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THESIS

OPTIMIZATION OF ESTERIFICATION AND TRANSESTERIFICATION OF HIGH FATTY ACID RAW MATERIAL USING BOX-BEHNKEN METHOD

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This study was the optimization of both esterification and transesterification of the mixture of palm fatty acid distillate (PFAD) and palm stearin (PS). The initial free fatty acid (FFA) contents of raw oil mixture were 5, 20, 40, 50, 60 and 87% for esterification and 1 and 2% for transesterification. Response surface methodology (RSM) three-level-three-factor and three-level-four-factor Box-Behnken designs were employed to predict the conversion of FFA and fatty acid methyl ester (FAME) as a function of reaction time, wt% of catalyst (based on FFA or triglyceride) and molar ratio of methanol to FFA or triglyceride in oil. In esterification, the experiments for initial 5, 20, 40, 50, 60 and 87% FFA were performed under fixed and varied conditions. The fixed conditions were molar ratio of methanol to triglycerides in oil of 3:1, 60 ºC reaction temperature and 500 rpm stirring rate. The varied parameters were reaction time, wt% of catalyst (based on FFA in oil) and molar ratio of methanol to FFA in oil.

The second order polynomial model was used to predict the optimum condition. The optimum condition parameters to reduce FFA from 5% initially to 1% were 4.5:1 molar ratio of methanol to FFA in oil, 3 wt% sulfuric acid (based on FFA) and 90 min reaction time respectively. The optimum condition for initial 20% FFA was molar ratio of methanol to FFA of 4.6:1 and 2.4 wt% sulfuric acid after 3 h of reaction. The predicted optimum condition for initial 40% FFA was 11.6:1 molar ratio of methanol to FFA, 3.3 wt% sulfuric acid and 5.4 h of reaction. In the optimization of initial 50% FFA esterification, the optimum condition was molar ratio of methanol to FFA of 11.8:1, sulfuric acid loading of 3.7 wt% (based on FFA) and reaction time of 5.3 h. The optimum condition for initial 60% FFA esterification was molar ratio of methanol of FFA of 11.6:1, reaction time of 5.4 h and catalyst based on FFA of 3.5 wt%. For transesterification of initial 1% FFA, this predicted optimum condition was molar ratio of methanol to triglyceride of 8.25:1, KOH concentration of 0.8 wt% (based on triglyceride) and reaction time of 75 min to get 96.7% FAME. For the initial 2% FFA, molar ratio of methanol to triglyceride of 12:1, KOH concentration of 0.82 wt% (based on triglyceride) and reaction time of 30 min to get 96% FAME of initial 2% FFA. The order of important factors were methanol amount > catalyst loading > time for both of 1 and 2% FFA transesterification. According to three-level-four-factor predicted design of initial 5-87% FFA, it can be used to predict the optimum conditions of initial 40, 50 and 60% but not for the initial FFA less than 40%.

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May Myat Khine

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LIST OF ABBREVIATIONS

PFAD	Palm fatty acid distillate
PS	Palm stearin
FFA	Free fatty acid
RSM	Response surface methodology
FAME	Fatty acid methyl ester
ANOVA	Analysis of variance
DF	Degree of freedom
CN	Cetane number
BBD	Box-Behnken design
DM	Doehlert design
CCD	Central composite design
CCRD	Central composite rotatable design
AV	Acid value
RSREG	Response surface regression

OPTIMIZATION OF ESTERIFICATION AND TRANSESTERIFICATION OF HIGH FATTY ACID RAW MATERIAL USING BOX-BEHNKEN METHOD

INTRODUCTION

Presently, the world's energy needs are through non-renewable resources such as, petrochemicals, natural gas and coal. These resources will be depleted in the few years as a result of continued increase in the demand of the present rate of consumption (Rhaeman and Phaveatare, 2004; Szybist *et al.*, 2005). It is also estimated that if the annual consumption increases to 24 billions barrels, all the existing crude oil deposited unederneath the earth with dry up by the year 2040. Oil consumption is increasing two percent annually. However, it is sure that one day, all the crude oil of the world will be gone. Due to the unstable political situation in the Middle East, the main crude oil producing region on earth, constant flow of oil to the markets is not grantee in the future. Thus, Asia which is depending on the Middle East for about 60% of its oil need is making plans to store crude oil as strategic reserve for emergency use (Knothe *et al*., 2005).

Petroleum fuels also deteriorate the ambiance and cause global warming. It is stronger threat to clean environment. Burning of fossil is associated with emissions like CO_2, CO, SO_x, NO_x and particulate matter which are currently the dominant global source of emissions. Hence, there are efforts around the globe to protect the environment from further deterioration. The harmful exhaust emissions from the engines, rapid increase in the prices of petroleum products and uncertainties of their supply have jointly created renewed interest among the researchers to search for suitable alternative fuels (Demirbas, 2008).

The extracted oil could not be used directly in diesel engines because of its high viscosity. High viscosity of pure vegetable oils would reduce the fuel atomization and increase fuel spray deposits and thickening of lubrication oil. Thus, efforts have already been made to develop vegetable oil derivatives with approximate properties and performance as fossil diesel. Predominately, four methods have been reported in the literatures to improve the properties of vegetable oils - dilution, thermal cracking, microemulsion, and transesterification (Srivastava and Prasad, 2000; Ma and Hana, 1999). Transesterification process is one of the most successful and promising processes to convert vegetable oils into diesel-like liquid, biodiesel. It does not require any modification in engine or injection system or fuel lines and directly possible in any diesel engine. Chemically, biodiesel is mono-alkyl esters (fatty esters) of long chain fatty acids (falls in carbon range from C_{12}-C_{20}) derived from renewable biological sources such as vegetable oils or animal fats (Srivastava and Prasad, 2000; Khan, 2002).

Selection of vegetable oil for biodiesel production is the key factor to reduce the cost of production. This selection depends on three factors. Firstly, the nature and availability of the vegetable oil is important. For example, soybean oil is being used in United State. Soybean oil and sunflower oil (both are edible oils) are being used in most of the European countries. In developing countries like India, land availability even for food grain is insufficient and edible oils are mostly being used in the food products (Sudha and Ravndranath, 1999; Subramanian *et al*., 2005). Secondly, the price of oils is very important. Costs of edible oils in developing countries are high because of high import bill. Moreover, non-edible oils availability is region specific and they find the usage in other sectors and hence the prices could be very high limiting the economic production of biodiesel. Lastly, the policies and planning to produce non edible vegetable oils from waste on the arid land in large excess from government and other non-government organizations. These will aid in production of non edible oils (Tiwari *et al*., 2006).

The amount of free fatty acid (FFA) in oil depends on the quality of the feedstock (Berchamans and Hirata, 2008). Generally, when free fatty acid (FFA) in raw oil material is less than 2%, one step transeserification can be applied (Kansendo, *et al*., 2008). If the high FFA raw material is used, biodiesel can be produced by two ways. The first one is to remove FFA from raw material using distillation, solvent extraction or membrane separation followed by transesterification to convert remained triglyceride to biodiesel. This method renders low yield of biodiesel because of FFA loss. The second one is to convert the FFA to biodiesel first using acid-catalyzed esterification then followed by transesterification. The latter method can get high yield of biodiesel as any loss does not occur (Al-Widyan and Al-Shyoukh, 2002; Serio *et al.,* 2005; Lotero *et al.*, 2005; Zheng *et al.,* 2006). Refined vegetable oils are the predominant feedstocks for the production of biodiesel. Usually, palm oil, soybean oil, rapeseed oil, corn oil, sesame oil and sunflower oil are common feedstocks for biodiesel production. The high value of refined vegetable oil as a food product makes the production of biodiesel fuel very challenging as the cost of raw material accounts for 60-70% of total production cost of biodiesel fuel (Krawczy, 1996; Ma and Hanna, 1999). Therefore, many researchers are looking for suitable raw materials to produce biodiesel. Inexpensive feedstocks are preferable to be used in biodiesel production to reduce its cost and to reduce waste oil from the industry. Then, biodiesel is conventionally produced from feedstock having FFA content less than 20% (Zhang, *et al*., 2003a; Tomasevic and Siler-Marinkovic, 2003; Ramadhas, *et al*, 2005; Wang, *et al*., 2006; Ghadge and Raheman, 2006; Warabi, *et al*., 2004; Royon, *et al*., 2006; Zheng, *et al*., 2006; Du, *et al*., 2004; He, *et al*., 2007). Palm fatty acid distillate (PFAD) and palm stearin (PS) are products from vegetable oil refining. This business improve in some regions of the world quickly, as they have the potential to replace biodiesel manufactured from other conventional sources. PFAD and PS are by-product from palm oil refining process.

Both esterification and transesterification are affected by many factors, such as methanol amount, catalyst loading, reaction temperature and reaction time etc. The factors can be optimized by conventional one-factor-at-a-time method and statistical methods. Conventional method, involve changing one independent variable at a time, while keeping other factors at a fixed level. The response surface methodology (RSM) is a powerful technique for testing multiple process variables because fewer experiment trials are needed comparing to the study of one-factor-at-a-time. Also, interactions between variables can be identified and quantified by such technique. This reaction conditions for pre-treatment and biodiesel production have been optimized using RSM by many authors (Vicente *et al*., 1998; Ghadge and Raheman, 2006; Yuan *et al*., 2008; Jeong *et al.*, 2009).

In this work, the mixture of PFAD and PS were used as raw material to reduce the biodiesel production cost and make value added of both raw materials. As the use of high free fatty acid, two-step reactions were used. According to study the optimization of the reaction conditions, the optimization values for the variables affecting the process were determined by application of Box-Behnken design and RSM. Box-Behnken design provides more information per experiment than unplanned approaches and it allows evaluating interaction among experimental variables within the range studied, leading to better knowledge of the process. Therefore, it can reduce research time and cost (Box and Wilson, 1951; Gopinath *et al*., 2010).

OBJECTIVES

1. To optimize the reaction parameters of esterification and transesterification reaction of raw materials with various free fatty acid content using response surface methodology

2. To find the correlation of esterification conditions of raw material with various initial FFA contents

LITERATURE REVIEW

1. Background

Since traditional fossil energy sources are limited and green house emissions are becoming a greater concern, research on alternative, renewable fuels has increased in recent years (Lopez *et al*., 2005). Biodiesel is renewable fuel comprised of fatty acid methyl esters (FAME) which are derived from vegetable oils or animals fats. Comparison between biodiesel and petroleum-based diesel has revealed that biodiesel is more effective in reducing exhaust emissions of carbon monoxide, hydrocarbons, particulate matter, and sulfur dioxide. In addition, carbon dioxide formed by combustion of FAME can be recycled by photosynthesis, which minimizes the impact of FAME combustion on green house effect (Zhang *et al*., 2003b).

Researchers are always looking for the suitable materials to produce biodiesel on a large scale (Dizge *et al*., 2009; Wen *et al*., 2010). Such soybean oil (Rosa, *et al*., 2009; Liang *et al*., 2009), rice bran oil (Shiu *et al*., 2010; Lin *et al*., 2009), sunflower oil (Guan *et al*., 2009), jatropha oil (Tamalampudi *et al*., 2008), waste edible oil (Maceiras *et al*., 2009; Li *et al*., 2009), canola oil (Dizge *et al*., 2009) and other raw materials (Gao *et al*., 2010; Anwar *et al*., 2010; Oner and Altun, 2009) as feed stock for biodiesel production have been reported previously.

The production of FAME at the industrial scale most frequently uses an alkali-catalyzed transesterification of oils to yield methyl esters (Zhang *et al*., 2003b). Conversion to FAME by this process is challenging if oil contains large amounts of free fatty acids (>1%) which soaps occurs (Ghadge and Raheman, 2005) and hence requires additional downstream operation. A number of researchers working with feedstocks with elevated FFA levels employed an excess of alkaline catalyst to neutralize the FFA, which could then be removed from the process stream as a waste product (Canakci and Gerpen, 2001). However, this approach increased the costs associated with higher catalyst usage and recovery (Dorado *et al*., 2002).

2. Biodiesel

Biodiesel (Greek, bio, life + diesel from Rudolf Diesel) refers to a diesel-equivalent, processed fuel derived from biological sources. Biodiesel is the name for a variety of ester-based oxygenated fuels from renewable biological sources. It can be made from processed organic oils and fats (Demirbas, 2008). Vegetable oil alkyl ester, commonly referred to as biodiesel, is prominent candidates as alternative diesel fuels. The name biodiesel has been given to transesterified vegetable oil to describe its use as a diesel fuel (Demirbas, 2002).

Chemically, biodiesel is defined as the monoalkyl esters of long chain fatty acids derived from renewable biolipids. Biodesel is typically produced through the reaction of a vegetable oil or animal fat with methanol or ethanol in the presence of a catalyst to yield methyl or ethyl ester (biodiesel) and glycerine (Demirbas, 2002). Fatty acid alkyl esters or biodiesels are produced from natural oils and fats. Generally, methanol is preferred for transesterification because it is less expensive than ethanol (Graboski and McCormick, 1998).

3. Biodiesel as an alternative to diesel engine fuel

In general, the physical and chemical properties and the performance of the ethyl esters are comparable to those of the methyl esters. Methyl and ethyl esters have almost the same heat content. The viscosities of ethyl esters are slightly higher and the cloud and pour points are slightly lower than that of methyl esters. Engine tests have demonstrated that methyl esters produced slightly higher power and torque than ethyl esters. The advantages of biodiesel as diesel fuel are its portability, ready availability, renewability, higher combustion efficiency, and lower sulfur and aromatic content (Ma and Hana, 1999 and Knothe *et al*., 2006), higher cetane number, biodegradability (Mudge, and Pereira, 1999, Speidel, *et al*. 2000 and Zang, *et al*. 2003a). The main advantages of biodiesel given in the literature include its domestic origin, which would help reduce a country's dependency on imported petroleum, its biodegradability, high flash point, and inherent lubricity in neat form (Mittelbach and Remschmidt, 2004 and Knothe, *et al*. 2005).

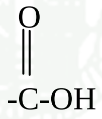
The major disadvantages of biodiesel are its higher viscosity, lower energy content, higher cloud point and pour point, higher nitrogen oxide (NO_x) emissions, lower engine speed and power, injector coking, engine compatibility, high price and greater engine ware. The technical disadvantages of biodiesel/fossil diesel blends include problems with fuel freezing in cold weather, reduced energy density, and degradation of fuel under storage for prolonged periods. Biodiesel blends loosen these deposits, causing them to block fuel filters. However, this is a minor problem, easily remedied by proper filter maintenance during the period following introduction of biodiesel blend (Wardle, 2003).

Biodiesel has significant potential for use as an alternative fuel in compression ignition engines (Demirbas, 2003 and Knothe, 1997). Biodiesel is a plant derive product and contains oxygen in its molecules, making it a cleaner-burning fuel than petrol and diesel (Sastry *et al*., 2006). Biodiesel has got better lubricant properties than fossil diesel. Its oxygen content improves the combustion process, leading to a decreased level tailpipe polluting emission. Biodiesel is non-toxic and quickly biodegrades. The risks of handling, transporting and storing biodiesel are much lower than those associated with fossil diesel. Biodiesel is technologically feasible alternative to fossil diesel, but nowadays biodiesel costs 1.5 to 3 times more than fossil diesel (Demirbas, 2008). The competitiveness of biodiesel relies on the prices of biomass feedstock and costs, which are linked to conversion technology. Depending on the feedstock used, byproducts may have more or less relative importance. Biodiesel is not competitive with fossil diesel under current economic conditions, where the positive externalities, such as impacts on the environment, employment, climate changes and trade balance, are not reflected in the price mechanism (Demirbas, 2008).

4. Fats and Oils

Fats and oils are simple lipids that are hydrophobic substances and can be found in animals and plants. Fats and oils are also known as triacylglycerols, glycerides, or triglyceride because normally they consist of three fatty acids bonded together with glycerol. Generally fats are solid at room temperature and oils are liquid at room temperature.

Fatty acids are long hydrocarbon chains that have a carboxyl group (COOH) at the end of the chain. Figure 1 below shows the structure for the carboxylic acid. A fatty acid can be denoted as (XX:Y) where XX represents the carbon atoms and Y represents the number of double bonds. A saturated fatty acid such as palmatic acid, C16:0 contains no double bond and is more stable oxidatively (i.e less reactive compared to oleic acid, which is unsaturated). Saturated fatty acids tend to occur more naturally in animal fats but can also be found in some vegetable oils such as palm oil.



$$-\overset{\overset{\displaystyle O}{\|}}{C}-OH$$

Figure 1 Carboxylic acid

Source: Khan (2002)

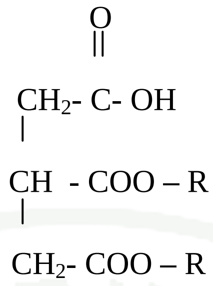
The suitability of fats and oils as diesel fuel (DF) results from their molecular structure and high energy content. Long chain, saturated, unbranched hydrocarbons are especially suitable for conventional diesel fuel as shown by CN scale. The long unbranched hydrocarbon chains in fatty acids meet this requirement. Saturated fatty compounds have higher CNs. Other observations are (i) that (a) double bond (s) decrease(s) quality (therefore, the number of double should be small rather large,) (ii) that a double bond, if present, should be near end of the molecule, and (iii) no aromatic compounds should be present. The technical properties of biodiesel were shown in Table 1.

Table 1 Technical properties of biodiesel.

Properties	Specifications
Common name	Biodiesel (bio-diesel)
Common chemical name	Fatty acid (m)ethyl ester
Chemical formula range	C_{14}-C_{24} methyl esters or C_{15-25} H_{28-48} O_2
Kinematic viscosity range (mm^2/s, at 313K)	3.3-5.2
Density range (kg/m^3, at 288K)	860-894
Boiling point range (K)	>475
Flash point range (K)	430-445
Distillation range (K)	470-600
Vapor pressure (mm Hg, at 295K)	<5
Solubility in water	Insoluble in water
Physical appearance	Light to dark yellow, clear liquid
Odor	Light musty/soapy odor
Biodegradability	More biodegradability than petroleum diesel
Reactivity	Stable, but avoid strong oxidizing agents

Source: Demirbas (2008)

The term (monoglyceride or diglyceride) refers to the number of fatty acids that are attached to the glycerol backbone i.e. a diglyceride would have one hydroxyl group and two fatty acid groups attached to the glycerol backbone as in Figure 2 (Khan. 2002). Table 2 shows the structural formula for fatty acids, the degree of saturation is dictated by the number of double bonds in the fatty acids, e.g 18:1 denotes a carbon length of 18 with one double bond. At high temperatures, there can be some problems with polymerization of unsaturated fatty acids, this is where cross linking starts to occur between other molecules, causing very large agglomerations to be formed and consequently gumming occurs.



$$\begin{array}{l} CH_2-\overset{\overset{O}{\parallel}}{C}-OH \\ | \\ CH-COO-R \\ | \\ CH_2-COO-R \end{array}$$

Figure 2 Diglyceride.

Source: Khan (2002)

Table 3 presents the general ranges of major fatty acid (in wt.%) of some oils and fats used or tested as alternative diesel fuels. The fatty acid compositions of vegetable oil samples were shown in Table 4. In this table, there are the typical of fatty acid composition of vegetable oils. Table 5 and 6 showed the fuel-related properties and iodine values of various fats and oils and esters. From these tables, some of properties are strongly decreased especially viscosities and flash points while some are increased such as pour points.

Table 2 Structural formula for fatty acids used in biodiesel.

Fatty Acid Name	No. of Carbon & Double Bonds	Chemical Structure
Caprylic	C8	$CH_3(CH_2)_6COOH$
Capric	C10	$CH_3(CH_2)_8COOH$
Lauric	C12	$CH_3(CH_2)_{10}COOH$
Myristic	C14	$CH_3(CH_2)_{12}COOH$
Palmitic	C16:0	$CH_3(CH_2)_{14}COOH$
Palmitoleic	C16:1	$CH_3(CH_2)_5CH=CH(CH_2)_7COOH$
Stearic	C18:0	$CH_3(CH_2)_{16}COOH$
Oleic	C18:1	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$
Linoleic	C18:2	$CH_3(CH_2)_2CH=CHCH_2CH=CH(CH_2)_7COOH$
Linolenic	C18:3	$CH_3(CH_2)_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_7COOH$
Arachidic	C20:0	$CH_3(CH_2)_{18}COOH$
Eicosenoic	C20:1	$CH_3(CH_2)_7CH=CH(CH_2)_9COOH$
Behenic	C22:0	$CH_3(CH_2)_{20}COOH$
Eurcic	C22:1	$CH_3(CH_2)_7CH=CH(CH_2)_{11}COOH$

Source: Tyson (2001)

Table 3 General ranges of major fatty acid (in wt%) of some oils and fats used or tested as alternative diesel fuels.

Oil or Fat	Fatty acid composition (wt%)							
	12:0	14:0	16:0	18:0	18:1	18:2	18:3	22:1
Babassu	44-45	15-17	5.8-9	2.5-5.5	12-16	1.4-3		
Fat or Oil	12:0	14:0	16:0	18:0	18:1	18:2	18:3	22:1
Canola			4-5	1-2	55-63	20-31	9-10	1-2
Coconut	44-51	13-18.5	7.5-10.5	1-3	5-8.2	1.0-2.6		
Corn			7-13	2.5-3	30.5-43	39-52	1	
Cottonseed		0.8-1.5	22-24	2.6-5	19	50-52.5		
Linseed			6	3.2-4	13-37	5-23	26-60	
Olive		1.3	7-18.3	1.4-3.3	55.5-84.5	4-19		
Palm		0.6-2.4	32-46.3	4-6.3	37-53	6-12		
Peanut		0.5	6-12.5	2.5-6	37-61	13-41		1
Rapeseed		1.5	1-4.7	1-3.5	13-38	9.5-22	1-10	40-64
Safflower			6.4-7	2.4-29	9.7-13.8	75.3-80.5		
Safflower, High-oleic			4-8	2.3-8	73.6-79	11-19		
Sesame			7.2-9.2	5.8-7.7	35-46	35-48		
Soybean			2.3-11	2.4-6	22-30.8	49-53	2-10.5	
Sunflower			3.5-6.5	1.3-5.6	14-43	44-68.7		
Tallow (beef)		3-6	25-37	14-29	26-50	1-2.5		

Source: Knothe *et.al* (1997)

Table 4 Fatty acid compositions of vegetable oil samples.

Sample	16:0	16:1	18:0	18:1	18:2	18:3	Others
Cottonseed	28.7	0	0.9	13.0	57.4	0	0
Poppyseed	12.6	0.1	4.0	22.3	60.2	0.5	0
Rapeseed	3.5	0	0.9	64.1	22.3	8.2	0
Safflowerseed	7.3	0	1.9	13.6	77.2	0	0
Sunflowerseed	6.4	0.1	2.9	17.7	72.9	0	0
Sesameseed	13.1	0	3.9	52.8	30.2	0	0
Linseed	5.1	0.3	2.5	18.9	18.1	55.1	0
Wheat grain	20.6	1.0	1.1	16.6	56.0	2.9	1.8
Palm	42.6	0.3	4.4	40.5	10.1	0.2	1.1
Corn marrow	11.8	0	2.0	24.8	61.3	0	0.3
Castor	1.1	0	3.1	4.9	1.3	0	89.6
Tallow	23.3	0.1	19.3	42.4	2.9	0.9	2.9
Soybean	13.9	0.3	2.1	23.2	56.2	4.3	0
Bay laurel leaf	25.9	0.3	3.1	10.8	11.3	17.6	31.0
Peanut kernel	11.4	0	2.4	48.3	32.0	0.9	4.0
Hazelnut kernel	4.9	0.2	2.6	83.6	8.5	0.2	0
Walnut kernel	7.2	0.2	1.9	18.5	56.0	16.2	0
Almond kernel	6.5	0.5	1.4	70.7	20.0	0	0.9
Olive kernel	5.0	0.3	1.6	74.7	17.6	0	0.8
Coconut	9.7	0.1	3.0	6.9	2.2	0	65.7

Source: Demirbas (2002)

Table 5 Fuel-related properties and iodine values of various fats and oils.

Oil or Fat	Iodine value	CN[a]	HC[b] (kj/kg)	Viscosity (mm^2/s)	CP[c] (˚C)	PP[d] (˚C)	FP[e] (˚C)
Babassu	10-18	38					
Castor	82-88		39500	297(38˚C)		-31.7	260
Coconut	6-12						
Corn	103-140	37.6	39500	34.9(38˚C)	-1.1	-40	277
Cottonseed	90-119	41.8	39468	33.5(38˚C)	1.7	-15	234
Crambe	93	44.6	40482	53.6(38˚C)	10.0	-12.2	274
Linseed	168-204	34.6	39307	27.2(38˚C)	1.7	-15	241
Olive	75-94						
Palm	35-61	42					
Peanut	80-106	41.8	39782	39.6(38˚C)	128	-6.7	271
Rapeseed	94-120	37.6	39709	37.0(38˚C)	-3.9	-31.7	246
Safflower	126-152	41.3	39519	31.3(38˚C)	18.3	-6.7	260
High-olive safflower	90-100	49.1	39516	41.2(38˚C)	-12.2	-20.6	293
Sesame	104-120	40.2	39349	35.5(38˚C)	-3.9	-9.4	260
Soybean	117-143	37.9	39623	32.6(38˚C)	-3.9	12.2	254
Sunflower	110-143	37.1	39575	37.1(38˚C)	7.2	-15	274
Tallow	35-48	-	40054	51.15(38˚C)	-		201
No.2DF		47	45343	2.7(38˚C)	-15	-33	52

(a) Cetane number; (b) Heat of combustion; (c) Cloud point; (d) Pour point; (e) Flash point

Source: Knothe *et.al* (1997)

Table 6 Fuel-related physical properties esters of fats and oils.

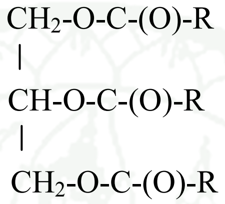
Ester	CN[a]	HC[b] (kj/kg)	Viscosity (mm/s)	CP[c] (˚C)	PP[d] (˚C)	FP[e] (˚C)
Methyl						
Cottonseed	51.2		6.8(˚21)		-4	110
Rapeseed	54.4	40449	6.7(˚40)	-2	-9	84
Safflower	49.8	40060			-6	180
Soybean	46.2	39800	4.08(˚40)	2	-1	171
Sunflower		39800	4.22(˚40)	0	-4	
Tallow		39949	4.11(˚40)	12	9	96
Ethyl	56.2					
Palm	48.2	39070	4.5(37.8˚)	8	6	19
Soybean		40000	4.41(˚40)	1	-4	174
Tallow				15	12	
Propyl						
Tallow				17	12	
Isopropyl						
Soybean	52.6			-9	-12	
Tallow				8	0	
n-Butyl						
Soybean	51.7	40700	5.24(˚40)	-3	-7	185
Tallow				13	9	
2-Butyl						
Soybean				-12	-15	
Tallow				9	0	

(a) Cetane number; (b) Heat of combustion; (c) Cloud point; (d)Pour point; (e) Flash point

Source: Knothe *et al.* (1997)

4.1 Triglyceride

Triglycerides are the tri-ester of glycerin with three equivalents of organic acid. Fatty acids are defined as those acids having alkyl of alkylene groups being C-5 and higher. All vegetable oils and animal fats consist primarily of triglyceride molecules shown in Figure 3 schematically below.



$$\begin{array}{l} CH_2\text{-}O\text{-}C\text{-}(O)\text{-}R \\ \ | \\ CH\text{-}O\text{-}C\text{-}(O)\text{-}R \\ \ | \\ CH_2\text{-}O\text{-}C\text{-}(O)\text{-}R \end{array}$$

Figure 3 Triglyceride.

Source: Khan (2002)

5. Feedstocks for Biodiesel

There are various feedstocks for biodiesel: almond, andirroba (*Carapa guianenis*), babassu (*Orbignia sp*), barley, camelina (*Camelina sativa*), coconut, copra, cumaru (*Dipteryx odorata*), *Cynara cardunculus*, fish oil, ground nut, *Jatropha curcas,* karanja (*Pongamia glabra*), laurel*, Lesquerella fendleri, Madjuca indica*, microalgae (*Chlorella vulgaris*), oat, piqui (*Caryocar sp*), poppy seed, rice, rubber seed, sesame, sorghum, tobacco seed, and wheat (Pinto *et al*., 2005).

Global vegetable oil production increased from 56 million tons in 1990 to 88 million tons on 2000, following a below-normal increase. The source of this gain was distributed among the various oils. Global consumption rose 56 million tons to 86 million tons, leaving world stocks comparatively tight. A variety of biolipids can be used to produce biodiesel. There are (a) virgin vegetable oil feedstocks; rape seed and soybean oils are most commonly used, though other crops such as mustard, palm oil, sunflower, bemp and even algae shows promise (b) waste vegetable (c) animal fats including tallow, lard and yellow grease; and (d) non-edible oil such as Jatropha, neem oil, caster oil tall oil, etc, (Demirsbas, 2008).

The main production cost for production of biodiesel is the price of raw material, which accounts for about 70% of total cost. Refine vegetable oil has a relatively high price, which keeps the price of biodiesel high (Shao *et al*., 2009). Many attempts have been made to produce biodiesel from non-edible plant oils such as tobacco (Veljkovic *et al*., 2006), rubber seed oil (Ramadhas *et al*., 2005), waste oils such as waste cooking oil (Leung and Guo, 2006) waste tallow (Bhatti *et al*., 2008) and animal fats (Canakci and Gerpen, 2001), palm fatty acid distillate (ChongKhong *et al*., 2007) as cheap feed stocks for biodiesel production. The amount of free fatty acid (FFA) in oil depends on the quality of feed stock. Generally, when the acid value is less than 2 mg KOH/g oil, corresponding to 1% FFA, one step transesterification can be applied. If the FFA content is higher, acid-catalyzed esterification, distillation, solvent extraction and membrane separation can be used for reducing FFA. For higher FFA raw materials, acid-catalyzed esterification is a typical method for the first step biodiesel production due to high rate of reaction. Then, it was followed by transesterification at the second step using alkaline catalyst to get high yield of biodiesel (Zhang and Jiang, 2008). So many researchers are looking for suitable raw material to produce biodiesel. The production of palm fatty acid distillate (PFAD) and palm stearin (PS) from industrial sources is growing in some regions of the world, as it has the potential to supplement biodiesel manufactured from more conventional sources. PFAD and PS are by product from palm oil refining process. The potential of PFAD and PS were used as a low cost feed stock in biodiesel production. The specifications of raw oil for biodiesel were shown in Table 7.

Table 7 Specifications of raw oil for biodiesel.

Raw oil	Item	Value
Raw vegetable oil	Free fatty acid (FFA, %)	<1%
	Water content % (moisture content)	<0.3%

Source: Demirbas (2002) and Markolwitz (2003)

5.1 High free fatty acid oil

In the case of using waste vegetable oil (yellow grease) as a feedstock, free fatty acids (FFA) may pose a problem. A free fatty acid is one that has already separated from the glycerol molecule. This is usually the result of the oil breaking down after many cycles of use. FFA creates four major problems.

(a) More catalyst will need to be used leading to higher cost

(b) Soap (fatty acid salt) is formed, making washing the finished product more difficult

(c) Water is formed which will retard the main reaction

(d) The FFA are not converted into fuel, reducing the yield

When the oil has less than 2.5% FFA, the problems listed previously are negligible using the single step (transesterification) only. Others have reported good results up to 4% FFA. (Gerpen *et al.*, 2004).

5.2 Palm oil

Palm oil is obtained from the mesocarp of the palm fruit while palm kernel oil is derived from the kernel found in the fruit. The oil found in the kernels is now known to be meant for the propagation of the species, but the biological purpose for the presence of the mesocarp oil is less certain. Due to this difference in functionality, the two kinds of oil are naturally endowed with different physical and chemical properties or characteristics. It is the chemical and physical properties of an oil that determine the types of application and processes to which it can be subjected to, either in its natural state or after processing and/or modifications. Hence, knowledge of the physical and chemical properties of both palm and palm kernel oil is the basis on which to build an understanding of the areas in which palm oil and palm kernel oil could be utilized (or could be potentially utilized).

Palm oil and palm kernel oil, like all oils and fats, are made up of mostly glyceride materials with some non-glyceride materials in small or trace quantities. It is this chemical composition that defines the chemical and physical characteristics of palm oil and palm kernel oil.

5.2.1 Origin and distribution

The oil palm, generic name *Elaeis guineensis Jacq* is indigenous to West Africa since about 5,000 years ago. It occurs wild and semi-wild groves along the African coast and is common throughout the Congo basin and extends as far east as Southern Sudan, Uganda and Tanganyika. It is also found in British Guiana., Brazil, Peru, Venezuela and West Indies. It is cultivated extensively in Malaysia and in Indonesia, particularly in Sumatra. The oil palm is today a prominent feature of the Malaysian landscape which was first introduced into Malaysia in 1870 as an ornamental plant.

5.2.2 Some botanical aspects

The palm grows to a height of 20-30 m, begins to bear when 4-6 years old and reaches full bearing at 12-15 years. The yield gradually declines after the age of 30 years and it has an economic life of about 35 years. Being a perennial (and a tree crop) its life span exceeds 100 years. However, as height becomes a limiting factor for economic harvests, palms are usually replaced after 25- 30 years under plantation conditions.

The fruit takes 6 months for ripening after pollination. The fruits are harvested when they are fully ripe and the oil content of the pericarp is at its maximum. The ripeness is indicated by a change in the color from red to orange. The oil palm tree is capable of bearing about 10-12 fresh fruit bunches (FFB) per year. The palm fruit is about the size of a small plam and grows in large bunches weighting 10-20 kg. The yield of FFB of mature palm is about 18-25 tonnes/ha/yr. The bunch can have up to 2,000 individual fruits. Each fruit consists of a hard kernel (seed) inside a shell (endocarp) which is surrounded by a fleshy mesocarp. The palm fruits were shown in Figure 4. The FFB are processed into palm oil and palm kernel in the palm oil mill.

Figure 4 Palm fruits.

Source: Kywe (2002)

5.2.3 Chemical properties of palm oil (triglyceride and fatty acid composition)

Triglycerides form the major component and bulk of the glyceride material present in palm oil and palm kernel oil with small amounts of monoglycerides and diglycerides which are mainly artifacts of the extraction process.

Triglycerides are esters formed glycerol and fatty acids with the hydrogen in all hydroxyl (carboxylic) end as shown in Figure 5.

(a) (b)

Figure 5 (a) Glycerol molecule (b) Triglyceride molecule.

Source: Kywe (2002)

R_1, R_2 and R_3 are hydrocarbon chains. These could vary in the number of carbons present in the chain (chain length) and in structure (presence of double bonds i.e. unsaturation). It is the variations in R_1, R_2 and R_3 that largely defines the chemical and physical properties of oils and fats.

5.2.4 Palm fatty acid distillate (PFAD)

During the refining of palm oil, a lower-value by-product known as palm fatty acid distillate (PFAD) is generated in the fatty acid stripping and deodorization stages. PFAD is potential a low cost valuable feedstock for the production of biodiesel. It also makes the much-debated ‘food vs fuel’ argument a non-issue as PFAD is generally sold as a source of industrial fatty acids for non-food applications. It has also been used as a fuel in power plants and industrial boilers. PFAD is always traded at a discount to crude or refined, bleached and deodorized (RBD) palm oil. PFAD contains 72.7-92.6% FFA with a small amount of unsaponifiable component (1-2.5%) and remainder neutral oil. Modern palm oil refineries consistently produce PFAD with FFA content higher than 88% and crude palm oil also contains non-glyceride minor components that have been associated with health benefits, some of which are distilled together with the FFA as in saponifiable components (Cheah *et al.*, 2010).

PFAD specification:

1. Free fatty acids (as palmitic) 70% minimum
2. Moisture & impurities 1.0% maximum
3. Saponifiable matter 95% minimum (basis 97%)

5.2.5 Palm stearin

Palm stearin is containing the more solid fraction obtained by fractionation of palm oil after crystallization at controlled temperatures. It is thus a co-product of palm olein. It is always traded at a discount to palm oil and palm olein; making it a cost effective ingredient in several applications. The physical characteristics of palm stearin differ significantly from those of palm oil and it is available in a wider range of melting points and iodine values. Palm stearin is a very useful source of fully natural hard fat component for products such as shortening and pastry and bakery margarines. In addition to palm olein and stearin, there are a dozen of other fractions, obtained from palm oil including various grades of double fractionated palm olein (aka superolein) and palm mid fractions. Where pourability and clarity can be issued for palm olein, especially in temperate countries, superolein finds uses as frying oil and cooking oil, usually in blends with seed oils. Palm mid fraction is commonly used as a highly versatile natural ingredient in the manufacture of tub margarine (Shen *et al.,* 1990). The fatty acid composition of PFAD and PS were shown in Table 8.

Palm stearin specification:

1. Free fatty acid (as palmitic) 0.2% maximum
2. Moisture & impurities iodine value 0.15% maximum

(3) Melting point° C (AOCS Cc3-25) 48 maximum- 44 minimum

(4) Colour (5¼" Lovibond Scale) 3 Red maximum

Table 8 Fatty acid composition of palm fatty acid distillate (PFAD) and palm stearin (PS).

	PFAD	palm stearin (PS)
Fatty acid	wt%*	wt%**
Palmitic	45.6	58.1
Oleic	33.3	28.6
Linoleic	7.7	6.3
Stearic	3.8	4.8
Myristic	1.0	1.3
Tetracosenoic	0.6	-
Linolenic	0.3	0.5
Ecosanoic	0.3	-
Ecosenoic	0.2	0.2
Palmitoleic	0.2	-
Mono, Di, Tri glycerides	<7%	<9%
Imputities	Traces	-

Source: ChongKhong *et al.* (2007)*, Shen *et al.* (1990)**

6. General Aspects of Transesterification

Transesterification is the general term used to describe the important class of organic reactions where an ester is transformed into another through interchange of the alkoxy moiety. When the original ester is reacted with an alcohol, the transesterification process is called alcoholysis as shown in Figure 6. In this review, the term transesterification will be used as synonymous for alcoholysis of carboxylic esters, in agreement with most publications in this field. The transesterification is an equilibrium reaction and the transformation occurs essentially by mixing the reactants. However, the presence of a catalyst (typically a strong acid or base) accelerates considerably the adjustment of the equilibrium. In order to achieve a high yield of the ester, the alcohol has to be used in excess (Schuchardt and Serchelt, 1997). KOH and methanol were selected due to economic reasons in transesterification (Dorado, *et al*., 2004a; Dorado, *et al*., 2004b).

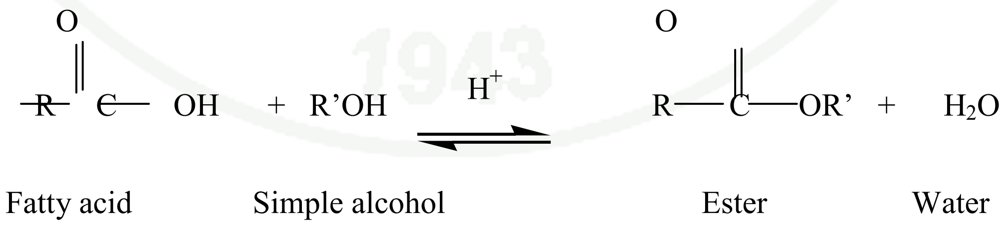
$$\mathrm{RCOOR' + R''OH \rightleftharpoons RCOOR'' + R'OH}$$

Figure 6 General equation for a transesterification reaction.

Source: Schuchard *et al*. (1998)

6.1 Esterification

The formation of esters occurs through a condensation reaction known as esterification. This requires two reactants, carboxylic acids (fatty acids) and alcohols. Esterification reactions are acid catalyzed and preceded slowly in the absence of strong acids such as sulphuric acid, phosphoric acid, organic sulfuric acids and hydrochloric acid (Schuchardt *et al*., 1998). The equation for an esterification reaction can be seen in Figure 7.



$$\mathrm{R-\overset{\overset{O}{\|}}{C}-OH + R'OH \xrightleftharpoons{H^+} R-\overset{\overset{O}{\|}}{C}-OR' + H_2O}$$

Figure 7 Esterification reaction with FFA.

Source: Khan (2002)

6.2 Transesterification of Vegetable Oils

Vegetable oils can be transesterified by heating them with a large excess of anhydrous methanol and a catalyst. The transesterification reaction can be catalyzed by alkalis (Gryglewicz, 1999; Zhang *et al*., 2003a), acids (Furuta *et al*., 2004), or enzymes (Shieh *et al*., 2003; Hama *et al*., 2004; Oda *et al*., 2004; Du *et al*., 2004; Noureddini *et al*., 2005). Various studies have been carried out using different oils as raw material, different alcohols (methanol, ethanol, butanol), as well as different catalysts, including homogenous ones such as sodium hydroxide, potassium hydroxide, sulfuric acid and supercritical fluids and heterogeneous ones such as lipases (Marchetti *et al*., 2007).

In the transesterification of vegetable oils, a triglyceride reacts with an alcohol in the presence of a strong acid or base, producing a mixture of fatty acid alkyl esters and glycerol as shown in Figure 8. The overall process is a sequence of three consecutive and reversible reactions, in which di- and monoglycerides are formed as intermediates. The stoichiometric reaction requires 1 mol of a triglyceride and 3 mol of the alcohol. However, an excess of the alcohol is used to increase the yields of the alkyl esters and to allow its phase separation from the glycerol formed.

Several aspects, including the type of catalyst (alkaline or acid), alcohol/vegetable oil molar ratio, temperature, purity of the reactants (mainly water content) and free fatty acid content have an influence on the course of the transesterification and will be discussed below, based on the type of catalyst used. (Schuchardt *et al*., 1998).

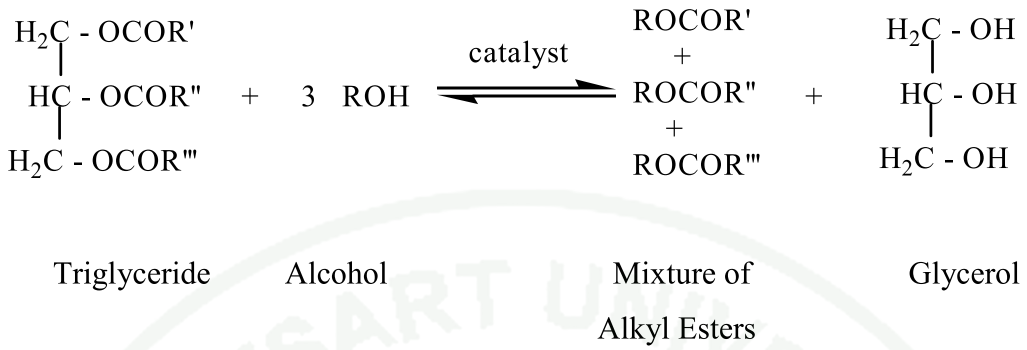


Figure 8 Transesterification of vegetable oils.

Source : Khan (2002)

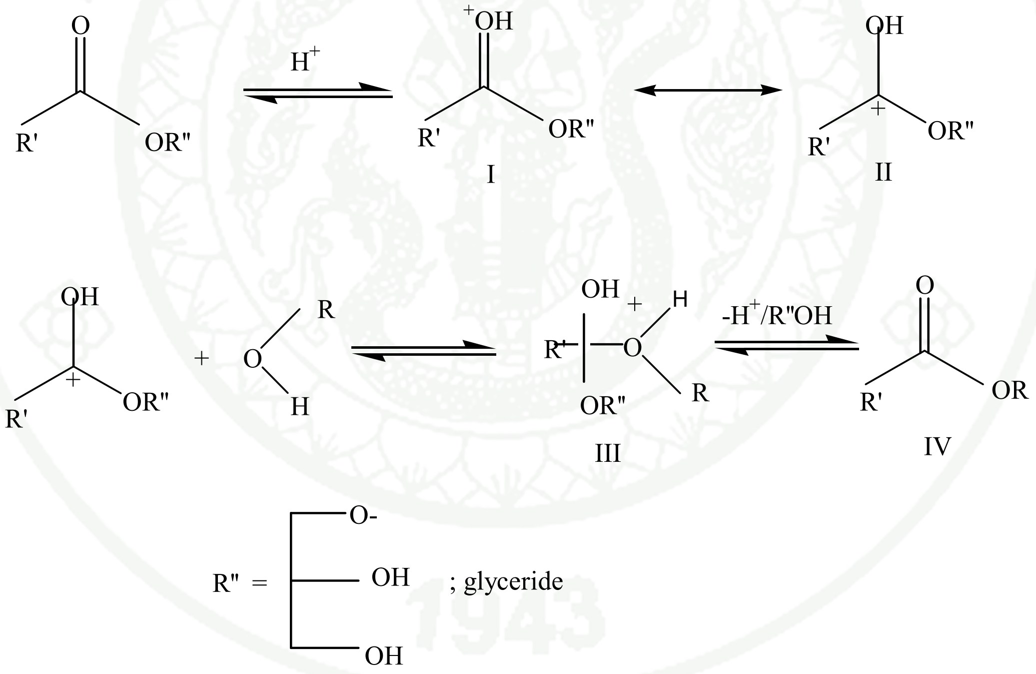
6.3 Acid-catalyzed Processes

The transesterification process is catalyzed by BrØnsted acids, preferably by sulfonic and sulfuric acids (Feedman *et al.*, 1986; Harrington and Evans, 1985). These catalysts give very high yields in alkyl esters, but the reactions are slow, requiring, typically, temperatures above 100 °C and more than 3 hours to reach the complete conversion (Freedman *et al.*, 1984). Pryde (1984) showed that the methanolysis of soyabean oil, in the presence of 1 mol% of H_2SO_4, with an alcohol/oil molar ratio of 30:1 at 65 °C, takes 50 hours to reach complete conversion of the vegetable oil (99%), while the butanolysis at 117 °C and ethanolysis at 78 °C, using the same quantities of catalyst and alcohol, take 3 and 8 hours, respectively.

The alcohol/ vegetable oil molar ratio is one of the main factors that influence the transesterification, an excess of the alcohol favours the formation of the products. On the other hand, an excessive amount of alcohol makes the recovery of the glycerol difficult, so that the ideal alcohol/oil ratio has to be established empirically, considering each individual process (Schuchardt *et al.*, 1998).

The mechanism of the acid-catalyzed transesterification of vegetable oils is shown in Figure 9, for a monoglyceride. However, it can be extended to di- and triglycerides. The protonation of the carbonyl group of the ester leads to the carbonation II which, after a nucleophilic attack of the alcohol, produces the tetrahedral intermediate III, which eliminates glycerol to form the new ester IV, and to regenerate the catalyst H^+.

According to this mechanism, carboxylic acids can be formed by reaction of the carbonation II with water present in the reaction mixture. This suggests that an acid-catalyzed transesterification should be carried out in the absence of water, in order to avoid the competitive formation of carboxylic acids which reduce the yields of alkyl esters (Schuchardt *et al.*, 1998



R' = carbon chain of the fatty acid, R = alkyl group of the alcohol

Figure 9 Mechanism of the acid-catalyzed transesterification of vegetable oils.

Source: Schuchardt *et al.* (1998)

6.4 Base-catalyzed Processes

The base-catalyzed transesterification of vegetable oils proceeds faster than the acid-catalyzed reaction (Freedman *et al*., 1986; Freedman *et al*., 1984). Due to this reason, together with the fact that the alkaline catalysts are less corrosive than acidic compounds, industrial processes usually favor base catalysts, such as alkaline metal alkoxides (Freedman *et al*., 1986; Harrington and Evans, 1985; Freedman *et al.,* 1984; Schwab *et al*., 1987) and hydroxides (Aksoy *et al*., 2003) as well as sodium or potassium carbonates (Bajwa and Bains, 1987).

The mechanism of the base-catalyzed transesterification of vegetable oils is shown in Figure 10. The first step equation 1 is the reaction of the base with the alcohol, producing an alkoxide and the protonated catalyst. The nucleophilic attack of the alkoxide at the carbonyl group of the triglyceride generates a tetrahedral intermediate equation 2, from which the alkyl ester and the corresponding anion of the diglyceride are formed equation 3. The latter deprotonates the catalyst, thus regenerating the active species equation 4, which is now able to react with a second molecule of the alcohol, starting another catalytic cycle. Diglycerides and monoglycerides are converted by the same mechanism to mixture of alkyl esters and glycerol (Schuchardt *et al*., 1998).

Alkaline metal alkoxides (as CH_3ONa for the methanolysis) are the most active catalysts, since they give very high yields (>98 %) in short reaction times (30 min) even if they are applied at low molar concentrations (0.5 mol%). However, they require the absence of water which makes them inappropriate for typical industrial processes (Freedman *et al.,* 1984). Alkaline metal hydroxides (KOH and NaOH) are cheaper than metal alkoxides, but less active. Nevertheless, they are a good alternative since they can give the same high conversions of vegetable oils just by increasing the catalyst concentration to 1 or 2 mol%. However, even if a water-free alcohol/ oil mixture is used, some water is produced in the system by the reaction of the hydroxide with the alcohol. The produced fatty acid is the system by the reaction of the hydroxide with the alcohol. The presence of water gives rise to hydrolysis of some of the produced ester, with consequent soap formation as shown in Figure 11. This undesirable saponification reaction reduces the ester yields and is considerably difficult the recovery of the glycerol due to the formation of emulsions (Freedman *et al.*, 1984).

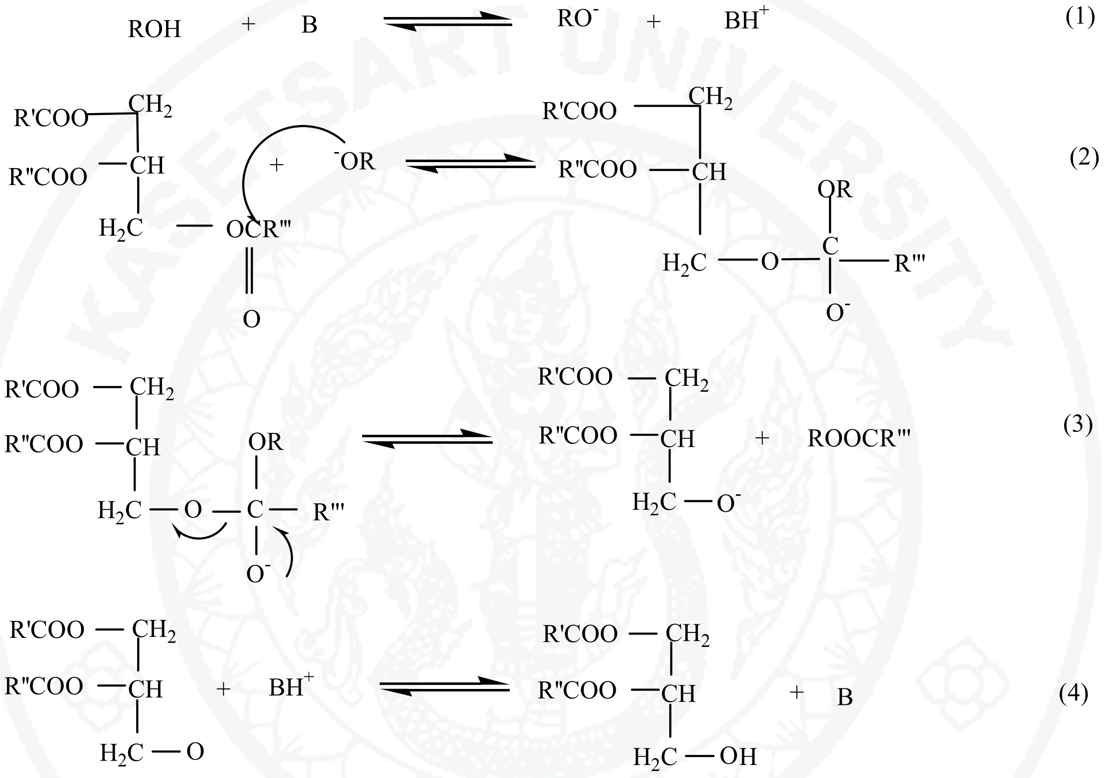
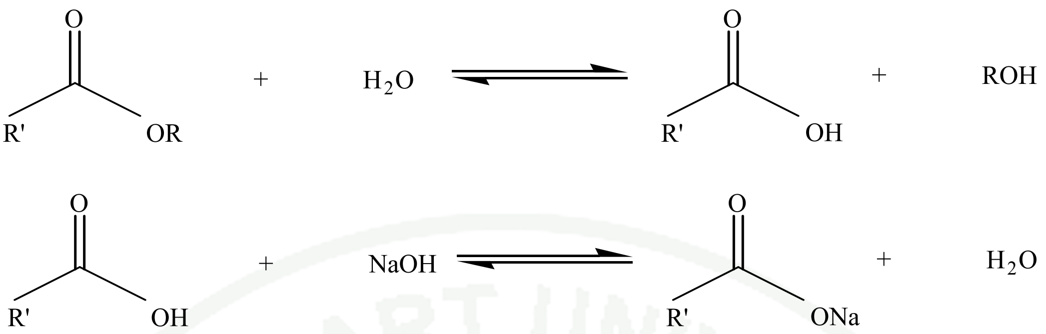


Figure 10 Mechanism of the base-catalysed transesterification of vegetable oils.

Source: Schuchardt *et al.* (1998)

Potassium carbonate, used in a concentration of 2 or 3 mol% gives high yields of fatty acid alkyl esters and reduces the soap formation. This can be explained by the formation of bicarbonate instead of water as shown in Figure 11, which does not hydrolyze the esters (Schuchardt *et al.*, 1998).



R' = carbon chain of the fatty acid; R = alkyl group of the alcohol

Figure 11 Saponification reaction of the produced fatty acid esters.

Source: Schuchardt *et al*. (1998)

6.5 Lipase-catalysed Processes

Due to their ready availability and the ease with which they can be handled, hydrolytic enzymes have been widely applied in organic synthesis. They do not require any coenzymes, are reasonably stable, and often tolerate organic solvents. Their potential for regioselective and especially for enantioselective synthesis makes them valuable tools.

Although the enzyme-catalyzed transesterification processes are not yet commercially developed, new results have been reported in recent article (McNeill *et al.*, 1991). The common aspects of these studies consist in optimizing the reaction conditions (solvent, temperature, pH, type of microorganism which generates the enzyme, etc) in order to establish suitable characteristics for an industrial application. However, the reaction yields as well as the reaction times are still unfavourable compared to the base-catalyzed reaction systems (Schuchardt *et al.*, 1998).

7. Factor Effecting in Esterification and Transesterification Reaction

7.1 Effect of alcohol to FFA or triglyceride molar ratio

The molar ratio of alcohol to vegetable oil is one of the important factors that affect the conversion efficiency as well as production cost of biodiesel. The conversion efficiency is defined as the yield of the process represented in terms of percentage. Molar ratio is the ratio of number of mole alcohol to number of moles of glycerides or free fatty acid in the oil. Theoretically, esterification reaction requires one mole of alcohol for each mole of FFA while transesterification needs three moles for each mole of triglyceride. However, in practice, the molar ratio should be used higher than that of stoichiometric ratio in order to drive the reaction towards completions.

7.2 Effect of catalyst amount

The amount of catalyst used in the process also affects the conversion efficiency of process. The catalyst is usually used to speed up the reaction. The acid catalyst is used in esterification while the base catalyst is in transesterification.

7.3 Effect of reaction temperature

At room temperature, the conversion efficiency is noted to be very low (about 10% only) even after 2 h of stirring in esterification (Ramadhas *et al*., 2005). With increase in temperature, the conversion takes place at a faster rate. However, at higher reaction temperature, there is a chance to loss the alcohol and also increase the production cost of biodiesel. Therefore, the reaction temperature at atmospheric pressure should be chosen lower than boiling point of alcohol. In this study, the methanol was used in both esterification and transesterification. As boiling point of methanol is about 65ºC, the reaction temperature was chosen at 60 ºC. Zullaikah *et al*., 2005; Veljkovic *et al*., 2006; Zhang and Jiang., 2008; Jarsri *et al*., 2007; Lin *et al*., 2009 also used reaction temperature 60 ºC in their esterification and transesterification.

7.4 Effect of reaction time

The longer the reaction time, the higher conversion of both esterification and transesterification can give. The reaction time for esterification depends on the initial FFA amount of its raw material. Therefore, if the high FFA was used, the long reaction time would be needed to complete reaction. Hancsok *et al*. (2004) used the reaction temperature up to 6h for above 40% initial FFA raw material. Houfang *et al*. (2009) also used up to 2 h for initial 7% FFA raw material to reduce final 2% FFA.

7.5 Effect of stirring rate

The reaction mixture formed two immiscible layers of oil and methanol. Thorough mixing of two layers was necessary for the reaction to proceed. For both esterification and transesterification, the stirring rate was used about 400-600 rpm (Velijkovic *et al*., 2006; Zhang *et al*., 2008; El-Mashad *et al*., 2008; Shiu *et al*., 2010). Therefore, in this study, the stirring rate was fixed at 500 rpm to all experiments in both reactions.

8. Statistical Design of Experiment

Design of experiment (DOE) is the process of planning the experiment so that appropriate data will be collected and analyzed by statistical methods, resulting in valid and objective conclusions. The statistical approach to experimental design is necessary if a meaningful conclusion from the data is to be drawn. When the problem involves data that are subject to be experimental errors, statistical methods are the only objective approach to analysis. Thus, there are two aspects to any experimental problem: the design of experiment and statistical analysis of data or analysis of variance (ANOVA). These two subjects are closely related because the method of analysis depends directly in the design employed. The three basic principles of experimental design are randomization, replication and blocking.

Randomization is the cornerstone underlying the use of statistical methods in experimental design. By randomization, both the allocation of the experimental material and the order in which the individual runs or trials of the experiment are to be performed are randomly determined. Statistical method requires that the observations (or errors) are independently distributed random variables.

Replication is an independent repeat of each factor combination. Replication has two important properties. First, it allows the experimenter to obtain an estimate of the experimental error. The estimate of error becomes a basic unit of measurement for determining whether observed differences in the data are really statistically different. Second, if the sample mean ($\bar{y}$) is used to estimate the true mean response for one of the factor levels in the experiment, replication permits the experiments to obtain a more precise estimate of the parameter.

Blocking is a design technique used to improve the precision with which comparisons among the factors of interest are made. Often blocking is used to reduce or eliminate the variability transmitted from nuisance factors- that is, factors that may influence the experimental response.

The above three basic principle experimental design, randomization, replication and blocking are part of every experiment (Myers *et al.*, 2009).

8.1 Using statistical technique in experimentation

Much of the research in engineering, science and industry is empirical and makes extensive use in experimentation. Statistical methods can greatly increase the efficiency of these experiments and often strengthen the conclusions so obtained. The experimenters have to know the proper use of following statistical techniques in experimentation.

(1) Use nonstatistical knowledge of the problem

Experimenters are usually highly knowledgeable in their fields. In some fields, there is a large body of physical theory on which to draw in explaining relationships between factors and responses. This type of nonstatistical knowledge is invaluable in choosing factors, determining factor levels, deciding how many replicates to run, interpreting the results of the analysis, and so forth. Using a designed experiment is no substitute for thinking about the problem.

(2) Keep the design and analysis as simple as possible

Simple design and analysis methods are almost always best.

(3) Recognize the difference between practical and statistical significance.

There is no assurance that this difference is large enough to have any practical value because two experimental conditions produce mean response that are statistically different.

(4) Experiments are usually iterative

In most situations, it is unwise to design too comprehensive experiment at the start of a study. Successful design requires knowledge of important factors, the ranges over which these factors are varied, the appropriate number of levels for each factors, and the proper methods and units of measurement for each factor and response (Montgomery, 2009).

8.2 Traditional one-variable-at-a-time method

One-variable-at-a-time method is the traditional one to approach the optimization problems (Nikerel *et al*., 2006). In this process, at each condition one parameter is varied while others are kept constant. Using this optimization, the optimization of the second is found. This process works if, and only if, there is no interaction between variables. In the case shown in Figure 12, the optimum found using the one-variable-at-a-time approach is 85%, far from real optimum of 90% (Vogel, 1997). The one-variable-at-a-time approach failed to the true optimum because of the interaction between the two nutrients. In order to find the optimum conditions, it would have been necessary to repeat the one-variable-at-a-time process at each step to verify that the true optimum was reached. This requires numerous sequential experimental runs, a time-consuming and ineffective strategy, especially when many variables need to be optimized. Because of the complexity of chemical reaction, interaction between the variables is inevitable. Therefore, since it is both time-consuming and inefficient, the one-variable-at-a-time approach is not satisfactory (Stanbury *et al.*, 1999). Therefore, since it is both time-consuming and inefficient, the one-variable-at-a-time approach is not satisfactory for esterification and transesterification. Fortunately, there are numerous statistical methods which will find the optimum quickly and efficiently.

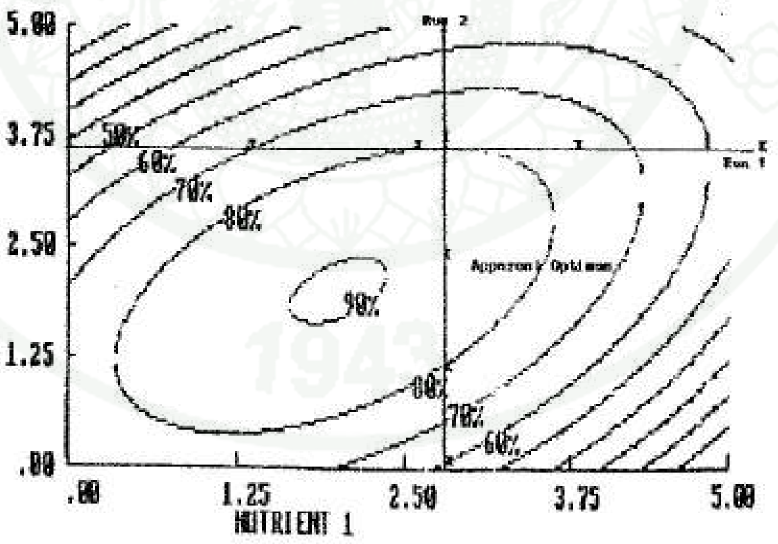


Figure 12 Sample of one-variable-at-a-time approach (contour plot of yield).

Source: Vogel (1997)

9. Response Surface Methodology (RSM)

RSM is a collection of mathematical and statistical techniques that are useful for modeling and analysis in applications where a response of interest is influenced by several variables and the objective is to optimize this response. For example; chemical engineers wish to find the level of temperature (x_1) and feed concentration (x_2) that maximize the yield (y) of a process. The process yield is a function of the levels of temperature and feed concentration as shown in equation 1;

$$Y = f(x_1, x_2) + \epsilon \tag{1}$$

Where ϵ represents the noise or error observed in the response Y. The expected response by $E(Y) = f(x_1,x_2) = \eta$, then the surface represented by $f(x_1,x_2) = \eta$ is called a response surface.

The form of the relationship between the response and the independent variables is unknown in most RSM problems. Thus, the first step in RSM is to find a suitable approximation for the true relationship between Y and the independent variables. If the response is well modeled by a linear function of the independent variables, the approximating function is the first-order model in equation 2;

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_k x_k + \epsilon \tag{2}$$

If there is curvature in the system, then a polynomial of height degree must be used, such as the second-order mode in equation 3.

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

The eventual objective of RSM is to determine the optimum operating conditions for the system or to determine a region of the factor space in which operation specifications are satisfied. The word ‘optimum’ in RSM is used in a special sense. The ‘hill climbing’ procedures of RSM guarantee convergence to a local optimum only (Myers *et al*., 2009).

RSM allows a more comprehensive analysis on the interactions between experimental variable than a single-factor experimental design. Consequently, this leads to a better understanding and knowledge of the process and subsequently maximizes the yield of biodiesel. Apart from, it also reduces the number of experimental runs required to generate statistically-validated results (Box and Hunter, 1976; Montgomery, 2001). RSM has been successfully applied in optimizing numerous biodiesel processing methods which include enzymatic canalization of tallow, kernel oil, homogeneous reaction involving animal fats and ultrasound-assisted methanolysis of soybean oil (Gao *et al*., 2009; Ghadge and Raheman, 2006; Jeong *et al*., 2009; Santos *et al*., 2009; Shieh *et al*., 2003).

9.1 Advantages and disadvantages of RSM

The response surface methodology approach has many advantages and disadvantages over optimization procedures. These are listed in Table 9.

Table 9 Advantages and disadvantages of RSM.

Advantages of RSM	Disadvantages of RSM
1. Greatest amount of information from experiment	1. Tells what happens, not why.
2. Forces you to plan	2. Notoriously poor for prediction outside the range of study.
3. Know how long project will take	
4. Gives information about the interaction between variables	
5. Multiple responses at the same time	
6. Give information necessary for design and optimization of a process	

Source: McNeil and Harvey (2008); Vogel (1997)

9.2 Important factors and effects on RSM

The use of RSM requires that certain criteria must be met (Lazic, 2004; McNeil and Harvey, 2008; Vogel, 1997). These are as following;

(a) The factors which are critical for the process are known

RSM programs are limited in the number of variables that they are designed to handle. If the number of variables increases, the number of experimental runs required by the designs increases exponentially. Therefore, the number of variables should be limited in RSM. Fortunately, the numbers of variables in esterification and transesterification to be optimized are limited. Some important variables in these reactions are listed as the following:

(i) Molar ratio of alcohol to free fatty acid / triglyceride
(ii) Catalyst loading
(iii) Reaction time
(iv) Reaction Temperature
(v) Stirrer rate

(b) The factors must vary continuously over the experimental range tested

For example, the variable of molar ratio of alcohol, catalyst loading and reaction time etc., are varied and can be used in RSM model.

(c) There exists a mathematical function which relates the response to the factors

The time-consuming and difficulties nature of the calculations have inhibited the wide spread use of RSM. However, now a day, numerous computer programs are available to solve these difficulties such as SAS^{TM}, SPSS^{TM}, E-Chip, X-STAT^{TM}, MINITAB^{TM} etc. (Lazic, 2004; McNeil and Harvey, 2008; Vogel, 1997; Ryan *et al.*, 2003). However, the users have to understand about the process or the meaning of the results (outputs from software).

10. Box-Behnken Design

In statistics, Box-Behnken design is experimental design for RSM to achieve the following goals:

(1) Each factor, or independent variable, is placed at one of three equally spaced values. (At least three levels are needed for the following goal).

(2) The design should be sufficient to fit a quadratic model, that is, on containing squared terms and products of two factors.

(3) The ratio of the number of experimental points to the number coefficients in the quadratic model should be reasonable.

(4) The estimation variance should more or less depend only on the istance from the centre (this is achieved exactly for the design with 4 and 7 factors), and should not vary too much inside the smallest (hyper) cube containing the experimental points (Box and Behnken, 1960).

Box and Behnken (1960) developed a family of efficient three-level design for fitting second-order response surfaces. The methodology for design construction is interesting and quite creative. The class of designs is based in the construction of balanced incomplete block designs.

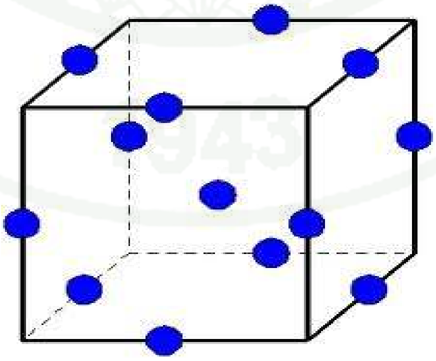


Figure 13 Displays the Box-Behnken design for number of variables (k=3).

Source: Box and Behnken (1960)

For example, a balance incomplete block design with three treatments and three blocks are given in Table 10.

Table 10 A balanced incomplete design.

	Treatment		
	1	2	3
Block 1	X	X	
Block 2	X		X
Block 3		X	X

Source: Box and Behnken (1960)

The Box-Behnken design is quite comparable in number of design points to central composite design (CCD) for k=3 and k=4. There is no Box-Behnken design for k=2. For k=3, the CCD contains 14+n_c runs while the Box-Behnken design contains 12+ n_c runs. For k=4, the CCD and Box-Behnken design both contain 24+ n_c design points (where n_c is center runs).

10.1 Characteristics of the Box-Behnken design

The Box-Behnken design is an efficient option and indeed an important alternative to the CCD. Box-Behnken design does not substantially deviate form rotatability, and in fact, for k=4 and k=7 is exactly rotatable. Another important characteristics of Box-Behnken is that it is a spherical design.

The spherical nature of Box-Behnken, combined with the fact that the designs are rotatable or near-rotatable, suggests that ample center runs should be used. In fact, for k=4 and 7, center runs are necessary to avoid singularity. The use of three to five center runs is recommended for the Box-Behnken (Myers *et al*., 2009).

10.2 Comparison between Box-Behnken design and other designs

A comparison between the Box-Behnken design and other response surface design (central composite, Doehlert matrix and three level full factorial design) has demonstrated that the Box-Behnken design and Doehlert matrix are slightly more efficient that the central composite design but much efficient than the three-level full factorial design where the efficiency of one experimental design is defined as the number of coefficients in the estimated model divided by the number of experiments. Table 11 established a comparison among the efficiencies of Box-Behnken design and other response surface designs for the quadratic model. This Table demonstrates also that the three-level full factorial designs are costly when the factor number is higher than 2. Another advantage of the Box-Behnken design is that its does not contain combinations for which all factors are simultaneously at their highest or lowest levels. So these designs are useful in avoiding experiments performed under extreme conditions, for which unsatisfactory results might occur (Ferreira *et al*., 2007). Not only the efficiency, but also the experimental number is less in Box-Behnken design.

Table 11 Comparison of efficiency of central composite design(CCD), Box-Behnken design (BBD) and Doehlert design (DM).

Factors (*k*)	Number of coefficient (*p*)	Number of experiment (*f*)			Efficiency (*p/f*)		
		CCD	DM	BBD	CCD	DM	BBD
2	6	9	7	-	0.67	0.86	-
3	10	15	13	13	0.67	0.77	0.77
4	15	25	21	25	0.60	0.71	0.60
5	21	43	31	41	0.49	0.68	0.61
6	28	77	43	61	0.36	0.65	0.46
7	36	143	57	85	0.25	0.63	0.42
8	45	273	73	113	0.16	0.62	0.40

Source: Ferreira *et al*. (2007)

11. Analysis of Variance (ANOVA)

The more reliable way to evaluate the quality of the model fitted is by application of analysis of variance (ANOVA). ANOVA is the test for significance of regression. The central idea of ANOVA is to compare the variation due to the treatment (change in the combination of variables levels) with the variables due to random errors inherent to the measurements of the generated responses. From this comparison, it is possible to evaluate the significance of the regression used to foresee responses considering the sources of experimental variance.

In ANOVA, the evaluation of data set variation is made by studying its dispersion. The evaluation of the divation (d_i) that each observation (y_i) or its replicates (y_{ij}) present in relation to the ($\bar{y}$) or more precisely, the square of this deviation is presented in equation (4):

$$d_i^2 = (y_{ij} - \bar{y})^2 \tag{4}$$

The sum of the square for all observation divations in relation to the mean is called the total sum of square (SS_T); it can be dismembered in the sum of square due to the fitted mathematical model, that is, due to regression (SS_R), and in the sum of square due residuals generated by the model (SS_E), as shown in below in equation 5:

$$SS_T = SS_R + SS_E \tag{5}$$

As replicates of the central point are made, it is possible to estimate the pure error associated with repetitions. Thus, the sum of the square for residuals can be dismembered into two more parcels: the sum of square doe to pure error (SS_{pe}) and sum of square due to the lack of fit (SS_{lof}), as shown in equation 6:

$$SS_E = SS_{pe}+SS_{lof} \tag{6}$$

When the division of the sum of square for each source of variation (total, regression, residual, lack of fit, and pure error) is made by its respective numbers of degree of freedom (df), the mean of the square (MS) are obtained. The numbers of degrees of freedom for these sources of variation are calculated by expressions presented in the third column of Table 12, where ‘p’ represents the numbers of coefficients of the mathematical model, ‘n’ represents the number of total observations, and m represents numbers of levels used in the investigation. Equations related to the source of variations for the calculation of SSs and MSs are also presented in Table 12.

The significance of regression can be evaluated by the ratio between the mean of the square of the regression (MS_R) and mean of square of residuals (MS_E) and by comparing these variation sources using the Fisher distribution (F test), taking into account its respective degrees of freedom associated to regression (v_R) and to residual (v_E) variances shown in equation 7:

$$F(v_R, v_E) = \frac{MS_R}{MS_E} \quad (7)$$

Thus, a statistically significant value for this ratio must higher than the tabulated value for F. this is an indication that the mathematical model is well fitted to the experimental data (Bezerra *et al*., 2008).

The coefficient of multiple determinations R^2 is defined as:

$$R^2 = \frac{SS_R}{SS_T} = 1 - \frac{SS_E}{SS_T} \quad (8)$$

R^2 is a measure of the amount of reduction in the variability of y obtained by using the regressor variables x_1, x_2, …, x_k in the model. From inspection of the analysis of the variance identify equation (8) that is $0 \leq R^2 \leq$. However, a large value of R^2 does not necessarily imply that the regression model is good one. Adding a variable to the model will always increase R^2, regardless of whether the additional variable is statistically significant or not. Thus, it is possible for models that have large values of R^2 to yield poor predictions of new observations or estimates of the mean response (Myers *et al*., 2008). Because R^2 always increases as the terms are added to the model, some regression model builders prefer to use an adjusted R^2 statistic defined as equation (9).

$$R^2_{adj} = 1 - \frac{SS_E/(n-p)}{SS_T/(n-1)} = 1 - \frac{n-1}{n-p}(1-R^2) \quad (9)$$

In general, the adjusted R^2 statistics will not always increase as variables are added to the model. ANOVA was shown in Table 11. In fact, if necessary terms are added, the value of R^2_{adj} will often decrease (Montgomery and Runger, 2011; Myers *et al.* 2008).

Table 12 Analysis of variance (ANOVA) for significance of regression in multiple regressions.

Source of variation	Sum of square	Degree of freedom	Mean square	$F(v_R, v_E)$
Regression	$SS_R=\sum_i^m \sum_j^{n_1}(\hat{y}_i - \bar{y})^2$	p-n	$MS_R=\frac{SS_R}{p-1}$	MS_R/MS_E
Residual	$SS_E=\sum_i^m \sum_j^{n_1}(y_{ij} - \hat{y}_i)^2$	n-p	$MS_E=\frac{SS_E}{n-p}$	
Lack of fit	$SS_{lof}=\sum_i^m \sum_j^{n_1}(\hat{y}_i - \bar{y}_i)^2$	m-p	$MS_{lof}=\frac{SS_{lof}}{m-p}$	
Pure error	$SS_{pe}=\sum_i^m \sum_j^{n_i}(y_{ij} - \bar{y}_i)^2$	n-m	$MS_{pe}=\frac{SS_{pe}}{n-m}$	
Total	$SS_T=\sum_i^m \sum_j^{n_i}(y_{ij} - \bar{y})^2$	n-1		

Source: Bezerra *et al.* (2008); Myers *et al.* (2008)

12. Some Optimization Studies with Box-Behnken Design and Other Designs

Shao *et al.* (2009) investigated process optimization for production of biodiesel from rape seed soap stock by a novel method of short path distillation. They used three-factor-three-level Box-Behnken statistical design. A short path distillation process was developed to separate free fatty acids (FFAs) and esterified fatty acids from rape seed soapstock. They found out that the most effective parameter was methanol quantity. Optimum performance occurred with a methanol to oil ratio of 0.33 v/v, an acid catalyst of 1.44%v/v and a reaction time 1.42 h at 60 ºC.

Chang *et al*. (2009) performed optimization of lipase-catalyzed biodiesel by isoproponolysis on a continuous packed-bed reactor using response surface methodology. They employed three-level-three-factor Box-Behnken design. In their study, the results showed that the flow rate and temperature have a significant effect on the percentage of molar conversion. The optimization conditions were at the flowrate of 0.1 ml/min, temperature of 51.5 ºC and substrate molar of 1:4.14.

Zhang *et al*. (2010) studied biodiesel production from high free fatty acid *Zanthoxylum bungeamum* oil (ZSO) using ferric sulfate as catalyst in esterification and calcium oxide as alkali catalyst. Acid value of ZSO 16.01 mg KOH /g of oil was reduced to less than 2 mg KOH/ g of oil by one-step estrification with methanol-to-FFA molar ratio 40.91:1, ferric sulfate 9.75% (based on the weight of FFA) reaction temperature of 95 ºC and reaction time of 2 h. For the second step, transesterification, they used a Box-Behnken factorial design with three factors and three levels including 12 factorial points and three centre points were for fitting a second-order response surface. The optimum combinations for transesterification which achieved the conversion to biodiesel above 96% were methanol-to-oil molar ratio of 11.69:1, catalyst amount of 2.52% and reaction time of 2.45 h.

Li and Yunyan (2010) conducted a new technique of biodiesel production from *Sapium sebiferum* oil catalyzed by immobilized lipase from *Pseudomonas cepacia* G63. They also employed the Box-Behnken design. The optimum condition to get biodiesel yield 97.07% was 4.1 methanol/oil molar ratio, 27% (w/w) lipase and temperature 41 ºC.

Liao and Chung (2011) investigated analysis of parameters and interaction between parameters of the microwave-assisted continuous transesterification process of Jatropha oil using response surface methodology. A Box-Behnken factorial model was employed in their study requiring 15 experimental runs. They got the optimal ratio of methanol to oil, amount of catalyst and flow rate of transesterification process were 10.74:1, 1.26% and 1.62 ml/min. the largest predicted and experimental conversion of alkyl esters (biodiesel) under the optimal conditions were 99.63% and 99.36% respectively.

Charoenchaitrakool and Thenmethangkoon (2011) undertook statistical optimization for production from waste frying oil through two-step catalyzed process. They reduced the initial acid value from 1.45 mg KOH/ g of oil to 1 mg KOH/ g of oil in esterification. The Box-Behnken design of experiment was carried out using the MINITAB Release 14, and the results were analyzed using response surface methodology. The optimum condition to get 90.56 ± 0.28 %FAME were obtained using methanol to oil molar ratio of 6.1:1, 0.68 wt% of sulfuric acid, at 51 ºC with a reaction time of 60 min in the first step, followed by molar ratio of methanol to product from the first step of 9.1:1, 1 wt% KOH, at 55 ºC with reaction time of 60 min in the second step.

Khan *et al*. (2010) conducted optimization and parametric analysis for acid esterification of high free fatty acid about 11.9 wt% crude palm oil and crude rubber seed oil blend using Taguchi experimental design. The optimal conditions for acid catalyzed esterification which reduced the FFA content in the feedstock to lower than 0.6% (95% reduction) were 65 ºC, 15:1 methanol to oil ratio (by mole) and 0.5 wt% H_2SO_4 after 3 h reaction time. Temperature was the most effect on the reduction of FFA.

WanOmar *et al*. (2009) studied a two-step biodiesel production from waste cooking oil (WCO). Central composite design (CCD) and RSM were used to determine the best operation condition for pre-treatment step. The optimum pre-treatment conditions were 60 ºC, 3 h and molar ratio of methanol to oil of 7:1 respectively with the maximum FAME yield of 81.3%. Based on the empirical model equation, the molar ratio was the most significant factor in the esterification process.

Rashid *et al*. (2011) used response surface methodology with composite rotatable design (CCRD), to explore optimum conditions for the transesterification of *Moringa oleifera* oil. They appraised the effect of 4 variables; reaction temperature

(25-65 ºC), time (20-90 min), methanol/oil molar ratio (3:1-12:1) and catalyst concentration (0.25-1.25 wt% KOH). Transesterification under the optimum conditions ascertained presently by RSM: 6.4:1 methanol/oil molar ratio, 0.8% catalyst concentration, 55 ºC reaction temperature and 71.08 min reaction time offered 99.3 %FAME. The catalyst concentration and molar ratio of methanol to oil exerted stronger effect on the ester formation than those exhibited by the reaction temperature and reaction time.

Tan *et al*. (2010) investigated the optimization study via RSM of a glycerol free process to produce biodiesel by supercritical methyl acetate technology using purified palm oil. The effects of three variables on the yield of biodiesel were studied concurrently in a rotatable central composite design (RCCD). The optimum condition were found to be 399 ºC for reaction temperature, 30 mol/mol of methyl acetate, methanol to oil molar ratio and reaction time of 59 min to achieve 97.6% biodiesel yield.

Abdullah *et al*. (2009) studied the optimization of mesoporous K/SBA-15 catalyzed transesterification of palm oil using central composite design (CCD). The optimum conditions were found to be reaction temperature of 70 ˚C, methanol to oil molar ratio of 11.6 (mol/mol), catalyst loading of 3.91% and reaction time of 5 h to achieve 93% of biodiesel yield. The effect of catalyst loading and reaction time was relatively more dominant effect to biodiesel yield.

Chen *et al*. (2008) introduced response surface optimization of biocatalytic biodiesel production of acid oil. They proved that the statistical optimization method is to be a powerful tool for the optimization of reaction condition catalyzed by soluble lipase NS 81020/NS81006. Under optimal condition (NS81020 amount 40.5U/g acid oil, NS81006 addition: 20.1U/g acid oil, temperature: 28.11 ˚C, molar ratio of methanol actual addition to theory addition: 2.4 and rate of stirring: 600 rpm), the predicted value of the yield of methyl ester was 0.915 (w/w). When they validated this predicted yield of methyl ester, the optimum value from the experiment was 0.887±0.0161 w/w. In their preliminary experiments, they found out that soluble lipase NS81006 had high activity towards triglyceride and free fatty acid when the two substrates were catalyzed separately with lipase NS81006. Also soluble lipase NS81020 had high activity towards free fatty acid and nearly no activity towards triglyceride.

Vieira *et al*. (2006) carried out the synthesis of ethyl hexadecanoate by esterification of palmitic acid with ethanol in solvent-free system using a commercial immobilized lipase (Lipozome RM.IM). They used central composite design 2^3 with six central points for statistical analysis. The best condition which gave values larger than 50% was palmitic acid/ethanol molar ratio of 0.5, temperature of 67 ˚C and enzyme concentration of 45% (w/w). The enzyme concentration and palmitic acid/ethanol molar ratio had been found to be the most significant variables affection the initial reaction rate.

WanOmar and Amin (2011) investigated the optimization of heterogeneous biodiesel production using Sr/ZrO_2 catalyst from waste cooking palm oil via using central composite 24 full factorial designs with two centre points. The maximum methyl ester yield of 79.7% was achieved at the optimum methanol to oil molar ratio of 29:1, catalyst loading of 2.7%, reaction time of 87 min and reaction temperature of 115.5 ºC. Interaction between reaction time and temperature gave the largest effect on FFA conversion and also methyl ester yield.

SathyaSelvabala *et al*. (2011) developed a two-step process to produce biodiesel from *Calophyllum inophyllum* oil. Central composite design (CCD) and RSM were utilized to determine the best operating condition for the pre-treatment step. Phosphoric acid modified β-zeolite was used as acid catalyzed in pre-treatment esterification process, preceded by transesterification which was done using conventional alkali-catalyzed KOH. In their study, the increase in parameter values will reduce the acid value in the following order: temperature > catalyst > oil methanol ratio The influence of the combination of parameters in the reduction of acid value is in the following order: temperature and oil methanol ratio> temperature and catalyst > catalyst and oil methanol ratio, where as the acid value reduced to a major extent under the influence of temperature and oil methanol ratio combination. They found out the exact global solution for the FFA reduction from 44 to 2 mg KOH/g of oil at the temperature of 60 ºC, oil methanol ratio of 0.33 (wt%) and catalyst amount of 0.1%.

Berrios *et al*. (2009) carried out an application of 3^2 factorial design of experiments to produce biodiesel from lard. A regression equation has been obtained as a result of the relation between the FAME concentration and the operational variables: catalyst concentration and agitation speed. Their statistical analysis showed that the two factors had a positive influence, with the catalyst concentration being the most important. However, the interaction had a slightly negative effect. The most suitable operational conditions were 0.9 wt% KOH and an agitation speed of 600 rpm, in operation terms.

Bouaid *et al*. (2007) proposed the optimization of biodiesel production from jojoba oil with acid value 0.8 mg KOH/g of oil using the factorial design and RSM. In their study, the maximum yield of esters (83.5%) was obtained at the maximum level of initial catalyst concentration (1.35%) and a medium level for the operation temperature (25 ºC). the most important factor was a initial catalyst concentration and it also had a positive influence on ester yield. Temperature equally had a positive influence on the ester yield. Synergetic effects of temperature and catalyst concentration were small and negatively influenced the process, which was due to the formation of by-products (soaps).

Yuan *et al*. (2008) studied the optimization of the conversion of waste rapeseed oil with high FFA to biodiesel using 2^4 full-factorial central composite design. In their pretreatment step, FFA was reduced to less than 2% by distillation refining method. The response surface methodology (RSM) was used to optimize the condition for the maximum conversion to biodiesel in alkaline-catalyzed transesterification process. The maximum conversion was obtained at methanol/oil molar ratio of 6.5:1, catalyst concentration of 1% (by weight of the oil), reaction time of 65.4 min and temperature of 48.2 ºC. Their results showed that catalyst concentration and reaction time were the limiting conditions and little variation in their value would alter the conversion. Moreover, there was a significant mutual interaction between catalyst concentration and reaction time.

Jeong *et al*. (2009) studied the optimization of transesterification of animal fat ester using five-level-three factors of central composite rotatable second order experimental design. From their statistical model, the highest conversion yield of lard biodiesel was 98.6% at the following optimization reaction conditions: reaction temperature of 65 ºC, catalyst amount of 1.26% and oil-to-methanol molar ratio of 7.5:1 and 20 min reaction time.

Vicente *et al*. (1998) investigated the application of the factorial design of experiments and response surface methodology to optimize biodiesel production. Optimum condition for the production of methyl esters was found at mild temperature (20-50 ºC) and large catalyst concentration (1.3%). Temperature and catalyst concentration were found to have a positive influence on conversion. Nevertheless, catalyst concentration had larger effect than temperature.

Bouaid *et al*. (2007) proved that a full two-factorial central composite design was effective in the study of the variable on the process. In their study, the experiment has been applied to optimize the synthesis process of ethyl esters from high oleic sunflower oil (HOSO) with 0.45 mg KOH/g of oil, high and low erucic *B. carinata* (HEBO and LEBO) with acid value 0.88 mg KOH/g of oil and 1.16 mg KOH/g of oil. According to their results, the maximum yield of ester 95.57% for HOSO, 91.31% for HEBO had been obtained, working at the maximum level of initial catalyst concentration (1.5%) and maximum level of operation temperature (32 ºC). Then, the maximum yield of ester for LEBO was achieved of 99.5% at the large catalyst concentration (1.5%) and mild temperature (22-32 ºC). In their observed statistical analysis, the most significant factor was the catalyst concentration.

Demirkol *et al*. (2006) performed the optimization of enzymatic methanolysis of soybean oil using *Rbizomucor miehei lip*ase, Lipozyme RMIM, in n-hexane. They used RSM based on three-level-three-factor (variable) face-centred cube design. Critical conditions for the response at which methyl ester content of the product was 76.9% were determined to be 50 ºC. 2.37 methanol/oil mole ratio, and 0.09 enzyme/oil weight ratio. The effects of temperature and enzyme amount were significant on methyl ester content. However, the interaction between temperature and enzyme amount was negative on the response while substrate molar ratio and enzyme amount was a positive effect in the response.

Casas *et al*. (2010) studied the optimization of reaction parameters for fast pseudo single-phase transesterification of refined sunflower oil with acid value 0.09 mg KOH/g of oil. In their study, transesterification of sunflower oil with methanol was carried out using potassium hydroxide and methoxide as catalyst and MTBE as cosolvent. The factorial design (2^3) design was used to optimize reaction parameters. The response variables were methyl ester (ME) and acid value (AV) for both catalysts. A ME content of 98 wt% and AV of 1.95 mg KOH/g of oil were obtained under the optimum values of variables: catalyst to oil molar ratio of 0.333, methanol to oil molar ratio of 10.9 and 5 min of reaction time for KOH catalyzed transesterification. For methoxide catalyzed transesterification, the optimum values were catalyst to oil molar ratio of 0.235, methanol to oil molar ratio of 12 and 5 min of reaction time which achieved methyl ester content of 99 wt% and AV of 0.2 mg KOH/g of oil. In their study, methanol to oil molar ratio was the most important effect in the variable range studied because of methanol amount was the only factor that helped entirely to the formation of methyl ester. The main effect was the catalyst amount for the methoxide catalyzed reaction and the methanol amount for the KOH catalyzed reactions. Moreover, the factors affection acid value showed up the catalyst had a positive on the acid value while methanol had a negative influence for both catalyst systems. The effect of the time variable was favorable for the KOH reaction and no significant for the methoxide catalyzed reaction since saponification reaction almost did not appear.

Alkabbashi *et al*. (2009) investigated optimization parameters to produce biodiesel from crude palm oil by transesterification process using five-level-five-factor experimental design by the design expert software. The optimal values of reaction time of 60 min, reaction temperature of 60 ºC, agitation speed of 250 rpm, molar ratio of methanol to oil of 10:1 and dosage of the KOH catalyst of 1.4 wt.% which gave the best possible yield of biodiesel at the end of the reaction which was 93.6%.

MATERIALS AND METHODS

In this section, it includes the statistical design of experiment and statistical analysis, esterification and transesterification to produce biodiesel and analysis of raw material and product. These experiments were conducted at Bioprocessing Engineering Laboratory (BEL) in chemical engineering department.

1. Experimental Design and Statistical Analysis

Statistical analysis of conversion of FFA and %FAME in biodiesel production was performed using MINITAB-14 software. The Box-Behnken design was used to study the interaction of process variables and to predict the optimum process condition for conversion of FFA and FAME % by applying RSM. The range and coded level of both esterification and transesterification are listed in Table 13. For three-level-three-factor design of specific FFA, the variables were reaction time (x_1), methanol amount (x_2), catalyst loading (x_3). On the other hand, the variables were), reaction time (x_1), methanol amount (x_2), catalyst (x_3), initial FFA (x_4) in four-factor-three-level design. Each variable consisted of three different levels from low (-1), to medium (0) and high (1). These variable parameters and their selected levels for both of esterification and transesterification were shown in Table 13. Total numbers of experiment for three-factor-three-level were 15 runs and 27 runs for four-factor-three-level individually. All runs were performed in random order to avoid bias.

The experimental data were analyzed by the response surface procedure to fit the following second-order polynomial model equation (10), as the following, predicted for optimization of both esterification and transesterification of mixture of PFAD and PS;

$$Y = \beta_{ko} + \sum_{i=1}^{3} \beta_{ki} x_i + \sum_{i=1}^{3} \beta_{kii} x_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{kij} \, x_i x_j \qquad (10)$$

Where *Y* was the response (% conversion) β_{ko}, β_{ki}, β_{kii}, and β_{kij} were constant coefficients and x_i is the uncoded independent variable. This ridge maximal option was used to compute the estimated ridge of maximum response for increasing radii from the centre of the origin design (Chang *et al*., 2009).

The empirical mathematical model was tested with the analysis of variance (ANOVA) with 95% confidence level. The ANOVA was used for checking the significance of the second-order models. The statistical significance of the second-order equation was determined by F-value. In general, the calculated F-value to reject the null hypothesis, where all the regression coefficients are zero. The calculated F-value is defined as the ratio between mean of square regression (MS_{SSR}) and mean of square residual (MS_{SSE}), where MS_{SSR} and MS_{SSE} are obtained by dividing sum of square (SS_R) and sum of residual (SS_E) over degree of freedom (df) respectively. Meanwhile, tabulated F-values are obtained from F-distribution based in (df) for regression and residual, respectively at a specified level of significance which is defined as α value (Cornell, 1990; Brown and Melemend, 1990; Montgomery, 1997). The molar ratio of methanol to FFA or triglyceride, catalyst loading and reaction time were selected. The level of molar ratio of methanol to FFA or triglyceride, catalyst loading and reaction time were started from theoretical limited as the minimum and the maximum levels were based on the optimum conditions and ranges of some literatures (El-Mashad *et al.,* 2008; Veljkovic *et al.,*2006; Lin *et al*., 2009; Wang *et al*., 2006; Chongkhong *et al*., 2007; Canakci and Gerpen, 2001; Hancsok *et al*., 2004; Houfang *et al.,* 2009). Besides, methanol (3:1 molar ratio of methanol to triglyceride) was added to all the experiments in all esterification reactions. After choosing the variable ranges, according to Table (13) the maximum limit of these conditions were checked by preliminary experimental tests. The results showed that those maximum optimum values were in the range of objective function. Levels and ranges of selected experimental parameters for the mixture of PFAD and PS with various initial FFA contents (1, 2, 5, 20, 40, 50, 60 and 87%) were also shown in Table 13.

2. Determination of Acid Value

2.1 Materials

Palm fatty acid distillate (PFAD) and palm stearin (PS) were used as raw materials in these experiments. These raw materials were obtained from Siam Fats and Oils Co.Ltd, located in Thailand.

The PFAD used in this experiment was brown in color and presented in solid form with ca. initial 87% FFA and PS was solid form and milky white in color with ca. 0.195% FFA. PFAD and PS were mixed together to get about initial 1, 2, 5, 20, 40, 50 and 60% FFA (the rest was assumed to be triglyceride) for further experiment. The molecular weight for oil mixture was assumed 820 g/gmol (based on palmitic acid) for all experiments. PFAD and PS were shown in Figure 14.

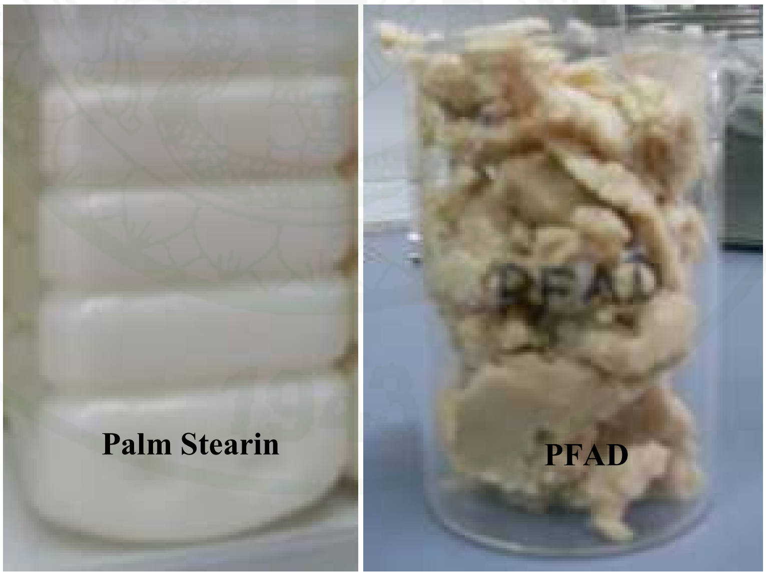


Figure 14 Sample of PFAD and palm stearin (PS).

2.1.1 Equipment

(1) Burette (25 ml)
(2) Conical flask (150 ml)
(3) Volumetric flask (500 ml)
(4) Volumetric flask (50 ml)
(5) Digital balance

2.1.2 Chemical

(1) KOH (85% purity, UNIVAR reagent)
(2) 2-propanol (99.7%, QReC^{TM} reagent)
(3) Phenolphthalein (Carlo Erba Reagent, 99.99% purity)
(4) Ethanol (95%, Zen Point reagent)

2.2 Method

The free fatty acid content in raw oil and product mixture were analyzed using titration method (Paquot, 1979). Approximately 1 g of the sample was added into a flask with 10 ml of 2-propanol and 2-3 droplets of phenolphthalein (Carlo Erba Reagent, 99.99% purity). The flask was then titrated with 0.1 N of KOH. When the solution turned into pink color, the titration was stopped. The acid value was then calculated using equation (11) and to calculate the conversion of FFA used equation (12):

$$Acid\ value\ \left(\frac{mgKOH}{g}\right) = \frac{volume\ of\ KOH\ used\ \times 56.1 \times 0.1}{weight\ of\ sample\ (g)} \tag{11}$$

The conversion of FFA in esterification was calculated as in equation (10).

$$\%\ Conversion\ of\ FFA\ = \frac{initial\ FFA - final\ FFA}{initial\ FFA} \times 100\% \tag{12}$$

3. Esterification and Transesterification Reaction

3.1 Materials

Mixture of PFAD and PS: initial 5, 20, 40, 50, 60 and 87% FFA were used as raw material in esterification. The initial FFA of 1 and 2% mixture of PFAD and PS were used as raw materials in transesterfication reaction.

3.1.1 Equipment

(1) Three-neck volumetric flask (500 ml)
(2) Condenser set
(3) Hot plate with temperature controller and magnetic stirrer
(4) Magnetic stirrer stick
(5) Beaker
(6) Measuring cylinder

3.1.2 Chemical

(1) Methanol (99%, ACS reagent)
(2) Sulfuric acid (98%, QReC^{TM} reagent))
(3) KOH (85%, UNIVAR reagent)

3.2. Method for esterification

The 100 g of oil mixture was poured in a round-bottomed flask equipped with reflux condenser and heated to reaction temperature of 60 ºC. The solution of H_2SO_4 (based on wt % of FFA in oil) in methanol due to statistical experimental design was also prepared and added to the flask. The mixture was stirred at the same speed of 500 rpm for all runs. According to statistically experimental design, the amount of methanol as a molar ratio of methanol to FFA in oil and as an additional amount of methanol (3:1 molar ratio of methanol to triglyceride in oil) were added to the flask for all runs. After finished the reaction, the cold distilled water (10% of total volume of reaction mixture) was added to stop the reaction. Then, this mixture was settled in separating funnel about 30 min. The esterification reaction set up was shown in Figure 15.

3.3 Method of transesterification

100 g of raw oil mixture was conducted into a round-bottomed flask equipped with a reflux condenser and heated at 60 ºC. According to statistically experimental design, KOH catalyst, based on triglyceride in oil, was mixed with methanol. Then this mixture was added to reaction flask and stirred with the speed of 500 rpm. After reaction finished, distilled water was added to the mixture to stop the reaction. The mixture was allowed to settle for about 1 h, in a separating funnel. The lower layer, water and glycerol rich phase was drawn off. The transesterification reaction set up was shown in Figure 15.

Figure 15 Esterification or transesterification reaction set up.

4. Washing and Drying

4.1 Materials

The mixtures from the both reactions were washed to remove catalyst and methanol.

4.1.1 Equipment

(1) Beaker (500 ml)
(2) Separation funnel
(3) Hot plate with temperature controller
(4) pH paper (range : 1-14)

4.2 Method

After the end of each reaction, distilled water (10% of total volume of reaction mixture) was added to the mixture to stop the reaction and then, the mixture was allowed to settle in a separating funnel. After that, lower layer, water-rich phase, was drawn out. The upper layer, ester-rich phase, was washed with warm distilled water at ca. 50 ºC until pH value of washed water reached 7. Finally, the neutral washed mixture was dried at 105±1 ºC for about 30 min. The washing set up was shown in Figure 16.

Figure 16 Washing set up.

5. Determination of fatty acid methyl ester (FAME) content

5.1. Materials

5.1.1. Equipment

(1) GC (Shimadzu, model 2010)
(2) Micro pipette (1000 μL)
(3) Micro pipette (100 μL)
(4) Vials
(5) Shaker
(6) Refrigerator

5.1.2. Chemical

1. Methyl Heptadecanoate (C17:0) (≥ 95.0%, Sigma ALDRICH reagent)
2. Heptane (99%, Sigma ALDRICH reagent)

5.2. Method

The FAME content in the product mixture was analyzed by gas chromatography (Shimadzu, model 2010) according to EN 14103 standard with the use of Methyl Heptadecanoate (C17:0) as an internal standard. The % of FAME was calculated using equation (13):

$$\% \, FAME = \frac{(\sum A) - A_{EI}}{A_{EI}} \times \frac{C_{EI} \times V_{EI}}{W} \times 100 \qquad (13)$$

Where,

$\sum A$ = sum of all areas under the curve from C_{14} to C_{24}

A_{EI} = area under the curve of $C\ 17{:}0$

C_{EI}= concentration of $C17{:}0$ (mg/ml)

V_{EI}= amount of $C17{:}0$ used (µl)

W= weight of product (mg)

Table 13 Levels and ranges of selected experimental parameters for the mixture of PFAD and PS with various initial FFA contents.

Reaction parameters	Symbols	Levels and ranges		
		-1	0	1
			Initial 5 % FFA	
Time (min)	x_1	30	90	150
Methanol:FFA	x_2	1:1	4.5:1	8:1
H_2SO_4 (wt%)	x_3	0.5	2.25	4
			Initial 20 % FFA	
Time (h)	x_1	2	3	4
Methanol:FFA	x_2	1:1	4.5:1	8:1
H_2SO_4 (wt%)	x_3	0.5	2.25	4
			Initial 40 % FFA	
Time (h)	x_1	2	4	6
Methanol:FFA	x_2	1:1	6.5:1	12:1
H_2SO_4 (wt%)	x_3	0.5	2.25	4
			Initial 50 % FFA	
Time (h)	x_1	1	3.5	6
Methanol:FFA	x_2	3:1	7.5:1	12:1
H_2SO_4 (wt%)	x_3	1	2. 5	4
			Initial 60 % FFA	
Time (h)	x_1	1	3.5	6
Methanol:FFA	x_2	3:1	7.5:1	12:1
H_2SO_4 (wt%)	x_3	1	2. 5	4
			Initial 5-87 % FFA	
Time (h)	x_1	0.5	3.25	6
Methanol:FFA	x_2	1.2:1	6.6:1	12:1
H_2SO_4 (wt%)	x_3	0.5	2.25	4
Initial FFA (%)	x_4	5	46	87

Table 13 (Continued).

Reaction parameters	Symbols	Levels and ranges		
		-1	0	1
			Initial 1% FFA	
Time (min)	x_1	30	75	120
Methanol:Triglyceride	x_2	4.5	8.25	12
KOH (wt%)	x_3	0.5	0.85	1.2
			Initial 2% FFA	
Time (min)	x_1	30	75	120
Methanol:Triglyceride	x_2	4.5	8.25	12
KOH (wt%)	x_3	0.5	0.85	1.2

-1: input minimum value; 0: medium value suggested by statistic; 1: the input maximum value.

RESULTS AND DISSCUSSION

According to Ramadhas *et al*., 2005; Ma and Hana, 1999; Yuan, 2008, the maximum limit of the initial FFA for transesterification was less than 2%. Therefore, the application of the two-step reaction to produce biodiesel, the initial FFA of raw material should be higher than that limit. However, if the FFA equals to 5%, the reaction can still be catalyzed with an alkali catalyst, but additional catalyst must be added to compensate for that lost to soap. The soap can be created during the reaction and is either removed with the glycerol or washed out during the purification. When the FFA level is more than 5%, the soap inhibits separation of the glycerol from the methyl ester and contributes to emulsion formation during the water wash (Knothe *et al*., 2005). Therefore, the following experiments were conducted at the specific initial FFA of 5, 20, 40, 50 and 60%. Moreover, to be able to propose the optimum condition of esterification for various initial FFA raw materials, four-factor-three-level design also was investigated. For optimization of transesterification reaction in the second step, initial 1 and 2% FFA of the mixture of PFAD and PS were used as raw material.

1. Optimization of esterification with specific initial FFA of the mixture of PFAD and PS

A three-level-three-factor and three-level-four-factor of Box-Behnken design with three replicates at the centers was used in this study. The variable parameters and their selected levels for the study of esterification were shown in Table 13.

1.1 Esterification of raw material with initial 5% FFA content

Table 14 showed the independent factor (x_i), levels and experimental design in term of coded and uncoded values for initial 5% FFA.

1.1.1 Model fitting of esterification of raw material with initial 5% FFA content

Response surface regression (RSREG) procedure was used to fit the second-order polynomial equation (10). Analysis of variance (ANOVA) was applied in order to check the experimental data whether they are adequate and fits the model as shown in Table 15. The significant of model equation was calculated by F-test for ANOVA, which showed that the regression was statistical significant at 95% confidence level (WanOmar *et al.*, 2011). The model F-value was 49.94 which implied that the model was statistically significant as this value was quite high compared with the tabulated F-value ($F_{0.05,\,9,\,5}$= 4.77; see Appendix D1). Moreover, P-values were used as a tool to check the significance of each coefficient, which also indicated the interaction strength of each parameter. The lower P-values were the higher the more significance of the predicted model was significant. The P-value of linear and square term of methanol quantity were not significant as these value were slightly higher than theoretically limit according to 95% confidence level as shown in Table 23. Although the P-values of the interaction terms were not significant was the predicted P-values were higher than 0.05 (Table 15 and 23), the predicted full model could still be useful to predict the optimum condition for initial 5% FFA esterification as all of terms included in this model were related each other. Besides, the other factor to check the predicted model was coefficient of determination (R^2). The closer its value to 1.0, the stronger the model and the better it predicted the response. The comparison between observed experimental data and the predicted data is shown in Figure 17. R^2 value is 0.989; that is 98.9% of variability in the assay reading that should be at least 0.75 (Haaland, 1989). According to these ANOVA results, the second-order polynomial predicted model (equation 14) was a good representative of experimental data and response. The photo of the products is shown in Appendix Figure C1.

$$Y = -22.0947 + 0.6322x_1 + 0.595x_2 + 28.9064x_3 - 0.0027x_1^2 + 0.0407x_2^2 - 4.2827x_3^2 - 0.0004x_1x_2 + 0.0444x_1x_3 + 0.2930x_2x_3 \qquad (14)$$

Where each variable was the following; Y: the % conversion of FFA, x_1: the reaction time (min), x_2: methanol-to-FFA molar ratio and x_3: H_2SO_4 catalyst loading.

Table 14 Experimental design matrix and result from esterification of the mixture of PFAD and PS with initial 5% FFA content.

Run no[a]	Factors			Results	
	Time (min) (x_1)[b]	Methanol : FFA (x_2)[b]	Catalyst (wt %) (x_3)[b]	Observed Conversion of FFA (%)	Predicted Conversion of FFA (%)
1	(90) 0	(1:1) -1	(0.5)-1	30.442	28.848
2	(150) 1	(4.5:1) 0	(4.0) 1	90.594	93.538
3	(90) 0	(1:1) -1	(4.0) 1	79.322	77.486
4	(90) 0	(8:1) 1	(4.0) 1	90.557	92.152
5	(150) 1	(8:1) 1	(2.25) 0	86.384	81.846
6	(30) -1	(8:1) 1	(2.25) 0	52.175	53.282
7	(30) -1	(4.5:1) 0	(0.5) -1	15.512	12.256
8	(150) 1	(1:1) 0	(2.25) 0	72.056	70.948
9	(90) 0	(8:1) 1	(0.5) 0	34.499	36.335
10	(90) 0	(4.5:1) 0	(2.25) 0	72.819	71.337
11	(30) -1	(1:1) -1	(2.25) 0	37.488	42.025
12	(90) 0	(4.5:1) 0	(2.25) 0	71.755	71.337
13	(30) -1	(4.5:1) 0	(4.0) 1	58.182	55.480
14	(90) 0	(4.5:1) 0	(2.25) 0	69.438	71.337
15	(150) 1	(4.5:1) 0	(0.5) -1	29.293	31.995

[a] the treatments were run in random order, [b] the values (-1), (0) and (1) are coded levels.

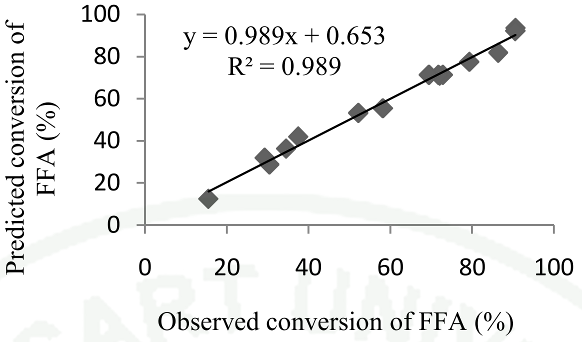


Figure 17 Correlation of experimental and predicted conversion of FFA (%).

Table 15 ANOVA for conversion for FFA (%) from esterification of the mixture of PFAD and PS with initial 5% FFA content.

Source	df	Sum of squares	F	P	R^2
Model	9	8394.13	49.94	0.000	98.9
Linear	3	7353.02	20.63	0.003	
Square	3	941.41	16.8	0.005	
Interaction	3	99.69	1.78	0.267	
Pure error	2	5.98			
Total error	14	8487.52			

1.1.2 The effect of parameters on % FFA conversion of raw material with initial 5% FFA content

The relationship between molar ratio of methanol to FFA and catalyst at 90 min shown in Figure 18a indicated that catalyst loading had more influence on conversion of FFA than that of methanol amount. Although the methanol amount was high, the conversion of FFA was still low. According to Figure 18b, the catalyst loading had more influence on the conversion of FFA than that of reaction time. The highest conversion was given at 3 wt% catalyst and 150 min. The reaction time was more influence on conversion of FFA than that of methanol amount was approved by Figure 18c. According to the behavior of Figure 18a-c, the catalyst loading was more important factor affected on conversion of FFA than the other two factors (reaction time and methanol amount).

The optimum point for initial 5% FFA esterficiation was shown in Figure 19. In this figure, the optimum value of reaction time and catalyst loading can be estimated. However, the methanol quantity could not show as it might be its range in the design was out of range. The optimum condition for esterification step was predicted to be 90 min for reaction time, 4.5:1 the molar ratio of methanol to FFA in oil and 2.9863 wt% H_2SO_4 catalyst wt% based on FFA to reduce 1% FFA (equivalent to 80% conversion of FFA).

Esterification reaction was taken by using this optimum condition (i.e. a condition that produces the best possible results) to confirm 80% conversion (equivalent to final 1% FFA). The experimental esterification product (% conversion of FFA) was 79.6% with 0.4% difference and could be neglected as shown in Table 16.

Table 16 Comparison of conversion of FFA (%) between predicted and experimental values for esterification of raw material with initial 5% FFA content.

Predicted value	Experimental value	Difference
80%	79.6%	0.4%

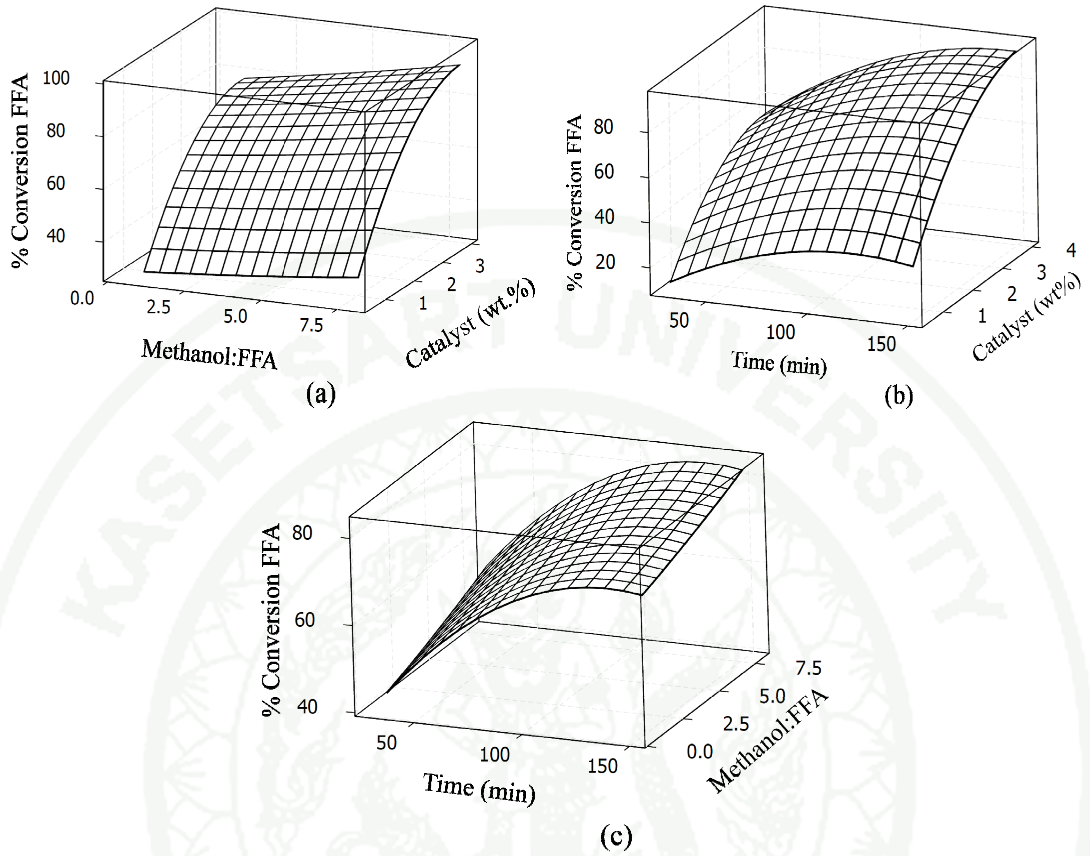
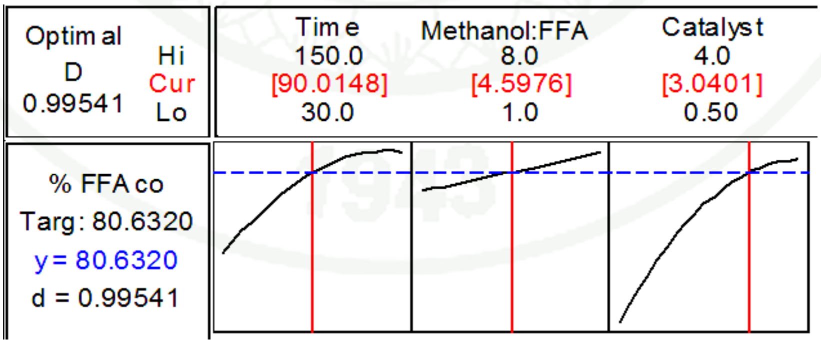


Figure 18 Response surface plots for % conversion of FFA of raw material with initial 5% FFA content.



```
Optimal          Time       Methanol:FFA   Catalyst
D        Hi      150.0      8.0            4.0
0.99541  Cur     [90.0148]  [4.5976]       [3.0401]
         Lo      30.0       1.0            0.50

% FFA co
Targ: 80.6320
y = 80.6320
d = 0.99541
```

Figure 19 Optimum condition for initial 5% FFA esterification.

1.2 Esterification of raw material with initial 20% FFA content

The independent factor (x_i), levels and experimental design in term of coded and uncoded values for initial 20% FFA are shown in Table 13.

1.2.1 Model fitting of raw material with initial 20% FFA content

Both experimental design and results were shown in Table 17. The final predicted response model equation (based on the actual value) by which the esterification of oil mixture with 20% was estimated as shown in equation (15). From Table 18, R^2 values were 98.4% for the oil mixture. These were high enough compared with theoretical limit as shown in esterification of raw material with initial 5% FFA content. Moreover, these results indicated that the second-order polynomial model was a good representative of experimental data and response (% conversion of FFA). Then, the F-values from each response were 34.74. These values were quite high compared with the reference F-value as described in esterification of raw material with initial 5% FFA content. Most of the P-values were lower than 0.05 as shown in Table 18. According to Table 23, the P-values of reaction time was slightly higher than 0.05. Moreover, all of P-values of interaction terms were not significant like initial 5% FFA esterification. However, this initial 20% FFA model could give the optimum condition that has high accuracy compared experimental value. According to these ANOVA data, the predicted model was high significant.

$$Y = 0.338 + 23.672x_1 + 7.762x_2 + 21.9968x_3 - 3.057x_1^2 - 0.3916x_2^2 - 2.5648x_3^2 - 0.2294x_1x_2 - 0.99x_1x_3 - 0.3549x_2x_3 \quad (15)$$

Where each variable is the following; Y: the % conversion of FFA, x_1: the reaction time, x_2: methanol-to-FFA molar ratio and x_3: H_2SO_4 catalyst loading. The photo of the products is shown in Appendix Figure C2.

Table 17 Experimental design matrix and result from esterification of mixture of PFAD and PS with initial 20% FFA content.

Run no	Time (h) (x_1)	Methanol : FFA (x_2)	Catalyst (wt%) (x_3)	Observed Conversion of FFA (%)	Predicted Conversion of FFA (%)
1	4	1.0:1	2.25	77.550	79.3660
2	3	4.5:1	2.25	93.991	93.9763
3	2	4.5:1	0.50	66.941	68.9581
4	3	4.5:1	2.25	93.878	93.9763
5	4	4.5:1	0.50	75.704	76.5634
6	2	1.0:1	2.25	72.962	73.6203
7	2	8.0:1	2.25	96.301	94.4850
8	3	4.5:1	2.25	94.060	93.9763
9	4	4.5:1	4.00	95.723	93.7059
10	3	8.0:1	0.50	83.026	82.8249
11	3	1.0:1	0.50	61.893	59.2176
12	3	1.0:1	4.00	83.972	84.1731
13	3	8.0:1	4.00	96.409	99.0844
14	4	8.0:1	2.25	97.678	97.0198
15	2	4.5:1	4.00	93.890	93.0306

Table 18 ANOVA for conversion of FFA (%) from esterification of the mixture of PFAD and PS with initial 20% FFA content.

Source	df	Sum of squares	F	P	R^2
Model	9	1969.35	34.74	0.001	98.4%
Linear	3	1625.46	23.36	0.002	
Square	3	310.4	16.43	0.005	
Interaction	3	33.49	1.77	0.269	
Pure error	2	0.02			
Total error	14	2000.84			

1.2.2 The effect of parameters on % FFA conversion of raw material with initial 20% FFA content

The predicted models were plotted in a three dimensional surface that represented the response (% conversion of FFA) as a function of other two factors. Figure 20 is the response surface plot for conversion of FFA from the oil mixture. The effects of methanol and reaction time on conversion of FFA at a constant catalyst concentration of 2.25 wt % are presented in Figure 20a. The methanol-to-FFA molar ratio had more influence on conversion of FFA than that of reaction time. The maximum conversion of FFA was found at methanol-to-FFA molar ratio of 5:1 after 3 h of reaction. The relationship between reaction time and catalyst loading with methanol-to-FFA molar ratio of 3:1 was shown in Figure 20b indicated that conversion of FFA also increased when catalyst loading and reaction time increased. However, the catalyst loading had higher influence on conversion of FFA than the reaction time. This reaction trend was very similar with Figure 20a. It might mean that methanol ratio and catalyst concentration had nearly equal effect on conversion of FFA. The effect of methanol ratio and catalyst concentration is shown in Figure 20c. When both methanol and catalyst concentration increased, the conversion of FFA also increased. However, methanol ratio had slightly more influence on conversion of FFA than catalyst amount. In conclusion, the methanol amount was more important factor than the rest of two factors. The optimal amount of catalyst loading was around 2.4 wt % with methanol-to-FFA molar ratio of 4.6:1 and reaction time of 3 h.

In Figure 21, the optimum graph for initial 20% FFA esterification was shown. According to this Figure, the optimum point for reaction time and catalyst loading can be seen clearly but the point for the methanol quantity was hard to estimate as its trend still continued. To verify the predicted optimum conduction and experimental value, esterification reaction was conducted using that optimum condition. The product from experimental esterification (% conversion of FFA) was 94.3% with only 0.7% difference (Table 19).

Table 19 Comparison of conversion of FFA (%) between predicted and experimental values for esterification of raw material with initial 20% FFA content.

Predicted value	Experimental value	Difference
95%	94.3%	0.7%

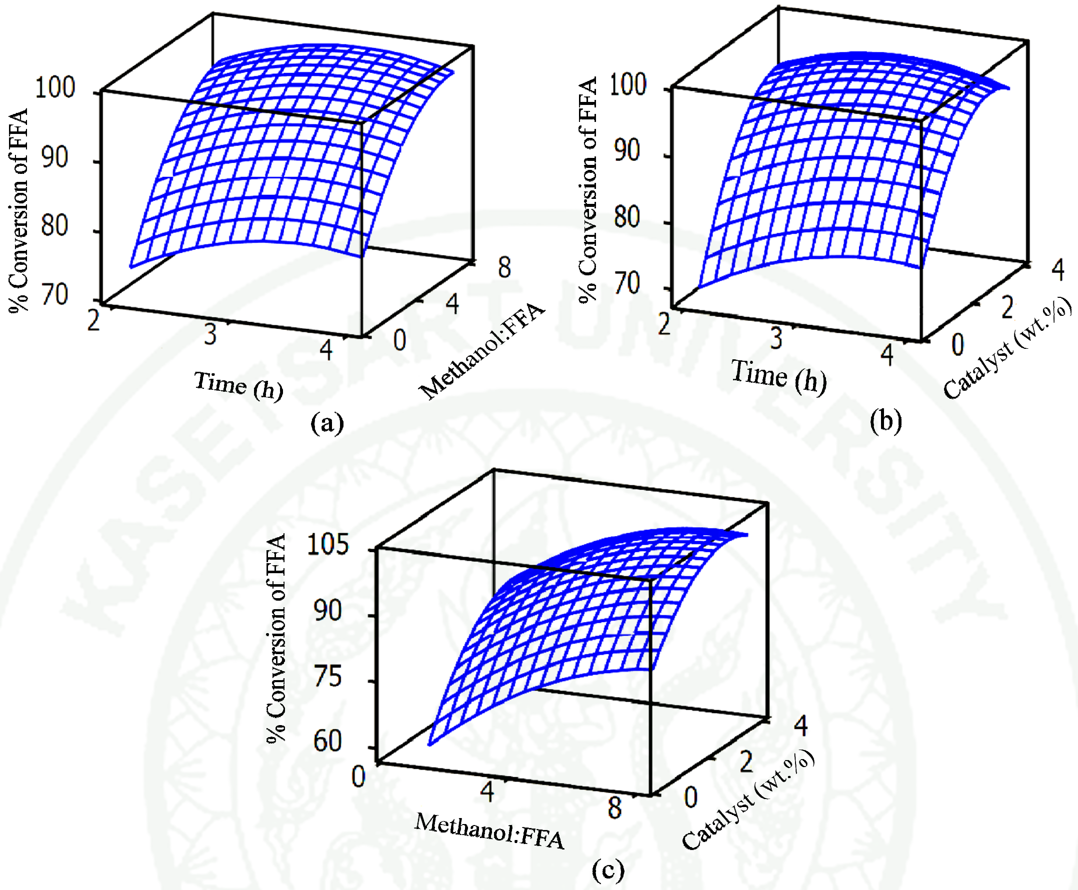
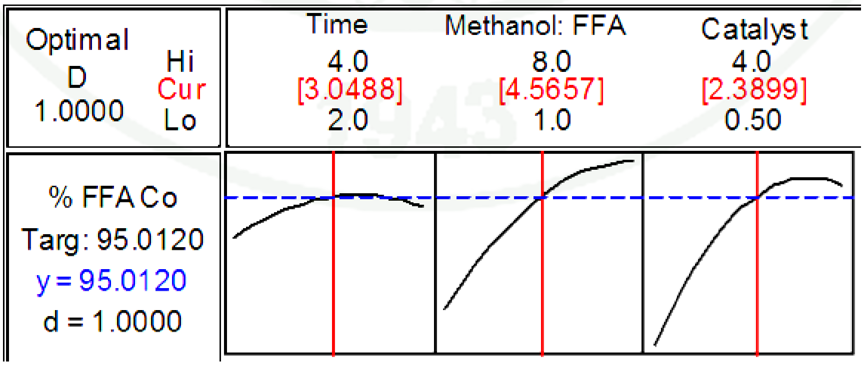


Figure 20 Response surface plots for % conversion of FFA of raw material with initial 20% FFA content.



```
Optimal     Hi    Time       Methanol: FFA   Catalyst
D           Cur   4.0        8.0             4.0
1.0000      Lo    [3.0488]   [4.5657]        [2.3899]
                  2.0        1.0             0.50

% FFA Co
Targ: 95.0120
y = 95.0120
d = 1.0000
```

Figure 21 Optimum condition for initial 20% FFA esterification.

1.3 Esterification of raw material with initial 40% FFA content

The ranges and interval of factors were shown in Table 13.

1.3.1 Model fitting of esterification of raw material with initial 40% FFA content

Table 20 shows both experimental design and results. The final predicted response model equation (based on the actual value) for esterification of raw material with initial 40% FFA content was estimated as shown in equation (16). From Table 21, R^2 value was 99.9% for the oil mixture. These were high enough compared with theoretical limit. Moreover, these results indicated that the second-order polynomial model was a good representative of experimental data and response (% conversion of FFA). Then, the F-value from each response was 460.19. These values were quite high compared with the tabulated F-value as presented in esterification of raw material with initial 5% FFA content. Beside, from Table 21 and 23, the P-values of the terms: linear, square, interaction and pure error were lower than 0.05. So, the predicted model of esterification of raw material with initial 40% FFA content was a good well fitted model between the experimental and predicted data.

$$Y = -45.7931 + 19.0424x_1 + 16.3094x_2 + 21.4769x_3 - 1.1074x_1^2 - 0.6331x_2^2 - 1.6015x_3^2 - 0.5973x_1x_2 - 1.4107x_1x_3 - 0.5766x_2x_3 \qquad (16)$$

Where each variable is the following; Y: the % conversion of FFA, x_1: the reaction time, x_2: methanol-to-FFA molar ratio and x_3: H_2SO_4 catalyst loading. The photo of the products is shown in Appendix Figure C3.

Table 20 Experimental design matrix and result from esterification of the mixture of PFAD and PS with initial 40% FFA content.

Run no	Time (h) (x_1)	Methanol : FFA (x_2)	Catalyst (wt%) (x_3)	Observed Conversion of FFA (%)	Predicted Conversion of FFA (%)
1	4	1.:1	0.5	34.35	33.174
2	6	1:1	2.25	60.4	60.561
3	2	6.5:1	0.5	66.6	66.412
4	4	6.5:1	2.25	95.45	95.477
5	2	1:1	2.25	33.55	34.914
6	6	6.5:1	0.5	87.78	88.795
7	2	6.5:1	4	94.38	93.365
8	6	12:1	2.25	97.1	95.736
9	4	1:1	4	61.7	61.351
10	4	12:1	4	97.39	98.566
11	4	6.5:1	2.25	95.49	95.477
12	4	6.5:1	2.25	95.49	95.477
13	2	12:1	2.25	96.53	96.369
14	4	12:1	0.5	92.24	92.589
15	6	6.5:1	4	95.81	95.998

Table 21 ANOVA for conversion of FFA (%) from esterification of the mixture of PFAD and PS with initial 40% FFA content.

Source	df	Sum of squares	F	P	R^2
Model	9	7383.74	460.19	0.000	99.9%
Linear	3	5564.84	526.99	0.000	
Square	3	1425.52	266.54	0.000	
Interaction	3	393.39	73.55	0.000	
Pure error	2	0.00			
Total error	14	7392.66			

1.3.2 The effect of parameters on % FFA conversion of esterification of raw material with initial 40% FFA content

Figure 22 is the response surface plot for conversion of FFA from the oil mixture with initial 40% FFA content. The effect of methanol ratio and reaction time on esterification was presented in Figure 22a. Although the conversion of FFA increased with the reaction time, it had less effect on the conversion of FFA than methanol ratio. Figure 22b showed the effect of catalyst loading and the effect of reaction time on the conversion of FFA. Effect of catalyst loading decreased with an increasing of reaction time. And also effect of time decreased with an increasing of catalyst concentration. The maximum conversion of FFA was about 3 wt.% catalyst after around 5 h. In addition, according to Table 21 and 23, there were significant interaction effects among variables. The effect of catalyst loading and methanol ratio on conversion of FFA is shown in Figure 22c. The effect of parameters on conversion of FFA was similar to the one from Figure 22b but more sensitive. For esterification of raw material with initial 40% FFA content, the order of importance factors which influenced on conversion of FFA were methanol amount was as the first one. The second and third one were reaction time and catalyst loading. According to the predicted model, the optimal amount of catalyst was around 3.3 wt% with methanol-to-FFA molar ratio of 11.6:1 and reaction time of 5.4 h. As the optimum points of initial 40% FFA were precise, this model was the high significant model to predict optimum and its location was also within the ranges as shown in Figure 23. To validate the predicted value and experimental value for esterification of raw material with initial 40% FFA content reaction was taken by using this optimum condition to confirm that whether it can reduce to final FFA 1 % (equivalence to conversion of FFA ca. 97.5%). The % conversion of predicted and experimental values had 0.5% difference as described in Table 22. This different value could be neglected.

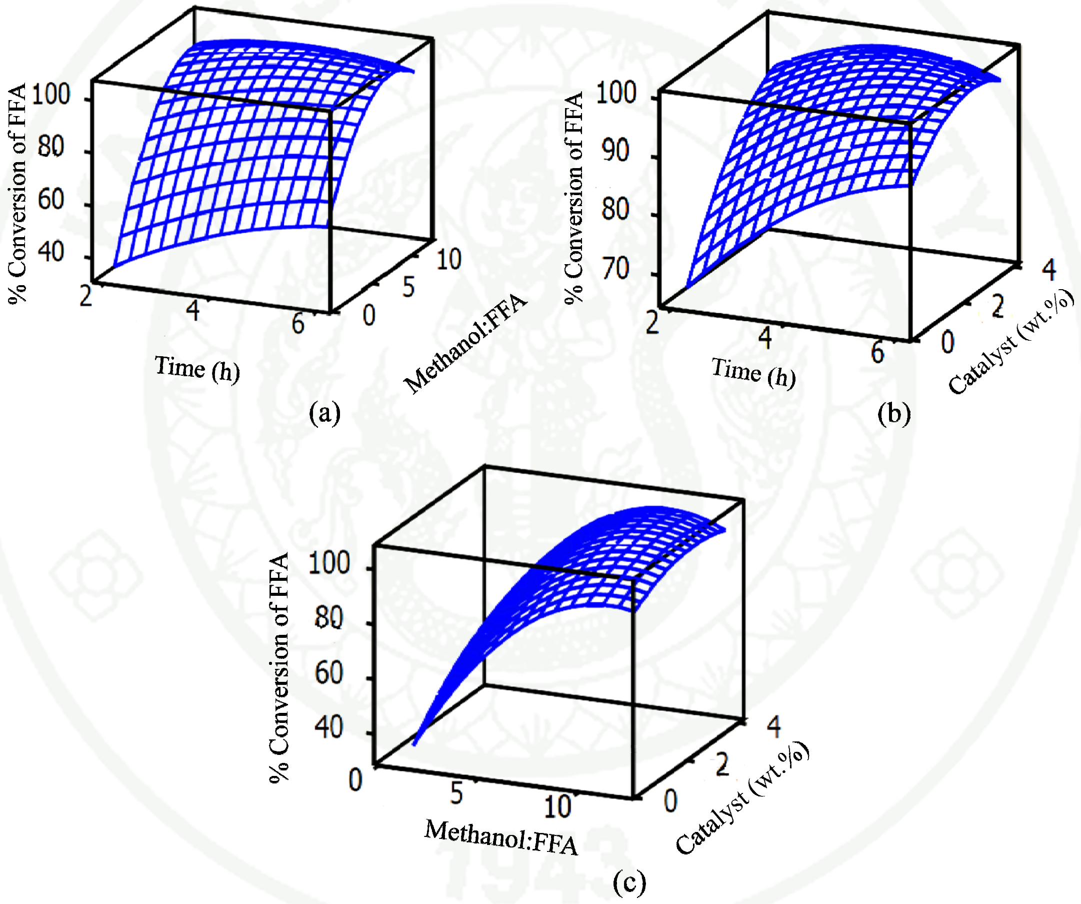


Figure 22 Response surface plots for % conversion of FFA of esterification of raw material with initial 40% FFA content.

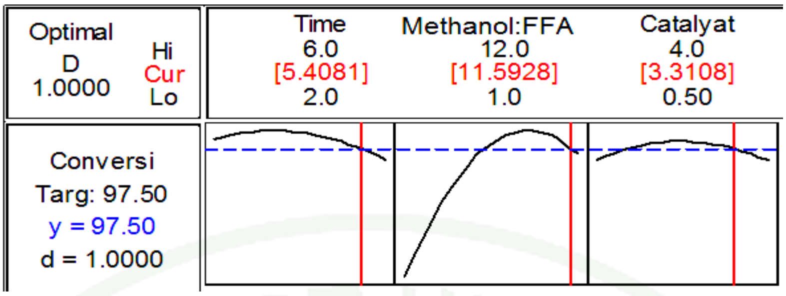


Figure 23 Optimum condition for initial 40% FFA esterification.

Table 22 Comparison of conversion of FFA (%) between predicted and experimental values for esterification of raw material with initial 40% FFA content.

Predicted value	Experimental value	Difference
97.5%	97%	0.5%

Table 23 Coefficient terms and P-values for specific initial 5, 20 and 40% FFA esterification models

Term	Initial 5% FFA		Initial 20% FFA		Initial 40% FFA	
	Coefficient	P	Coefficient	P	Coefficient	P
constant	-22.0949	0.071	0.3380	0.982	-45.7931	0.000
x_1	0.6322	0.005	23.6720	0.034	19.0424	0.000
x_2	0.5950	0.788	7.7620	0.004	16.3094	0.000
x_3	28.9064	0.001	21.9968	0.001	21.4769	0.000
x_1^2	-0.0027	0.007	-3.0570	0.066	-1.1074	0.001
x_2^2	0.0407	0.833	-0.3916	0.014	-0.6331	0.000
x_3^2	-4.2878	0.002	-2.5648	0.002	-1.6015	0.001
x_1x_2	-0.0004	0.968	-0.2294	0.550	-0.5973	0.000
x_1x_3	0.0444	0.084	-0.990	0.226	-1.4107	0.001
x_2x_3	0.2930	0.444	-0.3549	0.144	-0.5766	0.000

x_1: reaction time; x_2: methanol :FFA; x_3: catalyst

1.4 Esterification of raw material with initial 50% FFA content

1.4.1 Model fitting of esterification of raw material with initial 50% FFA content

Response surface regression procedure was used to fit second-order polynomial equation (10). Moreover, Experimental design and results are in Table 24. From Table 25, any P-value of terms; linear, square, interaction and pure error was lower than 0.05 which indicated that the model was suitable for use in this experiment. According to Table 30, the P-values for specific terms of initial 50% FFA were shown. From this table, the only square term of catalyst loading was not significant as it was higher than 0.05. The coefficient of determination (R^2) that indicated the accuracy and general availability of the polynomial model were adequate as shown in Table 25. R^2 value was 99.4% of variability in the assay that should be at least 75%. Then, F-value from the response was 89.85. Moreover, the F-value of linear term is the highest. This value is quite high compared with reference F-value. This indicated that the predicted model yielded good prediction on conversion of FFA at high confident level of 95%. The second order predicted model of initial 50% FFA is shown in equation (17). The photo of the products is shown in Appendix Figure C4.

$$\begin{aligned} Y = 22.406 + 10.2122x_1 + 8.1831x_2 + 10.0081x_3 - 0.5053x_1^2 - 0.267x_2^2 \\ - 0.4637x_3^2 - 0.3896x_1x_2 - 0.7227x_1x_3 - 0.407x_2x_3 \end{aligned} \qquad (17)$$

Where each variable was the following; Y: the % conversion of FFA, x_1: the reaction time, x_2: methanol-to-FFA molar ratio, x_3: H_2SO_4 catalyst loading.

Table 24 Experimental design matrix and result from esterification of the mixture of PFAD and PS with initial 50% FFA content.

Run no	Time (h) (x_1)	Methanol : FFA (x_2)	Catalyst (wt%) (x_3)	Observed Conversion of FFA (%)	Predicted Conversion of FFA (%)
1	6	3:1	2.5	88.54	88.851
2	1	7.5:1	4	93.57	93.060
3	1	7.5:1	1	81.74	81.318
4	3.5	3:1	1	76.63	75.809
5	3.5	7.5:1	2.5	96.27	96.257
6	3.5	12:1	1	96.75	97.484
7	3.5	7.5:1	2.5	96.24	96.257
8	6	12:1	2.5	97.51	96.266
9	3.5	3:1	4	88.36	87.626
10	6	7.5:1	1	95.96	96.470
11	1	3:1	2.5	69.11	70.354
12	3.5	12:1	4	97.49	98.311
13	3.5	7.5:1	2.5	96.26	96.257
14	1	12:1	2.5	95.61	95.299
15	6	7.5:1	4	96.95	97.373

Table 25 ANOVA for conversion for FFA (%) from esterification of the mixture of PFAD and PS with initial 50% FFA content.

Source	df	Sum of squares	F	P	R^2
Model	9	1065.9	89.85	0.000	99.4%
Linear	3	792.9	106.14	0.000	
Square	3	136.53	34.53	0.001	
Interaction	3	136.40	34.49	0.001	
Pure error	2	0.00			
Total error	14	1072.5			

1.4.2 The effect of parameters on % FFA conversion of esterification of raw material with initial 50% FFA content.

The predicted model was plotted as a three-dimensional surface that represented the response (% conversion of FFA) as a function of other two factors. In Figure 24a, % conversion of FFA caused by methanol was significantly higher than that of catalyst concentration especially at low molar ratio of methanol to FFA. According to Figure 24b, the molar ratio of methanol to FFA had more effect on the % conversion of FFA than that of reaction time. The influence of catalyst concentration and reaction time on % conversion was shown in Figure 24c. At low level of catalyst, reaction time had strong effect on % conversion. As level of catalyst increased, influence of reaction time decreased. Reaction trended to reverse at high level of variables, namely at high molar ratio of methanol to FFA after around 2.5 wt% catalyst (Figure 24a), at high molar ratio of methanol to FFA after around 4 h of reaction (Figure 24b) and at high wt% catalyst after around 4 h of reaction. According to Figure 24a-c, it was found out that the molar ratio of methanol to FFA was the most important factor influencing on conversion. The letter factor was catalyst loading and the last effected factor was reaction time. From the predicted model, the optimal condition for esterification of raw material with 50% initial FFA content were 11.8:1 molar ratio of methanol to FFA, 3.7 wt% of sulfuric acid (based on FFA) and 5.3 h of reaction time respectively. According to Figure 25, methanol quantity and reaction time could give the optimum point clearly. Although the catalyst loading factor had a little deviation, its optimum point could be still estimated. To check the predicted value and experimental value for esterification of raw material with 50% initial FFA content reaction was taken by using this optimum condition to confirm 96%. The product from experimental esterification (% conversion of FFA) was 95.4% with 0.6% difference which was neglectable value as presented in Table 26.

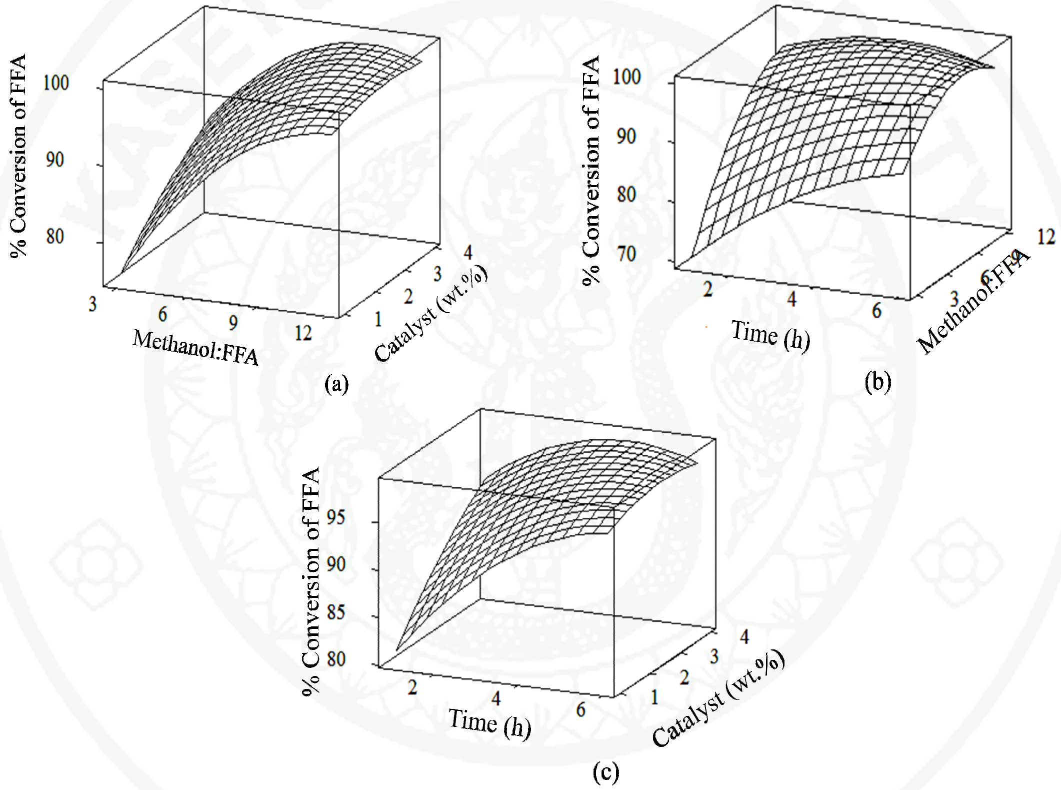


Figure 24 Response surface plots for % conversion of FFA of esterification of raw material with initial 50% FFA content.

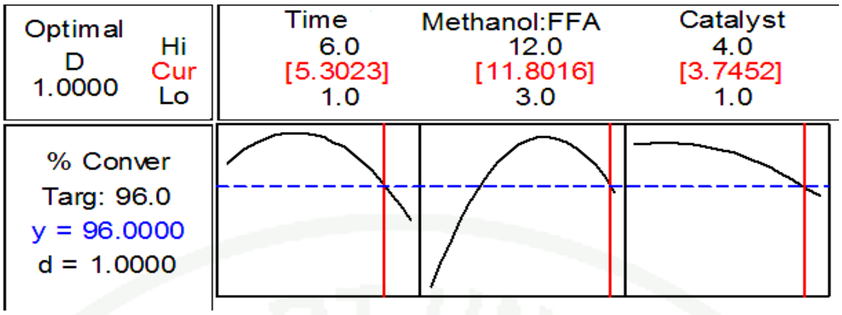


Figure 25 Optimum condition for initial 50% FFA esterification.

Table 26 Comparison of conversion of FFA (%) between predicted and experimental values for esterification of raw material with initial 50% FFA content.

Predicted value	Experimental value	Difference
96%	95.4%	0.6%

1.5 Esterification of raw material with initial 60% FFA content

1.5.1 Model fitting of esterification of raw material with initial 60% FFA content

The experimental design and results of esterification of raw material with initial 60% FFA content are shown in Table 27. The predicted model is expressed in equation (18) and the ANOVA is shown in Table 28. In this table, the suitability of the fit of the polynomial model equation (10) is tested by R^2. In our case, the R^2 was 99.3% which was high enough. The set of mathematical model was only considered satisfactory when the ANOVA data showed a high level of statistical significance (favorable adjustment values), with F-value within 95% confidence level and P-values <0.005. All of the P-values were lower than 0.05 as shown in Table 28 and 30 and F values were higher than theoretical limit as described in esterification of raw material with initial 5% FFA content. In Table 30, the P-values for all specific terms were lower than 0.05 except the square term of catalyst loading. Moreover, some of the P-value from that Table could give up to 99 % confidence level which is equivalent to P-value < 0.01. This indicated that the proposed model gave good prediction on conversion of FFA at high confidence level of 95%. The photo of products is shown in Appendix Figure C5.

$$Y = 31.4465 + 8.5062x_1 + 7.4903x_2 + 8.85x_3 - 0.3923x_1^2 - 0.2479x_2^2 - 0.3574x_3^2 - 0.3473x_1x_2 - 0.6193x_1x_3 - 0.3904x_2x_3 \quad (18)$$

Where Y is conversion of FFA and x_1, x_2, x_3 are reaction time (h), molar ratio of methanol to FFA, and catalyst (wt% based on FFA).

Table 27 Experimental design matrix and result from esterification of the mixture of PFAD and PS with initial 60% FFA content.

Run no	Time (h) (x_1)	Methanol : FFA (x_2)	Catalyst (wt%) (x_3)	Observed Conversion of FFA (%)	Predicted Conversion of FFA (%)
1	1	7.5:1	4	94.99	94.684
2	3.5	3:1	4	90.15	89.333
3	6	3:1	2.5	89.91	90.024
4	3.5	7.5:1	2.5	96.68	96.683
5	3.5	3:1	1	78.58	78.160
6	3.5	7.5:1	2.5	96.68	96.683
7	3.5	7.5:1	2.5	96.69	96.683
8	6	7.5:1	4	97.37	98.074
9	3.5	12:1	1	96.84	97.658
10	6	12:1	2.5	97.56	96.436
11	1	7.5:1	1	84.84	84.136
12	1	12:1	2.5	96.33	96.216
13	1	3:1	2.5	73.05	74.174
14	6	7.5:1	1	96.51	96.816
15	3.5	12:1	4	97.87	98.290

Table 28 ANOVA for conversion for FFA (%) from esterification of the mixture of PFAD and PS with initial 60% FFA content.

Source	df	Sum of squares	F	P	R^2
Model	9	823.206	84.39	0.000	99.3%
Linear	3	603.645	102.1	0.000	
Square	3	109.137	33.57	0.001	
Interaction	3	110.423	33.96	0.001	
Pure error	2	0.00	0.00		
Total error	14	828.625			

1.5.2 The effect of parameters on % FFA conversion of esterification of raw material with initial 60% FFA content

The response surface analysis was carried out by keeping two independent variables at constant level and changing the other two independent variables (Bauaid *et al*., 2007). The relationship between methanol amount and catalyst loading at 4 h is shown in Figure 26a. The methanol amount was highly influence in conversion of FFA than that of catalyst loading. When methanol amount used was low, the catalyst loading slightly affected on the conversion of FFA. Figure 26b shows that methanol amount had more influence on the conversion of FFA than that of reaction time. Especially, the molar ratio of methanol to FFA around 10:1 gives the highest conversion of FFA during 5 h of reaction. If both methanol amount and reaction time were increased, the conversion also increased. However, the reaction reversed after 5 h that it might due to water formation during esterification. The effect of reaction time and catalyst loading on the conversion of FFA is shown in Figure 26c. In this Figure, if both catalyst loading and reaction time were increased, the high conversions of FFA were obtained. However, catalyst loading was more influence on conversion of FFA than that of reaction time particularly at low catalyst loading. In conclusion, the methanol amount and catalyst loading were important factor compared with reaction time in esterification. In this Figure 27, the optimum points for initial 60% FFA esterification were shown. The optimum point for methanol quantity and reaction time were clearly seen but the catalyst loading was hard to estimate. The optimum condition for esterification of raw material with initial 60% FFA content to reduce FFA to 2% was molar ratio of methanol to FFA of 11.6:1, catalyst based on FFA of 3.5 wt% and reaction time of 5.4 h.

To adopt the different between the predicted optimal and experimental value, esterification reaction was taken by using this optimum condition to confirm 96.7%. The product from experimental esterification (% conversion of FFA) was 96.2% with 0.5% difference and could be considered as neglected value as shown in Table 29.

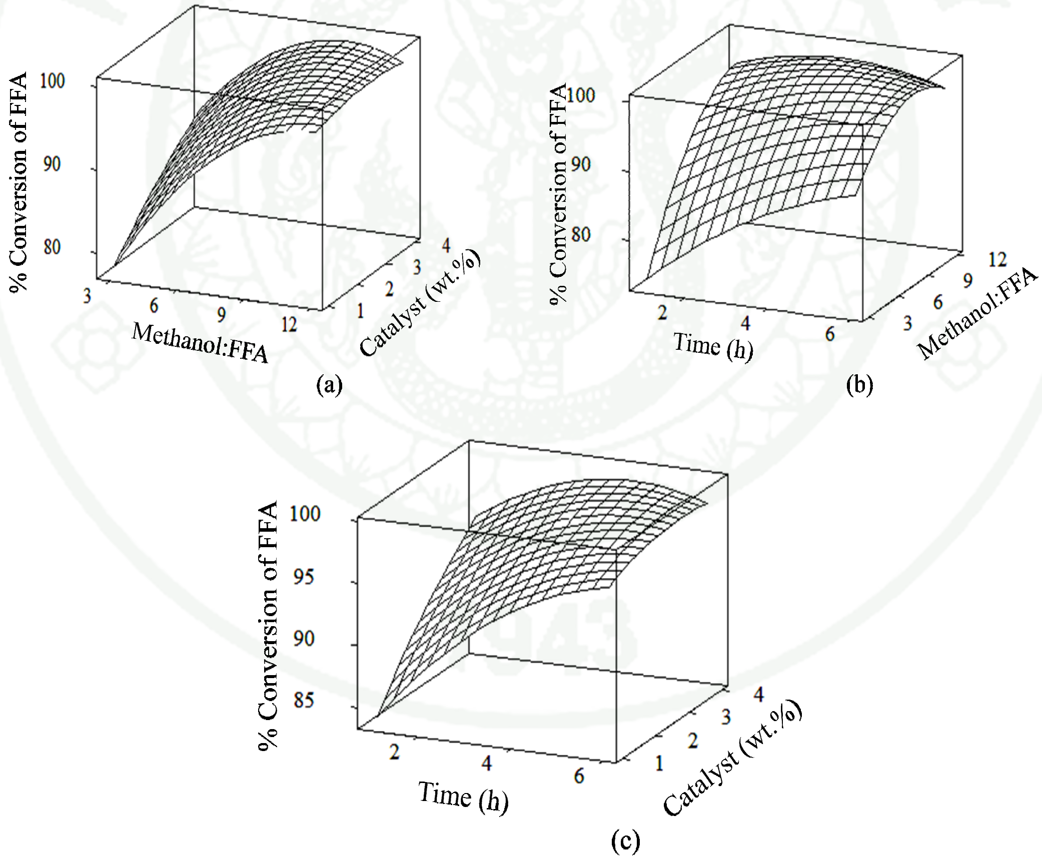


Figure 26 Response surface plots for % conversion of FFA of esterification of raw material with initial 60% FFA content.

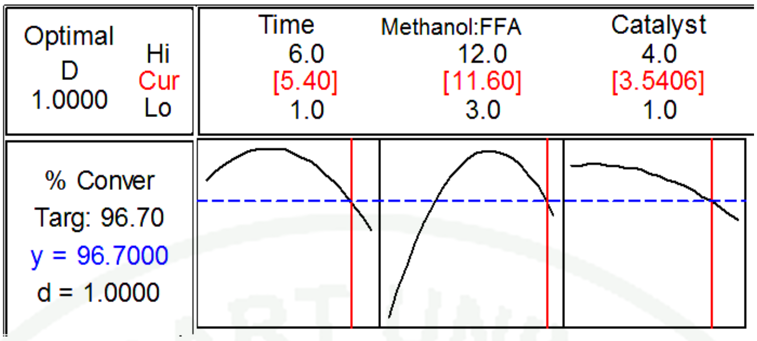


Figure 27 Optimum condition for initial 60% FFA esterification.

Table 29 Comparison of conversion of FFA (%) between predicted and experimental values for esterification of raw material with initial 60% FFA content.

Predicted value	Experimental value	Difference
96.7%	96.2%	0.5%

According to results from the esterification of raw material with specific initial FFA content, the order of the important factor effecting on conversion of FFA can be concluded as shown in Table 31. The methanol quantity is the most important factor for almost all of initial FFA level esterification except initial 5% FFA esterification as this reaction is reversible reaction so that it needs to force by using large quantity of methanol to become forward reaction.

Table 30 Coefficient terms and P-values for specific initial 50 and 60% FFA esterification models.

Term	Initial 50% FFA		Initial 60% FFA	
	Coefficient	P	Coefficient	P
constant	22.4060	0.001	31.4465	0.000
x_1	10.2122	0.000	8.5062	0.000
x_2	8.1831	0.000	7.4903	0.000
x_3	10.0081	0.001	8.8500	0.002
x_1^2	-0.5053	0.003	-0.3923	0.006
x_2^2	-0.2670	0.000	-0.2479	0.000
x_3^2	-0.4637	0.141	-0.3574	0.198
x_1x_2	-0.3896	0.001	-0.3473	0.001
x_1x_3	-0.7227	0.005	-0.6193	0.007
x_2x_3	-0.4070	0.005	-0.3904	0.004

x_1: reaction time; x_2: methanol :FFA; x_3: catalyst

Table 31 Order of the important factor affecting on conversion of FFA.

Initial FFA	Order of important factors
5%	Catalyst loading > Time > Methanol amount
20%	Methanol amount > Catalyst loading > Time
40%	Methanol amount > Time > Catalyst loading
50%	Methanol amount > Catalyst loading > Time
60%	Methanol amount > Catalyst loading > Time

2. Optimization of esterification with various levels of high FFA content of the mixture of PFAD and PS

2.1 Three-level-four-factor design for initial 5 to 87% FFA (overall model)

In order to get the optimum condition for various initial FFA content, the four-factor-three-level Box-Behnken design had been developed. Many researchers proposed the optimum condition of fixed initial FFA raw material but no report has been published in various initial FFA raw materials. Therefore, in this design, initial 5-87% FFA raw oil was used. The experimental design and results are shown in Table 32. The estimated model (based on actual value) is shown in equation (19). The ANOVA for conversion of FFA is shown in Table 33. In this table, every P-values were less than 0.05 which indicated that each term of this model was highly significant within 95% confidence level. Moreover, the F-values were high enough. Therefore, this model was good fitted model. The R^2 was 99.5% which indicated that the accuracy and general availability of the polynomial model were also good. Nevertheless, analysis of response trends using the model was considered to be reasonable. Finally, confirmatory runs at the estimated optimal operating condition were performed. The photo of products is shown in Appendix Figure C6.

$$\begin{aligned} Y = {} & -32.1093 + 18.6091x_1 + 10.4279x_2 + 18.2785x_3 + 0.8653x_4 - \\ & 1.0639x_1^2 - 0.4135x_2^2 - 1.4272x_3^2 - 0.0042x_4^2 - 0.3519x_1x_2 - \\ & 0.9558x_1x_3 - 0.0763x_1x_4 - 0.3889x_2x_3 + 0.0018x_2x_4 - \\ & 0.0509x_3x_4 \end{aligned} \tag{19}$$

Where Y is conversion of FFA, x_1, x_2, x_3 and x_4 are reaction time (h), molar ratio of methanol to FFA, catalyst (wt% based on FFA), and initial FFA (%).

From Figure 28a, the amount of methanol had positive effect on both conversion and initial FFA at initial FFA of 40-50%. The molar ratio of methanol to FFA of 10:1 at the 40% initial FFA gave the maximum conversion of FFA above 98%. Figure 28b presents that reaction time had strongly effect on the conversion of

FFA at lower initial FFA content of around 5-40%. However, effectiveness of FFA on time was reversed on the conversion of FFA at the higher level of initial FFA due to water formation during esterification reaction. Figure 28c shows the relationship between the catalyst loading and reaction time affecting on conversion of FFA at the initial 46% FFA and molar ratio of methanol to FFA of 6.6:1. From this figure, the reaction time and catalyst loading had slightly effect on conversion of FFA. The highest conversion of FFA was given at the amount 3% catalyst and 4.5 h. Figure 28d indicated that the methanol amount had greater influence on the conversion of FFA than that of reaction time. The maximum conversion of FFA was achieved under 9:1 molar ratio of methanol to FFA at 5 h. Figure 28e shows that methanol amount had geater influence on the conversion of FFA than that of catalyst loading. Then Figure 28f presents that the catalyst loading which had positive effect on the conversion of FFA when the initial FFA was 5-40%. However, the conversion of FFA was reversed when initial FFA was over 40%.

Table 32 Three-level-four-factor Box-Behnken design for initial 5-87% FFA.

Run No	Time (h) x_1	Methanol:FFA x_2	Catalyst (wt%) x_3	Initial FFA (%) (x_4)	Observed Conversion (%)	Predicted Conversion (%)
1	3.25	1.2:1	2.25	87	64.1	65.933
2	0.5	6.6:1	4.00	46	85.2	85.100
3	3.25	12:1	2.25	5	89.3	87.933
4	3.25	1.2:1	2.25	5	58.0	55.967
5	3.25	6.6:1	2.25	46	96.4	96.400
6	0.50	6.6:1	2.25	5	55.8	57.154
7	3.25	6.6:1	4	87	94.6	93.479
8	3.25	1.2:1	0.5	46	53.8	53.188
9	6	6.6:1	4	46	97.5	96.317
10	0.50	1.2:1	2.25	46	45.1	44.479
11	6	6.6:1	0.5	46	91.5	92.067
12	3.25	6.6:1	0.5	5	69.0	69.263
13	0.5	6.6:1	2.25	87	86.4	85.121
14	6	6.6:1	2.25	87	89.3	88.338
15	3.25	6.6:1	2.25	46	96.4	96.400
16	3.25	6.6:1	4	5	89.9	90.013
17	3.25	6.6:1	2.25	46	96.4	96.400
18	6	12:1	2.25	46	97.9	97.663
19	3.25	1.2:1	4	46	72.7	73.988
20	6	1.2:1	2.25	46	75.2	75.346
21	6	6.6:1	2.25	5	93.1	94.771
22	3.25	12:1	4	46	98.4	99.404
23	0.5	6:1	0.50	46	60.8	62.450
24	0.5	12:1	2.25	46	88.7	87.696
25	3.25	12:1	2.25	87	97.0	99.500
26	3.25	6.6:1	0.5	87	88.3	87.329
27	3.25	12:1	0.5	46	94.2	93.304

Table 33 ANOVA for the conversion of FFA (%) of initial 5-87% FFA.

Source	df	Sum of squares	F	P	R^2
Model	14	6864.87	168.84	0.000	99.5%
Linear	4	5361.96	173.34	0.000	
Square	4	905.28	77.93	0.000	
Interaction	6	597.64	34.30	0.000	
Pure error	2	0.00			
Total error	26	6899.72			

Table 34 Coefficient and P-value for initial 5-87 % FFA esterification overall model.

Term	Coefficient	P
Constant	-32.1041	0.000
x_1	18.6091	0.000
x_2	10.4279	0.000
x_3	18.2785	0.000
x_4	0.8653	0.000
x_1^2	-1.0639	0.000
x_2^2	-0.4135	0.000
x_3^2	-1.4272	0.000
x_4^2	-0.0042	0.000
x_1x_2	-0.3519	0.000
x_1x_3	-0.9558	0.000
x_1x_4	-0.0763	0.000
x_2x_3	-0.3889	0.001
x_2x_4	0.0018	0.647
x_3x_4	-0.0509	0.001

x_1: reaction time; x_2: methanol:FFA; x_3: catalyst; x_4: initial FFA (%)

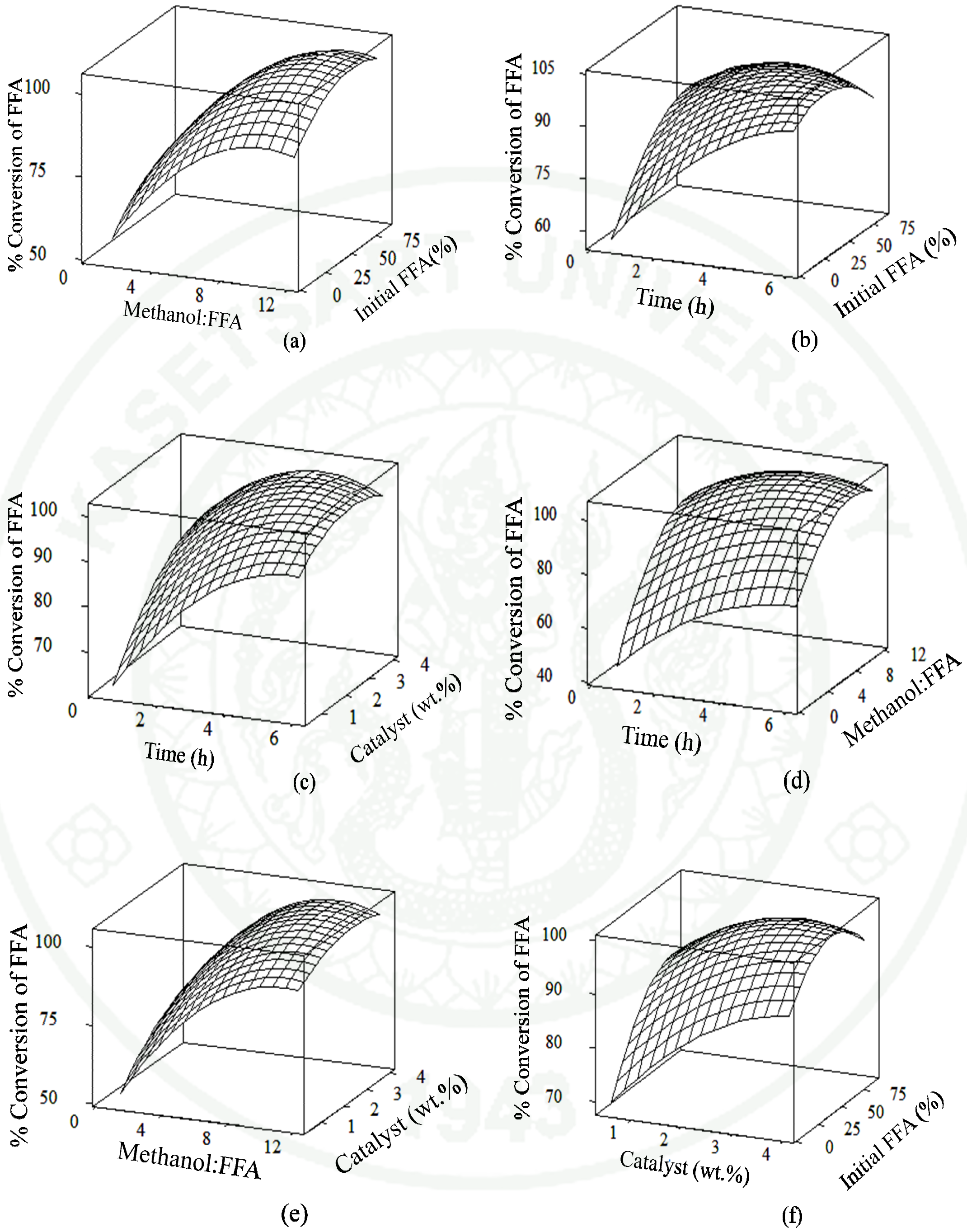


Figure 28 Response surface plots for % conversion of FFA of initial 5-87% FFA esterification.

3. Correlation of the specific initial FFA esterification models with four factors various initial FFA esterification overall model

According to the correlation of the specific initial FFA model with four factors various initial FFA overall model, the independent factors such as molar ratio of methanol to FFA, reaction time, catalyst loading of specific initial FFA model are put into the equation (19). Then, the calculated data are expressed in Table 36. From this Table, the values of the predicted models of specific initial FFA and the calculated values from predicted overall model of various FFA were all well fixed with the models of initial FFA over 40% up to 60%.

In this study, the 95% confidence level was chosen for all statistical models. According to Table 35, the percentage of error of initial 40, 50 and 60 % FFA esterification were less than 5%. Therefore, the overall model could be used to predict the optimum conditions for initial 40% FFA up to 60% esterification.

Table 35 Correlated the optimum conditions of specific FFA models with various FFA overall model.

Initial FFA (%)	Time (h)	Methanol:FFA	Catalyst (wt %)	Experimental Conversion (%)	Predicted Conversion (%) predicted with overall model	Error (%)
5	1.5	4.6	3	79.6	65	18.3
20	3	4.6	2.4	94.3	82	13
40	5.4	11.6	3.3	97	99	-2
50	5.3	11.8	3.7	95.4	96.6	-1.3
60	5.4	11.6	3.5	96.2	95.7	0.5

The overall model could not predict the optimum condition for the raw material with initial FFA under 40%. According to Table 31, changing in methanol quantity was the most important factor for almost all of esterifications from various raw materials. Large quantity of methanol was needed to ensure the completion of reaction.

If the minimum and maximum points of reaction factors were too far from an optimum point from ANOVA, these points could not affect on the reaction. From this reason, in model for raw material with initial 5% FFA content, used methanol quantity according to selected range (based on FFA) was low but the additional amount (based on triglyceride) was quite large amount, therefore, as the total quantity of methanol was too much, it could not affect on the reaction.

On the other hand, if one of the minimum and maximum points of reaction factors was near the optimum point but the other was far from there (ca. 0.025 wt% in the mixture), the changing in value between these two points would influence on the reaction. According to the previous reason, both maximum and minimum concentrations of catalyst were low but its maximum amount (ca. 0.2 wt%) was near the optimum point (ca. 2 wt%),therefore, the changing in catalyst loading would be the most important factor on the esterification reaction of raw material with initial 5% FFA content.

As long as the ranges of factors of both overall and specific models covered the optimum point, the same result could be obtained. The specific model could give the higher accuracy than the overall model did because the ranges of the factors of specific model were narrower than those of overall model. However, in the specific model for raw material with initial 20% FFA content, the methanol quantity range could not cover the optimum point; therefore, it could cause error.

Table 36 Correlation of the specific initial FFA esterification models with various initial FFA esterification overall model.

Run No	5%		20%		40%		50%		60%	
	Specific FFA Model	Overall FFA Model	Specific FFA Model	Overall FFA Model	Specific FFA Model	Overall FFA Model	Specific FFA Model	Overall FFA Model	Specific FFA Model	Overall FFA Model
1	28.847	14.419	79.366	65.696	33.174	55.426	88.851	85.868	94.684	93.3953
2	93.538	78.453	93.976	80.484	60.561	74.359	93.060	91.945	89.333	85.579
3	77.486	48.62	68.958	55.569	66.412	74.551	81.318	77.718	90.024	84.055
4	92.152	81.032	93.976	80.484	95.477	97.724	75.809	73.318	96.683	100
5	81.846	82.060	76.563	72.814	34.914	56.275	96.257	100	78.160	74.285
6	53.282	61.931	73.620	49.331	88.795	91.576	97.484	97.582	96.683	100
7	12.568	26.424	94.485	85.475	93.365	93.316	96.257	100	96.683	100
8	70.948	47.35	93.976	80.484	95.736	97.893	96.266	96.961	98.074	94.016
9	36.335	56.36	93.706	91.201	61.351	74.993	87.626	85.633	97.658	98.726
10	71.337	59.54	82.825	79.412	98.566	98.784	96.470	96.632	96.436	95.326
11	42.027	22.29	59.218	40.966	95.477	97.724	70.354	66.20	84.136	80.758
12	71.337	59.54	84.173	67.46	95.476	97.724	98.311	99.397	96.216	95.463
13	55.480	59.203	99.084	96.377	96.368	95.297	96.257	100	74.174	68.353
14	71.337	59.54	97.020	96.914	92.589	94.19	95.299	93.129	96.816	95.703
15	31.995	52.36	93.031	80.641	95.998	96.971	97.373	96.5345	98.299	98.951

4. Optimization for transesterification of final % FFA after esterification

According the results from the specific initial FFA esterification reaction, initial 5 – 40 % FFA esterifications could be reduced the FFA to 1% within only one step. The rest of two models 50% and 60% could only be reduced the FFA to 2% in one step, individually. Therefore, for transesterification optimization, the mixture of PFAD and PS of initial 1% and 2% FFA content were used.

4.1 Transesterification of raw material with initial 1% FFA

Both experimental design and results are shown in Table 37.

4.1.1 Model fitting of transesterification of raw material with initial 1% FFA

Based on the Box-Behnken design and results of experiments (Table 37), the quadratic regression model (based on the uncoded factors) of the experiment data was given in equation (20):

$$Y = 32.1549 + 0.202x_1 + 7.6907x_2 + 44.0025x_3 - 0.0004x_1^2 - 0.2563x_2^2 - 8.2786x_3^2 - 0.0097x_1x_2 - 0.0621x_1x_3 - 2.1453x_2x_3 \quad (20)$$

Where *Y* is the % conversion of triglyceride, x_1 is the reaction time, x_2 is methanol-to-triglyceride molar ratio and x_3 is KOH catalyst loading. The statistical significance of the model equation was evaluated by the F-test for ANOVA which showed that the regression was statistically significant at 95% confidence level as shown in Table 38. To test the accuracy of model, the regression equation and determination coefficient (R^2) were evaluated. The value of $R^2 = 0.954$ indicated that this model could explain 95.4% of the variability. All of P-values were lower than 0.05 which meant this predicted model gave the high accuracy but the almost all terms of reaction time were slightly higher than that limit presented in Table 42. The photo of the products is shown in Appendix Figure C7. GC results for initial 1% FFA transesterification are shown in Appendix A1-15.

Table 37 Experimental design matrix and result from transesterification of the mixture of PFAD and PS with initial 1% FFA content.

Run no	Time (min) (x_1)	Methanol:Triglyceride (x_2)	Catalyst (wt%) (x_3)	Observed FAME (%)	Predicted FAME (%)
1	75	8.25:1	0.85	97.247	97.449
2	30	8.25:1	1.2	98.297	98.868
3	75	4.5:1	0.5	82.317	83.936
4	120	12:1	0.85	94.073	94.073
5	120	8.25:1	1.2	97.13	97.641
6	120	8.25:1	0.5	94.87	94.299
7	30	8.25:1	0.5	92.123	91.612
8	75	8.25:1	0.85	97.23	97.449
9	75	8.25:1	0.85	97.87	97.449
10	75	12:1	1.2	97.713	96.094
11	30	12:1	0.85	96.66	97.708
12	120	4.5:1	0.85	92.627	91.579
13	75	12:1	0.5	96.963	96.426
14	30	4.5:1	0.85	88.7	87.592
15	75	4.5:1	1.2	94.33	94.867

Table 38 ANOVA for FAME (%) from transesterification of the mixture of PFAD and PS with initial 1% FFA content.

Source	df	Sum of squares	F	P	R^2
Model	9	248.379	11.59	0.007	95.4
Linear	3	151.315	17.01	0.005	
Square	3	50.912	7.12	0.03	
Interaction	3	46.152	6.46	0.036	
Pure error	2	0.266			
Total error	14	260.289			

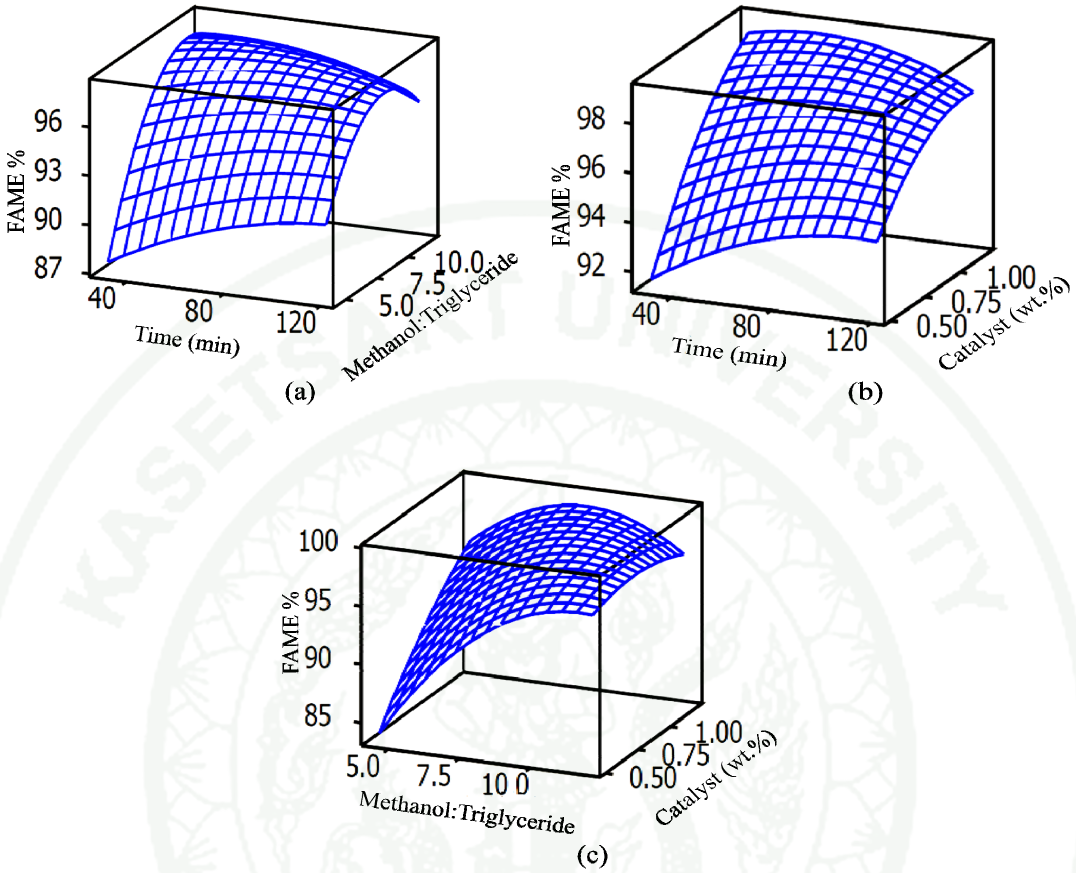
4.1.2 Relationship among the effects of parameters of transesterification of raw material with initial 1% FFA

All these plots in Figure 29 show similar relationships with respect to the effect of each variable. The response surface showing predicted response surface of % conversion of triglyceride as a function of methanol-to-triglyceride molar ratio and reaction time are shown on Figure 29a. It showed that the % conversion of triglyceride was sensitive to the methanol quantity especially at low level of reaction time. If methanol ratio increased at initial part of reaction time, the % conversion of triglyceride also increased. However, the % conversion of triglyceride decreased after methanol ratio and reaction time reached 7.5:1 and 80 min. It might be due to side reaction of saponification. Figure 29b shows the effect of catalyst loading and reaction time. At low reaction time, high catalyst concentration caused high %FAME content. The % conversion of triglyceride was affected slightly by reaction time and catalyst loading. The effect of catalyst concentration and methanol ratio on the % conversion of triglyceride is provided in Figure 29c. At low amounts of catalyst, % conversion of triglyceride was highly affected by the methanol ratio. There was no significant effect of catalyst loading on % conversion of triglyceride. Under such conditions, methanol-ratio about 8:1 gave the highest conversion. In addition, the interaction effects of all variables were significant as shown in Table 31. In conclusion, the molar ratio of methanol to triglyceride was more important factor than that of the rest two which the second important factor was catalyst and the last one was reaction time.

The optimum points of initial 1% FFA transesterification were shown in Figure 30. Form this Figure, optimum points of the reaction time and methanol quantity could be precisely seen but the catalyst loading might be needed more as it was still increasing. The optimal values of the selected variables were obtained by solving the regression equation (10) using Box-Behnken design for transesterification from the oil mixture with 1% FFA content as following: methanol-to-triglyceride molar ratio 8.25:1, KOH catalyst loading 0.8 wt % and reaction time of 75 min. Theoretical conversion to triglyceride (biodiesel) predicted under the condition was 97%. In order to confirm the reliability of the model, when the above optimal conditions were applied to the real experiment, the conversion of triglyceride was 96.7% which was much closed to the predicted response and verified the efficiency of the predicted model as expressed in Table 39.

Table 39 Comparison of %FAME between predicted and experimental values for transesterification of raw material with initial 1% FFA.

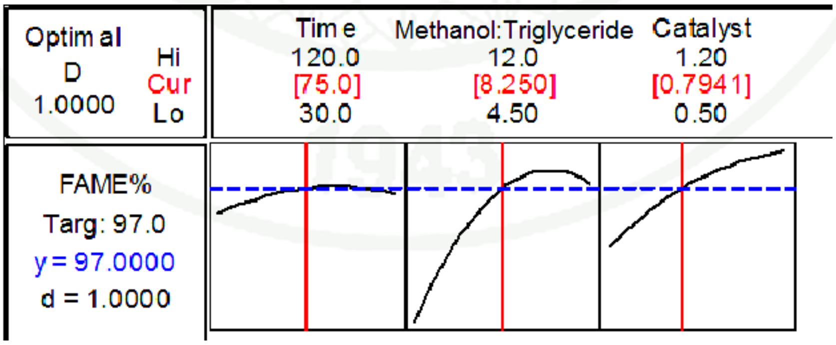
Predicted value	Experimental value	Difference
97%	96.7%	0.3%



(a) (b)

(c)

Figure 29 Response surface plots for FAME% from the mixture of PFAD and PS with initial 1% FFA content transesterification.


Optimal
D
1.0000
Hi
Cur
Lo

Time
120.0
[75.0]
30.0

Methanol:Triglyceride
12.0
[8.250]
4.50

Catalyst
1.20
[0.7941]
0.50

FAME%
Targ: 97.0
y = 97.0000
d = 1.0000

Figure 30 Optimum condition for initial 1% FFA transesterification.

4.2. Transesterification of raw material with initial 2% FFA

Both experimental design and results are shown in Table 40.

4.2.1 Model fitting of transesterification of raw material with initial 2% FFA

The ANOVA for the response surface linear model is provided in Table 41. The predicted model, equation (21), was the highly significant due to P-value of 0.007. However, the P-value for linear term of methanol quantity, square of methanol quantity and reaction time and interaction term of reaction time with methanol quantity and catalyst were slightly higher than 0.05 as shown in Table 43 . However, the full predicted should be used to estimate optimum value as all of the terms were related each other. To test the fitness of the model, the regression equation (21) and the determination coefficient (R^2= 0.954) indicate that the sample variation of 95.4% for the conversion of FFA was attributed to the independent variables selected (x_1, x_2) and 4.6% of the total variation were not explained by the model. Moreover, F-value of predicted model was quite high compared with tabulated value as expressed in esterification of raw material with initial 5% FFA. The photo of the products is shown in Appendix Figure C8. GC results for transesterification of raw material with initial 2% FFA are shown in Appendix B1-15.

$$Y = -189.554 + 0.62x_1 + 15.585x_2 + 378.441x_3 - 0.003x_1^2 - 0.168x_2^2 - 125.439x_3^2 - 0.22x_1x_3 - 11.49x_2x_3 \tag{21}$$

Where Y is %FAME and x_1, x_2, x_3 are reaction time (min), molar ratio of methanol to triglyceride and catalyst (wt% based on triglyceride).

Table 40 Experimental design matrixes and result from transesterification of the mixture of PFAD and PS with initial 2% FFA content.

Run no	Time (min) (x_1)	Methanol : Triglyceride (x_2)	Catalyst (wt%) (x_3)	Observed FAME (%)	Predicted FAME (%)
1	30	8.25:1	1.2	95.77	95.734
2	75	8.25:1	0.85	96.23	96.140
3	30	8.25:1	0.5	47.96	51.084
4	120	8.25:1	1.2	96.16	93.036
5	120	4.5:1	0.85	83.01	79.075
6	75	12:1	1.2	97.6	93.701
7	75	4.5:1	0.5	29.06	32.959
8	30	12:1	0.85	93.93	97.865
9	75	8.25:1	0.85	96.15	96.140
10	120	8.25:1	0.5	62.19	62.226
11	75	12:1	0.5	93.19	86.131
12	30	4.5:1	0.85	81.98	74.958
13	75	8.25:1	0.85	96.04	96.14
14	75	4.5:1	1.2	93.79	100
15	120	12:1	0.85	95.17	100

Table 41 ANOVA for FAME (%) for initial FFA 2% content.

Source	df	Sum of squares	F	P	R^2
Model	9	5835.81	11.61	0.007	95.4%
Linear	3	3941.92	13.22	0.008	
Square	3	936.38	5.59	0.047	
Interaction	3	957.52	5.57	0.045	
Pure error	2	0.02			
Total error	14	6115.00			

4.2.2 The effects of parameters of transesterification of raw material with initial 2% FFA

The relationship between the methanol amount and catalyst loading is shown in Figure 31a. Methanol amount had more influence on %FAME than that of catalyst loading. High %FAME was reached at low methanol amount of around 6:1 and 1.2% KOH. Moreover, although the catalyst loading was increased at over methanol amount of 7.5:1, the %FAME was decreased according to soap formation. The effect of reaction time and catalyst loading on %FAME was shown in Figure 31b. The catalyst loading had more influence on %FAME than that of reaction time. The highest conversion of triglyceride was reached at the catalyst loading of 1% KOH and reaction time of 90 min. Figure 31c presents the relationship between the reaction time and methanol amount affected on %FAME. From this Figure, methanol amount had more influence on %FAME than that of reaction time. According to Figure 31a-c, the orders of important factors were the same with transesterification of raw material with initial 1% FFA. At low temperature, if methanol amount increased, %FAME also increased. The reaction time of 90 min gave the highest %FAME at various ratio of methanol amount.

As shown in Figure 32, although the optimum points for reaction time and catalyst loading were clearly estimated, the methanol quantity might be the almost maximum rage as it could not be caught within the selected range. According to the predicted model, the optimum condition of transesterification of raw material with 2% initial FFA was methanol to triglyceride of 12:1, KOH amount of 0.82 wt % and reaction time of 30 min. To validate the deviation between the predicted optimal and experimental value, transesterification reaction was taken by using this optimum condition to confirm 97%. The product from experimental transesterification (%FAME) shown in Table 42 was 96% with 1% difference which can be neglected.

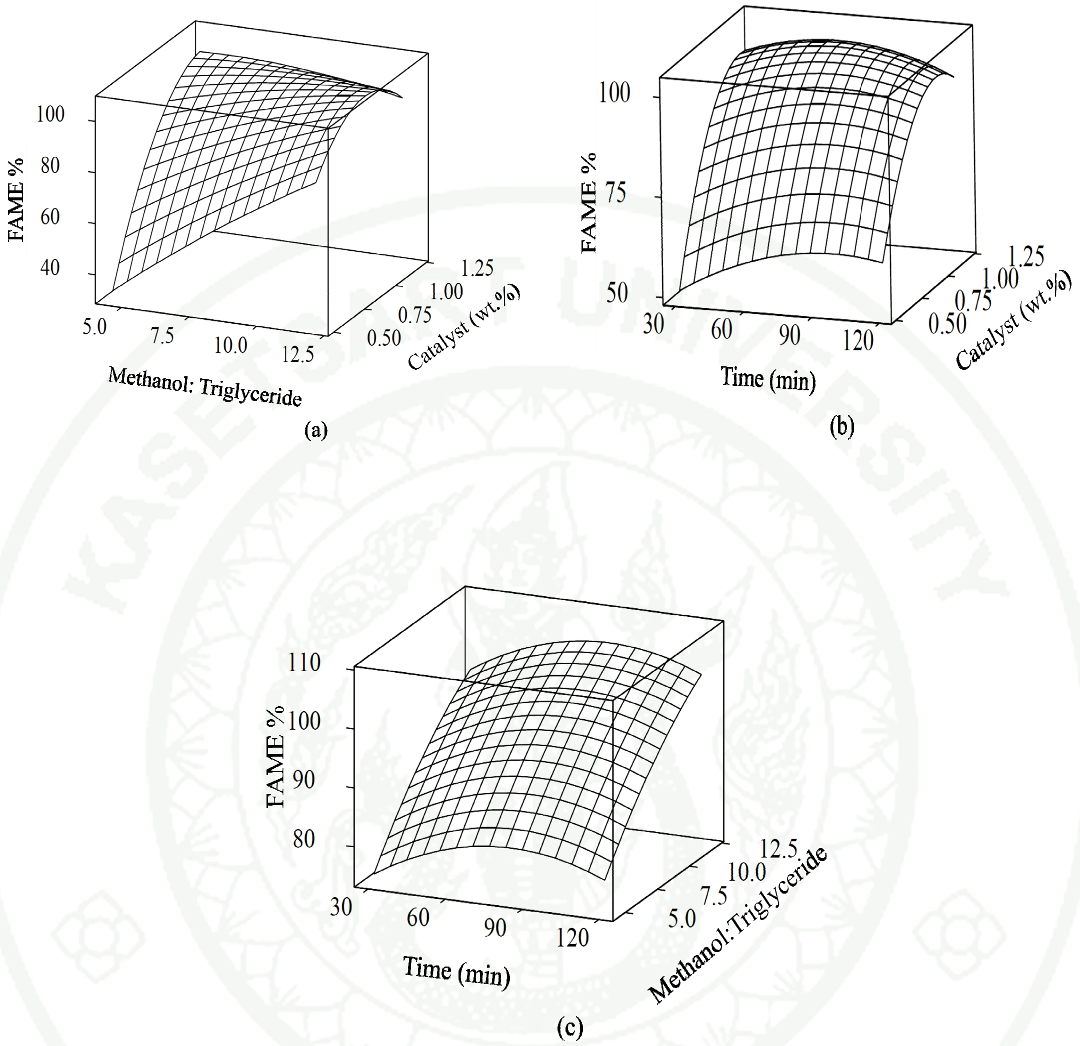
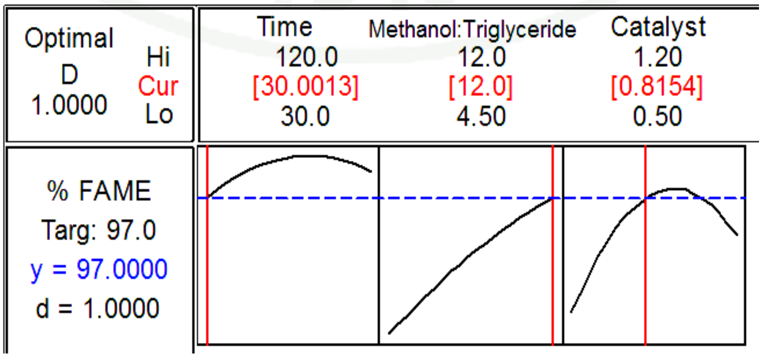


Figure 31 Response surface plots for %FAME from the mixture of PFAD and PS with initial 2% FFA content transesterification.



Optimal
D
1.0000

Hi
Cur
Lo

Time
120.0
[30.0013]
30.0

Methanol:Triglyceride
12.0
[12.0]
4.50

Catalyst
1.20
[0.8154]
0.50

% FAME
Targ: 97.0
y = 97.0000
d = 1.0000

Figure 32 Optimum condition for initial 2% FFA transesterification.

Table 42 Comparison of FAME (%) between predicted and experimental values for transesterification of raw material with initial 2% FFA.

Predicted value	Experimental value	Difference
97%	96%	1%

Table 43 Coefficient and P-value for initial 1 and 2% FFA transesterification.

Term	Initial 1% FFA		Initial 2% FFA	
	Coefficient	P	Coefficient	P
Constant	32.1549	0.015	-189.554	0.007
x_1	0.2020	0.059	0.620	0.182
x_2	7.6907	0.001	15.585	0.036
x_3	44.0025	0.018	373.411	0.002
x_1^2	-0.0004	0.349	-0.003	0.235
x_2^2	-0.2563	0.006	-0.168	0.570
x_3^2	-8.2786	0.262	-125.439	0.011
x_1x_2	-0.0097	0.089	0.000	0.989
x_1x_3	-0.0621	0.261	-0.220	0.397
x_2x_3	-2.1453	0.015	-11.490	0.010

x_1 : reaction time; x_2 : methanol :triglyceride; x_3: catalyst

The order of important factors for both reactions were the same like: methanol quantity > catalyst> reaction time. Therefore, the methanol quantity was the most important factor for both of them. According to the optimum conditions for both initial 1 and 2% FFA transesterification, the catalyst loading that was needed to get 97% FAME were almost the same. However, the reaction time for initial 1% FFA transesterification needed longer than 2% transesterification. On the other hand, the methanol quantity that was needed for initial 2% transesterification was higher than initial 1% transesterification. Compensation as methanol quantity is less in initial 1% FFA transesterification. This kind of nature might be caused as if high methanol quantity was used, the reaction time would be short to get desired product. Another possible reason might be the more methanol quantity is needed when the higher initial FFA transesterification is used instead of more reaction time. Moreover, the P-values of linear, square and interaction terms of reaction time for both reactions were not significant. Thus, both predicted models might not predict the suitable reaction time to get the desired product.

CONCLUSION

Three-level-three-factor Box-Behnken design and RSM were effective to determine the relationship among reaction time, molar ratio of methanol to FFA or triglyceride in the mixture of PFAD and PS and catalyst (wt% based on FFA or triglyceride), and to find the optimum conduction for both esterification and transesterification.

For the esterification, initial 5, 20, 40, 50 and 60% FFA were used to predict the optimum condition of each FFA level. For initial 5% FFA, based on statistical equation, the catalyst amount was the most significant factor. The optimum condition for the first step esterifiation was predicted to be 90 min of reaction time, 4.5:1 molar ratio of methanol to FFA, and 2.9863 wt% (ca. 3 wt%) sulfuric acid based on FFA to reduce FFA from 5% initially to 1%. The optimum condition to reduce FFA from initial 20% FFA to 1% was molar ration of methanol to FFA of 4.61:1 and 2.4 wt% sulfuric acid after 3 h of reaction. The methanol amount was the most important factor for esterification of raw material with initial 20% FFA content.

For esterification of raw material with initial 40% FFA content, the predicted optimum condition from the model to reduce final FFA of 1% was 11.6:1 molar ratio of methanol to FFA, 3.3 wt% sulfuric acid and 5.4 h of reaction. The methanol amount was also the most influence factor. The comparison between esterification of raw material with initial 20 and 40% FFA content, it was found that methanol ratio and time had more effect on the conversion of FFA (%) with increasing FFA content in the raw oil whereas the influence of catalyst dropped down.

In esterification of raw material with initial 50% FFA content, the optimum condition to reduce FFA up to final 2% FFA was molar ratio of methanol to FFA of 11.8:1, sulfuric acid loading of 3.7 wt% (based on FFA) and reaction time of 5.3 h. In this esterification, the methanol amount was the most important factor and catalyst was the second one. The optimum condition for esterification of raw material with initial 60% FFA content to reduce FFA to 2% was molar ratio of methanol of FFA of 11.6:1, reaction time of 5.4 h and catalyst based on FFA of 3.5 wt%. Similarly, the methanol amount of was the most influence factor.

For transesterification of raw material with initial 1 and 2% FFA content, the predicted optimum condition were molar ratio of methanol to triglyceride of 8.25:1, KOH concentration of 0.8 wt% (based on triglyceride) and reaction time of 75 min to achieve 97% FAME (actual 96.7%) of initial 1% FFA and molar ratio of methanol to triglyceride of 12:1, KOH concentration of 0.82 wt% (based on triglyceride) and reaction time of 30 min to get 97% FAME (actual 96%) of initial 2% FFA. Methanol was the most significant factor in initial 1% FFA transesterification. On the other hand, methanol and catalyst were the most influence factors on transesterification of raw material with initial 2% FFA content. The remarkable finding of the important factor for both initial 1 and 2% FFA transesterification were the same order like; methanol amount > catalyst loading > reaction time.

According to three-level-four-factor design of initial 5-87% FFA, the most of the run number of specific designs of esterification of raw material with initial 40, 50 and 60% FFA content were well fitted with the predicted model equation of esterification of raw material with initial 5-87% FFA content. The optimum condition might be use to predict the optimum condition of initial FFA higher than 40%.

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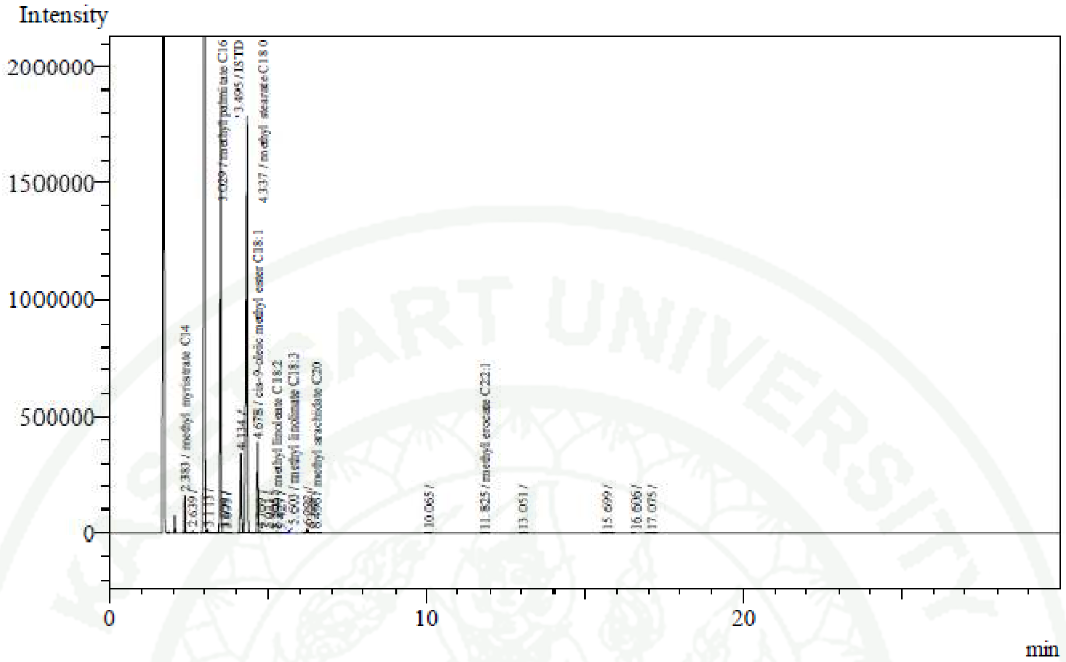
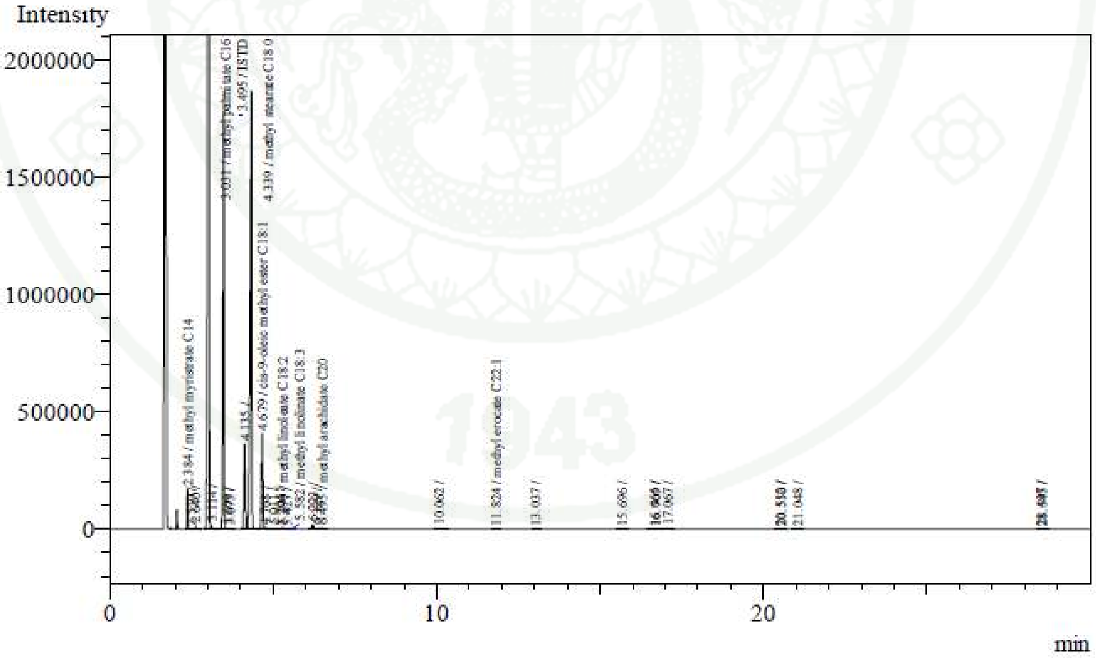
APPENDICES

Appendix A

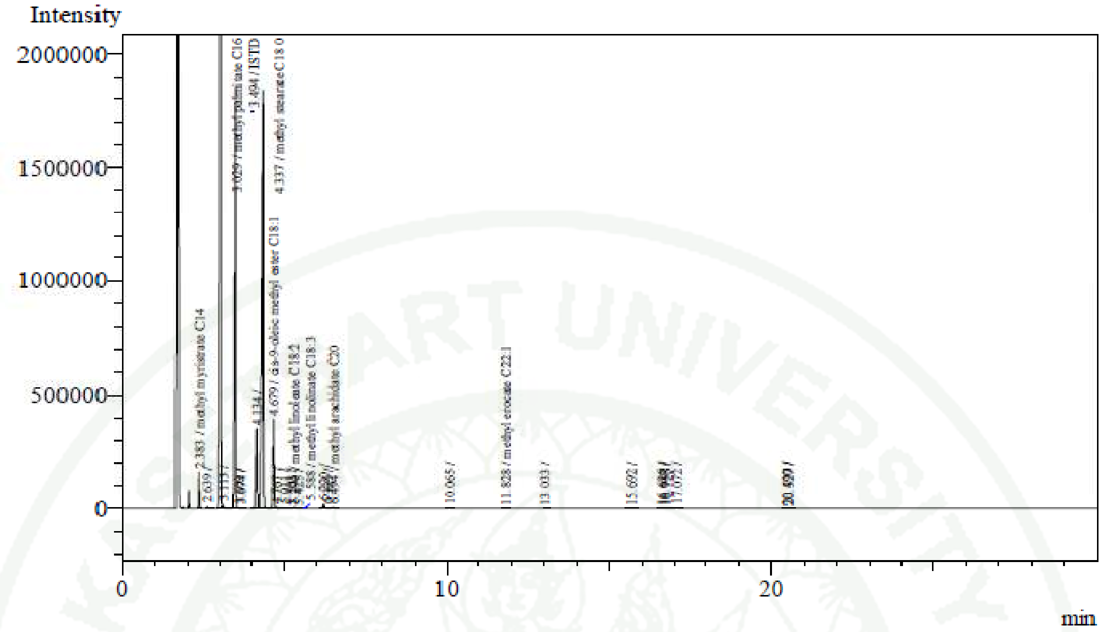
GC results for initial 1% FFA Transesterification



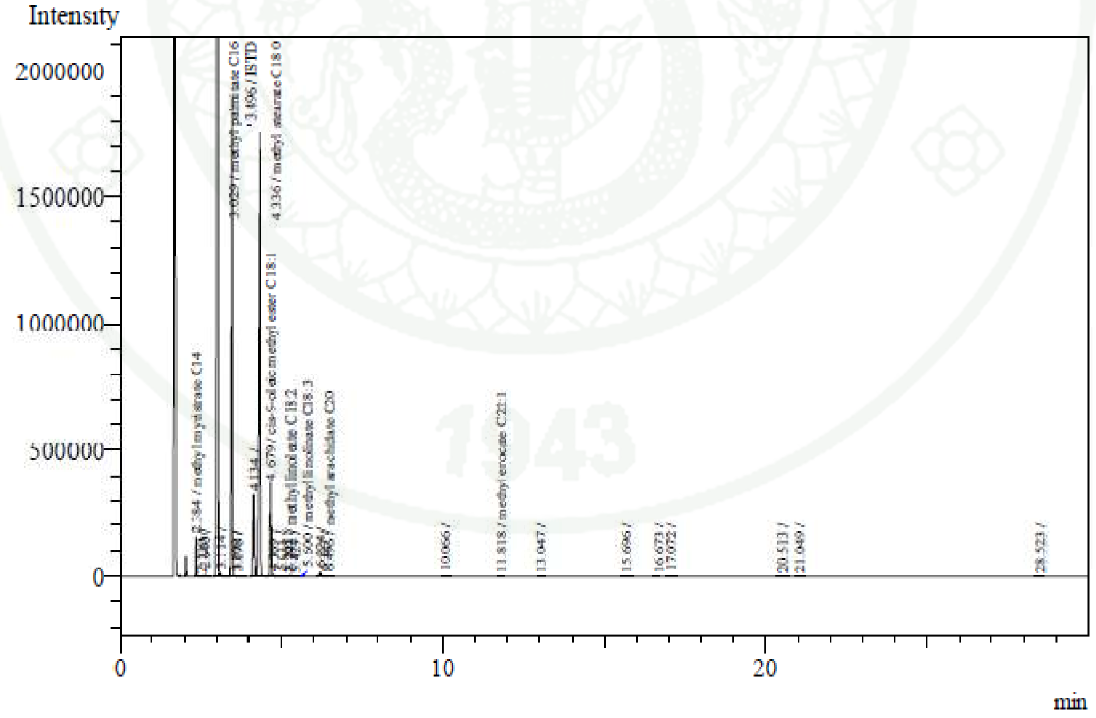
Appendix Figure A1 Chromatograph for run no. 1 of initial 1% FFA transesterification.



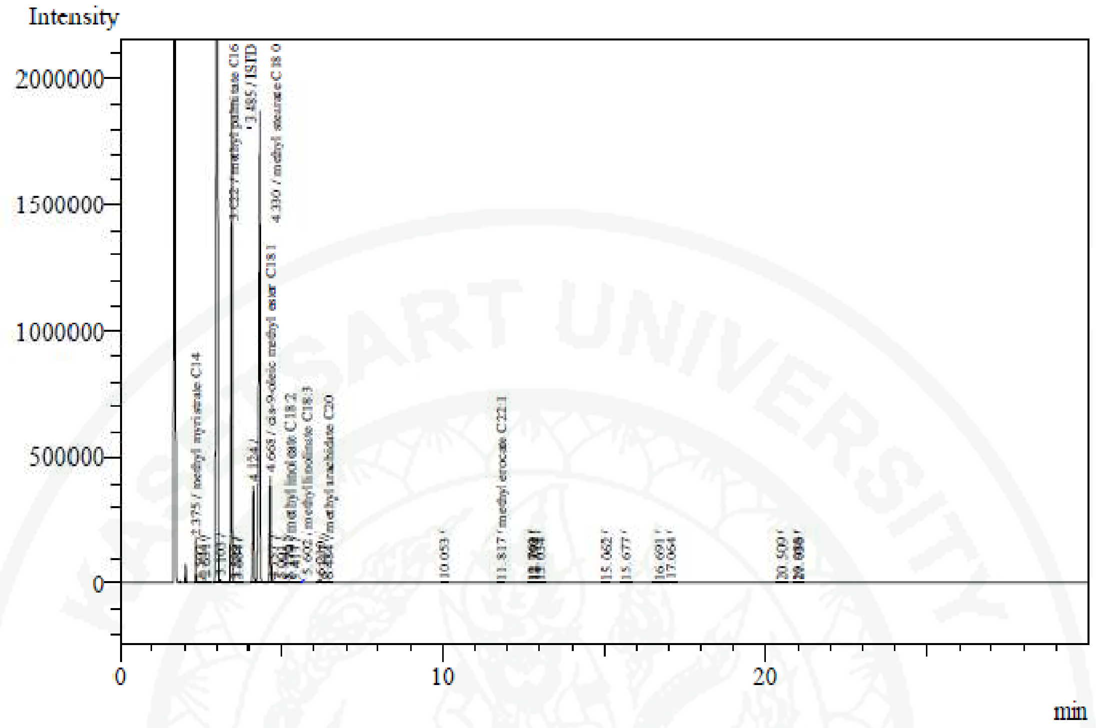
Appendix Figure A2 Chromatograph for run no. 2 of initial 1% FFA transesterification.



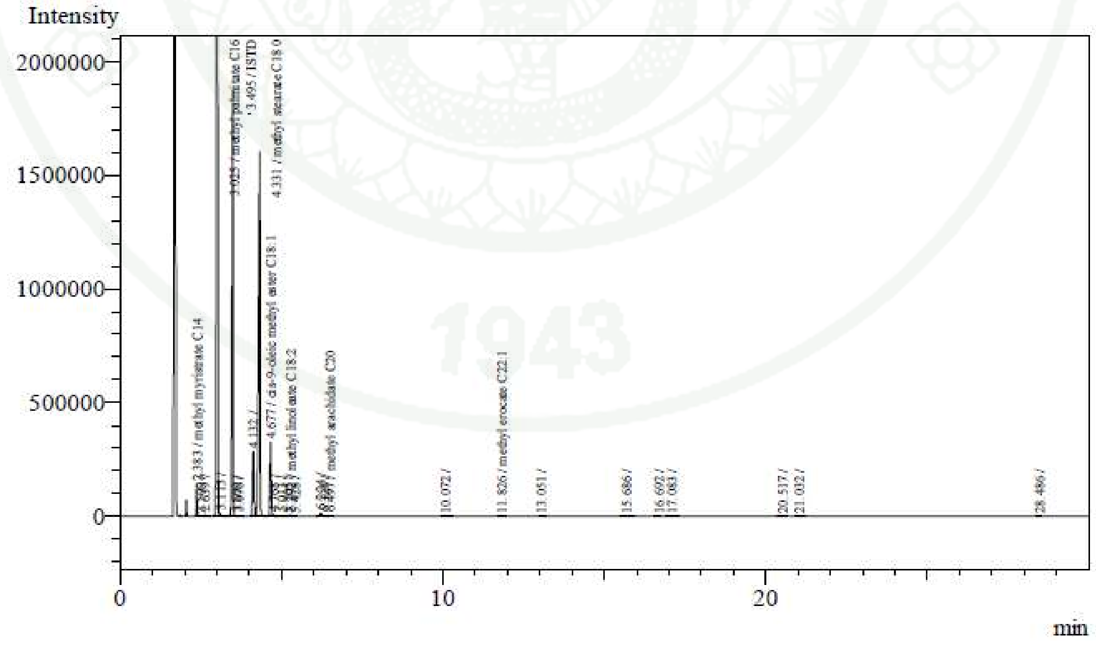
Appendix Figure A3 Chromatograph for run no. 3 of initial 1% FFA transesterification.



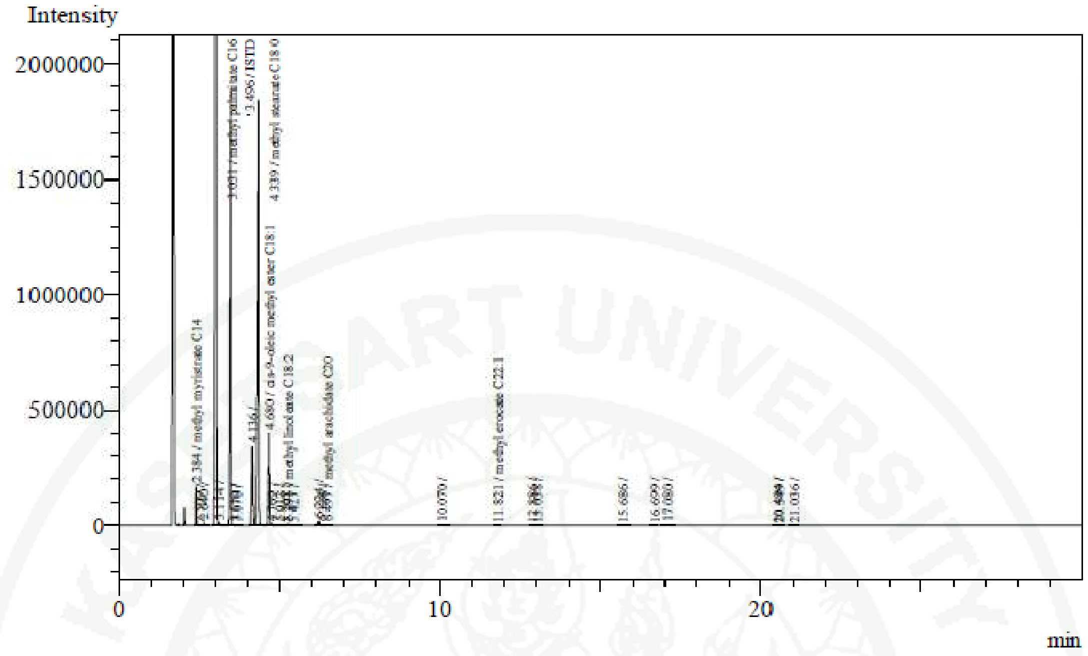
Appendix Figure A4 Chromatograph for run no. 4 of initial 1% FFA transesterification.



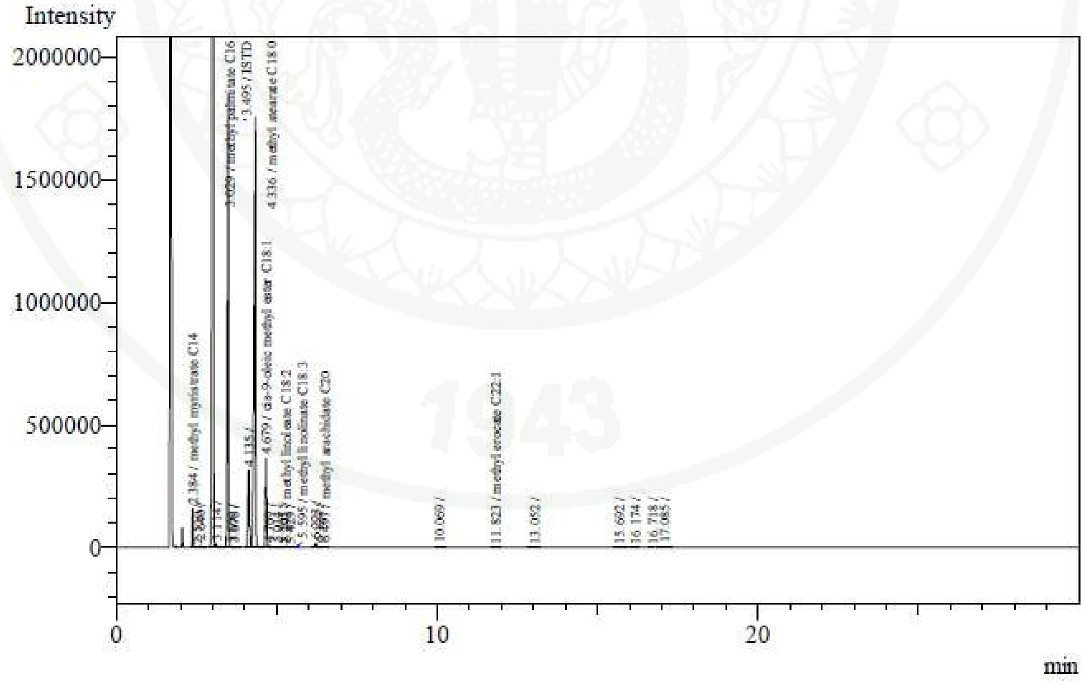
Appendix Figure A5 Chromatograph for run no.5 of initial 1% FFA transesterification.



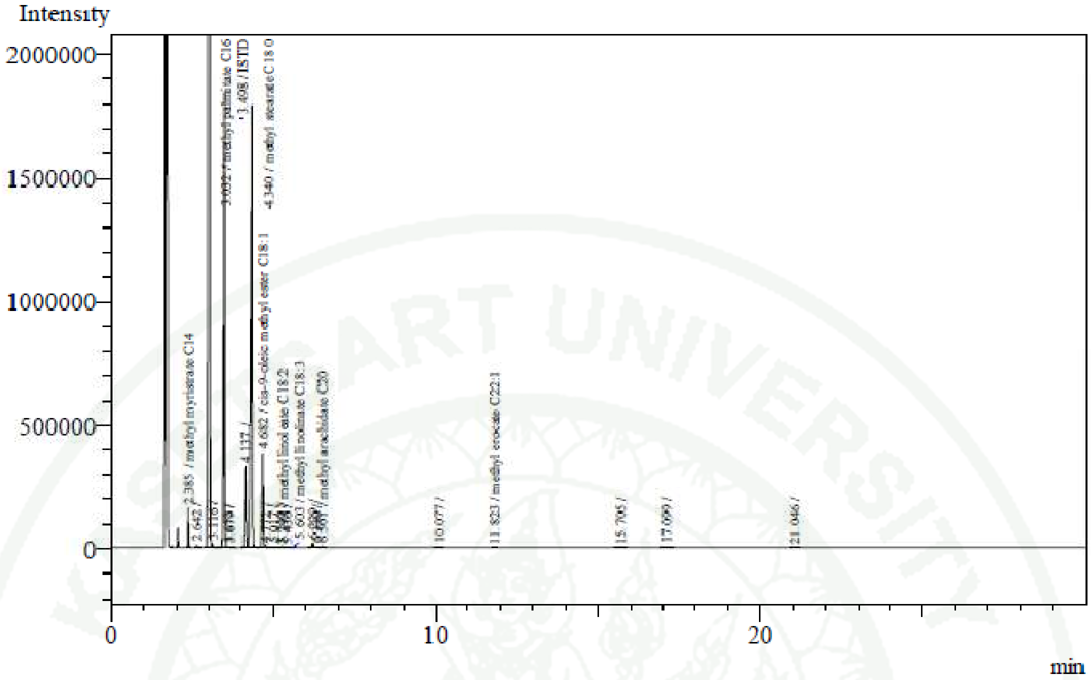
Appendix Figure A6 Chromatograph for run no. 7 of initial 1% FFA transesterification.



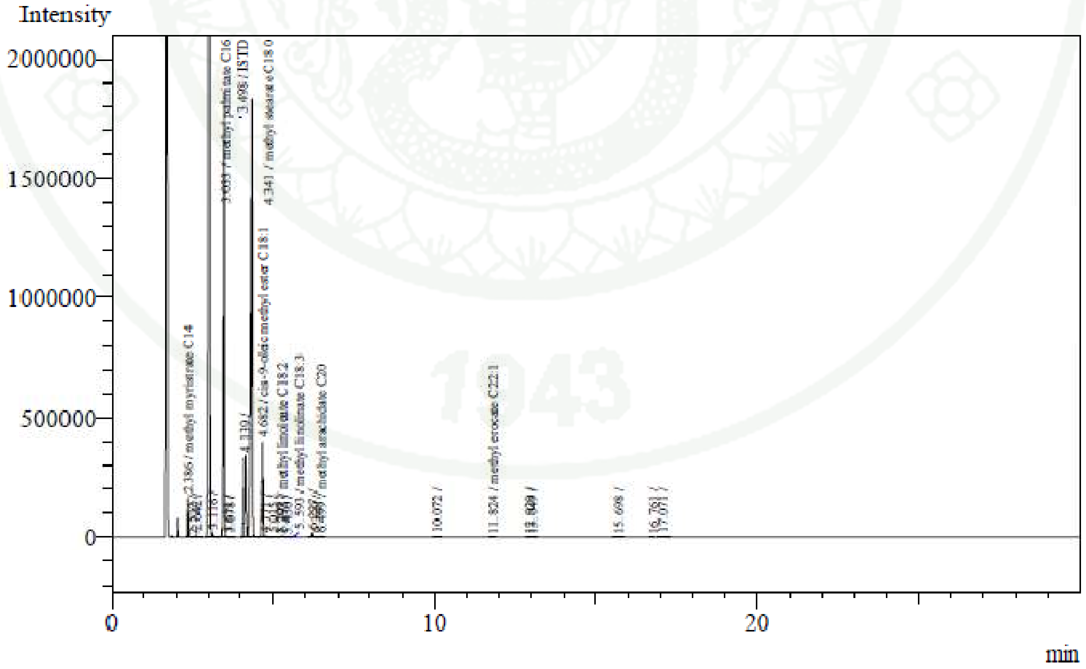
Appendix Figure A7 Chromatograph for run no. 7 of initial 1% FFA transesterification.



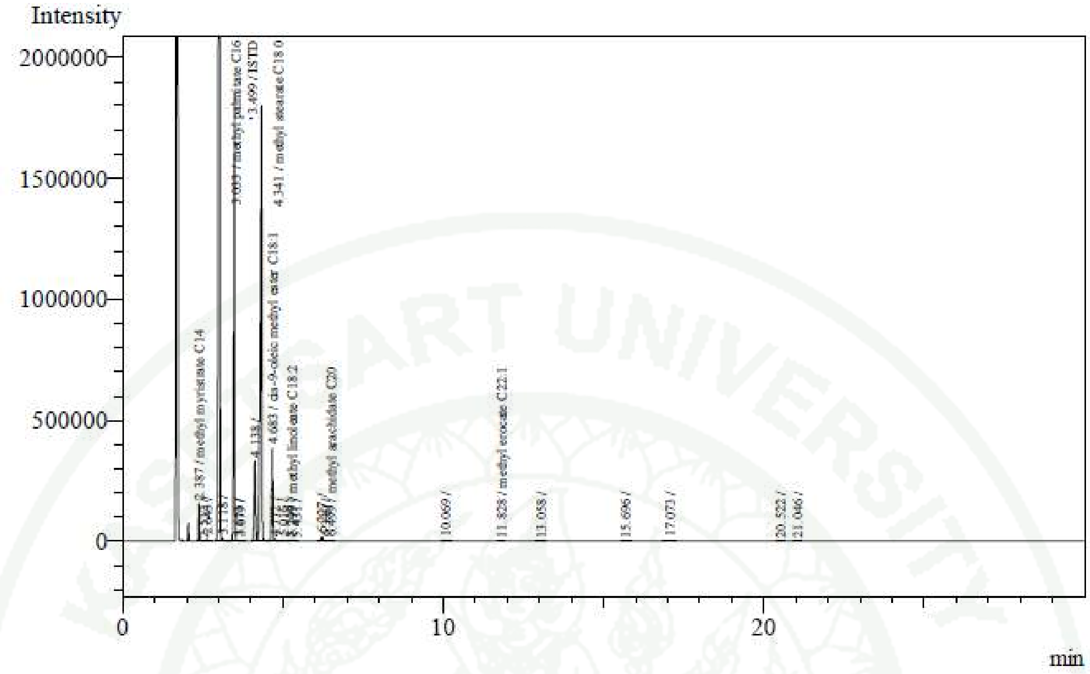
Appendix Figure A8 Chromatograph for run no. 8 of initial 1% FFA transesterification.



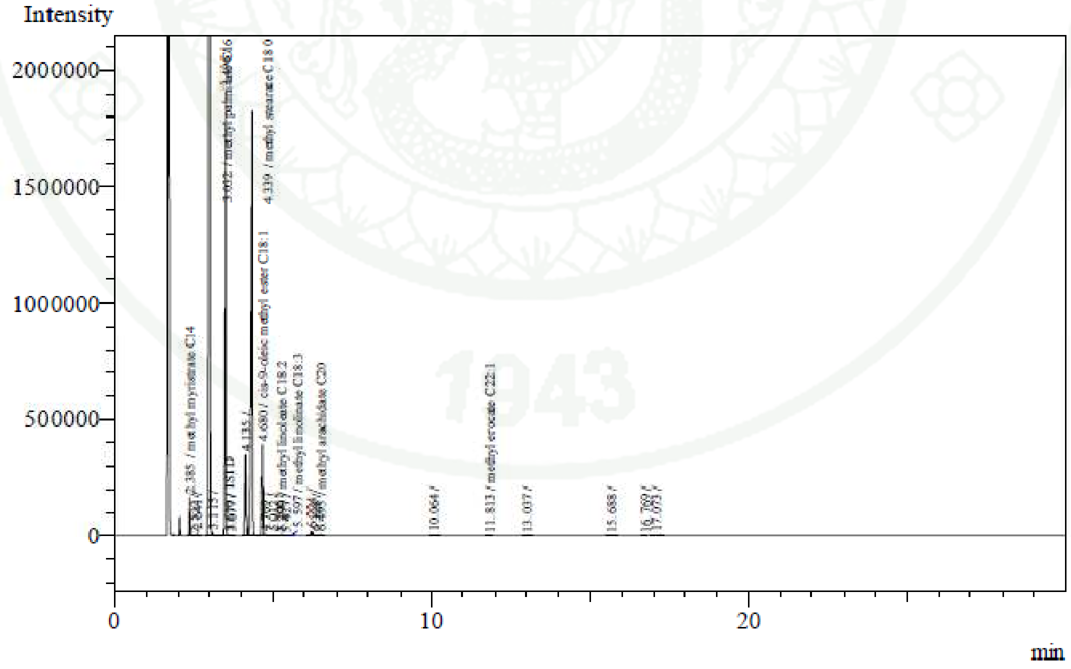
Appendix Figure A9 Chromatograph for run no. 9 of initial 1% FFA transesterification.



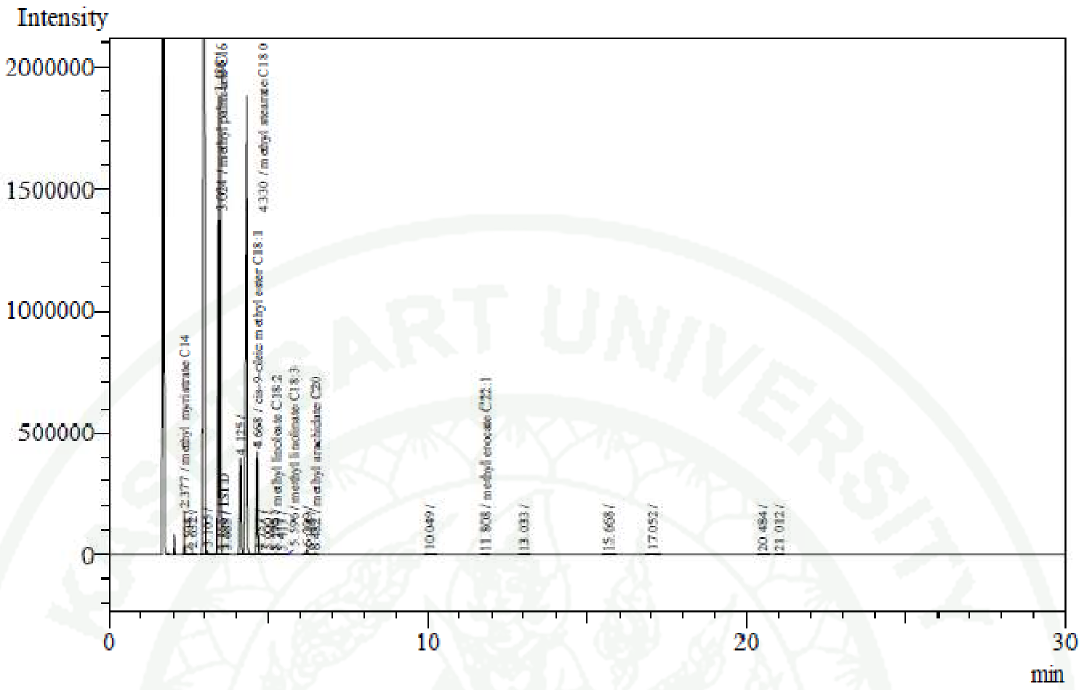
Appendix Figure A10 Chromatograph for run no. 10 of initial 1% FFA transesterification.



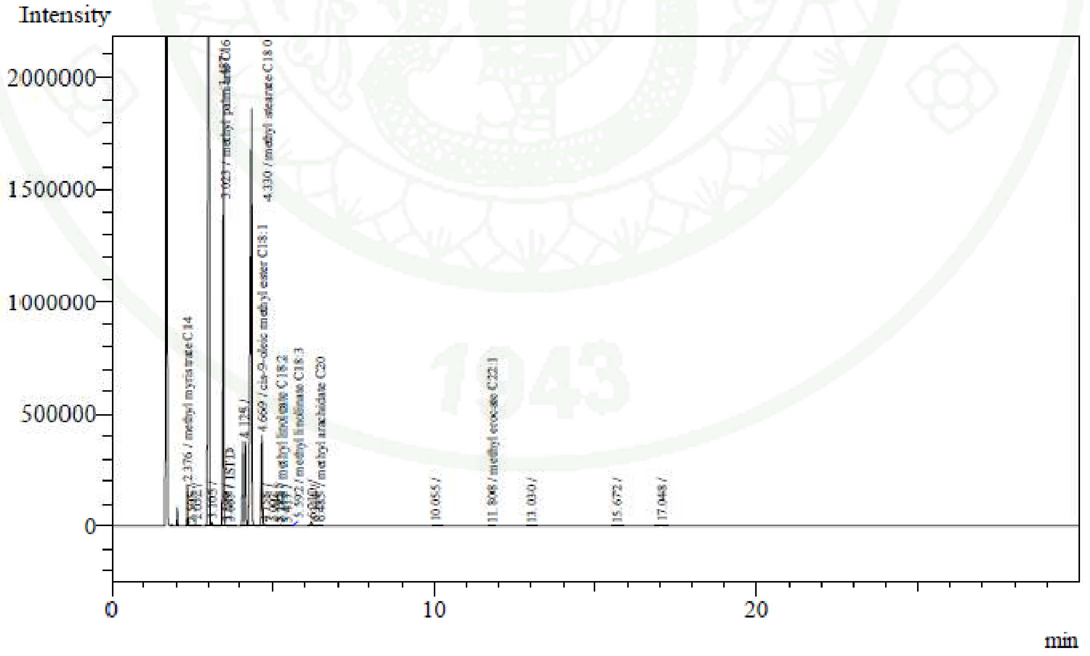
Appendix Figure A11 Chromatograph for run no. 11 of initial 1% FFA transesterification.



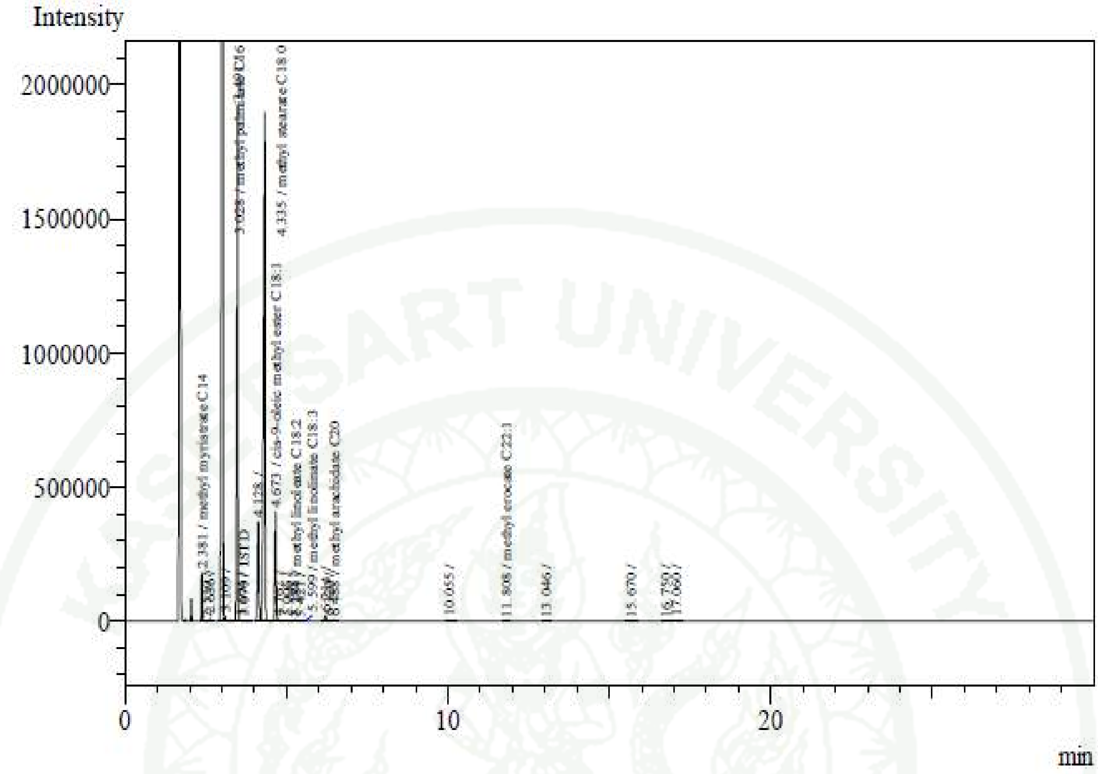
Appendix Figure A12 Chromatograph for run no. 12 of initial 1% FFA transesterification.



Appendix Figure A13 Chromatograph for run no. 13 of initial 1% FFA transesterification.



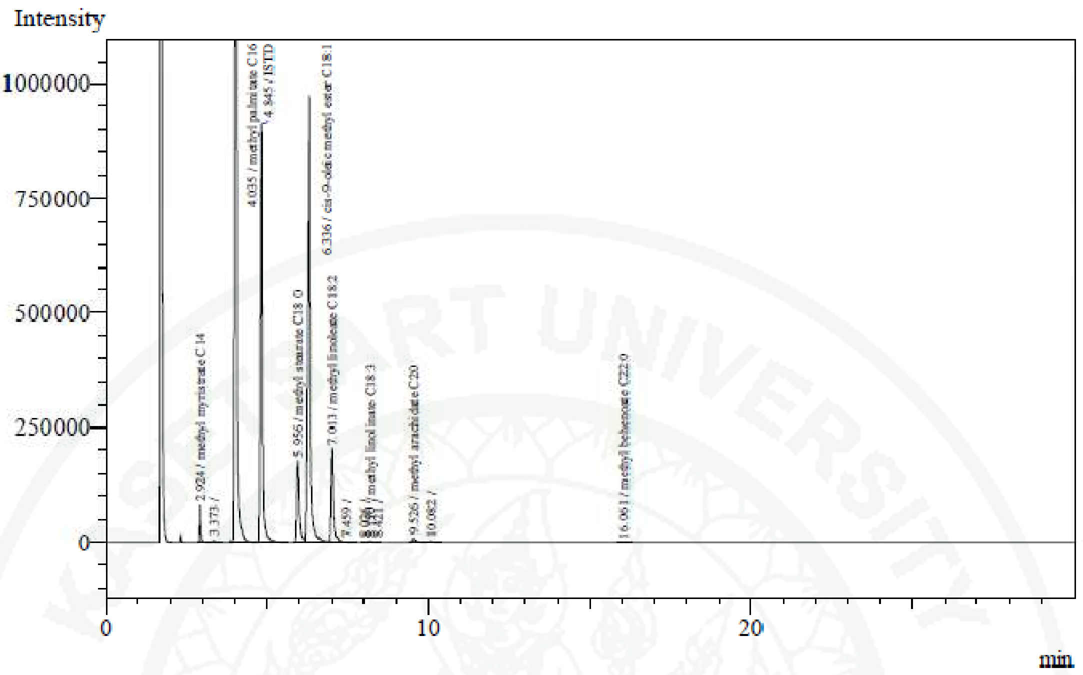
Appendix Figure A14 Chromatograph for run no. 14 of 1 initial % FFA transesterification.



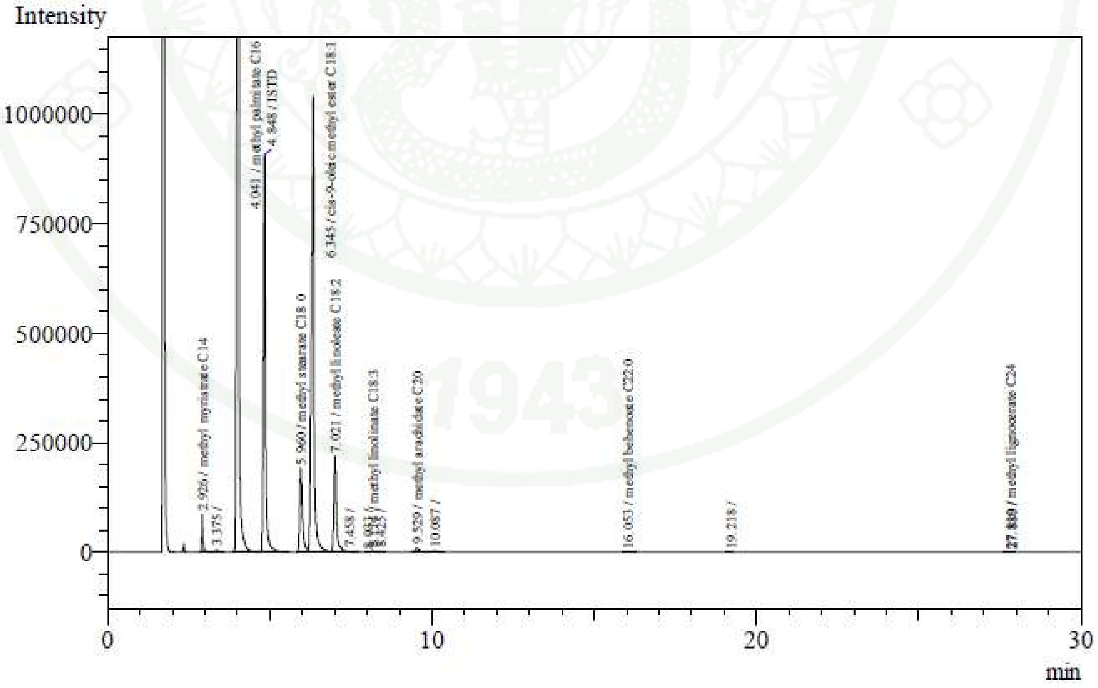
Appendix Figure A15 Chromatograph for run no. 15 of initial 1% FFA transesterification.

Appendix B

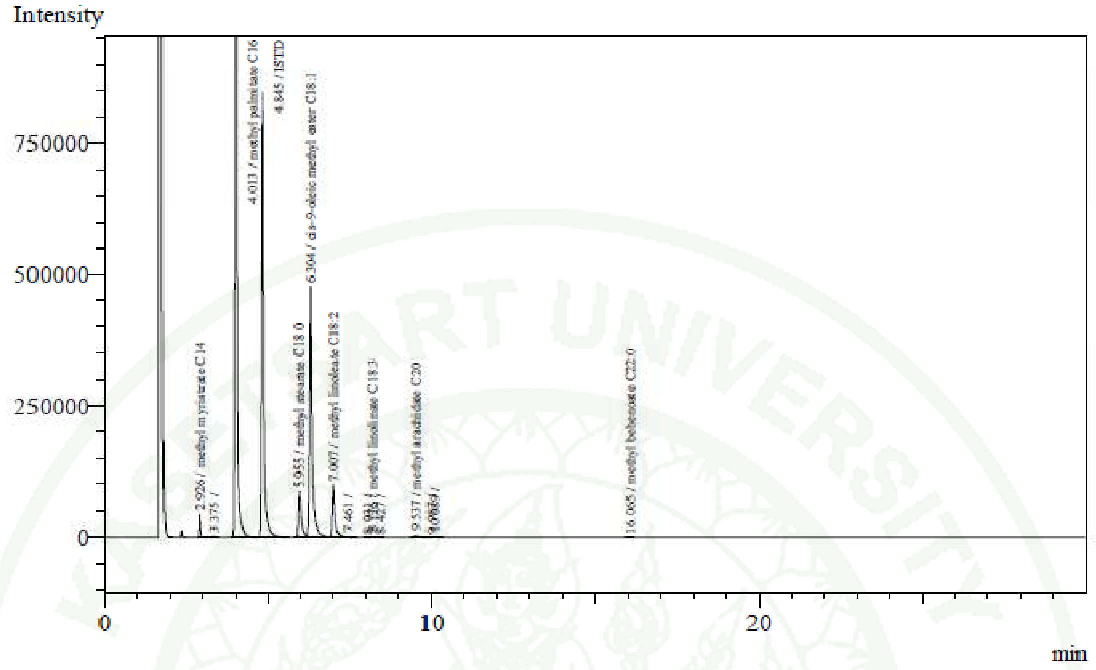
GC results for initial 2% FFA Transesterification



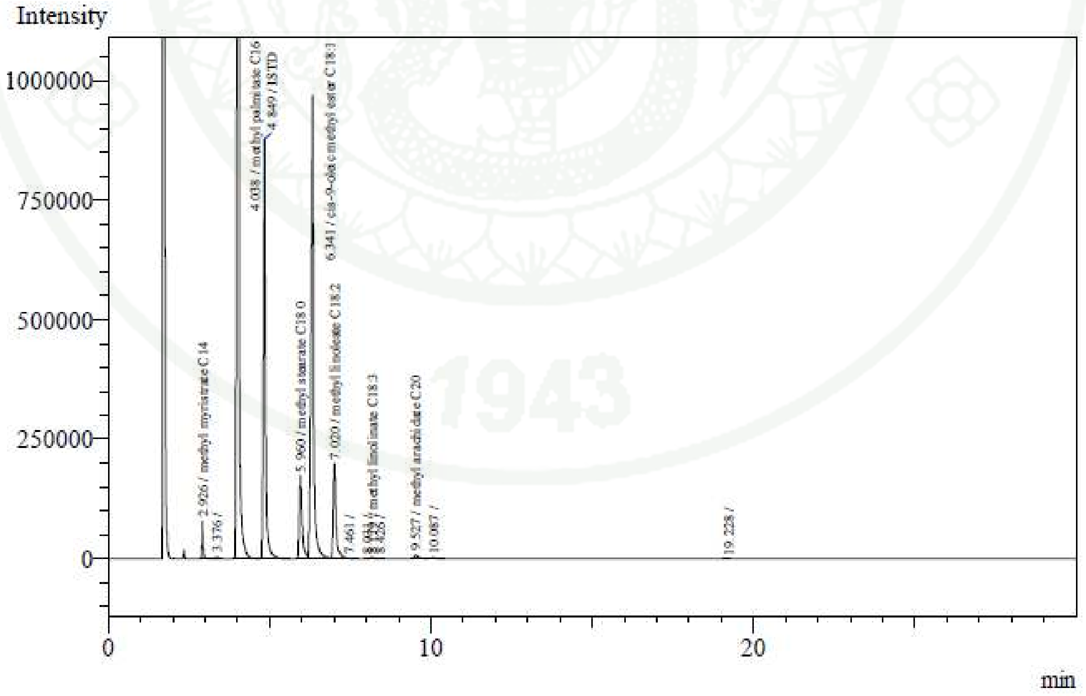
Appendix Figure B1 Chromatograph for run no. 1 of initial 2% FFA tansesterification.



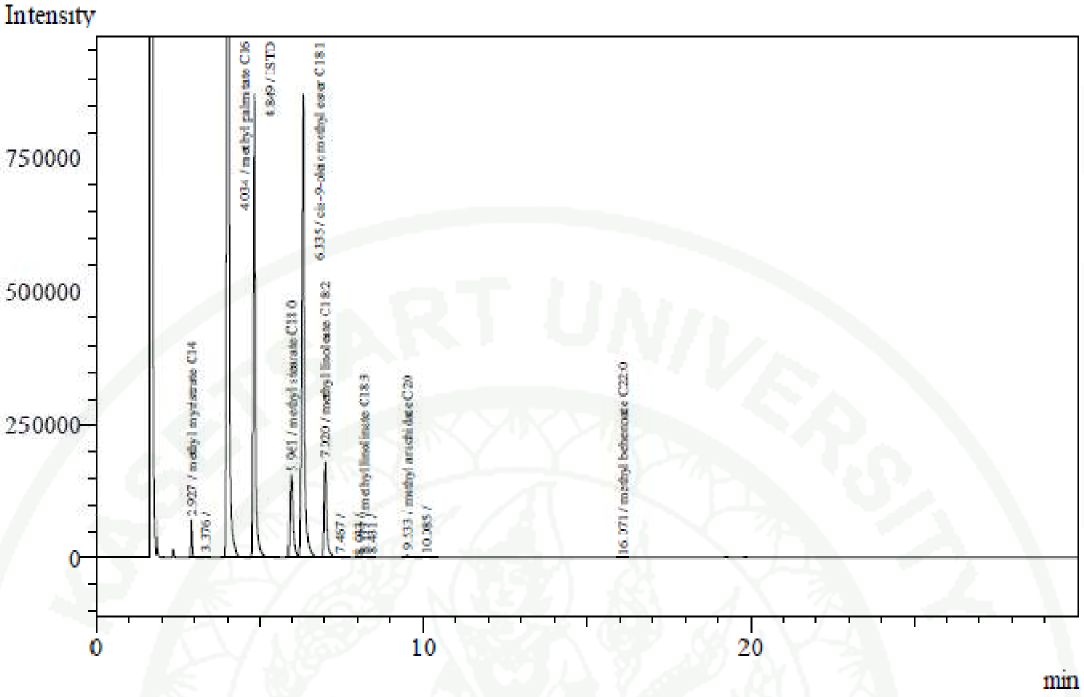
Appendix Figure B2 Chromatograph for run no. 2 of initial 2% FFA transesterification.



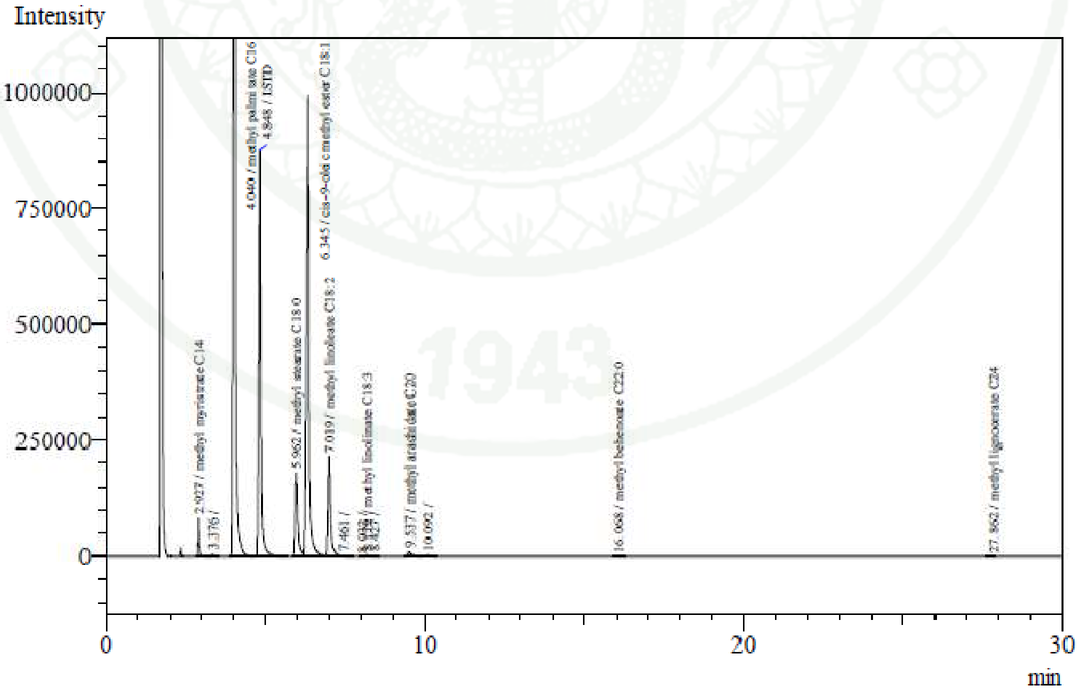
Appendix Figure B3 Chromatograph for run no. 3 of initial 2% FFA transesterification.



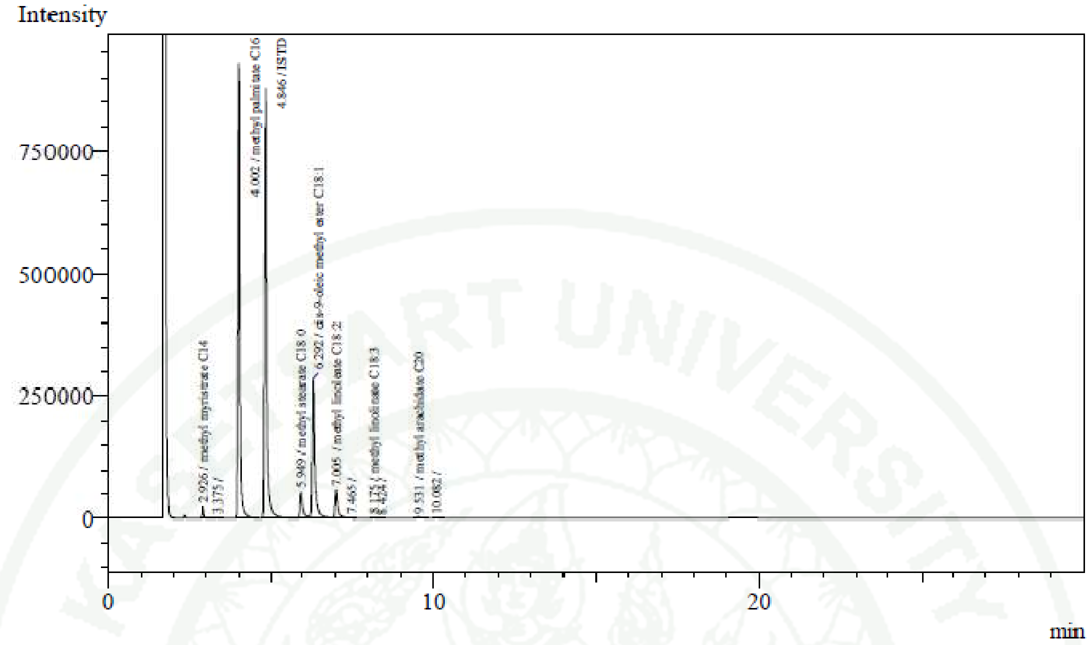
Appendix Figure B4 Chromatograph for run no. 4 of initial 2% FFA transesterification.



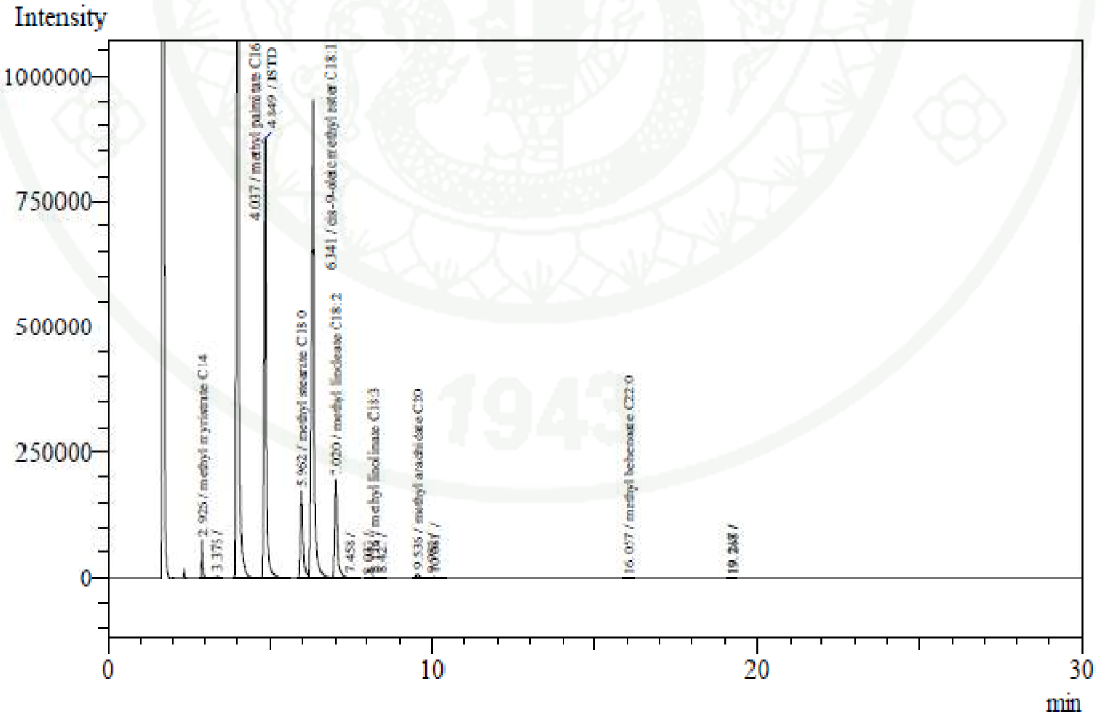
Appendix Figure B5 Chromatograph for run no. 5 of initial 2% FFA transesterification.



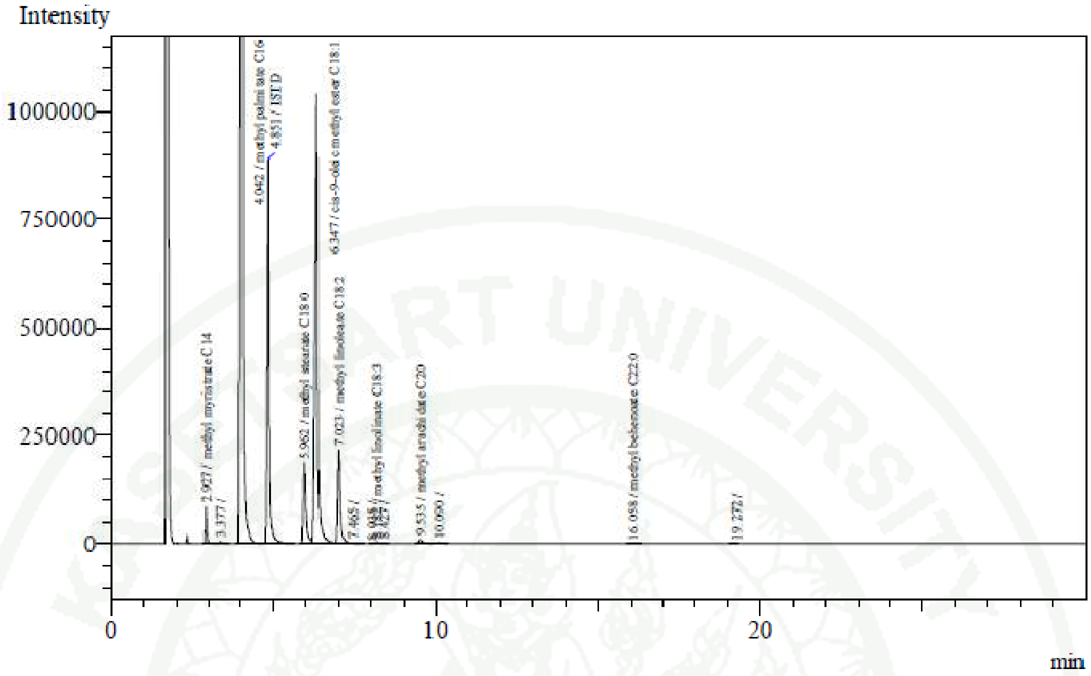
Appendix Figure B6 Chromatograph for run no.6 of initial 2% FFA transesterification.



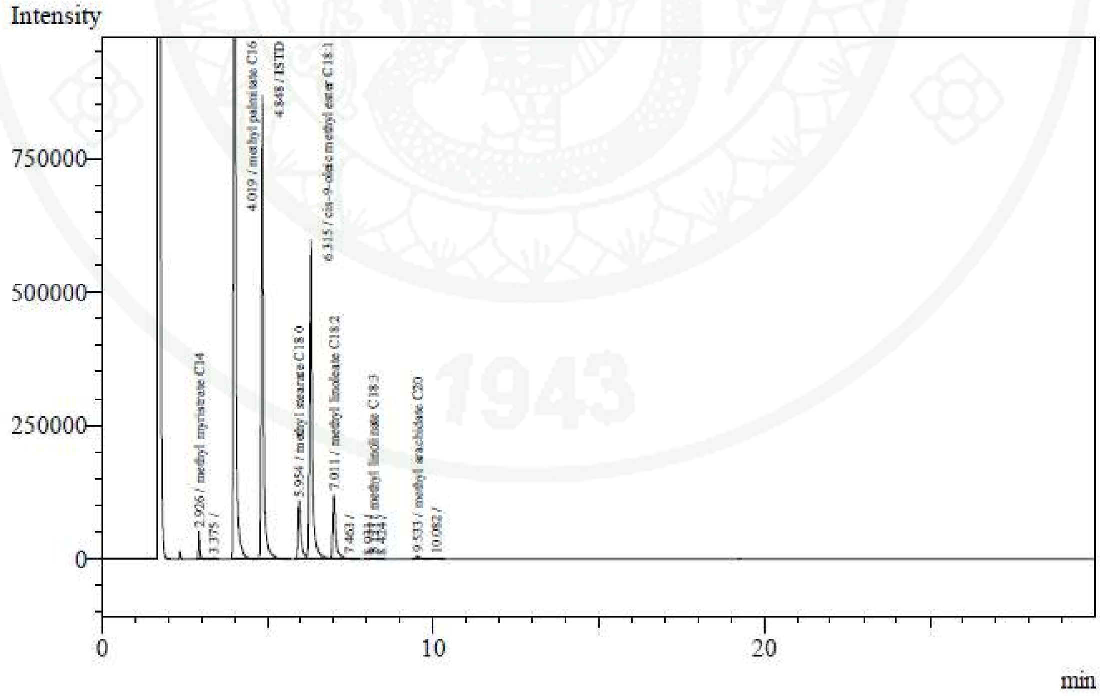
Appendix Figure B7 Chromatograph for run no.7 of initial 2% FFA transesterification.



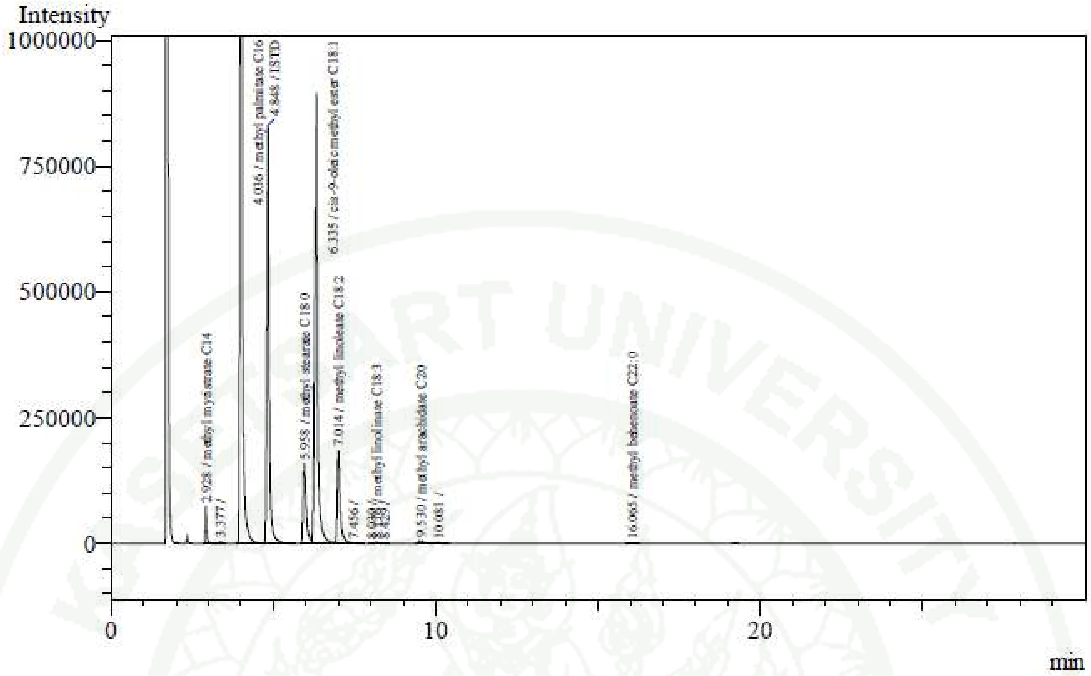
Appendix Figure B8 Chromatograph for run no. 8 of initial 2% FFA transesterification.



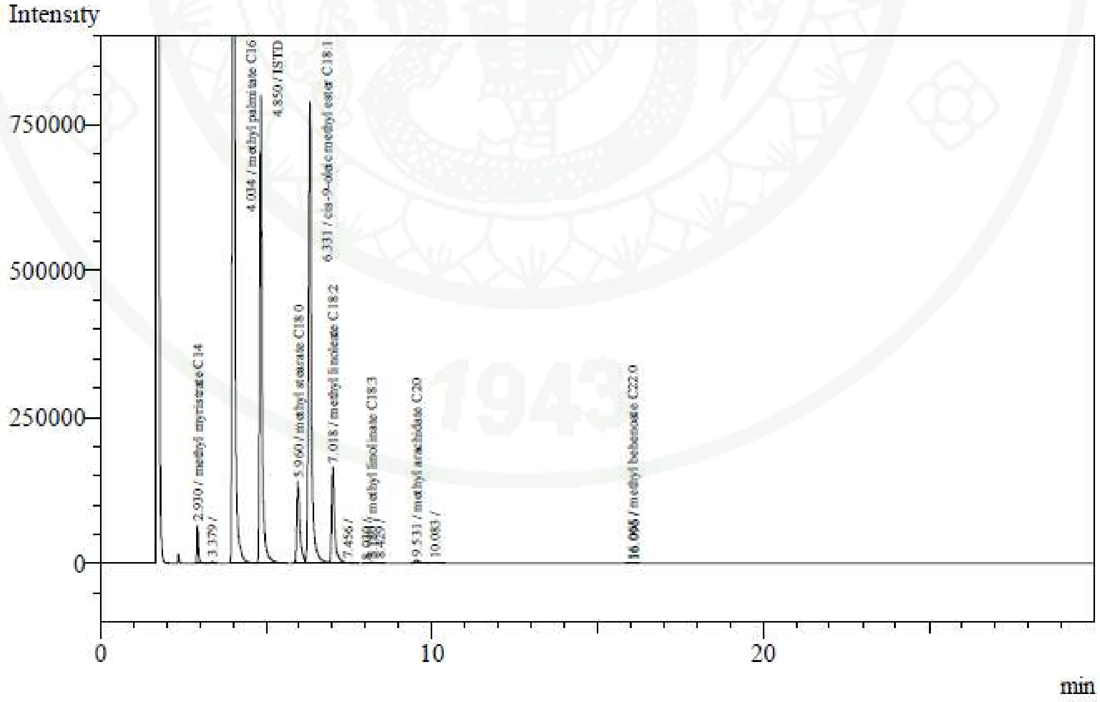
Appendix Figure B9 Chromatograph for run no. 9 of initial 2% FFA transesterification.



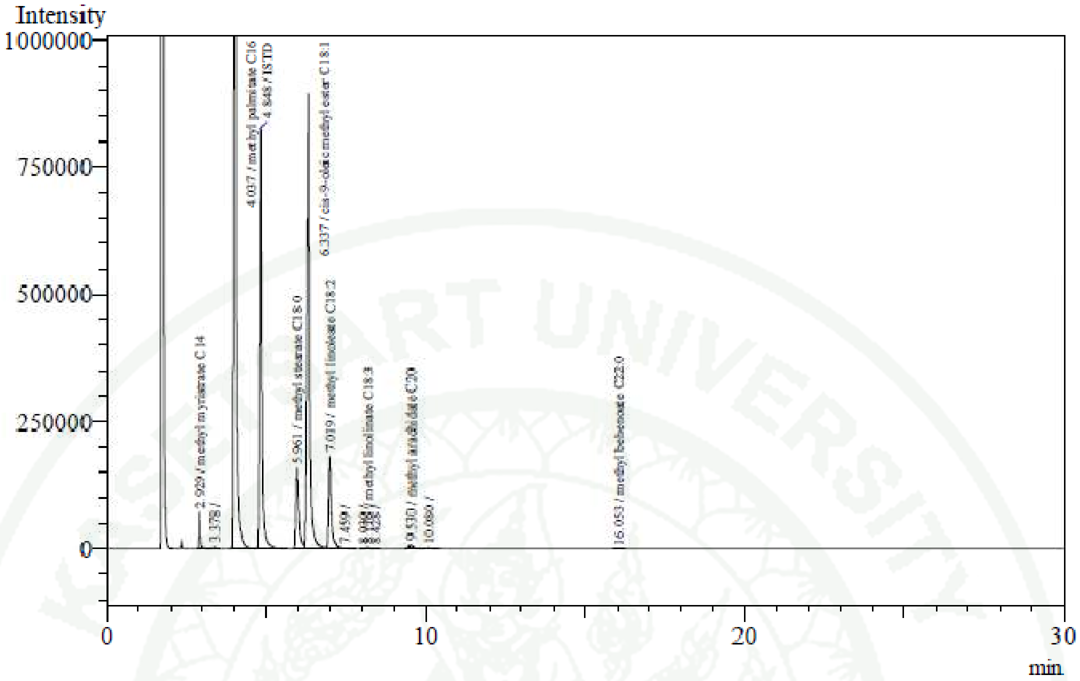
Appendix Figure B10 Chromatograph for run no. 10 of initial 2% FFA transesterification.



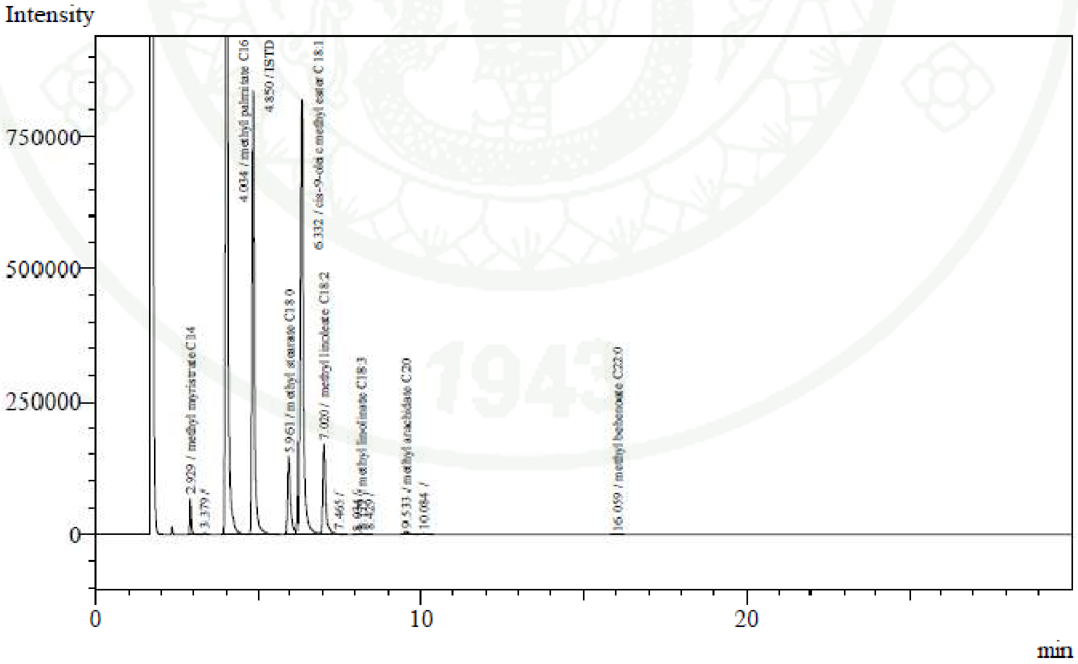
Appendix Figure B11 Chromatograph for run no. 11 of initial 2% FFA transesterification.



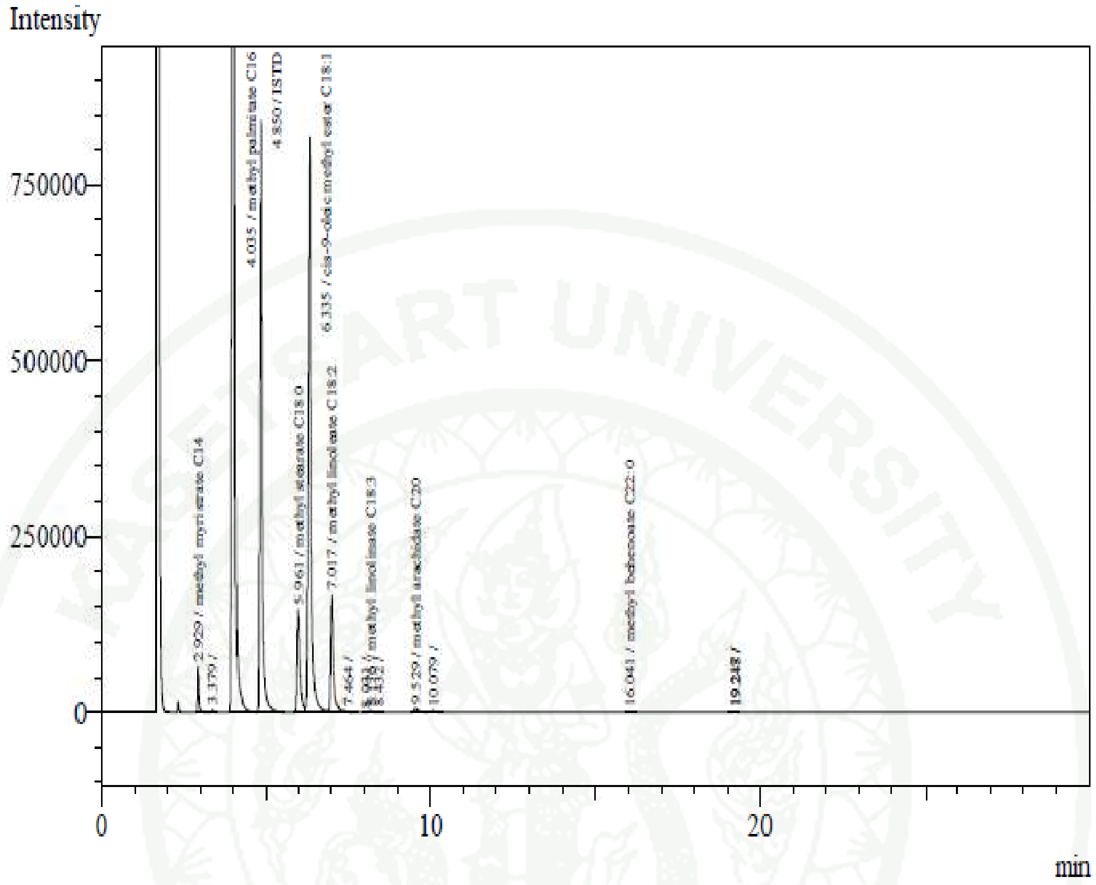
Appendix Figure B12 Chromatograph for run no. 12 of initial 2% FFA transesterification.



Appendix Figure B13 Chromatograph for run no. 13 of initial 2% FFA transesterification.



Appendix Figure B14 Chromatograph for run no. 14 of initial 2% FFA transesterification.



Appendix Figure B15 Chromatograph for run no. 15 of initial 2% FFA transesterification.

Appendix C

Products from Esterification and Transesterification

Appendix Figure C1 Products of initial 5% FFA Esterifiation.

Appendix Figure C2 Products of initial 20% FFA Esterifiation.

Appendix Figure C3 Products of initial 40% FFA Esterifiation.

Appendix Figure C4 Products of initial 50% FFA Esterifiation.

Appendix Figure C5 Products of initial 60% FFA Esterifiation.

Appendix Figure C6 Products of initial 5-87% FFA Esterifiation.

Appendix Figure C7 Products of initial 1% FFA Transesterifiation.

Appendix Figure C8 Products of initial 2% FFA Transesterifiation.

Appendix D

F-value table

Appendix Table D1 Percentage points of F distribution.

$P_{0.05,v1,v2}$

Degree of freedom (v_2)	Degree of Freedom (v_1)											
	1	2	3	4	5	6	7	8	9	10	12	15
1	161.4	199.5	215.7	224.6	230.2	234.0	236.8	238.9	240.5	241.9	243.9	245.9
2	18.51	19.00	19.16	19.25	19.30	19.33	19.35	19.37	19.38	19.40	19.41	19.43
3	10	9.55	9.28	9.12	9.01	8.94	8.89	8.85	8.81	8.79	8.74	8.70
4	13	6.94	6.59	6.39	6.26	6.16	6.09	6.04	6.00	5.96	5.91	5.86
5	7.71	5.79	5.41	5.19	5.05	4.95	4.88	4.82	4.77	4.74	4.68	4.62
6	6.61	5.14	4.76	4.53	4.9	4.28	4.21	4.15	4.10	4.06	4.00	3.94
7	5.99	4.74	4.35	4.12	3.97	3.87	3.79	3.73	3.68	3.64	3.57	3.51
8	5.59	4.46	4.07	3.84	3.69	3.58	3.50	3.44	3.39	3.35	3.28	3.22
9	5.12	4.26	3.86	3.63	3.48	3.37	3.29	3.23	3.18	3.14	3.07	3.01
10	4.96	4.10	3.71	3.48	3.33	3.22	3.14	3.07	3.02	2.98	2.91	2.85
11	4.84	3.98	3.59	3.36	3.20	3.09	3.01	2.95	2.90	2.85	2.79	2.72
12	4.75	3.89	3.49	3.26	3.11	3.00	2.91	2.85	2.90	2.75	2.69	2.62
13	4.67	3.81	3.41	3.18	3.03	2.92	2.83	2.77	2.71	2.67	2.60	2.53

Source: Montgomery and Runger (2011)

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