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Synthesis of Biodiesel from a Crude Blend of Palm Oil and Rubber Seed Oil

By

Modhar Ali Mohammed Khan

A THESIS

SUBMITTED TO THE POSTGRADUATE STUDIES PROGRAMME

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DEGREE OF MASTER OF SCIENCE IN CHEMICAL ENGINEERING

CHEMICAL ENGINEERING PROGRAMME

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Declaration

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTP or other institutions.

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Dedication

To my great mother who pursued me to further my knowledge and excel in life

To my lovely wife who offered me unconditional support, encouragement and patience throughout this journey, who is my source of motivation.

Acknowledgment

All praise to Allah, the most merciful; the most graceful with his blessings and enlightens I was able to finish this work and surviving everyday's life.

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Abstract

Biodiesel industry is expanding as new challenges arise from environmental impacts caused by hydrocarbon based fuels. However, the industry has shown some downfall recently due to the increasing demand of vegetable oils for human nutrition's need. Crude rubber seed and palm oils were used in this study to synthesize biodiesel since both can be extracted and produced here in Malaysia from their abundant plantations. The respective oils and the blends were characterized. An equi-volume blend of crude palm oil and rubber seed oil was chosen for biodiesel synthesis and exhibited the following properties: density of 0.91754 g/cm^3, viscosity of 39.35cP, heating value of 38.1 MJ/Kg, acid value 31.4, FFA 11.9%, refractive index of 1.465, mono-,di- and triglycerides were 2.5, 7.3 and 70 wt%. Sulfur content was acceptable at 0.031wt%. Fstty acid composition for the oil blend was established and the calculated iodine value was 106.3. Two stage transesterification is performed in this study to remedy the high acid value of the oil blend. Design of experiments using taguchi technique was used to study the interaction of parameters involved in the production. Temperature, alcohol and catalyst amount were studied independently for both acid and base reactions to find their relevant effects. The optimum operating conditions for acid esterification which could reduce the FFA% to lower than 0.6% were 65°C, 15/1 methanol to oil ratio and 0.5wt% catalyst. The optimum operating conditions for base transesterification were 55°C, 8/1 methanol to oil ratio and 2wt% potassium hydroxide which could achieve the highest possible conversion to methyl esters exceeding 98 %. The quality of biodiesel produced matched that of international standards. The kinetics of free fatty acids alcoholysis in acid esterification was investigated and the rate equation had a reaction order of (2.24). Rate constant was (6.769x10^3 lit/gmol.min) while the activation energy and Arrhenius constant were 60.289 and 14.06 x 10^9 KJ/g respectively. A nonlinear model was developed and with experimental data to predict FFA concentration change throughout the reaction. Kinetics of methyl ester formation in base transesterification was studied using differential method. Three techniques were applied, namely, graphical method, finite difference and linear regression. None of which could predict a rate law to represent the reaction. Consequently, a model was developed to represent the rate of reaction and the system using non linear regression of the experimental data.

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Nomenclature

CPO	Crude palm oil
RSO	Rubber seed oil
FFA	Free fatty acids
FAME	Fatty acids methyl esters
TG	Triglycerides
DG	Diglycerides
MG	Monoglycerides
ME	Methyl esters
V_A	Volume of KOH solution used in titration of the sample solution, ml
V_B	Volume of KOH solution used in titration of the blank solution, ml
N	Normality of the standard alkali
W	Mass, g
ADT	Atmospheric distillation temperature, °C
A	Atmospheric correction factor
VDT	Vacuum distillation temperature, °C
CI	Cetane Index
D	Density, g/cm^3
A_g	Peak area of glycerin
A_s	Peak area of internal standard 1
W_s	Weight of internal standard1 ,mg
a_g	Slope of the calibration function
b_g	Intercept of the calibration function
Gl_i	Mass percentage of individual glycerides in sample
A_{gli}	Peak area of individual glyceride
A_{is2}	Peak area of internal standard 2
W_{is}	Weight of internal standard 2 ,mg
a_{ol}	Slope of the calibration function for mono,di-,or triolein
b_{ol}	Intercept of the calibration function for mono,di-,or triolein
v	Kinematic viscosity, cSt
x	Mass fraction of each component in the blend.
VBI	Viscosity blending index
VBI_{blend}	Viscosity blending index for blend
TLC	Thin Layer Chromatography

GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometer
GL_{MG}	Bonded Glyercin per Monoglycerides from GC analysis,0.2591 X $\sum MG$
GL_{DG}	Bonded Glyercin per Diglycerides from GC analysis 0.1488 X $\sum DG$
GL_{TG}	Bonded Glyercin per Triglycerides from GC analysis 0.1044 X $\sum TG$
$-r_{ffa}$	Rate of reaction for FFA esterification
k_a	FFA esterification rate constant
C_{ffa}	Concentration of FFA
C_{MeOH}	Concentration of Methanol
α	Order of reaction with respect to FFA
β	Order of reaction with respect to methanol
k'_a	Modified rate of reaction constants for FFA esterification
k'_2	Reaction rate constant for FFA esterification at $45^{o}C$
k'_1	Reaction rate constant for FFA esterification at $65^{o}C$
E	Activation energy, KJ/Kmol
R	Global gas constant (8.314)
r_{ME}	Reaction rate for ME generated from base transesterification reaction
C_{ME}	Concentration of ME
a	Specific interfacial area

1 Introduction

1.1 Background

As the fossil fuel prices keep hiking and the irreversible effect that it has on our environment, the importance to research alternatives that can take off some of the economic burdens and industrial demands is taking higher priority in our world's agendas. Considerable work on alternative fuels had been going for some time (e.g. hydro, biomass, wind, solar, geothermal, hydrogen and nuclear) and while it was found possible to produce substitutes new challenges arises concerning possible implementation of these processes, economic feasibility, environmental impacts and the sustainability of their resources.

One of the most discussed solutions with a significant potential for large scale production compared to the other alternatives is biodiesel [1].

Biodiesel is the fuel that can be obtained from the triglycerides of vegetable and animal oils, fats and grease. It can be utilized either directly in the form of the source oils or by reducing its viscosity and breaking down the long chains using various methods and technologies [2] as long as the final product is in compliance either with European Standard (EN 14214) or the American Standard (ASTM D6751) [3,4].

The attraction trend toward increasing the production and the usage of biodiesel is based on the fact that it has renewable sources which inhibits qualifying properties that had brought biodiesel to a level where it can partially replaces or be blended with the diesel fuel to operate Compressed Ignition (CI) engines.

The idea of using biodiesel is not new. Rudolf C. Diesel, The inventor of the diesel engine, ran his diesel engine at the World Fair, France in 1900 using Peanut Oil. Researches dating back to the end of the 19th century showed as well the possibility of using Vegetable Oil sources as fuel. But large scale production and usage didn't take place only during crises such as WWII and the embargo on South Africa during 1980s, to power heavy duty vehicles, because the vegetable oil fuels were very expensive compared to petroleum fuels [5, 6].

Nowadays, many countries around the world including Austria, the Czech Republic, France, United States, Germany, Italy, Sweden and Malaysia are producing and using biodiesel on commercial levels. However, only recently the interest in the biodiesel industry increased as new challenges started to arise.

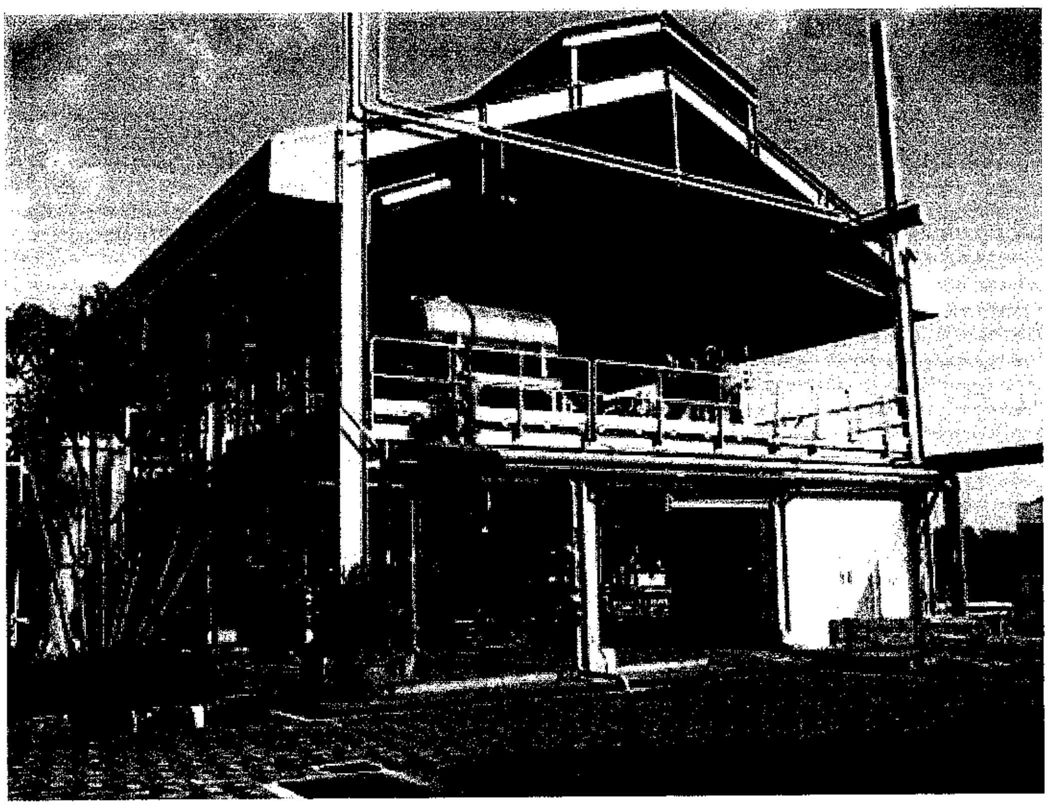


Figure 1-1 Palm based Biodiesel Continuous Pilot Plant (3,000 TPA) built and commissioned in 1985 Source: Malaysia Palm Oil Board (MPOB), Reference [7]

Biodiesel integration in the distribution chains may reduce and cut, although not eliminates a share of the crude oil import costs. This would result in a relief on the country's budget dedicated for energy use and the reduced cost can be used for in country aids and developments.

Increasing the usage of biodiesel will reduce the level of pollution that is brought by petroleum based diesel since biodiesel has a better combustion and emission profile compared to the later one. Research in this matter showed a substantial decrease of 45% in carbon monoxide and 70% in hydrocarbons emissions [8-12] released by conventional diesel fuel since that for biodiesel are being recycled and reused by their sources. Studies showed that using biodiesel will cut up to 70% of the greenhouse gas emissions compared to diesel fuel specially when it reaches the foreseen level of Integration in the transportation sector as it had been planned by the United States, European Union and some other countries [13].

Table 1-1 Change in emmission resulted from using 20% biodiesel (Soybean based) [14]

Emmision Type	***Percent Change in Emission***
NOx (nitrogen oxides)	2
HC (hydrocarbons)	-21.1
CO (carbon monoxide)	-11
PM (particulate matter)	-10.1

Biodiesel can be used neat or blended in existing diesel engines. It can be used alone and called B100 (100% biodiesel) or can be mixed with any portion of petroleum diesel from B5 to B80 as well. It can be used in the current diesel fuel operated machineries without having to go through sophisticated transitions or alterations. Some restrictions however exist in cold environment regarding the flow. Typically, B5 (5% biodiesel) to B20 is the current range of blending depending on the engine warranties. It has enhanced biodegradability, reduced toxicity, improved lubricity and free of sulfur and carcinogenic compounds in comparison with conventional diesel [15, 16].

It had been found that a 20 percent biodiesel fuel can reduce the physical wear and carbon depositions in the engines [17, 18] resulting in a longer improved engine life. It has cleaner burning fuel than petrol and diesel since biodiesel is a plant derived product that contains oxygen in its molecule [19]. 1-2% of biodiesel in blend with regular petroleum diesel had been proven to convert diesel fuel with poor lubricity and quality such as the ultra low sulfur diesel fuel into an acceptable grade [20].

Currently the technologies used to produce biodiesel can be concluded by the following techniques; Pyrolysis (thermal cracking), microemulsion with ionic or non ionic liquids, transesterification and direct use of vegetable/animal Oil sources or blending them straight with diesel fuel. The last process is by far not preferred since oils and waxes cause many problems, most of which are related to their high viscosity and low volatility [2]. Detailed description on each of the methods mentioned above will be discussed thoroughly in literature review.

Vegetable oils are currently the main resources for biodiesel production. With over 350 oil bearing crops being identified only sunflower, safflower, soybean, cottonseed, rapeseed, and peanut oils were found eligible to be used in the synthesis of the alternative fuel [21,22]. However as the research developed, other oils found its way such as castor oil and palm oil [23, 24].

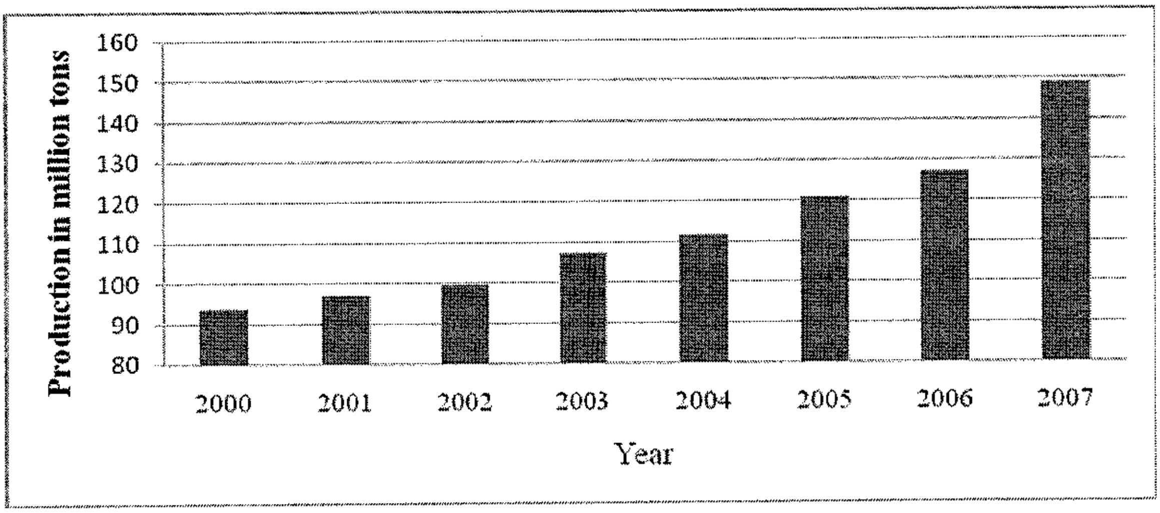


Figure 1-2 Total vegetable oil production worldwide
Source: Food and Agriculture Organization of the United Nations (FAO) [25]

At the present time, it had be shown that resources for biodiesel from vegetable oils comprises about 20 different species with soybean oil, palm/palm kernel oil, sunflower, rapeseed (colza), and coconut oils being the largest sources [26]. The Food and Agriculture Organization of the United Nations (FAO) reported in its *Food Outlook* series that 2008/2009 forecasted production of oils and fats is about 160 million tons versus utilization at around 161 million tons and a 13.2% stock to utilization ratio [25].

Vegetable oils world consumption for all purposes is increasing at about 4% per year. On the other hand, the growth in the production rates in vegetable oils comes mainly from palm oil which together with soy oil comprises 50% of the annual vegetable oil production.

Although biodiesel production is growing rapidly, the current statistics are not reliable enough for total production determination. Besides, the volume of commodities available to produce biodiesel is not enough to meet consumption levels. For instance, the total production capacity of vegetable and animal oils and fats in the United States had been estimated to be around 14.8 million tons per year. Assuming that the whole quantity would be used to produce biodiesel, it will give only 17.4 billion liters which can only displace 14% of the 125 billion liters of diesel consumed annually in the United Stated for on-highway demand [27].

Many countries around the world had adopted local mandates that will result in biodiesel production surge. One of which is Brazil with a nationwide energy policy implemented in 2008. The mandate enforces the usage of B2 (2 % biodiesel, 98 % petroleum diesel) throughout the country. India has past government legislation to achieve 5 % biodiesel by 2007 and 10 % 2010 [28]. The European Union on the other hand stated a 5.57 % biofuel mandate by 2010 in the transportation sector. These examples are driving a rapid growth of biodiesel in the pre-mentioned economies and the production levels have leapt in the last few years [26].

The estimated worldwide biodiesel output for 2006 is 7.5 million tons which is equivalent to 6.8 million tons of energy. The energy balance may vary according to the crops. While 4 units of energy is required for each unit of fossil input in rapeseed, it can be as high as 8 to 1 for high yielding palm oil biodiesel [26]. Malaysia and Indonesia are the largest producers of palm oil in the world. The two countries have already made future plans to establish a number of proposed biodiesel plants while maintaining an agreement signed in 2006 on the capacity of palm oil used. This agreement states a biofuel production of 6 million tons until further studies is ready to evaluate anticipated expansions impacts.

Almost all of the currently processed oils in the biodiesel industry come from edible vegetable oil sources. The domination of edible oils in biodiesel industry is a result of their ready availability in large volumes. Using edible oil sources has many downsides. It contributes in the rising prices of food sources worldwide and adds more burdens on the population. The human need to utilize vegetable oils will be unbalanced since using the edible oils in biodiesel production will create a shortage and will reduce the volume designated for this use since a big share will go for biofuel industry. Producers of vegetable oils might be more encouraged to sell off to biodiesel producers based on the large continuous demand over quantities.

Table 1-2 Biodiesel Production Worldwide *(Thousand tonnes)*

Country	**2004**	**2005**	**2006**
Germany	1,035	1,669	2,681
France	348	492	775
Italy	320	396	857
Malaysia		260	600
USA	83	250	826
Czech Republic	60	133	203
Poland		100	150
Austria	57	85	134
Slovakia	15	78	89
Spain	13	73	224
Denmark	70	71	81
Germany	9	51	445
France	6	36	430
Italy	***2,016***	***3,694***	***7,495***

Source: European Biodiesel Board, Malaysian Palm Oil Board, National Biodiesel Board (USA), Reference [26]

Research on nonedible vegetable oils as sources for biodiesel synthesis had been carried out. The interest had been growing over the past few years but practical investigations showed that in real-time implementations it cannot meet the desired levels of production.

Nonedible oils are defined as the vegetable oils that have no use in food processing for human needs. Jatropha, rubber seed and pongemia pinnata oils are examples of nonedible oil sources.

During the last few years, Malaysia had seen some major developments in biodiesel sector. Serious steps have been taken by the government to minimize the obstacles and to encourage the investments in this area. This is well illustrated by the government actions carried out. In March 2006, Malaysian parliament endorsed the first biodiesel policy by passing on the "Malaysian Biofuel Industry Bill". The national policy was launched to encourage the production of biofuel from palm oil for export as well as the utilization of palm based biodiesel through blending with petroleum based diesel locally. As of November 2006, 75 projects have been approved with a total investment of RM7.01 billion, out of which RM4.87 Billion (69%) are domestic investments and RM2.14 billion (31%) are foreign investments with major sources from Australia, Singapore, USA, India, Italy and Japan [29].

All of the current and future planned biodiesel projects are based on palm oil as the raw material source. In 2006 the grand total production of palm oil produced in Malaysia lies around 15 million tones. This production volume puts Malaysia on the top of the world palm oil producer's list and competitively outranking other countries.

Table 1-3 Malaysian Palm Oil Production Quantity

	Year										
	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
Million Tonnes	8.38	9.06	8.31	10.55	10.84	11.8	11.9	13.35	13.97	14.96	15.88

Source: Food and Agriculture Organization of the United Nations (FAO) [25]

It is estimated that a total of 8.601 million metric tonnes of palm oil will be consumed annually once the 75 previously planned projects are operating. The feedstock is mainly in the form of processed palm oil with around 5.134 million metric tonnes consumption per year. In 2007, it was expected that 8 companies will start production with 1.155 million metric tonnes capacity of biodiesel [29].

Malaysia Industrial Development Authority (*MIDA*) had approved 92 licenses to set up biodiesel plants in Malaysia. It's quite obvious that Malaysia is taking steady steps in building and developing of biodiesel sector and that it has already achieved accomplishments within few years. These are interpretations for the locally available resources that the country enjoys and more specifically the huge palm oil industry. This was one of the key factors for jump starting this alternative energy sector [29]. However, the latter was challenged recently with increasing palm oil prices and fluctuating hydrocarbon market which have led to shut down several producing mills and holding on others being built [30].

Palm is not the only vegetable oil that is abundant in Malaysia. Natural rubber trees that are widely spread and growing throughout the country represent another source of biodiesel raw material. It had been found that rubber seeds yields good amount of oil between 30-40 weight percent. Rubber seed oil exhibits qualifying properties that made it possible for other researchers to synthesis biodiesel from it [31].

Table 1-4 Planted hectarage of natural rubber in Malaysia

Year	P. Malaysia	Sabah & Sarawak	Malaysia Grand Total
1998	1,278.03	265.59	1,543.62
1999	1,206.00	258.8	1,464.80
2000	1,173.55	257.15	1,430.70
2001	1,142.93	246.37	1,389.30
2002	1,128.44	219.96	1,348.40
2003	1,094.74	220.26	1,315.00
2004	1,046.29	221.49	1,267.80
2005^{P}	1,038.44	222.3	1,260.70

Note: ^{P} (Provisional)

Source: Malaysia Rubber Board [32] Statistics for planted area in Sabah - figures provided by Lembaga Industri Getah Sabah (LIGS) for 1999- 2005 statistics for planted area in Sarawak - figures provided by Department of Agriculture Sarawak for 1996-2004 The data for total rubber planted area in Malaysia is estimated by Malaysian Rubber Board

With high amount of oil that can extracted from the seeds and possibility of synthesizing biodiesel from the oil, rubber seed oil can be regarded as a good nonedible source for biofuel production in Malaysia since it has widely spread rubber plantations. Currently, rubber seed and rubber seed oil are underutilized. It's estimated that each hectare of land planted with rubber trees can give an approximate amount of 150 kilogram of oil bearing seeds [31]. The seeds can be collected during their fall seasons which had been observed to occur twice a year.

Malaysia has around 1.2 million hectare of natural rubber trees planted throughout the country [32]. By comparing this area to the statistics and data on the amount of seeds and their yield, a conclusion can be drawn of the prospective and massive potential for rubber seed oil to be integrated in biofuel industry.

Nonetheless, more work needs to be done on statistical level by the designated authorities to identify many factors surrounding rubber seeds for the investors to develop an interest in this sector. These factors and parameters include exact amount of rubber seeds that can be obtained from each hectare of land, difference in amounts of seeds between areas, determination and identification of exact fall seasons. These data will certainly push the wheel in this field.

This study will introduce the possibility of producing biodiesel from a blend of rubber seed oil and crude palm oil. The advantages of blending are explained in section 2.8. The study includes the investigation of rubber seed oil extraction from the seeds, characterization of both oils and their respective blends, designing the experiments to find the optimum process conditions, studying each factor involved in the production process, analysis of the final products using standard methods of testing and finally a thorough kinetic study to establish related data.

1.2 Problem Statements

- Environmental problems are increasing worldwide caused by the emissions of fossil fuels. The need to find renewable fuels is inevitable to reduce these harmful emissions and balancing the environmental cycles.

- Biodiesel was found to be one of the possible replacements to the conventional petroleum diesel since it exhibits similar properties with no major changes that need to be done on technologies that utilize conventional diesel. However, the current sources for this renewable fuel are not preffered since most of it comes from edible sources. The human need versus industry requirement is unbalanced which increased the price and reduced the economy for biodiesel production processes.

- Increasing edible oils prices had led many biodiesel factories to either hold on their current production levels or even to shut down since it is not feasible to continue with current market conditions.

- Rubber seeds contain considerable amount of oil that can be extracted. The nonedible oil remains underutilized being side products of rubber plantation. The seeds are abundant over the wide rubber plantation in Malaysia and the oil can be converted to alkyl esters which can be used as biodiesel. However, the industry and the technology for biodiesel production from that feedstock are not well established.

1.3 Objectives

- To study the effect of operation parameters on solvent extraction process of the rubber seed kernel oil.

- To characterize rubber seed oil, crude palm oil and their blends.

- To find optimum operating conditions for acid and base esterification by applying design of experiment.

- To study the individual effect of temperature, reactants ratio, catalyst amount and reaction time over both acid esterification and base transesterification reactions.

- To characterize the fatty acid methyl esters produced for their suitability as biodiesel using international standards.

- To investigate acid and base transesterification reaction kinetics.

2 Literature Review

2.1 Historical Background

The idea of using and producing biodiesel as an alternative fuel was not born today. All of the technological breakthroughs that humankind made throughout the history came from their basic needs. Same applies for biodiesel, and hence, it's important to explore the early beginnings of its developments and the circumstances that led to the urge of using alternative fuels throughout the time.

In the summer of 1938, a commercial passenger bus between Brussels and Louvain (Leuven) was fueled with palm oil ethyl esters obtained by acid-catalyzed transesterification of palm oil. This is probably the first test of an urban bus operating on biodiesel with a satisfactory performance as its outcome was reported by Chavanne in 1942 [33]. This work was the fruit of his work on palm oil resources available in one of the former Belgian colonies, namely Zaire or Belgian Congo, and its potential use as a substitute for the diesel operating, internal combustion engines and was granted a Belgian patent [5].

The realm of power at that time needed to find other ways to sustain the operation and mobility of their colonies to relief the burden imposed on them from buying and importing coal and liquid fuels. This is well illustrated in a statement for Dr.Diesel on the diesel engine that was manufactured by Otto Company in France and presented at Paris exposition in 1900. The engine ran on arachide (earth-nut or peanut) oil. In his statement, the German inventor clearly indicates that the French government was behind the idea and they gave directions to the company to test the ability of diesel engine to run on the feedstock (groundnut oil) that is abundant in its African colonies. Groundnuts can easily be cultivated and contains high oil content. Thus, the colonies can rely on their own resources for fuel needs [5, 35, 36].

Commenting on the groundnut oil after more experiments were carried out, he affirmed that the oil has many other qualities besides being similar to the mineral oils. It had lubricating properties which can make the engine run independently in the tropics.

Furthermore, diesel referred in his statements to the similar experiments that were successfully carried out in St.Petersburg, Russia on other oils (castor and animal oil). He predicted the possible use of these renewable oils in the future by comparing them to natural mineral oils and tar. Stating that, the later seemed insignificant at that time. The fact was not far from what Diesel had stated. In the following decades and centuries, biodiesel and renewable oils utilization developed tremendously. Various reasons were behind the trends of its usage. Energy security, emergencies and environmental aspects were elements which played an important role in developing, researching and commercializing this industry. During World War II, it had been used to operate heavy machinery and vehicles when a shortage in supply was encountered [37, 18]. At the same era, Brazil, in a try to reduce its foreign expenditure and imported fuels, cut the export of cottonseed oil for it to be used as an alternative diesel fuel. Argentina followed the same path and employed all the potential to obtain the greatest advantage of their vegetable oils.

Soybean oil was used in YAMATO, a Japanese battle ship, as a bunker fuel. Tung among other oils was converted to various types of fuels using thermal cracking processes in China. Ongoing research in India during that period to substantiate the decrease in fuel incase of forced measure resulted in the identification of ten vegetable oils as a potential domestic sources. The United States was not far from the events. In the years following World War II, projects and developments were initiated to investigate local available resources to be used in this field as the prices hikes and increased demand worldwide resulted in local shortages [5].

The embargo on South Africa have led the country to consider redirecting local resources to be used in alternative diesel production to meet the local demand and keep the industrial cycle in the move for the country to survive. From the events noted above, we could see the role of biodiesel in the case of emergencies. However, the prices of petroleum based diesel dropped and it was inevitable for the vegetable oil based diesel to compete with it since the production process and the raw materials costs were big enough to redirect the eye sites from this industry, at least at that point of time.

As environmental awareness has been growing up and countries around the world had been taking serious steps to reduce their negative contribution toward the environment. Mandates were adopted and policies established in the European Union, United States, Brazil and other major industrial countries as an interpretation for these steps. Most of which aims for reduction in exhaust gases from industrial and transportation sectors [38, 22, 25].

Increasing fossil fuel prices were ignited by global incidents. Wars and reports speaking about near depletions of oil wells had sent a surge of warnings around the globe as the other goods prices were increasing accordingly. In parallel, governments have been taking serious steps in resolving these matters and looking into possible alternative solutions.

Once again, biodiesel surfaced among other green fuels that found grounds in many of the legislations and laws passed by countries. With the development in technologies and the ongoing research, it seems that this renewable source of energy has better chance to be sustained for the longer term as it has already been in use and operations worldwide.

2.2 Biodiesel Feedstock

Vegetable oils were historically used for nutritional purposes. But even then, it had other useful functions. Probably the most energy related utilization for them is the use of these oils and fats in lighting. Vegetable oils were the only mean human civilization knew to have longer lasting fire. There uses as fuel for lamps and lanterns was back before fossil and mineral liquid and solid fuels were discovered. Olive oil, fish oil and nut's oil were the most commonly used vegetable oils. Ancient Egyptians utilized castor oil as well while Indians used the sesame, groundnut and mustard as they were abundant.

This example illustrates how the potential use of oils and fats as an energy source was understood and discovered. As the ability to ignite is the first property to look for in possible sources, the sense grew with time to the extent of using these oils in what we currently know as biodiesel.

Vegetable and animal oils and fats may be obtained by extraction or pressing. They are substances that can be found in their resources consisting from one mole of glycerol and three of fatty acids. This unique composition has given them the name Triglycerides. As it can be seen from Table 2-1, there many oil carrying corps and sources where we can get the fatty acids from.

Table 2-1 Some oil bearing crops with amount of oil yielded per hectare [40]

Oil bearing crops *Quantities are in Kilogram Oil/Hectare*							
Corn	145	Linseed	402	Tung oil tree	790	jojoba	1528
Cashew nut	148	Hazelnut	405	Sunflower	800	Babassu palm	1541
Oat	183	Euphorbia	440	Cocoa	863	Jatropha	1590
Palm	189	Pumpkin seed	449	Peanut	890	Macadamia but	1887
Lupine	195	Coriander	450	Opium poppy	978	Brazil nut	2010
Rubber seed	217	Mustard	481	Rapeseed	1000	Avocado	2217
Kenaf	230	Camelina	490	Olive tree	1019	Coconut	2260
Calendula	256	Sesame	585	Piassava	1112	Oiticia	2520
Cotton	273	Crambe	589	Gopher plant	1119	Buriti palm	2743
hemp	305	Safflower	655	Castor bean	1188	Pequi	3142
Soybean	375	Buffalo gourd	665	Bacuri	1197	Macauba palm	3775
Coffee	386	Rice	696	Pecan	1505	Oil palm	5000

The fatty acids composition for the oil varies with its origin. These differences may shape the route taken to produce methyl esters and affect the properties of the biodiesel obtained. For instance, a higher content of unsaturated fatty acids leave an oil in liquid state while a higher saturated fatty acids content make it solid at room temperature.

Triglycerides are water-insoluble in nature. It exhibits a hydrophobic physical state to repel water (non-polar) molecules [39].

The choice of vegetable and animal oils and fats as an alternative fuel sources, alongside their environmental benefit, comes from the following advantages:

- Being renewable in sources, thus, depletion is not expected as in fossil fuels.
- Water insoluble, less pollution and destructive to water sources [39,2].
- Liquid in nature, or can be liquefied incase of solid fats, making it easier to store and transport [41]
- High heat content, been reported to be 80% of petroleum based diesel fuel [41,2] and 88% in comparison to No.2 Diesel [42]
- Nontoxic, and less hazardous to health than petroleum diesel
- Biodegradable, it doesn't harm the nature at the end of its cycle
- Ready availability from wide range of crops

Wide range of sources is available for oils and fats which are spread throughout the world. The volume of oil plantation and harvesting is growing numerously every day to meet the continuous demand and consumption. The production level increased from 93 million tons in 2000 to more than 127 million in 2006 as it was reported by FAO [25].

Many vegetable and animal oil and fats had been identified as possible sources in biodiesel production. Continuous research aims to find new starting material and increasing the productivity of the current ones. Further developments are being made to increase the amount of oil for biodiesel production by improving the yield per land and crop as the science is developing in the agriculture genetic field.

The diversity of the plantation is explained by the difference in the conditions surrounding them. While palm oil being the main cultivated crop in tropical areas in Asia, primarily Malaysia as the world leading exporter alongside Indonesia and Africa, it can found that rapeseed oil is dominating the European cultivation while soybean oil in Brazil and the United States.

Using nonedible oils for the production of biodiesel over edible oil sources have many advantages. It will minimize economical and food sacristy impacts resulted from using edible oils in producing biodiesel, be an added value to the relevant agricultural industry, contribute to the gross domestic product (GDP) while reducing expenditure over imported fuels, reduce deforestation rate, diminish the amount of carbon dioxide and provide more efficient utilization for the current plantation.

Methyl Esters were produced from pongamia, cotton seed and neem seed oils were verified to be suitable as fuel in climate weather [43]. Jatropha seems to be a promising source as one of the high oil yielding crops. Analysis on the fuel obtained from jatropha seed oil showed that methyl esters have met required standards [13].

Rubber Seed oil is among these none human nutritional oil resources. Ramadhas has shown in his work the possibility to synthesize and obtain alkyl esters from it and investigated its properties [31]. Biodiesel obtained from rubber seed oil can also be used in the current diesel engines with no required alteration since it confirmed to international standards [44].

2.3 Vegetable Oil Extraction

There are several methods to extract vegetable oils from oil bearing seeds. Of which, solvent and mechanical techniques are the most commonly used. Each one has its own advantages and disadvantages. In mechanical extraction, the capital involved is less than that for solvent extraction since it requires less machinery, material and operation cost. Mechanical extraction involves pressing of the seeds to obtain the oil [54].

On the other hand, solvent extraction gives higher oil yields that the later method. In practice, both techniques are employed in a combined method and used by the industries dealing with vegetable oil extraction. Commercial grade hexane had been used for long time as the solvent of choice since it's relatively less toxic and flammable and can be easily recovered when compared with other types of solvents [55].

Researchers investigated the kinetics of solvent extraction using various types of alcohols such as ethanol and iso-propanol. Hydrocarbon solvents like n-hexane and Iso-hexane were also investigated. Aqueous acid solutions were found to give better results when used in conjunction with solvents. Sulfuric acid, hydrochloric acid and phosphoric acid are examples of these acids that can be used [56].

The choice of solvent, type of oil to be extracted, time and conditions of the extraction process are judging factors that determines the efficiency of solvent extraction. The efficiency is defined by the capacity and yield of oil, extracted oil properties, process safety and economic feasibility [55].

2.4 Feedstock Properties and Analysis

The chemical and physical properties of the oils are important to be identified. They can be governing factors in choosing the right production route, technology, conditions and even the quality of biodiesel produced.

Properties, such as acidity or free fatty acid, are directly related to biodiesel production. For instance, vegetable oils with high acid value require additional treating to lower their free fatty acid content. Acidic esterification is usually conducted before alkali transesterification for that matter [44]. Increasing number of carbon atoms that is found in the carbon chains of vegetable oils increases the boiling and melting points of biodiesel obtained. On the other hand the boiling and melting temperature decreases with the increase of the double bonds between the molecules [2].

The American Oil Chemists' Society compiled a series of tests and established standard methods for testing the raw materials and assessing their properties to meet biodiesel production requirements. These are published in their standard methods book under Ck 1-07 "*Recommended Practices for Assessing Feedstock to ensure biodiesel quality*" [57]. It describes the standard analysis for oils and fats to be used in biodiesel production.

Table 2-2 AOCS Methods to assess biodiesel feedstock quality [57]

AOCS Methods to asses feedstock quality	
Test	***Method***
Sampling	AOCS C 1-47
Insoluble impurities	AOCS Ca 3a-46
Sediment by centrifugation	AOCS Ca 3d-02
Unsaponifiable matter	AOCS Ca 6b-53
Polar compounds in frying fats	AOCS Cd 20-91
Soap in oil	AOCS Cs 17-95
Polymerized triglycerides by gel-permeation HPLC	AOCS Cd 22-91
Water by modified Karl Fischer method	AOCS Ca 2e-84
Modified moisture and volatiles	AOCS Ca 2f-93
Acid Value	AOCS Cd 3d-63
Sulfur, elements in oil by ICP-OES	AOCS Ca 17-01
Phospholipids in vegetable oils	AOCS Ca 19-86
Phosphorus in oil by ICP-OES	AOCS Ca 20-99
Phosphorus content, colorimetric method	AOCS Ca 12a-02
Phosphorus	AOCS Ca 12-55
Fat stability, Oil Stability Index (OSI)	AOCS Cd 12b-92
Fat Stability, peroxide value	AOCS Cd 8b-90
p-Anisidine value	AOCS Cd 18-90
Fatty acid composition	AOCS Ce 1-62
Methyl ester preparation	AOCS Ce 2-66
Calculated iodine value	AOCS Cd 1c-85

Table 2-3, Table 2-4 and Table 2-5 provide important properties and characterizations for a number of vegetable oils as obtained by other researchers. The chemical and physical properties of the oil are crucial to be determined. As explained previously, they may serve as indicators to choose biodiesel production process or to predict the quality of biodiesel to be produced.

Table 2-3 Some chemical properties of vegetable oils

Oil Source	**Calorific Value (MJ/kg)**	**Acidic Value (mgKOH/gSample)**	**Cetane No. (Unitless)**	**References**
Almond Kernal	39.8	-	34.5	45
Castor	37.4	-	42.3	45
Corn	36.3	0.11**b**	38	46, **b** 21
Cotton Seed	36.8	0.07 **b**	38	46, **b** 21
Hazelnut Kernal	39.8	-	52.9	45
Jatropha	39.63	3.71	46.3	47
Linseed	39.3	-	27.6	48
Olive Kernal	37	-	39	46
Opium Poppy	39.6	-	36.7	45
Palm	33.5	-	62	49
Peanut	33.6	0.2 **b**	54	49, **b** 21
Rapeseed	37.6	1.14 **b**	37.6	50, **b** 21
Rice bran	41.1		50.1	51
Rubber Seed	37.5	34 **b**	-	31, **b** 48
Safflower Seed	39.5	-	42	45
Sesame	39.4	-	40.4	45
Soybean	39.6	0.2 **b**	38	50, **b** 21
Sunflower	39.5	0.15 **b**	37.1	50, **b** 21
Walnut Kernal	39.6	-	33.6	45

Table 2-4 Some physical properties of vegetable Oils

Oil Source	**Kinematic Viscosity (cSt)**	**Flash Point (°K)**	**Density (kg/m3)**	**Reference**
Alianthus	30.2	513	916	52
Almond Kernal	34.2	-	-	52
Brassica carinata	55.07		879	53
Babassu	-	400	875	49
Bay Laurel	23.2	499	921	52
Beech	34.6	515	915	52
Beechnut	38	533	912	52
Castor	29.7	-	-	45
Corn	34.9	550	909.5	21
Cotton Seed	34	509 [b]	910	46, [b] 21
Crambe	53.6	447	904.4	21
Hazelnut Kernal	24	503 [b]	920	45, [b] 52
Jatropha	46.82	235	914	47
Linseed	27.2	514	923.6	21
Olive Kernal	32	504 [b]	925	46, [b] 52
Palm	24.1	437	880	52,49
Peanut	39.6	544	902.6	21
Rapeseed	39.2	519 [b]	914	62, [b] 21
Rice bran	44	316	920	12
Rubber Seed	66.2	471	910	31
Safflower Seed	31.3	533	914.4	21
Soybean	32.6	527	913.8	21
Sunflower	33.9	447	916.1	21
Terminalia catappa L	39.8	-	910	48
Walnut Kernal	36.8	524 [b]	912	45, [b] 52

It can be seen from Table 2-3 that the acid value of the oils varies significantly. Some oils have higher acid value above the acceptable level which requires additional treatment [2]. However, it can be seen that some other properties are common for the vegetable oils. An example of which is the heating value which varies in a small range for the oils.

Table 2-4 shows physical properties for the oils. The viscosities are noted to be of high range for most of the oils. According to biodiesel standards [3,4], the viscosity needs to be with a range of 1.9-6.0 cSt for it to be accepted. The viscosity needs to be monitored the oil and biodiesel.

Table 2-5 shows the fatty acids composition for number of vegetable and animal oils as obtained by several researchers. It is important to identify the components of the oil since it can be related to other properties. For instance, knowing the compositional analysis for the oil enables the identification of saponification value and iodine number.

Table 2-5 Fatty acid composition of several oils (in weight percent)

	Caprylic C8/0	Capric C10/0	Lauric C12/0	Myristic C14/0	Palmitic C16/0	Palmitoleic C16/1	Stearic C18/0	Oleic C18/1	Linoleic C18/2	Linolenic C18/3	Arachidic C20/0	Behenic C22/0	Erucic acid C22/1	Lignoceric C24/0	*Reference*
Soybean	-	-	-	0.1	11	0.1	4	23.4	53.2	7.8	0.3	0.1	-	-	13
Sunflower	-	-	0.5	0.2	4.8	0.8	5.7	20.6	66.2	0.8	0.4	-	-	-	
Jatropha	-	-	-	-	14.2	1.4	6.9	43.1	34.4	-	-	-	21.1	78.9	
Karanjia	-	-	-	-	9.8	-	6.2	72.2	11.8	-	-	-	16	84	
Crambe	-	-	-	-	2.07	-	0.7	18.86	9	6.85	2.09	0.8	58.51	1.12	2
Coconut	-	-	46.5	19.2	9.8	-	3	6.9	2.2	-	-	-	-	-	
Peanut	-	-	-	-	11.38	-	2.39	48.28	31.95	0.93	1.32	2.52	-	1.23	
Corn	-	-	-		11.67	-	1.85	25.16	60.6	0.48	0.24		-	-	
Cottonseed	-	-	-	-	28.33	-	0.89	13.27	57.51	-	-	-	-	-	
Rapeseed	-	-	-	-	3.49	-	0.85	64.4	22.3	8.23	-	-	-	-	
Rubber Seed	-	-	-	-	10.2	-	8.7	24.6	39.6	16.3	-	-	-	-	31
Palm (mesocarp)	-	-	-	-	42.6	0.3	4.4	40.5	10.1	0.2	-	-	-	-	45
Safflowerseed	-	-	-	-	7.3	-	1.9	13.6	77.2	-	-	-	-	-	
Sesameseed	-	-	-	-	13.1	-	3.9	52.8	30.2	-	-	-	-	-	
Linseed	-	-	-	-	5.1	0.3	2.5	18.9	18.1	55.1	-	-	-	-	
Wheatgrain	11.4	-	-	0.4	20.6	1	1.1	16.6	56	2.9	-	-	-	-	
Tallow	-	-	-	-	23.3	0.1	19.3	42.4	2.9	0.9	-	-	-	-	
Bay Laurel	-	-	26.5	4.5	25.9	0.3	3.1	10.8	11.1	17.6	-	-	-	-	
Hazelnut Kernal	-	-	-	-	4.9	0.2	2.6	83.6	8.5	0.2	-	-	-	-	
Walnut kernel	-	-	-	-	7.2	0.2	1.9	18.5	56	16.2	-	-	-	-	
Almond Kernal	-	-	-	-	6.5	0.5	1.4	70.7	20	-	-	-	-	-	
Olive Kernal	-	-	-	-	5	0.3	1.6	74.7	17.6	-	-	-	-	-	

2.5 Biodiesel Quality

In order for biodiesel to be accepted, it needs to adhere to established quality standards. Those standards are instated by governing bodies in each country. In the United States, biodiesel produced needs to match the product specifications criteria set by the American Society for Testing and Materials (ASTM) in its established biodiesel testing standard numbered (*D6751-07be1*) [3].

Similarly, the European Committee for Standardization published their biodiesel quality criteria under the standard (*EN 14214*) [4]. India has (*IS 15607*) [166] as the official standard for biodiesel quality description. Table 2-6 shows each international standard with its corresponding criteria.

Table 2-6 Criteria of several international biodiesel standards

Property	**Unit**	**ASTM D6751 test method**	**ASTM D6751 limit range**	**EN 14214 test method**	**EN 14214 limit range**	**IS 15607 test method**	**IS 15607 limit range**
Density 15°C	kg/m^3	-	-	EN ISO 3675	860–900	-	-
Kinematic Viscosity, 40°C	mm^2/s	D 445	1.9-6.0	EN ISO 3104, ISO 3105	3.5–5.0	IS 1448 P:25	2.5-6.0
Flash point (closed cup)	°C	D 93	130.0 min	EN ISO 3679	120 min	IS 1448 P:21	120.0 min
Sulfur content S15 Grade	% mass (ppm)	D 5453	0.0015 max	EN ISO 20846; EN ISO 20884	0.001 max	D 5453	0.05 max
S500 Grade		D 5453	0.05 max				
Sulfated Ash	% mass	D 874	0.020 max	ISO 3987	0.02 max	IS 1448 P:4	0.02 max
Water and Sediment	% volume	D 2709	0.050 max	EN ISO 12937	0.050 max	D 2709	0.050 max
Copper strip corrosion	Degree of corrosion	D 130	No. 3 max	EN ISO 2160	No.1 max	IS 1448 P:15	No.1 max
Cetane number	-	D 613	47 min	EN ISO 5165	51 min	IS 1448 P:9	51 min
Cloud Point	°C	D 2500	To Report	-	-	IS 1448 P:10	-

Table 2-6 Continued

Carbon Residue	% mass	D 4530	0.050 max	EN ISO 10370	0.30 max	D 4530	0.050 max
Acid number	mg KOH/g	D 664	0.5 max	EN 14104	0.50 max	IS 1448 P:1/Sec.1	0.5 max
Glycerin Free	% mass	D 6584	0.020 max	EN 14105, EN 14106	0.020 max	D 6584	0.020 max
Total	% mass	D 6584	0.240 max	EN 14105	0.25 max	D 6584	0.250 max
Phosphorus content	% mass	D 4951	0.001 max	EN 14107	0.001 max	D 4951	0.001 max
Sodium/Potassium	ppm	-	To report	EN 14108, EN 14109	5.0 max	-	To Report
Distillation temperature,	°C	D 1160	360 max	-	-	-	-

American Society for Testing and Materials, European Committee for Standardization, Bureau of Indian Standards, Reference: [3,4,166]

2.6 Methods of Production

As the sources for the biodiesel and their properties had been presented previously, it is important to go through the various technologies that are used to produce biodiesel by processing oils and fats.

Currently, there are four techniques to substitute petroleum based diesel fuel with plant and animal based oil. These include the direct use of the oils and blending with conventional diesel, microemulsion, pyrolisis or thermal cracking and transesterification [2]. All will be illustrated in the following sections and the benefits and disadvantages of each process will be discussed.

2.6.1 Direct Use and Blending with Petroleum Diesel

The utilization of vegetable oils as a fuel source started with the direct use of these oils in engines and machinery designed to run on petroleum diesel without altering their structures and parts. There are many advantages of which direct use of the oils is favored for. The oils are generally available and their processing is not required, similar energetic properties for that of diesel, lower pollution tendency and comparatively more environmental friendly with lower sulfur and aromatic contents and biodegradable in nature. All of these were initiatives to use the vegetable and animal oils as diesel fuel alternative.

Cold flow seemed to be a consistent problem and initial heating of the engines was required for them to ignite when vegetable oils were used, this is caused by the high oils viscosity which resulted in mal fuel automization [45]. High viscosity of the vegetable oils had been reported by researchers to be 10-20 times higher than that of diesel and in some cases 100 times as for castor oil [21]. Higher injection pressure and advanced injection timing were methods introduced to solve the viscosity problem.

Lower volatility was among other reported constrains of the oils along with the reactivity of the unsaturated hydrocarbons chains. These have an extensive damage over diesel engines and higher impact on the direct injection models. Poor fuel automization, carbon deposits, oil ring sticking, cocking and carbon depositions on the injectors are encountered with direct use which may cause irreversible damage. Moreover, contamination of the vegetable oils results in thickening and gelling of the lubricating oil [41]. Processing of the oils to enhance its fuel like properties seems to be inevitable to match the diesel fuel standards.

Alternatively, another approach was developed to solve problems associated with direct use of vegetable oils. Blending was suggested to give better results. Blending is the mixing of the various types of the renewable oils (used or new) with different proportion of petroleum diesel or ethanol to minimize the problems related to the high viscosities of the oils. A Blend of 25% diesel with 75% crude soybean oil burned in a direct injection diesel engine [59]. Another with 25/75 ratio of degummed soybean oil and no.2 diesel fuel had shown to be suitable for agricultural machinery and was recommended for periods of shortages in fuel supply [60]. A single cylinder engine was operated with 70/30 rapeseed oil and no.1 diesel [61]. Blend of 25 to 75 parts of sunflower and diesel fuel gave a viscosity a bit higher than that of the standard [62]. The viscosity was high as well for the same blending ratio with safflower oil [63]. Even though the viscosity is reduced, blending could not yield a uniform product that meets the properties of diesel fuels that is set by the international recognized standards.

2.6.2 Microemulisification

Another way to reduce viscosity is microemulisification of vegetable and animal oils and fats with short chain alcohols. Microemulsion is defined as the colloidal equilibrium dispersion of optically isotropic micro-structured fluid that has nano-range diameter between 1-150nm. Microemulsions can be formed from any two immiscible, ionic and non ionic, surface active liquids. Many alcohols were studied to give such structures including methanol, ethanol, butanol, hexanol and octanol [64].

Improvement in the spray characteristics with the usage of Microemulsions had been reported [65]. Ziejewski confirmed the previous statement with his findings. He found that adding 1-butanol to refined sunflower and ethanol emulsion could result in a better spray patter and lower viscosity [66]. Goering prepared various emulsion using soybean oil with methanol, ethanol, butanol and octanol and several passed the tests of the Engine Manufacturers Association (EMA) [63, 67, 2].

Despite the successful performance of the Microemulsions, the blends had its downsides as well. Incomplete combustion, injector needle sticking, heavy carbon deposits and increase in viscosity were reported [68, 2]. In addition, cetane number and energy content of the vegetable oils microemulsion were lower than those of petroleum diesel [69]. Carbon deposits and lacquer accumulations were spread over the injector tips, valves and top of the cylinder lines [67,2].

2.6.3 Thermal Cracking

Thermal cracking or pyrolisis is the process that involves the decomposition of a substance into another by using high temperatures with/without the presence of catalysts. This definition of the process seems to be close to that of petroleum cracking processes which involves the breakdown of the heavy crude oil into lighter fractions. Similarly, thermal cracking of vegetable oils yields several products that are close in chemical nature to that of fossil fuels derivatives including alkanes, alkenes, alkadienes, carboxylic acids, aromatics and small amounts of gaseous products [70, 45]. Gasoline, diesel, olefins and other hydrocarbons could be obtained by this processing route of vegetable and animal oils [71].

The thermal process involves the heating of intended raw material to high temperatures in the absence of air or oxygen to break it down into number of substances with lower molecular weights and smaller molecules size. Although the work in this field had been ongoing for hundred years, researchers still find some difficulties in characterizing the oils and fats pyrolisis because of the variety in the reaction mechanisms and routes that may happen and cause the yield of a number of possible products. Schwab et al presented in his work a mechanism for the decomposition of vegetable oils in the pyrolisis reaction [70,2].

The mechanism can be seen in Figure 2-1. The reaction progress as the alkyl group is replaced with hydrogen then it goes on by dissociating molecular structure which releases molecules with free radicals. The reaction then continues until the final products are obtained.

Another advantage of thermal cracking is the variety of raw material that can be fed into the process and the many types of catalysts available. The catalyst that are used in the thermal fractionation are similar to that used in petroleum industry such as HZSM-5, Hydrogen-Zeolite Y, Silica-Alumina, Silica-alumina-pillared clay and H-modernite [71,72].

The extended advantage of the pyrolisis, besides biodiesel production, is the process subsidiaries that can be obtained. These include but not limited to petrochemical industry feedstock, production of hydrogen (H_2) that can be used in fuel cells and production of synthetic gas (H2,CO) in considerable amount that could be used to obtain biodiesel by Fischer-Tropsch catalysis and bio-methanol [73]. There are many types of reactors where the process can take place in industrial and laboratory scales. These include fractionating (pyrolisis) reactor, fluidized bed reactor, laboratory scale once-through micro riser reactor and transport riser reactor [74]. Based on the operating conditions involved in the thermal cracking, the process can be divided into conventional pyrolisis, fast pyrolisis and flash pyrolisis [70, 45].

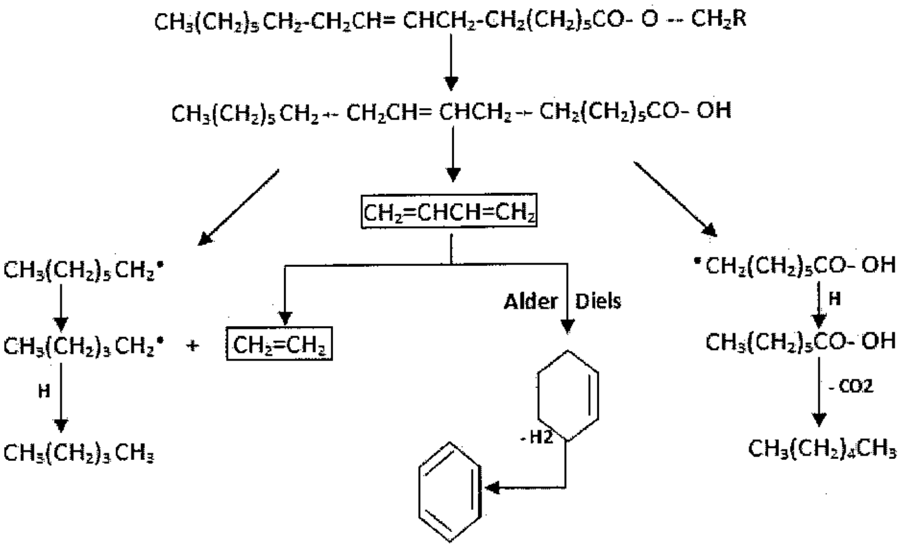


Figure 2-1 The mechanism of thermal cracking [70,2]

Pyrolisis of soybean and safflower oils was carried out using distillation apparatus. The distribution percentage of hydrocarbons obtained were 73-77 % and 80-88 % of alkanes and alkenes respectively as the major components [75, 76, 2]. Palm stearin and copra oils were catalytically cracked using Silica-Alumina catalyst at 450°C. The process was reported to be effective with no air or water contamination. Petroleum like products was obtained including biodiesel and bio-gasoline [77].

Thermal cracking of rapeseed oil was carried out in a tubular reactor with a range of temperature between 500-850°C under constant dilution of 13/1 molar ratio of nitrogen per feed in. The reaction gave a mixture of products including methyl esters and light hydrocarbons that favored high reaction temperature [78,1].

Another feed for thermal cracking is vegetable oil soaps. Tung oil was extracted by pressing seeds from tung tree nuts. The oil obtained was thermally cracked in 1947 by Chang and Wan. Initially, the oil was saponified in calcium soaps (lime) and then thermally fractionated in a tubular reactor. The reactor was heated with electrical tubular furnace which was inserted vertically into the reaction. Crude oil was obtained from pyrolisis and biodiesel was produced alongside. Other derivatives included low quantities of gasoline and kerosene. This process produced 50 liters of crude oil from each 68 kilograms of soap [79, 45,2]. The saponifcation and pyrolisis reactions are shown in Equations 2.1 and 2.2. The pyrolisis of vegetable and animal oils soaps can achieve higher yield of hydrocarbons by increasing the temperature [45].

Saponification:

$$\text{Vegetable/Animal Oils or fats} + \text{NaOH} \longrightarrow \text{RCOONa} + \text{Glycerin} \quad (2.1)$$

Pyrolysis of soaps:

$$2\text{RCOONa} + (1/2)\,\text{O}_2 \longrightarrow \text{R-R} + \text{Na}_2\text{CO}_3 + \text{CO}_2 \qquad (2.2)$$

The arguebility concerning the application of the thermal cracking process in the production of biodiesel is related to the high cost of process equipments and high energy consumption related to severe operating condition. Without some major decrease in the price of vegetable oils, pyrolisis has comparatively higher cost than the other processes involved in biodiesel production.

The other concern that surrounds thermal cracking is the environmental impact of the process. The removal of oxygen during thermal processing drops the environmental benefit of biodiesel as an oxygenated fuel [2].

2.6.4 Transesterification

Transesterification involves the reaction between the lipids that can be found in renewable oil sources with short chain alcohols. Like all the previously mentioned processes, the main aim of the transesterification reaction is to reduce the viscosity of the oils and fats for them to be used as proper alternative for diesel fuel.

The mechanism of the reaction involves three consecutive, reversible, reaction steps leading to the conversion of the triglycerides to the diesel like alkyl esters [2]. In transesterification, the complex molecule of fatty acids found in the vegetable and animal oils and fats is neutralized by alcohol while the glycerin is removed to obtain alkyl esters [42].

The reaction initiates with the formation of intermediates. Triglycerides are converted to diglycerides that react with the available alcohol to form monoglycerides. Mono glycerides in turn proceed to the final step to give the alkyl esters and glycerol as a byproduct. It is believed that the last reaction is the rate determining step since the transformation of the monoglycerides to alkyl esters is the slowest reaction step since monoglycerides are the most stable intermediates in transesterification [2, 64].

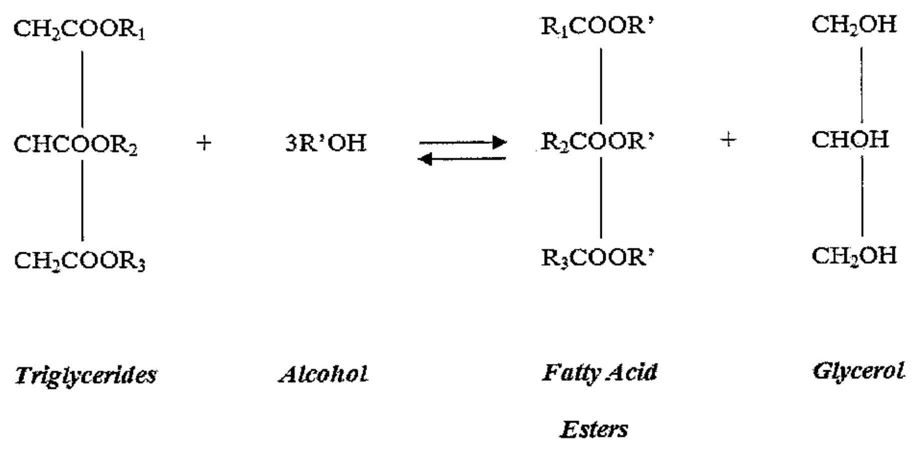


Figure 2-2 Transesterification of Oils and Fats with alcohols R', R_1, R_2 and R_3 represent alkyl groups [58]

The earliest reference to transesterification reaction could be found in the literature written by Rochieder dating back to 1846. The researchers described the alcoholysis of castor oil using ethanol to obtain glycerol. Intensive research on the reaction and its surroundings can be found in later studies on transesterification and significant developments had been made. These developments involved the identification of new process raw materials, studying reaction conditions and investigations of other routes where the reaction can take place [42].

$$\text{Triglyceride} + R'OH \rightleftarrows \text{Diglyceride} + R'COOR_1$$

$$\text{Diglyceride} + R'OH \rightleftarrows \text{Monoglyceride} + R'COOR_2$$

$$\text{Monoglyceride} + R'OH \rightleftarrows \text{Glycerol} + R'COOR_3$$

Figure 2-3 Three steps mechanism of the Transesterification Reaction R', R_1, R_2 and R_3 represent alkyl groups. [2,80]

There are a number of parameters and variables that had been found to have a noticeable effect on the reaction, kinetics and even properties of the final product. These include the type of transesterification route chosen, the temperature of reaction, type of alcohol used, ratio of alcohol to oil, type of catalyst and amount used, the properties of feedstock and the rate of mixing of reactants [49].

In biodiesel production, transesterification is generally fed with crude or purified vegetable or animal oils. However, Insitu transesterification had also been investigated by researchers. Insitu transesterification involves the production of alkyl ester by utilizing the original agricultural crops instead of crude or purified oils as the glycerides containing feedstock. The process is meant to eliminate the oil extraction process. Thus, reducing the amount of reagents and solvents required. Researchers also state that Insitu transesterification imposes less waste disposal [81].

Another advantage for transesterification reaction is glycerol. Glycerol is obtained as a byproduct from the transesterification reaction and it can be separated by settling, centrifuging or phase separation. Glycerol can be used in several industrial applications. It is currently involved in large production of Citric Acid, USP (i.e. United States Pharmacopeia recognized drugs) and dynamite alongside its other uses in food and detergent industries [2].

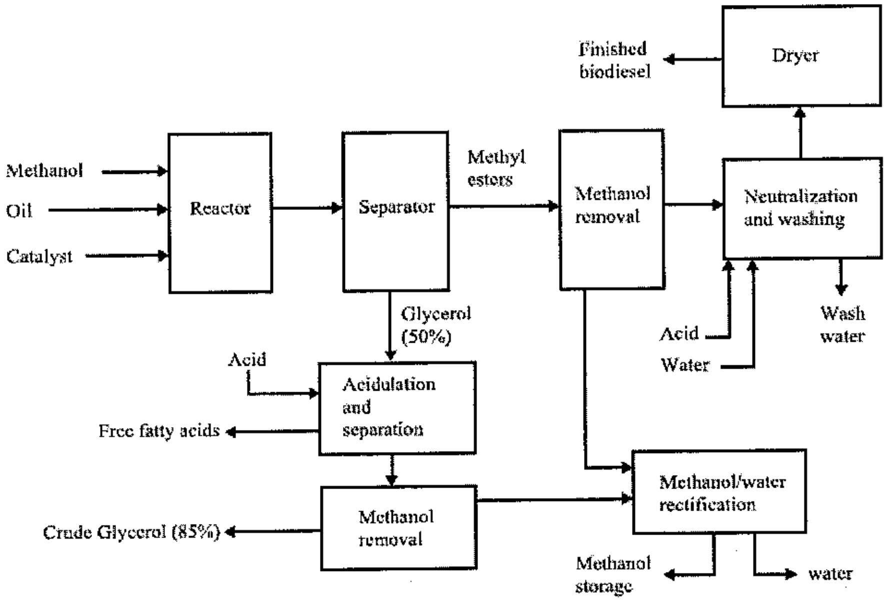


Figure 2-4 Proposed transesterification process [10]

Transesterification is widely used because of less production cost and environmental impacts than pyrolisis. The side product (i.e. Glycerol) of the reaction is an added advantage. Biodiesel production by transesterification can also be done using different process setups, raw materials, catalysts and other parameters. Figure 2-4 shows a proposed process setup for transesterification. In this study, each of these technologies will be discussed in details. The processes can be divided according to the type of alcohol that is fed into the transesterification reaction and the types of catalyst used.

2.6.4.1 Alcohols

One of the important aspects of the reaction is the type of alcohol used. Transesterification of oils proceeds using short chain, primary or secondary, monohydric aliphatic alcohols. The alcohols may range from one to eight carbon atoms to break the triglycerides and form alkyl esters. Alcohols including methanol, ethanol, propanol and butanol can be used. When comparing alcohols, methanol and ethanol are the most preferred in the process. As for methanol, the selectivity may be related to its physical and chemical properties such as shorter chain, polarity and the noticeably low cost of the alcohol. Ethanol advantage on the other hand is that it can be obtained from renewable, agriculturally cultivated, sources [45]. Table 2-7 shows a number of different production processes involved with the production of methanol and ethanol.

The usage of alcohol in transesterification varies according to the physical state in which it is fed into the reaction. Although the alcohols used in the reaction were always in liquid state, recent development showed the possibility and the potential of using them in supercritical form to transesterificate oils and produce biodiesel [45, 82, 83].

Based on reaction stoicheometry, 3 moles of alcohol are needed to transesterify 1 mol of triglycerides. Practically, more is needed to drive the reversible reaction to completion and to increase the yield of the esters formed. For instance, a range of 3.3-5.25 of methanol/oil molar ratio was proved to be acceptable for the transesterification of vegetable oils [2,84]. However, high amount of alcohol may cause saponification and difficult separation of products. The commonly used molar ratio of alcohol to glycerides is 6:1 [2].

Table 2-7 Methanol, Ethanol production processes [42]

Alcohol	*Production Process*
Methanol	Distillation of liquid from wood pyrolisis
	Gaseous products from biomass gasification
	Distillation of liquid from coal pyrolisis
	Synthetic gas from Biomass and coal
	Natural gas
	Petroleum gas
Ethanol	Fermentation of sugars and starches
	Bioconversion of cellulosic biomass
	Hydration of alkanes
	Synthesis from petroleum
	Synthesis from coal
	Enzymatic conversion of synthetic gas

2.6.4. 2 Catalysts

The reaction can progress with or without the presence of a catalyst. However, catalyzed transesterification is most preferred and widely used since the reaction rate is faster than the non-catalyzed processes. In addition, the catalyzed process provides higher yield and conversion. There are number of different catalyst types to choose from. They can be base, acid or enzymatic catalysts [42].

In the case of alkali and acidic catalyzed transesterification, the range of possible catalysts is expanded as they can be homogenous or heterogeneous. The right choice of the process whether to be catalytic or non-catalytic and the catalyst type depends on many factors of which are the available raw material and their properties. In addition, the product yield foreseen and the cost of plant erection [85,42]. Moreover, the excess amount of catalyst enhances the phase separation between the biodiesel and the glycerol [42].

2.7 Transesterification Techniques

2.7.1 Transesterification using liquid alcohol

2.7.1.1 Alkali catalyzed

At the current time, the widest spread biodiesel production process and most commercially used is alkali catalyzed transesterification. Many technologies have been developed for the reaction in which it can be carried out in batch or continuous processes. Base catalysts used may include alkali metal hydroxides like sodium and potassium hydroxides, alkoxides such as sodium methoxide, sodium ethoxide, sodium propoxide and sodium butoxide and carbonates [2].

Alkali catalyzed transesterification mechanism had been studied and was concluded to proceed in three steps. In the initial step, alcohol anion (Alkyl oxide ion) carries out a nucleophilic attack on the carbonyl carbon atom of the triglycerides molecule and gives an intermediate (tetrahedral). The intermediate proceeds to the next step where it reacts with alcohol and again, releases the alcohol anions. Finally the fatty acid esters and monoglycerides are formed as a result of the rearrangement of the tetrahedral intermediates [2,86]. Figure 2-5 shows the mechanism for base transesterification.

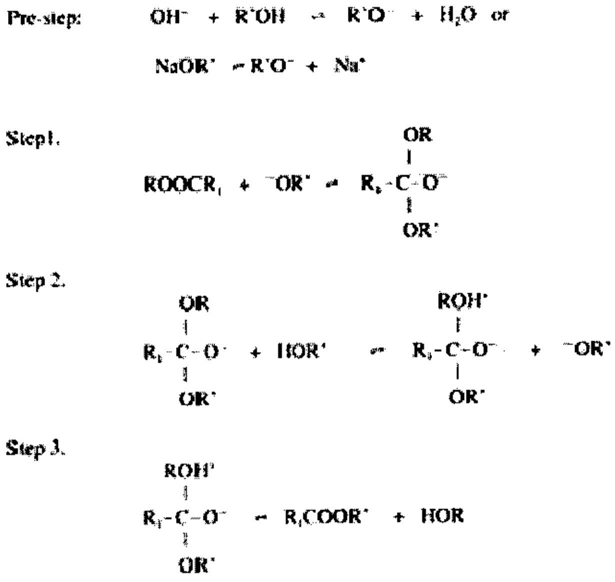


Figure 2-5 Alkali catalyzed Transesterification Mechanism.R_1 and R’ are long and short chain alkyl group[2,86]

Base catalysts have been found to be much faster and economic when compared to the other types of catalyst. However, some constrains surround their usage. The moisture content in vegetable or animal oils and fats needs to be at minimum. The presence of water tends to cause saponification of the oils which decreases the amount of esters produced and makes it more difficult and expensive to separate the products.

The amount of free fatty acids in oils and fats should not exceed 0.5% since this will favor soap formation as well. However, free fatty acid content up to 5 % could be transesterified and saponification could be avoided by adding additional amount of catalyst [87, 2]. The amount of catalyst used in transesterification is usually given in weight percent basis of catalyst to oils or fats and the advised range was found to be 0.1 to 1. However, the amount is usually dependant on the type of oil in use.

Homogenous alkali catalysts are widely used in the production of biodiesel by transesterification reaction but the use of homogenous alkali catalysts is not trouble free. The sensitivity toward water presence and difficult catalyst separation from final products result in additional costs added over to the total capital required since neutralization and separation equipments alongside additional water, solvents and energy are needed.

Heterogeneous catalysts have shown good potential to solve some of the pre mentioned problems and research is ongoing in that field. Heterogeneous catalysts can be separated easily from the final products by filtration without having to involve large amounts of solvents or even more water for catalyst washing and removal. Heterogeneous catalysts that have been researched include alkaline-earth oxides, zeolites and hydrotolacites [88].

Other advantages of heterogeneous catalysts are their noncorrosive effect, less waste disposal problems and other environmental benefits. They can be prepared to yield higher activity, selectivity and longer catalyst life cycles [89].

Many researchers have studied the alkali transesterification to understand the reaction and number of developments was achieved. In 1944, Bradshaw came to study transesterification through the production of soaps. He used based catalyzed transesterification to produce soap from natural oils and fats. Fatty acid esters obtained were used to produce soap after refining the feedstock to improve its properties and remove any undesired residues. The reaction parameters used were 1.1-1.75 methanol to oil ratio, temperature ranged from 25 to 100°C and 0.1-0.5% of catalyst (NaOH, KOH). He reported a successful separation of the final products with 98% conversion and obtained a high quality glycerol [84, 90, 2].

A continuous transesterification process was developed and reported by Trent in 1945 and Allen on the same year. Allen used refined coconut for the glycerides feed and ethanol. The amount of catalyst was 0.75% sodium hydroxide and the reaction took place at 100°C [91,92,2].Tanaka (1981) transesterified tallow, coconut and palm oils in a two step reaction with high conversion reaching 99.5% using alkali catalysts and alcohol to oil ratio between 6:1 and 30:1 [93]. A 6 to 1 molar ratio of methanol to oil with sodium and potassium hydroxides was found to give the best results for the alcoholysis of the rape seed oil by Nye in (1983) [94,2].

Freedman and his colleges (1984) used refined soybean oil to produce methyl ester at a molar ratio of 6:1 of methanol to oil and 1% NaOH over a number of temperatures. It was observed that higher temperature is favored by transesterification and gave higher reaction rate and conversion [95,2]. Wimmer (1992) used potassium hydroxide in alkali transesterification of unrefined rape oil with methanol and the product obtained was washed at room temperature by adding water [96]. Zheng (1994) used edible beef tallow and transesterified it using sodium hydroxide (1% by weight) and 6 to1 methanol to tallow ratio [97,2].

The alcoholysis of soybean oil was investigated by Noureddini and Zhu (1997). The study used 0.2 %wt NaOH as the alkaline catalyst and methanol for alcohol [80]. Darnoko and Cheryan (2000) used potassium hydroxide at an amount of 1.0 %wt in the transesterification of palm oil. The reaction was carried out in a continuous stirred reactor. Methanol to oil ratio was 6:1 with temperature held at 60°C and the purity of the products reached 97.3 %wt [24].

Alkali catalyst had been used in transesterification of used frying oil by Marinkovic (2003) where he reported that 1% of potassium hydroxide is the optimum amount of catalyst required for the process [98]. Lifka et al. (2004) reported the transesterification of rapeseed oil using NaOH at 0.5 wt% and compared the results obtained from ultrasonic and mechanical stirring at 45°C.The conversion obtained from both mixing methods yielded 80-85 % methyl esters after 30min [99].

Karmee et. al. [100] (2005) tried several heterogeneous catalysts including mono zinc oxide, Hb-Zeolite and montomorillonite K-10. The conversions were low in comparison with homogenous catalysts. They were 83%,59% and 47% respectively while those for KOH and NaOH were 92% and 99%.

Methanol and refined soybean oil were converted to methyl ester in a study carried out by Colucci et al. (2005) using potassium hydroxide at a concentration of 2.2 %wt and methanol to oil ratio of 6:1. The conversion reached was 95 % after 10 min and 60°C temperature [101]. Stavarache et al (2005) used NaOH at 1.0 wt% concentration to obtain alkyl esters using methanol as the alcohol. Ultrasonic agitation was used to mix the reactants at 28 kHz and the conversion reached 95% after 10 min at room temperature compared to 91 % after 10 min using mechanical stirring at 1800 rpm [102].

The amount of catalyst required for transesterification of oils and neutralization of unreacted acids was concluded from the study made by Ghadge (2005) and found to be equivalent to 0.7 %wt of KOH over vegetable oil [103]. Alkali transesterification with aid of ultrasonic agitation was studied by Ji and his colleagues (2005). The reaction was catalyzed using 0.2 % NaOH. The use of high speed mixing device had superiority over mechanical stirring [104,88].

Furuta et al. (2006) explored the possible use of new catalysts. Amorphous Zirconia, TiO_2/ZrO_2 and Al_2O_3/ZrO_2 were tested and conversion exceeded 95% when high temperatures were used [105,88]. Siatis et al. (2006) used methanol to transesterify cynara seed oil with aid of alkali catalyst and ultrasonic mixing and reached conversion of 95% within the first 30 min [106].

Neat and used frying oils were also used to produce biodiesel by Leung et. al. (2006). Sodium methoxide (CH_3Na 1.3 wt%), sodium hydroxide (NaOH 1.1 wt%) and potassium hydroxide (KOH 1.5 wt%) were the catalysts. All of which gave high conversions. CH_3Na had the advantage of not forming water over the others since it dissociates into CH_3O and Na^+. The amount of NaOH required was the least to accomplish the reaction [107,88].

Pongamia pinnata oil was transesterified (2006) using potassium hydroxide and amount of catalyst required was reported to be 1.0 wt % by Meher et al.[108,88]. Jitputti and his colleagues (2006) transesterified crude palm kernel oil and crude coconut oil using several heterogeneous catalysts. The yield for methyl ester from crude palm kernel oil was as high as 90.3 % for both SO_4^{2-}/SnO_2 and SO_4^{2-}/ZrO_2 with methyl ester content of 95.4% and 95.8% respectively. The yield from crude coconut oil was 80.6% and 86.3% by using the same catalysts [90].

Activated calcium oxide was used in the transesterification of sunflower oil [109]. Granados et al. (2007) studied the use of CaO for its availability and relatively low cost among other heterogeneous catalysts. The work showed that CaO can be used and stand for eight runs before it loses the activity. Calcium oxide (CaO) was investigated as well by Liu et al. (2007) in the transesterification of soybean oil. Alcohol (methanol) to oil molar ratio was 12:1 with temperature at 65°C and 8% catalyst. Biodiesel yield reached 95% at 1.5 h of reaction [89].

Sharma et. al. (2007) stated in his work that the use of potassium hydroxide is preferred over sodium hydroxide at industrial levels since potassium soaps formed by transesterification are softer than sodium ones [110,88]. Karanja oil transesterification was studied as well by Sirvastava et al. (2007). Methanol was used for alcohol and NaOH as alkali catalyst for the reaction [111].

Georgogianni et al. (2007) performed conventional transesterification of sunflower seed oil and Insitu over the seeds. Alcohols used were methanol and ethanol each with molar ratio of 7:1 to the oils. Temperatures were 60°C for methanol and raised to 80°C for ethanol. The catalyst used for both transesterifications was sodium hydroxide (NaOH) in amounts of 1.0,1.5 and 2.0 wt%. The achieved conversion of alkyl esters in both reactions reached 95% in 20 minutes with 2.0 wt% catalyst concentration [112].

Sarin et. al (2007) used NaOH and KOH ,1 wt% each, in the transesterification of palm oil and jatropha. The amount of catalyst mentioned was for neutralization of oil and excess acids. Qian et al. (2008) implemented Insitu transesterification for cotton seed oil. NaOH showed good result over 3 hours of reaction time. Methanol was used and reaction temperature was 40°C [81].

2.7.1.2 Acid catalyzed

The route of vegetable and animal oils esterification using an acid catalyst is one of the most important alcoholysis techniques for its ability to neutralize and process high free fatty acids content feedstock. When conventional alkali catalyst is used in the transesterification of low cost renewable oils having high content of free fatty acids, large amounts of soap formation is expected to be produced which inhibits the separation of the alkyl esters and glycerol. This form emulsion during water washes which is hard to separate.

Any vegetable or animal oils/fats with free fatty acids content larger than 5 % would have the same problem when transesterified with alkali catalyst [2]. This criterion of oils and fats covers a wide range of low cost, economically feasible, renewable lipids. Ignoring such feedstock would neglect valuable reduction in production cost. Figure 2-6 shows the saponification of oils and fats with alkali catalyst.

$$\mathrm{RCOOR'} + \mathrm{H_2O} \rightleftharpoons \mathrm{RCOOH} + \mathrm{R'OH}$$

$$\mathrm{RCOOH} + \mathrm{NaOH} \rightleftharpoons \mathrm{RCOONa} + \mathrm{H_2O}$$

Figure 2-6 Saponification of fatty acid alkyl esters

Acid catalyst has shown good ability to rectify this disability of base catalyst. Transesterification using acid catalyst can be used for the production of biodiesel from materials that was previously considered to be waste and of no value. Soapstock with 2-7% FFA, animal fats with FFA ranging between 5-30% and even trap grease with FFA approaching 100% can be treated using this method [10].

During acidic alcoholysis, the direct esterification of free fatty acids occurs simultaneously in parallel with triglycerides transesterification reaction. This direct esterification has the advantage of increasing the amount of alkyl esters obtained but slows down the reaction on the other hand [113]. The reaction is said to follow Brønsted-Lowry acid-base theory. The mechanism of acid transesterification can be seen in Figure 2-7. The initial step of the reaction mechanism involves protonation of acid to give an oxonium ion. An intermediate is formed from ion-alcohol exchange reaction. The ester is then obtained as a result of the proton expel from the intermediate. All the steps mentioned are reversible but the forward reaction can be maintained by providing excess alcohol to shift the equilibrium in order to reach completion [42].

The acid value, also referred to as acidity, is the term that refers to the number of acidic functional groups that can be measured and found in a sample. The method used to calculate the acid number involves using potassium hydroxide as a neutralizer and measuring the required quantity of strong base to yield water free, neutralized sample [88].

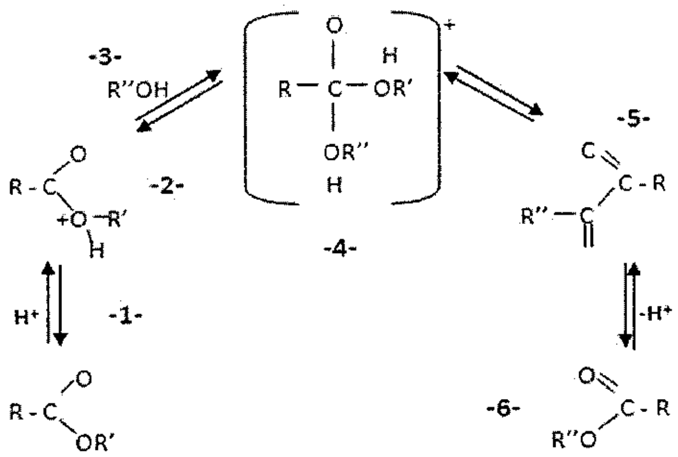


Figure 2-7 Mechanism of acid transesterification of vegetable oils. Numbers from 1 to 6 indicates the progress of reaction mechanism [42]

Acid catalysts that can be used in the process may include sulfuric acid, phosphoric acid, hydrochloric acid or organic sulfonic acid [114,115,2]. The same alcohols in alkali esterification can be used in acid alcoholysis. Oils or fats that are usually fed into base catalyzed transesterification may also be processed using acid approach as well. One aspect that has to be considered is alcohol to oil ratio. It has great effect on the outcomes of reaction. Excess amount of alcohol favors and increase the yield of alkyl esters. At the same time, extra amount of alcohol tends to make glycerol separation more difficult [42].

Suggested reaction parameters for acid esterification include alcohol to oil molar ratio of 30:1 and catalyst concentration from 0.5-1 %. Generally 1% is preferred but some other researchers used up to 3.5%. Temperature varies from 55°C to 80°C according to the type of alcohol and process consideration. The results showed that 1% sulfuric acid could yield a conversion of 99% over 50 h of time [49].

Production of biodiesel using acid catalyst may proceed in one or two stage transesterification. Oils and fats can be transesterified solely by an acid catalyst. The problem is that the resulting alkyl esters may have a high acid value exceeding the accepted acidity limit set by the standards [116, 2].

To solve this problem, another alkali treatment stage may be added to minimize the acidity draw back. Alkyl esters produced from acid esterification is fed into a base catalyzed alcoholysis reaction [114, 2]. Two step transesterification is useful as well when treating high acid value alkali process feedstock since acid value needs to be reduced to less than 2.0 mg KOH/g [88].

Freedman et al. [87] developed a kinetic model for acid esterification of soybean oil using Sulfuric acid (H_2SO_4) at 1% with alcohol to oil molar ratio of 30:1. They extended their experiments to five temperatures and they suggested that acid catalyzed transesterification follows a pseudo first order kinetics [49].

A literature survey showed that Keim et al. (1945) had used acid catalysts to transesterify palm oil with 50.8 % FFA into methyl esters. Methanol in 77% proportion to the weight of oil was used for alcohol. Sulfuric acid at 0.75% of the weight of oil was utilized to catalyze the reaction. The reaction continued for 1 hour at constant rate of mixing while temperature was held at 69°C. Once vegetable oil was neutralized, alkali transesterification was performed using sodium hydroxide with 1.25 wt% and methanol for a period of one hour at 50°C. The final conversion reached 97%. However, the acidic value of alkyl ester obtained was around 5% which is considerably high. This was related to the presence of water in the reaction mixture [116,10].

Spurles et al. (1950) used 15:1 alcohol to oil ratio in acid catalyzed transesterification of high free fatty acid content oils. Sulfuric acid was used in concentration of 5% wt of oil. The reaction was prolonged for 3-4 hours. The mixture was neutralized with calcium carbonate. Separation of transesterification products was done by using distillation to remove excess methanol then phase separation for glycerin. Alkyl esters were finally obtained by washing with warm water under vacuum [114,2].

Tanaka (1981) and his colleagues suggested a novel method for biodiesel production. The glycerides from oils and fats would be converted in a two-step process by using methanol as alcohol. The first step involves acidic treating (i.e. acid transesterification) to lower the FFA content of oils. The temperature was near alcohol boiling point. The reaction was carried out for 0.5-2 h. Temperature of 40-70°C was applied later to separate glycerol and lasted for 1-15min. The final products from first step were then subjected to a second alcoholysis, but this time using alkali catalyst. Catalyst amount was 0.2-0.5% with alcohol at 8-20% of the weight of oil. The conversion was equal to 98% within 5-60min. The final products were separated and methyl esters obtained [93, 2].

Freedman et al. (1984, 1986) used 1 mol% sulfuric acid and conversion reached 99% in 50 h reaction time. An alcohol (methanol) to oil ratio of 30:1 was used and 50°C for temperature. The researchers also investigated the use of other types of alcohol and their excess use had proven to give higher yields of alkyl ester. The same conversion obtained above could be done by using butanol at 117°C and reaction time of 3 hours while that for ethanol was at 78°C and 18 hours using the same catalyst concentration and soybean oil as the glycerides source [87,115,2].

Jeromin et al. (1987) patented a process for pretreatment of free fatty acid in fats and oils using acidic ion-exchange resins in a packed bed [117,10]. Stern et al. (1990) suggested that the use of sulfonic acid is more preferable for the catalysis of esterification [118]. Insitu acidic esterification of soybean oil had been studied by Kildiran et al. (1996). Sulfuric acid was used alongside several alcohols including methanol, ethanol, n-propanol and n-butanol. The study confirmed Freedman et al. [87,115] findings over alcohol effect on acid esterification in which high yield of alkyl esters could be obtained by using higher alcohol. Methanol showed poor results with soybean oil. The reaction was carried out at 78°C for 3 hours and conversions obtained were in a range of 80-85% [119].

Crabbe et al (2001) investigated transesterification of crude palm oil. The maximum yield reached 97%, but alkyl esters obtained could only serve as a No.2 diesel fuel since the cetane number and boiling point were of high values. The reaction parameters included methanol to CPO molar ratio of 40:1 with sulfuric acid concentration of 5% and temperature was set to 90°C for 9 hours [120].

Haas et al. (2002, 2003) developed an acid catalyzed process to convert soapstock to biodiesel. Soapstocks are low grade by-products of vegetable oil refining industry which consist of a mixture of oil, soaps and water. The feedstock is dried and neutralized then fed into transesterification were it reacts with methanol using an inorganic acid as a catalyst. The reaction required an excess amount of alcohol [121, 122, 10].

Ramadhas et al. (2005) studies the production of biodiesel from rubber seed oil using two steps transesterification. In the first stage of acid esterification, free fatty acid content was reduced to less than 2%. In the second step of alkali transesterification, products of the first step will be converted to its mono esters and glycerol. The acid esterification reaction was carried out at 50°C for 20-30min at atmospheric pressure using 0.5% sulfuric acid and methanol while alkali alcoholysis used NaOH and methanol with reaction temperature at 45±5°C for 30 min [31].

Veljkovic et al. (2006) used *Nicotiana tabacum* oil to obtain alkyl esters using acid-alkali transesterification. Sulfuric acid was used initially then potassium hydroxide for base catalyzed biodiesel production in concentration of 1.0% based on oil weight. The highest yield obtained reached 91% [123]. Sharma and Singh (2007) preferred acid esterification of karanja oil prior to alkaline transesterification since FFA content of the oil was 2.53%. H_2SO_4 was chosen for the acidic step [110].

Sahoo et al. (2007) developed a toluene aided transesterification after reducing polanga seed oil acidity from 22.0% to 2.0% using acidic esterification with sulfuric acid as the catalyst. The maximum yield of oil was found to be at KOH concentration of 1.5%wt [124]. Jatropha oil with 14% FFA was acid treated in the study performed by Tiwari et al. (2007) using H_2SO_4 as the catalyst for the FFA reduction step. The FFA content dropped to 1 % after the first stage which used alcohol to oil ratio of 6:1. The product was then fed to alkaline transesterification to produce biodiesel [125].

Wang et al. (2007) used acid esterification with sulfuric acid as the catalyst for waste cooking oil. Poor conversion of 82% was reported although high amount of alcohol with 200% excess of ethanol was used. The researchers then optimized the reaction by choosing a different catalyst and integrating it into the acidic transesterification. Ferric sulfate was used as the alternative catalyst which gave higher conversion of 97.02%. It was possible to recycle the catalyst since $Fe_2(SO_4)_3$ is insoluble with oil and could be separated easily using centrifuge [126,88].

Marchetti et al. (2008) synthesized an acidic oil model from refined sunflower oil and pure oleic acid. The final oil FFA% reached 10.684. It was fed into esterification reaction which used sulfuric acid at 2.261 wt% as the catalyzing agent and anhydrous ethanol for alcohol. The reaction took place at 55°C and final conversion was 96% [113].

2.7.1.3 Enzyme catalyzed

Another type of catalyst that had been developed recently to be used in transesterification reaction is lipase. Lipases are biological enzymes that are water soluble and may be developed from a number of sources like animal or plant enzymes. Beside their versatile usage in the medical field, these enzymes had proven to be of viable aids for various applications like food and detergent industries [127]. Moreover, these bio-catalysts are used in several reactions including hydrolysis of glycerol, alcoholysis and acidolysis.

It has been proven that extra and intracellular lipases can catalyze the transesterification reaction of vegetable/animal oils by immobilizing them with proper support. Enzymes that were found to fit into this criterion are obtained from micro-organisms such as Rhizopus orzyae, Candida antractica and Pseudomonas cepacia. The enzymatic transesterification reaction can proceed with the same short chained alcohols used in base/acid catalyzed reaction including methanol, ethanol, iso-propanol and iso-butanol with more preference over methanol [128].

The advantages of their usage may include, possible regeneration and reuse of immobilized residue, longer catalyst activation since high enzyme concentration can be allocated in the reactor, bigger thermal stability since they are native in state and protection from solvent used in the reaction as a result of immobilization of lipases. The final product separation is easier in comparison with other routes [49]. Lower operating temperature [128] and good tolerance for free fatty acid content in the feedstock [10] are among the attractions for its applicability.

Figure 2-8 shows a possible process scheme for enzymatic catalyzed transesterification.

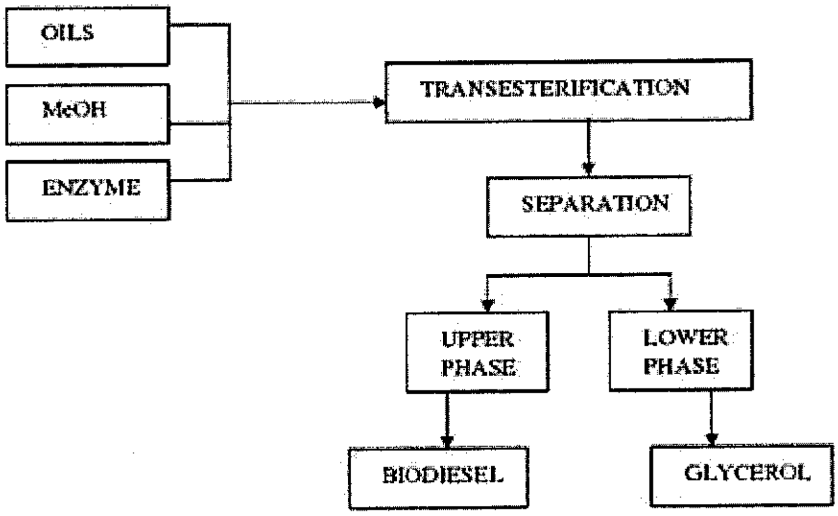


Figure 2-8 Enzymatic production of biodiesel [128]

Disadvantages of lipases can be concluded in several points. Among of which is that the catalyst initial activity is affected by amount of oil, dispersed number of support enzyme , high cost of the catalyst [49], the inability of enzymes to provide a satisfactory degree of reaction completion that complies with fuel standards and the need to develop a method to use multiple enzymes in sequence[10].

Most of the research done on transesterification using enzymatic catalyst involved finding the right reaction parameters and process variables to be implemented in industry. The use of lipase catalysts is less favorable when compared with base catalyzed transesterification since it had lower conversion and higher cost [129, 42].

Transesterification of vegetable oils and animal fats can be carried out using two types of enzymatic catalysts as it had been mentioned earlier. That includes Intracellular (Immobilized whole cell) and Extracellular (Immobilized enzymes) lipases. However, extracellular lipases need additional purification stages before immobilization which is not required in the case of intracellular. Figure 2-9 shows steps involved using both types of catalysts. The reaction using both catalysts had been investigated thoroughly by researchers. A review over these studies and developments is concluded below [128].

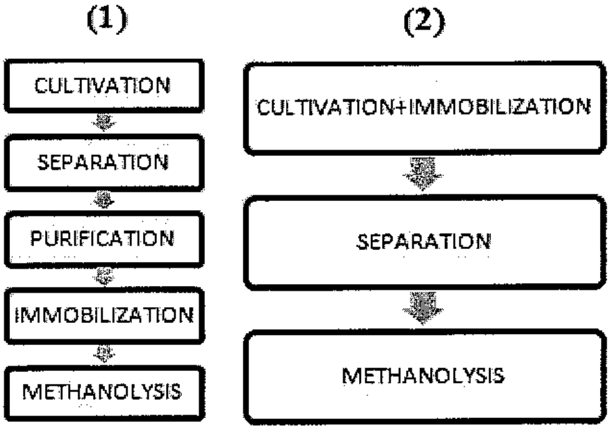


Figure 2-9 Steps involved in the immobilization of extracellular enzymes (1) and intracellular (2), [128]

A research by Mittelbach (1990) shown that it was possible to transesterificate sunflower oil using enzymatic catalyst. These catalyst included Pseudomonas fluorescens, Mucor miehei and aCandida sp. (extracellular).`Five alcohols were used in the study and the reaction seemed to prefer alcohols with longer chains.

The study reported as well that the use of solvent, such as petroleum ether, could be avoided. However, only ethanol and butanol could give high yields when no solvent was used while methanol gave only traces of methyl esters in the absence of solvent [130].

Nelson et al. (1996) from USDA investigated enzyme catalyzed alcoholysis of oils and fats with short chained alcohols. Lipases from Mucor miehei had the ability to catalyze the reaction with primary alcohols while that obtained from Candida Antarctica used secondary alcohols and gave branched esters [131].

Selmi et al. (1998) used ethanol in the transesterification of sunflower oil with Lipozyme (immobilized 1,3-specific Mucormiehei lipase). The optimum conditions reached was 1:3 ethanol to oil ratio with temperature at 50°C and 0.4g of catalyst per 5.7 mmol of the oil. The medium was solely composed of substrates. The recovery and recycle of enzyme could be done easily [132]. Linko et al. used a mixture of 2-ethyl-1-hexyl esters as a solvent for lipase transesterification of rapeseed oil and alkyl esters yield reached 97% [133].

Yuji Shimada (1999) et al. used three steps methanolysis since the catalyst (Immobilized Candida antarctica lipase) that was used would be inactivated by low methanol ratio. The yield reached more than 98%. The researchers reported that the catalyst could be reused for up to 50 cycle with conversion more than 95% [134]. Samukawa et al. and Abigor et al. (2000) shown that pretreatment of enzyme would reduce catalyst deactivation by methanol. Abigor et al. reported palm kernel oil conversion to alkyl esters using methanol and ethanol in 15% and 72% [128]. Kaieda et al. (2000) studied the use of different lipases in the stepwise alcoholysis and reported that Pseudomonas cepacia gave high conversion with lower methanol ratio in the reaction [135].

Noureddini et al. (2001) transesterified soybean oil using Pseudomonas flourescens. He reported conversions of 67% and 65% using methanol and ethanol respectively. Iso et al. (2001) used 1,4 dioxane as a solvent. He recommended that the use of methanol gave high conversion. The researcher then stated that the use of Pseudomonas flourescens gave 90% conversion at 60°C. Chen et al. (2002) developed a similar process with different acyl acceptor that improved the reaction [128].

Shimada et al. (2003) tried lipase for the production of biodiesel from waste oil. They discovered that an 0.5 higher molar ratio of methanol would reduce the activation of catalysts [49]. Xu et al. reported a novel enzymatic route for biodiesel production from soybean oil. Methyl acetates were used as the acyl acceptor while Novozym 435 gave the highest conversion of 92 %. Enzyme used was 30% based on oil. The reaction ran over 10 h at 40°C [136].

Chen et al. (2003) reported that ter-butanol and 2-butanol could regenerate the activity of inhibited catalyst up to 56% and 75% respectively. Fukuda et al. (2003) developed an enzymatic biodiesel process using intracellular catalyst. The enzyme cells were initially treated with short chain alcohols which increased the reaction rate up to 350-600 times [128]. Du et al. (2004) studied the transesterification of soybean oil using various acyl acceptors and concluded that a 12:1 methanol to oil ratio would give the best result since the activity of the catalyst was not reduced even after 100 batches [137]. Li et al. (2006) reported a yield of 95% of alkyl esters when tert-butanol was used as solvent for the alcoholyses of rapeseed oil. The catalysts used were Lipozyme TI LM and Novozyme 435 in 3:1 ratio [138].

Modi et al. (2006) researched the transesterification of jatropha, karanja and sunflower oils using bio catalysts. The conversion of were 92.8%,91.7% and 93.4% respectively. 2-propanol was used as an acyl acceptor with lipase as the catalyst [139]. Royon et al. (2007) reused tert-butanol as the solvent in the reaction of alcohol with cotton seed oil. Novozyme 435 was used as the catalyst at 55°C with 97% conversion within 24h. Modi et al. (2007) used ethanol to transesterify the oils used in his previous work with Novozyme 435 as the catalyst as well. Ethyl acetate was used as the solvent and reported a conversion of 90%. The solvent could keep the activity of catalyst up to 12 cycles with no major decrease in its operation level [128].

2.7.2 Transesterification using supercritical alcohol

A different approach for transesterification of vegetable and animal oils or fats is by using alcohols in supercritical state. A supercritical fluid may be defined as any substance above its critical thermodynamic temperature and pressure and share properties of gas (e.g. diffusivity) and liquid (e.g. viscosity) [140]. The supercritical conditions is based on the correlation between the temperature and pressure and their effect on the fluids thermo physical properties such as dielectric constant and viscosity. Glycerides from lipids can be dissolved in alcohol at critical conditions forming one phase mixture with no need for continuous stirring to keep a uniform mixture and higher conversion can be achieved this way [88]. Table 2-8 provides the critical conditions for different type of alcohols that can be used in transesterification to achieve supercritical state.

Table 2-8 Critical properties of some alcohols [42]

Alcohol	Critical Temperature (T_c)	Critical Pressure (P_c)
Methanol	512.2	8.1
Ethanol	516.2	6.4
1-Propanol	537.2	5.1
1-Butanol	560.2	4.9

Supercritical alcoholysis, unlike the other techniques, can be carried out without a catalyst and is not affected by the moisture or free fatty acid content[141,142]. A high yield of 95% is achievable within 10 min of reaction time but with large excess of alcohol. When methanol is used, a molar ratio of 40:1 is applicable to achieve that conversion within this short period of time and higher conversion can be achieved using ethanol. Temperature was also found to have positive effect over the conversion where increasing it shifted the yield from 78% to 96%. [88].

There are several advantages for supercritical transesterification. For high FFA content oils, there is no need for acidic treatment step or pre-esterification to reduce the acidity of the oils. Glycerides transesterification occurs alongside fatty acids esterification of the oils leading to higher conversion when compared to conventional methods. Moreover, in the catalyst free process, separation of the final products is not sophisticated as in alkaline alcoholysis without additional environmental impacts.

However, the downfall of this procedure is that it cannot be carried out without excess alcohol to enhance the forward reaction especially when there is no catalyst. Methanol to oil ratio ranges from 24 to 42:1. Additional cost is imposed by high temperatures required for the process reaching up to 513°K-623°K [143] and pressure to achieve supercritical state [83]. These factors have negative effect on process economy.

In conclusion, supercritical alcohol transesterification might be suitable from technical point of view but economic feasibility of the process is by far lower than the other technologies for biodiesel production since more capital is required due to high cost associated with energy and equipments required [144].

Kreutzer et al. (1984) mentioned in his work on the production of fatty alcohols that it is possible to transesterify oils and fats using high pressure and temperature reaching 90 bar and 240°C [145]. Saka et al. (1999-2004) performed extensive work on transesterification of oils and fats using supercritical alcohol. A non catalytic process was developed for alcoholysis of several oils including rapeseed, soybean and sunflower oils as it had been reported by the literature. High temperatures were used to increase the conversion which ranged from 573°K to 623°K. Excess methanol to oil ratio reached 42:1 to move the forward reaction. They reported that required reaction time was only 2-4 min. The transesterification of sunflower oil with ethanol had also been studied using the same temperatures [146-148]. A kinetic study was also performed [148] and the reaction was said to follow a first order kinetics.

$$\ln\frac{C_{MEt=t}}{C_{MEt=0}} = kt \tag{2.3}$$

First order kinetics proposed by Saka et al. [148]. *K: Kinetic constant, C_{ME}: Methyl Esters concentration*

Demirbas (2002) investigated supercritical transesterification of vegetable oils using catalytic and non catalytic approaches. He reported that the reaction rate and conversion to methyl esters increased with the increase of either pressure or temperature at supercritical point of alcohols. The yield increased from 50% to 95% during the first 8 min for the non catalytic process while that for catalytic process raised from 60% to 90% within the first 1 min. Alcohol to oil ratio ranged from 6:1 to 40:1 [42].

Rathore et al. (2007) studied the reaction over a series of edible and nonedible oils including palm and groundnut oils (edible) as well as pongamia pinnata and jatropha oils (non edible). Ethanol was used as alcohol which was subjected to supercritical condition where temperature reached from 200°C to 400°C while holding the pressure at 200 bar. The conversion was higher than 80% during the first 10 min of reaction time and reached completion after 40 min without the need to use a catalyst [140].

Yamazaki et al. (2007) used a bubble column reactor for the transesterification of sunflower oil to produce methyl ester. The non catalytic process used a temperature of 563°C as it was found to give the maximum yield of biodiesel while the pressure was kept at atmospheric level [149].

Mcneff et al. (2008) used soybean oil, acidulated soapstock, tall oil, algae oil and corn oil with different alcohols as the feedstock for their novel developed continuous fixed bed reactor. The reaction took place at elevated pressure of 2500 psi and temperature of 300-450°C. It only took 5.4 seconds to transesterify and esterify simultaneously the glycerides using heterogeneous metal-oxide based catalyst. These include porous zirconia, titania and alumina micro-particulate catalysts. The process scaled up for 115 hours of continuous operation without losing its conversion efficiency [150].

Refined palm oil was fed into a blowing bubble reactor developed by Joelianingsih et al. (2008) for supercritical transesterification. The non catalytic process used methanol for alcohol to produce methyl esters and the production of methyl esters reached a conversion of 95.17 % at the optimum temperature of 523°C [143].

Song et al. (2008) used batch reactor in non catalytic transesterification of refined, bleached and deodorized palm oil. Supercritical methanol at 473-673°K with molar ratio to oil of 3-80:1 was sufficient to enhance the forward reaction, The time required to give the final products was 0.5-20 min depending on the alcohol ratio. Thus increasing alcohol will decrease reaction time [83].

A comparison between several biodiesel processes that had been previously presented is given in Table 2-9. The adopted comparison was based on a review conducted by Marchetti et al. [49] to investigate the effect of process variables and related economy for different production technologies. In general, both supercritical and enzymatic transesterification give the best results in terms faster reaction time and conversion levels. However, these two processes are of high cost which is related to the conditions and materials involved respectively.

Table 2-9 Comparison between biodiesel production methods [49]

Parameter	Alkali Catalyzed	Acid Catalyzed	Enzyme Catalyzed	Supercritical Alcohol
Reaction temperature(^oK)	333-343	328-353	303-313	512-658
Products	Saponified Products	Esters	Methyl esters	Esters
Effect of water	Interference with the reaction	Interference with the reaction	No Influence	No Influence
Biodiesel Yield	Normal	Normal	Higher	Good
Glycerol Recovery	Difficult	Difficult	Easy	Easy
Purification of final products	Repeated Washing	Repeated Washing	None	None
Catalyst cost	Cheap	Cheap	Relatively Expensive	None/Medium*

*** depend on the type of process used (catalyzed or non catalyzed**

2.8 Blending Significance and Advantages

One of the problems encountered in biodiesel industry is the dependency on edible vegetable oils feedstock. Most of the studies that had been done were based on these types of vegetable oil. New feedstock alternatives and developments need to be explored for nonedible oil sources. Dependency on edible oil sources will leave no place for further expansions since its already on high demand by other food industries. Not to mention considerable high cost.

Most of biodiesel produced comes from edible sources like palm oil, soybean oil, sunflower oil. While biodiesel production from these sources in inevitable for their availability and large production levels, reducing their amounts will relieve them for other uses and encourage biodiesel production.

Blending edible/non-edible oils is a solution that will give big contribution toward the advancement of the industry by. This approach is getting more prospects. Jatropha-palm oil blend had been investigated [13]. These blends have shown good potential to be used in biodiesel production.

Although rubber seeds utilization did not evolve with time, a report published in the New York Times dating back to 1917 shows that the Agricultural authorities in the Federated Malay States were interested in investigating possible uses of these abundant seeds. Even then, experiments have proved that not only the oil extracted is suitable for various purposes, but that the residue can be used for cattle food or as fertilizer [152].

Biodiesel production from rubber (Hevea brasiliensis) seeds oil was investigated by Ramdhas et al. and was found that these crops inhibits the potential for alkyl ester production[31,151]. The reason to consider rubber seed oil is that it has no major application in the industry and none for the seeds as well.

However producing biodiesel at large volumes requires well established rubber seed oil industry. At the current time, there is no rubber seed oil extraction mill that could be found in Malaysia. Developing the industry will require some time. To reduce the amount of crude palm oil used in biodiesel production, it is suggested in this study to introduce rubber seed oil in the current crude palm oil/biodiesel mills.

Unrefined rubber seed oil contains around 17 % of FFA [31]. Production of alkyl esters from it requires two stages esterification/transesterification reactions since free fatty acids will react with base catalyst. This will produce soaps which will inhibit the reaction and make it difficult to separate products.

Similar with rubber seed oil, biodiesel production from crude palm oil requires two stage transesterification. This method is currently being used in factories constructed in Malaysia. It can then be understood that the production technology remains unchanged if rubber seed oil is introduced. Process conditions however need to be established.

2.9 Kinetic Studies

Studies had been conducted by researchers to understand the chemical kinetics of various transesterification reactions using different feed stocks. However, a common reaction model remains controversial. Research efforts were mostly focused on simplifying the reaction order by finding the best fit of empirical data. Only recently, few studies developed a kinetic model based on chemical mechanisms.

Moreover, these studies do not serve the industrial interest which in turn depends on the data obtained to enhance or increase the results of their production plant. Therefore it is important from a realistic point of view to conduct a kinetic study on selected reaction steps to benefit the industry from the results. A kinetic study over esterification and transesterification reactions need to include the investigation of rate equation, reaction rate constant and activation energies.

Biodiesel kinetic study began with Freedman and his colleagues at USDA in the early 1980's (Freedman 1984, 1986). Freedman mixed ethanol with soybean oil. At the beginning of his work he suggested a second order reaction model which he called a pseudo first order model. Due to data mismatch, he then suggested the reaction to be a "Shunt Reaction" of fourth order [95, 87].

Mittelbach and Trathnigg (1990) of Karl-Franzen University, Graz, investigated sunflower oil transesterification reaction using alkali catalyst. They studied the effect of various alcohol to oil ratios, temperature and catalyst concentration over the yield of methyl esters obtained. They found that the reaction tends to be of a second order at the beginning of transesterification. But then, the rate dropped rapidly as glycerol was produced which resulted in to phase reaction mixture. It was concluded that the byproduct leads to the loss of methanol and catalyst [153].

Noureddini and Zhu (1997) of the University of Nebraska also used soybean oil for their kinetic study. They utilized the same reaction model proposed by Freedman but their research was more focused on the effect of mixing over the reaction. They found that the change in mixing intensities have a similar effect with the variation of temperatures over the activation energy and reaction time [80].

$$\frac{d[TG]}{dt} = -k_1[TG][A] + k_2[DG][A] - k_7[TG][A]^3 + k_8[A][GL]^3$$

$$\frac{d[DG]}{dt} = k_1[TG][A] - k_2[DG][E] - k_3[DG][A] + k_4[MG][E]$$

$$\frac{d[MG]}{dt} = k_3[DG][A] - k_4[MG][E] - k_5[MG][A] + k_6[GL][E]$$

$$\begin{aligned}\frac{d[E]}{dt} = {} & k_1[TG][A] - k_2[DG][E] + k_3[DG][A] - k_4[MG][E] + k_5[MG][A] - k_6[GL][E] \\ & + k_7[TG][A]^3 - k_8[GL][E]^3\end{aligned}$$

$$\frac{d[GL]}{dt} = k_5[MG][A] - k_6[GL][E] + k_7[TG][A]^3 - k_8[GL][E]^3$$

$$\frac{d[A]}{dt} = -\frac{d[E]}{dt}$$

Figure 2-10 Transesterification reaction rate equations by Noureddini and Zhu [80]

Increasing mixing intensity led to decrease in the lag time and energy as well. Results obtained showed that the reaction was initially under a mass transfer controlled region followed by a kinetic controlled one. The model obtained was a good fit to a second order reaction mechanism and the product concentration distribution gave a sigmodial curve [80].

Table 2-10 Noureddini and Zhu calculated activation energies [80]

	N_{Re} = 6200		**N_{Re} = 12400**	
Reaction Step	**Arrhenius Constant**	**Modified Arrhenius Constant**	**Arrhenius Constant**	**Modified Arrhenius Constant**
TG-DG	13,145	11,707	13,600	12,130
DG-TG	9,932	8,482	9,783	8,313
DG-MG	19,860	18,439	18,769	16,767
MG-DG	14,639	13,433	11,177	9,710
MG-GL	6,421	7,937	5,182	8,036
GL-MG	9,588	10,992	9,873	11,365

Arrhenius equation, $k=Ae^{-Ea/RT}$. Modified Arrhenius, $k=ATe^{-Ea/RT}$. A is the constant,N_{Re}, Reynolds number

The researchers also provided transesterification mechanism in terms of successive reaction rate laws as shown in Figure 2-10. The equation shows as well the order for each reaction expressed by dependency of rate law and the concentrations power [80].

Table 2-10 and Table 2-11 show the kinetic study results. Arrhenius/Modified Arrhenius constants were calculated and are shown in Table 2-10. Rate constants are shown in Table 2-11.

Table 2-11 Noureddini and Zhu calculated reaction rate constants [80]

Rate constant	***Without shunt reaction***	***With shunt reaction***
k_1	0.050	0.049
k_2	0.110	0.102
k_3	0.215	0.218
k_4	1.228	1.280
k_5	0.242	0.239
k_6	0.007	0.007
k_7	-	7.84×10^{-5}
k_8	-	1.58×10^{-5}

At 50°C and $N_{Re} = 6200$, k1-k8 are reaction rate constants proposed in equation 2.4

Boocock (1998) of the University of Toronto disagreed with the second order kinetics proposed by the other researchers. Base catalyzed transesterification using methanol as alcohol was studied. The reaction was found to be slower than that of butanolysis and was related to the immiscibility of reactants. The reaction didn't give a second order data fit. The reaction rate drops over time as a result of the decrease in polarity of reactants. This is caused by mixing of non polar oils with alcohol. It was concluded that the reduced polarity reduces catalyst effectiveness [154].

Bikou (1999) and others at the National Technical University of Athens investigated effect of water on the kinetics of transesterification using cotton seed oil and ethanol. It was proposed that each of the three reaction steps follows third order kinetics. The rest of their work discussed the effect of water on the equilibrium constants. They found that presence of water will decrease the yield of alkyl esters since it will increase the backward reactions. However, increasing ethanol to oil ratio will give higher conversions [23].

Darnoko and Cheryan (2002) at the University of Illinois studied the kinetics of palm oil transesterification [24]. The data obtained from their work fitted a pseudo second order for the initial stages of the reaction followed by first or zero order kinetics. Potassium hydroxide was used for catalyst in the reaction that was conducted in a batch reactor with methanol as alcohol.

The reaction rate constants and activation energies were calculated for the forward reactions. Their results are presented in Table 2-12 and Table 2-13. Equation 2.4 shows the rate law proposed by the researchers. It can be seen that alcohol concentration was neglected since its present in large excess giving a pseudo order.

$$-\frac{dTG}{dt} = k[TG]^2, k_{TG}.t = \frac{1}{[TG_t]} - \frac{1}{[TG_0]} \qquad (2.4)$$

Table 2-12 Darnoko and Cheryan calculated reaction rate constants [24]

Reaction Step	**Temperature (^{0}C)**	**Rate constant (L.mol^{-1}.s^{-1})**
TG-DG	50	0.018
	55	0.024
	60	0.036
	65	0.048
DG-MG	50	0.036
	55	0.051
	60	0.070
	65	0.098
MG-GL	50	0.112
	55	0.158
	60	0.141
	65	0.191

Table 2-13 Darnoko and Cheryan calculated activation energies [24]

Reaction Step	Activation Energy (kcal/mol)
TG-DG	14.7
DG-MG	14.2
MG-GL	6.40

Komers (2002) and his colleagues at the University of Pardubice ,Czech Republic, researched proposed mechanisms for all progressive reactions occurring during esterification of rapeseed oil. Their kinetic model consisted of a two consecutive steps including the formation of methyl esters in the initial one while the second step describes the saponification of glycerides. They derived a system of six rate equations with eights reaction species and ten rate constants. The model explicitly treats the amount of water and catalyst present. The model also supports Bikou's research [155].

Foon et al. (2004) from Malaysian palm oil board performed a study on the production of biodiesel from locally abundant palm oil. Their kinetic work investigated the effect of several reaction parameters such as alcohol to oil ratio, catalyst concentration and temperature. Both sodium hydroxide and sodium methoxide gave high reaction rates. Fast formation of methyl esters with conversions above 99% was reported. The optimum conditions for the reaction were found to be 60°C with 10:1 methanol to the oil and catalyst concentration of 0.125 mol/kg oil [156]. Second order rate constants are shown in Table 2-14 for different catalyst concentrations.

Table 2-14 Foon et al. calculated reaction rate constants [156]

Catalyst Concentration (mol/kg oil)	*Rate constant (lit.mol^{-1}.min)*
0.1250	0.163
0.1875	0.313
0.2500	0.526

At a temperature of 60°C, 10:1 alcohol to oil ratio and 350rpm stirring speed

Theerayut Leevijit (2004) of King Mongkut's University of Technology Thonburi performed a kinetic study on biodiesel from palm oil. He found that a second-order mechanism is more convenient and more suitable than Darnoko and Cheryan pseudo second-order mechanism for predicting conversion at any reaction time. He stated that for second-order mechanism to be used all the initial concentrations of reactants are needed. In contrast, using a pseudo second order mechanism, initial concentrations of triglycerides, diglycerides and monoglycerides are only required. Finally, he stated that a pseudo second-order mechanism cannot predict the conversion over time unlike a second order mechanism [157].

Wenzel et al. (2006) included the reverse mechanism of soybean oil alcoholysis reaction in their kinetics study. Their proposed model had shown a good tendency to represent transesterification reaction after confirming its validity with the data obtained from various literatures. The model is based on three consecutive, forward and reverse, reactions with first order reaction rate order at both directions. They reported that their kinetic study gave successful results after comparing the simulation and experimental results performed [158]. Their first order constants are given in Table 2-15.

Table 2-15 Wenzel et al. calculated reaction rate constants [158]

Reaction rate constant	**Value (s^{-1})**
K_1 *(TG forward)*	3.15×10^{10}
K_4 *(TG backward)*	1.22×10^{-21}
K_2 *(DG forward)*	2.35×10^{11}
K_5 *(DG backward)*	2.47×10^{07}
K_3 *(MG forward)*	1.85×10^{-01}
K_6 *(MG backward)*	8.89×10^{-59}

Stamenkovic et al. (2006) from the University of Nis studied the transesterification reaction of sunflower oil at lower temperatures to confirm existence of mass transfer limitations in initial stage of the process. It was reported that the reaction was initially controlled by a mass transfer kinetics followed by chemical reaction controlled region [159]. This explained sigmoidal shape of kinetics curve obtained in their study. They found that the fast irreversible second-order reaction was followed by a slow reversible second-order reaction which led to a close completion of the methanolysis reaction. Equation 2.5 and 2.6 represent the two rate laws obtained by Stamenkovic to represent the reaction. Both of which are taken as pseudo orders assuming no effect from excess alcohol. The first law represent the initial reaction period which is controlled by mass transfer. The reaction then follows a second order kinetics as shown in Equation 2.6.

Phase 1 (Mass transfer controlled region)

$$-\frac{dC_{TG}}{dt} = k_C a C_{TG} \tag{2.5}$$

Phase 2 (Chemical reaction controlled region)

$$-\frac{dC_{TG}}{dt} = k_2 C_{TG}^2 \tag{2.6}$$

Berrios et al (2007) from the University of Cordoba performed experimental study on the production of alkyl esters from sunflower oil. Methanol was used to reduce the viscosity of oil at ratios ranging from 10 to 80:1. Sulfuric acid concentrations were 5 and 10 wt% and it was found that the energy of activation for the forward reaction was reduced by increasing the catalyst concentration [160].

The data obtained fitted a first order kinetic law for the forward reaction and a second order one for the reverse reaction. The study also reported that the best conditions were alcohol to oil mole ratio of 60:1 with 5% catalyst concentration and temperature of 60°C. These conditions gave methyl esters with acid value less than 1 mg KOH/g Oil within 120min [160]. Berrios rate law is shown in Equation 2.7 and calculated rate constants are shown in Table 2-16 with respect to catalyst amount.

$$-\frac{dC_{ffa}}{dt} = k_1 C_{ffa} - k_2 C_{ME} C_{H2O}$$

(2.7)

Table 2-16 Berrios et al. calculated reaction rate constants [160]

Catalyst Conc. (%wt of oil)	***K1* (s^{-1})**	***K2* (lit.mol^{-1}.s^{-1})**
5 %	2.869x10^{6}	37.068
10 %	3.913x10^{5}	707.166

2.10 Conclusion

Biodiesel has good prospect in replacing petroleum diesel. Many obstacles however lie on the way of implementations and increasing production levels. These are mostly caused by the fluctuating prices for petroleum oil and vegetable oil market prices. Developing the industry requires reduction in the capital involved. When breaking down the cost, it can be seen that most of which comes from the raw material.

Transesterification was found to be the best route for biodiesel production from the previous studies. Currently, methanol is the optimum alcohol to be used in methyl esters preparation since it has a comparatively lower price than the other alcohols which is used in the process. Homogenous base and acid catalyst still provide the highest yield when compared with the currently available heterogeneous ones.

Reaction kinetics have been investigated by many researchers. Despite the fact, a reaction rate model is still controversial and kinetics constants still differ and need to be established for further developments in process design. Reaction parameters including temperature, reactants ratio and amount of catalyst were found to be the most significant and have considerable effect over products conversion. A thorough study to find the optimum combination for these parameters is required to be used by the relevant industry.

3 Methodology

3.1 Oil extraction, blending and characterization

3.1.1. Rubber Seed Oil

3.1.1.1. Rubber seeds processing

Rubber seeds were purchased locally from Malaysian Rubber Board, formally known as RRIM. The seeds are of ellipsoidal shapes with varying brown shells covering kernels. As in most biological resources, the seeds were of different measurements in lengths and weights. Those characteristics were measured and calculated using analytical balance and length measurement tools. Seeds that had been collected are shown in a. **Accumulated rubber seeds** **b. Schematic diagram for a rubber seed**

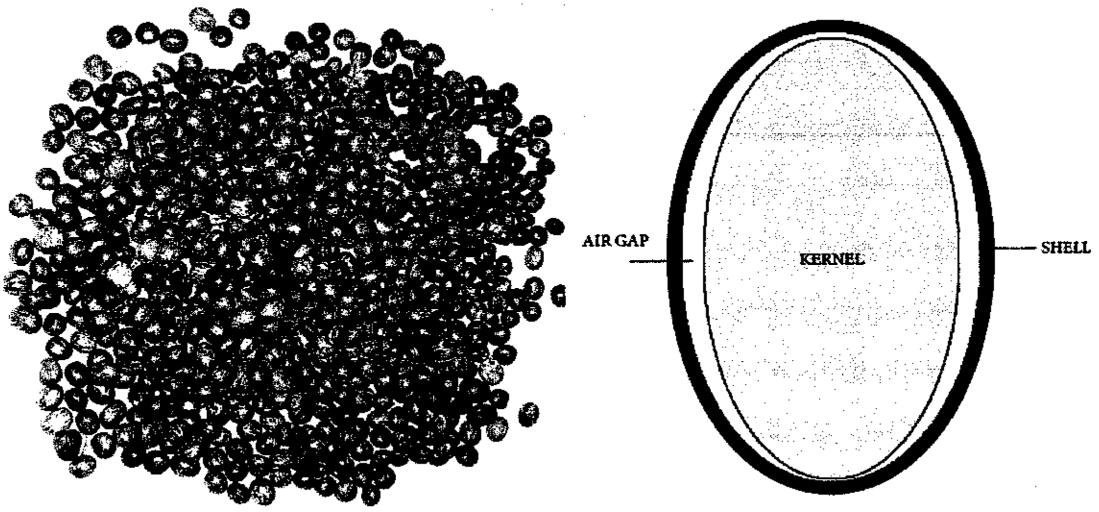


Figure 3-1.

a. Accumulated rubber seeds b. Schematic diagram for a rubber seed

Figure 3-1 Rubber seeds

Length and weights of the seeds were calculated as well as the weight for the kernels. These measurements are included in Table 3-1. Five different measurements were taken for each of seed length, total weight and kernel weight. The mean value was calculated for each and standard deviation was reported. The mean length of seeds was 2.6 mm with total weight of 4.5 g and that for kernels was 2 g which is equivalent to almost 46% of the total weight.

Table 3-1 Length and weight measurements of rubber seed

							Mean	***Standard deviation***
Length, mm	2.40	2.60	2.50	3.10	2.60	2.50	2.61	±0.206
Weight, g	5.31	3.06	3.78	5.08	5.18	4.63	4.50	±0.823
Weight (kernels),g	2.48	1.39	1.96	2.04	2.51	2.16	2.09	±0.376

Seeds were separated and shells were broken to obtain kernels which were collected and crushed into small sizes using a blender (Waring Commercial, US). The final size of kernel cake was determined using dry test sieves and a shaker (Retsch, Germany) to meet an upper fraction particle diameter of 2mm. The remaining kernels were further blended until the entire crushed kernel met the size criteria. Processed seed kernels were stored in air tight containers as shown in Figure 3-2 to prevent any contamination while samples were taking to determine the moisture content of the seeds as described in the moisture analysis procedure in following sections.

Figure 3-2 Dried and processed rubber seed kernels stored in an air tight container

Rubber seeds are seasonal products. It falls twice a year as it was observed from during visits to rubber plantations located in Sungai Buloh, Selangor and detailed discussions with workers and surveyors. Currently there is no utilization for the seeds except for small amounts for growing and embryonic of new crops. The seeds are collected manually by workers in the field. No available statistics were found referring to the amount of seeds produced from each hectare of plantation locally. However, Ramdhas reported that each hectare give 150 kg of seeds [2].

Rubber seed kernels that were not stored properly in air tight conditions had fungi forming inside and on its shells. This was noticed after 4-5 weeks from being freshly picked over rubber plantations. The oils extracted from those seeds had different physical properties of color and smell than that obtained from seeds with good storage conditions. Storage conditions need to be well maintained to avoid any losses in feedstock. Another solution for the problem is to process the seeds immediately to obtain kernels then dry and store them in air tight containers.

Moisture content of seed kernel was calculated using Moisture Analyzer, Mettler Toledo HR73. The analysis was based on the American Oil Chemist Society official method [57] for the determination of moisture and volatile matter in agricultural seeds.

The instrument operates on thermo-gravimetric principle. At the start of the measurement, the moisture analyzer determines the weight of sample. Samples are then quickly heated by an integral halogen dryer unit and moisture vaporizes. During drying operation, the instrument continuously determines the weight of sample and displays the loss of moisture. On completion of drying, the moisture content or dry substance content of samples is displayed as the final result.

Sample size of 5 g was used while holding the temperature at 130°C for two hours as it was proposed by the AOCS method [57]. The procedure was repeated three times for results confirmation. The quality of the instrument measurement is certified by ISO9001 and complies with the European Standard EN45501 [162]. The manufacturer provides the accuracy and repeatability for the tests in Table 3-2.

Table 3-2 Repeatability of the results obtained from moisture analysis

Standard deviation	**Minimum sample weight (g)**
±0.02%	10
±0.05%	4

±0.1%	2
±0.2%	1

3.1.1.2. Rubber seed oil extraction

One of the important aspects considered in the current study is the investigation of rubber seed oil extraction conditions. A parametric study was performed to conclude effects of different extraction variables.

Extraction time, amount of solvent and moisture effect were the key factors investigated. The chosen values for each variable are shown in Table 3-3. The selection of the parameters was based on literature review conducted alongside understanding of general variables involved in solvent extraction presented by related books [57].

Table 3-3 Oil extraction parameters

<table>
<tr><th>Parameter</th><th colspan="5">Value</th></tr>
<tr><td><i>Extraction time (hr)</i></td><td>0.5</td><td>1</td><td>2</td><td>3</td><td>4</td></tr>
<tr><td><i>Solvent/Seed (ml/g)</i></td><td>12</td><td>22</td><td>32</td><td>42</td><td>52</td></tr>
<tr><td><i>Drying time (hr)</i></td><td colspan="2">2</td><td colspan="2">3</td><td>4</td></tr>
</table>

Rubber seeds were dried at 100°C±5 at different time intervals starting with 1, 2, 3 and 4 hours. Samples from each drying period were analyzed for moisture and volatile components using the same established method described for non-dried rubber seed kernels in previous section. Rubber seed oil extraction was carried out using the setup shown in Figure 3-3. Analytical grade n-hexane (>98%, Systerm) was used and heat was supplied by a heating mantle (Ross ltd.). A measured quantity of processed rubber seeds (5 g) was packed in a thimble. The thimble was then placed in a soxhlet extractor installed on two necked flask while a condenser is placed on the top of it to prevent solvent losses and to condense n-Hexane on the top of the thimble. The round bottom container was then heated using temperature controlled heater. The solvent was added to the bottom of the two neck flask while temperature was held constant above the boiling point of solvent (approx. 70°C ±5) throughout the experiments.

The thermometer placed on the second neck helped in monitoring extraction temperature. Solvent vapors perforated the thimble and absorbed the oil from seeds. The extracted oil was then transferred into an evaporation flask to separate solvent/oil system by using a rotary evaporator (Heidolph Laborota4000, Germany) at elevated temperature of 70°C. The oil obtained was then weighed and percentage of oil yield was calculated using the Equation 3.1.

$$\text{Oil yield (wt\%)} = \frac{\text{mass, oil extracted}}{\text{mass, processed rubber seed kernel}} \times 100$$

(3.1)

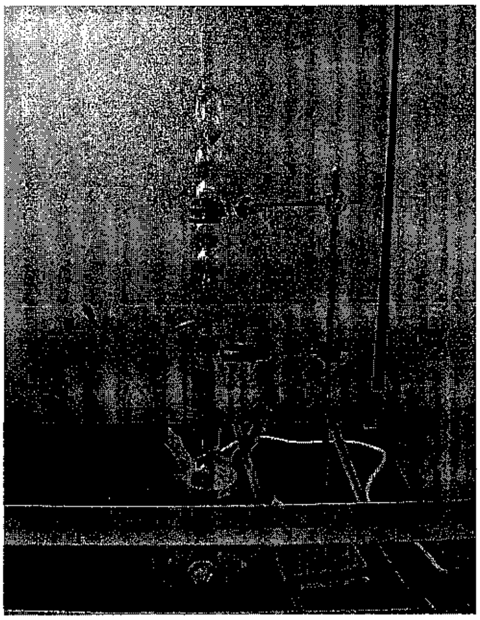


Figure 3-3 Oil extraction setup (Block 5, Level 1, Room 7)

3.1.2 Crude Palm Oil

Crude Palm Oil (CPO) was obtained from a palm oil processing mill located nearby Seri Iskandar, Perak, Malaysia. The factory is certified by Malaysian Palm Oil Board, thus, crude palm oil produced by the mill is similar in chemical and physical properties to that used by most industries including the one dealing with biodiesel.

Crude palm oil was to be heated and well mixed before any utilization. This was done to dissolve the solid particles in oil mixture and for the samples to be of a uniform composition.

3.1.3 RSO/CPO Blends

Rubber seed oil obtained from extraction stage was used in preparing crude palm oil and rubber seed oil blends. Predetermined proportions from the two oils were used in order to obtain blends that will be characterized and utilized at following stages for biodiesel production.

The blends were prepared at volumetric ratios of 3:10, 5:10 and 7:10 of rubber seed oil to crude palm. This is done to compare the effect of increasing rubber seed oil content on the blend properties. Each of the blends was labeled according to Table 3-4 below.

Table 3-4 Crude palm oil and rubber seed oil blends

RSO:CPO (Volumetric ratio)	**Blend Label**
3:10	BL1
5:10	BL2
7:10	BL3

3.1.4 Characterizations

All the oils were investigated thoroughly by testing several physical and chemical properties for each. Standard testing procedures were used throughout the characterization process in order to obtain the most accurate results. Analyses were performed over extracted rubber seed oil, crude palm oil, BL1, BL2 and BL3. The tests covered the following parameters as shown in Table 3-5.

Table 3-5 Tests conducted for oils characterization

Tests conducted for oils and blends characterization	
- Observation	- Refractive index
- Acid value	- Sulfur and nitrogen
- Calorific value	-Mono, di and trilgycerides
- Density	- Free fatty acids
- Viscosity	- Iodine value

3.1.4.1. Observation

One of the important aspects in characterization is the observation of oil's visible properties such as color, opacity, effect of heating and appearances of solids. These factors will help define the quality for oil in terms of visual observation.

3.1.4.2. Acid value

Acid value may be defined as the number of milligrams of potassium hydroxide required to neutralize free acids in 1 gram of sample. The acid number can be used with suitable factors to calculate the content of free fatty acids present in oils or fats.

The solvent mixture was initially prepared in two 250 ml Erlenmeyer flasks by mixing equal parts by volume of isopropyl alcohol and toluene and adding 125 ml of mixture in each container. Mass of oil sample required for the test was determined based on Table 3-6. The specified amount was then weighed, well mixed and added to one of the flasks where it was completely dissolved in solvent mixture. From previous literature review [31,60,126], it was noted that both of crude palm oil and rubber seed oil exhibit an acid value within the range of 15-75. For that reason the amount of oil sample dissolved in solvent mixture was 0.5g.

Table 3-6 Required amount of oil based on the estimation of acid value

Acid Value	**Mass of sample (g)**	**Weighing accuracy (±g)**
0-1	20	0.05
1-4	10	0.02
4-15	2.5	0.01
15-75	0.5	0.001
75 and over	0.1	0.0002

The indicator solution was prepared using 1% of phenolphthalein in Isopropyl alcohol. An approximate amount of one ml of solution was added to each of the sample and blank flaks. The titration of both solutions was carried out with potassium hydroxide solution 0.1N (*N=Normality)* by using burette while maintaining vigorous shaking during neutralization until the first permanent pink color was obtained and persisted for 30 seconds. The volume of KOH solution in burette was measured before and after each run. The test was repeated two times for each oil to get precise results.

The calculation of acid value was based on Equation 3.2

$$\text{AcidValue}\left(\frac{\text{mg KOH}}{\text{g Sample}}\right)=\frac{(V_A-V_B)\times N\times 56.1}{W}$$

(3.2)

To express results in terms of free fatty acids, the acid values were divided by a suitable factor for each oil based on the major fatty acid component present in them, whether oleic or palmitic. These factors for major fatty acid groups were obtained from literature [57] and that for blends were derived based on oil ratios. Table 3-7 shows these values which were obtained and that calculated for each blend. The precision of acid value result is obtained when two tests do not differ by more than 0.22 for acid values less than 4 nor by more than 0.36 for acid values in the range of 4-20 [57].

Table 3-7 FFA-Acid conversion value factors for the oils

Oil	Factor
CPO	2.56
RSO	2.81
BL1	2.63
BL2	2.68
BL3	2.73

Methanol was used as the base solution for potassium hydroxide in titration. Normally water would be used. However, in the case of high colored sample methanol is preferred over water since it gives a sharp end point [57]. Crude palm oil sample that was diluted in the solvent mixture was highly colored. Titration of crude palm oil resulted in a sharp reddish brown color. Rubber seed oil on the other hand was not highly colored. The same titration solution was used to obtain uniform results.

3.1.4.3. Calorific value

To calculate calorific value, oil samples were weighed to obtain a mass of 0.3-0.4g then filled in small containers. A short cotton thread was placed in each container in a position that allows contact between the thread and oil. Bomb calorimeter (C5000, IKA Werke, Germany) was turned on alongside water cooler attached. The instrument was stabilized before operation. Samples were then located inside in the Bomb calorimeter and the settings were adjusted to match sample weight before starting the experiments which lasted for 15 minutes.

The device screen displayed sample combustion graphs where the calorific value starts to increase until a constant value is shown at the end. Two test runs had been conducted for each sample to verify results and obtain average calorific value for oils.. Oxygen cylinder is connected to the bomb using a leak free plastic tube and connectors. Water bath is attached to the right wing of the instrument to maintain the temperature of device.

3.1.4.4. Density

A Pycnometer (JAYTEC UK, Confirm British Standard 733) of known volume (25ml) was used to calculate oils and blends densities. The glassware was cleaned and dried before each test to avoid inaccuracy of results obtained. In each experiment, pycnometer is filled with desired oil until it reaches top level of capillary tube inside the glass stopper. The weight of pycnometer is measured before and after filling the oil and the density is calculated according to the known volume of the glassware.

3.1.4.5. Viscosity

An efficient viscosity measurement device (Brookfield CAP2000+) was used to find the viscosity of oils and blends. It is certified to be used and recommended by several international standards including ASTM D4287, ISO 2884 and BS 3900. All of the tests were conducted at standard testing temperature of 40°C [3].

The method to analyze the samples had to be established since the manufacturer provides a specific range of viscosity for each cone to be used. The device was turned on. Test parameters had been set according to the pre-test assessment and calibration runs to obtain the most accurate results. The best conditions that were found to give the most accurate results for oils and blends were as follow. The temperature is set at 40°C with cone speed of 1200 rpm and hold time of 20 second while Run time was 50 second. Cone number two was used and allowed to equilibrate with the surface temperature of device. The sample was then added followed by held for another period of temperature normalizing for various equipment parts.

The sample had to meet a size of 1 mm around the cone. The test was started thereafter by rotation of the test cone at set speed. The viscosity change is displayed throughout the test until final result appears. Confirmation of result is based on the success percentage given by the device which needs to meet or exceed 10%.

In refinery engineering, a blend viscosity can be calculated from an established set of equations based on the initial viscosity of fluids being blended and their ratios [170]. Refutas equation was adapted in this work to provide such means in calculating the theoretical viscosity of vegetable oil blends which can be used for estimation purposes. The empirical results are compared with experimental one to check the accuracy of equation.

The calculation is carried out in several steps. Initially, viscosity blending index (or viscosity blending number) is established for both oils using the following equation:

$$\mathrm{VBI} = 14.534 \times \ln[\ln(v + 0.8)] + 10.975 \tag{3.3}$$

From above calculated values, we obtain the viscosity blending number for eacb blend using the following equation:

$$\mathrm{VBI}_{\mathrm{Blend}} = [x_A \times \mathrm{VBI}_A] + [x_B \times \mathrm{VBI}_B] + \ldots + [x_N \times \mathrm{VBI}_N] \tag{3.4}$$

Finally the viscosity of blend is determined using the initial equation using the later found viscosity blending index for blend, the equation becomes:

$$V_{\mathrm{Blend}} = e^{e^{\frac{\mathrm{VBI}_{\mathrm{Blend}} - 10.975}{14.584}}} - 0.8 \tag{3.5}$$

3.1.4.6. Refractive index

Refractive index is an optical measure of which the value obtained may provide visual identification for the tested substance. It may also be related to other values such as density. A refractometer (RX-5000,ATAGO) equipped with a temperature controller was used to carry out the experiments. One drop of each sample was spotted on the led of the temperature controlled refractometer. The test was initiated and values were obtained for each oil with repetitions to provide accuracy.

3.1.4.7. Sulfur and Nitrogen content

A CHNS Analyzer (LOCO Corp., USA) was used to determine sulfur and nitrogen contents in vegetable oils and their blends. The samples are initially prepared before introducing them into the testing vessel. Cotton threads are inserted in aluminum containers to absorb the sample need to be tested.

The cotton thread and aluminum cups total weight is measured before and after adding the sample to meet the test weight limit of 1-2 mg of sample. The aluminum cups are then tightly closed but with caution of not to expel the sample and inserted to the analyzer.

The test is started by ensuring the flow of helium, oxygen and compressed air from the cylindrical tubes. The pressure regulators are set to 40 psi. The ambient monitor is then calibrated for proper values. Carbon dioxide, hydrogen and sulfur infra-red cells need to be monitored for values to confirm a range of 7.5-9.2 volts.

The oxidation furnace temperature is then set to 1000°C while that for the reduction furnace is set to 650°C. The operating switch is then turned to manual to load samples individually and the gas switch is set to analyze position. Once the temperature is stable, the device injects the samples and weight percentage of each element is detected and displayed on the digital screen.

3.1.4.8. Mono, di and triglycerides

The procedure for glycerides determination in vegetable oils and blends involves the application of thin layer chromatography to find the composition of mono, di and triglycerides. The following procedure for chromatography analysis can also determine the composition of methyl esters and free fatty acid in the sample. For that reason it will used to test free fatty acids amount in acid esterification products and methyl esters conversion in base transesterification at later sections. TLC was reported to provide an accuracy of 0.1wt% [172].

TLC plates are cut and prepared to be of uniform measurements. The length of each plate was 12 centimeters with a width of 2.5 centimeters. The plates were marked by a horizontal line distanced by 1 cm from the bottom and was marked at the middle for sample spotting. The development solution was prepared using isopropyl alcohol, diethyl ether and acetic acid at the following ratio of 85:13.5:1.5. 10 ml of this solution was added in the TLC chamber which was closed tightly and left to be saturated before each test for a period of 30 minutes. The samples were prepared before testing by dissolving them in 2-propyl alcohol in 25/75 volumetric ratio of sample/alcohol. Capillary tubes with spotting diameter of 1mm were used to withdraw prepared samples and transfer them to designated spots on TLC plates.

The samples are left to be absorbed well on TLC plates before transferring plates to chamber. Twenty five minutes were sufficient for plate's development using the above mentioned solution. Plates are then transferred out to be dried. The iodine chamber is prepared by adding iodine granules in air tight desiccators. The chamber helps to render visible spots on TLC plates. Each spot represents a specific component that is present in the product. The yellow spots that can be seen on the plates represent different types of substances. The color resulted from the reaction of iodine with these substances.

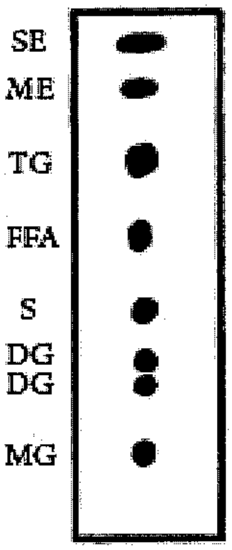


Figure 3-4 Typical lipid components by thin layer chromatography [173]

The distribution of components is compared with that provided by reference [173]. Cazes [173] provided that in any lipid analysis using thin layer chromatography, the component would be distributed on the plate as it can be seen in Figure 3-4. The only difference is that some oils and fats contain sterols and others do not. Thus S and SE which represent sterols and their methyl esters are not expected to be obtained in the current analysis since they are not contained in palm and rubber seed oils.

The component distribution in Figure 3-4 was compared with that of TLC obtained to identify each substance. Figure 3-5 shows a TLC plate which was obtained in this work with materials identified on it.

Finally, the plates are scanned, stored in image format and transferred to the computer. Image analysis software (ImageJ, National Institute of Health, USA) is used from this point to calculate the area of each component spot and the total area of the spots. By comparing the area of each spot to the total, the composition is obtained for each component.

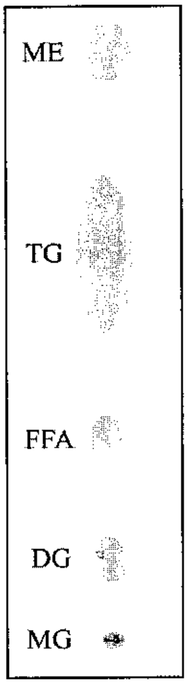


Figure 3-5 TLC plate obtained in the current work

3.1.4.9 Free fatty acids

In order to find the composition of fatty acids in vegetable oils, it is required to provide them in form of fatty acids methyl esters for the gas chromatography to be able to detect them. The composition of each component is then calculated using mass spectrometer installed for the gas chromatography unit (QP5000 series, Shimadzu, Japan).

A specific amount of oil, 3 grams, is measured in a round bottom glass which is placed in water bath to maintain uniform temperature and heated using a hotplate. Catalyst of 0.03g (0.016 ml) H_2SO_4 is dissolved in 5 ml methanol and added to reaction vessel once the desired temperature is reached. The reaction is stopped after 2 hours and products are washed with warm water at 50°C in separation funnel. The product, in the top layer, is then separated to obtain fatty acid methyl esters which is diluted in n-Heptane in a ratio of 1/10 of FAME to solvent and injected to the GC-MS.

Helium gas flow through the column was set at 40 ml per minute and the temperature was ramped to 270°C. For each injected sample, the test runs up to 25 min and the peaks for methyl esters are shown on the screen. The peaks were identified using retention time's library where they were compared to that of standard methyl esters. The mass spectrometer, alongside area integrator for the peaks were used to calculate the compositions.

3.1.4.10 Iodine value

The procedure for iodine value determination is based on AOCS official method Cd 1c-85 [57]. The method uses the compositions of free fatty acids obtained from gas chromatography-mass spectrometry analysis in section 3.1.4.10 and integrates them in the correlation provided in Equation 3.6 to obtain iodine value.

$$\begin{aligned} Iodinevalue = (\%hexadecenoic - acid \times 0.09976) + (\%octadecenoic - acid \times 0.8986) + \\ (\%octadecadienoic - acid \times 1.810) + (\%octadecatrienoic - acid \times 2.735) + \\ (\%ei\cos enoic - acid \times 0.0175) + (\%do\cos enoic - acid \times 0.7497) \end{aligned} \tag{3.6}$$

3.2 Design of Experiments

3.2.1 Reaction Parameters

In order to find optimum conditions for biodiesel production, a table of parameters was prepared for both of acid esterification and base transesterification and provided in Table 3-8 and Table 3-9. This is to ensure that the investigation scope will be focused on several factors of great importance and interest for both research and industrial applications.

Table 3-8 Acid esterification reaction variables

Parameter	**Value**		
Methanol:Oil Blend ratio (mole)	10:1	15:1	20:1
Temperature (°C)	45	55	65
Catalyst (H_2SO_4) amount (wt%)	0.5	1	2
Mixing speed (rpm)	350		

Table 3-9 Base transesterification reaction variables

Parameter	**Value**		
Methanol:Oil Blend ratio (mole)	6:1	8:1	10:1
Temperature (°C)	45	55	65
Catalyst (KOH) amount (wt%)	0.5	1	2
Mixing speed (rpm)	350		

The factors were chosen after reviewing related research and work in this field and selecting the most significant factors effecting conversion, process economics and dynamics.

Temperature, alcohol and catalyst used play a significant role in shaping and shifting the alkyl esters yielding reaction. They are important variables in designing the process, optimizing it and tuning control equipments and instrumentations.

For instance, methanol was selected as the alcohol of choice since it was the most economic reactant among the rest of possible oil emulsifying alcohols such as ethanol, propanol and butanol as it was seen in previous work [2, 31, 41].

Alcohol to oil ratios was based on the recommendations and actual biodiesel conversions obtained by previous studies [2, 95, 94, 97, 101]. Based on transesterification stoicheometry, three moles of alcohols are required to react with each mole of triglycerides to obtain alkyl esters. Despite the fact, excess amount of alcohol were found to be required by previous researchers to shift the reaction equilibrium toward the products. Six to one alcohol to oil ratio was recommended by several studies but increasing the amount of alcohol lead to saponification reaction which decreased the desired product.

Temperatures were selected based on the criteria of chosen alcohol. Methanol has a boiling point of 64.7°C. Exceeding this temperature will result in massive losses of alcohol and extra cost added to refluxing, condensation and substitution for evaporated alcohol. Boiling temperature were chosen alongside lower ones to investigate if they give feasible conversions when they interact with other variables.

The amount of catalyst required is related to vegetable oil chemical properties. More specifically, acid value and free fatty acid content of the vegetable oils. Knowing the previous properties will help us to decide whether excess amounts of catalyst might be needed to neutralize these free acids. The amount of catalyst also controls the breakdown of higher glycerides to lower ones by shifting reaction side. Lower catalyst amount is important to be studies as well since it may result in expenditure saving and might be more feasible to choose if the conversion is acceptable..

As it has been stated previously, investigation of reaction parameters is valuable for both research and industrial uses. It is in the sole objective of this study to present, investigate and analyze the effect of temperature, alcohol/oil ratio, catalyst amount and reaction time on reduction of free fatty acids in acid esterification and conversion of methyl esters in base transesterification, and on the top of which, to find the most suitable combination to give the optimum results in both reaction.

Transesterification of selected blend, number 2, was carried out in two stages. First stage involves acid esterification where acid content of raw material is being reduced by using acid catalyst, sulfuric acid. The product of the first stage is then subjected to the second stage which involves transesterification of triglycerides using base catalyst, potassium hydroxide. The catalyst was chosen since it is the industrial catalyst of choice and because of the comparative results obtained by other researchers when it was used in their studies [96, 88,110].

Two stage transesterification was chosen over conventional, one step alkyl production for several reasons. High acid content of the oil requires the additional treatment step. Moreover; if one stage alkyl esters production to be used, large excess in base catalyst may be required and biodiesel yield was reported to be lower in this case [31].

Additionally, in the current biodiesel industry where high free fatty acid materials are being used as the oil source, two stage transesterification is widely in use to produce biodiesel. By implementing the acid-base stages, results of the research would be more useful to the industry and for further developments in that area. Example of two stage transesterification industry is Malaysia's crude palm oil-biodiesel factories.

3.2.2 Taguchi method of Experimental Design

In order to find the optimum conditions for a given reaction, deign of experiment needs to be involved. Design of experiments is a crucial part in any practical engineering study or research. When a number of parameters are involved it becomes hard to identify the importance of each factor and the relation of each factor to one another. In statistics, several methods and approaches were developed to help solving these types of problems. One of those methods which is used in this study is Taguchi technique for design of experiments.

Proper testing strategy is always important in engineering. When typical approaches are used, especially in reaction engineering, the studies and tests are usually of high costs and time consumption. This is based on the fact of full factorial design of these experiments.

Statistical design of experiments provides potential saving in time and capital involved aside from the fact of providing fully developed process analysis. This is offered by more efficient testing strategies. Utilization of taguchi technique provides two main benefits. First of which is providing a better planning strategy to reduce the cost and increase the economy of tests. The second is proper representation of parameters involved in any study and providing means for results analysis and interpretation.Design of experiments using Taguchi method involves three stages. In the first one, experiments tables are being designed using orthogonal arrays by determining the number of factors and levels in hand. The information is then compiled and rearranged in fractional-factorial arrays. The second stage is where experiments are being conducted, at this level, sensitivity analysis may be applied when inconsistent results are found, or in other words, unexpected findings. Finally, the technique's third stage is concerned with the analysis and interpretation of results obtained. The use of orthogonal arrays provides the opportunity at this stage to utilize some analysis methods such as observation method and column effect method to obtain the optimum conditions and to better understand the interaction of parameters. In the current study, observation of results from experiments conducted will be the judging factor in determining the optimum conditions.

3.2.3 Building Experiment's Orthogonal Arrays

As it has been previously discussed in section 3.2.1, two stage transesterification is being investigated in this study. To have a thorough understanding and get most of the possible results, it is important to study each reaction separately to find the combination of optimum parameters for each. For that reason, two fractional-factorial arrays will be designed. But since the same number of parameters is involved, the method that will be developed will be used in both stages.In order to create the arrays, the number of factors and levels needs to be determined. The factors and levels are identified alongside numbers and letters for each and all are presented in Table 3-10 and Table 3-11.

Table 3-10 Determination of number of factors and levels involved in building orthogonal arrays

No. of Factors ↓	**No. of Levels ➡**		
Alcohol:Oil ratio (mole)	6:1	8:1	10:1
Temperature (°C)	45	55	65
Catalyst amount (wt%)	1	2	3

Total number of factors= 3 ***Total number of levels = 3***

Table 3-11 Assignment of letters and numbers for factors and levels

Letter Assigned for factors ***Letter(Factor)***	**Number assigned for levels** ***Number (level)***		
A (Alcohol:Oil ratio)	1 (6:1)	2(8:1)	3(10:1)
B (Temperature)	1(45)	2(55)	3(65)
C (Catalyst amount)	1(1)	2(2)	3(3)

As it can be concluded from table 3-10, three factors and three levels are involved. By referring to standard Taguchi orthogonal arrays [164], several orthogonal arrays may be used for three level factors which include L9, L18 and L27.

The selection can be extended to cover two levels arrays as well. Although three levels are involved, it can be converted to fit into two levels arrays. Each three-level factor is considered equivalent to three two-level factor. From that it is possible to convert three three-level factors into nine two-level factors. L16 and L32 may be used for that number of factors. However going to two-level orthogonal arrays is recommended only when there are multiple level factors, which is not the case in this study.

The orthogonal array name consists of a letter and a number. The number represents the number of tests involved by using the selected table. For instance if L16 table to be used, it means that the number of tests involved will be sixteen tests.

Another element to describe the table is the resolution number. The number is a measure of confounding for each column. Lower number of tests will result in lower resolution number while larger number of tests yields higher resolution number. The number ranges between 1-4, for example a full-factorial set of tests will have a resolution number of four.

Orthogonal arrays with lower resolution number may require additional experiments to understand the significance and effect of each factor separately. However, as in most fractional-factorial methods, this step is compulsory. Reducing the number of tests is feasible enough when compared with full experiments.

Table 3-12 Example of full factorial tests table for reaction parameter

Test No.	Alcohol:Oil Ratio	Temperature	Catalyst Amount
1	6:1	45	1
2	6:1	45	2
3	6:1	45	3
4	6:1	55	1
5	6:1	55	2
6	6:1	55	3
7	6:1	65	1
8	6:1	65	2
9	6:1	65	3
10	8:1	45	1
11	8:1	45	2
12	8:1	45	3
13	8:1	55	1
14	8:1	55	2
15	8:1	55	3
16	8:1	65	1
17	8:1	65	2
18	8:1	65	3
19	10:1	45	1
20	10:1	45	2
21	10:1	45	3
22	10:1	55	1
23	10:1	55	2
24	10:1	55	3
25	10:1	65	1
26	10:1	65	2
27	10:1	65	3

In the present case, selection will be from three-level orthogonal arrays. This limits the selection to L9, L18 and L27 arrays. Preliminary analysis of number of elements in hand showed that a full factorial experiment consists of 27 tests, or as in orthogonal array term, L27 table. The main objective of using design of experiment is to reduce the number of experiments involved in a full factorial plot so L27 was disregarded since it represents a full factorial in this case. Table 3-12 shows an example for a full factorial, L27, experiments table.

Table 3-13 Standard L9 Orthogonal array

	Column no.			
Trial no.	**1**	**2**	**3**	**4**
1	1	1	1	1
2	1	2	2	2
3	1	3	3	3
4	2	1	2	3
5	2	2	3	1
6	2	3	1	2
7	3	1	3	2
8	3	2	1	3
9	3	3	2	1

With only two orthogonal arrays to choose from which include L9 and L18, it is important to involve resolution number in choosing the proper one. Each one of the tables has a resolution number of 1. In addition, L18 table has no interaction between the columns contained that could be used in preceding results analysis. For all of these reasons, the orthogonal array of choice is L9. A standard L9 table is shown in Table 3-13.

As it can be seen from Table 3-13, there are 4 columns with numbers assigned for each. Each column represents a factor such as alcohol to oil ratio, temperature and catalyst amount. The table needs to be modified to fit the three factors in hand.

Column number 1,2 and 3 will be selected by referring back to interaction tables [164]. The modified L9 table that will be used in the current study is provided in Table 3-14. Each column represents a factor which has a letter assigned to. The factor's levels are represented by numbers. The interpretation for these letters and numbers for both reactions is given in Table 3-11.

Table 3-14 Modified L9 array

	Column no.		
Trial no.	**A**	**B**	**C**
1	1	1	1
2	1	2	2
3	1	3	3
4	2	1	2
5	2	2	3
6	2	3	1
7	3	1	3
8	3	2	1
9	3	3	2

3.3 Transesterification Reaction

3.3.1 Acid Esterification

Acid esterification is carried out to reduce the acidity of chosen oil before feeding it to alkali transesterification for biodiesel production. The catalyst is initially mixed, at the desired ratio, with alcohol separately. Sulfuric acid used is in liquid form. The amount required in weight is calculated in volume using density since it is easier to transfer liquids using volumetric apparatuses. The mixture needs to be mixed until uniformity is achieved.

The desired amount of oil is measured and added to three neck flask which is placed in a water bath. The condenser is placed on the top of three neck flask to prevent any alcohol losses by vaporization. Heat is then applied using a hotplate until the desired temperature is reached. Figure 3-6 shows the experimental setup that was used.

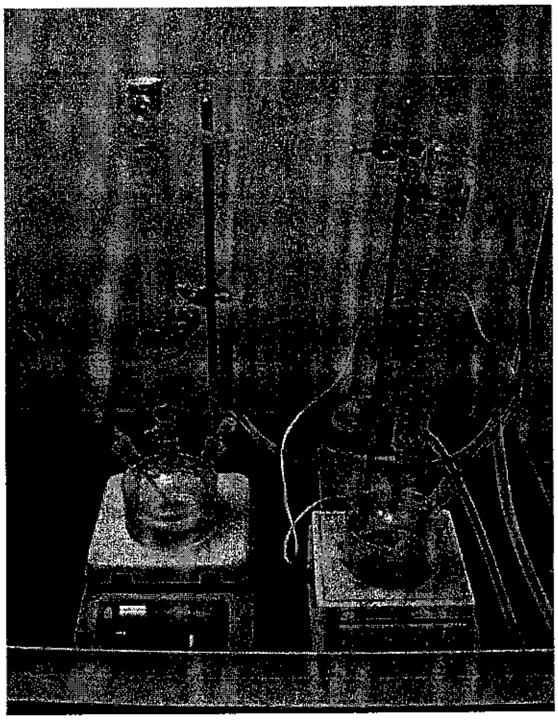


Figure 3-6 Transesterification Setup

(Location: Block 5, Level 1, Room 7)

The prepared alcohol/catalyst mixture is then added to heated oil blend and mixing rate is kept constant at 350rpm. The reaction is allowed to take place for three hours before moving the products to separation stage.

For kinetics investigation experiments, the same procedure is followed in general but the conditions used are based on the optimum conditions obtained. The time is extended to 5 hours as well for optimum reaction conditions to study the effect of time. The samples are withdrawn from the second neck quickly at specified time intervals.

Products obtained from the reaction stage are separated. Glycerol, excess alcohol and used catalyst are separated to obtain the final product. Initially, the products are transferred into a separation funnel. Warm, deionized, water is then added in a volume that is equivalent to double of products obtained. Hexane is then added to absorb and dissolve the desired product.

The mixture is left to settle and separate into two phases for 24 hours. The aqueous phase consists of deionized water containing spent catalyst, methanol and glycerol while the non-aqueous has the produced oil diluted in n-hexane. Figure 3-7 shows separation funnels with products obtained from a number of experiments.

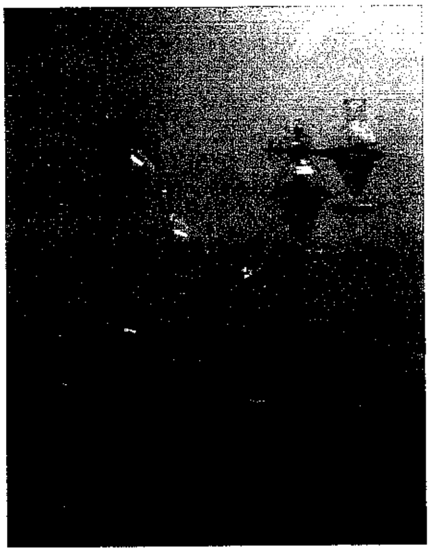


Figure 3-7 Phase separation funnels with the products in them (Location: Block 5, Level 1, Room7)

The phases are separated and plentiful quantities of drying agent Na_2SO_4, approximately 10g, is added to the non-aqueous phase to absorb and remove any water that might be present. Sodium sulfate particles that are saturated with water are then separated by filtration. The vacuum filtration was performed using air stream at elevated pressure of 4 psi and Pyrex glass filtration setup. Hexane is separated by rotary evaporation and the sample of each run is stored in 10 ml vials and labeled for further applications.

Each product obtained is analyzed for acid value and free fatty acid content. Titration was used to check for these values and the procedure was previously explained in section 3.1.4.2 in vegetable oil analysis. Thin layer chromatography was conducted to measure the amount of free fatty acids present in the products and samples that had been taken during time investigation. The procedure was described in section 3.1.4.8.

3.3.2 Base Transesterification

The same procedure and setup for acid transesterification is applied and used. However, the catalyst/alcohol mixture preparation is different since potassium hydroxide is available in the form of solid pellets. After weighing the desired amount of catalyst, magnetic stirrer is used to dissolve catalyst in methanol. The rest of reaction procedure involved is the same alongside the sampling for time effect investigation.

Another limitation that needs to be monitored during base transesterification is the presence of water in the reaction mixture. Moisture results in additional saponification of raw material which causes losses of oil. Thus, apparatuses involved in experiments need to be well dried before each use and monitored for any water contamination to prevent these losses.

Steps involved in separation of acid esterification products were implemented in the same order for the products of base transesterification. Few differences may be found however which is related to the difference in catalyst type. Washing with warm deionized water at 50°C is repeated for several times unlike single washing required in acid esterification. This repeated washing is needed to remove spent catalyst that couldn't be removed easily along with the saponified products which can't be found in acidic reaction.

The rest of separation procedure is continued in the same manner. The product of each trial is sampled and transferred to vials. The optimum product is found by analyzing each sample with thin layer chromatography to measure the amount of methyl esters obtained in base transesterification products through the same methodology which had been explained previously in section 3.1.4.8.

3.4 Methyl Esters Analysis

Final fatty acid methyl esters obtained from base transesterification reaction is analyzed using testing methods provided by international standards. This is to identify whether the final product is a suitable, biodiesel B100, fuel for commercial use or not. The tests conducted are cited from American Standard Testing and Material society standard D- 6751 which describe 12 standard tests to be conducted for the product [3] as well as the European biodiesel standards EN 14214 and 14213 [4].

The tests conducted are listed in Table 3-15 below and the results obtained will be compared to the acceptable ranges given in international standards.

Table 3-15 Tests to be conducted for analyzing methyl esters quality

Tests conducted on the fatty acids methyl esters obtained	
Density	Heating Value
Kinematic Viscosity	Acid number
Flash point (closed cup)	Monoglycerides
Sulfur content	Diglycerides
Water and Sediment	Triglycerides
Distillation temperature	Carbon Residue
Cetane number	Glycerin (Free & Total)
Methanol content	

3.4.1. Density

A pycnometer (25ml, JAYTEC, UK, Confirms British Standard 733) of known volume was filled with synthesized biodiesel until it reached the top of capillary tube located inside the glass stopper. The weight of pycnometer was measured before and after the filling to obtain the actual weight of each sample and by dividing over the known glass volume, density was found.

The test was repeated to confirm the result and standard deviation was calculated. The European standard EN 14214 and 14213 state that in order for biodiesel to be accepted its density needs to be within 860-900 kg/m^3.

3.4.2. Kinematic viscosity

Viscosity is one of the important properties to be tested for biodiesel since it directly affects vessels that utilize this renewable source of energy. Biodiesel of high viscosity may result in engine start problem in addition to residues that are tough to remove. Testing and confirming that the property matches international proposed standard is important.

An Ubbelohde viscometer was obtained with calibration certificate that confirms ASTM D445-446. Choosing the right viscometer was based on the range of kinematic viscosity that it serves. The test instrument (Tamson TVB445, ISL, France) was prepared and temperature of silicon bath was set to match standard testing condition at 40^{o}C. The viscometer was initially filled with the sample until it reached the designated level marked on the glass. It is then submerged in the temperature controlled bath.

Vacuum pump was turned on and suction was provided through the opening at the top of apparatus until the fluid level slightly exceeded the testing line. The suction was then stopped and the sample was allowed to be influenced by gravitational force and the time required for it to cross through the designated testing volume was calculated.

The time is totally converted into seconds and multiplied by factors provided with viscometer to obtain the kinematic viscosity. The test was repeated to confirm the result. Tamson viscosity instrument which had been used in the tests was equipped with suction pump installed on the top. The bath where viscometer was submerged is filled with silicone oil to provide uniform temperature for tests.

3.4.3. Flash point

A key property that determines the flammability of a fuel is its flash point. The flash point is the lowest temperature at which an applied ignition source will cause the vapors of a sample to ignite. Therefore, it is a measure of the tendency of a sample to form a flammable mixture with air. As a side note, the value of the flash point is used for the classification of flammable and combustible materials needed for safety and shipping regulations. The standard procedure for measuring the flash point for diesel and biodiesel fuels is ASTM D 93.

An automated flash point analyzer (FP93-5G2, ISL, France) was used in the test. The device work is based on the closed cup analyzer principle where the flash point is determined by heating a sample in a stirred container and passing a flame over the surface of the liquid. If the temperature is at or above flash point, this will lead to igniting the vapors and an easily detectable flash can be observed. The flash needs not to produce a sustainable flame. However in the current device, the flame is detected by a thermocouple or a ring-shaped ionization electrode. Once the flash point has been reached, the detector sends a signal to the control panel that sends an alarm signal and provides the temperature at the digital display.

The cup was filled with fatty acid methyl esters produced until it reached the marked spot at an approximate volume of 60ml. The test was then started and heat was applied while the agitator maintains stable mixing for uniform sample temperature. The temperature is measured using a platinum probe connected to control panel. An electrical ignition is provided using a plug which glows to provide the spark once an electrical current passes through. The plug is mounted over the test arm that holds the upper part over the testing cup.

3.4.4. Sulfur content

The procedure of analyses was discussed in section 3.1.4.7 where the test for sulfur was conducted using CHNS Analyzer (LOCO Corp., USA). An additional step was the calibration of device with standard material provided by the manufacturer to confirm results obtained. The tests were confirmed by repetition.

3.4.5. Water and sediments

Water and sediments may cause low fuel automation in diesel engines if the level does not conform to permitted extents. The fuel is confirmed to be clean by testing for the volume of water and sediments contamination. The American standard testing method ASTM D2709 established the methodology and procedure for analysis.

The test vials were marked down to 0.02 ml and filled initially with 10 ml of biodiesel then shaken for two minutes. After allowing the liquid to rest, the vials were introduced to the centrifuge (2-16, Sartorius, Germany) and each sample is placed in correspondence to one another to establish balanced distribution of force.

ASTM testing method D2709 describes the operating parameters of centrifuge and provides that the device needs to employ a relative centrifuging force of 800 rcf over the course of test which is extended for 10 minutes. Once the period of centrifugation is done, the vials are taken out carefully and the volume of each layer is recorded.

3.4.6. Atmospheric distillation temperature

American standard ASTM D6751 enforces that in order for biodiesel to be accepted in 100% form for vehicle use, it has to have an atmospheric distillation temperature that does not exceed 360^0C. Using conventional atmospheric distillation, it not possible to reach this temperature and the current available heating apparatus could not exceed 250°C. The standard testing method ASTM D1160 provides the mean for testing the sample under vacuum and establishes empirical correlations to convert the temperature obtained under vacuum distillation to its equivalent atmospheric one.

A vacuum distillation setup was assembled. Biodiesel sample was introduced to the distillation flask which was mounted on a heating mantle. A thermometer was installed on the distillation flask to measure the temperature of vapors passing through to the water cooled condenser. At the end of condenser, L-Shape connector was installed to lead the condensed sample to collection flask. The connector had an opening at the end which was connected to a vacuum pump with pressure control valve and a display for the amount of pressure being reduced.

The test was then initiated by turning on the heating mantle to increase the temperature which was monitored by the thermometer until the boiling point for liquid was reached. The distillation temperature was then recorded alongside the pressure at which the liquid started to evaporate.

Atmospheric distillation temperature was then calculated using Equation 3.7

$$ADT = \frac{748.1 \times A}{\frac{1}{VDT + 273.1} + (0.3861 \times A) - 0.00051606} - 273.1 \qquad (3.7)$$

Atmospheric correction factor (A) can be obtained from the following relation

For $P \geq 0.266\ kPa$:

$$A = \frac{5.143222 - 0.972546\ log_{10}P}{2579.329 - 95.761\ log_{10}P}$$

For $P < 0.266\ kPa$:

$$A = \frac{5.897249 - 0.987672\ log_{10}P}{2962.909 - 43.00\ log_{10}P}$$

3.4.7. Cetane number

Cetane index is a quantity that can be calculated using established equations to approximate the cetane number of fuel. Cetane number is a dimension less equity that is related to the ignition quality of diesel fuel in terms of delay time encountered with ignition once the fuel is injected to the combustion chamber.

The higher the cetane numbers the shorter the time that is required to ignite the fuel and vice versa. ASTM standard D976 gives the following empirical equation for the calculation of cetane index:

$$CI = 457.74 - 1641.416D + 774.74D^2 - 0.554ADT + 97.803(\log_{10} ADT)^2 \qquad (3.8)$$

3.4.8. Methanol content

The same procedure applied for flash point test is conducted. The flash point analyzer provides the mean to determine whether the amount of alcohol in the sample is acceptable by international standards or not. This is done by checking whether vegetable based diesel fuel passes a flash point limit of 97°C. The method is established by American standard testing method ASTM D93.

3.4.9. Heating value

The calorimetric testing procedure was discussed earlier for vegetable oils in section 3.1.4.3. The same methodology is applied to obtain the value for biodiesel using bomb calorimeter (C5000, IKA Werke, Germany).

3.4.10. Acid number

The AOCS method was explained in detail for the scope of testing acidity for vegetable oils and the same method at section 3.1.4.3 was used to check the acid value for methyl esters produced. All international standards for biodiesel recommend acid value testing.

3.4.11. Monoglycerides, diglycerides, triglycerides

Thin layer chromatography is used for the determination of glycerides and methyl esters. The method for TLC was discussed earlier in section 3.1.4.8. TLC has a detection accuracy of 0.1wt% [172] which is sufficient for testing these compounds [3, 4].

3.4.12. Carbon residue

The samples are prepared before the start of the test by adding 10g of methyl esters produced to soda-lime container. The container with the sample inside is then measured and the weight is recorded. The container is then transferred to a tubular furnace which serves as a coking oven.

The tubular furnace (Located at Block N, Nanotechnology Laboratory) was equilibrated and oxygen was removed by purging nitrogen gas at 600 ml/min while the temperature is still below 100°C for 10 min. The flow rate of nitrogen is decreased to 150 ml/min and heat was increased slowly thereafter by adding 10-15°C/min until it reached 500°C.

Once the testing temperature is reached, it had to be kept constant for the next 15 min to allow complete burning of the samples. After that period the oven is turned off and the medium is allowed to cool down under nitrogen flow rate of 600 ml/min. The sample is removed and weighed once the temperature dropped below 250°C. The percentage of carbon to the total sample was found and reported.

3.4.13. Free and total glycerin

GC (GC 2010, Shimadzu) is initially prepared and the column (HT-5 Column, 25 m, I.D. 0.32, Sigma-Alderich) is conditioned before the test by allowing hydrogen gas to flow at ambient temperature for 30 minutes. The ready to use standard solutions (ASTM D6584 standard solution kit, Sigma-Alderich) are transferred to vials based on the required volume for each and 100 μl of MSTFA (N-Methyl-N-trimethylsilyl-trifluoroacetamide) is added to each vial to provide volatility and thermal stability for analysis. The vials are shaken and left to settle for 15 minutes at room temperature. N-Heptane is then added to each vial in a volume of 8 ml. The same procedure is done for biodiesel sample where 10 ml of the FAME is placed in GC vial and the rest of the reagents are added accordingly.

The operating condition for the GC is programmed. Flame ionization detector is set at 360°C. The carrier gas, hydrogen, is set to flow through the column at 3 ml/min. The column temperature is held at 50°C for 1 minute then heated at an initial rate of 15°C/min until 180°C is reached followed by another period of heating at 7°C/min up to 230°C. The final temperature to be reached is 360°C and held for 10 min after heating up at a rate of 30°C/min. The gas chromatograph was attached to an auto sampler and auto injector mounted on the top.

At the end of test, the peaks for glycerin are obtained alongside the integration report as shown in Appendix (Page 177). The identification of peaks is based on the retention time for the reference material and the injected standards. The total glycerin is calculated based on the equation 3.9

$$\text{Total glycerin} = \text{Free glycerin} + \text{Bonded glycerin} \qquad (3.9)$$

And free glycerin can be obtained from equation 3.10

$$\text{Free Glycerine} = (a_g \times \frac{A_g}{A_s} + b_g) \times W_s \times \frac{100}{W} \qquad (3.10)$$

To calculate the bonded glycerin, it is required to calculate the individual glycerides (Mono,Di and Tri) using the following equation [7]:

$$Gl_i = (a_{ol} \times \frac{A_{gli}}{A_{is2}} + b_{ol}) \times W_{is2} + \frac{100}{W} \qquad (3.11)$$

3.5 Kinetic Study

The kinetic study is performed to postulate a reaction rate equation from the experimental data for both acid esterification and base transesterification. Both reactions were studied at optimum reaction conditions but at two different temperatures. Using data points obtained, the rate equation had been developed using two methods, namely, differential method and nonlinear modeling.

The two methods are well explained by a number of literatures. In the current study, Fogler approach was used from his book [174]. Differential method is used to establish a conventional rate equation. Graphical, finite difference, linear and non linear regression techniques were used to analyze the data. These techniques are used to find the concentration/time derivative which is used to find the rate law. In brief, the methods are explained below.

Graphical Method

In the graphical method, concentration difference over time is plotted against concentration to obtain a histogram. Using equal area differentiation, rate derivate can be evaluated. From graphical method, disparities in experimental data can be easily identified. Thus, the method can be used to analyze the data for errors as an added advantage before going to the next differential techniques. Once rate derivative is obtained, it is plotted against concentration to obtain reaction order and rate constant.

Finite difference Method

Finite difference is a numerical technique that can be used to the derivative of any function. Thus, it can be used here to find the rate derivative then apply it to obtain reaction order and constants. Finite difference method approximates the solutions for differential equation by replacing the derivative expressions with approximately equivalent difference quotients. Based on this approach the first derivative may be evaluated.

Regression Method

Another approach to find concentration/time derivative is to fit the concentration versus time data into an nth order polynomial. The polynomial is then differentiated to obtain the derivative and the calculation continues. To accept a polynomial as the source for differential data, it has to fit into the points of concentration visually with limited peaks and valleys as it goes through these points and beyond them. In addition, least square analysis and R^2 are used to verify the goodness of fit.

4 Results and Discussion

4.1 Characterization of Rubber seeds and extraction of Rubber seed oil

4.1.1 Characterization of Rubber seeds

The results of moisture analysis of rubber seeds are presented in Figure 4-1. Three repetitions were conducted for results confirmation. As it can be noticed from the figure, none dried seeds (0 hour) showed the largest drop in the results trend as it was recorded by the moisture analyzer. This major drop in the sample weight that had been recorded shows the high content of moisture contained by the seeds. Four hours dried seeds had shown the least drop in weight on the other hand meaning that it contained the lowest amount of moisture among the tested samples.

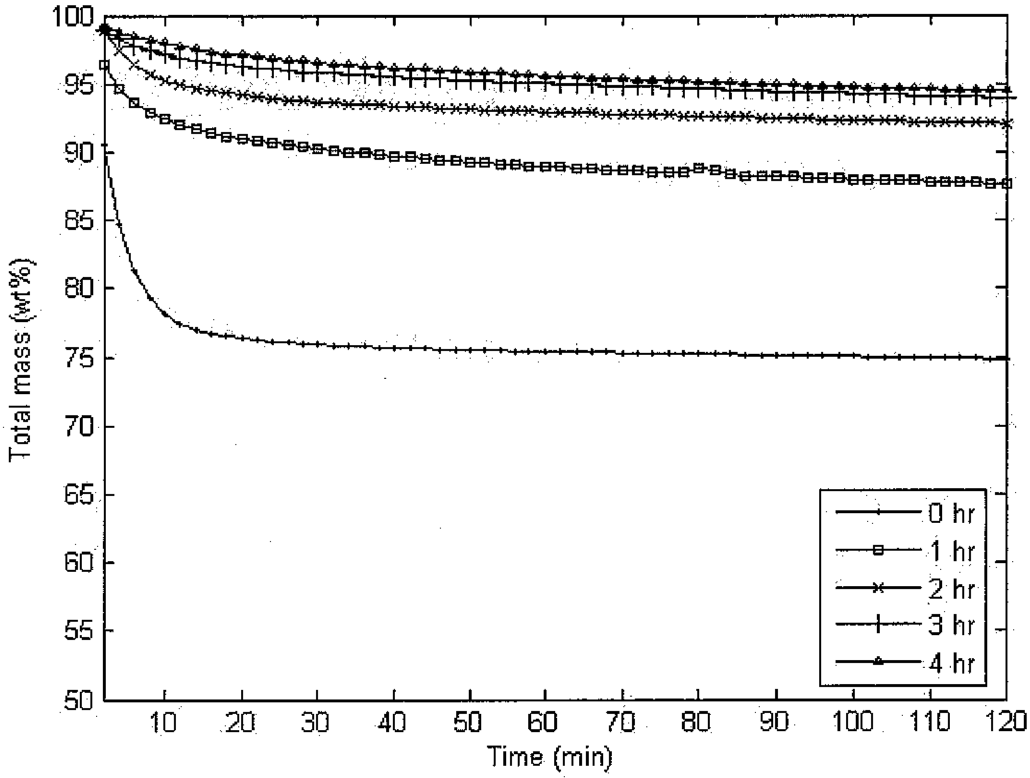


Figure 4-1 Drop in sample weight during moisture analysis while heating 5 grams of seeds at 120^{0}C

From the results obtained, the initial weight of rubber seed samples, 5 g each, is compared to the final weight reached at the end of each experiment. Moisture and volatile content are found based on AOCS method [57]. The average moisture and volatile matter content of fresh rubber seed samples was found to be 25.17% with standard deviation of 2.65. The moisture and volatile content of the rubber seeds had to be lowered for it to be utilized in oil extraction at later stages. Four drying hours gave a stable trend line that can be seen in Figure 4-1 after 20 minutes of initiating moisture analysis meaning that the moisture level is very low to show any change. On the other hand, the rest of trend lines for different drying times continued to drop as time proceeded. Three hours drying trend was close however to that of four hours. Any water or contaminant present in transesterification reaction may results in product losses.

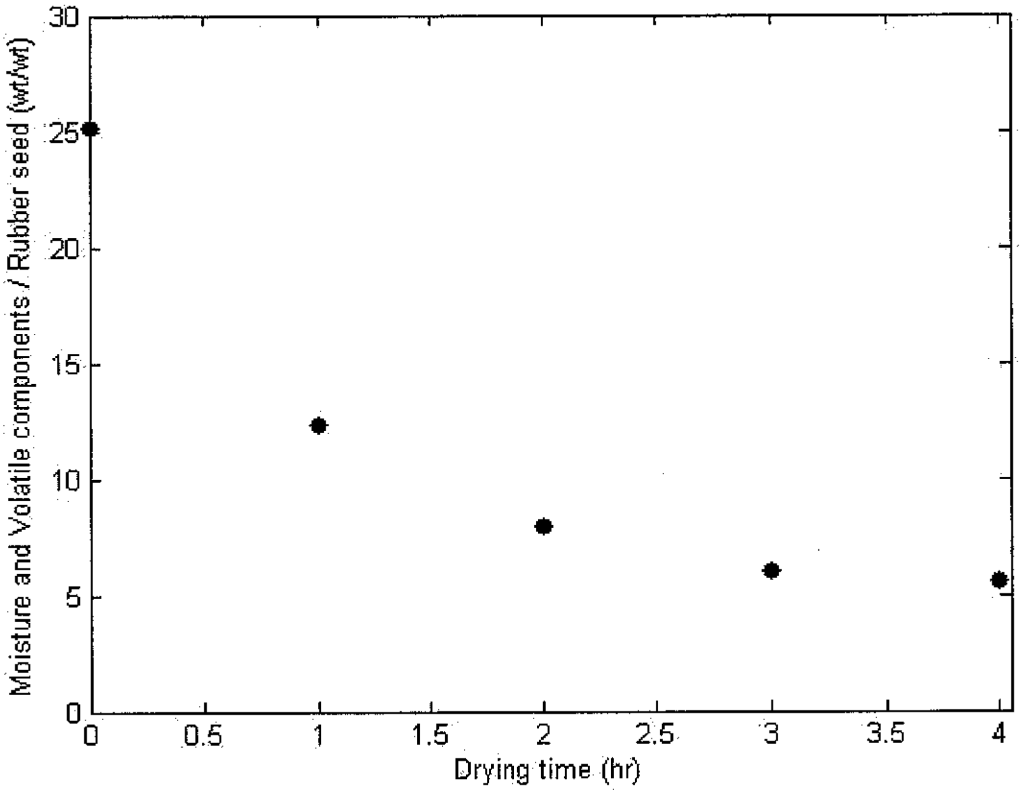


Figure 4-2 Moisture and volatile components amount in rubber seeds dried at 105°C for 0,1,2,3 and 4 hours

Figure 4-2 shows moisture and volatile components amount that was found in rubber seed samples which had been dried at different time extents. Elongated drying time had significant effect over the moisture content. After one hour of drying the moisture and volatile component dropped to 51%. On the other hand, four hours of drying resulted in 78% drop in moisture content of initial sample weight of 5 g.

Seeds were not subjected to longer drying times of more than 4 hours. The glycerides tend to decompose when high temperatures are being applied for long drying periods. This was observed as seeds started to form cokes and sludge after 5 hours of drying. This is to be avoided since it will cause losses in glycerides that are converted to biodiesel.

4.1.2 Extraction of Rubber seed oil

Rubber seed samples prepared for oil extraction study were subjected to 2,3 and 4 hours of drying over 110°C to study the effect of drying time over oil yield. These three drying periods were chosen since they gave the lowest amount of moisture and volatile components as explained in section 4.1.1. Five grams of processed seeds were used in each extraction test. Normal hexane (n-hexane) was used as solvent in oil extraction. It was reported to give the highest yield when compared to other available solvents [56]. Table 4-1 list all the parameters which had been used in investigating rubber seed oil extraction.

Table 4-1 Parameters involved in rubber seed oil extraction study

Parameter	**Value**				
Drying time (hr)	2		3		4
Extraction time (hr)	0.5	1	2	3	4
Solvent/Seed (v/w)	12	22	32	42	52

The temperature was kept constant at 70°C. The selection of the temperature was based on solvent's (n-hexane) boiling point that was 69°C. At this temperature, it was noticed that the amount of solvent condensed over thimble that was placed in the soxhlet extractor was enough to meet a drop rate of 150 drops of solvent per minute which was recommended by the literature [57].

Samples from each drying time were taken in an amount of five grams and packed in a thimble. The thimbles were then placed in a soxhlet and the extraction was initiated. Results are provided in Table 4-2. Standard deviation was calculated and is shown to be in an acceptable range of 0.01-0.05 g. The highest yield that could be obtained was 33.05%. This result was generated from samples which had been subjected for 4 hours of drying. The experiments were carried out at 70°C with 160 ml of hexane with 5g of seeds and 3 hours extraction time.

Table 4-2 Rubber seed oil yield at different drying periods

Drying time (hour)	Oil weight (g)		Mean	Standard Deviation	Oil Yield %
	Run 1	Run 2			
2	1.60	1.58	1.59	0.01	31.89
3	1.62	1.65	1.63	0.01	32.75
4	1.67	1.63	1.65	0.05	33.05

Lowering the mass of moisture and volatile component is important. Samples with lower moisture content as a result from longer drying time could yield higher amounts of oil. This factor may affect the overall process economy. It would take additional amounts of solvent or even longer extraction time to obtain more quantities of oil if not considered. The oil yield is calculated based on Equation 3.1.

Elongated extraction time favored the increase in amount of oil produced as shown in Table 4-3. From these results in Table 4-3, it can be seen that the amount of oil increased steadily with the increase of time where the highest yield was at four hours of extraction reaching 33.64%. The experiments were carried out at 70°C with 160 ml of hexane and 5 g of seeds dried for 4 hours. The standard deviation is shown in Table 4-3 and differences between results ranged from 1-4%.

However, there are some down sides of increasing extraction time above four hours. During preliminary tests conducted, longer periods of times resulted in gum formation when the extraction was left to continue for 5-6 hours.

The gum was stuck all around the wall of extraction vessel and it was very hard to clean and remove these accumulations by normal cleaning procedure. The utilization of nitric acid and ultra sonic cleaning was required to remove these formations from soxhlet extractor and glass extraction vessel.

Table 4-3 Rubber seed oil yield at different extraction time

Extraction time (hour)	Oil weight (g)		Mean	Standard Deviation	Oil Yield %
	Run 1	Run 2			
0.5	0.81	0.89	0.85	0.08	17.02
1	0.88	0.93	0.90	0.04	18.18
2	1.68	1.59	1.63	0.04	32.79
3	1.67	1.63	1.65	0.01	33.05
4	1.66	1.69	1.68	0.01	33.64

The effect of extraction time was also discussed by other researchers while studying that for sunflower seed oil [57] and rubber seed oil [162]. Although shorter extraction times of less than 1 hour were tested by the respective researchers, the same conclusions were obtained on the positive effect of increasing extraction time to increase oil yield.

Table 4-4 Rubber seed oil yield at different solvent amount

Solvent/Seed ratio (v/w)	Oil weight (g)		Mean	Standard Deviation	Oil Yield %
	Run 1	Run 2			
12	1.20	1.14	1.17	0.05	23.58
22	1.23	1.20	1.22	0.03	24.44
32	1.67	1.63	1.65	0.01	33.05
42	1.68	1.69	1.68	0.00	33.79
52	1.79	1.68	1.74	0.05	34.80

Oil yield was shown to increase in conjunction with increasing solvent to seed ratio as seen in Table 4-4. The maximum oil yield that could be obtained by using 260 ml of n-hexane was 34.8% with standard deviation between results obtained of 0.05. When solvent amount is increased, the amount of solvent vapors that perforates processed seeds placed in thimbles is increased as well. This will lead to more surface contact between oil containing matter and solvent.

Increasing amount of solvent seems to be the most significant variable due to the variation in oil yield obtained that reached 34.8%. This result is satisfactory with findings reported by Kamalu et al. who found the same significance of solvent amount over oil yield of rubber seed oil [162].

Attah et. al [56] reported that the dielectric constant of solvent as well as its polarity have noticeable effect on the amount of oil extracted and oil properties (e.g. acidity of oils). Based on all of the above mentioned and the results obtained, the solvent to seed ratio that gave the highest yield was found to be of 52/1 milliliters solvent over grams of seeds when compared to the rest of ratios investigated. Results obtained from experiments were tabulated in Table 4-4 .

4.2 Charecterization of RSO, CPO and Blends

4.2.1. Visual test

Extracted rubber seed oil was of yellow/golden color. It had a distinct smell that could be noticed once the solvent was evaporated and can be differentiated from other types of oils (e.g. crude palm oil). When the oil was cooled down to room temperature and stored for several days, no solid formation was noticed and no heating was required for further applications. Figure 4-3 shows rubber seed oil which was extracted.

Contrary to rubber seed oil, crude palm oil color was orange at room temperature while it turns to dark orange/red when it is heated up. In addition, the oil tends to solidify when kept at temperatures equal to or lower than 25°C for some time. The solid particles are dissolved in liquid phase by increasing the temperature. Thus, heating is always required during handling. Crude palm oil can be seen in Figure 4-3. Like rubber seed oil, crude palm oil has its own distinguished odor that can be noticed whether it's in solid or liquid form.

In crude palm oiland rubber seed oil blends, the dark orange/red color was dominant over all samples. The color is however diluted in blend number two where equal proportions of the oils were in place and had more recognizable yellow contrast in blend number three at 3/1 RSO to CPO volumetric ratio. Solidifications were not noticed even with blend number one where crude palm oil was in excess proportion and additional heating was not required. Odors were not apparent since they were mixtures of both oils.

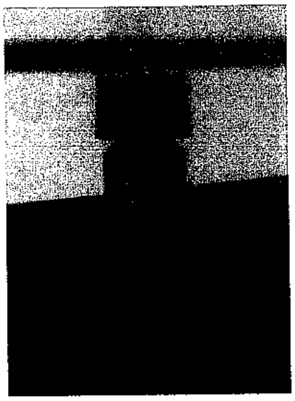


Figure 4-4 Rubber seed oil

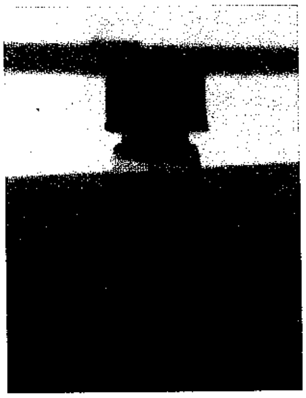


Figure 4-3 Crude palm oil

4.2.2. Acid value

Acid value is the number of milligrams of potassium hydroxide required to neutralize free acids in one gram of any given sample. Acid value may also be called acidity or acid number. It is a measure of the amount of carboxylic acid groups in a given oil or fat.

This value is of great importance in many applications especially in production of methyl esters. It can provide the means which is necessary to calculate amount of catalyst that is required to neutralize raw material to give higher yield. Observation of acid values for oils and fats is very important since any increase in the number for given oil may be caused by the decomposition of organic matter to form fatty acids by oxidation.

In this study methanol was used to dilute potassium hydroxide instead of water. The reason is that methanol gives a sharp end color when titrating. Table 4-5 shows the results for titration of oils and the free fatty acids content in each. It can be noted from results shown in Table 4-5 that rubber seed oil exhibits the highest acid value among other oils at almost 64. Crude palm oil's acidity was comparatively lower than that of RSO with a value of 18.5. The blends were of spectrum acidity where the lowest was blend 1 and increased steadily to Blend 3. The results show that the acidity of rubber seed oil is reduced by blending with crude palm oil. This will reduce the amount of free fatty acid that needs to be treated with acid esterification.

Table 4-5 Acid value of oils

Oil	**Acid value (mg,KOH/g,Oil)**		**Average**	**FFA %**
	Run 1	**Run 2**		
RSO	63.9	63.9	63.9	22.7
CPO	19.6	17.3	18.5	7.23
BL1 (3RSO/7CPO)	25.8	24.6	25.2	9.58
BL2 (1RSO/1CPO)	31.4	32.5	31.9	11.9
BL3 (7RSO/3CPO)	40.3	40.3	40.3	14.7

Free fatty acids percentages in oils were calculated. The calculations were based on relationship provided by AOCS [57] between acid number of oils and their free fatty acids. This is given in Equation 4.1.

$$\%\mathrm{FFA} = \frac{\text{Acid number}}{\text{Const.}} \tag{4.1}$$

The constant in Equation 4.1 depends on the main components of oils. For rubber seeds oil, it mainly consists of oleic acids. Then the constant is given in the reference as 2.81 while for Crude palm oil , which contains palmitic acids as the major component, the constant is 2.56 [57]. Based on these values, the constants for blends were derived using the ratios of the oils and are shown in section 3.1.4.2.

In the current study, rubber seed oil acidity reached a value of 64. Walton et al. reported an acid number of rubber seed oil equivalent to 34. However, the oil in literature [60] was refined and bleached before the test was conducted. Refining of oils removes free fatty acids which reduces the acid value. Refining of vegetable oils usually involves removal of gums, waxes, phosphatides, free fatty acids and smell. This will require additional process equipments like neutralizer, bleacher, deodorizer, heat exchanger and vacuum equipments [167].

Refining the oil add more cost to the process and hence, adding more economic barriers toward biodiesel production. With two stage transesterification, acid esterification is expected to reduce acidity of the oil toward using them in base esterification without the need to involve vegetable oil refining.

Malaysian palm oil board sets the limit for FFA content in crude palm oil produced to 5wt%. However, for the oil obtained prior final treatment the value can be a little higher. Crabbe et al. [120] reported that crude palm oil had an FFA content of 6.9%. The oil used in his study was obtained from a certified Malaysian palm oil board mill. In this study, crude palm oil was purchased from a certified mill as well and the FFA content was close to that of Crabbe et al. at 7.2%.

4.2.3. Heating value

Calorific value, or heating value of fuel or any combustible substance, is the amount of heat released during the combustion of a specific amount of substance. The calorific value is an internal characteristic of the substance that is measured in units of energy over units of mass.

Table 4-6 shows the difference in calorific values of crude palm oil, rubber seed oil and the blends. Rubber seed oil recorded the lowest value at 37.5 MJ/Kg while crude palm oil was the highest at 39.3 MJ/Kg. The oil blends heat content depended on amount of mixed oils. Blend with higher amounts of crude palm oil had more heat content while that with lower calorific values had higher amounts of rubber seed oil.

Table 4-6 Calorific value of oils

Oil	**Calorific value (J/g)**		**Average (J/g)**
	Run 1	**Run 2**	
RSO	37562	37412	37487
CPO	39372	39443	39407
BL1 (3RSO/7CPO)	38337	38445	38391
BL2 (1RSO/1CPO)	38141	38173	38157
BL3 (7RSO/3CPO)	37528	37976	37752

Marchetti et al. reported 33.5 MJ/Kg as the heating value for refined, bleached and deodorized palm oil [49]. This is related to removal of some components in vegetable oil that posses some significant heating value during refining process. This is not preferred in biodiesel industry since higher heating value is required.

Vegetable oils in general have lower heating value than that of diesel which reaches 45MJ/Kg[168]. Monitoring the heating value of biodiesel is important. It is shown that the energy content of biodiesel is almost 12.5% less than that of conventional diesel fuel on weight basis. This will lead to less energy generation when burned in compression ignition engines [169].

4.2.4. Density

Densities of oils and blends were calculated and results are shown in Table 4-7. Density is defined as the weight of a certain sample over its measured volume. As it can been from the results, crude palm oil had the highest density in comparison to the other oils which recorded lower densities. Characterization of vegetable oils densities is important since it is used in various engineering calculations. Establishing these properties will serve future applications.

Table 4-7 Density of oils

	Density (g/cm^3)		
Oil	**Run 1**	**Run 2**	**Average (g/cm^3)**
RSO	0.912	0.911	0.912
CPO	0.920	0.920	0.920
BL1 (3RSO/7CPO)	0.919	0.919	0.919
BL2 (1RSO/1CPO)	0.917	0.917	0.917
BL3 (7RSO/3CPO)	0.913	0.913	0.913

4.2.5. Viscosity

One of the most crucial properties of vegetable oils for its direct relation with biodiesel production is viscosity. Reduction of viscosity needs to take place for methyl esters produced to be utilized as alternative fuel. In general, vegetable oils have high viscosities as it was presented in Table 2-4. This disadvantage in its viscosity is rectified by transesterification reaction and the final viscosity of products needs to meet the industry standards [3,4].

Table 4-8 Dynamic viscosity of oils

Oil	Viscosity (cP)				Average (cP)
	Run 1	Accuracy	Run 2	Accuracy	
RSO	40.5	10.0	40.9	10.9	40.70
CPO	37.1	10.0	39.0	10.4	38.05
BL1 (3RSO/7CPO)	38.3	10.3	39.4	10.5	38.85
BL2 (1RSO/1CPO)	38.6	10.5	40.1	10.7	39.35
BL3 (7RSO/3CPO)	39.7	10.6	40.5	10.8	40.10

Viscosity of vegetable oil is related to its components and its degree of saturation. It's observed that when degree of saturation in oils increases the viscosity increases as well. Table 4-8 shows the viscosities obtained for all tested samples.

As it can be seen from Table 4-8, viscosity increased with the increase of RSO amount in the blends. This is well understood since rubber seed oil reported the highest viscosity among the oils at 40.7cP. Results of viscosity show that it is much higher than the criteria of biodiesel. ASTM set the range of biodiesel viscosity between 1.9-6.0cP. Esterification process is expected to reduce the viscosity of oils. Other researchers reported high viscosities for oils as well. Crude palm kernel oil viscosity was reported to be 28.65 cst by Jitputti et al [90]. Ramadhas et al. gave higher one for rubber seed oil at 66 cst [31]. All of the studies were successful however in reducing high viscosities using transesterification.

The property needs to be monitored since it may affect fuel atomization in engines utilizing biodiesel. If the viscosity of biodiesel exceeds the range provided by ASTM standard, it may cause low fuel consumption economy caused by incomplete combustion resulted from large droplets of fuel residing on the injection pumps. This has an extended effect on environment since mal combustion can cause increased emissions of harmful carbon gases [3]. Kinematic viscosities for oils were calculated as well and results are tabulated Table 4-9.

Table 4-9 Kinematic viscosity of oils

Oil	**Viscosity (N/m^2)x 10^{-4}**	**Density (kg/m^3)**	**Kinematic viscosity (m2/sec) x 10^{-5}**	**Kinematic viscosity (cSt)**
RSO	470	912	5.15	51.5
CPO	380	920	4.13	41.3
BL1 (3RSO/7CPO)	388	919	4.22	42.2
BL2 (1RSO/1CPO)	393	917	4.28	42.8
BL3 (7RSO/3CPO)	401	913	4.38	43.8

Methodology of Refutas technique for calculating blend viscosity was provided in section 3.1.4.5. Viscosity blending indexes were calculated for crude palm oil and rubber seed oil. They were 30.1 and 30.9 respectively. The two values were used in calculating the blends viscosity which is provided in Table 4-10.

Table 4-10 Calculated viscosities of oil blends from refutas equation

Blend	**x_{RSO} (wtRSO/wtBlend)**	**x_{CPO} (wtCPO/wtBlend)**	**VBI_{Blend}**	**v_{Blend} (cSt)**	**v_{actual} (cSt)**	**Deviation (v_{Blend}- v_{Actual})**
BL1 (3RSO/7CPO)	0.27	0.64	30.3	44.1	42.2	1.85
BL2 (1RSO/1CPO)	0.49	0.50	30.5	46.0	42.8	3.18
BL3 (7RSO/3CPO)	0.69	0.30	30.7	48.1	43.8	4.26

As it can be seen from Table 4-10, deviation from actual results was reported. Correction factors need to be investigated to obtain empirical relations that can be practically implemented. Blending of vegetable oils could not be found in literature except for that of jatropha with palm oil [13]. However, none considered performing these calculations for vegetable oil blends.

4.2.6. Refractive index

Refractive index, or index of refraction, is a fundamental physical property which may be used to identify a certain substance, determine its purity and even more, measure its concentration.

However, the main objective of calculating oil's refractive index in the current study is to provide identification for oils and blends in terms of optical measurements and to relate it to other properties. For instance, establishing the relation with density will help in measuring density be optical means. Refractive index data from optical sensors can be translated to densities with such relations.

Index of refraction may be defined as reduction in speed of light travelling in a certain medium in comparison to that in vacuum. The beam of light tends to change direction when the interface between two different materials is crossed and it reflects partially [171]. This phenomenon happens due to the change or difference in refractive index between two substances.

Table 4-11 Refractive index of oils and blends

Oil	**Refractive index**		**Average**
	Run 1	**Run 2**	
RSO	1.459	1.459	1.459
CPO	1.467	1.467	1.467
BL1 (3RSO/7CPO)	1.466	1.466	1.466
BL2 (1RSO/1CPO)	1.465	1.465	1.465
BL3 (7RSO/3CPO)	1.464	1.464	1.464

Table 4-11 shows the refractive index for oils and the blends obtained from the study. It can be noticed that refractive index decreased as RSO content increased. This is due to change in molecular optical properties. Figure 4-5 shows the relation between refractive index and densities of vegetable oil. The relation is obtained by fitting the data to a straight line equation and the values are shown in Table 4-12. Establishing such relation will enable the calculation of density by using refractive index.

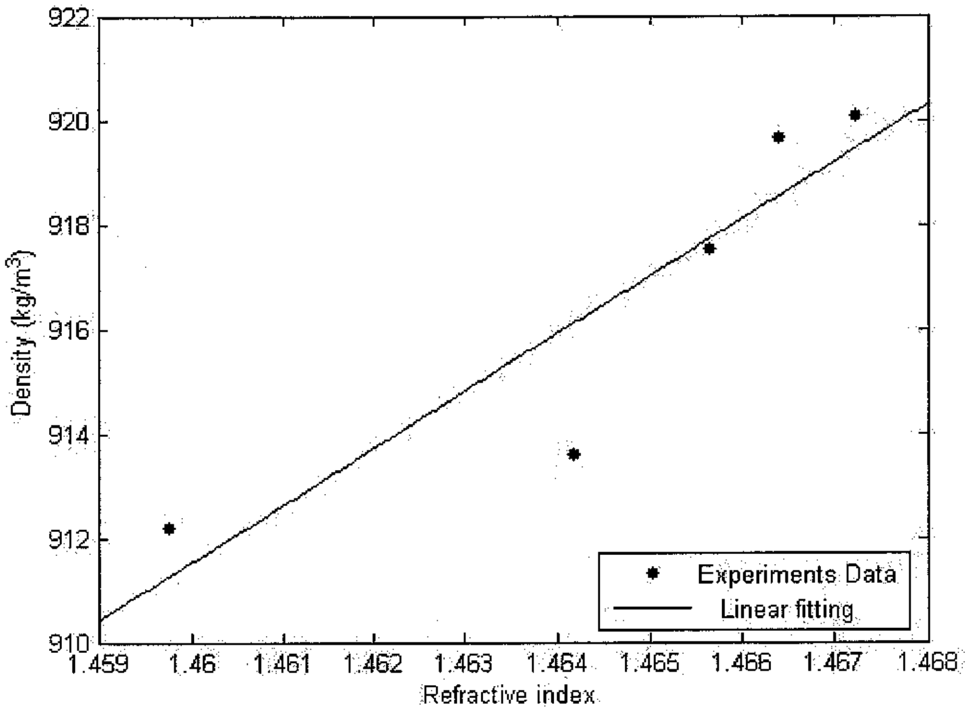


Figure 4-5 Refractive index vs. Density for vegetable oils

Table 4-12 Relation between refractive index and density

y = mx + c		
m = 1098	c = -692	R^2 = 0.825

y and x donate density and refractive index respectively, R^2 represents the goodness of the fit

4.2.7. Sulfur and Nitrogen content

Sulfur and nitrogen content for oils and blends were calculated to pre-assess pollution levels in raw material prior biodiesel production. The content of these elements were acceptable as feedstock for methyl esters when compared with international standard [3,4]. Sulfur and nitrogen content is provided in Table 4-13.

Table 4-13 Sulfur and nitrogen content of oils

Oil	Sulfur (wt%)	Nitrogen (wt%)
BL1 (3RSO/7CPO)	0.053	0.016
BL2 (1RSO/1CPO)	0.031	0.0134
BL3 (7RSO/3CPO)	0.029	0.0281

As it can be seen from Table 4-13, sulfur amount is below 0.05%wt for blend 1 (3RSO/7CPO) and blend2 (1RSO/1CPO) and is acceptable by international standards for production of biodiesel. However, it fails for blend 3 to meet S15 biodiesel but can meet S5000 biodiesel criteria in the same standard [3,4].

4.2.8. Mono, di and triglycerides

Glycerides compositions for vegetable oils were calculated using thin layer chromatography as shown in Table 4-14.

Table 4-14 Glycerides composition of oils

Oil	**Monoglycerides (wt%)**	**Diglycerides (wt%)**	**Triglycerides (wt%)**
RSO	5.94	10.1	60.3
BL1(3RSO/7CPO)	4.64	6.95	64.7
BL2(1RSO/1CPO)	2.50	7.28	70.1
BL3(7RSO/3CPO)	2.23	7.25	73.3
CPO	0.89	7.81	83.4

4.2.9. Free fatty acids

Gas chromatography/Mass spectrometer was used to analyze free fatty acid content of blend number two as the chosen blend for biodiesel production. This analysis is to help in understanding the change in fatty acid composition as a result of blending. Crude palm oil is said to be of palmitic base [120]. High content of palmitic acid is reported to result in low pouring point of biodiesel produced. Rubber seed oil is said to be of oleic basis on the other hand which preferred to increase the pouring point is. Blending of the oils is expected to increase the pouring point of biodiesel if the oleic acids content increases.Transesterification of the blend was required for the analysis which was conducted based on American oil chemist society official methods number Ce2-66 and Ce1-62 for preparation and analysis of vegetable oils with gas chromatography [57].

The identification, composition and percentage of each peak were calculated using integration software provided by the manufacturer (Shimadzu) and internal standards.

GC/MS report is provided in Appendix (Page 173). Table 4-15 shows free fatty acid analysis for blend number two. It can be seen from Table 4-15 that oleic acid content is more than that of palmitic acid although the oils were blended on equal ratio basis. This proves that blending resulted in higher oleic acid vegetable oil which is preferred for biodiesel production.

Table 4-15 Fatty acid composition of blend 2

Peak no.	Formula	Trivial name	Composition (%)
1	$C_{11}H_{20}O_4$	Azelaic acid	0.55
2	$C_{10}H_{22}O_2$	Capric acid	0.39
3	$C_{15}H_{30}O_2$	Myristic acid	0.44
4	$C_{20}H_{40}O_2$	Stearic acid	0.21
5	$C_{17}H_{34}O_2$	Palmitic acid	22.42
6	$C_{19}H_{34}O_2$	Linoleic acid	27.82
7	$C_{19}H_{36}O_2$	Elaidic acid	37.42
8	$C_{19}H_{38}O_2$	Margaric acid	10.51
9	Others	-	0.24

4.2.10. Iodine value

Iodine value was calculated based on AOCS method Cd 1c-85 [57]. The results obtained from gas chromatography analysis were used as explained in section 3.1.4.10. The iodine value for blend two was found to be 106.34 from compositions of hexadecenoic acid, octadecenoic acid and octadecadienoic acid. The result obtained is comparable to results obtained by other studies [165,175] since the iodine value obtained for blend number two falls in the range between that of crude palm oil and rubber seed oil. It was reported that rubber seed oil had an iodine value of 135.36 as it was determined by Aigobodian et al. [165]. Crude palm oil iodine value on the other hand was found to be 52.2 by other studies [175]. Lowering iodine value will lower the saponification tendency of oil which may result in lower biodiesel production yield. High iodine value makes the feedstock more sensitive towards water and humidity. Another important use for iodine value is a measure of how strong a tendency the oil has to oxidation and thus to drying. Oils with high iodine values are classified as drying oils which are hard to clean from their container.

4.2.11. Summary of findings of RSO, CPO and blends characterization

Analysis of vegetable oils was conducted to characterize the feedstock properties. Based on results, blend number two which contains equal proportion of CPO and RSO was found to have acceptable levels of sulfur (0.031wt%) by comparing it with international standards [3,4]. Thus, the blend was chosen for synthesizing biodiesel in the current study. Mono,di and triglycerides of all the oils were analyzed using thin layer chromatography.

High acid value of blend number two of 31.9 does not allow biodiesel production with single stage base transesterification since it will lead to feedstock saponification and loses of products. High viscosity must be reduced in the two stage transesterification for it to be accepted by international standards. Free fatty acids and iodine value were identified for blend number two and it was noticed that rubber seed had enhanced the blend property by increasing the oleic acid content over palmitic acid from palm oil. This is said to enhance biodiesel obtained since it will increase pouring point in cold environments. Iodine value had improved on the other hand as a result of crude palm oil, since rubber seed oil possesses high iodine value. Heating values of the oils were measured and found to be acceptable by European standards [3]. Refining of crude oil is not preferred since it will reduce the heating value of vegetable oil and energy content of biodiesel produced.

4.3 Acid Esterification

4.3.1 Determination of optimum reaction conditions

Constructed orthogonal array was followed in conducting experiments to find the optimum variables involved in the study of acid alcoholysis of free fatty acids. Taguchi L9 OA has been adopted in this study to find the set of reaction conditions which gave the lowest free fatty acid percentage or acid value in the product. Acid value and FFA were determined through titration and thin layer chromatography.

Design of experiments tested the interaction between several variables. That included alcohol to oil molar ratio, reaction temperature and catalyst amount while holding steady mixing at 350 rpm and reaction time for 3 hours. The results are shown in Figure 4-6 and Figure 4-7 in terms of acid value and FFA percentage in the products of each experiment. As it can be noticed from these figures, trial number six gave the best results since it had the lowest acidity and FFA percentage. The acid transesterification at this given trial yield a product with an acid value of 1.566 and FFA% of 0.583. This resembles a total drop in the acidity of CPO/RSO blend sample which was equivalent to more than 95% of the original value prior to acid transesterification.

The result shows that base transesterification can be carried out for the product obtained from trial number six since it meets the required criteria of alkali process in which the FFA% needs to be below 1% [2].

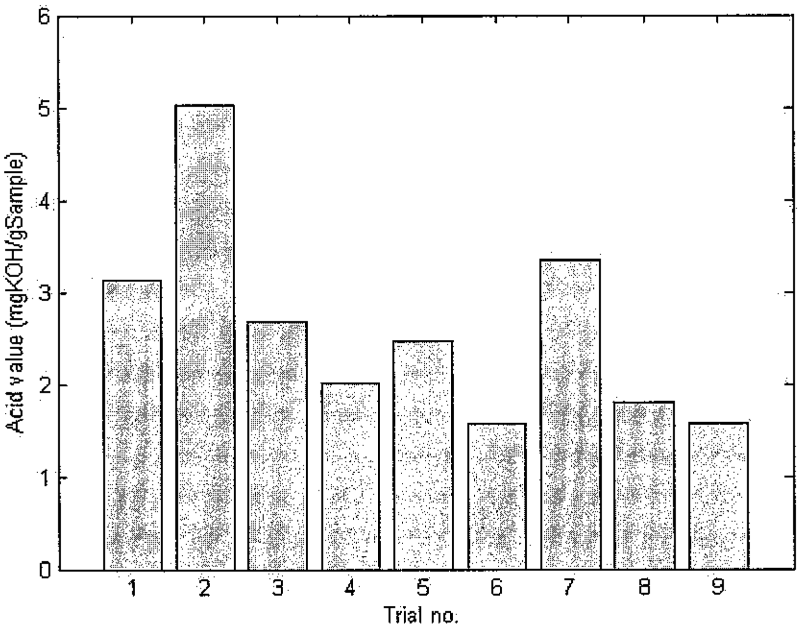


Figure 4-6 Acid value result for Acid esterification

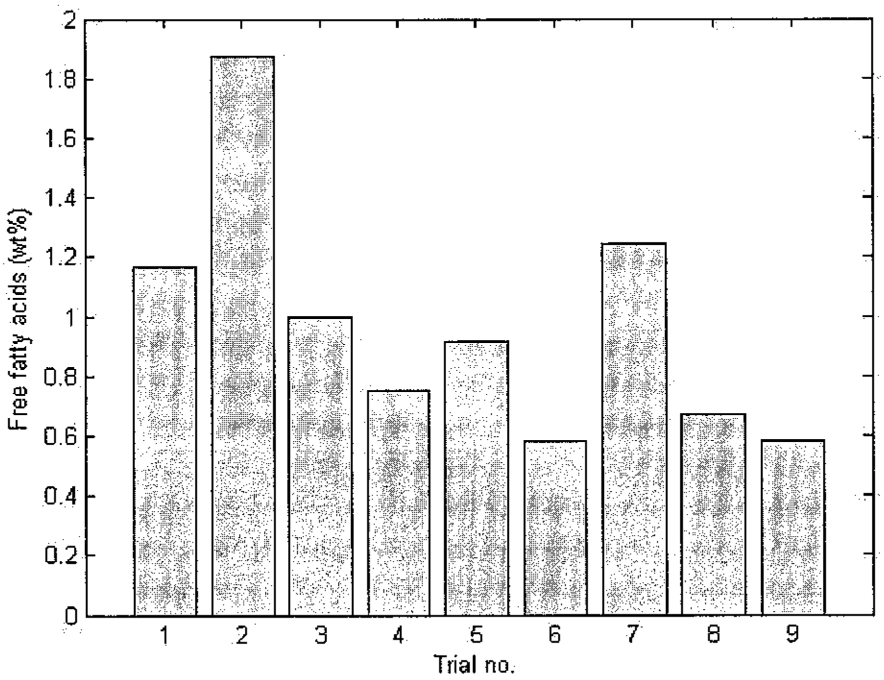


Figure 4-7 FFA Result for Acid esterification

4.3.2 Independent effect of reaction variables

Optimization of reaction conditions was studied and results were obtained following Taguchi design of experiments method. However, additional experiments were required to understand and study the effect and significance of each variable separately. In addition, time had to be investigated independently for optimum reaction conditions to study the change in reaction mixture concentration over time.

4.3.2.1. Effect of catalyst amount

Different catalyst amount were used in the study. Potassium hydroxide at 0, 0.5, 1 and 2%wt of blend were used. Results are given in Figure 4-8. The other reaction variables were held constant. Alcohol to oil ratio was set at 15/1 with reaction temperature of 55°C while the reaction was running for three hours. The reaction without catalyst could not lower the free fatty acids to acceptable levels. The result was found to be still considerably high with free fatty acids value of 4.27%wt.

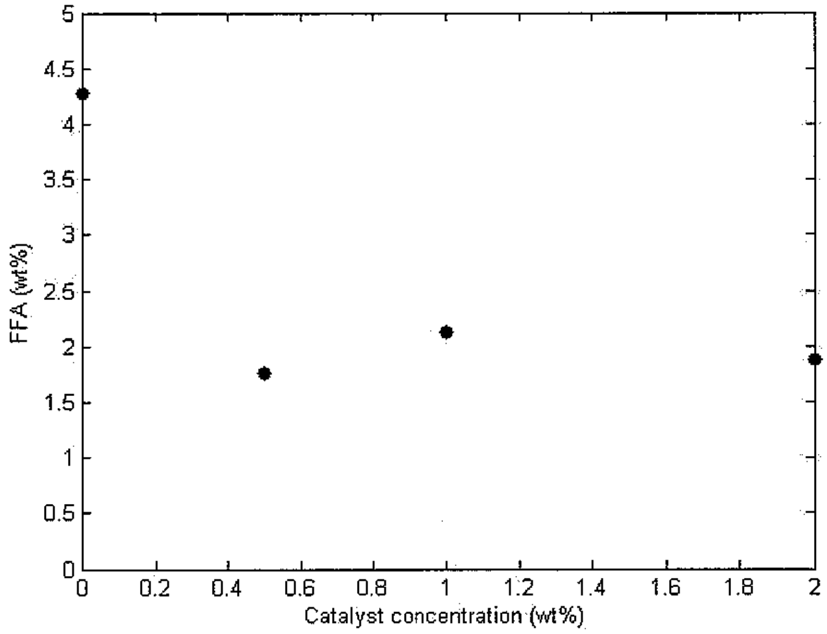


Figure 4-8 FFA% vs. Catalyst amount at 55°C, 15/1 aclohol to oil ratio and 3 hours

The best amount of catalyst was 0.5%. At this value, the lowest free fatty acid content could be obtained in the product. Acid value was higher when catalyst amount increased as it can be noticed from Figure 4-8. It was also noticed that the presence of triglycerides was reduced by increasing the amount of acid catalyst. It is then understood that increasing catalyst amount led to increasing the rate of triglycerides transesterification reaction over free fatty acid esterification. However, using lower catalyst amount catalyzed the reactions oppositely. At this stage, free fatty acid reduction in the main concern. Thus, lower amount of catalyst is preferred.

Choosing lower catalyst percentage is also preferable from economic point of view since fewer amounts will be involved in the reaction. It's totally feasible in this case since it doesn't affect the results. Catalyst amount of 5wt% was studied as suggested by other researchers [114,120]. From experiment observation, it resulted in reaction poisoning which could be understood from the dark black color of products. The darkening of products shows that the amount of sulfuric acid was sufficient to stop esterification reaction and destroy the oil.

The optimum catalyst amount concluded in this study was found to be sufficient in acid alcoholysis by other researchers as well. Ramadhas et al. [31] suggested 0.5% sulfuric acid for acid esterification of rubber seed oil. Keim et al [116] proposed sulfuric acid amount which is close to the one found here. He found that 0.75% of catalyst is sufficient for acid value reduction of palm oil that has FFA% which exceeded 50.

4.3.2.2. Effect of temperature

Increasing the temperature resulted in decreasing the amount of free fatty acids in acid esterification products. As it can be seen from Figure 4-9, the lowest free fatty acid result could be noticed at 65°C. This was the highest temperature to be achieved at atmospheric pressure. The rest of the variables were kept constant. These include three hours reaction time with 1wt% Catalyst and 15/1 molar ratio of methanol to oil. Increasing the heat increases the conversion rate of free fatty acids to methyl esters which led to acid value reduction.

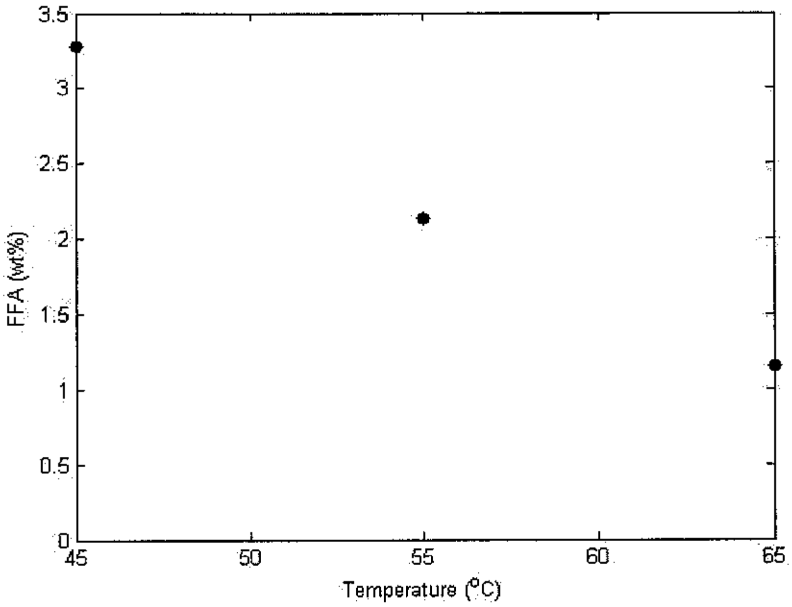


Figure 4-9 FFA% vs. Temperature at 1wt% catalyst, 15/1 aclohol to oil ratio and 3 hours

4.3.2.3. Effect of alcohol/oil ratio

The molar ratio of alcohol to oil was varied across experiments to test the effect of different reactants proportions. Methanol was selected at molar amounts of 10/1, 15/1 and 20/1 with respect to crude palm/rubber seed oil blend. The excess amount of alcohol was introduced to shift the reaction equilibrium toward the forward reaction. This is to increase production of methyl esters from free fatty acids which will reduce acid value accordingly. The results can be seen in Figure 4-10.

However, increasing the alcohol amount has its draw back. The product mixture takes longer separation time particularly for the products of acid esterification when high amount of alcohols were introduced at 20/1 alcohol to oil ratio. This effect was also concluded by Freedman et al. [87] when methanol was used in acid esterification of soybean oil.

Molar ratio of 15/1 was found to be sufficient in reducing the amount of free fatty acid and would ease product separation. Spurles et. al [114] recommended the same alcohol to oil ratio in his study where acid esterification of high free fatty acid oil sources was conducted.

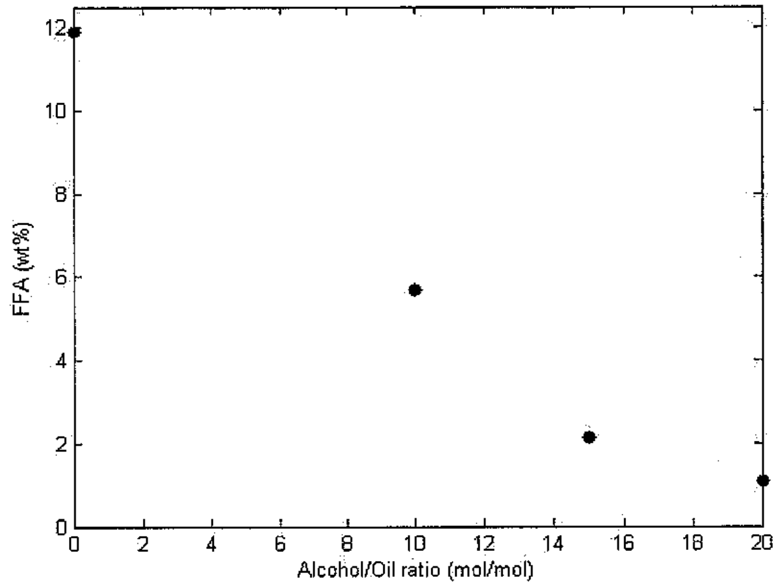


Figure 4-10 FFA% vs. Alcohol/Oil ratio at 55°C, 1wt% catalyst and 3 hours

4.3.2.4.Effect of time

Effect of time was studied for the set of optimum reaction conditions obtained using design of experiments. The optimum conditions at which time was studied were alcohol to oil ratio of 15/1 with temperature at 65°C and sulfuric acid of 0.5 wt%. Eleven readings were taken at 0, 3,5,10,25,60,90,120,180,240 and 300 minutes. At these time intervals, samples were withdrawn quickly from reaction chamber and analyzed with thin layer chromatography. The test was repeated to verify the data that was plotted in Figure 4-11.

Free fatty acids were reduced as the time proceeded. The initial 25 minutes of acid esterification were sufficient to reduced FFA below 1wt%. At the end of five hours of reaction time, free fatty acids almost reached 0.1 wt% in the products, accounting for a total reduction of 98.9% in free fatty acids.

Keim et al. [116] found that one hour is sufficient for the reduction of free fatty acids from 50.8% to 0.75% using the same catalyst. Tanaka et. al maintained the temperate at 65°C while the reaction time ranged between 0.5 to 2 hours to reduce the FFA content to acceptable amount [93]. Rubber seed oil acid treatment was carried out using sulfuric acid at the same concentration and 20-30min reaction time was found sufficient to reduce acid value by Ramadhas et al.[31].

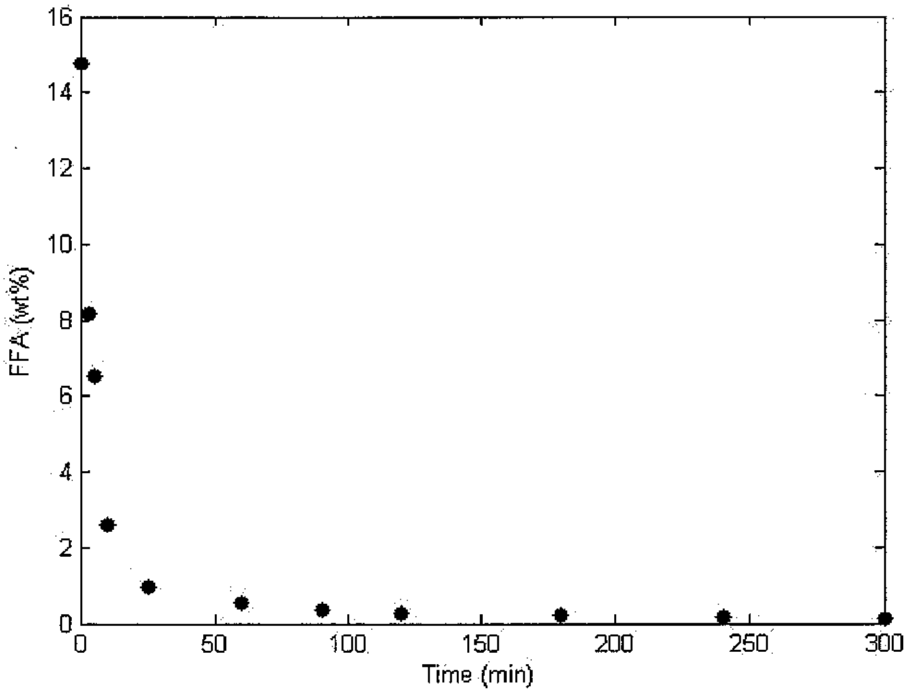


Figure 4-11 FFA% vs. Time at 55°C, 1wt% catalyst and15/1 alcohol to oil ratio

The reaction mixture composition was monitored for five hours. By looking at Figure 4-12, it can be seen that the formation of methyl esters was divided into two regions. The initial region covers the first 10 minutes of acid esterification reaction while the second one covers the remaining time up to 300 minutes of total reaction time.

In the first region, it can be noticed that methyl esters formation was accompanied by noticeable drop in free fatty acids. This leads to understand that the reaction in the initial region is controlled by free fatty acid alcoholysis reaction. Figure 4-13 shows the surge in methyl esters formation as the amount of free fatty acid drops down during the first ten minutes of acid esterification.

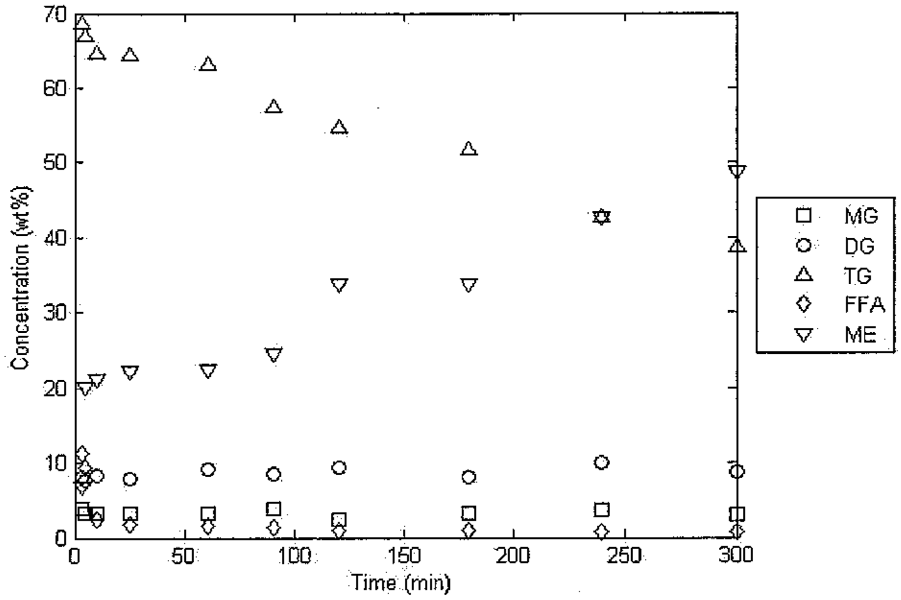


Figure 4-12 Product composition change with respect to time

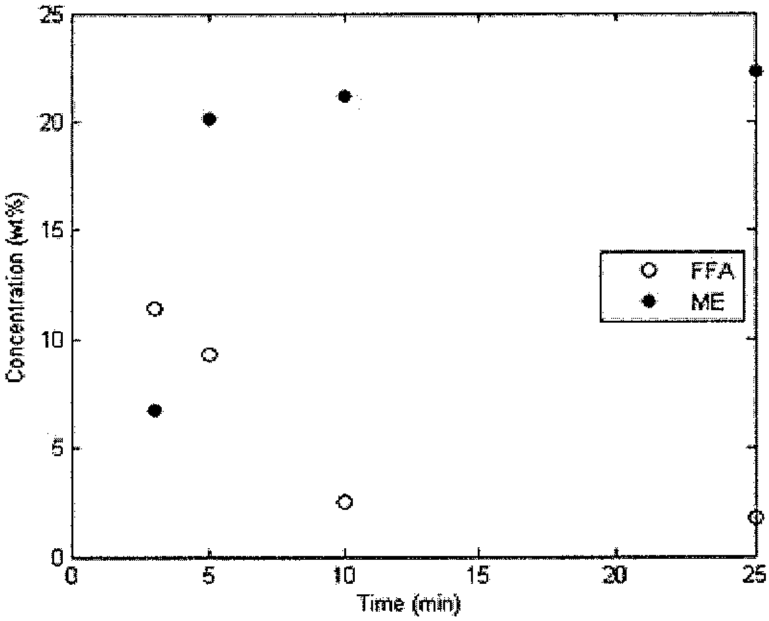


Figure 4-13 Region one in Acid esterification

On the other hand, methyl esters formation in the second region is totally dependent on triglycerides since free fatty acids are not left in the reaction mixture. Mono and diglycerides remain at constant levels throughout the five hours of reaction time which was not sufficient for total conversion of triglycerides to methyl esters. The second region is shown in Figure 4-14.

Freedman et al (1986) found that 99% conversion of triglycerides to methyl esters occurred after 50 hours of reaction time when sulfuric acid and methanol were used [87]. This fact resembles the importance to carry out base transesterification for the products of Acid esterification. Base transesterification was reported to give higher conversion of trilgycerides to methyl esters at shorter reaction time.

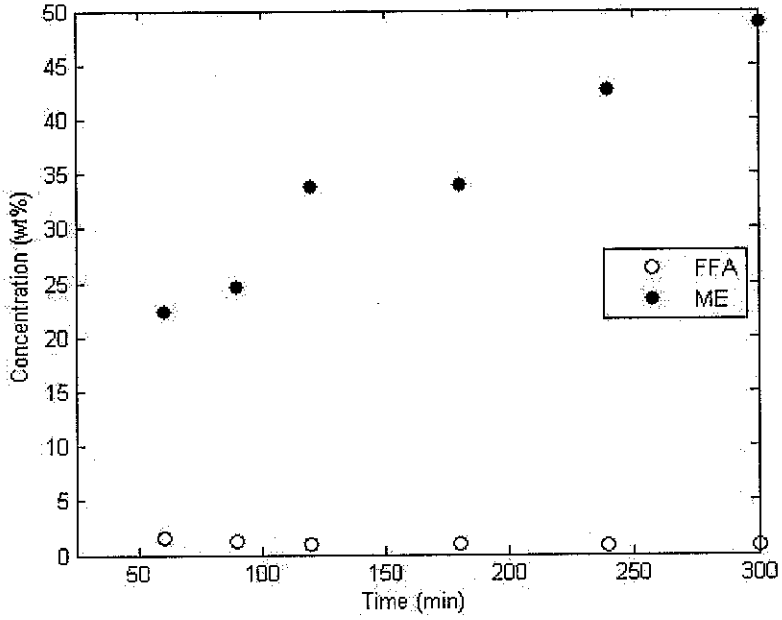


Figure 4-14 Region two in Acid esterification

Table 4-16 Optimum acid esterification sample composition analysis

Compound	**TLC (wt%)**	**GC (wt%)**
MG	4.70	3.02
DG	7.30	5.41
TG	39.3	33.0
ME	49.1	56.1

The method for TLC and GC is given in the Appendix (Page 170,173).

4.4 Base Transesterification

4.4.1 Determination of optimum reaction conditions

Taguchi technique for design of experiments was used as well in base transesterification to investigate optimum reaction conditions that will lead to the highest conversion of methyl esters. Reaction variables which had been covered in the study include alcohol to oil ratio, temperature and catalyst amount. The variables, their levels and L9 experiments table were presented in section 3.2.

After analyzing the products of experiments with thin layer chromatography, trial number five was found to give the highest conversion of methyl esters in the product obtained. The conversion exceeded 96% as it is shown in Figure 4-15. Base transesterification was conducted for three hours while mixing rate was held constant at 350 rounds per minute. The optimum conditions were at temperature of 55°C, alcohol to oil molar ratio at 8/1 and catalyst concentration of 2wt% of weight of oil.

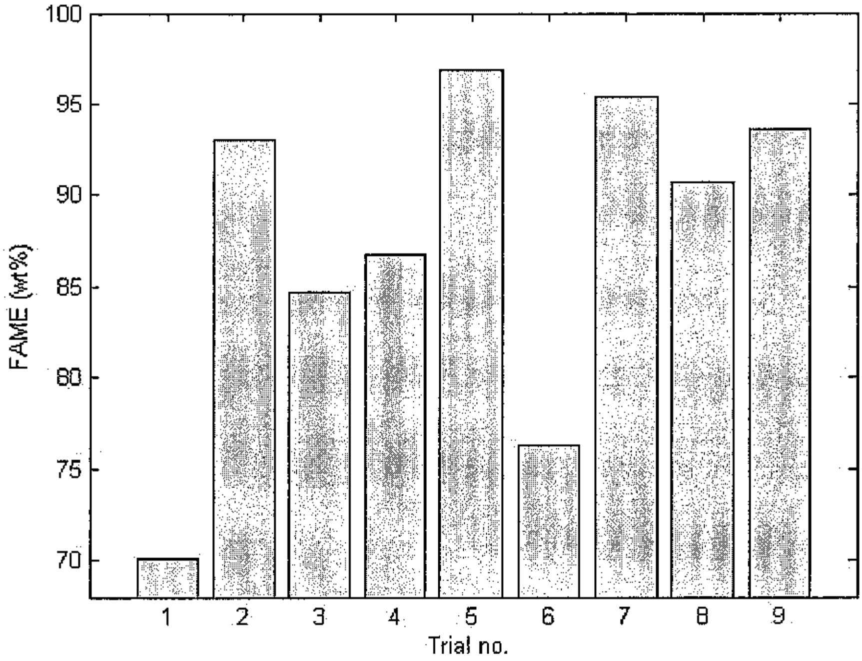


Figure 4-15 FAME results in base transesterification

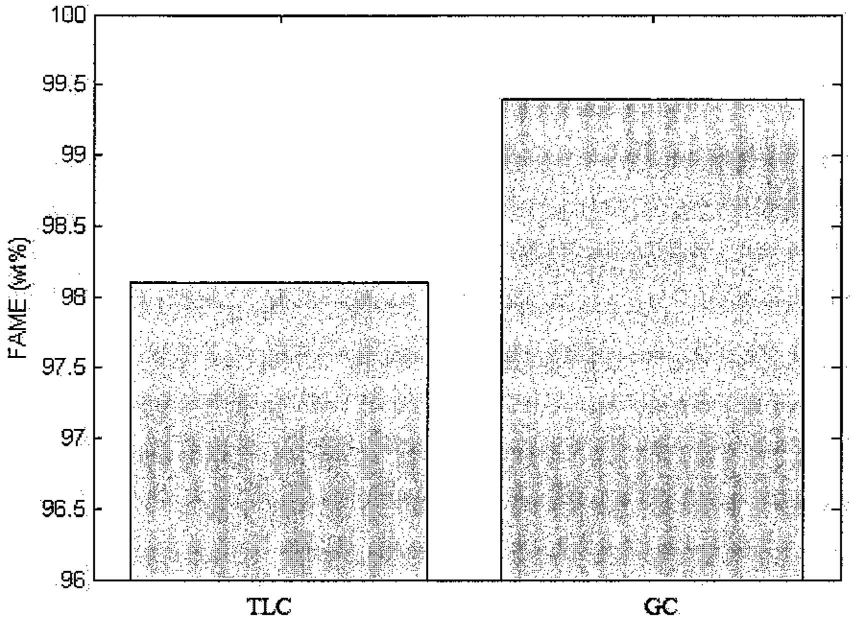


Figure 4-16 Comparison between the result obtained from TLC and GC for FAME content in the optimum product from base transesterification after 5 hours of reaction time

Figure 4-16 compares the result obtained from the analysis of product for fatty acid methyl esters by using thin layer chromatography and gas chromatography. Although this layer chromatography is less accurate from gas chromatography, it was successful in providing good results as presented in the figure.

4.4.2 Independent effect of reaction variables

Further experiments were conducted to show the significance of each variable over the conversion of methyl esters in base transesterification. The effect of time is also studies to find the best reaction time at optimum conditions which had been found in section 4.4.1.

4.4.2.1. Effect of catalyst amount

The catalyst amount was varied at 0, 0.5,1 and 2 %wt of weight of acid treated oil. The non-catalyzed reaction did not give any significant result when compared with conversions obtained when catalyst is present. This can lead to understand that triglyceride transesterification may not proceed to give good conversion without catalyst.

The highest conversion of fatty acid methyl esters was obtained when 2wt% of catalyst was used. This is clearly shown in Figure 4-17. Increasing catalyst amount was shown to shift the conversion of products positively while holding the rest of conditions with temperature at 55°C and alcohol to oil ratio of 8/1.

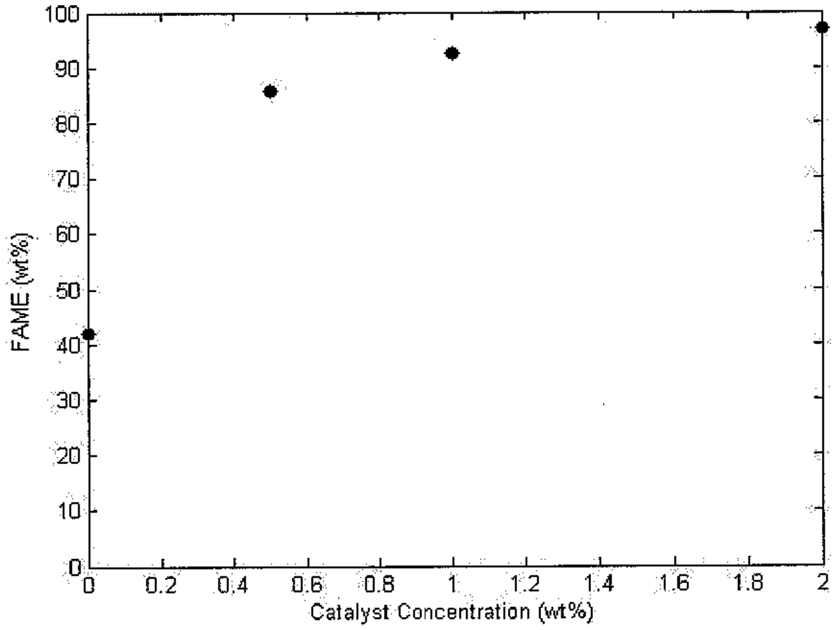


Figure 4-17 FAME% vs. Catalyst amount at 55°C, 8/1 alcohol to oil ratio and 3 hours

Potassium hydroxide was the catalyst of choice since it is the mostly used alkali catalyst in industry. Potassium hydroxide is preferred over the rest because it gives softer soap in transesterification as it had been found by other researchers [110,88]. Others had proposed that it gave the highest conversion in their studies [96,112,101].

The amount of catalyst that was found to give the highest conversion of methyl esters was compared to different results obtained from literature. Colucci et al. prepared methyl esters with conversion reaching 95% using 2.2% potassium hydroxide [101]. Georgogianni et al. used the same catalyst when they transesterified sunflower seed oil at a catalyst amount of 2.0 wt % [111].

4.4.2.2. Effect of temperature

The results obtained from the study on effect of temperature are shown in Figure 4-18. Fatty acid methyl esters conversion increased by increasing temperature. The temperature was varied from 45°C to 65°C which is methanol boiling temperature.

The highest conversion was observed at that temperature as well. This shows that increasing heat applied will increase methyl esters conversion rate and would shift equilibrium of reaction towards products side.

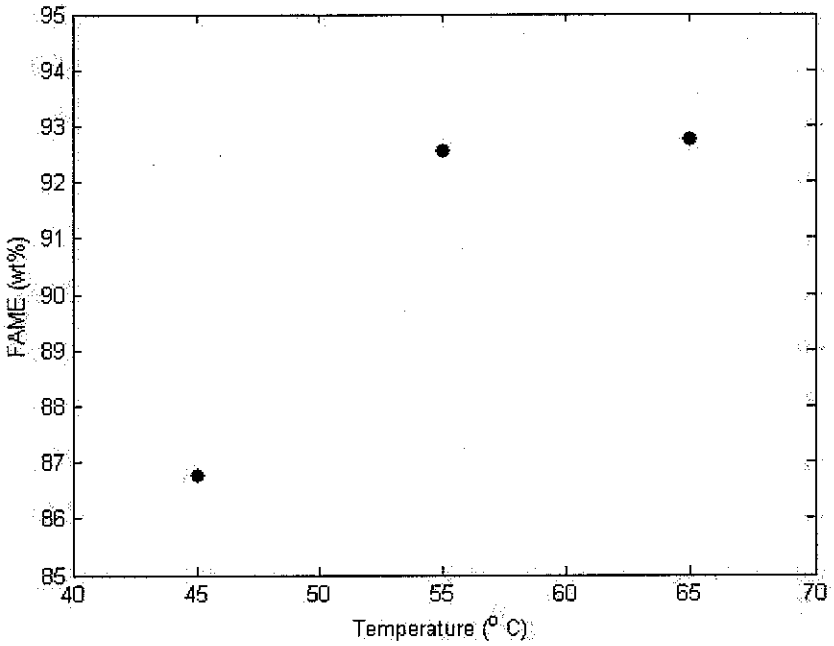


Figure 4-18 FAME% vs. Temperature at 2wt% catalyst, 8/1 alcohol to oil ratio and 3 hours

Freedman and his colleagues (1984) experimented on refined soybean oil using methanol for transesterification. They made a number of runs over different temperatures and commented in their research that higher temperatures are favored by the reaction and gave higher reaction rate and conversion [95].

4.4.2.3. Effect of alcohol/oil ratio

Methanol was fed at different molar ratios with oil including 6/1, 8/1 and 10/1. As discussed previously in literature review chapter, the actual ratio needs to be maintained higher than the stoicheometry one (3/1). It had been shown that base transesterification can't proceed to give high conversions of methyl esters without increasing the amount of alcohol to shift reaction equilibrium [2,84]. Based on the results obtained in this study, 6 to 1 was the best ratio to give products without most conversion of methyl esters. Ratios exceeding this value resulted in lower conversions due to saponification reaction which increased at these higher molar ratios. In addition, large excess in the amount of alcohol made the phase separation of products and unreacted material much harder than lower amounts of alcohols.

The same molar ratio of 6 to 1 was found to be the optimum by several other researchers [94,95]. Nye et al. [94] transesterified rape seed oil with methanol and commented that 6/1 alcohol to oil ratio gave the highest yield. Soybean oil was used by Freedman and his colleagues with the same ratio of reactants [95,2]. Zhang et al. experimented different alcohol ratios and found that 6/1 was the best molar ratio for Tallow and methanol [97]. Colucci et al. carried out a study on refined soybean oil as well and proposed the same molar ratio of 6/1 [101].

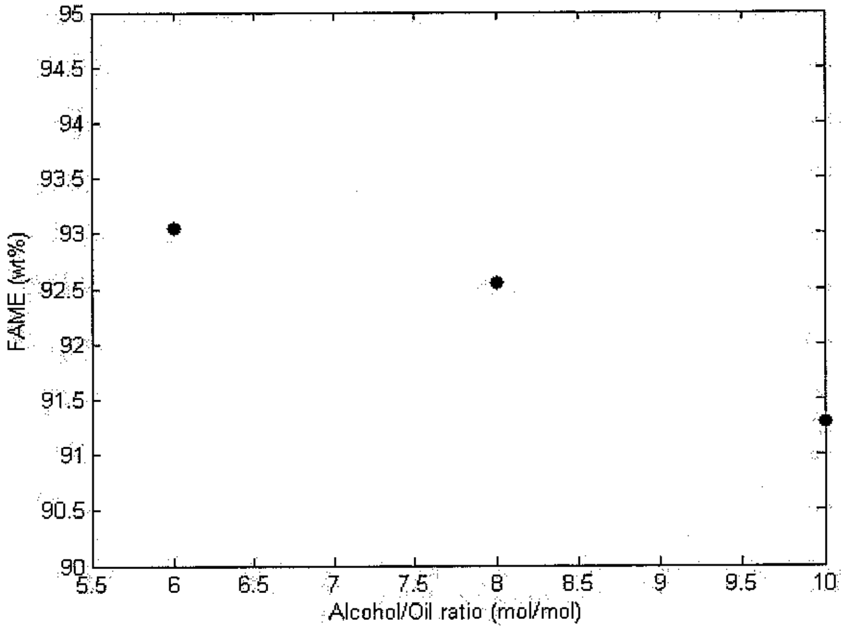


Figure 4-19 FAME% vs. Alcohol/Oil ratio at 55°C, 2wt% catalyst and 3 hours

Figure 4-19 shows methyl ester conversions that were obtained from different alcohol to oil ratios. It is clear that 6 to 1 ratio proved to be the optimum reactants ratio to give the highest conversion to FAME.

4.4.2.4. Effect of time

In conjunction with other reaction parameters being investigated under base transesterification, time represents a very important factor that can be used independently to optimize the results obtained. Reaction time was elongated while the rest of reaction conditions that gave the highest conversion of methyl esters were kept constant. These included temperature at 55°C with alcohol to oil molar ratio at 6/1 and catalyst amount of 2 wt% of oil.

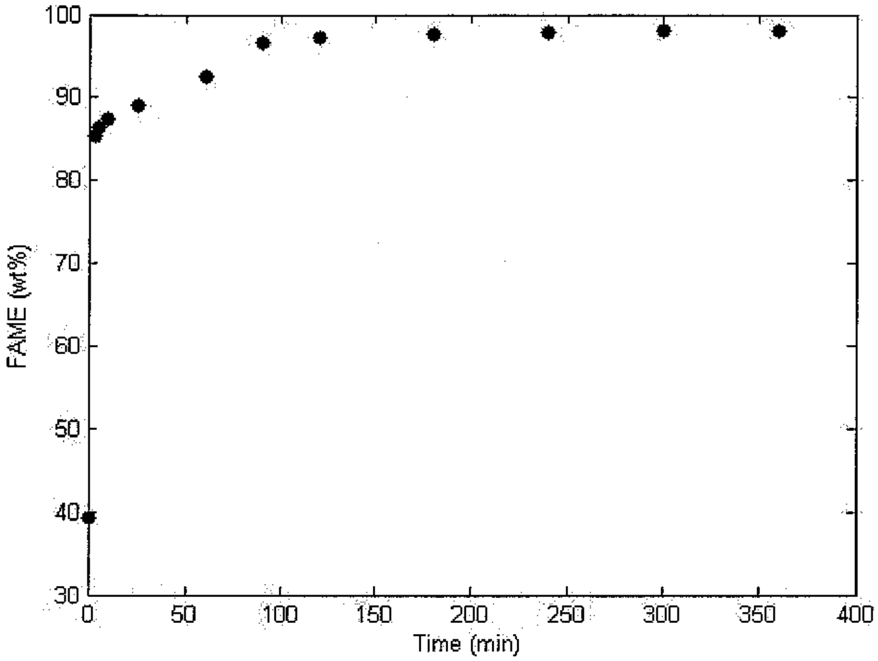


Figure 4-20 FAME% vs. Time at 55°C, 2wt% catalyst and 8/1 alcohol to oil ratio

During the course of reaction, twelve readings were obtained by rapidly taking samples from reaction vessel and spot them on silica plates for thin layer chromatography analysis of products. The time intervals at which samples were withdrawn were 0 , 3 , 5 , 10 , 25, 60, 90 , 120 , 180, 240, 300 and 360 minutes. The experiments and analysis were repeated to minimize errors. Figure 4-20 shows the result for time investigation.

Fatty acid methyl esters conversion surged during the initial reaction period that covered the first 25 minutes while it started to slow down in the period thereafter until a steady conversion rate was noticed after 3hr of reaction time. The conversion curve had minimum change in the last three hours. The conversion achieved after six hours of alkali catalyzed transesterification exceeded 98.1 wt%. The conversion after five hours was 98.05 wt% which is not far from the final time. Both conversions exceeded the limit set by European standard which enforce 96% methyl esters content in biodiesel [3].

Results obtained were compared with other researchers. Lifka et al. [99] stated that methyl esters obtained from rapeseed oil by base transesterification reached 80-85% conversion after 30 minutes. In the current study, methyl esters conversion could reach 89% within the first 25 minutes. Liu et al. reached 95% conversion after 1.5 hour using the same alcohol while maintaining higher temperature at 65°C [89]. The conversion of methyl esters reached 96% within 1.5 hours in the current study

It can be seen from the comparisons that higher conversion could be reached at shorter reaction time in the current study. In addition, lower temperature was used to reach similar conversion. This will result in energy saving for biodiesel process.

Alkali medium has shown to provide faster conversion of triglycerides to methyl esters when compared with that of acid. Looking at Figure 4-20 for base transesterification and Figure 4-12 for acid esterification, it can be noticed that methyl ester conversion is much faster in alkali catalyzed reaction than that of acid catalyzed alcoholysis.

4.5 Biodiesel Quality

The quality of biodiesel obtained confirmed to that of ASTM and EN criteria [3,4]. Table 4-17 shows the results for quality analysis of biodiesel generated at 50:50 blend ratio of rubber seed oil to crude palm oil.

Table 4-17 Properties for biodiesel produced with comparison to international standards.

Property	Unit	Value	ASTM Criteria	EN Criteria
Esters	% mass	99.4	-	96.5 min
Density	g/cm^3	0.874	-	0.860-0.900
Kinematic Viscosity	cSt	4.22	1.9-6.0	3.5-5.0
Flash point (closed cup)	°C	150	130 min	120 min
Sulfur content	% mass	0.01	0.05 max (S500)	0.1 max
Water and Sediment	% volume	< 0.02	0.05 max	0.05 max
Distillation temperature	°C	311	360° max	-
Cetane number	No units	50.1	47 min	51 min
Methanol content	Pass/Fail	Pass	97°C min	-
Heating Value	KJ/g	38.5	-	35 min
Acid number	mgKOH/g	0.46	0.5max	0.5 max
Monoglycerides	% mass	0.063	-	0.8 max
Diglycerides	% mass	0.128	-	0.2 max
Triglycerides	% mass	0.095	-	0.2 max
Carbon Residue	% mass	0.03	0.05 max	0.3 max
Free Glycerin	% mass	0.002	0.02 max	0.02 max
Total Glycerin	% mass	0.0484	0.24 max	0.25 max

ASTM: American society for testing and material standard [3]
EN: European standard [4]

Biodiesel was obtained by two stage transesterification where acid esterification was followed by base transesterification. The optimum conditions of acid esterification were temperature of 65°C with alcohol to oil ratio of 8:1 and catalyst amount of

0.5wt%. The optimum conditions for base transesterification were temperature at 55^{0}C, alcohol to oil ratio of 6:1 and catalyst amount of 2wt%. The reaction time to reach 98.1% conversion was 6 hours. Five hours were sufficient however to reach 98.05% conversion.

4.6 Kinetic Study

4.6.1 Acid Esterification

Acid esterification consists of two parallel reactions taking place simultaneously. The first of which is where methanol esterifies triglyceride to yield methyl esters and glycerol while simultaneously the alcohol also esterifies the free fatty acids to give methyl esters and water.

Most of kinetic studies [160,153] involved in biodiesel production using acid esterification route consider the first reaction and neglect the second one. None of the published literature had studied and developed rate equation particularly for free fatty acid esterification. It is very important to understand the kinetics of this reaction knowing that free fatty acids are the limiting reactant for the second reaction. In addition, studying this reaction will help shaping the kinetics for biodiesel production for material containing high FFA.

Free fatty acids are esterified using methanol based on the following reversible reaction:

$$\text{FFA} + \text{Methanol} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{Methyl Ester} + \text{Water} \tag{4.2}$$

The concentration of free fatty acids, on equal oleic and palmitic basis, was calculated at different time intervals. This is provided in Table 4-18. The first derivative of free fatty acid concentration with respect to time $-dCffa/dt$ was evaluated using several techniques as it was explained in section 3.5. A comparison between all of the values obtained is provided in Table 4-19.

Table 4-18 FFA concentration throughout the reaction

Time (min)	Concentration $\times 10^{-1}$(mol/ml)
0	1.010
3	0.560
5	0.440
10	0.170
25	0.065
60	0.038
90	0.023
120	0.016
180	0.013
240	0.010
300	0.009

Table 4-19 -dCffa/dt for FFA esterification from all analysis techniques

Time (min)	Differential method (mol/ml.min)		Regression (mol/ml.min)
	Graphical	Finite difference	
0	0.012	0.080	0.018
3	0.004	2.83×10^{-3}	0.010
5	1.56×10^{-3}	2.69×10^{-3}	0.006
10	5.25×10^{-4}	3.77×10^{-4}	0.002
25	5.09×10^{-5}	3.86×10^{-5}	3.30×10^{-4}
60	1.50×10^{-5}	2.54×10^{-5}	2.87×10^{-5}
90	4.53×10^{-6}	1.07×10^{-5}	8.08×10^{-6}
120	3.50×10^{-6}	2.65×10^{-6}	3.07×10^{-6}
180	1.50×10^{-6}	2.46×10^{-6}	6.60×10^{-7}
240	5.96×10^{-7}	1.32×10^{-6}	1.62×10^{-7}
300	N/A	N/A	2.05×10^{-8}

Using the values tabulated in Table 4-19, a plot of natural logarithm for free fatty acid concentrations against its derivative was made and shown in Figure 4-21. GM, FDM and NLRM represent graphical method, finite difference method and regression method. The residuals were plotted to verify the results. The scattered distribution of residual data points confirms that the results of plots are acceptable since the error function is not uniform [163]. Data points are then linearly fitted. From fitting results, reaction order can be obtained. Table 4-20 shows the fitted results

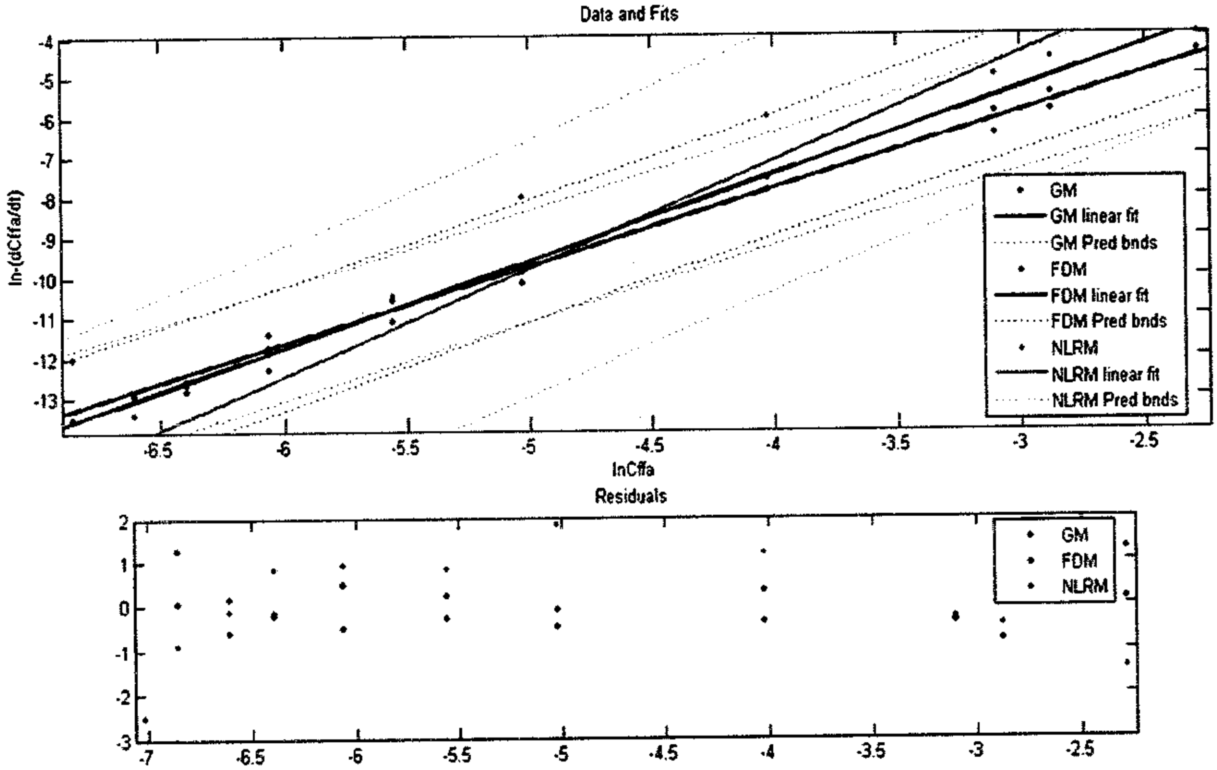


Figure 4-21 Natural logarithmic plot of –dCffa/dt vs. Cffa and the residuals for all analysis techniques

The general fit equation is of the form of y = mx + c, where m represents the slope. When compared to the linear fits shown in Figure 4-21, the slope represents reaction order (α). The fit was produced at 95% confidence interval and the goodness of fit is provided by four methods. Least square analysis, R^2, Modified R^2 and root mean square analysis. As it can be seen from results in Table 4-20, the reaction order ranged from 1.9 to 2.6 with an average value of 2.24.

Table 4-20 FFA esterification reaction order from all analysis techniques

Method of Analysis	Equation constants		Goodness of fit			
	m	c	SSE	R^2	Mod.R^2	RMSE
Graphical	1.91	-0.16	2.62	0.97	0.96	0.57
Finite difference	2.14	1.11	3.14	0.97	0.97	0.62
Regression	2.67	3.57	16.5	0.92	0.91	1.35

By taking the average value of concentration derivative $-dCffa/dt$ from three methods presented in Table 4-19 with free fatty acid concentrations in Table 4-18 and average reaction order obtained from Table 4-20, it is possible to calculate the reaction rate constant k' using Equation 4.3. The results are shown in Table 4-21

$$k' = \frac{dC_{ffa}/dt}{C_{ffa}^{\alpha}} \tag{4.3}$$

Table 4-21 FFA esterification reaction rate constant

Average dC_{ffa}/dt (mol/ml.min)	C_{ffa} (mol/ml)x10^{-3}	k' x10^3(lit/mol.min)
5.99 x10^{-2}	101	10.2
5.36 x10^{-3}	56.0	3.44
4.02 x10^{-3}	44.8	4.29
1.07 x10^{-3}	17.8	9.00
1.36 x10^{-4}	6.54	10.8
2.66 x10^{-5}	3.84	7.04
9.88 x10^{-6}	2.31	8.19
2.79 x10^{-6}	1.66	4.83
1.86 x10^{-6}	1.34	5.20
9.40 x10^{-7}	1.05	4.57

The average rate constant was found to be 6.769x10^3. Using all the values that had been calculated previously, the rate equation for free fatty acid esterification to methyl esters may be written based on power law in the following form:

$$-r_{ffa} = -\frac{dC_{ffa}}{dt} = \left(6.769\times10^{3}\right)C_{ffa}^{2.24} \tag{4.4}$$

Nonlinear modeling, discussed previously in section 3.5, was used to establish another rate model to represent free fatty acid esterification. The rate equation which had been obtained is shown in Equation 4.5.

$$-r_{ffa} = -\frac{dC_{ffa}}{dt} = \frac{0.5265\,C_{ffa}^{2} + 0.1406\,C_{ffa} + 0.0003107}{C_{ffa}^{4} + 0.2034\,C_{ffa}^{3} + 0.6715\,C_{ffa}^{2} + 1.2\,C_{ffa} + 0.9041} \tag{4.5}$$

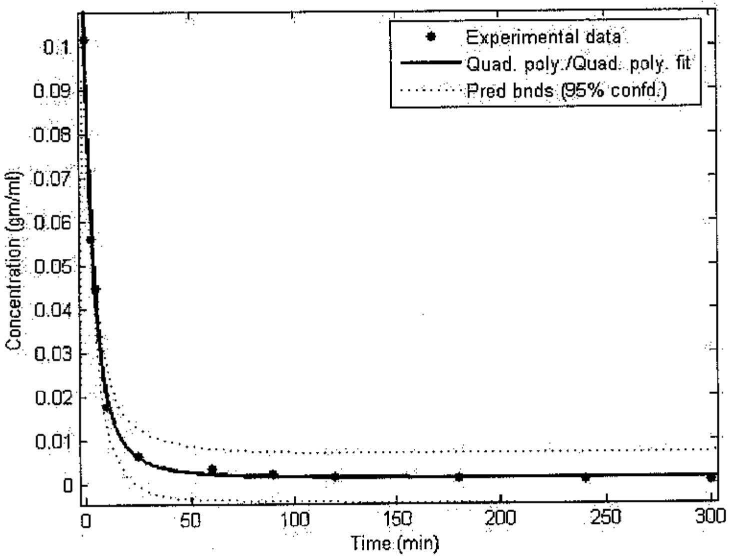


Figure 4-22 Nonlinear modelling result for optimum reaction conditions at 55°C ,8:1 alcohol to oil ratio and 0.5wt% catalyst

The nonlinear rate law was verified by fitting experimental data to the curve obtained from Equation 4.5 with 95% confidence prediction bounds. This is shown in Figure 4-22. The goodness of fit was verified with sum of squares and R^2 analysis. Both gave good results with values of $2.67\text{x}10^{-5}$ and 0.997 respectively.

When comparing previous rate laws obtained from differential method and nonlinear modeling, it is important to state that nonlinear modeling gave better representation for experimental data. If goodness of fit is compared for both terms, it can be seen that R^2 and sum of square analysis results provide that nonlinear modeling term is more accurate statistically. Activation energy and Arrhenius constant were calculated for free fatty acid esterification based on the methodology described in section 3.5. The average rate constant for FFA esterification at 45°C was found to be 26.094x10^{3}. Activation energy and Arrhenius constant were then obtained with values of 14.06 x 10^{9} and 60.289 KJ/mole respectively.

4.6.2 Base Transesterification

Base transesterification was studied using the same methodology explained in section 3.5. The differential method could not yield an acceptable rate equation that can represent concentration data which is shown in

Table 4-22. Nonlinear modeling was used to obtain a mathematical expression that resembles methyl esters conversion. The conditions of reaction were at temperature of 55°C with alcohol to oil ratio of 6 to 1 and catalyst amount of 2wt%.

This model obtained is shown in Equation 4.6. In addition, Figure 4-23 shows the fit of this equation to experimental data obtained. The fitness was verified with least sum of squares analysis, R^2, Adjusted R^2 and Root mean square analysis. The results were 6.948 x 10^{-5}, 0.999, 0.999 and 0.002 respectively. It can be seen that the plot passes all the statistical tests.

$$r_{ME} = \frac{dC_{ME}}{dt} = \frac{k_1 C_{ME}^{\beta'}}{1 + k_1 C_{ME}^{\beta'}} \qquad (4.6)$$

The values for k_1 and β' in Equation 4.6 were 1.304 x 10^{-4} and -6.706 respectively.

Table 4-22 Methyl esters concentration throughout base transesterification

Time (min)	**Concentration (mol/ml)**
0	0.270
3	0.586
5	0.594
10	0.601
25	0.612

60	0.635
90	0.664
120	0.668
180	0.671
240	0.673
300	0.674
360	0.675

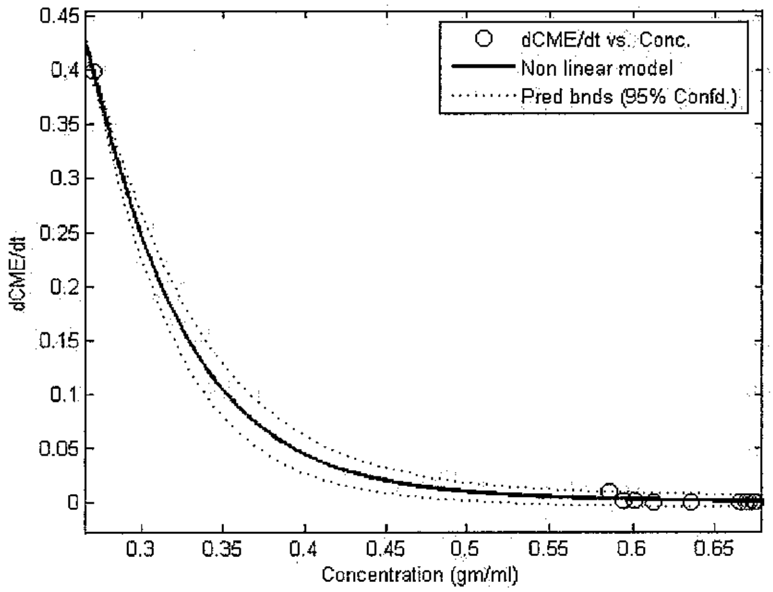


Figure 4-23 Methyl esters concentration time derivative vs. concentration as obtained by the proposed model at 55°C, Alcohol to oil ratio of 6 to 1 and catalyst amount of 2wt%

As it can be seen from Figure 4-23, time derivative for methyl ester concentration had to be utilized to obtain the plot. Advanced curve fitting toolbox ® in MATLAB 7.0 was used to obtain the values of derivative from concentration data. A plot of concentration versus time had to be established first, the plot is shown in Figure 4-24.

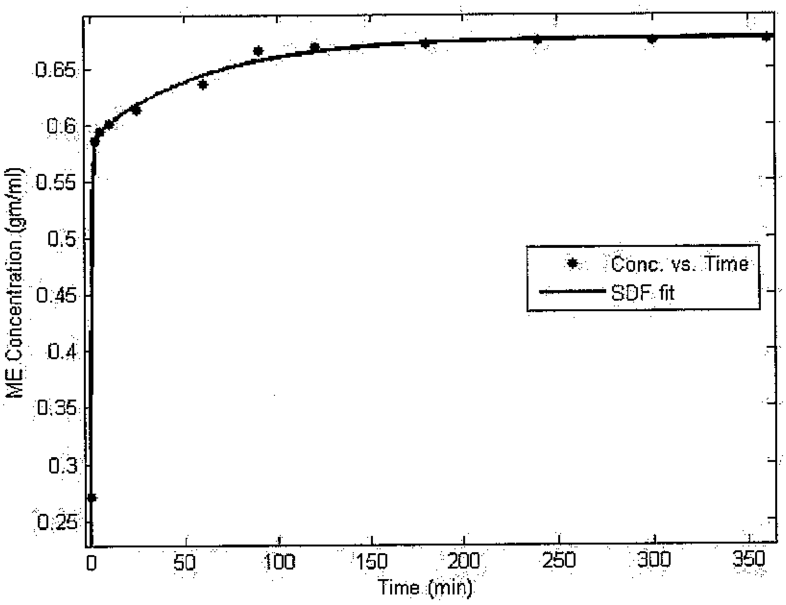


Figure 4-24 Methyl esters conversion vs. Reaction time in base transesterification

4.7 Summary over finding for Acid Esterification and Base Transesterification of CPO/RSO blend

The optimum conditions for acid esterification and base transesterification were found for both reactions in section 4.3 and 4.4 respectively. The independent effect of each variable over the output of each reaction was studied and reported in the same sections. Fatty acid methyl esters that was obtained from the optimum conditions were analyzed based on international standards [3, 4] and was found to be compatible with biodiesel quality criteria as it was provided in section 4.5.

In section 4.6, the kinetics for both reactions was investigated to obtain a rate equation that represents each of fatty acid esterification in acid esterification and methyl esters formation in base transesterification. Activation energy, Arrhenius constant and rate constants could be obtained by studying the reaction at two different temperatures. The validity of the rate equations were verified statistically by fitting experimental data to mathematical models that had been developed.

5 Conclusion

Vegetable oils can be used as an alternative for petroleum diesel either by blending them directly with the fuel or by enhancing their properties through a number of available technologies. The later is more preferred since it has less problems associated with its application. Transesterification, or alcoholysis, enhances the properties of oils and is more economical when compared with thermal cracking process that requires massive use of energy due to high process temperature and pressure. In addition, blending of vegetable oils directly with conventional diesel and microemulsion may result in engine malfunction.

One stranding factor that faces the use of transesterification is the source of raw material fed to the process. Increased demand of vegetable oils for human consumption has decreased its utilization in other applications such as biodiesel. One solution is to look for nonedible oils which may eventually replace edible sources.

A blend of crude rubber seed and crude palm oils had been investigated in this research. It has been proved in the current study that it is possible to produce biodiesel that meets international specification from such blend. Rubber seed oil is a nonedible oil that is under utilized in Malaysia. Rubber plantations are widely abundant and can produce sufficient amount of oil for the industry. But establishment of oil extraction facility and designated biodiesel mills can take some time. A solution is to blend and utilize the oil in the currently available, crude palm oil based biodiesel factories.

Rubber seed oil extraction was studied and the yield of oil had shown to be affected by several factors. Increased drying time would increase the amount of oil produced, but high temperature and longer time of drying would lead to destruction of triglycerides. Drying reduce the amount of surface moisture in the bulk solid. Presence of moisture may interfere with the solvent extraction of the oil. Drying from 1 to 3 hours resulted in oils with acceptable moisture level. The oil yield increased from 31% to 33% when drying time was increased from 1 to 3 hours. Extending drying time might be not be feasible when comparing energy consumption with the small increase in oil yield.

Increasing extraction time resulted in increasing the oil yield significantly. The period of extraction was studied from half an hour to 4 hours in which the yield was 17% and reached 33.6% eventually. n-Hexane was the solvent of choice since it gave the highest yield with rubber seed oil as proposed by other researchers [56,162]. Solvent to seed ratio was also investigated. The oil yield increased from 23% to almost 35% at solvent to seed ratio of 52 (ml/gm).

Crude vegetable oils and their blends were characterized. Acid value was recorded to be the highest for crude rubber seed oil and decreased toward crude palm oil. The acidity is an important in biodiesel production. It can identify the best route for biodiesel production since it's related to the amount of free fatty acid present in the oils. High content of free fatty acid in vegetable oils can yield significant amount of soap if base transesterification was solely used to produce methyl esters from high acid value stock.

Rubber seed oil had the highest density and viscosity values. Viscosity can lead to engine malfunction and high densities are not recommended by European standards. Transesterification of the oil blend had shown to rectify these properties to acceptable range. For instance, the final value for viscosity was reduced from 41 cSt to 4 cSt. The calorific values of the oils were acceptable. The refractive index was established for all samples. Gas chromatography and Mass spectrometer analysis for the selected oil, blend number two, was conducted to investigate the fatty acids that are present in the oil and their amount. Elemental analysis using CHNS showed that sulfur was found to be present in an acceptable amount based on international standards with its value below 0.05wt%.

Based on the characterization of oils, two stage transesterification was chosen to produce biodiesel from blend number two. Acid esterification was done to reduce the amount of free fatty acids of the oil and prepare the feedstock for the second stage of base transesterification where glycerides are converted to fatty acid methyl esters. Methanol was the alcohol of choice since it is the most economic alcohol that can be used in transesterification when compared with the others.

Several parameters that may affect transesterification reaction had been investigated. These include temperature, reactants ratio, catalyst amount and reaction time. Taguchi technique was adopted to find the optimum reaction conditions from the interaction of temperature, alcohol and catalyst amount while the time was studied independently for the optimum conditions obtained.

Acid and base alcoholysis were both studied and the optimum conditions for them were obtained. The optimum parameters for acid esterification were that with the highest reduction of free fatty acid. The optimum parameters had been found that could achieve high free fatty acid reduction with only 0.5% FFA in the product. In base transesterification, the optimum conditions are defined as that which gave the highest conversion of methyl esters. Methyl esters conversion exceeded 97wt% at the optimum conditions after three hours of reaction time and reached 98.1wt% after six hours as it was provided by TLC and 99.4wt% by GC.

Furthermore, the effect and significance of each variable over the reduction of acidity and the conversion to methyl esters was studied independently. In acid esterification, lower catalyst amount was preffered to reduce the amount of free acids while increasing the catalyst amount made no major change in amount of FFA present. The acid catalyzed reaction favored higher temperature and gave the lowest acid value. Time investigation showed that the first 20 minutes of reaction was sufficient to reduce FFA in the product to near 1%.

However, higher amount of catalyst were required to give the highest conversion of methyl esters in base transesterification. The best temperature was found to be at.55°C Increasing alcohol to the maximum ratio of 10/1 led to production of more soaps than that of 6/1 which gave good conversion results.

The quality of methyl esters produced was analyzed to match the criteria set by international standards. The properties obtained from the tests had shown that free fatty acid methyl esters obtained from the two stage transesterification matches the criteria of biodiesel.

In acid esterification, the kinetics for free fatty acids reduction was studied. Differential method and nonlinear modeling were used to obtain the rate law. From differential method, the average order was found to be equal to 2.24 with a rate constant of $6.769\text{x}10^3$ lit/gmol.min.. The activation energy and Arrhenius constant were calculated by further investigations and were found to be 60.289 KJ/mole and $14.06 \text{ x } 10^9$ respectively. Nonlinear modelling resulted in another mathematical term to represent free fatty acid esterification.

An empirical correlation was established for base transesterification to represent the concentration change in the reaction mixture using nonlinear modeling. No results could be obtained however from differential method since it failed to give acceptable results.

6 Recommendations and future developments

- Mixing effect over both acid and base transesterification needs to be investigated. A range of different mixer types and mixing speeds needs to be used with a suitable experimental setup.

- Different types of homogenous catalyst, acid and base, with the same operating conditions can be experimented to see the optimum catalyst to give best results in both reactions. Heterogeneous catalyst may also be developed and used for that matter which will enhance the separation of the final product and the economy of the process as whole.

- Other types of alcohol may be considered to explore their effect on the acidity of the product from the acid esterification and the conversion of methyl esters in base transesterification.

- Studying the two stage transesterification and the products of different blending ratios of crude palm oil and rubber seed oil and using different types of oils.

- Design of process is required for both oil extraction and biodiesel production. A turning point in the process for oil extraction would be to accept different type of raw materials to be processed using the parameters established in this research. For acid esterification process caution needs to be taken since acidic medium is involved. Design of separation process needs to be considered carefully for separation and recycling of unreacted material, catalyst and water used for washing.

- Kinetic study finding may be used to establish a pilot scale production unit and to integrate the models into process control studies. The pilot plant may serve future testing for the proposed process, using oil blend as the raw material, compatibility with large scale production before application in the industrial . The calculated kinetics parameters can be used as well to design the process reactor and help optimizing it.

- The kinetic study can be extended further more by dedicating a suitable analysis unit for monitoring the concentration of all reactants and products. This will help expand the work and make better prediction for the change in system. The proposed non linear model for the production of fatty acid methyl esters in base transesterification may be tested as well using other's data obtained for the alkali stage from two step alcoholysis.

7 References

1. Campbell, C.J., *The Coming Oil Crisis*,. Essex, England: Multi-Science Publishing Company and Petroconsultants, S.A., (1997).
2. Fangrui Maa, Milford A. Hanna, *Biodiesel production: a review*, Bioresource Technology 70 (1999) 1-15.
3. European Committee for standardization , *European Standard (EN 14214)*, Jan 2003.
4. American Society for Testing and Material, *American standard (ASTM D6751)* ,Jan 2002.
5. Gerhard Knothe, *Historical perspectives on vegetable oil-based diesel fuels*, J. Industrial Oils , Volume 12 • November 2001, 1103-1107
6. Nitschke WR, Wilson CM., *Rudolph diesel, pionier of the age of power*, Norman,: The University of Oklahoma Press;1965.
7. Dato Dr.Mohd Basri Wahid (MPOB), Mohd Zain Omar (MPIC), *Technological progress and commercialization of Biodiesel in Malaysia,* Presentation, USDA, August 2007.
8. Peterson CL, Hustruid T., *Carbon cycle for rapeseed oil biodiesel fuel,* Biomass Bioenergy 1998;14:91–101.
9. Tan RR, Culaba AB, Purvis MRI., *Carbon balance implications of coconut biodiesel utilization in the Philippine automotive transport sector,* Biomass Bioenergy 2004;26:579–85.
10. Gerpan JV., *Biodiesel processing and production,* Fuel Process Technol 2006;86:1097–107.
11. Carraretto C, Macor A, Mirandola A, Stoppato A, Tonon S., *Biodiesel as alternative fuel: experimental analysis and energetic evaluations*, Energy 2004;29:2195–211.
12. Sinha S et al., *Biodiesel Biodiesel development from rice bran oil: Transesterification process optimization and fuel characterization,* Energ Convers Manage V.49,5,2008, 1248-1257
13. Sarin R., *Jatropha-Palm Biodiesel blends: An optimum mix for Asia*, fuel 86 (2007) 1365-1371

14. Chiu C-W, Schumacher LG, Suppes GJ., *Impact of cold flow improvers on soybean biodiesel blend,* Biomass Bioenergy 2004;27:485–91.

15. Pahl, Greg. *Biodiesel: growing a new energy economy.* White River Junction, VT: Chelsea Green Publishing Co. 66, (2005).

16. Venkataraman NS, editor. *Focus on bio-diesel.* Nandini Chem J 2002;9(IX):19–21.

17. Agarwal AK, Bijwe Jayashree, Das LM., *Wear assessment In biodiesel fuelled compression ignition engine,* J Eng Gas Turbine Power (ASME J) 2003;125(3):820–6.

18. Agarwal AK, Bijwe J, Das LM., *Effect of biodiesel utilisation on wear of vital parts in compression ignition engine*, J Eng Gas Turbine Power (ASME J) 2003;125(2):604–11

19. Sastry GSR, Krishna Murthy ASR, Ravi Prasad P, Bhuvaneswari K, Ravi PV. *,Identification and determination of bio-diesel in Diesel*, Energy Sources Part A 2006;28:1337–42.

20. Sheehan J., Camobreco V., Duffield J., Graboski M., and Shapouri H., *Life cycle inventory of biodiesel and petroleum diesel for use in an urban bus*, final report for USDOE, Office of Fuel Development, and the USDA Office of Energy, by the National Renewable Energy Laboratory, NREL/ SR-580-24089 (May 1998).

21. Goering E, Schwab W, Daugherty J, Pryde H, Heakin J., *Fuel properties of eleven vegetable oils.*, Trans ASAE 1982;25: 1472–83.

22. Pryor RW, Hanna MA, Schinstock JL, Bashford LL., *Soybean oil fuel in a small diesel engine*, Trans ASAE 1982;26:333–8.

23. Bikou, *The Effect of water on transesterification kinetics of cotton seed oil with ethanol*, Chemical Engineering and Technology 22(1): 70-75, (1999),.

24. Darnoko, D. and Cheryan, M., *Kinetics of palm oil transesterification in a batch reactor*, JAOCS, 77(12): 1263-1267, (2000).

25. Food and Agriculture Organization of United Nations, Statistics Division. July 2008, *http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567*

26. World Energy Council, Survey of Energy Resources, 2007, *http://www.worldenergy.org/publications/survey_of_energy_resources_2007/ bioenergy/714.asp*
27. Pearl G.G., *Animal fat potential for bioenergy use*, Bioenergy 2002, The Tenth Biennial Bioenergy Conference, Boise, ID, Sept. 22–26, 2002.
28. Rajagopal D., Khan A., Yoo KJ, *India's Unique Sources of Fuel for Electricity and Transportation,* RAEL, University of Berkeley, October 2005.
29. Y.B. Datuk Peter Chin Fah Kui, Minister of plantation industries and commodities, Special ministerial address on Malaysia's biodiesel master plan , *An update on the advances in biodiesel production and challenges in bringing the clear-burning alternative fuel to a wider market,* The international biofuel & alternative energy conference, 5th december 2006, Kuala Lumpur.
30. Lopez GP., Laan T,*Report: Biofuels at What Cost/Government support for biodiesel in Malaysia*, September 2008, the International Institute for Sustainable Development (IISD),Geneva, Switzerland
31. Ramadhas A.S., Jayaraj S., Muraleedharan C., *Biodiesel production from high FFA rubber seed oil*, Fuel 84 (2005) 335–340.
32. Malaysia Rubber Board Statistics, *http://www.lgm.gov.my/nrstat/nrstatframe.html* as of Jan 2009.
33. Chavanne, G., *Sur un Mode d'Utilisation Possible de l'Huile de Palme à la Fabrication d'un Carburant Lourd (A Method of Possible Utilization of Palm Oil for the Manufacture of a Heavy Fuel)*, Bull. Soc. Chim. 10: 52–58 (1943).Chem. Abstr. 38:2183 (1944).
34. Ryan, T.W., Dodge, L.G., The effect of vegetable oil properties on injection and combustion in two different diesel engines. JAOCS,61,(1984), 161.
35. Diesel, R., *The Diesel Oil-Engine*, Engineering 93:395–406 (1912).Chem. Abstr. 6:1984 (1912).
36. Diesel, R., *The Diesel Oil-Engine and Its Industrial Importance Particularly for Great Britain*, Proc. Inst. Mech.Eng.: 179–280 (1912). Chem. Abstr.7:1605 (1913).
37. Diasakou, M., Louloudi, A. and Papayannakos, N., *Kinetics of the Non-catalytics Transesterification of Soybean Oil, Fuel*, 77, (1998) pp. 1297-1302.

38. International Energy Agency, *Biofuel for transport - an international prospective*, , 2004, http://www.iea.org/textbase/nppdf/free/2004/biofuels2004.pdf.
39. Sonntag, N.O.V., *Structure and composition of fats and oils*, Bailey's industrial oil and fat products, 1979a, vol. 1, 4th edition, D. John Wiley and Sons, New York, p. 1.
40. Tickell J., *From the Fryer to the Fuel Tank: The Complete Guide to Using Vegetable Oil as an Alternative Fuel*, 3rd ed., 2000.,Tickell Energy Consulting, Tallahassee, FL. 162 p
41. Pryde, E.H., *Vegetable oil as diesel fuel: Overview.* JAOCS 60, 1983.,1557-8.
42. Demirbas A., *Biodiesel production from vegetable oils via catalytic and non-catalytic supercritical methanol transesterification methods*, Progress in Energy and Combustion Science 31 (2005) 466–487
43. Eevera T. et al., *Biodiesel production process optimization and characterization to assess the suitability of the product for varied environmental conditions*, Renewable Energy , V34,3, 2009, 762-765
44. Ramadhas AS, et al., *Dual fuel mode operation in diesel engines using renewable fuels: Rubber seed oil and coir-pith producer gas,* Renew Energy V33, 9,2008, Pages 2077-2083
45. Demirbas A., *Biodiesel fuels from vegetable oils via catalytic and non-catalytic supercritical alcohol transesterifications and other methods: a survey,* Energy Conversion and Management 44 (2003) 2093–2109
46. Rakopoulos C.D et al., *Comparative performance and emissions study of a direct injection Diesel engine using blends of Diesel fuel with vegetable oils or bio-diesels of various origins*, Energy Conversion and Management 47 (2006) 3272–3287
47. Achten WMJ et al., *Jatropha bio-diesel production and use,* Biomass and Bioenergy (2008), doi:10.1016/j.biombioe.2008.03.003
48. Dos Santos I.C.F et al., *Studies of Terminalia catappa L. oil: Characterization and biodiesel production,* Bioresource Technology 99 (2008) 6545–6549

49. Marchetti J.M., Miguel V.U., Errazu A.F., *Possible methods for biodiesel production*, Renewable and Sustainable Energy Reviews 11 (2007) 1300–1311
50. Bala BK. *Studies on biodiesels from transformation of vegetable oils for diesel engines*. Energy Edu Sci Technol 2005;15:1–43
51. Stamenkovic O.S. et al., *Kinetics of sunflower oil methanolysis at low temperatures*, Bioresour. Technol, V99,5,2008, Pages 1131-1140.
52. Demirbas A., *Relationships derived from physical properties of vegetable oil and biodiesel fuels*, Fuel 87 (2008) 1743–1748.
53. Bouaid A., Diaz Y., Martinez M., Aracil J., *Pilot plant studies of biodiesel production using Brassica carinata as raw material,* Catalysis Today 106 (2005) 193–196.
54. Nut, Idris Mohammed, J. Assoc. Adv. Agric. Sci. Afr. 3:46 (1976).
55. Attah J.C. , Ibemesi J.A. , *Solvent Extraction of the Oils of Rubber, Melon, Pumpkin and Oilbean Seeds* , JAOCS, Vol. 67, no. 1 (January 1990) P:25-2
56. Topallar H., Gecgel U.. *Kinetics and Thermodynamics of Oil Extraction from Sunflower Seeds in the Presence of Aqueous Acidic Hexane Solutions*, Turk J Chem 24 (2000) , 247 - 253.
57. Official Methods and Recommended Practices of the AOCS, 5th Edition, 2nd Printing, Champaign, Ill, ISBN: 0-935315-37-7) (2007)
58. Fukuda H., Kondo A. ,Noda H. *Biodiesel Fuel Production by Transesterification of Oils*. J. Biosci. Bioeng., Vol. 92, 405-416 (2001)
59. Schlautman, N.J., Schinstock, J.L., Hanna, M.A., *Unrefined expelled soybean oil performance in a diesel engine*, Trans. ASAE 29 (1), 1986, 70-3.
60. Adams C. et al., *Investigation of soybean oil as a diesel fuel extender: Endurance tests,* JAOCS 60, 1983, 1574-9.
61. Peterson, C.L., Auld, D.L., Korus, R.A., *Winter rape oil fuel for diesel engines: Recovery and utilization,* JAOCS 60, 1983, 1579-87.
62. Ziejewski, M., Goettler, H., Pratt, G.L., Paper No. 860301, International Congress and Exposition, Detroit, MI, 24-28 February 1986..
63. Goering, C.E., *Final report for project on Effect of nonpetroleum fuels on durability of direct-injection diesel engines*, USDA, ARS, Peoria, IL. 1984.

64. Schwab, A.W., Bagby, M.O., Freedman, B., *Preparation and properties of diesel fuels from vegetable oils,*. Fuel 66, 1987,1372-8.
65. Pryde, E.H., *Vegetable oils as fuel alternatives – symposium overview,* JAOCS 61, 1984, 1609-10.
66. Ziejewski, M., Kaufman, K.R., Schwab, A.W., Pryde, E.H., *Diesel engine evaluation of a nonionic sunflower oil-aqueous ethanol microemulsion.* JAOCS 61, 1984, 1620-6.
67. Goering, C.E., Fry, B., *Engine durability screening test of a diesel oil/soy oil/alcohol microemulsion* fuel. JAOCS 61, 1984a, 1627-32.
68. Ziejewski, M.Z., Kaufman, K.R., Pratt, G.L., *Vegetable oil as diesel fuel,* USDA, Argic, Rev. Man., 1983, ARM-NC-28, pp. 106-111.
69. Goering, C.E., Camppion, R.N., Schwab, A.W., Pryde, E.H., *vegetable oil fuels,* proceedings of the international conference on plant and vegetable oils as fuels, Fargo, North Dakota. American Society of Agricultural Engineers, St Joseph, MI 4, 1982, 279-286
70. Sonntag, N.O.V. *Reactions of fats and fatty acids. Bailey's industrial oil and fat products,* vol. 1, 4th edition, 1979., John Wiley & Sons, New York, p. 99.
71. Idem, R.O., Katikaneni, S.P.R., Bakhshi, N.N., *Catalytic conversion of canola oil to fuels and chemicals: Roles of catalyst acidity, basicity and shape selectivity on product distribution,* Fuel Process. Technol., 51, (1997), 101-125.
72. Weisz, P.B., Haag, W.O., Rodewald, P.G., *Catalytic production of high-grade fuel (gasoline) from biomass compounds by shape selective catalysts, Science,* 206, (1979). 57-58.
73. Billaud F., Gornay J., Coniglio L., *Pyrolysis of secondary raw material from used frying oils,* Récents Progrès en Génie des Procédés, Numéro 94 - 2007 ISBN 2-910239-68-3, Ed. SFGP, Paris, France
74. Tian H., Chunyi L. , Chaohe Y. , Honghong S., *Alternative Processing Technology for Converting Vegetable Oils and Animal Fats to Clean Fuels and Light Olefins,* Chinese Journal of Chemical Engineering, 16(3) 394-400 (2008)

75. Schwab, A.W.et al., *Diesel fuel from thermal decomposition of soybean oil,* JAOCS 65, 1988, 1781-6.
76. Niehaus, R.A., Goering, C.E., Savage, L.D., Jr., Sorenson, S.C., *Cracked soybean oil as a fuel for a diesel engine,* Trans. ASAE 29, 1986, 683-689.
77. Pioch, D. et al., *Biofuels from catalytic cracking of tropical vegetable oils,* Oleagineux- 48, 1993, 289-291
78. Billaud, F., Dominguez, V., Broutin, P., Busson, C*., Production of hydrocarbons by pyrolysis of methyl esters from rapeseed oil.* JAOCS 72, 1995, 1149-54.
79. Chang, C.C., Wan, S.W., *China's motor fuels from tung oil,* Ind.Eng. Chem. 39, 1947, 1543-8.
80. Noureddini, H., Zhu, D., *Kinetics of transesterification of soybean oil,* JAOCS, 74(11), (1997), 1457-63.
81. Qian, J. et al., *In situ alkaline transesterification of cottonseed oil for production of biodiesel and nontoxic cottonseed meal,* Bioresour. Technol.V99, 18, 2008, 9009-9012
82. Saka S., Kusdiana D., *Biodiesel fuel from rapeseed prepared in supercritical methanol,* Fuel 80 (2001) 225–231.
83. Song ES, Lim JW, Lee HS, Lee YW., *Transesterification of RBD palm oil using supercritical methanol,* J. of Supercritical Fluids 44 (2008) 356–363
84. Bradshaw, G.B., Meuly, W.C., 1944. Preparation of detergents. US Patent 2, 360-844.
85. West A., Posarac D., Ellis N., *Assessment of four biodiesel production processes using HYSYS.Plant,* Bioresource Technology 99 (2008) 6587–6601
86. Eckey, E.W., Esterification and interesterification. JAOCS 33, 1956, 575-79.
87. Freedman, B., *Transesterification kinetics of soybean oil,* JAOCS, 63(10), (1986), 1375-1380.
88. Sharma Y.C., Singh B., Upadhyay S.N., *Advancements in development and characterization of biodiesel: A review,* Fuel 87 (2008) 2355–2373
89. Liu X et al., *Transesterification of soybean oil to biodiesel using CaO,* Fuel (2007), doi:10.1016/ j.fuel.2007.04.013

90. Jitputti J. et al., *Transesterification of crude palm kernel oil and crude coconut oil by different solid catalysts*, Chemical Engineering Journal 116 (2006) 61–66
91. Trent, W.R., 1945. Process of treating fatty glycerides. US Patent 2, 383±632.
92. Allen, H.D., Rock, G., Kline, W.A., 1945. Process for treating fats and fatty oils. US Patent 2, 383-579.
93. Tanaka, Y., Okabe, A., Ando, S., 1981. Method for the preparation of a lower alkyl ester of fatty acids. US Patent 4, 303±590.
94. Nye, M.J., Southwell, P.H., *Esters from rapeseed oil as diesel fuel,* Proc. Vegetable Oil as Diesel Fuel Seminar III. Peoria: Northern agricultural energy center, 1983, 78-83.
95. Freedman B, Pryde EH, Mounts TL . *Variables affecting the yields of fatty esters from transesterified vegetable oils.* JAOCS 61, 1984,1638–43.
96. Wimmer, T., *Transesterification process for the preparation of C1-5-alkyl fatty esters from fatty glycerides and monovalent lower alcohols.* PCT Int. Appl. WO, 1992, 9200-68.
97. Zhang, D., 1994. *Crystallization characteristics and fuel properties of tallow methyl esters,* Master thesis, Food Science and Technology, University of Nebraska-Lincoln.
98. Marinkovic SS, Tomasevic A. *Methanolysis of used frying oil.* Fuel Process Technol 2003;81:1–6.
99. Lifka J., B. Ondruschka, *Influence of mass transfer on the production of biodiesel*, Chemical Engineering & Technology 27 (2004) 1156–1159.
100. Karmee SK, Chadha A. *Preparation of biodiesel from crude oil of Pongamia pinnata.* Bioresour Technol 2005;96:1425–9.
101. Colucci J.A., E.E. Borrero, F. Alape, *Biodiesel from an alkaline transesterification reaction of soybean oil using ultrasonic mixing*, JAOCS 82 (2005) 525–530.
102. Stavarache C., M. Vinatoru, R. Nishimura, Y. Maeda, *Fatty acids methyl esters from vegetable oils by means of ultrasonic energy*, Ultrasonics Sonochemistry 12 (2005) 367–372.

103. Ghadge SV, Raheman H. *Biodiesel production from mahua (Madhuca indica) oil having high free fatty acids*. Biomass Bioenergy 2005;28:601–5.

104. Ji J. et al., *Preparation of biodiesel with the help of ultrasonic and hydrodynamic cavitation,* Ultrasonics 2006;44:411–4.

105. Siatis N.G. et al., *Improvement of biodiesel production based on the application of ultrasound: monitoring of the procedure by FTIR spectroscopy,* JAOCS 83 (2006) 53–57.

106. Leung DYC, Guo Y., *Transesterification of neat and used frying oil:Optimization for biodiesel production.*, Fuel Process Technol2006;87:883–90.

107. Meher LC, Dharmagadda VSS, Naik SN., *Optimization of alkali catalyzed transesterification of Pongamia pinnata oil for production of biodiesel.* Bioresour Technol 2006;97:1392–7.

108. Granados ML. et al., *Biodiesel from sunflower oil by using activated calcium oxide*, Appl Catal B: Environ 2007;73:317–26.

109. Sharma YC, Singh B., *Development of biodiesel from karanja, a tree found in rural India.*, Fuel 2007. doi:10.1016/j.fuel.2007.08.001.

110. Srivastava PK, Verma M., *Methyl ester of karanja oil as an alternative renewable source energy,* Fuel 2007. doi:10.1016/ j.fuel.2007.08.018.

111. Georgogianni K.G. et al., *Conventional and in situ transesterification of sunflower seed oil for the production of biodiesel,* Fuel Processing Technology (2007), doi:10.1016/j.fuproc.2007.10.004

112. Marchetti JM, Errazu AF., *Esterification of free fatty acids using sulfuric acid as catalyst in the presence of triglycerides,* Biomass and Bioenergy (2008), doi:10.1016/j.biombioe.2008.01.001

113. Sprules, F.J., Price, D., 1950. Production of fatty esters. US Patent 2, 366-494.

114. Mittelbach M, Pokits B, Silberholz A, *Diesel fuel derived from vegetable oils, IV: Production and fuel properties of fatty acid methyl esters from used frying oil,* liquid fuels from renewable resources, Proceedings of the alternatives energy conference, Michigan (USA): American Society of Agricultural Engineers, 1994. P,74

115. Keim, G.I., 1945. Process for treatment of fatty glycerides. US Patent 2, 383-601.

116. Jeromin L., Peukert E., Wollman G., Process for the pre-esterification of free fatty acids in fats and gils, US Patent No. 4,698,186 (1987).

117. Stern R, Hillion G, Eur P. Appl EP 1990;356:317 [Cl.C07C67/56]; Stern R, Hillion G, Eur P. Chem Abstr 1990;113:P58504k.

118. Kildiran, G., Ozgul-Yucel, S., Turkay, S., In-situ alcoholysis of soybean oil.JAOCS 73, 1996., 225–228.

119. Furuta S, Matsuhashi H, Arata K., *Biodiesel fuel production with solid amorphous-zirconia catalysis in fixed bed reactor.* Biomass Bioenergy 2006;30:870–3.

120. Crabbe E. et al., *Biodiesel production from crude palm oil and evaluation of butanol extraction and fuel properties,* Process Biochemistry 37 (2001) 65–71

121. Haas M.J. et al., *Production of FAME from acid oil, a byproduct of vegetable oil refining,* JAOCS 80 (1) (2003) 97- 102.

122. Haas M.J., Bloomer S., Scott K., Process for the production of fatty acid alkyl esters, US Patent No.6,399,800 (2002).

123. Veljkovic VB et al.. *Biodiesel production from tobacco (Nicotiana tabacum L.) seed oil with a high content of free fatty acids,* Fuel 2006;85:2671–5.

124. Sahoo PK, Das LM, Babu MKG, Naik SN. *Biodiesel development from high acid value polanga seed oil and performance evaluation in a CI engine.* Fuel 2007;86:448–54.

125. Tiwari AK, Kumar A, Raheman H. *Biodiesel production from jatropha oil (Jatropha curcas) with high free fatty acids: an optimized process.* Biomass Bioenergy 2007;31:569–75.

126. Wang Y, Ou S, Liu P, Zhang Z. *Preparation of biodiesel from waste cooking oil via two-step catalyzed process.* Energy Conservat. Manage. 2007;48:184–8.

127. Guo Z, Xu X, *New opportunity for enzymatic modification of fats and oils with industrial potentials.* Org Biomol Chem 3 (14): (2005). 2615–9. doi:10.1039/b506763d

128. Srivathsan VR., Srinivasan LN., Karuppan M., *An overview of enzymatic production of biodiesel* ,Bioresource Technology 99 (2008) 3975–3981

129. Schuchardt U, Ricardo Sercheli R, Vargas RM. *Transesterification of vegetable oils: a review.* J Braz Chem Soc 1998;9:199–210.

130. Mittelbach,M., *Lipase catalyzed alcoholysis of sunflower oil.* J. Am. Oil Chem. Soc. 67, 1990. 168–170.

131. Nelson, L.A., Foglia, T.A., Marmer, W.N., *Lipase-catalyzed production of biodiesel.* J. Am. Oil Chem. Soc. 73 (8), 1996, 1191–1195.

132. Selmi B. , Thomas D. , *Immobilized lipase-catalyzed ethanolysis of sunflower oil in a solvent-free medium,* JAOCS, doi:10.1007/s11746-998-0207-4

133. Linko, Y.et al., *Biodegradable products by lipase biocatalysis,* J. Biotechnol. 66, 1998. 41–50.

134. Shimada, Y et al., *Conversion of vegetable oil to biodiesel using immobilized Candida antarctica lipase,* .JAOCS 76, 1999, 789–793.

135. Kaieda, M., Samukawa, T., Kondo, A., Fukuda, H., *Effect of methanol and water contents on production of biodiesel fuel from plant oil catalyzed by various lipases in a solvent-free system.* J. Biosci. Bioeng. 91, 2000.,12–15.

136. Xu Y. , Du W. , Liu D. , Zeng J. , *A novel enzymatic route for biodiesel production from renewable oils in a solvent-free medium,* 2003, Biotech. Letters, V25:15:1239-1241.

137. Du W, Xu Y, Liu D, Zeng J. *Comparative study on lipase-catalyzed transformation of soybean oil for biodiesel production with different acyl acceptors.* J Mol Catal B: Enzym 2004;30:125–9.

138. Li, L., Du, W., Liu, D., Wang, L., Li, Z., *Lipase-catalyzed transesterification of rapeseed oils for biodiesel production with a novel organic solvent as the reaction medium,* J. Mol. Catal. B: Enzym. 43, 2006, 58–62.

139. Modi, M.K., Reddy, J.R.C., Rao, B.V.S.K., Prasad, R.B.N., *Lipase mediated transformation of vegetable oils into biodiesel using propane-2-ol as acyl acceptor.* Biotechnol. Lett. 28, 2006, 637–640.

140. Vivek R., Giridhar M., *Synthesis of biodiesel from edible and non-edible oils in supercritical alcohols and enzymatic synthesis in supercritical carbon dioxide,* Fuel 86 (2007) 2650–2659

141. Komers K, Machek J, Stloukal R., *Biodiesel from rapeseed oil and KOH Part two: Composition of solution of KOH in methanol as reaction partner of oil.* Eur J Lipid Sci Technol 2001;103:359.

142. Kusdiana D, Saka S., *Effects of water on biodiesel fuel production by supercritical methanol treatment.* Bioresour Technol 2004;91:289.

143. Joelianingsih et al. *Biodiesel fuels from palm oil via the non-catalytic transesterification in a bubble column reactor at atmospheric pressure: A kinetic study,* Renewable Energy 33 (2008) 1629–1636

144. Marchetti J.M., Errazu A.F., *Technoeconomic study of supercritical biodiesel production plant,* Energy Conversion and Management 49 (2008) 2160–2164

145. Kreutzer U.R., *Manufacture of fatty alcohols based on natural fats and oils,* JAOCS 61 (2) (1984) 343–348.

146. Warabi Y, Kusdiana D, Saka S. *Reactivity of triglycerides and fatty acids of rapeseed oil in supercritical alcohols.* Bioresour Technol 2004;91:283.

147. Saka S, Kusdiana D. *Kinetics of transesterification in rapeseed oil to biodiesel fuel as treated in supercritical methanol.* Fuel 2001;80: 693.

148. Saka S., Kusdiana D, *Transesterification of rapeseed oils in supercritical methanol to biodiesel fuels,* Proceedings of the 4th Biomass Conference of the Americas, Oakland, CA, 1999 (Aug. 29–Sept. 2).

149. Yamazaki R. et al., *Noncatalytic alcoholysis of oils for biodiesel fuel production by semi-bach process.* JPN J Food Eng 2007;8:11–9.

150. Clayton V. McNeff et al., *A continuous catalytic system for biodiesel production* ,Applied Catalysis A: General 343 (2008) 39–48

151. Canakci M, Van Gerpan J. *Biodiesel production from oils and fats with high free fatty acids.* Trans, ASAE 2001; 44:1429–36.

152. The New York Times, *To make Rubber-Seed Oil: Experiments being made in Middle East,* Published August 26,1917

153. Mittelbach, M. and Trathnigg, B., *Kinetics of alkaline catalyzed methanolysis of sunflower oil,* Fat Science and Technology, 92(4): 145-148, (1990).

154. Boocock D. et al., *Fast formation of high-purity methyl esters from vegetable oils,* JAOCS,75:12, 1998 , 1167-1172(6)

155. Komers, K., *Kinetics and mechanism of the KOH - catalyzed methanolysis of rapeseed oil for biodiesel production*, Eur. J. of Lip. Sci. and Tech., 104(11), , (2002), 728-737.

156. Foon CS. et al, *Kinetics study on transesterification of palm oil,* Journal of Oil Palm Research Vol. 16 No. 2, December 2004, p. 19-29

157. Leevijit T., *A Second Order Kinetics of Palm Oil Transesterification,* The Joint International Conference on Sustainable Energy and Environment (SEE)" -3 December 2004, Hua Hin, Thailand

158. Wenzel B. et al., *Modelling Chemical Kinetics of Soybean Oil Transesterification Process for Biodiesel Production: An Analysis of Molar Ratio between Alcohol and Soybean Oil Temperature Changes on the Process Conversion Rate*, Bioautomation, 2006, 5, 13 – 22

159. Stamenkovic O.S. et al., *Kinetics of sunflower oil methanolysis at low temperatures*, Bioresour. Technol. (2007), doi:10.1016/j.biortech.2007.02.028

160. Berrios M., *A Kinetic study of the esterification of free fatty acid (FFA) in sunflower oil*, Fuel 86 (2007) 2383-2388

161. Mettler Toledo, , Moisture Analyzer HR73, User manual.

162. Kamalu C.I.O., *Extraction and characterization of variable parameters on rubber seed oil*, Inter. Res. Jour, in Engr. Sc. & Tech (IREJEST), Vol. 2 No. 1 2005 54-66

163. NIST/SEMATECH e-Handbook of Statistical Methods, http://www.itl.nist.gov/div898/handbook/, July 2006.

164. Phillip J. Ross, *Taguchi Technqiues for Quality engineering*, 2nd Edition, McGraw-Hill Professional, 1995,ISBN 0070539588

165. Aigbodion A.I., Pillai C.K.S. , *Preparation, analysis and applications of rubber seed oil and its derivatives in surface coatings*,Progress in Organic Coatings 38 (2000) 187–192

166. Bureau of Indian Standards, Indian Standard IS 15607, 2005

167. Troika refining technology, http://www.troikaindia.com/refinery-plant.html, as of 14/02/2008

168. GREET Transportation Fuel Cycle Analysis Model, GREET 1.8b, developed by Argonne National Laboratory, Argonne, IL, released September 5, 2008. http://www.transportation.anl.gov/modeling_simulation/GREET/index.html

169. Gerpen JV, *The basics of diesel engines and diesel fuels*, University of Idaho Department of Biological and Agricultural Engineering publications, http://www.uidaho.edu/bioenergy/BiodieselEd/publication/03.pdf.

170. Al-Besharah J.M., Mumford.J., Akashah S.A. and Salman O. , *Prediction of the viscosity of lubricating oil blends* Fuel V68, I6, 1989, 809-811

171. Wooten, Frederick (1972). *Optical Properties of Solids*. New York City: Academic Press.ISBN 0127634509.

172. Cvengros J, Cvengroaovf Z , "*Quality Control of Rapeseed Oil Methyl Esters by Determination of Acyl Conversion*", JAOCS, (1994),Vol. 71, no. 12

173. Jack Cazes, *Encyclopedia of chromatography*, 2nd Edition, Marcel Dekker, 2005, ISBN 0824727878

174. H.Scott Fogler, *Elements of Chemical Reaction Engineering:International Edition 4th Edition,* Prentice Hall PTR, Oct 2005, ISBN: 9780131278394

175. Pocklington, W. D., *Determination of the iodine value of oils and fats,* Pure 84 Appl. Chem., Vol. 62, No. 12, pp. 2339-2343,1990,

Appendix

Thin Layer Chromatography Method and Analysis

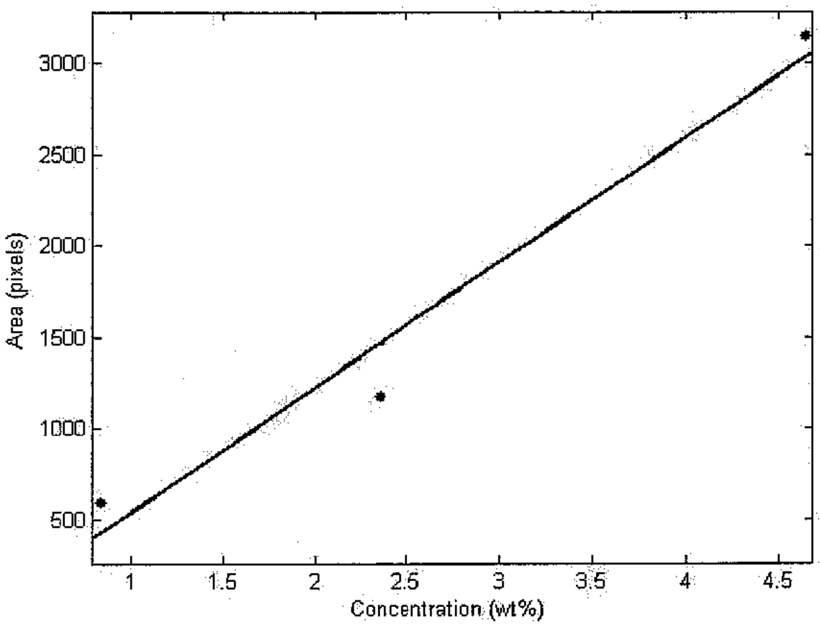
A- Standard preparation and quantification

Mono-,di- and triolein were used as the standards for quantification of mono-,di-, and triglycerides present in the samples. A pre-weighted amounts of the standards were used to establish the calibration curve. The standard sample was diluted in isopropyl alcohol and spotted on the TLC plate. The TLC-plate development solvent was added to the development chamber at 10ml. The solvent mixture consists of isopropyl alcohol, diethyl ether and acetic acid at the following volumetric ratio of 85:13.5:1.5.

The plates were then dried and placed in an Iodine chamber to render the spots. The plates were then scanned using a digital scanner and converted to a digital image. ImageJ ®, image processing software, was used to calculate the area of the spot. The calibration curve which consists of the area of spot of standard versus its concentration was established and fitted using MATLAB® to obtain the mathematical relation. This relation is used later on to determine the concentration of glycerides from the area of spots obtained. Below are the calibration curves for mono-,di-and triolein.

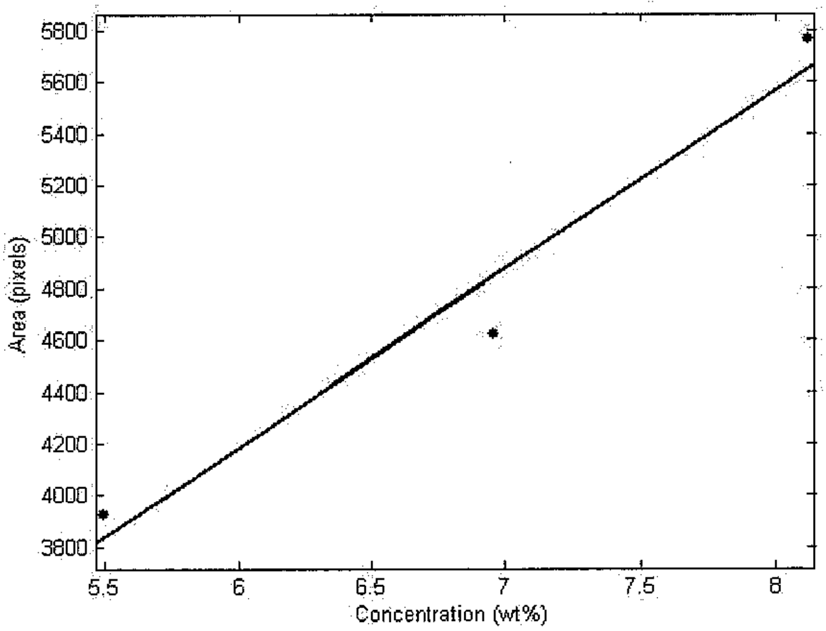
Monoolein calibration curve

Linear model fit: Y =MX+C
Y = area of spot (pixels)
X= concentration (wt%)
M = 686.6
C= -154.8
Goodness of fit (R^2) = 0.9639



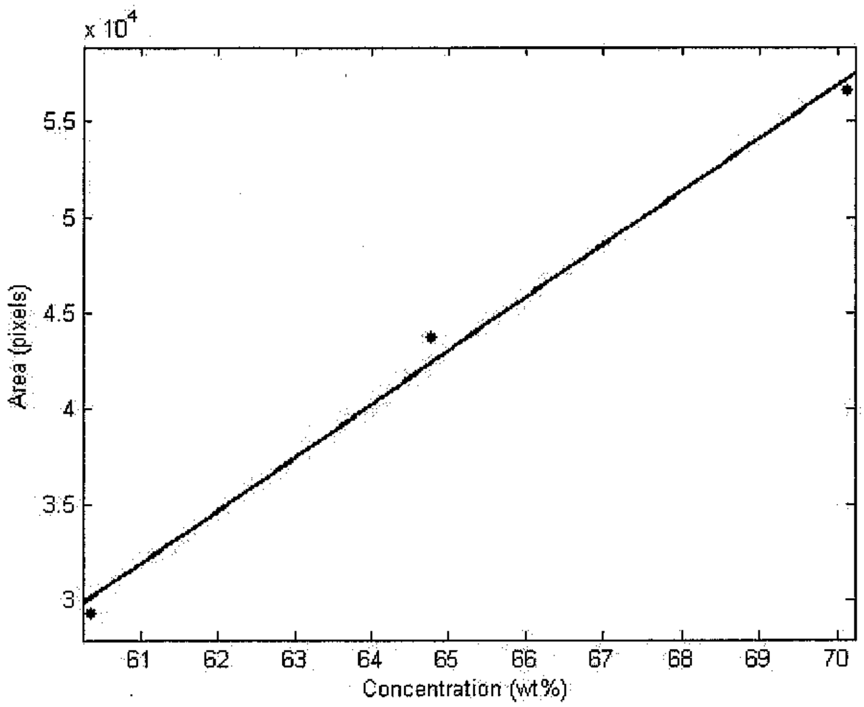
Diolein calibration curve

Linear model fit: Y =MX+C
Y = area of spot (pixels)
X= concentration (wt%)
M = 8.116
C= 31.93
Goodness of fit (R^2) = 0.9603



Triolein calibration curve

Linear model fit: Y =MX+C
Y = area of spot (pixels)
X= concentration (wt%)
M = 2783
C= -1.379e+005
Goodness of fit (R^2) = 0.9925



B- Sample preparation and quantification

Sample preparation and development is the same as that for standard. The quantification of the spots is done by calculating their area using the image analysis software, then by using the relation of area of spot with concentration obtained from standard calibration curves, mono-, di- and triglycerides are quantified.

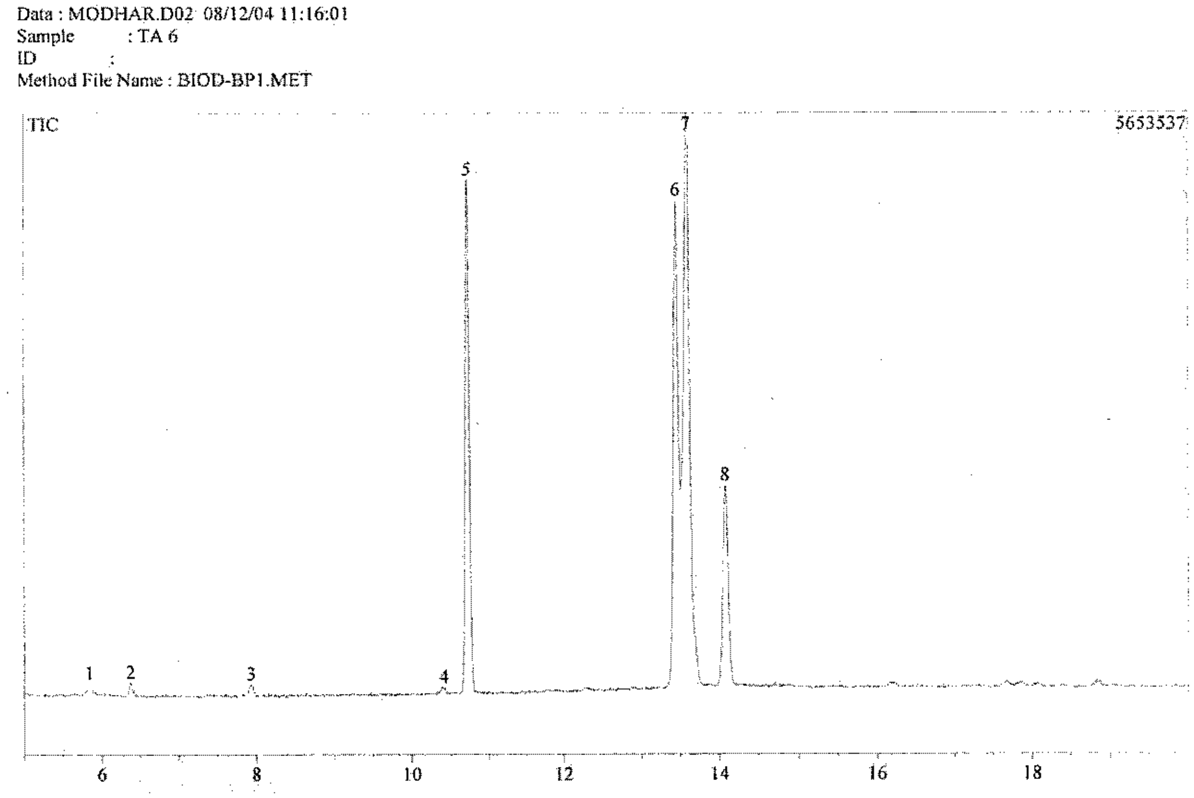
Free fatty acids concentration in the sample is obtained from chemical titration technique. Methyl esters are calculated as the remaining constituent in the sample.

GC / GC-MS REPORTS

A- GC/MS report: Fatty acids determination in oil blend number 2 (1:1 volumetric ratio of crude palm oil to rubber seed oil)

The report includes the peaks obtained from the gas chromatography analysis; each peak represents certain fatty acid specie contained in the vegetable oil in the form of fatty acid methyl esters. Mass spectrometer analysis shows the identification and compositional analysis for each peak.

Gas chromatography peaks



Data : MODHAR.D02 08/12/04 11:16:01
Sample : TA 6
ID :
Method File Name : BIOD-BP1.MET

Mass Spectrometer – Composition analysis

Peak no.	*Compound*	*Retention time (min)*	*%wt of sample*
1	Azelaic acid	*5.842*	*0.55*
2	Capric acid	*6.371*	*0.39*
3	Myristic acid	*7.933*	*0.44*
4	Stearic acid	*10.419*	*0.21*
5	Palmitic acid	*10.724*	*22.42*
6	Linoleic acid	*13.592*	*27.82*
7	Elaidic acid	*14.084*	*37.42*
8	Margaric acid	*23.833*	*10.51*
9	*Others*	-	*0.23*
		Total	*100*

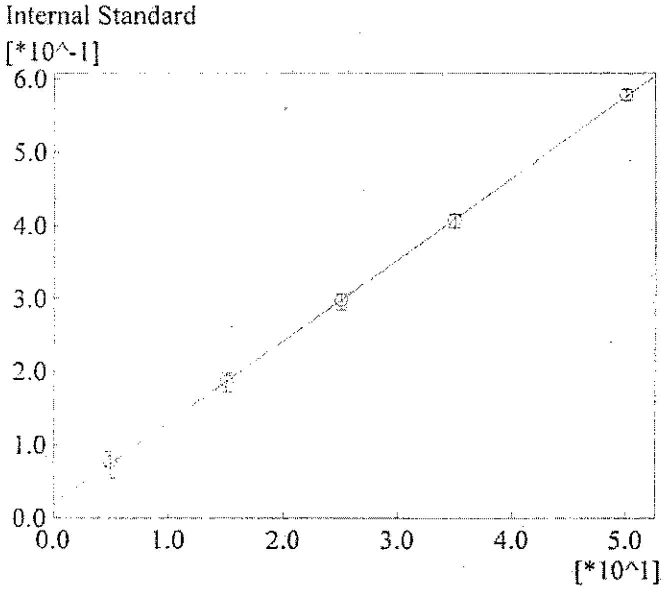
B- GC report: Determination of Mono-Di-Triglycerides and Glycerine in biodiesel sample based on ASTM method D 6584

The report includes the calibration curves established from standard solution to calculate the compositions for mono-di-triglycerides and glycerine that is found in biodiesel sample. The peaks obtained from gas chromatography analysis for two biodiesel samples alongside the concentration analysis are included.

Glycerin Calibration curve

```
                                   Calibration Curve - Analytical Line 1 - Channel 1
ID#:1   Name:Glycerin

f(x)=1.11549022563e-002*x+1.92702254095e-002
 R=0.999967249902  R^2=0.999934500877
MeanRF:1.25610707373e-002  RFSD:2.08967792675e-003   RFRSD:16.6361448834
CurveType:Linear
ZeroThrough:Not through
WeightedRegression:None
```



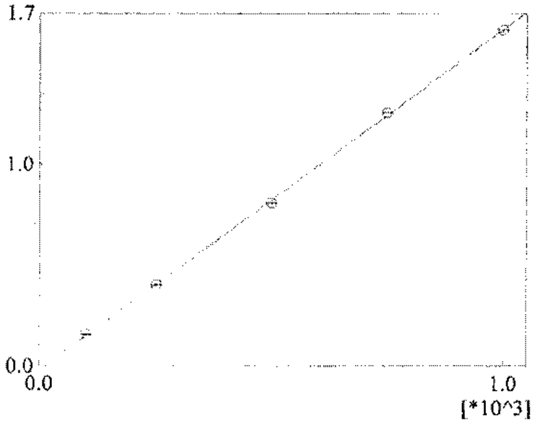
No.	Conc.	Area
1	5.01	0.0764
2	15.01	0.1865
3	25.03	0.2977
4	35.01	0.4077
5	50.02	0.5790

Monoglycerides calibration curve

```
ID#:3   Name:Monoglyceride

f(x)=1.68663616107e-003*x-2.23747811983e-002
 R=0.99984318816   R^2=0.99968640091
MeanRF:1.61244758047e-003  RFSD:5.59393831111e-005   RFRSD:3.46922180842
CurveType:Linear
ZeroThrough:Not through
WeightedRegression:None

Internal Standard
```



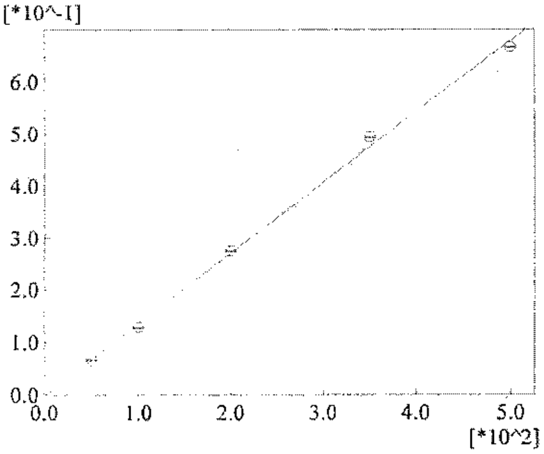
```
No.   Conc.      Area
 1     100.00    0.1526
 2     250.10    0.3969
 3     499.90    0.8061
 4     750.50    1.2577
 5    1000.00    1.6609
```

Diglycerides calibration

```
ID#:5   Name:Diglyceride

f(x)=1.35965974314e-003*x-1.28118025551e-003
 R=0.998933429478   R^2=0.997867996528
MeanRF:1.33758581162e-003  RFSD:4.98394111918e-005   RFRSD:3.72607211327
CurveType:Linear
ZeroThrough:Not through
WeightedRegression:None

Internal Standard
```



```
No.   Conc.     Area
 1     50.02    0.0655
 2     99.90    0.1267
 3    200.20    0.2742
 4    350.00    0.4925
 5    500.00    0.6664
```

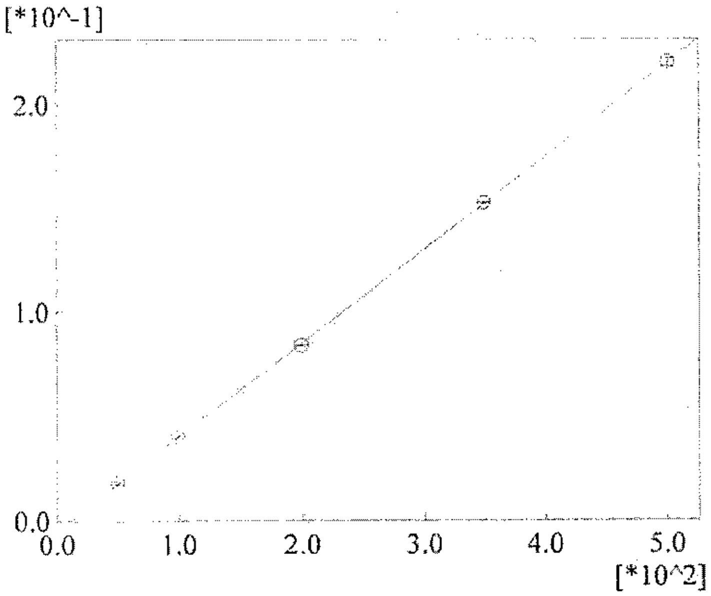
curve

Triglycerides calibration curve

ID#:6 Name:Triglyceride

f(x)=4.48921518458e-004*x-5.00020954905e-003
R=0.999973640619 R^2=0.999947281932
MeanRF:4.10396555458e-004 RFSD:3.0689714798e-005 RFRSD:7.47806344616
CurveType:Linear
ZeroThrough:Not through
WeightedRegression:None

Internal Standard



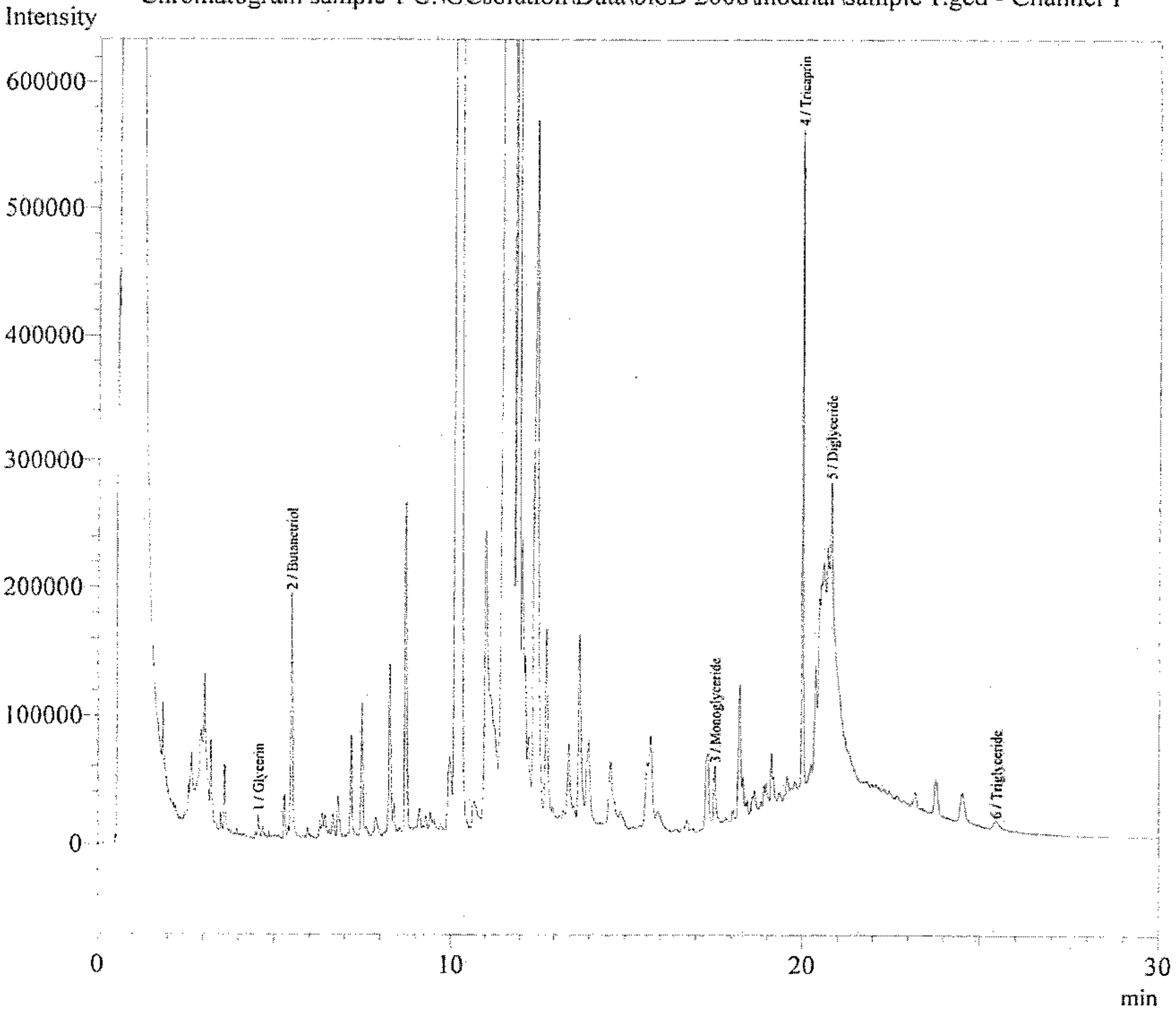
No.	Conc.	Area
1	50.02	0.0180
2	99.90	0.0397
3	199.90	0.0838
4	349.50	0.1524
5	501.00	0.2199

Gas chromatography peaks for Sample no.1

Sample Information

Analysis Date & Time	: 3/13/2009 3:59:11 PM
Sample Name	: sample 1
Data Name	: C:\GCsolution\Data\bioD 2008\modhar\sample 1.gcd
Method Name	: C:\GCsolution\Data\bioD 2008\modhar\standard curve.gcm

Chromatogram sample 1 C:\GCsolution\Data\bioD 2008\modhar\sample 1.gcd - Channel 1

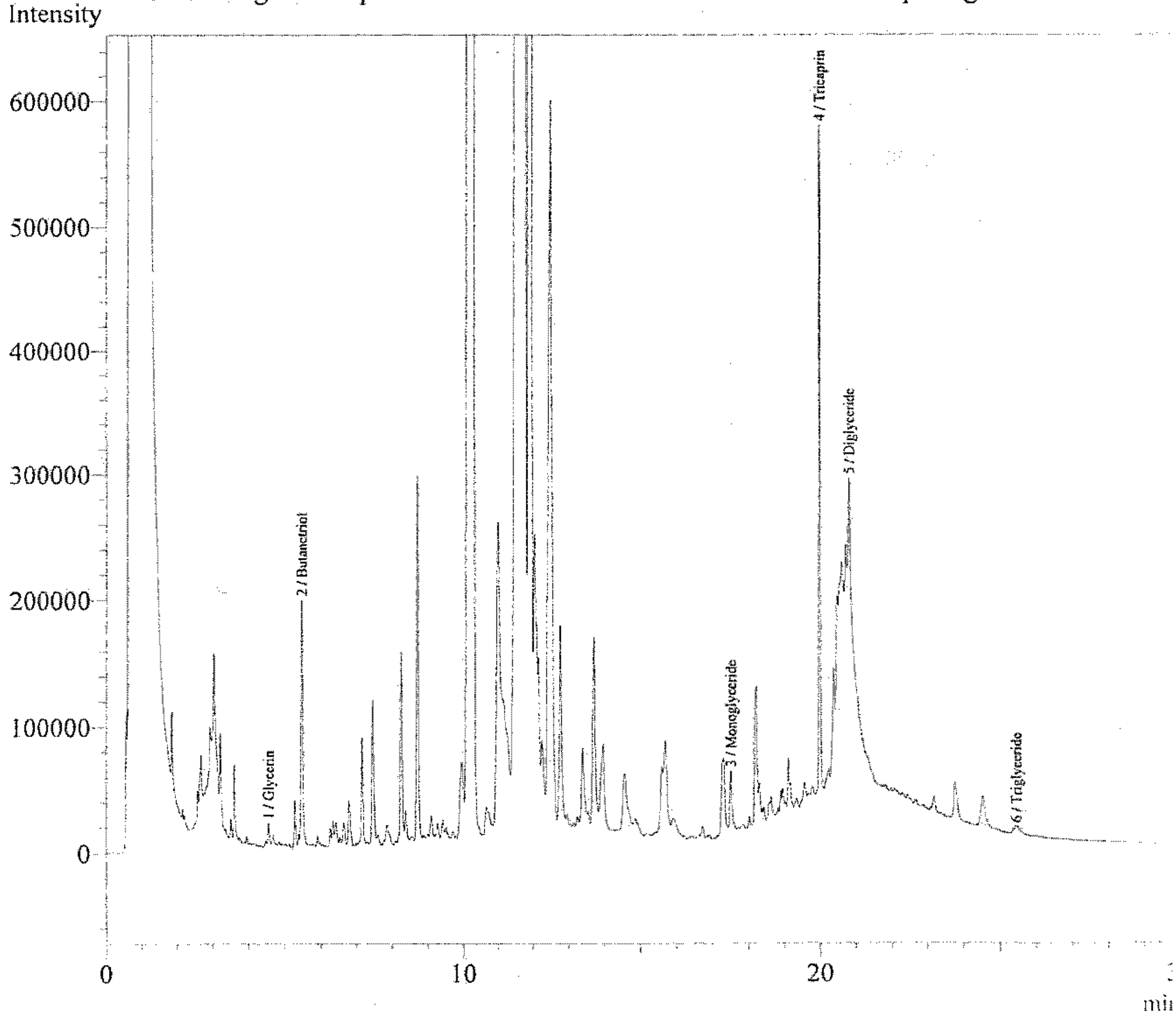


Gas chromatography peaks for Sample no.2

Sample Information
Analysis Date & Time : 3/13/2009 4:52:21 PM
Sample Name : sample 2

Data Name : C:\GCsolution\Data\bioD 2008\modhar\sample 2.gcd
Method Name : C:\GCsolution\Data\bioD 2008\modhar\standard curve.gcm

Chromatogram sample 2 C:\GCsolution\Data\bioD 2008\modhar\sample 2.gcd - Channel 1



Concentration analysis for sample no. 1 (mass = 0.135g)

Peak no.	*Compound*	*Peak area*	*Concentration (µg)*
1	*Glycerin*	*41135*	*3.804*
2	*Butanetriol (IS)*	*666691*	-
3	*Monoglyceride*	*146464*	*84.031*
4	*Tricaprin (IS)*	*1227104*	-
5	*Diglyceride*	*278900*	*168.104*
6	*Triglyceride*	*66654*	*132.135*

Concentration analysis for sample no. 2 (mass = 0.145g)

Peak no.	*Compound*	*Peak area*	*Concentration (µg)*
1	*Glycerin*	*43952*	*3.997*
2	*Butanetriol (IS)*	*688327*	-
3	*Monoglyceride*	*176839*	*94.784*
4	*Tricaprin (IS)*	*1286179*	-
5	*Diglyceride*	*335706*	*192.910*
6	*Triglyceride*	*71077*	*134.238*