PRODUCTION OF FATTY ACID METHYL ESTER VIA SUBCRITICAL ETHYL ACETATE

DARREN PATRESE ANAK PADA

Project report submitted in partial fulfillment of the requirements for the award of Bachelor of Engineering (HONS) Petrochemical Engineering

Faculty of Engineering and Green Technology
Universiti Tunku Abdul Rahman

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DECLARATION

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

Signature :

Name : Darren Patrese Anak Pada

ID No. : 10AGB06401

Date :

APPROVAL FOR SUBMISSION

I certify that this project report entitled "**PRODUCTION OF FATTY ACID METHYL ESTER VIA SUBCRITICAL ETHYL ACETATE**" was prepared by **DARREN PATRESE ANAK PADA** has met the required standard for submission in partial fulfilment of the requirements for the award of Bachelor of Engineering (Hons.) Petrochemical at Universiti Tunku Abdul Rahman.

Approved by,

Signature : _________________________

Supervisor : Dr. Tan Kok Tat

Date : _________________________

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ABSTRACT

Mimusops Elengi (*M. Elengi*) is among the underutilized seed of forest origin that hold a potential as biodiesel feedstock. In good intention, using seed oil as feedstock was supposed to reduce the dependency on fossil fuels but the heavy link between deforestation and climate change requires alternatives which can helps environmentally as well as fulfilling the demand for fuels in the future. In this study, subcritical ethyl acetate was employed to produce FAME from *M. Elengi* seed oil. Performing subcritical ethyl acetate transesterification by using HPR at 240 ^{o}C reaction temperature, 20 minutes reaction time and 1:40 oil to ethyl acetate molar ratio obtain 31.12% of FAME yield. Comparatively, performing reflux condenser method yields 76.2% of FAME under the operating parameters of 80 ^{o}C reaction temperature, 4 hours of reaction time and 0.17g of potassium hydroxide as catalyst.

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LIST OF SYMBOLS / ABBREVIATIONS

FAME	fatty acid methyl esters
FFA	free fatty acid
SCF	supercritical fluid
SCM	supercritical method
THF	tetrahydrofuran
CCD	central composite design
HPR	high pressure reactor
KOH	potassium hydroxide

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CHAPTER 1

INTRODUCTION

1.1 Background

Biodiesel is an alternative fuel that can be used in existing diesel engines which are made from edible or non-edible vegetable oil instead of the conventional petroleum. Biodiesel can also be obtained from any other type of oil, including animal fats.

The usage of biodiesel produces far less hydrocarbons, carbon oxides emission and particulates into the atmosphere. The superior properties of biodiesel as fuel in environmental control have caught the attention of researchers and developers to further improve the production of biodiesel as it is biodegradable and essentially non-toxic.

Early production of biodiesel started using peanut oil as the feedstock. It requires no engine modification and was available ever since (Lam et al., 2009). Implementation of blending with petroleum diesel was taken into considerations in several countries, including Malaysia. Production of biodiesel requires edible or non-edible vegetable oil as the feedstock, alcohol as the reaction medium for the conversion and catalyst which is usually acids. Oil undergoes transesterification process where the triglycerides break off the glycerol molecules with the assistance of catalyst. The triglycerides then combine with methanol molecules to create methyl esters, which is the biodiesel than we've known these days.

Since biodiesel is produced from renewable sources, many researchers and developers are trying to promote worldwide usage as a new generation of operational alternative energy fuels to reduce petroleum oil reliance.

1.2 *Mimusops Elengi*

Mimusops Elengi (*M. elengi*) is evergreen trees belongs to the Sapotaceae which are 12-15 meter high when fully grown. *M. elengi* is originated from regions of Asia, mainly originating from India, Sri Lanka and most parts of South East Asia. Mostly are grown as ornament and shade tree along walkways and roadsides.

M. elengi is known by many names. Different region gives different vernacular names. Kadam et al (2012) have listed the vernacular name used to identify the tree as shown in Table 1.1.

Table 1.1: Vernacular names for *M. elengi.* (Kadam et al, 2012)

Language	**Vernacular names**
English	Bullet wood, Spanish cherry, Maulsari
Hindi	Sinha kasakara, Bakula, Kesara
Sanskrit	Madhuganda, Vagulam
Tamil	Magadam, Muhunain

The trees have many uses as the almost the whole parts of the trees have its own quality and beneficial purposes. Apart from being an ornament or shade tree, it was originally utilized as a traditional Ayurvedic medication. *M. elengi* seeds are mainly used to fix loose teeth by rubbing the seed powder on the teeth as well as to cure headache (Gami et al, 2012). The oil extracted from the seed can also be used as cooking oil.

Medicinal purpose of *M. elengi* is slowly deteriorating in the modern medicinal era but it is still used for ornament or made into garlands for prayer purposes throughout Asia. Underutilized, *M. elengi* provides good potential for edible feedstock to produce biodiesel. Extractable seed oil from the kernel is within the range 17.9 – 22 % (Dutta & Deka, 2014; Lemmens, 2005). Figure 1.1 shows *M. elengi* behavioural and its physical properties.

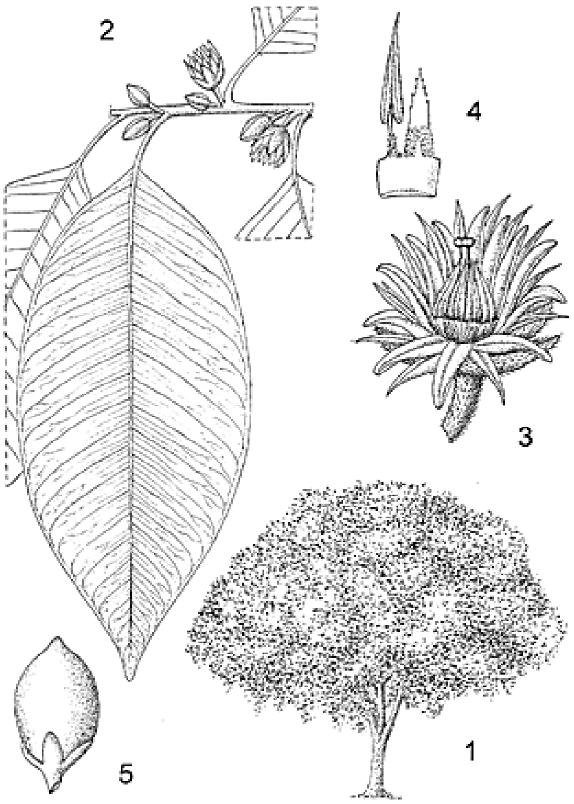


Figure 1.1: *M. elengi* tree habit (1), leaf and flowering twig (2), flower (3), stamen and staminode (4), fruit (5).

1.3 Super and Subcritical Fluid Method

Supercritical fluids (SCF) are an indefinite state of matter where its temperature and pressure was pressed beyond its critical point. SCFs physical and thermal properties are an intermediate between pure liquid and gas. It possessed a strong function of fluid density in relative to its temperature and pressure. These properties led to a high diffusivity value, resulting to an excellent catalytic activity. This distinctive phase is achievable in the yellow region pass the critical point shown in Figure 1.2.

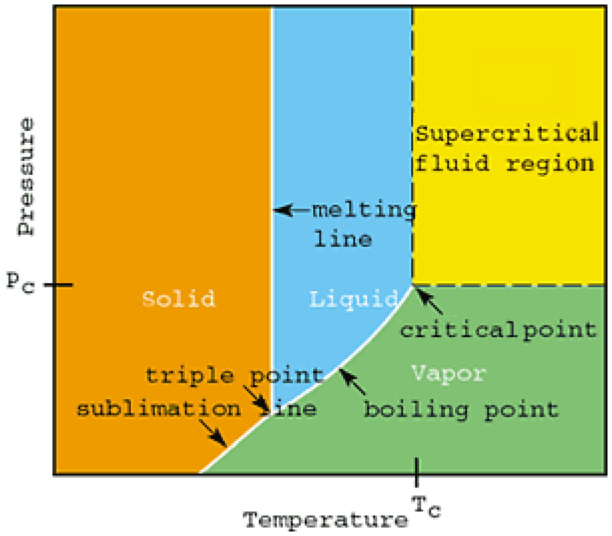


Figure 1.2: Pressure-temperature phase diagram. (www.slideshare.com)

As SCF are highly compressible, it is highly utilized in most industries, ranging from pharmaceuticals, cosmetics, polymers, biotechnology and even petrochemicals. The development towards green technology had opened the opportunities for SCF to be utilized. SCF was also utilized in biodiesel production. The supercritical condition allows SCF to acts as the solvent as well as providing catalytic activity towards the reaction, removing the requirement of introducing catalyst in the reaction. Wen et al (2009) have listed the critical properties of several SCFs as shown in Table 1.2.

Table 1.2: Critical properties of several SCFs (Wen et al, 2009)

SCF	Critical Temperature (K)	Critical Pressure (MPa)
Carbon dioxide	304.1	7.38
Water	647.3	22.12
Methane	190.4	4.60
Ethane	305.3	4.87
Methanol	512.6	8.09
Ethanol	513.9	6.14
Acetone	508.1	4.70

Furthermore, other applications of SCFs within the petrochemical industries generally involves around extraction of residual organics from kerogen shale and petroleum recoveries. Other applications are extraction of essential oils from agricultural products, as used in this study, environmental controls and many other applications.

Although SCM was utilized in transesterification processes, subcritical condition was approached mildly. Reacting under subcritical conditions can save operating cost as it operates under lower temperature and pressure. Similar to SCM, subcritical method was also used in other field such as purification of water.

1.4 Problem statement

"Food vs. Fuel" debate has been going on since researchers and developers began to incorporate green technologies. Edible oil is vastly used as the main feedstock for biodiesel production throughout the world give rise to many issues such as ethical usage of edibles, rise in food prices and most importantly, climate changes.

In Malaysia's scenario, 42.3% of worldwide oilseed production was contributed mainly from palm oil (Zhou & Thomson, 2009). In good intention, using seed oil as feedstock was supposed to reduce the dependency on fossil fuels but the heavy link between deforestation and climate change requires alternatives which can helps environmentally as well as fulfilling the demand for fuels in the future.

A study was done by Shingwekar (2015) shows that underutilized seeds of forest origin hold a potential as a source of renewable energy. It is an effective alternative as it retains the natural forestry, reducing the amount of deforestation.

This study investigates the condition for fatty acid methyl ester (FAME) production with the employment of subcritical ethyl acetate as to the conventional alcohol and the analysis of products obtained from *M. Elengi* as the feedstock.

1.5 Objectives

The objective of this thesis is to achieve the statements bellows:

- To determine the operating parameters to produce FAME from subcritical ethyl acetate.
- Analyze products obtained from subcritical transesterification of *M. Elengi* seed oil.

CHAPTER 2

LITERATURE REVIEW

2.1 Feedstock

2.1.1 Edible and Non-edible Feedstock

The discovery of biodiesel has made diesel engine as the “engine of choice” due to its power reliability and high fuel economy as for the wide range of feedstock selection. Dr. Rudolph Diesel early works revolved around using agricultural product, which is widely vegetable oil; to support the agriculture works itself.

Biodiesel has become the main alternative for diesel engines but the setback was the new diesel engine could not run on traditional vegetable oil due to its high viscosity. Naik et al (2010) studies shows that lowering the viscosity can be achieved via transesterification process to produce fatty acid methyl esters (FAME) which popularly known as biodiesel. As it grown its reputation as an environmental-friendly renewable energy sources, edible feedstock which is also known as the ‘first-generation’ feedstock appear to be unsustainable as the conflict of using food for fuels is morally debatable.

Globally, edible feedstocks were the most relied upon for the production of biodiesel. High oil yield from crops such as rapeseed and palm oil mill makes it a suitable choice for mass produced feedstock. Romano and Sorichetti (2011) studies shows that different countries and region need to rely on home-grown feedstocks which are natives to the geographical area.

Alternative feedstocks for biodiesel production were specifically grown as energy crops. Known as the ‘second-generation’ feedstock, it was mass produced for the sole reason of producing energy in form of bioethanol and biodiesel. Non-edibles oils like Jatropha yields high amount of oil and its ability to adapt in arid environment, these characteristics makes it as one of the sustainable option for non-edible feedstocks alongside other high oil yield feedstock such as jojoba, cotton, tung and sea mango (Romano & Sorichetti, 2011).

2.1.2 Minor Seed as Feedstock

Amongst the development of both conventional petroleum and bioenergy, there were issues regarding depletion as well as increased environmental problems. Minor seed oils from forest origin can boost the range of feedstock options for biodiesel production. Shingwekar (2015) founds that biofuel from minor seed oils shows potentials and resemblances in its properties.

Subjected to the studies, several feedstocks was taken into consideration and listed in Table 2.1. As an effective alternative, it works similarly compared to conventional petroleum diesel but with lower carbon emission, completing the carbon cycle as well as emitting low carbon emission to the environment.

Table 2.1: Potential biodiesel feedstock from minor seed oil. (Shingwekar, 2015)

Genus	Species	Common name
Actinodapne	*Angustifolia*	Pisa
Bombax	*Ceiba* Syn.	Semul / Kapok
Calophyllum	*Inophyllum*	Undi
Celastnis	*Paniculata*	Malkajguni
Citrilus	*Colocynthis*	Thumba
Hydnocarpus	*Wightianum*	Maroti
Litsea	*Chinesis*	Maida lakdi
Michelia	*Champaca*	Champa
Mimusops	*Elengi*	Maulsari
Mimusops	*Hexandra*	Khirni
Perilla	*Frutcens*	Bhujira

M.Elengi traditionally used as medicinal remedy of many purposes (Gopalkrishnan & Shimpi, 2013). Underutilized, *M.Elengi* can be used as a potential alternative as biodiesel feedstock. Dutta and Deka (2014) studies shows that *M.Elengi* contains palmitic acid (53.55%) and oleic acid (28.52%) as the dominant fatty acid within these seed kernels.

As these feedstocks came mostly from the forest itself, there is no need for deforestation to make way for commercial plantation. Casually, some of the tree species can be grown as ornaments or shade tree.

2.2 Transesterification

Biodiesel is produced via transesterification, also known as alcoholysis; by reacting triglycerides from vegetable or animal fats and alcohols. The chemistry involved in the reaction is to produce either methyl or ethyl fatty acid esters, depending on solvent employed. Transesterification process lowers the viscosity of the oil to cater for modern diesel engines (Abuhayaba et al, 2010). The main by-product from transesterification is glycerol. The reaction can be represented as shown in Figure 2.1.

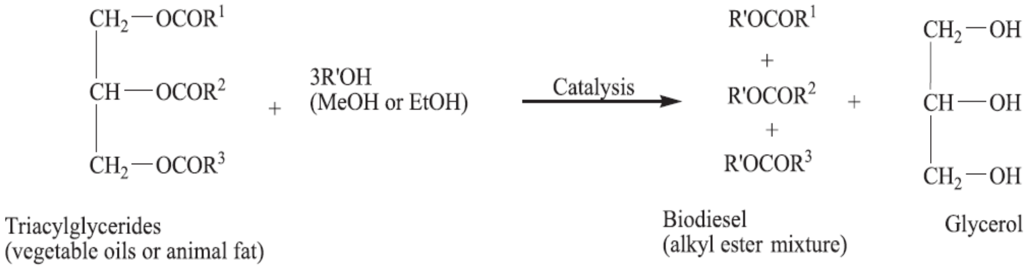


Figure 2.1: Biodiesel production via transesterification with glycerol by-product (Meireles & Pereira, 2013)

Transesterification is a reversible reaction which depends on the raw materials quality as fatty acid chains can appear in triglyceride, diglyceride and monoglyceride. The carbon chain length differs between the three chain mentioned. Nieves-Soto et al. (2012) generalized the transesterification represented in Figure 2.2 and compares it to the general chemical reaction.

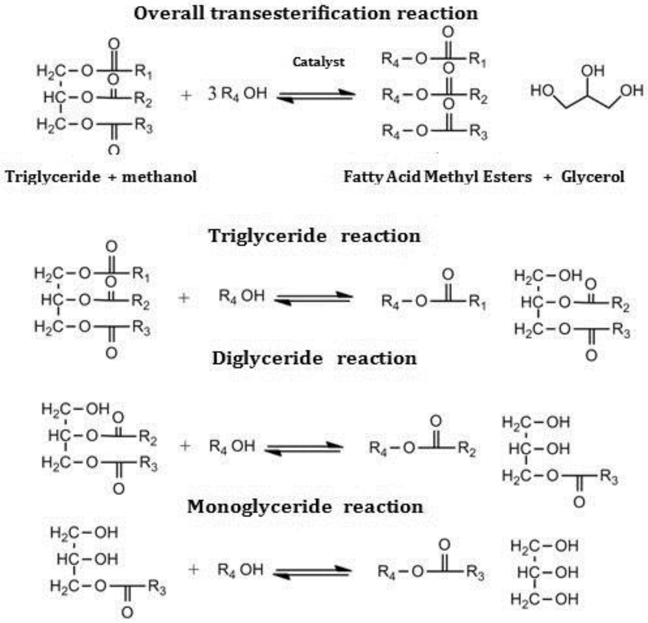


Figure 2.2: Generalized transesterification pathways (Nieves-Soto et al, 2012)

2.3 Catalytic Transesterification

Transesterification of vegetable oils or animal fats can be done by chemically reacting it with an alcohol in the presence of a catalyst. As the major part of the reaction is the biomass and the alcohol, the variable that can be worked on is the type and ratio of catalyst used was continued to be researched and developed. The influence of the catalyst amount on rate of conversion was the main concern as catalyst needed to be recovered and optimize biodiesel production as high as possible although it might cause viscosity of the FAME to increase (Yap, Abdullah and Basri, 2011).

2.3.1 Acid-Catalyzed Transesterification

Acid-catalyzed process can employed either homogeneous or heterogeneous acid catalyst. This reaction path has more tolerance to free fatty acids (FFA) and water as liquid acid catalyst isn’t too subtle to FFA. Lotero et al. (2005) studies show that the reaction pathway requires higher reaction temperature as well as longer reaction time compared to alkali catalyst.

Soriano, Venditti and Argyropoulos (2009) deploy Lewis acids to catalyze the transesterification of canola oil with methanol. $AlCl_3$ or $ZnCl_2$ was used alongside co-solvent tetrahydrofuran (THF). The study shows that the optimization of each catalyst gave different level of effectiveness. $AlCl_3$ catalyzed reaction was optimized at 110 ^{o}C and 18 hour reaction time with molar ratio of 24:1 to achieve 98% conversion. Soriano, Venditti and Argyropoulos (2009) also show that $ZnCl_2$ was less effective which contributed from its lower acidity value.

Effective acidic transesterification continued to be researched due to is low-cost compared to base-catalyzed pathway (Miao, Li & Yao, 2009; Letero et al, 2005). Trifluoroacetic acid catalyzed process is highly effective to produce methyl ester at the combination of 2.0 M catalyst concentration with 1:20 oil to methanol ratio. The reaction parameter is 120 ^{o}C and about 5 hour reaction time to achieve biodiesel content of 98.4%. The simplicity of the method shows an industrial potential for mass production of biodiesel in the future (Miao, Li & Yao, 2009).

A study by Bhatti et al (2008) shows that acid-catalyzed transesterification can also be done on animal fats. The study emphasised on varying the process parameters to obtain the maximum yield of biodiesel. The optimum process parameters for the acid-catalyzed transeseterification were 2.5 g of concentrated sulphuric acid, reaction time of 24 hours and temperature at 50 ^oC for dairy cow, yielding 94.1 ± 2.43 % of biodiesel. On the other hand, beef tallow requires the same amount of acid but relatively higher process temperature (60 ^oC) and faster reaction time (6 hour) with biodiesel yield of 98.4 ± 2.3 %.

2.3.2 Alkali-Catalyzed Transesterification

Alkali-catalyzed transesterification commonly employed potassium or sodium hydroxide dissolved into an alcohol medium. Generally, alkali-catalyzed transesterification is widely used process route to produce biodiesel. Letero et al (2005) studies states that using homogenous acid catalyst is 4000 times slower than transesterification where alkali homogeneous catalyst was used. Industrial production of biodiesel have utilized alkali-catalyzed transesterification due to it is the most economical process, requiring low temperatures, pressure and producing over 98% conversion yield (Kar, Gupta & Kumar Das, 2012).

Liu et al (2008) studies the transesterification of soybean oil with a solid catalyst which was calcium oxide. The molar ratio of methanol and oil, reaction temperature, mass ratio of catalyst to oil were all the parameters to study its separate effect. The optimum result obtained is when a 1:12 molar ratio of oil to methanol was used with the presence of 8% calcium oxide catalyst, process temperature at 65 ^oC and 2.03% water content in methanol. The biodiesel yield was reported to exceed 95% at 3 hours. Furthermore, Liu et al (2008) also discovers that the catalyst have longer lifetime which lasting for 20 cycles.

Another study done by Helwani et al (2009) focuses on the utilization of solid heterogeneous catalyst. The study suggested that solid alkali catalyst is more viable than alkaline metal alkoxides due to its better economic potential and lesser environmental effects.

Rashid and Anwar (2008) studies on alkali-catalyzed transesterification on rapeseed oil also shows better process parameters compared to those to acid-catalyzed. In the study, Rashid and Anwar (2008) manipulated variables such as oil to methanol molar ratio (1:3 – 1:21), catalyst concentration (0.25 – 1.50%), temperature (35 – 65 ^{o}C), mixing intensity (180 – 600 rpm) and its catalyst type. The best biodiesel yield obtained from this study was 95 -96% under the process parameters of 1:6 oil to methanol ratio, 1% potassium hydroxide catalyst concentration, 600 rpm of mixing intensity and temperature at 65 ^{o}C.

2.3.3 Enzyme-Catalyzed Transesterification

Recently introduced enzyme catalyst is to overcome problems associated with the before mentioned catalyst processes. Usage of enzyme catalyst can be classified as hydrolysis and synthesis. It also can be used in nearly anhydrous condition and react in lower temperatures.

A study by Jothiramalingam and Wang (2009) focuses on the current status on enzymatic transesterification for biodiesel production. It was reported that there are an improvement on the results. Increased yield, reaction time and stability are the strength of the process but due to its performance and price, enzymatic transesterification must improve to become industrially feasible. Jothiramalingam and Wang (2009) listed down the limitations which are critical to the reaction. Limitations in forms of mass transfer, solvent usage and water activity were further studied if the production is to be industrially scaled up.

Similarly, Gog et al (2012) also focuses on enzyme which is lipase in their studies. The study shows that although enzymatic transesterification has favourable conversion rate under nearly anhydrous and low temperature condition, there are still few limitations that restrict this process. Gog et al (2012) listed the main limitations are the cost of lipase, slower reaction rate and lipase inactivity due to methanol and glycerol.

Although with limitations, enzymatic transesterification has certain advantage compared to acid and alkali-catalyzed transesterification. Bajaj et al (2010) listed the parameters that enzyme catalyst has advantages over chemical catalyst which are lower energy required, easier glycerol recovery and able to perform transesterification with high FFA content.

Albeit, recent studies proposed that enzyme catalyst can be deployed alongside acid or alkali catalyst. Waste cooking oil contains high amount of FFA which required treatment prior to the process. The usage of enzyme and acid catalyst was reported to be most favorable option to produce biodiesel from waste cooking oil (Lam et al, 2010). Limitations problem can be overcome by using the latest technological advancement. Using oscillatory flow reactor, ultrasonification, microwave reactor and co-solvent were listed as the potential solution to overcome the limitations (Lam et al, 2010).

2.4 Supercritical and Subcritical Transesterification

Supercritical transesterification is potential technique which can boost the biodiesel production in the industry. Silva and Oliveira (2014) studies shows that the supercritical method (SCM) is better in terms of rate of reactions, flexibility of feedstocks, efficiency of production and its environmental concerns. SCM requires its feedstock to react under high pressure and temperature thus the scenario where the product might degrade is a possibility. However, Silva and Oliveira (2014) also shows that the SCM method is developing and shows potential for the future.

Reacting under high pressure and temperature allows the reaction to occur without the presence of catalyst. SCM is robust in nature thus self-providing the catalytic activity needed for transesterification to take place. Wei et al (2012) carried a study on transesterification of oil and lipids with supercritical methanol. The reaction results in 97.83% biodiesel yield under process parameters of 1:60 ratio of oil to methanol, 1:3 ratio of oil to acetic acid, 20 MPa operating pressure and 90 minutes reaction time. Wei et al. (2012) also obtained 30.2% of glycerol as compared to 10% theoretically.

Saka et al (2006) researches on SCM show that supercritical methanol is able to react with FFA without the presence of catalyst or pre-treatment. The studies shows that the FFA undergoes one-step method which reacts under shorter period of time and have greater yield compared to other catalyzed transesterification. However, to further improve the quality and standards, Saka et al (2006) developed a two-step process which hydrolyzed the FFA.

As SCM develops, environmental concerns over this process also follow. As study done by Khalid et al (2011) shows concern upon the usage of acid and alkali catalyst for transesterification process. Supercritical alcohol is benign towards the environment, thus leading to a cleaner biodiesel production path. Similarly, Wen et al (2009) reported that SCM is a promising technique that can be utilized in industrial scale. The study also listed the advantages of SCM method such as fast reaction kinetics, high production rate, feasibility to operate continuously as well as no presence of catalyst required.

For subcritical reaction, the reaction was performed at temperature higher than the solvent's boiling point but not exceeding its critical temperature. Sanchez et al (2012) performed the reaction by using non-edible vegetable oils as the feedstock can obtain product with high ester yield, reaching 94.7 wt %. The optimum operating condition includes the presence of small amount of potassium methoxide at 1:24 oil to methanol ratio, 220 ^{o}C, 36 bar and 4 hour of reaction time. Sanchez also stated that increment of operating temperature enhances the ester content. This is due to the solvent is approaching its critical point, where the catalytic value is higher.

CHAPTER 3

METHODOLOGY

3.1 Materials

M. Elengi appears in bright red-orange color when ripe. The fruit has a cherry-like looks and produce a sweet smell. All of the fruits and seed were collected in Kampar Perak, Malaysia. Ethyl acetate was employed as the solvent in supercritical condition and n-hexane for extracting oil from *M. Elengi* kernels. Potassium hydroxide (KOH) is an alkaline catalyst which was later used in subcritical transesterification by using the Reflux Condenser Method.

3.2 Equipment

Table 3.1 shows the equipments utilized for experimental and research purpose of this study.

Table 3.1 Equipments Utilized for Experimental and Research Purpose

Equipment	Brand
Blender	Waring, USA
Rotary Evaporator	Buchi, Switzerland
Gas Chromatography	Shimadzu, Japan
High Pressure Reactor	Amar Equipments, India

3.3 Pre-treatment of *M. Elengi* Seeds

M. Elengi kernels collected were oven dried at 80 ^{o}C for 24 hours to remove excess moisture prior to extraction. A study by Dutta and Deka (2014) shows that *M. Elengi* contain low percentage of moisture in the seeds which leads to resistance against contamination and rancidity. Thus, the temperature was controlled to not exceed 100 ^{o}C as it might decompose the seeds and lowering the yield of oil extraction later. The seed was then processed using a blender into fine particles.

3.4 Extraction of *M. Elengi* Seed Oil

M. Elengi seed oil was subjected to solvent extraction by using n-hexane as the solvent. Extraction was done by deploying thimble to accommodate the seed cake while it was soaked in n-hexane. The extraction process was performed for 24 hours and the oil was recovered from the solvent-oil mixture via rotary evaporator.

3.5 Subcritical Ethyl Acetate Transesterification

3.5.1 High Pressure Reactor (HPR)

The reaction was conducted by using Amar Equipments' High Pressure Stirred Lab Autoclaves. The set-up was equip with a reactor tube which can withstand high temperature and pressure which required by the reaction design parameters. The material of construction is SS-316 (Stainless Steel – 316) which can endure the extreme conditions. The HPR can operate up to 500 ^{o}C and 50 bar and the rest of the specification is shown in Table 3.2.

Table 3.2: Specification of HPR

Property	**Dimension**
Volume	50ml
Body & Head Sealing	Split clamp type quick opening system with clamp bolts and flat gasket
Heating System	Electrical ceramic band heater with ceramic wool insulation cladding
Control Panel	Control panel with microprocessor based programmable PID temperature controller cum indicator, variable motor speed drive and other digital indicators and controllers.

Source: Amar Equipments

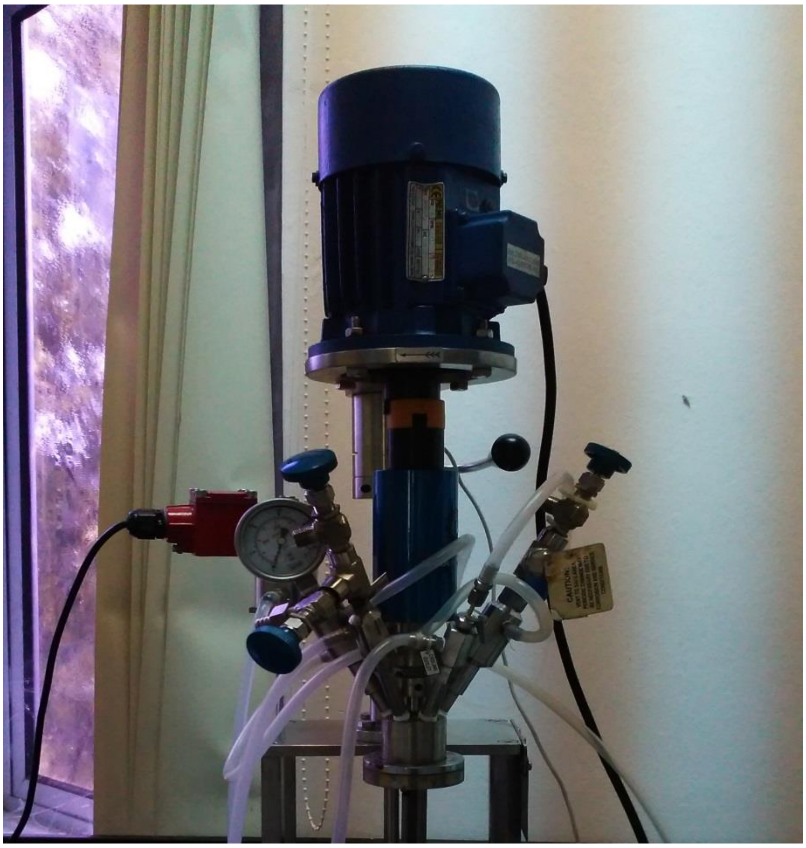


Figure 3.1: HPR Table Top Rig

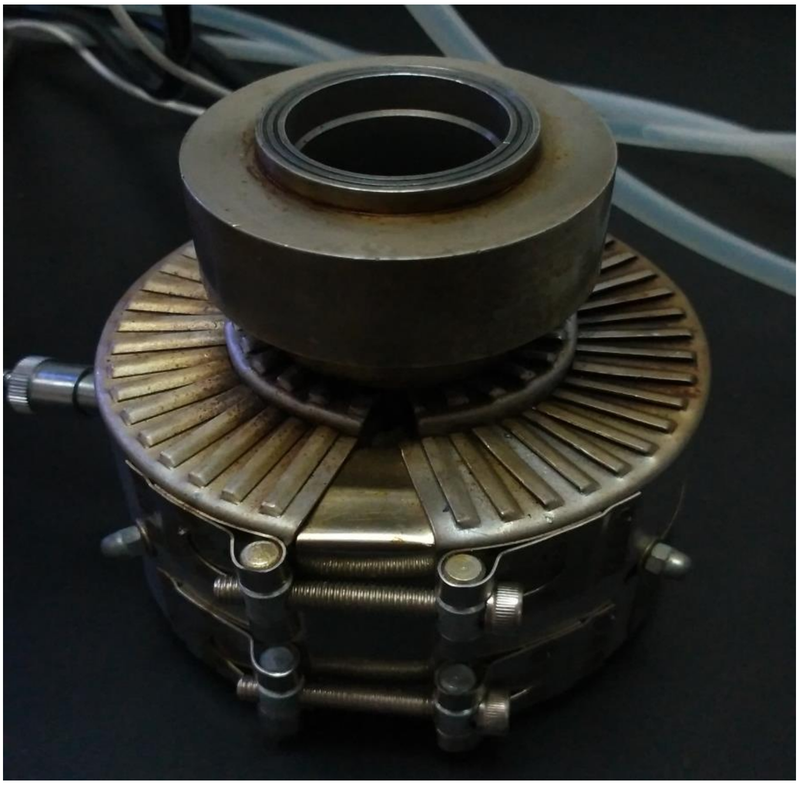


Figure 3.2: HPR with heating coil

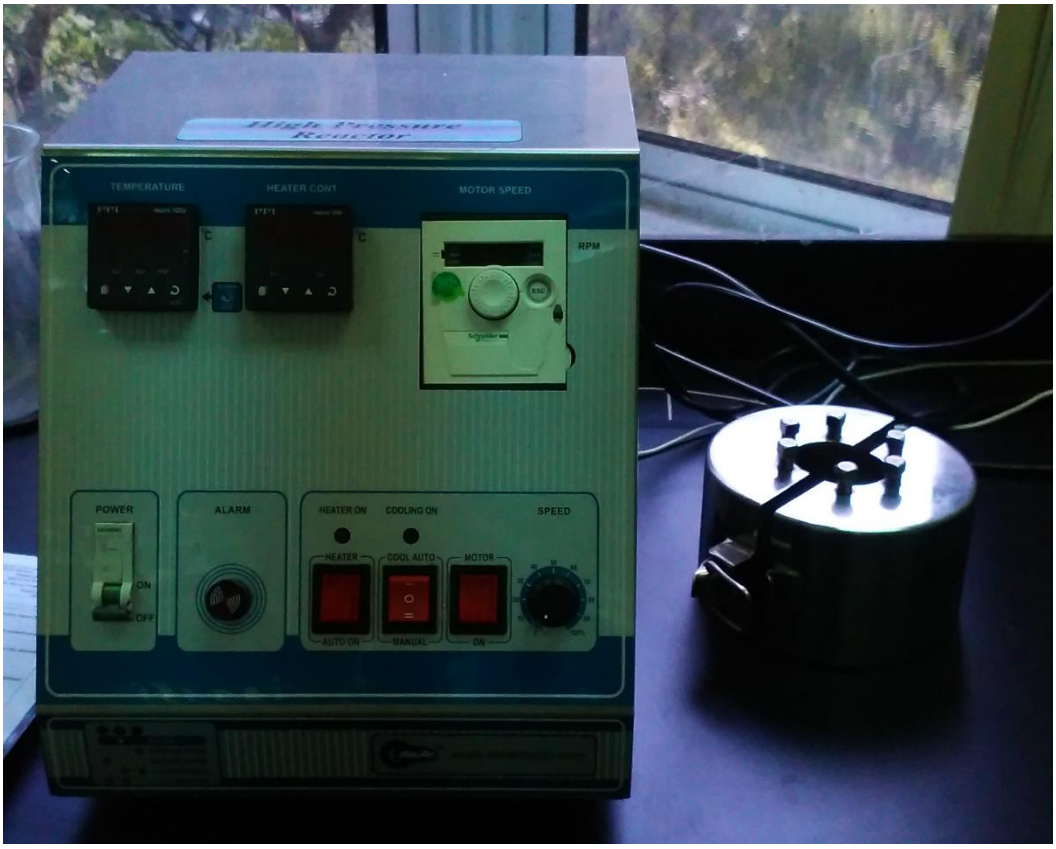


Figure 3.3: HPR control panel (left) and Split Clamps with bolts and gasket (right)

3.5.2 Procedures for Subcritical Ethyl Acetate Transesterification

The transesterification process was performed under the parameters ranging from temperature between 200 ^{o}C to 240 ^{o}C with oil to ethyl acetate molar ratio from 1:20 to 1:60. The reaction was conducted within the time range of 10 to 30 minutes excluding temperature build-up period. To start with, the seed oil of M. Elengi and ethyl acetate was fed into the 50 ml reactor. Then, the reactor was placed firmly onto the table top rig and fastened securely using the split clamps. For safety precautions, the sealing and fastenings of bolts and gasket was checked thoroughly to avoid leakages of reactants and loss in pressure. After securing the reactor and the heating coil onto the rig, the reactor was heated to the designated temperature which was set on the control panel of the HPR.

After the reaction was completed, the reactor was allowed to cool down to a workable temperature prior to removing the split clamps as the clamps will be very hot. The product was then collected and evaporated to remove excess solvent to obtain pure FAME sample. Finally, the FAME sample from each run was analysed by using gas chromatography (GC) to determine the product yield.

3.5.3 Reflux Condenser Method

For comparison purposes, a separate subcritical ethyl acetate transesterification was performed. Experimental set up for this method was by using 2-neck round bottom flask with a Graham condenser on top. The other neck was fitted with thermocouple to monitor the temperature of the mixture.

Employment of catalyst was utilized in this method to compare the effect on it FAME yield. The catalyst employed for this reaction was KOH and after the reaction was completed, the mixture was then transferred to a separating funnel for washing. This step is important to remove the catalyst and other impurities. The FAME sample was then sent for gas chromatograpy analysis to determine its yield.

3.6 Product Analysis

3.6.1 Gas Chromatography

The diluted samples were analysed by using GC-2010 Plus Shimadzu to identify the amount of FAME yielded from the transesterification. The gas chromatography was equipped with SGE Analytical Science BPX70 column (60m × 0.25 mm, 0.25 µm) and the Flame Ionization Detector (FID) as the detector. Helium gas was employed as the carrier with initial oven temperature at 110 °C held for 0.5 minute and then raised to 220 °C (held for 8 minutes) at a rate 10 °C per minute. Furthermore, temperatures of the injector and detector were set at 220°C and 250°C respectively.

3.6.2 Dilution of FAME Sample

The dilution of FAME sample requires dissolving methyl heptadecanoate in hexane as an internal standard to obtain the concentration of 1 g/L. Dilution factor (volume of FAME to total volume) of the sample was 19. The measurement for sample product and hexane used was 80 µL and 1420 µL respectively. For sampling procedures, 1 µl of diluted sample was injected into the column of gas chromatography. The peaks obtained from the gas chromatography were then used to determine the content of each individual components of sample. By using EN 14103 standards, all of the content was calculated by referring to its individual peaks in the following Equation 3.2.

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

Where

C_i = content of different component in the sample in %
A_i = component peak area
ΣA = total peak area
A_{IS} = internal standard (methyl heptadecanoate) peak area

3.6.3 Determination of FAME Yield

According to modified EN 14103 methods, the yield of FAME produced was calculated by expressing FAME content obtained as a mass fraction percentage using methyl heptadecanoate as the internal standard. The total content of FAME obtained was determined using Equation 3.3 shown below.

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

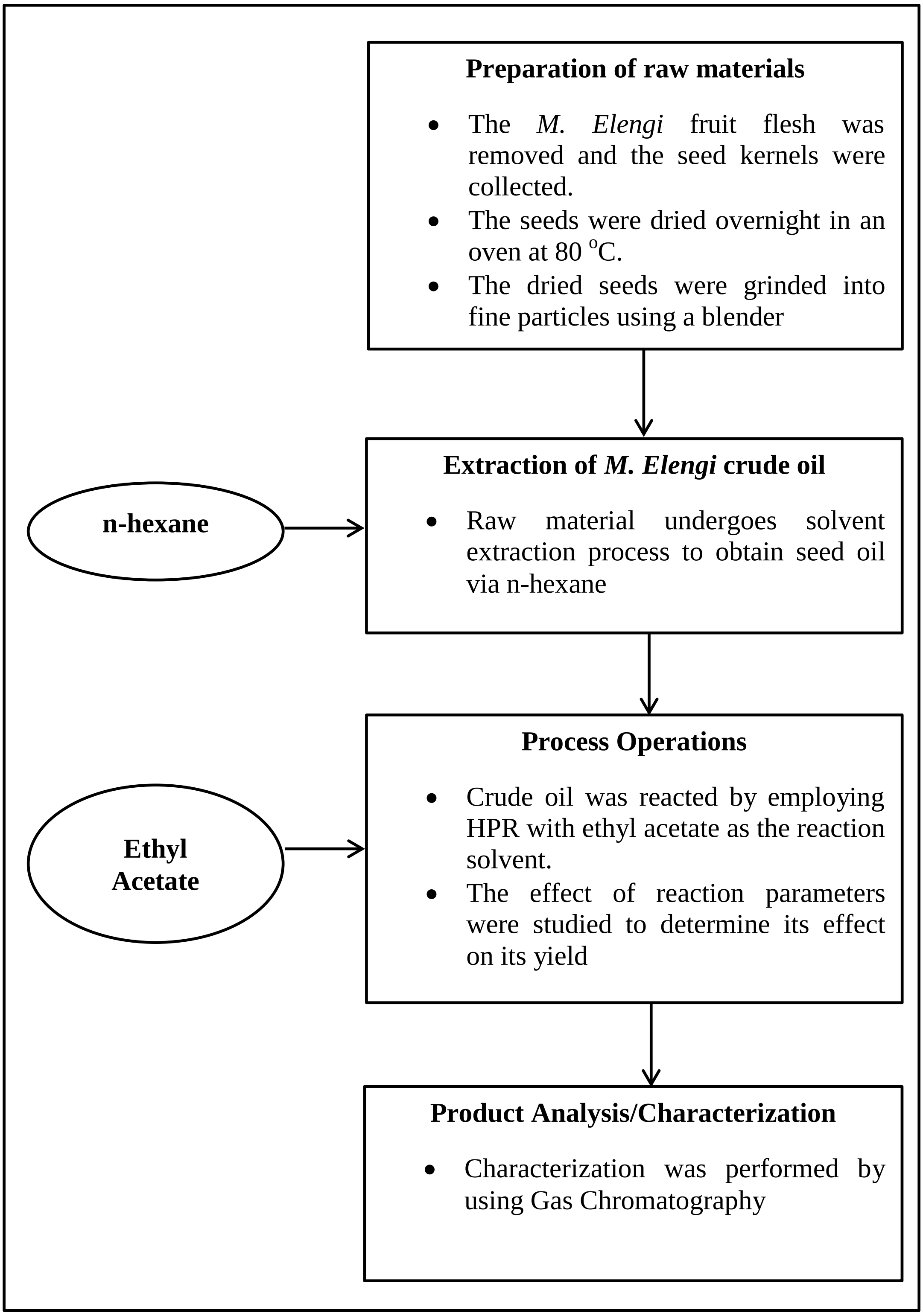
Where

ΣA = total peak area
A_{IS} = internal standard (methyl heptadecanoate) peak area
C_{IS} = concentration of the internal standard solution, in mg/mL
V_{IS} = volume of the internal standard solution used, mL
m = mass of the sample, mg

Appendix B shows the gas chromatogram of the methyl esters in the product sample and sample calculation to determine the yield of FAME in SCM reaction.

3.7 Research Flow Diagram

The figure below shows the project's work flow.



Preparation of raw materials

- The *M. Elengi* fruit flesh was removed and the seed kernels were collected.
- The seeds were dried overnight in an oven at 80 ^{o}C.
- The dried seeds were grinded into fine particles using a blender

Extraction of *M. Elengi* crude oil

- Raw material undergoes solvent extraction process to obtain seed oil via n-hexane

Process Operations

- Crude oil was reacted by employing HPR with ethyl acetate as the reaction solvent.
- The effect of reaction parameters were studied to determine its effect on its yield

Product Analysis/Characterization

- Characterization was performed by using Gas Chromatography

Figure 3.4: Work flow chart of project

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Optimization of *M. Elengi* Seed Oil Extraction

Table 4.1: Oil Percentage Extracted From Different Mass of *M. Elengi* Seed

Sample no.	Mass of sample (g)	Mass of extracted seed oil (g)	Oil percentage (%)
1	10	1.0038	10.038
2	15	1.8012	12.008
3	20	2.6128	13.064
4	25	3.4869	13.947
5	30	4.1331	13.777

Table 4.1 shows the result of *M. Elengi* seed oil extraction. All of the extraction process undergoes the same amount of time which was 24 hours. Increasing amount of seed was used to determine the efficiency of the extraction. The oil percentage increases proportionally from 10.008% to 13.947% as the mass of seed increases from 10g to 25g. Further increment in the amount of seed from 25g to 30g results in a drop of percentage in the oil content.

By using solvent extraction method, extraction yield increases if the surface area to volume ratio is high. The extraction was performed in a 250ml beaker. For Sample 4, 25g to 250ml sized beaker can yield approximately 14% of oil, thus showing it is the maximum mass should be used in a 250ml beaker. Since Sample 4 shows it was a maximum, 30g in 250ml beaker lowers the surface area to volume ratio. Although the surface area of seed sample is higher, the solvent, n-hexane was saturated. The volume of hexane was unable to extract the oil in optimum condition. Figure 4.1 shows the relationship of oil content with its mass for the extraction process.

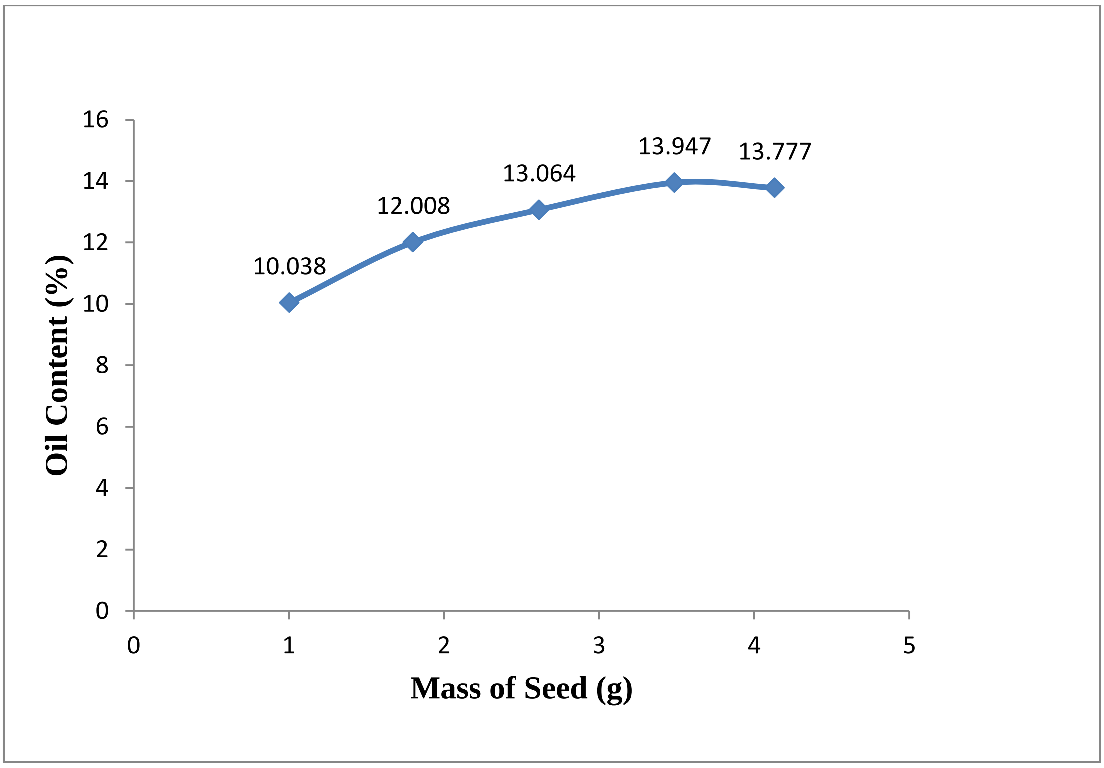


Figure 4.1: Effect of Mass of Seed on Oil Content of *M. Elengi* Seed

Regarding the oil percentage from the extracted seed sample, the percentage is lower compared to theoretical value. One of the variables leading to this was the geographical factor where the sample was collected. Inadequate fertilizers, dry soil condition of urban areas and inconsistent hours of sun available influence the optimal condition of the plants growth as well as the oil content of the seed.

M. Elengi fatty acid profile shown by Dutta and Deka (2014) shows that the seed oil contains palmitic acid, oleic acid, stearic acid and linoleic acid. The percentage of the fatty acid component is as show in Figure 4.2. The major saturated fatty acids were palmitic and stearic acid where its weight percentage was 53.55%

and 10.26% respectively. Likewise, the unsaturated fatty acids were oleic and linoleic acids were present with weight percentage 28.52% and 7.65% respectively.

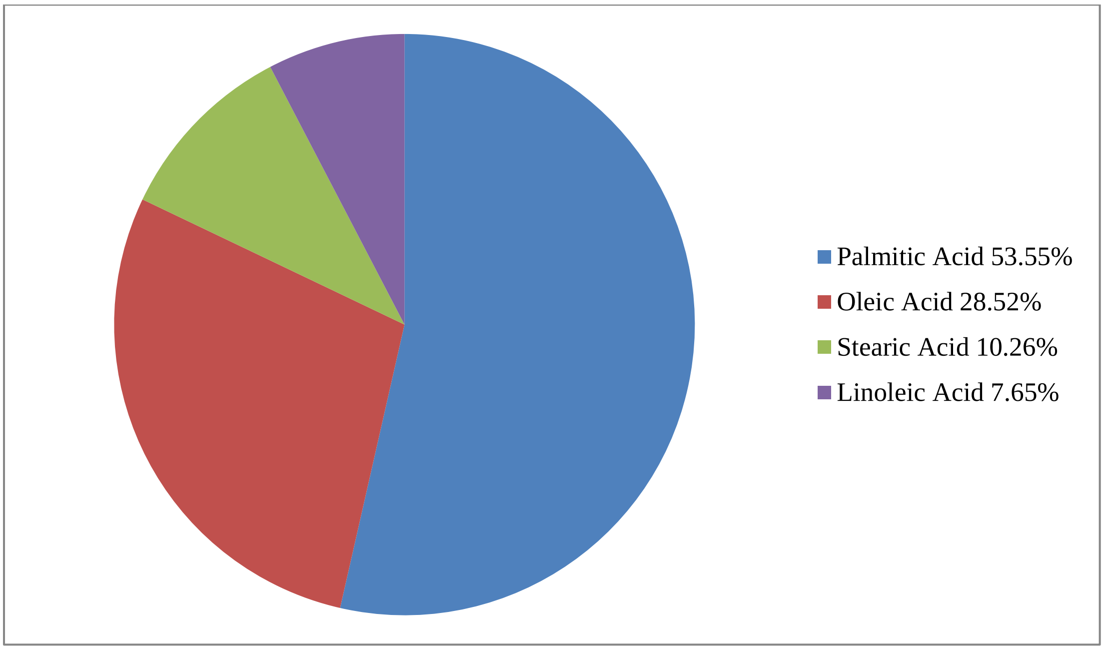


Figure 4.2: Weight Percentage of Fatty Acid in *M. Elengi*

From Figure 4.2, the saturated fatty acids accounts for more than 60% of *M. Elengi* seed oil fatty acid content. Thus for the FAME product, 4 peaks were expected from the Gas Chromatography analysis.

4.2 Subcritical Ethyl Acetate Transesterification

4.2.1 Design of Experiment (DOE)

Table 4.2 shows the complete experimental design matrix for the transesterification of *M. Elengi* seed oil. All of the samples were sent for analysis to check for their FAME content. The highest yield of FAME content was achieved during Run 10 with operating parameters of 20 minutes of reaction time, reaction temperature of 240 ^{o}C and molar ratio of oil to ethyl acetate of 1:40.

Table 4.2: Experimental Design Matrix and Results for SCM Reaction

Run No.	Reaction Time, A (minute)	Reaction Temperature, B (°C)	Molar Ratio, C (mol/mol)
1	10	220	40
2	15	210	30
3	15	230	30
4	15	210	50
5	15	230	50
6	20	200	40
7	20	220	20
8	20	220	40
9	20	220	60
10	20	240	40
11	25	210	30
12	25	210	50
13	25	230	30
14	25	230	50
15	30	220	40
16	20	220	40
17	20	220	40
18	20	220	40
19	20	220	40
20	20	220	40

For Run 10, the yield of FAME obtained was 31.12% which is arguably low. This is caused by insufficient activation energy for the transesterification to occur. Run 10 operate at a temperature closer to the critical temperature of ethyl acetate (260 ^{o}C) which provides better catalytic activities compared to the other samples, showing that the employment of catalyst is critical to obtain higher yield in performing subcritical transesterification.

For subcritical reaction, research did by Encinar et al (2010) shows that employment of potassium hydroxide catalyst yields 90% of FAME. The experimental design matrix was limited with the maximum operating temperature of the HPR, which was in the range of 240 ^{o}C to 250 ^{o}C. Although the heating coil's maximum temperature can be set to 500 ^{o}C, the heat transfer between the heating coil and the reaction vessel could not obtain readings of higher temperature.

4.2.2 Comparison with Reflux Condenser Method

A preliminary run was performed using the same temperature of 240 ^{o}C but longer reaction time. The product was thermally degraded due to prolonged thermal overexposure (Tan et al, 2010).

A separate run of subcritical ethyl acetate transesterification was performed by using reflux condenser method operating at 80 ^{o}C, 4 hours of reaction time and under atmospheric pressure with the employment of potassium hydroxide as the catalyst. The reaction temperature for this run exceeded the boiling point of ethyl acetate but lower than its critical temperature. These operating parameters conventionally lowers the operating cost as lower temperature uses less energy required as well as shortening its reaction time. The operation yields 76.2% of FAME which is more than double the yield of subcritical ethyl acetate reaction using the HPR. Appendix B shows the gas chromatogram of both samples obtained from HPR and reflux condenser method.

Employment of catalyst must cater for the reactions conditions and parameters. Selection of catalyst can affect the operations if it is a strong acid of alkaline. A catalyst which is not corrosive, having high conversion rate and shorten reaction time is favorable. Strong alkaline catalyst will react with FFA in the seed oil forming soap via saponification which reduces the yield of FAME and causes difficulties to obtain pure FAME product as more washing is required.

Addition of catalyst for the reflux condenser run effectively reduces the activation energy required for the reaction (Encinar et al, 2012). Therefore, lower energy is required to complete the reaction, allowing lower temperature reaction for high FAME yield. However, studies done by Leung et al (2010) stated that further addition of catalyst will reduce FAME yield. This is due to the catalyst's sensitivity towards FFA content in the seed oil.

4.3 Product Analysis

4.3.1 Gas Chromatography Analysis

Gas chromatography was performed to identify the contents within the FAME samples obtained from the subcritical ethyl acetate transesterification. Based on the research Dutta and Deka, the fatty acid component present in *M. Elengi* seed oil is palmitic acid, linoleic acid, oleic acid and stearic acid. Table 4.3 shows the FAME weight percentage profile obtained after subcritical transesterification.

Table 4.3: Content of FAME

Component	**Retention Time (min)**	**Area**	**Content, C_i (%)**
Methyl Palmitate	14.556	417400	7.29
Methyl Linoleate	16.384	92710	1.62
Methyl Oleate	16.630	693982	12.12
Methyl Stearate	16.943	268305	4.69
Methyl Heptadecanoate (IS)	15.325	3943042	68.88

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Extraction of *M. Elengi* seed oil was optimized by using solvent extraction method. Optimum condition is where n-hexane was employed to extract oil from 25g of *M. Elengi* seeds within 24 hours.

Subcritical ethyl acetate transesterification was performed and converted *M. Elengi* seed oil to biodiesel using the HPR. The operating conditions of this subcritical ethyl acetate transesterification are 240 ^{o}C reaction temperature, 20 minutes reaction time and 1:40 oil to ethyl acetate molar ratio yielding 31.12% of FAME.

Comparing to Reflux Condenser Method, the yield of FAME is more double than the yield from the HPR reaction due to the employment of catalyst, potassium hydroxide. The operating conditions are 80 ^{o}C reaction temperature, 4 hours reaction time and 0.17mL of potassium hydroxide yielding 76.2% of FAME.

5.2 Recommendations

Subcritical ethyl acetate transesterification converted *M. Elengi* seed oil to biodiesel but the yield was arguably low. *M. Elengi* has a potential to become an alternative feedstock given if the process was performed in an appropriate condition. The results in this study were not as expected from the literature review. This is due to the varying parameters employed in the research. Therefore, several improvements can be made to fully unravel the potential of *M. Elengi* as biodiesel feedstock. Listed below are some recommendations that can be taken into consideration for future research and development in this field.

- Performing a catalytic subcritical transesterification under similar process parameters can greatly improves the yield of FAME as it lowers the reactions activation energy. It also saves on operating cost as it can perform in lower temperatures, reducing energy required for heating.

- Employment of heterogeneous alkali catalyst to ease the purification process. Homogeneous alkali can lead to emulsions occurring during washing step as well as high tendency to undergo saponification during transesterification. Heterogeneous alkali can be easily recovered during washing steps, thus lowers the generation of waste water.

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APPENDIX

APPENDIX A: Gantt Charts

Gantt Chart for First Long Trimester

Details/Week	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Project Proposal/Selection	🟩	🟩												
Preliminary Research Work		🟩	🟩											
Project Work				🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
Progress Report Submission												🟩		
Oral Presentation													🟩	🟩

Gantt Chart for Second Long Trimester

Details/Week	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Project Work	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩		
Progress Report Submission								🟩						
Final Report Submission													🟩	
Oral Presentation													🟩	🟩
Hard Bound Submission														🟩

APPENDIX B: FAME ANALYSIS

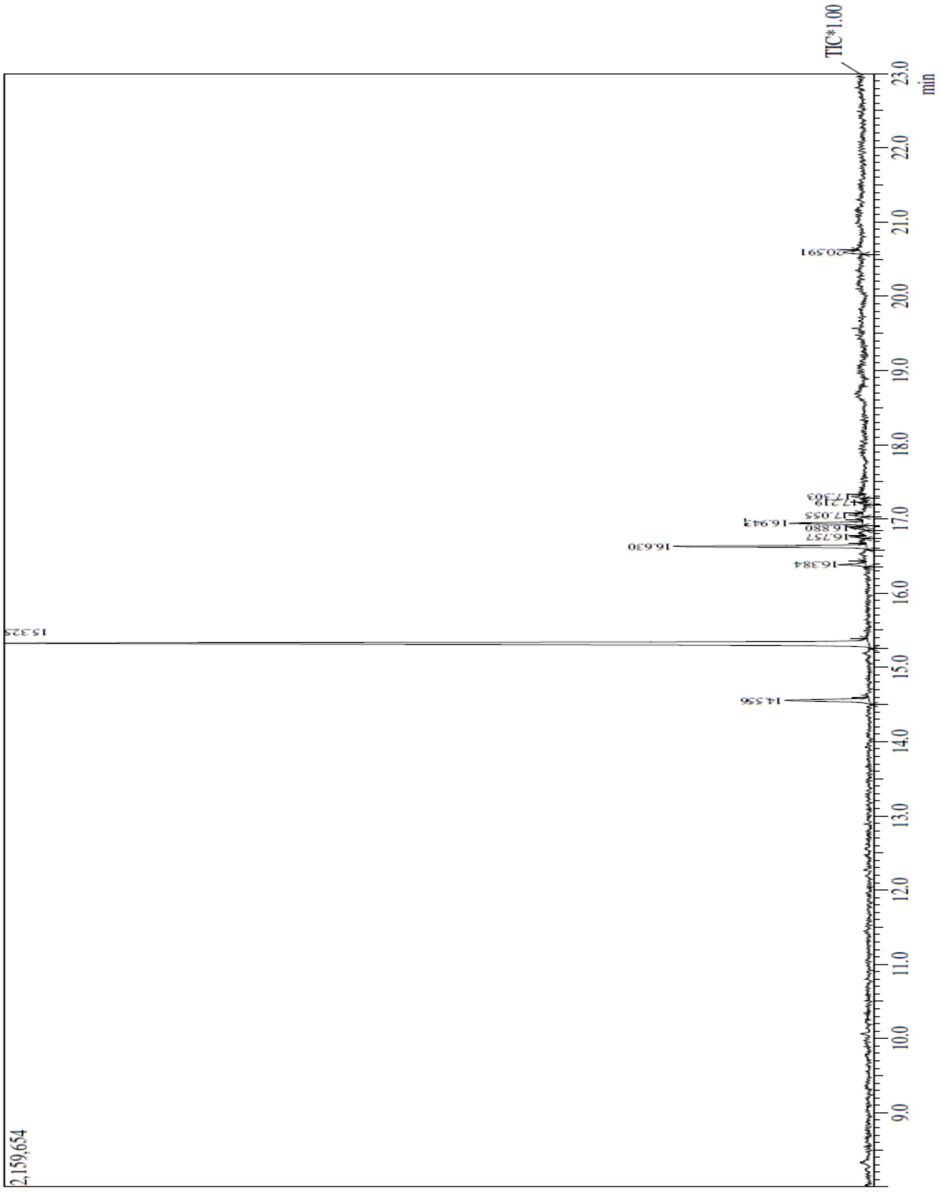

Figure B.1: Gas Chromatography analysis on FAME with methyl heptadecanoate as internal standard

Sample Calculation

Based on Figure B, the area and retention time of FAME obtained for Run 10 in Table 4.2 was summarized in Table B.1. It is given that,

Total peak area = 5724810
Concentration of internal standard solution = 1 mg/ml
Volume of internal standard = 1.8 ml
Mass of the sample for GC test = 82 mg

Table B.1: Retention Time and Area of FAME obtained in Figure B

Component	Retention Time (min)	Area	Content, C_i (%)
Methyl Palmitate	14.556	417400	7.29
Methyl Linoleate	16.384	92710	1.62
Methyl Oleate	16.630	693982	12.12
Methyl Stearate	16.943	268305	4.69
Methyl Heptadecanoate (IS)	15.325	3943042	68.88

According to modified EN 14103 standard, the content of each individual component of sample was calculated by using Equation 3.2.

$$C_i = \frac{A_i}{\sum A - A_{IS}} \times 100\% \qquad (3.2)$$

Example of calculation:

Content of methyl heptadecanoate $= \dfrac{A_i}{\sum A - A_{IS}} \times 100\%$

$= \dfrac{417400}{5724810 - 3943042} \times 100\%$

= 7.29 %

Furthermore, the yield of FAME produced was calculated according to modified EN 14103 method. The yield of FAME produced was calculated by expressing FAME content obtained as a mass fraction in percent using methyl heptadecanoate (C_{17}) as internal standard. The total content of FAME obtained was determined by using Equation 3.3.

$$C = \frac{\sum A - A_{IS}}{A_{IS}} \times \frac{C_{IS} \times V_{IS}}{m} \times 100\% \qquad (3.3)$$

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

$= \dfrac{5724810 - 3943042}{3943042} \times \dfrac{1 \times 1.8}{82} \times 100\%$

= 0.3112 ×100%

= 31.12%

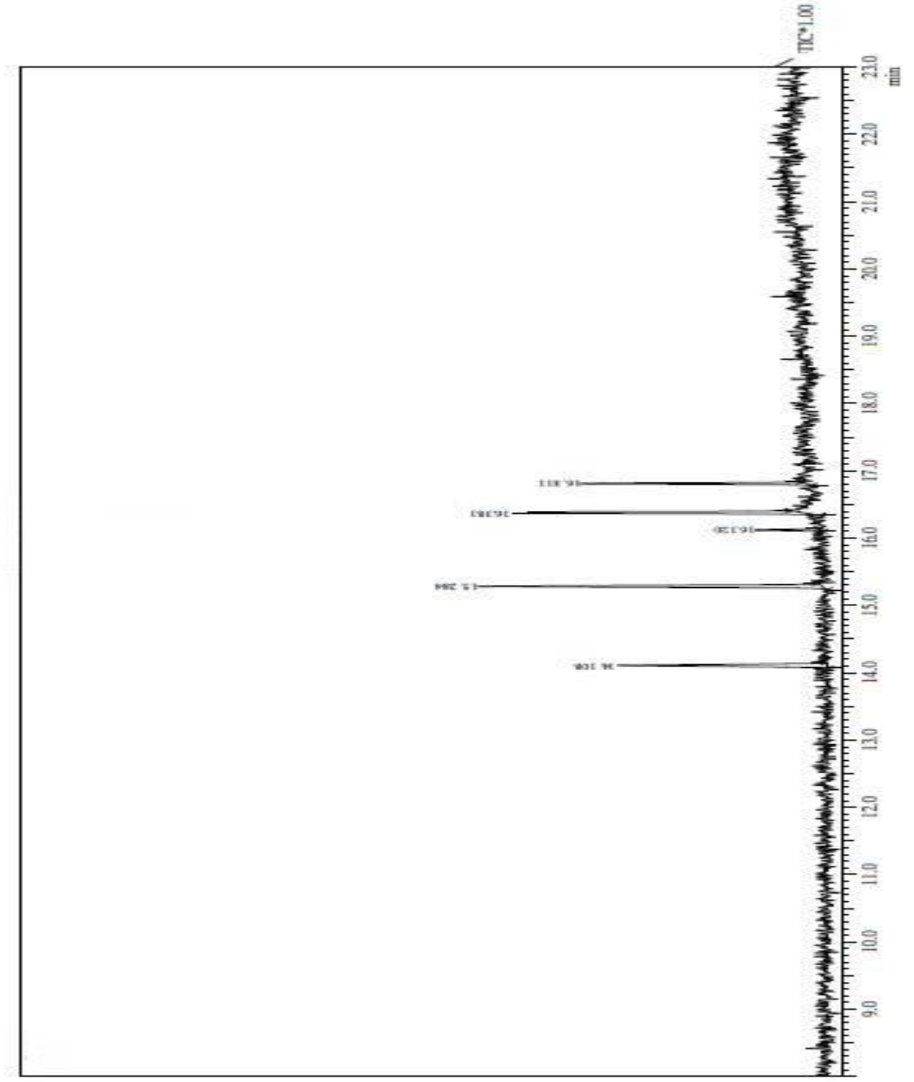

Figure B.2: Gas Chromatography analysis on FAME obtained from Reflux Condenser Method