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Optimization Using Response Surface Methodology

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THESIS

**BIODIESEL PRODUCTION FROM TRAP GREASE TREATING
CAFETERIA AND RESTAURANT WASTEWATER VIA
TWO-STEP CATALYZED PROCESS OPTIMIZATION USING
RESPONSE SURFACE METHODOLOGY**

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**A Thesis Submitted in Partial Fulfillment of
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The main purpose of this research was to develop a two-step biodiesel production technique from trap grease as a raw material to prevent water pollution and producing the environmentally friendly renewable energy reducing green house gas emission causing global warming. The developed technique was acid catalyzed esterification and alkali catalyzed transesterification. The special attention was paid to optimize the first step, the acid catalyzed esterification to reduce the free fatty acid content and the second step, the alkali catalyzed transesterification for converting be fatty acid methyl ester.

Trap grease contained high free fatty acid of 26.19 % and molecular weight of 846 g/mol with highest oleic acid component, could be reduced to less than 2 % of free fatty acid and amount of larger trap grease methyl ester by optimized two-step reaction conditions, which applied response surface methodology with using central composite design in acidic catalyzed esterification and alkali catalyzed transesterification of the two step catalyze process. The Optimum condition for the acidic catalyzed esterification was found as 0.43 v/v or 10:1 of methanol to oil molar ratio, 2.5 % v/v sulphuric acid concentration and 4 hours of reaction time. This optimum value gave a predicted acid value of 2.66 mg KOH/g or 1.30 % of free fatty acid. The optimum condition for the alkali catalyzed transesterification was as follows; 0.26 v/v or 6:1 of methanol to oil molar ratio, 1 % w/v potassium hydroxide concentration and 1 hour of reaction time. The maximum fatty acid methyl ester content under the optimum conditions of the variables was 95.49 %. Properties of trap grease biodiesel was 880.13 Kg/m³ of density (at 15°C), 4.83 mm²/s of viscosity (at 40°C), 165°C of flash point, 0.54 mg KOH/g of acid value, 73.45 g Iodine/100 g of Iodine value which indicated that the trap grease biodiesel with this optimum condition in the two-step catalyzed process had properties satisfying both biodiesel standard of ASTM D 6751 and EN 14214.

This developed biodiesel production technique could used for biodiesel production in pilot plant from the low-cost feedstock as trap grease, hence this could decrease biodiesel cost and solving environmental problem on wastewater and the technique has encouraged environmental concern. Overall, the trap grease biodiesel is environmentally friendly renewable energy.

Student's signature

Thesis Advisor's signature

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LIST OF TABLES

Table		Page
1	Urban waste grease resources in 30 metropolitan areas	11
2	Prices of rendering plant products	13
3	The United States of America USA production of fats and oils (billion pounds)	13
4	Some economic assessment studies for biodiesel based on the feedstock	14
5	Properties of crude waste fryer grease	14
6	Chemical analysis results of restaurant grease and animal fat samples	15
7	Yields of the reaction	25
8	American standard specification for biodiesel (ASTMD 6751)	32
9	European standard specifications for biodiesel (EN 14214)	33
10	Some properties of diesel fuel and biodiesel fuels produced from different feedstocks	34
11	Independent variables and levels used for central composite design in acid catalyzed esterification of two-step catalyzed process	39
12	Central composite design (CCD) arrangement for acid catalyzed esterification	40
13	Independent variables and levels used for central composite design for alkali catalyzed transesterification	45
14	Central composite design (CCD) arrangement for for alkali catalyzed transesterification	46
15	Physical and chemical properties of trap grease	51

LIST OF TABLES (Continued)

Table		Page
16	Central composite design arrangement and response for acid catalyzed esterification	54
17	Analysis of variance (ANOVA) for quadratic polynomial model	55
18	Regression coefficients of predicted quadratic polynomial model for regression equation	55
19	Central composite design arrangement and response for alkali catalyzed transesterification	62
20	Analysis of variance (ANOVA) for quadratic polynomial model	63
21	Regression coefficients of predicted quadratic polynomial model for regression equation	63
22	Fuel properties of trap grease biodiesel	70
23	Properties of trap grease biodiesel in comparison with various biodiesel	72
Appendix Table		
B1	Average molecular weight of trap grease	98

LIST OF FIGURES

Figure		Page
1	Exhaust emissions of diesel engine operating with biodiesel compared to diesel fuel. (a) Moment versus engine speed. (b) Engine power versus engine speed. (c) Brake-specific fuel consumption versus engine speed. (d) Smoke density versus engine speed. (e) CO emissions versus engine speed. (f) CO ₂ emissions versus engine speed. (g) Exhaust temperatures versus engine speed. (h) Exchange of NO _x	7
2	Flow sheet of the transesterification process	17
3	Chromatogram of waste fryer grease methyl esters	24
4	Treatment process of trap grease. (A) Collection of trap grease sample in shop (B) Heating at 105°C for removing water (C) Filtration of trap grease (D) Treated trap grease	38
5	An experiment set up of acid catalyzed esterification	43
6	Two- layers separation of trap grease after acid catalyzed esterification experiments	44
7	Two- layers separation of sample after alkali catalyzed transesterification experiments	49
8	Kinematic viscosity of trap grease biodiesel using Ubbelohde glass capillary viscometer	50
9	Response surface plots and contour plots representing the effect of methanol to oil ratio and catalyst concentration on acid value predicted from quadratic polynomial model	56

LIST OF FIGURES (Continued)

Figure		Page
10	Response surface plots and contour plots representing the effect of methanol to oil ratio and reaction time on acid value predicted from quadratic polynomial model	57
11	Response surface plots and contour plots representing the effect of catalyst concentration and reaction time on acid value predicted from quadratic polynomial model	58
12	Response surface plots and contour plots representing the effect of methanol to oil ratio and catalyst concentration on %FAME predicted from quadratic polynomial model	64
13	Response surface plots and contour plots representing the effect of methanol to oil ratio and reaction time on %FAME predicted from quadratic polynomial model	65
14	Response surface plots and contour plots representing the effect of catalyst concentration and reaction time on %FAME predicted from quadratic polynomial model	66
15	A trap grease biodiesel from two-step catalyzed process	71
16	Process flow schematic for trap grease biodiesel production of the two step catalyzed process	73

LIST OF FIGURES (Continued)

Appendix Figure		Page
A1	Standard curve of octanoic acid	82
A2	Standard curve of decanoic acid	83
A3	Standard curve of lauric acid	84
A4	Standard curve of myristic acid	85
A5	Standard curve of palmitic acid	86
A6	Standard curve of palmitoleic acid	87
A7	Standard curve of heptadecanoic acid	88
A8	Standard curve of stearic acid	89
A9	Standard curve of oleic acid	90
A10	Standard curve of linoleic acid	91
A11	Standard curve of linolenic acid	92
A12	Standard curve of arachidic acid	93
A13	Standard curve of behenic acid	94
A14	Standard curve of erucic acid	95
A15	Standard curve of lignoceric acid	96

LIST OF FIGURES (Continued)

Appendix Figure		Page
C1	GC chromatogram of trap grease methyl ester in transesterification condition at 0.22 v/v (5:1) methanol to oil molar ratio, 0.5 % v/v catalyst concentration and 0.67 hour reaction time (experimental treatment no.1)	100
C2	GC chromatogram of trap grease methyl ester in transesterification condition at 0.22 v/v (5:1) methanol to oil molar ratio, 0.5 % v/v catalyst concentration and 1.33 hour reaction time (experimental treatment no.2)	101
C3	GC chromatogram of trap grease methyl ester in transesterification condition at 0.22 v/v (5:1) methanol to oil molar ratio, 1.5 % v/v catalyst concentration and 0.67 hour reaction time (experimental treatment no.3)	102
C4	GC chromatogram of trap grease methyl ester in transesterification condition at 0.22 v/v (5:1) methanol to oil molar ratio, 1.5 % v/v catalyst concentration and 1.33 hour reaction time (experimental treatment no.4)	103
C5	GC chromatogram of trap grease methyl ester in transesterification condition at 0.30 v/v (7:1) methanol to oil molar ratio, 0.5 % v/v catalyst concentration and 0.67 hour reaction time (experimental treatment no.5)	104
C6	GC chromatogram of trap grease methyl ester in transesterification condition at 0.30 v/v (7:1) methanol to oil molar ratio, 0.5 % v/v catalyst concentration and 1.33 hour reaction time (experimental treatment no.6)	105

LIST OF FIGURES (Continued)

Appendix Figure		Page
C7	GC chromatogram of trap grease methyl ester in transesterification condition at 0.30 v/v (7:1) methanol to oil molar ratio, 1.5 % v/v catalyst concentration and 0.67 hour reaction time (experimental treatment no.7)	106
C8	GC chromatogram of trap grease methyl ester in transesterification condition at 0.30 v/v (7:1) methanol to oil molar ratio, 1.5 % v/v catalyst concentration and 1.33 hour reaction time (experimental treatment no.8)	107
C9	GC chromatogram of trap grease methyl ester in transesterification condition at 0.19 v/v (5:1) methanol to oil molar ratio, 1 % v/v catalyst concentration and 1 hour reaction time (experimental treatment no.9)	108
C10	GC chromatogram of trap grease methyl ester in transesterification condition at 0.33 v/v (7:1) methanol to oil molar ratio, 1 % v/v catalyst concentration and 1 hour reaction time (experimental treatment no.10)	109
C11	GC chromatogram of trap grease methyl ester in transesterification condition at 0.26 v/v (6:1) methanol to oil molar ratio, 0.16 % v/v catalyst concentration and 1 hour reaction time (experimental treatment no.11)	110

LIST OF FIGURES (Continued)

Appendix Figure		Page
C12	GC chromatogram of trap grease methyl ester in transesterification condition at 0.26 v/v (6:1) methanol to oil molar ratio, 1.84 % v/v catalyst concentration and 1 hour reaction time (experimental treatment no.12)	111
C13	GC chromatogram of trap grease methyl ester in transesterification condition at 0.26 v/v (6:1) methanol to oil molar ratio, 1 % v/v catalyst concentration and 0.45 hour reaction time (experimental treatment no.13)	112
C14	GC chromatogram of trap grease methyl ester in transesterification condition at 0.26 v/v (6:1) methanol to oil molar ratio, 1 % v/v catalyst concentration and 1.55 hour reaction time (experimental treatment no.14)	113
C15	GC chromatogram of trap grease methyl ester in transesterification condition at 0.26 v/v (6:1) methanol to oil molar ratio, 1 % v/v catalyst concentration and 1 hour reaction time (experimental treatment no.15)	114
C16	GC chromatogram of trap grease methyl ester in transesterification condition at 0.26 v/v (6:1) methanol-to-oil ratio, 1 % v/v catalyst concentration and 1 hour reaction time (experimental treatment no.16)	115
C17	GC chromatogram of trap grease methyl ester in transesterification condition at 0.26 v/v (6:1) methanol-to-oil ratio, 1 % v/v catalyst concentration and 1 hour reaction time (experimental treatment no.17)	116

LIST OF FIGURES (Continued)

Appendix Figure		Page
C18	GC chromatogram of trap grease methyl ester in transesterification condition at 0.26 v/v (6:1) methanol-to-oil ratio, 1 % v/v catalyst concentration and 1 hour reaction time (experimental treatment no.18)	117
C19	GC chromatogram of trap grease methyl ester in transesterification condition at 0.26 v/v (6:1) methanol-to-oil ratio, 1 % v/v catalyst concentration and 1 hour reaction time (experimental treatment no.19)	118
C20	GC chromatogram of trap grease methyl ester in transesterification condition at 0.26 v/v (6:1) methanol-to-oil ratio, 1 % v/v catalyst concentration and 1 hour reaction time (experimental treatment no.20)	119
C21	GC chromatogram of trap grease methyl ester in transesterification condition at 0.26 v/v (6:1) methanol-to-oil ratio, 1 % v/v catalyst concentration and 1 hour reaction time (experimental treatment no.21)	120
C22	GC chromatogram of trap grease methyl ester in transesterification condition at 0.26 v/v (6:1) methanol-to-oil ratio, 1 % v/v catalyst concentration and 1.55 hour reaction time (experimental treatment no.22)	121
C23	GC chromatogram of trap grease methyl ester in transesterification condition at 0.26 v/v (6:1) methanol-to-oil ratio, 1 % v/v catalyst concentration and 1 hour reaction time (experimental treatment no.23)	122

LIST OF FIGURES (Continued)**Appendix Figure**

D1	Correlation of the experimental and predicted acid value in the acid catalyzed esterification	124
D2	Correlation of the experimental and predicted fatty acid methyl ester in the alkali catalyzed transesterification	124

BIODIESEL PRODUCTION FROM TRAP GREASE TREATING CAFETERIA AND RESTAURANT WASTEWATER VIA TWO-STEP CATALYZED PROCESS OPTIMIZATION USING RESPONSE SURFACE METHODOLOGY

INTRODUCTION

Currently, water pollutions causing majority from waste fats and oils from household and restaurant sources in the city, have been a growing environmental problem in Thailand and worldwide. For instance, in the United States of America (USA), the waste restaurant fats and oils were collected approximately 2.5 billion pounds/year from restaurants and fast-food establishments (Canakci, 2007). This residue was regularly poured down to sanitary sewer system where it could cause blockages, resulting in problems for wastewater treatment plants and energy loss. Furthermore, global climate change into global warming, has been effecting to natural resource, including human across the world because of green house gas emission from combustion of fossil fuel.

Trap grease is waste fats and oils in wastewater that is collected by the special traps before they poured down to the drain. Urban waste grease resources was high average 13 pounds/year/ person (Canakci, 2007). Hence, Biodiesel production from trap grease could reduce environmental problem on water pollution and air pollution with using trap grease as low cost raw feedstock for biodiesel production being renewable energy for community.

However, Trap grease contained high free fatty acids (FFA) and high moisture levels effect the transesterification reaction, which was main biodiesel production process in converting the feedstocks into biodiesel (Cvengros and Cvengrosova, 2004; Canakci, 2007). The conversion was complicated if trap grease contained a large amounts of free fatty acid (FFA) as more than 1% w/w that will form soap with

alkaline catalyst. The soap could prevent separation of the biodiesel from the glycerin fraction and contributed to emulsion formation during washing the biodiesel with water. Acid catalyst as sulfuric acid could esterify the free fatty acid to methyl ester in esterification reaction (Gerpen, 2005). Nevertheless, the main factors affecting transesterification are the amount of alcohol, catalyst, reaction temperature, pressure, time, the contents of free fatty acid (FFA) and water content of feedstock.

Biodiesel prepared from the high free fatty acids (FFA) fats and oils by the two step process (esterification and transesterification reactions) is better than the one step process (a transesterification reaction) in terms of lower reaction temperature, reduced catalyst and alcohol used and high yields obtained (Zhang *et al.*, 2003; Wang *et al.*, 2007; Veljkovic *et al.*, 2006). This method could be easily adapted to a continuous process.

Biodiesel production with the two step catalyzed processes consist of alkali catalyzed process and acid catalyzed process that have proved to be more practical nowadays. An alkali catalyzed process could achieve high purity and yield of biodiesel product in a short time as 30–60 minutes. The refined vegetable oil with having less than 0.5 % free fatty acid (FFA) could be used as the reactant in the alkali catalyzed process. While, waste cooking oil (WCO) with more than 10 % free fatty acids (FFA) content was used, an acid catalyzed process was preferred, but it requires more excess of methanol, high pressure of 170–180 kPa and high cost stainless steel equipment. In addition, the yield of product is low as 82% of mass conversion with 200% excess of ethanol and the most common sulfuric acid catalyst was used (Zhang *et al.*, 2003).

Response surface methodology (RSM) is a useful statistical technique which has been applied in research into complex variable processes. The multiple regression and correlation analyses are used as tools to assess the effects of two or more independent factors on the dependent variables. Furthermore, the central composite design (CCD) of response surface methodology has been applied in the optimization

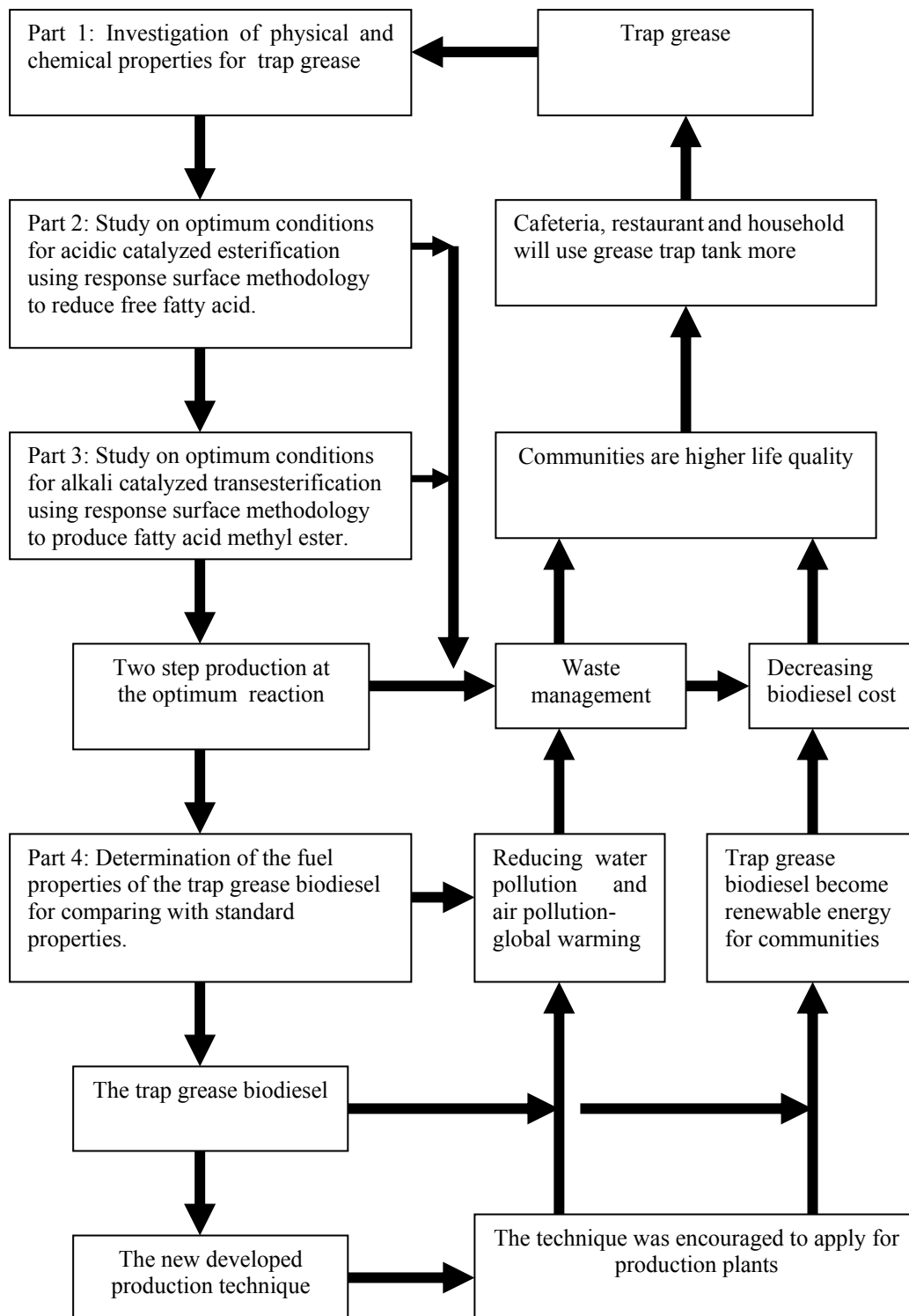
of several biotechnological and chemical processes. Its main advantage is the reduction number of experimental runs that required to generate sufficient information for a statistically acceptable result. Response surface methodology has been successfully applied for optimization of biodiesel production in several fats and oils for instance mahua oil (*Madhuca indica*) (Ghadge and Raheman, 2006), jatropha oil (*Jatropha curcas*) (Tiwari *et al.*, 2007), waste rapeseed oil (Yuan *et al.*, 2008), animal fat (Jeong *et al.*, 2009), palm oil (Kansedo *et al.*, 2009).

The present studies concentrate on developing a two-step biodiesel production technique from trap grease as a raw material. The developed process is esterification and transesterification. The special attention is paid to optimize the first step being the acid catalyzed process (esterification) to reduce the free fatty acid (FFA) content and the second step is the alkali catalyzed process (transesterification) for producing fatty acid methyl ester (FAME). The fuel properties of trap grease biodiesel is also determine. The optimized biodiesel production technique from trap grease with the two step catalyzed process could be evaluated for the utilization of biodiesel plant and to solve the environmental problem on waste water. The present research is shown by the research framework

The objectives of this research are as follows:

1. To investigate physical and chemical properties of crude trap grease.
2. To study optimum conditions for acid catalyzed esterification using response surface methodology to reduce free fatty acid (FFA) of trap grease.
3. To study optimum conditions for alkali catalyzed transesterification using response surface methodology to produce fatty acid methyl ester (FAME).
4. To determine the properties of the biodiesel produced from trap grease at the optimum reaction conditions for comparing with standard properties.

The research framework



LITERATURE REVIEW

1. Biodiesel

Renewable energy was derived from natural resources generating with natural processes into various energy forms such as solar power, geothermal power, hydro power, tidal power, wave power, wind power and bioenergy. Bioenergy consist of several categories such as biogases, bioethanol, biomass energy, biohydrogen including biodiesel (Celiktas *et al*, 2009). Main outstanding advantages of the the renewable energy, were as follow

1. Environmentally friendly energy was waste utilization with producing as renewable energy and solving environmental pollution or global warming.
2. Self sufficiency economy for country development into energy security.
3. Agricultural value Adding lead to being higher life quality of farmers.
4. Life of human beings were healthy worldwide because renewable energy could reduce air pollution and wastes effecting to human.

Hence, biodiesel is environmentally friendly alternative diesel fuel, which as a biodegradable, non-toxic due to exhaust emissions of diesel engine operating with biodiesel could decrease approximately 20% in carbonmonoxide (CO) emission, 30% in hydrocarbon (HC) emission, 40% in particulate matter (PM) emission and 50% in soot emission, compared to the diesel fuel. Lubricity proparty of biodiesel was much better than that of diesel fuel, especially low-sulfer diesel fuel. Little biodiesel additive, even as little as 15%, was enough to significantly improve the conventional diesel fuel's lubricity (Canakci and Sanli, 2008). Since, waste frying oil methyl ester has a lower heating value, higher density and viscosity, waste frying oil methyl ester 's specific fuel consumption was increased as 14.34%. Emission values were decreased as 17.14% carbonmonoxide (CO), 1.45% nitrogendioxide compounds (NO_x). Smoke intensity was increased in average 22.46% for the utilization of waste frying oil methyl ester compared to diesel fuel. Exhaust temperatures of waste frying oil

methyl ester was decreased on average 6.5% than diesel fuel (Figure 1) (Utlu and Kocak, 2008). Moreover, biodiesel contained higher oxygen content than petroleum diesel and its usage in diesel engines had shown great reductions in emission of particulate matter, carbon monoxide, sulfur, polyaromatics, hydrocarbons, smoke and noise. In addition, burning of vegetable-oil based fuel did not contribute to net atmospheric carbondioxide (CO₂) levels because such fuel was made from agricultural materials which were produced via photosynthetic carbon fixation (Zullaikah *et al.*, 2005).

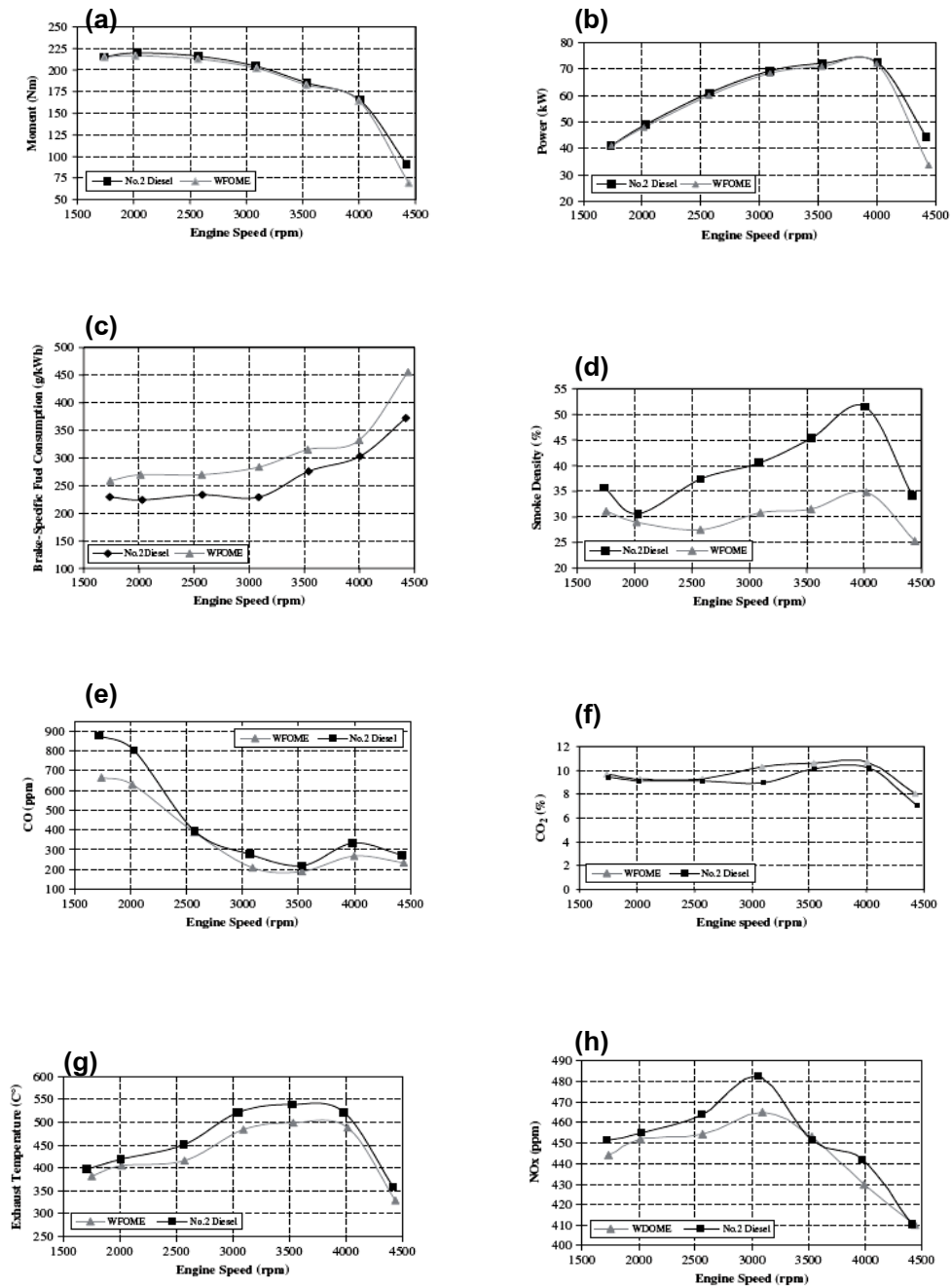


Figure 1 Exhaust emissions of diesel engine operating with biodiesel compared to diesel fuel. (a) Moment versus engine speed. (b) Engine power versus engine speed. (c) Brake-specific fuel consumption versus engine speed. (d) Smoke density versus engine speed. (e) CO emissions versus engine speed. (f) CO₂ emissions versus engine speed. (g) Exhaust temperatures versus engine speed. (h) Exchange of NO_x.

Source: Utlu and Kocak (2008)

2. Trap Grease in Wastewater

Trap grease is material that was collected in special traps in restaurants to prevent the grease from entering the sanitary sewer system where it could cause blockages. Trap grease have high free fatty acids (FFA) and high moisture levels effect the transesterification reaction in converting these feedstocks into biodiesel (Cvengros and Cvengrosova, 2004). The high free fatty acids (FFA) level exceeds 15%, it was called trap grease, sometimes referred to as brown grease, and it might be sold at a discount. yellow grease was required to have a free fatty acid (FFA) level of less than 15% (Canakci, 2007).

According to previously overseas research like United States of America (USA) reported that approximately 2.5 billion pounds of waste restaurant fats were collected annually from restaurants and fast-food establishments in United States of America (USA). The US Department of Energy's National Renewable Energy Laboratory (NREL) sponsored a study on urban trap grease resources in 30 randomly selected metropolitan areas in the United States of America (USA). This study showed that an average of 13 pounds/year person of trap grease and 9 pounds/year person of yellow grease were produced in 1998. Table 1 showed quantitative data for trap grease and yellow grease from 30 metropolitan areas in the United States of America (USA). The cities ranged in population size from 83,831 to 3,923,574. These data also showed that the cities had an average of 1.4 restaurants per 1000 people. Although the range of the data was fairly wide, the National Renewable Energy Laboratory (NREL) study the volume of the waste restaurant grease produced correlated to general population as well as to the number of restaurants (Canakci, 2007).

Furthermore, Waste vegetable oils and fats were generally low in cost and were collected from large food processing and service facilities. They were then rendered and used almost exclusively in animal feed. The prices of rendering plant products are shown in Table 2. The price of yellow grease varies widely from \$0.09 to \$0.20/lb. Brown grease was usually discounted \$0.01–\$0.03. Brown grease was often

cited as a potential feedstock for biodiesel because it currently had very low value. However waste vegetable oil from restaurants and rendered animal fats were inexpensive compared with food-grade vegetable oil (Canakci, 2007).

In addition, Department of Agriculture of the United States of America (USA) had reported on a combination of greases and animal fats represents one-third of the United States of America (USA) total fats and oils production (Table 3), but soybean oil alone represents more than half of USA production. However, biodiesel production from the grease could be expected to benefit from a raw material cost advantage and it would help to reduce overall biodiesel cost. One pound of most fats and oils can be converted to a pound of biodiesel. If all of the 11.638 billion pound/year of greases and animal fats were converted to biodiesel, it would replace about 1.5 million gallons of diesel fuel (1 gallon is 3.78 liter). The low-cost and profitable biodiesel could be produced from low-cost feedstocks such as waste frying oils, animal fats, soapstocks, and grease, shown in Table 4 (Canakci and Sanli, 2008).

Properties of crude waste fryer grease (WFG) had Initial water content of waste fryer grease (WFG) was 7.3% w/w. The water content was reduced to $0.20 \pm 0.10\%$ w/w after silica gel treatment. The crude waste fryer grease (WFG) contains 5.6% free fatty acids which made alkaline catalyzed transesterification impractical. The waste fryer grease (WFG) was contaminated with various other chemicals due to exposure of cooking oil to a high temperature for a long period. Hence, the total polar content of waste fryer grease (WFG) was 22%, which was much higher than that in the neat vegetable oil (0.40–6.40 mg/100 g). Due to the exposure of the cooking oil to a higher temperature for a long period of time some triglycerides got oxidized. Oxidized triglyceride content of the waste fryer grease (WFG) was 4.72%. The same reason, some of the triglycerides got polymerized and the content of polymerized triglycerides in waste fryer grease (WFG) was 1.43% (Table 5) (Issariyakul *et al.*, 2007).

Feedstocks from food-grade vegetable oils for biodiesel production were more expensive than diesel fuel. Therefore, biodiesel produced from food-grade vegetable oil is currently not economically feasible. Waste cooking oils, restaurant grease and animal fats were potential feedstocks for biodiesel. These inexpensive feedstocks represent one-third of the United States of America (USA) total fats and oil production, but are currently devoted mostly to industrial uses and animal feed. The characteristics of feedstock were very important during the initial research and production stage. Free fatty acids and moisture reduce the efficiency of transesterification in converting these feedstocks into biodiesel. Hence, the study was conducted to determine the level of these contaminants in feedstock samples from a rendering plant. Levels of free fatty acids varied from 0.70% to 41.80%, and moisture from 0.01% to 55.38%. These wide ranges indicate that an efficient process for converting waste grease and animal fats must tolerate a wide range of feedstock properties. These data showed on Table 6 (Canakci, 2007).

Table 1 Urban waste grease resources in 30 metropolitan areas.

No	Metro area	State	Population	Number of restaurants	Urban waste grease resources (pounds/year)			Urban waste grease resources (pounds/year/ person)			
					Yellow grease	Trap grease	Total grease	Restaurant/ 1000 P	Yellow grease	Trap grease	Total grease
1	Sacramento	CA	1,481,102	2200	4,500,000	16,600,000	21,100,000	1.49	3.04	11.21	14.25
2	Olympia	WA	161,238	240	1,080,000	1,200,000	2,280,000	1.49	6.70	7.44	14.14
3	Provo	UT	263,590	400	4,380,000	7,000,000	11,380,000	1.52	16.62	26.56	43.17
4	Denver	CO	1,848,319	2670	17,000,000	15,900,000	32,900,000	1.44	9.20	8.60	17.80
5	Lincoln	NE	213,641	350	4,500,000	2,600,000	21,600,000	1.64	21.06	12.17	101.10
6	Bismarck	ND	83,831	133	430,000	400,000	830,000	1.59	5.13	4.77	9.90
7	Bloomington	IL	129,180	200	500,000	2,300,000	2,800,000	1.55	3.87	17.80	21.68
8	Battle Creek	MI	135,982	211	1,500,000	1,500,000	3,000,000	1.55	11.03	11.03	22.06
9	Mansfield	OH	126,137	244	650,000	190,000	840,000	1.93	5.15	1.51	6.66
10	Elmira	NY	95,195	140	950,000	1,500,000	2,450,000	1.47	9.98	15.76	25.74
11	Boston	MA	1,950,855	3000	10,400,000	33,600,000	44,000,000	1.54	5.33	17.22	22.55
12	Harrisburg	PA	587,986	900	6,000,000	10,800,000	16,800,000	1.53	10.20	18.37	28.57
13	Altoona	PA	130,542	143	1,300,000	1,000,000	2,300,000	1.10	9.96	7.66	17.62
14	Hagerstown	MD	121,393	170	1,200,000	1,000,000	2,200,000	1.40	9.89	8.24	18.12
15	Washington	DC	3,923,574	5000	39,000,000	50,000,000	89,000,000	1.27	9.94	12.74	22.68
16	Richmond	VA	865,640	1480	8,700,000	17,300,000	26,000,000	1.71	10.05	19.99	30.04
17	Danville	VA	108,711	157	1100,000	1,900,000	3,000,000	1.44	10.12	17.48	27.60
18	Fayetteville	NC	274,566	384	2,700,000	2,100,000	4,800,000	1.40	9.83	7.65	17.48
19	Florence	SC	114,344	185	1,100,000	900,000	2,000,000	1.62	9.62	7.87	17.49
20	Greenville	SC	640,861	1017	6,400,000	4,600,000	11,000,000	1.59	9.99	7.18	17.16

Table 1 (Continued)

No	Metro area	State	Population	Urban waste grease resources (pounds/year)				Urban waste grease resources (pounds/year/ person)			
				Number of restaurants	Yellow grease	Trap grease	Total grease	Restaurant/ 1000 P	Yellow grease	Trap grease	Total grease
21	Lexington	KY	348,428	562	3,500,000	3,600,000	7,100,000	1.61	10.05	10.33	20.38
22	Memphis	TN	981,747	1128	9,800,000	18,500,000	28,300,000	1.15	9.98	18.84	28.83
23	Decatur	AL	131,556	245	1,300,000	2,400,000	3,700,000	1.86	9.88	18.24	28.12
24	Macon	GA	281,103	348	2,800,000	5,900,000	8,700,000	1.24	9.96	20.99	30.95
25	Lakeland	FL	405,382	445	4,100,000	4,600,000	8,700,000	1.10	10.11	11.35	21.46
26	Bradenton	FL	211,707	360	2,100,000	3,000,000	5,100,000	1.70	9.92	14.17	24.09
27	Baton Rouge	LA	528,264	657	5,300,000	5,800,000	11,100,000	1.24	10.03	10.98	21.01
28	Shreveport	LA	334,341	442	3,300,000	4,700,000	8,000,000	1.32	9.87	14.06	23.93
29	Beaumont	TX	361,226	383	3,600,000	3,900,000	7,500,000	1.06	9.97	10.80	20.76
30	Bryan	TX	121,862	198	1,200,000	2,000,000	3,200,000	1.62	9.85	16.41	26.26
							Weighted average	1.41	8.87	13.37	23.09

Source: Canakci (2007)

Table 2 Prices of rendering plant products.

Bulk	Price range (usdollar per pound)	Average price (usdollar per pound)
Animal/ livestock feed	0.09–0.14	0.12
Tallow	0.10–0.20	0.17
Grease	0.07–0.20	0.16

Source: Canakci (2007)

Table 3 The United States of America (USA) production of fats and oils (billion pounds).

Vegetable oil*		Animal fat*	
Soybean	18.340	Edible tallow	1.625
Corn	2.420	Inedible tallow	3.859
Peanuts	0.220	Lard and grease	1.306
Sunflower	1.000	Yellow grease	2.633
Cottonseed	1.010	Poultry fat	2.215
Others	0.669		
Total	23.659		11.638

*National Renderers Association (USDA averages, 1995–2000).

Source: Canakci (2007)

Table 4 Some economic assessment studies for biodiesel based on the feedstocks.

Feedstock type /year	Biodiesel price (US \$1)
Canola/2003	0.72
Soybean /2005	0.53
Waste cooking oil/2003	0.54-0.74
Waste grease/1999	0.34-0.42
Yellow grease/2001	0.324
Brown grease/2001	0.246

Source: Canakci and Sanli (2008)

Table 5 Properties of crude waste fryer grease.

Property	Value	Method
1. Solid portion	19 %	–
2. Total polar compounds	22 %	AOCS Cd 20-91
3. Polymerized triglycerides	1.43 %	IUPAC, 2.508 (1987)
4. Oxidized triglycerides	4.72 %	IUPAC, 2.508 (1987)
5. Acid value	11.2 mg KOH g ⁻¹	AOCS Te 1a-64
6. Free fatty acid (FFA)	5.6 %	–
7. Water content	7.3 %	Karl Fischer method
8. Saponification value	177.87 mg KOH g ⁻¹	AOCS Cd 3-25

Source: Issariyakul *et al.* (2007)

Table 6 Chemical analysis results of restaurant grease and animal fat samples

Test/sample	Soy oil 1	Soy oil 2	SIM-01	SIM-02	SIM-03	SIM-04	SIM-05	SIM-07	SIM-08	SIM-09	SIM-10	SIM-11	SIM-24
MIU (%)	0.44	0.41	24.11	0.74	2.85	3.54	0.98	6.42	58.14	1.71	1.06	1.97	2.37
Moisture and volatiles by hot plate	0.01	<0.10	18.06	0.31	0.11	3.11	0.35	0.26	55.38	1.26	0.65	1.42	0.35
Insoluble impurities (%)	<0.10	<0.10	1.22	<0.10	0.11	0.09	0.11	3.83	2.51	0.03	0.03	0.08	1.03
Unsaponifiable matter (%)	0.43	0.41	4.83	0.43	2.63	0.34	0.52	2.33	0.25	0.42	0.38	0.47	0.99
Peroxide value (meq/kg)	66	7.3	0.8	4.0	<0.2	3.7	3.4	<0.2	0.6	4.6	3.1	1.7	1.0
FFA (%)	0.02	0.01	41.8	9.7	25.7	2.6	1.1	25.5	14.8	0.7	1.3	0.7	10.5
<i>Fatty acid profile (%)</i>													
C14:0 Tetradecanoic (myristic)	<0.10	<0.10	2.79	0.45	1.52	0.59	0.19	1.56	0.43	0.20	0.49	0.18	1.45
C16:0 Hexadecanoic (palmitic)	10.29	10.40	25.92	12.84	22.39	13.44	11.49	22.46	12.74	10.92	13.67	10.91	20.51
C16:1 Hexadecenoic (palmitoleic)	<0.10	<0.10	3.79	1.12	3.14	1.24	0.64	3.06	0.88	0.44	0.95	0.28	3.58
C17:0 Heptadecanoic (margaric)	0.11	<0.10	1.09	0.22	0.53	0.28	0.13	0.57	0.24	0.14	0.22	0.14	0.49
C17:1 Heptadecenoic (margaroleic)	<0.10	<0.10	0.71	0.15	0.39	0.19	<0.10	0.41	0.14	<0.10	0.16	<0.10	0.39
C18:0 Octadecanoic (stearic)	4.28	4.51	16.49	7.18	12.81	7.59	5.69	13.33	8.22	5.05	7.45	6.61	11.43
C18:1 Octadecenoic (oleic)	21.55	24.37	39.54	43.49	42.53	42.11	29.37	42.74	41.52	33.47	37.70	37.22	41.87
C18:2 Octadecadienoic (linoleic)	53.68	51.90	4.40	29.47	12.07	29.33	44.77	10.89	30.86	42.64	33.48	38.33	13.98
C18:3 Octadecatrenoic (linolenic)	8.16	6.52	0.57	2.66	0.81	2.86	5.38	0.67	2.96	4.90	3.58	4.22	1.41
C18:4 Octadecatetraenoic	<0.10	<0.10	0.25	0.30	0.10	0.27	<0.10	0.12	0.20	0.21	0.19	0.23	0.25
C20:0 Eicosanoic (arachidic)	0.34	0.37	0.18	0.32	0.20	0.33	0.35	0.21	0.34	0.35	0.33	0.37	0.22
C20:1 Eicosenoic (gadoleic)	0.19	0.28	0.66	0.57	1.01	0.53	0.39	1.04	0.46	0.45	0.46	0.45	1.06
C22:0 Docosanoic (behenic)	0.37	0.39	<0.10	0.33	<0.10	0.33	0.35	<0.10	0.34	0.37	0.30	0.36	<0.10
Unknown components (%)	0.91	1.13	2.16	0.90	0.89	0.70	1.14	1.38	0.67	0.74	1.02	0.58	1.02

SIM-01: Unprocessed restaurant grease. As delivered. Collected from the tops of three separate barrels. Water mostly at the bottom of the barrel. SIM-02: Restaurant grease. Boiled and settled. This is the final product before mixing with animal fat. SIM-03: Animal fat. This was the final product for animal fat but before it is mixed with any restaurant grease. SIM-04: Processed restaurant grease. Skimmed from top of barrel. SIM-05: Restaurant grease. Skimmed from top of barrel. SIM-07: Mixed fat from storage tank. This is the rendered product as sold. SIM-08: Restaurant grease, partially processed. This material had been through the first stage of processing, where the solids and free water are removed but it had not been cooked. SIM-09—SIM-11: Restaurant grease. Skimmed from top of barrel. SIM-24: Finished grease, with some restaurant grease.

Source: Canakci (2007)

3. Biodiesel Production

There were at least four ways in which oils and fats could be converted into biodiesel, namely transesterification, blending, micro-emulsions and pyrolysis. Transesterification was the most commonly used method. In 2005, Bouaid *et al.* study about transesterification for biodiesel production from *B. carinata* oil in the pilot plant. A scheme of the main reaction unit (Figure 2). *B. carinata* oil was stored in a 1000 kg cone-bottom tank at room temperature. For the transesterification reaction, the oil was transferred to the main reaction tank. The main transesterification reaction was carried out in a continuous stirred tank reactor of 200 liters glass tank with a turbine agitator, under fixed pressure and temperature conditions. The reactor was also equipped with stationary baffles attached along the surface. A load cell mounted on one leg of the reaction tank measures the reactant amounts. After preparing the alcohol solution with the catalyst, it was added to the reaction tank. The reactants were agitated for 60 min and then the reaction mixture was transferred to a 400 liters decanter for glycerine and methyl ester separation, allowing glycerol to separate by gravity for 2 hours. Temperature, pressure and stirring speed controllers were provided. Nevertheless transesterification could use as one step process for low free fatty acid of raw material which like *B. carinata* oil. This pilot plant using *B. Carinata* oil as raw materials with methanol and using potassium hydroxide as catalyst could produce the biodiesel quality that was European specifications defined by EN 14214:2002. The obtained results had been used for industrial scale up of the process.

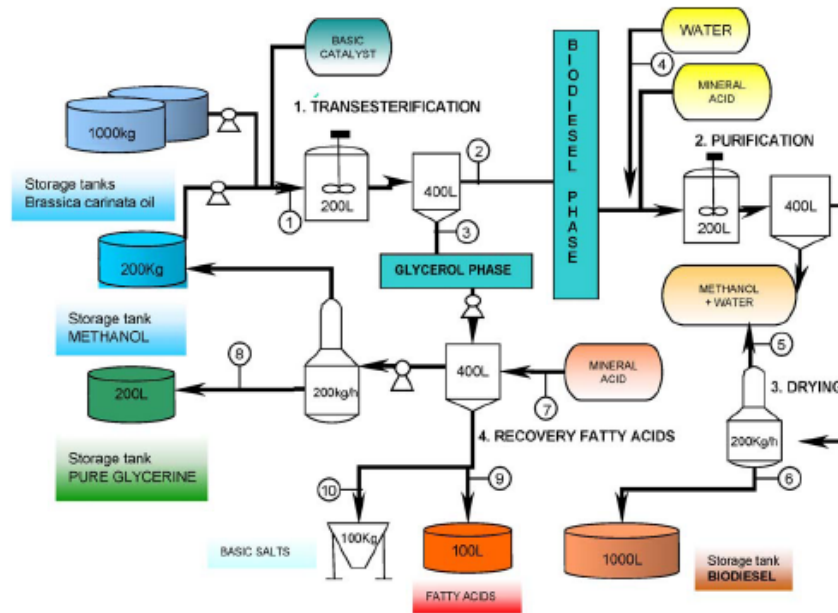
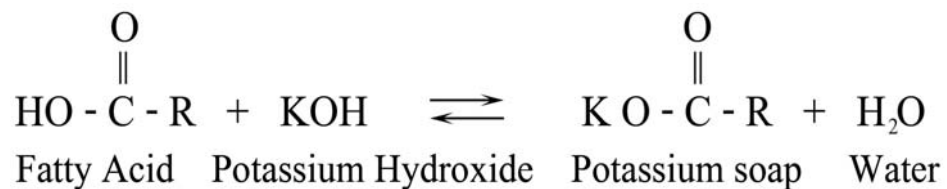


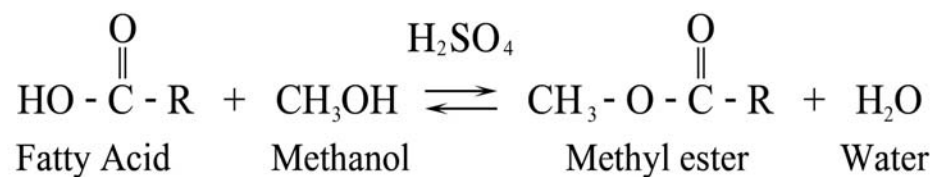
Figure 2 Flow sheet of the transesterification process.

Source: Bouaid *et al.* (2005)

Conversion was complicated if oil contains a large amounts of FFA that would form soap with alkaline catalyst, shown in the reaction below.

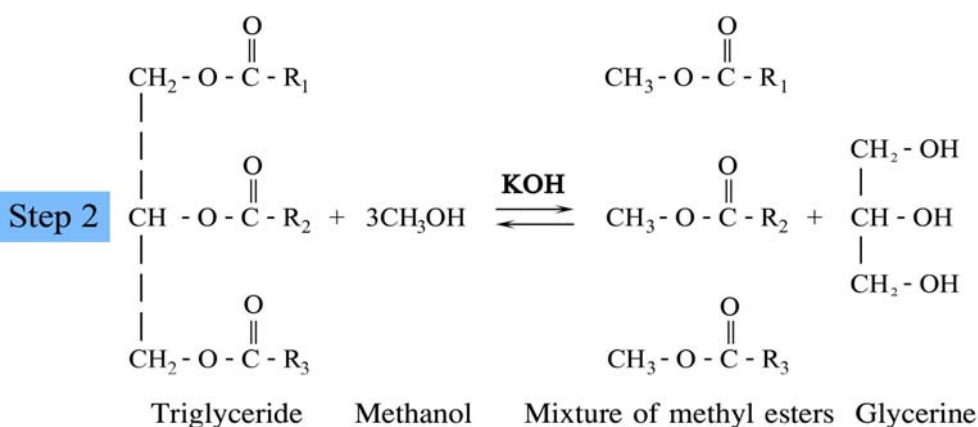
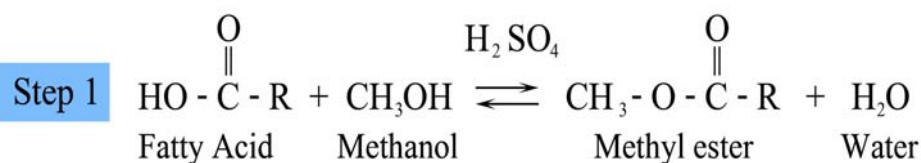


The soap could prevent separation of the biodiesel from the glycerin fraction and contributes to emulsion formation during the water wash for these cases, an acid catalyst, such as sulfuric acid, could be used to esterify the free fatty acid to methyl ester, as shown in the following reaction (Gerpen, 2005).



Problem avoidance of the saponification, the two-step process method was used for biodiesel production from high free fatty acid (FFA) feedstock like waste fryer grease (WFG). In the first step esterification carried out with using sulphuric acid as a catalyst and in the second step transesterification performed using potassium hydroxide as a catalyst. The esterification of free fatty acid (FFA) was carried out as follows: A 50 g waste fryer grease (WFG) was placed in a batch type reactor equipped with glass stopper, thermometer, and a magnetic stirrer. In another flask 2 wt.% of sulphuric acid with respect to waste fryer grease (WFG) was mixed with alcohol (3:1 alcohol to waste fryer grease (WFG) ratio plus 0.0088 mol extra alcohol for esterification of free fatty acid (FFA) in waste fryer grease (WFG)) and then poured to the waste fryer grease (WFG). The reaction temperature of 50°C was selected for methanol and mixture of alcohol and 60°C for ethanol to avoid the evaporation of alcohols. Depending on the decrease in acid value of waste fryer grease (WFG), the esterification reaction was continued for 5 hours. Water formed during the esterification reaction of free fatty acid and alcohol had an adverse effect during the alkali catalyzed transesterification reaction and removed prior to the transesterification reaction. In order to remove water, the product of the esterification reaction was mixed with 10 wt.% silica gel and stirred for 15 minutes. After stirring, the mixture was filtered to remove the silica gel. The transesterification of neutral waste fryer grease (WFG) was carried out using KOH as a catalyst. 1% KOH for the transesterification with 1.144 g was properly mixed in alcohol to neutralize sulphuric acid and then poured to the reaction mixture. The transesterification reaction was carried out for 1 hour at room temperature for methanol, and for mixture of alcohols and ethanol at 50 and 60 °C, respectively (Issariyakul *et al.*, 2007).

The feedstocks having higher free fatty acid (FFA) levels using the two-step process to reduce the free fatty acid (FFA) of yellow grease from 12% and brown grease from 33% to less than 1%, the transesterification reaction was completed with an alkaline catalyst to produce biodiesel (Ghadge and Raheman, 2005). The two-step catalyzed process could be represented by the following reaction scheme.



The main factors affecting the reaction of biodiesel production are the contents of free fatty acid (FFA), reaction temperature, methanol to oil Ratio, catalyst concentration and reaction time.

3.1 Effect of Free Fatty Acid

Zullaikah *et al.* (2005) investigated the effect of temperature, moisture and storage time on the accumulation of free fatty acid in the rice bran oil. The rice bran oil stored at room temperature with most triacylglyceride was hydrolyzed and free fatty acid (FFA) content was raised up to 76% in six months. Hence, A two-step acid-catalyzed methanolysis process was employed for the efficient conversion of rice bran oil into fatty acid methyl ester (FAME). The first step was carried out at 60°C, depending on the initial free fatty acid (FFA) content of oil, 55–90% FAME content in the reaction product was obtained. More than 98% FFA and less than 35% of triglyceride were reacted in 2 hours. The organic phase of the first step reaction product was used as the substrate for a second acid-catalyzed methanolysis at 100°C.

By this two-step methanolysis reaction, more than 98% fatty acid methyl ester (FAME) in the product could be obtained in less than 8 hours. Distillation of reaction product gave 99.8% fatty acid methyl ester (FAME) or biodiesel with recovery of more than 96%. The residue contains enriched nutraceuticals such as 16–18% of α -oryzanol, mixture of phytosterol, 19–21% of tocol and steryl ester.

3.2 Effect of Temperature

Zheng *et al.* (2006) studied the reaction kinetics of acid-catalyzed transesterification of waste frying oil in excess methanol to form the fatty acid methyl esters (FAME), for possible used as biodiesel. Rate of mixing, feed composition (molar ratio of oil:methanol:acid) and temperature were independent variables. There was no significant difference in the yield of fatty acid methyl ester (FAME) when the rate of mixing was 100 to 600 rpm for turbulent range. The oil:methanol:acid molar ratios and temperature were the most significant factors affecting the yield of FAME. At 70°C with oil:methanol:acid molar ratios of 1:245:3.8, and at 80°C with oil:methanol:acid molar ratios in the range 1:74:1.9–1:245:3.8, the transesterification was a pseudo-first-order reaction as a result of the large excess of methanol which drove the reaction to completion as 99±1% at 4 hours. In the large excess of methanol, free fatty acids present in the waste oil were very rapidly converted to methyl esters in the first few minutes under the above conditions. Little or no monoglycerides were detected during the course of the reaction, and diglycerides present in the initial waste oil were rapidly converted to fatty acid methyl ester (FAME).

Leung and Guo (2006) found that higher reaction temperature more than 50 °C had a negative impact on the product yield for neat oil, but it had a positive effect for waste oils with higher viscosities. Optimal reaction for the transesterification of used frying oil (UFO) with an acid value of 2 mg KOH/g and viscosity of 35 cSt could be achieved at 60 °C for a reaction time of 20 minutes, 1.1 wt.% NaOH and 7:1 molar ratio of methanol to UFO. For commercial edible Canola

oil, the optimal conditions were 40–45°C for a reaction time of 60 minutes, 1.0 wt.% NaOH and 6:1 methanol to oil molar ratio.

At higher reaction temperatures, there was a chance of loss of methanol and increased in darkness of the product. However, high reaction temperature increased the production cost of biodiesel too (Ramadhas *et al.*, 2005).

3.3 Effect of Methanol to Oil Ratio

Felizardo *et al.* (2006) studied waste frying oils transesterification with the purpose of achieving the best conditions for biodiesel production. The transesterification reactions were carried out for 1 hour using waste frying oil (WFO), methanol, and sodium hydroxide as catalyst. In order to determine the best conditions for biodiesel production, a series of experiments were carried out, using methanol to WFO molar ratios between 3.6 and 5.4 and catalyst to waste frying oil (WFO) weight ratios between 0.2% and 1.0%. For oils with an acid value of 0.42 mgKOH/g, results showed that a methanol to waste frying oil (WFO) ratio of 4.8 and a catalyst to waste frying oil (WFO) ratio of 0.6% gave the highest yield of methyl esters. Furthermore, an increase in the amount of methanol or catalyst quantity saw to simplify the separation/purification of the methyl esters phase, as showed by a viscosity reduction and an increasing purity to values higher than 98% for methyl esters phase. In addition, Alamu *et al.* (2008) studied the laboratory scale production and testing of palm kernel oil (PKO) biodiesel, as well as the effect of ethanol to palm kernel oil (PKO) ratio on biodiesel yield. Nigerian palm kernel oil (PKO) biodiesel gave promising results as alternative diesel fuel with fuel properties in good agreement with previous studies and within limits set by international biodiesel standards. For ethanol to palm kernel oil (PKO) ratios: 0.1, 0.125, 0.15, 0.175, 0.2, 0.225 and 0.25 converted as palm kernel oil (PKO) biodiesel yield of 29.5%, 54%, 75%, 89%, 96%, 93.5% and 87.2% were obtained under typical transesterification reaction conditions of 60°C temperature, 120 minutes reaction duration and 1.0% alkali catalyst concentration like KOH. A maximum palm kernel oil (PKO) biodiesel yield of 96%

was obtained with ethanol to palm kernel oil (PKO) ratio 0.2 under typical transesterification reaction conditions of 60°C temperature, 120 minutes of reaction time and 1.0% alkali catalyst (KOH) concentration. Nevertheless, this research result indicated that increase in the proportion of ethanol in the feedstock did not increase palm kernel oil (PKO) biodiesel yield.

3.4 Effect of Catalyst Concentration

Ghadge and Raheman (2005) reported that 19% of high free fatty acid (FFA) level of crude mahua oil could be reduced to less than 1% in a two-step using acid catalyzed esterification reaction of 1% v/v sulphuric acid with methanol (0.30–0.35 v/v) at 60°C temperature and 1 hour reaction time. After each step, a settling time of minimum 1 hour was required for removal of methanol and water mixture. The second step product having acid value less than 2 mg KOH/g was used for the final alkali-catalyzed transesterification reaction of 0.70% w/v potassium hydroxide with methanol of 0.25 v/v to produce biodiesel. This process gave an yield of 98% mahua biodiesel, which has comparable fuel properties with that of diesel and are within the limits prescribed by the American and European biodiesel standards. Therefore, the catalyst concentration concern with both esterification and transesterification in two step production of biodiesel from high free fatty acid (FFA) feedstocks.

3.5 Effect of Reaction Time

Issariyakul *et al.* (2007) found that the acid value of crude waste fryer grease (WFG) of 11.2 mg KOH/g was decreased by esterification reaction of two step process after 5 hours of reaction time due to there was no significant decrease in acid value after 5 hours, hence the first-step esterification was stopped after 5 hours.

4. Two Step Catalyzed Process

Biodiesel of good quality could be prepared from used cooking oil by two step base catalyzed reaction. The two step process was better than one step process in terms of lower reaction temperature, reduced catalyst and alcohol used and high yields obtained. This method could be easily adapted to a continuous process.

Issariyakul *et al.* (2007) studied two step process. For transesterification of waste fryer grease (WFG) containing 5–6 wt.% free fatty acid (FFA) was carried out with methanol, ethanol and mixtures of methanol/ethanol maintaining alcohol to oil molar ratio of 6:1, and potassium hydroxide as a alkaline catalyst. Formation of soap by reaction of free fatty acid (FFA) in waste fryer grease (WFG) with potassium hydroxide showed difficulty in the separation of glycerol from biodiesel. To solve this problem, the two step process as acid catalyzed esterification and alkali catalyzed transesterification, was used for biodiesel production. More than 90% ester was obtained when two-stage method was used compared to 50% ester in single stage alkaline catalyst. In the case of mixed alcohol, a relatively smaller amount of ethyl esters was formed along with methyl esters. Acid value, viscosity, and cetane number of all esters prepared from waste fryer grease (WFG) within the range of the ASTM standard. Esters obtained from waste fryer grease (WFG) showed good performance as a lubricity additive. Gas chromatography spectrometry, which gave the results as chromatogram could be used to determine the yield of methyl esters from waste fryer grease methyl ester (Figure 3).

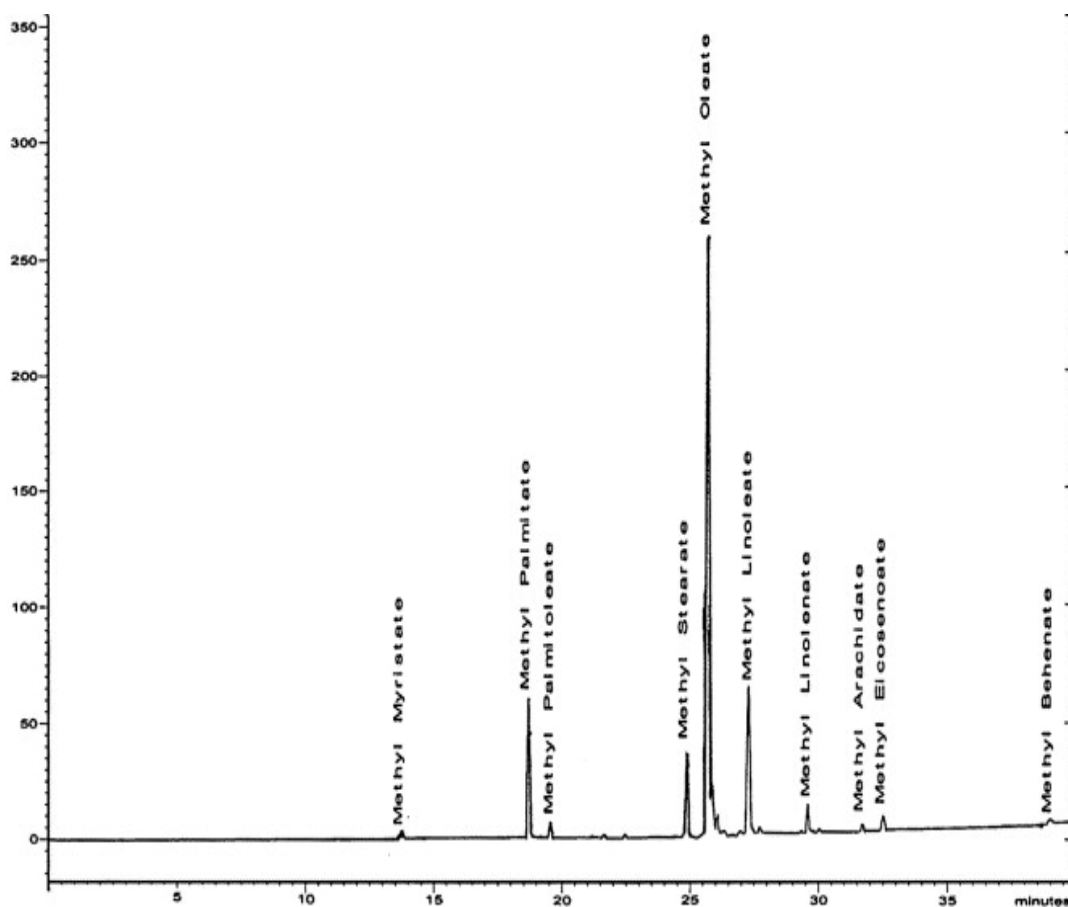


Figure 3 Chromatogram of waste fryer grease methyl esters.

Source: Issariyakul *et al.* (2007)

Short chain alcohol esters of fatty acids could be used as diesel fuel. In previous studies, one step and two step base catalyzed at room temperature for transesterification reaction of used cooking oil was compared. In the two step base catalyzed process, 1000 g of used cooking oil, 4.20 g sodium hydroxide and 140 ml methanol was used in the first step and 1.80 g sodium hydroxide and 60 ml of methanol was used in the second step. All reactions were done at 25°C; the effects of water content and suspended particles on the yield were studied. The yields were easily determined by Thermo Gravimetric Analysis (TGA) instead of Gas chromatography and viscosity of products was measured by Ubbelohde type viscosimeter. It was found that two step processes gave a better yield (94%) than the one step process (86%) (Table 7) (Leung and Guo, 2006; Wang *et al.*, 2007; Cayli and Kusefoglu, 2008).

Table 7 Yields of the reaction.

Type of oil	Yields for one step process (%)	Two step process	
		Yields for the first step of the two step process (%)	Yields of the two step process (%)
Used cooking oil	78%	60%	86%
Filtered and dried used cooking oil	86%	73%	94%

Source: Cayli and Kusefoglu (2008)

The traditional acid and the new two-step catalyzed processes for synthesis of biodiesel expressed as fatty acid methyl ester (FAME) were comparatively studied to achieve an economic and practical method for utilization of waste cooking oil (WCO) from Chinese restaurants. Waste cooking oil (WCO) samples with the acid value of 75.92 ± 0.04 mg KOH/g mixed with methanol were catalyzed under 95°C for various reaction time, followed by methanol recovery under vacuum of 10 ± 1 mmHg at 50°C with a rotational evaporation. fatty acid methyl ester (FAME) analyzed by gas chromatography was obtained directly from sulphuric acid catalyzed reaction in the traditional acid method, whereas in the two-step method it was produced from ferric sulfate (2.0%) catalyzed reaction followed by alkali (1.0% potassium hydroxide) transesterification. The waste cooking oil (WCO) can be converted into biodiesel directly by one-step sulphuric acid catalyzed process. The conversion of waste cooking oil (WCO) was $\geq 90\%$, at the reaction time of 10 hours and mole ratio of methanol to waste cooking oil (WCO) 20:1. The disadvantages with this process are acidic effluent, no reusable catalyst and high cost of equipment. The conversion of FFA of the waste cooking oil (WCO) into fatty acid methyl ester (FAME) in the two-step method reached 97.22% at the reaction time of 4 hours, methanol to waste cooking oil (WCO) molar ratio of 10:1 and 95°C of reaction temperature. This two-step catalyzed process provides a simple and economic method to produce biodiesel from the waste cooking oil (WCO). Glycerine by-products and soap stock in this

process could be easily handled compared with the acid catalyzed one (Wang *et al.*, 2006).

Furthermore, Ramadhas *et al.* (2005) had studied a two-step transesterification process for developing to convert the high free fatty acid (FFA) oils to its esters. The first step being acid catalyzed esterification reduces the free fatty acid (FFA) content of the oil to less than 2%. The alkaline catalyst transesterification process converted the products of the first step to its esters and glycerol. The effects of alcohol to oil molar ratio, catalyst amount, reaction temperature and reaction time were analyzed in each step. Excess addition of sulfuric acid darkened the product. It had been also found that the conversion efficiency was strongly affected by molar ratio of alcohol to oil. The methanol to oil molar ratio of 6:1 favored the completion of alkaline catalyzed transesterification process within half an hour. The maximum ester conversion was achieved at the reaction temperature of $45\pm 5^{\circ}\text{C}$. The viscosity, flash point and calorific value were agreeable for biodiesel standard.

In addition, Investigation of Veljkovic *et al.* (2006) on the production of fatty acid methyl esters (FAME) from crude tobacco seed oil with high free fatty acid (FFA) contained high free fatty acid (FFA) content, hence the tobacco seed oil was processed in two step process. The first step was acid-catalyzed esterification followed by base-catalyzed transesterification. The first step reduced the free fatty acid (FFA) level to less than 2% in 25 min for methanol to oil molar ratio of 18:1. The second step converted the product of the first step into fatty acid methyl esters (FAME) and glycerol. The maximum yield of fatty acid methyl esters (FAME) was about 91% in about 30 minutes. The tobacco biodiesel had the fuel properties within the limits prescribed by the latest American as ASTM D 6751-02 and European as DIN EN 14214 standards, except a somewhat higher acid value than that prescribed by the latter standard as less than 0.5. Hence, tobacco seeds, as agricultural wastes, might be a valuable renewable raw material for the biodiesel production with two step catalyzed process.

Moreover, Mashad *et al.* (2008) studied biodiesel production from animal oil like by-product with salmon oil from salmon processing, was used as a feedstock for biodiesel production via transesterification in a two-step process. Two different types of salmon oil as the salmon oil extracted from acidified salmon hydrolysate and the salmon oil extracted from salmon by-products, were tested. Optimal amounts of chemicals reagent required to give the highest biodiesel yield from each oil were determined using batch production procedures. It was found that due to the high acid value of salmon oil, alkaline-catalysed transesterification was not an effective method for producing biodiesel from the salmon oil. Therefore a two-step process was applied, in which a sulphuric acid-catalysed pretreatment was used in the first step to reduce the acid value from 12.0 to 3.0 mg KOH/g and then, in the second step, KOH-catalysed transesterification was applied. All experiments were performed at a temperature of $52 \pm 2^\circ\text{C}$ with a stir rate of 600 rpm. Based on the total weight of salmon oil used, the maximum biodiesel yield of 99% was achieved using a total methanol to oil molar ratio of 9.2% and 0.5% (w/w) KOH. Ester loss due to the formation of emulsion during the washing and drying steps was 15% maximum. This loss could be reduced in practical applications by better design of washing and drying techniques. A preliminary economic analysis showed that the cost of biodiesel production from salmon oil was almost twice compared with soybean oil.

Wang *et al.* (2007) studied biodiesel production as two step catalyzed process from waste cooking oil (WCO) with using ferric sulfate as new catalyst. The two step catalyzed process was adopted to prepare biodiesel from waste cooking oil (WCO) which acid value was 75.92 ± 0.036 mg KOH/g. The free fatty acids of waste cooking oil (WCO) were esterified with methanol catalyzed by ferric sulfate in the first step and the triglycerides in waste cooking oil (WCO) were transesterified with methanol catalyzed by potassium hydroxide in the second step. The results showed that ferric sulfate had high activity to catalyze the esterification of free fatty acids (FFA) with methanol, The conversion rate of free fatty acids (FFA) reached 97.22% when 2 wt.% of ferric sulfate was added to the reaction system containing methanol to triglyceride molar ratio in 10:1 and reacted at 95°C for 4 hours. The methanol was evaporated under vacuum pressure, and further transesterified the remained triglycerides at 65°C

for 1 hour in a reaction system containing 1 wt.% of potassium hydroxide and 6:1 of methanol to triglyceride molar ratio. The final product with 97.02% of biodiesel, obtained after the two step catalyzed process which analyzed by gas chromatography. Although The new two-step process showed advantages as no acidic wastewater, high efficiency, low equipment cost, and easy recovery of catalyst but, the ferric sulfate catalyst had been still expensive for large scale production of biodiesel.

5. Response Surface Methodology (RSM)

Response surface methodology (RSM) was a useful statistical technique which had been applied in research into complex variable processes. The multiple regression and correlation analyses were used as tools to assess the effects of two or more independent factors on the dependent variables. Furthermore, the central composite design (CCD) of response surface methodology had been applied in the optimization of several biotechnological and chemical processes. Its main advantage was to reduced the number of experimental runs required to generate sufficient information for a statistically acceptable result. Response surface methodology had been successfully applied for optimization of biodiesel production in several fats and oils as these details.

Yuan *et al.* (2008) studied waste rapeseed oil with high free fatty acids (FFA) that used as raw material for biodiesel production. In the pretreatment step, free fatty acids (FFA) was reduced by distillation method. Then, biodiesel was produced by alkaline-catalyzed transesterification process, which was designed according to the 24 full-factorial central composite design. The response surface methodology (RSM) was used to optimize the conditions for the maximum conversion to biodiesel and understand the significance and interaction of the factors affecting biodiesel production. The results showed that catalyst concentration and reaction time were the limiting conditions and little variation in their value would altered the conversion. At the same time, there was a significant mutual interaction between catalyst concentration and reaction time. The biodiesel produced in the experiment was analyzed by gas chromatography/mass spectrometry, which showed that it mainly

contained six fatty acid methyl esters. In addition, diesel indexes analysis showed that most of the fuel properties were in reasonable agreement with diesel standard of China (GB252-2000) and biodiesel standard of America (ASTM D6751).

Tiwari *et al.* (2007) studied response surface methodology (RSM) based on central composite rotatable design (CCRD) which used to optimize three important reaction variables: methanol quantity (M), acid concentration (C) and reaction time (T) for reduction of free fatty acid (FFA) content in *Jatropha curcas* oil to around 1%. The optimum condition for reducing the FFA of *Jatropha curcas* oil from 14% to less than 1% was found to be 1.43% v/v sulphuric acid catalyst, 0.28 v/v methanol to oil ratio and 88 minutes reaction time at a reaction temperature of 60°C. This process gave an average yield of biodiesel more than 99%. The fuel properties of jatropha biodiesel were found to be comparable to those of diesel and confirming to the American and European standards.

Despite, a central composite design (CCD) had been used to study the effect of methanol quantity, acid concentration and reaction time on the reduction of free fatty acids content of mahua oil during its pretreatment for making biodiesel by Ghadge and Raheman, 2006. All three variables significantly affected acid value of product, methanol being the most effective followed by reaction time and acid catalyst concentration. Using response surface methodology, a quadratic polynomial equation was obtained for acid value by multiple regression analysis. Verification experiments confirmed the validity of the predicted model. The optimum combinations for reducing the acid level of mahua oil to less than 1% after pretreatment was 0.32 v/v methanol to oil ratio, 1.24% v/v sulphuric acid catalyst and 1.26 hours of reaction time at 60°C. After the pretreatment of mahua oil, transesterification reaction was carried out with 0.25 v/v methanol to oil ratio or 6:1 molar ratio and 0.7% w/v KOH as an alkaline catalyst to produce biodiesel. The fuel properties of mahua biodiesel were agreement with requirements of both American and European standards for biodiesel properties.

Moreover, Kansedo *et al.* (2009) reported that the study of transesterification of palm oil via heterogeneous process using montmorillonite KSF as heterogeneous catalyst. This study was carried out using a design of experiment, specifically response surface methodology (RSM) based on four-variable central composite design (CCD). The transesterification process variables were reaction temperature, 50–190°C, reaction time, 60–300 minutes, ethanol to oil ratio of 4–12 and amount of catalyst, 1–5 wt.%. It was found that the yield of palm oil fatty acid methyl esters (FAME) could reach up to 79.6% followed this optimum reaction conditions: reaction temperature of 190°C, reaction time of 180 minutes, methanol to oil molar ratio of 8:1 and amount of catalyst of 3%.

However, The optimization of reaction conditions of biodiesel production from lard applied response surface methodology (RSM), could employed five-level-three-factors and their reciprocal interactions were assessed. The total of 20 individual experiments were conducted and designed to study reaction temperature, catalyst concentration and methanol to oil molar ratio. A statistical model predicted that the highest conversion yield of lard biodiesel would be 98.6%, at the following optimized reaction conditions: a reaction temperature of 65°C, a catalyst amount of 1.26%, and an oil to methanol molar ratio of 7.5:1, with a 20 minutes of reaction time. Using these optimum factor values under the experimental conditions in three independent replicates, an average content of 97.8± 0.6% was achieved. This value was fixed well within the range predicted by model. The quality of biodiesel produced from lard at the optimum reaction conditions satisfied the relevant quality of biodiesel standards, with the exception of cold filter plugging point (Jeong *et al.*, 2009).

6. Properties of Biodiesel

Meng *et al.* (2008) found that waste cooking oil (WCO) after pretreatment could had a transesterification reaction. Compared to pure oils, the conversion rate could be reduced. With the appropriate quality upgrading treatment, biodiesel obtained from waste cooking oil (WCO) could be used as a fuel in diesel engines. Through the feasible analysis of orthogonal test, biodiesel of good quality could be

produced from waste cooking oil (WCO) in the following reaction conditions: methanol to oil molar ratio of 9:1, with 1.0 wt.% NaOH concentration at 50°C for 90 minutes. Verified experiments showed methanol to oil molar ratio of 6:1 was more suitable in the process. Engine testing results showed without any modification to diesel engine, under all conditions dynamical performance kept normal. The B20 and B50 blend fuels could lead to satisfied emissions whilst the B20 blend fuels reduced significantly 20.58% of particles emission, 26.7% of hydrocarbon (HC) emission and 18.6% of carbon monoxide (CO) emission.

Some specifications were put into execution so as to standardize the quality of biodiesel fuel worldwide in both the United States of America (USA), ASTM D 6751 and in Europe, EN 14214 (Canakci and Sanli, 2008). By means of these mentioned standards shown in Tables 8 and 9, respectively, both the security of users and the opportunity in producing biodiesel from various feedstocks, just in case these specifications were provided. Diesel engine producers approve the biodiesel usage on the condition that it was complied with these standards. At the end of the transesterification reaction, glycerol phase separation could be obtained, but all features defined in the standards must be measured in order to determine fuel quality of the produced fatty acid methyl ester. On the condition that these standard values were met, biodiesel could be produced either from high-quality vegetable oils or from inexpensive poor quality feedstocks.

The best current measure for biodiesel quality in the United States is the ASTM standard, ASTM D 6751: Standard Specification for Biodiesel Fuel (B100) Blend Stock for Distillate Fuels. This standard specifies the properties required for a fuel to be used in an engine without problems. Even if fuel was blended with diesel fuel, most people in the industry expected that the biodiesel blending stock would meet the standard before being blended. While some properties in the standard, such as cetane number and density, reflected the properties of the chemical compounds that make up biodiesel, other properties provide indications of the quality of the

production process. This discussion would focus on the most important issues for assuring product quality for biodiesel.

Table 8 American standard specification for biodiesel (ASTMD 6751).

Property	Test method	Limits	Unit
Kinematic viscosity (at 40°C)	D 445	1.9-6.0	Mm ² /s
Cetane number	D 613	47 min	-
Flash point (closed cup)	D 93	130.0 min	°C
Cloud point	D 2500	Report	°C
Water and sediment	D 2709	0.050 max	Volume(%)
Sulfated ash	D 874	0.020 max	Mass (%)
Sulfur	D 5453	0.05 max	Mass (%)
Copper strip corrosion	D 130	No. 3 max	-
Carbon residue (100% sample)	D 4530	0.050 max	Mass (%)
Acid value	D 664	0.80 max	mg KOH/g
Free glycerol	D 6584	0.020 max	Mass (%)
Total glycerol	D 6584	0.240 max	Mass (%)
Phosphorus content	D 4951	0.001 max	Mass (%)
Distillation temperature (90% recovered)	D 1160	360 max	°C

Source: Canakci and Sanli (2008)

Table 9 European standard specifications for biodiesel (EN 14214).

Property	Test method	Limits	Unit
Kinematic viscosity (at 40°C)	EN ISO 3104	3.5-5.0	3mm ² /s
Density (at 15 °C)	EN ISO 3675/EN ISO 12185	860-900	Kg/m ³
Cetane number	EN ISO 5165	51 min	-
Flash point	ISO/CD 3679	120.0min	°C
Water	EN ISO 12937	500 max	mg/kg
Sulfated ash	ISO 3987	0.02 max	Mass (%)
Sulfur	NF T 60-71/DIN 51680	10.0 max	mg/kg
Copper strip corrosion (3 hours,at 50°C)	EN ISO 2160	No.1	-
Carbon residue (10% sample)	EN ISO 10370	0.3 max	Mass (%)
Acid value	Pr EN 14104	0.5 max	mgKOH/g
Free glycerol	Pr EN 14105/Pr EN 14106	0.02 max	Mass (%)
Total glycerol	Pr EN 14105	0.25 max	Mass (%)
Phosphorus content	Pr EN 14107	10 max	mg/kg
Iodine number	Pr EN 14111	120max	-
Oxidative stability (at 110°C)	Pr EN 14112	6 min	hr
Monoglyceride content	Pr EN 14105	0.8 max	Mass (%)
Diglyceride content	Pr EN 14105	0.2 max	Mass (%)
Triglyceride content	Pr EN 14105	0.2 max	Mass (%)

Source: Canakci and Sanli (2008)

Some critical properties of various vegetable oils commonly used in biodiesel production and some methyl ester or biodiesel fuels produced from vegetable oils and low cost feedstocks. In this section, the fuel properties of fatty acid alkyl monoesters were compared to each other (Canakci and Sanli, 2008) (Table 10).

Table 10 Some properties of diesel fuel and biodiesel fuels produced from different feedstocks.

Fuel	Kin. Viscosity (mm ² /s, at 40°C)	Density (g/cm ³ , at 21°C)	Cetane number	Flash Point (°C)	Cloud Point (°C)	Pour Point (°C)
1. Diesel fuel	2.0-4.5	0.820-0.860	51.0	55	-18	-25
2. Yellow grease methyl ester	5.16	0.873	62.6	-	9	12
3. Waste frying oil methyl ester	4.318	0.888	52	156	+3	-2.5
4. Soybean methyl ester	4.08	0.884	50.9	131	-0.5	-4
5. Rapeseed methyl ester	4.83	0.882	52.9	155	-4	-10.8
6. Sunflower methyl ester	4.60	0.880	49.0	183	1	-7
7. Tallow methyl ester	5.00	0.877	58.8	150	12	9
8. Soapstock methyl ester	4.30	0.885	51.3	169	6	-

Source : Canakci and Sanli (2008); Utlu and Kocak (2008)

7. Recovery Process in Biodiesel Production

Nowaday, management of chemical reagent wastes from processes of biodiesel production, could be emitted to generate various utilized compound reagents and using by product ,such as excess methanol, potassium hydroxide alkali catalyst , sulfuric acid catalyst, glycerine by product. Hence, The biodiesel production has been the environmental friendly and zero-waste process plant.

Potassium hydroxide alkali catalyst was neutralized by phosphoric acid to generate as potassium phosphate fertilizer (Cayli and Kusefoglu, 2008). Sulfuric acid catalyst was completely removed in a neutralization reaction by adding calcium oxide (CaO) to produce CaSO₄ and H₂O. Calcium oxide was used primarily due to its low-cost relative to other alkali substances. the water produced would also be absorbed by the resulting CaSO₄ to form CaSO₄·2H₂O (Zhang *et al.*, 2003).

The by-product glycerol is glycerine or 1,2,3-propane-triol, which would be produced by a biodiesel plant about 10% of the biodiesel production level. Glycerol was a very common industrial chemical with a multitude of utilization. The glycerol

produced by transesterification is only about 50% pure. The glycerol could be raw material for soap production and the desirable grade of glycerin could be used for food and pharmaceutical products. It contained a significant amount of contaminants including methanol, soap, and catalyst. It was relatively easy to raise the purity level of the crude glycerol to 80%-90%. This could be accomplished by adding hydrochloric acid to the crude glycerol until the pH was acidic around 4.5. This splits the soaps into fatty acids and salt. The fatty acids would rise to the top of the glycerol where they can be removed. Then, the methanol could be removed by evaporation to yield 80%-90% purity glycerol. The actual level would depend on the purity of the original oil because contaminants tend to concentrate in the glycerol. However, some of the excess methanol in the production process could be removed by flash evaporation, at 90°C and 100 mmHg. The methanol was recovered and was re-used (Bouaid *et al.*, 2005).

MATERIALS AND METHODS

Materials

1. Trap Grease

Trap grease was collected from the grease trap in cafeterias and restaurants at Kasetsart university. Food remaining in trap grease was removed by trap grease treatment process.

2. Chemical Compounds

N-heptane(C_7H_{16}), sodium sulphate (Na_2SO_4), sodium chloride ($NaCl$) and boron trifluoride (BF_3), methanol (CH_3OH), potassium hydroxide(KOH) in pellet, sulphuric acid (H_2SO_4), phosphoric acid (H_3PO_4 , 85%), methyl heptadecanoate is analytical reagent (AR) grade. Methyl heptadecanoate was used as reference standards for fatty acid methyl ester calculation. All of these chemical compounds obtained from Supelco.

Methods

The present study consist of 4 parts. Part 1 is physical and chemical properties of trap grease. Part 2 is the optimum conditions for acid catalyzed esterification using response surface methodology. Part 3 is the optimum conditions for alkali catalyzed transesterification using response surface methodology. Part 4 is properties of trap grease biodiesel at the optimum reaction conditions.

Part 1 Physical and Chemical Properties of Trap grease

1.1 Trap Grease Treatment Process

Trap grease had contained the small wastewater, solid particles, free fatty acid (FFA) and many other chemical compounds, hence trap grease was treated before experiment (Figure 4). Water in the trap grease was removed by heating process at 105°C. The solid portion of trap grease was removed using filtration process using Whatman filter paper No.1 quantitative, 11 µm pore size (Issariyakul *et al.*, 2007).

1.2 Properties Investigation

The treated trap grease after treatment process was investigated acid value (AV). Kinematic viscosity of trap grease was determined using an Ubbelohde glass capillary viscometer (Leung and Guo, 2006). The trap grease was investigated water content and fatty acid compositions of trap grease were determined by gas chromatography spectrometry (GC). Identification of fatty acids contained in trap grease was performed by comparison retention times with fatty acids reference standard. The molecular weight of trap grease was calculated by the summation of each molecular weight of fatty acid multiple area peak from chromatogram and divided by 100 that follow experiment of Meng *et al.* (2008).

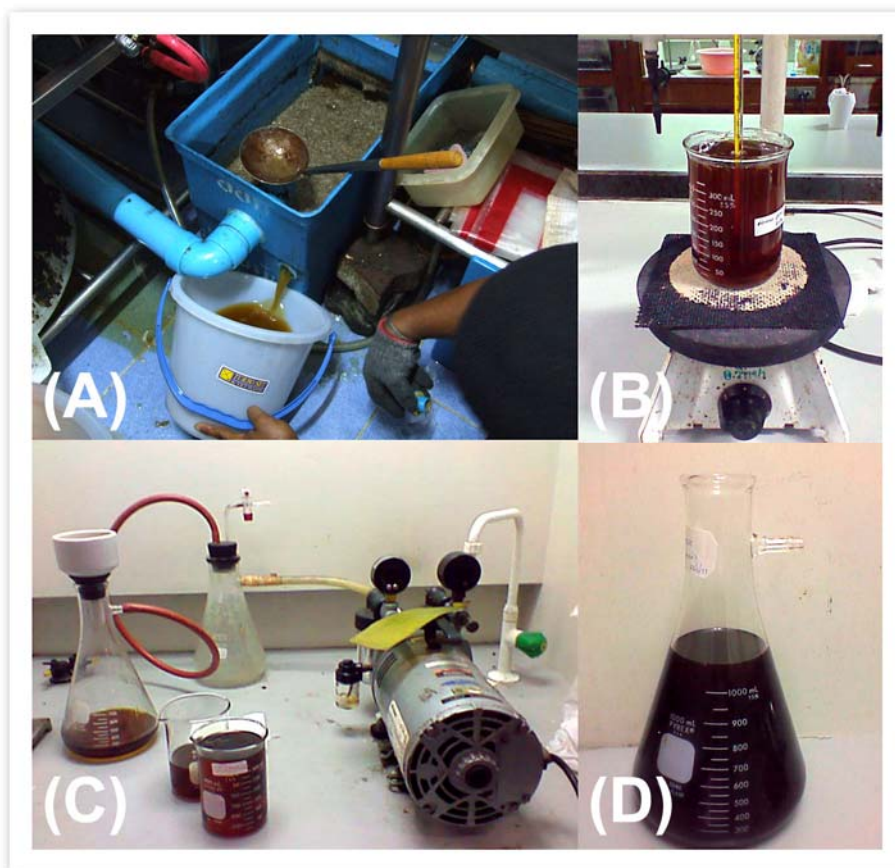


Figure 4 Treatment process of trap grease

- (A) Collection of trap grease sample in shop
- (B) Heating at 105°C for removing water
- (C) Filtration of trap grease
- (D) Treated trap grease

Part 2 The Optimum Conditions for Acid Catalyzed Esterification Using Response Surface Methodology

These studies used a response surface methodology (RSM) to optimize the variables effect on acid value (AV) (mg KOH/g).

2.1 Experimental Design in Acid Catalyzed Esterification

A five-level-three-factor central composite design (CCD) was employed in this optimization study, requiring 23 experiments. Methanol-to-oil molar ratio (M), H₂SO₄-catalyst concentration (C) and reaction time (T) were the independent variables selected to optimize the reduction of acid value (AV) of trap grease after acid catalyzed esterification.

The coded and uncoded levels of the independent variables were given in Table 11. Two replications were carried out for all design points. The central values (zero level) chosen for experimental design, were methanol to oil molar ratio = 10:1 (0.43 v/v), H₂SO₄-catalyst concentration = 2.5% (v/v), reaction time = 4 hours. The central composite design (CCD) experimental plan was shown on Table 12.

Table 11 Independent variables and levels used for central composite design in acid catalyzed esterification of two-step catalyzed process.

Variables	Symbols	Levels ^a				
		-1.68 (-α)	-1	0	1	+1.68 (+α)
Methanol-to-oil ratio (v/v)	M	0.28	0.34	0.43	0.51	0.58
H ₂ SO ₄ -Catalyst concentration (% v/v)	C	0.02	1	2.5	4	5.02
Reaction time (hr)	T	0.64	2	4	6	7.36

^a Transformation of variable levels from coded (X) to uncoded could be obtained as: $M = 0.43 + 0.09X$, $C = 2.5 + 1.5X$ and $T = 4 + 2X$.

Table12 Central composite design (CCD) arrangement for acid catalyzed esterification.

Design points	Coded independent variable levels		
	Methanol to oil ratio (v/v) M	H ₂ SO ₄ concentration (% v/v) C	Reaction time (hr) T
1	-1	-1	-1
2	-1	-1	1
3	-1	1	-1
4	-1	1	1
5	1	-1	-1
6	1	-1	1
7	1	1	-1
8	1	1	1
9	-1.68	0	0
10	1.68	0	0
11	0	-1.68	0
12	0	1.68	0
13	0	0	-1.68
14	0	0	1.68
15	0	0	0
16	0	0	0
17	0	0	0
18	0	0	0
19	0	0	0
20	0	0	0
21	0	0	0
22	0	0	0
23	0	0	0

2.2 Quantitative Analysis of Acid Value

Acid value (AV) was determined by acid–base titration technique. Potassium hydroxide (KOH) was used as standard alkali solution. The acid value which was free fatty acid types of trap grease, was calculated by the following equation 1.

$$\text{Acid value} = \frac{(A-B) \times N \times 56.1}{W} \quad (1)$$

Where

A is the volume, in milliliters of standard alkali used in the titration.

B is the volume, in milliliters of standard alkali used in the titrating the blank.

N is the normality of standard alkali.

W is the mass, in grams of sample.

2.3 Statistical Analysis in Acid Catalyzed Esterification

The experimental datum were analyzed by response surface regression procedure using the following second-order polynomial equation (2)

$$Y = b_0 + \sum_{i=1}^k b_i X_i + \sum_{i=1}^k b_{ij} X_i^2 + \sum_{i>j}^k \sum_j^k b_{ij} X_i X_j \dots\dots\dots(2)$$

where y was response (acid value, mgKOH/g); xi and xj were the uncoded independent variables; b0, bi, bii and bij were intercept, linear, quadratic and interaction constant coefficients respectively and k was the number of factors studied and optimized in the experiment. SPSS package was used for regression analysis and analysis of variance (ANOVA). Response surfaces and contour plots were developed using the fitted quadratic polynomial equation obtained from regression analysis, holding one of the independent variables at a constant value corresponding to stationary point and changing the other two variables. Confirmation experiments were carried out to validate equation, using combinations of independent variables which were not part of the original experimental design but within the experimental region (Ghadge and Raheman, 2006).

2.4 Analytical Procedure in Acid Catalyzed Esterification

Ten gram of the trap grease mixed with methanol and sulphuric acid. It was carried out in a three-necked round-bottomed flask equipped with a reflux condenser. The reaction flask was immersed in a chamber placed on hot plate with a magnetic stirrer for stirring at 400 rpm. The temperature was maintained at 60°C by water circulating from a thermostated bath by means of a pump to condenser for condensing methanol (Figure 5). The mixture product was left to settle into two layers.

The lower layer was water with insoluble catalyst that was recovered for further using. The upper layer was fatty acid methyl ester (FAME) or biodiesel and unreacted triglyceride (Figure 6) (Ghadge and Raheman, 2006). The reaction progress was monitored by measuring acid value (AV) which was determined follow biodiesel standard method.

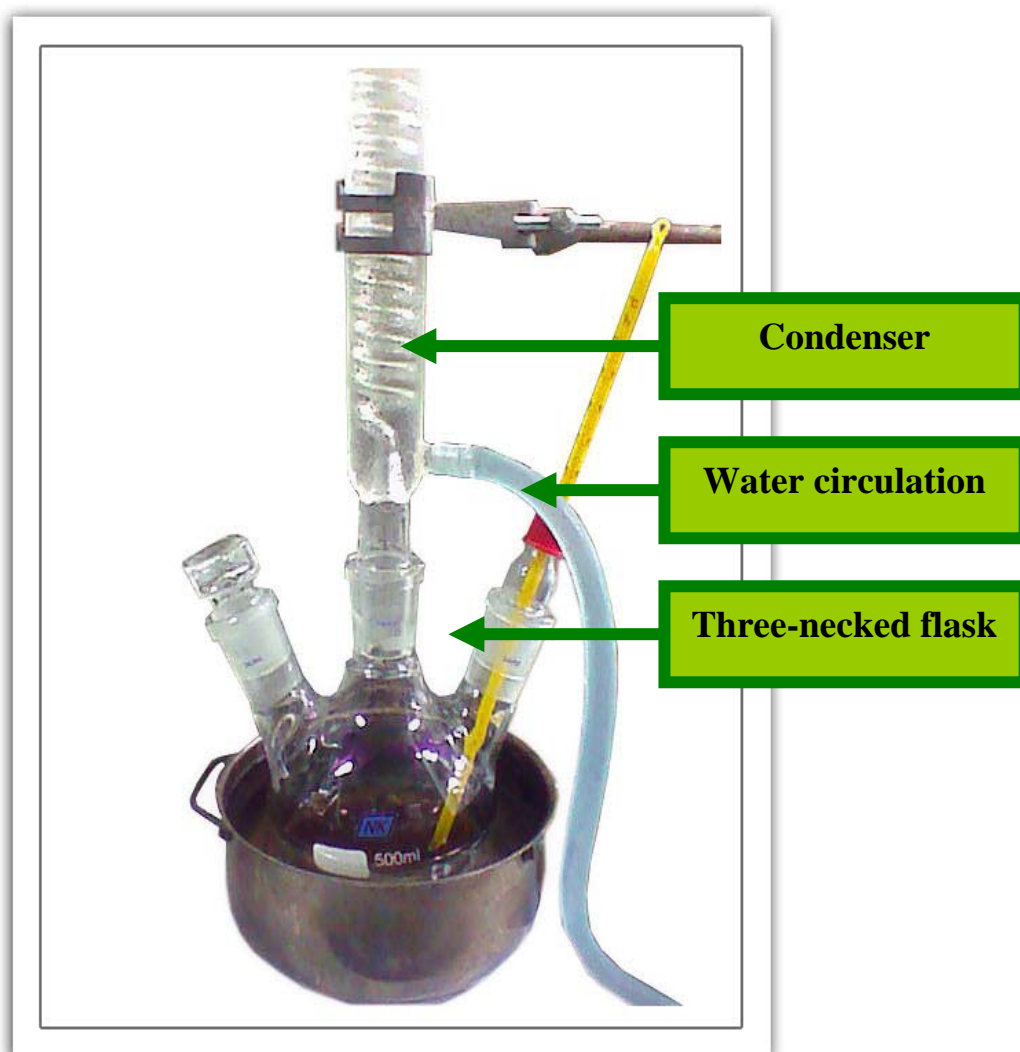


Figure 5 An experiment set up of acid catalyzed esterification.



Figure 6 Two- layers separation of trap grease after acid catalyzed esterification experiments.

Part 3 The Optimum Conditions for Alkali Catalyzed Transesterification Using Response Surface Methodology

This studies used a response surface methodology (RSM) to optimize the variables effect on % fatty acid methyl ester (FAME).

3.1 Experimental Design in Alkali Catalyzed Transesterification

A five-level-three-factor central composite design (CCD) was employed in this optimization study, requiring 23 experiments (Ghadge and Raheman 2006). Methanol-to-oil ratio (M'), KOH-catalyst concentration (C') and reaction time (T') were the independent variables selected to optimize condition for fatty acid methyl ester (FAME) production.

The coded and uncoded levels of independent variables were given in Table 13. Two replications were carried out for all design points. The central values (zero level) chosen for experimental design were methanol to oil molar ratio = 6:1 (0.26 w/v), KOH-catalyst concentration = 1% (v/v), reaction time = 1 hour. The central composite design (CCD) experimental plan was shown in Table14.

Table 13 Independent variables and levels used for central composite design for alkali catalyzed transesterification.

Variables	Symbols	Levels ^a				
		-1.68 (- α)	-1	0	1	+1.68 (+ α)
Methanol-to-oil ratio (v/v)	M'	0.19	0.22	0.26	0.30	0.33
KOH-Catalyst concentration (% w/v)	C'	0.16	0.5	1	1.5	1.84
Reaction time (hr)	T'	0.45	0.67	1	1.33	1.55

^a Transformation of variable levels from coded (X) to uncoded could be obtained as: $M' = 0.26 + 0.04X$, $C' = 1 + 0.5X$ and $T' = 1 + 0.33X$.

Table 14 Central composite design (CCD) arrangement for alkali catalyzed transesterification.

Design points	Coded independent variable levels		
	Methanol to oil ratio (v/v) M'	KOH concentration (% v/v) C'	Reaction time (hr) T'
1	-1	-1	-1
2	-1	-1	1
3	-1	1	-1
4	-1	1	1
5	1	-1	-1
6	1	-1	1
7	1	1	-1
8	1	1	1
9	-1.68	0	0
10	1.68	0	0
11	0	-1.68	0
12	0	1.68	0
13	0	0	-1.68
14	0	0	1.68
15	0	0	0
16	0	0	0
17	0	0	0
18	0	0	0
19	0	0	0
20	0	0	0
21	0	0	0
22	0	0	0
23	0	0	0

3.2 Quantitative Analysis of Fatty Acid Methyl Ester Content

The content of fatty acid methyl ester was measured by gas chromatography. Chromatographic analysis was performed on a Agilent Technologies GC-6890 gas chromatograph equipped with a DB-WAX 127-7012 column (30 m x 0.32 mm x 0.25 μ m; Agilent Technologies, USA) and a flame ionization detector (FID). The operating conditions were following: both temperatures of injector and detector were set at 250°C. The split ratio was 1:50. Helium was used as carrier gas. The analysis of biodiesel for each sample was performed by dissolving 0.05 g of

biodiesel sample and 1 ml of methyl heptadecanoate (internal standard) after that injected 1 μ l of this solution into gas chromatograph spectrometer. Fatty acid methyl ester content was calculated by the following equation 3.

$$C = \frac{[\Sigma A - A_{SI}]}{A_{SI}} \times \frac{[C_{SI} \times V_{SI} \times 100]}{m} \quad (3)$$

Where

ΣA is the total peak area from the methyl ester

C is the weight, in percent of fatty acid methyl ester content.

A_{SI} is the peak area of methyl heptadecanoate.

C_{SI} is concentration, in milligram per milliliter of methyl heptadecanoate solution used.

V_{SI} is the volume, in milliliters of methyl heptadecanoate solution used.

m is the mass, milligram in of sample.

3.3 Statistical Analysis in Alkali Catalyzed Transesterification

The experimental datum were analyzed by response surface regression procedure using the second-order polynomial equation 2. SPSS package was used for regression analysis and analysis of variance (ANOVA). Response surfaces and contour plots were developed using the fitted quadratic polynomial equation obtained from regression analysis, holding one of the independent variables at a constant value corresponding to the stationary point and changing other two variables. Confirmation experiments were carried out to validate the equation, using combinations of independent variables which were not part of the original experimental design but within the experimental region (Ghadge and Raheman, 2006).

3.4 Analytical Procedure in Alkali Catalyzed Transesterification

Trap grease obtained from acid catalyzed esterification having FFA level less than 2% was used to study the optimum condition of alkali catalyzed transesterification. 10 g of oil layer from the first step was transferred to a three-necked round-bottomed flask equipped with a reflux condenser. The reaction flask was immersed in a chamber placed on hot plate with a magnetic stirrer for stirring at 400 rpm. The temperature was maintained at 60°C by water circulating from a thermostated bath by means of a pump to condenser for condensing methanol. The mixture product was left to settle into two layers (Figure 7). The lower layer was glycerol. The upper layer was fatty acid methyl ester (FAME) with lighter color. It was washed with 5% phosphoric acid at 50°C and followed with distilled water at 80°C for several times until the wash water was neutral, which this process was modified from Cayli and Kusefoglu (2008). The biodiesel was dried on hot plate at 100°C for 15 minutes. The reaction was monitored by measuring percentage of fatty acid methyl ester (FAME) which was determined by gas chromatography spectrometer (GC).



Figure 7 Two- layers separation of sample after alkali catalyzed transesterification experiments.

Part 4 Properties of Trap Grease Biodiesel at the Optimum Reaction Conditions

The analysis of biodiesel quality contained density (at 15 °C), viscosity (at 40 °C) (Figure 8), flash point, acid value, iodine value, cloud point, and were carried out using the methods developed by Center of Excellence oil palm, Kasetsart university and compared with some of ASTM D 6751 and EN 14214 biodiesel standards (Canakci and Sanli, 2008).



Figure 8 Kinematic viscosity of trap grease biodiesel using Ubbelohde glass capillary viscometer

RESULTS AND DISCUSSION

Part 1 Physical and Chemical Properties of Trap Grease

The initial free fatty acid (FFA) in trap grease from waste water contained 26.19% corresponding to acid value of 52.13 mg KOH/g, 29.8 cSt of kinematic viscosity at 40°C. Water content of trap grease was 1.156 %. The fatty acids of the trap grease were octanoic acid (C8:0), decanoic acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0), behenic acid (C22:0), lignoceric acid (C24:0). The mean molecular weight of trap grease obtained from fatty acid composition was 846 g/mol. Properties of trap grease were shown in Table 15.

Table 15 Physical and chemical properties of trap grease.

Property	Amount
Acid value (mg KOH/g)	52.13
Kinematic viscosity at 40°C (cSt)	29.8
Water content (%wt)	1.156
Fatty acid composition (%wt)	
octanoic acid (C8:0)	0.4
decanoic acid (C10:0)	0.4
lauric acid (C12:0)	3.0
myristic acid (C14:0)	1.9
palmitic acid (C16:0)	25.7
palmitoleic acid (C16:1)	2.0
stearic acid (C18:0)	5.5
oleic acid (C18:1)	39.4
linoleic acid (C18:2)	19.8
linolenic acid (C18:3)	1.0
arachidic acid (C20:0)	0.2
behenic acid (C22:0)	0.08
lignoceric acid (C24:0)	0.09
Average molecular weight (g/mol)	846

Part 2 The Optimum Conditions for Acid Catalyzed Esterification Using Response Surface Methodology

Experimental values obtained for acid value responses at the design points are given in Table 16. Statistical analysis of the model was performed to evaluate the analysis of variance (ANOVA) which showed F value of 6.324, imply that there was statistical significant in the model (Table 17) and multiple regression coefficients indicated in Table18 were obtained by employing a least square technique to predict quadratic polynomial model for the acid value. The model was tested for adequacy by analysis of variance. The regression model for data was found to be highly significant with a coefficient of determination as 0.842. Using the coefficients determined, the predicted model for acid value in equation 4.

The regression equation could be use for prediction of acid value because the high R square ($R^2 = 0.842$), which imply that the model is accurate. Prediction was conduct by putting the methanol to oil ratio value (M), acid catalyst concentration value (C) and reaction time value (T) on the equation 4 to become the predicted acid value.

The relationship between independent and dependent variables of the developed model indicated that high methanol to oil ratio, there was reduction in acid value with increasing in catalyst concentration (Figure 9) and reaction time (Figure 10). For low catalyst concentration found that acid value decrease with increasing reaction time (Figure 11). The response surface and contour plots were generally graphical representations of the regression equation. Each contour curve represents an infinite number of combinations of two test variables with the other two maintained at their respective zero level.

The response surface and contour plots, the optimum levels of three variables were found to be 0.43 v/v or 10:1 of methanol to oil molar ratio, 2.5 % v/v of sulphuric acid concentration and 4 hours of reaction time locating the stationary point

in the experimental region. The model predicted that the minimum acid value could be obtained by putting the optimum levels of X_i which was M, C and T in the regression equation 4 under the above optimum conditions of the variables. It was 2.661 mg KOH/g of acid value.

Many published papers suggested that alkaline catalysts could be applied when free fatty acid (FFA) content in the oils or fats is less than 2 %. The optimum combinations for reducing the acid level of mahua oil to less than 2 % after pretreatment was 0.32 v/v of methanol-to-oil ratio, 1.24% v/v of sulphuric acid concentration and 1.26 of hours reaction time at 60°C (Ghadge and Raheman, 2006). Moreover the optimum combination for reducing the free fatty acid (FFA) of Jatropha oil from 14% to less than 1% was found to be 1.43% v/v of sulphuric acid concentration, 0.28 v/v of methanol to oil ratio and 88 minutes of reaction time at 60°C (Tiwari *et al.*, 2007).

Table 16 Central composite design arrangement and response for acid catalyzed esterification.

Treatment	M	C	T	Methanol/oil ratio(v/v)	Catalyst concentration (%v/v)	Reaction time (hr)	Acid value (mg KOH/g)	
							Experimental	Predicted
1	-1	-1	-1	0.34	1.00	2.00	14.77	16.30
2	-1	-1	1	0.34	1.00	6.00	4.92	8.21
3	-1	1	-1	0.34	4.00	2.00	5.35	10.52
4	-1	1	1	0.34	4.00	6.00	3.74	7.78
5	1	-1	-1	0.51	1.00	2.00	14.69	13.78
6	1	-1	1	0.51	1.00	6.00	12.14	10.06
7	1	1	-1	0.51	4.00	2.00	2.29	1.88
8	1	1	1	0.51	4.00	6.00	2.21	3.52
9	-1.68	0	0	0.28	2.50	4.00	17.11	10.37
10	+1.68	0	0	0.58	2.50	4.00	2.81	5.10
11	0	-1.68	0	0.42	0.02	4.00	20.28	20.64
12	0	+1.68	0	0.42	5.02	4.00	15.70	11.09
13	0	0	-1.68	0.42	2.50	0.64	7.22	5.45
14	0	0	+1.68	0.42	2.50	7.36	2.29	-0.19
15	0	0	0	0.42	2.50	4.00	2.47	2.60
16	0	0	0	0.42	2.50	4.00	2.55	2.60
17	0	0	0	0.42	2.50	4.00	2.55	2.60
18	0	0	0	0.42	2.50	4.00	2.49	2.60
19	0	0	0	0.42	2.50	4.00	2.51	2.60
20	0	0	0	0.42	2.50	4.00	2.47	2.60
21	0	0	0	0.42	2.50	4.00	2.51	2.60
22	0	0	0	0.42	2.50	4.00	2.45	2.60
23	0	0	0	0.42	2.50	4.00	2.49	2.60

Table 17 Analysis of variance (ANOVA) for quadratic polynomial model.

Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	633.165	9	70.352	6.324	0.002
Residual	144.630	13	11.125		
Total	777.796	22			

Table 18 Regression coefficients of predicted quadratic polynomial model for regression equation.

Terms	Coefficients	Standard error
Linear		
β_0	78.215	25.762
β_1	-217.419	99.601
β_2	-9.392	4.660
β_3	-4.680	3.546
Quadratic		
β_{11}	237.241	107.397
β_{22}	2.129	0.377
β_{33}	2.544E-03	0.210
Interaction		
β_{12}	-11.979	9.243
β_{13}	6.441	6.932
β_{23}	0.446	0.393

$$\begin{aligned}
 AV = & 78.215 - 217.419M - 9.392C - 4.680T \\
 & + 237.241M^2 + 2.129C^2 + 2.544E-03T^2 \\
 & - 11.979MC + 6.441MT + 0.446CT \quad \dots\dots\dots(4)
 \end{aligned}$$

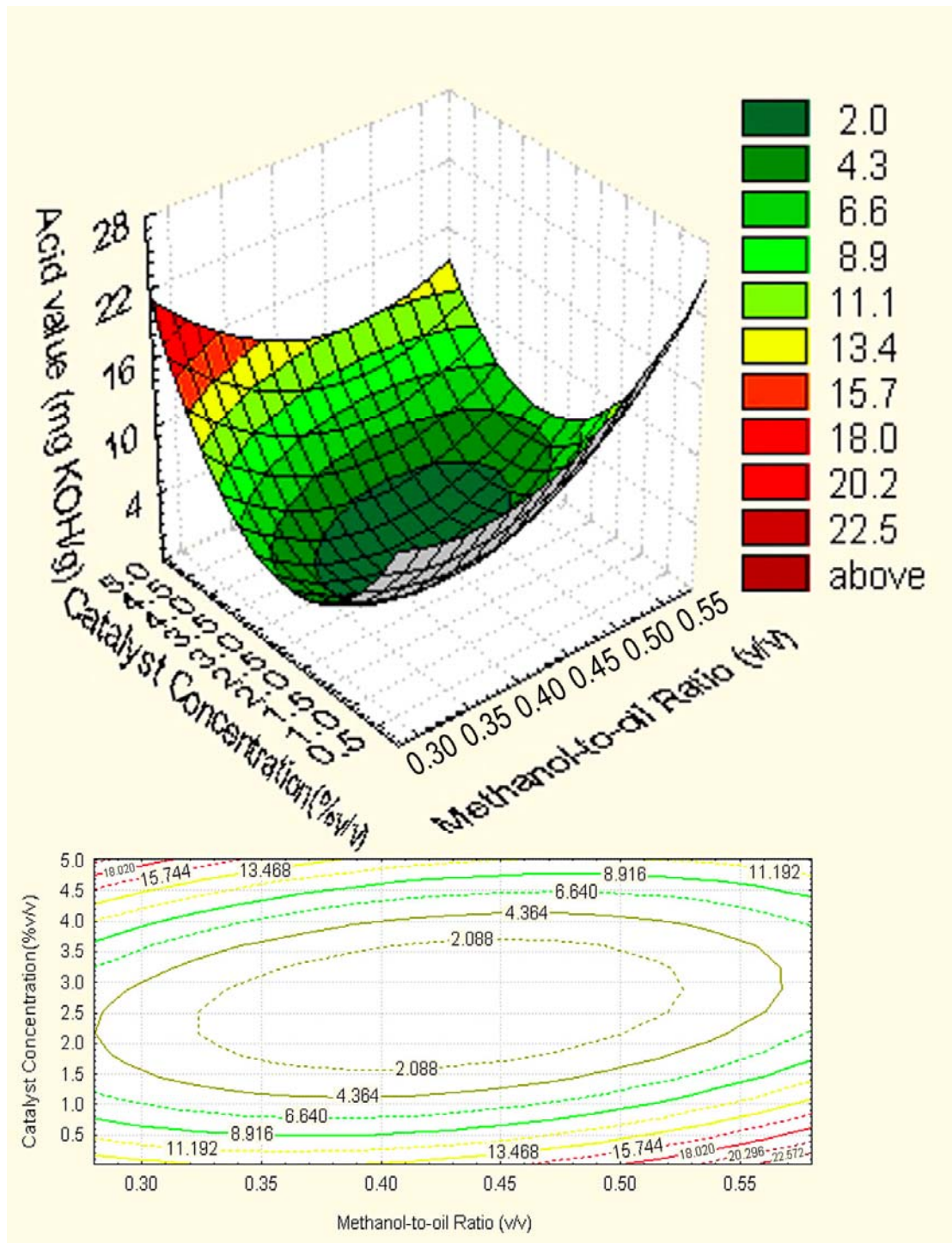


Figure 9 Response surface plots and contour plots representing the effect of methanol to oil ratio and catalyst concentration on acid value predicted from quadratic polynomial model.

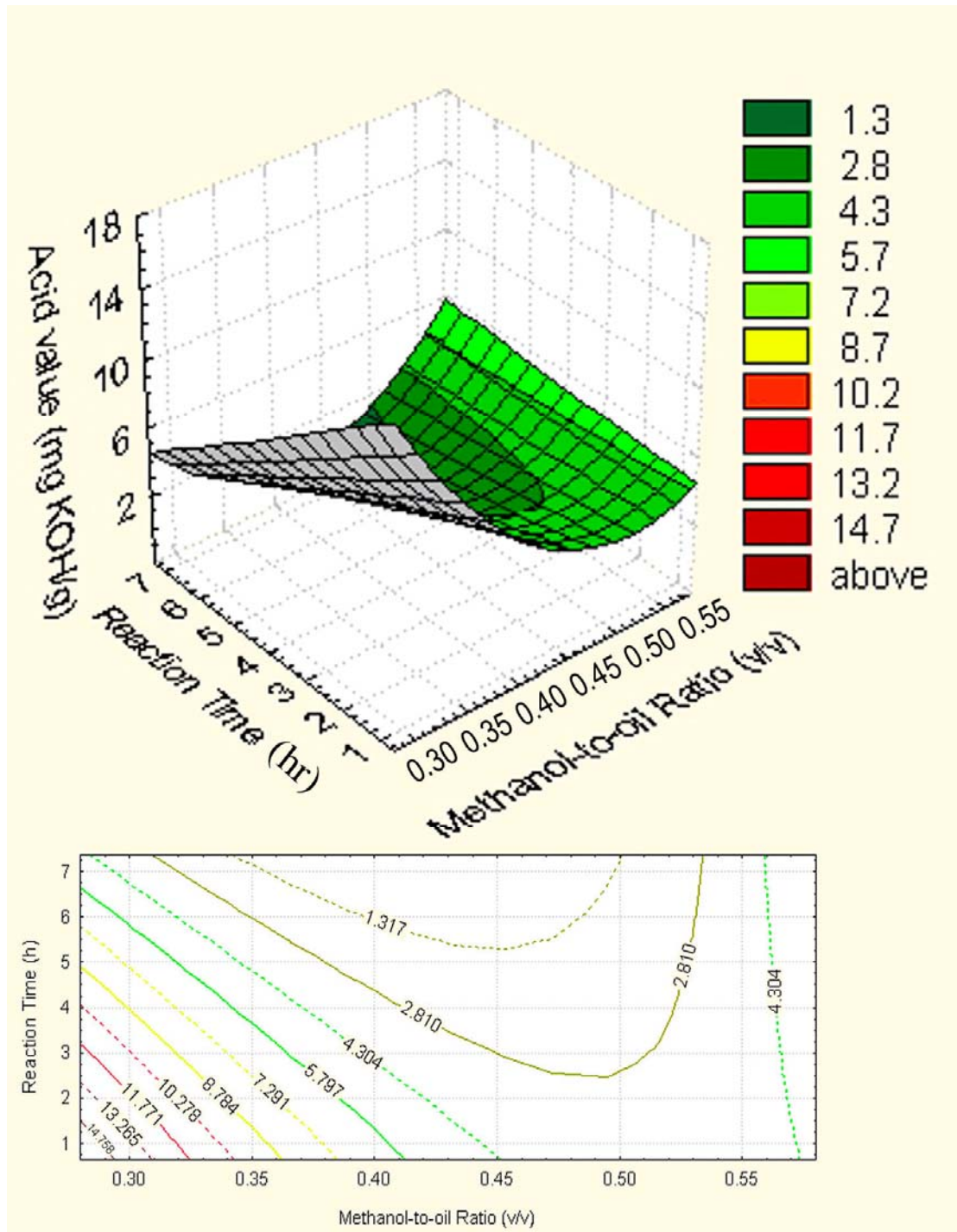


Figure 10 Response surface plots and contour plots representing the effect of methanol to oil ratio and reaction time on acid value predicted from quadratic polynomial model.

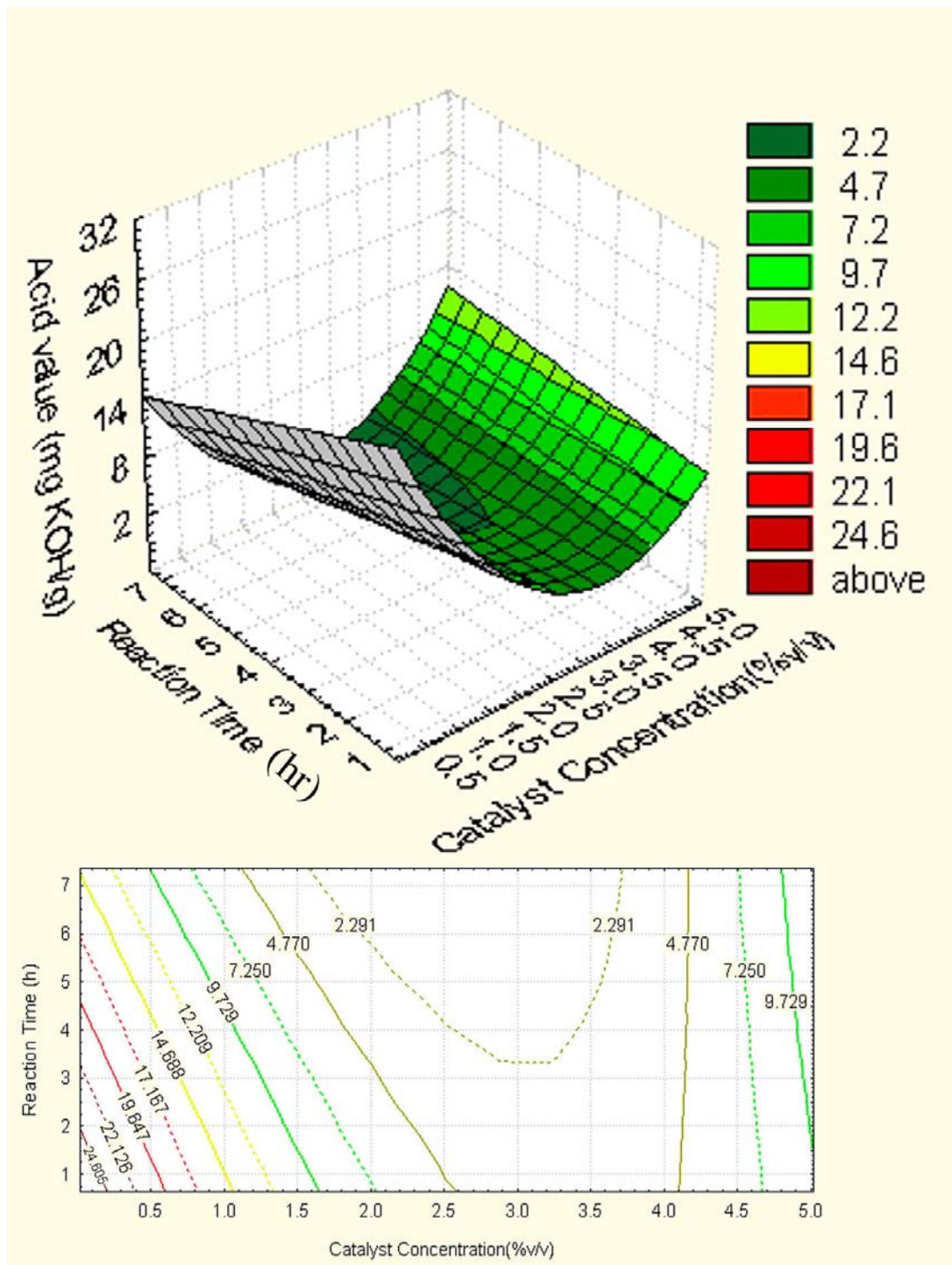


Figure 11 Response surface plots and contour plots representing the effect of catalyst concentration and reaction time on acid value predicted from quadratic polynomial model

Part 3 The Optimum Conditions for Alkali Catalyzed Transesterification Using Response Surface Methodology

Experimental values obtained for fatty acid methyl ester (FAME) responses at design points were given in Table 19. Statistical analysis of model was performed to evaluate the analysis of variance (ANOVA). F value as 7.830, indicated that there was statistical significant in the model (Table 20) and multiple regression coefficients indicated in Table 21 were obtained by employing a least square technique to predict quadratic polynomial model for the fatty acid methyl ester content (%). The model was tested for adequacy by analysis of variance. The regression model for data was found to be highly significant with a coefficient of determination as 0.844. Using the coefficients determined, the predicted model for fatty acid methyl ester (FAME) in equation 5.

The regression equation could be use for prediction of fatty acid methyl ester because the high R square ($R^2 = 0.844$), which imply that the model is accurate. Prediction was conduct by putting the methanol to oil ratio value (M'), alkali catalyst concentration value (C') and reaction time value (T') on the equation 5 to become the predicted fatty acid methyl ester value.

The response surface plots and contour plots were generally graphical representations of the regression equation. Each contour curve represented an infinite number of combinations of two test variables with other two maintained at their respective zero level. From the plots, the levels of three variables were found to be 0.26 v/v or 6:1 of methanol to oil ratio, 1 % v/v of catalyst concentration in 1 hour of reaction time locating the stationary point in experimental region. The model predicted that the maximum fatty acid methyl ester content that could be obtained by putting the optimum levels of X_i which was M' , C' and T' in regression equation 5 under the above optimum conditions of the variables is 95.49 %. While, in the literature, model predicts that the maximum conversion to biodiesel for alkali transesterification of

waste rapeseed, under the optimum conditions of the variables, was 83.34 wt% (Yuan *et al.*, 2008).

The result of this present study similar to Jeong *et al.* (2009) that studied response surface methodology, and the effects of five-level-three-factors for optimizing the reaction conditions of animal fat biodiesel production. The referred study contain 20 individual experiments, which were designed to study reaction temperature, catalyst amount, and oil to methanol molar ratio. A statistical model predicted that the highest conversion yield of animal fat biodiesel would be 98.6%, at the following optimized reaction conditions: a reaction temperature of 65°C, a catalyst amount of 1.26%, and an oil to methanol molar ratio of 7.5:1, and 20 minutes of reaction time. Using these optimal factor values under experimental conditions in three independent replicates, an fatty acid methyl ester content of $97.8 \pm 0.6\%$ that the value was well within the range predicted by the model, was achieved.

The literatures discussed that conversion was complicated if oil contains large amounts of free fatty acid (FFA) that would form soap with alkaline catalyst. The soap could prevent separation of the biodiesel from the glycerine fraction and contributes to emulsion formation during the water wash for these cases, an acid catalyst, such as sulphuric acid, could be used to esterify the free fatty acid to methyl ester (Gerpen, 2005). Previous studies (Ghadge and Raheman, 2006; Tiwari *et al.*, 2007; Yuan *et al.*, 2008; Jeong *et al.*, 2009) and this present study indicated that more than 90% ester was obtained when two-step process was used compared to 50% ester in one step alkali catalyzed process.

Furthermore, the results of the relationship between independent and dependent variables of the developed model from the response surface plots and contour plots showed that a low methanol to oil ratio, % fatty acid methyl ester (FAME) increased with increasing catalyst concentration (Figure 12). At short reaction time, the % fatty acid methyl ester (FAME) increase with increasing methanol to oil

ratio (Figure 13). For high catalyst concentration, % fatty acid methyl ester (FAME) increased with increasing reaction time (Figure 14).

The response surface methodology (RSM) was used to optimize the conditions for the maximum conversion to biodiesel and understand the significance and interaction of the factors affecting the biodiesel production. The results showed that catalyst concentration and reaction time were the limiting conditions and little variation in their value would alter the conversion. While, there was a significant mutual interaction between catalyst concentration and reaction time.

This present study has found that trap grease methyl ester contained fatty acid methyl ester which consisted of the major fatty acid esters as palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1) and linoleic (C18:2), which they were requirement of biodiesel standard and oleic acid was the predominant compound in the trap grease methyl ester. In contrast, The analysis of waste rapeseed biodiesel indicated that the biodiesel contains large amount of C19 and the amount of 9,12-octadecadienoic acid methyl ester was the highest in the biodiesel. The produced biodiesel in the literature, was analyzed by gas chromatography/mass spectrometry (GC/MS), which showed that it mainly contained six fatty acid methyl esters. The biodiesel analysis showed that most of the fuel properties were in reasonable agreement with the diesel standard of China (GB252-2000) and the biodiesel standard of America (ASTM D6751) (Yuan *et al.*, 2008).

Table 19 Central composite design arrangement and response for alkali catalyzed transesterification.

Treatment	Methanol/oil ratio (v/v)			Catalyst Concentration (%w/v)	Reaction time (hr)	fatty acid methyl ester (%)		
	M'	C'	T'			Experimental	Predicted	
1	-1	-1	-1	0.22	0.50	0.67	90.30	89.83
2	-1	-1	1	0.22	0.50	1.33	84.30	82.00
3	-1	1	-1	0.22	1.50	0.67	92.30	90.57
4	-1	1	1	0.22	1.50	1.33	87.40	91.28
5	1	-1	-1	0.30	0.50	0.67	92.70	89.18
6	1	-1	1	0.30	0.50	1.33	82.00	84.10
7	1	1	-1	0.30	1.50	0.67	86.10	88.77
8	1	1	1	0.30	1.50	1.33	91.40	92.23
9	-1.68	0	0	0.19	1.00	1.00	86.80	87.33
10	+1.68	0	0	0.33	1.00	1.00	88.60	87.59
11	0	-1.68	0	0.26	0.16	1.00	80.00	82.67
12	0	+1.68	0	0.26	1.84	1.00	93.30	90.12
13	0	0	-1.68	0.26	1.00	0.45	92.10	94.11
14	0	0	+1.68	0.26	1.00	1.55	93.00	90.47
15	0	0	0	0.26	1.00	1.00	95.90	95.49
16	0	0	0	0.26	1.00	1.00	96.10	95.49
17	0	0	0	0.26	1.00	1.00	95.00	95.49
18	0	0	0	0.26	1.00	1.00	95.40	95.49
19	0	0	0	0.26	1.00	1.00	95.70	95.49
20	0	0	0	0.26	1.00	1.00	95.50	95.49
21	0	0	0	0.26	1.00	1.00	95.10	95.49
22	0	0	0	0.26	1.00	1.00	95.10	95.49
23	0	0	0	0.26	1.00	1.00	95.50	95.49

Table 20 Analysis of variance (ANOVA) for quadratic polynomial model.

Model	Sum of Squares	df	Mean Square	F	Sig.
regression	424.395	9	47.155	7.830	0.001
Residual	78.291	13	6.022		
Total	502.686	22			

Table 21 Regression coefficients of predicted quadratic polynomial model for regression equation.

Terms	Coefficients	Standard error
Linear		
β_0	-17.471	33.008
β_1	815.371	204.150
β_2	20.985	13.453
β_3	-8.678	21.340
Quadratic		
β_{11}	-1636.963	360.843
β_{22}	-12.884	2.467
β_{33}	-10.563	5.736
Interaction		
β_{12}	-14.375	43.382
β_{13}	52.083	65.730
β_{23}	12.955	5.258

$$\begin{aligned} \%FAME = & -17.471 + 815.371 M' + 20.985 C' - 8.678 T' \\ & - 1636.963 M'^2 - 12.884 C'^2 - 10.563 T'^2 \\ & - 14.375 M' C' + 52.083 M' T' + 12.955 C' T' \dots\dots\dots(5) \end{aligned}$$

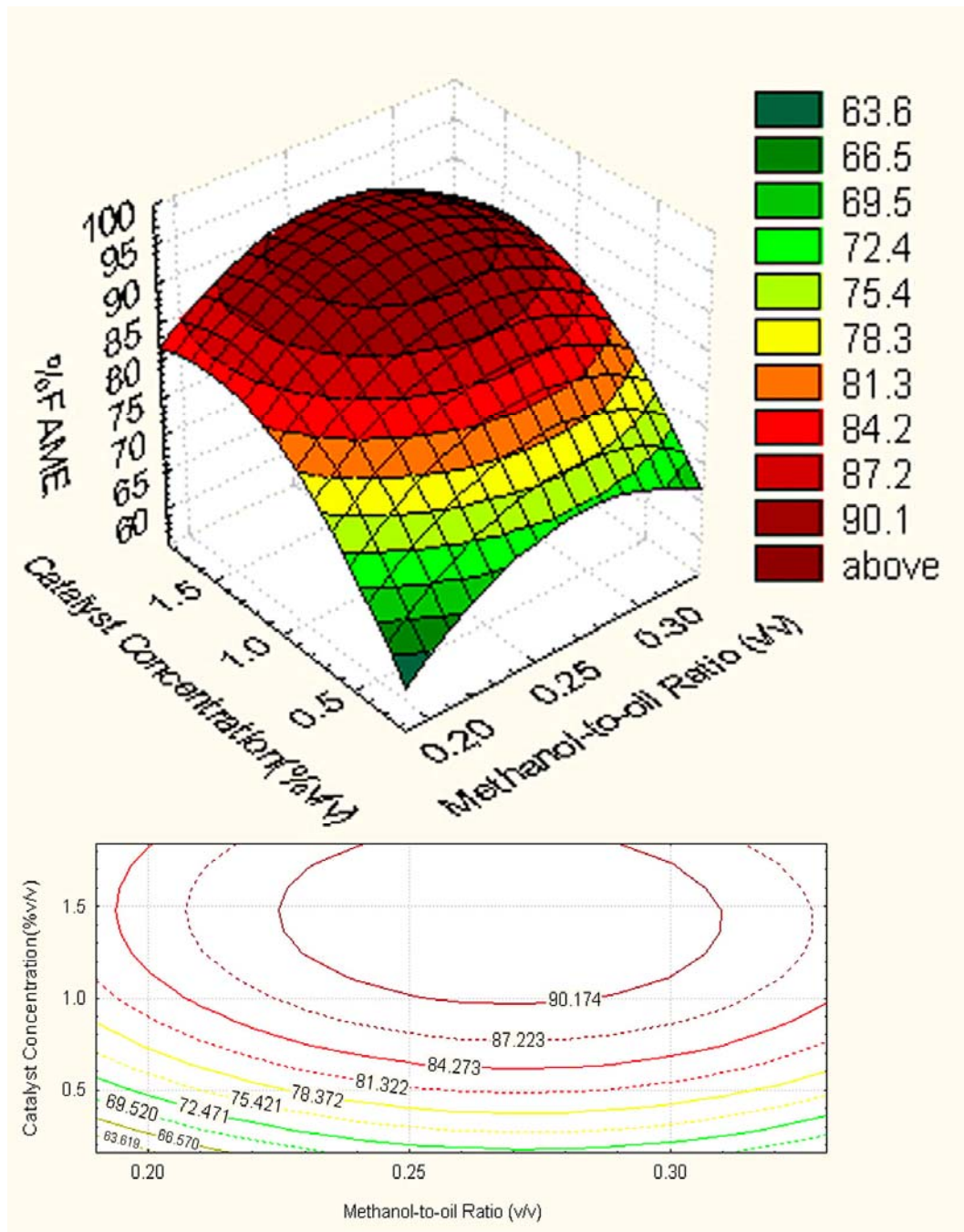


Figure 12 Response surface plots and contour plots representing the effect of methanol to oil ratio and catalyst concentration on %FAME predicted from quadratic polynomial model.

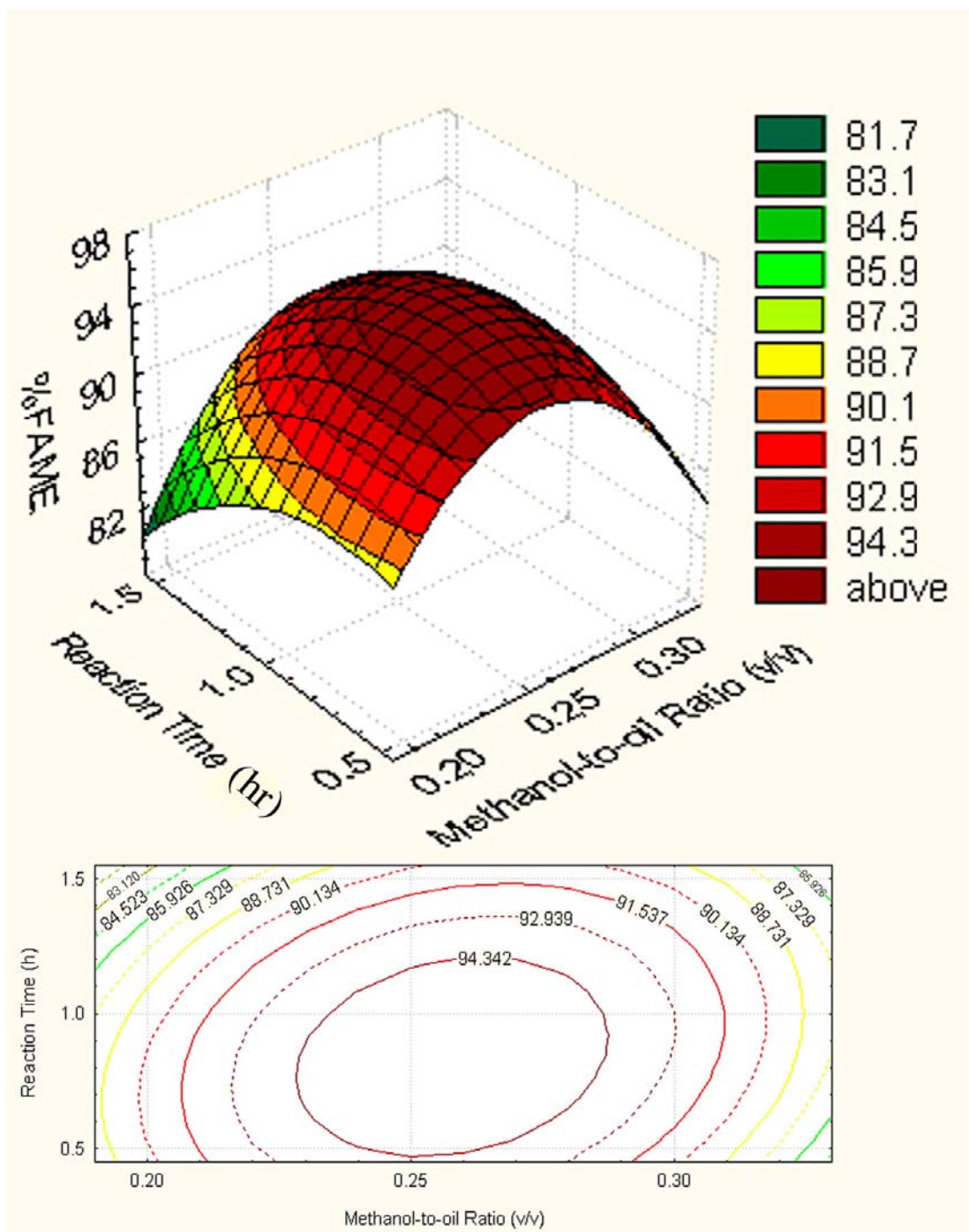


Figure 13 Response surface plots and contour plots representing the effect of methanol to oil ratio and reaction time on %FAME predicted from quadratic polynomial model

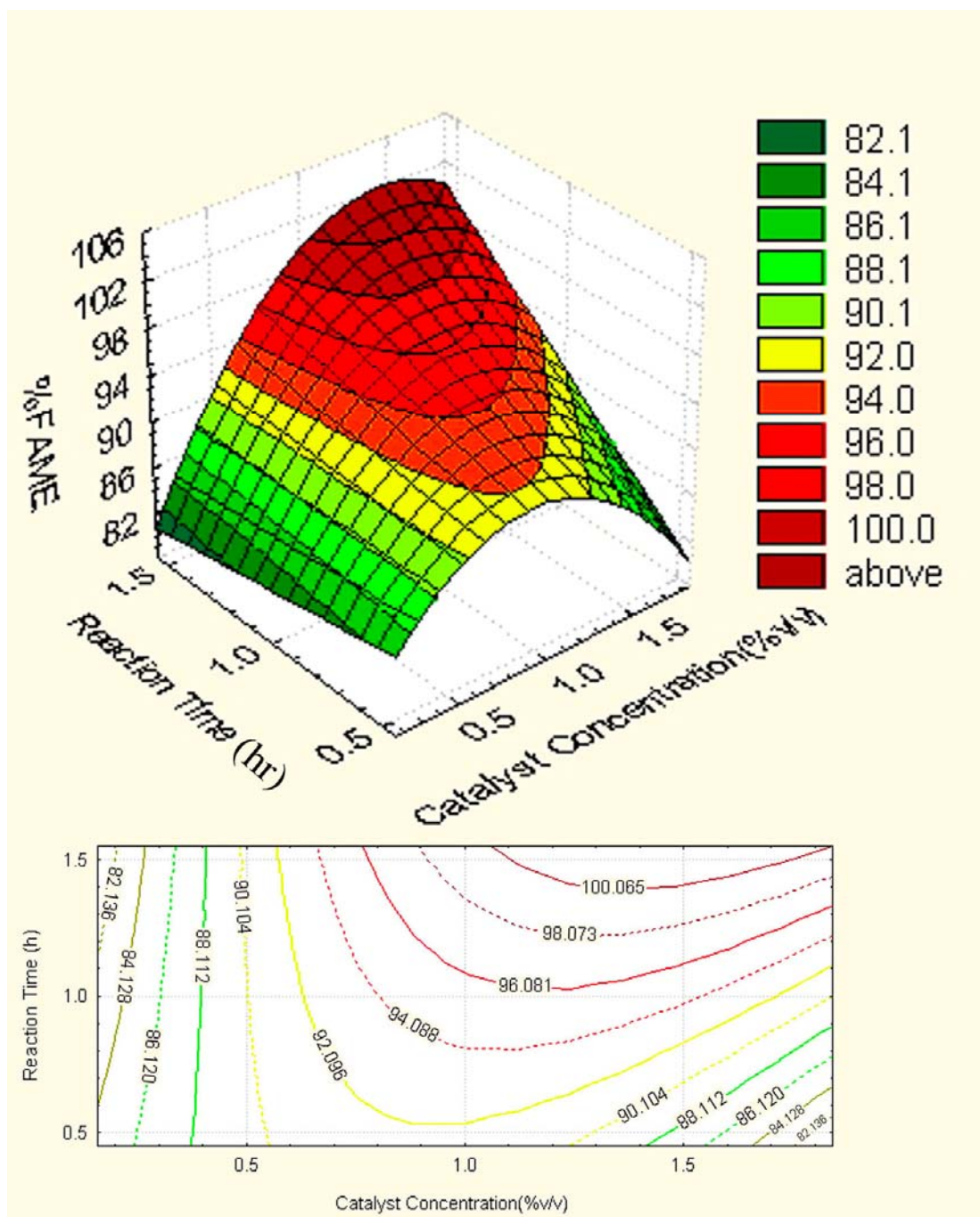


Figure 14 Response surface plots and contour plots representing the effect of catalyst concentration and reaction time on %FAME predicted from quadratic polynomial model

Part 4 Properties of Trap Grease Biodiesel at the Optimum Reaction Conditions

The properties of biodiesel produced from trap grease in waste water at the optimum conditions were analyzed and compared with ASTM D 6751 and EN 14214 biodiesel standards in Table 22. A trap grease biodiesel was produced by optimum condition of two step catalyzed processes (Figure 15). Most of the biodiesel properties from trap grease were found to be in reasonable agreement with ASTM D 6751 and EN 14214.

Moreover, other biodiesel were compared to the biodiesel produced from trap grease for some properties of fuel in Table 23. Acid value is defined as milligrams of potassium hydroxide necessary to neutralize fatty acids in 1 g of sample. Acid value of trap grease was very high but by using two step process, hence the acid value could be reduced to 0.54 mg KOH/g, which was like previous studies that acid value of esters were within the limit of the ASTM D 6751 standard (0.8 mg KOH g⁻¹) (Veljkovic *et al.*, 2006; Ghadge and Raheman, 2006; Tiwari *et al.*, 2007; Yuan *et al.*, 2008; Utlu and Kocak, 2008; Bouaid *et al.*, 2005; Ramadhas *et al.*, 2005). Kinematic viscosity at 40 °C of trap grease was very high at 29.8 mm²/s. Higher viscosity of trap grease was due to the higher molecular weight molecules such as triglycerides, polymerized triglycerides and many other unknown heavier compounds formed during frying. After two step process, trap grease methyl ester reduced to be 4.84 mm²/s, while several oils showed reduction of viscosity in the range of 3.98–5.81 mm²/s. They were within the limit of the ASTM D 6751 standard of 1.9–6.0 mm²/s) (Veljkovic *et al.*, 2006; Ghadge and Raheman, 2006; Tiwari *et al.*, 2007; Jeong *et al.*, 2009; Utlu and Kocak, 2008; Bouaid *et al.*, 2005; Ramadhas *et al.*, 2005). Most of the biodiesel properties from trap grease were found to be in reasonable agreement with ASTM D 6751 and EN 14214 standards.

The production process of trap grease biodiesel from this research consists of a filtration process, heating process, acid catalyzed esterification and alkali catalyzed transesterification, which these were two step catalyzed process using optimized condition from this studies, separation process, washing process, recovery process

and dehydration process. The reaction temperature was maintained at 60°C and stirring at 400 rpm. A process flow schematic for trap grease biodiesel production of the two step catalyzed process was shown in Figure 16.

Since methanol and ethanol including the alkali catalysts was more soluble in the polar glycerol phase, most would be removed when the glycerol was separated from the biodiesel. However, the biodiesel might still contain 2%-3% methanol after the separation, which might constitute as much as 40% of the excess methanol from the reaction. Several plants would recover this methanol by heating the biodiesel. Any methanol remaining after this heating process should be removed by the water washing process. Therefore, the residual alcohol level in the biodiesel should be very low. The allowable alcohol level was specified in European biodiesel standards. Tests had shown that even as little as 1% methanol in the biodiesel could be lower the flashpoint of the biodiesel from 170°C to less than 40°C. Therefore, a flashpoint specification of 130°C, the ASTM standard limits the amount of alcohol to a very low level as less than 0.1%. Residual alcohol left in the biodiesel would not affect its use in the engine. The amount is too small to negatively impact the fuel's performance. However, lowering the flashpoint presents a potential safety hazard, as the fuel might need to be treated like gasoline, which also had a low flashpoint, than diesel fuel. Most of the residual catalyst was removed with the glycerol. Like the alcohol, remaining catalyst should be removed during the water washing. Although a value for residual catalyst was not included in the ASTM standard. It would be limited by the specification on sulfated ash. Excessive ash in the fuel could lead to engine deposits and high abrasive wear levels (Canakci and Sanli, 2008).

Water and sediment contamination was basically housekeeping issues for biodiesel. Water could be present in two forms, either as dissolved water or as suspended water droplets. While biodiesel was generally considered to be insoluble in water, it actually took up considerably more water than diesel fuel. Biodiesel could contain as much as 1500 ppm of dissolved water while diesel fuel usually only took up about 50 ppm. The standards for diesel fuel (ASTMD 975) and biodiesel (ASTM D 6751) both limit the amount of water to 500 ppm. For petroleum-based diesel fuel,

this actually allowed a small amount of suspended water. However, biodiesel must be kept dry. This was a challenge because many diesel storage tanks have water on the bottom due to condensation. Suspended water was a problem in fuel injection equipment because it contributed to the corrosion of the closely fitting parts in the fuel injection system. Sediment might consist of suspended rust and dirt particles or it might originate from the fuel as insoluble compounds formed during fuel oxidation (Canakci , 2007; Canakci and Sanli, 2008).

On the condition that the biodiesel standard values were met, biodiesel could be produced either from high-quality vegetable oils or from inexpensive poor quality feedstocks (Canakci , 2007) like trap grease which was also environmentally friendly alternative biodiesel fuel.

Table 22 Fuel properties of trap grease biodiesel.

Property	Unit	Trap grease	Biodiesel standards	
			ASTMD 6751	EN 14214
Density at 15 °C	Kg/m ³	880.13	-	860-900
viscosity at 40°C	mm ² /s	4.835	1.9-6.0	3.5-5.0
Flash point	°C	165	>130	>120
Acid value	mg KOH/g	0.54	<0.8	<0.5
Iodine value	g iodine/100g	73.45	-	<120
Cloud point	°C	7.2	-	-



Figure 15 A trap grease biodiesel from two-step catalyzed process.

Table 23 Properties of trap grease biodiesel in comparison with various biodiesel.

Biodiesel	Property							References
	Density at 15°C (kg/m ³)	Viscosity at 40°C (mm ² /s)	Flash point (°C)	Acid value (mg KOH/g)	Iodine value (g iodine/100g)	Cloud point (°C)		
Jatropha methyl ester	880	4.80	135	0.40	NA	NA	Tiwari et al., 2007	
Waste Rape-seed methyl ester	NA	6.35	NA	0.31	NA	NA	Yuan et al., 2008	
Mahua methyl ester	880	3.98	208	0.41	NA	NA	Ghadge and Raheman, 2006	
Tobacco-seed methyl ester	882	5.20	NA	0.66	109.80	NA	Veljkovic et al., 2006	
Waste cooking oil methyl ester	888	4.32	156	0.48	NA	NA	Utlu and Kocak, 2008	
Brassica carinata methyl ester	810	4.88	NA	0.06	109.40	NA	Bouaid et al., 2005	
Rubber-seed methyl ester	874	5.81	130	0.12	NA	4.0	Ramadhas et al., 2005	
Animal fat methyl ester	NA	4.20	NA	0.08	72.00	NA	Jeong et al., 2009	
Trap grease methyl ester	880	4.84	165	0.54	73.45	7.2	This study	

*NA-not available

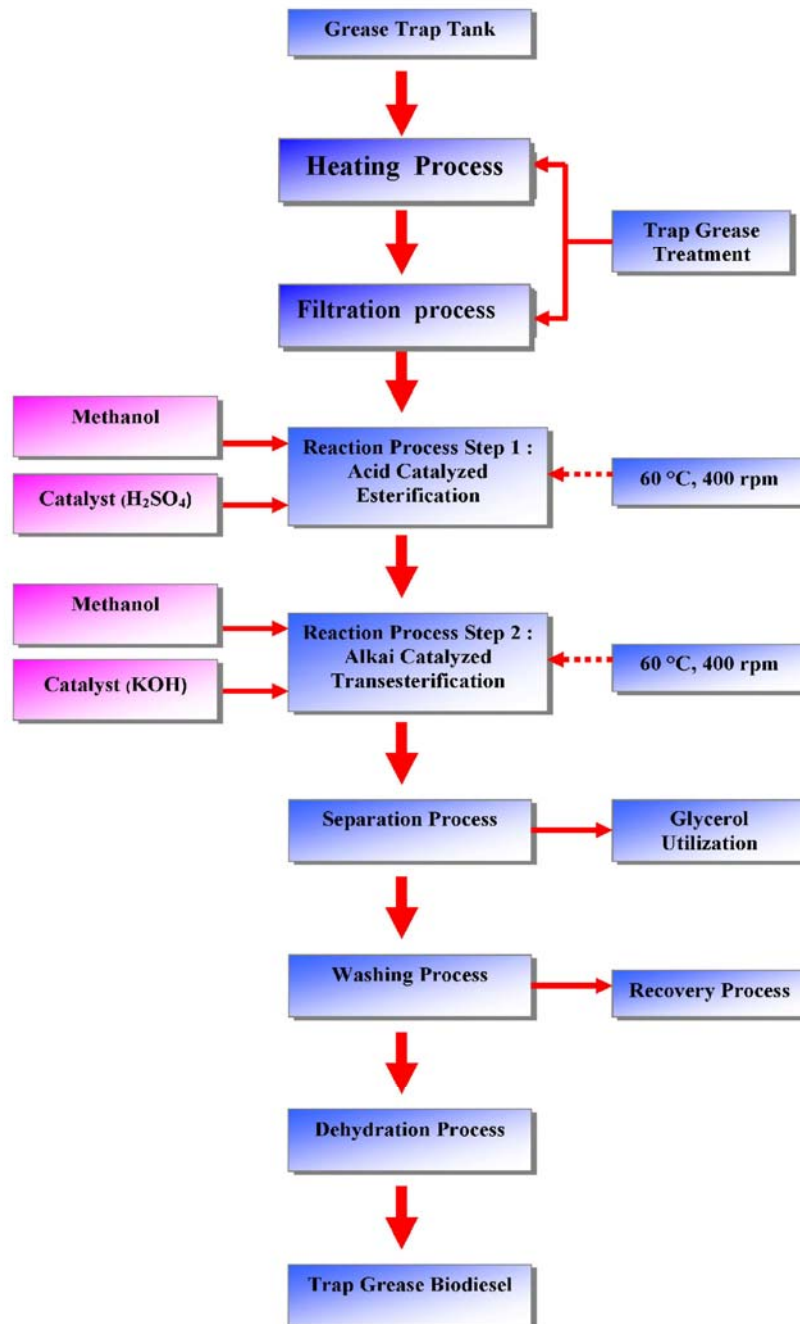


Figure 16 Process flow schematic for trap grease biodiesel production of the two step catalyzed process.

CONCLUSIONS

1. Properties of trap grease were 52.13 mg KOH/g of acid value or 26.19 % of free fatty acid (FFA) which there was the highest oleic acid content. The trap grease contained 29.8 cSt of viscosity and 1.156 % of water content and molecular weight of the trap grease was 846 g/mol.

2. The trap grease could be reduced to less than 2% of free fatty acid using response surface methodology in optimization of acidic catalyzed esterification. The optimized esterification was found as 0.43 v/v or 10:1 methanol to oil molar ratio, 2.5 % v/v of sulphuric acid concentration and 4 hours of reaction time. This optimum value gave an minimum acid value was 2.661 mg KOH/g (or 1.3%FFA).

3. The optimum condition for the alkali catalyzed transesterification was as follows using 0.26 v/v or 6:1 methanol to oil molar ratio, 1 % w/v potassium hydroxide concentration in 1 hour of reaction time. The maximum fatty acid methyl ester content under this optimum conditions was 95.45 %.

4. Trap grease biodiesel from the two-step catalyzed process which consist of the acidic catalyzed esterification and the alkali catalyzed transesterification in optimum condition contained properties satisfying both the biodiesel standard of ASTM D 6751 and EN 14214.

5. This developed biodiesel production technique from trap grease was several advantage. Firstly, trap grease was low-cost feedstock for biodiesel production, hence this could decrease biodiesel cost. Secondly, trap grease was used as the feedstock, could help wastewater treatment and the trap grease utilization has encourage environmental concern. In addition, the optimized production technique increase amount of fatty acid methyl ester and zero-waste emission. In brief, the trap grease biodiesel is environmentally friendly renewable energy.

6. The two step process optimized technique could apply for community plant with using the low-cost feedstock as the trap grease to reduce saponification in alkali catalyzed process and to produce the higher biodiesel purity. In addition, the trap grease biodiesel can be used various such as pick-up truck and agricultural vehicle. Furturmore, this research has been grease trap tank–using promotion to prevent water pollution for household, restaurant, including communities in Thailand and worldwide.

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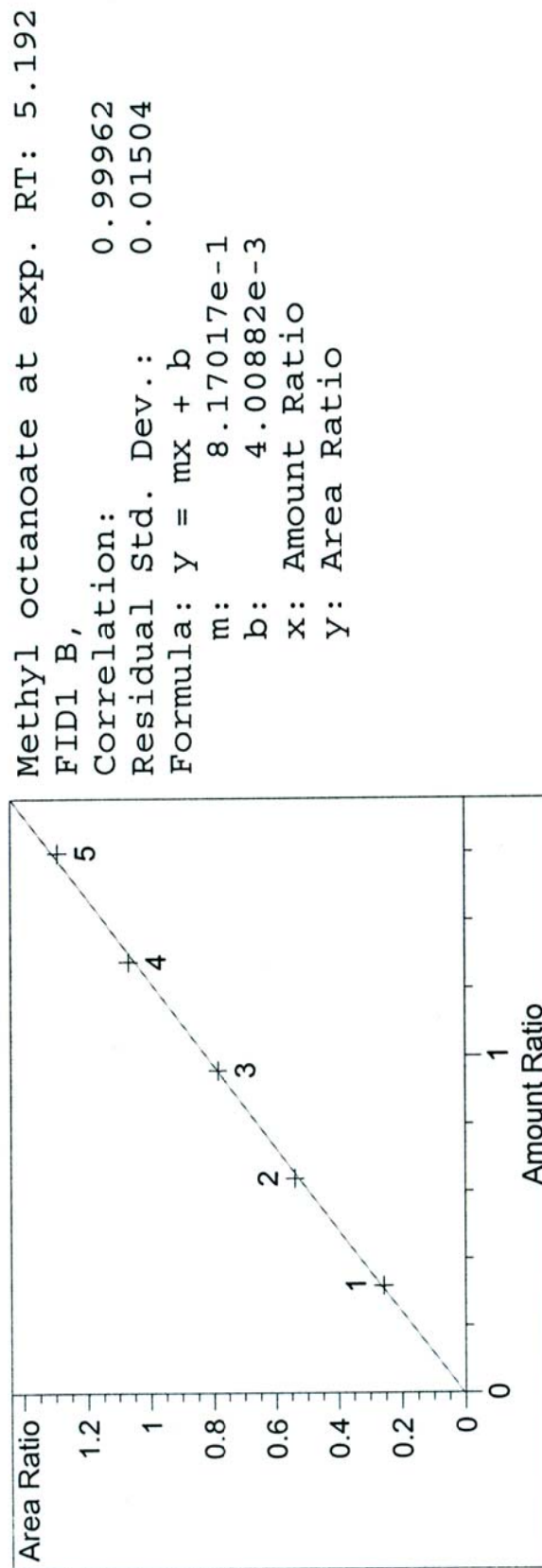
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Appendix

Appendix A
Standard curve of fatty acid methyl ester

The standard curve was prepared as 0, 2000, 4000, 6000, 8000, and 10000 ppm to make peak areas that were plotted against various standard concentration from appendix Figure 1-15.



Appendix Figure A1 Standard curve of octanoic acid

Methyl decanoate at exp. RT: 7.470

FID1 B,

Correlation: 0.99973

Residual Std. Dev.: 0.01396

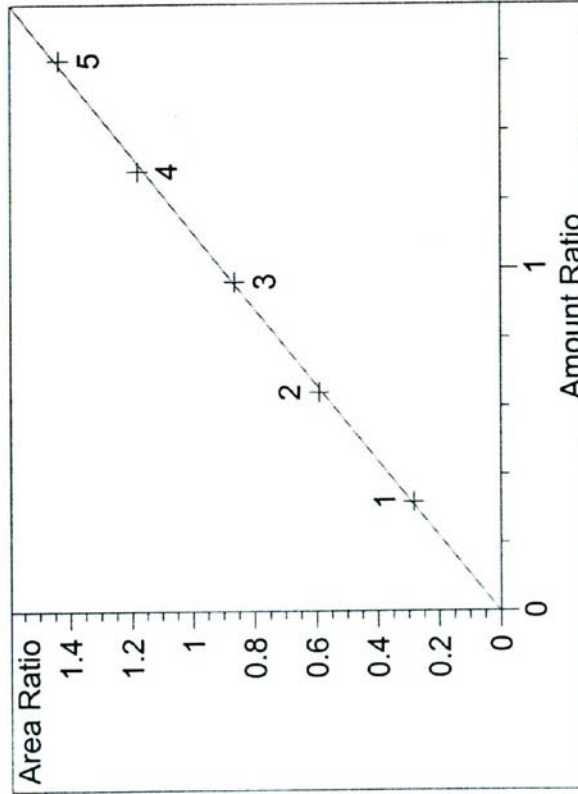
Formula: $y = mx + b$

m: $9.04370e-1$

b: $1.04904e-3$

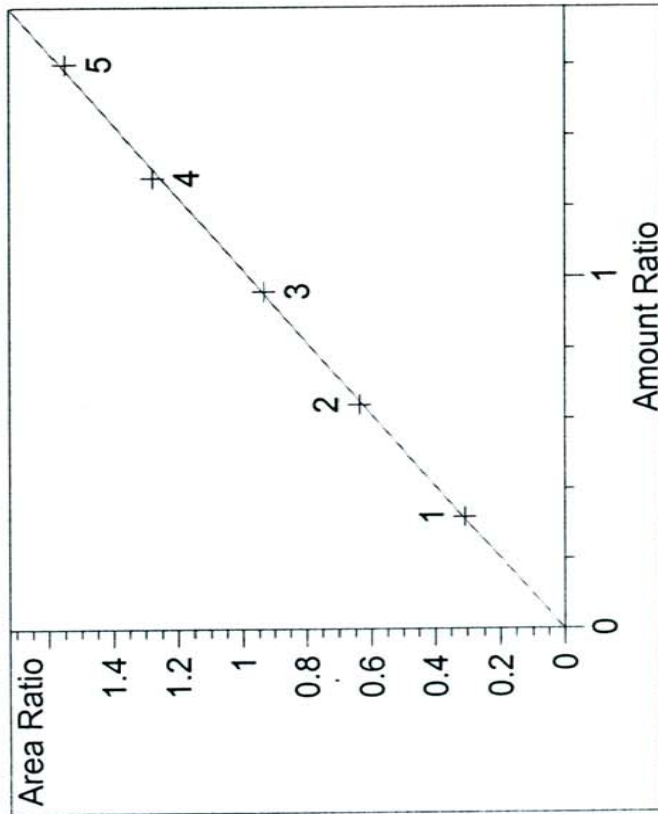
x: Amount Ratio

y: Area Ratio



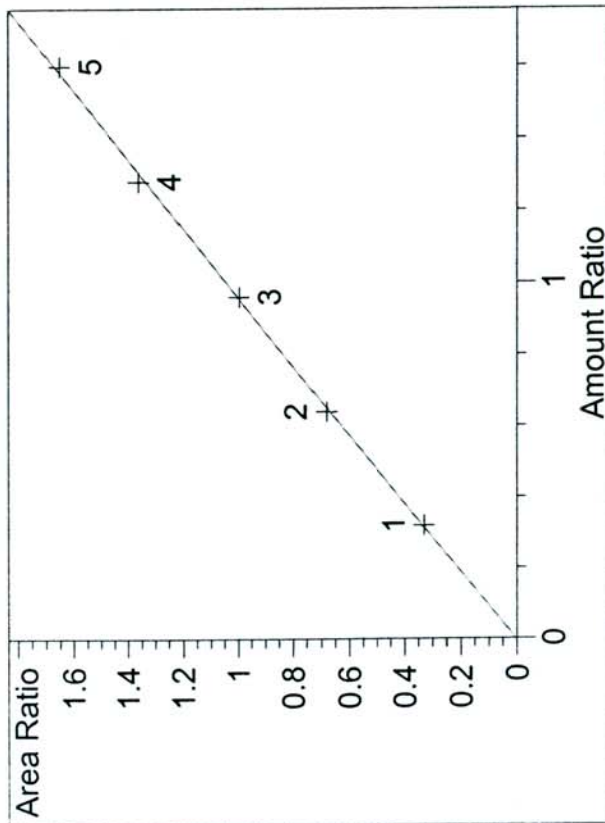
Appendix Figure A2 Standard curve of decanoic acid

Methyl Laurate at exp. RT: 9.709
FID1 B,
Correlation: 0.99969
Residual Std. Dev.: 0.01622
Formula: $y = mx + b$
m: 9.77319e-1
b: 1.33014e-3
x: Amount Ratio
y: Area Ratio



Appendix Figure A3 Standard curve of lauric acid

Methyl myristate at exp. RT: 11.795
FID1 B,
Correlation: 0.99969
Residual Std. Dev.: 0.01730
Formula: $Y = mx + b$
m: 1.04216
b: 3.06666e-3
x: Amount Ratio
y: Area Ratio



Appendix Figure A4 Standard curve of myristic acid

Methyl palmitate at exp. RT: 13.721

FID1 B,

Correlation: 0.99967

Residual Std. Dev.: 0.02600

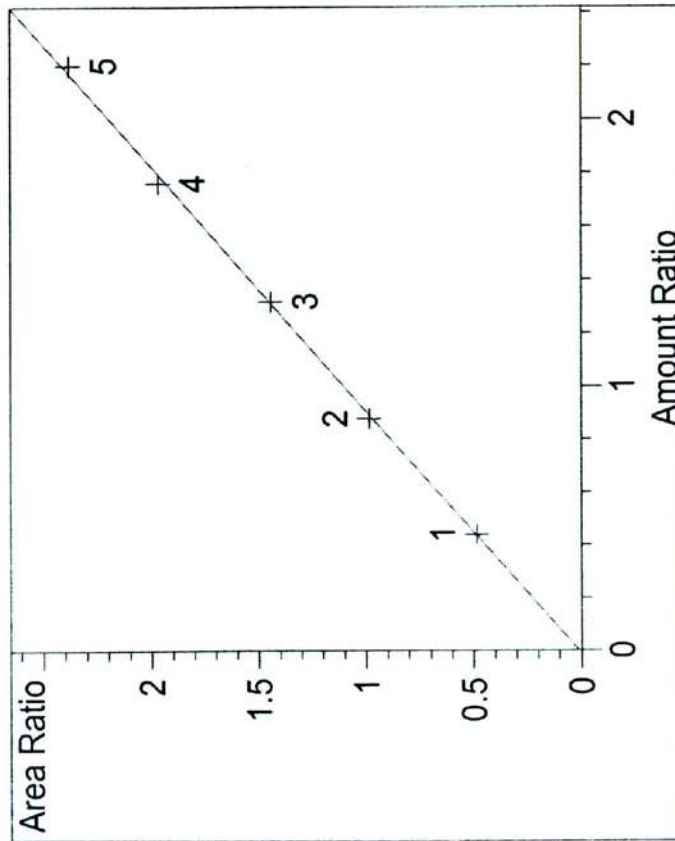
Formula: $y = mx + b$

m: 1.09213

b: $9.61911e-3$

x: Amount Ratio

y: Area Ratio



Appendix Figure A5 Standard curve of palmitic acid

Methyl palmitoleate at exp. RT: 13.969

FID1 B,

Correlation: 0.99969

Residual Std. Dev.: 0.01076

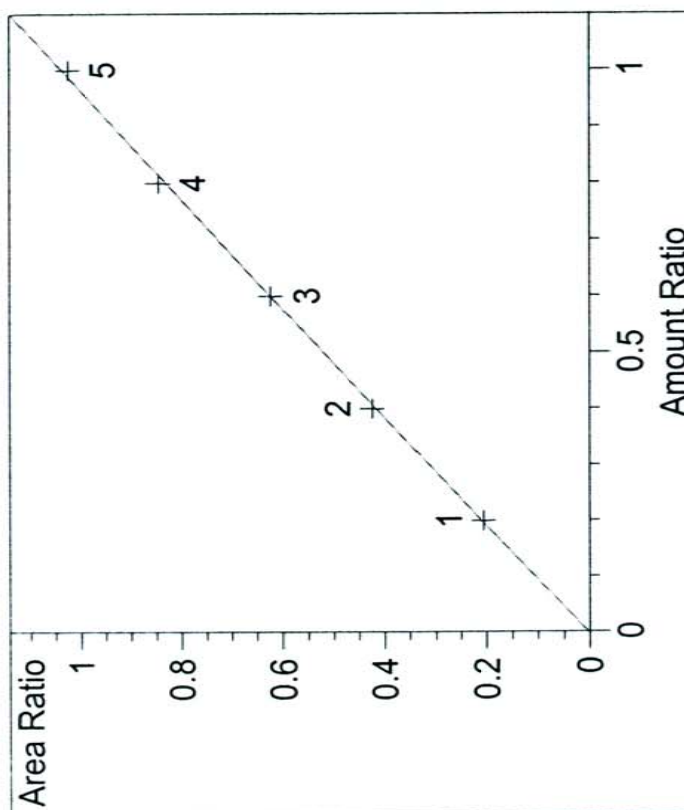
Formula: $y = mx + b$

m: 1.03495

b: $3.10713e-3$

x: Amount Ratio

y: Area Ratio



Appendix Figure A6 Standard curve of palmitoleic acid

Methyl heptadecanoate at exp. RT: 14.628

FID1 B,

Correlation: 1.00000

Residual Std. Dev.: 0.00000

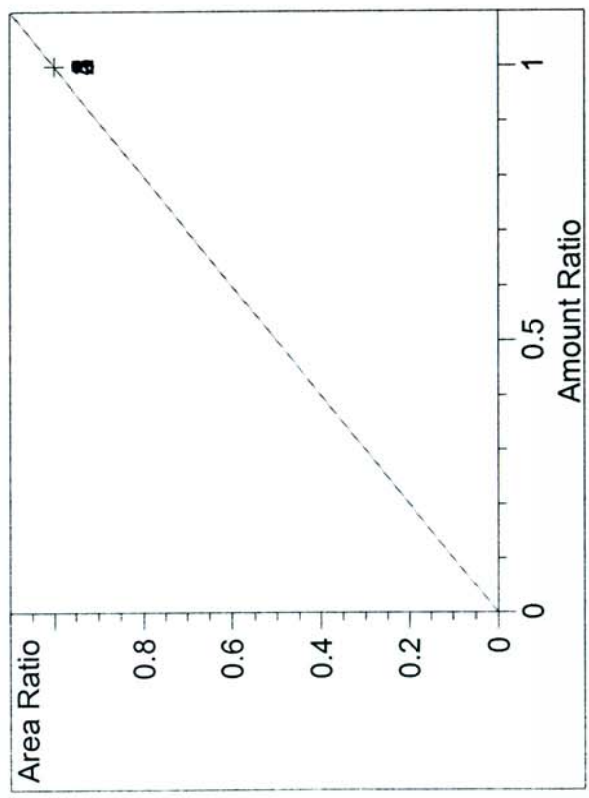
Formula: $y = mx + b$

m: 1.00000

b: 0.00000

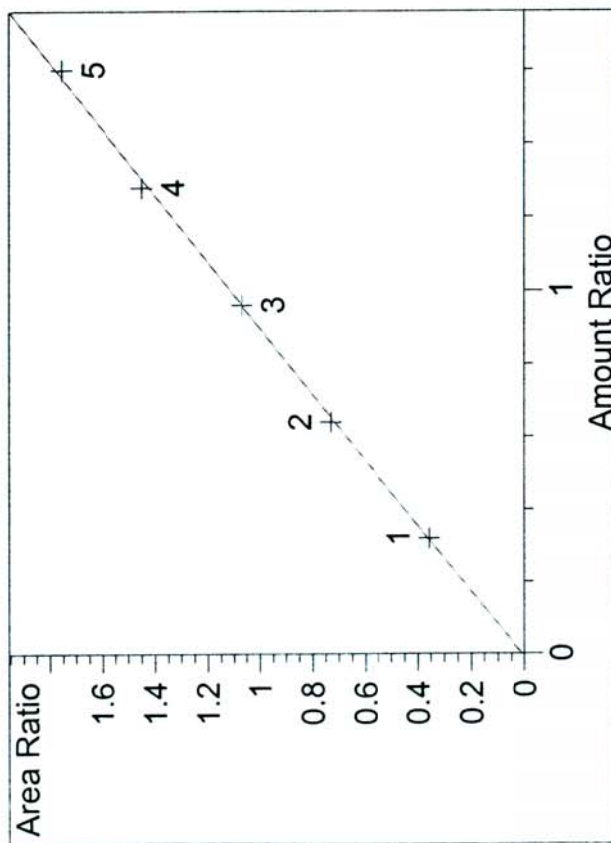
x: Amount Ratio

y: Area Ratio



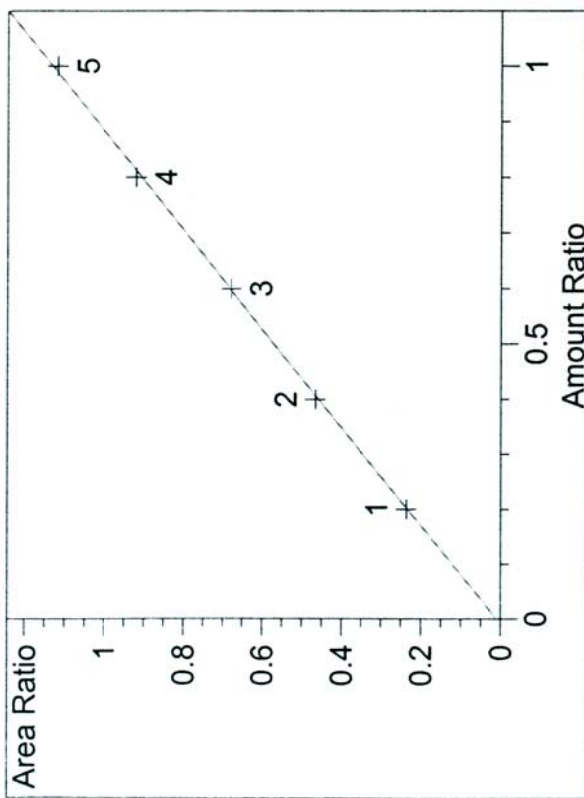
Appendix Figure A7 Standard curve of heptadecanoic acid

Methyl stearate at exp. RT: 15.497
FID1 B,
Correlation: 0.99967
Residual Std. Dev.: 0.01905
Formula: $y = mx + b$
m: 1.10711
b: 7.93234e-3
x: Amount Ratio
y: Area Ratio

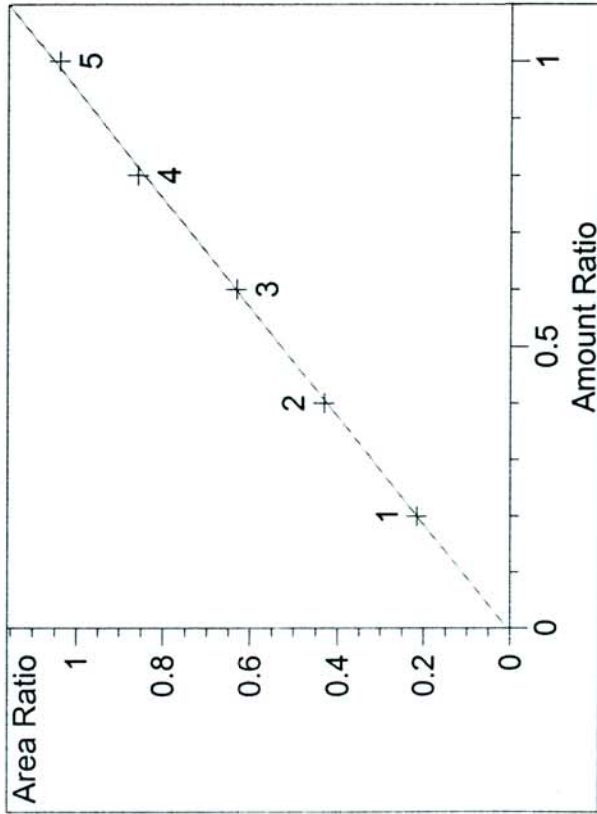


Appendix Figure A8 Standard curve of stearic acid

Methyl oleate at exp. RT: 15.680
FID1 B,
Correlation: 0.99970
Residual Std. Dev.: 0.01157
Formula: $y = mx + b$
m: 1.12225
b: 8.40573e-3
x: Amount Ratio
y: Area Ratio



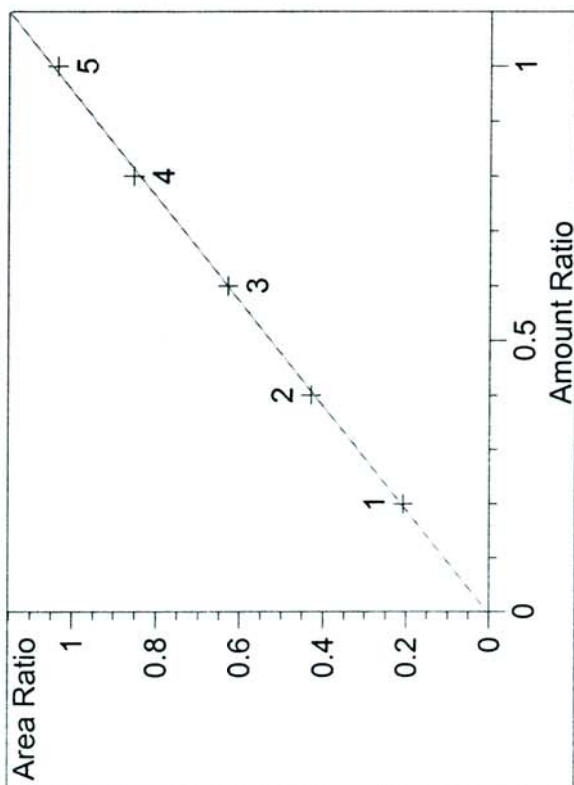
Appendix Figure A9 Standard curve of oleic acid



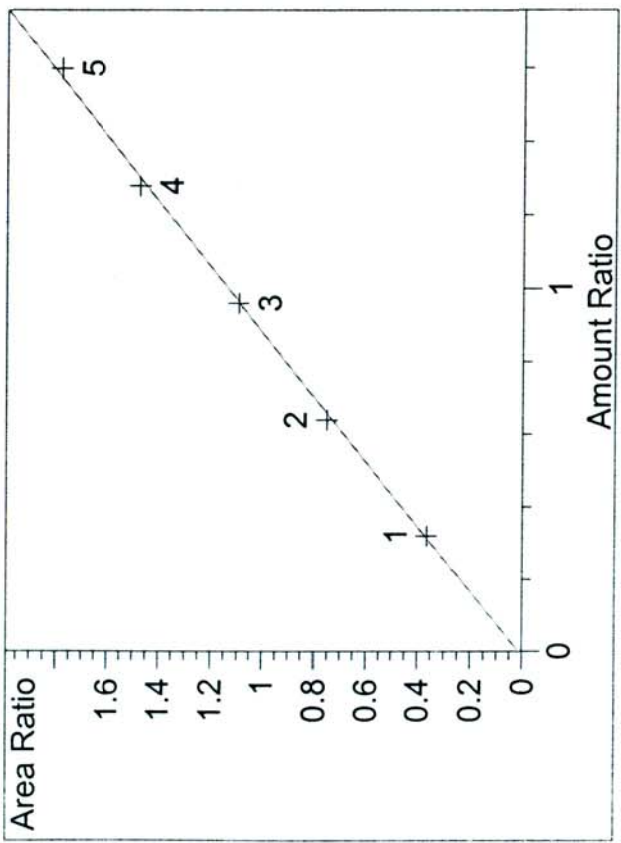
Methyl linoleate at exp. RT: 16.078
 FID1 B,
 Correlation: 0.99971
 Residual Std. Dev.: 0.01056
 Formula: $Y = mx + b$
 m: 1.04618
 b: 5.83291e-3
 x: Amount Ratio
 Y: Area Ratio

Appendix Figure A10 Standard curve of linoleic acid

Methyl linolenate at exp. RT: 16.621
FID1 B,
Correlation: 0.99969
Residual Std. Dev.: 0.01091
Formula: $y = mx + b$
m: 1.04487
b: $3.52557e-3$
x: Amount Ratio
y: Area Ratio



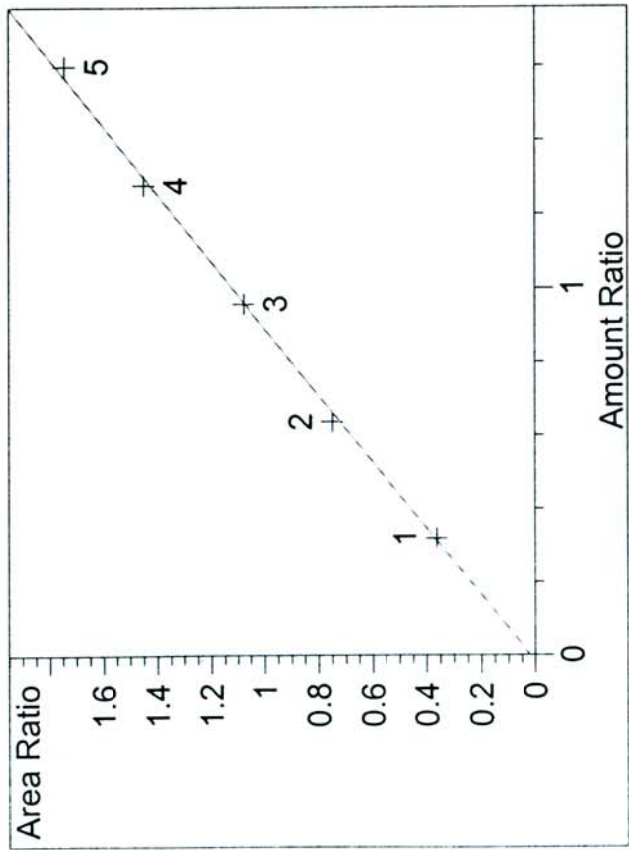
Appendix Figure A11 Standard curve of linolenic acid



Methyl arachidate at exp. RT: 17.146
 FID1 B,
 Correlation: 0.99954
 Residual Std. Dev.: 0.02273
 Formula: $y = mx + b$
 m: 1.12299
 b: 1.24265e-2
 x: Amount Ratio
 y: Area Ratio

Appendix Figure A12 Standard curve of arachidic acid

Methyl behenate at exp. RT: 18.739
FID1 B,
Correlation: 0.99936
Residual Std. Dev.: 0.02625
Formula: $y = mx + b$
m: 1.10027
b: 1.79413e-2
x: Amount Ratio
y: Area Ratio



Appendix Figure A13 Standard curve of behenic acid

Methyl erucate at exp. RT: 18.952

FID1 B,

Correlation: 0.99928

Residual Std. Dev.: 0.01747

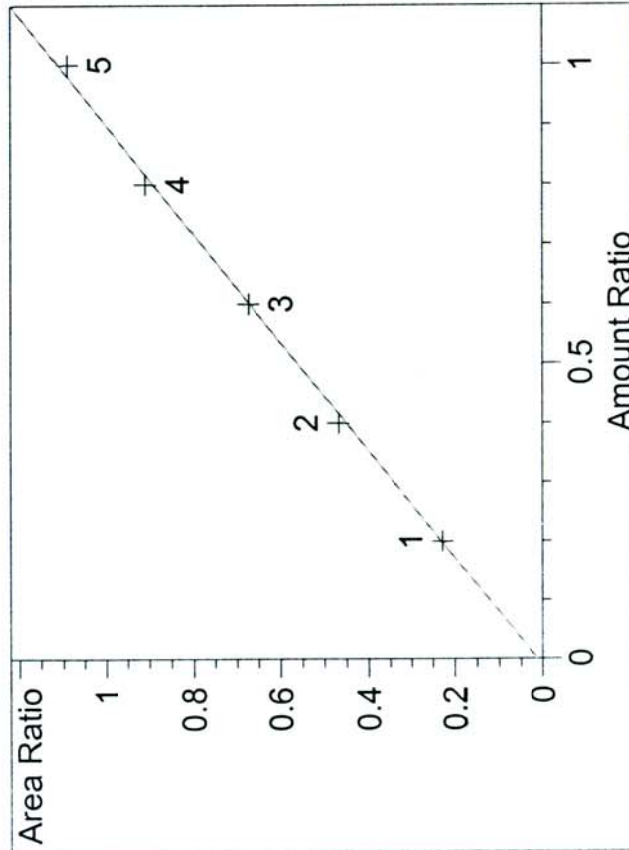
Formula: $y = mx + b$

m: 1.09984

b: 1.15859e-2

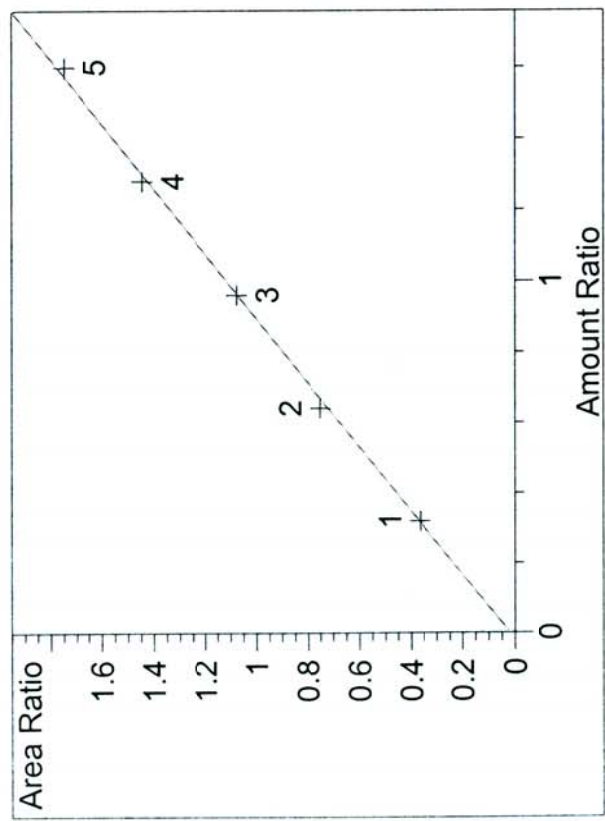
x: Amount Ratio

y: Area Ratio



Appendix Figure A14 Standard curve of erucic acid

Methyl lignocerate at exp. RT: 20.789
FID1 B,
Correlation: 0.99944
Residual Std. Dev.: 0.02456
Formula: $y = mx + b$
m: 1.09944
b: 1.83197e-2
x: Amount Ratio
y: Area Ratio



Appendix Figure A15 Standard curve of lignoceric acid

Appendix B

Average molecular weight of trap grease

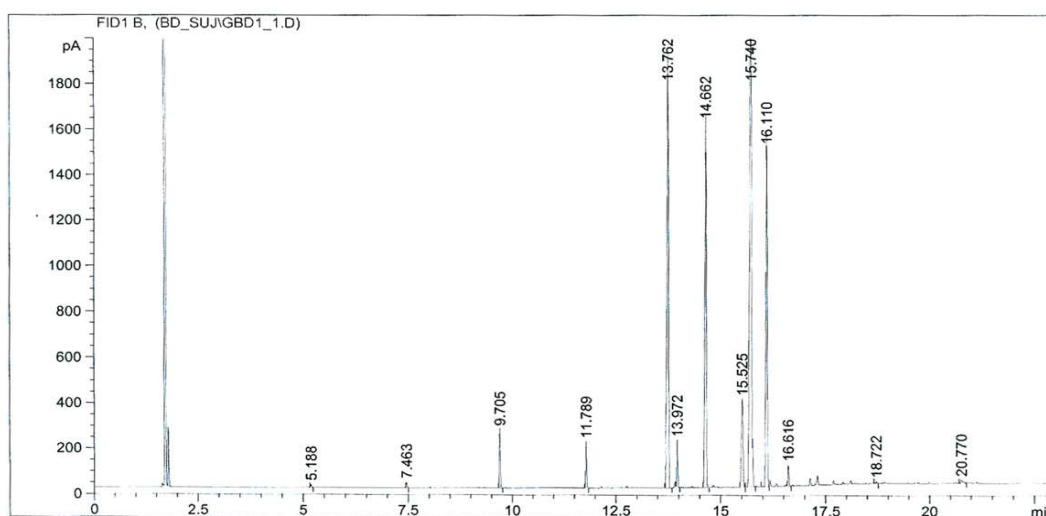
Appendix Table B1 Molecular weight of trap grease

Peak #	Retention Time (min)	Fatty acid	Chemical structure	Molecular Weight (MW)	Area%	(Area%)(MW)	Weight %	(Weight%)(MW)	
1	5.194	Octanoic (C8:0)	CH ₃ (CH ₂) ₆ COOH	144	0.35688	51.391	0.466	67.075	
2	7.471	Decanoic (C10:0)	CH ₃ (CH ₂) ₈ COOH	172	0.33214	57.128	0.398	68.418	
3	9.713	Lauric (C12:0)	CH ₃ (CH ₂) ₁₀ COOH	200	2.71155	542.310	3.020	604.078	
4	11.795	Myristic (C14:0)	CH ₃ (CH ₂) ₁₂ COOH	228	1.85599	423.166	1.934	441.058	
5	13.758	Palmitic (C16:0)	CH ₃ (CH ₂) ₁₄ COOH	256	25.87614	6624.292	25.799	6604.599	
6	13.977	Palmitoleic (C16:1)	CH ₃ (CH ₂) ₅ CH=CH(CH ₂) ₇ COOH	226	1.97437	446.208	2.073	468.386	
7	15.520	Stearic (C18:0)	CH ₃ (CH ₂) ₁₆ COOH	284	5.62743	1598.190	5.524	1568.768	
8	15.731	Oleic (C18:1)	CH ₃ (CH ₂) ₇ CH=CH(CH ₂) ₇ COOH	282	40.61959	11454.724	39.424	11117.590	
9	16.109	Linoleic (C18:2)	CH ₃ (CH ₂) ₄ CH=CHCH ₂ CH=CH(CH ₂) ₇ COOH	280	19.11226	5351.433	19.895	5570.569	
10	16.622	Linolenic (C18:3)	CH ₃ CH ₂ CH=CHCH ₂ CH=CHCH ₂ CH=CH(CH ₂) ₇ COOH	278	0.99176	275.709	1.027	285.582	
11	17.143	Arachidic (C20:0)	CH ₃ (CH ₂) ₁₈ COOH	312	0.29018	90.536	0.259	80.698	
12	18.734	Behenic (C22:0)	CH ₃ (CH ₂) ₂₀ COOH	340	0.11826	40.208	0.083	28.341	
13	20.779	Lignoceric (C24:0)	CH ₃ (CH ₂) ₂₂ COOH	368	0.13346	49.113	0.098	35.979	
					SUM	27004.408	SUM	26941.142	
				Average Molecular Weight of waste grease					846.234

APPENDIX C

GC chromatogram of trap grease biodiesel

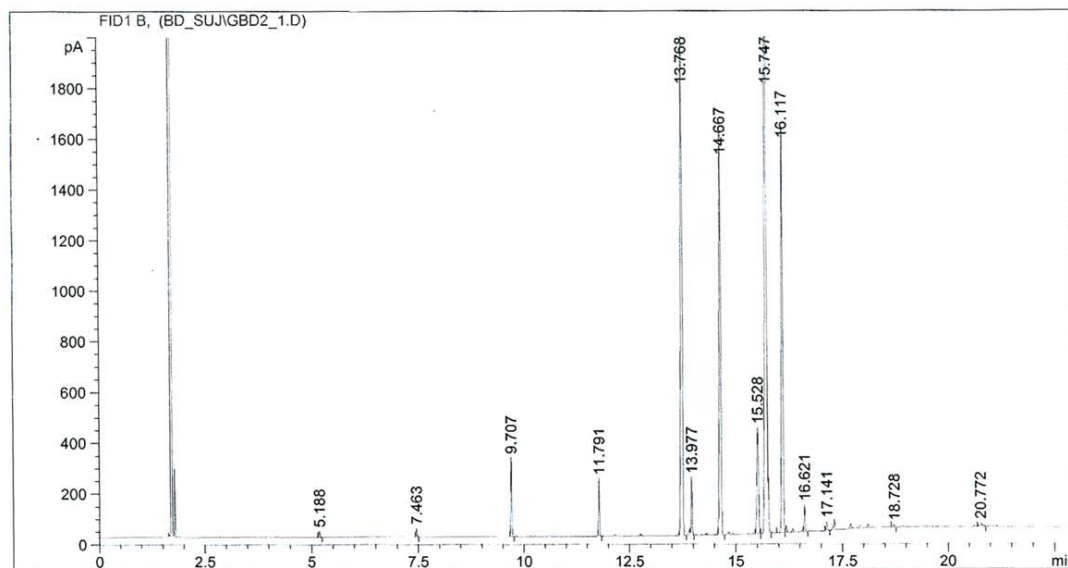
Trap grease biodiesel from alkali catalyzed transesterification was detected by gas chromatograph analysis. It showed main fatty acid components were methyl myristate, methyl palmitate, methyl palmitoleate, methyl stearate, methyl oleate, methyl linoleate, methyl linolenate, methyl arachidate, methyl eicosenate, and methyl behenate. methyl oleate and methyl palmitate were major components.



Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
1	5.188	BB	0.0233	18.90631	0.08517	Methyl octanoate
2	7.463	BB	0.0235	35.55930	0.16018	Methyl decanoate
3	9.705	BB	0.0268	412.52304	1.85827	Methyl Laurate
4	11.789	BP	0.0271	328.98795	1.48198	Methyl myristate
5	13.762	BB	0.0389	4889.30371	22.02460	Methyl palmitate
6	13.972	VB	0.0278	349.49771	1.57436	Methyl palmitoleate
7	14.662	BP	0.0381	3841.15381	17.30305	Methyl heptadecanoate
8	15.525	BP	0.0422	1065.21985	4.79844	Methyl stearate
9	15.740	VV	0.0462	7669.83105	34.54990	Methyl oleate
10	16.110	BV	0.0364	3344.60962	15.06629	Methyl linoleate
11	16.616	VP	0.0286	161.31049	0.72665	Methyl linolenate
12	17.146		0.0000	0.00000	0.00000	Methyl arachidate
13	18.722	BP	0.0436	23.52556	0.10597	Methyl behenate
14	18.952		0.0000	0.00000	0.00000	Methyl erucate
15	20.770	BB	0.0623	58.85806	0.26446	Methyl lignocerate

Totals : 2.22561e4

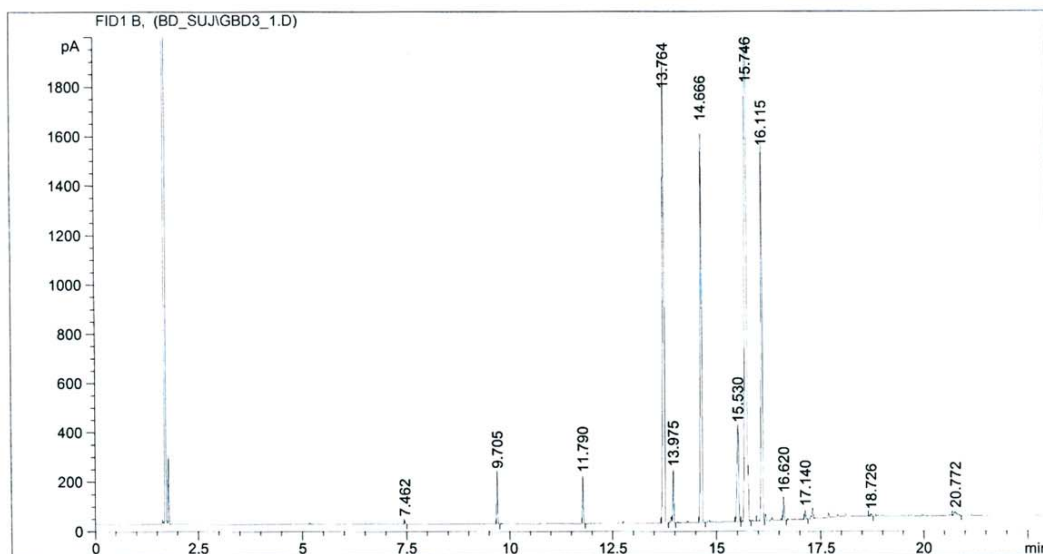
Appendix Figure C1 GC chromatogram of trap grease methyl ester in transesterification condition at 0.22 v/v (5:1) methanol to oil molar ratio, 0.5 % v/v catalyst concentration and 0.67 hour reaction time (experimental treatment no.1)



Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
1	5.188	PB	0.0254	31.89606	0.13398	Methyl octanoate
2	7.463	BB	0.0235	48.67882	0.20448	Methyl decanoate
3	9.707	BP	0.0269	500.99332	2.10448	Methyl Laurate
4	11.791	BP	0.0272	369.98425	1.55416	Methyl myristate
5	13.768	BP	0.0396	5264.92871	22.11589	Methyl palmitate
6	13.977	VB	0.0258	383.67877	1.61168	Methyl palmitoleate
7	14.667	BP	0.0388	3908.77490	16.41922	Methyl heptadecanoate
8	15.528	BP	0.0439	1138.52136	4.78248	Methyl stearate
9	15.747	VV	0.0425	8196.57910	34.43059	Methyl oleate
10	16.117	BV	0.0355	3631.82227	15.25585	Methyl linoleate
11	16.621	VP	0.0279	178.06786	0.74799	Methyl linolenate
12	17.141	BB	0.0291	60.95027	0.25603	Methyl arachidate
13	18.728	BP	0.0429	24.97836	0.10492	Methyl behenate
14	18.952		0.0000	0.00000	0.00000	Methyl erucate
15	20.772	VB	0.0702	66.24055	0.27825	Methyl lignocerate

Totals : 2.38061e4

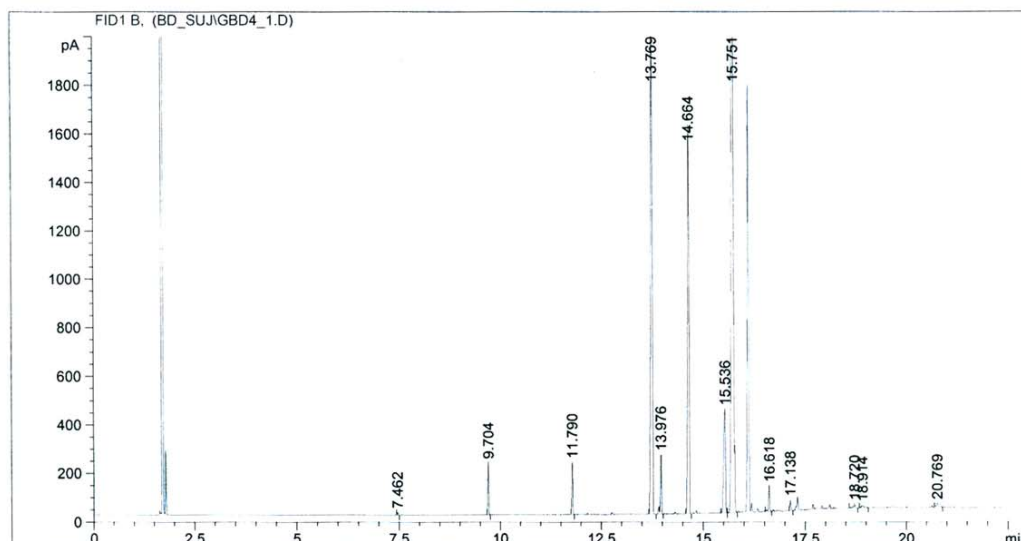
Appendix Figure C2 GC chromatogram of trap grease methyl ester in transesterification condition at 0.22 v/v (5:1) methanol to oil molar ratio, 0.5 % v/v catalyst concentration and 1.33 hour reaction time(experimental treatment no.2)



Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
1	5.192		0.0000	0.00000	0.00000	Methyl octanoate
2	7.462	BP	0.0235	21.88409	0.09630	Methyl decanoate
3	9.705	BP	0.0266	331.12903	1.45715	Methyl Laurate
4	11.790	BP	0.0247	303.89041	1.33729	Methyl myristate
5	13.764	BB	0.0448	4886.31152	21.50246	Methyl palmitate
6	13.975	VB	0.0254	349.95184	1.53998	Methyl palmitoleate
7	14.666	BB	0.0389	3981.52246	17.52089	Methyl heptadecanoate
8	15.530	PP	0.0477	1105.36707	4.86422	Methyl stearate
9	15.746	VV	0.0502	7967.77783	35.06261	Methyl oleate
10	16.115	BV	0.0369	3453.59985	15.19774	Methyl linoleate
11	16.620	VP	0.0286	164.60425	0.72435	Methyl linolenate
12	17.140	PP	0.0283	59.96614	0.26388	Methyl arachidate
13	18.726	BP	0.0409	27.15647	0.11950	Methyl behenate
14	18.952		0.0000	0.00000	0.00000	Methyl erucate
15	20.772	VB	0.0723	71.26775	0.31362	Methyl lignocerate

Totals : 2.27244e4

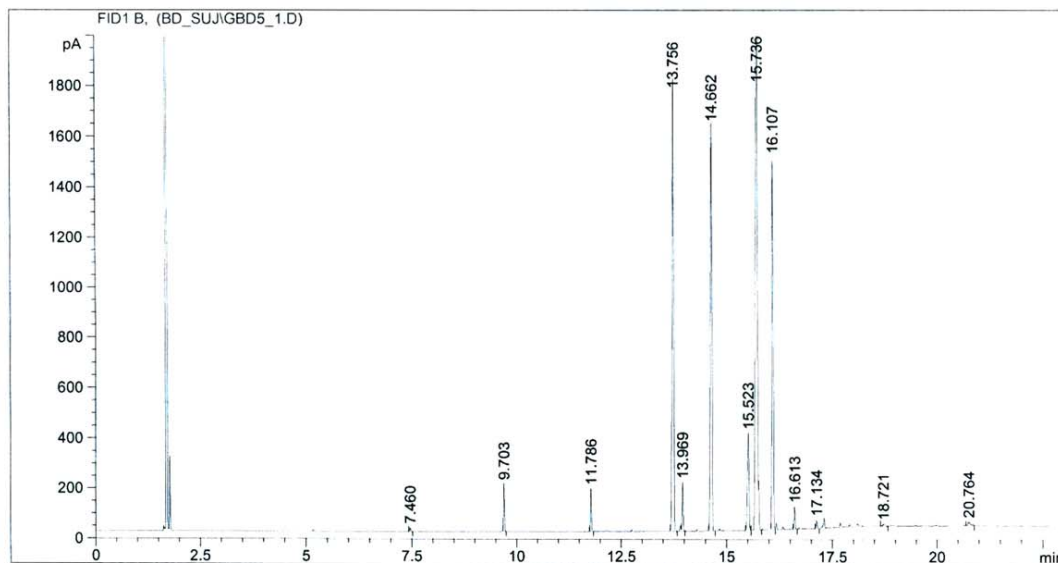
Appendix Figure C3 GC chromatogram of trap grease methyl ester in transesterification condition at 0.22 v/v (5:1) methanol to oil molar ratio, 1.5 % v/v catalyst concentration and 0.67 hour reaction time(experimental treatment no.3)



Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
1	5.192		0.0000	0.00000	0.00000	Methyl octanoate
2	7.462	BP	0.0235	14.84493	0.06677	Methyl decanoate
3	9.704	BB	0.0243	333.48349	1.49991	Methyl Laurate
4	11.790	BP	0.0251	349.36624	1.57135	Methyl myristate
5	13.769	BB	0.0435	5826.38086	26.20537	Methyl palmitate
6	13.976	VB	0.0259	414.59067	1.86471	Methyl palmitoleate
7	14.664	BV	0.0381	3917.55225	17.62001	Methyl heptadecanoate
8	15.536	PP	0.0528	1343.07520	6.04076	Methyl stearate
9	15.751	VV	0.0516	9578.16602	43.07981	Methyl oleate
10	16.078		0.0000	0.00000	0.00000	Methyl linoleate
11	16.618	VP	0.0302	198.54395	0.89299	Methyl linolenate
12	17.138	BP	0.0283	72.79429	0.32741	Methyl arachidate
13	18.720	BB	0.0452	49.06820	0.22069	Methyl behenate
14	18.914	PB	0.0719	48.68793	0.21898	Methyl erucate
15	20.769	VB	0.0747	86.98474	0.39123	Methyl lignocerate

Totals : 2.22335e4

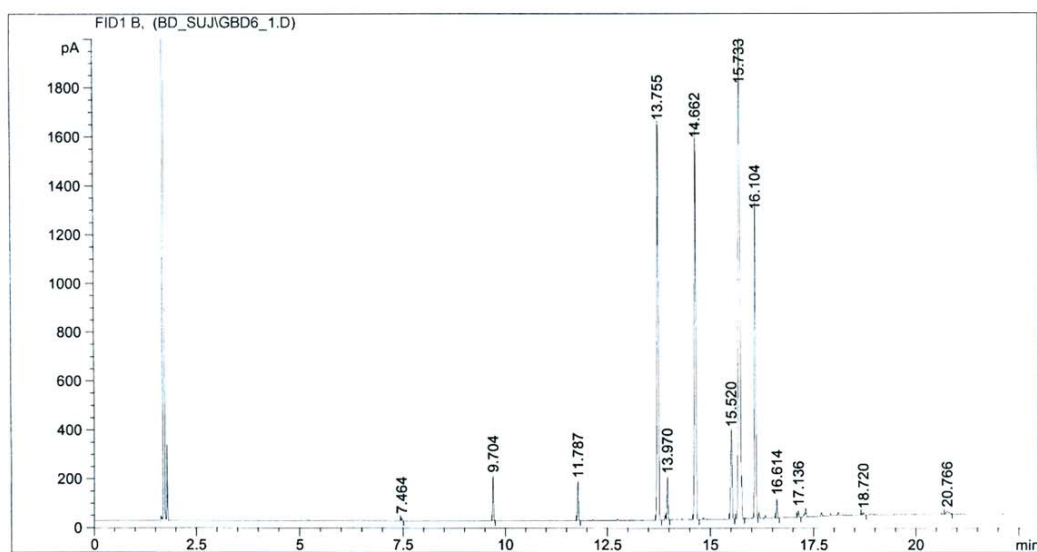
Appendix Figure C4 GC chromatogram of trap grease methyl ester in transesterification condition at 0.22 v/v (5:1) methanol to oil molar ratio, 1.5 % v/v catalyst concentration and 1.33 hour reaction time (experimental treatment no.4)



Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
1	5.192		0.0000	0.00000	0.00000	Methyl octanoate
2	7.460	PB	0.0261	20.34999	0.09550	Methyl decanoate
3	9.703	BP	0.0245	299.33759	1.40471	Methyl Laurate
4	11.786	BP	0.0273	277.95551	1.30437	Methyl myristate
5	13.756	BP	0.0362	4502.98047	21.13122	Methyl palmitate
6	13.969	VB	0.0254	320.00101	1.50167	Methyl palmitoleate
7	14.662	BB	0.0359	4116.99854	19.31992	Methyl heptadecanoate
8	15.523	PP	0.0448	1019.53424	4.78439	Methyl stearate
9	15.736	VV	0.0433	7295.92578	34.23773	Methyl oleate
10	16.107	BV	0.0329	3157.08521	14.81531	Methyl linoleate
11	16.613	VP	0.0279	151.29924	0.71000	Methyl linolenate
12	17.134	PP	0.0285	55.84293	0.26206	Methyl arachidate
13	18.721	BB	0.0468	25.34434	0.11893	Methyl behenate
14	18.952		0.0000	0.00000	0.00000	Methyl erucate
15	20.764	VB	0.0707	66.95291	0.31419	Methyl lignocerate

Totals : 2.13096e4

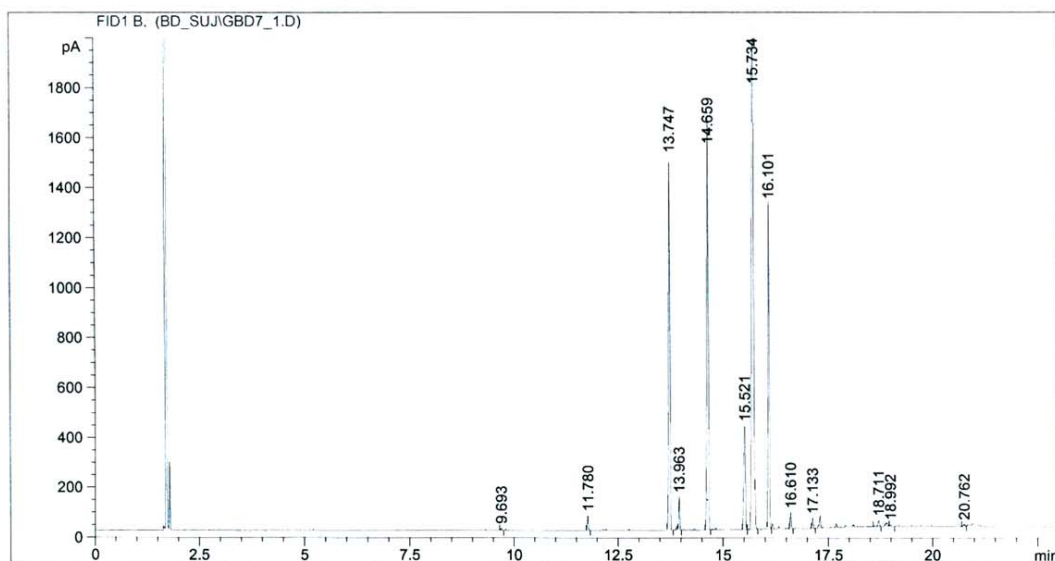
Appendix Figure C5 GC chromatogram of trap grease methyl ester in transesterification condition at 0.30 v/v (7:1) methanol to oil molar ratio, 0.5 % v/v catalyst concentration and 0.67 hour reaction time (experimental treatment no.5)



Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
1	5.192		0.0000	0.00000	0.00000	Methyl octanoate
2	7.464	BP	0.0234	17.66466	0.09219	Methyl decanoate
3	9.704	BP	0.0242	280.86142	1.46572	Methyl Laurate
4	11.787	BP	0.0272	257.12997	1.34188	Methyl myristate
5	13.755	BP	0.0371	4036.31274	21.06421	Methyl palmitate
6	13.970	VB	0.0255	287.53851	1.50057	Methyl palmitoleate
7	14.662	BP	0.0397	3924.74609	20.48198	Methyl heptadecanoate
8	15.520	BP	0.0394	901.18085	4.70297	Methyl stearate
9	15.733	BV	0.0488	6435.41553	33.58434	Methyl oleate
10	16.104	BV	0.0339	2760.77686	14.40760	Methyl linoleate
11	16.614	VP	0.0282	130.56111	0.68136	Methyl linolenate
12	17.136	PP	0.0284	48.02074	0.25060	Methyl arachidate
13	18.720	BP	0.0393	21.43973	0.11189	Methyl behenate
14	18.952		0.0000	0.00000	0.00000	Methyl erucate
15	20.766	VB	0.0652	60.30232	0.31470	Methyl lignocerate

Totals : 1.91620e4

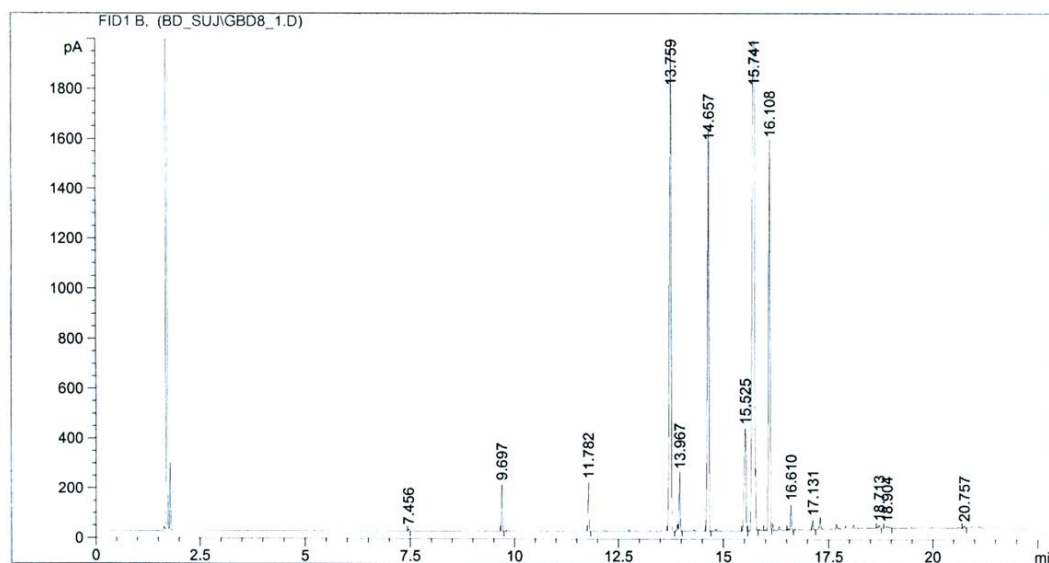
Appendix Figure C6 GC chromatogram of trap grease methyl ester in transesterification condition at 0.30 v/v (7:1) methanol to oil molar ratio, 0.5 % v/v catalyst concentration and 1.33 hour reaction time (experimental treatment no.6)



Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
1	5.192		0.0000	0.00000	0.00000	Methyl octanoate
2	7.470		0.0000	0.00000	0.00000	Methyl decanoate
3	9.693	PP	0.0265	14.97939	0.07779	Methyl Laurate
4	11.780	BP	0.0270	95.58972	0.49641	Methyl myristate
5	13.747	BP	0.0370	3446.77856	17.89969	Methyl palmitate
6	13.963	VB	0.0256	220.99167	1.14765	Methyl palmitoleate
7	14.659	BV	0.0407	4016.60425	20.85889	Methyl heptadecanoate
8	15.521	BP	0.0428	1099.82947	5.71160	Methyl stearate
9	15.734	VV	0.0451	7352.41650	38.18232	Methyl oleate
10	16.101	BV	0.0322	2704.63208	14.04560	Methyl linoleate
11	16.610	VP	0.0263	112.18228	0.58258	Methyl linolenate
12	17.133	BP	0.0283	69.80590	0.36251	Methyl arachidate
13	18.711	BV	0.0441	74.14905	0.38507	Methyl behenate
14	18.992	VB	0.0394	20.82341	0.10814	Methyl erucate
15	20.762	BV	0.0479	27.29359	0.14174	Methyl lignocerate

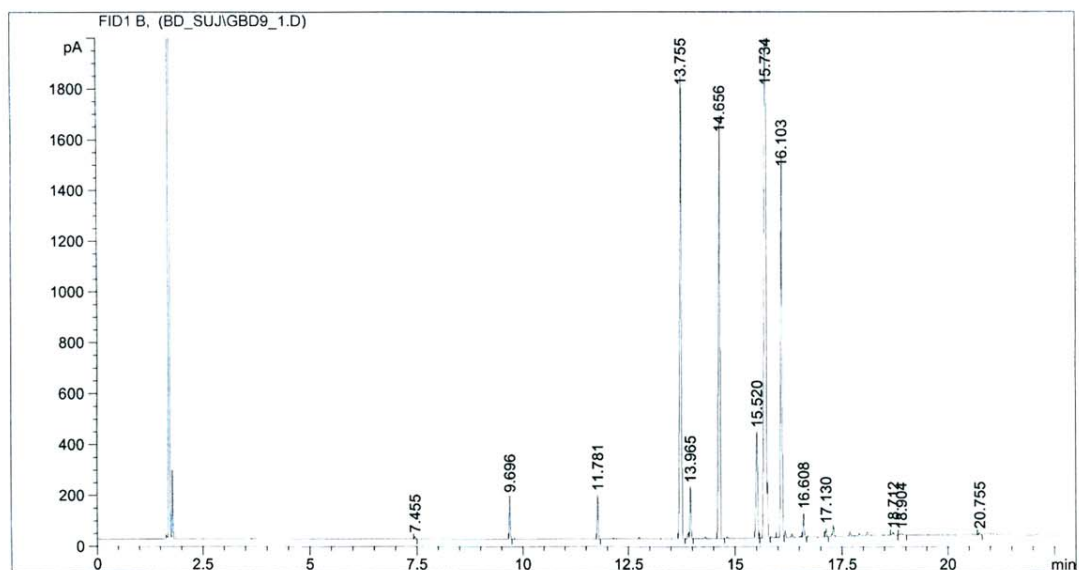
Totals : 1.92561e4

Appendix Figure C7 GC chromatogram of trap grease methyl ester in transesterification condition at 0.30 v/v (7:1) methanol to oil molar ratio, 1.5 % v/v catalyst concentration and 0.67 hour reaction time (experimental treatment no.7)



Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
1	5.192		0.0000	0.00000	0.00000	Methyl octanoate
2	7.456	BB	0.0234	12.89162	0.05249	Methyl decanoate
3	9.697	BP	0.0242	294.17340	1.19781	Methyl Laurate
4	11.782	BP	0.0249	317.99597	1.29481	Methyl myristate
5	13.759	BB	0.0455	5370.02295	21.86553	Methyl palmitate
6	13.967	VB	0.0271	382.30228	1.55665	Methyl palmitoleate
7	14.657	BV	0.0379	3958.03247	16.11622	Methyl heptadecanoate
8	15.525	PP	0.0512	1236.64746	5.03535	Methyl stearate
9	15.741	VV	0.0442	8864.17969	36.09295	Methyl oleate
10	16.108	BV	0.0362	3764.72241	15.32910	Methyl linoleate
11	16.610	VP	0.0273	187.62370	0.76396	Methyl linolenate
12	17.131	BP	0.0282	67.72202	0.27575	Methyl arachidate
13	18.713	BP	0.0437	34.34303	0.13984	Methyl behenate
14	18.904	VB	0.0748	39.99883	0.16287	Methyl erucate
15	20.757	BV	0.0481	28.65693	0.11668	Methyl lignocerate
Totals :				2.45593e4		

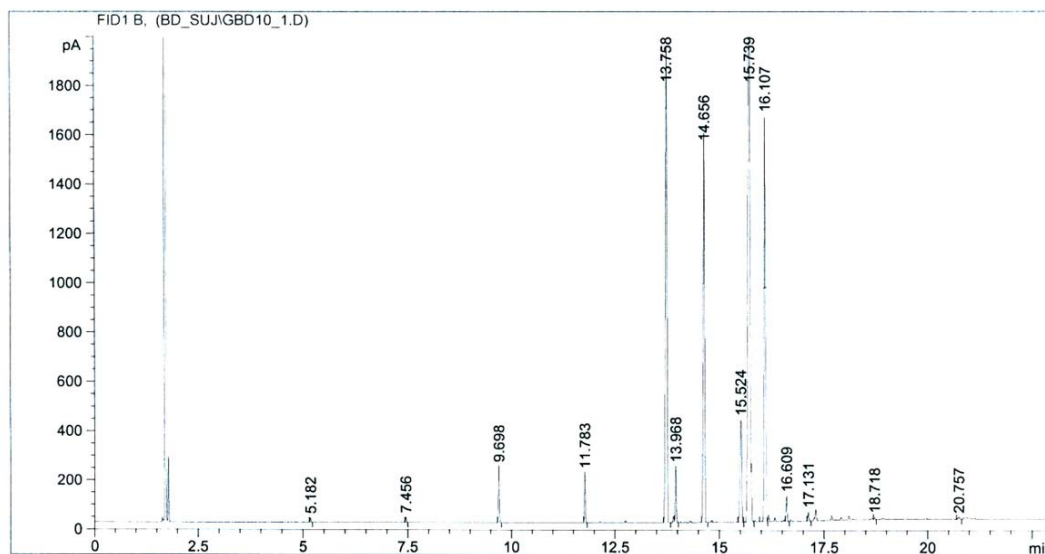
Appendix Figure C8 GC chromatogram of trap grease methyl ester in transesterification condition at 0.30 v/v (7:1) methanol to oil molar ratio, 1.5 % v/v catalyst concentration and 1.33 hour reaction time (experimental treatment no.8)



Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
1	5.192		0.0000	0.00000	0.00000	Methyl octanoate
2	7.455	BP	0.0237	12.21845	0.05452	Methyl decanoate
3	9.696	BB	0.0243	261.98929	1.16900	Methyl Laurate
4	11.781	BP	0.0273	284.33441	1.26871	Methyl myristate
5	13.755	BB	0.0395	4810.43506	21.46430	Methyl palmitate
6	13.965	VB	0.0277	340.50018	1.51932	Methyl palmitoleate
7	14.656	BB	0.0356	4004.38354	17.86767	Methyl heptadecanoate
8	15.520	BP	0.0409	1103.30725	4.92299	Methyl stearate
9	15.734	VV	0.0437	7893.60986	35.22151	Methyl oleate
10	16.103	BV	0.0374	3388.12256	15.11790	Methyl linoleate
11	16.608	VP	0.0279	161.29509	0.71970	Methyl linolenate
12	17.130	BP	0.0265	59.77165	0.26670	Methyl arachidate
13	18.712	BB	0.0473	31.46943	0.14042	Methyl behenate
14	18.904	BB	0.0724	34.70971	0.15488	Methyl erucate
15	20.755	BV	0.0518	25.18746	0.11239	Methyl lignocerate

Totals : 2.24113e4

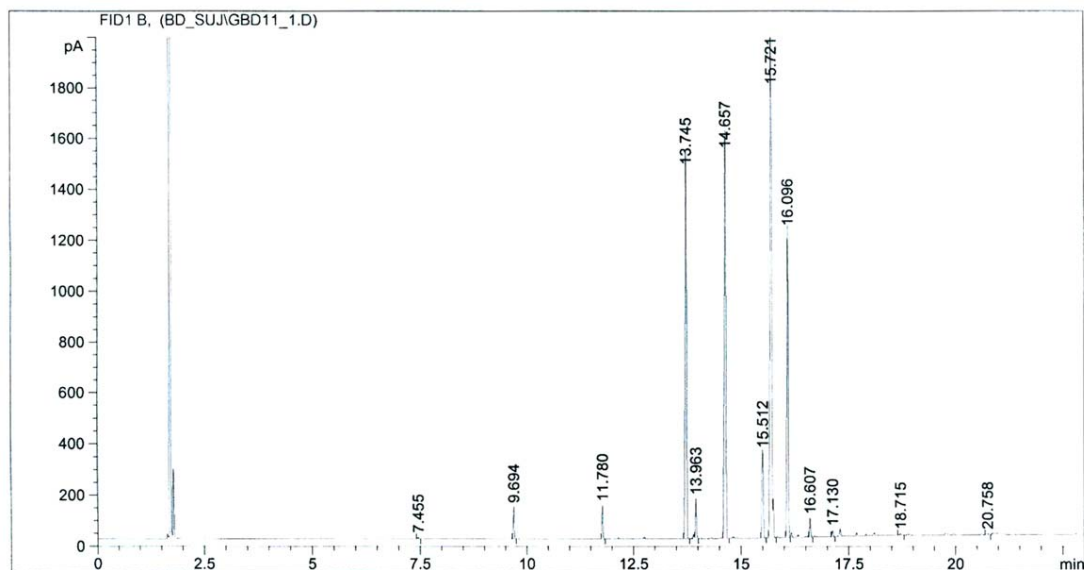
Appendix Figure C9 GC chromatogram of trap grease methyl ester in transesterification condition at 0.19 v/v (5:1) methanol to oil molar ratio, 1 % v/v catalyst concentration and 1 hour reaction time (experimental treatment no.9)



Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
1	5.182	BB	0.0231	24.44839	0.10262	Methyl octanoate
2	7.456	BB	0.0231	31.96153	0.13415	Methyl decanoate
3	9.698	BP	0.0267	367.69351	1.54329	Methyl Laurate
4	11.783	BP	0.0247	326.83240	1.37179	Methyl myristate
5	13.758	BB	0.0412	5238.44727	21.98697	Methyl palmitate
6	13.968	VB	0.0270	370.74792	1.55611	Methyl palmitoleate
7	14.656	BB	0.0355	3865.99536	16.22647	Methyl heptadecanoate
8	15.524	PV	0.0469	1175.13965	4.93233	Methyl stearate
9	15.739	VV	0.0478	8441.45801	35.43074	Methyl oleate
10	16.107	BV	0.0343	3694.85815	15.50817	Methyl linoleate
11	16.609	VP	0.0285	178.81262	0.75052	Methyl linolenate
12	17.131	BP	0.0285	63.53138	0.26666	Methyl arachidate
13	18.718	VP	0.0321	17.63744	0.07403	Methyl behenate
14	18.952		0.0000	0.00000	0.00000	Methyl erucate
15	20.757	BV	0.0498	27.67587	0.11616	Methyl lignocerate

Totals : 2.38252e4

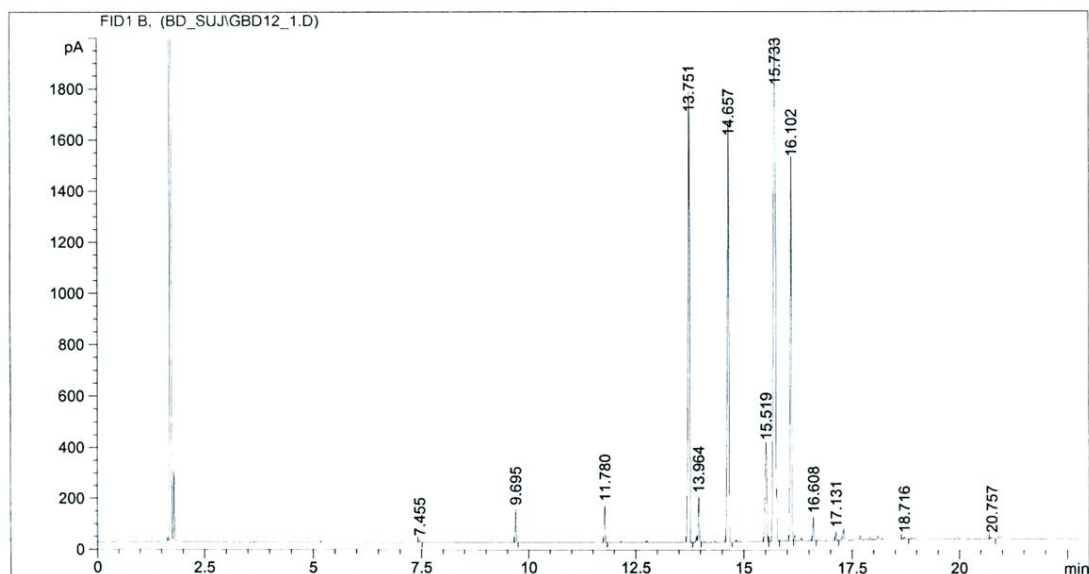
Appendix Figure C10 GC chromatogram of trap grease methyl ester in transesterification condition at 0.33 v/v (7:1) methanol to oil molar ratio, 1 % v/v catalyst concentration and 1 hour reaction time (experimental treatment no.10)



Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
1	5.192		0.0000	0.00000	0.00000	Methyl octanoate
2	7.455	PB	0.0257	10.36147	0.05923	Methyl decanoate
3	9.694	BP	0.0265	196.64030	1.12399	Methyl Laurate
4	11.780	BP	0.0268	208.82646	1.19365	Methyl myristate
5	13.745	BP	0.0362	3500.05835	20.00625	Methyl palmitate
6	13.963	VB	0.0254	255.53922	1.46066	Methyl palmitoleate
7	14.657	BB	0.0388	3987.60742	22.79307	Methyl heptadecanoate
8	15.512	BP	0.0384	812.12482	4.64209	Methyl stearate
9	15.721	BV	0.0404	5769.05029	32.97575	Methyl oleate
10	16.096	BV	0.0327	2550.73999	14.57997	Methyl linoleate
11	16.607	VP	0.0281	127.13538	0.72670	Methyl linolenate
12	17.130	BP	0.0260	41.90630	0.23954	Methyl arachidate
13	18.715	BB	0.0462	18.02454	0.10303	Methyl behenate
14	18.952		0.0000	0.00000	0.00000	Methyl erucate
15	20.758	BV	0.0480	16.81247	0.09610	Methyl lignocerate

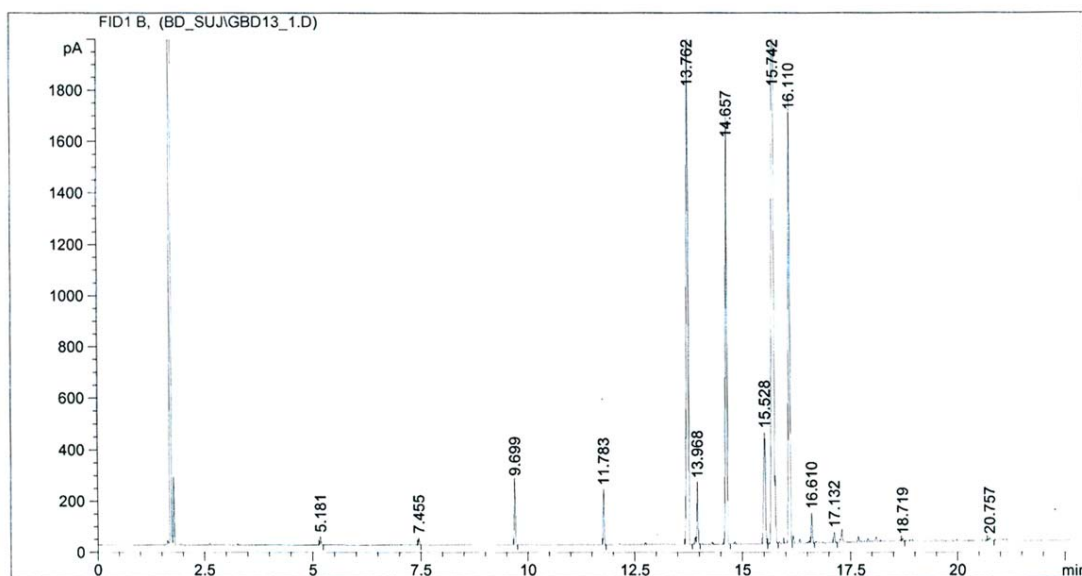
Totals : 1.74948e4

Appendix Figure C11 GC chromatogram of trap grease methyl ester in transesterification condition at 0.26 v/v (6:1) methanol to oil molar ratio, 0.16 % v/v catalyst concentration and 1 hour reaction time (experimental treatment no.11)



Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
1	5.192		0.0000	0.00000	0.00000	Methyl octanoate
2	7.455	PB	0.0260	11.72903	0.05562	Methyl decanoate
3	9.695	BP	0.0260	200.94275	0.95283	Methyl Laurate
4	11.780	BP	0.0268	233.38284	1.10666	Methyl myristate
5	13.751	BP	0.0384	4323.01514	20.49896	Methyl palmitate
6	13.964	VB	0.0261	304.21033	1.44251	Methyl palmitoleate
7	14.657	BB	0.0377	3979.75342	18.87127	Methyl heptadecanoate
8	15.519	BP	0.0438	1042.74817	4.94452	Methyl stearate
9	15.733	VV	0.0412	7482.41699	35.48028	Methyl oleate
10	16.102	BV	0.0315	3243.45581	15.37988	Methyl linoleate
11	16.608	VP	0.0279	156.49931	0.74209	Methyl linolenate
12	17.131	BP	0.0280	58.39598	0.27690	Methyl arachidate
13	18.716	BB	0.0481	26.51013	0.12571	Methyl behenate
14	18.952		0.0000	0.00000	0.00000	Methyl erucate
15	20.757	BV	0.0503	25.88875	0.12276	Methyl lignocerate
Totals :				2.10889e4		

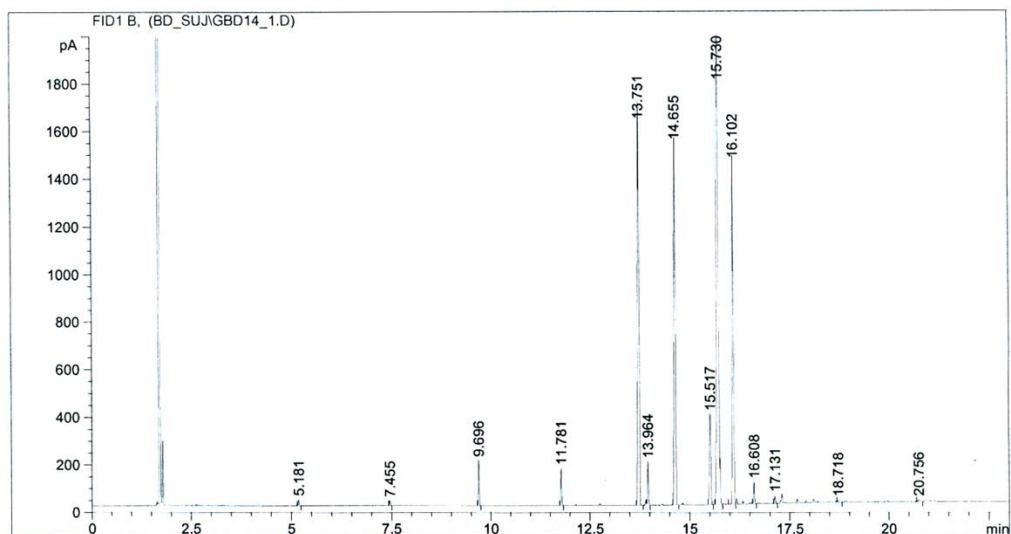
Appendix Figure C12 GC chromatogram of trap grease methyl ester in transesterification condition at 0.26 v/v (6:1) methanol to oil molar ratio, 1.84 % v/v catalyst concentration and 1 hour reaction time (experimental treatment no.12)



Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
1	5.181	BB	0.0251	46.07547	0.17999	Methyl octanoate
2	7.455	BB	0.0235	42.27747	0.16516	Methyl decanoate
3	9.699	BP	0.0273	418.91074	1.63646	Methyl Laurate
4	11.783	BP	0.0253	356.25867	1.39171	Methyl myristate
5	13.762	BB	0.0408	5647.67676	22.06240	Methyl palmitate
6	13.968	VB	0.0275	404.36966	1.57965	Methyl palmitoleate
7	14.657	BB	I 0.0369	3867.98437	15.11011	Methyl heptadecanoate
8	15.528	BP	0.0463	1274.08557	4.97716	Methyl stearate
9	15.742	VV	0.0449	9173.77344	35.83694	Methyl oleate
10	16.110	BV	0.0383	4050.07275	15.82143	Methyl linoleate
11	16.610	VB	0.0260	198.22227	0.77435	Methyl linolenate
12	17.132	BP	0.0280	69.35078	0.27092	Methyl arachidate
13	18.719	VP	0.0345	17.74753	0.06933	Methyl behenate
14	18.952		0.0000	0.00000	0.00000	Methyl erucate
15	20.757	BB	0.0516	31.84397	0.12440	Methyl lignocerate

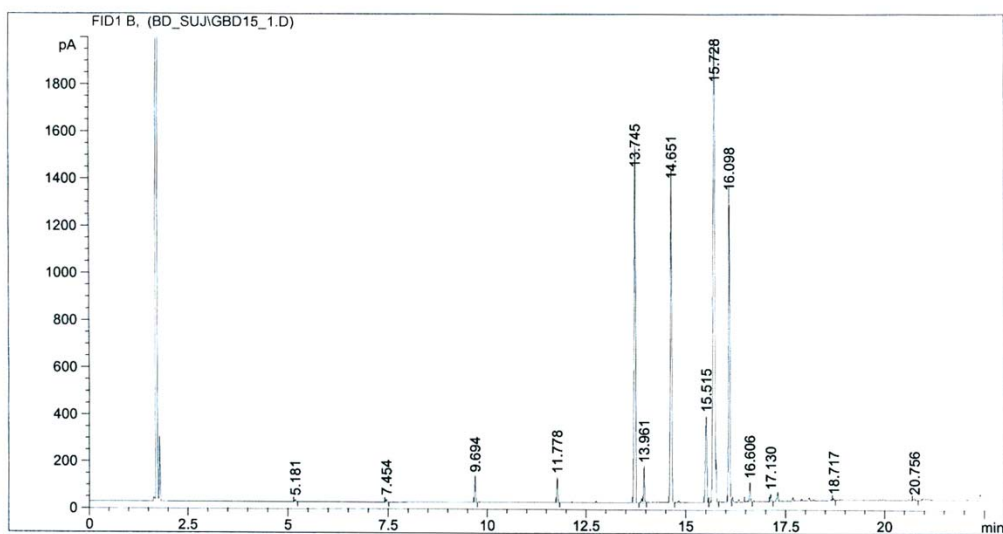
Totals : 2.55986e4

Appendix Figure C13 GC chromatogram of trap grease methyl ester in transesterification condition at 0.26 v/v (6:1) methanol to oil molar ratio, 1 % v/v catalyst concentration and 0.45 hour reaction time (experimental treatment no.13)



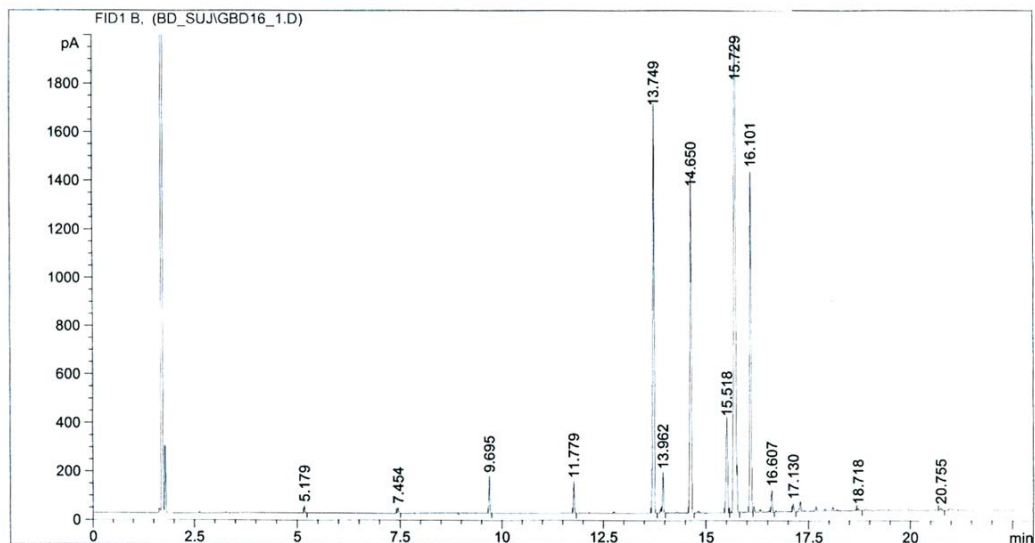
Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
1	5.181	BB	0.0249	34.50816	0.16725	Methyl octanoate
2	7.455	BP	0.0237	30.52970	0.14797	Methyl decanoate
3	9.696	BP	0.0238	287.75662	1.39464	Methyl Laurate
4	11.781	BP	0.0274	255.43399	1.23799	Methyl myristate
5	13.751	BB	0.0433	4295.10303	20.81664	Methyl palmitate
6	13.964	VB	0.0256	303.92310	1.47299	Methyl palmitoleate
7	14.655	I	0.0379	3883.36182	18.82110	Methyl heptadecanoate
8	15.517	BB	0.0407	993.25983	4.81393	Methyl stearate
9	15.730	BV	0.0450	7146.67676	34.63708	Methyl oleate
10	16.102	BV	0.0337	3153.55981	15.28404	Methyl linoleate
11	16.608	VP	0.0280	154.01698	0.74646	Methyl linolenate
12	17.131	BP	0.0283	54.04859	0.26195	Methyl arachidate
13	18.718	VB	0.0346	16.07059	0.07789	Methyl behenate
14	18.952		0.0000	0.00000	0.00000	Methyl erucate
15	20.756	BB	0.0519	24.77586	0.12008	Methyl lignocerate
Totals :				2.06330e4		

Appendix Figure C14 GC chromatogram of trap grease methyl ester in transesterification condition at 0.26 v/v (6:1) methanol to oil molar ratio, 1 % v/v catalyst concentration and 1.55 hour reaction time (experimental treatment no.14)



Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
1	5.181	PB	0.0268	13.83861	0.07505	Methyl octanoate
2	7.454	PP	0.0259	18.99342	0.10301	Methyl decanoate
3	9.694	BP	0.0264	176.11890	0.95517	Methyl Laurate
4	11.778	BP	0.0270	173.78050	0.94249	Methyl myristate
5	13.745	BB	0.0422	3630.95630	19.69224	Methyl palmitate
6	13.961	VB	0.0273	250.97354	1.36114	Methyl palmitoleate
7	14.651	BP	0.0384	3301.49414	17.90542	Methyl heptadecanoate
8	15.515	BP	0.0423	953.44385	5.17094	Methyl stearate
9	15.728	VV	0.0447	6755.63281	36.63870	Methyl oleate
10	16.098	BV	0.0341	2912.70410	15.79685	Methyl linoleate
11	16.606	BP	0.0299	154.21330	0.83637	Methyl linolenate
12	17.130	BP	0.0261	54.78144	0.29710	Methyl arachidate
13	18.717	VV	0.0362	17.17698	0.09316	Methyl behenate
14	18.952		0.0000	0.00000	0.00000	Methyl erucate
15	20.756	BB	0.0511	24.40863	0.13238	Methyl lignocerate
Totals :				1.84385e4		

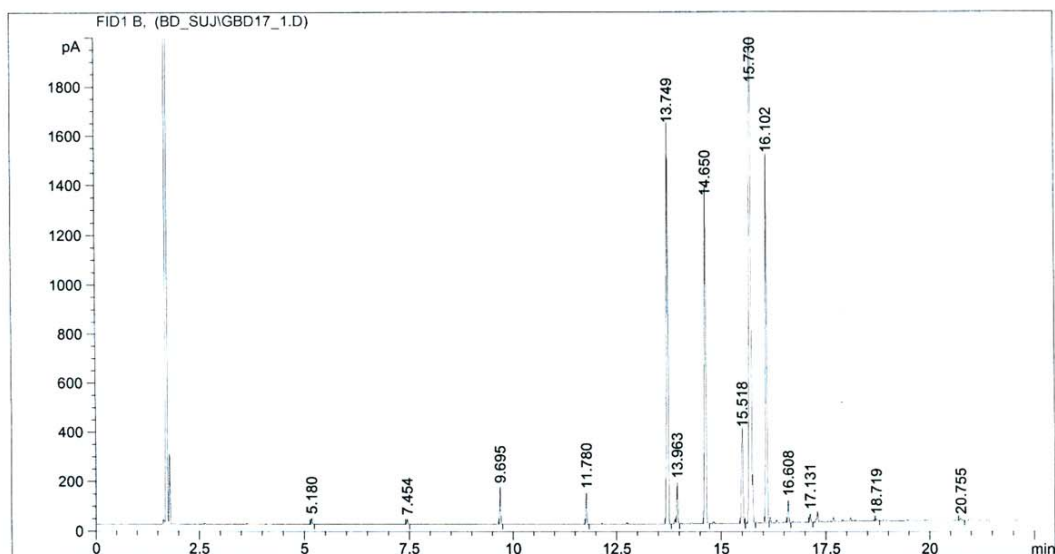
Appendix Figure C15 GC chromatogram of trap grease methyl ester in transesterification condition at 0.26 v/v (6:1) methanol to oil molar ratio, 1 % v/v catalyst concentration and 1 hour reaction time (experimental treatment no.15)



Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
1	5.179	BB	0.0249	41.32462	0.20964	Methyl octanoate
2	7.454	BP	0.0256	32.83493	0.16657	Methyl decanoate
3	9.695	BP	0.0264	238.29471	1.20885	Methyl Laurate
4	11.779	BP	0.0271	207.23149	1.05127	Methyl myristate
5	13.749	BP	0.0340	3984.83887	20.21472	Methyl palmitate
6	13.962	VB	0.0279	278.01285	1.41033	Methyl palmitoleate
7	14.650	BP	0.0363	3225.82886	16.36433	Methyl heptadecanoate
8	15.518	BP	0.0434	1019.03052	5.16945	Methyl stearate
9	15.729	VV	0.0500	7252.53516	36.79143	Methyl oleate
10	16.101	BV	0.0343	3170.37427	16.08301	Methyl linoleate
11	16.607	VP	0.0284	155.60811	0.78939	Methyl linolenate
12	17.130	BP	0.0286	59.18933	0.30026	Methyl arachidate
13	18.718	VB	0.0386	19.15267	0.09716	Methyl behenate
14	18.952		0.0000	0.00000	0.00000	Methyl erucate
15	20.755	BB	0.0497	28.30802	0.14360	Methyl lignocerate

Totals : 1.97126e4

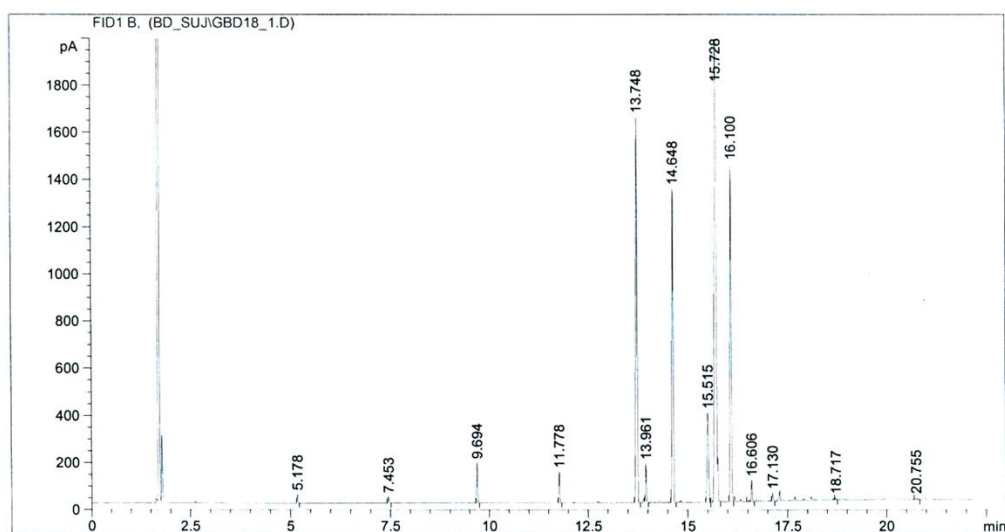
Appendix Figure C16 GC chromatogram of trap grease methyl ester in transesterification condition at 0.26 v/v (6:1) methanol to oil molar ratio, 1 % v/v catalyst concentration and 1 hour reaction time (experimental treatment no.16)



Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
1	5.180	BB	0.0253	34.72917	0.17630	Methyl octanoate
2	7.454	BP	0.0259	30.98742	0.15731	Methyl decanoate
3	9.695	BP	0.0267	237.60472	1.20619	Methyl Laurate
4	11.780	BP	0.0275	208.48209	1.05835	Methyl myristate
5	13.749	BP	0.0391	3999.01440	20.30088	Methyl palmitate
6	13.963	VB	0.0257	278.45477	1.41357	Methyl palmitoleate
7	14.650	BP	0.0385	3185.34375	16.17030	Methyl heptadecanoate
8	15.518	BP	0.0442	1022.71881	5.19180	Methyl stearate
9	15.730	VV	0.0496	7268.75293	36.89961	Methyl oleate
10	16.102	BV	0.0331	3170.94336	16.09720	Methyl linoleate
11	16.608	VP	0.0283	155.31253	0.78844	Methyl linolenate
12	17.131	BP	0.0282	59.55196	0.30231	Methyl arachidate
13	18.719	VB	0.0343	17.73702	0.09004	Methyl behenate
14	18.952		0.0000	0.00000	0.00000	Methyl erucate
15	20.755	BB	0.0498	29.09335	0.14769	Methyl lignocerate

Totals : 1.96987e4

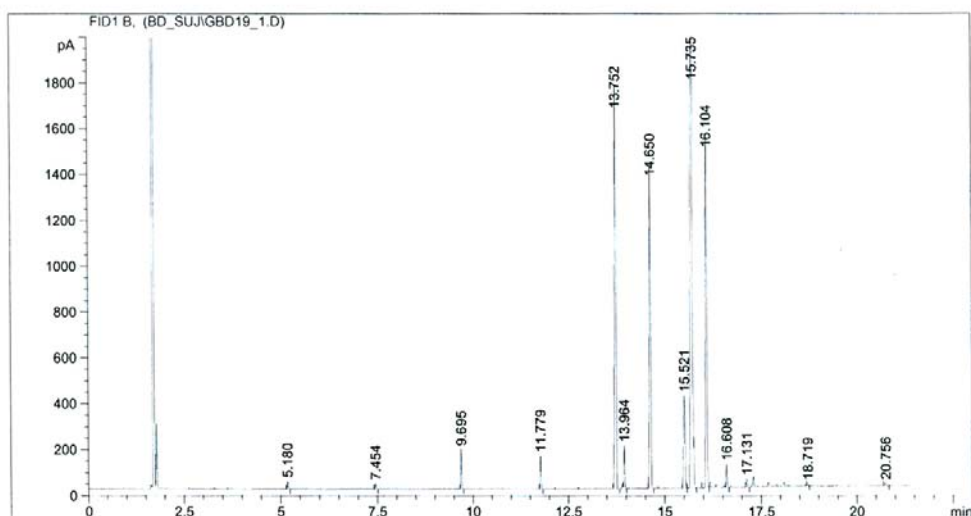
Appendix Figure C17 GC chromatogram of trap grease methyl ester in transesterification condition at 0.26 v/v (6:1) methanol to oil molar ratio, 1 % v/v catalyst concentration and 1 hour reaction time (experimental treatment no.17)



Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
1	5.178	BB	0.0227	52.55765	0.27013	Methyl octanoate
2	7.453	BP	0.0258	38.37978	0.19726	Methyl decanoate
3	9.694	BP	0.0262	266.09329	1.36765	Methyl Laurate
4	11.778	BP	0.0270	215.41249	1.10717	Methyl myristate
5	13.748	BP	0.0364	3981.80566	20.46548	Methyl palmitate
6	13.961	VB	0.0276	278.52887	1.43157	Methyl palmitoleate
7	14.648	BP	0.0346	3026.34839	15.55467	Methyl heptadecanoate
8	15.515	BP	0.0429	1007.30017	5.17727	Methyl stearate
9	15.728	VV	0.0438	7178.02588	36.89325	Methyl oleate
10	16.100	BV	0.0336	3141.45239	16.14628	Methyl linoleate
11	16.606	BP	0.0297	167.14685	0.85909	Methyl linolenate
12	17.130	BP	0.0262	58.46627	0.30050	Methyl arachidate
13	18.717	VP	0.0331	15.92647	0.08186	Methyl behenate
14	18.952		0.0000	0.00000	0.00000	Methyl erucate
15	20.755	BB	0.0492	28.76075	0.14782	Methyl lignocerate

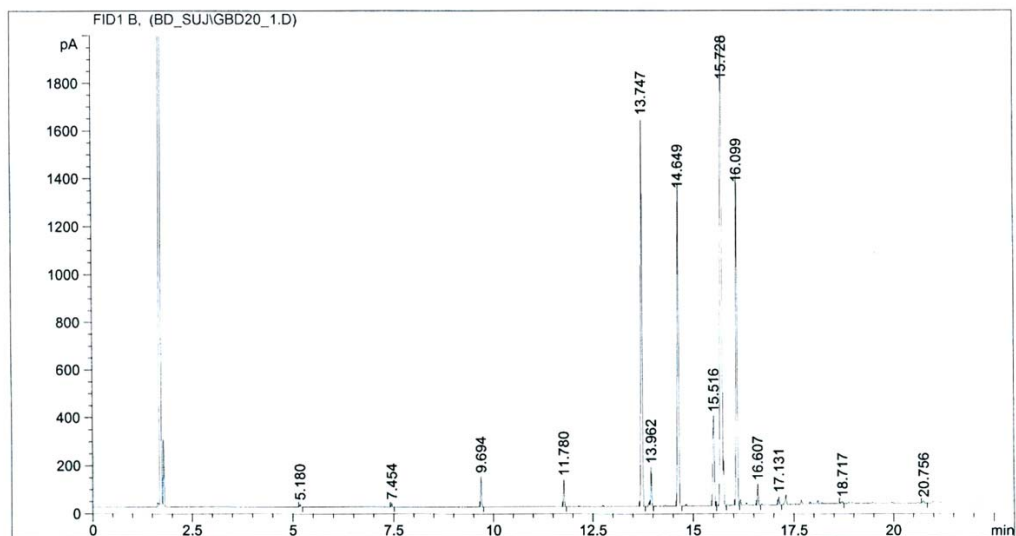
Totals : 1.94562e4

Appendix Figure C18 GC chromatogram of trap grease methyl ester in transesterification condition at 0.26 v/v (6:1) methanol to oil molar ratio, 1 % v/v catalyst concentration and 1 hour reaction time (experimental treatment no.18)



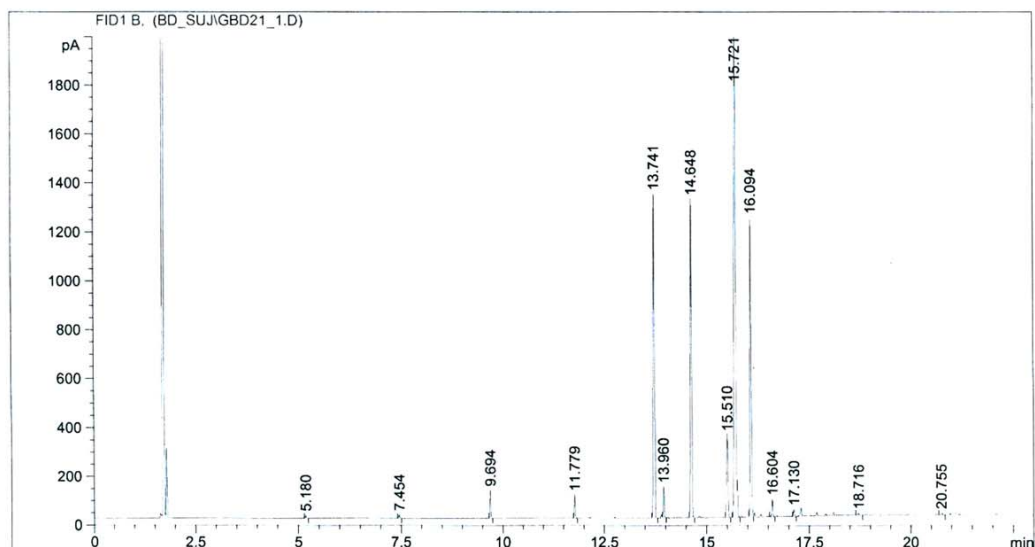
Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
1	5.180	BB	0.0251	47.50721	0.22263	Methyl octanoate
2	7.454	BB	0.0259	36.57838	0.17142	Methyl decanoate
3	9.695	BP	0.0264	270.90372	1.26954	Methyl Laurate
4	11.779	BP	0.0273	235.39157	1.10312	Methyl myristate
5	13.752	BP	0.0394	4416.65869	20.69784	Methyl palmitate
6	13.964	VB	0.0253	308.26318	1.44462	Methyl palmitoleate
7	14.650	BP	0.0358	3196.04614	14.97767	Methyl heptadecanoate
8	15.521	BP	0.0443	1113.46765	5.21806	Methyl stearate
9	15.735	VV	0.0451	7938.94775	37.20438	Methyl oleate
10	16.104	BV	0.0378	3491.52954	16.36240	Methyl linoleate
11	16.608	VP	0.0280	170.77174	0.80029	Methyl linolenate
12	17.131	BP	0.0288	64.09798	0.30038	Methyl arachidate
13	18.719	VV	0.0319	17.63133	0.08263	Methyl behenate
14	18.952		0.0000	0.00000	0.00000	Methyl erucate
15	20.756	BB	0.0493	30.94875	0.14504	Methyl lignocerate
Totals :				2.13387e4		

Appendix Figure C19 GC chromatogram of trap grease methyl ester in transesterification condition at 0.26 v/v (6:1) methanol to oil molar ratio, 1 % v/v catalyst concentration and 1 hour reaction time (experimental treatment no.19)



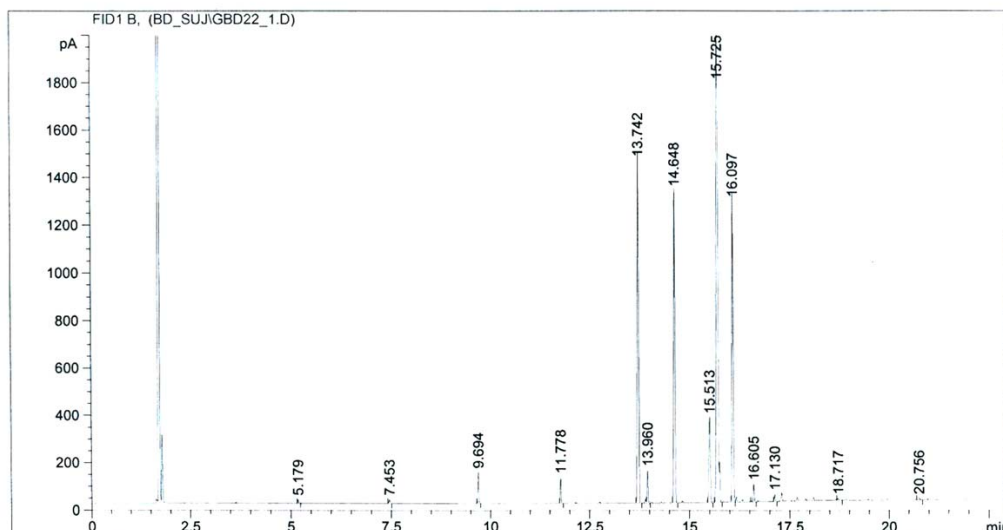
Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
1	5.180	PB	0.0254	15.69805	0.08378	Methyl octanoate
2	7.454	PP	0.0258	21.19086	0.11309	Methyl decanoate
3	9.694	BP	0.0264	195.73399	1.04460	Methyl Laurate
4	11.780	BP	0.0275	185.01271	0.98739	Methyl myristate
5	13.747	BP	0.0330	3738.52710	19.95198	Methyl palmitate
6	13.962	VB	0.0249	258.77271	1.38103	Methyl palmitoleate
7	14.649	BP	0.0359	3133.31909	16.72207	Methyl heptadecanoate
8	15.516	BP	0.0429	982.82062	5.24517	Methyl stearate
9	15.728	VV	0.0498	6945.66650	37.06802	Methyl oleate
10	16.099	BV	0.0344	3005.46753	16.03975	Methyl linoleate
11	16.607	VP	0.0280	146.61104	0.78244	Methyl linolenate
12	17.131	BP	0.0284	57.71017	0.30799	Methyl arachidate
13	18.717	BP	0.0424	22.63609	0.12081	Methyl behenate
14	18.952		0.0000	0.00000	0.00000	Methyl erucate
15	20.756	BB	0.0525	28.45737	0.15187	Methyl lignocerate
Totals :				1.87376e4		

Appendix Figure C20 GC chromatogram of trap grease methyl ester in transesterification condition at 0.26 v/v (6:1) methanol to oil molar ratio, 1 % v/v catalyst concentration and 1 hour reaction time (experimental treatment no.20)



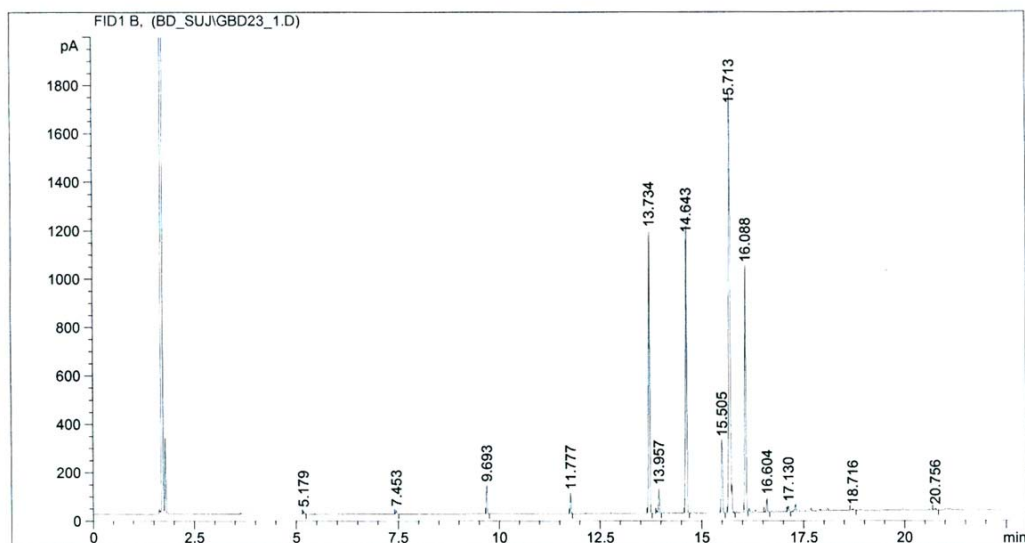
Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
1	5.180	PB	0.0250	15.85378	0.09988	Methyl octanoate
2	7.454	PB	0.0254	20.61012	0.12984	Methyl decanoate
3	9.694	BP	0.0262	178.53432	1.12475	Methyl Laurate
4	11.779	BP	0.0270	153.49858	0.96703	Methyl myristate
5	13.741	BP	0.0370	3055.49121	19.24938	Methyl palmitate
6	13.960	VB	0.0275	211.82556	1.33449	Methyl palmitoleate
7	14.648	BP	I 0.0345	2950.88135	18.59035	Methyl heptadecanoate
8	15.510	BP	0.0382	816.06476	5.14115	Methyl stearate
9	15.721	BV	0.0411	5758.79346	36.28000	Methyl oleate
10	16.094	BV	0.0318	2492.07886	15.69992	Methyl linoleate
11	16.604	BB	0.0288	126.99506	0.80006	Methyl linolenate
12	17.130	BP	0.0266	48.60553	0.30621	Methyl arachidate
13	18.716	BB	0.0442	20.27891	0.12776	Methyl behenate
14	18.952		0.0000	0.00000	0.00000	Methyl erucate
15	20.755	BB	0.0500	23.67849	0.14917	Methyl lignocerate
Totals :				1.58732e4		

Appendix Figure C21 GC chromatogram of trap grease methyl ester in transesterification condition at 0.26 v/v (6:1) methanol to oil molar ratio, 1 % v/v catalyst concentration and 1 hour reaction time (experimental treatment no.21)



Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
1	5.179	BB	0.0251	22.52194	0.12986	Methyl octanoate
2	7.453	BP	0.0260	25.21039	0.14536	Methyl decanoate
3	9.694	BP	0.0262	202.92314	1.17007	Methyl Laurate
4	11.778	BP	0.0268	173.14720	0.99838	Methyl myristate
5	13.742	BP	0.0357	3416.87256	19.70186	Methyl palmitate
6	13.960	VB	0.0283	237.76711	1.37098	Methyl palmitoleate
7	14.648	BP	0.0348	3017.36597	17.39829	Methyl heptadecanoate
8	15.513	BP	0.0393	897.49695	5.17501	Methyl stearate
9	15.725	BV	0.0445	6361.15283	36.67874	Methyl oleate
10	16.097	BV	0.0354	2752.18384	15.86923	Methyl linoleate
11	16.605	BP	0.0300	140.95674	0.81276	Methyl linolenate
12	17.130	BP	0.0263	52.71521	0.30396	Methyl arachidate
13	18.717	VP	0.0384	17.33486	0.09995	Methyl behenate
14	18.952		0.0000	0.00000	0.00000	Methyl erucate
15	20.756	BB	0.0496	25.24173	0.14555	Methyl lignocerate
Totals :				1.73429e4		

Appendix Figure C22 GC chromatogram of trap grease methyl ester in transesterification condition at 0.26 v/v (6:1) methanol to oil molar ratio, 1 % v/v catalyst concentration and 1 hour reaction time (experimental treatment no.22)

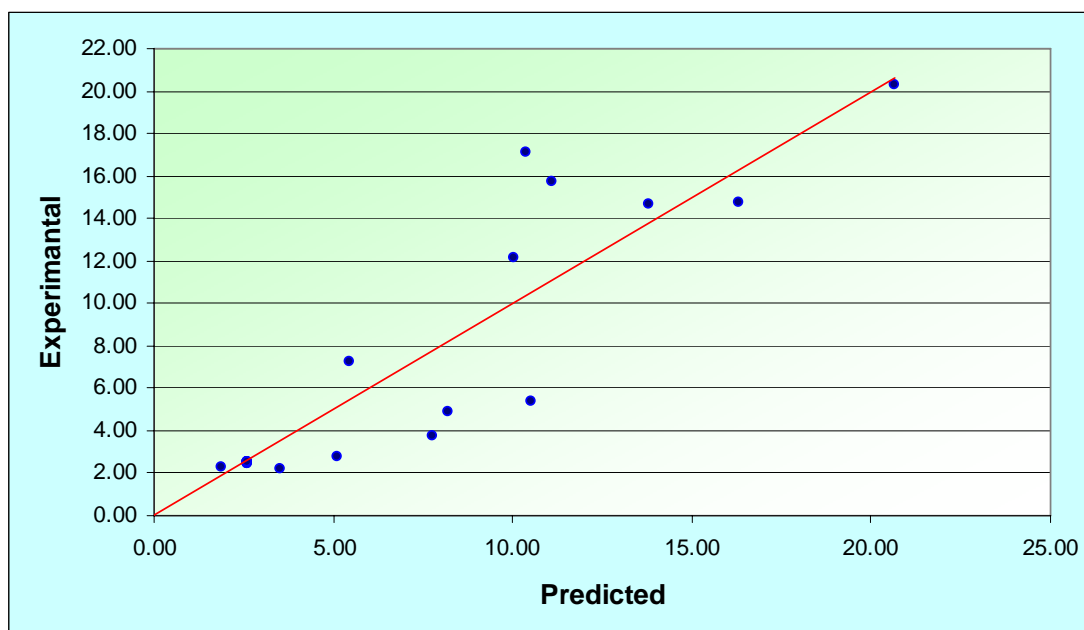


Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
1	5.179	BB	0.0249	17.23981	0.13250	Methyl octanoate
2	7.453	BP	0.0259	22.48565	0.17282	Methyl decanoate
3	9.693	BP	0.0264	183.49957	1.41030	Methyl Laurate
4	11.777	BP	0.0246	130.13211	1.00014	Methyl myristate
5	13.734	BP	0.0322	2417.59644	18.58062	Methyl palmitate
6	13.957	BB	0.0276	185.34578	1.42449	Methyl palmitoleate
7	14.643	BP	0.0317	2515.45776	19.33274	Methyl heptadecanoate
8	15.505	BP	0.0363	668.72113	5.13951	Methyl stearate
9	15.713	BV	0.0427	4687.02197	36.02246	Methyl oleate
10	16.088	BV	0.0311	2005.66895	15.41472	Methyl linoleate
11	16.604	BB	0.0293	101.14211	0.77734	Methyl linolenate
12	17.130	PP	0.0285	41.34282	0.31774	Methyl arachidate
13	18.716	BB	0.0425	16.37998	0.12589	Methyl behenate
14	18.952		0.0000	0.00000	0.00000	Methyl erucate
15	20.756	BB	0.0482	19.35268	0.14874	Methyl lignocerate
Totals :				1.30114e4		

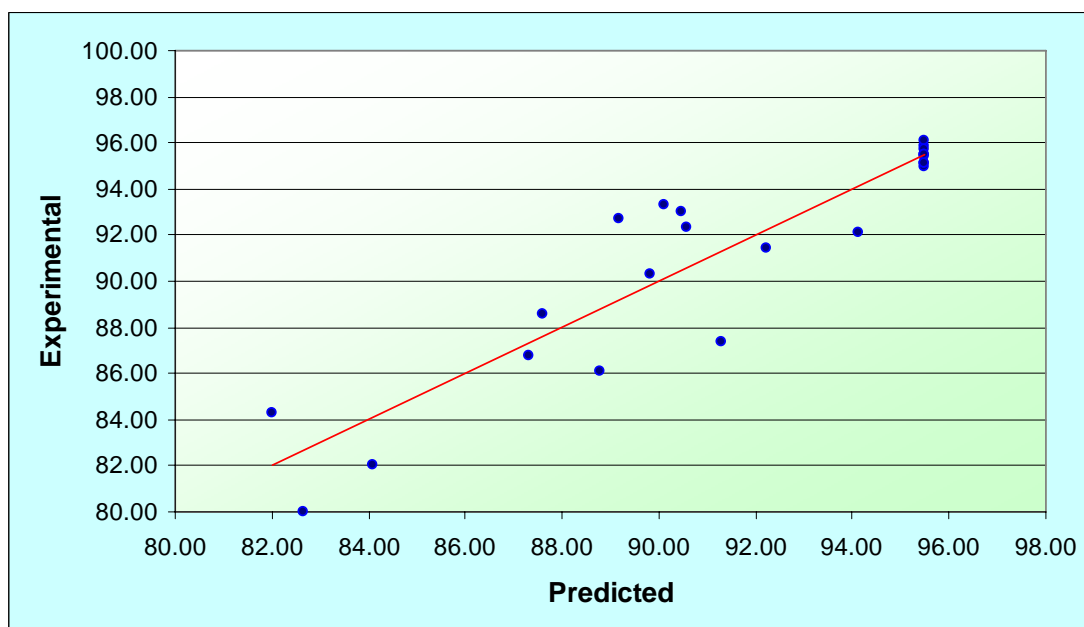
Appendix Figure C23 GC chromatogram of trap grease methyl ester in transesterification condition at 0.26 v/v (6:1) methanol to oil molar ratio, 1 % v/v catalyst concentration and 1 hour reaction time (experimental treatment no.23)

Appendix D

Correlation of the experimental and predicted responses



Appendix Figure D1 Correlation of the experimental and predicted acid value in the acid catalyzed esterification



Appendix Figure D2 Correlation of the experimental and predicted fatty acid methyl ester in the alkali catalyzed transesterification

Appendix E

Calculation of central composite design

The central composite design was carried out with increasing two experimental point along each coordinate axis to opposite side of the origin and at a distance equal to the semi diagonal of the hyper cube of the factorial design and new extreme values as low and high for each factor added in the central composite design model. The calculation was shown below

$$\alpha = [2^K]^{1/4}$$

Optimization for acid catalyst esterification and alkali catalyst transesterification concerned with methanol to oil molar ratio, catalyst concentration and reaction time. The central composite design (CCD) could be performed by this following calculation

$$N = 2^K + 2K + X_0$$

N = Total numbers of experimental run

K = Numbers of variables

X₀ = Numbers of central values or zero level

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