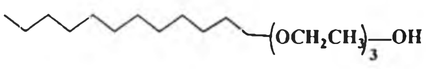
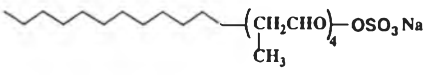
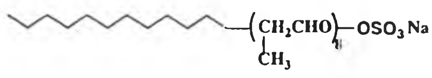
CHAPTER III
EXPERIMENTAL

3.1 Materials

3.1.1 Surfactants

Nonionic surfactants used in this research were fatty alcohol ethoxylate with 3 Ethylene oxide (EO) groups (dehydol LS3) and fatty alcohol ethoxylate with 9 EO groups (dehydol LS9) manufacture reported purity of 99.7%, obtained from Thai Ethoxylate Co., Ltd. Anionic extended surfactants were sodium alkyl polypropylene oxide sulfate surfactant with 4 propylene oxide (PO) groups and 8 PO groups (Alfoterra 123-4PO and Alfoterra 123-8PO) which contain 30% activity (provided by Sasol North America Inc.). Properties of the surfactants are summarized in Table 3.1.

Table 3.1 Properties and characterization of selected surfactant

Chemical formula	Structure	Type	MW
$C_{12,14}H_{25,29}{-}(EO)_3{-}OH$		Nonionic	333
$C_{12,14}H_{25,29}{-}(EO)_9{-}OH$		Nonionic	597
$C_{12,13}H_{25,27}{-}(PO)_4{-}SO_4Na$		Anionic	527
$C_{12,13}\ H_{25,27}{-}(PO)_8{-}SO_4Na$		Anionic	766

3.1.2 Oil

Crude palm oil is conducted as a model oil before bleaching process, in this work supplied by Bangchak Biofuel Co., Ltd.

3.1.3 Spent Bleaching Earth (SBE)

Spent bleaching earth used in this study is a solid waste after bleaching process, which was obtained from Bangchak Biofuel Co., Ltd.

3.2 Equipments

- Surface tension tensiometer (Krüss, K10T)
- Spinning drop tensionmeter (Dataphysics, Model SVT20)
- Gas chromatography coupled with a flame ionized detector (GC-FID)
- Ultraviolet-visible spectrophotometry (UV/VIS, Shimadzu 2550)
- High Performance Liquid Chromatography with Evaporative Light Scattering Detector (HPLC-ELSD, model Shimadzu-10Avp)
- Scanning electron microscopy (SEM, HITACHI S-3400N)
- Fourier transform infrared spectroscopy (FTIR)
- Soxhlet extractor
- Vortex
- Hot plate with stirrer
- Centrifuge
- Incubator

3.3 Chemicals and Solvents

- Sodium Chloride, analytical grade with 99% purity, was purchased from Lab Scan Co., Ltd.
- Hexane, analytical grade with 99% purity, was purchased from Lab Scan Co., Ltd.
- Ethyl alcohol with 95% purity
- 1% phenolphthalein solution in alcohol

- Sodium hydroxide
- Isopropanol (HPLC grade)
- Methanol (HPLC grade)

3.4 Experimental Procedures

This research experiment was divided into two studies. The first part is the microemulsion formation and another part is the oil extraction. The overall of experimental process is illustrated in Figure 3.1 and 3.2.

1. Microemulsion formation

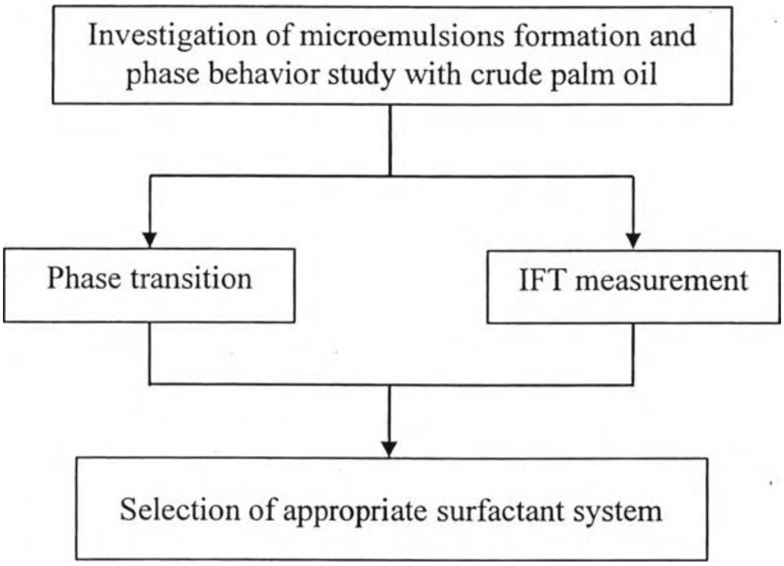


Figure 3.1 Flow chart of surfactant selection for oil extraction.

2. Oil extraction

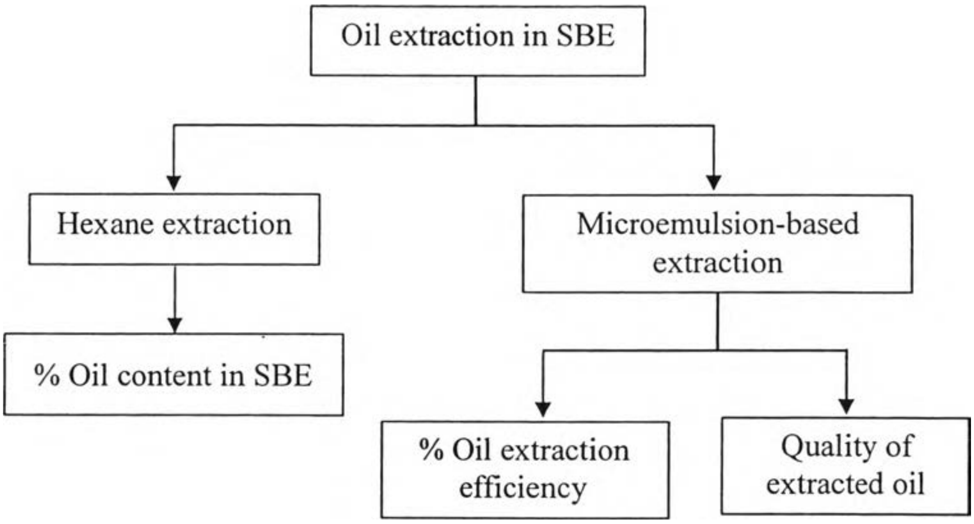


Figure 3.2 Flow chart of residual oil extraction in SBE.

3.4.1 Microemulsion Formation

3.4.1.1 CMC Measurement

The surface tension and critical micelle concentration (CMC) of all surfactant systems were determined with and without electrolyte concentration using a Wilhelmy plate tensiometer (DCAT 11, DataPhysics, Filderstadt, Germany) with a platinum plate at room temperature (25 °C).

3.4.1.2 Microemulsion Phase Bevaior

Dynamic interfacial tension measurements were conducted to evaluate the interaction between the aqueous surfactant solution and crude palm oil using spinning drop tensiometer (Dataphysics, Model SVT20). These experiments were measured the optimum salt concentration for anionic surfactant solution of 0.5 wt% maintained at a constant temperature (25±2°C), temperature scans were not evaluated for nonionic surfactants. The CμC point of each surfactant was also determined by varying surfactant concentration. For each sample was continually recorded the IFT value until reached equilibrium at constant value within 20 min because the system reached equilibrium. Furthermore, the phase behavior study was observed by placing equal volume (5 ml) of aqueous surfactant solution and crude palm oil. The sample were hand-shaken for 1 minute in the first 3 days and left to reach a state of equilibrium at 30 °C for 2 weeks (Nguyen *et al.*, 2010 and Kadioglu *et al.*, 2011).

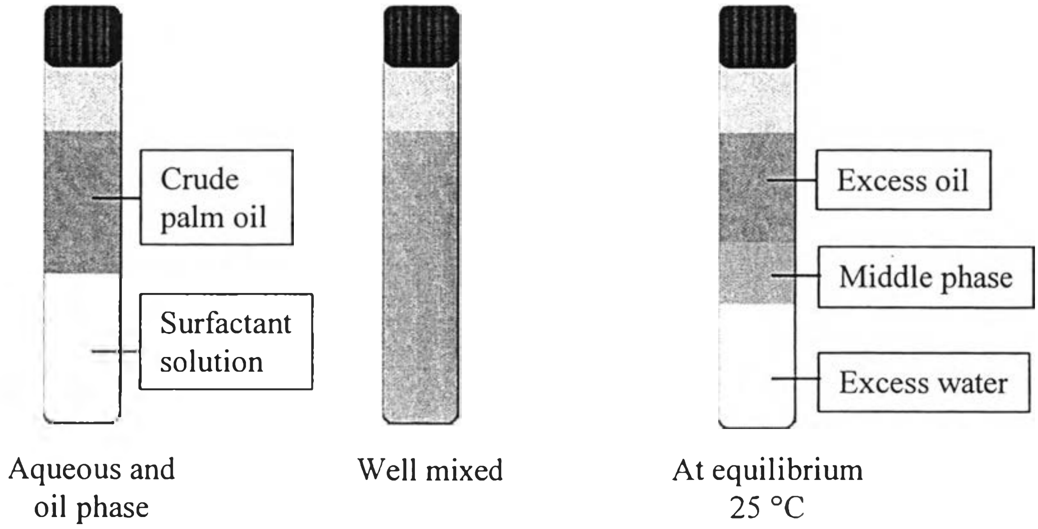


Figure 3.3 Experimental procedure of phase transition.

3.4.2 Oil Extraction

3.4.2.1 Oil Content in SBE

The moisture content was determined by weighing the SBE before and after drying in the oven until the weight remains constant. The range of moisture level was about 7 to 8%. The amount of total oil adsorbed in SBE (TO) and residual oil (RO) after through microemulsion extraction process was measured by hexane extraction. The procedure was carried out using 30 ml of hexane, stirring 1000 rpm, 20 min contact time and 3 g SBE loading at room temperature. For scale-up and extracted oil quality, the amount of oil was measured by soxhlet extraction with hexane. The SBE (20 g) was packed in thimble and 200 ml of hexane was used. The extraction process heated continually to 70 °C until the extracted hexane was colorless. The mixer between extracted oil and hexane was evaporated in a rotary vacuum evaporator. Percentage of total oil content in SBE was calculated based on weight of dried SBE for initial oil extraction.

3.4.2.2 Microemulsions-Based Extraction

Dried spent bleaching earth (3 g) was put in 30 ml of surfactant solution. The mixture was mixed for 30 min, 1,000 rpm and room temperature (25±2°C). The slurry was centrifuged for 20 min at 4,500 rpm. The sample was separated into two fractions; the aqueous solution and solid fraction. The liquid part was then centrifuged for 20 min at 4500 rpm. After centrifuge, excess oil was obtained on top. For quantification purpose only, 30 ml of hexane was added to recover the extracted oil place in separatory funnel for 10 min. The two immiscible phases were obtained. The upper phase (extracted oil and hexane) was evaporated to remove hexane and weighed the remaining residual oil. This method was shown in Figure 3.4. For solid part, the sample was washed with 30 ml of water and then was centrifuged for 10 min at 4,500 rpm. It was heated to 110 °C for removing water. For oil extraction experiments, each sample was conducted in triplicate (Tuntiwiwattanapun *et al.*, 2013).

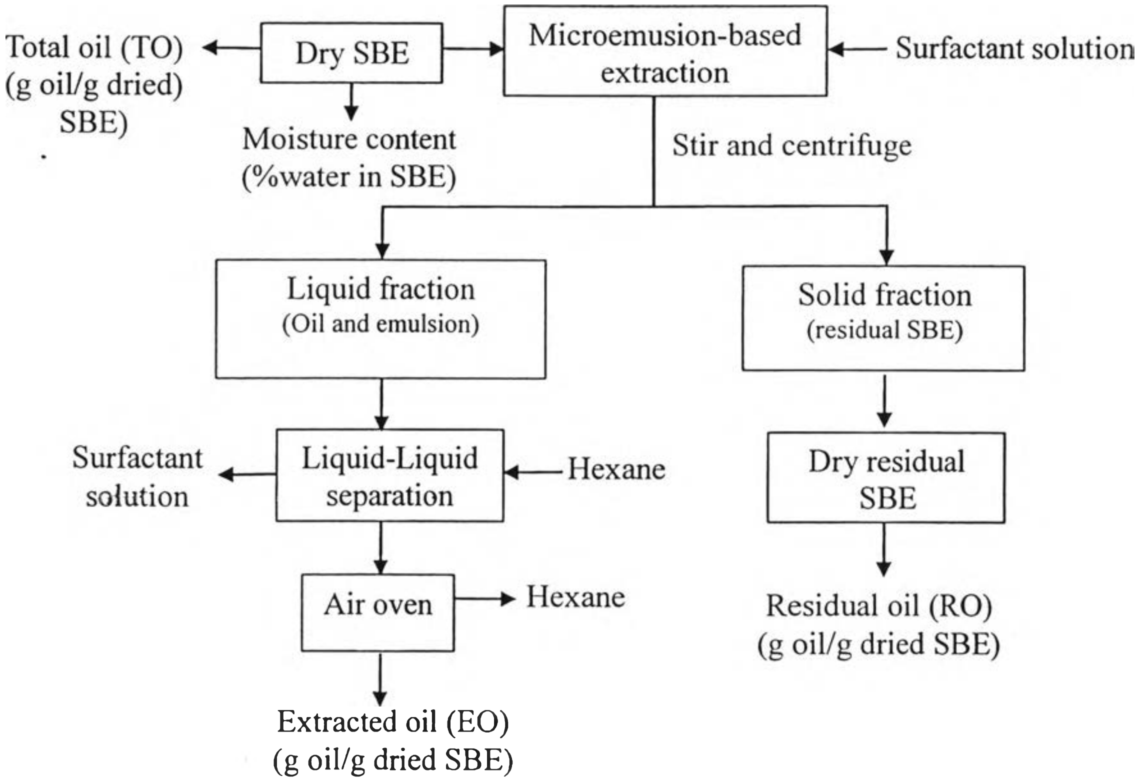


Figure 3.4 Diagram of microemulsion based extraction.

The amounts of dried SBE and extracted SBE were extracted via hexane extraction defined as the total oil (TO) and residual oil (RO), respectively. The oil extraction efficiency was calculated based on the weight of extracted oil (EO) relative to the weight of total oil as shown in Equation (3.1). In addition, the total oil extraction efficiency was determined following Equation (3.2).

$$\text{Free oil extraction efficiency (\%)} = \frac{\text{EO}}{\text{TO}} \times 100\ \% \qquad (3.1)$$

$$\text{Total oil extraction efficiency (\%)} = \frac{\text{(TO-RO)}}{\text{TO}} \times 100\ \% \qquad (3.2)$$

The extraction process was studied in three effects and all extraction experiments were conducted in triplicates.

- Salt concentration

The effect of salt concentration on oil extraction efficiency was evaluated in the range of 1 to 8 wt%. The salt concentration that yields the highest oil extraction efficiency was selected for the subsequent experiments.

- Surfactant concentration

The effect of aqueous surfactant on oil extraction efficiency was evaluated in the range of 0.08 to 2 wt%. The surfactant concentration that yields the highest oil extraction efficiency was selected for the subsequent experiments.

- Contact time

The effect of extraction times on oil extraction efficiency was evaluated at four levels; 5, 10, 20, 30, 60 and 120 min. The optimum contact time that provides the highest oil extraction efficiency was selected for the subsequent experiments.

- Solid to liquid ratio

The effect of solid-to-solvent ratio (g SBE/mL solvent) on oil extraction efficiency was investigated at five levels; 1/10, 2/15, 1/6, 1/5, 3/10, 4/10 and 1/2 (w/v).

3.4.3 Quality of Extracted Oil and Residual SBE

The extracted oils from solvent and microemulsions-based extraction were analyzed and characteristics of extracted oil from two extraction methods were compared.

3.4.3.1 Color and Carotene Content

Physical color appearance of extracted oil was analyzed by visual observation. Carotene content of extracted oil was also analyzed. The concentration of β-carotene was measured using UV/VIS spectrophotometer (Shimadzu, 2550). Prior to analysis, The samples were melted at 60–70 °C and homogenised thoroughly. About 20 mg of extracts were diluted with 7 mL of hexane. The solution was transferred to a 1-cm quartz cuvette and the absorbance was read at 450 nm, which corresponds to the maximum absorption of carotenoids. The concentration of β-carotene content is expressed as ppm using the following formula; (Mustapa, 2011)

$$\text{Concentration of } \beta\text{-carotene} = V \times \frac{383}{1000\ W} \times (a_s - a_b) \tag{3.3}$$

Where: a_s = adsorbence of sample at 446 nm

a_b = cuvette error (based on three measurement)

383 = extinction coefficient for carotenoids

V = volume used for analysis

W = weight of sample in grams

3.4.3.2 Free Fatty Acid (FFA)

Ethyl alcohol can be neutralized by adding 2-3 drops of phenolphthalein and then add 0.5M sodium hydroxide (NaOH) drop-wise to give a faint permanent pink color. The sample of 10 g into a clean, dry conical flask and add 100 ml of neutralised ethanol. The mixture was heated on the hotplate until it is almost boiling. A few of phenolphthalein was added to the mixture and titrate with 0.5M NaOH with swirling the flask. The end-point of titration was a pink color of the same color as in the neutralized ethanol developed and persisted for 30 second.

$$\%\ \text{Free fatty acid (as oleic acid)} = \frac{T \times M \times 28.2}{W} \tag{3.4}$$

where: T = Titration value (ml of NaOH)

M = Molarity of NaOH

W = Weight of sample (g)

3.4.3.3 Triglyceride

The triglyceride composition in extracted oil was analyzed using HPLC-ELSD. The column used in this research was C18 column, 5 µm, 4.6×250 mm (Inertsil® ODS-3, Japan). The mobile phase system consisted of methanol and isopropanol (gradient elution: starting at 100% methanol and ending at 15% methanol after 30 min) with a flow rate of 0.75 mL/min. The detector temperature and pressure of ELSD were 40 °C and 2.1 - 2.2 bar, respectively.

3.4.3.4 Fatty Acids Composition

Fatty acids composition was analyzed using gas chromatography with a flame ionized detector (GC-FID) after their derivatization from nonvolatile fatty acids chemically converted to volatile methyl esters. The methyl ester from extracted oil was reacted through tranesterification following method of Lepage and Roy (1986).

3.4.3.5 SBE Analysis

The microstructure of SBE, residual SBE obtained microemulsion based extraction and residual SBE from hexane extraction was characterized by Scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR).