SYNTHESIS ON BIO REACTION KINETICS OF BIODIESEL PRODUCTION FROM RUBBER SEED OIL USING LIPASE AS THE CATALYST

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ABSTRACT

Energy is fundamental to the quality of life on the earth. Meeting the growing demand for energy sustainably is one of the major challenges of the 21st century. The number of human also continuously grows bigger, causing energy request become higher. That is why our energy such as petroleum depleting very fast. The situation has led to the search for an alternative source of energy. The purpose of this study is to study on bio reaction kinetics of biodiesel production from rubber seed oil using lipase as the catalyst. Biodiesel is an alternative fuel that produced by chemical reaction of vegetable oil or animal fat with an alcohol which used for diesel engines. Biodiesel have advantages to use as a renewable energy like fossil fuel such as it is non toxic, biodegradable and greenhouse gas neutral. Biodiesel produced by using transesterification process. Transesterification process is triglycerides react with methanol and aid of catalyst produce methyl ester and glycerol as by product. In this study, we only focus the biodiesel production using enzymatic process. In this process, catalyst that will use is lipase *Pseudomonas cepacia* (P. Cepacia). The process optimization consisted of (a) Alcohol to oil molar ratio, (b) Catalyst amount, (c) Reaction temperature, and (d) Reaction time. ^{1}H NMR spectrum of the RSO and biodiesel samples are analysed which confirms the conversion of RSO to biodiesel. The biodiesel properties have been investigated and are found to be comparable with diesel.

Keywords: Rubber seed oil; Biodiesel; Transesterification; Enzyme; Lipase; Pseudomonas cepacia: Free Fatty Acids: Kinetics Reaction

SINTESIS KE ATAS TINDAKBALAS BIO KINETIK TERHADAP PENGELUARAN BIODIESEL DARI MINYAK BIJI GETAH MENGGUNAKAN LIPASE SEBAGAI PEMANGKIN

ABSTRAK

Tenaga adalah asas kepada kualiti kehidupan di bumi. Memenuhi permintaan yang semakin meningkat untuk tenaga mampan adalah salah satu cabaran utama abad ke-21. Bilangan manusia juga terus membesar, menyebabkan permintaan tenaga menjadi lebih tinggi. Itulah sebabnya tenaga seperti petroleum semakin berkurangan dengan sangat cepat. Keadaan ini telah membawa kepada pencarian sumber tenaga alternatif. Tujuan kajian ini adalah untuk mengkaji tindakbalas bio kinetik pengeluaran biodiesel daripada minyak biji getah menggunakan lipase sebagai pemangkin. Biodiesel adalah bahan api alternatif yang dihasilkan oleh tindak balas kimia minyak sayuran atau lemak haiwan dengan alkohol yang digunakan untuk enjin diesel. Biodiesel mempunyai kelebihan untuk digunakan sebagai tenaga yang boleh diperbaharui seperti bahan api fosil iaitu ia bukan toksik, mesra alam dan gas rumah hijau neutral. Biodiesel dihasilkan menggunakan proses transesterifikasi. Proses transesterifikasi adalah apabila trigliserida bertindak balas dengan metanol dengan bantuan pemangkin menghasilkan metil ester dan gliserol sebagai hasil. Dalam kajian ini, kami hanya menumpukan pengeluaran biodiesel menggunakan proses enzim. Dalam proses ini, pemangkin yang akan digunakan lipase *Pseudomonas cepacia* (P. cepacia). Proses pengoptimuman terdiri daripada (a) nisbah molar alkohol terhadap minyak, (b) amaun Pemangkin, (c) Reaksi suhu, dan (d) Reaksi masa. Spektrum ^{1}H NMR RSO dan sampel biodiesel dianalisis yang mengesahkan penukaran RSO kepada biodiesel. Ciri-ciri biodiesel telah disiasat dan didapati setanding dengan diesel.

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CHAPTER 1

INTRODUCTION

1.1 Background of Proposed Study

As the number of human continuously grows bigger, energy request become higher. That is why our energy such as petroleum depleting very fast. Figure 1.1 showed that Malaysian population increase each year. The situation has led to the search for an alternative source of energy. The alternative source of energy includes solar energy, wind energy, geothermal energy, tidal energy, ocean thermal energy, hydropower, nuclear energy and biodiesel that can be used as our alternative sources. But as the accident of nuclear energy power plant that happen in Japan, Three Mile Island in United State and Chernobyl, the uses of nuclear energy have become limited. Solar energy, wind energy, geothermal energy, tidal energy, ocean thermal energy, and hydropower are not very effective as the alternative source. It is because the uses of it would only be efficient when the energy present at the time of using it. Which mean, as the sun not present at night, the solar energy will become much low.

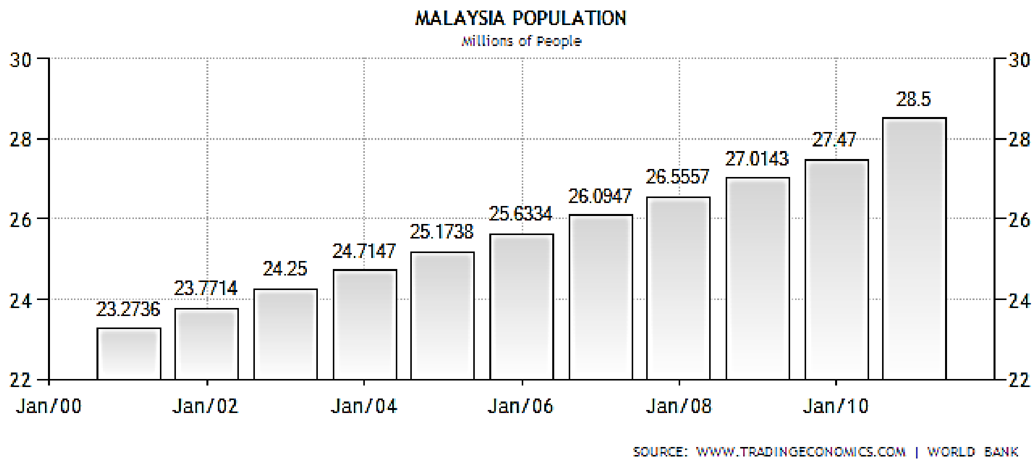

Figure 1.1 Malaysian population increases each year
Source: www.tradingeconomics.com | World Bank (2010)

In a few past decades, the experts have been studied about biodiesel oil that can be our alternative sources. Biodiesel can replace the fossil fuel as it is renewable, biodegradable and as effective as fossil fuel. Biodiesel is produce from vegetable oil or animal fats. Vegetable oils are becoming a promising alternative to diesel fuel because they are renewable in nature and can be produced locally and environmental friendly as well. As said by Ramadhas A. S., Jayaraj S., and Muraleedharan C. (2005), vegetable oils have practically no sulfur content, offer no storage difficulty, and they have excellent lubrication properties.

In Malaysia, biodiesel has been widely accepted in the country. The National Biofuel Policy which was launch by the prime minister in 2006 has boosted the industry to the next level. The demand for biofuel in Europe is projected to increase from 3 million tons in 2005 to 10 million tons in 2010 (The National Biofuel Policy, 2006).Other country such as India, Japan, Brazil and United States has started to invest heavily in the biofuel industry. There are many vegetables have been used in producing biodiesel. Among that are palm oil, soybean oil, sunflower oil, coconut oil, rubber seed oil, tung oil microalgae and Jatropha oil. The main vegetable oil use in commercial scale to produce biodiesel in Malaysia is palm oil. But, palm oil is edible oil. Using edible oil to produce biodiesel, it may cause the competition between biodiesel and food supply product. In this study, rubber seed oil will be used to produce biodiesel. Rubber seed oil is non-edible oil. It has high free fatty acid. Rubber seed oil is produce from the seed and kernel of the rubber seed. In Malaysia, the second largest plantation is rubber that is produce from rubber tree. The purpose of the present study is to develop a method for transesterification of high FFA vegetable oils. Rubber seed oil, typical non-edible high FFA oil is considered as a potential feedstock for biodiesel production in this study.

To produce biodiesel using rubber seed oil, there are a few methods that can be used. There are base catalysts, supercritical catalyst, solid catalyst or enzyme catalyst method. Enzyme catalyst method can be very effective method. That is because it is not producing soap. But it kinetic reaction is very slow. In the enzymatic catalyst process, the example of catalyst that we can use is lipase. So, this review is to investigate the kinetic reaction of enzyme catalysts process using lipase as the catalyst.

1.2 Problem Statement

Enzyme catalyst process has gaining more attention nowadays. That is because there is no soap formation during the production of biodiesel. As said by Vasudevan P. T., Briggs M. (2008), since no glycerol is produced in the process, this method is very convenient for recycling the catalyst, and byproduct triacetylglycerol shows no negative effect on the fuel property. But, this process has very slow kinetic reaction. As said by Lam M. K., Lee K. T. and Mohamed A. R. (2010), the reaction of biodiesel production using enzymatic process is from 2.47 hours to 72 hours. The reaction time very slow than the other methods. According to Juan J. C., Kartika D. A., Wu T. Y. and Hin Y. Y. (2011), the problem faced using enzymatic process is long reaction time and denaturation problem. Lipase will denatured when reach it optimum temperature. This is because lipase is made from protein.

1.3 Objectives

The objectives for this thesis are:

1. To investigate the kinetic reaction of enzyme catalysts process using lipase as the catalyst.
2. To determine the effect of different parameter to the kinetic reaction of enzyme catalyst process.
3. To optimize parameter or kinetic study.

1.4 Scope of Study

This thesis is focus on:

1. The kinetic reaction of biodiesel production from rubber seed oil using lipase as the catalyst.
2. Comparatively study the characteristics of biodiesel produced using HPLC, NMR or GC.

1.5 Rationales and Significances

Significance of this study is to find the advantages using enzyme transesterification process and to study the yield or length of the process reaction. To eliminate the dependence on the foreign alternative like other vegetable oil those not currently grow in Malaysia. To investigate the biodiesel production using non-edible and high free fatty acid oil.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The world today is faced with serious global warming and environmental pollution due to ozone depletion. Diesel fuel will release CO, NO_2, and SO_2. All these emission will cause the air pollution. So, the uses of diesel should be decrease. Table 2.1.1 showed that the emission of pollutants to the atmosphere by sources in Malaysia.

Table 2.1 The Emission of Pollutants to the Atmosphere by Sources, 2008 – 2010, Malaysia

Tahun *Year*	**Punca Tetap**[1] *Stationary Sources*[1]		**Punca Bergerak** *Mobile Sources*	**Lain-lain**[2] *Others*[2]	**Jumlah** *Total*
	Industri *Industrial*	**Stesen Janakuasa** *Power Plant*			
2008	148.7	221.4	1,630.79	54.4	2,055.30
2009	166.3	595.9	1,762.80	60.3	2,585.26
2010	113.9	619.2	1,829.70	60.5	2,623.23

Source: Department of Statistics, Malaysia (2010)

Besides that, fossil fuel will become diminish and faces serious shortage in the near future. The World Energy Forum predicted that fossil oil will be exhausted in less than 10 decades, if new oil wells are not found (Sharma and Singh, 2009).The main reason that caused the fast diminishing of energy resources is due to rapid population and industrialization growth globally (Pimentel D. and Pimentel M., 2006). Due to this phenomenon, the era of cheap crude oil no longer exists leading to high sky rocketing price of petroleum, bellicose conflicts and increasing the number of undernourished people especially from undeveloped countries. Therefore, this has triggered the awareness to find alternative energy as their sustainable energy. As said by Sharma Y. C. and Singh B. (2009), various renewable sources of energy have successfully been tried and used by different nations to limit the use of fossil fuels. This renewable source of energy includes solar energy, wind energy, geothermal energy, tidal energy, ocean thermal energy, hydropower and others. But neither of that is as important as fossil fuel. Figure 2.1 and 2.2 showed total energy demand of Malaysian peoples and total energy production per year respectively.

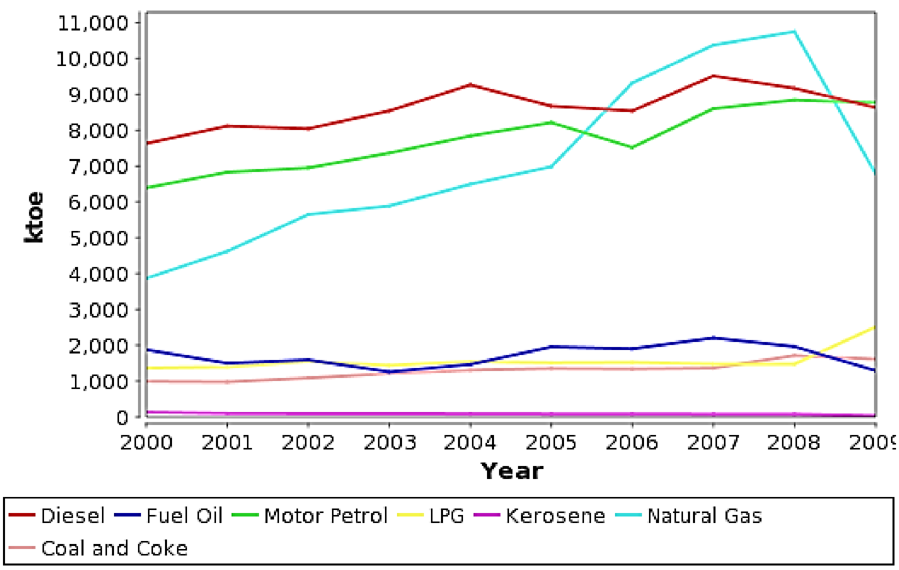

Figure 2.1 Total energy demand of Malaysian peoples per year
Source: Malaysia Energy Information Hub (2010)

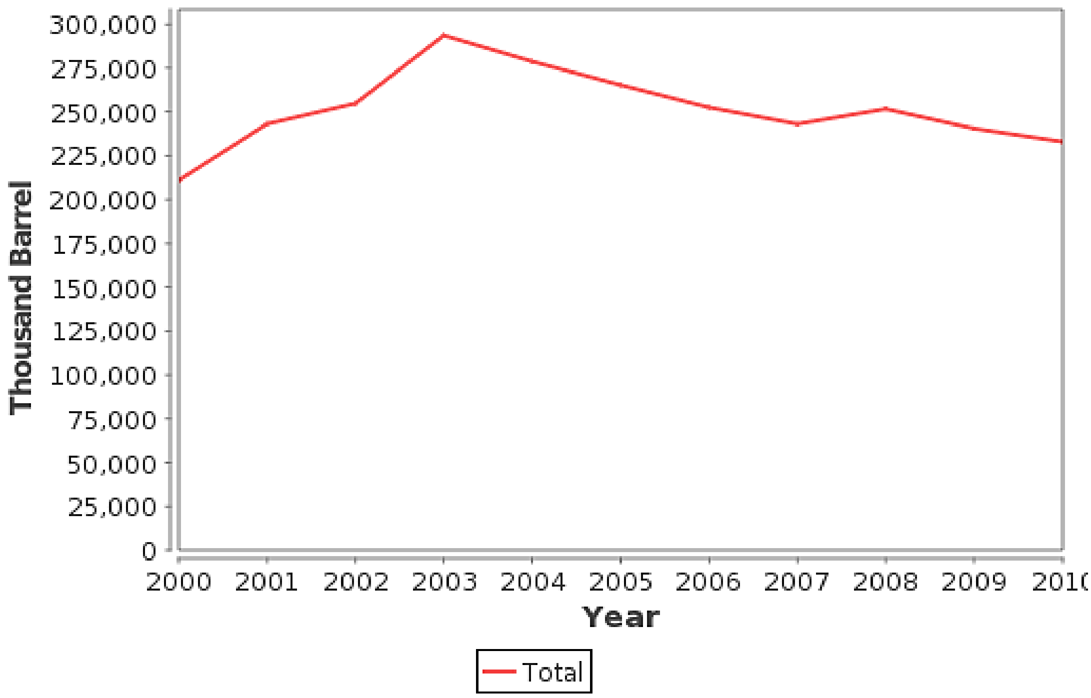

Figure 2.2 Total energy production per year
Source: Malaysia Energy Information Hub (2010)

As we can see from the Figure 2.1, the diesel demand increase by year. But, Figure 2.2 showed that the total diesel production decrease by year. So, new energy need to be developed as the diesel decrease. Biodiesel is considered as better option as an alternative energy because of its environmental friendly while giving almost same functional properties like fossil fuels. It is also renewable. Global warming and environmental pollution due to ozone depletion problems can be decrease.

2.2 The characteristic of biodiesel

Biodiesel is an alternative fuel which produced by chemical reaction of vegetable oil or animal fat with an alcohol which used for diesel engines (Yusuf N.N.A.N., Kamarudin S.K. , Yaakub Z., 2011). According to Canoira L., Alcántara

R.,Torcal S., Tsiouvaras N., Lois E. and Korres D. M. (2007), biodiesel consists of alkyl esters, which are produced from the transesterification reaction between triglycerides and alcohol. In experimental studies, the fatty acid alkyl esters or fatty acid methyl esters (FAME) is the final product instead of biodiesel. According to Ma and Hanna, (1999), the advantages of biodiesel as fuel are liquid nature portability, ready availability, renewability, higher combustion efficiency, lower sulfur and aromatic content. The production of biodiesel can reduce the typical waste product's harmful environmental effects. That because biodiesel is a biodegradability which is content oxygen that will improve the biodegradation process. That means leading to an increased level of quick biodegradation will become biodiesel is non-toxic and degrades about four times faster than petroleum diesel (Demirbas A., 2007). Next, biodiesel is less polluting than petroleum because it produces less soot, carbon monoxide, unburned hydrocarbons and sulfur dioxide. (Kmyhr, 2001). As said by Sharma Y. C. and Singh B. (2009), biodiesel can be termed clean fuel as it does not contain carcinogens and its sulphur content is also lesser than the mineral diesel. Table 2.2 shown the technical properties of biodiesel:

Table 2.2 Technical properties of biodiesel

Properties	Characteristics / Value
Common name	Biodiesel (bio-diesel)
Common chemical name	Fatty acid (m)ethyl ester
Chemical formula range	C_{14}–C_{24} methyl esters or C_{15} $25H_{28}$ $48O_2$
Kinematic viscosity range (mm^2/s, at 313 K)	3.3–5.2
Density range (kg/m3, at 288 K)	860–894
Boiling point range (K)	>475
Flash point range (K)	420–450
Distillation range (K)	470–600
Vapor pressure (mm Hg, at 295 K	<5
Solubility in water	Insoluble in water
Physical appearance	Light to dark yellow, clear liquid

Table 2.2 Technical properties of biodiesel (continue)

Properties	Characteristics / Value
Biodegradability	More biodegradable than petroleum diesel
Reactivity	Stable, but avoid strong oxidizing agents
Odor	Light musty/soapy odor

Source: Demirbas, A. (2009)

2.3 Non-edible oil as resource of biodiesel

The availability of fossil fuel and the environmental problems caused by the use of fossil fuels have been given much attention. Due to the concern, the biodiesel production was led as an alternative to fuel. However, as the biodiesel is produced from vegetable oils and animal fats, there are concerns that biodiesel feedstock may compete with food supply in the long-term. In Malaysia, oil that have been used to produce biodiesel is palm oil, soy bean oil, sunflower oil, coconut oil, rubber seed oil, tung oil, microalgae and Jatropha oil. Palm oil, soy bean oil sunflower oil and microalgae oil are made from edible oil. There are two main crops in Malaysia which is palm and rubber tree. These two crops are suitable for biodiesel production. But palm oil is edible oil. Malaysian Palm Oil Berhad (MPOB, 2011), said that the main uses of palm oil is in food products. Besides that, palm oil also used in cosmetics, soap and etc. Table 2.3 showed the application of edible oil sources:

Table 2.3 The application of edible oil sources

Type of vegetables	Uses
Palm oil	Cooking oil, food additives, cosmetics, toilet soaps, fertilizer and furniture.
Soybean	Drinks, cooking oil, food additives, foam and animal foods.
Coconut	Drinks, food additives, hair gel, toothpaste and sunscreen
Sunflower	Snack food, cooking oil, food additives and fertilizer.

So, edible oil is not suitable for biodiesel production. According to Ramadhas et. al. (2005), rubber seed oil is non-edible oil and it has high free fatty acid content. Since rubber seed oil not produced as food product, it can be produce to biodiesel.

2.4 The uses rubber seed oil in biodiesel production

According to the Association of Natural Rubber Producing Countries, Kuala Lumpur, Malaysia has an estimated acreage of 999,327 hectares of rubber plantation in 2010 (Malaysian Rubber Board, 2010).Rubber (*hevea brasiliensis*) tree starts to bear fruits at four years of age. Each fruit contain three or four seeds, which fall to the ground when the fruit ripens and splits. Each tree yields about 800 seeds (1.3 kg) twice a year (Eka, H. D., Tajul Aris, Y. and Wan Nadiah, W. A., 2010). A rubber plantation is estimated to be able produce about 800-1200 kg rubber seed per year (Siriwardene and Nugara, 1972), and these are normally regarded as waste. Based on an estimated average of 1000 kg seeds per year, the projected annual production of rubber seeds in Malaysia would be 1.2 million metric tons. Despite Malaysia being a major rubber growing country, to date, there is a dearth of information on the chemical composition of the Malaysia rubber seed. According to Bressani et al (1983), the rubber seed kernel (hull has been removed) contains 29.6% fat and 11.4% protein. Thus, it is estimated that Malaysia wastes about 355,200,000 kg fat and 136,800,000 kg protein per year. Table 2.4 showed the principal statistic of rubber industry for 2008 – 2010 in Malaysia:

Table 2.4 Principal statistic of rubber industry for 2008 – 2010

Tahun *Year*	Bilangan estet *Number of estates*	Keluasan bertanam getah (hektar) *Rubber planted area (hectares)*		
		Jumlah *Total*	Estet *Estates*	Kebun kecil *Smallholdings*
2008[a]	196	1,247,030	50,890	1,196,140
2009	185	1,008,647	49,740	958,907 [b]
2010[a]	190	999,327	50,737	948,590

Source: Department of Statistics, Malaysia (2010)

From the table, it shows that total rubber planted area. Even though it is decrease from 2008 to 2010, but it is still a very large area of plantation. From this, many rubber seed can be collect. It is also giving advantages to the plant owner. This will become their side income beside the income from the rubber itself. Malaysia also does not have to import the alternative non-edible oil such as *Jatropha curcas* from other country. The precious time also can be saved from waiting the *Jatropha curcas* to produce the seed if we want to plant it in Malaysia.

2.5 Free fatty acid in oil

Fat is a lipid family. It can be in solid or liquid form at room temperature. This will depend on their structure. Fat usually consist of the ester of glycerol which is monoglyceride, diglyceride or triglyceride. It also has free fatty acid content which is carboxylic acid. According to Suwannakarn K. (2008), free fatty acid can contain from 4-24 of carbon atom. Figure 2.3 shows the chemical structure of vegetable oils.

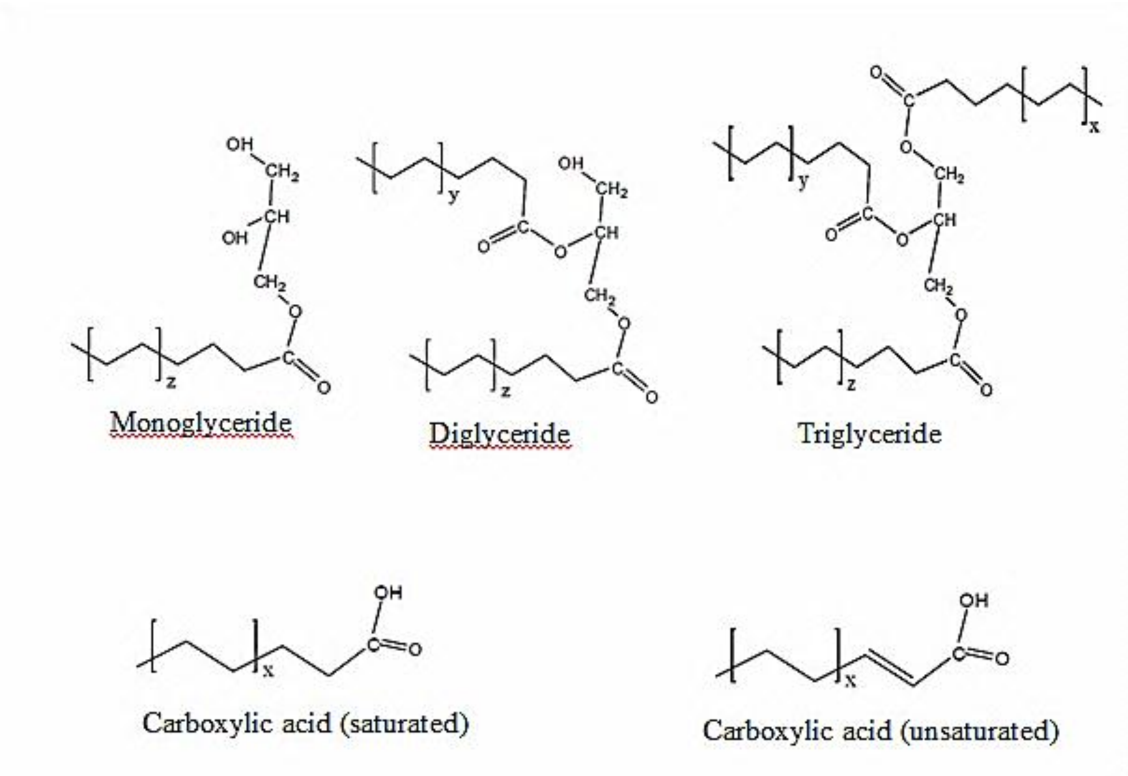

Figure 2.3 The chemical structure of vegetable oils
Source: Suwannakarn K. (2008)

Rubber seed oil consist high free fatty acids content. The fatty acid composition and the important properties of rubber seed oil in comparison with other oils is given in Table 2.5.

Table 2.5 Properties of rubber seed oil in comparison with the other oils

Property	Rubber seed oil	Sunflower oil	Rapeseed oil	Cotton seed oil	Soybean oil
Fatty acid composition (%)					
(i) Palmitic acid $C_{16:0}$	10.2	6.8	3.49	11.67	11.75
(ii) Stearic acid $C_{18:0}$	8.7	3.26	0.85	0.89	3.15
(iii) Oleic acid $C_{18:1}$	24.6	16.93	64.4	13.27	23.26
(iv) Linoleic acid $C_{18:2}$	39.6	73.73	22.3	57.51	55.53
(v) Linolenic acid $C_{18:3}$	16.3	0	8.23	0	6.31
Specific gravity	0.91	0.918	0.914	0.912	0.92
Viscosity (mm^2/s) at 40 °C	66.2	58	39. 5	50	65
Flash point (°C)	198	220	280	210	230
Calorific value (MJ/kg)	37.5	39.5	37.6	39.6	39.6
Acid value	34	0.15	1.14	0.11	0.2

Source: Ramadhas A. S., Jayaraj S., and Muraleedharan C. (2005)

2.6 Process of Synthesizing Biodiesel

There are many different processes which can be applied to synthesize biodiesel such as direct use and blending, micro-emulsion process, thermal cracking process and transesterification process. Transesterification process is the most conventional way among all mentioned above. However, there are also a lot of methods can be done through transesterification process such as based-catalyzed transesterification, acid-catalyzed esterification, integrated acid-catalyzed pre-esterification of FFAs and based-catalyzed transesterification, enzyme-catalyzed transesterification, hydrolysis and supercritical alcohol transesterification (Saka et al., 2001).

2.6.1 Direct use and blending

Vegetable oil usage as a fuel has been discussed since 1980. Vegetable oil can directly use or blending with petroleum diesel in engine. Used cooking oil and a blend of 95% used cooking oil and 5% diesel fuel were used. Anon(1982) said, a diesel fleet was powered with filtered, used frying oil. In this method, blending or preheating was used as needed to compensate for cooler ambient temperatures. Ma. F. and Hanna M. A. (1999) said, there were no coking and carbon build-up problems and the key was suggested to be altering and the only problem reported was lubricating oil contamination (viscosity increase due to polymerization of polyunsaturated vegetable oils). Furthermore, the lubricating oil had to be changed every 4,000±4,500 miles. Vivek and Gupta A. K said, the advantages of vegetable oils as diesel fuel are:

i. Liquid nature-portability
ii. High heat content (80% of diesel fuel)
iii. Ready availability
iv. Renewability.

However, the disadvantages are:

i. Higher viscosity
ii. Lower volatility
iii. The reactivity of unsaturated hydrocarbon chains lower

2.6.2 Micro-emulsion process

To solve the problem of the high viscosity of vegetable oils, micro-emulsions with solvents such as methanol, ethanol and 1-butanol have been studied (Ma, F. and Hanna, M., A., 1999). Schwab et al., (1987) defined micro-emulsion as colloidal equilibrium dispersion of optically isotropic fluid microstructures with dimensions generally in the 1±150 nm range formed spontaneously from two normally immiscible liquids and one or more ionic or non-ionic amphiphiles. The lower viscosity will make the atomization is really easy. Goering et al., (1982) stated that short term performances of both ionic and non-ionic micro-emulsions of aqueous ethanol in soybean oil were nearly meet the specification for biodiesel (ASTM D6751 biodiesel specification) except for the lower cetane number and energy content.

2.6.3 Pyrolysis (thermal cracking)

Pyrolysis is an alternative to gasification and is becoming an increasingly popular option for converting biomass to solid, liquid, and gaseous fuels (Maher K. D and Bressler D. C, 2007). Sonntag (1979) defined pyrolysis as the conversion of one substance into another by means of heat or by heat with the aid of a catalyst. Pyrolysis involves heating in the absence of air or oxygen and cleavage of chemical bonds to yield small molecules (Weisz et al., 1979).The advantages of pyrolysis include the fact that it is simple and inexpensive to construct (Onay and Kockar, 2004). The pyrolyzed material can be vegetable oils, animal fats, natural fatty acids and methyl esters of fatty acids. The equipment for thermal cracking and pyrolysis is expensive for modest throughputs. In addition, while the products are chemically similar to petroleum-derived gasoline and diesel fuel, the removal of oxygen during the thermal processing also removes any environmental benefits of using an oxygenated fuel. It produced some low value materials and, sometimes, more gasoline than diesel fuel. (Ma, F. and Hanna, M., A., 1999)