STUDY OF PREPARATION OF FATTY ACID METHYL ESTERS (FAMEs) FROM VEGETABLE OIL BY TRANSESTERIFICATION REACTION USING GAS CHROMATOGRAPHY

WANVISA CHOMSIRI

A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE
(APPLIED ANALYTICAL AND INORGANIC CHEMISTRY)
FACULTY OF GRADUATE STUDIES
MAHIDOL UNIVERSITY
2009

COPYRIGHT OF MAHIDOL UNIVERSITY

Copyright by Mahidol University

Thesis
Entitled

STUDY OF PREPARATION OF FATTY ACID METHYL ESTERS (FAMEs) FROM VEGETABLE OIL BY TRANSESTERIFICATION REACTION USING GAS CHROMATOGRAPHY

……..
Miss Wanvisa Chomsiri
Candidate

……..
Lect. Tinnakorn Tiensing,
Ph.D. (Environmental Science)
Major advisor

……..
Asst. Prof. Atitaya Siripinyanond,
Ph.D. (Analytical Chemistry)
Co-advisor

……..
Prof. Banchong Mahaisavariya,
M.D.
Dean
Faculty of Graduate Studies

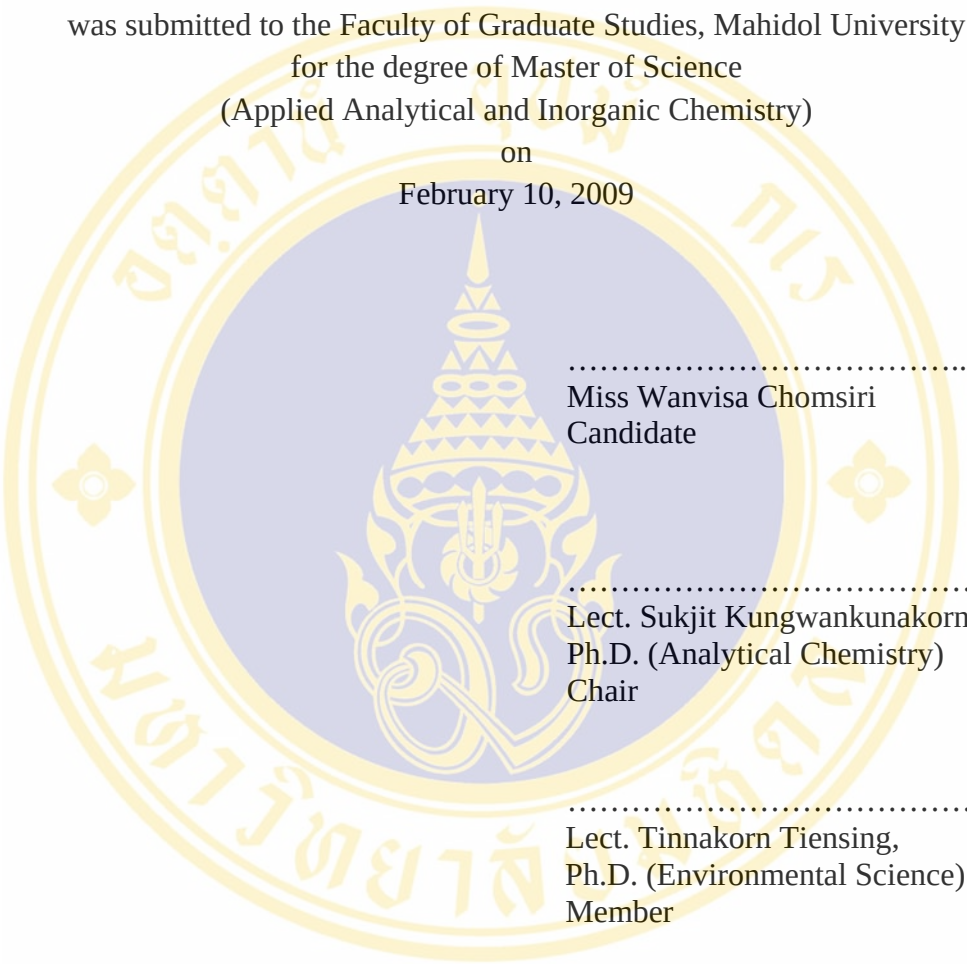
……..
Asst.Prof. Duangjai Nacapricha,
Ph.D. (Analytical Chemistry)
Chair
Master of Science Program in Applied Analytical and Inorganic Chemistry
Faculty of Science

Copyright by Mahidol University

Thesis
Entitled

STUDY OF PREPARATION OF FATTY ACID METHYL ESTERS (FAMEs) FROM VEGETABLE OIL BY TRANSESTERIFICATION REACTION USING GAS CHROMATOGRAPHY

was submitted to the Faculty of Graduate Studies, Mahidol University
for the degree of Master of Science
(Applied Analytical and Inorganic Chemistry)
on
February 10, 2009



…….....
Miss Wanvisa Chomsiri
Candidate

…….....
Lect. Sukjit Kungwankunakorn,
Ph.D. (Analytical Chemistry)
Chair

…….
Lect. Tinnakorn Tiensing,
Ph.D. (Environmental Science)
Member

…….....
Lect. Jonggol Tantirungrotechai,
Ph.D. (Chemistry)
Member

…….
Asst. Prof. Atitaya Siripinyanond,
Ph.D. (Analytical Chemistry)
Member

…….....
Prof. Banchong Mahaisavariya,
M.D.
Dean
Faculty of Graduate Studies
Mahidol University

…….....
Prof. Skorn Mongkolsuk,
Ph.D. (Biological Science)
Dean
Faculty of Science
Mahidol University

Copyright by Mahidol University

ACKNOWLEDGEMENTS

I would like to express my deepest appreciation to Dr. Tinnakorn Tiensing, for him suggestion, assistant and encouragement throughout my M.Sc. research. My gratitude is also expressed to Asst. Prof. Dr. Atitaya Siripinyanond and Dr. Jonggol Tantirungrotechai for their comments and suggestions in completion of the thesis. My grateful acknowledgement is expressed to Dr. Sukjit Kungwankunakorn, the external examiner for her comment and suggestion.

I am grateful to the Department of Chemistry, Faculty of Science, Mahidol University for the opportunity to provide teaching assistantship. I also appreciate the financial support provided by the Center of Excellence for Innovation in Chemistry (PERCH-CIC).

My sincere thankfulness is expressed to all staff members in Applied Analytical and Inorganic Chemistry Program (AAICP) and particularly TTS Lab, for their friendship and kind help for learning and research. My gratitude is also to all the staff members of the Department of Chemistry for their generous help.

Finally, I would like to express my deepest gratitude is given to my parents and my family for their understanding, encouragement and support throughout my life.

Wanvisa Chomsiri

Copyright by Mahidol University

STUDY OF PREPARATION OF FATTY ACID METHYL ESTERS (FAMEs) FROM VEGETABLE OIL BY TRANSESTERIFICATION REACTION USING GAS CHROMATOGRAPHY

WANVISA CHOMSIRI 4936467 SCAI/M

M.Sc. (APPLIED ANALYTICAL AND INORGANIC CHEMISTRY)

THESIS ADVISORY COMMITTEE: TINNAKORN TIENSING, Ph.D. (ENVIRONMENTAL SCIENCE), ATITAYA SIRIPINYANOND, Ph.D. (ANALYTICAL CHEMISTRY)

ABSTRACT

In general, biodiesel is fatty acid methyl ester (FAME) which is usually obtained from transesterification of vegetable oil with methanol using acid or base catalyst. This reaction exchanges the alcohol group of triglyceride with methanol to create a mono alkyl ester. Fatty acid (FA) compositions of an individual vegetable oil affect on FAME properties and its production. This research studied preparation of FAMEs from various vegetable oils for biodiesel production and determination of FAMEs in order to explore the FA profiles in vegetable oils.

The optimum procedure for FAMEs preparation was separated into two conditions including one-step and two-step methods. The optimum condition of one-step transesterification of vegetable oils using a base catalyst was performed under low free fatty acid (FFA) content in the vegetable oil (acid value < 1). This condition gave conversion in the range of 80.29-93.97%. The two-step method was introduced for vegetable oils with high FFA contents (acid value > 1). The two-step method of acid-base transesterification gave a high conversion within the range of 74.12-96.62%. The FAME product was investigated by the optimum GC-FID condition, which consists of using split injection (40:1), DB-wax column, He carrier gas (0.5 mL/min), column temperature (210 °C), injector temperature (250 ^{o}C) and detector temperature (250 ^{o}C). This condition gave a low detection limit level of FAME compounds, in the range of 0.19-0.30 mg/L. This method was applied to determine the FA composition in vegetable oil samples using GC-FID. The percentage conversion evaluated from GC and ^{1}H-NMR techniques showed no significant difference at 95% confidence interval.

KEY WORDS: BIODIESEL/FATTY ACID METHYL ESTERS/ TRANSESTERIFICATION/GAS CHROMATOGRAPHY

90 pp.

Copyright by Mahidol University

การศึกษาการเตรียมเอสเทอร์ของกรดไขมันจากน้ำมันพืชโดยปฏิกิริยาทรานส์เอสเทอริฟิเคชั่นโดยใช้เทคนิคแก๊สโครมาโทกราฟี
(STUDY OF PREPARATION OF FATTY ACID METHYL ESTERS (FAMEs) FROM VEGETABLE OIL BY TRANSESTERIFICATION REACTION USING GAS CHROMATOGRAPHY)

วันวิสาข์ ชมศิริ 4936467 SCAI/M

วท.ม. (เคมีวิเคราะห์และเคมีอินทรีย์ประยุกต์)

คณะกรรมการที่ปรึกษาวิทยานิพนธ์: ทินกร เตียนสิงห์, Ph.D. (Environmental Science), อทิตยา ศิริภิญญานนท์, Ph.D. (Analytical Chemistry)

บทคัดย่อ

โดยทั่วไปไบโอดีเซลประกอบด้วยเอสเทอร์ของกรดไขมัน(เฟลม) ซึ่งปกติไบโอดีเซลได้มาจากทรานส์เอสเทอริฟิเคชั่นของน้ำมันพืชกับเมทานอล โดยใช้กรดหรือเบสเป็นตัวเร่งปฏิกิริยา ปฏิกิริยานี้จะเปลี่ยนหมู่แอลกอฮอล์ของไตรกลีเซอไรด์กับเมทานอลเป็นโมโนอัลคิลเอสเทอร์ องค์ประกอบของกรดไขมันในน้ำมันพืชแต่ละชนิดจะส่งผลต่อคุณสมบัติและการผลิตของเอสเทอร์ของกรดไขมัน ดังนั้นในงานวิจัยนี้จึงศึกษาการเตรียมเฟลมจากน้ำมันพืชแต่ละชนิดสำหรับการผลิตไบโอดีเซล และศึกษาการวิเคราะห์เอสเทอร์ของกรดไขมันในการหาโปรไฟล์ของกรดไขมันในน้ำมันพืช

วิธีการที่เหมาะสมสำหรับการเตรียมเฟลมมี 2 สภาวะประกอบด้วย วิธีหนึ่งขั้นตอนและสองขั้นตอน สภาวะที่เหมาะสมของการทำทรานส์เอสเทอริฟิเคชั่นหนึ่งขั้นตอนของน้ำมันพืชโดยใช้ตัวเร่งปฏิกิริยาที่เป็นเบส สามารถทำได้กับน้ำมันพืชที่มีกรดไขมันปริมาณต่ำ (ค่าความเป็นกรด<1) ซึ่งวิธีนี้ได้การเปลี่ยนแปลงอยู่ในช่วง 80.29-93.97 เปอร์เซ็นต์ วิธีสองขั้นตอนใช้สำหรับน้ำมันพืชมีกรดไขมันปริมาณสูง (ค่าความเป็นกรด>1) สภาวะที่เหมาะสมของการทำทรานส์เอสเทอริฟิเคชั่นสองขั้นตอนของวิธีกรด-เบส ได้การเปลี่ยนแปลงอยู่ในช่วง 74.12-96.62 เปอร์เซ็นต์ ผลิตภัณฑ์เอสเทอร์ของกรดไขมันสามารถติดตามโดยสภาวะที่เหมาะสมของแก๊สโครมาโทกราฟีที่มีเครื่องตรวจวัดเป็นเฟลมไอออไนเซชัน ประกอบด้วยการฉีดแบบแบ่งส่วนที่อัตราส่วน 40 ต่อ 1 คอลัมน์ดีบี-แวกซ์ ฮีเลียมเป็นแก๊สตัวพา (0.5 มิลลิลิตรต่อนาที) อุณหภูมิคอลัมน์ (210 องศาเซลเซียส) อุณหภูมิของเครื่องฉีดและเครื่องตรวจวัด (250 องศาเซลเซียส) สภาวะที่ศึกษาให้ค่าการตรวจวัดค่าต่ำสุดของสารประกอบเอสเทอร์ของกรดไขมันอยู่ในระดับต่ำในช่วง 0.19-0.30 มิลลิกรัมต่อลิตร วิธีนี้สามารถใช้ในการวิเคราะห์หาองค์ประกอบของกรดไขมันในตัวอย่างน้ำมันพืชโดยใช้เทคนิคแก๊สโครมาโทกราฟีที่มีเครื่องตรวจวัดแบบเฟลมไอออไนเซชัน เปอร์เซ็นต์การเปลี่ยนแปลงที่คำนวณโดยแก๊สโครมาโทกราฟีและเทคนิคโปรตอนนิวเคลียร์แมกเนติกเรโซแนนซ์ให้ผลไม่แตกต่างกันอย่างมีนัยสำคัญที่ความเชื่อมั่น 95 เปอร์เซ็นต์

90 หน้า

Copyright by Mahidol University

CONTENTS

Copyright by Mahidol University

CONTENTS (cont.)

Copyright by Mahidol University

CONTENTS (cont.)

Copyright by Mahidol University

CONTENTS (cont.)

Copyright by Mahidol University

CONTENTS (cont.)

Copyright by Mahidol University

LIST OF TABLES

Copyright by Mahidol University

LIST OF TABLES (cont.)

Copyright by Mahidol University

LIST OF FIGURES

Copyright by Mahidol University

LIST OF FIGURES (cont.)

Copyright by Mahidol University

LIST OF FIGURES (cont.)

Copyright by Mahidol University

LIST OF ABBREVIATIONS

^{o}C	Celsius degree
et al.	*Eet alia* (Latin), and others
etc.	Et cetara
FA	Fatty acid
FFA	Free fatty acid
FAME	Fatty acid methyl ester
g	Gram
g/L	Gram per liter
g/mol	Gram per mol
i.d.	Internal diameter
kPa	Kilopascal
LOD	Limit of detection
mg	Milligram
mg/kg	Milligram per kilogram
mg/L	Milligram per liter
mm	Millimeter
mL	Milliliter
mL/min	Milliliter per minute
µm	Micrometer
µL	Microliter
µg/L	Microgram per liter
µg/mL	Microgram per milliliter
nm	Nanometer
r^2	Correlation coefficient
RSD	Relative standard deviation
SD	Standard deviation
TG	Triglyceride

Copyright by Mahidol University

THE RELEVANCE OF THE RESEARCH WORK TO THAILAND

Biodiesel is available as an alternative diesel oil substitution because it contains almost no sulfur, no aromatics, non-toxic, biodegradable and environmentally friendly fuel. The raw materials for biodiesel production are content vegetable oils and alcohols. Each type of vegetable oils has different fatty acids (FAs) which affect to the biodiesel properties. Therefore, the relationship between an individual of FAs and biodiesel quality can be evaluated. The possibilities of this research to Thailand are that the biodiesel can be easily produced by appropriate condition and the method can be applied to use waste cooking oils. In addition, the optimized GC-FID condition can be applied to determine FA contents in each type of vegetable oils which are commonly used as raw material for biodiesel production in Thailand.

Copyright by Mahidol University

CHAPTER I
INTRODUCTION

Biodiesel contains mono alkyl ester of a long chain fatty acid (FA). It is a renewable and alternative fuel substituted for petroleum diesel. Biodiesel has low emission profiles and environmental benefit fuel because of biodegradable and non-toxic. Most of the commercialize biodiesels are produced from bio-energy sources such as vegetable oil and animal fat which primarily contain with triglyceride (TG) and free fatty acid (FFA). These compounds can be used as reactants for the biodiesel production [Ma, *et al.*, 1999]. The interesting in the use of a renewable fuel started when scientists discovered the direct use of vegetable oils as a substitution for diesel. However, their direct use to be renewable oil in compressed ignition engines is restricted because high viscosity affects on a poor fuel atomization and an incomplete combustion. Therefore, these renewable resources have been chemically modified in order to reduce the viscosity and to improve the combustion [Sharma, *et al.*, 2008]. In a general process, the vegetable oil was converted by transesterification reaction between triglycerides and low molecular weight alcohol (methanol or ethanol) in the presence of a catalyst to fatty acid methyl esters (FAMEs) and glycerol as by-product [Shahid, *et al.*, 2008]. The properties of FAME are low viscosity and high combustion which are similar to diesel fuel properties [Chongkhong, *et al.*, 2007].

In this research, the proposed procedures of FAME (biodiesel) preparation were performed by transesterification of vegetable oil with methanol using acid or base catalysts. Investigation and optimization of the reaction parameters of the transesterification considered in the reaction including types of catalyst, catalyst concentration, molar ratio of alcohol to oil, reaction temperature and reaction time [Marchetti, *et al.*, 2007; Meher, *et al.*, 2006]. The types of acid catalyst were H_2SO_4, BF_3 and HCl. The types of base catalyst were NaOH, KOH and $NaOCH_3$. These catalysts were dissolved in methanol, methanolic solution. The general preparation of

Copyright by Mahidol University

FAMEs via base-catalyzed transesterification was applied to the renewable sources with low free fatty acid (FFA) contents but the reaction was not employed with the sources of high FFA contents because of soap formation. The soap formation in the reaction reduced the efficiency of the reaction. Therefore, the FAMEs generated from vegetable oil with high FFA contents were prepared by the two-step methods of transesterification. In this work, the procedure of FAMEs preparation by the two-step methods was studied. The studied procedure consisted of (1) acid-base and (2) base-acid methods. In acid-base method, the FFAs were firstly converted to FAMEs by acid-catalyzed and then followed by base-catalyzed transesterification of TG to FAMEs. In base-acid method, TG and FFAs were preliminary converted to soap and then the esterification was applied to convert soap products to become FAMEs. The FAME concentrations from those two procedures were compared. The FAMEs were optimized and separated under the appropriate GC-FID condition. The transesterification reaction can be monitored by using ^{1}H-NMR to confirm the conversion efficiency. The properties of the biodiesel fuel are strongly influenced by the structure and variation of the FA components in the oil depends on the source. Consequently, a suitable composition of FA in oils will give desirable biodiesel properties [Pinto, *et al.*, 2005].

Copyright by Mahidol University

CHAPTER II
OBJECTIVES

The purpose of this work was to study parameters for preparation and determination of FAMEs using optimum gas chromatography (GC) condition equipped with flame ionization detector (FID). Palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2) were used as interested FA compounds. These compounds are major components in the vegetable oil. Methyl heptadodecanoate (C17:0) was used as internal standard. Transesterification and esterification reaction were used for the preparation of FAMEs. This method was then applied to prepare and to determine FAMEs in the vegetable oil samples.

Copyright by Mahidol University

CHAPTER III
LITERATURE REVIEWS

3.1 Introduction

Vegetable oils or animal fats are contained with complex mixture of triglyceride (TG) and other minor components such as free fatty acids (FFA), gums, waxes and etc. There are reactants for biodiesel production which has many methods to produce suitable biofuels. The most commonly used method is transesterification of vegetable oils and animal fats with alcohol. In this chapter was studied the important parameters affecting on biodiesel production including types of catalyst, catalyst concentration, molar ratio of alcohol to oil, reaction temperature and reaction time.

3.2 Vegetable oil

3.2.1 Triglyceride (TG)

Chemically oils/fats consist of triglyceride (triacylglyceride or triacylglycerol) molecule that is composed of one molecule of glycerol and three an ester bond linked of fatty acids (FA) molecules [Seniha, *et al.*, 2006]. The structure of triglyceride is shown in Figure 3.1. The FAs contribute in the range from 94–96% of the total weight of one triglyceride molecule. The most common FAs in natural oil are detailed in Table 3.1. Saturated FAs have no double bonds in structure. Unsaturated FAs have one or more than one double bond. Individual of FA has various physical properties, as shown in Table 3.2. Consequently, properties of triglyceride or oils are depended on the FA components.

Copyright by Mahidol University

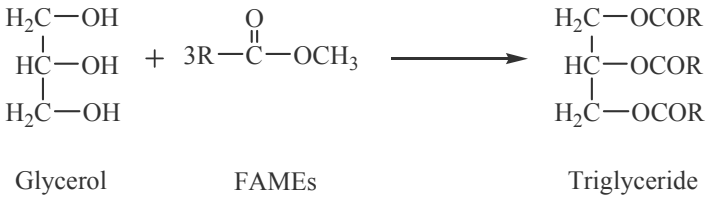


Figure 3.1 The equation for synthesis of triglyceride; where, R is hydrocarbon chain.

3.2.2 Fatty acid (FA)

FAs consisting of long chain carbon are varied with the number of carbon atoms and the number of double bonds. These FAs are usually found in the oil which consisting of free fatty acid (FFA) and FA bonded with triglyceride. [Antonio, 2005; Barnwal, *et al.*, 2005]. The chain length and degree of unsaturation influence on a variety of FA differing in oils. The long chains of C16 to C18 are interested FA in this work. The FA compositions in each type of vegetable oils that can be used in the biodiesel production are illustrated in Table 3.1. The common structure of FA is indicated by the conventional symbol (X:Y). Where, "X" is the number of carbon atoms in the chain and "Y" is the number of unsaturations. For example, palmitic acid is a 16-carbon FA with no unsaturation and it is designated by 16:0. Palmitoleic acid is a 16-carbon FA with one site of unsaturation, and is designated by 16:1. Additionally, the FAME is designated by the same numerical symbol.

The different of the carbon chain length and degree of unsaturation are strongly influenced on the biodiesel properties. Saturated FA gives higher cetane number and cloud point, whereas unsaturated FAs have lower cetane number and cloud point and also easy to oxidize. Therefore, a suitable composition of FA in oils will be give desirable biodiesel properties [Pinto, *et al.*, 2005].

Copyright by Mahidol University

Table 3.1 Fatty acid compositions in vegetable oils.

Vegetable oil	Fatty acid composition (% w/w)						
	Palmitic C16:0	Palmitoleic 16:1	Stearic C18:0	Oleic C18:1	Linoleic C18:2	Linolenic C18:3	Other
Soybean	13.9	0.3	2.1	23.2	56.2	4.3	-
Sunflower	6.4	0.1	2.9	17.7	72.9	-	-
Palm	42.6	0.3	4.4	40.5	10.1	0.2	1.1
Rice barn	15.0	-	1.9	42.5	39.1	1.1	0.7
Castor	1.1	-	3.1	4.9	1.3	-	89.6
Olive	5.0	0.3	1.6	74.7	17.6	-	0.8
Coconut	7.8	0.1	3.0	4.4	0.8	-	65.7
Cottonseed	28.7	-	0.9	13.0	57.4	-	-
Rapeseed	3.5	-	0.9	64.1	22.3	8.2	-
Jatropha	16.0	3.5	6.0	42.0	34.4	0.8	0.7

Table 3.2 Characteristics of common FAs in oils and their methyl esters.

Fatty acid ***Methyl ester***	**Formula**	**Molecular weight**	**Boiling point (°C)**	**Melting point (°C)**
Palmitic acid	$C_{16}H_{32}O_2$	256.43	352	63.0
Methyl palmitate	$C_{17}H_{34}O_2$	270.46	185	30.5
Palmitoleic acid	$C_{16}H_{30}O_2$	254.41	162	33.0
Methyl palmitoleate	$C_{17}H_{32}O_2$	268.44	180	-0.1
Stearic acid	$C_{18}H_{36}O_2$	284.48	383	70.0
Methyl stearate	$C_{19}H_{38}O_2$	298.51	181	39.0
Oleic acid	$C_{18}H_{32}O_2$	282.47	286	16.0
Methyl oleate	$C_{19}H_{34}O_2$	296.50	218	-20.0
Linoleic acid	$C_{18}H_{30}O_2$	280.43	229	-5.0
Methyl linoleate	$C_{19}H_{32}O_2$	294.48	192	-35.0

Copyright by Mahidol University

3.3 Source for biodiesel production

More than 100 years ago, vegetable oils have the potential to replace a fraction of the petroleum distillated because vegetable oils have many advantages. For examples, renewability, higher heat content, lower sulfur content, lower aromatic content and biodegradability. But there have many problems when use vegetable oils directly in diesel engines. The problems are high viscosity and lower volatility of the vegetable oil, resulting in decreasing of thermal efficiency and carbon deposits in the engine due to incomplete combustion. However, vegetable oils can be converted by a transesterification (alcoholysis) reaction with methanol into FAME (biodiesel). This method can resolve the problem of high viscosity and increase in combustion efficiency. In the present, the tendency of petroleum prices increases and uncertainties of surrounding petroleum availability. Therefore, vegetable oils have become more attractive recently as primary raw materials used in the production of biodiesel [Arzamendi, *et al.*, 2006]. Each country in various regions utilizes different types of plant oil feedstock depending on their abundance or availability. For examples, using of soybean oil in US and Brazil [Bernard, *et al.*, 1986; Winayanuwattikun, *et al.*, 2008], rapeseed oil and soybean oil in European countries [Rashid, *et al.*, 2008], sunflower oil in Brazil [Rashid, *et al.*, 2008], palm oil and coconut oil in tropical countries [Winayanuwattikun, *et al.*, 2008] and jatropha oil in India [Berchmans, *et al.*, 2008]. The potential feedstocks for the biodiesel production in Thailand are mostly use palm oil because of large amount of agricultural products and lower costs. The selection of a suitable source to produce biodiesel must consider in costs and large production scale [Pinto, *et al.*, 2005]. Soybean oil, sunflower oil, rice barn oil, coconut oil, jatropha oil, used oil and used oil are primarily considered as source of the biodiesel production in Thailand.

Copyright by Mahidol University

3.4 Determination method

Various analytical methods were developed for analyzing mixtures containing fatty acid methyl esters or biodiesel obtained from the transesterification. The standard procedures are chromatographic techniques including HPLC and GC. The chromatographic and the other techniques for monitoring transesterification reaction are given in this section.

3.4.1 High performance liquid chromatography (HPLC)

HPLC is one of the most suitable techniques for the analysis of fats and oils because of high resolution, speed, sensitivity and reproducibility. There are many detectors of HPLC used for detecting FA derivatives such as ultraviolet–visible spectrophotometer (UV-Vis) [Ramadan, *et al.*, 2006], fluorescence detector (FLD) [Fang, *et al.*, 2007], refractive index detector (RID) [Kadam, *et al.*, 2006], mass spectrometer (MS) [Rezanka, *et al.*, 2007] and evaporative light scattering detector (ELSD) [Isbell, *et al.*, 2008]. The HPLC instruments are summarized in Table 3.3. A general advantage of HPLC compared with GC is that usually time and reagent consuming derivatizations are not necessary, thereby reducing analysis times.

3.4.2 Gas chromatography (GC)

Generally, GC has been the most widely used method for the analysis of biodiesel or FAMEs because of high resolution, sensitivity, precision and rapid technique. The most reports of common used of gas chromatographic detectors for FAME analysis are included flame ionization detector (FID) and mass spectrometer (MS). The GC instruments are summarized in Table 3.4.

Copyright by Mahidol University

Table 3.3 Analytical technique for the determination of FAMEs using HPLC.

Instrument	Condition		Sample	References
	Column	Mobile phase		
HPLC-UV	Li Chrospher-Si 60, (250×4 mm i.d., 5 µm particle size)	Isooctane/ethyl acetate (96:4, v/v) at 1.0 mL/min and detection at 295 nm	Crude seed oil	Ramadan, *et al.*, 2006
	Hypersil ODS (250×4.0 mm i.d., 5 µm particle size)	93% of MeOH in H_2O at 1.0 mL/min and detection at 246 nm	Soil	Sitthirakan, 2007
HPLC-RID	Hypersil ODS (250×4.6 mm i.d., 5 µm particle size)	Acetone/ acetonitrile/MeOH (50:50:10, v/v/v) with a gradient elution at 30 °C	Rice barn oil	Kadam, *et al.*, 2006
	A Spheri-5, C-18 (220×4.6 mm i.d., 5 µm particle size)	MeOH (1.0 mL/min) at temperature 40 °C	Pongamia pinnata oil	Meher, *et al.*, 2006
HPLC-FLD	Eclipse XDB-C8 (150×4.6 mm i.d., 5 µm particle size)	50% acetonitrile and 100% acetonitrile, emission wavelengths at 250 nm	Free fatty acids	Fang, *et al.*, 2007
HPLC APCI-MS	Hichrom HIRPB-250 AM (250×2.1 mm i.d., 5 µm particle size)	Gradient elution acetonitrile/ propionitrile/ dichloromethane (60:30:10, v/v/v) at 0.37 mL/min	Ximenia oil	Rezanka, *et al.*, 2007
HPLC-ELSD	Rainin (250×4.6 mm i.d., 5 µm particle size)	Hexane/acetone 50:50 at 1.0 mL/min	Lesquerella seeds	Isbell, *et al.*, 2008

Copyright by Mahidol University

Table 3.4 Analytical technique for the determination of FAMEs using GC-FID.

Instrument	Conditions		Sample	References
	Column	GC conditions		
GC-FID	MFE-1000	N_2(1.8 mL/min); split ratio (50:1); oven temperature, 80°C, 1 min →(5°C/min)→230°C, 10 min; injector temperature, 290°C and detector temperature, 300°C	Cider	Blanco, *et al.*, 2001
	Omega wax 250	He(103.4 kPa); oven temperature, 50°C, 2 min→(4°C/min)→220°C, 35 min; injector temperature, 250°C and detector temperature 260°C	Fish oil and cod liver oil	Indarti, *et al.*, 2005
	A FFAP–CB	He(2.0 mL/min); oven temperature, 70°C→(15°C/min) →200°C→(8°C/min)→250°C; injector temperature, 250°C and detector temperature was 260°C	Olive mill waste waters	Procida, *et al.*, 2006
	A BPX-5	H_2(11.0 mL/min); oven temperature, 220°C→(5°C/min) →320°C, 10 min; injector and detector were 320°C	Mixture of FAs (C24:0–C36:0)	Antoln, *et al.*, 2008
	HP-5	He(1.0 mL/min); oven temperature, 60°C, 2 min →(20°C/min)→180°C, 0.5 min →(5°C/min)→240°C, 10 min; injector temperature, 250°C and detector temperature, 280°C	Culture bacteria and soil	Chaidacho, 2008
GC-MS	Fused Silica	He(1.0 mL/min); oven temperature, 50°C→(4°C/min) →290°C; injector temperature, 290°C and detector temperature, 300°C	Crude wax sunflower and seed oil	Kanya, *et al.*, 2007
	HP Innowax	He(2.7 mL/min); split ratio, (40:1); 60°C, 3 min→(10°C/min) →185°C, 1 min→(5°C/min)→ 200°C, 10 min→(5°C/min)→ 220°C, 20 min; ion source temperature, 280°C	Sorghum seed oils	Mehmood, *et al.*, 2008

Copyright by Mahidol University

Flame ionization detection (FID) is the most sensitive GC detector for hydrocarbon compounds. FID has a high sensitivity, high stability, excellent resolution capabilities and has linear response [Eder, 1995; Shantha, 1992]. FID consists of a hydrogen/air flame and a collector plate. The analyst molecules can be ionized when the effluent from the GC column passes through the flame to produce ions. The ions are collected at the jet and cylindrical electrode to produce an electrical signal. The current is amplified and sent to a recorder or to the converter of a computer data.

MS has high resolution of separation with selective and sensitive detection. This detection is a powerful technique for diagnostic fragmentation. There was used for analysis of geometric and positional isomers of FAs from diagnostic fragmentation of saturated and unsaturated FAs [Brondz, 2002; Pinto, *et al.*, 2005]. The individual compounds are eluted from the GC column and entered to the electron ionization generator where the vapor phase can be ionized with electron into fragment. The fragments ions are moved through a magnetic or electrostatic field and separated according to their mass to charge ratio (*m/z*).

3.4.3 Gel permeation chromatography (GPC)

GPC is a method for simultaneous analysis of transesterification reaction products such as ethyl esters, mono-, di- and triglycerides and glycerol. The common detector of GPC for detecting compounds is refractive index detector [Arzamendi, *et al.*, 2006; Darnoko, *et al.*, 2000].

3.4.4 ^{1}H-Nuclear magnetic resonance (^{1}H-NMR)

The determination of the yield of transesterification reaction or percentage of methyl esters can be quantified form ^{1}H-NMR spectrum. The yield was investigated from the signal of methylene protons and methoxy protons [Jin, *et al.*, 2007; Meher, *et al.*, 2006].

Copyright by Mahidol University

3.5 FAME preparation/Biodiesel production

Chemically, biodiesel is referred to mono alkyl esters of long chain fatty acids or FAME derived from vegetable oil or animal fats via a transesterification process. In general, FAME is a form used as a volatile derivative for the determination of FA. The most reports of analysis of FA or FAME is gas chromatographic analysis which is required volatize compound for the analysis. Therefore, the derivatization of FA to FAME as volatize derivatives is needed. The FAMEs preparations which are explained in the following section are performed by transesterification and esterification reaction.

3.5.1 Transesterification reaction

Transesterification or alcoholysis is the reaction of triglycerides with alcohols to produce ester and glycerol as a by product. A catalyst is usually used to improve the reaction rate and yield. The common catalysts are alkaline or acidic catalysts. The alkaline catalyst is used such as NaOH, KOH and $NaOCH_3$. The reaction requires excess of alcohols to increase the efficiency of the transesterification reaction. The general equation of transesterification reaction is presented in Figure 3.2 [Meher, *et al.*, 2006]. The triglyceride is converted stepwise to diglyceride, monoglyceride and finally glycerol, respectively. Individual reaction step produces one mol of ester. The three steps of the transesterification reaction are shown in Figure 3.3 [Om, *et al.*, 2008].

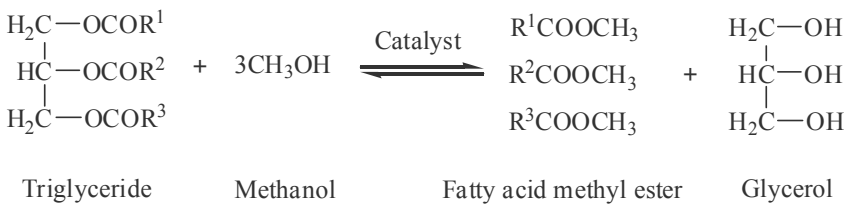


Figure 3.2 The equation for transesterification of triglyceride.

Copyright by Mahidol University

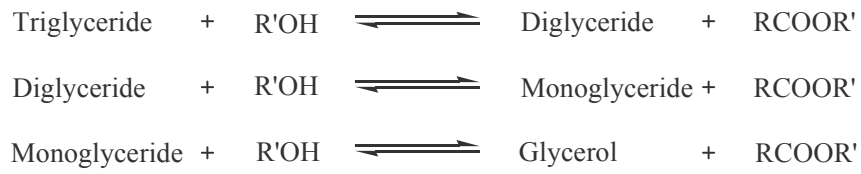


Figure 3.3 The three steps of transesterification reaction of triglyceride.

3.5.1.1 Acid-catalyzed transesterification

The transesterification can be catalyzed by acids such as HCl, H_2SO_4, BF_3, H_3PO_4, and organic sulfonic acids. When acid catalyst is used to catalyze the reaction, the direct esterification of the FFA occurs simultaneously with the transesterification reaction. The esterification reaction is the reaction of FFA with alcohols to produce FAME and water as a by product. The direct esterification reaction is important in increasing of the biodiesel product. The general equation of esterification reaction is presented in Figure 3.4 [Marchetti, *et al.*, 2008].

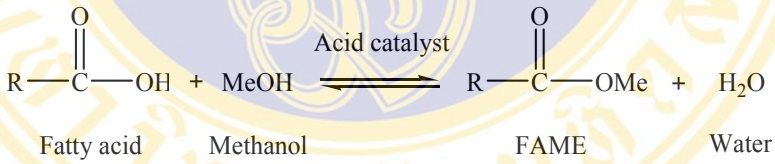


Figure 3.4 The equation for esterification reaction of FFA with methanol.

The transesterification reaction using acid catalyst are very slow, requiring long time more than the alkaline catalysis, also needs more extreme temperature and pressure condition for complete the reaction [Furuta, *et al.*, 2004; Marchetti, *et al.*, 2007; Pinto, *et al.*, 2005]. Usually, the acid catalyst reaction is applied in the oil with high FFA content to increase FAME products. The acid catalyzed transterification conditions are summarized in Table 3.5. The mechanism of acid catalyzed transesterification of vegetable oil or triglyceride is shown in Figure 3.5 [Meher, *et al.*, 2006].

Copyright by Mahidol University

Table 3.5 The acid-catalyzed transterification conditions for FAME preparation.

Sample	Catalyst (%w/w)	MeOH : oil	Reaction condition	% Conversion	References
Lipids	25.0% BF_3	55 %	100 °C; 4-5 h	-	William, *et al.*, 1964
Soybean oil	1.0% H_2SO_4	30:1	65 °C; 50 h	90.00	Freedman, *et al.*, 1984
Canola oil	3.0% H_2SO_4	30:1	65 °C; 24 h	90.00	
Olive oil	1.0% H_2SO_4	30:1	67 °C; 45 h	95.00	Aksoy, *et al.*, 1988
Adipose tissue	5.0% HCl	-	70 °C; 45 min	-	Carrapiso, *et al.*, 2000
Waste cooking oil	3.0% H_2SO_4	50:1	80 °C; 4 h	97.00	Zhang, *et al.*, 2003
Refined soybean oil	0.5% H_2SO_4	9:1	100 °C; 24 h	98.00	Goff, *et al.*, 2004

Copyright by Mahidol University

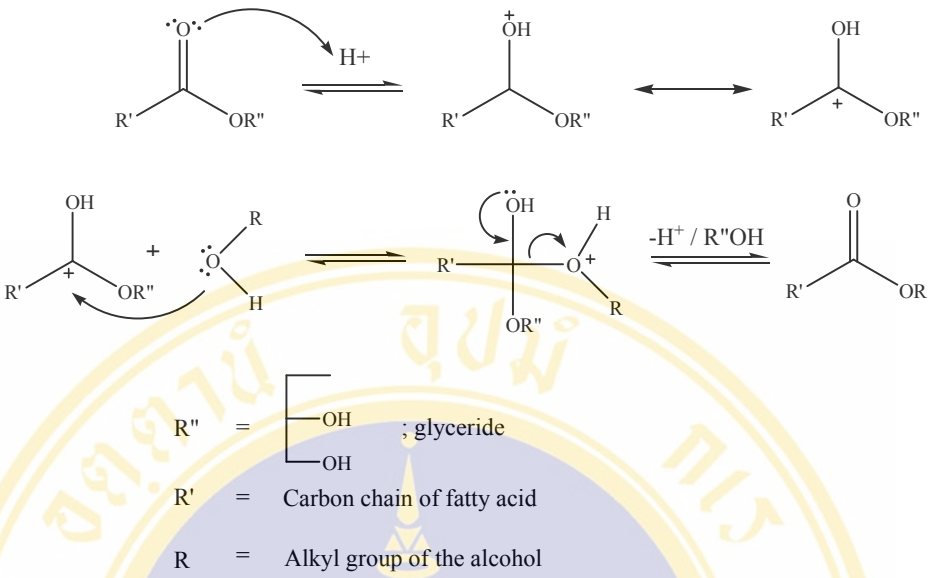


Figure 3.5 The mechanism of acid-catalyzed transesterification reaction.

3.5.1.2 Base-catalyzed transesterification

Biodiesel is currently produced by the transesterification using base catalysts because the reaction is faster than acid catalyzed transesterification reaction. In addition, base catalysts are less corrosive than acid catalyst. The most common basic catalysts are NaOH, KOH, $NaOCH_3$, Na_2CO_3 and K_2CO_3 [Pinto, *et al.*, 2005]. The base-catalyzed transterification conditions are summarized in Table 3.6. The mechanism of base catalyzed transesterification of vegetable oil or triglyceride is shown in Figure 3.6.

Copyright by Mahidol University

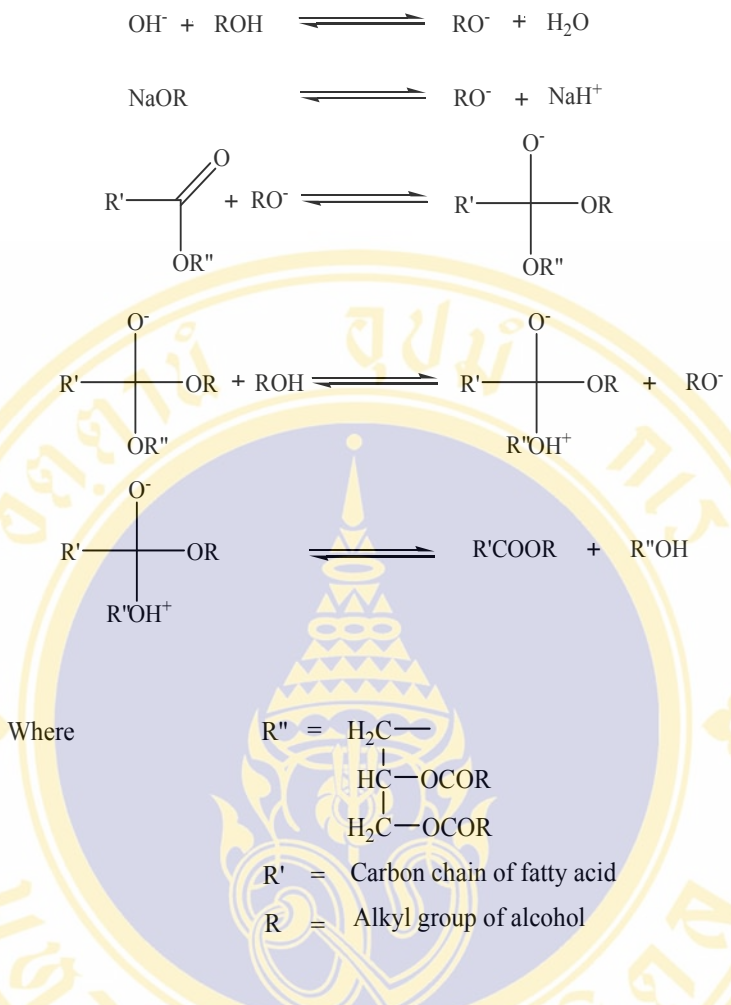


Figure 3.6 The mechanism of base-catalyzed transesterification reaction.

The problem of FAME preparation when used base-catalyst transesterification with high FFA contents in oil is the formation of soap form saponification of FFAs with base catalyst. The soap prevents the separation of the glycerin and FAME fraction. Therefore, the two-step method was applied in the oil with high FFA contents to resolve this problem [Ozbay, *et al.*, 2008]. The two-step methods for FAME preparation are given in section 3.5.4.

Copyright by Mahidol University

Table 3.6 The base-catalyzed transterification conditions for FAME preparation.

Sample	Catalyst (% w/w)	MeOH: oil	Reaction condition	% Conversion	References
Soybean oil	0.2% NaOH	6:1	70 °C; 90 min	90.00	Noureddini, *et al.*, 1997
Palm oil	1.0% KOH	6:1	65 °C; 60 min	82.00	Darnoko, *et al.*, 2000
Sun-flower oil	1.0% KOH	6:1	65 °C; 4 h	90.10	Vicente, *et al.*, 2004
Palm oil	1.5% NaOH	6:1	65 °C; 1 h	94.40	Kusdiana, *et al.*, 2004
Crude pongamia oil	1.0% KOH	10:1	60 °C; 1.5 h	92.00	Karmee, *et al.*, 2005
Used frying oil	1.1% NaOH	7:1	60 °C; 15 min	88.80	Leung, *et al.*, 2006
Karanja oil	1.5% KOH	6:1	65 °C; 3 h	98.00	Meher, *et al.*, 2006
Used frying oil	1.0% KOH	12:1	60 °C; 2 h	96.40	Encinar, *et al.*, 2007
Used oil	0.5% NaOH	9:1	60 °C; 1 h	78.00	Cayll, *et al.*, 2008

Copyright by Mahidol University

3.5.2 Saponification reaction

The problem of soap formation was observed during the base catalyzed transesterification reaction with high FFA contents in vegetable oil. Figure 3.7 is saponification reaction of FFAs with base catalyst. This reaction affects on the neutralization of one part of the base catalyst so no longer available for catalyze the transesterification and give less FAME product. Therefore, the process to reduce FFA content is required for high FFAs in feed stock for biodiesel production.

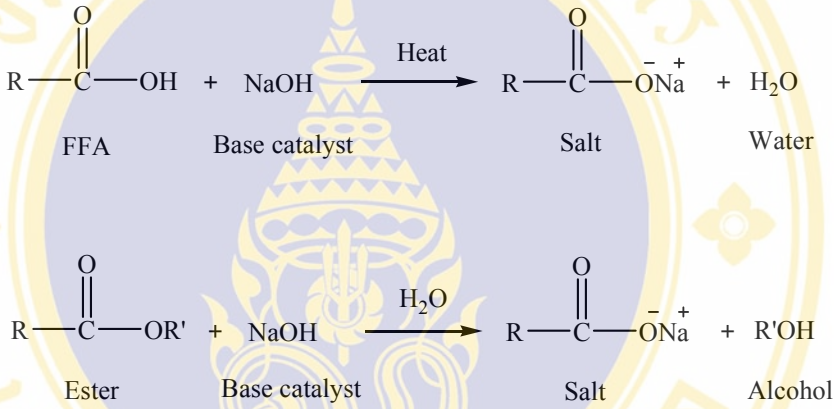


Figure 3.7 The saponification reaction of FFA and fatty acid alkyl ester with base catalyst.

3.5.3 Esterification reaction

This process can be used as pretreatment step which converts FFAs with high FFA content in the oil to FAME. This step can reduce the soap formation from the oil and also increase FAME yield. Therefore, this reaction was included in the two-step method to produce biodiesel. Then, low FFAs in the pretreated oil can be transesterified with an alkali catalyst by converting triglycerides to methyl esters [Gerpen, 2005].

Copyright by Mahidol University

3.5.4 Two-step method of FAME preparation

FAMEs can also be prepared by the two-step method which consist of acid-base and base-acid method. This two-step method can be employed for the oil with high FFA contents.

3.5.4.1 Acid-base catalyzed method

This method is usually employed in feedstock with high FFA contents. In the first step, the esterification or pretreatment step is applied in oil with high FFA contents. Result in FFA contents is reduced by using acid catalyst. Then, base-catalyzed transesterification reaction can be applied in the second step to produce FAMEs from triglyceride. The summary reactions of acid-base catalyzed methods are illustrated in Figure 3.8. The acid-base catalyzed conditions from some literature are summarized in Table 3.7.

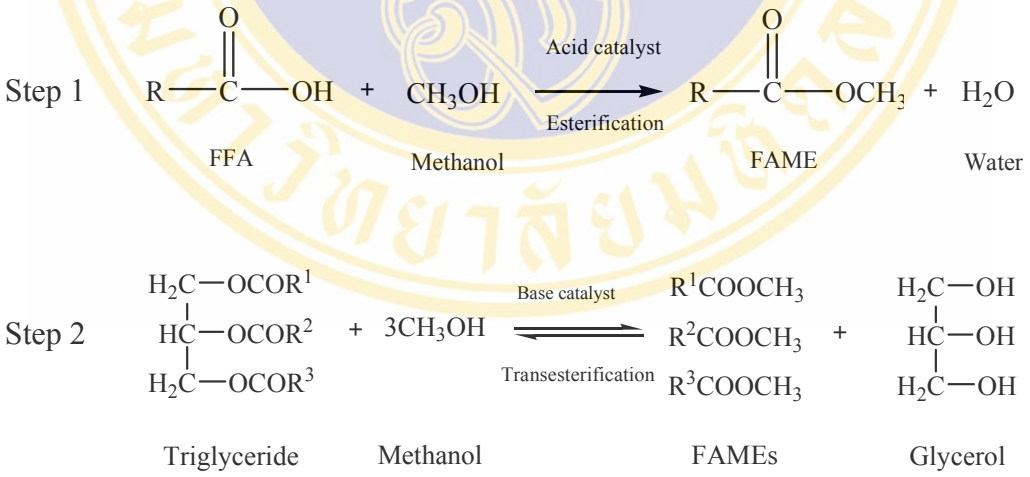


Figure 3.8 The acid-base catalyzed reaction for FAME preparation.

Copyright by Mahidol University

Table 3.7 The acid-base catalyzed reaction conditions for FAME preparation.

Sample	Catalyst (% w/w)	MeOH : oil	Reaction condition	% Conversion	References
Mahua oil	1.0% H_2SO_4	0.35 (% v/v)	60 °C; 2 h		Ghadge, *et al.*, 2005
	0.7% KOH	0.25 (% v/v)	60 °C; 30 min	98.00	
Waste cooking oils	2.0% $Fe_2(SO_4)_3$	10:1	95 °C; 4 h	97.22	Wang, *et al.*, 2005
	1.0% KOH	6:1	65 °C; 1 h	97.02	
Palm fatty acid distillate	1.83% H_2SO_4	8:1	70 °C; 1 h		Chongkhong, *et al.*, 2007
	0.4 M NaOH	8:1	65 °C; 15 min	99.48	
Salmon oil	1.0% H_2SO_4	9:1	65 °C; 1 h		Hamed, *et al.*, 2008
	0.8% KOH	4.7:1	52 °C; 30 min	97.60	
Pongamia pinnata oil	0.5% H_2SO_4	6:1	65 °C; 15 min		Naik, *et al.*, 2008
	1.0% KOH	6:1	65 °C; 3 h	96.60	
Jatropha curcas seed oil	1.0% H_2SO_4	0.60 (% w/w)	50 °C; 1 h		Berchmans, *et al.*, 2008
	1.4% NaOH	0.24 (% w/w)	65 °C; 2 h	90.00	

Copyright by Mahidol University

3.5.4.2 Base-acid catalyzed method

This method is usually applied for the lipid analysis. In the first step, the FFAs and triglycerides are saponified by base catalyst to form salt of ester or soap. Then, the esterification of soap is employed in the second step to convert salt of ester to become FAMEs. The summary reaction of base-acid catalyzed method is illustrated in Figure 3.9. The base-acid catalyzed conditions for FAME preparation are summarized in Table 3.8.

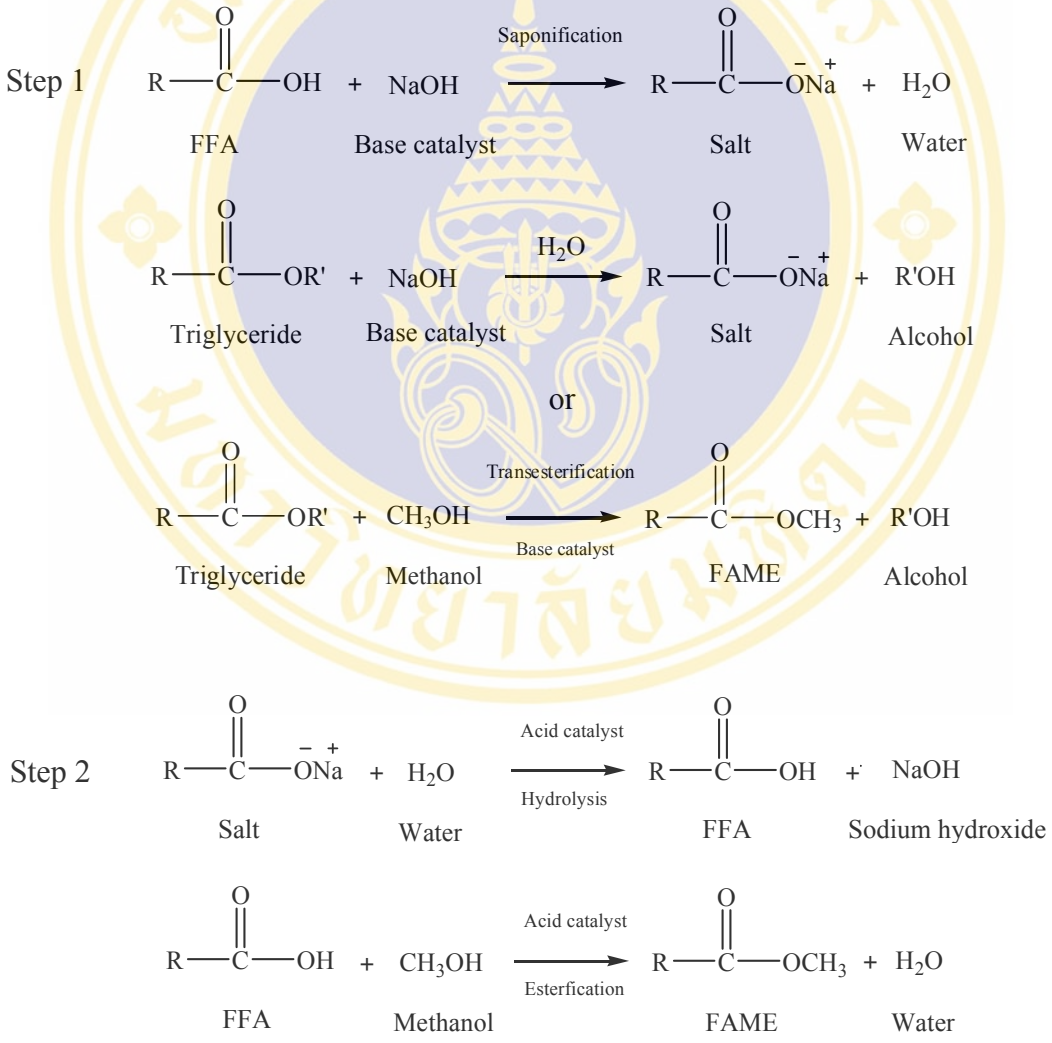


Figure 3.9 The base-acid catalyzed reaction for FAME preparation.

Copyright by Mahidol University

Table 3.8 The base-acid catalyzed reaction conditions for FAME preparation.

Sample	Reaction temperature (°C)	Catalyst	Reaction time	References
Menhaden oil	reflux	0.5 N NaOH	1 h	Vorbeck, *et al.*, 1961
		5.0% HCl	25 min	
Lipids	reflux	0.5% w/w $NaOCH_3$	5 min	Metcalfe, *et al.*, 1966
		BF_3/CH_3OH (3mL)	2 min	
Fat	reflux	0.5 N $NaOCH_3$	5 min	Van, *et al.*, 2001
		14.0% v/v BF_3	2 min	
Cider	60	0.5 M NaOH	30 min	Blanco, *et al.*, 2001
		BF_3/CH_3OH (5 mL)	20 min	
Low-fat milk and plain milk	95	10.0 mg K_2CO_3	30 min	Lu, *et al.*, 2002
		1.0 M H_2SO_4	30 s	
Cod liver oil	100	0.5 M NaOH	5 min	Carvalho, *et al.*, 2005
		12.0% v/v BF_3	30 min	

Copyright by Mahidol University

CHAPTER IV
MATERIALS AND METHODS

The description of instruments, operating conditions used for the determination of fatty acid methyl ester, chemical reagents, materials, procedures for preparation of reagents, standard solutions and samples are presented in this chapter.

4.1 Instrumentation

4.1.1 Gas chromatography

A GC, model GC-14B (Shimadzu, Japan) equipped with split/splitless injector and a flame ionization detector (FID) was used. The detector output was recorded by the PowerChrom system. The optimum GC conditions for the analysis of FAMEs were illustrated in section 5.1.4.

4.1.2 Vortex

A vortex-2 Genie, model G-560E with speed controlling unit (New York, USA) was used for preparation of standard solutions and samples.

4.1.3 Heating block

A heating block, model Denver Instrument was used to heat chemicals in the derivatization of standard FAs and samples.

Copyright by Mahidol University

4.1.4 Micropipette

A micropipette, model Eppendorf Model Research (Hamburg, Germany) was used for preparation of standard solutions and samples.

4.1.5 Analytical balance

Analytical balance, model Sartorius CP 225D from Germany was used to weigh samples and chemicals in the preparation of standard solutions and samples.

4.1.6 Hot plate

A hotplate, model Barnstead/Thermolyne USA was used to heat chemicals in the preparation of standard solutions.

4.1.7 Stirrer

A stirrer, model Heidolph MR 3001 was used to mix chemicals in the preparation of standard solutions.

4.2 Chemical reagents and materials

All chemical reagents and materials used in this work are detailed in Table 4.1.

Copyright by Mahidol University

Table 4.1 List of chemical reagents, formula and suppliers.

Chemicals	Formula	Supplier
Methyl oleate	$C_{19}H_{36}O_2$	Aldrich, U.S.A.
Methyl orange	$C_{14}H_{14}N_3NaO_3S$	
Methyl palmitate	$C_{17}H_{34}O_2$	
Phenophthalein	$C_{20}H_{14}O_4$	
Boron trifluoride	BF_3	BDH, England
Palmitic acid	$C_{16}H_{32}O_2$	
Sodium thiosulfate	$Na_2S_2O_3$	
Acetic acid	CH_3COOH	E. Merck, Germany
Barium hydroxide	$Ba(OH)_2$	
Hydrochloric acid	HCl	
Iodine chloride	ICl	
Nitric acid	HNO_3	
Potassium hydrogen phthalate	$KHC_8H_4O_4$	
Potassium hydroxide	KOH	
Sodium hydroxide	NaOH	
Methyl heptadodecanoate	$C_{18}H_{36}O_2$	Fluka, Switzerland
Methyl linoleate	$C_{19}H_{32}O_2$	
Methyl palmitoleate	$C_{17}H_{32}O_2$	
Methyl stearate	$C_{19}H_{38}O_2$	
Oleic acid	$C_{18}H_{36}O_2$	
Palmitoleic acid	$C_{16}H_{30}O_2$	
Sodium hydrogen carbonate	$NaHCO_3$	
Stearic acid	$C_{18}H_{36}O_2$	
Ethanol	CH_3CH_2OH	Lab Scan, Ireland
Hexane	C_6H_{14}	
Methanol	CH_3OH	
Sulfuric acid	H_2SO_4	
Margaric acid	$C_{17}H_{34}O_2$	Sigma-Aldrich, Germany

Copyright by Mahidol University

4.3 Preparation of reagents and solutions

4.3.1 Standard fatty acid solution

Standard solutions of 1000 mg/L palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2) were prepared by dissolving 10.00 mg of pure compounds and made up final volume to 10.00 mL in a volumetric flask with hexane to give a concentration of 1000 mg/L. These stock solutions were stored in dark glass bottles and kept at 4 °C.

4.3.2 Internal standard solution

Standard solution of 1000 mg/L margaric acid and methyl heptadecanoate (C17:0) was prepared by dissolving 10.00 mg of pure compound and made up final volume to 10.00 mL in a volumetric flask with hexane. This solution was stored in a dark glass bottle and kept at 4 °C. The stock solution was diluted to give a suitable working solution for preparation of the internal calibration standard and for oil samples.

4.3.3 Standard fatty acid methyl ester solution

Standard solution of 1000 mg/L of methyl palmitate (C16:0), methyl palmitoleate (C16:1), methyl stearate (C18:0), methyl oleate (C18:1) and methyl linoleate (C18:2) were prepared by dissolving 10.00 mg of a pure compounds and made up final volume to 10.00 mL in a volumetric flask with hexane to give a concentration of 1000 mg/L. These stock solutions were stored in dark glass bottles and kept at 4 °C. The FAME solution was diluted to give a suitable working solution of the calibration curve. The working solutions were prepared by diluting standard solutions of FAMEs (1000 µg/mL) in a 10.00 mL volumetric flask and making up volume with hexane to give the concentration of 5 µg/mL.

Copyright by Mahidol University

4.3.4 Sulfuric acid in methanol solution

Aliquot of 0.25, 1.25, 2.50, 3.75 and 5.00 mL of concentrated H_2SO_4 were diluted with MeOH for a final volume of 25.00 mL to obtain the concentration of 1, 5, 10, 15 and 20% (v/v), respectively.

4.3.5 Sodium hydroxide in methanol solution

Sodium methoxide solution prepared by dissolving 1.00 g of NaOH in MeOH 25.00 mL to give a concentration of 1.0 M.

4.3.6 Potassium hydroxide in methanol solution

Potassium methoxide solution was prepared by dissolving 1.40 g of KOH in MeOH 25.00 mL to give a concentration of 1.0 M.

4.3.7 Potassium hydroxide solution for acid value

Potassium hydroxide solution of 0.1 M was prepared by dissolving 6.0 g of KOH to 1000 mL of distilled water. Then, the mixture was boiled for 10 minutes with stirring and added 2.0 g of reagent grade $Ba(OH)_2$. The mixture was boiled additionally about 5-10 minutes and cooled down to room temperature. Then, the mixture was filtered through glass funnel and stored in an alkali resistant bottle protecting CO_2. Potassium hydroxide solution was standardized by primary standard potassium hydrogen phthalate using phenolphthalein as indicator.

4.3.8 Hydrochloric acid solution for saponification value

HCl of 0.5 M was prepared by adding 42.00 mL of HCl and made up volume to 1000 mL

Copyright by Mahidol University

4.3.9 Saturated sodium hydrogen carbonated solution

$NaHCO_3$ was dissolved in deionized water until it cannot dissolve to give a saturated concentration of $NaHCO_3$ and stored in a plastic bottle at room temperature.

4.3.10 Glassware cleaning

All glassware, pipette tips, vials and other materials were carefully cleaned by washing with liquid detergent to remove dust or particulate remaining and then soaking in 10% HNO_3 solution overnight. Then, glassware was rinsed with deionized water and left to dry in an oven.

4.4 Procedures and methods

4.4.1 Characterization of vegetable oils

Characterization of vegetable oils is summarized in Table 4.2.

Table 4.2 Summary of standard method for basic properties of biodiesel.

Properties	Unit	Standard method
Acid value	mg KOH/g	A.O.C.S., 1989
Saponification value	mg KOH/g	A.O.C.S., 1989
Iodine value	g I_2/100 g	A.O.C.S. Official Method Cd 3d-63
% FFA	%	A.O.C.S., 1989

4.4.1.1 Acid value (AV)

The acid value was determined by acid-base titration technique (A.O.C.S., 1989). In a typical procedure, the mixture solvent of toluene and isopropanol (1:1) was neutralized by a standard KOH solution of 0.1 M to a faint

Copyright by Mahidol University

permanent pink color of phenolphthalein. The sample of 2.0 g was weighed into an Erlenmeyer flask and added 125.00 mL of the neutralized solvent mixture. The sample was vigorously shaken while titrated with standard KOH of 0.1 M to the permanent pink color. The color must persist for 30 seconds. The acid value is calculated by following equation:

$$\text{Acid value} = [(A - B) \times N \times \text{MW of KOH}]/W \quad (4.1)$$

Where; A = Volume of standard alkali used to titrate sample (mL)
B = Volume of standard alkali used to titrate blank (mL)
N = Concentration of standard alkali (Normal)
W= Weight of sample (g)

4.4.1.2 Saponification value (SV)

The saponification value was determined by acid-base titration technique (A.O.C.S., 1989). The sample about 2 g ± 0.1 mg was weighed into the Erlenmeyer flask and added 25.00 mL of toluene. Then, the alcoholic KOH of 50.00 mL was added. The conduct blank was prepared similarly in all respects. The mixture solution was completely saponified by reflux about 1 hour. Then, phenolphthalein about 1.00 mL was added and the mixture was titrated with HCl of 0.5 N until the pink color just disappears. The volume of 0.5 N HCl for the titration was recorded. The saponification value is calculated by following equation:

$$\text{Saponification value} = [(B - S) \times (N)]/ W \times \text{MW of KOH} \quad (4.2)$$

Where; B = Volume of 0.5 N HCl required to titrate blank (mL)
S = Volume of 0.5 N HCl required to titrate sample (mL)
N = Concentration of HCl solution (Normal)
W= Weight of sample (g)

Copyright by Mahidol University

4.4.1.3 Free fatty acid measurement

Percent FFA in vegetable oil was measured by titration method according to the procedure as describe in acid value (A.O.C.S., 1989). Percent free fatty acid (% FFA) is calculated by following equation:

$$\% \text{ FFA} = \frac{\text{volume of base solution} \times \text{base concentration} \times \text{MW of FFA}}{\text{weight of oil}} \quad (4.3)$$

Where; MW = Molecular weight (g/mol)

4.4.1.4 Molecular weight

Molecular weight of vegetable oils (triglyceride) can be calculated from saponification value (amount of alkali necessary to saponify a definite quality of the sample). A substraction of saponification value and acid value refers to the amount of KOH for saponification of a triglyceride and free fatty acid molecules, respectively. Three molars of KOH are equal to one molar of triglyceride. This value can be converted to a molecular weight of vegetable oil. Summary method for calculation of molecular weight of triglyceride is shown as following:

KOH for saponification of a triglyceride = SV - AV

3 mol of KOH = mol of triglyceride

$$\text{MW of triglyceride} = \frac{\text{weight of oil}}{\text{mol of triglyceride}}$$

Where; SV = Saponification value (mg KOH/g oil)

AV = Acid value (mg KOH/g oil)

MW = Molecular weight (g/mol)

Copyright by Mahidol University

4.4.1.5 Iodine value

Iodine values of the oil were determined by Wijs (cyclohexane-acetic acid solvent) method. In a typical procedure, Wijs solution of 25.00 mL was prepared by dissolving 16.5 g of ICl in 1.00 L of acetic acid. The sample about 1.50 g was weighed into conical flask and added 15.00 mL of 1:1 (v/v) cyclohexane-acetic solvent to dissolve the sample. The mixture was stirred for 1 hour and stored the flasks in a dark place at 25 ± 5 °C for the duration of the reaction. The flasks was removed from the dark place. Then, 20.00 mL of 15.0% (w/v) KI solution and 150.00 mL of H_2O were added. The mixture was titrated with 0.1 N standard $Na_2S_2O_3$ solution. Titration was performed until yellow color has almost disappeared and added 0.5% (w/v) about 1.00-2.00 mL of soluble starch indicator solution. Then, the solution was titrated until blue color has just disappeared. The iodine value is calculated by following equation:

$$\text{Iodine value} = \frac{(\text{mL of } Na_2S_2O_3 \text{ for blank - mL of } Na_2S_2O_3 \text{ for sample}) \times N \text{ of } Na_2S_2O_3 \times 0.127 \text{ g/meq} \times 100}{\text{weight of samples (g)}} \quad (4.4)$$

4.4.1.6 Density

The density of the biodiesel was determined by accurate weight 1.00 mL of FAME. This value can be converted to density of biodiesel by following equation:

$$\text{Density of biodiesel} = \frac{\text{weight of biodiesel (g)}}{\text{volume of biodiesel (mL)}} \quad (4.5)$$

4.4.1.7 Viscosity

The viscosity of biodiesel is calculated by following equation:

$$D = 33.107\ V + 745.39 \quad (4.6)$$

Where; D is the density (g/mL) and V is the viscosity of the biodiesel samples (Kcst).

Copyright by Mahidol University

4.4.2 FAME preparation using acid-catalyzed esterification

Standard solution of FAs (C16:0, C16:1, C18:0, C18:1 and C18:2) 5 mg/L and internal standard (C17:0) 10 mg/L in hexane were used to optimize and develope the method and instrumental studies.

Standard solution 5 μL of 1000 mg/L of FAs (C16:0, C16:1, C18:0, C18:1 and C18:2), and 10 μL of 1000 mg/L of FAs C17:0 were pipetted into screw-capped tube. The hexane layer was dried under N_2 gas. Solution of acid catalyst 10% (v/v) such as H_2SO_4, HCl, BF_3 and HNO_3 in MeOH, 1.00 mL was added. The mixture solutions were performed in a heating block at 50 ^oC for 10 minutes. Then, the solution was cooled down to room temperature and 1.00 mL of saturated $NaHCO_3$ was added to neutralize the reagent. FAMEs products were then removed by extracting with 1.00 mL hexane and repeated the step within 3 times. The organic layer was carefully transferred to a test tube and evaporated under N_2-stream. The FAME residue fraction was dissolved in 1.00 mL of hexane prior to GC injection. The final concentration of each FAMEs were 5 mg/L for optimizing the method.

4.4.3 FAME preparation using base-catalyzed transesterification

Vegetable oil 500 μL (0.44 g) was pipetted into a screw-capped tube. NaOH of 1.0 M and MeOH were added. The mixture solutions were performed in a heating block at 60 °C for 30 minutes and stirred vigorously in a vortex for 10 seconds every 5 minutes. The solution was cooled down to room temperature. FAMEs products were then removed by extracting with 1.00 mL hexane repeatable 3 times. The organic layer was separated into a test tube. Then, 1.00 mL of H_2O was added to wash the catalyst and the phases were allowed to separate. The organic layer was carefully transferred to a test tube and evaporated under N_2-stream. The FAME residue fraction was weighed and diluted to 10,000 times with hexane. Methyl heptadodecanoate (C17:0) of 10 μL was added as internal standard prior to GC injection.

Copyright by Mahidol University

4.4.4 Two-step method for FAME preparation

4.4.4.1 Acid-base catalyzed method

Vegetable oil 500 μL (0.44 g) was pipetted into a screw-capped tube. In the first step, 1.00 mL of 10% (v/v) H_2SO_4 in MeOH was added. The mixture solutions were performed in a heating block at 50°C for 10 minutes. The solution was cooled down to room temperature. The oil fraction was removed by extracting with 1.00 mL hexane repeatable 3 times and 1.00 mL of H_2O was added. The organic layer was separated into a screw-capped tube for dryness under N_2-stream. Then, followed by transesterification process in the second step, 0.1 M NaOH and MeOH were added. The mixture solutions were heated in a heating block at 60 °C for 30 minutes and stirred vigorously in a vortex for 10 seconds every 5 minutes. The solution was cooled down to room temperature. The FAME products were extracted by 1.00 mL of hexane repeatable 3 times. The organic layer was separated into a test tube. Then, 1.00 mL of H_2O was added to wash the catalyst and the phases were allowed to separate. The organic layer was carefully transferred to a test tube and evaporated under N_2-stream. The FAME residue fraction was weighed and diluted to 10,000 times with hexane. Methyl heptadodecanoate (C17:0) of 10 μL was added as internal standard prior to GC injection.

4.4.4.2 Base-acid catalyzed method

Vegetable oil 500 μL (0.44 g) was pipetted into a screw-capped tube. In the first step, 1.0 M NaOH and MeOH were added. The mixture solutions were performed in a heating block at 60 °C for 30 minutes and stirred vigorously in a vortex for 10 seconds every 5 minutes. The solution was cooled down to room temperature. Then, 1.00 mL of 10% (v/v) H_2SO_4 in MeOH was added. The mixture solutions were heated in a heating block at 50 °C for 10 minutes and stirred vigorously in a vortex for 10 seconds every 5 minutes. The solution was cooled down to room temperature and then FAME products were extracted by 1.00 mL of hexane with 3 times. The organic layer was separated into a test tube. Then, 1.00 mL of H_2O was added to wash the

Copyright by Mahidol University

catalyst and the phases were allowed to separate. The organic layer was carefully transferred to a test tube and evaporated under N_2-stream. The FAME residue fraction was weighed and diluted to 10,000 times with hexane. Methyl heptadodecanoate (C17:0) of 10 μL was added as internal standard prior to GC injection.

4.5 Analytical performance of gas chromatography

The analytical performance was investigated in term of a detection limit and a linearity range. The experiments were carried out in triplicate of the performance and the GC analysis. Variation of values was shown as percentage relative standard deviation (% RSD) value.

4.5.1 Linearity range

The standard mixtures of FAMEs were prepared in the range of concentrations between 0.5-100 mg/L. The linear ranges of studied compound were obtained by plotting peak area versus the concentration. Correlation coefficient value (r^2) was used to be reliable the linear ranges.

4.5.2 Detection limit

In this work, the limit of detection was determined at the ratio of peak height to noise at the level of three. This value refers to lowest concentration of an analyte that can be detected in this studied condition.

4.5.3 Data analysis

The statistical test of the methods for determining FAMEs were performed using *t*-test (paired two samples for mean) at 95% confidence interval.

Copyright by Mahidol University

4.5.4 Confirmation of the method

4.5.4.1 Calculation of the conversion from GC-FID

The conversion of vegetable oil to FAME can be calculated from the content of methyl esters analyzed by GC with the following equation [Kim, *et al.*, 2004]:

$$\text{Conversion (\%)} = \frac{(\text{weight of biodiesel product}/\text{MW of biodiesel}) \times \text{biodiesel concentration}}{(\text{weight of oil}/\text{MW of oil}) \times 3 \times 100} \quad (4.7)$$

The conversion from GC-FID in this work is implied to the percent yield of the reaction.

4.5.4.2 Calculation of the conversion from ^{1}H-NMR

The conversion of vegetable oil to FAME can be calculated from the integration values (I) of the selected signals in ^{1}H-NMR by following the equation [Knothe, 2000]:

$$\text{Conversion (\%)} = \frac{5 \times I_{ME}}{(5 \times I_{ME}) + (9 \times I_{TAG})} \times 100 \quad (4.8)$$

Where; I_{ME} and I_{TAG} are the integration values of the methyl ester and the glyceridic protons, respectively.

Copyright by Mahidol University

CHAPTER V
RESULTS AND DISCUSSION

In this chapter, optimization of the GC-FID conditions and appropriately optimized conditions for the preparation of FAMEs are described in the following section.

5.1 Chromatographic studies

The study of the optimum GC conditions for detection and determination of FAMEs are detailed in the following section (column temperature, injection temperature and split ratio). The mixture solution of 10 mg/L FAMEs in hexane was injected to study these parameters.

5.1.1 Effect of column temperature

The optimum column temperature depends on boiling point of the sample, but it must not higher than limit of the column temperature (260 °C for DB-Wax). If use higher temperature, the organic coating in the column will be lost, leading to shorten of the usage and reduce the efficiency. The column temperature was investigated by fixing injection volume (1 μL), injection temperature (at 250 °C) and detector temperature (at 250 °C). The effects of varying column temperature on peak area, peak height and chromatograms are shown in Figure 5.1-5.2. Retention times and resolution of FAMEs obtained from varying column temperature are shown in Table 5.1-5.2. Retention time, peak area and resolution decreased with the increasing of column temperature. Considering of good resolution and short times for the analysis, the optimum column temperature at 210 °C was chosen for the study.

Copyright by Mahidol University

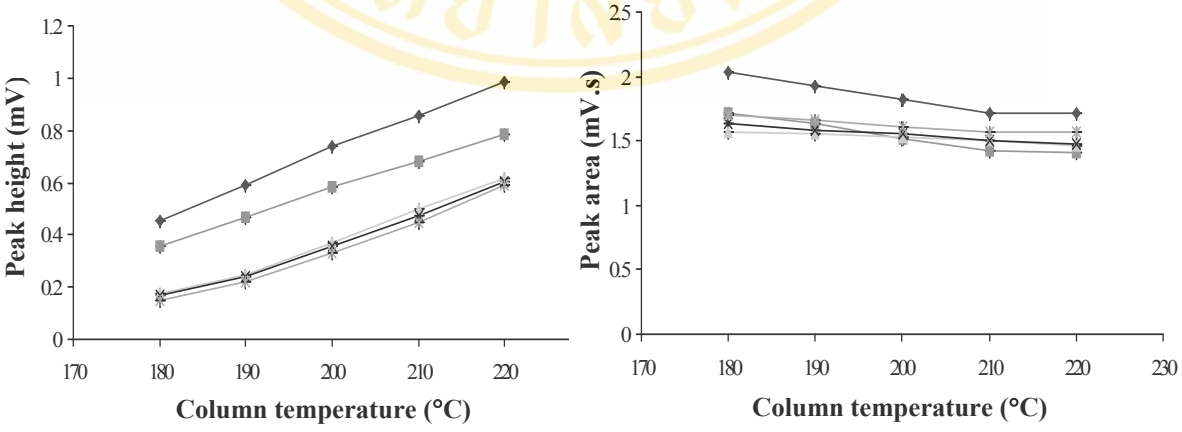
Table 5.1 Retention times of FAMEs obtained from varying column temperatures.

Column temp °C)	Retention time (minutes)				
	C16:0	C16:1	C18:0	C18:1	C18:2
180	3.70	3.98	6.44	6.84	7.86
190	2.95	3.15	4.75	5.03	5.72
200	2.46	2.60	3.68	3.88	4.34
210	2.13	2.24	2.97	3.12	3.44

S.D. values of the retention time for all compounds were less than 0.01 and RSD values were lower than 0.3%. Therefore, the variation of retention time in every injection was very low and the retention time of the analyte was acceptable for the quantitative analysis.

Table 5.2 Resolution of FAMEs obtained from varying column temperatures.

Column temp °C)	Resolution (mean ± SD)				
	C16:0	C16:1	C18:0	C18:1	C18:2
180	2.15 ± 0.08	5.80 ± 0.15	8.13 ± 0.12	1.62 ± 0.06	3.75 ± 0.10
190	1.98 ± 0.04	4.90 ± 0.16	7.15 ± 0.09	1.59 ± 0.03	3.60 ± 0.08
200	1.77 ± 0.02	4.02 ± 0.05	6.10 ± 0.09	1.51 ± 0.05	3.23 ± 0.04
210	1.58 ± 0.05	3.31 ± 0.03	5.27 ± 0.05	1.46 ± 0.03	2.84 ± 0.04



(◆) C16:0, (■) C16:1, (▲) C18:0, (×) C18:1 and (*) C18:2

Figure 5.1 Effect of column temperature of FAME on peak height (mV) and peak area (mV.s).

Copyright by Mahidol University

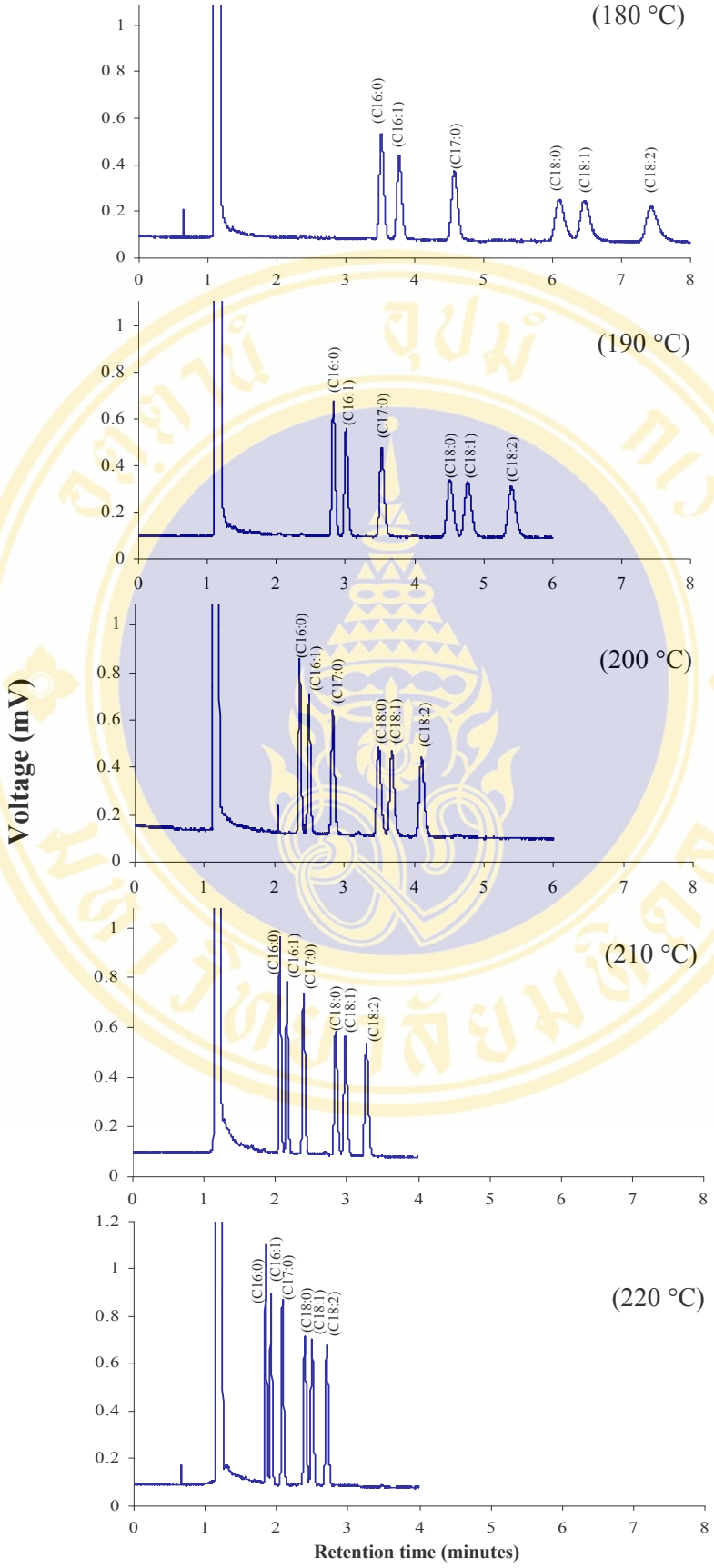
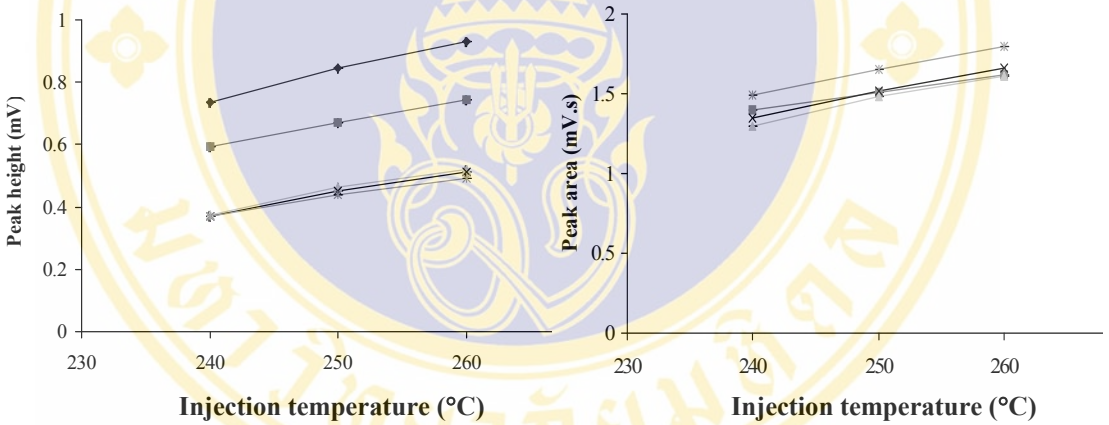


Figure 5.2 Chromatograms of FAMEs (10 mg/L) at various column temperatures.

Copyright by Mahidol University

5.1.2 Effect of injection temperature

The injector may be set at the same temperature or higher than the column temperature. The injection temperature was investigated in the range of 240-260 °C. The column temperature was an isothermal fixed at 210 °C. The effects of varying injection temperatures on peak area, peak height and chromatograms are shown in Figure 5.3-5.4, respectively. Retention times and resolution of FAMEs obtained from varying injection temperature are shown in Table 5.3-5.4. The injection temperature should be high enough to rapidly vaporize the sample, not decompose the sample and not higher than the limit of column temperature (260 °C), therefore the optimum injection temperature at 250 °C was chosen for the study.



(◆) C16:0, (■) C16:1, (▲) C18:0, (×) C18:1 and (*) C18:2

Figure 5.3 Effect of injection temperature for FAMEs on peak height (mV) and peak area (mV.s).

Table 5.3 Retention times of FAMEs obtained from varying injection temperatures.

Injection temp (°C)	Retention time (minutes)				
	C16:0	C16:1	C18:0	C18:1	C18:2
240	2.15	2.25	2.99	3.13	3.45
250	2.14	2.24	2.98	3.12	3.44
260	2.14	2.24	2.98	3.12	3.44

Copyright by Mahidol University

Table 5.4 Resolution of FAMEs obtained from varying injection temperatures.

Column temp °C)	Resolution (mean ± SD)				
	C16:0	C16:1	C18:0	C18:1	C18:2
240	1.48 ± 0.04	3.18 ± 0.09	5.12 ± 0.11	1.38 ± 0.05	2.78 ± 0.08
250	1.56 ± 0.02	3.34 ± 0.02	5.39 ± 0.01	1.46 ± 0.02	2.93 ± 0.02
260	1.61 ± 0.01	3.43 ± 0.05	5.57 ± 0.08	1.52 ± 0.01	3.01 ± 0.02

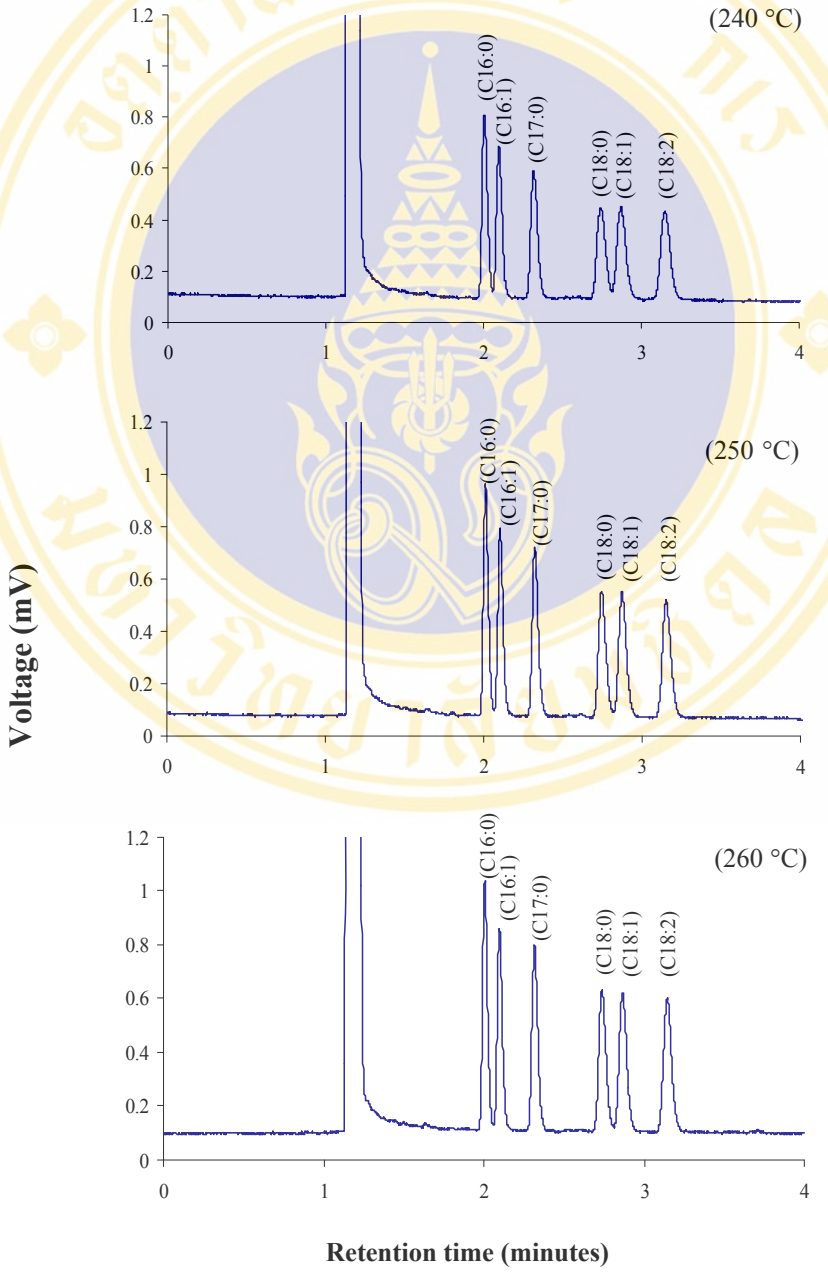
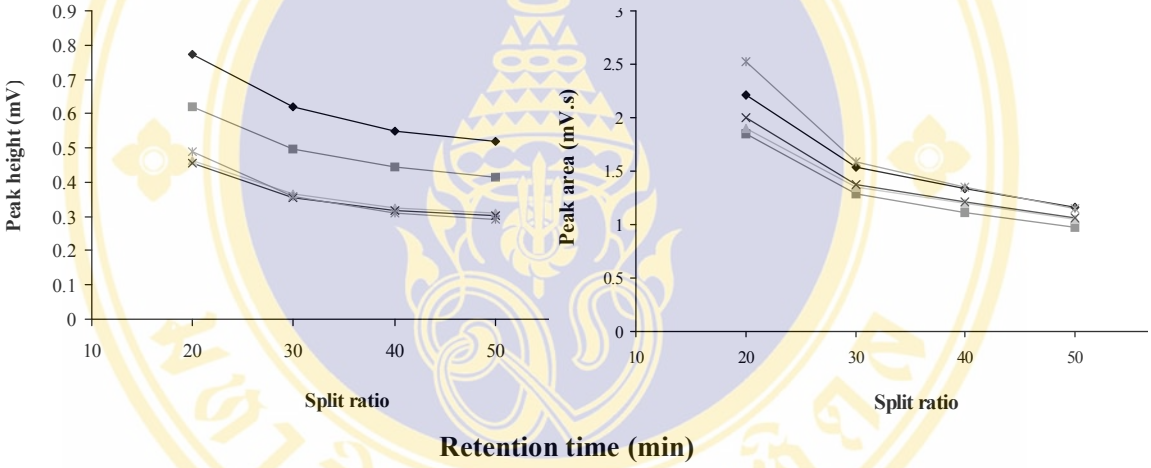


Figure 5.4 Chromatograms of FAMEs (10 mg/L) at various injection temperatures.

Copyright by Mahidol University

5.1.3 Effect of split ratio

The amount of sample vapor that introduces into the column depends on the split ratio. The low split ratio refers to high amount of sample are introduced into the column. In this section, the split ratio was varied from 20:1-50:1. The effects of varying split ratios on peak area, peak height and chromatograms are illustrated in Figure 5.5-5.6, respectively. Retention times and resolution of FAMEs obtained from varying split ratio are shown in Table 5.5-5.6. High of sensitivity and resolution of FAME therefore, the optimum split ratio of 40:1 was chosen.



(◆) C16:0, (■) C16:1, (▲) C18:0, (×) C18:1 and (*) C18:2

Figure 5.5 Effect of split ratio for FAMEs on peak height (mV) and peak area (mV.s).

Table 5.5 Retention times of FAMEs obtained from varying split ratios.

Split ratio	Retention time (minutes)				
	C16:0	C16:1	C18:0	C18:1	C18:2
20:1	2.14	2.25	2.98	3.12	3.44
30:1	2.14	2.25	2.98	3.12	3.43
40:1	2.14	2.24	2.97	3.12	3.43
50:1	2.14	2.24	2.97	3.12	3.43

Copyright by Mahidol University

Table 5.6 Resolution of FAMEs obtained from varying split ratios.

Split ratio	Resolution (mean ± SD)				
	C16:0	C16:1	C18:0	C18:1	C18:2
20:1	1.34 ± 0.03	2.67 ± 0.04	4.50 ± 0.05	1.30 ± 0.03	2.41 ± 0.02
30:1	1.55 ± 0.03	3.17 ± 0.05	5.13 ± 0.09	1.43 ± 0.02	2.77 ± 0.07
40:1	1.58 ± 0.03	3.37 ± 0.08	5.40 ± 0.05	1.46 ± 0.01	2.94 ± 0.06
50:1	1.65 ± 0.02	3.53 ± 0.02	5.62 ± 0.05	1.54 ± 0.01	3.06 ± 0.04

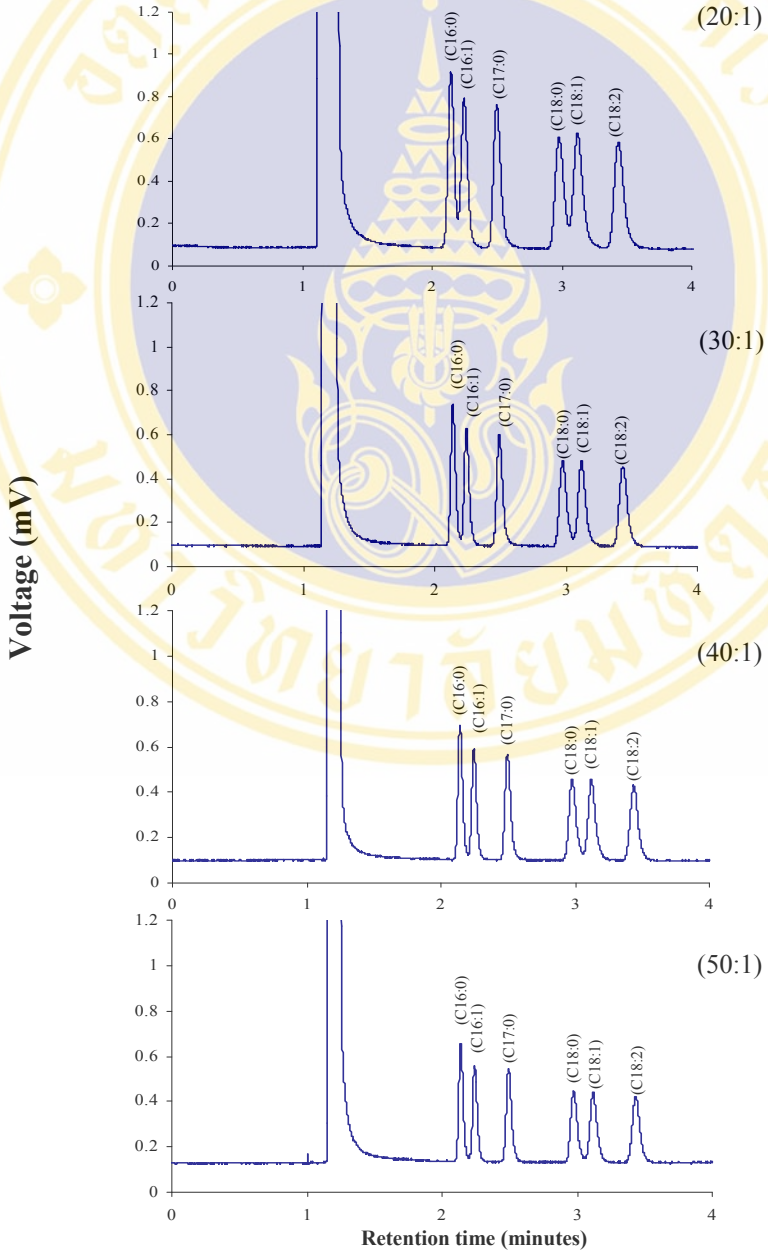


Figure 5.6 Chromatograms of FAMEs (10 mg/L) at various split ratios.

Copyright by Mahidol University

5.1.4 GC conditions

The optimum GC conditions for the analysis of FAMEs are illustrated in Table 5.7. The chromatogram and chromatographic data of FAMEs are illustrated in Figure 5.7 and Table 5.8, respectively.

Table 5.7 The optimum condition of the GC-FID.

Parameters	Conditions
Column	DB-WAX capillary column (30 m × 0.32 mm i.d., 0.25 µm film thicknesses, polyethylene glycol)
Oven temperature	210 °C
Injection port	split mode (40:1)
Injector temperature	250 °C
Detector temperature	250 °C
Injection volume	1 µL
Carrier gas	He (125 kPa)
Make up gas	N_2 (100 kPa)
Fuel gas	H_2 (70 kPa)
Oxidant gas	Air (50 kPa)
Carrier gas flow rate	0.5 mL/min

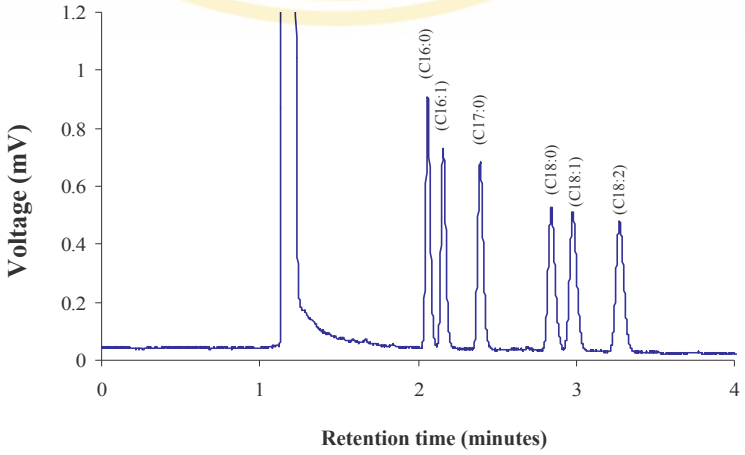


Figure 5.7 Chromatogram of FAMEs (10 mg/L) C16:0, C16:1, C18:0, C18:1 and C18:2 from the optimum GC-FID condition.

Copyright by Mahidol University

Table 5.8 Chromatographic data of FAMEs using the optimum condition.

FAME	Retention time (min)	Peak width at half height (min)	Peak asymmetry	Resolution
Methyl palmitate ($C16:0\text{-}CH_3$)	2.01	0.07	1.31	-
Methyl palmitoleate ($C16:1\text{-}CH_3$)	2.10	0.07	1.37	1.55
Methyl heptadodecanoate ($C17:0\text{-}CH_3$)	2.31	0.09	1.48	3.28
Methyl stearate ($C18:0\text{-}CH_3$)	2.73	0.11	1.33	5.27
Methyl oleate ($C18:1\text{-}CH_3$)	2.86	0.11	1.47	1.42
Methyl linoleate ($C18:2\text{-}CH_3$)	3.13	0.13	1.40	2.87

5.1.5 Analytical performance

Method validation of the GC was investigated to confirm the method performance for determination of FAMEs. All validation parameters were evaluated using the optimum GC-FID condition as given in the section 5.1.4. Results of the analytical performances including linearity, limit of detection and precision are described in the Table 5.9.

The limit of detection was determined using signal-to-noise ratio value at 3 to 1 as described in section 4.5.2. Methyl stearate gave the lowest value of the detection limit. However, limit of detection of all methyl esters were in the same order of magnitude.

The internal calibration curves of FAMEs obtained from the optimum GC conditions are shown in Table 5.9. The linear ranges of all FAMEs were in the range of 0.5-100 mg/L and showed high linearity with high linear regression ($r^2 >$ 0.9990).

Copyright by Mahidol University

Table 5.9 The analytical data of FAME obtained from the optimum condition.

FAMEs	t_R (min)	Calibration equation	r^2	LOD (mg/L)	Linear range (mg/L)	% RSD [(a)]
C16:0	2.01	y = 0.1071x - 0.0804	0.9997	0.30	0.5-100	5.86
C16:1	2.10	y = 0.1054x - 0.0908	0.9998	0.29	0.5-100	1.33
C18:0	2.73	y = 0.0969x - 0.1109	0.9996	0.03	0.5-100	4.77
C18:1	2.86	y = 0.0999x - 0.1147	0.9995	0.28	0.5-100	5.20
C18:2	3.13	y = 0.1065x -0.0636	0.9997	0.19	0.5-100	2.50

[(a)] % RSD = Relative standard deviation (n=10) at the concentration of 0.5 mg/L

5.2 Characterization of vegetable oils

Vegetable oils must be characterized prior to prepare biodiesel because different source of oils will affect the quality of the product. Common characteristics are acid value (AV) and saponification value (SV). The acid value refers to FFA content in the oil and it will directly affect the usage of NaOH/MeOH because of occurring of soap formation. The saponification value refers to total FA in the oil which consists of FFA and FA bonded with triglyceride. This value can be used to calculate molecular weight of oil and to estimate the ratio of methanol to oil for the biodiesel production.

5.2.1 Acid value (AV)

The acid value is a measurement of the FFA content in vegetable oil. Its can be determined by standard methods (A.O.C.S. Official Method Cd 3d-63). The general of vegetable oil have acid value less than 1 mg KOH/g. Used oil has high amount of FFAs leading to show the acid value more than 1 mg KOH/g. The high amount of FFA affects in the FAME preparation. Results of acid value of vegetable oils are also summarized in Table 5.10.

Copyright by Mahidol University

5.2.2 Saponification value (SV)

Saponification value is a measurement of the average molecular weight (or chain length) of all FAs in vegetable oil. Its can be determined by the standard methods (A.O.C.S. Official Method Cd 3d-63). The results of saponification value are also summarized in Table 5.10.

5.2.3 Molecular weight

Molecular weight of vegetable oils (triglyceride) can be calculated by using SV of triglyceride molecules in vegetable oil and acid value of oil. Results of molecular weight of vegetable oils are presented in Table 5.10.

Table 5.10 Common properties of vegetable oils obtained from the experiment.

Vegetable oil	**Saponification value** [(b)] (mg KOH/g)	**Acid value** [(b)] (mg KOH/g)	**% FFA** [(b)]	**Molecular weight of oil** [(b)]
Soybean oil	198.85	0.27	0.16	847.68
Palm oil	192.10	0.96	0.42	880.69
Sunflower oil	198.96	0.27	0.14	847.23
Rice barn oil	194.22	0.55	0.28	869.13
Coconut oil	200.23	0.87	0.47	844.97
Used palm oil	198.59	2.20	1.07	857.11
Jatropha oil	228.75	36.35	18.34	873.32

[(b)] SV, AV, % FFA and MW of vegetable oil determined by the acid-base titration technique (A.O.C.S, 1989)

Copyright by Mahidol University

5.3 Derivatization methods

FAMEs can be produced from both parts of FFA and FA bonding with triglyceride by esterification and transesterification, respectively. FAs and triglyceride are major component of vegetable oil. The reaction conditions of esterification and transesterification were studied to optimize the best condition of the FAME preparation. Adjusted parameters of the reaction were types of catalysts, catalyst concentration, reaction temperature and reaction time.

5.3.1 Esterification

The esterification reaction is the reaction of carboxylic acid with alcohols to produce FAME and water. Summary of esterification reaction is shown as following Figure 5.8.

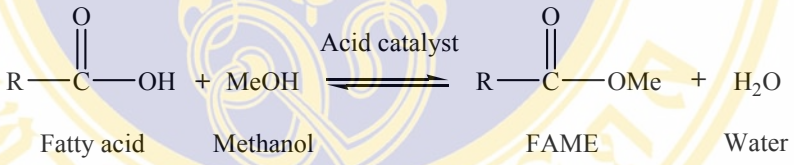


Figure 5.8 The esterification reaction of FA with methanol.

5.3.1.1 Effect of acid catalyst for esterification

Methanolic reagents containing BF_3, H_2SO_4, HCl and HNO_3 with the concentration of 10% (v/v) were compared in the esterification reaction of FAs with methanol using the reaction temperature at 50 °C for 10 minutes. Results of acid catalyst are shown in Figure 5.9 by comparing a ratio of peak area of FAMEs and C17:0 internal standard. The commonly used acid catalysts for esterification are HCl, H_2SO_4 and BF_3 [Liu, 1994]. The results showed no significant difference between H_2SO_4 and BF_3. BF_3 is more toxic and expensive. Therefore, it does not a suitable catalyst. Otherwise, undesirable side reaction probably occurred such as hydrolysis of FAME to FFA. HCl is a very strong acid, low pK_a (pK_a = -8) thus it has high potential

Copyright by Mahidol University

to hydrolyze FA. HNO_3 has higher pK_a value (pK_a = -1.5) than HCl and H_2SO_4, that does not enough to esterify FA. H_2SO_4 has moderate pK_a value (pK_a = -3) which is a better choice of the catalyst in this study. Using H_2SO_4 catalyst in the esterification gave the maximum ratio of peak area. Therefore, H_2SO_4 in MeOH was used for the esterification reaction of FAs.

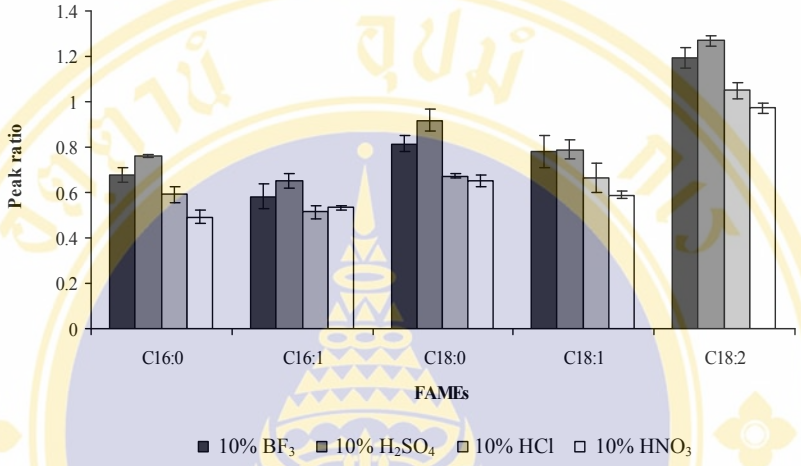


Figure 5.9 Peak ratio of FAMEs and C17:0 on types of acid catalyst.

5.3.1.2 Effect of H_2SO_4 concentration

The concentration of H_2SO_4 was varied in the range of 1-20% (v/v). The reaction was performed at 50 °C for 10 minutes. Results of the H_2SO_4 concentration are shown in Figure 5.10.

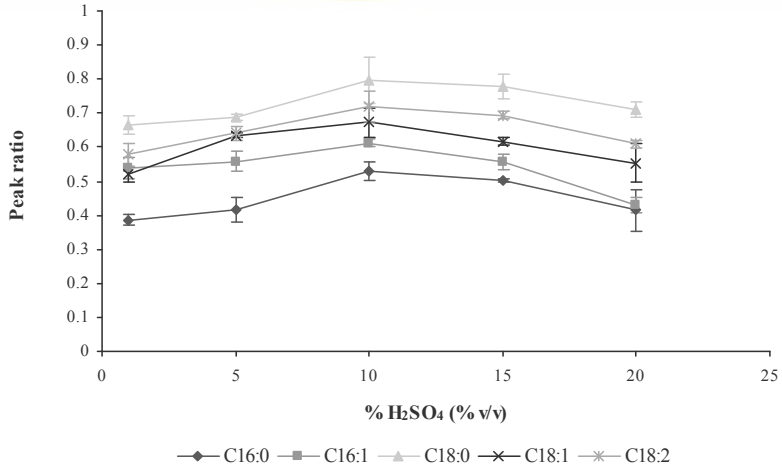


Figure 5.10 Peak ratio of FAMEs and C17:0 on H_2SO_4 concentration.

Copyright by Mahidol University

The peak area of FAMEs increased with the increasing of the concentration of H_2SO_4. However, the excess of acid catalyst can convert FAMEs to FFAs leading to the decreasing of peak area. At higher concentration of H_2SO_4, lower ratio of peak area was observed. The optimum concentration of H_2SO_4 was 10% (v/v).

5.3.1.3 Effect of reaction temperature

In order to study effects of temperature on a ratio of peak area of FAMEs, the reaction temperature was varied from 40-70 °C using the reaction time for 10 minutes. Results of the reaction temperature are presented in Figure 5.11. It was shown that maximum peak areas of FAMEs were obtained at the reaction temperature of 50 °C. Lower reaction temperature (40 °C) gave lower peak area because the reaction was not completed. However, the reaction temperature is limited by the boiling point of methanol (65 °C). Then, higher temperature than 60 °C showed the decreasing of the peak area. The optimum reaction temperature was selected at 50 ºC for the esterification reaction of FA using 10% (v/v) H_2SO_4 in MeOH.

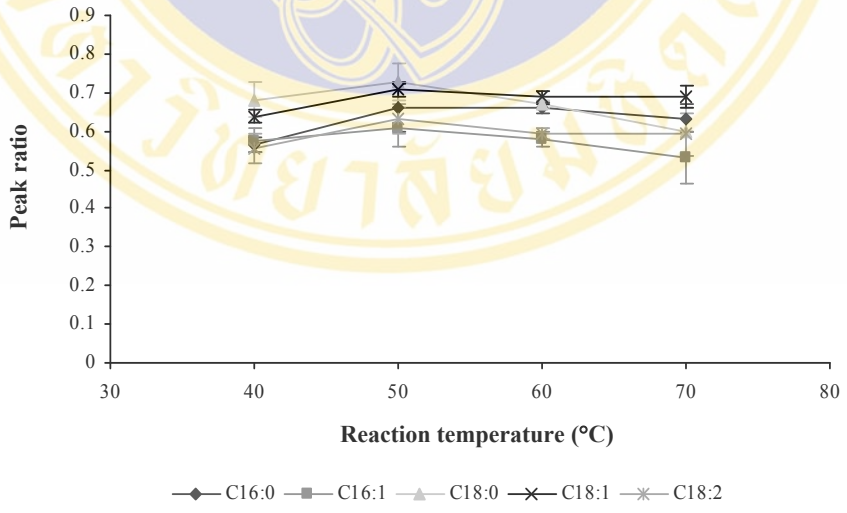


Figure 5.11 Peak ratio of FAMEs and C17:0 on reaction temperature.

Copyright by Mahidol University

5.3.1.4 Effect of reaction time

The reaction time of the esterification was varied from 0-20 minutes by using H_2SO_4 10% (v/v), reaction temperature at 50 °C. Results of the reaction time are shown in Figure 5.12. It was shown that peak areas of FAMEs increased with the increasing of reaction time. Therefore, the optimum reaction time of 10 minute was selected for the esterification reaction.

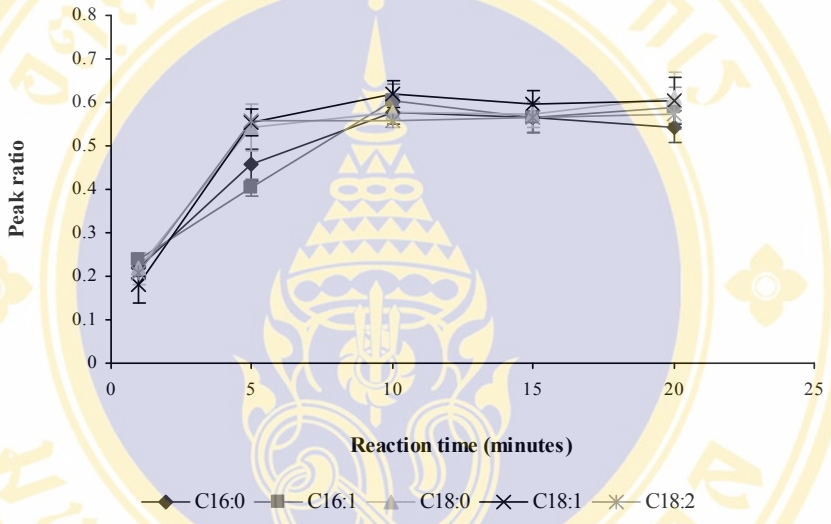


Figure 5.12 Peak ratio of FAMEs and C17:0 on reaction time.

5.3.2 Transesterification

Transesterification is the reaction of oil or triglyceride with an alcohol to form esters and glycerol. Summary of transesterification reaction is shown in Figure 5.13.

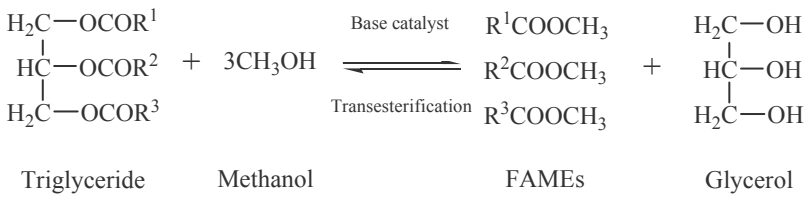


Figure 5.13 The transesterification reaction of triglyceride.

Copyright by Mahidol University

5.3.2.1 Effect of base catalyst for transesterification

NaOH, KOH and $NaOCH_3$ were compared in the transesterification reaction of soybean oil with methanol using 1.0% (w/w) of base catalyst and the reaction temperature was performed at 60 °C for 30 minutes. Results of the catalyst types on the ratio of peak area of FAMEs/C17:0 are shown in Figure 5.14. No significant results of each base were observed in the peak ratio of FAME from the transesterification reaction. The reaction can be performed by using NaOH, KOH and $NaOCH_3$ in methanol for preparing FAMEs from vegetable oils. The base catalyst of NaOH was selected for the transesterfication reaction because of the lowest cost. The product cost will be considered from all variable parameter. So, cheap base is an alternative source of the production.

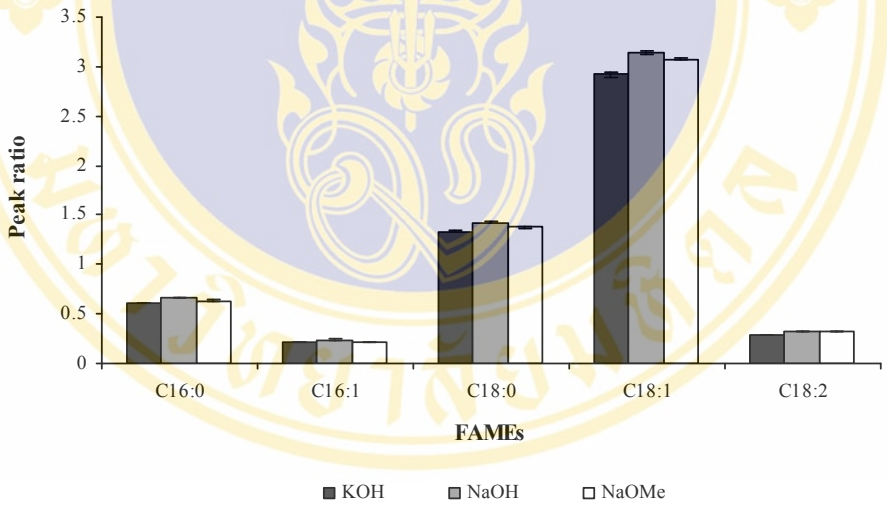


Figure 5.14 Peak ratio of FAMEs and C17:0 on types of acid catalyst.

5.3.2.2 Effect of NaOH concentration

The effect of NaOH concentration on the transesterification of soybean oil was investigated by varying the concentration in the range of 0.1-1.5% (w/w_{oil}). The reaction condition was performed at the reaction time for 30 minutes, molar ratio of methanol to oil of 6:1 and the reaction temperature at 60 ºC. Results of NaOH concentration on the ratio of peak area of FAMEs/C17:0 are shown in Figure

Copyright by Mahidol University

5.15. NaOH of 0.1% (w/w_{oil}) was insufficient to perform the reaction completely by showing the lowest of peak area. Higher soap formation was observed at the higher base concentration, 1.25-1.5% (w/w_{oil}). The optimum concentration of 1.0% (w/w_{oil}) NaOH was selected for the transesterification reaction of vegetable oil.

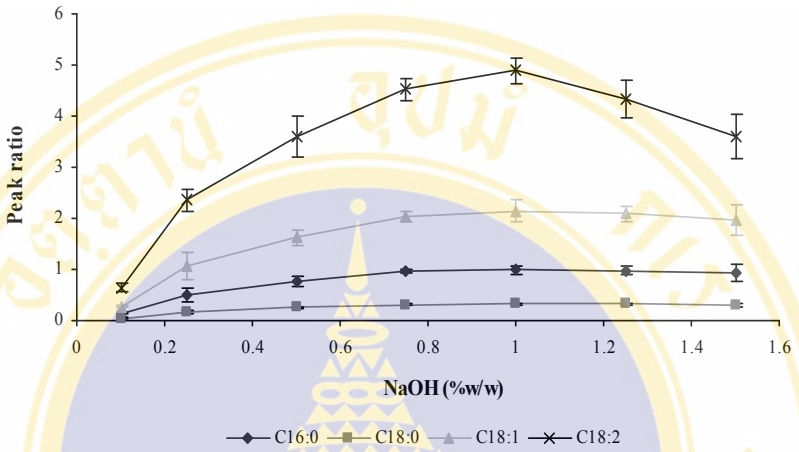


Figure 5.15 Peak ratio of FAMEs and C17:0 on NaOH concentration.

5.3.2.3 Effect of molar ratio of methanol to oil

Molar ratio of alcohol to vegetable oil is one of the most important parameter for the transesterification. Theoretically, the stoichiometric ratio of alcohol to oil for transesterification is 3:1 to produce 3 mol of FAME and 1 mol of glycerol. However, the excess usage of alcohol is required to perform the reaction completely because several factors in the real performance influences on the reaction yield. In this work, molar ratios of MeOH to oil were varied from 4:1 to 10:1. The reaction was performed at the temperature of 60 °C for 30 minutes using 1.0% (w/w) NaOH. The results are shown in Figure 5.16. Lower ratio of peak area was observed at the molar ratio of 4:1 and 5:1 which indicated the incomplete reaction. Moreover, higher molar ratio than 6:1 also affected the separation of glycerol and FAME fraction from the solution. The maximum ratio of peak area was obtained at the molar ratio of 6:1 (MeOH:oil). Therefore, the optimum molar ratio of methanol to oil at 6:1 was selected for the transesterification reaction.

Copyright by Mahidol University

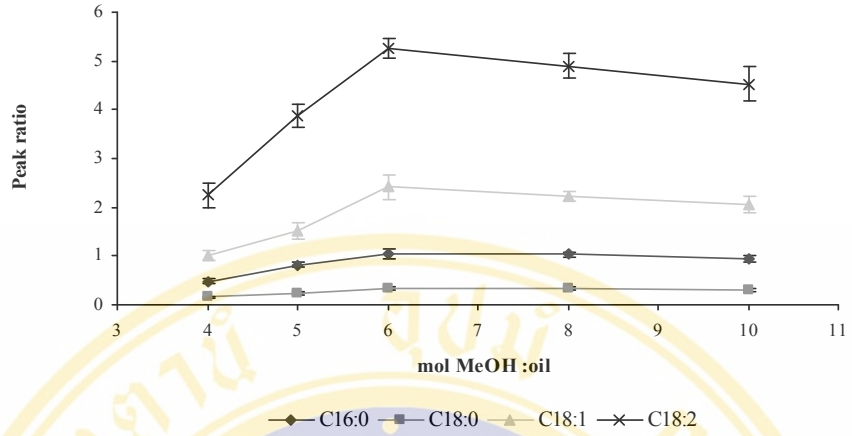


Figure 5.16 Peak ratio of FAMEs and C17:0 on molar ratio of methanol to oil.

5.3.2.4 Effect of reaction temperature

The effect of reaction temperature on the transesterification of soybean oil was investigated by varying the temperature from 40-70 °C. The reaction was performed at the reaction time of 30 minutes, molar ratio of methanol to oil at 6:1 and using 1.0% (w/w) NaOH as catalyst. Results of the reaction temperature are presented in Figure 5.17. The reaction temperature at 60 °C was selected for the reaction.

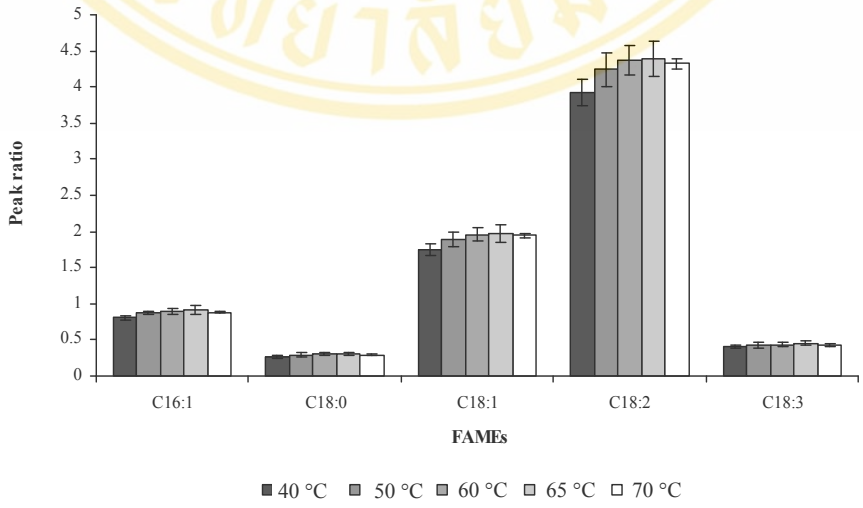


Figure 5.17 Peak ratio of FAMEs and C17:0 on reaction temperature.

Copyright by Mahidol University

5.3.2.5 Effect of reaction time

The reaction time of transesterification was varied from 5-90 minutes. The reaction condition was performed at the reaction temperature of 60 ºC, molar ratio of methanol to oil at 6:1 and using NaOH 1% (w/w) as catalyst. Results of the reaction time are shown in Figure 5.18. Ratio of peak areas of FAMEs increased with the increasing of the reaction time. The optimum reaction time of 30 minutes was selected for the transesterification reaction.

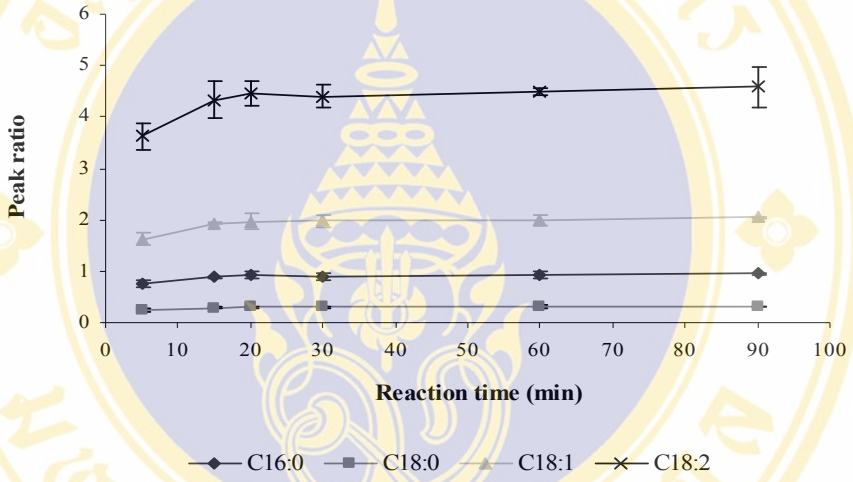


Figure 5.18 Peak ratio of FAMEs and C17:0 on reaction time.

5.3.3 FAME preparation using two-step method

Acid-base and base-acid methods for FAME preparation were operated by using used palm oil with high FFA contents (acid value > 1 mg KOH/g). The results of FAME concentration from the two step methods are shown in Figure 5.19. There were no significantly different between comparing datas at 95% confidence interval ($P > 0.05$, $t_{stat} < t_{critical}$). The comparing data of FAME concentration from the two methods are summarized in Appendix B. Therefore, acid-base and base-acid method for FAME preparation were successfully performed in the used palm oil or acidic oil.

Copyright by Mahidol University

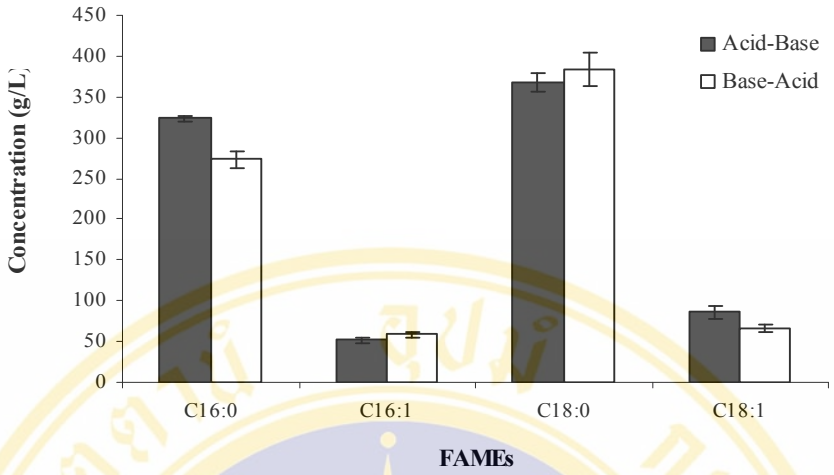


Figure 5.19 Comparison between acid-base and base-acid catalyzed methods of used palm oil.

On the other hand, jatropha oil gave different results in the two-step methods. The base-acid method was produced less FAMEs concentration than the acid-base method. The results are shown in Figure 5.20. The higher amount of FFA contents in jatropha oil produce more water in the reaction. As a result, water contents affected in the two-step method of base-acid method. Then, effect of the water content was studied in the following section.

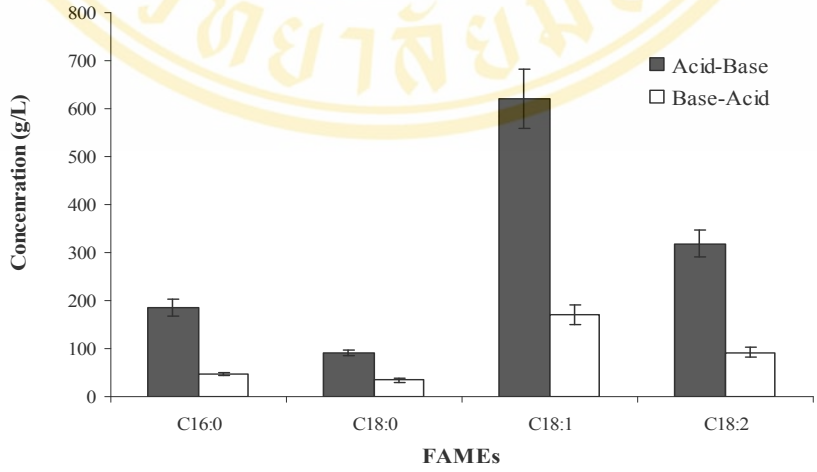


Figure 5.20 Comparison between acid-base and base-acid catalyzed methods of jatropha oil.

Copyright by Mahidol University

In order to study the effect of water content, the reaction was investigated by adding water into the reaction after base catalyzed transesterification. Results are shown in Figure 5.21. High water content will affect in the second step of using acid catalyst due to FAME possibly hydrolyzes to become FFA. This effect occurred more than the FAME produced. Therefore, the more water generated from the saponification reaction in the first step of base catalyzed will affect the reverse reaction in the second step.

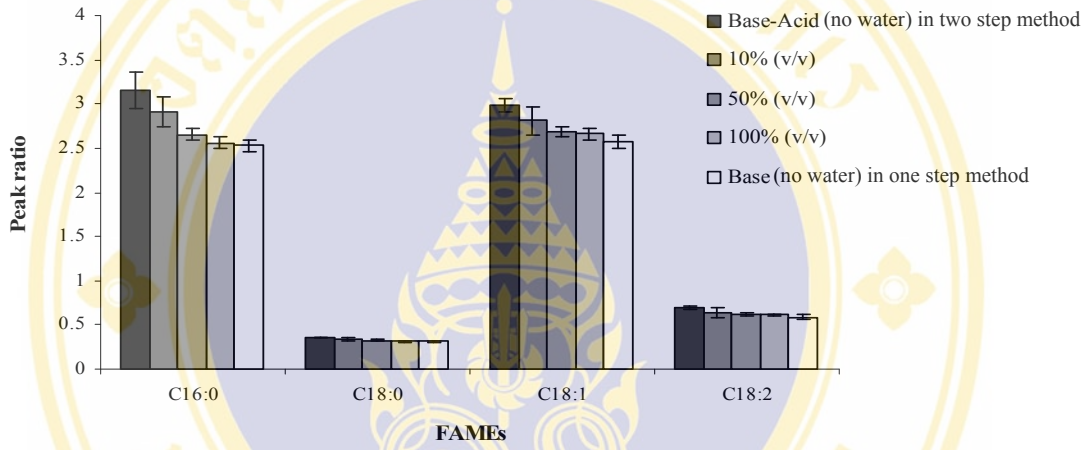


Figure 5.21 Effects of water content on base-acid catalyzed method (peak ratio of FAMEs and C17:0).

5.4 Determination of FAMEs in vegetable oil

5.4.1 Comparison of FAMEs calibration

Comparison results of standard FAME and FAME obtaining from the derivatization of FFAs with are shown in Figure 5.22. The calibration method was performed by using internal standard method. It can be seen that the internal calibration of FAME from two methods are shown no significant difference results at 95% confidence interval ($P > 0.05$, $t_{stat} < t_{critical}$). Statistical test data for comparing standard calibration curves is presented in Table 5.11. The internal calibration of FAME compounds was used for determination of FAME in vegetable oil.

Copyright by Mahidol University

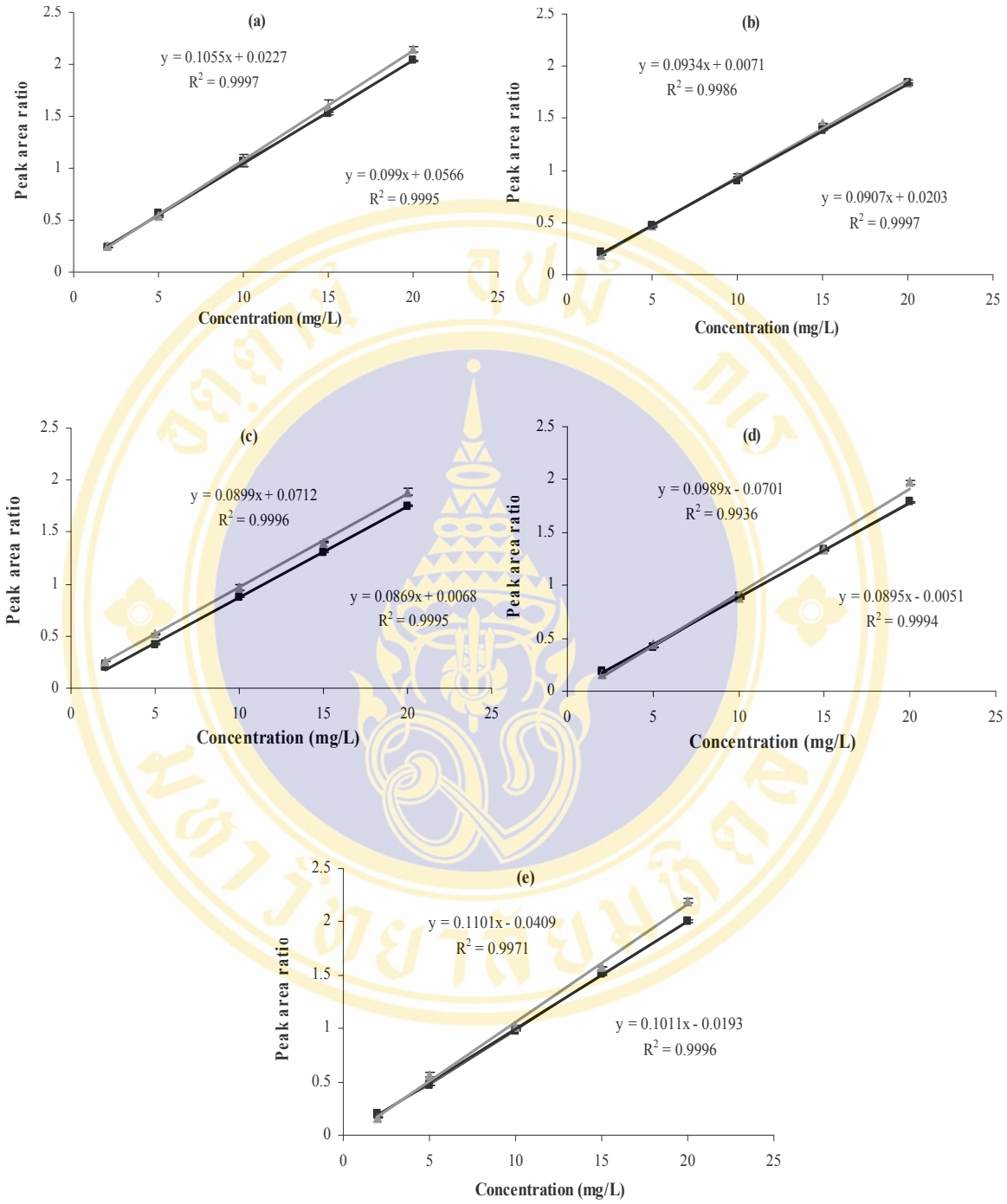


Figure 5.22 Comparison of internal calibration curves of FAMEs from (■) standard FAMEs and (▲) derivatized of FFAs using 10% (v/v) H_2SO_4 in MeOH; (a) C16:0, (b) C16:1, (c) C18:0, (d) C18:1 and (e) C18:2.

Copyright by Mahidol University

Table 5.11 Statistical test data for comparing the internal calibration of FAMEs obtained from standard FAMEs and derivative of FFAs

FAME	*t* Stat	P (T≤t) two-tail	*t* Critical two-tail
C16:0	0.51	0.61	2.05
C16:1	-0.07	0.95	2.05
C18:0	-1.02	0.31	2.05
C18:1	-0.21	0.84	2.05
C18:2	-0.35	0.73	2.05

5.4.2 Calibration of FAs

External calibration curve was performed by preparing standard FAMEs in the same range of FAME concentration in the sample. The major components of FAME in vegetable oils were such as C16:0, C18:0, C18:1 and C18:2. Table 5.12 shows the concentration values of FAME in vegetable oil samples comparing the values obtained from the external and internal standard curves. The calibration curves are shown in Figure 5.23 for comparing the external calibration (left column) and internal calibration (right column).

Internal standard method is widely used in the chromatographic techniques. The internal standard must be similar to the chemical properties of the analyte and completely separate from other compound in the chromatogram. In this work, the FAME of C17:0 was used as an internal standard by adding into the samples and the standard solutions with a given concentration. The internal standard curve is plotted between of the ratio of analyte signal and internal standard signal with the analyte concentration.

The concentration values of FAMEs in the vegetable oils using external standard curves were compared with the concentrations from internal standard curves by using *t*-test at 95% confidence interval as summarized in Table 5.13. The values of external standard method were significantly different comparing with internal standard method ($P < 0.05$, $t_{stat} > t_{critical}$). However, the internal standard method was used for

Copyright by Mahidol University

determination of FAME from vegetable oil for correction the loss of analytes during the preparation and the analysis steps.

Table 5.12 The concentration values of FAMEs (g/L) obtained from external and internal standard curves.

Vegetable oils	C16:0		C18:0		C18:1		C18:2	
	External	Internal	External	Internal	External	Internal	External	Internal
Soybean oil	123.34 ±5.44	104.98 ±0.37	46.53 ±3.99	45.93 ±3.04	269.53 ±12.26	223.60 ±6.09	582.78 ±12.69	410.62 ±9.78
Sun-flower oil	69.32 ±3.41	55.85 ±1.90	39.29 ±1.71	38.26 ±1.31	468.77 ±16.74	362.29 ±8.47	637.23 ±6.93	433.74 ±7.98
Rice barn oil	259.07 ±16.97	182.65 ±8.46	30.88 ±4.65	32.34 ±2.67	502.10 ±32.05	399.41 ±9.81	418.96 ±11.78	293.28 ±6.70
Palm oil	504.87 ±20.17	330.17 ±9.92	53.84 ±3.36	45.72 ±2.48	580.76 ±17.67	431.61 ±6.86	156.27 ±6.84	96.67 ±4.12
Coconut oil A	336.36 ±21.22	315.74 ±9.05	52.94 ±2.67	44.87 ±1.14	372.12 ±5.16	358.58 ±9.49	116.67 ±4.45	115.13 ±4.99
Coconut oil B	83.48 ±11.15	68.28 ±7.75	45.57 ±4.15	34.92 ±2.22	62.94 ±4.38	56.76 ±6.41	13.76 ±3.27	15.12 ±2.40
Used palm oil	445.8 ±10.56	382.82 ±3.94	56.03 ±5.24	56.14 ±2.97	453.45 ±2.66	433.51 ±7.33	126.43 ±5.83	103.02 ±5.45
Jatropha oil	308.45 ±10.25	183.84 ±8.94	103.71 ±6.40	92.16 ±5.84	707.28 ±22.38	621.94 ±40.29	551.20 ±4.93	300.86 ±3.08

Coconut A was prepared by heating.

Coconut B was prepared by cool extraction.

Copyright by Mahidol University

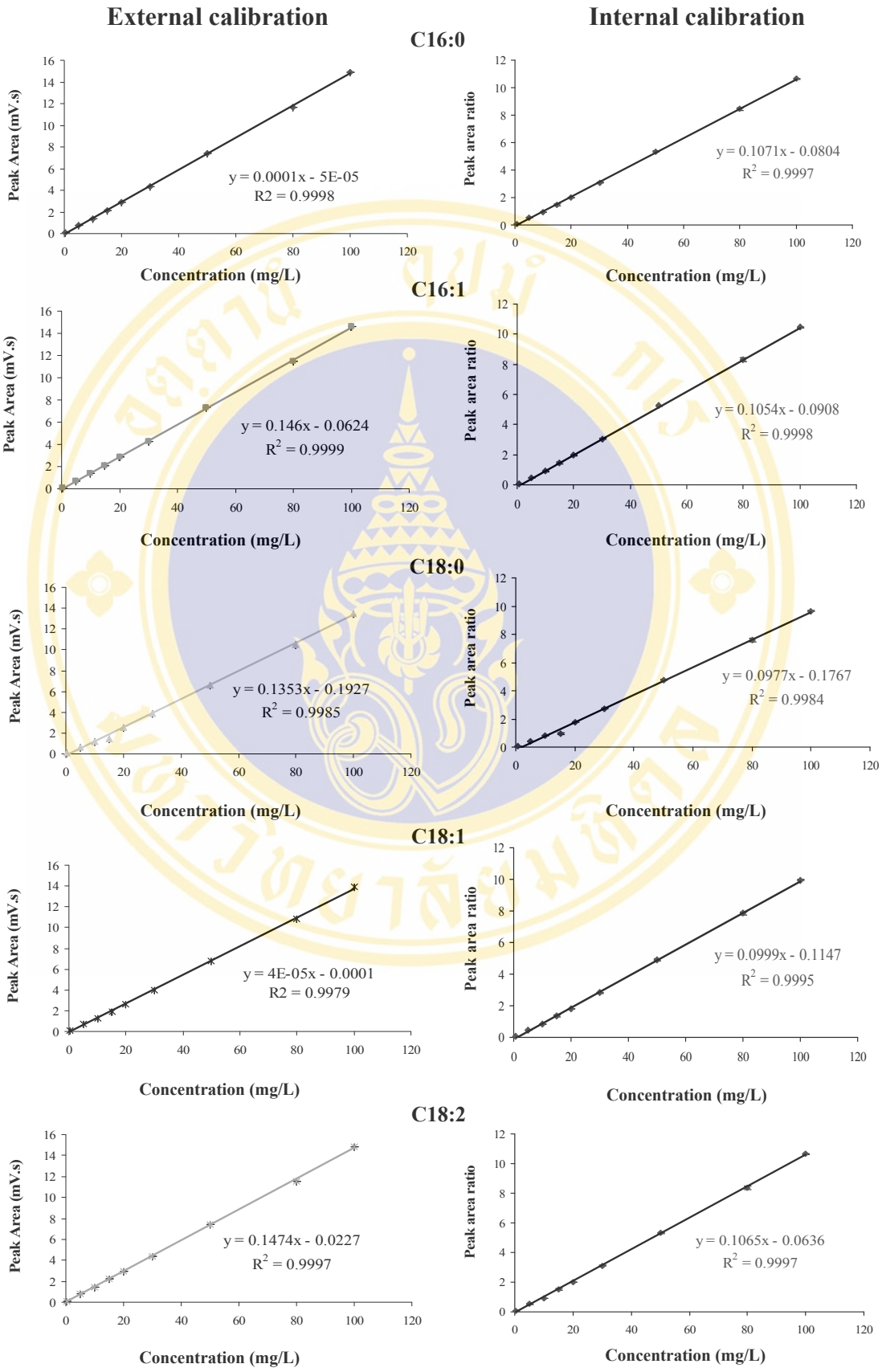


Figure 5.23 Comparison of external and internal calibration of FAMEs.

Copyright by Mahidol University

Table 5.13 Statistical test data of pair *t*-test between external standard and internal standard methods at 95% confidence interval.

Vegetable oils	FAMEs	*t* Stat	*P* (T≤t) two-tail	*t* Critical two-tail
Soybean oil	C16:0	2.97	0.10	4.30
	C18:0	-0.63	0.59	4.30
	C18:1	25.61	0.01	4.30
	C18:2	10.73	0.01	4.30
Sunflower oil	C16:0	11.84	0.01	4.30
	C18:0	2.17	0.16	4.30
	C18:1	13.37	0.01	4.30
	C18:2	17.00	0.01	4.30
Rice barn oil	C16:0	14.24	0.01	4.30
	C18:0	-1.28	0.33	4.30
	C18:1	9.17	0.01	4.30
	C18:2	11.56	0.01	4.30
Palm oil	C16:0	7.92	0.02	4.30
	C18:0	9.83	0.01	4.30
	C18:1	7.57	0.02	4.30
	C18:2	35.78	0.01	4.30
Used palm oil	C16:0	3.60	0.07	4.30
	C18:0	0.80	0.51	4.30
	C18:1	1.65	0.24	4.30
	C18:2	3.74	0.06	4.30
Jatropha oil	C16:0	4.24	0.05	4.30
	C18:0	16.74	0.01	4.30
	C18:1	4.43	0.05	4.30
	C18:2	5.53	0.03	4.30
Coconut oil A	C16:0	3.21	0.94	4.30
	C18:0	7.45	0.02	4.30
	C18:1	1.23	0.35	4.30
	C18:2	2.42	0.14	4.30
Coconut oil B	C16:0	7.72	0.02	4.30
	C18:0	27.83	0.01	4.30
	C18:1	5.67	0.03	4.30
	C18:2	-2.71	0.11	4.30

Copyright by Mahidol University

5.4.3 Comparison of FAME conversion derived from GC-FID and ^{1}H-NMR

The conversion values efficiency of FA to FAME were also studied by using ^{1}H-NMR technique. This technique was chosen to check the conversion of the triglyceride to the FAME compound. The conversion from ^{1}H-NMR technique was compared with the GC techniques, as presented in Table 5.14. It shown that the FAME conversion from two techniques were no significant difference result at 95% confidence interval ($P > 0.05$, $t_{stat} < t_{critical}$), as summarized in Table 5.15. On the other hand, the percent conversion from coconut oil B showed significantly different result ($P < 0.05$) because the other FAs which are not in the range of interest were found in the profile. This could affect the calculation of conversion by using GC-FID. Therefore, the percent conversion from GC-FID was less than ^{1}H-NMR technique which calculated from all FAMEs in the reaction.

Table 5.14 The FAME conversions derived from GC-FID and ^{1}H-NMR.

Vegetable oil	% Conversion from GC	% Conversion from ^{1}H-NMR
Soybean oil	80.29±5.75	100.00
Sunflower oil	80.77±2.93	100.00
Rice barn oil	93.97±11.34	100.00
Palm oil	83.93±0.57	100.00
Coconut oil A	80.20±5.34	100.00
Coconut oil B	16.47±1.49	100.00
Used palm oil	74.12±1.96	100.00
Jatropha oil	96.62±4.73	100.00

Copyright by Mahidol University

Table 5.15 Statistical test data of pair *t*-test between percent conversions derived from GC-FID and ^{1}H-NMR at 95% confidence interval.

Vegetable oil	*t* Stat	P (T≤t) two-tail	*t* Critical two-tail
Soybean	-1.30	0.32	4.30
Sunflower	-3.65	0.07	4.30
Rice barn	-0.04	0.97	4.30
Palm oil	-4.30	0.05	4.30
Used palm oil	-3.16	0.09	4.30
Jatropha	-2.68	0.12	4.30
Coconut A	-4.46	0.05	4.30
Coconut B	-65.16	$1.18E^{-4}$	4.30

5.4.4 FA profile in different source of coconut oils

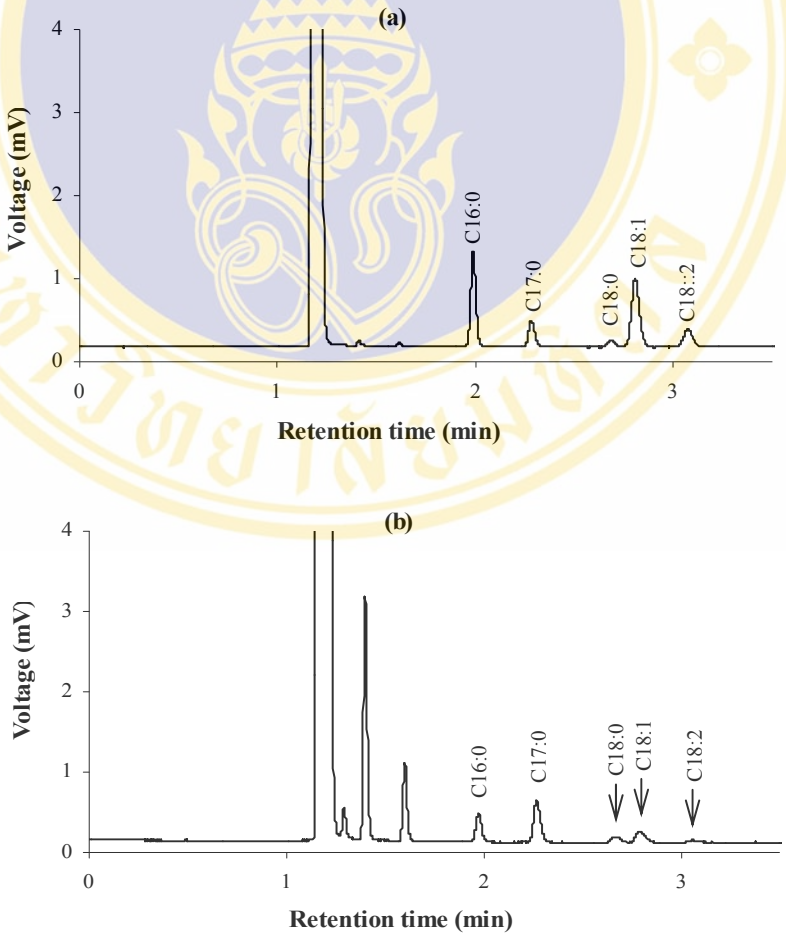


Figure 5.24 FA profiles of (a) coconut oil A was prepared by heating, and (b) coconut oil B was prepared by cool extraction.

Copyright by Mahidol University

The FA profile can be investigated in the different sources of vegetable oil. In this study, coconut oils were used to study the FA profile. The samples were obtained from the different source of the method preparation of the oils. Coconut A and B were prepared by heating and cool extraction, respectively. The FA profiles of individual sources of coconut oil were shown different, as presented in Figure 5.24. Some types of FA in coconut oil could not be identified because these FA are not in the range of interested or there are no standard FAs to confirm. However, the unknown FAs can be predicted by using the correlation between retention time and carbon numbers of FAMEs.

5.4.5 Characterization of FAMEs

Biodiesel has different fuel properties in comparison to the conventional diesel fuels. For example, it contains almost no sulfur, no aromatics, non-toxic, biodegradable and environmentally friendly fuel. Consequently, these advantages of biodiesel make it possible to be a candidate for a diesel substitution. Biodiesel can be blended with petroleum diesel fuels and used in diesel engines. Therefore, characterization of the properties of biodiesel fuel is needed before using in a diesel engine [Alptekin, *et al.*, 2009]. Viscosity and density are important parameters of biodiesel and diesel fuel standards because of being key fuel for determining efficiency of a fuel for diesel engines as fuel introduced into the combustion chamber. High density causes poor fuel atomization [Knothe, 2005]. The density of biodiesel usually varies between 0.86 and 0.90 g/mL in addition to the standard viscosity varies between 3.5 and 5.0 KcSt [Alptekin, *et al.*, 2008]. In this study, the results of density and viscosity were presented in Table 5.16. The density values were obtained from the experiment. The viscosity values were calculated from an empirical correlation as described in section 4.4.1.7. The densities of methyl esters produced in this study were in the range of standard value. Moreover, the density is relate to the cetane number and heating value. Because of the high density fuel exhibits lower combustion engine. Some of the other important values were also studied such as acid value and iodine value, as presented in Table 5.16. The acid value refers to acidity of fuel which affects to the corrosion and engine deposits ["Biofuel testing," 2007]. The iodine value (or

Copyright by Mahidol University

iodine number) is commonly used as a measure of the chemical stability properties of the biodiesel. This value has been used to indicate the total amount of unsaturation contained in the biodiesel [Ramos, *et al*., 2009]. A higher iodine value indicates a higher quantity of double bonds in the sample therefore, biodiesel has lesser stability and high potential to polymerise leads to deterioration of the lubricating system [Jon, *et al.*, 2004]. The results of basic properties of FAMEs/biodiesel from the preparation in the term of small scale are followed by the standard method of ASTM D664.

Table 5.16 Basic properties of FAMEs derived from vegetable oil.

Biodiesel	**Acid value** [(c)] mg KOH/g	**Density** (g/mL)	**Viscosity** (KcSt)	**Iodine value** [(d)]
Soybean oil	0.29	0.87	4.13	8.64
Rice barn oil	0.29	0.87	4.13	8.84
Sunflower oil	0.15	0.86	3.79	8.82
Palm oil	0.29	0.86	3.79	8.86
Coconut oil	0.29	0.86	3.79	8.87
Used palm oil	0.29	0.87	4.13	8.78
Jatropha oil	0.29	0.87	4.13	8.85
Standard palm biodiesel	0.44	0.86	3.79	8.79
Standard soybean biodiesel	0.29	0.87	4.13	8.50

[(c)] ASTM D664 (Acid value < 0.5 mg KOH/g)

[(d)] ASTM D664 (Iodine value < 120 g I_2/100 g)

Copyright by Mahidol University

CHAPTER VI
CONCLUSION

Vegetable oils are more attractive source of substitution fuel because most of currently using energy comes from fossil fuels known as non-renewable fuel. Due to many advantages of biodiesel therefore, it have been many studied. Biodiesels (FAMEs) were derived from FFAs and triglyceride presenting in vegetable oil via the transesterification with alcohol in the presence of a catalyst. In this research, important parameters affecting to the preparation of FAMEs from vegetable oil and the appropriate instrumental condition were optimized. The FAME products were investigated by GC-FID comparing to ^{1}H-NMR in order to confirm the result. The FA in vegetable oil was determined as FAME compounds under the appropriate GC-FID condition.

In the analysis, gas chromatography equipped with FID detector was optimized for determination of FAMEs which were separated on of DB-wax column using split injection at ratio of 40:1 and Helium carrier gas at a flow rate of 0.5 mL/min. The column temperature was set at 210 °C as isothermal. The injector and detector temperatures were maintained at 250 °C. A good linearity and a low detection limit level of FAMEs were in the range of 0.5-100 mg/L and 0.19-0.30 mg/L, respectively. An internal standard calibration of FAMEs was used for the determination these compounds in the vegetable oil for correction the loss of analytes in the preparation and the analysis.

The vegetable oils are converted into their methyl esters (biodiesel) by transesterification which can reduce their viscosity of the biodiesel. The FAMEs preparation are based on transesterification of triglyceride and esterification of FFAs. The optimum conditions of FAMEs preparation from vegetable oil have two

Copyright by Mahidol University

conditions depending on FA contents in vegetable oil. Generally, refined vegetable oil has the acid value less than 1 mg KOH/g. Therefore, the direct transesterification reaction can perform [Alptekin, *et al.*, 2008]. The optimum condition for vegetable oil with low FFA contents which was optimized in soybean oil was employed at 60 ºC of reaction temperature for 30 minutes, using 6:1 molar ratio of methanol to oil, and 1.0% (w/w) NaOH. The percentage conversions from GC were 80.29-93.97 %. Due to high cost of the refined oil, the production of biodiesel from used cooking oil was utilized instead. However, high FFA content in used cooking oil is an impediment to produce biodiesel by direct transesterification because FFAs easily react with base catalysts and form soap which prohibits the separation of biodiesel. The appropriate condition for vegetable oil with high FFA contents was used two steps of acid-base catalyzed method which was optimized in used palm oil and non-extracted of jatropha oil. In the optimum acid-base catalyzed method, the esterification reaction was preliminarily performed at reaction temperature of 50 °C for 10 minutes and using 10% (v/v) H_2SO_4/methanol as catalyst. Then, the reaction was followed by the transesterification which proceeded at reaction temperature of 60 °C for 30 minutes, 6:1 molar ratio of methanol to oil and using 1.0% (w/w) NaOH as catalyst. This condition gave high conversion in the range of 74.12-96.62%. The base-acid method was not suitable for vegetable oil with high FFA because water generated in the reaction affected to the next reaction.

The preparation and determination of FAs as FAME compounds in vegetable oil samples were successfully performed under the optimum GC-FID conditions. The major FAs in vegetable oils are palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2). The FA component in vegetable oil affects in the properties of biodiesel as in the fuel. The properties of biodiesel are controlled by the standard method. The biodiesel have an oxygen content which can improve its combustion efficiency. In fact, highly saturated FAs in vegetable oil affecting to biodiesel are exhibited as high viscosity, cetane number and heating of combustion. The density and the viscosity are key determination efficiency of diesel fuel because the density relates to cetane number and heating value. The high density contributes to lower combustion engine. The basic properties of FAME from the experiment were

Copyright by Mahidol University

determined by following the standard method. The acid value was less than 0.5 mg KOH/g, the density in the range of 0.86-0.87 g/mL, viscosity values were 3.74-4.13 KcSt and iodine value less than 120 g I_2/100g. The properties values of FAMEs from the study were in the range of standard values of biodiesel. The FAMEs from all vegetable oil samples have a tendency to use as fuel for substituted a diesel fuel. However, the suitable sources for biodiesel production are considered in the term of low production cost and large production scale.

Suggestion for the future work

In the present, biodiesel is an alternative energy synthesized from a vegetable oil or animal fat for substitution of diesel fuel because of non-toxic and biodegradable. The future work can be carried out in many aspects of the biodiesel production. Generally, efficiency of the production and properties of the product should be concerned in the study from lab-scale extended to industrial-scale. The efficiency of the preparation focuses on the high conversion yield of the reaction. So, various analytical techniques must be applied to compare the results such as GC, HPLC, IR, NMR and so on. The comparison of the analytical technique used well help the researcher for their evaluation of the products. The production of the product can be performed by following the standard method for quality control of the product.

Copyright by Mahidol University

REFERENCES

Aksoy, H. A., Kahraman, I., Karaosmanoglu, F., & Civelekoglu, H. (1988). Evaluation of Turkish sulphur olive oil as an alternative diesel fuel *J Am Oil Chem Soc, 65*, 936-938.

Alptekin, E., & Canakci, M. (2008). Determination of the density and the viscosities of biodiesel-diesel fuel blends. *Renew Energ, 33*(12), 2623-2630.

Alptekin, E., & Canakci, M. (2009). Characterization of the key fuel properties of methyl ester-diesel fuel blends. *Fuel, 88*(1), 75-80.

Antoln, E. M., Delange, D. M., & Canavaciolo, V. G. (2008). Evaluation of five methods for derivatization and GC determination of a mixture of very long chain fatty acids (C24:0-C36:0). *J Pharm Biomed, 46*(1), 194-199.

Antonio, Z. 2005, from *http://www.scientificpsychic.com/fitness/fattyacids.html.*

Arzamendi, G., Arguiarena, E., Campo, I., & Ganda, L. M. (2006). Monitoring of biodiesel production: Simultaneous analysis of the transesterification products using size-exclusion chromatography. *Chem Eng J, 122*(1-2), 31-40.

Barnwal, B. K., & Sharma, M. P. (2005). Prospects of biodiesel production from vegetable oils in India. *Renew Sust Energ Rev, 9*(4), 363-378.

Berchmans, H. J., & Hirata, S. (2008). Biodiesel production from crude Jatropha curcas L. seed oil with a high content of free fatty acids. *Bioresource Technol, 99*(6), 1716-1721.

Bernard, F., Royden O., B., & Everett H., P. (1986). Transesterification kinetics of soybean oil 1. *J Am Oil Chem Soc, 63*(10), 1375-1380.

Biofuel testing. (2007). from *http://www.biofueltesting.com/quality_control.asp.*

Blanco, G. D., Mangas, A. J. J., Cabrales, I. M., & Abrodo, P. A. (2001). Gas chromatographic analysis of total fatty acids in cider. *J Agr Food Chem, 49*(3), 1260-1263.

Copyright by Mahidol University

Brondz, I. (2002). Development of fatty acid analysis by high-performance liquid chromatography, gas chromatography, and related techniques. *Anal Chim Acta, 465*(1-2), 1-37.

Carrapiso, A. I., Luisa, T. M., Jess, P. M., Tejeda, J. F., & Garca, C. (2000). In situ transesterification of fatty acids from Iberian pig subcutaneous adipose tissue. *Meat Sci, 56*(2), 159-164.

Carvalho, A. P., & Malcata, F. X. (2005). Preparation of fatty acid methyl esters for gas-chromatographic analysis of marine lipids: Insight studies. *J Agr Food Chem, 53*(13), 5049-5059.

CaylI, G., & Kusefoglu, S. (2008). Increased yields in biodiesel production from used cooking oils by a two step process: Comparison with one step process by using TGA. *Fuel Process Technol, 89*(2), 118-122.

Chaidacho, I. (2008). Determination of phospholipid fatty acids as fatty acid methyl esters using gas chromatography. *Mahidol University, Bangkok*, 1-68.

Chongkhong, S., Tongurai, C., Chetpattananondh, P., & Bunyakan, C. (2007). Biodiesel production by esterification of palm fatty acid distillate. *Biomass Bioenerg, 31*(8), 563-568.

Darnoko, D., & Cheryan, M. (2000). Kinetics of palm oil transesterification in a batch reactor. *J Am Oil Chem Soc, 77*(12), 1263-1267.

Darnoko, D., Cheryan, M., & Perkins, E. G. (2000). Analysis of vegetable oil transesterfication products by gel permeation chromatography. *J Liq Chromatogr R T, 23*(15), 2327-2335.

Eder, K. (1995). Gas chromatographic analysis of fatty acid methyl esters. *J Chrom B Biomed Sci Appl, 671*(1-2), 113-131.

Encinar, J. M., Gonzlez, J. F., & Rodrguez, R. A. (2007). Ethanolysis of used frying oil. Biodiesel preparation and characterization. *Fuel Process Technol, 88*(5), 513-522.

Fang, Z., Wen, C. Z., Xian, E. Z., You, R. S., Su, J. L., & Jin, M. Y. (2007). Determination of Free Fatty Acids by High Performance Liquid Chromatography with Fluorescence Detection and Identification with Mass Spectrometry. *Chinese J Anal Chem, 35*(4), 489-494.

Copyright by Mahidol University

Freedman, B., Pryde, E., & Mounts, T. (1984). Variables affecting the yields of fatty esters from transesterified vegetable oils. *J Am Oil Chem Soc, 61*(10), 1638-1643.

Furuta, S., Matsuhashi, H., & Arata, K. (2004). Biodiesel fuel production with solid superacid catalysis in fixed bed reactor under atmospheric pressure. *Catal Commun, 5*(12), 721-723.

Gerpen, J. V. (2005). Biodiesel processing and production. *Fuel Process Technol, 86*(10), 1097-1107.

Ghadge, S. V., & Raheman, H. (2005). Biodiesel production from mahua (Madhuca indica) oil having high free fatty acids. *Biomass Bioenerg, 28*(6), 601-605.

Goff, M., Bauer, N., Lopes, S., Sutterlin, W., & Suppes, G. (2004). Acid-catalyzed alcoholysis of soybean oil. *J Am Oil Chem Soc, 81*(4), 415-420.

Hamed, M. E. M., Ruihong, Z., & Roberto, A. B. J. (2008). A two-step process for biodiesel production from salmon oil. *Biosyst Eng, 99*(2), 220-227.

Indarti, E., Majid, M. I. A., Hashim, R., & Chong, A. (2005). Direct FAME synthesis for rapid total lipid analysis from fish oil and cod liver oil. *J Food Compos Anal, 18*(2-3), 161-170.

Isbell, T. A., Mund, M. S., Evangelista, R. L., & Dierig, D. A. (2008). Method for analysis of fatty acid distribution and oil content on a single Lesquerella fendleri seed. *Ind Crop Prod, 28*(2), 231-236.

Jin, F., Kawasaki, K., Kishida, H., Tohji, K., Moriya, T., & Enomoto, H. (2007). NMR spectroscopic study on methanolysis reaction of vegetable oil. *Fuel, 86*(7-8), 1201-1207.

Jon, V. G., Rudy, P., Clements, Gerhard, K., Brent, S., Paul, J., *et al.* (2004). Biodiesel education.from *http://www.3.me.iastate.edu/biodiesel/Pages/ biodiesel19. html.*

Kadam, M., & Bhowmick, D. N. (2006). HPLC Analysis of rice bran oil. *J Food Lipids, 13*(4), 354-361.

Kanya, T. C. S., Rao, L. J., & Sastry, M. C. S. (2007). Characterization of wax esters, free fatty alcohols and free fatty acids of crude wax from sunflower seed oil refineries. *Food Chem, 101*(4), 1552-1557.

Copyright by Mahidol University

Karmee, S. K., & Chadha, A. (2005). Preparation of biodiesel from crude oil of Pongamia pinnata. *Bioresource Technol, 96*(13), 1425-1429.

Kim, H. J., Kang, B. S., Kim, M. J., Park, Y. M., Kim, D. K., Lee, J. S., *et al.* (2004). Transesterification of vegetable oil to biodiesel using heterogeneous base catalyst. *Catal Today, 93-95*, 315-320.

Knothe, G. (2000). Monitoring a processing transesterification reaction by fiber-optic near infared spectroscopy with correlation to ^{1}H-nuclear magnetic resonance spectroscopy. *J Am Oil Chem Soc, 77*, 489-493.

Knothe, G. (2005). Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. *Fuel Process Technol, 86*(10), 1059-1070.

Kusdiana, D., & Saka, S. (2004). Effects of water on biodiesel fuel production by supercritical methanol treatment. *Bioresource Technol, 91*(3), 289-295.

Leung, D. Y. C., & Guo, Y. (2006). Transesterification of neat and used frying oil: Optimization for biodiesel production. *Fuel Process Technol, 87*(10), 883-890.

Liu, K. S. (1994). Preparation of fatty acid methyl esters for gas-chromatographic analysis of lipids in biological materials. *J Am Oil Chem Soc, 71*(11), 1179-1187.

Ma, F., & Hanna, M. A. (1999). Biodiesel production: a review. *Bioresource Technol., 70*(1), 1-15.

Marchetti, J. M., & Errazu, A. F. (2008). Esterification of free fatty acids using sulfuric acid as catalyst in the presence of triglycerides. *Biomass Bioenerg, 32*(9), 892-895.

Marchetti, J. M., Miguel, V. U., & Errazu, A. F. (2007). Possible methods for biodiesel production. *Renew Sust Energ Rev, 11*(6), 1300-1311.

Meher, L. C., Dharmagadda, V. S. S., & Naik, S. N. (2006). Optimization of alkali-catalyzed transesterification of Pongamia pinnata oil for production of biodiesel. *Bioresource Technol, 97*(12), 1392-1397.

Meher, L. C., Vidya, S. D., & Naik, S. N. (2006). Technical aspects of biodiesel production by transesterification-a review. *Renew Sust Energ Rev, 10*(3), 248-268.

Copyright by Mahidol University

Mehmood, S., Orhan, I., Ahsan, Z., Aslan, S., & Gulfraz, M. (2008). Fatty acid composition of seed oil of different Sorghum bicolor varieties. *Food Chem, 109*(4), 855-859.

Naik, M., Meher, L. C., Naik, S. N., & Das, L. M. (2008). Production of biodiesel from high free fatty acid Karanja (Pongamia pinnata) oil. *Biomass Bioenerg, 32*(4), 354-357.

Noureddini, H., & Zhu, D. (1997). Kinetics of transesterification of soybean oil. *J Am Oil Chem Soc, 74*(11), 1457-1463.

Om, T., Neyda, C., Gomes, A., Donato, A., De, M. C., Jos, W., *et al.* (2008). Transesterification of Jatropha curcas oil glycerides: Theoretical and experimental studies of biodiesel reaction. *Fuel, 87*(10-11), 2286-2295.

Ozbay, N., Oktar, N., & Tapan, N. A. (2008). Esterification of free fatty acids in waste cooking oils (WCO): Role of ion-exchange resins. *Fuel, 87*(10-11), 1789-1798.

Pinto, A. C., Guarieiro, L. L. N., Rezende, M. J. C., Ribeiro, N. M., Torres, E. A., Lopes, W. A., *et al.* (2005). Biodiesel: an overview. *J Brazil Chem Soc, 16*, 1313-1330.

Procida, G., & Ceccon, L. (2006). Gas chromatographic determination of free fatty acids in olive mill waste waters. *Anal Chim Acta, 561*(1-2), 103-106.

Ramadan, M. F., Sharanabasappa, G., Seetharam, Y. N., Seshagiri, M., & Moersel, J. T. (2006). Characterisation of fatty acids and bioactive compounds of kachnar (Bauhinia purpurea L.) seed oil. *Food Chem, 98*(2), 359-365.

Ramos, M. J., Fernandez, C. M., Casas, A., Rodriguez, L., & Perez, A. (2009). Influence of fatty acid composition of raw materials on biodiesel properties. *Bioresource Technol, 100*(1), 261-268.

Rashid, U., & Anwar, F. (2008). Production of biodiesel through optimized alkaline-catalyzed transesterification of rapeseed oil. *Fuel, 87*(3), 265-273.

Rashid, U., Anwar, F., Moser, B. R., & Ashraf, S. (2008). Production of sunflower oil methyl esters by optimized alkali-catalyzed methanolysis. *Biomass Bioenerg, 32*(12), 1202-1205.

Copyright by Mahidol University

Rezanka, T., & Sigler, K. (2007). Identification of very long chain unsaturated fatty acids from Ximenia oil by atmospheric pressure chemical ionization liquid chromatography-mass spectroscopy. *Phytochem, 68*(6), 925-934.

Seniha, G. F., YagcI, Y., & Tuncer, E. A. (2006). Polymers from triglyceride oils. *Prog Polym Sci, 31*(7), 633-670.

Shahid, E. M., & Jamal, Y. (2008). A review of biodiesel as vehicular fuel. *Renew Sust Energ Rev, 12*(9), 2484-2494.

Sharma, Y. C., Singh, B., & Upadhyay, S. N. (2008). Advancements in development and characterization of biodiesel: A review. *Fuel, 87*(12), 2355-2373.

Shantha, N. C. (1992). Review gas chromatography of fatty acids. *J Chromatogr A, 624*, 37-51.

Sitthirakan, W. (2007). Delopment of high performance liquid chromatography for long chain fatty acids determination in environmental sample. *Mahidol University, Bangkok*, (1-84).

Van, D. B. J. D. J., Van, D. B. K. J., & Boon, J. J. (2001). Determination of the degree of hydrolysis of oil paint samples using a two-step derivatisation method and on-column GC/MS. *Progr Org Coating, 41*(1-3), 143-155.

Vicente, G., Martinez, M., & Aracil, J. (2004). Integrated biodiesel production: a comparison of different homogeneous catalysts systems. *Bioresource Technol, 92*(3), 297-305.

Vorbeck, M. L., Mattick, L. R., Lee, F. A., & Pederson, C. S. (1961). Preparation of methyl esters of fatty acids for gas-liquid chromatography quantitative comparison of methylation techniques. *Anal Chem, 33*(11), 1512-1514.

Wang, H. L., Zhao, C. H., & Wang, C. Z. (2005). Comparative study of sex pheromone composition and biosynthesis in Helicoverpa armigera, H. assulta and their hybrid. *Insect Biochem Molec, 35*(6), 575-583.

William, R. M., & Llotd, M. S. (1964). Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. *J Lipid Res, 5*, 600-608.

Winayanuwattikun, P., Kaewpiboon, C., Piriyakananon, K., Tantong, S., Thakernkarnkit, W., Chulalaksananukul, W., *et al.* (2008). Potential plant

Copyright by Mahidol University

oil feedstock for lipase-catalyzed biodiesel production in Thailand. *Biomass Bioenerg*, *32*(12), 1279-1286.

Zhang, Y., Dube, M. A., McLean, D. D., & Kates, M. (2003). Biodiesel production from waste cooking oil: 1. Process design and technological assessment. *Bioresource Technol*, *89*(1), 1-16.

Copyright by Mahidol University

APPENDIX

Copyright by Mahidol University

APPENDIX A

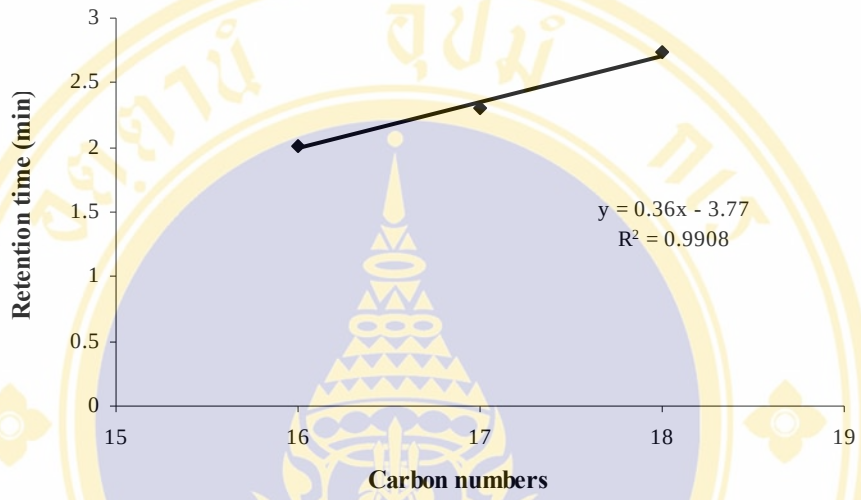


Figure A1 Correlation of retention time with carbon numbers of FAMEs.

Copyright by Mahidol University

APPENDIX B

Table B1 Statistical test data of pair *t*-test between FAME concentrations from acid-base and base-acid method at 95% confidence interval.

Vegetable oil	*t* Stat	P (T≤t) two-tail	*t* Critical two-tail
Used palm oil	1.19	0.36	4.30
Jatropha oil	22.74	0.01	4.30

Copyright by Mahidol University

APPENDIX C

The chromatograms of FAMEs were found in each type of vegetable oil, as shown following in this section.

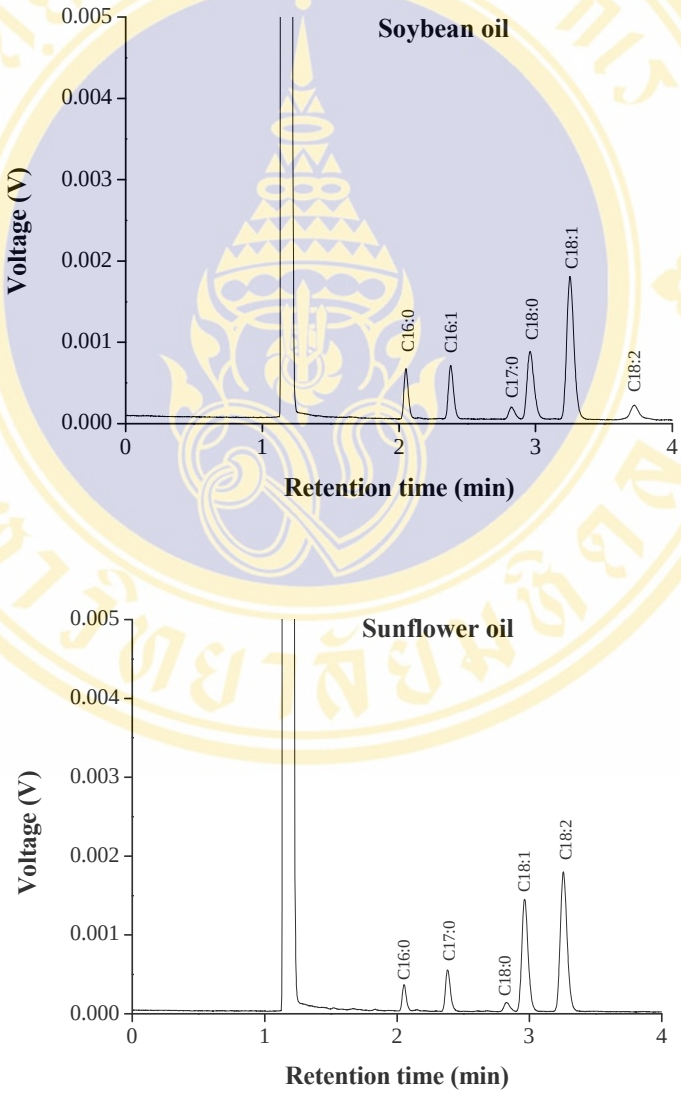


Figure C1 Chromatograms of vegetable oil samples.

Copyright by Mahidol University

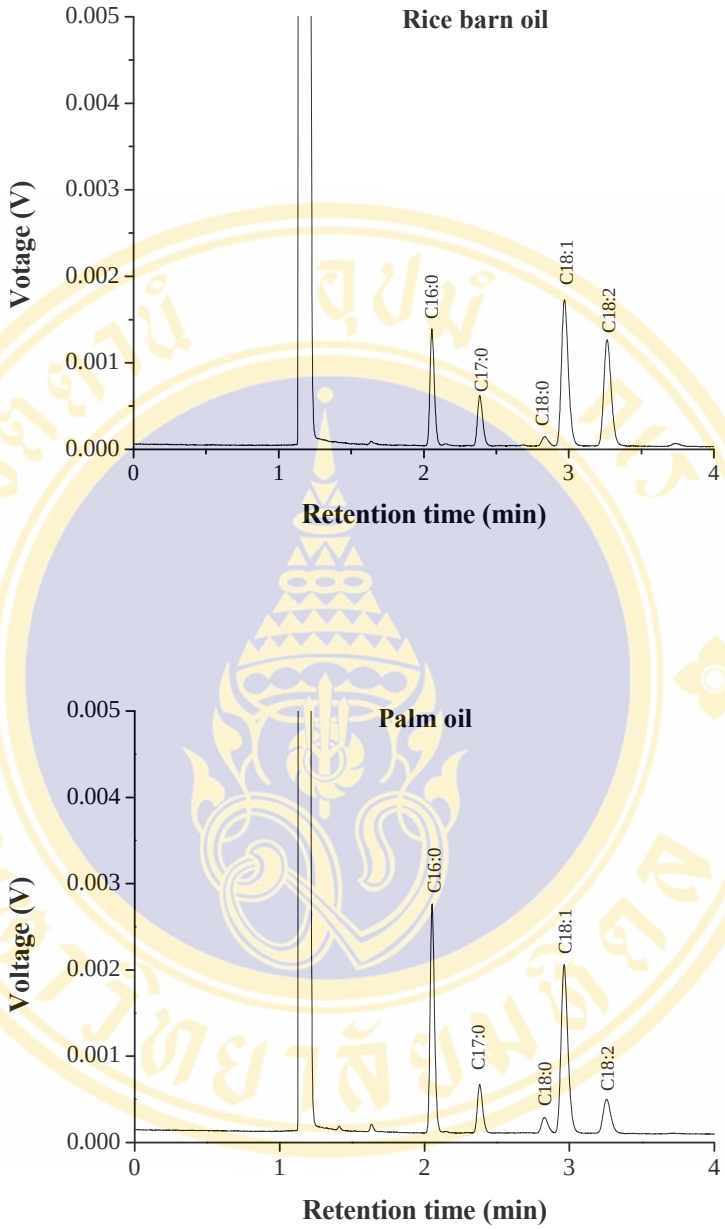


Figure C1 (cont.) Chromatograms of vegetable oil samples.

Copyright by Mahidol University

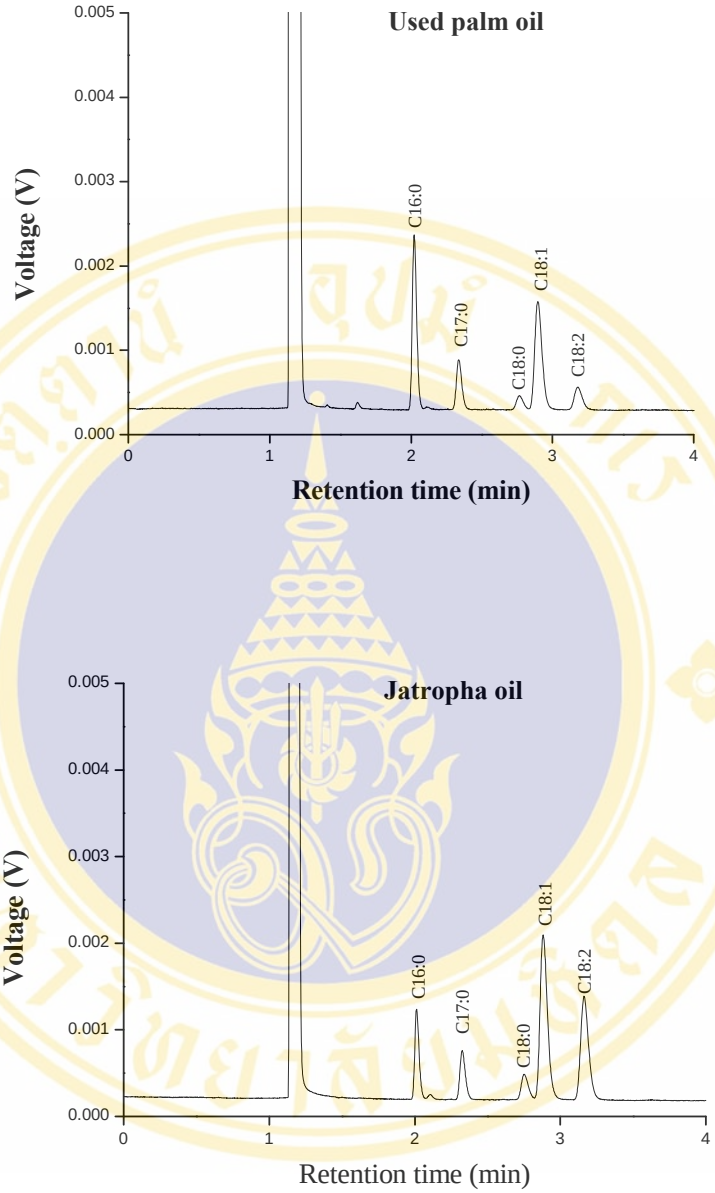


Figure C1 (cont.) Chromatograms of vegetable oil samples.

Copyright by Mahidol University

^{1}H-NMR spectra of FAMEs from transesterification reaction of individual of vegetable oils with methanol in optimum condition.

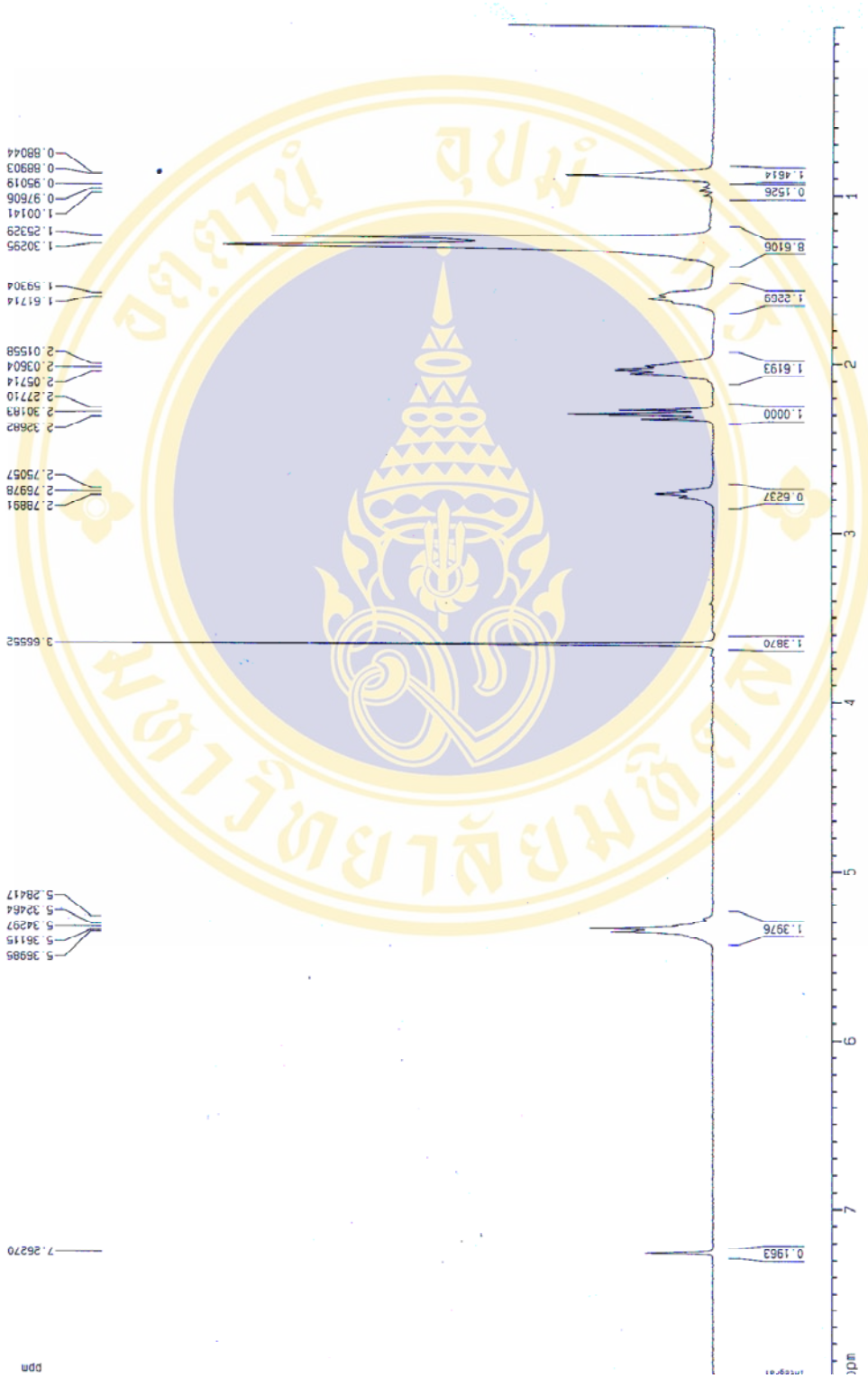


Figure C2 ^{1}H-NMR spectra of FAMEs from soybean oil.

Copyright by Mahidol University

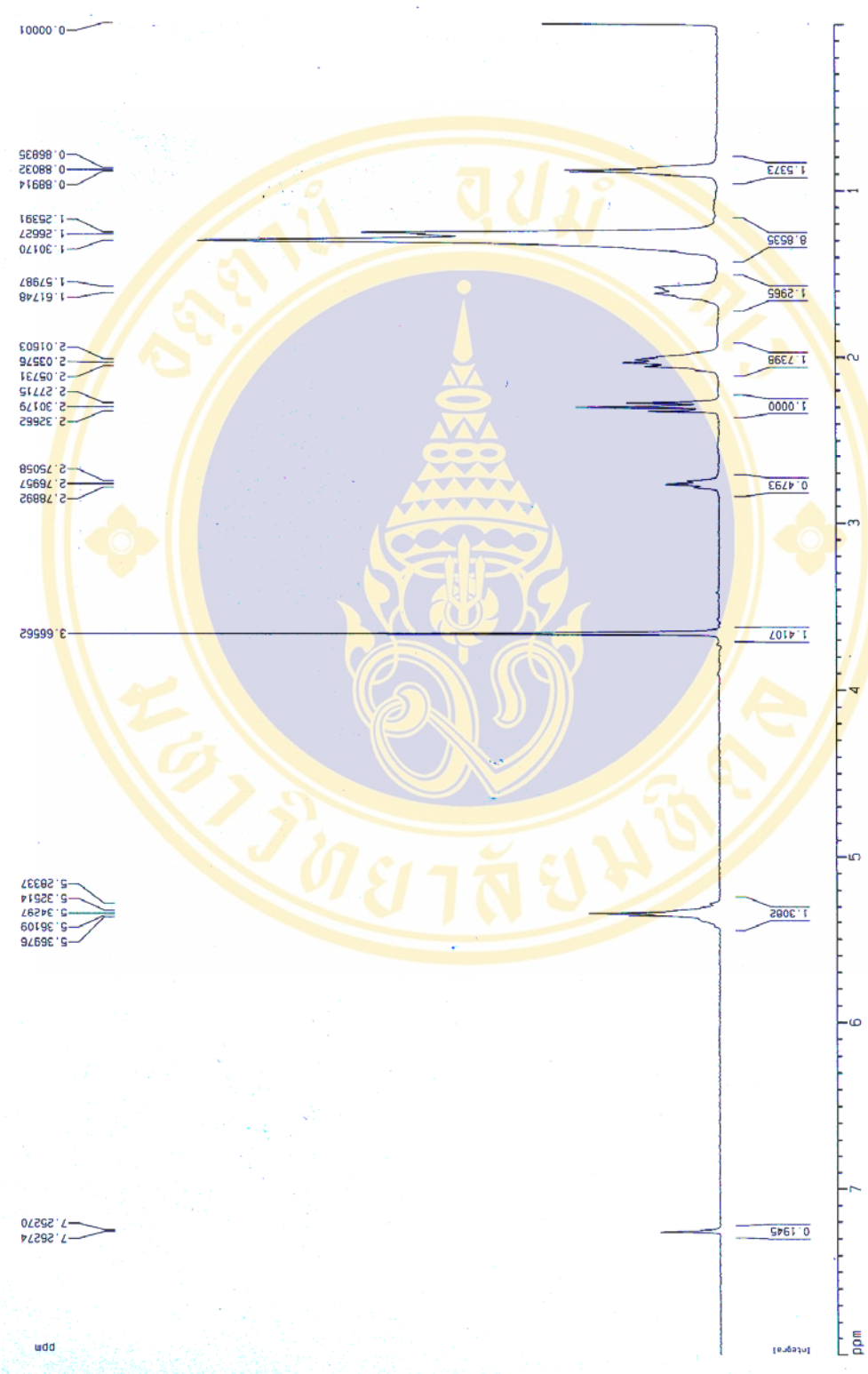


Figure C3 ^{1}H-NMR spectra of FAMEs from sunflower oil.

Copyright by Mahidol University

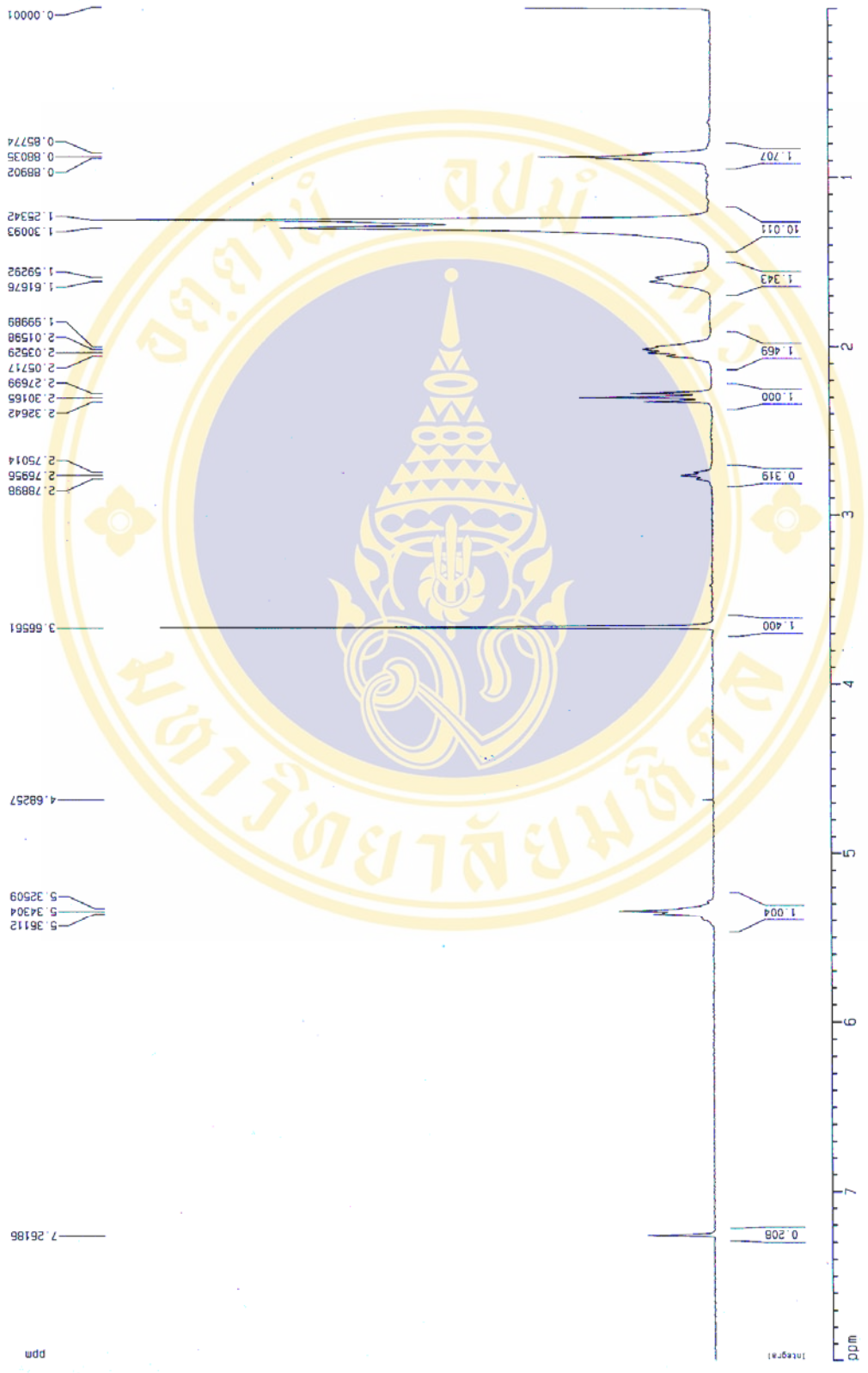


Figure C4 ^{1}H-NMR spectra of FAMEs from rice bam oil.

Copyright by Mahidol University

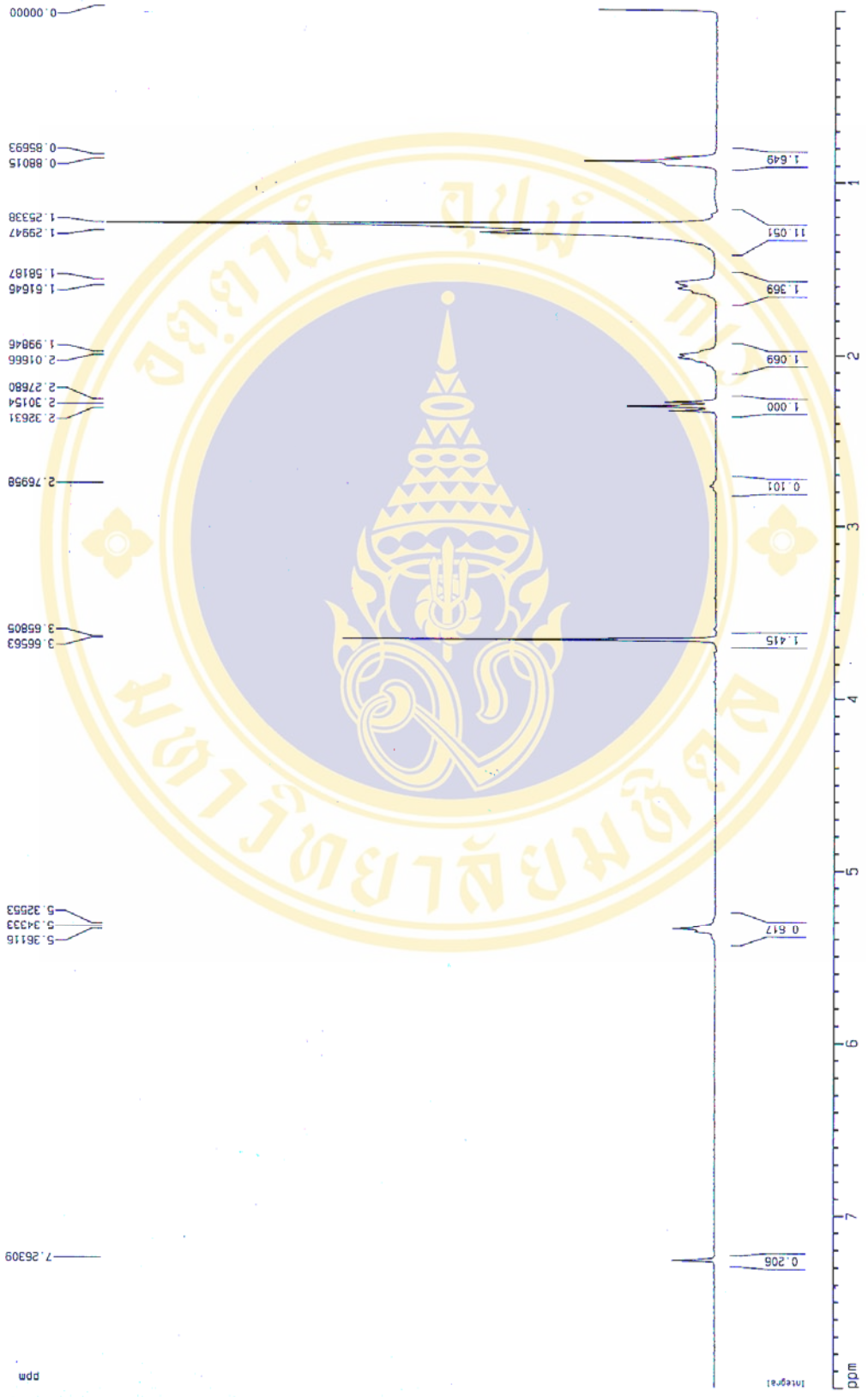


Figure C5 ^{1}H-NMR spectra of FAMEs from palm oil.

Copyright by Mahidol University

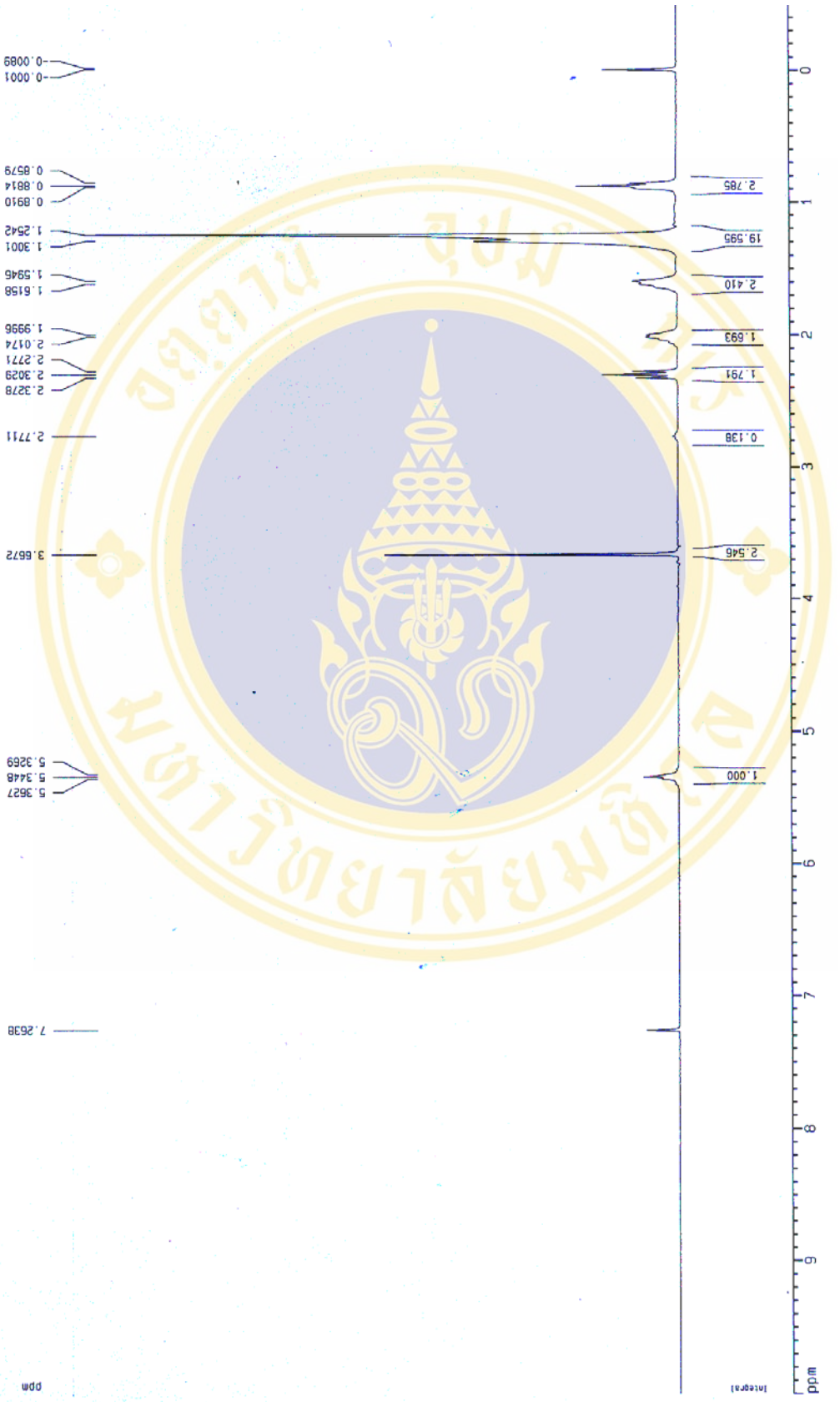


Figure C6 ^{1}H-NMR spectra of FAMEs from used palm oil by two- step method of acid-base catalyzed transesterification.

Copyright by Mahidol University

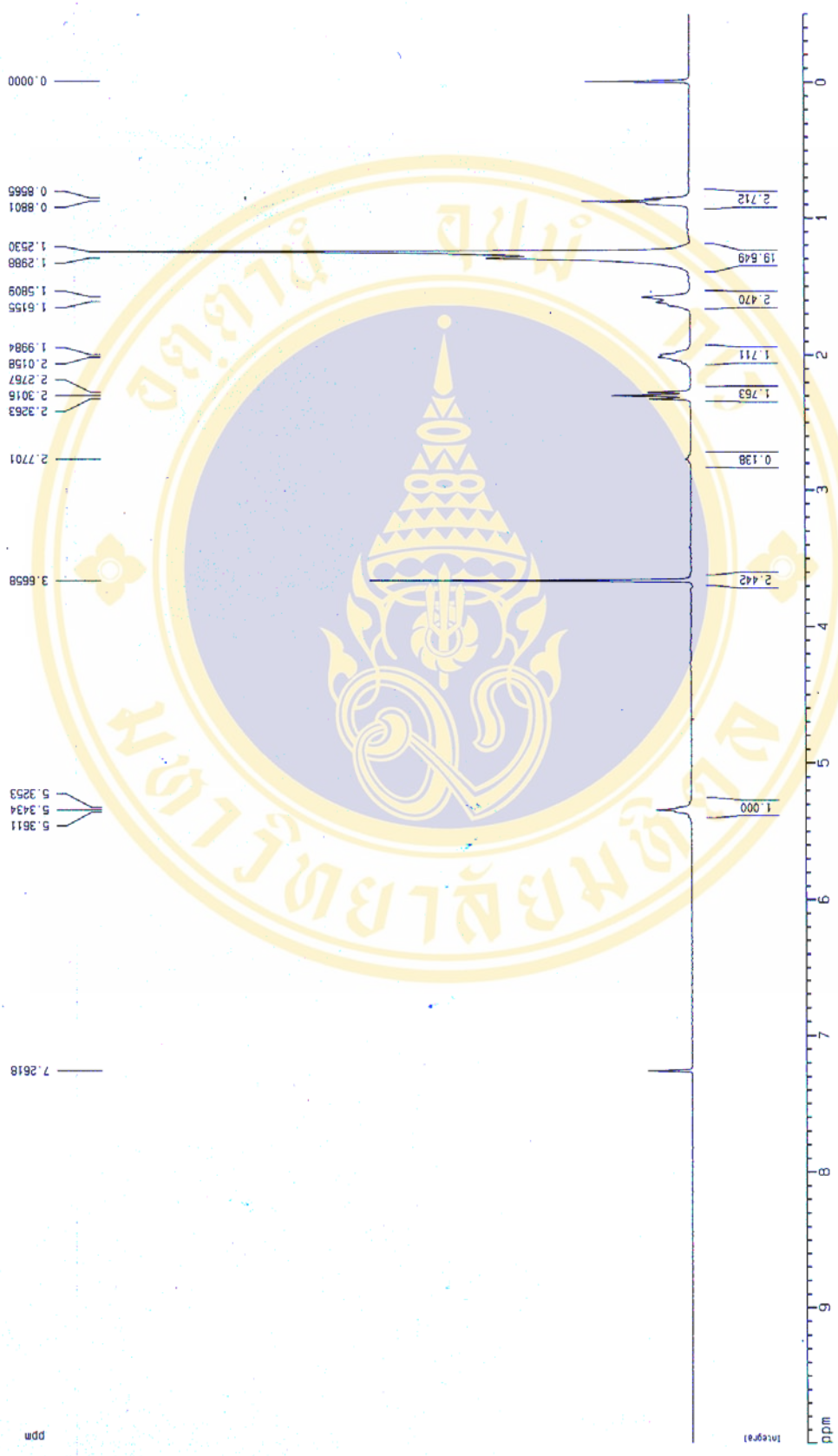


Figure C7 ^{1}H-NMR spectra of FAMEs from used palm oil by two-step method of base-acid catalyzed transesterification.

Copyright by Mahidol University

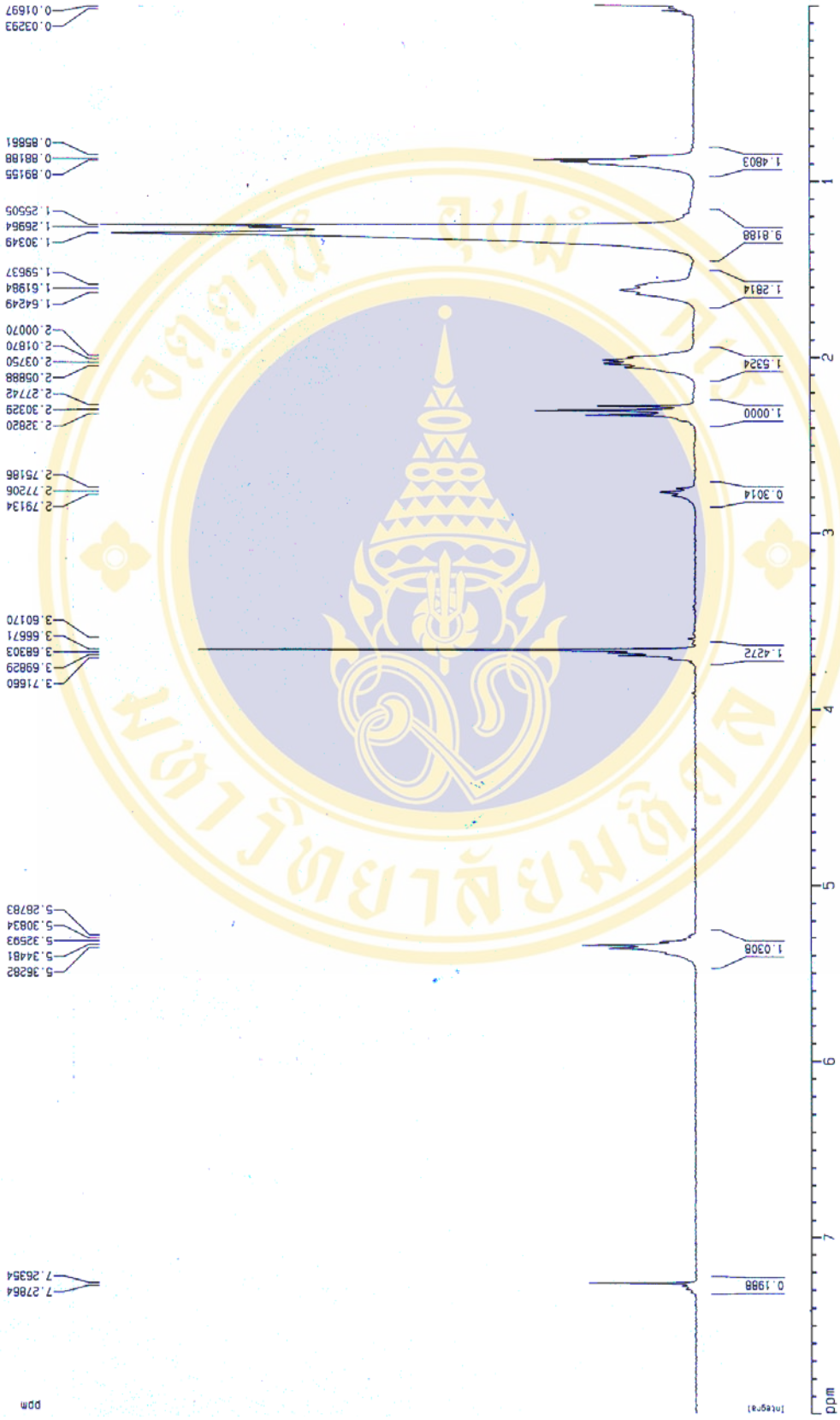


Figure C8 ^{1}H-NMR spectra of FAMEs from jatropha oil by two-step method of acid-base catalyzed transesterification.

Copyright by Mahidol University

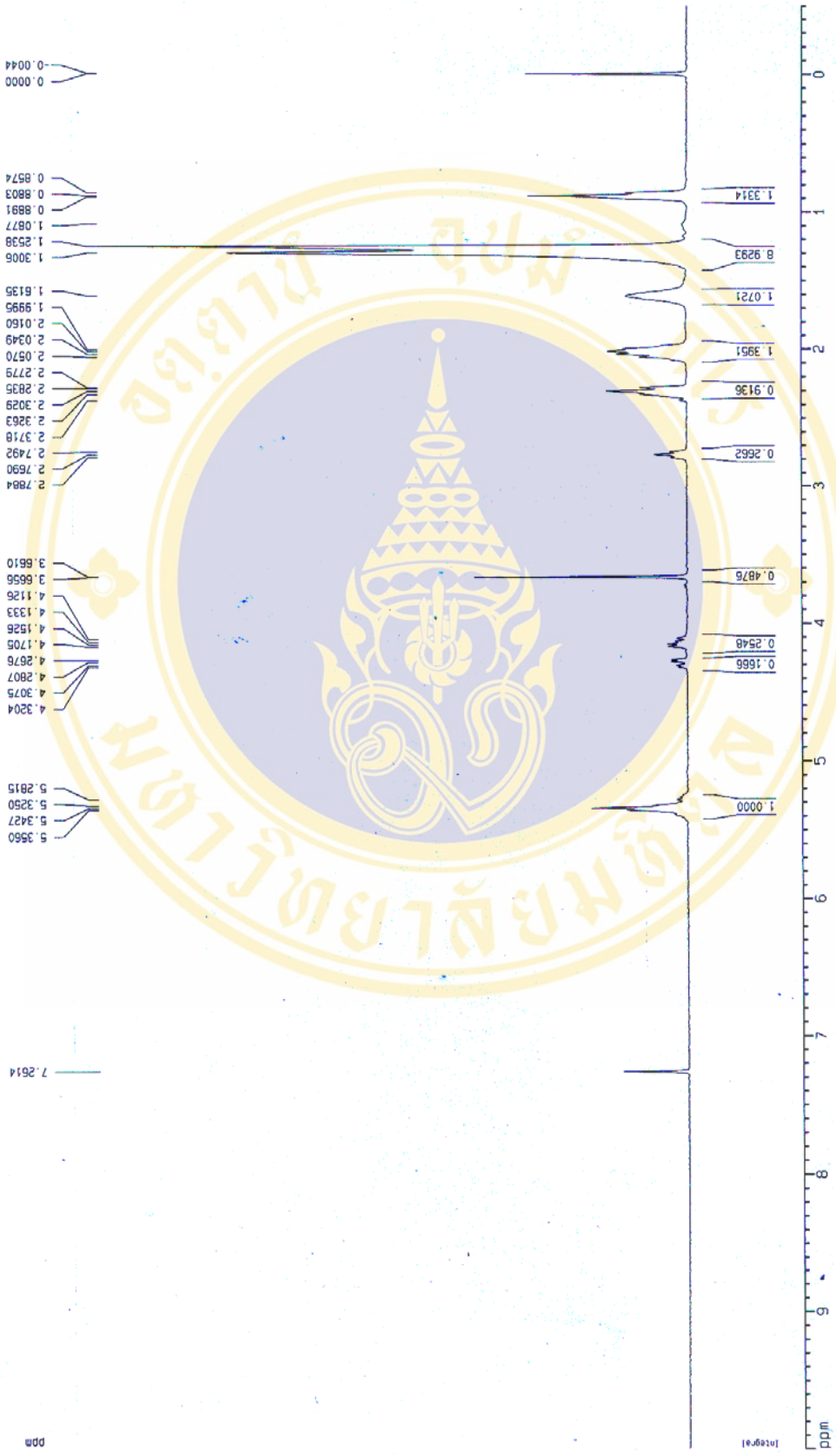


Figure C9 ^{1}H-NMR spectra of FAMEs from jatropha oil by two-step method of base-acid catalyzed transesterification.

Copyright by Mahidol University

BIOGRAPHY

NAME	Miss Wanvisa Chomsiri
DATE OF BRITH	May 26, 1983
PLACE OF BIRTH	Nakhonphanom, Thailand
INSTITUTIONS ATTENDED	Thammasat University, 2002-2005: Bachelor of Science (Chemistry) Mahidol University, 2006-2008: Master of Science (Applied Analytical and Inorganic Chemistry)
RESEARCH GRANTS	Recipient of scholarship from the Center of Excellence for Innovation in Chemistry (PERCH-CIC), 2007-2008 Recipient of a Teaching Assistant Scholarship from the Department of Chemistry, Faculty of Science, Mahidol University, 2007

Copyright by Mahidol University