OPTIMIZATION OF BIODIESEL PRODUCTION VIA REFLUX CONDENSER METHYL ACETATE REACTION FROM *CERBERA ODOLLAM* (SEA MANGO)

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A project report submitted in partial fulfilment of the
requirements for the award of Bachelor of Engineering
(Hons.) Petrochemical Engineering

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DECLARATION

I hereby declare that this project is based on my original work except for citations and quotations which have been duly acknowledge. I also declare that it has not been previously and concurrently submitted for any other degree or award at UTAR or other institutions.

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APPROVAL FOR SUBMISSION

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ABSTRACT

Cerbera Odollam (sea mango) is a proven promising feedstock for the production of biodiesel due to its high content and its advantage of being a non-edible feedstock. In this study, extracted sea mango oil has been used as triglyceride for the reflux condensation reaction. Fatty acid methyl esters (FAME) were produced as final reaction product in the transesterification reflux condensation reaction of sea mango oil and methyl acetate. Since methyl acetate is used in place of methanol as solvent, it is a glycerol-free process. In this study, potassium methoxide was used as catalyst to study its reacting potential as a homogeneous base catalyst. The initial part of this project studied the optimum conditions to extract crude sea mango oil. It was found that the content of sea mango oil was 55%. This optimum amount was obtained by using 18g of grinded sea mango seeds in 250 ml hexane for 24 hours using solvent extraction method. Extracted oil was then used in the reflux condensation method for reaction to form FAME with its experimental conditions. Response surface methodology (RSM) was used to determine the optimum conditions of the reaction. The three manipulated variables in this reaction were reaction time, oil to solvent molar ratio, and catalyst wt%. Statistical analysis of the design expert software was used to study the influence of each of these variables on the FAME yield. The optimum condition for this reaction determined was 5 hours reaction time, 0.28wt% of catalyst and 1:35mol/mol of oil:solvent molar ratio to achieve FAME yield of 66%. A series of test were conducted on the final FAME product of this study, namely the FTIR test, GC-FID, calorimeter bomb and vicometry test.

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A	oil to solvent molar ratio, mol/mol
A_i	peak area, *i* = component
A_{IS}	peak area of internal standard (methyl heptadecanoate)
B	catalyst ratio, wt%
C	reaction time, hours
C	total content, %
C_i	content of components, %
C_{IS}	concentration of internal standard, in mg/mL
F-value	ratio of model mean square to residual mean square
m	mass of sample
V_{IS}	volume of internal standard used, mL
x	independent variable
Y	yield of FAME content
β	constant in quadratic model

CCD	central composite design
DG	diglycerides
DOE	design of experiment
FAME	fatty acid methyl esters
FFA	free fatty acid
FTIR	fourier transform infrared spectroscopy
GC	gas chromatography
IS	internal standard
MA	methyl acetate
MG	monoglycerides
RBO	rice brand oil
RBDPO	refined, bleached, deodorized palm olein
RSM	response surface methodology
TG	triglyceride

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CHAPTER 1

INTRODUCTION

1.1 Background

Due to the rapid growth in population, depletion in fossil fuels as well as the hike in petroleum prices around the globe calls for a need to search for alternative methods to replace these geologic deposits of organic materials. In addition, pollution emission has been a massive problem with millions of dollars spent to discover new ways to reduce them, which then lead to new studies on the possibility of having alternative renewable energy sources as a substitute to fossil fuels.

From the many renewable energy sources, biodiesel has been receiving much attention due to its potential in substituting diesel fuel in terms of vehicle engine usage. Before, biodiesel was produced using edible feedstocks, but due to food vs fuel debate, new researches were carried out only to discover many non-edible feedstocks for the production of biodiesel. The several advantages of non-edibles as diesel fuels are, its biodegradability, it is readily available, it has lower sulphur and aromatic content and it is renewable (Bhuiya et al., 2014).

Biodiesel, also known as fatty acid methyl esters (FAME) is a mixture of monoalkyl esters of long chain fatty acids. They are derived from lipid substances originated from oil, fats, waste oil and many more (Palash et al., 2015; Masjuki et al., 2015). The properties of FAME may vary according to the types of vegetable oil used.

In the biodiesel industry, selecting a feedstock that would yield good productivity cost with a large scale production is vital. Generally, biodiesel feedstocks can be categorized into four main categories, which are non-edible vegetable oil, edible vegetable oil, waste or recycle oil, and animal fats. An example of edible vegetable oils are, soybean palm oil, sunflower, safflower, rapeseed, coconut, and peanut (Bhuiya et al., 2014). Fruits like *Cerbera Odollam* (sea mango), *Jatropha, Karanja, Mahua,* linseed, cottonseed, neem, *Camelina* and *Polanga* are non-edible biodiesel feedstocks (Bhuiya et al., 2014). Amongst edible vegetable oils, palm oil is the most common oil produced in industries these days as its oil content is exceptionally high, with 30-60% oil content. Non-edible oils are regarded as second generation biodiesel while edible oils are of first generation. However, due to many economic, environmental and financial issues, second generation biodiesels are gaining popularity over the first generation.

1.2 Second generation Biodiesel

Second generation biodiesel is referred to as biodiesel produced from non-edible oil, waste or recycle oils or even animal fats. Second generation biodiesel has grabbed a great amount of attention worldwide due to problems associated with edible oil feedstocks like the food vs fuel debate. Biodiesel production for the second generation is a growing industry since it has no impact on food issues (Harch et al., 2014). The typical oil content in some non-edible feedstock is shown in Table 1.1. Amongst all these feedstock, Jatropha and *Cerbera Odollam* has the highest oil content recorded.

Table 1.1: Estimated oil contents in second generation biodiesel feedstocks (Atabani et al., 2012)

Feed Stock	Oil content %
Cottonseed	18-25
Jatropha	Seed : 35-40 Kernel : 50-60
Jojoba	45-50
Karanja (*Pongamia Pinnata*)	27-39
Rubber seed	40-50
Sea mango(*Cerbera Odollam*)	54

1.3 *Cerbera Odollam*

Cerbera Odollam or commonly known as sea mango, belongs to the family “Apocynaceae”. They are used for medicinal purposes in countries like India (Gokushankar and Sadananda Rai., 2009) and are commonly known as suicide trees. These suicide trees bear fruits containing high toxicity with composition like cerberin as the main active cardeonolide. The fruits are injurious to health when ingested due to its extreme poisonous content.

The tree measuring to about 8-15m tall with dark green fleshy leaves is found mainly in southern Asian countries (Ong et al., 2014). The flowers of this tree are of jasmine fragrance containing a white tubular 5 lobed corolla about 3-5cm in diameter. In Malaysia, the sea mango plant could be found by lakes or rivers and are grown as ornamental plants which fruits are available throughout the year.

Having a similar shape and colour to a small mango, the sea mango is protected by a green fibre shell which contains two cross matching white fleshy halves. The seed of the fruit is contained in the centre, where the fruit needs to be dissected into half to obtain the white fleshy seed. If exposed to air for too long, the green fibre covering the fruit slowly turns dark red then brown and finally black. Figure 1.1 visually describes the *Cerbera Odollam* plant and its flower while Figure 1.2 describes the fruit.

Figure 1.1: ***Cerbera Odollam*** **tree**

Figure 1.2: ***Cerbera Odollam*** **fruit**

Despite its high contents of poisonous composition, *Cerbera Odollam* has been proven to be one of the most promising non-edible feedstock in the biodiesel industry.

1.4 Reflux Condenser Methyl Acetate Method

A typical lab experiment set up equipped with a reflux condenser where the solvent, methyl acetate (MA) is refluxed to the reactor when it is condensed. The reflux method helps reduce loss of MA solvent used (Casas et al., 2013). In the set up experiment, the 3 neck round bottom flask serves as the reactor where triglycerides, solvent and catalyst react to form FAME and triacetin. Heater provides desired heat to the reaction for an optimum conversion to take place.

A thermometer as well as a magnetic stirrer is used to monitor the temperature and enhance a proper mix to the solution respectively. This conventional reflux condenser method uses low temperature and pressure but would produce optimal results in a longer period of time whereas a method using supercritical reactors and subcritical reactors produces FAME in a shorter period of time while having to use extremely high temperatures and pressures (Goembira and Saka, 2013).

In processes involving MA, it replaces the use of methanol as solvent to produce triacetin as a side product instead of glycerol (Wu et al., 2014). The triacetin produced can be used as an addition to biodiesel without any negative side effects (Wu et al., 2014). Up to this date, many researches had been carried out using supercritical fluid method where no catalyst is used. There's less documentation on reflux condensation using MA as a solvent.

There are several advantages to the reflux condenser method. Firstly, this method saves a whole load of energy since it operates at atmospheric pressure and considerably low temperatures. Next, a considerably high yield of biodiesel is produced in this process using the right catalyst and a properly recorded time of reaction.

1.5 Problem Statement

Today's leading industries produce biodiesel from edible feedstocks, for an instance palm oil, rapeseed oil and even soybeans. However, these industries have been getting negative feedbacks due to the food vs fuel debate where in fact, a valid argument has been put in place between the importance of food and the demand in biodiesel in these coming days. The lands used to grow these crops are indeed used for the production of biodiesel more vastly than to harvest them for human consumption.

On the other hand, producing biodiesel using a glycerol-free method and having another side product that could be of better use in the industry is another issue. During biodiesel production, triglycerides are converted to fatty acids methyl esters (FAME) by a transesterification reaction with methanol (Calero et al., 2015).

Alternative ways has been discovered where non-edible feedstocks are used. Common feedstock's like Jatropha, cotton seeds and sea mango are used. Nevertheless, Tan et al. (2010) has researched on palm oil's conversion and its biodiesel properties using supercritical method where instead of methanol, MA was employed in the reaction. MA was used to avoid the formation of glycerol as side product and instead, producing triacetin in the transesterification process.

Previous study by Tan et al. (2010) shows a conversion of 97.6% of biodiesel from palm oil using the response surface methodology (RSM) at 399 ° C. Since edible feedstock like palm oil has been widely used in the biodiesel industry, in this research non-edible feedstock like sea mango will be used.

In this current study, the optimum conditions for biodiesel conversion from sea mango oil will be investigated by using the Reflux Condensation method.

1.6 Aims and Objectives

This thesis is aimed to achieve the following objectives:

i. To determine the optimum condition for the extraction of sea mango oil
ii. To investigate the optimum conditions for biodiesel production via Reflux Condenser Methyl Acetate Method
iii. To investigate the quality of the biodiesel produced from the production process using various characterization tests.

CHAPTER 2

LITERATURE REVIEW

2.1 Transesterification

Biodiesel is produced via various methods like, pyrolysis, hydrodeoxygenation, dilution, microemulsification and transesterification (Calero et al., 2015). Amongst all these methods, transesterification being the most common method used in industries these days require low cost of production as well as its high conversion efficiency. Transesterification provides solutions to high viscosity problems faced by other conversion methods (Bhuiya et al., 2014).

Biodiesel, also known as fatty acid methyl ester (FAME) is produced via transesterification reaction with vegetable oil and animal fats in the presence of a short chain alcohol usually methanol or ethanol, and are catalysed by either acid or base catalyst. The reaction, with the presence of a catalyst also produces glycerol as its side product.

However, methanol is preferred as a solvent over ethanol due to its economic advantages. Methanol is easier to recover, while ethanol forms an *azeotrope* with water making it expensive to purify during recovery. Since edible oils create a global imbalance to market demand and food supply, lower cost non edible oil like Jatropha and sea mangoes are used for biodiesel production (Nizah et al., 2014). A general idea on transesterification is described in Figure 2.1.

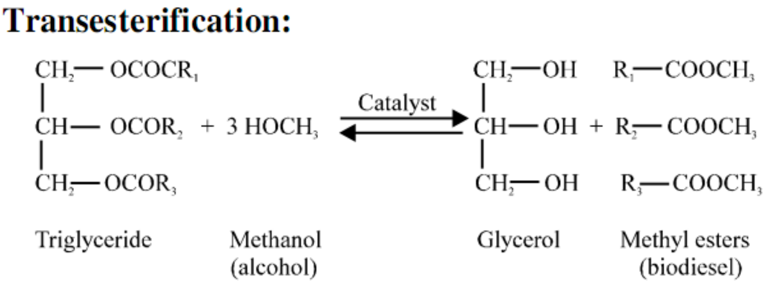


Figure 2.1: Transesterification process

Figure 2.1 generally describes transesterification when raw vegetable oil (triglyceride) reacts with methanol under the aid of a certain catalyst to produce biodiesel and side product glycerol. The triglyceride in this reaction is converted to diglycerides, then monoglycerides and finally into glycerol in a step wise manner. Transesterification reaction variables include the methanol/oil ratio, catalyst concentration, reaction temperature and also reaction time (Bhuiya et al., 2014).

2.2 Methyl Acetate

Different processes using MA and methanol respectively produce different side products in a transesterification reaction. Reaction involving methanol as the solvent produces glycerol and FAME whereas a reaction with MA produces triacetin and FAME. Used in many research, the MA's function is mainly to produce glycerol-free biodiesel product. Tan et al (2010) successfully produced FAME using supercritical fluid method from triglycerides and MA instead of alcohol. A reaction temperature of 399^{o}C and time of 59 minutes were the optimum condition to achieve a biodiesel yield of 97.7%.

In another research, Casas et al (2011) used MA and sunflower oil only to produce a yield of 76.7% biodiesel and 17.2% triacetin at a temperature of 50^{o}C and a compatible base catalyst. Figure 2.2 describes the reaction of triglycerides and MA to produce triacetin and FAME.

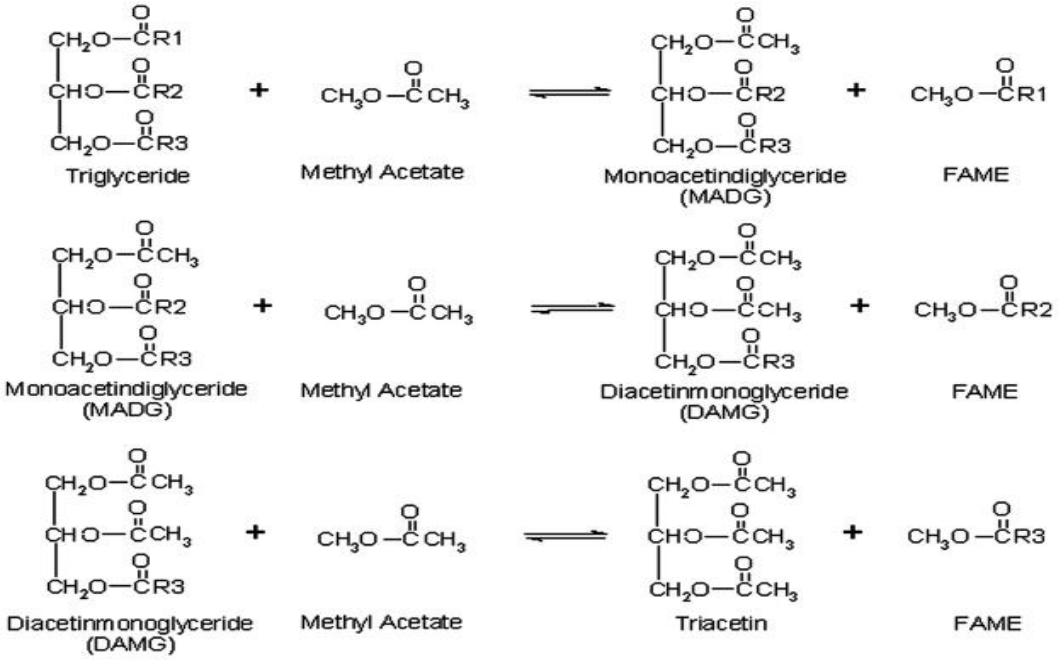


Figure 2.2: Transesterification reaction of triglycerides and Methyl Acetate (MA)

2.3 Catalytic Transesterification

The term “catalyst” was first used by a Berzelius in 1836 to identify a new chemical used in promoting a reaction. The main reason to the employment of catalyst in transesterification is to increase the rate of reaction and maximize the biodiesel yield by enhancing the solubility of alcohol of methyl acetate solvent in the reaction (Taufiq Yap et al., 2014). Two common catalyst used in transesterification are acid and alkaline (base) catalyst. Adding on to that, the acid and base catalyst can be either a heterogeneous catalyst transesterification or a homogeneous catalyst transesterification process. Homogeneous catalysts are the catalyst in the same phase as when the reaction occurs whereas heterogeneous catalysts are catalyst of different phase with the reactants.

2.3.1 Acid Catalysed Transesterification

Generally acid catalyst requires a higher alcohol to oil molar ratio and catalyst concentrations to achieve a desired conversion during transesterification. When compared to base catalysts, acid catalyst undergoes a more vigorous reaction (Istadi et al., 2015). However, there is a back drawn in using base catalyst due to the formation of soap and other undesired by-products in reaction process (Istadi et al., 2015)

Istadi et al (2015) suggests that the usage of heterogeneous catalyst has a process which is more environmental friendly. He also suggested that by using heterogeneous catalysts, the efficiency of reactor design can be enhanced, continuous process will be enabled and also the economics of biodiesel production can be improved. Heterogeneous catalyst does not produce soap through free fatty acid neutralization, thus it simplifies the separation and purification process.

Istadi et al (2015) used an acid catalysed reaction with soybean and methanol to achieve 80.14% FAME at 4h of reaction time and the methanol to oil molar ratio being 6:1. Sulphated zinc oxide has been proven as a promising acid catalyst for transesterification of soybean with methanol to produce biodiesel.

Thompson et al (2014) studied various acid catalysed transesterification with Jatropha –like bio-oil. Homogenous acid catalyst like sulphuric acid was used and a FAME yield of 74.4% was obtain with oil to alcohol molar ratio of 1:15. A similar transesterification reaction was carried out using a zeolite catalysed system. It was found that at a reaction time of 3h and oil to alcohol molar ratio of 1:15, FAME yield obtained was 61%.

Miao et al (2009) used Trifluoroacetic acid for biodiesel production. Using a 2.0M concentration of acid, and oil to methanol molar ratio of 1:20, biodiesel yield of 98.4% was achieved. The temperature used in this reaction was as high as 120°C with a reaction time of 5h. Different catalyst concentrations were also used in the reaction to determine the ideal concentration in producing the highest yield of FAME.

2.3.2 Base Catalysed Transesterification

Many researches were carried out using base /alkali catalyst and they have shown promising results in transesterification process. Some experimental studies reveal that base catalyst requires more reaction time a higher reaction temperature. Nevertheless, there are many advantages to base catalysed transesterification reaction and one of them is producing high yield FAME (Deshmane et al., 2013).

Deshmane and Adewuyi (2013) in their research used calcium methoxide solid base catalyst, methanol and soybean oil to produce a biodiesel yield of 90% within 90 minutes. The optimum yield was achieved using 9:1 methanol to oil molar ratio and a 1% catalyst loading. The reaction temperature was set to 65˚C. The research was done in the absence and presence of ultrasound.

Metallic hydroxides are frequently used as catalysts due to its low price but they have lower catalytic activity when compared to alkoxides. However catalyst like potassium methoxide being a homogeneous base catalyst has to be separated from FAME after the reaction.

Casas et al (2013) used phosphoric acid to neutralize the basic catalyst which would eventually form salt when contacted with acid. Then solution is centrifuged to remove the salts. Casas et al (2013) used prepared potassium methoxide as catalyst and methyl acetate as solvent in the reaction instead of methanol. A FAME yield of 77% was produced and 12.1% of triacetin. A mass fraction of 6.8% of glycerol and monoacetin was also produced in the reaction.

Meddikeri et al (2013) performed a reaction of waste cooking oil with methyl acetate and potassium methoxide catalyst using ultrasonic horn (22Hz and 740W). The reaction was carried out using different parameters like reaction temperature, oil to solvent ratio, catalyst concentration and also varying ultrasonic amplitude. The experiment results revealed that a maximum yield 90% biodiesel from waste cooking oil using sonochemical reactors was obtained using a molar ratio of 1:12mol/mol, catalyst concentration of 1.0% and a temperature of 40 °C.

2.3.2 Enzyme Catalysed Transesterification

From all the catalytic transesterification processes, enzymatic transesterification is the most preferred one since there is no soap formation thus there is no hassle in purification or neutralization of FAME. In addition, enzyme catalysts have high tolerance towards free fatty acids (FFA). It has high conversion ability of almost 90% of feed stock with high FFA content into biodiesel. Only a few plants have employed enzymatic process for transesterification in industries due to its high cost when compared to chemical catalysed transesterifications like NaOH and KOH (Zhao et al., 2015).

Debnath et al (2011) reported an optimisation of enzyme Interesterification with rice brand oil (RBO) and refined, bleached, deodorized palm olein (RBDPO) blend using immobilized 1,3-specific lipase. In his study, for variables for selected. They are RBO (20–80%) in RBO–RBDPO blend, reaction time of (1-13h), reaction temperature ranging from 25-65 ̊ C, and enzyme concentration of 1-13% w/w. Since the research was mainly focused on reaction for modulating rheological and heat transfer properties of frying oil, maximum heat transfer coefficient obtained were at 62% RBO, temperature 65°C, enzyme concentration of 10% w/w and time of 6.4 h.

Another research carried out by Sun et al (2012) using Lipozyme TL IM-catalysed transesterification in a solvent free system with coconut oil. Flavour esters, especially octanoic acid esters were formed during the reaction. In this reaction, a molar ratio of 3:1 alcohol to oil and enzyme loading of 15% w/w were used. The reaction temperature maintained at 23°C with a stirring speed of 130 rpm and 20h of reaction time. In addition, the operational stability of Lipozyme TL IM was improved by washing it with solvents, so that the enzyme could be continuously used for at least 100 h within 5 batches reactions without significant loss of activity.

Lara Pizarro and Park (2003) performed transesterification with *Rhizopus oryzae* lipase using oil to molar ratio of 1:4, 67 IU/g of substrate and shaking it with 175 rpm for 96h at 35 ̊ C. A conversion yield of 55% w/w was obtained using palm oil at 96h of reaction time.

2.3.4 Non-Catalytic Transesterification

Non-catalytic transesterification processes are usually referred to a process called supercritical transesterification where very high temperatures and pressures are used for biodiesel production. Absence of catalyst in this process leads to simpler separation and purification steps of biodiesel (Ghoreishi and Moein, 2013). Different solvents used in this reaction yields different final by-products, like the usage of methanol produces glycerol and by using methyl acetate, triacetin is produced with FAME.

Tan et al (2010) studied transesterification using supercritical methyl acetate. This non-catalytic supercritical reaction produced triacetin as a product with FAME and triacetin is a valuable biodiesel additive. Response Surface Methodology (RSM) analysis was used to optimize the various important parameter for the production of biodiesel. Using this mathematical model, the optimum conditions for reaction temperature was 399 ˚ C, 30mol/mol of methyl acetate to oil molar ratio and reaction time of 59 min to achieve a 97.6% biodiesel yield.

Nan et al (2015) researched on supercritical methanol and ethanol for the production of biodiesel using microalgae oil. RSM combined with a five-parameter-five-level central composite design (CCD) was employed to optimize the 270-350 ˚ C, using a pressure range of 80-200 bar, alcohol to oil molar ratio of 10:1 – 42:1mol/mol and a residence time of 10-50 minutes range. Thirty two experiment runs were conducted for each alcohol type. Finally, the optimum biodiesel yield obtained from methanol process was 90.8% and ethanol 87.8%.

In addition, Joelianingsih et al (2012) used a laboratory scale continuous flow bubble column reactor with a 200mL of palm oil in the reactor. Different methanol feed flow rates and reaction temperatures were used in this study. It was found that the biodiesel yield increased with the increase in methanol feed flow rate at a higher temperature which was 6.0mL/min and 290 ˚ C respectively. But with this higher flowrate and temperature, the purity of methyl ester in the biodiesel production decreased.

Ghoreishi and Moein (2013) also used RSM in their study to discover the yield of biodiesel in non-catalytic supercrital methanol system. Waste vegetable oil was used in this reaction with 33.8:1 mol/mol methanol to oil molar ratio, temperature of 271.1 ^{o}C, a high pressure of 23.1MPa and 20 minutes of reaction time. A maximum predicted yield of 95.27% biodiesel was expected from this reaction. Moreover, central composite rotatable design was used in this research to maximize the yield of the biodiesel.

Kwon et al (2014) performed transesterification of coconut oil into biodiesel using dimethyl carbonate (DMC) using a non-catalytic system under ambient pressure. The reaction was carried out using liquid oil and gas DMC. The reaction was enhanced with the presence of porous materials due to its intrinsic physical property. In this study, the highest yield of biodiesel produced was 98% within 1-2 minutes at a temperature of about 360-450 ^{o}C. without the increase in pressure, this high yield is achieved. This way, supercritical method could also be avoided. There are also other methods of non-catalytic transesterification without using supercritical method.

2.4 Solvent Extraction

Solvent extraction in a process of extracting oil from an oil-bearing materials leaving behind only 0.5-0.7 % residual oil in the raw material (Oilgae ., 2015). Commonly, n-hexane is used as solvent. Hexane is a preferred solvent in oil extraction because it is non-reactive and a non-polar solvent. Hence it is also known as an inert solvent. Hexane also has a low boiling point of 67 ^{o} C, hence the extraction process is only carried out at room temperature to prevent solvent lost. In a previous study by Ang et al (2015), n-Hexane was used to extract sea mango oil. As much as 55.99% oil was extracted.

CHAPTER 3

METHODOLOGY

3.1 Materials

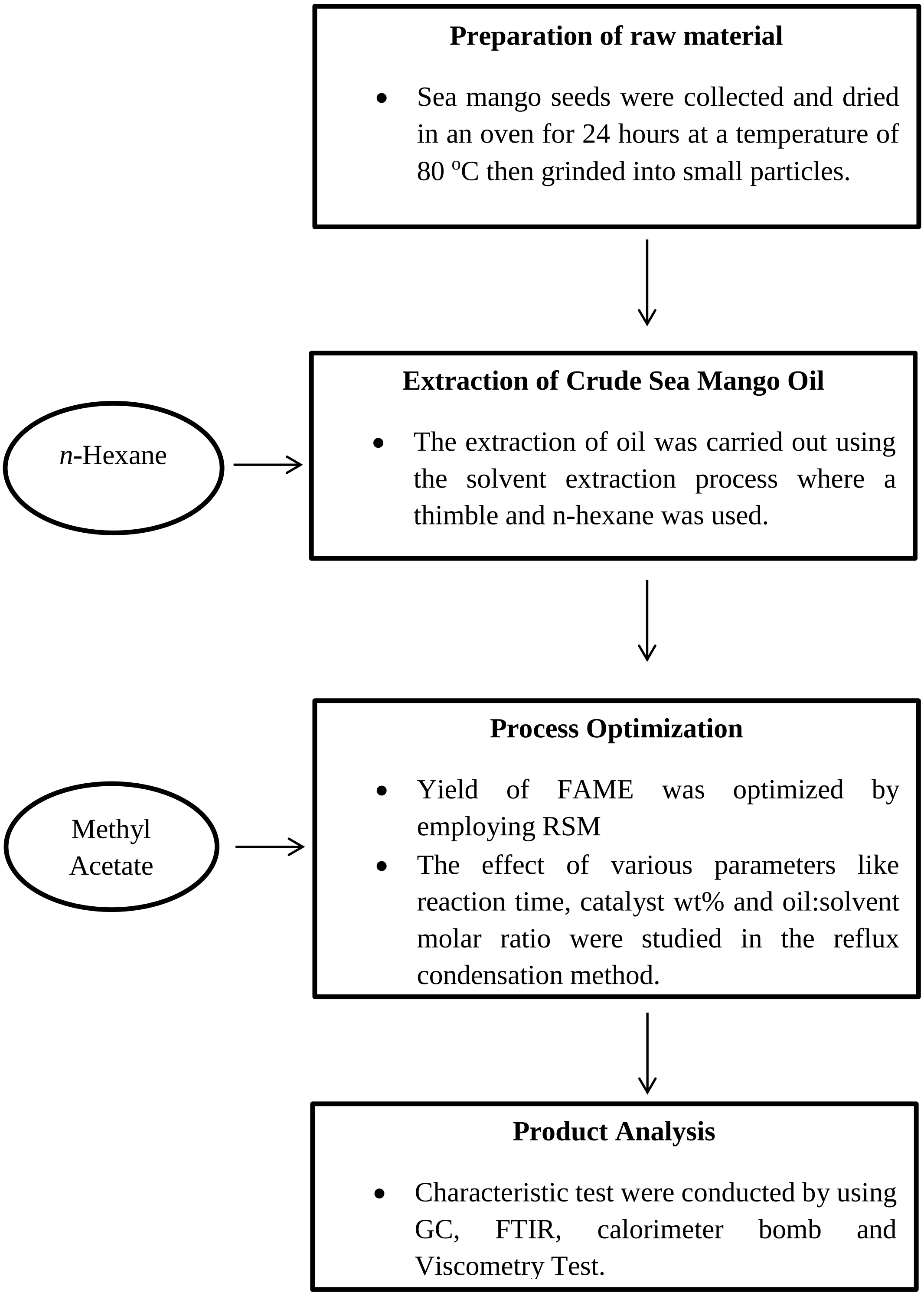
Reddish or brownish *Cerbera Odollam* fruits indicate that the fruits are matured enough to be used for research purposes. This fruits were collected from around Kampar area as well as some areas in Kuala Lumpur, Malaysia. Chemicals that were employed throughout this research are listed in Table 3.1.

Table 3.1: List of Chemicals Used throughout the Research

Chemicals	Supplier	Purpose
n-hexane	CHEMSOLn	Solvent used for oil extraction
Methyl Acetate	Fisher Chemicals, UK	Solvent used during reaction
Methanol	Fisher Chemicals, UK	Added as a component in catalyst
Potassium Hydroxide	GENE Chemicals	Added as a component in catalyst
Phosphoric acid	RCI Labscan	Neutralize the reaction

3.2 Research flow diagram

The research was carried out according to the flow diagram shown in Figure 3.1.



Preparation of raw material

- Sea mango seeds were collected and dried in an oven for 24 hours at a temperature of 80 ^{o}C then grinded into small particles.

Extraction of Crude Sea Mango Oil

- The extraction of oil was carried out using the solvent extraction process where a thimble and n-hexane was used.

Process Optimization

- Yield of FAME was optimized by employing RSM
- The effect of various parameters like reaction time, catalyst wt% and oil:solvent molar ratio were studied in the reflux condensation method.

Product Analysis

- Characteristic test were conducted by using GC, FTIR, calorimeter bomb and Viscometry Test.

Figure 3.1: Research Flow Diagram

3.3 Equipment

Table 3.2 displays the type of equipment used in this experimental research.

Table 3.2: List of Equipment used in Research

Equipment	Brand
Gas Chromatography (GC)	Perkin Elmer, USA
Fourier Transform Infrared Spectroscopy	Perkin Elmer, USA
Grinder	Panasonic
Micropipette	Hmbg, Germany
Rotary Evaporator	Buchi, switzerland
Reflux Condenser	Hmbg, Germany
Heating Mantle	Favorit, Malaysia

3.4 Pre-treatment of *Cerbera Odollam* Seeds

First the seeds were placed in a metal tray after being cleaned, and dried in an oven at a constant temperature of 80˚C for 24 hours to remove its moisture content. The oven temperature was set at a moderate temperature to prevent the seeds from decomposing at high temperatures (>100˚C). Once the seed were obtained from the fruit, it was instantly placed for drying to prevent oxidation from occurring. As a result, keeping the seed for too long might show a less promising reading in the oil extraction amount. Once dried, the seed was grinded in a mechanical grinder into small powder form particles. Oil is then extracted from these seed particles.

3.5 Extraction of *Cerbera Odollam* oil

The extraction method used was solvent extraction process, where *n*-hexane was employed in this process. Several thimbles were used to store the grinded seeds and it was placed in a 250ml beaker. The solvent n-hexane was added in the beaker. All beakers were covered to prevent solvent lost and the process was left to occur for 24 hours. In this study, mass range of 8-20g seeds are used to identify the optimum weight of seeds needed to achieve maximum amount of oil. Two sets of data were obtained with one set using 250ml *n*-hexane and another set using 200ml. After an interval of 4 hours, the colour of seed particles becomes lighter, which indicates that the extraction process is successfully taking place. After 24 hours, the dark brown seeds becomes white, indicating successful oil extraction from the seeds. Oil was recovered via the employment of a rotary evaporator where the hexane was recollected and reused for other extraction processes. Figure 3.2 pictures a typical rotary evaporator used to evaporate hexane from raw oil extracted.

Figure 3.2: Rotary Evaporator

3.6 Design of Experiment (DOE)

Software employed in this research was Design-Expert software version 6.0.6 (STAT-EASE Inc., Minneapolis, USA) to use Response Surface Methodology (RSM) for the transesterification of *Cerbera Odollam* oil. This design method analyses statistical data and optimizes desired responses for experimental runs. The three major steps that are involved in the design of these experiments are statistical design experiment, validation of mathematical model with response prediction and optimization of response. To study the influence of operating parameters (independent variable) on the transesterification of *Cerbera Odollam* into FAME (response), the central composite design (CCD) was chosen as the experimental design. Optimum operating parameters were generated and used from the software to determine the maximum yield of FAME from the transesterification process performed. Three independent variables studied in this experimental design were reaction time, oil to Methyl Acetate molar ratio and catalyst wt%. Table 3.3 lists the levels and range of the independent variable studied. Alpha (α) value of this CCD was set at two with (-α) as the lowest, (-1) being low, (0) as the centre point, (1) being high and (+α) at highest point. The range and levels of independent variables are shown in Table 3.3.

Table 3.3: Range and Levels of Independent Variables

Variable	**Coding**	**Unit**	**Level**				
			-α	**-1**	**0**	**+1**	**+α**
Reaction time	*A*	Hours	3.32	4	5	6	6.68
Oil/Methyl Acetate molar ratio	*B*	mol/mol	10	20	35	50	60.23
Catalyst %	*C*	wt%	0.12	0.15	0.20	0.25	0.28

Hence, 20 runs were carried out and the first 15 runs were organized in a factorial design and the additional 5 repeated runs were conducted at centre points to estimate effect of curvature. Design expert software was used to conduct statistical analysis and regression analysis of the experimental data obtained from the transesterification process. The yield of FAME was calculated and inserted into the software for the analysis to be generated. The mathematical model produced from the response as a function to independent variables allows the desired optimum yield of reflux condenser process to be determined. Equation 3.1 portrays the general equation for 3 independent variables.

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i x_i + \sum_{i=1}^{3} \beta_{ii} x_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} x_i x_j \tag{3.1}$$

Where,

Y = predicted yield of FAME

x_i = i^{th} independent variable

x_j $= j^{\text{th}}$ independent variable

β_0 $=$ intercept

β_i $=$ first order model coefficient

β_{ii} $=$ quadratic coefficient for the variable i

β_{ij} $=$ linear model coefficient for the interaction between variables i and j

ANOVA which is the analysis of variance and correlation coefficient (R^2) was used to check the quality of the proposed model and test the lack of fit of the model respectively. Furthermore there are two-dimensional and three-dimensional plots available to ease the study between the interactions of independent variables used.

3.7 Reflux Condenser Methyl Acetate Transesterification

3.7.1 Condenser Reaction

The condenser method was carried out by employing a coil condenser together with a 250ml round bottom flask since the sample size used does not exceed 200ml in volume. Since temperature of the reaction was maintained at 60 ˚C throughout the experiment, a thermometer was placed in an opening of the bottom flask to monitor the temperature and control the fluctuations. Since homogeneous base catalyst was used, the optimum temperature of 60 ˚C would be sufficient for this transesterification process (Casas et al., 2011). To prevent loss of MA due to heat, the condenser was sealed properly during each runs.

3.7.2 Product Separation

Once products are produced from the performed reaction, it is further boiled to evaporate excess MA. FAME and some impurities like salts remain after excess MA was evaporated. Salts are formed due to the addition of acid to neutralize the final product. Next, product FAME and boiling water was poured into a separating funnel and left to settle for at least 10 minutes. This is to wash away remaining impurities. Once the mixture has settled, it is stirred vigorously and left aside to settle down again. Once settled, 2 layers are observed. The top layer, which is FAME, is further sent for centrifugation while the bottom layer containing water and impurities are disposed.

Figure 3.3: Condenser Tube

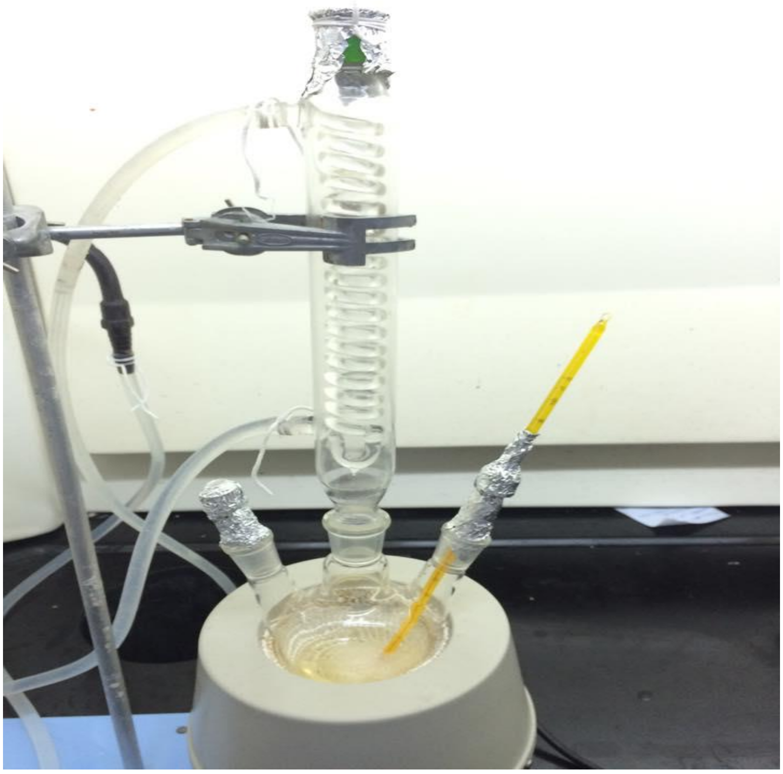


Figure 3.4: Reflux Condenser set-up

From the set up observed in Figure 3.4, a thermometer is used to monitor the reaction temperature where it remains constant at 60 ̊ C. Cool water flows in from the bottom of the coil condenser and exits the top through a hose. Evaporated methyl acetate is fluxed back into the round bottom flask as it touches the condenser coil. The top of the condenser is covered to prevent solvent (MA) from evaporating.

3.7.3 Procedure of Condensation Reaction

For the condensation process to take place, a 250ml round bottom flask, thermometer, retort stand and the coil condenser was set up accordingly. While the experimental setup is in progress, the catalyst used in this reaction was prepared by diluting 3g of potassium hydroxide in methanol. Potassium hydroxide is prepared in access for complete dilution in methanol forming potassium methoxide. The catalyst preparation was done using a magnetic stirrer to provide agitation for a faster dissolving rate.

Depending on the oil to solvent molar ratio, required amount of solvent was added into the round bottom flask to initiate a reaction with 10ml of *Cerbera Odollam* oil extracted earlier. In some reactions, molar ratio of 1:35mol/mol is used, proposing that for 10ml of oil, 32ml of solvent is used. This is calculated using molecular weights of MA and stoichiometric equations. Once the oil and solvent are mixed in the round bottom flask, heater is turned on and the reaction starts to occur. Catalyst is added in immediately after the solvent and the reaction column is sealed to avoid loses of MA due to evaporation.

A thermometer was fitted into the round bottom flask to monitor its boiling temperature. Once the reaction starts to take place, running tap water is turned on to complete the reflux reaction. As methyl acetate evaporates and comes in contact with the cool water running through the coil condenser, it refluxes back in to the round bottom flask where reaction is taking place. This way, there is minimum solvent loss.

After each run, phosphoric acid is added to neutralize the reaction since a strong base is used as catalyst. Based on the amount of catalyst used, a calculated amount of phosphoric acid is added immediately after the reaction to quench the heated solution, producing salts. After quenching, the solution is placed into a centrifuging tube and centrifuged to remove salts. The solution is then heated to evaporate remaining solvent, leaving behind the final product of FAME. Impurities are important to be removed to avoid further reaction from taking place while evaporating access solvent. The amount of phosphoric acid used in each run differs due to the catalyst amount used.

3.8 Product Analysis

3.8.1 Gas Chromatography Procedures

GC-2010 Plus Shimadzu was used to carry out the analysis of diluted samples to identify the amount of FAME produced. The gas chromatography was equipped with Nukol^{TM} capillary column (15 m x 0.53 mm, 0.5 µm film) and Flame Ionization Detector (FID) as the detector. Nitrogen gas was used as the carrier with an initial oven temperature of 120˚C (held for 5 minutes) and raised to 260˚C (held for 8 minutes) at a rate of 20˚C/minute. Apart from that, the temperature of injector and detector were set at 230˚C and 260˚C respectively.

3.8.2 Preparation of Diluted FAME sample

Once converted to FAME, the samples are kept in a universal bottle until further test are conducted. Dilution for Gas Chromatography test was done using hexane, methyl heptadecanoate (C17) and the FAME sample. 20 samples were prepared in a 1.5 ml vial and a dilution factor (total volume/ total volume of FAME) of 19 was used. The internal standard, methyl heptadecanoate was dissolved in hexane to achieve a 1g/L mixture. Then a sample of 80µL was added into the 1420µL of internal standard and hexane mixture. The diluted samples were injected into the GC column and the peak areas of each individual components and internal standard were recorded. According to the modified EN 14103 standards, the content of all the components could be calculated using its areas from the peak as shown in Equation 3.2.

$$C_i = \frac{A_i}{\sum A - A_{IS}}\ x\ 100\% \tag{3.2}$$

Where

C_i = content of different component in the sample in %

A_i= compound peak area

$\sum A$= total peak area

A_{IS} = peak area of internal standard (methyl heptadecanoate)

3.8.3 Determination of FAME yield

According to modified EN 14103 method, the yield of FAME produced was calculated using FAME content as mass fraction and also methyl heptadecanoate (C17) as internal standard. The total FAME content was determined using Equation 3.3.

$$C = \frac{\sum A - A_{IS}}{A_{IS}}\ x\ \frac{C_{IS}\ x\ V_{IS}}{m}\ x\ 100\% \tag{3.3}$$

Where,

$\sum A$= total peak area

A_{IS}= peak area of internal standard (methyl heptadecanoate)

C_{IS}= concentration of internal standard solution, in mg/mL

V_{IS}= volume of internal standard solution used, mL

m= mass of sample, in mg

The mass of FAME content can be obtained while the yield is calculated based on Equation 3.4.

$$Yield\ of\ FAME = \frac{Mass\ of\ FAME\ Content\ (g)}{Mass\ of\ sample\ (g)}\ x\ 100\% \tag{3.4}$$

3.8.4 Fourier Transform Infrared Spectroscopy (FTIR)

To identify the functional group of each component in FAME produced from Cerbera Odollam, the qualitative analysis of product was conducted using Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR) (PerkinElmer Inc., USA). The FTIR was equipped with beam splitter and deuterated triglycine sulphate (DTGS) detector. By putting in contact with ATR on a multi-bounce plate of crystal at 25^{o} C the sample was analysed and subsequently the spectrum ranged from 4000-650 cm^{-1}, co-adding 32 interferograms at resolution of $4cm^{-1}$, was collected.

3.8.5 Calorimeter Bomb

IKA C200 Calorimeter system was used to identify the calorific value of product. The calorimeter system was equipped with measuring cell C200, decomposition vessel C5010, oxygen filling station C248 and consumables for calibrations and instillation. The sample was subjected to decomposition vessel by putting inside gelatin capsule. With a measuring time of 17 minutes isoperibolic mode was chosen for sample analysis. Calorific value was obtained.

3.8.6 Viscometry

A CANNON Instrument Ubbelohde Viscometer was used to determine the kinematic viscosity of the FAME product and was done in accordance to ASTM D445 method. Firstly, the viscometer is cleaned using octane as a suitable solvent and subsequently dried to remove the solvent used earlier. The viscometer was then charged with enough FAME products until the level reached between the upper and lower marking of the lower reservoir. The viscometer was then secured to its holder and placed in a water bath of constant temperature of 40 ° C for 20 minutes to allow the sample to reach the water bath temperature.

Later, tube M was covered and suction was applied using pipette pump to tube N until the sample reaches the centre of bulb D. Once the suction was removed, the cover from tube M was lifted and placed onto tube N. Tube N was then covered until all excess sample dropped away from the lower end of the capillary tube. Finally, the kinematic viscosity was calculated by multiplying the resultant time with the viscometer constant which was at 0.004828 mm^2/s^2. Figure 3.5 shows the diagram of a viscometer.

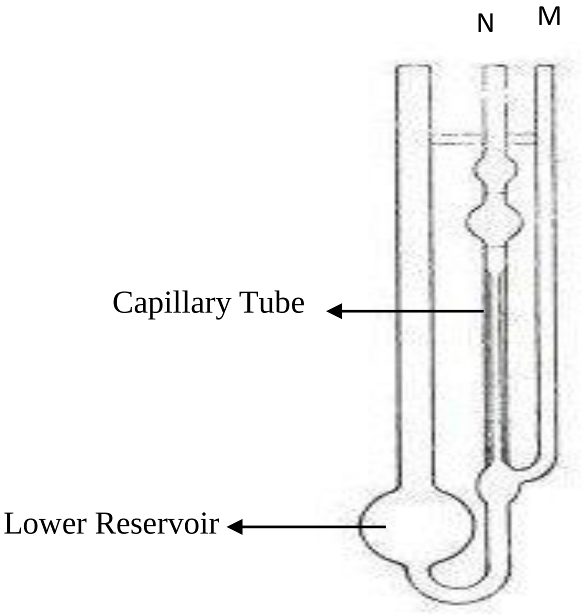


Figure 3.5: Diagram of Viscometer

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Optimization of Sea Mango Oil Extraction

In this research, three variables were manipulated to determine the optimum crude oil extracted namely, the mass of seeds, amount of hexane and extraction time. The first process was carried out using 8g, 12g, 15g, 16g, and 20g of grinded seed in a fixed 200ml of hexane and 24 hours of extraction time.

Table 4.1: Results of Oil Extraction Using a Smaller Volume of 200ml Hexane and Fixed Time of 24 Hours

Sample No.	Mass of seed (g)	Mass of sea mango oil extracted (g)	Oil content (%)
1	8	3.25	40.63
2	12	5.01	41.75
3	15	6.40	42.70
4	16	6.9	43.20
5	20	8.10	40.05

Table 4.1 illustrates the results from the first attempt of extracting sea mango oil. From the table, the increment in percentage of oil extracted becomes lesser and even drops at 20g of seeds used. Since the thimble only accommodates 20g of sample, the hexane is not sufficient enough to extract oil from higher mass of grinded seed. Then another attempt was done with higher volume of hexane.

Table 4.2: Results of Oil Extraction for 250ml Hexane used within 24 Hours

Sample No.	Mass of seed (g)	Mass of Sea Mango Oil Extracted (g)	Oil Content (%)
1	8	3.50	43.75
2	10	4.82	48.00
3	12	6.10	50.80
4	15	8.00	53.00
5	18	9.91	55.05
6	20	11.00	55.00

Table 4.2 displays the results using higher volume of hexane with different masses. In accordance with this table, the oil content increases from 8g to 20g smoothly. Comparing with the earlier results obtained in Table 4.1, the 8g sample is higher in oil content during the second attempt. Oil content increased from 43.75% to 55.05% as the mass of seed increases from 8g to18g. The extraction time was fixed at 24 hours. At 20g of seed mass, the oil content remained at 55% without any further increment. Hence it shows that the sea mango has reached its optimum oil content when 18g of seed is used.

Kansedo (2009) researched that the sea mango seed contains 54% oil content. However the oil content in this study fluctuated to about 55%. This is because of the use of thimbles to extract the sea mango seeds, with high volume of hexane (250ml) used for each thimble. The solubility of soil and area at which the sea mango tree was planted also makes a slight difference in its oil content. When the seeds are grinded to smaller particles, it is most likely to be exposed to a larger surface area when it comes in contact with hexane, thus better extraction results are obtained.

4.2 Reflux Condensation Methyl Acetate

4.2.1 Design of Experiment (DOE)

Table 4.3 shows the entire experiment designed using design expert software together with FAME yield of the research conducted. From this table, the range of yield obtained is from 15% to 66%, with three operating variables in the experiment. This shows that the yield obtained is moderately high. The highest FAME yield was achieved with oil to MA ratio of 1:35mol/mol, reaction time of 5 hours and a catalyst percentage of 0.28%.

Table 4.3: Experimental Design Matrix and Results for Reflux Condensation Reaction

Run No.	Reaction Time (hours)	Catalyst Ratio (wt%)	Oil: Solvent molar ratio (mol/mol)	Yield (%)
1	3.30	0.20	35.00	44
2	4.00	0.15	20.00	25
3	4.00	0.25	20.00	35
4	4.00	0.15	50.00	45
5	4.00	0.25	50.00	48
6	5.00	0.12	35.00	45
7	5.00	0.20	35.00	58
8	5.00	0.28	35.00	66
9	5.00	0.20	9.77	15
10	5.00	0.20	60.23	41
11	6.00	0.25	50.00	40
12	6.00	0.15	20.00	32
13	6.00	0.25	20.00	30

14	6.00	0.15	50.00	38
15	6.40	0.20	35.00	53
Repeated Runs				
16	5.00	0.20	35.00	55
17	5.00	0.20	35.00	59
18	5.00	0.20	35.00	60
19	5.00	0.20	35.00	58
20	5.00	0.20	35.00	62

From Table 4.3, the optimum condition patterns on the yield of FAME can be observed clearly. For example, when the oil: solvent molar ratio is too low, the yield of FAME is relatively low but it increases with the increment in oil: solvent molar ratio. When the molar ratio is at 1:9.77mol/mol, the FAME yield is at its lowest. As the molar ratio increases to 20mol to 35mol, the yield of FAME increases but when the molar ratio is too high, the FAME yield starts decreasing again.

Runs 7 and 8 both contain the same solvent ratio, 1:35mol/mol, when the ratio increases to 1:50mol/mol, yield decreases. Hence the optimal solvent ratio is about 1:35mol/mol. With the molar ratio, reaction temperature and catalyst percentage aids in determines the optimum yield. Too low percentage of catalyst gives lower yield and too much time of reaction lowers the FAME yield. The optimum reaction time in this research is 5 hours. For runs that exceeds 5 hours give lower FAME yield.

For runs 11 to 15, the time was fixed to 6 hours with different oil to solvent ratio and catalyst percentage. A drop in FAME yield was observed when lower solvent ratios were used as well as lower catalyst percent, in addition to that, runs that has high solvent ratios and low catalyst percent also resulted in lower FAME yields ranging from 30% to 36%. The run had obtained highest FAME yield is run 8 with 5 hours reaction time, 0.28% catalyst 1:35mol/mol solvent ratio, obtaining 66% FAME yield.

4.2.2 Statistical Analysis

Statistical analysis includes sequential model sum of squares and variance analysis using ANOVA. This analysis is conducted mainly to determine the conditions of variables which influence the response. Table 4.4 shows the sequential model sum of squares.

Table 4.4: Sequential Model Sum of Square for Reflux Condensation Reaction

Source	**Sum of Squares**	**DF**	**Mean Square**	**F Value**	**Prob > F**	
Mean	41587.20	1	41587.20			
Linear	815.53	3	271.84	1.61	0.2272	
2FI	58.38	3	19.46	0.095	0.9612	
Quadratic	2331.75	3	777.25	24.51	>0.0001	Suggested
Cubic	129.97	4	32.49	1.04	0.4583	Aliased
Residual	187.18	6	31.20			
Total	45110.00	20	2255.50			

Once yield of FAME is obtained and keyed in the software, a model will be generated together with its optimized analysis of the data entered. From Table 4.4, it is known that the quadratic model was suggested as the best model to optimize this experimental reaction as it fits the studied range of operating parameters with a ‘Prob>F’ value equal to 0.0001.

Then, an analysis of variance model (ANOVA) is used to evaluate the fitness of the model and also to determine the interactions of the models from the F value. The model is considered to be significant if the ‘Prob>F’ value is smaller than 0.05. Hence in this research conducted, the ‘Prob>F’ value is calculated to be 0.0004. This indicates that the models A, A^2, C^2, are significant model terms. Values of ‘Prob>F’ larger than

0.1 is insignificant and will not be taken into account of it will be eliminated. There are many insignificant terms in a model, thus model reduction improves a model.

Table 4.5: Analysis of Variance (ANOVA) Reflux Condensation Reaction

Source	**Sum of Squares**	**DF**	**Mean Square**	**F Value**	**Prob > F**	
Model	3205.65	9	356.18	11.23	0.0004	significant
A	629.59	1	629.59	19.85	00012	
B	183.05	1	183.05	5.77	0.0372	
C	2.88	1	2.88	0.091	0.7693	
A2	2181.45	1	2181.45	68.78	< 0.0001	
B2	109.57	1	109.57	3.45	0.0927	
C2	250.78	1	250.78	7.91	0.0184	
AB	1.12	1	1.12	0.035	0.8544	
AC	36.12	1	36.12	1.14	0.3109	
BC	21.13	1	21.13	0.67	0.4334	
Residual	317.15	10	31.75			
Lack of Fit	295.65	5	59.13	13.75	0.0664	not significant
Pure Error	21.5	5	4.30			
Cor Total	3522.80	19				

From Table 4.5, the model F-value of 11.23 implies the model is significant. This means, there is only 0.04% chance that a “Model F-Value” this large could occur due to noise. In addition, The "Lack of Fit F-value" of 13.75 implies the lack of fit is insignificant. There is only a 6% chance that a "Lack of Fit F-value" this large could occur due to noise. A significant lack of fit is not a good sign for the model. In order for the model to fit, the lack of fit has to be insignificant.

Through a predicted vs experimental plot, the quality of the model which was developed is determined by the correlation coefficient (R^2). In this study, the R^2 value is 0.9100 which means that 91% of the total variation of results was from the studied variables in this research. Figure 4.1 shows the predicted yield vs experimental yield of this research. The straight line slope compares the regression model with the theoretical one. There is a fairly good achievement in experimental yield although not very promising, since the regression model is almost nearing the value 1. The higher the value of R^2, the higher the accuracy of the model generated.

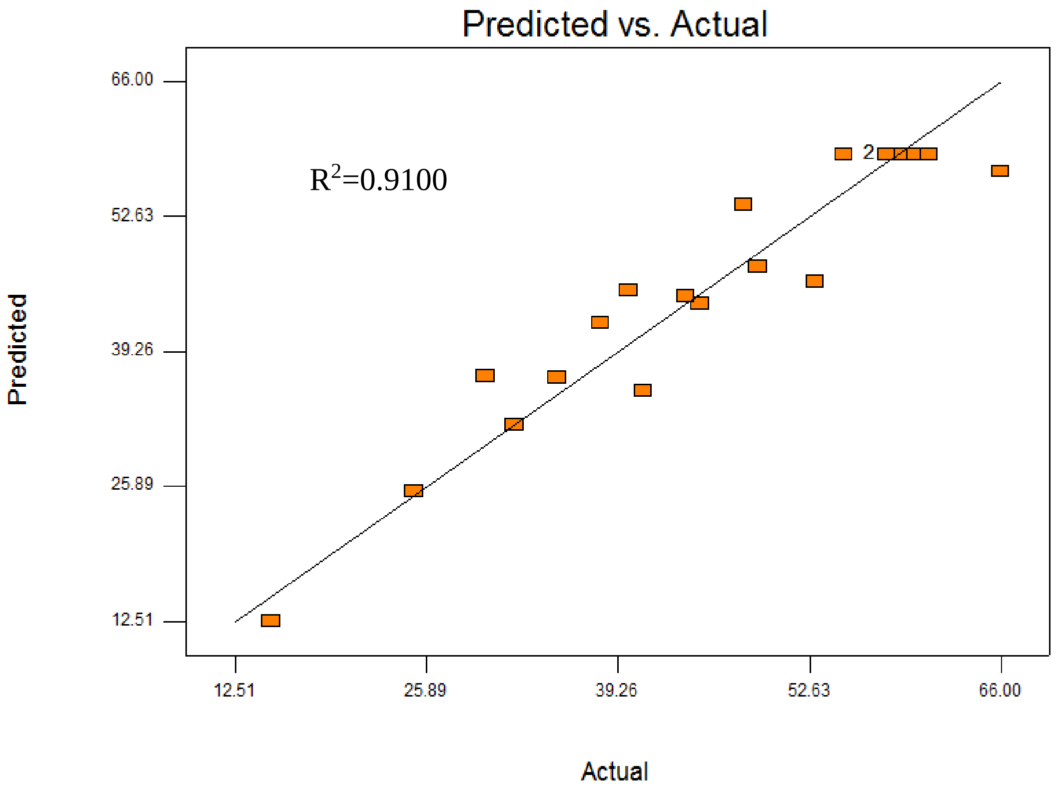


Figure 4.1: Predicted Yields (%) against Actual Yields for Reflux Condensation Method

The final equation of coded factors after elimination of insignificant terms is equated in Equation 4.1.

$$\text{Yield} = 58.73 + 6.79\,(A) + 3.66\,(B) - 0.46\,(C) - 12.30\,(A^2) - 2.76\,(B^2) - 4.17\,(C^2) - 0.37\,(AB) - 2.13\,(AC) - 1.63\,(BC) \tag{4.1}$$

From Equation 4.1 it is known that significant terms existing has a great influence on the FAME content. Those insignificant is eliminated and neglected. From the equation, there are both positive values and negative values existing. The positives means synergistic effect while the negative is antagonistic effect. From the equation, molar ratio of solvent to oil (A) and catalyst percentage (%) have high coefficient, which means they play an important role as a variable affecting the FAME content in this research. While Equation 4.1 shows coded factors, Equation 4.2 equates actual factor interpretations.

$$\text{Yield} = -245.64080 + 5.08867\,(\text{Molar ratio}) + 694.39767\,(\text{Catalyst}) + 52.71460\,(\text{Time}) - 0.054681\,(\text{Molar ratio})^2 - 1102.93796\,(\text{Catalyst})^2 - 4.17156\,(\text{Time})^2 - 0.50000(\text{Molar ratio})\,(\text{Catalyst}) - 0.14167\,(\text{Molar ratio})\,(\text{Time}) - 32.50000\,(\text{Catalyst})\,(\text{Time}) \qquad (4.2)$$

4.2.3 Variable Interaction

Interactions amongst each variable are important and significant in the study of biodiesel production as it affects the yield of FAME produced. Single variable studies are not necessary when there is optimization of variables involved.

Figures 4.2 (a) and (b) illustrates the effects of molar ratio (A) and catalyst ratio (B) on the FAME content. The figures are shown in two-dimensional and three-dimensional interaction plots. Increment in molar ratio of MA helps to increase the yield of FAME. Limitation of reaction equilibrium and difficulties in separating excessive MA from FAME and triacetin results in a decrement in FAME yield (Tan et al., 2009).

In this research, the illustration in Figure 4.2 (b) shows an increment in yield as the catalyst amount increases from 0.15% to 0.25% and methyl acetate molar ratio of 20-35 mol/mol. As the molar ratio increases beyond 1:35 mol/mol, FAME yield gradually decreases regardless of the catalyst amount. Tan et al. (2009) explains the effects in productivity when there is too much access of methyl acetate in the reaction. Higher temperatures degrade the catalyst being used (Casas et al., 2012).

The three-dimensional graph distinguishing two variables and its effect on FAME yield gives a quadratic curve. This is due to its rise in FAME yield with increment in catalyst amount and molar ratio up to 1:35 mol/mol, and gradually drop from 1:35mol/mol onward to 1:50mol/mol ratio.

The trend observed in Figure 4.2 differs from Figure 4.3 and Figure 4.4. It shows that there is no decrease in yield of FAME with an increasing amount of catalyst at an optimum oil:solvent molar ratio amount. As the molar ratio increases with incresing amount of catalyst, yield of FAME gradually decreases. This trend can be explained with Tan et al., (2009) previous study that describes the difficulties in separating excessive MA from FAME and triacetin which results in a decrement in FAME yield.

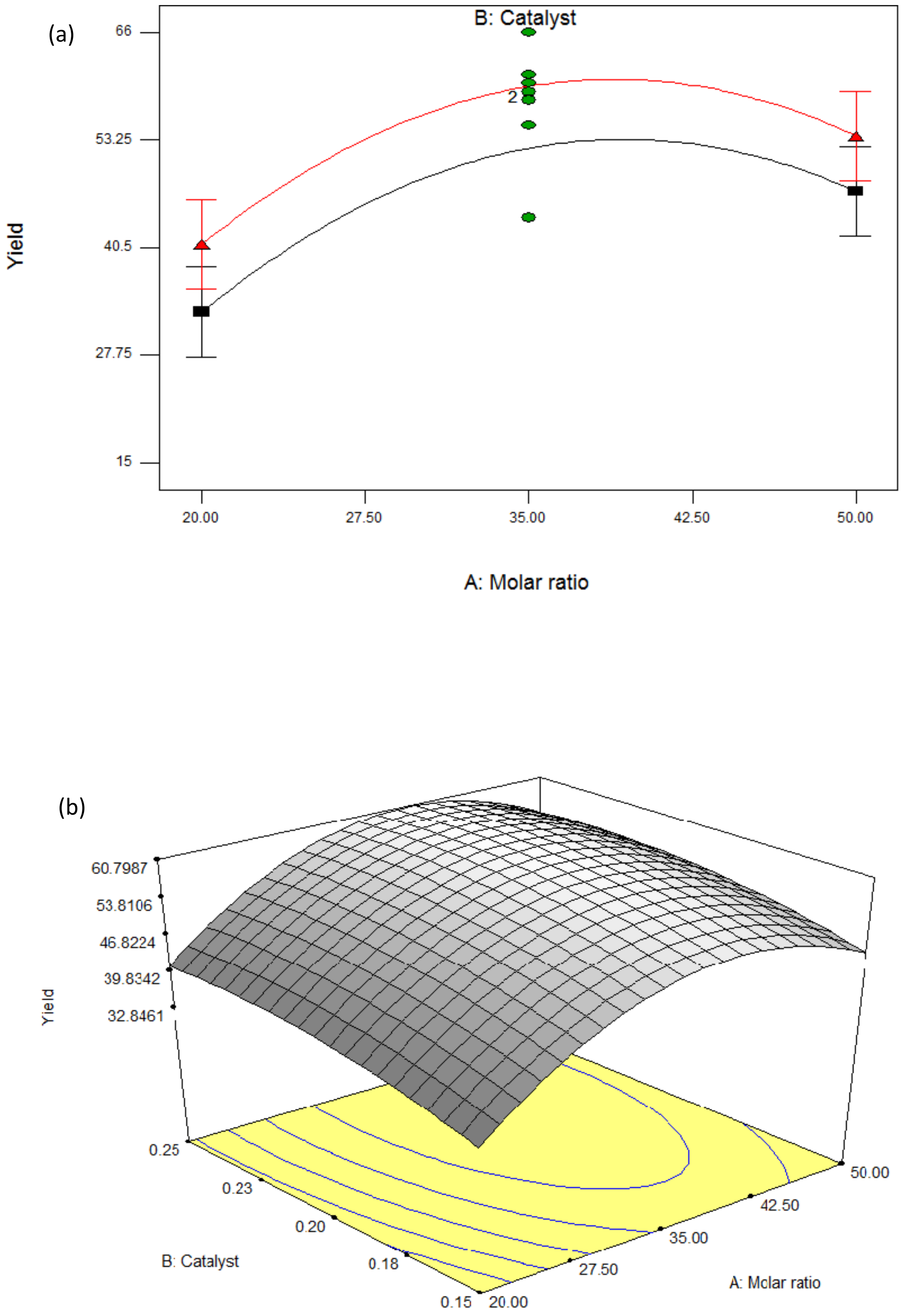


Figure 4.2: Effects of Oil to Solvent Molar Ratio (A) and Catalyst (B) on the FAME content in (a) and (b) Two-dimensional and Three-dimensional graph respectively in Reflux Condensation Reaction

Next, Figure 4.3 (a) and (b) shows the effect of oil: methyl acetate molar ratio (A) and time of reaction (C) on FAME yield for reflux condensation method used in this study. Illustration is shown in two-dimensional and three-dimensional figures respectively. From previous researches it is stated that longer time in a reaction promotes higher activity till it reaches equilibrium (Tan et al., 2010). Once it reaches equilibrium at an optimum reaction time, the activity starts to decrease. Similarly in this study, reaction time with highest FAME yield is at 5 hours.

From Figure 4.3 (a) and (b), as the molar ratio increases from 1:20mol/mol to 1:50mol/mol, the yield in FAME increases. The lowest yield was obtained with 4 hours of reaction time and 1:20mol/mol oil: solvent molar ratio. From this interaction, it is observed that molar ratio plays a bigger role in this research as compared to reaction time.

At reaction time of 4 hours and molar ratio of 1:50mol/mol, the yield of FAME obtained is higher when compared to the same reaction time and a molar ratio of 1:20mol/mol. It is also observed that the FAME content decreases when molar ratio exceeds 1:35mol/mol and reaction time of 5 hours. The effect of molar ratio on the reaction time is most prominent at 1:35mol/mol in 5 hours of reaction time.

A trend is observed in Figure 4.3 (a) and (b) where the yield of biodiesel decreases after its optimum reaction time and molar ratio. The reaction time has reached its equilibrium at which the reaction rate is high. No further reaction in an increment in reaction time and molar ratio would result in degradation of product. Thus the FAME yield decreases when the reaction time is beyond its optimum condition. This trend shows that there is a good interaction between these two variables.

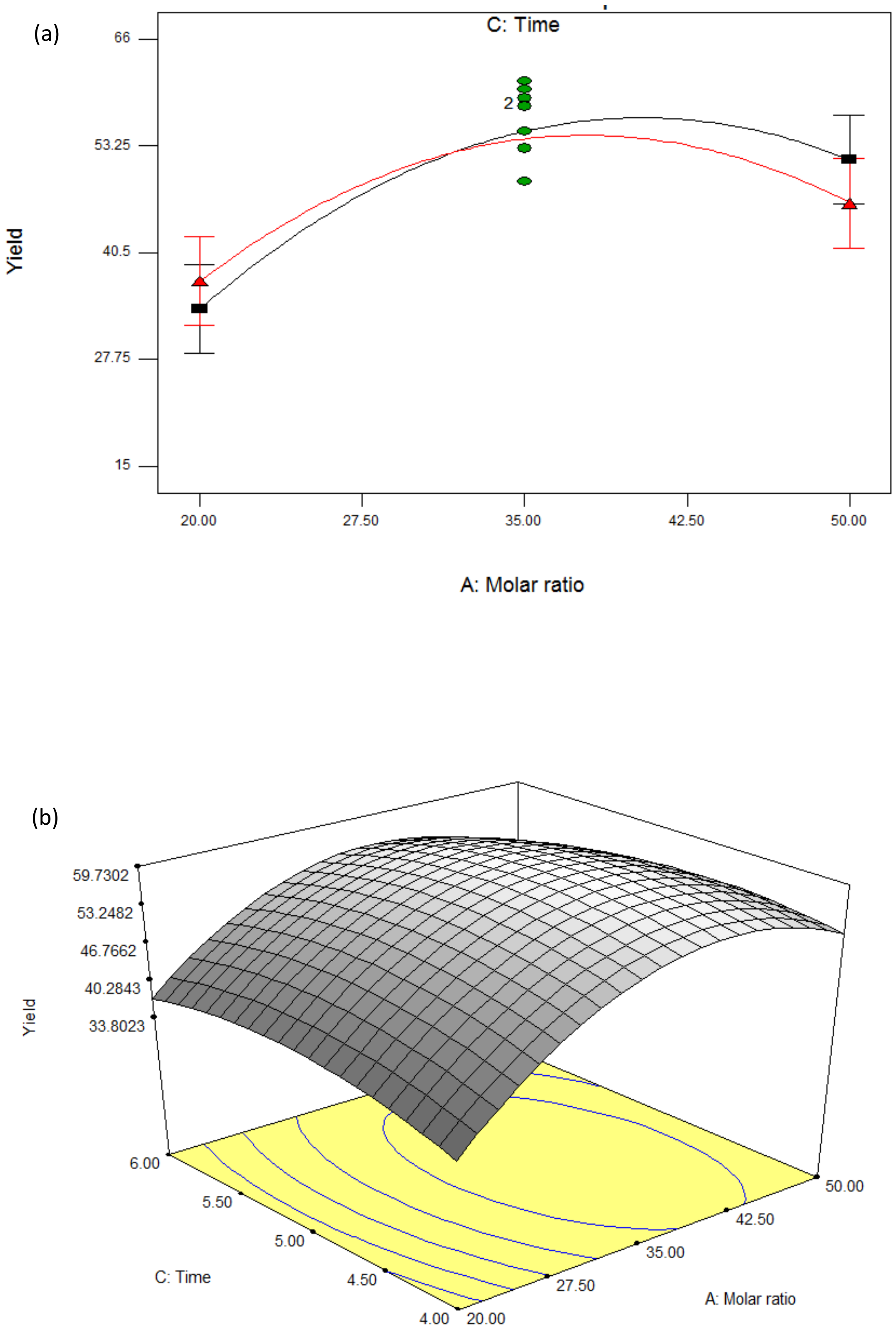


Figure 4.3: The Effects of Oil to Solvent Molar Ratio (A) and Reaction Time (C) on the Yield of FAME in (a) Two-Dimensional and (b) Three-Dimensional graph for Reflux Condensation Reaction.

Furthermore, the effect of amount of catalyst used (A) and reaction time (C) is further illustrated in Figure 4.4 (a) and (b) both in two-dimensional and three-dimensional graph respectively. Wu et al (2014) stated that an insufficient amount of catalyst would result in a longer reaction time in which an equilibrium conversion would be obtained. Also, the effect of an increasing catalyst decreases for a long reaction time. Based on Wu et al (2014) study which comes in agreement with this research, a high concentration of catalyst was chosen and a medium reaction time for the optimum yield of FAME.

From Figure 4.4 (a) and (b), it is observed that the FAME yield deteriorates with a longer reaction time. It is not at its highest performance even when the reaction time is too short for a complete conversion. At 4 hours of reaction time, the yield is at its lowest. As the reaction time increased from 4 hours to 4.5 hours and finally 5 hours, FAME yield increased with the increment in catalyst amount. As the reaction time increased further beyond 5 hours, FAME yield starts deteriorating.

Comparison of the same amount of catalyst used at different reaction time can be done from Figure 4.4 as well. It is observed that at 5 hours and 6 hours of reaction time, the same amount of catalyst used (0.20%), has a different effect on FAME yield. For the run with 5 hours of reaction time and 0.20% catalyst, FAME yield is observed to be higher than the 6 hours run reaction with the same amount of catalyst. Hence the optimum time of reaction suggested is at 5 hours and 0.28% catalyst. As stated by Wu et al (2014), higher amount of catalyst could result in a shorter reaction time.

In contrast, when a smaller amount of catalyst is used at a shorter time, the reaction would not be completed. For a large amount of catalyst at a short reaction time, the reaction may not be completed either. Thus it is important to determine an optimum reaction time for the concentration of catalyst chosen. From the trend observed in Figure 4.4, a longer reaction time causes catalyst activity to deteriorate, resulting in lower FAME yield for an increasing reaction period.

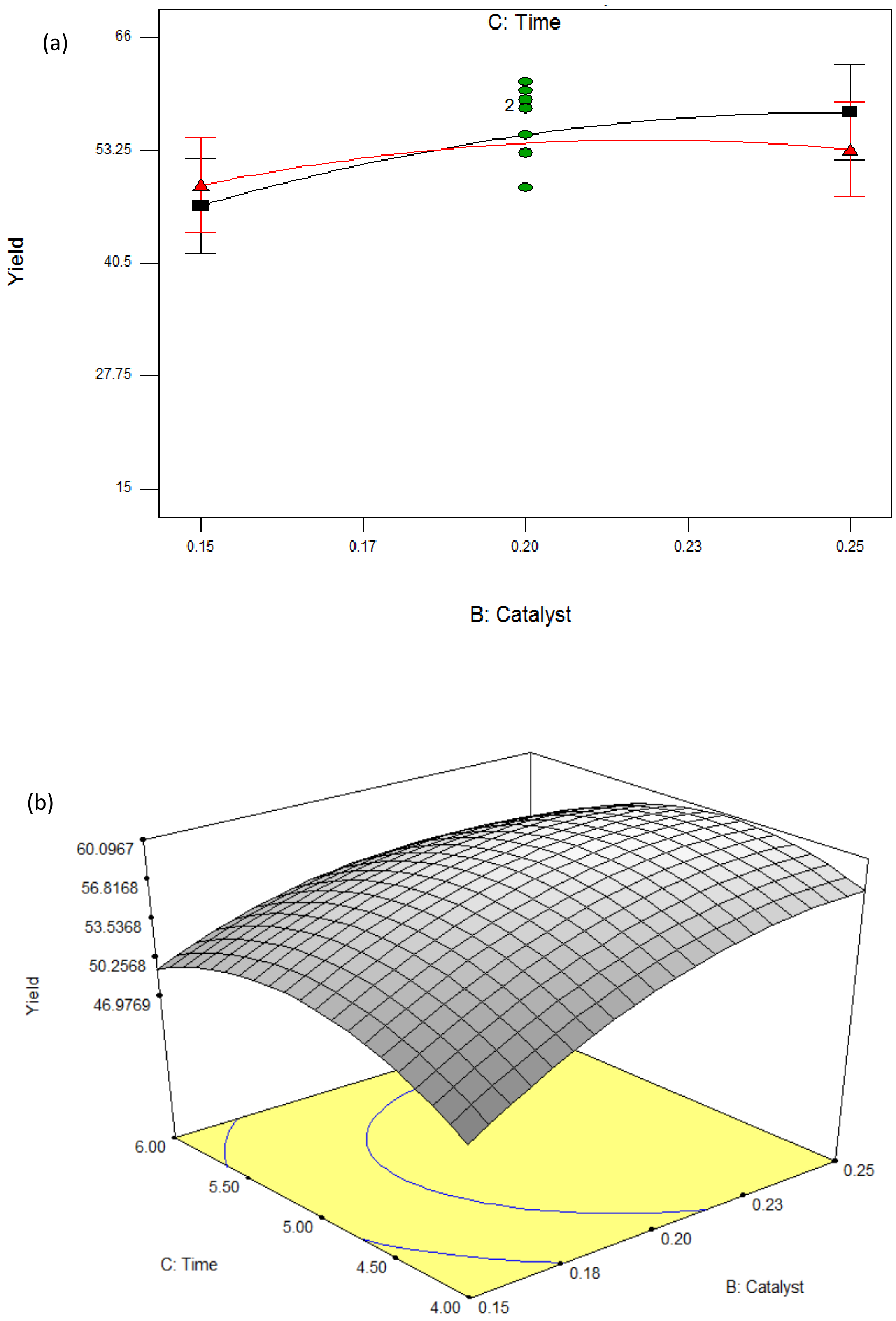


Figure 4.4: The Effect of Catalyst (B) and Reaction Time (C) on FAME Yield in (a) Two-dimensional and (b) Three-dimensional graph for Reflux Condensation Reaction.

4.2.4 Process Optimization

RSM analysis is employed in this research to aid in the optimization of process variables in FAME production of *Cerbera Odollam*. Numerical features of the design software used to identify a perfect combination of optimizing variables needed to achieve a desired FAME yield throughout this research. The variables used in this study are (A) reaction time in hours, (B) the oil: methyl acetate molar ratio (mol/mol) and (C) the amount of catalyst used (%). All these variables are fixed between the lowest range of (-1) and highest range of (+1) while the FAME content was set to a maximum value. The optimum yield content and proposed optimum conditions to achieve this desired yield is shown in Table 4.6.

Table 4.6: Proposed Optimum Conditions and Optimum Yield of Reflux Condensation Reaction

Conditions	Reflux Condensation Reaction
Reaction Time (A), hours	5
Oil: Solvent Molar Ratio, mol/mol	1:35
Catalyst, %wt	0.28
Predicted FAME yield, %	62

Table 4.6 summarizes the optimum conditions to achieve a FAME yield of 62%, where 5 hours of reaction time, 1:35mol/mol oil: solvent molar ratio and 0.28%wt catalyst was used in this reaction study. After conducting 20 experimental runs with different reaction condition parameters in each reaction, the highest yield was observed to be at these conditions. In addition, the experimental yield produced with these parameters is 66% slightly higher than the predicted yield produced by the design software. A small percentage error of 6.45% indicates that the experimental and predicted yield is not much of a gap. Nevertheless, the smaller the percentage error obtained results in a better experiment. Smaller percentage error value indicates that the experimental and

predicted values are at agreement to each other. A conclusion can be made that the regression model developed in this research was reliable in predicting the FAME yield in the given condition range provided. Table 4.7 compares the predicted and experimental yield in this research.

Table 4.7: Comparison of Predicted and Experimental Yield in Reflux Condensation Method

Run	Predicted FAME Content (%)	Experimental FAME Content (%)	Error (%)
1	62	66	6.45

4.3 Characterization Tests

In order to verify the various components in the reflux condensation reaction process to produce FAME, characterization tests were conducted. The sample with the highest FAME yield was chosen for the series of tests. Table 4.8 shows each component found when GC-FID test was conducted. Together is the calculation of weight percent for each component.

Table 4.8: Weight percent of each component in FAME Content

Component	Content, C_i (%)
Methyl Palmitate	32.11
Methyl Palmitoleate	2.58
Methyl Stearate	4.29

Methyl Oleate	38.45
Methyl Linoleate	14.18
Methyl Heptadecanoate (IS)	2.78

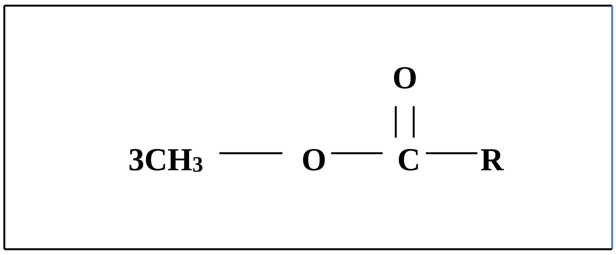
4.3.1 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) is used to do a qualitative analysis on the FAME sample to determine present functional groups in the sample. An FTIR spectrum is showed in Appendix A. Results obtained from FTIR analysis is shown in Table 4.9.

Table 4.9: Absorption Frequency for Respective Functional Group in FTIR test Results

Functional Group	**Molecular Motion**	**Absorption Frequency (cm^{-1})**	**Absorption Frequency of Peak Exist in Sample (cm^{-1})**
Alcohols	O-H stretch	3200-3600	3474
Alkanes	C-H stretch	2850-3000	2926
Esters	C=O stretch	1735-1750	1744
Alkanes	-C-H bend	1350-1480	1439, 1452
Esters	C-O stretch	1000-1320	1116, 1244
Alkenes	=C-H stretch	650-1000	810

The chemical structure of fatty acid methyl ester (FAME) is shown in Figure 4.5.



$$3CH_3 — O — \overset{\overset{O}{||}}{C} — R$$

Figure 4.5: Fatty Acid Methyl Ester (FAME) Chemical Structure

The ester peak from range 1735 cm^{-1} to 1750 cm^{-1} and range 1000 cm^{-1} to 1320 cm^{-1} is the most prominent functional group. From Table 4.9, it is shown that there were esters present in the sample where it had molecular motion C=O stretch at the peak of 1744 cm^{-1} and C-O vibration stretch at peaks 1116 cm^{-1} and 1244 cm^{-1}. The presence of esters indicates that the reaction to produce FAME is successful. From the analysis, alkanes were also found to be present in the sample at peaks 2926 cm^{-1} where it was the C-H vibration stretch. On the other hand, at peaks 1439 cm^{-1} and 1452 cm^{-1}, alkanes with -C-H bend was observed. These observations were made with the aid of the FTIR spectrum provided in Appendix A. alcohols were also observed at peak 3474 cm^{-1}. Finally methyl palmitoleate, methyl oleate and methyl linoleate was present and observed using GC-FID analysis. These components contains C=C double bonds which leads to alkenes observation in the FTIR spectrum at peak 810 cm^{-1}.

4.3.2 Calorific Value

FAME sample was tested with calorimeter bomb to identify its calorific value or its heating value. In previous studies conducted by Ong et al. (2014), the calorific value obtained was 39.9 MJ/Kg. In this study conducted, the calorific value obtained was 39.76 MJ/Kg. In another study by Silitonga et al. (2013), the calorific value obtained is 40.226 MJ/Kg. from all three test results, the calorific value does have a huge difference. The average calorific value from these three results is 39.96 MJ/Kg. A small percentage error of 0.5% was obtained. This percentage error is too small and insignificant, hence the calorific value from this study and previous studies are well in agreement. Table 4.10 summarises the calorific value in this current study and two other previous studies. Comparing with test methods used to determine the calorific value of petroleum diesel, it is found that the calorific values for FAME are not far behind the calorific value of petroleum diesel. Using test method EN14214, the calorific value for petroleum diesel is 45.825 MJ/Kg. This shows that the FAME produced is suitable and able to replace petroleum diesel, hence promoting renewable energy.

Table 4.10: Comparison of Calorific Value between Previous Studies and Current Study

	Previous Study by Silitonga et al. (2013)	**Previous Study by Ong et al. (2014)**	**Current Study**	**Average Calorific Values**	**Percentage Error (%)**	**Petroleum Diesel Calorific Value**
Calorific Value (MJ/Kg)	40.226	39.90	39.76	39.96	0.5%	45.825

4.3.3 Kinematic Viscosity

One sample is chosen to undergo the kinematic viscosity test. In previous studies by Silitonga et al. (2013) the kinematic viscosity at 40 ^{o}C obtained was 4.86 mm^2/s. The ASTM standard requires the kinematic viscosity of FAME to be at 1.9 mm^2/s to 6 mm^2/s. Test method D 6751 was used to determine the kinematic viscosity range of FAME. A lower kinematic viscosity means the product is not viscous hence it is able to flow easily. In this current study, the kinematic viscosity was recorded to be at 5.1 mm^2/s. The kinematic viscosity obtained from this study is within the ASTM standard range.

As for petroleum diesel, the kinematic viscosity is at 2.91 mm^2/s (Ong et al., 2014). Comparing from previous studies and this current study, a slight percentage error of 4.9 % is obtained.

Table 4.11: Kinematic Viscosity Comparisons

	Previous Study by Silitonga et al. (2013)	**Current Study**	**Petroleum Diesel**	**ASTM Standard (D 6751)**
Viscosity (mm^2/s)	4.86	5.10	2.91	1.9-6.0

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In conclusion, the extraction of sea mango oil was carried out successfully after a few trials and comparisons with previous studies. The highest percent weight of oil extracted using solvent extraction method in this study is 55wt%. Theoretically, sea mango contains 54wt% of oil. This ensures that the extraction process was successful.

On the other hand, the reflux condensation method was optimized using the response surface methodology (RSM) method. The three parameters used to determine an optimum yield of FAME in this study is oil to methyl acetate molar ratio, percentage weight of catalyst and reaction time. It was found that the parameters used in this study are sufficient to predict the yield of FAME in the reflux condensation method used. Nevertheless, the optimum conditions used in this study is reaction time of 5 hours, oil to methyl acetate molar ratio of 1:35mol/mol and catalyst amount of 0.28wt%. With these optimum parameters used, FAME yield of 66% was obtained.

Also, various characterization test were conducted for the analysis of FAME content produced in this study. The test conducted were FTIR, calorimeter bomb and also the viscosity test. For FTIR test, the presence of ester in the spectrum indicates a good quality of FAME produced with C=O vibration stretch and C-O vibration stretch. Furthermore, the calorific value of FAME sample was found to be at 39.76 MJ/Kg which is near the calorific value of petroleum diesel. Lastly, the viscosity of FAME sample was found to be at 5.10 mm^2/s which is in the range of the ASTM standard (1.9-6 mm^2/s).

5.2 Recommendations

The reflux condensation method has proved to be a good technology in the production of biodiesel. New catalyst have been tried out instead of just a single heterogeneous catalyst, potassium hydroxide is further reacted with methanol in the presence of methyl acetate in this study. The results have been shown to be a successful conversion process to biodiesel. Nevertheless there is always room for improvement to further increase the yield of biodiesel. Several recommendations can be carried out like:

- In this research, the temperature of the reaction was fixed to 60 ^{o}C due to a reference study conducted previously. In the future, reaction temperature can be another parameter to further compare the optimized reaction temperature from previous studies and this current study.
- References on biodiesel production using potassium methoxide and reflux condensation method are limited. Further literature on this homogeneous catalyst and its exact mechanism on the reaction would be useful for future researches.
- A better design of the condensation process with no escaping solvent during reaction would definitely result in a higher productivity. When there is loops holes for solvents with low boiling points to escape, the ratio of oil to solvent becomes imbalance, hence a not so accurate reading is acquired. A ready-made thermal sensor installed in the heating mental would solve the problem of using a thermometer where solvent could escape from small holes surrounding the thermometer. With this thermal sensor, the bottom flask would be sealed completely with no solvent to escape to the atmosphere.

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APPENDICES

APPENDIX A: Qualitative Analysis of Fatty Acid Methyl Ester (FAME)

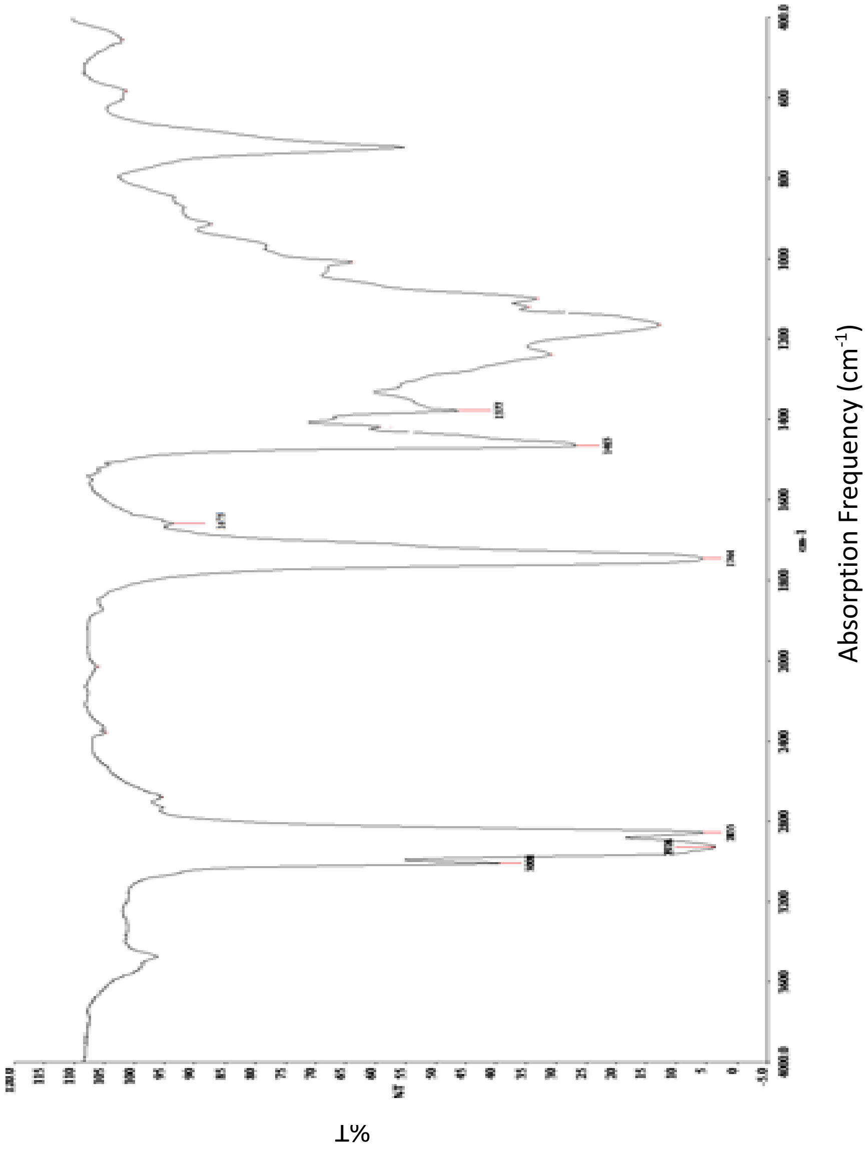

Figure A: FTIR Spectrum of FAME produced

APPENDIX B: Fatty Acid Methyl Esters (FAME) Analysis by GC

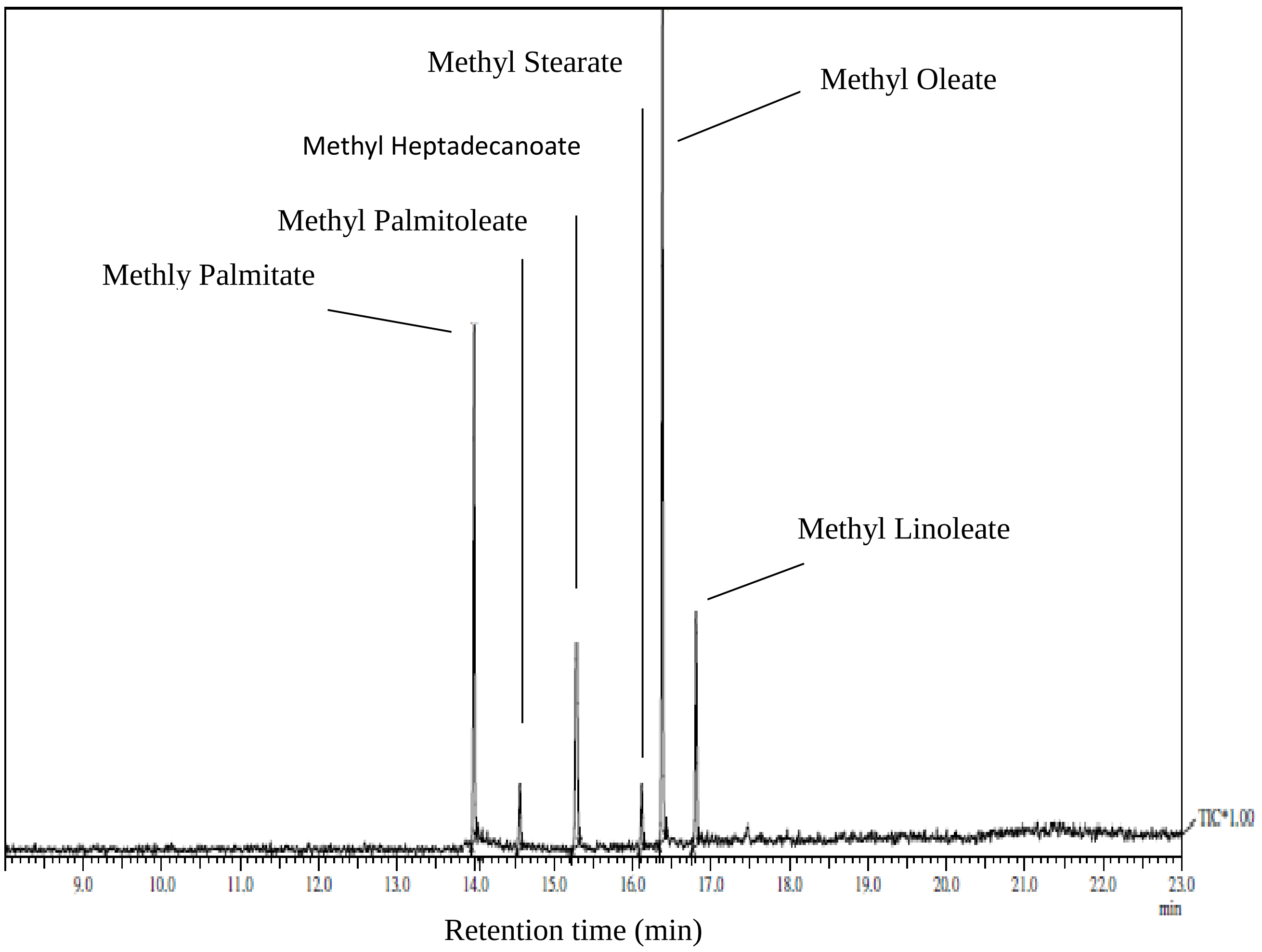

Figure B: Chromatogram for FAME analysis obtained from Reflux Condensation Method

Sample Calculation

Based on Figure B, the area and retention time of each peaks can be known. Table B.1 summarises the peak area, retention time, as well as the individual peak content in %.

Total peak area = 324789

Concentration of internal standard solution = 1mg/ml

Volume of internal standard = 1.5 ml

Mass of the sample for GC test = 82 mg

Table B.1: Retention Time, Area of Peak and Content of Individual Component

Component	Retention Time (min)	Area	Content, C_i (%)
Methyl Palmitate	14.11	101478	32.11
Methyl Palmitoleate	14.51	8152	2.58
Methyl Stearate	16.12	13554	4.29
Methyl Oleate	16.38	121504	38.45
Methyl Linoleate	16.81	44810	14.18
Methyl Heptadecanoate (IS)	15.38	8782	2.78

According to the modified EN 14103 standard, the content of each individual component can be calculated using Equation 3.2

$$C_i = \frac{A_i}{\sum A - A_{IS}}\, x\, 100\% \tag{3.2}$$

The sample calculation for component methyl palmitate is:

Content of methyl palmitate $= \frac{A_i}{\sum A - A_{IS}}\ x\ 100\%$

$$= \frac{101478}{324789 - 8782}\ x\ 100\%$$

= 32.1%

In addition, the yield of FAME was also calculated using the EN 14103 method. The yield of FAME was calculated by using methyl heptadecanoate (C_{17}) as a reference. The total FAME yield is calculated using Equation 3.3.

$$C = \frac{\sum A - A_{IS}}{A_{IS}}\ x\ \frac{C_{IS}\ x\ V_{IS}}{m}\ x\ 100\% \tag{3.3}$$

Total Content, C $= \frac{\sum A - A_{IS}}{A_{IS}}\ x\ \frac{C_{IS}\ x\ V_{IS}}{m}\ x\ 100\%$

$$= \frac{324789 - 8782}{8782}\ x\ \frac{1x1.5}{82}\ x100\%$$

= 0.6582 x 100%

= 65.82%

The mass of FAME could be obtained at the end of the reaction and the yield of FAME was calculated using Equation 3.4.

$$Yield\ of\ FAME = \frac{Mass\ of\ FAME\ Content\ (g)}{Mass\ of\ sample\ (g)}\ x\ 100\% \tag{3.4}$$

Yield of FAME $= \frac{Mass\ of\ FAME\ Content\ (g)}{Mass\ of\ sample\ (g)}\ x\ 100\%$

$$= \frac{0.6582x2.822g}{2.822g}x100\%$$

$$= \frac{1.8574g}{2.822g}x100\%$$

$$= 65.82\%$$

APPENDIX C: Calorific Data Graph of FAME Sample using Calorimeter Bomb

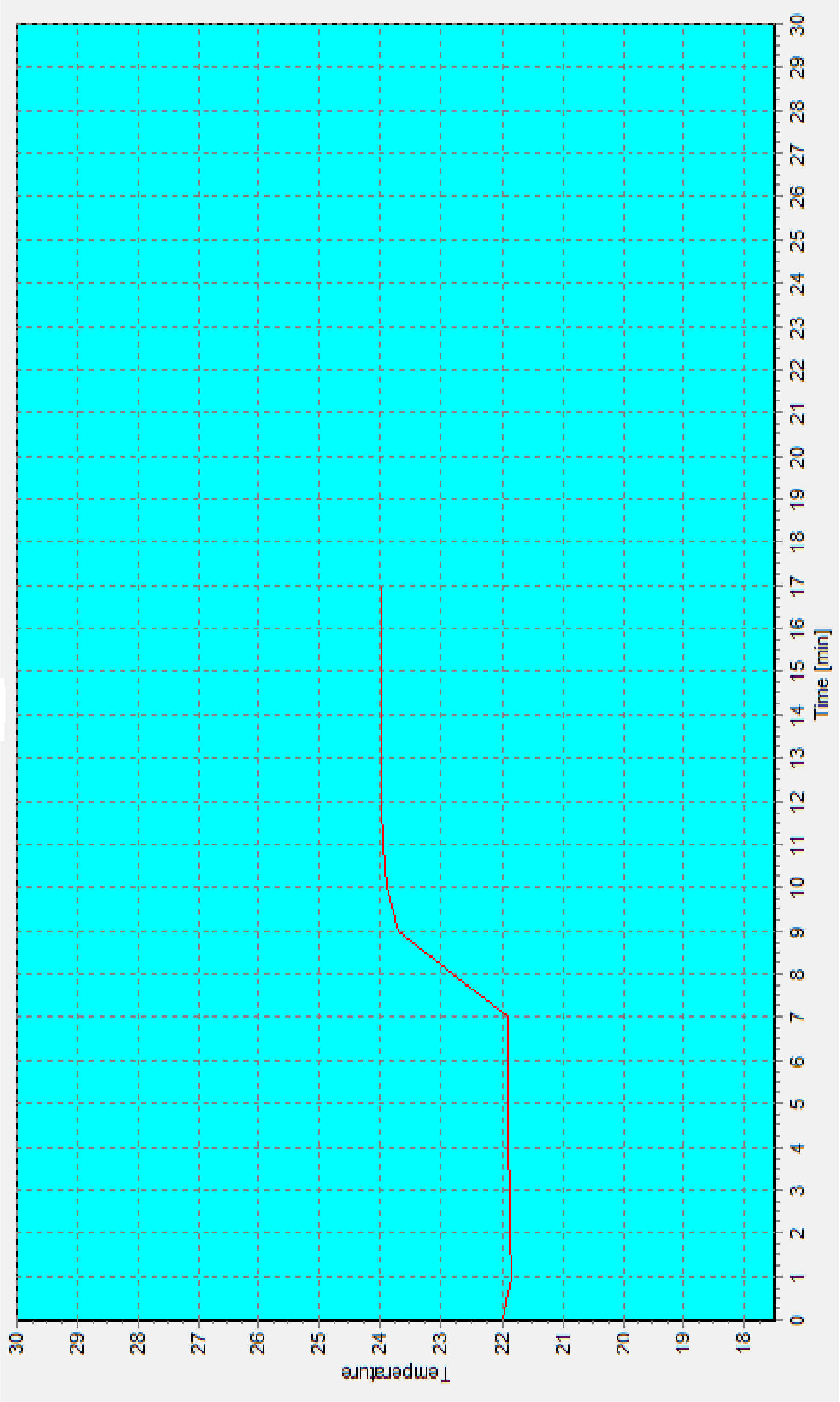

Figure C: Calorific Data of FAME sample