical and chemical components of the oils.
4. Generate biodiesel from the oils of the substrates.
5. Evaluate the biodiesel yield from the oils.
6. Determine the properties of the biodiesels obtained.

CHAPTER TWO

LITERATURE REVIEW

2.1 Fossil Fuels

These are fuels formed by natural processes such as anaerobic decomposition of buried dead organisms. The age of the organisms and their resulting fossil fuels is typically millions of years, and sometimes exceeds 650 million years (Paul *et. al.,* 2009) Fossil fuels contain high percentages of carbon and include coal, petroleum, and natural gas. They range from volatile materials with low carbon-hydrogen ratios like methane, to liquid petroleum and nonvolatile materials composed of almost pure carbon, like anthracite coal.

2.1.1 Challenges associated with fossil fuels

Anthropogenic factors, mostly associated with the use of fossil fuels, have been identified as the main cause of global warming, which is responsible for the adverse change in climate that is currently a serious global environmental concern. Concentrations of carbon dioxide in the atmosphere are projected to double with future energy use based on today's trend (Vaughn, 2011). Also, as the Arctic thaws, methane, a more potent greenhouse gas than CO_2, would further increase global warming (Stoddard *et. al*., 2006).

But since the realization of the need by world leaders to save the planet from further environmental degradation, the world is daily seeking to substitute petrochemicals in general, most especially diesel and other engine combustion fuels with cleaner and environmentally-friendly ones.

The Kyoto Protocol to reduce greenhouse gas emissions became effective in 2005 as Russia became the 55th country to ratify the agreement. The goal was for the participating countries to collectively reduce emissions of greenhouse gases by 5.2% below the emission levels of 1990 by 2012 (Vaughn, 2011). However, carbon dioxide emissions will still increase, even if nations reduce their emissions to 1990 levels, because of population growth and increase in energy use in the under-developed world.

Nigeria, as a nation, has been described as one of the major leaders in electric generator imports in Africa. This is probably due to the failed attempts to find lasting solution to the power sector, which from all indications, is tending towards a virtual collapsed in spite of the money already pumped into it. A whooping sum of about $103.1 million was spent importing generators between January and June 2010 (Ibitoye and Adenikinju, 2007).

In addition, due to the lack of reliable electricity (Figure 2.1), many people and companies complement the electricity provided by the national grid with their own generators. In fact, almost everyone who can afford a generator owns one. According to one estimate, well over 90% business ventures in Nigeria have generators (Oparaku, 2003). A study conducted by Stanley *et al*., (2010) showed that small household generators in Nigeria operate an average of six (6) hours daily, while average distance of generator away from building was 5.6m. Therefore, continuous efforts have to be made towards the solution of the energy supply depletion problem and the environmental impacts caused by these human activities (Li *et al*., 2009a).

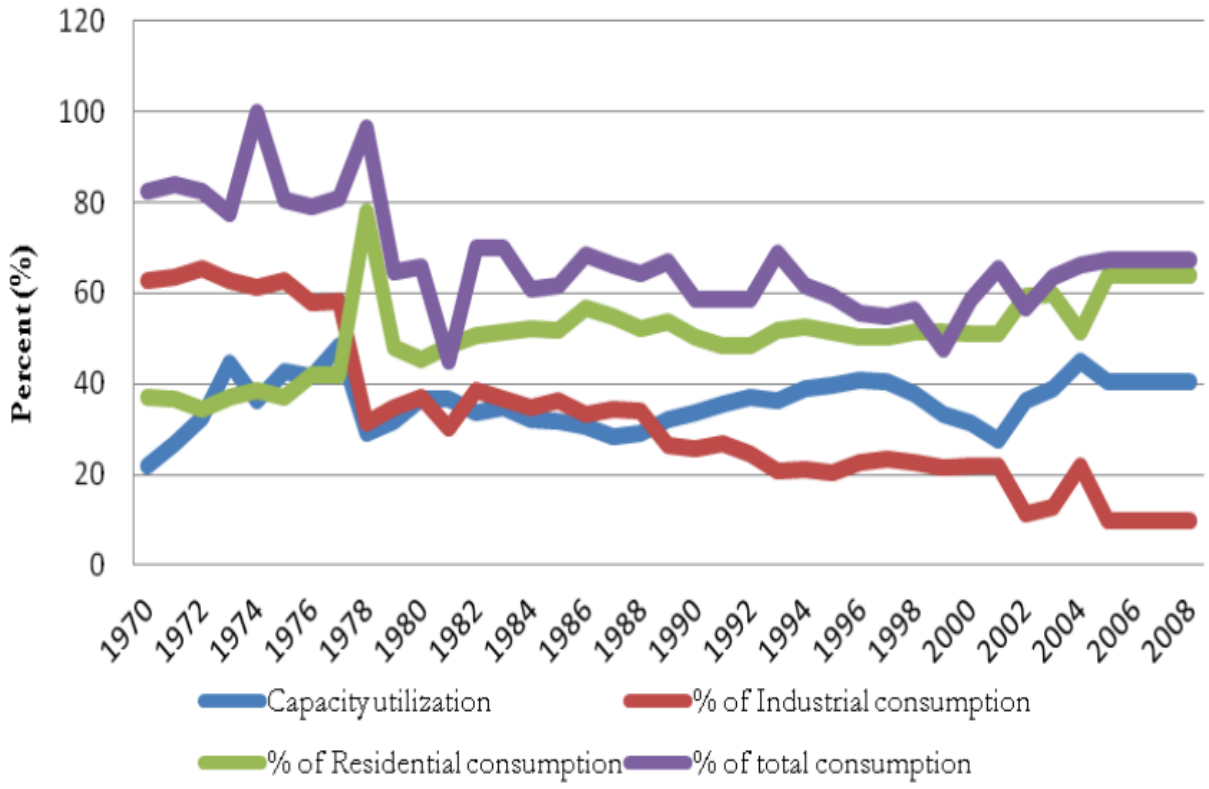


Fig. 2.1: Indicators of Electricity crises in Nigeria (1970 – 2008)
Source: Godwin and Usenobong, 2012

2.1.2 Quality of Emissions from Fossil Fuels

The World's energy demand is increasing geometrically as observed in the increased need of fuels for transportation, industrial as well as domestic operations. This has resulted in the unsuccessful war against the sky-rocketing energy demand despite the attendant environmental pollution and global warming effect resulting from the use of petroleum-based fuels (Rodrigues *et al*., 2009). Within the last 20 years about 75% of human made CO_2 emissions were from burning of fossil fuels. Nigeria's oil, for example, has not guaranteed ecologically and socially acceptable development in the country.

There are over 11 oil companies operating 1,481 wells from 159 oil fields in the Niger Delta, producing 2.7 million barrels of crude oil each day and flaring about 17 billion cubic metres of associated gas. These companies spew 2,700 tons of particulates, 160 tons of sulphur oxides, 5,400 tons of carbon monoxide, 12 and 3.5 million tons of methane and carbon dioxide respectively in the process (Olaniyi, 2007).

Nigeria currently stands as the largest emitter of these undesirable gases from the sub-saharan Africa and particularly the second worlds' biggest gas 'flarer'-contributing immensely to the global atmospheric pollution (Galadima *et. al*., 2011). The country is also one of the world's 10 largest emitters of methane (which is known to be more prevalent in flares that burn at lower efficiency and more harmful than carbon dioxide), with 38 per cent of it coming from oil and gas exploration, coal mining and landfills (Shaad and Wilson, 2009).

Plate 2.1: Picture showing gas flaring activity in a part of the Niger Delta region of Nigeria
Source: http://viiphoto.com/articles/nigeria/

Gas flares release toxic substances, including benzene and particulates, which damage the human immune system and increase the acidity of rain. It is common to see women drying *kpokpo* garri (as shown in Plate 2.1) and fish at flare sites, bearing the searing heat and reaping a benefit of snacks dried by the infernal flames. This act, which may be considered as an economic benefit to the people, is in fact harmful to human health because the products of these processes (i.e. the *kpokpo* garri and the dried fish) are all poisoned (Bassey, 2008).

Households that rely on traditional livelihoods such as fishing and crop production have also suffered due to negative impacts of gas flaring on fish and vegetation (Shaad and Wilson, 2009). The health risks associated with these flaring activities include child respiratory illnesses, asthma and cancer. According to Bassey (2008), gas flaring from Bayelsa State in Nigeria alone is believed to be responsible annually for 49 premature deaths, 4,960 children's respiratory illnesses and 120 asthma cases.

The current trajectory of fossil fuel use and its related emission of greenhouse gases are unsustainable (IEA, 2008). The environment and various life-forms are threatened by exploration of oil. For example, emissions of hazardous gases from the exhausts of heavy duty vehicles have increased tremendously over the years, which have resulted in intense air pollution-identified to be one of the reasons for climatic change that results in frequent heavy rains, hurricanes and floods threatening lives and properties (Bamgboye and Hansen, 2008).

Cooking is the most important energy need for most Nigerians; sixty-seven per cent of the population use wood or charcoal as a cooking fuel, and this wood fuel is inefficient and is believed to be responsible for about 79,000 deaths annually from indoor air pollution (Shaad and Wilson, 2009). Kerosene is also used for cooking, but is polluting, hazardous and expensive. Kerosene lamps provide poor lighting and are expensive, inefficient, highly polluting and dangerous. Small diesel generators are an option for those with sufficient cash, but these carry high fuel costs and require maintenance. They produce polluting fumes and noise and they often generate excess unused power (Shaad and Wilson, 2009).

2.2 Biofuels

These could be defined as organic primary and/or secondary fuels derived from biomass, which can be used for the generation of thermal energy by combustion or by other technology. They comprise purpose-grown energy crops, as well as multipurpose plantations and by-products (residues and wastes) (FAO, 2000).

The term *biofuel* here is used to mean any liquid fuel made from plant materials that can be used as a substitute for petroleum-derived fuel. Biofuels can include relatively familiar ones, such as ethanol made from sugar cane or diesel-like fuel (biodiesel) that can be made from soybean oil and several other plant materials (Bugaje, 2006; Bobboi *et. al.*, 2006), to less familiar fuels such as dimethyl ether (DME) or Fischer-Tropsch liquids (FTL) made from lignocellulosic biomass.

Biodiesel and bioethanol, which are the main primary sources of biofuels, both currently account for more than 95 percent of global biofuels usage (Bugaje and Mohammed, 2008). Biodiesel is a light to dark yellow liquid immiscible with water, with high boiling point and low vapour

pressure. The '*bio*' in biodiesel represents its renewable and biological source in contrast to traditional petroleum-based diesel (i.e. fossil diesel), and the '*diesel*' refers to its use in diesel engines (Zhang *et. al.*, 2003).

A variety of biolipids can be used to produce biodiesel. These include (a) virgin vegetable oil feedstock; rapeseed and soybean oils are most commonly used, though other crops such as mustard, palm oil, sunflower, hemp, and even algae show promise; (b) waste vegetable oil; (c) animal fats including tallow, lard, and yellow grease; and (d) non-edible oils such as algal oil, jatropha oil, neem oil, castor oil, and tall oil (Demirbas, 2008).

Bioethanol (also known as ethyl alcohol), on the other hand, is a biofuel produced from renewable feedstocks such as cassava, sugarcane, maize, sorghum, and potatoes by fermentation; and it can be used in either neat form in specially designed engines, or blended with petroleum fuel.

2.3 Classes of Biofuels

"*First-generation*" and "*second-generation*" fuels are the two relatively recent classifications for biofuels that have been popularized. There are no strict technical definitions for these terms, and the main distinction between them is the feedstock used.

2.3.1 First Generation Biofuels

A first-generation fuel is generally one made from sugars, grains, or seeds, i.e. one that uses only a specific (often edible) portion of the above-ground biomass produced by a plant, and relatively simple processing is required to produce a finished fuel (Naik et al, 2010).

Biodiesel, made from oil-seed crops, is a well-known first generation biofuel. The other well-known first-generation biofuel is ethanol made by fermenting sugar extracted from sugar cane or sugar beets, or sugar extracted from starch contained in maize kernels or other starch-laden crops (Naik et al, 2010). Similar processing, but with different fermentation organisms, can yield another alcohol, butanol. First-generation fuels are already being produced in significant commercial quantities in a number of countries.

2.3.2 Second Generation Biofuels

Second-generation fuels are generally those made from non-edible lignocellulosic biomass, either non-edible residues of food crop production (e.g. corn stalks or rice husks) or non-edible whole plant biomass (e.g. grasses or trees grown specifically for energy). Second-generation fuels are not yet being produced commercially in any country (Eric, 2008).

2.4 Prospects of Biofuel Production

Research on improving biofuels production has been accelerating for both ecological and economic reasons, primarily for its use as an alternative to petroleum based fuels to help address energy cost, energy security and global warming concerns associated with liquid fossil fuels (Prasad *et al*., 2007). Biofuels are environmentally friendly fuels that are similar to petrol, diesel or LPG in combustion properties (El Diwani *et al*., 2009).

Biofuels may be of special interest in many developing countries for several reasons. Climates in many of the countries are well suited to growing biomass which can be converted to biofuels. The potential for producing rural income by production of high-value products (such as liquid fuels) is attractive. The potential for export of fuels to industrialized-country markets also may be appealing (Eric, 2008). In addition, the potential for reducing greenhouse gas emissions may offer the possibility for monetizing avoided emissions of carbon, e.g., via Clean Development Mechanism credits.

Nigeria has an abundance of biomass resources which could be used as feedstock for biofuels. Although biomass can be produced continuously over a long term, the amount that can be produced at a given time is limited by the availability of the natural resources that support biomass production (Ololade, 2007 and EBB, 2005). Also, most arable lands in Nigeria are already being used for food, feed, and fiber production (Highina *et. al.*, 2011).

2.5 Biodiesel

Biodiesel can be thought of as a solar collector that operates on carbon dioxide (CO_2) and water (H_2O) through the process of photosynthesis (Plate 2.2 and Formula 2.1). The photosynthesis process captures the energy from sunlight to produce the hydrocarbon (vegetable oil). CO_2 is

used by the plant in the creation of the organic material and then the CO_2 is released in the combustion process when the fuel is used by a diesel engine.

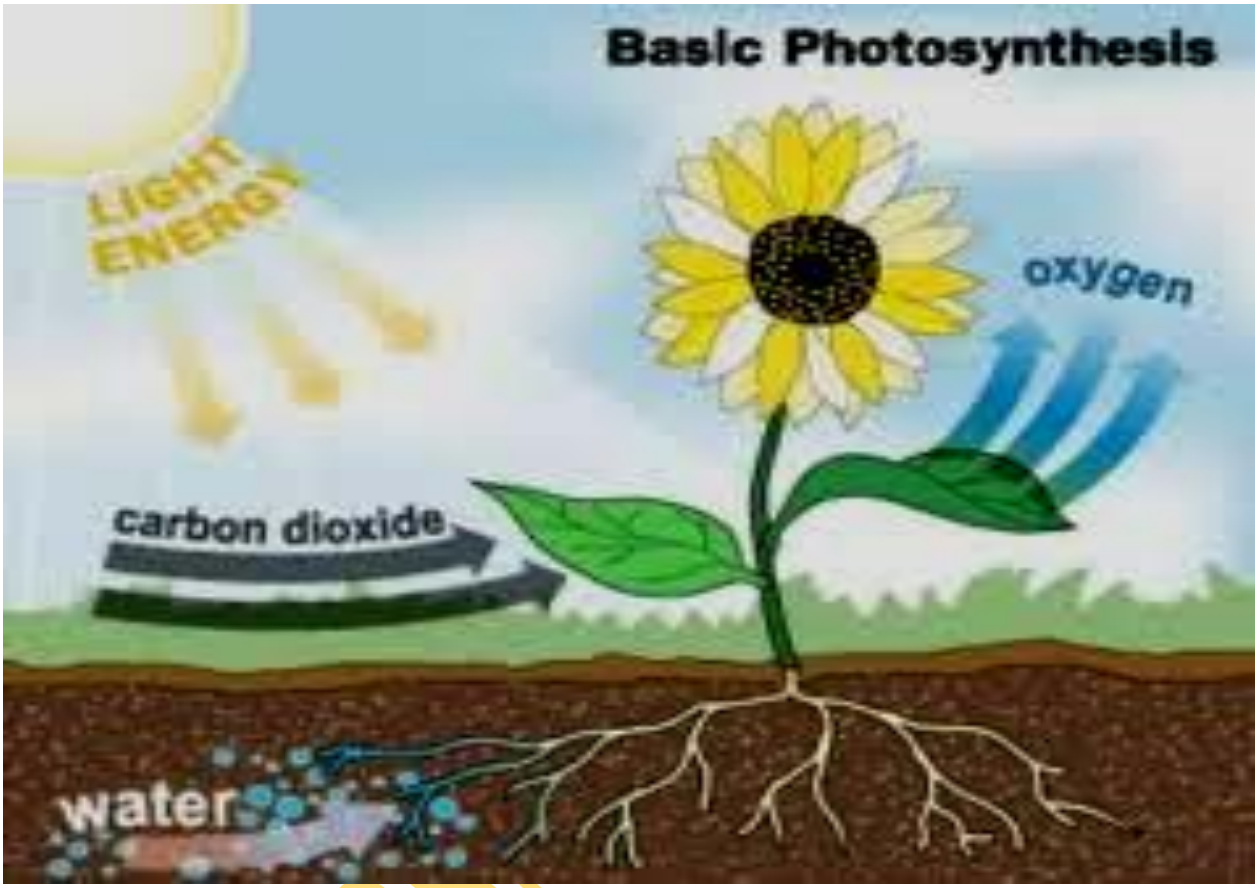


Plate 2.2: Schematic representation of the overview of photosynthesis
Source: http://static.ddmcdn.com/gif/irrigation-photosynthesis.gif

$$6CO_2 + 12H_2O + \lambda\nu \longrightarrow C_6H_{12}O_6 + 6O_2 + 6H_2O$$

Formula 2.1: Photosynthesis reaction where $\lambda\nu$ is energy of photons

Photosynthesis is carried out by many different organisms, ranging from plants to bacteria. Energy for the process is provided by light, which is absorbed by pigments such as chlorophylls and carotenoids. Photosynthesis produces organic matter as vegetables such as sugarcane, sorghum, soybean, castor oil plant, oil palm tree, eucalyptus, water hyacinth, water lily and others.

From these plant biomasses it is possible to produce biofuels such as: ethanol, biodiesel, methanol from wood, charcoal, biogas and hydrogen (Israel, 2005). Thus, through the process of photosynthesis, the energy of sunlight could be converted to a liquid fuel that, with some additional processing, can be used to power a diesel engine.

The photosynthesis process requires one major element, which is land. The crop must be planted over a wide area and to be economically feasible must compete advantageously with other crops which the landowner might choose to plant (Peterson, 2005).

Different vegetal species can be converted into solid, liquid and gaseous fuels by means of different processes of conversion, economically adequate to each application, with biodiesel fuel being an example of such as shown in the following table.

Table 2.1: Examples of different plant species and their possible biofuel derivative

Biomass or its derivatives	**Process**	**Biofuel**
Sugarcane	Mechanical	Bagasse
Fermented of sugarcane, sorghum, etc	Distillation	Ethanol
Eucalyptus and other forest species	Mechanical	Wood, chips, etc
Vegetal oils	Transesterification	Biodiesel
Crop residues, Urban residues, etc	Anaerobic digestion	Methane
Water hyacinth, Water lily, etc	Anaerobic digestion	Methane
Crop residues and from wood industry	Pyrolysis and reform	Hydrogen
Ethanol	Direction reform	Hydrogen
Green algae	Transesterification	Biodiesel

(Source: Israel, 2005)

2.5.1 Quality of Emissions from Biodiesel

Biodiesel has a superior lubricity to petrodiesel and hence its addition allows the overall reduction of sulphur in the fuel to almost nil (Drown *et. al*., 2001). Infact, most emissions are greatly reduced (Lapuerta *et. al*., 2008). Typical biodiesel produces about 65% less net carbon monoxide, 78% less carbon dioxide, 90% less sulphur dioxide and 50% less unburnt hydrocarbon emission (Margaroni, 1998; Knothe and Steidley, 2005b; Krahl *et al.*, 2005).

When biodiesel is used as a blend for petrodiesel up to 20%, no changes are required for existing diesel engines (ASTM International, 2009). On the other hand, while raw vegetable oils have some environmental and cost advantages over biodiesel, engine modifications are required (Hossain and Davies, 2010).

2.6 Conventional Feedstock for Biodiesel Production

Vegetable oils, which are renewable fuels, have become more attractive recently not only because they are renewable resources but also because of their environmental benefits. These oils, which are present in a huge variety of plants commonly called *oil crops*, are liquid substances at room temperature, with low fusion point as a result of unsaturated fatty acids. They are an important source of liquid biomass and are the main input in biodiesel production (Franz *et. al*., 2005).

The renewable nature of vegetable oils makes them a potentially inexhaustible source of energy, with energy content close to that of diesel fuel. Global vegetable oil production increased from 56 million tons in 1990 to 88 million tons in 2000, following a below-normal increase. The source of this gain was distributed among the various oils. Global consumption rose to about 56–86 million tons, leaving world stocks comparatively tight (Demirbas, 2005).

Various oils have been in use in different countries as raw materials for biodiesel production owing to their availability. Soybean oil is commonly used in United States and rapeseed oil is used in many European countries for biodiesel production, whereas, coconut oil and palm oils are used in Malaysia and Indonesia for biodiesel production (Sarin *et. al*., 2007; Demirbas, 2006 and Ghadge & Raheman, 2005). In India and Southeast Asia, Jatropha tree (Jatropha curcas) (Tiwari *et. al.*, 2007), Karanja (Pongamia pinnata) (Srivastava & Verma, 2008; Sharma & Singh,

2008; Karmee & Chadha, 2005) and Mahua (*M. indica*) (Ghadge & Raheman, 2005) is used as a significant fuel source.

Generally, a considerable amount of research has been done on alternative feedstocks. Table 2.2 lists those for which only physico-chemical laboratory tests have been done. Table 2.3 lists those plant species for which engine tests have been conducted. While every effort has been made to make these lists complete and the classifications accurate, the lists are almost certainly incomplete nonetheless.

For the most widely studied species, only review papers and some representative studies are included. Not included in these lists are those that have long been used as biodiesel feedstocks: soybean, palm, rapeseed, coconut, sunflower, peanut and cottonseed oil. The 'plant type' classifications follow those of the United States Department of Agriculture (USDA) (Plants, 2009). When there is no classification available from the USDA database, the 'plant type' is derived from the cited literature.

Table 2.2: Alternative biodiesel feedstock that have been physico-chemically tested

Scientific name	Common name	Plant type	Plant part	References
Aleurites (Vernicia) fordii	Tung	Tree	Nut	Park et al., 2008
Asclepias syriaca	Milkweed	Herbaceous perennial	Seed	Holser& Harry-O'Kuru, 2006
Astrocaryum vulgare[1]	Tucum	Tree	Kernel	Lima *et al*., 2008
Canarium ovatum[1]	Pili	Tree	Pulp	Bicol & Razon, 2007
Cerbera odollam[1]	Sea mango	Tree	Seed	Kansedo *et al.*, 2009
Coffea spp.	Coffee	Shrub/tree	Defective beans coffee grounds	Oliveira *et al.*, 2008; Kondamudi *et al.*, 2008
Cucurbita pepo	Pumpkin	Annual vine	Seed	Schinas *et al*., 2009
Cuphea viscosissima× *Cuphea Lanceolata*	Cuphea	Herbaceous annual	Seed	Knothe *et al*., 2009
Cynara cardunculus	Cardoon	Herbaceous perennial	Seed	Encinar *et al*., 1999
Cyperus esculentus	Yellow nutsedge	Herbaceous perennial	Tuber	Barminas *et al*., 2002; Pascual *et al.*, 2000
Guizotia abyssinica	Niger	Herbaceous annual	Seed	Sarin *et al*., 2009
Hura crepitans	Sandbox tree	Tree	Seed	Sunandar *et al.*,2005
Idesia polycarpa[1]		Tree	Fruit	Yang *et al*.,2009
Kosteletzkya virginica	Seashore mallow	Herbaceous perennial	Seed	Ruan *et al*., 2008
Melia azedarach	Syringa	Shrub/tree	Berries	Stavarache *et al*.,2008
Michelia champaca[1]	Champaca	Tree	Seed	Hosamani *et al*., 2009
Moringa oleifera	Moringa	Shrub/tree	Seed	Rashid *et al*.,2008b
Orbignya oleifera[1]	Babassu	Tree	Nut	Lima *et al*., 2007
Psophocarpus tetragonolobus[1]	Winged bean	Perennial vine	Seed	Bicol & Razon, 2007
Raphanus sativus	Radish	Herbaceous annual	Seed	Domingos *et al.*, 2008
Sclerocarya birrea[1]	Marula	Tree	Seed	Mariod *et al*.,2006
Simmondsia chinensis	Jojoba	Shrub	Seed	Canoira *et al.*,2006
Terminalia catappa	Tropical almond	Tree	Nuts	dos Santos *et al.*, 2008
Zanthoxylum bungeanum	Chinese pepper	Shrub/tree	Seed	Yang *et al*., 2008; Zhang & Jiang, 2008

[1]No USDA classification available. Classification is derived from cited literature.

Source: Luis, 2009

Table 2.3: Alternative biodiesel feedstock which have been engine-tested

Scientific name	Common name	Plant type	Plant part	References
Azadirachta indica	Neem	Tree	Seed	Nabi *et al.*, 2006
Camelina sativa	Camelina	Herbaceous annual	Seed	Frohlich and Rice, 2005
Eruca vesicaria ssp.sativa	Rocket	Herbaceous annual	Seed	Li X. *et al.*,2009
Olea europaea	Olive	Tree/shrub	Pomace	Caynak *et al.*,2009
Salvadora oleoides[1]	Peehl	Tree	Seed	Kaul *et al.*,2007
Brassica carinata[1]	Ethiopian mustard	Herbaceous annual	Seed	(a)
Calophyllum inophyllum	Polanga	Tree	Seed	(b)
Hevea brasiliensis	Rubber	Tree	Seed	(c)
Jatropha curcas	Physic	Tree/shrub	Seed	(d)
Linum usitatissimum	Linseed	Herbaceous annual	Seed	(e)
Madhuca indica[1]	Mahua	Tree	Seed	(f)
Oryza sativa	Rice	Grass annual	Bran	(g)
Nicotiana tabacum	Tobacco	Herb	Seed	(h)
Pongamia (Millettia) pinnata/*Pongamia glabra*	Koroch, karanja	Tree	Seed	(i)
Ricinus communis	Castor	Tree/shrub	Seed	(j)
Balanites aegyptiaca[1]	Desert date	Tree	Kernel	(k)
Carthamus tinctorius	Safflower	Herbaceous annual	Seed	(l)
Corylus avellana	Hazelnut	Tree	Kernel	(m)
Sesamum indicum	Sesame	Herbaceous annual	Seed	(n)
Simarouba glauca	Paradise tree	Tree	Seed	(o)
Sterculia foetida	Poon	Tree	Seed	(p)
Thevetia peruviana	Yellow oleander	Shrub	Seed	(q)

[1]No USDA classification available. Classification is derived from cited literature.

Source: Luis, 2009

(a) Bouaid *et al*. (2005 and 2009), Cardone *et al*. (2002 and 2003), Vicente *et al*. (2005).
(b) Banapurmath *et al*. (2008), Sahoo *et al*. (2007), Sahoo and Das (2009).
(c) Ikwuagwu *et al*. (2000), Ramadhas *et al*. (2005a, 2005b and 2005c).
(d) Achten *et al*. (2008), Carels (2009), Foidl *et al*. (1996), Gubitz *et al*. (1999), Kumar and Sharma (2008), Makkar *et al*. (2009), Pramanik (2003).
(e) Agarwal *et al*. (2003 and 2008), Sendzikiene *et al*. (2005).

(f) Agarwal *et al*. (2008), Kapilan and Reddy (2008), Puhan *et al*. (2005a and 2005b), Raheman and Ghadge (2007).
(g) Agarwal *et al*. (2008), Lin *et al*. (2009), Saravanan *et al*. (2009), Sinha *et al*. (2008), Zullaikah *et al*. (2005).
(h) Giannelos *et al*. (2002), Usta (2005a and 2005b), Veljkovic *et al*. (2006).
(i) Das *et al*. (2009), Karmee and Chada (2005) Sahoo and Das (2009), Sahoo *et al*. (2007), Sarma *et al*. (2005).
(j) Albuquerque *et al*. (2009), Ali *et al*. (2008), Conceição *et al*. (2007), Goodrum and Geller (2005), Scholz (2008).
(k) Chapagain *et al*. (2009); Deshmukh and Buyar (2009)
(l) Rashid and Anwar (2008a); Xin *et al*. (2009)
(m) Gumus (2008); Xu and Hanna (2009)
(n) Banapurmath *et al*. (2008);Saydut *et al*. (2008)
(o) Devan and Mahalakshmi (2009b and 2009c)
(p) Devan and Mahalakshmi (200a)
(q) Balusamy and Marappan (2007); Oluwaniyi and Ibiyemi (2007)

The Nigerian government has recently embraced the production of biofuels, particularly bioethanol and biodiesel, as a good option. The production of these fuels would enhance fuel use in automotive industry, electric power generation and rural development, including agricultural mechanization and light industrial goods development; and ensuring that the common man is fully benefiting from the country's economy (Azih, 2007). These positive attributes prompted the *Biofuels Policy* of year 2007, where the necessary framework to ensure a successful biofuels production and utilization in Nigeria was designed (Oniemola and Sanusi, 2009).

2.7 Prospect of Biodiesel Production

2.7.1 Effects of Biodiesel Production on the Economy

Modern biofuels have even been reported as a promising long term renewable energy source, which has potential to address both environmental impacts and security concerns posed by current dependence on fossil fuels (Batidzirai *et al.*, 2006; Alamu *et al.*, 2007a; Gupta *et al.*, 2007). In comparison with petroleum-based fuels, biodiesel offers reduced exhaust emissions, improved biodegradability (Prince *et. al*., 2008), reduced toxicity (Lapinskiene *et. al.*, 2006) and higher cetane rating which can improve performance and clean up emissions (Gerpen, 2005; and Pahl, 2005).

At the moment, there is no commercial biodiesel plant that exists in Nigeria, except for maybe a few production facilities that are notably not well documented. Production and consumption are still at their infancy stage. There is now an increasing emphasis on renewable energy following the global trend in the automobile industry, and biodiesel is gaining increasing popularity. This global trend is paving the way for increased consumer confidence in automobile engines' ability to utilize biodiesel of which Nigeria cannot be isolated. With an estimated population of about 150 million people and a population growth rate of 2.38% (2007 estimate) and an average of 12 vehicles to 1000 people (1997 estimate), the potentials of biodiesel cannot be underestimated (Idusuyi *et. al*., 2012).

With the rise in oil prices and the adverse effects of global climate change, Sub-Saharan Africa has an unprecedented opportunity in choosing a cleaner development pathway via low-carbon energy alternatives that can reduce greenhouse gas (GHG) emissions; and at the same time, meeting current suppressed energy demand and future needs more efficiently and affordably (Christophe *et. al*., 2008).

The current need for renewable fuel sources stresses what Rudolf Diesel (in 1912) said at the World Exhibition in Paris: "The use of vegetable oils for engine fuels may seem insignificant today, but such oils may become in the course of time as important as the petroleum and coal tar products in the present time" (Leray, 2006). Therefore, in order to have a real impact on the country's total GHG emissions, certain types of biofuels must be targeted, such as biodiesel and cellulose-based ethanol (Forge, 2007).

The oil crises of the 1970's has in fact rekindled interest in the use of renewable fuels such as biodiesel and the following main factors have sustained this interest to date:

i. Prices of petroleum products have been on the increase since the time of the oil crises (EPA, 2008).
ii. Uncertainties in oil supplies due to political instability and conflicts in some oil producing areas of the world (Bobboi *et. al.*, 2006).
iii. Growing anxiety over the future security of the world's supply of crude oil (USDA, 2005)

The production of biodiesel from oilseeds is potentially going to create a new window of opportunity for agriculture and at the same time mitigate GHG emissions and generate environmental benefits for agriculture itself. In terms of effects on the agricultural frontier, if the cultivation of energy crops replaces intensive agriculture, impacts can range from neutral to positive; if it replaces natural ecosystems or displaces other crops into protected areas, the effects will be mostly negative.

In terms of energy balances, emissions and air quality, the evidence suggests wide variation in greenhouse gas (GHG) savings from biofuel use depending on feedstock, cultivation methods, conversion technologies, and energy efficiency assumptions. For example, the greatest GHG reductions can be derived from sugarcane-based bioethanol and the forthcoming 'second generation' of biofuels such as lignocellulosic bioethanol and Fischer-Tropsch biodiesel. On the other hand, maize-derived bioethanol shows the worst GHG emission performance and, in some cases, the GHG emissions can even be higher than those related to fossil fuels (Peskett et. al., 2007).

Contrary to crude oil as feedstock for fossil-based diesel, the feed stocks for biodiesel (plants and animal fats) are more uniformly dispersed, being available in every country, albeit in varying quantities and at different costs. The concerns over having to rely on a limited number of countries for crude oil supply and their enormous market power also make biofuels like biodiesel attractive as a means of enhancing security of energy supply (Bugaje and Mohammed, 2007).

Biodiesel as a fuel can be handled and used safely, thanks to extensive experience in handling stems from the oils used in the food sector and the esters employed as feedstocks in the detergent, cosmetics and soap industries. Biodiesel causes less health risk to humans and animals than fossil diesel and present less danger to the environment because of its biodegradability. Due to the automobile fuel consumption profile, which has diesel oil as the main item, biodiesel is the biomass by-product with the best potential to be used as a replacement for fossil fuels (Franz et. al., 2005).

2.7.2 Challenges associated with Biodiesel Production

Regarding soil and water management, the production of some biofuels (e.g. biodiesel via the common homogenous catalysis, which is described in Section 2.3) requires large volumes of water for washing the product-fatty acid esters; and this would be problematic in semi-arid areas. In addition, processing of some feedstocks requires large volumes of water and tends to generate large volumes of effluent.

The introduction and enforcement of appropriate technologies, regulations and standards can help to mitigate most of these problems, but this would be slow to materialize where policy environments are weak. As regards environmental management, biodiesel has been tested for the bioremediation of petroleum spills (Pereira and Mudge, 2004 & Ferna´ ndez-A´ lvarez *et. al*., 2007).

Contrary to the benefits accruable from biodiesel production from oilseeds such as the new window of opportunity for agriculture amongst others, the production of biodiesel from edible oilseeds, like palm oil and soya bean oil grown for traditional markets may prove too expensive for use as fuel and may bring about rising cost of food (Highina *et. al.*, 2011).

The IEA (2008) World Energy Outlook stated in a report that rising oil demand, if left unchecked, would accentuate the consuming countries' vulnerability to a severe supply disruption and resulting price shock. This report suggested that biofuels may one day offer a viable alternative, but also that "the implications of the use of biofuels for global security as well as for economic, environmental, and public health need to be further evaluated (EEA, 2006).

2.7.3 Challenges associated with Biodiesel Production from Edible Vegetable oils

The use of edible vegetable oils from biomasses like those of soybean (de Oliveira, 2005), sunflower (Vicente *et. al.* 2004), cotton seed (Öznur *et. al.*, 2002), safflower (Meka *et. al.*, 2007), canola (Singh *et. al.*, 2006), palm (Oghenejoboh & Umukoro, 2011; Alamu *et. al.*, 2008; Cheng *et. al.*, 2004; Crabbe *et. al*,. 2001; Darnoko & Cheryman 2000; and Abigor *et. al.*, 2000), fish oil (El Mashad *et. al.*, 2006) and also animal fats for biodiesel production has recently been of great concern. This is because of the major criticism against large-scale fuel production from

agricultural crops that it will consume vast expanse of farmlands and native habitat, compete with food materials, and drive up food prices (Patil *et. al.*, 2008).

Infact, the demand for vegetable oils for food has increased tremendously in recent years. For example, meeting only half the existing US transport fuel by biodiesel would require unsustainably 54% and 24% of the US cropping land using coconut and oil palm, respectively (Chisti, 2007). Researchers have even questioned whether the net energy benefits of biofuels production may be negative for many crops because their energy outputs are less than the fossil energy inputs required to produce them. Peskett *et. al.* (2007) stated that biofuels will be a "Pandora's box" and questioned whether large-scale biofuel production can be environmentally, socially and economically sustainable and efficient.

Amongst the more than 350 known oil bearing crops, those with the greatest production potential are sunflower, safflower, soybean, cottonseed, rapeseed, canola, corn, and peanut oil (Peterson, 2005). Unfortunately though, most of these oil sources are commodities whose prices are strongly dependent on the international market. Asides this, the food industry also imposes a direct competition for these feedstock and this may be critical for a world with an exponentially increasing population.

In view of these underlying factors, the production of biodiesel is preferably carried out, especially on commercial scale using non-edible oil sources, particularly those that require low agronomic demand for cultivation, a reasonable plant cycle, favorable geographic adaptability, high oil content and a low cost for cultivation and harvesting. (Domingos *et. al.*, 2008).

2.7.4 Effects of biodiesel use on different factors

2.7.4.1 Environmental benefits of biodiesel use

i. **Reduction in Life-Cycle Greenhouse Gas Emissions:** When biodiesel displaces petroleum, it significantly reduces greenhouse gas (GHG) emissions. By one estimate, GHG emissions [including carbon dioxide (CO_2), methane (CH_4), and nitrogen oxide (NOx)] are reduced by 41%, if biodiesel is produced from crops harvested from fields that were already in production (Sheehan *et. al.*, 1998b). When plants, such as oil crops grow, they take CO_2 from the air to make the stems, roots, leaves, and seeds (soybeans). After oil is extracted from the crop, the

oil is converted into biodiesel. When the biodiesel is burnt, CO_2 and other emissions are released and return to the atmosphere. This cycle does not add to the net CO_2 concentration in the air because the next oil crop will reuse the CO_2 as it grows. When fossil fuels such as coal or diesel fuel are burned however, 100% of the CO_2 released add to the CO_2 concentration levels in the air.

ii. **Biodiesel Reduces Tailpipe Emissions:** Biodiesel reduces tailpipe PM, hydrocarbon (HC), and carbon monoxide (CO) emissions from most modern four-stroke combustion ignition (CI) or diesel engines. These benefits occur because biodiesel contains 11% oxygen by weight. The fuel oxygen allows the fuel to burn more completely, so fewer unburnt fuel emissions result. This same phenomenon reduces air toxics, which are associated with the unburnt or partially burnt HC and PM emissions. Testing has shown that PM, HC, and CO reductions are independent of the biodiesel feedstock. The EPA reviewed 80 biodiesel emission tests on CI engines and has concluded that the benefits are real and predictable over a wide range of biodiesel blends.

An investigation into the Environmental Protection Agency's (EPA) database confirms the positive impact of B20 (blend of biodiesel and petrodiesel i.e. 20% biodiesel and 80% petrodiesel) on emissions of HC, CO, and PM. However, examination of the NOx results shows that the effect of biodiesel can vary with engine design, calibration, and test cycle. At this time, there is insufficient data for users to conclude anything about the average effect of B20 on NOx, other than that it is likely very close to zero.

When biodiesel is used in boilers or home heating oil applications, NOx tends to decrease because the combustion process is different (open flame for boilers, enclosed cylinder with high-pressure spray combustion for engines). The NOx reduction seen with biodiesel blends used in boilers appears to be independent of the type of biodiesel used. In blends with heating oil up to 20% biodiesel, NOx is reduced linearly with increasing biodiesel content. For every 1% biodiesel added NOx decreases by 1%. A B20 heating oil fuel will reduce NOx by about 20% (Krishna, 2003 and Batey, 2002).

Sulfur dioxide (SO_2) emissions are also reduced when the two fuels were blended, because biodiesel contains much less sulfur than typical heating oil does. A 20% blend of biodiesel in heating oil will reduce SO_2 by about 20%. Heating oil and diesel fuel dyed red for off-road use (agriculture, power, boiler fuels, construction, forestry, and mining) can contain as much as 500 ppm sulfur. Blending biodiesel into off-road diesel fuel can significantly reduce SO_2 emissions.

2.7.4.2 Health Benefits of biodiesel use

iii. **Reduction of toxic emissions entering human respiratory system:** Some PM and HC emissions from diesel fuel combustion are toxic or carcinogenic. Using B100 (i.e. unblended biodiesel) can eliminate as much as 90% of these air toxics. B20 reduces air toxics by 20% to 40%. The positive effects of biodiesel on air toxics have been shown in numerous studies.

Recently, the U.S. Department of Labor Mining Safety Health Administration (MSHA) has implemented rules for underground mines that limit workers' exposure to diesel PM. MSHA found that switching from petroleum diesel fuels to high blend levels of biodiesel (B50 to B100) significantly reduced PM emissions from underground diesel vehicles and substantially reduced workers' exposure. However, even low concentrations of biodiesel reduce PM emissions and provide significant health and compliance benefits wherever humans receive higher levels of exposure to diesel exhaust.

2.7.4.3 Other Benefits of biodiesel use

iv. **Provision of a High Energy Return and Displacement of Imported Petroleum:** Life-cycle analyses show that biodiesel contains 2.5 to 3.5 units of energy for every unit of fossil energy input in its production, and because very little petroleum is used in its production, its use displaces petroleum at nearly a 1-to-1 ratio on a life-cycle basis (Hill *et. al*. 2006 and Huo *et. al.* 2008). This value includes energy used in diesel farm equipment and transportation equipment (trucks, locomotives); fossil fuels used to produce fertilizers, pesticides, steam, and electricity; and methanol used in the manufacturing process. Because biodiesel is an energy-efficient fuel, it can extend petroleum supplies.

v. **Improves Engine Operation:** Even in very low concentrations, biodiesel improves fuel lubricity and raises the cetane number of the fuel. Diesel engines depend on the lubricity of the fuel to keep moving parts, especially fuel pumps, from wearing prematurely. One unintended side effect of the federal regulations, which have gradually reduced allowable fuel sulfur to only 15 ppm and lowered aromatics content, has been to reduce the lubricity of petroleum diesel. The hydro-treating processes used to reduce fuel sulfur and aromatics content also reduces polar impurities such as nitrogen compounds, which provide lubricity. To address this, the ASTM D975 diesel fuel specification was modified to add a lubricity requirement (a maximum wear scar diameter on the high-frequency reciprocating rig [HFRR] test of 520 microns). Biodiesel can impart adequate lubricity to diesel fuels at blend levels as low as 1%.

vi. **Is Easy To Use:** Finally, one of the biggest benefits to using biodiesel is that it is easy. Blends of B20 or lower are literally a "drop in" technology. No new equipment and no equipment modifications are necessary. B20 can be stored in diesel fuel tanks and pumped with diesel equipment. B20 does present a few unique handling and use precautions, but most users can expect a trouble-free B20 experience.

vii. **Lower Energy Density:** Biodiesel contains 8% less energy per gallon than typical No. 2 diesel in the United States and 12.5% less energy per pound. The difference between these two measurements is due to the higher density of biodiesel compared with diesel fuel. All biodiesel, regardless of its feedstock, provides about the same amount of energy per gallon or per pound. Typical values are as follows: Btu/lb Btu/gal Typical Diesel No. 2 18,300 129,050 Biodiesel (B100) 16,000 118,170. The difference in energy content between petroleum diesel and biodiesel can be noticeable with B100. For B20, the differences in power, torque, and fuel economy are 1% to 2%, depending on the base petroleum diesel. Most users report little difference in fuel economy between B20 and No. 2 diesel fuel. As the biodiesel blend level is lowered, differences in energy content become proportionally less significant; blends of B5 or lower cause no noticeable differences in performance in comparison to No. 2 diesel.

viii. **Low-Temperature Operability:** In some areas of the country, the cold flow properties of biodiesel are important. Unlike gasoline, petroleum diesel and biodiesel both freeze or gel at common winter temperatures; however, biodiesel's freeze point may be 20º to 30ºF higher than that of petroleum diesel. If the fuel begins to gel, it can clog filters and eventually become so thick that it cannot be pumped from the fuel tank to the engine. However, with proper handling, B20 has been used successfully all year in the coldest U.S. climates. Soy biodiesel, for example, has a cloud point of 32ºF (0ºC). In contrast, most petroleum diesels have cloud points of about 10º to 20ºF (-12º to -5ºC).

Blending of biodiesel can significantly raise the cloud point above that of the original diesel fuel. For example, a recent study (Coordinating Research Council, 2006) showed that, when soy biodiesel was blended into a specially formulated cold weather diesel fuel (cloud point of -36ºF [-38ºC]) to make a B20 blend, the cloud point of the blend was -4ºF (-20ºC). In very cold climates, this cloud point may still not be adequate for wintertime use. To accommodate biodiesel in cold climates, low cloud point petroleum diesel or low-temperature flow additives, or both, are necessary.

ix. **Storage Stability** Although biodiesel blends have adequate storage stability for normal use, special precautions must be taken if they are to be stored for extended periods. This might occur in a snow plow or farm implement used seasonally, or in the fuel tank of a backup generator. If the fuel will be stored for more than a few months, a stability additive is recommended, and acidity should be measured monthly.

Finally, biodiesel is generally more susceptible than petroleum diesel to microbial degradation. In the case of spills in the environment, this is a positive attribute because it biodegrades more rapidly. However, microbial contamination of fuel storage tanks can plug dispensers and vehicle fuel filters and cause vehicles to stall. This is not unheard of for petroleum diesel, but anecdotal evidence suggests it is a greater problem for biodiesel blends. The best way to deal with this issue (for both petroleum diesel and biodiesel) is adequate fuel storage tank housekeeping and monitoring, especially minimizing water in contact with the

fuel. Water bottoms must be removed from tanks, and standing tanks should be sampled and tested for microbial contamination.

2.7.5 Future Outlook for Biodiesel Production

Biodiesel production at the present day volume is relatively recent, but that notwithstanding, it is experiencing very dramatic expansion in the developed countries; and for classification of national development, it is almost an index. There is already considerable experience in the oleochemicals industry in biodiesel manufacture and handling.

Some countries and territories have been able to move quickly and actually require that a certain percentage of diesel fuel be from biodiesel (Colares, 2008 and Republic Act 9367, 2006). The need for the commodity, which is preferred to the conventional diesel, serves as a great driver for success in the sector. The drive is greatly supported by high price arising from artificial scarcity and fear for future real scarcity of petroleum diesel.

In view of the continued epileptic power situation plaguing the country and the increase in the use of diesel generators by individuals and corporate organizations, an alternative fuel source such as biodiesel with a proven higher efficiency and environmentally friendly nature, becomes necessary. Biodiesel demands in 2007 alone was 480 million liters, with a projected demand of 900 million liters in 2020 stressing the need for an intensified effort into developing plans for the sustainable production of biodiesel.

According to the US National Biodiesel Board, the number of active and proposed biodiesel plants grew by more than 67% in six months in 2005 (Frank, 2006). Projected production capacity for 2005 was 545 million gallons per year. As the capacity of biodiesel production increases, there shall be a corresponding increase in demand for oils and fats. In USA, soybean is the favorite oil, because it is easily available and the ease of processing it into biodiesel. Even in the developed nations, there is an aggressive drive for alternate seed oil feedstock for biodiesel in particular. This is in anticipation of a major need of biodiesel by internal combustion engines, a justification for source in unorthodox new oil seed crops world over.

The United States of America is the largest consumer of oil in the world. As of 2008, the US consumed 19.5 million barrels of oil per day, on average, with a production of only 8.154 million barrels per day (Central Intelligence Agency, 2008). As shown in Figure 2.2, in recent history, 2008 was the last year that global oil supplies were greater than the demands globally.

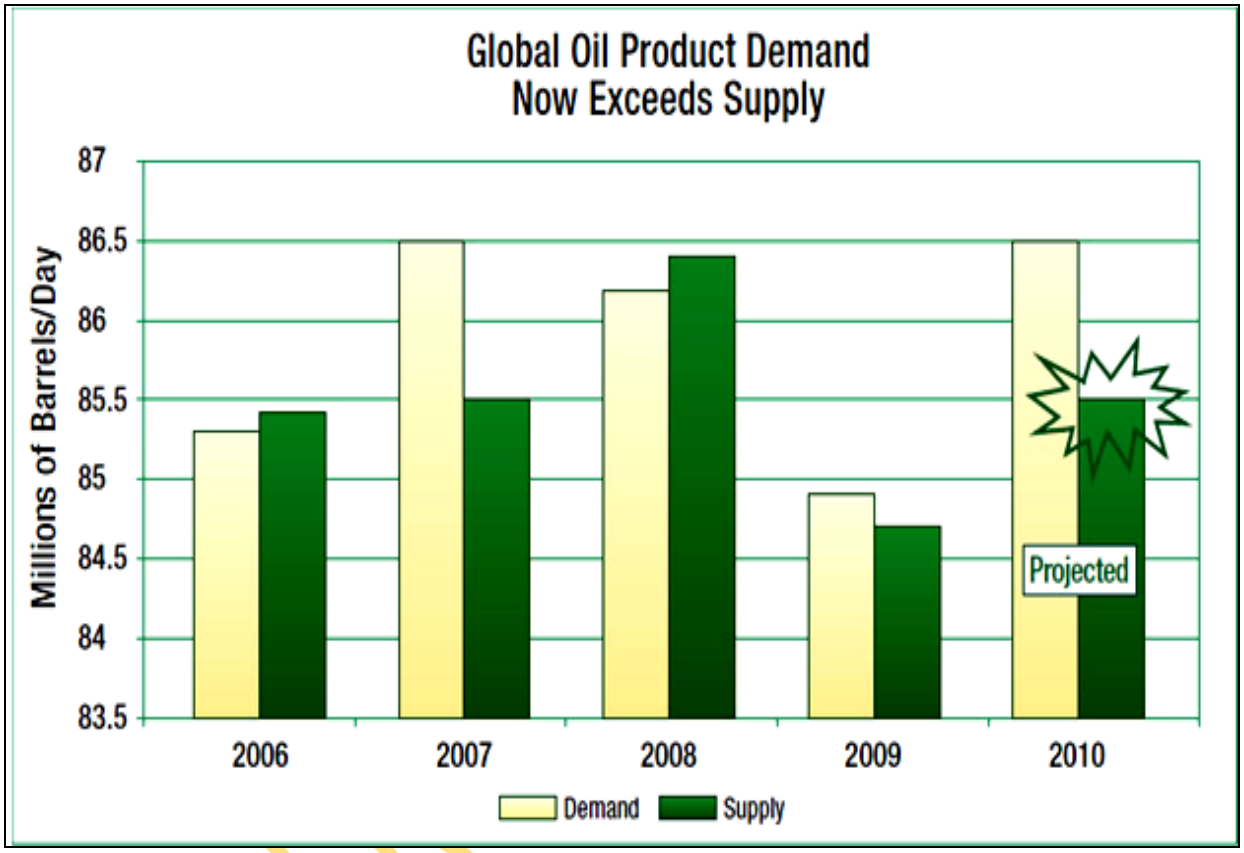


Fig 2.2: Global Oil Product Demand
Source: IEA Oil Market Report (2010)

As of 2009 around 84.9 million barrels of oil were consumed daily, with a supply of 84.6 million. For 2010, staggering predictions were made that the oil demand will exceed availability by nearly 1 million barrels of oil per day, and the IEA Oil Market Report (OMR) later reported a global oil demand of 86.5mb/d. For year 2013, the IEA OMR projects that the global oil demand would increase from 89.9mb/d in 2012 to 92 mb/d in 2014 (Table 2.4). In 2013, the world oil demand estimate was 90.8mb/d but total OPEC supply estimate for that same year was 30.41mb/d as released in July 2013 by the IEA Market Report, and this is clearly a source of concern to the world.

Table 2.4: Global Oil demand Projection for year 2014

Global Oil Demand, 2012-2014 (million barrels per day)			
	2012	**2013**	**2014**
Africa	3.6	3.7	3.9
America	30.1	30.4	30.5
Asia/Pacific	29.7	30.1	30.7
Europe	14.4	14.2	14.1
FSU	4.5	4.6	4.7
Middle East	7.6	7.8	8.1
World	89.9	90.8	92.0

Source: IEA Oil Market Report, 2013

*FSU = Former Soviet Union

This difference has a great effect on oil prices and certainly contributes to the reason prices have skyrocketed in recent history. Such high oil consumption has many implications. The first major global implication is that, since astronomically high amounts of oil are being consumed, there is an ever increasing release of polluting emissions to the atmosphere. The second concern is how the oil demand gap can be met in order to keep providing affordable energy. Hence, there is need for more research works to develop sustainable protocols in the use of non-edible oils as major non-edible raw materials for biodiesel production that are adaptable to specific local conditions.

2.8 Local Oil Biomasses for Biodiesel Production

2.8.1 Biomass

Biomass is a biological material derived from living, or recently living organisms (Van Wyk, 2001). It most often refers to plants or plant-derived materials which are specifically called *lignocellulosic* biomass (Boerrigter & van der Drift, 2003; and Kartha & Larson, 2000). It contains carbon, hydrogen and oxygen (oxygenated hydrocarbon), sometimes with high level of moisture and volatile matter, low bulk density and calorific value (Lal and Reddy, 2005).

The use of biomass for energy can complement solar, wind, and other intermittent energy resources in the renewable energy mix and reduce fossil fuel greenhouse gas emissions (Li *et al*., 2009b). In Nigeria for example, the estimated total energy consumption in 2009 was about 4.6 EJ or 111 MTOE (IEA, 2012) (Figure 2.3). Out of this, traditional biomass (wood fuel and charcoal) accounted for 85% of total energy consumption. However, this has contributed to desertification, deforestation and erosion in the country.

The high percent share of biomass represents its use to meet off-grid heating and cooking, mainly in rural areas and by the urban poor. It has been estimated that about 80% of Nigerian households living in the rural and urban areas use wood fuel and charcoal for cooking and heating (Sambo, 2006).

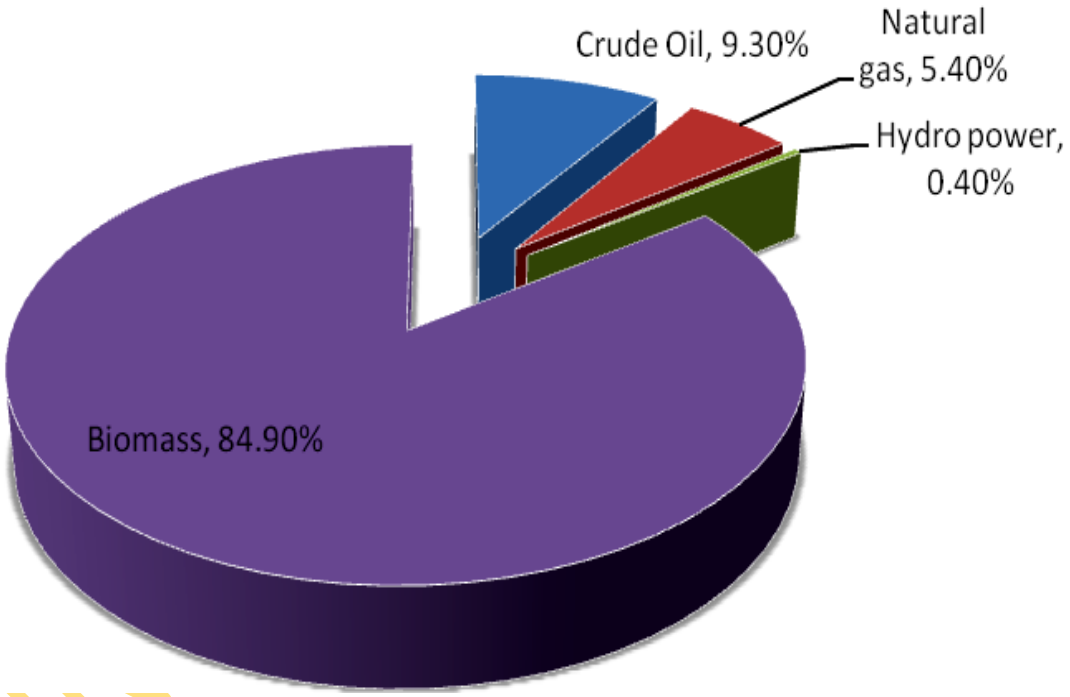


Fig. 2.3: Energy consumption in Nigeria, 2009. Source: IEA (2012)

As a renewable energy source, biomass can either be used directly via combustion to produce heat, or indirectly after converting it to various forms of biofuels. This conversion of biomass to biofuel can be achieved by different methods which are classified into: *physicochemical*, *biochemical*, and *thermochemical* methods (Figure 2.4).

Biomass is one of the better sources of energy (Kulkarni and Dalai, 2006), and is the only renewable source of carbon, which makes it the only renewable resource for producing carbon-bearing liquid fuels (Eric, 2008).

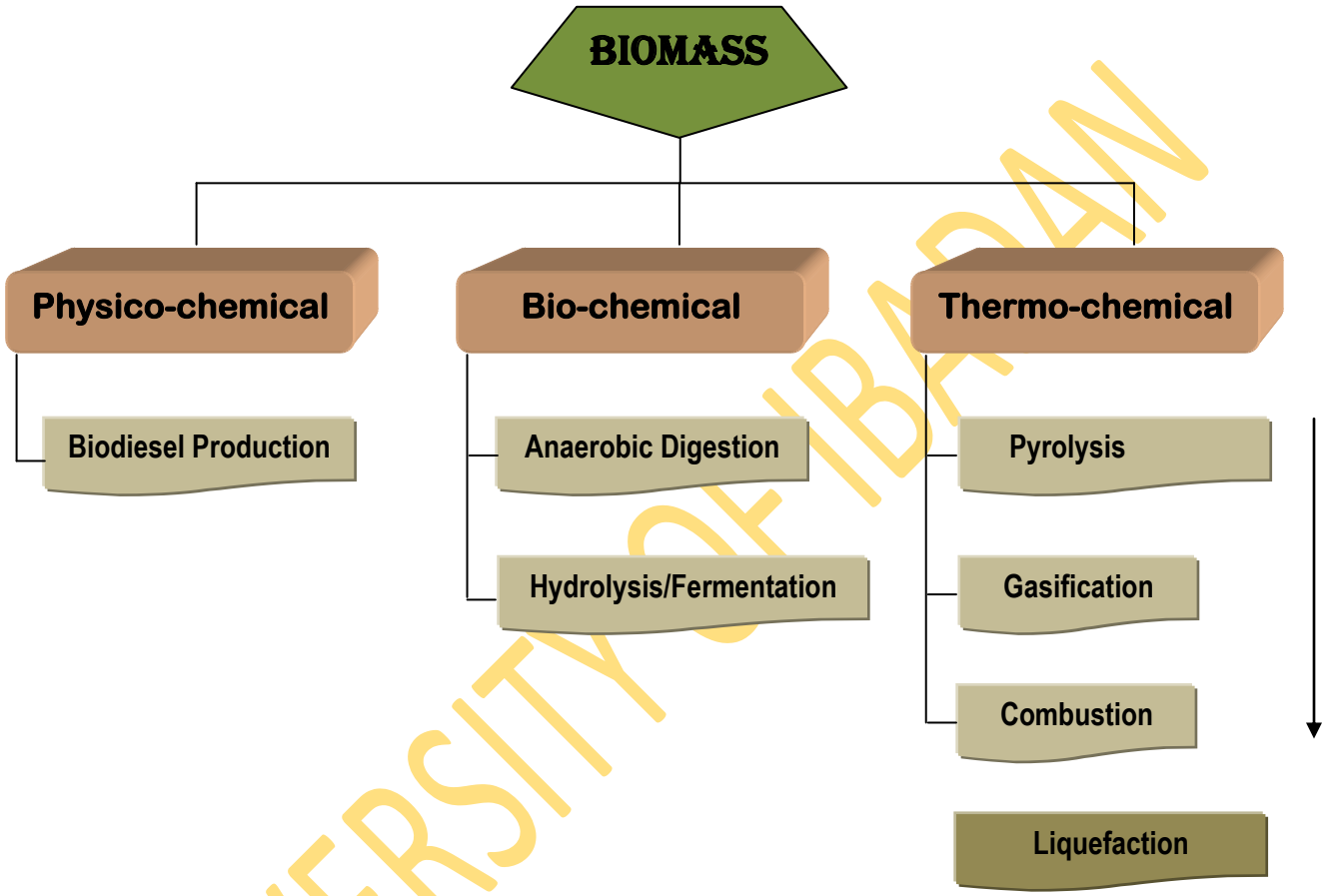


Fig. 2.4: Schematic diagram of bioenergy conversion
Source: Eric, 2008

Oil crops are important biomasses that are widely grown in different parts of the world due to their wide application. World oilseed stocks were estimated at 39.8 million tons for 2003/2004 (USDA, 2004). On an impressive note, as of 2005, Germany led the world in production of biodiesel (primarily from rapeseed and sunflower) with about 2.3 billion litres produced (EBB, 2006); and production worldwide has been growing rapidly since that year.

Asides from the fact that oil seeds are a major source of vegetable protein and oils for human and animal nutrition, they also constitute an essential part of industrial raw materials. For example, interest in palm biodiesel is growing, especially in South-East Asia (Malaysia, Indonesia and

Thailand) where the majority of the world's palm oil for food use is made. Also, Jatropha- a non-edible oil tree is drawing attention for its ability to produce oil seeds on lands of widely varying quality. In India, Jatropha biodiesel is being pursued as part of a wasteland reclamation strategy (Government of India Planning Commission, 2005).

Oil seeds that are commonly used as industrial raw materials include soybean, cotton seed, rape seed, sunflower seed and peanut (Usman *et. al*., 2009). In Nigeria, notable among the non-edible lesser known oil seeds are Castor, *Jatropha curcas*, *Jatropha gossipifolia* and *Thevetia peruviana*. Apart from these crops, other seeds that are used in the production of oils include linseed and sesame seed (O'Brien *et al*., 2000). When these seeds are defatted for oil and/or biodiesel production, the seed cakes could be used in animal feed formulation.

2.8.2 Challenges of Biofuel Production from Biomasses

According to the Food and Agriculture Organization (FAO), traditional oil crops like ground nut and sesame seeds continue to be important in the food supply and food security of many countries, e.g. Sudan and Myanmar. As the evident role of agrofuels as a suitable and sustainable means to meet regional and global energy needs increases, this raises serious questions about biodiversity conservation (habitat fragmentation and degradation), increased green-house gas emissions from degraded carbon sinks and deforestation. Concerns are also raised about water pollution and eutrophication, overexploitation caused by land conflicts, food security and human livelihoods. All these face increasing threats from the demands placed on limited land resources (COPESCO, 2008 and SBSTTA, 2007).

MDG 7 calls for environmental sustainability, emphasizing that the current and future wellbeing of ecosystems are not negatively affected in the long run by inappropriate development practices or technologies. Currently, there are concerns about biofuel production from edible oil crops competing with food supply; and with no reliable prospects for a massive compensatory scale-up in food production capacity (especially in developing countries like Nigeria). Hence, there is the need to intensify effort in exploring other potentially viable inedible oil biomasses that can be employed in liquid biofuel production such as algae, thevetia, jatropha, rice bran, neem, castor, etc.

2.9 Algal Biomass

2.9.1 Basic Algae Biology

Algae are a large and diverse group of simple, typically autotrophic organisms, ranging from unicellular to multicellular forms. The study of algae is called *Phycology* or *Algology*. By modern definitions algae are eukaryotes that conduct photosynthesis within membrane-bound organelles called *chloroplasts*. Chloroplasts contain circular DNA and are similar in structure to Cyanobacteria, presumably representing reduced cyanobacterial endosymbionts. The exact nature of the chloroplasts is different among the different lines of algae, reflecting different endosymbiotic events. Algae are not a monophyletic group because they do not all descend from a common algal ancestor (Louise and Richard, 2004).

Algae generally contain three main components: *Carbohydrates*, *Protein* and *Natural oils*. They are photosynthetic-like "simple" plants because they lack the many distinct organs that characterize land plants such as phyllids (leaves) and rhizoids in nonvascular plants; or leaves, roots, and other organs that are found in tracheophytes (vascular plants) (Zhiyou and Michael, 2009). Nearly all algae have photosynthetic machinery ultimately derived from the cyanobacteria, and so produce oxygen as a by-product of photosynthesis, unlike other photosynthetic bacteria such as purple and green bacteria.

Although many are *photoautotrophic*, some groups however contain members that are *mixotrophic*, deriving energy both from photosynthesis and uptake of organic carbon either by *osmotrophy*, *myzotrophy*, or *phagotrophy*. Some unicellular species even rely entirely on external energy sources and have limited or no photosynthetic apparatus. Fossilized filamentous algae from the Vindhya basin have been dated back to 1.6 to 1.7 billion years ago.

The green algae and land plants are closely related based on the structure and pigment composition of their plastids. This hypothesis was put forward a long time before molecular and ultrastructural data were available. Just as many green algae are single cells, others form groups of cells or grow as seaweeds (Thomas, 2002).

Like plants, most green algae use sunlight to make their own food. The green algae contain two forms of chlorophyll (a and b), which they use to capture light energy to fuel the manufacture of sugars, but unlike plants they are primarily aquatic. They also contain the accessory pigments-beta carotene and xanthophylls, and have stacked thylakoids (Graham and Wilcox, 2000). Because they are aquatic and manufacture their own food, these organisms are called *"algae"*, along with certain members of the Chromista, the Rhodophyta, and photosynthetic bacteria, even though they do not share a close relationship with any of these groups.

2.9.2 Classes of Algae

There are two general classifications of algae: *macroalgae* and *microalgae*.

Macroalgae are the large (measured in inches), multi-cellular algae often seen growing in ponds. The largest and most complex marine forms are called seaweed, and can grow in a variety of ways. An example is the giant kelp plant which can be more than 100 feet long.

Microalgae, on the other hand, are tiny (measured in micrometers), unicellular algae that normally grow in suspension within a body of water (Zhiyou and Michael, 2009).

The term '*algae*' is not phylogenetically meaningful without qualifiers. There are about 6,000 species of green algae (singular: green alga) (Thomas, 2002). Algae in general and green algae in particular are difficult to define to the exclusion of other phylogenetically related organisms that are not algae. This difficulty is a reflection of recent data on algae as well as the way phylogenetic thinking has permeated classification (Louise and Richard, 2004). The green algae are one of the most diverse groups of eukaryotes, showing morphological forms ranging from flagellated unicells, coccoids, branched or unbranched filaments, to multinucleated macrophytes and taxa with parenchymatic tissues (Plate 2.3).

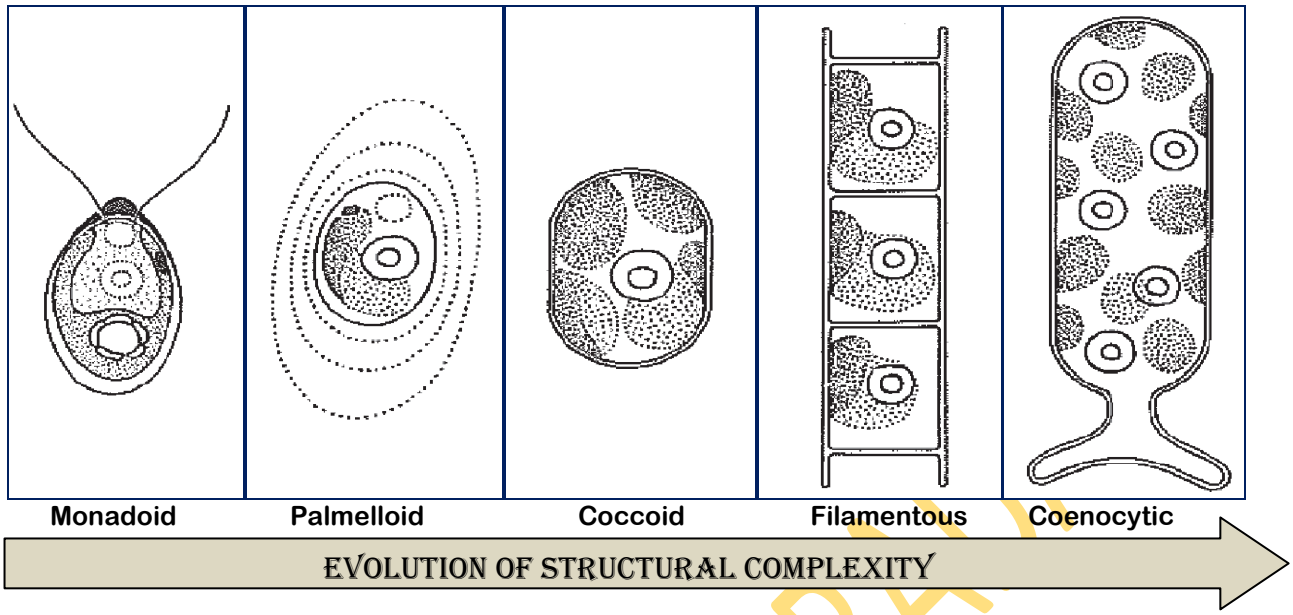


Plate 2.3: Different morphological organization of green algae (Parenchymatous and Siphonocladous levels of organization/morphological forms are not illustrated).
Source: Pröschold and Leliaert, 2007.

2.9.3 Important Functions of Algae in the Environment

The various forms of algae play significant roles in aquatic ecology. Microscopic forms that live suspended in the water column (phytoplankton) provide the food base for most marine food chains. Algae are also variously sensitive to different factors, which have made them useful as biological indicators in the *Ballantine Scale* (a biologically defined scale for measuring the degree of exposure level of wave action on a rocky shores) and its modifications.

However, in very high densities where there is rapid or excessive reproduction (*algal blooms*) these algae may discolor the water and outcompete, poison, or asphyxiate other life forms. Examples of algal blooms are the ones on Liberty Lake in Spokane County, Washington and Waughop Lake, Pierce County in the city of Lakewood, Washington (Plate 2.4). Another smaller example of algal bloom on a water course in the University of Ibadan is shown in plate 2.5.

Also, some algae may harm other species by producing protective toxins (e.g. microcystins and anatoxin), which can kill aquatic animals and even sometimes terrestrial animals that come in contact with the water (Department of Ecology, Washington, 2009). Dinoflagellates, for

example, secrete a compound that turns the flesh of fish into slime, and then the algae consume this nutritious liquid.

(a) **(b)**

Plate 2.4: Showing algal blooms on water bodies (a) Aerial view of a blue-green bloom on Liberty Lake near Spokane. Photo source: Liberty Lake Water and Sewer District **(b) Blue-green algal bloom on Waughop Lake.** Photograph by Don Russell (Water Quality Program, Washington State Department of Ecology, Olympia, Washington; www.ecy.wa.gov/biblio/0910082.html)

(c) **(d)**

Plate 2.5: Showing an algal (Spirogyra) bloom (c) Watercourse with several clusters of Spirogyra filaments showing algal scum with bubbles of oxygen gas (yellow arrow); (d) Arrow shows a sample of scooped-out spirogyra filaments from the water course. Snapshot of photos (c) and (d) were taken at a water course sandwiched between Obafemi Awolowo Hall, CBT (Computer-based Test) centre and the New Sport Complex, University of Ibadan.

2.9.4 Spirogyra

This is one of the commonest of green algae abundant in spring (Fuad *et. al*., 2010). It is one of the three species representatives of key freshwater macroalgae genera viz: *Oedogonium*, *Cladophora* and *Spirogyra* (Lawton *et. al.*, 2013). It is a genus of filamentous green algae of the order Zygnematales, named for the helical or spiral arrangement of the chloroplasts that is diagnostic of the genus (Plate 2.6). The scientific classification of Spirogyra (Lewis and McCourt, 2004) is presented below:

Domain:	Eukaryote
(unranked):	Archaeplastida
Kingdom:	Plantae
(unranked):	Streptophyta
Phylum:	Charophyta
Class:	Zygnematophyceae
Order:	Zygnematales
Family:	Zygnemataceae
Genus:	***Spirogyra***
Species:	***africana*** **(Fritsch)**

There are more than 400 species of Spirogyra in the world (John and Brook, 2002) and they measure approximately 10 to 100µm in width, and may stretch centimeters long. According to Gerrath (2003), Spirogyra is extremely common and occasionally an abundant genus in standing water bodies with most species collected as large floating masses or flimsy aggregates or long strings of cells from permanently or temporarily stagnant aquatic habitats that have neutral or slightly acidic pH values such as ponds, lakes and ditches.

a **b** **c**

Plate 2.6: Pictures of Spirogyra filament clusters: (a) Freshly collected spirogyra filaments from the stream (b) Lump of Spirogyra filaments for drying (c) Dry Spirogyra filaments

The large groups of Spirogyra cells are slimy and often called "*pond scum*" (Plate 2.5c). All the cells are bright green and morphologically similar; and capable of growth, division and reproduction. The only exception to the fact that all the cells are capable of reproduction is because of a unique cell, that is, the apical basal cells of the attached forms of spirogyra filaments. Unlike in the free floating forms that do not show apical basal polarity, the attached species (such as *S. jogensis* and *S. adnata*) have polarity. This is because the apical basal cell is colourless or dull green coloured and is incapable of dividing or reproducing. It only functions in helping to attach the filament to the substratum, and hence this unique cell is known as *Holdfast* or *Hapteron*.

Spirogyra filaments are distinguishable by their unbranched filaments with the cells connected end to end in long male reproductive system, and with their chloroplasts forming a spiral ribbon just under the cell surface. This gives a coiled or twisted texture to the cells, and it is from this appearance that the organism gets its name (Greek: *speira* = "coil" + *gyros* = "twisted"). The cell wall is characteristically straight and parallel-sided. It has two layers *viz*: the outer wall, which is composed of pectin that dissolves in water to make the filaments slimy to touch; and the inner wall which made up of cellulose.

Spirogyra is very common in relatively clean eutrophic water, developing slimy filamentous green masses. In spring Spirogyra grows under water, but when there is enough sunlight and warmth they produce large amounts of oxygen, adhering as bubbles between the tangled filaments. The filamentous masses come to the surface and become visible as slimy green mats. *Mougeotia* and *Zygnema* are often found tangled together.

2.9.5 Cultivation and Reproduction of Spirogyra biomass

The suitable season for the growth of Spirogyra is spring. In other unfavorable seasons, the filament gets converted to resistant spores (Sharma *et. al*., 2013). *Spirogyra* can reproduce *vegetatively, sexually* and *rarely asexually*.

In **vegetative reproduction**, fragmentation takes place due to mechanical injury by water currents, aquatic animal movements and bitings and gelatinization of middle lamellum. The fragmentation causes *Spirogyra* to simply undergo intercalary mitosis and form new fragment of cell(s). Each fragment then develops into a filament by repeated divisions.

Sexual reproduction involves conjugation where neighboring filaments and/or cells send out processes which fuse into tubes. There are two types of sexual reproduction: *Scalariform conjugation and Lateral conjugation.*

Asexual Reproduction is uncommon in Spirogyra. It takes place by non-motile spores known as *Akinetes* and *Aplanospores*. Akinetes are resting spores formed due to thickening of the cell wall of vegetative cells to overcome unfavourable conditions. In favourable conditions however, each akinete germinates and forms a new Spirogyra filament. An example is in *S. farlowii*. Aplanospores, unlike the akinetes, are formed in favourable conditions, e.g. *S. aplanospora*.

2.9.6 Prospects of Algal Oil as a Biofuel

Microalgae have the potential to produce more biofuel per acre than any other potential source. In fact algae are the highest yielding feedstock for biodiesel, and biodiesel from algae may be the only way to produce enough automobile fuels to replace current gasoline usage (Hossain *et. al*.,

2008). The basic idea with algae is that some algae have a high lipid (fat) count, which can be turned into biodiesel.

Algae can grow very fast, so the productivity is faster than corn or other possibilities to biofuels. It can be grown on water bodies, so it doesn't compete for prime agriculture land. It can grow in both fresh and salt water, even if the water is polluted. Many areas of the world/country are ideally suited for algae growth since algae needs large amounts of sunlight, brackish water and carbon dioxide. Those conditions are typical in the coastal regions (Scott *et. al.*, 2010).

Algae offer a diverse spectrum of valuable products and pollution solutions (Pienkos and Darzins, 2009). Because of environmental conditions such as temperature, the locally occurring strains of algae are preferred in most cases (Sheehan *et. al.*, 1998a). The best algae for biodiesel would be microalgae (Bajhaiya *et. al.*, 2010). Microalgae have much more oil than macroalgae and it is much faster and easier to grow and harvest.

The use of microalgae can be a suitable alternative because algae are the most efficient biological producer of oil on the planet and a versatile biomass source and may soon be one of the Earth's most important renewable fuel crops (Yang *et. al.*, 2010). Higher photosynthetic efficiency, higher biomass production, a faster growth rate than higher plants, highest CO fixation and O_2 production, growing in liquid medium which can be handled easily make the algae to stand high in front of other oil seed crops.

Microalgae are generally sunlight-driven cell factories of which convert fractional carbondioxide to prospective biofuels, food items, feeds and high value bioactive products. Their production is not seasonal and can be harvested throughout the year (Chisti, 2008 and Chisti, 2007). Infact, average oil yield from microalgae can be 10 to 20 times higher than the yield obtained from oleaginous seeds and/or vegetable oils (Chisti, 2007 and Tickell, 2000) as shown in Table 2.5.

Different types of biofuels can be derived from microalgae. These include *methane* produced by anaerobic digestion of algal biomass (Spolaore *et.al.*, 2006), *biodiesel* derived from microalgal oil (Thomas, 2006 and Banerjee *et. al.*, 2002) and photo-biologically produced *bio-hydrogen* (Gavrilescu and Chisti, 2005 and Fedorov *et. al*, 2005) etc.

Table 2.5: Comparison of the oil yield from some biodiesel sources

Crop	Oil yield(L ha-1)
Soybean	446
Canola	1,190
Jatropha	1,892
Palm	5,950
Microalgae	136,900

Source: Chisti, 2007.

The great ability of algae to fix CO makes it an interesting method for the removal of gases emitted from power plants; and can be used to reduce greenhouse gases with higher production of microalgal biomass and consequently higher biodiesel yield (Maeda, 1995). Few microalgae have convenient fatty acid profile and unsaponifiable fraction allowing biodiesel production with high oxidation stability (Minowa, 1995). The physical and fuel properties of biodiesel from microalgal oil in general (e.g. density, viscosity, acid value, heating value etc.) are comparable to those of fuel diesel (Gouveia and Oliveira, 2009; Rana and Spada, 2007; and Miao and Wu, 2006).

Animals and plants were mostly used for production of oil but nowadays microalgae are mostly preferred. In United States soybean is used for the production of biodiesel, but "Biofuels, if done right" must be derived from feed stocks with low greenhouse gas emissions and little or no competition with food production. Algae are likely to win on both counts (Hill *et. al.,* 2006*).* Microalgae can produce valuable co-products such as proteins and residual biomass after oil extraction, which may be used as feed or fertilizer, or fermented to produce ethanol or methane (Hirano *et. al*., 1997).

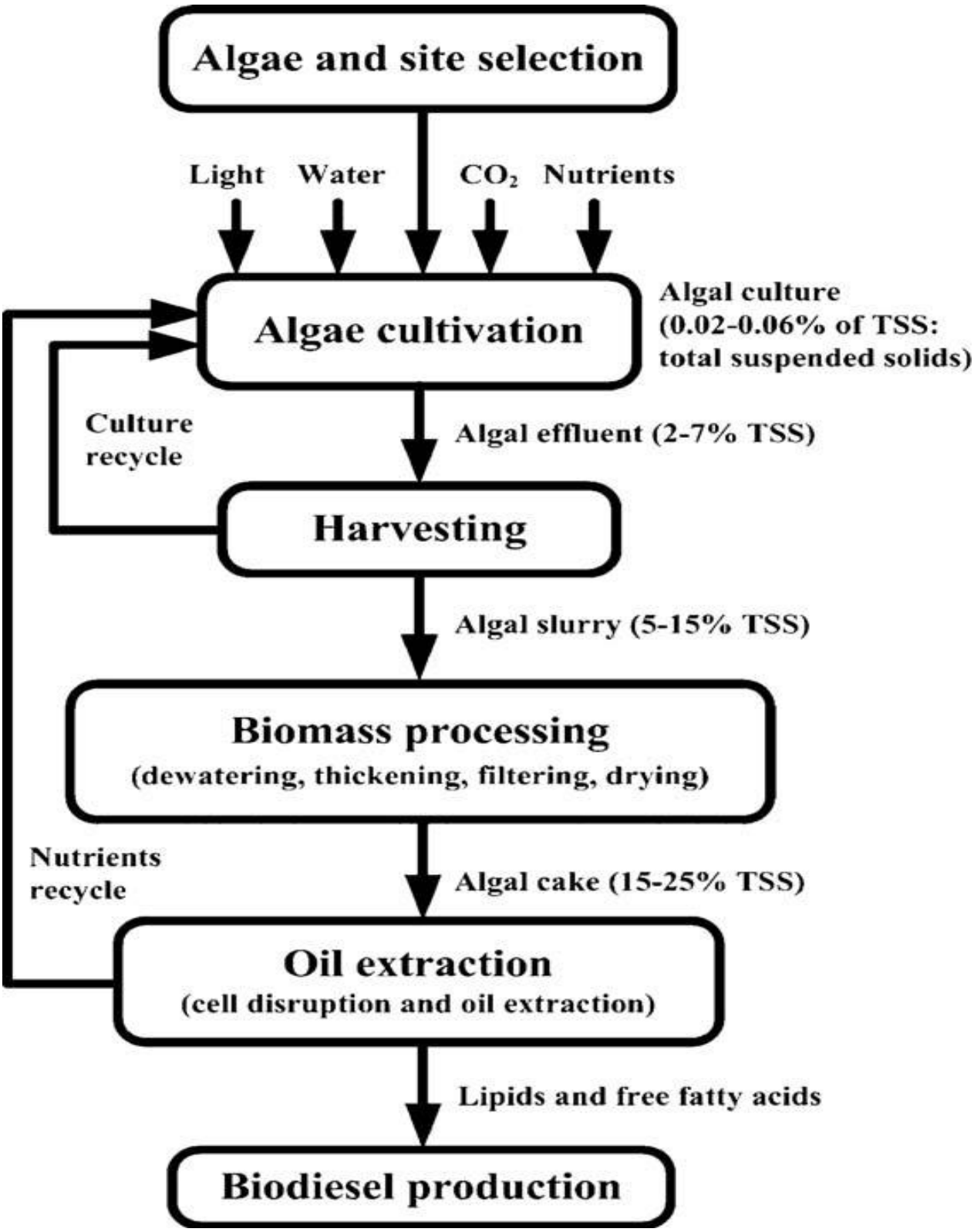


Fig 2.5: Microalgae biodiesel value chain stages.

Source: Emad, 2011

In the past 2-3 years the production of biodiesel from algae has been an area of considerable interest (Miao and Wu, 2006). This is due to algae higher productive abilities compared to land plants, with many species obtaining doubling times of some hours while many species could accumulate very large amounts connected with triacylglycerides (TAGs), the major feedstock regarding biodiesel production and also high good quality agricultural land is just not required growing the biomass.

Figure 2.5 above shows a schematic representation of the algal biodiesel value chain stages, starting with the selection of microalgae species depending on local specific conditions and the design and implementation of cultivation system for microalgae growth. Then, it follows the biomass harvesting, processing and oil extraction to supply the biodiesel production unit.

Despite all the positive attributes accruable to biodiesel generation from algal biomass, several challenges have to be tackled permitting commercial production of algal biodiesel in a scale sufficient enough to make significant contributions to our transport and energy needs. For example, algal oil could be sometimes highly viscous with viscosities ranging from 10-20 times (those of No. 2 Diesel fuel) as a result of large molecular mass and chemical structure of the oils. This could lead to problems in pumping, combustion and atomization in the injector systems of a diesel engine. Therefore, this viscosity could and should be reduced to make the highly viscous oil a suitable alternative fuel for diesel engines.

2.10 *Moringa oleifera* Biomass

2.10.1 Moringa plant

Moringa oleifera Lam (syn. *M. ptreygosperma*) is one of the best known and most widely distributed and naturalized species of the monogeneric family *Moringaceae* (Garima *et. al.*, 2011). It is also commonly called ‘Miracle Tree’, ‘Drumstick Tree’ (arising from the shape of the pods - Plate 2.7), and ‘Horseradish-tree’ (arising from the taste of a condiment prepared from its roots). In addition, it is sometimes addressed as ‘Ben oil’ or ‘behen oil’ due to its content of behenic (docosanoic) acid, which makes it possesses significant resistance ability to oxidative degradation; and hence has been extensively used in the enfleurage process. The plant has a host of other country specific vernacular names, an indication of the significance of the tree around the world.

M. oleifera is found either wild growing or cultivated throughout the plains, especially in hedges and in house yards. Native to Western and sub-Himalayan tracts, India, Pakistan, Asia, and Africa (Kumar *et al*., 2010), the plant is well distributed in the Philippines, Cambodia, America, and the Caribbean Islands; and has an impressive range of medicinal uses with high nutritional value throughout the world.

Plate 2.7: Picture of *Moringa oleifera* branch with dry pods

The taxonomic classification of *M. oleifera* is given below:

Kingdom	-	Plantae
Sub kingdom	-	Tracheobionta
Super Division	-	Spermatophyta
Division	-	Magnoliophyta
Class	-	Magnoliopsida
Subclass	-	Dilleniidae
Order	-	Capparales
Family	-	Moringaceae
Genus	-	***Moringa***
Species	-	***oleifera***

2.10.2 Plant Morphology

M. oleifera is a slender softwood perennial tree species that thrives best under the tropical insular climate, and is plentiful near the sandy beds of rivers and streams. It has a very fast growth rate and usually grows as high as 9 m, with a soft and white wood and corky and gummy bark. It commonly reaches about four metres in height just 10 months after the seed is planted and can bear fruit within its first year (International Centre for Underutilized Crops, 2008).

The roots have the taste of horseradish and the leaves are longitudinally cracked, it has 30-75 cm long main axis and its branches are jointed, glandular at joints. The leaflets, which are glabrous and entire, are finely hairy, green and almost hairless on the upper surface, paler and hairless beneath, with red-tinged mid-veins. The leaflets are also with entire (not toothed) margins, and are rounded or blunt-pointed at the apex and short-pointed at the base.

The twigs are finely hairy and green. The flowers are white, scented in large axillary down panicles; the pods are pendulous and ribbed. These pods are triangular in cross-section (30 to 50 cm long) and legume-like in appearance (Brockman, 2008) (Plate 2.8). These pods contain oil-rich black-winged or brown-winged seeds that are 3-angled (Roloff *et. al.*, 2009) (Plate 2.10), and the seeds have the potential to produce oil for biodiesel production (Rashid *et. al.*, 2008b and Hsu *et. al*., 2006).

Plate 2.8: Showing matured dried *M. oleifera* pods

Plate 2.9: Longitudinally divided pods showing Moringa seeds

Plate 2.10: Picture of brown-winged *M. oleifera* seeds

Plate 2.11: Freshly removed *M. oleifera* seeds from pod

2.10.3 Cultivation of *Moringa oleifera*

Moringa can be grown easily from seeds or cuttings. In the Philippines, moringa is propagated by planting 1–2m long limb cuttings, preferably from June to August. The plant starts bearing pods 6–8 months after planting, but regular bearing commences after the second year, continuing for several years. It can also be propagated by seeds, which are planted an inch below the surface and can be germinated year-round in well-draining soil. Seeds should be planted 2cm (approximately 1 inch) deep and ought to germinate within 1-2 weeks. Germination rates are usually very good, but can drop to 0% after 2 years.

M. oleifera and *M. stenopetala* for example, can be started from cuttings. Cuttings 45-100 cm (18-40 inches) long with stems 4-10 cm (2-4 inches) wide should be taken from the woody parts of the branches. It should be wood from the previous year. Cuttings can be cured for 3 days in the shade and then planted in a nursery or in the field. However, one should note that trees grown from cuttings are known to have much shorter roots. Where longer roots are an advantage for stabilization or access to water, seedlings are clearly preferable.

2.10.4 Prospects of *M. oleifera*

Moringa oleifera plant is highly esteemed such that almost every part of it have long been consumed by humans and used for various domestic purposes as for alley cropping, animal forage, biogas, domestic cleaning agent, blue dye, fertilizer, foliar nutrient, green manure, gum (from tree trunks), honey and sugar cane juice-clarifier (powdered seeds), ornamental plantings, bio-pesticide, pulp, rope, tannin for tanning hides, water purification, machine lubrication (oil), manufacture of perfume, and hair care products (Anwar *et. al.*, 2007 and Fahey, 2005).

Generally, various parts of this plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, possess antitumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal activities, and are being employed for the treatment of different ailments in the indigenous system of medicine (Kumar *et al*., 2010 and Nadkarni, 2009).

M. oleifera is variably labeled as Miracle Tree, Tree of Life, God's Gift to Man, Savior of the Poor, etc (Majambu, 2012). In many regions of Africa, it is widely consumed for self-medication by patients affected by diabetes, hypertension, or HIV/AIDS (Dieye *et al*., 2008; Kasolo *et al*., 2010; Monera and Maponga, 2010). The fruit (pod)/drum sticks and leaves have been used to combat malnutrition, especially among infants and nursing mothers (Estrella *et. al.*, 2000). Infact, in the Philippines, it is known as "*mothers' best friend*" because of its utilization to increase women's breast milk production (Kumar *et al*., 2010). It also functions to regulate thyroid hormone imbalance (Tahiliani and Kar, 2000). The sap can also be used as a potential dye.

Moringa plants are generally highly tolerant to salinity, water logging, frost and drought. A large amount of Nigerian salinity affected land and drought-prone areas could be potentially used to grow these plants to increase its productivity. Amongst the several advantages accruable from the production of biodiesel from *Moringa oleifera* plants, when compared with some other crops for example, is the fact that Moringa tree plantations can potentially increase green coverage to sequester more CO_2 than other vegetable oil crops (Hsu *et. al*., 2006). This is because when their pods are harvested, the trees keep on growing to produce more pods. In the process, it uses water, thereby reducing high water table whilst sequestrating carbon.

2.11 *Thevetia Peruviana* (Yellow oleander) Biomass

2.11.1 Thevetia Plant

This is a tropical shrub which grows in the wild and remains ornamental, despite the abundance of the plant around our homes, schools and other buildings. The plant is grown as hedges and kept for its bright and attractive flowers. In Nigeria specifically, the plant has been grown for over fifty years as an ornamental plant in homes, schools and churches by missionaries and explorers (Ibiyemi, 2007).

Thevetia plant is recorded to be more than 2000 years in its native countries-West Indies, Brazil and Mexico. It was taken to Europe about three hundred years ago, and today it has naturalized in virtually all countries in the tropics. Thevetia plant thrives very well in all the climatic and vegetation belts of Nigeria, it is readily found in Port Harcourt and in Maiduguri or Sokoto.

To date, despite the fact that there is high level of oil content of its kernel, about 60-65% (Azam *et al*., 2005) and valuable protein content in the seed, about 40-45% (Ibiyemi *et al*., 2002), it remains non-edible because of the presence of cardiac glycoside (toxins), hence the plant remains a plant of no significant economic value whereas it has a lot of potentials.

The botanical classification of *Thevetia peruviana* Juss is given below:

Kingdom	-	Plantae
Subkingdom	-	Tracheobionta
Superdivision	-	Spermatophyta
Division	-	Magnoliophyta
Class	-	Magnoliopsida
Subclass	-	Asteridae
Order	-	Gentianales
Family	-	Apocynaceae
Genus	-	***Thevetia***
Species	-	**Thevetia *peruviana* Juss**
Common names	-	Yellow oleander, Kolke (Bengal), Mexican oleander, Lucky nut, etc

2.11.2 Thevetia Plant Morphology

The plant is a dicotyledon which belongs to the Apocynaceae family. It is a composite, evergreen shrub, which is found to have a milky sap. It is commonly found in the tropics and sub-tropics but it is native to Central and South America. There are two varieties of the plant, one with yellow flowers (i.e. yellow oleander), and the other with purple flowers (i.e. nerium oleander). Both varieties flower and fruit all the year round hence provide a steady supply of seeds (Kokate *et. al.*, 2005).

However, thevetia plants are generally addressed as Yellow oleander (or Nerium oleander), Gum bush, Bush milk, Be-still, Trumpet flower, Flor Del Peru, Lucky beans (in Sri Lanka and are worn as talismans or charms to attract luck), Exile tree (in India), Cabalonga (in Puerto Rico), Ahanai (in Guyana), and Olomi ojo (by the Yorubas in Nigeria)

The plant is a shrub that can reach a height of 3.0-3.9metres (Plate 2.12). It is perennial and the evergreen leaves are spirally arranged, linear, narrow/sword-like and about 13-15 cm in length. The plant starts flowering after one and a half year; and after that it blooms thrice a year (Balusamy & Manrappan, 2007).

Plate 2.12: Picture of a *Thevetia peruviana* Juss (Yellow oleander) plant
(Photo taken at the Chemistry department, University of Ibadan)

The plant produce fruits virtually ten out of the twelve months of the year. The flowers are yellow flutes (or funnel-shaped) with petals that are spirally twisted (Plate 2.13). These flowers develop to fruits that have a pair of follicles or drupes. They can produce between 400-800 fruits per annum depending on the rainfall pattern and plant age (Ibiyemi, 2007).

Plate 2.13: Picture of the funnel-shaped flowers of Yellow Oleander plant

The fruits of *T. peruviana* are drupes and are globular in shape with a fissure on the ventral side where it can be opened up (Plate 2.14). It consists of deep green-waxy pericarp, fleshy mesocarp (that has a diameter of 4-5 cm) and a bony endocarp. The fruits have varying masses (2.0-6.1g) which are dispersed by man or propagated by seed or stem. The fruits are usually hard and green in colour when unripe but after drying or when they fall-off/plucked from the parent plant, the pulpy meso-pericarp (hull) becomes soft, turns dark and shrinks to expose the endocarp (Plate 2.15).

However, the bony endocarp (shell) has been referred as 'kernel' (Plate 2.16) in some texts for convenience and would be used as such in the course of this write-up for easy understanding. The kernels, which are longitudinally and transversely divided, have one to four compartments, each containing a light brown or white seed (especially in the matured and bigger ones) (Plate 2.17).

Plate 2.14: Picture of matured Yellow Oleander fruits

Plate 2.15: Picture of soft, ripe and dark Oleander fruits

Plate 2.16: Picture of matured *T. peruviana* kernels
Inset: Longitudinally divided kernels showing two compartments with one seed each covered with seed coat

Plate 2.17: Showing freshly removed Yellow Oleander seeds
Inset: Showing the seeds still covered with their seed coat

These seeds are also covered with a seed coat, which is very fragile (Insets: Plates 2.16 and 2.17). With utmost care one can get intact seeds but normally the seed coat ruptures or at least the wing shaped structure gets separated. During manual/mechanical dehulling, hardly 1 to 2% seed coat remains intact (Sahoo *et. al.*, 2009). The seed contains about 60-65% oil and the cake comprise of 30-37% protein on dry matter basis (Usman *et. al.*, 2009).

Generally, the estimated physical parameters of the fruits and kernels of *Thevetia* are summarized in the table below:

Table 2.6: Physical properties of *Thevetia* fruit and kernel

Physical properties	**Number of sample**	**Fruit**	**Kernel**
Length (mm)	100	31.08 ± 3.47	13.35 ± 1.05
Width (mm)	100	15.87 ± 1.13	10.75 ± 0.59
Thickness (mm)	100	14.27 ± 1.17	5.40 ± 0.46
1000 unit mass (g)	20	2586.63 ± 69.65	330.92 ± 11.68
Kernel fraction (%)	20	16.14	N.A.
Shell fraction (%)	20	83.86	N.A.
Arithmetic mean diameter (mm)	100	20.41 ± 1.71	9.83 ± 0.52
Geometric mean diameter (mm)	100	19.14 ± 1.47	9.17 ± 0.48
Sphericity (decimal)	100	0.62 ± 0.03	0.69 ± 0.04
Surface area (mm^2)	100	1157.60 ± 179.53	264.86 ± 26.86
Aspect ratio (%)	100	51.44 ± 4.33	80.85 ± 6.00
Bulk density (kg m^{-3})	20	591.7 ± 8.91	657.73 ± 5.23
True density (kg m^{-3})	20	1106.68 ± 38.85	942.05 ± 79.87
Nos per m^3	-	222 364	1 840 515
Porosity (%)	20	46.51 ± 1.15	29.82 ± 6.48
Angle of repose (°)	20	44.05 ± 2.04	43.28 ± 0.90

N.A - not applicable.

Source: Sahoo *et al*, 2009

2.11.3 Cultivation of Thevetia plant

Thevetia peruviana is cultivated as large flowering shrub or small ornamental standards in gardens and parks in temperate climates. It is mostly planted as a container plant in frost-prone areas, and in the winter season, it is brought inside a greenhouse or a plant house. It tolerates most soils and it is drought tolerant.

The plant generally grows best when overwintering period is short. Overwinter is a cool location (40s F), such as basement or garage, with moderate light and very little water or as a houseplant in a bright sunny but cool room with reduced water. It grows well in average, medium moisture soils in full sun to part shade. It is drought tolerant and tolerates most soils (Bandara *et. al*., 2010) but thrives a little better in rich, sandy soils. Container plants do best in fertile soils with good drainage. Water regularly but let plant soils dry out between watering.

The plant could be propagated by seed in spring by putting a clean seed coat in a glass of water containing 10% bleach and 90% warm water for 2-3mins; after which the seed is washed and soaked in warm water for 24hours. It can also be propagated from cuttings in spring-early summer with hardwood cuttings. For both, it is advisable to use seed cutting compost that contains perlite (Singh *et. al.*, 2012) and by extension, this same procedure could be scaled-up to cultivate a thevetia plantation.

2.11.4 Prospects of Thevetia Plant

The plant produces white milky juice or latex (sap) in all its organs, which is highly poisonous and the seed is also highly poisonous. This attribute accounts solely for the lack of interest in the development of the plant. The seed on the basis of its protein content (40-45%) should be preferred to most orthodox protein sources in the formulation of animal feeds. Bisset (1963) was among the first few to report on the seeds for its toxins, cause of death as recorded for two children, horses and other animals.

The seed, which is cardiotonic, has been shown to contain between 3.6-4.0% of the cardenolides-*thevetin A and B* (cerebroside) (Figure 2.6), the major glycosides of the seed, and the most lethal toxins (Ibiyemi, 2007). Some of the other compounds that have been identified asides from

thevetin are theveside, theveridoside, neriifolin, digitoxigenin (thevetigenin), cerberin, ruvoside, and perusitin (Perez-Amador *et. al*., 1994). These cardenolides are not destroyed by drying or heating and they are very similar to digoxin from *Digitalis purpurea* (Sangodare *et. al.*, 2012). They produce gastric and cardiotoxic effects.

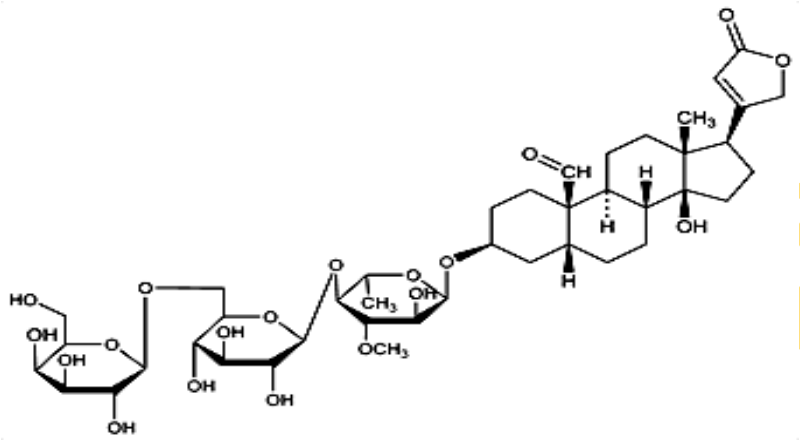


Fig. 2.6: Structure of Thevetin A
Source: www.drugfuture.com/chemdata/structure/Thevetin-A.gif

In spite of the toxicity of the plant, it has found useful applications in several spheres of life. Its latex is used as analgesic for toothache when the stem part is chewed in Juccata, and also as an insecticide. The latex or extract of the stem is used as vesicant; and the bark as a febrifuge and an effective abortifacient. The wood is used by some as handle for axes (Usman *et. al.*, 2009).

There is a myriad of edible and non-edible oils that could be used as bio-diesel feedstocks, but the appropriate technology would be to utilize the abundantly available native non-edible oil feedstocks rather than edible ones. One of these feedstocks could be *Thevetia peruviana* J. oil. This is because, amongst other benefits, in a hectare of land, 3000 saplings can be planted and out of which 52.5 tons of seeds (3,500kg of kernels) can be collected. Hence, about 1,750litres of oil can be obtained from a hectare of wasteland (Balusamy and Marappan, 2007).

However, most of the research works on *Thevetia* has revolved around aspects such as the clinical, toxicological, pharmacological, e.t.c. This is probably the reason for limited research on the oil and biodiesel yielding potential of *Thevetia* seeds that would have promoted its industrial and domestic potentials. Though some literatures are available on *Thevetia* plant and its oil characteristics (Ibiyemi *et al*., 2002 and Usman *et. al*., 2009), there is only a few studies available on its biodiesel properties (see Table 2.8).

2.12 Palm Kernel Biomass

2.12.1 Characteristics of Oil Palm Tree

Palm kernels are nuts that are obtained from the fruits of oil palm trees (Plate 2.18). The oil palm tree is a tropical plant, which commonly grows in warm climates at altitudes of less than 1,600 feet above sea level. The species, *Elaeis oleifera (H.B.K) Cortes* is native of America; and the species *Elaeis guineensis Jacq*. (Binomial name of *Elaeis guineensis*), which originated in the Gulf of Guinea in West Africa (hence its scientific name) is better known as the African oil palm. This tree produces one of the most popular edible oils (palm oil) in the world-a versatile oil of superb nutritional value. Oil from the African oil palm (*Elaeis guineensis Jacq.*) has long been recognized in West and Southwest African countries.

Oil palm grows best in areas with a mean maximum temperature of 30-32 ºC and on an average of at least five hours of sunlight. It can be grown in areas, which receive well-distributed annual rainfall of 200 cm or more. However, it can tolerate 2-4 months of dry spell. The oil palm grows on wide range of tropical soils. The adult palms can withstand occasional water-logging, but frequently waterlogged, extremely sandy and hard lateritic soils should be avoided.

Plate 2.18: Left picture-Palm oil plantation; Right picture-Enlarged single Palm oil tree
(Photos taken at the Teaching and Research Farm, University of Ibadan)

The Scientific classification of African oil palm is given thus:

Kingdom: Plantae

Subkingdom Viridaeplantae

Division Tracheophyta

Subdivision Spermatophyta

Class Magnoliopsida

Superorder Lilianae

Order Arecales

Family: Arecaceae

Subfamily: Arecoideae

Genus: ***Elaeis* Jacq.**-Oil palm

Species: ***Elaeis guineensis***-African oil palm

Oil palms have both male and female flowers on the same tree. They produce thousands of fruits, in compact bunches whose weight varies between 10-40 kilograms (Plate 2.19). Each fruit is almost spherical, ovoid or elongated in shape. Generally, the fruit is dark purple, almost black before it ripens and orange red when ripe (Plate 2.20). The fruit has a single seed (i.e. the palm kernel) (Plate 2.22) protected by a wooden endocarp or shell, surrounded by a fleshy mesocarp or pulp (Plate 2.21).

Palm kernel fruit produces two types of oil: one extracted from the pulp (palm oil) and the other from the kernel (palm kernel oil-PKO) (Figure 2.20). Both palm oil and palm kernel oil are two of the highly saturated vegetable fats. These oils give the name to the 16-carbon saturated fatty acid, palmitic acid that they contain.

Plate 2.19: Bunches of freshly harvested palm kernel fruits

Plate 2.20: Showing palm nuts detached from the bunch

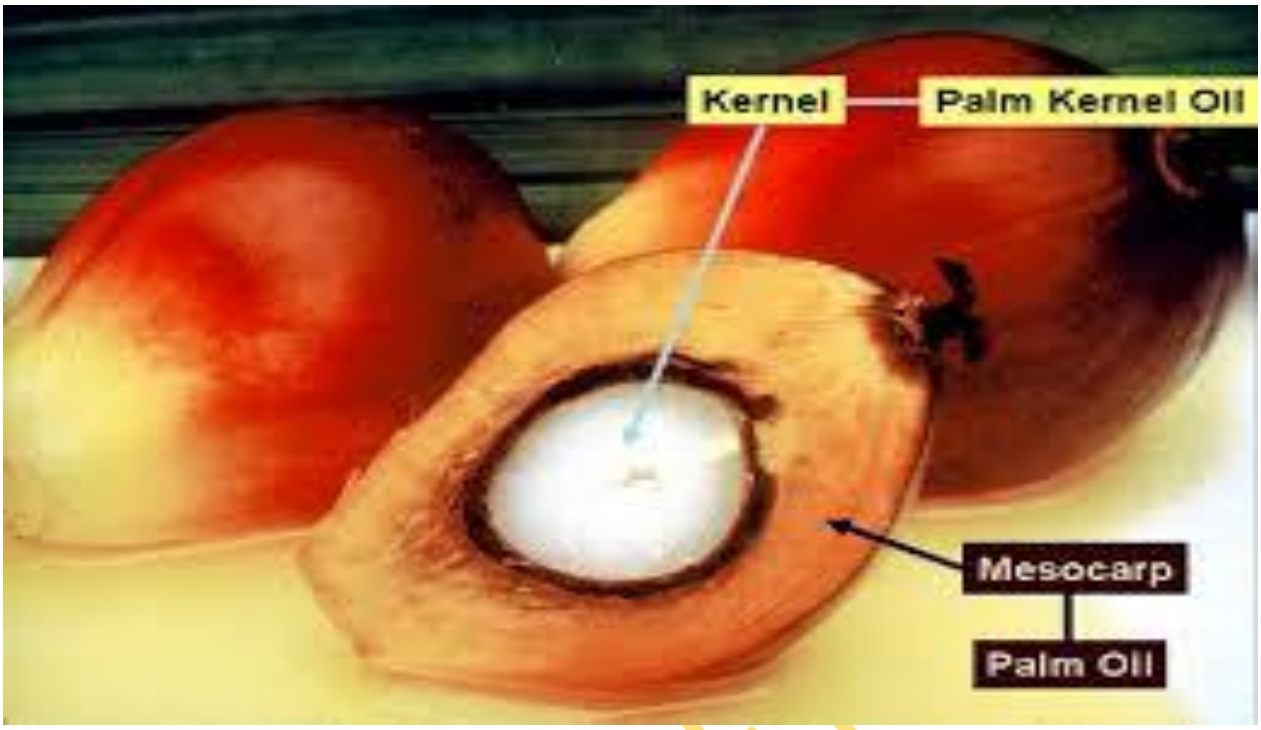


Plate 2.21: Longitudinal section through a palm fruit

Plate 2.22: Picture of Palm kernel seeds

2.12.2 Cultivation of Oil palm tree

1. Planting

African oil palms are indigenous to the tropical rain forest region in the coastal belt of West Africa from Liberia to Angola. Oil palms grow on a wide range of tropical soils, require adequate water supply and are best cultivated on lowlands, with a 2-4 month dry period. In commercial cultivation 75 to 150 palm trees are planted per hectare, yielding about 2.5 MT of palm fruits per hectare per year.

Oil palms are propagated by seed or seedlings and could be planted in the main field in triangular system at spacing of 9 meters accommodating 140 palms per ha. For seedling, the polythene bag is torn open and the entire ball of earth is buried in the pit (50 × 50 × 50 cm) and leveled. Planting is preferably done at the onset of monsoon during May-June. A commercial plantation of 410 ha would sustain about 50,000 trees. Each tree produces on average 5 bunches of fruit, equivalent to 5 kg oil per year. The total annual yield of such a plantation can be 250,000 kg oil per annum.

2. Leaf pruning

Dead and diseased leaves and all inflorescences should be cut off regularly up to three years after planting. When the palms are yielding, judicious pruning to retain about 40 leaves on the crown is advocated. It is necessary to remove some of the leaves while harvesting. In such cases, care should be taken to avoid over pruning. In addition, all dead and excess leaves should be cut off and crown cleaned at least once in a year, usually during the dry season.

3. Pollination

Oil palm is a cross-pollinated crop, and so assisted pollination is done to ensure fertilization of all female flowers. However, this is not necessary if the pollination weevil *Elaedobius kamerunicus* is introduced in the plantation. They congregate and multiply on male inflorescence during flower opening. The weevils also visit the female flowers and pollinate them effectively.

2.12.3 Harvesting and Processing of Palm Oil Fruits

The first harvest of the oil palm tree can be taken 3.5-4.0 years after planting. When a few ripe fruits are loose/fall off, this indicates that the bunch is ready for harvesting. Processing over-ripe fruits reduces quantity and quality of oil. A chisel is used for harvesting bunches from young palms. The stalk of the bunch is struck hard with the chisel to cut off and push the bunch out. When the palms become taller (from 10 year onwards) a harvesting hook has to be used. When the palms are too tall, it is necessary to climb the palms for harvesting.

For mature plantations not exceeding 40 ha, a hand-operated hydraulic press will be enough for extraction of oil. In the case of large-scale plantations, the hydraulic press will not be economical and as such, mechanically driven oil mills have to be established. The fruit bunches brought to the factory are first quartered by means of a chisel. They are then sterilized in steam or boiling water for 30-60 minutes. The objective of this process is to soften the fruits for easy pounding and to inactivate the fat splitting enzymes which are present in the fruit. This is because these enzymes may raise the free fatty acid content of the oil if this is not done.

The sterilized fruits are stripped off from the bunch and then pounded. The pounded fruit mass is then reheated and squeezed using a hydraulic press. It is then boiled in a clarification drum where the sludge will deposit and pure oil float over the water. The oil is then drained out.

2.12.4 Potentials of Palm Kernel Oil

Palm kernel oil is high in saturated fats and is more saturated than palm oil (Chow, 2007). The oil is high in lauric acid, which has been shown to raise blood cholesterol levels, both as LDL-C (cholesterol contained in low density lipoproteins) and HDL-C (cholesterol contained in high density lipoprotein) (Rakel, 2012). Palm kernel oil, which is semi-solid at room temperature, is commonly used in commercial cooking because it is lower in cost than other oils and remains stable at high cooking temperatures. It can be stored longer than other vegetable oils (Bjorklund, 2010).

Palm kernel oil (PKO) is one vegetable oil in Nigeria which had hitherto been underutilized as edible oil. Available records ranked Nigeria as one of the world's best producer of palm kernel.

Between 1995 and 1998, Nigeria's share in the world production of palm kernel were 0.27, 0.26 and 0.25MMT for 1995/96, 1996/97 and 1997/98 production seasons respectively; and in 2002, the country's production of palm kernel was approximately 0.61MMT. This record placed Nigeria next to Malaysia and Indonesia, and ahead of PKO producing countries like Thailand, China, Ivory Coast, Congo and Brazil (Alamu *et al.*, 2007a, b and FAO, 2006) as shown in Table 2.7 below.

Malaysia purchased its first palm oil seedling in mid 50s from NIFOR (Nigerian Institute for Oil Palm Research). In less than 50 years after, Malaysia and Indonesia led the world in the production of palm oil and palm kernel oil. In 2002, Malaysia produced approximately 3 MMT crude palm kernel oil and 12 MMT of crude palm oil (FAO, 2006 and Salmiah, 2003). Today, Malaysian oil palm industry is one of the most highly organized sectors of any national agriculture system of the world and the world's largest producer of palm oil (Yusof, 2007). The country has the largest oleochemicals capacity of any country in the world with her capacity representing 25% of the world capacity in 2002. Oleochemicals from Malaysia have been exported to over 100 countries, including North America, European Union countries, Japan and China.

Although the volume of trade to Nigeria on the oleochemicals and crude vegetable oils is negligible, Nigeria however imports a lot of its oleochemicals for the few oleochemical industries in the country possibly mainly from Malaysia and Indonesia. But unfortunately, Nigeria has greater potentialities to have been producers of oleochemicals which now compete effectively with petrochemicals. If certain measures are not taken, sooner than later, the country shall have to import biodiesel to supplement, if not replace, her petroleum diesel (Ibiyemi, 2007). This would however most likely then be at a high price and a major drain on the economy of the nation, and this would be nothing but an unfortunate circumstance for a country with such potentials.

Table 2.7: Palm oil and Palm kernel production in the world

	Palm Oil Production (x1000 tons)				Palm Kernel Production (x1000 tons)			
	1969-71	**1980**	**1990**	**2002**	**1969-71**	**1980**	**1990**	**2002**
World	1,983,034	5,052,641	11,163,308	-	1,178,651	1,812,081	3,511,624	7,059,000
Africa	1,108,647	1,365,350	1,683,454	-	731,005	733,927	672,208	1,018,000
S. America	46,752	134,759	505,660	-	248,489	330,549	325,701	312,000
Asia	769,583	3,461,300	8,687,410	-	177,683	730,405	2,420,034	5,520,000
Malaysia	457,298	2,573,000	6,094,700	11,909,000	98,996	557,000	1,844,700	3,269,000
Indonesia	217,900	676,800	2,186,210	9,350,000	48,980	121,105	477,824	2,053,000
Nigeria	528,330	675,000	820,000	908,000	287,100	345,000	356,000	608,000
Thailand	-	9,500	226,000	590,000	-	1,900	50,000	126,000
China	114,333	190,000	133,000	220,000	28,333	48,000	33,500	56,000
Ivory Coast	46,467	170,000	207,714	216,000	19,333	30,000	36,800	40,000
Congo	232,433	108,300	180,000	170,000	99,100	69,300	74,000	81,000
Brazil	7,166	16,000	65,000	118,000	218,599	265,988	229,000	120,000

(Source: FAO, 2006; www.unctad.org)

2.13 Available Methods for Biodiesel Production

There are different approaches for the conversion of vegetable oils or fats to biodiesel. These include the *homogenous catalysis* viz: base catalysis/transesterification, acid catalysis method & enzymatic conversion method; the *heterogeneous catalysis*, which involves the use of solid catalysts; and the *non-catalytic conversion* method, which does not require any catalyst.

2.13.1 Homogenous Catalysis

The homogenous catalysis involves the use of catalysts that are soluble in alcohol. The catalyst could either be a base, an acid or an enzyme. In the homogenous system, the catalyst ends up in the byproducts, and it is not recovered for re-use.

2.13.1.1 Base Transesterification and Acid Catalysis

The base catalysis/transesterification and the acid catalysis are the commonest amongst the different approaches available for the conversion of oils/fats to biodiesel. However, most of the current biodiesel production operations use base transesterification. In 1977, the Brazillian scientist-Expedito Parente, produced biodiesel by transesterifying palm oil with ethanol (Addison, 2005). In the experiment, he heated a mixture of ethanol, sodium hydroxide, and palm oil for two hours and later separated a layer of fatty acid methyl ester for use as biodiesel.

Biodiesel produced by transesterification involves the conversion of large, branched triglycerides into smaller, straight chain molecules of methyl or ethyl esters using an alkali or acid or enzyme as catalyst. Many studies have been done on the transesterification of vegetable oils such as Jatropha and Palm oil to produce biodiesel (Shweta *et. al*, 2004 and Cheng *et. al.*, 2004). In each of these studies, transesterification of vegetable oils is an important reaction that produces fatty acid methyl esters (FAME) which are excellent substitute for diesel fuel.

The base-catalysed transesterification is much faster, and less corrosive, than the acid catalyzed reaction. Thus alkali hydroxides are the most commonly used catalyst. However, if the feedstock has a high free fatty acid (FFA) content (as is common with rendered fats and spent restaurant oils), excess of alkali causes loss of the free fatty acids as their insoluble soaps. This decreases the final yield of ester and consumes alkali. As an alternative in these cases one can conduct an

acid catalysed reaction via esterification (Figure 2.6), which requires higher reaction temperature (100^{o}C) and longer reaction times than alkali catalysed reaction (Shweta *et. al.*, 2004).

Drewette and Dwyer documented in their research work titled "*Biofuels for Transport*" where they explained that there are three basic routes to production of fatty acid methyl esters (FAME) viz: *esterification of fatty acid distillates to fatty acid methyl esters, base-catalyzed transesterification of triglyceride oils, and acid catalyzed transesterification* (Drewette and Dwyer, 2005).

Igbokwe (2005) reported in her research work titled "*Optimization and Characterization of Palm and Kernel Oils for use as Biodiesels in Compression Ignition Engines*", that biodiesel of good ignition qualities could be successfully produced by transesterifying palm oil with a mixture of sodium hydroxide and ethanol.

Transesterification, which involves chemical conversion of the oil into its corresponding fatty ester, also serves as the most common method used in the biodiesel industry to reduce vegetable oil viscosity. Other methods of producing biodiesel from raw feedstock oils that have been considered to reduce the high viscosity of the oil are:

- Dilution of 25 parts of plant with 75 parts of diesel fuel
- Micro-emulsions with short chain alcohols (e.g. Ethanol or Methanol)
- Thermal decomposition, which produces alkanes, alkenes, carboxylic acids and aromatic compounds.
- Catalytic cracking, which produces alkanes, cycloalkanes and alkybenzenes

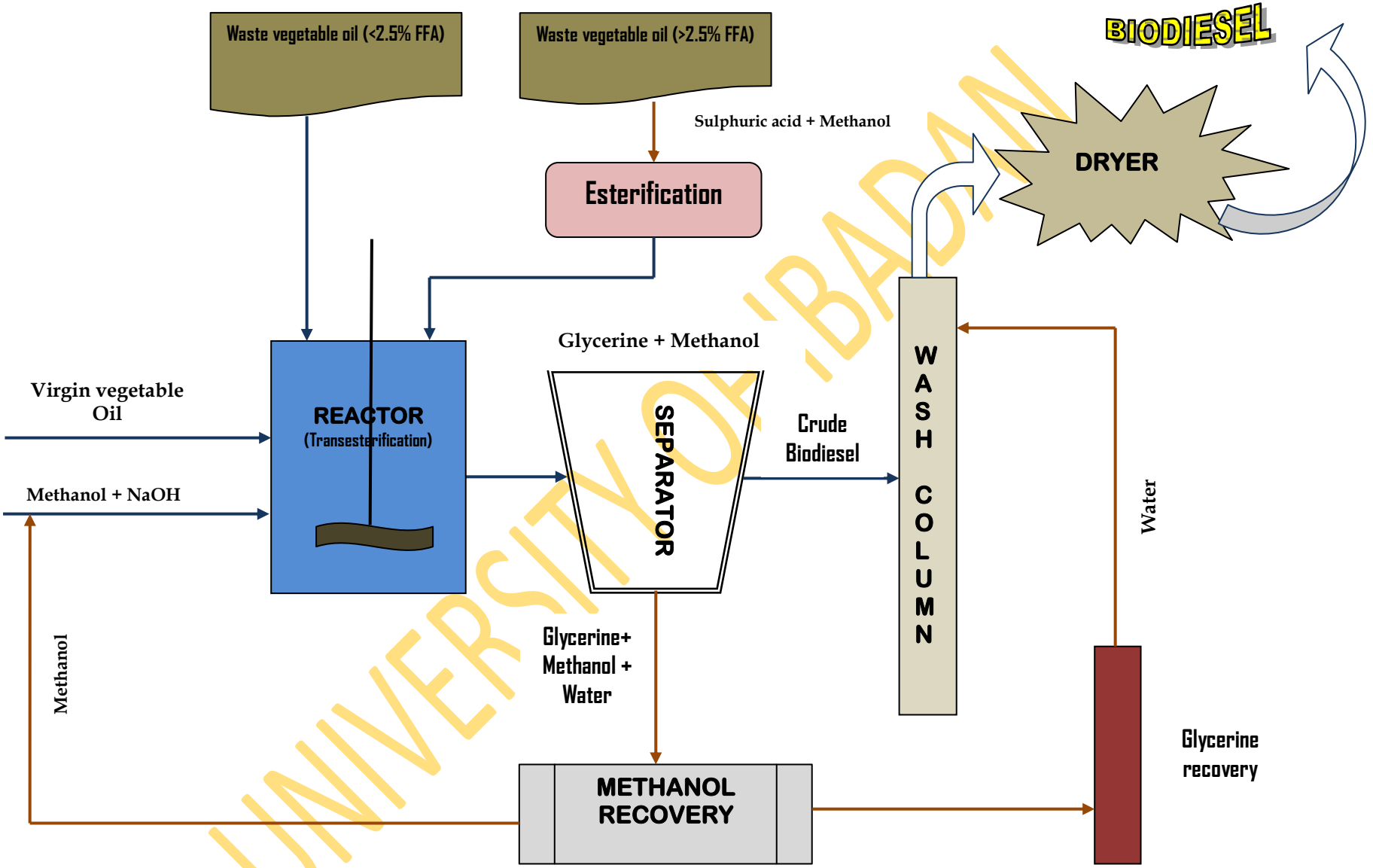


Fig. 2.6: Process Flowchart for typical Biodiesel Production
Flowchart design by Udofia and Ana, 2014 (unpublished dissertation)

2.13.1.2 Enzymatic Catalysis

In the enzymatic method, lipase catalysed transesterification is carried out in non-aqueous environments. Although chemical transesterification is efficient in terms of reaction time, however, the utilization of the method in synthesizing biodiesel from triglyceride has draw backs such as difficulty in the recovery of glycerol and the energy intensive nature of the process. In contrast, biocatalyst allows synthesis of specific alkyl esters and usually the recovery of glycerol and transesterification of glycerides with high free fatty acid content (Highina *et. al.*, 2011).

One common draw back with the use of enzymes based process is the high cost of the enzymes. Immobilization of enzymes has generally been used to obtain reliable enzymes derivative. There are three stepwise reactions with intermediate formation of diglycerides and monoglycerides resulting in the production of three moles of methyl esters and one mole of glycerol from triglycerides.

2.13.2 Heterogeneous Catalysis

Heterogeneous catalysis on the other hand involves the use of solid catalysts such as Alkaline earth metal oxides, various alkaline metal compounds supported on alumina or zeolite and sulfonic resins (where the catalyst stays on fixed-bed reactors and is used for an extended time).

2.13.3 Non-catalytic conversion

The non-catalytic conversion technique involves the use of a co-solvent that is soluble in both methanol and oil and can improve reaction rates. The BIOX Process (www.bioxcorp.com) is a typical example of such which utilizes either tetrahydrofuran (THF) or methyl tert-butyl ether (MTBE) as a co-solvent to generate a one-phase system. In the presence of a co-solvent, the reaction is 95 percent complete in 10 minutes at ambient temperatures and does not require a catalyst.

2.14 Effect of Different Parameters on the Production of Biodiesel

Several works have established that the parameters affecting methyl ester formation are reaction temperature, pressure, molar ratio, water content, and free fatty acid content. It is evident that at subcritical states of alcohol, the reaction rate is very slow and gradually increases as either

pressure or temperature rises. The most important variables affecting the methyl ester yield during transesterification reaction are molar ratio of alcohol to vegetable oil and reaction temperature.

2.14.1 Effect of molar ratio

According to Demirbas (2002), the yield of alkyl ester increases when the molar ratio of oil to alcohol is increased. In the supercritical alcohol transesterification method, the yield of conversion rises from 50% to 95% in the first 10 min. The stoichiometric ratio for transesterification reaction requires 3 mol of alcohol and 1 mol of triglyceride to yield 3 mol of fatty acid ester and 1 mol of glycerol. Ramadhas *et. al*. (2004) and Sahoo *et. al*., (2007) have reported 6:1 molar ratio during acid esterification and 9:1 vegetable oil-alcohol molar during alkaline esterification to be the optimum amount for biodiesel production from high FFA rubber seed oil and polanga seed oil, respectively.

Atu *et. al*. (2011) studied the optimum requirements of temperature, retention time, mole ratio of reactants and catalyst for the direct synthesis of biodiesel from fatty acid distillates of palm kernel oil using tetraoxosulphate (VI) acid as catalyst. Their result showed that the optimal conditions for the acid catalyzed esterification of palm kernel oil fatty acid distillates are: eight moles of methanol per mole of fatty acid; 0.06 moles of tetraoxosulphate (VI) acid per mole of fatty acid; a retention time of sixty (60) minutes and a reaction temperature of 65^{o}C.

Veljkovic *et al*. (2006) employed 18:1 molar ratio during acid esterification and 6:1 molar ratio during alkaline esterification. Meher *et al*. (2006) took 6:1 molar ratio during acid esterification and 12:1 molar ratio during alkaline esterification. Instead of taking molar ratio, Tiwari *et al*. (2007) and Ghadge and Raheman (2005) utilized volume as a measure of ratio. Demirbas (2002) also reported in an experiment where he transesterified vegetable oils between 1:6 to 1:40 vegetable oil-alcohol molar ratios in catalytic and supercritical alcohol conditions that higher molar ratios result in greater ester production in a shorter time.

2.14.2 Effect of temperature

It was observed that increasing the reaction temperature, especially to supercritical conditions, had a favorable influence on the yield of ester conversion. In the alkali (NaOH or KOH) transesterification reaction, the temperature maintained by researchers during different steps range between 45-65^{o}C. The boiling point of methanol is 65^{o}C. Temperature higher than this will burn the alcohol and will result in much lesser yield.

A study by Leung & Guo (2006) showed that temperature higher than 50^{o}C had a negative impact on the product yield for neat oil, but had a positive effect for waste oil with higher viscosities. Demirbas (2002) also reported that increasing the reaction temperature, especially to supercritical temperatures, had a favorable influence on ester conversion.

2.14.3 Effect of water and free fatty acid (FFA) contents on the yield of biodiesel

In the transesterification process, the vegetable oil should have an acid value less than 1 and all materials should be substantially anhydrous. If the acid value is greater than 1, more NaOH or KOH is injected to neutralize the free fatty acids. Water can cause soap formation and frothing. The resulting soaps can induce an increase in viscosity, formation of gels and foams, and make the separation of glycerol difficult (Ghadge & Raheman, 2005). Water content is an important factor in the conventional catalytic transesterification of vegetable oil.

In the conventional transesterification of fats and vegetable oils for biodiesel production, free fatty acids and water always produce negative effects since the presence of free fatty acids and water causes soap formation, consumes catalyst, and reduces catalyst effectiveness. Kusdiana and Saka (2004) are of the opinion that water can pose a greater negative effect than presence of free fatty acids and hence the feedstock should be water free. Canakci and Gerpen (1999) insist that even a small amount of water (0.1%) in the transesterification reaction will decrease the ester conversion from vegetable oil.

In conventional catalyzed methods, the presence of water and FFA has negative effects on the yields of methyl esters. Presence of water and FFA in raw material cause soap formation, a decrease in yield of the alkyl ester, greater consumption of catalyst and a reduced catalyst

effectiveness (Demirbas, 2006). However, Demirbas in 2006 also reported that the presence of water had a positive effect on the yield of methyl esters when methanol at room temperature was substituted by supercritical methanol. The presence of water had negligible effect on the conversion while using lipase as a catalyst (Madras *et. al*., 2004).

2.14.4 Effect of catalyst content

It has been reported that CaO can accelerate the methyl ester conversion from sunflower oil at 252^{o}C and 24 MPa even if a small amount of catalyst (0.3% of the oil) was added. The transesterification speed obviously improved as the content of CaO increased from 0.3% to 3%. However, further enhancement of CaO content to 5% produced little increase in methyl ester yield (Demirbas, 2008).

2.15 Summary of Available Literatures

There is currently no commercial biodiesel plant that exists in Nigeria, except for a few production facilities that are notably not well documented. Production and consumption are still at their infancy stage. This work sought to evaluate the oil- and biodiesel-yielding potential of the seeds of Palm kernel (*Elaeis guineensis*), Yellow oleander (*Thevetia peruviana*), Moringa (*Moringa oleifera*) and also Spirogyra biomass (*Spirogyra africana* Fritsch).

Generally, a few amount of experimental work have been carried out by Nigerian researchers on some of the local plant feedstocks used for biodiesel production in this work *viz* Palm kernel (*Elaeis guineensis*), Yellow oleander (*Thevetia peruviana*), Moringa (*Moringa oleifera*) and also Spirogyra biomass (*Spirogyra africana* Fritsch) as highlighted in Table 2.8 below. No online publication was found for the production of oil and biodiesel from Spirogyra filaments by any Nigerian researcher.

From available literature, it is clear that researches in Nigeria and even West Africa are still evolving, with so much work left to be done in evaluating the full potential of locally available biomasses for oil and biodiesel production. This is because the parameters that have been evaluated in the works highlighted in Table 2.8 are not exhaustive. This work was therefore designed to further give more insight into some of these and other parameters as they affect the biodiesel production process.

Table 2.8: A chronology of publications on biodiesel research works in Nigeria

Year	Investigators	Title of Publication	Source
		Palm Kernel	
2000	Abigor R.D., Uadia P.O., Foglia T.A., Hass M.J., Jones K.C., Okpefa E., Obibuzor J.U. & Bafor M.E.	Lipase-catalyzed production of biodiesel fuel from some Nigerian lauric oils.	*PubMedAbstract; BiochemSoc Trans*, 28:979-981
2004	Igbokwe P.K., Effiong E.E., Nwafor O.M.I. and Ngochindo R.I.	Factors affecting the transesterification of Palm olein	*Nigerian Journal of Engineering Management; Vol 5, N0 2, pp. 25-30*
2008	Igbokwe P.K., Effiong E.E., Mgbemena C. and Obike I.J.	Kinetics of the transesterification of Nigeria Palm and Palm kernel oils	*Journal of Science, Engineering and technology (JSET); vol 15, N0 1, pp. 7998-8003*
2007a	Alamu O.J., Waheed M.A., and Jekayinfa S.O	Biodiesel production from Nigerian palm kernel oil: effect of KOH concentration on yield	*Energy for Sustainable Development. Journal; 11(3): 77-82*
2007b	Alamu O.J., Waheed M.A., Jekayinfa S.O	Alkali-catalysed laboratory production and testing of biodiesel fuel from Nigerian palm kernel oil	*Agricultural Engineering International: the CIGR Journal of Scientific Research and Development. 9(EE 07-009)*
2008	AlamuO.J., Akintola T. A., Enweremadu C. C. &Adeleke A. E.	Characterization of palm-kernel oil biodiesel produced through NaOH-catalysed transesterification process.	*Academic Journals, Scientific Research and Essay;*Vol.3 (7), pp. 308-311
2011	Atu A.A., Emeka C.U and Akunna E.E.	Optimum Requirements for the Synthesis of Biodiesel Using Fatty Acid Distillates	*Journal of Emerging Trends in Engineering and Applied Sciences (JETEAS) 2 (6): 897-900*
2011	Oghenejoboh K. M. &Umukoro P. O.	Comparative analysis of fuel characteristics of biodiesel produced from selected oil-bearing seeds in Nigeria	*European Journal of Scientific Research, ISSN 1450-216X, Vol.58, No.2 (2011), pp.238-246*
2012	Igbum O.G., Asemave K. and Ocheme P. C	Evaluation of the biodiesel potential in Palm kernel Oil	*International Journal of Natural Products Research; 1(3):57-60*
2012	Ojolo S.J., Adelaja A.O. and Sobamowo G.M.	Production of Biodiesel from Palm Kernel Oil and Groundnut Oil	*Advanced Materials Research Vol. 367; pp 501-506*

		Yellow Oleander	
2007	Balusamy T. and MarappanR.	Performance evaluation of direct injection diesel engine with blends of *Thevetia peruviana* seed oil and diesel	*Journal of Scientific and Industrial Research; 66:1035–40*
2007	Oluwaniyi O.O. and Ibiyemi S.A. 2007	Efficacy of catalysts in the batch esterification of the fatty acids of *Thevetia peruviana* seed oil	*Journal of Applied Science and Environmental Management; 66:1035–40*
2009	Olisakwe H.C.,Tuleun L.T. and Eloka-Eboka A.C.	Comparative Study of *Thevetia peruviana* and *Jatropha curcas* seed oils as feedstock for grease production	*International Journal of Engineering Research and Applications (IJERA); Vol. 1, Issue 3, pp.793-806*
2009	Usman L.A., Oluwaniyi O.O., Ibiyemi S.A., Muhammad N.O. and Ameen O.M	The potential of Oleander (*Thevetia peruviana*) in African agricultural and industrial development: A case study of Nigeria	*Journal of Applied Biosciences 24: 1477-1487*
2013	Chindo I. Y., Danbature W. and Emmanuel M	Production of Biodiesel from Yellow Oleander (*Thevetia peruviana*) Oil and its Biodegradability	*Journal of the Korean Chemical Society 2013; Vol. 57, No. 3*
		Moringa Oleifera	
2008	Rashid U., Anwar F., Moser B.R. and Knothe G.	Moringa oleifera oil: a possible source of biodiesel	*Bioresource Technology; 99:8175–9*
2011	Uzama D., Thomas S.A., Orishadipe A.T. and Clement O.A.	The Development of a Blend of *Moringa Oleifera* Oil with diesel for Diesel Engines	*Journal of Emerging Trends in Engineering and Applied Sciences (JETEAS) 2 (6): 999-1001*
2013	Aliyu A. O., Nwaedozie J. M. and Ahmed A.	Quality Parameters of Biodiesel Produced from Locally Sourced *Moringa oleifera* and *Citrullus colocynthis L.* Seeds found in Kaduna, Nigeria	*International Research Journal of Pure & Applied Chemistry 3(4): 377-390*

CHAPTER THREE

METHODOLOGY

3.1 Study Design

The study was an experimental study which involved field and laboratory components. The field component involved exploring and sourcing for the substrates while the laboratory component involved oil extraction, oil processing to biodiesel and analysis for specific parameters in the different substrates, oils and biodiesels produced. Different types of plant-based biomasses such as palm kernel seeds, moringa seeds, yellow oleander seeds and spirogyra filaments were utilized in the experiment. The experiment employed a complete randomized design with three (3) replicates of most of the analyses carried out on the biomass samples unless where otherwise stated.

3.2 Description of Study Area

The study area for this research work was Ibadan, the capital city of Oyo state, located in south-western Nigeria. The city, which is located on coordinates $7^{o}23'47''$N $3^{o}55'0''$E on the global map, is the third largest metropolitan area (by population) in Nigeria after Lagos and Kano, with a population of 2,258,625 according to the 2006 census (Omonijo *et. al.*, 2007). At independence, Ibadan was the largest and most populous city in Nigeria and the third in Africa after Cairo and Johannesburg.

Ibadan, which has a total area of 1,190 sq mi (3,080 km^2) is located in the southeastern part of Oyo state about 120 km east of the border with the Republic of Benin in the forest zone close to the boundary between the forest and the savanna. The city has a tropical wet and dry climate with a lengthy wet season and relatively constant temperatures throughout the course of the year. Basically, the city experiences an annual rainfall of about 2,500 mm and temperature below 53^{o}F.

The choice of Ibadan city as a study area is because of large scale agricultural activities, which is evident by the presence of Research Institutes like International Institute of Tropical Agriculture (IITA), Institute of Agricultural Research and Training (IAR&T), Cocoa Research Institute of Nigeria (CRIN), National Institute for Horticultural Research and Training (NIHORT) and

Agricultural plantations (government and private-owned). The city also serves as a commercial nerve centre for agricultural produce such as grains, tuber crops, plants seeds and seedlings of various types and so on that are brought from other states in the country, most especially from the Northern states.

3.3 Laboratory Management Practices

The motive was to avoid any source of contamination of the equipment and/or samples under study by mineral oils, greases, plasticizers from plastics and detergents. Therefore, the glassware that was used for this study were thoroughly washed with detergent, rinsed properly with distilled water and then allowed to dry in hot-air oven. The container used in handling any one of the biomasses at any particular time was also properly cleaned before being used to hold another biomass. This was to forestall any cross-contamination of one substrate by another, which may give false results in oil yield and biodiesel yield afterwards.

The following gears were utilized during the biodiesel production process for safety purposes:

i. Chemical-resistant gloves (butyl rubber is best for methanol and lye)
ii. Chemistry goggles (indirect vented) and face shield
iii. Eyewash bottle with saline solution
iv. Fire extinguishers (ABC or CO_2)
v. Access to running water

3.4 Sourcing for Materials

3.4.1 Sample Source

The biomasses utilized for these experiments were: Palm kernel (*Elaeis guineensis*) seeds, Moringa seeds (*Moringa oleifera*), Yellow oleander (*Thevetia peruviana*) seeds and Green algae (*Spirogyra africana* Fritsch) as shown in Plate 3.1 below. It should be noted at this point that "substrates" and/or "biomass" are used interchangeably in this write-up.

(a) **(b)**

(c) **(d)**

Plate 3.1: Showing an array of the biomasses utilized in the study-(a) Palm kernel seeds (b) Moringa seeds (c) Thevetia seeds (d) Spirogyra filaments

Palm kernel seeds and Moringa seeds were obtained from the Teaching and Research Farm, University of Ibadan, Thevetia seeds harvested from a Thevetia plantation grown as hedges around the Faculty of Education, University of Ibadan; while the Spirogyra filaments were harvested from a water course sandwiched between Obafemi Awolowo Hall, CBT centre and the New Sport Complex, University of Ibadan.

3.4.2 Field Sampling of Substrates

About four (4) polythene bags each of matured dry Moringa pods and fresh matured Thevetia fruits were harvested from their parent trees and gathered for this work; while one (1) polythene bag of decorticated dry palm kernel nuts was also collected from the oil milling section of the Teaching and Research farm, University of Ibadan (Plate 3.2). Identification of all the plant biomasses utilized for the experiment was done at the Herbarium unit of Botany Department, University of Ibadan for validation.

Palm kernel seeds were identified as the African oil palm seeds (*Elaeis guineensis*), Moringa seeds identified as *Moringa oleifera*, and Yellow oleander fruits identified as *Thevetia peruviana*. In the case of the Spirogyra biomass, the genus of the different samples taken randomly from the different spirogyra biomasses that were collected from the same source at different points were identified to be majorly comprised (over 90%) of *Spirogyra africana* (Fritsch) Czurda under binocular light microscope (x10 magnification) (Plate 3.3) based on morphological studies with reference to the published algal monograms (Smith, 1950; Transeau, 1951; Randhawa, 1959 and Zaman *et. al*., 2009).

Present amongst some of the Spirogyra biomass collected was a mixture of few cells of Oedogonium, Zygnema, Zygnemopsis and epiphytic diatom species. A sieve was used to scoop the filaments from the water course into a clean large bowl. All the collected biomass samples were taken home for preparation (as described shortly) prior to laboratory processing.

(a)

(b)

(c)

(d)

Plate 3.2: Showing an array of the samples in their natural state when collected from their different sources- (a) Matured dry Moringa pods; (b) Showing some of the polythene bags that were used to collect fresh matured Thevetia fruits; (c) Decorticated dry palm kernel nuts (Inset: Oil mill, Teaching and Research farm, University of Ibadan); (d) Spirogyra filaments being harvested from the water course.

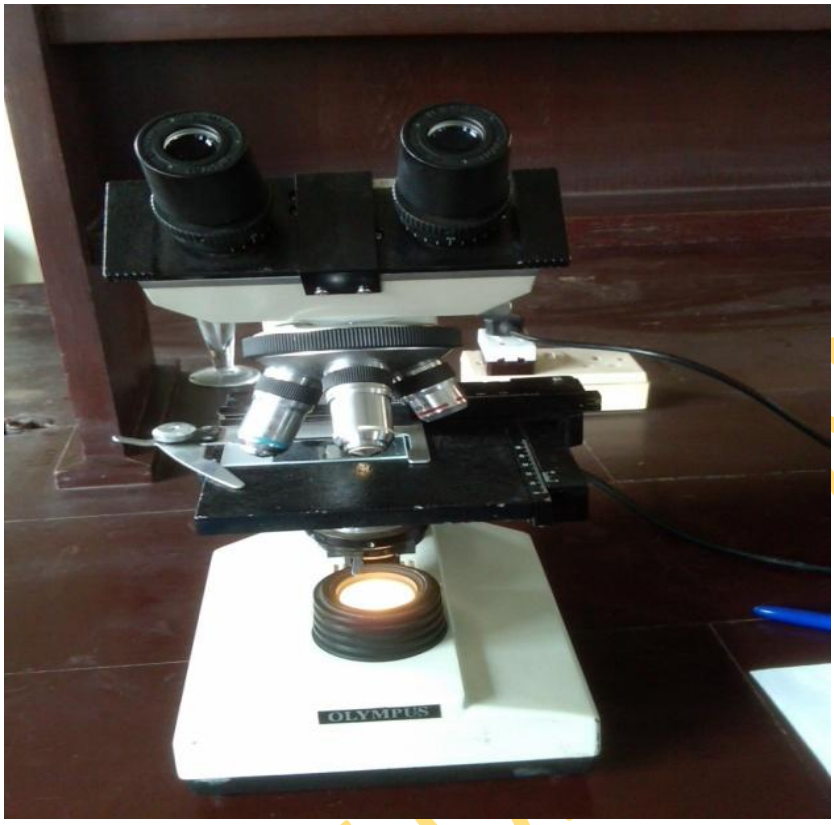


Plate 3.3: Picture of the Light Microscope used for morphological assessment of the Spirogyra filaments

3.5 Materials and Methods

3.5.1 Materials

The following materials were utilized in this study: Miller, Mortar & pestle, Weighing balance, Oven, Dessicator, Filter papers, Soxhlet extractor, Muslin fabric, Centrifuge, 20ml and 50ml air tight plastic bottles, Erlenmeyer flasks, Water bath, Conical flasks, Test tubes, Beakers, Spatula, Cotton wool, Aluminum foil, Retort stand, pH meter, Magnetic stirrer with hot plate, Separating glass funnel, Rota-evaporator, etc.

3.5.2 Consumables

The following consumables were utilized in the course of the experiment: n-Hexane (99.0% purity; 0.659g wt per mL @ 20^{o}C), 99.5% methanol, 0.5M NaOH and Sulphuric acid. Other consumables include Detergent and Distilled water.

All reagents that were used for the experiments were of analytical grade and were purchased as industrial-sealed products from Juliemak (Nig) Enterprises, N0 100, Yemetu-Adeoyo road, opposite Kitchenette Palladium, Yemetu, Ibadan, Oyo state, while some few others were obtained from the Department of Environmental Health Sciences Laboratory, Faculty of Public Health, College of Medicine, University of Ibadan, Oyo state.

3.6 Laboratory Methods

The operations that were undertaken in this experimental work (summarized in Fig. 3.1) include:

- Sample processing
- Substrate preparation and characterization
- Oil extraction
- Characterization of the extracted oils
- Transesterification process
- Phase separation and Purification process
- Determination of biodiesel yield
- Characterization of the biodiesels

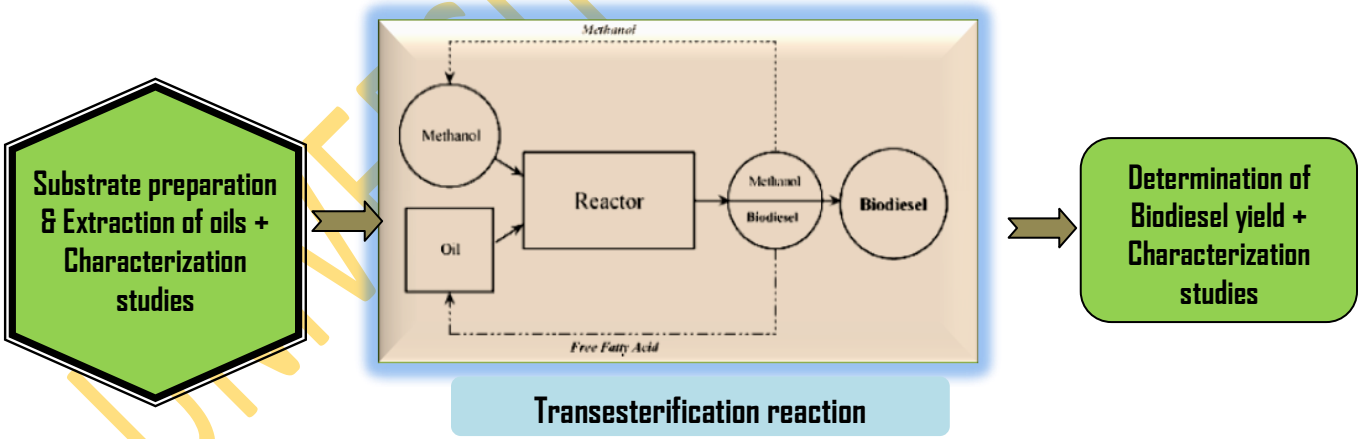


Fig 3.1: A simple flow chart of the major steps involved in the experimental work

3.6.1 Sample Processing

3.6.1.1 Decortications and Sun-drying

The dry moringa pods were manually unshelled with hand, while the brown-winged seeds were decorticated with knife (while taking care not to cut the seeds in the process). The thevetia fruits

were manually decorticated with knife to reveal the kernels, which were dried in continuous sunlight for about 5 hours and subsequently decorticated with stones to reveal the seeds. The decorticated dry palm kernel nuts were unshelled manually with stone to reveal the embedded seeds. The pods/shafts obtained from the decorticated biomasses (Plate 3.4) were discarded. The weight of the seeds from each of the substrates was measured using a Top-loading balance and recorded as ***wet weight*-W_1**.

All the decorticated/unshelled seeds were then individually spread on trays or drying slab, subjected to sundrying for about 48 hours intermittent sunlight, and then allowed to air-dry for about a week (Plate 3.5).

(a) **(b)**

(c) **(d)**

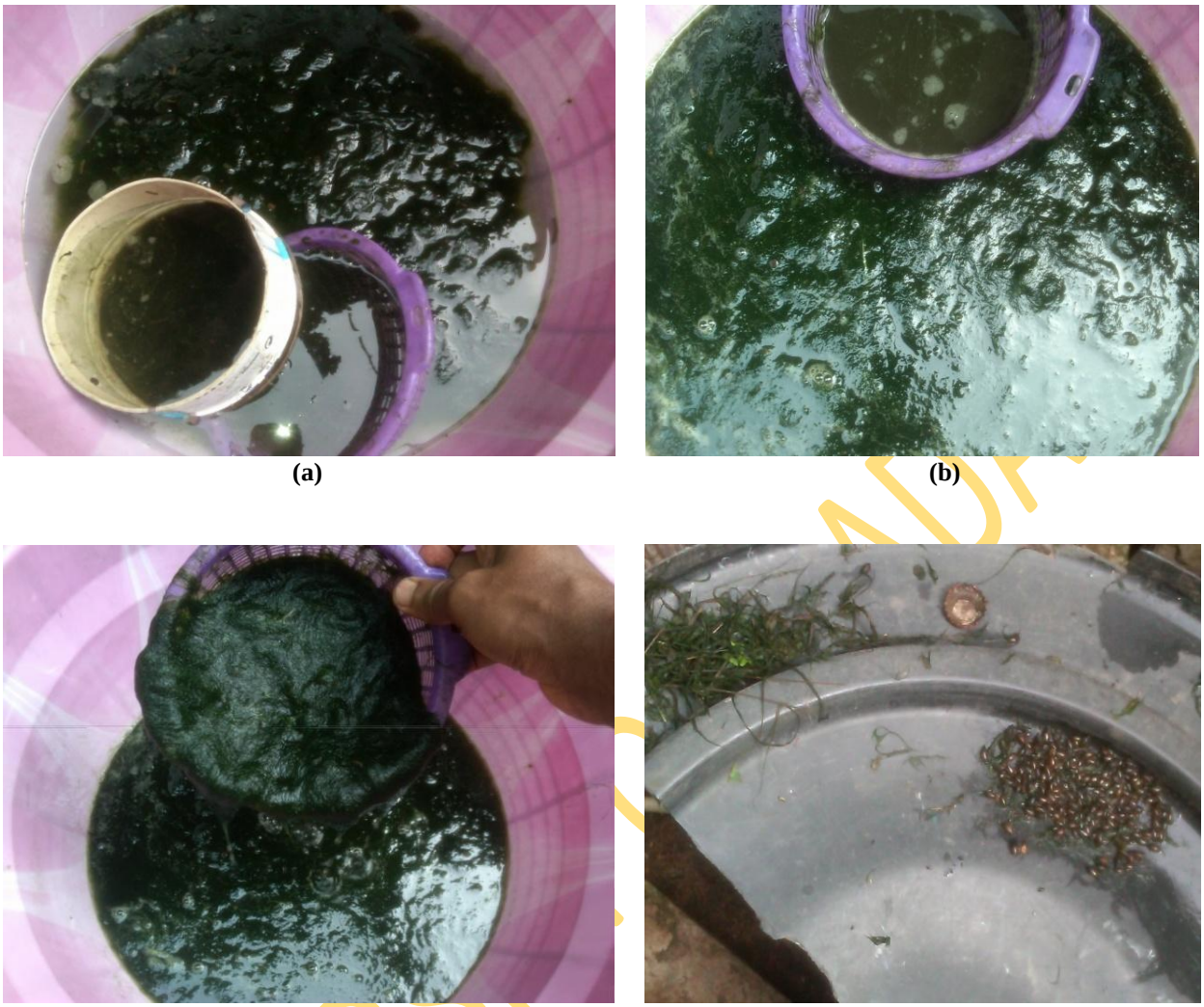
Plate 3.4: Pictures of decorticated biomasses-(a) Detached moringa pods/shafts (b) Detached moringa brown-winged shafts (c) Decorticated thevetia flesh (d) Broken/empty thevetia kernels

Plate 3.5: Showing an array of the different substrates prepared for sundrying

In the case of the Spirogyra biomass, the sample was prepared according to the method of Fuad *et. al*., 2010. The filaments were gradually rinsed with fresh water in a basin to remove all extraneous materials/debris/sediments e.g. plant materials or residues, sand particles, scum, and macro-invertebrates like water snails, tadpoles, insects, etc. (Plate 3.6).

The clean filaments were then drained off water by packing them in the sieve and pressing down gently until water stopped dripping. These were then spread on a slab and air-dried for about a day. The weight of these filaments was then measured and also recorded as *wet weight*-W_1. The filaments were also subsequently spread on a drying slab (Plate 3.5), sundried for about 24 hours intermittent sunlight and allowed to air-dry for about a week.

All the sundried samples were thereafter taken to the laboratory and the weight of the individual samples were measured using a Top-loading balance and recorded as ***sundried weight*-W_s.**



(a) **(b)**

(c) **(d)**

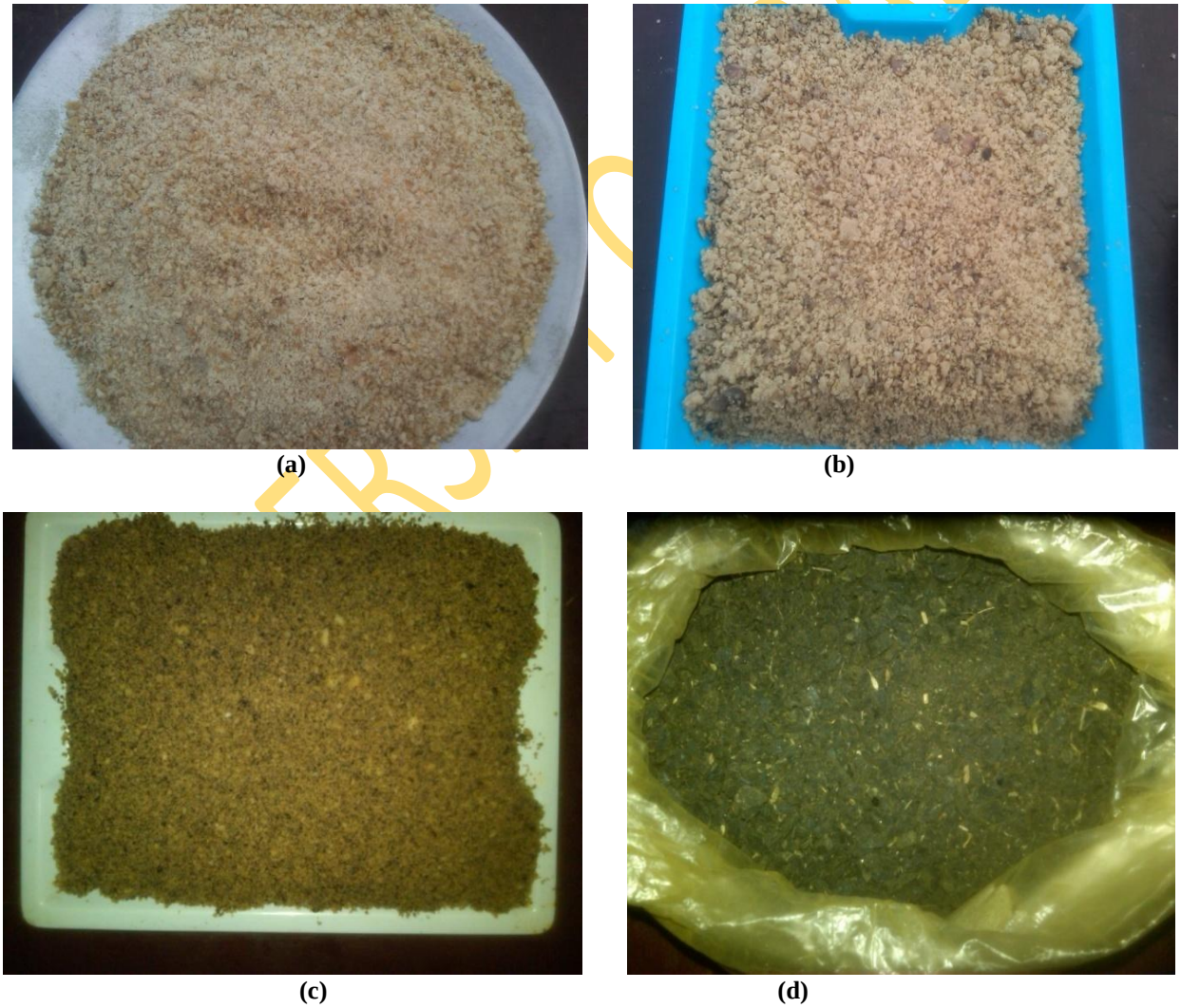
Plate 3.6: Showing the rinsing of Spirogyra biomass to remove extraneous materials-(a) Commencement of the filaments rinsing; (b) Showing the nature dirt in the biomass from the colour of water in the purple sieve; (c) Relatively clean spirogyra biomass (d) Part of the extraneous materials removed from the biomass

3.6.1.2 Milling and Oven-drying

The sundried samples of moringa and thevetia seeds were ground using a hand-powered bench grinder (Plate 3.8b); the sundried sample of palm kernel seeds were ground using a fuel-powered domestic grinder (Plate 3.8c); while the sundried spirogyra filaments were pulverized using a dry mill blender (IKA® A11 BS2 model) that is equipped with a stainless steel cutting blade (Plate 3.8a); to granular form that would expose a larger surface area of the substrates for enhanced extraction of oil from them (Plate 3.7).

The ground/pulverized substrates were respectively weighed (in clean empty crucibles that have been tare) and then subjected/left to oven-dry at 105^{o}C for an intermittent period of 48 hours. The drying temperature of 105^{o}C and the extended period of 48 hours were chosen to ensure that the weight loss was because of water losses and not losses of organic matter through volatilization (NEH, 2000).

During the oven-drying period, the weights of the respective substrates were measured at intervals until there was no longer loss of water-weight. When this point of nil water-weight loss was reached, the oven-dried substrates were put in a dessicator for about 30 minutes to cool. Thereafter, the weight of the substrates were taken again and recorded as ***dry* weight-W_2**.



(a) **(b)** **(c)** **(d)**

Plate 3.7: Showing an array of sundried milled substrates (a) Milled moringa seeds (b) Milled thevetia seeds (c) Milled palm kernel seeds (d) Milled spirogyra biomass

(a) **(b)** **(c)**

Plate 3.8: Showing the instruments used for pulverization/milling-(a) Miller (IKA® A11 BS2 model); (b) Hand-powered bench grinder (c) Fuel-powered domestic grinder

3.6.2 Substrate Preparation and Characterization

Each of the milled substrates (i.e. seeds of Palm kernel, Moringa, Thevetia and Spirogyra biomass) utilized in this experiment was weighed using a Top-loading balance (AИD EK-410*i* model) which has a maximum limit of 400 g and sensitive enough to read as low as 0.01g of a substance (Plate 3.9). Once the balance was tare with a measuring container, the respective biomasses were scooped using a spatula in a stepwise mode unto the aluminum foil or any other container placed on the balance until the meter reads the desired quantity.

All weighing were carried out in the balance room of the laboratory where the ambient environmental conditions such as temperature, pressure, relative humidity, air speed, etc, were relatively stable.

Plate 3.9: Showing the AHD EK-410*i* model Top-loading balance

3.6.2.1 Determination of Moisture Content

Moisture content can be expressed on a *wet basis, dry basis, or as the fixed solids content* (NEH, 2000). The moisture content as expressed on a ***wet basis*** gives the percentage of the original wet sample that is water. This is useful for determining whether a dry matter is sufficiently dry.

Moisture content expressed on a ***dry basis*** denotes the moisture content as a percentage of the sample after it has been dried. The content remaining after a sample has been dried is known as the total solids. Because a dry sample is defined as the total solids of a sample, the dry basis moisture can also be expressed as units of moisture per unit of total solids. Dry basis moisture is useful when calculating moisture changes.

Fixed solids are defined as the weight remaining after ignition of the total solids at 600 degrees Celsius until complete combustion (NEH, 2000).

For the purpose of this work, the percentage moisture in the substrates was determined using two different methods: First, the oven-drying method (Section 3.6.1.2) was used for the moisture content determination (% wet basis), where the formula below was used for the calculation:

$$\textbf{Moisture content} = \frac{(W_1 - W_2)}{W_1} \times 100\% \quad \textbf{Formula 3.1}$$

where W_1 = Original weight of the fresh sample before any drying,

W_2 = Weight of the sample after oven drying.

N.B: The weight of the container used in measurement is negligible because the weighing balance is always tare with the weight of the container before any measurement takes place.

Secondly, the moisture content was determined using a Moisture Analyzer device, which contains a Super Hybrid Sensor (SHS) (Plate 3.10). It is an automated device which is simple to use, and displays both the percentage moisture content of the biomass, the temperature at which the reading was done and the time range of exposure of the sample to the heating process.

The substrate was measured unto the aluminum foil sample holder of the device in a stepwise fashion until the LCD screen displayed 5 g (which was the required quantity of substrate to be loaded on the sample holder). The sample holder containing the substrate was then covered with a thin glass fiber material to ensure that the sample did not get burnt in the heat-to-dryness operation of the device.

This is because, if burning (carbonization) occurs during drying, the results are not valid because organic matter is also lost in addition to the water. Then the lid of the device was closed and the start button was pressed. Once the device was through with the moisture content determination, it displayed the results of the analysis on the screen to be read. The process was done in triplicate for each of the biomasses

Plate 3.10: Picture of the Moisture Analyzer (АИД MX-50 model) device

3.6.2.2 Determination of Relative density

The density (and hence, Relative density) of the biomasses was determined by using the simple weight to volume ratio estimation using weighing balance and measuring cylinder.

Principle: All matter has mass and volume. Mass and volume are the physical properties of matter and may vary with different objects. The amount of matter contained in an object is called mass. Its measure is usually given in grams (g) or kilograms (kg). Volume is the amount of space occupied by an object. The units for volume include liters (L), meters cubed (m^3), and gallons (gal).

The mass of a unit volume of a substance is called its density.

$$Density = \frac{Mass\ of\ the\ substance}{Volume\ of\ the\ subsatnce} \quad \text{.....Formula 3.2}$$

If D is the density of a body of mass M and volume V, then

$$D = \frac{M}{V}$$ In S.I unit, density is expressed in kg/m^3 or g/cm^3.

Relative density of a substance is defined as the ratio between the density of the substance to the density of water at 4^{o}C. Relative density is also known as ***specific gravity*** but the term "*relative density*" is often preferred in modern scientific usage. The relative density of a substance is a pure number *without any unit*. It tells how many times a substance is heavier than water.

The density of the biomasses was determined at room temperature using the weight to volume (w/v) ratio, wherein a measuring cylinder was used to determine the compacted volume of the milled biomasses and a weighing balance was used to determine their weights. This was done in triplicates for each of the substrates using different weight to volume ratios, and the results expressed in g/cm^3.

Relative density (R.D) of a substance can be calculated by dividing density of a substance with the density of water. In SI units, the density of water is (approximately) 1000 kg/m^3 or 1 g/cm^3, which makes relative density calculations particularly convenient: the density of the object only needs to be divided by 1000 or 1, depending on the units.

$$Relative\ density\ of\ a\ substance = \frac{Density\ of\ the\ substance}{Density\ of\ water}$$ .....**Formula 3.3**

3.6.2.3 Elemental composition determination (Proximate Analysis)

Plant analysis may be regarded as the study of the relationship of the nutrient/elemental composition of plant with respect to certain predefined parameters such as the effects that these elements could mediate *in vitro* when the plant materials are utilized in experiments.

Phosphorous, calcium, and magnesium, for example, are minor components typically associated with phospholipids and gums that may act as emulsifiers (ASTM Standard D6751, 2009) or cause sediment, lowering yields during the transesterification process (Gerpen *et. al.*, 2004). Hence, plant analysis (in the context of biofuels such as biodiesel) requires the determination of

the level of certain mineral element constituents of the plant tissues that are to be used for the production of the biodiesel.

Such mineral elements might have been implicated to affect any of the stages in the biofuel production processes, hence their percentage composition need to be measured so as to determine the best methods that could be used to derive optimal yield from processing the biomasses. The procedures used in plant analysis include: the conversion of the organic form of the nutrient to the inorganic forms; and the determination of the nutrient element in the extract by an appropriate method.

The conversion of the organic form of the element to the inorganic form is generally done by either dry digestion method or by wet digestion method. Dry ashing involves the sample being heated to a high temperature without the addition of any reagent. Dry ashing is not however suitable for the determination of volatile elements such as Sulphur, Arsenic and Selenium. However for this work, wet digestion was used for organic matter destruction in the biomasses prior to elemental analysis as described below.

3.6.2.4 Wet (organic matter) digestion

Principle: The wet digestion procedure was carried out according to the method described by Owen, 1992. Wet digestion involves the destruction of organic matter through the use of both heat and acids. Acids that have been used in these procedures include H_2SO_4, HNO_3, and $HClO_4$, either alone or in combination. Hydrogen peroxide (H_2O_2) is also used to enhance reaction speed and complete digestion. Most laboratories have eliminated the use of $HClO_4$ due to risk of explosion. Safety regulations require specially designed hoods where $HClO_4$ is utilized. Hot plates or digestion blocks are utilized to maintain temperatures of 80 – 125 ^{o}C. After digestion is complete and the sample is cooled, the vessel is filled to volume and dilutions are made to meet analytical requirements.

Apparatus: Hot plate, block digester, fume hood and 200 mL tall-form beakers or digestion tubes.

Reagents: Deionized water, conc. Nitric acid (HNO_3), conc. Sulphuric acid H_2SO_4) and 30% Hydrogen peroxide (H_2O_2).

Procedure: 1 g of dried plant material that has been ground (0.5-1.0 mm) and thoroughly homogenized was weighed and placed in a digestion tube. 5.0 mL concentrated HNO_3 was added and a funnel was placed in the mouth of digestion tube and allowed to stand overnight or until frothing subsided. The digestion tube was placed into a block digester and heated at 125^oC for 1 hour. The digestion tube was removed and allowed to cool. 2 mL of 30 % H_2O_2 was added and digested at the same temperature (i.e. 125 ^oC). Heating and 30 % H_2O_2 additions were repeated until digest was clear. Additional HNO_3 was added as needed to maintain a wet digest. After sample digest was clear, the funnel was removed and the temperature lowered to 80 ^oC. The heating was continued until near dryness. The residue became clear white indicating that digestion was completed. Dilute HNO_3 and deionized water was then added to dissolve the digest residue and bring sample to final volume depending upon requirements of subsequent analytical procedures.

The percentage composition of the following elements were determined in the fresh milled substrates: Total Organic Carbon (T.O.C), Total Nitrogen (TN), Total Phosphorus (TP), Sodium content (Na), Calcium content (Ca) & Sulphur content (S). Samples were generally analyzed chemically according to the official methods of analysis described by the Association of Official Analytical Chemists (A.O.A.C., 1998). All analysis and/or readings were carried out in triplicates.

3.6.2.5 Total Organic Carbon determination

This was measured using the Walkley-Black Wet Oxidation method (1934).

Apparatus: Automatic burette, Conical flask and Pipette.

Reagents: Std. Normal $K_2Cr_2O_7$, Std. Normal $Fe(NH_4)(SO_4)_2$ and Diphenylamine indicator.

i. **Preparation of Standard Normal Potassium dichromate:** $K_2Cr_2O_7$ was oven dried at 130-150^oC for 2-3 hours. It was cooled in a dessicator; 49.035 g of the dried salt was

weighed out; this was dissolved in about 950 ml of distilled water, and placed in a cool place overnight. When cool, it was made up to 1000 ml with cold distilled water.

ii. **Preparation of Standard Normal Ferrous Ammonium Sulphate:** 156.86 g of $Fe(NH_4)(SO_4)_2$ was weighed out and dissolved in about 900 ml of distilled water. 25 ml of conc. H_2SO_4 was added and allowed to cool. It was made up to mark with distilled water and standardized using normal Potassium dichromate.

iii. **Preparation of Diphenylamine indicator:** 1g of Diphenylamine was dissolved in 200 ml of 1:1 solution of water and H_2SO_4.

Procedure: 3 g of the sample was weighed (depending on how deep the colour of the analyte was), and unto this was added 10 ml of 1N $K_2Cr_2O_7$ from an automatic burette. 20 M conc. H_2SO_4 was then gently added into the mixture from a dispensing burette. The mixture was shaken gently and left to cool. Afterwards, distilled water was added to make up to the 150 ml mark on the conical flask. Thereafter, about 8-10 drops of diphenylamine indicator was added, and the colour changed to dark violet. This solution was then titrated against 0.4N $Fe(NH_4)(SO_4)_2$ until the violet colour changed to green. A duplicate blank determination was carried out on 10ml of the Normal $K_2Cr_2O_7$ using all the reagents each time a set of determination was done.

Calculations: Let y be the volume in milliliters of the 0.4 N $Fe(NH_4)(SO_4)_2$ used to react with the remaining $K_2Cr_2O_7$ which is 0.4y. For example, since 10 ml of $K_2Cr_2O_7$ was used in the first place, then the amount used to oxidize any carbon in the sample will be (10-0.4y). 1 ml of $K_2Cr_2O_7$ = 0.003 g carbon. However, the reaction is only approximately 75 % complete.

$$\text{Therefore, 1 ml of } K_2Cr_2O_7 = \frac{0.003 \times 100}{75} = 0.004 \text{ g}$$

$$\text{That is, } \textbf{\% Total Organic Carbon (TOC) in sample (hydrosylate)} = \frac{(10 - 0.4 \times T.V) \times 0.004 \times 100}{\text{Wt of sample taken}} \quad \textbf{....Formula 3.4}$$

where T.V = Titre value

3.6.2.6 Total Nitrogen determination

The Total Nitrogen in the substrates was determined by the routine Semi-micro Kjeldahl technique. This technique consists of three major stages *viz*: Digestion, Distillation and Titration.

Apparatus: Weighing balance, Digestion tubes, Digestion block heater, 50 ml burette, 5 ml pipette, 10 ml Measuring cylinder, 100 ml Beakers, and Fume cupboard.

Reagents: Conc. H_2SO_4, 0.01 N HCl, 40 % (w/v) NaOH, 2 % Boric acid solution, Methyl red Bromocresol green mixed indicator, Kjeldahl catalyst tablet.

Procedure:

a. **Digestion:** 0.5 g of each milled substrate was weighed carefully into the Kjeldahl digestion tubes to ensure that all the sample materials got to the bottom of the tubes. To these were added one (1) Kjeldahl catalyst tablet and 10 ml of conc. H_2SO_4. These were set in the appropriate holes of the Digestion block heater that have been positioned in a fume cupboard. The digestion was left on for four (4) hours, after which a clear colourless solution was left in the tube. The digest was cooled and transferred into 100 ml volumetric flask, thoroughly rinsing the digestion tube with distilled water and the flask was made up to mark with distilled water.

b. **Distillation:** This was done with Markham distillation apparatus, which allows volatile substances such as ammonia to be steam-distilled with complete collection of the distillate. The apparatus was steamed out for about ten (10) minutes. The steam generator was then removed from the heat source to the developing vacuum to remove condensed water. The steam generator was then placed on the heat source (i.e. heating mantle) and each component of the apparatus was fixed up appropriately.

   A 5 ml portion of the digest above was pipette into the body of the apparatus via the small funnel aperture. To this was added 5 ml of 40 % (w/v) NaOH through the same opening with the 5 ml pipette. The mixture was steam-distilled for 2 minutes into a 50 ml conical flask containing 10 ml 0f 2 % Boric Acid plus mixed indicator solution placed at the receiving tip

of the condenser. The Boric Acid plus indicator solution changed colour from red to green showing that all the ammonia liberated had been trapped.

c. **Titration:** The green colour solution obtained was then titrated against 0.01 N HCl contained in a 50 ml burette. At the end point or equivalent point, the green colour turned to wine colour, which indicated that all the Nitrogen trapped as Ammonium borate $\{(NH_4)_2BO_3\}$ was removed as Ammonium chloride (NH_4Cl).

The percentage Nitrogen in the respective biomasses was calculated from the formula:

$$\%N = \frac{\text{Titre value} \times \text{Normality of HCl used} \times \text{Atomic mass of N} \times \text{Volume of flask containing the digest}}{2000} \times 100 \quad \text{.....Formula 3.5}$$

3.6.2.7 Total Phosphorus determination

Phosphorus was determined the Vanadomolybdate (Yellow) Colorimetric Method or Spectrophotometric method.

Apparatus: Colorimeter/Spectrophotometer, 50 ml Volumetric flask, 10 ml Pipette, Whatman filter paper, Funnel, Wash bottle, Glass rod, Heating mantle, Crucibles, Weighing balance and Flame photometer.

Reagents: Vanadomolybdate yellow solution, 2 M HCl

i. **Preparation of Standard Phosphate solution:** 219.5 mg anhydrous KH_2PO_4 was dissolved in distilled water and diluted to 1000 ml; 1 ml = 10 ug $PO_4^{3-}P$

ii. **Preparation of Standard Calibration Curve:** 10 ml of the standard Phosphate solution was placed in a 50 ml volumetric flask. 10 ml Vanadate-molybdate yellow solution was added and diluted to the mark with distilled water, stoppered and left for 10 mins for full yellow development. After 10 mins or more, the absorbance was measured versus a blank solution (using 15 ml, 20 ml, 25 ml and 30 ml). A graph of Absorbance against Concentration was drawn and the slope was calculated.

Procedure: 20 mg (0.02 g) of each milled substrate was digested by adding 5 ml of 2 M HCl solution to the hydrosylate in the crucible and heated to dryness on a heating mantle. 5 ml of 2 M HCL was added again, heated to boil, and filtered through a No 1 Whatman filter paper. 10 ml of the filterate solution was pipette into 50 ml standard flask and 10 ml of vanadate yellow solution was added; and the flask was made up to mark with distilled water, stoppered and left for 10minutes for full yellow development.

The concentration of phosphorus was obtained by taking the optical density (OD) or absorbance of the solution on a Spectronic 20 spectrophotometer at a wavelength of 470 nm. It is pertinent to note that the wavelength of 470 nm was used because ferric ion causes interference at lower wavelengths, especially at 400 nm.

The Percentage Phosphorus was calculated from the formula below:

$$\%P = \frac{\text{Absorbance reading} \times \text{Slope} \times \text{Dilution factor}}{1000} \quad \text{.....Formula 3.6}$$

But Absorbance × Slope × Dilution factor = ppm/10,000

Hence, **%P** = ppm/10,000

Where, Absorbance = Reading obtained from the Spectrophotometer

Slope = Result of the Standard curve

Dilution factor = Volume of the extract/weight of the sample

3.6.2.8 Calcium and Sodium determination

Wet ashing was used to digest the samples prior to the determination of the percentage calcium and percentage sodium present in the respective samples. Wet ashing is suitable for the determination of Ca, Mg, Na, K, Cu, Fe, Mn, Se and Zn in plant tissues, and may be applicable for the determination of other elements as well.

Apparatus: Fume cupboard, Berzelius beaker, 50 ml volumetric flask, Flame photometer

Reagents: Nitric acid (HNO_3), 70 % Perchloric acid ($HClO_4$) solution, 5 % (w/v) Lanthanum solution and a watch glass.

Plate 3.11: Picture showing the Jenway® Model PFP7 Flame Photometer

Procedure: 1 g of milled dried substrate was weighed into 100 ml Berzelius beaker; and 5ml of HNO_3 and 2 ml $HClO_4$ were added into the beaker. The mixture was covered with a watch glass, and digested in a fume cupboard, heating to dryness (since no volatile elements was required in this stage). 15 ml of deionized water was added and the digest solution was filtered through an acid-washed No 1 Whatman filter paper into a 50 ml volumetric flask. The filter paper was washed with deionized water and the filtrate made up to volume with the water.

Note: Because of contaminations from reagents used, it is advisable to add the same reagents in the blank. Also, as a precaution, nitric acid was added to the substrate sample before adding Perchloric acid to avoid any explosive reaction of Perchloric acid with the untreated organic material.

The filtrates were read with Jenway® Model PFP7 Flame Photometer (Plate 3.11) to determine the proportion of Ca and Na. This was done by setting up the flame photometer, aspirating the

blank solution into it and zeroing. Thereafter, a standard curve of calcium concentration against intensity was plotted. Then the sample solution was aspirated into the flame and the reading obtained recorded. But specifically (for Calcium estimation), the final solution of filtrate has 1 % (w/v) Lanthanum added to it

The sample's concentration was determined from the recorded reading on the calibration graph, and the determined concentration was multiplied with the dilution factor to obtain Percentage Calcium thus (and same calculation used to obtain Percentage Sodium):

$$\textbf{\% Ca} = \frac{\text{Absorbance reading} \times \text{Slope} \times \text{Dilution factor}}{1000} \quad \text{.....Formula 3.7}$$

But Absorbance × Slope × Dilution factor = ppm/10,000

Hence, **% Ca** = ppm/10,000

Where, Absorbance = Reading obtained from the spectrophotometer

Slope = Result of the Standard curve

Dilution factor = Volume of the extract/weight of the sample

3.6.2.9 Total Sulphur determination

This procedure was a modification of the Massoumi and Cornfield (1963) and the Chaudry and Cornfield (1966) methods. Sulfate-sulfur was precipitated in aqueous solution by adding barium chloride. The finely divided barium sulfate crystals remained suspended in the solution, diffracting light. The effect on light transmission through the solution was measured with a spectrophotometer.

Apparatus: A spectrophotometer with digital display capable of measuring absorbance to 0.001 was used. A vortex stirrer was used for uniform mixing.

Reagents:

i. Acetic/phosphoric acid solution: 75 mL concentrated acetic acid was mixed with 25 mL concentrated H_3PO_4 and diluted to 1 L.

ii. Gum acacia solution: 5 g gum acacia was dissolved in 500 mL hot water. This was filtered hot through a Whatman No. 42 filter paper on a Buchner funnel using suction. This was then cooled and diluted to 1 L with acetic acid.

iii. Barium sulfate seed suspension: 18 g $BaCl_2.2H_2O$ was dissolved in 44 mL hot water. Unto this was added 0.5 mL of the 2,000 mg S L-1 standard. The mixture was boiled and cooled quickly. 4 mL of the gum acacia solution was added and mixed well. This suspension was always prepared fresh whenever it is to be used.

iv. Barium chloride solution: 200 g $BaCl_2.2H_2O$ was added to a 1 L volumetric flask. Enough hot water was added to dissolve. The solution was then cooled and diluted to volume.

v. Standard sulfate solution (2,000 mg S L^{-1}): 1.0875 g of oven-dried K_2SO_4 was dissolved in 0.1 M HCl and diluted to 100 mL. Working standards containing 10, 20, 30, 40, 50, and 100 mg S L^{-1} were prepared by diluting appropriate aliquots of this stock with demineralized water. New working standards were prepared fresh on each day of use.

Procedure: 1 mL aliquots of each standard and digested sample were pipetted into standard test tubes. Not more than 30 samples with a single set of standards were run. Unto this was added 22 mL of the acetic/phosphoric acid solution. The solution was mixed on a vortex mixer. Exactly 0.5 mL of the barium sulfate seed suspension was added. Thereafter, 1 mL of the barium chloride solution was added and each tube was mixed exactly the same length of time on a vortex mixer. 1mL of the gum acacia solution was added, and the solution was mixed again. The mixtures were allowed to set for 30minutes. Each sample was mixed uniformly just prior to reading absorbance or transmittance on a spectrophotometer set on a wavelength of 440nm. The wavelength was not critical since only light blockage and not absorbance by the barium sulfate suspension was measured. Absorbance or transmittance was plot against S concentration.

3.6.3 Oil Extraction

Materials: Measuring cylinder**,** Conical flask**,** Muslin fabric, Soxhlet extractor, Cotton wool, Weighing balance, Rotary evaporator, Oven, Dessicator, 50 ml and 100 ml Plastic bottles.

Reagents: n-Hexane (99 % purity) and Petroleum ether

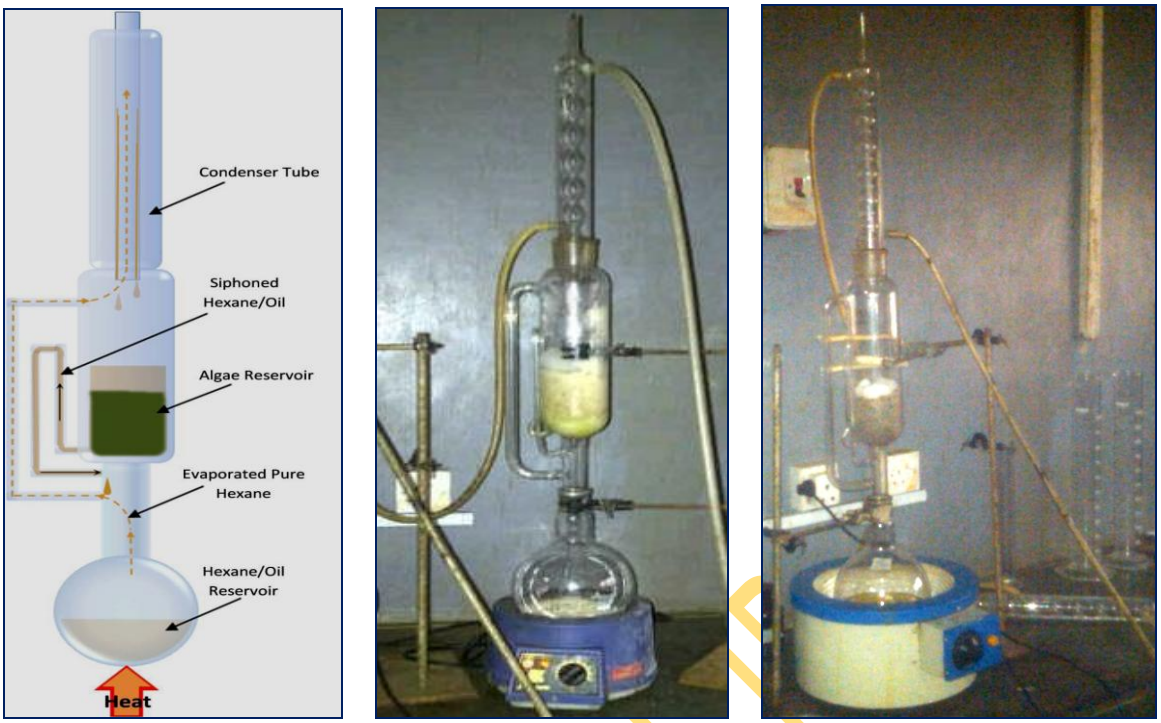
Procedure: The milled, oven-dried biomass samples were used for the extraction process and two extraction methods were experimented *viz*: Soxhlet extraction and Cold solvent extraction. For the Soxhlet extraction, 250 g each of palm kernel seeds, moringa seeds and oleander seeds were respectively placed in the thimble of a Soxhlet extractor with the use of about 800 ml hexane (as extracting solvent) (Plates 3.12 b and c).

In the case of the algal biomass, a dual-phase Soxhlet procedure was used to extract 40 g of the Spirogyra biomass (20 g in each thimble) using 300 ml n-hexane (i.e. 150 ml n-hexane for each extraction set-up) (Plate 3.12d). The spirogyra biomasses were wrapped in a muslin fabric, and put into their separate thimbles respectively (Awolu *et. al*., 2013).

A round bottom flask containing the estimated sufficient n-hexane (800 ml as estimated from literatures) was fixed to the end of the extractor and a condenser was tightly fixed at the bottom end of the extractor. Once the respective sample for a particular extraction period was placed in the thimble of the extractor, the flask was heated at 60 ºC with the use of an electric mantle.

As the solvent was heated in the boiler, the pure vapor rose through a by-pass and into the top part of the Soxhlet container (thimble) where the sample to extract was contained. In the condenser, the vapors condensed and drip into the sample-containing thimble. When the level of liquid reaches the same level as the top of the siphon, the liquid containing the extracted material was siphoned back into the boiler.

Soxhlet extraction is recognized by the A.O.A.C as the standard method for crude fat analysis (Celine *et. al*., 2012). Extraction by Soxhlet is not a continuous procedure, but a batch system with repeated extractions. Each of the extraction processes carried out underwent a minimum of 40 cycles within the 8 hours period, which is considered necessary to complete an extraction (Barthet and Daun, 2004). After the extraction period, the residual biomass was weighed and recorded. The solvent was recovered at 65 ^{o}C under vacuum using a rotary evaporator (Buchi Rotavapor:R-210 model) (Plate 3.19), and the respective residual oils obtained thereafter were also measured and recorded.



(a) (b) (c)

(d) (e)

Plate 3.12: Showing the Soxhlet Extraction Systems-(a) Schematic diagram showing some parts of a Soxhlet extraction system; (b) & (c) Extraction of oil from the milled moringa and palm kernel seeds respectively; (d) Simultaneous extraction of algal biomass oil using two Soxhlet apparatus; (e) Picture of the round bottom flask containing a mixture of the extracted algal oil and little hexane solvent after the Soxhlet extraction.

For the Cold Solvent extraction, the method used by Hossain *et. al*., 2008, which was also used by Abd El-Moneim *et. al*., 2010, Emad, 2011 and Sangodare *et. al*., 2012 was modified. Two extraction-solvent systems (Figure 3.2 below) were experimented to compare the oil yield in each case and report the more suitable solvent system for the highest biodiesel yield (Afify *et al*., 2010).

A known weight of each of the ground dried palm kernel, moringa and thevetia substrates (250 g dry weight) was mixed with the extraction solvent mixtures *viz*: hexane/ether (600 ml, 1:1, v/v) and hexane only (600 ml). In the case of the algal substrate, 30 g dry weight of the biomass was mixed with the extraction solvent mixtures *viz*: hexane/ether (200 ml, 1:1, v/v) and hexane only (200 ml) (Plate 3.13 below).

All the different sample/solvent mixtures were kept to settle in their respective labeled and well-sealed plastic containers (cover lids further held air-tight with sellotape) for 48 hours, with intermittent shaking (every 3-5 hours) of the containers to enhance a better percolation/breakage of the solvent into the cell wall of the plant biomasses.

After the 48hour period was followed by the separation of the sample/solvent mixtures by "squeeze-filtration" using two muslin cloths inserted into each other as a precaution to better reduce the amount of sediment that may probably be small enough to pass through the sieve pores into the solvent/oil mixture (Plate 3.14 below).

The residual biomass (Plate 3.15 below) was collected, weighed and recorded after the complete "squeezing-out"/filtering-out of the oil/solvent mixture. The extracted oil/solvent mixture (which was still rather cloudy) was left to settle and air-dry for 24 hours. After this settling period, the extracted oil, which was still mixed with the extraction solvent, was seen on the upper layer of the sediments (that were in form of paste, possibly a mixture of gums, tannins, etc) (Plate 3.16 below).

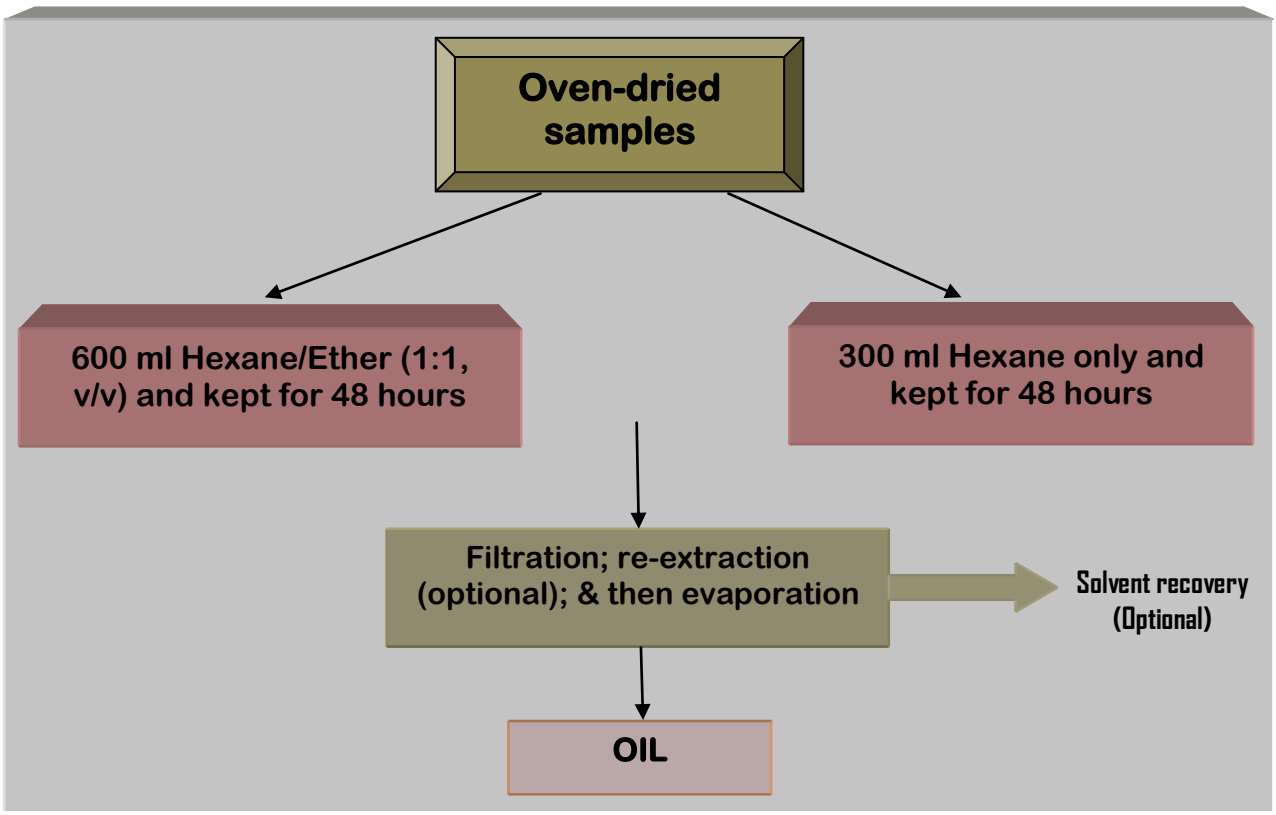


Fig 3.2: Schematic representation of the steps involved in Cold solvent extraction using two solvent systems.

Plate 3.13: Showing an array of the different sample/solvent mixtures

Plate 3.14: Picture of the Muslin sieves used

Plate 3.15: Showing an array of some of the residual biomasses obtained after the squeeze-filtration process

Plate 3.16: Oils suspended on the paste of sediments

Plate 3.17: Part of the decanted oils in labeled bottles

Plate 3.18: Residual paste of sediments left over after decanting the respective oils

Plate 3.19: Picture of the Buchi Rotavapor (R-210 model) concentrating the residual oil

The clear oils were then decanted through a No 1 Whatman filter paper into labeled bottles (Plate 3.17 above), leaving behind the residual paste/sediments (Plate 3.18 above). Each of the decanted oils were individually evaporated under vacuum for about 5 minutes at 60 ^{o}C using the Buchi type Rotavapor (R-210 model) (Plate 3.19 above). This was to ensure that all the extraction solvents in the oils are evaporated off.

The residual oils obtained after evaporation were left to air-dry for about 2 hours; the volume and weight of the oils were subsequently measured and recorded. The weight and volume of the oils obtained from the different extraction methods; alongside the weight of the residual biomasses left-over from the extractions and the quantity of solvent used in each of the extraction methods for each substrate were measured and recorded. The oils were kept for characterization and further processing via transesterification process.

The proportion (%) of oil extracted from the different substrates by both the Soxhlet extraction and Cold extraction systems respectively was determined using equation below:

% Oil content = (W_o/W_u) 100 %.....**Formula 3.8**

where: W_o = weight of oil extracted

W_u **=** Weight of the oven-dried biomass used for the extraction process (g)

The weights of the residual oils obtained were taken and they were also characterized for: ***pH, Relative density, Free Fatty Acid (FFA) level, Fatty Acid Composition-FAC (otherwise called Fatty Acid Profile-FAP), Kinematic viscosity and Saponification value***.

3.6.4 Characterization of Extracted Oils

3.6.4.1 Determination of pH

The pH of the sample oils was read using a calibrated Jenway® 3520 pH meter (Plate 3.20 below). The pH meter probe was inserted into the containers holding the respective oils, making sure it did not touch the inside wall of the containers. The pH reading was then taken from the LCD display after it had stabilized.

Plate 3.20: Showing the pH of one of the oils being determined using Jenway® 3520 model pH meter

3.6.4.2 Determination of Relative density

The Relative density of the oils was determined at 25 ^{o}C following the same method that was described in Section 3.5.2.2.

3.6.4.3 Determination of Free Fatty Acid level

Free Fatty Acid (FFA) level is a critical parameter that needs to be determined in oils because they can react with the catalyst during transesterification and lead to soap formation, emulsions, increased catalyst consumption and reduced catalyst efficiency; and these are undesirable factors in the production process (Knothe et. al, 2005).

Apparatus: Micropipette, 20 mL capacity screw-capped tubes, Centrifuge, and PerkinElmer® Clarus® 600 Gas chromatography.

Reagents: Arachidic acid, Chloroform, Dichloromethane, Diisopropylethylamine, Diethylamine, Bis (2-methoxyethyl) aminosulfur trifluoride, Hexane, Distilled water and Substrate oil sample.

Procedure: The FFA content was determined by selective formation of diethyl amide derivatives according to Kangani *et. al*., 2008. To do this, 0.45 mg arachidic acid (C20:0) in chloroform (150 µL) was added as internal standard before extraction. The extracted lipids were then dissolved in 750 µL dichloromethane and transferred into a screwcapped tube. After addition of 10 µL diisopropylethylamine and 30 µL diethylamine, the solution was cooled to 0 ^{o}C. Bis (2-methoxyethyl) amino sulfur trifluoride (10 µL) was added dropwise and the solution was vortex mixed for 5 seconds.

The solution was kept at 0 ^{o}C for 5 min, subsequently warmed to room temperature, and kept there for 15 min. Water (2 mL) and hexane (4 mL) were added and the tubes were vortex mixed for 1min. After centrifugation for 10 minutes at 2,000 rpm, the organic layer was collected and transferred into a vial for GC analysis. A blank analysis was performed by use of the same method, but without addition of bis (2-methoxyethyl) amino sulfur trifluoride.

The diethyl amide derivatives were analyzed with a Perkin Elmer® Clarus® 600 GC-FID equipped with a Supelco SP 2340 fused silica column (Sigma-Aldrich Co.), 60 m, 0.25 µm ID, 0.2 µm film thicknesses based on AOCS Method Ce 1c-89. The GC oven was heated to 150°C, ramped to 200 °C at 1.3 °C/min and held at 200 °C for 20 minutes.

A total volume of 1.0 µL was injected and split at a 100:1 ratio, the helium flow was 2.0 ml/min at 1.6 psi and the FID temperature was 210 °C. Samples were prepared and measured separately in triplicate. The area percentages from the output reading corresponding to the proportion (%) of each fatty acid were recorded with a TotalChrom® chromatography data system.

3.6.4.4 Determination of Fatty Acid Composition

The determination of Fatty acid composition, otherwise known as *Fatty Acid Profile (FAP)* was done according to the method described by Christie (2003) with little modifications.

Apparatus: Micropipette, 20 mL capacity screw-capped tubes, and PerkinElmer® Clarus® 600 Gas chromatography.

Reagents: Toluene, Sulphuric acid, Methanol, Sodium chloride (NaCl) solution, Hexane, Distilled water and Substrate oil sample.

Procedure: In the Methylation stage, which preceded GC analysis, 5 mg oil sample was dissolved in 1 mL toluene, and 2 mL of 1 % sulfuric acid in methanol was added. The mixture was left overnight in a stoppered tube at 50 ^{o}C. Aqueous sodium chloride solution (5 %, 5 mL) was then added and the required methyl esters were extracted with 3mL hexane. Necessary dilutions were made before injection for GC analysis.

The fatty acid methyl esters (FAMEs) obtained were separated by gas chromatography in a PerkinElmer® Clarus® 600 GC-FID equipped with a Supelco SP 2340 fused silica column (Sigma-Aldrich Co.), 60 m, 0.25 µm ID, 0.2 µm film thicknesses based on AOCS Method Ce 1c-89. The GC oven was heated to 150 °C, ramped to 200 °C at 1.3°C/min and held at 200 °C for 20 minutes. A total volume of 1.0 µL was injected and split at a 100:1 ratio, the helium flow was 2.0 ml/min at 1.6 psi and the FID temperature was 210 °C. Samples were prepared and measured separately in triplicate. Peak areas were quantified with TotalChrom® chromatography data system.

3.6.4.5 Determination of Viscosity

Kinematic viscosity (ν) is the measure of an oil's resistance to flow and shear under the forces of gravity. **Dynamic viscosity (η)** of oil is the ratio between the applied shear stress and rate of shear of the oil, and its value could be determined from the value of Kinematic viscosity once the density of the oil is known for a specific working temperature. Oil has a unique molecular structure, and larger molecules create greater resistance (higher kinematic viscosity). Highly viscous liquid flows less readily under the force of gravity.

Oil and/or biodiesel viscosity are one of the most important properties of these liquids because it brings out a fuel's capacity to lubricate moving parts. Incorrect viscosity leads to poor lubrication, and poorly lubricated machinery can quickly break down. The viscosity of the oils was predetermined for an easy comparison with that which was obtained for their corresponding biodiesels.

Apparatus: Cannon-ubbelohde viscometer, Temperature-controlled bath, and Temperature measuring device (in the range of 0 ^{o}C-100 ^{o}C).

Reagent: Chromic Acid Cleaning Solution, biodiesel samples

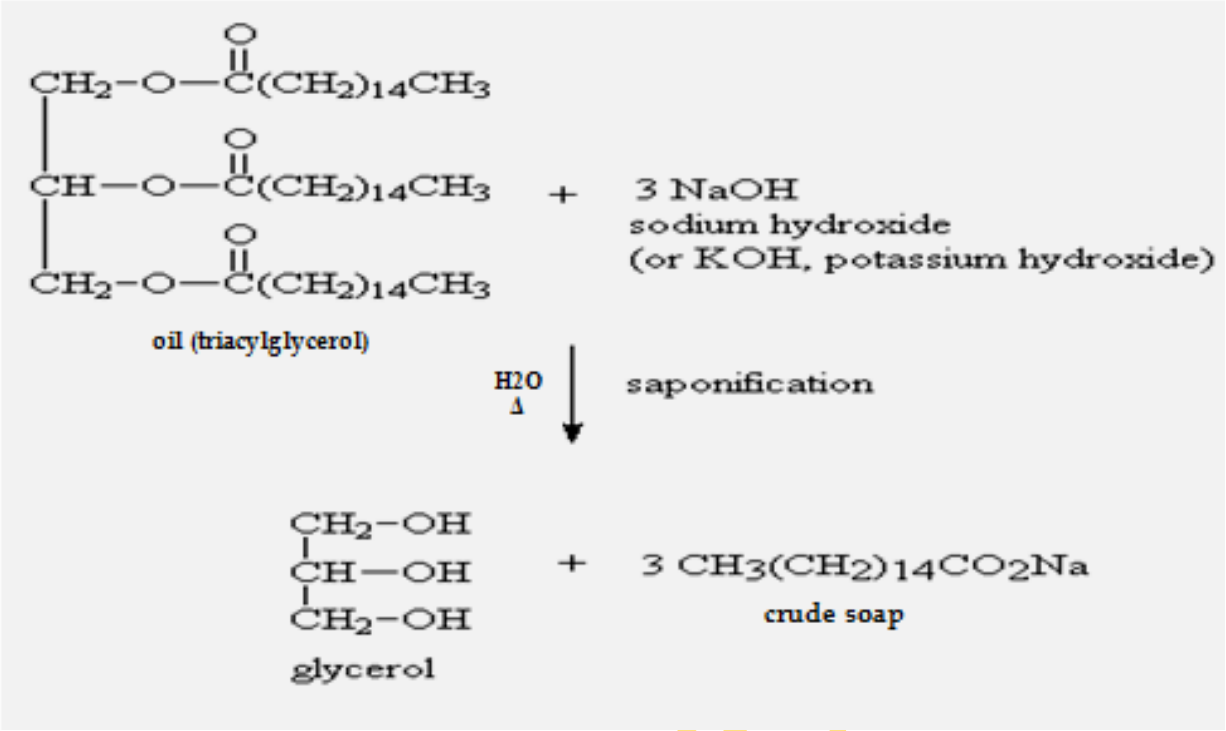
Procedure: The **kinematic viscosity (v)** was measured following the established procedure in the ASTM D445. It was determined with the use of a calibrated Cannon-ubbelohde viscometer at a temperature of 40 ^{o}C. The viscometer was placed in a temperature-controlled vessel equipped with a thermostat, which maintained the temperature with an accuracy of $\pm$0.1 ^{o}C.

The density vs. temperature measurement was taken using a 25 CC pycnometer immersed in a temperature-controlled circulating water bath. The kinematic viscosity value at 40 ^{o}C was determined by multiplying the measured flow time of the oil through the viscometer capillary with the calibration constant of the viscometer.

The Dynamic viscosity (η) was estimated by the product of Kinematic viscosity (v) and the corresponding density (ρ) of the biodiesels at 40^{o}C using the following equation for the temperature: $\boldsymbol{\eta = v \times \rho}$

3.6.4.6 Determination of Saponification value

Saponification is defined as the reaction of triacylglycerol (fatty acid esters) with an alkali (such as Sodium hydroxide or Potassium hydroxide) to produce Sodium or Potassium salt of the fatty acid and glycerol (Formula 3.9 below).



Formula 3.9: Showing a Saponification reaction process

Saponification value is the number of milligrams of KOH required to neutralize the fatty acids resulting from the complete hydrolysis of 1 g of fat or oil. It gives an indication of the nature of the fatty acids constituent of oil and thus, depends on the average molecular weight of the fatty acids constituent of the oil. The greater the molecular weight (longer carbon chain), the smaller the number of fatty acids that is liberated per gram of fat hydrolyzed and therefore, the smaller the saponification number and vice versa.

Apparatus: 3 ground neck Erlenmeyer flasks (250 mL capacity), Reflux condenser, Heating mantle, 50 ml volumetric Pipette, and 50 mL volumetric Burette.

Reagents: 0.7 N Alcoholic potassium hydroxide (KOH), Phenolphthalein indicator (1.0 % in Isopropanol), 1.0 N Sulfuric acid (H_2SO_4) and Substrate oil sample.

Procedure: 5 g of the substrate oil was weighed into a 250 mL ground neck Erlenmeyer flask. A second flask, which was left empty (i.e. without the sample) was also provided that served as blank. 50 mL alcoholic KOH was pipette into each of the 2 flasks and approximately 10 mL deionized (D.I) water was added to each of them. Then a boiling stone was put in the sample

flask. A condenser was attached to the sample flask and heat was applied to reflux on the heating mantle for 30 minutes. The blank flask was left to stand at room temperature.

At the end of the refluxing period, the flask was allowed to cool to 60^{o}C and the condenser was rinsed with about 10 mL D.I. water. The flask was thereafter removed from the condenser and the ground glass neck was also rinsed with about 10 mL D.I. water. 10 mL D.I. was then added to the blank flask. 1 mL Phenolphthalein indicator was added to the sample flask and blank flask and each of them was titrated with 1.0 N Sulphuric acid until a colourless endpoint was reached.

$$\textbf{Saponification value} = \frac{[\text{mL(blank)} - \text{mL(sample)}] \times \text{N}(H_2SO_4) \times 56.1}{\text{Wt of sample (in grams)}} \quad \text{.....Formula 3.10}$$

3.6.5 Transesterification Process

Materials: 200 ml Erlenmeyer flasks, 100 ml Conical, Beakers, Measuring cylinder, Weighing balance, Aluminum foil, Glass stirrer, Thermometer, and a Magnetic stirrer with hot plate.

Reagents: 99.5 % Methanol, 90 % Ethanol, 0.5 M NaOH, Distilled water

Procedure: The transesterification of Palm kernel, Moringa seed, Thevetia and Spirogyra oils were carried out with methanol-only and methanol/ethanol mixture (1:1) in the presence of NaOH as catalyst respectively (i.e. identical reaction conditions and production protocols would be used for each of the oils). This implies that each of the extracted oils was allowed to undergo a transesterification reaction using methanol-only (as the alcohol) and another one using methanol/ethanol (1:1 v/v) mixture (as the alcohol) respectively, with all other reaction conditions remaining the same.

The transesterification reaction (Section 2.12.1.2) for each of the oils was carried out at a 6:1 alcohol to oil molar ratio, 1 % weight of the oil of NaOH catalyst and 65^{o}C reaction temperature. The transesterification is a reversible reaction, thus the alcohol quantity is required to shift the equilibrium favorably. The alcohol to oil molar ratio, the weight percent of catalyst and the reaction temperature were chosen since they have been found to give optimal yields of alkyl ester from seed oils (Berchmans and Hirata, 2008).

An Erlenmeyer flask (500 ml capacity) was charged with about 100 g of the individual oils respectively (i.e. one substrate oil per production process) and warmed to a desired temperature of about 55^{o}C, which is less than the boiling point of methanol (65^{o}C) in a water bath (Plate 3.21a). While the oil was being warmed, a methanol quantity of 6:1 molar ratio of methanol to oil and an optimal weight of NaOH pellets (1 % weight of the oil) were mixed and heated in a separate flask to a desired temperature of 50^{o}C on the magnetic stirrer until the NaOH pellets were completely dissolved (Plate 3.21b).

The weight and volume of each of the oils used for the transesterification reactions were measured to enable a definite estimation of the quantity of alcohol (methanol and/or ethanol) and NaOH pellets that would be used in the respective reactions.

(a) **(b)** **(c)**

Plate 3.21: Showing some of the preparatory stages preceding the transesterification reaction-(a) Picture of the water bath set at 55^{o}C (b) NaOH pellets mixed with alcohol placed on the hot plate (c) R-Warm oil placed on the magnetic stirrer with hot plate; L-Sealed flask with reaction mixture about undergoing transesterification**.**

After the complete dissolution, the beaker was taken-off the magnetic stirrer, and the Erlenmeyer flask containing the warm oil was removed from the water bath and placed on the stirrer (Plate 3.21c-R). The methanol-NaOH mixture (i.e. sodium methoxide) in the beaker was then added to the oil in the flask (including a corrode-resistant stir bar), the temperature of the hot plate was immediately increased to 65^{o}C and the revolution of the stirrer was set at level four (i.e. 400 rpm). The mouth of the flask was sealed with an aluminum foil to minimize alcohol evaporation during the conversion process (Plate 3.21c-L).

The reaction was allowed to continue for 1 hour, after which the stirrer was turned off, the stir bar was removed, and the content of the flask was immediately poured into a separatory funnel (Plate 3.22a). This procedure was repeated for each of the oils using the specific alcohol or alcohol-mixture and all other reaction parameters.

3.6.6 Phase separation and Purification process (washing and drying)

Materials: Separatory flask, Measuring cylinder, LabPro pH tester, Wash bottle, Retort stands with clamp.

Reagents: Hot distilled water (about 60^{o}C) and 1M H_2SO_4 solution

Procedure: The transesterification reactions produced glycerol and methyl esters when they were completed as was later observed after phase separation (Plate 3.22b). These, being completely insoluble with one another, separated into two distinct phases when poured into a separatory funnel.

(a) **(b)**

Plate 3.22: Showing a typical example of the reaction mixture obtained after transesterification reaction in a separatory flask-(a) Before separation (b) After separation

The impure glycerol settled at the bottom part of the funnel (as shown in Plate 3.22b above) and was thus drained out by the stopper at the bottom of the separator. The quantity of the glycerol impurity was measured using a measuring cylinder.

A sample of the biodiesel remaining in the flask was thereafter taken and the pH determined (Plate 3.23). If found to be caustic i.e. alkaline (pH 8 and above), the biodiesel in the flask was washed with hot water (about 55^{o}C) and 0.1% acid solution. However, if found to be in normal pH range (of say like 7.0-7.5), then only warm distilled water was used in washing.

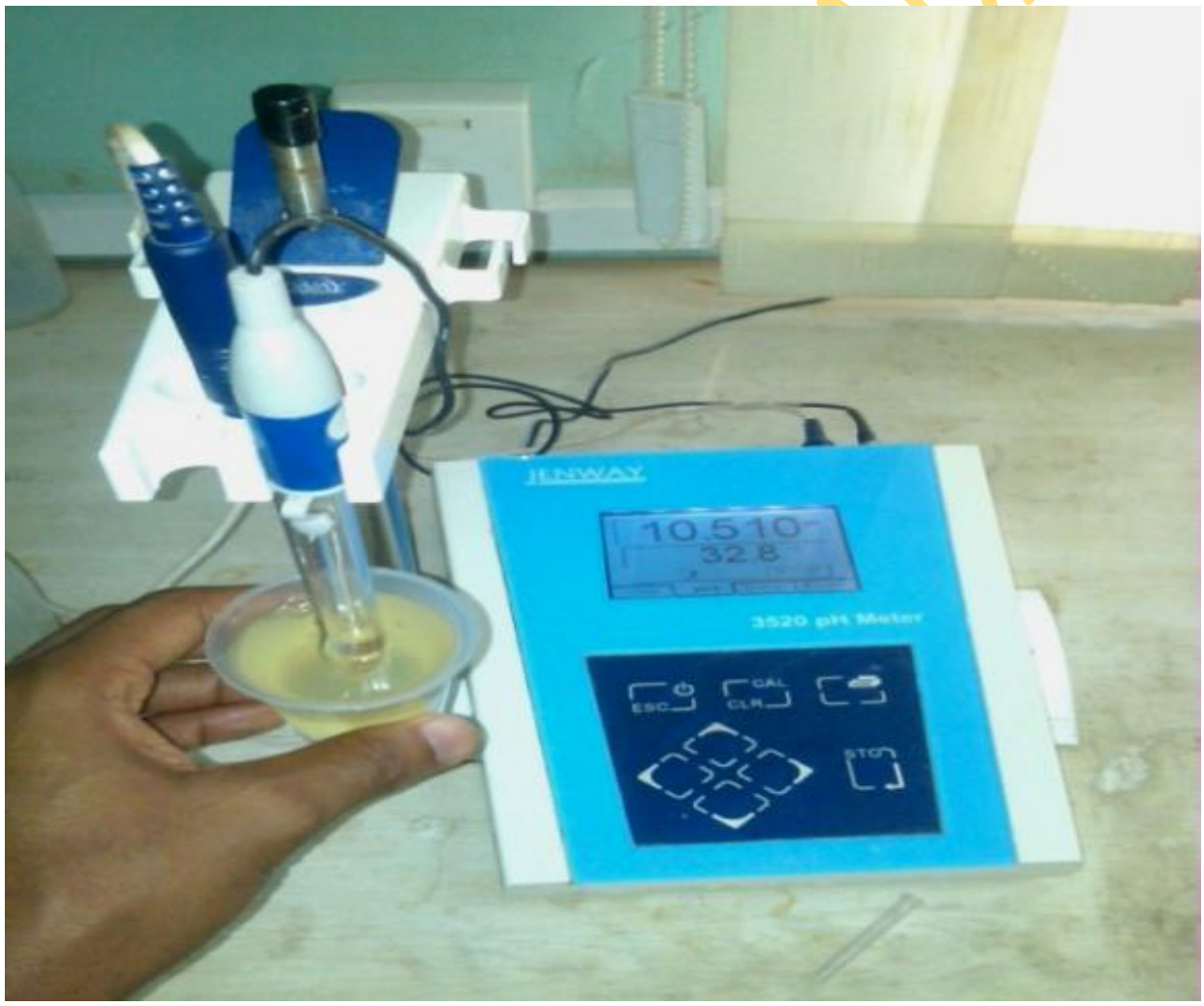


Plate 3.23: Showing the pH testing of a sample of one of the biodiesels

One third ($^1/_3$) as much hot distilled water as there is biodiesel was added in a stepwise fashion to the biodiesel in the flask. The water settles quickly at the bottom of the flask and was subsequently drained out as it settles. The washing continued in the stepwise fashion until the water settling at the bottom of the flask was visibly clear; and until the time it took for the water to separate from the biodiesel was $\leq$ 30 minutes.

A sample of the biodiesel was again taken and the pH determined using a pH meter to verify that the biodiesel is neutral (pH 7 $\pm$ 0.1) as exemplified in Plate 3.23 above. Thereafter, the biodiesel was observed from all angles to make sure there were no particles in the fuel. The biodiesel was then heated at 100^{o}C for 15 minutes, air-dried for about 30minutes, and then bottled and kept for characterization studies.

3.6.7 Determination of Biodiesel Yield

The biodiesel yield (% wt) after the post-treatment stage, relative to the amount of the different substrate oils poured into the flask for each of the alcohol parameters used *viz*: Methanol-only transesterification yield and Methanol/Ethanol mixture transesterification yield was calculated from the equation below:

$$\textbf{Biodiesel yield} = \frac{\text{Volume of biodiesel produced}}{\text{Volume of oil used}} \times 100\ \% \quad \text{.....} \textbf{Formula 3.11}$$

The biodiesels obtained were characterized for ***Relative density, Flash point, Cloud and Pour points (using the Freezer test), Viscosity, Acid value and Elemental composition***. These parameters were compared with European (EN) standard and American Standard for Testing and Materials (ASTM) (Table 3.1 below); while Table 3.2 highlights some key parameters of conventional diesel fuels as compared to unblended (or B100) biodiesel.

Table 3.1: Showing the ASTM and EN Guidelines for Biodiesel Fuels

Fuel properties	**ASTM guideline (D6751)**		**EN standard (EN 14214)**	
	Limits	**Method**	**Limits**	**Method**
Density (g/cm^3)	Unspecified	D287	0.860-0.900 @ 15^oC	EN ISO 3675/12185
Kinematic Viscosity @ 40^oC (mm^2/s)	1.9-6.0	D445	3.5-5.0	EN ISO 3104
Flash point (^oC) min.	130	D93	101	ISO/CD 3679
Acid value (mg KOH/g) max.	0.8	D664	0.5	EN 14104
Phosphorus content max.	0.001% **or** 10 mg/kg	D4951	0.001 % **or** 10 mg/kg	EN 14107
Alkaline earth metal content (Ca + Mg) max.	-	-	0.00005 % **or** 5 mg/kg	EN 14108 EN 14109
Alkaline metal content (Na + K) max.	-	-	0.00005 % **or** 5 mg/kg	EN 14108 EN 14109
Sulphur content max.	0.05% **or** 500 mg/kg	D5453	0.001 % **or** 10 mg/kg	EN ISO 14596
Cloud point (^oC)	Report to customer	D2500	-	-
Pour point (^oC)	-	-	-	-

Sources: ASTM D6751, 2009 and EN 14214 standards, 2008

Table 3.2: Comparison of certain key Parameters of Conventional Petroleum-based Diesel fuel with B100 Biodiesel fuel

Fuel Property	Diesel	Biodiesel	
	ASTM D975	ASTM D6751	EN 14214
Kinematic Viscosity 40^{o}C (mm^{2}/s)	1.3-4.1	1.9-6.0	3.5-5.0
Flash point (^{o}C)	60-80	130 min.	101 min.
Sulphur content (wt %)	0.0015	0.05	0.001
Cloud point (^{o}C)	-15 to 5	-3 to 12	-
Pour point (^{o}C)	-35 to -15	-15 to 10	-

Source: US Department of Energy, Biodiesel Handling and Use Guidelines (2nd Edition, March 2006)

3.6.8 Characterization studies for the biodiesels

3.6.8.1 Determination of Relative density

The Relative density of the biodiesels was determined at 25^{o}C following the same method that was described in Section 3.5.2.2.

3.6.8.2 Determination of Flash point

A minimum flash point for diesel fuel is required for fire safety. Flash point is used in shipping and safety regulations to define flammable and combustible materials. The flash point is the lowest temperature at which fuel emits enough vapors to ignite (ASTM D93, 2003).

Biodiesel has a high flash point; usually more than 150 °C, while conventional diesel fuel has a flash point of 55-66 °C (Knothe *et. al*., 2005). If methanol, with its flash point of 12 °C is present in the biodiesel the flash point can be lowered considerably. Hence, a manually operated Pensky-Martens closed cup flash point test was used to ensure that the methanol has been adequately stripped from the biodiesel according to ASTM D93, 2003.

The apparatus and method consist of the controlled heating of the biodiesel in a closed cup, introducing an ignition source, and observing if the heated biodiesel flashes. The temperature at which the biodiesel flashes is recorded as the flash point.

Apparatus: Manual Pensky-Martens closed cup apparatus-This apparatus consists of the test cup, test cover and shutter, stirring device, heating source, ignition source device, air bath, and top plate.

Reagent: Cleaning solvent (toluene)

Procedure: The test cup was filled with the biodiesel sample to the filling mark inside the cup. The temperature of the test cup and biodiesel sample was ensured to be at least 18 ^{o}C or 32 ^{o}F below the expected flash point for biodiesels. The test cover was placed on the test cup and this assembly was placed into the apparatus. The test flame was lighted and adjusted to a diameter of about 3.2 mm (0.126 inches). The heat was subsequently applied at such a rate that the

temperature (as indicated by the temperature measuring device) increased to 5 ^{o}C (9 ^{o}F)/min. The stirring device was turned at about 90 rpm, stirring in a downward direction.

The observed flash point was recorded as the reading on the temperature measuring device at the time ignition source application caused a distinct flash in the interior of the test cup. The sample was deemed to have flashed when a large flame appeared and instantaneously propagated itself over the entire surface of the test specimen. The test cover and the test cup were removed when the apparatus has cooled down to a safe handling temperature (less than 55 ^{o}C or 130 ^{o}C), and the apparatus was cleaned in readiness for another round of flashpoint determination for another sample.

3.6.8.3 Determination of Cloud and Pour points (using the Freezer test)

The Freezer test is a simple test using jars, a freezer, and a thermometer and is effective in determining proper winter blending rates. **Cloud point** is the temperature at which small solid crystals are first visually observed as the fuel is cooled. Below cloud point, these crystals might plug filters or could drop to the bottom of a storage tank. However, fuels can usually be pumped at temperatures below cloud point.

Pour point is the temperature at which the fuel contains so many agglomerated crystals that it is essentially a gel and will no longer flow. Distributors and blenders use pour point as an indicator of whether the fuel can be pumped, even if it would not be suitable for use without heating or taking other steps.

A deep freezer which is capable of measuring as low as -10 ^{o}F and which has been completely defrosted was used for the tests. The biodiesel fuels of varying proportions were made up in two jars respectively and then placed in the freezer. By frequently checking the temperature of each jar, the temperature at which clouding and gelling occurred for the biodiesels was roughly estimated. Knowing the expected low temperature, users can then predict if a biodiesel fuel would be trouble free.

3.6.8.4 Determination of Viscosity

The viscosities of the respective biodiesels were determined according to the method earlier described in Section 3.5.4.5.

3.6.8.5 Determination of Acid value

The acid number for biodiesel is primarily an indicator of Free Fatty Acids (natural degradation products of fats and oils) and can be elevated if a fuel is not properly manufactured or has undergone oxidative degradation. Acid numbers higher than 0.50 mgKOH/g have been associated with fuel system deposits and reduced life of fuel pumps and filters. By definition, acid value is the number of mg of potassium hydroxide required to neutralize the free fatty acids in 1g of the biodiesel.

Apparatus: Titration vessels (Burette, Pipette, Conical flasks)

Reagents: Solvent mixture 1/1 (v/v) of 95 % ethanol and diethyl ether; 0.5 N Potassium hydroxide (KOH), about 0.1 mol/L solution in ethanol; 10g/L Phenolphthalein solution in 95 % (v/v) ethanol.

Procedure: 2.8 g of the biodiesel sample was weighed into a 125 ml Erlenmeyer flask and 50ml of the solvent mixture (ethanol/diethylether) was added and the mixture swirled for few minutes.1mL of Phenolphthalein solution was added into the Erlenmeyer flask. The content of the flask was then titrated (while shaking) with the solution of KOH in ethanol contained in a burette until a pink colour (that persisted for 30 seconds or more) was obtained. The burette reading was then taken as accurately as possible to two (2) decimal places.

Calculation:

$$\textbf{Acid value} = \frac{\text{mL sample} \times \text{N KOH} \times 56.1}{\text{g sample}} \quad \text{……..….}\textbf{Formula 3.12}$$

3.6.8.6 Determination of Elemental composition (Proximate analysis)

Sodium (Na), Potassium (K), Calcium (Ca), and Magnesium (Mg) can cause deposits to form, catalyze undesired side reactions, and poison emission control equipment. The Group I and II metals are limited as the combination of metals in each category, Na+K and Ca+Mg. For each combination, the limit is 5 ppm. Phosphorus for example is limited to 10 ppm maximum in biodiesel because it can damage catalytic converters; phosphorus above 10 ppm can be present in some plant oils.

Biodiesel produced in the United States generally has phosphorus levels of about 1 ppm. Also, sulfur content is limited in biodiesels by standard to reduce sulfate and sulfuric acid pollutant emissions and to protect exhaust catalyst systems when they are deployed on diesel engines in the future. Sulfur content of 15 ppm or lower is also required for proper functioning of diesel particle filters. Biodiesel generally contains less than 15 ppm sulfur.

Prior to elemental analysis, the biodiesel samples were digested according to EPA Method 3031, 1996 for the determination of Calcium (Ca) and Sodium (Na) metals. Schematic summary of the digestion procedure is presented in Figure 3.3 below.

Apparatus: Beakers (250 ml capacity), Thermometer, Filter paper-Whatman No 41, Funnels, Heating mantle, Volumetric flasks, Volumetric pipette, glass rod.

Reagents: Nitric acid (conc. HNO_3), Hydrochloric acid (conc. HCl), Sulfuric acid (conc. H_2SO_4), Potassium permanganate ($KMNO_4$), Ammonium hydroxide (NH_4OH), Ammonium phosphate (NH_4PO_4), Distilled water and Biodiesel samples.

Procedure: The biodiesel sample to be digested was homogenized and then a representative sample of 0.5 g was taken and placed in a beaker. 0.5 g of potassium permanganate powder and 1 mL of conc. H_2SO_4 (in a dropwise fashion) were added, and the mixture was stirred with a glass rod. A grey-white vapor was emitted from the beaker (SO_3) and splattering or bubbling occurred. The beaker became very hot. This step was deemed to be complete when no more gases were given off and the sample was a thick black lumpy paste. The beaker was allowed to cool to room temperature.

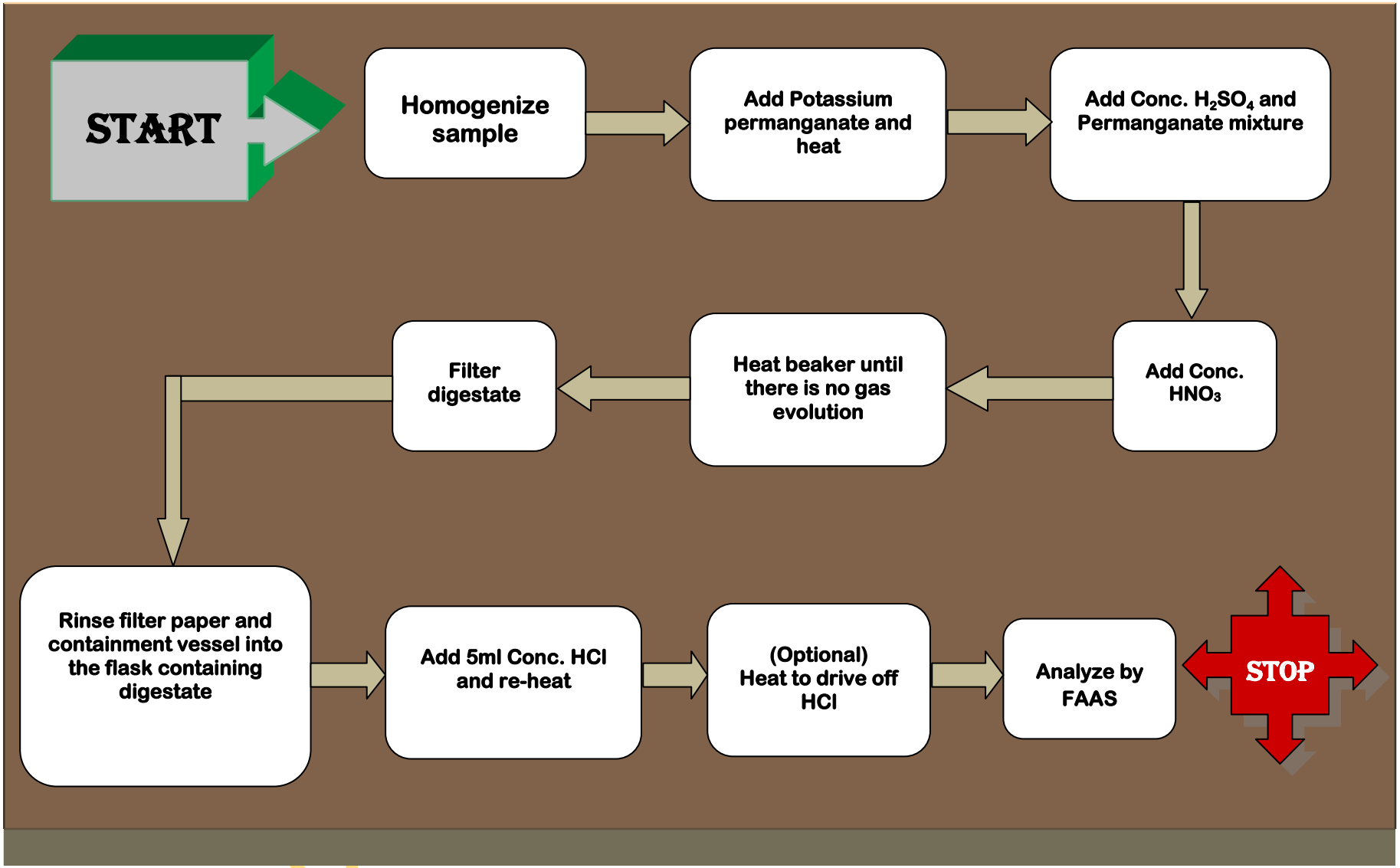


Fig 3.3: Schematic Representation of EPA Method 3031-Acid Digestion of Oils for Metal Analysis by AAS

2 ml of concentrated HNO_3 was then added to the beaker and stirred. Some reddish-brown vapor (NO_2) was given off, and the reaction was allowed to continue until complete (which was determined by the point at which the digestate gave off no more fumes). The beaker was again allowed to cool to room temperature. 10 ml of concentrated HCl was subsequently added and the mixture stirred. The beaker was heated to about 120 ^oC until there was no further evolution of gas. The final digestate was observed to be a clear yellow liquid with black to dark reddish-brown particulates.

The digestate was filtered through a No 41 Whatman filter paper, and the filtrate was collected in a volumetric flask. The digestion beaker was washed with about 5 ml hot HCl into the filter paper and the filter paper was also washed while still in the funnel with the same acid solution. The final filtrate obtained was thereafter analyzed with an Atomic Absorption Spectrophotometer (Plate 3.24).

Plate 3.24: Showing the Buck Scientific® Model 210 VGP AAS machine

3.7 Data Management and Statistical Analysis

Data was recorded in tabular formats and other details were taken at each step of the production process. These included measurement of weight (or specific gravity), volumes, relative density, moisture content of biomasses, etc.

- All data were analyzed using the SPSS statistical software version 15. Descriptive statistics such as proportions, means and standard deviations were used to summarize the data.

- The results obtained from the proximate analysis and physicochemical properties of oils and biodiesels were subjected to Inferential statistics such as Student t-test, while One-way Analysis of Variance (ANOVA) with Least Significance Difference (LSD) at 5% level of precision ($\alpha = 5\%$) was used to test for significant differences in the mean relative densities across the test groups.

- Spearman-rank correlation was used to check if a relationship exists between the biodiesel yield and the levels of the elements in the substrates.

CHAPTER FOUR

RESULTS

This chapter presents the results of the exploration study which includes evaluation of the oil and the biodiesel yielding potentials of the selected plant biomasses; and characterization studies on the plant biomasses, the oils from these biomasses and the biodiesel obtained from the processed oils.

4.1 Characteristics of the Plant Biomasses

4.1.1 Physical Characteristics of the Plant biomasses

Table 4.1 below shows the quantity of the respective plant-based biomasses that were used in each of the experimental setup for oil extraction *viz*: Soxhlet extraction, Cold extraction using Hexane/Ether solvent mixture and Cold extraction using Hexane as the only extraction solvent. The mean percentage moisture contents of the biomasses for each of the three (3) methods of oil extraction are presented in Table 4.1.

A comparison between the Moisture content determined by the two methods [i.e. Moisture analyzer equipment method (Table 7.2 in appendix) and Oven-drying method (Table 7.3 in appendix)] is presented in Figure 4.1 below. The mean relative density estimated for the biomasses is presented in Table 4.1 and the triplicate readings shown in Table 7.4 (appendix).

Table 4.1: Showing the different physical parameters that were determined in the biomasses

Biomass	Weight of biomass (for Soxhlet extraction) (g)				Weight of biomass (for Cold extraction: Hexane/Ether)(g)				Weight of biomass (for Cold extraction: Hexane only) (g)				Mean Moisture content using the Moisture analyzer equipment (%)	Mean Moisture content through oven-drying method (%)	Relative density (R.D)
	W_1	W_s	W_2	W_{bu}	W_1	W_s	W_2	W_{bu}	W_1	W_s	W_2	W_{bu}			
Moringa	300	292.20	271.85	250	300	295.00	270.90	250	300	292.50	271.92	250	9.37 @160°C	9.48 @105°C	0.604 @25°C
P.K	300	295.50	275.20	250	300	292.15	274.90	250	300	294.50	275.15	250	8.25 @160°C	8.31 @105°C	0.572 @25°C
Thevetia	300	292.02	280.01	250	300	289.44	280.05	250	300	290.10	280.05	250	6.63 @160°C	6.64 @105°C	0.750 @25°C
Spirogyra	77.80	52.00	48.25	40	48.39	41.40	30	30	48.39	41.31	30	30	39.65 @160°C	39.71 @105°C	0.641 @25°C

Key: W_1 = Wet weight of biomass (g)
W_s = Sundried weight of biomass (g)
W_2 = Oven-dried weight of biomass (g)
W_{bu} = Weight of oven-dried Biomass Used for the extraction process (g)

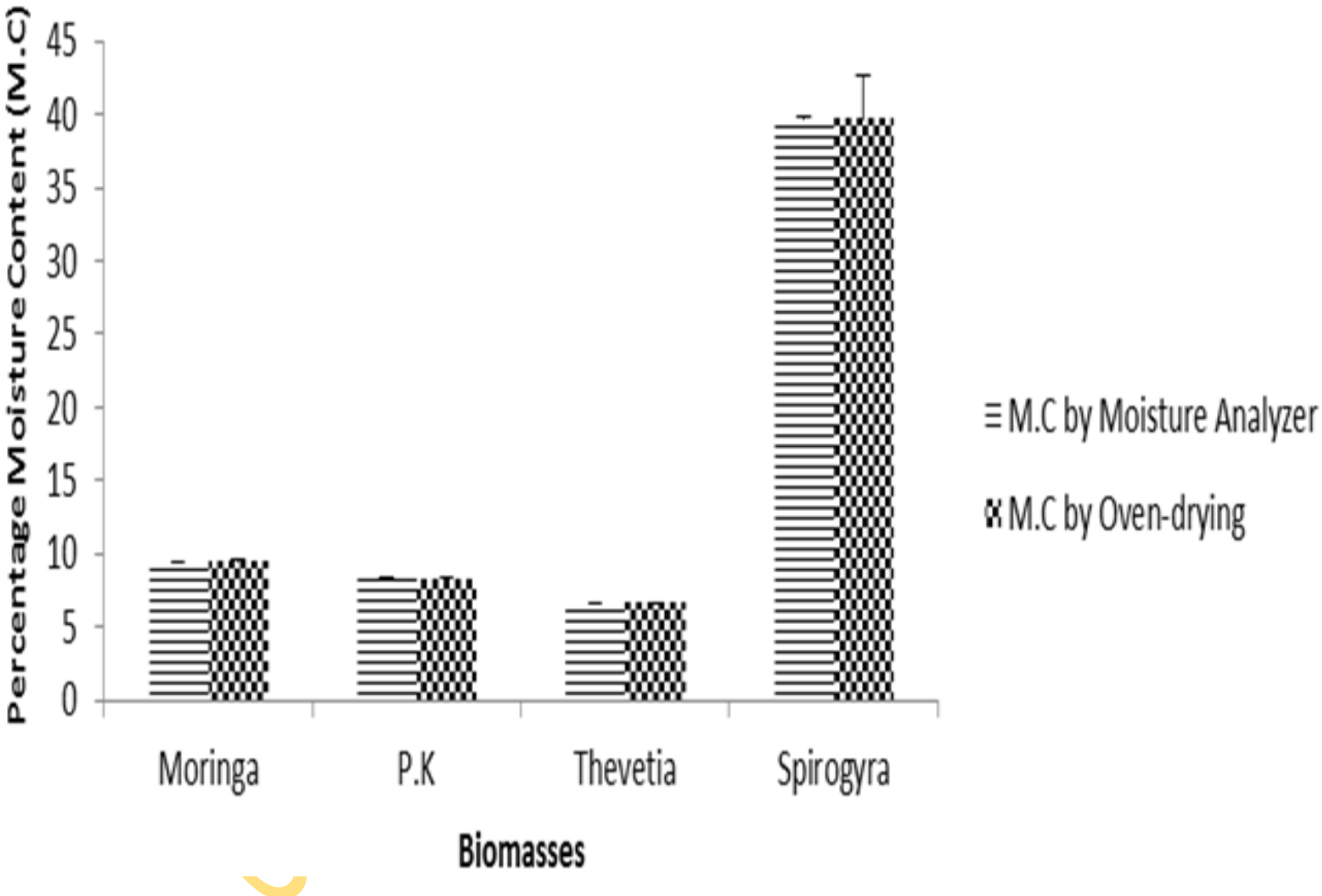


Fig. 4.1: Comparison of the Moisture content of biomasses determined using two different methods

4.1.2 Chemical Characteristics of the Plant biomasses

Table 4.2 shows that the Total Organic Carbon contained in these substrates were considerably high as compared to other elemental composition *viz*: Total Nitrogen, Total Phosphorus, Calcium, Sodium and Sulphur. Moringa seeds were observed to contain the highest T.O.C (60.9%) closely followed by Palm kernel and Thevetia seeds (60.8% and 60.7%) respectively, with Spirogyra biomass having the lowest percentage of (50.9%).

The percentage Total Nitrogen (T.N) content of *Moringa oleifera* seeds was seen to surpass all the other substrates with Palm kernel seeds having the least percentage T.N value. Moringa seeds were also shown to have a high percentage Total Phosphorus (T.P) value that was surpassed by that of Spirogyra biomass (0.28%). In the same vein, the Spirogyra biomass was again seen to possess the highest percentage Calcium (Ca), Sodium (Na) and Sulphur (S) content of 0.05%, 1.35% and 0.88% respectively.

The proximate analysis carried out to estimate the percentage elemental composition of the biomasses were all carried out in duplicate as shown in Table 7.5 (appendix) and represented pictorially in Figure 4.2 below.

Table 4.2: Different chemical parameters that were determined in the biomasses

Biomass	Elemental Composition					
	T.O.C (%)	T.N (%)	T.P (%)	Ca (%)	Na (%)	S (%)
Moringa	60.85	0.210	0.211	0.050	0.016	0.038
P.K	60.84	0.091	0.118	0.045	0.016	0.047
Thevetia	60.73	0.147	0.046	0.040	0.017	0.080
Spirogyra	50.96	0.112	0.281	0.054	1.350	0.882

Key: **T.O.C** = Total Organic Carbon; **T.N** = Total Nitrogen; **T.P** = Total Phosphorus; **Ca** = Calcium content; **Na** = Sodium content; **S** = Sulphur content

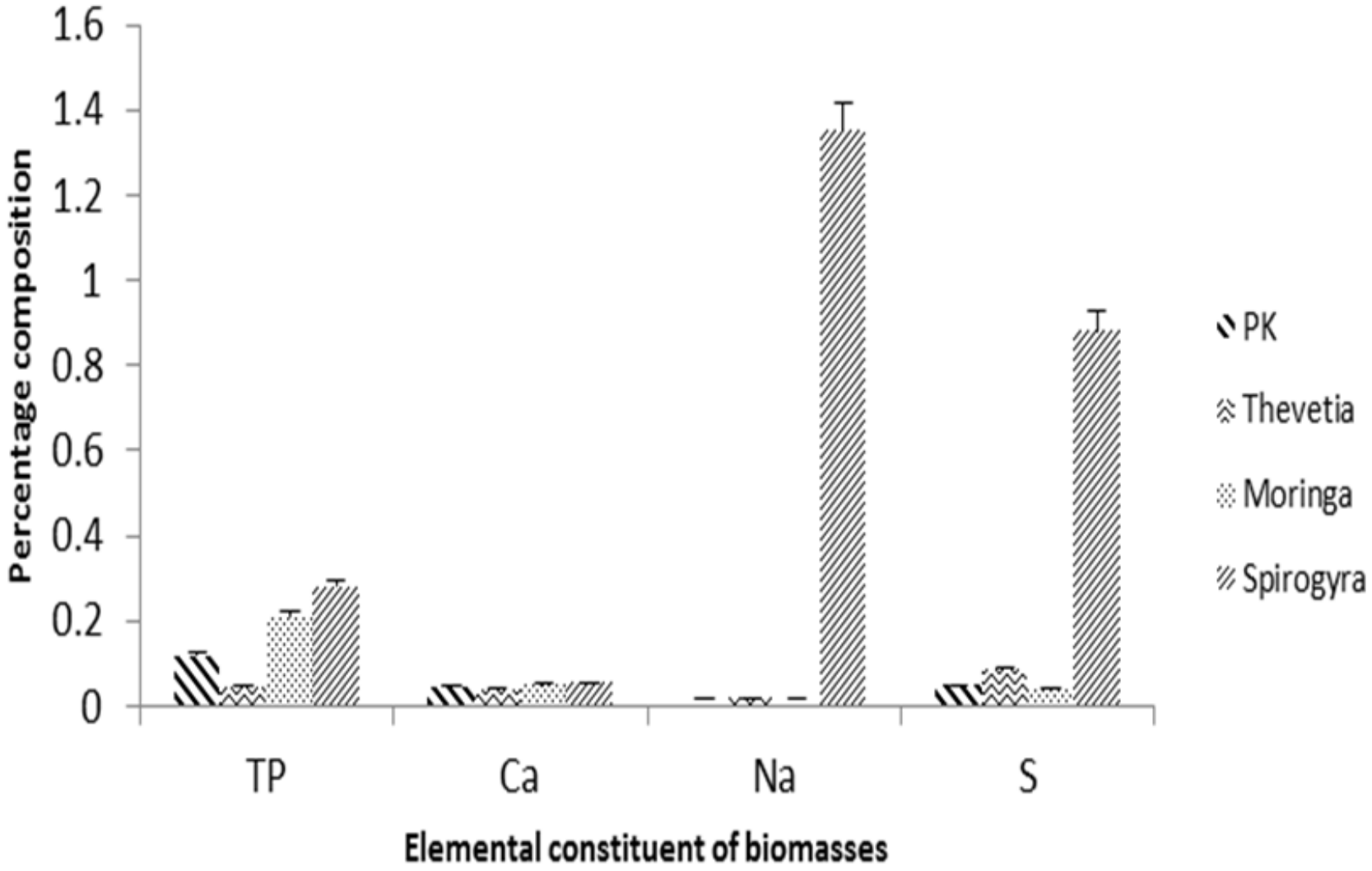


Fig 4.2: Showing the proportion of elements in the biomasses

4.2 Characteristics of the Extracted Oils

4.2.1 Physical Characteristics of the Extracted Oils

The results of the measurements made on each of the oils from the different extraction methods described in this work are presented in Table 4.3 below. All the oils from each of the three (3) extraction processes, after undergoing evaporation in a Rotavapor apparatus and left to air-dry for about 24hours, were put together respectively and their total weight and volume measured as presented in Table 4.3. This latter measurement was done to know exactly what weight or volume of each of the oils was necessary for the transesterification reaction process so as to remain some quantity of oil sufficient enough for characterization. A comparison of the oil yields across the three (3) extraction procedures performed is presented in Figure 4.3 below.

The pH of each of the oils was determined in triplicate (shown in Table 7.6-appendix) and the mean pH value presented in Table 4.3. Also, the density of the oils at 25^{o}C was determined in triplicate as shown in Table 7.7 and the mean relative density presented in Table 4.3. The Kinematic viscosity of the oils was determined in duplicate (Table 7.9-appendix) and the result presented in Table 4.3. The dynamic viscosity of the oils was also determined using the values of both the Kinematic viscosity and density @ 40^{o}C and the result shown in Table 7.8 (appendix).

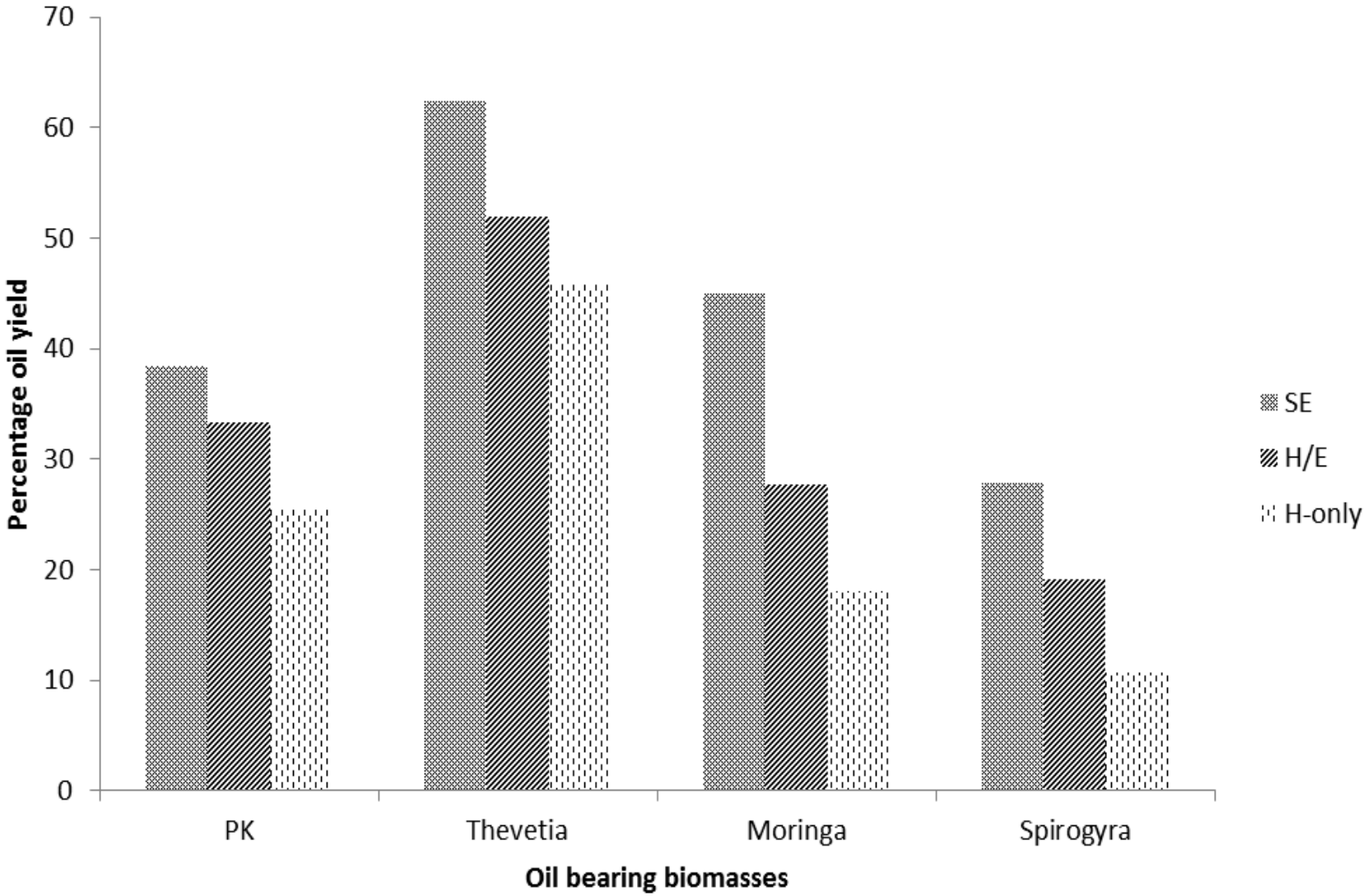


Fig. 4.3: Shows the percentage oil yield from the biomasses via three extraction methods

Table 4.3: Showing the different physical parameters estimated for in the extracted oils

Test parameter	Soxhlet extraction						Cold extraction (Hexane/Ether solvent mixture; 1:1 v/v)						Cold extraction (Hexane only)										
	W_{bu} (g)	W.O (g)	V.O (mL)	W.R (g)	Q.S (mL)	O.Y (%)	W_{bu} (g)	W.O (g)	V.O (mL)	W.R (g)	Q.S (mL)	O.Y (%)	W_{bu} (g)	W.O (g)	V.O (mL)	W.R (g)	Q.S (mL)	O.Y (%)	W.O.T (g)	V.O.T (mL)	W_{ou} (g)	V_{ou} (ml)	Relative density (R.D)	pH	Kinematic viscosity (mm^2/s)
Moringa	250	112.44	140.00	122.o0	800	44.98	250	69.17	91.00	175.50	600	27.67	250	45.10	55.00	195.40	300	18.04	226.71	276.10	200	252.30	0.803 @25°C	6.63 @26.9°C	44.50 @ 40°C
P.K	250	95.87	108.67	149.50	800	38.35	250	83.09	102.00	162.00	600	33.24	250	63.57	80.00	160.92	300	25.43	258.96	290.67	200	224.49	0.881 @25°C	6.02 @24.8°C	4.85 @ 40°C
Thevetia	250	155.79	179.16	85.20	800	62.32	250	129.68	163.00	116.50	600	51.87	250	114.52	140.00	115.45	300	45.81	359.99	482.16	200	267.87	0.871 @25°C	6.64 @26.3°C	21.50 @ 40°C
Spirogyra	40	8.90	14.94	29.60	300	22.25	30	3.44	2.06	24.50	200	11.47	30	1.92	1.15	27.40	100	6.40	14.26	18.15	10	12.73	0.531 @25°C	6.68 @25.2°C	4.50 @ 40°C

Colour of Extracted Oils

Moringa	Light orange	Deep yellow	Deep yellow
P.K	Light orange	Deep orange	Deep orange
Thevetia	Deep yellow	Deep yellow	Deep yellow
Spirogyra	Deep green	Deep green	Deep green

KEY: $\mathbf{W_{bu}}$ = Weight of oven-dried Biomass Used for the extraction process (g)
W.O = Weight of Oil (g)
V.O = Volume of Oil (ml)
W.R = Weight of Residual biomass (g)
Q.S = Quantity of solvent used (ml)
O.Y = Oil Yield (%)
W.O.T = Weight of Oil Total (g)
V.O.T = Volume of Oil Total
$\mathbf{W_{ou}}$ = Weight of Oil Used for the transesterification reaction
$\mathbf{V_{ou}}$ = Volume of Oil Used for the transesterification reaction

4.2.2 Chemical Characteristics of Extracted Oils

The chemical characterization of the oils revealed Palm kernel oil as having the highest Saponification value (230.2 mgKOH/g) amongst the other substrate oils. However, the extracted algal (spirogyra) oil was only sufficient for few physicochemical characterizations and processing into biodiesel but insufficient for the three chemical characterizations under this section (i.e. saponification value, FFA value and FAP. This was followed by Moringa seed oil (192.5 mgKOH/g) with Thevetia oil having the least value (120.1 mgKOH/g) (Table 4.4).

The analysis of the Free Fatty Acid content of the biomass oils revealed Moringa oil to possess a significant highest level of the these free molecules (3.0 %) as compared to the other two substrates *viz*: Palm kernel (1.9 %) and Thevetia (0.6 %). A pictorial comparison of some physicochemical properties of the extracted oils is presented in Figure 4.4 below.

Table 4.4: Showing the chemical parameters estimated for in the extracted oils

Oil	Sap. value (mgKOH/g)	FFA content (%)
Moringa	192.5	3.0
P.K	230.2	1.9
Thevetia	120.1	0.6
Spirogyra	-	-

Key: **Sap. value** = Saponification value **FFA** = Free Fatty Acid content

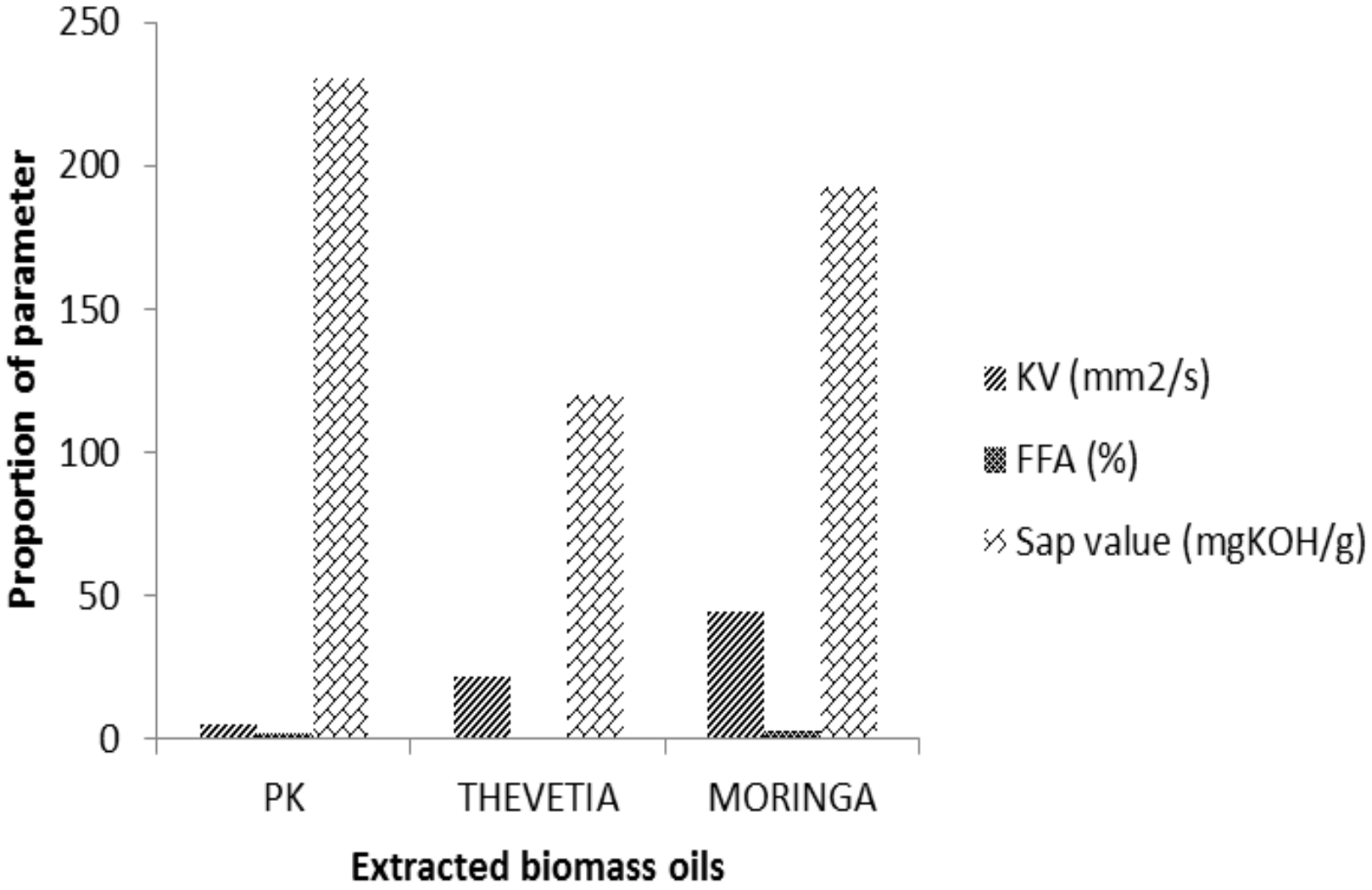


Figure 4.4: Comparison of the physicochemical parameters of biomass oils

The fatty acid profile of the oils, which was read in triplicates (Table 7.10-appendix) and summarized in Table 4.5 below, shows a divergent range of composition of fatty acids in the oils, and a varying degree of saturation and unsaturation across the various substrate oils. From the results, it was clear that the major fatty acid component of Palm kernel oil is Lauric acid (C12:1) while Moringa seed oil, *Thevetia peruviana* (Yellow oleander) seed oil and Spirogyra oil have Oleic acid (C18:1) as their major component fatty acid.

The fatty acid profile of Moringa seed oil shows a high level of unsaturation followed by that of Spirogyra and Thevetia while Palm kernel oil shows the lowest level of unsaturated fatty acid composition. This invariably indicates that Palm kernel oil contains the highest level of saturated fatty acids (79.99%).

It is pertinent to note that while a higher number of the total fatty acid composition of the oils of moringa, palm kernel and spirogyra were accounted for, the result obtained for thevetia analysis showed that there was about 17.30% of the fatty acids that their values were not accounted for. These unaccounted fatty acids may belong to the group of uncommon fatty acids, but which may probably find some usefulness in some areas if they could be identified and their beneficial value explored.

Table 4.5: Showing the Fatty Acid Profile (FAP) or Percentage Fatty Acid Composition (FAC) of the Extracted Oils

		Fatty Acid Profile of Oils			
Test parameter [a]	**Name**	**Moringa (%) $\bar{x} \pm$ S.D**	**Palm kernel (%) $\bar{x} \pm$ S.D**	**Thevetia (%) $\bar{x} \pm$ S.D**	**Spirogyra (%) $\bar{x} \pm$ S.D**
C8:0	Caprylic	0.04±0.01	3.28±0.01	-	-
C10:0	Capric	-	3.41±0.01	-	-
C12:0	Lauric	-	47.60±0.01	-	0.99±0.01
C14:0	Myristic	0.15±0.01	16.12±0.02	0.19±0.01	7.50±0.00
C15:0	Pentadecanoic	-	-	-	0.50±0.01
C16:0	Palmitic	6.10±0.01	8.35±0.00	19.50±0.01	25.05±0.01
C16:1	Palmitoleic	1.35±0.01	0.31±0.01	0.25±0.01	8.50±0.01
C17:0	Margaric	0.05±0.01	-	0.10±0.01	0.20±0.01
C18:0	Stearic	5.80±0.01	2.49±0.01	6.39±0.01	4.50±0.00
C18:1	Oleic	71.19±0.6	15.50±0.01	42.25±0.01	33.47±0.06
C18:1-9c, 12 (OH)	Ricinoleic	-	-	0.05±0.01	-
C18:2	Linoleic	0.69±0.00	2.10±0.00	10.50±0.00	10.80±0.01
C18:3	Linolenic	3.00±0.02	0.15±0.01	0.50±0.01	0.50±0.01
C18:3-9c,11t, 13t	α-Eleostearic	-	-	0.01±0.01	-
C20:0	Arachidic	3.60±0.01	0.20±0.01	1.25±0.00	1.20±0.01
C20:1	Gadoleic	2.00±0.06	0.05±0.01	0.13±0.01	0.50±0.01
C20:1-11c,14(OH)	Lesquerolic	-	-	-	0.15±0.01
C20:2	Eicosadienoic	-	-	-	-
C20:5	Timnodonic	-	-	-	0.05±0.01
C22:0	Behenic	4.57±0.01	0.10±0.00	0.82±0.01	1.50±0.01
C22:1	Erucic	-	-	-	0.39±0.01
C24:0	Lignoceric	0.50±0.01	-	1.15±0.00	-
C24:1	Nervonic	-	-	-	0.85±0.01
Unknown	**=**	**1.21**	**2.62**	**17.30**	**3.93**
Total known	**=**	**98.79**	**97.38**	**82.70**	**96.07**
Total saturated	**=**	**20.79**	**79.99**	**29.02**	**41.01**
Total unsaturated	**=**	**78.00**	**17.41**	**53.68**	**55.06**

[a] Numbers denote the number of carbon atoms and double bonds in one molecule. For example, in Linoleic acid, 18:2 indicates that each molecule contains eighteen carbon atoms and two double bonds.

4.3 Characteristics of the Biodiesels

4.3.1 Physical Characteristics of the Biodiesels

The results of the measurements made on each of the biodiesel obtained from the two (2) different alcohol system described in this work (Section 3.5.5) are presented in Table 4.6a below. The glycerine content of the oils is presented in Table 4.6a. The pH of the biodiesels is also presented in the same table 4.6a and duplicate readings shown in Table 7.11-appendix.

There was a significant difference ($p<0.05$) between the all oils and their respective biodiesels (both M-only and M/E biodiesels) except the pH of Moringa M/E biodiesel, where there was no significant difference ($p > 0.05$) between the biodiesel and its parent oil (Table 4.6b below). The chart showing the comparison between the pH of oils and the pH of the biodiesels is presented in Figure 4.5 below. The colour of the cleaned/refined biodiesels, which were visually inspected, is also reported in Table 4.6a.

The Relative density (R.D.) of the individual biodiesels obtained from each of the alcohol system reactions was also determined in triplicate (Table 7.12-appendix). The biodiesel yield expressed in percentage v/v is presented in Table 4.6a. A comparison of the R.D. of the biodiesels to that of the oils, and a comparison of the biodiesel yield using the different alcohol systems are presented pictorially in Figure 4.6 and Figure 4.7 below respectively.

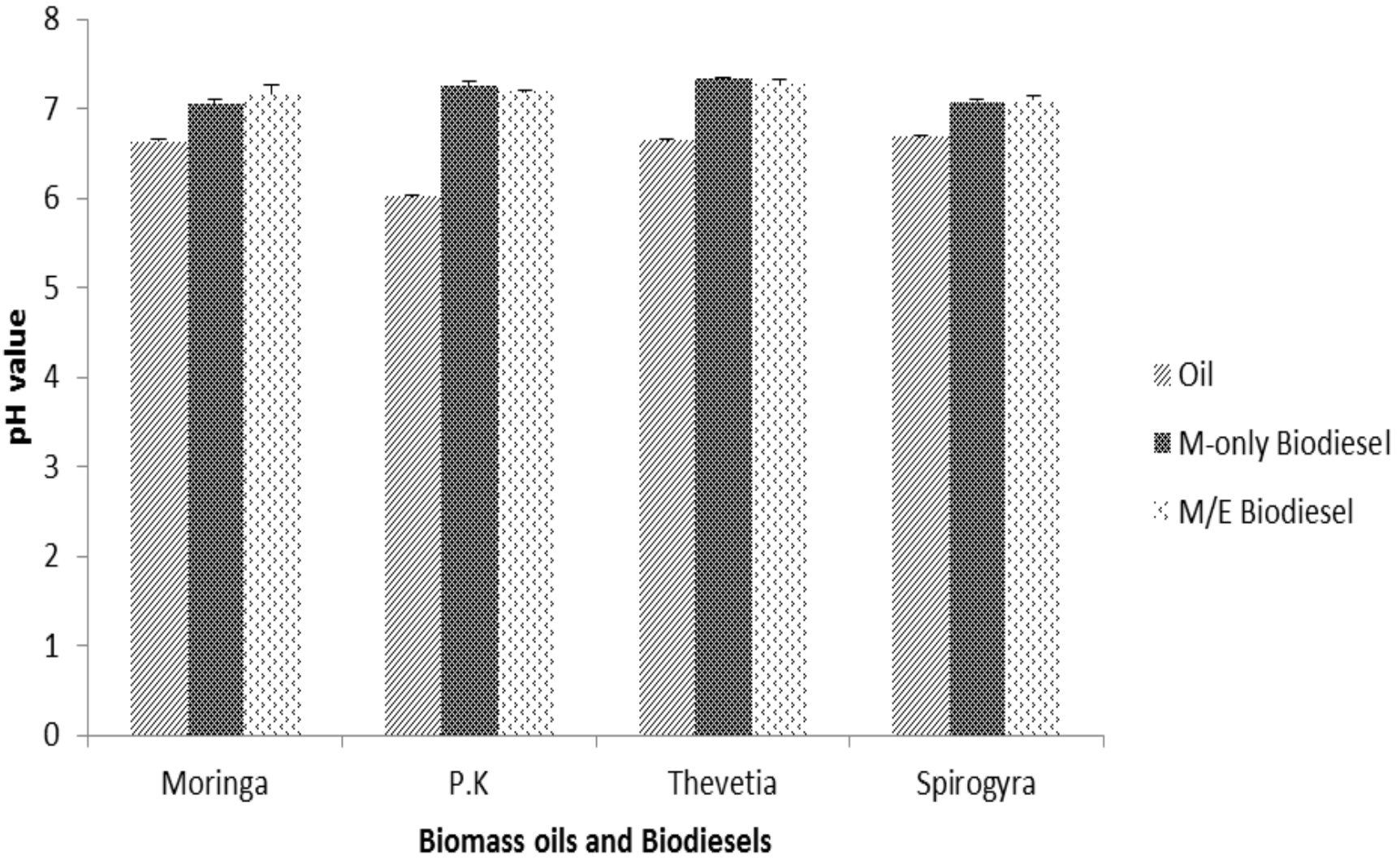


Figure 4.5: Comparison of the pH of oils to the pH of biodiesels

Table 4.6a: Physical characteristics of Biodiesels

Oil	Parameter	Q.O (g and ml)	Q.M (g and ml)	Q.E (g and ml)	Q.NaOH (g)	Q.D (ml)	G.C (ml)	C.B	pH	R.D @25°C	B.Y (g, ml & %)
Moringa	**M-only transesterification**	100g 126ml	16.67g 20.9ml	-	1.00	29.0	26.05	Light yellow	7.05	0.877	72.21g, 82.53ml, 65.50%
	M/E (1:1) transesterification	100g 126ml	8.37g 10.5ml	8.29g 10.5ml	1.00	27.0	28.50	Light yellow	7.17	0.878	67.59g, 77.16ml, 61.24%
P.K	**M-only transesterification**	100g 145ml	19.30g 24.2ml	-	1.00	41.0	14.40	Light orange	7.25	0.913	99.50g, 109.11ml, 75.25%
	M/E (1:1) transesterification	100g 145ml	9.57g 12.0ml	9.47g 12.0ml	1.00	40.0	16.80	Light orange	7.19	0.899	94.38g, 104.98ml, 72.40%
Thevetia	**M-only transesterification**	100g 134ml	17.90g 22.4ml	-	1.00	41.0	17.50	Light yellow	7.34	0.839	95.65g, 114.20ml, 85.20%
	M/E (1:1) transesterification	100g 134ml	8.77g 11.0ml	8.68g 11.0ml	1.00	38.0	27.00	Light yellow	7.27	0.842	88.05g, 105.10ml, 78.43%
Spirogyra	**M-only transesterification**	5.00g 6.3ml	0.89g 1.3ml	-	0.05	0.60	2.10	Light green	7.08	0.881	1.46g, 1.65ml, 26.19%
	M/E (1:1) transesterification	5.00g 6.3ml	0.45g 0.65ml	0.51g 0.65ml	0.05	0.40	2.45	Light green	7.12	0.885	1.06g, 1.20ml, 19.05%

KEY:
M-only transesterification = Transesterification reaction using Methanol
M/E transesterification = Transesterification using Methanol/Ethanol mixture in ratio 1:1
Q.O = Quantity of Oil used (expressed in g and ml)
Q.M = Quantity of Methanol used (expressed in g and ml)
Q.E = Quantity of Ethanol used (expressed in g and ml)
Q.NaOH = Quantity of NaOH pellets used (expressed in g)
Q.D = Quantity of Distilled water used (expressed in ml)
G.C = Glycerine content of oil (expressed in ml)
C.B = Colour of Biodiesel obtained
R.D = Relative density of biodiesel (no unit)
B.Y = Biodiesel yield (expressed in g, ml and % v/v)

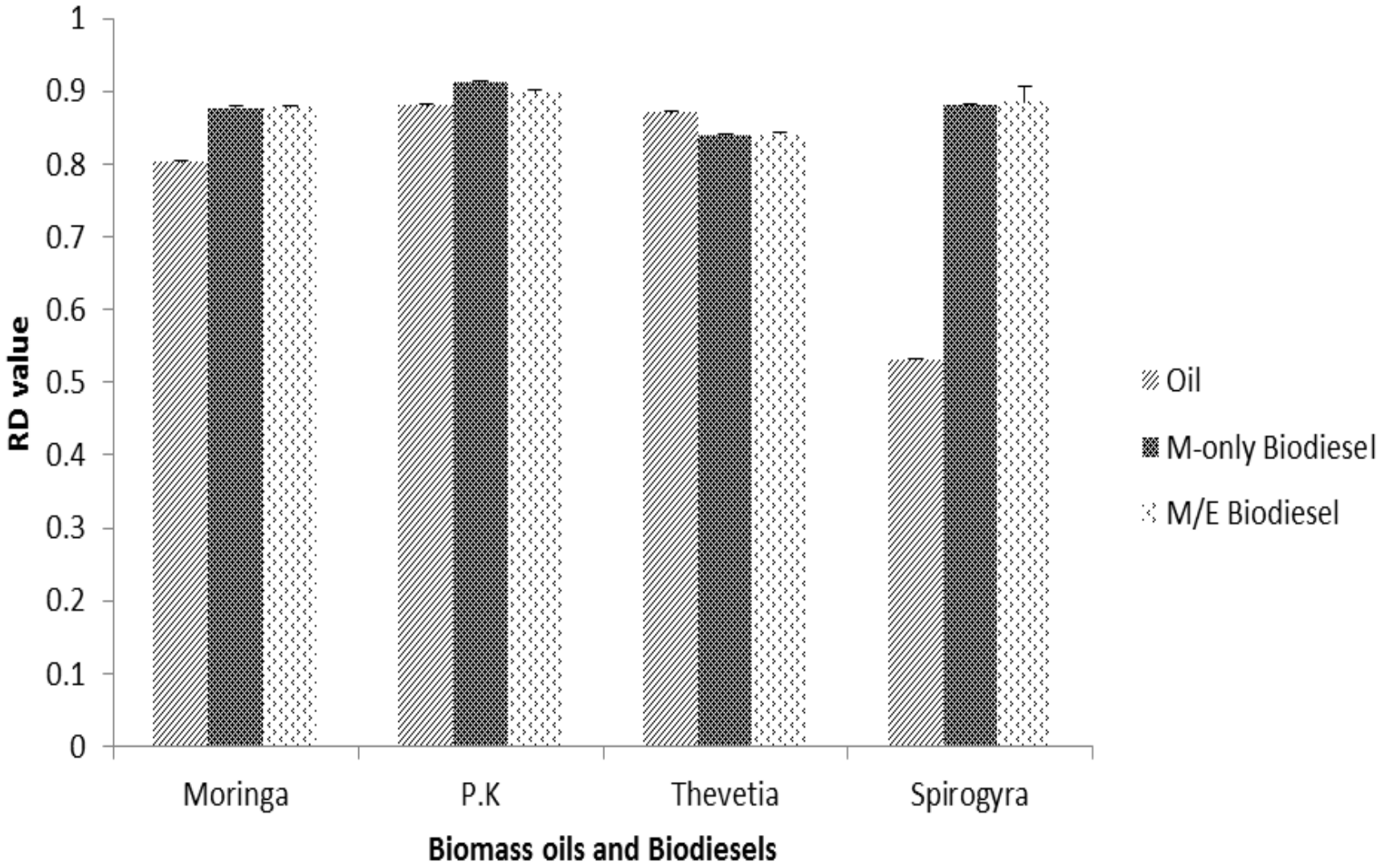


Figure 4.6: Comparison of the Relative density of oils to the Relative density of biodiesels

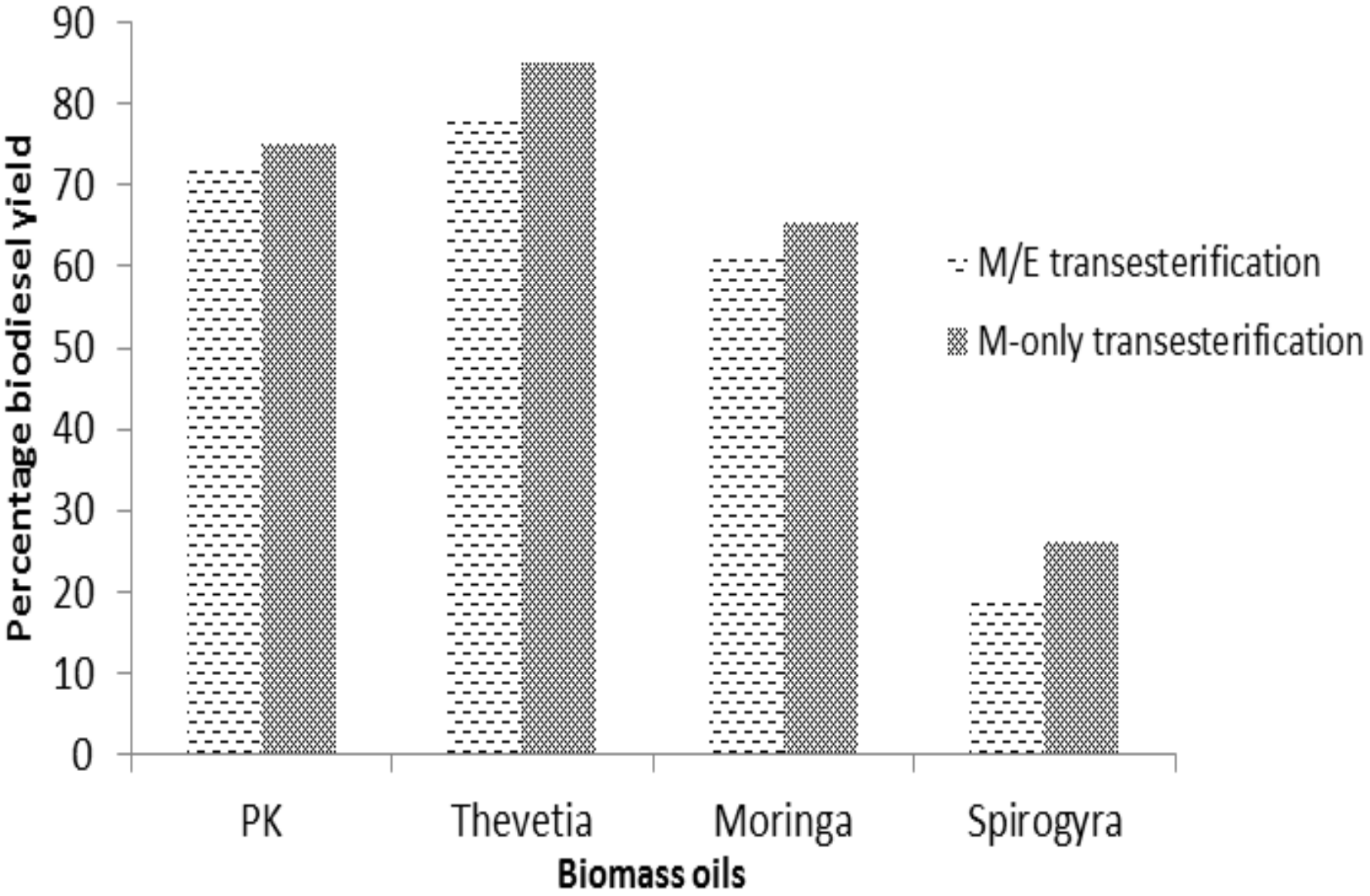


Figure 4.7: Comparison of Percentage biodiesel yield from the oils using two transesterification processes

Table 4.6b: Comparison of pH of oils and pH biodiesels

Parameter	Test Group	Mean ± S.D	T-test	p-value
Moringa	pH Oil	6.63 ± 0.02	14.70	0.00
	pH (M-only BD)	7.05± 0.05	11.60	0.04
	pH (M/E BD)	7.17 ± 0.10	7.70	*0.08
P.K	pH Oil	6.02 ±0.02	34.68	0.00
	pH (M-only BD)	7.25 ± 0.06	26.79	0.02
	pH (M/E BD)	7.19 ± 0.01	88.00	0.00
Thevetia	pH Oil	6.64 ± 0.01	81.72	0.00
	pH (M-only BD)	7.34 ± 0.01	66.72	0.00
	pH (M/E BD)	7.27 ± 0.06	15.78	0.04
Spirogyra	pH Oil	6.68 ± 0.01	34.84	0.00
	pH (M-only BD)	7.08 ± 0.01	33.01	0.00
	pH (M/E BD)	7.10 ± 0.04	15.91	0.03

KEY: **pH Oil** = pH of oil; **pH M-only BD** = pH Methanol-only biodiesel; **pH M/E BD** = pH Methanol/Ethanol biodiesel; * p > 0.05 is not significant

4.3.2 Chemical Characteristics of the Biodiesels

Table 4.7a below shows the results of the proximate analysis carried out on the biodiesel obtained from the transesterification of each of the substrate oils. There was a significant reduction in the percentage content of each of the elements that were initially analyzed for in the substrate oils as compared to their corresponding biodiesel result (duplicate readings presented in Table 7.13-appendix). Moringa seed biodiesel recorded the highest percentage value of Total Phosphorus (T.P) and Sulphur (S) contents amongst the other biodiesels; and had an equal proportion of Sodium (Na) content with Thevetia biodiesel.

A comparison of the mean percentage elemental composition of the biodiesels is shown in a chart format (Figure 4.10 below). The correlation between mean elemental compositions in biomasses to their biodiesel yield is presented in Table 4.8 below. The percentage T.P in the biomasses negatively correlated with the biodiesel yield in both M-only ($r = 0.99$, $p = 0.00$) and M/E ($r = 0.99$, $p = 0.00$) transesterification processes. In the same vein, the percentage Calcium in the biomasses negatively correlated with the biodiesel yield in both M-only ($r = 0.80$, $p = 0.02$) and M/E ($r = 0.80$, $p = 0.02$) transesterification processes. There was also a negative correlation between the percentage sodium ($r = 0.30$) and percentage sulphur ($r = 0.29$) with the biodiesel yield in both M-only and M/E transesterification processes respectively although both correlations were not significant ($p > 0.05$). Figures 4.8 and 4.9 below show the strength of linear relationship between the T.P content of the biomasses and the yield of biodiesel in the M-only ($R^2 = 85.5\%$) and M/E ($R^2 = 82.2\%$) transesterification processes respectively.

Also, Moringa biodiesel was observed to have the highest flash point (176^{o}C) with Thevetia biodiesel having the lowest (130^{o}C). P.K biodiesel was observed to have the highest Cloud and Pour points (14.1^{o}C and 8.6^{o}C respectively), closely followed by Moringa biodiesel with Thevetia biodiesel having the lowest temperature points for the two parameters. Moringa biodiesel was also observed to have the highest acid value of 0.657mgKOH/g, which is slightly above the $\leq$ 0.5mgKOH/g limit set by the EN standard. The Flash point, Cloud and Pour points and the Acid value for the respective biodiesels were all determined in duplicate as presented in Table 7.14, Table 7.15 and Table 7.16 respectively (appendix).

There was a reduction in the density of all the biodiesels at 40^{o}C (Table 4.7a below) when compared to their density at the room temperature of 25^{o}C (Table 7.12-appendix). The oils of Moringa, Palm kernel and Thevetia seeds all witnessed a significant reduction in their resistance to flow and sheer under the forces of gravity (i.e. Kinematic viscosity, whose readings were determined in duplicate, Table 7.18-appendix). This reduction was after the oils underwent transesterification and purification processes as presented in Table 4.7a and represented pictorially in Figure 4.12 below. Moringa seed oil witnessed the highest reduction in kinematic viscosity (88.72%) followed by Thevetia oil (78.14%) with Palm kernel oil, which experienced the least but significant reduction of 50.72%. A comparison of certain physicochemical parameters (KV, FP and AV) of the different biodiesels is presented in Figure 4.11 below.

In the same vein, the estimated values of Dynamic viscosity (and hence Kinematic viscosity) of the biodiesel fuels obtained revealed a significant reduction in these values as compared to their corresponding precursor oils. Again, Moringa biodiesel witnessed the highest significant reduction ($p<0.05$) with a decline of 87.7% from its parent oil (i.e. Moringa oil). Thevetia oil also reduced by 79.2% though the t-test and p-value could not be computed due to equal standard deviation of the test groups. Palm kernel oil witnessed the lowest reduction value (though again significant, $p<0.05$) but with a value of 50.35% (Table 4.7b below).

Some parameters of the biodiesels produced in this work were compared with ASTM and EN standards/limits (Table 4.9 below) and the comparison is shown pictorially (Figures 4.13 and 4.14 below). There was a significant decrease ($p<0.05$) in the proportion of elements in the biomasses when compared to their respective biodiesels (shown in Figure 4.12). The elemental composition of the biodiesels as compared with ASTM and EN guidelines is also shown pictorially in Figure 4.15 below.

Table 4.7a: Chemical characteristics of Biodiesels

Biodiesel	Elemental composition				Fuel properties						
	T.P (%)	**Ca** (%)	**Na** (%)	**S** (%)	**F.P** (^oC)	**C.P** (^oC)	**P.P** (^oC)	**A.V** (mgKOH/g)	**Density @ 40^{o}C**	**K.V @ 40^{o}C** (mm^2/s)	**D.V** (g/ms)
Moringa	0.020	0.005	0.002	0.035	176^{o}C	13.6	6.5	0.657	0.872	5.02	4.38
P.K	0.002	0.004	0.001	0.002	166^{o}C	14.1	8.6	0.417	0.881	2.39	2.11
Thevetia	0.001	0.003	0.002	0.008	130^{o}C	8.5	5.1	0.441	0.825	4.70	3.88

N.B: Analyses of the parameters in the above table were not carried out on Spirogyra biodiesel

Table 4.7b: Comparison of the KV of oils to the KV of biodiesels

Parameter	Kinematic viscosity (mm^2/s)	Mean	T-test	p-value
Moringa	KV Oil	44.50 ± 0.01	2791.658	0.000
	KV Biodiesel	5.02 ± 0.01	2791.658	0.000
P.K	KV Oil	4.85 ± 0.00	246.000	0.000
	KV Biodiesel	2.39 ± 0.01	246.000	0.003
Thevetia	KV Oil	21.50 ± 0.00[(a)]	-	-
	KV Biodiesel	4.70 ± 0.00[(a)]	-	-
Spirogyra	KV Oil	-	-	-
	KV Biodiesel	-	-	-

[(a)]**t** cannot be computed because the standard deviations of both groups are 0; $p < 0.05$ is significant; The KV of Spirogyra oil and biodiesel was not determined due to limited quantity of each.

Key: **KV Oil** = Kinematic viscosity of Oil; **KV Biodiesel** = Kinematic viscosity of biodiesel

Table 4.8: Spearman correlation between mean elemental composition of biomasses and biodiesel yield

Parameter		**TP** (%)	**Ca** (%)	**Na** (%)	**S (%)**	**M-only biodiesel yield** (%)	**M/E biodiesel yield** (%)
TP (%)	Correlation coefficient	1.00					
	Sig. (2-tailed)	.					
Ca (%)	Correlation coefficient	0.79*	1.00				
	Sig. (2-tailed)	0.02	.				
Na (%)	Correlation coefficient	0.27	-0.20	1.00			
	Sig. (2-tailed)	0.52	0.64	.			
S (%)	Correlation coefficient	0.34	0.02	0.39	1.00		
	Sig. (2-tailed)	0.40	0.60	0.34	.		
M-only biodiesel yield (%)	Correlation coefficient	-0.99**	-0.80*	-0.30	-0.29	1.00	
	Sig. (2-tailed)	0.00	0.02	0.47	0.48	.	
M/E biodiesel yield (%)	Correlation coefficient	-0.99**	-0.80*	-0.30	-0.29	1.00**	1.00
	Sig. (2-tailed)	0.00	0.02	0.47	0.48	.	.

* Correlation is significant at p<0.05
** Correlation is significant at p<0.01

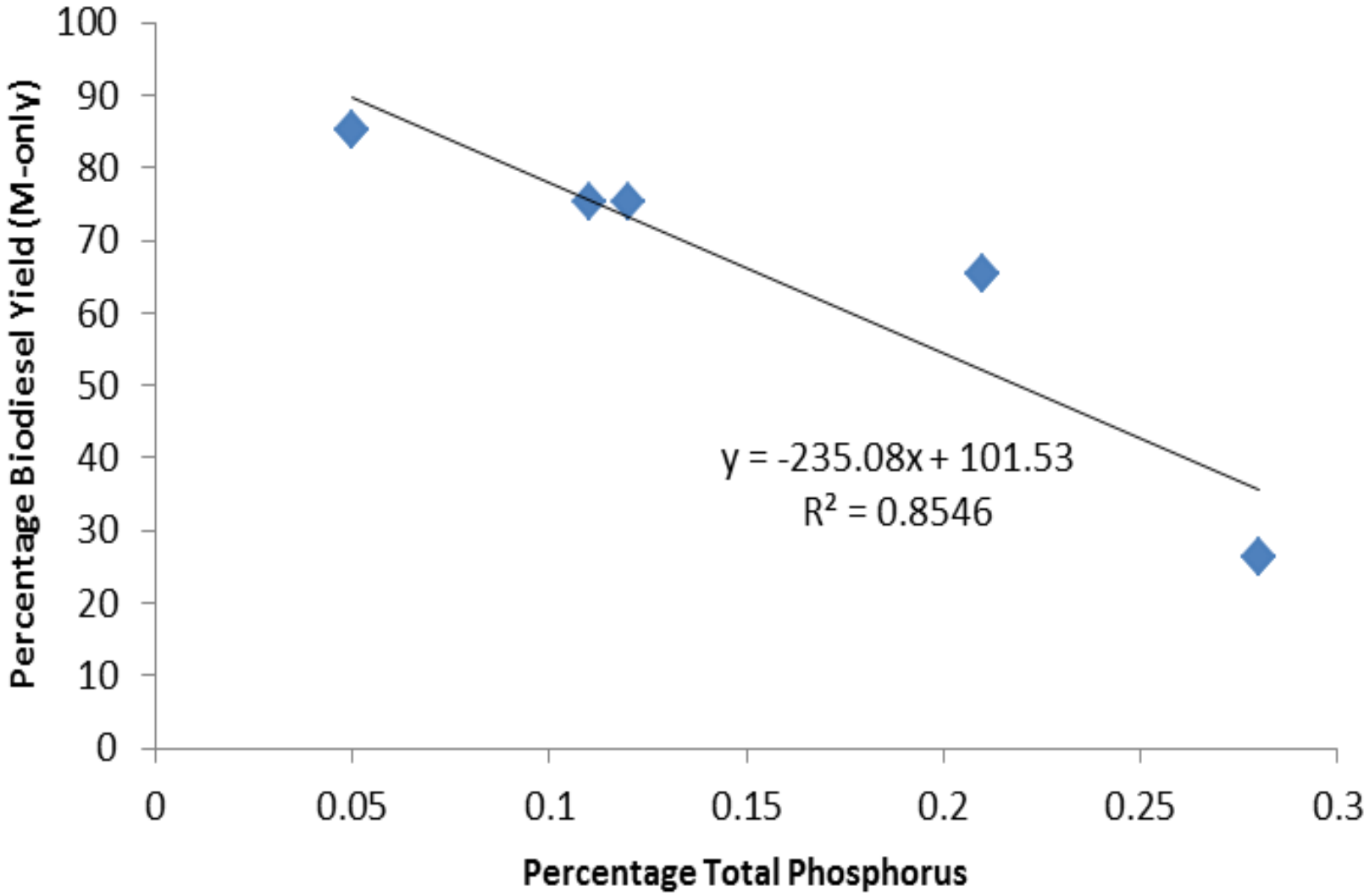


Fig 4.8: Relationship between percentage Total Phosphorus in biomasses and Biodiesel yield (M-only transesterification)

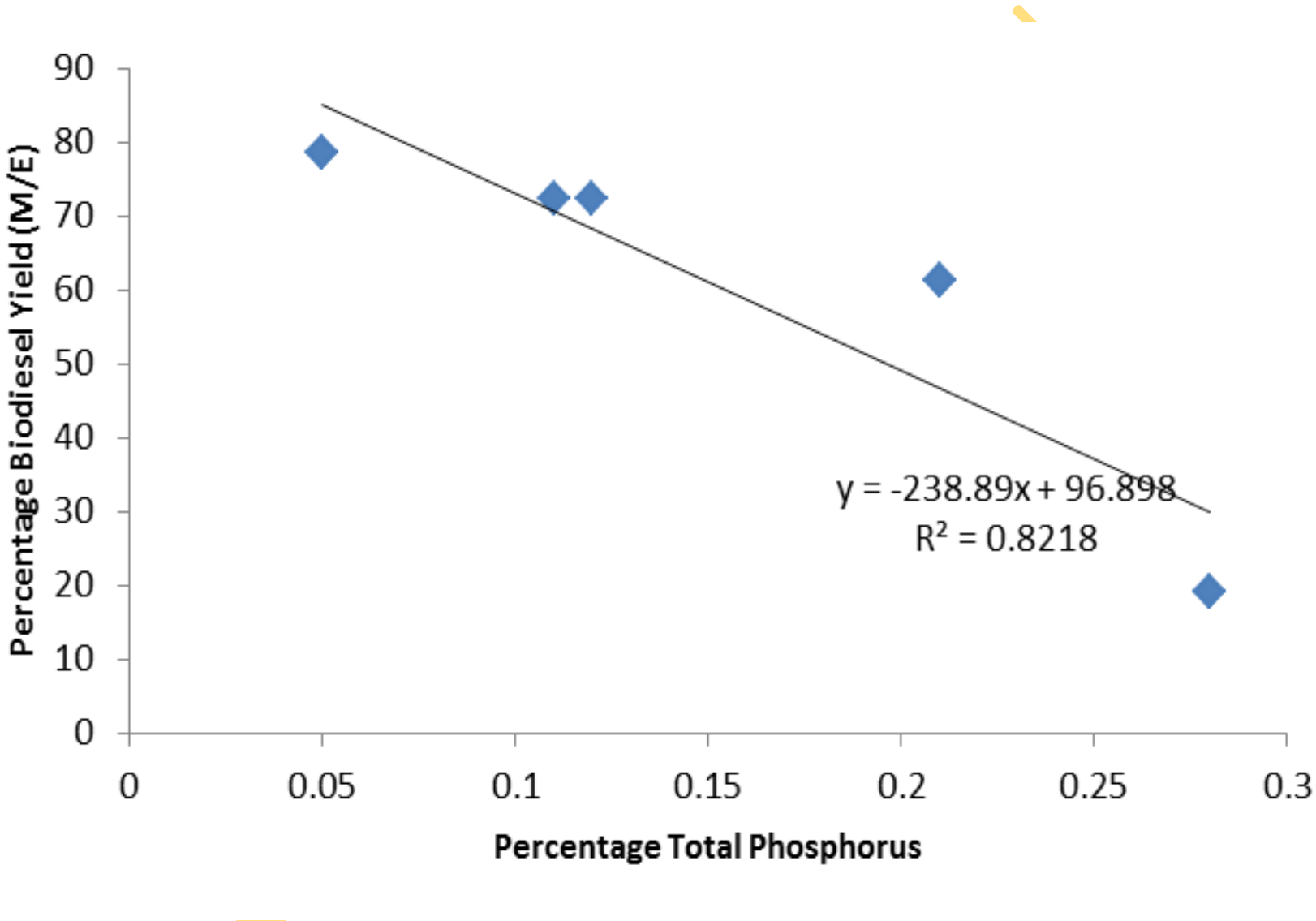


Fig 4.9: Relationship between percentage Total Phosphorus in biomasses and Biodiesel yield (M/E transesterification)

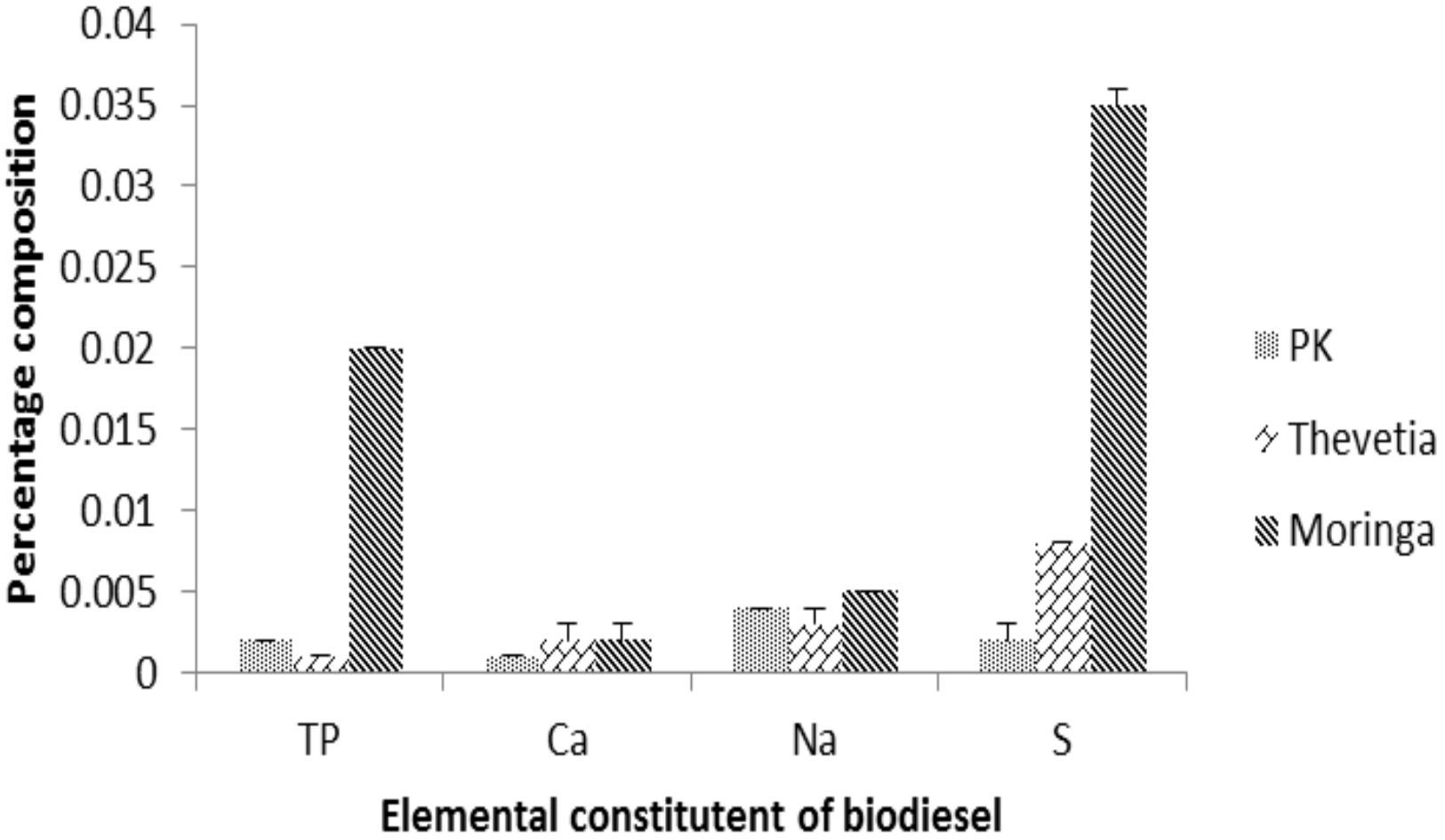


Fig 4.10: Showing the proportion of elemental constituent of the biodiesels

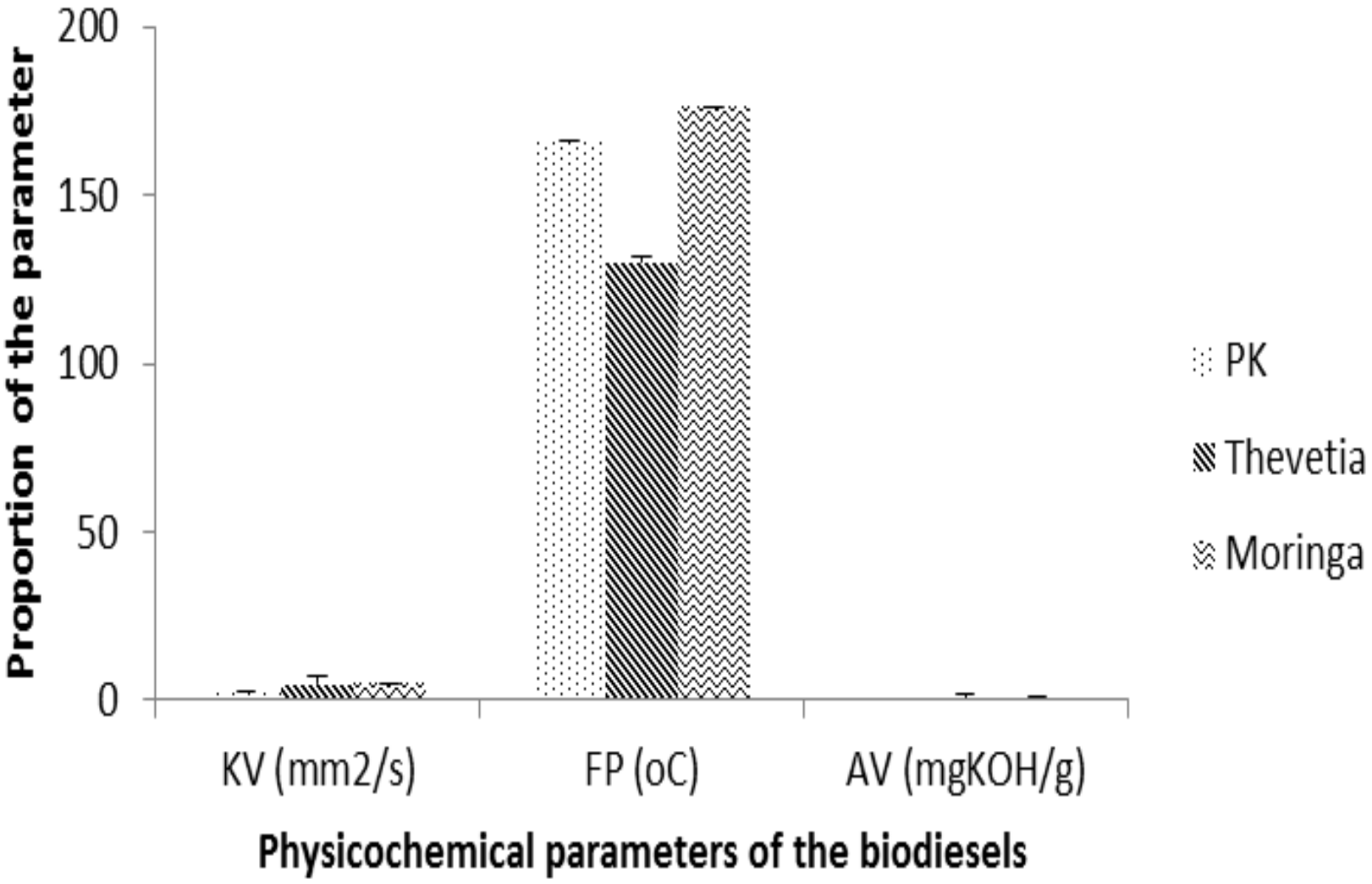


Fig 4.11: Comparison of some physicochemical parameters of each biodiesel

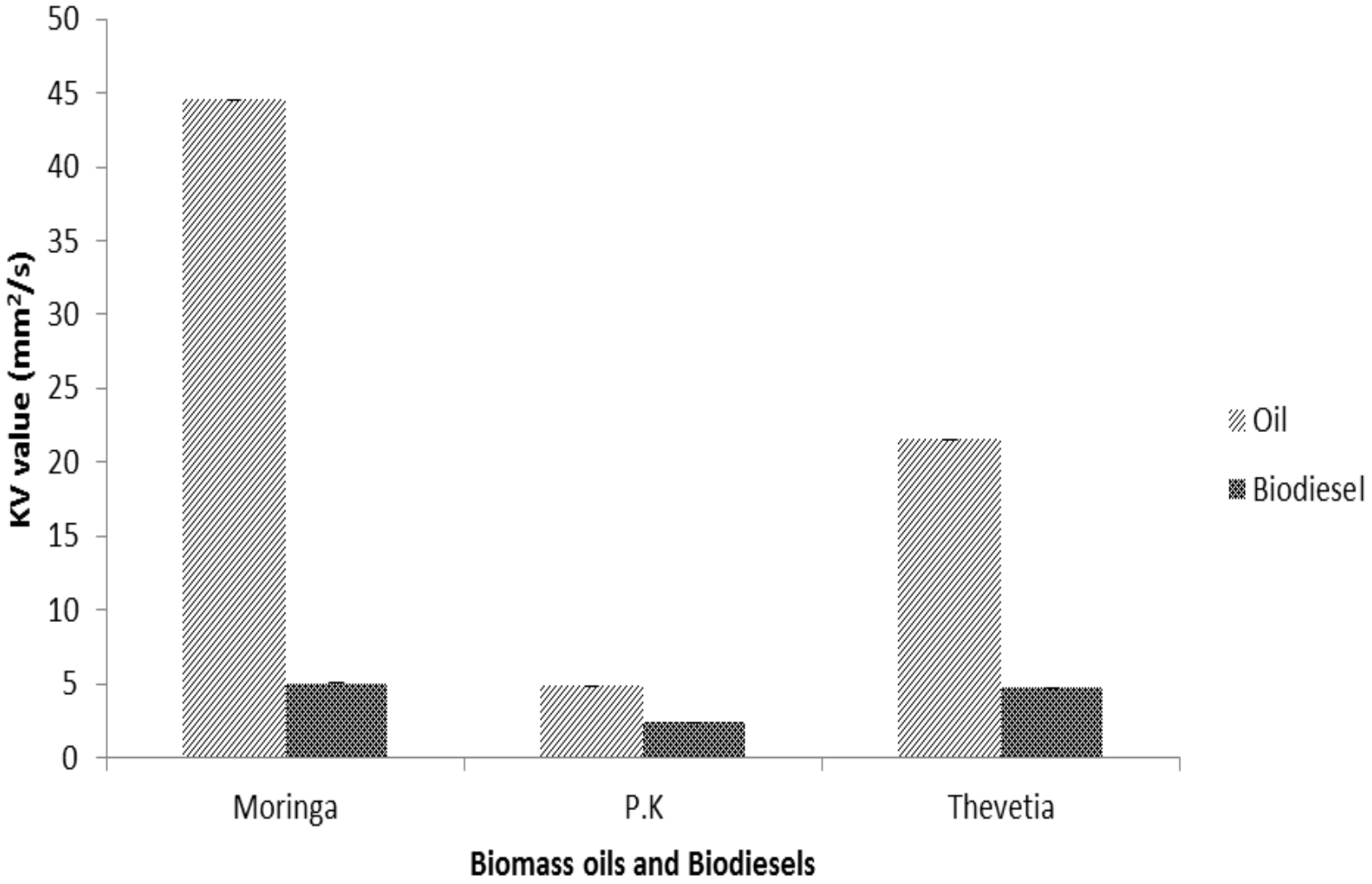


Figure 4.12: Comparison of the Kinematic viscosity of oil to the Kinematic viscosity of biodiesel

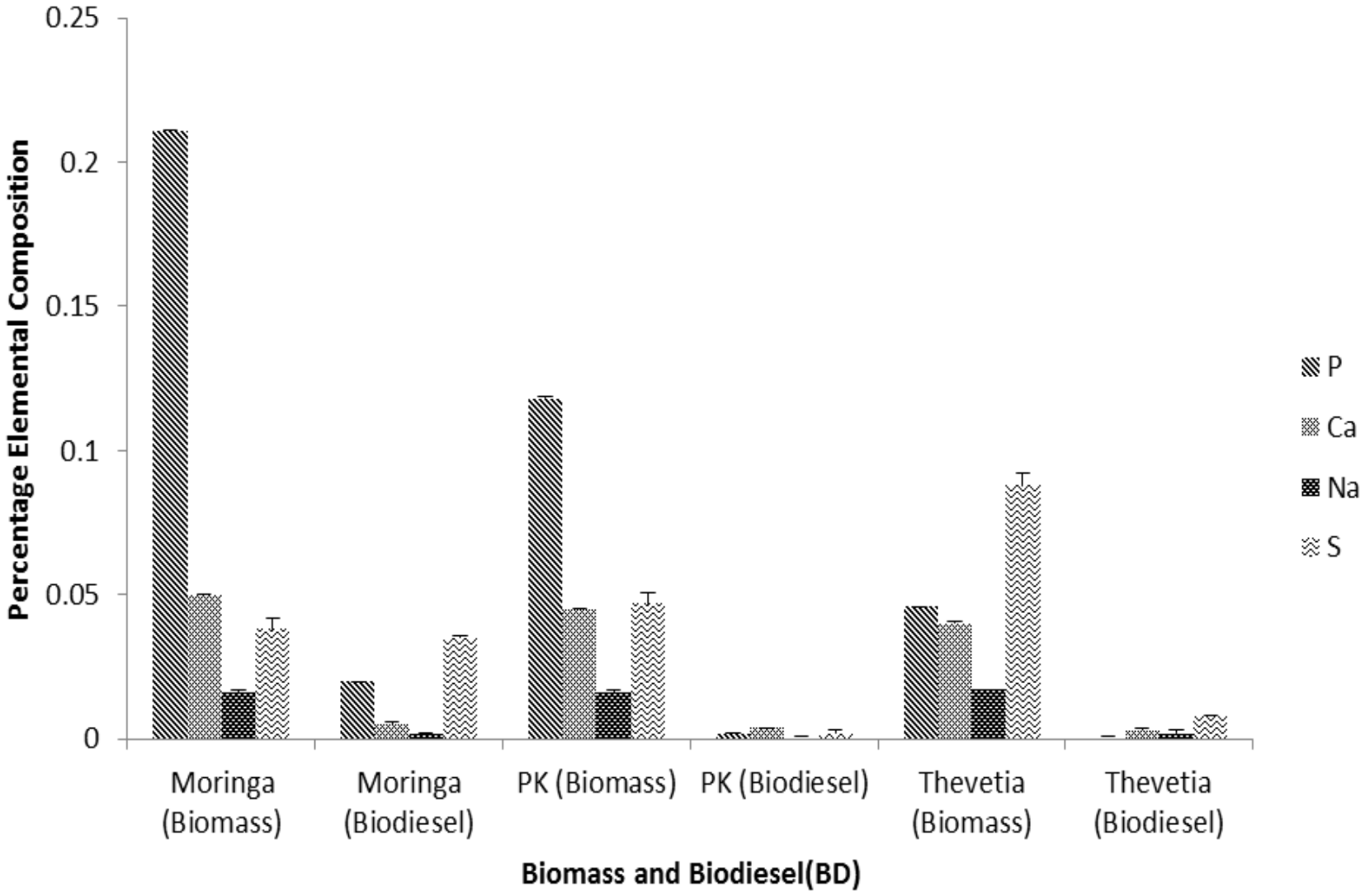


Figure 4.13: Comparison of the Percentage Elemental composition of biomasses to those of biodiesels

Table 4.9: Showing a comparison between properties of the biodiesels obtained in this work with ASTM and EN guidelines respectively

Fuel properties		Moringa biodiesel	PK biodiesel	Thevetia biodiesel	Spirogyra biodiesel	ASTM guideline (D6751) Limits	EN standard (EN 14214) Limits
Density @ 25^{o}C (g/cm^3)	M-only transesterification	0.877	0.913	0.839	0.881	Unspecified	0.860-0.900 @ 15^{o}C
	M/E (1:1) transesterification	0.878	0.899	0.842	0.885		
Kinematic viscosity @ 40^{o}C (mm^2/s)		5.02	2.39	4.70	Undetermined	1.9-6.0	3.5-5.0
Flash point (^{o}C) min.		176	166	130	Undetermined	130	101
Acid value (mg KOH/g) max.		0.657	0.417	0.441	Undetermined	0.8	0.5
Phosphorus content max.		0.020	0.002	0.001	Undetermined	0.001% **or** 10 mg/kg	0.001 % **or** 10 mg/kg
Alkaline earth metal content (Ca) max.		0.005	0.004	0.003	Undetermined	-	0.00005 % **or** 5 mg/kg
Alkaline metal content (Na) max.		0.002	0.001	0.002	Undetermined	-	0.00005 % **or** 5 mg/kg
Sulphur content max.		0.035	0.002	0.008	Undetermined	0.05% **or** 500 mg/kg	0.001 % **or** 10 mg/kg
Cloud point (^{o}C)		13.6	14.1	8.5	Undetermined	Report to customer	-
Pour point (^{o}C)		6.5	8.6	5.1	Undetermined	-	-

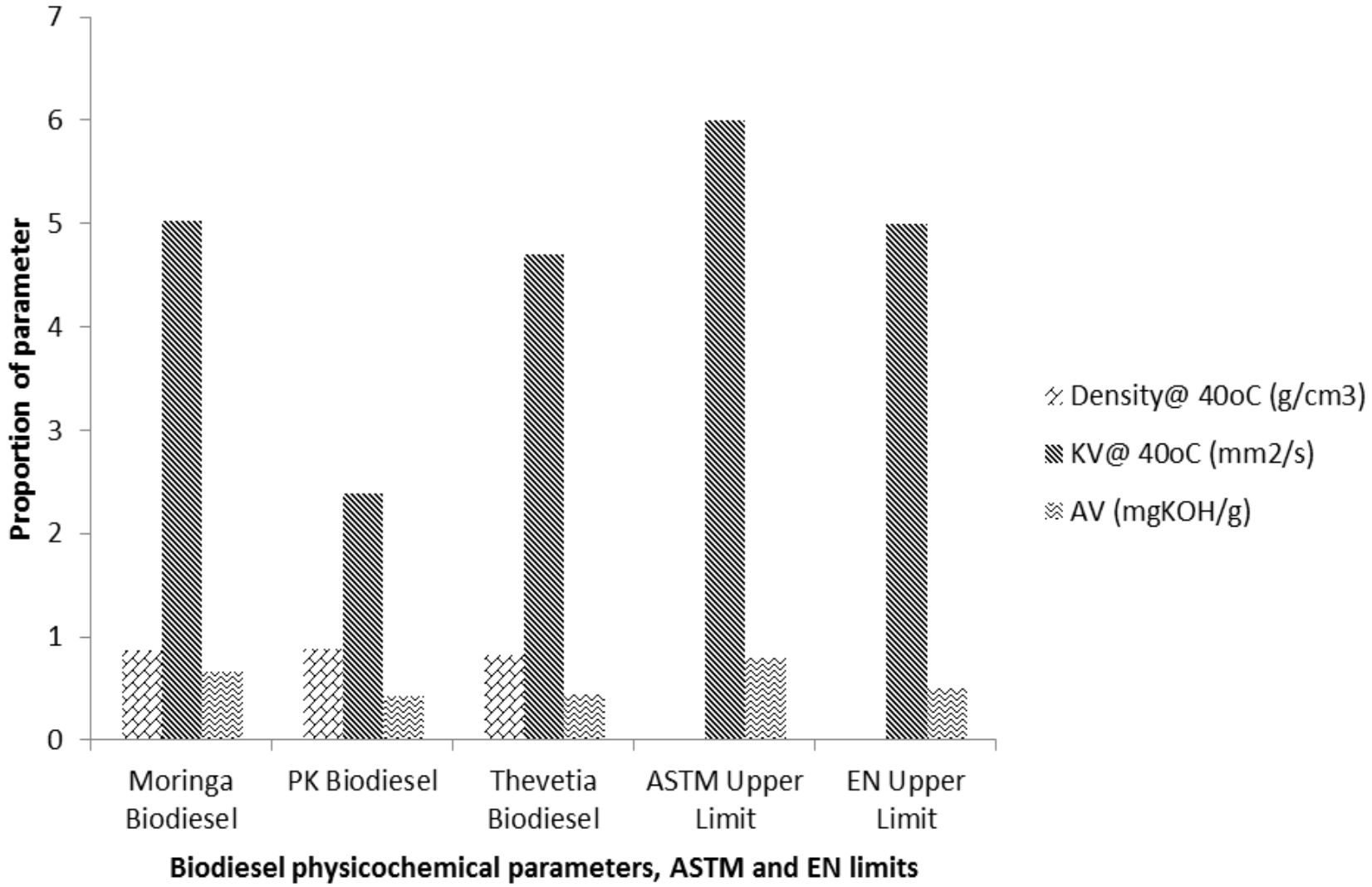


Figure 4.14: Shows the comparison of some biodiesel physicochemical parameters to ASTM and EN standards

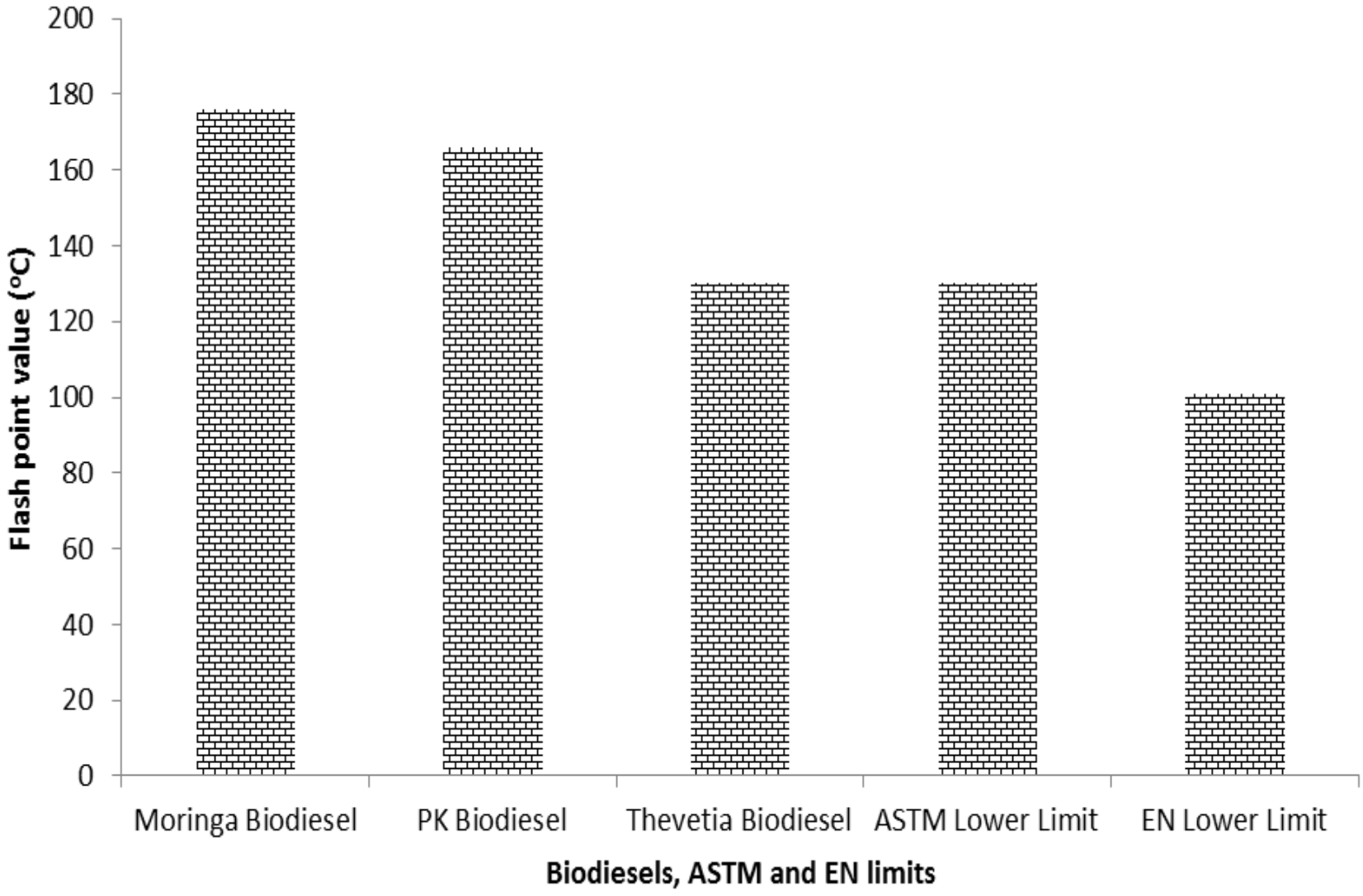


Fig 4.15: Comparison of Flash point to ASTM and EN standards

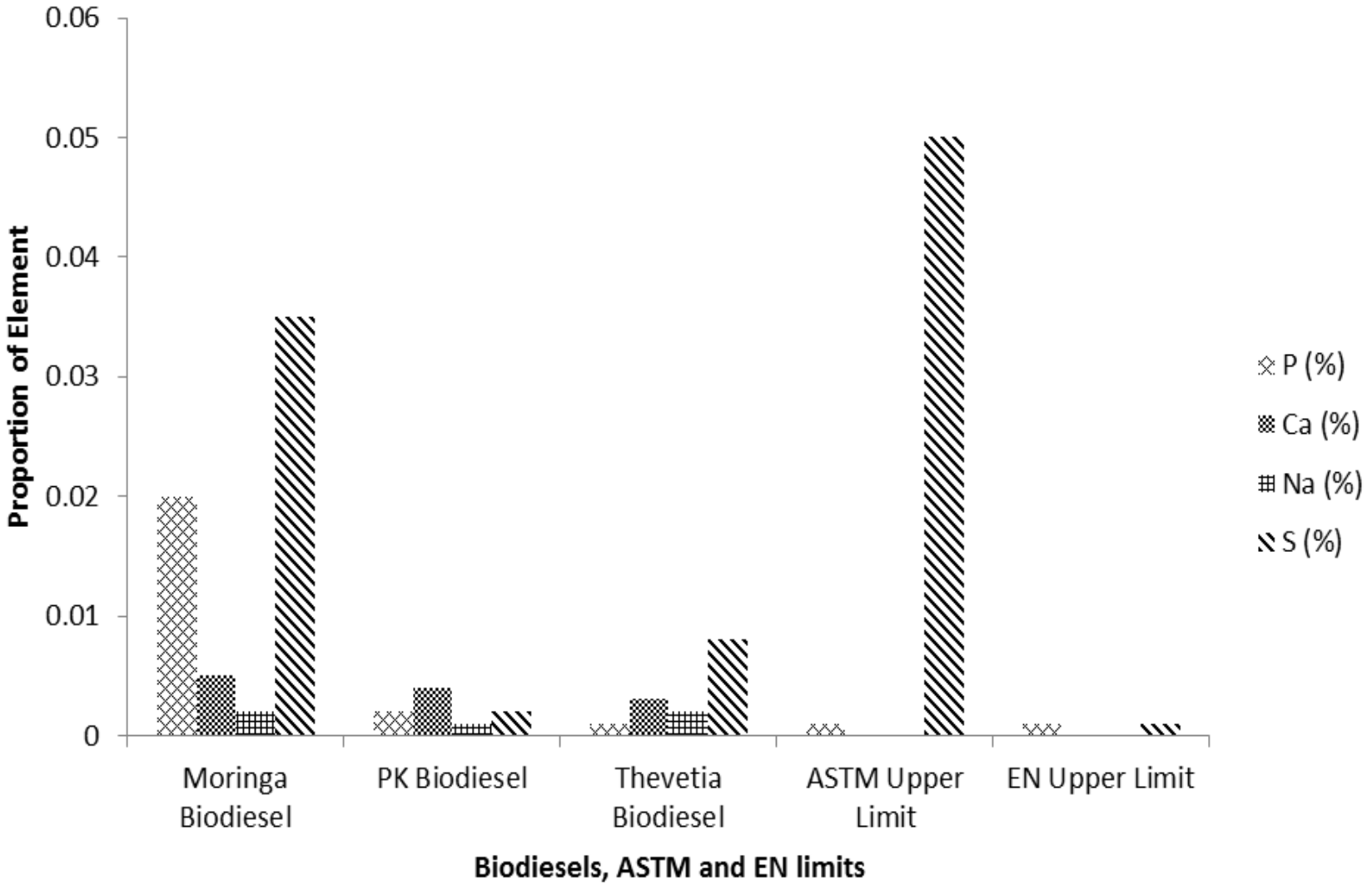


Fig 4.16: Comparison of elemental composition of biodiesels to ASTM and EN limits

CHAPTER FIVE

DISCUSSION

5.1 Sources of Substrates

An assessment of the availability of the biomasses used in this work when prospecting for them indicated that Moringa and Palm kernel seeds were readily available in commercial quantities. However, the former was relatively expensive in commercial quantities because of the high demand for it in some areas. However, this study sought to characterize the biomasses derived directly from their natural source.

There is no known supply of Thevetia and Spirogyra biomasses in commercial quantities in the state. Thevetia plants are majorly grown as hedges in homes, schools, offices, etc, and most are constantly trimmed to shape and to maintain the plant for its aesthetic appearance. The seeds are, therefore available on the plants since the plant produces fruits virtually in ten out of the twelve months of the year.

Spirogyra filaments are extremely common and occasionally an abundant genus in standing water bodies. Most species are collected as large floating masses or flimsy aggregates or long strings of cells from permanently or temporarily stagnant aquatic habitats. These habitats usually have neutral or slightly acidic pH values such as ponds, lakes and ditches. They are mostly found anywhere there is a relatively slow flowing or stagnant water body with a relatively sufficient sunlight. That is why the green filaments could even grow in a container of water left in a household environment for a long period of time. However, there is the need for sunlight, a favorable pH and certain essential nutrients to support their growth.

Most of the substrates used in this experimental work, especially Thevetia and Spirogyra biomass are relatively available in the environment but not utilized for the economic growth and development of the society. Based on available records that ranks Nigeria as one of the world's top producers of palm oil and hence palm kernel (FAO 2006, see Table 2.7), it would not be out of place to say that palm kernel oil has just relatively found application in few industrial uses (such as soap-making) but its full potentials are yet to be explored. Moringa plant has just

recently been gaining increased popularity across board in Nigeria as a plant that has every part of it with their own potentials. However, majority of the research works on *Moringa oleifera* has centered on its nutritional and therapeutic value, with just very few works done to explore its oil and biodiesel potential in the country.

5.2 Physical Characteristics of the Plant Biomasses

The 24 hours intermittent sundrying period (for the biomasses used in each of the 3 different extraction process) was observed to remove approximately a mean moisture of 2.3±0.4 %, 2.0±0.2 %, 3.2±0.4 % and 20.7±8.8 % from the fresh biomass samples of milled Moringa seeds, Palm kernel seeds, Thevetia seeds and Spirogyra biomass respectively as presented in Table 7.0-appendix.

Also, Table 4.1, shows that Spirogyra biomass had a significantly high moisture content (39.7±0.1 % and 39.7±3.0 %) from the two methods used for moisture content determination (i.e. Moisture analyzer equipment and Oven-drying method respectively) in comparison with the three (3) other substrates.

Fuad *et. al*., (2010) reported 40% moisture content in Spirogyra biomass, Rutikanga (2011) also reported 10.7±0.4 % while Kanyaporn *et. al.*, (2012) reported 8.5 % moisture in Spirogyra biomass. However, it was only the report of Fuad *et. al*., (2010) that was in agreement with the moisture content of Spirogyra biomass obtained in this work. Also, Moringa seeds (9.37±0.03 % and 9.48±0.19 %) were shown to have slightly higher moisture content (%) than Palm kernel seeds (8.3±0.0 % and 8.3±0.1 %), with Thevetia seeds having the lowest moisture content (6.6±0.1 % and 6.6±0.0 %) from the two methods used respectively.

However, the results of moisture content obtained for Moringa seeds were at variance with the 4.7±0.3 % reported for Moringa seeds by Dalen *et. al*. (2009). Also, Chindo *et. al*. (2013) reported 2.2 % moisture content for Thevetia seeds, which was also at variance with the results obtained for the moisture content of fresh milled Thevetia seeds used in this work.

Milled Thevetia seeds were observed to have the highest mean relative density (0.750±0.001) amongst the substrates while Milled Palm kernel seeds had the lowest value of 0.572±0.002 as shown in the Table 4.1 and Table 7.3-appendix.

5.3 Chemical Characteristics of the Plant biomasses

The results of the proximate analysis carried out on the biomasses (Table 4.2, with the duplicate readings presented in Table 7.4-appendix) shows the percentage Total Organic Carbon (T.O.C) present in Moringa seeds, Palm kernel seeds and Thevetia seeds to be very close, but Moringa seeds had the highest T.O.C content (60.9±0.5 %) and Spirogyra had the least (51±0.7 %). *Moringa oleifera* seeds were found to have the highest proportion of Total Nitrogen (T.N) (i.e. 0.210±0.007 %) with Palm kernel seeds having the lowest (0.091±0.001%).

Spirogyra biomass, unlike in the T.O.C where it recorded the lowest value, was observed to surpass the other three substrates (i.e. Moringa, Palm kernel and Thevetia seeds) in the levels of Percentage T.P (0.28±0.00), Percentage Calcium (0.05±0.00), Percentage Sodium (1.35±0.00) and Percentage Sulphur (0.882±0.01) respectively (Table 4.2).

Phosphorous, calcium, and magnesium are minor components typically associated with phospholipids and gums that may act as emulsifiers (ASTM Standard D6751, 2009) or cause sediment, lowering yields during the transesterification process (Gerpen *et. al.*, 2004), hence, their percentage composition in the respective biomasses were determined to establish if there would be a significant reduction in their composition upon taking the substrates through the solvent extraction process and the oils through the transesterification process respectively.

5.4 Physical Characteristics of the Extracted Oils

The oven-dried biomasses used in this experimental work were all observed to give a significantly different yield of oil across the three different extraction methods that were employed. The Soxhlet extraction gave the highest oil yield across the four substrates followed by Cold extraction (using Hexane/Ether mixture as the extraction solvent) while the Cold extraction (using Hexane-only) gave the lowest yield.

All the extracted oils were observed to have a slightly acidic pH (Table 4.2 with the triplicate readings presented in Table 7.5-appendix). Palm kernel oil recorded the lowest pH (6.02±0.02) while Spirogyra oil had an almost neutral pH (6.68±0.01). Palm kernel oil was observed to have the highest specific gravity (0.88±0.002) at a room temperature (25.0±0.4 ^{o}C), which somewhat agrees with the 0.85g/cm^3 value that was reported by Ojolo *et. al.* (2012), while Spirogyra oil had the least value (0.53±0.001) at the same temperature (Table 7.6-appendix).

The test for oil viscosity showed Moringa oil to have the highest kinematic viscosity (44.5±0.014) with Spirogyra oil having the least value (4.50±0.000) (Table 4.2 with duplicate readings presented in Table 7.8-appendix). Viscosity, from a physicochemical point of view, means the measure of resistance to flow that a liquid offers when it is subjected to sheer stress. Hence, it must be closely correlated with the structural parameters of fluid particles.

Viscosity is one of the important properties of oil which needs to be determined as it influences the ease of handling, transport and nature of storage. The viscosity of oils is strongly dependent on temperature as an increase in temperature causes a decrease in viscosity (Abramovic and Klofutar, 1998). The kinematic viscosity observed for Moringa seed oil corroborates what was reported by Sanford *et. al*, (2009). Also, Uzama *et. al*., (2011) reported a Kinematic viscosity of 43.5mm^2/s, which further agrees with the result obtained for moringa seed oil in the present work.

The oils extracted from each of the biomasses via the different extraction processes employed in this work were observed to have characteristic colour, as reported in Table 4.2. The oils were also observed to possess a characteristic odour. Generally Moringa oils were perceived to have a sweet fruity smell, while Palm kernel oils had a sweet nutty smell, Thevetia had a very sweet butter fragrance and Spirogyra oil possessed a sweet forest tree smell.

5.5 Chemical Characteristics of the extracted Oils

Saponification value indicates the average molecular weight of a fat or oil. It gives us information whether an oil or fat contains high proportion of lower or higher fatty acids. The greater the molecular weight (longer carbon chain), the smaller the number of fatty acids that is

liberated per gram of oil hydrolyzed and therefore, the smaller the saponification value and vice versa.

Palm kernel oil was observed to have the highest Saponification value (Table 4.3) indicating a high molecular weight as a result of fatty acids with long carbon chains. It should be noted at this point that Saponification value (alongside some other parameters) was not estimated for in the Spirogyra oil (and by extension in the Spirogyra biodiesel) due to insufficient quantity that was available to undergo transesterification and yet be enough for those analyses.

FFA (or fatty acids that have been unbound from the original triglyceride) occur in vegetable oils either because of contact with water or poor storage or because of the presence of enzymes that rapidly cleave the fatty acids from the glycerol backbone. A good example is rice bran oil (Zullaikah *et. al*., 2005), which would have been a nutritionally desirable oil if not for its very high content of FFA caused by naturally occurring lipases. When a homogeneous alkali catalyst is used, Gerpen (2005) recommends that the maximum FFA content of the feed oil should be 5%. Otherwise, soaps will form, making separation of the glycerine difficult. Hence, an additional step to remove the FFA or to convert them via an esterification step is necessary before using the feed oil in transesterification reaction.

Stavros and John (2002) had reported a saponification value of 188.4mgKOH/g for the degummed oil of *Moringa oleifera* seeds. Also, Sanford *et. al.* (2009) and Uzama *et. al*. (2011) reported 195.0mgKOH/g and 191.4mgKOH/g saponification values respectively for moringa seed oil. The result of the saponification analysis (Table 4.3) seemed closer to that of Sanford *et. al.* (2009) and Uzama *et. al.* (2011), but was in considerable variance with that of Stavros and John (2002). Also, the result of the FFA analysis carried out on Moringa oil agrees with the 2.9% value reported by Sanford *et. al.* (2009) but does not agree with the 1.12$\pm$0.20% value reported by Stavros and John (2002).

Palm kernel oil was observed to have a lower FFA content (1.9 %) compared to that of Moringa seed oil. However, it had a higher saponification value (230.2 mgKOH/g) than both Moringa and Thevetia seed oils (Table 4.3). Igbum *et. al.* (2012) reported a value of 210.3 mgKOH/g for palm

kernel oil, which seemingly conflicted with the 230.2 mgKOH/g value that was recorded in this work. The saponification value obtained in this work for PK oil however was found to fall between the 230-254 mgKOH/g range that was estimated by CODEX-STAN 210 (1999) as the general Saponification range of value for Palm kernel oils.

The "soap-formation value" (Saponification value) of Thevetia oil was observed to be the least amongst the three different oils that were analyzed where a value of 120.1 mgKOH/g was recorded. Also, and as expected though, the FFA value of the oil was also the least among the three oils where it recorded a percentage value of 0.58 (Table 4.3).

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The analysis of the Fatty acid composition (Table 4.4) of the respective biomass oils revealed a diverse array of fatty acid types in each of the oils. Palm kernel oil, which was observed to be a high Lauric acid (C12:1)-containing oil, was shown to contain the highest level of saturation (79.99%) amongst the substrate oils and consequently the lowest unsaturation level of 17.41%. The oils of Moringa seeds, Thevetia seeds and Spirogyra biomass were found to be majorly composed of Oleic acid (C18:1). They were observed to be relatively more unsaturated, with Moringa seed oil having the highest level of unsaturation (78%).

5.6 Physical Characteristics of the Biodiesels

Table 4.5a shows that the different transesterification reaction processes, which employed two different alcohol systems *viz*: Methanol-only and Methanol/Ethanol mixture for each of the extracted oils, gave different biodiesel parameters such as pH, relative density and biodiesel yield. Also, the quantity of glycerine that was obtained from each of the transesterification process using the two alcohol systems gave different yields for each system.

Generally, the alcohol system where methanol-only was utilized was observed to have a higher conversion of oil to biodiesel efficiency as opposed to the methanol/ethanol (M/E) mixture, which gave a considerable lower yield across the substrate oils. It could then be said that unlike in the cold solvent extraction process where two extraction solvent systems were evaluated *viz*: Hexane-only and Hexane/Ether mixture and the latter gave a higher oil yield across all the biomasses, this could not be said of the alcohol systems used in the transesterification processes

as the Methanol/Ethanol mixtures were observed to give a lower biodiesel yield compared to Methanol-only. The M/E transesterification process gave an expectedly higher yield of the by-product (glycerine) than the methanol-only transesterification process. This suggests the lower conversion efficiency of the M/E transesterification compared to the methanol-only transesterification process.

There was no significant difference ($p > 0.05$) in the pH of all the biodiesels obtained from the different transesterification processes suggesting that the washing (or cleaning) step was effective in considerably bringing the pH of the biodiesels within neutral range. The results of ANOVA showed that there was significant difference ($p<0.05$) in the density of almost all the oils when compared to their respective biodiesels (Table 7.18-appendix). Density is temperature dependent, so the density of biodiesel varies with temperature. Since biodiesel is typically sold by volume, the density of biodiesel as a function of temperature is therefore an important factor in biodiesel commerce (Biodiesel Handling and Use Guide, 2008).

5.7 Chemical Characteristics of the Biodiesels

There are specifications that govern biodiesel quality, and the differences in key performance parameters of biodiesels versus conventional diesel. ASTM International (*www.astm.org*) is a consensus-based standards group that comprises engine and fuel injection equipment companies, fuel producers, and fuel users whose standards are recognized in the United States by most government entities and in some other countries.

The specification for biodiesel (B100) is ASTM D6751. This specification is a compilation of efforts from researchers, engine manufacturers, petroleum companies and distributors, and many other fuel-related entities, and it is intended to ensure the quality of biodiesel used as a blend stock at 20% and lower blend levels. In the United States for example, any biodiesel used for blending should meet ASTM D6751 standards (ASTM D6751, 2009) Also, the German Institute of Standardization (DIN EN 14214) is another notable regulatory body that issues specifications for all biodiesels produced or sold for use in the European Union.

The result of the elemental analyses carried out on the respective biodiesel fuels produced (except for the Spirogyra biodiesel where there was insufficient quantity to run the analyses) showed a significant reduction ($p < 0.05$) in the levels of all the elements that were assessed in the biodiesel compared to their corresponding parent biomass. The negative correlation observed between the percentage elemental composition of the biomasses and the biodiesel yield in the two transesterification processes (Table 4.7) indicates that an increase in the proportion of the elements in the biomasses results in the decrease of biodiesel yield after transesterification. This is in agreement with the findings of Gerpen *et. al.* (2004). There was also a significant decrease ($p < 0.05$) in the kinematic viscosity of the biodiesels when compared to their parent oils. This suggests a significant increase in the fluidity of the fuels and a greater performance in diesel engines in terms of fluid operability.

Table 3.1 shows the specifications made by ASTM and DIN EN. It can be seen from the table that the Phosphorus content of Moringa biodiesel clearly exceeded the 0.001% maximum limit set by both regulatory bodies. Palm kernel biodiesel slightly surpassed the limit but Thevetia biodiesels was within the maximum set value. The Calcium and Sodium levels of the biodiesels of Moringa, Palm kernel and Thevetia all considerably surpassed the EN standard for these elemental compositions in biodiesel fuels. However, the Sulphur content in all the biodiesel fuels were clearly within the guideline set by ASTM, except that they slightly exceeded the guideline set by DIN EN.

A minimum flash point for diesel fuel is required for fire safety. B100's flash point should be at least 93ºC (200ºF) to ensure it is classified as nonhazardous under the National Fire Protection Association (NFPA) code. The biodiesels from all the respective oils in this work were in definite conformity with both ASTM and DIN EN guidelines, indicating a good level of safety handling with much less danger of inflaming accidentally.

Aliyu *et. al*, (2013) reported 186^{o}C as the flash point for moringa biodiesel, while Sanford *et. al.* (2009) also reported a value of >160^{o}C as the flash point for moringa biodiesel. Also, Alamu *et. al*. (2008) reported 167^{o}C, Atu *et. al.* (2011) reported 209^{o}C, while Oghenejoboh and Umukoro, (2011) reported 152^{o}C as the flash point of Palm kernel biodiesel respectively. In the same vein,

Balusamy and Marappan (2007) reported a flash point value of 128^{o}C for Thevetia biodiesel whereas Olisakwe *et. al.* (2009) and Chindo *et. al.* (2013) reported a flash point of 168^{o}C and 175^{o}C respectively.

The results of all the flash point values recorded in this work for the biodiesels from the different substrate oils clearly show all these biodiesels to have a significantly higher "ignitability point" as compared to that of conventional diesel fuel as shown in Table 3.1, which compares certain parameters of B100 biodiesel to conventional petroleum-based diesel.

The low-temperature properties of biodiesel and conventional petroleum diesel are extremely important. Unlike gasoline, petroleum diesel and biodiesel can freeze or gel as the temperature drops. If the fuel begins to gel, it can clog filters on dispensing equipment and may eventually become too thick to pump. Cloud point is the most commonly used measure of low-temperature operability; fuels are generally expected to operate at temperatures as low as their cloud point.

The B100 cloud point is typically higher than the cloud point of conventional diesel. Cloud point must be reported to indicate biodiesel's effect on the final blend cloud point. Thevetia oil had the lowest cloud point (8.5$\pm$0.1^{o}C) amongst the three biodiesels indicating a substantially very good cold property while Moringa and Palm kernel biodiesel fuels were observed to start containing small solid crystals at 13.6$\pm$0.1^{o}C and 14.1$\pm$0.1^{o}C respectively. At the same time, Thevetia, Moringa and Palm kernel oils were observed to essentially become a gel/solid (i.e. Pour point) at 5.1$\pm$0.1^{o}C, 6.5$\pm$0.0^{o}C and 8.6$\pm$0.1^{o}C respectively.

Balusamy and Marappan (2007) had reported a Cloud point value of -4^{o}C and a Pour point of -7^{o}C for Thevetia biodiesel while Olisakwe *et. al.* (2009) reported 8^{o}C Pour point value for Thevetia biodiesel. Also, Alamu *et. al.* (2008) had reported a Cloud point value of 6^{o}C and a Pour point value of 2^{o}C for Palm kernel biodiesel, whereas Oghenejoboh and Umukoro (2011) reported a Cloud point value of 8^{o}C and a Pour point value of-15^{o}C. Furthermore, Igbum *et. al.* (2012) reported -13.19^{o}C as the Pour point value for Palm kernel biodiesel. These results generally show that the biodiesel from these substrate oils considerably conform to ASTM D6751 and/or EN 14214 standards.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The purpose of this study was to evaluate the biodiesel yielding potential of certain plant biomasses *viz-a-viz* the characteristics of the biomasses and their respective products (i.e. extracted oil and biodiesel). The oils were extracted by Solvent extraction processes: Soxhlet extraction and Cold extraction respectively; while these oils were processed to biodiesel by transesterification reaction using two alcohol systems.

The results of the experimental work show that Thevetia seeds produced the highest oil yield across the different extraction processes utilized while Spirogyra biomass produced the lowest yields. In the same vein, Thevetia oil gave the highest biodiesel yield across the two transesterification reaction systems in this work while Spirogyra oil produced the least biodiesel yields.

The extraction of oil from the plant biomasses via Solvent system proved that a mixture of the organic solvent (n-hexane) with another non-polar solvent (pet ether) in ratio 1:1 was more effective than the use of one organic solvent alone. However, the transesterification experiment showed that the use of a single alcohol such as methanol alone proved to be more effective than the combination of two alcohol systems (such as methanol/ethanol mixture). The physical and chemical characteristics of the biomasses and their respective products showed that they conformed to set standards that are stipulated by some regulatory bodies such as ASTM and DIN EN.

There was significant reduction in the level of certain undesirable parameters in the extracted oils when they were processed to their respective biodiesels through the base-catalyzed transesterification process. This suggests that undesirable qualities of biodiesels (such as high viscosity and high content of certain elements) could be significantly reduced by processing the oils to biodiesels via the base-catalyzed transesterification process.

The results of analyses that were carried out in this work also revealed that there was a significant difference ($p<0.05$) between the relative density of the oils and that of the biodiesels that were produced from the oils. In the same vein, there was a significant difference ($p<0.05$) between the pH of the oils and their respective biodiesels. There was also a significant difference ($p<0.05$) between the Kinematic viscosity of the oils and their respective biodiesels.

Conclusively, the biodiesels derived from the respective extracted oils are acceptable substitutes for petrodiesel based on the plausible results from the analyses that were carried out to assess certain physicochemical properties of the oils and biodiesels respectively. Although the analyses carried out were somewhat limited to the available resources, but the major physicochemical properties analyzed for in the oils make them an attractive alternative feedstocks for biodiesel production. However, same cannot be said for Spirogyra biomass due to its significantly low oil and biodiesel yield respectively.

6.2 Recommendations

The need for an alternative biofuel such as biodiesel that is environmentally friendly and sustainable in today's economy cannot be over-emphasized. This is because there is an increasing awareness of renewable energy as a viable option to mitigate against the woes that are persistently posed by the use of conventional biofuels. Hence, there is the need to propose, develop and implement modalities that would support and sustain the growth of biodiesel production, especially in Nigeria.

Arising from this work, the following recommendations are therefore proffered for biodiesel production:

1. Further investigations to develop other solvent mixtures that could prove to be more efficient in extracting oils from the oil-bearing biomasses using desirable equipment.

2. Research into processing the substrate oils to biodiesel via transesterification (or esterification if required) using other alcohol systems and catalysts.

3. The list of biodiesel guideline parameters by International regulatory bodies such as the American Standard for Testing and Material, which is used in accessing biodiesel quality,

was not exhausted in this work. Hence, further oil and biodiesel characterization studies could be carried out to access the conformity of the substrate oils and their respective biodiesels to other guideline parameters by these regulatory bodies.

4. Biodiesels produced from the biomasses and/or their blends with petrodiesel could be subjected to comprehensive ignition testing operations to access their suitability for use in direct ignition diesel engines.

5. Engineering systems or automobile engines that would be locally adaptable and suitable for the efficient utilization of biodiesels produced from the substrate oils or their blends could be developed.

6. Life Cycle Assessment (LCA) studies of the biomasses could be carried out to establish the detailed agronomic and environmental requirements for maximal production output right from the farm to the industry, and up to the point of sale of the biodiesel products for either profit-making or other purposes.

7. There could be further studies to evaluate the oil and/or biodiesel potential of other locally available oil bearing biomasses in Nigeria asides from the ones explored in this work.

8. Considering the low yield of oil and biodiesel from spirogyra filaments, a microalga could be explored for its oil and/or biodiesel potentials rather than a macroalga (like the spirogyra that was used in this work).

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APPENDICES

Appendix 1: Supplementary result of Plant biomass characteristics

Table 7.1: Showing the Percentage moisture content removed by sundrying from each of the substrates used in the different extractions

Biomass	Soxhlet biomass moisture	Cold extraction biomass (H/E) moisture	Cold extraction biomass (H-only) moisture	Mean (%) Moisture content removed	Standard deviation
Moringa	2.60	1.67	2.50	**2.26**	0.42
P.K	1.50	2.62	1.83	**1.98**	0.22
Thevetia	2.66	3.52	3.30	**3.16**	0.36
Spirogyra	33.16	14.45	14.45	**20.68**	8.82

Table 7.2: Showing triplicate moisture content determination in the biomasses using the Moisture analyzer equipment

Biomass	Soxhlet biomass reading @ 160^{o}C	Cold extraction biomass (H/E) reading @ 160^{o}C	Cold extraction biomass (H-only) reading @ 160^{o}C	Mean (%) Moisture content	Standard deviation
Moringa	9.34	9.38	9.40	**9.37**	0.03
P.K	8.29	8.22	8.25	**8.25**	0.04
Thevetia	6.65	6.58	6.67	**6.63**	0.05
Spirogyra	39.70	39.74	39.50	**39.65**	0.13

Table 7.3: Showing triplicate moisture content determination in the biomasses using the Oven-drying method

Biomass	Soxhlet biomass reading @ 105°C	Cold extraction biomass (H/E) reading @ 105°C	Cold extraction biomass (H-only) reading @ 105°C	Mean (%) Moisture content	Standard deviation
Moringa	9.38	9.70	9.36	**9.48**	0.19
P.K	8.27	8.37	8.28	**8.31**	0.06
Thevetia	6.63	6.65	6.65	**6.64**	0.01
Spirogyra	43.12	38.00	38.00	**39.71**	2.96

Table 7.4: Showing triplicate readings for the density of the respective milled biomasses

Biomass	1st reading @ 25°C (g/cm^3)	2nd reading @ 25°C (g/cm^3)	3rd reading @ 25°C (g/cm^3)	Mean density (g/cm^3)	Standard deviation
Moringa	0.606	0.603	0.604	**0.604**	0.002
P.K	0.570	0.573	0.574	**0.572**	0.002
Thevetia	0.750	0.750	0.749	**0.750**	0.001
Spirogyra	0.642	0.641	0.641	**0.641**	0.001

Table 7.5: Showing duplicate readings for Elemental composition (Proximate readings) of the biomasses

Biomass	T.O.C (%)				T.N (%)				T.P (%)				Ca (%)				Na (%)				S (%)			
	R_1	R_2	$\bar{x}$	S.D	R_1	R_2	$\bar{x}$	S.D	R_1	R_2	$\bar{x}$	S.D	R_1	R_2	$\bar{x}$	S.D	R_1	R_2	$\bar{x}$	S.D	R_1	R_2	$\bar{x}$	S.D
Moringa	60.50	61.20	**60.85**	0.49	0.210	0.200	**0.210**	0.007	0.211	0.211	**0.211**	0.000	0.050	0.050	**0.050**	0.000	0.015	0.016	**0.016**	0.001	0.035	0.041	**0.038**	0.004
P.K	60.87	60.80	**60.84**	0.05	0.091	0.090	**0.091**	0.001	0.117	0.118	**0.118**	0.001	0.045	0.045	**0.045**	0.000	0.016	0.015	**0.016**	0.001	0.044	0.049	**0.047**	0.004
Thevetia	61.00	60.45	**60.73**	0.39	0.147	0.146	**0.147**	0.001	0.046	0.046	**0.046**	0.000	0.040	0.039	**0.040**	0.001	0.017	0.017	**0.017**	0.000	0.085	0.090	**0.088**	0.004
Spirogyra	50.50	51.42	**50.96**	0.65	0.112	0.112	**0.112**	0.000	0.280	0.281	**0.281**	0.001	0.054	0.053	**0.054**	0.001	1.350	1.350	**1.350**	0.000	0.874	0.890	**0.882**	0.011

Key: R_1 = 1st reading
R_2 = 2nd reading
$\bar{x}$ = Mean
S.D = Standard deviation

Appendix 2: Supplementary result of Extracted oil characteristics

Table 7.6: Showing triplicate readings for the pH determination of the respective extracted oils

Oil	1st reading	2nd reading	3rd reading	Mean pH value	Standard deviation
Moringa	6.61@ *26.9^oC*	6.63@ *26.9^oC*	6.64@ *25.8^oC*	**6.63**@ *26.6^oC*	0.02
P.K	6.00@ *24.9 ^oC*	6.02@ *25.1 ^oC*	6.03@ *24.8^oC*	**6.02**@ *24.9 ^oC*	0.02
Thevetia	6.63@ *26.3 ^oC*	6.64@ *26.4 ^oC*	6.64@ *26.3 ^oC*	**6.64**@ *26.3 ^oC*	0.01
Spirogyra	6.69@ *24.9 ^oC*	6.67@ *25.1 ^oC*	6.69@ *25.5 ^oC*	**6.68**@ *25.2 ^oC*	0.01

Table 7.7: Showing triplicate readings for the density of the respective extracted oils

Oil	1st reading @ 25^{o}C (g/cm^3)	2nd reading @ 25^{o}C (g/cm^3)	3rd reading @ 25^{o}C (g/cm^3)	Mean density (g/cm^3)	Standard deviation
Moringa	0.803	0.803	0.802	**0.803**	0.001
P.K	0.879	0.883	0.881	**0.881**	0.002
Thevetia	0.872	0.870	0.871	**0.871**	0.001
Spirogyra	0.532	0.531	0.531	**0.531**	0.001

Table 7.8: Showing the determination of dynamic viscosity of oils

Oil	Density @ 40°C	Kinematic viscosity @ 40°C	Dynamic viscosity @ 40°C
Moringa	0.798	44.50	35.51
P.K	0.877	4.85	4.25
Thevetia	0.868	21.50	18.66
Spirogyra	0.450	4.50	2.03

Table 7.9: Showing the duplicate determination for Kinematic viscosity of oils

Biodiesel	1st reading @ 40°C (mm^2/s)	2nd reading @ 40°C (mm^2/s)	Mean @ 40°C (mm^2/s)	Standard deviation
Moringa	44.51	44.49	**44.50**	0.014
P.K	4.85	4.85	**4.85**	0.000
Thevetia	21.50	21.50	**21.50**	0.000
Spirogyra	4.50	4.50	**4.50**	0.000

Table 7.10: Showing the triplicate readings for the Fatty Acid Profile (FAP) of the extracted oils

Fatty Acid Profile of Oils (Triplicate Readings)																	
Test parameter	**Name**	**Moringa** (%)				**Palm kernel** (%)				**Thevetia** (%)				**Spirogyra** (%)			
		R1	**R2**	**R3**	**Mean ± S.D**	**R1**	**R2**	**R3**	**Mean ± S.D**	**R1**	**R2**	**R3**	**Mean ± S.D**	**R1**	**R2**	**R3**	**Mean ± S.D**
C8:0	Caprylic	0.03	0.03	0.05	0.04±0.01	3.28	3.27	-	3.28±0.01	-	-	-	-	-	-	-	-
C10:0	Capric	-	-	-	-	3.42	3.41	3.40	3.41±0.01	-	-	-	-	-	-	-	-
C12:0	Lauric	-	-	-	-	47.60	47.59	47.60	47.60±0.01	-	-	-	-	1.00	0.99	0.99	0.99±0.01
C14:0	Myristic	0.15	0.15	0.14	0.15±0.01	16.10	16.13	16.13	16.12±0.17	0.20	0.19	0.19	0.19±0.01	7.50	7.50	7.50	7.50±0.00
C15:0	Pentadecanoic	-	-	-	-	-	-	-	-	-	-	-	-	0.51	0.50	0.50	0.50±0.01
C16:0	Palmitic	6.10	6.11	6.10	6.10±0.01	8.35	8.35	8.35	8.35±0.00	19.51	19.50	19.50	19.50±0.01	25.05	25.05	25.04	25.05±0.01
C16:1	Palmitoleic	1.35	1.36	1.35	1.35±0.01	0.30	0.32	0.31	0.31±0.01	0.25	0.26	0.25	0.25±0.01	8.50	8.51	8.50	8.50±0.01
C17:0	Margaric	0.04	-	0.05	0.05±0.01	-	-	-	-	0.10	0.11	0.09	0.10±0.01	0.19	0.21	0.20	0.20±0.01
C18:0	Stearic	5.79	5.80	5.81	5.80±0.01	2.49	2.49	2.50	2.49±0.01	6.39	6.39	6.40	6.39±0.01	4.50	4.50	4.50	4.50±0.00
C18:1	Oleic	71.52	70.50	71.56	71.20±0.60	15.51	15.50	15.50	15.50±0.01	42.25	42.24	42.25	42.25±0.01	33.50	33.40	33.50	33.47±0.06
C18:1-9c, 12 (OH)	Ricinoleic	-	-	-	-	-	-	-	-	0.04	0.05	-	0.05±0.01	-	-	-	-
C18:2	Linoleic	-	0.69	0.69	0.69±0.00	2.10	-	2.10	2.10±0.00	10.50	10.50	10.50	10.50±0.00	10.81	10.80	10.81	10.80±0.01
C18:3	Linolenic	2.98	3.01	2.98	2.99±0.02	0.16	0.15	0.15	0.15±0.01	0.51	0.50	0.50	0.50±0.01	0.50	-	0.49	0.50±0.01
C18:3-9c,11t,	α-Eleostearic	-	-	-	-	-	-	-	-	0.01	0.02	0.01	0.01±0.01	-	-	-	-
C20:0	Arachidic	3.61	3.60	3.60	3.60±0.01	0.21	0.20	0.20	0.20±0.01	1.25	1.25	1.25	1.25±0.00	1.21	1.20	1.20	1.20±0.01
C20:1	Gadoleic	2.01	2.01	1.99	2.03±0.06	0.05	0.05	0.04	0.05±0.01	0.14	0.13	0.13	0.13±0.01	0.50	0.50	0.49	0.50±0.01
C20:1-11c,14(OH)	Lesquerolic	-	-	-	-	-	-	-	-	-	-	-	-	-	0.01	0.02	0.02±0.01
C20:5	Timnodonic	-	-	-	-	-	-	-	-	-	-	-	-	0.05	0.04	0.05	0.05±0.01
C22:0	Behenic	4.56	4.58	4.57	4.57±0.01	0.10	-	0.10	0.10±0.00	0.82	0.81	0.82	0.82±0.01	1.50	1.49	1.50	1.50±0.01
C22:1	Erucic	-	-	-	-	-	-	-	-	-	-	-	-	0.40	0.39	0.39	0.39±0.01
C24:0	Lignoceric	0.50	0.51	0.50	0.50±0.01	-	-	-	-	1.15	-	1.15	1.15±0.00	-	-	-	-
C24:1	Nervonic	-	-	-	-	-	-	-	-	-	-	-	-	0.86	0.85	0.85	0.85±0.01
Unknown	**=**	**1.36**	**1.65**	**0.61**	**1.21**	**0.84**	**2.92**	**4.10**	**2.62**	**16.88**	**18.05**	**16.96**	**17.30**	**3.42**	**4.91**	**3.47**	**3.93**
Total known	**=**	**98.64**	**98.35**	**99.39**	**98.79**	**99.16**	**97.08**	**95.90**	**97.38**	**83.12**	**81.95**	**83.04**	**82.70**	**96.58**	**95.09**	**96.53**	**96.07**
Total saturated	**=**	**20.78**	**20.78**	**20.82**	**20.79**	**81.12**	**81.06**	**77.80**	**79.99**	**29.42**	**28.25**	**29.40**	**29.02**	**41.01**	**40.59**	**41.43**	**41.01**
Total unsaturated	**=**	**77.86**	**77.57**	**78.57**	**78.00**	**18.12**	**16.02**	**18.10**	**17.41**	**53.70**	**53.70**	**53.64**	**53.68**	**55.57**	**54.50**	**55.10**	**55.06**

R1 = 1st Fatty acid value reading from GC analysis (expressed in percentage)
R2 = 2nd Fatty acid value reading from GC analysis (expressed in percentage)
R3 = 3rd Fatty acid value reading from GC analysis (expressed in percentage)

Appendix 3: Supplementary result of Biodiesel characteristics

Table 7.11: Showing duplicate readings for the pH determination of the respective biodiesels

Biodiesel	Parameter	1st reading	2nd reading	Mean pH value	Standard deviation
Moringa	**M-only transesterification**	7.01@ *25.4°C*	7.08@ *25.7°C*	***7.05***@ *25.6°C*	0.050
	M/E (1:1) transesterification	7.10@ *26.1°C*	7.24@ *24.9°C*	**7.17**@ *25.5°C*	0.099
P.K	**M-only transesterification**	7.20@ *25.7°C*	7.29@ *25.3°C*	**7.25**@ *25.5°C*	0.064
	M/E (1:1) transesterification	7.18@ *24.6°C*	7.20@ *25.9°C*	**7.19**@ *25.3°C*	0.014
Thevetia	**M-only transesterification**	7.33@ *26.3°C*	7.35@ *25.4°C*	**7.34**@ *25.8°C*	0.014
	M/E (1:1) transesterification	7.23@ *25.5°C*	7.31@ *25.6°C*	**7.27**@ *25.6°C*	0.057
Spirogyra	**M-only transesterification**	7.09@ *24.9°C*	7.07@ *25.1°C*	**7.08**@ *25.2°C*	0.014
	M/E (1:1) transesterification	7.12@ *25.7°C*	7.07@ *25.3°C*	**7.10**@ *25.5°C*	0.035

Table 7.12: Showing triplicate determination of density for the respective biodiesels

Biodiesel	Parameter	1st reading @ 25°C (g/cm^3)	2nd reading @ 25°C (g/cm^3)	3rd reading @ 25°C (g/cm^3)	Mean density (g/cm^3)	Standard deviation
Moringa	**M-only transesterification**	0.875	0.877	0.879	**0.877**	0.002
	M/E (1:1) transesterification	0.876	0.880	0.877	**0.878**	0.002
P.K	**M-only transesterification**	0.912	0.914	0.912	**0.913**	0.001
	M/E (1:1) transesterification	0.899	0.902	0.897	**0.899**	0.003
Thevetia	**M-only transesterification**	0.838	0.839	0.839	**0.839**	0.001
	M/E (1:1) transesterification	0.842	0.841	0.843	**0.842**	0.001
Spirogyra	**M-only transesterification**	0.880	0.882	0.882	**0.881**	0.001
	M/E (1:1) transesterification	0.884	0.884	0.886	**0.885**	0.022

Table 7.13: Showing duplicate determinations for the elemental composition (Proximate analysis) of the biodiesels

Biodiesel	T.P (%)				Ca (%)				Na (%)				S (%)			
	R_1	R_2	$\bar{x}$	S.D	R_1	R_2	$\bar{x}$	S.D	R_1	R_2	$\bar{x}$	S.D	R_1	R_2	$\bar{x}$	S.D
Moringa	0.020	0.020	**0.020**	0.000	0.004	0.006	**0.005**	0.001	0.002	0.002	**0.002**	0.000	0.034	0.036	**0.035**	0.001
P.K	0.002	0.002	**0.002**	0.000	0.004	0.004	**0.004**	0.000	0.001	0.001	**0.001**	0.000	0.001	0.003	**0.002**	0.001
Thevetia	0.001	0.001	**0.001**	0.000	0.002	0.004	**0.003**	0.001	0.001	0.003	**0.002**	0.001	0.008	0.008	**0.008**	0.000
Spirogyra	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 7.14: Showing the duplicate determination of Flash point for the biodiesels

Biodiesel	1st reading (^oC)	2nd reading (^oC)	Mean (^oC)	Standard deviation
Moringa	175	177	**176**	1.41
P.K	164	168	**166**	2.83
Thevetia	129	131	**130**	1.41
Spirogyra	-	-	-	-

Table 7.15: Showing duplicate readings for the Cloud and Pour points of the biodiesels respectively

Biodiesel	Cloud point (^{o}C)				Pour point (^{o}C)			
	1st reading	2nd reading	Mean	S.D	1st reading	2nd reading	Mean	S.D
Moringa	13.5	13.7	**13.6**	0.1	6.5	6.5	**6.5**	0.0
P.K	14.0	14.2	**14.1**	0.1	8.6	8.5	**8.6**	0.1
Thevetia	8.5	8.4	**8.5**	0.1	5.0	5.1	**5.1**	0.1
Spirogyra	-	-	-		-	-	-	-

Table 7.16: Showing the duplicate determination of acid number for the biodiesels

Biodiesel	1st reading (mgKOH/g)	2nd reading (mgKOH/g)	Mean (mgKOH/g)	Standard deviation
Moringa	0.603	0.711	**0.657**	0.076
P.K	0.410	0.423	**0.417**	0.009
Thevetia	0.440	0.442	**0.441**	0.001
Spirogyra	-	-	-	-

Table 7.17: Showing the determination of dynamic viscosity of the biodiesels

Biodiesel	Density @ 40°C	Kinematic viscosity @ 40°C	Dynamic viscosity @ 40°C
Moringa	0.689	5.02	3.46
Palm kernel	0.772	2.39	1.85
Thevetia	0.760	4.70	3.57
Spirogyra	-	-	-

Table 7.18: Showing the duplicate determination of Kinematic viscosity for the biodiesels

Biodiesel	1st reading @ 40°C (mm^2/s)	2nd reading @ 40°C (mm^2/s)	Mean @ 40°C (mm^2/s)	Standard deviation
Moringa	5.01	5.03	**5.02**	0.01
Palm kernel (P.K)	2.38	2.40	**2.39**	0.01
Thevetia	4.70	4.70	**4.70**	0.00
Spirogyra	-	-	-	-

Table 7.19: Comparison of the Relative densities of test parameters using ANOVA with Least Significance Difference (LSD)

Test Group		Mean ± S.D	Test Parameter	ANOVA (F-value)	p-value
MORINGA	RD Biomass	0.60 ± 0.00	RD Oil	18140.48	0.00
			RD M-only BD		0.00
			RD M/E BD		0.00
	RD Oil	0.80 ± 0.00	RD Biomass		0.00
			RD M-only BD		0.00
			RD M/E BD		0.00
	RD M-only BD	0.88 ± 0.00	RD Biomass		0.00
			RD Oil		0.00
			RD M/E BD		*0.64
	RD M/E BD	0.88 ± 0.00	RD Biomass		0.00
			RD Oil		0.00
			RD M-only BD		*0.64
P.K	RD Biomass	0.57 ± 0.00	RD Oil	19971.72	0.00
			RD M-only BD		0.00
			RD M/E BD		0.00
	RD Oil	0.88 ± 0.00	RD Biomass		0.00
			RD M-only BD		0.00
			RD M/E BD		0.00
	RD M-only BD	0.91 ± 0.00	RD Biomass		0.00
			RD Oil		0.00
			RD M/E BD		0.00
	RD M/E BD	0.90 ± 0.00	RD Biomass		0.00
			RD Oil		0.00

			RD M-only BD		0.00
THEVETIA	RD Biomass	0.75 ± 0.00	RD Oil	12399.67	0.00
			RD M-only BD		0.00
			RD M/E BD		0.00
	RD Oil	0.87 ± 0.00	RD Biomass		0.00
			RD M-only BD		0.00
			RD M/E BD		0.00
	RD M-only BD	0.84 ± 0.00	RD Biomass		0.00
			RD Oil		0.00
			RD M/E BD		0.00
	RD M/E BD	0.84 ± 0.00	RD Biomass		0.00
			RD Oil		0.00
			RD M-only BD		0.00
SPIROGYRA	RD Biomass	0.64 ± 0.00	RD Oil	112880.00	0.00
			RD M-only BD		0.00
			RD M/E BD		0.00
	RD Oil	0.53 ± 0.00	RD Biomass		0.00
			RD M-only BD		0.00
			RD M/E BD		0.00
	RD M-only BD	0.88 ± 0.00	RD Biomass		0.00
			RD Oil		0.00
			RD M/E BD		0.00
	RD M/E BD	0.89 ± 0.00	RD Biomass		0.00
			RD Oil		0.00
			RD M-only BD		0.00

* $p > 0.05$ is not significant