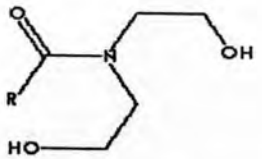
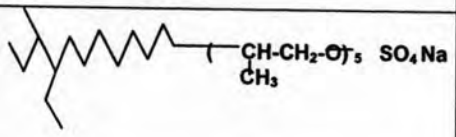
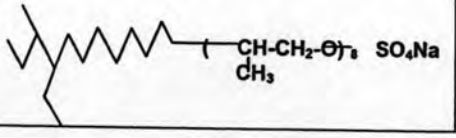
CHAPTER III
METHODOLOGY

3.1 Materials

3.1.1. Surfactants

Surfactants used in this research were coconut fatty acid diethanolamide (Comperlan KD), which is a nonionic surfactant with 97% activity (obtained from Henkel) and anionic surfactants Alfoterra145-5PO and Alfoterra5-8PO which were obtained from Sasol Co. Alfoterra145-5PO and Alfoterra5-8PO used in this study contain 28.7% and 29.1% active surfactants, respectively. Properties of these three surfactants are shown in Table 3.1

Table 3.1 Properties and selected characterization of surfactant

Chemical name	Structure	Type	MW
Coconut fatty acid diethanolamide (Comperlan)		Nonionic	280
Propoxylate sulfate with branched C14-C15 alkyl 5 Propoxy group (Alfoterra145-5PO)		Anionic	595
Propoxylate sulfate with branched C15 alkyl 8 Propoxy group (Alfoterra5-8PO)		Anionic	712.8

3.1.2 Oils

Oils used in this study were soybean oil and palm kernel oil .The commercial soybean oil was purchased from Loxley Trading. The raw palm kernel oil was obtained from LamSoong Thailand Co. Ltd.

3.1.3 Seeds

The seeds used in this study were soybean and palm kernel seeds. The commercial soybean seed was purchased from Krungsri Ayuthaya International Industrial Food. The palm kernel seed was obtained from LamSoong Thailand Co. Ltd.

3.1.4 Electrolytes

The electrolyte used in this research was sodium chloride, analytical grade with 99% purity, purchased from Lab Scan Co. Ltd.

3.1.5 Solvent

Solvents used in this research were n-hexane and methanol, Hexane, analytical grade with 99% purity, was purchased from Lab Scan Co.Ltd. Methanol (C_2H_5OH), HPLC grade with 99.9% purity was purchased from Carlo Erba reagent Co. Ltd.

3.1.6 Trifluoroaeitic acid

Trifluoroacetic acid ($C_2F_3O_2H$), analytical grade with 99.5% purity, was purchased from Fluka Co. Ltd. This chemical used as mobile phase for HPLC analysis.

3.1.7 Total Protein Kit

The components of protein kit set are protein standard solution which containing 100 mg/ml of bovine serum, Folin Ciocalteu's phenol reagent and Biuret reagent. The kit was purchased from Sigma Aldrich.

3.1.8 Dyed oil

Oil red O (solvent Red 27, Cl No. 261265) was purchased from Aldrich Chemical company, Inc. It was used for preparing the dyed oil.

3.1.9 Water

Ultra pure water was used throughout this research for preparation of aqueous surfactant solution and other chemical solutions. Furthermore, it used as rinse water and glassware cleaning.

3.2 Methodology

This research was divided into two parts. The first part is the phase behavior study and the second part is extraction study. Figure 3.1 and 3.2 show overall of the experimental procedure diagram.

1. Microemulsion formation and phase study

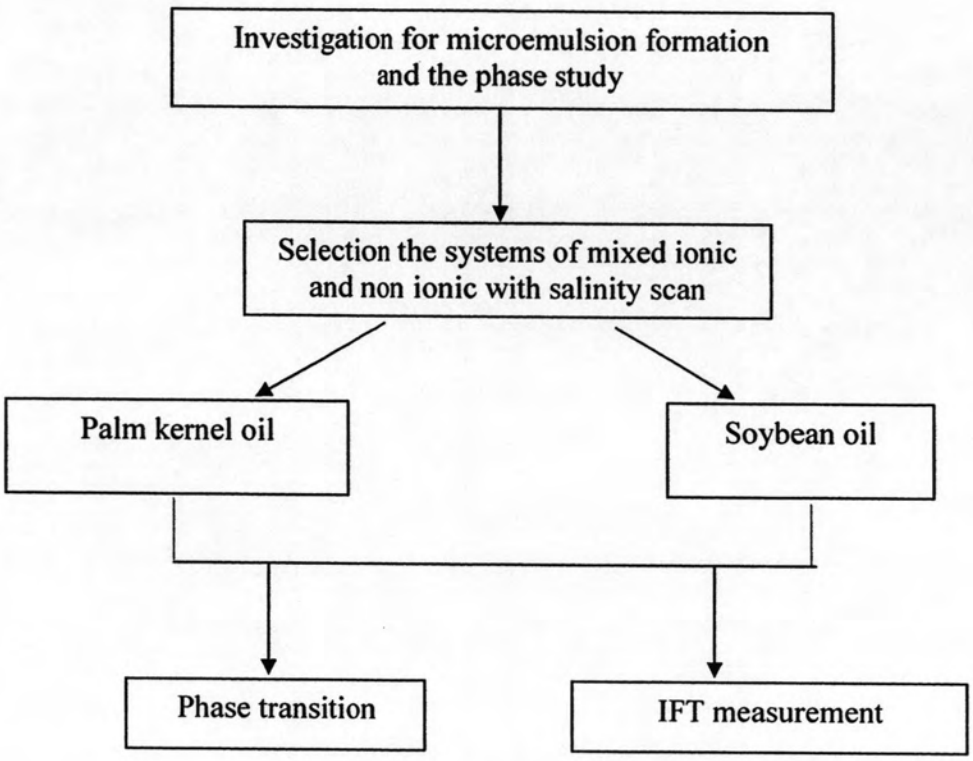


Figure 3.1 Flow chart of the step for the study on microemulsion and phase behavior

2. Vegetable oil extraction

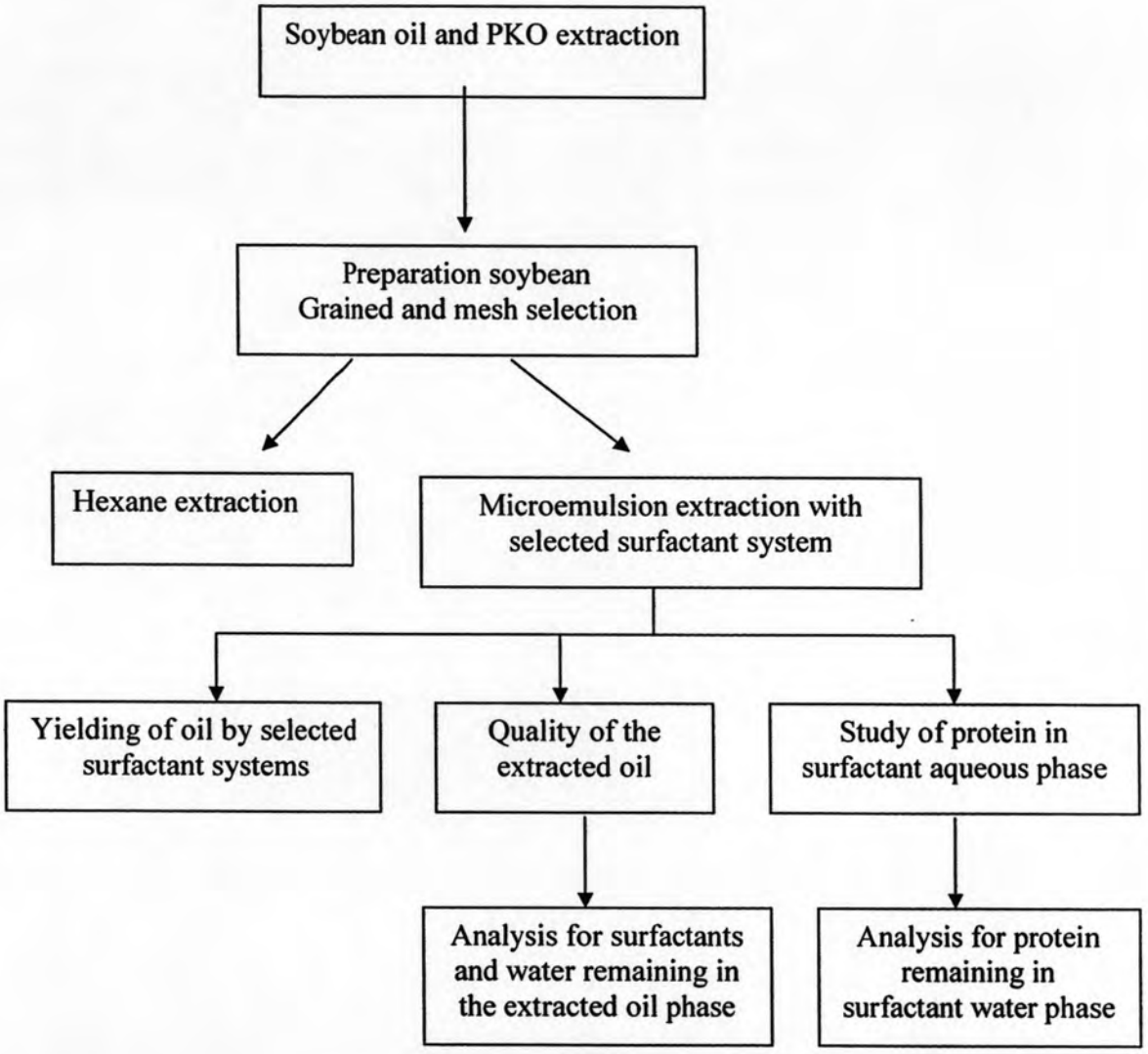


Figure 3.2 Flow chart of the step for the study on oil extraction and its quality

3.2.1 Microemulsion formation and phase study

The microemulsion transition was conducted by using salinity scan for mixed surfactant systems. Oil and surfactant ratio at unity was carried out in flat-bottomed test tube 15 mL by containing 5 mL each. Then the experimental tubes were gently shaken

with vortex in order to form microemulsion and were left to reach equilibrium at 30^0C. Since the oil and surfactant have similar color, the oil was dyed with the O-red dye before using in order to be easily to distinguish the phase transition. The phase transition was observed from the change of the volume ratio and by the interfacial tension. Then, the interfacial tension (IFT) between oil and surfactant solution phase was measured to determine the behavior of the system by spinning drop tensiometer (Dataphysics, Model SVT20). Table 3.2 shows the condition for surfactant systems for palm kernel oil. In the soybean oil case, the surfactant system used for the extraction was already investigated by the previous study. (Table B-1 in Appendix B)

Table 3.2 Surfactant system for phase behavior study which kept at 30°C

No	System	Salinity scan (%wt NaCl)
1	0.1% Alfoterra5PO + 3% Comperlan KD	0-20
2	0.2% Alfoterra5PO + 3% Comperlan KD	0-20
3	0.3% Alfoterra5PO + 3% Comperlan KD	0-20
4	0.1% Alfoterra8 PO + 3% Comperlan KD	0-20
5	0.2% Alfoterra8PO + 3% Comperlan KD	0-20
6	0.3% Alfoterra8PO + 3% Comperlan KD	0-20

3.2.2 Interfacial Tension Measurement in Batch Study

The interfacial tension (IFT) between lower phase or surfactant solution and upper phase (soybean oil or palm kernel oil) of each system were measured by a spinning drop tensiometer (Dataphysics, Model SVT20) to select the suitable surfactant solution system for extraction oil. Figure 3.3 shows the experimental procedure for interfacial tension measurement.

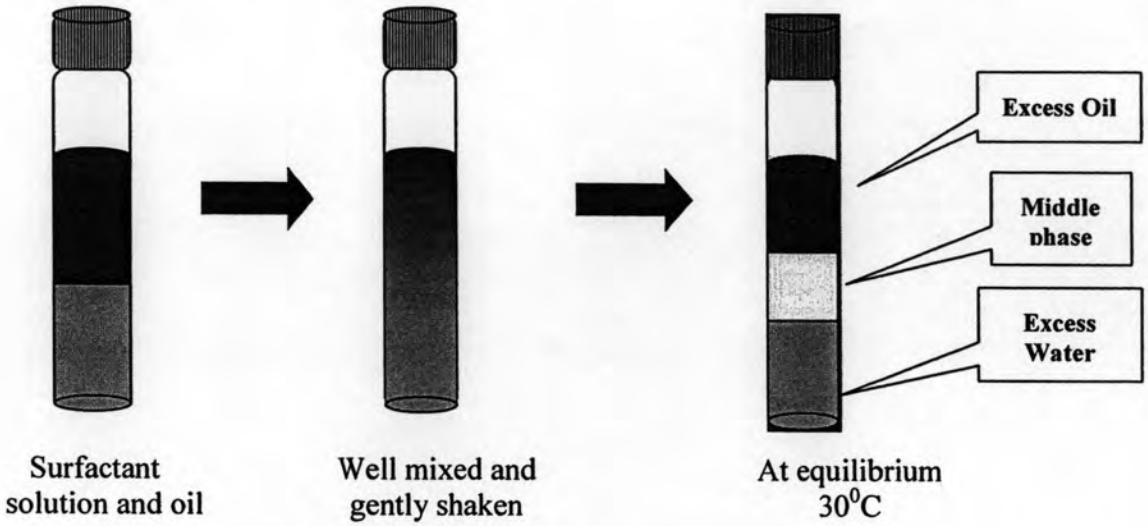


Figure 3.3 Experimental procedure of microemulsion formation

Figure 3.4 Spinning drop tensiometer

3.2.3 Vegetable oil extraction

3.2.3.1 Hexane extraction

Soybean and palm kernel were extracted with hexane by the soxlet method in order to investigate the extraction efficiency and compared with microemulsion system by using the same condition. The volume of extracted oil was measured to determine the yield of extraction. The Figure 3.5 shows the experimental set up for hexane extraction. After oil extraction by the soxlet, the solvent was evaporated out left the solely oils in the soxlet.

Figure 3.5 Apparatus set up for hexane extraction

3.2.3.2 Surfactant aqueous based extraction

Soybean oil extraction

The surfactant system able to form microemulsion with soybean oil was selected for this study. The extraction condition followed the previous study by using grain size of soybean: 0.212-0.425 mm or 35 Mesh – 65 Mesh, contact time 30 minutes and soybean load 1 g. The extraction study was conducted by 10 mL of mixed surfactant solution in the flat-bottomed test tube 15 mL. The soybean was dyed before extraction as mentioned earlier. The solution was gentle mixed after shaking in a vortex for 30 seconds at 180 rpm and was centrifuged for 15 minutes in order to separate the oil phase. Then the volume of extracted oil was measured to determine the yield. Three replications were conducted to examine the precision.

Palm kernel oil extraction

Palm kernel from Lamsoong Co. Ltd. was used for palm kernel oil extraction by the selected surfactant aqueous solution from the phase behavior study. To determine the optimum for palm kernel oil extraction, the following variables were studied; the salinity, grain size, contact time and palm kernel load in the extraction systems. In extraction stage, there two mixed surfactant systems of 0.1% Alfoterra 5PO and 3% Comperlan KD and 0.1% Alfoterra 8PO and 3% Comperlan KD with salinity scans from 1-20% NaCl were carried out.

The extraction study was conducted by graining palm kernel and mixed the meal with 10 mL of mixed surfactant solution in the flat-bottomed screw-capped test tube 15 mL by vortex for 30 second. Then the mix of three phases; free oil, aqueous surfactant and residue palm kernel meal were separated by using a centrifuge for 15 minutes. The

volume of free oil phase was measured to examine for the oil extraction yield. Three replications were conducted to examine the precision.

3.2.4 Oil quality

3.2.4.1 Soybean oil quality

In order to promote this extraction method, additional to an efficiency of extracted oil yielding, the quality of extracted oil was another indicator needed to be determined to compared with the oil extracted by hexane extraction. The parameters selected for determination of the quality of soybean extracted oil in this study are the surfactant partition in the extracted oil and amount of protein in water phase. The surfactant concentration in the aqueous phase was analyzed by using HPLC-ELSD for Comperlan KD. While concentration of Alfoterra in aqueous phase was analyse by using titration method ASTMD1681-92. The fatty acid composition was not determined in this oil due to the high volume of oil usage for analysis and the collection sample problems.

3.2.4.2 Palm kernel oil quality

For the Palm kernel oil in addition to the surfactant partition in the extracted oil, water content, fatty acid composition was determined. However, amount of protein was not determined for the palm kernel oil extraction because the mainly composition in kernel meal was not protein then the lost of valuable for usage as feedstock was not considered for this oil. The amount of water was measured by using Karl Fisher Titration Coulometer. The fatty acid composition was measured by GC-FID while the surfactant in the aqueous phases determination using the same method as mention for soybean quality.

3.2.5 Analytical method

3.2.5.1 GC-FID

GC-FID is the common technique method for analysis of fatty acids after their derivatization from nonvolatile fatty acids chemically converted corresponding to volatile methyl esters. There resulting volatile mixture can be analyzed by gas chromatography with a flame ionization detector (GC-FID). Capillary column supelco sp2560 (250 μm x 100 m).was used. The film thickness was 0.2 μm. The injection volume was 2 μL. The

operation condition was set firstly at initial temperature 175 ^{0}C at the oven, at inlet initial temperature 220^{0}C by Split mode at spit ratio 100:1 while the initial flow at column 1.2 mL/min and the temperature at detector was set at 230 ^{0}C.

3.2.5.2 HPLC-ELSD

HPLC-ELSD is the technique for analysis of surfactant remaining in aqueous phase. HPLC Shimadzu Model was used in this research. C_{18} reverse phase column (J'Sphere ODS-HSD 250 mm x 4.6 mm, diameter 4 μm) was used. The mobile phase system composed of Methanol and 0.2% Trifluoroacetic acid (TFA). The flow rate was used at 0.8 mL/min. The injection volume was 20 μL. The operation condition firstly mixed 70% Methanol and 30% of 0.2% Trifluoroacetic acid for 3 minutes after that increasing Methanol 95 for 42 minutes and to 100% at the last.

3.2.5.3 Spectrophotometer

Total protein kit, Micro Lowry, Onishi& Barr Modification methods, was used for protein determination in aqueous phase in this research. Protein in sample with peptide bonds formed complex under alkaline conditions and reacted with Folin reagent. The absorbance was read at suitable wavelength 750 nm. Protein concentrations are determined from calibration curve. This method is suitable for final protein concentration in the range of 150 – 1000 μg/mL.

3.2.5.4 Titration Method

ASTMD1681-92

ASTMD1681-92 is the method for determination concentration of Alfoterra in the aqueous phase. This method use titration technique with cationic surfactant, Hyamine 0.005 M for quantified Alfoterra, anionic surfactant concentration in sample.

Karl Fisher Titration

Karl Fisher Titration is the widely technique for water determination in oil phase. This method has advantages such as high specificity and precision, work over the wide range from ppm up to % and shorter determination time. This instrument analyzes together with pH and acid-base titration.