# 学位論文全文に代わる要約

## Extended Summary in Lieu of Dissertation

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学位論文題目：　　　　　　　A Study of Food Nano-Emulsification Using Low-Energy Method in Aqueous Phase/Polyoxyethylene Sorbitan Fatty Acid Ester/Vegetable Oil Systems
Title of Dissertation　　　（ポリオキシエチレンソルビタン脂肪酸エステル水溶液/食用油系における省エネルギーなナノエマルション調製に関する研究)

学位論文要約：

Dissertation Summary

Nano-emulsions are emulsions with droplet sizes from 20 nm up to 200-500 nm, are thermodynamically unstable, and appear transparent or translucent to the naked eye. Usually, nano-emulsions are highly stable to gravitational separation because the relatively small particle size means that Brownian motion effects dominate gravitational forces. Low-energy emulsification method uses energy from the chemical potential of the components. It is suggested that remarkable changes in the curvature of the surfactant assembly plays an important role in low-energy nano-emulsification methods. However, the studies of this emulsification methods using food ingredients are limited.

This work tries to use food ingredients in the study of nano-emulsification using low-energy method in aqueous phase/polyoxyethylene sorbitan fatty acid ester (Tween®)/vegetable oil systems. Water or 20% sucrose solution was used as an aqueous phase, four types of polyoxyethylene sorbitan fatty acid esters i.e. Tween20, Tween40, Tween60 and Tween80 were used as surfactants, and vegetable oil was used as oil phase. The fatty acid content of vegetable oil was C16:0 7.78%, C18:0 3.13%, C18:1 45.3%, C18:2 34.21%, C18:3 6.47%, and others 3.11%.

The triangular phase diagram (Fig. 1) was used to study phase behavior of surfactant existing at equilibrium with desired properties. The phases in the diagrams were determined using visual methods, crossed plate polarizers, polarized light microscopy (Olympus, BX51, Japan), consistency index of viscosity evaluated by viscometers (DV-III Ultra; Brookfield, USA , TV-20 and TV-30; Toki Sangyo, Japan), Small Angle X-ray Scattering (SAXS) techniques using a Nano-viewer SAXS instrument (Rigaku Co., Tokyo, Japan), and the conductivity measurement using a conductivity meter (Eutech Instruments, Fisher Scientific, Singapore).

Each arrow in the diagram represented an emulsification course. The letters A-H are codes for emulsification courses that starting from A-H point at ratio of aqueous phase to surfactant are 0.1:0.9, 0.2:0.8, 0.3:0.7, 0.4:0.6, 0.5:0.5, 0.6:0.4, 0.7:0.3, and 0.8:0.2, respectively. Surfactant and aqueous phase were mixed at the desired ratio by a stirrer at a rotational speed of 300 rpm, followed by dropwise addition of vegetable oil. Water was then added dropwise, until the ratio of aqueous phase, surfactant, and vegetable oil of prepared emulsion equal to 0.8:0.1:0.1at the point shown as the star in the phase diagram. The droplet size of the prepared emulsions was measured using a laser diffraction particle-size analyzer (SALD-7100; Shimadzu, Kyoto, Japan). By overlaying the emulsification course onto each phase diagram, phase behavior along the emulsification process could be predicted. It is clear that the phase diagram or phase behavior along the emulsification process correlates with the droplets size of emulsions which prepared by different pathway.
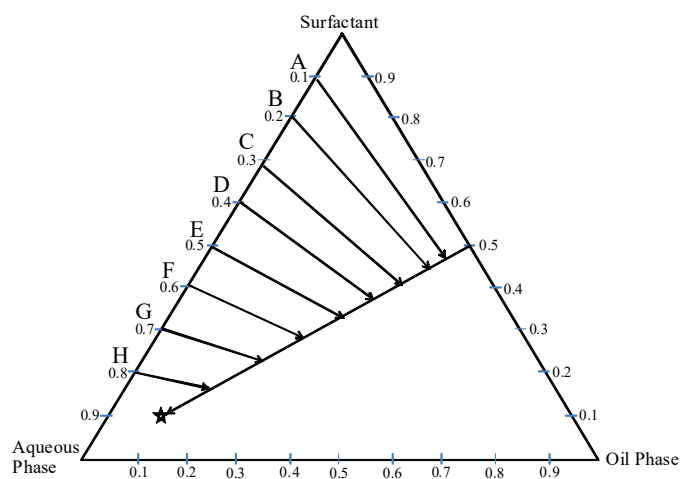


**Fig. 1** Phase diagram and emulsification courses using a low-energy method.

The effect of sucrose on phase behavior of aqueous phase/Tween[®]/vegetable oil systems and food nano-emulsification using low-energy methods were studied. The results show as Fig. 2. In the water/Tween20/vegetable oil system, there were two phase types; surfactant phase coexisting with vegetable oil (S + O), and micellar phase coexisting with vegetable oil ($W_m$ + O). In the sucrose solution/Tween20/vegetable oil system, there was minimal expansion in the boundary of S + O towards a higher aqueous content instead of $W_m$+O region in the water/Tween20/vegetable oil system and the re-crystalized sucrose (Suc) could be seen. This expansion of the S+O phase might be due to the increasing hydrophobicity of Tween20.

In binary compositions of Tween40 and water system showed a lamellar liquid crystalline phase ($L_a$). On the other hand, in a sucrose-Tween40 binary system, a liquid crystalline phase ($L_c$) was formed at high

sucrose solution content (higher than 50%). A sponge phase ($L_3$) was formed at the ratio of sucrose solution to Tween40 was 0.3:0.7 and 0.4:0.6 (C- and D- point). In ternary composition, water/Tween40/vegetables oil system showed coexisting of cubic phase and vegetable oil ($I_1$ + O), and a small area of $L_3$ phase. In the sucrose system, the boundary of the S+O phase did not change; however, the $L_3$ region expanded to lower vegetable oil content at the same Tween40 concentration and to lower Tween40 content at the same sucrose solution content. The expansion of an $L_3$ phase has been attributed to the decrease in the cloud point of the surfactant by sucrose. It is known that the effective HLB of the surfactant shifts toward the hydrophobic side in the presence of sugar (Ikeda et al., 2013; Wakisaka et al., 2014). In addition, re-crystallization of sucrose (Suc) and region of three coexisting phases, $W_m$ + O + $L_c$, could be seen in the diagram of sucrose solution/Tween40/vegetables oil system.

In Tween60 systems, solid Tween60 (Solid T60) existed with other phases in both phase diagrams of water and sucrose systems. Adding sucrose caused re-crystallization of sucrose and all $L_c$ phases existing in water/Tween60/vegetable oil system changed to $L_a$ phase in sucrose solution/Tween60/vegetable oil system. In sucrose system, $L_a$ phase could have be seen for all area in phase diagram in term of sole $L_a$, $L_a$ + $I_1$, $L_a$ + $W_m$ + O, $L_a$ + O, and $L_a$ + O + Suc phases.

In Tween80 systems, the phases of binary compositions of water and Tween80 were a hexagonal crystalline phase ($H_1$) at D- (0.4:0.6) and E-point (0.5:0.5), and coexisting phases of $H_1$ and $L_3$ ($H_1$+$L_3$) at C-point (0.3:0.7). Adding sucrose transformed $H_1$ into $L_a$ phase at D-point and an $L_3$ phase appeared at F-point (0.6:0.4). For the ternary compositions system, the cubic phase ($I_1$) region in the sucrose solution/Tween80/vegetable oil system was smaller than that in the water/Tween80/vegetable oil system. Moreover, $I_1$ and $I_1$ + O phases transformed to $L_c$ + $W_m$ + O phases, $W_m$ + O region expanded towards a lower aqueous content, and some part of $W_m$ + O and $I_1$ phase changed into an $L_3$ phase leading to the expansion of the $L_3$ region ($L_3$ or $L_3$ + O) in the sucrose solution/Tween80/vegetable oil system.

The smallest nano-emulsion droplet sizes using Tween20 as surfactant for both the water and sucrose systems were produced by emulsification courses near the inversion point, the boundary between S + O and $W_m$ + O, which was located at a ratio of aqueous phase to Tween20 of 3.5:6.5. The results of Tween40 and Tween80 systems indicated that small droplet sizes could be prepared in both water and sucrose systems when emulsification course passed through an $L_3$ phase. The big droplet sizes could have been a result of passing through the $H_1$ and/or $I_1$ phase, a highly viscous phase, during emulsification process. On the other hand, the low-energy emulsification methods using Tween60 as surfactant were unsuccessful both in water and sucrose system at 25 °C. It was thought that presented of Solid T60 was a reason of this problem.
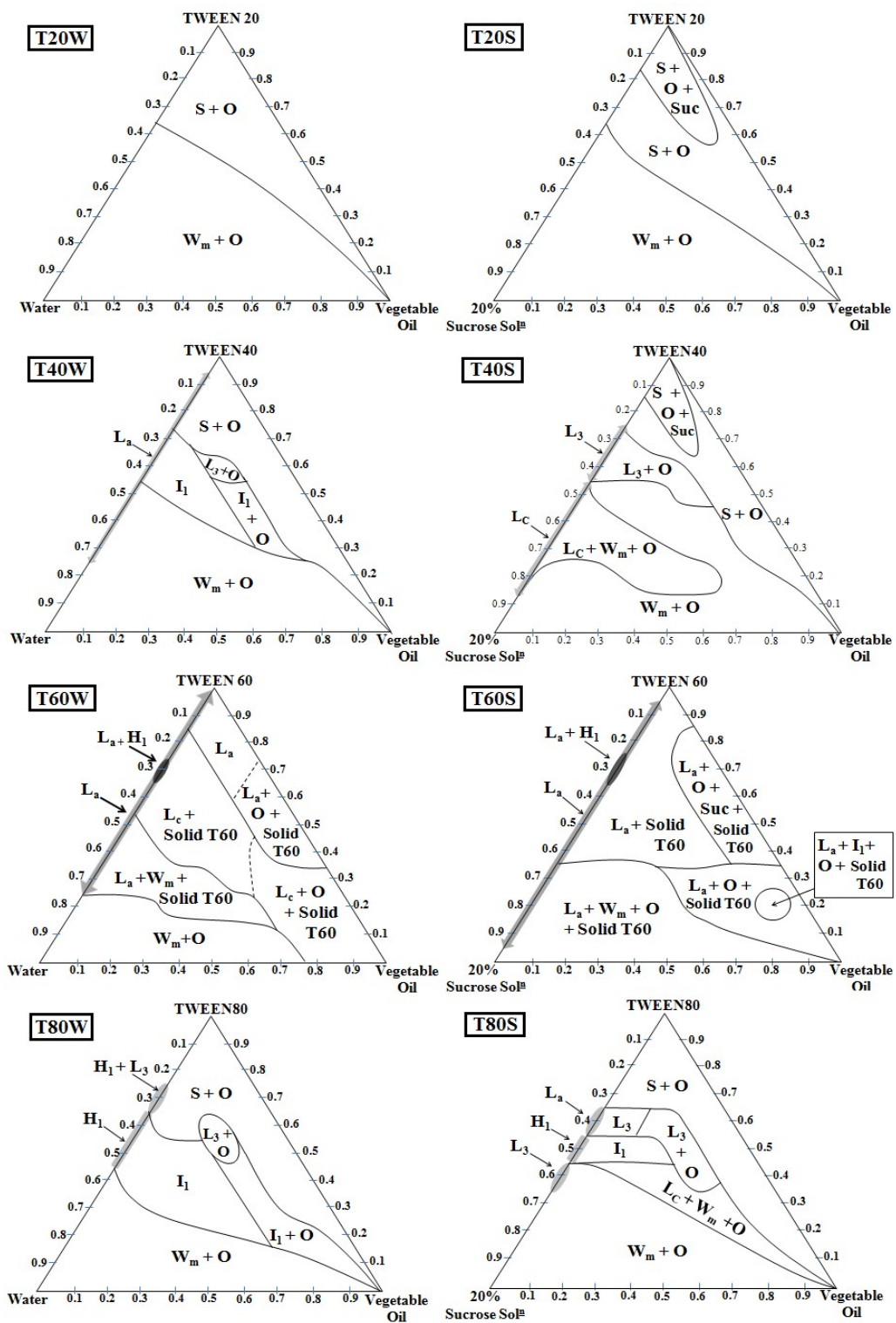
**Fig. 2** Phase diagram of the aqueous phase/Tween®/vegetable oil systems at 25°C, where Tween® are Tween20 (T20), Tween40 (T40), Tween60 (T60), and Tween80 (T80). Water (W) or 20% sucrose solution (S) was used as the aqueous phase. Phase types are denoted as micellar ($W_m$), sponge phase ($L_3$), hexagonal phase ($H_1$), lamellae phase ($L_a$), surfactant phase (S), cubic phase ($I_1$), liquid crystal phase ($L_c$), vegetable oil (O) and solid Tween60 (Solid T60).

Therefore, the next part will study the effect of temperature on phase behavior of the surfactants to solve this problem. The effect of temperature (25, 40, 50, 60, and 70 °C) on food nano-emulsification using low-energy method in water/Tween60/vegetable oil systems was studied. The result is shown as Fig.3.
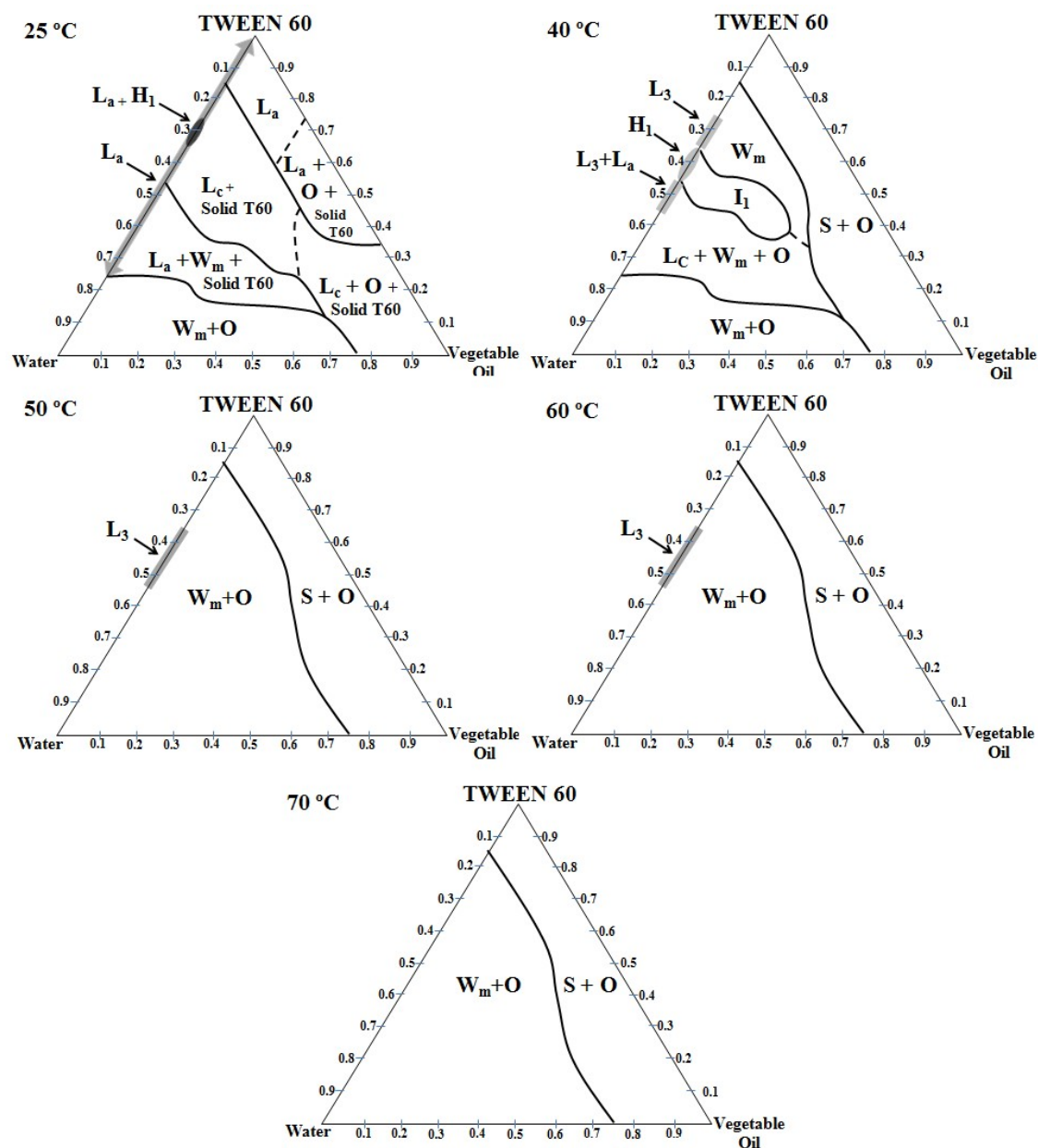


**Fig.3** Phase diagram of the water/Tween60/vegetable oil systems at 25, 40, 50, 60, and 70 ˚C.

The results of the Tween60 system can be concluded that solid Tween60 at 25 °C disappeared by increased temperature from 25 to 40 °C due to an increase in solubility of surfactant. Increasing temperature from

25 °C to 40, 50, 60, and 70 °C caused phase transformation of $L_a$ to $H_1$ or $L_3$ or $L_c$ phase, $H_1$ to $L_3$, $L_3$ to $S + O$ or $W_m$ phase, and $L_c$ and $I_1$ phase to $W_m$ phase as shown in Fig. 3.

Solid T60 existing at 25 °C is a disadvantage to nano-emulsification using the low-energy method resulting in unsuccessful emulsification, while nano-emulsification was successful at higher temperature. At 40 °C, the emulsification courses that started from $H_1$ phase and passed through $I_1$ phase for D-course resulted in the formation of very big $(1,424.89 \pm 82.79 \text{ nm})$ droplet sizes of emulsion. For C-course, droplet size of emulsion was smallest (179.56 nm), while the standard deviations of droplet distribution $(SD_d)$ which was used as representative of polydispersity index (PDI) in this study was high (130.33). It is considered that the emulsification C-course started at $L_3$ phase promoted small droplet size formation and then, passed through $I_1$ and $L_c + W_m + O$ phase that led to high $SD_d$. Droplet size of emulsions prepared at 50 ºC and 60 ºC were not different compared within the same emulsification course. Both phase diagrams of these temperatures are same. Smallest emulsions were prepared passing through D- and E-course for both temperature started with $L_3$ phase. However, emulsions prepared at 60 ºC showed lower $SD_d$ than that prepared at 50 ºC. Increasing prepared temperature to 70 ºC resulted in bigger droplet size than those at 50 ºC and 60 °C. At this temperature (70 ºC), an $L_3$ phase had already transformed to $W_m$ phase and viscosity of the system was very low.

To extend the study of the effect of temperature, other Tween[®]; Tween20, 40, and 80 were selected, since the hydrophilic head group of these surfactants are same as Tween60 while the structures of hydrophobic tail are different with each other. The effect of temperature (25, 40, and 60 ºC) on nano-emulsification and the phase types along the emulsification course (C- and D-course) in water/Tween20, 40, or 80/vegetable oil system were studied. Moreover, emulsions prepared using low-energy emulsification method were compared with the emulsions prepared using high-energy emulsification method.

The results of low-energy emulsification methods showed that temperature did not show obviously difference effect on phase behavior in Tween20 system. The boundary between $W_m + O$ and $S + O$ at 40 ºC and 60 °C were similar with that at 25 ºC in this system. Increasing temperature resulted in minimized the properties difference between $W_m + O$ and $S + O$ phases. Increasing temperature caused increased in droplet size of prepared emulsion in Tween20 system. At 60 °C, very big droplet size emulsion (more than 1,000 nm) could be produced, consequently leading to phase separation. However, the phenomenon of phase behavior of Tween20 system should be more studied in the future.

In Tween40 and Tween80 system, increasing temperature resulted in phase transformation from $L_a$ to $H_1$ or $L_3$, $H_1$ to $L_3$, $L_3$ and $I_1$ to $W_m$ or $S$ phase. The changes in phase behavior along the emulsification process

resulted in differences in droplet size and homogeneity of prepared emulsions of Tween40 and Tween80 systems. It is clear that $L_3$ structure gives an advantage in the formation of small droplet sized emulsions with low $SD_d$, while $H_1$ and $I_1$ structures do not favor the formation of small emulsion droplets.

The emulsion droplet size for all emulsions prepared by the low-energy method was smaller than that emulsions prepared by high-energy method. Very large size of droplets, bigger than 10,000 nm, was obtained using the high-energy method. These results pointed that emulsification using low-energy method had higher ability to form small droplet sized emulsions than using high-energy method in water/Tweens[®]/vegetable oil systems.

The emulsion stability was studied in prepared emulsions using low-energy emulsification method of Tween20, 40, 60, and 80 systems. The results showed that prepared emulsions which had composition of water:Tween[®]:vegetable oil equal to 0.8:0.1:0.1 exhibited good stability without increase in the average of droplet size over 4 months of storage period. However the fluctuation of droplet size could be seen in the samples whose droplet size bigger than 500 nm, especially the prepared emulsion using B- and D-course at 40 °C in Tween60 system, and using D-course at 25 °C in Tween80 and Tween40 systems. All of these emulsification courses involved with $H_1$ and/or $I_1$ phases. Moreover, emulsion separation (creaming) was visually observed in the samples whose droplet sizes bigger than 500 nm. This could be attributed to the creaming velocity of oil droplets which increases with an increase in the droplet radius, hence emulsions containing larger droplets will cream more rapidly than those containing smaller ones (Yang et al., 2012).

It can be noted that $H_1$ and $I_1$ phases led to very big droplet size and heterogeneity of emulsions, resulted in fluctuation of droplet size and creaming during storage.

The surfactant concentration of all samples in this study was fixed at 10%, which might be high content for emulsion stabilization. In order to study the stability of emulsions at lower surfactant concentrations, emulsion prepared by C- and D-course in all Tween[®] systems at 25, 40 and 60 °C were diluted to 1.0% and 0.5% by pure water and stored at 25 °C for 4 weeks. The results showed that most emulsion samples showed high stability through storage time (4 weeks), especially nano-emulsions with droplet sizes smaller than 500 nm. The average droplet sizes did not vary significantly during storage time. Surfactant concentration of 0.5 and 1.0% did not significantly affect droplet size of all samples.

The representative emulsions in sucrose solution were the prepared emulsion using D-course for Tween20 system, and the prepared emulsion using C-course for Tween40 and Tween80 systems. Each emulsion was selected from the smallest droplet size emulsion in each sucrose solution/Tween[®]/vegetable oil system. The

results were as same as in water system that all samples had high stability without changing their droplet size and surfactant concentration of 0.5 and 1.0% did not significantly affect droplet size of all samples through storage time. These small droplet emulsions significantly improved emulsion stabilities by against creaming and flocculation due to the substantially reduced rates of gravitational separation and enhanced Brownian motion of oil droplets (Solans et al., 2005). Moreover, sucrose molecules increased viscosity of continuous phase compared with water, then decreased the opportunities of droplets collisions which resulted in enhance of stability.

It is known that Ostwald ripening is one of the most common mechanisms for emulsion destabilization. The solubility of oil in water is an important parameter on the Ostwald ripening rate in the Lifshitz-Slezov and Wagner (LSW) theory. Ostwald ripening is prevented by long chain triglycerides of the oil phase. The oil phase in the present study was vegetable oil consisting of long chain triglycerides which have low solubility in water, resulted in high stability of emulsion in all system. In addition, it is assumed that all Tweens[®] had high ability to completely adsorb at the surface of oil droplets. Low-energy emulsification facilitated surfactant adsorption and orientation along the interface between oil and water due to the formation of suitable aggregate structures in the water, surfactant and oil mixtures.

In overall results indicate that food nano-emulsification using low-energy method is useful and successively lead to high emulsion stability. Therefore, it is important to select conditions such as stating composition, preparation pathway, and temperature that involve with $L_3$ phase and avoid $H_1$ and $I_1$ phases during the emulsification process.

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