CHAPTER III

EXPERIMENTAL AND ANALYTICAL METHODS

3.1 Materials

3.1.1 Palm Fatty Acid and Palm Stearin

Palm fatty acid and palm stearin were derived from a process palm oil. This process was established mainly to add value to byproducts from the refinery. The raw materials Palm Fatty Acid Distillate (PFAD) and Refined Bleached Deodorized (RBD) were obtained from refinery.

3.1.2 Chemicals

All chemical used in the experiment are shown in Table 3.1. They were used without purification.

Table 3.1 Chemicals used in the research

Name	Source	Purity
Methanol	Fisher Scientific	99.8% (analytical reagent grade)
Potassium hydroxide	Ajax Chemicals	98% (analytical reagent grade)
Sulfuric acid	Ajax Chemicals	98% (analytical reagent grade)
Tetrahydrofuran	Fisher Scientific	99.9% (analytical reagent grade)
Ethanol	Fisher Scientific	95% (analytical reagent grade)
Diethyl ether	Fisher Scientific	99.8% (analytical reagent grade)
Oleic acid	Aldrich	90%
Stearic acid	Sigma-aldrich	95%
Methyl palmitate	Acros	95%

3.2 Equipment

3.2.1 Transesterification and esterification of palm fatty acids and palm stearin using potassium hydroxide and sulfuric acid catalysts.

A 250-ml flask equipped with a reflux condenser, thermometer and a sampling port was used in the experiment. The flask was immersed in a water bath on a magnatic stirrer used to provide agitation. The experimental set-up is shown in Figure 3.1
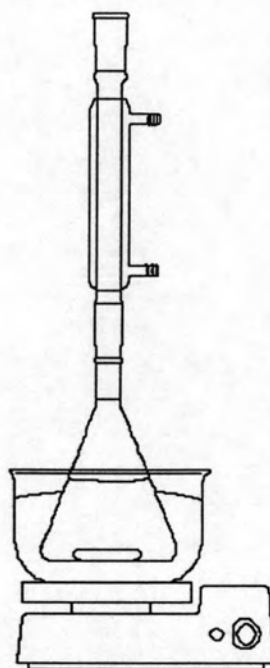
Hot plate and magnetic stirrer

Figure 3.1 Experimental set-up used for the transesterification and esterification.

3.2.2 Analysis of fatty acid composition of the palm fatty acid and palm stearin using gas chromatography.

After the reaction of triglycerides, the products were the mixtures of methyl ester, glycerol, methanol and the catalyst. After the products were cooled to ambient temperature the methyl ester layer and the glycerol were separated from one another. The top phase was the methyl ester layers and the bottom phase was the glycerol layer. Since the methyl ester was our interest in the study, it was further purified by washing with distilled water, which was recommended for the best purification. The sample of 0.5g was dissolved in 1.5 g of THF, 0.5 µl of the solution was injected into the column of gas chromatograph Perkin elmer 8700 shown in Figure 3.2 for analysis of composition. The composition was studied as a function of time.

Figure 3.2 Gas chromatograph for analysis of methyl ester

3.3 Methodology

3.3.1 Analysis of Palm Fatty Acids and Palm Stearin

The palm fatty acids and palm stearin were analyzed based on the density at 60 °C

3.3.1.1 Acid Value (AOCS Cd – 3D – 63)

3.3.1.2 Saponification Value (AOCS Cd – 3B – 76)

3.3.1.3 Free Fatty Acid Content (AOCS Ca 5A – 40)

3.3.2 Investigation the effect of base and acid catalyst on transesterification and esterification

3.3.2.1 Base catalysts for transesterification

1. The free fatty acid content in palm stearin was firstly determined so that KOH can be used for its neutralization. (It was found 0.31% FFA.)

2. The refined palm stearin was then heated above 100°C to reduce the water content. The experiment set up is shown in Figure 3.3.

3. Weigh 500 g of palm stearin was into a 1000 ml conical flask about one necked glass flask with a reflux condenser to avoid methanol, 100%mol excess of methanol from its stoichometric and 1%KOH as catalyst are added to the flask.

4. The transesterification reactions were carried out at 60°C under stirring at 750 rpm for 120 min.

5. About 10 ml of the sample was collected at every 10 mins for gas chromatography analysis.

6. The samples are separated of the methyl ester and glycerol phase.

7. The methyl ester production in the top layer was washed with distilled water until the water layer was pH 7.

8. The water present is eliminated by heating to above 100°C to boil off water to reduce water content.

9. The samples were collected for gas chromatography analysis.

3.3.2.2 Acid catalysts for esterification

    1. The refined palm fatty acid was then heated above 100°C to reduce the water content. The experimental set up is shown in Figure 3.3.

    2. Weigh 500 g of palm fatty acid was into a 1000 ml conical flask about one necked glass flask with a reflux condenser to avoid methanol, 100%mol excess of methanol from its stoichometric and 1%H_2SO_4 as catalyst are added to the flask.

    3. The reactions were carried out at 60°C under stirring at 750 rpm for 120 min.

    4. About 10 ml of the sample was collected at every 10 mins for gas chromatography analysis.

    5. The samples were separated of the methyl ester and water phase.

    6. The methyl ester production in the top layer was washed with distilled water until the water layer was pH 7.

    7. The water present was eliminated by heating to above 100°C to boil off water to reduce water content.

    8. The samples were collected for gas chromatography analysis.

3.3.3 Transesterification and esterification of palm stearin and palm fatty acid use base and acid catalyst.

    1. The refined palm stearin and palm fatty acid were then heated above 100° C to reduce the water content.

    2. Weigh 100 g of palm stearin and palm fatty acid were into a 250 ml conical flask about one necked glass flask with a reflux condenser to avoid methanol, different amount of methanol (100%mol, 300%mol excess of methanol from its stoichometric ratio different amount of catalyst (0.5 and 10.0 wt% of catalyst) are added to the flask.

    3. The reactions were carried out at 60°C under stirring at 750 rpm for 60 min.

    4. Samples are taken out from the reaction were collected for gas chromatography analysis.

5. The samples were separated of the methyl phase

6. The methyl ester production in the top layer was washed with distilled water until the water layer was pH 7.

7. The water present is eliminated by heating to above 100°C to boil off water to reduce water content.

8. The samples were collected for gas chromatography analysis.

Before   After

Figure 3.3 Heating palm stearin and palm fatty acid

Table 3.2 Conditions used in transesterification and esterification of palm stearin and palm fatty acid with methanol

Reactant	Catalysts	Amount of catalyst (wt%)	% mol excess of methanol
25%FFA	KOH	0.5	100
25%FFA	KOH	0.5	300
25%FFA	KOH	1.0	100
25%FFA	KOH	1.0	300
25%FFA	H_2SO_4	0.5	100
25%FFA	H_2SO_4	0.5	300
25%FFA	H_2SC_4	1.0	100
25%FFA	H_2SO_4	1.0	300

Reactant	Catalysts	Amount of catalyst (wt%)	% mol excess of methanol
50%FFA	KOH	0.5	100
50%FFA	KOH	0.5	300
50% FFA	KOH	1.0	100
50%FFA	KOH	1.0	300
50%FFA	H_2SO_4	0.5	100
50%FFA	H_2SO_4	0.5	300
50% FFA	H_2SO_4	1.0	100
50%FFA	H_2SO_4	1.0	300
75%FFA	KOH	0.5	100
75%FFA	KOH	0.5	300
75%FFA	KOH	1.0	100
75%FFA	KOH	1.0	300
75%FFA	H_2SO_4	0.5	100
75%FFA	H_2SO_4	0.5	300
75%FFA	H_2SO_4	1.0	100
75%FFA	H_2SO_4	1.0	300
100%FFA	KOH	0.5	100
100%FFA	KOH	0.5	300
100%FFA	KOH	1.0	100
100%FFA	KOH	1.0	300
100%FFA	H_2SO_4	0.5	100
100%FFA	H_2SO_4	0.5	300
100%FFA	H_2SO_4	1.0	100
100%FFA	H_2SO_4	1.0	300
Palm Stearin	KOH	0.5	100
Palm Stearin	KOH	0.5	300
Palm Stearin	KOH	1.0	100
Palm Stearin	KOH	1.0	300

Reactant	Catalysts	Amount of catalyst (wt%)	% mol excess of methanol
Palm Stearin	H_2SO_4	0.5	100
Palm Stearin	H_2SO_4	0.5	300
Palm Stearin	H_2SO_4	1.0	100
Palm Stearin	H_2SO_4	1.0	300

3.3.4 Analysis of methyl esters

The samples of methyl esters were analyzed by Perkin elmer 8700 gas chromatograph with a flame ionization detection system. There were separations carried out on a OV-5 fused silica column (30 m × 0.32 mm × 0.25 micron; Ohio Valley. Marietta, Ohio). The operating conditions are in Table 3.3. The temperatures of injector and detector were set at 310°C, and the carrier gas was 9.5 Psig. The split ratio was 1:2 and carrier gas used was helium. The samples of 0.5 g were dissolved in 1.5 g of THF and 0.5 µl sample was injected onto the column.

Table 3.3 Conditions analyzed by Perkin elmer 8700 gas chromatograph

Oven temperature	Iso time.	Ramp rate
1=120°C	1=0.0	1=1.0
2=130°C	2=0.0	2=15.0
3=200°C	3=0.0	3=10.0
4=310°C	4=5.0	4=0.0

Analysis of methyl ester in product by gas chromatography (GC). The reaction time of each methyl ester is different. Therefore, for find the type of methyl ester by compare retention time of each methyl ester with methyl ester standard. The retention time are shown in Table 3.4.

Table 3.4 Retention time of methyl ester in GC chromatogram

Number of peak	Retention time (min)	Peak of sample
1	3.33	Tetrahydrofuran
2	20.76	Methyl palmitate
3	22.69	Methyl oleate + Methyl linoleate
4	22.94	Methyl stearate

The concentration of methyl esters is defined as a ratio of weight of methyl ester, which was determined by using gas chromatography (GC) to weight of sample, which may consist of mono-, di- and triglycerides as shown in Equation 3.1.

$$\% \text{ Methyl esters} \quad = \quad \frac{\text{weight of methyl esters}}{\text{Weigh of sample}} \times 100 \qquad (3.1)$$

By ; weight of methyl esters calculate from calibration curve determined by GC

3.4 Experimental and Analytical Error

3.4.1 Experimental Error

In this section, the experiments were conducted to verify repeatability, an average, and a standard deviation value of the experiment. In Equation 3.1 and Equation 3.2 define an average value and percent deviation respectively.

$$\text{Average value, } \overline{X} \quad = \quad \frac{\sum x}{n} \qquad (3.2)$$

$$\text{Standard deviation from average value} \quad = \quad \sqrt{\frac{\sum (X - \overline{X})^2}{n}} \qquad (3.3)$$

Methyl ester is prepared from palm stearin with 100%mol excess of methanol. The condition was kept constant at 1%KOH on the basis of the palm stearin at 60°C for 120 minutes from three experiments.

The concentration of methyl esters (wt%) were measured as a function of time in transesterification.

Table 3.5 Results of concentration of methyl ester (wt%) from 3 experiments.

Time.(min.)	Concentration of methyl ester total (wt %)				
	Ex.No.1	Ex.No.2	Ex.No.3	Average	Y Error bar
0	0.00	0.00	0.00	0.00	0.00
10	80.19	87.55	67.54	78.43	6.55
20	85.47	94.28	85.25	88.33	6.27
30	76.62	93.27	81.11	83.67	5.82
40	77.11	90.00	73.93	80.35	5.38
50	78.11	88.10	69.36	78.52	5.04
60	72.85	86.66	81.20	80.24	4.26
75	76.39	85.04	77.53	79.65	3.74
90	71.32	84.91	78.44	78.23	3.67
105	77.56	81.85	78.22	79.21	1.67
120	77.57	80.02	79.20	78.93	1.25

The results shows that concentration of methyl ester average are almost constant. It indicates that transesterification of part stearin section it equilibrium conversion with in 40-60 minutes and methyl ester concentration of appropriate 80% was obtained. Based on this study, a section time of 60 minutes was used in subsequent study.
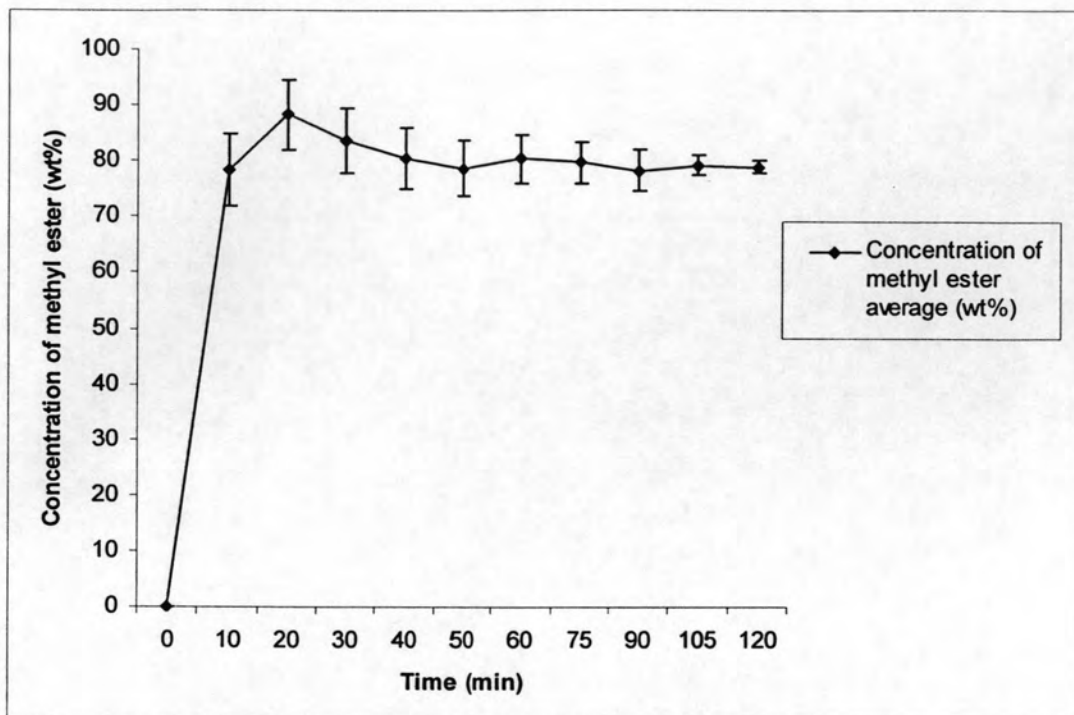
Figure 3.4 Percent deviation from mean on experiment of transesterification of palm stearin with methanol.

3.4.2 Analytical Error

In this section, from experiment the same sample was analyzed for 3 times by gas chromatography at the same condition. The condition constant at 0.5% KOH on the basis of the palm stearin, 100%mol excess methanol from its stoichiometric ratio and 60°C for 1 hour. Average percents methyl esters and deviation of these analysis are shown in Table 3.6.

Table 3.6 Concentration of methyl esters average (wt%) and % deviation from analysis of transesterification of palm stearin with methanol.

Concentration of methyl ester (wt %)			Concentration of methyl ester average (%)	Deviation from mean (%)
Analysis 1	Analysis 2	Analysis 3		
64.84	65.28	68.27	66.13	1.52

The result shows that the percents deviation concentration of methyl esters analysis is 1.52%.