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ORIGINAL ARTICLE

Engkabang Fat Esters for Cosmeceutical Formulation

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Abstract Engkabang fat esters were synthesized from engkabang fat using an enzyme as catalyst. The main composition of the fat esters were oleyl palmitate, oleyl stearate and oleyl oleate. The percentage yield was 93.67%. Ternary phase diagrams systems containing fat esters/surfactant/water were constructed. Several regions appeared in the ternary phase diagrams such as isotropic, homogenous, liquid crystal, two-phase and three-phase regions. Increasing the hydrophilic-lipophilic balance value of the used surfactants gave a larger homogenous and isotropic region in ternary phase diagrams of engkabang fat esters/nonionic surfactant/deionized water. Isotropic and homogenous regions in the ternary phase diagram of engkabang fat esters: PEG-40 hydrogenated castor oil (2:1)/polyoxyethylene(20) sorbitan tri-oleate/deionized water, was the largest when compared to the other ternary phase diagrams. The isotropic and homogenous region can be used as a medium in formulation of cosmetics and pharmaceutical products such as creams, lotions, balms and lipsticks.

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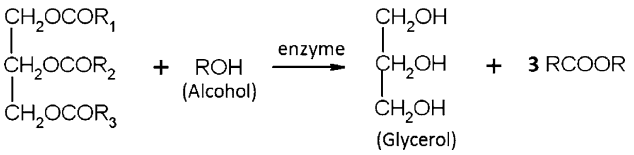
Introduction

In many industries, wax esters or fat esters have a wide range of applications as lubricants, polishes, plasticizers, antifoaming agents and coating materials in the medical and food industries, and as raw materials in cosmetics and other chemical industries [1, 2]. Particularly in cosmetics, fat esters are formulated in numerous personal care products due to their non-greasy, non-toxic and excellent emollient behavior [3]. Scheme 1 depicts the chemical reaction in the production of wax or fat esters from triglycerides and alcohol using an enzyme as a catalyst. Engkabang fat that was used as a starting material came from the seeds of engkabang trees. This engkabang tree is from the genus of '*Shorea*' which can be found in the forest of Borneo (Sarawak and Kalimantan) [4]. Engkabang fat which is also known as illipe butter is classified as an exotic butter that can moisturize the skin and restores elasticity. The physico-chemical properties of engkabang fat are very close to cocoa butter [4]. Engkabang fat has been used by the natives in Borneo for many centuries for food, therapeutic and cosmetic purposes [5].

The most common types of delivery systems used for cosmetics and pharmaceuticals products are emulsions. They are mixtures of two insoluble materials containing a water and an oil phase, which are stabilized against separation using an emulsifier surfactant. Emulsions are defined as heterogeneous systems in which at least one immiscible or barely miscible liquid is dispersed in another liquid in the form of tiny droplets of various sizes [6]. Emulsions



Scheme 1 Synthesis of engkabang fat esters (EFE)

designed by the emulsification process can be approximately tailored by the HLB system. Their behavior is influenced by the hydrophilic-lipophilic properties of the surfactants which are correlated with the affinity of their polar and nonpolar moieties towards the water and the oil, respectively [7]. The physical or physicochemical significant of the HLB scale could be ascertained by associating the HLB value with some surfactant properties [8]. The system indicates the relative strength of the hydrophilic and hydrophobic portions of the surfactant molecule and can be used to characterize the relative affinity of surfactants for aqueous and oil phases. A high HLB number generally indicates good surfactant solubility in water, while a low HLB number indicates a high relative affinity for the oil phase. Surfactant molecules exhibit a very peculiar behavior, which is inherent to their structure [8].

Surfactants with a high HLB tend to stabilize O/W emulsions whereas surfactants with a low HLB tend to stabilize W/O emulsions. An accurate determination of the hydrophilic-lipophilic nature of surfactants plays an essential role in guiding the formulation of emulsion [9]. HLB value smaller than 8 usually promote W/O emulsions, meanwhile HLB value within 10–16 is suitable for preparation of O/W emulsions. At an intermediate HLB value ranging with 8–11, the surfactant is balanced in favor for oil and water phases [10]. For the stabilization of oil-in-water emulsions, surfactants with an HLB value in the range 9–12 are optimal [11] and good for emulsification. Sulaiman et al. [9] had constructed ternary phase diagrams of oleyl oleate/surfactants/water. Through the construction of a ternary phase diagram, the phase behavior of the emulsion system was determined. Ternary phase diagrams of oil/surfactant/water exhibit stable emulsions which could then be used in many industries such as in the formulation of cosmetics and pharmaceutical products.

In this work, engkabang fat esters were synthesized by an enzymatic reaction of engkabang fat. The newly synthesized esters were used as the oil phase in the determination of the phase behavior of systems containing engkabang fat esters/non-ionic surfactant/water. The non-ionic surfactants used are in the group of fatty acid esters of sorbitan and their ethoxylated derivatives [12]. The toxicity of these surfactants is very low and thus they have wide applications in the food, pharmaceutical and cosmetic industries.

Experimental Procedures

Materials

Engkabang fat was obtained from Sarawak, Malaysia. Fatty acid compositions of engkabang fat are 43.7% stearic acid, 35.7% oleic acid, 19.9% palmitic acid, 0.4% linoleic: acid and 0.1% palmitoleic acid [13]. Sorbitan mono-oleate (Span80), sorbitan monolaurate (Span20), polyoxyethylene(20) sorbitan tri-oleate (Tween85) and polyoxethylene(20) sorbitan mono-oleate (Tween80) were purchased from Merck, Germany. The HLB values of sorbitan mono-oleate (Span80), sorbitan monolaurate (Span20), polyoxyethylene(20) sorbitan tri-oleate (Tween85) and polyoxethylene(20) sorbitan mono-oleate (Tween80) are 4.3, 8.6, 11.0 and 15.0, respectively. Oleyl alcohol (*cis*-9-Octadecen-1-ol) and *n*-heptane were also purchased from Merck, Germany. The used catalytic enzyme Lipozyme® RM IM was purchased from Novozymes, Denmark. PEG-40 hydrogenated castor oil (solubilisant gamma®2429/SG) was purchased from Gattefosse, USA. Deionized water was produced in the laboratory.

Synthesis of Engkabang Fat Esters

Engkabang fat esters were synthesized through alcoholysis reaction using an enzyme as catalyst. In the alcoholysis reaction, 10 mmol engkabang fat and 30 mmol oleyl alcohol and 1.5 g enzyme were placed in a capped reaction bottle and *n*-heptane was added to give a total volume of 100 mL. The reaction mixture was incubated in a horizontal water bath shaker at 150 rpm, at 40 °C for 5 h. The mixture was then filtered to separate the product mixture from the enzyme. *n*-Heptane was removed using a rotary vacuum evaporator at 98 °C. Engkabang fat esters were purified by adding ethanol in a separation funnel in a ratio of engkabang fat esters to ethanol of 1:3. The funnel was shaken to remove the glycerol which is soluble in the ethanol. The mixture was left to stand until two layers appeared. The engkabang fat esters were in the bottom layer and the ethanol containing glycerol was at the top. This step was repeated three times. Then, engkabang fat esters were collected from the separation funnel and kept for further use.

Thin Layer Chromatography (TLC)

Samples containing engkabang fat, oleyl alcohol and engkabang fat esters were separated on silica gel plates (Merck, DC-Aluminiumfolien Kieselgel 60 F_{254}). The eluent, leading to the best achievable separation of the different components, consisted of a mixture of *n*-heptane/diethyl ether (80:20 vol/vol). After elution, the plates were

dried and stored in an iodine chamber. The presence of the oleyl alcohol, engkabang fat and engkabang fat esters were detected as brown spots.

Gas Chromatography (GC)

GC analysis was conducted by injecting 0.5 μL aliquot of samples into a Shimadzu GC-9A gas chromatography apparatus in a split mode equipped with a flame-ionization detector and a RTX65 capillary column (30 m × 0.25 mm i.d.; film thickness 0.25 μm; Restex Corporation, USA). Injector and detector temperature were set at 300 and 320 °C, respectively. Oven temperature was maintained at 180 °C for 2 min, increased to 300 °C with the ramp of 20 °C/min and held for 10 min. Nitrogen was used as the carrier gas with a flow rate 50 mL/min. The product composition was quantified by an internal standard method with methyl arachidate as the internal standard.

Analysis of Yield of Engkabang Fat Esters

The product composition was quantified by an internal standard method with methyl arachidate as the internal standard. The amount of engkabang fat esters produced was obtained by the equation below;

$$C_X = \left(\frac{A_X}{A_{IS}}\right) \times C_{IS} \left(\frac{D_{R_{fIS}}}{D_{R_{fX}}}\right)$$

where C_X, amount of engkabang fat esters (oleyl palmitate, oleyl stearate and oleyl oleate); C_{IS}, amount of internal standard (methyl arachidate); A_X, area of engkabang fat esters; A_{IS}, area of internal standard; $D_{R_{fX}} = \left(\frac{A_X}{C_X}\right)$, detector response factor of engkabang fat esters; $D_{R_{fIS}} = \left(\frac{A_{IS}}{C_{IS}}\right)$, detector response factor of internal standard.

Thus, the percentage yield $(\%) = \left(\frac{\text{mmol ester produced}}{3 \times \text{mmol engkabang fractions used}}\right) \times 100\%$

Construction of Ternary Phase Diagrams

Engkabang fat esters/nonionic surfactant/deionized water were weighed at various proportions ranging from 0:100 to 100:0 [fat esters/nonionic surfactant: water (w/w)]. The mixture with a total weight of 0.5 g was placed in a 10-mL screw-cap glass tube. The mixture was then vortexed/shaken for 5 min. The samples were then centrifuged for 15 min at 4,000 rpm. The phase behaviors of the samples were examined through cross-polarized light. The experiment was repeated with the addition of deionized water according at percentages from 0 to 100%. The phase behaviors that were obtained from the observation were shown in the ternary phase diagrams. Five ternary phase diagrams were constructed using four nonionic surfactants. Comparison among the ternary phase diagrams were made to see the changes happen when different HLB value of the nonionic surfactants were used.

Results

Analysis of Products

Figure 1 shows the TLC analysis of engkabang fat esters. Lane 1 represented oleyl alcohol with R_f value 0.11 whilst engkabang fat was shown at lane 2 with R_f value of triglyceride at 0.35. Trace amount of diglycerides and monoglycerides were detected in engkabang fat esters with the R_f value 0.05 and 0.01 respectively. The formation of a new product was shown in lane 3 which was the spot of engkabang fat esters. Figure 2 depicts the chromatogram of engkabang fat esters after the alcoholysis reaction catalyzed by the enzyme.

Phase Behavior of Engkabang Fat Esters (EFE)

Figures 3, 4, 5, 6 and 7 depict the ternary phase diagram of engkabang fat esters EFE/sorbitan mono-oleate/deionized water, EFE/sorbitan mono-laurate/deionized water, EFE/polyoxyethylene (20) sorbitan tri-oleate/deionized water, EFE/polyoxethylene (20) sorbitan mono-oleate/deionized water, and EFE:PEG-40 hydrogenated castor

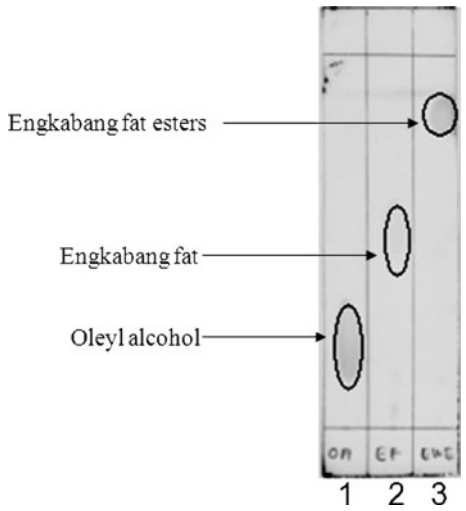


Fig. 1 Thin layer chromatogram of oleyl alcohol (*lane 1*), engkabang fat (*lane 2*), and engkabang fat esters (*lane 3*)

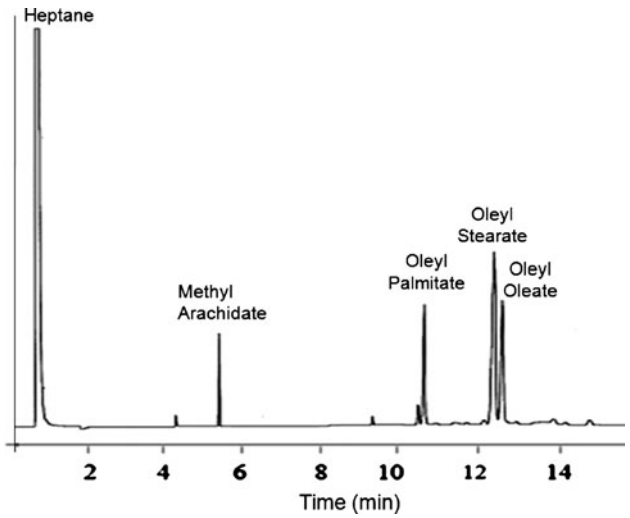


Fig. 2 A representative chromatogram of purified engkabang fat esters

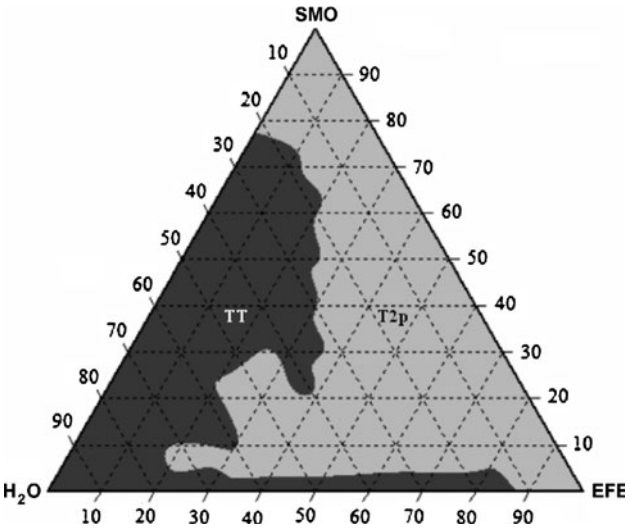


Fig. 3 Ternary phase diagram of EFE/sorbitan mono-oleate SMO/deionized water. *T2p* two-phase region and *TT* three-phase region

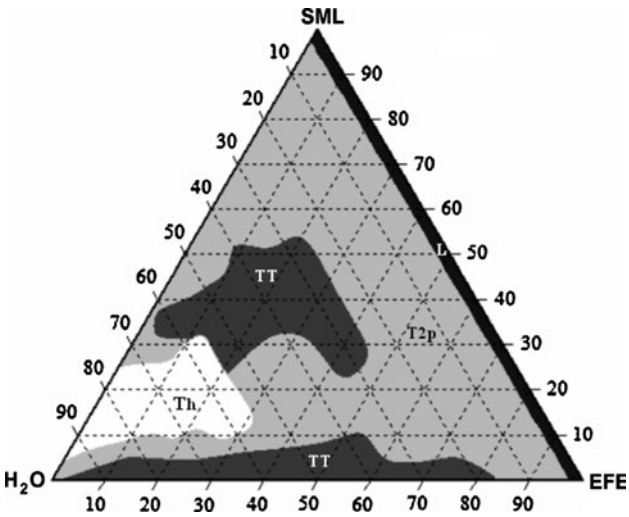


Fig. 4 Ternary phase diagram of EFE/sorbitan mono-laurate SML/deionized water. *L* isotropic region, *Th* homogenous milky region, *T2p* two-phase region and *TT* three-phase region

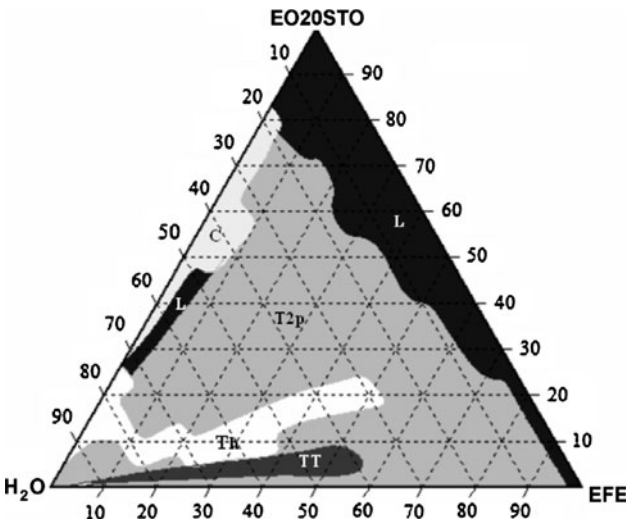


Fig. 5 Ternary phase diagram of EFE/polyoxyethylene (20) sorbitan tri-oleate EO20STO/deionized water. *C* liquid crystal region, *L* isotropic region, *Th* homogenous milky region, *T2p* two-phase region and *TT* three-phase region

oil/polyoxyethylene (20) sorbitan tri-oleate/deionized water. The ratio of EFE:PEG-40 hydrogenated castor oil) was 2:1.

Discussion

Analysis of Products

The compounds were separated on the basis of polarity. Lane 1 is oleyl alcohol with a retention factor value (R_f) of 0.11 and lane 2 is engkabang fat with an R_f value of 0.35. There were trace amounts of diglycerides and monoglycerides found in engkabang fat with the R_f values of 0.05 and 0.01, respectively. The formation of a new product is shown in lane 3 which was the spot of engkabang fat esters with an R_f value of 0.76. Oleyl alcohol with higher polarity was absorbed to the stationary phase longer than triglycerides in engkabang fat, resulting in less time spent in the mobile phase and therefore moved through the stationary phase particles more slowly.

Five high peaks appeared in the gas chromatogram of the alcoholysis products. The first peak was *n*-heptane (solvent), and the second peak was methyl arachidate which was the internal standard used in the sample. Three other peaks are exhibited. The corresponding esters were oleyl palmitate, oleyl stearate and oleyl oleate. They were

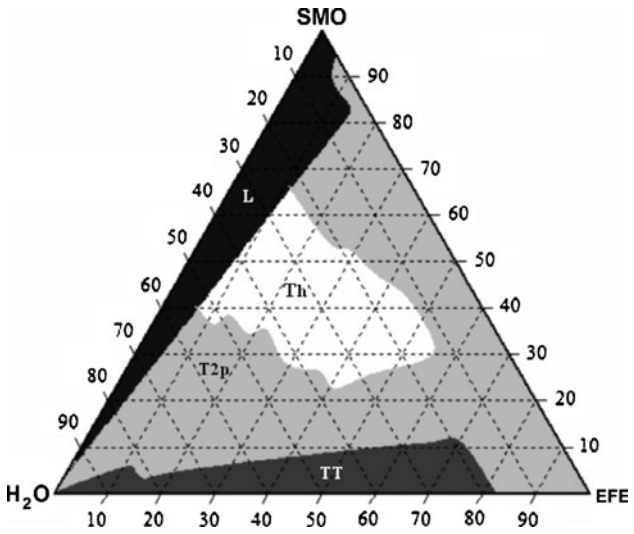


Fig. 6 Ternary phase diagram of EFE/polyoxethylene (20) sorbitan mono-oleate EO20SMO/deionized water. *L* isotropic region, *Th* homogenous milky region, *T2p* two-phase region and *TT* three-phase region

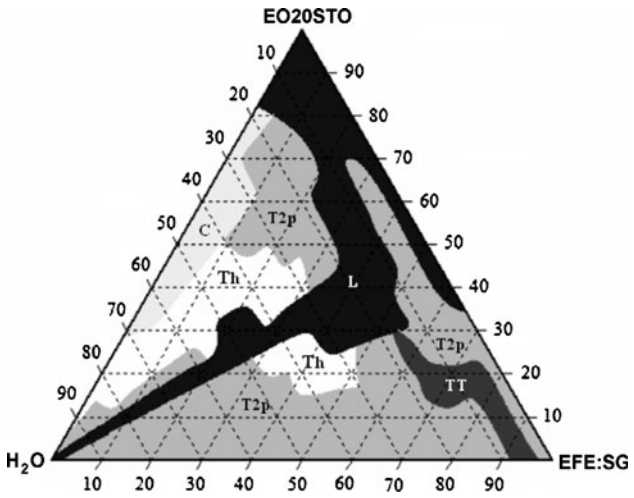


Fig. 7 Ternary phase diagram of EFE: PEG-40 hydrogenated castor oil SG (2:1)/polyoxyethylene (20) sorbitan tri-oleate EO20STO)/ deionized water. *C* liquid crystal region, *L* isotropic region, *Th* homogenous milky region, *T2p* two-phase region and *TT* three-phase region

identified at t_R 10.28, 12.06 and 12.27 min, respectively. Oleyl alcohol reacted with the triglycerides in engkabang fat to produce engkabang fat esters. The percentage yield of oleyl palmitate, oleyl stearate and oleyl oleate were 17.51, 40.84 and 35.32%, respectively. The total percentage yield of engkabang fat esters was 93.67%.

Phase Behavior of Engkabang Fat Esters

Two regions appeared in the ternary phase diagram of EFE/ sorbitan mono-oleate /deionized water; two-phase (T2P) and three-phase (TT) regions. Sorbitan mono-oleate is a C18 lipophilic surfactant which has a low HLB value of 4.3. It was observed that the two-phase region (T2P) was formed along the apex line of sorbitan mono-oleate and EFE. The two-phase region (T2P) appeared at the oil rich corner of ternary phase diagram where the percentages of deionized water were low. In addition, the three-phase region (TT) appeared at the water rich corner of the ternary phase diagram where the percentages of deionized water were high. The three-phase region (TT) appeared at water rich corner of the corner of the ternary phase diagram. It referred to a region with three layers which consists of two layers of transparent or isotropic phases and one layer of a homogenous milky phase, that may be a very stable emulsion.

In the ternary phase diagram of EFE/sorbitan monolaurate/deionized water, the isotropic or transparent region (L) appeared along the apex line of sorbitan mono-laurate and EFE. Sorbitan mono-laurate is a C12 lipophilic surfactant with the HLB value of 8.6. The homogenous milky region (Th) was found at the water-rich corner of the ternary system which could be classified as stable O/W emulsions, whereby the emulsions did not separate after being subjected to centrifugation at 4,000 rpm for 15 min. The two-phase region (T2P) was the largest region exhibited in this ternary phase diagram. The three-phase region (TT) appeared at the percentages of deionized water at 16–100% at low percentage of sorbitan monolaurate. The three-phase region (TT) also appeared at the middle of ternary phase diagram.

In the ternary phase diagram of EFE/polyoxyethylene (20) sorbitan tri-oleate/deionized water, a liquid crystal region (C) appeared at the low percentage of EFE, where the percentage of deionized water was 20–70%. The molecular weight of lipophilic and hydrophilic portions of polyoxyethylene (20) sorbitan tri-oleate were quite similar, thus giving the HLB value of 11 and approaching neutral. It also appeared at 40–50% of deionized water. It was observed that the isotropic region (L) was formed along the apex line of polyoxyethylene (20) sorbitan tri-oleate and EFE. The homogenous region (Th) was found at the water-rich corner in the system. Most of the two-phase region (T2P) appeared at the middle of the system. The three-phase region (TT) started to appear at the low percentage of polyoxyethylene (20) sorbitan tri-oleate where the percentage of deionized water was 40% and above.

In the ternary phase diagram of EFE/polyoxethylene (20) sorbitan mono-oleate/deionized water, the isotropic region (L) was observed along the apex line of polyoxethylene (20) sorbitan mono-oleate and deionized water at the low percentage of EFE. Polyoxyethylene (20) sorbitan mono-oleate is a C12 hydrophilic surfactant with the HLB value of 15. The homogenous milky region appeared at the

middle of ternary phase diagram where the percentage of deionized water was around 20–50%. The two phase region (T2P) was the largest region observed in this ternary phase diagram. The three-phase region (TT) appeared at the low percentage of polyoxethylene(20) sorbitan mono-oleate.

In the ternary phase diagram of EFE:PEG-40 hydrogenated castor oil /polyoxyethylene (20) sorbitan tri-oleate/ deionized water, isotropic or transparent (L) and homogenous milky (Th) regions were found to be larger than the isotropic and homogenous regions in the ternary phase diagram of EFE/polyoxyethylene (20) sorbitan tri-oleate/ deionized water. The three-phase region (TT) appeared at low percentages of deionized water and polyoxyethylene (20) sorbitan tri-oleate but the area was quite small. The combination of EFE:PEG-40 hydrogenated castor oil/ polyoxyethylene (20) sorbitan tri-oleate/deionized water produced larger isotropic (L) and homogenous milky (Th) regions as compared to the other ternary phase diagrams. PEG-40 hydrogenated castor oil could act as co-surfactant in the ternary phase diagram that helps to reduce the interfacial tension of the EFE. Emulsion particles in a dispersion medium always exhibit Brownian motion and hence collide with each other frequently. The stability of emulsion is thus determined by the interaction between the particles during such a collision [14].There are two basic interactions; one being attractive and the other repulsive. When attraction dominates, the particles will adhere with each other and finally the entire dispersion may coalesce. When repulsion dominates, the system will be stable and remain in a dispersed state [15]. In this case, perhaps the PEG-40 hydrogenated castor oil may contribute in the repulsive interaction of the system and thus make it more stable.

From the results obtained, the increase of hydrophilic lipophilic balance (HLB) values of the non-ionic surfactants used gave larger isotropic and homogenous regions in the ternary phase diagrams. Sulaiman et al. [9] studied the ternary phase diagrams of oleyl oleate with nonionic surfactants. The presence of a larger percentage of high HLB value surfactant (polyoxyethylene sorbitan mono-stearate) with HLB = 14.9 contribute to the enlargement of the isotropic region [9]. The HLB value plays the important roles in the determination of phase behavior of the emulsion system. The nonionic surfactant with the high HLB value is better for producing an isotropic and homogenous emulsion as compared to the nonionic surfactant with the low HLB value.

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Zahariah Ismail graduated with a diploma in food technology in 1980 from Universiti Technology Mara. She earned a B.Sc. in 1995 from Universiti Sains Malaysia and a Ph.D. in surfactant chemistry from Universiti Putra Malaysia in 2002. She obtained her MBA from Universiti Teknologi, Malaysia in 2007. She is currently a Chief Chemist 1 at Sime Darby Plantation Sdn. Bhd., Malaysia.