BIODIESEL ANALYTICAL DEVELOPMENT AND CHARACTERISATION

By

Ebenezer Prah

Thesis Submitted In Partial Fulfilment for the Requirement of

MASTER OF SCIENCE IN ENGINEERING

(CHEMICAL ENGINEERING)

In the Department of Process Engineering

Stellenbosch University

March 2010

Supervisors:

Dr. Linda Callanan & Prof. Leon Lorenzen

DECLARATION OF ORIGINALITY

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and I have not previously in its entirety or in part submitted it at any university for a degree.

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Signature Date

SUMMARY

Development of analytical methods to characterise biodiesel has become central to the overall success of the marketing of biodiesel fuel. In this regard, different bodies including the American Society for Testing and Materials (ASTM) and the European normalization (EN) have come up with various methods to determine important biodiesel parameters such as total glycerol, methanol and the fatty acid methyl esters (FAMEs), etc. Various studies have been conducted on the parameters mentioned above using a variety of instrumentation and sample preparations. The best methods reported are those that have been adopted by both the ASTM and EN standards.

The purpose of this study was to develop alternative analytical methods to both the recommended ASTM and EN methods and, in some cases, to make modifications to both standards (ASTM D 6571 and EN 14214) and methods to determine total and bound glycerol, the ester content and also methanol content in biodiesel. Moreover, water washing after transesterification and the effect this practice has on biodiesel cold flow properties such as kinematic viscosity, cloud and pour point and density were evaluated. The possibility of using the iodine value to predict the feedstock source of an unknown biodiesel was also investigated. Six different vegetable oil samples were transesterified with methanol and used for this study. The six samples used were palm, crown, sunflower, waste vegetable oil (wvo), peanut and rapeseed biodiesel.

Quantitative results indicated that the use of programmable temperature volatilisation (PTV) for total glycerol did not produce the required repeatability of between 1-4% relative standard deviation(RSD) for total glycerol analyses in biodiesel with precision of 25%, 86%, 25% and 56% for free glycerol (FG), monoglycerides (MG), diglycerides (DG), and triglycerides (TG) respectively. The standard requires a relative standard of between 1-4%

As an alternative to the method using gas chromatography, normal phase high performance chromatography (HPLC) with binary gradient elution was used to determine the bound glycerol content. This method proved accurate and repeatable with RSD % of 0.33, 1.12, and 1.2 for TG, DG and MG respectively.

Following the EN14103 protocol (European standard ester determination), the Zebron ZB-WAX column which is comparable to the specification recommended by EN14103 but afforded the determination of ester content from the esters of myristic acid ($C_{14:0}$) to behenic

acid ($C_{22:0}$) with reproducibility with RSD % of 6.81, 1.91, 7.27, 0.64, 1.18, 1.55, 6.03, 1.96, and 5.21 for methyl esters of myristic, palmitic, stearic, oleic, linoleic, linolenic, arachidoic, gadoleic and behenic acid respectively.

Solid phase micro extraction (SPME) using GC-MS was developed as an alternative to both the EN14110 and ASTM D93 protocols for determining the methanol content in biodiesel. For this method, polyethylene glycol fibre (PEG) was used together with a deuterated methanol internal standard and a DB-FFAP (60m×0.25um×0.25um) column. Less volume of sample was required as compared to the EN14214 method. This method was found to be sensitive, accurate and repeatable with a RSD % of 4.82.

The Iodine number of biodiesel decrease compared to their corresponding feed stock and therefore predicting the feed stock of an unknown biodiesel was going to be difficult .Results from this study indicated that it is not possible to predict the feed stock source of an unknown biodiesel from its iodine value.

The effect of water washing after phase separation on biodiesel cold flow properties such as kinematic viscosity, density, cloud and pour point depended on the type of biodiesel produced. We observed that water washing after transesterification caused an increase in all the cold flow properties of sunflower biodiesel, whereas only the densities and kinematic viscosities increased in the case of palm and waste vegetable oil biodiesel. The cloud and pour point of the latter two diesel samples remained unchanged after water washing. Thus, the effect of water washing on biodiesel cold flow depended on the type of biodiesel.

Blending a highly saturated biodiesel (fewer numbers of double bonds) with a less saturated biodiesel (higher number of double bonds) resulted in an improvement of both the pour and cloud points of the resultant biodiesel blend.

OPSOMMING

Die ontwikkeling van analitiese metodes om biodiesel te karakteriseer word tans as ‘n kernmaatstaf gesien om biodiesel suksesvol te bemark. Hiervoor het verskeie liggame wat die Amerikaanse Vereniging vir Toetsing van Materiale (AVTM) en die Europese Normalisering (EN) insluit met verskeie standaard analitiese metodes vorendag gekom om belangrike biodiesel parameters soos bv. totale gliserol, metanol en vetsuur metielesters te meet. Om hierdie parameters te bepaal is van ‘n wye verskeidenheid toetse met verskillende instrumente en monsterbereidings gebruik gemaak. Die beste metodes is deur beide die AVTM en EN aanvaar.

Die doel van hierdie studie was om metodes te ontwikkel wat as alternatiewe kan dien tot die wat deur die AVTM en EN voorsgeskryf is. In sommige gevalle is aanpassings tot beide die standaarde (AVTM en EN) en metodes aangebring om die totale en gebonde gliserol-, ester- en metanolinhoud te bepaal. Verder is die effek van ‘n water wasstap na transesterifikasie op biodiesel se kouevloei eienskappe gevalueer wat eienskappe soos kinematiese viskositeit, vertroebelingspunt, gietingspunt en digtheid insluit. Die moontlike gebruik van die Jodiumpunt om die bron van die voerstof van ‘n onbekende diesel te bepaal is ook ondersoek. In hierdie studie is ses verskillende oliemonsters van plantaardige oorsprong gebruik wat d.m.v. metanol getransesterifiseer is. Hierdie monsters het palm-, kroon-, sonneblom-, afvalplant-, grondboontjie- en raapsaadolie ingelsuit.

Tydens die studie is programmeerbare temperatuur vervlugtiging (PTV) vergelyk met in-kolom inspuiting soos deur AVTM D6584/EN14214 vir totale gliserol analise voorgeskryf. Kwantitatiewe resultate het getoon dat die PTV metode nie die verlangde akkuraatheid van ‘n relatiewe standaardafwyking (RS) van 1-4% vir beide vrye en gebonde gliserol kon handhaaf nie. Die akkuraatheid was in die omgewing van 25%, 86%, 25% en 56% vir vrye gliserol (VG), monogliseriede (MG), digliseriede (DG) en trigliseriede (TG), onderskeidelik.

Normale fase hoë werkverrigting vloeistofchromatografie met ‘n binêre elueeringsgradiënt is as alternatief tot gaschromatografie (GC) ondersoek om die gebonde gliserolinhoud te bepaal. Al was die GC metode meer sensitief, het die vloeistofchromatografie metode ‘n hoë graad van akuraatheid en herhaalbaarheid getoon met RS% waardes van 0.33, 1.12 en 1.2 wat vir TG, DG en MG, onderskeidelik, verkry is.

‘n Zebron ZB-WAX kolom is vir die EN14103 protokol gebruik. Behalwe vir ‘n groter lengte kon hierdie kolom met spesifikasies soos deur EN14103 voorgeskryf vergelyk word. Met die gebruik van hierdie kolom kon die esterinhoud van miristiensuur ($C_{14:0}$) tot behensuur ($C_{14:0}$) bepaal word. ‘n Hoë graad van herhaalbaarheid met RS% waardes van 6.81, 1.91, 7.27, 0.64, 1.18, 1.55, 6.03, 1.96 en 5.21 vir die metielesters van miristien-, palmitien-, stearien-, oleïn-, linoleïn-, linoleen-, aragidoon-, gadoleïen- en behensuur is onderskeidelik verkry.

Om die metanolinhoud van die biodiesel te bepaal is soliede fase mikroekstraksie (SFME) m.b.v. gaschromatografie-massaspektrometrie (GC-MS) as alternatiewe tot EN14110 en AVTM D93 ontwikkel. In hierdie metode is daar van poliëtileenglikolvesels (PEG) en gedeutereerde metanol saam met ‘n DB-FFAP kolom (60 mm x 0.25 mm x 0.25 mm) gebruik gemaak. Hierdie metode het ‘n kleiner monstervolume as die EN14214 metode benodig en was sensitief, akkuraat en hehaalbaar wat tot ‘n RS% waarde van 4.82 gelei het.

Op grond van die Jodiumwaarde van biodiesel en hul ooreenstemmende voerstowwe het hierdie studie bevind dat die Jodiumwaarde nie gebruik kan word om die voerstof van ‘n onbekende diesel kan voorspel nie.

Die effek van ‘n water wasstap na faseskeiding op verskeie kouevloei eienskappe soos kinematiese viskositeit, vertroebelingspunt, gietingspunt en digtheid het van die tipe diesel afgehang. Dit is bevind dat ‘n water wasstap na transesterifikasie ‘n toename in al die kouevloeieienskappe van sonneblomdiesel tot gevolg gehad het. In teenstelling hiermee het slegs die kinematiese viskositeit en digtheid van palm- en afvalplantdiesel vermeerder terwyl hul vertroebelings- en gietingspunte onveranderd gebly het. Die hipotese dat ‘n water wasstap na transesterifikasie tot swak kouevloei eienskappe lei is dus as onwaar bevind aangesien hierdie eienskappe deur die tipe biodiesel bepaal word.

Deur ‘n hoogs versadigde biodiesel (lae aantal dubbelbindings) met ‘n minder versadigde biodiesel (hoë aantal dubbelbindings) te vermeng het tot ‘n verbetering van beide die vertroebelings- en gietingspunte gelei.

ACKNOWLEDGEMENTS

I would like to thank God Almighty for the ability He graciously bestowed upon me. I will remain forever thankful to Him. Moreover, I would like to thank my supervisors, Prof. Leon Lorenzen and Dr. Linda Callanan for an interesting topic and also for the attention and time. Mention also goes to SANERI for the funding provided me for my sustenance on Stellenbosch campus and Mrs. A Van Zyl and H. Botha for the guidance through GC and HPLC techniques. Lastly, and certainly not the least my parents Mr and Mrs Prah, for their sweat and toil to get me where I am today.

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ANN	Artificial Neural Network
APCI-MS	Atmospheric Pressure Chemical Ionization-Mass Spectrometer
ASTM	American Society of Testing and Materials
B100	Hundred Percent Biodiesel
CFPP	Cold Filter Plugging Point
IV	Iodine Value
CN	Cetane Number
CP	Cloud Point
^{13}C-NMR	Carbon 13-Nuclear Magnetic Resonance
DG	Diglycerides
DIN	German biodiesel standard
EN	European Normalization
FAMES	Fatty Acid Methyl Esters
FFA	Free Fatty Acids
FID	Flame Ionization Detection
FTIR	Fourier Transform Infra Red
FTNIR	Fourier Transform Near Infra Red
GC	Gas Chromatography
GHG	Green House Gas

GPC	Gel Permeation Chromatography
H-MNR	Proton- Nuclear Magnetic Resonance
HPLC	High Performance Liquid Chromatography
HT	High Temperature
IS	Internal Standard
RSD	Relative Standard Deviation
LC	Liquid Chromatography
MERO	Methyl Esters of Rapeseed Oil
MG	Monoglycerides
MS	Mass Spectrometry
NARP-HPLC	Non-Aqueous Reverse Phase High Performance liquid Chromatography
NIR	Near Infra Red
NO_x	Nitrogen Oxides
ONORM	Austrian biodiesel standard
PCA	Principal Component Analysis
PLS	Partial Least Square
PP	Pour Point
PTV	Programmable Temperature Volatilization
MSTFA	N-methyl-N-trimethylsilyltrifluoroacetamide
RP-HPLC	Reverse Phase High Performance Liquid Chromatography

SABS	South African Bureau of Standards
SD	Standard Deviation
SIM	Selected Ion Monitoring
THF	Tetra Hydro Furan
TG	Triglycerides
TLC	Thin Layer Chromatography
UV	Ultra Violet
VOMEs	Vegetable Oil Methyl Esters

1 INTRODUCTION

The recent increases (the pre-financial crisis of 2008-09) in crude oil prices and the dwindling petroleum reserves have led to a considerable debate among world leaders about the future of petroleum based fuels and the need for alternative energy sources. This has come about because of the total dependence on petroleum as the only major energy source and also because of the instability in the Middle East which has majority of the world's crude oil reserve (Byron, 2007). More recently, the issue of the environment with regard to petrochemical emissions and their contributions to problems such as global warming and acid rain have all necessitated the need for alternative energy sources.

Research has been conducted and is still ongoing for alternative renewable energy sources such as solar energy, wind and hydro energy and most importantly on biofuels (Meher *et al.,* 2006). Among the biofuels, biodiesel seems to be at the forefront because of its environmental credentials such as renewability, biodegradability and clean combustion behaviour (Hanna, 1999). Biodiesel has gained increasing support as an alternative to fossil diesel due to the fact that it is non toxic, has a closed carbon cycle, and is essentially free of sulphur and aromatics. Moreover, its use will shift total dependence on fossil fuels and help save expenditure on petroleum for nations that rely heavily on petroleum for their energy needs of which the majority of nations do (Tickell, 2003).

Apart from the fact that biodiesel can be a diesel fuel substitute, it can also be used in any mixture with petrol diesel since it has properties that are similar in characteristics to mineral diesel. Biodiesel and mineral diesel mixtures are denoted by Bxx, where xx refers to the volume percentage of biodiesel in the mixture (Monteiro, 2008). For instance, B20 refers to a biodiesel and mineral diesel mixture with a 20 volume of biodiesel.

The manufacture of biodiesel is simple and uncomplicated. Any oil bearing seed, and also animal fat, can be used as a feed stock for the production of biodiesel. Since oils have different characteristic compositions, biodiesel produced from different oils will likely have different chemical and physical compositions and more importantly different properties. For instance the presence of fatty acids in feed stocks may differ in percentage composition leading to differences in properties such as cloud and pour points.

Table 1:1 lists the fatty acid composition of some different feed stocks that can used in the production of biodiesel.

Table 1-1 Component acids of the major oils, wt %,(Padley, 1994).

Oil Source	16:0	18:0	18:1	18:2	18:3	Other
Corn	13	3	31	52	1	-
Cottonseed	27	2	18	51	Trace	2
Groundnut	13	3	38	41	Trace	$C_{20\text{-}24}5$
Linseed	6	3	17	14	60	-
Olive	10	2	78	7	1	2
Palm	44	4	40	10	Trace	2
Palm olein	40	4	43	11	Trace	2
Palm stearin	47-69	~5	20-38	4-9	Trace	-
Rape (low erucic)	4	2	56	26	10	20:1 2
Rice bran	16	2	42	37	1	2
Safflower(high linolenic)	7	3	14	75	-	1
Safflower (high oleic)	6	2	74	16	-	2
Sesame	9	6	38	45	1	1
Soybean	11	4	22	53	8	2
Sunflower (high linoleic)	6	5	20	69	Trace	-
Sunflower (high oleic)	4	5	81	8	Trace	2
Tall oil	5	3	46	41	3	2

16:0-palmitic acid *18:0-stearic acid* *18:1 oleic acid*

18:2-Linoleic acid *18:3 linolenic acid* *other- % of other fatty acids*

The presence of other factors like saturated and unsaturated bonds in the feed stocks may also differ in terms of percentage compositions in most feed stocks used in the production of the biodiesel and this can result in differences in chemical behaviour between biodiesel samples. Biodiesel composition and therefore its properties, is completely dependent on the feed stock source used to produce it (Stauffer, 2007).

Currently, there are no regulations in place regarding the type of feed stock that can be used in the production of biodiesel although the inclusion of certain parameters such as the iodine

and acid values indirectly limit the use of feed stocks with high degree of unsaturation and free fatty acid respectively.

The quality of biodiesel produced is of great importance to consumer confidence and its commercialisation. Currently, there is a debate within the biodiesel industry over how much quality control is necessary and whether current test methods for the end product biodiesel are rigid enough (Weiksner, 2007). It should be emphasized that poorly produced biodiesel can operate diesel fuelled equipment in the short term without noticeable effect but with possible engine damage or breakdown in the long term. Once a poorly produced biodiesel starts to deteriorate, nothing can be done to stop it.

1.1 PROJECT MOTIVATION

Since biodiesel can be produced from varied feed stocks resulting in biodiesel with different properties, it has become necessary to have a standard that will serve as a point of reference for biodiesel that is produced from all feed stocks to guarantee engine performance without difficulty. The biodiesel produced is not classified as diesel fuel substitute unless they meet the requirements established by standards such as the ASTM D6571and EN14214. This has led to the establishment of standards in different parts of the world. Some of these standards are the ASTM (America), ONORM (Austria), and DIN (Germany). European countries have unified their standards and have come out with a single standard called the EN 14214. South Africa currently uses the SAN 1935 Automotive diesel fuel standard. This standard (SAN 1935 automotive standard) document is a slight modification of the EN14214 standard and the South African Bureau of Standards (SABS) noticed some discrepancies with this method. According to the SABS, the SANS 1935 has the following weakness/limitations:

- It specifies the iodine value of the biodiesel. This specification will eliminate certain biodiesel feed stocks which have high degrees of unsaturation putting pressure on biodiesel producers regarding the kind of feed stocks that could be used. It specifies an Iodine value (IV) of 140 g I_2/100g sample.
- It defines biodiesel as fatty acid methyl esters although there are transesterification reactions that involve the use of ethanol and propanol as the alcohol for the reaction forming ethyl and propyl esters thus making the definition of biodiesel as methyl esters very narrow.

- It indicates the properties of biodiesel meant to be used directly as a pure fuel without blending. However, it does not take into account the dilution effects of blends; it requires that the same requirements be applied to the Biodiesel that are meant for blending (Nolte, 2007).

With these loopholes encountered in the SANS 1935 automotive standard applied to biodiesel and due to the current upsurge of interest in biodiesel in South Africa and Africa, there is an urgent need for a well defined biodiesel standard in South Africa and Africa in general that will be comparable to both the American and European standards.

Studies have been carried out regarding the qualitative and quantitative characteristics of biodiesel. Most of these studies were carried out using chromatographic and spectroscopic methods and in some cases wet chemistry with chromatography being the most extensively used in the study and analysis of biodiesel components. Most of the ASTM and EN14214 standards recommend the use of Gas Chromatography in the determination of biodiesel parameters such as free and total glycerol accompanied by complex sample preparation and lengthy analysis time. The extensive use of especially gas chromatography (GC) is due to its ability to quantify minor components in biodiesel at the level required by the standards (Knothe, 2001). Since there are problems associated with the methods recommended in both the American society of testing and materials (ASTM) and European normalization (EN) standards, there is the need for alternatives to these methods recommended by ASTM and EN.

The main disadvantage of biodiesel, aside, the nitrogen oxides (NOx) emissions are its unfavourable cold flow properties since it begins to gel at low temperatures which can clog filters or even become so thick that it cannot be pumped from the fuel tank to the engine (Joshi *et al.,* 2007). This can have dangerous effects on the engine such as filter blockage and engine breakdown. Therefore, there is the need for an investigation into transesterification practices such as washing of the ester phase as a purification step and their subsequent effect on biodiesel cold flow properties such as cloud point, pour point, kinematic viscosity and density.

1.2 RESEARCH OBJECTIVES/HYPOTHESIS

This study has set as it goals using six different kinds of biodiesel originating from palm, rapeseed, crown, sunflower, waste vegetable oil (wvo), and crown oils to test the hypotheses that:

- The repeatability afforded by on-column injectors in GC analysis of total glycerol in biodiesel is achievable with the programmable temperature volatilisation (PTV) injector when following the procedure recommended by the ASTM D 6584 protocol.

- Normal phase – high performance liquid chromatography with binary gradient elution is suitable for the determination of bound glycerol and free fatty acids that occur in biodiesel after transesterification.

- A Zebron ZB-WAX column with similar column specifications to those recommended by EN14103 is suitable for the determination of ester and linolenic acid content.

- Headspace solid phase micro extraction (SPME) coupled to GC-MS offers a better alternative to headspace GC-FID for the determination of methanol content in biodiesel.

- The iodine value (IV) could be used to predict the feed stock source of an unknown biodiesel.

- Water washing of biodiesel after phase separation leads to poor cold flow properties such as kinematic viscosity, pour and cloud points as well as density of biodiesel.

- Blending a highly saturated biodiesel with a least saturated biodiesel may improve the cloud and pour points of the least saturated biodiesel.

1.3 STUDY OUTLINE

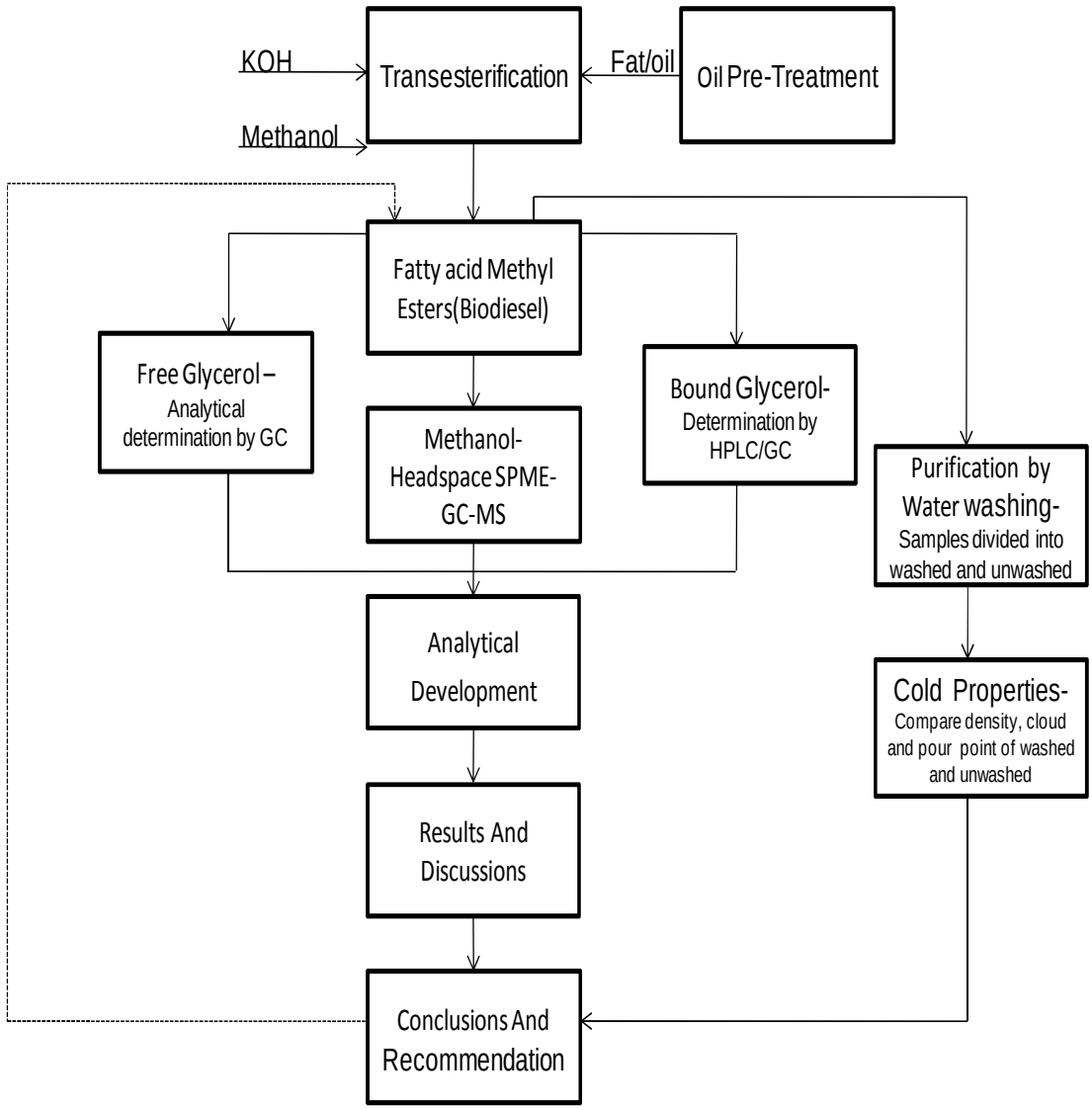


Figure 1-1: Proposed analytical plan for Biodiesel samples

The next chapter discusses the some literature information on the chemical compositions and reactions of oil and biodiesel respectively. It also discusses the transesterification reaction, compares and tabulates the differences in composition of mineral diesel and biodiesel. Analytical methods so far employed in the biodiesel analysis and characterisation are looked at.

2 LITERATURE REVIEW

The literature review looks at the chemistry of oils and possible reactions that they undergo which can affect and alter the chemical compositions of biodiesel. It also discusses the parameters that define the quality of biodiesel. Analytical methods so far employed in the analysis of the constituents of biodiesel and their shortcomings are evaluated. The manufacturing process of biodiesel is out of the scope of this work and will therefore just be mentioned in certain sections but not expanded on.

2.1 Introduction

Biodiesel, known and defined as the mono alkyl esters of fatty acids, is derived from the transesterification of vegetable oils with monohydric alcohol, usually methanol even though other alcohols such as ethanol and propanol have been considered (Joshi et al., 2007). There are considerable analytical challenges associated with the control of the product quality during and after production, and a variety of analytical methods have been used (Ingvar, 2007).

Quality standards are necessary for the commercial use of biodiesel, as sceptics are not too keen to have their vehicles/equipment run on the fuel. These standards serve as a guideline for the production process, guarantee customers that the fuel they are buying has passed the necessary quality checks and therefore, should not entertain any fears regarding damages to their equipment, and provide authorities with approved tools for the assessment of safety risks and environmental pollution (Prankl, 1999). Car manufacturers see these standards as a means by which they could issue warranties for their vehicles and/ or equipment to be run on biodiesel.

2.2 Benefits of biodiesel pursuit

One of the major benefits of biodiesel is in their environmental friendliness. Biodiesel has been described as having a closed carbon cycle. This is due to the fact that, the carbon dioxide released as a result of their use in combustion engines is absorbs by another sets of crops that are grown to be used as feed stocks for the next batch of fuels (Fig 2-1). In the process, there is no net significant contribution to the atmospheric carbon dioxide and this therefore helps in the maintenance of the carbon dioxide gas concentration (a major green house gas and a facilitator of global warming) in atmosphere.

This situation of no net release of carbon into the atmosphere is seen by environmentalists as a positive step in resolving environmental pollution issues such as global warming which is mainly caused by mineral diesel emission.

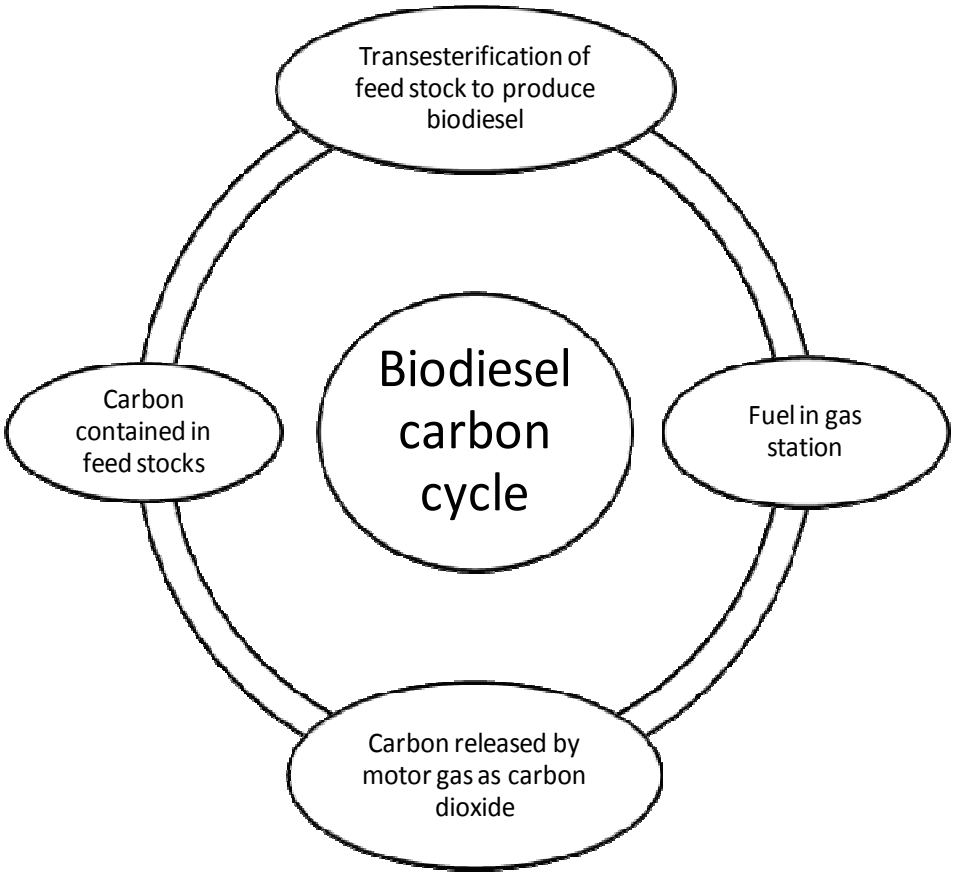


Figure 2-1 Biodiesel carbon cycle (redrawn from Tickell, 2003)

Another crucial benefit of the pursuit of biodiesel is the development of the economies and agriculture of the various countries that pursue biofuels especially biodiesel. Jobs are created right from the farmer who grows the crops to the attendant at the gas station. These auxiliary workers pay taxes to the government which it uses in the provision of vital social infrastructure to its people. Unlike the use of mineral diesel where the income is sent to overseas where the fuel was purchased. The creation of jobs and the development of agriculture will lead to a decrease in the trade deficit of countries since a third of the trade deficit of most countries that import petroleum comes from petroleum. More so, biodiesel provides a means of putting to good use waste materials such as waste cooking oil.

2.3 Background

2.3.1 Chemistry of lipids (A brief Overview)

Lipids (fats and oils) are made up of building blocks, called triglycerides, which results from the combination of one unit of glycerol and three units of fatty acid. The triglyceride molecule is the major component of oils even though monoglycerides and diglycerides may be/are present as minor components (Gunstone, 1996). The monoglycerides are fatty acid monoesters of glycerol. They exist in two isomeric forms, α- and β monoglyceride (Fig 2-2).

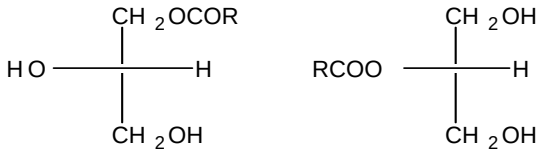


Figure 2-2 α-monoglyceride and β monoglyceride respectively.

The presence of acid or alkali determines the isomeric form that will be present as it is in the case of transesterification where an acid or alkali could be used as catalyst for the reaction. Although, the effects of these isomeric forms on biodiesel quality are unknown, reversed phase high performance liquid chromatography (RP-HPLC) with acetone/acetonitrile and a ultraviolet detection (UV) was used to separate the different isomeric forms that formed during the lipase catalysed transesterification reaction of sunflower oil with methanol (Turkan *et al*., 2006).

Diglycerides are fatty acid diesters of glycerol and like monoglycerides occur in two isomeric forms with the 1, 3-diacylglycerol (Fig 2-3) being the most stable.

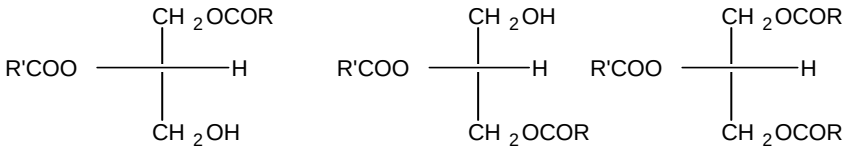


Figure 2-3: 1,2-diacyl-sn-glycerol; 2,3-diacyl-sn-glycerol and 1,3-diacylglycerol respectively.

Technically, and also for the purpose of this study, an oil will be referred to as a triglyceride as this will help in giving a proper insight into the chemistry of the transesterification reaction which leads to the formation of the fatty acid methyl esters (biodiesel).

The main atoms present in a triglyceride molecule are carbon, hydrogen and oxygen as depicted in Fig 2-4.

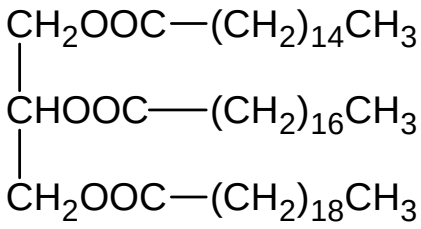


Figure 2-4: Structure of a triglyceride molecule.

The triglyceride molecule is made up of a glycerol backbone of interlinked carbon atoms bound to oxygen atoms. Attached to each of these oxygen atoms is long chain fatty acid of approximately 20 carbon atoms. These fatty acids can separate from the triglyceride molecule in the presence of water to form free fatty acids (FFA). For biodiesel production purposes, the presence of water and FFA in the feedstock presents a major problem to the transesterification reaction. The water deactivates the catalyst and the presence of FFA in the feed stock consumes the catalyst (Nye 1983). Both of these substances affect the yield of the biodiesel. There are different types of fatty acids (usually in terms of percentage composition) in each type of feed stock used in the production of biodiesel. The differences between the different fatty acids occur in the chain length and also the presence of saturated and unsaturated bonds.

Biodiesel produced from oil feed stocks with high percentage composition of saturated fatty acids have unpleasant properties such as a higher cloud and pour points than those with lower percentages of saturated fatty acids. The cloud and pour points are the temperatures at which crystals begin to form in the fuel and the crystallisation becomes so intense the fuel no longer can be poured respectively (Imahara *et al*, 2006). Likewise, biodiesel from feed stocks with a high number of unsaturated fatty acids are more prone to oxidation than their counterparts with fewer unsaturated fatty acids (Knothe, 2007).

The major obstacles encountered when using vegetable oils and fat (Lipids) as diesel fuel substitutes are their high viscosity and very low volatility. Other problems such as their high flash point and the tendency of the oil to polymerize at high temperatures also exist.

In order to circumvent these problems, processes such as micro emulsification, pyrolysis and transesterification are performed on the oils so that their properties conform to that of mineral diesel (Schwab *et al.,* 1987)

Micro emulsions are heterogeneous mixture of an immiscible liquid dispersed in each other. They are transparent or at least translucent and thermodynamically stable and is mostly stabilized by the use of a mixture of surface active agents (Becher, 2001). Micro emulsification of vegetable oils for use as a diesel fuel substitute involves mixing the oil with an alcohol such as methanol and ethanol etc. It was concluded that micro emulsions of vegetable oils with alcohol could not be recommended for long term use in diesel engines based on the same reasons as that for neat oils (Pryde, 1984). For these, Pryde cited reasons such as incomplete combustion and the formation of carbon deposits.

Pyrolysis refers to thermal degradation either in complete absence of an oxidizing agent or with such limited supply that gasification does not occur to an appreciable extent or may be described as partial gasification. Pyrolysis of vegetable and fish oils, optionally in the presence of metallic salts has been employed since World War II as a means of finding alternative to diesel fuel (Knothe, 2001).

Mixtures such as alkanes, alkenes and alkadienes have been produced. Usually the cetane numbers of the oils are increased when they are subjected to pyrolysis. The process has been abandoned because the viscosities of pyrolysed oils were considered too high. Moreover, environment concerns have been raised since the removal of oxygen during pyrolysis eliminates one of the main ecological benefits of oxygenated fuels (Ma and Hanna, 1999).

Transesterification has become the most ideal and effective means to date of modifying vegetable oils to lower their viscosity to the level comparable to mineral diesel so that they can be suitable for use as a diesel fuel substitute. Thus, biodiesel is currently being produced mainly by the use of this process (Demirbas, 2005)

Transesterified vegetable oils are suitable for use in mineral diesel fuelled equipment after minor adaptations and in some other cases without any adaptation at all. The principles of transesterification will be looked at in a more detailed manner in section 2.5.1 of this chapter.

There are other properties of the vegetable oil aside the viscosity that can have a possible effect on diesel equipments and these should be monitored even after the transesterification

reaction. Some of these properties are the level of the free fatty acids, the amount of water that remains after the transesterification among many other properties.

2.4 Biodiesel oxidation

Biodiesel oxidation occurs naturally between unsaturated fatty acids and atmospheric oxygen. The reaction is catalysed by substances such as metals, light, heat and several other elements. Because metals enhance biodiesel oxidation, the storage of biodiesel in metallic containers is strictly discouraged. Antioxidants such as tocopherols which occur naturally in vegetable oils can inhibit biodiesel oxidation but unfortunately are mostly removed during refining processes that take place before the transesterification reaction. The oxidative degradation reactions of biodiesel are mainly influenced by olefinic unsaturation present in the fatty acid chain.

The fatty acid chain is unaffected during the transesterification reaction and, therefore the oxidation chemistry of the biodiesel and the feedstock oil from which it was derived are basically the same (Gunstone, 1996).

In most fatty acids, there are two kinds of arrangements for the unsaturation; the methylene interrupted and the conjugated unsaturation. The conjugated unsaturation is the most thermodynamically stable arrangement due to the delocalisation of the pi electrons and is therefore more likely to resist oxidation than the methylene interrupted unsaturation. Figures 2-5 and 2-6 indicate both methylene interrupted and conjugated structures of linolenic acid.

Figure 2-5: Methylene Interrupted Linolenic Acid.

Figure 2-6: Conjugated linolenic acid

The oxidation of biodiesel occurs by a series of chemical reactions categorised as the initiation step, propagation step and the termination step as explained in the proceeding paragraphs.

2.4.1 Initiation Step

This stage involves the abstraction of a hydrogen atom from a carbon atom to form a carbon based free radical (Eqn 2-1). The hydrogen atoms most easily abstracted are those bonded allylic and bis allylic to the olefinic unsaturation. Hydrogen atoms non allylic to the olefinic unsaturation are difficult to abstract due to the resonance stabilization imparted by the pi electron system in the adjacent olefin group.

$$RH^* \longrightarrow R^* \quad . \qquad \text{Eqn [2-1]}$$

2.4.2 Propagation step

From the carbon based free radical formed in Eqn 2-1, if diatomic oxygen is present, the carbon based free radical reacts with it to form the peroxy radical (see Eqn 2-2).

$$R^* + O_2 \longrightarrow RO_2^* \quad \text{Fast reaction} \qquad \text{Eqn [2.2]}$$

This reaction is so fast that it prevents the carbon based free radical from following alternative reaction routes. The peroxy free radical, though not as reactive as the carbon based free radical, is sufficiently reactive to abstract another hydrogen atom to form the hydroperoxide (Eqn 2-3).

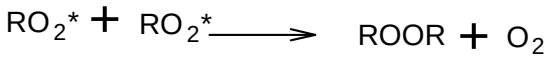
$$RO_2^* + RH \longrightarrow ROOH + R \quad \text{Rate determining step} \qquad \text{Eqn [2-3]}$$

At the initial stages of oxidation, the concentration of the hydroperoxide remains low until an interval of time has passed. As the oxidation reaction continues the concentration of the hydroperoxide (ROOH) increases. The concentration of the hydroperoxide depends on

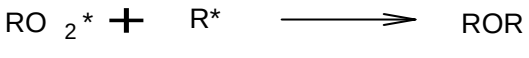
oxygen availability and the presence of metals that catalyse the decomposition of the hydroperoxide into aldehydes such as hexenal, propanal and heptenals and other short chain aliphatic alcohols which increase the rancidity of the biodiesel ((Waynick, 2005).

2.4.3 Termination Step

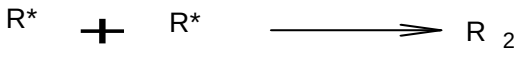
The oxidation reaction ceases when two free radicals combine (Eqn 2-4 to Eqn 2-6). This combination could be a reaction between two carbon based free radical or a peroxy radical. When this happens, the cycle is broken and the chain is ended. Such termination steps occur infrequently, however, because the concentration of radicals in the reaction at any given moment is very small (Mcmurry, 2004).



Eqn [2-4]



Eqn[2-5]



Eqn [2-6]

As hydroperoxide decomposes, oxidative linkage of the fatty acid chain results with the formation of higher molecular weight species (polymers) which results in an increase in viscosity of the biodiesel. The result of this is the clogging of filters.

The process of biodiesel/lipid oxidation from the initiation stage to the final terminal stage is illustrated in Fig 2.7.

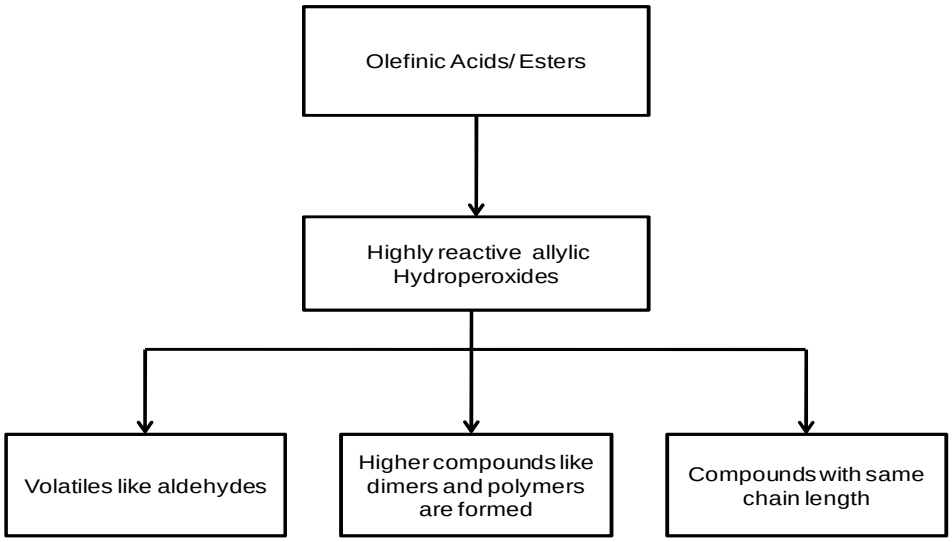


Figure 2-7 Flowchart of lipid/biodiesel oxidation

2.5 Feed stock pre- treatment

Vegetable oils are obtained by the extraction or expression of the oil from the oil seed source. This extraction is done by solvent extraction or pre-press/solvent extraction. The oil at this stage could be referred as “crude” oil. ”Crude” oils at this stage contain varying amounts of naturally occurring non-glyceridic materials. In order to achieve a biodiesel product that meets standard specification, these substances should be removed or reduced prior to the transesterification reaction. It should however be noted that, not all non-glyceridic materials should be considered as undesirable elements in the biodiesel. For instance, tocopherols act as an anti-oxidant in the biodiesel. Pre-treatment of the oil is necessary so as to ensure that the biodiesel meets the required standard as set in bodies like the ASTMD6751 or EN14214. Some of the pre-treatment techniques employed include the following:

2.5.1 Degumming

This involves the removal of high levels of phosphatides in the feed stock. It includes the treatment of the crude oil with a limited amount of water to hydrate the phosphatides and make them separable by centrifugation. High levels of phosphatides in the final product

increase the turbidity of the product. (Brunner *et al.*, 2001) recommended the addition of methanol to the feed stock as this makes the phosphatides swell and precipitate.

2.5.2 Neutralization

This is performed on the feed stock to reduce its content of the free fatty acids (FFA). Higher levels of FFA inactivate the catalyst for the transesterification reaction and thus reduce the mass percent (%) ester yield. An alkali glycerol phase of a subsequent transesterification step is employed to neutralise the FFA (Turck, 1999).This results in the FFA being converted to high specific gravity soaps. After this, the oils are washed with water to remove the residual soaps.

2.5.3 Hydrogenation/partial hydrogenation

Hydrogenation is intended to reduce the amount of unsaturation in the oil as this relate to the stability of the fuel. This process can have detrimental consequences especially in temperate climates as the conversion of unsaturation in the oil will lead to an increase in the presence of saturated fatty acids giving rise to biodiesel with poor cold flow properties a situation that is unwanted in cold zones. The technique involves the passing of H_2(g) through the oil at elevated temperatures in the presence of a suitable catalyst, such as platinum (Mcmurry, 2004). The unsaturation is destroyed and a saturated fatty acid is created (Eqn 2-7).

$$CH_3CH_2CH{=}CHCOOH \xrightarrow[\text{High T,P}]{H_2,Pt} CH_3CH_2CH_2CH_2COOH \qquad \text{Eqn [2-7]}$$

The hydrogenation process is easily controlled and could be stopped at any desired point. If the hydrogenation is stopped after only a small amount of hydrogenation has taken place, the oils remain a liquid.

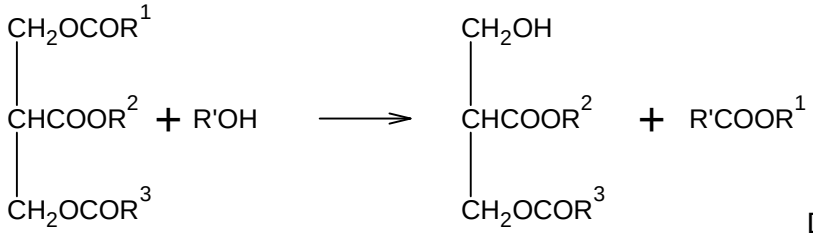
2.5.4 Dehydration

The final stage in the pre- treatment of the feed stock before transesterification is dehydration. This involves the removal of traces of water from the feed stock. The presence of water in the feed stock decreases the conversion rates and may therefore result in the inability of the biodiesel to meet the minimum requirement of 96.5% conversion rate. Dehydration is done by passing nitrogen gas through the oil.

2.6 The chemistry of biodiesel production

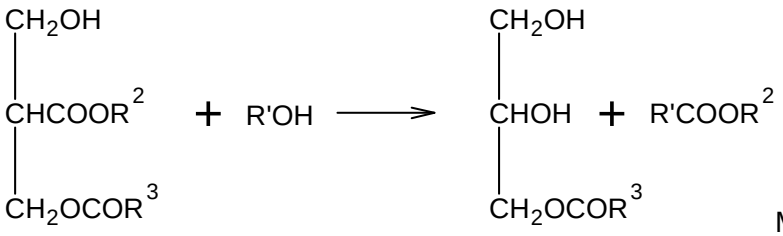
2.6.1 Transesterification

The production of biodiesel from vegetable oils is by means of a transesterification reaction. This involves the transformation of one type of ester into another type of ester (Tickell, 2003). Transesterification has the sole aim of lowering the viscosity of the biodiesel so that problems such as poor fuel atomization and high flash points of the final product can be avoided. The reaction involves a triacylglycerol reacting with a low chain alcohol, catalysed by an acid or a base to form the biodiesel and glycerol as the secondary product. The base catalysed process is quicker, being complete in few minutes at high levels. Moreover, its yields are higher and selective besides showing less corrosion problems (Ferrari *et a.l*, *2005).* The transesterification reaction is a step wise reaction that involves the transformation of the triglyceride (TG) into the diglyceride (DG) (Eqn 2-8)



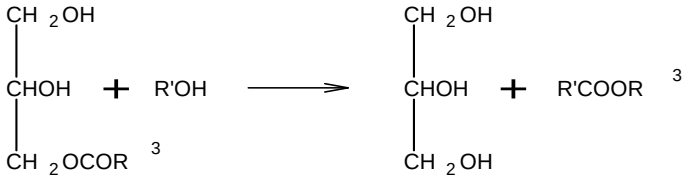
DG formation Eqn[2-8]

The diglyceride formed reacts with more of the alcohol to form the monoglyceride as seen in (Eqn 2-9).



MG formation Eqn [2-9]

The final stage of the transesterification reaction involves the transformation of the monoglyceride formed in Eqn into the desired fatty acid methyl esters and glycerol as a by product of the reaction (Eqn 2-10).



Final product Eqn [2-10]

Thus, an incomplete transesterification reaction will have traces of triglyceride (TG), diglyceride (DG), and monoglyceride (MG) in the final biodiesel. The alcohol used for the transesterification reaction is mostly either ethanol or methanol**.** Methanol has become the more popular choice due to the fact that it is cheaper, produces a more stable biodiesel reaction, has high reactivity, and gives an ester yield of more than 80% even after as little time as five minutes (Mittelbach, 1989) and proceeds at low reaction temperatures. However, in countries like Brazil, anhydrous ethanol is the preferred alcohol because it is produced on a large scale to be mixed with gasoline (Schuchart *et al*, 1984) and is thus affordable. The activation energy of the transesterification reaction depends on several experimental parameters such as heating rate, particle size distribution of the sample, presence of impurities and atmosphere around the sample, amongst others (Dantas, 2007). The properties of biodiesel and mineral diesel are compared in Table 2-1.

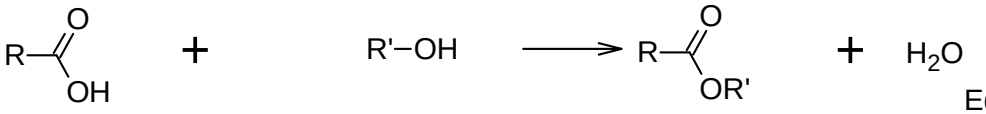
Table 2-1 Specification of Diesel and Biodiesel fuels (Tyson, 2001)

Fuel property	**Diesel**	**Biodiesel**	**Units**
Fuel standard	**ASTM D975**	**ASTM PS 121**	
Fuel composition	C10-21HC*	C12-22	Not applicable
Lower heating value	$36.6x10^3$	$32.6x10^3$	Calories
Kinematic viscosity@40^oC	1.3-4.1	1.9-6	^oC
Specific gravity @15.5^oC	0.85	0.88	No units
Density @ 15^oC	848	878	g/cm^3
Carbon	87	77	Wt %
Hydrogen	13	12	Wt %
Sulphur	0.05	0.0-0.0024	Wt %
Boiling point (^oC)	188-343	182-338	^oC
Flash point	60-80	100-170	^oC
Cloud point	-15 to5	-3 t0 12	^oC
Pour point	-35 to -15	-15 to 10	^oC
Cetane number	40-55	48-65	Not applicable
Stoichiometric air/fuel	15	13.8	

HC*- Hydrocarbon

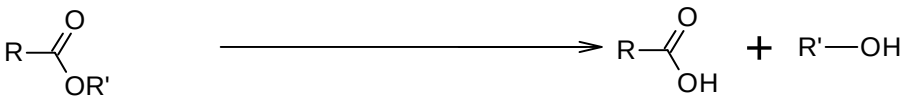
2.6.2 Esterification

This involves the reaction of a fatty acid with an alcohol to form esters and water. Both fatty acids and alcohol are likely components of the final biodiesel if the purification stage is not properly carried out. The reaction is catalysed by a dilute mineral acid like dilute hydrochloric acid (HCl) (Eqn 2-11).



Eqn [2-11]

It should be noted that the reverse reaction (Eqn 2-12) which produces fatty acid and an alcohol is called hydrolysis of the esters.

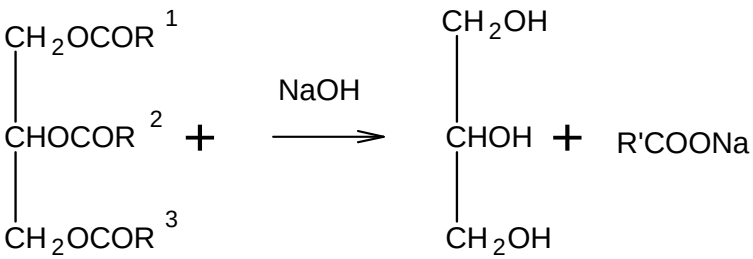


Eqn [2-12]

Therefore, in the presence of water in biodiesel, there is a possibility of an increase in free fatty acid and this may affect properties such as its cold flow properties.

2.6.3 Soap Formation

The alkaline hydrolysis of triglyceride results in the formation of soaps, a common occurrence in the production of biodiesel, a situation that arises when excess of the catalyst is used. This problem (Eqn 2-13) makes glycerol separation quite difficult and also decreases the amount of the Biodiesel that could be formed.



Eqn [2-13]

An example of a transesterification reaction that formed soap due to excess catalyst is shown in Fig 2-8.

Figure 2-8 A transesterification reaction that formed soap due to excess of catalyst

2.6.4 Acidolysis

Due to the constituents of oils, one likely reaction that can occur is acidolysis reaction: an interaction between an ester and a carboxylic acid leading to an exchange of acyl groups in the presence of a catalyst usually a metallic oxide (zinc, calcium, magnesium, aluminium) at about 150^{o}C (Eqn 2-14).

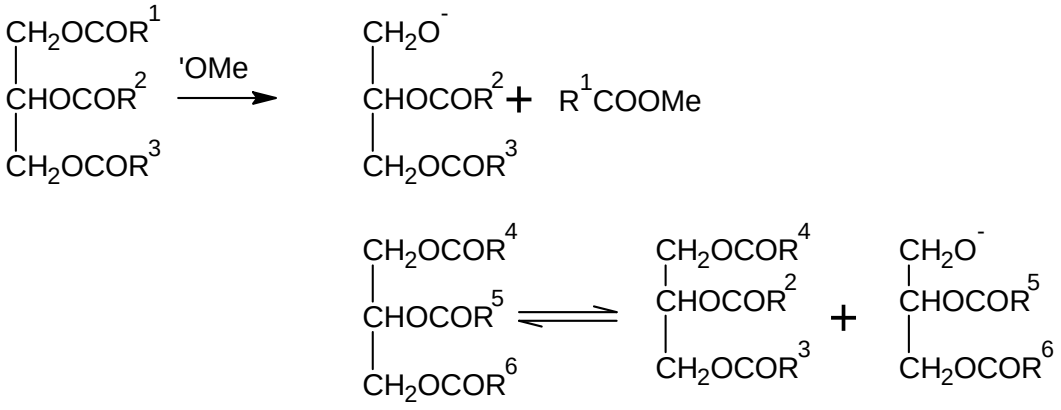
$$CH_3COOH + RCOOR \longrightarrow RCOOR + CH_3COOH$$

Eqn [2-14]

2.6.5 Interesterification

This involves the interaction between two esters. The aim of Interesterification is to produce esters which have their acyl groups randomised since natural esters do not show this

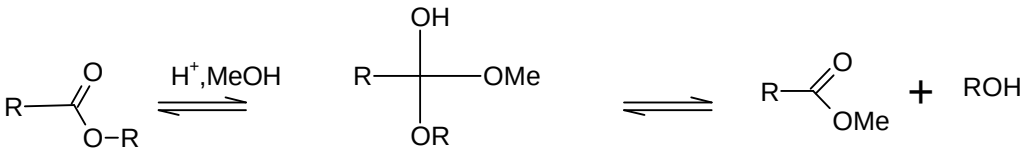
phenomenon. When applied to single oils, the redistribution of the acyl groups from non-random to random changes the triacylglycerol composition and thus leads to changes in certain properties such as the melting point of the oil. For instance, the melting point of soybean oil is raised from -7 to + 6^{o} C. Interesterification reactions are catalysed by such substances as sodium methoxide and sodium hydroxide (Eqn 2-15).



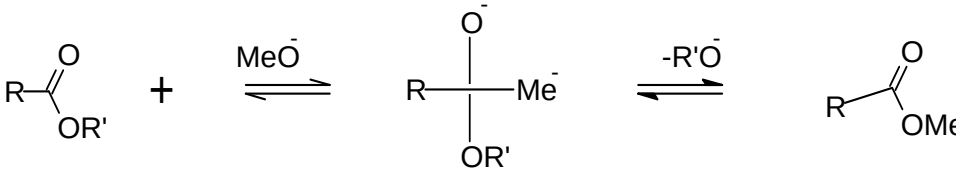
Eqn [2-15]

2.6.6 Alcoholysis

This is a catalysed reaction between an ester and an alcohol which leads to the exchange of the alkyl portions of the ester. Particularly important is the fact that it is an effective means of converting triglyceride to methyl esters by reacting with methanol or MG and DG by reacting with glycerol. The catalyst employed is either acidic (Eqn-2-16) or basic (Eqn 2-17).



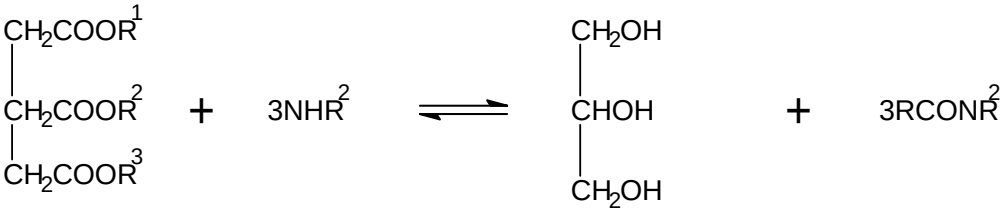
Eqn [2-16]



Eqn [2-17]

2.6.7 Aminolysis

Esters react with amines, mostly the primary and secondary amines. This is a nucleophilic substitution at their acyl carbon atoms [Eqn 2-18]. These reactions are slow but are synthetically useful (Solomons, 1996).



Eqn [2-18]

2.7 Separation and purification of biodiesel

Having a good and complete reaction is usually not enough. The production process yields with it certain impurities and residues which are left in the final Biodiesel. These impurities and residues could be detrimental to the combustion system and, therefore, have to be removed.

2.7.1 Phase Separation

This involves the separation of the glycerine layer from the ester layer. This process occurs naturally especially when methanol or absolute ethanol is used as a reacting partner in alkaline-catalysed transesterification process since the glycerol has a higher density than the ester formed and therefore settles to the bottom. It can be quite a slow process (around 3 hours for complete separation) and, therefore, to facilitate the separation, centrifugation has been suggested though it is not economical (Mittelbach, 2006). Other means of facilitating the phase separation includes the addition of water. The addition of hexane and extra glycerol to the reaction mixture has also been proved to be helpful.

2.7.2 Purification of Biodiesel

Once phase separation has been achieved, the purification of the ester phase is necessary to ensure that the biodiesel meet specifications. After the phase separation of glycerol, the biodiesel still has an excessive amount of soaps, aggressive pH, catalyst, FFAs, water, methanol, glycerides and other impurities. These substances, if not reduced to their minimum, will have effects on the biodiesel. There are various means of removing the impurities mentioned that are left in the ester phase after transesterification.

One of the means of removing these impurities is by washing the ester phase with water. The effect of this process on biodiesel cold flow properties such as kinematic viscosity, pour

and cloud point are discussed in section 4.7 of chapter 4. In the water washing process, a certain percentage of water mostly 50 volume% is added to the biodiesel and this is allowed to settle. As the water passes through the ester phase, it attaches to the impurities such as MG, DG, TG, catalyst etc. Once settled, the contaminated water is drained off together with the impurities. This process continues until clear water is obtained. Once all the water is removed, the remaining biodiesel is dried and ready for final quality check. Traces of glycerol are removed by water or acid washing solutions (Karaosmanoglu *et al*., 1996).

Free fatty acids (FFA) are removed by distilling the ester phase making use of the fact that the boiling points of methyl esters are generally 30^{o}C to 50^{o}C lower than the FFAs (Farris, 1979). Methanol is removed by heating the ester phase to a temperature of 70^{o}C.

Partial glycerides (MG, DG) can be removed from the ester phase by converting them into triglyceride which can then be separated from the methyl ester product. This is done by adding an extra alkaline catalyst to the ester phase and the reaction is heated to about 100^{o}C (*Klok et al*., 1990). In the process, the glycerols and the partial glycerides react with the methyl esters and thus are converted to triglycerides which were then reintroduced into the transesterification reactor together with new oils

Catalysts are generally removed by using an adsorbent such as bleaching earth (Wimmer, 1991), and also by the use of silica gel or magnesium silicate (Cooke, 2004). The method employed to purify biodiesel depends on the manufacturers and also the scale of the biodiesel produced.

The effects of some of these substances on diesel equipment and the environment are listed in Table 2.2.

Table 2-2 Effects of Impurities in biodiesel on Diesel Engine Performance (Berrios, 2008).

Impurity	**Effects**
FFAs	Corrosion, low oxidation stability.
Water	Hydrolysis (free fatty acid and alcohols formation), corrosion, bacteriological growth (filter blockage).
Methanol	Low values of density and viscosity, low flash point (transport, storage and use problems).
Glycerides	High viscosity, deposits in the injectors (carbon residue), crystallization.
Metals(soap, catalyst)	Deposit in the injectors, filter blockage (sulphated ashes), engine weakening,
Glycerol	Settling problems, increased aldehyde and acrolein emissions.

The flow chart in Fig 2-9 shows the steps involved in the purification process:

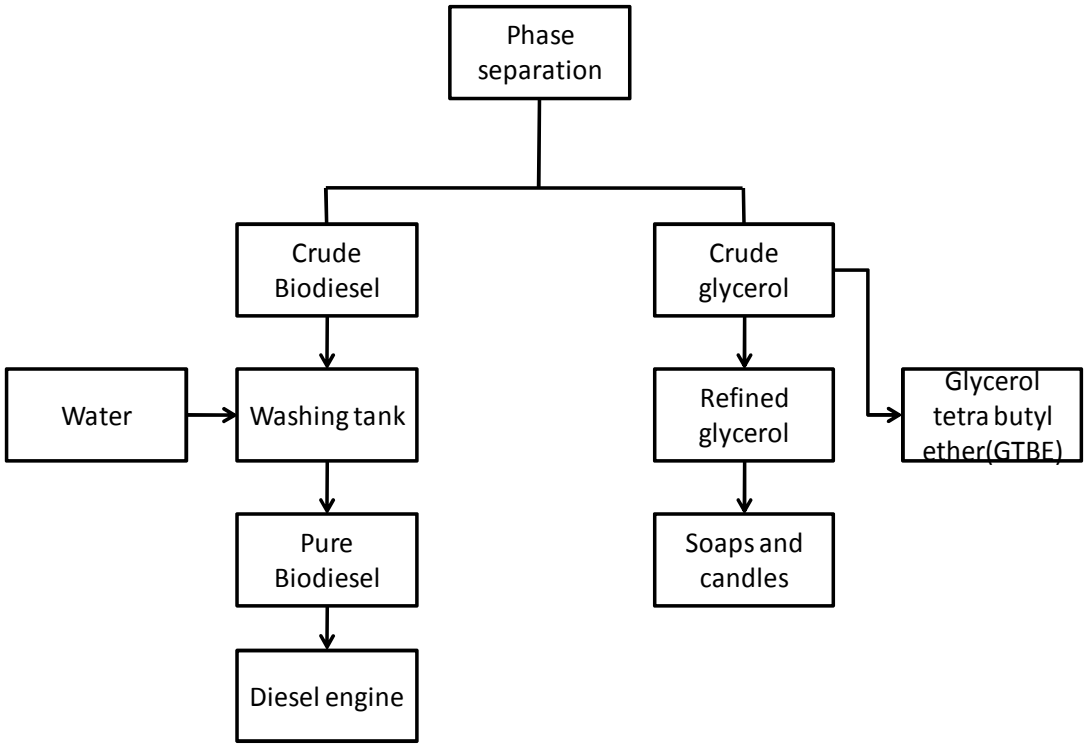


Figure 2-9 Flow chart of biodiesel purification.

2.8 Important biodiesel quality parameters

The parameters that define biodiesel quality can be divided into two groups. One group contains parameters that are applicable to both biodiesel and mineral diesel fuels and the other contains parameters that describe the chemical composition and purity of fatty acid methyl esters (Mittelbach, 1996), which is applicable only to biodiesel. Table 2-3 lists both parameters as a means of comparing the properties of both biodiesel and mineral diesel. It is worth noting that the extent of reaction as well as the experimental conditions used in the production of biodiesel greatly influences the fuel properties, discussed in the following paragraph.

2.8.1 Amount of Ester

This happens to be the main parameter that defines and distinguishes biodiesel. Limits have been established by the American society for testing and materials (ASTM) and the European normalization (EN). They define the minimum to be 96.5 %(m/m) for fatty acid methyl esters. This is the most important component of biodiesel. The limit allows the detection of illegal mixtures of biodiesel with fossil diesel. The amount of esters in the final product is affected mainly by the extent of transesterification reaction. Moreover, inappropriate analytical procedures can also compromise the amount of esters in the biodiesel. A high concentration of the mono, di and triglycerides as well as the of unsaponifiable matter could be an indication of the low level of esters in the biodiesel. The type of ester formed depends critically on the type of feed stock oil and the alcohol used. For instance, methyl esters are formed when methanol is the alcohol used in the transesterification reaction, and ethyl esters when ethanol is used.

2.8.2 Total Glycerol

This includes the free glycerol and the bound glycerol. Bound glycerol is a function of the residual amount of the triglycerides and partial glycerides that remain in the final biodiesel product (Foglia *et al.,* 2004). The amount of free glycerol is largely dependent on the production and the separation process.

High values of free glycerol could be attributed to improper purification methods and also the hydrolysis of partial glycerides such as the MG, DG etc. Bound glycerol is affected by factors such as incomplete transesterification reaction and moreover oils naturally contain MG, DG as constituent. High levels of total glycerol are the source of carbon deposits in the engine

because of incomplete combustion (Knothe, 2006). Free glycerol can collect at the bottom of fuel tank where they attract other polar substances such as the partial glycerides and water.

2.8.3 Alcohol Content

Some amount of the alcohol used in the transesterification reaction can remain in the final product after the reaction. The alcohol content has been set at 120^0C minimum (ASTM) and 0.2 mass% (EN14214) High alcohol content in biodiesel pose safety risks especially during transportation and may cause deterioration of rubber components of the vehicles fuel system (Paraschivescu *et al.*, 2007). The alcohol in biodiesel is indicated by its flash point; the lowest temperature at which application of an ignition source causes the vapours of a specimen to ignite under the specified conditions of the test.

2.8.4 Acid Number/Value

Free fatty acids occur naturally in vegetable oils and thus are carried over into the final product after transesterification. The fatty acids present in biodiesel depend primarily on the type of feed stock used, although most of them are removed during the refining of the feed stock oil before the transesterification reaction. High free fatty acid levels in biodiesel can cause fuel system deposits and is also an indication that the fuel will act as a solvent resulting in the deterioration of the rubber components of a fuel system (Mittelbach and Remschmidt, 2004). One major cause of high level free fatty acids in biodiesel even with refined feed stocks is the presence of moisture in biodiesel. The moisture hydrolyses the methyl ester to its component free fatty acids and alcohol, a reverse process of esterification. The amount of free fatty acids in the Biodiesel is indicated by the acid number which is an expression of the milligrams of KOH per gram of sample required to titrate a sample to a specified end point. The standard established by the ASTM is a maximum of 0.80mgKOH/g.

2.8.5 Water Content

Water can affect the transesterification reaction when present in the feed stock oil and also the final product. It decreases the ester yield in the transesterification reaction and also promotes bacteria growth in biodiesel. Moisture facilitates the rapid disintegration of the methyl ester leading to an increase in the flash point and the acid number of the biodiesel. Water in biodiesel can lead to corrosion of zinc and chromium parts within the engine and injection systems (Kobmehl and Heinrich, 1997). Water is usually introduced into the

biodiesel product during the final washing step and also due to the hygroscopic nature of biodiesel. A limit of maximum 0.05 volume % is set as a standard for B100 in ASTM D6751.

2.8.6 Conradson Carbon Residue

This is the part of the fuel remaining after combustion. This may be due to the fact that the majority of the constituents of biodiesel contain carbon skeletons such as the glycerides. While it is only a minor relevance in fossil fuels, carbon residue is considered to be one of the most important quality criteria as it is linked with many other limited parameters such as the total glycerol (Mittelbach *et al*, 2006). The ASTM standard requires a maximum of 0.050 wt %.

2.8.7 Cetane Index

This indicates the ignition quality of the biodiesel fuel. Fatty acid methyl esters (FAMEs) have a higher cetane number (CN) compared to petrol diesel. For conventional diesel fuel, high CN is correlated with reduced nitrogen oxides (NO_x) exhaust emissions. For FAMEs, there is an increase CN when the alkyl chain increases but a decrease in the CN when the unsaturation increases in the feed stock. However for unsaturated FAMEs, a longer period of storage leads to an increase in the CN as a result of oxidation to form the hydroperoxides substances which are discussed as cetane improvers (Van Gerpen, 1996).The EN14214 requires a minimum of 51 CN. The reference substances are hexadecane, a high quality with an arbitrary 100 CN and a low quality reference compound nonane compound which is assigned a CN of 15.

2.9 Biodiesel analysis

Biodiesel can be contaminated with various compounds such as monoglyceride (MG), diglycerides (DG), glycerol, the alcohol for transesterification, and the catalyst. Although it is almost technically impossible to completely remove all these contaminants, the establishments of standards have ensured they are reduced to their minimum. There are two major standards currently in use. These are the ASTM and the EN standards. Although these standards have a lot in common, there are still some parameters that they do not agree on. For instance, the ASTM standard does not include the iodine value since it is believe that the inclusion of this parameter will lead to the exclusion of some potential feed stock that could be used in the production of biodiesel.

Table 2.3 displays some important biodiesel parameters and the recommended methods for both the ASTM and EN14214 standards used in the analysis of some of the contaminants and the maximum levels expected in a good biodiesel in these standards, (EN14214 and ASTMD 6751).

Table 2-3 ASTM D6571 and EN14214 standards for biodiesel.

Property	**Test method ASTM [EN14214]**	**Limits ASTM [EN14214]**	**Units ASTM [EN14214]**
Flash point (closed cup)/ methanol content	D93/ EN EN14110	130.0min [0.20max]	^{o}C [%mol/mol]
Water and sediment	D2709 [EN ISO12937]	0.050max [500max]	Volume % [mg/kg]
Kinematic viscosity	D445 [EN ISO 3104]	1.9-6.0 [3.5-5.0]	mm^2/s
Sulphated ash	D874/I SO 3987	0.020 max [0.02max]	mass% [%mol/mol]
Iodine value	*NA [EN14111]	[120max]	%mass
Copper strip corrosion	D130 [EN ISO 2160]	No. 3 max [1]	[degree of corrosion]
Cetane number	D613 [EN ISO 5165]	47 min [51min]	
Cloud point/CFPP	D2500 [EN116]	*NSV	^{o}C
Carbon residue	D4530 [EN ISO 10370]	0.05 max [0.30max]	Mass % [mol%]
Acid number/Value	D664 [EN14204]	0.50 max	mgKOH/g
Free glycerin	D6584 [EN14105]	0.02 max	Mass%
Total glycerin	D6584 [EN14105]	0.240 max [0.25max]	Mass%
Linolenic acid content	NA [EN14103]	12.0max	%(mol)
Content of FAME with $\geq^{=}$ double bonds		1max	%(mol)
Ester content(min)	NA [EN14103]	96.5min	%(mol/mol)

**NA-not applicable* **NSV-No specific value*

Several analytical techniques have been used in the analysis of the impurities and by-products of biodiesel. The analytical techniques should be accurate, reproducible, reliable, relatively quick and simple. Some of the analytical techniques are looked at. Techniques so far employed in the analysis of biodiesel could be grouped into the chromatographic

methods, spectroscopic methods, and some physical/wet properties based methods. The most intensively studied methods are the chromatographic methods whilst the spectroscopic methods have also been studied in some detail. Physical property based methods have been explored less and is an area that requires further study (Knothe, 2001).

2.9.1 Chromatographic Methods

Chromatography has been the most extensively used method in the study of lipids and biodiesel. Thin layer chromatography/flame ionization detector (TLC/FID**)**, gas chromatography (GC), gel permeation chromatography (GPC), and high performance liquid chromatography (HPLC) have all been used in the analysis of Biodiesel. TLC/FID was first developed to basically replace the time-consuming column chromatography so as to obtain faster results (Hamilton, 1998). In addition to that, it requires less sample, low maintenance cost of the instrumentation and is relatively simple. TLC/FID combines the separation power of the TLC and the quantitation ability of the flame ionisation detector. To increase the capabilities of the TLC/FID, Chromarods are treated with special reagents one of which is the impregnation of the rods with silver nitrate ($AgNO_3$). A synthetic mixture of FAMES was first analysed using chromarods impregnated with $AgNO_3$ (Sebidio, 1981). The results obtained were comparable with GC analyses for all methyl esters except for methyl linolenate where the results were over estimated by the TLC/FID method. TLC/FID with Iatroscan has been used in the analyses and quantitation of transesterification reaction mixtures (Freedman, 1984).

A solvent system of PE/DE/AA (90:10:1) was found suitable for the transesterified products analyses because it afforded a better separation of DG and MG. However an increased in the polar system resulted in the decrease in the separation of ME and TG. In another study (Cvengros, 2007), TLC/FID with Iatroscan was used as a reference to provide information on bound glycerol content in methyl esters of rapeseed oil (MERO) samples. The major drawback for the TLC/FID compared with GC and HPLC is its low accuracy. TLC/FID analyses of biodiesel have been abandoned because of lower accuracy and material inconsistencies (Knothe, 2006).

Gas chromatography (GC) is perhaps the most extensively applied in the study of biodiesel analysis with various detection methods such as FID and mass spectrometer (MS). This is due to its ability to quantify minor components required in biodiesel analyses (Knothe, 2006). Most standards (EN and ASTM) recommend the use of GC for the determination of the major parameters such as total glycerol**,** FAMES and methanol as part of biodiesel

characterisation. GC analyses usually deal with the determination of a specific contaminant or class of contaminants in methyl esters. Plank and Lorbeer (1995) developed a rapid and reliable GC procedure for the simultaneous determination of MG, DG, and TG in vegetable oil methyl esters (VOMES). The method was especially developed for rapeseed oil methyl esters. In this method, trimethylsilylation of the free hydroxy groups of glycerol, MG, DG and TG was followed by GC analyses using a short thin film capillary column enabling the determination of all analytes. Trimethylsilylation of the free hydroxy groups improves their chromatographic properties. Calibration was done by the analyses of a standard solution containing monoolein, diolein, triolein and glycerol.

However, this method cannot be applied to methyl esters from the transesterification of lauric acid without the necessary changes because of the superimposition of peaks of long chain fatty acids esters (Plank, 1995). GC analyses have mostly been applied to methyl esters and not the higher esters. Freedman investigated the methyl and butyl esters of transesterified soy bean oil. Not all the individual compounds were separated in the butyl esters but classes of compounds were analysed (Freedman, 2007).

In the determination of glycerol esters, both the ASTM 6751and EN 14214 employs GC with FID as is the case with other parameters such as methanol. For instance, in the analyses of the ester content in Biodiesel, GC method with a 30-m carbowax column is employed (Knothe, 2006). Hyphenated GC methods have been used in the analysis of biodiesel. Methods such as gas chromatography-mass spectrometer (GC-MS) and gas chromatography-liquid chromatography (GC-LC) have been used. The purpose of these combinations is to reduce the complexities of the chromatogram and therefore obtain very comprehensive peaks (Demirbas, 2006). Lechner *et al* (1997) used a fully automated LC-GC to determine acylglycerols in vegetable oil methyl esters (VOMEs). Hydroxy groups were acetylated and then the methyl esters and the acylglycerols were pre-separated by LC (variable wavelength detector). The solvent system for the LC was hexane/methylene chloride/acetonitrile. In another method (Mariani *et al*., 1991), GC-MS was used in the determination of free glycerol in biodiesel. In this method selective ion monitoring (SIM) was used to track the ions m/z 116 and 117 of bis-O-trimethylsilyl-1,4 butanediol and m/z 147 and 205 of tris-O-trimethylsilyl-1,2,3-propanetriol.The problem with GC analyses are that, a standard is required for each feed stock used in the transesterification reaction. Moreover, the accuracy is affected by factors such as overlapping signals, aging of standards and samples and also baseline drift.

Gel permeation chromatography (GPC) has been reported in the analyses of transesterification products using tetra hydro furan (THF) as a mobile phase to determine the amount of MG, DG and TG of transesterified palm oil (Darnoko *et al.*, 2000).

HPLC is perhaps the most convenient means of analysing biodiesel although few reports exist in literature about biodiesel analysis as compared to GC. The general advantages of HPLC as an analytical procedure are that it allows for viable direct analysis without derivatisation. Analyses time in HPLC analyses are reduced because reagent consuming derivatisation are completely eliminated. Traithnigg and Mittelbach (1990) reported on the use of an isocratic solvent (Chloroform with 0.6% ethanol) to determine MG, DG, TG as well as methyl esters as classes of compounds.

In another method (Di Nicola *et al*., 2008) developed a strategy for optimizing a non-aqueous reverse phase (NARP- HPLC) for analysing biodiesel mixtures. This was based on the use of a fast and efficient chromatographic linear elution suitable for analysing biodiesel and its related substances. In this method, acetonitrile/methanol 4:1 (vol/vol) with isocratic elution was considered a suitable mobile phase for determining FAMES. Moreover, hexane/isopropanol system with isocratic elution was considered a good mobile phase for separating acylglycerols. A reverse phase (RP-HPLC) procedure with universal detection (UV) at 210nm was an efficient means of separating the major compounds during lipase catalysed transesterification of sunflower oil with methanol. The identification of the individual compounds was done by atmospheric pressure chemical ionisation (APCI-MS) in the positive-ion and negative ion modes (Turkan, 2006).

In the determination of bound glycerol content in biodiesel, Foglia *et al* (2004) compared the statistical accuracy of GC and HPLC methods in ascertaining the bound glycerol content in biodiesel fuels from different feed stocks. They found that there was no statistical difference between the two methods even though they concluded that the HPLC was superior due to the fact that it was applicable to most biodiesel fuels.

Hyphenated HPLC techniques such as HPLC/MS have also been used in the analysis of biodiesel. Even though HPLC seems to have a good upper hand because of the issue of derivatisation, one major problem is its inability to include into a single protocol determination of free and bound glycerol like GC does. In concluding a preview of chromatographic techniques used in the analysis of transesterification products, it should be emphasised that although chromatographic methods have become the method of choice for analysis of transesterification products, issues such as the time wasting derivatisation of samples before

analysis during GC analysis and the fact that HPLC analyses are not able to include into a single protocol the determination of bound and free glycerols are issues that are that need to be looked at.

2.9.2 Spectroscopic Methods

Spectroscopy has been used in the analysis of transesterification products and also the monitoring of the transesterification reaction. The most widely used spectroscopic methods for the analysis of transesterification products are the Nuclear Magnetic Resonance spectroscopy (NMR), Near Infra Red (NIR) and Fourier Transforms.

There are reports of the use of carbon-13 nuclear magnetic resonance (^{13}C- NMR) and proton nuclear magnetic resonance (^{1}H-NMR) and near infra red (NIR) with fibre optic probe. The fibre optic probe facilitated the acquisition of the spectra and made it more time efficient (Gelbard, 1995).

^{1}H NMR analysis was used to determine the degree of fatty acid unsaturation in methyl esters and also to provide initial rates of the methyl ester formation. In the determination of the degree of unsaturation of soy bean oil, a comparison of the ^{1}HNMR integration of the methyl group and olefin protons in the methyl ester was made and value of 1.52 DU comparable to what has been reported in literature of similar work was obtain (Morgenstern *et a.l*, 2006).

NIR is described as an effective, inexpensive method of analysing biodiesel and allows multi-component analysis in a fast non-destructive way without the need for complex sample pre-treatment (Jefferson *et al.*, 2006). Together with well established methods like principal component analysis (PCA) and partial least square (PLS), near infra-red (NIR) has been used in the analysis and quantification of Biodiesel. PCA and PLS were used for qualitative analysis and development of calibration models between analytical and spectral data respectively to determine the Iodine value, kinematic viscosity and CFPP and density (Baptista, 2008).

The use of NIR in determining the Iodine value has been described as interesting because the recommended GC or Wijs method (a titrimetric method using iodine monochloride in glacial acetic as the Wijs reagent) is very expensive and time- consuming (Morgenstein *et al,* 2006). Further calibration model was developed for the quantification of the content of methanol and water in Biodiesel (Felizardo *et al*, 2008). PLS and artificial neural network

(ANN) combined with Fourier transform infra-red (FTIR-ATR) and Fourier transform near infra-red (FTNIR) were used to design a calibration model for the determination of methyl ester content (%, w/w) in biodiesel blends (Jefferson, 2006). In this work, two sets of samples were used, one sample(I) consisted of binary mixtures of one part diesel and the other a particular type of methyl ester and in the other, sample(II) , they had quaternary mixtures consisting of one part diesel and three parts of three different types of methyl esters. A precise and accurate FTNIR model was obtained for both sample I and sample II. The drawback with these spectroscopic methods especially NIR is that they cannot quantify the constituents of biodiesel at the level required by most of the standards and also are suitable for rapid online analysis to determine the extent of reaction.

2.10 Selecting a method for biodiesel analyses

In selecting a method for analysing Biodiesel parameters, there are certain considerations that should be taken into account. Some of these considerations are;

2.10.1 Precision and accuracy of methodology

Precision and accuracy are at the heart of any analytical procedure or methodology. Accuracy reflects the closeness of the readings to its true value and precision indicates the reproducibility of the measurements with same instrumentation by the same personnel under the same conditions. In this study, development and utilisation of analytical methods were based on how precision and accuracy of the methods.

2.10.2 Flexibility of instrumentation

Since there are varied feed stocks from which biodiesel could be made, the analytical method of choice should be one that could be applied to all or most biodiesel from different feedstock. In the case where this realisation is not possible, there should be allowance of minor adaptation for its applicability to the particular biodiesel. Currently, the ASTM D6584 which is used in the determination of free and total glycerol is not applicable to biodiesel from lauric acid sources such as coconut and palm kernel oil due to the superimposition of peaks.

2.10.3 Analyses time

The most reliable method so far employed in the analyses of biodiesel components and impurities has been the use of GC/FID with most standards (ASTMD6571 and EN14214) recommending it for its characterisation. Most GC analyses require complex sample preparations and derivatisation thus increasing the time for such analyses. HPLC offers a better alternative since it requires a shorter analyses time because there issue of derivatisation is completely eliminated. Normal HPLC analyses time takes less than far less than 30 minutes as compared with GC.

2.10.4 Instrument availability

Instrument availability depends on the cost and the amount of training required for personnel operating the equipment.

In conclusion, no method can simultaneously satisfy all criteria of simultaneously determining all trace contaminants with minimal investment of time, cost and labour (Knothe, 2001). Thus, it behoves on the analyst to adjust the analytical method or equipments base on time, cost and labour and to manipulate these factors to as to ensure the of an efficient analytical method.

.

3 EXPERIMENTAL METHODS

Biodiesel from six selected oils originating from rapeseed, sunflower, palm, waste vegetable, peanut and crowns were transesterified. Analytical methods were developed to establish and characterise the content of the following parameters of each biodiesel sample produced;

- Total glycerine
- Bound glycerol and free fatty acids
- Ester and linolenic acid methyl ester
- Methanol

Moreover, comparisons were made regarding the following features of biodiesel;

- Iodine value of the biodiesel and its feedstock
- Cold flow properties of the washed and unwashed biodiesel. The cold flow properties looked at included the kinematic viscosity, density and cloud and pour points.
- And lastly, the effect of blends of unsaturated and saturated biodiesel on cold flow properties such as cloud and pour points were evaluated.

3.1 Biodiesel samples production.

3.1.1 Materials and transesterification reaction

The rapeseed, crown, sunflower and peanut oils were purchased from Shoprite, Stellenbosch. Waste vegetable and palm oil as well as the methanol and potassium hydroxide (KOH) were all obtained from the biofuels process laboratory, University of Stellenbosch.

Laboratory scaled biodiesel samples (approximately 400mL from each sample) were produced from the above mentioned vegetable oils. This involved the measurement of a litre of each of the feed stocks, except the palm and peanut oils where approximately 750mL were used. The transesterification reaction involved the dissolution of 18.8g of potassium hydroxide catalyst (KOH) in approximately 400mL of methanol. For both palm

and peanut oils, proportionately lesser amounts of the catalyst/alcohol combination were used. After the transesterification reaction, the samples were divided into washed, unwashed biodiesel. Washing of the samples involved the use of 50% by volume warm water and 50% volume of biodiesel. After about 3 hours, the water was separated and the process repeated three times until final clear, non- cloudy water was obtained. The biodiesel was then heated to about 70^0C for the residual alcohol to evaporate.

3.2 Analysis of bound and total glycerol.

The analysis of bound and total glycerol in biodiesel is normally achieved by the use of a gas chromatography/flame ionization detector (GC/FID) with an on-column injector as stipulated in ASTM D6584. In this study, the analyses were conducted using the DANI GC/FID equipped with the PTV injector instead of the recommended on-column injector.

3.2.1 Chemicals and reagents.

The standards used for the analysis of total glycerol are listed in Table 3-1.

Table 3-1 Standards used in the analysis of total glycerol and their cass numbers

Standard	Supplier	Cass No.
Glycerol		
(S)-1-1, 2 ,4-Butanetriol	Fluka	1341947
1-mono [cis-9-octadecenoyl]-rac-glycerol (monoolein),	Sigma	111-03-5
1, 3-di[cis-9-octadecenoyl] glycerol (diolein)	Sigma	25637-84-7
1, 2 ,3-tri-[cis-9-octadecenoyl] glycerol (triolein)	Sigma	122-32-7
1, 2, 3-tridecanoylglycerol (tricaprin)	Fluka	91022
N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA).	Fluka	

3.2.2 Preparation of stock and calibration standards.

Five GC standards (S1-S5) were prepared by mixing aliquots of the individual stock solutions consisting of glycerol, monoolein, diolein, and triolein together with the two internal standards, 1,2,4-butanetriol (IS1) and tricaprin(IS2) in proportions as specified in Appendix A1. 100µL of each internal standard was added to each of the five standards.

The stock and calibration solutions that were prepared for the setting up of calibration curves and the further determination of each glyceride in samples are shown in Appendix A1.

These were then used to set up calibration curves for glycerol, monoglyceride, diglyceride and triglyceride. 1µL of the standards (from S1-S5) were injected onto the GC column for analysis. The ratio of glycerol response to butanetriol response (defined as the response ratio for glycerol) and the ratios of responses of the glycerides (mono, di and triglycerides) to the tricaprin response also defined as the response ratios for the glycerides were determined for each of monoolein, diolein and triolein. Moreover, the amount ratios of glycerol (ratio of amount of glycerol to 1,2,4- butanetriol) and amount ratios of each glyceride (ratio of amount of each glyceride to that of tricaprin) were also determined. A graph of amount ratios (vertical axis) and responses ratios (horizontal axis) were plotted for the glycerol, monoolein, diolein and triolein (Appendix A2 and A3). These graphs (see Appendix A5- A7) were used later in the determination of the glycerol and the glycerides in the samples.

3.2.3 Sample analysis

About 85mg each of the six biodiesel samples were taken.100µL each of the internal standards tricaprin and 1, 2, 4-butanetriol were added to the samples. 100µL of a derivatisation agent, MSTFA (N-methyl-N-trimethylsilyltrifluoroacetamide), was added to the samples. This derivatisation was in essence to ‘cap’ the polar groups by substituting the active protons of the glycerides so that their volatility is increased and also to prevent their adsorption to the column (Handley, 2001). In this analysis, the derivatisation converted the glycerides into the trimethylsilylether (TMS) derivatives which are more volatile and do not interact with the column. The mixture was then allowed to stand for about 20 minutes after which 8mL of n-Heptane was added before being injected onto the GC column for analysis. 1µL (as stipulated in ASTM D6584) of each sample was injected into the column. Each sample was run three times. The results of the sample analysis are shown and discussed in chapter 4.2

3.2.4 Method repeatability

Palm vegetable oil biodiesel was run five times and the relative standard deviation (RSD) for glycerol, monoglycerides, diglycerides and triglycerides determined. From this, an analytical error limit was obtained for each of the glycerides and glycerol and these were

used as a general error for each glyceride in this method. The results of the repeatability are also shown and discussed in chapter 4.2.1.

3.2.5 Instrumentation

Gas chromatography (GC) analyses were performed following the ASTM D6584 protocol with the DANI MASTER GC (Fig 3-1) which was equipped with a programmable temperature volatilisation (PTV) and a flame ionisation detector (FID).

Figure 3-1 Dani Master GC instrument for glycerol and glyceride analyses.

The PTV temperature was immediately ramped to 360^{o}C at a rate of 999^{o}/min. The difference in instrumentation between the ASTM D6584 and the one used in this study is use of the PTV injector instead of the cool on-column injector as recommended by the ASTMD 6584 protocol. The GC was fitted with a Zebron 5HT inferno column with specification 15m ×0.32mm × 0.1µm with stationary phase of 5%-diphenyl-95%dimethyl polysiloxane copolymer. Samples were injected at a PTV temperature of 50^{o}C and an oven temperature of 50^{o}C. After an isothermal period at 50^{o}C for 1min, the oven was heated to a temperature of 180^{o}C at the rate of 15^{o}C/min and then to 230^{o}C at 7^{o}C/min and finally at 30^{o}C/min to 380^{o}C for 10min. The carrier gas used was Helium. Data acquisition was by means of the clarity chromatography software. The detector temperature was set at 380^{o}C. Analysis time took approximately 40mins.

3.3 Bound glycerol by normal phase high performance liquid chromatography with binary elution.

3.3.1 Chemicals and reagents.

The chemicals and reagents used in the analysis of bound glycerol (a function of the amount of mono, di and triglycerides that remain in biodiesel after glycerol separation) in the samples are the same as those used in the GC determination of total glycerol in 3.2.1 except that here oleic acid (Sigma) was included as one of the calibration standards in the quantitation of free fatty acids present in the samples. Moreover, there was no derivatisation since in HPLC analysis of bound glycerol, derivatisation is not necessary and therefore the use of MSTFA was not included. All the solvents and chemicals used were HPLC grade and were therefore used without purification.

3.3.2 Calibration standards

Four standards were used to set up calibration curves. These standards are oleic acid (for quantitation of free fatty acids), monoolein, diolein, and triolein. The table showing the concentration of the standards is displayed in Appendix B1.

3.3.3 Sample analysis

About 100mg of each sample was weighed into a 10ml volumetric flask and diluted to the mark with hexane/ ethanol (9:1). This was then filtered through a 0.45µm filter syringe. 10µL of the resulting solution was injected onto the HPLC column. The amount of individual glycerides present in each was sample was determined based on the response obtained from the chromatogram and the calibration function for each glyceride. The results of the sample analysis are discussed in section 4.3 of chapter

3.3.4 Validation of analytical method

The suitability of the analytical method for the analysis of the glycerides was determined by a statistical analysis of the results obtained after running the rapeseed biodiesel sample five times successively and determining the glyceride content. The mean and precision (SD) were determined of these runs were determined. The results obtained are discussed in 4.3.1.

3.3.5 HPLC instrumentation

HPLC analyses of the bound glycerol content were performed using the Thermo separations HPLC consisting of a binary gradient pump, AS1000 auto sampler and a helium degassing unit. The HPLC was equipped with an evaporative light scattering detector (ELSD) set to an evaporative temperature of 40^{o}C, a nebulizer temperature of 30^{o}C and a nitrogen flow of 1.50SPLM (See Fig 3-2). Elution of the glycerides was by means of a binary gradient set out in Table 3-2. The flow rate was set at 0.8mL/min. Data acquisition was by the Delta Chromatography software. The HPLC instrument was equipped with a Supelco discovery Cyano column of 250×4.6mm, 5µ and a 20× 4mm, 5µ Supelco guard column.

Figure 3-2 HPLC instrumentation

Table 3-2 Method of elution for binary solvents

Time	%A	%B
0	100	0
7	100	0
17	20	80
25	20	80
25.1	100	0
45	100	0

Where;

A= Hexane /0.4% acetic acid,B=Methyl-tert—butyl ether/5%ethanol/0.4% acetic acid

3.4 Ester and linolenic acid methyl ester content.

The ester and linolenic acid content of biodiesel defines the actual biodiesel. A limit of over 90 mass% of FAMES and between 1%- 15% and linolenic acid methyl ester respectively is required to define a quality Biodiesel.

3.4.1 Chemicals and reagent

Reagent grade Methyl heptadecanoate (99.5%) was used as the internal standards.

3.4.2 Sample analysis

Approximately 250mg of each sample was accurately weighed in a 10mL vial after which 5ml of the internal standard methyl heptadecanoate (10mg/L) was added. 1µL of this solution was injected into the GC for analysis. The results of the analysis are discussed

in 4.4.

3.4.3 Repeatability

To ensure that the results are repeatable, an appropriate liner was used. Moreover, the four standards used in this study were each run successively four times and their response ratios determined. The results are shown in 4.4.1.

3.4.4 Instrumentation for ester and linolenic acid methyl ester.

Samples were analysed on a Varian C-3380 gas chromatograph equipped with a split/split less injector and a flame ionisation detector. Chromatography was performed on a Zebron ZB-WAX (30m× 0.32×0.25µm). For instrument conditions, see Table 3-3.

Table 3-3 Instrument conditions

Parameters	**Mode in operation**
Oven	210^{o}C, isothermal
Injector	Split/ split less
Carrier gas	Helium, 8psi
detector	FID, 280^{o}C

3.5 Determination of methanol by headspace solid phase micro extraction.

Flash point is among the most important parameters that determines the quality of the final biodiesel product. The property is directly related to the amount of alcohol that is retained in the fuel after the transesterification reaction. EN14214 has set a maximum limit of 0.2 mass% and the ASTM D93 method places a 0.2 volume %.

3.5.1 Chemicals and reagents

Analytical grade methanol (99%, Sigma), analytical grade sodium chloride (99%, Sigma) and biodiesel samples from the Stellenbosch University were used. The 10mL SPME vials, fibre assembly, and the 60µm polyethylene glycol (PEG) fibre were purchased from Supelco (Stable flex).

3.5.2 Calibration standards

Calibration standards were prepared by using a thoroughly washed waste vegetable biodiesel as a reference biodiesel and spiking them with weighted amount of methanol. Deuterated methanol was added as an internal standard. A stock solution of methanol of concentration 4000 ppm (parts per million) was prepared and this was serially diluted with distilled water to give concentrations of 0 ppm, 40 ppm, 80 ppm, 120 ppm and 160 ppm (see Appendix D1). The washed waste vegetable biodiesel which was not spiked with any methanol was used to check for the presence of methanol in the biodiesel sample after it had been washed with warm water three times.

3.5.3 Samples analysis

The samples comprised unwashed biodiesel which had high levels of methanol and therefore had to be diluted to ensure that their methanol concentration levels were within the range of the standard methanol concentration used in the calibration.

The process of dilution involved diluting a known volume of the actual samples for analyses, in this case the unwashed biodiesel, with a known volume of the three times washed biodiesel samples. During this dilution, 0.5mL of the unwashed biodiesel samples was diluted with 9.5mL of the washed sample to which 1mL of the deuterated

methanol internal standard (IS) and about 2g of sodium chloride added. The resulting mixture was vortexed for a minute.

1mL of the resulting solution was analysed by exposing the polyethylene glycol fibre (PEG) into the headspace of the vial. In order to determine the accuracy of this method, the 120ppm standard was run five times and the relative standard deviation determined.

The results of the methanol analysis and the repeatability of this analytical method are discussed in 4.4 and 4.4.1.

3.5.4 Instrumentation

The analysis was performed with the Agilent 6890N GC with CTC CombiPAL auto sampler and Agilent 5975 mass spectrometer (MS).The instrument was fitted with a DB-FFAP column (60m×0.25µm × 0.25µm film thickness). For oven temperature program see Table 3-4:

Table 3-4 Oven temperature program

Oven temp	*^{o}C/min*	*Temp*	*Hold (min)*
Initial		35	5
Ramp 1	5	80	0
Ramp 2	30	240	0

Helium was used as a carrier gas at a constant flow rate of 1.5mL/min. The injector was in a split ratio of 1:5 at an injector temperature of 220^{o}C.The scanning mass range of 29 to 300m/z was used.

3.6 Iodine values

The iodine value (IV) indicates the amount of unsaturation or the number of double bonds present in the biodiesel. This unsaturation may stem from the free fatty acids present or it may come from the TG, DG and MG and various other components such as carotenes and squalenes and other steroids present in the fuel. The IV was determined by using the EN ISO 3961(1996). In this method, the sample was reacted with excess of Wijs solution (Iodine chloride in acetic acid solution) followed by determination of the

excess Wijs solution by reacting with potassium iodide which liberated iodine. The amount of iodine liberated was by a back titration using the titrator in Fig 3-3.

Figure 3-3 Equipment for iodine value determination.

3.7 Cold temperature properties of biodiesel

The major disadvantage of biodiesel apart from the emission of nitrogen oxides (NOx) is its unfavourable cold flow properties since it begins to gel at low which clog the filters or can even become so thick that it cannot be pumped from the fuel tank to the engine (Joshi *et al*, 2006). The cold flow properties that characterise biodiesel are its viscosity, density and cloud and pour points.

In this study, the kinematic viscosity, the cloud and pour points and densities of the biodiesel were investigated for both the unwashed and washed samples with regard to temperature changes.

3.7.1 Kinematic viscosity

The main purpose of the transesterification reaction was to reduce the viscosity of the oil. In this study, the viscosity of each of the six biodiesel samples was taken from temperatures of 20^{o}C to 40^{o}C intervals of 5^{o}C (293K-313K) for both the washed and the unwashed samples. This measurement was done according to the ASTM D445 procedure. Viscosity measurements for the samples were made from the temperature range 293K to 313K. Duplicates readings were taken and the results averaged. The changes in viscosity with regard to the temperatures indicated in this study are discussed under the cold flow properties in 4.7.1.

3.7.2 Cloud point and pour point

The Cloud and Pour point characterise the low temperature operability of biodiesel and are strongly influenced by the presence of saturated fatty acids in the fuel. The cloud point is defined as the lowest temperature at which a cloud of wax crystals first appear in a liquid when it is cooled under controlled conditions during a standardised test (Bhale 2009) and the Pour point is the temperature at which the fuel can no longer be poured due to gel formation. The Cloud point is determined by the presence of a haze in the normally clear fuel. The equipment usually used for the determination of both the Cloud and Pour point is shown in the Fig 3-4.

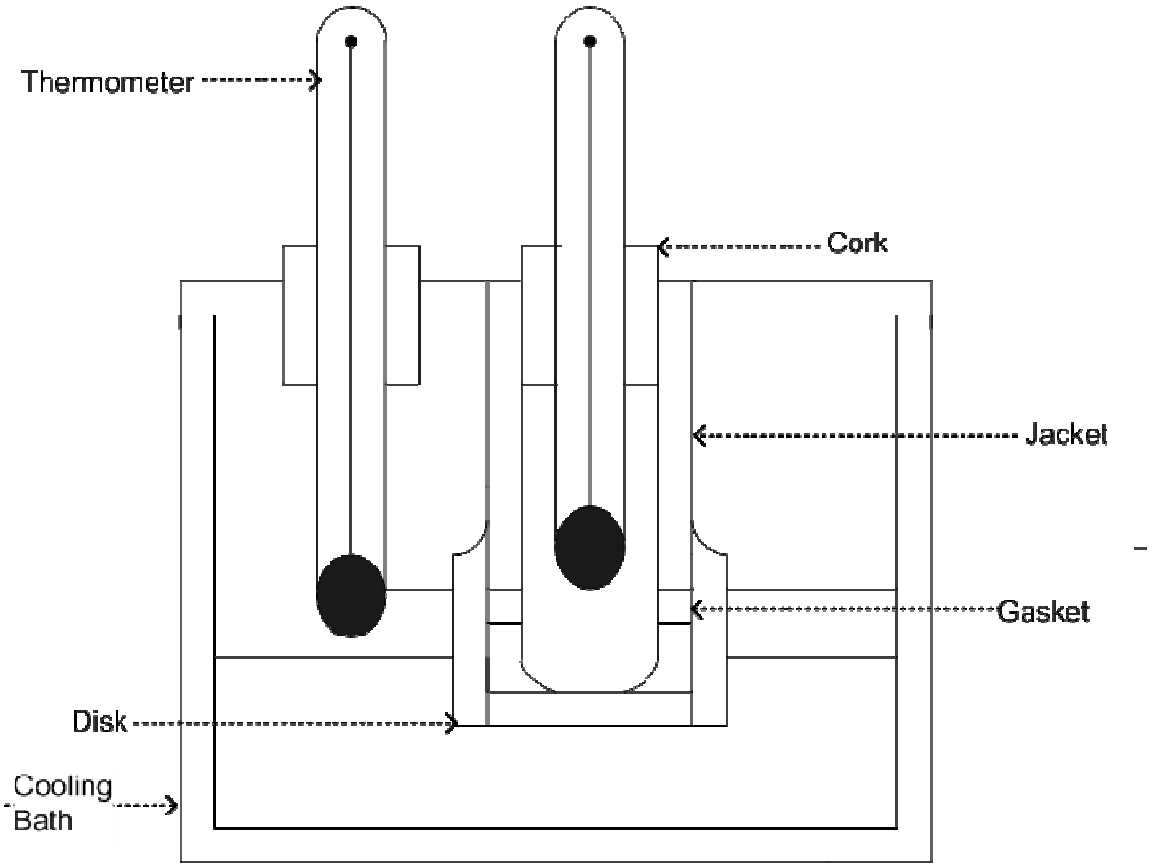


Figure 3-4 Set-up for the determination of Cloud and Pour points.

Before the Cloud and Pour points measurements were made methanol and glycerol in the washed samples were completely removed to ensure an effective comparison between the washed and unwashed biodiesel. In measuring the Cloud point and Pour point of the biodiesel samples made up of washed, unwashed, and blended biodiesel (the blending was 50vol% for both the saturated and unsaturated biodiesel). Samples were cooled at a specified rate and examined at specific temperature intervals following

the procedure prescribed by ASTM D97 and D2500 for the Pour point and Cloud point respectively. Three different readings were taken for each sample and the results averaged. The difference between each measurement was not more 3^{o}C for both the Cloud and Pour points indicating a consistency in the measurements. The readings obtained from this study are presented and discussed in section 4.7.2- 4.7.5 of the next chapter.

3.7.3 Density

Density is a fundamental physical property that can be used in conjunction with other properties to characterise biodiesel. The digital density analyzer was calibrated at 20^{o}C for the determination of the density of the various biodiesel samples at 20^{o}C. The procedure for the determination was done according to ASTM D4052. A small volume of each of the samples (0.7mL) was introduced into an oscillating tube and the change in the oscillating frequency of the U-tube as a result of the change in mass of the tube was used together with calibration data to determine the density of the biodiesel samples. The density of the sample was recorded when the instrument displayed a steady reading.

The results obtained from the methodology presented in this chapter will be presented and discussed in the next chapter in 4.7.6.

4

4.1 RESULTS AND DISCUSSIONS

4.2 Determination of total glycerol (Using GC).

The response and the amount ratios (see Appendix A2 and Appendix A4) of glycerol, monoolein, diolein and triolein (of the standards S1-S5) were used to set-up a calibration curve for each of monoolein, diolein and triolein and glycerol. These calibration curves were used to quantitate the amount of monoglyceride, diglyceride and triglyceride and glycerol respectively. The calibration curves for all the glycerol and glycerides (MG, DG and TG) displayed excellent linearity. The standard used in setting up the response and amount ratios is in appendix A1. Using the slope and y-intercept of each calibration function (see Appendix A5, A6 and A7), the mass percent (%) of the glycerides and glycerol in each sample was determined according to the ASTM D6584 protocol. The following equation was used for the determination of the mass percent (%) of the glycerol as detailed in the ASTM D6584 protocol:

$$G = \left(a_g \times \frac{A_g}{A_{IS1}} + b_g \right) W_{IS1} \times \frac{100}{W}$$ for glycerol determination Eqn [4-1]

Where, G = Mass percent of glycerol in the sample,

A_g = Peak area of the glycerol in sample.

A_{IS1} = Peak area of internal standard 1(1, 2, 4-butanetriol).

W = Weight of the biodiesel sample, milligrams (mg).

a_g = Slope of glycerol calibration curve.

b_g = Intercept of the glycerol calibration curve

W_{IS1} = Weight of internal standard 1 (mg)

As an example, the determination of glycerol was based on Eqn 4-1 and the glycerol calibration function shown in Fig 4-1.

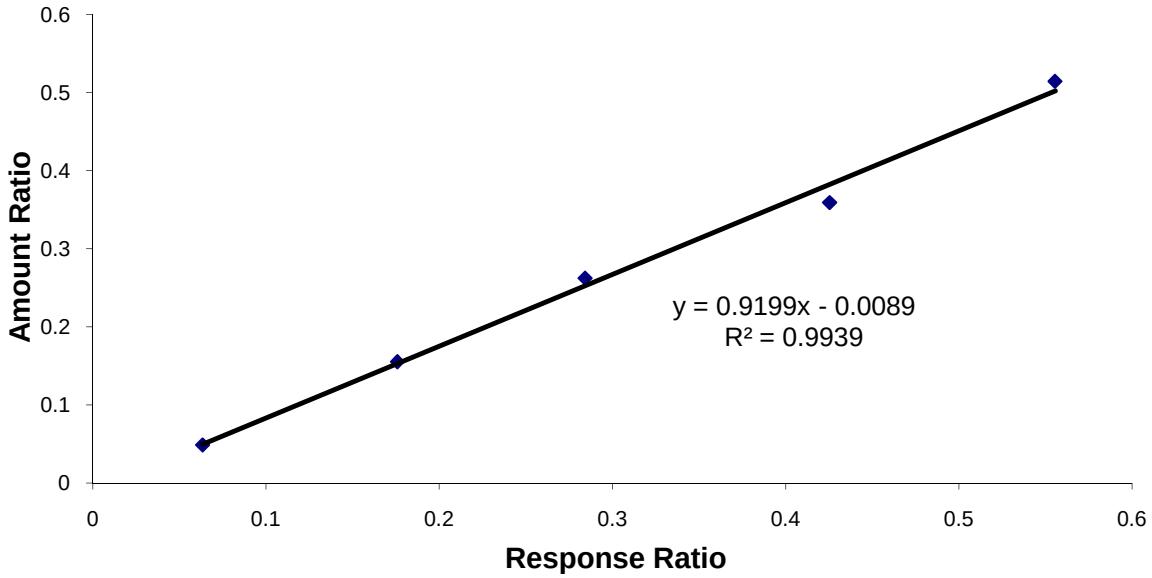


Figure 4-1 Glycerol calibration curve

This example is based on responses obtained from the run of rapeseed biodiesel chromatogram.

Thus, for glycerol; $G = \left(a_g \times \left(\frac{A_G}{A_{IS1}} \right) + b_g \right) \times W_{IS1} \times \frac{100}{W}$

$$a_g = 0.9199, b_g = -0.0089, W_{IS1} = 0.103mg, A_g = 35.2670, A_{IS1} = 153.0340, W = 85.7mg$$

$$G = \left(0.9199 \times \frac{35.2670}{153.034} - 0.0089 \right) \times 0.103mg \times \frac{100}{85.7mg}$$

$$G = 0.0244, mass\,\%$$

Therefore, 85.7mg of rapeseed biodiesel contained, 0.0244 mass % of free glycerol.

For the glycerides of mono, di and triglycerides (MG, DG and TG) the mass percent (%) of each sample was determined using Eqn 4-2 and the slopes and intercepts of their respective calibration functions (See Appendix A5-A7).

$$G_{lm} = \left(a_o \times \frac{A_{glm}}{A_{IS2}} + b_{o1} \right) W_{IS2} \times \frac{100}{W}$$ for glyceride determination Eqn [4-2]

$$G_{lm} = \left(a_{o1} \times \frac{A_{glm}}{A_{IS2}} + b_{o1} \right) W_{IS2} \times \frac{100}{W}$$

Where, A_{glm} = Peak area of monoglycerides, and G_{lm} is the mass percent of all identified monoglyceride,

A_{IS2} =Peak area of internal standard 2 (tricaprin)

a_{ol} = Slope of monoglyceride calibration function

b_{o1} = Intercept of monoglyceride calibration function.

W_{IS2} = Weight of internal standard 2

The response obtained from the run of palm biodiesel is used as an example to determine the mass percent (%) of monoglycerides (MG).

For monoglycerides (MG):

$$a_{o1} = 1.0120, b_{o1} = 0.0463, A_{glm} = 103.072, A_{IS2} = 480.3630, W_{IS2} = 0.8mg, W = 83.8000mg$$

Using Eqn 4-2, $G_{lm} = \left(a_{o1} \times \frac{A_{glm}}{A_{IS2}} + b_{o1} \right) \times W_{IS2} \times \frac{100}{W}$

$$G_{lm} = \left(1.0120 \times \frac{103.0720}{480.3630} - 0.0463 \times \frac{100}{83.8000mg} \right) = 0.16mass\%$$

According to the ASTM D6584 protocol, the mass% determine above is multiplied a response factor (also for DG and TG) to arrive at the total mass percent (%) present in the biodiesel. The response factors for the glycerides are:

$$MG = 0.2951 \quad , DG = 0.1488 \quad , TG = 0.1044$$

Thus, for the total percentage monoglyceride G_{lm_t} ; $G_{lm_t} = 0.2951 \times \sum G_{lm}$

$$\text{Where } 0.2591 = \frac{mm(glycerol)}{mm(monoolein)} = \frac{92.09}{355.42} = 0.2951$$

$$\text{Thus, } G_{lm_t} = 0.2591 \times 0.16\% = 0.04 mass\%$$

The mass percent (%) of DG and TG were determined in a similar manner using the slope and y-intercept of their respective calibration functions together with response factor mentioned above. All the results for the glycerols and the glycerides for each biodiesel sample are the average of three runs for each sample (Appendix A8). The average mass percent (%) of the glycerol and glycerides (MG, DG and TG) for all the various samples are shown in Fig 4-2 and the actual mass % are displayed in Appendix A9.

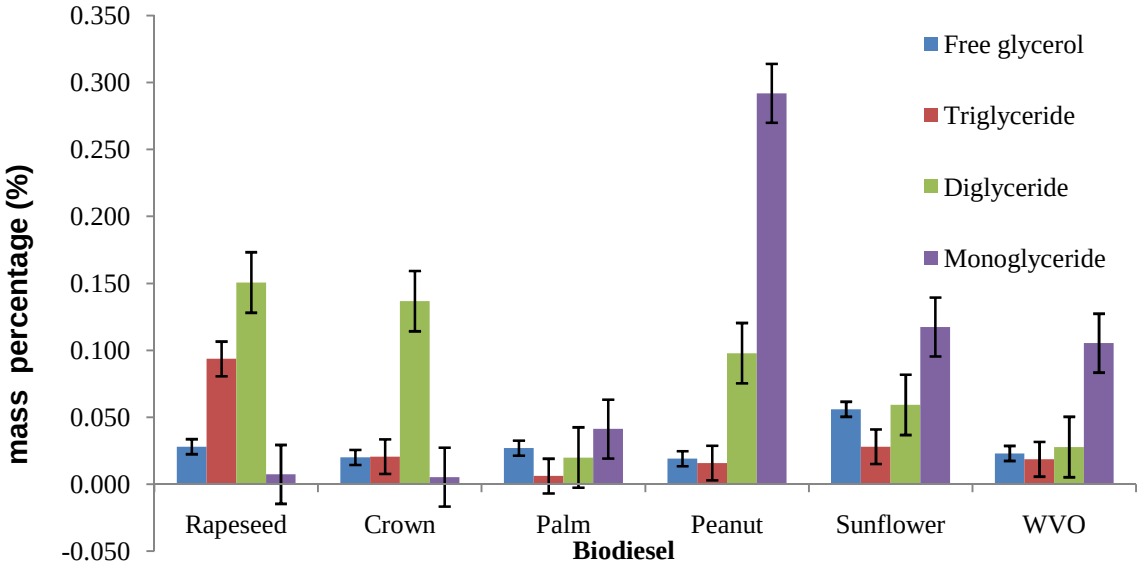


Figure 4-2 Percentage mass of free and bound glycerol in biodiesel samples.

The levels of free glycerol (See Appendix A9), in all the samples except sunflower biodiesel (0.056%) meets the maximum limit allowed for a standard biodiesel according to both the ASTM D6571 and EN 14214 both of which have set a 0.02 mass percent (%) maximum. The levels of total glycerol (sum of glycerol and MG, DG and TG) in the biodiesel samples of crown (0.183%), palm (0.094) and wvo (0.175) were within the required maximum limits of 0.240 mass percent (%). However, the total glycerol in peanut biodiesel (0.425%) (See Appendix A9) was higher than the required maximum limits as per ASTM D6584/EN14105 protocol. This maybe as a result of an inefficient transesterification compared to the other biodiesel used in this study.

4.2.1 Repeatability

The mass percent (%) of glycerol and the glycerides (MG,DG and TG) in palm biodiesel (random choice) was determined through a series of successive runs (n=5) and from the results obtained, a statistical analysis comprising the determination of the mean, standard deviation (SD) and relative standard deviation (RSD) was used to assess this analytical method (Table 4-1).

Table 4-1 Repeatability of the mass % glycerol and glycerides in palm biodiesel (. $n = 5$)

Biodiesel	Free Glycerol	Monoglyceride	Diglyceride	Triglyceride
Palm 1	0.0480	0.0155	0.0255	0.0124
Palm 2	0.0234	0.0220	0.0203	0.0073
Palm 3	0.0524	0.0194	0.0321	0.0137
Palm 4	0.0437	0.1072	0.0354	0.0226
Palm 5	0.0436	0.0730	0.0201	0.0049
Mean	0.0422	0.0474	0.0267	0.0122
S.D.	0.0111	0.0409	0.0069	0.0068
RSD (%)	26.3041	86.1295	25.8533	56.2128
St error	0.0050	0.0183	0.0031	0.0031

A standard error for this analytical method (for both glycerol and the glycerides was determined from the successive run of palm biodiesel $(n = 5)$ and from this, an error bar was generated for the glycerol and glycerides (See Fig 4-3).

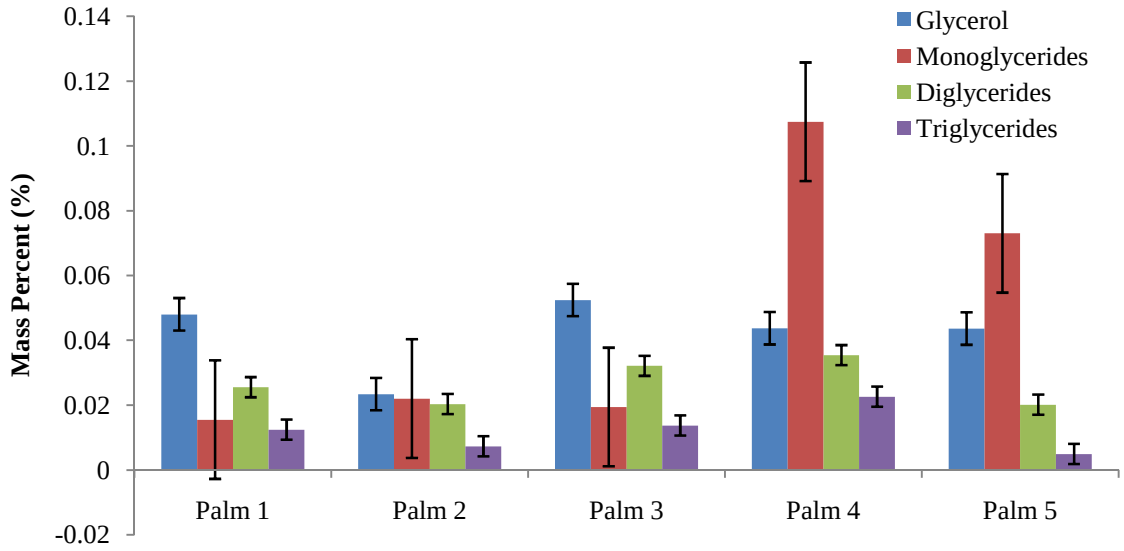


Figure 4-3 Percentage mass of free glycerol and glycerides.

The error associated with this method is responsible for the negative mass indicated on the mass percent axis as the mass percent of some of the glycerides were smaller than the error associated in analysing the particular compound. For instance, the level of monoglycerides in palm 1(0.0155) was smaller than the error associated with analysing monoglycerides using this method (0.0183).

In the ASTM method, a cool on-column injector is recommended as the suitable injector in determining the bound and free glycerol content in biodiesel samples to achieve good repeatability. Moreover, Klee (1990) affirmed that the highest precision and accuracy attainable by GC analysis is afforded by direct on-column injection techniques (Klee, 1990) during repeated determinations.

The results of the precision of this method expressed as RSD% (26, 86, 25, and 56 respectively for glycerol, MG, DG, and TG) indicate a poor repeatability when using the PTV as a substitute injector in analysing glycerides and glycerol content in biodiesel. The poor repeatability may be due to the malfunctioning of the PTV injector, which always ramped to a very high temperature leading to the melting of the liner and this affected the results obtained in this study and also caused the poor repeatability. Therefore, in this study, the use of the DANI MASTER GC equipped with PTV injector did not afford the accuracy and precision required when following the ASTM D6584 protocol. Thus, according to the results obtained, the hypothesis that the repeatability afforded by the cool- on-column injector in glycerol and glyceride analysis biodiesel is achievable by the PTV injector is false.

4.3 Determination of bound glycerol by normal phase high performance liquid chromatography with a binary gradient.

Five standards S1-S5 (Appendix B1) of oleic acid, monoolein, diolein, and triolein of different concentrations in microgram per millilitre (µg/mL) were used to set up a calibration curve for the determination of free fatty acids (in the form of oleic acid) and bound glycerol which is a function of residual amount of triglyceride and partial glycerides (MG and DG) in biodiesel (Foglia et al, 2004). After identifying the peaks and their responses (area), a calibration graph of peak (x-axis) and the concentration (ug/mL) was set up for each of oleic acid (OA), monoglyceride (MG), diglyceride (DG) and triglyceride (TG) (Appendix B2-B6). The amount (ug/mL) of these substances (MG, DG, and TG) in each of the biodiesel was determined from their respective functions and these were further converted to their mass percent (%) using Eqn 4-3:

$$\sum AO = \frac{C \times V}{M} \qquad \text{Eqn [4-3]}$$

Where, $\sum OA =$ Percentage mass of oleic acid and also each glyceride as the case may be.

$C =$ Concentration of analytes in microgram per millilitre (µg/mL) obtained from the calibration curves

$V =$ Volume of the volumetric flask (10mL)

$M =$ Mass of the sample in microgram (µg)

For instance, WVO was used as an example with the following responses to determine the mass percent (%) of the free fatty acid (oleic acid) and the glycerides with the responses (a.u.) indicated;

Oleic acid =1477892, triglyceride =302427V, diglyceride =4085659, monoglyceride = 1172723. Thus, for oleic acid (OA);

$y = 1E - 5x + 26.576$, (calibration function of OA), as displayed in Appendix B2.

Where x is the response obtained from the chromatogram and y is the concentration in ug/mL.

$x = 1477892$ (response for OA), $y = 1E-5(1477892) + 26.576 = 41.790 ug/mL$

From the y value obtained, the mass percent (%) of OA from the above concentration (41.790µg/mL) was determined by using Eqn [4-3];

$$\sum OA = \frac{C \times V}{M}$$

$$\sum OA = \left(\frac{41.790\,ug}{1mL}\right) \times \left(\frac{10\,mL}{111500\,ug}\right) \times 100 = 0.375\,mass\,\%$$

For monoglyceride (MG): using the calibration function in Fig 4-4;

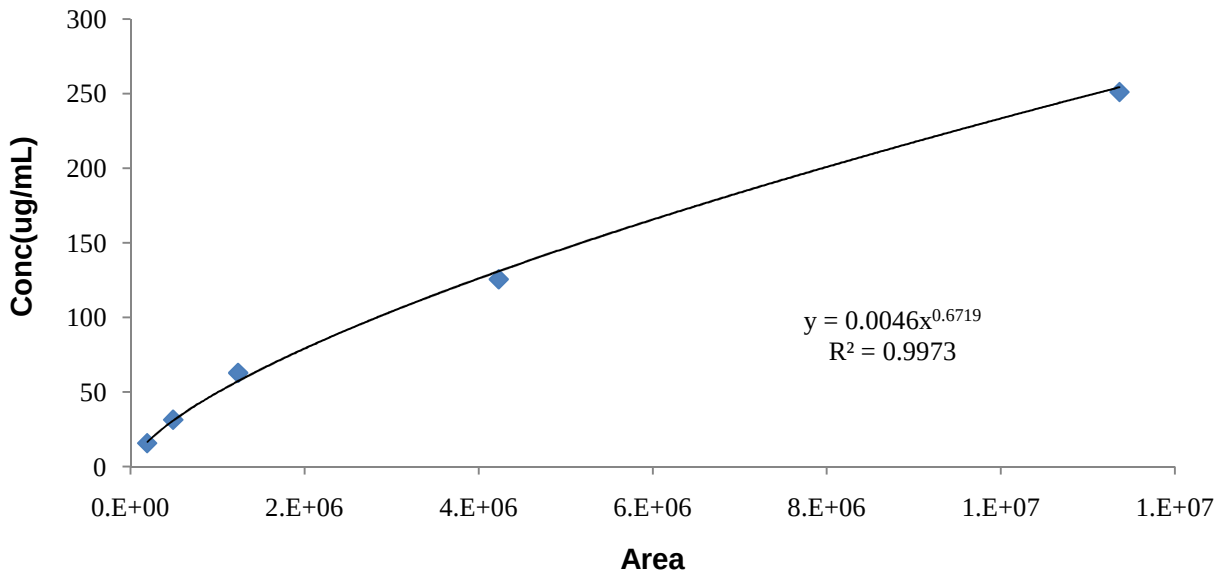


Figure 4-4 Calibration curve for monoglycerides

$y = 0.0046x^{0.6719}$ (MG calibration function)

$y = 0.0046(1172723)^{0.6719} = 55.036 \mu g / mL$

$$\sum MG = \left(\frac{55.036ug}{1mL}\right) \times \left(\frac{10mL}{111500ug}\right) \times 100 = 0.494mass\%$$

$$MG_t = 0.494mass\% \times 0.2591 = 0.13mass\%$$

Therefore, $111500\mu g$ wvo samples contain 0.375% free fatty acid in the form of oleic acid and 0.013% MG.

The components in the various biodiesel were separated into fractions of oleic acid (OA), triglycerides (TG), diglycerides (DG) and monoglycerides(MG) in ascending order of elution times (Fig 4-5). The presence of ethanol in the chromatogram in Fig 4-5 was due to the fact the biodiesel samples were diluted in with a hexane/ethanol.

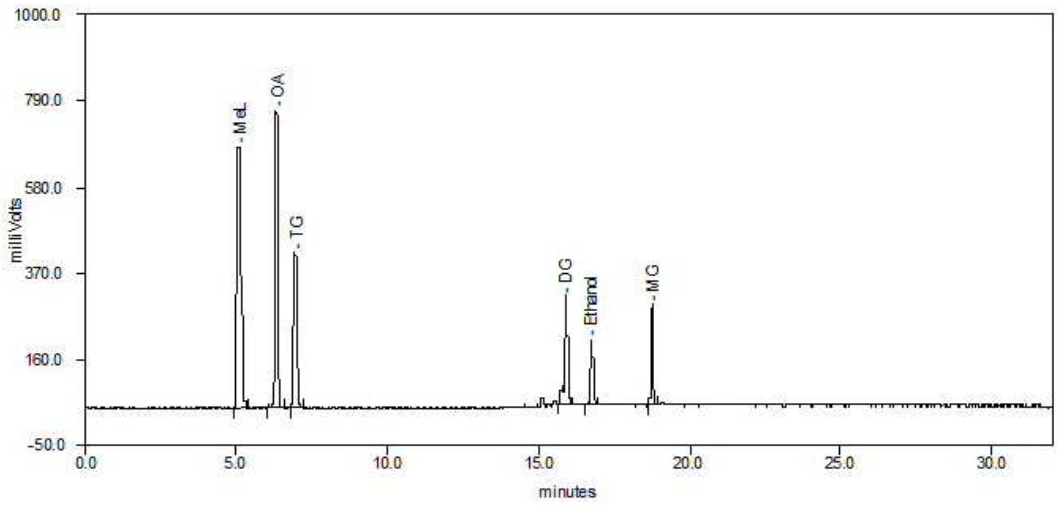


Figure 4-5 Chromatogram of palm biodiesel

The presence of the free fatty acids in the form of oleic acid in palm and wvo biodiesel samples was to be expected (Fig 4-6).

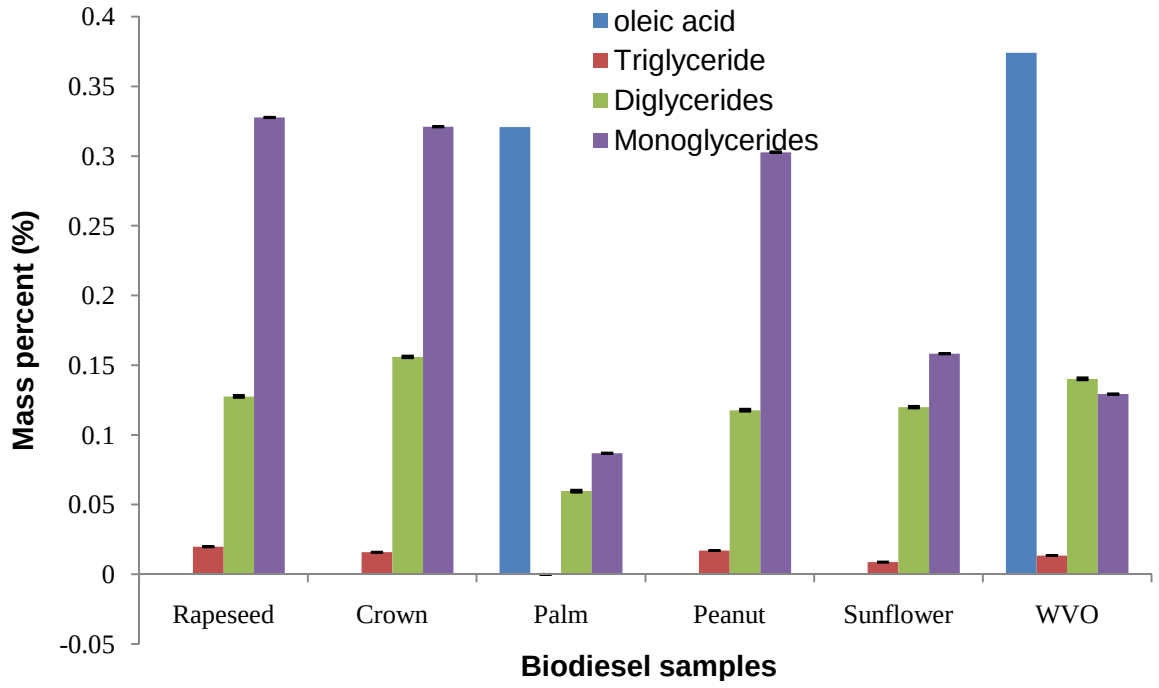


Figure 4-6 Mass percent (%) of the glycerides in all the biodiesel samples.

The mass percent % in Fig 4-6 are the average of 3 determinations. Refining of feed stocks by neutralization removes/reduces the amount of free fatty acids by treating the feed stock with an alkali solution. Refining may be responsible for the lack of free fatty acid in the biodiesel samples of crown, peanut, sunflower and rapeseed oils since the feed stock oils were purchased refined whilst the wvo and palm were not. The amount (mass %) of TG in all the samples indicates the degree of the transesterification reaction. The mass% of TG in all samples ranged between 0.020 and 0.009. For the actual amount of the oleic acid and the glycerides see appendix B6. All the samples displayed a high concentration of monoglyceride. An analytic error could not be generated for free fatty acids in the biodiesel since the sample used for the generation of the error associated with this method did not contain free fatty acid and this is the reason why there was no error bar on free fatty acids in Fig 4-6.

4.3.1 Repeatability

To check on the suitability of this method for the determination of the glycerides, rapeseed biodiesel was run successively for five times by the same operator and under the same analytical conditions and the results obtained evaluated statistically as shown in Table

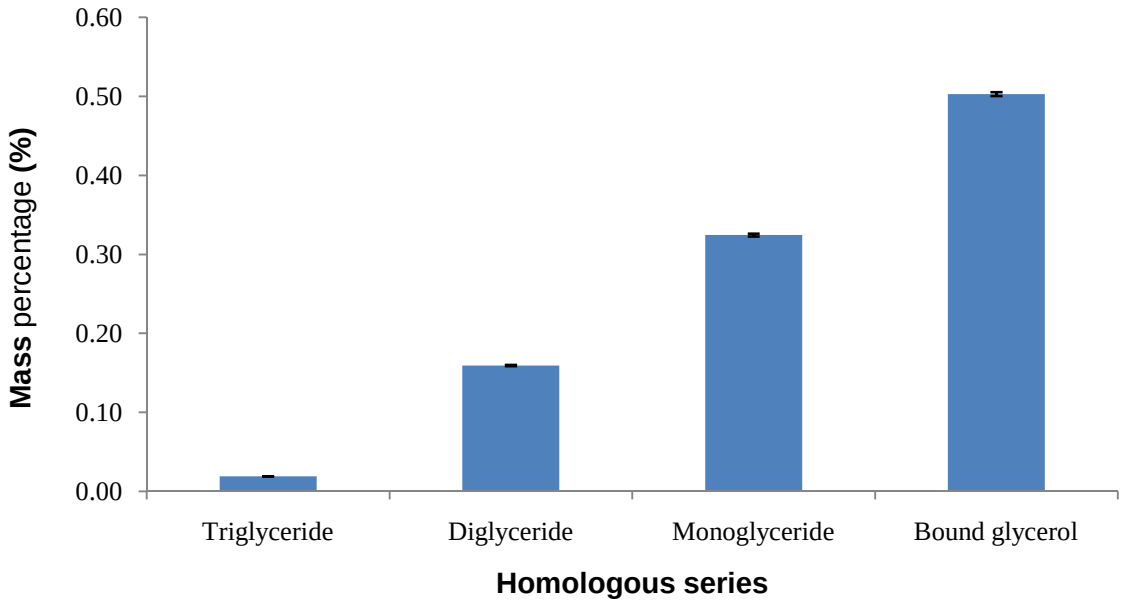
4.2.The results indicate a good repeatability for all the glycerides since the RSD obtained for all he glycerides were within the recommended 1-4%.

Table 4-2 Mass percentage of MG, DG, TG and BG in rapeseed biodiesel.

Biodiesel Sample	**TG**	**DG**	**MG**	**BG**
Rapeseed 1	0.183	1.068	1.236	1.487
Rapeseed 2	0.183	1.065	1.248	2.496
Rapeseed 3	0.183	1.058	1.240	2.482
Rapeseed 4	0.184	1.070	1.275	2.529
Rapeseed 5	0.185	1.090	1.260	2.535
Mean	0.184	1.070	1.252	2.506
Stdev	0.001	0.012	0.016	0.025
%RSD	0.331	1.117	1.264	0.978
Std. error	0.0003	0.0053	0.0071	0.0110

The error bar for each glyceride is indicated in Figure 4-7.

Figure 4-7 Mass % of MG, DG and TG in rapeseed biodiesel.



Therefore, the results obtained from this analysis indicate that, a normal phase – HPLC with binary solvents of hexane/ 0.4% acetic acid and methyl tert-butyl ether / 5% ethanol/ 0.4% acetic acid affords the analysis of free fatty acids and bound glycerol with repeatable results when analysing biodiesel contaminants. This method is simple, relatively quick and requires no complex sample preparation.

4.4 Ester and linolenic acid methyl ester content.

The ester and linolenic acid methyl ester content was determined according to the EN14103 where the fatty acid methyl esters (FAMES) content was expressed as mass percent (%) fraction using the methyl heptadecanoate ($C_{17:0}$) as the internal standard. Five standards namely Methyl Palmitate (MeP)**,** Methyl Oleate (MeO), Methyl Linoleate (MeL) and Methyl Stearate (MeS) were run and their retention times were used in identifying the chromatogram produced by the biodiesel samples. The following formula, according to EN14103 protocol for ester determination was used in the determination of the ester:

$$C = \frac{\sum A - A_{IS}}{A_{IS}} \times \frac{C_{IS} \times V_{IS}}{m} \times 100 \qquad \text{Eqn [4-4]}$$

And the determination of the linolenic acid methyl ester content was also determined according to the equation;

$$L = \frac{A_L}{A_{IS}} \times \frac{C_{IS} \times V_{IS}}{m} 100 \qquad \text{Eqn [4-5]}$$

Where , $\sum A =$ Total peak area from $C_{14:0,}$ $C_{16:0,}$ $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$, $C_{20:0}$, $C_{20:1}$, $C_{22:0}$ (i.e. methyl esters of myristic acid, palmitic acid, stearic acid, oleic acid, linolenic acid, linolenic acid, arachidic acid, gadoleic acid, and behenic acid).

$A_{IS} =$ Peak area of the internal standard (methyl heptadecanoate).

$C_{IS} =$ Concentration of the internal standard in milligram per millilitre (mg/mL)

$V_{IS} =$ Volume of the internal standard in mL.

$m =$ Mass of the sample.

A_L = Peak area of linolenic acid methyl ester ($C_{18:3}$).

L = Percentage linolenic acid methyl ester

For instance, using Eqn [4-4], the ester content (in mass percent), of wvo with the following responses was determined as follows:

$C_{14:0,}$ = 2931, $C_{16:0,}$= 175242, A_{IS}=36378, $C_{18:0,}$= 100607 $C_{18:1,}$= 810296, $C_{18:2,}$=1072865, $C_{20:0,}$ = 6656 $C_{20:1,}$= 7759, $C_{22:0}$ = 11389, C_{IS}= 1.012mg/mL, V_{IS}= 5mL

$$\sum A(C_{14:0-22:0}) = 2224447.2$$

$$\text{Using, } C = \frac{\sum A - A_{IS}}{A_{IS}} \times \frac{C_{IS} \times V_{IS}}{m} \times 100$$

$$C = \frac{2224447.2 - 36378}{36378} \times \frac{5mL \times 1.012mg/mL}{324.2mg} \times 100 = 93.88mass\%$$

Each sample was run three times and the content of the ester determined in a similar manner as above. The percentage mass conversions of the oil as indicated in Fig 4-8 showed sunflower and palm biodiesel having the highest mass % conversions. Data for the setting up of this graph could be found in appendix C1. The results as seen in Fig 4-8, are the averages of the three determinations for each sample analysed.

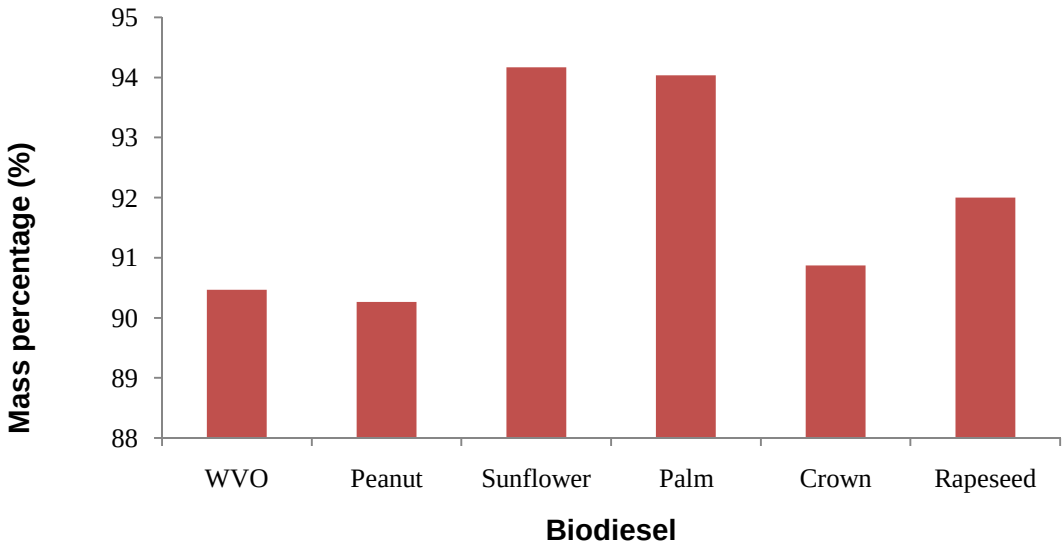


Figure 4-8 mass % of ester in Biodiesel samples.

Identification of the individual fatty acid methyl esters (FAMEs) was based on the retention times of the standard FAMEs which were run prior to the sample analysis. Oil conversion to the fatty acid methyl ester (biodiesel) depends on the reaction conditions employed for the transesterification reaction. These reaction conditions include the molar ratio of the oil to the alcohol, the presence of free fatty acid (FFA) and water in the feed stock. The low percentage conversion of wvo may be due to the presence of free fatty acids (FFA). The FFA consumes the catalyst and this may have impacted on the percentage conversions observed for the wvo.

Moreover, since the wvo was not refined, the likelihood of water being present could account for the low percentage conversions observed since water inactivates the catalyst thus affecting the effectiveness/efficiency of the transesterification reaction. Sunflower, rapeseed and palm oil had the highest conversion rates compared to the other samples. The reasons for their high conversion rate may be due to a low level of FFA and lack of water in the feed stock.

It is understandable that all the samples failed to make the 96.5% minimum conversions since the transesterification reaction was conducted in an open beaker in the laboratory at 70^{o}C and therefore some of the methanol may have evaporated thus affecting the molar ratio of the methanol to the oil.

For the linolenic acid methyl ester content in the biodiesel, the response of the linolenic acid methyl ester $(C_{18:3})$, and the internal standard $(C_{17:0})$ was used. For instance, the mass % of linolenic acid methyl ester in 259.7mg of wvo with the following responses was determined using Eqn [4-5] as follows;

$$A_L = 30201, A_{IS} = 37486, C_{IS} = 1.012 mg/mL, V_{IS} = 5mL, m = 259.7mg$$

$$L = \frac{30201}{37486} \times \frac{1.012 mg/mL \times 5mL}{259.7} \times 100 = 1.56 mass\%$$

The results as seen in Fig 4-9 are the average of three determinations for each biodiesel.

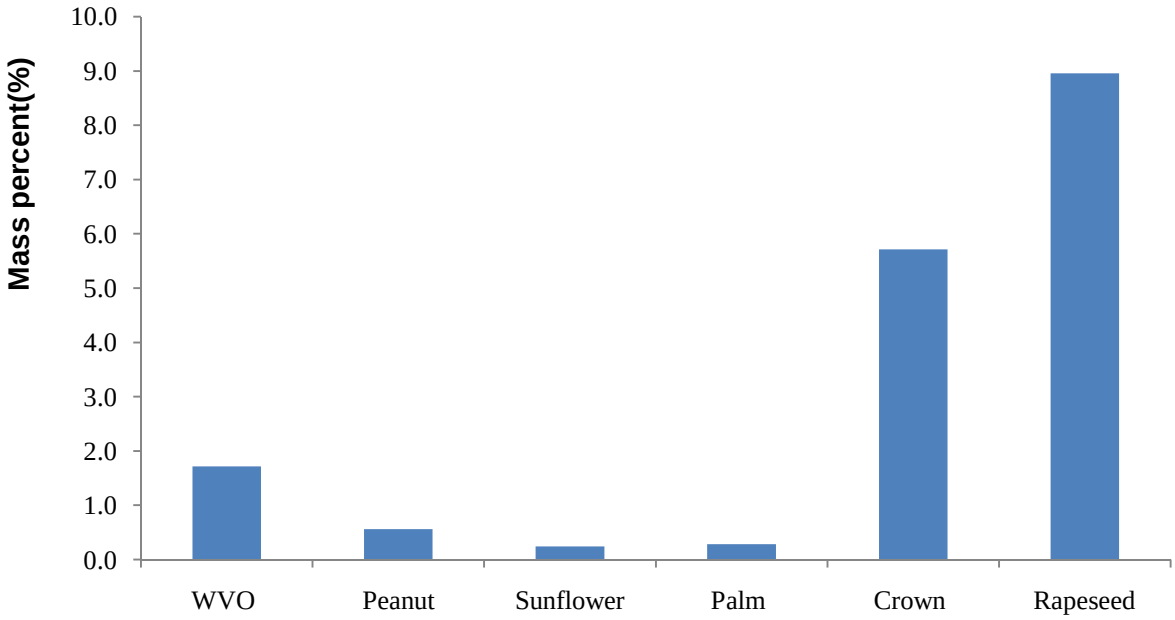


Figure 4-9 Percentage mass of linolenic acid methyl ester.

Rapeseed methyl ester had the highest mass percent (%) of linolenic acid methyl ester compared to the other biodiesel samples. WVO, crown and rapeseed methyl esters had average linolenic acid methyl ester content of 1.716%, 5.714% and 8.956% respectively. These fall within the stipulated values of between 1% and 15% that is recommended by both the ASTM D6751and EN14214 for a standard biodiesel.

In this study, it was observed that the amount of linolenic acid methyl ester present in a given biodiesel depend on the percentage composition of linolenic acid naturally occurring in the feedstock and also the extent of transesterification.

An efficient transesterification reaction coupled with a high degree of naturally occurring linolenic acid in the feed stock are responsible for a high percentage of linolenic acid methyl ester in the biodiesel sample. The above mentioned factors may be responsible for the high linolenic acid methyl ester content of rapeseed biodiesel. Naturally, rapeseed oil contains 10%wt of linolenic acid (refer to Table1.1) whilst others like peanut and palm biodiesel contain trace amounts of linolenic acid and this could be a good reason why the linolenic acid methyl ester content was higher in rapeseed, peanut and palm biodiesel samples aside

the extent of transesterification. In this study, the identification of the ester was from $C_{14:0}$ (methyl myristate) to $C_{22:0}$ (methyl ester of behenic acid) even though only wvo, peanut and sunflower showed small quantities of $C_{14:0}$. The mass % of the esters from $C_{14:0}$- $C_{22:0}$ are shown in Table 4-3.

Table 4-3 Percentage FAMEs Composition, m/m, of the various Biodiesel

Biodiesel Sample	**C14:0**	**C16:0**	**C18:0**	**C18:1**	**C18:2**	**C18:3**	**C20:0**	**C20:1**	**C22:0**
WVO	0.901	6.777	4.129	33.037	43.621	0.642	0.299	0.292	0.476
Peanut	0.021	11.617	3.128	35.383	34.565	0.050	1.232	0.971	2.644
Sunflower	0.062	5.769	2.638	34.080	50.352	0.023	0.165	0.184	0.506
Palm	0.629	36.657	4.902	40.066	10.706	0.026	0.355	0.157	0.116
Crown	0.000	8.228	3.237	28.186	44.793	0.519	0.371	0.311	0.380
Rapeseed	0.000	4.416	2.017	54.029	21.375	0.824	0.533	0.903	0.315

C14:0-Myristicric acid *C16:0-palmitic acid* *C18:0-steric acid* *C18:1-oleic acid*

C18:2-linoleic acid *C18:3Linolenic acid* *C20:0-arachidic acid*

C20:1-gadoleic acid *C22:0-behenic acid*

The elution of the esters took place in order of increasing number of carbon with esters having the least number of carbons eluting first as depicted in Fig 4-10.

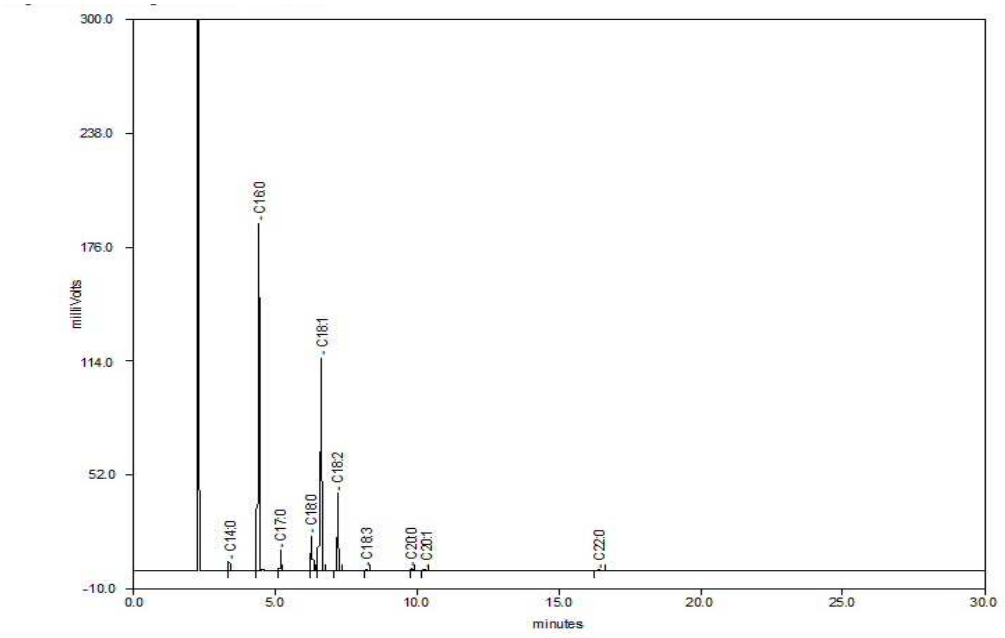


Figure 4-10 Chromatogram of wvo biodiesel.

The compositions of the fatty acid methyl ester content in each biodiesel reflected the percentage weight composition of the fatty acids naturally occurring in each feed stock. For instance, for palm biodiesel, the composition of the methyl esters making up the total FAMEs reflects the amount (%) and composition of the fatty acid present.

4.4.1 Repeatability of FAMEs and linolenic acid methyl esters

In order to ensure repeatable results and linear responses for the fatty acid methyl esters, it is imperative to ensure that the appropriate injection technique and also that the appropriate liner is used. Failure to adhere to the above mentioned factors will lead to a compromise of the responses and the reproducibility of the analytical results. This study ensured appropriate injection technique and that the appropriate liner was in place.

Moreover, retention time reproducibility was also found to be consistent in this study. The four standards used Methyl Palmitate (MeP)**,** Methyl Oleate (MeO), Methyl Linoleate (MeL) and Methyl Stearate (MeS) were each run four times in succession to determine retention time and response reproducibility. The repeatability of the results obtained after the standards were run was determined statistically by the use of the RSD% as shown in Table 4.4.

An average of the responses of the various standards was used to determine the average response for the standards (Table 4-4).

Table 4-4 Response ratios of the four FAMEs standards

Peak Response						**Response Ratio**			
	MeP	IS	MeS	MeO	MeL	MeP	MeS	MeO	MeL
1	22044	283694	35023	53133	44420	0.078	0.123	0.187	0.157
2	21653	276018	33971	51772	43367	0.078	0.123	0.188	0.157
3	21598	275735	33635	51130	42860	0.078	0.122	0.185	0.155
4	21781	278261	34110	52172	43698	0.078	0.123	0.187	0.157
5	21060	265641	32312	47443	41438	0.079	0.122	0.186	0.156
Mean						0.078	0.123	0.187	0.156
S.D						0.001	0.001	0.001	0.001
St Error						0.00025	0.00034	0.00043	0.00032
%RSD						0.722	0.612	0.510	0.456

There was a good reproducibility of the response ratio of the standards as could be seen from their RSD % which was less than 1 for all the esters.

For the samples, wvo biodiesel was run six times consecutively to check for the repeatability of the responses given (Table 4-5).

Table 4-5 Percentage mass composition in WVO Biodiesel

Sample	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0
WVO1	0.11	6.88	4.16	33.31	44.04	1.57	0.31	0.33	0.40
WVO2	0.11	6.88	3.52	33.31	44.45	1.56	0.39	0.33	0.36
WVO3	0.10	6.86	4.16	32.79	44.14	1.61	0.28	0.33	0.40
WVO4	0.12	6.88	4.16	33.31	44.18	1.56	0.29	0.33	0.40
WVO5	0.10	7.01	4.11	33.31	44.18	1.54	0.29	0.33	0.36
WVO6	0.11	7.20	4.42	33.31	42.15	1.56	0.25	0.33	0.40
Mean	0.11	6.95	4.09	33.22	44.52	1.57	0.28	0.33	0.39
Stdev	0.007	0.13	0.30	0.21	0.53	0.02	0.02	0.01	0.02
%RSD	6.81	1.91	7.27	0.64	1.18	1.55	6.03	1.96	5.21

The reproducibility of the mass percent (%) of the methyl esters of the fatty acid present in wvo biodiesel was also very good as indicated by the RSD %.

In conclusion, the analytical column, Zebron ZB-WAX, 30 m× 0.32 mm ×0.25 µm, is suitable for the determination of the ester and linolenic acid methyl ester content of biodiesel when following the procedure recommended by the EN142103 method and the results obtained were repeatable.

4.5 Methanol analysis

Concentration of the five methanol standards spiked with a deuterated methanol internal standard as and their response ratios (methanol/D-methanol) was used to set up a calibration curve from which the concentrations of methanol in all the biodiesel were determined (Figure 4-11). The calibration graph gave an excellent fit for a second degree polynomial and the calibration function obtained was used to determine the amount of methanol in mass percentage present in each biodiesel.

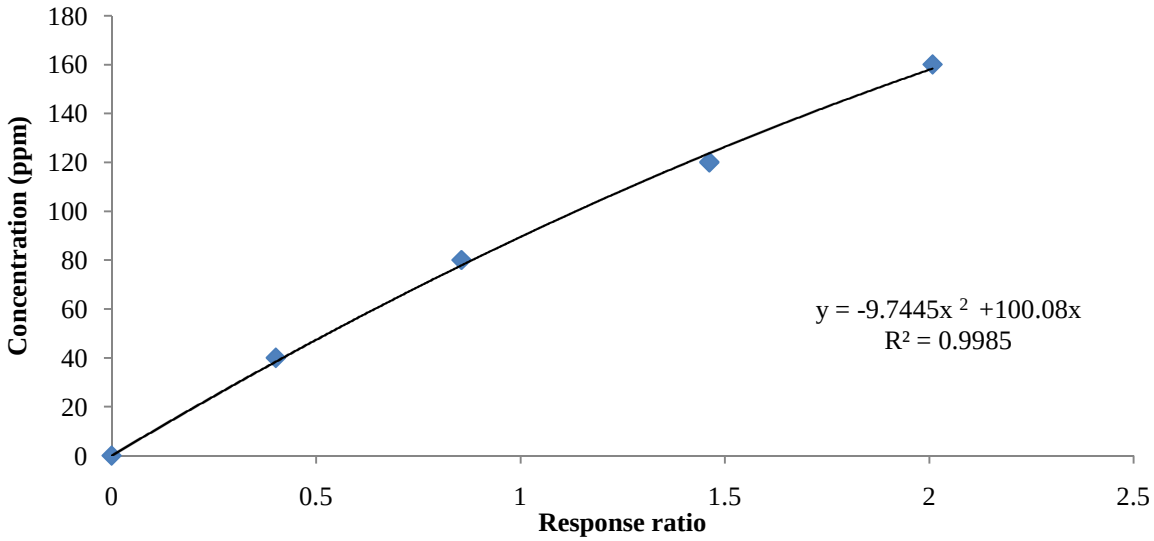


Figure 4-11 Methanol calibration curve

A table of standard concentration and response ratio can be found in Appendix D1.The reference biodiesel used for the calibration was waste vegetable biodiesel (wvo). This reference biodiesel was washed three times with warm water to ensure that there was no methanol presents and thereafter, spiked with both methanol and deuterated. To ensure the actual amount of methanol in the reference biodiesel (wvo) since it could not be ascertained that three times warm water washing removed the entire methanol in wvo biodiesel, one of the standards was left without being spiked (0ppm) with methanol but only the internal standard. This standard (0 ppm) was run and the response from the methanol present was subtracted from all the other standards and the calibrated curve zeroed. In the determination of methanol in the biodiesel which were all unwashed, all the biodiesel samples were diluted with their washed samples to ensure that their methanol concentration did not exceed the

concentrations used in the calibration. In determining the mass percent (%) of methanol in all the biodiesel, the concentration in parts per million (ppm) obtained from their respective response ratio using the calibration function $y = -9.7445x^2 \oplus 100.08x$ was converted to milligram per millilitres (mg/mL) by dividing by 1000.

Based on the EN14110 protocol for the determination of methanol in biodiesel which stipulates that 1milligram methanol in 1millitre of biodiesel gives 0.11mass percent (%) methanol in biodiesel (Paraschivescu *et al.,* 2007), the methanol concentration in mg/mL was multiplied by the factor 0.11 to arrive at the actual concentration of methanol in mass percent (%). For instance, the mass (%) of methanol in palm biodiesel with the following responses (a.u) was determined as follows;

Methanol = 671831a.u

Internal standard = 248689, Response ratio = 671831 / 248689 = 2.70

where 2.70 = response ratio = x in the calibration equation

$$y = -9.744(2.70)^2 + 100.08(2.70) = 199.19\,ppm = 0.1992mg/mL$$

$$1mg\,(methanol)/mL(biodiesel) = 0.11mass\%$$

$$\frac{0.1992mg/ML}{1mg/mL} \times 0.11mass\% = 0.022mass\%$$

All the analyses were done three times for each sample and the results of the mass percent (%) methanol averaged for each sample as shown in Table 4-6.

Table 4-6 Average methanol concentration in biodiesel samples in mass percentage (%)

Biodiesel	**Average methanol (mass%)**
Rapeseed	0.0128
Crown	0.024
Palm	0.006
Peanut	0.012
Sunflower	0.023
WVO	0.020

From the mean concentrations of methanol in each of the samples (Table4-6), the methanol concentrations in all the samples were within the recommended limit of 0.2 mass% as

stipulated in the EN 141214. This was as a result of diluting the unwashed biodiesel samples with the washed biodiesel samples at least twenty times to bring the methanol level within the concentration level of the calibration standards.

4.5.1 Repeatability of methanol analysis

The reproducibility of this analytical method was evaluated using the standard deviation (SD) and relative standard deviation (RSD). In this regard, the 120ppm standard was run five times and for each run the mass percent (%) was calculated from the response based on the calibration function, and from these results, the mean mass percent (%) and the standard deviation and standard error was determined as displayed in Table 4.7.

Table 4-7 Repeatability of analytical method for the standard 120ppm

Standard Concentration	**Methanol**	**D-methanol**	**Response ratio**	**mass %**
120A	388913	240744	1.6155	0.0149
120B	368984	248767	1.4833	0.0139
120C	381953	274955	1.3891	0.0131
120D	372185	252622	1.4733	0.0138
120E	401312	258307	1.5536	0.0144
Mean	382669	255079	1.5030	0.0140
SD	13079	12814	0.0857	0.0006
%RSD	3.42	5.02	5.71	4.82
Standard error				0.0003

The analytical limit of error expressed as a standard error i.e. the difference between the estimated and the actual methanol concentration value was determined to be 0.0003%. A graph showing the mean methanol concentration together with the standard error is indicated in the Fig 4-12.

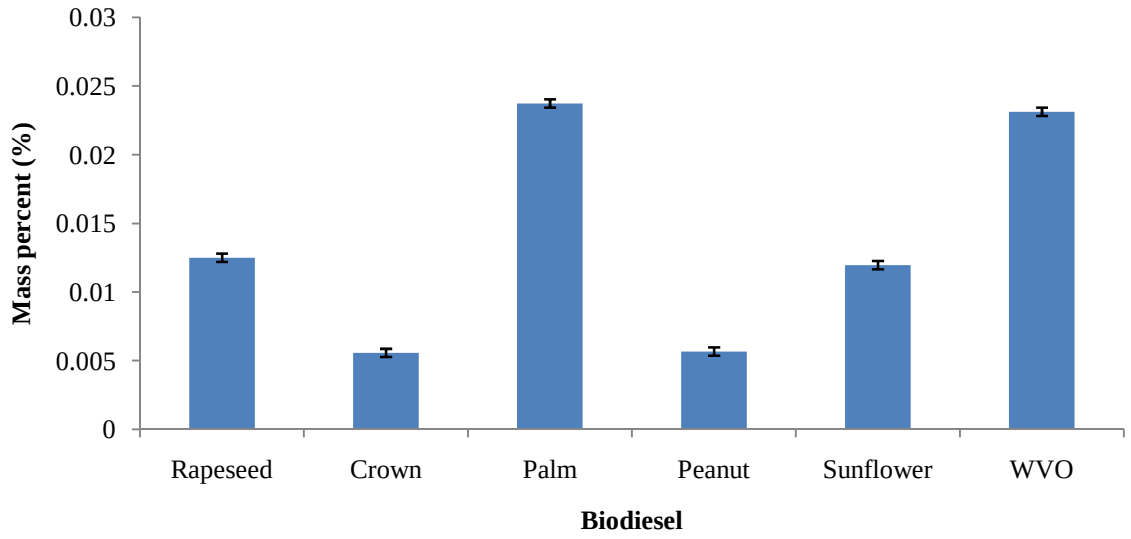


Figure 4-12 Methanol concentration (mass %) with standard error.

Thus, **Solid phase micro extraction (SPME) using PEG** (polyethylene glycol fibre) and deuterated methanol as internal standard with Gas Chromatography-Mass Spectrometry affords a repeatable and accurate analysis of methanol in biodiesel and could be a viable alternative to the EN14214 method which recommends the use of GC-FID to determine methanol concentration in biodiesel. This method uses only 1mL sample as compared to the flash point method that uses approximately 70 mL of sample. In the **EN14110**, a biodiesel sample **(B100)** is heated at 80^{o}C in a hermetically sealed vial and a sample of the gaseous methanol is introduced into a GC-FID by means of a preheated syringe. The method developed during this study is fast, accurate and sensitive with a relative standard deviation of 4.82%. The use of deuterated methanol as internal standard (IS) is recommended.

4.6 Iodine value (IV)

Except for wvo and partially/fully hydrogenated feed stocks, it was expected that the iodine value of oil and its corresponding biodiesel produced from methanol should be nearly identical since the transesterification reaction does not affect the unsaturation present in the feedstock. Results obtained from this study indicated that there was a general decrease in

the iodine value of the biodiesel as compared to their parent or corresponding feed stock oil (Fig 4-13).

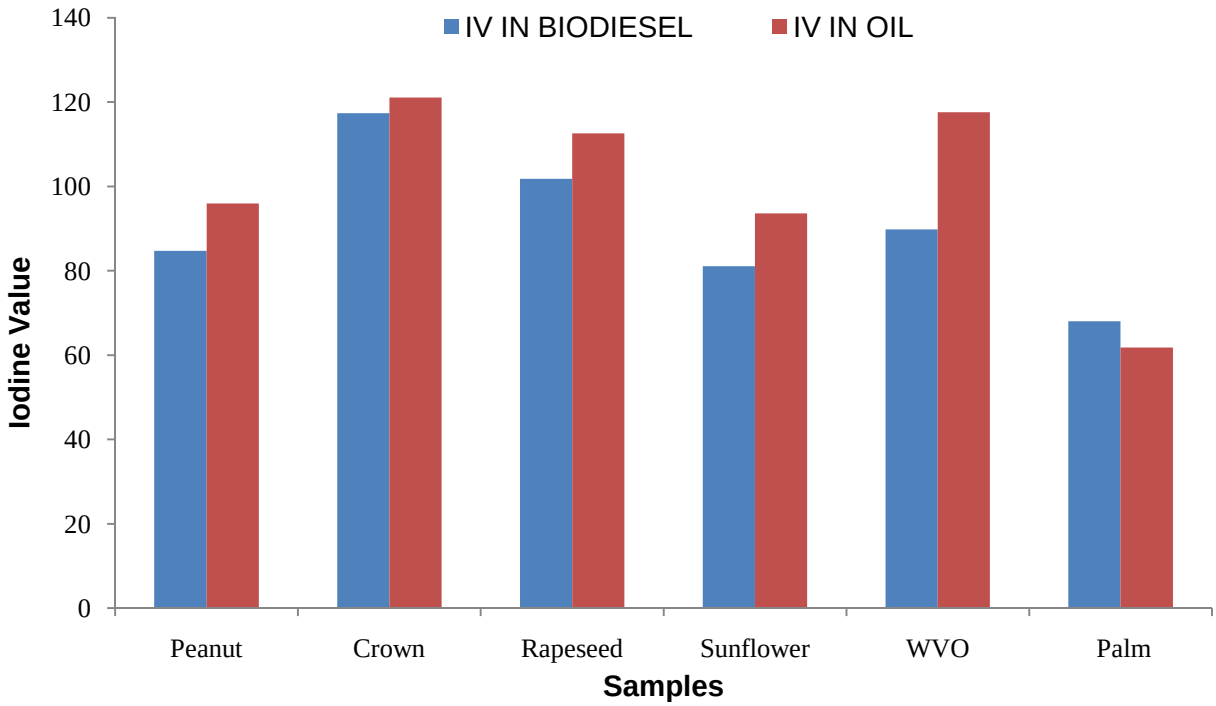


Figure 4-13 Iodine value of biodiesel and corresponding feed stock oil.

There was however, an unexpected increase in the iodine value of biodiesel made from palm oil. The reason for this is could not be established and therefore needs further studies.

Removal of the glycerol phase and further washing of the ester phase after transesterification may have caused the iodine value (IV) of the biodiesel to decrease. The reason is that since the iodine value is an average amount of unsaturation in the feedstock with contribution coming from sources such as unsaturated free fatty acids, unsaturated steroids, carotenes and squalenes amongst others, phase separation of the glycerine phase may have led some of the unsaturated compounds/ components into the glycerine phase leading to a drop in the iodine value of the biodiesel as compared to it corresponding feed stock oil. The drop in iodine value (IV) from the feed stock oil to its corresponding biodiesel may not always be a consistent amount since factors such as the extent of purification may dictate the extent of the drop in iodine value (IV). Therefore predicting the feedstock from

which a biodiesel fuel was derived may prove a daunting task and may not be a feasible idea. Thus, for instance one cannot say for certain that a particular biodiesel is always likely to produce a consistent iodine value.

The iodine values recorded in this study were compared with different batches of reagent and were found to be consistent and in line with literature values except sunflower oil which gave an unexpectedly low iodine value suggesting that the sunflower oil may have been partially hydrogenated or blended (See Appendix E). The crown oil gave a more typical value for sunflower oil suggesting that the crown oil is actually sunflower oil with crown as its trade name.

Although, the substances mentioned above such as the steroids and the carotenes may affect the iodine value, they are present in the feed stocks in very small amounts and thus may not react with the reagent during the measurement of the iodine value thus explaining the minor drop in the iodine value.

From this study, it was observed that there is a general drop in the iodine value of a biodiesel sample as compared to its feed stock/parent oil. This drop in the iodine value may not always be consistent and therefore it is impossible to predict the feed stock source of an unknown biodiesel using the iodine value.

4.7 COLD TEMPERATURE PROPERTIES

4.7.1 Kinematic viscosities

The kinematic viscosity of both the washed and unwashed samples (at 40^oC), were within the recommended range of between 1.6 -6 mm^2/s set up by the ASTM standard (Appendix F1-A). At the temperature of 40^oC, it was found that, the kinematic viscosity of the washed biodiesel was higher than the kinematic viscosity of the unwashed biodiesel from the same sample. The viscosities of the washed and unwashed biodiesel for the six samples investigated were then taken at temperatures of 20^OC, 25^OC, 30^OC, 35^oC. It was found that at each of these temperatures, the viscosities of the washed biodiesel were higher than the viscosities of the unwashed biodiesel drawn from the same sample. Thus, for each temperature point at which the viscosity was investigated (20^OC, 25^OC, 30^OC, 35^oC), the washed biodiesel showed an increase in kinematic viscosity as compared to the unwashed biodiesel from the same sample even though such increments were very small. The kinematic viscosity of peanut biodiesel (See Fig 4-14) is used here as an example.

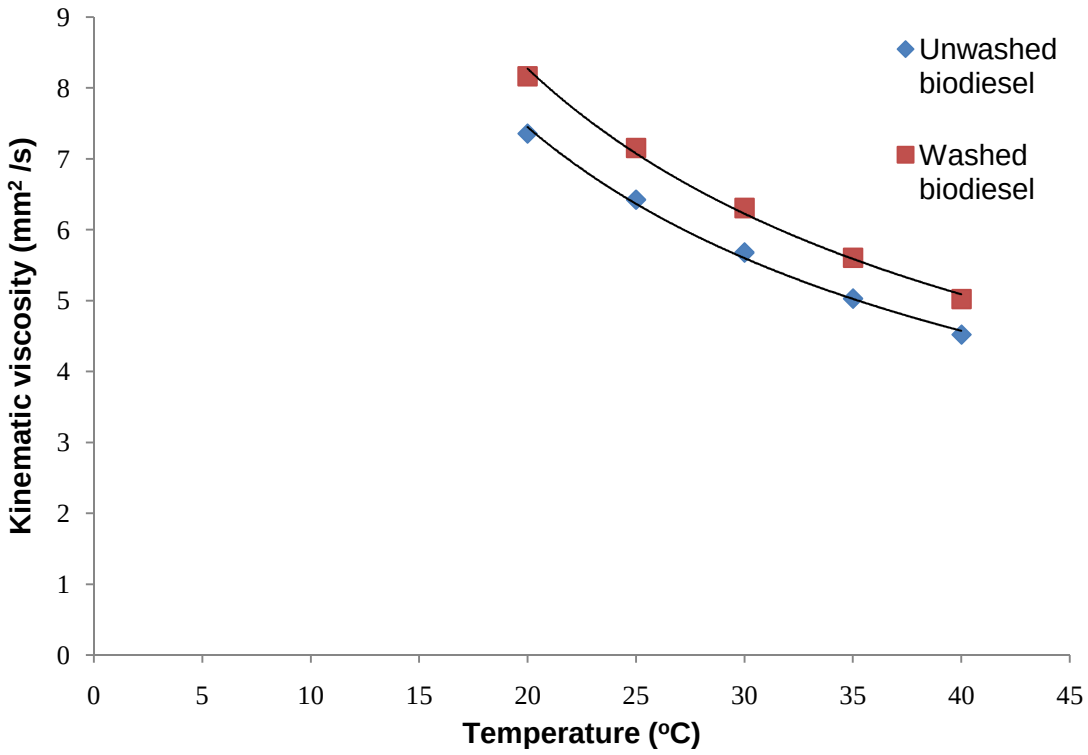


Figure 4-14 Kinematic viscosity of washed and unwashed peanut biodiesel

The viscosity of any biodiesel depends largely on the extent of the transesterification reaction. The presence of triglycerides, diglycerides, monoglycerides and glycerol can lead to an increase in viscosity. In this regard, one would have expected the unwashed biodiesel to have had a higher viscosity than the washed biodiesel since washing the biodiesel may remove the glycerol, mono and possibly the diglycerides from the fuel. The effect of methanol as a viscosity reducing agent has yet to be established in literature even though ethanol has been reported as a pour point depressant (Bhale *et al*.,2008). The presence of methanol in the unwashed sample may have contributed to reducing its viscosity. In terms of cold flow behaviour, it could be concluded that, water washing a biodiesel sample after transesterification, leads a poor cold flow property in terms of its viscosity .Since fuel atomization is affected by viscosity (Rushang *et al*., 2006), there is a likelihood of a poor fuel atomization for the washed biodiesel fuel in compression ignition (CI) engines. This phenomenon was observed for all the samples used in this study.

4.7.2 Pour Point

A high amount of saturated fatty acid results in an increase in pour point. The pour points of the washed and unwashed biodiesel were also investigated in this study. From the results obtained for the washed and unwashed biodiesel, it was observed that there were increases in the pour points of the washed samples of peanut (from 0 ^{o}C to 15 ^{o}C), sunflower (from -7^{o} C to -2 ^{o}C) and crown (- 7 ^{o}C to -5 ^{o}C) biodiesel whereas there was no change in the pour point of both the washed and unwashed samples of wvo, rapeseed and palm biodiesel samples (Fig 4-15).

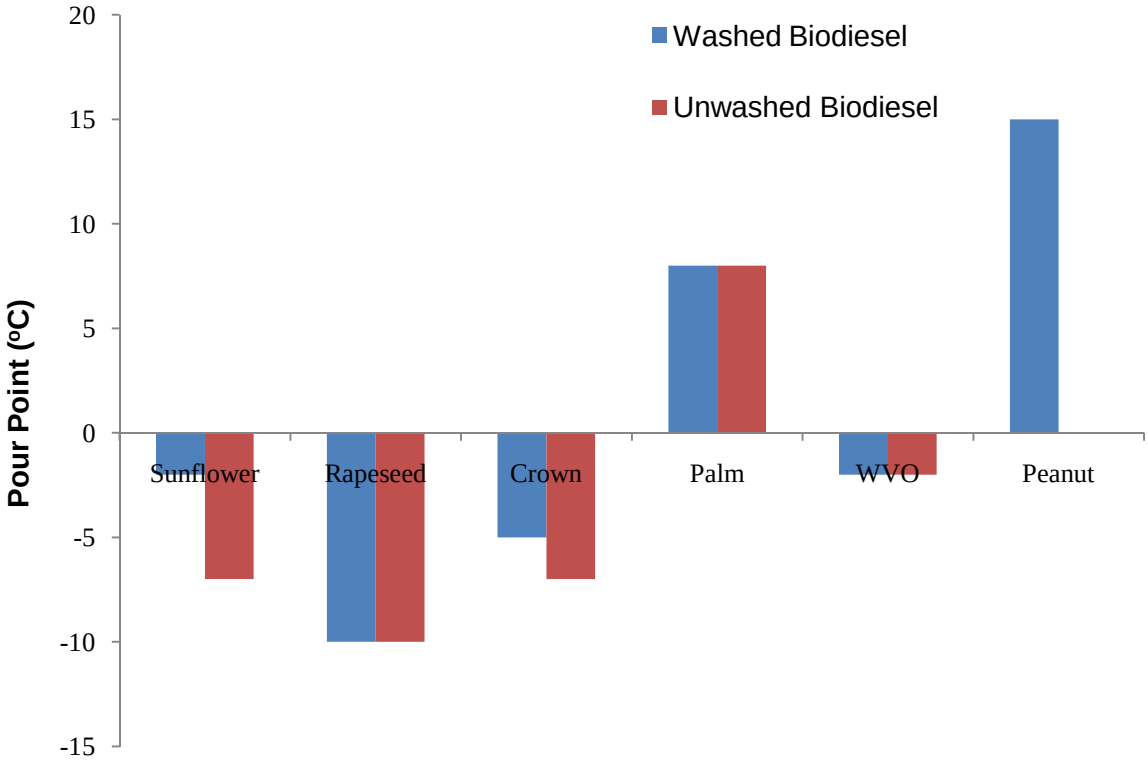


Figure 4-15 pour point of washed and unwashed biodiesel.

The pour of sunflower, rapeseed and palm biodiesel were compared with the literature values and were found to be consistent.

Those of wvo and crown biodiesel were not obtained. There was no consistency in the increase in pour point for sunflower, peanut and crown biodiesel. Whereas, there was a sharp increase in the pour point of peanut biodiesel from the unwashed to the washed (0^{o}C

to 15^{o}C), the increase in pour for crown (-7^{o}C to -5^{o}C) were marginal and that of sunflower moderate (-7^{o}C to -2^{o}C) (Fig 4-15).

The increase in pour point for the above mentioned biodiesel (sunflower, peanut and crown) may be as a result of the hydrolysis of the methyl esters (biodiesel) as can be seen in this equation:

Eqn [4-7]

This hydrolysis reaction in the presence of a basic catalyst like potassium hydroxide (catalyst used in biodiesel production) may have led to an increase in the free fatty acid concentration in the biodiesel during the water washing and therefore increasing the pour point of the biodiesel. For the washed and unwashed wvo, palm and rapeseed biodiesel, both the pour point of the washed and unwashed sample stayed the same and the reason for this could not be established even though they were expected to follow the trend observed for peanut, crown and sunflower biodiesel. It could also be that, this observed increase in pour point as a result of water washing is dependent on the type of biodiesel used.

From the results of this study, a generalisation regarding the effect of water washing on biodiesel cold flow property like the pour point could no be made since a general trend was not observed.

4.7.3 Pour points of blended biodiesel

It has been established that higher amounts of saturated compounds increase the cloud and pour point of biodiesel (Knothe, 2005). Therefore, biodiesel obtained from feed stocks with high percentage of saturated fatty acids and therefore low degree of unsaturation was blended with biodiesel with low amount of saturated fatty acid (one of high degree of unsaturated fatty acid) and the effect on the pour point of the resultant blend formed observed. The blending was 50 vol% of the unsaturated and 50 vol% of the saturated biodiesel samples for all the blends used.

For instance, peanut biodiesel which has a high level of saturated fatty acid was blended with biodiesel obtained from feed stock with a high level unsaturated fatty acid (Fig 4-16).

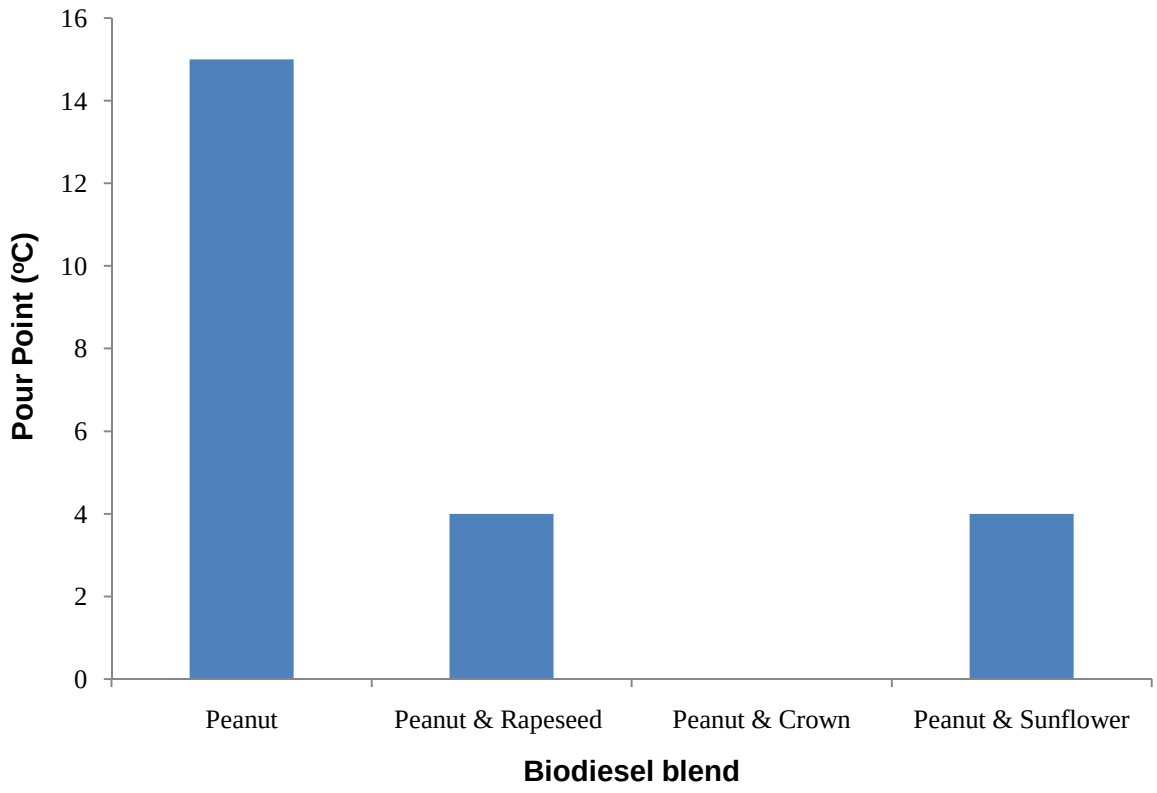


Figure 4-16 Pour point of blended peanut biodiesel

It was observed that, the resultant blends had reduced points of 4°C for rapeseed and peanut biodiesel blend, 0°C for peanut and crown blend (reason for no bar indicated on the chart) and 4°C for peanut and sunflower blend (See Appendix G3). The crown and peanut blends produce the greatest reduction in pour point. Therefore, the introduction of the more unsaturated biodiesel in the less saturated biodiesel had an effect on the pour point of the resultant biodiesel.

In the same vein, wvo biodiesel was blended with the more unsaturated biodiesel such as sunflower, crown and rapeseed (Appendix G2). The resultant blends had a reduced pour point but there was considerable reduction in the pour point of the wvo biodiesel when blended with sunflower biodiesel with the pour point changing from -2°C of the wvo biodiesel to -8° C of the blends of wvo and sunflower blends as can be seen in the Fig 4-17.

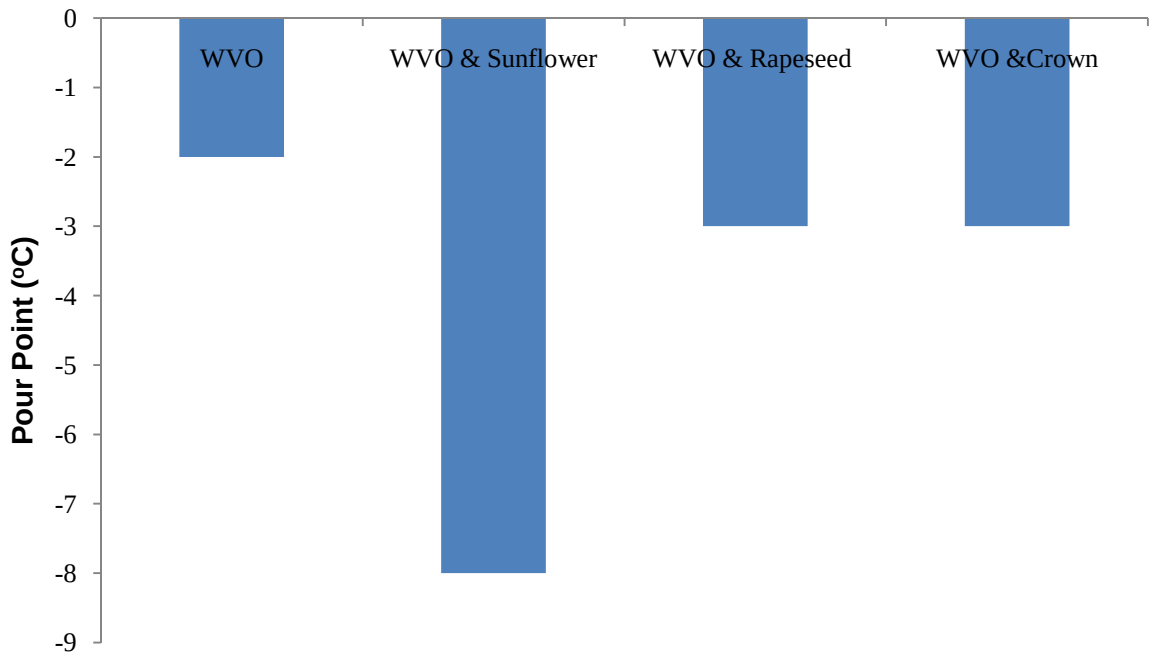


Figure 4-17 Pour point of blended wvo biodiesel

For the palm biodiesel, there was considerable change in the pour point when it was blended with crown, rapeseed with the pour point changing from 8°C to -1°C, 0°C and -1°C respectively (Appendix G4). Therefore, there was a general reduction in pour points of the resultant blend when a highly unsaturated biodiesel is blended with a more saturated biodiesel blend.

4.7.4 Cloud point

The results obtained from the cloud point investigations indicated a decrease in cloud point for both peanut and rapeseed biodiesel when the samples were washed after the transesterification reaction.

Sunflower biodiesel displayed an increase in its cloud point after water washing (from1°C to 10°C). The same observation was made regarding its pour point. Increase in cloud point for sunflower biodiesel after water washing maybe due to the hydrolysis of the fatty acids to free fatty acid which are responsible for the increase as explain earlier in their pour point.

As it has been observed in this study for sunflower biodiesel, all the cold flow properties investigated in this study (kinematic viscosity, density, cloud and pour points) showed an increase in temperature after water washing.

Moreover, as happen in their pour points, the cloud points of palm and wvo biodiesel did not changed after water washing (Fig 4-18) and also Appendix H1. The reason for cloud point staying unchanged after washing could not be established and this should be an area for further investigation.

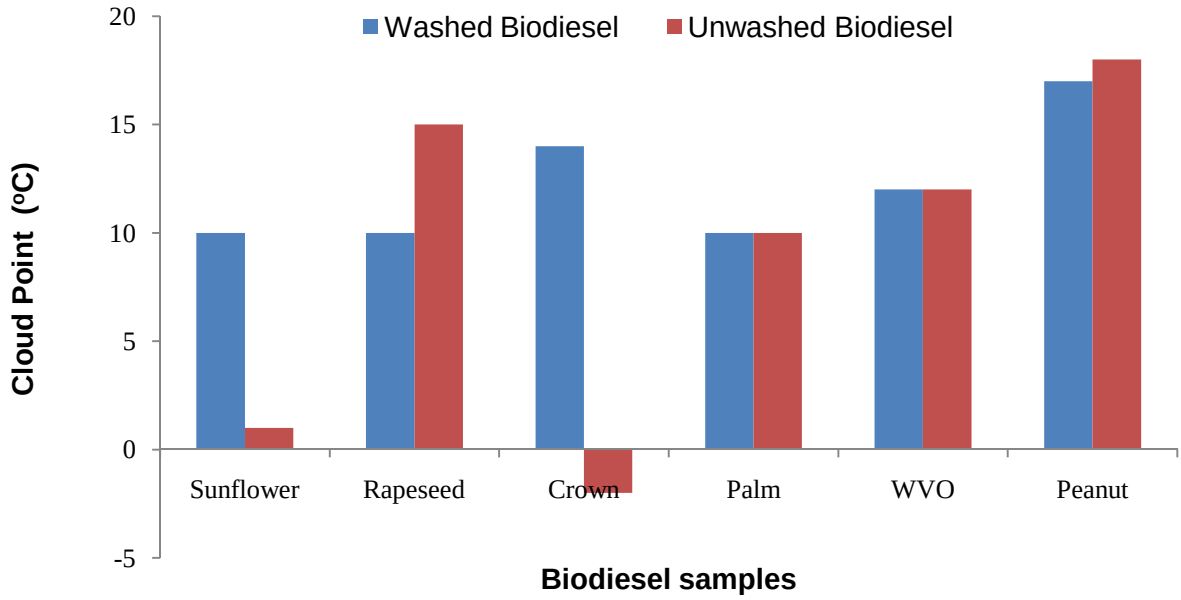


Figure 4-18 Cloud point of washed and unwashed biodiesel

Thus, it could be inferred based on the results obtained from this study that, water washing after transesterification, helped in improving the cloud point of peanut, rapeseed and biodiesel since there was a drop in their cloud points after water washing which is a good sign since most cold flow improvers are targeted at improving the cloud point because cloud point triggers the pour since it occurs before the pour point. However, for sunflower biodiesel, water washing may not be a good idea after transesterification. In the case of palm and wvo biodiesel, their cloud points did not change as a result of washing after transesterification. Therefore, in summary the effect of water washing after transesterification on biodiesel cloud points may depend on the type of biodiesel since there was no

consistency in the observations regarding the cloud point of the six feed stocks used in this study.

4.7.5 Cloud point of blended biodiesel

Blending a biodiesel containing few unsaturated fatty acids with one containing a higher percentage of unsaturated fatty acids should lower the cloud point of biodiesel since the cloud point is mainly affected by the presence of saturated fatty acid which tends to increase the cloud point of the fuel. Therefore, when palm biodiesel (which has high levels of saturated fatty acid) was blended with crown, rapeseed and sunflower, the resulting blend had a reduced cloud point than palm biodiesel (See Fig 4-19). The introduction of unsaturation fatty acid may have contributed to the reduction of cloud points.

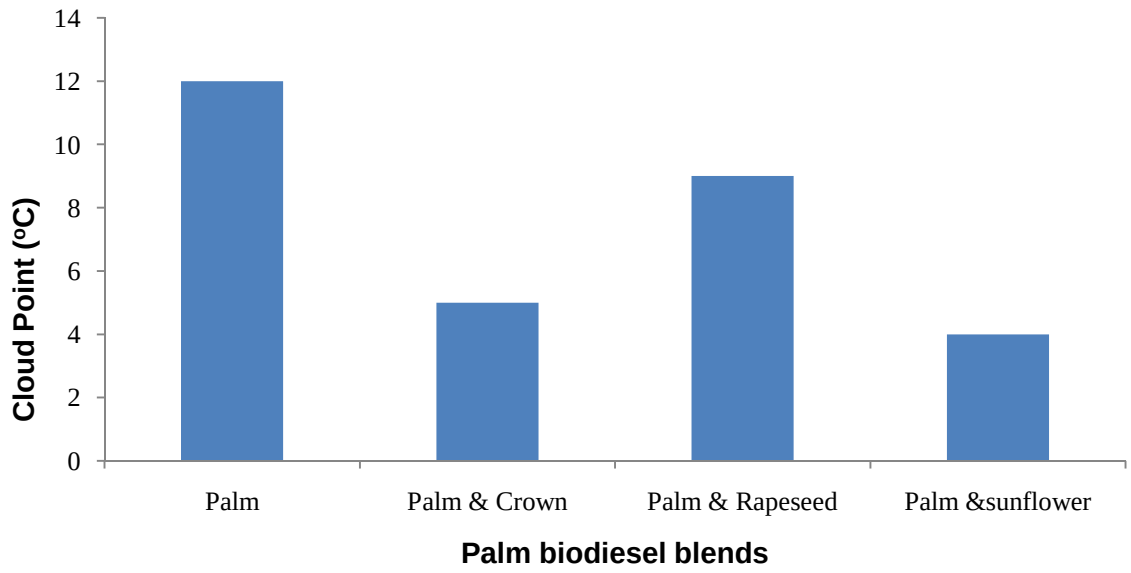


Figure 4-19 Cloud point of blended palm biodiesel

Although, rapeseed biodiesel has a high degree of unsaturated fatty compounds, it did not have the same effect as crown and sunflower biodiesel. All the unsaturated biodiesel, namely crown and rapeseed had a reducing effect on the cloud point of the palm biodiesel.

For the wvo biodiesel blends (Fig 4-20), there was an increase in the cloud points, when it was blended with unsaturated samples such as sunflower, rapeseed and crown.

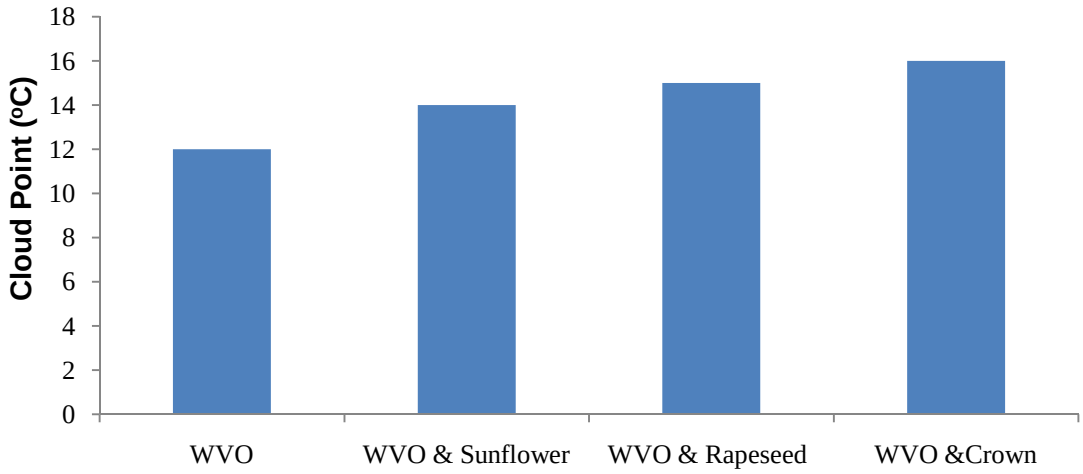


Figure 4-20 Cloud Point of Blended WVO Biodiesel

There was a reduction of the cloud point of peanut biodiesel when blended with biodiesel samples such as rapeseed, crown, sunflower (Fig 4-21) with all these blends having a temperature of 14^{o}C (Appendix H3).

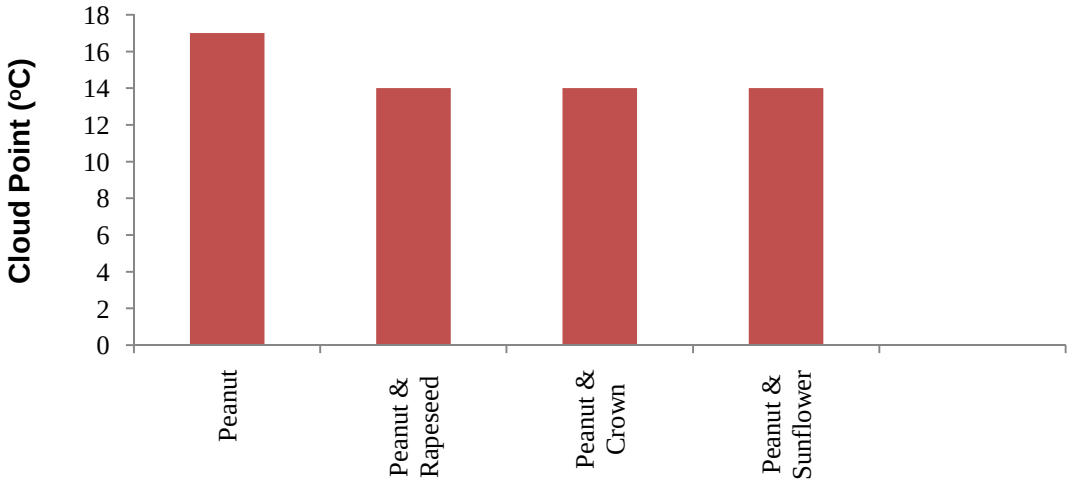


Figure 4-21 Cloud point of blended peanut biodiesel

From the results of the cloud points of the blended biodiesel samples, blending unsaturated biodiesel (rapeseed, crown and sunflower) samples gave an improved cloud point for palm and peanut biodiesel samples although the improvement was marginal. The blends of wvo biodiesel with sunflower, rapeseed and crown produced unexpected results, since it was

expected that blending wvo biodiesel (which has high levels of saturated fatty acids) with rapeseed, crown and sunflower biodiesel (all with high unsaturation) was going reduce the cloud points of the resulting blend.

4.7.6 Density

The densities of biodiesel of both the washed and unwashed samples were taken at 20^{o}C. It was observed that, there was an increase in the densities of all the biodiesel samples after water washing at the temperature (20^{o}C) at which the density was taken (See Fig 4-22) and also appendix I.

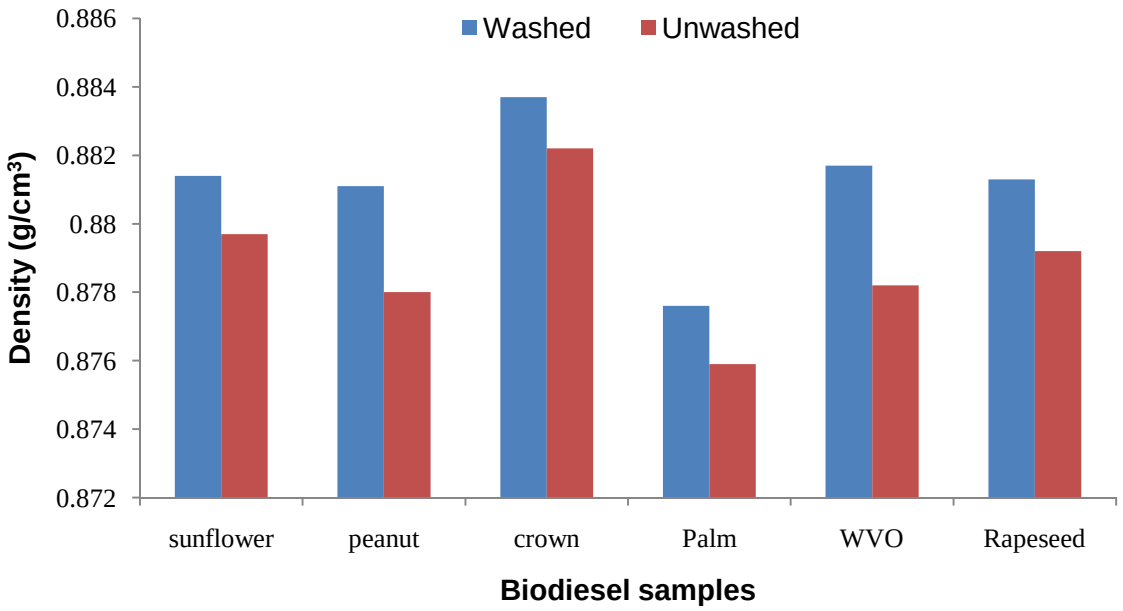


Figure 4-22 Density of washed and unwashed biodiesel at 20^{o}

It was observed that the increment between the washed and unwashed biodiesel was marginal. According to Worgetter (1998), the density of biodiesel increases with increasing unsaturation and therefore, it was expected that, the densities of the unwashed biodiesel was going to higher than the washed biodiesel since washing may remove unsaturated compounds from the washed biodiesel thus decreasing their densities. The effect of methanol on decreasing the densities of biodiesel was also explained by Worgetter (1998) and also Mittelbach (2004). Therefore, the reason for the reduced density of the unwashed biodiesel could be as a result of the presence of methanol. Therefore, it is to be expected in

terms of density that, a washed biodiesel will have poor cold flow (density) behaviour and this may lead to poor fuel atomization than an unwashed biodiesel.

In summarizing the effect of water washing on biodiesel cold flow properties such as kinematic viscosity, density, pour and cloud points, water washing after transesterification could lead to poor cold flow properties such as poor kinematic viscosity and density as observed for all the samples used in this study. However, for cloud and pour points, only sunflower biodiesel had an increase in both properties. For the other samples, cloud and pour may be sample dependent as there was not a consistent trend observed.

5 CONCLUSIONS

- The use of a programmable temperature volatilization as a substitute injector for the recommended on-column injector when following ASTM D6584 protocol to determine mass % of free and bound glycerol did not afford the repeatability required when using ASTM D6584 protocol. Therefore, the hypothesis that the PTV affords the same repeatability as the on-column injector was not true according to the results obtained from this study.

- Normal phase high performance liquid chromatography with binary gradient elution is a suitable, time saving technique for the qualitative and quantitative determination of the monoglycerides, diglycerides, triglycerides and free fatty acids which are found in biodiesel during the transesterification of vegetable oils. The repeatability afforded in this method is very good and falls within the RSD% required for the quantitation of glycerides in biodiesel of 1-4%.
  The %RSD obtained for the glycerides are 0.33, 1.12 and 1.12 for TG, DG and MG respectively. Therefore, the hypothesis that this method is suitable for bound glycerol and free fatty acids analysis in biodiesel is true.

- The Zebron ZB-WAX column (30m× 0.32×0.25µm), with similar specification to the recommended standard method EN14103 affords the detection and quantitation of methyl esters from $C_{14:0}$ –$C_{22;0}$ and the results are repeatable with RSD % of 6.81, 1.91, 7.27, 0.64, 1.18, 1.55, 6.03, 1.96, and 5.21 for methyl esters of myristic, palmitic, stearic, oleic, linoleic, linolenic, arachidoic, gadoleic and behenic acids respectively.

- Headspace solid phase micro extraction using deuterated methanol as internal and a polyethylene glycol fibre (PEG) offers a direct, quantitative and repeatable determination of methanol in Biodiesel and could serve as a viable alternative to both the EN14110 and the ASTM D93 methods. This method had a very good repeatability with RSD of 4.82%.

- The iodine value (IV) cannot be used to predict the feedstock from which the biodiesel was made (according to this study) since there is drop in the iodine value

when a feed stock is transesterified to form the biodiesel. This drop in the IV may not be consistent and therefore makes it difficult to predict the IV of an unknown biodiesel from its corresponding feed stock oil.

- The hypothesis that water washing of biodiesel after phase separation leads poor cold flow properties such as kinematic viscosity, density, cloud and pour points is true only for the kinematic viscosity and the density. However, for flow properties such as cloud and pour point, the hypothesis that water washing leads to poor cloud and pour points depends on the type of biodiesel. For instance, for sunflower biodiesel, the hypothesis was true.

- Blending an unsaturated biodiesel (one with a high degree of unsaturation) and a more saturated biodiesel resulted in an improvement of the pour and cloud points of the resulting biodiesel blend formed according to this study. Therefore, the hypothesis that blend of unsaturated biodiesel with a highly saturated leads to improvement in pour and cloud was found to be true.

6 FUTURE WORK AND RESEARCH

Further studies on the use of the programmable temperature volatilisation (PTV) in place of the recommended on-column injector for quantifying free glycerol and glycerides should be carried out. This is because, there was malfunctioning of the PTV injector and this might have affected the results obtained in this study.

Studies should be conducted regarding the detection and quantitation of free fatty acids in biodiesel using the normal phase HPLC with gradient elution to determine the suitability of this method for free fatty acids. This is because of the six samples used in this study only two samples contained free fatty acids for quantitation.

More investigation should be conducted on the iodine value of palm oil and its corresponding biodiesel as it was the only sample used in this study that showed an increase in IV from the feed stock oil to its corresponding biodiesel. This trend was not consistent with observations made in this study and therefore needs further investigation.

Further studies should be carried out on more samples about the cold flow properties of their washed and unwashed samples such as kinematic viscosity, cloud and pour points and density to confirm or disprove the observations made in this study since very few samples were used for this study. More especially, studies should be conducted on the effect of methanol on the kinematic viscosity and densities (at 20^0C) of biodiesel to see if the reduced viscosity of the unwashed samples was due to the presence of methanol. Moreover, the effect of water washing on the cloud and pour points of wvo and palm biodiesel should be investigated further to prove or disprove the observations made in this study.

For blending unsaturated and saturated samples, the scope of samples should be increased for both the saturated and unsaturated biodiesel so that a proper generalisation could be made regarding the effect they both have on each other's pour point.

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APPENDICES

APPENDIX A - Gas chromatographic determination of total glycerol.

APPENDIX A1: Standards used in setting up the calibration curve for glycerol, MG, DG, TG

Standards	*Glycerol*	*Butanetriol*	*Monolein*	*Tricaprin*	*Diolein*	*triolein*
Stock(ug/mL)	0.534	1.028	5.020	8.800	4.900	4.990
		Amount (mg)				
S1	0.005	0.103	0.100	0.880	0.049	0.050
S2	0.016	0.103	0.251	0.880	0.098	0.100
S3	0.027	0.103	0.502	0.880	0.196	0.200
S4	0.037	0.103	0.753	0.880	0.343	0.349
S5	0.053	0.103	1.004	0.880	0.490	0.499

APPENDIX A2: Amount ratios of calibration standards

Standards	*Glycerol*	*Monolein*	*Diolein*	*Triolein*
S1	0.0485	0.1250	0.0612	0.0625
S2	0.1553	0.3137	0.1225	0.1250
S3	0.2621	0.6275	0.2450	0.2500
S4	0.3592	0.9412	0.4287	0.4362
S5	0.5145	1.2560	0.6125	0.6237

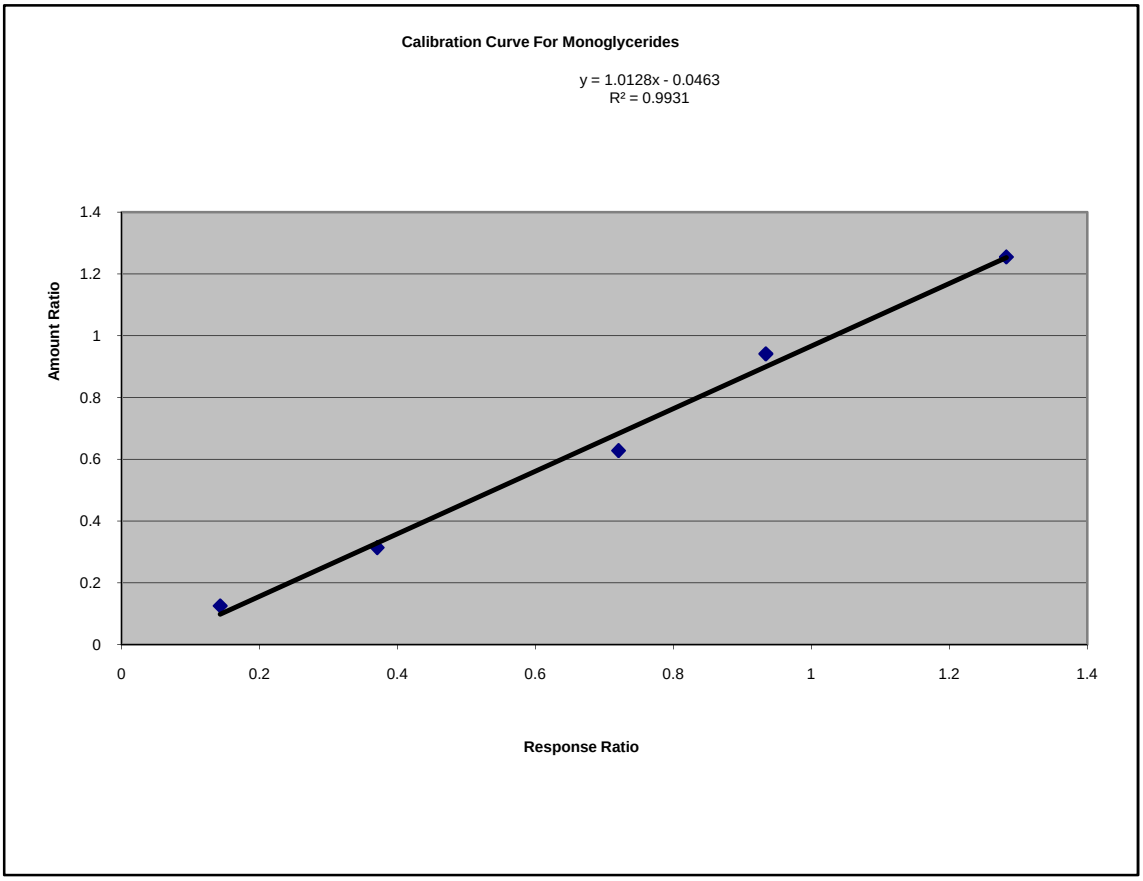
APPENDIX A3: Responses of the calibration standards

Standards	*Glycerol*	*Butanetriol*	*Monolein*	*Tricaprin*	*Diolein*	*Triolein*
S1	13.254	209.025	106.827	748.918	38.155	42.414
S2	32.5	184.766	269.142	726.693	71.548	67.835
S3	55.598	195.535	521.529	724.086	147.667	180.814
S4	78.335	184.129	633.925	678.879	252.490	280.617
S4	92.035	165.648	853.752	665.669	359.850	365.116

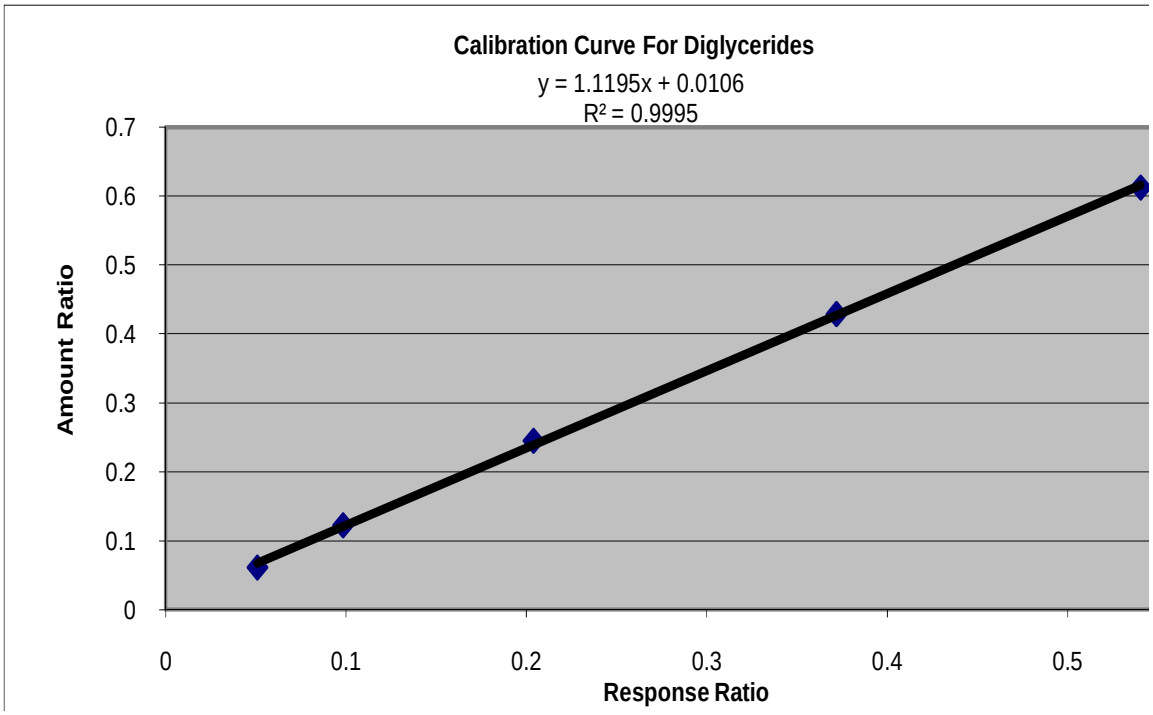
APPENDIX A4: Response ratio of calibration standards.

	Glycerol	*Monolein*	*Diolein*	*Triolein*
S1	0.0634	0.1426	0.0509	0.0566
S2	0.1758	0.3704	0.0984	0.0933
S3	0.2843	0.7202	0.2039	0.2497
S4	0.4254	0.9337	0.3719	0.4133
S5	0.5656	1.2825	0.5406	0.5484

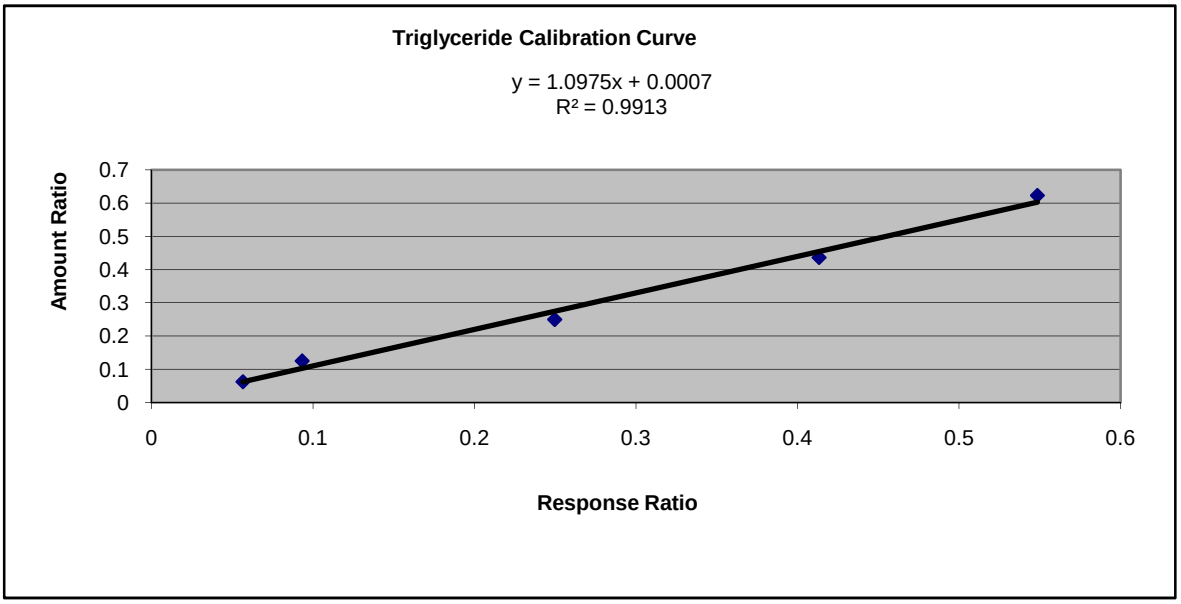
APPENDIX A5: Monoglyceride calibration for glycerol analysis using DANI Master Gas chromatograph.



APPENDIX A6: Calibration curve for diglyceride analysis using DANI Master Gas Chromatograph.



APPENDIX A7: Calibration Curve for triglycerides



APPENDIX A8: Raw data for %mass of FG, MG, DG, and TG in all triplicated analysis of the samples.

Sample	*FG*	*MG*	*DG*	*TG*
Rapeseed	0.026548382	0.667007823	1.590544514	0.899685221
Rapeseed	0.030681207	1.241580392	0.862313367	1.535423392
Rapeseed	0.027611471	1.01060129	0.584540253	0.254066805
Crown	0.029090013	0.81493701	0.992392494	0.252632707
Crown	0.016400738	1.426367877	0.816258528	0.173206146
Crown	0.015957057	1.005246843	0.948839547	0.166439172
Palm	0.026913983	0.1632626	0.138984804	0.048938779
Palm	0.021336422	0.104801058	0.133493751	0.083057124
Palm	0.033411568	0.209409071	0.129299351	0.043070529
Peanut	0.021650826	0.983287723	0.663108724	0.158100378
Peanut	0.010959383	1.152868224	0.666865786	0.156925157
Peanut	0.023113732	1.243879684	0.642845302	0.139872402
Sunflower	0.059466636	1.136507023	0.468811842	0.391154835
Sunflower	0.054880173	0.07671714	0.504937255	0.21019984
Sunflower	0.05481621	0.145571487	0.221836308	0.203913877
WVO	0.017628333	0.344340934	0.19798785	0.191957767
WVO	0.028662576	0.440917998	0.179980778	0.139623763
WVO	0.021832368	0.434455568	0.182087387	0.202913127
Palm 1	0.050153762	0.059931206	0.171240577	0.118503436
Palm 2	0.025619142	0.085194183	0.136634161	0.069569264
Palm 3	0.05458967	0.075049144	0.215838777	0.131219936
Palm 4	0.045891458	0.414247931	0.23769096	0.216080877
Palm 5	0.045803611	0.281867552	0.134991599	0.04711401

APPENDIX A9 –Average mass % for samples n=3

Sample	FG	TG	DG	MG	*Total glycerol*
Rapeseed	0.028	0.094	0.151	0.007	0.280
Crown	0.020	0.021	0.137	0.005	0.183
Palm	0.027	0.006	0.020	0.041	0.094
Peanut	0.019	0.016	0.098	0.292	0.425
Sunflower	0.056	0.028	0.059	0.117	0.261
WVO	0.023	0.019	0.028	0.105	0.175

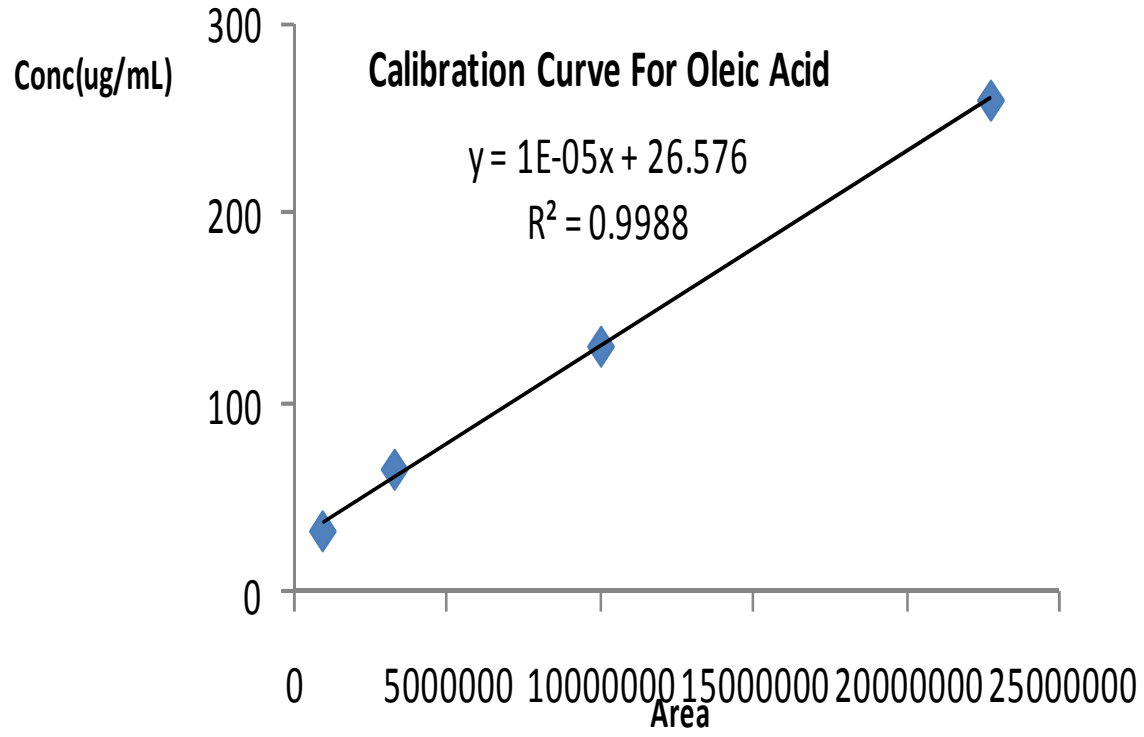
APPENDIX B- Determination of bound glycerol using normal phase HPLC with Binary solvents.

APPENDIX B1

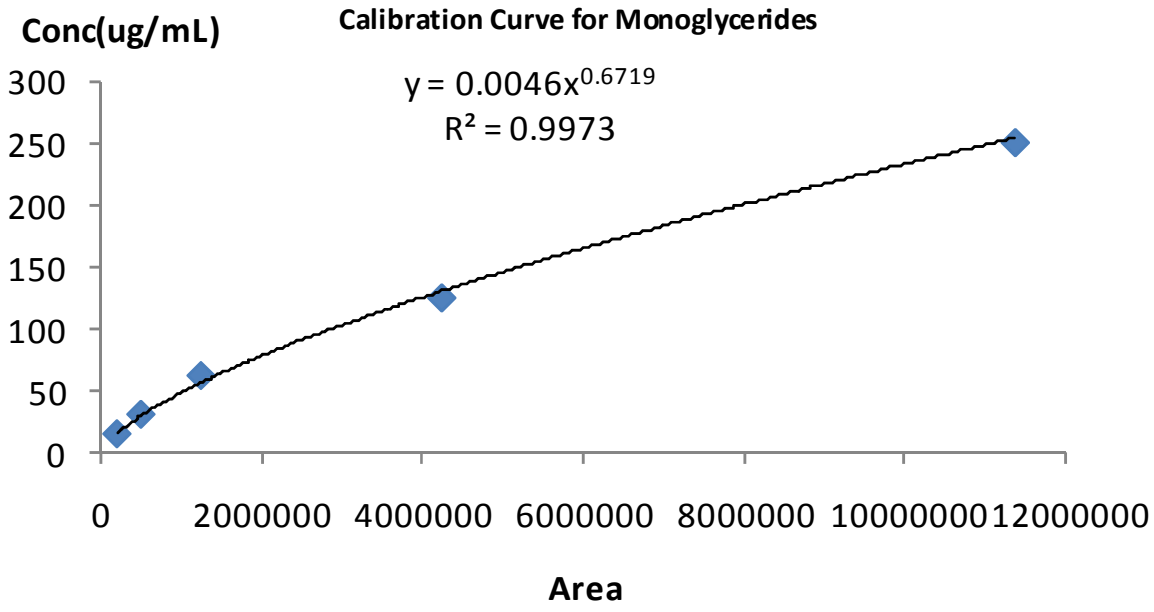
Calibration standards.

Standards	*Concentration(ug/ml*	*Calculated concentration*	*Bias*
S1	259	260.42	0.55
S2	129.5	129.7	0.16
S3	64.75	60.51	-6.54
S4	32.38	36.42	12.48
S5			

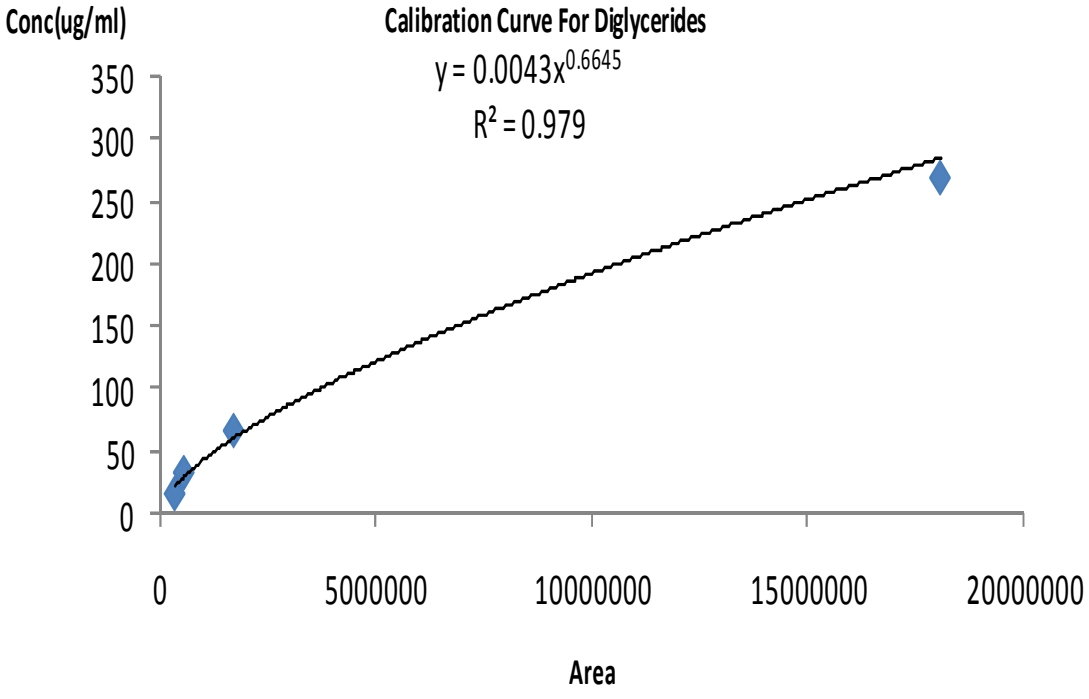
APPENDIX B2: Oleic acid calibration curve for free fatty acid determination



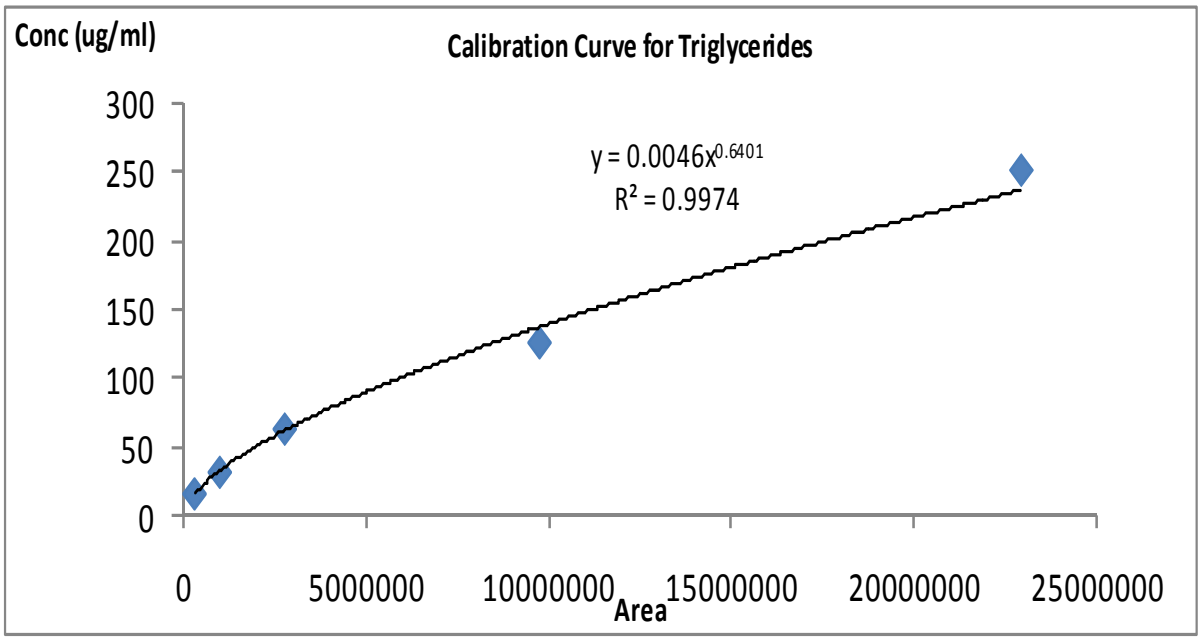
APPENDIX B3: Monoglyceride calibration curve.



APPENDIX B4: Diglyceride calibration curve.



APPENDIX B5: Triglyceride calibration curve.



APPENDIX B6:

Mass % of OA, MG, DG, TG in Biodiesel samples

Sample Biodiesel	*OA*	*TG*	*DG*	*MG*
Peanut	0.000	0.017	0.112	0.290
Peanut	0.000	0.017	0.123	0.315
Sunflower	0.000	0.009	0.116	0.157
Sunflower	0.000	0.009	0.124	0.160
Rapeseed	0.000	0.019	0.126	0.324
Rapeseed	0.000	0.020	0.129	0.331
Crown	0.000	0.016	0.156	0.331
Crown	0.000	0.016	0.156	0.311
Palm	0.296	0.000	0.058	0.085
Palm	0.346	0.000	0.061	0.089
WVO	0.375	0.014	0.142	0.128
WVO	0.397	0.013	0.139	0.131

Average mass percent(%) of MG,DG, and TG in HPL C method

Biodiesel	*TG*	*DG*	*MG*
Rapeseed	0.020	0.128	0.328
Crown	0.016	0.156	0.331
Palm	0.000	0.060	0.087
Peanut	0.017	0.118	0.303
Sunflower	0.009	0.120	0.158
Wvo	0.013	0.140	0.129

APPENDIX C: Ester and linolenic acid methyl ester content

Appendix C1: Average mass % of ester and linolenic acid methyl ester.

Biodiesel	*Ester content (mass %)*	*Linolenic acid methyl ester (mass %)*
WVO	90.4677	1.7000
Peanut	90.2658	0.5575
Sunflower	94.1679	0.2398
Palm	94.0361	0.2813
Crown	90.8714	5.7145
Rapeseed	90.9990	8.9569

APPENDIX D: Methanol determination using Headspace SPME with GC-MS

APPENDIX D1: Calibration standards for methanol

Standards (ppm).	*Methanol*	*D- methanol (IS)*	*Response ratio*
0	0	239387	0
40	140352	349592	0.401473718
80	225956	263974	0.85597824
120	372958	255079	1.462127419
160	482990	240514	2.008157529

APPENDIX D2: Raw data for methanol analysis

Biodiesel samples	*Methanol response*	*IS response*	*Response ratio*	*Conc. (ppm)*	*Conc(mg/mL)*	*%mass*
Rapeseed	335442	240514	1.3947	119.0590	0.1191	0.0131
Rapeseed	339630	255688	1.3283	114.3219	0.1143	0.0126
Rapeseed	344898	255470	1.3501	115.8844	0.1159	0.0127
Crown	160945	269011	0.5983	56.1000	0.0561	0.0062
Crown	159071	314567	0.5057	47.9109	0.0479	0.0053
Crown	120015	285555	0.4203	40.1987	0.0402	0.0044
Palm	671831	248689	2.7015	193.3707	0.1934	0.0213
Palm	736827	275952	2.6701	192.0093	0.1920	0.0211
Palm	734884	295659	2.4856	183.5778	0.1836	0.0202
Peanut	136351	293639	0.4643	44.1973	0.0442	0.0049
Peanut	159475	263931	0.6042	56.6196	0.0566	0.0062
Peanut	148835	314094	0.4739	45.0545	0.0451	0.0050
Sunflower	390449	304137	1.2838	111.0943	0.1111	0.0122
Sunflower	432682	308235	1.4037	119.6977	0.1197	0.0132
Sunflower	398820	342661	1.1639	102.1907	0.1022	0.0112
WVO	747981	331785	2.2544	172.0027	0.1720	0.0189
WVO	698325	292454	2.3878	178.8198	0.1788	0.0197
WVO	763501	255330	2.9903	204.9305	0.2049	0.0225

APPENDIX E: Iodine values

Sample	IV in Oil	Literature value	IV in Biodiesel
Peanut	96	123.22	84.7
Crown	121.1	Not available	117.4
Rapeseed	112.6	100-120	101.8
Sunflower	93.6	120-135	81.1
WVO	117.6	Not available	89.8
Palm oil	61.8	55-65	68

APPENDIX F. Cold temperature properties

APPENDIX F1: Kinematic viscosity of washed and unwashed Biodiesel.

APPENDIX F1-A- kinematic viscosity of peanut Biodiesel

Sample	*unwashed Biodiesel*	*Unwashed Biodiesel*
Temperature (^{o}C)	Kinematic viscosity	
20	7.353	8.164
25	6.421	7.152
30	5.676	6.305
35	5.025	5.604
40	4.518	5.021

APPENDIX F1-B- Kinematic viscosity of crown Biodiesel

Sample	*Washed Biodiesel*	*Unwashed Biodiesel*
Temperature (^{o}C)	Kinematic viscosity	
20	6.951	7.142
25	6.166	6.263
30	5.439	5.545
35	4.887	4.936
40	4.408	4.449

APPENDIX F1-C kinematic viscosity palm Biodiesel

Sample	*Washed Biodiesel*	*Unwashed Biodiesel*
Temperature (^{o}C)	Kinematic viscosity	
20	7.140	7.141
25	6.216	6.321
30	5.441	5.528
35	4.894	4.926
40	4.409	4.429

APPENDIX F1-D- Kinematic viscosity of WVO

Sample	*Washed Biodiesel*	*Unwashed Biodiesel*
Temperature (^{o}C)	Kinematic viscosity	
20	6.130	7.164
25	5.501	6.494
30	4.911	5.592
35	4.351	4.992
40	3.887	4.504

APPENDIX F1-E kinematic viscosity of rapeseed Biodiesel

Sample	*Washed Biodiesel*	*Unwashed Biodiesel*
Temperature (^{o}C)	Kinematic viscosity	
20	7.122	7.147
25	6.240	6.262
30	5.567	5.581
35	4.944	4.981
40	4.453	4.472

APPENDIX F1-F- Kinematic viscosity of Sunflower Biodiesel

Sample	*Washed Biodiesel*	*Unwashed Biodiesel*
Temperature (^{o}C)	Kinematic viscosity	
20	6.445	7.455
25	5.686	6.563
30	5.066	5.681
35	4.542	5.177
40	4.170	4.651

APPENDIX G- Pour points

APPENDIX G1- Pour points of washed and unwashed Biodiesel samples

Sample	*Washed*	*Unwashed*
	Pour points(^{0}C)	
Sunflower	-2	-7
Rapeseed	-10	-10
Crown	-5	-7
Palm	8	8
WVO	-2	-2
Peanut	15	0

APPENDIX G2- Pour points of blended WVO Biodiesel samples

Sample	*Pour points(^{0}C)*
WVO	-2
WVO& sunflower	-8
WVO& Rapeseed	-3
WVO & Crown	-3

APPENDIX G3- Pour points of blended peanut Biodiesel samples.

Sample	*Pour points(^{0}C)*
Peanut	15
Peanut & Rapeseed	4
Peanut & Crown	0
Peanut & Sunflower	4

APPENDIX G4- Pour points of blended Palm Biodiesel.

Sample	*Pour points(^{0}C)*
Palm	8
Palm & Rapeseed	0
Palm & Crown	-1
Palm & Sunflower	8

APPENDIX H- Cloud Points: APPENDIX H1- Cloud points of washed and unwashed Biodiesel

Sample	*Washed*	*Unwashed*
	Cloud points(^{0}C)	
Sunflower	10	1
Rapeseed	10	15
Crown	14	-2
Palm	10	10
WVO	12	12
Peanut	17	18

APPENDIX H2- Cloud points of blended palm Biodiesel

Sample	*Cloud points(^{0}C)*
Palm	12
Palm & Rapeseed	5
Palm & Crown	9
Palm & sunflower	4

APPENDIX H3- Cloud points of blended peanuts Biodiesel

Sample	*Cloud points (^{0}C)*
Peanut	17
Peanut & Rapeseed	14
Peanut & Crown	14
Peanut & Sunflower	14

APPENDIX-H4- Cloud points of blended WVO

Sample	*Cloud points(^{0}C)*
WVO	12
WVO & Sunflower	14
WVO & Rapeseed	15
WVO & Crown	16

APPENDIX I Density @20^{o}C

Biodiesel	*Washed*	*Unwashed*
Peanut	0.8811	0.8780
Rapeseed	0.8813	0.8792
Palm	0.8714	0.8717
WVO	0.8817	0.8782
Crown	0.8813	0.8792
Sunflower	0.8814	0.8797