THESIS APPROVAL

GRADUATE SCHOOL, KASETSART UNIVERSITY

Master of Science (Chemistry)
DEGREE

Chemistry	Chemistry
FIELD	**DEPARTMENT**

TITLE: Synthesis of Wax Esters from Palm Fatty Acid Distillate and Oleyl Alcohol over Amberlyst 15

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THESIS

SYNTHESIS OF WAX ESTERS FROM PALM FATTY ACID DISTILLATE AND OLEYL ALCOHOL OVER AMBERLYST 15

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A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
Master of Science (Chemistry)
Graduate School, Kasetsart University
2010

Vimonvan Chobset 2010: Synthesis of Wax Esters from Palm Fatty Acid Distillate and Oleyl Alcohol over Amberlyst 15. Master of Science (Chemistry), Major Field: Chemistry, Department of Chemistry. Thesis Advisor: Associate Professor Vittaya Punsuvon, Ph.D. 135 pages.

Wax esters are long chain ester that were derived from long chain fatty acids and long chain alcohols with chain lengths of 12 carbons or more on each compound. Wax esters have high potential for various applications used as important ingredients in cosmetic formulations, pharmaceuticals, plasticizers, polishes, fruit coating, and lubricants. The present works focused on the synthesis wax esters through esterification reaction of palm fatty acid distillate (PFAD) with oleyl alcohol using Amberlyst 15 as a catalyst. The purpose of this work was to study effect of several of reaction parameters such as reaction time, reaction temperature, amount of catalyst, molar ratios of oleyl alcohol to PFAD, and stirring speed on percentage conversion of free fatty acid(FFA), and study the physicochemical properties of wax ester products. The results from experiment showed that the optimum reaction conditions were at 60ºC for 1 hour of reaction time, 30% wt of catalyst, 2:1 molar ratios of oleyl alcohol to PFAD, and stirring speed at 250 rpm. The percentage conversion of FFA under the optimum conditions was 98%. The gas chromatography (GC) analysis showed that about 60% of wax esters were produced at this condition. In addition, FT-IR analysis was used to confirm wax ester products. The absorption band at 1699 cm^{-1} was assigned to C=O stretching of the starting material and the band slightly shifted to 1738 cm^{-1} after the formation of esterification wax esters. The physicochemical properties of synthesized wax esters showed the acid value of 4 mg KOH/g of sample, the refractive index of 1.460 (at 25ºC), the iodine value of 83.18 g I_2/100 g sample, the water content of 0.05 % wt, the flash point of 228 ºC, the saponification value of 69.36 mg KOH/g of sample, and the kinematic viscosity of 16.49cSt (at 40ºC).

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Student’s signature Thesis Advisor’s signature

ACKNOWLEDGEMENTS

I would like to gratefully thank and give gratitude and appreciation to my advisor, Assoc. Prof. Dr.Vittaya Punsuvon, for his valuable suggestions, guidance in to complete writing this thesis and his encouragement for the research presentation at International Symposium on Engineering, Energy and Environment 2009, ISEEE 2009. I would sincerely like to thank Dr. Pinsuda Viravathana and Dr. Potjanart Suwanruji my committee for their helpful comments and consulting in the experiment, Asst. Prof. Dr. Wirunya Keawwattana for her support for my study, and Dr. Siriporn Larpkittaworn from Thailand Institute of Scientific and Technological Research (TISTR) for her valuable comments and suggestions.

I would like to thank the members of Material Innovation Department and Analytical Chemistry Department, Thailand Institute of Scientific and Technological Research (TISTR), for their support and assistance on sample preparation, EDS, SEM, surface analysis, and physicochemical properties analysis of product and raw materials.

I am thankful to the Department of Chemistry, Kasetsart University for the laboratory, instrumental supports and making this research possible. Also, I would like to thank my friends and personals at Department of Chemistry, Faculty of Science for their help in various ways during this work and study.

This research was supported by the Thailand Research Fund (TRF) and Faculty of Science under Project MRG-WII 515S058, Center of Excellence Oil Palm, Kasetsart University, and partially funded from Thai Lube Base Public Co., LTD.

Lastly, I am so grateful to my family for their continuous support and encouragement on my Master studies.

Vimonvan Chobset
April 2010

TABLE OF CONTENTS

Page

TABLE OF CONTENTS i
LIST OF TABLES ii
LIST OF FIGURES iv
LIST OF ABBREVIATIONS viii
INTRODUCTION 1
LITERATURE REVIEW 6
MATERIALS AND METHODS 30
  Materials 30
  Methods 32
RESULTS AND DISCUSSION 45
CONCLUSIONS AND RECOMMENDATION 74
  Conclusions 74
  Recommentdation 75
LITERATURE CITED 76
APPENDICES 89
  Appendix A Calibration curve of standard methyl esters 90
  Appendix B Calibration curve of standard mono, di, and triolein 94
  Appendix C The results of variable parameters for esterification reaction 100
  Appendix D Calibration curve of standard oleyl esters 105
  Appendix E Analysis method 118
  CURRICULUM VITAE 135

LIST OF TABLES

Table **Page**

1	Characteristics of PFAD	16
2	Properties of Amberlyst 15	24
3	The physicochemical properties of palm fatty acid distillate	45
4	The relation of percentage area of fatty acid with retention time of PFAD	47
5	The relation of percentage weight of glycerides to the retention time of PFAD	49
6	The physical properties of Amberlyst 15	50
7	Elemental compositions of Amberlyst 15 by EDS	51
8	Detector response factor of standard oleyl palmitate	62
9	Detector response factor of standard oleyl oleate	64
10	Detector response factor of standard oleyl linoleate	66
11	Summarize of response factor for three standard oleyl esters	67
12	Amount and percentage yield of oleyl esters in wax esters	69
13	Physicochemical properties of wax ester products	71

LIST OF TABLES (Continued)

Appendix Table		Page
A1	The relation of retention time and fatty acid compounds	91
A2	The relation of retention time and composition compound of fatty acid in PFAD	93
C1	The relation of catalyst amount and percent conversion of free fatty acid by titration with 0.1N NaOH	101
C2	The relation of reaction time and percent conversion of free fatty acid by titration with 0.1 N NaOH	101
C3	The relation of reaction temperature and percent conversion of free fatty acid by titration with 0.1N NaOH	102
C4	The relation of molar ratio of oleyl alcohol to PFAD and percent conversion of free fatty acid by titration with 0.1 N of NaOH	103
C5	The relation of stirring speed and percent conversion of free fatty acid by titration with 0.1 N NaOH	103
C6	The relation of reusability of catalyst and percent conversion of free fatty acid by titration with 0.1 N NaOH	104
D1	Detector response factor of standard oleyl palmitate	109
D2	Detector response factor of standard oleyl oleate	113
D3	Detector response factor of standard oleyl linoleate	117

LIST OF FIGURES

Figure		Page
1	Process of refined oil and PFAD production	2
2	Esterification reactions of palm fatty acid distillate to produce wax esters	3
3	Esterification of PFAD and oleyl alcohol	9
4	Oil refining processing	15
5	Structure of tocopherol	18
6	Structure of tocotrienol	18
7	Structure of squalene	19
8	The copolymerization of sytrene and divinylbenzene	22
9	Sulphonation reaction of polymer matrix	23
10	Palm fatty acid distillate (PFAD)	30
11	Amberlyst 15	30
12	The esterification reaction of palm fatty acid distillate (PFAD)	33
13	Analysis of wax esters by GC	39
14	Summary diagram of experiment concept	44
15	Chromatogram of fatty acid composition in PFAD	46
16	Chromatogram of triglycerides composition in PFAD	48
17	Effect of the catalyst amount on conversion of FFA. The reaction mixture consisted of molar ratio of oleyl alcohol to PFAD of 2:1, reaction temperature of 60˚C, reaction time of 3 hours, and stirring speed at 250 rpm	52
18	Effect of reaction time on conversion of FFA. The reaction mixture consisted of molar ratio of oleyl alcohol to PFAD of 2:1, reaction temperature of 60 ˚C, catalyst amount of 30% wt, and stirring speed at 250 rpm	53

LIST OF FIGURES (Continued)

Figure **Page**

19 Effect of reaction temperature on percentage conversion of FFA. The reaction mixture consisted molar ratio of oleyl alcohol to PFAD of 2:1, reaction time of 1 hour, catalyst amount of 30% wt, and stirring speed at 250 rpm 55

20 Effect of molar ratios of oleyl alcohol to PFAD on percentage conversion of FFA. The reaction temperature of 60 ºC, reaction time of 1hour, amount of catalyst of 30%, and stirring speed at 250 rpm 56

21 Effect of stirring speed on percentage conversion of FFA. The reaction temperature of 60 ºC, reaction time of 1 hour, catalyst amount of 30% wt, and molar ratio of oleyl alcohol to PFAD of 2:1 57

22 Percentage of conversion of FFA and reuse number of catalyst. The reaction mixture consisted molar ratio of oleyl alcohol to PFAD of 2:1, reaction time of 1 hour, amount of catalyst of 30%, stirring speed 250 rpm, and reaction temperature of 60 ˚C 59

23 Chromatogram of standard oleyl palmitate and methyl laurate (an internal standard) 61

24 Chromatogram of standard oleyl oleate and methyl laurate (an internal standard) 63

25 Chromatogram of standard oleyl linoleate and methyl laurate (an internal standard) 65

26 Chromatogram of oleyl esters in the final product at the optimum condition 68

27 Infrared spectrums of palm fatty acid distillate (a) and wax esters (b) 69

LIST OF FIGURES (Continued)

Appendix Figure **Page**

A1 The chromatogram of standard fatty acid methyl esters mixture (C_8- C_{24}) 91
A2 Chromatogram of fatty acid compositions in PFAD 92
B1 Chromatogram of standard mono,di, and triolein 1 95
B2 Chromatogram of standard mono,di, and triolein 2 95
B3 Chromatogram of standard mono,di, and triolein 3 96
B4 Chromatogram of standard mono,di, and triolein 4 96
B5 Chromatogram of standard mono,di, and triolein 5 97
B6 Calibration curve of standard monoolein 97
B7 Calibration curve of standard diolein 98
B8 Calibration curve of standard triolein 98
B9 Chromatogram of triglyceride compositions in PFAD 99
D1 Chromatogram of standard oleyl palmitate concentration 500 mg/L 106
D2 Chromatogram of standard oleyl palmitate concentration 1000 mg/L 106
D3 Chromatogram of standard oleyl palmitate concentration 2000 mg/L 107
D4 Chromatogram of standard oleyl palmitate concentration 3000 mg/L 107
D5 Chromatogram of standard oleyl palmitate concentration 4000 mg/L 108
D6 Chromatogram of standard oleyl palmitate concentration 5000 mg/L 108
D7 Chromatogram of standard oleyl oleate concentration 512.5 mg/L 110

LIST OF FIGURES (Continued)

Appendix Figure **Page**

D8 Chromatogram of standard oleyl oleate concentration 1025 mg/L 110

D9 Chromatogram of standard oleyl oleate concentration 2050 mg/L 111

D10 Chromatogram of standard oleyl oleate concentration 3075 mg/L 111

D11 Chromatogram of standard oleyl oleate concentration 4100 mg/L 112

D12 Chromatogram of standard oleyl oleate concentration 5125 mg/L 112

D13 Chromatogram of standard oleyl linoleate concentration 528.6 mg/L 114

D14 Chromatogram of standard oleyl linoleate concentration 1057.2 mg/L 114

D15 Chromatogram of standard oleyl linoleate concentration 1585.8 mg/L 115

D16 Chromatogram of standard oleyl linoleate concentration 2114.4 mg/L 115

D17 Chromatogram of standard oleyl linoleate concentration 3176.6 mg/L 116

D18 Chromatogram of standard oleyl linoleate concentration 4228.6 mg/L 116

LIST OF ABBREVIATIONS

AOAC = American Oil Associate Chemist
ASTM = American Society Testing and Material
BET = Brunauer-Emmett-Teller
CPO = Crude Palm Oil
cSt = Centistokes
DSC = Differential Scanning Calorimeter
FFA = Free Fatty Acid
EDS = Energy-Dispersive X-Ray Spectroscopy
FA = Fatty Acid
FAME = Fatty Acid Methyl Esters
FT-IR = Fourier Transform Infrared Spectrometer
GC = Gas Chromatography
IS = Internal Standard
PFAD = Palm Fatty Acid Distillate
mg = Milligram
nm = Nanometer
ppm = Part per million
rpm = Round per minute
RT = Retention Time
RF = Response Factor
RPO = Refined palm oil
TISTR = Thailand Institute of Scientific and Technological Research

SYNTHESIS OF WAX ESTERS FROM PALM FATTY ACID DISTILLATE AND OLEYL ALCOHOL OVER AMBERLYST 15

INTRODUCTION

Thailand is an agricultural country which cultivates oil plants such as oil palm for industrial uses in producing vegetable oil which is in highly consumer demand. Currently, Thailand is able to produce about 39,583.36 tons per year of refined palm oil (Source: Office of Agricultural Economics, (2009)). The product in palm oil refinery process is not only edible oil but also by-products which consist of free fatty acids called palm fatty acid distillate (PFAD). The PFAD fraction is found about 5-7% of crude oil in distillation process and the process to produce refined palm oil and PFAD was shown in Figure 1. The comparison of the cost between PFAD and crude palm oil shown that PFAD is about 5 baht per kilogram lower than palm oil, such as palm oil price of 25 baht, thus PFAD cost 20 baht (Source: Ministry of Commerce (2010)). Generally, the PFAD was exported to developing countries which use this raw material in oleo chemical industries. Thus, PFAD, a by-product of physical refining of crude palm oil, composed mainly of free fatty acids, glycerides, minor amount of squalene, vitamin E, sterols, and other substances; there were gently need to study for increasing value-added of this by-product. Usually, PFAD is used as a raw material in animal feed, laundry soap industries as well as a raw material for the oleo chemicals industries. In addition, vitamin E (tocopherol and tocotrienol), squalene, and phytosterols can be extracted from PFAD to produce value-added materials for the nutraceutical and cosmetic industries (Yujaroen *et al*., 2009). Wax esters are one of value-added products that are very interesting because of their high price. Therefore, the objective of this research is to investigate the synthesis of wax esters starting from PFAD.

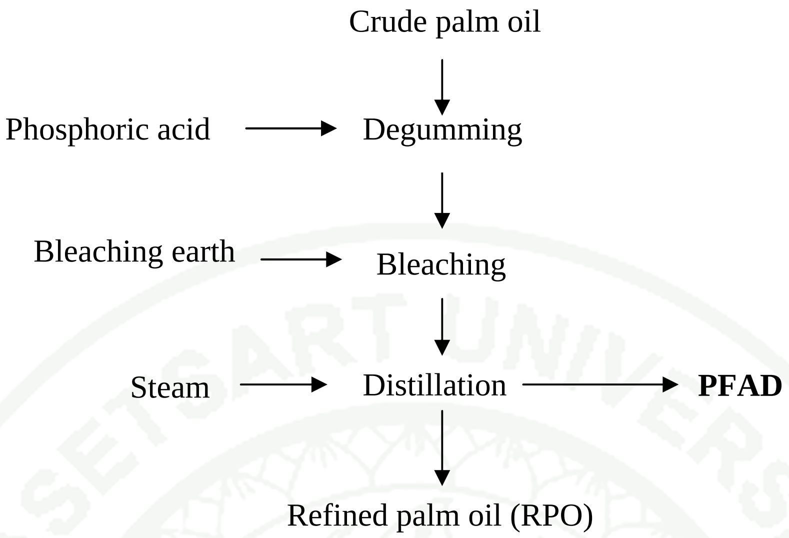


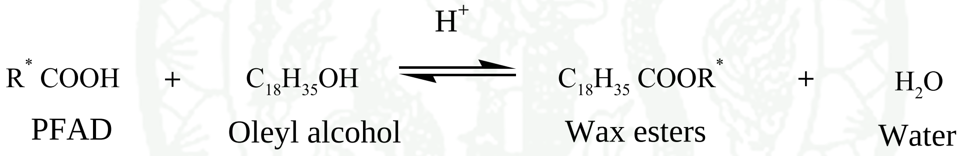
Figure 1 Process of refined oil and PFAD production.

Source: Gunstone (2004)

Wax esters are long chain ester that was derived from fatty acids and alcohol with chain lengths of 12 carbons or more. Wax esters can be classified as solid wax esters (solid at room temperature) and liquid wax esters (liquid at room temperature). The properties were depending on the carbon chain length and degree of unsaturation of esters. Increasing the carbon chain length and saturation will increase the melting point of the esters. Wax esters have high potential in various applications to use as important ingredient; in cosmetics formulations such as cleansers, conditioners and moisturizers (Rit and Behrer, 2001); pharmaceuticals such as anti-foaming agents, penicillin production, a timed release, pharmaceuticals tablets (Kline, 1956); plasticizers and polishes (Chen *et al.*, 1997); foods; personal cares and synthetic lubricants. This is due to their unique property of having excellent wetting behavior at interfaces (Hadzir, 2001) without oily feeling and it is not greasy when applied on human skin.

Natural waxes are usually obtained from animals, vegetables, fruits and inorganic minerals which is mostly containing of high saturated wax esters such as beeswax. The starting materials in saturated and unsaturated waxes are sperm whale and jojoba oil. Because natural waxes are high price and have limitation quantity, so it is necessary to find an alternative way for increasing wax esters product from the reaction between fatty acid and fatty alcohol. Thus, PFAD, a by-product from refined palm oil distillation process is selected as a fatty acid raw material to react with oleyl alcohol in wax esters production. This way is possible to replaces natural waxes and also increases value of PFAD.

Wax esters can be produced by the esterification reaction between long chain fatty acid which from PFAD and long chain alcohol (oleyl alcohol) as shown in Figure 2. Many researchers tried to synthesize wax esters through esterification reaction using oleic acid and oleyl alcohol with immobilized lipase enzyme as a catalyst (Radzi *et al*., 2005).



$$\underset{\text{PFAD}}{R^{*}COOH} + \underset{\text{Oleyl alcohol}}{C_{18}H_{35}OH} \overset{H^{+}}{\rightleftharpoons} \underset{\text{Wax esters}}{C_{18}H_{35}COOR^{*}} + \underset{\text{Water}}{H_2O}$$

Where

$R^{*} = C_{16}H_{32}O_2, C_{18}H_{32}O_2, C_{18}H_{34}O_2$

Figure 2 Esterification reaction of palm fatty acid distillate to produce wax esters

Synthetic wax esters can be synthesized by using chemicals and enzymatic catalysts (Gunawan *et al*., 2004). The chemical substances in homogeneous and heterogeneous catalysts are used for catalysis in esterification and transesterification reaction. The use of homogenous acid catalyst such as sulfuric acid (H_2SO_4) for esterification may lead to several problems such as the corrosion of equipment, hazard of handling, high-energy consumption, and degradation of wax esters (Yadav, 2003; Knox, 1984). The use of enzymatic catalysts was high cost of investment and difficult to control (Wang *et al*., 2008). In addition, this method cannot be used in industry today due to the high price of enzyme and the problems related to its deactivation caused by feed impurities (Kulkarni *et al*., 2006, Fukuda *et al*., 2001, and Haas, 2002).

Heterogeneous acid catalysts used for ion-exchange resins that are composed of copolymers of divinyl-benzene, styrene and sulfonic acid groups grafted on benzene (being the active site) (Ozbay *et al*., 2008). Furthermore, ion-exchange resins can offer better selectivity towards the desired product and better reusability compared to homogeneous acid catalysts (Liu *et al*., 2006). From the viewpoint of cost savings, ion-exchange resins used as heterogeneous catalyst might have greater advantage over enzyme and homogeneous catalyst. Thus, heterogeneous acid catalyst has high potential to replace homogeneous and enzyme catalyst because it can eliminate separation and corrosion problems, including easy to recovery and reuse as well as being compatible with environmental regulations (Kim *et al*., 2004, Lopez *et al*., 2005 and Park *et al*., 2008).

In this work, the synthesis of wax esters was conducted through esterification reaction of palm fatty acid distillate and oleyl alcohol using Amberlyst 15 as a solid acid catalyst. The Amberlyst 15 is a commercial sulfonated resin with good properties in terms of its esterification efficiency. This catalyst is reusable and easy to separate from liquid products.

The effect of various parameters such as amount of catalyst, reaction time, reaction temperature, molar ratios of alcohol to PFAD, and the stirring speed on esterification reactions was investigated to determine the optimum conditions. The percentage conversion of PFAD was determined by free fatty acid calculation and the percentage product yield of wax esters was also determined by GC analysis. In addition FT-IR analysis was used to confirm the produced wax esters of wax esters from the optimum condition were also determined, lastly, the physicochemical properties.

The main objects of this experiment are:

1. To study the chemical compositions of PFAD.

2. To study the effect of parameters on esterification reaction of wax esters synthesis. (Such as amount of catalyst, reaction time, reaction temperature, molar ratios of PFAD to oleyl alcohol, and stirring speed).

3. To study the optimum condition of wax esters synthesis.

4. To investigate the physicochemical properties of wax esters.

LITERATURE REVIEW

1. Wax esters

Wax esters are high-molecular weight esters derived from long-chain acids and long-chain alcohols. The properties depend on the carbon chain length and degree of unsaturation of esters. Furthermore, wax esters can be extracted from animals and plant material such as beeswax (Nelson *et al.*, 2000), insect, lanolin, sperm whale, jojoba oil and carnauba oil. However, they are expensive for commercial use and the main disadvantage to the large-scale use is its availability (Sanchez, 1992). Wax esters are important for ingredients in cosmetic formulations such as cleansers, conditioners and moisturizers (Rit *et al.*, 2001), in the pharmaceutical products (as an anti-foaming agent) in the production penicillin and a timed release in the production of pharmaceutical tablet, lubricants, plasticizers and polishes. This is due to the unique properties of wax esters in having non-toxicity, good fat soluble properties and excellent wetting behavior without oily feeling.

1.1 Source of wax esters

There are two types of wax esters: natural wax and synthesis wax. The detail of both waxes can be explained as follows:

1.1.1 Beeswax is an abdominal secretion of bees (*Apis mellifera*). It is a hard amorphous solid, usually depending on the source and manufacturing process. Pure beeswax consists of about 0-80% of long chain esters. Beeswax is used as a component of seals, coatings, polishes and candles.

1.1.2 Spermaceti wax is extracted by cooling from adipose tissues and also collected from a big cavity in the head of a cachalot (*Physeter acrocephalus*) known as sperm whale. This product contains fatty esters 65-95%. Spermaceti wax is used in cosmetics, pharmacy and candles.

1.1.3 Chinese insect wax is secreted by insects (Coccus ceriferus). Its content in esters is about 83%. The purified wax is used to make candles and polish.

1.1.4 Shellac wax is produced by a cochineal insect (Tachardia lacca) native of India. It contains 70-82% of esters; this wax is used in the varnish industry and may replace carnauba wax.

1.1.5 Carnauba wax is secreted by leaves of a Brasilian palm tree (*Copernicia prunifera cerifera*), which can be harvested from the dried leaves. It contains mainly 80-85% wax esters. It is not only used mainly mixed to beeswax to make various polishes for shoes, floor and furniture but also in cosmetics (lipsticks and creams) and in the food industry (glazes for candies, gums, fruit coatings).

1.1.6 Jojoba oil wax is fluid (melting point about 7ºC) and it is produced by pressing from seeds of the jojoba tree (*Simmondsia chinensis*, *Euphorbiacae*). It is formed quite exclusively of alcohols esterified with long-chain fatty acids (more than 98%) with a total of 38 to 44 carbon atoms. Jojoba oil is very resistant to oxidation and it is largely used in cosmetics, lubricants, polishes and candles.

1.1.7 Candelilla wax is a vegetable wax produced mainly in Mexico. It is used chiefly in the manufacturing of chewing gum and cosmetics. The chemical composition of candelilla wax is 28-29% of esters.

1.1.8 Wool wax is secreted by sheep sebaceous glands and it is collected from crude wool by dilute alkali or detergent washing. Lanolin contains fatty esters about 14-24%.

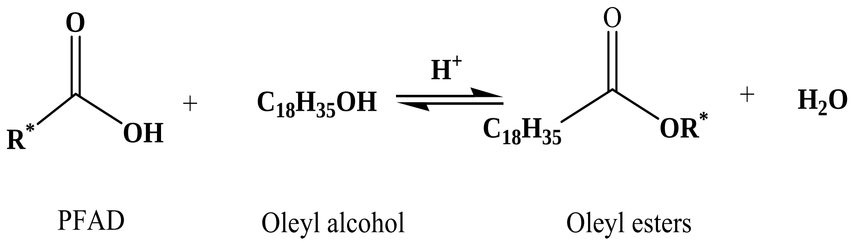
1.1.9 Synthetic wax esters are waxes only in the sense that they possess physical properties customarily attributed to the natural waxes**.** Improvement in the art of oxidizing the paraffin to acids with the same carbon content, and then combining these wax acids with alcohols of a high molecular weight, would be the means of yielding the type of esters that are found in the natural wax. The source of wax alcohols would be derived from the reduction of mixed fatty acids by means of hydrogen with the aid of a catalyst**.** Typically, synthetic wax, such as halogenated straight chain hydrocarbons, unsaponifiable wax, emulsifiable wax, synthetic hydrocarbon wax, hydrogenated wax and symmetrical ketone is chemically synthesized compounds that have no close relation to the natural waxes in chemical structures, but which are similar to them in physical characteristics.

1.2 Synthesis of wax esters from vegetable oils can be synthesized from two methods.

1.2.1 Chemical methods; these methods use homogeneous catalyst to produce esters, For example, acid and basic homogeneous catalysts for esterification and transesterification reaction.

1.2.2 Enzymatic methods; these methods use lipase enzyme for catalysis through esterification and transesterification reaction.

Several literatures reported the synthesis of wax esters from vegetable oil or fatty acid with oleyl alcohol using enzyme as catalyst. Because of enzyme catalyst was high price and difficult for controlling, thus, in this research Amberlyst 15 was selected as a solid acid catalyst to replace enzyme and homogeneous catalysts. This catalyst was supposed to reduce percent free fatty acid in esterification reaction of palm fatty acid distillate. The main product was oleyl esters and water was by-product as shown in Figure 3.



Where:

R*; $C_{16}H_{32}O_2$ (Palmitic acid), $C_{18}H_{34}$ O_2 (Oleic acid) and $C_{18}H_{32}O_2$ (Linoleic acid)

Oleyl esters; Oleyl palmitate, Oleyl oleate, Oleyl linoleate

Figure 3 Esterification reaction of PFAD and oleyl alcohol.

From Figure 3 the direction of the shift of an equilibrium can be predicted qualitatively using LeChâtelier's principle. LeChâtelier's principle states that if a stress is applied to a system at equilibrium, the equilibrium will tend to shift in a direction to relieve that stress. A stress is a change of conditions, such as the change in the temperature of the system, pressure, catalyst amount, reaction time, stirring speed and concentration of the reactant and product. In this experiment these various effects was studied.

1.3 Factors that effect equilibrium condition of esterification reaction of the synthesis of wax esters

1.3.1 Effect of reaction temperature

Increasing the temperature always favors the endothermic change, and decreasing the temperature always favors the exothermic change. In this case, the reaction is forced to the right by an increase in temperature. A decrease in temperature causes the position of equilibrium to shift to the left. Such as the thermo chemical in Figure 3, the synthesis of wax esters when the reaction is exothermic (ΔH is negative), we include heat as a product. If heat is added (the temperature of system is raised). The position of equilibrium will shift to the left, the direction in which heat is absorbed. If the mixture is cooled, the position of equilibrium will shift to the right, the direction in which heat is evolved.

1.3.2 Effect of adding or removing reactants or products

Increasing the concentration of one of the reactants or decreasing the concentration of one the products shifts the equilibrium in favor of the products. On the other hand, decreasing the concentration of one of the reactants or increasing the concentration of one of products shifts the equilibrium in favor of reactants. The effect of concentration on equilibrium state can be demonstrated by Figure 3. The position of equilibrium is said to have shifted to the right. If the concentration of wax esters is increase, the position of equilibrium will shift to the left. In this way some wax esters will be used up. When equilibrium is established again, the concentration of oleyl alcohol and PFAD will be higher than they were initially.

1.3.3 Effect of addition of a catalyst

Catalysts can not change the position of equilibrium. A catalyst functions by lowering the activation energy for a reaction. A catalyst speeds up both the forward and reverse reactions, and therefore it has no net effect on the position of the equilibrium. However, the lowered activation energy allows equilibrium to be established more quickly than if the catalyst were absent.

1.3.4 Effect of reaction time

The production yield is nearly independent of reaction time but the oleyl esters concentration increases with increasing reaction time.

1.3.5 Effect of stirring speed

Stirring speed plays a major role in a molecule movement. Higher stirring speed can increase the movement of molecules, thus resulting in the high combination of catalysts to substrates. Moreover, it will also increase external mass transfer rates between the bulk phase of the reaction mixture and the surface of catalyst.

1.4 Application of wax esters

1.4.1 Coatings for Fruits and Plants (Apples, Lemons, Bananas, Melons, Oranges, Cucumbers, Tomatos)
1.4.2 Candles
1.4.3 Plasticizers
1.4.4 Paper
1.4.5 Lubricant and slip/release agents
1.4.6 Adhesives
1.4.7 Cosmetics
1.4.8 Pharmaceuticals
1.4.9 Inks andcoatings
1.4.10 Fertilizers

Many researchers have been working on the synthesis of wax esters. Their works were summarized as follows:

Sanchez *et al*. (1992) studied the esterification of oleic acid and oleyl alcohol using zeolite as catalyst and compared with the Jojoba oil. The variables studied were reaction temperature, catalyst concentration and reduced pressure. A full 2^3 factorial design at two levels was used in the synthesis. The result showed that the best condition was at 180 ºC, using 0.6 wt% of zeolite and reduced pressure of 16 mmHg. The reaction gave 47% of ester yield. The properties of oleyl oleate and jojoba oil were compared. Solidifying temperature of 6 and 8 ºC, Saponification values of 94 and 92, Iodine value of 94 and 82, and viscosity at 25 ºC of 29 and 35.2 cp for oleyl oleate and jojoba oil, respectively. Thus, the synthesis oleyl oleate has properties like from jojoba oil.

Abdul Rahman *et al*. (2001) studied esterification reaction between oleic acid and oleyl alcohol to produced oleyl oleate as wax esters product. The result showed that the optimum conditions for ester synthesis were at 50 ºC for 2 hours using 100 mg of catalyst and 1:1 of molar ratio of oleyl alcohol to oleic acid and the initial water activity of 0.120. The results showed a maximum yield at 90%.

Steinke *et al*. (2001) studied the preparation of wax esters through esterification reaction of long chain fatty acids and oleyl alcohols from Crambe and Cramelina oil by using lipase as biocatalyst. The result showed the highest percentage conversion to wax esters was obtained at 95% after 4-6 hours of reaction time. Wax esters product had the compositions approaching to jojoba (*Simmondsia chinensis*) oil, especially Crambe fatty acids in combination with Cramelina alcohols or Cramelina fatty acids in combination with crambe alcohols were used as substrates.

Gunawan *et al*. (2004) studied the synthesis of wax esters from palm oil and oleyl alcohols that was catalyzed by lipase enzyme. The effects of various reaction parameters such as reaction time, temperature, amount of enzyme, molar ratio of substrates, various organic solvents and initial water activity (a_w) of the reaction system were investigated. The optimum conditions to produce wax esters were: 5-7 hours incubation time, 40-50 ºC, 1.5% (w/v) of enzyme and 3:1 molar ratio of oleyl alcohol to palm oil. Hexane was the best solvent for this reaction. At the optimum condition, 78-83% wax esters were produced.

Radzi *et al*. (2005) studied the synthesis of oleyl oleate in large scale reaction by using a stirred tank reactor. The oleyl oleate was synthesized from oleic acid and oleyl alcohol catalyzed by Novozym 435 (immobilized Candida antartica Lipase B as biocatalyst. The effect of various reaction parameters were optimized to obtain a high yield of liquid wax esters. Investigation in large scale production was performed in batch mode of a stirred tank reactor with one multi-bladed impeller. The optimum condition to produce liquid wax esters was, reaction time at 30 minute, reaction temperature at 50 ºC, 90 g catalyst, agitation speed of 400 rpm, number of impeller tip(N) of 2 and molar ratio of oleyl alcohol to oleic acid of 2:1. Analysis of the yield showed that at optimum condition, >90% liquid wax esters were produced. The stability of catalyst showed at high percentage (>80%) up to 4 cycles.

Radzi *et al*. (2005) studied the synthesis of oleyl oleate through esterification reaction between oleic acid and oleyl alcohol using lipase-catalysed. Various reaction parameters were optimized to obtain a high yield of oleyl oleate. The optimum condition to produce oleyl oleate was: reaction time at 5 min, organic solvents of log P ≥ 3.5, temperature; 40-50ºC, amount of enzyme; 0.2- 0.4 g and molar ratio of oleyl alcohol to oleic acid; 2:1. The operational stability of enzyme was maintained at >90% yield up to 9 cycles. At the optimum condition, >95% liquid wax esters were produced.

Gunawan *et al*. (2008) studied the synthesis of wax esters through alcoholysis reaction between palm kernel oil and oleyl alcohol. In this work, the effects of various parameters were studied. The optimum conditions were reaction time for 10 hours, reaction temperature of 40 ºC, amount of catalyst of 1.5%, molar ratio of substrates 1: 3 (PKO: oleyl alcohol) and hexane was used as an organic solvent. The reaction mixture products were analyzed by a gas chromatograph (GC) which the product composition was quantified by an internal standard method with methyl linoleate as an internal standard. The total amount of wax esters product from this experiment was more than 80% yield.

Keng *et al*. (2008) studied the synthesis of wax esters through alcoholysis reaction of palm oil, palm olein, palm stearin, palm kernel oil, palm kernel olein with oleyl alcohol. The analysis of the product composition of all fraction samples was determined by gas chromatography (GC). The optimum condition of this reaction was 5 hours of reaction time, 50°C of reaction temperature, 16 % of catalyst concentration; 3:1 of molar ratio of oleyl alcohol to palm oil. This condition gave more than 80 % of wax esters yield. The result of physicochemical properties of wax ester was compared together. These properties of wax esters such as refractive index, density, surface tension, slip melting point, Saponification value, iodine value and acid value were analyzed following the American Oil Chemists' Society standards.

Vijayakuma *et al*. (2009) studied the wax esters synthesis by using a long chain fatty acid of spermaceti mainly palmitic acid and fatty alcohol with acid activated Indian bentonite as a catalyst. In addition, microwave was used to produce cetyl palmitate too. The optimum condition showed that molar ratio of cetyl alcohol to palmitic acid was at 1:1.2, using 300 mg of catalyst and 120 min of reaction time. This condition gave 81% of wax ester yield. In addition, jojoba oil was used to synthesize oleyl oleate because it had mainly contained oleic acid to react with oleyl alcohol using p-toluene sulphonic as a catalyst. The optimum condition showed that 1:1.5 molar ratio of oleyl alcohol to oleic acid, 150 mg catalyst and 2 min of reaction time. This condition gave 44% of wax ester yield.

2. Palm Fatty Acid Distillate (PFAD)

The PFAD was a by-product from the physical refining of crude palm oil (CPO) in refined palm oil industry. The diagram of refined palm oil production is in Figure 4. The deodorization stage produced palm fatty acid distillate. This deodorization stage removed free fatty acids, aldehydes, ketones and other short-chain compounds from oxidation reaction, tocopherols (vitamin E), sterols, carotenes, degradation products, nitrosamines, residual extraction solvents, organo-chlorine pesticides and volatile sulfur compounds. This stage was feasible because of the great differences in volatility between these undesirable substances and triglyceride. This stage was carried out by steam distillation process at temperature between 170 and 250 ºC under reduced pressure and by using a stripping media, such as steam or nitrogen. Since the substances responsible for odors and flavors are usually volatile so the distilled fatty acid was known as PFAD. A high odor product also contained some glycerol esters along with minor components of the feed stocks. It was a light brown solid at room temperature and was melted to a brown liquid on heating. The physical and chemical properties of PFAD are showed in Table 1. Palm fatty acid distillate was usually used as a raw material in soap industries, animal feed industries and oleo chemical industries.

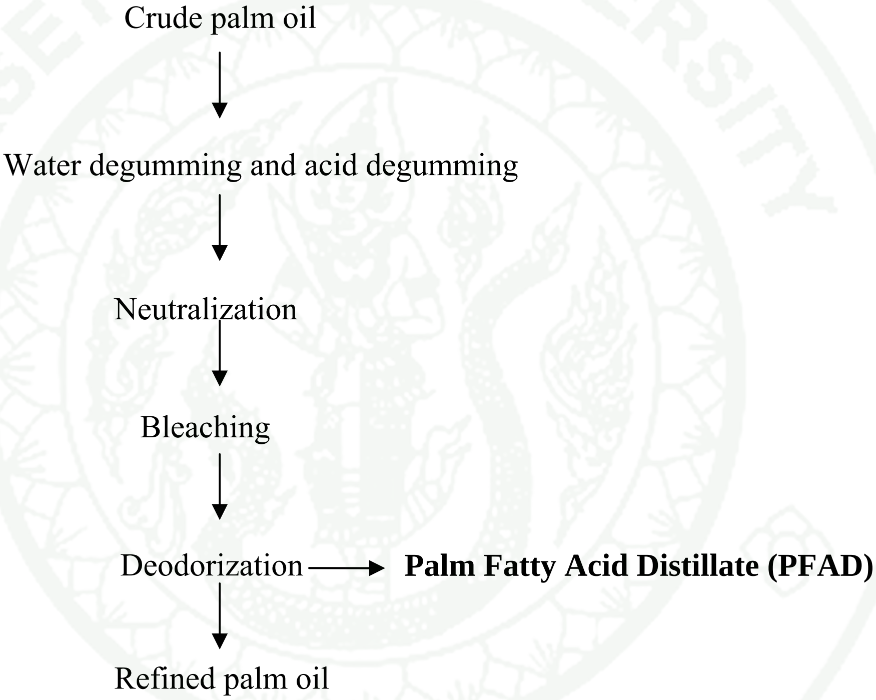


Figure 4 Oil refined processing steps.

Source: Gunstone (2004)

Table 1 Characteristics of PFAD.

Properties	Value
Iodine value (g I_2/100 g of sample)	55.3
Free fatty acid (%) (as palmitic acid)	75-89
Moisture content (%)	0.08
Saponification value (mg KOH/g of sample)	198
Unsaponification matter (%)	2.5
Vitamin E (%)	0.5
Squalene (%)	0.8
Sterols (%)	0.4
Fatty acid composition (%)	
Palmitic acid (C16:0)	47.1
Stearic acid (C18:0)	4.5
Oleic acid (C18:1)	36.6
Linoleic acid (C18:2)	9.6
Linolenic acid (C18:3)	0.47

Source: Hamirin (1989)

Applications of palm fatty acid distillate (PFAD) in oleo chemical industries

1. Ingredients in feed fat formulation for feed animals. PFAD has been traditionally and widely used as an ingredient in an animal feed industry in the form of both free acid and calcium soap. Calcium soap is composed of long-chain fatty acids which are reacted with calcium oxide or calcium hydroxide to yield a calcium salt or soap product as shown in Equation 1. This calcium salt is known in the industry as “CaPFAD”. This product is relatively inert in the first digestive compartment (rumen) of ruminants (e. g. cows) but it is broken down to free fatty acids in the abomasum, or true stomach. The fatty acids are then absorbed from the small intestine and utilized by the cow for milk fat production or as an energy source. The advantages of PFAD as a feed fat are: a) PFAD is probably one of the cheapest and the most reliable sources of feed fat. b) PFAD is a source of essential fatty acid (EFA) as PFAD contains about 10% of linoleic acid (C 18:2). c) PFAD is also less prone to rancidity than some other fats. PFAD contains low amount of linolenic acid (C18:3) and high amount of vitamin E which acts as an antioxidant. d) On hydrogenation, PFAD is converted to hydrogenate PFAD (HPFAD) which is also used in the animal feed industry.

$$2\mathrm{RCOOH} + \mathrm{Ca(OH)_2} \longrightarrow 2\mathrm{RCOO^-Ca^{2+}} + 2\mathrm{H_2O} \qquad \text{Eqn. 1}$$

2. PFAD contains high amount of Vitamin E (mostly tocopherols and tocotrienols), which acts as a powerful antioxidant. The concentration of vitamin E in PFAD is about 2160-4240 ppm (Md Top, 1981). There is an increasing interest in nutraceutical feed and in the functional food industry, which promotes food-derived phytochemicals as their active ingredients. There is a growing body of evidence that vitamin E is important for antioxidant activities in foods and biological systems. Palm vitamin E is a tocopherol and tocotrienol-rich product that has the chemical structure as shown in Figures 5 and 6, respectively.

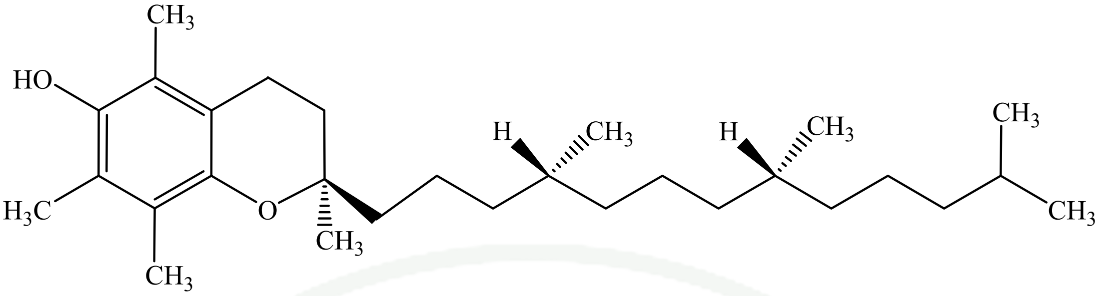


Figure 5 Chemical structure of tocopherol.

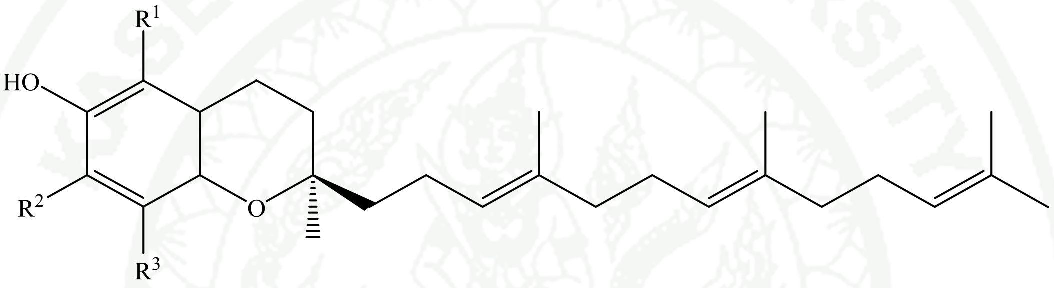


Figure 6 Chemical structure of tocotrienol.

3. Squalene is a natural emollient and finds wide utilizations in the cosmetic industry in the form of squalene or as fully hydrogenated squalane. Other areas of squalene utilizations are in health food and pharmaceutical industries. However, because of concerning on sustainability and availability of squalene from the traditional sources, a new source such as PFAD is much desirable. It is necessary desirable to isolate useful phytochemicals compounds from PFAD and deliver them in concentrated and pure forms to relevant industries for the benefit of consumers. Extraction of the high value compounds, such as vitamin E, squalene and phytosterols from PFAD, will represent a value added of PFAD itself and palm oil industry. The chemical structure of squalene is shown in Figure 7.

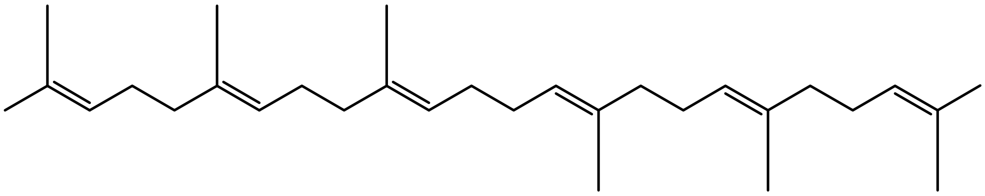


Figure 7 Structure of squalene.

4. PFAD is used for the manufacture of laundry and toilet soaps.

5. Fatty acids of PFAD could also be esterified and the product is used as a feedstock for oleo chemicals industry and biofuel.

The application of PFAD has been studied by many researchers. Their work is summarized as follows:

Chu *et al.* (2003) studied the separation of tocopherols and tocotrienols from palm fatty acid distillate by comparison chromatography method and hydrolysis-neutralization-adsorption method. They used three methods for separating vitamin E. The first method used neutralization and adsorption column chromatography. The results showed 98.30 % recovery of vitamin E was obtained. The second method used only adsorption column chromatography. The results showed 90.40 % recovery of vitamin E was obtained. The last method used hydrolysis, neutralization and adsorption column chromatography. The results showed 96.71 % recovery of vitamin E was obtained. From comparing all of these experiments, the method of neutralization gave the highest percent recovery of vitamin E from palm fatty acid distillate.

Chongkhong *et al*. (2007) used palm fatty acid distillate as a raw material to react with methanol and sulfuric acid was used as catalyst to produced biodiesel. They studied two methods, the first method studied esterification reaction in batch process. The result showed that the optimum conditions were 4.3:1 molar ratio of methanol to

PFAD, 90 min of reaction time, 100ºC of reaction temperature and 1.834 % wt of catalyst amount. The second method was the continuous esterification process which showed that the optimum conditions was 70ºC of reaction temperature, 60 min of reaction time, 1.834 wt % of H_2SO_4 catalyst, 8:1 molar ratio of MeOH to PFAD. The purification processes of fatty acid methyl ester were performed by neutralized with 3 M NaOH in water at 80ºC for 15 min. After that it was followed by transesterification process with 0.396 M sodium hydroxide in methanol solution at a reaction temperature of 65 ºC for 15 min. This method could reduce the amount of free fatty acid from 93 % wt in PFAD to 2 % wt in fatty acid methyl esters.

Boonnoun *et al*. (2008) used palm fatty acid distillate (PFAD) which composed of free fatty acid (FFA) about 90-93 %wt to produced biodiesel through esterification reaction. They used PFAD to react with methanol having sulfuric acid as catalyst. The results were discussed in two parts. Part I was esterification step and part II was the transesterification step. The optimum conditions of esterification process were 120 min of reaction time, 60-70 ºC of reaction temperature, 16:1 molar ratio of methanol to oil and 1% w/v of H_2SO_4. It could reduce the acid value of raw material from 183 mg/g KOH to less than 0.5 mg/g KOH. The purity of biodiesel was between 80-90 % and viscosity was in the range of 4-6 mm^2/s. In transesterification step, the optimum condition of reaction was 0.6 % wt of KOH, 8:1 molar ratio of methanol to PFAD and 40 min of reaction time. After transesterication step, the acid value of biodiesel could be reduced to below 0.8 % with the purity of biodiesel higher than 98 % wt of methyl ester content.

Suwanwong *et al*. (2008) studied the production of fatty acid methyl ester (FAME) from palm fatty acid distillate (PFAD) that contained high free fatty acid by using esterification reaction and ferric sulfate was used as a solid catalyst. The percentage free fatty acid (FFA) conversion of the reaction mixture was determined by standard titration method (Ozbay *et al*., 2008). This experiment showed that the optimum conditions were 40 % w/w catalyst, 12:1 molar ratios of methanol to PFAD, 360 min of reaction time and 95ºC reaction temperature. This condition gave 98% wt of fatty acid methyl ester content.

Yujaroen *et al*. (2009) studied the production of biodiesel through esterification reaction of palm fatty acid distillate (PFAD) by using supercritical methanol. They used PFAD that contained high free fatty acid (about 93%). Their FAMEs analysis in products was carried out by gas chromatography with cosine that was used as an internal standard. The optimum conditions were at 300 ºC reaction temperature, 30 min of reaction time, 6:1 of molars ratio of methanol to PFAD. The yield of biodiesel at the optimum condition was 95 % wt of fatty acid methyl ester content.

3. Amberlyst 15 (an acidic ion exchange resin)

In this study, Amberlyst 15 was selected as a solid acid heterogeneous catalyst. It had acid properties to reduce free fatty acid (FFA) in esterification reaction. The advantage of this solid catalyst was its easy separation from the reaction products. Amberlyst 15 was used in several application reactions, such as dehydration of alcohol and etherification of oleins with alcohol. Amberlyst 15 which was ion-exchange resin containing acidic styrene divinyl benzene sulfonated, was used to reduce free fatty acid in esterification reaction in the feed stocks which contained high free fatty acid. In addition, the catalyst was recycled and reused with negligible loss in activity.

Amberlyst 15 was a bead form, strongly acidic ion exchange resin developed particularly for heterogeneous acid catalysis of a wide variety of organic reactions. It was also useful in non aqueous ion exchange systems for the removal of cationic purity. It could be described as an insoluble polymer matrix that could exchange ions with the adjacent mixture. The resin could be formed by the copolymerization of styrene with divinylbenzene. These acts as cross linking agent. The reaction is shown in Figure 8. The amount of cross linking had a pronounced effect on the resin's ability to swell when the resin was immersed in solution (Laatikainen *et al*., 2002).

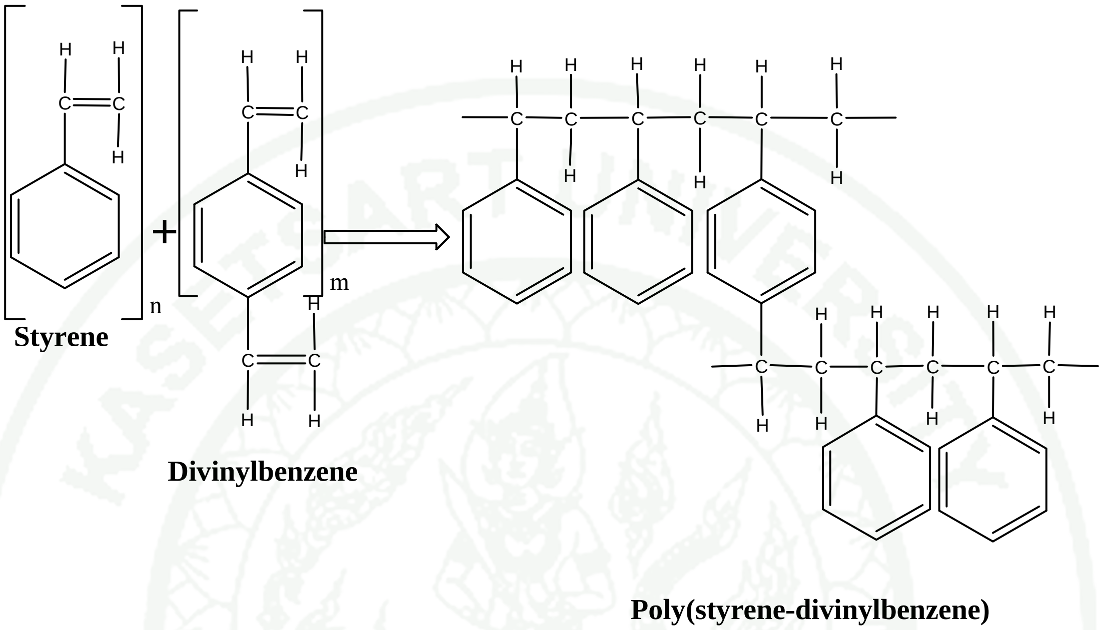
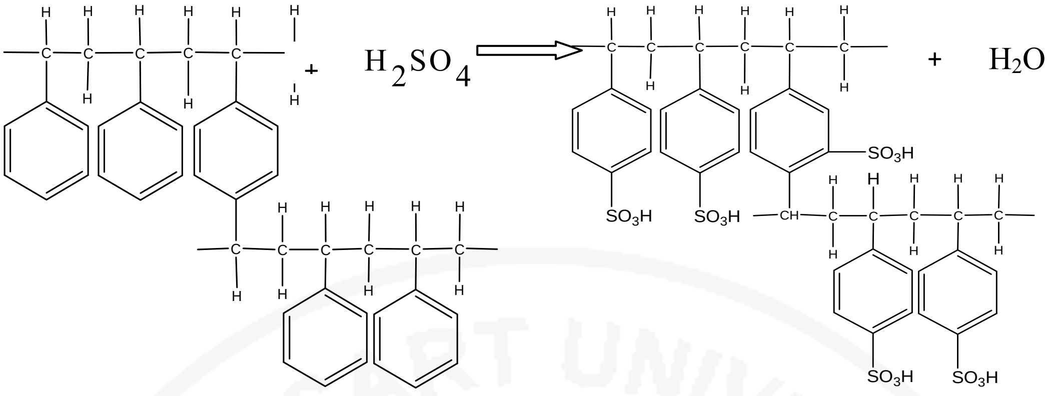


Figure 8 The copolymerization of sytrene and divinylbenzene.

Source: Helfferich (1962)

For the reaction to proceed on catalyst surface, active sites needed to be placed on the resin matrix. For cation exchange resins, acid sites were deposited on the polymer matrix by the treatment of the polymer matrix with a strong acid. For the formation of sulphonated cation exchange resins the polymer matrix was treated with concentrated sulfuric acid. The reaction was shown in Figure 9. The acid loading of the resin was a measure catalytic activity of the polymer matrix, and it plays an important part in catalysis reaction (Chakrabarti *et al*., 1993).



Poly(styrene-divinylbenzene) **Sulfonated cation exchange resins**

Figure 9 Sulphonation reaction of polymer matrix.

Amberlyst 15 was more environmentally friendly catalyst. The advantages of heterogeneous catalysts were found more than their homogenous counterparts (Harmer *et al*., 2001). The examples of advantages were reduced equipment corrosion; ease of product separation, less potential contamination in waste streams and recycles of the catalyst. The selectivity may also be improved in going to a solid acid catalyst. The properties of Amberlyst 15 were shown in Table 2.

Table 2 Properties of Amberlyst 15.

Properties	Values
Crosslinked structure	High
Form	H^+
Acidity (mmol/g)	4.7
Average pore diameter (nm)	30
Surface area (m^2/g)	53
Particle size (mm)	16-50 mesh
Moisture (%)	<1.6
T_{max} (ºC)	120

Source: Klepacova *et al*. (2005)

Many researchers studied reactions that used Amberlyst 15 as a catalyst in their reactions. The summary of works is as explain follows:

Yadav *et al*. (2002) used two heterogeneous catalysts such as Amberlyst 15 and Amberlyst 36 for esterification reaction. Both catalysts were used in the reaction between maleic acid and ethanol to produce diethyl maleate. The analysis reaction was titrated with alcoholic KOH solution. The quantitation of diethyl maleate was conducted by gas chromatography (GC). For purification of the product, catalyst was filtered off and excess unreacted ethanol was removed from the reaction mixture by using an evaporator. The remaining mass was treated with sodium carbonate to remove unreacted maleic acid and then treated with diethyl ether to extract ester product in an organic phase. The product was further purified by vacuum distillation.

The result showed that Amberlyst 15 gave more than 60% fatty acid conversions when compared to Amberlyst 36.

Izci *et al*. (2007) studied adsorption equilibrium constants and the kinetic parameters of reaction between isobutanol and propionic acid by using Amberlyst 15 as catalyst to produced isobutyl propionate. The product was analyzed by a gas chromatography and water contents in product were analyzed by Karl Fischer method. They showed that the kinetic experiment data was obtained in a batch reactor within the temperature range of 318–348 K. The reaction took place between an adsorbed alcohol molecule and an acid molecule in the bulk phase. It was also observed that water had inhibiting effect on reaction. The activation energy of esterification reaction was at 42.5kJ/mol. The heat of adsorption for isobutanol and water was also calculated as -36.9 kJ/ mol and - 48.7kJ/mol, respectively.

Marchetti *et al*. (2007) studied esterification reaction of frying oil that contained high free fatty acid with ethanol by using Amberlyst 15 as a catalyst to produce biodiesel. The results showed that the optimum condition was 6:1 of molar ratio of alcohol to acid, 2-5% catalyst, 45-55ºC of reaction temperature. This condition gave the percentage conversion of free fatty acid more than 75%.

Lathi *et al*., (2007) reported the reusability of Amberlyst 15 by repeating the reaction four times. At the end of the reaction, the catalyst was filtered and further refluxed with 50 ml alcohol for 30 min to remove any adsorbed materials from the catalyst surface. After that it was dried at 110 ºC. The result showed that catalyst could be reusable up to four times with little difference in percent conversion.

Ozbay *et al*. (2008) studied esterification reaction of free fatty acids that contained in waste cooking oils by using ion-exchange resins. The amount of free fatty acid (FFA) in waste cooking oil caused saponification problems during transesterification reaction. Acidic ion-exchange resins could be used to decrease the amount of free fatty acid in waste cooking oil by esterification reaction with methanol. They found that the optimum conditions were 60˚C of reaction temperature, 2 % wt of solid catalysts. The result showed that FFA conversion was about 45.7%.

Kolah *et al*. (2008) studied the kinetic reaction of succinic acid and ethanol to form monoethyl and diethyl succinate by using Amberlyst 15 as catalyst. The occurring of monoethyl succinate (MES), and diethyl succinate (DES) was confirmed by GC-MS. Experimental data were obtained between 78 and 120 ºC, molar ratio of ethanol to saccinic acid from 10:1 to 20:1 and Amberlyst 15 varied from 1% to 5% of solution. Kinetics esterification was described using a mole fraction based on pseudohomogeneous model that includes intraparticle mass transfer limitations expressed as effectiveness factor for the first order irreversible reaction. The rate expressions described the kinetics of MES and DES formation over a wide range of catalyst concentration, reactant molar ratios, and temperature. The model presented can be conveniently used for design and scale-up of integrated processes like reactive distillation for synthesis of DES.

Ozbay *et al*. (2008) studied the comparison of different strong acidic ion-exchange resin that affected on biodiesel synthesis from waste cooking oil. The highest FFA conversion (45.7%) was obtained over strong acidic ion-exchange resin of Amberlyst 15 at 60ºC with 2 wt% of catalyst amount. In terms of the level of catalytic activities, the order was found to be Amberlyst 15 > Amberlyst 35 > Amberlyst 16. Comparable performance level of Amberlyst 15 catalyst with conventional catalyst, Amberlyst 15 made it the best probable.

Feng *et al*. (2009) investigated the activities of three cation-exchange resins, NKC-9(the same properties as Amberlyst 15), 001 - 7 and D 61, for esterification of waste fry oil with an acid value of 13.7 KOH/g. They studied the effect of the amount of catalyst, reaction temperature, reaction time and methanol/oil molar ratio on the FFA conversion. Moreover, the characterizations of feedstock and production were also analyzed with titration to measure the acid value. The feedstock and product were analyzed by GC-MS. The comparison of different cation-exchange resins with FFA conversions were 79.7%, 32.2% and 10.3% by using NKC-9, 001-7 and D 61 as catalysts, respectively. Thus, the NKC-9 ion exchange resins catalyst that had properties like Amberlyst 15 catalytic activity showed excellent potential applications to esterification in frying oil.

Talukder *et al*. (2009) studied the synthesis of biodiesel from (PFAD and methanol using enzyme and Amberlyst 15 as catalyst to compare the performance of both catalysts in biodiesel synthesis. They investigated the effect of various parameters and the result showed that the amount of enzyme and Amberlyst 15 were 1% and 30 % wt on PFAD, respectively, reaction time of enzyme and Amberlyst 15 were 2 h, 6-8 h, respectively. Water usually inhibited esterification and accelerates hydrolysis reaction. When increase water content, biodiesel yield from Amberlyst 15 catalytic system dropped from 97 to 80% but enzyme catalyst system is the most active for esterification and the least active for hydrolysis reaction. Therefore the effect of water on the esterification activity of enzyme was not significant.

The wax esters could be analyzed and identified by gas chromatography (GC) and identified and ATR-FT-IR. The summary of works in this field is as follows:

Ke *et al*. (1976) reported the determination of wax esters in lube oil by infrared spectrometry. They found that IR was a simple and rapid method for estimating the content of wax esters in lubricating oils. The ratio of the absorption bands at 1740 and 1385 cm^{-1} could be used directly for determination of wax esters content in lube oil samples.

Isbell *et al*. (1996) studied the composition of wax esters and other composition in meadow foam oil. They analyzed hydrolysis product of wax esters as fatty acid methyl esters and fatty alcohols. The result showed a mixture of saturated fatty acid methyl esters from C_{16} to C_{32} including the small amounts of the odd-chain methyl esters C_{21}-C_{29} atom with the predominately methyl ester being C_{24} by gas chromatography. The analysis conditions with polar column: 172 KPa He head pressure, programmed ramp 150°C to 250°C at 3°C /min with a 2 min hold at 250ºC, injector and detector set at 250°C. The analysis condition with non-polar column analysis conditions: 172 KPa He head pressure, programmed ramp 250°C to 350°C at 3°C /min with a 2 min hold at 350ºC, injector and detector set at 250°C.

Bertran *et al*. (1999) determined free fatty acid in olive oil by Fourier Transform Infrared Spectroscopy (FT-IR). Two spectral ranges were studied; the first range (1775–1689 cm^{-1}) exhibited a strong band at 1748 cm^{-1} that corresponded to absorption by carbonyl bonds in acyl glycerides; another at 1710 cm^{-1}, due to the carbonyl bonds in free fatty acids, strongly overlapped with the previous one. In order to complement the spectral information and assess its potential influence on quantitation, an additional range (1480–1050 cm^{-1}) was selected. It provided information about asymmetric stretching in methyl and methylene groups (~1465 cm^{-1}), a band due to stretching in the C–O bonds of aliphatic esters (~1160 cm^{-1}).

Sejal *et al*. (2001) reported the analysis of wax esters from the synthesis of alcohol and acid chloride that was dissolved in diethyl ether or tetrahydrofuran to obtain solution mixture which analyzed by GC-MS equipped with a pressure programmable cool on column injection port. The column consisted of a 1 m retention gap connected to a capillary column and coupled to a quadrupole mass selective detector. Helium was used as carrier gas and the initial column temperature was between 150 and 200 ºC. The temperature program was increased to 320 ºC at 3 or 4º/min of the rate and held constant at 320 ºC. The result showed the purity of wax esters greater than 98%.

Stransky *et al*. (2006) analyzed wax esters from acetate synthesis by using several alcohols (C_{22}, C_{24}, C_{26}, C_{28} and C_{30}). After synthesis the reaction mixture was picked up to analyze wax ester composition by GC equipped with a flame ionization detector. The injector was used in a split mode with a SGE 4 mm I.D. Chromatographic conditions were: injector and detector temperatures 260 ºC, oven temperature 240 ºC (0 min), rate of 1ºC min^{-1} to 340 ºC, hydrogen gas as a carrier gas, 130 kPa, the rate at 60 cm/s at 240ºC.

MATERIALS AND METHODS

Materials

1. Palm fatty acid distillate samples were obtained from Patum Vegetable Oil Co., Ltd (Thailand)

Figure 10 Palm fatty acid distillate (PFAD).

2. Oleyl alcohol (65%) (Commercial grade, Aldrich, USA)
3. Amberlyst 15 from Rohm and Haas (Commercial grade, Aldrich, USA)

Figure 11 Amberlyst 15.

4. Reagents
    - Anhydrous sodium sulphate (Analytical grade, APS, Australia)
    - Boron trifluoride in methanol ($BF_3$14%v/v, Supelco Analytical, USA)
    - Ethanol 95% (Analytical grade, Merck, Germany)
    - Hexane (Analytical grade, Merck, Germany)
    - n-Heptane (Analytical grade, Merck, Germany)
    - Methanol 99% (Analytical grade, Merck, Germany)
    - Potassium hydroxide (Analytical grade, J.T. Baker, USA)
    - Sodium chloride (Analytical grade, APS, Australia)
    - Sodium hydroxide (Analytical grade, J.T. Baker, USA)
5. Standard chemical compounds
    - Oleyl palmitate 99% (GC grade, Chemos GmbH, USA)
    - Oleyl oleate 99% (GC grade, Sigma, USA)
    - Oleyl linoleate 99.5% (GC grade, Sigma, USA)
    - Methyl heptadecanoate 99.5% (GC grade, Fluka, Switzerland)
    - Fatty acid methyl esters mixture (C_8-C_{24}) (GC grade, Supelco, USA)
    - Methyl laurate 99.5% (GC grade, Aldrich, USA)

Equipments

1. A 4-digit balance (Percisa, 120A, USA)
2. Gas chromatography instrument (Agilent Technique, 6890N, USA)
3. Surface area analysis (Quanta Chrome, Autosorpb-1)
4. Rota evaporator (BÜCHI, R114, Switzerland)
5. Hot air oven (Binder, German)
6. Fourier transforms infrared spectrophotometer (EQUINOX 55, BRUKER)
8. Water bath (Memmert, WB14 and Germany)
9. Hot plate stirrer (SCHOTT, GmbH D-55122 Mainz)
10. Thermometer
11. Reaction flasks, 25 and 50 ml
12. Reflux apparatus

Methods

Esterification of PFAD

The parameters studied in the esterification of PFAD were varied as follows; Six levels of the amount of catalyst which were 10, 20, 30, 40, 50, 60 (%, w/w, PFAD); six levels of reaction time which were 0.5, 1, 3, 5, 7, and 9 hours; five levels of reaction temperature which were 50, 60, 70, 80, and 90 ºC; seven levels of molar ratios of oleyl alcohol to PFAD which were 1, 1.5, 1.8, 2, 2.5, 2.8, 3, and six levels of stirring speed which were 125, 250, 500, 750, 1000, and 1250 rpm. The reusability of Amberlyst 15 was also studied.

Esterification reactions were carried out in a three-necked round bottom flask (100 ml) equipped with a reflux condenser to condense alcohol vapor. The three-necked reactor was immersed in a constant temperature oil bath equipped with a temperature controller. Experimental equipment was shown in Figure 12. A hotplate stirrer was used to control the temperature and stirring speed. The PFAD was preheated to the reaction temperature. Amberlyst 15 was mixed with preheated PFAD and the reaction was initiated by adding oleyl alcohol. After the reaction was completed, the product was poured into a separating funnel. The upper oil layer was wax esters and the lower layer contained water, Amberlyst 15 and unreacted PFAD. The FFA percentage value in wax esters was analyzed by standard titration method (Ozbay *et al*., 2008). Wax ester content at the optimum condition was analyzed by comparing each type of wax esters with standard esters. Methyl laurate was used as an internal standard for quantify wax ester product by gas chromatography.

After the reaction was completed, Amberlyst 15 catalyst was filtered, refluxed with excess hexane for 30 min, and washed with excess methanol to remove any adsorbed non-polar and polar materials from the surface and pore of catalyst. Amberlyst 15 was further dried in an oven at 100 ±5 ºC for four hours. After all of these steps, Amberlyst 15 can be reused as a catalyst.

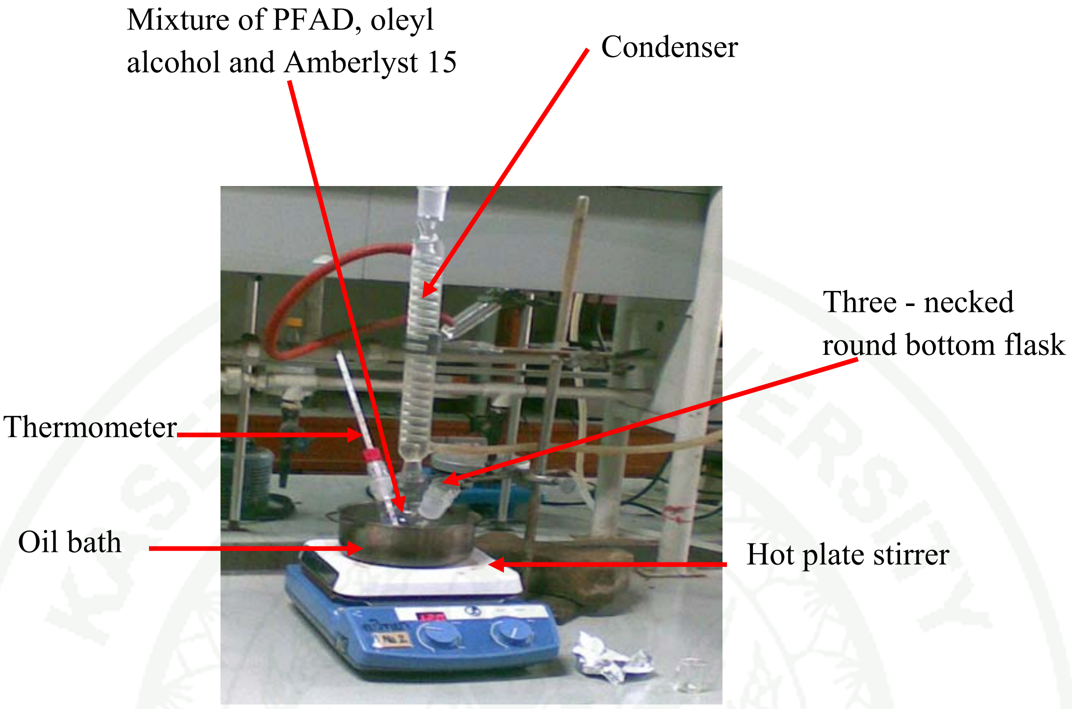


Figure 12 The esterification reaction of palm fatty acid distillate (PFAD).

Analytical Methods

1. Analysis of fatty acid compositions analysis in palm fatty acid distillate using AOCS Ce 2-66 method.

100 mg of PFAD was weighed into a round bottom flask; 5 ml of 0.5 M methanolic sodium hydroxide solution was added into the solution and the mixture was refluxed at 90 ºC for 15 min. Then, 5 ml of borontrifluoride (BF3, 14% v/v) in methanol was added into the flask and the mixture continuously was refluxed at 90 ºC for 5 min. 10 ml of n-heptane was added into the flask and the mixture continuously was refluxed at 90 ºC for 1 min. When the reaction was completed, the mixture was cooled down to room temperature and 10 ml of saturated sodium chloride solution was added and the flask was shaken for 5 min. Then, the solution was allowed to

separate into two layers. The upper layer of solution was analyzed for the fatty acid compositions by GC.

GC analysis was conducted by injecting 1 µl of aliquot into DB-wax capillary column (30m x 0.32mm i.d.; film thickness 0.1µm; Agilent Technologies, USA) in a split mode equipped with a flame-ionization detector. Both injector and detector temperatures were set at 250ºC. Oven temperature was maintained at 80ºC for 2 min and increased to 250ºC with the ramping rate of 20ºC /min and held constant at this temperature for 10 min. Helium was used as a carrier gas with a flow rate of 2 ml/min. The fatty acid compositions were quantified by percentage peak area of each fatty acid.

Response factor calibration for standard fatty acid

The standard fatty acid methyl esters mixture C_8 – C_{24} was dissolved in heptane. The standard fatty acid were prepared in five concentrations (1000, 2000, 3000, 4000, 5000 ppm); methyl heptadecanoate (C_{17}) at the concentration 10 mg/ml was used as the internal standard, added 1 ml into each standard fatty acid. The relation of the peak area of standard fatty acid to the peak area of the internal standard and fatty acid concentrations to concentration of the internal standard were plotted on y-axis and x-axis, respectively. After that the composition of each fatty acid was calculated by the response factor using Equation 2 (Keng *et al*., 2008).

$$R_F = \left(\frac{A_{std}}{A_{is}}\right) \times \left(\frac{C_{is}}{C_{std}}\right) \qquad \text{Eqn. 2}$$

Where:

R_F = Response factor

A_{std} = Area of a standard

A_{is} = Area of an internal standard

C_{is} = Concentration of an internal standard

C_{std} = Concentration of a standard

The average response factor between 1000-5000 ppm of each fatty acid was calculated for further uses in the calculation of the sample concentration.

2. Analysis of Mono, di and triglyceride compositions in palm fatty acid distillate using ASTM D 6584-07 method.

PFAD was weighed about 100 mg into a septa vial; 100 µl of tricaprin was added, then 100 µl of N-Methyl-N- trimethylsilytrifluoroacetamide (MSTFA) was added, the mixtures solution was shaken, and the mixture was allowed to settle for 15 to 20 min at room temperature, 8 ml of n-heptane was added to the vial and shakes. The mixtures solution was analyzed for the composition of mono,di and triglyceride by GC.

GC analysis was conducted by injecting 1 µl of the mixtures into DB-5HT capillary column (30 m x 0.32 mm i.d.; film thickness 0.1µm; Agilent Technologies, USA) in a cool on column mode equipped with a flame-ionization detector and temperatures of detector was set at 380ºC. Oven temperature conditions was maintained at 50ºC, for 2 min, isothermal 50-270 ºC with the rate of 20ºC/min, isothermal 270-380ºC with the rate of 3ºC/min and held for 10 min. Helium was used as a carrier gas with a flow rate of 3 ml/min. The mono, di and triglyceride were quantified by peak area of each compound.

Response factor calibration for standard mono, di, and triglyceride

The mixture standard of mono, di and triglyceride contained monoolein, diolein and triolein. These standard concentrations were prepared at five levels of concentration at 1000, 2000, 3000, 4000, and 5000 ppm. The tricaprin used as an internal standard at the concentration 8 mg/ml was added 100 µl into each mixture of mono, di, and tricaprin standard component. The relation of the peak area of each standard to the peak area of the internal standard and concentrations standard of mono,di and triglyceride to concentration of the internal standard were plotted on y-axis and x-axis, respectively. After that the composition of each standard was calculated using calibration curve.

The average response factor between 1000-5000 ppm of monoolein, diolein and triolein was calculated and further used for calculating concentration of the sample by Equation 3, 4, 5.

$$\text{Monoglyceride (\%)} = \frac{(a_m \times (\frac{A_m}{A_{is}}) + b_m) \times m_{is} \times 100}{m_s} \qquad \text{Eqn. 3}$$

Where:

a_m = Slope of the calibration function

A_m = Peak area of monoglyceride

A_{is} = Peak area of an internal standard

b_m = Intercept of the calibration function

m_{is} = Weight of an internal standard (mg)

m_s = Weight of a sample (mg)

$$\text{Diglyceride (\%)} = \frac{(a_d \times (\frac{A_d}{A_{is}}) + b_d) \times m_{is} \times 100}{m_s} \qquad \text{Eqn. 4}$$

Where:

a_d = Slope of the calibration function

A_d = Peak area of diglyceride

A_{is} = Peak area of an internal standard

b_d = Intercept of the calibration function

m_{is} = Weight of an internal standard (mg)

m_s = Weight of a sample (mg)

$$\text{Triglyceride (\%)} = \frac{(a_{tri} \times (\frac{A_{tri}}{A_{is}}) + b_{tri}) \times m_{is} \times 100}{m_s} \qquad \text{Eqn. 5}$$

Where:

a_{tri} = Slope of the calibration function

A_{tri} = Peak area of triglyceride

A_{is} = Peak area of an internal standard

b_{tri} = Intercept of the calibration function

m_{is} = Weight of an internal standard (mg)

m_s = Weight of a sample (mg)

3. Determination of acid value in PFAD and wax esters using AOCS Cd 3d-63 method.

The samples taken at an established time were analyzed by titration to measure the acid value of the product and PFAD. The measurement of acid value was carried out as the following procedure. Sixty five milliliters of hot ethanol was firstly neutralized by 0.1 N KOH solutions using 0.5 ml of phenolphthalein as an indicator. The sample was added into the neutralized ethanol to fully dissolve by heating. The sample was then titrated by 0.1 N KOH. The volume of KOH consumed by the FFA was recorded and the acid value was calculated using Equation 6 (Ozbay *et al*., 2008).

$$S = \frac{56.1 \times N \times (A - B)}{W} \qquad \text{Eqn. 6}$$

Where:

A = Volume, in milliliters of standard alkali used in the titration (ml)

B = Volume, in milliliters of standard alkali used for titrating blank (ml)

N = Normality of standard alkali (mg/L)

W = Weight of a sample (g)

S = Acid value (mg KOH/g sample)

The conversion of acid value can be calculated from Equation 7 (Marchetti and Errazu, 2008).

$$\text{Conversion of acid value (\%)} = \frac{(S_i - S_t) \times 100}{S_i} \qquad \text{Eqn. 7}$$

Where:

S_i = The initial acid value

S_t = The acid value at established reaction time

The quantity of the produced wax ester was analyzed by GC as shown in Figure 13.

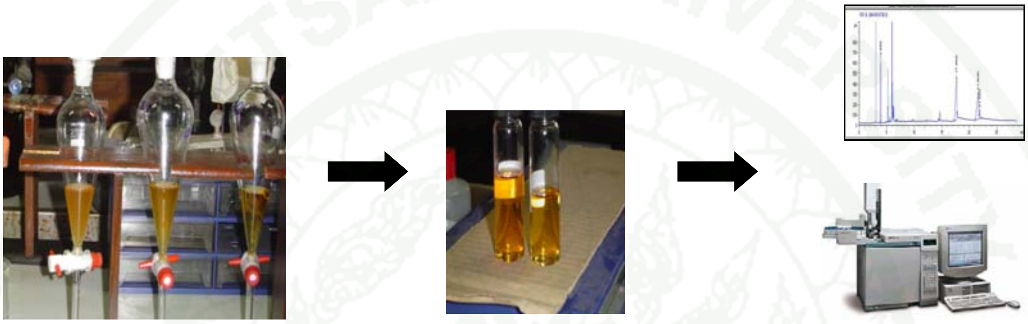


Figure 13 The process of wax esters analysis by GC.

4. Analysis of wax esters composition in reaction mixture

Wax esters was weighed about 50 mg and methyl laurate (C_{12}) used as internal standard concentration 3000 mg/L was added 0.5 ml into septa vials and was shaken. The composition of wax esters was analyzed by GC.

GC analysis was conducted by injecting 1 µl of of aliquot into DB-5HT capillary column (30 m x 0.32 mm i.d; film thickness 0.1µm; Agilent Technologies, USA) in a split mode equipped with a flame-ionization detector and injector temperatures were set at 300 and 250ºC. Oven temperature conditions was maintained at 150ºC, held constant for 2 min, increased temperature to 270ºC with the ramping rate of 3ºC /min, held for 0 min and then increased to the final temperature of 290ºC, held constant for 16 min. Helium was used as a carrier gas with the flow rate of 0.8 ml/min. The wax esters detected in the chromatograms were identified by comparing with the retention time of standards oleyl esters. The product composition was quantified by an internal standard method with methyl laurate used as an internal standard for calibration. The concentrations of oleyl esters were calculated by Equation 8 (Keng *et al*., 2008).

$$\mathrm{Cs} = \left(\frac{\mathrm{As}}{\mathrm{Ais}}\right) \times \left(\frac{\mathrm{Cis}}{\mathrm{R_F}}\right) \qquad \text{Eqn. 8}$$

Where:

R_F = Response factor

A_s = Area of a sample

A_{is} = Area of an internal standard

C_{is} Concentration of an internal standard

C_s = Concentration of a sample

The percentage yield of wax esters was calculated by Equation 9 (Keng *et al*., 2008).

$$\text{Percentage yield} = \left(\frac{\text{Weight of wax ester from GC (g)}}{\text{Weight of the initial PFAD(g)}}\right) \times 100 \qquad \text{Eqn. 9}$$

Response factor calibration for standard oleyl esters

1. Three stock solutions were prepared from oleyl palmitate, oleyl oleate, and oleyl linoleate standards at the original concentrations of 10000, 10,250, and 52,860 mg/L, respectively.
2. A stock solution of an internal standard (methyl laurate) was prepared for the concentration of 3,000 mg/L.

3. Six levels of standard concentration were prepared by dilution of the stock solution. The concentrations of oleyl palmitate were 512.5, 1025, 2050, 3075, 4100, and 5125 mg/L. The concentrations of oleyl oleate were 500, 1,000, 2,000, 3,000, 4,000, and 5,000 mg/L. The concentrations of oleyl linoleate were 528.6, 1057.2, 1585.8, 2114.4, 3171.6, and 4228.6 mg/L.

4. Each standard concentration was put into the vial and added 0.5 ml of internal standard was added into the vial.

5. Adjusted the final volume of solution was adjusted for 1 ml with n-heptane; inject the standards and calculate the response factor using Equation 1.

The average response factor was further calculated to determine the sample concentration.

5. Fourier Transform Infrared Spectroscopy (FT-IR) Analysis

The functional groups of a PFAD molecule and the reaction mixture of wax esters product were analyzed by FT-IR. A Bruker Equinox 55 FT-IR spectrometer equipped with Attenuated Total Reflectance (ATR) apparatus was used to analyze films, pastes, solids, liquids and powders without sample preparation. One or two drops of the samples were applied on Zn-Se crystal; the sample background was collected; and the sample was measured. The spectra were scanned from 4000 to 400 cm^{-1}. All spectra were collected at 4 cm^{-1} of spectral resolution by using 1 minute sample and 1 minute background collection times.

6. Physicochemical properties analysis of wax esters and palm fatty acid distillate.

These parameters are very important properties to indicate the quality of wax esters for specific application.

6.1 Saponification value

Saponification value is the number of milligrams of potassium hydroxide or sodium hydroxide that reacts with 1 g of sample. This value indicated that oils are normal triglycerides. This number is very useful in production of liquid soap and shampoo industries. Saponification value of wax esters and palm fatty acid distillate were determined according to AOCS Official Method Cd 3-25.

6.2 Specific gravity

Specific gravity is the ratio of the weight per unit volume of sample to the weight per unit volume of water at 25 ºC. Specific gravity is one of the most important properties because it can be correlated to some important performance indicators such as cetane number and heating value (Tat and Van Gerpen, 2000). Specific gravity value of wax esters was determined according to AOCS Official Method Cc 10b-25.

6.3 Iodine value

The iodine value is a measure of the unsaturation containing in fatty acids and expressed in terms of the number of grams of iodine absorbed per 100 of sample. Iodine value of wax esters and palm fatty acid distillate was determined according to AOCS Official Method Cd 1b-25.

6.4 Refractive index

The refractive index is the ratio of the speed of light in a vacuum to the speed of light in the substance. This can be used in conjunction with other properties to characterize pure hydrocarbon and their mixtures. Refractive index of wax esters and palm fatty acid distillate was determined according to AOCS Official Method Cc 7-25.

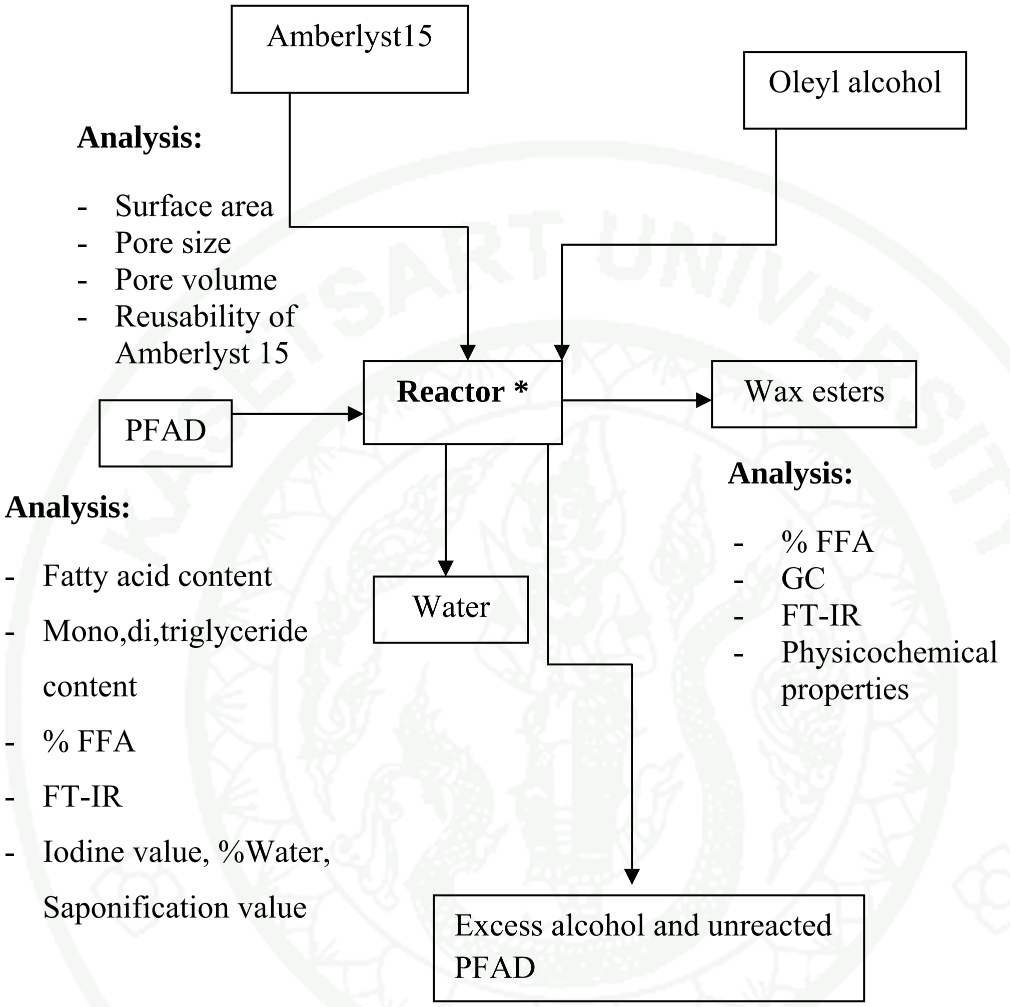
6.5 Water content (%)

The actual water content in fats and oils was determined by titration with Fischer reagent. Water content of wax esters and palm fatty acid distillate was determined according to AOCS Official Method Ca 2e-84.

6.6 Flash point

The flash point is the temperature at which a flash will occur when a small flame is passed over the surface of the sample. Flash point of wax esters was determined according to AOCS Official Method Cc 9c-95.

The summary of the experiment and analysis was shown in Figure 14



* Parameters for esterification reaction of synthetic wax esters were studied as following:

- Amount of catalyst (10, 20, 30, 40, 50, and 60%)
- Reaction time (0.5, 1, 3, 5, 7, and 9 hours)
- Reaction temperature (50, 60, 70, 80, and 90 ºC)
- Molar ratio of PFAD to oleyl alcohol (1:1, 1:1.5, 1:1.8, 1:2,1:2.5, and 1:3)
- Stirring speed (125, 250, 750, 1000, and 1250 rpm))

Figure 14 The summary diagram of experiment concept.

RESULTS AND DISCUSSION

1. Physicochemical properties of palm fatty acid distillate (PFAD)

The data collected from the study of physical and chemical properties of the test sample was shown in Table 3.

Table 3 The physicochemical properties of PFAD

Parameters	Values
% FFA (as palmitic acid)	85
Total triglycerides (%)	4.10
Acid value (mg KOH/g sample)	186.15
Viscosity at 60°C (cSt)	10.75
Saponification value (mg KOH/g sample)	200.57
Iodine value (g I_2/100 g sample)	57.57
Density at 40 ºC (g/cm^3)	0.87
Water content (wt %)	0.05

PFAD is a starting raw material for synthetic wax esters. The initial free fatty acid (FFA) was 85% giving the acid value of 186.15 mg KOH/g sample. The value of 2.19 from the ratio of the acid value and the %FFA could be multiplied with percentage free fatty acid and calculated for the other acid values.

Iodine value is a measured of the unsaturation of PFAD. Higher iodine value indicated higher unsaturation of fats and oils content (Akbar *et al*., 2009). The iodine value of PFAD was determined of 57.57 g I_2/100g sample. This value indicated that the content of unsaturated of fatty acid in PFAD.

Viscosity of PFAD is defined as the resistance of liquid to flow. Viscosity was increased with molecular weight but decreased with increasing unsaturated level and temperature (Nouredini *et al*., 1992). At 60 ºC, viscosity of the sample was detected at 10.75 cSt.

Saponification value of PFAD was 200.57 mg KOH/g sample, corresponded to the value of Hamirin, 1998. This value indicated that PFAD have composition of triglycerides. Triglycerides, which is the major component in palm oil are esters formed from glycerol and three fatty acids of different chain lengths. Hence, the bulky molecules have higher Van der Waals interaction per unit weight (Keng *et a*l., 2008).

Water content in PFAD was 0.05%. Water content has significant effects on the esterification reaction because water reacted with esters to produced carboxylic acid and alcohol. In addition, higher water content could reduce the activity of Amberlyst 15 due to the poisoning of acid sites as well as the poor accessibility of reactants to acid sites due to the presence of water (Rat *et al*., 2008).

The result of fatty acid compositions in PFAD was determined by gas chromatography (GC). The chromatogram of fatty acids composition in PFAD was shown in Figure 15.

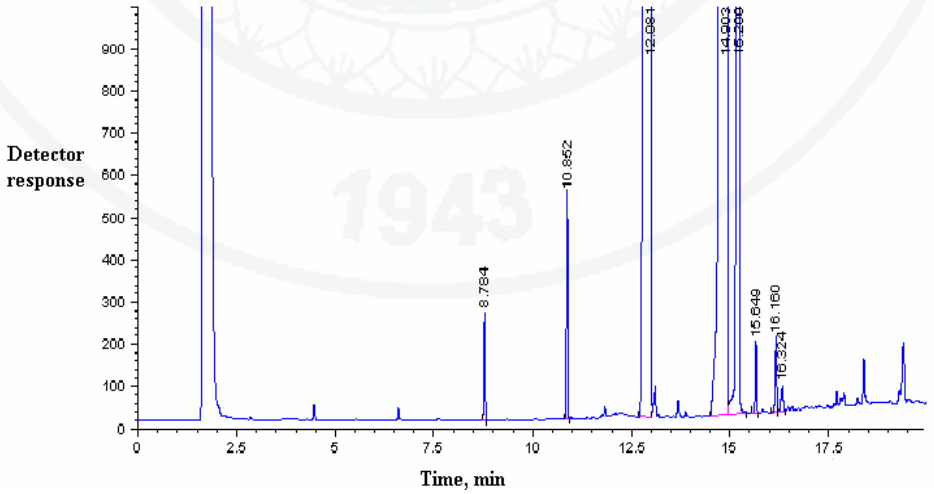


Figure 15 Chromatogram of fatty acid compositions in PFAD

The fatty acid composition in PFAD was known by comparing the retention time of each peak with the retention time of the standard, From Figure 15, the result showed that PFAD was consisted of lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2).

The relation of percentage area of each fatty acid with retention time was showed in Table 4.

Table 4 The relation of percentage area of fatty acid with retention time of PFAD

Retention time (min)	Fatty acid	% Peak area
8.78	Lauric acid (C12:0)	0.25
10.85	Myristic acid (C14:0)	0.86
12.08	Palmitic acid (C16:0)	42.49
14.90	Heptadecanoic acid (C17:0) (an internal standard)	-
15.65	Stearic acid (C18:0)	3.35
16.20	Oleic acid (C18:1)	34.77
16.16	Linoleic acid (C18:2)	8.25
-	Others	10.03

Table 4 showed that the fatty acid compositions in PFAD were lauric, myristic, oleic, linoleic, palmitic and stearic acid. Palmitic acid showed the highest percentage area of 42.49 % followed by oleic acid of 34.77 % and linoleic acid of 8.25 %. Thus, in this research palmitic, oleic and linoleic acid were selected to calculate the amount of wax esters that produced from these three fatty acids, in addition, the average molecular weight of PFAD could be calculated from the relation of molecular weight and percentage weight of each fatty acid as shown in Equation 10.

Average molecular weigh of PFAD $= \frac{\sum(\%\ \text{Area} \times \text{MW})}{100}$ Eqn. 10

Where:

Σ = The summation

%Area = The area peak of each fatty acid

MW = Molecular weight of each fatty acid

Therefore, the average molecular weight of PFAD is equal to 263 g/mol. This molecular weight of PFAD was used to calculate stoichiometric ratio of esterification reaction of synthesis wax esters between PFAD and oleyl alcohol.

PFAD not only contained fatty acids but also contained another compounds such as mono, di, and triglyceride that could be analyzed by GC. The chromatogram of mono, di and triglyceride in PFAD was shown in Figure 16.

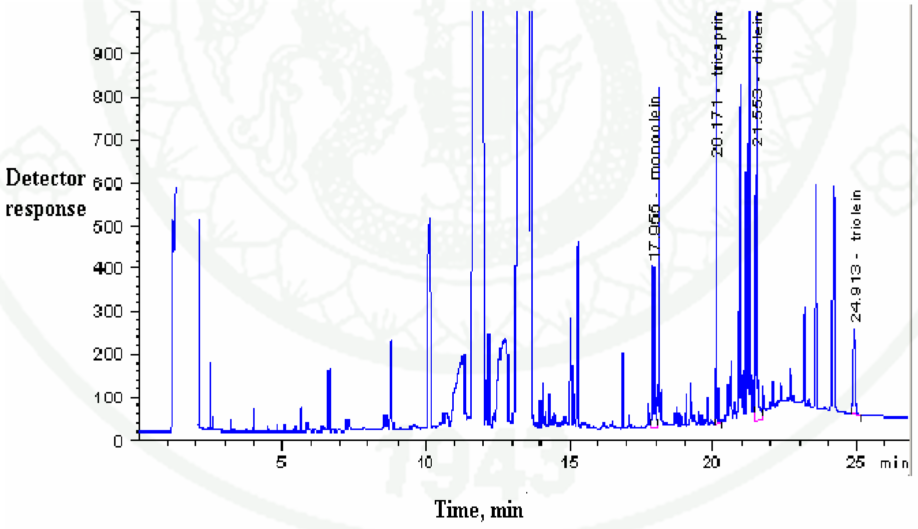


Figure 16 Chromatogram of triglycerides composition in PFAD

From Figure 16, the relation of retention time of mono, di and triglyceride compared with the standard compound and the amount of all compounds based on tricaprin internal standard was shown in Table 5.

Table 5 The relation of percentage weight of glycerides to the retention time of PFAD.

Retention time (min)	Compounds	wt %
19.169	monoglyceride	0.7492
20.171	tricaprin (an internal standard)	-
21.559	diglyceride	2.7726
24.913	triglyceride	0.5751

Table 5 showed the relation of percentage weight of glycerides with to the retention time. The compositions of total glycerides in PFAD were mono, di, and triglyceride analyzed by GC. The results showed 0.7492% monoglyceride, 2.7726% diglycerides, and 0.5751% triglyceride.

2. The physical properties of Amberlyst 15

In this research, Amberlyst 15 was used as a catalyst for wax esters synthesis from PFAD. Amberlyst 15 is a strong acidic sulfonic acid, macroreticular polymeric resin based on crosslinked styrene divinylbenzene copolymers. The properties of Amberlyst 15 are given in Table 6. The elemental compositions in Amberlyst 15 were analyzed by Energy-Dispersive X-Ray Spectroscopy (EDS) as shown in Table 7.

Table 6 The physical properties of Amberlyst 15

Properties	Result
Matrix	Styrene – divinylbenzene[a]
shape	Bead[a]
form	H^{+a}
Crosslinking level	high[a]
Exchange capacity (acid site) (eq/kg)	4.74[a]
Pore diameter (nm)	30[a]
Moisture (%)	< 2%[a]
Temperature $_{max}$ (ºC)	120[a]
Pore volume (cc/g)	0.30[b]
Surface area (m^2/g)	48.02[b]
Particle size (mm)	0.3-1.2[b]

Source: [a] Kunin, 1962
[b] Analysis by TISTR

The data from Table 6 showed the physical properties of Amberlyst 15. It was found that the functional group was the sulfonic acid group; the matrix was formed of a copolymer of styrene and divinylbenzene, indicating high crosslink between molecules by a covalent bond. The exchange capacity represents a number of chemical equivalents of ions that can be taken up by a unit amount of the resin cross-linking (decreases the capacity measured on the dry basis or fewer functional groups may be attached to highly cross-linked polymer molecules). The exchange capacities of 4.74eq/kg to indicate of the substitution of one sulfonic acid group per aromatic ring (Malik, 2009). The surface area was 48.02 m^2 / g, this showing a possible contact between the molecules of reactant and active sites of catalyst; increasing the surface area generally increases the rate of a chemical reaction. Pore diameter of 30 nm was classified in macroporous and indicated that FFA molecules entered into the inner surface of the catalyst and would increase esterification rate (Klepacova *et al.*, 2005). The maximum temperature for reaction testing was 120 ºC.

Table 7 Elemental compositions of Amberlyst 15 by EDS

Elemental	Wt %
C	43.50
O	22.60
Mg	0.43
Al	5.01
S	26.8
Ca	2.17
Cr	0.91
Cu	6

Source: Analysis by TISTR

The data from Table 7 showed the results of elemental compositions of Amberlyst 15 analyzed by Energy-Dispersive X-Ray Spectroscopy (EDS). This method provided types and amount of elements in Amberlyst 15. From Table 7, the result showed the high carbon atom of 43.50 % obtained from styrene divinylbenzene. In addition, the main of composition of catalyst was sulfur obtained from treating polymer matrix with concentrated sulfuric acid. Thus, the Amberlyst 15 to support with sulfuric acid this call sulfonic acid (SO_3H) from Table 7 gave 26.8 % of sulfur. Moreover, this catalyst has also aluminum (Al) in composition that it has properties of acid in the same way as sulfur. This result to indicate the related species are the main active sites for esterification reaction.

3. The results of optimization of esterification reaction

3.1. Effect of the catalyst amount

For this studied, a reaction was carried out with a molar ratio of oleyl alcohol to PFAD of 2:1. The reaction was performed at 60 ºC, the reaction time of 3 hours, and stirring speed of 250 rpm. By varying the amount of catalyst, the percentage conversion of FFA was studied. The result of analysis can be seen on the Figure 17.

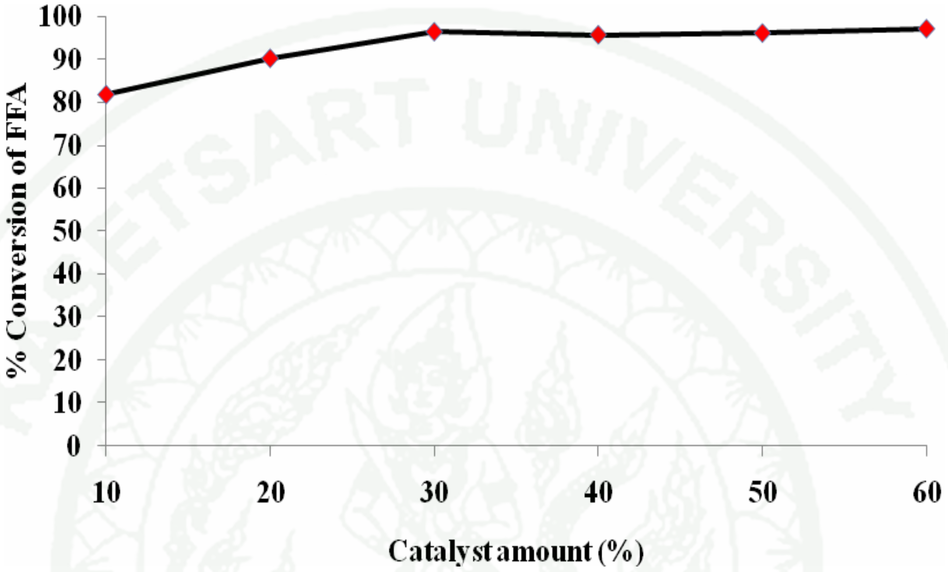


Figure 17 Effect of the catalyst amount on percentage conversion of FFA. The reaction mixture consisted of molar ratio of oleyl alcohol to PFAD of 2:1, the reaction temperature of 60 ºC, the reaction time of 3 hours, and stirring speed at 250 rpm.

Figure 17 show the effect of catalyst amount on the percentage conversion of FFA. The excess catalyst did not contribute to the increase in the percentage conversion of FFA. By increasing 10 to 60 % wt of catalyst, the percentage conversion of FFA was initially increased to 81.92% when using 10 % wt catalyst. The percentage conversion was gradually increased to 96.52 % when using 30 % wt catalyst. When increasing %wt of catalyst to 60 %, the percentage conversion did not show any significant increase. There was also similar reported by Radzi *et al.*, (2005) that an excess enzyme did not increase the percentage conversion of FFA and sometime it would decrease the product yield. At the equilibrium point, all the substrates were bound to the catalyst and added catalyst molecule could not find any substrate to serve as a reactant. Thus, the appropriate of the catalyst amount was at 30 % wt, giving the percentage conversion of FFA was 96.52%.

3.2. Effect of reaction time

For this studied, the reaction time was varied in the range of 0.5 to 9 hours. At 30 %wt of catalyst, the reaction temperature of 60 ºC, the stirring speed of 250 rpm, and molar ratio of oleyl alcohol to PFAD of 2:1, % conversion of FFA at various reaction time were shown in Figure 18.

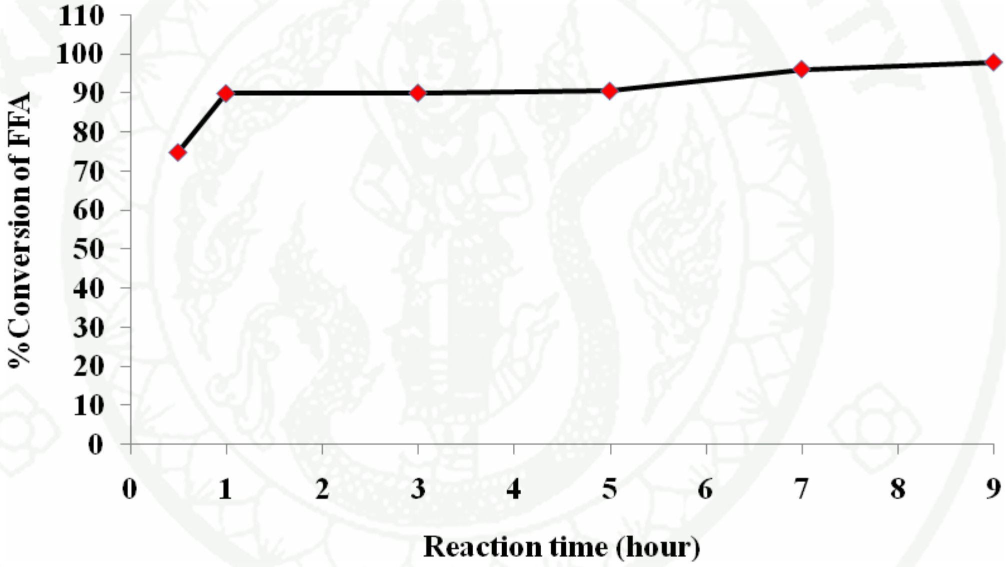


Figure 18 Effect of reaction time on percentage conversion of FFA. The reaction mixture consisted of molar ratio of oleyl alcohol to PFAD of 2:1, reaction temperature of 60 ºC, catalyst amount of 30 % wt, and stirring speed at 250 rpm.

The reaction time helps the reaction of PFAD and oleyl alcohol reacting on the catalyst surface where they need to diffuse to the interior surface of the catalyst. Ganapati *et al*., 1994 reported a heterogeneous catalytic reaction between substrate and a heterogeneous catalyst in seven steps. Thus, the reaction time course was a good indicator of catalyst performance and reaction progress. The effect of reaction time on esterification reaction with a solid acid catalyst (Amberlyst 15) was presented in Figure 18, by increasing 0.5 to 9 hours of the reaction time; the percentage conversion of FFA was initially increased to 78 % when using 0.5 hour. The percentage conversion was gradually increased to 90.06% when using 1 hour. At 1 hour was gave the equilibrium point. The reaction time from 3 to 5 hours gave percentage conversion of FFA about the same as at 1 hour and after 5 hours the percent conversion of FFA slightly increased. This may be due to some mass-transfer limitations, which inevitably arise in a reaction mixture containing a high proportion of products; the reactions achieved the equilibrium state where the rate of forward reaction was equal to the reverse reaction (Gunawan *et al*., 2008). Sometime increasing the reaction time did not show any increase in the conversion of FFA, this may be due to catalyst deactivation from the reaction in longer time that the change in catalyst structure and state occurs. On the other hand, the concentration of products and synthesis water had gradually increased and may have so promoted the reverse hydrolysis reaction (Razdi *et al*., 2005). The optimum of reaction time was 1 hour which gave 90.06% conversion of FFA.

3.3. Effect of reaction temperature

The reaction was carried out reaction temperature was used in the range between 50 to 90 ºC, the catalyst amount of 30 % wt, the stirring speed at 250 rpm, the reaction time of 1 hour, and molar ratio of oleyl alcohol to PFAD of 2 to 1, % conversion of FFA at various reaction temperature were shown in Figure 19.

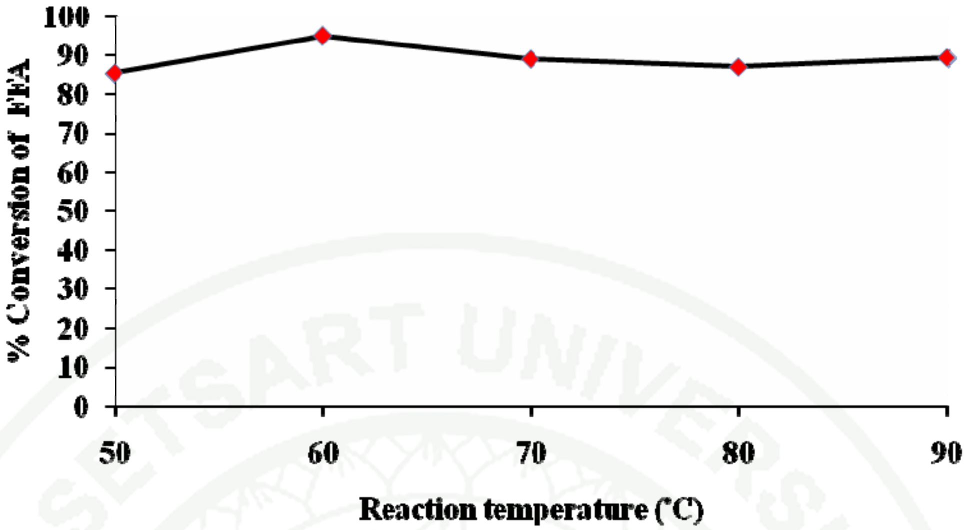


Figure 19 Effect of reaction temperature on conversion of FFA. The reaction mixture consisted of molar ratio of oleyl alcohol to PFAD was 2:1, reaction time 1 hour, catalyst amount of 30 % wt , and stirring speed at 250 rpm.

The reaction temperature was one of the factors that could influence the esterification reaction; changing the reaction temperature could affect the activity and stability of the enzyme and the rate of reaction (Liu *et al*., 2006). In addition, the effect of reaction temperature can be affect on substrate solubility as well as the reaction and the enzyme (Faciolli *et al*., 2001). Effect of reaction temperature on esterification reaction was shown in Figure 19. Initially, the percentage conversion of FFA was increased from 0 % to 95.15% when increase temperature from increasing temperature from 37ºC to 60ºC. The percent conversion of FFA reached the maximum of 95.15% at the temperature of 60ºC. This is probably because beyond a critical temperature, the catalyst may have been deactivated. The result is similar in the findings by most reviewed papers that Novozym 435 was optimally used at 40ºC to 60ºC (Lozano *et al*. 2003). The conversion decreased slightly after 60ºC probably caused by the vibration and movement of the enzyme molecule, which would affect the hydrogen bonds and other bonds in the lipase structure. Hence, the enzyme molecule will unfold and alter its tertiary and quaternary structure or globular structure (three-dimensional conformation). Consequently the catalytic power of lipase will be reduced, because denaturation process has occurred. At high temperature, a higher water evaporation rate may shift the position of the equilibrium to the product side and increases the yield (Chen *et al*., 1995). Thus, the optimum of reaction temperature was 60ºC and the percentage conversion of FFA at 60ºC temperature was 95.15%.

3.4. Effect of molar ratios of oleyl alcohol to PFAD

The reaction was carried out at the constant temperature of 60ºC and stirring speed at 250 rpm, catalyst amount of 30 % wt, and reaction time for 1 hour, % conversion of FFA at various molar ratios of oleyl alcohol was shown in Figure 20.

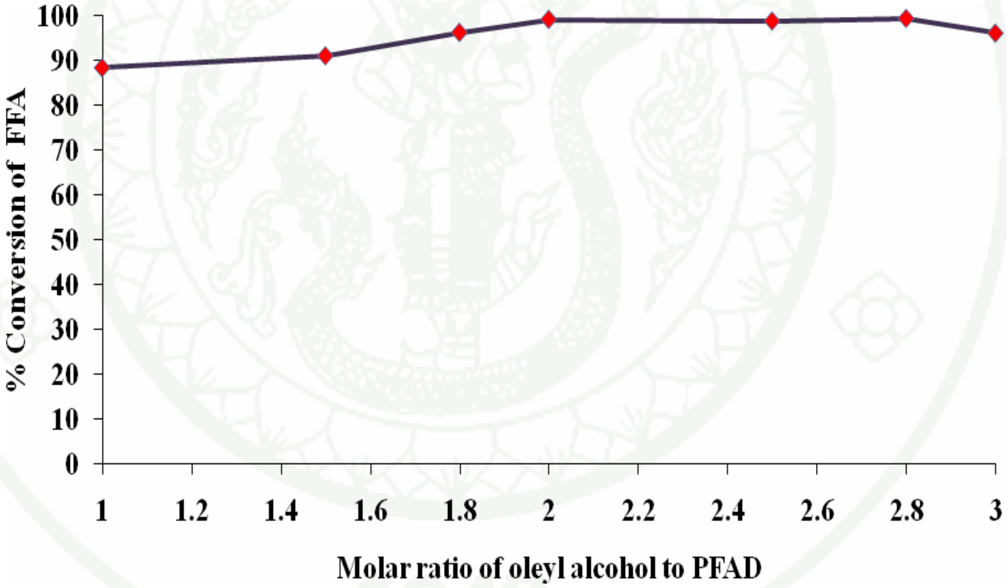


Figure 20 Effect of molar ratios of oleyl alcohol to PFAD on percentage conversion of FFA. The reaction temperature was 60 ºC, reaction time of 1hour, catalyst amount of 30 % wt, reaction time of 1 hour, and stirring speed at 250 rpm.

The effect of molar ratios of oleyl alcohol to PFAD on the esterification reaction was shown in Figure 20. The percentage conversion of FFA was increased from 0% to 99.26% when increase molar ratio from increasing molar ratio from 1:1 to 2:1, and kept constant from 2:1to 2.8:1. However, from 2.8:1 to 3:1 the percentage conversion of FFA decreases. Increasing the oleyl alcohol cause produced more water content which can be adsorbed on the active sites of Amberlyst 15. The catalyst can be poisoned and the efficiency of accelerating the esterification reaction rate is reduced (Park *et al*., 2010). At the same time, the excess of oleyl alcohol will hinder the interaction frequency between reactants (oleyl alcohol and oleic acid) and lipase catalyst (Gunawan *et al*., 2005). Thus, the optimum molar ratio of oleyl alcohol to PFAD was 2:1 which gave 99.26% the percentage conversion of FFA.

3.5. Effect of stirring speed

For this studied, the reaction was carried out with a molar ratio of oleyl alcohol to PFAD of 2:1. The reaction was set at 60ºC; reaction time for 1 hour and amount of catalyst was 30 wt% based on weight of PFAD. The initial percentage of FFA was 85%. This analysis can be seen on Figure 21. The speed of agitation was varied in a range at 125-1250 rpm.

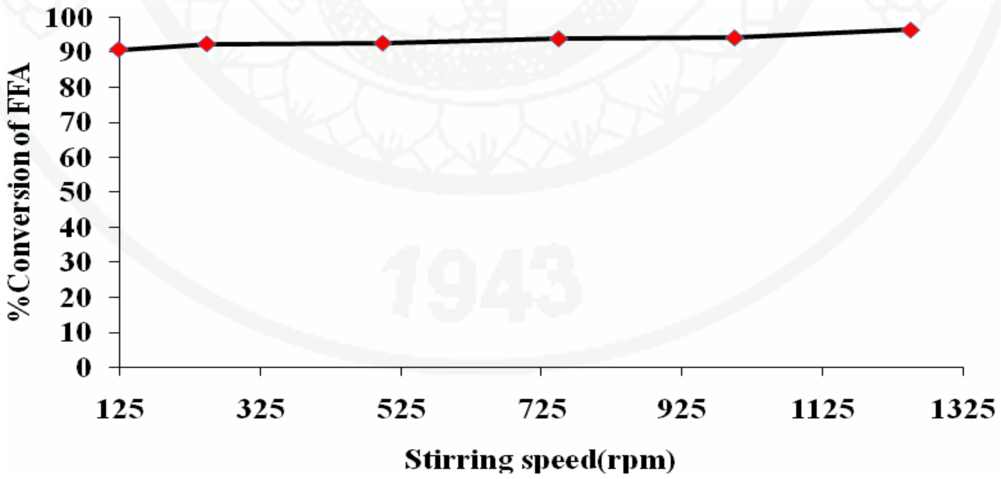


Figure 21 Effect of stirring speed on percentage conversion of FFA. The reaction mixture consisted of molar ratios of oleyl alcohol to PFAD of 2:1,

reaction time for 1 hour, amount of catalyst 30 %wt, and reaction temperature at 60 ºC.

Stirrer plays a major role in a molecule movement. Higher stirring speed can increase the movement of molecules, resulting in the high interaction between catalyst and reactant. Moreover, it increases external mass transfer rate between the bulk phase of the reaction mixture and the surface of catalyst (Radzi *et al.*, 2005). From Figure 21, the effect of stirring speed on the percentage conversion of FFA was studied in the range at 125 to 1250 rpm. The result showed that the percentage conversion of FFA was increased slightly from 90.86% at the stirring speed 125 to 92.47% at 250 rpm. When increased stirring speed from 500 to 1250 rpm, the percentage conversion of FFA unchanged. Thus, there was no limitation of external mass transfer beyond the stirring speed were 500 to 1250 rpm. Therefore, this studied performed at 250 rpm of stirring speed for the optimum condition giving the percentage conversion of FFA of 92.47%.

From the studies, the optimum conditions for wax esters production was at 30% amount of catalyst based on weight of PFAD, the reaction time of 1 hour, the reaction temperature of 60ºC, molar ratios of oleyl alcohol to PFAD of 2:1, and stirring speed at 250 rpm.

One important benefit of heterogeneous catalyst is reusability. Amberlyst 15 could be reused in order to decrease the cost of production after and washing regenerating.

3.6. Reusability of the Amberlyst 15

Reusability of the catalyst was also investigated since the cost of the process could be reduced. After the reaction completed, the catalyst was recovered by filtering, washing with hexane followed by methanol, and drying at 105 ºC for 4 hours. The condition used for studied, were carried out at 30 % of catalyst amount, the reaction time of 1 hour, reaction temperature of 60 ºC, molar ratio of reactant (oleyl alcohol to PFAD) of 2:1, and stirring speed at 250 rpm. The activity of Amberlyst 15 was determined in terms of percentage conversion of FFA.

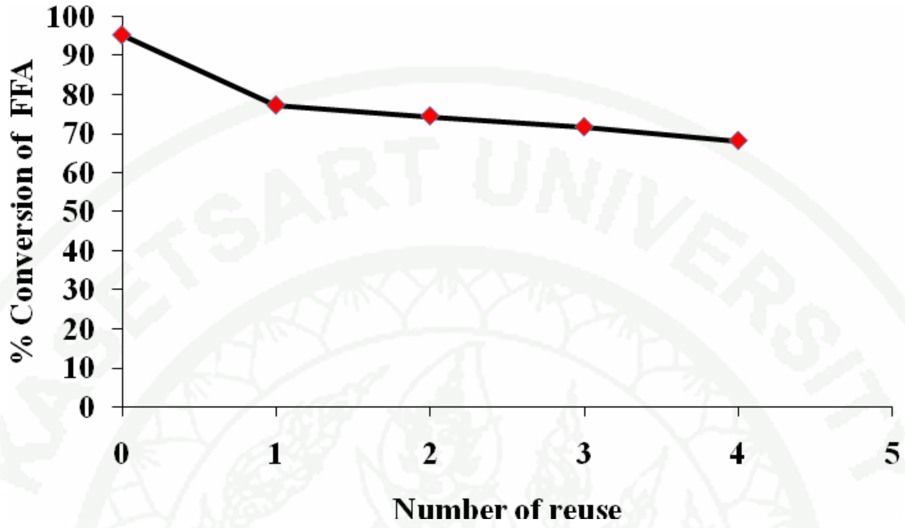


Figure 22 Percentage conversion of FFA and number of reuse. The reaction mixture consisted of molar ratios of oleyl alcohol to PFAD of 2:1, reaction time for 1 hour, catalyst amount of 30 % wt, stirring speed at 250 rpm, and reaction temperature of 60 ˚C.

The catalyst was reused for the purpose of process economy. The result of reusability of Amberlyst 15 was shown in Figure 22. It was revealed the percentage conversion of FFA decreased when increased the number of reuse. A fresh catalyst (0 time) gave percentage conversion of FFA 98%, after to reuse again that used the same condition was found decrease percentage free fatty acid from 98% to 80%. This cause may be it had efficiency of acid site of catalyst to decrease, deactivation to be related to the reversible water poisoning, but it was attributed to the gradual blocking Amberlyst 15 sites with heavy molecular weight hydrophobic products that could become entangled within the polymer matrix. Moreover, not only water but other minor compounds such as carotene and vitamin E present in PFAD might be adsorbed onto catalysts, and the solvent washing is therefore, necessary for prolong use of the catalyst.

4. Quantitative analysis at the optimum condition

Quantitative analysis of wax esters product was performed by Gas Chromatography (GC). For this analytical measurement methyl laurate (C12) was used as internal standard with concentration of 3000 mg/L. Wax esters samples were analyzed in duplicate. Response factor (R_F) and peak area of each standard oleyl esters compound were used to relate with area peak and concentration of methyl laurate (C12) that acted as internal standard calculate followed by Equation 1. The amount of each oleyl esters compound was calculated and expressed in terms of milligrams per liter (mg/L) calculate followed by Equation 8. The yield of each oleyl esters calculate followed by Equation 9.

In this research, selected three standard oleyl esters for comparison standard that it cause to select these standard because of the PFAD as raw material were three main fatty acids. The chromatograms of each standard oleyl esters determined quantitative by GC. These were shown in Figure 23-25 and calculate R_F of each standard was showed in Table 8-10.

The result of response factor calibration for oleyl palmitate was showed chromatogram in Figure 23 and the response factors for six levels of concentration in Table 8.

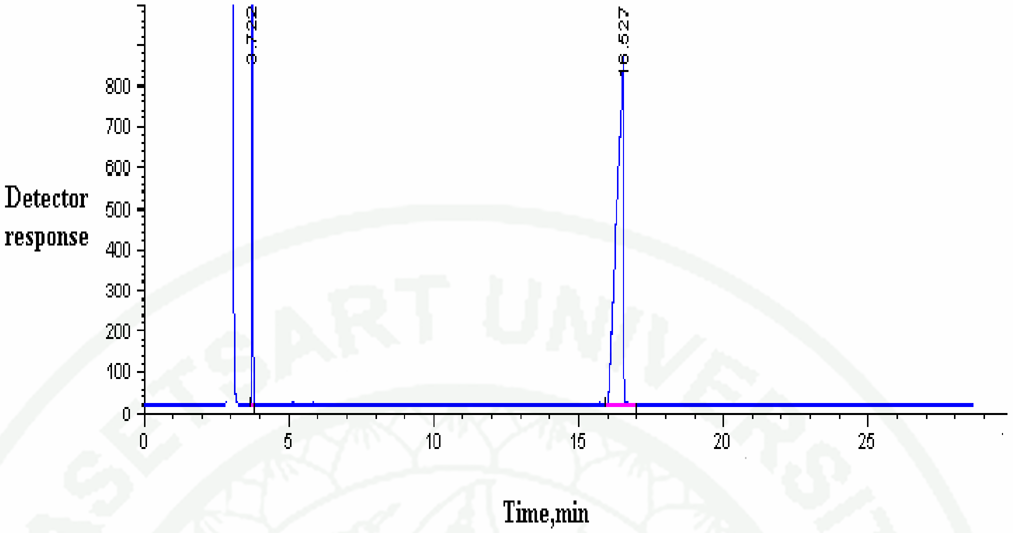


Figure 23 Chromatogram of standard oleyl palmitate and methyl laurate (an internal standard).

From Figure 23 presented the relation of detector response of peak area standard oleyl palmitate and methyl laurate as an internal standard and retention time at 16.527 and 3.722 min.

Table 8 Detector response factor of standard oleyl palmitate.

Conc of oleyl palmitate(mg/L) (C_x)	Peak area of oleyl palmitate (A_x)	Peak area of methyl laurate (A_{is})	Conc of methyl laurate (mg/L) (C_{is})	Response factor (R_F)
500	1350.08	4034.85	1606	1.07
1000	2755.70	4249.33	1606	1.04
2000	5653.88	4326.86	1606	1.04
3000	8642.70	4258.49	1606	1.08
4000	11767	4339.47	1606	1.08
5000	14436.5	4236.73	1606	1.09
mean	-	-	-	**1.07**
sd	-	-	-	0.02
%cv	-	-	-	1.88

Table 8 showed calculation of the response factor of oleyl palmitate, the response factors (R_F) were determined average value of response factor of concentration of this standard in the range 500 to 5000 mg/L and calculated response factor by Equation 2. The response factor of oleyl palmitate was 1.0725, standard deviation (sd) of 0.0202% and coefficient of variation (% cv) of 1.8829 %. This response factor value was used to calculate the concentration of oleyl palmitate in the final product.

The result of response factor calibration for oleyl oleate was showed chromatogram in Figure 24, the response factors at six levels of concentration in Table 9.

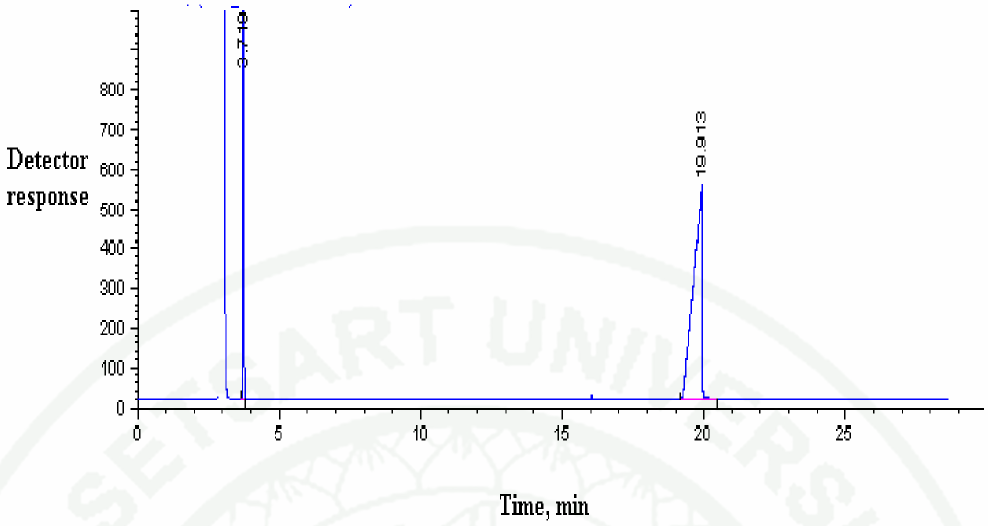


Figure 24 Chromatogram of standard oleyl oleate and methyl laurate (an internal standard).

From Figure 24 presented the relation of detector response of peak area of oleyl palmitate standard and methyl laurate as an internal standard and retention time at 19.913 and 3.719 min.

Table 9 Detector response factor of standard oleyl oleate.

Conc of oleyl oleate (mg/L) (C_x)	Peak area of oleyl oleate (A_x)	Peak area of methyl laurate (A_{is})	Conc of methyl laurate (mg/L) (C_{is})	Response factor (R_F)
512.5	1397.46	4298.12	1606	1.01
1025	2947.80	4348.86	1606	1.06
2050	5369.64	4367.16	1606	0.96
3075	8883.46	4330.50	1606	1.07
4100	11576.10	4297.79	1606	1.05
5125	14742.90	4304.21	1606	1.07
Mean	-	-	-	**1.04**
sd	-	-	-	0.03
%cv	-	-	-	3.75

Table 9 showed the result of response factor of standard oleyl oleate that the response factors (R_F) were determined at average value of response factor of concentration of this standard in the range 512.5 to 5125 mg/L and calculated response factor of oleyl oleate by Equation 2. The response factor of oleyl oleate was 1.0407, standard deviation (sd) was 0.0390% and coefficient of variation (%cv) was 3.7516%. This response factor value was used to calculate the concentration of oleyl oleate contained in final product.

The result of response factor calibration for oleyl linoleate was showed as a chromatogram in Figure 25 and the response factors at six levels of concentration was in Table 10.

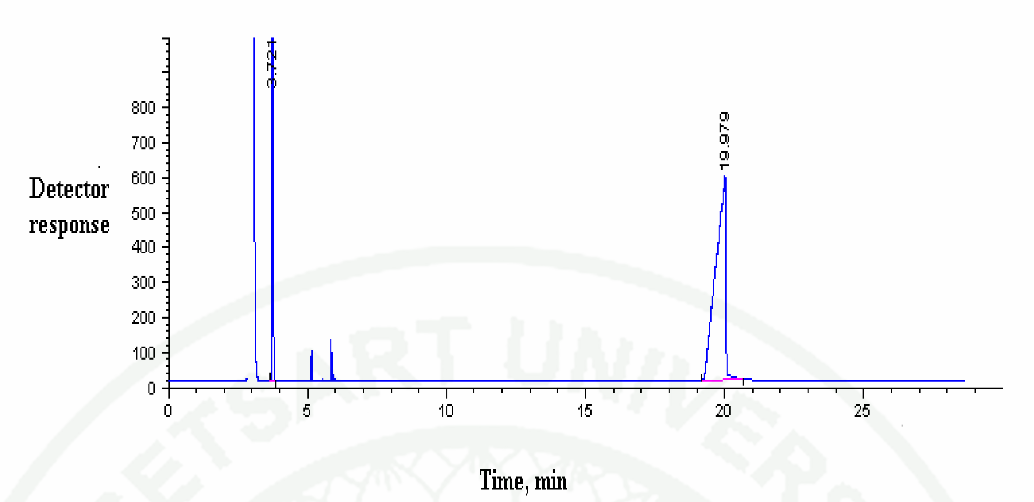


Figure 25 Chromatogram of standard oleyl linoleate.

From Figure 25 presented the relation of detector response of peak area peak of oleyl linoleate standard and methyl laurate as an internal standard at 19.979 and 3.721 min.

Table 10 Detector response factor of standard oleyl linoleate.

Conc of oleyl linoleate (mg/L) (C_x)	Peak area of oleyl linoleate (A_x)	Peak area of methyl laurate (A_{is})	Conc of methyl laurate (mg/L) (C_{is})	Response factor (R_F)
528.6	2098.98	4429.97	1606	1.44
1057.2	3620.53	4410.26	1606	1.25
1585.8	5302.11	4411.00	1606	1.22
2114.4	7661.86	4526.66	1606	1.29
3171.6	11225.90	4674.47	1606	1.22
4228.6	14819.20	4639.51	1606	1.21
mean	-	-	-	**1.27**
sd	-	-	-	0.08
%cv	-	-	-	6.30

Table 10 showed the result of response factor of standard oleyl linoleate that the response factor (RF) was determined at average value of response factor of concentration in the range 528.6 to 4228.6 mg/L and calculated response factor of oleyl linoleate by Equation 2. The response factor of oleyl linoleate was 1.2698, standard deviation (sd) was 0.0800% and coefficient of variation (%cv) was 6.3031%. This response factor value was used to calculate the concentration of oleyl linoleate contained in the final product.

From the data above, the response factor for each standard oleyl esters could be summarized as shown in Table 11.

Table 11 Summary of the response factor for three standard oleyl esters.

Standard oleyl esters	Retention time (min)	Average Response factor
Oleyl palmitate	16.572	1.0725
Oleyl oleate	19.913	1.0407
Oleyl linoleate	19.979	1.2698

5. The result of esterification reaction at the optimum conditions

The esterification reaction of palm fatty acid distillate was carried out using the optimum condition. This optimum reaction conditions involves reaction time for 1 hour, amount of catalyst was 30 %wt, reaction temperature of 60 ºC, molar ratios of oleyl alcohol to PFAD of 2:1, and stirring speed 250 rpm. The result of percentage conversion of FFA was 98%. Compositions of oleyl esters in the final product at the optimum condition were analyzed by gas chromatography (GC) as shown in Figure 26.

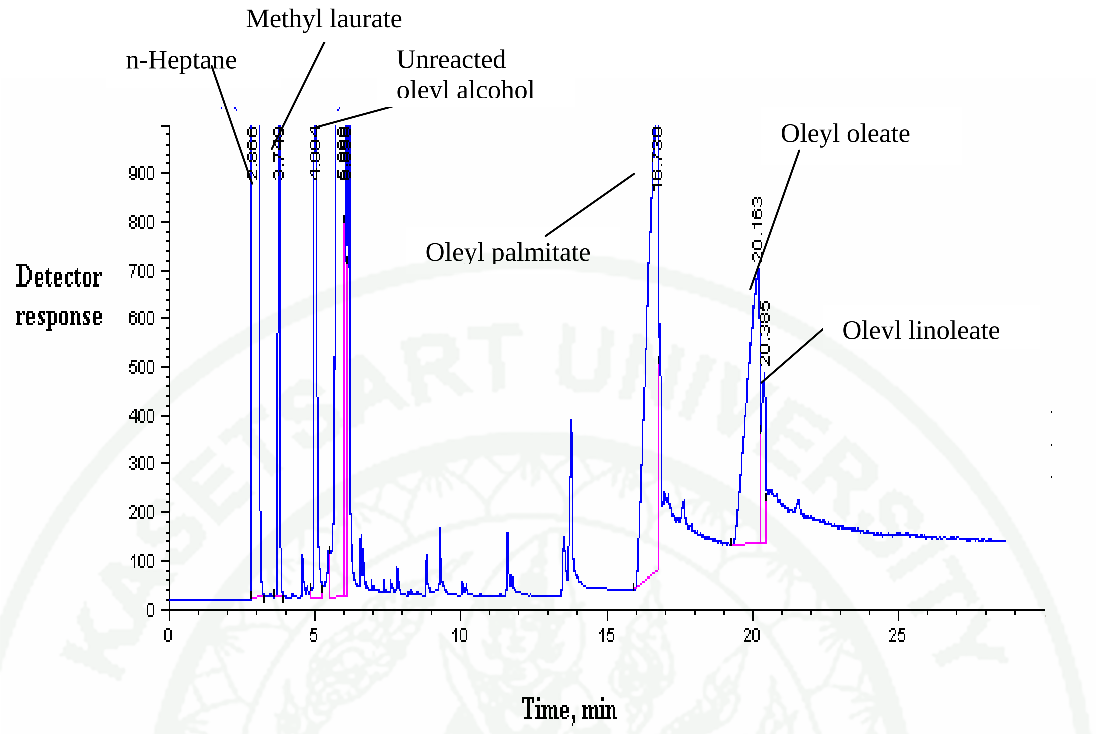


Figure 26 Chromatogram of oleyl esters in the final product at the optimum condition.

The chromatograms of the analytical method were shown in Figure 26. The analysis was carried out by comparing the retention time of peak chromatogram to the peak of authentic standard esters. Methyl laurate as an internal standard at retention time 3.749 min was added to the sample for quantitative analysis of oleyl esters yield determination. The three main fatty acids found in palm fatty acid distillate were palmitic acid, oleic acid and linoleic acid. These fatty acids reacted with oleyl alcohol to produce fatty acid oleyl esters. They were identified at retention time 16.730 min, 20.163 and 20.385 min, respectively. The highest peak at retention time 2.800 was identified as the solvent peak (n-heptane). While, the unreacted of oleyl alcohol was observed at retention time 4.60 to 6.801 min. Other minor peaks observed in the chromatogram are most probably due to the trace impurities that are present in oleyl alcohol.

The result of the amount and percent yield of three oleyl esters was shown in Table 12. Oleyl palmitate had the highest percent yield followed by oleyl oleate and oleyl linoleate, respectively. The total percentage yield of the oleyl esters that contained in wax esters product was 55.73%.

Table 12 Amount and percentage yield of oleyl esters in wax esters.

Compound	Retention time(min)	Detector response (Area peak)	Amount (mg/L)	%Yield
Methyl laurate(is)	3.749	6751.00	-	-
Oleyl palmitate	16.739	26137.80	5800	32.76
Oleyl oleate	20.163	16617.70	3800	19.61
Oleyl linoleate	20.385	3456.40	2238.15	3.36

6. Fourier Transform Infrared Spectrophotometer analysis

The confirmation of wax ester products was experimented on Fourier transform infrared spectrophotometer (FT-IR). The spectrum of PFAD and wax esters was shown in Figure 27.

(a)

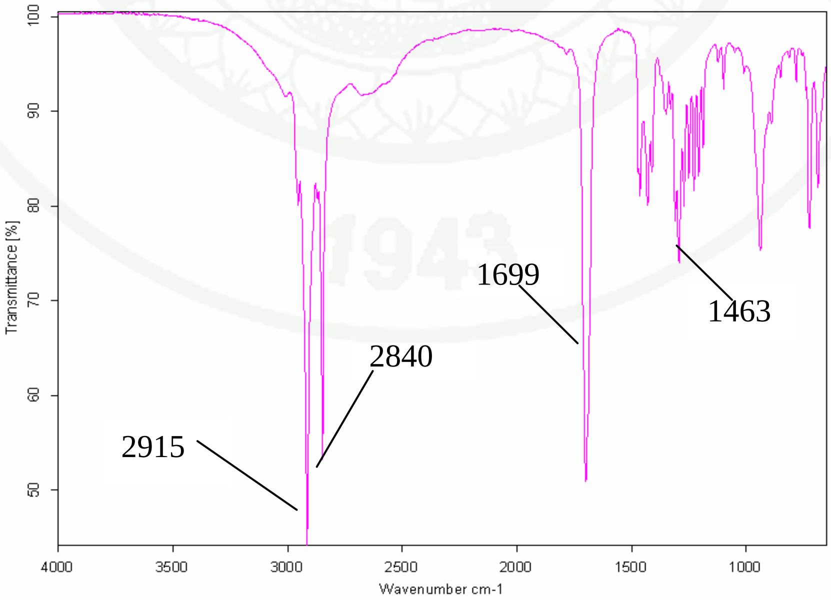


Figure 27 Infrared spectrums of palm fatty acid distillate (a) and wax esters (b)

(b)

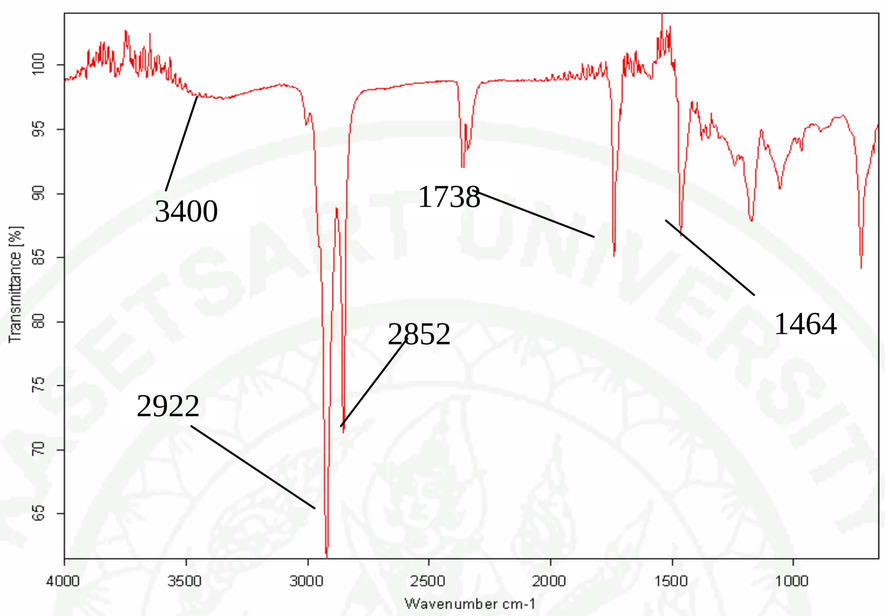


Figure 27 (continued)

To confirm the esterification reaction had occurred, infrared (IR) spectra of the starting materials (PFAD) and final products (wax esters) were compared. The absorption band at 1699 cm^{-1} , assigned to C=O stretching of acid in the IR spectrum of the starting material (PFAD), had shifted slightly to 1738 cm^{-1} which was assigned to C=O stretching of ester after esteritication reaction. This suggested the fact that the reaction has already taken place. Other absorption peaks were quite similar. The result of infrared spectrum confirmed that the product of reaction was esters product.

7. Physicochemical properties of wax esters

The physicochemical properties are important for the industrial applications. The physicochemical properties of wax esters were determined by standard procedures. Flash point, refractive index, saponification value, specific gravity, iodine value, % water, and acid value were analyzed according to AOCS Test Methods AOCS Cc 9c-95, AOCS Cc 7-25, AOCS Cc 10b-25, AOCS Cd 1-25, AOCS Ca 2e-

84, AOCS Cd 3d-63, respectively. All tests were carried out in duplicate. The physicochemical properties of wax esters were presented in Table 13.

Table 13 The physicochemical properties of wax esters.

Properties	Wax esters from PFAD	Wax esters from palm stearin*	Wax ester from palm olein*
Acid value (mg KOH/g sample)	4	0.7	0.80
viscosity at 40ºC (cSt)	16.49	-	-
Saponification value (mg KOH/g sample)	69.36	96.5	84
Iodine value (g I_2/100 g sample)	83.18	62.1	71.6
Water content (%)	0.05	-	-
Refractive index	1.460	1.453	1.457
Density (g/cm^3)	0.856	0.841	0.838
Flash point (ºC)	228	-	-

(*Keng *et al.*, 2008)

The physicochemical properties of wax esters were presented in Table 13. The corresponding physicochemical values of wax esters from palm olein and palm stearin were compared with as raw materials (Keng *et al.*, 2008).Wax ester products exhibited the highest acid value of 4 mg KOH/g of sample. The acid value indicated amount of free fatty acids formed in the samples. Higher value of FFA is a disadvantage as it will reduce the oxidative stability of the compounds and promotes rancidity (Frega *et al.*, 1999).

Refractive index was 1.460. This value increased with alkyl carbon chain length, unsaturation and conjugation but decreased with increasing temperature of measurement (Thin *et al*., 2001).

Iodine value of wax esters was 83.18 g I_2/100 g of sample. This value indicated unsaturated or double bond in wax esters. The higher iodine value indicated higher unsaturation or double bond of fats and oils such as linoleic and oleic acid (Knothe, 2002; Kyriakidis and Katsiloulis, 2000). This value is also important to increase the efficiency of wax esters as moisturizer as they can quickly permeate the skin and exhibit softening ability from within.

Water content was 0.05%. The higher water can promote a host of chemical reactions (hydrolysis) with compounds and atomic species including oil additives, base stock and suspended contaminants.

Flash point was 228 ºC performed on engine oil to measure and oil flammability limit for storage and handing. The lowest temperature to which a lubricant must be heat before its vapor, when mixed with air will ignite but not continue to burn.

Saponification value was 69.36 mg KOH/g sample. This value indicated the long chain fatty acids found in fats. Saponification value has low value because it had a relative number of carboxylic functional groups per unit mass of the fat as compared to short.

Kinematic viscosity of wax esters which was 16.49cSt (at 40ºC) defined as resistance liquid to flow.

From the overall physicochemical properties of synthesized wax esters from palm fatty acid distillate is comparable to the result of Keng *et al*., (2008) that synthesized wax esters for the purpose in cosmetic industry. Thus, wax esters from this experiment showed able to use in cosmetic industry. Moreover, the wax ester products can be used to replace the natural wax, fossil wax, petroleum wax in several applications.

CONCLUSIONS AND RECOMMENDATION

Conclusions

The esterification reaction of palm fatty acid distillate with oleyl alcohol using Amberlyst 15 as a catalyst produced wax esters. These products use for many applications such as ingredient in cosmetic, lubricant, polish, and coating. This method could replace wax esters from natural wax such as jojoba oil, bees and carnauba.

Palm fatty acid distillate (PFAD) was used as a staring raw material for synthetic wax esters. The properties of PFAD are: the initial free fatty acid (FFA) of 85%, iodine value of 57.57 g I_2/100g sample, kinematic viscosity of 10.75cSt (60 ºC), saponification value of 200.57 mg KOH/g sample, and water content of 0.05%. In addition, their compositions fatty acids are 42.49 %, 34.77% oleic acid, and 8.25% linoleic acid and 4% of total glyceride.

Five parameters were chosen to optimize the synthesis of oleyl esters, namely reaction time, reaction temperature, molar ratios of oleyl alcohol to PFAD, stirring speed, and amount of Amberlyst 15. The optimum percentage conversion of free fatty acid (>90%) was obtained within 1 hour, reaction temperature of 60ºC, molar ratios of oleyl alcohol to PFAD of 2:1, stirring speed 250 rpm, and amount of Amberlyst 15 of 30 %wt. The GC analysis gave nearly 60% of wax ester production.

FT-IR analysis was used to confirm wax ester production. The absorption band at 1699 cm^{-1} that assigned to C=O stretching of acid in the IR spectrum of the starting material (PFAD) shifted slightly to 1738 cm^{-1} that assigned to C=O stretching of ester. The result of infrared spectrum confirmed that the product of reaction was ester product.

The result of physicochemical properties was found acid values at 4 mg KOH/g sample, refractive index of 1.460, iodine value of 83.18 g I_2/100 g sample, water content of 0.05 %, flash point of 228ºC, saponification value of 69.36 mg KOH/g sample, and kinematic viscosity of 16.49cSt (40 ºC).

Reusability of Amberlyst 15 was tested by conducting three runs. After the reaction, the catalyst was filtered and then refluxed with methanol for 30 min to remove any adsorbed material from the catalyst surface and pores and dried at 105 ºC after every use. It was observed that percent conversion of free fatty acid decrease from 98 % to 80%. Thus, the catalyst could reusable up to three times.

This work indicated suggestion that wax esters can be produced at high percentage of FFA by esterification of palm fatty acid distillate and oleyl alcohol using Amberlyst 15 as catalyst. Application of this process will produced high value-added product derivative of palm fatty acid distillate.

Recommendation

This research used for application in oleo chemical industry that it can be replace wax from natural and petrochemical wax. The recommend for this experiment therefore: the final product of wax esters still has color and odor which should be studied to eliminate both problems; studied the optimization of wax ester using statistical experiment designs; this method could reduce the amount of acid value of wax ester from 4 to < 2 mg KOH /g ; studied the vitamin E, sterol, and squalene in PFAD; Studied synthesis wax ester from PFAD and selected other alcohol which has the production in Thailand (to replace oleyl alcohol).

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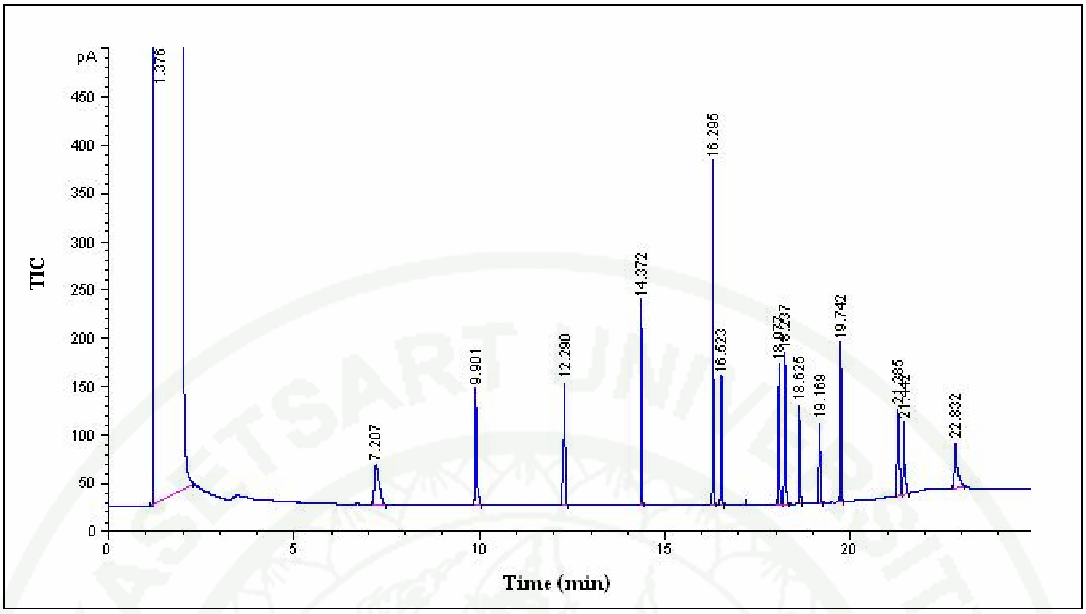
APPENDICES

Appendix A

Calibration curve of standard methyl esters



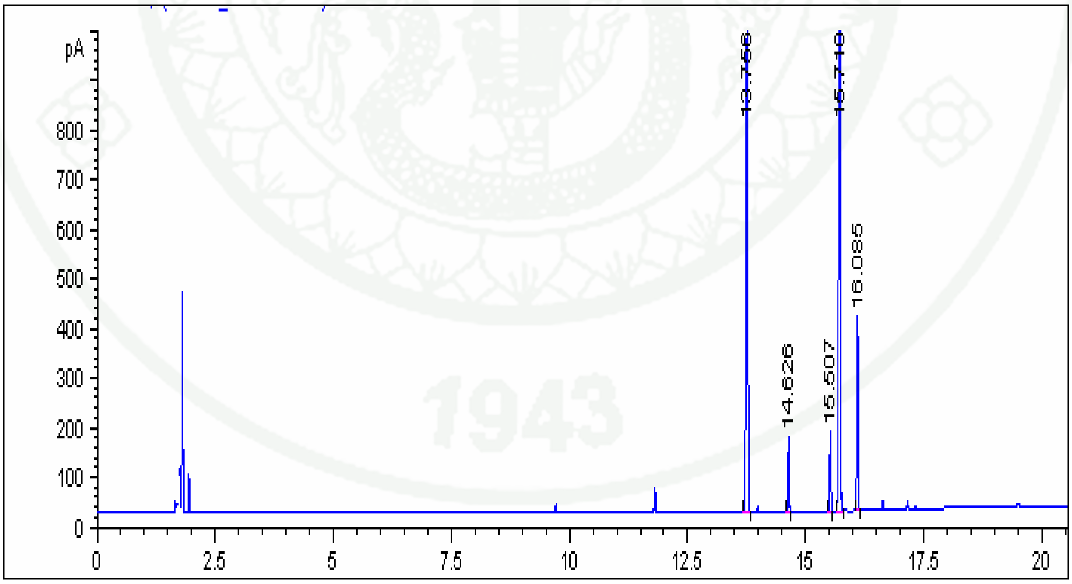
Appendix Figure A 1 The chromatogram of standard fatty acid methyl esters mixture (C_8- C_{24}).

Appendix Table A1 The relation of retention time and fatty acid compounds.

Retention time, (min)	Fatty acid compound
7.207	Methyl octanoate
9.901	Methyl decanoate
12.290	Methyl laurate
14.372	Methyl myristate
16.295	Methyl palmitate
16.523	Methyl palmitoleate
18.077	Methyl stearate

Appendix Table A1 (Continued)

Retention time, (min)	Fatty acid compound
18.237	Methyl oleate
18.625	Methyl linoleate
19.169	Methyl linolenate
19.724	Methyl arachidate
21.285	Methyl behenate
21.442	Methyl erucate
22.832	Methyl lignocerate



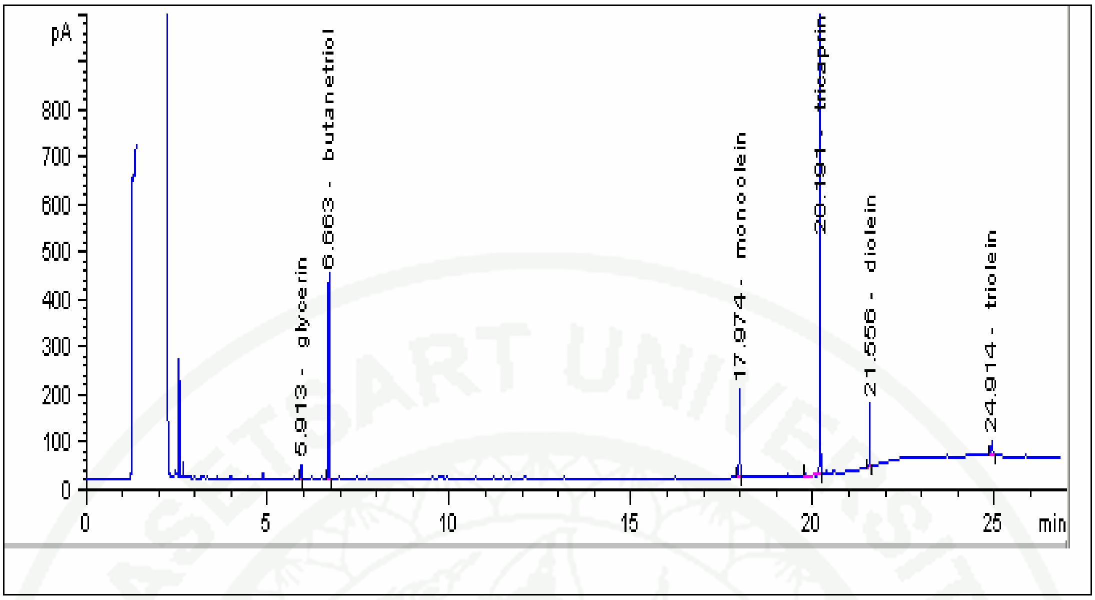
Appendix Figure A2 Chromatogram fatty acid compositions of PFAD.

Appendix Table A2 The relation of retention time and fatty acid composition compound in PFAD.

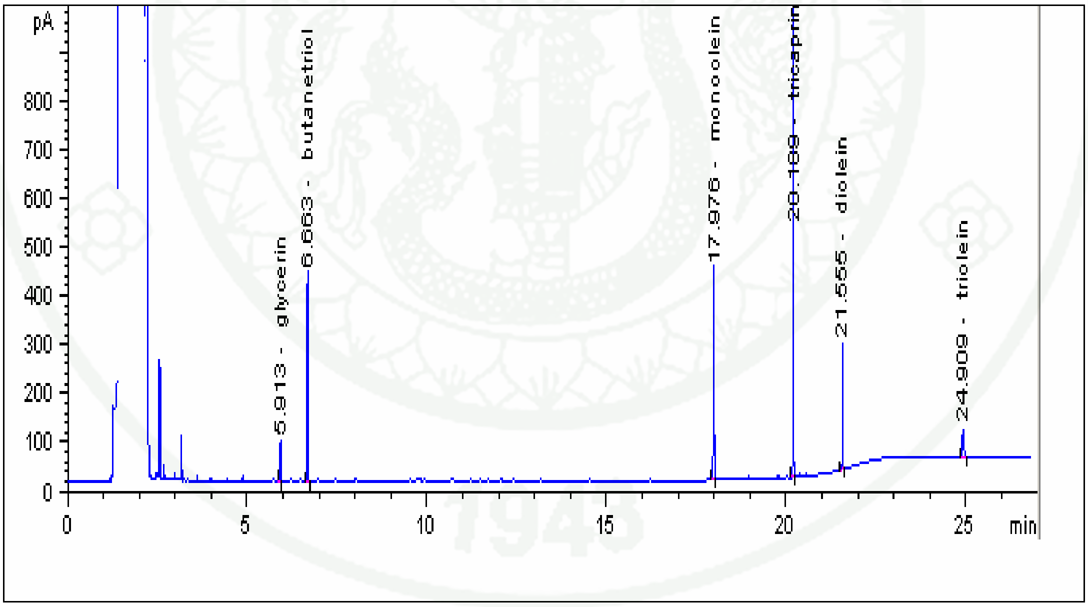
RT, (min)	Name
13.760	Methyl palmitate
13.976	Methyl palmitoleate
15.507	Methyl stearate
16.716	Methyl oleate
16.085	Methyl linoleate

Appendix B

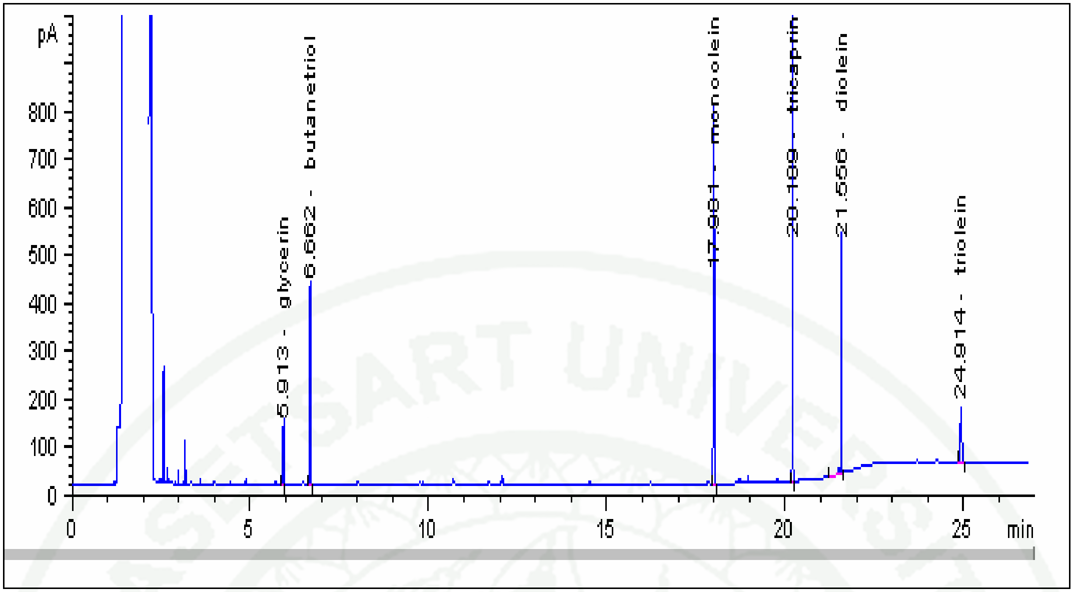
Calibration curve of standard mono, di and triolein



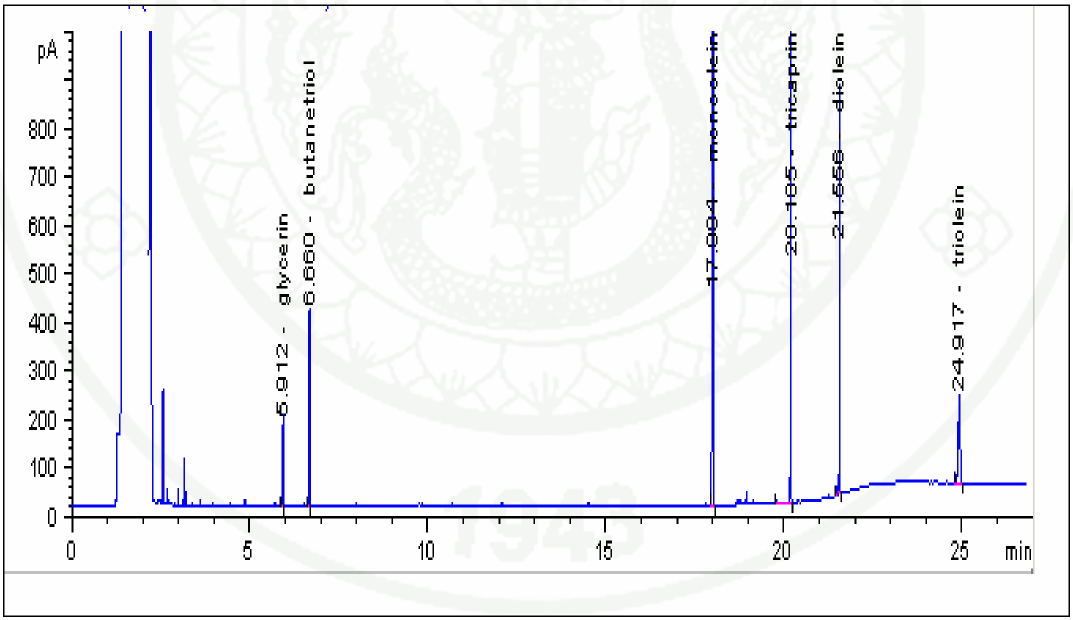
Appendix Figure B1 Chromatogram of standard mono,di, and triolein 1.



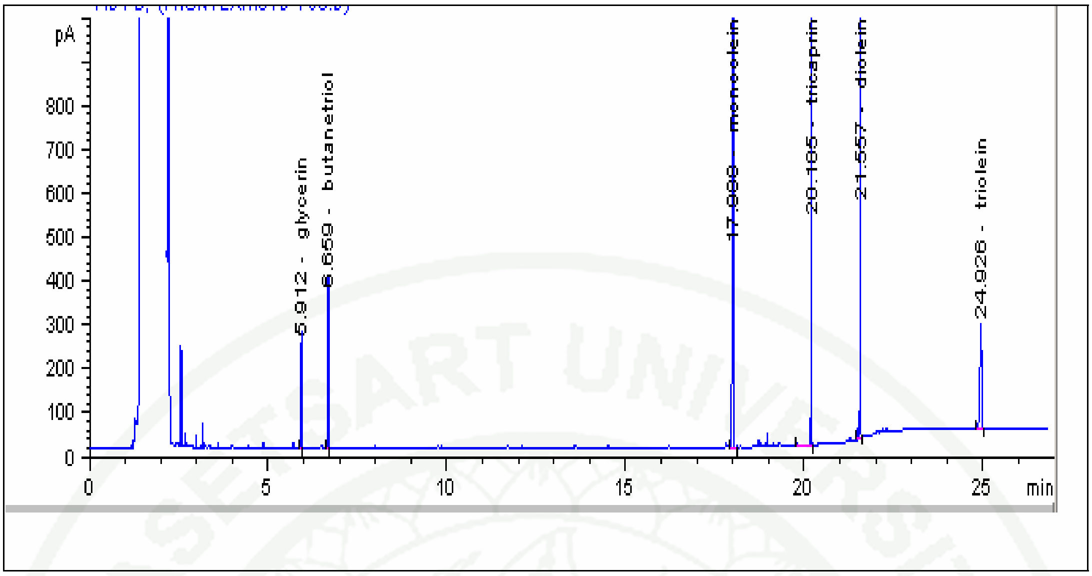
Appendix Figure B2 Chromatogram of standard mono,di, and triolein 2.



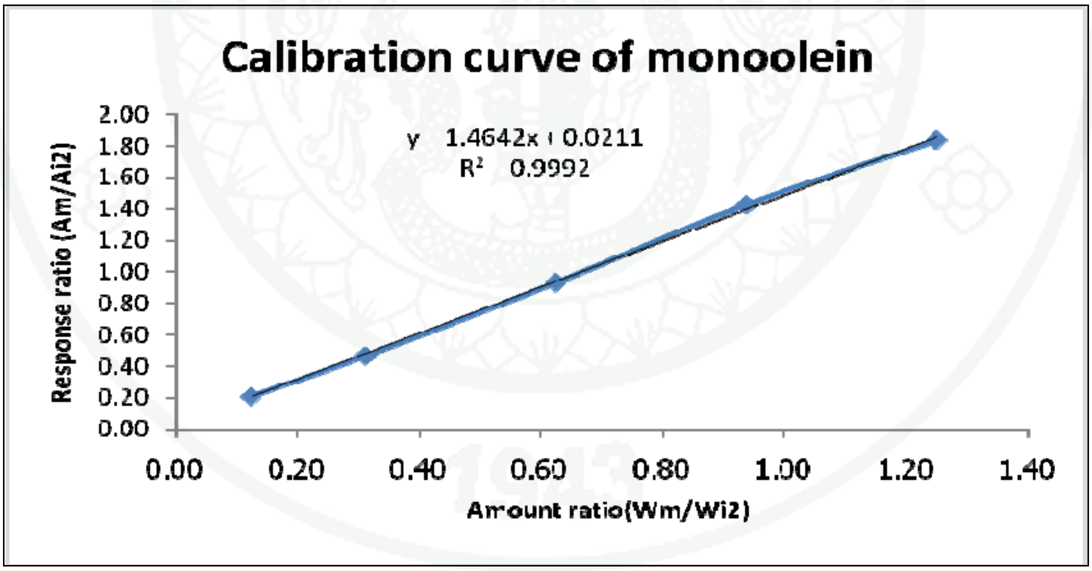
Appendix Figure B3 Chromatogram of standard mono,di, and triolein 3.



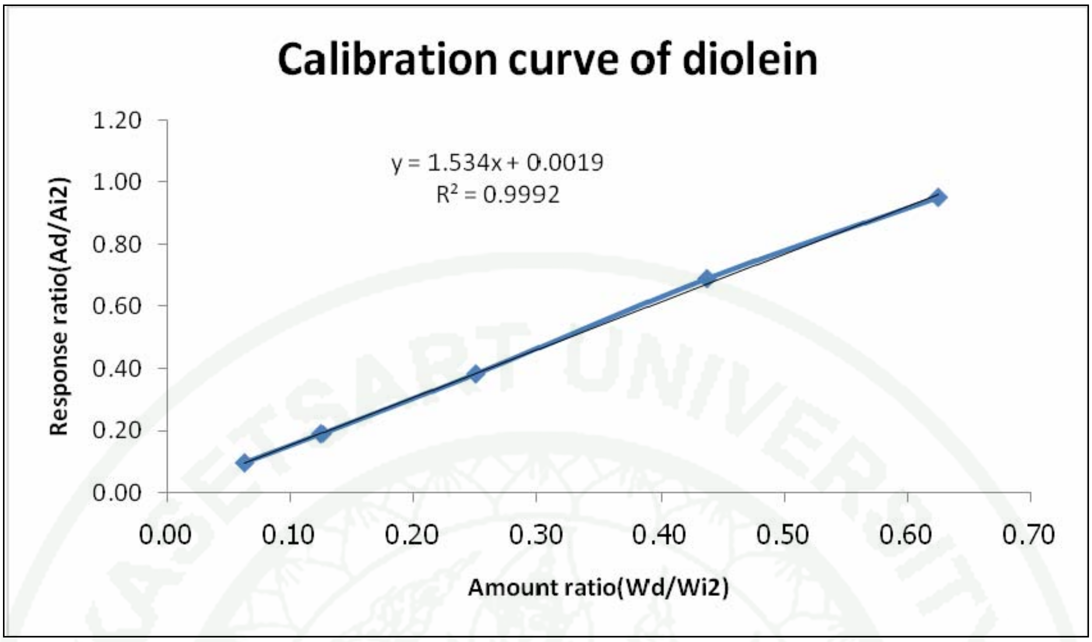
Appendix Figure B4 Chromatogram of standard mono,di, and triolein 4.



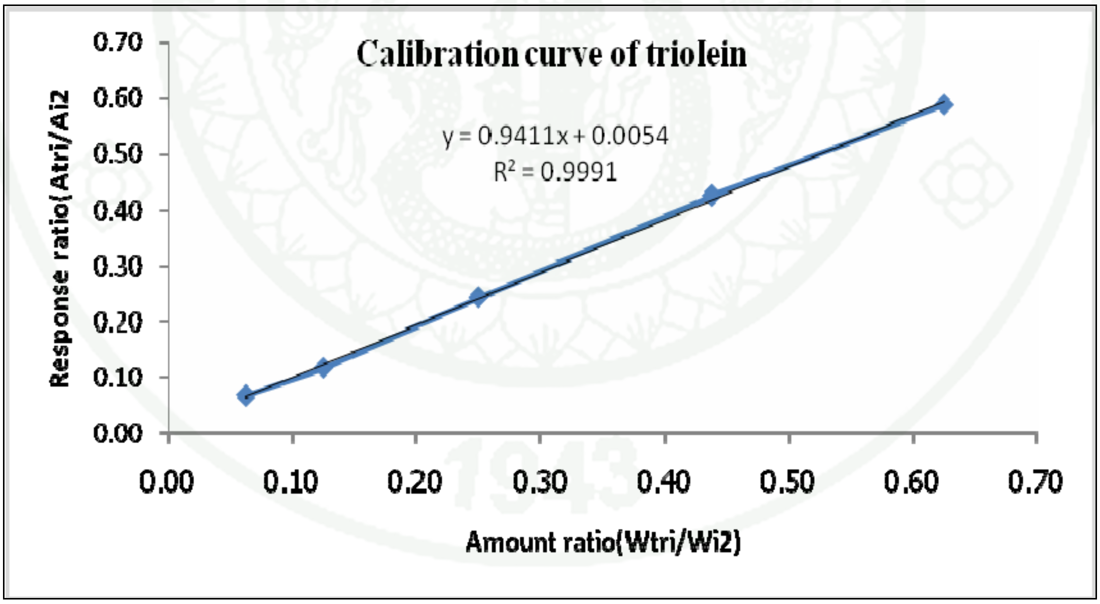
Appendix Figure B5 Chromatogram of standard mono,di, and triolein 5.



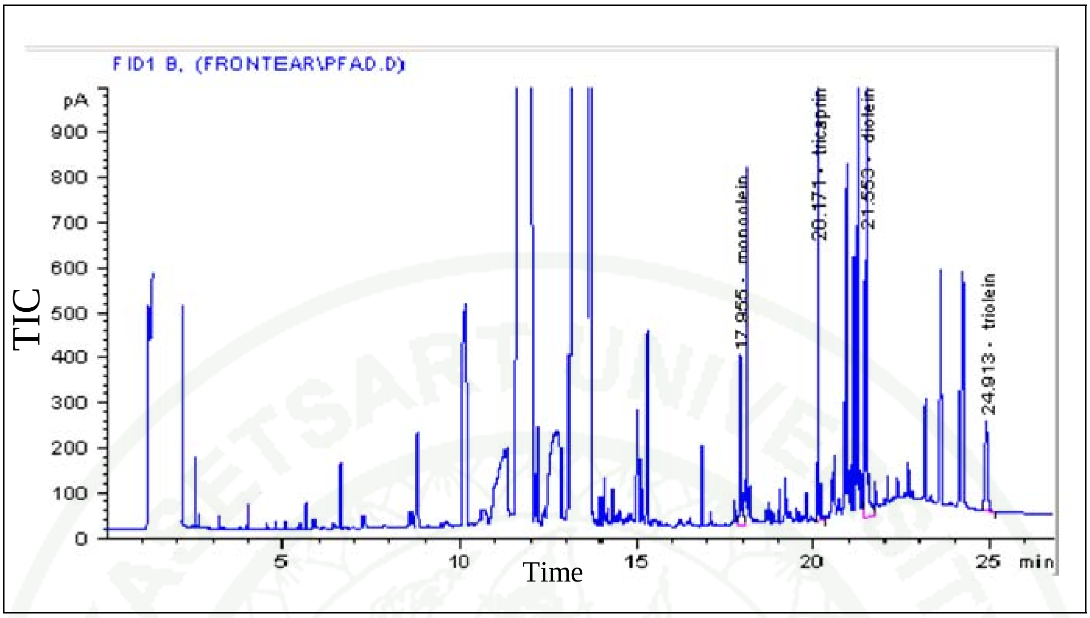
Appendix Figure B6 Calibration curve of standard monoolein.



Appendix Figure B7 Calibration curve of standard diolein.



Appendix Figure B8 Calibration curve of standard triolein.



Appendix Figure B9 Chromatogram of triglyceride compositions in PFAD.

Appendix C

The results of variable parameters for esterification reaction

Appendix Table C1 The relation of catalyst amount and percent conversion of free fatty acid by titration with 0.1N NaOH.

Amount of catalyst (%)	Initial FFA (%)	Final FFA (%)	% Conversion of FFA (as palmitic acid)
10	85	9.60	81.92
20	85	9.60	90.33
30	85	8.75	96.52
40	85	5.37	95.81
50	85	2.77	96.21
60	85	0.88	97.21

Appendix Table C2 The relation of reaction time and percent conversion of free fatty acid by titration with 0.1 N NaOH.

Reaction time (hr)	Initial FFA (%)	Final FFA (%)	% Conversion of FFA (as palmitic acid)
0.5	85	21.22	75.00
1	85	8.45	90.06
3	85	8.34	90.19
5	85	7.91	90.69

Appendix Table C2 (Continued)

Reaction time (hr)	Initial FFA (%)	Final FFA (%)	% Conversion of FFA (as palmitic acid)
7	85	3.18	96.26
9	85	1.66	98.05

Appendix Table C3 The relation of reaction temperature and percent conversion of free fatty acid by titration with 0.1 N NaOH.

Reaction temperature (˚C)	Initial FFA (%)	Final FFA (%)	% Conversion of FFA (as palmitic acid)
50	85	12.34	85.48
60	85	4.12	95.15
70	85	9.23	89.14
80	85	11.03	87.02
90	85	8.92	89.51

Appendix Table C4 The relation of molar ratios of oleyl alcohol to PFAD and percent conversion of free fatty acid by titration with 0.1 N NaOH.

Molar ratio of oleyl alcohol to PFAD	Initial FFA (%)	Final FFA (%)	%Conversion of FFA (as palmitic acid)
1	85	9.85	88.41
1.5	85	7.57	91.10
1.8	85	10.20	96.34
2	85	3.11	99.26
2.5	85	0.63	98.89
2.8	85	0.94	99.47
3	85	0.45	96.18

Appendix Table C5 The relation of stirring speed and percent conversion of free fatty acid by titration with 0.1 N NaOH.

Stirring speed (rpm)	Initial FFA (%)	Final FFA (%)	% Conversion of FFA (as palmitic acid)
125	85	7.77	90.86
250	85	6.4	92.47
500	85	6.2	92.71

Appendix Table C5 (Continued)

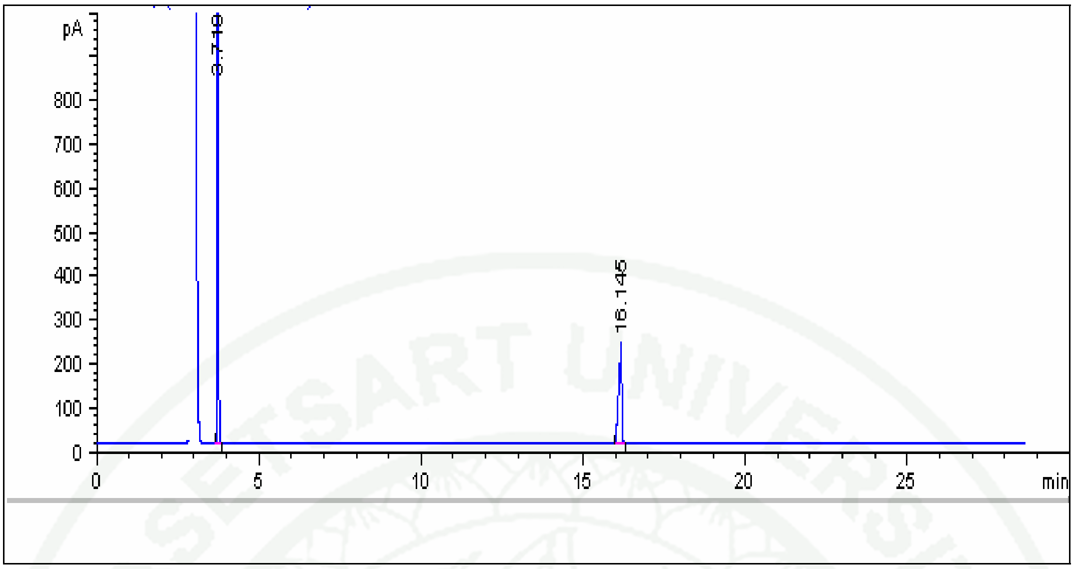
Stirring speed (rpm)	Initial FFA (%)	Final FFA (%)	%Conversion of FFA (as palmitic acid)
750	85	5.1	94
1000	85	4.9	94.24
1250	85	2.94	96.54

Appendix Table C6 The relation of reusability of catalyst and percent conversion of free fatty acid by titration with 0.1 N NaOH.

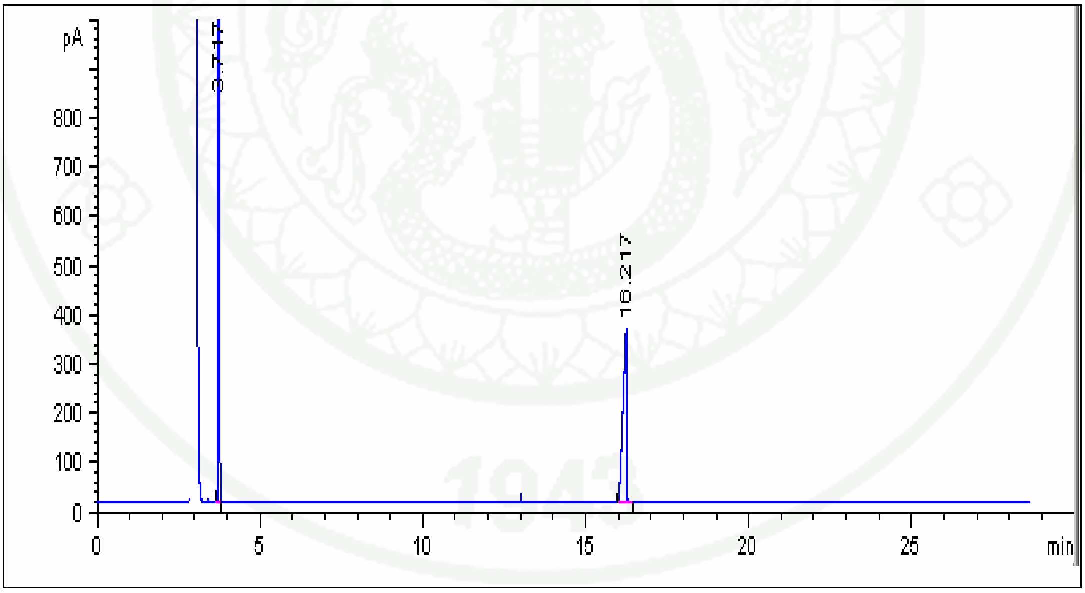
Reuse No	Initial FFA (%)	Final FFA (%)	%Conversion of FFA (as palmitic acid)
1	85	7.77	90.86
2	85	6.4	92.47
3	85	6.2	92.71
4	85	5.1	94

Appendix D

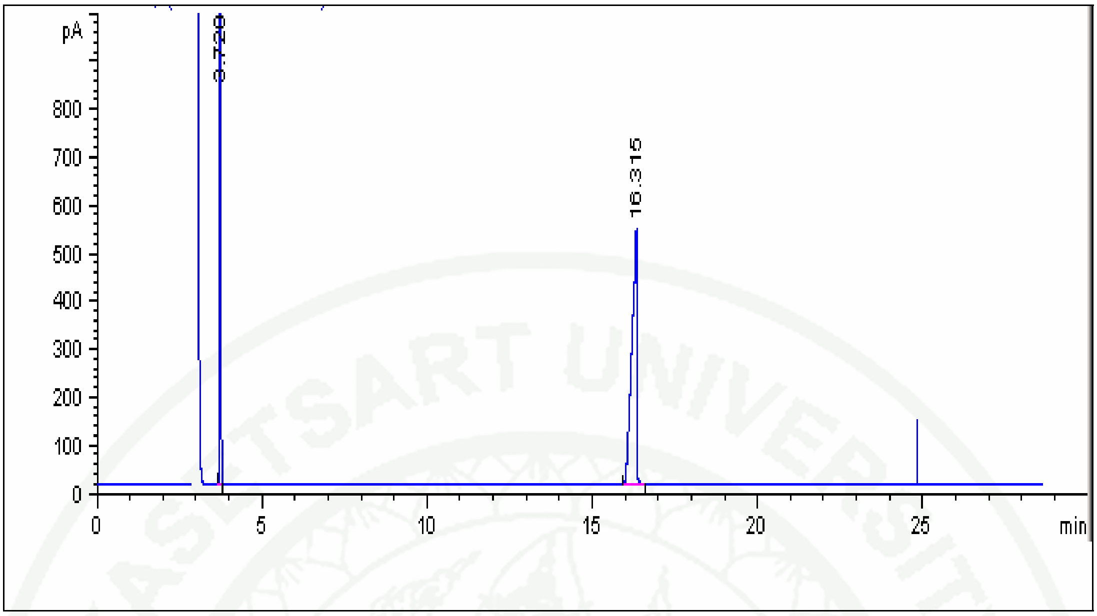
Calibration curve of standard oleyl esters



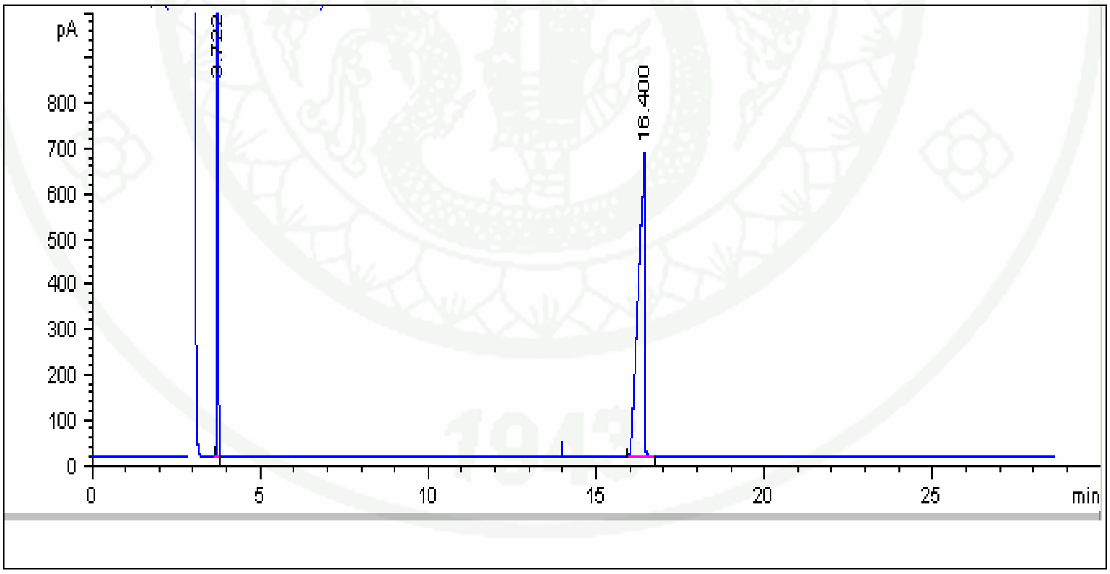
Appendix Figure D1 Chromatogram of standard oleyl palmitate concentration 500 mg/L.



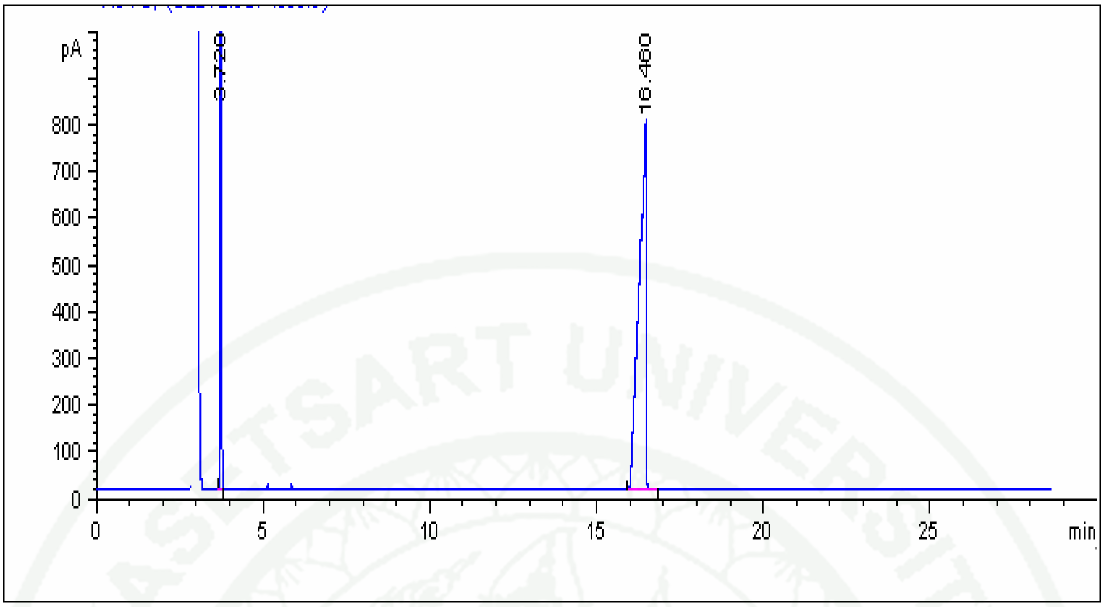
Appendix Figure D2 Chromatogram of standard oleyl palmitate concentration 1000 mg/L.



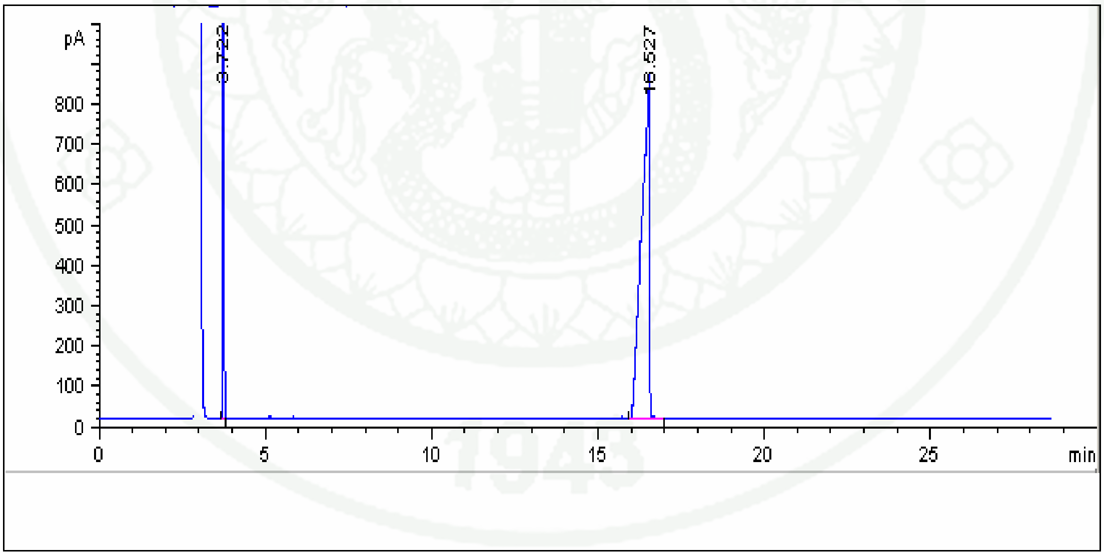
Appendix Figure D3 Chromatogram of standard oleyl palmitate concentration 2000 mg/L.



Appendix Figure D4 Chromatogram of standard oleyl palmitate concentration 3000 mg/L.



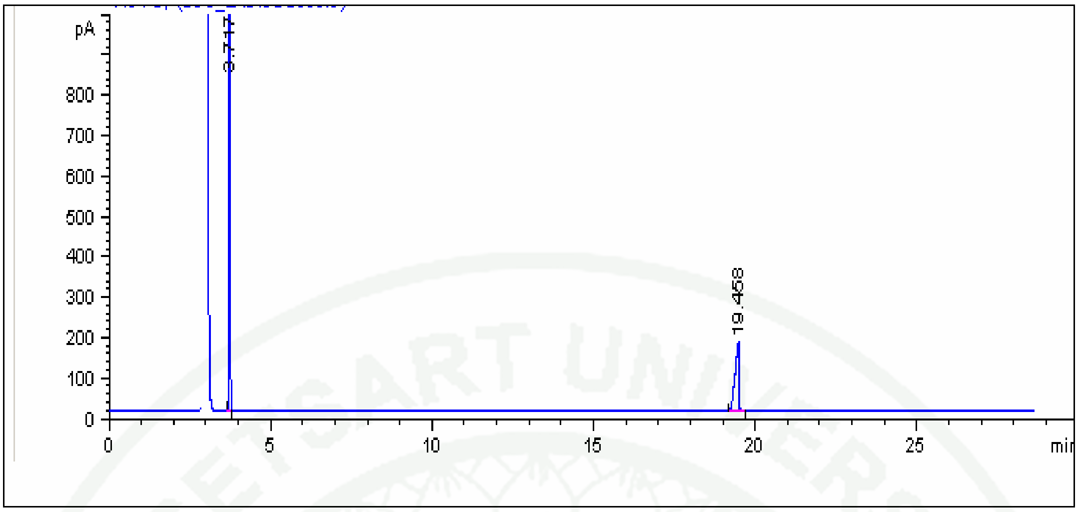
Appendix Figure D5 Chromatogram of standard oleyl palmitate concentration 4000 mg/L.



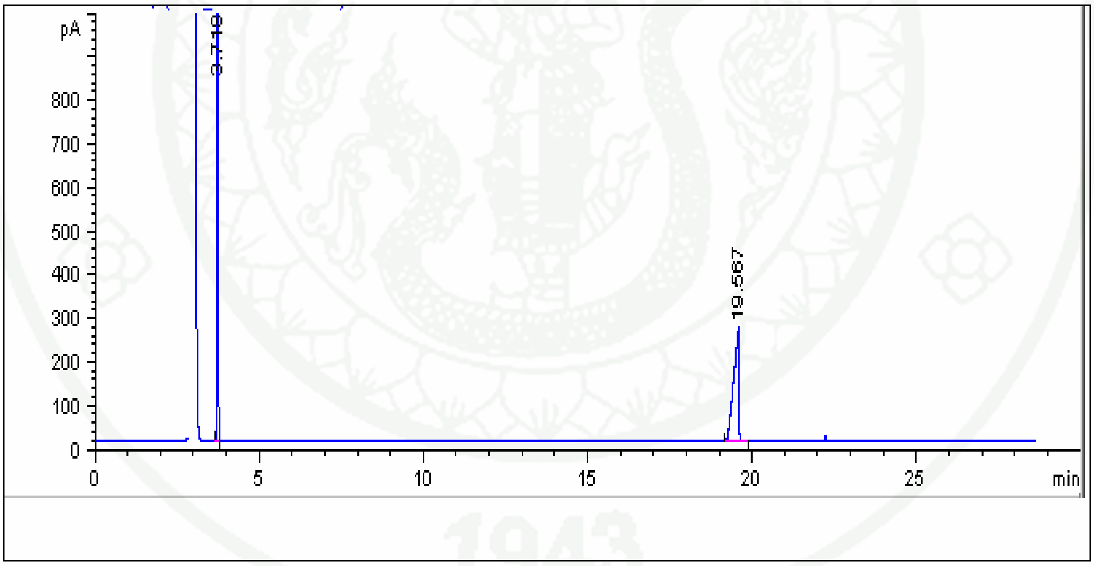
Appendix Figure D6 Chromatogram of standard oleyl palmitate concentration 5000 mg/L.

Appendix Table D1 Detector response factor of standard oleyl palmitate.

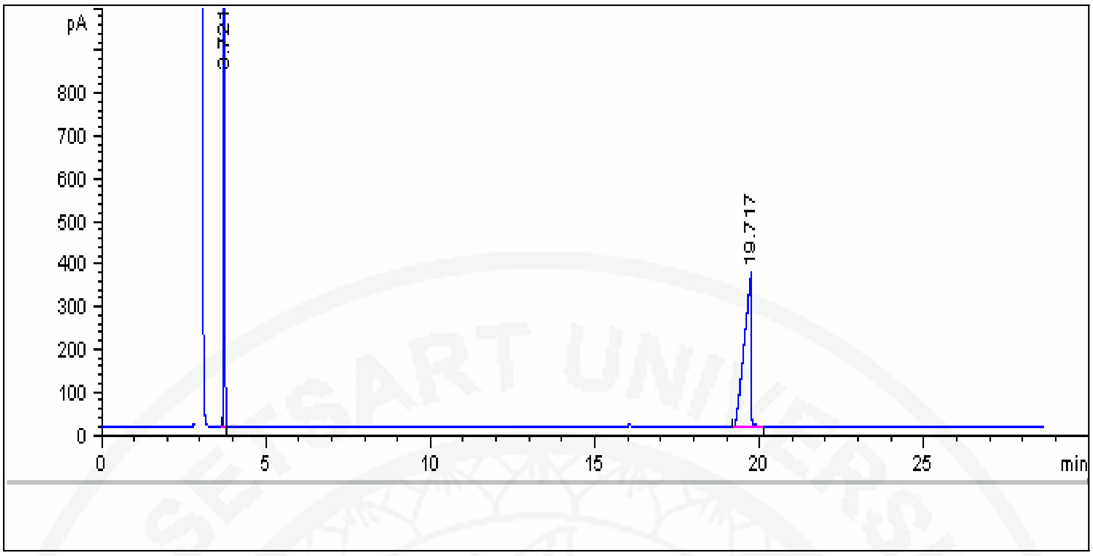
Conc of Oleyl palmitate (mg/L) (C_x)	Peak area of Oleyl palmitate (A_x)	Peak area of methyl laurate (A_{is})	Conc of methyl laurate (mg/L) (C_{is})	Response factor (R_F)
500	1350.08	4034.85	1606	1.0747
1000	2755.70	4249.33	1606	1.0415
2000	5653.88	4326.86	1606	1.0493
3000	8642.70	4258.49	1606	1.0865
4000	11767.00	4339.47	1606	1.0887
5000	14436.50	4236.74	1606	1.0945
mean	-	-	-	**1.0725**
sd	-	-	-	0.0202
%cv	-	-	-	1.8809



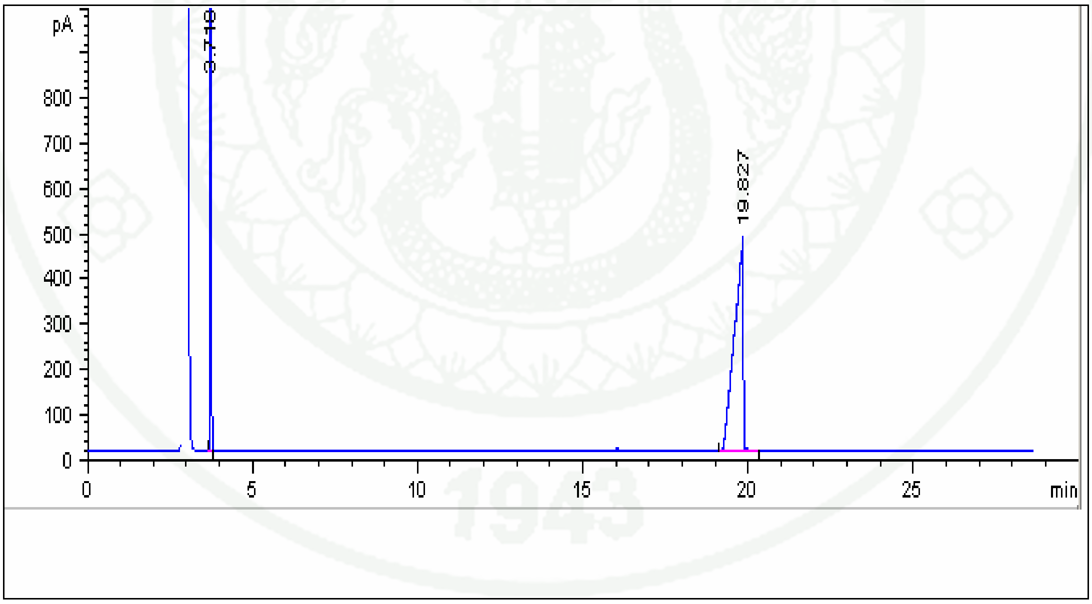
Appendix Figure D7 Chromatogram of standard oleyl oleate concentration 512.5 mg/L.



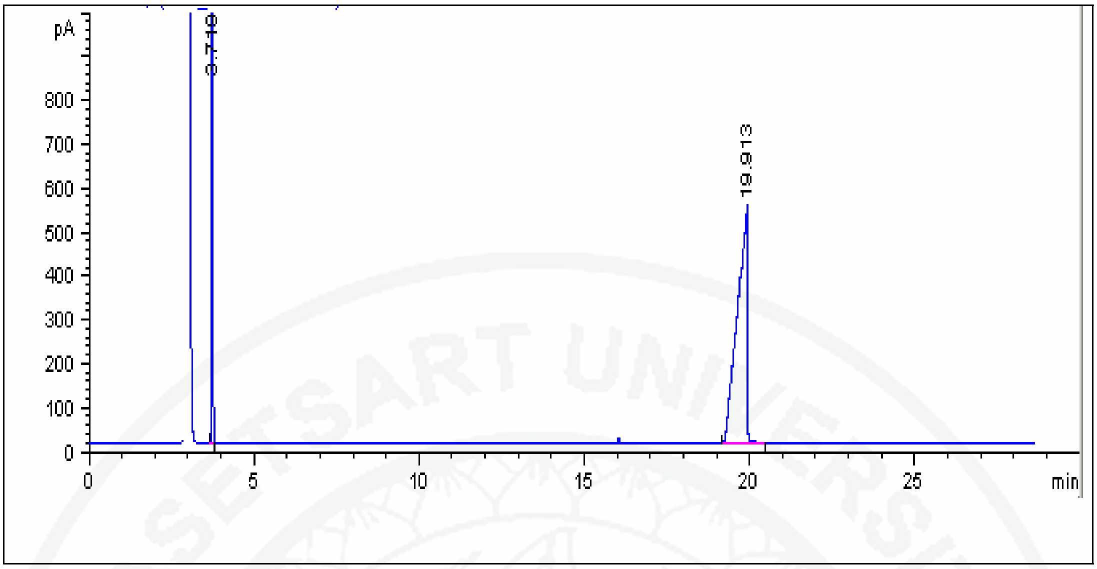
Appendix Figure D8 Chromatogram of standard oleyl oleate concentration 1025 mg/L.



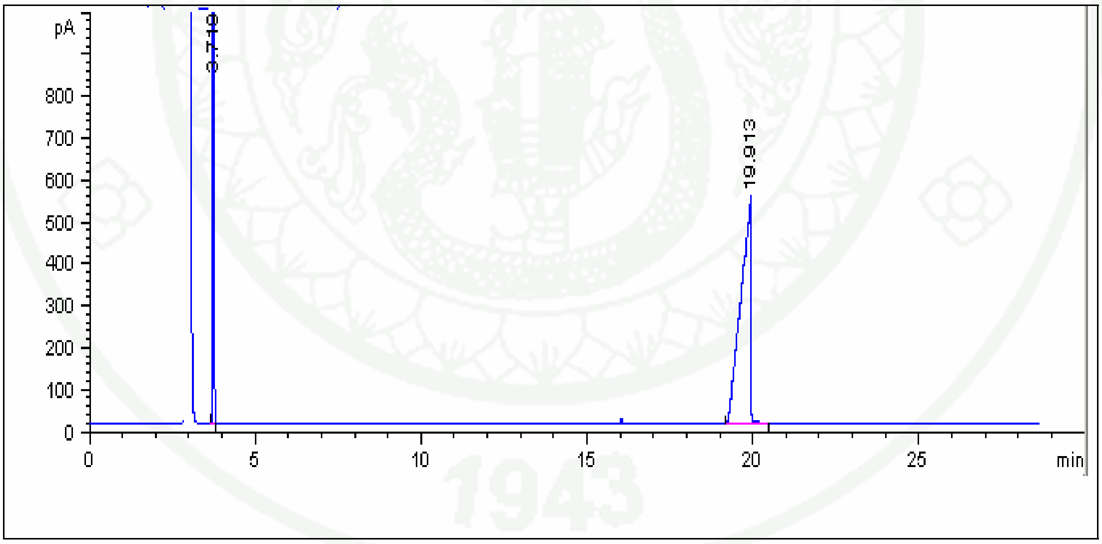
Appendix Figure D9 Chromatogram of standard oleyl oleate concentration 2050 mg/L.



Appendix Figure D10 Chromatogram of standard oleyl oleate concentration 3075 mg/L.



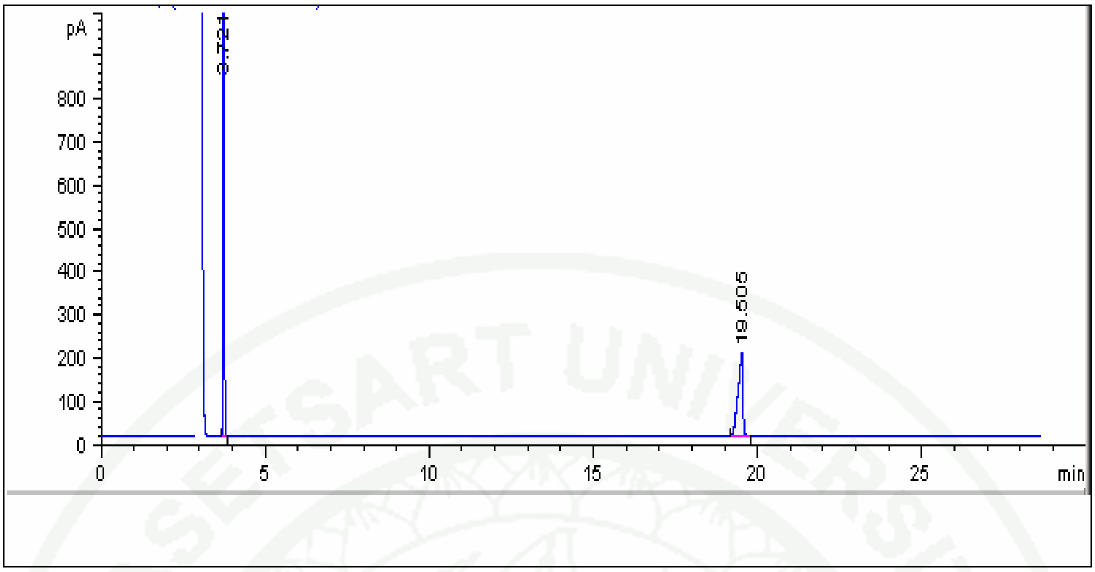
Appendix Figure D11 Chromatogram of standard oleyl oleate concentration 4100 mg/L.



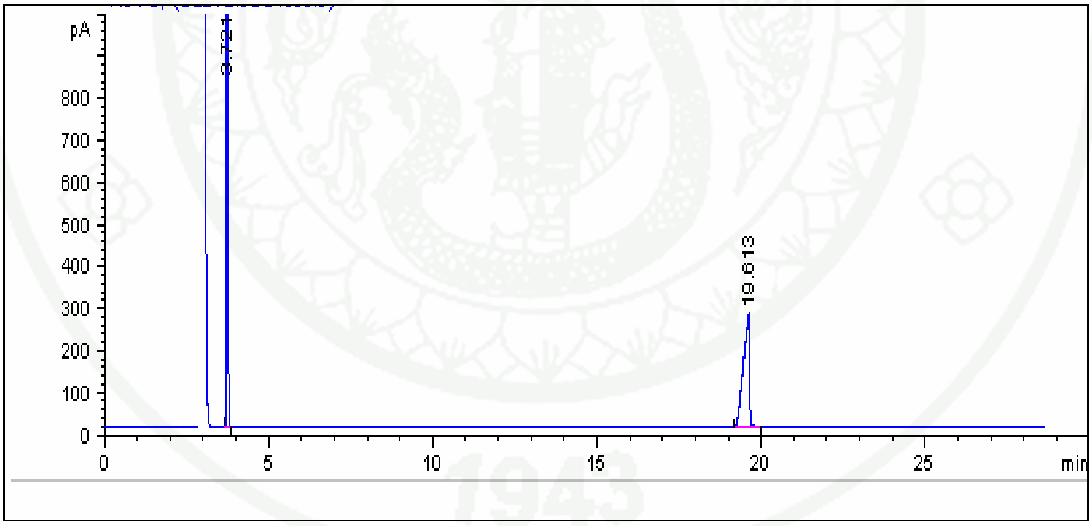
Appendix Figure D12 Chromatogram of standard oleyl oleate concentration 5125 mg/L.

Appendix Table D2 Detector response factor of standard oleyl oleate.

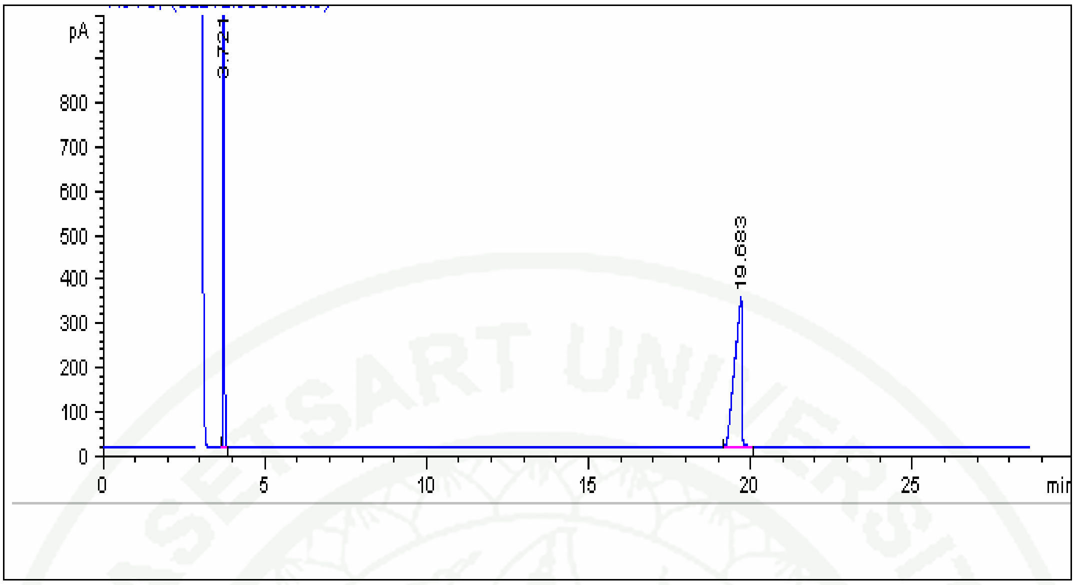
Conc of Oleyl oleate(mg/L) (C_x)	Peak area of Oleyl oleate (A_x)	Peak area of methyl laurate (A_{is})	Conc of methyl laurate (mg/L) (C_{is})	Response factor (R_F)
512.5	1397.46	4298.12	1606	1.0189
1025	2947.80	4348.86	1606	1.0620
2050	5369.64	4367.16	1606	0.9632
3075	8883.46	4330.50	1606	1.0714
4100	11576.10	4297.79	1606	1.0551
5125	14742.90	4304.21	1606	1.0734
mean	-	-	-	**1.0407**
sd	-	-	-	0.0390
%cv	-	-	-	3.7475



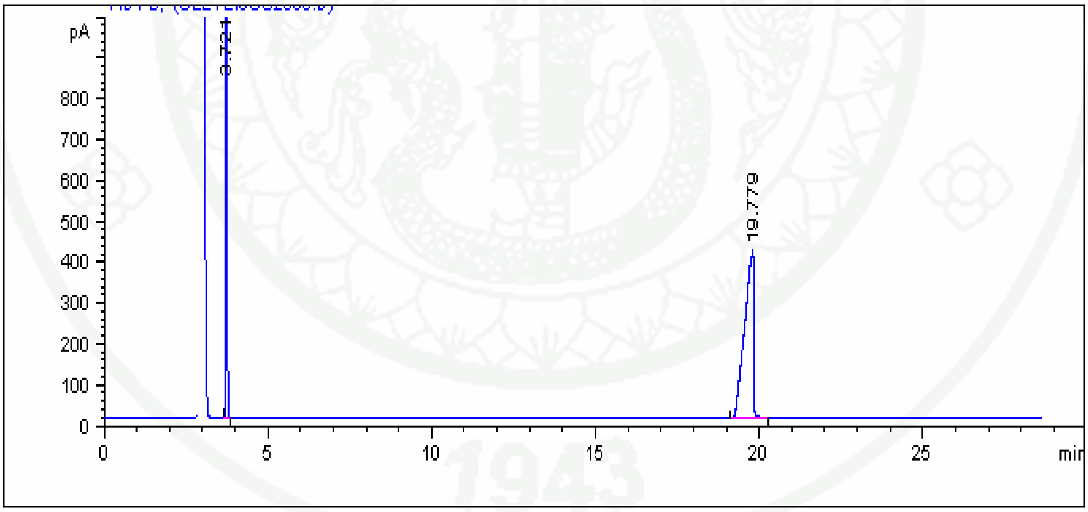
Appendix Figure D13 Chromatogram of standard oleyl linoleate concentration 528.6 mg/L.



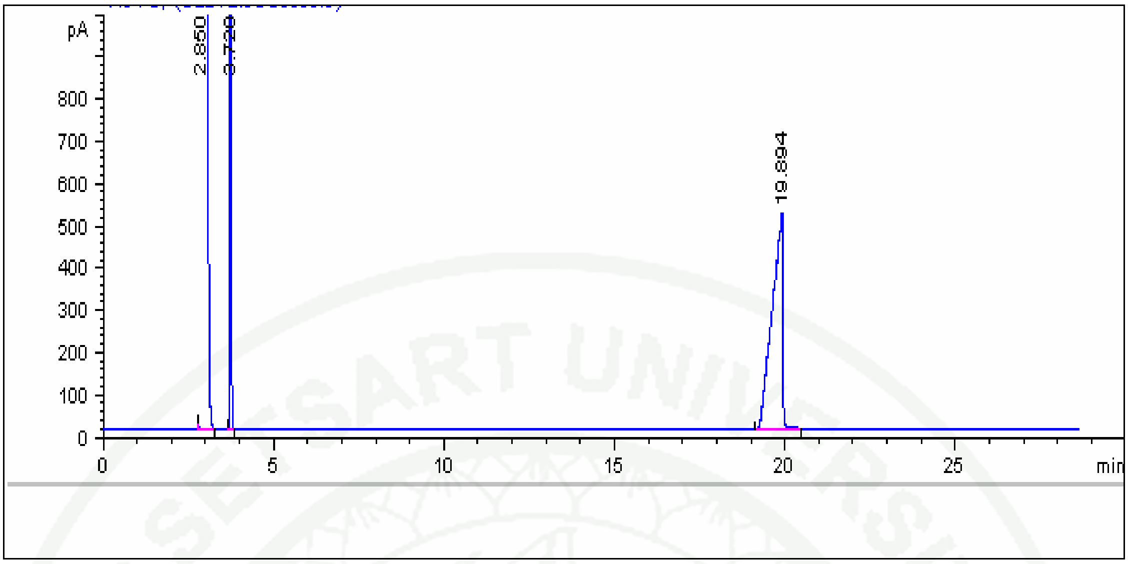
Appendix Figure D14 Chromatogram of standard oleyl linoleate concentration 1057.2 mg/L.



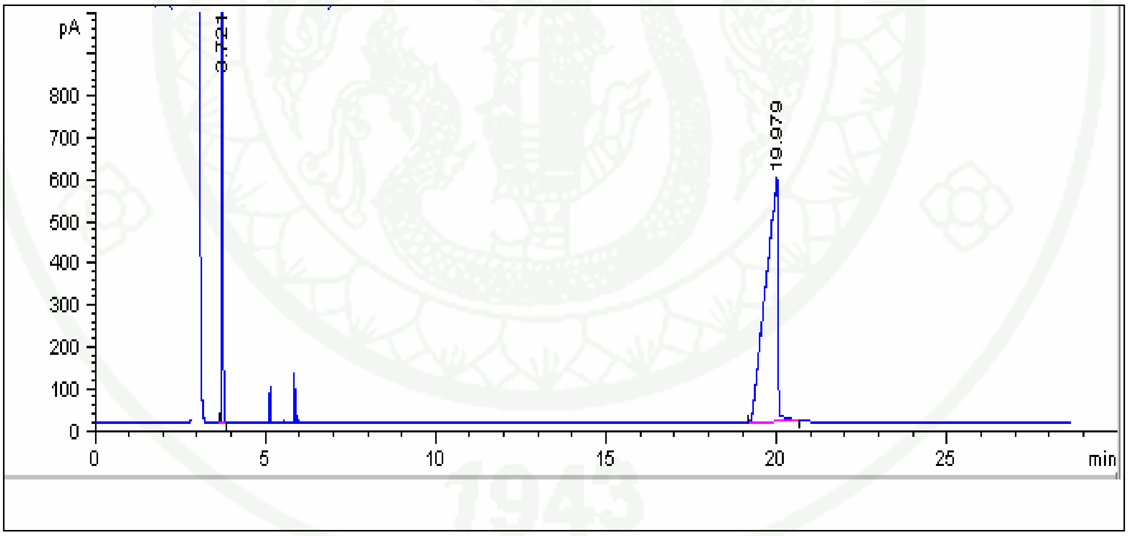
Appendix Figure D15 Chromatogram of standard oleyl linoleate concentration 1585.8 mg/L.



Appendix Figure D16 Chromatogram of standard oleyl linoleate concentration 2114.4 mg/L.



Appendix Figure D17 Chromatogram of standard oleyl linoleate concentration 3176.6 mg/L.



Appendix Figure D18 Chromatogram of standard oleyl linoleate concentration 4228.6 mg/L.

Appendix Table D3 Detector response factor of standard oleyl linoleate.

Conc of oleyl linoleate (mg/L) (Cx)	Peak area of oleyl linoleate (Ax)	Peak area of methyl laurate (Ais)	Conc of methyl laurate (mg/L) (Cis)	Response factor (R_F)
528.6	2098.98	4429.97	1606	1.4395
1057.2	3620.53	4410.26	1606	1.2471
1585.8	5302.11	4411.00	1606	1.2173
2114.4	7661.86	4526.66	1606	1.2856
3171.6	11225.90	4674.47	1606	1.2161
4228.6	14819.20	4639.51	1606	1.2131
mean	-	-	-	**1.2698**
sd	-	-	-	0.0800
%cv				6.3002

Appendix E

Analysis method

Appendix E1 Acid value by AOCS Official Method Cd 3d-63.

Procedure

1. Add indicator solution to the required amount of solvent in ratio of 2 mL to 125 mL and neutralize with alkali to a faint but permanent pink color.

2. Determine the sample size from the following table below.

Acid value	Mass, g of sample (±10%),g	Weighing accuracy, ±g
0-1	20	0.05
1-4	10	0.02
4-15	2.5	0.01
15-75	0.5	0.001
75 and over	0.1	0.002

3. Weigh the specified amount of a well-mixed liquid sample into an Erlenmeyer flask.

4. Add 125 mL of the neutralized solvent mixture. Be sure that the sample is completely dissolved before titrating. Warming may be necessary in some cases.

5. Shake the sample vigtorously while titrating with standard alkali to the first permanent pink color of the same intensity as that of the nertralized solvent before the latter was added to the sample. The color must persist for 30 s.

6. Perform a blank titration using 125 mL of the neutralized solvent mixture.

Calculation

Acid value, mg KOH/g of sample = $\frac{A - B \times N \times 56.1}{W}$

Where:

A = volume, mL of standard alkali used in the titration
B = volume, mL of standard alkali used in the titrating the blank
N = normality of standard alkali
W = mass, grams of sample

7. To express in terms of free fatty acids as percent lauric, oleic, or palmitic, divide the acid value by 1.99, 2.81, or 2.56, respectively.

Appendix E2 Free Fatty Acids by AOCS Official Method Ca 5a-40.

Table Free fatty acid range, alcohol volume and strength of alkali.

FFA range (%)	Sample (g)	Alcohol (mL)	Strength of alkali
0.00 to 0.2	56.4 ± 0.2	50	0.1 N
0.2 to 1.0	28.2 ± 0.2	50	0.1 N
1.0 to 30.0	7.05 ± 0.05	75	0.25 N
30.0 to 50.0	7.05 ± 0.05	100	0.25 or 1.0 N
50.0 to 100	3.525 ± 0.001	100	1.0 N

Procedure

1. Sample must be well mixed and entirely liquid before weighing; however, do not heat the sample more than 10ºC over the melting point.

2. Use Table above to determine the sample weight for various ranges of fatty acids. Weigh the designated sample size into an oil sample bottle or Erlenmeyer flask.

3. Add the specified amount of hot neutralized alcohol and 2 mL of indicator.

4. Titrate with standard sodium hydroxide, shaking vigorously until the appearance of the first permanent pink color of the same intensity as that of the neutralized alcohol before the addition of the sample. The color must persist for 30 seconds.

Calculations

1. The percentage of free fatty acids in most types of fats and oils is calculated as oleic acid, although in coconut and palm kernel oils it is frequently expressed as lauric acid and in palm oil in terms of palmitic acid.

(a) Free fatty acids as oleic, % $= \dfrac{\text{ml of alkali} \times N \times 28.2}{\text{weight sample (g)}}$

(b) Free fatty acids as lauric, % $= \dfrac{\text{ml of alkali} \times N \times 20}{\text{weight sample (g)}}$

(c) Free fatty acids as palmitic, % $= \dfrac{\text{ml of alkali} \times N \times 28.2}{\text{weight sample (g)}}$

2. The free fatty acids are frequently expressed in terms of acid value instead of percentage free fatty acids. The acid value is defined as the number of milligrams

of KOH necessary to neutralize 1 g of sample. To convert percentage free fatty acids (as oleic) to acid value, multiply the percentage free fatty acids by 1.99.

Appendix E3 Saponification value by AOCS Official Method Cd 3-25.

Procedure

1. Melt the sampleif it is not already liquid and filter through dry filters paper to remove and impurities and moisture. The sample must be completely dry.

2. Weigh a test portion of such size that the back titration is 45-55% of the blank. This usually requires a test portion of 1.0-2.2 g if 25 mL of KOH are used (Table 1) or 4-5 g if 50 mL of KOH are used. Add the alcoholic KOH with a pipet and allow the pipet to drain for a definite period of time.

3. Prepare and conduct blank determinations simultaneously with the sample and similar in all respects, except omitting the fat or oil.

Expected saponification value	Mass of test portion
150 to 200	2.2 g – 1.8 g
200 to 250	1.7 g – 1.4 g
250 to 300	1.3 g – 1.2g
>300	1.1g – 1.0 g

3. Connect the condenser and boil gently, but steadily, until the sample is completely saponified. This usually requires about 1 hr for normal samples (see Notes, 3). Make certain that the vapor ring in the condenser does not rise to the top of the condenser, or loss may occur.

4. After the flask and condenser have cooled somewhat, but not sufficiently to form a gel, wash the inside of the condenser with a small quantity of distilled water. Disconnect the condenser, add about 1 mL of phenolphthalein indicator and titrate with 0.5 M HCl until the pink color just disappears. Record the volume of 0.5 M HCl required for the titration.

Calculation

$$\text{Saponification value} = \frac{(B - S) \times (M)}{W} \times 56.1$$

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

M = molarity of HCl solution

W = mass of test portion in grams

Appendix E4 Iodine value by AOCS Official Method Cd 1-25.

Procedure

1. Melt the sample, if it is not already liquid (the temperature during melting should not exceed the melting point of the sample by more than 10°C), and filter through two pieces of filter paper to remove any solid impurities and the last traces of moisture. The filtration may be performed in an air oven at 80-85°C, but should be completed within 5 min ± 30 sec. the sample must be absolutely dry.

2. After filtration, allow the filtered sample to achieve a temperature of 68-71 ± 1°C before weighing the sample.

3. Once the sample has achieved a temperature of 68-71 ± 1°C, immediately weigh the sample into a 500-mL iodine flask.

4. Add 15 mL of carbon tetrachloride on top of the sample and swirl to ensure that the sample is completely dissolved.

5. Dispense 25 mL of Wijs solution using the pipet into flask containing the sample, stopper the flask, and swirl to ensure an intimate mixture. Immediately set the timer for 30 min.

6. Immediately store the flasks in the dark for the required reaction time at a temperature of 25±5°C.

7. Remove the flasks from storage and add 20 mL of KI solution, followed by 100 mL of distilled water.

8. Titrate with 0.1 N $Na_2S_2O_3$ solution, adding it gradually and with constant and vigorous shaking. Continue the titration until the yellow color has almost disappeared. Add 1-2 mL of starch indicator solution and continue the titration until the blue color just disappears.

9. Prepare and conduct at least one blank determination with each group of samples simultaneously and similar in all respects to the samples.

Appendix E5 %Water content by Karl Fischer Reagent by AOCS Official Method Ca 2e-84.

Determination of the water equivalent of the karl fisher reagent

1. Accurately weigh (to the nearest 0.0001 g) a drop of water, approximately 50 mg, and transfer to the titration vessel. For this calibration of the reagent, 250 mg of crystalline sodium tartrate ($Na_2C_4H_4O_6.2H_2O$) may also be used instead of a drop of water.

2. Add the stirring rod by means of a pair of tweezers and connect the titration vessel with the apparatus.

3. Add 20 mL of methanol (Reagent, 1) by means of a pipet or a burette. Close the aperture and dissolve by stirring gently.

4. Titrate with the Karl Fischer reagent up to the electrometric end point is reached when the deflection remains constant for 30 sec after dropwise adding the Karl Fischer reagent. Carry out a blank test.

5. For direct reading apparatus, the calibration factor is inserted into the equipment in accordance with manufacturer's instruction, and a blank determination is not required.

Procedure

1. Weigh (to the nearest 0.01 g) 5-25 g of the sample into the titration vessel, but make certain that it does not contain more than 100 mg of water dilute the Karl Fischer solution in case of a low water content and/or poor solubility of the sample in order to get a sufficiently high titration.

2. Put into the titration vessel the stirring rod by means of a pair of tweezers and connect it with the apparatus. Add, if necessary, exactly 10 mL chloroform or

another suitable solvent and dissolve completely. These are first dissolved in 10 mL of dry chloroform and then 10 mL of methanol are added.

3. The amount of solvent used must allow the electrode to dip 2-3 mm.
4Add 10 mL of methanol and titrate with the Karl Fishcher reagent up to the electrometric end point.

4. Carry out a blank test following the same procedure and using the same quantity of all of the reagents as used for the determination but omitting the test portion. This is not required for direct reading instruments.

Calculation

The water equivalent, T, of the Karl Fischer reagent in mg H_2O/mL is given by the formula

$$T = \frac{m_2}{v_0 - v_1}$$

Where:

m_1 = Mass, in mg, of the water added

V_0 = Volume, in mL, of the Karl Fischer reagent use for the determination of the water equivalent of the Karl Fischer reagent

V_1 = Volume, in mL, of the Karl Fischer reagent used for the blank test

The water content of the test sample is given by the formula

$$\%\ \text{Water (wt/wt)} = \frac{(V_2 - V_3) \times T}{m_2} \times 100$$

Where:

m_2 = mass, in mg, of the test portion

V_2 = volume, in mL, of the Karl Fischer reagent used for the determination (procedure, 4)

V_3 = volume, in mL, of the Karl Fischer reagent used for the blank (procedure, 5)

Appendix E6 Flash point by Pensky-Martens Closed cup Flash Tester AOCS Official Method Cc 9c-95.

Procedure

1. Preparation of test portion

(a) If the fat is a solid at room temperature it should be liquefied by warming slowly to a temperature not exceeding 5°C over its melting point. The flashpoint determination will then start at this elevated temperature.

(b) Carefully transfer (see Notes, 2) approximately 90 g of fat or oil to the stoppered tube and add 5 g of anhydrous sodium sulphate. Shake the mixture vigorously for 1 min with the stopper secured and allow it to stand for 30 min with, if necessary at an elevated temperature

(c) Centrifuge the treated oil as prepared in (see Procedure, 1, b) at 2,500 rpm for 3 min or until a sufficient amount of clear oil is available to make the flashpoint determination. Maximum time of centrifuging-5 min.

2. Determination:

(a) Fill the cup with the liquefied oil or fat sample (see Procedure, 1, c) so that the top of the meniscus is exactly at the filling line marked in the cup. Place the lid on the cup and engage the locating devices. Insert the thermometer (see Apparatus, 2) and suspend it so that the lower surface of the portion of the lid inside the rim.

(b) Light the test flame and adjust it so that it is the size of a bead (4 mm) in diameter.

(c) Heat the sample so that the temperature increased not less than 5°C and not more that 6°C per minute. During the heating, turn the stirring device one to two revolutions per second.

(d) At the specified temperature (usually 121°C) discontinue stirring and apply the test flame by operating the device that controls the shutter and lowers the test flame into the shutter opening. Lower the test flame within 0.5 sec and observe whether this causes a distinct flash in the interior of the cup. The true flash must not be confused with the bluish halo that sometimes surrounds the test flame.

(e) It is not permitted to open the cup more than once during each determination because solvent vapor, although present in too low a concentration to cause a flash, might escape. This would lead to an incorrect result at the measuring temperature.

(f) If information is needed about the precise level of the flashpoint, the whole procedure should be repeated at temperatures other than 121°C. Fresh portions of the test samples should be used for each temperature determination.

(g) Carry out the test in duplicate.

Appendix E7 Reflective index by AOCS Official Method Cc 7-25.

Procedure

1. Melt the sample, if it is not already liquid, and filter through filter paper to remove any impurities and the last traces of moisture. The sample must be completely dry.

2. Check the repeatability requirement of the method by carrying out two single determinations as described below.

3. The instrument is standardized, following the manufacturer’s instructions, with ethyl laurate or with a glass prism of known refractive index. Distilled water, which has a refractive of 1.3330 at 20.0°C, is satisfactory in some cases.

4. Measure the refractive index of the sample at one of the following temperatures.

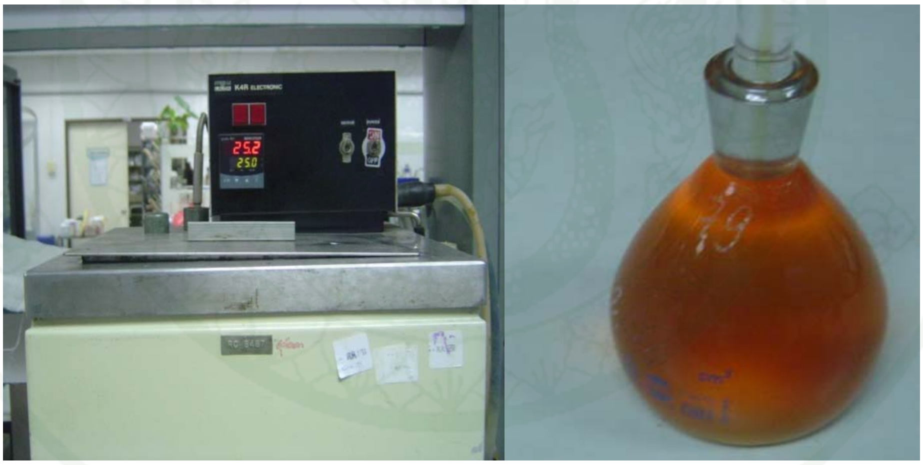
20°C Completely liquid at 20°C
40°C Completely liquid at 40°C but not at 20°C
50°C Completely liquid at 50°C but not at 40°C
60°C Completely liquid at 60°C but not at 50°C

80°C or above for other fats and oils.

5. Be sure the prisms are clean and completely dry. Place several drops of the sample on the lower prism. Close the prisms and tighten firmly with the screwhead. Allow to stand for 1-2 min or until the sample comes to the temperature of the instrument.

6. Adjust the instrument and light to obtain the most distinct reading possible and determine the refractive index to the nearest 0.0001 and record the temperature of the prism. Take at least three separate readings and calculate the average of all readings.

Appendix E8 Specific Gravity by AOCS Official Method Cc 10b-25.



Procedure

1. Prepare 7 solutions (50 to 100 mL each) of alcohol and water with densities ranging from 0.940 to 1.000 at 25 ºC, varying by 0.01 specific gravity (sp.gr.) units. Use the Westphal balance to determine the sp. gr. Store in glass stoppered bottles, appropriately numbered, from 1 to 7, in order of increasing sp. gr.

2. Melt 5 g of the sample, making certain the melted sample is clear, free of moisture and air bubbles. If the sample is turbid and/or contains moisture, filter through dry filter paper with the aid of a hot water funnel. Allow the sample to cool slowly to room temperature.

3. Cut a small pellet (3 to 5 mm in diameter) from the solidified sample and brush over with a camel hair brush (wet with distilled water) to avoid the development of air bubbles on the surface.

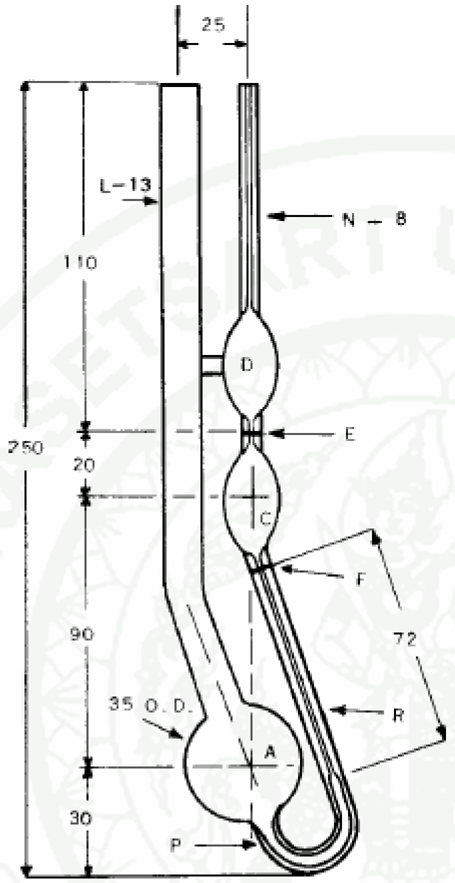
4. Pour alcohol solution 1 into a beaker, adjust to 25 ºC ± 0.1 ºC and immerse the pellet with the aid of forceps. If the pellet sinks, repeat with solution 2, and so on, until a solution is found in which the pellet just floats.

5. When the solution is found in which the pellet floats, slowly add alcohol at 25 ºC from a buret, stirring gently with a glass stirring rod to prevent the formation of air bubbles around the sample pellet. Continue adding the alcohol until the pellet just sinks. If the correct amount of alcohol is exceeded, distilled water may be added dropwise and the endpoint again determined.

6. Determine the sp. gr. of the solution generated in step with the Westphal balance, making certain the solution is at 25 ºC ± 0.1 ºC. The sp. gr. of this solution is identical with the sp. gr. of the sample.

7. The result is confirmed by adding 1 or more pellets to the same solution. If the sample floats, it should slowly sink upon the addition of a drop of alcohol. If the sample sinks, slowly add water dropwise until it just rises and then determine the sp. gr. of the solution.

Appendix E10 Kinematic viscosity by ASTM D 445.



Procedure

1. Clean the viscometer using suitable solvents, and by passing clean, dry, filtered air through the instrument to remove the final traces of solvents. Periodically, traces of organic deposits should be removed with chromic acid or non-chromium cleaning solution.

2. If there is a possibility of lint, dust, or other solid material in the liquid sample, filters the sample through a sintered glass filter or fine mesh screen.

3. To charge the sample into the viscometer, invert the instrument and apply suction to tube L, immersing tube N in the liquid sample, and draw liquid to mark F. Wipe clean arm N, and turn the instruments to its normal vertical position.

4. Place the viscometer into the holder, and insert it into the constant temperature bath. A viscometer holder which fits the Cannon-Fenske Opaque viscometer and the Cannon-Manning Semi-Micro viscometer will also fit the Cannon-Fenske Routine viscometer. Align the viscometer vertically in the bath by means of a small plumb bob in tube, if a self-aligning holder is not used.

5. Allow approximately 10 minutes for the sample to come to the bath temperature at 40^{o}C and 15 minutes at 100^{o}C.

6. Apply suction to tube and draw the liquid slightly above mark.

7. To measure the efflux time, allow the liquid sample to flow freely down past mark F, measuring the time for the meniscus to pass from mark E to mark F.

8. A check run may be made by repeating steps 6 and 7.

9. Calculate the kinematic viscosity in mm^2/s (cSt) of the sample by multiplying the efflux time in seconds by the viscometer constants.

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SCHOLARSHIP/AWARDS : Thai Lube Base (Public Company), Ltd 2008-2009, Thailand Research Fund and Faculty of Science, Kasetsart University, Thailand 2009-2010.