การผลิตไบโอดีเซลจากเมล็ดพืชน้ำมันโดยใช้เฟร์ริกซัลเฟตและโซเดียมไฮดรอกไซด์

นายสุเมธา อิสริยะเนตร

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BIODIESEL PRODUCTION FROM OILSEED PLANTS USING
FERRIC SULFATE AND SODIUM HYDROXIDE

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A Thesis Submitted in Partial Fulfillment of the Requirements
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Thesis Title	BIODIESEL PRODUCTION FROM OILSEED PLANTS USING FERRIC SULFATE AND SODIUM HYDROXIDE
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Field of study	Petrochemistry and Polymer Science
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สุเมธา อิสริยะเนตร : การผลิตไบโอดีเซลจากเมล็ดพืชน้ำมันโดยใช้เฟร์ริกซัลเฟตและโซเดียมไฮดรอกไซด์ (BIODIESEL PRODUCTION FROM OILSEED PLANTS USING FERRIC SULFATE AND SODIUM HYDROXIDE) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร.สมใจ เพ็งปรีชา, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ดร. กันย์ กังวานสายชล, 142 หน้า.

งานวิจัยนี้ศึกษาความเป็นไปได้ของพืชน้ำมัน 30 ชนิดในประเทศไทยเพื่อใช้เป็นวัตถุดิบในการผลิตไบโอดีเซล ปริมาณน้ำมัน ปริมาณกรดไขมันอิสระ ค่าสปอนนิฟิเคชัน และค่าไอโอดีนของพืชน้ำมันเหล่านี้อยู่ในช่วง 13-69 เปอร์เซ็นต์ 0.64-30.13 เปอร์เซ็นต์ 161.23-209.54 มิลลิกรัมของโพแทสเซียมไฮดรอกไซด์ต่อน้ำมันหนึ่งกรัม และ 45.13-164.38 มิลลิกรัมของไอโอดีนต่อน้ำมันหนึ่งกรัมตามลำดับ ไบโอดีเซลของพืชน้ำมันเหล่านี้สามารถสังเคราะห์ได้จากปฏิกิริยาทรานส์เอสเทอริฟิเคชัน และปฏิกิริยาเอสเทอริฟิเคชัน ปฏิกิริยาทรานส์เอสเทอริฟิเคชันใช้สัดส่วนโดยโมลของเมทานอลต่อน้ำมันเป็น 12:1 และ 1 เปอร์เซ็นต์โดยน้ำหนักของโซเดียมไฮดรอกไซด์ ที่อุณหภูมิ 65 องศาเซลเซียส เป็นเวลา 1.5 ชั่วโมงสำหรับน้ำมันที่มีปริมาณกรดไขมันอิสระน้อยกว่า 3 เปอร์เซ็นต์ ปฏิกิริยาเอสเทอริฟิเคชันใช้สัดส่วนโดยโมลของเมทานอลต่อน้ำมันเป็น 10:1 และเฟร์ริกซัลเฟต ที่อุณหภูมิ 65 องศาเซลเซียสสำหรับน้ำมันที่มีปริมาณกรดไขมันอิสระมากกว่า 3 เปอร์เซ็นต์ สำหรับเวลาในการทำปฏิกิริยา และปริมาณตัวเร่งปฏิกิริยาของปฏิกิริยาเอสเทอริฟิเคชันคือ 3 เปอร์เซ็นต์โดยน้ำหนักของเฟร์ริกซัลเฟต เป็นเวลา 1 ชั่วโมง 3 เปอร์เซ็นต์โดยน้ำหนักของเฟร์ริกซัลเฟต เป็นเวลา 2 ชั่วโมง และ 5 เปอร์เซ็นต์โดยน้ำหนักของเฟร์ริกซัลเฟต เป็นเวลา 3 ชั่วโมง ใช้สำหรับน้ำมันที่มีปริมาณกรดไขมันอิสระ 10 เปอร์เซ็นต์ 20 เปอร์เซ็นต์ และ 30 เปอร์เซ็นต์ตามลำดับ พืชน้ำมันเหล่านี้ทุกชนิดให้ไบโอดีเซลที่มีจุดวาบไฟสูงกว่า 120 องศาเซลเซียส และค่าความหนืด ค่าความหนาแน่น ค่าความเป็นกรดและปริมาณเอสเทอร์อยู่ในช่วง 3.2-5.5 เซนติสโตรกส์ 866.95-887.64 กรัมต่อลูกบาศก์เซ็นติเมตร 0.1252-0.4404 มิลลิกรัมของโพแทสเซียมไฮดรอกไซด์ต่อน้ำมันหนึ่งกรัม และ 91.71-96.83 เปอร์เซ็นต์ตามลำดับ จากการพิจารณาเปอร์เซ็นต์น้ำมัน ค่าสปอนนิฟิเคชัน ค่าไอโอดีน ค่าความหนืด ค่าความหนาแน่น จุดวาบไฟ ค่าความเป็นกรดและปริมาณเอสเทอร์ พบว่า ไบโอดีเซลที่ได้จากพืชน้ำมันจำนวน 15 ชนิด มีศักยภาพในการนำไปใช้และมีสมบัติส่วนใหญ่เป็นไปตามมาตรฐานของไบโอดีเซล

สาขาวิชา ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์ ลายมือชื่อนิสิต...สุเมธา อิสริยะเนตร......
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5072533423 : MAJOR PETROCHEMISTRY AND POLYMER SCIENCE
KEYWORDS : BIODIESEL / TRANSESTERIFICATION / FERRIC SULFATE

SUMETHA ISSARIYANATE: BIODIESEL PRODUCTON FROM OILSEED PLANTS USING FERRIC SULFATE AND SODIUM HYDROXIDE. THESIS ADVISOR: ASSOC. PROF. SOMCHAI PENGPRECHA, Ph.D., THESIS CO-ADVISOR: KUNN KANGVANSAICHOL, Ph.D., 142 pp.

This research was aimed to study possibility of 30 oilseed plant species in Thailand to use as raw materials to produce biodiesel. The amount of oil, free fatty acid (FFA), saponification number (SN) and iodine value (IV) of these oilseed plants were in the range of 13-69%, 0.64-30.13%, 161.23-209.54 mgKOH/g and 45.13-164.38 mgI_2/g, respectively. Biodiesel from these oilseed plant species could be synthesized by both transesterification reaction and esterification reaction. Transesterification reaction was carried out by using 12:1 molar ratio of methanol to oil and 1%wt of NaOH at 65°C for 1.5 h for oils having FFA content less than 3%. Esterification reaction was carried out by using 10:1 molar ratio of methanol to oil and $Fe_2(SO_4)_3$ at 65°C for oils having FFA content more than 3%. For the reaction time and amount of catalyst of esterification reaction, 3% wt of $Fe_2(SO_4)_3$ for 1 h, 3% wt of $Fe_2(SO_4)_3$ for 2 h and 5% wt of $Fe_2(SO_4)_3$ for 3 h were used for oils having FFA 10%, 20% and 30%, respectively. All of these oilseed plant species gave biodiesel having flash point more than 120°C and viscosity, density, acid value and ester content in the range of 3.2-5.5 cSt, 866.95-887.64 g/cm^3, 0.1252-0.4404 mgKOH/g and 91.71-96.83%, respectively. According to the %oil content, saponification number, iodine value, viscosity, density, flash point, acid value and ester content, the biodiesel from 15 oilseed plant species could be potentially used and they met the major specification of biodiesel standards.

Field of Study : Petrochemistry and Polymer Science Student's Signature Sumetha Issariyanate
Academic Year :...........2009............................... Advisor's Signature...........
Co-Advisor's Signature........

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LIST OF ABBREVIATIONS

µl	microliter
µm	micrometer
ASTM	American Standard Test Method
°C	Degree Celsius
cSt	Centistroke
°F	Degree
FFA	Free fatty acid
FID	Flame Ionization Detector
FT-IR	Fourier Transform Infrared Spectroscopy
g	gram
GC	Gas-liquid chromatography
h	hour
NMR	Nuclear Magnetic Resonance Spectroscopy
kg/cm^3	Kilogram per cubic metre
L	Liter
MJ/kg	Millijule per kilogram
min	Minute
mg	Miligram
ml	Milliter
mm	Millimeter
nm	Nanometer
ppm	parts per million
rpm	Revolution per minute
v/v	Volume by volume
w/v	Weight by volume
%wt	percent weight
δ_H	Chemical shift of ^{1}H NMR
BA	*Basella alba*
BC	*Brassica chinensis*

BEH	*Benincasa hispida*
BJ	*Brassica juncea*
BP	*Brassica pekinensis*
CAC	*Caesalpinia crista*
CAO	*Camellia oleifera*
CAS	*Cassia suratlensi*
CC	*Crassocephalum crepidioides*
CIL	*Citrullus lanatus*
CIM	*Citrus maxima*
CIR	*Citrus reticulata*
CUM	*Cucurbita moschata*
CV	*Chukrasia velutina*
DO	*Dalbergia oliveri Gamble*
GJ	*Gardenia jasminodes*
HIS	*Hibiscus sabdariffa Linn.*
LS	*Lagenaria siceraria*
MAI	*Macadamia integrifolia*
ME	*Mimusops elengi*
MH	*Millingtonia hortensis*
ML	*Millettia kangensis Craib*
MO	*Moringa oleifera Lamk.*
MYF	*Myristica fragrans*
OC	*Ocimum canum*
PAF	*Passiflora foetida*
PE	*Phyllanthus emblica*
PF	*Perilla frutescens*
SEI	*Sesamum indicum*
TC	*Terminalia chebula*

CHAPTER I

INTRODUCTION

As the fossil fuels are depleting day by day, there is a need to find out an alternative fuel to fulfill the energy demand of the world. The petroleum fuels play a very important role in the development of industrial growth, transportation, agricultural sector and to meet many other basic human needs but these fuels are limited. Therefore, the biodiesel is one of the alternative fuels can be used nowadays[1,2,3]. The main advantages of using biodiesel are its renewability, better-quality exhaust gas emissions, it given that all the organic carbon present is photosynthetic in origin, it does not contribute to a rise in the level of carbon dioxide (CO_2) in the atmosphere and consequently to the greenhouse effect [4].

The most wildly used technique for producing biodiesel was transesterification using alkali (e.g. NaOH, KOH) or acid (e.g. H_2SO_4) as catalyst. Because of the shorter reaction time and lower cost of catalyst as compared with acid catalytic process, the alkali catalytic process has received much more attention. However, it is limited for its high sensitivity to both water and free fatty acids (FFA) in the raw materials. During the process, FFA can react with the alkali catalyst to release soap and water, while it does not react with acid catalyst in the acid catalytic process. The saponification would not only hinder separation of ester from glycerin but also leads to the low yield as well as the formation rate of FAME. As for vegetable oil with a high concentration of FFA and water, it is not suitable to adopt alkali process for production of biodiesel[5].

Two-step catalytic process combined with acid and alkali catalyst was developed to produce biodiesel from these oils. By using sulfuric acid as a catalyst, FFA is generally esterified with methanol and when the FFA concentration decreases, sulfuric acid is then removed. The transesterification was then completed with alkali catalyst. However, it still has some drawbacks: difficult recovery of acid catalyst, corrosiveness of sulfuric acid, and high cost of equipments required for the reaction system [6]. Therefore, solid acid catalyst such as ferric sulfate is turned out to be a good alternative to sulfuric acid in the esterification reaction.

Objectives of the research:

1. To synthesize methyl ester from oilseed plants for using as biodiesel.
2. To study the properties of biodiesel from oilseed plants.

CHAPTER II

THEORY AND LITERATURE REVIEWS

2.1 Alternative renewable energy[7, 8, 9, 10]

In the industrial economy diesel fuels have an essential function of a developing country and used for transport of industrial, agricultural goods and operation of diesel tractor in agricultural sector. The high energy demand in the industrialized world and in the domestic sector, and pollution problems caused due to the widespread use of fossil fuels making it increasingly necessary to develop the renewable energy sources of limitless duration and smaller environmental impact than the traditional one. This has stimulated recent interest in alternative sources for petroleum-based fuels. An alternative fuel must be technically feasible, economically competitive, environmentally acceptable, and readily available. One possible alternative to fossil fuel is the use of oils of plant origin like vegetable oils and tree borne oil seeds. This alternative diesel fuel can be termed as biodiesel. This fuel is biodegradable and non-toxic and has low emission profiles as compared to petroleum diesel. Usage of biodiesel will allow a balance to be sought between agriculture, economic development and the environment.

The advantages of using vegetable oils as fuels are:

- Vegetable oils are liquid fuels from renewable sources.
- They do not over-burden the environment with emissions.
- Vegetable oils have potential for making marginal land productive by their property of nitrogen fixation in the soil.
- Vegetable oil's production requires less energy input in production.
- The current prices of vegetable oils in world are nearly competitive with petroleum fuel price.
- Vegetable oil combustion has cleaner emission spectra
- Simpler processing technology.

Because the rapid decline in crude oil reserves, in many countries the use of vegetable oils as diesel fuels is again promoted. Depending upon climate and soil conditions, different vegetable oils for diesel fuels are looked into from different nations. For example, soybean oil in the USA, rapeseed and sunflower oils in Europe, palm oil in Southeast Asia (mainly Malaysia and Indonesia), and coconut oil in Philippines are being considered as substitutes for mineral diesel.

2.2 Vegetable oil chemistry

Chemically the oils/fats consist of triglyceride molecules of three long chain fatty acids that are ester bonded to a single glycerol molecule. These fatty acids differ by the length of carbon chains, the number, orientation and position of double bonds in these chains and the structure notation as shown in Figure 2.1.

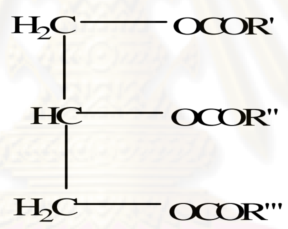

Figure 2.1 Structure of a triglyceride.

where R', R'', R''' represent hydrocarbon chain of fatty acids. Fatty acids vary in carbon chain length and in the number of unsaturated bonds (double bonds). The structures of common fatty acids are given in Table 2.1, and fatty acid compositions of some vegetable oils are given in Table 2.2.

Vegetable oils have about 10% less heating value than diesel due to the oxygen content in the molecule. In addition, the viscosity of mineral diesel is several times lower than that of vegetable oil due to high molecular weight and complex chemical structure in vegetable oil. Physical and thermal properties of some of the vegetable oils are listed in Table 2.3.

Table 2.1 Chemical structure of common fatty acids [9]

Fatty acid	Systermatic name	Structure	Formular
Lauric	Dodecanoic	12:0	$C_{12}H_{24}O_2$
Myristic	Tetradecanoic	14:0	$C_{14}H_{28}O_2$
Palmitic	Hexadecanoic	16:0	$C_{16}H_{32}O_2$
Stearic	Octadecanoic	18:0	$C_{18}H_{36}O_2$
Arachidic	Eicosanoic	20:0	$C_{20}H_{40}O_2$
Behenic	Docosanoic	22:0	$C_{22}H_{44}O_2$
Linoceric	Tetracosanoic	24:0	$C_{24}H_{48}O_2$
Oleic	cis-9-Octadecenoic	18:1	$C_{18}H_{34}O_2$
Linoleic	cis-9,cis-12Octadecadienoic	18:2	$C_{18}H_{32}O_2$
Linolenic	cis-9,cis-12,cis-15-Octadecatrienoic	18:3	$C_{18}H_{30}O_2$
Erucic	cis-13-Docosenoic	22:1	$C_{22}H_{42}O_2$

Table 2.2 Chemical composition of vegetable oils [9]

Vegetable oil	Fatty acid composition (wt%)									
	C14:0	C16:0	C18:0	C20:0	C22:0	C24:0	C18:1	C22:1	C18:2	C18:3
Corn	0	12	2	*Tr*	0	0	25	0	6	*Tr*
Cottonseed	0	28	1	0	0	0	13	0	58	0
Crambe	0	2	1	2	1	1	19	59	9	7
Linseed	0	5	2	0	0	0	20	0	18	55
Peanut	0	11	2	1	2	1	48	0	32	1
Rapeseed	0	3	1	0	0	0	64	0	22	8
Safflower	0	9	2	0	0	0	12	0	78	0
Sesame	0	13	4	0	0	0	53	0	30	0
Soya bean	0	12	3	0	0	0	23	0	55	6
Rice-bran	0.4-0.6	11.7-16.5	1.7-2.5	0.4-0.6	—	0.4-0.9	39.2-43.7	—	26.4-35.1	—
Mahua	—	16-28.2	20-25.1	0.0-3.3	—	—	41.0-51.0	—	8.9-13.7	—
Neem	0.2-0.26	13.6-16.2	14.4-24.1	0.8-3.4	—	—	49.1-61.9	—	2.3-15.8	—
Karanja	—	3.7-7.9	2.4-8.9	—	—	1.1-3.5	44.5-71.3	—	10.8-18.3	—

Tr : Trace

Table 2.3 Physical and thermal properties of vegetable oils [9]

Vegetable oil	Kinematic viscosity	Cetane number	Heating value (MJ/kg)	Cloud point (°C)	Pour point (°C)	Flash point (°C)	Density (Kg/l)	Carbon residue (wt%)	Ash (wt%)
Corn	34.9	37.6	39.5	−1.1	−40.0	277	0.9095	0.24	0.01
Cotton seed	33.5	41.8	39.5	1.7	−15.0	234	0.9148	0.24	0.01
Cramble	53.6	44.6	40.5	10.0	−12.2	274	0.9044	0.23	0.05
Linseed	22.2	34.6	39.3	1.7	−15.0	241	0.9236	0.22	<0.01
Peanut	39.6	41.8	49.8	12.8	−6.7	271	0.9026	0.24	0.005
Rapeseed	37.0	37.6	39.7	−3.9	−31.7	246	0.9115	0.30	0.054
Safflower	31.3	41.3	39.5	18.3	−6.7	260	0.9114	0.25	0.006
Sesame	35.5	40.2	39.3	−3.9	−9.4	260	0.9133	0.25	<0.01
Soyabean	32.6	37.9	39.6	−3.9	−12.2	254	0.9138	0.27	<0.01
Sunflower	33.9	37.1	39.6	7.2	−15.0	274	0.9161	0.23	<0.01
Palm	39.6	42.0	—	31.0	—	267	0.9180	—	—
Tallow	—	—	40.0	—	—	201	—	6.21	—

The high viscosity of vegetable oil, 35–60 cSt compared to 4 cSt for diesel at 40 ℃, leads to problem in pumping and spray characteristics (atomization and penetration etc.). The inefficient mixing of oil with air contributes to incomplete combustion. High flash point attributes to its lower volatility characteristics. This results in high carbon deposit formation, injector coking, piston ring sticking and lubrication oil dilution and oil degradation. The combination of high viscosity and low volatility of vegetable oils cause poor cold starting, misfire and ignition delay.

2.2.1 Utilization of vegetable oil as engine fuel

Vegetable oils can be used through at least four ways: dilution, micro-emulsion, thermal cracking and transesterification.

2.2.1.1 Dilution

Diesel fuels are derived from the dilution of vegetable oils with a solvent or ethanol. The dilution of sunflower oil with diesel fuels in the ratio of 1:3 by volume has been studied and engine tests. The viscosity of this blend was 4.88 cSt at 40^{o}C. They concluded that the blend could not be recommended for long-term use in the direct injection diesel engines because of severe injector nozzle coking and sticking. High oleic safflower oil was compared with blended and also tested. It gave pleasant results, however, its use in the long term is not applicable as it leads to thickening of lubricant.

2.2.1.2 Micro-emulsion

A microemulsion is designed to tackle the problem of the high viscosity of pure vegetable oils by reducing the viscosity of oils with solvents such as simple alcohols. They are defined as a colloidal equilibrium dispersions of optically isotropic fluid microstructures, with dimensions generally in the 1-150 nm range. These are formed spontaneously from two normally immiscible liquids and one or more ionic or non-ionic amphiphile. The performances of ionic and non-ionic microemulsions where found to be similar to diesel fuel, over short term testing. They also achieved good spray characteristics, with explosive vaporization which improved the combustion characteristics.

2.2.1.3 Thermal cracking

Thermal cracking also known as pyrolysisis the conversion of one substance into another by means of heat or by heat in presence of a catalyst. The pyrolyzed material can be vegetable oils, animal fats, natural fatty acids or methyl esters of fatty acids. The pyrolysis of fats has been investigated for more than 100 years, especially in those areas of the world that lack deposits of petroleum. Many investigators have studied the pyrolysis of triglycerides to obtain products suitable for diesel engine. Thermal decomposition of triglycerides produces alkanes, alkenes, aromatics and carboxylic acids.

2.2.1.4 Transesterification

Transesterification, also called alcoholysis, is the displacement of alcohol from an ester by another alcohol in a process similar to hydrolysis, except than an alcohol is used instead of water. This process has been widely used to reduce the viscosity of triglycerides.

2.3 Sources of biodiesel [11]

There are more than 350 oil-bearing crops identified, among which only soybean, palm, sunflower, safflower, cottonseed, rapeseed and peanut oils are considered as potential alternative fuels for diesel engines. Worldwide consumption of soybean oil is the highest in 2003 (27.9 million metric tons). Vegetable oil is one of the renewable fuels. Concerning about environmental benefits and the fact that these are made from renewable resources vegetable oils have become more attractive. Vegetable oils are a renewable and potentially inexhaustible source of energy with energy content close to diesel fuel. However, extensive use of vegetable oils may cause other significant problems such as starvation in developing countries. The vegetable oil fuels were more expensive than petroleum fuels so they were not acceptable.

In Malaysia and Indonesia palm oil is used as a significant biodiesel source. In Europe, rapeseed is the most common base oil used in biodiesel production. In India and Southeast Asia, the Jatropha tree is used as a significant fuel source. Soybeans are commonly used in the United States for food products which has led to soybean biodiesel becoming the primary source for biodiesel in this country.

2.4 Transesterification [7, 9, 12, 13]

Transesterification or alcoholysis is the displacement of alcohol from an ester by another in a process similar to hydrolysis, except than alcohol is used instead of water. This process has been widely used to reduce the high viscosity of triglycerides.

The transesterification reaction is represented by the general equation as Figure 2.2

$$RCOOR' + R''OH \xrightleftharpoons{catalyst} RCOOR'' + R'OH$$

Figure 2.2 General equation of transesterification.

In the transesterification of vegetable oils, a triglyceride reacts with an alcohol in the presence of strong acid or base to give a mixture of fatty acids alkyl esters and glycerol as shown in Figure 2.3

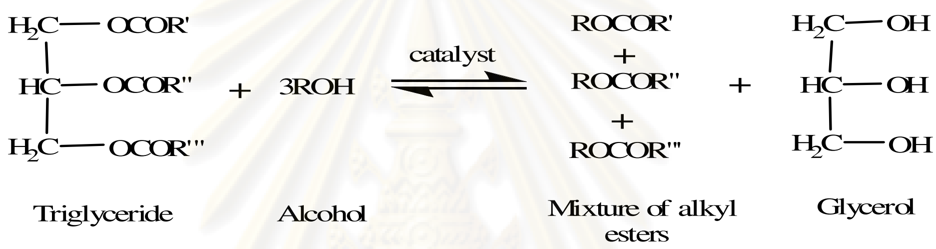

Figure 2.3 General equation for transesterification of vegetable oils.

2.4.1 Alkali-catalyzed transesterification

The transesterification reaction can be catalyzed by both homogeneous and heterogeneous catalysts. In turn, the homogeneous catalysts include alkalis and acids. Sodium hydroxide, sodium methoxide and potassium hydroxide are the most commonly used as alkali catalysts. Sulfuric acid, hydrochloric acid and sulfonic acid are usually preferred as acid catalysts. Finally, the heterogeneous catalysts include enzymes, titanium silicates, alkaline earth metal compounds, anion exchange resins and guanadines heterogenized on organic polymers. The Alkali-catalyzed transesterification is much faster than acid –catalyzed transesterification and is most often used commercially.

The mechanism of alkali-catalyzed transesterification is described in Figure 2.4. The first step involves the attack of the alkoxide ion to the carbonyl carbon of the triglyceride molecule, resulting in the formation of a tetrahedral intermediate. The reaction of this intermediate with an alcohol produces the alkoxide ion in the second step. In the last step the rearrangement of the tetrahedral intermediate gives rise to an ester and a diglyceride.

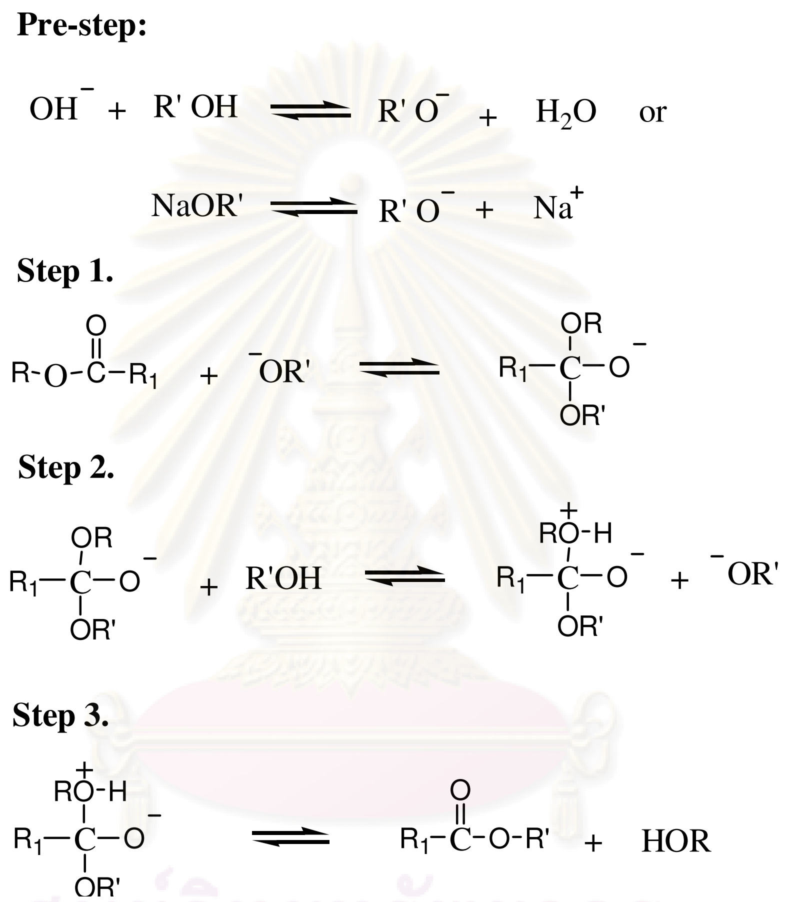

Figure 2.4 Mechanism of base catalyzed transesterification.

2.4.2 Acid-catalyzed transesterification

Transesterification can be catalyzed by Brownsted acids, preferably by sulfonic and sulfuric acids. The mechanism of acid catalyzed transesterification of vegetable oil (for a monoglyceride) is shown in Figure 2.5. However, it can be extended to di-and tri-glycerides. The protonation of carbonyl group of the ester leads to the carbocation, which after a nucleophilic attack of the alcohol produces a tetrahedral intermediate. This intermediate eliminates glycerol to form a new ester and to regenerate the catalyst. The process of transesterification is affected by various factors depending upon the reaction condition used. The effects of these factors are described below.

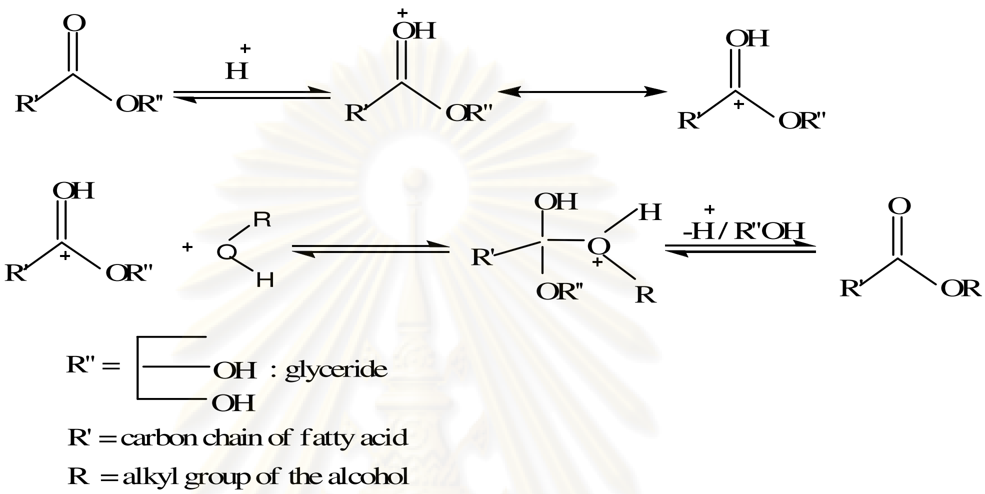

Figure 2.5 Mechanism of acid catalyzed transesterification.

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2.4.3 Heterogeneous catalyst[24]

Heterogeneous catalysts are potentially useful for the production of biodiesel and chemicals. The ability to achieve high yields of biodiesel esters without the need to neutralize and wash the biodiesel products helps make biodiesel production more attractive in countries where conservation of water resources is critical. Research is also ongoing to further identify nanocrystalline oxides that have enhanced surface reactivity that stems from reactive surface sites, such as edges, corners, and defect sites capable of coordinating with Lewis acids or Lewis bases. Example for heterogeneous catalyst : Alkali earth or transition metal oxides like CaO, MgO, BaO, and ZnO/Al_2O_3, Alkaline metal oxides supported on zeolites and MCM-41, Ion exchange resins such as clay minerals with acidic reaction sites, Sulfated metal oxides such as ferric sulfate ($Fe_2(SO_4)_3$).

The advantages of heterogeneous catalyst were reusable and do not form soaps, more tolerant to water and free fatty acids in the feedstock, improve product yield and purity, simpler purification process for glycerol and ease of separating biodiesel product.

Disadvantages of heterogeneous catalyst were require elevated temperatures and pressures to work well, those on solid support tend to show less activity than the active species in solution and there is the possibility of leaching which might contaminate the biodiesel.

2.4.4 Effect of free fatty acid and moisture [14, 15, 16]

The free fatty acid and moisture content are key parameters for determining the viability of the vegetable oil transesterification process. To carry the base catalyzed reaction to completion; free fatty acid (FFA) value lower than 3% is needed. The higher the acidity of the oil, smaller is the conversion efficiency. Both, excess as well as insufficient amount of catalyst may cause soap formation. If the oil with high FFA are used to make biodiesel fuel, they have to be refined by saponification using NaOH solution to remove free fatty acids. In the other hand, the acid catalyzed process can also be used for esterification of these free fatty acids. All material should be substantially anhydrous and triglycerides should have lower acid value. The addition of more sodium hydroxide catalyst compensates for higher acidity, but the resulting soap causes an increase in viscosity or formation of gels that interferes in the reaction and with separation of glycerol. When the reaction conditions do not meet the above requirements, ester yields are significantly reduced. The methoxide and hydroxide of sodium or potassium should be maintained in anhydrous state. Prolonged contact with air will diminish the effectiveness of these catalysts through interaction with moisture and carbon dioxide. Most of the biodiesel is currently made from edible oils by using methanol and alkaline catalyst. However, there are large amounts of low cost oils and fats that could be converted to biodiesel. The problems with processing are low cost oils and fats are that they often contain large amounts of free fatty acids that cannot be converted to biodiesel using alkaline catalyst. Therefore, two-step esterification process is required for these feed stocks. Initially the FFA of these can be converted to fatty acid methyl esters by an acid catalyzed pretreatment and in the second step transesterification is completed by using alkaline catalyst to complete the reaction.

2.4.5 Molar ratio of alcohol to oil

From Scheme 2.3, it is necessary to use either a large excess of alcohol or remove one of the products from the reaction mixture continuously to shift the transesterification reaction to the right. When 100% excess methanol is used, the reaction rate is at its highest. A molar ratio of 6:1 is normally used in industrial processes to obtain methyl ester yields higher than 98% by weight.

2.4.6 Effect of reaction time and temperature

The conversion rate increases with reaction time. Freedman et al. [17] the reaction condition used as 6:1 molar of methanol to oil, 0.5%NaOH by weight of oil at 60 °C, transesterified peanut, cotton-seed, sunflower and soybean oil gave approximate yield of 80% after 1 min for soybean and sunflower oils. After 1 h, the conversion was almost the same for all four oils (93–98%). Ma et al. [18] studied the effect of reaction time on transesterification of beef tallow with methanol. The reaction was very slow during the first minute due to mixing and dispersion of methanol into beef tallow. From one to 5 min, the reaction proceeds very fast. The production of beef tallow methyl esters reached the maximum value at about 15 min. Transesterification can occur at different temperatures, depending on the oil used. For the transesterification of refined oil with methanol (6:1) and 1% NaOH, the reaction was studied with three different temperatures. After 0.1 h, ester yields were 94, 87 and 64% for 60, 45 and 32 °C, respectively. After 1 h, ester formation was identical for 60 and 45 °C runs and only slightly lower for the 32°C run. Temperature clearly influenced the reaction rates and yield of esters [7].

2.4.7 Catalyst type

Catalysts used in transesterification reaction can be classified as alkali, acid, or enzymes. Alkali-catalyzed transesterification is much faster than acid-catalyzed reaction. In contras, acid-catalyzed transesterification reaction is suitable when a vegetable oil has high free fatty acid and water content. Partly due to faster transesterification and partly because alkaline catalysts are less corrosive to industrial equipment than acidic catalysts, most commercial transesterification reactions are conducted with alkaline catalysts. Sodium alkoxides was found to be more effective than sodium hydroxide and are among the most efficient catalysts used for this purpose, although NaOH, due to its low cost, has attracted its wide use in large-scale transesterification. Methanol can quickly react with triglycerides and NaOH is easily dissolved in it. The reaction can be catalyzed by alkalis, acids, or enzymes. The alkalis include NaOH, KOH, carbonates and corresponding sodium and potassium alkoxides such as sodium methoxide, sodium ethoxide, sodium propoxide and sodium butoxide. Acid catalysts usually use as sulfuric acid, sulfonic acids and hydrochloric acid.

2.5 Advantages of biodiesel [11, 19]

Main advantages of biodiesel given in the literature include:

- Availability and renewability of biodiesel

Biodiesel can be made from domestically produced, renewable oilseed crops such as soybean, rapeseed and sunflower. The risks of handling, transporting and storing biodiesel are much lower than those ones, associated with petrodiesel. Biodiesel is safe to handle and transport because it is as biodegradable as sugar and has a high flash point compared to petroleum diesel fuel. Biodiesel can be used alone or mixed in any ratio with petroleum diesel fuel. The most common blend is a mix of 20% biodiesel with 80% petroleum diesel, or B20 under recent scientific investigations; however, in Europe the current regulation foresees a maximum 5.75% biodiesel.

- Higher combustion efficiency of biodiesel

The combustion process and decreased oxidation potential could be improved by oxygen content of biodiesel. Structural oxygen content of a fuel improves combustion efficiency due to the increase of the homogeneity of oxygen with the fuel during combustion. Biodiesel contains 11% oxygen by weight and contains no sulfur therefore, the use of biodiesel can extend the life of diesel engines because it is more lubricating than petroleum diesel fuel. Biodiesel has got better lubricant properties than petrodiesel. The higher heating values (HHVs) of biodiesels are relatively high. The HHVs of biodiesels (39–41 MJ/kg) are slightly lower than that of gasoline (46 MJ/kg), petrodiesel (43 MJ/kg) or petroleum (42 MJ/kg), but higher than coal (32–37 MJ/kg).

- Lower emissions by using biodiesel

Combustion of biodiesel alone provides over a 90% reduction in total unburned hydrocarbons (HC), and a 75–90% reduction in polycyclic aromatic hydrocarbons (PAHs). Biodiesel further provides significant reductions in particulates and carbon moNOxide than petroleum diesel fuel. Biodiesel provides a slight increase or decrease in nitrogen oxides depending on engine family and testing procedures.

Sulfur content of petrodiesel is 20–50 times those of biodiesels. Several municipalities are considering mandating the use of low levels of biodiesel in diesel fuel on the basis of several studies which have found HC and particulate matter (PM) benefits from the use of biodiesel. The use of biodiesel to reduce N_2O is attractive for several reasons. Biodiesel contains little nitrogen, as compared with petrodiesel which is also used as a reburning fuel. The N_2O reduction was strongly dependent on initial N_2O concentration and only slightly dependent upon temperature, where increased temperature increased N_2O reduction. This results in lower N_2O production from fuel nitrogen species for biodiesel. In addition, biodiesel contains virtually trace amount of sulfur, so SO_2 emissions are reduced in direct proportion to the petrodiesel replacement. One of the most common blends of biodiesel contains 20 vol% biodiesel and 80 vol% conventional diesel. The use of blends of biodiesel and diesel oil is preferred in engines, in order to avoid some problems related to the decrease of power and torque and to the increase of NOx emissions (a contributing factor in the localized formation of smog and ozone) with increasing content of pure biodiesel in the blend. Emissions of all pollutants except NOx appear to decrease when biodiesel is used. The fact that NOx emissions increase with increasing biodiesel concentration could be a detriment in areas that are out of attainment for ozone. Reductions in net carbon dioxide emissions are estimated at 77–104 g/MJ of diesel displaced by biodiesel.

These reductions increase as the amount of biodiesel blended into diesel fuel increases. The best emissions reductions are seen with biodiesel.

- Biodegradability of biodiesel

Biodiesel is non-toxic and degrades about 4 times faster than petrodiesel. Its oxygen content improves the biodiesel biodegradation process, leading to a decreased level of quick biodegradation. In comparison with petrodiesel, biodiesel shows better emission parameters. It improves the environmental performance of road transport, including decreased greenhouse emissions (mainly of carbon dioxide). Chemicals from biodegradation of biodiesel can be released into the environment. With the increasing interest in biodiesel, the health and safety aspects are of utmost importance, including determination of their environmental impacts in the transport, storage or processing [7]. The biodegradabilities of several biodiesels in the aquatic environment show that all biodiesel fuels are readily biodegradable. After 28 days all biodiesel fuels were 77%–89% biodegraded, diesel fuel was only 18% biodegraded. The enzymes responsible for the dehydrogenation/oxidation reactions that occur in the process of degradation recognize oxygen atoms and attack them immediately.

2.5.1 Disadvantages of biodiesel as diesel fuel

Major disadvantages of biodiesel are higher viscosity, lower energy content, higher cloud point and pour point, higher nitrogen oxides (NOx) emissions, lower engine speed and power, injector coking, engine compatibility, high price and higher engine wear. Important operating disadvantages of biodiesel in comparison with petrodiesel are cold start problems, the lower energy content, higher copper strip corrosion and fuel pumping difficulty from higher viscosity. This increases fuel consumption when biodiesel is used in comparison with application of pure petrodiesel, in proportion to the share of the biodiesel content.

2.6 Properties and specification of biodiesel [20]

The characteristics of biodiesel are close to mineral diesel, therefore, biodiesel becomes a strong candidate to replace the mineral diesel if the need arises. The quality of the biodiesel was evaluated by the determinations of important properties such as viscosity, flash point, density, acid value and ester content according to ASTM and EN standards. The values of these properties of methyl esters of these oilseed plants were shown in Table 2.4.

Table 2.4 Specification for quality of biodiesel

Property	Value	Method
Viscosity at 40 °C	3-5 cSt	ASTM D445
Flash point	>120 °C	ASTM D93
Density at 15 °C	860-900 kg/cm^3	ASTM D4052
Acid number	< 0.5 mg KOH/g	ASTM D974
Ester content	> 96.5 %wt	EN 14103

Kinematic viscosity – "the resistance to flow of a fluid under gravity". [Equal to the dynamic viscosity/density.] The kinematic viscosity is a basic design specification for the fuel injectors used in diesel engines. Too high a viscosity and the injectors do not perform properly.

The flash point is defined as the "lowest temperature corrected to a barometric pressure of 101.3 kPa (760 mm Hg), at which application of an ignition source causes the vapors of a specimen to ignite under the specified conditions of test." This test, in part, is a measure of residual alcohol in the B100. The flash point is also a determinant for flammability classification of materials. B100's typical flash point is > 200 ° C, classifying it as "non-flammable".

Density – "the mass per unit volume of a substance at a given temperature."

Acid number – "The quantity of base, expressed as milligrams of potassium hydroxide per gram of sample, required to titrating a sample to a specified end point." The acid number is a direct measure of free fatty acids in B100. The free fatty acids can lead to corrosion and may be a symptom of water in the fuel.

Methyl ester: The purpose of this EN is to determine the ester content of fatty acid methyl ester intended for use as pure biofuel or as a blending component for heating and diesel fuels. Determination of the percentage of methyl ester of fatty acid present in the sample by gas chromatography with internal calibration.

2.7 Literature reviews

In 2002, Antolin *et al*. [27] the optimization of biodiesel production of sunflower oil transesterification were studied. The results showed that 96% of methyl ester was achieved at 70°C using 0.28% w/w of potassium hydroxide as a catalyst with an excess amount of methanol. According to European standard, the sunflower methyl ester could be used as diesel fuels.

In 2004, Vincente *et al*. [28] studied biodiesel production by used different basic catalyst (sodium methoxide, potassium methoxide, sodium hydroxide and potassium hydroxide) for transesterification of sunflower oil. The condition reactions were used as 1% basic catalyst with 6:1 molar ratio of methanol to oil at 65 °C. All the experiments were carried out under the same reaction condition. Using sodium hydroxide, potassium hydroxide, sodium methoxide and potassium methoxide 85.9, 91.67, 99.33 and 98.46 % yield of biodiesel were obtained, respectively. The yield of biodiesel from methoxide catalyst was higher because the basic methoxides only have the trace hydroxide ion as an impurity. In this sense, they do not produce soap through triglyceride saponification.

In 2005, Mohibbe Azam *et al*. [21] studied fatty acid profiles of seed oils of 75 plant species in India having 30% oil in their seed or kernel. By using fatty acid compositions, saponification number, iodine value and cetane number to predict the quality of fatty acid methyl esters of oil for use as biodiesel. The results showed that 4 plant species including *Azadirachta indica*, *Calophyllum inophyllum*, *Jatropha curcus* and *Pongamia pinnata* were found most suitable for use as biodiesel.

In 2006, Meher *et al.* [22] synthesized biodiesel from *Pongamia pinnata* oil. The results showed that 97-98% of methyl ester was obtained by using the optimum condition, 1% KOH as catalyst, 9:1 molar ratio of methanol to oil, stirring speed of 360 rpm at 65 °C for 2 h.

In 2006, Gan et al. [5] studied the esterification of free fatty acid (FFA) in waste cooking oil with methanol by uses $Fe_2(SO_4)_3/C$ (ferric sulfate/active carbon).

The results showed that 96% of free fatty acid (FFA) in waste cooking oil can be effectively eliminated under optimum conditions, 3%$Fe_2(SO_4)_3$/C, methanol/FFA mole ratio 18:1 at 368.15 K.

In 2006, Yong et al. [6] synthesized biodiesel from waste cooking oil by two different processes, traditional acid (sulfuric acid) and two-step catalyzed processes. For traditional acid, the results showed that 90% of methyl ester was obtained by using 20:1 molar ratio of methanol to oil at 95^oC for 10 h. In contrast, two-step catalyzed processes gave 97.22% of methyl ester by using 2%ferric sulfate, 10:1 molar ratio of methanol to oil at 95^oC for 4 h followed by alkali transesterification. Ferric sulfate showed good activity to catalyze the methanolysis of FFA in waste cooking oil, environmental friendly and easy to be separated from system.

In 2007, Yong et al. [29] studied preparation of biodiesel from waste cooking oils which contain large contents of FFAs via two-step catalyzed process. The results showed that the conversion rate of FFA reached 97.22% when the parameters were as follows: 2 %wt $Fe_2(SO_4)_3$, 10:1 molar ratio of methanol to triglycerides at 95 ^oC for 4 h. After that the remained triglycerides were transerterified at 65 ^oC for 1 h in reaction system containing 1 %wt KOH and 6:1 molar ratio of methanol to triglycerides. The final product after the two-step catalyzed process gave 97.02% of biodiesel.

In 2008, Kittipong, M. [25] studied biodiesel production from 25 oilseed plants in Thailand. The results showed that 86.65-95.4 %methyl ester content was obtained by using 1%NaOH, 6:1 molar ratios of methanol to oil at 65 ^oC for 1 h (transesterification reaction) and 1%H_2SO_4, 10:1 molar ratios of methanol to oil at 65 ^oC for 1 h (esterification reaction) followed by transesterification reaction. In addition, 16 plant species were found to have great potential of biodiesel and they meet the major specification of biodiesel standard.

In 2010, Prafulla et al. [23] studied transesterification of waste cooking oil from two different processes, using ferric sulfate and supercritical methanol. The results showed that 96% of biodiesel conversion was obtained by using ferric sulfate ($Fe_2(SO_4)_3$), 9:1 molar ratio of methanol to oil, at 100 ^oC for 2 h. The 50-65%yield of biodiesel was obtained by using supercritical methanol in only 15 min, reaction was carried out at pressure of 1450 psi (100 bar) and 300 ^oC in PARR micro-reactor.

Ferric sulfate showed good catalyst activity and easy recovery of the catalyst. The supercritical methanol method has high potential for both tranesterification of triglycerides and methyl esterification of high free fatty acid for petro-diesel substitute.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials and equipments

3.1.1 Raw material

30 oilseed plants from different parts in Thailand are listed in Table 3.1.

Table 3.1 Oilseeds and sources

Oilseeds	Sources
Basella alba	Jatujak market, Bangkok; Thailand
Brassica chinensis	Jatujak market, Bangkok; Thailand
Benincasa hispida	Jatujak market, Bangkok; Thailand
Brassica juncea	Jatujak market, Bangkok; Thailand
Brassica pekinensis	Jatujak market, Bangkok; Thailand
Caesalpinia crista	Sumpeng market, Bangkok; Thailand
Camellia oleifera	Jatujak market, Bangkok; Thailand
Cassia suratlensi	Department of Forestry, Bangkok; Thailand
Crassocephalum crepidioides	Jatujak market, Bangkok; Thailand
Citrullus lanatus	Sumpeng market, Bangkok; Thailand
Citrus maxima	Khlong Toei market, Bangkok; Thailand
Citrus reticulate	Khlong Toei market, Bangkok; Thailand
Cucurbita moschata	Jatujak market, Bangkok; Thailand
Chukrasia velutina	Department of Forestry, Bangkok; Thailand
Dalbergia oliveri Gamble	Department of Forestry, Bangkok; Thailand
Gardenia jasminodes	Sumpeng market, Bangkok; Thailand
Hibiscus sabdariffa Linn	Sumpeng market, Bangkok; Thailand
Lagenaria siceraria	Jatujak market, Bangkok; Thailand
Macadamia integrifolia	Doitung, Chiangrai, Thailand
Mimusops elengi	Jatujak market, Bangkok; Thailand
Millingtonia hortensis	Department of Forestry, Bangkok; Thailand
Millettia kangensis Craib	Jatujak market, Bangkok; Thailand
Moringa oleifera Lamk	Sumpeng market, Bangkok; Thailand
Myristica fragrans	Sumpeng market, Bangkok; Thailand

Table 3.1 Oilseeds and sources (*Continued*)

Oilseeds	Sources
Ocimum canum	Khlong Toei market, Bangkok; Thailand
Passiflora foetida	Jatujak market, Bangkok; Thailand
Phyllanthus emblica	Department of Forestry, Bangkok; Thailand
Perilla frutescens	Jatujak market, Bangkok; Thailand
Sesamum indicum	Sumpeng market, Bangkok; Thailand
Terminalia chebula	Department of Forestry, Bangkok; Thailand

3.1.2 Chemicals

Anhydrous sodium sulfate: analytical grade; Carlo Erba

Carbon tetrachloride;analytica grade; Mallinckrodt

Chloroform-D: NMR spectroscopy grade; Merck

Dichloromethane: analytical grade; Lab-Scan

Ethanol: analytical grade; Merck

Glacial acetic acid: analytical grade; Merck

Heptane: analytical grade; Merck

Hexane: analytical grade; Lab-Scan

Hydrochloric acid: analytical grade; Merck

Methanol: analytical grade; Merck

Methyl heptadecanoate: analytical grade; Fluka

Methyl stearate; analytical grade; Merck

Potassium hydroxide: analytical grade; Lab-Scan

Potassium dichromate: analytical grade; Lab-Scan

Potassium iodide: analytical grade; Lab-Scan

Potassium hydrogen phthalate

Sodium hydrogen carbonate: analytical grade; Carlo Erba

Sodium hydroxide: analytical grade; ACS

Sodium thiosulfate: analytical grade; Lab-Scan

Sulfuric acid: analytical grade; Carlo Erba

Toluene: analytical grade; Merck

Wijs solution: analytical grade; Merck

37 Components FAME standard; Supelco
2-Propanol: analytical grade; Merck

3.1.3 Equipments

Fourier-Transform NMR Spectrometer: Mercury (400MHz); Varian
Gas-liquid Chromatography; Model 3800; Varian
Cannon Automatic Viscometer: Model CAV-3; Cannon
Density meters: Model DMA 4500 ; Anton parr
Mini Flash: FLA; Grabner instruments
Rotary evaporator: Model ; Buchi

3.2 Methods

3.2.1 Solvent extraction of oilseed plants[25]

500 ml of hexane was added to 500 g of the crushed kernel in 2000 ml of Erlenmeyer flask and allowed to shake overnight. The organic extract was filtered and evaporated to dry by rotary evaporator. The percentage of oil was determined. The physical and chemical properties of oils were determined according to 3.2.2**.**

3.2.2 Determination of physical and chemical properties of crude oil from oilseed plants

3.2.2.1 Determination of free fatty acids contained in crude oils

The free fatty acid was determined according to ASTM D 5555; Standard test method for determination of free fatty acids contained in animal, marine, and vegetable fats and oils used in fat liquors and stuffing compounds.

To the 250 ml of Erlenmeyer flask, oil sample (1 g), ethanol (75 ml) and 2 ml of 1% phenolphthalein were added. The mixture was titrated with 0.25 N sodium hydroxide solutions until the pink color occurred. The volume of alkali solution used was recorded.

The percentage of free fatty acid was calculated as follows:

% free fatty acids = (ml of alkali x N x 28.2) / weight of sample
N = normality of alkaline solution
ml of alkali = ml of sodium hydroxide solution

3.2.2.2 Determination of the saponification value of crude oils

The saponification value was determined according to ASTM D 5558; Standard test method for determination of the saponification value of fats and oils.

To the 250 ml of Erlenmeyer flask, oil sample (1 g), alcoholic potassium hydroxide (25 ml) and 1 ml of 1% phenolphthalein were added. The mixture was titrated with 0.5 N of hydrochloric acid until the pink color disappeared. The volume of acid solution used was recorded.

The saponification value was calculated as follows:

saponification value = $56.1 \times N \times (A - B)$ / weight of sample
A = titration of blank
B = titration of sample
N = normality of hydrochloric acid solution
Alcoholic KOH = 40 g of potassium hydroxide dissolved in 1 L of ethanol

3.2.2.3 Determination of the iodine value of crude oils

The iodine value was determined according to ASTM D 5554; Standard test method for determination of the iodine value of fats and oils.

To the 500 ml of glass-stopper flask, oil sample (0.1 g), carbon tetra chloride (20 ml) and Wijs solution (25 ml) were added. Store the flasks in a dark place for 30 min. From storage, removed the flasks and add 20 ml of KI solution followed by 100 ml of distilled water. The mixture was titrated with 0.1 N of sodium thiosulfate until the yellow color has almost disappeared, add 2 ml of starch indicator solution, and continued the titration until the blue color has just disappeared. The volume of sodium thiosulfate used was recorded.

The iodine value was calculated as follows:

Iodine value = $(B - S) \times N \times 12.69$ / weight of sample
B = titration of blank
S = titration of sample
N = normality of $Na_2S_2O_3$ solution

3.2.2.4 Determination of the fatty acid composition of crude oils

The fatty acid compositions of the oils from oilseed plants were analyzed by gas chromatography (GC).

Sample (250 mg) was dissolved in heptadecanoic solution (5 ml) (500 mg heptadecanoate was dissolved in heptane (50 ml)) and 1 µl of solution was injected into using 1:100 split ratios.

The condition of GC used was as follows:

Column: ZB-Wax plus; Zebron, 30m, 0.25mm ID, 0.25µm
Injector temperature: 240 ℃
Detector: Flame ionization
Detector temperature: 250 ℃
Column Oven: 50 ℃ (hold 2 min), rate of 4 ℃/min to 220 ℃ (hold 15 min)
Carrier gas: N_2
Flow rate: 1.2 ml/min
Volume injected: 1 µl

3.2.3 Biodiesel production

3.2.3.1 Optimization for base catalyzed process[25]

- Effect of the amount of free fatty in palm oil to palm oil methyl ester.

20 g of palm oil with various amount of free fatty acid (1, 2, 3, 4, 5, and 6%wt) was added into 100 ml of round bottom flask equipped with condenser. After the oil was heated to 65 °C, 1%NaOH by wt. of oil (sodium hydroxide 0.2 g in methanol 5.79 ml) was added and then the mixture was heated to 65°C for 1.50 h. The mixture was transferred to a separatory funnel, allowed glycerol to separate for 2 h. The methyl ester layer (upper layer) was washed with hot water (5 x 100 ml), dried by rotary evaporator. The percent conversion of methyl ester was analyzed by ^{1}H-NMR.

- Effect of methanol to oil molar ratio and reaction time

The palm oil methyl ester synthesized following the method described in 3.2.3.1, by using molar ratios of methanol to oil equal 3:1, 6:1, 9:1 and 12:1, the reaction times equal 15, 30, 45, 60, 90 and 120 min. The methyl ester (5 mg) was subjected to ^{1}H-NMR analysis. The calculation conversion of product was shown in Appendix C.

3.2.3.2 Optimization for two-step catalyzed process (Acid – base catalyzed)

- Effect of reaction time, amount of free fatty acid and amount of catalyst [6]

20 g of palm oil with 10, 20 and 30% free fatty acid was added into 100 ml of round bottom flask equipped with condenser. After the oil was heated to 65 °C, various amount of ferric sulfate (1, 2, 3 and 4%wt for 10 and 20%FFA in palm

oil; 1, 2, 3, 4, 5 and 10%wt for 30%FFA in palm oil) and methanol (1:10 molar ratios of oil to methanol) was added, then the mixture was heated to 65^{o}C for 1, 2 and 3 h. The excess of methanol was removed by rotary evaporator, and the mixture was left to separate. The upper oil layer was subjected to determine the amount of FFA according to ASTM D 5555 and used in the second step following the method described in 3.2.3.1

- Effect of methanol to oil molar ratio and reaction time

The palm oil methyl ester with 10, 20 and 30% free fatty acid synthesized following the method described in 3.2.3.2, by using molar ratios of methanol to oil equal 6:1, 10:1 and 20:1, the reaction times equal 1, 2 and 3 h. The methyl ester (5 mg) was subjected to ^{1}H-NMR analysis. The calculation conversion of product was shown in Appendix C.

3.2.3.3 Synthesis of methyl ester from oilseed plants via base-catalyzed process (FFA< 3%)

The fatty acid methyl ester was synthesized following the method described in 3.2.3.1, using oils of 22 species with less than 3 % FFA. The product was characterized by ^{1}H-NMR and GC techniques.

3.2.3.4 Synthesis of methyl ester from oilseed plants via two-step catalyzed process (FFA> 3%)

This process involved an esterification in first step and transesterification in second step. At first step, the fatty acid methyl ester was synthesized following the method described in 3.2.3.2, using 8 species of oils with more than 3 % FFA. The lower layer was methyl ester and unreacted triglyceride was used in the second step following the method described in 3.2.3.1. The product was characterized by ^{1}H-NMR and GC techniques.

3.2.4 Characterization and determination of the biodiesels

3.2.4.1 Characterization of the biodiesels

The biodiesels were characterized by:

3.2.4.1.1. Fourier-transform NMR spectrometer (FT-NMR)

^{1}H NMR spectra were recorded in $CDCl_3$ using $CHCl_3$ as an internal standard.

3.2.4.2 % Ester contents

The %ester content was determined by ^{1}H-NMR and GC.

3.2.4.2.1. Fourier-transform NMR spectrometer (FT-NMR)

The conversion of methyl esters from oilseed plants was determined using ^{1}H NMR. The conversion of methyl esters was calculated by comparing the peak area of methoxy and methylene protons using the following equation:

$$\% \text{ conversion} = [(2I_{CH3}) / (3I_{CH2})] \times 100$$

3.2.4.2.2. Gas-liquid chromatography (GC)

The fatty acid compositions and methyl esters content of the oil from oilseed plants were also determined by GC.

The GC condition for the determination of methyl ester was set as 3.2.2.4. Methyl heptadecanoate was used as an internal standard. The methyl ester content of biodiesel was calculated by the following equation:

$$C = \left(\frac{\sum A - Ai}{Ai} \right) \times \left(\frac{Ci \times Vi}{m} \right) \times 100$$

C = Methyl ester content

$\sum A$ = Total area of fatty acid methyl esters

A_i = Area of methyl heptadecanoate

C_i = Concentration of methyl heptadecanoate solution

V_i = Volume of methyl heptadecanoate solution

m = Mass of the sample

The results were shown in appendix A.

3.2.4.3 Determination of the properties of biodiesel

The physical properties of biodiesel were determined according to the test methods shown in Table 3.2.

Table 3.2 Test method of biodiesel fuels

Property	Method
Viscosity at 40 °C (cSt)	ASTM D445
Flash point (℃)	ASTM D93
Density at 15 °C (kg/cm^3)	ASTM D4052
Acid number (mg KOH/g)	ASTM D974
Ester content (%wt)	EN 14103

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Solvent extraction of oilseed plants

The oils from 30 oilseed plants in different part of Thailand were extracted from the kernel or seed using hexane as a solvent. The amount of oils were shown in the Table 4.1. The percent oil contents were in range of 13-69 %wt. The highest and lowest oil content could be extracted from *Dalbergia oliveri Gamble* (DO) and *Sesamum indicum* (SEI), respectively. Moreover, some oilseed plants in this study may be a new source of raw materials for biodiesel production because of its high percentage of oil content.

4.2 Physical and chemical properties of crude oil from oilseed plants for biodiesel production

The physical and chemical properties, % free fatty acid, saponification value, iodine value and fatty acid profiles, of crude oil from oilseed plants were listed in Table 4.1.

Table 4.1 Summary of physical and chemical properties of crude oils from oilseed plants

Oilseed plants	Family	Codes	% Oil	% FFA	SN	IV	Fatty acid composition (%)
Basella alba	Basellaceae	BA	27	0.65	179.05	76.78	C14:0(0.15), C16:0(22.47), C16:1(0.41), C18:0(4.15), C18:1n9c(44.25), C18:2n6t(23.54),C18:3n3(0.23), C20:0(1.12), C20:1n9(0.32), C22:0(0.63), C24:0(0.20), C24:1n9c(0.49), Unk (2.03)
Brassica chinensis	Cruciferae	BC	44	1.44	161.23	56.29	C16:0(1.61), C18:0(0.77), C18:1n9c(14.58), C18:2n6t(10.09), C18:3n3(7.05), C20:0(0.62), C20:1n9(5.74), C20:2(0.31), C22:0(0.66), C22:1n9(54.67), C22:2(0.64), C24:0(0.24), C24:1n9(1.48), Unk (2.18)
Benincasa hispida	Cucurbitaceae	BEH	25	1.39	180.57	128.96	C16:0(9.82), C18:0(4.96), C18:1n9c(6.40), C18:2n6t(77.06), C18:3n3(0.22), C20:0(0.19), C20:1n9(0.15), C20:2(0.36), Unk (1.44)
Brassica juncea	Cruciferae	BJ	36	2.79	165.29	102.06	C16:0(2.19), C16:1(0.15), C18:0(0.86), C18:1n9c(7.39), C18:1n9t(0.71),C18:2n6c(13.39), C18:3n3(10.13), C20:0(0.78), C20:1n9(4.95), C20:2(0.70), C20:4n6(0.15), C20:5(1.01), C22:1n9(50.47), C22:2(1.37), C24:0(0.55), C24:1n9c(1.95), Unk(2.37)
Brassica pekinensis	Cruciferae	BP	26	2.17	166.56	96.36	C16:0(1.96), C18:0(0.96),C18:1n9c(12.50), C18:1n9t(0.61), C18:2n6c(11.81), C18:3n3(8.01), C20:0(0.84), C20:1n9(7.20), C20:2(0.56), C20:5(0.99), C22:1n9(50.05), C22:2(0.84), C24:0(0.44), C24:1n9c(1.60), Unk (1.90)
Caesalpinia crista	Caesalpiniaceae	CAC	21	1.07	173.51	125.03	C16:0(7.91), C18:0(3.63), C18:1n9c(13.17), C18:1n9t(0.41), C18:2n6t (73.91), C20:0(0.26), C20:1n9(0.36), C22:1n9(0.41)
Camellia oleifera	Theaceae	CAO	37	0.66	168.23	115.68	C16:0(6.35), C18:0(2.92), C18:1n9c(13.79), C18:2n6t(58.75), C18:3n6(0.52), C18:3n3(15.43), C20:0(0.68), C20:1n9(0.34), C22:0(0.24), C24:0(0.15), Unk(0.83)
Cassia suratlensi	Leguminosae	CAS	15	0.73	173.09	95.46	C16:0(22.08), C16:1(0.22), C18:0(4.77), C18:1n9c(22.91), C18:2n6t(42.47), C18:3n6(0.09), C18:3n3(0.64), C20:0(1.88), C20:1n9(0.30), C22:0(0.95), C23:0(0.15), C24:0(0.57), C24:1n9c(0.24), Unk(0.47)

SN* = Saponification values, IV* = Iodine values

Table 4.1 Summary of physical and chemical properties of crude oils from oilseed plants *(continued)*

Oilseed plants	Family	Codes	% Oil	% FFA	SN*	IV*	Fatty acid composition (%)
Crassocephalum crepidioides	Compositae	CC	37	0.70	163.30	94.00	C16:0(1.85), C16:1(0.15), C18:0(0.82), C18:1n9c(6.98), C18:1n9t(0.90), C18:2n6c(12.31), C18:3n3(12.41), C20:0(0.76), C20:1n9(4.16), C20:2(0.72), C20:3n3(0.19), C20:5n3(1.31), C22:0(50.73), C22:1n9(0.32), C22:2(1.58), C23:0(0.59), C24:0 (0.64), C24:1n9(2.25), Unk(1.34)
Citrullus lanatus	Cucurbitaceae	CIL	56	13.86	204.59	138.42	C16:0(9.94), C18:0(5.94), C18:1n9c(10.97), C18:1n9t(0.49), C18:2n6t (71.92), C20:0(0.23), C20:4n6(0.13), C22:0(0.15), C22:1n9(0.15), C24:0(0.20), Unk(0.22)
Citrus maxima	Rutaceae	CIM	62	2.60	194.37	97.71	C16:0(22.49), C18:0(5.98), C18:1n9c(26.28), C18:1n9t(0.54), C18:2n6t(40.61), C18:3n3(3.58), C20:0(0.33), C24:0(0.18)
Citrus reticulata	Rutaceae	CIR	56	0.64	176.74	95.43	C16:0(21.17), C16:1(0.51),C18:0(4.45), C18:1n9c(19.57), C18:1n9t(1.96), C18:2n6c(45.96), C18:3n3(4.49), C20:0(0.52), C20:1n9(0.13), C22:0(0.13), C23:0(0.63), C24:0(0.27), Unk(0.14)
Cucurbita moschata	Cucurbitaceae	CUM	38	5.82	173.72	99.60	C14:0(0.13), C16:0(20.48), C18:0(8.28), C18:1n9c(20.15), C18:1n9t(0.42), C18:2n6c (48.01), C18:3n3(0.18), C20:0(0.43), C24:0(0.21), C24:1n9(0.13), Unk(0.74)
Chukrasia velutina	Meliaceae	CV	33	12.01	171.25	110.11	C16:0(8.29), C18:0(5.19), C18:1n9c(7.63), C18:1n9t(0.53), C18:2n6t(55.73), C18:3n3(22.22), C20:0(0.36), C20:1n9(0.22), Unk(0.32)
Dalbergia oliveri Gamble	Papilionaceae	DO	13	1.40	173.28	60.44	C16:0(11.81), C18:0(6.76), C18:1n9c(53.90), C18:2n6c(11.39), C18:3n3(0.59), C20:0(1.99), C20:1n9(1.63), C22:0(3.64), C22:1n9(0.28), C23:0(0.20), C24:0(4.45), Unk(0.57)
Gardenia jasminodes	Rubiaceae	GJ	22	1.59	187.78	112.53	C14:0(0.16), C16:0(19.71), C16:1(0.33), C18:0(3.22), C18:1n9c(0.99), C18:2n6c(49.89), C18:3n3(1.34), C20:0(0.41), C20:1n9(0.16), C24:0(0.53), Unk(23.53)

SN* = Saponification values
IV* = Iodine values

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Table 4.1 Summary of physical and chemical properties of crude oils from oilseed plants *(continued)*

Oilseed plants	Family	Codes	% Oil	% FFA	SN*	IV*	Fatty acid composition (%)
Hibiscus sabdariffa Linn.	Malvaceae	HIS	22	1.39	181.33	81.83	C14:0(0.24), C16:0(20.16), C18:0(4.97), C18:1n9c(37.66), C18:2n6c(31.61), C18:3n3(0.19), C20:0(0.84), Unk(2.34)
Lagenaria siceraria	Cucurbitaceae	LS	54	2.72	183.74	119.20	C16:0(11.71), C18:0(6.48), C18:1n9c(5.51), C18:1n9t (0.31), C18:2n6t(74.79), C18:3n3(0.15), C20:0(0.31), C20:1n9(0.11), C20:5n3(0.10), C22:1n9(0.11), Unk(0.42)
Macadamia integrifolia	Proteaceae	MAI	60	0.67	178.06	67.49	C14:0(0.87), C16:0(8.39), C16:1(19.95), C18:0(3.36), C18:1n9c(55.43), C18:1n9t(3.25), C18:2n6c(1.47), C18:3n3(0.12), C20:0(2.45), C20:1n9(2.08), C22:0(0.68), C22:1n9(0.21), C24:0(0.30), Unk(1.74)
Mimusops elengi	Sapotaceae	ME	22	7.85	195.70	67.81	C16:0(11.91), C16:1(0.12), C18:0(8.55), C18:1n9c(64.24), C18:2n6c(11.85), C18:3n3(0.13), C20:0(1.18), C20:1n9(0.80), C22:0(0.54), C24:0(0.36), C24:1n9c(0.32)
Millingtonia hortensis	Bignoniaceae	MH	40	2.70	175.88	54.16	C16:0(11.32), C16:1(0.18), C18:0(9.53), C18:1n9c(67.70), C18:2n6c(3.04), C18:3n3(0.39), C20:0(2.97), C20:1n9(1.69), C20:5n3(3.17), C22:1n9(0.47), C24:0(0.94), Unk(5.90)
Millettia kangensis Craib	Leguminosae	ML	31	1.35	176.48	78.40	C16:0(5.67), C18:0(2.80), C18:1n9t(46.26), C18:2n6t(29.25), C18:3n6(0.10), C18:3n3(1.82), C20:0(1.17), C20:1(2.87), C22:0(7.12), C22:1(0.58)
Moringa oleifera Lamk.	Moringaceae	MO	37	3.17	180.14	61.58	C16:0(5.57), C16:1(1.16), C18:0(4.80), C18:1n9c(71.14),C18:1n9t(3.73), C18:2n6c(0.67), C18:3n3(0.15), C20:0(3.09), C20:1n9(2.45), C22:0(6.34), C22:1n9(0.16), C24:0(0.85)
Myristica fragrans	Myristicaceae	MYF	42	18.62	209.54	45.13	C10:0(0.41), C12:0(0.50), C14:0(70.17), C14:1(0.16), C16:0(7.35), C18:0(0.83), C18:1n9c(8.86), C18:2n6c(0.57), Unk(9.26)

SN* = Saponification values
IV* = Iodine values

Table 4.1 Summary of physical and chemical properties of crude oils from oilseed plants *(continued)*

Oilseed plants	Family	Codes	% Oil	% FFA	SN*	IV*	Fatty acid composition (%)
Ocimum canum	Labiatae	OC	21	5.47	181.93	113.30	C16:0(6.27), C16:1(0.11), C18:0(3.42), C18:1n9c(12.67), C18:2n6c(21.34), C18:3n3(54.52), C20:0(0.22), C20:1n9(0.15), C20:2(0.25), C23:0(0.15), C24:0(0.15), Unk(0.60)
Passiflora foetida	Passifloraceae	PAF	23	1.38	179.66	126.16	C16:0(10.64), C16:1(0.19), C18:0(2.45), C18:1n9c(0.53), C18:2n6c(70.84), C18:3n3(0.48), C20:0(0.15), C20:1n9(0.14), Unk(14.98)
Phyllanthus emblica	Euphorbiaceae	PE	17	2.10	186.00	164.38	C16:0(10.64), C18:0(6.64), C18:1n9c(11.54), C18:1n9t(0.86), C18:2n6c(17.10), C18:3n3(51.01), C20:0(0.22), C20:1n9(0.23), Unk(0.28)
Perilla frutescens	Labiatae	PF	42	1.17	172.40	118.04	C16:1(7.22), C18:0(2.93), C18:1n9c(14.06), C18:1n9t(0.83), C18:2n6c(28.03), C18:3n3(46.02)
Sesamum indicum	Pedaliaceae	SEI	69	1.34	179.26	94.09	C16:0(8.99), C18:0(5.43), C18:1n9c(40.08), C18:1n9t(0.52),C18:2n6c(43.59), C20:0(0.58)
Terminalia chebula	Combretaceae	TC	49	30.13	185.31	91.65	C16:0(17.28), C18:0(7.18), C18:1n9c(33.42), C18:2n6t(39.25), C20:0(1.08), C20:1n9(0.26), C20:2(0.17), C22:0(0.70), C24:0(0.18)

SN* = Saponification values
IV* = Iodine values

From Table 4.1, the results showed that %free fatty acid were varied from 0.64-30.13%wt, *Citrus reticulata* and *Terminalia chebula* gave the lowest and highest %free fatty acid, respectively. The value of free fatty acid content could be depended on type of plants and duration of oil storage.

Saponification numbers were in the range of 161.23-209.54 mgKOH/g. The highest and lowest saponification numbers were belonged to *Myristica fragrans* (MYF) and *Brassica chinensis* (BC), respectively. Oils having high percentages of high molecular weight fatty acids will have lower saponification numbers than oils having high percentages of lower molecular weight fatty acids[26]. Thus, *Myristica fragrans* (MYF) oil containing 70.17 percent of myristic acid (C14:0) has high saponification number of 209.54 mg KOH/g. *Brassica chinensis* (BC) oil containing 54.67% of erucic acid (C22:1n9), has low saponification number of 161.23 mg KOH/g.

In addition, iodine values were in the range of 45.13-164.38 mgI_2/g. The highest and lowest iodine values were belonged to *Phyllanthus emblica* (PE) and *Myristica fragrans* (MYF), respectively. Because iodine absorption occurs at double bond positions, thus a higher iodine value indicates a higher quantity of double bonds in the oil[27]. The result showed that most oilseed plants contained unsaturated fatty acid such as BEH, CAC, CIL, PAF and PE. In contrast, MYF seed oil contains mostly saturated fatty acids.

According to ASTM standard, maximum value of iodine value was set to 120 mg I_2/g. Therefore the oil of 25 species including BA, BC, BJ, BP, CAO, CAS, CC, CIM, CIR, CUM, CV, DO, GJ, HIS, LS, MAI, ME, MH, ML, MO, MYF, OC, PF, SEI and TC oils could be used to produce biodiesel.

Fatty acid profiles of these oilseed plants were determined by the comparison of 37 FAMEs standard. The GC chromatogram of these standards and these fatty acid profiles were shown in Figure A1-A31.

4.3 Biodiesel production

4.3.1. Optimization for base catalyzed process

- Effect of the amount of free fatty acid in palm oil.

The synthesis of biodiesel from palm oil with various amount of free fatty acids (FFA) content (1, 2, 3, 4, 5 and 6 % wt) was investigated. The reaction conditions were carried out by using 6:1 molar ratio of methanol to oil, 1% NaOH by weight of oil at 65°C for 1.50 h. The results were shown in Figure 4.1

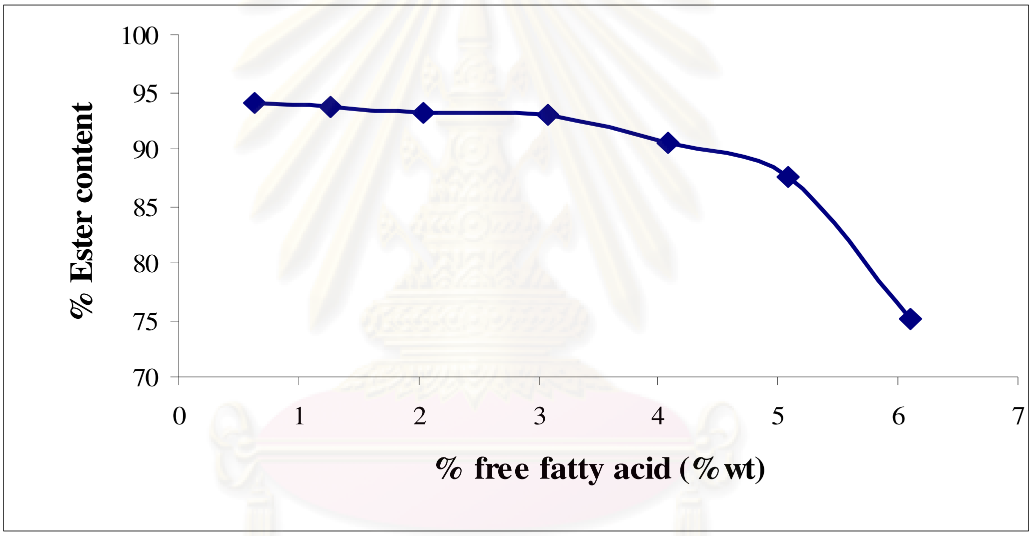

Figure 4.1 Effect of the amount of free fatty acid in palm oil.

As seen in Figure 4.1, the results showed that %ester content more than 93% could be achieved when FFA lower than 3%. Therefore, this process was suitable to synthesis biodiesel from oil containing FFA less than 3%.

- Effect of methanol to oil molar ratio and reaction time

The synthesis of biodiesel from palm oil by using molar ratios of methanol to oil equal 3:1, 6:1, 9:1 and 12:1, the reaction times equal 15, 30, 45, 60, 90 and 120 min, 1% NaOH by weight of oil at 65°C were investigated. The results were shown in Figure 4.2.

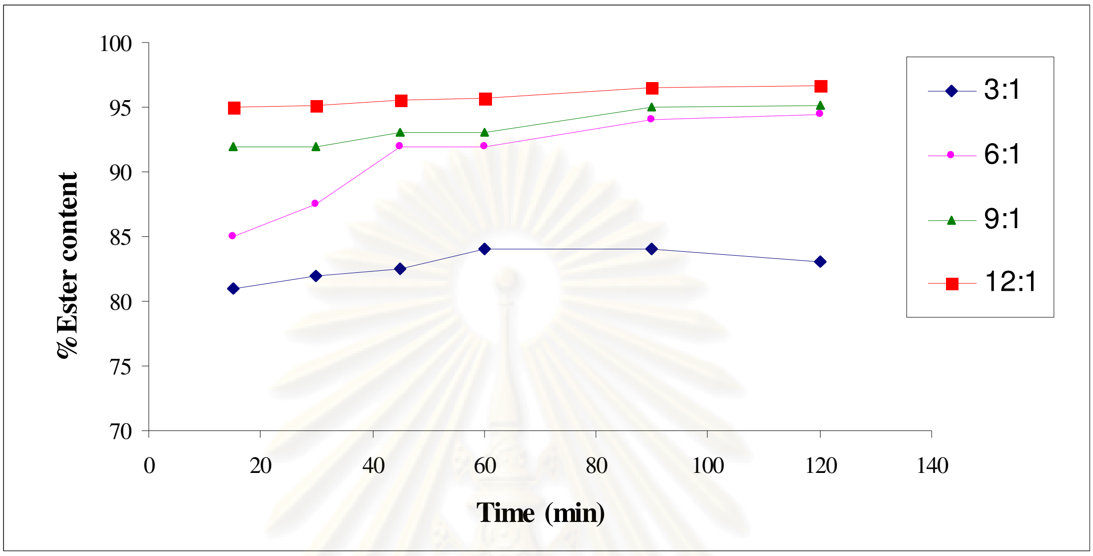

Figure 4.2 Effect of methanol to oil molar ratio and reaction time.

From Figure 4.2, at higher molar ratio, the ester content was increased. In addition, %ester content of each molar ratios of methanol to oil at 15 to 120 minutes gave 81-84% for 3:1 ratios, 85-94.5 % for 6:1 ratios, 92-95.2% for 9:1 ratios and 95-96.7% for 12:1 ratios. Hence, 12:1 molar ratio of methanol to oil was used to synthesize biodiesel in this process for 1.5 h to obtain the high ester content.

Therefore, oilseed plants in table 4.1 including BA, BC, BEH, BJ, BP, CAC, CAO, CAS, CC, CIM, CIR, DG, GJ, HIS, LS, MAI, MH, ML, PAF, PE, PF and SEI having FFA less than 3% could be synthesized by using base catalyzed process, 1%NaOH by weight of oil, 12:1 molar ratio of methanol to oil at 65°C for 1.5 h.

4.3.2. Optimization for two-step catalyzed process (Acid – base catalyzed)

- Effect of reaction time, amount of free fatty acid and amount of catalyst.

The conditions for synthesis of biodiesel from palm oil with various amount of free fatty acids content (10, 20 and 30 %wt) were investigated by varying amount of ferric sulfate (1, 2, 3, 4, 5 and 10 %wt) and reaction time (1, 2 and 3 h). The results were shown in Figures 4.3, 4.4, and 4.5.

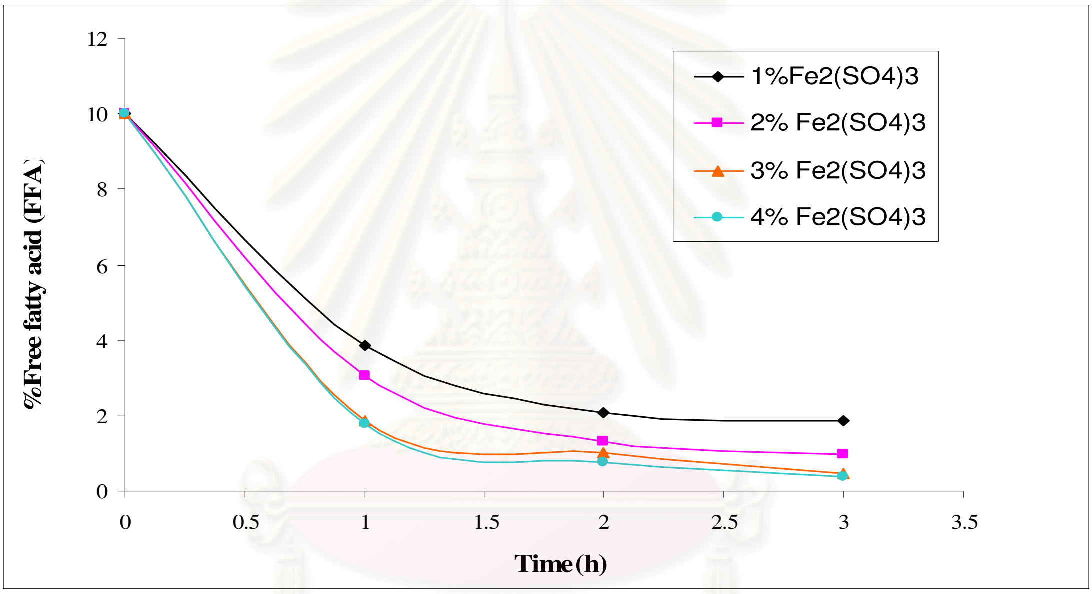

Figure 4.3 Effect of reaction time and amount of ferric sulfate to palm oil having 10%FFA (10:1 molar ratio of methanol to oil at 65 ℃).

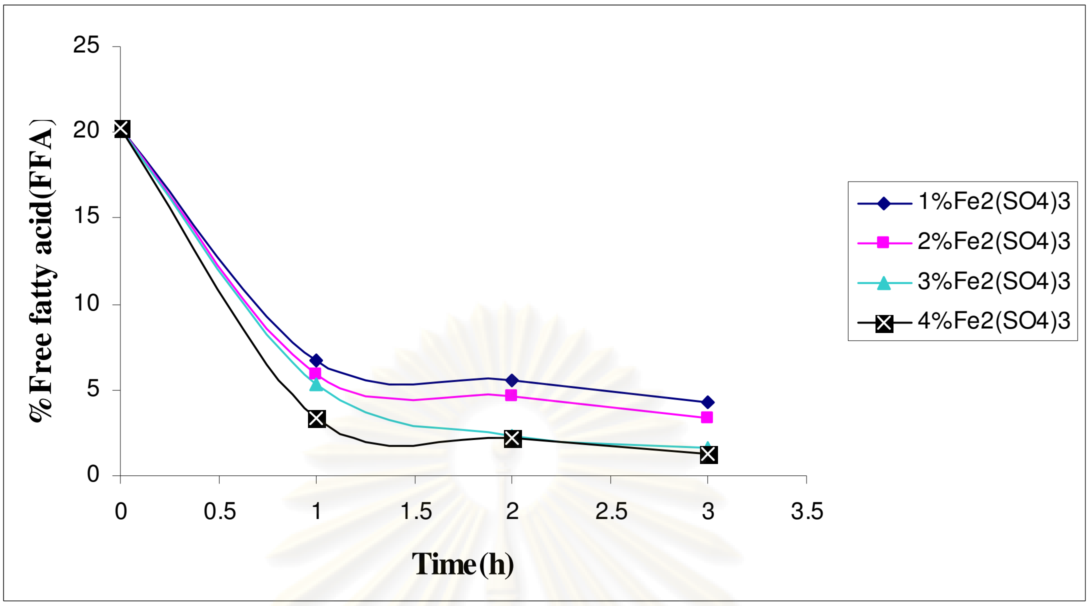

Figure 4.4 Effect of reaction time and amount of ferric sulfate to palm oil having 20%FFA (10:1 molar ratio of methanol to oil at 65℃).

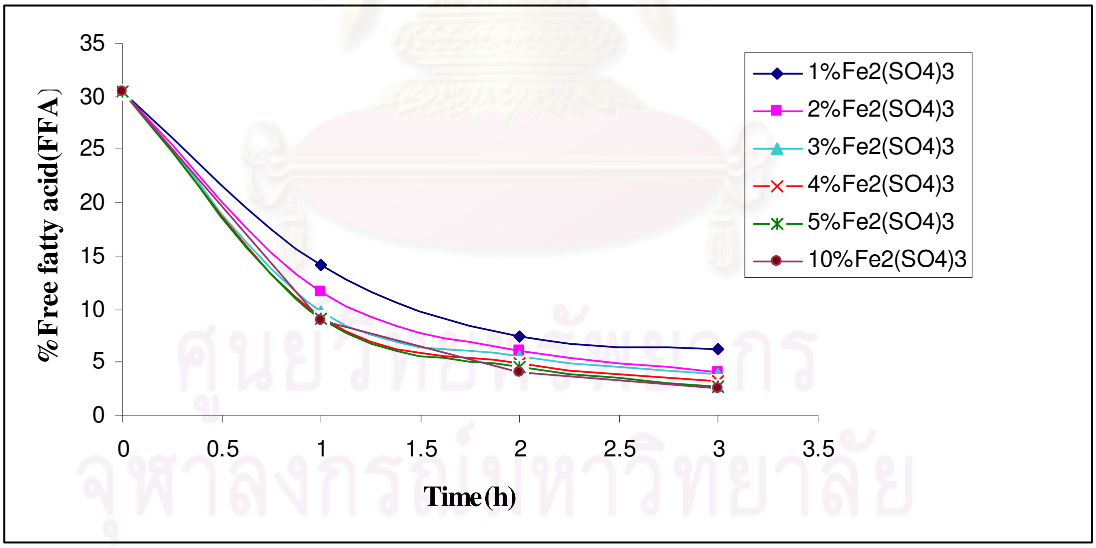

Figure 4.5 Effect of reaction time and amount of ferric sulfate to palm oil having 30%FFA (10:1 molar ratio of methanol to oil at 65℃).

It could be seen in the Figure 4.3, FFA could be decreased by using higher amount of $Fe_2(SO_4)_3$ and longer period of reaction time. To obtain oil having FFA less than 3%, 1%$Fe_2(SO_4)_3$ for 3 h, 2%$Fe_2(SO_4)_3$ for 2 h, 3 and 4%$Fe_2(SO_4)_3$ for 1 h must be used. Therefore, the optimal condition for synthesis of biodiesel of oil having FFA 10% or less was 3%$Fe_2(SO_4)_3$ for 1 h.

Similarly, the optimal conditions for synthesis of biodiesel of oils having FFA 20% or less and 30% or less were 3%$Fe_2(SO_4)_3$ for 2 h and 5%$Fe_2(SO_4)_3$ for 3 h, respectively.

- Effect of methanol to oil molar ratio and reaction time

The conditions for synthesis of biodiesel from palm oil with various amount of free fatty acids content (10, 20 and 30 %wt) were investigated by varying molar ratio of methanol to oil (6:1, 10:1 and 20:1) and reaction time (1, 2 and 3 h). The results were shown in Figure 4.6, 4.7 and 4.8.

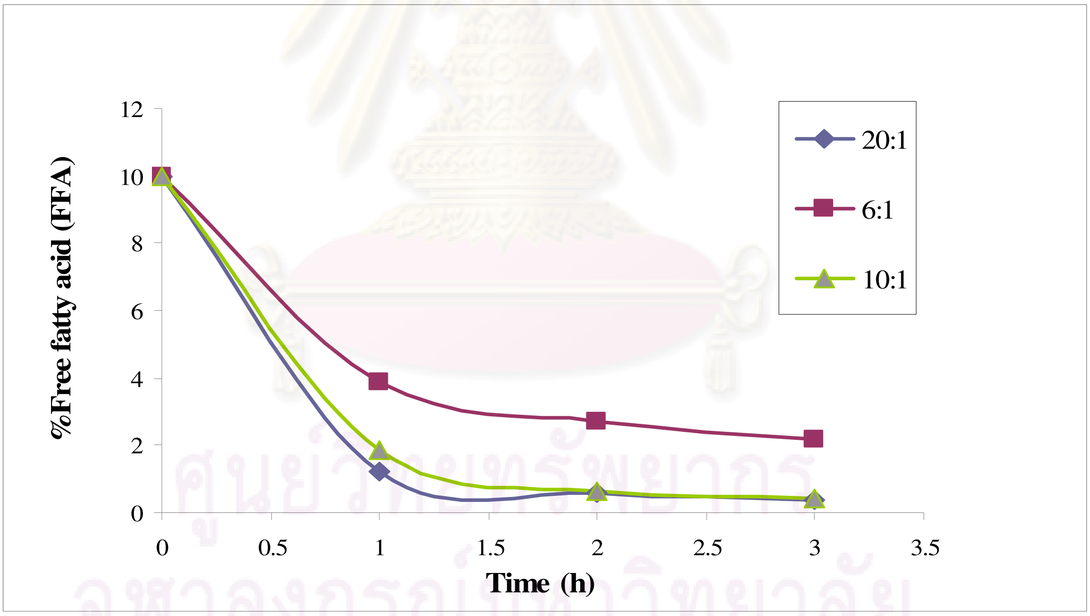

Figure 4.6 Effect of methanol to oil molar ratio and reaction time to palm oil having 10%FFA (3% $Fe_2(SO_4)_3$ at 65℃).

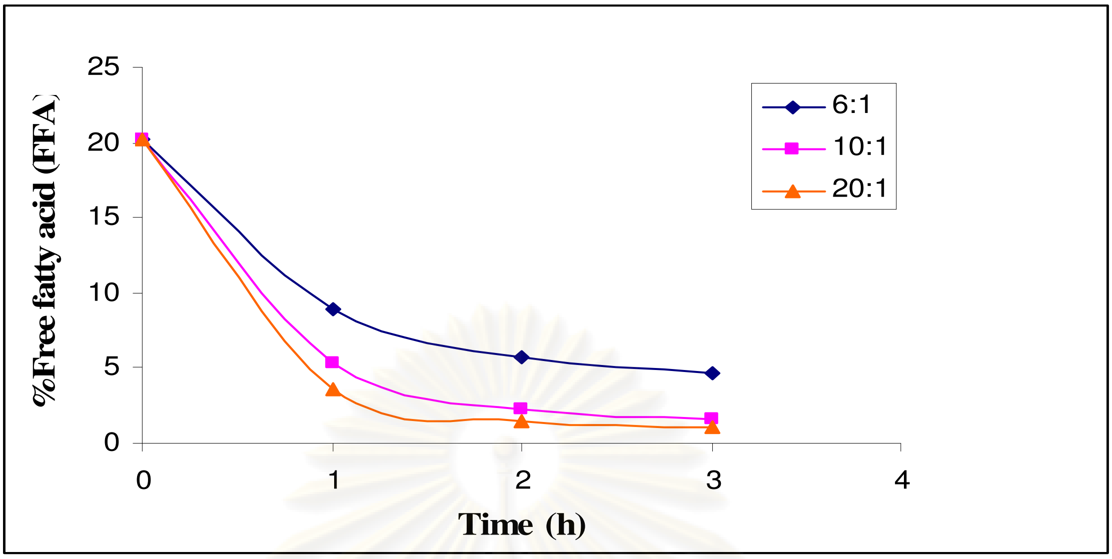

Figure 4.7 Effect of methanol to oil molar ratio and reaction time to palm oil having 20%FFA (3% $Fe_2(SO_4)_3$ at 65℃).

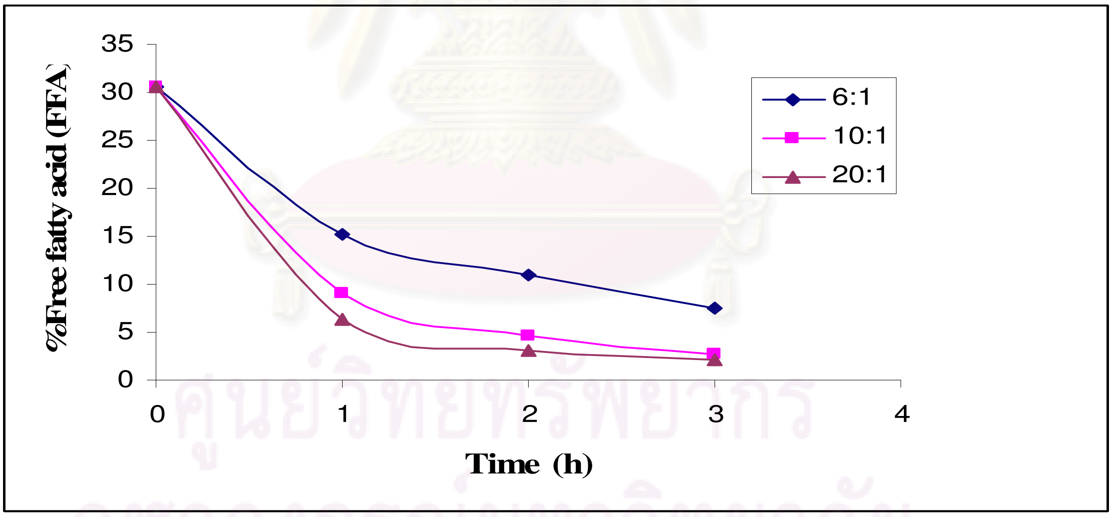

Figure 4.8 Effect of methanol to oil molar ratio and reaction time to palm oil having 30%FFA (3% $Fe_2(SO_4)_3$ at 65℃).

As seen in the Figure 4.6, FFA of oil was decreased lower than 3% by using 6:1 molar ratio of methanol to oil for 2 h, 10:1 and 20:1 for 1 h. Therefore, 3% $Fe_2(SO_4)_3$, 10:1 molar ratio for 1 h were found to be the optimal conditions for synthesis of biodiesel of oil having 10%FFA or less.

By using 10:1 molar ratio of methanol to oil, the optimal condition of oil having 20%FFA or less was 3% $Fe_2(SO_4)_3$ for 2 h, whereas, oil having 30%FFA or less was 5% $Fe_2(SO_4)_3$ for 3 h.

It could be concluded that the optimal conditions of oils having FFA less than 10% such as CUM, ME, MO and OC were 3% $Fe_2(SO_4)_3$, 10:1 molar ratio for 1 h. For oils having FFA less than 20% such as CIL, CV and MYF were synthesized by using 3% $Fe_2(SO_4)_3$, 10:1 molar ratio for 2 h. In addition, the conditions for TC oil which had 30%FFA was found to be 5% $Fe_2(SO_4)_3$, 10:1 molar ratio for 3 h.

4.3.3 Synthesis of methyl ester from oilseed plants via base-catalyzed process (FFA< 3%)

The methyl ester of oilseed plants having %FFA less than 3% were synthesized by using the method and conditions as described in 3.2.3.1. The results of % ester content were shown in Table 4.2.

Table 4.2 Summary of synthetic results of methyl ester of oilseed plants

Oilseed plants	**Codes**	**% FFA**	**% Ester content**
Basella alba	BA	0.65	94.68
Brassica chinensis	BC	1.44	94.57
Benincasa hispida	BEH	1.39	95.32
Brassica juncea	BJ	2.79	94.63
Brassica pekinensis	BP	2.17	95.08
Caesalpinia crista	CAC	1.07	94.68
Camellia oleifera	CAO	0.66	96.83
Cassia suratlensi	CAS	0.73	91.17
Crassocephalum crepidioides	CC	0.70	95.10
Citrus maxima	CIM	2.60	95.01

Table 4.2 Summary of synthetic results of methyl ester of oilseed plants (continued)

Oilseed plants	**Codes**	**% FFA**	**% Ester content**
Citrus reticulata	CIR	0.64	95.51
Dalbergia oliveri Gamble	DO	1.40	95.31
Gardenia jasminodes	GJ	1.59	94.74
Hibiscus sabdariffa Linn.	HIS	1.39	92.06
Lagenaria siceraria	LS	2.72	96.76
Macadamia integrifolia	MAI	0.67	95.96
Millingtonia hortensis	MH	2.70	93.77
Millettia kangensis Craib	ML	1.35	92.90
Passiflora foetida	PAF	1.38	95.16
Phyllanthus emblica	PE	2.10	92.43
Perilla frutescens	PF	1.17	93.95
Sesamum indicum	SEI	1.34	96.20

As discussed in 4.3.1, %ester content was effected by % FFA. Thus, oilseed plants with %FFA less than 3% was chosen to synthesize biodiesel via base catalyzed process. %ester content was in range of 91.17-96.83 %.

4.3.4 Synthesis of methyl ester from oilseed plants via two-step catalyzed process (FFA>3%)

The methyl ester of oilseed plants with %FFA less than 3 % were synthesized by using the method and conditions as described in 3.2.3.2. The results of %ester content were shown in Table 4.3.

Table 4.3 Summary of methyl ester of oilseed plant from synthesize with two-step catalyzed process (FFA>3%)

Oilseed plants	**Codes**	**% FFA**	**% Ester content**
Citrullus lanatus	CIL	13.86	95.83
Cucurbita moschata	CUM	5.82	93.60
Chukrasia velutina	CV	12.01	94.32
Mimusops elengi	ME	7.85	96.34
Moringa oleifera Lamk.	MO	3.17	96.56
Myristica fragrans	MYF	18.62	94.24
Ocimum canum	OC	5.47	96.61
Terminalia chebula	TC	30.13	94.20

As discussed in 4.3.1 and 4.3.2, %ester content was effected by % FFA. Thus, oilseed plants with %FFA more than 3% was chosen to synthesize biodiesel via two-step catalyzed process. %ester content was in range of 93.60-96.61 %.

4.4 Characterization of the biodiesels.

4.4.1 ^{1}H-NMR (Nuclear magnetic resonance)

Fatty acid methyl esters of various oilseed plants were characterized by ^{1}H-NMR. The ^{1}H-NMR spectra of crude oils and fatty acid methyl esters of oils of 30 species were shown in Figures A32-A91. For the example, the ^{1}H-NMR spectra of crude BEH oil and BEH oil methyl ester were shown in Figures 4.9 and 4.10, respectively.

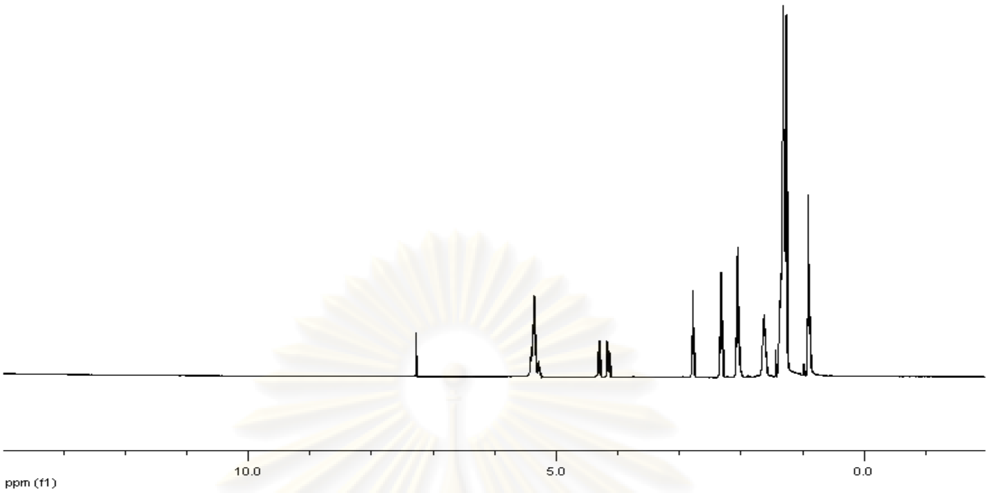

Figure 4.9 [1]H-NMR spectrum of crude BEH oil.

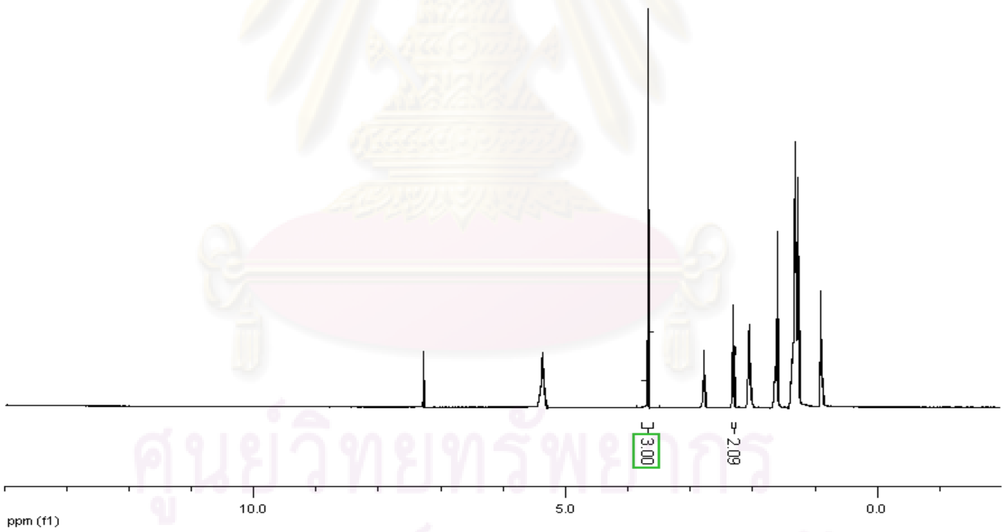

Figure 4.10 [1]H-NMR spectrum of BEH oil methyl ester.

From Figure 4.9, the characteristic peaks of crude BEH oil were observed at δ_H 4.05- 4.35 ppm which were the protons of glycerol moiety and Figure 4.10, the signal at δ_H 3.7 ppm was the protons of methoxy group in BEH oil methyl ester.Therefore, ^{1}H-NMR could be used to analyze two different oils with the signal at

δ_H 4.05- 4.35 ppm in Figure 4.9 was disappeared in Figure 4.10. In addition, signal of methoxy group at δ_H 3.7 ppm in Figure 4.10 was not shown in Figure 4.9.

4.5 Properties of biodiesel

The properties of biodiesel such as viscosity, flash point, density, acid value and ester content were determined according to ASTM and EN standards in Table 4.4. The values of these properties of methyl esters of these oilseed plants were shown in Table 4.4.

Table 4.4 Specification for quality of biodiesel

Property	**Value**	**Method**
Viscosity at 40 °C	3-5 cSt	ASTM D445
Flash point	>120 °C	ASTM D93
Density at 15 °C	860-900 kg/cm^3	ASTM D4052
Acid number	< 0.5 mg KOH/g	ASTM D974
Ester content	> 96.5 %wt	EN 14103

Table 4.5 Summary of properties of biodiesel from various oilseed plants

Oilseed plants	**Codes**	**Viscosity (cSt)**	**Flash point (°C)**	**Density (kg/cm^3)**	**Acid number (mg KOH/g)**	**conversion (^{1}H-NMR)**	**Ester content (GC)**
Basella alba	BA	4.5	>120	882.97	0.1878	94.34	94.68
Brassica chinensis	BC	5.0	>120	872.14	0.2502	94.79	94.57
Benincasa hispida	BEH	3.9	>120	881.00	0.1255	95.69	95.32
Brassica juncea	BJ	5.5	>120	858.37	0.3140	94.79	94.62
Brassica pekinensis	BP	5.2	>120	851.72	0.3144	95.24	95.08
Caesalpinia crista	CAC	3.5	>120	862.37	0.1889	94.79	94.68
Camellia oleifera	CAO	3.8	>120	880.55	0.1881	97.09	96.83
Cassia suratlensi	CAS	4.4	>120	882.81	0.3143	92.59	91.71
Crassocephalum crepidioides	CC	5.0	>120	866.95	0.1262	94.79	95.10
Citrullus lanatus	CIL	3.5	>120	880.37	0.3143	94.79	95.83

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Table 4.5 Summary of properties of biodiesel from various oilseed plants *(continued)*

Oilseed plants	Codes	Viscosity (cSt)	Flash point (°C)	Density (kg/cm^3)	Acid number (mg KOH/g)	Conversion (^{1}H-NMR)	Ester content (GC)
Citrus maxima	CIM	4.1	>120	881.57	0.2524	96.15	95.01
Citrus reticulata	CIR	3.6	>120	880.01	0.1877	95.69	95.51
Cucurbita moschata	CUM	4.5	>120	882.97	0.1256	93.90	93.60
Chukrasia velutina	CV	3.9	>120	861.71	0.1888	94.79	94.32
Dalbergia oliveri Gamble	DO	4.8	>120	873.05	0.1260	95.24	95.31
Gardenia jasminodes	GJ	4.8	>120	884.27	0.4396	95.24	94.74
Hibiscus sabdariffa Linn.	HIS	4.4	>120	882.62	0.1888	92.17	92.06
Lagenaria siceraria	LS	3.5	>120	879.95	0.2503	95.69	96.76
Macadamia integrifolia	MAI	4.3	>120	882.34	0.1890	95.69	95.96
Mimusops elengi	ME	4.5	>120	887.64	0.1879	96.15	96.34

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Table 4.5 Summary of properties of biodiesel from various oilseed plants *(continued)*

Oilseed plants	**Codes**	**Viscosity (cSt)**	**Flash point (℃)**	**Density (kg/cm^3)**	**Acid number (mg KOH/g)**	**conversion (^{1}H-NMR)**	**Ester content (GC)**
Millingtonia hortensis	MH	5.3	>120	885.84	0.4390	94.34	93.77
Millettia kangensis Craib	ML	4.6	>120	879.97	0.1252	94.34	92.90
Moringa oleifera Lamk.	MO	4.7	>120	883.75	0.3132	95.69	96.56
Myristica fragrans	MYF	3.9	>120	880.96	0.1254	93.46	94.24
Ocimum canum	OC	3.6	>120	879.72	0.1877	96.15	96.61
Passiflora foetida	PAF	3.9	>120	880.80	0.1886	96.62	95.16
Phyllanthus emblica	PE	3.6	>120	867.41	0.4404	93.02	92.43
Perilla frutescens	PF	3.2	>120	886.87	0.4383	94.79	93.95
Sesamum indicum	SEI	4.1	>120	881.49	0.1262	96.62	96.72
Terminalia chebula	TC	4.7	>120	880.74	0.1253	94.34	94.20

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4.5.1 Viscosity

From Table 4.5, the viscosity of 30 oils methyl esters of oilseed plants were in the range of 3.2-5.5 cSt. The viscosity of methyl esters of BJ and BP were above the standard values. Moreover, the results showed that the viscosity values were depended on chain length of fatty acids degree of unsaturation of fatty acid and unreacted triglycerides (including mono- and di-glycerides) [25]. For example, the viscosity value of PF was 3.2 cSt and the fatty acid compositions were in the range of C16-C18 atoms with C18:3 was the main composition (46.02%). While the viscosity values of BJ was 5.5 cSt and the fatty acid compositions were in the range of C16-C24 atoms, with C22:1 was the main composition (50.47%). It could be concluded that oil with long chain carbon should have high viscosity.

4.5.2 Density

The standard for biodiesel states that the fuel should have a density between 860 and 900 kg /cm^3. Density is an important parameter for diesel fuel injection systems. In Table 4.5, the results showed that the density values of all methyl esters of 30 species of oilseed plants were in range of 887.64-866.95 kg/cm^3, which were within the specification limits.

4.5.3 Flash point

As seen in Table 4.5, the flash point of all methyl esters of 30 species of oilseed plants were higher than 120 ℃, which was in the specification of ASTM standards. According to the relationship between viscosity and flash point, as flash point increase with viscosity. Moreover, all of seed oils have the length of free fatty acid chain from C12-C24 atoms, which are higher than diesel fuel (C9-C16 atoms). Thus, the biodiesel obtained from oil seed plants was safer than diesel fuels.

4.5.4 Acid number

From Table 4.5, It could be seen that the acid number of all methyl esters of 30 species of oilseed plants were in range of 0.1252-0.4404 mg KOH/g, which were within standard values (0.5 mg KOH/g). Therefore, the most of oils and free fatty acid contents were essentially converted to methyl esters.

4.5.5 Methyl ester content

Ester content could be calculated from ^{1}H-NMR and GC. The ^{1}H-NMR spectra of crude oils and fatty acid methyl esters were shown in Figure A32-A91, GC chromatogram of fatty acid methyl ester were shown in Figure A2-A31. From Table 4.5, it could be seen that both techniques gave a similar values of ester contents, 92.17-97.09 % (^{1}H-NMR) and 91.71-96.83 % (GC).

According to EN 14103 standard, ester content should be higher than 96.5%wt. Therefore, biodiesel from 5 plant species including *Camellia oleifera, Lagenaria siceraria*, *Moringa oleifera Lamk., Ocimum canum* and *Sesamum indicum* could be fulfilled the specification of biodesel standards.

CHAPTER V

CONCLUSION AND SUGGESTION

5.1 Conclusion

In this study, seed oils of 30 species were investigated as an alternative source for biodiesel production**.** The amount of oil, %free fatty acid, saponification number and iodine value were in range of 13 to 69%, 0.64-30.13%, 161.23-209.54 mg KOH/g and 45.13-164.38 mg I_2/g, respectively.

According to fatty acid compositions, oils of these plants could be classified into 3 groups

- C14 composition: MYF.
- C16-C18 composition: BA, BEH, CAC, CAO, CAS, CIL, CIM, CIR, CUM, CV, DO, GJ, HIS, LS, MAI, ME, MH, ML, MO, OC, PAF, PE, PF, SEI and TC.
- over C20 composition: BC, BJ, BP and CC.

From the optimization condition for biodiesel production, either base catalyzed process (transesterification reaction) or two-step catalyzed process (esterification and transesterification reaction) were used to synthesize biodiesel of oils of these 30 species.

In base catalyzed process (for oils containing FFA < 3%), the biodiesel of BA, BC, BEH, BJ, BP, CAC, CAO, CAS, CC, CIM, CIR, DO, GJ, HIS, LS, MAI, MH, ML, PAF, PE, PF and SEI oils were synthesized by using 1%wt of NaOH, 12:1 molar ratio of methanol to oil at 65°C for 1.5 h.

In two-step catalyzed process (for oils containing FFA > 3%) could be achieved by using esterification reaction followed by transesterification reaction. The optimum conditions of esterification reaction were found to be 3 %$Fe_2(SO_4)_3$, 10:1 molar ratio of methanol to oil at 65 °C for 1 h which were used to synthesize oils having 10%FFA or less such as CUM, ME, MO and OC. With the same conditions, oils having 20%FFA or less such as CIL, CV and MYF were synthesized for 2 h. In addition, TC oil which had 30%FFA was synthesized by using 5 %$Fe_2(SO_4)_3$, 10:1 molar ratio of methanol to oil at 65°C for 3 h. The reaction conditions of transesterification reaction were used the same as described in base catalyzed process.

For the properties of biodiesel of 30 plant species are as follow:

1. Viscosity: in range of 3.2-5.5 cSt.
2. Density: in range of 866.95-887.64 g/cm^3.
3. Flash point: >120°C
4. Acid number: in range of 0.1252-0.4404 mg KOH/g.
5. Methyl ester content: in range of 91.71-96.83%.

According to %oil content, saponification number, iodine value, viscosity, density, flash point, acid number and methyl ester content, the biodiesel from 15 plant species including BC, CAO, CC, CIM, CIR, CUM, CV, LS, MAI, ML, MO, MYF, PF, SEI and TC could be potentially used and they meet the major specification of biodiesel standards.

5.2 Suggestion

1. Soap could be produced by using NaOH as catalyst. Large amount of water was consumed to purify biodiesel which caused basic wastewater. For further study, heterogeneous base catalyst such as MgO and ZnO should be used as catalyst to decrease basic wastewater in biodiesel production.

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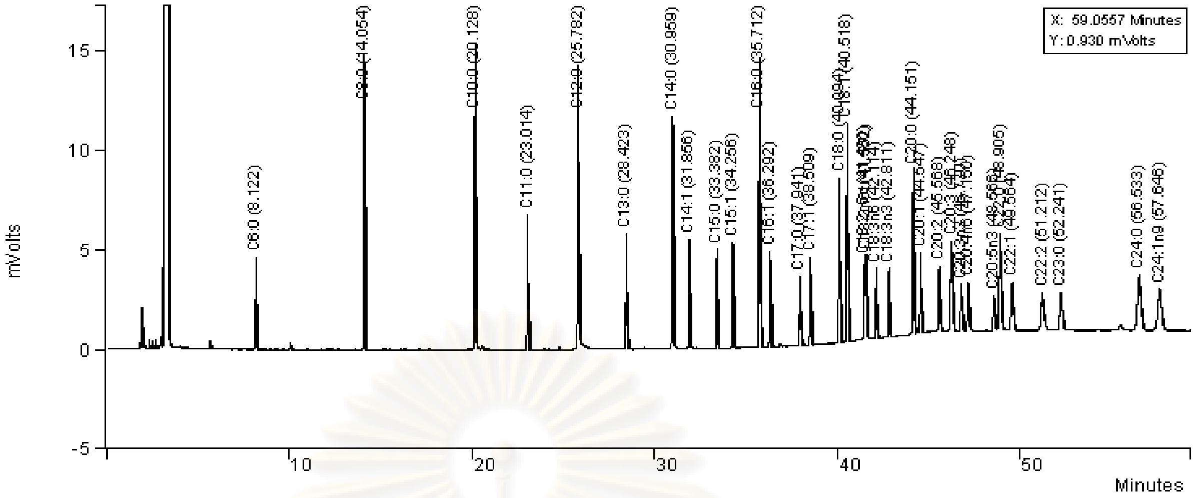
APPENDICES

APPENDIX A

GC CHROMATOGRAMS and ^{1}H-NMR SPECTRA OF CRUDE OILS AND METHYL ESTERS

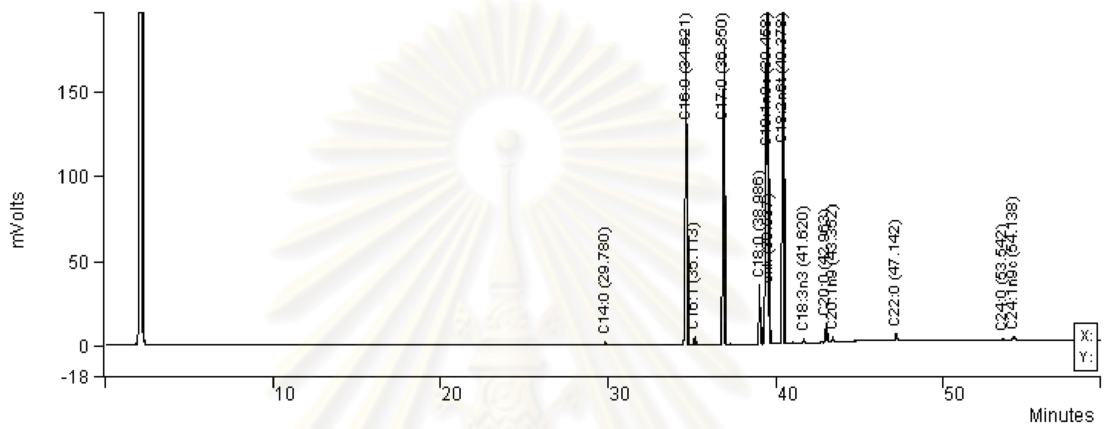


Identification (Peak Name)	Retention time (min)
C6:0 (Caproic)	8.122
C8:0 (Caprylic)	14.054
C10:0 (Capric)	20.128
C11:0 (Undecanoic)	23.014
C12:0 (Lauric)	25.782
C13:0 (Tridecanoic)	28.423
C14:0 (Myristic)	30.959
C14:1 (Myristoleic)	31.856
C15:0 (Pentadecanoic)	33.382
C15:1 (cis-10-Pentadecenoic)	34.256
C16:0 (Palmitic)	35.712
C16:1 (Palmitoleic)	36.292
C17:0 (Heptadecanoic) (Internal standard)	37.941
C17:1 (cis-10-Heptadecenoic)	38.509
C18:0 (Stearic)	40.094
C18:1n9c (Oleic)	40.518
C18:1n9t (Elaidic)	40.612
C18:2n6c (Linoleic)	41.480
C18:2n6t (Linolelaidic)	41.552
C18:3n6 (γ-Linolenic)	42.114
C18:3n3 (α-Linolenic)	42.811
C20:0 (Arachidic)	44.151
C20:1n9 (cis-11-Eicosenoic)	44.547
C20:2 (cis-11,14-Eicosadienoic)	45.568
C20:3n6 (cis-8,11,14-Eicosatrienoic)	46.248
C20:3n3 (cis-11,14,17-Eicosatrienoic)	46.770
C20:4n6 (Arachidonic)	47.150
C20:5n3 (cis-5,8,11,14,17-Eicosapentaenoic)	48.566
C22:0 (Behenic)	48.905
C22:1n9 (Erucic)	49.564
C22:2 (cis-13,16-Docosadienoic)	51.212
C23:0 (Tricosanoic)	52.241
C24:0 (Lignoceric)	56.533
C24:1n9 (Nervonic)	57.646

Figure A1 GC chromatogram of 37 FAMEs standard.

Data File: c:\star\joe\pakpung2 30.8.52 5;46;20
Channel: Front = FID RESULTS
Sample ID: BA 2
Operator (Inj): jo
Injection Date: 27/02/2010 17:46:20
Injection Method: c:\labchem07\new method jo-1.2.mth
Run Time (min): 59.440
Workstation:
Instrument (Inj): gc

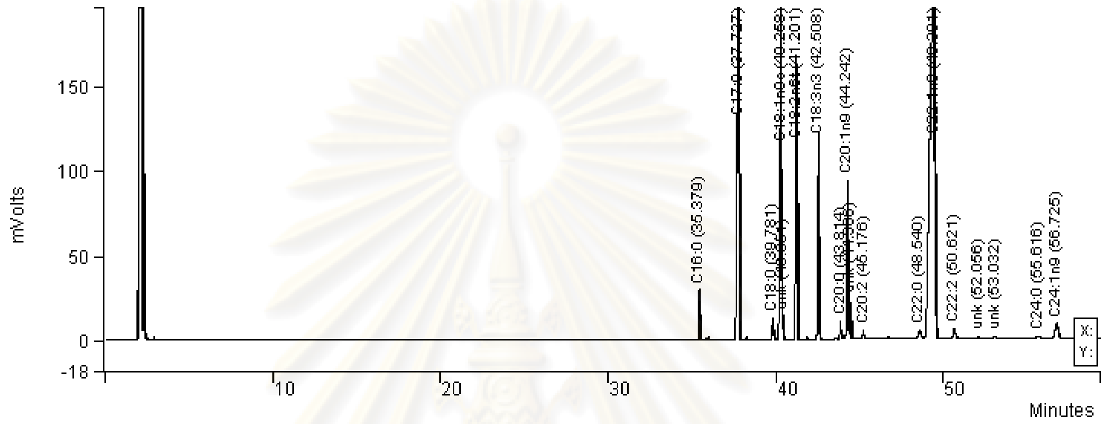
Operator (Calc): jo
Calc Date: 05/04/2010 13:34:58
Times Calculated: 6
Calculation Method: ba 2 5;46;20 pm-front.mth
Instrument (Calc): gc
Run Mode: Analysis
Peak Measurement: Peak Area
Calculation Type: Percent
Calibration Level: N/A
Verification Tolerance: N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C14:0	0.1206	29.780	-0.025	6954	0.00	BB	4.1		0
2	C16:0	18.6132	34.621	0.068	1072968	0.00	BB	5.5		0
3	C16:1	0.3437	35.113	-0.005	19810	0.00	BB	4.2		0
4	C17:0	17.1687	36.850	0.080	989697	0.00	BB	5.3		0
5	C18:0	3.4369	38.986	0.058	198121	0.00	BV	5.3		0
6	C18:1n9c	36.6557	39.458	0.000	2113035	0.00	VV	6.6		0
7	unk	1.6808	39.537	0.037	96889	0.00	VB	3.8		0
8	C18:2n6t	19.5033	40.378	0.007	1124277	0.00	BB	5.3		0
9	C18:3n3	0.1921	41.620	-0.014	11075	0.00	BB	4.5		0
10	C20:0	0.9313	42.963	-0.023	53687	0.00	BB	4.6		0
11	C20:1n9	0.2612	43.352	-0.019	15056	0.00	BB	4.9		0
12	C22:0	0.5234	47.142	-0.034	30170	0.00	BB	6.7		0
13	C24:0	0.1648	53.542	-0.065	9500	0.00	BB	11.3		0
14	C24:1n9c	0.4042	54.138	-0.415	23302	0.00	BB	11.6		0
	Totals	**99.9999**		**-0.350**	**5764541**					

Figure A2 GC chromatogram of BA oil methyl ester.

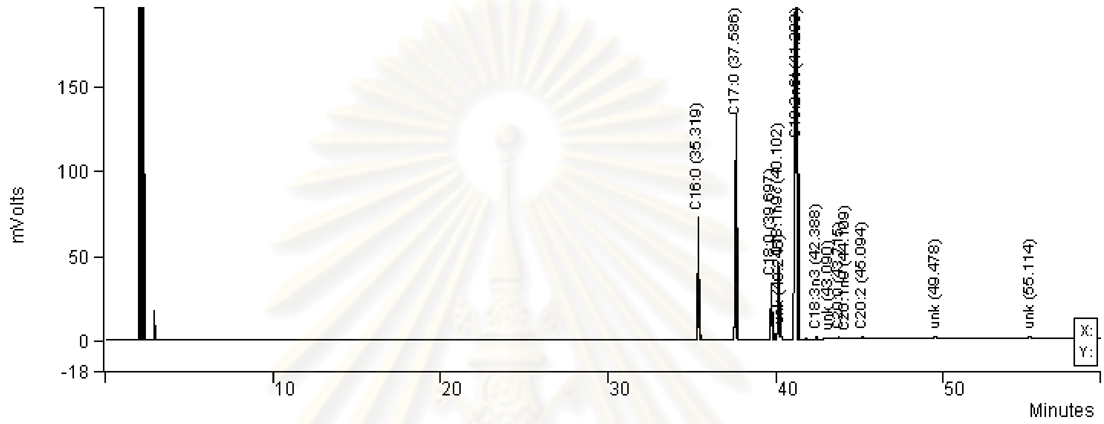
Data File:	c:\star\joe\kwangtung1 7;11;57	Operator (Calc):	jo
Channel:	Front = FID RESULTS	Calc Date:	05/04/2010 17:58:37
Sample ID:	BC	Times Calculated:	6
Operator (Inj):	jo	Calculation Method:	bc 7;11;57 pm-front.mth
Injection Date:	21/02/2010 19:11:57	Instrument (Calc):	gc
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	gc	Calibration Level:	N/A
		Verification Tolerance:	N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	1.3422	35.379	-0.012	136078	0.00	BB	4.3		0
2	C17:0	16.1349	37.727	0.113	1635761	0.00	BB	5.9		0
3	C18:0	0.6448	39.781	0.014	65375	0.00	BP	4.9		0
4	C18:1n9c	12.1454	40.258	0.066	1231313	0.00	PV	5.3		0
5	unk	0.4917	40.351	-0.000	49847	0.00	VB	3.9		0
6	C18:2n6t	8.4112	41.201	-0.021	852735	0.00	BP	4.8		0
7	C18:3n3	5.8748	42.508	0.030	595590	0.00	VB	4.6		0
8	C20:0	0.5184	43.814	-0.009	52555	0.00	PV	4.7		0
9	C20:1n9	4.7861	44.242	0.026	485221	0.00	VV	4.8		0
10	unk	1.0292	44.386	-0.000	104340	0.00	VB	4.3		0
11	C20:2	0.2578	45.176	-0.019	26131	0.00	BB	4.9		0
12	C22:0	0.5496	48.540	0.139	55720	0.00	BV	11.1		0
13	C22:1n9	45.5548	49.381	0.360	4618376	0.00	VP	15.3		0
14	C22:2	0.5324	50.621	0.021	53979	0.00	PB	8.5		0
15	unk	0.1514	52.056	0.000	15348	0.00	VV	9.1		0
16	unk	0.1454	53.032	-0.000	14740	0.00	VB	9.8		0
17	C24:0	0.1970	55.616	-0.061	19974	0.00	BB	12.1		0
18	C24:1n9	1.2327	56.725	-0.006	124976	0.00	BB	13.3		0
	Totals	**99.9998**		**0.641**	**10138059**					

Figure A3 GC chromatogram of BC oil methyl ester.

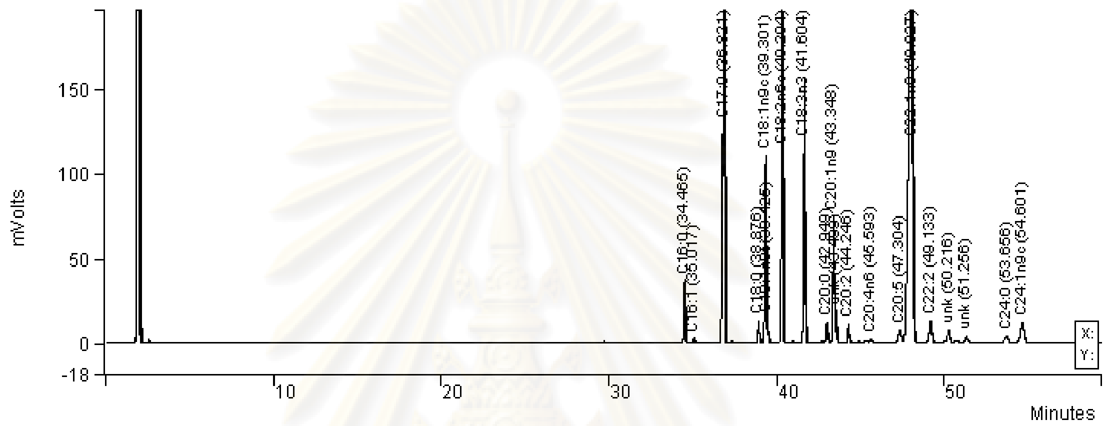
Data File:	c:\star\joe\greenfug1 11;45;58 am.run	Operator (Calc):	jo
Channel:	Front = FID RESULTS	Calc Date:	05/04/2010 13:56:14
Sample ID:	BEH 1	Times Calculated:	9
Operator (Inj):	io	Calculation Method:	beh1 11;45;58 am-front.mth
Injection Date:	2/02/2010 11:45:58	Instrument (Calc):	gc
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	gc	Calibration Level:	N/A
		Verification Tolerance:	N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	8.1381	35.319	-0.072	338483	0.00	BB	4.4		0
2	C17:0	16.6603	37.586	-0.028	692943	0.00	BB	4.9		0
3	C18:0	4.1122	39.697	-0.070	171037	0.00	BP	4.8		0
4	C18:1n9c	5.2990	40.102	-0.090	220400	0.00	PV	4.5		0
5	unk	0.5423	40.246	-0.049	22555	0.00	VB	4.6		0
6	C18:2n6t	63.8349	41.203	-0.018	2655050	0.00	BB	6.7		0
7	C18:3n3	0.1842	42.388	-0.090	7661	0.00	BB	4.2		0
8	unk	0.1256	43.090	-0.001	5225	0.00	BB	24.1		0
9	C20:0	0.1588	43.715	-0.108	6606	0.00	BV	4.7		0
10	C20:1n9	0.1234	44.109	-0.107	5132	0.00	VV	5.8		0
11	C20:2	0.2945	45.094	-0.101	12248	0.00	VB	23.8		0
12	unk	0.1671	49.478	-0.000	6951	0.00	BV	7.3		0
13	unk	0.3596	55.114	-0.000	14956	0.00	BB	11.2		0
	Totals	**100.0000**		**-0.734**	**4159247**					

Figure A4 GC chromatogram of BEH oil methyl ester.

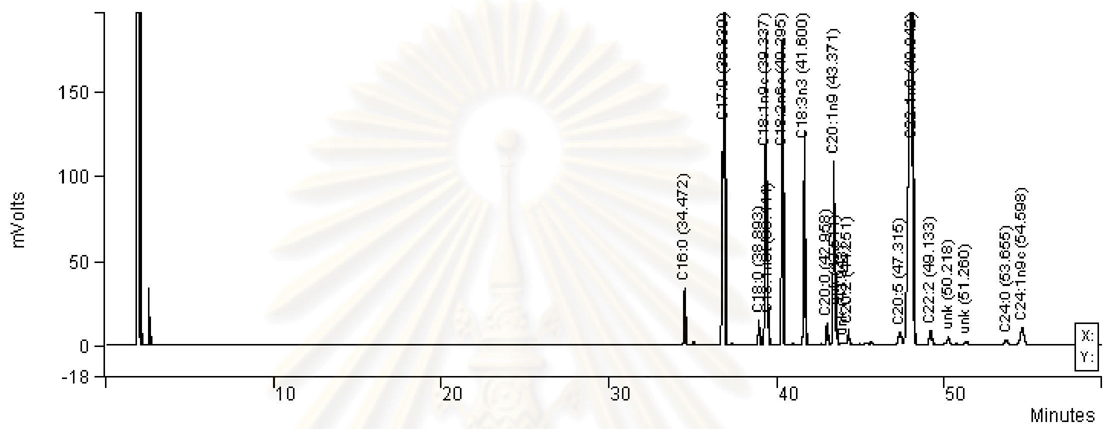
Data File:	c:\star\joe\4. bj 5;09;31 pm.run	Operator (Calc):	keak
Channel:	Front = FID RESULTS	Calc Date:	06/04/2010 18:23:41
Sample ID:	BJ	Times Calculated:	3
Operator (Inj):	keak	Calculation Method:	bj 8;51;46 pm-front.mth
Injection Date:	06/04/2010 17:09:31	Instrument (Calc):	gc
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	gc	Calibration Level:	N/A
		Verification Tolerance:	N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	1.8291	34.465	-0.088	192547	0.00	BV	4.9		0
2	C16:1	0.1238	35.017	-0.101	13030	0.00	VV	5.1		0
3	C17:0	17.1556	36.821	0.051	1805904	0.00	BB	6.5		0
4	C18:0	0.7161	38.876	-0.052	75379	0.00	BV	5.4		0
5	C18:1n9c	6.1797	39.301	-0.046	650508	0.00	VV	5.4		0
6	C18:1n9t	0.5958	39.425	-0.015	62716	0.00	VV	5.2		0
7	C18:2n6c	11.1931	40.294	-0.013	1178252	0.00	BB	5.6		0
8	C18:3n3	8.4633	41.604	-0.030	890902	0.00	BB	5.4		0
9	C20:0	0.6510	42.949	-0.037	68530	0.00	PP	5.5		0
10	C20:1n9	4.1356	43.348	-0.023	435342	0.00	PV	5.5		0
11	unk	0.9168	43.499	-0.012	96509	0.00	VB	5.2		0
12	C20:2	0.5847	44.246	-0.065	61550	0.00	BB	5.3		0
13	C20:4n6	0.1248	45.593	-0.073	13139	0.00	VV	6.5		0
14	C20:5	0.8448	47.304	-0.011	88928	0.00	BV	11.6		0
15	C22:1n9	42.1820	48.027	0.000	4440325	0.00	VB	13.7		0
16	C22:2	1.1487	49.133	0.012	120922	0.00	BB	9.0		0
17	unk	0.7334	50.216	0.045	77201	0.00	BV	9.3		0
18	unk	0.3318	51.256	-0.004	34925	0.00	VP	10.5		0
19	C24:0	0.4620	53.656	0.049	48633	0.00	BB	13.5		0
20	C24:1n9c	1.6278	54.601	0.048	171355	0.00	BB	13.8		0
	Totals	**99.9999**		**-0.365**	**10526597**					

Figure A5 GC chromatogram of BJ oil methyl ester.

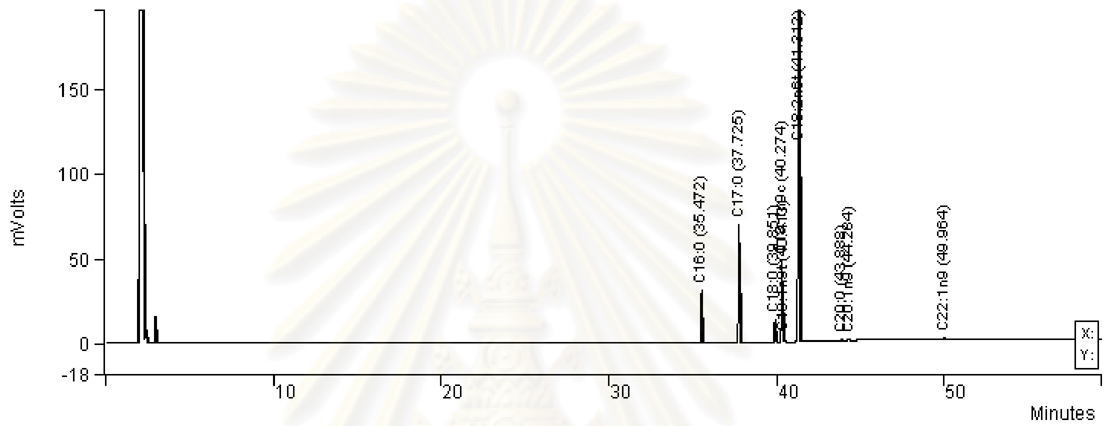
Data File:	c:\star\joe\5. bp 3;56;03 pm.run	Operator (Calc):	keak
Channel:	Front = FID RESULTS	Calc Date:	06/04/2010 17:24:51
Sample ID:	BP	Times Calculated:	5
Operator (Inj):	keak	Calculation Method:	bp 11;39;53 am-front.mth
Injection Date:	06/04/2010 15:56:03	Instrument (Calc):	gc
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	gc	Calibration Level:	N/A
		Verification Tolerance:	N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	1.6403	34.472	-0.081	178821	0.00	BB	5.0		0
2	C17:0	16.1994	36.830	0.060	1766035	0.00	BB	6.7		0
3	C18:0	0.8025	38.893	-0.035	87483	0.00	BV	5.8		0
4	C18:1n9c	10.4472	39.337	-0.010	1138934	0.00	VV	5.8		0
5	C18:1n9t	0.5069	39.444	0.004	55261	0.00	VV	4.7		0
6	C18:2n6c	9.8701	40.295	-0.012	1076020	0.00	BB	5.6		0
7	C18:3n3	6.6965	41.600	-0.034	730042	0.00	BB	5.4		0
8	C20:0	0.7061	42.958	-0.028	76977	0.00	PP	5.7		0
9	C20:1n9	6.0211	43.371	-0.000	656410	0.00	PV	5.6		0
10	unk	0.8407	43.511	0.000	91652	0.00	VV	5.0		0
11	unk	0.1315	43.943	-0.000	14333	0.00	VV	16.0		0
12	C20:2	0.4681	44.251	-0.060	51029	0.00	VB	5.5		0
13	C20:5	0.8282	47.315	0.000	90287	0.00	BV	11.7		0
14	C22:1n9	41.8282	48.042	0.317	4560040	0.00	VB	14.0		0
15	C22:2	0.7012	49.133	0.012	76440	0.00	BB	8.8		0
16	unk	0.4152	50.218	0.047	45265	0.00	BV	9.3		0
17	unk	0.1984	51.260	0.000	21632	0.00	VP	10.7		0
18	C24:0	0.3654	53.655	0.048	39834	0.00	BB	13.6		0
19	C24:1n9c	1.3331	54.598	0.045	145335	0.00	BB	13.8		0
	Totals	**100.0001**		**0.273**	**10901830**					

Figure A6 GC chromatogram of BP oil methyl ester.

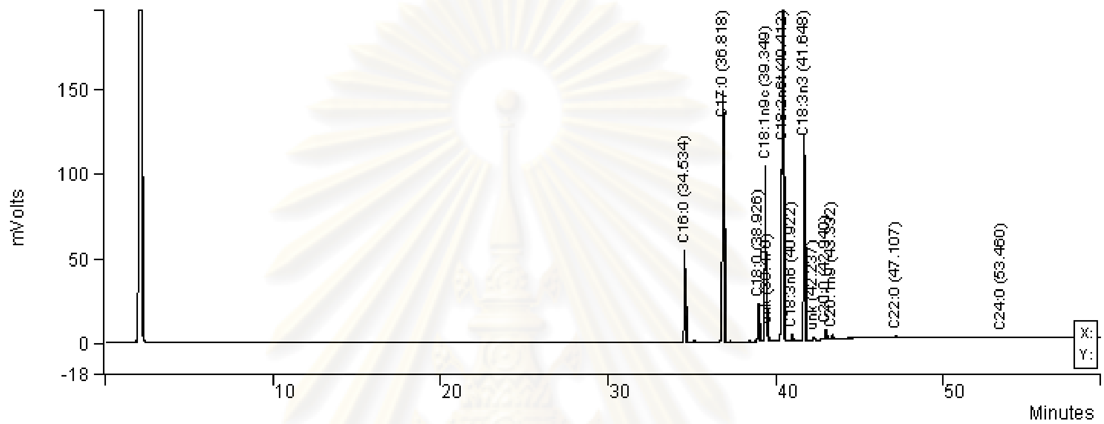
Data File:	c:\star\joe\12(16.2.52) 3;39;18 pm.run	Operator (Calc):	jo
Channel:	Front = FID RESULTS	Calc Date:	05/04/2010 18:53:36
Sample ID:	CAC	Times Calculated:	4
Operator (Inj):	io	Calculation Method:	cac 3;39;18 pm-front.mth
Injection Date:	5/02/2010 15:39:18	Instrument (Calc):	GC3800
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	GC3800	Calibration Level:	N/A
		Verification Tolerance:	N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	6.6249	35.472	-0.019	136675	0.00	BB	4.2		0
2	C17:0	16.2370	37.725	-0.007	334976	0.00	BB	4.5		0
3	C18:0	3.0389	39.851	-0.017	62694	0.00	BP	4.5		0
4	C18:1n9c	11.0260	40.274	-0.035	227471	0.00	PB	4.4		0
5	C18:1n9t	0.3401	40.413	-0.021	7016	0.00	TS	0.0		0
6	C18:2n6t	61.8714	41.312	-0.034	1276439	0.00	PB	5.3		0
7	C20:0	0.2197	43.888	-0.017	4532	0.00	VV	5.9		0
8	C20:1n9	0.2978	44.284	-0.019	6145	0.00	VV	8.9		0
9	C22:1n9	0.3442	49.964	-0.030	7101	0.00	VB	7.7		0
	Totals	**100.0000**		**-0.199**	**2063049**					

Figure A7 GC chromatogram of CAC oil methyl ester.

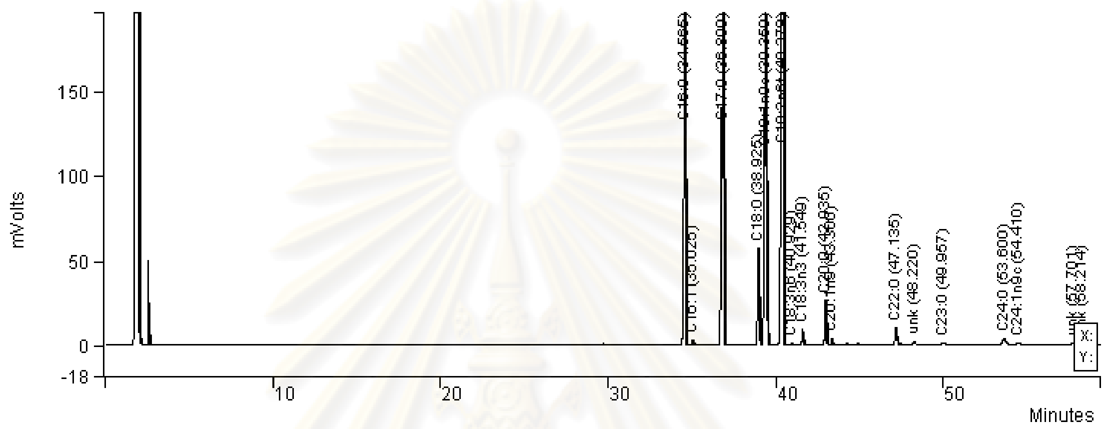
Data File:	c:\star\joe\hoymaw2 7;44;51 pm.run	Operator (Calc):	jo
Channel:	Front = FID RESULTS	Calc Date:	05/04/2010 16:56:42
Sample ID:	CAO	Times Calculated:	2
Operator (Inj):	jo	Calculation Method:	cao 6;39;17 pm-front.mth
Injection Date:	02/01/2010 19:44:51	Instrument (Calc):	gc
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	gc	Calibration Level:	N/A
		Verification Tolerance:	N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	5.2633	34.534	-0.019	248839	0.00	BB	4.3		0
2	C17:0	17.0581	36.818	0.048	806476	0.00	BB	5.1		0
3	C18:0	2.4228	38.926	-0.002	114544	0.00	BV	4.8		0
4	C18:1n9c	11.4359	39.349	0.002	540666	0.00	VV	4.8		0
5	unk	0.5184	39.470	0.000	24509	0.00	VB	4.5		0
6	C18:2n6t	48.7311	40.412	0.041	2303913	0.00	BB	6.5		0
7	C18:3n6	0.4276	40.922	-0.014	20217	0.00	BB	4.7		0
8	C18:3n3	12.8016	41.648	0.014	605236	0.00	PB	4.7		0
9	unk	0.1673	42.237	0.000	7907	0.00	BB	4.5		0
10	C20:0	0.5668	42.940	-0.046	26797	0.00	PP	4.8		0
11	C20:1n9	0.2793	43.332	-0.039	13204	0.00	PB	5.2		0
12	C22:0	0.2014	47.107	-0.069	9524	0.00	BB	6.6		0
13	C24:0	0.1263	53.460	-0.146	5972	0.00	BB	15.2		0
	Totals	**99.9999**		**-0.230**	**4727804**					

Figure A8 GC chromatogram of CAO oil methyl ester.

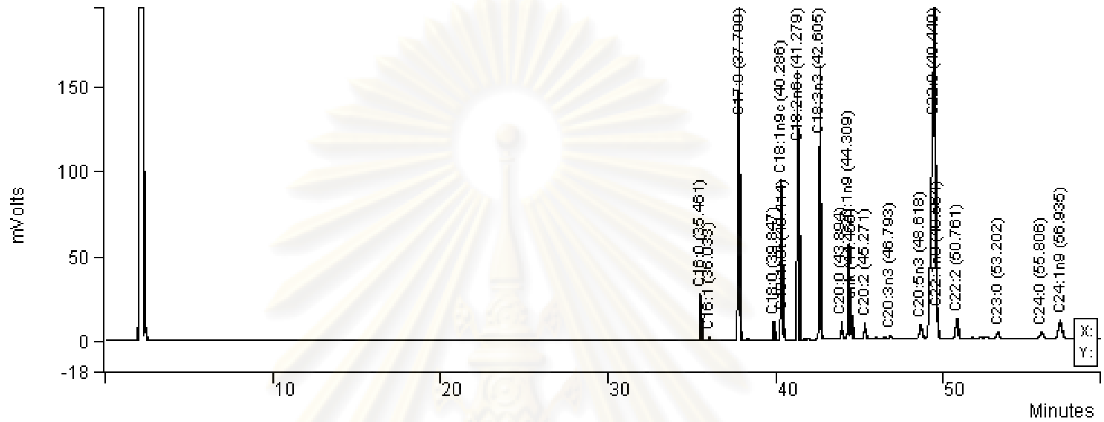
Data File:	c:\star\joe\8. cas 11;39;53 am.run	Operator (Calc):	keak
Channel:	Front = FID RESULTS	Calc Date:	06/04/2010 18:11:54
Sample ID:	CAS	Times Calculated:	13
Operator (Inj):	keak	Calculation Method:	8. cas 11;39;53 am-front.mth
Injection Date:	06/04/2010 11:39:53	Instrument (Calc):	gc
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	gc	Calibration Level:	N/A
		Verification Tolerance:	N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	18.4557	34.565	0.012	1790743	0.00	BB	6.4		0
2	C16:1	0.1844	35.025	-0.093	17891	0.00	VB	4.9		0
3	C17:0	18.3153	36.809	0.039	1777118	0.00	BB	6.6		0
4	C18:0	3.9876	38.925	-0.003	386916	0.00	BV	6.4		0
5	C18:1n9c	19.1498	39.359	0.012	1858091	0.00	VB	6.4		0
6	C18:2n6t	35.4965	40.379	0.008	3444200	0.00	BB	7.5		0
7	C18:3n6	0.0748	40.929	-0.007	7257	0.00	BB	5.2		0
8	C18:3n3	0.5347	41.549	-0.085	51886	0.00	BB	5.1		0
9	C20:0	1.5689	42.935	-0.051	152227	0.00	PV	5.4		0
10	C20:1n9	0.2501	43.300	-0.071	24269	0.00	VV	5.6		0
11	C22:0	0.7981	47.135	-0.041	77438	0.00	BV	7.4		0
12	unk	0.1758	48.220	-0.000	17057	0.00	VB	7.7		0
13	C23:0	0.1243	49.957	-0.032	12059	0.00	BB	10.9		0
14	C24:0	0.4724	53.600	-0.007	45832	0.00	BV	13.3		0
15	C24:1n9c	0.1974	54.410	-0.144	19156	0.00	VB	15.3		0
16	unk	0.0834	57.701	-0.000	8097	0.00	BV	14.5		0
17	unk	0.1307	58.214	0.000	12678	0.00	VB	14.6		0
	Totals	**99.9999**		**-0.463**	**9702915**					

Figure A9 GC chromatogram of CAS oil methyl ester.

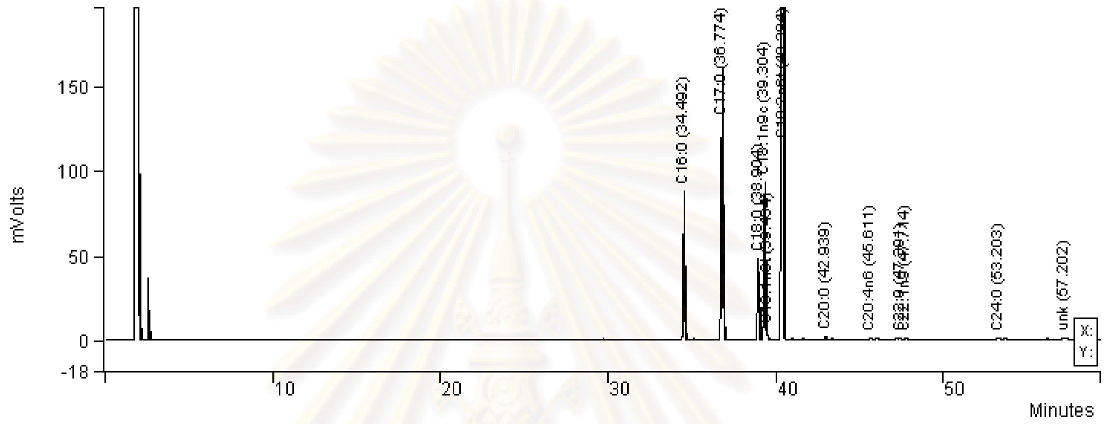
Data File:	c:\star\joe\18(16.2.52) 11;34;30 pm.run	Operator (Calc):	jo
Channel:	Front = FID RESULTS	Calc Date:	05/04/2010 19:07:31
Sample ID:	CC	Times Calculated:	6
Operator (Inj):	io	Calculation Method:	cc 11;34;30 pm-front.mth
Injection Date:	7/02/2010 23:34:30	Instrument (Calc):	GC3800
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	GC3800	Calibration Level:	N/A
		Verification Tolerance:	N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	1.5442	35.461	-0.030	122184	0.00	BB	4.2		0
2	C16:1	0.1262	36.033	-0.000	9986	0.00	VV	4.2		0
3	C17:0	16.4224	37.790	0.058	1299445	0.00	BB	5.5		0
4	C18:0	0.6887	39.847	-0.021	54492	0.00	BB	4.6		0
5	C18:1n9c	5.8300	40.286	-0.023	461306	0.00	BV	4.5		0
6	C18:1n9t	0.7482	40.414	-0.020	59198	0.00	VB	4.2		0
7	C18:2n6c	10.2902	41.279	0.000	814222	0.00	BB	4.8		0
8	C18:3n3	10.3732	42.605	0.029	820791	0.00	BB	4.8		0
9	C20:0	0.6364	43.894	-0.011	50360	0.00	VP	4.7		0
10	C20:1n9	3.4796	44.309	0.006	275330	0.00	PV	4.6		0
11	unk	1.1172	44.465	0.000	88402	0.00	VB	4.4		0
12	C20:2	0.6010	45.271	0.224	47557	0.00	BB	5.0		0
13	C20:3n3	0.1563	46.793	0.000	12365	0.00	VB	5.8		0
14	C20:5n3	1.0946	48.618	0.101	86610	0.00	BV	9.9		0
15	C22:0	42.4029	49.449	0.386	3355178	0.00	VB	13.4		0
16	C22:1n9	0.2646	49.564	-0.430	20935	0.00	TS	0.0		0
17	C22:2	1.3180	50.761	-0.000	104285	0.00	BB	8.7		0
18	C23:0	0.4907	53.202	0.000	38829	0.00	VB	9.8		0
19	C24:0	0.5356	55.806	-0.058	42382	0.00	BB	12.6		0
20	C24:1n9	1.8800	56.935	-0.000	148759	0.00	BB	13.3		0
	Totals	**100.0000**		**0.211**	**7912616**					

Figure A10 GC chromatogram of CC oil methyl ester.

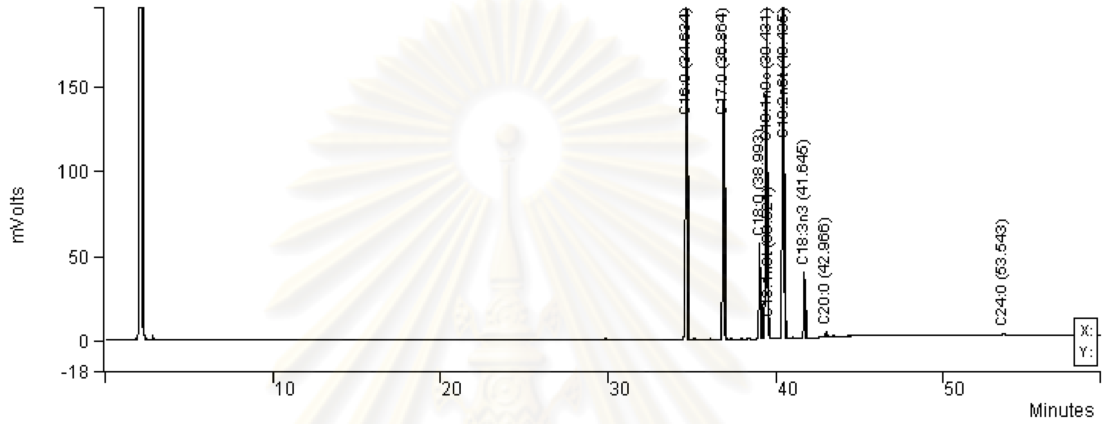
Data File:	c:\star\joe\watermellon_k1 9;52;28	Operator (Calc):	jo
Channel:	Front = FID RESULTS	Calc Date:	01/04/2010 21:32:42
Sample ID:	CIL	Times Calculated:	7
Operator (Inj):	jo	Calculation Method:	cil 9;52;28 am-front.mth
Injection Date:	28/01/2010 09:52:28	Instrument (Calc):	gc
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	gc	Calibration Level:	N/A
		Verification Tolerance:	N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	8.2608	34.492	-0.061	489426	0.00	BB	5.2		0
2	C17:0	16.6108	36.774	0.004	984132	0.00	BB	5.8		0
3	C18:0	4.9403	38.904	-0.024	292693	0.00	BP	5.7		0
4	C18:1n9c	9.1186	39.304	-0.043	540247	0.00	PV	5.4		0
5	C18:1n9t	0.4072	39.434	-0.000	24127	0.00	VV	5.6		0
6	C18:2n6t	59.7725	40.394	0.023	3541319	0.00	BB	7.8		0
7	C20:0	0.1871	42.939	-0.047	11084	0.00	BP	5.3		0
8	C20:4n6	0.1092	45.611	-0.055	6471	0.00	BP	6.7		0
9	C22:0	0.1250	47.391	0.215	7405	0.00	VV	7.5		0
10	C22:1n9	0.1211	47.714	-0.011	7173	0.00	VB	7.6		0
11	C24:0	0.1622	53.203	-0.404	9612	0.00	BV	11.6		0
12	unk	0.1851	57.202	0.000	10969	0.00	BV	15.7		0
	Totals	**99.9999**		**-0.403**	**5924658**					

Figure A11 GC chromatogram of CIL oil methyl ester.

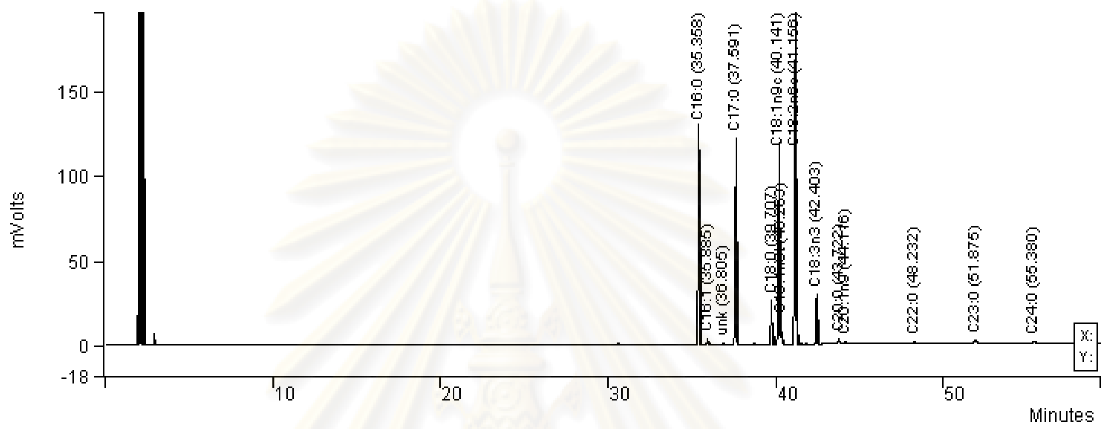
Data File:	c:\star\joe\somoh1 30.8.52 2;27;34	Operator (Calc):	jo
Channel:	Front = FID RESULTS	Calc Date:	05/04/2010 15:28:19
Sample ID:	CIM	Times Calculated:	4
Operator (Inj):	jo	Calculation Method:	cim 2;27;34 pm-front.mth
Injection Date:	05/02/2010 14:27:34	Instrument (Calc):	gc
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	gc	Calibration Level:	N/A
		Verification Tolerance:	N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	18.6654	34.634	0.081	1172800	0.00	BB	5.5		0
2	C17:0	17.0061	36.864	0.094	1068541	0.00	BB	5.6		0
3	C18:0	4.9652	38.993	0.065	311979	0.00	BV	5.2		0
4	C18:1n9c	21.8103	39.431	0.084	1370403	0.00	VV	5.8		0
5	C18:1n9t	0.4492	39.521	-0.000	28223	0.00	VV	4.2		0
6	C18:2n6t	33.7068	40.435	0.064	2117898	0.00	BB	6.3		0
7	C18:3n3	2.9735	41.645	0.011	186831	0.00	VB	4.5		0
8	C20:0	0.2742	42.966	-0.020	17228	0.00	BP	4.8		0
9	C24:0	0.1495	53.543	-0.064	9392	0.00	BB	11.7		0
	Totals	**100.0002**		**0.315**	**6283295**					

Figure A12 GC chromatogram of CIM oil methyl ester.

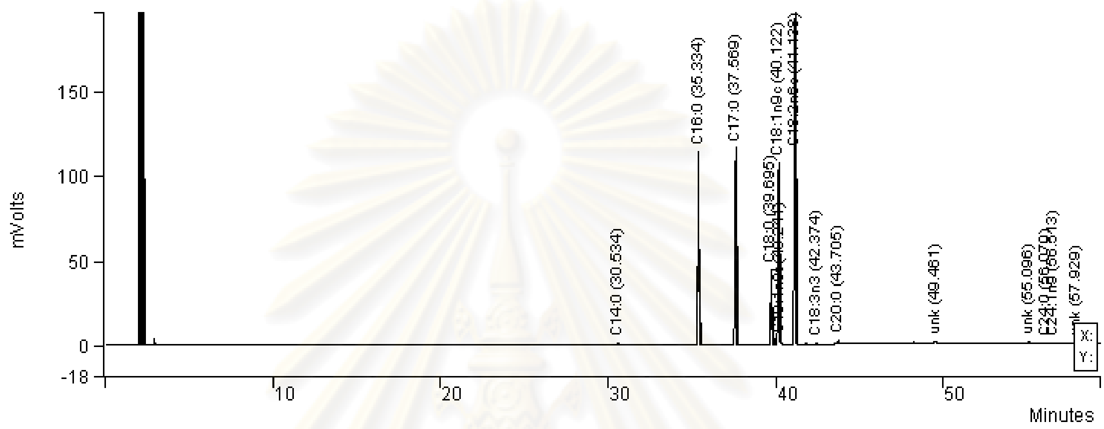
Data File:	c:\star\joe\orange1 2;10;02 pm.run	Operator (Calc):	keak
Channel:	Front = FID RESULTS	Calc Date:	05/04/2010 16:47:50
Sample ID:	CIR	Times Calculated:	6
Operator (Inj):	keak	Calculation Method:	cir 2;10;02 pm-front.mth
Injection Date:	06/02/2010 14:10:02	Instrument (Calc):	gc
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	gc	Calibration Level:	N/A
		Verification Tolerance:	N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	17.5593	35.358	-0.033	654014	0.00	BB	4.7		0
2	C16:1	0.4209	35.885	-0.079	15676	0.00	BV	4.1		0
3	unk	0.1184	36.805	0.000	4409	0.00	BB	4.4		0
4	C17:0	17.1254	37.591	-0.023	637852	0.00	BB	4.9		0
5	C18:0	3.6889	39.707	-0.060	137398	0.00	BV	5.0		0
6	C18:1n9c	16.2289	40.141	-0.051	604463	0.00	VV	4.8		0
7	C18:1n9t	1.6218	40.263	0.000	60405	0.00	VV	4.1		0
8	C18:2n6c	38.1217	41.156	0.003	1419882	0.00	BB	5.4		0
9	C18:3n3	3.7267	42.403	-0.075	138805	0.00	VB	4.4		0
10	C20:0	0.4336	43.722	-0.101	16150	0.00	BV	4.8		0
11	C20:1n9	0.1103	44.116	-0.100	4108	0.00	VV	5.8		0
12	C22:0	0.1040	48.232	-0.169	3875	0.00	BB	7.2		0
13	C23:0	0.5187	51.875	0.294	19321	0.00	VB	8.9		0
14	C24:0	0.2214	55.380	-0.297	8246	0.00	BB	12.2		0
	Totals	**100.0000**		**-0.691**	**3724604**					

Figure A13 GC chromatogram of CIR oil methyl ester.

Data File:	c:\star\joe\pumpkin1 1;50;02 pm.run	Operator (Calc):	keak
Channel:	Front = FID RESULTS	Calc Date:	05/04/2010 14:30:23
Sample ID:	CUM 1	Times Calculated:	4
Operator (Inj):	keak	Calculation Method:	cum1 1;50;02 pm-front.mth
Injection Date:	4/02/2010 13:50:02	Instrument (Calc):	gc
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	gc	Calibration Level:	N/A
		Verification Tolerance:	N/A

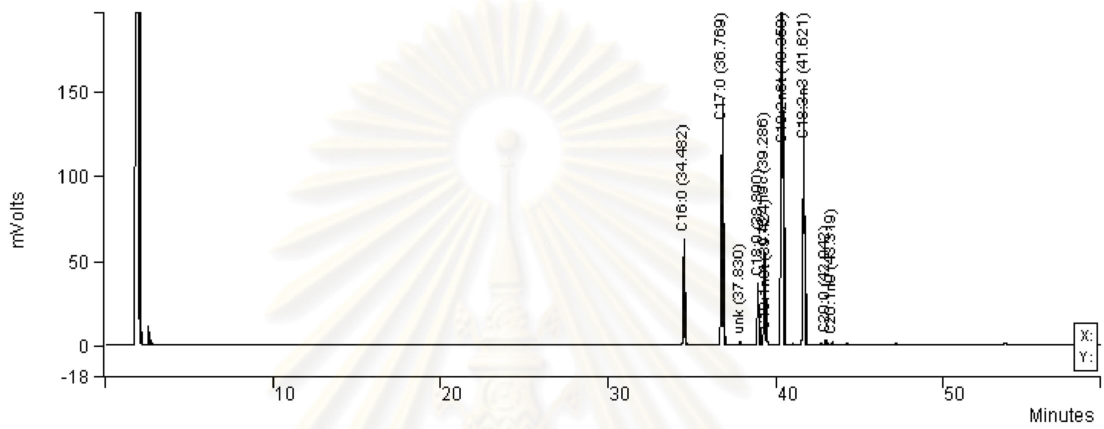


Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C14:0	0.1093	30.534	-0.108	3564	0.00	BB	4.1		0
2	C16:0	17.0255	35.334	-0.057	555138	0.00	BB	4.6		0
3	C17:0	17.5644	37.569	-0.045	572709	0.00	BB	4.6		0
4	C18:0	6.8864	39.695	-0.072	224539	0.00	BP	4.7		0
5	C18:1n9c	16.7514	40.122	-0.000	546201	0.00	PV	4.7		0
6	C18:1n9t	0.3453	40.241	0.000	11260	0.00	VB	4.6		0
7	C18:2n6c	39.9125	41.138	-0.015	1301399	0.00	BB	5.2		0
8	C18:3n3	0.1528	42.374	-0.104	4981	0.00	BB	4.4		0
9	C20:0	0.3563	43.705	-0.118	11619	0.00	BB	4.8		0
10	unk	0.3250	49.461	0.000	10598	0.00	VV	7.6		0
11	unk	0.1837	55.096	0.000	5990	0.00	BV	11.5		0
12	C24:0	0.1741	56.070	0.393	5677	0.00	BV	13.6		0
13	C24:1n9	0.1097	56.513	-0.218	3575	0.00	VB	13.8		0
14	unk	0.1035	57.929	-0.000	3376	0.00	BB	20.4		0
	Totals	**99.9999**		**-0.344**	**3260626**					

Figure A14 GC chromatogram of CUM oil methyl ester.

Data File: c:\star\joe\yomhin2 2;12;33 pm.run
Channel: Front = FID RESULTS
Sample ID: CV 2
Operator (Inj): jo
Injection Date: 27/01/2010 14:12:33
Injection Method: c:\labchem07\new method jo-1.2.mth
Run Time (min): 59.440
Workstation:
Instrument (Inj): gc

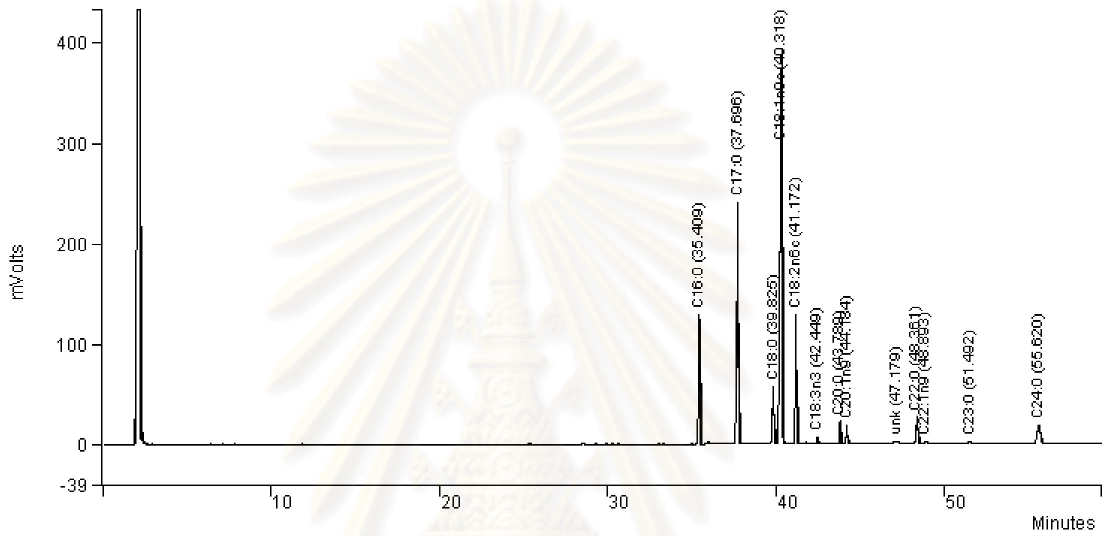
Operator (Calc): jo
Calc Date: 01/04/2010 21:43:44
Times Calculated: 8
Calculation Method: cv 2;12;33 pm-front.mth
Instrument (Calc): gc
Run Mode: Analysis
Peak Measurement: Peak Area
Calculation Type: Percent
Calibration Level: N/A
Verification Tolerance: N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	6.8182	34.482	-0.071	336032	0.00	BB	5.0		0
2	C17:0	17.3512	36.769	-0.001	855149	0.00	PP	5.6		0
3	unk	0.2592	37.830	0.000	12775	0.00	BB	5.2		0
4	C18:0	4.2669	38.890	-0.038	210295	0.00	BV	5.4		0
5	C18:1n9c	6.2795	39.286	-0.061	309481	0.00	VV	5.3		0
6	C18:1n9t	0.4322	39.424	-0.009	21303	0.00	VV	5.8		0
7	C18:2n6t	45.8396	40.350	-0.021	2259192	0.00	BP	6.6		0
8	C18:3n3	18.2787	41.621	-0.013	900859	0.00	BB	5.5		0
9	C20:0	0.2947	42.942	-0.044	14523	0.00	VP	5.5		0
10	C20:1n9	0.1799	43.319	-0.052	8866	0.00	PB	6.0		0
	Totals	**100.0001**		**-0.310**	**4928475**					

Figure A15 GC chromatogram of CV oil methyl ester.

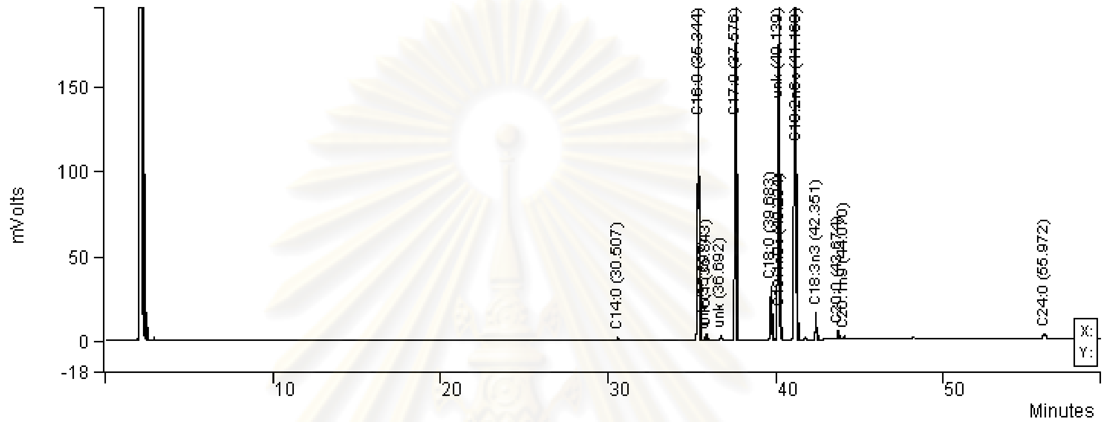
Data File:	c:\star\joe\chingchan1 12;42;28	Operator (Calc):	jo
Channel:	Front = FID RESULTS	Calc Date:	05/04/2010 17:34:14
Sample ID:	DO	Times Calculated:	9
Operator (Inj):	jo	Calculation Method:	do 11;15;54 am-front.mth
Injection Date:	06/02/2010 00:42:28	Instrument (Calc):	GC3800
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	GC3800	Calibration Level:	N/A
		Verification Tolerance:	N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	9.4830	35.409	-0.000	638043	0.00	BB	4.7		0
2	C17:0	21.9205	37.696	0.000	1474875	0.00	BB	5.9		0
3	C18:0	5.4304	39.825	-0.000	365371	0.00	BP	6.1		0
4	C18:1n9c	43.2866	40.318	-0.000	2912456	0.00	PB	7.1		0
5	C18:2n6c	9.1437	41.172	0.000	615214	0.00	BB	4.5		0
6	C18:3n3	0.4773	42.449	0.000	32114	0.00	VB	4.4		0
7	C20:0	1.6001	43.789	-0.000	107662	0.00	PV	4.6		0
8	C20:1n9	1.3094	44.184	0.000	88103	0.00	VB	4.6		0
9	unk	0.4594	47.179	-0.000	30907	0.00	BB	20.5		0
10	C22:0	2.9238	48.361	0.004	196722	0.00	BB	7.2		0
11	C22:1n9	0.2286	48.893	-0.003	15380	0.00	TF	0.0		0
12	C23:0	0.1626	51.492	0.002	10938	0.00	BB	9.5		0
13	C24:0	3.5747	55.620	0.002	240518	0.00	BB	12.2		0
	Totals	**100.0001**		**0.005**	**6728303**					

Figure A16 GC chromatogram of DO oil methyl ester.

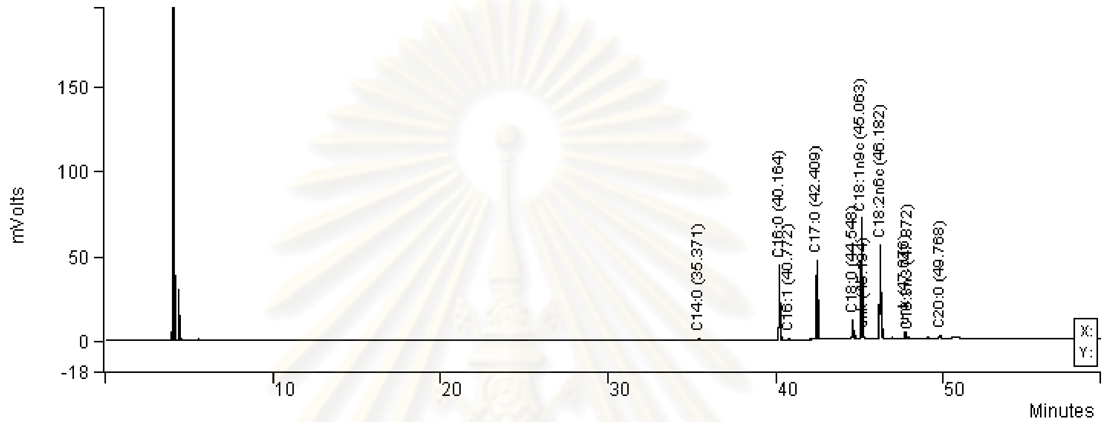
Data File:	c:\star\joe\lukpud1 4;43;37 pm.run	Operator (Calc):	jo
Channel:	Front = FID RESULTS	Calc Date:	05/04/2010 15:16:25
Sample ID:	GJ 1	Times Calculated:	5
Operator (Inj):	jo	Calculation Method:	gj 1 4;43;37 pm-front.mth
Injection Date:	04/02/2010 16:43:37	Instrument (Calc):	gc
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	gc	Calibration Level:	N/A
		Verification Tolerance:	N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C14:0	0.1345	30.507	-0.135	8886	0.00	BB	4.1		0
2	C16:0	16.3573	35.344	-0.047	1080860	0.00	BB	5.2		0
3	unk	0.1298	35.697	-0.000	8574	0.00	BV	4.1		0
4	C16:1	0.2748	35.843	-0.121	18156	0.00	VB	4.1		0
5	unk	0.1659	36.692	0.000	10964	0.00	BV	4.1		0
6	C17:0	16.7782	37.576	-0.038	1108674	0.00	BB	5.3		0
7	C18:0	2.6728	39.683	-0.084	176616	0.00	BP	5.3		0
8	unk	19.2357	40.139	0.000	1271062	0.00	PV	5.5		0
9	C18:1n9c	0.8227	40.234	0.042	54363	0.00	VB	4.1		0
10	C18:2n6c	41.4051	41.168	0.015	2735970	0.00	BB	6.7		0
11	C18:3n3	1.1103	42.351	-0.127	73367	0.00	VB	4.3		0
12	C20:0	0.3403	43.674	-0.149	22487	0.00	VP	4.7		0
13	C20:1n9	0.1292	44.070	-0.146	8536	0.00	PB	5.0		0
14	C24:0	0.4434	55.972	0.296	29301	0.00	BB	12.4		0
	Totals	**100.0000**		**-0.494**	**6607816**					

Figure A17 GC chromatogram of GJ oil methyl ester.

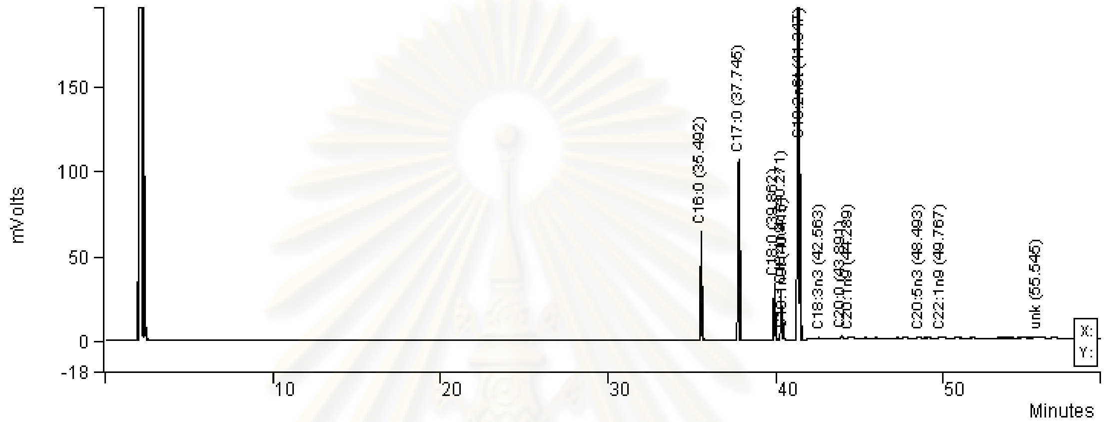
Data File:	c:\star\joe\24(14.1.09) 10;22;14 am.run	Operator (Calc):	jo
Channel:	Front = FID RESULTS	Calc Date:	05/04/2010 19:13:59
Sample ID:	HIS	Times Calculated:	17
Operator (Inj):	io	Calculation Method:	his 10;22;14 am-front.mth
Injection Date:	13/02/2010 10:22:14	Instrument (Calc):	GC3800
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	GC3800	Calibration Level:	N/A
		Verification Tolerance:	N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C14:0	0.1997	35.371	-0.026	1885	0.00	BB	3.2		0
2	C16:0	16.7242	40.164	-0.002	157871	0.00	BB	3.3		0
3	C16:1	0.2571	40.772	-0.022	2427	0.00	BB	3.2		0
4	C17:0	18.4519	42.409	0.003	174181	0.00	BB	3.5		0
5	C18:0	4.1196	44.548	-0.013	38887	0.00	BB	3.5		0
6	C18:1n9c	31.2381	45.063	0.000	294878	0.00	BB	3.9		0
7	unk	0.4119	45.194	0.000	3889	0.00	TS	0.0		0
8	C18:2n6c	26.2159	46.182	0.000	247471	0.00	BB	4.2		0
9	unk	1.5273	47.676	0.000	14417	0.00	BV	4.9		0
10	C18:3n3	0.1606	47.872	-0.034	1516	0.00	VB	4.6		0
11	C20:0	0.6937	49.768	-0.043	6548	0.00	BB	5.4		0
	Totals	**100.0000**		**-0.137**	**943970**					

Figure A18 GC chromatogram of HIS oil methyl ester.

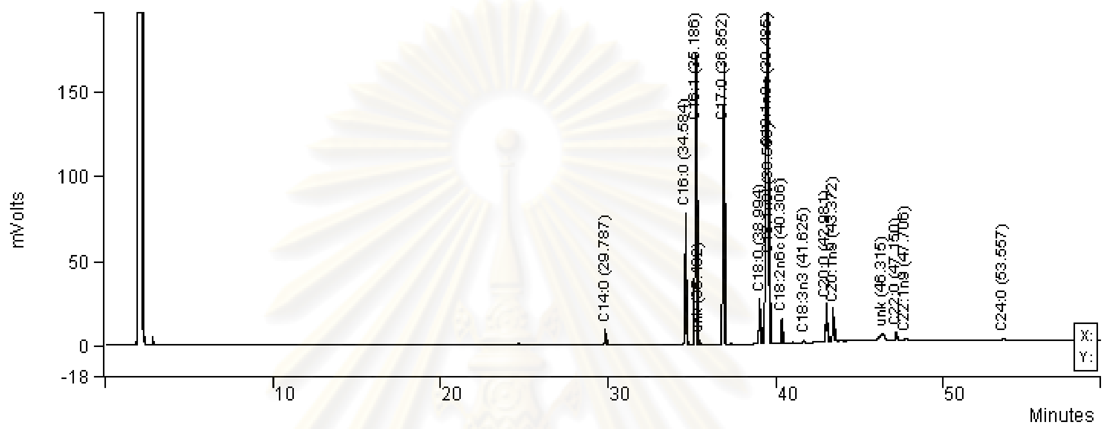
Data File:	c:\star\joe\14(16.2.52) 5;47;14 pm.run	Operator (Calc):	keak
Channel:	Front = FID RESULTS	Calc Date:	05/04/2010 17:50:21
Sample ID:	LS	Times Calculated:	3
Operator (Inj):	keak	Calculation Method:	ls 5;47;14 pm-front.mth
Injection Date:	20/01/2010 17:47:14	Instrument (Calc):	GC3800
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	GC3800	Calibration Level:	N/A
		Verification Tolerance:	N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	9.7410	35.492	0.001	296898	0.00	BB	4.3		0
2	C17:0	16.8479	37.745	0.013	513510	0.00	BB	4.5		0
3	C18:0	5.3844	39.862	-0.006	164112	0.00	BB	4.5		0
4	C18:1n9c	4.5819	40.271	-0.037	139653	0.00	BB	4.4		0
5	C18:1n9t	0.2561	40.415	-0.019	7806	0.00	TS	0.0		0
6	C18:2n6t	62.1903	41.347	0.000	1895513	0.00	BB	6.0		0
7	C18:3n3	0.1218	42.563	-0.013	3714	0.00	BB	4.4		0
8	C20:0	0.2614	43.891	-0.014	7967	0.00	BB	4.6		0
9	C20:1n9	0.0915	44.289	-0.014	2789	0.00	BB	6.7		0
10	C20:5n3	0.0823	48.493	-0.024	2508	0.00	BB	7.0		0
11	C22:1n9	0.0950	49.767	-0.227	2894	0.00	BB	7.7		0
12	unk	0.3465	55.545	-0.037	10562	0.00	BV	11.5		0
	Totals	**100.0001**		**-0.377**	**3047926**					

Figure A19 GC chromatogram of LS oil methyl ester.

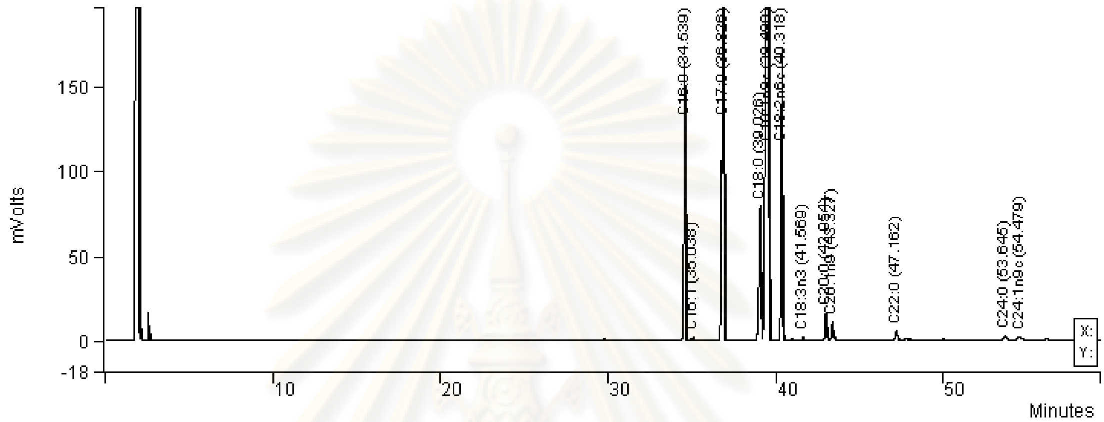
Data File:	c:\star\joe\maccadamia1 30.8.52	Operator (Calc):	jo
Channel:	Front = FID RESULTS	Calc Date:	05/04/2010 14:20:56
Sample ID:	MAI 1	Times Calculated:	9
Operator (Inj):	jo	Calculation Method:	mai 1 30.8.52 12;04;43 pm-front.mth
Injection Date:	02/02/2010 12:04:43	Instrument (Calc):	gc
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	gc	Calibration Level:	N/A
		Verification Tolerance:	N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C14:0	0.7233	29.787	-0.018	40346	0.00	BB	4.1		0
2	C16:0	6.9582	34.584	0.031	388132	0.00	BB	4.7		0
3	C16:1	16.5512	35.186	0.068	923233	0.00	BB	5.0		0
4	unk	0.2281	35.402	0.000	12726	0.00	TS	0.0		0
5	C17:0	16.7995	36.852	0.082	937079	0.00	BB	5.3		0
6	C18:0	2.7901	38.994	0.066	155633	0.00	BV	5.5		0
7	C18:1n9c	45.9767	39.485	-0.000	2564591	0.00	VV	7.2		0
8	C18:1n9t	2.6965	39.560	0.000	150410	0.00	VB	3.8		0
9	C18:2n6c	1.2171	40.306	-0.001	67889	0.00	BB	4.4		0
10	C18:3n3	0.1001	41.625	-0.009	5581	0.00	BB	4.6		0
11	C20:0	2.0298	42.981	-0.005	113224	0.00	BP	4.7		0
12	C20:1n9	1.7247	43.372	0.001	96202	0.00	PV	4.7		0
13	unk	1.2155	46.315	-0.000	67803	0.00	BB	18.6		0
14	C22:0	0.5671	47.150	-0.026	31634	0.00	BB	6.5		0
15	C22:1n9	0.1739	47.706	-0.019	9698	0.00	BV	7.1		0
16	C24:0	0.2482	53.557	-0.050	13847	0.00	BB	11.4		0
	Totals	**100.0000**		**0.120**	**5578028**					

Figure A20 GC chromatogram of MAI oil methyl ester.

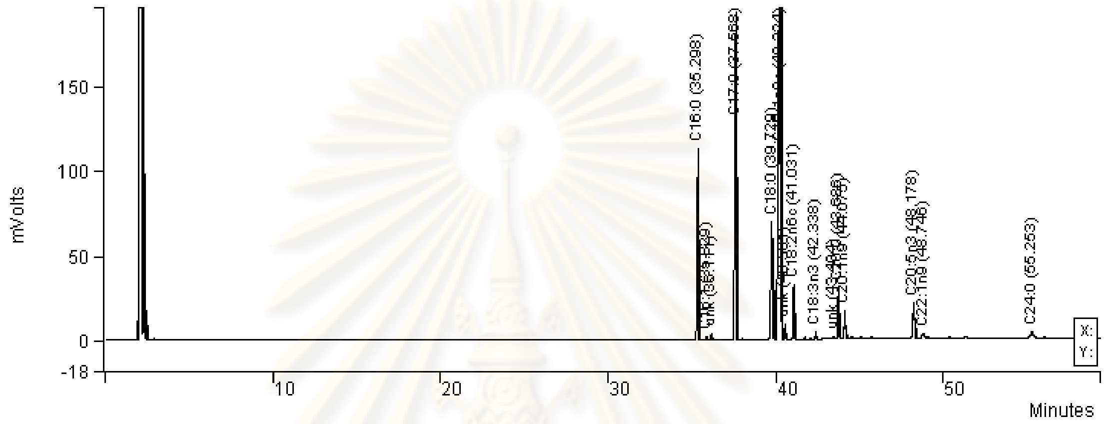
Data File:	c:\star\joe\pikul keak 6;51;23 pm.run	Operator (Calc):	jo
Channel:	Front = FID RESULTS	Calc Date:	01/04/2010 21:13:38
Sample ID:	pikul keak	Times Calculated:	9
Operator (Inj):	jo	Calculation Method:	c:\labchem07\new method jo-1.2.mth
Injection Date:	01/04/2010 18:51:23	Instrument (Calc):	gc
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	gc	Calibration Level:	N/A
		Verification Tolerance:	N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	9.9000	34.539	-0.014	978519	0.00	BB	5.6		0
2	C16:1	0.1030	35.038	-0.080	10180	0.00	VB	4.8		0
3	C17:0	16.8969	36.826	0.056	1670098	0.00	BB	6.6		0
4	C18:0	7.1051	39.026	0.098	702267	0.00	BV	8.3		0
5	C18:1n9c	53.3877	39.498	0.151	5276855	0.00	VV	9.6		0
6	C18:2n6c	9.8492	40.318	0.011	973498	0.00	VB	5.4		0
7	C18:3n3	0.1043	41.569	-0.065	10308	0.00	VB	5.3		0
8	C20:0	0.9782	42.954	-0.032	96681	0.00	BV	5.4		0
9	C20:1n9	0.6655	43.327	-0.044	65780	0.00	VV	5.5		0
10	C22:0	0.4460	47.162	-0.014	44085	0.00	BV	7.5		0
11	C24:0	0.2992	53.645	0.038	29576	0.00	BV	13.0		0
12	C24:1n9c	0.2649	54.479	-0.075	26186	0.00	VV	16.9		0
	Totals	**100.0000**		**0.030**	**9884033**					

Figure A21 GC chromatogram of ME oil methyl ester.

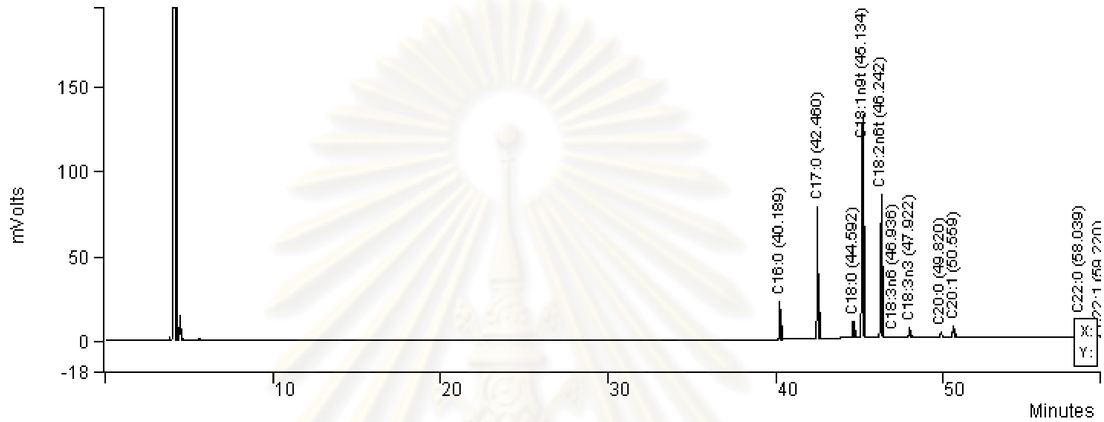
Data File:	c:\star\joe\peep1 1;57;00 pm.run	Operator (Calc):	jo
Channel:	Front = FID RESULTS	Calc Date:	05/04/2010 13:03:59
Sample ID:	peep1	Times Calculated:	14
Operator (Inj):	jo	Calculation Method:	peep1 1;57;00 pm-front.mth
Injection Date:	19/02/2010 13:57:00	Instrument (Calc):	gc
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	gc	Calibration Level:	N/A
		Verification Tolerance:	N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	9.0871	35.298	-0.093	544816	0.00	BB	4.5		0
2	C16:1	0.1408	35.829	-0.135	8442	0.00	VV	4.1		0
3	unk	0.2684	36.111	0.000	16090	0.00	VB	4.2		0
4	C17:0	17.5724	37.568	-0.046	1053558	0.00	BB	5.2		0
5	C18:0	7.6501	39.729	-0.037	458664	0.00	BV	6.2		0
6	C18:1n9c	54.3411	40.224	0.032	3258029	0.00	VV	7.3		0
7	unk	0.6679	40.501	-0.042	40045	0.00	VB	4.1		0
8	C18:2n6c	2.4406	41.031	-0.122	146330	0.00	BB	4.3		0
9	C18:3n3	0.3116	42.338	-0.140	18683	0.00	BB	4.2		0
10	unk	0.1009	43.404	-0.000	6049	0.00	BV	4.5		0
11	C20:0	2.3853	43.686	-0.137	143014	0.00	VB	4.4		0
12	C20:1n9	1.3530	44.075	-0.141	81119	0.00	BV	4.5		0
13	C20:5n3	2.5448	48.178	0.114	152573	0.00	BB	7.0		0
14	C22:1n9	0.3796	48.746	-0.275	22759	0.00	BV	9.5		0
15	C24:0	0.7563	55.253	-0.424	45345	0.00	BB	12.0		0
	Totals	**99.9999**		**-1.446**	**5995516**					

Figure A22 GC chromatogram of MH oil methyl ester.

Data File:	c:\star\joe\ml 6;08;25 pm.run	Operator (Calc):	keak
Channel:	Front = FID RESULTS	Calc Date:	06/04/2010 09:06:34
Sample ID:	ML	Times Calculated:	7
Operator (Inj):	keak	Calculation Method:	ml 7;13;31 pm-front.mth
Injection Date:	28/01/2010 18:08:25	Instrument (Calc):	GC3800
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	GC3800	Calibration Level:	N/A
		Verification Tolerance:	N/A

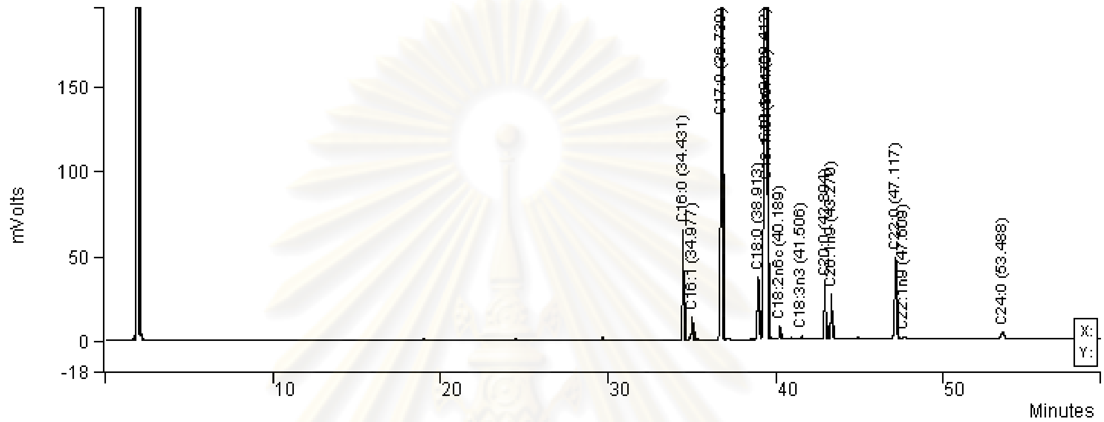


Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	4.7348	40.189	0.023	77714	0.00	BB	3.2		0
2	C17:0	18.0481	42.460	0.054	296231	0.00	BB	3.5		0
3	C18:0	2.3386	44.592	0.031	38384	0.00	BB	3.7		0
4	C18:1n9t	38.6646	45.134	-0.000	634617	0.00	BB	4.5		0
5	C18:2n6t	24.4458	46.242	0.000	401239	0.00	BB	4.4		0
6	C18:3n6	0.0834	46.936	-0.049	1369	0.00	BB	4.3		0
7	C18:3n3	1.5170	47.922	0.016	24900	0.00	BB	4.6		0
8	C20:0	0.9785	49.820	0.009	16061	0.00	BB	5.5		0
9	C20:1	2.3993	50.559	0.011	39381	0.00	BB	5.7		0
10	C22:0	5.9531	58.039	0.045	97711	0.00	BB	9.1		0
11	C22:1	0.4825	59.220	0.008	7919	0.00	BB	9.9		0
	Totals	**99.6457**		**0.148**	**1635526**					

Figure A23 GC chromatogram of ML oil methyl ester.

Data File:	c:\star\joe\marum kikky 12;04;41
Channel:	Front = FID RESULTS
Sample ID:	marum kikky
Operator (Inj):	io
Injection Date:	19/12/2009 12:04:41
Injection Method:	c:\labchem07\new method jo-1.2.mth
Run Time (min):	59.440
Workstation:	
Instrument (Inj):	gc

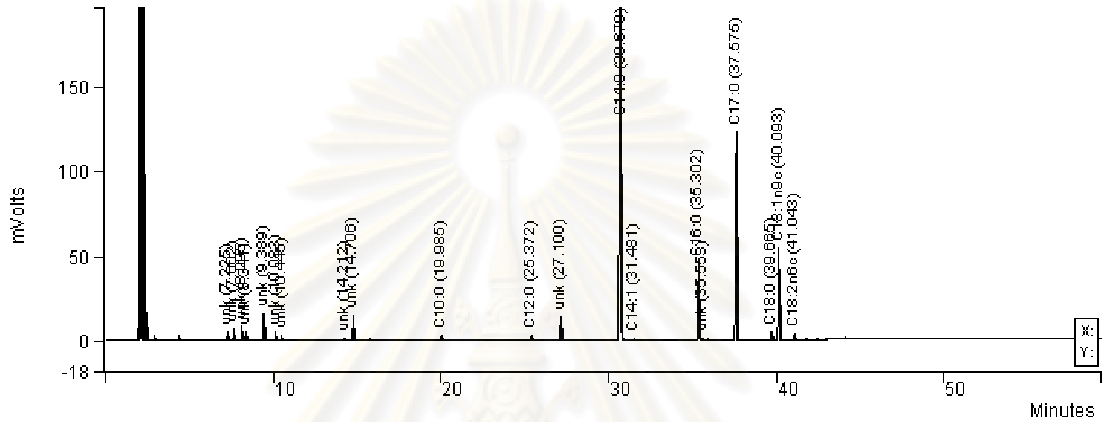
Operator (Calc):	jo
Calc Date:	01/04/2010 21:21:58
Times Calculated:	6
Calculation Method:	c:\labchem07\new method jo-1.2.mth
Instrument (Calc):	gc
Run Mode:	Analysis
Peak Measurement:	Peak Area
Calculation Type:	Percent
Calibration Level:	N/A
Verification Tolerance:	N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	4.6147	34.431	-0.122	344698	0.00	BB	5.0		0
2	C16:1	0.9637	34.977	-0.141	71982	0.00	VV	5.0		0
3	C17:0	17.0697	36.739	-0.031	1275037	0.00	BB	6.0		0
4	C18:0	3.9761	38.913	-0.015	296999	0.00	BV	7.6		0
5	C18:1n9c	58.9249	39.412	0.065	4401455	0.00	VV	8.9		0
6	C18:1n9t	3.0916	39.479	-0.000	230934	0.00	VV	4.0		0
7	C18:2n6c	0.5576	40.189	-0.118	41650	0.00	VB	5.0		0
8	C18:3n3	0.1257	41.506	-0.000	9387	0.00	VV	5.4		0
9	C20:0	2.5595	42.894	-0.092	191184	0.00	PV	5.6		0
10	C20:1n9	2.0311	43.270	-0.101	151716	0.00	VV	5.4		0
11	C22:0	5.2477	47.117	-0.059	391986	0.00	BV	7.7		0
12	C22:1n9	0.1352	47.609	-0.116	10096	0.00	VV	8.1		0
13	C24:0	0.7026	53.488	-0.119	52484	0.00	BB	12.9		0
	Totals	**100.0001**		**-0.849**	**7469608**					

Figure A24 GC chromatogram of MO oil methyl ester.

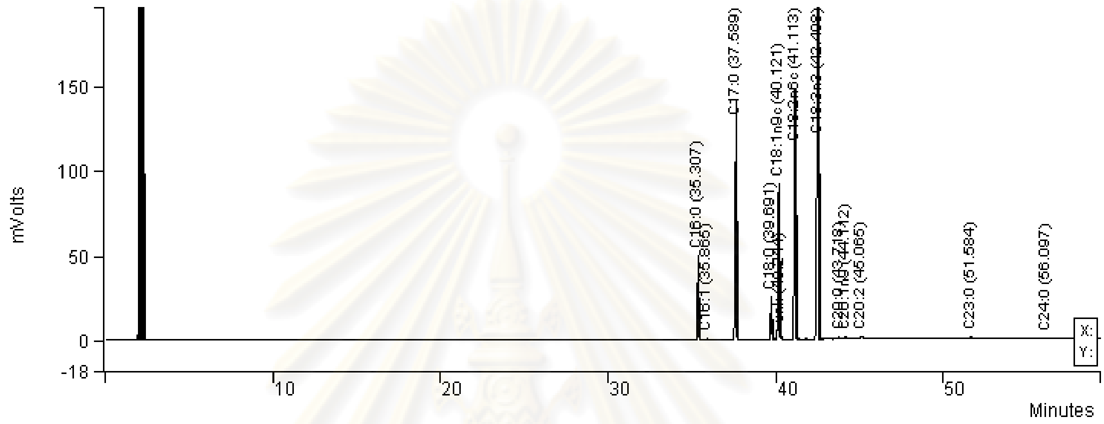
Data File:	c:\star\joe\jantet1 1;01;03 pm.run	Operator (Calc):	keak
Channel:	Front = FID RESULTS	Calc Date:	05/04/2010 15:03:47
Sample ID:	MYF 1	Times Calculated:	4
Operator (Inj):	keak	Calculation Method:	myfl 2;20;51 pm-front.mth
Injection Date:	5/02/2010 13:01:03	Instrument (Calc):	gc
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	gc	Calibration Level:	N/A
		Verification Tolerance:	N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	unk	0.4397	7.225	0.000	14892	0.00	BB	2.9		0
2	unk	0.5557	7.602	-0.001	18821	0.00	BV	2.6		0
3	unk	0.7219	8.095	0.000	24450	0.00	BB	2.7		0
4	unk	0.4028	8.341	0.000	13643	0.00	BV	2.8		0
5	unk	1.4585	9.389	0.000	49401	0.00	BB	2.9		0
6	unk	0.4206	10.083	-0.000	14245	0.00	BB	2.9		0
7	unk	0.3257	10.445	-0.000	11032	0.00	BB	3.0		0
8	unk	0.1570	14.212	0.000	5317	0.00	BB	3.4		0
9	unk	1.5503	14.706	0.000	52510	0.00	BB	3.3		0
10	C10:0	0.3419	19.985	0.147	11579	0.00	PB	3.4		0
11	C12:0	0.4129	25.372	-0.101	13984	0.00	BB	3.8		0
12	unk	1.5089	27.100	-0.000	51107	0.00	VB	3.5		0
13	C14:0	58.2049	30.679	0.037	1971477	0.00	BB	6.0		0
14	C14:1	0.1326	31.481	-0.054	4491	0.00	BB	4.6		0
15	C16:0	6.0993	35.302	-0.089	206592	0.00	BV	4.3		0
16	unk	0.1425	35.553	-0.000	4827	0.00	VB	3.7		0
17	C17:0	18.6207	37.575	-0.039	630709	0.00	BB	4.8		0
18	C18:0	0.6875	39.665	-0.102	23288	0.00	VB	4.5		0
19	C18:1n9c	7.3454	40.093	-0.099	248798	0.00	BB	4.3		0
20	C18:2n6c	0.4715	41.043	-0.110	15971	0.00	PB	4.6		0
	Totals	**100.0003**		**-0.411**	**3387134**					

Figure A25 GC chromatogram of MYF oil methyl ester.

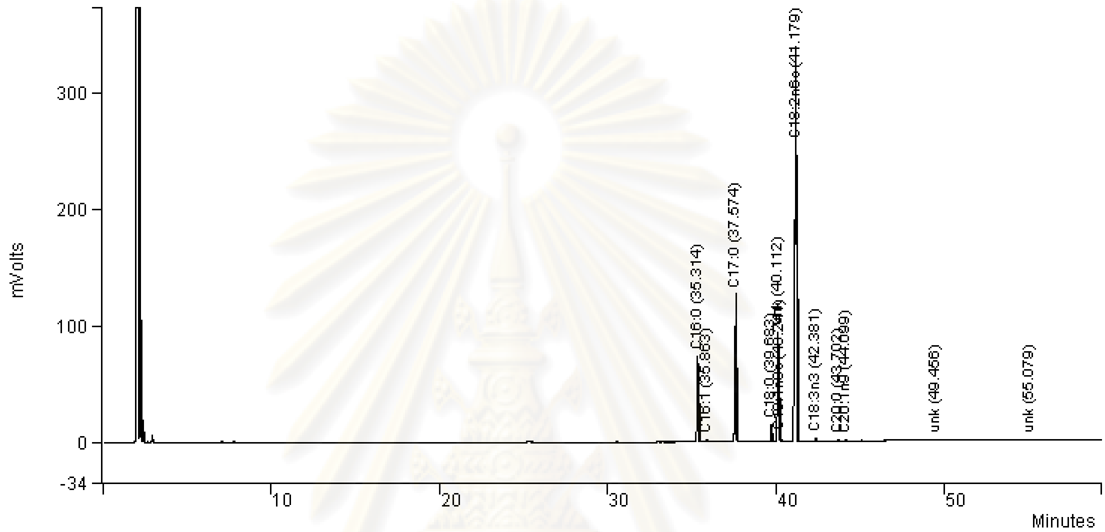
Data File:	c:\star\joe\maengluk2 10;41;18 am.run	Operator (Calc):	keak
Channel:	Front = FID RESULTS	Calc Date:	04/02/2010 11:40:39
Sample ID:	OC 2	Times Calculated:	1
Operator (Inj):	keak	Calculation Method:	c:\labchem07\new method jo-1.2.mth
Injection Date:	04/02/2010 10:41:18	Instrument (Calc):	gc
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	gc	Calibration Level:	N/A
		Verification Tolerance:	N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	5.2109	35.307	-0.084	223661	0.00	BB	4.2		0
2	C16:1	0.0943	35.865	-0.099	4047	0.00	VB	4.3		0
3	C17:0	16.9916	37.589	-0.025	729312	0.00	BB	4.9		0
4	C18:0	2.8428	39.691	-0.076	122020	0.00	BB	4.6		0
5	C18:1n9c	10.5339	40.121	-0.071	452134	0.00	BV	4.6		0
6	unk	0.4962	40.244	-0.050	21298	0.00	VB	4.3		0
7	C18:2n6c	17.7429	41.113	-0.040	761561	0.00	BP	4.8		0
8	C18:3n3	45.3208	42.498	0.020	1945259	0.00	BP	5.8		0
9	C20:0	0.1850	43.718	-0.105	7939	0.00	PB	4.7		0
10	C20:1n9	0.1265	44.112	-0.104	5431	0.00	BB	4.5		0
11	C20:2	0.2047	45.065	-0.130	8786	0.00	BB	9.0		0
12	C23:0	0.1258	51.584	0.004	5399	0.00	BV	8.1		0
13	C24:0	0.1247	56.097	0.420	5352	0.00	BB	11.9		0
	Totals	**100.0001**		**-0.340**	**4292199**					

Figure A26 GC chromatogram of OC oil methyl ester.

Data File:	c:\star\joe\katokrok1 3;39;48 pm.run	Operator (Calc):	jo
Channel:	Front = FID RESULTS	Calc Date:	05/04/2010 13:49:37
Sample ID:	PAF 1	Times Calculated:	18
Operator (Inj):	jo	Calculation Method:	paf 1 3;39;48 pm-front.mth
Injection Date:	28/01/2010 15:39:48	Instrument (Calc):	gc
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	gc	Calibration Level:	N/A
		Verification Tolerance:	N/A

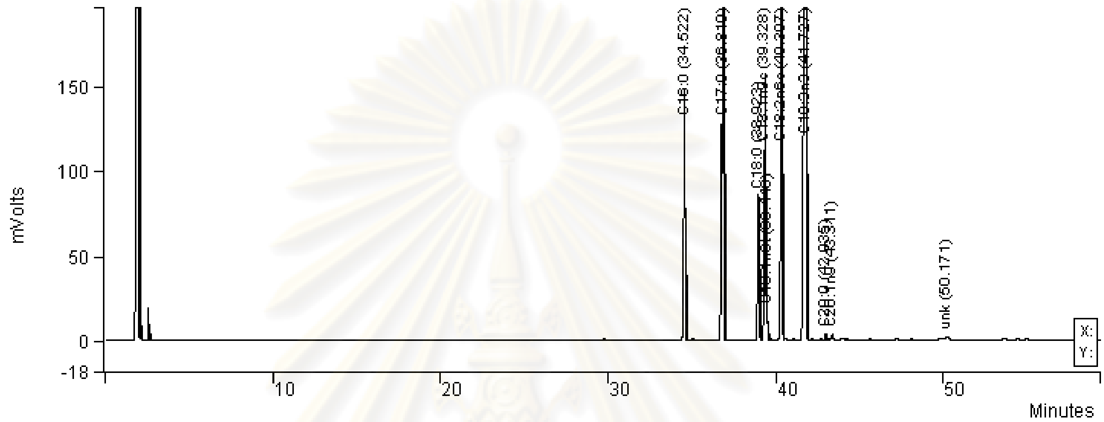


Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	8.8319	35.314	-0.077	337619	0.00	BB	4.3		0
2	C16:1	0.1544	35.863	-0.101	5902	0.00	VB	4.3		0
3	C17:0	16.6794	37.574	-0.040	637606	0.00	BB	4.7		0
4	C18:0	2.0326	39.683	-0.084	77699	0.00	BB	4.9		0
5	unk	12.1543	40.112	0.000	464623	0.00	BV	4.6		0
6	C18:1n9c	0.4421	40.241	0.049	16899	0.00	VB	4.7		0
7	C18:2n6c	58.7909	41.179	0.026	2247407	0.00	BB	6.3		0
8	C18:3n3	0.3987	42.381	-0.097	15241	0.00	VB	4.4		0
9	C20:0	0.1212	43.702	-0.121	4634	0.00	BP	4.8		0
10	C20:1n9	0.1152	44.099	-0.117	4404	0.00	PB	5.4		0
11	unk	0.1341	49.456	-0.000	5127	0.00	BB	7.4		0
12	unk	0.1453	55.079	0.000	5553	0.00	BB	11.5		0
	Totals	**100.0001**		**-0.562**	**3822714**					

Figure A27 GC chromatogram of PAF oil methyl ester.

Data File:	c:\star\joe\27 pe 1;11;33 pm.run
Channel:	Front = FID RESULTS
Sample ID:	PE
Operator (Inj):	keak
Injection Date:	06/04/2010 13:11:33
Injection Method:	c:\labchem07\new method jo-1.2.mth
Run Time (min):	59.440
Workstation:	
Instrument (Inj):	gc

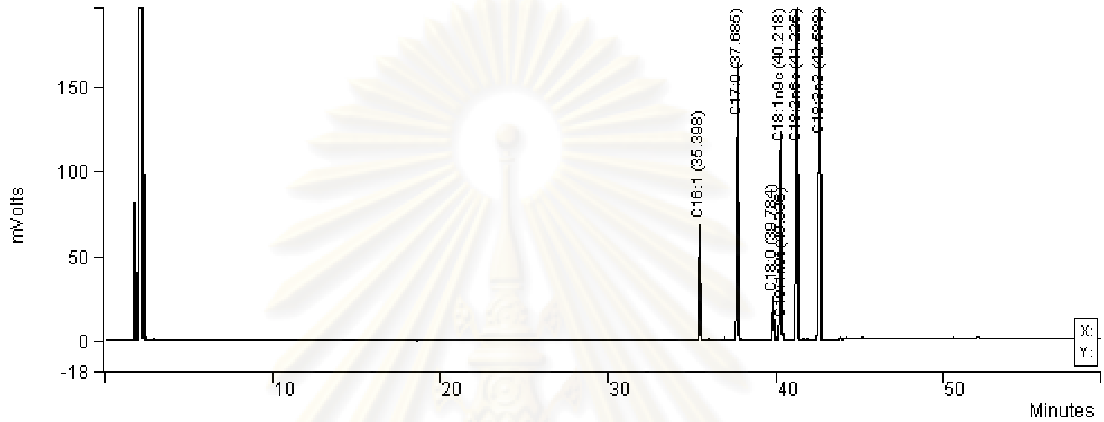
Operator (Calc):	keak
Calc Date:	06/04/2010 17:16:13
Times Calculated:	4
Calculation Method:	27 pe 1;11;33 pm-front.mth
Instrument (Calc):	gc
Run Mode:	Analysis
Peak Measurement:	Peak Area
Calculation Type:	Percent
Calibration Level:	N/A
Verification Tolerance:	N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	8.8947	34.522	-0.031	876065	0.00	BB	5.5		0
2	C17:0	17.6670	36.819	0.049	1740073	0.00	BB	6.5		0
3	C18:0	5.5501	38.923	-0.005	546640	0.00	BV	6.0		0
4	C18:1n9c	9.6412	39.328	-0.019	949588	0.00	VV	5.6		0
5	C18:1n9t	0.7147	39.440	0.000	70396	0.00	VV	4.8		0
6	C18:2n6c	14.2901	40.307	0.000	1407472	0.00	BB	5.8		0
7	C18:3n3	42.6305	41.727	0.093	4198794	0.00	BP	7.9		0
8	C20:0	0.1858	42.935	-0.051	18296	0.00	TS	0.0		0
9	C20:1n9	0.1890	43.311	-0.060	18613	0.00	PB	0.0		0
10	unk	0.2368	50.171	0.000	23325	0.00	BB	10.7		0
	Totals	**99.9999**		**-0.024**	**9849262**					

Figure A28 GC chromatogram of PE oil methyl ester.

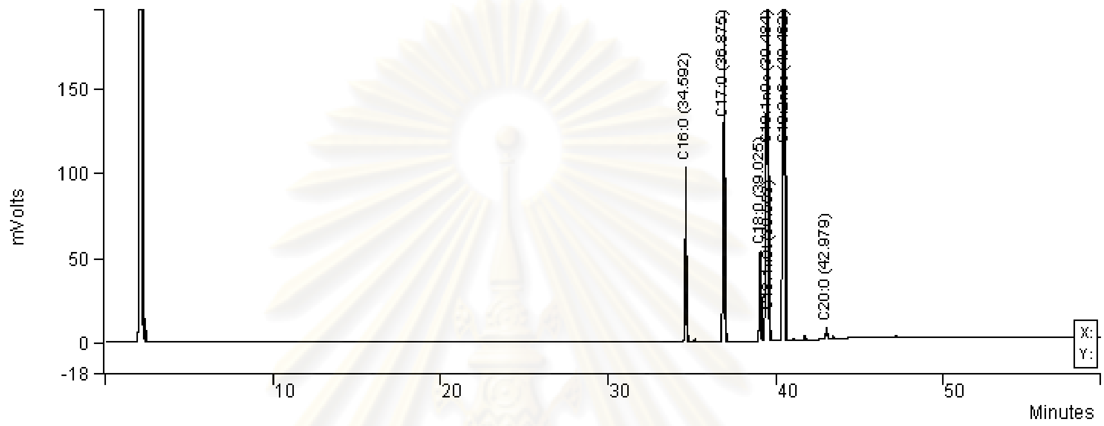
Data File:	c:\star\joe\pf 2;07;41 pm.run	Operator (Calc):	keak
Channel:	Front = FID RESULTS	Calc Date:	06/04/2010 09:50:17
Sample ID:	PF	Times Calculated:	21
Operator (Inj):	keak	Calculation Method:	pf 29.8.52 2;32;08 pm-front.mth
Injection Date:	03/03/2010 14:07:41	Instrument (Calc):	gc
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	gc	Calibration Level:	N/A
		Verification Tolerance:	N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:1	6.0405	35.398	0.280	323133	0.00	BB	4.4		0
2	C17:0	17.0570	37.685	-0.000	912453	0.00	BB	5.3		0
3	C18:0	2.4490	39.784	-0.000	131009	0.00	BV	5.0		0
4	C18:1n9c	11.7686	40.218	-0.000	629556	0.00	VV	4.8		0
5	C18:1n9t	0.6940	40.336	0.000	37124	0.00	VB	4.2		0
6	C18:2n6c	23.4630	41.225	0.000	1255138	0.00	BB	5.2		0
7	C18:3n3	38.5279	42.588	-0.000	2061022	0.00	BB	5.6		0
	Totals	**100.0000**		**0.280**	**5349435**					

Figure A29 GC chromatogram of PF oil methyl ester.

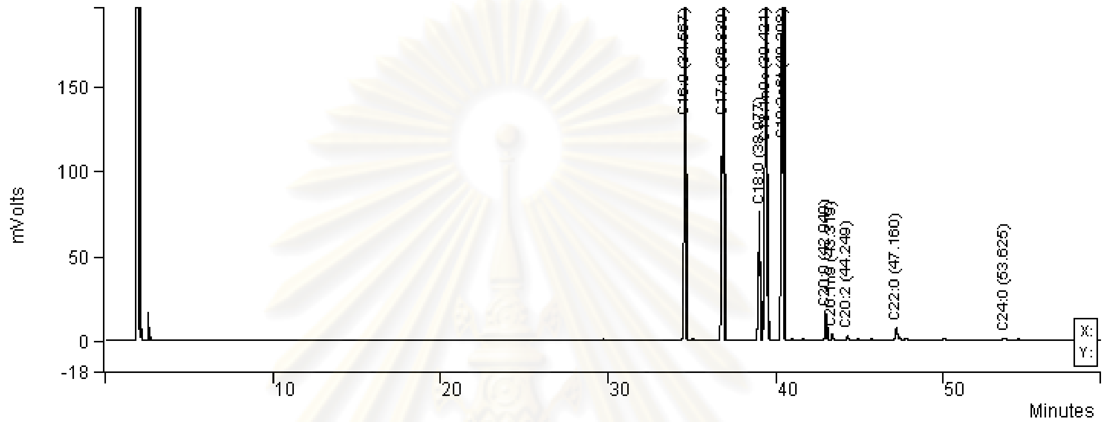
Data File:	c:\star\joe\sei 3;37;52 pm.run	Operator (Calc):	keak
Channel:	Front = FID RESULTS	Calc Date:	06/04/2010 09:58:51
Sample ID:	SEI	Times Calculated:	12
Operator (Inj):	keak	Calculation Method:	sei 2;32;08 pm-front.mth
Injection Date:	05/03/2010 15:37:52	Instrument (Calc):	gc
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	gc	Calibration Level:	N/A
		Verification Tolerance:	N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	7.5242	34.592	0.039	508346	0.00	BB	4.7		0
2	C17:0	16.9608	36.875	0.105	1145904	0.00	BB	5.5		0
3	C18:0	4.5427	39.025	0.097	306913	0.00	BV	5.3		0
4	C18:1n9c	33.5566	39.484	0.000	2267146	0.00	VV	6.7		0
5	C18:1n9t	0.4380	39.553	0.000	29589	0.00	VB	0.0		0
6	C18:2n6c	36.4923	40.462	-0.000	2465490	0.00	BB	6.5		0
7	C20:0	0.4855	42.979	-0.007	32800	0.00	PV	4.8		0
	Totals	**100.0001**		**0.234**	**6756188**					

Figure A30 GC chromatogram of SEI oil methyl ester.

Data File:	c:\star\joe\samohthai keak 7;57;23	Operator (Calc):	jo
Channel:	Front = FID RESULTS	Calc Date:	01/04/2010 21:07:46
Sample ID:	samohthai keak	Times Calculated:	7
Operator (Inj):	io	Calculation Method:	samohthai keak 7;57;23 pm-front.mth
Injection Date:	01/04/2010 19:57:23	Instrument (Calc):	gc
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	gc	Calibration Level:	N/A
		Verification Tolerance:	N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	14.3363	34.567	0.014	1407740	0.00	BB	6.1		0
2	C17:0	17.4484	36.830	0.060	1713337	0.00	BB	6.6		0
3	C18:0	5.9531	38.977	0.049	584564	0.00	BV	7.2		0
4	C18:1n9c	27.7198	39.421	0.074	2721934	0.00	VP	7.4		0
5	C18:2n6t	32.5531	40.398	0.027	3196529	0.00	PB	7.3		0
6	C20:0	0.8979	42.949	-0.037	88164	0.00	BP	5.3		0
7	C20:1n9	0.2164	43.319	-0.051	21252	0.00	PV	5.7		0
8	C20:2	0.1406	44.249	-0.063	13803	0.00	BB	5.4		0
9	C22:0	0.5844	47.160	-0.016	57383	0.00	VV	7.6		0
10	C24:0	0.1501	53.625	0.018	14738	0.00	VV	13.9		0
	Totals	**100.0001**		**0.075**	**9819444**					

Figure A31 GC chromatogram of TC oil methyl ester.

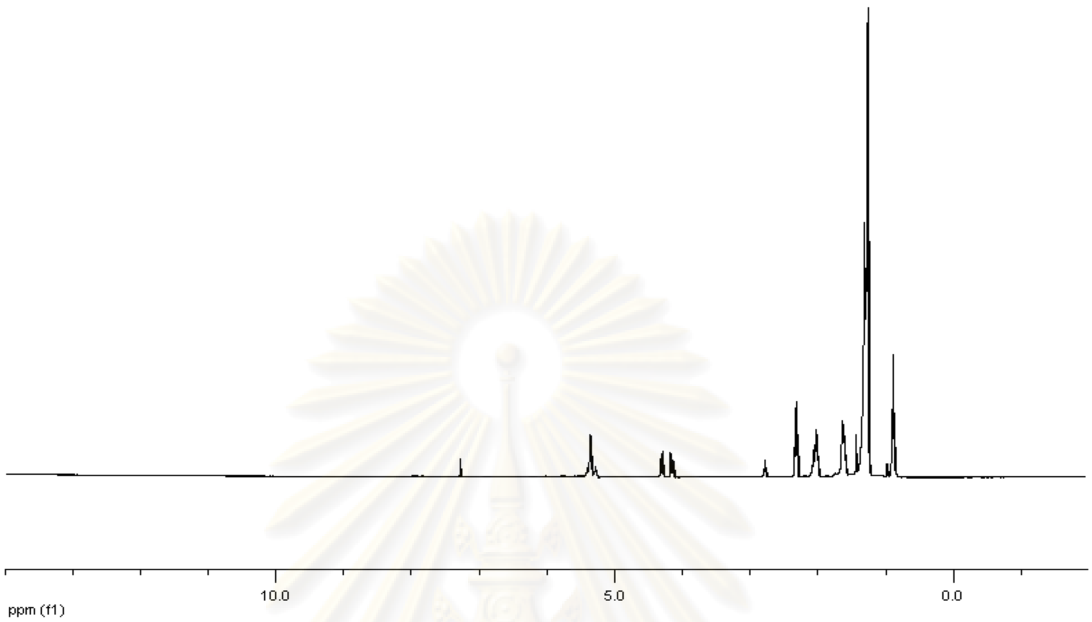


Figure A32 [1]H-NMR spectrum of crude BA oil.

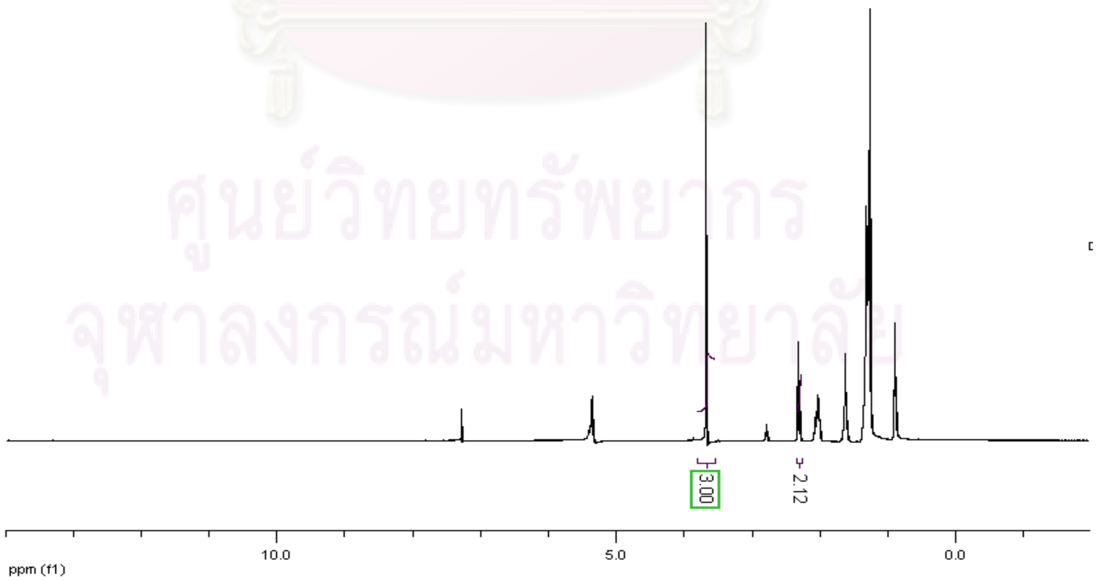


Figure A33 [1]H-NMR spectrum of BA oil methyl ester.

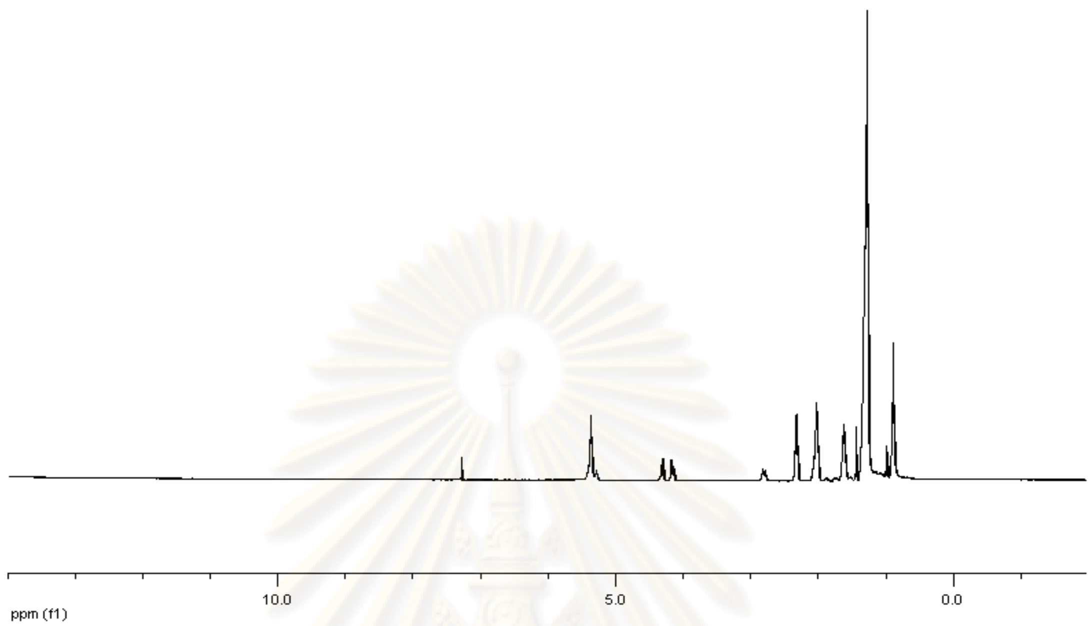


Figure A34 ¹H-NMR spectrum of crude BC oil.

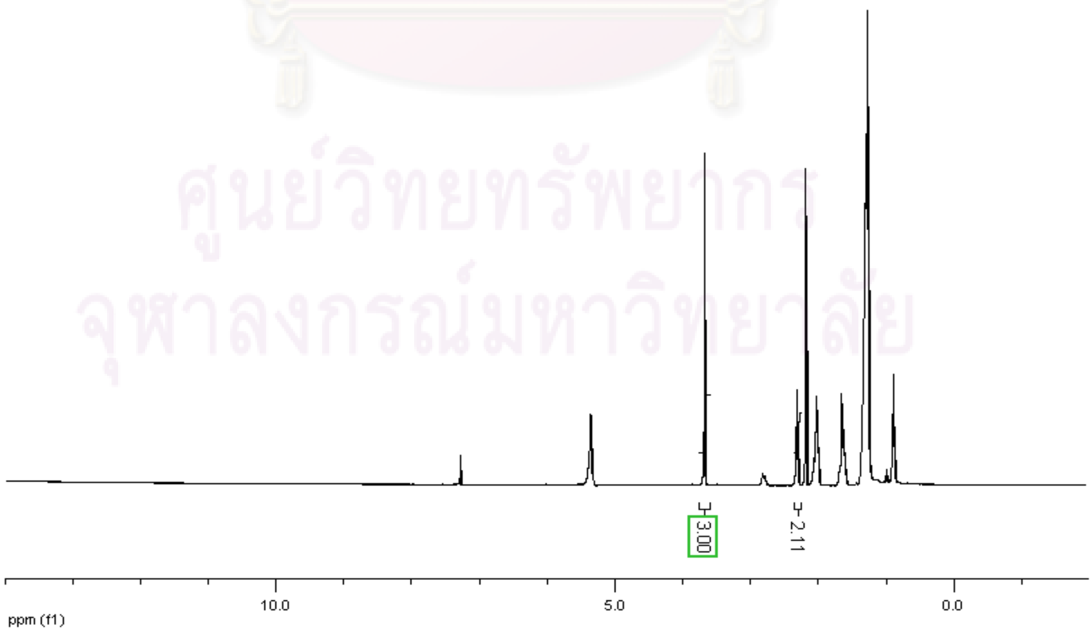


Figure A35 ¹H-NMR spectrum of BC oil methyl ester.

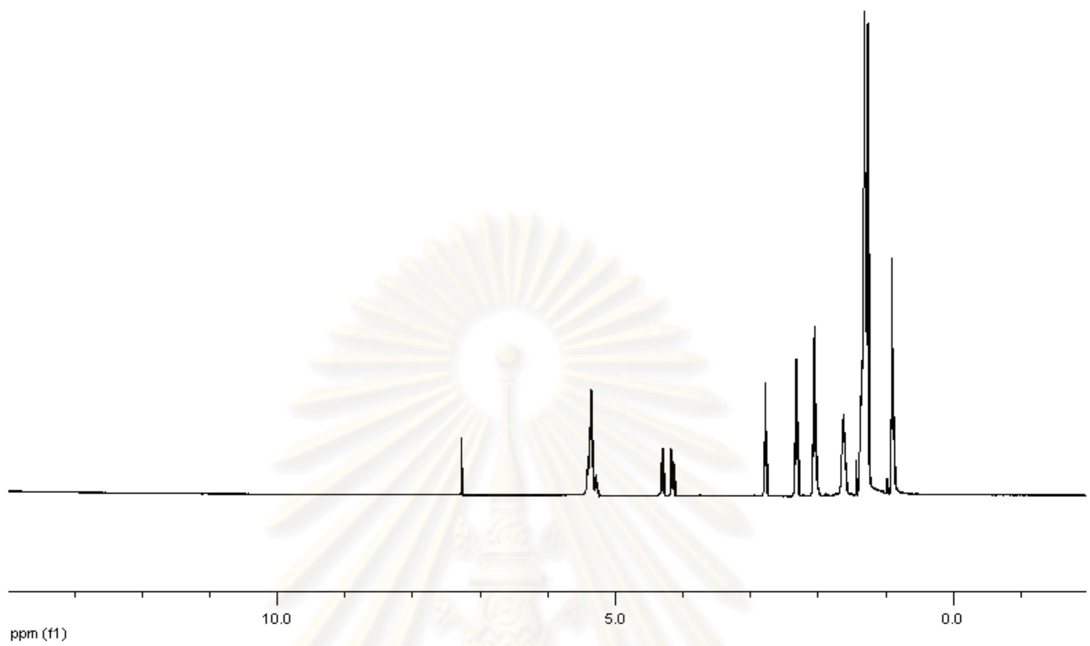


Figure A36 ^{1}H-NMR spectrum of crude BEH oil.

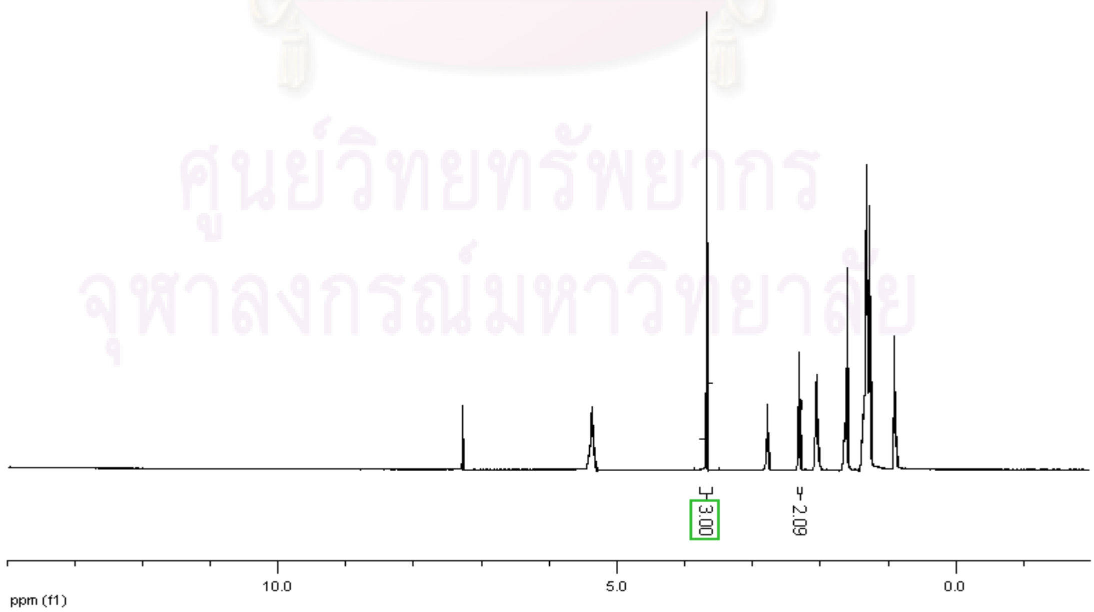


Figure A37 ^{1}H-NMR spectrum of BEH oil methyl ester.

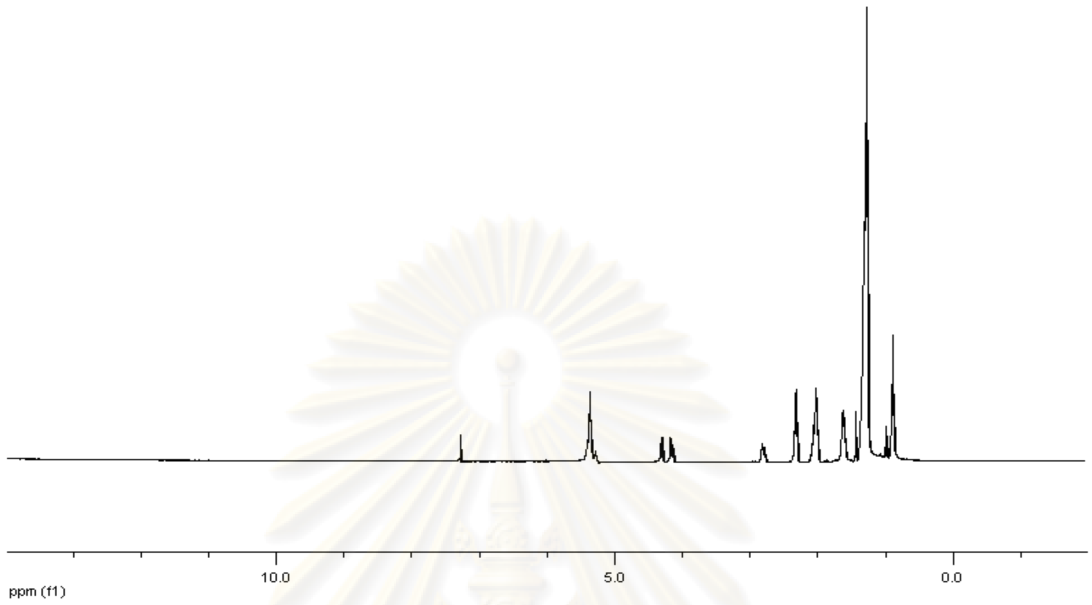


Figure A38 ^{1}H-NMR spectrum of crude BJ oil.

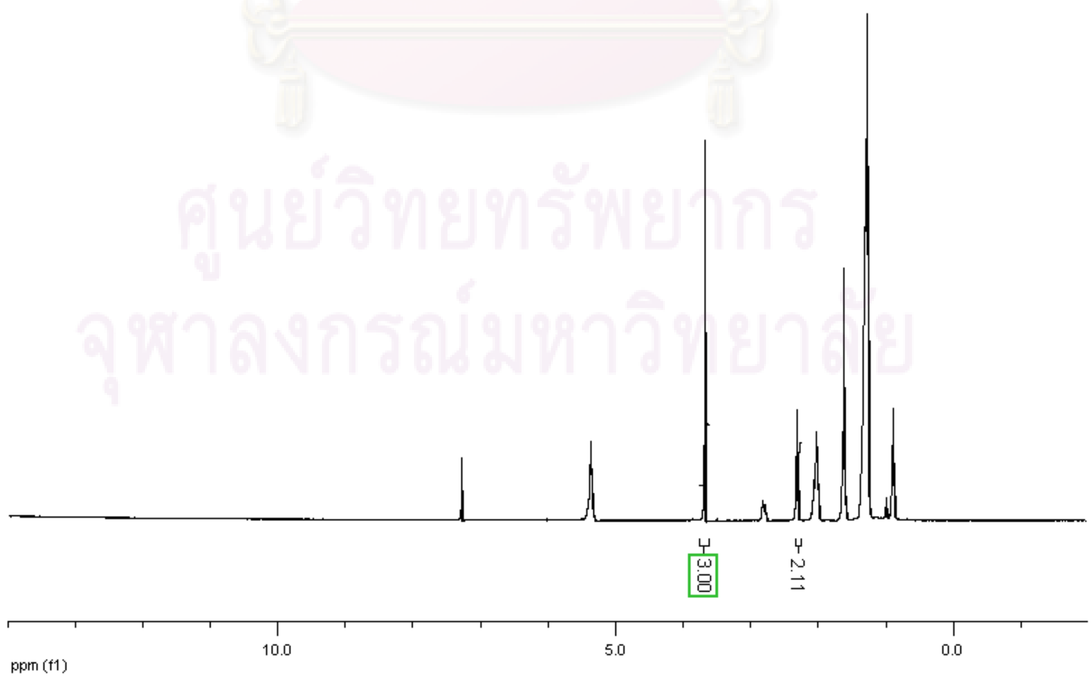


Figure A39 ^{1}H-NMR spectrum of BJ oil methyl ester.

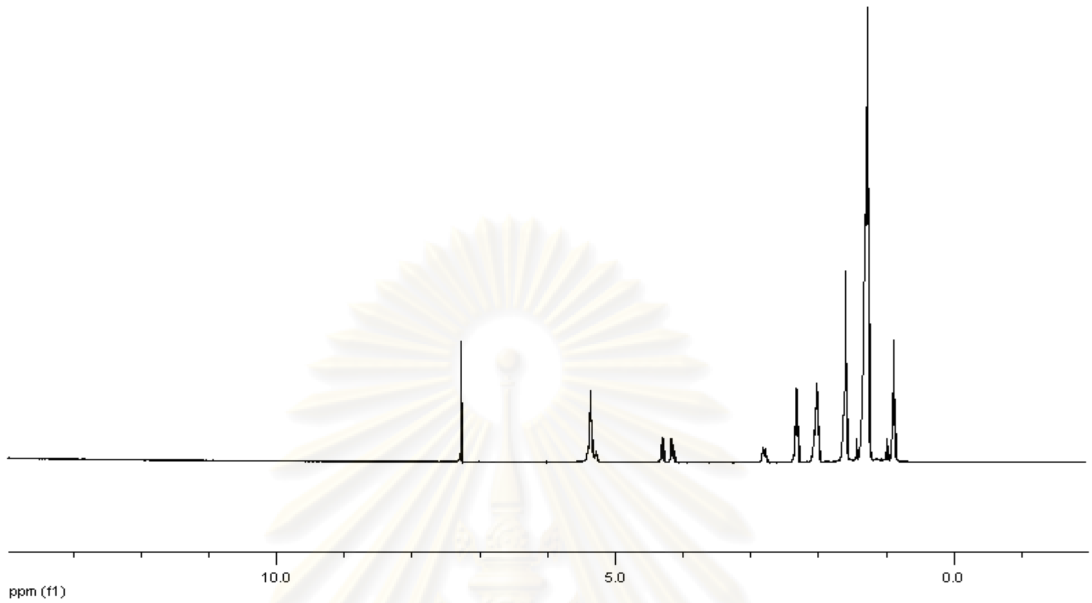


Figure A40 ^{1}H-NMR spectrum of crude BP oil.

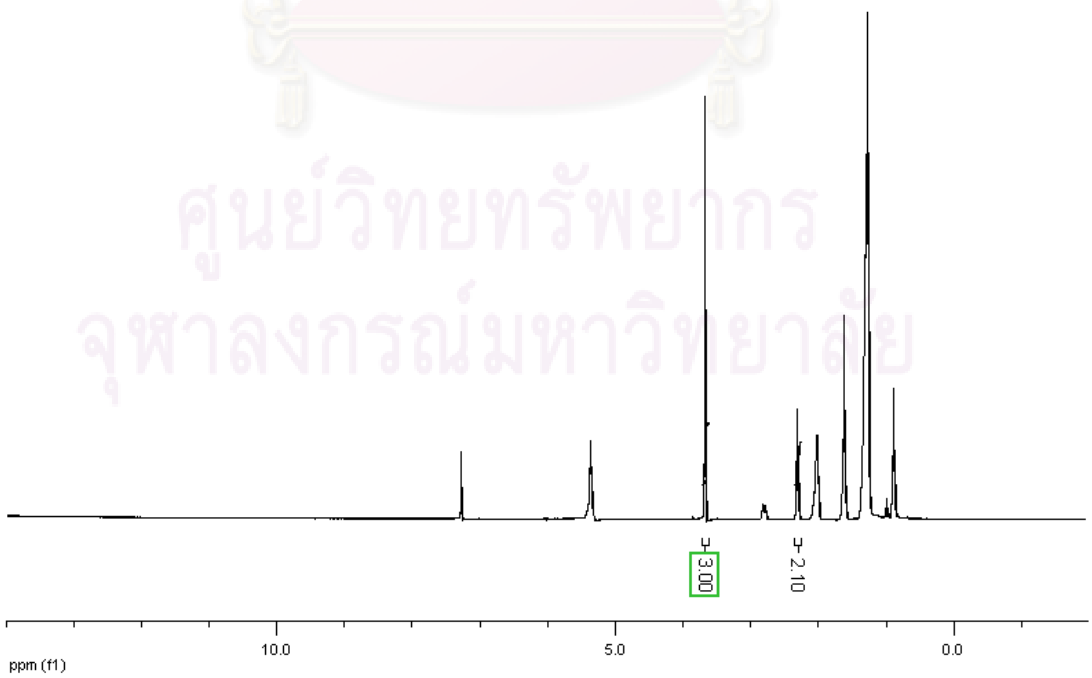


Figure A41 ^{1}H-NMR spectrum of BP oil methyl ester.

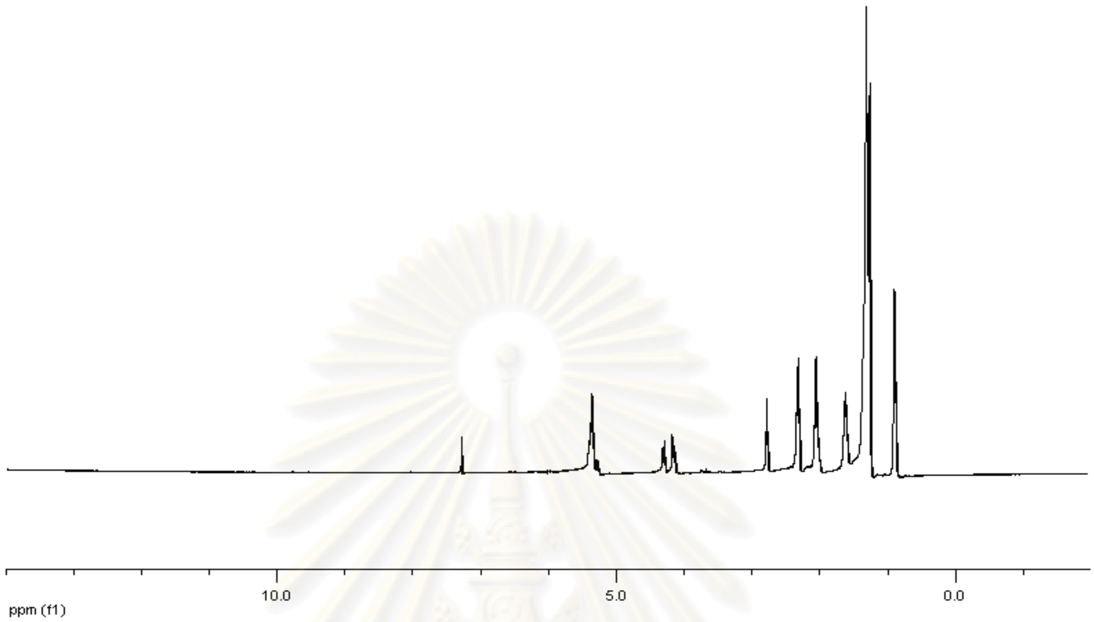


Figure A42 ^{1}H-NMR spectrum of crude CAC oil.

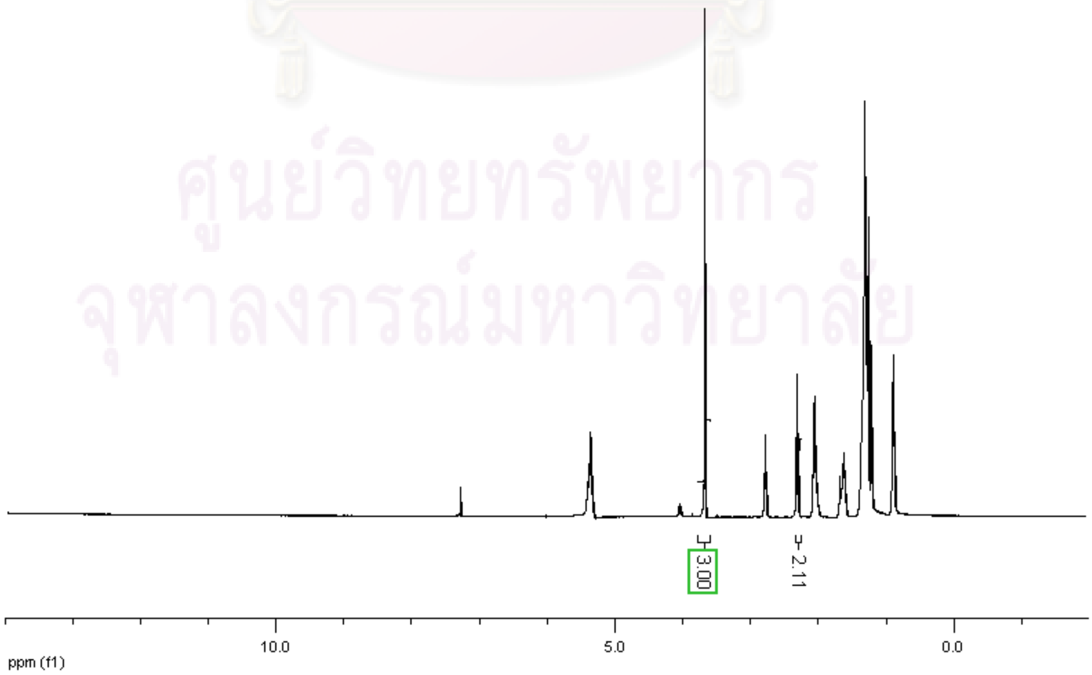


Figure A43 ^{1}H-NMR spectrum of CAC oil methyl ester.

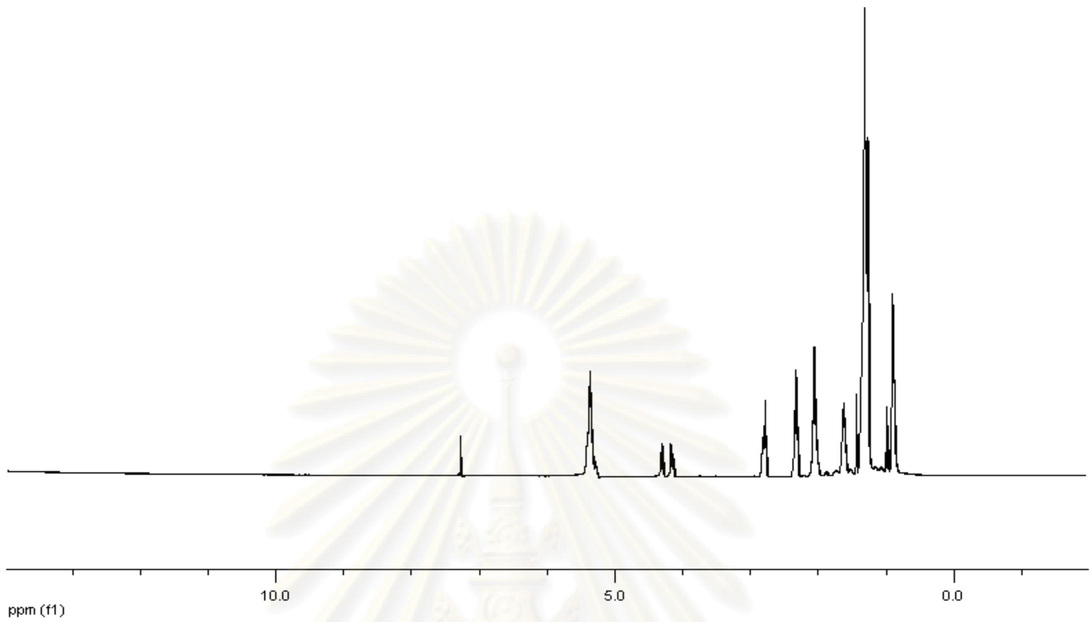


Figure A44 ^{1}H-NMR spectrum of crude CAO oil.

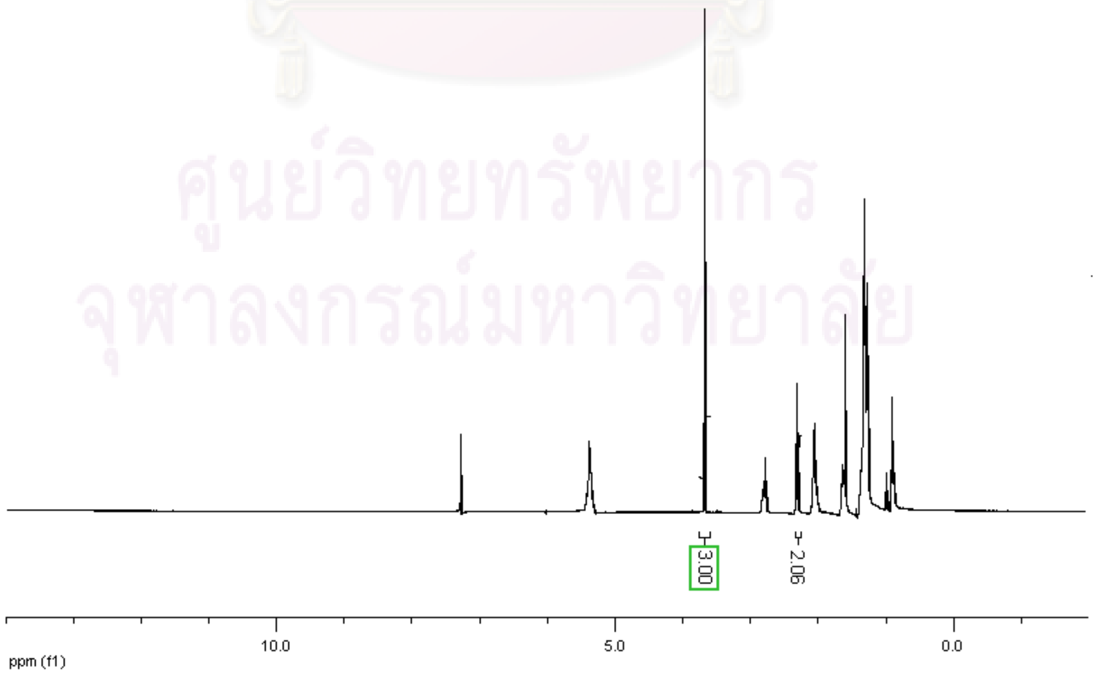


Figure A45 ^{1}H-NMR spectrum of CAO oil methyl ester.

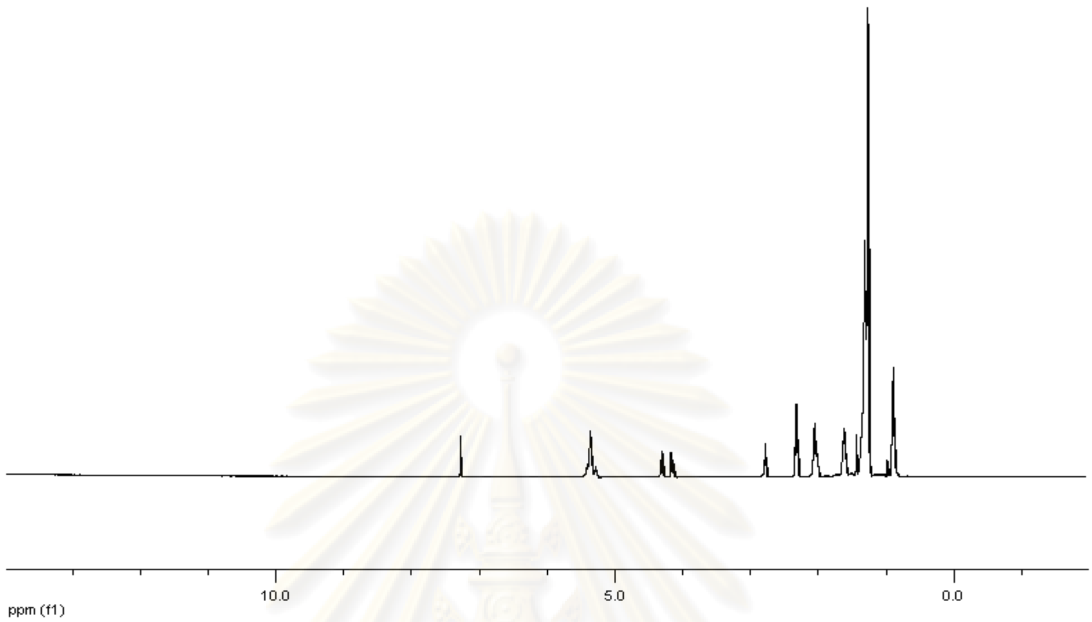


Figure A46 ^{1}H-NMR spectrum of crude CAS oil.

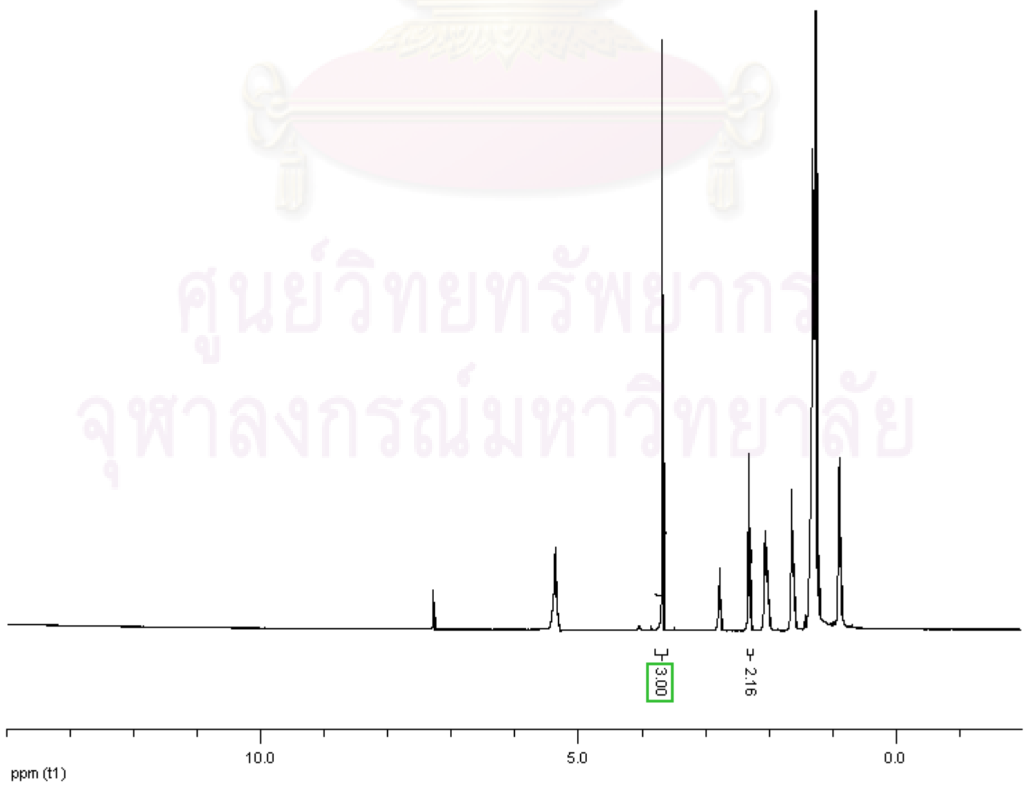


Figure A47 ^{1}H-NMR spectrum of CAS oil methyl ester.

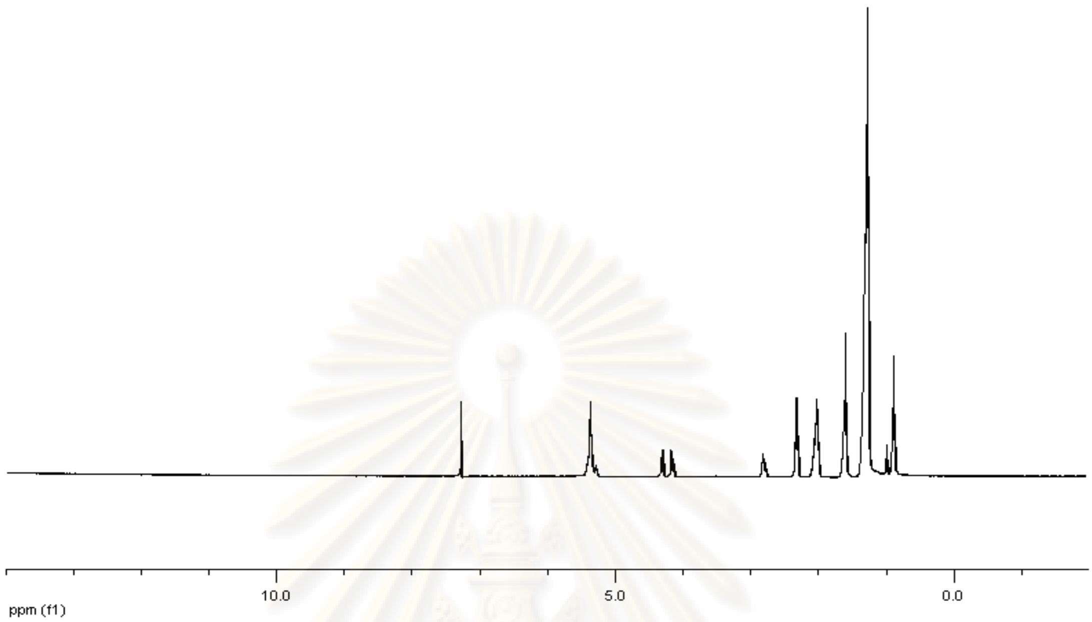


Figure A48 ^{1}H-NMR spectrum of crude CC oil.

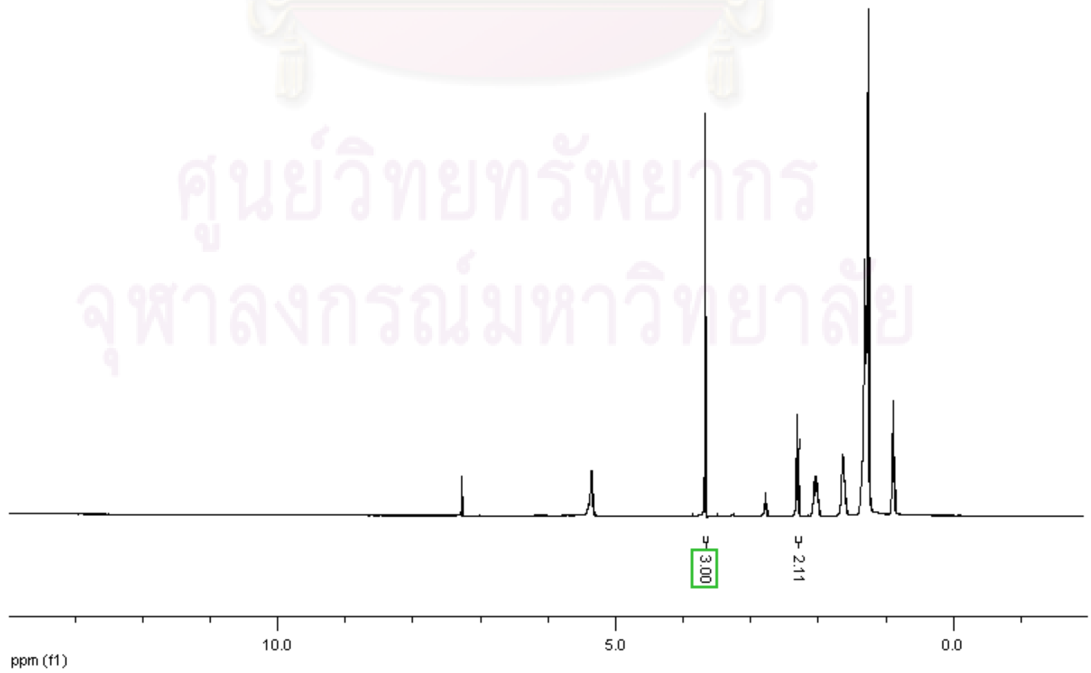


Figure A49 ^{1}H-NMR spectrum of CC oil methyl ester.

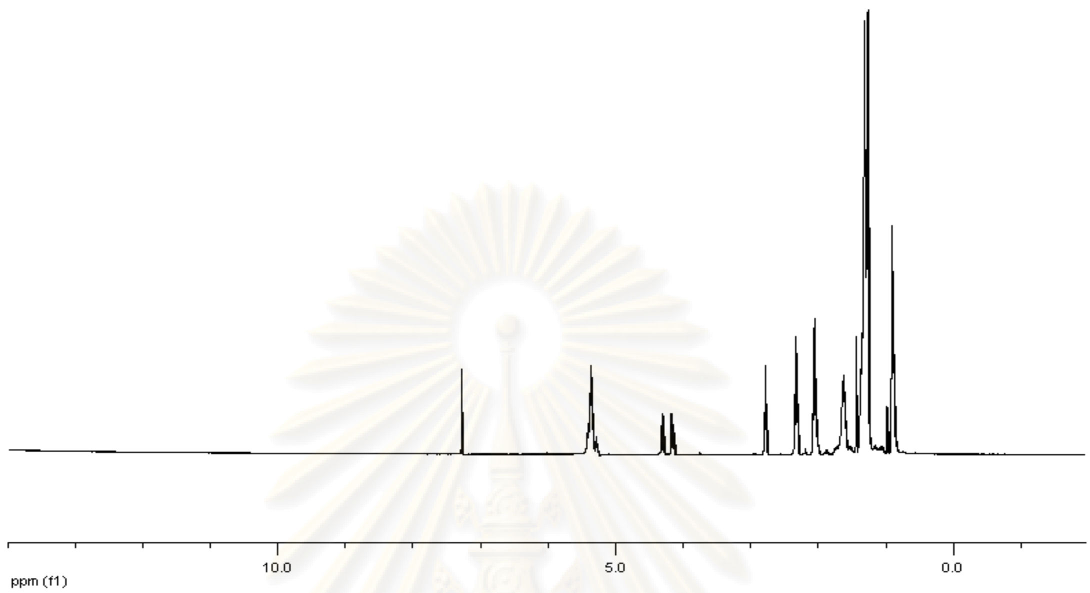


Figure A50 ^{1}H-NMR spectrum of crude CIL oil.

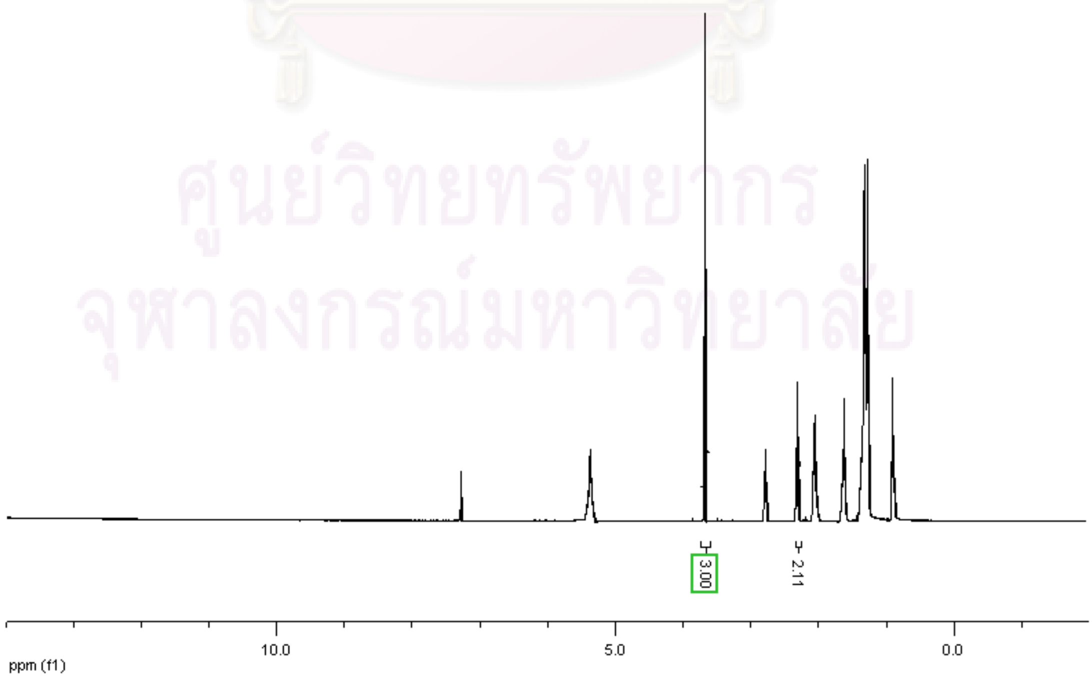


Figure A51 ^{1}H-NMR spectrum of CIL oil methyl ester.

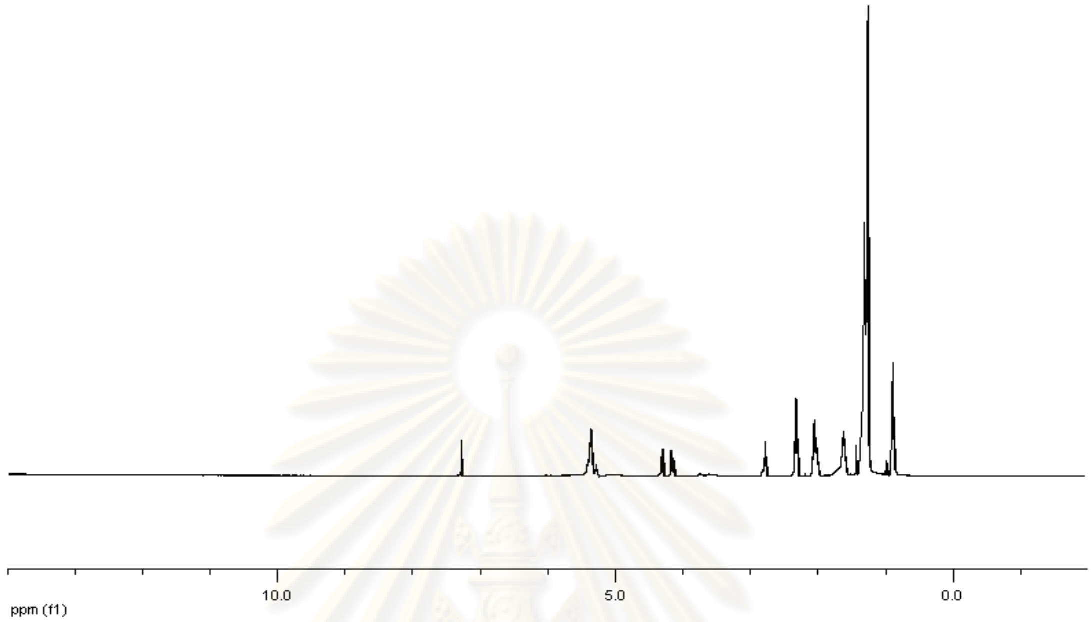


Figure A52 [1]H-NMR spectrum of crude CIM oil.

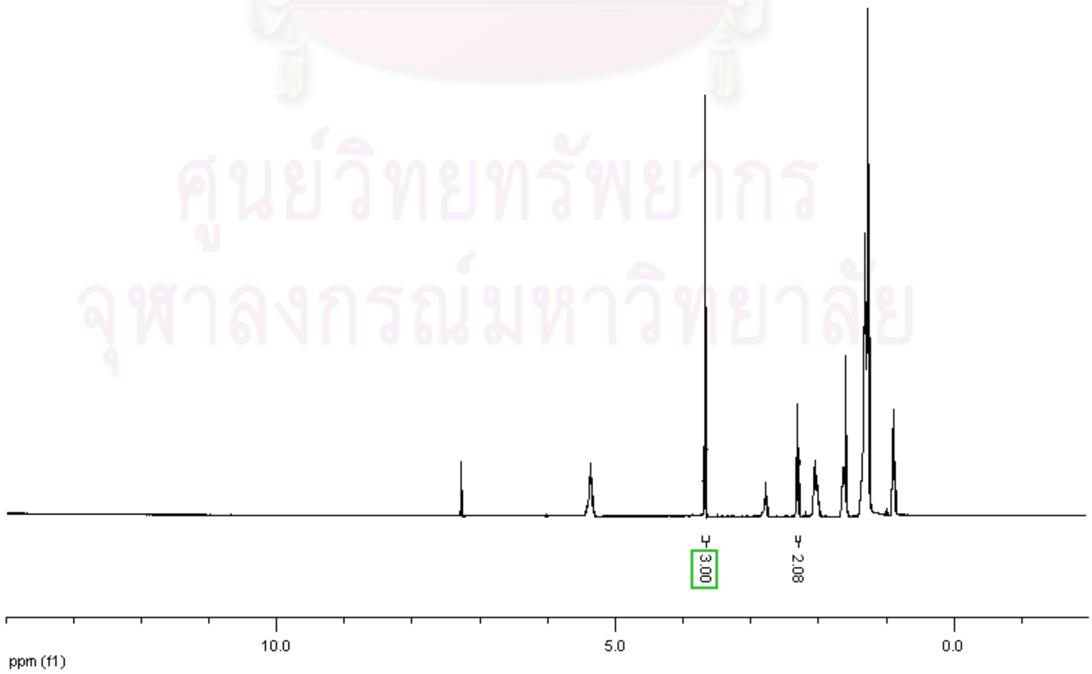


Figure A53 [1]H-NMR spectrum of CIM oil methyl ester.

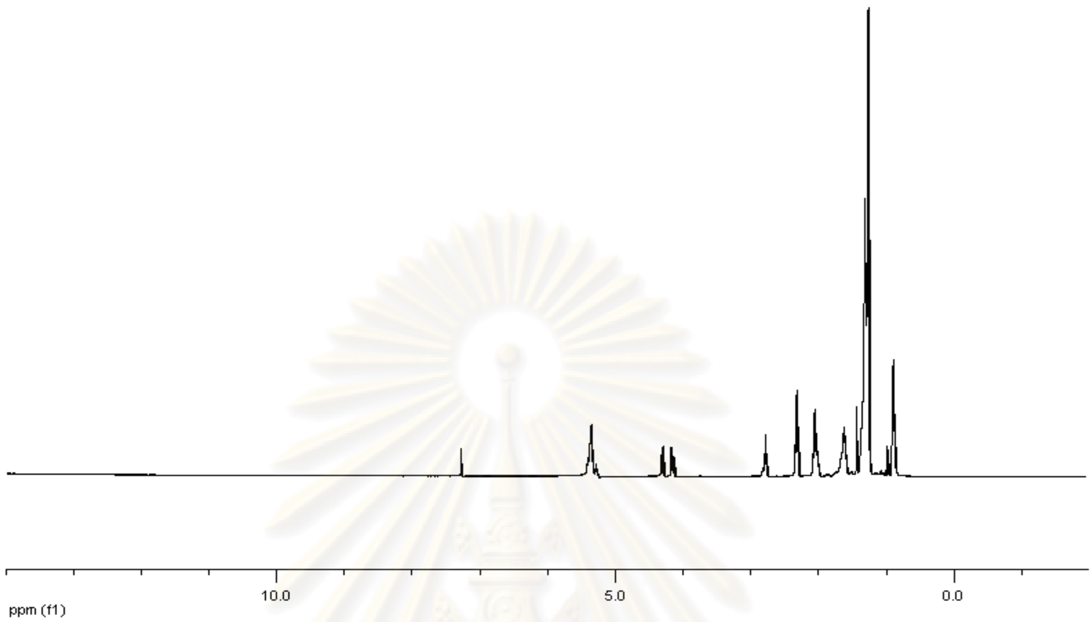


Figure A54 [1]H-NMR spectrum of crude CIR oil.

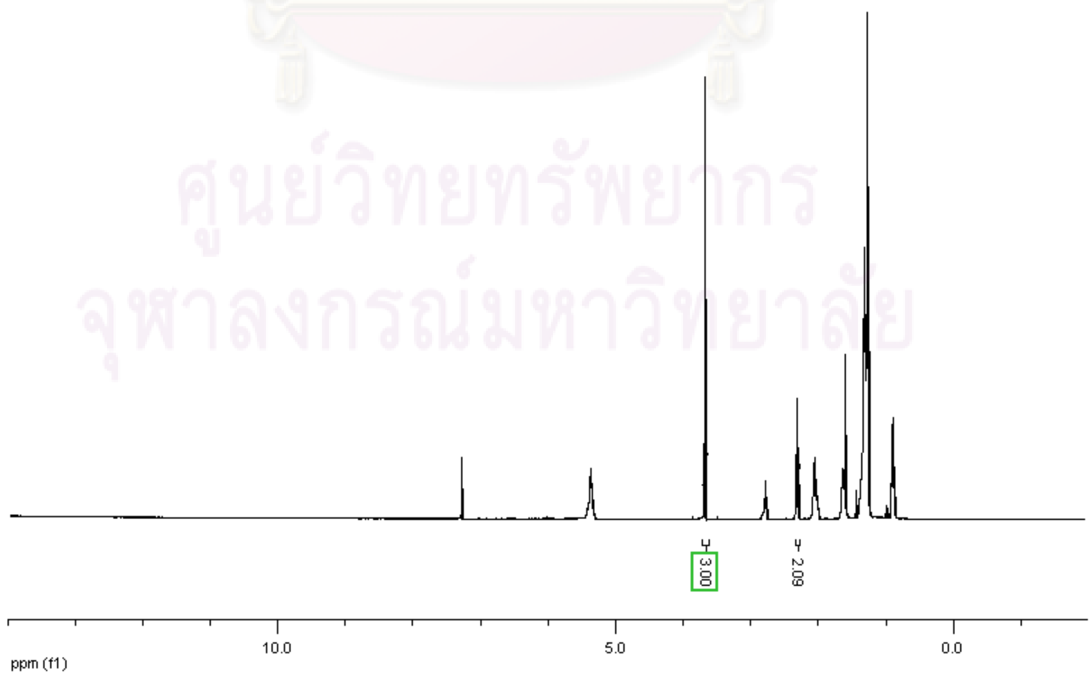


Figure A55 [1]H-NMR spectrum of CIR oil methyl ester.

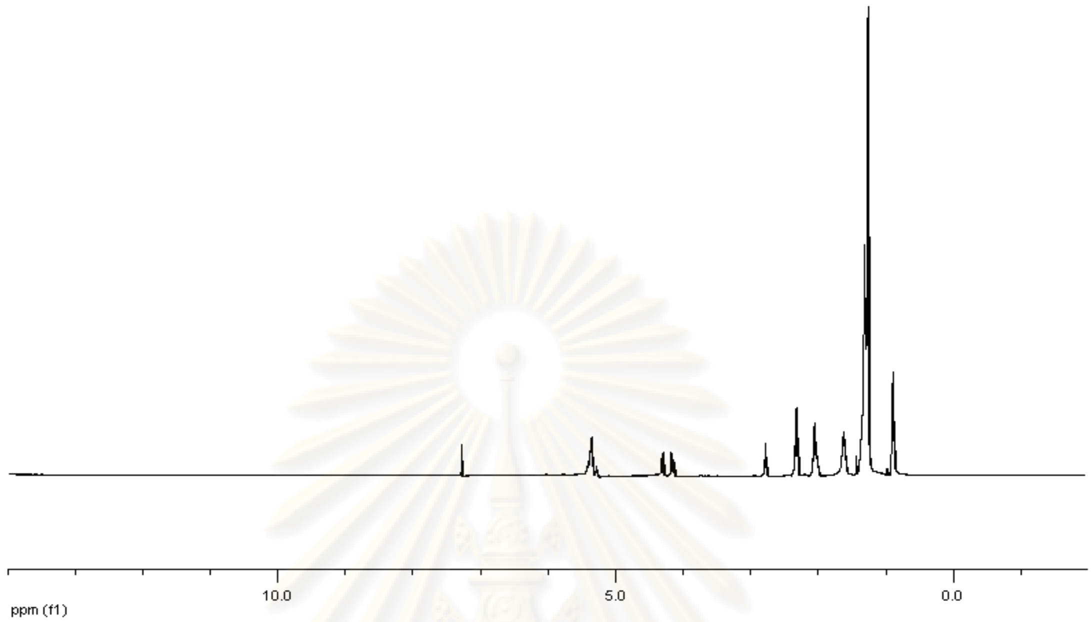


Figure A56 ^{1}H-NMR spectrum of crude CUM oil.

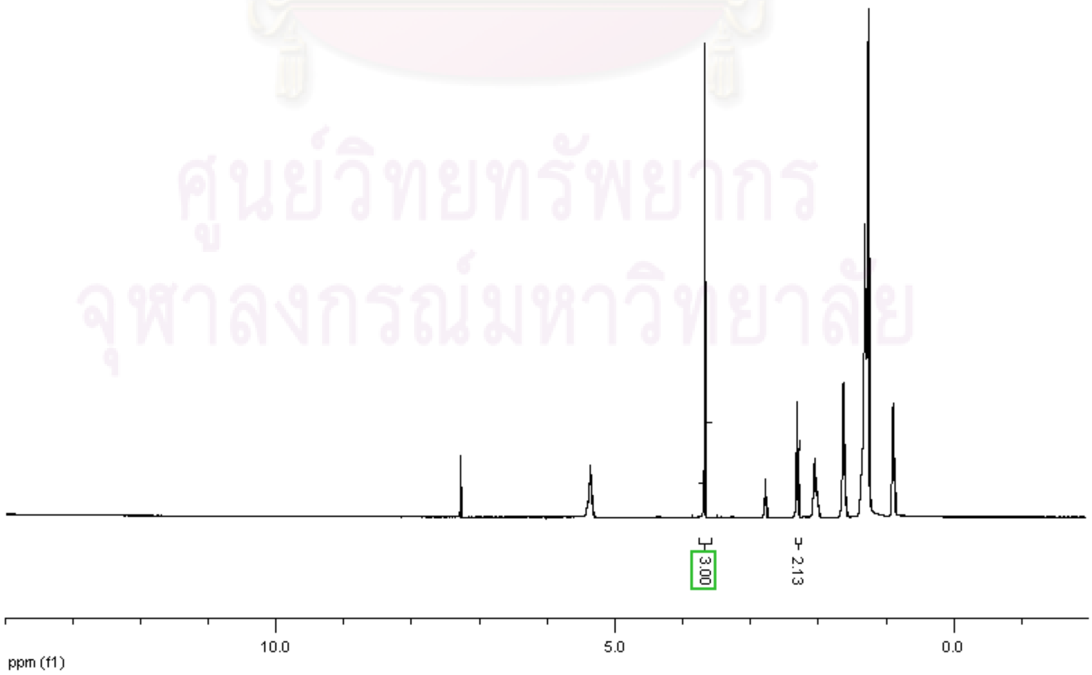


Figure A57 ^{1}H-NMR spectrum of CUM oil methyl ester.

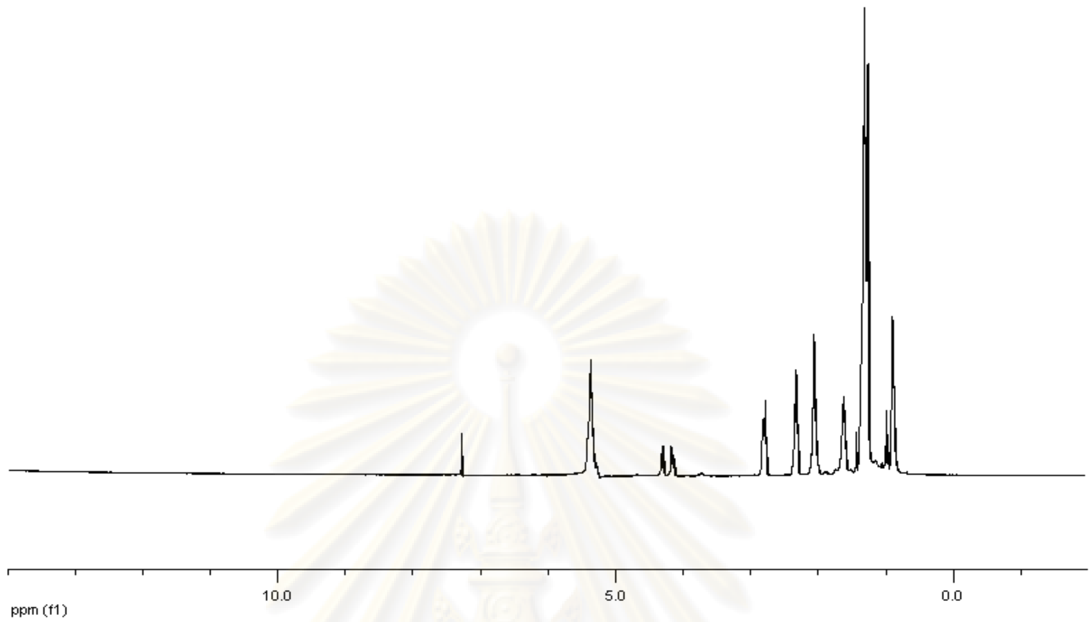


Figure A58 ^{1}H-NMR spectrum of crude CV oil.

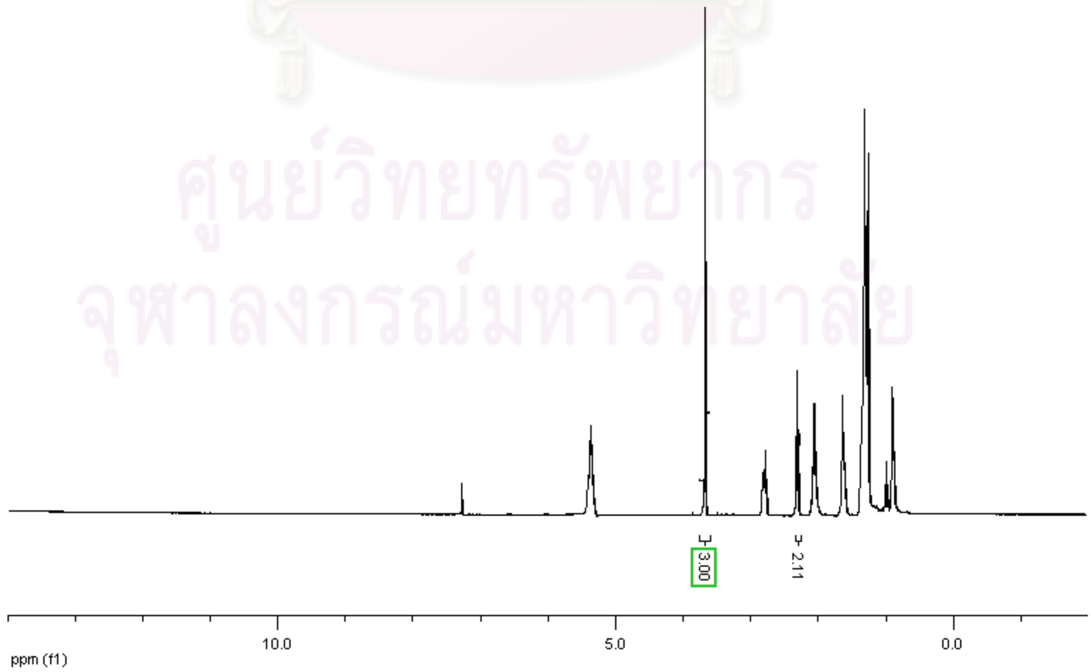


Figure A59 ^{1}H-NMR spectrum of CV oil methyl ester.

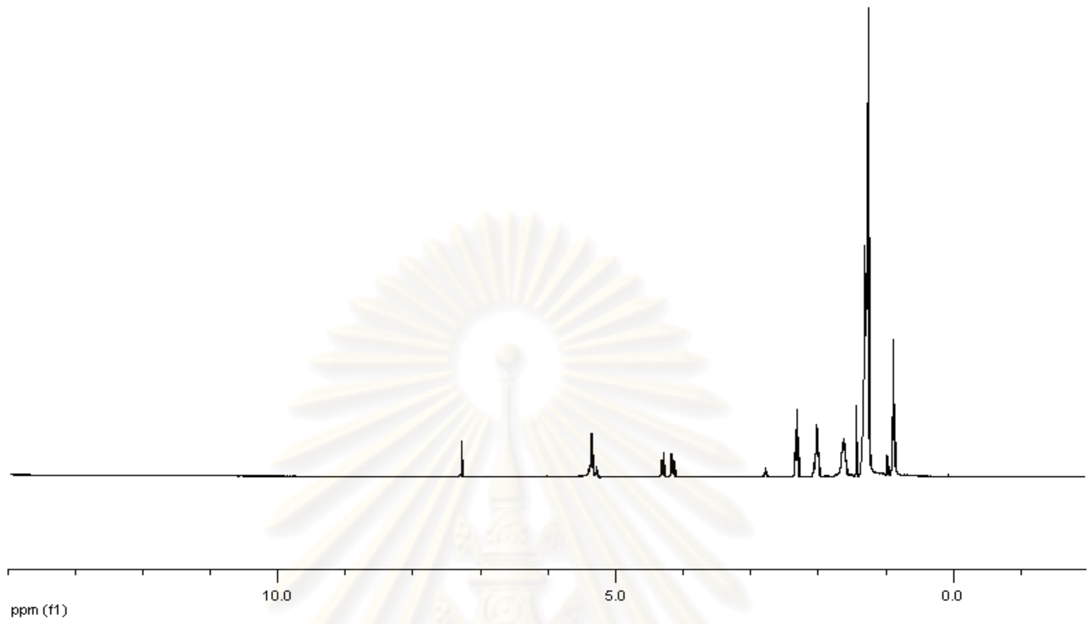


Figure A60 [1]H-NMR spectrum of crude DO oil.

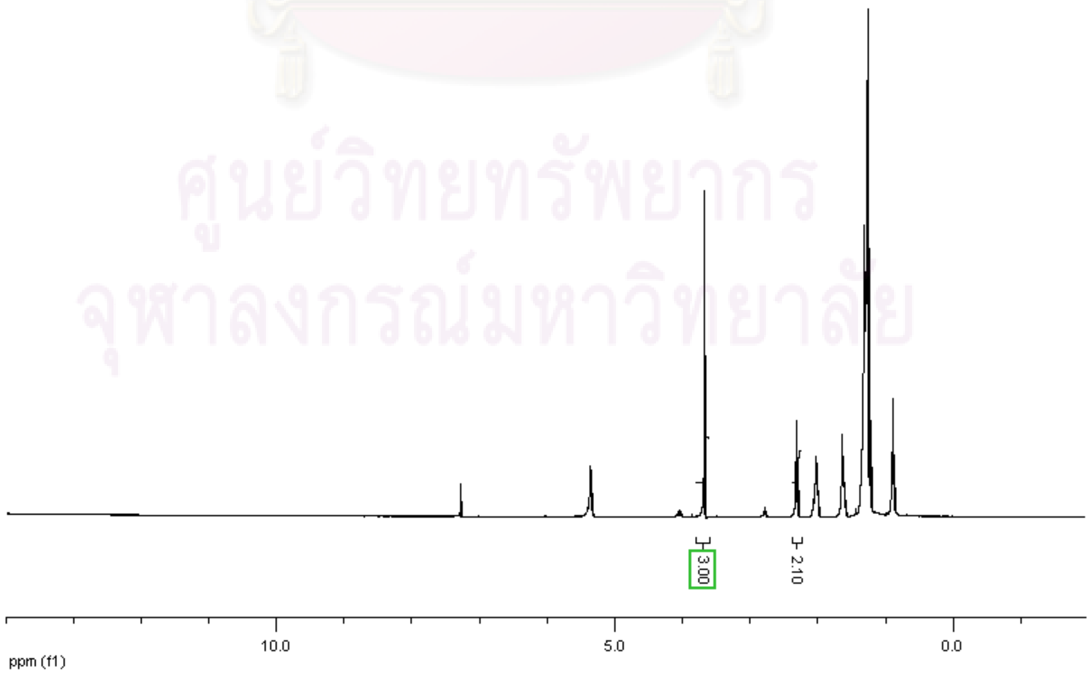


Figure A61 [1]H-NMR spectrum of DO oil methyl ester.

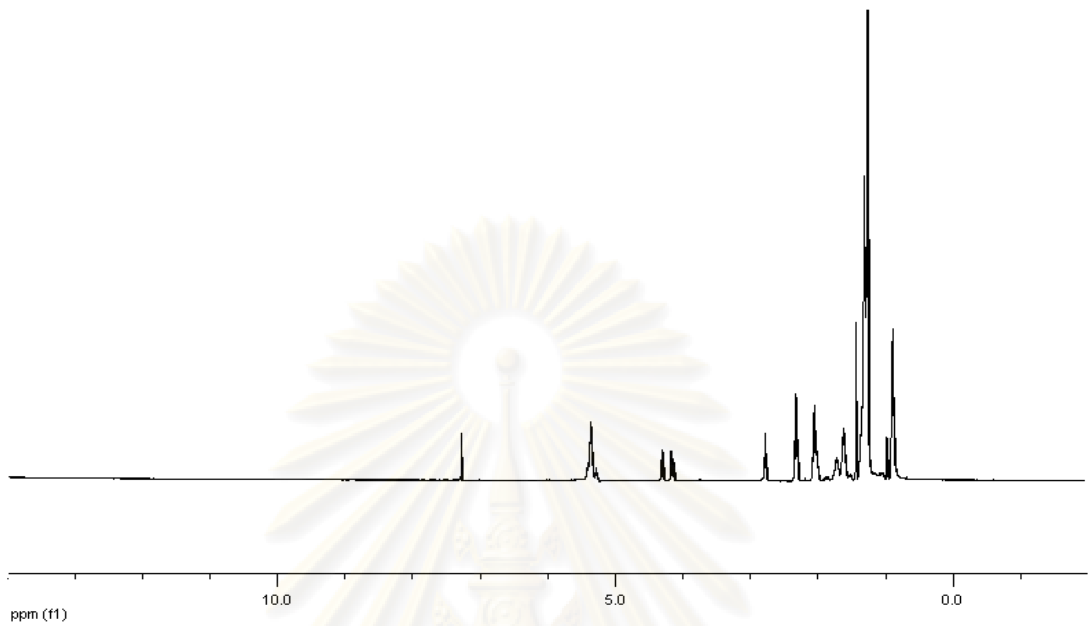


Figure A62 ^{1}H-NMR spectrum of crude GJ oil.

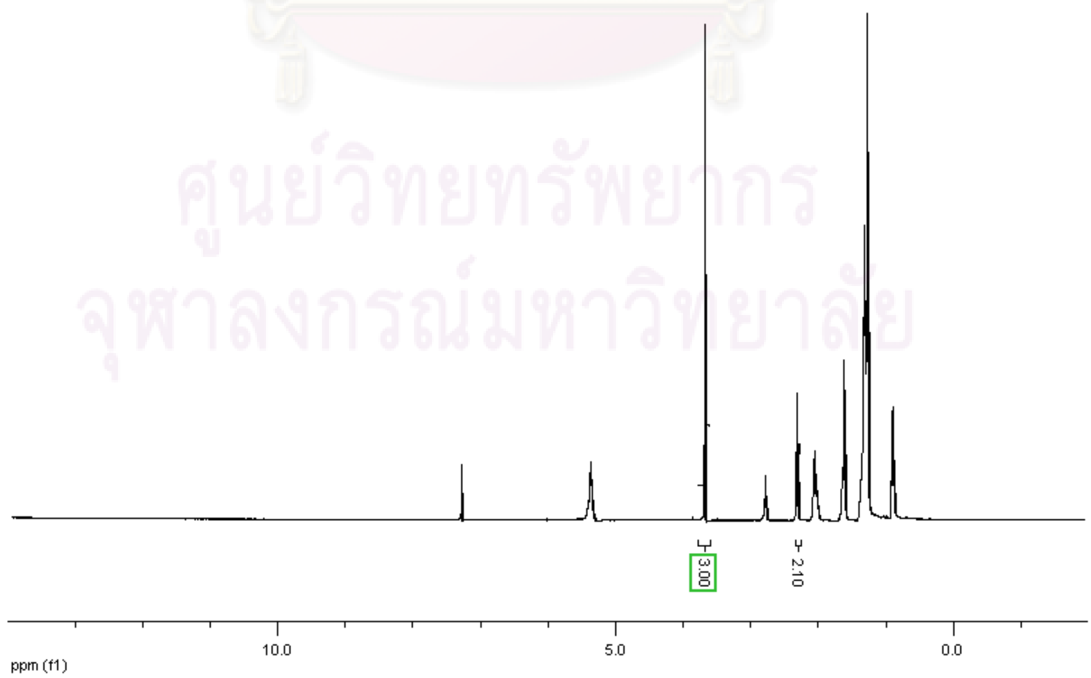


Figure A63 ^{1}H-NMR spectrum of GJ oil methyl ester.

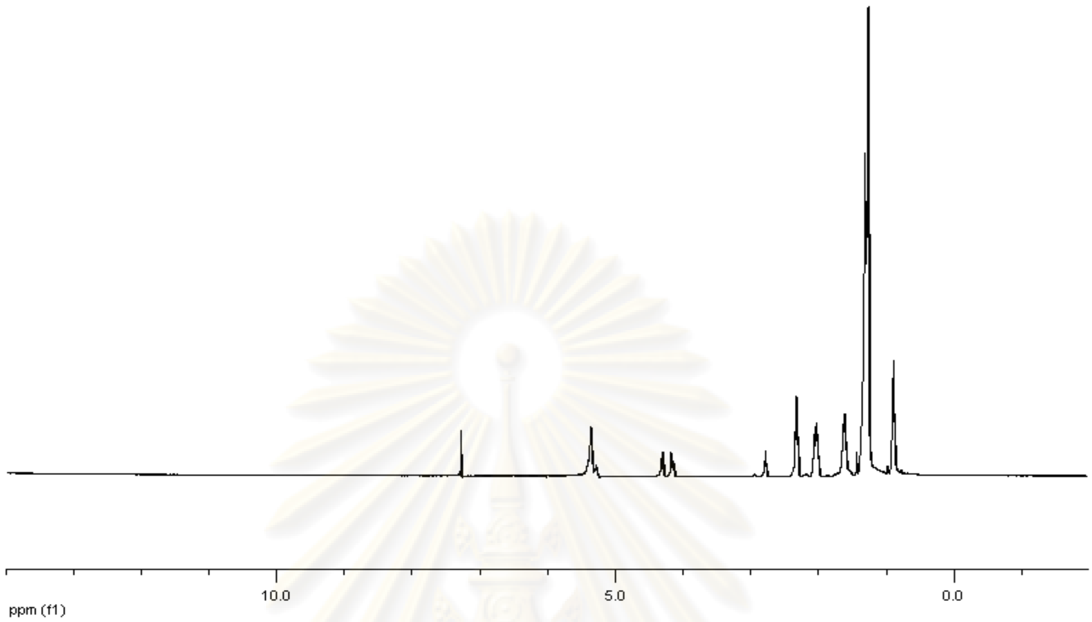


Figure A64 ^{1}H-NMR spectrum of crude HIS oil.

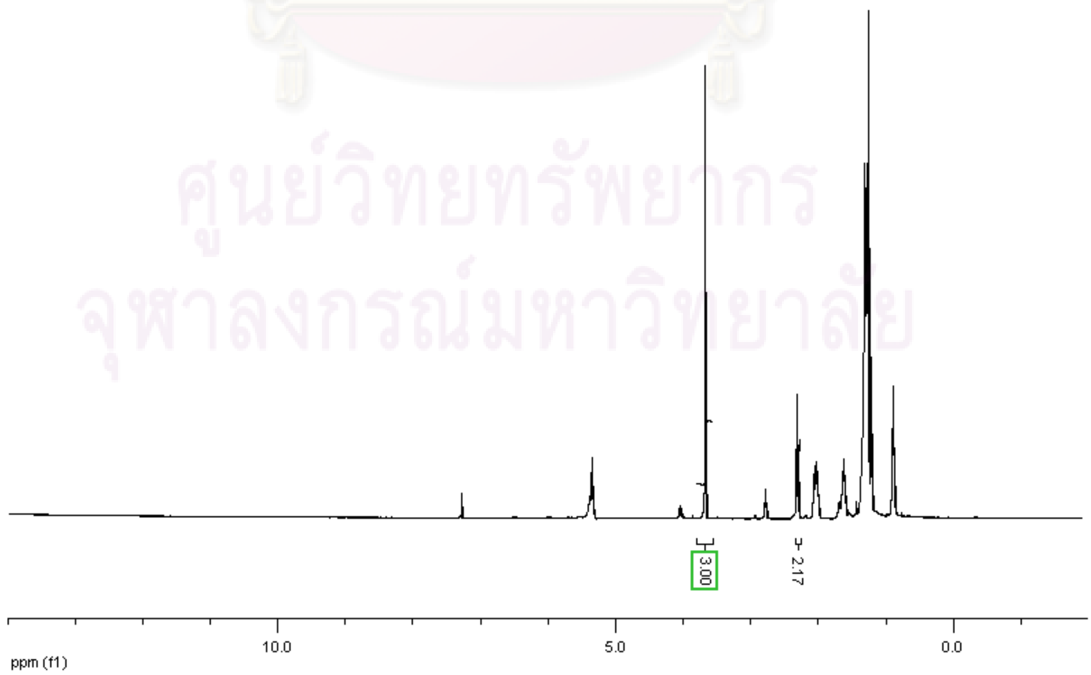


Figure A65 ^{1}H-NMR spectrum of HIS oil methyl ester.

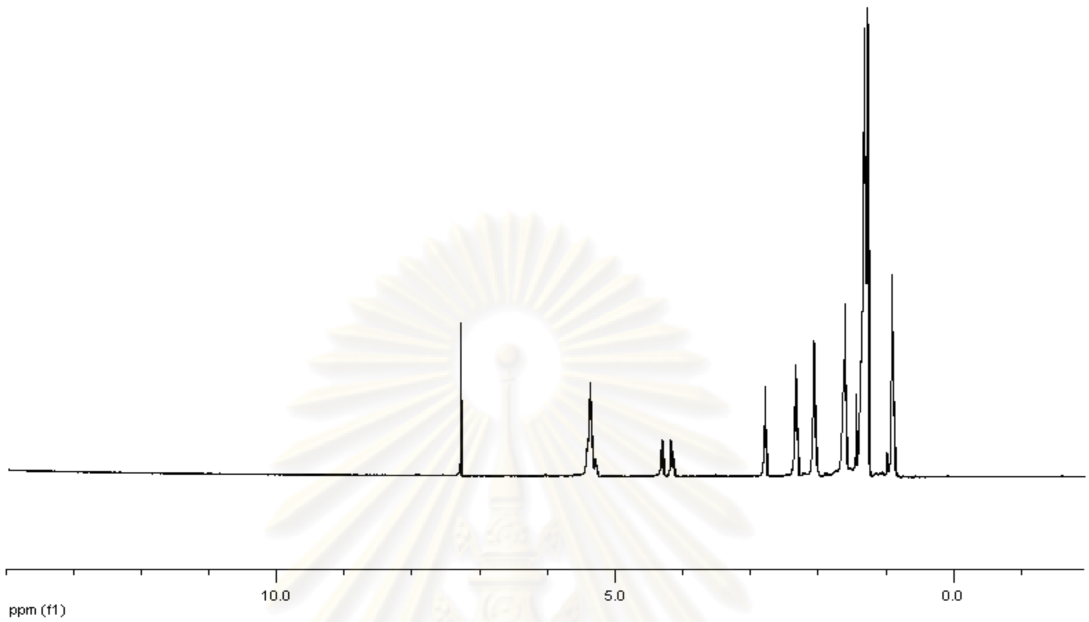


Figure A66 [1]H-NMR spectrum of crude LS oil.

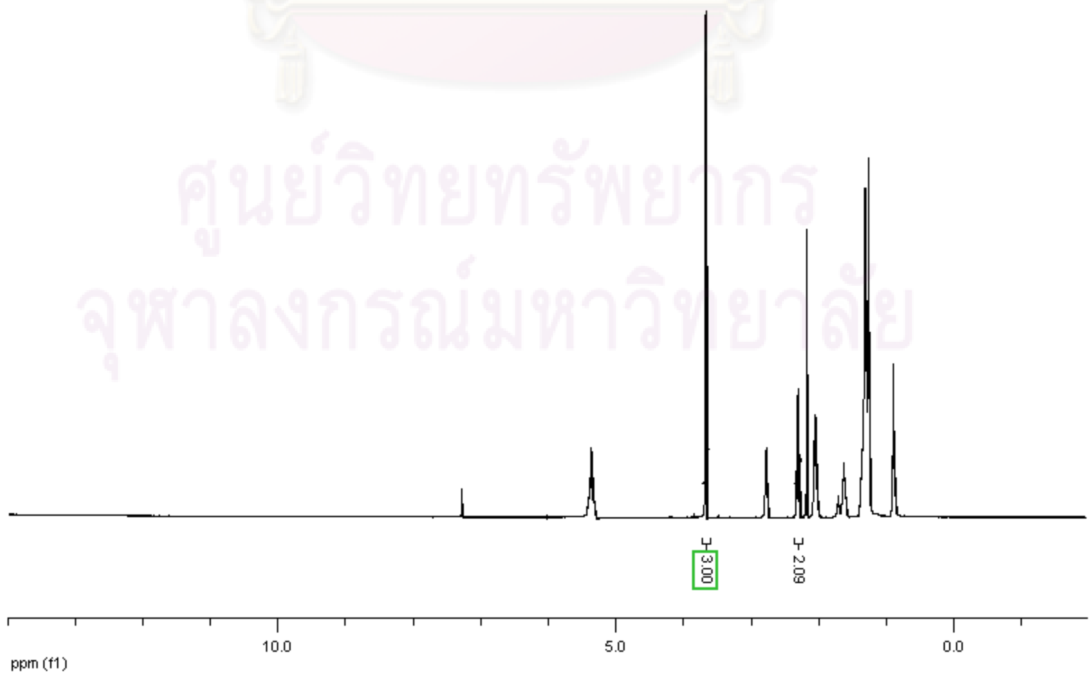


Figure A67 [1]H-NMR spectrum of LS oil methyl ester.

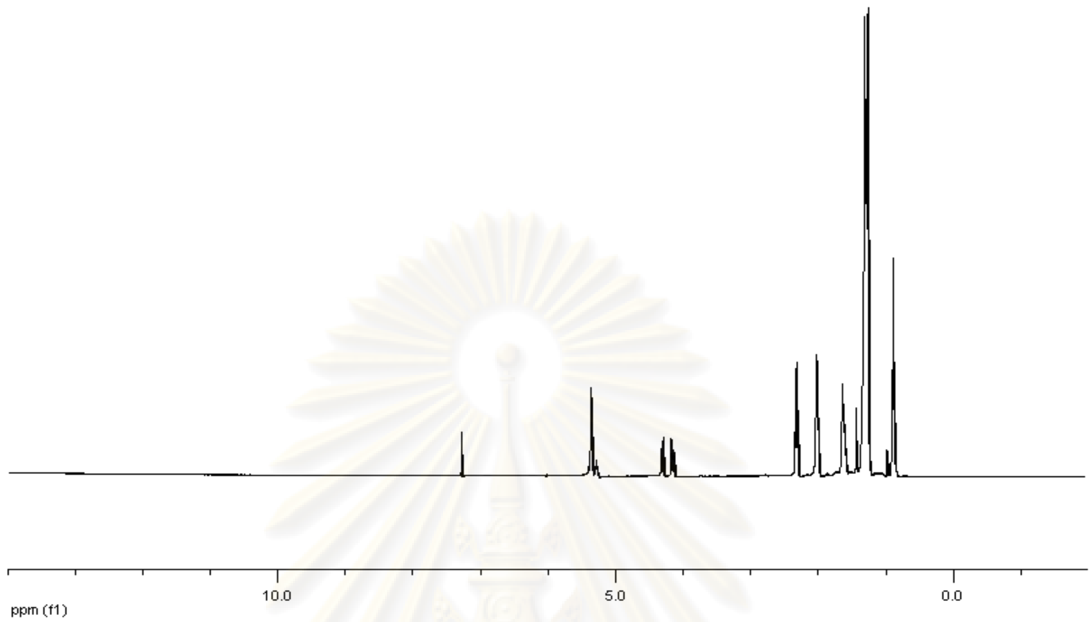


Figure A68 ^{1}H-NMR spectrum of crude MAI oil.

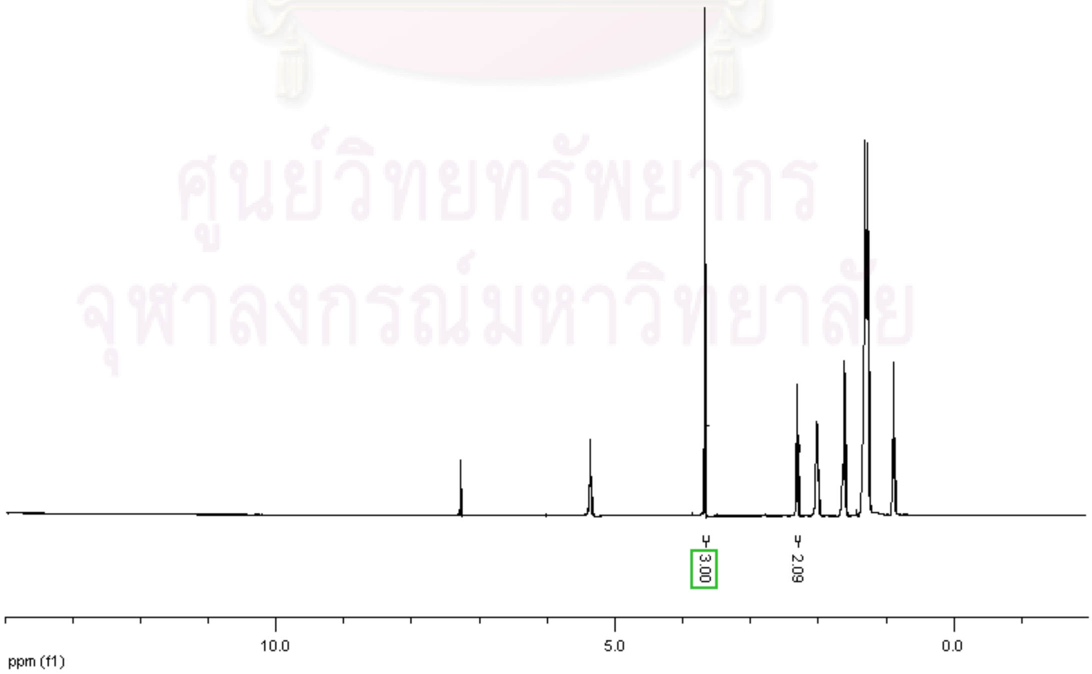


Figure A69 ^{1}H-NMR spectrum of MAI oil methyl ester.

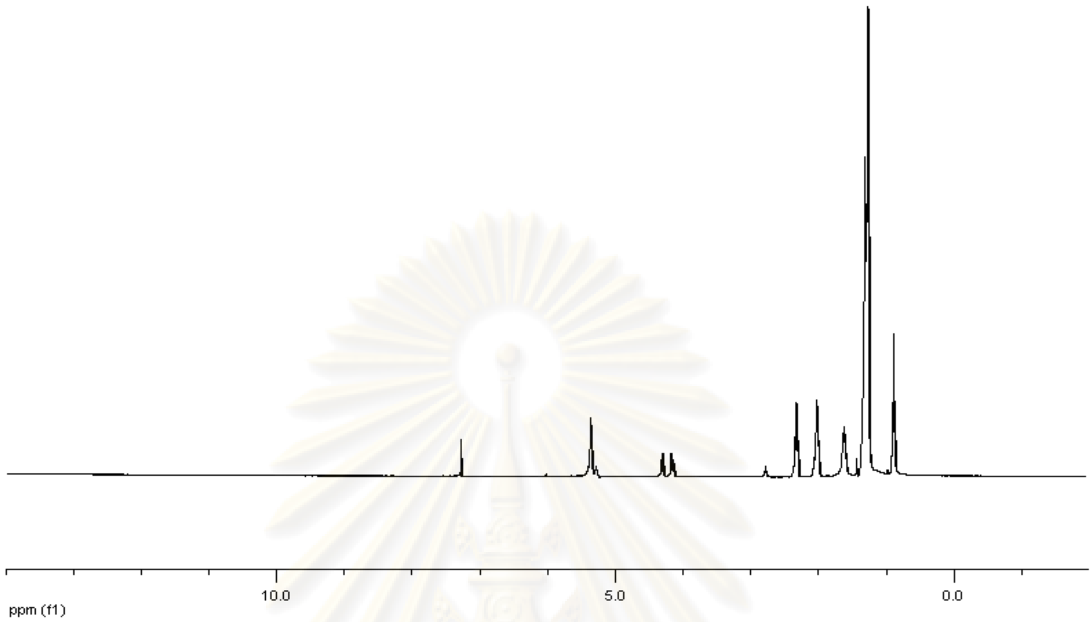


Figure A70 [1]H-NMR spectrum of crude ME oil.

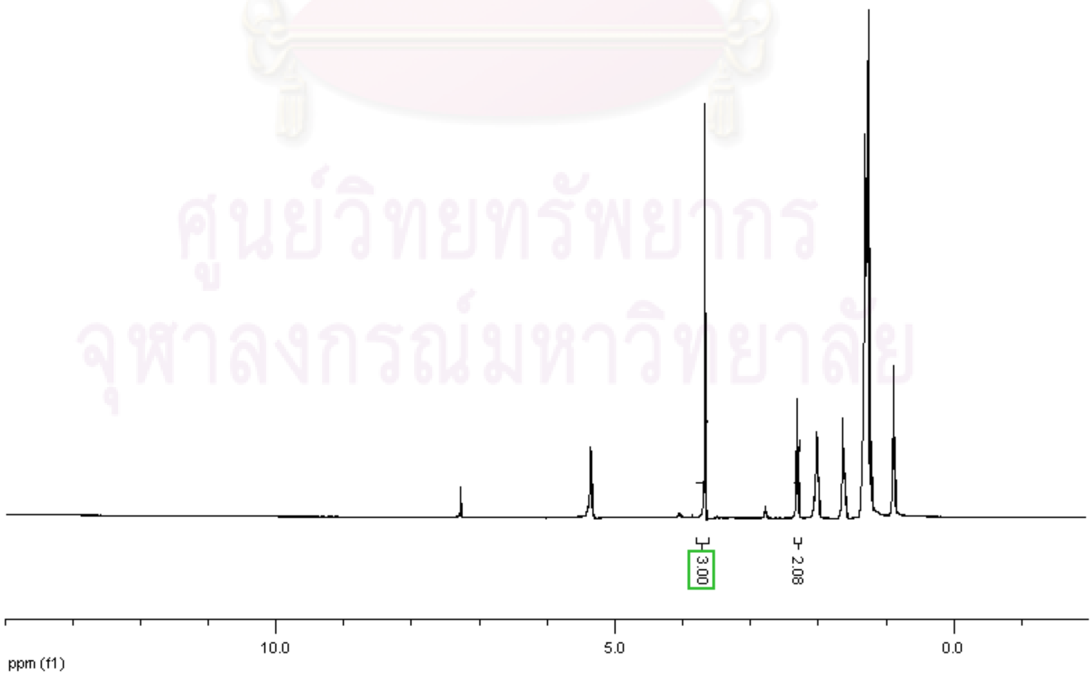


Figure A71 [1]H-NMR spectrum of ME oil methyl ester.

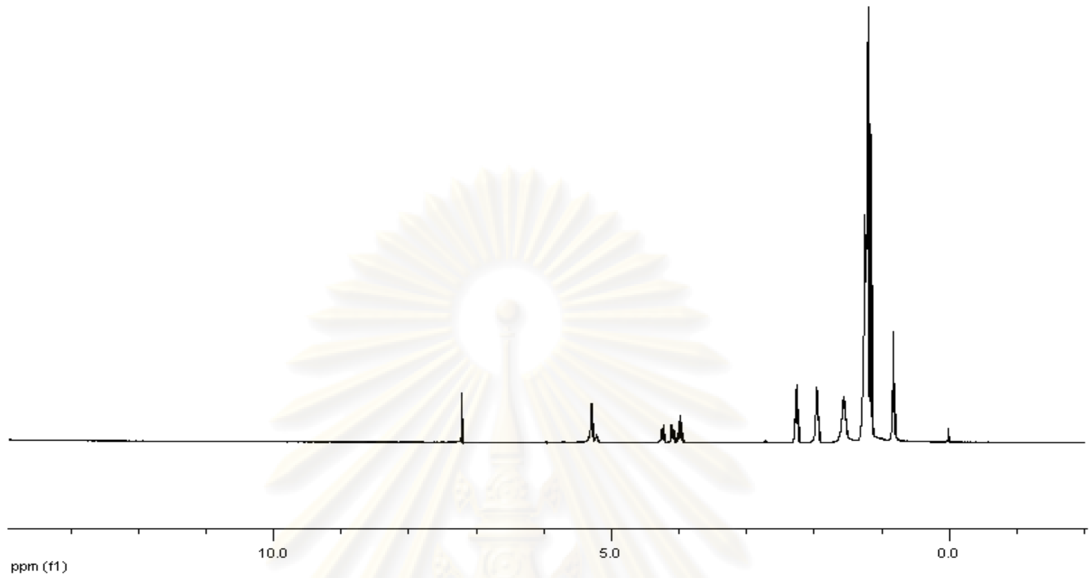


Figure A72 [1]H-NMR spectrum of crude MH oil.

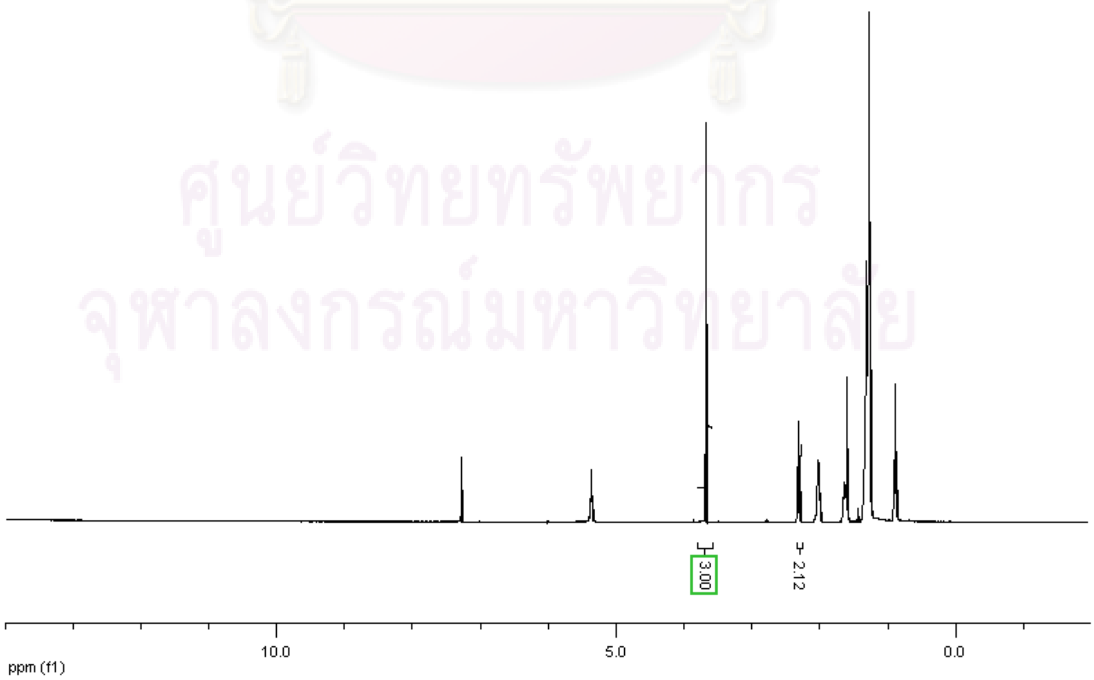


Figure A73 [1]H-NMR spectrum of MH oil methyl ester.

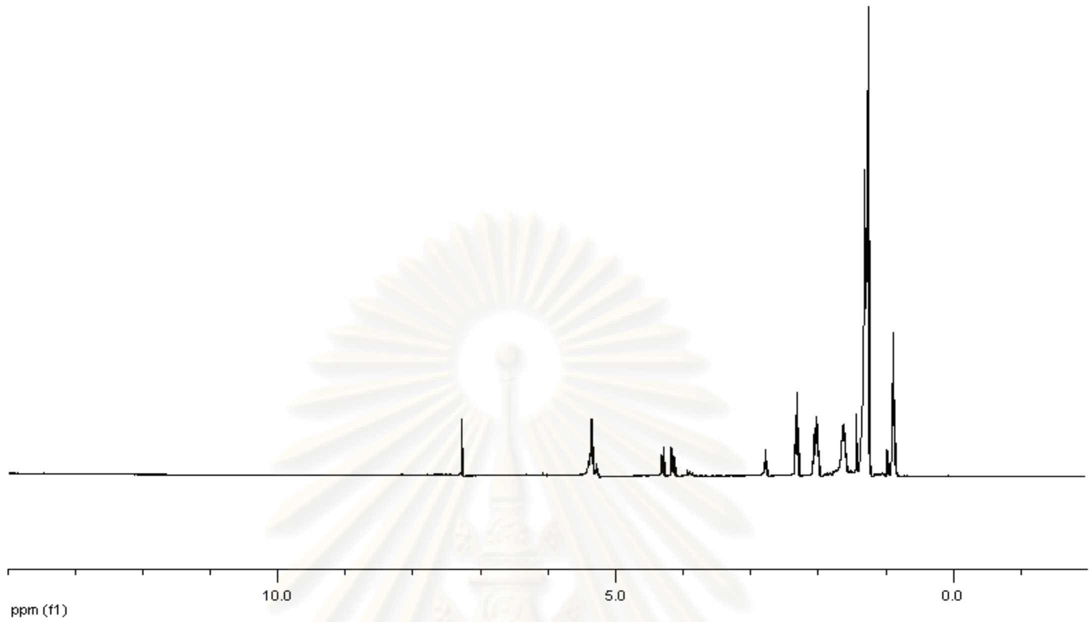


Figure A74 [1]H-NMR spectrum of crude ML oil.

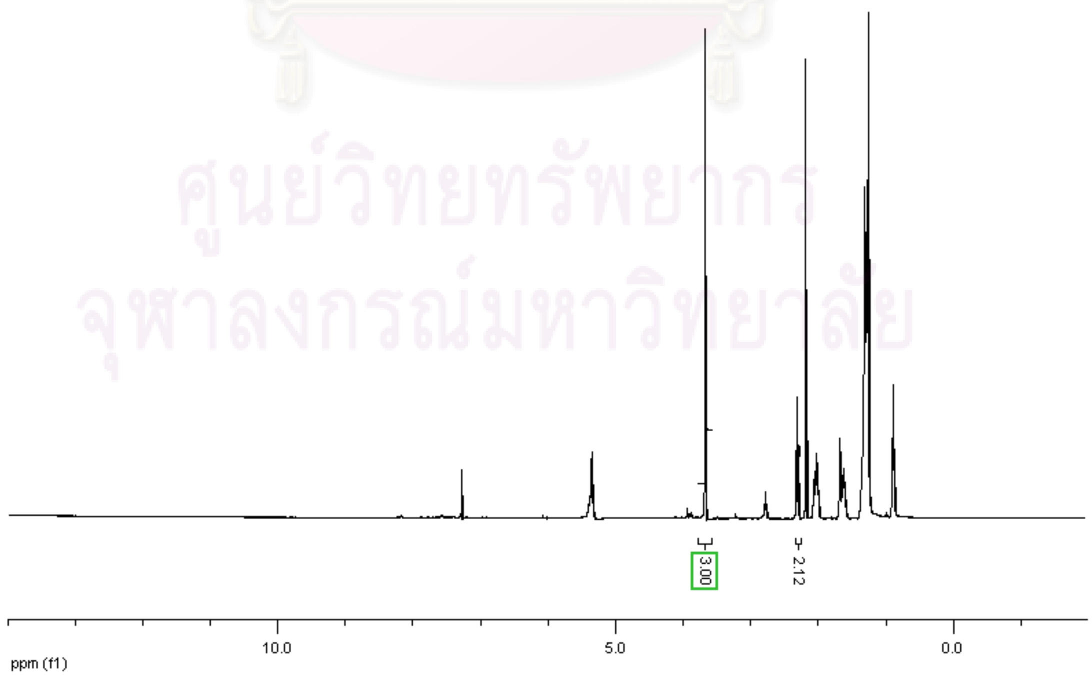


Figure A75 [1]H-NMR spectrum of ML oil methyl ester.

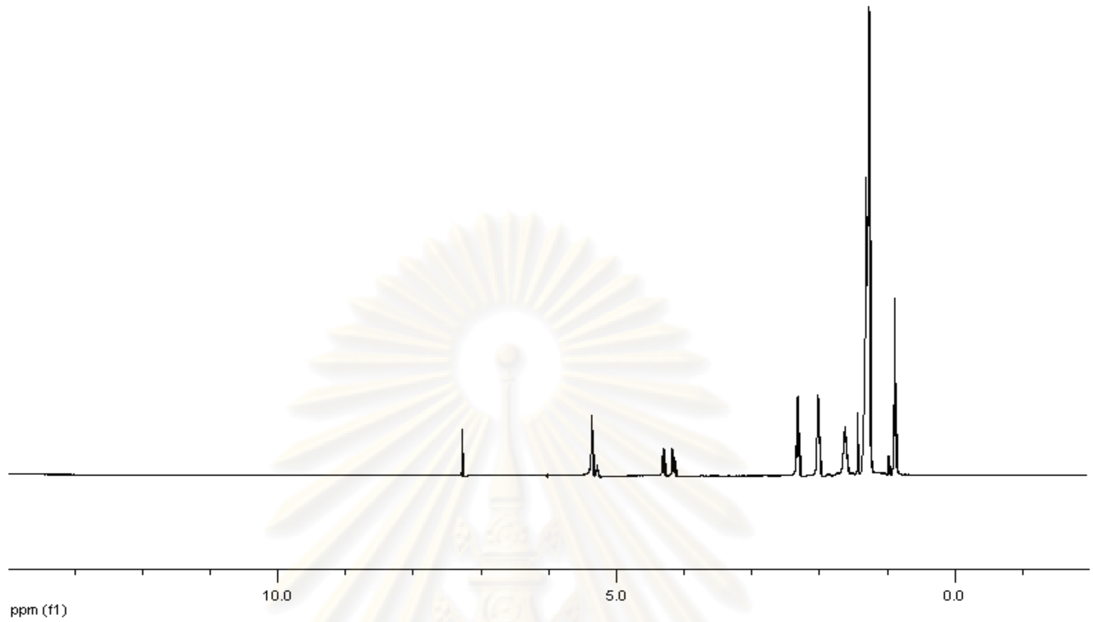


Figure A76 [1]H-NMR spectrum of crude MO oil.

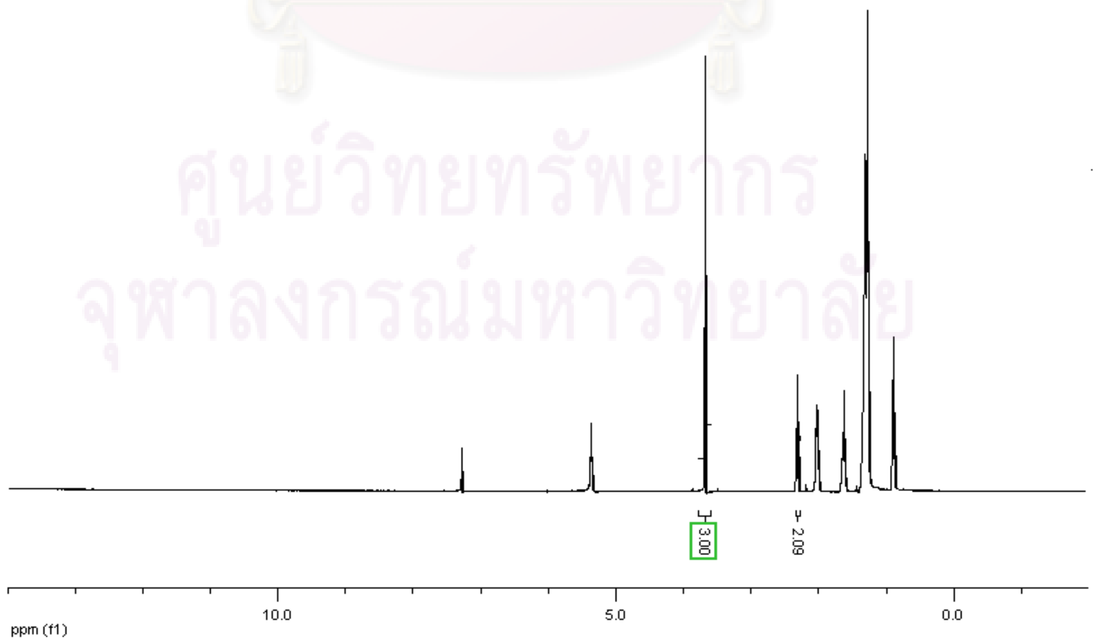


Figure A77 [1]H-NMR spectrum of MO oil methyl ester.

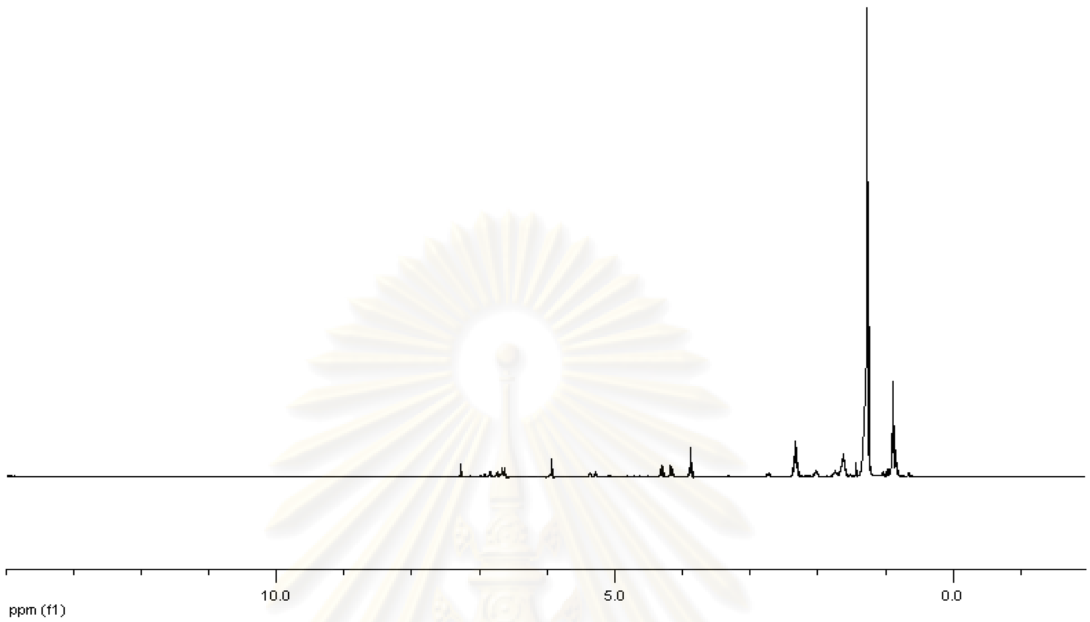


Figure A78 ^{1}H-NMR spectrum of crude MYF oil.

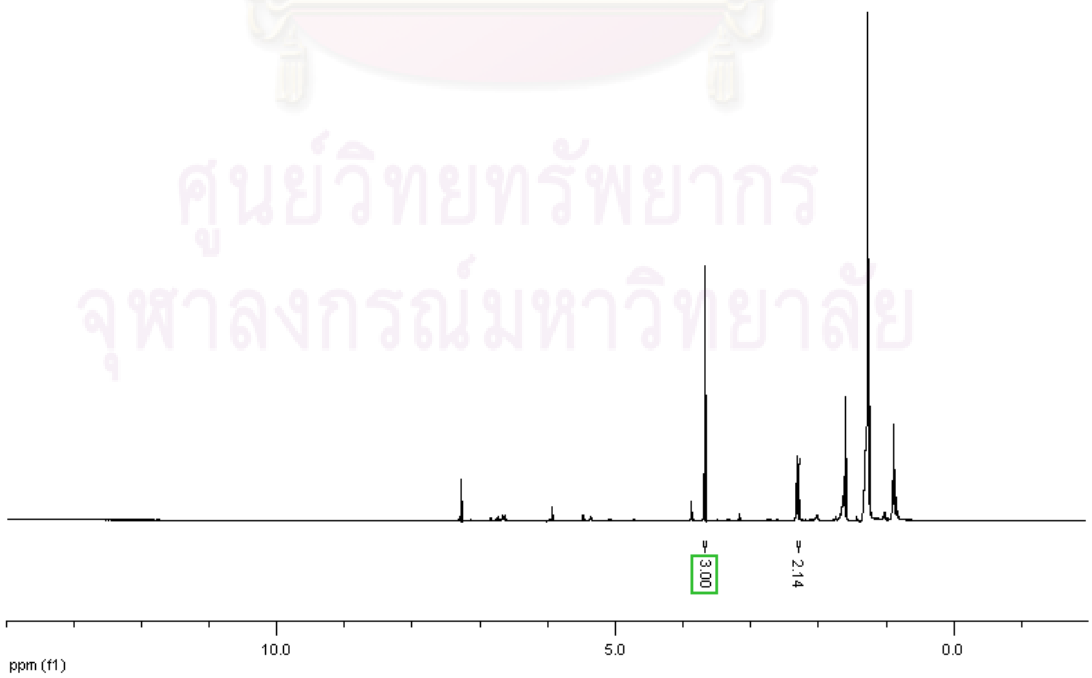


Figure A79 ^{1}H-NMR spectrum of MYF oil methyl ester.

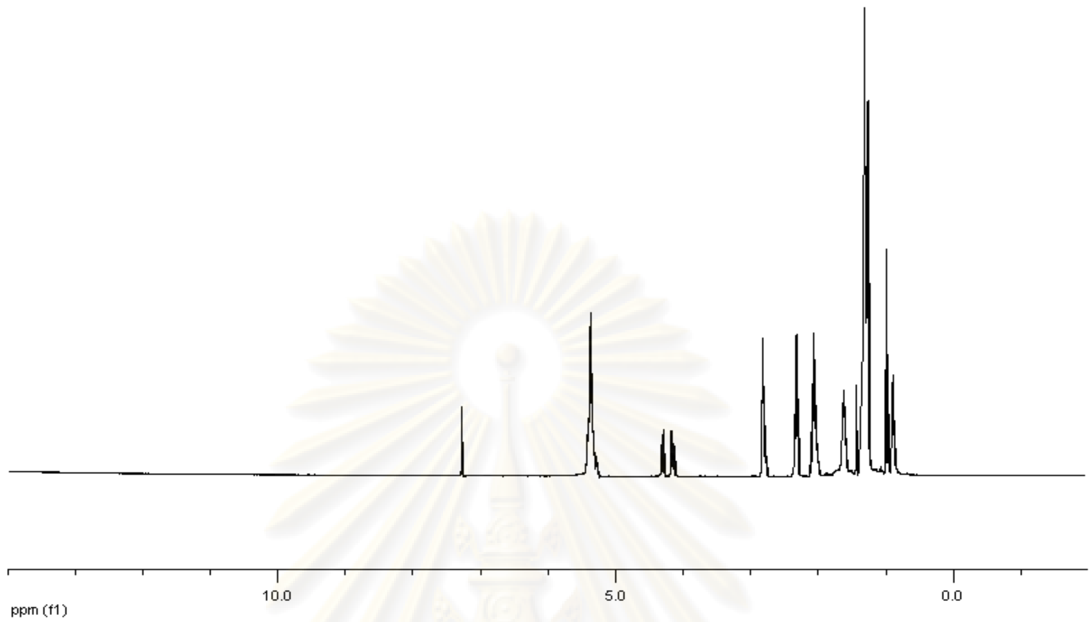


Figure A80 ^{1}H-NMR spectrum of crude OC oil.

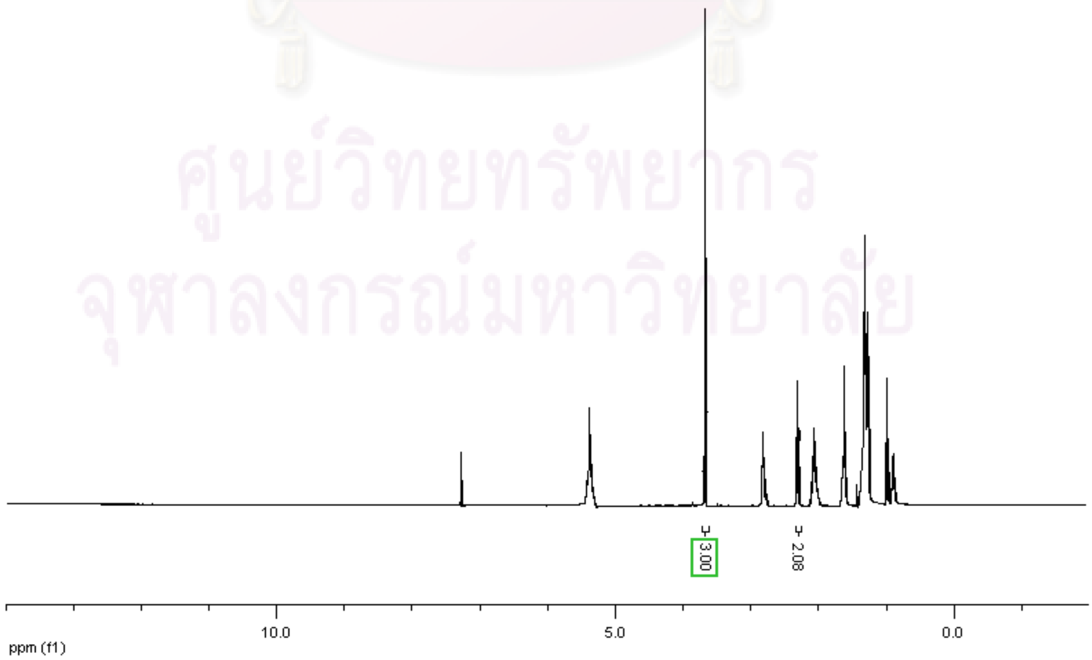


Figure A81 ^{1}H-NMR spectrum of OC oil methyl ester.

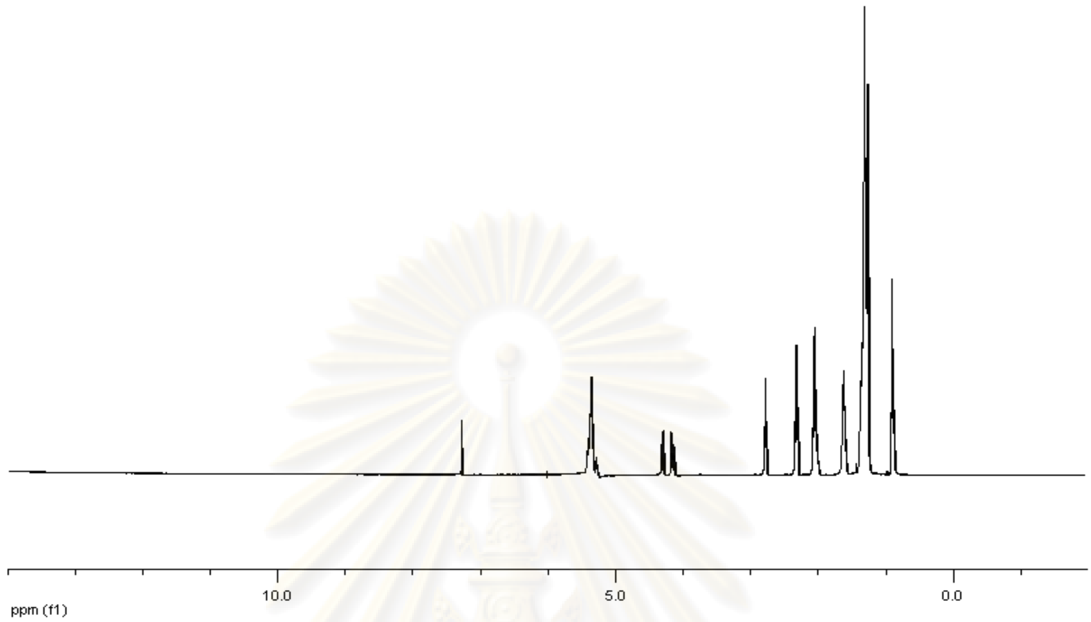


Figure A82 ^{1}H-NMR spectrum of crude PAF oil.

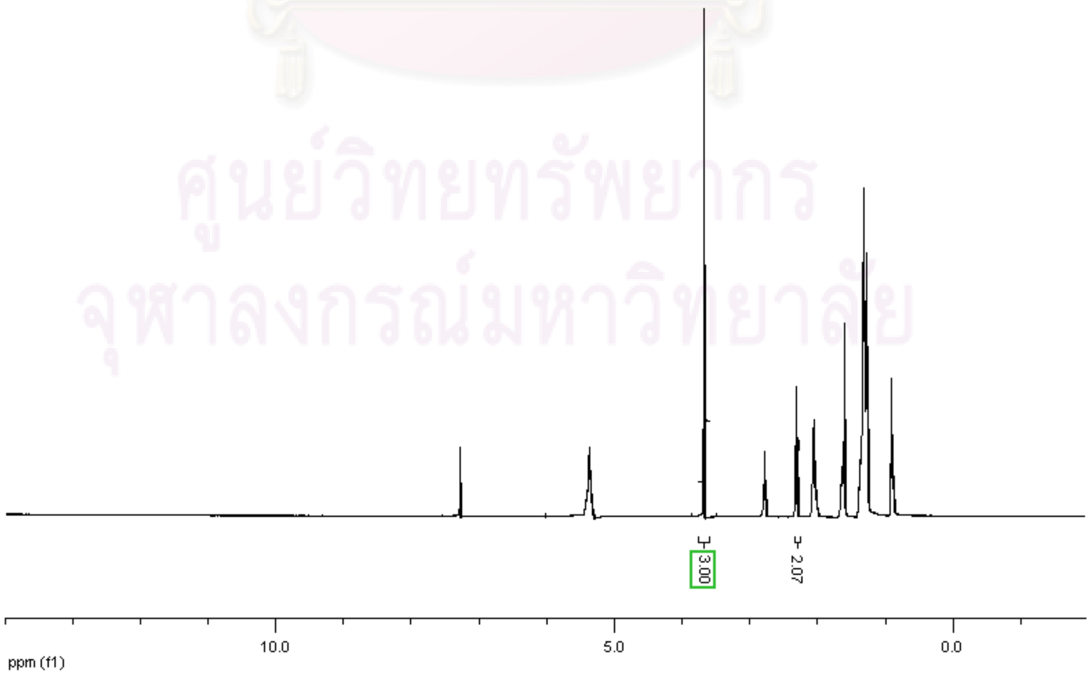


Figure A83 ^{1}H-NMR spectrum of PAF oil methyl ester.

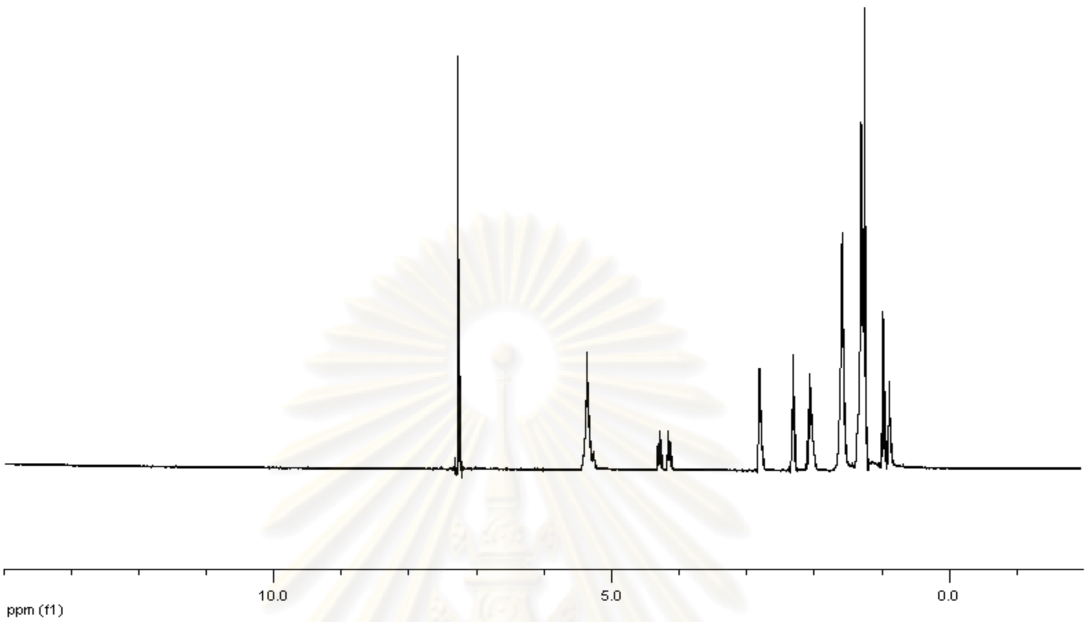


Figure A84 ^{1}H-NMR spectrum of crude PE oil.

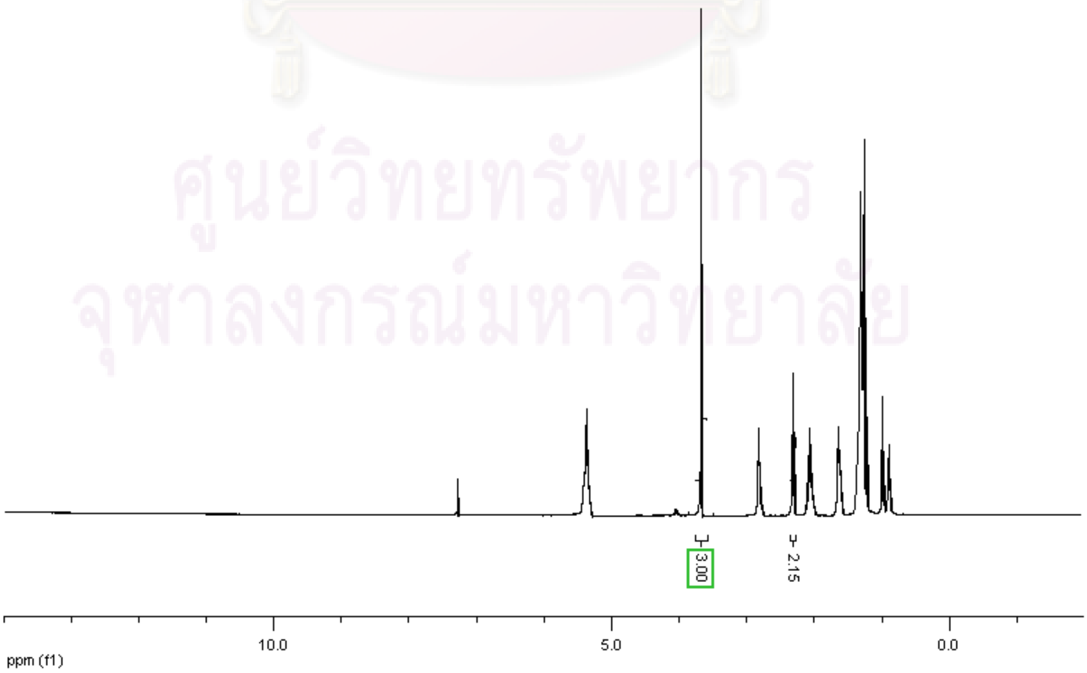


Figure A85 ^{1}H-NMR spectrum of PE oil methyl ester.

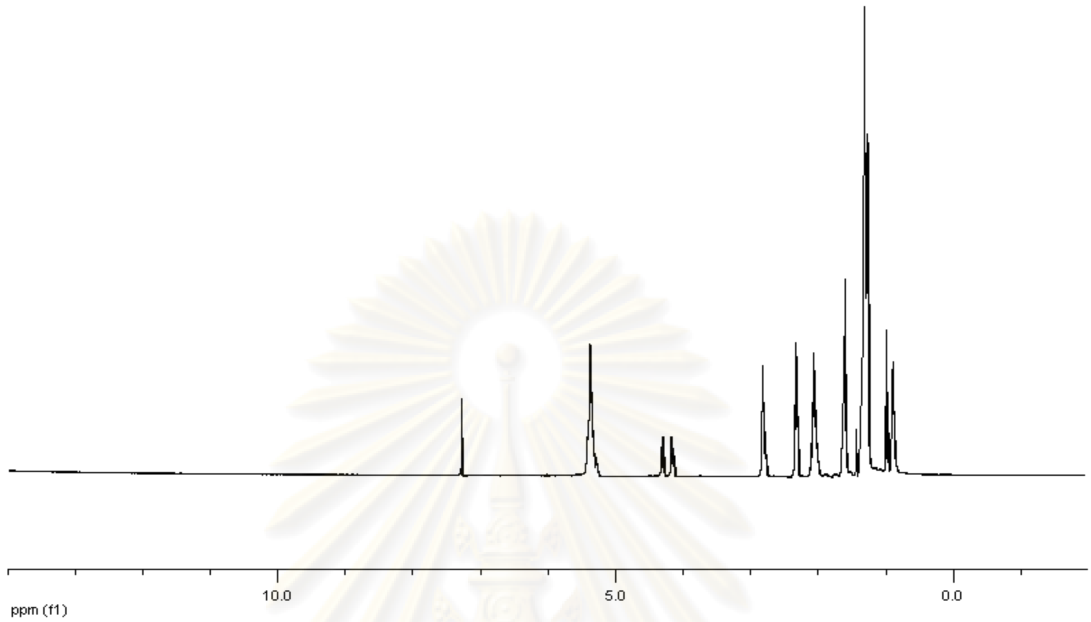


Figure A86 ^{1}H-NMR spectrum of crude PF oil.

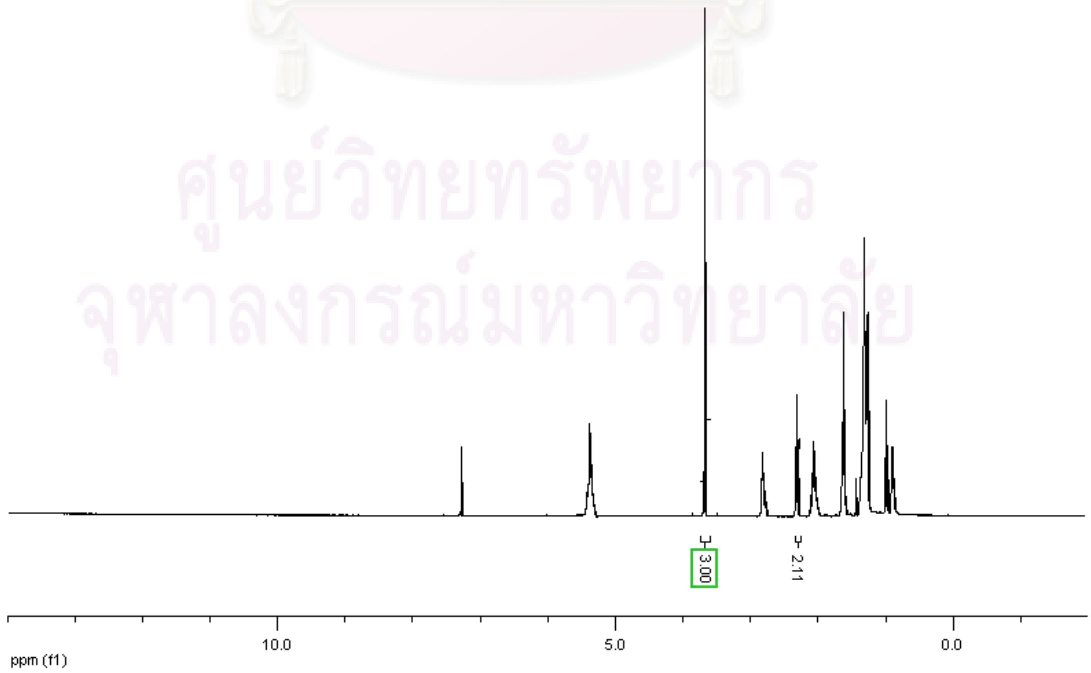


Figure A87 ^{1}H-NMR spectrum of PF oil methyl ester.

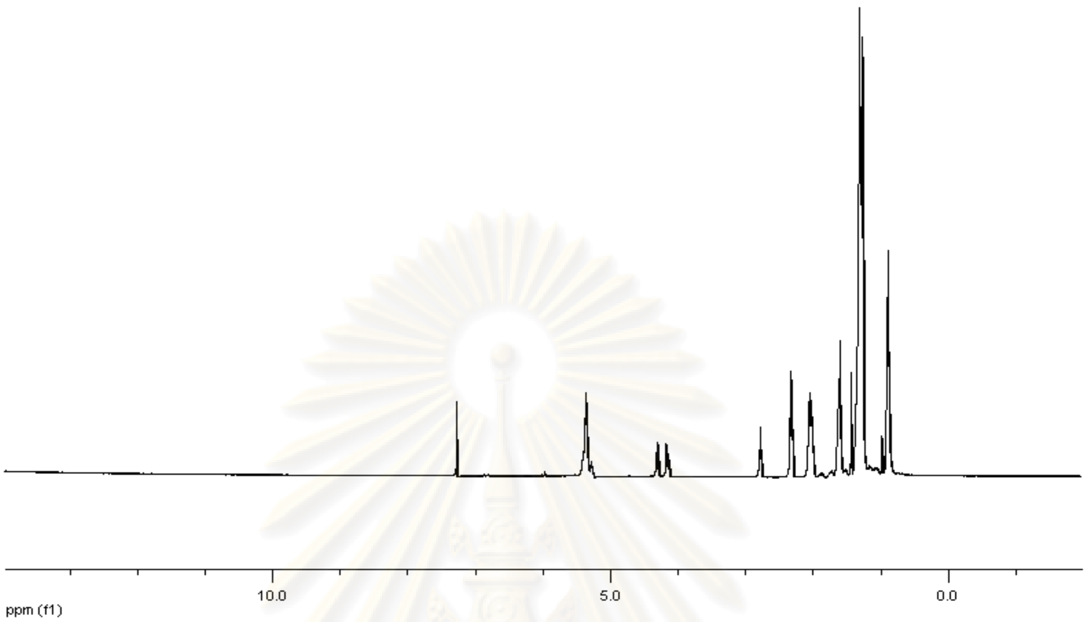


Figure A88 ^{1}H-NMR spectrum of crude SEI oil.

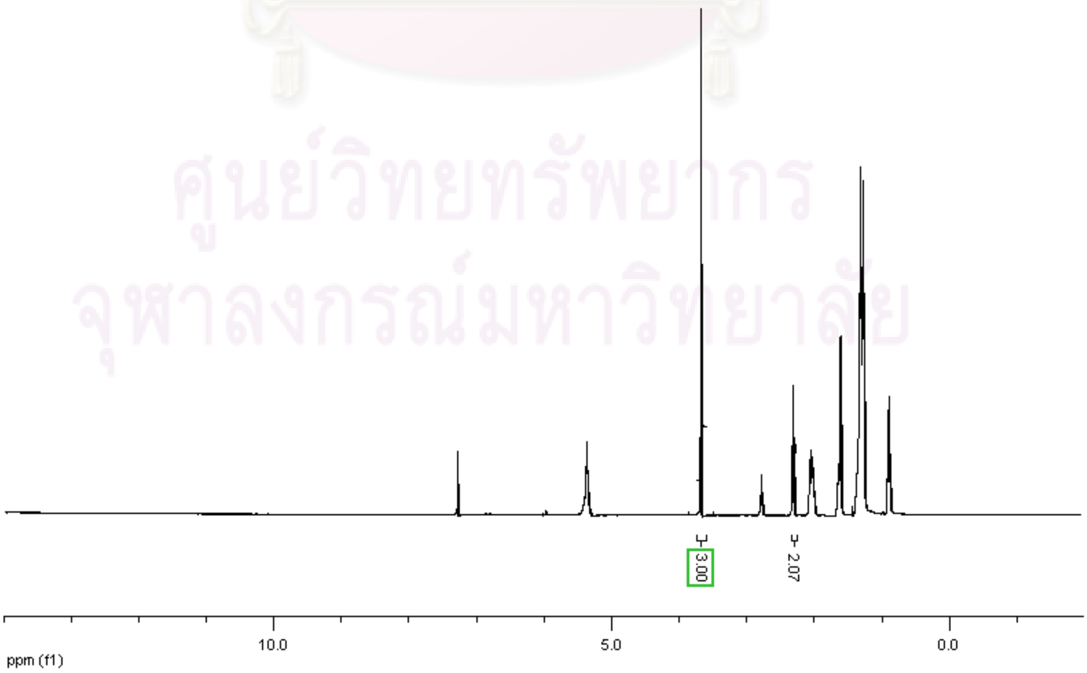


Figure A89 ^{1}H-NMR spectrum of SEI oil methyl ester.

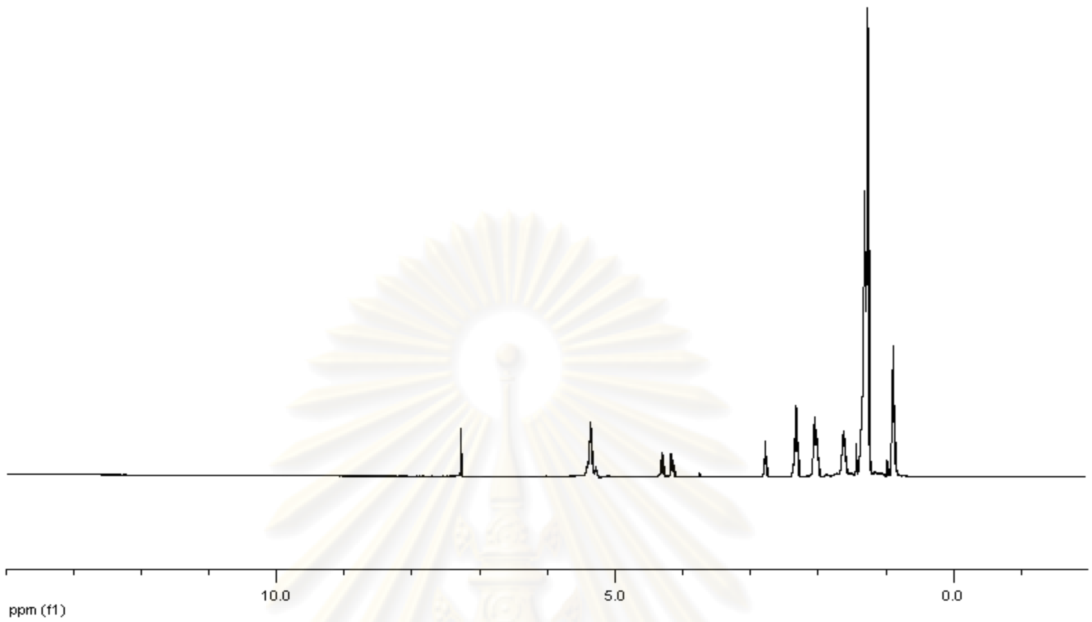


Figure A90 [1]H-NMR spectrum of crude TC oil.

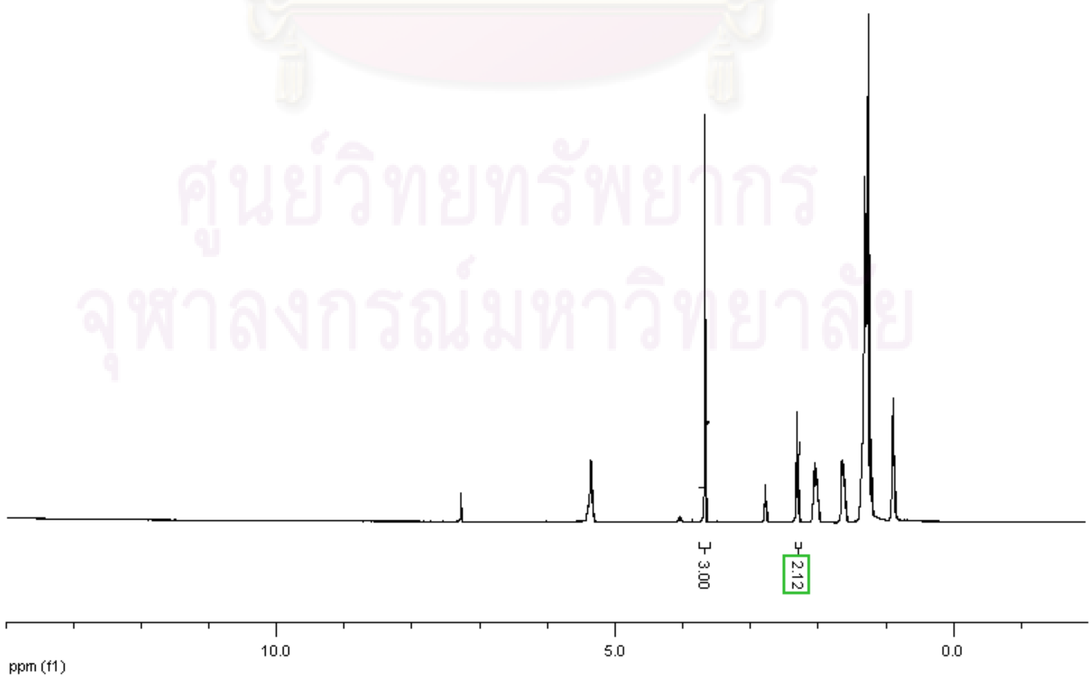


Figure A91 [1]H-NMR spectrum of TC oil methyl ester.

APPENDIX B

VALUES OF FREE FATTY ACID, SAPONIFICATION NUMBER, IODINE VALUE, ACID VALUE, VISCOSITY and METHYL ESTER CONTENT

Table B1 Values of Free Fatty Acids contained in crude oils

Sample	Weight of sample (g)	Volume of titrant (ml)	Concentration of NaOH (N)	% FFA
Basella alba (BA oil)	1.0012	0.10	0.23	0.65
	1.0027	0.10	0.23	
Brassica chinensis (BC oil)	1.0253	0.25	0.23	1.44
	1.0036	0.20	0.23	
Benincasa hispida (BEH oil)	1.0087	0.25	0.22	1.39
	1.0028	0.20	0.22	
Brassica juncea (BJ oil)	1.0006	0.40	0.22	2.79
	1.0027	0.50	0.22	
Brassica pekinensis (BP oil)	1.0138	0.35	0.24	2.17
	1.0172	0.30	0.24	
Caesalpinia crista (CAC oil)	1.0180	0.20	0.22	1.07
	1.0125	0.15	0.22	
Camellia oleifera (CAO oil)	1.0208	0.10	0.24	0.66
	1.0172	0.10	0.24	
Cassia suratlensi (CAS oil)	1.0058	0.10	0.22	0.73
	1.0115	0.15	0.22	
Crassocephalum crepidioides (CC oil)	1.0240	0.10	0.25	0.70
	1.0111	0.10	0.25	
Citrullus lanatus (CIL oil)	1.1098	2.35	0.23	13.86
	1.1137	2.40	0.23	
Citrus maxima (CIM oil)	1.0173	0.40	0.25	2.60
	1.0158	0.35	0.25	
Citrus reticulata (CIR oil)	1.0000	0.10	0.23	0.64
	1.0397	0.10	0.23	
Cucurbita moschata (CUM oil)	1.0174	0.90	0.24	5.82
	1.0189	0.85	0.24	
Chukrasia velutina (CV oil)	1.0174	1.75	0.25	12.01
	1.0083	1.70	0.25	
Dalbergia oliveri Gamble (DO oil)	1.0100	0.20	0.25	1.40
	1.0110	0.20	0.25	

Table B1 Values of Free Fatty Acids contained in crude oil *(continued)*

Sample	Weight of sample (g)	Volume of titrant (ml)	Concentration of NaOH (N)	% FFA
Gardenia jasminodes (GJ oil)	1.0200	0.25	0.23	1.59
	1.0190	0.25	0.23	
Hibiscus sabdariffa Linn. (HIS oil)	1.0092	0.25	0.22	1.39
	1.0012	0.20	0.22	
Lagenaria siceraria (LS oil)	1.0168	0.45	0.23	2.72
	1.0125	0.40	0.23	
Macadamia integrifolia (MAI oil)	1.0079	0.10	0.24	0.67
	1.0052	0.10	0.24	
Mimusops elengi (ME oil)	1.0137	1.25	0.23	7.85
	1.0110	1.20	0.23	
Millingtonia hortensis (MH oil)	1.0226	0.40	0.23	2.70
	1.0194	0.45	0.23	
Millettia kangensis Craib (ML oil)	1.0010	0.20	0.24	1.35
	1.0009	0.20	0.24	
Moringa oleifera Lamk. (MO oil)	1.0192	0.50	0.24	3.17
	1.0107	0.45	0.24	
Myristica fragrans (MYF oil)	1.0105	2.90	0.23	18.62
	1.0098	2.90	0.23	
Ocimum canum (OC oil)	1.0075	0.85	0.23	5.47
	1.0071	0.85	0.23	
Passiflora foetida (PAF oil)	1.0180	0.25	0.22	1.38
	1.0057	0.20	0.22	
Phyllanthus emblica (PE oil)	1.0010	0.30	0.25	2.10
	1.0100	0.30	0.25	
Perilla frutescens (PF oil)	1.1182	0.20	0.23	1.17
	1.1029	0.20	0.23	
Sesamum indicum (SEI oil)	1.0157	0.20	0.24	1.34
	1.0104	0.20	0.24	
Terminalia chebula (TC oil)	1.0113	4.50	0.24	30.13
	1.0105	4.50	0.24	

Table B2 Saponification value of crude oils

Sample	Weight of sample (g)	Volume of titrant (ml)	Volume of blank (ml)	SN
Basella alba (BA oil)	1.0049	24.30	31.40	179.05[a]
	1.0032	24.50	31.40	
Brassica chinensis (BC oil)	1.0075	24.60	30.90	161.23[a]
	1.0092	24.60	30.90	
Benincasa hispida (BEH oil)	1.0008	24.40	31.40	180.57[a]
	1.0000	24.40	31.40	
Brassica juncea (BJ oil)	1.0035	24.50	30.90	165.29[a]
	1.0027	24.45	30.90	
Brassica pekinensis (BP oil)	1.0072	26.80	33.60	166.56[b]
	1.0083	26.80	33.60	
Caesalpinia crista (CAC oil)	1.0054	26.60	33.60	173.51[b]
	1.0005	26.50	33.60	
Camellia oleifera (CAO oil)	1.0040	24.50	30.60	168.23[c]
	1.0058	24.50	30.60	
Cassia suratlensi (CAS oil)	1.0101	24.55	31.10	173.09[d]
	1.0083	24.40	31.10	
Crassocephalum crepidioides (CC oil)	1.0013	26.30	32.50	163.30[d]
	1.0009	26.30	32.50	
Citrullus lanatus (CIL oil)	1.0040	23.30	31.10	204.59[d]
	1.0065	23.30	31.10	
Citrus maxima (CIM oil)	1.0045	23.20	30.30	194.37[c]
	1.0037	23.20	30.30	
Citrus reticulate (CIR oil)	1.0197	24.50	31.40	176.74[a]
	1.0100	24.40	31.40	
Cucurbita moschata (CUM oil)	1.0110	24.60	31.40	173.72[a]
	1.0093	24.60	31.40	
Chukrasia velutina (CV oil)	1.0023	26.70	33.60	171.25[b]
	1.0012	26.60	33.60	
Dalbergia oliveri Gamble (DO oil)	1.0102	24.80	31.50	173.28[a]
	1.0004	24.70	31.50	

****** *X^a was titrate with 0.46 N HCl*
X^b was titrate with 0.44 N HCl
X^c was titrate with 0.49 N HCl
X^d was titrate with 0.47 N HCl

Table B2 Saponification value of crude oils *(continued)*

Sample	Weight of sample (g)	Volume of titrant (ml)	Volume of blank (ml)	SN
Gardenia jasminodes (GJ oil)	1.0100	23.40	30.30	187.78^c
	1.0102	23.40	30.30	
Hibiscus sabdariffa Linn. (HIS oil)	1.0192	25.60	32.50	181.33^d
	1.0022	25.50	32.50	
Lagenaria siceraria (LS oil)	1.0090	24.45	31.60	183.74^a
	1.0065	24.40	31.60	
Macadamia integrifolia (MAI oil)	1.0130	23.85	30.30	178.06^c
	1.0094	23.65	30.30	
Mimusops elengi (ME oil)	1.0124	23.60	31.10	195.70^d
	1.0086	23.60	31.10	
Millingtonia hortensis (MH oil)	1.0201	23.80	30.30	175.88^c
	1.0118	23.80	30.30	
Millettia kangensis Craib (ML oil)	1.0201	24.55	31.50	176.48^a
	1.0198	24.50	31.50	
Moringa oleifera Lamk. (MO oil)	1.0019	24.60	31.60	180.14^a
	1.0037	24.60	31.60	
Myristica fragrans (MYF oil)	1.0060	23.40	31.40	209.54^d
	1.0074	23.40	31.40	
Ocimum canum (OC oil)	1.0046	24.40	31.40	181.93^d
	1.0100	24.50	31.40	
Passiflora foetida (PAF oil)	1.0309	24.35	31.40	179.66^d
	1.0311	24.40	31.40	
Phyllanthus emblica (PE oil)	1.0047	23.80	30.90	186.00^d
	1.0083	23.80	30.90	
Perilla frutescens (PF oil)	1.0100	24.50	31.10	172.40^d
	1.0088	24.50	31.10	
Sesamum indicum (SEI oil)	1.0002	23.75	30.30	179.26^c
	1.0010	23.80	30.30	
Terminalia chebula (TC oil)	1.0103	23.60	30.85	185.31^a
	1.0090	23.60	30.85	

****** *X^a was titrate with 0.46 N HCl*
X^b was titrate with 0.44 N HCl
X^c was titrate with 0.49 N HCl
X^d was titrate with 0.47 N HCl

Table B3 Iodine value of crude oils

Sample	Weight of sample (g)	Volume of titrant (ml)	Volume of blank (ml)	IV
Basella alba (BA oil)	0.1015	43.20	49.60	76.78[a]
	0.1008	43.25	49.60	
Brassica chinensis (BC oil)	0.1027	43.30	48.20	56.29[b]
	0.1073	43.30	48.20	
Benincasa hispida (BEH oil)	0.1092	37.95	49.60	128.96[a]
	0.1114	37.90	49.60	
Brassica juncea (BJ oil)	0.1682	30.25	43.60	102.06[c]
	0.1626	30.35	43.60	
Brassica pekinensis (BP oil)	0.1506	32.40	43.60	96.36[c]
	0.1458	32.30	43.60	
Caesalpinia crista (CAC oil)	0.1107	36.80	47.50	125.03[d]
	01020	36.85	47.50	
Camellia oleifera (CAO oil)	0.1007	40.70	50.65	115.68[e]
	0.1105	40.80	50.65	
Cassia suratlensi (CAS oil)	0.1047	41.70	49.70	95.46[c]
	0.1067	41.80	49.70	
Crassocephalum crepidioides (CC oil)	0.1126	42.20	50.50	94.00[e]
	0.1063	42.10	50.50	
Citrullus lanatus (CIL oil)	0.1051	38.20	50.15	138.42[a]
	0.1048	38.25	50.15	
Citrus maxima (CIM oil)	0.1187	39.50	48.80	97.71[e]
	0.1169	39.40	48.80	
Citrus reticulata (CIR oil)	0.1076	40.85	49.05	95.43[a]
	0.1013	40.90	49.05	
Cucurbita moschata (CUM oil)	0.1064	40.80	49.60	99.60[a]
	0.1089	40.80	49.60	
Chukrasia velutina (CV oil)	0.1074	38.40	48.20	110.11[b]
	0.1072	38.40	48.20	
Dalbergia oliveri Gamble (DO oil)	0.1445	36.00	43.10	60.44[e]
	0.1437	36.05	43.10	

****** *X^a was titrate with 0.096 N $Na_2S_2O_3$*
X^b was titrate with 0.095 N $Na_2S_2O_3$
X^c was titrate with 0.100 N $Na_2S_2O_3$
X^d was titrate with 0.098 N $Na_2S_2O_3$
X^e was titrate with 0.097 N $Na_2S_2O_3$

Table B3 Iodine value of crude oils *(continued)*

Sample	Weight of sample (g)	Volume of titrant (ml)	Volume of blank (ml)	IV
Gardenia jasminodes (GJ oil)	0.1819	31.00	47.50	112.53[d]
	0.1839	30.90	47.50	
Hibiscus sabdariffa Linn. (HIS oil)	0.1080	40.20	47.50	81.83[d]
	0.1125	40.30	47.50	
Lagenaria siceraria (LS oil)	0.1017	40.20	50.50	119.20[e]
	0.1104	40.30	50.50	
Macadamia integrifolia (MAI oil)	0.1066	44.20	50.15	67.49[a]
	0.1073	44.25	50.15	
Mimusops elengi (ME oil)	0.1050	43.20	49.05	67.81[a]
	0.1052	43.20	49.05	
Millingtonia hortensis (MH oil)	0.1002	44.20	48.80	54.16[e]
	0.1081	44.25	48.80	
Millettia kangensis Craib (ML oil)	0.1441	33.80	43.10	78.40[e]
	0.1472	33.85	43.10	
Moringa oleifera Lamk. (MO oil)	0.1006	43.80	49.05	61.58[a]
	0.1053	43.90	49.05	
Myristica fragrans (MYF oil)	0.1472	55.00	60.40	45.13[c]
	0.1539	55.10	60.40	
Ocimum canum (OC oil)	0.1087	39.60	49.60	113.30[a]
	0.1053	39.70	49.60	
Passiflora foetida (PAF oil)	0.1211	36.40	49.05	126.16[a]
	0.1242	36.30	49.05	
Phyllanthus emblica (PE oil)	0.1481	41.40	60.40	164.38[c]
	0.1449	41.45	60.40	
Perilla frutescens (PF oil)	0.1036	40.45	50.5	118.04[c]
	0.1055	40.50	50.5	
Sesamum indicum (SEI oil)	0.1047	42.40	50.65	94.09[a]
	0.1077	42.50	50.65	
Terminalia chebula (TC oil)	0.1123	41.50	49.70	91.65[a]
	0.1134	41.60	49.70	

****** *X^a was titrate with 0.096 N $Na_2S_2O_3$*
X^b was titrate with 0.095 N $Na_2S_2O_3$
X^c was titrate with 0.100 N $Na_2S_2O_3$
X^d was titrate with 0.098 N $Na_2S_2O_3$
X^e was titrate with 0.097 N $Na_2S_2O_3$

Table B4 Acid value of biodiesels

Sample	Weight of sample (g)	Volume of titrant (ml)	Volume of blank (ml)	AV
Basella alba (BA oil)	2.0182	0.175	0.10	0.1878
	2.0144	0.175	0.10	
Brassica chinensis (BC oil)	2.0176	0.200	0.10	0.2502
	2.0185	0.200	0.10	
Benincasa hispida (BEH oil)	2.0104	0.150	0.10	0.1255
	2.0128	0.150	0.10	
Brassica juncea (BJ oil)	2.0105	0.225	0.10	0.3140
	2.0097	0.225	0.10	
Brassica pekinensis (BP oil)	2.0084	0.225	0.10	0.3144
	2.0058	0.225	0.10	
Caesalpinia crista (CAC oil)	2.0052	0.175	0.10	0.1889
	2.0036	0.175	0.10	
Camellia oleifera (CAO oil)	2.0157	0.175	0.10	0.1881
	2.0125	0.175	0.10	
Cassia suratlensi (CAS oil)	2.0076	0.225	0.10	0.3143
	2.0085	0.225	0.10	
Crassocephalum crepidioides (CC oil)	2.0004	0.150	0.10	0.1262
	2.0013	0.150	0.10	
Citrullus lanatus (CIL oil)	2.0075	0.225	0.10	0.3143
	2.0093	0.225	0.10	
Citrus maxima (CIM oil)	2.0005	0.200	0.10	0.2524
	2.0013	0.200	0.10	
Citrus reticulata (CIR oil)	2.0164	0.175	0.10	0.1877
	2.0188	0.175	0.10	
Cucurbita moschata (CUM oil)	2.0103	0.150	0.10	0.1256
	2.0095	0.150	0.10	
Chukrasia velutina (CV oil)	2.0076	0.175	0.10	0.1888
	2.0051	0.175	0.10	
Dalbergia oliveri Gamble (DO oil)	2.0057	0.150	0.10	0.1260
	2.0043	0.150	0.10	

****** *All of oils were titrated with 0.09 N alcoholic KOH*

Table B4 Acid value of biodiesels *(continued)*

Sample	Weight of sample (g)	Volume of titrant (ml)	Volume of blank (ml)	AV
Gardenia jasminodes (GJ oil)	2.0105	0.275	0.10	0.4396
	2.0097	0.275	0.10	
Hibiscus sabdariffa Linn. (HIS oil)	2.0014	0.175	0.10	0.1888
	2.0098	0.175	0.10	
Lagenaria siceraria (LS oil)	2.0187	0.200	0.10	0.2503
	2.0153	0.200	0.10	
Macadamia integrifolia (MAI oil)	2.0014	0.175	0.10	0.1890
	2.0053	0.175	0.10	
Mimusops elengi (ME oil)	2.0147	0.175	0.10	0.1879
	2.0169	0.175	0.10	
Millingtonia hortensis (MH oil)	2.0137	0.275	0.10	0.4390
	2.0120	0.275	0.10	
Millettia kangensis Craib (ML oil)	2.0136	0.150	0.10	0.1252
	2.0197	0.150	0.10	
Moringa oleifera Lamk. (MO oil)	2.0154	0.225	0.10	0.3132
	2.0148	0.225	0.10	
Myristica fragrans (MYF oil)	2.0152	0.150	0.10	0.1254
	2.0138	0.150	0.10	
Ocimum canum (OC oil)	2.0182	0.175	0.10	0.1877
	2.0175	0.175	0.10	
Passiflora foetida (PAF oil)	2.0064	0.175	0.10	0.1886
	2.0098	0.175	0.10	
Phyllanthus emblica (PE oil)	2.0071	0.275	0.10	0.4404
	2.0056	0.275	0.10	
Perilla frutescens (PF oil)	2.0149	0.275	0.10	0.4383
	2.0172	0.275	0.10	
Sesamum indicum (SEI oil)	2.0006	0.150	0.10	0.1262
	2.0015	0.150	0.10	
Terminalia chebula (TC oil)	2.0137	0.150	0.10	0.1253
	2.0152	0.150	0.10	

****** *All of oils were titrated with 0.09 N alcoholic KOH*

Table B5 Viscosity of biodiesels

Sample	Time 1 (s)	Time 2 (s)	Constants of tube	Viscosiy (cSt)
Basella alba (BA oil)	311.40	311.52	0.01434[a]	4.5
Brassica chinensis (BC oil)	345.63	345.12	0.01434[a]	5.0
Benincasa hispida (BEH oil)	271.40	271.45	0.01434[a]	3.9
Brassica juncea (BJ oil)	385.52	385.87	0.01434[a]	5.5
Brassica pekinensis (BP oil)	363.19	363.34	0.01434[a]	5.2
Caesalpinia crista (CAC oil)	464.25	464.39	0.00757[b]	3.5
Camellia oleifera (CAO oil)	501.09	500.83	0.00757[b]	3.8
Cassia suratlensi (CAS oil)	309.85	309.01	0.01434[a]	4.4
Crassocephalum crepidioides (CC oil)	351.78	351.65	0.01434[a]	5.0
Citrullus lanatus (CIL oil)	241.07	241.35	0.01434[a]	3.5
Citrus maxima (CIM oil)	538.61	539.01	0.00757[b]	4.1
Citrus reticulata (CIR oil)	254.19	253.72	0.01434[a]	3.6
Cucurbita moschata (CUM oil)	311.35	311.49	0.01434[a]	4.5
Chukrasia velutina (CV oil)	520.62	520.03	0.00757[b]	3.9
Dalbergia oliveri Gamble (DO oil)	332.97	332.49	0.01434[a]	4.8

****** X^a = *constant values of no.100 of viscometer tube*
X^b = *constant values of no.75 of viscometer tube*

Table B5 Viscosity of biodiesels *(continued)*

Sample	Time 1 (s)	Time 2 (s)	Constants of tube	Viscosiy (cSt)
Gardenia jasminodes (GJ oil)	637.80	637.84	0.00757[b]	4.8
Hibiscus sabdariffa Linn. (HIS oil)	585.69	585.21	0.00757[b]	4.4
Lagenaria siceraria (LS oil)	246.28	246.33	0.01434[a]	3.5
Macadamia integrifolia (MAI oil)	567.03	567.30	0.00757[b]	4.3
Mimusops elengi (ME oil)	594.22	594.10	0.00757[b]	4.5
Millingtonia hortensis (MH oil)	695.70	696.00	0.00757[b]	5.3
Millettia kangensis Craib (ML oil)	613.78	614.38	0.00757[b]	4.6
Moringa oleifera Lamk. (MO oil)	326.60	326.72	0.01434[a]	4.7
Myristica fragrans (MYF oil)	516.31	516.11	0.00757[b]	3.9
Ocimum canum (OC oil)	470.30	470.40	0.00757[b]	3.6
Passiflora foetida (PAF oil)	269.20	269.36	0.01434[a]	3.9
Phyllanthus emblica (PE oil)	107.31	107.59	0.03370[c]	3.6
Perilla frutescens (PF oil)	223.29	223.78	0.01434[a]	3.2
Sesamum indicum (SEI oil)	282.65	282.75	0.01434[a]	4.1
Terminalia chebula (TC oil)	615.85	615.41	0.00757[b]	4.7

****** X^a = *constant values of no.100 of viscometer tube*
X^b = *constant values of no.75 of viscometer tube*
X^c = *constant values of no.150 of viscometer tube*

Table B6 Methyl ester content of biodiesels

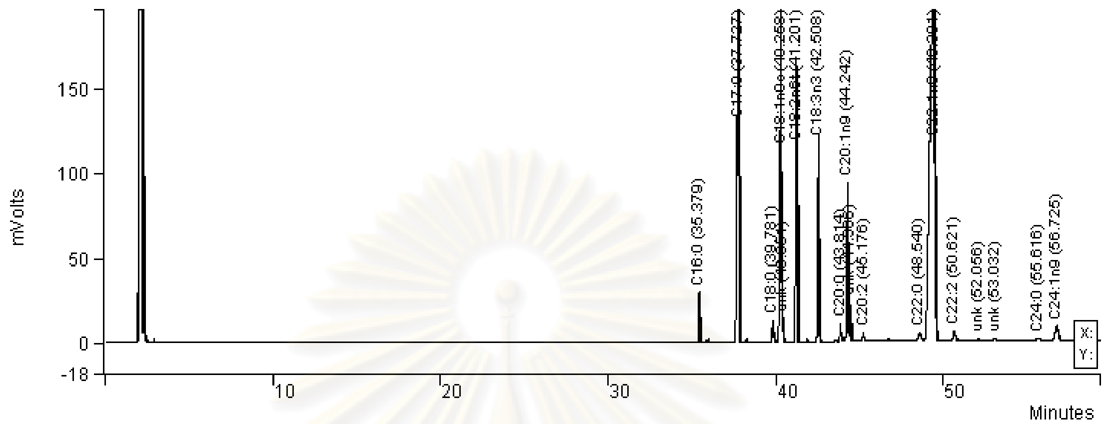
Codes	C17:0 (mg)	Oil (mg)	Area FAME	Area Ins.	%Ester
BA	506.40	249.10	5764541	989697	94.685
BC	496.70	273.00	10138059	1635761	94.569
BEH	500.00	260.70	4159247	692943	95.320
BJ	508.70	259.60	10526597	1805904	94.627
BP	497.20	270.50	10901830	1766035	95.085
CAC	500.00	263.00	2063049	334976	94.681
CAO	508.70	246.60	4727804	806476	96.831
CAS	510.30	248.16	9702915	1777118	91.711
CC	500.00	258.30	7912616	1299445	95.104
CIL	506.40	256.10	5924658	984132	95.831
CIM	506.40	251.10	6283295	1068541	95.015
CIR	500.00	251.70	3724604	637852	95.512
CUM	500.00	249.10	3260626	572709	93.598
CV	508.70	248.00	4928475	855149	94.323
DO	500.00	258.00	8728303	1474875	95.310
GJ	507.09	256.30	6607816	1108674	94.739
HIS	510.30	244.10	958970	172427	92.061
LS	507.09	249.70	3047926	513510	96.760
MAI	506.40	252.30	5578028	937079	95.964
ME	508.70	259.70	9884033	1670098	96.338
MH	507.09	250.00	5995516	1053558	93.765
ML	510.30	248.70	1685526	296231	92.900
MO	506.30	253.10	7469680	1275037	96.560
MYF	507.90	248.30	3837134	680709	94.237
OC	500.00	251.20	4292199	729312	96.611
PAF	500.00	259.50	3822714	637606	95.155
PE	508.70	247.60	9849262	1740073	92.432
PF	500.00	258.80	5349435	912453	93.947
SEI	507.09	247.80	6756188	1145904	96.721
TC	508.70	255.50	9819444	1713337	94.198

APPENDIX C

CALCULATIONS

A. Calculated % Methyl ester content from GC

% Methyl ester content from GC of BC oil methyl ester was calculated as follow:



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	1.3422	35.379	-0.012	136078	0.00	BB	4.3		0
2	C17:0	16.1349	37.727	0.113	1635761	0.00	BB	5.9		0
3	C18:0	0.6448	39.781	0.014	65375	0.00	BP	4.9		0
4	C18:1n9c	12.1454	40.258	0.066	1231313	0.00	PV	5.3		0
5	unk	0.4917	40.351	-0.000	49847	0.00	VB	3.9		0
6	C18:2n6t	8.4112	41.201	-0.021	852735	0.00	BP	4.8		0
7	C18:3n3	5.8748	42.508	0.030	595590	0.00	VB	4.6		0
8	C20:0	0.5184	43.814	-0.009	52555	0.00	PV	4.7		0
9	C20:1n9	4.7861	44.242	0.026	485221	0.00	VV	4.8		0
10	unk	1.0292	44.386	-0.000	104340	0.00	VB	4.3		0
11	C20:2	0.2578	45.176	-0.019	26131	0.00	BB	4.9		0
12	C22:0	0.5496	48.540	0.139	55720	0.00	BV	11.1		0
13	C22:1n9	45.5548	49.381	0.360	4618376	0.00	VP	15.3		0
14	C22:2	0.5324	50.621	0.021	53979	0.00	PB	8.5		0
15	unk	0.1514	52.056	0.000	15348	0.00	VV	9.1		0
16	unk	0.1454	53.032	-0.000	14740	0.00	VB	9.8		0
17	C24:0	0.1970	55.616	-0.061	19974	0.00	BB	12.1		0
18	C24:1n9	1.2327	56.725	-0.006	124976	0.00	BB	13.3		0
	Totals	**99.9998**		**0.641**	**10138059**					

$$C = \frac{(\sum A - A_i)}{A_i} \times \frac{(C_i \times V_i)}{m} \times 100$$

C = Methyl ester content

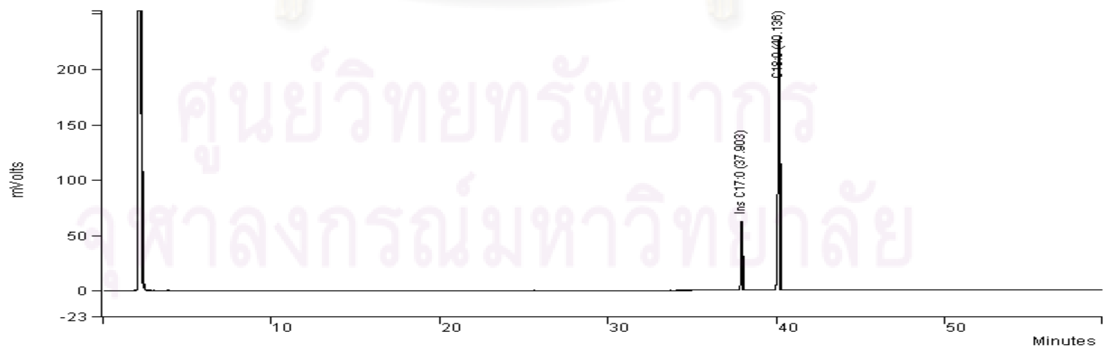
∑A	=	Total area of fatty acid methyl esters
Ai	=	Area of methyl heptadecanoate
Ci	=	Concentration of methyl heptadecanoate solution
Vi	=	Volume of methyl heptadecanoate solution
m	=	Mass of the sample

$$\text{C of BC oil} = \frac{(10138059 - 1635761)}{1635761} \times \frac{(9.934 \times 5)}{273.00} \times 100$$

$$= 94.57\%$$

B. ***% precision from GC***

Methyl sterate (250 mg) was dissolved in heptadecanoic solution (5 ml) (500 mg heptadecanoate dissolved in heptane (50 ml)) and 1 µl of solution was injected in to GC using 1:100 split ratios. The GC condition for the determination of methyl ester was set as 3.2.2.4. Methyl heptadecanoate was used as an internal standard. The methyl ester content of biodiesel was calculated by the following equation:



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	Ins C17:0	16.6413	37.903	-0.035	292380	0.00	BB	4.4		0
2	C18:0	83.3587	40.136	0.061	1464574	0.00	BB	6.0		0
	Totals	**100.0000**		**0.026**	**1756954**					

$$\text{C of Methyl Stearate} = \frac{(\sum A - Ai)}{Ai} \times \frac{(Ci \times Vi)}{m} \times 100$$

$$= \frac{(1756954-292380)}{292380} \times \frac{(10.0346 \times 5)}{254.45} \times 100$$

$$= 98.77\ \%$$

$$\%\ \text{precision} = 98.77 \times \text{purity of C18 (99.5\%)}$$

$$= 99.26\ \%$$

C. Calculated % methyl ester of transesterification from ^{1}H-NMR spectrum

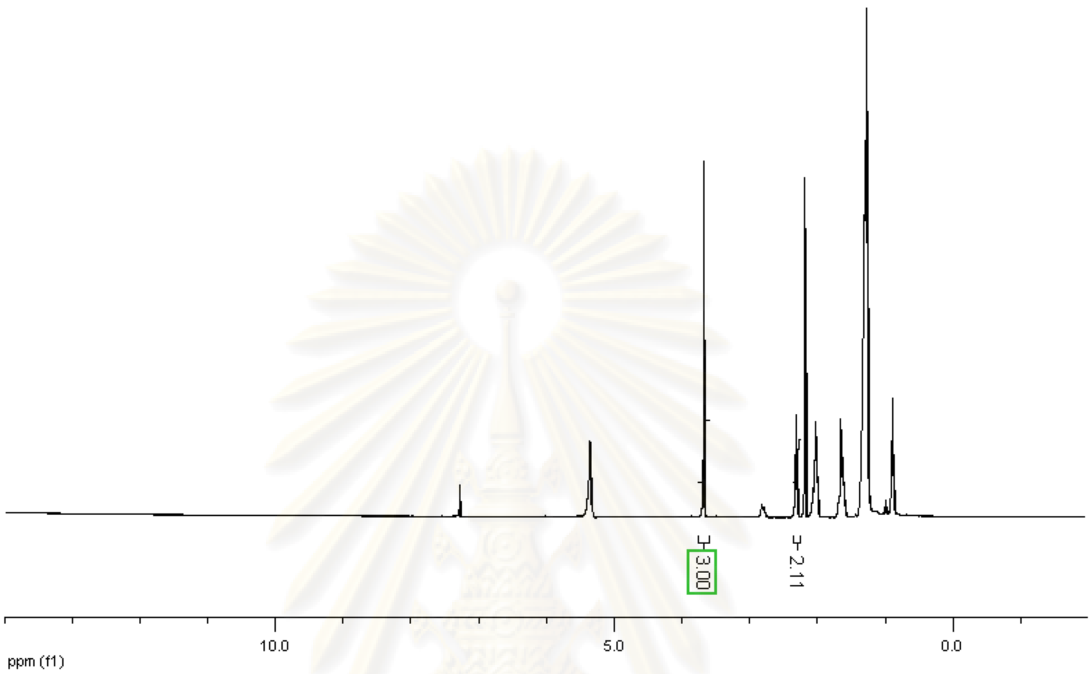
The % methyl ester of transesterification was calculated in following:

$$\%\ \text{Methyl ester} = [(2I_{CH3}) / (3I_{CH2})] \times 100$$

I_{CH3} = Integration value of the protons of the methyl esters (the strong singlet), appear at δ 3.7 ppm

I_{CH2} = Integration value of the methylene protons, appear at δ 2.3 ppm

Sample: BC oil methyl ester



% Methyl ester of BC oil = [(2×3) / (3×2.11)] × 100

= 94.79 %

D. Determine the % free fatty acid (ASTM D5555)

Reagent

1. Ethanol
2. Phenolphthalein
3. 0.25 N NaOH

To the 250 ml of Erlenmeyer flask, oil sample (1 g), ethanol (75 ml) and 2ml of 1% phenolphthalein were added. The mixture was subject to titrate with 0.25 N sodium hydroxide solutions until the pink color was occurred. The ml of alkali solution used was recorded.

The percentage of free fatty acids was calculated as follows:

$$\% \text{ free fatty acids} = (\text{ml of alkali x } N \text{ x } 28.2) / \text{weight of sample}$$

$$N = \text{normality of alkaline solution}$$

$$\text{ml of alkali} = \text{ml of sodium hydroxide solution}$$

Example:

$$\% \text{ FFA of BC oil} = \frac{0.25 \times 0.23 \times 28.2}{1.0253}$$

$$= 1.58 \%$$

E. Determination of the Saponification value (ASTM D5558)

Reagent

1. Phenolphthalein
2. Alcoholic KOH solution
3. 0.5 N HCl

To the 250 ml of Erlenmeyer flask, oil sample (1 g), alcoholic potassium hydroxide (25 ml) and 1 ml of 1% phenolphthalein were added. The mixture was subject to titrate with 0.5 N of hydrochloric acid until the pink color has disappeared. Prepare a blank determination and carried out same with the sample. The ml of acid solution used was recorded.

The saponification value was calculated as follows:

saponification value = $56.1 \times N \times (A - B)$ / weight of sample

A = titration of blank

B = titration of sample

N = normality of hydrochloric acid solution

Alcoholic KOH = 40 g of potassium hydroxide dissolved in 1 L of ethanol

Example:

$$\text{SN of BC oil} = \frac{56.1 \times 0.46 \times (30.90\text{-}24.60)}{1.0075}$$

$$= 161.37 \text{ mg KOH/g of oil}$$

F. Determination of the Iodine value (ASTM D5554)

Reagent

1. KOH solution
2. Wijs solution
3. CCl_4
4. 0.1 N $Na_2S_2O_3$
5. Starch

To the 500 ml of Glass-Stopper flask, oil sample (0.1 g), carbon tetra chloride (20 ml) and Wijs solution (25 ml) were added. Store the flasks in a dark place for 30 min. From storage, removed the flasks and add 20 ml of KI solution followed by 100 ml of distilled water. The mixture was subject to titrate with 0.1 N of sodium thiosulfate until the yellow color has almost disappeared, add 2 ml of starch indicator solution, and continued the titration until the blue color has just disappeared. Prepare a blank determination and carried out same with the sample. The ml of sodium thiosulfate used was recorded.

The iodine value was calculated as follows:

$$\text{Iodine value} = (B - S) \times N \times 12.69 \, / \, \text{weight of sample}$$

B = titration of blank

S = titration of sample

N = normality of $Na_2S_2O_3$ solution

Example:

$$\text{IV of BC oil} = \frac{(48.20-43.30) \times 0.095 \times 12.69}{0.1027}$$

$$= 57.52 \text{ mg } I_2 \text{ /g of oil}$$

G. Determination of the acid value (ASTM D974)

Reagent

1. p-naphtholbenzein indicator solution
2. 0.1 M Alcoholic KOH solution
3. Titration solvent (250 ml toluene + 250 ml isopropyl alcohol)

To the 250 ml of Erlenmeyer flask, oil sample (2 g), titration solvent (25 ml) and 0.125 ml of 1% p-naphtholbenzein indicator solution were added. The mixture was subject to titrate with 0.1 M of alcoholic KOH until the green color was occured. Prepare a blank determination and carried out same with the sample. The ml of acid solution used was recorded.

The acid value was calculated as follows:

Acid value = $[(A - B) \times N \times 56.1]$ / weight of sample

A = titration of sample

B = titration of blank

N = normality of alcoholic KOH solution

Alcoholic KOH = 0.6 g of potassium hydroxide dissolved in 100 ml of isopropyl alcohol

Example:

$$\text{Acid value of BC oil methyl ester} = \frac{[(0.20\text{-}0.10) \times 0.09 \times 56.1]}{2.0176}$$

$$= 0.250 \text{ mg KOH/g of oil}$$

H. Determination of viscosity (ASTM D445)

Viscosity is the unit specifying the resistance to flow; therefore viscosity is normally a specific value.

7 ml of each sample were added into the Viscometer tube, and insert the viscometer into the bath. After insertion, allow the viscometer to reach bath temperature, Use suction to adjust the head level of the test sample to a position in the capillary arm of the instrument about 7 mm above the first timing mark. With the sample flowing freely, measure, in seconds to within 0.1 s, the time required for the meniscus to pass from the first to the second timing mark. The time of sample used was recorded.

The viscosity was calculated as follows:

$$\text{viscosity} = Ct$$

C = Constant of viscometer tube (mm^2/s^2)

time = measured flow times for $t1$ and $t2$, respectively(s)

Example:

$$\text{Viscosity of BC oil methyl ester} = 0.01434 \times \frac{(345.63 + 345.12)}{2}$$

$$= 4.95 \text{ cSt}$$

VITA

Mr. Sumetha Issariyanate was born on August 25, 1984 in Bangkok, Thailand. He graduated with Bachelor Degree of Science, from Department of Chemistry, Faculty of Science, Chulalongkorn University in 2007. He was admitted to the Master degree of Science in Petrochemistry and Polymer Science, Faculty of Science, Chulalongkorn University in 2007 and completed the program in 2010.

Conference

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Pure and Applied Chemistry International Conference 2010

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