Separation of Free Fatty Acid (FFA) from High-FFA Crude Palm Oil Using Vacuum Distillation and Production of Glycerides from the FFA Distillate by Lipase-catalyzed Reaction

Nantanit Tohpong

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biotechnology

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This is to certify that the work here submitted is the result of the candidate's own investigations. Due acknowledgement has been made of any assistance received.

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I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

...……Signature

(Nantanit Tohpong)

Candidate

ชื่อวิทยานิพนธ์ การแยกกรดไขมันอิสระออกจากน้ำมันปาล์มดิบกรดสูงด้วยวิธีกลั่นภายใต้สภาวะสุญญากาศและการผลิตกลีเซอไรด์จากกรดไขมันอิสระที่แยกได้โดยใช้เอนไซม์ไลเปสเป็นตัวเร่งปฏิกิริยา

ผู้เขียน นางสาวนันทนิตย์ โต๊ะปอง

สาขาวิชา เทคโนโลยีชีวภาพ

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บทคัดย่อ

งานวิจัยนี้ต้องการศึกษาการแยกกรดไขมันอิสระ (free fatty acid, FFA) ออกจากน้ำมันปาล์มดิบกรดสูง (high free fatty acid crude palm oil, HFFA-CPO) โดยให้ความร้อนที่สภาวะสุญญากาศและนำ FFA ที่แยกได้มาเป็นวัตถุดิบในการศึกษาสภาวะที่เหมาะสมในการผลิตกลีเซอไรด์โดยใช้เอนไซม์ไลเปสเป็นตัวเร่งปฏิกิริยา HFFA-CPO ที่ใช้ทดลองแยก FFA มี 2 ชนิดคือ HFFA-CPO จากกระบวนการสกัดน้ำมันปาล์มดิบแบบเปียกซึ่งเป็นน้ำมันที่ได้จากเปลือกผลปาล์ม (crude palm oil, CPO) และ HFFA-CPO จากกระบวนการสกัดน้ำมันปาล์มดิบแบบแห้งซึ่งเป็นน้ำมันผสมของน้ำมันจากเปลือกผลปาล์มและเมล็ดในปาล์ม (mixed crude palm oil, MCPO) โดยใช้ถังปฏิกรณ์ขนาด 40 ลิตร

ในการแยก FFA ออกจาก CPO ที่สกัดแบบเปียก ได้ทำการทดลอง 4 ชุดการทดลอง ได้แก่ชุด CPO1, CPO2, CPO3 และ CPO4 แต่ละชุดการทดลองใช้ CPO 28 ลิตร กรดไขมันหลักใน CPO ที่ใช้ประกอบด้วยกรดปาล์มมิติก (42.07%), กรดโอเลอิก (38.86%), กรดลิโนเลอิก (7.98%) และกรดสเตียริก (3.95%) เวลาเฉลี่ยที่ใช้ทดลองสำหรับชุด CPO1, CPO2 และ CPO3 คือ 7.2 ชั่วโมง ขณะที่ชุด CPO4 ใช้เวลา 15 ชั่วโมง อุณหภูมิเริ่มต้น (initial temperature) และอุณหภูมิทดลอง (setting temperature) ของ CPO แต่ละชุดการทดลองคือ 27.4 ºC และ 230 ºC, 29.5 ºC และ 250 ºC, 30.5 ºC และ 250 ºC และ 27.6 ºC และ 250 ºC ตามลำดับ ความดันสุญญากาศที่ใช้คือ 20, 160, 30 และ 40 mmHg ตามลำดับ ปริมาณ FFA ใน CPO ก่อนและหลังกระบวนการกลั่นแยกเท่ากับ 25.70% และ 14.34%, 14.95% และ 10.60%, 10.29% และ 5.22% และ 20.61% และ 10.43% ตามลำดับ ผลการกลั่นพบว่า FFA ที่แยกได้ของแต่ละชุดการทดลองมี %FFA เท่ากับ 73.37%, 76.70%, 89.28% และ 51.62% ตามลำดับ

สำหรับ CPO จากกระบวนการหีบแบบแห้ง ได้ทำการทดลอง 7 ชุดการทดลอง ได้แก่ชุด MCPO1, MCPO2, MCPO3, MCPO4, MCPO5, MCPO6 และ MCPO7 กรดไขมันหลักใน MCPO ที่ใช้ประกอบด้วยกรดโอเลอิก (40.41%), กรดปาล์มมิติก (38.63%), กรดลิโนเลอิก (10.18%) และ

กรดสเตียริก (4.27%) เวลาเฉลี่ยที่ใช้ทดลองสำหรับชุด MCPO1-MCPO7 คือ 6.62 ชั่วโมง อุณหภูมิเริ่มต้น และอุณหภูมิทดลองของ MCPO แต่ละชุดการทดลองคือ 31.4 ºC และ 220 ºC, 27.1 ºC และ 240 ºC, 29.6 ºC และ 250 ºC, 31.2 ºC และ 220 ºC, 29.3 ºC และ 230 ºC, 27.8 ºC และ 240 ºC และ 29.0 ºC และ 250 ºC ตามลำดับ ความดันสุญญากาศที่ใช้คือ 20 mmHg (สำหรับ MCPO1-MCPO3) และ 30 mmHg (สำหรับ MCPO4-MCPO7) ปริมาณ FFA ใน MCPO ก่อนและหลังกระบวนการกลั่นแยกเท่ากับ 12.57% และ 7.36%, 16.20% และ 12.11%, 10.36% และ 8.79%, 14.06% และ 10.80%, 14.19% และ 11.76%, 14.05% และ 11.49% และ 19.57% และ 11.49% ตามลำดับ ผลการกลั่นพบว่า FFA ที่แยกได้ของแต่ละชุดการทดลองมี %FFA เท่ากับ 98.88%, 98.87%, 79.90%, 100.00%, 99.50%, 98.21% และ 99.94% ตามลำดับ เปอร์เซ็นต์การลดลงของปริมาณ FFA มีค่าสูงสุดอยู่ที่ความดันและอุณหภูมิในการกลั่นเป็น 20 mmHg และ 220 ℃ ตามลำดับ

ส่วนชุดปฏิกรณ์ทั้ง 4 แบบดังที่แสดงในรูปที่ 3.1 ที่ใช้ในการทดสอบนั้นนั้นมีการใช้หม้อระเหยตัวเดียวกัน ดังนั้นอัตราการให้ความร้อนและพื้นที่ของการระเหยจึงเท่ากันเนื่องจากใช้ฮีตเตอร์ไฟฟ้าเดียวกันและขนาดเส้นผ่าศูนย์กลางของหม้อระเหยเท่ากัน ค่าความดันสุญญากาศที่ได้จากปั๊มสุญญากาศขนาดเล็ก (0.75 kW) และขนาดใหญ่ (2.20 kW) มีค่าไม่แตกต่างกัน ดังนั้นอัตราการระเหยจึงขึ้นอยู่กับค่าความดันสุญญากาศที่ใช้แ ชนิดและคุณภาพของน้ำมัน สามารถสรุปได้ว่าชุดปฏิกรณ์แบบ D ดีที่สุดเนื่องจากไม่มีหม้อควบแน่นที่หนักอยู่ด้านบนของหม้อระเหยทำสามารถใช้งานได้ง่าย นอกจากนี้ชุดปฏิกรณ์แบบ D สามารถใช้ปั๊มสุญญากาศขนาดเล็กแทนปั๊มสุญญากาศขนาดใหญ่ได้เพื่อเป็นการลดการใช้พลังงาน

ในส่วนของการผลิตกลีเซอไรด์ [monoglycerides (MG), diglyceride (DG) และ triglycerides (TG)] จาก FFA ที่แยกได้ร่วมกับกลีเซอรอลดิบด้วยปฏิกิริยาเอสเทอริฟิเคชันโดยใช้ไลเปสเป็นตัวเร่งปฏิกิริยา ในขั้นแรกได้ศึกษาการคัดเลือกเชื้อจุลินทรีย์ที่ผลิตเอนไซม์ไลเปสเพื่อนำไปใช้เป็นตัวเร่งปฏิกิริยาในการผลิตกลีเซอไรด์ จากเชื้อ 3 ชนิดคือ *Bacillus subtilis*, *Bacillus coagulans* และ *Psuedomonas* sp. โดยเลี้ยงในอาหารเลี้ยงเชื้อที่มีกรดไขมันเป็นองค์ประกอบ (ใช้ FFA ที่แยกได้) พบว่ากิจกรรมเอนไซม์ไลเปสของเชื้อทั้ง 3 ชนิดมีค่า 0.08, 0.07 และ 0.07 U/ml ตามลำดับ ซึ่งมีค่าต่ำมาก ในการศึกษาการผลิตกลีเซอไรด์จึงเปลี่ยนไปใช้เอนไซม์ทางการค้าจากเชื้อ *Camlicla lipolytica* แทน การศึกษาสภาวะที่เหมาะสมในการผลิตกลีเซอไรด์ เริ่มต้นจึงใช้กรดโอเลอิกและกลีเซอรอลทางการค้าศึกษาอัตราส่วนโดยโมลของกรดไขมันต่อกลีเซอรอล (1:4, 1:3, 1:2, 1:1, 2:1, 3:1 และ 4:1) โดยใช้อุณหภูมิที่ 45 ºC เป็นเวลา 24 ชั่วโมง ความเข้มข้นเอนไซม์ 300 U/g FA ภายใต้สภาวะการเขย่าด้วยความเร็ว 200 รอบต่อนาที พบว่าที่อัตราส่วนกรดไขมันต่อกลีเซอรอล 1:3 ได้ผลดีที่สุด (10.28% MG, 24.34% DG และ 6.41% TG

ตามลำดับ) จึงนำอัตราส่วนของกรดไขมันต่อกลีเซอรอล 1:3 มาศึกษาผลของอุณหภูมิ (40, 45, 50, 55, 60 , 65 และ 70 ºC) พบว่าที่อุณหภูมิ 50 ºC ได้ผลดีที่สุด (15.51% MG, 28.51% DG และ 9.16% TG ตามลำดับ) จากนั้นจึงนำค่าอัตราส่วนของกรดไขมันต่อกลีเซอรอล 1:3 และอุณหภูมิ 50 ºC มาศึกษาผลของความเข้มข้นเอนไซม์ (50, 100, 300, 500 และ 700 U/g FA) พบว่าที่ความเข้มข้น 100 U/g FA ได้ผลดีที่สุด (17.71% MG, 26.02% DG และ 4.83% TG ตามลำดับ) จากนั้นจึงนำค่าอัตราส่วนของกรดไขมันต่อกลีเซอรอล 1:3 อุณหภูมิ 50 ºC และความเข้มข้นเอนไซม์ที่ 100 U/g FA มาศึกษาผลของเวลาที่ใช้ในการทำปฏิกิริยา (12, 24, 36 และ 48 ชั่วโมง) พบว่าที่เวลา 24 ชั่วโมง ได้ผลดีที่สุด (25.72% MG, 34.74% DG และ 5.52% TG ตามลำดับ) หลังจากนั้นได้นำสภาวะที่เหมาะสมดังกล่าวทั้งหมดมาทดลองกับ FFA ที่แยกได้ร่วมกับกลีเซอรอลดิบ โดยเปรียบเทียบกับกรดโอเลอิกกับกลีเซอรอลทางการค้า, กรดโอเลอิกกับกลีเซอรอลดิบ และ FFA ที่แยกได้กับกลีเซอรอลทางการค้า พบว่าปริมาณ DG ของชุดการทดลองที่ใช้ FFA ที่แยกได้กับกลีเซอรอลทางการค้า (19.54%) มีค่าสูงกว่าชุดการทดลองที่ใช้กรดโอเลอิกกับกลีเซอรอลดิบ (1.99%) และ FFA ที่แยกได้กับ กลีเซอรอลดิบ (4.68%) แต่ต่ำกว่ากรดโอเลอิกกับกลีเซอรอลทางการค้า (33.45%) ซึ่งเป็นผลมาจากความไม่บริสุทธิ์ของกลีเซอรอลดิบ

Thesis Title Separation of free fatty acid (FFA) from high-FFA crude palm oil using vacuum distillation and production of glyceride from the separated FFA by lipase-catalyzed reaction

Author Miss Nantanit Tohpong

Major Biotechnology

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ABSTRACT

This research aimed to study separation of free fatty acid (FFA) from high free fatty acid crude palm oil (HFFA-CPO) by heating under vacuum condition in a 40-liter reactor and then use the obtained FFA to produce glycerides using lipase catalyzed esterification. There were two types of HFFA-CPO used in this research, the mesocarp oil from palm oil wet milling process (called crude palm oil, CPO) and the mixed oil between mesocarp oil and kernel oil from dry milling process (called mixed crude palm oil, MCPO).

Experimental separations of FFA from CPO obtained from wet milling process had 4 batches, called CPO1, CPO2, CPO3 and CPO4. Each batch had 28 liter working volume. The main fatty acid components in this CPO were palmitic (42.07%), oleic (38.86%), linoleic (7.98%) and stearic (3.95%). The average times used for each batch were 7.2 h for CPO1-CPO3 and 15 h for CPO4. Initial temperatures and setting temperatures for each batch were 27.4 ^{o}C & 230 ^{o}C, 29.5 ^{o}C & 250 ^{o}C, 30.5 ^{o}C & 250°C and 27.6 ^{o}C & 250 °C. Vacuum pressure for each batch were 20, 160, 30 and 40 mmHg, respectively. FFA in the CPO before and after separation process for each batch were 25.70% & 14.34%, 14.95% & 10.60%, 10.29% & 5.22% and 20.61% & 10.43%, respectively. The product FFAs obtained from separation for each batch were 73.37%, 76.70%, 89.28% and 51.62% %, respectively.

Separations of FFA from MCPO obtained from dry milling process had 7 batches, called MCPO1, MCPO2, MCPO3, MCPO4, MCPO5, MCPO6 and MCPO7, respectively. The main fatty acid components in MCPO were oleic (40.41%), palmitic (38.63%), linoleic (10.18%) and stearic (4.27%). The average time used for each batch was 6.62 h. Initial and

setting temperatures were 31.4 °C & 220°C, 27.1 °C & 240°C, 29.6 °C & 250 °C, 31.2 °C & 220 °C, 29.3 °C & 230 °C, 27.8 °C & 240 °C and 29.0 °C & 250 °C, respectively. Vacuum pressures were 20 mmHg (for MCPO1-MCPO3) and 30 mmHg (for MCPO4-MCPO7). FFA in the MCPO before and after separation process for each batch were 12.57% & 7.36%, 16.20% & 12.11%, 10.36% & 8.79%, 14.06% & 10.80%, 14.19% & 11.76%, 14.05% & 11.49% and 19.57% & 11.49%, respectively. The product FFAs obtained from separation for each batch were 98.88%, 98.87%, 79.90%, 100.00%, 99.50%, 98.21% and 99.94%, respectively. The highest percentage reduction of FFA content was obtained at distillation pressure and temperature of 20 mmHg and 220°C, respectively.

Four configurations of the reactor as shown in Figure 3.1 were tasted they used the same evaporator. Thus, the heating rate and the evaporation area were the same due to the same electric heater and the same dimeter of the evaporation tank, respectively. The vacuum pressures made by both the smaller (0.75 kW) and bigger (2.20 kW) vacuum pumps were not difference. Therefore, the evaporation rate depends on vacuum pressure used and type and quality of the oil. It could be concluded configuration D is the best due to it has no heavy condenser on the top of the evaporator, easy operation. In addition, the vacuum pump of configuration D can be replaced by the smaller one to reduce energy consumption.

Production of glycerides [monoglycerides (MG), diglyceride (DG) and triglycerides (TG)] using the separated FFA and crude glycerol as substrates by means of lipase catalized esterification was investigated. Beginning with screening of lipase producing bacteria for using as catalyst in glyceride production. Three bacterial strains were chosen, *Bacillus subtilis*, *Bacillus coagulans* and *Psuedomonas* sp. The bacterial cultivations were performed in the FFA-added nutrient broth. The lipase activities of three strains were found 0.08, 0.07, 0.07 U/ml, respectively, which were very low. To save the amount of the FFA distillate from strain cultivation for further using in glyceride production, the study of glyceride esterification began with using commercial lipase from *Camlicla lipolytica*, oleic acid and glycerol as catalyst and substrates, respectively. Optimization of FFA to glycerol molar ratio (1:4, 1:3, 1:2, 1:1, 2:1, 3:1 and 4:1) was firstly

investigated, each ratio were done at 45°C for 24 h with lipase concentration of 300 U/g FA and shaking of 200 rpm. The ratio of 1:3 gave the best result (10.28% MG, 24.34% DG and 6.41% TG, respectively). Then the FFA to glycerol molar ratio of 1:3 was used for finding the optimal temperature. Seven temperatures (40, 45, 50, 55, 60, 65 and 70 °C) were studied and found that the temperature of 50°C gave the best result (15.51% MG, 28.51% DG and 9.16% TG, respectively). After that, the FFA to glycerol ration of 1:3 and the temperature of 50°C were used to find the optimal enzyme concentration. Five lipase concentrations (50, 100, 300, 500 and 700 U/g FA) were studied and found that at 100 U/g FA the esterification gave the best result (17.71% MG, 26.02% DG and 4.83% TG, respectively). Effect of reaction time was studied using FFA to glycerol ration of 1:3, temperature of 50°C and lipase concentration of 100 U/g FA. Four reaction times (12, 24, 36 and 48) were chosen and found that 24 h gave the best result (25.72% MG, 34.74% DG and 5.52%, respectively). Finally, the synthesis of glycerides was investigated using all above obtained optimum conditions with 4 cases of substrates for comparison: case 1 (commercial oleic & commercial glycerol), case 2 (commercial oleic & crude glycerol), case 3 (FFA distillate & commercial glycerol) and case 4 (FFA distillate & crude glycerol). Noting that the crude glycerol was from biodiesel plant. Considering DG, it was found that case 3 gave amount of DG (19.54%) higher than case 2 (1.99%) and 4 (4.68%) whereas case 1 showed the highest amount of DG (33.45%). These results represented effect of glycerol purity.

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Nantanit Tohpong

CONTENTS

CONTENTS (CONT.)

CONTENTS (CONT.)

CONTENTS (CONT.)

LIST OF TABLES

LIST OF TABLES (CONT.)

LIST OF TABLES (CONT.)

LIST OF FIFURE

LIST OF FIFURE (CONT.)

LIST OF FIFURE (CONT.)

LIST OF FIFURE (CONT.)

LIST OF ABBREVIATIONS

CPO	Crude Palm Oil
MCPO	Mixed Crude Palm Oil
FFAs	Free Fatty Acids
HFFA-CPO	High Free Fatty Acid - Crude Palm Oil
HFFA-MCPO	High Free Fatty Acid - Mixed Crude Palm Oil
DG	Diglyceride
MG	Monoglyceride
TG	Triglyceride
CG	Crude Glycerol
PKO	Palm Kernel Oil

CHAPTER 1

INTRODUCTION

1.1 Rationale

Palm oil extraction process can be classified into two major processes. The first one is called standard process or wet process which generates the crude palm oil (CPO) from mesocarp of the palm fruit. The second one is called dry process which gives the mixed crude palm oil (MCPO) of the mesocarp oil and the kernel oil of the palm fruit. The CPO is generally used to produce edible oil, whereas both CPO and MCPO are further used as raw material for many downstream industries such as artificial cream, margarine, sweetened condensed milk, snacks, instant noodles, etc. In addition, they can also be used to produce biodiesel by transesterification reaction, directly mixed with diesel oil or replaced the diesel oil. CPO generally consists of about 95% triglyceride, 3-5% free fatty acids (FFA) and 1% other components. FFA content is important for quality of CPO. High FFA content affects the refining process for edible oil and biodiesel production, which leads to high cost in the acid elimination step (Somnuk *et al*., 2013).

Based on the standard of CPO in Thailand (Department of Agriculture, 2017), good quality CPO has maximum 5 % FFA and 0.5 % moisture content. The CPO having FFA content greater than 5% is called high free fatty acid crude palm oil (HFFA-CPO). The HFFA-CPO can be obtained due to poor harvested palm fruit, improper transportation and long-term storage. Also, it can be obtained as a recovery oil from palm oil mill effluent. It is very essential to control %FFA in the CPO to be not exceed the standard to get high price for selling to the refinery. The palm oil mill usually adjusts %FFA in the HFFA-CPO by mixing with the low FFA CPO until the FFA value meet the standard.

Other method to reduce FFA content in the CPO is separation which can be done by using many techniques, such as solvent extraction (Fornasero *et al*., 2013; Rodrigues *et al*., 2014; Gonçalves *et al*., 2016), membrane filtration (Rao *et al*., 2013; Azmi *et al*., 2015; Purwasasmita *et al*., 2015), supercritical fluid extraction (Markom

et al., 2001; Zacchi *et al*., 2006; Julian and Ana, 2014) and vacuum distillation (Molekul *et al*, 2016). Distillation under very high vacuum pressure can diminish the evaporation temperature of FFA to be lower than 200°C which avoid thermal cracking of the oil (Watkins, 1979). Normally, FFA in CPO will be separated in the palm oil refinery in the deodorization step after degumming and bleaching steps. In this present work, the CPO from the milling process is used directly to investigate the FFA separation and CPO quality.

Separation of FFAs from CPO provides two main products, FFAs and low FFA CPO. FFAs can be applied as a substrate in various industries, such as washing industry, animal food, cosmetic, biodiesel production (FFA methyl esterification), bioplastics, bio-lubricant and glyceride production, etc. (Molekul *et al*, 2016). In addition, some FFAs which are also essential to body can be used as food supplements, such as linoleic acid (18: 2 (n-6)) from safflower oil etc.

Glycerides are used in many industries as emulsifiers and stabilizers in food products such as bread, ice cream and margarine, or used as main ingredient in the production of cosmetics and drug. Monoglyceride containing n-3-polyunsaturated fatty acid such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) can help to prevent various diseases. Some monoglycerides such as monolaurin, monomyristin, monolinolein and monolinolenin have antimicrobial properties, which can destroy bacteria (i.e., *Listeria monocytogenes* and *Helicobacter pylori*), protozoa (i.e., *Giardia lamblia*) and virus (i.e., HIV virus, herpes, Cytomegalovirus and influenza) (Freitas *et al*., 2010). At present, study of nutritional properties and effects of consumption of diglyceride (DG) oil (with DG content at least 80% by weight) found that the DG oil with 1,3 -DG has health benefits compared to general edible oils. Consuming of DG oil shows the reduction of triglyceride accumulation in liver (Kupongsak and Lucharit, 2014). Production of DG oil can be classified according to types of chemical reactions and the catalysts used. Esterification is a well-known reaction in the production of DG that use enzymes as catalysts. The use of enzymes as catalysts at mild conditions to produce the desired product yield and purity is quite suitable for production of DG than using various alkalis or inorganic substances at high temperatures (Lo *et al*., 2008).

From above, HFFA-CPO is a good source of FFA whereas FFA itself can be used for diglyceride synthesis. Therefore, this research will consist of 2 parts. The first part is to study the separation of FFAs from HFFA-CPO using distillation under vacuum conditions. The second part is production of DG from FFA distillate using lipase catalyzed esterification.

1.2 Objectives of the study

1. To design and improve reactor for separating FFA from CPO.
2. To separate FFAs from HFFA CPO using the maximum ability of the existing reactor.
3. To screening lipase producing microbe.
4. To synthesis diglycerides from FFA distillate using lipase catalyzed esterification.
5. To study the optimal conditions for the synthesis of diglycerides from FFA distillate using lipase catalyzed esterification.

CHAPTER 2

LITERATURE REVIEWS

2.1 Introduction

This chapter reviews some important backgrounds relating to the topic of the research, including palm oil, fatty acid, glycerides and lipase.

2.2 Palm oil

Oil palm tree (*Elaeis guineensis*) is a potential oil plant for producing edible oil. Palm fruit from palm tree contains 56% oil and can be extracted by using screw press. As shown in Figure 2.1, palm fruit is composed of flesh, shell and kernel. The oil appears in both flesh and kernel. However, physical and chemical properties may be different due to the difference of fatty acid composition. (Nithiya Rattanapanon, 2017)

Figure 2.1 Illustration of palm fruit structure: flesh, shell and kernel.
Source: Nithiya Rattanapanon (2017)

2.2.1 Types of palm oil

Palm oil can be extracted from two parts of palm fruit, the flesh or mesocarp and the seed or kernel.

2.2.1.1 Palm oil from the flesh of palm fruit

In general, crude palm oil (CPO) is named for the oil obtained from the flesh of palm fruit surrounding the palm shell. When the palm fruit being crushed, the CPO containing water is released from the flesh. After separating dirt and fiber, the moisture is removed to reduce hydrolysis reaction during storage and transportation.

CPO is red-orange in color because of pigments called carotene, lycopene and xanthophyll. It can appear in clear part together with wax part, and forms solid at the temperature between 25-50 ˚C depending on type and amount of fatty acid composition. Generally, it can melt at about 40 ˚C.

2.2.1.2 Palm oil from seed or kernel (palm kernel oil)

Palm seeds or palm kernel oil (PKO) contains 46-57% oil. PKO extraction can be done with a high compressive strength or solvent extraction. PKO is different from CPO. It is clear, colorless to pale yellow. Most fatty acids are saturated fatty acids similar to those found in coconut oil. (Suratthani oil palm research center, 2017)

2.2.2 Composition of crude palm oil

CPO consists of 95% triglycerides (TG), 3-5% FFA and 1% of other nutritious elements such as tocopherols, tocotrienols and sterols. Type of free fatty acids originated from the natural elements of fats and oils and the composition of fatty acids in fats and oils is different depending on the source. CPO contains high amount of palmitic (16: 0) (44.0%) and oleic (18: 1) (39.1%), while PKO contains high amount of lauric (12: 0) (48.2%), myristic (14:0) (16.2%) and palmitic (8.4%), as shown in Table 2.1. (Cermak *et al*., 2012) CPO and PKO have saturated FFA to unsaturated FFA about 50:50 and 82:18 respectively. (Department of Agriculture, 2017)

Table 2.1. Fatty acid composition of fats and oils.

	Fatty acid length and unsaturation												
Fat/Oil	8:0	10:0	12:0	14:0	16:0	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1
Canola	-	-	-	0.1	4.1	1.8	60.9	21.0	-	0.7	-	0.3	-
Coconut	7.8	6.7	47.5	18.1	8.8	2.6	6.2	1.6	-	0.1	-	-	-
Cottonseed	-	-	-	0.7	21.6	2.6	18.6	54.4	0.7	0.3	-	0.2	-
Crambe	-	-	-	-	1.7	0.8	16.1	8.2	2.9	3.3	-	2.2	59.5
Cuphea (PSR-23)	0.8	81.9	3.2	4.3	3.7	0.3	3.6	2.0	0.3	-	-	-	-
Palm	-	-	0.2	1.1	44.0	4.5	39.1	10.1	0.4	0.4	-	-	-
Palm kernel	3.3	3.4	48.2	16.2	8.4	2.5	15.3	2.3	-	0.1	0.1	-	-
Rapeseed	-	-	-	-	2.7	1.1	14.9	10.1	5.1	10.9	-	0.7	49.8
Soybean	-	-	0.1	0.2	10.7	3.9	22.8	50.8	6.8	0.2	-	-	-
Sunflower	-	-	-	-	3.7	5.4	81.3	9.0	-	0.4	-	-	-
Lard	-	0.1	0.1	1.5	26.0	13.5	43.9	9.5	0.4	0.2	0.7	-	-
Tallow	-	-	0.1	3.2	23.4	18.6	42.6	2.6	0.7	0.2	0.3	-	-

Source: Cermak *et al*. (2012)

Melting point and boiling point of each type of fatty acid are different and depend on pressure. As shown in Table 2.2, melting point at atmospheric pressure of lauric, palmitic and oleic are 44.8, 62.9 and 16.3 °C, respectively whereas their boiling points at a pressure of 10 mmHg are 173, 212 and 223 °C, respectively. (Cermak *et al*., 2012) Table 2.3 also shows boiling points at different pressures of saturated fatty acids including palmitic, myristic and lauric. Obviously, boiling point of palmitic, myristic and lauric will be less than 200 °C at boiling pressure below 8, 16 and 32 mmHg, respectively.

Table 2.2. Melting point and boiling point of each type of fatty acid.

Symbol	Systematic Name	Trivial Name	Melting point (°C)		Boiling point (°C)/ (at 10mmHg)	
Saturated fatty acids			Acid	Methyl	Acid	Methyl
10:0	Decanoic	Capric	31.0	- 13.5	150	108
12:0	Dodecanoic	Lauric	44.8	4.3	173	133
14:0	Tetradecanoic	Myristic	54.4	18.1	193	161
16:0	Hexadecanoic	Palmitic	62.9	28.5	212	184
18:0	Octadecanoic	Stearic	70.1	37.7	227	205
20:0	Eicosanoic	Arachidic	76.1	46.4	248	223
22:0	Docosanoic	Behenic	80.0	53.2	263	240
24:0	Tetracosanoic	Lignoceric	84.2	58.6	-	198
Unsaturated fatty acids						
16:1	9-hexadecenoic	Palmitoleic	0.5	- 34.1	180	182
18:1	9-octadecenoic	Oleic	16.3	- 20.2	223	201
18:2	9,12-octadecadienoic	Linoleic	- 6.5	- 43.1	224	200
18:3	9,12,15-octadecatrienoic	Linolenic	- 12.8	- 52.4	225	202
20:1	9-eicosenoic	Gadoleic	23.0	-	170	154
20:4	5,8,11,14-eicosatetraenoic	Arachidonic	- 49.5	-	163	194
22:1	13-docosenoic	Erucic	33.5	-3.5	255	242

Source: Cermak *et al*. (2012)

Table 2.3. Boiling point of saturated fatty acids at different pressure.

Pressure	Boiling point (°C)						
(mmHg)	Caproic	Caprylic	Capric	Lauric	Myristic	Palmitic	Stearic
1	61.7	87.5	110.3	130.2	149.2	167.4	183.6
2	71.9	97.9	121.1	141.8	161.1	179.0	195.9
4	82.8	109.1	132.7	154.1	173.9	192.2	209.2
8	94.6	121.3	145.5	167.4	187.6	206.1	224.1
16	107.3	134.6	159.4	181.8	202.4	221.5	240.0
32	120.8	149.2	174.6	197.4	218.3	238.4	257.1
64	136.0	165.3	191.3	214.6	236.3	257.1	276.8
128	156.5	183.3	209.8	234.3	257.3	278.8	299.7
256	171.5	203.0	230.6	256.6	281.3	303.6	324.8
512	192.5	225.6	254.9	282.5	309.0	332.6*	255.2*
760	205.8	239.7	270.0	298.9	326.2*	351.5*	376.1*

*Values obtained by extrapolation.
Source: Oreopoulou *et al*. (2006)

2.2.3 Palm oil production process

2.2.3.1 Milling process

Harvested palm fruit bunches will be transported to a palm oil mill. Three oil extraction processes can be classified: (i) the standard or wet process which is a steam-based production process for extracting CPO from the palm flesh, the mill has high production capacity of 30 - 80 tons / hour. The CPO obtained is usually classified as “A” grade oil. (ii) the production process of grilled palm fruit for extracting blended oil (CPO+PKO), and (iii) the production process of fried fruit for extracting blended oil (CPO+PKO). For blended oil production, the mill has relatively low production capacity, and oil is extracted as a mixture of oil from flesh (CPO) and oil from kernel (PKO). As shown in Figure 2.2, standard CPO milling process has 4 main steps:

(1) sterilization: steamed at 130 - 135 °C, pressure 2.5 - 3.0 bars for 50 - 75 minutes. Steaming will help stopping the lipolysis reaction that develop FFAs in the palm fruit and help to soften the palm fruit to be easily stripped from the bunch.

(2) stripping: by sending the palm fruit bunches into the separator. Empty bunches will be separated. Subsequently, the palm fruit is digested with a palm fruit shredder to separate the shell from the seed.

(3) oil extraction: take the palm fruit after digestion and then bake at 90 - 100°C for 20 - 30 minutes, then pass through the twin-screw press, the crude palm oil obtained is composed of 66% oil, 24% water and 10% solids.

(4) clarification: The crude palm oil obtained from the extraction is sent to the filter tank to separate the water and solids. After that, send it to the oil storage tank to wait for further refining or distribution. The crude palm oil separates into two parts, the top part is crude palm olein (red orange liquid) (about 30 -50%) and the lower part is crude palm stearin (yellow-orange wax) (about 50 - 70 %).

Palm fruit pulp will be extracted from the seeds. The seeds are dried and cleaned. Then the shells are cracked to separate the kernel. The kernel was dried to get the moisture content of not more than 7%, bag packing and wait for sale or bring to extract the kernel oil. Crude palm oil and palm kernel oil obtained from the extraction process can be sent to refine in the refinery (Suratthani oil palm research center, 2017).

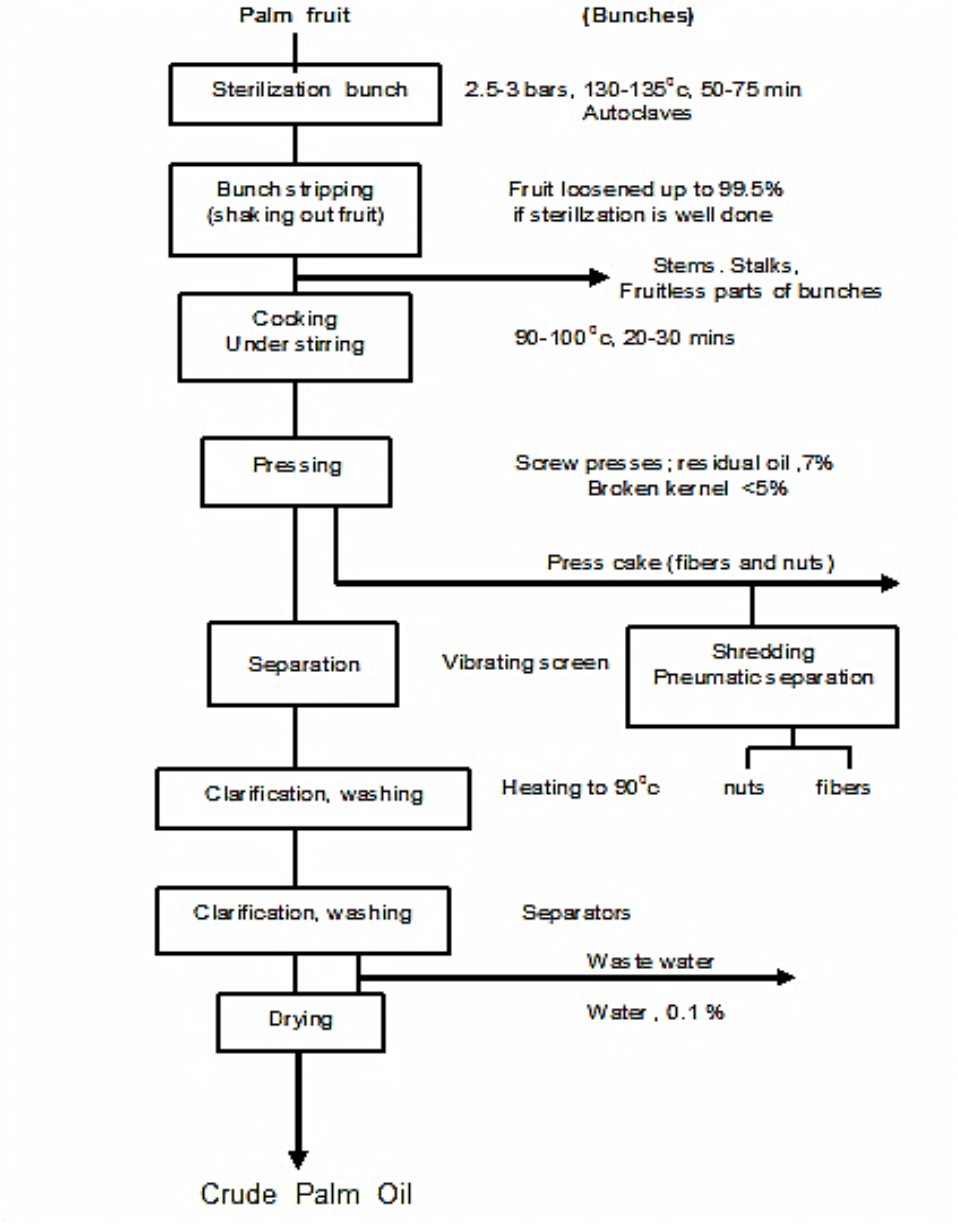


Figure 2.2 Palm oil production process, standard oil mill.

Source: (Suratthani oil palm research center, 2017)

2.2.3.2 Refine processing

Palm oil distillation is the process of removing impurities and other unwanted components in order to achieve the desired quality, taste, smell, color and shelf life. Palm oil refining process is divided into 2 methods: physical and chemical methods, as schematically shown in Figure 2.3.

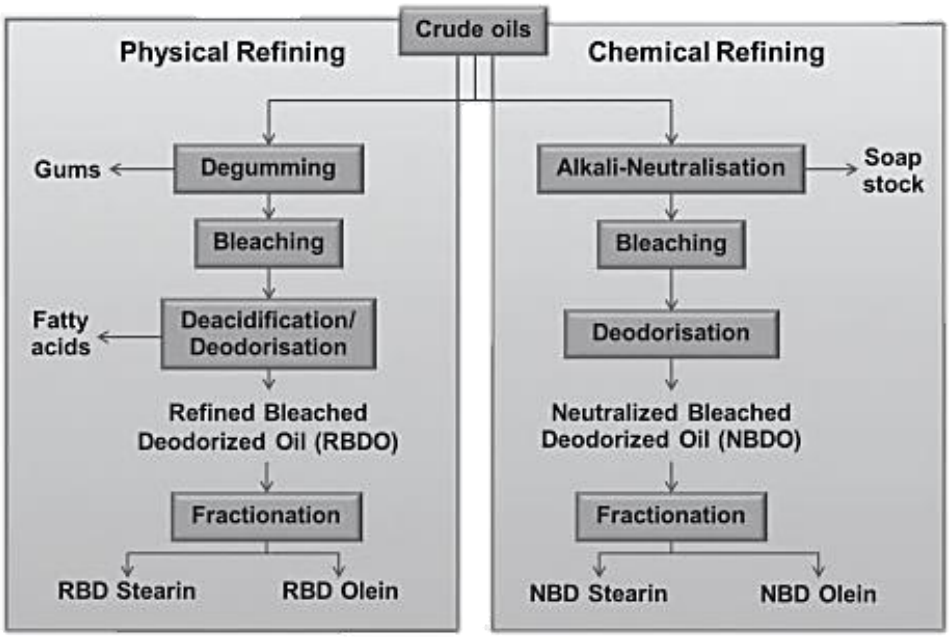


Figure 2.3 Diagram of palm oil refining process by physical and chemical methods. Source: Sirikarn Sattawitchayapit (2015)

2.2.3.2.1 Physical or Steam refining

(1) Degumming

Degumming is the removal of gum or phospholipids which are organic compounds of phosphorus in oil. There are 6 methods of degumming: dry degumming, water degumming, acid degumming, enzymatic degumming, EDTA-degumming and membrane degumming. Suitable method is varying, depending on the amount of phospholipid contained in the vegetable oil. Palm oil contains less phospholipid, therefore dry method is chosen, which uses 80-85% phosphoric acid or citric acid, heats the oil 90°C for 15-30 minutes to make gum precipitation and then filters without the need of centrifugal separation before entering the bleaching process.

(2) Bleaching

Palm oil is bleached by using absorbents such as activated bleaching earth (or bleaching clay), natural bleaching earth, activated carbon, synthetic silicates and synthetic resins. The absorbents are used for absorbing the substances that cause color in the oil. Bleaching of palm oil often uses bleaching clay (hydrated aluminum-silicates) (Figure 2.4), which has good absorption properties. The bleaching clay can absorb chlorophyll and other colored substances, including phospholipids and free fatty acids that may occur during the bleaching process. The method begins with adding bleaching clay about 0.8-2.0% of the amount of crude palm oil into the palm oil, and stirring for 30-45 minutes at 90°C, and then passing into the filter before entering the next step.

Figure 2.4 Bleaching clay.
Source: http://www.foodnetworksolution.com/wiki/word/4546/bleaching

(3) Deodorization

Deodorization is an important process in the elimination of free fatty acids, other volatile compounds (that cause odor and have lower boiling points than triglycerides) and color of some oils (that cannot be eliminated in the previous process). The method is to distill the oil with steam at high temperatures under vacuum conditions. The temperature is an important parameter in distillation. The effect of temperature and vapor pressure of some elements in the oil is shown in Figure 2.5

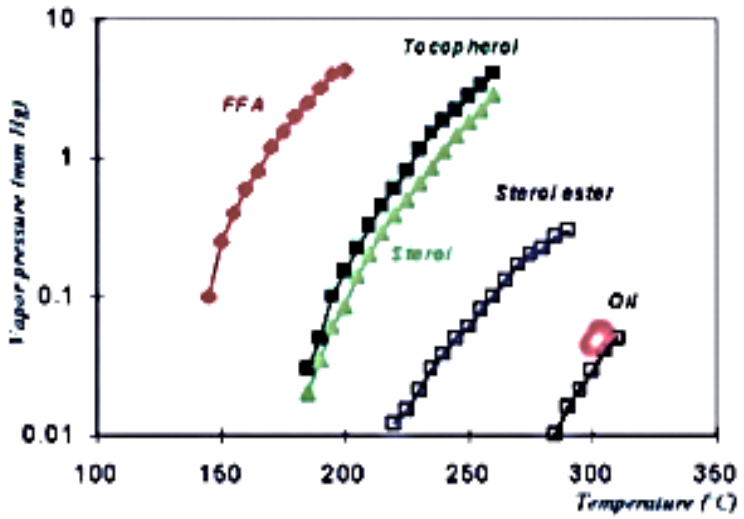


Figure 2.5 Diagram showing temperature and vapor pressure of certain elements in palm oil.

Source: Sirikarn Sattawitchayapit (2015)

At temperatures above 270 °C, the oil will evaporate. Therefore, the optimum distillation temperature range is 240-270 °C for 1-2 hours and the pressure is not more than 10 mmHg. After refining, the palm oil is called refined bleached and deodorized palm oil (RBDPO) which is the main product. The free fatty acid is released in the form of palm fatty acid distillate (PFAD) which is a valuable by-product as well.

2.2.3.2.2 Chemical refining

(1) Degumming and neutralization

This step begins with the removal of gum by adding phosphoric or citric acid as well as physical methods, but after that, sodium hydroxide (NaOH) or potassium hydroxide (KOH) is added to produce soap from saponification with free fatty. Then the soap is separated with centrifugal separator before entering the next process.

(2) Bleaching

Palm oil, after separating the soap, is bleached by using bleachng clay as the same condition as physical methods.

(3) Deodorization

The oil after the bleaching process enters the deodorizer to remove odors and other contaminants by steam distillation at a temperature of about 200°C under vacuum pressure, and then is passed through the filter to get pure palm oil, called neutralized bleached and deodorized palm oil (NBD PO) (Madya *et al*., 2006)

2.2.4 Quality of crude palm oil

Crude palm oil extracted from fresh palm bunches must contain no more than 5%FFA. Free fatty acid affects the physical properties of the oil such as melting point and crystallization point. In addition, the CPO for refining of edible oil must have FFA content does not exceed 0.2%. In distillation process, removing of FFA results in the decreasing of amount of the clear oil. Therefore, to increase the quality of crude palm oil, it is necessary to know the factors affecting the formation and inhibition of FFA. These factors are as following.

(1) Physical impact from harvesting and transportation can break the cells in the mesocarp of palm fruit, causing hydrolysis reaction which develops the FFA formation.

(2) The harvested palm fruit bunches are too ripe. The cell wall covering the oil (oil bags) is brittle and easily breaks from sudden impact, resulting in more free fatty acid formation.

(3) Microorganisms are able to destroy the oil palm fruits and this will help to accelerate the formation of free fatty acids.

(4) After harvesting, the transportation time which takes too long to the palm oil mill cooperated with the waiting time before milling cause important effect on the formation of FFA. (Department of Agriculture, 2017)

2.2.5 Grade of palm oil

The amount of FFA content will be one of factors used to control the standard of crude palm oil. The classification areas follow (Department of Agriculture, 2017).

(1) Crude palm oil grade A (CPO - A). The FFA content is not more than 5% the DOBI value is not less than 2.0. The characteristics of the oil are yellow until red color and viscous.

(2) Crude palm oil grade B (CPO-B) The FFA content is not exceed 8 -10%. The oil color will be between yellow, red and dark brown depending on the production in the palm baking process of each manufacturer that will affect the burned fruit or the cleanliness of the palm before entering the extraction process.

2.3 Fatty acids

Fatty acids are organic acids that consist of carbon, hydrogen and oxygen, usually with a number of carbons in pairs, from 2 or more. There is a general formula, R - COOH. The structure of fatty acid formulas consists of 2 parts: the part called carboxyl (COOH) which contains carbon, hydrogen and oxygen (Figure 2.6). Another part is called Carbon chain (R) with a carbon chain together. Normally, carbon has four arms, which the remaining arms from the carbon capture together will catch with the hydrogen element. There are many types of fatty acids. Every type has a carboxyl unit as well. The difference is that the chemical composition of the carbon chain causes different fatty acids.

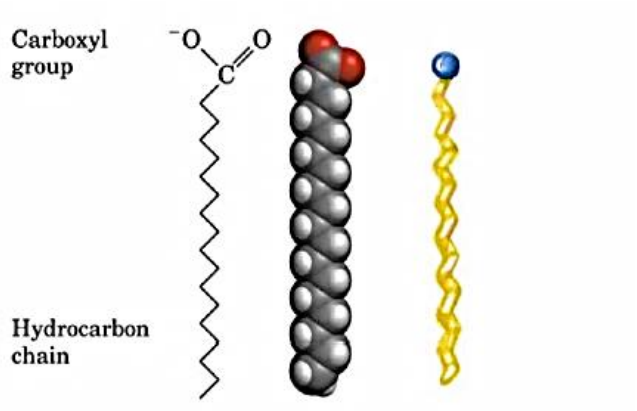


Figure 2.6 Structure of fatty acids.
Source: Godfray *et al*. (2010)

Fatty acids can be divided into 2 types according to the needs of the body: essential fatty acids and non-essential fatty acid. Essential fatty acids are the fatty acids that the body cannot synthesize. It must be obtained from food only. Essential fatty acids act as the structure of various cell membranes and organisms, regulating the metabolism of lipoprotein, allowing to control blood sugar levels, which helps to reduce arteriosclerosis and ischemic heart disease, and is also a key to create prostaglandins, a hormone-like substance that controls the functions of various organs in the body, such as the emission of salt in the stomach, contraction and relaxation of smooth muscles of internal organs, regulating body temperature, and including the aggregation of the blood sheet, etc.

2.3.1 Classification of fatty acid types

The fatty acid obtained from hydrolyzing triglycerides is a straight chain and has an even number of carbons between 12-24 atoms. Natural fatty acids contain about 40 species, of which the fatty acid structure consists of hydrocarbon chains and carboxyl group. Fatty acids may have a single bond or double bond. Therefore, the types of fatty acids are classified into 2 types:

2.3.1.1 Saturated fatty acids

Saturated fatty acids are fatty acids that are saturated molecules, in which carbon atoms are arranged together by a single bond (as shown in Figure 2.7). There is a general formula, $C_nH_{2n}O_2$ or $C_nH_{2n+1}COOH$, or can be formulated as $CH_3(CH_2)_nCOOH$ when n is an integer. Saturated fatty acid can be classified into 4 groups, as shown in Table 2.4, according to the length of the carbon atoms connected together (Godfray *et al*., 2010).

Table 2.4. Classify of saturated fatty acid.

Group	Number of carbon atoms	Fatty acid type
1	3-7	short chain fatty acids
2	8-13	medium chain fatty acids
3	14-20	long chain fatty acids
4	from 20 atoms or more	very long chain fatty acids

Saturated fatty acids are stable, not reacting with oxygen, causing no rancidity, found in both plants and animals but most of the animals have saturated fats rather than plants, for examples, there were found in pork, beef, fat from coconut milk, butter and egg yolk. Oils derived from some plants are also sources of saturated fats, such as palmitic acid: 16 carbon, which is abundant in palm oil, coconut oil, animal fat, and dairy mill-butter products. Saturated fatty acid is abundant in nature and found in the body due to it can be synthesized by body. Nutritional, it is classified as non-essential fatty acids. The most common types of saturated fatty acids are palmitic acid and stearic acid as shown in Figure 2.7.

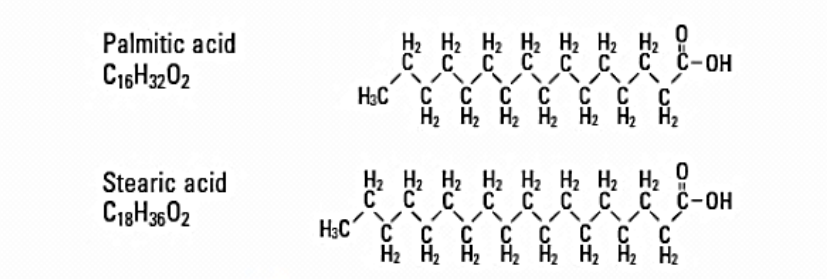


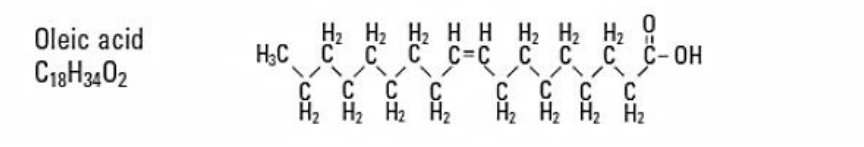
Figure 2.7 Samples of saturated fatty acids.
Source: Godfray *et al*. (2010)

2.3.1.2 Unsaturated fatty acids

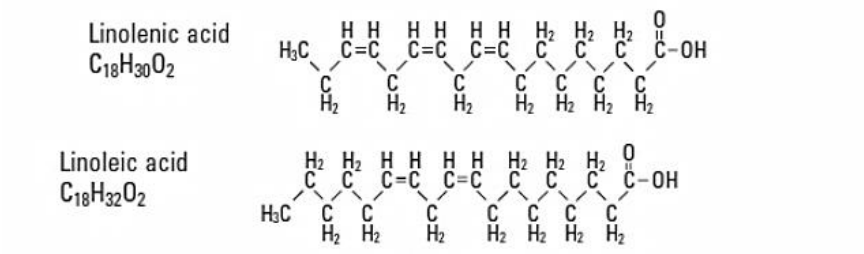
Unsaturated fatty acids have carbon atoms that are formed together, with some positions that are not fully hydrated, causing a double bond in some positions or more. Normally, the body cannot synthesize the unsaturated fatty acids themselves, therefore it is considered as essential fatty acids. For examples, linoleic acid (C18:2), oleic acid (C18:1), linolenic acid (C18:3) and arachidonic acid (C20:4). Double bonds in unsaturated fats or oils will be oxidized by oxygen in the air or may be hydrolyzed with water with microorganisms as catalysts, resulting in small molecular fatty acids that can easily evaporate, causes rancidity, and causes free radicals when ingested into the body. Unsaturated fatty acids can be divided into 2 types (as shown in Figure 2.8).

(1) Monounsaturated fatty acids (MUFA) is a fatty acid that has carbon elements together with only one double bond. The fatty acids in this group, which are found in nature, are oleic acid and palmitoleic acid.

(2) Polyunsaturated fatty acids (PUFA) is a fatty acid that contains carbon together with multiple bonds in many locations. The fatty acids in this group can be found in nature, such as linoleic acid which is an essential fatty acid for the body.



Monounsaturated fatty acids



Polyunsaturated fatty acids

Figure 2.8 Samples of unsaturated fatty acids.
Source: Godfray *et al*. (2010)

2.3.2 Physical and chemical properties of fatty acids

2.3.2.1 Physical properties of fatty acids

(1) The structure of fatty acids consists of a polar component, namely the carboxyl group, and the non-polar part is the hydrocarbon chain that is attached to the carboxyl group. Therefore, if the chain length is increased, fatty acids will have less polarity.

(2) The boiling point and melting point of saturated fatty acids will increase according to the length of the hydrocarbon chain or increase according to the number of carbon atoms. For unsaturated fatty acids, there is a lower boiling point and lower melting point of saturated fatty acids that have the same amount of carbon atoms when comparing between unsaturated fatty acids that have the same amount of carbon

but with different bonds. Unsaturated fatty acids which more double bonds have lower boiling point and melting point.

(3) For fatty acids having the same amount of carbon atoms but different amounts of double bonds, there melting points reduced with increasing of number of double bonds.

(4) Fatty acids in triglycerides are large molecules and insoluble, but in the form of fatty acids salts, for examples, sodium (Na) and potassium (K), of fatty acids, such as soap, can be soluble (Rustan and Drevon, 2005).

2.3.2.2 Chemical reactions of fatty acids

The reaction of fatty acids is caused by the exchange of carboxylic acids in fatty acids. These reaction as follow.

(1) Hydrolysis: is a reaction that occurs with an acid-alkali catalyst or lipase. The reaction can occur without the need of catalyst when the fat and water dissolving in the layer of fat have the appropriate temperature and pressure. The occurrence of hydrolysis in conditions such as the alkaline digestion of grease and oils into soap is called the sponification reaction. At the laboratory level, the alkalinity used in the reaction is low and the reaction is mild. Unlike industrial scale, the production of fatty acids occurs directly from the reaction between water and fat under conditions with appropriate temperature and pressure (temperature ~ 260°C, pressure 20-60 bar) without catalysts. At the end of the process, 99% fatty acids is obtained while glycerol is a by-product (Griffiths, 2005).

(2) Esterification: is an ester bonding reaction, reacting between fatty acids and alcohol with acid or lipase as a catalyst.

(3) Acidolysis: is a reaction of decompose oil with acids or enzymes as catalysts to form medium chain fatty acids.

(4) Alcoholysis or transesterification: is a change in triglyceride molecules, which are the main components of vegetable oil or animal fat, to be a new esters or mono-alkyl ester and glycerol.

(5) Glycerolysis: is a reaction that occurs between triglycerides and glycerol, with catalysts such as sodium hydroxide or sodium methoxide. The product of this reaction are monoglyceride and diglyceride which are used as additives in the food industry and other industrial applications. Various reactions as shown in Figure 2.9

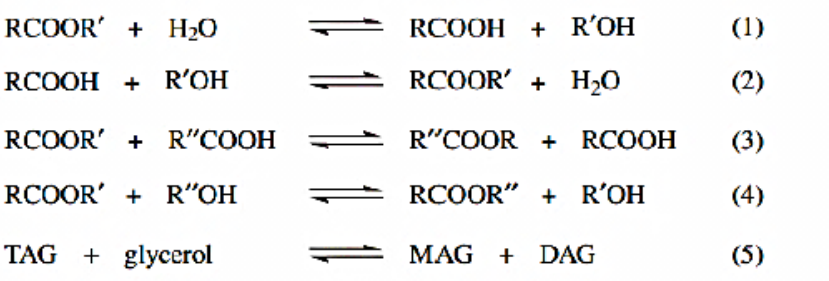


Figure 2.9 Carboxyl exchange reaction in fatty acids when (1) Hydrolysis reaction, (2) Esterification reaction, (3) Acidolysis reaction, (4) Alcoholysis reactions, (5) Glycerolysis reaction, MAG: monoacylglycerol, DAG: diacylglycerol and TAG: triacylglycerol.
Source: Griffiths *et al*. (2005)

2.3.3 Utilization of free fatty acids

Fatty acids are widely used as substrates in industries such as washing industry, animal feed industry, cosmetic industry, biodiesel production, and in other industries such as glyceride production, bio-plastics, bio-lubricants etc. (Molekul, 2016). In addition, some free fatty acids are also essential for body and can be used to produce dietary supplements such as linoleic acid (18: 2 (n-6)), which is found in safflower oil and so on.

2.3.4 Separation of free fatty acids from palm oil

Separation of free fatty acids by distillation method under vacuum conditions is a process of separating substances that are not stable to high temperatures or high boiling point. This method takes short distillation and condensation times which are faster than traditional distillation. Under low pressure or vacuum conditions, pure substances can be extracted in a temperature lower than the actual boiling point of that substance, causing no degradation or destruction of important substances or changes in chemical structure with high temperatures. (Weera Piyatheerawong, 2012)

Martins *et al*. (2006) studied the separation of free fatty acids from soybean oil extraction using molecular distillation methods. The conditions used for distillation were the rate of soybean oil 1.5 - 23 g / min and evaporation temperature 100-180 °C and 10^{-6} bar pressure. The results showed that the rate of soybean oil of 11 g/min evaporated at 160 °C can reduce free fatty acids in soybeans oil with 96.16% separation.

Molekul (2016) studied the separation of free fatty acids from HFFA-CPO using molecular distillation methods. The conditions used for distillation were 2.3 g/min of crude palm oil feed rate, 250 rpm wind speed, 150-265 °C temperature and 10 torr pressure. It was found that the free fatty acids can be separated and reduced from 9.4% to 0.1% (equivalent to 99.89%).

2.4 Glyceride

Glyceride or acylglycerol is generally synthesized by the process of esterification between fatty acids and glycerol (Deshman *et al*., 2008). Glycerides are important components and are widely used in products that obtained from the food and cosmetics industry. It also includes benefits in medicine and pharmaceutical because it has properties to reduce water-oil interface tension (Nandi *et al*., 2004). There are 3 types of glycerides: monoglycerides (MG), diglyceride (DG) and triglycerides (TG), which contains 1,2 and 3 fatty acids molecule per one molecule of glycerol, respectively. Esterification reaction of MG, DG and TG are shown in Figure 2.10. Most glycerides are in the form of TG

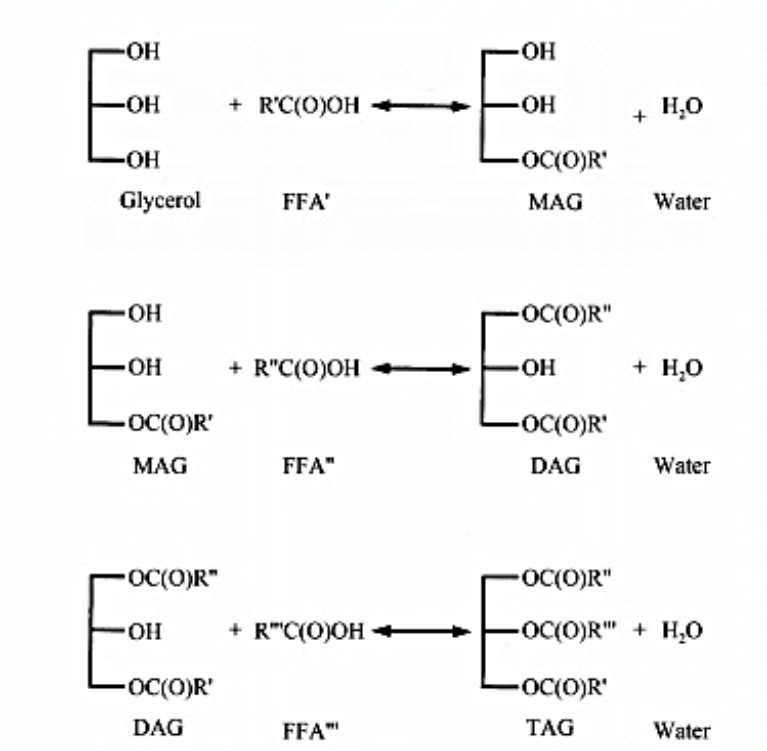


Figure 2.10 Esterification reaction. (MAG: monoacylglycerol, DAG: diacylglycerol, TAG: triacylglycerol and FFA: free fatty acids).
Source: Mostafa *et al*. (2013)

2.4.1 Triglyceride (TG)

The TG or triacylglycerol is the most common glycerol, containing ester-linked fatty acids and the three hydroxyl groups of glycerol. TG molecule having the same type of fatty acid is simple triacylglycerol such as tripalmitoyl glycerol. In general, TG contain 2 or more type of fatty acids and is called mixed triacylglycerol, such as 1-palmitoyl distearoyl glycerol (Yajai Wittayapong, 2005). Palm oil contains 34.6% of TG molecules which have 2 saturated fatty acids (as shown in Table 2.5). The structural characteristics of TG affect the crystallization of oil.

Table 2.5. Classification of the arrangement of the fatty acids in the triacyl glycerides of palm oil saturation properties.

Triglyceride Type	Composition (%)
Trisaturated (GS_3)	10.2
Disaturated (GS_2U)	48.0
Monosaturated (GSU_2)	34.6
Triunsaturated (GS_3)	6.8

Source: Hui (1996)

2.4.2 Diglyceride (DG)

DG or Diacylglycerol is a glycerol ester with two fatty acids and one free hydroxyl group. This glyceride is rarely found in nature of oil and fat. In general, different types of edible fats or oils contain less than 10% of dichloride content (Lo *et al*., 2008). Diglyceride content in various types of edible oils is shown in Table 2.6.

Table 2.6. Glyceride content in various types of edible oil.

Types of edible oil	MG	DG	TG	Other
Soybean	-	1.0	97.9	1.1
Cotton seed	0.2	9.5	87.0	3.3
Palm	-	5.8	93.1	1.1
Corn	-	2.8	95.8	1.4
Safflower	-	2.1	96.0	1.9
Olive	0.2	5.5	93.3	2.3
Rapeseed	0.1	0.8	96.8	2.3
Lard	-	1.3	97.9	0.8

Source: Modify from Flickinger and Matsuo (2003)

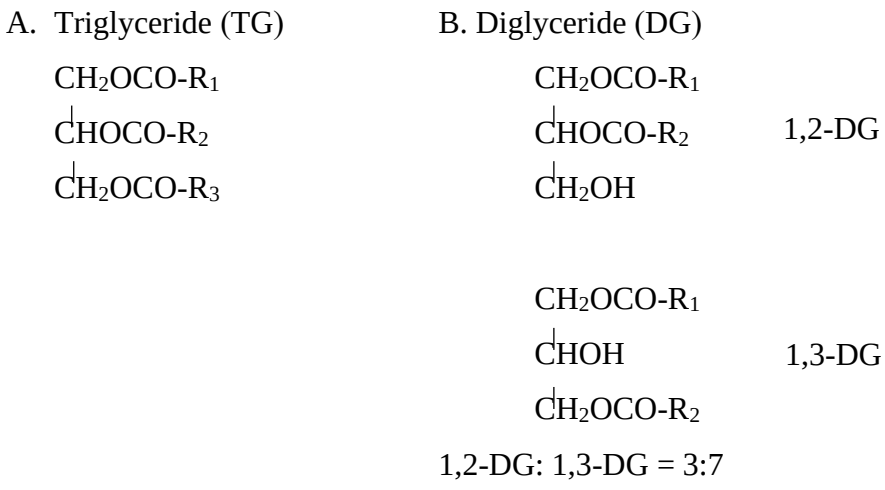


Figure 2.11 The structure of the TG and the various isomers of DG. R1, R2 and R3 are fatty acids.
Source: Yanai *et al*. (2012)

DG has 2 isomers, 1,2-DG (or 2,3) and 1,3-DG, as shown in Figure 2.11, These isomers will move the group of fatty acids to enter the equilibrium at 1,2- DG: 1,3-DG ratio of 3:7 when acid or alkalinity occurs in the system (Lo *et al*., 2008). For the new oil from commercial production, the proportion of 1,2-DG to all DGs is high. 1,3-DG will increase when the raw material is stored (Watanabe *et al*., 2003), freeze or destroyed fungal and insect, etc. (Katsuta *et al*., 2008)

Physico-chemical properties such as melting point, smoke point and polymorphic forms of DG are different from TG. 1,3-DG has a better thermodynamic stable than 1,2-DG, due to different steric effects. Generally, 1,3-DG has a melting point greater than TG about 10°C and 1,2-DG has a melting point less than TG about 10°C when having the same fatty acid composition. The difference of this melting point comes from the strength of the hydrogen bond of the hydroxyl group and fatty acid chain arrangement of DG isomer. 1,3-DG is a V shape, while 1,2-DG is a hairpin shape.

DG is used in many industries as emulsifiers and stabilizers (EEC code: E471) in many products such as bread, ice cream and margarine (Plou *et al*., 1996) or used as the main raw material for the production of cosmetics and medicines (Lai *et al*., 2011) In addition, it is found that the DG oil with major 1,3-DG is health benefits compared to conventional oils. Based on studies in animals and humans, when consuming DG oil,

it shows the reduction of TG accumulation of body fat and liver. (Kupongsak and Lucharit, 2014)

2.4.3 Monoglyceride (MG)

MG or monoacylglycerol is an ester of glycerol and one molecule of fatty acids and has two independent hydroxyl groups. This type of glyceride is rarely found in nature but is found in fat that from incomplete hydrolysis. It is useful for the synthesis or modification of structures of TG that has economic value (Wipawadee Paripatanapairod, 2003). MGs is widely used in the food, pharmaceutical and cosmetic industries (Thude et al., 1997), including other industries such as toothpaste (Sontag, 1982). In the food industry, MG is used as emulsifiers in bakery products, margarines, dairy products, confectionery, and food seasoning (Jackson and King, 1997). In pharmaceutical industry, MG is used as adhesives in tablets, and mixed in long-active drugs (Jackson and King, 1997). In cosmetic industry, MG is use as a texturing agent to provide concentrated creams or lotions and improve the viscosity of creams or lotions (Stevenson *et al*., 1993). Also, MG is used in textile industry, mixed in oil for using in machinery (since MG has lubicant and plasticizing properties) (Coteron *et al*., 1998).

Besides being used as an emulsifier in food, pharmaceutical and cosmetic industries, MG containing n-3-polyunsaturated fatty acid such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) can help preventing many diseases in human body. Some MG such as monolaurin, monomyristin, monolinolein and monolinolenin, which have antimicrobial properties, can destroy virus that is wrapped in fat such as HIV, herpes, cytomegalovirus and influenza, and various pathogenic bacteria including *Listeria monocytogenes* and *Helicobacter pylori* and protozoa, such as *Giardia lamblia* (Freitas *et al*., 2010).

2.4.4 Synthesis of glyceride

Glyceride synthesis can be divided according to the use of catalysts and reactions. Based on using catalysts, glyceride synthesis can be divided into 2 ways: enzyme method (using enzyme as catalysts) and chemical method (using alkalis or inorganic substances as catalysts) (Kupongsak and Lucharit, 2014).

2.4.4.1 Glyceride synthesis using enzymes as catalysts

(1) Selective / partial hydrolysis reaction

Selective / partial hydrolysis reaction is a reaction that separates the bonds between the molecules of fat or oil with water and enzymes or inorganic substances as catalysts.

(2) Esterification

Esterification is an ester bonding reaction between fatty acids or free fatty acids with glycerol. In general, production of glyceride often used the esterification of fatty acid and glycerol catalyzed by immobilized 1,3-specific lipase, resulting in a small amount of water or alcohol. Water will change the reaction equilibrium. Thus, it is needed to remove water from the system by reducing the pressure. At the end of the reaction, DG and TG will be obtained. Excessive use of enzymes will increase the yield of the reaction but will reduce the purity of DG due to increase of TG. Therefore, synthesis of DG by esterification must consider about ending point of reaction that give high yield of DG. The attention should be paid based on factor such as substrate ratio, reaction temperature and concentration of enzyme used. Increasing the ratio of fatty acid to glycerol will increase the rate of reaction and yield. Using longer time will reduce amount of DG but increase TG.

The ratio of fatty acids to glycerol is most commonly used at a 1.6-2.5. Water caused by the reaction should be eliminated. Especially, state of the reaction without water should be controlled, except water from enzyme preparation. Water removal methods such as reducing pressure, using absorbents such as zeolites, etc, will be used. The temperature of the reaction depends on the melting point of the raw material or product or the heat tolerance of the enzyme used. The reaction temperature

is most commonly used at 30-70°C, based on enzyme activity. The reaction time should be within 10 h (Watanabe *et al*., 2003).

Lo *et al.* (2004c) studied the optimum conditions for the most DG production with minimum TG, using immobilized Lipozyme RM IM (Immobilized *Rhizomucor miehei* lipase) as a catalyst and using RSM to track DG and TG (% by weight). The studied factors were reaction time of 3-8 h, enzyme concentration of 4-10% of fatty acids, reaction temperature of 55-75°C, and fatty acids to glycerol molar ratio of 2-3. When comparing the main influences of variables affecting DG and TG values, it was found that the reaction temperature factor had the most significant influence on the DG yield followed by the molar ratio of the substrate. The increase in the concentration of enzymes does not result in increased of DG production. In particular, when the system is related to reversible reactions that have multiple products, the increased amount of lipase concentration in the system of the esterification reaction results in an increase in the reaction rate of all parts of the esters and may change the reaction balance to proceed forward, resulting in more TG, as well as increasing the lipase concentration and longer time resulting in more TG. Therefore, the increase in the molar ratio of the substrate, the concentration of the enzyme and the reaction temperature resulted in a decrease in DG and TG. As already mentioned, this system is related to reversible reactions where the desired component is produced as a product in a reaction, and then used to continue to be substrate to another reaction in the system. DG is caused by the esterification reaction of MG and FFA with glycerol and is used as a substrate to synthesize TG. This causes DG to decrease but will increase TG. Therefore, the experiment to find the optimal conditions for the production of DG for the most efficiency should use the amount of lipase to the minimum and to get the most DG and get the least TG in the shortest time. It was found that the optimal temperature for producing DG was 66.29 ◦C, enzyme concentration of 4% (wt.), molar ratio of 2.14 and 4.14 h, which gave the maximum DG of 48% (wt.) and TG of 14% (wt.).

Lo *et al.* (2004c) studied the synthesis of DG by using lipase to catalyze esters of glycerol and fatty acids obtained from soybean oil deodorizer distillate (SODD), corn oil deodorizer distillate (CODD) and palm oil deodorizer distillate (PODD). Various factors affect the reaction, reaction time (0.5-72 h), enzyme concentration (1-13% of fatty acids), temperature (50-70°C), the ratio of fatty acids to glycerol (1-3:1), and the amount of water (0-10%), were studied. It was found that the optimal condition for producing the highest DG (69.9% (wt.)) from SODD was at of 65°C, 10% (wt.) enzyme content, fatty acids to glycerol molar ratio of 2.5:1, time of 4 h. While the production of DG from CODD under the same condition was 70% (wt.) DG at 5 h (Lo *et al*., 2004b). For DG production from PODD at the same condition, the amount of 52% DG was obtained at 6 h (Lo *et al*., 2004a). The influence of time is important in tracking the changes in the substrate and the products of enzymatic reaction to find the shortest time that gives high yields and reduces production costs. The influence of temperature is important to the rate of reaction and enzyme activity. In general, the higher temperature of the reaction will reduce the viscosity of the mixture, increasing the rate of interaction between molecules of substrates and enzymes. The optimal temperature of the immobilized lipase in the reaction is in the range of 30-65°C. Water content effects of the structure of enzyme in terms of synthesis reaction flexibility, only a small amount of water is necessary for proper enzyme activity. However, too much water reduces enzyme activity, thus, it is necessary to eliminate water that occurs during the reaction as much as possible.

(3) Glycerolysis reaction

Glycerolysis is a fatty acid exchange reaction between fat or oil (TG) and glycerol. Kristensen *et al.* (2005) studied the optimal conditions of glycerolysis in the production of DG in order to obtain the highest DG yield and the least cost by using RSM. Their studies 5 important factors: time, enzyme concentration, temperature, water content and molar ratio of the substrate (oil and glycerol). Factor that affected the most cost of production was the concentration of enzymes used. In addition, time and temperature are also effective. Controlling the amount of water is difficult, large amount of water, resulted in high amount of FFAs

in the product. It is necessary to have as little water as possible. Considering the molar ratio, especially of glycerol, large an amount of glycerol will cause high viscosity. Small quantity of glycerol is often used. The conditions of study were time (3-14 h), enzyme content (3-15% (wt.)), temperature (40-75^{o}C), water content (0-6% of glycerol weight) and the oil to glycerol molar ratio (0.25-2.00). It was found that the factors affecting the highest DG yield were temperature. The optimal temperature made the enzyme work well, which the enzyme function depended on the heat inhibition and the influence of temperature on the reaction. Increasing the temperature has the advantage of reducing viscosity and increasing the solubility of glycerol in the oil phase, and time also has a positive effect on DG production. The influence of the molar ratio of the substrate has a positive effect on the DG yield, but the excess glycerol is not common. The amount of glycerol is as small as about 5% of oil weight (which is equal to the oil to glycerol molar ratio of 2) which is good for production at a larger level. For the concentration of enzymes used, increase the amount of enzyme does not increase the yield but may increase the rate of reaction due to restrictions in mass transfer. Therefore, in a system with high viscosity, the important factor to consider is to mix them together and have a good mass transfer, so the appropriate amount of enzymes should be used in order to increase productivity and help to reduce costs. For water content factors, there was no need to add water to the reaction mixture because there were 1-2% of water in the reaction already. This amount of water was enough to cause good enzyme activity. Therefore, the optimum conditions for producing DGs (60% (wt.)) without adding water were molar ratio is 2, temperature of 60-65°C, time of 4-5 h and only 5% (wt.) lipase.

Fregolente *et al.* (2008) studied of the factors of water to be added or not added to the reaction by selecting various enzymes in both the form of aqueous solution and immobilized enzyme which were capable for producing DG and MG via glycerolysis of soybean oil, and found that *Candida antartica* had the best capable. Therefore, this enzyme was used to study influences of various factor. The result showed that enzyme content (10% of oil weight), oil to glycerol ratio (1:8), and water content (1% of glycerol) at temperature of 50 $^{\circ}$C, tracking time from 0-24 h, can produce

maximum DG and MG in the range of 45-48% and 28-30% (wt.), respectively. Using immobilized lipase, resulted in the FFA content about 5%, but when using independent lipase with 3.5% extra water in the system, the amount of FFA increased due to the amount of water added to the system will caused competition between the hydrolysis and glycerolysis reactions.

(4) Partial hydrolysis reaction followed by esterification reaction

Partial hydrolysis reaction followed by esterification is the production of glycerides by using two common reactions between partial hydrolysis of fat or oil in order to obtain FFAs, followed by the esterification reaction of FFA with glycerol. Yamada *et al.* (2001) reported that the production of DGs with single step esterification, resulted in expensive raw material costs due to the use of pure fatty acids. The hydrolysis reaction can use oil directly with applying a steam separation method at high temperature of 250-260 ^{o}C, resulting in products that did not have the desired color. Further distillation was needed and may cause the yield to be reduced by about 10% and may lose important phytosterols. Most glycerolysis reactions take about 10 h or more, which is not suitable for industrial production. Therefore, it is needed to study the production of DG that contains partial hydrolysis of fat or oil, which steam (at 190-240^{o}C) is used to separate the components, and adding 20-180 parts (wt.) of water per 100 part (wt.) of fat or oil, followed by the reaction of esters with glycerol in order to obtain the high purity of the DG with a low cost (from the use of inexpensive fats or oils as raw materials) and give higher efficiency than the production using conventional esterification and glycerolysis, and does not affect the quality of oil, such as discoloration and loss of important substances that are useful in fat or oil.

The first step of production is to partially hydrolyze the reaction of fat or oil, which may be used to separate the components with steam at temperatures from 190-240^{o}C. Mostly, the reaction are made at 200-230^{o}C or enzymatic methods are used at 20-70°C. Due to the need to produce DG, it is not necessary to separate the components with steam to 100% in the hydrolysis of fat or oil in the first step, resulting in the mixture of partial glyceride and TG. The reaction will control the amount of fatty acids about 67-96% (wt.) (commonly 75-93% (wt.)), that will help to reduce the

reaction time of esterification in the second stage due to the product obtained from the first step contains some glycerides. After some hydrolysis reaction, the oil is separated from the liquid by centrifugation. For glycerol distributed in water, after water is removed, the glycerol can be used in the reaction of esterification. Alternatively, the glyceride-TG mixture can be used in the second stage without separating the oil. Most of the products obtained from this method of separating some components with steam often do not have to go through a refining process, which has the advantage that when using vegetable oil as a raw material, it can still be a useful substance in DG products.

For the second step of the reaction of esterification, the related factor is similar to the general reaction of esterification. Most molar ratio of the fatty acids obtained from the separation of components with steam and glycerol is used 0.8-2.5. Esterification reaction can be carried out when the system does not have water (or has minimal) or does not add water, except the water that comes from enzyme preparation. Removal of water containing or occurring in the reaction can be done, such as using water absorbent material or vacuum drying. Commonly, vacuum is used drying, because it helps to avoid contamination in the reaction system. After that, the enzyme is separated from the reaction product of the second step. In addition, the non-reacting fatty acids and MG are separated by a general purification process.

2.4.4.2 Synthesis of glycerides by chemical methods

Glyceride production can use chemical methods with various catalysts as previous mentioned. The advantages of the chemical method compared to the use of enzymes is that it makes a variety of glyceride products that have different physical properties. Due to the use of enzymes is limited on the ability of the enzyme's heat resistance and the movement of the acyl group when using higher temperatures in the reaction, and the limitation of use in high melting point fats, therefore the production of commercial glycerides uses chemical methods rather than enzymatic methods (Jacobs *et al*., 2006).

2.5 Distillation methods for fatty acids

Although the present work is subjected to split free fatty acids from triglyceride (crude palm oil), which is different from the separation of fatty acid from contaminated fatty acids, the idea of using distillation for separating fatty acids is accepted for the present work to be used to separate free fatty acid from crude palm oil. Types of fatty acids distillation are concluded by Cermak *et al*. (2012) as follows.

Reported by Gunstone *et al.* (2007a), industrial production of fatty acids begins with the hydrolysis of triglycerides (fats or oils) with water to obtain about 10% of glycerol and 96% of fatty acid mixture. A Twitchell batch process (Ackelsberg, 1958; Twitchell, 1898) or a Colgate-Emery continuous process at high pressure and temperature (Barnebey & Brown, 1948) can be used for the fatty acid splitting process. The product fatty acids from this process are comparatively dusky in color and comprise several impurities. Consequently, the fatty acids are distilled into fractions for purification.

Distillation of fatty acids to get purified fatty acids has been widely performed for many years. This method eliminates the odors and the contaminants of both low and high boiling points. The batch or continuous distillations at vacuum or barometric pressure can be possible. Lausberg *et al.* (2008) concluded that the new distillation units are based on heating at high vacuum in a small interaction time with good mass transfer and less used of steam.

2.5.1 Batch distillation

Batch distillation can be done at atmospheric pressure with 260-316°C direct heating and 149°C steam dispersing. The steam to fatty acid vapor is commonly 5:1. Huge mass of steam is used, and high amount of fatty acids go with the condense steam. Decarboxylation and polymerization often occur due to the uncertainty of fatty acids with heat. Distillation of 95% fatty acid mixture obtained from hydrolysis gives 15-20% separated fatty acids and 10-15% residue (Muckerheide, 1952). Potts (1956) improved this technique by doing at low vacuum pressure about 5-50 mmHg and reducing steam used.

2.5.2 Continuous distillation

Wecker (1927) improved the first continuous distillation of fatty acid as the diagram shown in Figure 2.12. The fatty acids feed was warmed before entering the system at point c and then flowed through tube b into an interconnected reaction tanks which were heated by burners. Gas or oil was used as fuel of the burners. The feed was sparged (points m and n showed the sparger) by superheated steam through tube l. Vacuum pressure about 30-35 mmHg was introduced in the system causing the superheated steam to vaporized forcefully, resulting in an immediate evaporation of fatty acids from the reaction tank to the header g, condensed at condenser h and collected at collector tank I, whereas steam flowed to the condenser k which worked at atmospheric pressure. Other gases, which not condensed, continue flowed to vacuum pump. The last reaction tank ejected the residue to be cooled in d and kept in e. The reaction tanks had temperature of 196-260°F and the time used was about 30 min.

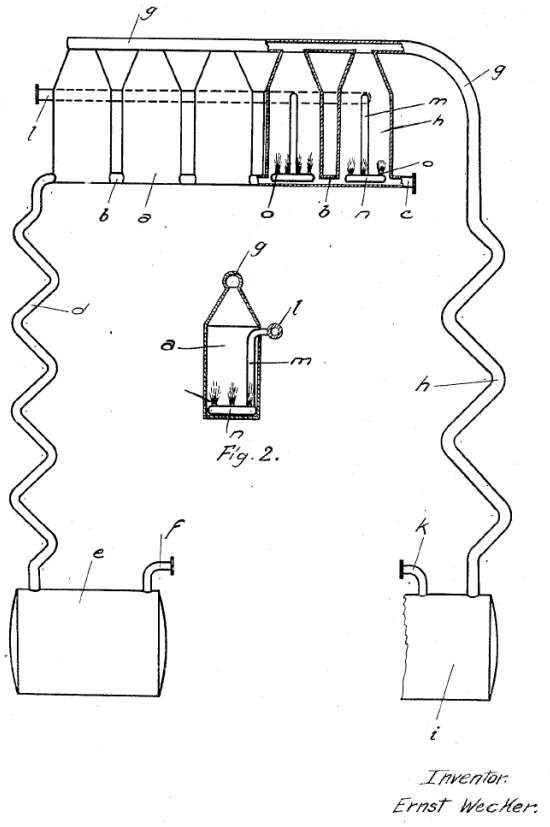


Figure 2.12 Continuous distillation

Source: Wecker (1927)

Mills (1942) developed a continuous process of a mix of dry and flash distillations to evade a creation of emulsion such as found in the wet process which fatty acids reacted with calcium and magnesium salts in water to form soaps. Figure 2.13 showed the Mill's process which the crude fatty acids mixture was heated by heat exchanger (no. 4) to their corresponded boiling points at the vacuum pressure of equal or smaller 12.7 mm Hg which was produced in chamber no. 10. At the inlet point no. 13, the heated fatty acids evaporated suddenly due to vacuum pressure and lifted the liquid in evaporator no. 10 up through the tube no. 12. At the top exit no. 14, the liquid hit the baffle no. 15 and formed an umbrella-shape thin layer. The fatty acids vapor condensed at the condensers no. 17 and 18. The un-evaporated liquid flowed through tube no. 30 back to the heat exchanger or to be collected at tank no. 31.

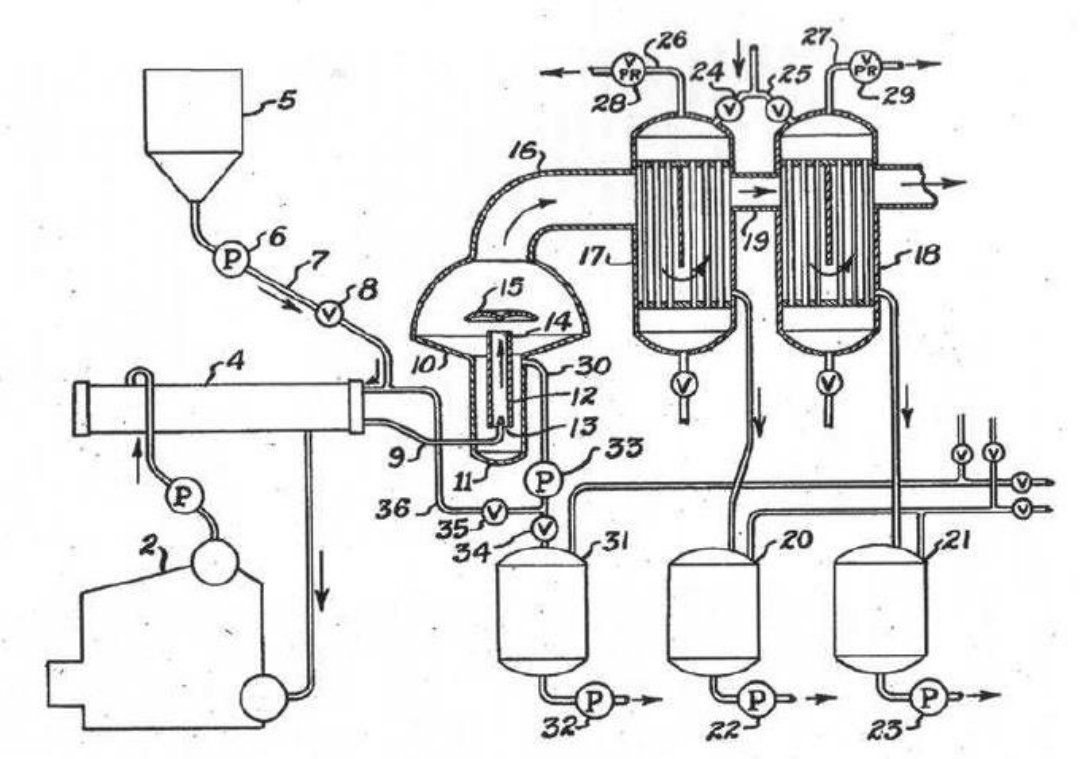


Figure 2.13 Continuous dry distillation
Source: Mills (1942).

2.5.3 Fractional distillation

In general, known properties and reliable composition of pure and mixed fatty acids are needed. Fatty acids with 2 carbon different chain lengths are simply separated to get 90% or more purity by fractional distillation (Potts & White, 1953; Ruston, 1952). Basically, fractional distillation, which separates fatty acids based on their boiling points, is performed like continuous distillation. However, this technique has the fractional column fixed with a number of bubble cap trays for elimination of the adjacent fatty acids stream distillates and return some part back as a reflux (Muckerheide, 1952; Stage, 1984). Marketable fatty acid products that can be taken by fractional distillation are shown in Fig. 2.14

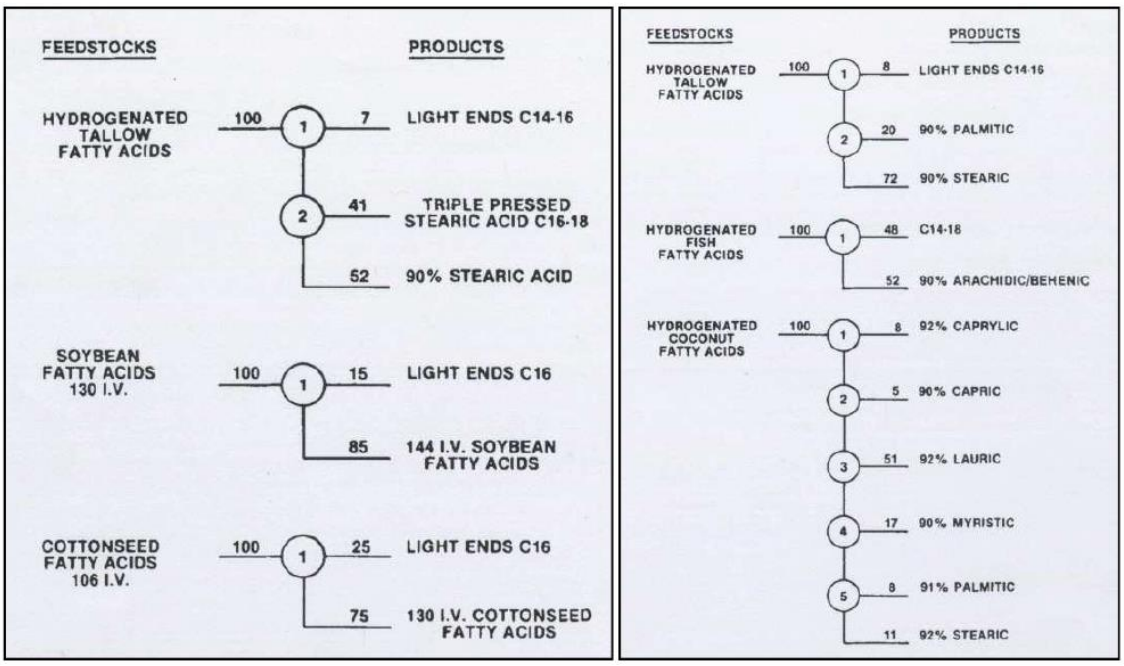


Figure 2.14 Partially fractionated hydrogenated tallow, soybean and cottonseed fatty acids and fully fractionated hydrogenated tallow, fish, and coconut fatty acids.
Source: Berger and McPherson (1979)

In 1933, Armour and Company were the first who built the continuous fractional distillation unit for the separation of a fatty acid mixture, as shown in Figure 2.15. The system comprised a direct-fired fatty acid heater, coolers, condensers, conventional air ejectors and boosters, the major fractionating tower and two minor side stripping towers. The direct-fired heater was vulnerable to coking and corrosion from the fatty acids. Shell and tube heaters were substituted the direct-fired heater in subsequent installations (Potts & White, 1953).

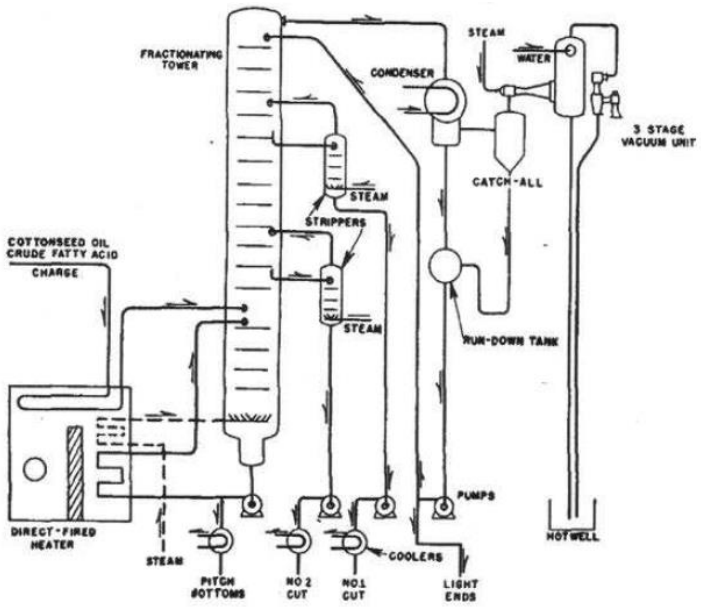


Figure 2.15 Flow diagram of fractional distillation employed by Armour and Company in 1933.
Source: Potts and White (1953)

In 1948, General Mills installed fractional distillation in their fat and oil processing plant which produce fractionated fatty acids, fatty acid esters, and their derivatives from low grade fats, oils, acid oils, and tall oil, as shown in Figure 2.16. To eliminate fouling in the heating tube during warming the feed, the rising vapor was used to heat the feed stock in the first distillation tower.

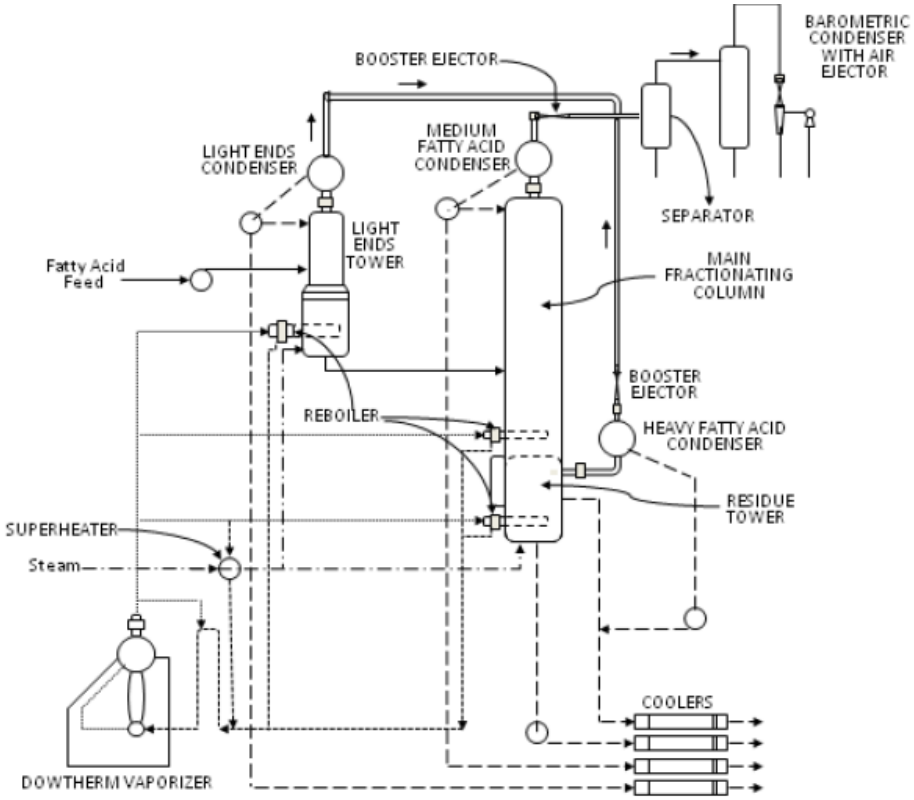


Figure 2.16 Flow diagram of fractional distillation employed by General Mills in 1948.

Source: Potts and White (1953)

2.5.4 Molecular distillation

Molecular distillation, conducted at vacuum conditions to decrease evaporating temperature and danger of oxidative destruction, is useful in the refining of extreamly oxidatively unstable fats, oils and their derivatives. Splitting the oil's components by mass permits pollutes to be diminished far beneath commerce standards. Existing industrial applications cover natural and herbal products that have to be distilled on an industrial scale. Molecular distillation comprises as a minimum the following types: wiped film molecular distillation unit (Figure 2.17) and centrifugal molecular distillation unit (Figure 2.18).

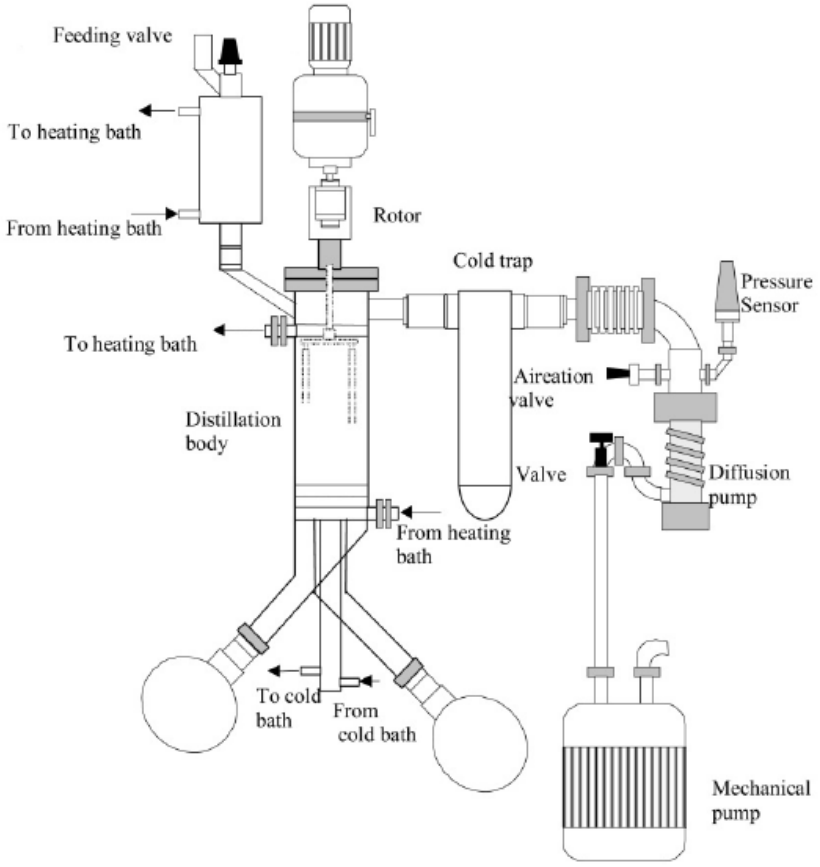


Figure 2.17 Wiped film molecular distillation unit.

Source: Marttinello *et al.* (2008)

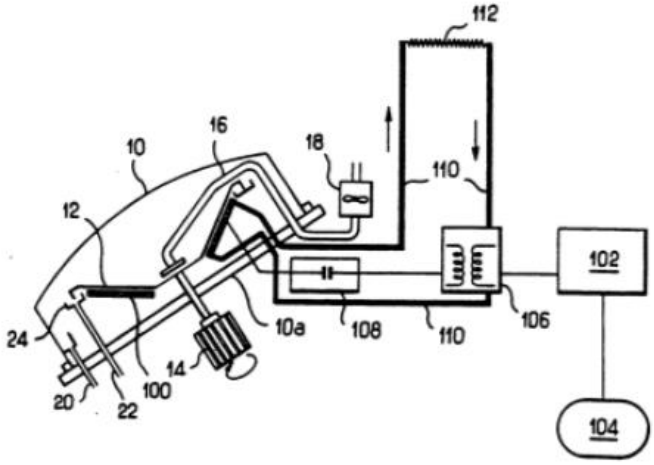


Figure 2.18 Centrifugal film molecular distillation unit.

Source: Nuns *et al.* (1994)

2.6 Lipase

Lipase (triacylglycerol acyl hydrolase: EC 3.1.1.3) is classified in the hydrolase group, used to catalyzes the degradation of oils and fats, providing fatty acids and glycerol. In addition, the reaction can be reversed such as ester synthesis (esterification) between glycerol and FFA (Sarothon Tantaseemant, 2013).

2.6.1 Lipase source

Lipase is an important enzyme in the metabolism of fat, which is important for the growth of organisms, found in animals, plants and microorganisms.

2.6.1.1 Animal lipases

Animal derived lipases can be found in animal tissues and organs such as the pancreas, stomach, intestines, heart, kidney, muscles and brain. In addition, lipases are found in animal milk (Homanics, 1995).

2.6.1.2 Plant lipases

Plant lipases can be found in plant tissues, vegetables, fruits and seeds. The most common plant lipases are found in various types of seeds, such as oatmeal (Kaur *et al*., 1993), rice bran, palm seed, rubber seed, soybean, wheat and castor seed etc. (Homanics, 1995).

2.6.1.3 Microbial lipases from microorganisms

Lipase is produced from many types of microorganisms such as yeast, fungi and bacteria etc. Lipases from microorganisms have advantages over lipases derived from plants and animals. Microorganisms grow faster and are easier to cultivate than plants and animals. It can grow in climate-free culture, the production space is save, as well as the labor in cultivation and harvesting. The higher concentration of enzymes is obtained. Moreover, lipases obtain from different types of microorganisms have different properties. There are two type of lipase produced by microorganisms: lipase that is produced and then deposited in the cells (intracellular lipase) and lipases produced outside the cell (extracellular lipase). In addition, there are some microorganisms that accumulate lipase in the cell first and lipase is excreted outside the cell at appropriate environment, such as lipase from *Yarrowia lipolytica*, which is

expelled out of the cell when medium is fed with a high carbon concentration. (Pereira-Meirelles *et al*., 2000)

2.6.2 Specificity of lipase

Lipases in the hydrolase group act as a catalyst for the decomposition of oil and fat to give product as fatty acids and glycerol. In addition, it can accelerate the revers reaction such as the ester synthesis (esterification) between fatty acids and alcohol. This reaction occurs in systems with less water. Transesterification can be divided into 3 groups: (i) ester - alcohol (alcoholysis), (ii) esters - fatty acids (acidolysis), and (iii) between various ester (interesterification) as shown in Figure 2.19 (Weera Piyatheerawong, 2012).

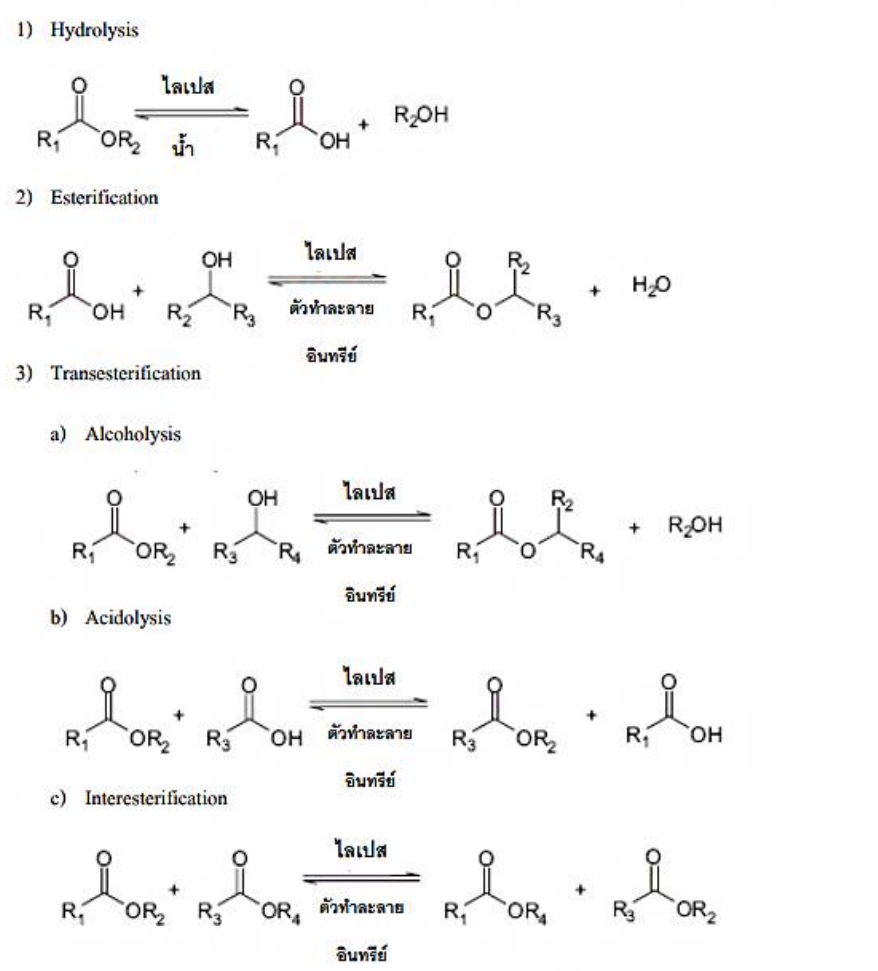


Figure 2.19 Reactions catalyst by lipase.

Source: Weera Piyatheerawong (2012)

There are three lipase specificity patterns, regio-selectivity, stereo-selectivity and fatty acid specificity.

2.6.2.1 Regio-selectivity

Regio-selectivity means that this type of enzyme is specific to the reaction with the position of the ester bond in different triglyceride oils such as 1,3-specific lipase (is an enzyme that is specific to the reaction with position 1 or 3, rather than the position 2 of the ester bond in triglyceride) or non-specific lipase (is an enzyme that is not specific to the reaction with any position of the ester bond in the triglyceride oil).

2.6.2.2 Stereo-selectivity

Stereo-selectivity means that an enzyme has specificity in reacting with a substance that is a type of enantiomer, such as R-configuration or S-configuration or L-configuration or D-configuration, etc., causing the catalytic rate with substances that are enantiomer are different.

2.6.2.3 Fatty acid specificity

Fatty acid specificity means the enzyme is specific to the reaction with the type of acid. For example, some lipases will accelerate the degradation of oil with short-chain fatty acids (the number of carbon atoms is less than 6 atoms), or catalyze the degradation of oil containing medium fatty acids (the number of carbon atoms between 6 - 12 atoms), or catalyze the decomposition of oil with long-chain fatty acids as well (the number of carbon atoms more than 12 atoms), as shown in Table 2.7 (Schmid & Verger, 1998; Reetz, 2002; Hayes, 2004; Ghanem, 2007; Veera, 2012).

Table 2.7. Source of lipase catalyst esterification reaction.

Lipase	Acid	Alcohol
Aspergillus niger *Rhizopus delemar* *Penicillium cyclopium* *Geotrichum candidum*	C_2-C_{18}, Benzoic, Oleic, Ricinoleic, etc.	Glycerol
Rhizomucor miehei	High free fatty acid rice bran oil, C_{10}, C_{10}-C_{18}, n-3 fatty acid	Glycerol
Candida rugosa	Oleic, Linoleic, Linolenic	Glycerol
Candida viscosum	Oleic, Linoleic, Linolenic, n-3 fatty acid	Glycerol
Rhizopus nodosus	C_8-C_{16}	Glycerol
Pseudomonas (CES) *Pseudomonas* (PS-30) *Penicillium* sp. *Mucer* sp.	n-3 fatty acid	Glycerol

Source: Modify from Gandhi *et al*., 1997

2.6.3 Industrial microbial lipase utilization

The use of lipase increases rapidly and plays a huge role in industries related to biotechnology, especially, food industry, medical science, pharmaceutical industry, chemical industry, and biofuels (Stehr *et al*. 2003; Steiner and Williams, 2002; Pandey *et al*, 1999). The important advantages of lipase are stability in organic solvents and specificity of substrates (Sharma *et al*., 2001). The use of lipase at industrial levels are at following...

2.6.3.1 Fat and oil processing

Lipase is used to modify the properties of fats and oils by changing the position and type of fatty acids that are binding to glycerol in fat or oil molecules to achieve the desired properties, resulting in new type of oil. (Davidson, 1996). In addition, lipase is also used to eliminate phospholipids in vegetable oil (de-gumming)

by using microbial phospholipases. The lipase can be used in the form of pure or immobilized cells (Singh *et al*., 2012).

2.6.3.2 Food industry

There are many applications in the food industry such as cheese ripening, flavor development and EMC technology. In addition, lipase is also used to adjust the taste of food by synthesizing the ester of short fatty acids and alcohol, which is a well-known flavor and fragrance (Singh *et al*., 2012)

2.6.3.3 Medical industry

There are many lipase applications in medical industry. One of example is lipase from *Candida rugosa* that are used to synthesize Lovastatin, which is a drug that reduces blood cholesterol levels (Matsumae *et al*., 1993).

2.6.3.4 Detergent

The use of enzymes in detergent formulas increases the ability to clean dirt and stains, making the detergent safer for the environment (Jeon *et al*., 2009).

2.6.3.5 Pulp and paper industry

Lipase is used to remove stains from paper pulp. Lipase from *Candida rugosa* can remove stains up to 90% (Jaeger *et al*., 1998).

2.6.3.6 Tea processing

The quality of black tea depends on drying, rolling and fermentation. During fermentation of tea leaves, lipase will decompose the fatty membrane tissue, resulting in a unique flavor, and found that lipase produced from *Rhizomucor miehei* increases the amount of unsaturated fatty acids that affect the flavor (Hasan *et al*., 2006).

2.6.3.7 Environmental management

Lipase is wildly used in biological treatment processes. Mainly, it is used in fat processing plants and restaurants, which will help to reduce environmental pollution in contaminated soils (Lin *et al*., 2012).

CHAPTER 3
MATERIALS AND METHODS

3.1 Materials

3.1.1 High free fatty acid mixed crude palm oil (HFFA-MCPO)

MCPO is a mixture between CPO and PKO. HFFA-MCPO with initial FFA content about 14% was obtained from Rungruangkit Vegetable Oil Partnership Limited (1981), Songkhla Province, Thailand.

3.1.2 Crude glycerol (CG)

CG with 28.8% methanol content was obtained from a biodiesel pilot plant, Faculty of Engineering, Prince of Songkla University, Songkhla Province, Thailand. Prior to use, the CG was heated at 70°C for eliminating methanol. High-performance liquid chromatography (HPLC) was used to ensure the smallest remaining amount of methanol in CG.

3.1.3 Glycerol

Glycerol (analytical grade) was from Loba Chemie, Mumbai, India.

3.1.4 Oleic acids

Oleic acids (commercial grade) was from Panreac Applichem, Milan, Italy.

3.1.5 Lipase

Lipase (from *Camlicla lipolytica*) (food grade) was from Power-Tech Chemical Industry, Bangkok, Thailand.

3.1.6 Lipase producing microorganism

To find the suitable microorganism, three potential lipase producing strains (*Bacillus subtilis*, *Bacillus coagulans* and *Psuedomonas* sp.) from Microbiology Laboratory, Department of Industrial Biotechnology, Faculty of Agro-Industry, Prince of Songkla University, were chosen for screening.

3.1.7 Molecular sieve

Molecular sieves, 3A, 2-5 mm (0.08-0.20 in) beads were from Alfa Aesar, Heysham, England. Prior to use, the molecular sieves were dried in an oven at temperatures of 190-210°C for 24 h and then put in the desiccators before use (Al-Asheh *et al*., 2004). In this study, molecular sieves were used to prevent reversed hydrolysis of the products due to the presence of water (Chong *et al*., 2007). 1 g molecular sieves per 5% moisture content was used (Yesiloglu *et al*., 2004).

3.1.8. Chemicals and reagents

Chemicals and reagents used in this research were shown in Table 3.1. Details of all chemical preparation methods were explained in appendix A.

Table 3.1. List of chemicals and reagent.

For FFA analysis	For I.V. analysis	For P.V. analysis	For S.V. analysis
- 95% ethyl alcohol - Phenolphthalein - 0.1 N Sodium hydroxide (NaOH)	- Carbon tetrachloride (CCl_4) - Potassium iodide (KI) - 0.01 N Sodium thiosulphate ($Na_2S_2O_3$) - 1% Starch - Wijs solution	- Acetic-chloroform solution - Potassium iodide (KI) - 0.01 N Sodium thiosulphate ($Na_2S_2O_3$) - 1% Starch	- Alcoholic Potassium hydroxide (KOH) - Phenolphthalein - 0.5 N Hydrochloric acid (HCl)

3.1.9 Instruments

All instruments used in this work were listed and shown in Table 3.2

Table 3.2. List of instruments.

Instruments	Series	Suppliers
Balance 4 digit	PA214	OHAUS, USA
Centrifuge	CF-10	Wise Spine, Korea
Color meter	ColorFlex	HunterLab, USA
High-performance liquid chromatograph (HPLC)	1200	Agilent, USA
Moisture Analyzer	MA35	Sartorius, Germany
pH meter	EF-201	Mettler Toledo, China
Rotary evaporator	RV10	IKA, Germany
Incubator shaker	-	LMS, USA
Vortex	VM-19	Wise Mix, Korea
Viscometer	RV DVII+	Brookfield, USA

3.2 Experimental methods

3.2.1 Design and improve reactor for separating FFA from CPO

In this work, vacuum distillation was used to separate FFAs from HFFA-CPO and HFFA-MCPO. There were four configurations of reactor used for separation FFA from CPO as shown in Figure 3.1. Each configuration has evaporation tank of 40-L made from stainless steel heated by an electric heater (2.50 kW), condenser, vacuum pump and cooling water pump (0.37 kW). Configuration A, B, C and D used the same evaporator (No.4) and cooling water system (No.8,9,10 and 11). There were two condensers, the bigger one (No.7) and the smaller one (No.12). Configuration A used only condenser No.7 and configuration D used only condenser No.12, whereas configurations B and C used both condenser No.7 and 12. There were two vacuum pumps used in this work. Configuration C and D used the same bigger vacuum pump (2.20 kW), (No.13 and 14, respectively). Configuration A and B used the same smaller vacuum pump (0.75 kW), (No.14). Configuration D used a new cover (No.7) instead of a bigger condenser. The idea of the evaporator build in this work was from the evaporator of the continuous dry distillation (No.10 in Figure 2.13). The improve direct heating using electric heater was added instead of indirect heat exchanger.

3.2.2 Separation of FFAs from HFFA-CPO and HFFA-MCPO using distillation under vacuum conditions

3.2.2.1 Free fatty acid separation

The oil working volume was 28 L. The experimental conditions include the evaporation pressure of 20-30 mmHg, cooling water temperature of 15-20°C and evaporation temperature of 220-250°C. After distillation, the distillate and the remaining oil were collected, weighed and analyzed.

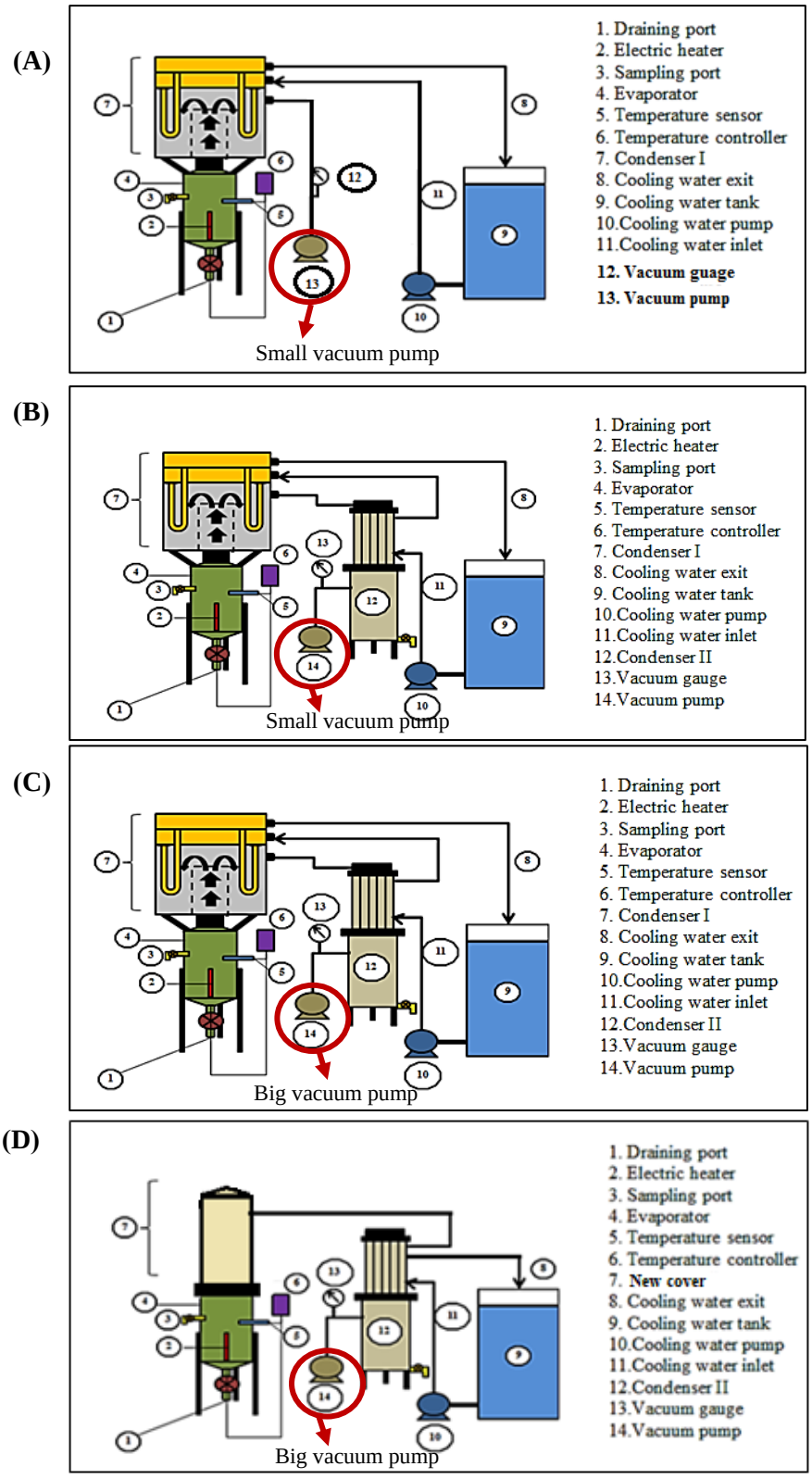


Figure 3.1 Four configurations (A, B, C and D) of reactor used for separating FFA from CPO.

3.2.2.2 Fatty acid composition

Fatty acid compositions or fatty acid profiles of HFFA-CPO before and after experiments and of the distillate were determined using an Agilent 6890 series gas chromatograph (GC) with a flame ionization detector (FID). In addition, free fatty acid content of above oil and distillate were determined using titration.

3.2.3 Production of glyceride from FFA distillate using lipase as a catalyst

3.2.3.1 Screening of lipase producing bacteria

3.2.3.1.1 Inoculum preparation

For inoculum preparation, the following nutrients medium were used (g/L): malt extract 10.0, glucose 4.0, yeast extract 4.0, K_2HPO_4 1.0, $NaNO_3$ 2.5. Cultivation was performed in 250 ml Erlenmeyer flask, containing 100 mL nutrient medium with pH 7. The flasks were incubated at 37°C for 24 h at 150 rpm shaking (Bharathi *et al*., 2013).

3.2.3.1.2 Cultivation in medium contain FFAs

FFA distillate of amount 10% (wt./wt.) was added into medium prepared from 3.2.2.1.1, and then incubated at 37 ^{o}C for 24 h at 150 rpm shaking.

3.2.3.1.3 Esterification Activity

Enzymatic esterification was performed using 3:1 molar ratio of a mixture of oleic acid to glycerol and 1 ml of lipase solution in a 50 ml conical flask. The esterification reactions were carried out for 1 h at 45 ^{o}C and 200 rpm shaking.
The esterification activity was determined by the following relation:

$$\text{Esterification activity} = \frac{V \times N \times 100}{E \times T} \text{ Units} \qquad (3.1)$$

where V is the difference in volume in ml of NaOH between the blank and samples after time T (period of incubation in min), which is a measure of the fatty acid consumed due to esterification, N is the Normality of NaOH and E is the amount of the enzyme employed in ml (Modified from Kiran *et al*,. 2012). One unit of esterification activity is defined as 1 µmol fatty acid consumed in the esterification reaction per min per ml lipase.

The esterification yield [EY (in %)] was measured by titrating 0.1 g of the sample with a standardized 0.1 N sodium hydroxide solution in the presence of phenolphthalein indicator. EY was calculated according to the following (Wang *et al*., 2012; Guebara *et al*., 2018):

$$EY = [(V_B - V_A) \div V_B] \times 100 \quad (3.2)$$

where V_o and V_i are the volumes of sodium hydroxide consumed respectively before and after the esterification. Every experiment was conducted in triplicate.

3.2.3.2 Preliminary experiment

This topic presents the preliminary investigate of the effects of concentration of enzyme used and effect of using solvent in esterification reaction before studying the optimal conditions in detail.

3.2.3.2.1 Effect of enzyme concentration

The enzyme lipase concentrations of 100, 300, 1000 and 2000 U/g fatty acids were used. The reaction was performed in a 250 ml flask with FFA distillate to crude glycerol molar ratios of 3:1 and reaction temperature of 45°C. The flask was incubated for 24 h with 200 rpm shaking together with using molecular sieves for removing water in the reaction. The enzyme concentration that gave the highest amount of DG was chosen to be used in the next experiment.

3.2.3.2.2 Effect of using organic solvent

The reaction was performed in the 250 ml flask, molar ratios of fatty acid to glycerol of 3:1, enzyme concentration according to 3.2.2.2.1 and reaction temperature 45°C and incubated for 24 h with 200 rpm shaking together with using molecular sieves for removing water in the reaction. The condition that gave the highest amount of DG was chosen to be used in the next experiment.

3.2.3.3 Study the optimal conditions for the synthesis glyceride using lipase as a catalyst

3.2.3.3.1 Effect of molar ratio of free fatty acid to glycerol

In order to see effect of free fatty acid to glycerol ratio on production of glycerides, the experiments were performed with the ratios of 4:1, 3:1, 2:1, 1:1, 1:2, 1:3 and 1:4. The enzyme content used in the reaction was 300 U/g fatty acids for all free fatty acid to glycerol ratios. The reaction was performed in the 250 ml flask, incubated at 45°C for 24 h with 200 rpm shaking together with using molecular sieves for removing water in the reaction. After completion of esterification reaction, the mixture was filtrated to separated molecular sieves and then separatory funnel was used to separate the oil (ester phase) and glycerol (glycerol phase) layers. Then, the ester was washed with hot distilled water (ester per water volume ratio of 1:1) in the following order: (i) washing two times without air injection (1^{st} and 2^{nd} washing) and (ii) washing three times with air injection (3^{rd} -5^{th} washing). After that, glyceride analysis was made using thin layer chromatography (TLC) and the ratio of fatty acid to glycerol that gave the highest amount of DG was chosen to be used in the next experiment.

3.2.3.3.2 Effect of temperature

Effect of temperature on production of glyceride was investigated. Seven temperatures were observed, 40, 45, 50, 55, 60, 65 and 70 °C. According to 3.2.2.3.1, the fatty acid to glycerol ratio that gave the highest amount of DG was selected for this study. The enzyme used was 300 U/g fatty acids. The reaction was performed in the 250 ml flask, incubated for 24 h with 200 rpm shaking together with using molecular sieves for removing water in the reaction. After that, glyceride analysis and temperature that gave the highest amount of DG was chosen to be used in the next experiment.

3.2.3.3.3 Effect of enzyme concentration

The enzyme lipase concentrations of 50, 100, 300, 500 and 700 U/g fatty acids were used. The best fatty acid to glycerol ratio and the best temperature were chosen from section 3.2.2.3.1 and 3.2.2.3.2, respectively. The reaction was performed in the 250 ml flask, incubated for 24 h with 200 rpm shaking together with using molecular sieves for removing water in the reaction. After TLC analysis, the enzyme

concentration that gave the highest amount of DG was selected to be used in the next experiment.

3.2.3.3.4 Effect of time

To study the effect of reaction time, the times used were 12, 24, 36 and 48 h. The best fatty acid and glycerol ratio, temperature and enzyme concentration were chosen from sections 3.2.2.3.1 - 3.2.2.3.3, respectively. The reaction was performed in the 250 ml flask, incubated with 200 rpm shaking together with using molecular sieves for removing water in the reaction. After TLC analysis, the reaction time that gave the highest amount of DG was selected. Figure 3.3 shows the scheme of the glyceride production (Modify from Karaosmanoğlu *et al*., 1996).

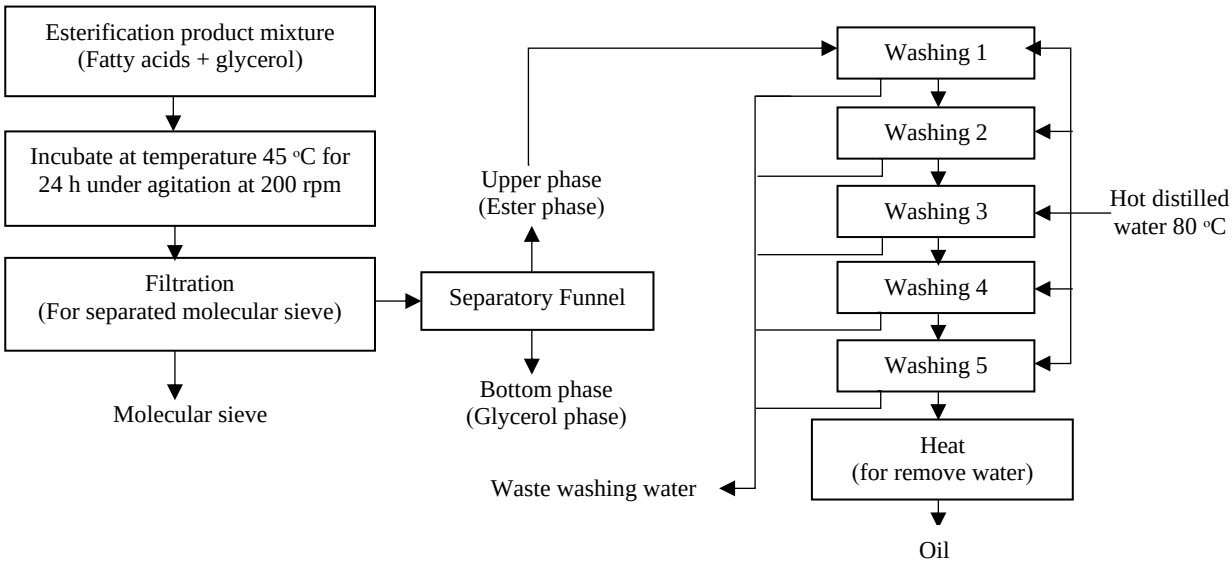


Figure 3.2 Scheme of the glyceride production.

3.3 Analytical and calculation methods

3.3.1. Determination of FFA using titration method

The free fatty acid in oil was determined according the American Oil Chemists' Society (AOCS) method Ca 5a–40 (1989) (Japir *et al*., 2016). Start from weighing 1-10 g of sample and then putting into flask, adding 50 mL of neutralized ethyl alcohol (neutralized ethyl alcohol by add 3-5 drops of phenolphthalein and then addition of

sodium hydroxide (NaOH, 0.1 N) until a permanent pink color) into the flask and shaking. After shaking the mixture, titration was made with sodium hydroxide solution (NaOH, 0.1 N) until a pink color permanently appeared for at least 30 seconds.

Percent of FFA (% FFA) in CPO based on palmitic acid was calculated using the following equations:

$$\% \text{ FFA as palmitic acid} = \frac{25.6 \times N \times V}{W} \quad (3.3)$$

where the value of 25.6 is an equivalence factor for palmitic acid, the predominant fatty acid in palm oil. N is normality of NaOH, V is volume of NaOH solution used (mL) and W is weight of sample (g).

Acid value was calculated using the following equations:

$$A.V. = \%FFA \times 2.19 \quad (3.4)$$

where, AV= acid value; 2.19 is a conversion factor for palmitic acid.

3.3.2. Determination of fatty acid composition

The fatty acid compositions (FAC) or fatty acide profiles of the CPO before and after experiments were determined using an Agilent 6890 series gas chromatograph (GC) with a flame ionization detector (FID). A capillary column size was 30 x 0.25 x 0.25 mm. The FID was set at 240°C with a flow rate of 0.8 ml/min. The injector temperature was also set at 240°C. Hydrogen was used as the carrier gas. The peaks were identified by measuring the retention time of the samples and comparing them with authentic standards analyzed under the same conditions.

3.3.3. Determination of iodine value

The iodine value was determined according the American Oil Chemists' Society (AOCS) method Cd 1-25 (1989) (Abdullah *et al*., 2013), by weighing 0.3 g of sample and putting into a 500 mL flask, adding 15 mL of carbon tetrachloride (CCl_4) to dissolve the oil, and 25 mL of the wijs solution. After gently shaking the mixture, the flask was kept in the dark for 1 hour. After 1 hour, 20 mL of potassium iodide (KI, 10% v/v) solution and 150 mL of water were added, then the mixture was titrated with the sodium thiosulphate ($Na_2S_2O_3$, 0.1 N) solution until the yellow color appeared. After that, 1 mL

of indicator solution (starch, 1% v/v) was added and the titration was continued until the blue color just disappeared. The blank test was prepared in the same way of sample.

The iodine value was calculated as recommended by the (AOCS 1989) standard methods Cd 1-25 as given in the equation below:

$$\text{I.V.} = \frac{(\text{Vb-Vs}) \times \text{N} \times 12.69}{\text{W}} \quad (3.5)$$

where N is the normality of the $Na_2S_2O_3$ solution, V_b is the volume (mL) of $Na_2S_2O_3$ solution used for the blank test, V_s is the volume (mL) of $Na_2S_2O_3$ solution used for the determination of the sample, W is the weight (g) of the sample. The value of 12.69 are used to transfer equivalent thiosulphate per g of iodine. The value of 126.9 is molecular mass unit or relative molecular unit of iodine.

3.3.4. Determination of peroxide value

The peroxide value was determined according the American Oil Chemists' Society (AOCS) method Cd 8-53 (1997) (Fiebig *et al.*, 2003), by weighing 5 g of sample, putting into a 250 mL flask, adding 25 mL of acetic acid-chloroform (ratio 3:2v/v) and 1 mL of potassium iodide, shaking the mixture gently, and then the flask was kept in the dark for 5 minutes. After 5 minutes, 75 mL of water was added and the mixture was titrated with the sodium thiosulphate ($Na_2S_2O_3$, 0.01 N) solution until the yellow color appeared. After that, 0.5 mL of the indicator solution (starch, 1% v/v) was added, and the titration was continued until the blue color just disappeared. The blank test was prepared in the same way of sample.

The iodine value was calculated as recommended by the (AOCS 1997) standard methods Cd 8-53 as given in equation below:

$$\text{P.V.} = \frac{(\text{Vb-Vs}) \times \text{N} \times 1000}{\text{W}} \quad (3.6)$$

where N is the normality of the $Na_2S_2O_3$ solution, V_b is the volume (mL) of $Na_2S_2O_3$ solution used for the blank test, V_s is the volume (mL) of $Na_2S_2O_3$ solution used for the determination of the sample and W is the weight (g) of the sample.

3.3.5. Determination of saponification value

The saponification value was determined according the American Oil Chemists' Society (AOCS) Method cd 3-25 (1993) (Odoom and Edusei, 2015), by weighing 2 g of sample, putting into a 250 mL flask, then adding 25 mL of 1.0 N Alcoholic Potassium hydroxide (KOH), mixing for 1 min. The mixture sample was boiled gently for 45 min for completing saponification. After that, the mixture was cooled down and then added 1 mL of phenolphthalein indicator solution and titrated with 0.5 N hydrochloric acid (HCl). The titration was continued until the pink color just disappeared. The blank test was prepared in the same way of sample.

The saponification value (S.V.) was calculated as recommended by the (AOCS 1993) standard method Cd 3-25 using the formula below:

$$S.V. = \frac{(Vb\text{-}Vs)\times N \times 56.1}{W} \qquad (3.7)$$

where, N is normality of HCl, V_b is volume (mL) of HCl used for the blank test, V_s is volume (mL) of HCl used for the determination of the sample and W is weight (g) of sample.

3.3.6. Determination of moisture content

The moisture content of oil was determined according the American Oil Chemists' Society (AOCS) method Cd 1-25 (1989) (Molekul *et al*., 2016). The moisture content of oil was determined using infrared radiation (IR) moisture analyzer model MA35 Sartorius. Approximately 5g of sample was weighed into a moisture dish, and dried in the moisture analyzer for 30 minutes at 105°C.

3.3.7. Determination of viscosity

The viscosity of the oil was determined using a Brook field model RV DVII+ (U.S.A) Spindle of LV-2 (62). The viscosity was read in Pascal second (Pa.s) directly from the viscometer, which was maintained at 1 min and 50 rpm at room temperature.

3.3.8. Determination of color

The oil color was determined using a HunterLab model ColorFlex. The semisolid oil samples were melted at a temperature of 45°C. The liquid oil sample was

put into glass sample cup. Results were expressed as L*, a* and b*, which corresponded to lightness, the green–red component, and the blue–yellow component, respectively. (Rossi *et al*., 2001).

3.3.9. Determination of crude glycerol (CG)

The methanol in CG was removed by rotary evaporator at 90°C. Finally, the pretreated glycerol was diluted with water (1:100 ratio) and analyzed. Agilent 1200 series high-performance liquid chromatography (HPLC) with a UV 215 nm detector and a BIORAD Aminex HPX-87 H Ion Exclusion column (300 mm × 7.8 mm) was used. The flow rate of 0.6 ml/min. H_2SO_4 (0.005 M) was used as the mobile phase. The peaks were identified by measuring the retention time of the samples and comparing them with authentic standards analyzed under the same conditions.

For soap content, the pH of 50 mL crude glycerol solution was lowered to pH 1 by adding 85% of H_3PO_4 to convert soap to free fatty acids (FFA). The solution obtained was centrifuged at 5000 rpm for 20 min. The top layer (FFA) was collected and weighed (Chen *et al*., 2017).

The amount and content of soap were calculated according equations (3.8) and (3.9) below:

$$\text{Soap amount (g)} = \frac{304 \times \text{FFA amount}}{282} \quad (3.8)$$

$$\text{Soap content (g)} = \frac{\text{g of soap}}{\text{50 mL of crude glycerol solution}} \times 100 \quad (3.9)$$

3.3.10. Determination of ash

The ash content was analyzed according to the standard method ISO 2098-1972 by burning 1 g glycerol in muffle furnace at 750°C for 3 h (Kongjao *et al*., 2010). The content of ash was calculated according equation below:

$$\% \text{ Ash} = \frac{\text{A-B}}{\text{W}} \times 100 \quad (3.10)$$

where A is weight of sample (g) after burn at 750°C for 3 h, B is weight of crucible (g) and W is weight of sample (g).

3.3.11. Determination of glyceride

Mono-, di- and triglyceride were analyzed by Thin-Layer Chromatography-Flame Ionization Detection (TLC-FID). Chromarods S-lI (quartz rod) was soaked in 3% HNO_3 for 3 minute and air ovened at 105 °C for 5 minutes. The scanning rate was 30 sec/rod, the hydrogen flow was 160 mL/min (the Iatroscan was a model MK-6). Drop of 1-3 µl of sample solution (0.1 g of sample dissolve in chloroform and dilute 100x) was applied on quartz rod, brought to soak in solvent (benzene: chloroform: acetic acid, 70:30:2) until the height of solvent reached 10 cm. Read the analysis results from the program Chrom Star light. The results were shown in the form of peak areas. Peak areas were quantitated by cutting and weighing. Weight percentage values were computed after converting peak weights to grams of sample employing (Intarat *et al*., 2014). The conversion of product was calculated according equations below:

$$\% \text{ Conversion} = \frac{\text{mol of FA consumed}}{\text{mol of FA initial}} \times 100 \quad (3.11)$$

3.3.12 Energy consumption

The energy consumption was calculated according equations below:

$$\text{Energy consumption (kW-h)} = \text{electric power x time (h)} \quad (3.12)$$

3.3.13 Evaporation efficiency

The evaporation efficiency was calculated according equations below:

$$\% \text{ Evaporation efficiency} = \frac{\text{the loss mass}}{\text{the initial mass}} \times 100 \quad (3.13)$$

3.3.14 Manually scraped efficiency

The manually scraped efficiency was calculated according equations below:

$$\% \text{ Manually scraped efficiency} = \frac{\text{the mass of solid FFA trapped by condensor}}{\text{the loss mass of the evaporator}} \times 100 \quad (3.14)$$

3.3.15 Statistical analysis

The data will be calculated with mean values and standard deviations (mean + SD) will be determined from triplicate trials. Statistical significance of the results will be evaluated by one-way ANOVA (analytical of variance) and Duncan's multiple range tests ($P < 0.05$) using SPSS version software 22.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Separation of FFAs from HFFA-CPO and HFFA-MCPO using distillation under vacuum conditions

Many previous investigators reported that the efficient fatty acids separation using vacuum distillation was usually obtained at pressure lower than 10 mmHg (especially 3-4 mmHg), which the boiling point of fatty acids was lower than 200°C. In this present investigation, the operation of the proposed reactor (Figure 3.1) could not meet the above ranges of desired pressure. The experimental conditions were shown in Table 4.1. The advantages and disadvantages of each reactor configurations were explained in Table 4.2.

Table 4.1. The temperature of oil, vacuum pressure and FFA content of each batch.

Batch	Initial FFA content (%)	Temperature of oil in evaporator (°C)		Pressure in evaporator and condenser (mmHg)	Heating to reach the desire temperature (h)	Operating time at the desired temperature (h)	Configurations of reactor used
		initial	setting				
CPO 1	25.70 ± 0.31	27.4	230.0	20	3.50	4.00	**A**
CPO 2	14.95 ± 0.13	29.5	250.0	160	2.80	4.00	**B**
CPO 3	10.29 ± 0.24	30.5	250.0	30	3.30	4.00	**C**
CPO 4	20.61 ± 0.16	27.6	250.0	40	3.58	11.62	**D**
MCPO 1	12.57 ± 0.39	31.4	220.0	20	2.00	4.00	**D**
MCPO 2	16.20 ± 0.27	27.1	240.0	20	3.00	4.00	**A**
MCPO 3	10.36 ± 0.41	29.6	250.0	20	2.67	4.00	**B**
MCPO 4	14.06 ± 0.08	31.2	220.0	30	2.50	4.00	**C**
MCPO 5	14.19 ± 0.37	29.3	230.0	30	2.33	4.00	**C**
MCPO 6	14.05 ± 0.46	27.8	240.0	30	2.78	4.00	**C**
MCPO 7	19.57 ± 0.14	29.0	250.0	30	3.08	4.00	**C**

Table 4.2. Advantages and disadvantages of each reactor configurations.

Configurations	Advantages	Disadvantages
A	- The condensing tank (No. 7) was designed to easily trap the FFA vapor.	- Difficult to harvest due to complex desire of the ring coil. - The condensing tank and evaporating tank were very heavy.
B	- The condensing tank (No. 7) was designed to easily trap the FFA vapor. - There are 2 condensers that can trap more free fatty acid.	- Difficult to harvest due to complex desire of the ring coil. - The condensing tank and evaporating tank were very heavy. - Took a long time to set up the devices (evaporating and condensing tanks)
C	- The condensing tank (No. 7) was designed to easily trap the FFA vapor. - There are 2 condensers that can trap more free fatty acid.	- Difficult to harvest due to complex desire of the ring coil. - The condensing tank and evaporating tank were very heavy. - Took a long time to set up the devices (evaporating and condensing tanks) - Used too much of energy due to a large vacuum pump.
D	- The condenser (No.12) was separated from evaporating tank, easily harvesting of the FFA.	- Used too much of energy due to a large vacuum pump.

Profiles of oil temperatures and vacuum pressures during separation of FFA from HFFA-CPO and HFFA-MCPO were shown in Figures 4.1 and 4.2, respectively. Which 28 L of oil, the heater (2.5 kW) used in this reactor can increase the oil temperature in the rate of 1.21 ^{o}C/minute. Firstly, the vacuum pump was turned on to produce the vacuum pressure in the reactor. However, the pressures obtained as shown in Figures 4.2 and 4.3, respectively were the lowest that the vacuum pump can make for each batch, which were not meet the desired pressure (< 10 mmHg), due to some small leakage may occur at rubber flange and the joints of several valves.

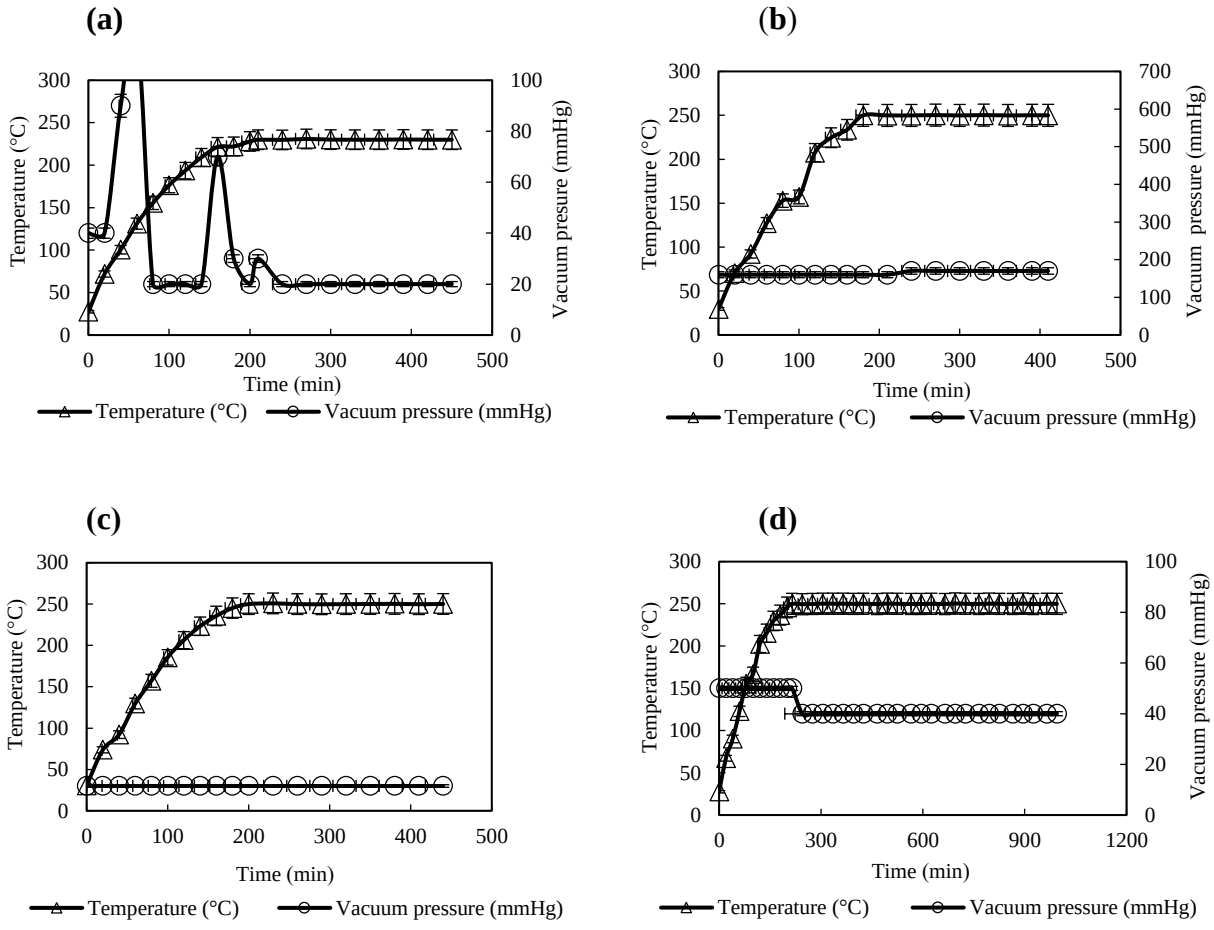


Figure 4.1. Profiles of oil temperature and vacuum pressure during separation of FFA from HFFA-CPO. (a) CPO 1; (b) CPO 2; (c) CPO 3 and (d) CPO 4.

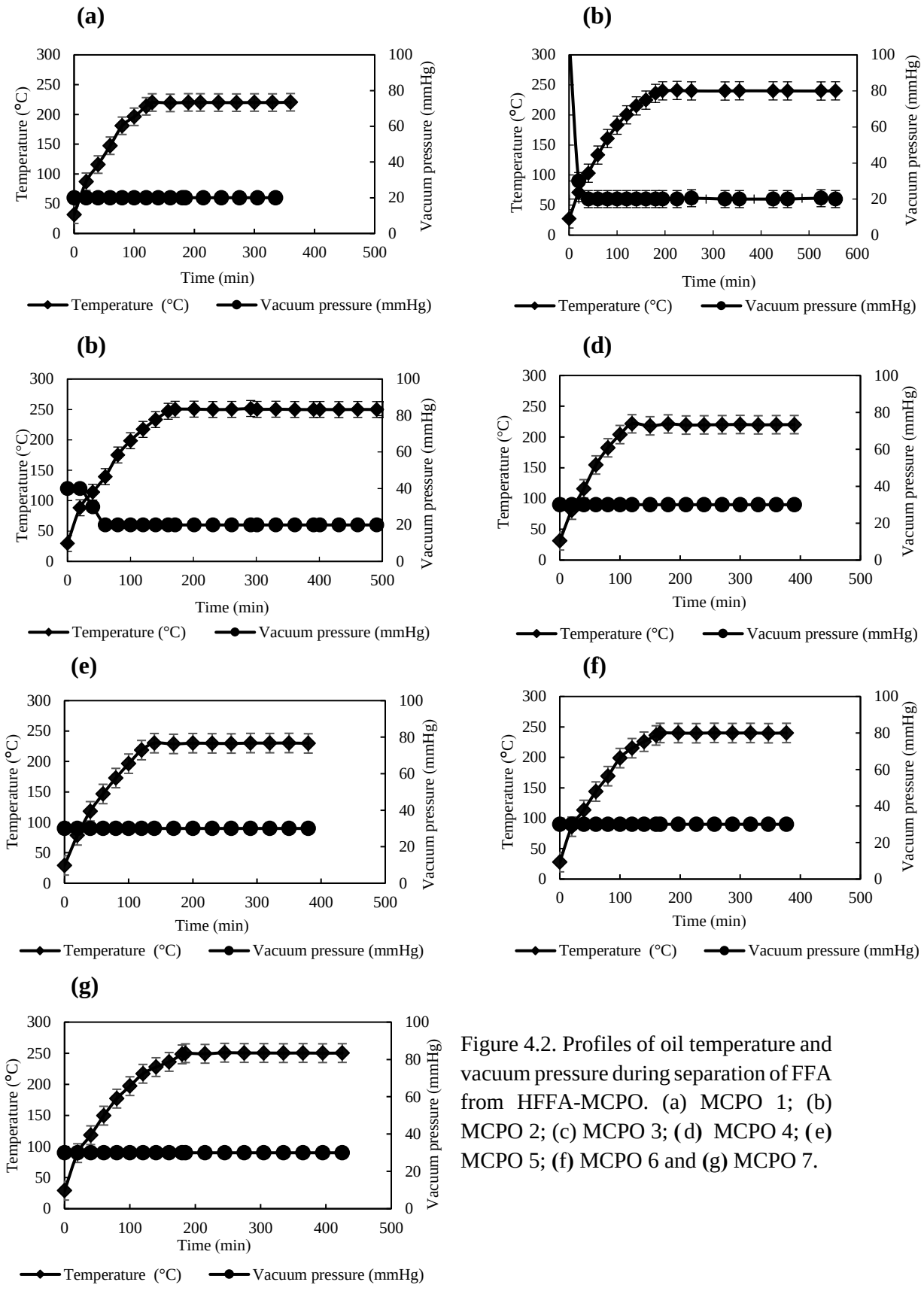


Figure 4.2. Profiles of oil temperature and vacuum pressure during separation of FFA from HFFA-MCPO. (a) MCPO 1; (b) MCPO 2; (c) MCPO 3; (d) MCPO 4; (e) MCPO 5; (f) MCPO 6 and (g) MCPO 7.

FFA content and acid values of HFFA-CPO and HFFA-MCPO before and after experiments corresponding to Figures 4.1 and 4.2 were shown in Table 4.3. Data of HFFA-MCPO from Table 4.3 at various temperatures and vacuum pressures were used to plot the relationships between evaporating pressure and percent reduction of FFA content, the results was shown in Figure 4.3. Different values of percentage reductions of FFA content may be due to the initial FFA content and distillation conditions. It can be compared that at vacuum pressures of 20 and 30 mmHg and the temperature of 220-240^{o}C, the percentage reduction of FFA reduced when pressure increased. However, at temperature of 250^{o}C, the percentage reduction of FFA content increased when pressure increased, which of opposite to the results at 220-240^{o} C. Extra experiments at 250^{o} C, and at though three vacuum pressures should be repeated in order to confirm the result. Nevertheless, the results of 250^{o} C at pressure 30 and 40 mmHg showed that the percentage reduction of FFA content were quite constant in the range of 40-50%, expressing the limitation of vaporization of the component in the oil at pressure higher than 30 mmHg. The natural fatty acids have sensitivity on heat (Lausberg *et al*., 2008), at the high temperature fatty acids in the molecule of TG are broken during heating. this process is called “thermal cracking” or “pyrolysis” of TG (Maher and Bressler, 2007., Seifi and Sadrameli, 2016).

Table 4.3. FFA contents and acid values of HFFA-CPO and HFFA-MCPO before and after experiments.

Batch	FFA contents (%)			Acid values (mg of NaOH/g)		
	Before	After	FFA reduction (%)	Before	After	Acid value reduction (%)
CPO 1	25.70 ± 0.31	14.34 ± 0.15	44.20	56.32 ± 0.31	31.43 ± 0.15	44.19
CPO 2	14.95 ± 0.13	10.61 ± 0.52	29.03	32.77 ± 0.13	23.24 ± 0.52	29.08
CPO 3	10.29 ± 0.24	5.22 ± 0.36	49.27	22.55 ± 0.24	11.44 ± 0.36	49.27
CPO 4	20.61 ± 0.16	10.43 ± 0.31	49.39	45.17 ± 0.16	22.86 ± 0.31	49.39
MCPO 1	12.57 ± 0.39	7.36 ± 1.28	41.45	27.51 ± 0.39	16.13 ± 1.28	41.37
MCPO 2	16.20 ± 0.27	12.11 ± 0.18	25.25	35.50 ± 0.27	26.53 ± 0.18	25.27
MCPO 3	10.36 ± 0.41	8.79 ± 0.06	15.15	22.71 ± 0.41	19.28 ± 0.06	15.10
MCPO 4	14.06 ± 0.08	10.80 ± 0.14	23.19	30.79 ± 0.08	23.66 ± 0.14	23.16
MCPO 5	14.19 ± 0.37	11.76 ± 0.19	17.12	31.08 ± 0.37	25.77 ± 0.19	17.08
MCPO 6	14.05 ± 0.46	11.49 ± 0.27	18.22	30.78 ± 0.46	25.17 ± 0.27	18.23
MCPO 7	19.57 ± 0.14	11.49 ± 0.27	41.29	42.86 ± 0.14	25.17 ± 0.27	41.27

Abbreviations: ±, standard deviation

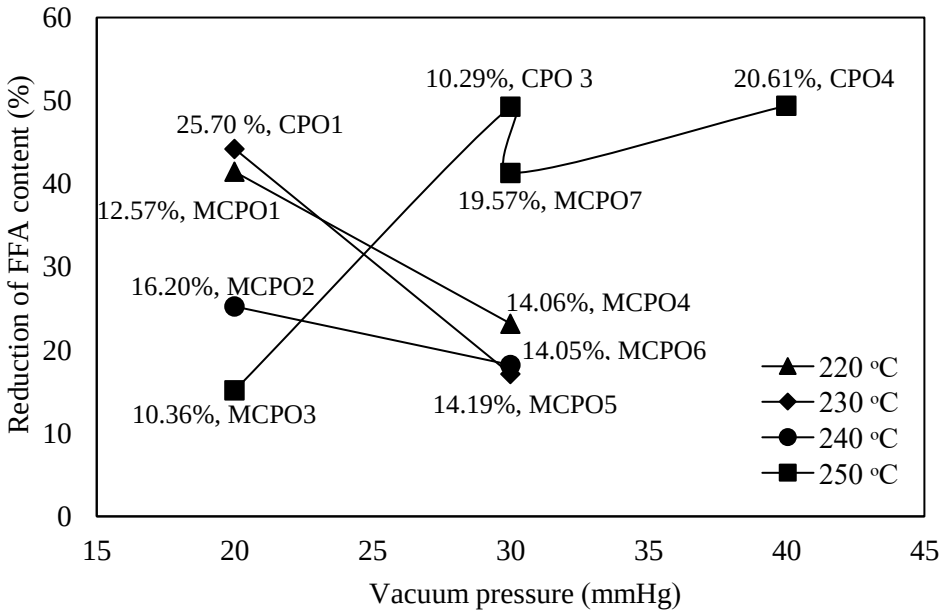


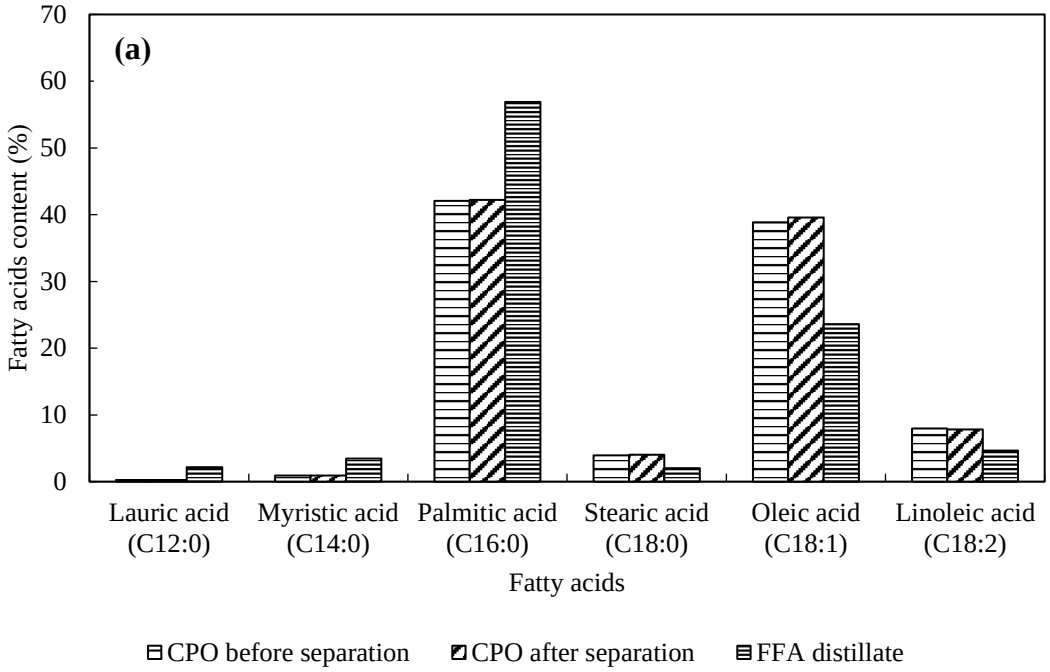
Figure 4.3 Relationships between evaporating pressure and percent reduction of FFA content (the percentage values shown in the graph are the initial values of %FFA of each batch).

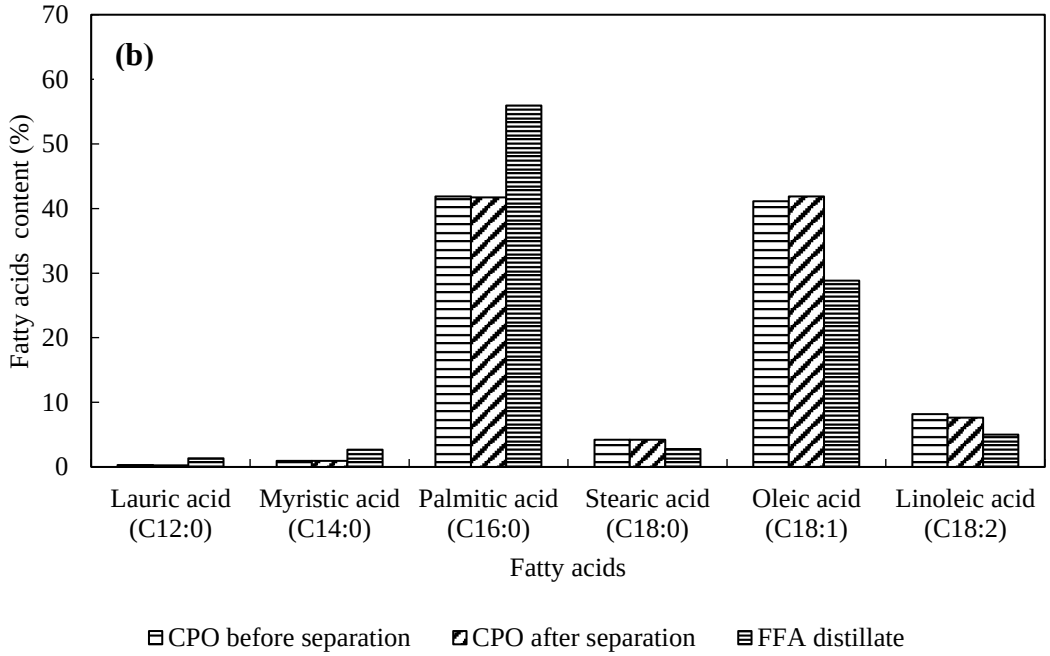
4.1.1 Fatty acid composition

The fatty acid compositions in the HFFA-CPO before and after experiment, and in the fatty acid distillates collected from the condenser were shown in Figure 4.4

For CPO1, the CPO before and after experiment mainly contained palmitic acid (44.25% and 44.08%), oleic acid (40.87% and 41.31%), linoleic acid (8.40% and 8.16%), stearic acid (4.15% and 4.20%), myristic acid (0.97% and 0.95%) and lauric acid (0.25% and 0.24%), respectively. For CPO2, the CPO before and after experiment mainly contained palmitic acid (42.80% and 42.74%), oleic acid (42.02% and 42.91%), linoleic acid (8.34% and 7.80%), stearic acid (4.29% and 4.29%), myristic acid (0.98% and 0.968%) and lauric acid (0.28% and 0.27%), respectively. For CPO3, the CPO before and after experiment mainly contained palmitic acid (41.80% and 44.25%), oleic acid (42.91% and 41.17%), linoleic acid (8.70% and 7.80%), stearic acid (4.28% and 4.51%), myristic acid (0.97% and 0.99%) and lauric acid (0.26% and 0.25%), respectively. The distillates obtained from batches CPO1-3 contained FFA content more than 90%. They were mainly composted of palmitic acid (60.28%, 57.42%, 58.12%), oleic acid (25.01%, 29.59%, 27.29%), linoleic acid (4.95%, 5.12%, 5.08%), stearic acid (2.14%, 2.86%, 2.72%), myristic acid (3.65%, 2.74%, 3.24%) and lauric acid (2.30%, 1.40%, 2.02%), respectively.

It can be seen in the FFA distillate that the content of palmitic acid is higher than that of oleic acid. This is because of the boiling point of palmitic acid (212 ^{o}C at 10 mmHg) is lower than that of oleic acid (223 ^{o}C at 10 mmHg). Similar to the research of Bono *et al*., (2010), which reported that fatty acid composition in CPO mainly contained palmitic acid (40.93%), oleic acid (41.51%), linoleic acid (11.64%) and stearic acid (4.18%) respectively. Cermak *et al.*, (2012) reported that fatty acid composition in CPO mainly contained palmitic acid (44.00%), oleic acid (39.10%), linoleic acid (10.10%) and stearic acid (4.5%), respectively. Furthermore, Lamaisri *et al*., (2015) reported that fatty acid composition in CPO mainly contained palmitic acid (44%), oleic acid (37.8%), linoleic acid (9.3%) and stearic acid (7.1%), respectively.


(a)
70
60
50
40
30
20
10
0
Fatty acids content (%)
Lauric acid (C12:0)
Myristic acid (C14:0)
Palmitic acid (C16:0)
Stearic acid (C18:0)
Oleic acid (C18:1)
Linoleic acid (C18:2)
Fatty acids
CPO before separation
CPO after separation
FFA distillate


(b)
70
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50
40
30
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10
0
Fatty acids content (%)
Lauric acid (C12:0)
Myristic acid (C14:0)
Palmitic acid (C16:0)
Stearic acid (C18:0)
Oleic acid (C18:1)
Linoleic acid (C18:2)
Fatty acids
CPO before separation
CPO after separation
FFA distillate

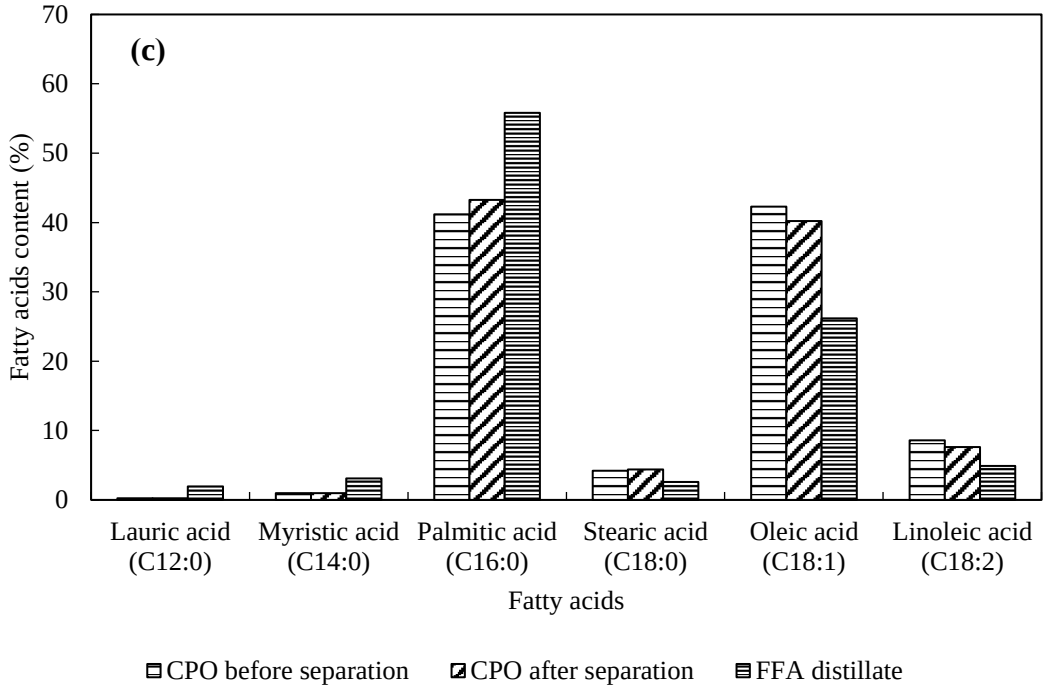
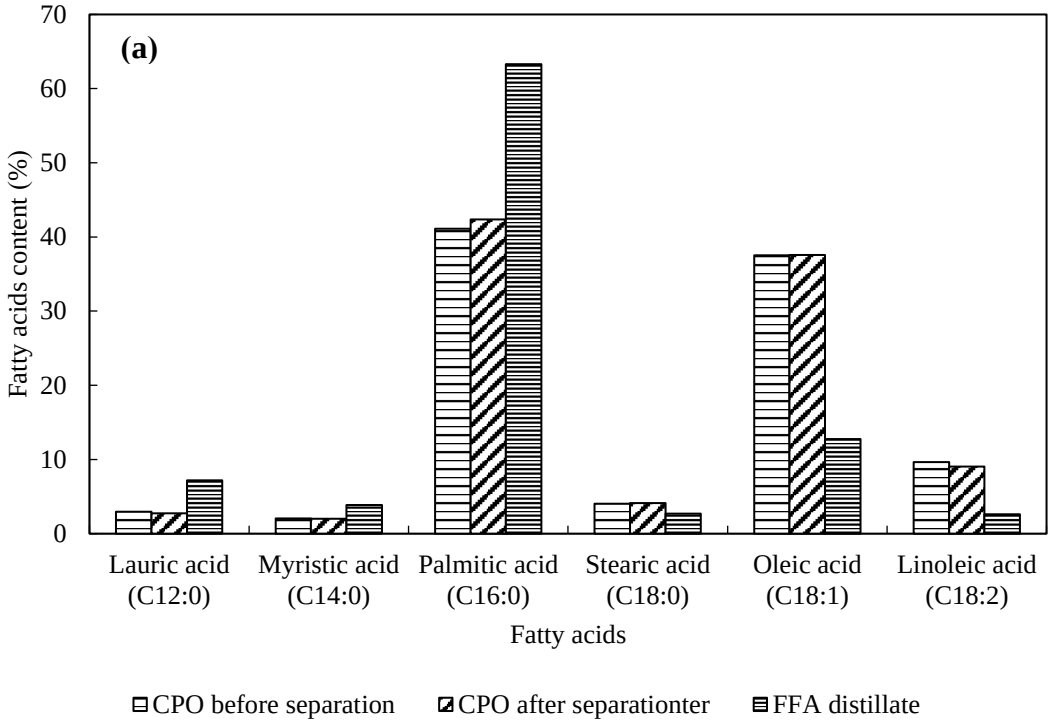


Figure 4.4 The fatty acid compositions in the HFFA-CPO before and after experiment, and in the fatty acid distillates. (a) CPO1. (b) CPO2 and (c) CPO3.

The fatty acid compositions in the HFFA-MCPO before and after experiment, and in the distillates collected from the condenser were shown in Figure 4.5. For MCPO 1, the MCPO before and after experiment mainly contained palmitic acid (37.80% and 40.67%), oleic acid (40.75% and 39.00%), linoleic acid (10.45% and 9.76%), stearic acid (4.16% and 4.20%), myristic acid (2.05% and 2.02%) and lauric acid (3.05% and 2.84%), respectively. For MCPO2, the MCPO before and after experiment mainly contained palmitic acid (38.99% and 41.27%), oleic acid (40.79% and 39.68%), linoleic acid (10.28% and 9.49%), stearic acid (4.32% and 4.31%), myristic acid (1.77% and 1.78%) and lauric acid (2.31% and 2.25%), respectively. For MCPO3, the MCPO before and after experiment mainly contained palmitic acid (40.40% and 41.10%), oleic acid (39.73% and 40.15%), linoleic acid (9.60% and 8.86%), stearic acid (4.43% and 4.45%), myristic acid (1.80% and 1.80%) and lauric acid (2.32% and 2.32%), respectively. For MCPO4, the MCPO before and after experiment mainly contained

palmitic acid (41.53% and 42.28%), oleic acid (37.95% and 38.14%), linoleic acid (9.74% and 9.29%), stearic acid (4.07% and 4.20%), myristic acid (2.07% and 1.99%) and lauric acid (2.99% and 2.69%), respectively. For MCPO5, the MCPO before and after experiment mainly contained palmitic acid (41.53% and 42.38%), oleic acid (37.95% and 38.02%), linoleic acid (9.74% and 9.35%), stearic acid (4.07% and 4.21%), myristic acid (2.07% and 1.99%) and lauric acid (2.99% and 2.66%), respectively. For MCPO6, the MCPO before and after experiment mainly contained palmitic acid (41.53% and 42.73%), oleic acid (37.95% and 37.89%), linoleic acid (9.72% and 9.13%), stearic acid (4.07% and 4.17%), myristic acid (2.07% and 2.01%) and lauric acid (2.99% and 2.76%), respectively. For MCPO7, the MCPO before and after experiment mainly contained palmitic acid (41.53% and 39.95%), oleic acid (37.95% and 40.07%), linoleic acid (9.42% and 9.52%), stearic acid (4.07% and 4.05%), myristic acid (2.07% and 2.00%) and lauric acid (2.99% and 2.92%), respectively. The distillates obtained from batches MCPO1-**7** contained FFA content more than 90%. They were mainly composted of palmitic acid (57.38%, 59.15%, 59.35%, 56.27%, 69.45%, 65.97%, 50.83%), oleic acid (13.12%, 23.45%, 24.55%, 22.95%, 14.14%, 13.32%, 20.68%), linoleic acid (2.06%, 5.12%, 5.07%, 5.77%, 1.29%, 2.73%, 4.91%), stearic acid (2.70%, 2.47%, 2.66%, 2.40%, 3.34%, 2.83%, 2.37%), myristic acid (2.78%, 3.74%, 3.67%, 3.74%, 4.53%, 4.01%, 4.62%) and lauric acid (5.10%, 4.23%, 3.62%, 6.22%, 3.99%, 7.51%, 11.84%), respectively. It can be seen in the FFA content of FFA distillate that the palmitic acid is higher than oleic acid. This is because of the boiling point of palmitic acid (212 ^{o}C at 10 mmHg) is lower than that of oleic acid (223 ^{o}C at 10 mmHg), which was similar to fatty acid compositions in the HFFA-CPO. While the lauric acid and myristic acid of fatty acid compositions in the HFFA-MCPO are higher than the lauric acid and myristic acid of fatty acid compositions in the HFFA-CPO. This is because of the difference between the two CPOs. HFFA-MCPO had the lauric acid and myristic acid more than HFFA-CPO, and the lauric acid is higher than myristic acid due to the boiling point of lauric acid (173 ^{o}C at 10 mmHg) is lower than that of myristic acid (193 ^{o}C at 10 mmHg).


(a)
Fatty acids content (%)
70
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10
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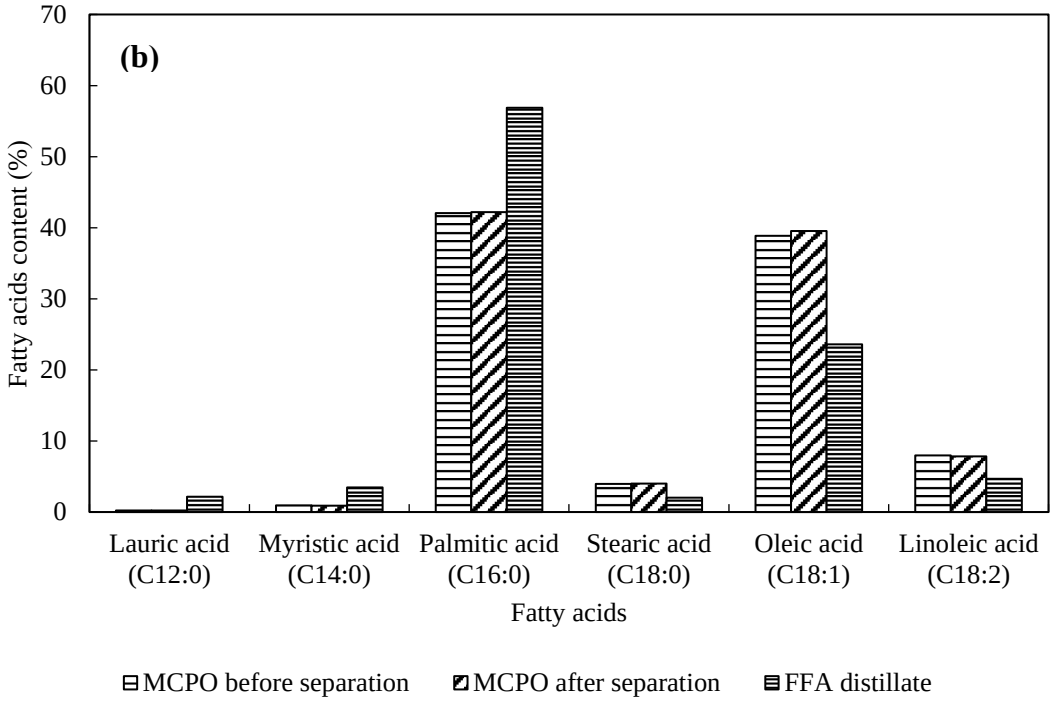
Lauric acid (C12:0)
Myristic acid (C14:0)
Palmitic acid (C16:0)
Stearic acid (C18:0)
Oleic acid (C18:1)
Linoleic acid (C18:2)
Fatty acids
CPO before separation
CPO after separationter
FFA distillate


(b)
Fatty acids content (%)
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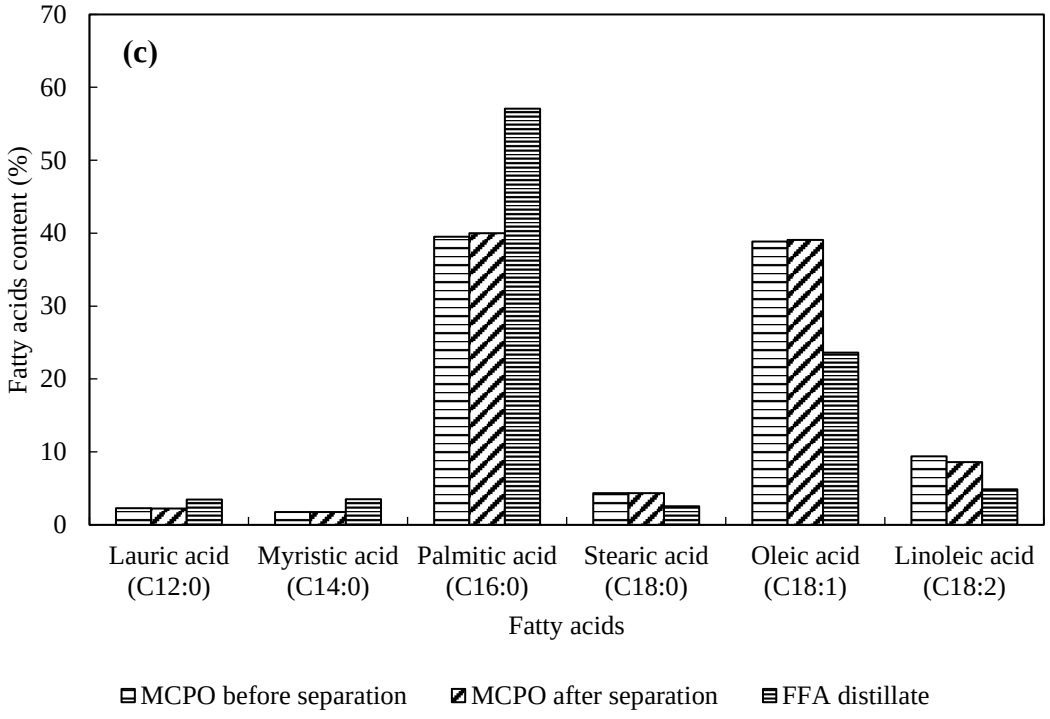
Lauric acid (C12:0)
Myristic acid (C14:0)
Palmitic acid (C16:0)
Stearic acid (C18:0)
Oleic acid (C18:1)
Linoleic acid (C18:2)
Fatty acids
MCPO before separation
MCPO after separation
FFA distillate


(c)
Fatty acids content (%)
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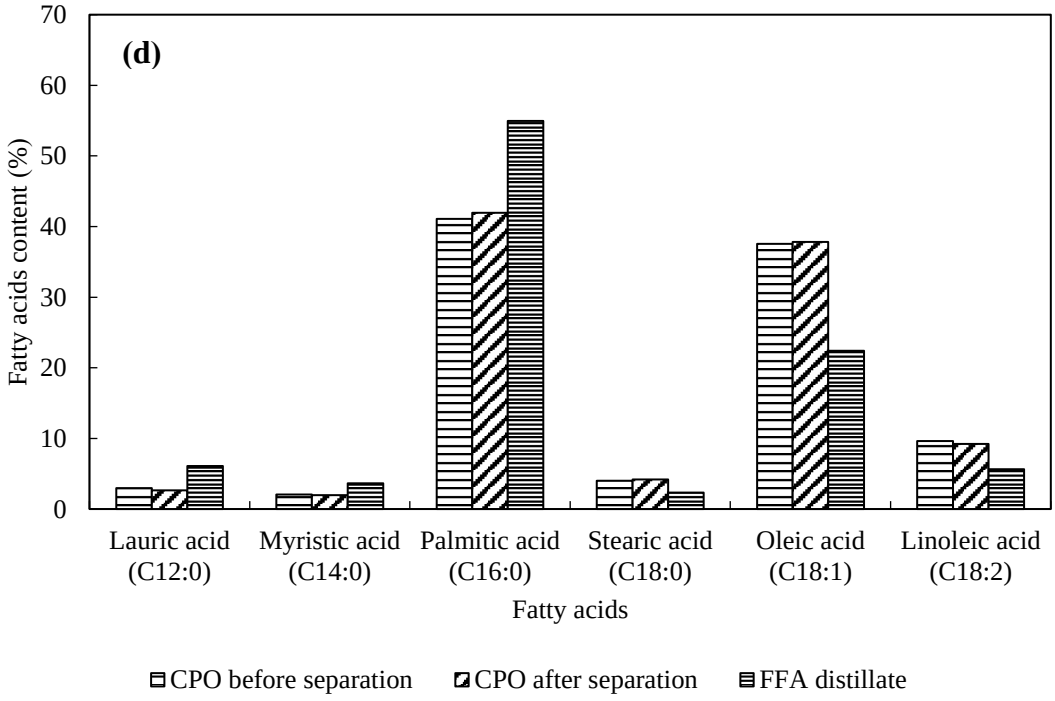
Lauric acid (C12:0)
Myristic acid (C14:0)
Palmitic acid (C16:0)
Stearic acid (C18:0)
Oleic acid (C18:1)
Linoleic acid (C18:2)
Fatty acids
MCPO before separation
MCPO after separation
FFA distillate


(d)
Fatty acids content (%)
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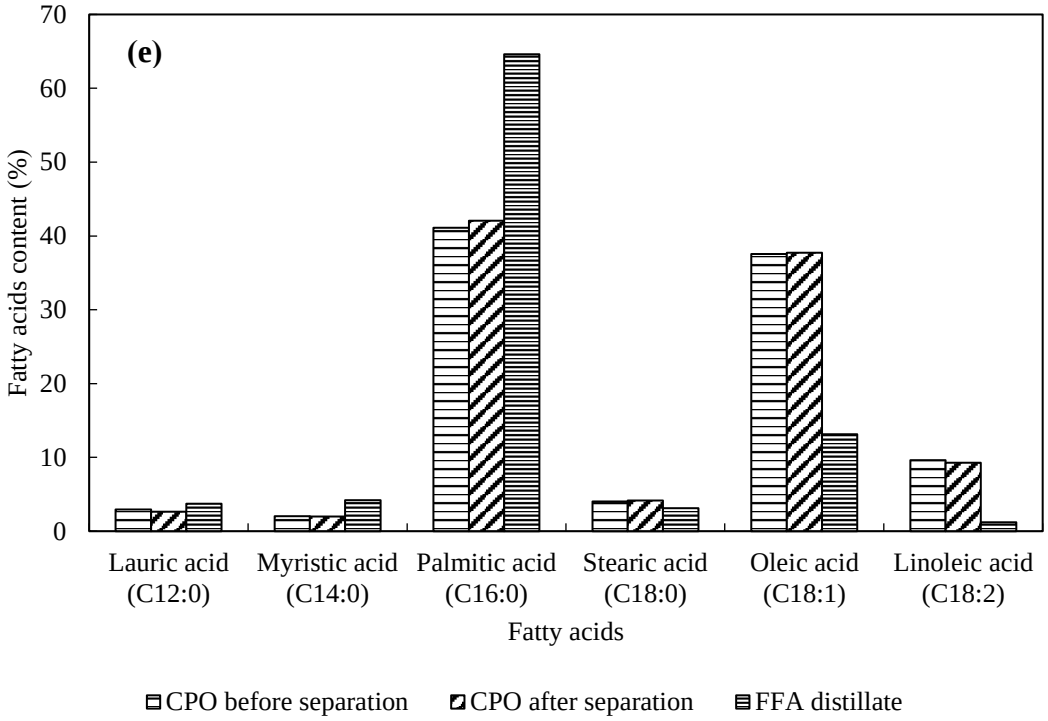
0
Lauric acid (C12:0)
Myristic acid (C14:0)
Palmitic acid (C16:0)
Stearic acid (C18:0)
Oleic acid (C18:1)
Linoleic acid (C18:2)
Fatty acids
CPO before separation
CPO after separation
FFA distillate


(e)
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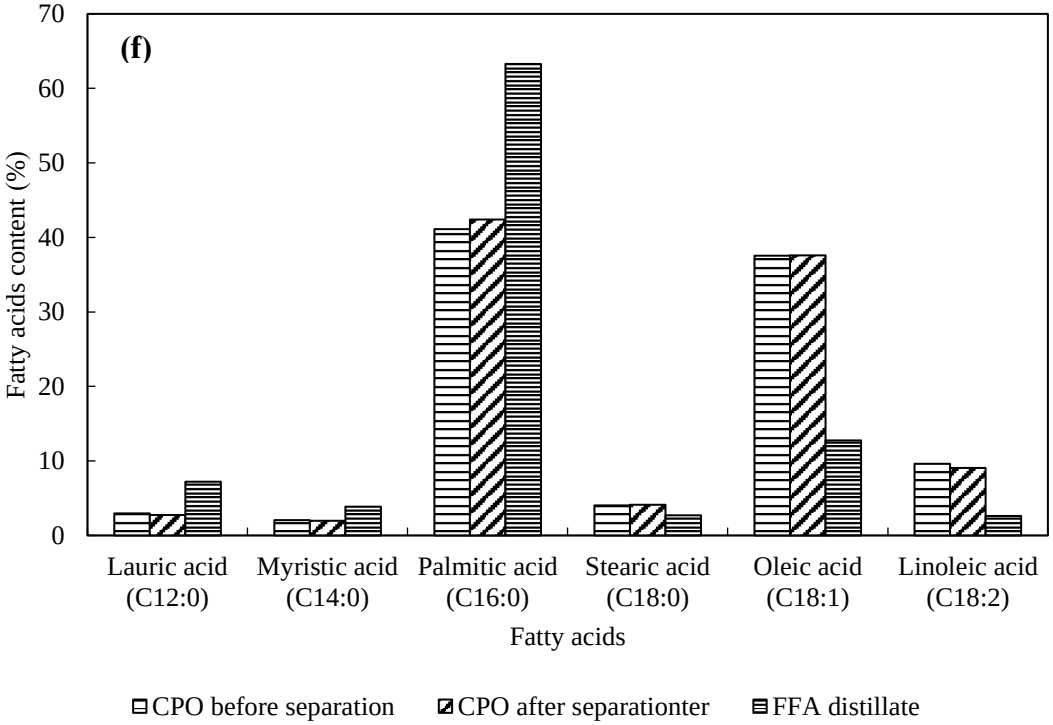
Fatty acids content (%)
Lauric acid (C12:0)
Myristic acid (C14:0)
Palmitic acid (C16:0)
Stearic acid (C18:0)
Oleic acid (C18:1)
Linoleic acid (C18:2)
Fatty acids
CPO before separation
CPO after separation
FFA distillate


(f)
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Fatty acids content (%)
Lauric acid (C12:0)
Myristic acid (C14:0)
Palmitic acid (C16:0)
Stearic acid (C18:0)
Oleic acid (C18:1)
Linoleic acid (C18:2)
Fatty acids
CPO before separation
CPO after separationter
FFA distillate

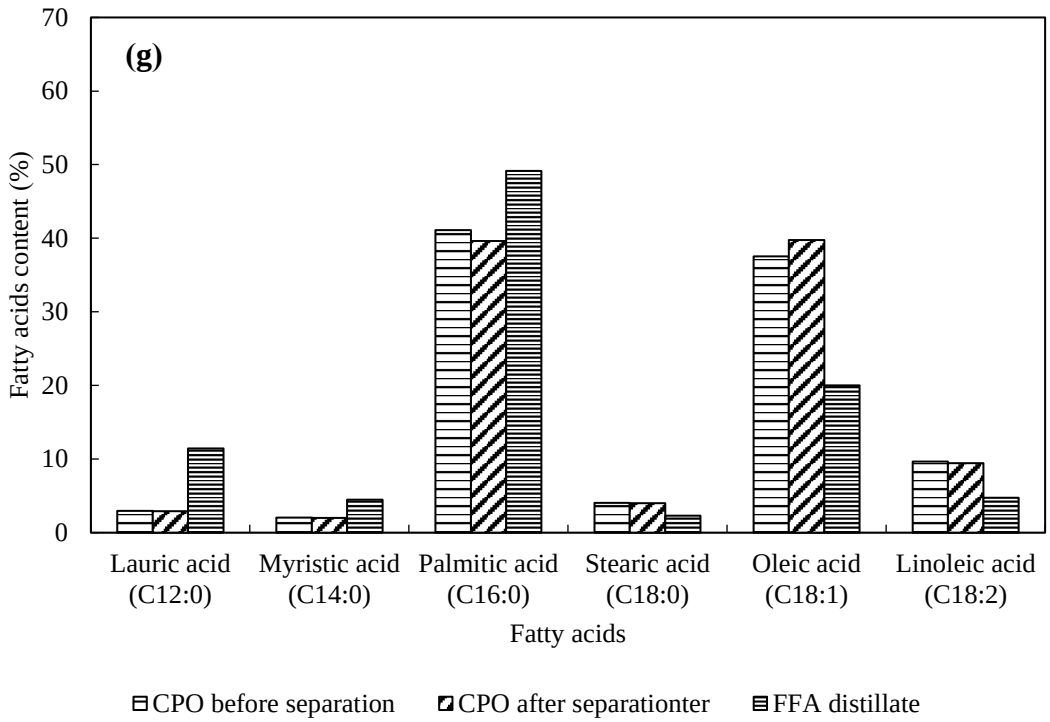

Figure 4.5 The fatty acid compositions in the HFFA-MCPO before and after experiment, and in the fatty acid distillates. (a) MCPO1, (b) MCPO2, (c) MCPO3, (d) MCPO4, (e) MCPO5, (f) MCPO6 and (g) MCPO7.

4.1.2 Physical properties of CPO

Tables 4.4 and 4.5 showed the physical properties and compositions of CPO and MCPO before and after separation of each batch. Before separation, both of these oils were different in terms of FFA content and acids value. However, there has similar physical properties, just slightly different, in moisture content, iodine value, peroxide value and saponification value. After separation, the FFA content reduced very low from initial. This was due to at the high temperature, fatty acids were formed, the heat caused thermal cracking (pyrolysis) of triglycerides in CPO and fatty acids, resulting to the bound cleavage took place along the triglyceride molecules (Figure 4.6 and 4.7). The pyrolysis of fatty acids proceeds through the breaking of C/C and C/O bonds (Asmaning *et al*., 2014), which is accordant with the reports of Molekul (2016) and Japir *et al.* (2017), saying that the peak of C/O of carboxylic acid disappeared after separations. The acid value varied with the FFA content.

Table 4.4. Physical properties and composition of crude palm oil (CPO) before and after separation of each batch.

Property	Batch							
	CPO1		CPO2		CPO3		CPO4	
	before	after	before	after	before	after	before	after
Weight of oil (kg)	25.50	24.85	25.45	24.68	24.92	24.38	25.61	24.68
Free fatty acid content (wt. %)	25.70±0.31	14.34±0.15	14.95±0.13	10.60±0.52	10.29±0.24	5.22±0.36	20.61±0.16	10.43±0.31
Moisture content (wt. %)	0.20±0.01	0.13±0.005	3.57±0.57	0.28±0.09	0.18±0.02	0.11±0.05	0.13±0.04	0.05±0.01
Acid value (mg of NaOH/g)	56.32±0.52	31.43±3.59	32.77±0.24	23.24±0.36	22.55±0.16	11.44±0.31	45.17±0.28	22.86±0.18
Iodine value (g I_2/100g)	51.37±0.43	52.31±0.51	52.11±0.29	51.58±0.13	53.19±0.26	52.84±0.57	53.87±0.45	52.61±0.38
Peroxide value (mg of KOH/g)	9.73±0.02	10.48±0.10	9.98±0.02	10.18±0.10	9.47±0.12	9.98±0.20	9.96±0.02	11.63±0.02
Saponification value (mg of KOH/g)	198.17±0.39	200.15±0.28	199.30±0.52	195.64±0.35	198.54±0.50	201.92±0.37	198.67±0.52	199.48±0.45

Abbreviations: ±, standard deviations

Table 4.5. Physical properties and composition of mixed crude palm oil (MCPO) before and after separation of each batch.

Property	Batch							
	MCPO1		MCPO2		MCPO3		MCPO4	
	before	after	before	after	before	after	before	after
Weight of oil (kg)	25.45	24.95	26.70	25.85	28.50	27.81	24.65	24.53
Free fatty acid content (wt. %)	12.57 ± 0.39	7.36 ± 1.28	16.20 ± 0.27	12.11 ± 0.18	10.36 ± 0.41	9.79 ± 0.06	14.06 ± 0.08	10.80 ± 0.14
Moisture content (wt. %)	0.11 ± 0.01	0.08 ± 0.01	0.12 ± 0.02	0.09 ± 0.01	0.25 ± 0.12	0.12 ± 0.01	0.10 ± 0.01	0.08 ± 0.01
Acid value (mg of NaOH /g)	27.51 ± 0.39	16.13 ± 1.28	35.50 ± 0.27	26.53 ± 0.18	22.71 ± 0.41	19.28 ± 0.06	30.79 ± 0.08	23.66 ± 0.14
Iodine value (g I_2/100g)	57.31±0.43	55.32 ±0.51	53.71±0.39	51.38±0.33	55.14±0.23	57.14±0.17	53.87±0.45	54.15±0.28
Peroxide value (mg of KOH/g)	9.75±0.01	10.11±0.13	9.82±0.02	11.29±0.13	9.54±0.20	10.97±0.13	9.59±0.10	11.18±0.20
Saponification value (mg of KOH/g)	198.17±0.39	200.15±0.28	189.35±0.25	190.02±0.31	189.32±0.33	191.10±0.42	198.87±0.32	199.78±0.35
	MCPO5		MCPO6		MCPO7			
	before	after	before	after	before	after		
Weight of oil (kg)	25.47	25.19	27.38	27.20	25.22	24.95		
Free fatty acid content (wt. %)	14.19 ± 0.37	11.76 ± 0.19	14.05 ± 0.46	11.49 ± 0.27	19.57 ± 0.14	11.49 ± 0.27		
Moisture content (wt. %)	0.11 ± 0.01	0.07 ± 0.02	0.12 ± 0.01	0.07 ± 0.02	0.10 ± 0.01	0.08 ± 0.01		
Acid value (mg of NaOH /g)	31.08 ± 0.37	25.77 ± 0.19	30.78 ± 0.46	25.17 ± 0.27	42.86 ± 0.14	25.17 ± 0.27		
Iodine value (g I_2/100g)	53.37±0.13	56.12±0.11	54.51±0.19	56.53±0.19	54.16±0.29	55.64±0.17		
Peroxide value (mg of KOH/g)	9.47±0.21	11.02±0.02	10.20±0.20	10.95±0.10	10.17±0.01	11.76±0.02		
Saponification value (mg of KOH/g)	201.13±0.19	200.16±0.18	199.87±0.52	197.84±0.25	198.57±0.15	201.82±0.31		

Abbreviations: ±, standard deviation

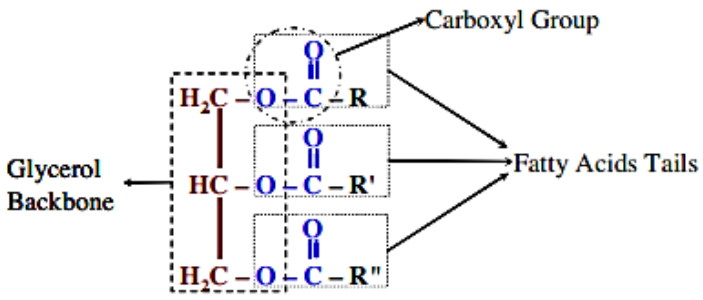

Figure 4.6 Molecule structure of triglyceride.
Source: (Seifi and Sadrameli, 2016)

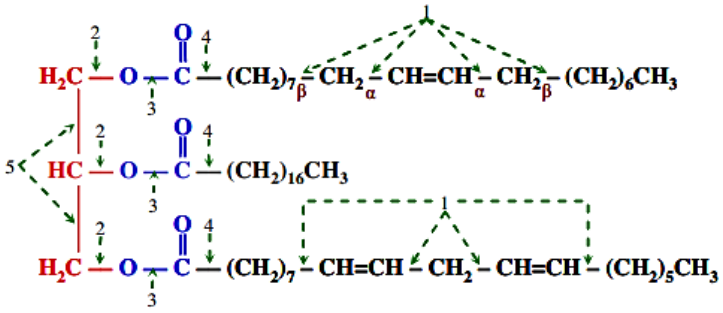

Figure 4.7 Probable position of bound cleavage along triglyceride during thermal cracking.
Source: (Seifi and Sadrameli, 2016)

The iodine value is a measure of the total number of unsaturated double bonds present in an oil molecule, whereas the peroxide value is a measure of rancidity due to oxidation of oil or fat, which can be analyzed from the amount of peroxide caused by the reaction of oxygen at the double bond position of unsaturated fatty acids, including substances created from free radicals of fatty acids as well. The differences in iodine values of the oil are indicative of the increased rate of oxidation. Both iodine and peroxide values indicate the deterioration in the oil. The oxidation reaction can always occur continuously when the oil is exposed to oxygen in the air. At the same time, heat and light also influence the oxidation reaction (Man and Jaswir, 2000). Saponification value is expressed by potassium hydroxide in mg required to saponify one gram of fat or oil. It is a measure of the average molecular weight (or chain length) of all the fatty acids present in molecule of fat or oil, which depends on the kind of fatty acid contained in the fat or oil.

The iodine values of the CPO and MCPO before and after separation were in the ranges 51-54, 51-53 g I_2/100g and 53-58, 51-58 g I_2/100g, respectively. Peroxide values of the CPO and MCPO before and after separation were in the ranges 9-10, 10-11 mg of KOH/g and 9-10, 10-12 mg of KOH/g, respectively. In addition, the saponification value of the CPO and MCPO before and after separation were ranged 198-200, 195-201 mg of KOH/g and 197-202 mg of KOH/g, respectively. The results showed that the iodine and peroxide values increased after the separation due to oxidation by heat. However, these three properties still follow the Thailand's standard of CPO as shown in Table 4.6.

Table 4.6. Thailand's standard of CPO.

Parameter	Value
Free fatty acid content (%)	5 Max.
Moisture content (%)	0.5 Max.
Acid value (mg of NaOH /g)	10 Max.
Iodine value (g I_2/100g)	45-60
Peroxide value (mg of KOH/g)	10 Max.
Saponification value (mg of KOH/g)	190-209

Source: Standard for edible palm oil (1992)

In addition, from Table 4.4 and 4.5, small residual moisture content after separation still remained in the oil. This may be because the carboxyl groups in triglyceride was disappeared in the form of water, CO_2 and other compounds (Seifi and Sadrameli, 2016). Thus, continuous heating at high temperatures for a long time will cause undergoes several chemical and physical changes, depending on the reaction conditions. Various reactions such as oxidation, polymerization and hydrolysis accumulated with prolonged heating time (Hein *et al*., 1998).

Table 4.7. Color of palm oil before and after separation of each batch.

Batch	L*		a*		b*	
	before	after	before	after	before	after
CPO1	43.37 ± 0.12	45.78 ± 0.22	16.85 ± 0.34	17.54 ± 0.20	60.87 ± 0.36	60.35 ± 0.17
CPO2	50.15 ± 0.10	56.34 ± 0.02	7.03 ± 0.16	7.82 ± 0.13	43.84 ± 0.29	43.75 ± 0.21
CPO3	64.08 ± 0.21	54.30 ± 0.14	2.95 ± 0.25	5.91 ± 0.32	60.03 ± 0.15	42.32 ± 0.35
CPO4	42.45 ± 0.09	28.48 ± 0.11	21.17 ± 0.33	26.61 ± 0.18	69.32 ± 0.23	41.28 ± 0.07
MCPO1	45.68 ± 0.05	47.10 ± 0.31	5.87 ± 0.16	6.13 ± 0.09	70.01 ± 0.05	46.74 ± 0.28
MCPO2	56.71 ± 0.12	56.99 ± 0.14	14.21 ± 0.13	16.49 ± 0.11	73.28 ± 0.36	69.55 ± 0.31
MCPO3	59.11 ± 0.35	62.34 ± 0.37	9.35 ± 0.21	11.36 ± 0.21	69.51 ± 0.22	65.09 ± 0.04
MCPO4	61.32 ± 0.28	62.35 ± 0.18	11.32 ± 0.09	15.39 ± 0.16	55.88 ± 0.09	46.37 ± 0.14
MCPO5	57.35 ± 0.16	59.72 ± 0.21	8.45 ± 0.10	10.53 ± 0.12	62.65 ± 0.07	59.05 ± 0.27
MCPO6	49.28 ± 0.13	50.79 ± 0.14	17.30 ± 0.21	17.74 ± 0.08	65.95 ± 0.14	56.91 ± 0.05
MCPO7	49.95 ± 0.21	50.94 ± 0.11	11.95 ± 0.17	13.12 ± 0.11	73.17 ± 0.12	55.34 ± 0.13

Abbreviations: ±, standard deviation

When L* is the brightness value

a* is the red value

b* is the yellow value

The main pigment substances in palm oil are carotenoids, which are yellow and red-oranges. The color of CPO and MCPO before and after separation of each batch were shown in Table 4.7. As explained in Figure 4.10, L* showed the brightness of product. The positive and negative value of a* indicates the red and green color, respectively. Finally, the positive and negative value of b* indicates the yellow and blue color, respectively.

The use of high separation temperature affected the color value of palm oil after separation. Which showed the value of a* increased (more red), whereas the value of b* decrease (more blue), corresponding to the final a darker color. Within experimental time about 6-7 h, the value of L* increase. However, CPO4, which used 15 h, showed L* decreased. It's was found that long time of high separation temperature, i.e. CPO4 which the time was more than 7h, cause the oil became more dark (Figure 4.11).

The change in color of the oil is because the carotenoids in palm oil decomposes during the process by thermal destruction. (Gibon *et al*., 2007). Consistent with the research of Bonnie and Choo (1999) which reported that thermal process caused carotenoids to produce isomerization and oxidation reaction, which introduce carotenoid molecules to decay. In addition, Rodriguez-Amaya (1999) has reported that carotenoids underwent a thermal process will changes the structure from trans to cis, resulting in loss of color and ability to convert carotenoids into vitamin A. However, that is not the only reason that causes color changes. Another reason is phospholipids, which are substances found in gum and contaminated in crude palm oil, also cause the oil to become dark color when heated (Promwungkwa *et al*., 2003).

The black color of oil comes from the decomposition of glycerol (glycerol has a boiling point of 290 ^{o}C at atmospheric pressure), which forms black acrolein (Figure 4.8). At temperatures greater than 200 ^{o}C (Nong-nga Kongsit, 2014).

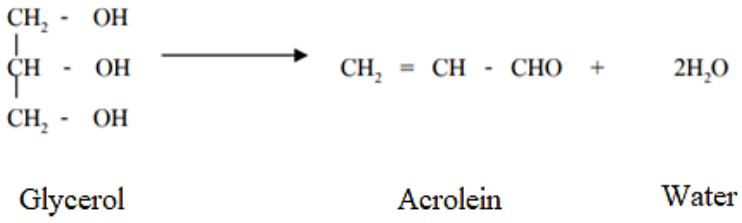

Figure 4.8 Glycerol disintegration reaction
Source: Nong-nga Kongsit (2014)

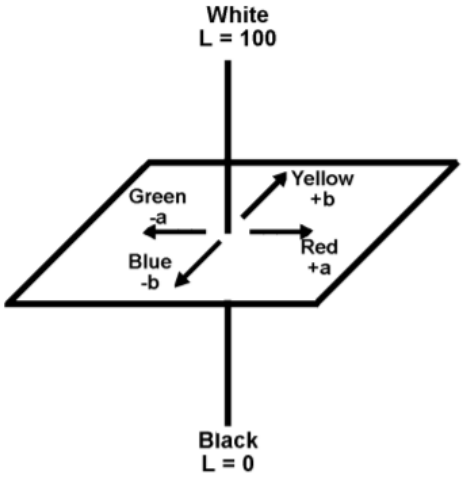

Figure 4.9 Diagram of the Hunter lab color space shows the color values in the system L * a * b *. Positive L is white, negative L is black, positive a is red, negative a is green, positive b is yellow, negative b is blue.
Source: Hunter Associates Laboratory (1996)

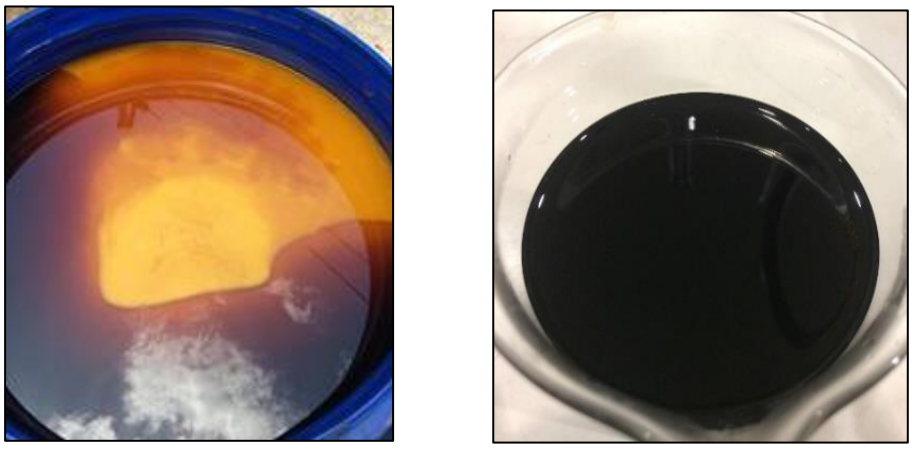

Figure 4.10 Color of the oil before (left) and after (right) separation of CPO4.

4.1.3 Recovery of FFA distillated

Table 4.8 showed evaporation efficiency of evaporator. The mass loss was the difference between the initial and final mass. The evaporation efficiency was determined from the ratio of the loss to the initial mass. The mass of solid FFA distillated trapped by condenser, presume may be less than the loss mass, has not been measure. However, part of it was collected manually by scraping. The manually scraped efficiency was calculated from the ratio of the mass of solid FFA distillated trapped by condenser to the loss mass of the evaporator. Table 4.8 show that the evaporation efficiency of the evaporator was in the range of 0.49-3.63%. Whereas manually scraped efficiency of the condenser was in the range of 0.8-26% of the lost mass. More than 80 % (wt.) of vapors cannot be harvested, some were loss to the surrounding air, and some were catch to the condenser. The evaporation efficiency was too small this may be because (i) the limitation of top surface of the oil in the cylindrical evaporator, (ii) the evaporation (vacuum)pressure was not reach the desired value (<10mmHg).

There would be note that this work used two sized of vacuum pumps separately. The batches CPO1, CPO2, MCPO2 and MCPO3 use a 1-hp vacuum pump, whereas the remaining batches use a 3-hp vacuum pump. The same heater of 3.35 hp (2500W) and the same water pump of 0.5 hp were used for all batches. There for, difference power consumptions mainly depending on the vacuum pump used. The average time for CPO1-3 were 7.2 h (neglecting CPO4) and 6.62 h for MCPO1-7. The times used for heating the oil were different, depending on the setting temperature.

Table 4.8. Recovery efficiency and energy consumption of all batches of experiment.

Batch	Initial FFA Content (%)	Separation time (h)	Mass (kg)			Evaporation efficiency (%)	FFA distillates manually scraped from condenser (kg)	Manually scraped efficiency (%)	Energy consumption (kW-h)
			Initial	Final	Lost				
CPO1	25.70 ± 0.31	7.5	25.50	24.85	0.65	2.55	0.054	8.31	27.15
CPO2	14.95 ± 0.13	6.8	25.45	24.68	0.77	3.01	0.085	11.11	24.62
CPO3	10.29 ± 0.24	7.3	24.92	24.38	0.54	2.16	0.103	19.13	26.45
CPO4	20.61 ± 0.16	15.2	25.61	24.68	0.93	3.63	0.215	23.21	77.32
MCPO1	12.57 ± 0.39	6.0	25.45	24.95	0.50	1.96	0.004	0.80	30.42
MCPO2	16.20 ± 0.27	7.0	26.70	25.85	0.85	3.18	0.018	2.13	25.34
MCPO3	10.36 ± 0.41	6.7	28.50	27.81	0.69	2.42	0.035	5.00	24.15
MCPO4	14.06 ± 0.08	6.5	24.65	24.53	0.12	0.49	0.031	26.49	32.95
MCPO5	14.19 ± 0.37	6.3	25.47	25.19	0.28	1.10	0.016	5.86	32.11
MCPO6	14.05 ± 0.46	6.7	27.38	27.20	0.18	0.66	0.012	6.39	34.39
MCPO7	19.57 ± 0.14	7.1	25.22	24.95	0.27	1.07	0.011	4.07	35.91

Abbreviations: ±, standard deviation

4.2 Production of glyceride from FFA distillate using lipase as a catalyst

4.2.1 Screening of lipase producing bacteria.

The screening microbial lipase production from microorganism list of the basic microbiology laboratory, Department of Industrial Biotechnology, Faculty of Agro-Industry. Focus on the bacteria because microbial lipase from bacteria can grow faster, easier to cultivate and easier to harvested than fungi and yeast. From the microorganism list, found that there are three strain (such as *Bacillus subtilis*, *Bacillus coagulans* and *Psuedomonas* sp. that can lipase production.

The results showed that the growth rate of microbial lipase in NB, *Bacillus subtilis* had the best growth (Figure 4.11a). And when all three microbial lipase cultures were cultured in medium with FFA. Found that the rate of growth is similar (Figure 4.11b). After that, measuring lipase activity showed that the *Bacillus subtilis* has the highest enzyme activity, followed by *Bacillus coagulans* and *Psuedomonas* sp., respectively. After 15 hours, the enzyme activity decreased. And all three strain of microbial lipase have very low enzyme activity (< 0.1 U/ml) (Figure 4.12). This may be because the microbial can be used directly, so it does not stimulate the production of enzymes or inhibit by free fatty acid to produce less and have low enzyme activity. Which, consistent with the research of Dobrev *et al*., (2015) found that the use of free fatty acid (free stearic acid) with a high concentration more than 6.6% inhibits the synthesis of enzymes (< 0.25 U/ml). Therefore, changed to use commercial enzymes in next experiment.

4.2.1.1 Growth curve of lipase producing bacteria

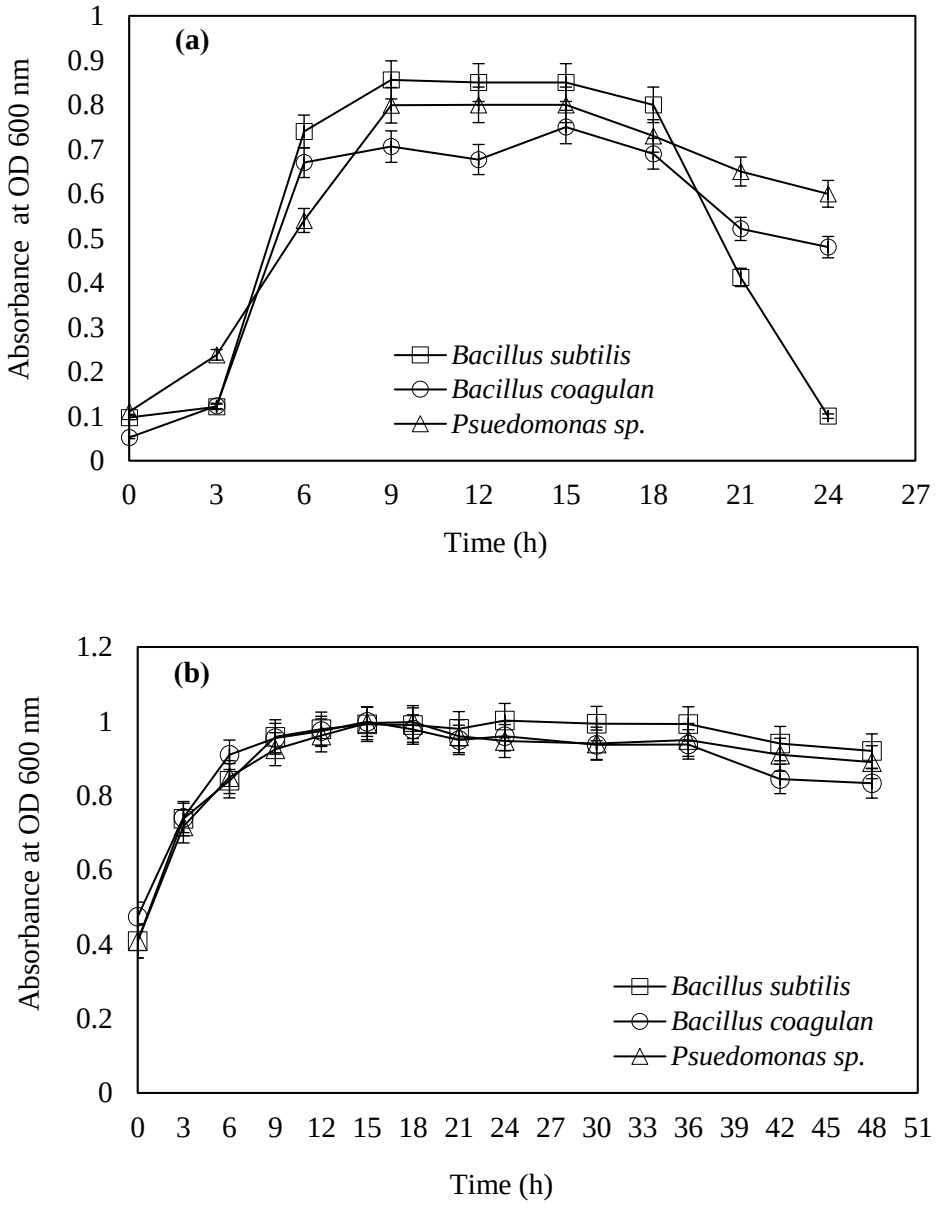

Figure 4.11 Growth curve of *Bacillus subtilis*, *Bacillus coagulans and Psuedomonas* sp. incubated at 37 °C for 24 h at 150 rpm. **(**a**)** cultivation in nutrient broth **(**NB**)** and **(**b**)** cultivation in medium with 10 % FFA.

4.2.1.2 Enzyme activity of lipase producing bacteria.

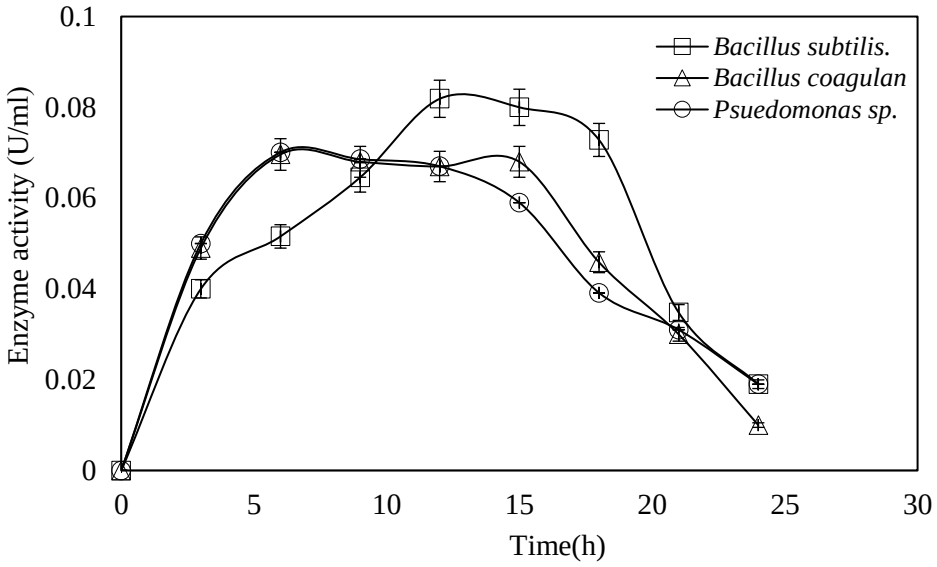

Figure 4.12 Time course of enzyme production by *Bacillus subtilis, Bacillus coagulans and Psuedomonas* sp. Lipase production medium was seeded with 10 % FFA inoculum and incubated at 37 ^{o}C for 24 h at 150 rpm.

4.2.2 Preliminary experiment

This topic presents the preliminary investigate of the effects of concentration of enzyme used and effect of using solvent in esterification reaction before studying the optimal conditions in detail.

4.2.2.1 Effect of enzyme concentration

Synthesis of glycerides from FFA distillate and crude glycerol to obtain the product having a high content of DG, normally used enzymes that are specific (1,3-specific lipase, analytical grade). In order to save cost, due to the specific enzymes are very expensive, this experiment intended to use the nonspecific enzyme (food grade, which is made from *Camlicia lipolytica*). Since there were no reports on the concentration of food grade enzyme, it is necessary to have a preliminary test for this enzyme. Based on Hu *et al.* (2012), the enzyme concentration will be used in a unit per gram of fatty acids (U/g FA). The enzyme concentration that gave the highest amount of DG was chosen to be used in the next experiment. The results showed in Figure 4.13 that the enzyme concentration of 300 U/g FA could be used for glyceride synthesis.

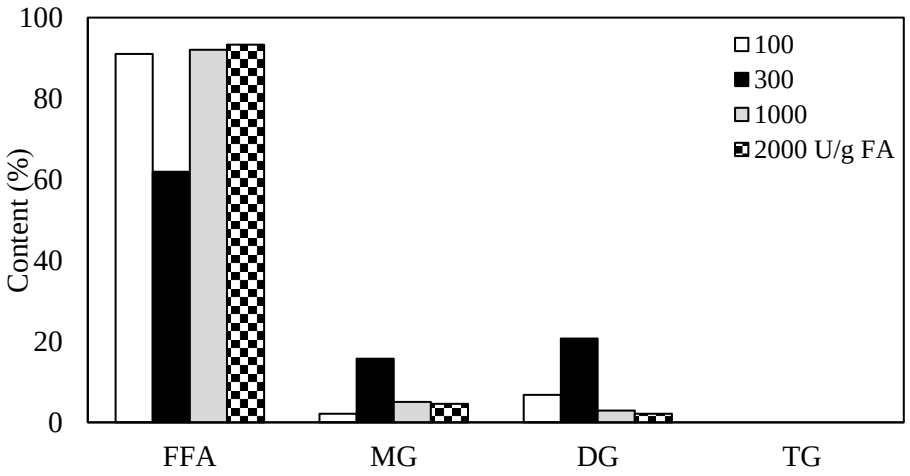

Figure 4.13 Effect of concentration of enzyme on the production of glyceride (F/G molar ratios of 3:1, reaction temperature of 45°C, reaction time of 24 h under shaking speed 200 rpm)

4.2.2.2 Effect of using organic solvent

The use of organic solvent can improve the poor solubility in water of a hydrophobic nature (Yesiloglu *et al*.,2004). Hexane is a hydrophobic solvent with high log P value that results in less inactivation of lipase biocatalyst than solvents with lower log P values. For that reason, hexane is used as the reaction medium for esterification of fatty acids and glycerol (Chong *et al*., 2007). In the present work, effect of hexane on the production of glycerides was examined and the result was shown in Figure 4.14.

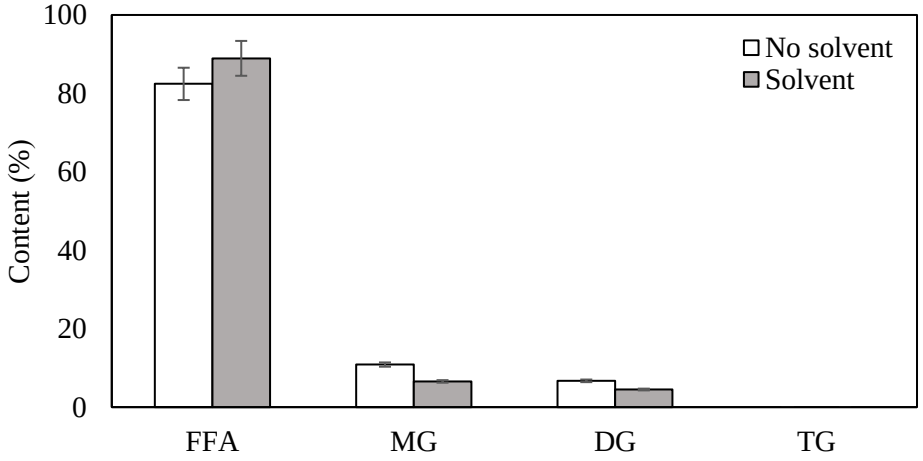

Figure 4.14 Effect of hexane on the production of glycerides (F/G molar ratios of 3:1, enzyme concentration of 300 U/g FA, reaction temperature of 45°C, reaction time of 24 h under shaking speed 200 rpm)

From Figure 4.14, the esterification with free solvent (hexane) is better. MG and DG content in the products of free solvent reaction were higher than that using solvent, whereas FFA content was lower. This was because of organic solvent produced various physicochemical effects on enzyme molecules, and the effect difference depend on the kinds of organic solvents and enzymes used. The main disadvantage of using solvent is the difficulty to complete removing of the toxic solvent **(**Hasenhuettl, 2019**)**. This reaction can also be conducted without solvent, which is an advantage if the products are intended for usind in foods. Thus, the enzyme concentration at 300 U/g FA and with solvents free esterification were selected for starting the investigation of the optimal condition for the synthesis of glyceride.

4.2.3 Study the optimal conditions for the synthesis glyceride using lipase as a catalyst.

4.2.3.1 Effect of substrate molar ratio

The first parameter to study was the optimal esterification molar ratio of fatty acid to glycerol (FA/G molar ratio). Firstly, Oleic acid and glycerol were used as substrates. The interaction between substrate molar ratio also significantly affected the glyceride content. This work focus on the DG content of the product. The esterification process was carried out at oleic acid: glycerol molar ratios of 4:1, 3:1, 2:1, 1:1, 1:2, 1:3 and 1:4. The enzyme concentration for each molar ratio, reaction temperature and reaction time and shaking speed were 300 U/g FA, 45°C, 24 h and 200 rpm, respectively, as shown in Figure 4.15.

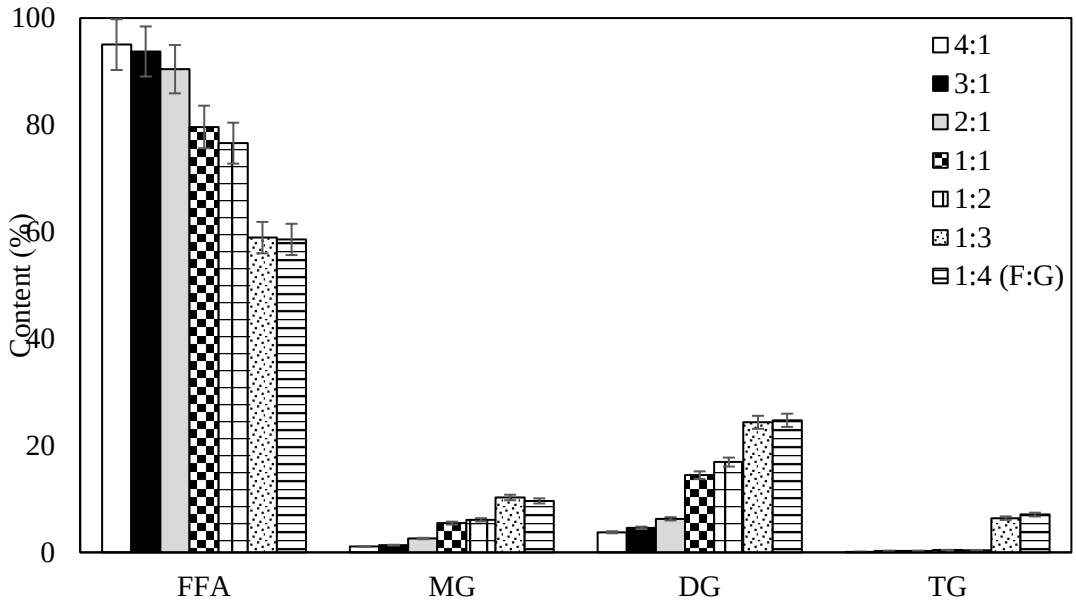

Figure 4.15 Effect of FA/G molar ratio on the production of glyceride (enzyme concentration of 300 U/g FA, reaction temperature of 45°C, reaction time of 24 h under shaking speed 200 rpm)

From Figure 4.15, The maximum total glyceride (41.43%) and maximum DG (24.72%) were received at the FA/G molar ratio of 1:4, which was quite similar to the result of 1:3 oleic acid/glycerol molar ratio (24.34%). The nearly closed results between molar ratio of 1:3 and 1:4 demonstrated that there was no significant for effect excess glycerol amount in the FA/G molar ratio. In accordance with Le Chatelier's principle, in the case of increasing glycerol content, making the esterification process obtain more product formation, which can be seen appearance of TG. Corresponding to Lo *et al.* (2004), which found that the increase in the molar ratio of glycerol resulted in increased of DG and TG. Hasenhuettl **(**2019) reported that the FA/G molar ratio determines the concentrations of MG, DG and TG in the final product. Higher levels of glycerol produce higher concentrations of MG.

The higher glycerol ratio causes the higher DG content, because of (i) only glycerol be able to adsorbed on the catalyst by reason of its polarity **(**hydrophilic**)** (Freitas *et al*., 2007 and Sudibyo *et al*., 2017) and (ii) the greater the high glycerol to fatty acid ratio, the greater the absorption of total fatty acid, if glycerol is esterified as part of glyceride molecules (Roll *et al*., 2018); Kristensen *et al*. (2005) and Tangkam

et al. (2008). In accordance with work of Yesiloglu *et al*. (2004) which used 1:10 oleic to glycerol molar ratio and gave 39.6% maximum DG, 17.1% MG and 22.3% TG, and Zhao *et al*. (2011) which used 1:5 oleic to glycerol molar ratio and gave 53.3% maximum DG and 22.3% TG. Besides, Singh *et al*. (2013) stated that the significant reaction rate of oleic-glycerol esterification increased when the FA/G molar ratio increased from 1:2 to 1:4. It can be concluded that of oleic to glycerol molar ratio of 1:3 gave the maximum DG under the required lower amount of glycerol, which helped to save costs and to reduce the unreacted glycerol removal after the completion of the reaction (Konwar *et al*., 2016). Therefore, the oleic/glycerol molar ratio of 1:3 was selected for studying the optimal reaction temperature in the next experiments.

4.2.3.2 Effect of reaction temperature

The second studied parameter was the optimum esterification temperature. The influence of temperature is important on the rate of reaction and enzyme activity. In general, high temperature of reaction, helps to reduce the viscosity and increase the mass transfer of the reactants in the reaction (Zhang *et al*.,2017). Therefore, it increases the rate of interaction between the molecules of the reactants. The optimum temperature of most lipases will be in the range of 45-65 °C (Chong *et al*., 2007). Experiments were carried out at temperature of 40, 45, 50, 55, 60, 65 and 70 °C, respectively. The esterification process was use under the shaking speed of 200 rpm, molar ratio of oleic acid to glycerol at 1:3, enzyme concentration of 300 U/g FA and reaction time of 24 h.

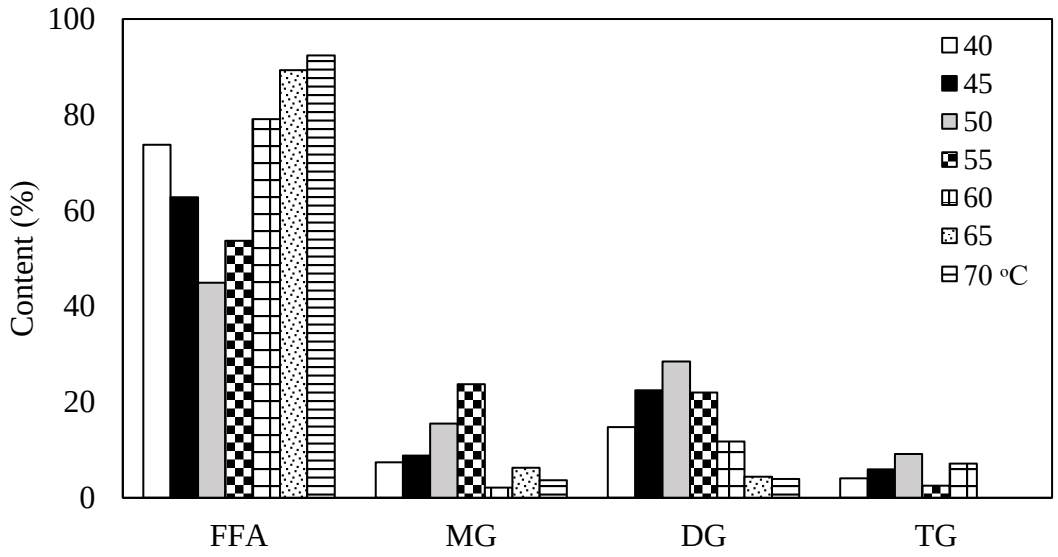

Figure 4.16 Effect of temperature on the production of glyceride (F/G molar ratios of 1:3, enzyme concentration of 300 U/g FA, reaction temperature of 45°C, reaction time of 24 h under shaking speed 200 rpm)

The production of glyceride by lipase at various temperatures were shown in Figure 4.16. The total glyceride obtained at 40, 45, 50, 55, 60, 65 and 70 °C were 26.27, 37.25, 55.07, 46.35, 20.94, 10.71 and 7.62%, respectively. The optimal temperature for the glyceride production (55.07%) and the highest DG content (28.51%) was 50 °C. The content of MG (23.71%) was highest at 55 °C. In the range of 40-55 °C MG, DG and TG increased, this was because the activity of lipase was increased and thermodynamic equilibrium of reaction between MG and DG was shifted in support of

the formation of DG along with the increasing temperature. **(**Zhao *et al*., 2011**)**. However, at the temperature above 65 °C MG and DG are generated low, this may be due to lipase may denaturation and decrease its activity. Therefore, the temperature of 50 °C was selected as the optimal reaction temperature to be used in the next experiments.

4.2.3.3 Effect of enzyme concentration

The replacement of inorganic catalysts by lipase in the synthesis of glyceride, has advantages of catalysis at lower temperatures which prevents the discoloration and avoids undesired byproduct creating, less pollution and energy consumption, moreover, it can produce glycerides with unsaturated fatty acids that is difficult by chemical method (Zhao *et al*., 2011). It is therefore important to predicate the optimum enzyme concentration for an enzymatic reaction to achieve the highest level of efficiency.

The effect of enzyme concentration on glyceride production was carried out at 50, 100, 300, 500 and 700 U/g FA. Initial water content in reaction was about 0.5-0.8 %. In this study, molecular sieve was used to prevent reversed hydrolysis of the products due to the presence of water (Chong *et al*., 2007). Result were shown in Figure 4.17. With the increase of enzyme concentration, MG, DG and TG content decreased. It may be because of the mass transfer limitations caused by enzyme catalysis agglomeration and possible diffusion problem **(**Sun *et al*., 2009**)**. Similar results of Zhao *et al*. **(**2011**)** reported that excess enzymes presented in the reaction decreases the efficiency per mass unit of enzyme in other enzymatic reactions. According to Valério *et al*. (2009), the decrease in MG and DG contents when increasing of enzyme concentration may be due to a poor mixing of the reaction and occurrence of mass transfer limitations. This result is also in agreement with previous reports of Watanabe *et al*. (2003) and Yang *et al*. (2005), showing that an increase in enzyme loading above a certain volume may not increase the product, while might increase the reaction rate, showing that high enzyme concentrations may lead to the formation of aggregates, thus not making the enzyme active site available to the substrates. The total glyceride **(**48.56%**)** and highest DG **(**26.02%**)** were obtained at enzyme concentration of 100 U/g FA. These results indicate that a high enzyme concentration is not superior for the

esterification reaction. To obtain the highest DG, enzyme concentration of 100 U/g FA was selected for further experiments.

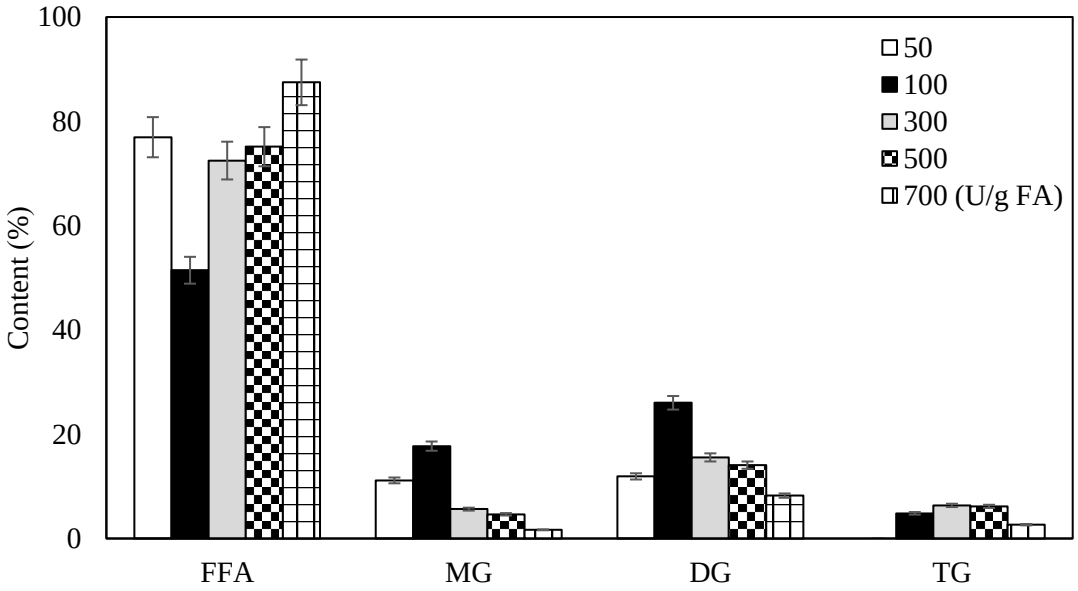

Figure 4.17 Effect of enzyme concentration on the production of glyceride (F/G molar ratios of 1:3, reaction temperature 50°C and reaction time 24 h under shaking speed 200 rpm).

4.2.3.4 Effect of reaction time

The effect of time is important in tracking the changes in the substrates and the reaction products using enzymes to find the shortest time that gives high yields and reduces production costs. The reaction times used in this study was 12, 24, 36 and 48 h, the molar ratio of oleic acid to glycerol at 1:3, reaction temperature 50°C and enzyme concentration of 100 U/g FA. The results were shown in Figure 4.18. The total glyceride **(**65.99%**)** and highest DG **(**34.74%**)** were obtained at 24 h. When the reaction time increased more than 24 h, DG decreased, and TG increased **(**MG converted to DG and DG converted to TG**)**. Von Der Haar *et al*. (2015) reported that elongations of the reaction time rather led to a decrease of DG content. Duan *et al*. (2012) and Zhong *et al*. (2013) also supported this development also supports the hypothesis mentioned above, that further esterification of the FFA occurs with DG as the acyl-group acceptor to become TG. After obtaining all optimum parameters, they were used to investigate the esterification of the FFA distillate obtained from section 4.1 and crude glycerol.

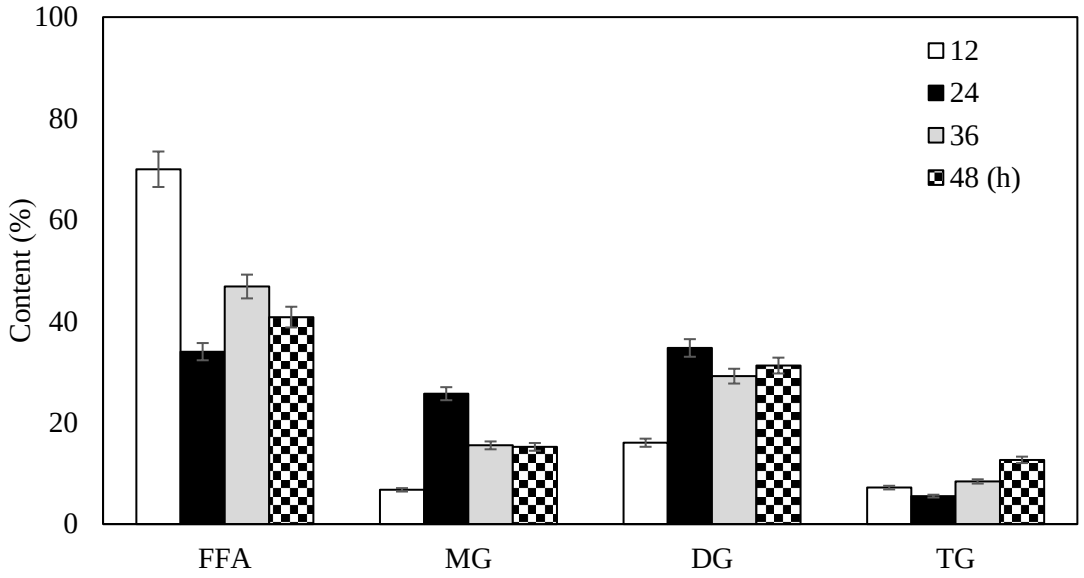

Figure 4.18 Effect of time on the production of glyceride (F/G molar ratios of 1:3, enzyme concentration 100 U/g FA and reaction temperature 50°C and shaking speed 200 rpm).

4.2.3.5 Esterification of the FFA distillate and crude glycerol using optimal conditions.

The growing of biodiesel production in recent years has generated a crude glycerol as a byproduct. Most of the crude glycerol is used as substrate for production of other value-added chemicals. Crude glycerol can also be chemically converted into many values-added products, such as acrolein, glycerides, polyols, polyglycerol, polyurethanes, lactic acid, and bio-oil, using catalysts (Luo *et al*., 2016). In this study, crude glycerol was used as precursors in the production of glyceride with the FFA distillate in order to increase the value of crude glycerol. The crude glycerol used in the study has physical properties as shown in the Table 4.9.

Table 4.9. Physical properties of crude glycerol used in this study and commercial glycerol.

Property	Crude glycerol (PSU Biodiesel plant)	Commercial glycerol (Loba chemie)
Free glycerol (wt.%)	24.9	99.98
Methanol (wt.%)	1.17	-
Moisture content (wt.%)	1.70	0.01
Soap (wt %)	20.11	-
FAME (wt %)	22.91	-
FFAs (%)	0.38	-
Ash (%)	4.72	-
pH	9.26	6.97
Density (g/cm3)	1.64	1.25
Viscosity at 28 ^{o}C (cP)	349.50	369.23
Appearance	Dark brown and viscous liquid	Clear and viscous liquid

Table 4.10. Comparison of DG, MG and TG in the products of lipase-catalyzed esterification from various substrate at optimal condition*.

Case	Substrate	% FFA	% MG	% DG	% TG
1	Oleic acids + commercial glycerol	35.09	18.72	33.45	12.74
2	Oleic acids + Crude glycerol	92.47	4.02	1.99	1.51
3	FFAs distillate + commercial glycerol	73.88	5.02	19.54	1.56
4	FFAs distillate + Crude glycerol	82.41	11.63	4.68	1.29

*Compositions of DG, MG and TG on esterification when using the optimal conditions (molar ratios of fatty acid to glycerol of 1:3, enzyme concentration 100 U/g FA and reaction temperature 50°C and reaction time 24 h under shaker speed 200 rpm)

Table 4.10 Compared amounts of DG, MG and TG in the products of lipase-catalyzed esterification from various substrates at optimal conditions. The glycerol undergoes esterification with fatty acids, leading to the formation of glycerides. Commercial glycerol showed high conversion to glycerides, while crude glycerol showed significantly low yields for both reactions with oleic acids and FFAs distillate. When comparing between case1 and case 2 **(**reactions of oleic acids with commercial glycerol and crude glycerol**)**, case 1 gave the highest amount of DG **(**33.45%**)** whereas case 2 gave the lowest amount of DG **(**1.99%**)**. In addition, when comparing between case 3 and case 4 **(**reaction of FFA distillate with commercial glycerol and crude glycerol**)**, case 3 gave the highest amount of DG **(**19.54%**)** but smaller than case 1 whereas case 4 gave the lowest amount of DG **(**4.68%**)** but higher than case 2. This is because

1) Effect of impurities in the crude glycerol on glyceride production. These all results represented effect of glycerol purity since crude glycerol has a significantly different composition, and contains various impurities such as methanol, water, soap, free fatty acids (FFAs), and fatty acid methyl esters (FAMEs) (Luo *et al*., 2013). In accordance with Chetpattananondh and Tongurai (2008), the lower amount of DG came from the impurities in the crude glycerol itself, which were not removed before the esterification reaction. Luo *et al*. (2016) reported that the impurities in crude glycerol have effects on the value-added conversion process and may participate in reactions as a reactant

or catalyst for the production of chemicals, which depending on the reaction mechanism.

2) Crude glycerol has a lower concentration of glycerol than commercial glycerol. Because in the experiment, the amount of glycerol is used based on the molecular weight, causing the amount of glyceride to be produced in case 1 is greater than in case 2. Same as case 3 and 4, case 3 is greater than in case 4.
3) Effect of pH **(**Alkaline, ~9.26**)** in crude glycerol on lipase activity.
4) The high viscosity of the excessive crude glycerol in the system, the glycerol forms a layer around the lipase, make it not disperse in the system as well as lipase power. **(**Zhao *et al*., 2011**)**

Therefore, FFA distillate can be used as substrate in the production of glycerides. However, crude glycerol should be purified before being used in the production of glycerides.

CHAPTER 5

CONCLUSIONS AND SUGGESTIONS

5.1 Conclusions

5.1.1 The suitable reactor configuration

Four configurations of the reactor as shown in Figure 3.1 were tasted they used the same evaporator. Thus, the heating rate and the evaporation area were the same due to the same electric heater and the same dimeter of the evaporation tank, respectively. The vacuum pressures made by both the smaller (0.75 kW) and bigger (2.20 kW) vacuum pumps were not difference. Therefore, the evaporation rate depends on vacuum pressure used and type and quality of the oil. It could be concluded configuration D is the best due to it has no heavy condenser on the top of the evaporator, easy operation. In addition, the vacuum pump of configuration D can be replaced by the smaller one to reduce energy consumption.

5.1.2 The suitable operation condition of the proposed reactor

The operation of the proposed reactor could not meet the standard distillation vacuum pressure (<10 mmHg) which the boiling point of FFAs was lower than 200°C. Eleven batches were performed: four batches for CPO and seven batches for MCPO. The initial FFA contents in CPO and MCPO were 10.2-25.7% and 10.3-19.5%, respectively. Whereas, the final FFA contents were 5.2-10.6% and 7.3-12.1%, respectively. The operating pressures and temperature were 20-160 mmHg and 220-250°C, respectively. the evaporation efficiency of the evaporator was in the range of 0.49-3.63%. Whereas manually scraped efficiency of the condenser was in the range of 0.8-26% of the lost mass. The highest percentage reduction of FFA content was obtained at distillation pressure and temperature of 20 mmHg and 220°C, respectively.

5.1.3 The suitable condition for production of glyceride

The production of glycerides by lipase-catalyzed esterification of crude glycerol with FFA distillate were conducted at the obtained optimal conditions at molar ratio of 1:3 (FFA: glycerol), reaction temperature at 50°C, enzyme concentration of 100 U/g FA and shaking speeds 200 rpm for 24 h. The TG, DG and MG content of case 1 (12.74, 33.45 and 18.72%), case 2 (1.51, 1.99 and 4.02%)), case 3 (1.56, 19.54 and 5.02%) and

case 4 (1.29, 4.68 and 11.63%) , respectively. Thin-Layer Chromatography-Flame Ionization Detection (TLC-FID) was used for glyceride analysis.

5.2 Suggestions

(1) The efficiency of the evaporator and condensers should be improved to be more efficient.

(2) The condenser should be designed to be easy collecting FFA distillate.

(3) Using nitrogen sparging or mechanical agitation in the evaporator to increase surface area, may provide more FFA evaporation.

(4) The temperature of cooling water should be lower than 15 ^{o}C to increasing the heat transfer rate for better condensation.

(5) Crude glycerol should be purified before using in the esterification reaction.

(6) Further investigation at 250°C of the proposed reactor should be performed in order to explain about thermal cracking of the oil.

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APPENDIX

APPENDIX A

Preparation of crude palm oil samples

Preheat the crude palm oil to be liquid before.

Preparation of 0.1 N sodium hydroxide (NaOH) concentration

Prepared by weighing 4 grams of NaOH dissolved in distilled water and adjusting the volume to 1 liter, stored in a brown bottle.

Preparation of alcohol solution to neutralize

Prepared by adding 5 drops of phenolphthalein and dropping NaOH 0.1 N down by one drop and then shaking it until it gets permanent pink.

Preparation of 10% Potassium iodide (KI) solution

Prepared by weighing 50 grams of KI dissolved in distilled water and adjusting the volume to 500 ml, stored in a brown bottle.

Preparation of 0.01 N Sodium Thiosulfate Solution ($Na_2S_3O_3$) solution

Prepared by weighing 25 grams of $Na_2S_3O_3$ dissolved in distilled water and adjusting the volume to 1 liter, boil for 5 min and stored in a brown bottle.

Preparation of 1% starch solution

Prepared by weighing 1 grams of starch dissolved in 100 ml distilled water. Heat until the starch is clear.

Preparation of wijs solution

Prepared by weighing 9 grams of Iodine monochloride (ICl) dissolved in solution (700 ml of acetic + 300 ml of CCL_4).

Preparation of acetic-chloroform

Prepared by mixed acetic: chloroform in a ratio of 3:2

Preparation of Alcoholic Potassium hydroxide (KOH)

Prepared by weighing 35-40 grams of Potassium hydroxide dissolved in 100 ml distilled water and adjusting the volume with 95 % Ethyl alcohol to 1 liter

The temperature of oil, temperature of cooling water and vacuum pressure of each batch of HFFA-CPO.

Table 1. Data of CPO1

Time (min)	Temperature (°C)	Temperature of cooling water (°C)	Vacuum pressure (mmHg)
0	27.4	15	40
20	71.9	13	40
40	100.5	14	90
60	131.2	14	120
80	155.6	14	20
100	176.4	14	20
120	193.6	15	20
140	209.2	15	20
160	221.5	15	70
180	222.1	16	30
200	228.0	17	20
210	230.0	17	30
240	229.8	18	20
270	230.9	19	20
300	230.1	18	20
330	230.0	20	20
360	230.0	18	20
390	230.3	18	20
420	230.0	19	20
450	230.0	18	20

Table 2. Data of CPO2

Time (min)	Temperature (°C)	Temperature of cooling water (°C)	Vacuum pressure (mmHg)
0	29.5	28	160
20	70.5	30	160
40	92.2	30	160
60	127.3	30	160
80	152.9	24	160
100	157.1	22	160
120	207.5	22	160
140	224.5	22	160
160	233.6	22	160
180	250.0	22	160
210	249.9	22	160
240	249.9	20	170
270	250.3	20	170
300	249.9	19	170
330	250.3	18	170
360	249.9	18	170
390	250.1	18	170
410	250.0	17	170

Table 3. Data of CPO3

Time (min)	Temperature (°C)	Temperature of cooling water (°C)	Vacuum pressure (mmHg)
0	30.5	23	30
20	73.7	19	30
40	92.1	18	30
60	129.7	18	30
80	157.2	19	30
100	185.6	20	30
120	206.4	20	30
140	223.2	20	30
160	235.7	20	30
180	245.2	20	30
200	250.0	20	30
230	250.9	17	30
260	250.0	19	30
290	249.8	20	30
320	250.0	20	30
350	250.1	20	30
380	250.3	20	30
410	249.9	20	30
440	250.2	21	30

Table 4. Data of CPO4

Time (min)	Temperature (°C)	Temperature of cooling water (°C)	Vacuum pressure (mmHg)
0	27.6	20	50
20	67.3	18	50
40	90.1	18	50
60	122.8	18	50
80	155.4	18	50
100	166.8	18	50
120	202.5	19	50
140	215.3	19	50
160	229.8	20	50
180	236.7	19	50
200	245.8	19	50
215	250.0	19	50
245	249.6	19	40
275	249.9	18	40
305	250.2	19	40
335	250.2	19	40
365	250.2	20	40
395	250.0	19	40
425	250.1	19	40
465	249.8	20	40
495	250.3	20	40
525	250.1	20	40
565	249.9	19	40
595	249.9	19	40
625	250.1	18	40
665	249.8	18	40
695	250.4	19	40
725	250.1	19	40
765	249.9	19	40
795	250.3	20	40
825	250.0	17	40
865	249.9	18	40
895	250.3	18	40
925	249.8	19	40
965	250.1	19	40
995	250.0	19	40

The temperature of oil, temperature of cooling water and vacuum pressure of each batch of HFFA-MCPO.

Table 5. Data of MCPO1

Time (min)	Temperature (°C)	Temperature of cooling water (°C)	Vacuum pressure (mmHg)
0	31.4	21	20
20	86.9	18	20
40	115.7	17	20
60	147.4	17	20
80	180.9	17	20
100	196.0	17	20
120	213.6	18	20
130	220.0	18	20
160	219.3	18	20
190	220.2	18	20
210	220.1	17	20
240	219.9	16	20
270	219.8	15	20
300	220.0	14	20
330	219.9	15	20
360	220.4	15	20

Table 6. Data of MCPO2

Time (min)	Temperature (°C)	Temperature of cooling water (°C)	Vacuum pressure (mmHg)
0	27.1	20	110
20	71.1	19	30
40	103.1	18	20
60	133.3	17	20
80	160.8	17	20
100	183.0	18	20
120	200.2	19	20
140	215.1	20	20
160	224.7	20	20
180	235.9	19	20
195	240.1	17	20
225	240.8	17	20
255	240.0	16	20.5
325	239.9	15	20
355	240.2	15	20
425	240.1	16	20
455	240.0	15	20
525	239.9	16	20.5
555	240.1	16	20

Table 7. Data of MCPO3

Time (min)	Temperature (°C)	Temperature of cooling water (°C)	Vacuum pressure (mmHg)
0	29.6	18	40
20	88.2	18	40
40	113.8	19	30
60	139.5	19	20
80	175.1	20	20
100	198.5	20	20
120	217.2	20	20
140	233.3	20	20
160	247.0	19	20
171	250.3	18	20
201	250.5	18	20
231	249.9	18	20
261	250.1	18	20
291	251.6	19	20
301	250.1	19	20
331	250.4	19	20
361	249.9	18	20
391	249.8	18	20
401	250.0	18	20
431	249.7	18	20
461	250.0	18	20
491	249.8	19	20
501	249.9	19	20

Table 8. Data of MCPO4

Time (min)	Temperature (°C)	Temperature of cooling water (°C)	Vacuum pressure (mmHg)
0	31.2	21	30
20	81.1	19	30
40	115.9	19	30
60	154.6	19	30
80	182.5	19	30
100	204.0	19	30
120	221.5	20	30
150	218.2	19	30
180	221.3	20	30
210	219.5	20	30
240	219.8	18	30
270	220.1	16	30
300	220.4	16	30
330	219.8	17	30
360	220.1	17	30
390	220.2	17	30

Table 9. Data of MCPO5

Time (min)	Temperature (°C)	Temperature of cooling water (°C)	Vacuum pressure (mmHg)
0	29.3	25	30
20	78.6	23	30
40	118.2	21	30
60	146.6	19	30
80	172.8	19	30
100	196.4	18	30
120	218.7	18	30
140	230.1	18	30
170	228.9	17	30
200	230.1	16	30
230	229.8	16	30
260	229.6	16	30
290	230.3	16	30
320	230.2	16	30
350	230.4	16	30
380	229.7	16	30

Table 10. Data of MCPO6

Time (min)	Temperature (°C)	Temperature of cooling water (°C)	Vacuum pressure (mmHg)
0	27.8	24	30
20	86.1	22	30
40	113.6	20	30
60	143.8	18	30
80	169.1	18	30
100	198.8	18	30
120	215.1	18	30
140	225.7	18	30
160	236.0	18	30
167	240.1	18	30
197	239.9	16	30
227	239.7	15	30
257	240.2	15	30
287	239.9	16	30
317	240.1	17	30
347	239.7	16	30
377	240.1	16	30
407	239.6	16	30

Table 11. Data of MCPO7

Time (min)	Temperature (°C)	Temperature of cooling water (°C)	Vacuum pressure (mmHg)
0	29.0	22	30
20	89.4	19	30
40	118.2	17	30
60	149.6	17	30
80	177.0	18	30
100	197.1	18	30
120	217.0	18	30
140	227.7	17	30
160	236.1	17	30
180	248.2	18	30
185	250.0	18	30
215	249.1	18	30
245	250.8	17	30
275	250.4	16	30
305	250.4	14	30
335	250.2	14	30
365	250.4	15	30
395	250.1	15	30
425	250.3	15	30

Fatty acid composition in the HFFA-CPO (analyzed by GC)

Table 12. Fatty acid composition of CPO1

Fatty acids	CPO_{BS}	CPO_{AS}	Distillate
Caprylic acid (C8:0)	0	0.007843585	0.490723658
Nonanoic acid (C9:0)	0	0	0
Capric acid (C10:0)	0.017081347	0.016512262	0.433694556
Undecanoic acid (C11:0)	0	0	0
Lauric acid (C12:0)	0.246522542	0.238681033	2.298319422
Tridecanoic acid (C13:0)	0	0	0
Myristic acid (C14:0)	0.969997519	0.954306363	3.65343345
Pentadecanoic acid (C15:0)	0.051980306	0.052158276	0.173312521
Palmitic acid (C16:0)	44.24911335	44.08055268	60.2774611
Palmitoleic acid (C16:1)	0.12402909	0.124766272	0.215528043
Heptadecanoic acid (C17:0)	0.104244599	0.105465412	0.092452418
Stearic acid (C18:0)	4.151377336	4.199545406	2.140933088
Oleic acid (C18:1)	40.86959412	41.31408567	25.01068368
Linoleic acid (C18:2)	8.397533008	8.161025987	4.954538312
Linolenic acid (C18:3)	0.230692845	0.159691637	0.141544916
Arachidic acid (C20:0)	0.332497252	0.336960844	0.071408234
Eicosenoic acid (C20:1)	0.113279622	0.101862167	0.029605966
Behenic acid (C22:0)	0.064864942	0.064471974	0.016360634
Erucic acid (C22:1)	0.018175226	0.019332192	0
Lignoceric acid (C24:0)	0.059016895	0.062738238	0
Nervonic acid (C24:1)	0	0	0

Abbreviation:

CPO_{BS} = Crude palm oil before separation.

CPO_{AS}= Crude palm oil after separation.

Condition of separation:

Temperature at 230 ^{o}C and vacuum pressure at 20 mmHg.

Table 13. Fatty acid composition of CPO2

Fatty acids	CPO_{BS}	CPO_{AS}	Distillate
Caprylic acid (C8:0)	0	0	0.018439484
Nonanoic acid (C9:0)	0	0	0.029158204
Capric acid (C10:0)	0	0	0.1239429
Undecanoic acid (C11:0)	0	0	0.026242383
Lauric acid (C12:0)	0.282142677	0.269164844	1.390497239
Tridecanoic acid (C13:0)	0	0	0
Myristic acid (C14:0)	0.982704294	0.982474731	2.74323255
Pentadecanoic acid (C15:0)	0.055046245	0.05507251	0.114045538
Palmitic acid (C16:0)	42.79842472	42.73972011	57.41760574
Palmitoleic acid (C16:1)	0.199174572	0.135243169	0.21084256
Heptadecanoic acid (C17:0)	0.108241938	0.110636739	0.101273423
Stearic acid (C18:0)	4.290417199	4.288638549	2.864044023
Oleic acid (C18:1)	42.02434201	42.90580839	29.5874145
Linoleic acid (C18:2)	8.344444312	7.795331457	5.115262687
Linolenic acid (C18:3)	0.172520493	0.094338308	0.086817525
Arachidic acid (C20:0)	0.369077711	0.372487266	0.139148287
Eicosenoic acid (C20:1)	0.226789711	0.100638456	0
Behenic acid (C22:0)	0.070024467	0.070510433	0.032032956
Erucic acid (C22:1)	0	0	0
Lignoceric acid (C24:0)	0.076649647	0.079935044	0
Nervonic acid (C24:1)	0	0	0

Abbreviation:

CPO_{BS} = Crude palm oil before separation.

CPO_{AS}= Crude palm oil after separation.

Condition of separation:

Temperature at 250 ^{o}C and vacuum pressure at 160 mmHg.

Table 14. Fatty acid composition of CPO3

Fatty acids	CPO_{BS}	CPO_{AS}	Distillate
Caprylic acid (C8:0)	0.019296188	0	0.138977298
Nonanoic acid (C9:0)	0	0	0.21302907
Capric acid (C10:0)	0.021113135	0	0.323148263
Undecanoic acid (C11:0)	0	0	0.035536099
Lauric acid (C12:0)	0.260168639	0.250816933	2.016650189
Tridecanoic acid (C13:0)	0	0	0
Myristic acid (C14:0)	0.970899705	0.987116372	3.239744173
Pentadecanoic acid (C15:0)	0.05355428	0.056151059	0.152135343
Palmitic acid (C16:0)	41.80320589	44.25428098	58.12448365
Palmitoleic acid (C16:1)	0.139833919	0.134011267	0.318355942
Heptadecanoic acid (C17:0)	0.108123614	0.111892704	0.098180064
Stearic acid (C18:0)	4.277308039	4.51479692	2.721100484
Oleic acid (C18:1)	42.9090368	41.16796864	27.28661932
Linoleic acid (C18:2)	8.703280971	7.80339023	5.08076623
Linolenic acid (C18:3)	0.218957423	0.098985944	0.128184158
Arachidic acid (C20:0)	0.371611481	0.383221253	0.123089713
Eicosenoic acid (C20:1)	0	0.106621506	0
Behenic acid (C22:0)	0.067775194	0.07105371	0
Erucic acid (C22:1)	0	0	0
Lignoceric acid (C24:0)	0.075834728	0.080163159	0
Nervonic acid (C24:1)	0	0	0

Abbreviation:

CPO_{BS} = Crude palm oil before separation.

CPO_{AS}= Crude palm oil after separation.

Condition of separation:

Temperature at 250 ^{o}C and vacuum pressure at 30 mmHg.

Fatty acid composition in the HFFA-MCPO (analyzed by GC)

Table 15. Fatty acid composition of MCPO1

Fatty acids	$MCPO_{BS}$	$MCPO_{AS}$	Distillate
Caprylic acid (C8:0)	0.151752742	0.071046711	10.22781768
Nonanoic acid (C9:0)	0	0	0.51533975
Capric acid (C10:0)	0.201697705	0.166025052	3.570561174
Undecanoic acid (C11:0)	0	0	0.034388366
Lauric acid (C12:0)	3.050145328	2.83802644	5.098795623
Tridecanoic acid (C13:0)	0	0	0
Myristic acid (C14:0)	2.053820038	2.020963987	2.776703256
Pentadecanoic acid (C15:0)	0.047552692	0.048934977	1.423082222
Palmitic acid (C16:0)	37.79795002	40.66722032	57.37695037
Palmitoleic acid (C16:1)	0.175493759	0.167440527	0.159908663
Heptadecanoic acid (C17:0)	0.095983559	0.098597912	0.098892772
Stearic acid (C18:0)	4.164368186	4.199187194	2.704173059
Oleic acid (C18:1)	40.7532524	38.99522136	13.1158443
Linoleic acid (C18:2)	10.45127616	9.763761345	2.06335717
Linolenic acid (C18:3)	0.350321316	0.277220687	0.082885346
Arachidic acid (C20:0)	0.377554847	0.376475784	0.143238861
Eicosenoic acid (C20:1)	0.154881873	0.141881101	0
Behenic acid (C22:0)	0.077239057	0.073078928	0.121413564
Erucic acid (C22:1)	0	0	0.075212821
Lignoceric acid (C24:0)	0.096710325	0.094917678	0
Nervonic acid (C24:1)	0	0	0.411434998

Abbreviation:

$MCPO_{BS}$ = Mixed crude palm oil before separation.

$MCPO_{AS}$= Mixed crude palm oil after separation.

Condition of separation:

Temperature at 220 ^{o}C and vacuum pressure at 20 mmHg.

Table 16. Fatty acid composition of MCPO2

Fatty acids	$MCPO_{BS}$	$MCPO_{AS}$	Distillate
Caprylic acid (C8:0)	0.131297318	0.022863186	0.245115838
Nonanoic acid (C9:0)	0	0	0.187993692
Capric acid (C10:0)	0.154843836	0.116831823	0.579241413
Undecanoic acid (C11:0)	0	0	0
Lauric acid (C12:0)	2.305903474	2.24661987	4.227551392
Tridecanoic acid (C13:0)	0	0	0
Myristic acid (C14:0)	1.768562462	1.779386925	3.743887322
Pentadecanoic acid (C15:0)	0	0.047770535	0.121367207
Palmitic acid (C16:0)	38.9934969	41.27031466	59.15421634
Palmitoleic acid (C16:1)	0.134991285	0.130553928	0.283517639
Heptadecanoic acid (C17:0)	0	0.095991835	0.091113507
Stearic acid (C18:0)	4.315098626	4.308898098	2.473029035
Oleic acid (C18:1)	40.78654451	39.67973509	23.44730068
Linoleic acid (C18:2)	10.27964441	9.492331398	5.121087416
Linolenic acid (C18:3)	0.333759014	0.171133199	0.137866312
Arachidic acid (C20:0)	0.393357036	0.368830045	0.121516713
Eicosenoic acid (C20:1)	0.173989889	0.095467691	0.029837217
Behenic acid (C22:0)	0.079238623	0.076063859	0.022831772
Erucic acid (C22:1)	0	0.014319629	0
Lignoceric acid (C24:0)	0.092672148	0.082888221	0.012526505
Nervonic acid (C24:1)	0.056600458	0	0

Abbreviation:

$MCPO_{BS}$ = Mixed crude palm oil before separation.

$MCPO_{AS}$= Mixed crude palm oil after separation.

Condition of separation:

Temperature at 240 ^{o}C and vacuum pressure at 20 mmHg.

Table 17. Fatty acid composition of MCPO3

Fatty acids	$MCPO_{BS}$	$MCPO_{AS}$	Distillate
Caprylic acid (C8:0)	0.072158859	0.0778539	0.024473022
Nonanoic acid (C9:0)	0	0	0.120805655
Capric acid (C10:0)	0.145810589	0.145761571	0.286814666
Undecanoic acid (C11:0)	0	0	0
Lauric acid (C12:0)	2.323071479	2.321694328	3.615914949
Tridecanoic acid (C13:0)	0	0	0
Myristic acid (C14:0)	1.802908052	1.802517629	3.666170934
Pentadecanoic acid (C15:0)	0.049898715	0.049155742	0.1163976
Palmitic acid (C16:0)	40.40438	41.10479379	59.35241099
Palmitoleic acid (C16:1)	0.204441579	0.140880932	0.185616538
Heptadecanoic acid (C17:0)	0.10261957	0.102113245	0.091415157
Stearic acid (C18:0)	4.431128577	4.448430243	2.656414474
Oleic acid (C18:1)	39.73428525	40.15226772	24.55167015
Linoleic acid (C18:2)	9.595921639	8.855965867	5.065364488
Linolenic acid (C18:3)	0.317787327	0.113590453	0.130786157
Arachidic acid (C20:0)	0.414404736	0.408771505	0.135745219
Eicosenoic acid (C20:1)	0.218296652	0.093739096	0
Behenic acid (C22:0)	0.085778754	0.084686794	0
Erucic acid (C22:1)	0	0	0
Lignoceric acid (C24:0)	0.097108216	0.097777182	0
Nervonic acid (C24:1)	0	0	0

Abbreviation:

$MCPO_{BS}$ = Mixed crude palm oil before separation.

$MCPO_{AS}$= Mixed crude palm oil after separation.

Condition of separation:

Temperature at 250 ^{o}C and vacuum pressure at 20 mmHg.

Table 18. Fatty acid composition of MCPO4

Fatty acids	$MCPO_{BS}$	$MCPO_{AS}$	Distillate
Caprylic acid (C8:0)	0.142639337	0.024182968	0.942265993
Nonanoic acid (C9:0)	0	0	0.107727021
Capric acid (C10:0)	0.193056359	0.13361619	0.775859731
Undecanoic acid (C11:0)	0	0	0
Lauric acid (C12:0)	2.989765789	2.688613794	6.221555333
Tridecanoic acid (C13:0)	0	0	0
Myristic acid (C14:0)	2.070160522	1.991239092	3.741694656
Pentadecanoic acid (C15:0)	0.050275515	0.048144166	0.150623358
Palmitic acid (C16:0)	41.53405413	42.27606184	56.27128376
Palmitoleic acid (C16:1)	0.183474295	0.157305217	0.231093652
Heptadecanoic acid (C17:0)	0.094446001	0.098173374	0.096212257
Stearic acid (C18:0)	4.068586961	4.201032136	2.395777305
Oleic acid (C18:1)	37.95375825	38.13548208	22.9490899
Linoleic acid (C18:2)	9.742846908	9.29111848	5.768129485
Linolenic acid (C18:3)	0.321635937	0.241204693	0.209159304
Arachidic acid (C20:0)	0.350301269	0.36966978	0.101186635
Eicosenoic acid (C20:1)	0.153120983	0.183786525	0.038341608
Behenic acid (C22:0)	0.067013804	0.066762733	0
Erucic acid (C22:1)	0	0	0
Lignoceric acid (C24:0)	0.084863937	0.093606937	0
Nervonic acid (C24:1)	0	0	0

Abbreviation:

$MCPO_{BS}$ = Mixed crude palm oil before separation.

$MCPO_{AS}$= Mixed crude palm oil after separation.

Condition of separation:

Temperature at 220 ^{o}C and vacuum pressure at 30 mmHg.

Table 19. Fatty acid composition of MCPO5

Fatty acids	$MCPO_{BS}$	$MCPO_{AS}$	Distillate
Caprylic acid (C8:0)	0.142639337	0.017729519	0.856589838
Nonanoic acid (C9:0)	0	0	0.193277179
Capric acid (C10:0)	0.193056359	0.127876297	0.448471853
Undecanoic acid (C11:0)	0	0	0
Lauric acid (C12:0)	2.989765789	2.659871325	3.987938128
Tridecanoic acid (C13:0)	0	0	0
Myristic acid (C14:0)	2.070160522	1.992217356	4.531221144
Pentadecanoic acid (C15:0)	0.050275515	0.047403029	0.237113905
Palmitic acid (C16:0)	41.53405413	42.38280299	69.44859676
Palmitoleic acid (C16:1)	0.183474295	0.155423071	0.22167753
Heptadecanoic acid (C17:0)	0.094446001	0.097366204	0.133541846
Stearic acid (C18:0)	4.068586961	4.208508012	3.343942437
Oleic acid (C18:1)	37.95375825	38.02425426	14.14110653
Linoleic acid (C18:2)	9.742846908	9.353928872	1.292097715
Linolenic acid (C18:3)	0.321635937	0.223821317	0
Arachidic acid (C20:0)	0.350301269	0.371110379	0.165855039
Eicosenoic acid (C20:1)	0.153120983	0.177093603	0.998570093
Behenic acid (C22:0)	0.067013804	0.066916587	0
Erucic acid (C22:1)	0	0	0
Lignoceric acid (C24:0)	0.084863937	0.093677174	0
Nervonic acid (C24:1)	0	0	0

Abbreviation:

$MCPO_{BS}$ = Mixed crude palm oil before separation.

$MCPO_{AS}$= Mixed crude palm oil after separation.

Condition of separation:

Temperature at 230 ^{o}C and vacuum pressure at 30 mmHg.

Table 20. Fatty acid composition of MCPO6

Fatty acids	$MCPO_{BS}$	$MCPO_{AS}$	Distillate
Caprylic acid (C8:0)	0.142639337	0.037377026	1.323751765
Nonanoic acid (C9:0)	0	0	0.197965428
Capric acid (C10:0)	0.193056359	0.143004683	1.111869336
Undecanoic acid (C11:0)	0	0	0
Lauric acid (C12:0)	2.989765789	2.75650274	7.507716378
Tridecanoic acid (C13:0)	0	0	0
Myristic acid (C14:0)	2.070160522	2.007480043	4.007074632
Pentadecanoic acid (C15:0)	0.050275515	0.04810515	0.192502875
Palmitic acid (C16:0)	41.53405413	42.73084397	65.98616181
Palmitoleic acid (C16:1)	0.183474295	0.155053657	0.183652288
Heptadecanoic acid (C17:0)	0.094446001	0.097379908	0.104413993
Stearic acid (C18:0)	4.068586961	4.169549934	2.829279368
Oleic acid (C18:1)	37.95375825	37.88586587	13.31713123
Linoleic acid (C18:2)	9.742846908	9.133081874	2.725814025
Linolenic acid (C18:3)	0.321635937	0.174392512	0.082219765
Arachidic acid (C20:0)	0.350301269	0.363536195	0.164095514
Eicosenoic acid (C20:1)	0.153120983	0.142440045	0.26635159
Behenic acid (C22:0)	0.067013804	0.066415557	0
Erucic acid (C22:1)	0	0	0
Lignoceric acid (C24:0)	0.084863937	0.088970833	0
Nervonic acid (C24:1)	0	0	0

Abbreviation:

$MCPO_{BS}$ = Mixed crude palm oil before separation.

$MCPO_{AS}$= Mixed crude palm oil after separation.

Condition of separation:

Temperature at 240 ^{o}C and vacuum pressure at 30 mmHg.

Table 21. Fatty acid composition of MCPO7

Fatty acids	$MCPO_{BS}$	$MCPO_{AS}$	Distillate
Caprylic acid (C8:0)	0.142639337	0.081297576	2.433406848
Nonanoic acid (C9:0)	0	0	0.060530296
Capric acid (C10:0)	0.193056359	0.181029726	1.524655959
Undecanoic acid (C11:0)	0	0	0
Lauric acid (C12:0)	2.989765789	2.921340614	11.84491625
Tridecanoic acid (C13:0)	0	0	0
Myristic acid (C14:0)	2.070160522	2.001577371	4.623740909
Pentadecanoic acid (C15:0)	0.050275515	0.046948191	0.190972463
Palmitic acid (C16:0)	41.53405413	39.94788126	50.83323283
Palmitoleic acid (C16:1)	0.183474295	0.176544551	0.204894845
Heptadecanoic acid (C17:0)	0.094446001	0.098200135	0.086306355
Stearic acid (C18:0)	4.068586961	4.047986333	2.370528573
Oleic acid (C18:1)	37.95375825	40.07269007	20.67722975
Linoleic acid (C18:2)	9.742846908	9.523104951	4.905033013
Linolenic acid (C18:3)	0.321635937	0.174377556	0.100104614
Arachidic acid (C20:0)	0.350301269	0.367471898	0.107448516
Eicosenoic acid (C20:1)	0.153120983	0.194213116	0.036998781
Behenic acid (C22:0)	0.067013804	0.07122861	0
Erucic acid (C22:1)	0	0	0
Lignoceric acid (C24:0)	0.084863937	0.094108042	0
Nervonic acid (C24:1)	0	0	0

Abbreviation:

$MCPO_{BS}$ = Mixed crude palm oil before separation.

$MCPO_{AS}$= Mixed crude palm oil after separation.

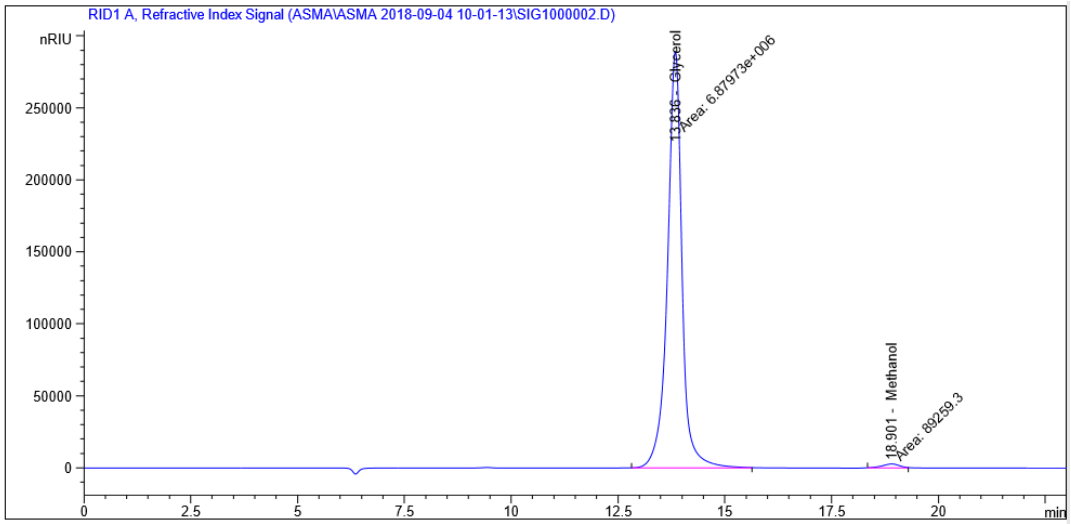
Condition of separation:

Temperature at 250 ^{o}C and vacuum pressure at 30 mmHg.

Table 22. Energy of electrical equipment used.

Electrical equipment	Energy (kW-h)
Vacuum pump (big)	2.20
Vacuum pump (small)	0.75
Water pump	0.37
Heater	2.50

APPENDIX B



RetTime [min]	Type	Area [nRIU*s]	Amt/Area	Amount [g/l]	Grp	Name
10.320		-	-	-		Xylose
11.340		-	-	-		Arabinose
12.739		-	-	-		Succinic acid
13.498		-	-	-		Lactic acid
13.836	MF T+	6.87973e6	4.50684e-6	310.05863		Glycerol
15.617		-	-	-		Acetic acid
18.243		-	-	-		Propionic acid
18.901	MF T+	8.92593e4	3.22305e-5	28.76874		Methanol
21.569		-	-	-		Ethanol
36.166		-	-	-		Butanol
Totals :				338.82737		

Figure 1 Representative HPLC chromatogram for the determination of glycerol and methanol in crude glycerol.

APPENDIX C

Experiment picture

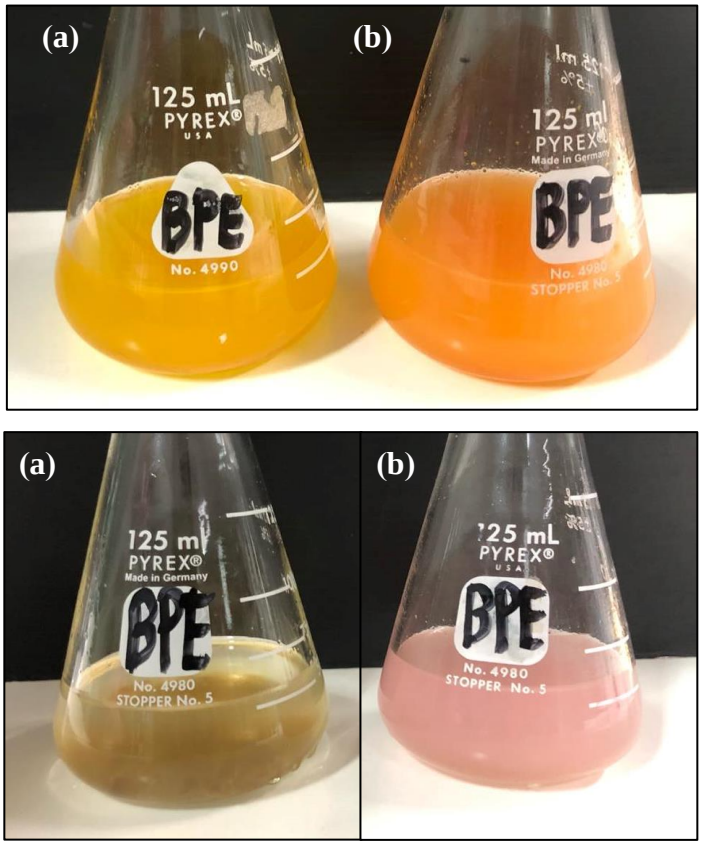


Figure 2 Color of sample before (a) and after (b) titration. (top: the oil before separation and below: the oil after separation)

Figure 3 FFA distillate.

Figure 4 Structure of internal free fatty acid separation reactor.

Figure 5 Photo of a reactor for separating FFA from CPO.

Figure 6 Ester (oil) washing with distilled hot water.

(a) **(b)**

Figure 7 Color of (a) crude glycerol and (b) commercial glycerol.

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The Higher Graduate Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission (AGR570600S), Prince of Songkla University

The Thailand Research Fund (RTA6080010)

List of Publications and Proceedings

International conference

Tohpong, N. Duangsuwan, W. and Prasertsan, P. 2018. Feasibility of Vacuum Distillation of Free Fatty Acids (FFAs) from Crude Palm Oil (CPO) Recovered from Palm Oil Mill Effluent (POME): Preliminary Test Using the Very High FFA CPO. Water and Environment Technology Conference 14th-15th July 2018 Ehime University, Johoku campus (Oral and poster presentation)

Proceedings

Tohpong, N. Duangsuwan, W. and Prasertsan, P. 2018. Vacuum separation of free fatty acids (FFA) from high-free fatty acids crude palm oil (HFFA-CPO). The 2nd Graduate School Conference 2018 of Suan Sunandha Ralabhat University on Nov 30, 2018, Bangkok, Thailand (Excellence oral presentation award)