Biodiesel production from plant oils of African origin

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ABSTRACT

As the search for alternative sources of energy to supplement traditional fossil-derived energy has intensified across the globe, biodiesel derived from biolipids has emerged as a promising alternative to diesel fuel. This study investigated the feasibility of biodiesel production from Adansonia digitata L. (baobab), Calodendrum capense (L.f.) Thunb, and Moringa oleifera Lam. plant seed oils of African origin. Biodiesel was synthesized from the three plant seed oils via transesterification process catalyzed by biocatalysts known as lipases. *Candida antarctica lipase-B*, *Porcine pancreas*, *Candida sp.*, *Candida rugosa* and *Pseudomonas fluorescens* lipases were screened to identify the ideal biocatalyst for methanolysis of baobab seed oil. The effects of varying reaction conditions including oil to methanol molar ratio, temperature, and time on transesterification of baobab seed oil were also assessed to establish optimum operating conditions. Once the optimal operating conditions for transesterification of baobab seed oil had been determined, the same operating conditions were employed in methanolysis of calodendrum capense and moringa oils.

Results for lipase screening showed that the highest conversion of baobab seed oil to biodiesel can be achieved when *Candida antarctica lipase-B* is employed as catalyst. Methanolysis of baobab seed oil catalysed by *C. rugosa*, *P. pancreas*, *P. fluorescence*, *Candida sp.* and C. antarctica lipase-B lipases respectively yielded 0.28±0.73, 0.43±0.73, 0.84±0.73, 0.98±0.73% and 87.3±0.73% conversions at operating conditions of 10 wt% catalyst loading (based on mass of baobab oil), 1:3 oil to methanol molar ratio, 40°C, and 6 hours reaction time. Studies on the effects of oil to methanol molar ratio, temperature, and reaction time on methanolysis of baobab seed oil catalysed by 10 wt% Candida antarctica lipase-B revealed that the optimum operating conditions for this reaction system are 1:3 oil to methanol molar ratio, 50°C, and 6 hours reaction time. Under these conditions, 91.8±2.6% of baobab seed oil was converted to biodiesel. Methanolysis of moringa and calodendrum capense seed oils at the same operating conditions yielded 80.4±2.6% and 89.6±2.6% biodiesel yields respectively. Hence, biodiesel production from baobab, moringa, and calodendrum capense plant seed oils via biocatalytic transesterification is highly feasible.

Keywords: Biodiesel, baobab seed oil, calodendrum capense seed oil, moringa seed oil, transesterification, lipase.

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“Yet, not I but the grace of God within me.” 1 Corinthians 15: 10

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NOMENCLATURE

Abbreviation	**Description**
IEA	International Energy Agency
BP	British Petroleum
AfDB	African Development Bank
CO	Carbon monoxide
CO_2	Carbon dioxide
NO_x	Nitrogen oxide
SO_X	Sulphur oxide
H_2	Hydrogen gas
He	Helium
$NaOCH_3$	Sodium methoxide
NaOH	Sodium hydroxide
KOH	Potassium hydroxide
CH_3OH or MeOH	Methanol
H_2SO_4	Sulphuric acid
HCl	Hydrochloric acid
CaO	Calcium oxide
MgO	Magnesium oxide
SrO	Strontium oxide
TG	Triglyceride
FAAE	Fatty Acid Alkyl Ester
FAME	Fatty Acid Methyl Ester
FFA	Free Fatty Acid
%	Percent
Wt%	Weight percent
M	Metre
°C	Degrees Celsius
H	Hour
Min	Minute
Vol.	Volume
Ml	Millilitre
µl	Microliter
G	Gram
mol.	Mole

Mmol	Millimole
MW	Molecular weight
Rpm	Revolutions per minute
SANS	South African National Standard
TMSH	Trimethylsulphonium hydroxide
$CDCl_3$	Deuterated chloroform
GC	Gas chromatograph
^{1}HNMR	Proton Nuclear Magnetic Resonance Spectroscopy

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CHAPTER 1. GENERAL INTRODUCTION

1.1 Introduction

In this chapter, a general overview of the current study is presented. Section 1.2 provides the background and motivation behind this study. The aim and objectives are presented in section 1.3, and the scope of the dissertation is given in section 1.4.

1.2 Background and motivation

About 81% of the energy used globally is generated from fossil fuels including oil, natural gas, and coal (IEA, 2017). Global consumption of fossil fuels has on average continuously been increasing since 2006 as can be seen in Figure 1-1. Increasing human population, urbanization, and modernization are some of the factors contributing to the growing energy demand (Asif & Muneer, 2007). The current rate of fossil fuel consumption is raising concerns over long-term global energy security since fossil fuels are finite resources. Estimates show that fossil fuel reserves are at risk of getting depleted in the next 50 years (Vohra *et al.*, 2014; Zabed *et al.*, 2017). Heavy usage of fossil fuels is also raising some environmental concerns since production and use of fossil fuels generate toxic gases such as carbon monoxide (CO), carbon dioxide (CO_2), nitrogen oxides (NO_x) and sulphur oxides (SO_X) (Aransiola *et al.*, 2012).

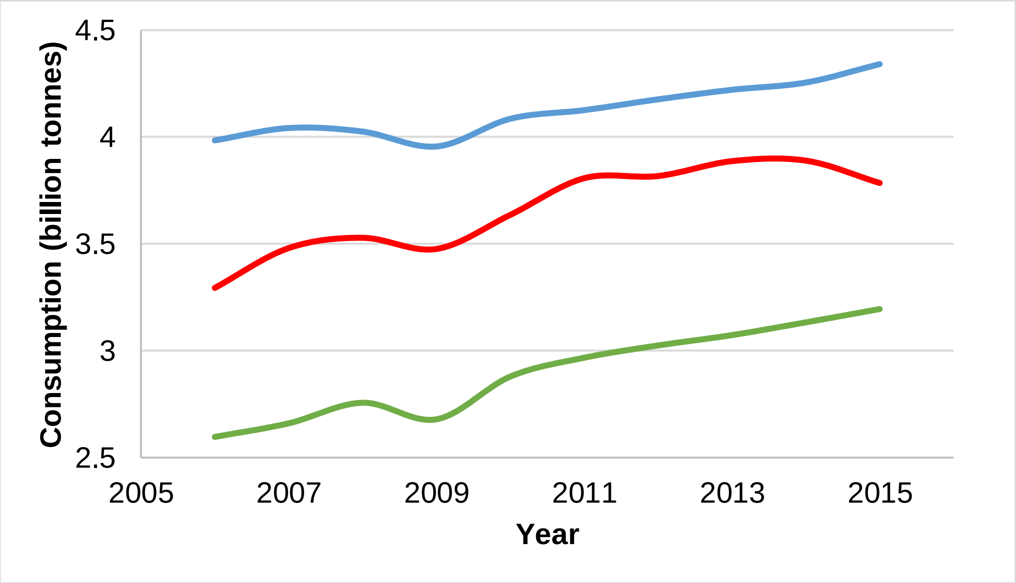

Figure 1-1: Global consumption of natural gas (—), coal (—) and oil (—) between 2006 and 2015 (BP, 2017)

In efforts to reduce overexploitation of fossil fuels and combat environmental pollution, the search for alternative sustainable and clean energy sources has intensified across the globe. Biomass is among the promising alternative sources of energy discovered so far. Biomass resources can be used as raw materials for producing various renewable fuels commonly known as biofuels.

Biofuels have potential to replace or supplement the traditional fossil-based fuels such as diesel and petrol. Biomass is considered ideal for energy production because it is renewable and it is found in abundance (Yang *et al.*, 2016). Furthermore, substitution of fossil energy with biomass energy has potential to reduce emissions of some toxic gases including CO_2 (Petrou & Pappis, 2009).

Just like the rest of the world, various countries in Africa are showing interest in the development of biofuels. Biodiesel is among the major biofuels receiving increasing attention on the continent. Biodiesel can be used in place of fossil diesel and as such, its utilization has potential to reduce depletion of fossil fuel reserves and assist in mitigating some of the environmental issues arising from fossil energy use. Raw materials for biodiesel preparation include biomass-derived lipids such as plant oils and animal fats (Karmee, 2016). Currently, Jatropha is the most dominant energy crop being promoted for biodiesel production in Africa (Von Maltitz *et al.*, 2009; Walimwipi *et al.*, 2012; Yang *et al.*, 2014). Other potential feedstocks being considered include palm, castor, coconut, sunflower and soybean oils (Von Maltitz *et al.*, 2009; Walimwipi *et al.*, 2012).

In order for the biodiesel sector in Africa to be sustainable, it is imperative that more feedstock sources are identified. Diversification of feedstock is crucial to avoid overexploitation of certain biomass resources. Africa has a vast distribution of oil-bearing plants which are currently underutilized and hence could serve as raw materials for biodiesel preparation. However, for most of the available plant seed oils, limited research has been conducted regarding their application as raw materials for biodiesel production. As such, extensive research is needed in this area of study. Along this line, this study focuses on biodiesel production from Adansonia digitata (A. digitata or baobab) plant seed oil.

The baobab tree (family Malvaceae) grows naturally in Africa and is widely distributed across the continent particularly in the sub-Saharan region. In Southern Africa alone, baobab trees cover about 93 000 km^2 (Modiba *et al.*, 2014). A. digitata produces fruits containing oil-rich seeds. Baobab seed oil was selected for this study because currently, the oil has minimal commercial application. The oil is mainly used in traditional medicine (Sidibe *et al.*, 2002; Kamatou *et al.*, 2011), although its application as a raw material for the cosmetics industry is also reported in the literature (Komane *et al.*, 2017). Baobab seed oil is also ideal for this study because its application as feedstock for biodiesel synthesis has not been extensively researched.

Biodiesel is mainly produced via transesterification. Transesterification involves the reaction of oil with an alcohol. The reaction can be conducted in the presence of a catalyst. Biodiesel production from A. digitata seed oil via transesterification method has previously been reported by Modiba *et al.* (2014). Modiba and co-authors catalysed methanolysis of baobab seed oil with sodium methoxide ($NaOCH_3$). The use of chemical catalysts is however not only energy intensive and time-consuming but also generates large volumes of wastewater (Sarin, 2012). Furthermore, biodiesel synthesis catalysed by homogeneous alkaline catalysts such as sodium methoxide is often hampered by side reactions which affect product yield and purity (Rathore *et al.*, 2016).

In this study, biocatalysts known as lipases were used for biodiesel preparation. Biocatalytic transesterification is considered environmentally benign because not only are lipases biodegradable, but the process generates no wastewater (Gorji & Ghanei, 2014; Yan *et al.*, 2014). A low energy requirement also makes the lipase-catalysed transesterification process attractive (Gog *et al.*, 2012). Besides, biodiesel produced via the biocatalytic route is of high quality because side reactions are not encountered (Vyas *et al.*, 2010; Guldhe *et al.*, 2015).

1.3 Aim and objectives

The aim of this research was to study the feasibility of lipase-catalysed biodiesel production from plant oils of African origin.

The research aim was achieved through the following objectives:

- Assess the catalytic activity of lipases from various sources during transesterification of baobab seed oil.
- Optimize process parameters for lipase-catalysed methanolysis of baobab seed oil, viz., oil to methanol molar ratio, temperature, and reaction time.
- Determine conversion of baobab, calodendrum capense and moringa plant seed oils to biodiesel.

1.4 Scope of dissertation

This dissertation is organized as follows:

Chapter 1: General introduction

Provides a general introduction to biomass energy, biofuels, and biodiesel. The chapter also provides the background and motivation behind this study.

Chapter 2: Literature review

Provides literature on A. digitata plant, biodiesel, and its production methods, as well as the operating conditions affecting production efficiency during biodiesel synthesis.

Chapter 3: Experimental

Provides details of the experimental procedure and analytical techniques followed in this study.

Chapter 4: Results and discussion

In this chapter, results on lipase screening and effects of oil to methanol molar ratio, reaction temperature and time on lipase-catalysed methanolysis of baobab seed oil are presented and discussed. Results on biodiesel production from calodendrum capense and moringa plant seed oils are also discussed.

Chapter 5: Conclusion and recommendations

This chapter gives the main conclusion of this study. Several recommendations are also given.

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CHAPTER 2. LITERATURE REVIEW

2.1 Introduction

In general, this chapter presents available literature on biodiesel and its preparation. The chapter begins with a brief discussion on the energy situation in Africa (section 2.2), followed by a description of biofuels in section 2.3. A description of biodiesel and its properties is given in section 2.4, while section 2.5 presents the feedstock commonly used for biodiesel preparation. A description of A. digitata is also given in this section. Biodiesel production methods and transesterification process are respectively discussed in sections 2.6 and 2.7, followed by a discussion on reaction parameters that affect lipase-catalysed transesterification process in section 2.8. Lastly, a brief discussion on biodiesel fuel quality is given in section 2.9.

2.2 Energy overview in Africa

Oil, coal, natural gas, biomass, solar, geothermal, wind and hydropower constitute some of the energy sources available in Africa (AfDB, 2012; Mohammed *et al.*, 2013). Among these, traditional biomass (i.e. unprocessed biomass such as firewood and crop residues) serves as the major source of energy. As of 2014, biomass accounted for about 48% of the total primary energy supplied on the continent while shares of oil, coal and natural gas were 21%, 15% and 14% respectively (IEA, 2016b) (Figure 2-1). Hydropower resources also supply a substantial amount of energy. Exploitation of non-traditional sources of energy such as solar, wind and geothermal is however currently limited in Africa.

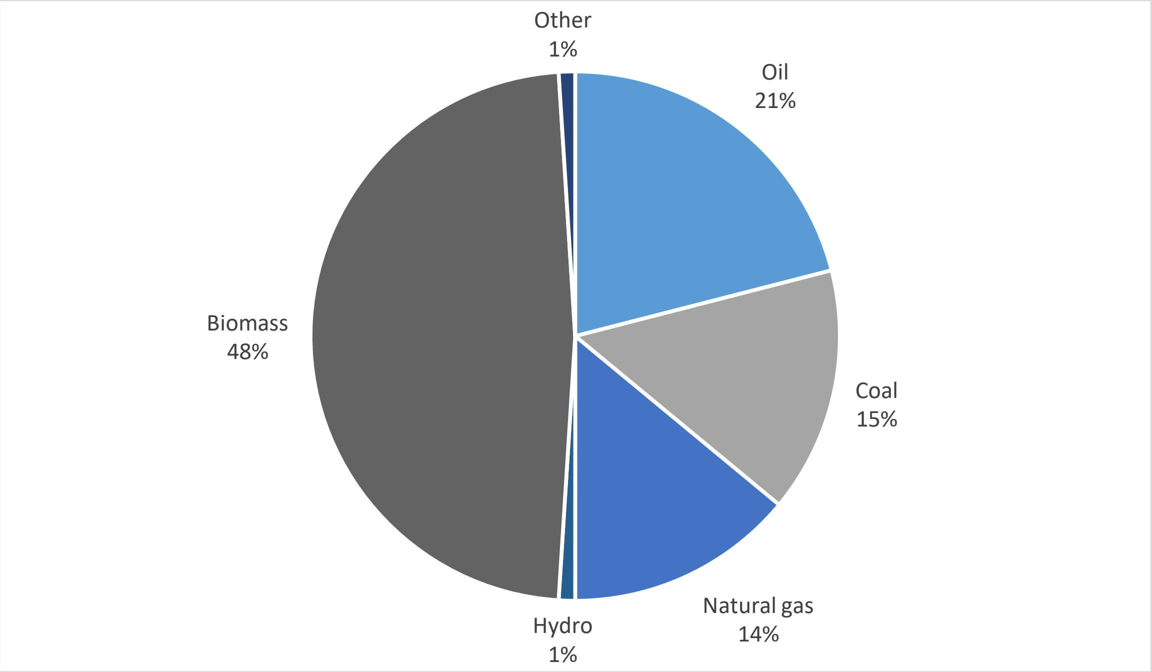

Figure 2-1: Shares of total primary energy supply by fuel in Africa (2014) (IEA, 2016b)

Energy insecurity is one of the major issues in Africa. As of 2014, about 69% of the total African population relied on traditional biomass for domestic energy needs such as cooking and heating (IEA, 2016a). Uneven distribution of fossil fuel reserves is one of the contributing factors to the energy insecurity in Africa (Yang, 2014). Africa accounts for 7.6%, 7.5% and 3.6% of the total world proven reserves of oil, natural gas, and coal respectively (BP, 2016). Of the total oil reserves available on the continent, about 45% are located in North Africa, 32% in West Africa and the remaining 23% are distributed across Southern Africa, East Africa and Central Africa (Table 2-1) (AfDB, 2012).

Table 2-1: Distribution of oil and natural gas reserves in Africa (AfDB, 2012)

Region	Oil (%)	Gas (%)
North Africa	45%	51%
Southern Africa	12	11
East Africa	4	1
West Africa	32	35
Central Africa	7	2

As can be seen in Table 2-1, North and West Africa regions also account for the majority of Africa's natural gas reserves, with 51% located in North Africa and 35% in West Africa. Furthermore, about 95% of the African coal reserves are located in South Africa alone (AfDB, 2012). Due to the irregular distribution of fossil fuel reserves, more than 70% of the countries in Africa are net importers of energy (Amigun *et al.*, 2011). Dependence on foreign energy affects local availability and affordability of energy sources.

2.3 Biofuels

Any solid, liquid or gaseous fuel derived from biomass is commonly known as a biofuel (Demirbaş, 2001; Nigam & Singh, 2011). Some examples of biofuels include biodiesel, bioethanol, and biogas. Biofuels are increasingly becoming popular across the globe mainly due to the environmental benefits which they present (Demirbas, 2009). Biofuels are considered eco-friendly because they are biodegradable and renewable (Balat, 2007a; Demirbas, 2009). Biofuels are also found attractive because their utilization has the potential to reduce dependence on imported fuels thereby enhancing national energy security (Amigun *et al.*, 2008; Gasparatos *et al.*, 2015). In developing countries, development of the biofuels industry is perceived as a catalyst for poverty alleviation since it creates job opportunities (Von Malititz & Brent, 2008; Amigun *et al.*, 2011).

2.4 Biodiesel

Biodiesel is one of the important liquid biofuels to have been discovered so far. As it can be seen in *Table 2-2*, some of the physical and chemical properties of biodiesel and fossil diesel are similar. For this reason, biodiesel is widely recognized as the most feasible substitute for petroleum diesel (Atadashi *et al.*, 2010).

Table 2-2: Physical properties of biodiesel and standard diesel fuel (Demirbas, 2003)

Property	**Biodiesel**	**Standard diesel fuel**
Specific gravity, kg/L	0.87 to 0.89	0.84 to 0.86
Cetane number	46 to 70	47 to 55
Cloud point, K	262 to 289	256 to 265
Pour point, K	258 to 286	237 to 243
Flash point, K	408 to 423	325 to 350
Sulphur, wt%	0.0000 to 0.0024	0.04 to 0.01
Ash, wt%	0.002 to 0.01	0.06 to 0.01
Iodine number	60 to 135	-
Kinematic viscosity, 313 K	3.7 to 5.8	1.9 to 3.8
Higher heating value, MJ/kg	39.3 to 39.8	45.3 to 46.7

Due to the similarities in fuel properties, biodiesel can be used to power the conventional diesel engine without a requirement for significant adjustments in the engine (Balat, 2007a; Escobar *et al.*, 2009). Biodiesel is commonly used as a transport fuel and to generate heat and electricity (Mushrush *et al.*, 2001; Amigun *et al.*, 2011).

2.4.1 Advantages of biodiesel

Biodiesel is advantageous because it can be produced locally from renewable resources (Knothe, 2009). Biodiesel fuel is also environmentally favorable due to its biodegradable nature (Yusuf *et al.*, 2011). In addition, biodiesel has reduced emission profiles of particulate matter, unburned hydrocarbons, CO and SO_2 (Al-Zuhair, 2007; Marchetti, 2010). According to Helwani *et al.* (2009), emissions of CO, particulate matter and unburned hydrocarbons can be reduced by 46.7%, 66.7%, and 45.2% respectively when biodiesel is used in place of fossil diesel. Biodiesel is also non-flammable and hence easier to handle, transport and store compared to fossil diesel (Balat, 2006).

2.4.2 Disadvantages of biodiesel

Even though biodiesel is considered as a clean fuel, it has higher NO_x emissions compared to fossil diesel (EPA, 2002; Dincer, 2008). Biodiesel also has a higher viscosity compared to petroleum diesel, which causes difficulties in fuel pumping (Mahmudul *et al.*, 2017). For this reason, a higher fuel consumption is observed when biodiesel is used as fuel (Demirbas, 2007; Yusuf *et al.*, 2011). Biodiesel also has a poor cold-flow property and as such the fuel tends to crystallize or thicken at low temperatures, causing operating problems such as filter and tube plugging (Kerschbaum *et al.*, 2008; Boshui *et al.*, 2010). Biodiesel is also highly susceptible to oxidative degradation, which affects engine performance (Pullen & Saeed, 2012). Furthermore, it has been widely reported that lower engine speed and power, excessive engine wear and higher copper and brass corrosion are experienced when biodiesel is used as a fuel (Demirbas, 2007; Mahmudul *et al.*, 2017).

2.5 Feedstock for biodiesel production

Chemically, biodiesel consists of a mixture of fatty acid alkyl esters (FAAEs) which are synthesized from biolipids (Knothe, 2005). The majority (>90%) of biolipids used to produce biodiesel also form part of the human diet. (Gui *et al.*, 2008). Agricultural crops such as rapeseed, sunflower, and soybean constitute some of the major sources of feedstock used for biodiesel preparation. This presents a major challenge in the commercialization of biodiesel because the diversion of food material for fuel production is considered unethical. Utilization of food grade materials in the biodiesel industry has potential to induce food insecurity and increments in food prices (Lam *et al.*, 2009; Caetano *et al.*, 2014). Furthermore, the high cost of edible vegetable oils escalates biodiesel production costs, making biodiesel less economically competitive against fossil-derived diesel fuel (Demirbas, 2008).

Substitution of edible oils with low-value lipids is considered as one of the solutions to the challenges mentioned above. Along this line, inedible plant oils from plants such as Jatropha curcas, Pongamia pinnata, and Azadirachta indica are being exploited for biodiesel production (Sawangkeaw & Ngamprasertsith, 2013; Karmee, 2015). Other promising feedstocks being considered include waste frying oil, grease, animal fats and microbial oils (Phan & Phan, 2008; Encinar *et al.*, 2011; Marx & Venter, 2014). In Africa, there is a variety of non-agricultural plant seed oils which have insignificant commercial application and hence can serve as raw materials for biodiesel preparation. Examples of marginalized oil-bearing plants available in Africa are presented in Table 2-3.

Table 2-3: Oil-bearing plants of African origin

Species name	Common name	Distribution	Traditional uses	Reference
Adansonia digitata L.	Baobab	Angola, Cameroon, Sudan, Zimbabwe, Malawi, South Africa, Namibia	Source of food and folk medicine, seed oil used as raw material for cosmetics	Sidibe *et al.* (2002); Nkafamiya *et al.* (2007); Donkor *et al.* (2014); Komane *et al.* (2017)
Calodendrum capense (L.f.) Thumb.	Cape chestnut	South Africa, Kenya, Lesotho, Swaziland, Botswana, Uganda, Swaziland, Tanzania	Leaves used as insecticide, seed oil used for making soap	Orwa *et al.* (2009); Wagutu *et al.* (2009); Lall and Kishore (2014)
Ceiba pentandra (L.) Gaertn	Kapok	Ethiopia, Gambia, Ghana, Kenya, South Africa, Tanzania, Uganda	Wood used for paper production, fruit fiber used as material for filling mattresses, e.tc, seed oil used for soap manufacturing and as lubricant	Sivakumar *et al.* (2013) Ong *et al.* (2013) Orwa *et al.* (2009)
Moringa oleifera Lam.	Moringa	Ghana, Kenya, Tanzania, Ethiopia	Source of food and traditional medicine, seed oil used as lubricant and ingredient in cosmetics and perfumes	Lim (2012); Yang *et al.* (2014) (Orwa *et al.*, 2009)

2.5.1 Botany and morphological description of A. digitata

A. digitata trees belong to the genus Adansonia of the Malvaceae family (Venter & Witkowski, 2011; Rahul *et al.*, 2015). Common English names for A. digitata include baobab, monkey bread tree and upside-down tree (Sidibe *et al.*, 2002). As can be seen in Figure 2-2, A. digitata is a tree that stands out in nature due to its massive size of 23 m or more in height (Chadare *et al.*, 2008; Kamatou *et al.*, 2011). The tree is distinguishable by its short, swollen trunk (10m diameter) which may be conical, cylindrical or bottle shaped depending on the maturity of the tree (Gebauer *et al.*, 2002; Yusha'u *et al.*, 2010; Sharma & Jain, 2015). A. digitata has thick, short branches which are irregularly distributed either along the trunk or concentrated at the apex (Sidibe *et al.*, 2002). Baobab leaves are 2-3-foliate when young, and 5-7(-9) foliate when mature (Sharma & Jain, 2015). Baobab trees are however deciduous, hence the leaves are only available for 3 to 4 months per year (Gebauer *et al.*, 2002; Kamatou *et al.*, 2011).

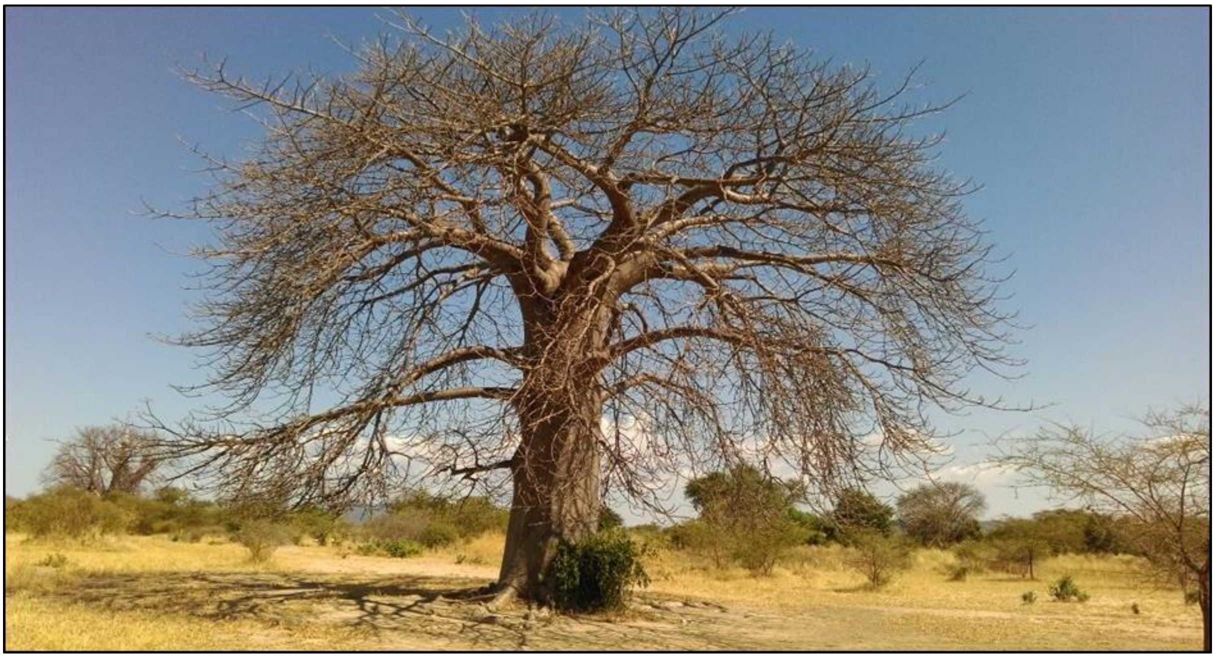

Figure 2-2: Baobab tree (Figure courtesy: Madalitso Mwenemurupa)

Depending on the method of cultivation, flowering in baobab trees commences when the trees are between 8 to 23 years old (Sidibe *et al.*, 2002; Sacande *et al.*, 2006). Between 5 to 6 months after flowering, fruits start to develop (Sidibe *et al.*, 2002). Mature fruits can be globose or ovoid in shape, 12 cm or more in length, and consist of a yellow-brown hard woody shell which can grow up to 8-10 mm in thickness (Baum, 1995; Sidibe *et al.*, 2002; Sacande *et al.*, 2006). According to Sacande *et al.* (2006), mature trees produce about 200 kg of fruits per annum. The baobab fruit capsule has numerous dark brown seeds embedded in a yellowish-white acidic powder commonly known as fruit pulp. 1 kg of fruit may yield about 1700 to 2500 seeds (Sacande *et al.*, 2006). The seeds can contain about 45% oil (Nkafamiya *et al.*, 2007). Figure 2-3 shows the baobab fruit (before and after it is cracked open), seeds and seed oil of A. digitata.

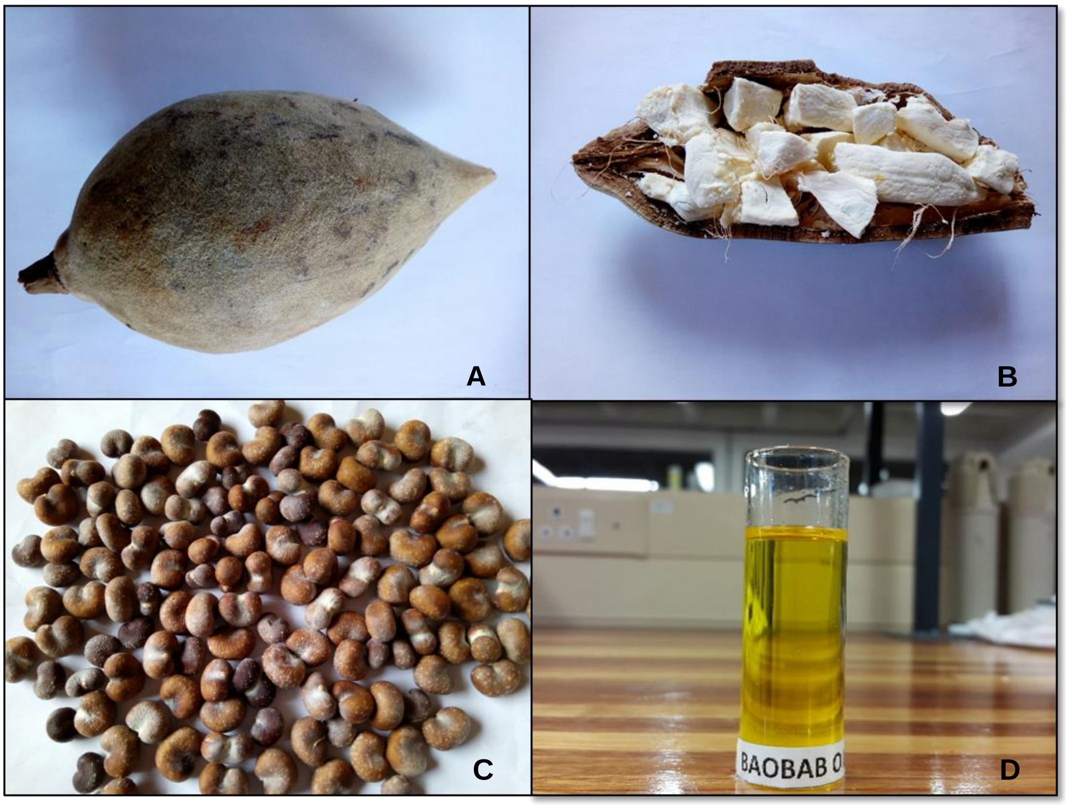

Figure 2-3: (A) Fruit of A. digitata; (B) Baobab fruit cut open, showing seeds embedded in fruit pulp; (C) A. digitata seeds; (D) baobab seed oil

2.5.2 Extraction of baobab seed oil

Traditional methods for extracting baobab seed oil involve pounding or boiling the seeds (Wickens, 1982; Sidibe *et al.*, 2002). Various researchers have used soxhlet extraction method to obtain baobab seed oil. As it can be seen in Table 2-4, the choice of extraction solvent influences the oil yield. Donkor *et al.* (2014) reported that soxhlet extraction with hexane yielded 13 wt% baobab seed oil, whereas extraction with petroleum ether yielded about 29 wt% oil. Chindo *et al.* (2010) and Nkafamiya *et al.* (2007) respectively obtained 33 wt% and 45 wt% baobab seed oil using soxhlet extraction with petroleum ether (Table 2-4).

Table 2-4: Effect of extraction solvent on baobab oil yield

Extraction solvent	Baobab oil yield	Researcher
Hexane	13 wt%	Donkor *et al.* (2014)
Petroleum ether	29 wt%	Donkor *et al.* (2014)
	33 wt%	Chindo *et al.* (2010)
	45 wt%	Nkafamiya *et al.* (2007)

2.5.3 Fatty acid profile of baobab seed oil

The distribution of fatty acids in the starting material largely influences biodiesel fuel properties (Razon, 2009; Lin *et al.*, 2011). As such, the fatty acid profile of the starting material is one of the crucial factors to consider when selecting feedstock for biodiesel production. Generally, palmitic, stearic, oleic, linoleic and linolenic acids constitute the dominant fatty acids in traditional biodiesel feedstock such as sunflower, rapeseed and soybean oils (Table 2-5) (Lin *et al.*, 2011). Baobab seed oil has also been reported to be rich in C16:0, C18:0, C18:1, and C18:2 fatty acids by Modiba *et al.* (2014) (Table 2-5).

Table 2-5: Distribution of fatty acids in sunflower, rapeseed, soybean, and baobab seed oils (Lin et al., 2011; Modiba et al., 2014)

Fatty Acid	Sunflower (wt%)	Rapeseed (wt%)	Soybean (wt%)	Baobab (wt%)
Lauric (C12:0)	0.5	-	-	-
Myristic (C14:0)	0.2	-	0.1	-
Palmitic (C16:0)	4.8	3.49	11	21
Palmitoleic (C16:1)	0.8	-	0.1	-
Stearic (C18:0)	5.7	0.85	4	20.3
Oleic (C18:1)	20.6	64.40	23.4	22.1
Linoleic (C18:2)	66.2	22.30	53.2	27.5
Linolenic (C18:3)	0.8	8.23	7.8	8.8
Arachinid (C20:0)	0.4	-	0.3	0.29

2.6 Methods for biodiesel production

Due to high viscosity, vegetable oils cannot be used directly to power the conventional diesel engine (Vyas *et al.*, 2010). When used as fuel in their natural form, the high viscosity of vegetable oils causes problems such as carbon deposition and injector coking (Ma & Hanna, 1999; Balat, 2007b). Hence, vegetable oils require modification prior to use as a substitute for diesel fuel.

Modification of vegetable oils can be achieved through techniques such as dilution, micro-emulsification, pyrolysis, and transesterification (Vyas *et al.*, 2010; Abbaszaadeh *et al.*, 2012). Dilution of vegetable oils with mineral diesel helps to improve the viscosity of vegetable oils (Mahmudul *et al.*, 2017). Mixing the oil with solvents such as methanol and ethanol to form micro-emulsions also helps to improve the viscosity (Ma & Hanna, 1999; Abbaszaadeh *et al.*, 2012). Thermal decomposition of vegetable oils via pyrolysis also helps to lower the viscosity (Gorji &

Ghanei, 2014). However, transesterification (also known as alcoholysis) is by far the most common technique employed to produce biodiesel (Lin *et al.*, 2011; Mahmudul *et al.*, 2017). Some of the merits and demerits of the 4 techniques mentioned above are listed in Table 2-6.

Table 2-6: Techniques for biodiesel production (Lin et al., 2011)

Technique	Merits	Demerits
Dilution or micro-emulsion	Simplified technique	Viscous product Product has poor volatility and stability
Pyrolysis	Simplified process	High energy requirements Equipment is highly costly Poor product quality
Transesterification	Product has similar properties to fossil diesel High production yields Inexpensive Can be utilized for large-scale production.	Efficiency influenced by feedstock quality (depends on choice of catalyst) Generates wastewater streams Production yield affected by undesirable side reactions Complicated downstream processes

2.7 Transesterification

Modification of vegetable oils via transesterification process involves reacting the oil with an alcohol to produce FAAEs (main product) and glycerol (Ma & Hanna, 1999). Oils consist of triglycerides (TGs) which are composed of fatty acid chains attached to a glycerine molecule (Marchetti, 2010). During transesterification, the TGs are broken down, yielding three alkyl ester molecules and a glycerol molecule. Transesterification reaction proceeds in a series of 3 steps and in each step an ester is synthesized. Figure 2-4 shows an illustration of the transesterification reaction.

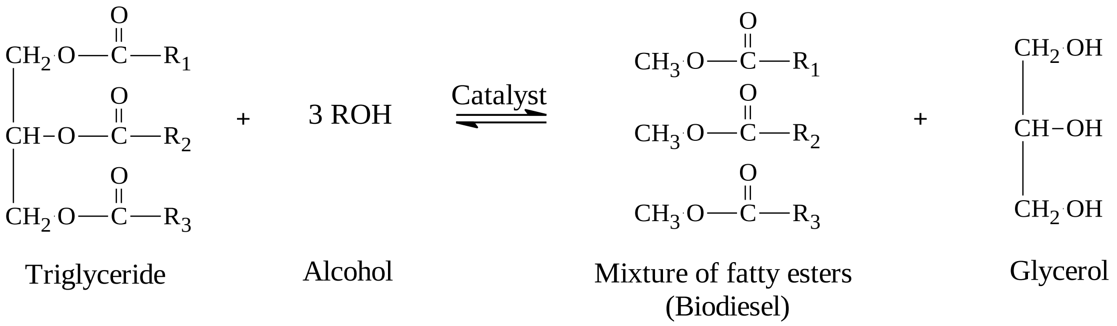

Figure 2-4: Transesterification reaction (Leung et al., 2010)

Oils can be reacted with different types of alcohols to produce biodiesel. Among the various alcohols available for transesterification, methanol is preferred in transesterification because it is highly reactive and cheap (Mittelbach & Remschmidt, 2010). During transesterification, catalysts are usually employed in order to enhance reaction rate and production yield. (Aransiola *et al.*, 2014). Non-catalytic transesterification process has also been developed. However, non-catalytic transesterification process is limited by the requirement for extreme operating conditions and the high cost of equipment (Tan & Lee, 2011). Catalysts used in transesterification reaction can be classified as chemical or biocatalysts.

2.7.1 Chemical catalysts

Conventionally, homogeneous alkaline catalysts are used to catalyse transesterification reaction. Examples of homogeneous alkaline catalysts commonly used in transesterification include sodium hydroxide (NaOH) and potassium hydroxide (KOH) (Atadashi *et al.*, 2012). Homogeneous alkaline catalysts are however selective and perform best when high-quality feedstock i.e. refined oil is used (Atadashi *et al.*, 2013). The catalytic activity of homogeneous alkaline catalysts is negatively affected when the feedstock contains more than 0.5 wt% FFA and 0.06 wt% water. Presence of FFA and water above these concentrations promotes two un-wanted side reactions, viz., saponification and hydrolysis (Figure 2-5) (Ma *et al.*, 1998; Canakci & Van Gerpen, 2001).

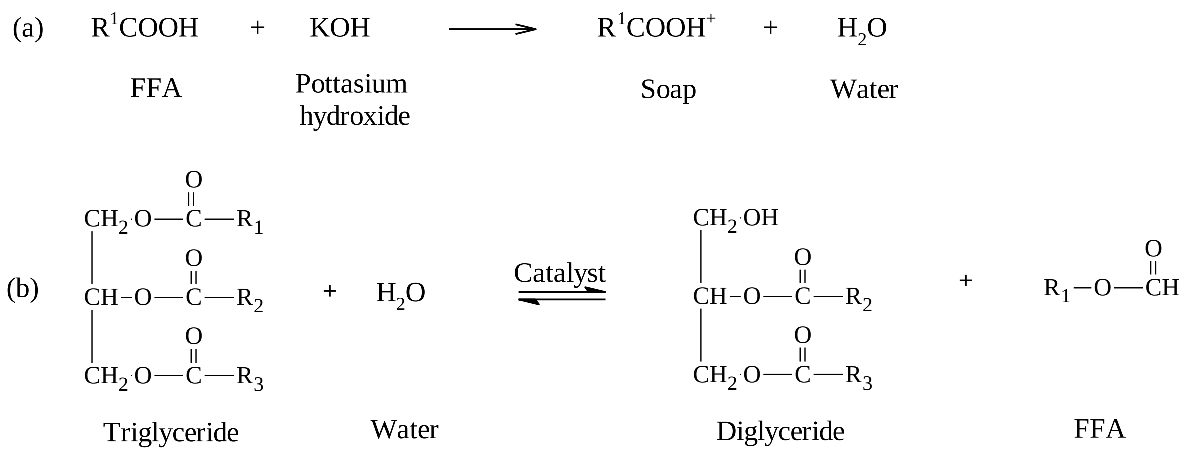

Figure 2-5: Saponification (a) and hydrolysis (b) reactions (Leung et al., 2010; Rathore et al., 2016)

Saponification is undesirable because it leads to catalyst consumption, which consequently results in low biodiesel yield (Fukuda *et al.*, 2001; Al-Zuhair, 2007). In addition, the soap formed during saponification reaction complicates downstream product recovery processes (Guldhe *et al.*, 2015). High water content in feedstock promotes hydrolysis of TGs to diglycerides and FFA (Leung *et al.*, 2010), which subsequently promotes saponification.

To avoid saponification, transesterification of feedstock with high FFA content is usually catalysed by homogeneous acid catalysts (Aransiola *et al.*, 2014). Some of the acid catalysts commonly employed in transesterification include sulphuric acid (H_2SO_4) and hydrochloric acid (HCl). In addition to catalysing transesterification reaction, acid catalysts simultaneously catalyse esterification reactions in which FFAs are converted to FAAEs as shown in Figure 2-6 (Yan *et al.*, 2014). Thus, acid catalysts are not susceptible to FFA content and hence can ably catalyse alcoholysis of low-quality feedstock.

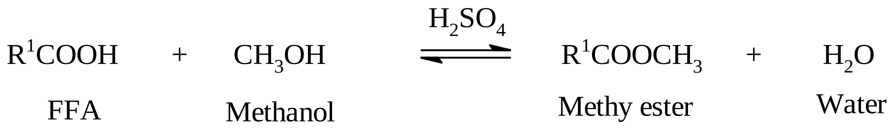

Figure 2-6: Esterification of FFAs to FAAEs (Rathore et al., 2016)

Acid catalysts are however characterised by slow reaction rates, sometimes up to 4000 times slower than alkaline catalysts (Gorji & Ghanei, 2014) and as such are seldom used for commercial biodiesel production. Alternatively, transesterification can be catalysed by heterogeneous alkaline and acidic catalysts such as calcium oxide (CaO), tungsten oxide zirconia (WO_3/ZrO_2), and magnesium-aluminium hydrotalcites (Amini *et al.*, 2016).Table 2-7 lists the benefits and limitations of using homogeneous and heterogeneous catalysts in biodiesel preparation.

Table 2-7: Advantages and drawbacks of homogeneous catalysts (Leung et al., 2010)

Catalyst	Advantages	Drawbacks
Homogeneous, alkaline	Highly efficient, moderate operating conditions, high reaction rates.	Feedstock must be of high quality, undesirable side reactions, difficult to recover and reuse, tedious downstream processes, generation of large volumes of wastewater.
Homogeneous, acid	Catalyse both esterification and transesterification reactions, saponification not an issue.	Corrosive in nature, generation of wastewater requiring treatment, difficult to recover and reuse, slow reaction rates, long reaction times.
Heterogeneous, alkaline	Noncorrosive, eco-friendly, easy to recover and hence can be reused, high selectivity, simplified product recovery processes.	Susceptible to high FFA and water contents, produces wastewater requiring treatment, the requirement for high temperature, pressure and alcohol loading, diffusion limitations, high cost.
Heterogeneous, acid	Eco-friendly, catalyse both transesterification and esterification reactions, easy to recover and reuse, product recovery is simple.	Low acid site concentrations, low microporosity, diffusion limitations, high cost.

2.7.2 Biocatalysts

Application of biocatalysts known as lipases in transesterification addresses some of the challenges presented by chemical catalysts. Lipases are a group of naturally occurring enzymes with the ability to catalyse numerous reactions including transesterification and esterification (Pandey *et al.*, 1999), and hence their application in biodiesel preparation. Lipases commonly used in biodiesel synthesis are obtained from microbial sources including bacteria, yeast, and fungi (Yan *et al.*, 2014). Examples of commercialized lipases used to catalyse transesterification reaction include *Candida antarctica lipase-B* (Novozyme 435), *Candida rugosa (C. rugosa)*, *Candida sp*., *Pseudomonas fluorescens (P. fluorescens)*, *Porcine pancreas (P. pancreas)* and *Rhizomucor miehei (R. miehei)*.

Biodiesel synthesis reaction catalysed by lipases may proceed in a single step i.e. direct alcoholysis of TGs to FAAEs (Al-Zuhair *et al.*, 2007), or in 2 reaction steps encompassing hydrolysis of TGs to FFAs (step 1) and esterification of the liberated FFAs to FAAEs (step 2) (Sun *et al.*, 2013). Several authors noted that addition of organic solvents such as hexane to a transesterification system catalysed by lipases helps to enhance the reaction rate (Yan *et al.*, 2014; Guldhe *et al.*, 2015). Organic solvents are however volatile, flammable, and toxic, which makes solvent-free biocatalytic transesterification more attractive (Bharathiraja *et al.*, 2014). Moreover, the requirement for removal of the organic solvents during downstream processes entails additional production costs (Tongboriboon *et al.*, 2010).

2.7.2.1 Free and immobilized lipases

Different forms of lipases are available on the market for biodiesel production including free and immobilized forms. Free lipases can be found in powder form (freeze-dried enzyme powder) or in liquid enzyme formulations (Nielsen *et al.*, 2008; Aguieiras *et al.*, 2015).The major benefit of using free lipases in transesterification is that they are easy and inexpensive to prepare (Gog *et al.*, 2012). Free lipases are however highly unstable and are easily inactivated by organic solvents and process conditions such as temperature and pH (Yan *et al.*, 2014). Furthermore, free lipases are difficult to recover and reuse (Ribeiro *et al.*, 2011).

To make lipase-catalysed biodiesel production cost-effect, researchers have devised techniques for ensuring easy recovery and hence reuse of lipases. Immobilization is one of the techniques commonly employed. Immobilization involves confining lipases to solid supports through techniques such as covalent bonding, cross-linking and entrapment (Zhao *et al.*, 2015). Immobilized lipases are insoluble in the reaction system and hence can easily be recovered through simple separation techniques such as filtration and centrifugation (Aguieiras *et al.*, 2015). Immobilization also enhances enzyme stability towards various lipase denaturing agents including temperature (Fjerbaek *et al.*, 2009). Thus, Immobilization allows for multiple reuses of lipases with minimal loss in catalytic activity. Immobilized lipases such as *Candida antarctica lipase-B* immobilized on macroporous acrylic resin are more commonly employed in biodiesel production compared to free lipases (Robles-Medina *et al.*, 2009).

2.7.2.2 Advantages of biocatalysts

Biocatalytic transesterification is advantageous because of its simplified downstream operations. This is especially the case when immobilized lipases are used. Since immobilized lipases can easily be recovered from the product stream, product recovery and purification steps are simple compared to when chemical catalysts are employed (Motasemi & Ani, 2012; Aguieiras *et al.*, 2015). Reusability of immobilized lipases also makes transesterification via the biocatalytic route attractive. Lipases perform efficiently under moderate operating conditions which makes lipase-catalysed biodiesel production less energy intensive compared to the alkali or acid catalysed process (Abbaszaadeh *et al.*, 2012; Yan *et al.*, 2014). Lipases are also attractive because they do not have stringent feedstock-quality requirements owing to their ability to catalyse esterification reactions (Antczak *et al.*, 2009; Guldhe *et al.*, 2015). Biocatalytic transesterification is considered as green technology because the process produces minimal wastewater (Yan *et al.*, 2014). Additionally, lipases are biodegradable in nature (Gorji & Ghanei, 2014).

2.7.2.3 Disadvantages of biocatalysts

Even though biocatalysts are attractive alternatives to chemical catalysts, their application has several limitations. Lipases are generally expensive compared to chemical catalysts and as such, lipases have limited application in industrial biodiesel production. The development of immobilized lipases, however, provides a solution to this problem since immobilized lipases can be reused in a number of cycles (Ranganathan *et al.*, 2008). Lipases also have slow reaction rates which necessitate longer reaction times in order to achieve high production yields (Robles-Medina *et al.*, 2009). In addition, lipases are prone to inactivation by short chain alcohols and glycerol (Tan *et al.*, 2010; Abbaszaadeh *et al.*, 2012).

2.8 Parameters affecting biocatalytic transesterification

During transesterification process, operating parameters have a significant influence on the efficiency of the process (Motasemi & Ani, 2012; Amini *et al.*, 2017). Some of the reaction parameters that influence biocatalytic transesterification reaction include the alcohol to oil molar ratio, the concentration of catalyst, reaction temperature and time. Effects of operating parameters on transesterification reaction vary with reaction system and as such, to maximize yield, it is essential that the operating parameters are optimised for each system.

2.8.1 Effect of alcohol to oil molar ratio

Stoichiometrically, three moles of methanol are required to achieve complete conversion of one mole of triglycerides to FAME (Figure 2-4) (Meher *et al.*, 2006). Transesterification is, however, an equilibrium reaction and as such, excess amounts of methanol are often required to maximise yield (Ma & Hanna, 1999). For biocatalytic transesterification process, lipase deactivation may, however, be observed at high alcohol loading especially when the reaction system contains methanol (Nelson *et al.*, 1996; Shimada *et al.*, 1999). According to Shimada *et al.* (2002), lipase inactivation may be observed when more than 0.5 moles of methanol is added to a transesterification system.

Biocatalytic transesterification is normally conducted at substrate ratios near the stoichiometry ratio of 1:3 (Rathore *et al.*, 2016). Köse *et al.* (2002) evaluated the influence of methanol loading on methanolysis of cotton seed oil catalysed by Novozyme 435 by varying the substrate ratio from 1:1 to 1:6 (oil to methanol). Köse and co-authors noticed a gradual increase in FAME yield as the substrate ratio increased from 1:1 to 1:4. The reaction was optimized at 1:4 molar ratio, yielding 87.4% conversion, and further increment in substrate ratio resulted in a gradual decrease in conversion. Various authors have also investigated the effect of substrate ratio on lipase catalysed methanolysis (Table 2-8).

Table 2-8: Studies on effect of substrate ratio on biocatalytic transesterification

Feedstock	Enzyme and load	Temperature	Time	Oil:Alcohol range	Observation	References
Cotton seed oil	Novozyme 432; 30 wt%	40°C	7 h	1:1-1:6	Reaction optimized at 1:4 molar ratio.	Köse *et al.* (2002)
Soybean oil	Novozyme 435; 15 wt%	30°C	6 h	1:3-1:12	Optimum yield at 1:5 molar ratio.	Rodrigues *et al.* (2008)
Lipid from food waste	Novozyme 435; 10 wt%	40°C	6 h	1:3-1:10	Highest conversion at 1:5 substrate ratio	Karmee *et al.* (2015)
Pongamia oil	Mixture of Novozyme 435; *C. rugosa*, *R. oryzae*, *P. cepacia* & *P. pancreas*; 10 wt%	40°C	6 h	1:1-1:12	Reaction optimized at 1:4 molar ratio.	Karmee (2016)

2.8.2 Effect of catalyst loading

The rate of transesterification reaction and product yield can be improved by increasing the catalyst loading (Mathiyazhagan & Ganapathi, 2011; Ribeiro *et al.*, 2011). However, a limit is often reached beyond which further increment in catalyst loading has no substantial effect on conversion efficiency or lowers the biodiesel yield (Ribeiro *et al.*, 2011; Taher & Al-Zuhair, 2017). Karmee (2017) studied the effect of varying the dosage of Novozyme 435 on methanolysis of Manilkara Zapota (L.) seed oil within the range of 5 wt% to 25 wt%. At operating conditions of 1:3 substrate ratio (oil to methanol), 40°C, 200 rpm and 4 hours, the optimum FAME yield was obtained at 10 wt% lipase loading. Any additional increase in lipase loading above 10 wt% resulted in lower biodiesel yield. In a study by Amini *et al.* (2017), Novozyme 435 loading was optimised at 30 wt% in methanolysis of sweet basil seed oil. High lipase loading is uneconomic considering the high cost of lipases.

2.8.3 Effect of operating temperature

Generally, the conversion efficiency of a transesterification reaction increases as the operating temperature is increased (Guldhe *et al.*, 2015). However, for lipase-catalysed transesterification reaction, low operating temperatures are preferred because lipases are prone to thermal inactivation (Guldhe *et al.*, 2015; Amini *et al.*, 2017). Various authors have optimized lipase catalysed transesterification of different oils at temperatures within the range of 30 to 50°C as presented in Table 2-9. The optimum temperature for biocatalytic transesterification is influenced by factors such as thermal stability of lipase, substrate molar ratio, the rate of reaction and choice of organic solvent (Antczak *et al.*, 2009).

Table 2-9: Studies on effect of reaction temperature on lipase-catalysed transesterification

Feedstock	Enzyme and load	Alcohol:Oil	Time	Temperature range	Observation	References
Soybean oil	Novozyme 432; 15 wt%	5:1	6 h	20-50°C	Highest conversion at 30°C.	Rodrigues *et al.* (2008)
Sweet basil oil	Novozyme 435; 5 wt%	12:1	72 h	30-70°C	Highest FAME yield at 40°C.	Amini *et al.* (2017)
Lipid from waste oil	Novozyme 435; 10 wt%	4:1	6 h	30-60°C	Highest conversion at 40°C.	Karmee *et al.* (2015)
Cotton seed oil	Novozyme 435; 30 wt%	4:1	7 h	30-70°C	Optimum yield at 50°C.	Köse *et al.* (2002)
soybean and rapeseed oils (mixture)	Novozyme 432; 4 wt%	1:1	6 h	20-60°C	Highest conversion at 50°C.	Shimada *et al.* (1999)

2.8.4 Effect of reaction time

During transesterification, conversion of lipids to FAAEs increases with reaction time (Mathiyazhagan & Ganapathi, 2011). This has been demonstrated in a study by Lai *et al.* (2005). Lai and co-authors observed that during transesterification of refined rice bran oil, concentrations of TGs and FAME in the reaction product varied with reaction time. While the FAME content increased progressively as the reaction time was extended, the concentration of TG decreased gradually (Table 2-10). The reactions were conducted at 50°C in the presence of 5 wt% Novozyme 435 (based on lipid weight), and a substrate molar ratio of 3.6:1 (methanol to oil) was employed.

Table 2-10: Product composition of lipase-catalysed transesterification of rice bran oil (Lai et al., 2005)

Time (h)	FAME (wt%)	TG (wt%)
0	0	98.16
1	32.72	57.51
2	59.05	34.24
3	80.60	16.14
4	89.77	8.48
5	92.00	6.75
6	95.84	2.86
7	98.74	-

As mentioned in section 2.7.2.1, biocatalytic transesterification process is generally characterised by long reaction times. Optimal reaction times ranging from 4 to 72 hours have been reported for lipase catalysed methanolysis of various oils (Maceiras *et al.*, 2009; Amini *et al.*, 2017).

2.9 Biodiesel fuel quality

The quality of biodiesel fuel is influenced by its chemical and physical properties (Monteiro *et al.*, 2008). Various countries and regions have established biodiesel standard specifications to which biodiesel fuel must adhere to be used for commercial purposes. Table 2-11 lists the biodiesel standard specifications according to South African standard SANS 1935.

Table 2-11: SANS 1935 biodiesel standard specifications (SABS, 2011)

Property	Requirement	Test method
Ester content, % mass fraction, min.	96,5%	SANS 54103
Density at 15°C, kg/m^3	860 - 900	ISO 3675, ISO 12185
Kinematic viscosity at 40°C, mm^2 /s	3,5 - 5,0	ISO 3104
Flash point, °C, min	101	ISO 2719, ISO 3679
Sulphur content, mg/kg, max.	10,0	ISO 20846, ISO 20884
Cetane Number, min.	51,0	ISO 5165
Water content, mg/kg, max.	500	ISO 12937
Oxidation stability, at 110°C, h, min.	6	SANS 54112, EN 15751
Acid value, mg KOH/g, max.	0,5	SANS 54104
Monoglyceride content, % mass fraction, max.	0,8	SANS 54105
Diglyceride content, % mass fraction, max.	0,2	SANS 54105
Triglyceride content, % mass fraction, max.	0,2	SANS 54105
Free glycerol, % mass fraction, max	0,02	SANS 54105, SANS 54106

2.10 Concluding remarks

Biodiesel could play a significant role in offsetting environmental pollution and enhancing global energy security. Biodiesel is attractive because it is renewable, non-toxic, and can be produced locally.

Conventionally, biodiesel is produced via transesterification process catalysed by homogeneous chemical catalysts. This method is however not environmentally friendly due to the generation of alkaline and/or acidic wastewater requiring treatment prior to disposal. The product recovery and purification processes are also energy intensive, complex, and time-consuming.

Utilization of biocatalysts in biodiesel production presents several advantages over chemical catalysis. Lipase catalysed transesterification process is less energy intensive, has simplified downstream processes and produces no wastewater. Furthermore, the process is eco-friendly because unlike chemical catalysts, lipases are biodegradable in nature.

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CHAPTER 3. MATERIALS AND METHODS

3.1 Introduction

This chapter focusses on the experimental aspects of this study. Details of all the materials and chemicals used are given in section 3.2 and the experimental procedure is outlined in section 3.3. The analytical equipment and analytical methods used for analyses are discussed in section 3.4.

3.2 Materials

3.2.1 Feedstock

Baobab seed oil was purchased from Nautica Organic Trading Company, Durban, South Africa. The oil was analysed for its fatty acid profile using Gas chromatography (GC) technique. The fatty acid profile was then used to compute the molecular weight of the oil. Using this procedure, the molecular weight of the baobab seed oil used in this study was calculated to be 577.58 g/mol. All the calculations are shown in Appendix B.1.

3.2.2 Chemicals

Details of all the materials and chemicals used in the present study are summarized in Table 3-1.

Table 3-1: List of materials and chemicals

Component	**Purity**	**Supplier**	**CAS no./ Product code**	**Purpose**
Candida antarctica lipase-B (immobilized on acrylic resin)		Sigma-Aldrich	9001-62-1	Catalyst
Candida rugosa lipase Type VII		Sigma-Aldrich	9001-62-1	Catalyst
Candida sp. lipase		Sigma-Aldrich	1001847943	Catalyst
Amano lipase from Pseudomonas fluorescens		Sigma-Aldrich	9001-62-1	Catalyst
Porcine pancreas lipase Type II		Sigma-Aldrich	1002139403	Catalyst
Methanol	99.5%	Rochelle Chemicals		Reagent
Diethyl ether	99%	Associated Chemicals Enterprise (ACE)	60-29-7	Solvent for separating catalyst
Deuterated chloroform ($CDCl_3$)	99%	Sigma-Aldrich	865-49-6	Solvent for ^1H NMR analysis
Trimethylsulphonium hydroxide (TMSH)	0.25 M	Sigma-Aldrich	101712061	Oil sample derivative

3.3 Experimental procedure

Experiments carried out in this study involved screening of lipases from different sources and optimization of reaction conditions viz., oil to methanol molar ratio, temperature, and reaction time. Figure 3-1 shows a representation of the experimental procedure followed in this study.

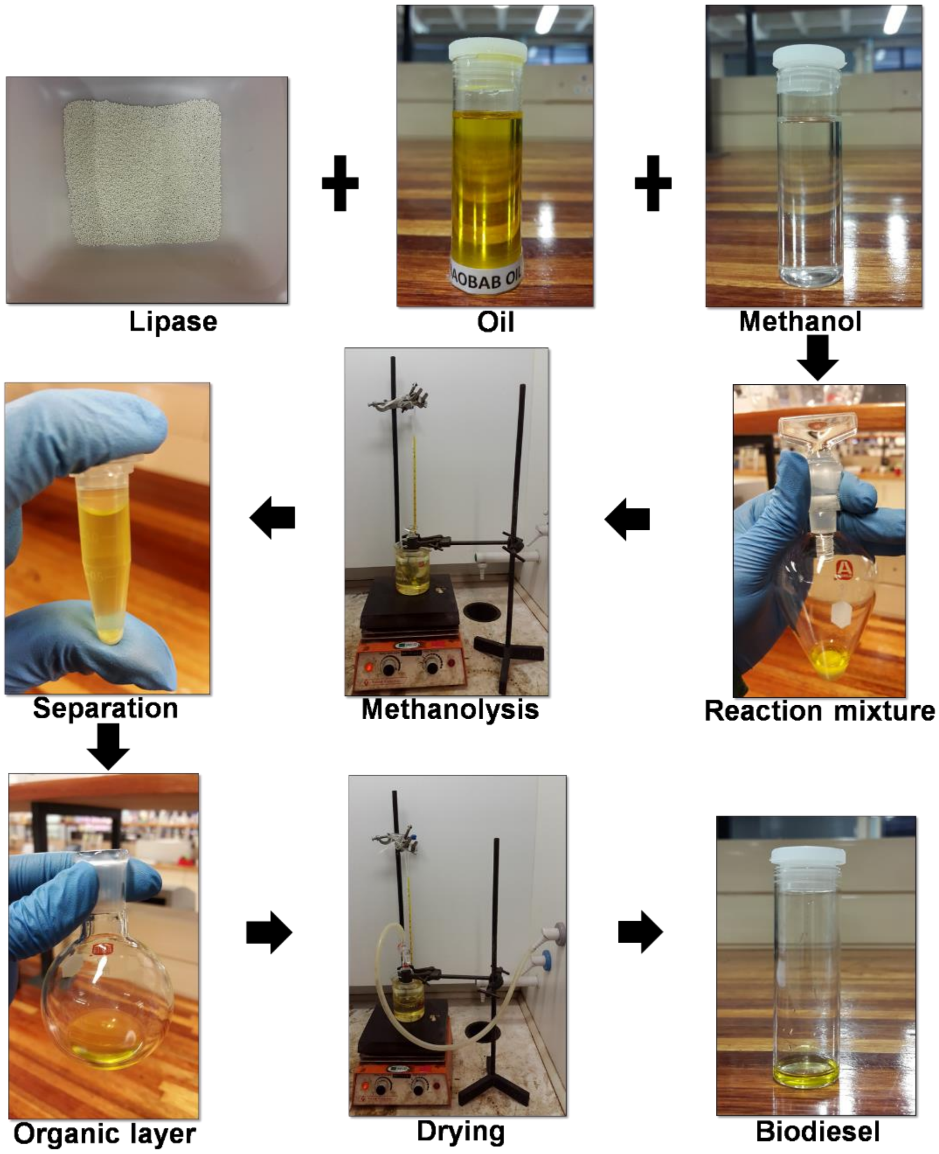

Figure 3-1: Experimental procedure for biodiesel preparation

3.3.1 Lipase-catalysed transesterification

Each experiment commenced with the addition of lipase into a 50 ml pear-shaped reaction flask, followed by addition of oil. To this mixture was then added an appropriate amount of methanol depending on the desired oil to alcohol molar ratio. The reaction vessel was then capped, clamped to a stand, and immersed into a pre-heated oil bath. The specific operating conditions used in the experiments conducted are given in sections 3.3.1.1-3.3.1.4. The transesterification protocol was adopted from Karmee *et al.* (2015).

3.3.1.1 Lipase screening

Lipases from *Candida antarctica* (immobilized on acrylic resin) (Novozyme 435), *Porcine pancreas*, *Candida sp*., *Candida rugosa* and *Pseudomonas fluorescens* were individually screened for their efficiency in catalysing methanolysis of baobab seed oil. In these reactions, 0.1 g (10 wt% based on oil weight) of lipase was mixed with 1 g (1.73 mmol) of oil and methanol was added at a molar ratio of 1:3 (210 µl, 5.2 mmol) (based on oil weight). Methanolysis was conducted at 40°C for 6 hours while stirring constantly at 700 rpm.

3.3.1.2 Oil to alcohol molar ratio optimization

The oil to methanol molar ratio was varied from 1:1 to 1:10 to establish the optimum substrate ratio. Reaction mixtures comprised of 1 g (1.73 mmol) of baobab seed oil, 0.1 g (10 wt%) Novozyme 435 lipase and methanol at 1:1 (70 µl, 1.73 mmol), 1:3 (210 µl, 5.2 mmol), 1:4 (281 µl, 6.9 mmol), 1:5 (351 µl, 8.7 mmol), 1:6 (421 µl, 10.4 mmol), 1:8 (561 µl, 13.8 mmol) and 1:10 (701 µl, 17.3 mmol) oil to methanol molar ratios. Other operating conditions were kept constant at 6 hours, 40°C and 700 rpm stirring rate.

3.3.1.3 Temperature optimization

To determine the optimum operating temperature, methanolysis of baobab seed oil was conducted at 30, 40, 50 and 60°C. In these reactions, 0.1 g (10 wt%) of C. antarctica lipase-B was mixed with 1 g (1.73 mmol) of baobab seed oil and 210 µl (5.2 mmol, 1:3 molar ratio) of methanol. The experiments were carried out for 6-hours while stirring constantly at 700 rpm.

3.3.1.4 Reaction time optimization

Reactions were performed for 0.5, 1, 1.5, 3, 6, 9, 12, 15 and 18 hours to ascertain the optimum reaction time for lipase-catalysed methanolysis of baobab seed oil. Reaction mixtures comprised of 1 g (1.73 mmol) of oil, 210 µl (5.2 mmol, 1:3 molar ratio) of methanol and 0.1 g (10 wt%) of Novozyme 435. The temperature was kept constant at 50°C and reactions were stirred constantly at 700 rpm.

3.3.2 Product separation and drying

Once the desired reaction time had elapsed, 2 ml of diethyl ether was transferred into the reaction vessel to facilitate removal of Novozyme 435 from the reaction product. The resulting mixture was then centrifuged to recover the liquid product. The liquid layer was then heated under vacuum at 80°C for 30 minutes to evaporate the diethyl ether. The method employed for product recovery was adopted from Karmee (2016).

3.4 Analyses

3.4.1 Gas Chromatography (GC) analysis

Baobab seed oil was analysed using a gas chromatograph equipped with a flame ionization detector (GC-FID) to determine its molecular weight and fatty acid profile. The Agilent 7820A GC system used for the quantification analysis is shown in Figure 3-2.

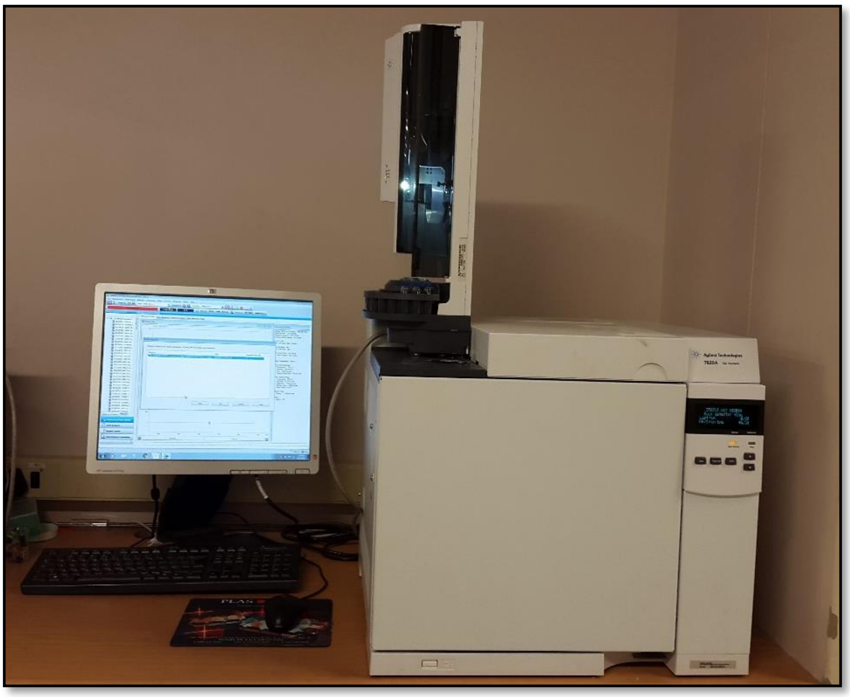

Figure 3-2: GC-FID instrument used for fatty acid composition analysis

The instrument is equipped with a 100 m HP-88 capillary column and uses helium as carrier gas. The operating conditions used for GC-FID analysis are listed in *Table 3-2* while the calibration curves are given in Appendix A.

Table 3-2: Operating conditions for GC-FID analysis

Parameter	**Value**
Carrier gas linear velocity	30 cm/s
Split ratio	1:150
Sample injection volume	1.0 µl
Inlet temperature	250°C
Inlet pressure	400 kPa
Oven temperature programming	100°C; hold for 5 minutes; ramp at 10°C/min to 120°C, hold for 1 minute; ramp at 10°C/min to 175°C, hold for 10 minutes; ramp at 5°C/min to 210°C, hold for 5 minutes; ramp at 5°C/min to 230°C, hold for 5 minutes
FID temperature	350°C
Detector gas flows	H_2: 40 ml/min; Air: 400 ml/min; Make-up He: 1.0 ml/min

3.4.1.1 Oil sample preparation

100 µl of baobab seed oil was mixed 100 µl of TMSH in a GC sample vial and vortexed for 1 hour to improve mixing. The mixture was then diluted with an appropriate volume of isooctane to make 1 ml and injected into the GC-FID system.

3.4.2 ^{1}H NMR Analysis

The degree of conversion achieved in each methanolysis reaction was determined by ^{1}H NMR spectroscopy (Knothe, 2000). ^{1}H NMR spectra of all transesterification products were obtained using a Bruker 600 MHz NMR spectrometer (Figure 3-3). The instrument is equipped with a 5 mm PA BBO 1H/DZ-GRD probe and a B-ACS 60 autosampler. Deuterated chloroform was used as a solvent for all ^{1}H NMR analyses. The analysis was conducted at ambient temperature and a soft pulse set to 30° flip angle was employed for proton excitation. Data acquisition and processing were done with Topspin version 3.5. A sample calculation showing how conversion of baobab seed oil to FAME (%) was calculated is given in Appendix B.4.

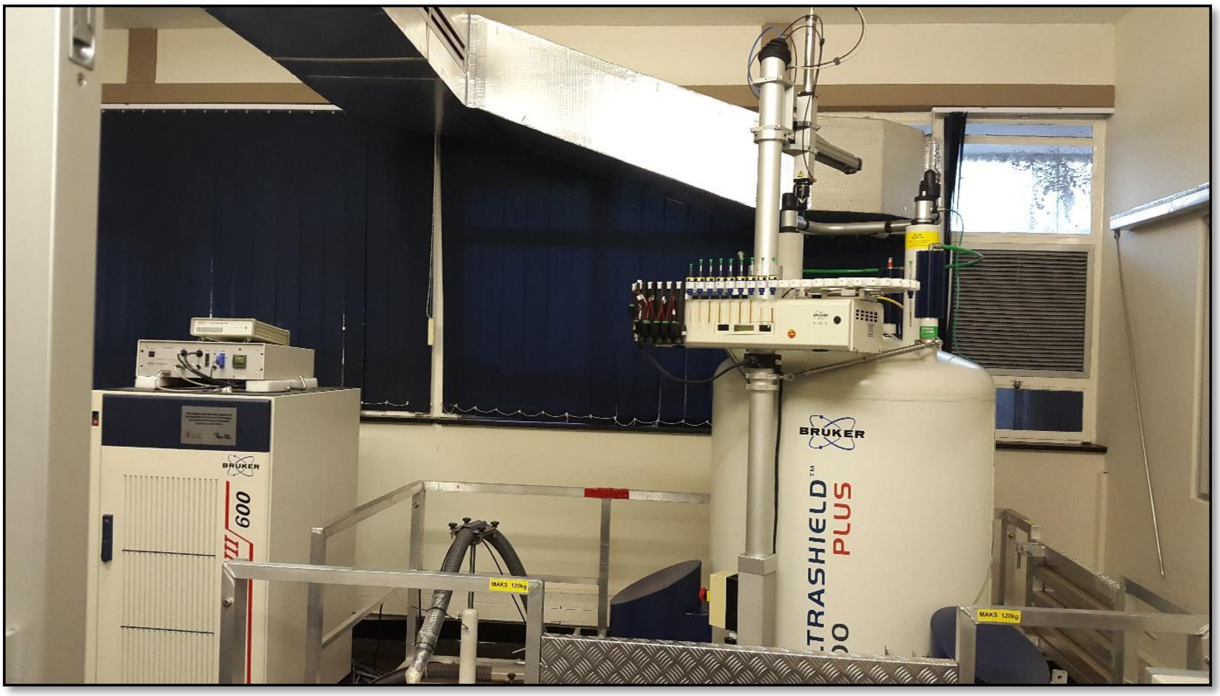

Figure 3-3: NMR instrument

3.4.2.1 Biodiesel sample preparation

30 mg of biodiesel was transferred into an NMR tube. The biodiesel sample was then dissolved in 650 µl of $CDCl_3$ and submitted for ^1H NMR analysis.

3.5 References

Karmee, S.K. 2016. Preparation of biodiesel from nonedible oils using a mixture of used lipases. Energy Sources, Part A: Recovery, Utilization, and Environmental Effects, 38(18):2727-2733.

Karmee, S.K., Linardi, D., Lee, J. & Lin, C.S.K. 2015. Conversion of lipid from food waste to biodiesel. Waste Management, 41:169-173.

Knothe, G. 2000. Monitoring a progressing transesterification reaction by fiber-optic near infrared spectroscopy with correlation to 1H nuclear magnetic resonance spectroscopy. Journal of the American Oil Chemists' Society, 77(5):489-493.

CHAPTER 4. RESULTS AND DISCUSSION

4.1 Introduction

In this chapter, the results obtained in this study are discussed. Results on baobab seed oil characterization are presented in section 4.2, followed by results on lipase screening in section 4.3. Results on effects of oil to methanol molar ratio, temperature, and reaction time on biocatalytic transesterification of baobab seed oil are presented in sections 4.4, 4.5, and 4.6 respectively. Seed oils from Moringa oleifera and Calodendrum capense plants were also transesterified under the optimum operating condition obtained for baobab seed oil and the results are presented in section 4.7.

4.2 Baobab seed oil characterization

Baobab seed oil was analysed for its fatty acid composition and the results are presented in Table 4-1. The GC spectrum obtained for the oil is given in Figure B- 1. Physiochemical properties of the seed oil including water and FFA contents and density were also analysed and are presented in the table.

Table 4-1: Properties of baobab seed oil

Property	Baobab Oil
Fatty acid composition (wt%)	
C16:0	16.4
C18:0	5.4
C18:1	21.9
C18:2	21.5
FFA (%)	2.09
Water content (%)	0.05
Density at (g/mL)	0.89

Quantitative analysis of the oil with GC-FID revealed that the main fatty acid constituents of the seed oil were C16:0 (16.4%), C18:1 (21.9%) and C18:2 (21.5%). The oil also contained 5.4% of C18:0 fatty acid. A qualitative analysis of the oil with a gas chromatograph equipped with a mass spectrometer detector (GC-MS) also showed that the oil was rich in C16:0, C18:1 and C18:2 fatty acids, thus confirming the results obtained by GC-FID analysis. The GC-MS analysis also showed that the baobab seed oil had small compositions of other fatty acids such as C14:0, C16:1, C20:0, C20:1 and C22:0 which were possibly below the detection limit of the GC-FID system. The library search report obtained from the GC-MS analysis is presented in Appendix D. The fatty acid profile obtained for baobab seed oil in the current study is slightly similar to that obtained by Modiba *et al.* (2014) who reported that the oil is rich in C16:0 (20.96%), C18:0 (20.29%), C18:1 (22.14%) and C18:2 (27.47%) fatty acids. The major difference is that the concentration of C18:0 in the oil used in the current study was about 15% lower than that reported by Modiba and co-authors. Modiba *et al.* (2014) also reported that baobab seed oil had 8.84% C18:3 and 0.29% C20:0 fatty acids which were however not detected in this study. The distribution of fatty acids in plant oils is influenced by factors such as the environmental and climatic conditions under which the plant is grown (Stefanoudaki *et al.*, 1999; Issaoui *et al.*, 2010). Hence, the differences in the fatty acid profile of baobab seed oil determined in the current study and that reported by Modiba *et al.* (2014) can be attributed to the fact that the oils were obtained from different sources.

While the fatty acid profile of starting materials influences some critical fuel properties of biodiesel including cetane number and cold flow properties, other characteristics of the feedstock such as the FFA and water contents affect the biodiesel production process (Karmakar *et al.*, 2010). High concentrations of FFA and water are undesirable especially when transesterification is to be catalysed by alkaline catalysts (Ma *et al.*, 1998; Canakci & Van Gerpen, 2001). The baobab seed oil had a free fatty acid content of 2.09% (Table 4-1) which is above the maximum requirement for transesterification via alkaline catalysis. Hence, transesterification with biocatalysts was ideal for the feedstock under study.

4.3 Lipase screening

Lipases from distinct sources exhibit different catalytic activity even when operating under similar reaction conditions (Yahya *et al.*, 1998; Guldhe *et al.*, 2015). Furthermore, the catalytic activity of a specific lipase may vary from one reaction system to another (Bharathiraja *et al.*, 2014). Hence, to maximize conversion, it is essential to identify the ideal lipase for a specific transesterification system. Along this line, *Candida antarctica lipase-B, Candida rugosa, Candida sp., Pseudomonas cepacia, and Porcine pancreas lipases* were screened to evaluate their efficiency in catalysing methanolysis of baobab seed oil. Results obtained for the lipase screening reactions are presented in Table 4-2. For these experiments, the experimental error was calculated as 0.73% at a 95% confidence level. The data used to calculate the experimental error and the calculation steps are presented in Appendix B.5.1.

Table 4-2: Transesterification of baobab seed oil catalysed by different lipases

Lipase	Conversion (%)
Candida rugosa	0.28±0.73
Porcine pancreas	0.43±0.73
Pseudomonas fluorescens	0.84±0.73
Candida sp.	0.98±0.73
Candida antarctica lipase-B	87.3±0.73

Among the 5 lipases screened, *C. antarctica* lipase-B showed the highest efficiency in catalysing methanolysis of baobab seed oil. Methanolysis of the oil at operating conditions of 10 wt% C. antarctica lipase-B loading, 1:3 substrate ratio (oil to methanol), 40°C and 6 hours reaction time yielded 87.3±0.73% conversion. Under the same operating conditions, lipases from *C. rugosa*, *P. pancreas*, *P. fluorescence* and *Candida sp.* showed very little catalytic activity, yielding 0.28±0.73, 0.43±0.73, 0.84±0.73 and 0.98±0.73% conversions respectively.

The catalytic activity of lipases in transesterification reaction depends on factors such as the method of their preparation (Stergiou *et al.*, 2013). In this study, free forms of *C. rugosa*, *P. pancreas*, *P. fluorescence* and *Candida sp. lipases* were used, while *C. antarctica lipase-B was* used in its immobilized form. The high catalytic activity exhibited by *C. antarctica lipase-B* may be attributed to immobilization. Immobilization enhances the stability of lipases towards temperature, pH, chemical and shear denaturation (Fjerbaek *et al.*, 2009). Hence, even though all the lipase screening experiments were conducted in a similar reaction media and under the same operating conditions, immobilized *C. antarctica lipase-B* was more capable of withstanding any potential denaturation effects present in the reaction system compared to the free lipases from *C. rugosa*, *P. pancreas*, *P. fluorescence* and *Candida sp*. It is widely reported that immobilized lipases exhibit higher catalytic efficiency than free lipases (Iso *et al.*, 2001).

Immobilization also prevents the formation of lipase aggregates arising from interactions between lipase molecules (Garcia-Galan *et al.*, 2011; Rodrigues *et al.*, 2012). Aggregation of lipases during transesterification is undesirable because it reduces the number of available active sites on the enzyme (Iso *et al.*, 2001; Aguieiras *et al.*, 2015). When methanolysis of baobab seed oil was catalysed by lipases from *C. rugosa*, *P. pancreas,* and *P. fluorescence, a*ggregates of enzyme powder formed around the reaction vessel despite continuous stirring of the reaction mixtures. This could, therefore, explain the low conversions obtained in reactions catalysed by *C. rugosa*, *P. pancreas,* and *P. fluorescence* lipases*.* It can be assumed that due to aggregation of the lipases, a limited number of enzyme active sites were available for interaction with the substrate, which subsequently led to low conversion. Lipase aggregation was not observed with immobilized C. antarctica lipase-B, which may thus also explain the high conversion obtained in methanolysis of baobab seed oil catalysed by Novozyme 435 lipase.

Lipase catalytic activity may also be affected by the concentration of water in the reaction system (Bajaj *et al.*, 2010; Gog *et al.*, 2012). Lipases are activated by oil/water interfaces and some lipases only become active if the water concentration is above a specific value (Bajaj *et al.*, 2010; Vyas *et al.*, 2010; Yan *et al.*, 2014). Studies conducted by Kaieda *et al.* (2001) and Lu *et al.* (2009) showed that the catalytic activity of lipases from *C. rugosa*, *P. fluorescens,* and Candida sp. increases as the moisture content of the reaction system is increased. On the contrary, the catalytic efficiency of *C. antarctica lipase-B* is higher when the transesterification system has a low moisture content (Deng *et al.*, 2005). The feedstock used in this study contained 0.05 wt% water (Table 4-1). The water content of the transesterification system was thus possibly below the critical amount required to initiate high catalytic activity in lipases from *C. rugosa*, *P. pancreas*, *P. fluorescence* and *Candida sp.*, but ideal for transesterification catalysed by *C. antarctica lipase-B*.

Reaction conditions such as the type of alcohol used, substrate ratio and operating temperature also have effects on lipase catalytic activity. Since the lipase screening experiments were not optimized, it is possible that the selected process parameters were not ideal for lipases from *C. rugosa*, *P. pancreas*, *P. fluorescence* and *Candida sp.* Since *Candida antarctica lipase-B* showed the highest efficiency in catalysing methanolysis of baobab seed oil, the lipase was used to catalyse all subsequent methanolysis reactions carried out in this study. C. antarctica lipase-B also exhibited the highest catalytic activity compared to *C. rugosa* and *P. pancreas* in methanolysis of Manilkara Zapota (L.) seed oil (Karmee, 2017).

4.4 Effect of oil to methanol molar ratio

Lipases are susceptible to inactivation by alcohols especially at high alcohol loading (Shimada *et al.*, 1999). For this reason, the amount of alcohol to be added to the reaction system is one of the critical issues to consider during biocatalytic transesterification. The influence of oil to methanol molar ratio on methanolysis of baobab seed oil was thus investigated in this study. The molar ratios under study were 1:1, 1:3, 1:4, 1:5, 1:6, 1:8 and 1:10 (oil to methanol). The substrate ratio was varied while keeping the C. antarctica lipase-B loading, temperature, and reaction time constant at 10 wt%, 40°C and 6 hours respectively. Figure 4-1 shows how conversion of baobab seed oil to FAME varied with substrate ratio. The experimental error was calculated as 2.5% at a 95% confidence level and the results in Figure 4-1 are expressed with percentage error bars. The data used for experimental error calculations and the associated calculation steps are shown in Appendix B.5.2.

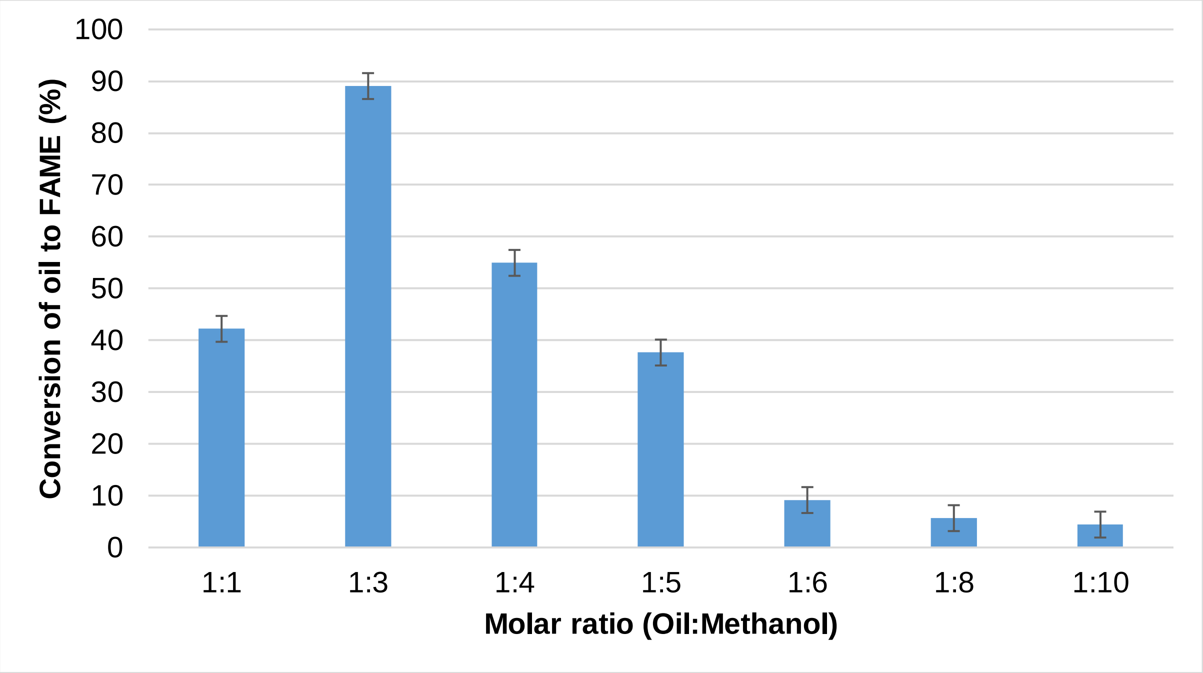

Figure 4-1: Effect of oil to methanol molar ratio on methanolysis of baobab seed oil

As shown in Figure 4-1, the alcohol loading had an influence on methanolysis of baobab seed oil. FAME yield increased from 42±2.5% to 89±2.5% with increment in substrate ratio from 1:1 to 1:3. The low conversion observed when a molar ratio of 1:1 was employed can be attributed to incomplete transesterification. Since each TG is made up of three fatty acid esters, correspondingly, 3 moles of methanol are required to completely break down the TG molecule into three fatty acid alkyl esters and a glycerol molecule. As such, transesterification was incomplete when 1 mole of methanol was reacted with an equivalent of 1 mole of baobab oil triglycerides. When the stoichiometry ratio of 1:3 was employed, a high conversion of baobab oil to FAME could be achieved due to the increased concentration of methanol relative to the concentration of baobab oil triglycerides. Conversion of baobab seed oil to FAME was optimized at 1:3 molar ratio and further increment in alcohol loading beyond the stoichiometry ratio resulted in a gradual decrease in FAME yield.

The decrease in conversion efficiency observed at 1:4 molar ratio can be attributed to lipase deactivation by methanol. Activation of lipases at oil/water interfaces is associated with conformation changes in the lipase which result in the exposure of the enzyme catalytic site, thus making it easily accessible for interaction with the substrate (Paiva *et al.*, 2000; Kapoor & Gupta, 2012). In order to maintain the active conformation state, a critical amount of water must be available in the reaction system (Yahya *et al.*, 1998). Removal of the essential water molecules by hydrophilic solvents such as methanol leads to conformation changes in the lipase structure which are detrimental to catalytic activity (Zaks & Klibanov, 1985; Lima *et al.*, 2004). The detrimental effect of high alcohol loading on lipase catalytic activity has also been observed in previous studies (Shimada *et al.*, 1999; Köse *et al.*, 2002; Du *et al.*, 2004; Rodrigues *et al.*, 2008; Karmee *et al.*, 2015; Karmee, 2016).

Lipase deactivation became increasingly pronounced as the alcohol loading was increased further between 1:4 and 1:10 substrate ratios. The lowest conversion of baobab oil to FAME (4.4±2.5%) was observed at the highest substrate ratio under study i.e. 1:10. According to Shimada *et al.* (2002), methanol and oil are not completely miscible with each other. For this reason, when a high volume of methanol is added to a transesterification system, some of the methanol remains undissolved. The interaction between a lipase and the insoluble methanol molecules leads to loss of lipase catalytic efficiency (Shimada *et al.*, 1999; Shimada *et al.*, 2002). As such, increasing the methanol loading resulted in the high accumulation of insoluble methanol molecules in the system and hence the low conversion observed at higher alcohol loading.

A substrate ratio of 1:3 was thus found to be optimal for the system under study. Biodiesel yields lower than 89±2.5% have however been reported at this alcohol loading in previous studies. In a study by Karmee *et al.* (2015), methanolysis of waste food lipid at the same operating conditions used in this study yielded about 57% conversion. A conversion of 83.6% at 1:3 substrate ratio was however reported by Köse *et al.* (2002) who transesterified cotton seed oil. Operating conditions of 30 wt% C. antarctica lipase-B loading, 40°C and 7 hours reaction time were employed by Köse and co-authors. In their studies, Köse *et al.* (2002) and Karmee *et al.* (2015) obtained the highest biodiesel yields at 1:4 and 1:5 substrate ratios respectively. The optimal substrate ratio is influenced by the nature of catalyst and oil properties (Amini *et al.*, 2017). As such, even when the same type of lipase is used, the optimum molar ratio may vary when different feedstocks are employed.

4.5 Effect of operating temperature

Overall, an increase in temperature has a positive effect on transesterification reaction. High operating temperatures increase the reaction rate, making it possible to obtain high yields within short reaction times (Mathiyazhagan & Ganapathi, 2011). However high operating temperatures are unattractive due to high energy requirements which increase production costs. Besides, for biocatalytic transesterification, high operating temperatures may cause thermal deactivation of lipases which negatively affects conversion efficiency (Guldhe *et al.*, 2015). The influence of operating temperature on methanolysis of baobab seed oil was evaluated in this study. The temperature was varied between 30 and 60°C and other operating conditions were kept constant at 10 wt% C. antarctica lipase-B loading, 1:3 (oil to methanol) substrate ratio, and 6 hours reaction time. The results obtained are presented in Figure 4-2. The experimental error was determined to be 1.9% at 95% confidence level and the results are expressed with percentage error bars. The data used for experimental error calculations together with the associated calculations are given in Appendix B.5.3.

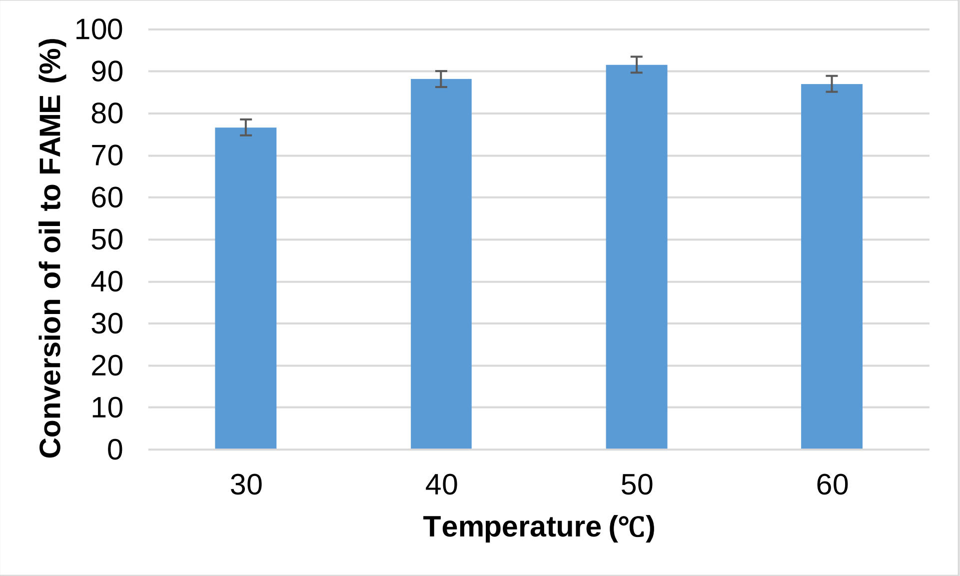

Figure 4-2: Effect of reaction temperature on methanolysis of baobab seed oil

For the system under study, the highest increase in FAME yield was observed when the operating temperature was raised from 30°C to 40°C. With this increment in temperature, biodiesel yield increased by about 11% (from 76.7±1.9% to 88.2±1.9%). Conversion slightly improved to 91.6±1.9% (about 3% increment) when the reaction temperature was increased further to 50°C. Increasing the reaction temperature between 30 and 50°C enhanced the movement of the substrate and C. antarctica lipase-B molecules which consequently increased the frequency of collisions between the substrate and lipase active sites (Eed, 2012), thus leading to the higher conversion of baobab seed oil to FAME. The increase in conversion with temperature can also be attributed to improved mass transfer between methanol and baobab seed oil. The viscosity of oil is known to decrease with increase in temperature (Leung *et al.*, 2010). As such, baobab seed oil became less viscous as the temperature was being increased, which consequently led to increased miscibility between the reactants, and hence improved conversion.

It has been reported that high operating temperatures induce undesirable conformational changes in lipases which reduce their catalytic efficiency (Gianfreda & Scarfi, 1991; Taher & Al-Zuhair, 2017). This phenomenon may hence explain the decrease in conversion efficiency observed at temperatures between 40 and 60°C. Conversion efficiency started to decrease when the temperature was increased from 40°C to 50°C due to thermal deactivation of C. antarctica lipase-B which reduced its efficiency in catalysing the transesterification reaction. The negative effect of high operating temperature on the catalytic activity of Novozyme 435 was more pronounced at 60°C as conversion decreased to 87.05±1.9%. According to Ribeiro *et al.* (2011), thermal inactivation of lipases is more pronounced at temperatures above 60°C. The decrease in conversion observed at 60 °C can also be as a result of the loss of methanol due to evaporation since the boiling point of methanol is around 64.7 °C (Karmee, 2016). A high concentration of methanol in the transesterification system is desirable because it pushes the reaction equilibrium towards the product side.

Transesterification of baobab seed oil conducted at 1:3 oil to methanol molar ratio, 10 wt% Novozyme 435 loading and 6 hours reaction time was optimized at 50°C in this study. Methanolysis of cotton seed oil catalysed by Novozyme 435 has also been optimized at 50°C in previous work by Köse *et al.* (2002). Köse *et al.* (2002) obtained a 91.5% methyl ester yield which corresponds well to the 91.6±1.9% yield obtained at 50°C in the current study.

4.6 Effect of reaction time

Long reaction times favour high conversions of triglycerides to FAME (Ferrari *et al.*, 2011). However, an optimum reaction time is often reached beyond which the FAME yield stays constant or starts to decrease (Eevera *et al.*, 2009). To ascertain the effect of reaction time on methanolysis of baobab seed oil, reactions were performed for 0.5, 1, 1.5, 3, 6, 9, 12, 15 and 18 hours. Other operating conditions were kept constant at 10 wt% Novozyme 435 loading, 1:3 substrate ratio, and 50°C. Figure 4-3 shows the influence of reaction time on methanolysis of baobab seed oil. The experimental error in these results was calculated as 2.6% at a 95% confidence level and the results are expressed with percentage error bars. The data used to calculate the experimental error and the calculation steps are presented in Appendix B.5.4.

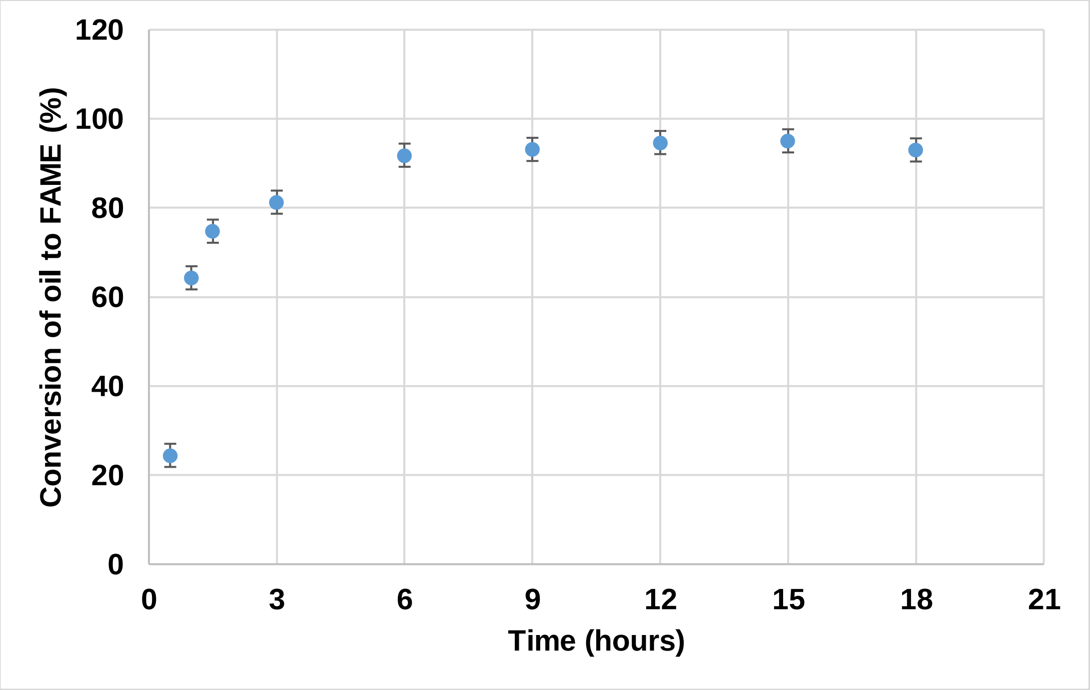

Figure 4-3: Effect of reaction time on transesterification of baobab seed oil

Conversion of baobab seed oil to FAME increased with reaction time especially within the time range of 0.5 to 6 hours. Within this time range, more triglycerides could be converted to FAME by prolonging the reaction time as the biodiesel yield increased from 24.4±2.6% to 91.8±2.6% with increment in incubation time from 30 minutes to 6 hours.

There was, however, no significant change in conversion when the incubation time was extended beyond 6 hours as can be seen in Figure 4-3. Increasing the reaction time from 6 hours to 15 hours resulted in only about 3% increment in biodiesel yield. An additional extension of reaction time to 18 hours resulted in a decrease in FAME yield from 95±2.6% to 93±2.6%. The decrease in conversion efficiency observed when the reaction time was extended beyond 6 hours can be due to several factors including inhibition of C. antarctica lipase-B by glycerol. Owing to its low solubility in FAME, glycerol tends to adsorb to surfaces of immobilized lipases (Christopher *et al.*, 2014). Presence of a glycerol layer around the lipase makes it difficult for the substrate to access the enzyme catalytic sites (Watanabe *et al.*, 2000; Antczak *et al.*, 2009; Guldhe *et al.*, 2015). Consequently, unreacted methanol accumulates in the system, leading to lipase inactivation (Watanabe *et al.*, 2000; Xiao *et al.*, 2009). Along these lines, it can be assumed that longer reaction times resulted in the high accumulation of glycerol in the reaction system which negatively affected the catalytic activity of the lipase. Another possible contributing factor to the decrease in conversion may be the loss of methanol through evaporation as the reaction time was extended. Furthermore, a long reaction time favours hydrolysis reaction which results in loss of esters (Eevera *et al.*, 2009; Mathiyazhagan & Ganapathi, 2011).

As mentioned in section 2.7.2.3, one of the major drawbacks of biocatalytic transesterification is the requirement for long reaction times. In several studies, biodiesel yields of greater than 90% could only be achieved when lipase-catalysed transesterification reaction was conducted for 10 hours or more (Karmee, 2015; Amini *et al.*, 2017; Karmee, 2017). The optimum reaction time for biocatalytic transesterification is influenced by several factors including operating temperature, catalyst loading and substrate ratio (Christopher *et al.*, 2014). In the present study, C. antarctica lipase-B could withstand thermal deactivation at temperatures between 30°C and 50°C which made it possible to conduct the study on the influence of reaction time on methanolysis of baobab seed oil at 50°C. A reaction temperature of 50°C is relatively high in terms of biocatalytic transesterification which is usually carried out at temperatures between 30 and 50°C (Antczak *et al.*, 2009). As such, the operating temperature may have influenced the reaction to proceed at a fast rate, hence making it possible to achieve >90% conversion within 6 hours of reaction.

The results obtained can be correlated to results obtained by Maceiras *et al.* (2009) who optimized the reaction time for transesterification of waste frying oil at 4 hours. At optimum operating conditions of 25:1 oil to methanol molar ratio, 10% Novozyme 435 loading and 50°C, Maceiras and co-authors obtained 89.1% biodiesel yield when they transesterified waste frying oil for 4 hours. Maceiras *et al.* (2009) employed a higher methanol loading (25:1) than the one used in the present study (3:1) which possibly accounts for the shorter reaction time. The results obtained in this study also do not differ significantly from results obtained by Köse *et al.* (2002). Köse and co-authors obtained a 91.5% FAME yield in methanolysis of cotton seed oil catalysed by C. antarctica lipase-B (30% loading) conducted for 7 hours at 50°C and 1:4 oil to methanol molar ratio.

Even though the highest conversion (95±2.6%) of baobab seed oil to FAME was achieved after 15 hours, 6 hours was adopted as the optimum reaction time for the system under study in order to economise the process. A summary of the optimum operating condition determined for biocatalytic transesterification of baobab seed oil in this study is given in Table 4-3.

Table 4-3: Optimum operating conditions for biocatalytic methanolysis of baobab seed oil

Catalyst	C. antarctica lipase-B
Catalyst loading	10 wt% (based on the.weight of oil)
Oil to methanol molar ratio	1:3
Temperature	50°C
Reaction time	6 hours

4.7 Lipase-catalysed transesterification of calodendrum capense and moringa oils

The study was extended to other oil-bearing plants of African origin viz., Calodendrum capense (L.f.) Thunb (cape chestnut or yangu) and Moringa oleifera Lam. (moringa). Calodendrum capense (family Rutaceae) trees produce seeds containing about 60-63wt% oil (Wagutu *et al.*, 2009). Moringa oleifera (family Moringaceae) seeds contain about 33-41wt% oil (Yang *et al.*, 2014). The seed oils were purchased from Nautica Organic Trading Company, Durban, South Africa.

Transesterification of yangu and moringa seed oils was conducted under the optimum operating conditions obtained for methanolysis of baobab seed oil in this study i.e. 10 wt% Novozyme 435 loading, 210 µl methanol loading, 50°C and 6 hours reaction time. Transesterification and analyses protocols proceeded as presented in chapter 3. The results obtained are presented in Table 4-4. For these experiments, the experimental error was calculated as 2.6% at a 95% confidence level. The data used to calculate the experimental error and the calculation steps are presented in Appendix B.5.5.

Table 4-4: Biodiesel production from seed oils of African origin

Oil	Conversion (%)
Moringa	80.4±2.6
Yangu	89.6±2.6

Methanolysis was a success especially with yangu oil, yielding about 90% conversion. A lower conversion (80.4±2.6%) was however obtained in methanolysis of moringa oil. GC-FID analysis showed that yangu oil contained 11.1% C16:0, 3.4% C18:0, 29.4% C18:1 and 17.7% C18:2 fatty acids while the moringa oil contained 2.6% C16:0, 2.35% C18:0, 35.5% C18:1 and 1.5% C18:2 fatty acids. The GC spectra obtained for the two oils are given in Figure C- 29 and Figure C- 30. The differences in biodiesel yield obtained in methanolysis of the two oils may thus be attributed to the variations in fatty acid compositions. On the other hand, distribution of fatty acids in yangu oil was relatively similar to the distribution of fatty acids in baobab seed oil which may thus account for the similar FAME yields obtained in methanolysis of yangu and baobab seed oils. Considering that methanolysis of moringa and yangu oils was not optimized in the current study, the operating conditions may not have been ideal for moringa oil. As such, there is a possibility of obtaining higher conversions from biocatalytic transesterification of M. oleifera seed oil if the operating conditions are optimized.

While biocatalytic methanolysis of yangu and moringa seed oils has not been reported in literature, the results obtained do not differ significantly from results obtained by various authors who catalysed transesterification of the two plant seed oils with homogeneous and heterogeneous chemical catalysts. Wagutu *et al.* (2009) reported that NaOH-catalysed methanolysis of calodendrum capense seed oil yielded 93.3% conversion. Kafuku and Mbarawa (2010) obtained 82% FAME yield in KOH-catalysed methanolysis of moringa seed oil. Kafuku *et al.* (2010) also reported that methanolysis of moringa oil catalysed by sulphated tin oxide enhanced with SiO_2 gave 84% FAME yield. The results obtained in this study show that biocatalytic transesterification is as highly efficient as the conventional chemical transesterification process.

4.8 References

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CHAPTER 5. CONCLUSION

5.1 Overview

The aim of this research was to study the feasibility of lipase-catalysed biodiesel production from plant oils of African origin. To achieve this, first, the catalytic activity of *Candida antarctica lipase-B, Candida rugosa, Candida sp., Pseudomonas cepacia, and Porcine pancreas* lipases on transesterification of baobab seed oil was evaluated to identify the best lipase for the reaction system. *C. antarctica lipase-B* exhibited the best catalytic efficiency and hence was selected to catalyze various reactions on optimization of operating conditions for methanolysis of baobab seed oil viz., oil to methanol molar ratio, reaction temperature and time. Methanolysis of seed oils from calodendrum capense and moringa oleifera plants was then performed under the identified optimum conditions. Conclusions made from the results obtained are listed in section 5.2. Recommendations were also made, and these are listed in section 5.3.

5.2 Conclusions

- C. antarctica lipase-B is the most efficient biocatalyst for methanolysis of baobab seed oil among the lipases screened in this study.
- Optimum operating conditions for methanolysis of baobab seed oil catalysed by C. antarctica lipase-B are 10 wt% lipase loading (based on the weight of oil), 1:3 oil to methanol molar ratio, 50°C and 6 hours reaction time.
- Lipase-catalysed transesterification of seed oils from baobab, calodendrum capense, and moringa oleifera plants can respectively yield 91.8±2.6%, 89.6±2.6%, and 80.4±2.6% conversions.
- Lipase-catalysed methanolysis of baobab, calodendrum capense, and moringa plant seed oils is highly feasible.
- Baobab, calodendrum capense, and moringa plant seed oils have promising potential as feedstock for biodiesel production and hence commercial application should be considered in future.

5.3 Recommendations

- Tests should be conducted to evaluate the effect of lipase preparation method and reaction conditions including moisture content, type and amount of alcohol, reaction temperature and time on efficiency of *C. rugosa*, *P. pancreas*, *P. fluorescence* and *Candida sp.* lipases in catalysing transesterification of baobab seed oil.
- Methanolysis of moringa oil catalysed by C. antarctica lipase-B needs to be optimized to improve the yield.

- Analyses of fuel properties of FAMEs produced from baobab, calodendrum capense, and moringa seed oils via the biocatalytic route need to be done to ensure that the fuel properties are within SANS biodiesel standard specifications.
- Evaluation of the economic feasibility of biodiesel production from baobab and calodendrum capense seed oils needs to be done. Currently, data on how much of these oils are produced in Africa is scarce in literature.
- Similar studies need to be done to expand the feedstock base for biodiesel production in Africa.
- Future studies should evaluate the relationship between the fatty acid composition of plant seed oils and biodiesel yield.

Appendix A Calibration curves

Appendix A.1 GC calibration curves

Calibration of the GC-FID system was done by injecting pure and mixtures of FAME standard samples into the gas chromatograph. Mass fractions of FAME standards added to the GC sample vial were then plotted against the signal area to obtain the calibration curves. A trendline was fitted to the data to obtain K values which were used to determine compositions of fatty acids in baobab seed oil. Calibration curves of C16:0, C18:0 and C18:1 FAME standards are given in Figure A- 1 to Figure A- 3.

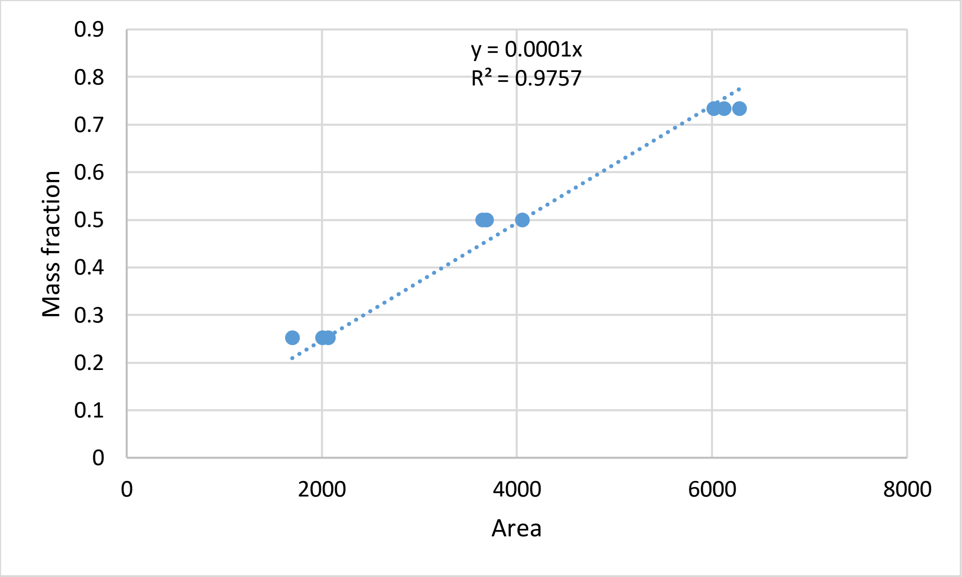

Figure A- 1: Calibration curve of C16:0 methyl ester

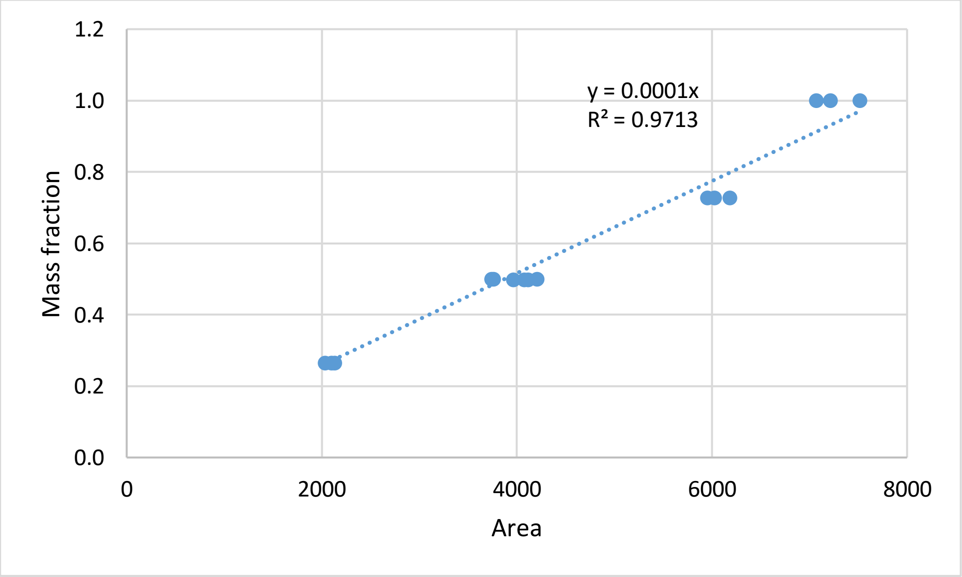

Figure A- 2: Calibration curve of C18:0 methyl ester

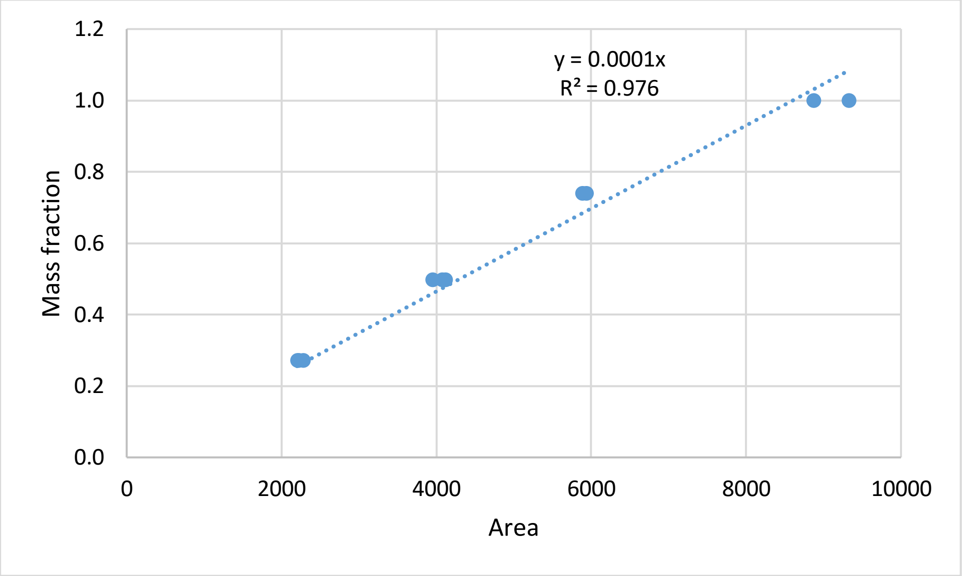

Figure A- 3: Calibration curve of C18:1 methyl ester

Appendix B Calculations

Appendix B.1 Molecular weight of baobab oil

The mass fraction of each fatty acid present in baobab seed oil was calculated by using the equation:

$$x_i = K * A_{FA}$$

Where: x_i = Mass fraction of fatty acid

K = K value obtained from GC calibration graph

A_{FA} = signal area of fatty acid

Signal areas of fatty acids were obtained from the GC spectrum of baobab seed oil shown in in Figure B- 1.

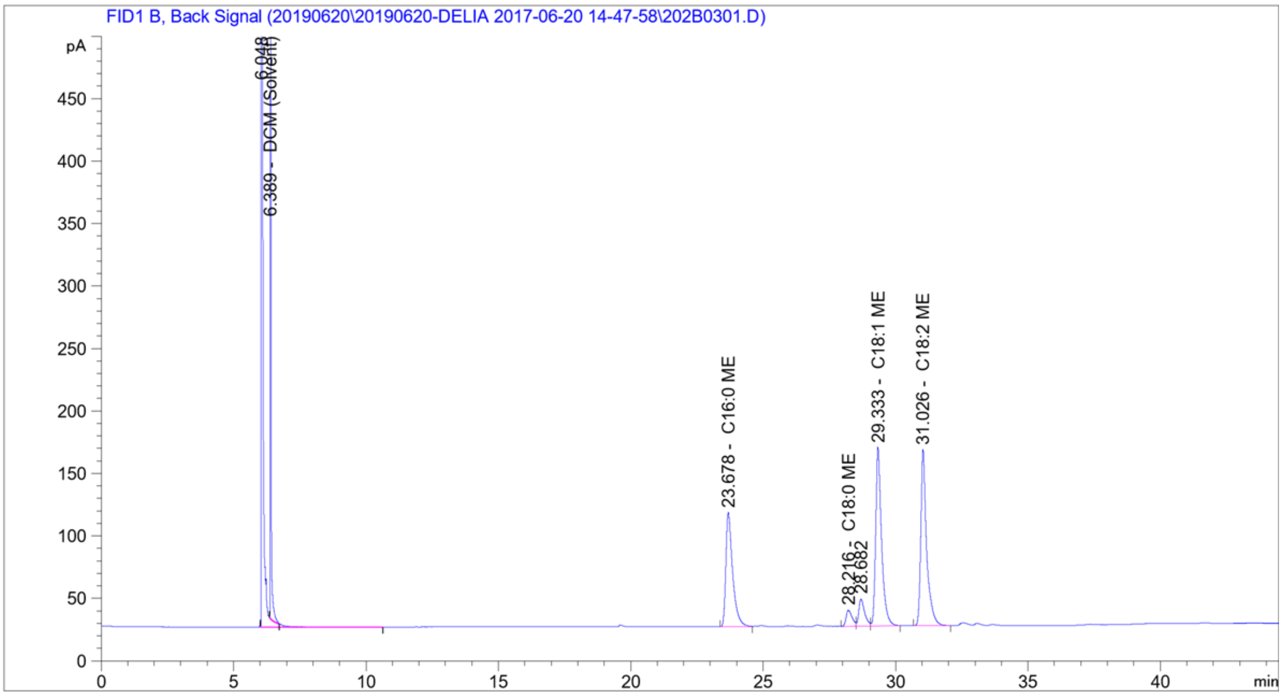

Figure B- 1: Chromatogram of baobab seed oil

The calculated mass fractions of baobab seed oil fatty acids are given in Table B- 1 together with the data used for calculations. The K value obtained for C18:1 was also used for quantification of C18:2 fatty acid.

Table B- 1: Data used to calculate the fatty acid content of baobab seed oil

FAME	K value	Area	X_i
C16:0	0.0001	1638.38	0.16
C18:0	0.0001	542.73	0.05
C18:1	0.0001	2194.41	0.22
C18:2	0.0001	2153.74	0.22

The average molecular weight of the fatty acids was then calculated using the equation:

$$MW_{FA} = (x_{c16:0} * MW_{C16:0}) + (x_{c18:0} * MW_{C18:0}) + (x_{c18:1} * MW_{C18:1}) + (x_{c18:2} * MW_{C18:2})$$

$$= (0.16 * 256.43) + (0.05 * 284.49) + (0.22 * 282.49) + (0.22 * 280.49)$$

$$= 179.85$$

Finally, the molecular weight of baobab seed oil was calculated using the equation:

$$MW_{baobab\ oil} = 3MW_{FA} + MW_{glycerol} - 3MW_{water}$$

$$= (3 * 179.85) + 92.02 - (3 * 18.02)$$

$$= 577.58\ g/mol$$

Appendix B.2 Volume of methanol

The volume of methanol used in each reaction was calculated based on the mass of oil to be transesterified.

For each reaction, 1 g of oil was used. The number of moles, n, in 1 g of baobab seed oil was thus calculated using the equation:

$$n_{oil} = \frac{mass_{oil}}{MW_{oil}} = \frac{1\ g}{577.58\ g/mol} = 0.0017\ mol$$

The required number of moles of methanol was then calculated depending on the desired molar ratio. Assuming 1:3 molar ratio, the relationship below was used:

$$\frac{n_{oil}}{n_{MeOH}} = \frac{1}{3}$$

$$n\ MeOH = 3 * n_{oil} = 3 * 0.0017\ mol = 0.0052\ mol$$

Required mass of methanol was then calculated according to the following equation:

$$n_{MeOH} = \frac{mass_{MeOH}}{MW_{MeOH}}$$

Thus,

$$mass_{MeOH} = 0.0052\ mol * 32.04 \frac{g}{mol} = 0.166\ g$$

The required volume of methanol was then calculated using the relationship between density (p), mass and volume (V):

$$\rho_{MeOH} = \frac{mass_{MeOH}}{V_{MeOH}}$$

$$V_{MeOH} = \frac{0.166\ g}{0.791\ g/ml} = 0.210\ ml * 1000 \frac{\mu l}{ml} = 210\ \mu l$$

Table B- 2 shows the calculated volumes of methanol at all oil to methanol molar ratios under study.

Table B- 2: Calculated volumes of methanol

Oil:MeOH	**n_{MeOH}**	**$mmol_{MeOH}$**	**$Vol._{MeOH}$ (ml)**	**$Vol._{MeOH}$ (µl)**
1:1	0.002	0.06	0.07	70
1:3	0.005	0.17	0.21	210
1:4	0.007	0.22	0.28	281
1:5	0.009	0.28	0.35	351
1:6	0.010	0.33	0.42	421
1:8	0.014	0.44	0.56	561
1:10	0.017	0.55	0.70	701

Appendix B.3 Catalyst loading

Amount of catalyst added to the reaction system was also calculated based on the weight of the oil. Catalyst loading was kept constant at 10 wt% based on the mass of oil in all the experiments. Hence, for 1 g of oil,

$$mass_{catalyst} = 1\ g * 0.1 = 0.1\ g$$

Appendix B.4 Conversion of oil to FAME

Conversion (C_{ME}) of oil to FAME was determined by integration of glyceridic and methyl ester peaks observed at approximately 3.6-3.7 ppm and 4.0-4.3 ppm respectively. Figure B- 2 gives the ^{1}H NMR spectrum for baobab biodiesel. The peaks labelled G correspond to the signals of glyceridic protons attached to triglycerides of oil while the peak labelled ME correspond to the signal of methoxylic protons present in methyl esters.

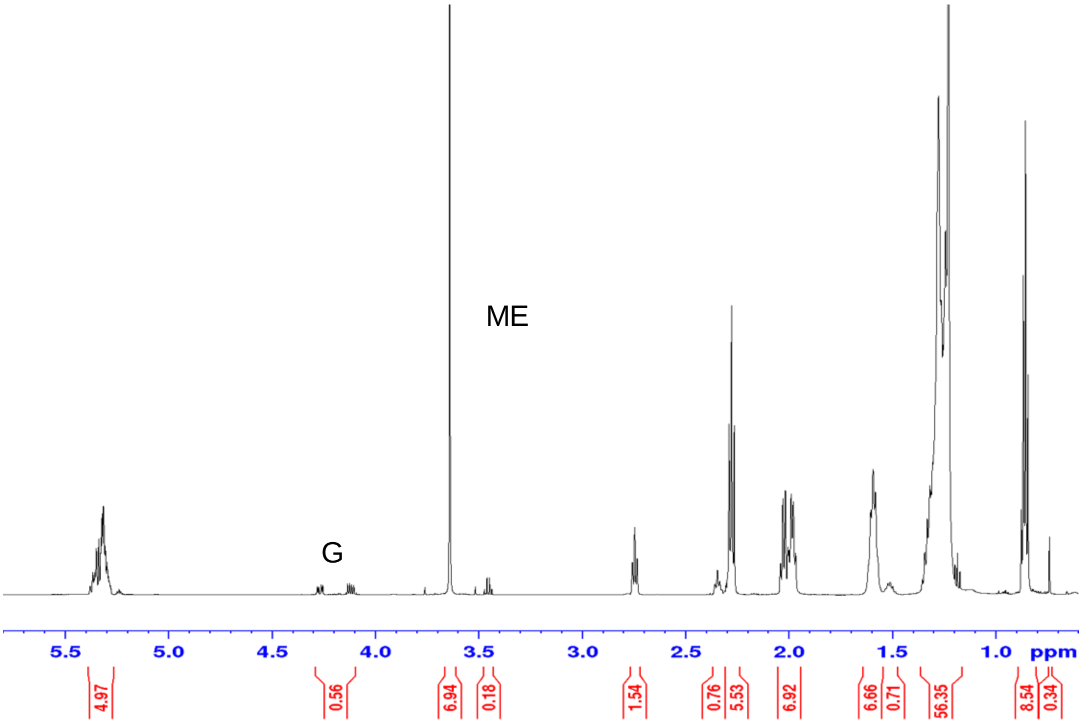

Figure B- 2: ^{1}HNMR spectra of baobab biodiesel at 10 wt% Novozyme 435 loading, 1:3 oil to methanol molar ratio, 40℃, and 6 hours

Conversion of oil to FAME was determined using the equation:

$$C_{ME} = 100 * \frac{5 * I_{ME}}{(5 * I_{ME}) + (9 * I_{TAG})}$$

Where I_{ME} is the integration value of methyl ester peak and I_{TAG} is the integration value of glyceridic peaks.

Using the ^{1}H NMR spectrum shown in FIGURE Y, I_{ME} = 6.94 and I_{TAG} = 0.56. Hence,

$$C_{ME} = 100 * \frac{5 * 6.94}{(5 * 6.94) + (9 * 0.56)}$$

$$= 87.3\%$$

Appendix B.5 Error calculations

For each parameter under study, 1 experiment was performed in triplicate and the results were used to determine the experimental error. Experimental errors were calculated using the equation:

$$Experimental\ error\ (\%) = \frac{Confidence\ limit}{\bar{x}} * 100$$

Where $\bar{x}$ corresponds to the average of the 3 results obtained.

To determine the experimental error, the confidence limit and $\bar{x}$ must be determined first. $\bar{x}$ was calculated using the equation:

$$\bar{x} = \frac{1}{n} * \sum_{i=1}^{n} x_i$$

Where n is the number of experimental runs and x_i is the result obtained in each experiment.

The confidence limit was calculated using the equation:

$$\bar{x} \pm 1.96(\frac{\sigma}{\sqrt{n}})$$

Where σ is the standard deviation. The standard deviation was calculated using the equation:

$$\sigma = \sqrt{\frac{\sum_{i=1}^{n}(x - \bar{x})^2}{n-1}}$$

Where *x* is the sample mean.

Appendix B.5.1 Lipase screening

The experimental error was determined by performing methanolysis of baobab seed oil catalysed by C. rugosa in triplicate at operating conditions of 1:3 oil to methanol molar ratio, 10 wt% catalyst loading, 40°C, 6 hours, and 700 rpm stirring rate. The data used for the statistical analysis is given in Table B- 3.

Table B- 3: Experimental error for lipase screening

	Conversion (%)	Average	Standard deviation	Confidence limit	Error (%)
Experiment 1	0.29	0.29	0.002	0.002	0.73
Experiment 2	0.28				
Experiment 3	0.29				

Appendix B.5.2 Effect of oil to methanol molar ratio

Transesterification of baobab seed oil catalysed by 10 wt% C. antarctica lipase-B at 1:3 oil to methanol molar ratio, 40°C, 6 hours, and 700 rpm stirring rate was conducted in triplicate and the results were used to calculate the experimental error. The data used for the statistical analysis is given in Table B- 4.

Table B- 4: Experimental error for effect of oil to methanol molar ratio

	Conversion (%)	Average	Standard deviation	Confidence limit	Error (%)
Experiment 1	42.31	42.74	0.95	1.07	2.5
Experiment 2	42.08				
Experiment 3	43.82				

Appendix B.5.3 Effect of operating temperature

Data for experimental error calculation was generated by repeating the reaction conducted at 1:3 oil to methanol molar ratio, 10 wt% Novozyme 435 loading, 50°C, 6 hours, and 700 rpm stirring rate twice. The data used for the statistical analysis is given in Table B- 5.

Table B- 5: Experimental error for effect of operating temperature

	Conversion (%)	Average	Standard deviation	Confidence limit	Error (%)
Experiment 1	81.29	79.80	1.37	1.55	1.94
Experiment 2	79.52				
Experiment 3	78.60				

Appendix B.5.4 Effect of reaction time

Transesterification of baobab seed oil carried out for a duration of 1 hour at 1:3 oil to methanol molar ratio, 10 wt% C. antarctica lipase-B loading, 50°C and 700 rpm stirring rate was conducted in triplicate and the results were used to calculate the experimental error. The data used for the statistical analysis is given in Table B- 6.

Table B- 6: Experimental error for effect of reaction time

	Conversion (%)	Average	Standard deviation	Confidence limit	Error (%)
Experiment 1	91.62	90.54	2.05	2.31	2.6
Experiment 2	88.18				
Experiment 3	91.82				

Appendix B.5.5 Methanolysis of yangu, kapok, and moringa

Methanolysis of yangu oil at 1:3 oil to methanol molar ratio, 10 wt% C. antarctica lipase-B loading, 50°C, 6 hours reaction time and 700 rpm stirring rate was carried out in triplicate to generate data for experimental error calculations. The data used for the statistical analysis is given in Table B-7.

Table B- 7: Experimental error for methanolysis of moringa and yangu oils

	Conversion (%)	Average	Standard deviation	Confidence limit	Error (%)
Experiment 1	89.65	88.77	2.05	2.32	2.6
Experiment 2	90.22				
Experiment 3	86.42				

Appendix C Experimental data

This section presents the ^{1}H NMR spectra generated in this study.

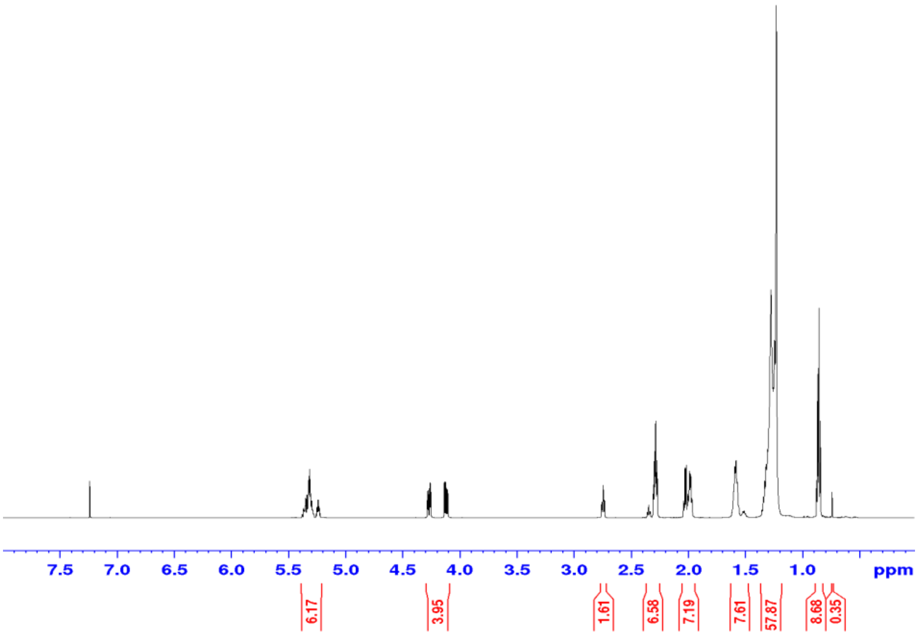

Figure C- 1: ${}^{1}H$ NMR spectrum of baobab seed oil

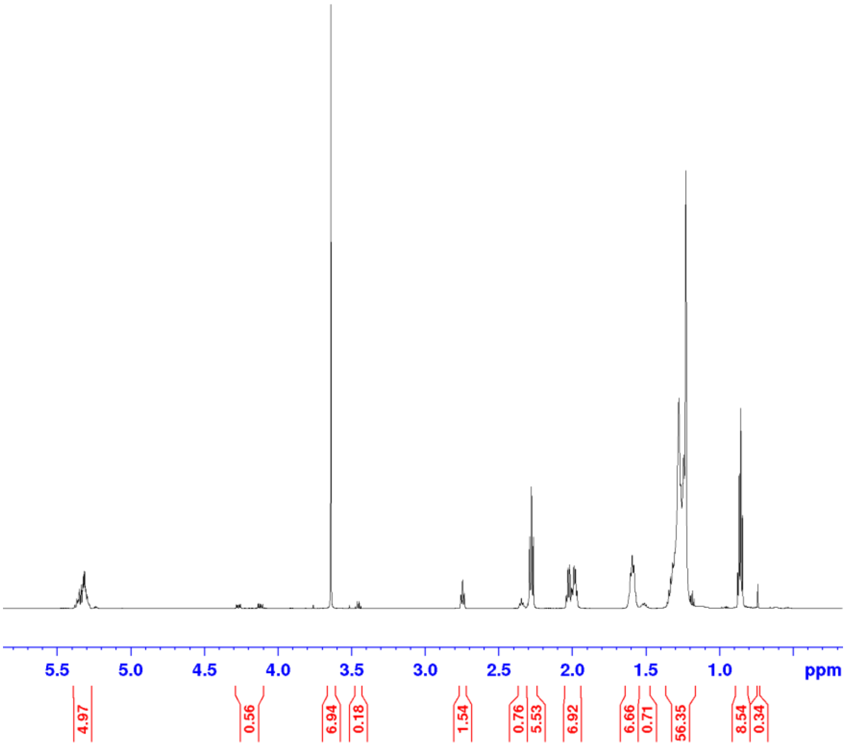

Figure C- 2: ${}^{1}H$ NMR spectrum of baobab biodiesel at 10 wt% Novozyme 435 loading, 1:3 oil to methanol molar ratio, 40℃, 6 hours (lipase screening)

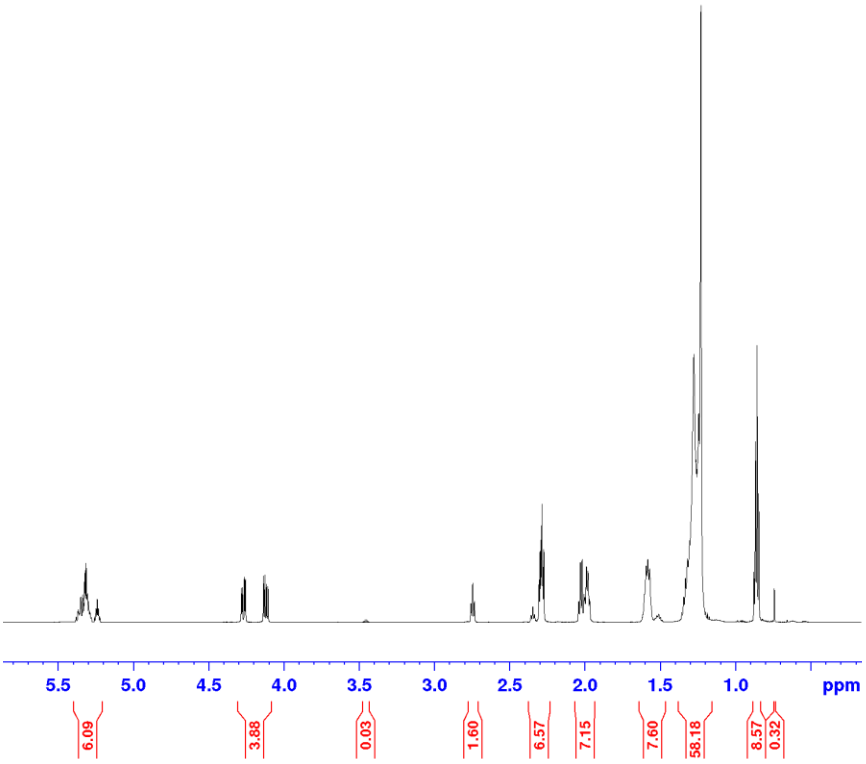

Figure C- 3: ^{1}H NMR spectrum of baobab biodiesel at 10 wt% P. pancreas loading, 1:3 oil to methanol molar ratio, 40℃, 6 hours

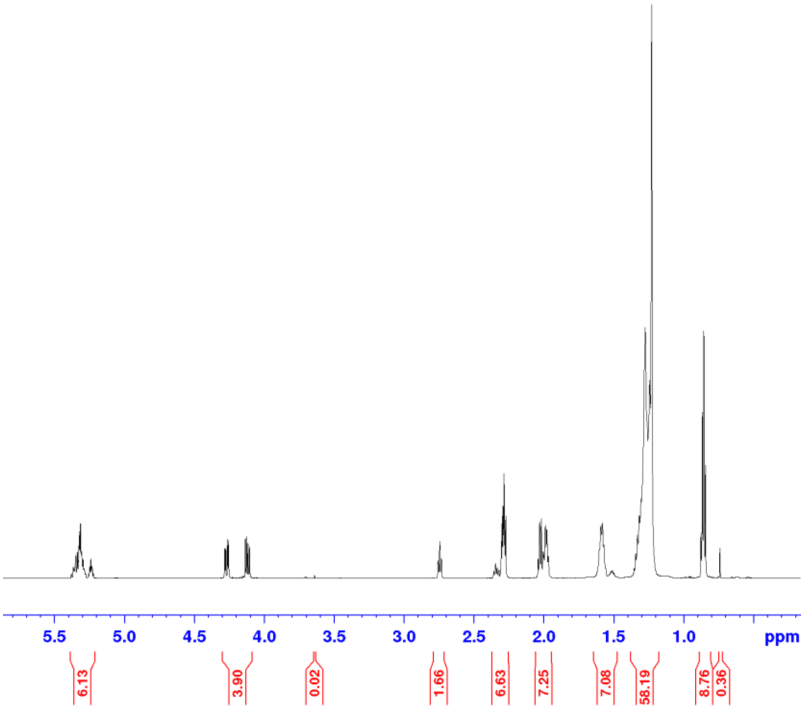

Figure C- 4: ^{1}H NMR spectrum of baobab biodiesel at 10 wt% C. rugosa loading, 1:3 oil to methanol molar ratio, 40℃, 6 hours

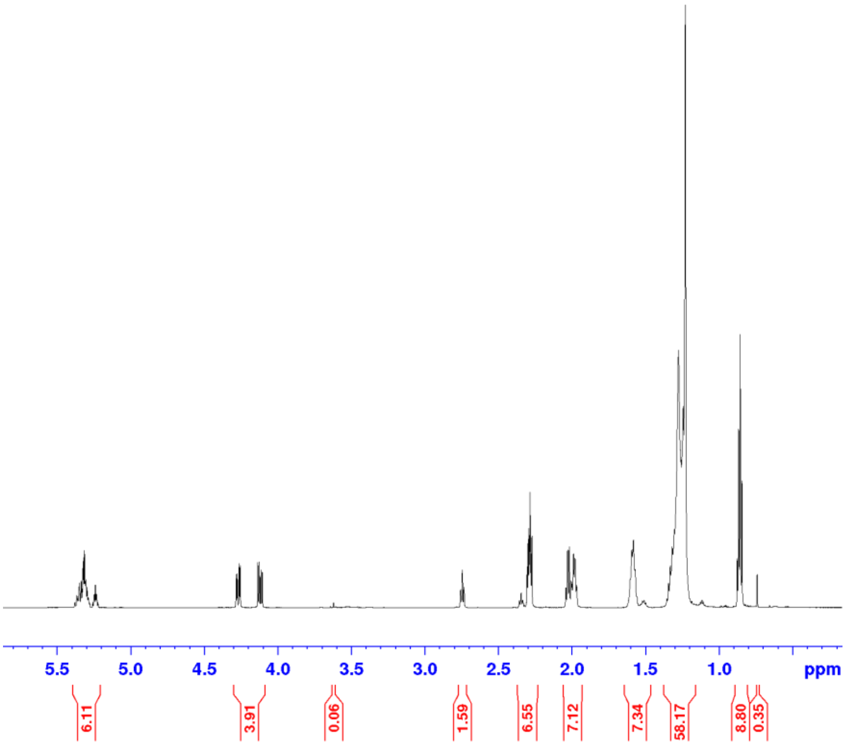

Figure C- 5: ^{1}H NMR spectrum of baobab biodiesel at 10 wt% P. cepacia loading, 1:3 oil to methanol molar ratio, 40°C, 6 hours

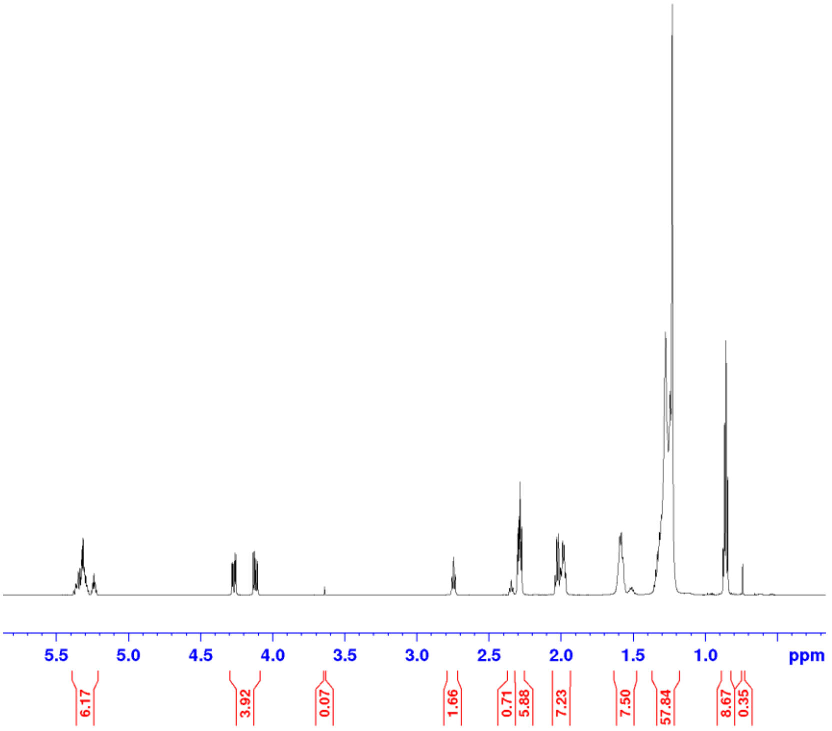

Figure C- 6: ^{1}H NMR spectrum of baobab biodiesel at 10 wt% Candida sp. loading, 1:3 oil to methanol molar ratio, 40°C, 6 hours

The ^{1}H NMR spectra presented in Figure C- 2 to Figure C- 6 were used to determine the catalytic activity of *Novozyme 435, Candida rugosa, Candida sp., Pseudomonas cepacia, and Porcine pancreas* lipases on methanolysis of baobab seed oil. The processed data is presented in Table C- 1.

Table C- 1: ^{1}H NMR data generated from lipase screening experiments

Lipase	**I_{ME}**	**I_{TAG}**	**Conversion (%)**
Novozyme 435	6.94	0.56	87.32
P. pancreas	0.03	3.88	0.43
C. rugosa	0.02	3.9	0.28
P. cepacia	0.06	3.91	0.85
Candida sp.	0.07	3.92	0.98

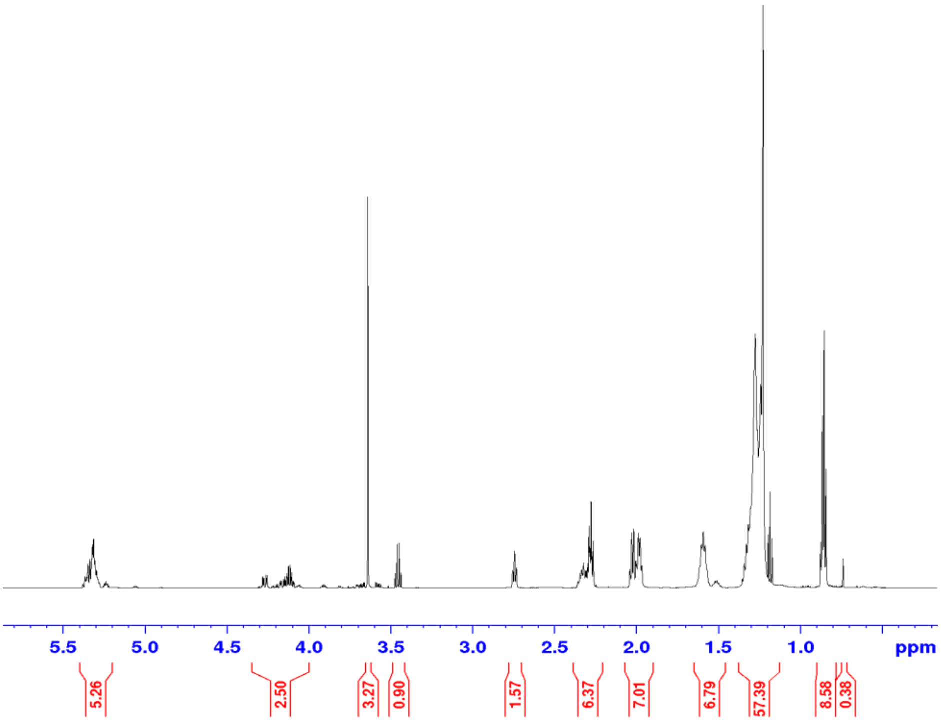

Figure C- 7: ^{1}H NMR spectrum of baobab biodiesel at 10 wt% Novozyme 435 loading, 1:1 oil to methanol molar ratio, 40℃, 6 hours

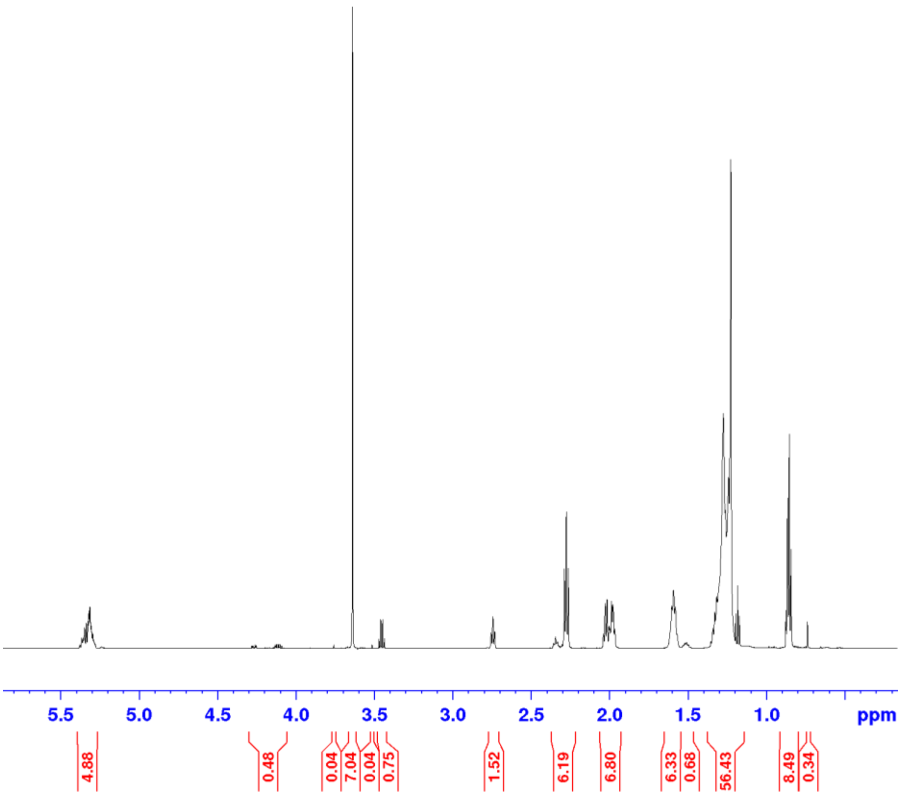

Figure C- 8: ^{1}H NMR spectrum of baobab biodiesel at 10 wt% Novozyme 435 loading, 1:3 oil to methanol molar ratio, 40℃, 6 hours

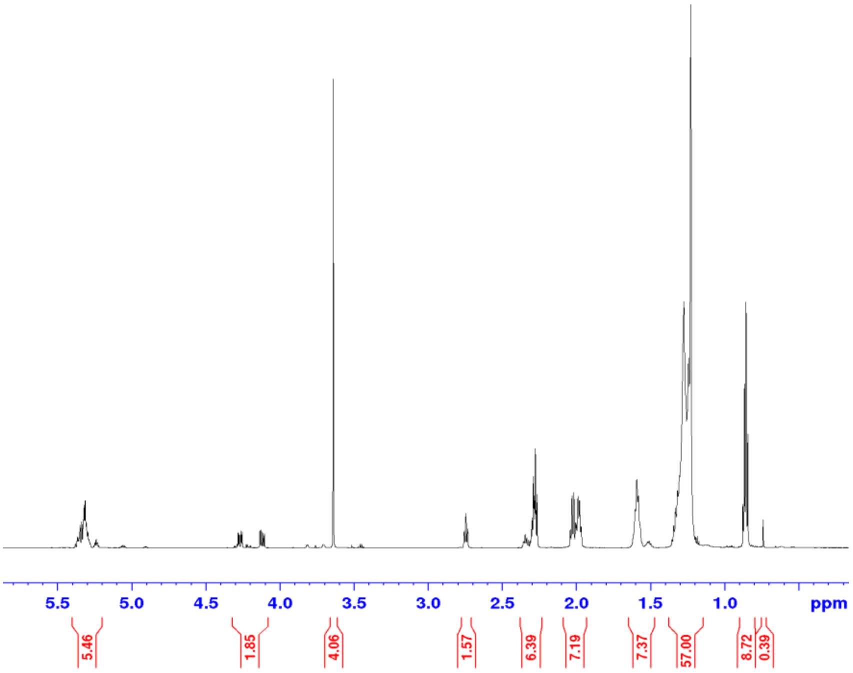

Figure C- 9: ^{1}H NMR spectrum of baobab biodiesel at 10 wt% Novozyme 435 loading, 1:4 oil to methanol molar ratio, 40℃, 6 hours

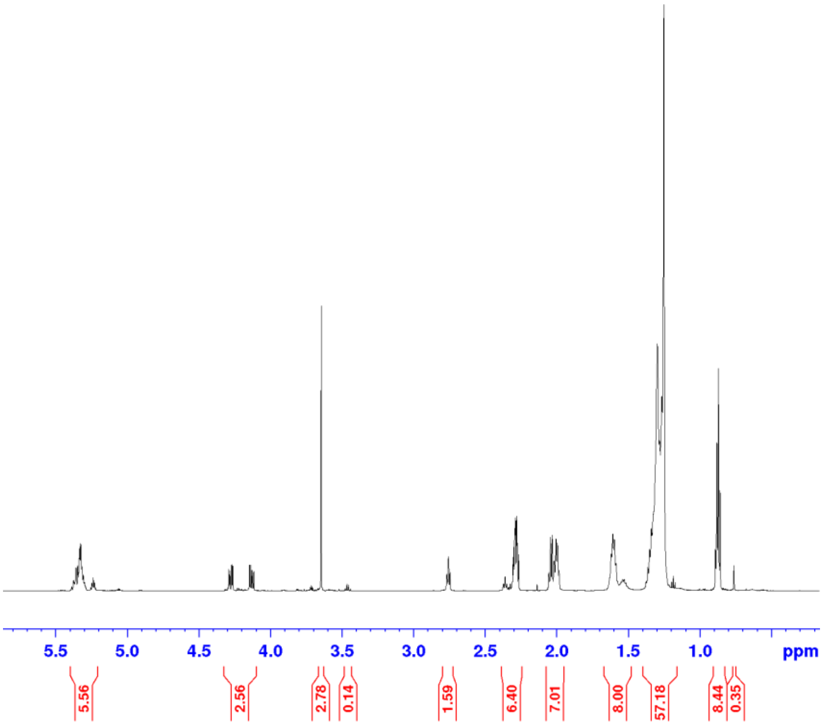

Figure C- 10: ^{1}H NMR spectrum of baobab biodiesel at 10 wt% Novozyme 435 loading, 1:5 oil to methanol molar ratio, 40℃, 6 hours

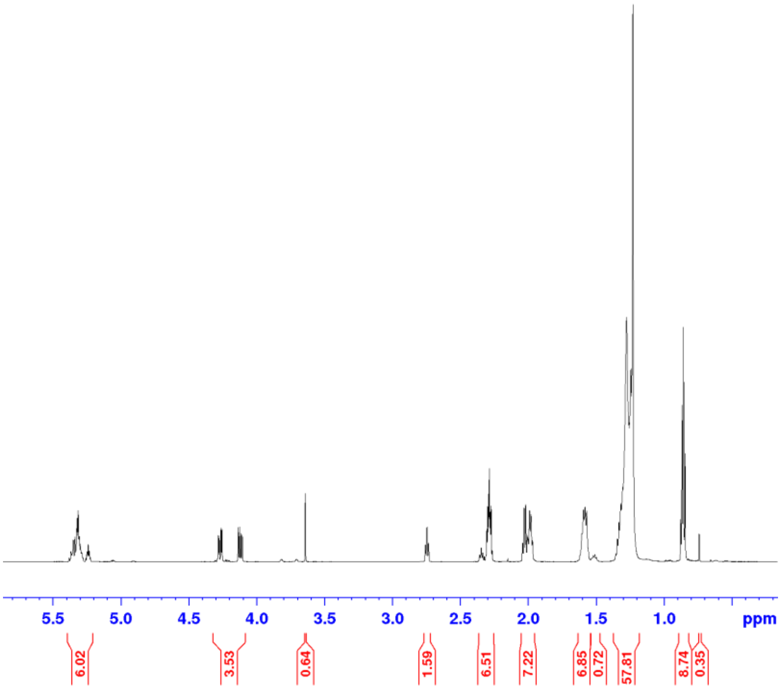

Figure C- 11: ^{1}H NMR spectrum of baobab biodiesel at 10 wt% Novozyme 435 loading, 1:6 oil to methanol molar ratio, 40℃, 6 hours

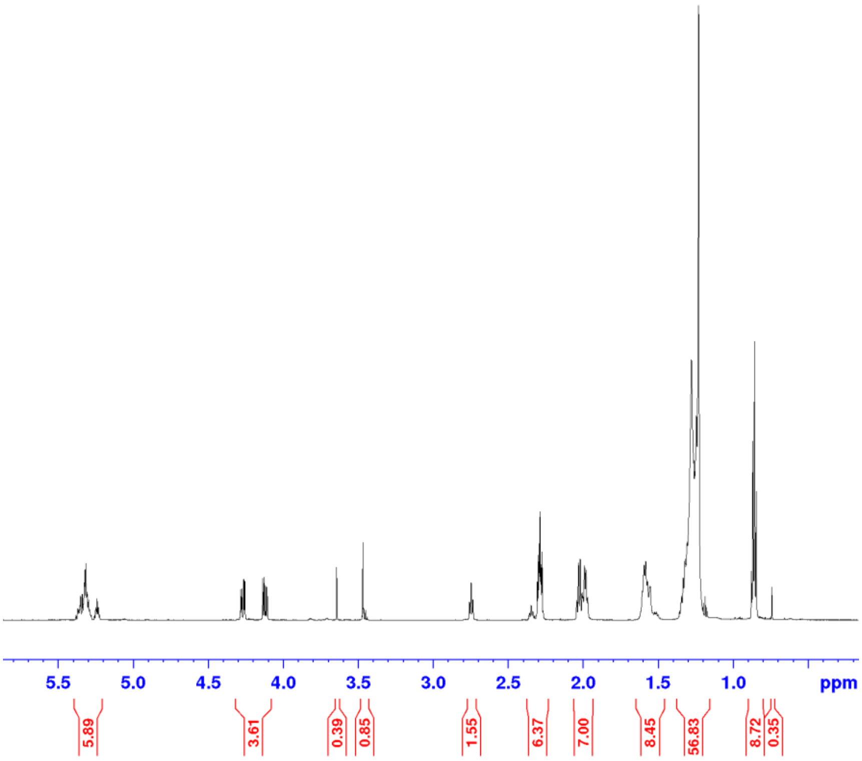

Figure C- 12: ^{1}H NMR spectrum of baobab biodiesel at 10 wt% Novozyme 435 loading, 1:8 oil to methanol molar ratio, 40℃, 6 hours

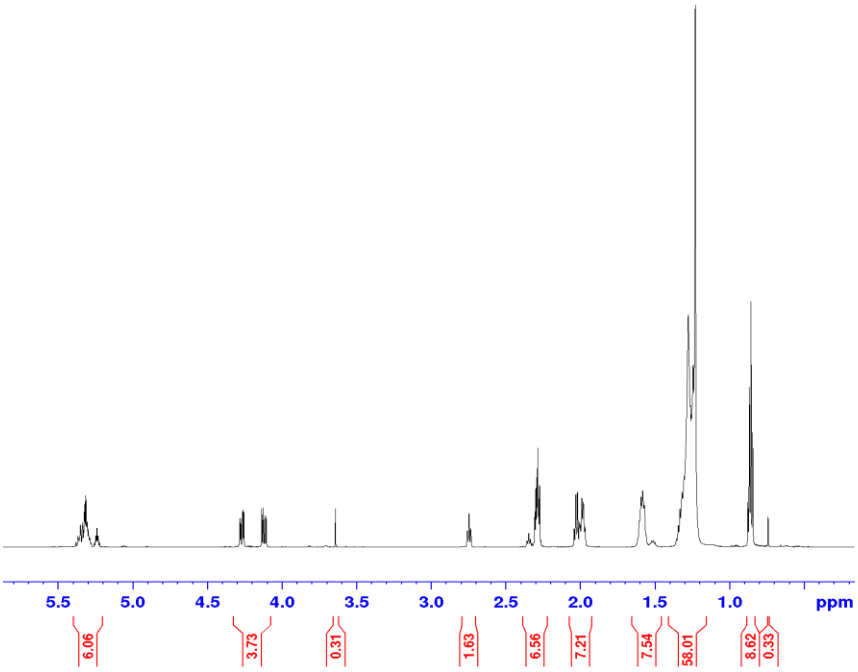

Figure C- 13: ^{1}H NMR spectrum of baobab biodiesel at 10 wt% Novozyme 435 loading, 1:10 oil to methanol molar ratio, 40℃, 6 hours

The ^{1}H NMR spectra presented in Figure C- 7 to Figure C- 13 were used to determine the effect of oil to methanol molar ratio on transesterification of baobab seed oil. The processed data is presented in Table C- 2.

Table C- 2: ^{1}H NMR data generated from experiments on effect of oil to methanol molar ratio

Molar ratio	**I_{ME}**	**I_{TAG}**	**Conversion (%)**
1:1	3.27	2.5	42.08
1:3	7.04	0.48	89.07
1:4	4.06	1.85	54.94
1:5	2.78	2.56	37.63
1:6	0.64	3.53	9.15
1:8	0.39	3.61	5.66
1:10	0.31	3.73	4.41

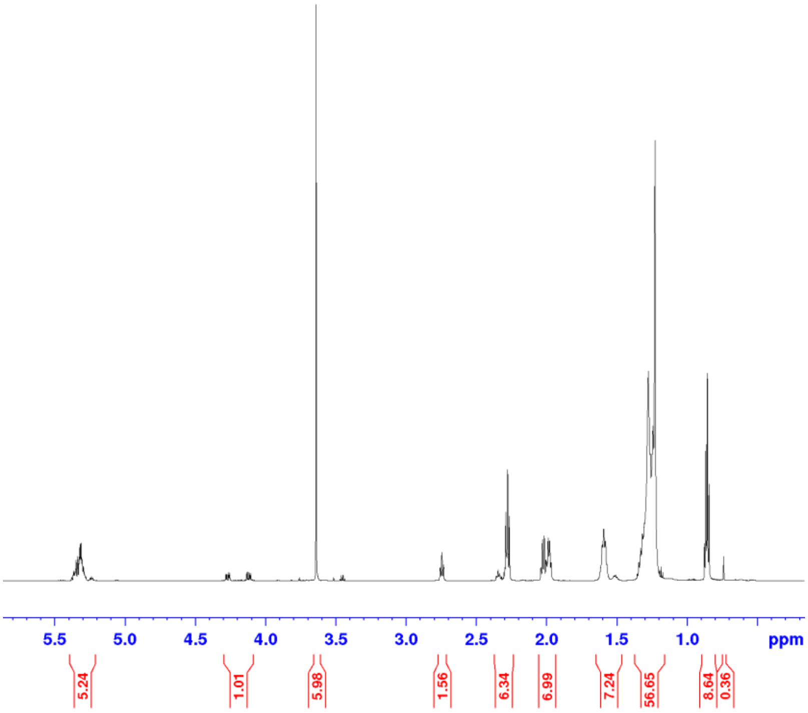

Figure C- 14: ^{1}H NMR spectrum of baobab biodiesel at 10 wt% Novozyme 435 loading, 1:3 oil to methanol molar ratio, 30℃, 6 hours

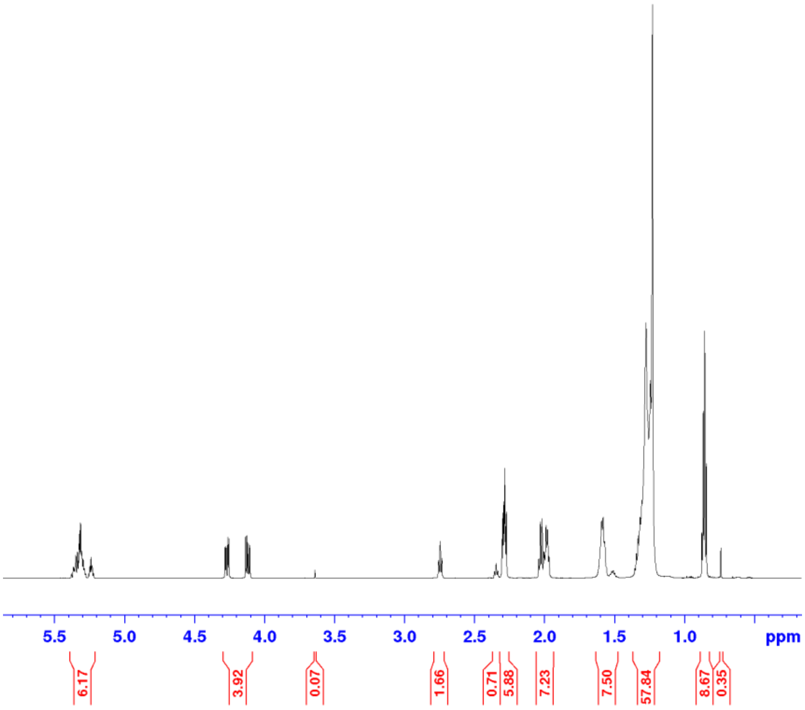

Figure C- 15: ^{1}H NMR spectrum of baobab biodiesel at 10 wt% Novozyme 435 loading, 1:3 oil to methanol molar ratio, 40°C, 6 hours

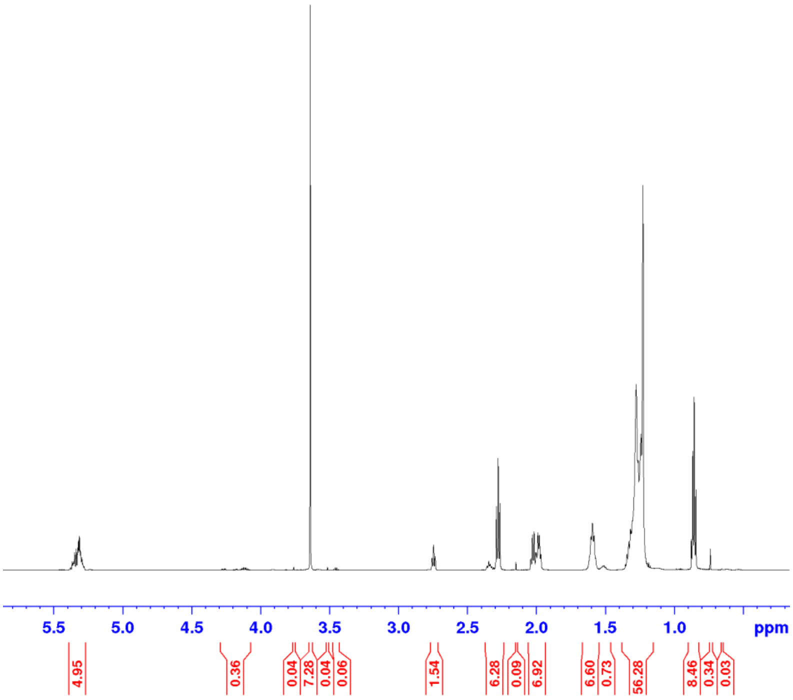

Figure C- 16: ^{1}H NMR spectrum of baobab biodiesel at 10 wt% Novozyme 435 loading, 1:3 oil to methanol molar ratio, 50°C, 6 hours

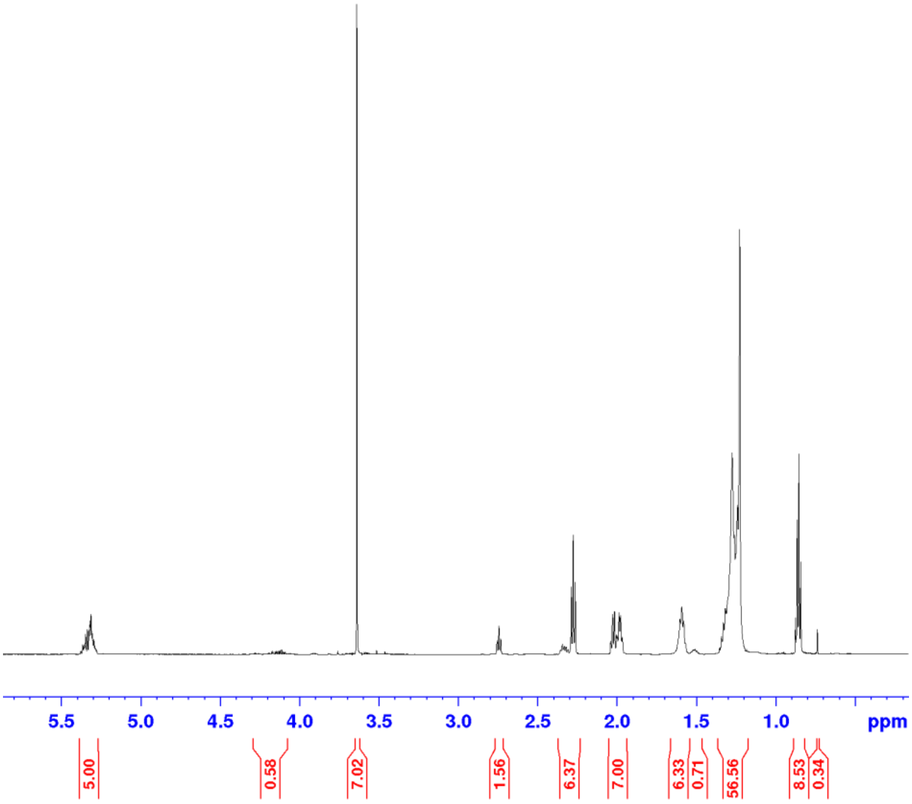

Figure C- 17: ^{1}H NMR spectrum of baobab biodiesel at 10 wt% Novozyme 435 loading, 1:3 oil to methanol molar ratio, 60℃, 6 hours

The ^{1}H NMR spectra presented in Figure C- 14 to Figure C- 17 were used to evaluate the effect of operating temperature on methanolysis of baobab seed oil. The processed data is presented in Table C- 3.

Table C- 3: ^{1}H NMR data generated from experiments on effect of reaction temperature

Temperature (°C)	**I_{ME}**	**I_{TAG}**	**Conversion (%)**
30	5.98	1.01	76.69
40	6.94	0.56	87.32
50	7.28	0.37	91.62
60	7.02	0.58	87.05

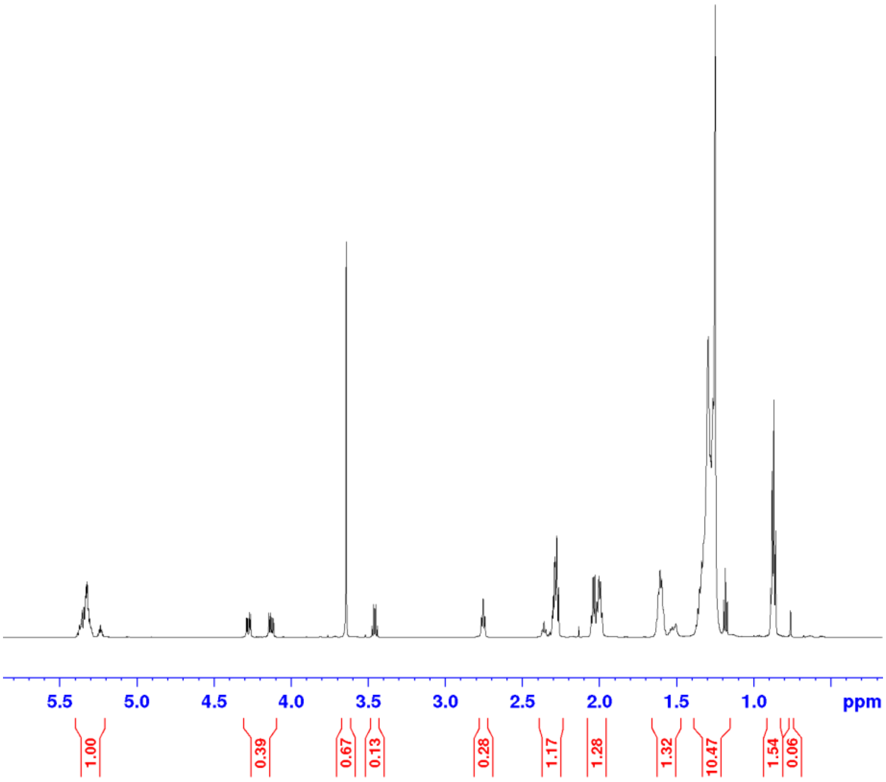

Figure C- 18: ^{1}H NMR spectrum of baobab biodiesel at 10 wt% Novozyme 435 loading, 1:3 oil to methanol molar ratio, 50°C, 0.5 h

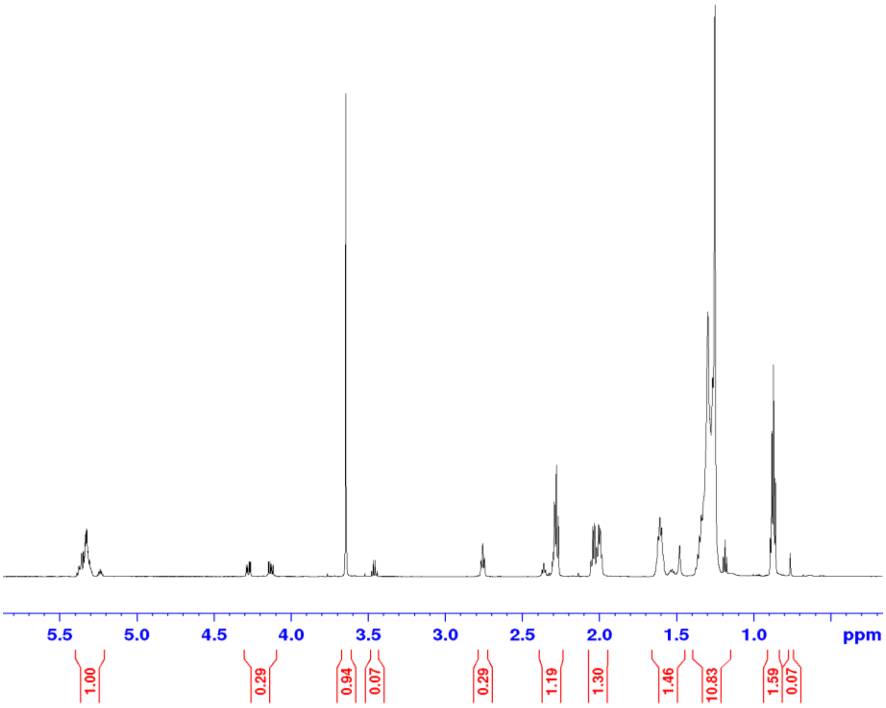

Figure C- 19: ^{1}H NMR spectrum of baobab biodiesel at 10 wt% Novozyme 435 loading, 1:3 oil to methanol molar ratio, 50°C, 1 hour

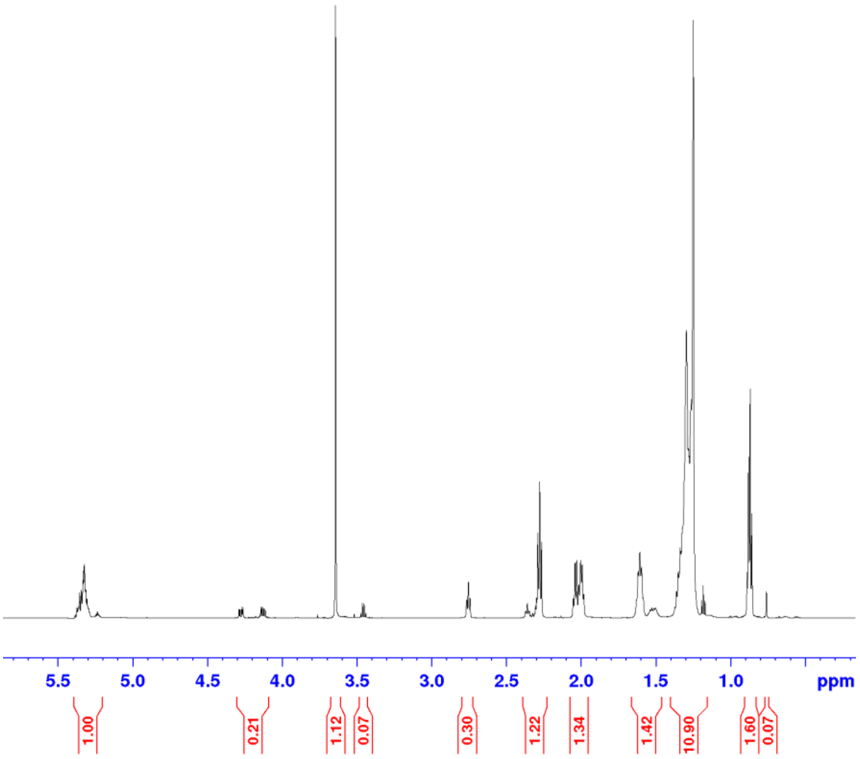

Figure C- 20: ^{1}H NMR spectrum of baobab biodiesel at 10 wt% Novozyme 435 loading, 1:3 oil to methanol molar ratio, 50℃, 1.5 hours

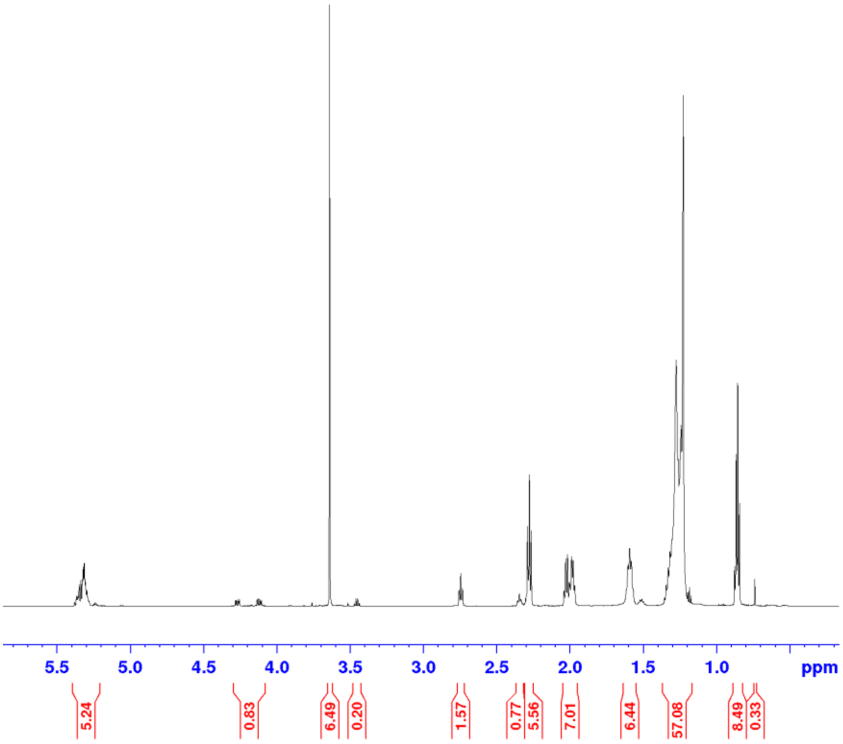

Figure C- 21: ^{1}H NMR spectrum of baobab biodiesel at 10 wt% Novozyme 435 loading, 1:3 oil to methanol molar ratio, 50℃, 3 hours

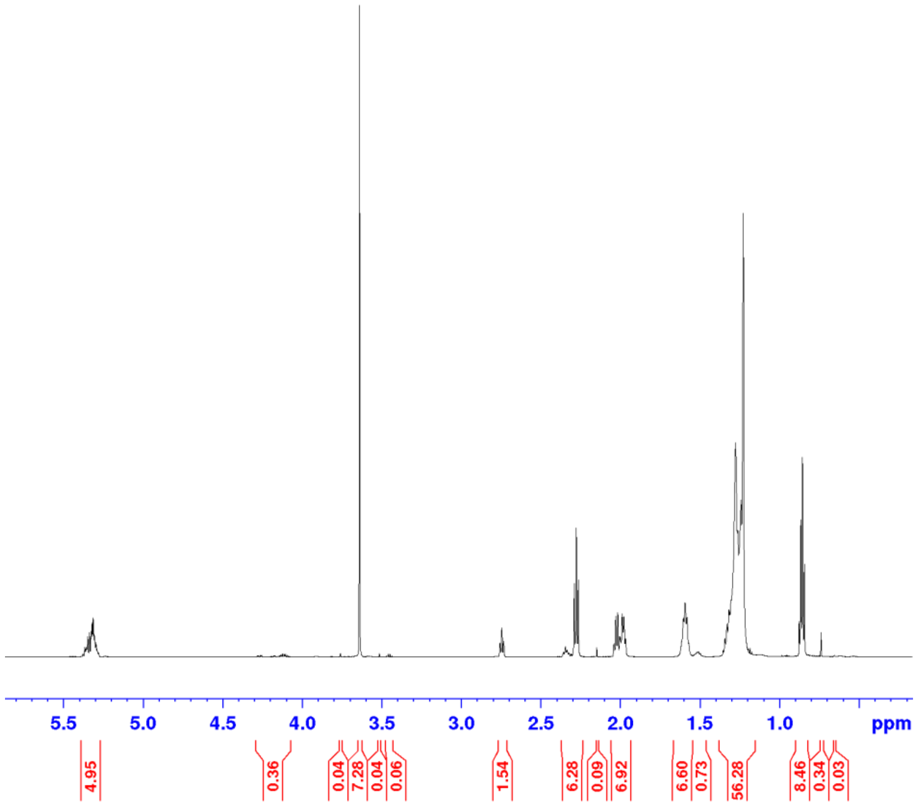

Figure C- 22: ^{1}H NMR spectrum of baobab biodiesel at 10 wt% Novozyme 435 loading, 1:3 oil to methanol molar ratio, 50℃, 6 hours

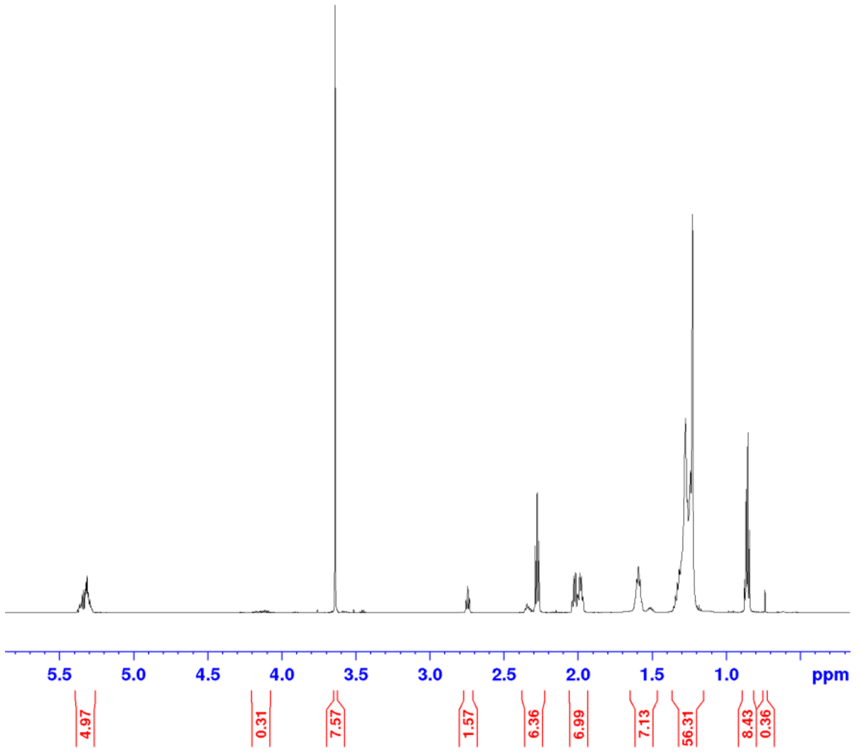

Figure C- 23: ^{1}H NMR spectrum of baobab biodiesel at 10 wt% Novozyme 435 loading, 1:3 oil to methanol molar ratio, 50℃, 9 hours

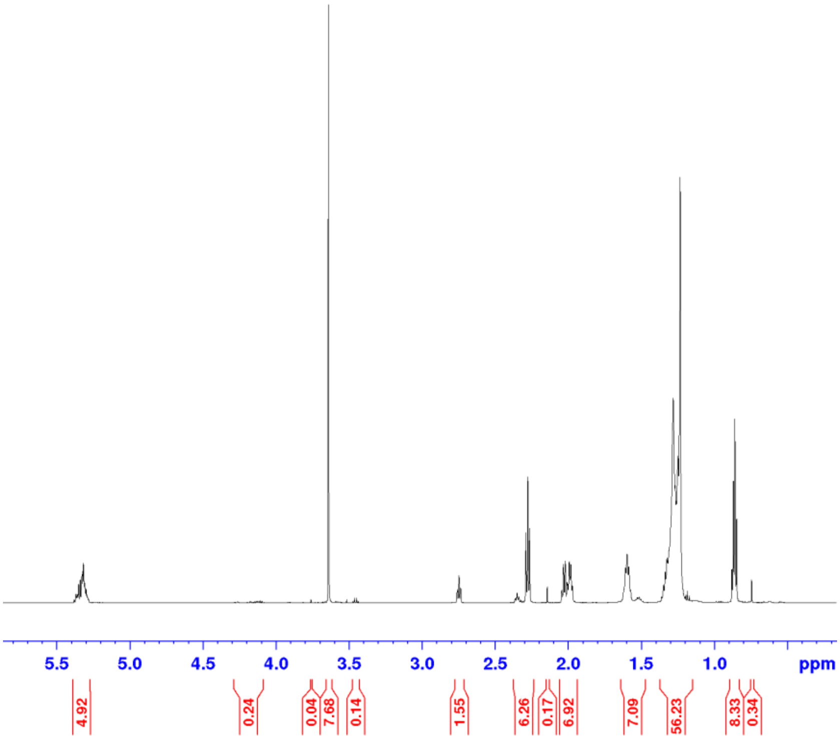

Figure C- 24: ^{1}H NMR spectrum of baobab biodiesel at 10 wt% Novozyme 435 loading, 1:3 oil to methanol molar ratio, 50℃, 12 hours

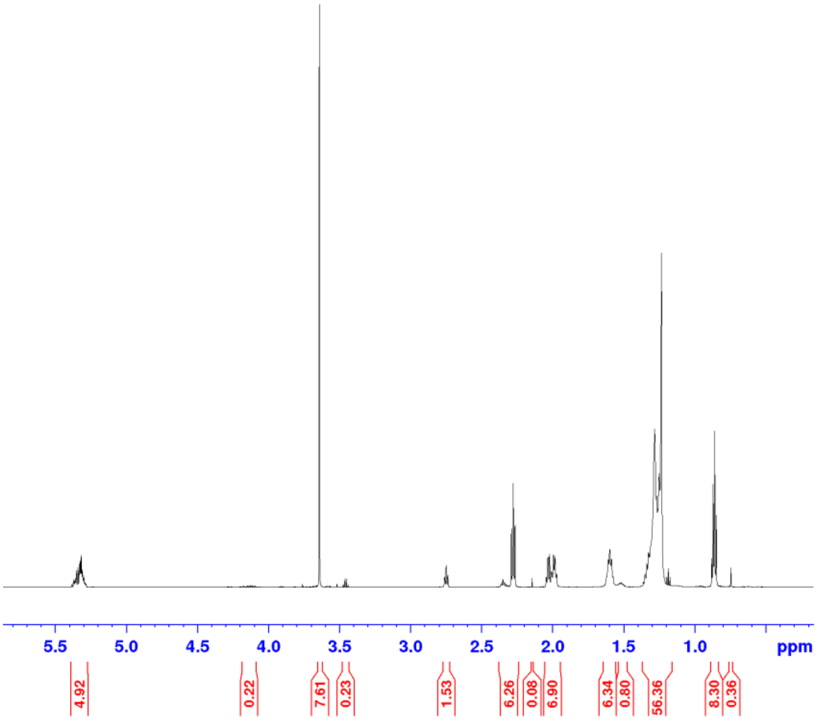

Figure C- 25: ^{1}H NMR spectrum of baobab biodiesel at 10 wt% Novozyme 435 loading, 1:3 oil to methanol molar ratio, 50℃, 15 hours

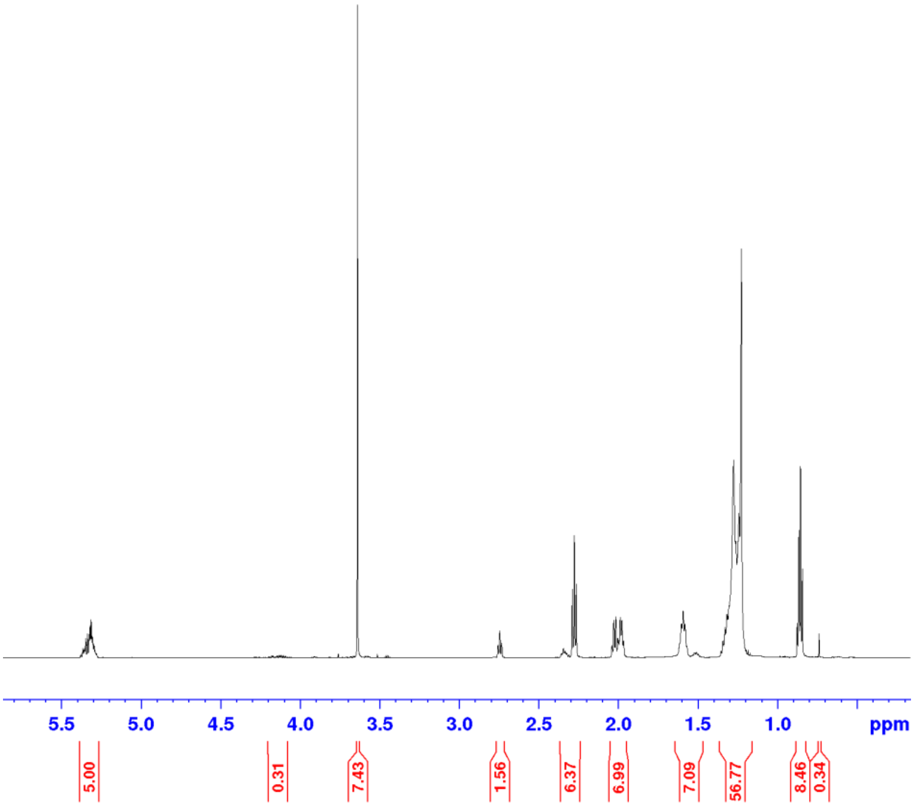

Figure C- 26: ^{1}H NMR spectrum of baobab biodiesel at 10 wt% Novozyme 435 loading, 1:3 oil to methanol molar ratio, 50℃, 18 hours

The ^{1}H NMR spectra presented in Figure C- 18 to Figure C- 26 were used to determine the effect of reaction time on methanolysis of baobab seed oil. The processed data is presented in Table C- 4.

Table C- 4: ^{1}H NMR data generated from experiments on effect of reaction time

Time (hr)	I_{ME}	I_{TAG}	Conversion (%)
0.5	0.39	0.67	24.44
1	0.94	0.29	64.30
1.5	1.12	0.21	74.77
3	6.49	0.83	81.29
6	7.28	0.36	91.83
9	7.57	0.31	93.13
12	7.68	0.24	94.67
15	7.61	0.22	95.05
18	7.43	0.31	93.01

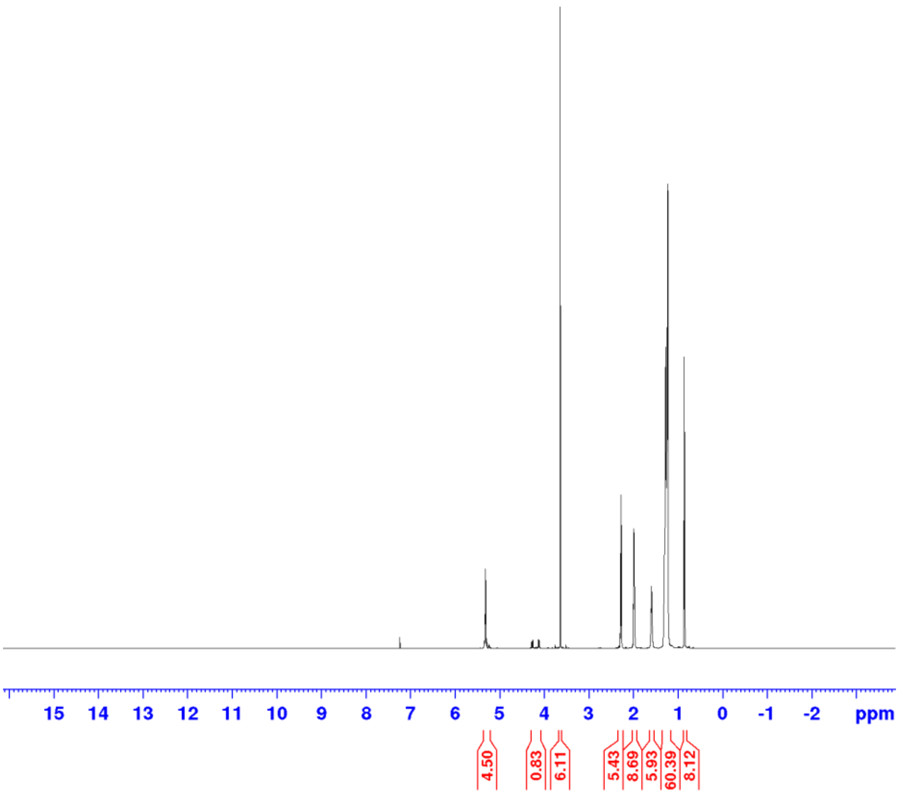

Figure C- 27: ^{1}H NMR spectrum of moringa biodiesel at 10 wt% Novozyme 435 loading, 210 µl methanol loading, 50℃, 6 hours

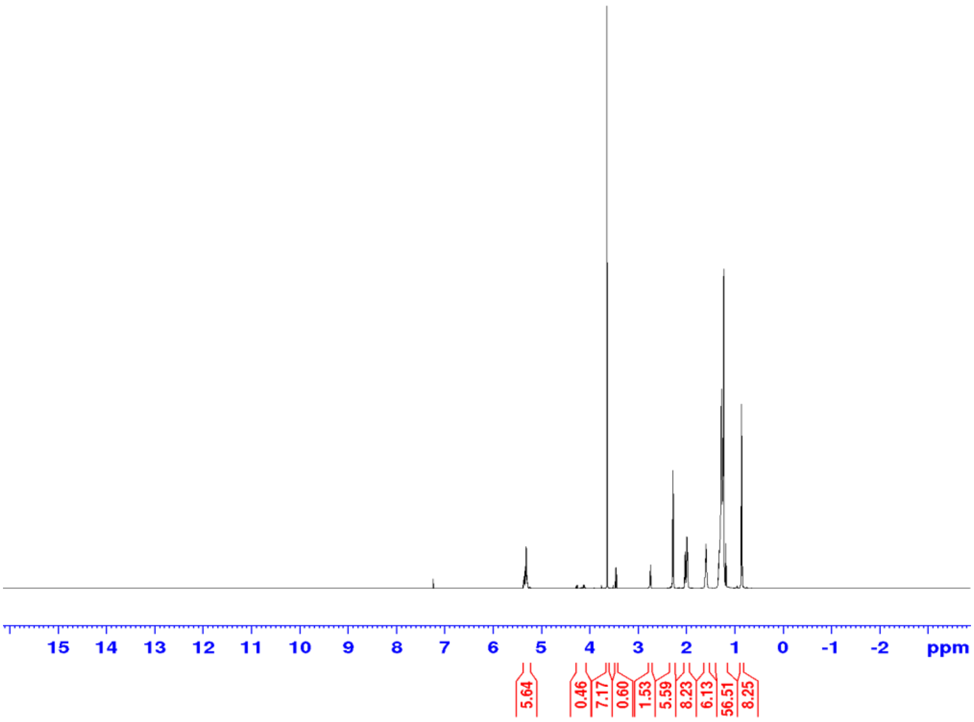

Figure C- 28: ^{1}H NMR spectrum of yangu biodiesel at 10 wt% Novozyme 435 loading, 210 µl methanol loading, 50°C, 6 hours

The ^1H NMR spectra presented in Figure C- 27 to Figure C- 28 were used to determine the conversion of moringa, calodendrum capense and ceiba pentandra oils to biodiesel. The processed data is presented in Table C- 5.

Table C- 5: ^1H NMR data generated from methanolysis of moringa and yangu oils

Oil source	**I_{ME}**	**I_{TAG}**	**Conversion (%)**
Moringa	6.11	0.83	80.4
Calodendrum capense	7.17	0.46	89.6

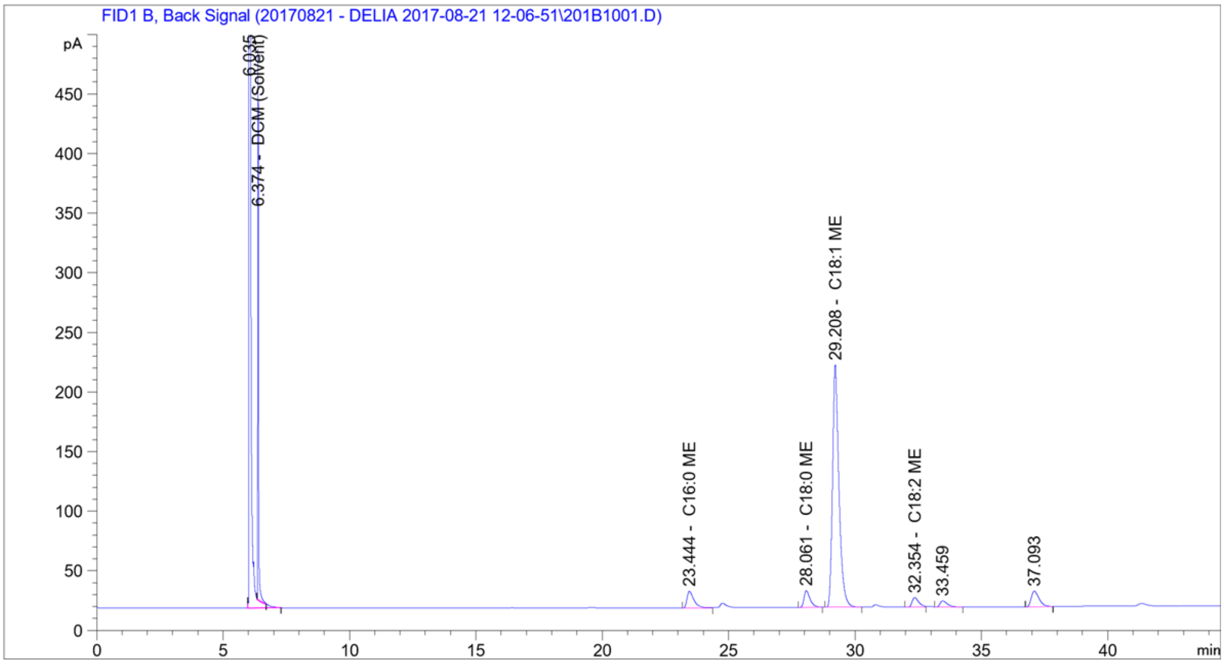

Figure C- 29: GC chromatogram of moringa oil

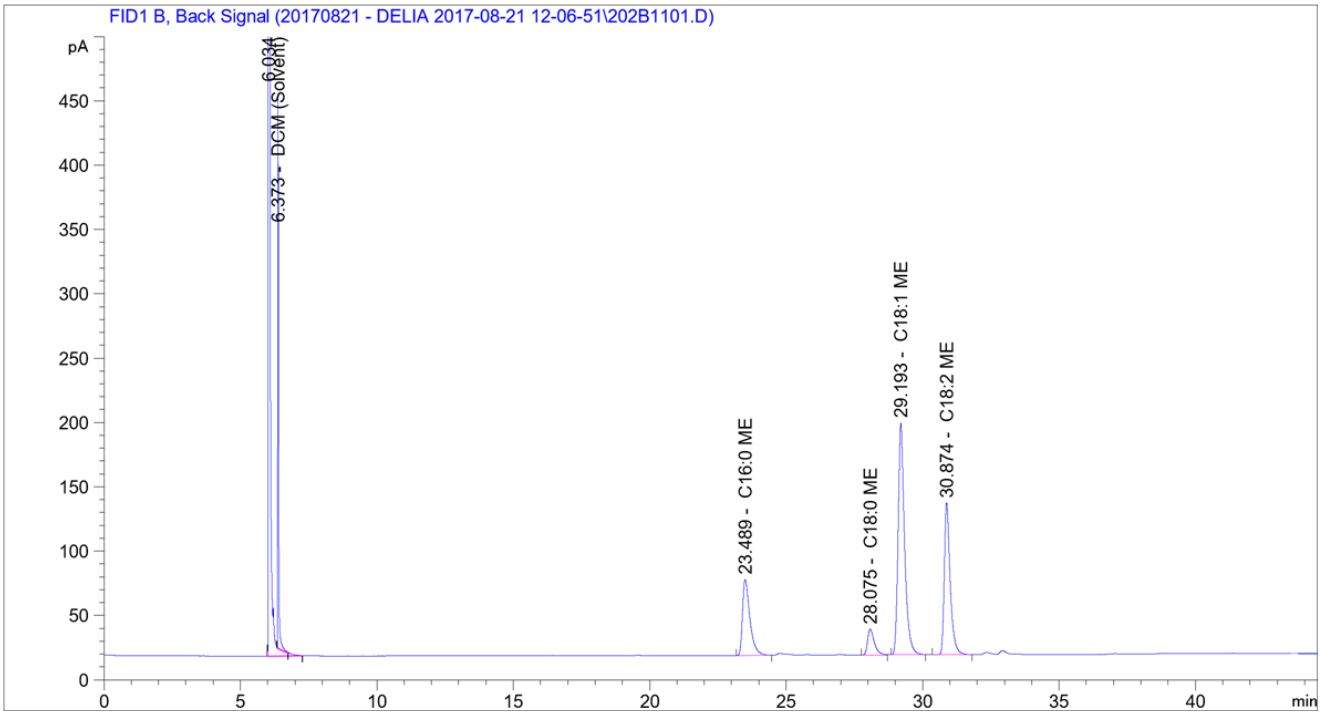

Figure C- 30: GC chromatogram of yangu oil

Appendix D GC-MS Report

The GC-MS report obtained for baobab oil is given below:

```
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Library Search Report

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  Data File : Run 1 Delia.D
  Acq On    : 19 Oct 2017  12:54
  Operator  : Gideon
  Sample    : Run 1Delia
  Misc      :
  ALS Vial  : 1   Sample Multiplier: 1

  Search Libraries:    C:\Database\NIST11.L                Minimum Quality:    0

  Unknown Spectrum:    Apex minus start of peak
  Integration Events: ChemStation Integrator - autoint1.e

Pk#      RT  Area%           Library/ID                     Ref#      CAS#     Qual
_____________________________________________________________________________________
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                   Tridecanoic acid, 12-methyl-, meth       95899 005129-58-8 94
                   yl ester
                   Methyl 8-methyl-nonanoate                50078 1000336-43-6 76

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                   Pentadecanoic acid, methyl ester        107595 007132-64-1 96
                   Methyl 13-methyltetradecanoate          107583 1000336-31-4 93
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                    (Z)-
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                    (Z)-
                   9-Octadecenoic acid (Z)-, methyl e      141303 000112-62-9 93
                   ster

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                   Pentadecanoic acid, 14-methyl-, me      119425 005129-60-2 97
                   thyl ester
                   Hexadecanoic acid, methyl ester         119408 000112-39-0 93
                   Dodecanoic acid, 10-methyl-, methy       84513 005129-65-7 64
                   l ester

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                   Methyl 9,12-heptadecadienoate           127645 1000336-36-2 91
                   9-Hexadecyn-1-ol                         92512 1000342-40-3 89

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                   cis-10-Heptadecenoic acid, methyl       129385 1000333-62-1 99
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                   -, methyl ester
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                   Hexadecanoic acid, 15-methyl-, met      131321 006929-04-0 97
                   hyl ester
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                   Cyclohexene, 1,2-dimethyl-                5976 001674-10-8 58

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                   9,12-Octadecadienoic acid, methyl       139708 002462-85-3 99
                                    Page 1
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                    methyl ester
                    8,11-Octadecadienoic acid, methyl   139707 056599-58-7 99
                    ester

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                     ester
                    9,12-Octadecadienoic acid, methyl  139708 002462-85-3 96
                    ester
                    10,13-Octadecadienoic acid, methyl 139716 056554-62-2 96
                     ester

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                    13-Octadecenoic acid, methyl ester 141286 056554-47-3 86
                    14-Octadecenoic acid, methyl ester 141287 056554-48-4 86

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                    Isobutyl 3-(perhydro-5-oxo-2-furyl  72329 093306-10-6 25
                    )propionate
                    4-Hydroxylamino-6-methylpyrimidin-  18847 006220-22-0 22
                    2(1H)-one

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                    yl ester
                    Heptadecanoic acid, 16-methyl-, me 143185 005129-61-3 93
                    thyl ester

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                    1-Methyl-2-methylenecyclohexane      6010 002808-75-5 70
                    Cyclohexanol, 1-ethynyl-            10506 000078-27-3 49

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                    -, methyl ester
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                    -, methyl ester, cis-
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                    ster

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                    trans-13-Octadecenoic acid, methyl 141314 1000333-61-3 90
                     ester

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                    Eicosanoic acid, methyl ester      166219 001120-28-1 98
                    Heptadecanoic acid, methyl ester   131301 001731-92-6 94

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                    en-2-one
                    E-2-Octadecadecen-1-ol             117619 1000131-10-2 38
                    (S,S,S,S)-1,1'-Bicyclopentyl-2,2'-  56254 1000099-28-5 30
                    dicarboxaldehyde

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                    Hexadecanoic acid, 2-hydroxy-1-(hy 169234 023470-00-0 68
                    droxymethyl)ethyl ester
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                       Glycerol 1-palmitate                   169227 000542-44-9 47

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                       Eicosanoic acid, methyl ester          166218 001120-28-1 95
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                       2-hydroxy-1-(hydroxymethyl)ethyl e
                       ster
                       E,Z-1,3,12-Nonadecatriene              112663 1000131-11-3 87
                       n-Propyl 9,12-octadecadienoate         162978 1000336-77-8 70

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                       9-Octadecenoic acid (Z)-, 2-hydrox 166116 004500-01-0 91
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                       Methyl stearate                        143126 000112-61-8 83
                       Nonadecanoic acid, methyl ester        154941 001731-94-8 80

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                       Methyl tetradecanoate                   95859 000124-10-7 76
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                       .beta.-Sitosterol                      217432 000083-46-5 97
                       17-(1,5-Dimethylhexyl)-10,13-dimet 217457 1000210-86-9 44
                       hyl-4-vinylhexadecahydrocyclopenta
                       [a]phenanthren-3-ol

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