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Formulation and stability of water-in-virgin coconut oil microemulsion using ternary food grade nonionic surfactants

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Abstract: This study was intended to obtain formulation of water-in-virgin coconut oil (w/o) microemulsions and to determine its stability. The stable w/o microemulsions were prepared based on the hydrophilic lipophilic balance (HLB) concept by using ternary nonionic surfactants combination having low, medium, and high HLB values. Microemulsions were formulated with ternary surfactants mixture consisting of Span 80, Span 20 and Tween 20 to achieve predetermined HLB values of 6.0, 6.5, 7.0, 7.5, and 8.0. These values were subsequently used to determine the proportion of water, surfactant, and oil to obtain microemulsions. These microemulsions were subjected to stability tests which include centrifugation, heating treatment, and storage at room temperature. The results indicated that a clear w/o microemulsion was successfully formulated containing 75% of oils portion. It required surfactant and water ratio of 4.5:1 or higher. When the microemulsions were formulated to contain 77.78% oils, the surfactant and water ratio is at least 5.5:1 or higher. These microemulsions remained stable during storage, even after centrifugation, but they were not stable when subjected to heating at 70 °C or higher. This study confirmed that water-in-virgin coconut oil microemulsion is obtainable and suitable for carrying hydrophilic bioactive compound in food application.

Keywords: HLB value, w/o microemulsion, virgin coconut oil, nonionic surfactant

Introduction

Water-in-oil (w/o) microemulsion is nano-scale droplets of water that are surrounded by oil medium. According to Lim (2006), these droplets are stabilized by a monolayer of surfactant and/or co-surfactant molecules enveloped around these droplets. Nano-droplets formed when correct compositions of surfactant, co-surfactant, water, and hydrocarbon are mixed in the right sequence. Surfactants are aggregated in a polar solvent with their polar heads directed toward the core of the aggregates and their hydrophobic tails directed outward, shielding the inner polar (water) core from the nonpolar (oil) medium. The co-surfactant is often required to lower the interfacial tension of the interface between water and oil because a low interfacial tension is essential for producing microemulsions (Lv *et al*., 2006). Moreover, the chain length compatibility of the surfactant and the oil is an important factor regarding the formation of microemulsions (Bayrak and Iscan, 2005).

Microemulsions are thermodynamically stable, transparent isotropic solutions with particle sizes ranging from 5 to 10 nm, and arise from the spontaneous self-assembly of the hydrophobic or hydrophilic parts of surfactant molecules (Flanagan and Singh, 2006). Microemulsions have found numerous applications but the application in foods is limited by the types of surfactants which are used to facilitate microemulsion formation. Many surfactants are not permissible in foods; many more may only be added at low levels. Microemulsion using co-surfactants may not be suitable used in foods because short- or medium-chain alcohols can cause toxicity and irritation (Flanagan and Singh, 2006). Also, co-surfactants can lead to destruction of a microemulsion upon dilution due to partitioning of the co-surfactant out of the interfacial region into the continuous phase (Warisnoicharoen *et al*., 2000).

Several studies have resulted in co-surfactant free oil-in-water (o/w) microemulsions using nonionic surfactants. Polyoxyethylene *n*-alkyl ether surfactants have been extensively studied for preparing o/w microemulsions without the aid of co-surfactants for pharmaceutical applications (Warisnoicharoen *et al*., 2000). However, polyoxyethylene ether surfactants, such as Brij 96, are not food grade (Flanagan and Singh, 2006). Cho *et al*. (2008) reported that a co-surfactant free of o/w microemulsion can be formulated by using nonionic surfactant mixtures. They used a combination of hydrophobic (Span 20, 40, 60, and 80) and hydrophilic (Tween 20, 40, 60, and 80) surfactants.

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Polyoxyethylene sorbitan esters (Tweens) have minimal toxicity and are commercially inexpensive, so they are widely used in food, cosmetics, and pharmaceutical applications (Yaghmur *et al*., 2002; Campo *et al*., 2004). Microemulsion containing Tween 20 in combination with sorbitan monolaurate (Span 20) has been investigated as potential bioactive compound delivery systems (Lv *et al*., 2006). Moreover, the combined use of surfactants showed desirable self-emulsifying microemulsions with small particle size, increased bioactive compound loading, and improved physical stability (Li *et al*., 2005). This study was intended to obtain water-in-virgin coconut oil (w/o) microemulsion based on the hydrophilic-lipophilic balance (HLB) concept by using ternary nonionic surfactants combination having low, medium, and high HLB values and to determine their stability.

Materials and Methods

Materials

A freshly prepared virgin coconut oil (VCO) obtained from a local VCO producer was filtered using filter paper in the presence of anhydrous Na_2SO_4 to obtain the dehydrated VCO. It was then used as the continuous phase. Low HLB surfactant, i.e. sorbitan monooleate (Span 80, HLB = 4.3) and medium HLB surfactant, i.e. sorbitan monolaurate (Span 20, HLB = 8.6) were purchased from Sigma Chemical Company (St. Louis, MO), and high HLB surfactant, i.e. polyoxyethylene sorbitan monolaurate (Tween 20, HLB = 16.7) was purchased from Merck (Darmstadt, Germany). Deionized water was used as the disperse phase.

Determination of HLB value of w/o microemulsion

In this study, the microemulsion systems were prepared with ternary surfactants mixture consisting of Span 80, Span 20 and Tween 20 to achieve predetermined HLB values of 6.0, 6.5, 7.0, 7.5, and 8.0. Briefly, deionized water and a combination of ternary surfactants with ratio of 1:4 (w/w) were mixed on a hot-plate stirrer (SRS 710 HA, Advantec, Japan) at medium speed and the temperature was kept at 40 ± 2°C. After 10 min, dehydrated VCO was added dropwise while stirring at high speed. The formation of microemulsion system was characterized by the presence of clear and transparent solutions.

Determination of water/surfactant/oil ratio

Based on the selected HLB value of surfactant mixtures, the ratio of water/surfactant/oil was determined. The microemulsion was prepared by mixing water and a combination of surfactants and then dehydrated VCO was added dropwise as previously described. The presence of transparent microemulsion mixtures revealed that the optimum ratio of water/surfactant/oil was obtained. These microemulsion samples were allowed to equilibrate at room temperature (30 ± 1°C) for at least 24 h before further measurements on viscosity, conductivity, and interfacial tension were performed. The viscosity measurements were taken using a Brookfield viscosimeter model LVT. The conductivity measurements were taken with a conductivity probe (Vernier Con-BTA) and the drop method was used to perform the interfacial tension measurements.

Stability test of w/o microemulsion

The stability of microemulsions during storage at room temperature and under extreme conditions was examined according to Cho *et al*. (2008). All of the microemulsions (10 g) samples were stored in disposable conical tubes. Microemulsions stability was routinely monitored by turbidity measurements every two weeks at room temperature (30 ± 1°C).

Stability tests under extreme condition were conducted by subjecting the microemulsion samples to the heating or centrifugation test. For the heating stability test, 10 g microemulsions were subjected to mild up to high temperature (60 -105°C) for 5 h in a drying oven (MOV-112, Sanyo, Japan). For the centrifugation stability test, 10 g microemulsions were centrifuged at 2,300 g for 15 min in a centrifuge (EBA 3S Hettich, Germany). After the heating stability test or centrifugation test, the microemulsion was monitored by visual inspection, and turbidity measurement using a UV/Vis spectrophotometer (UV-1650 PC, Shimadzu, Japan). Samples were put into quartz cuvettes with a path length of 1 cm and all measurements were observed at a wavelength of 502 nm. The turbidity was calculated as described by Fletcher and Morris (1995).

Statistical analysis

The data were represented as the mean value of three replicates. Significant differences ($P < 0.05$) between means were determined using Duncan's multiple range tests.

Results and Discussion

Suitable HLB value for w/o microemulsion

The water-in-VCO microemulsions in this study was prepared by using surfactants combination which matches the fatty acid of the VCO, having low, medium, and high HLB value. It was conducted

because of choosing surfactant mixtures which is the best match with the oil and proper HLB is among necessary conditions for a microemulsion formation. The HLB is one of the most common methods to correlate surfactant structure with their effectiveness as emulsifiers. The HLB value indicates how the surfactant will behave in a solution (Pilemand, 2002) and it's closely related to its capability to solubilize substances (Kruglyakov, 2000).

According to Flanagan and Singh (2006), the surfactant, also called emulsifier or amphiphilic compound, plays an important role in microemulsion formation by reducing the interfacial tension. They also reported that the surfactants mixture increased the solubility of the bioactive compound in the microemulsion. Moreover, the chemical structure of surfactant must be considered because the chain length compatibility of a surfactant and oil is an important factor in the formation of microemulsion (Bayrak and Iscan, 2005; Fanun, 2009). The combination of high and low HLB values of surfactants provides the necessary conditions for the formation of a stable w/o microemulsion (Osborne *et al*., 1988; Pilemand, 2002; Garti *et al*., 2005; Li *et al*., 2005; Cho *et al*., 2008).

It was found that the w/o microemulsion formed only at the HLB value of 7.0 which was composed of 16.6% of Tween 20, 15.0% of Span 20, and 68.4% of Span 80. The transparent appearance of the w/o microemulsion using surfactants having the HLB value of 7.0 was selected for further experiments.

Optimum formula of w/o microemulsion

The microemulsion system can be formulated by appropriate proportions of water, surfactants, and oil. In order to determine the optimum formula of water-in-VCO microemulsion, the combinations of water/surfactants/VCO at various ratios were prepared (Table 1). Transparent w/o microemulsions were formed when the ratios of water and surfactants mixture were of at least 1:4.5. Upon addition of dehydrated VCO as the continuous phase, clear appearance of the microemulsions were obtained when the ratio of water/surfactant mixture and VCO was 1:3 (the system containing 75% of VCO) as indicated in formulae 5, 6, 7, and 8. However, when the VCO was added at a higher ratio (i.e. 1:3.5 or 77.78%), transparent w/o microemulsion was not achievable as indicated by formulae 9 and 10. The higher proportion of VCO addition could only form clear microemulsions when the ratio of water and surfactants mixture was also increased to 1:5.5 or higher as indicated in formulae 11 and 12.

Table 1. Evaluation of water-in-virgin coconut oil microemulsion formulation

Formula	Ratio of water/ surfactants (w/s)	Ratio of (w/s) : VCO	Appearance
1	1 : 1.5	1 : 3	Cloudy
2	1 : 2	1 : 3	Cloudy, separate phase
3	1 : 3	1 : 3	Cloudy, separate phase
4	1 : 4	1 : 3	Cloudy
5	1 : 4.5	1 : 3	Transparent
6	1 : 5	1 : 3	Transparent
7	1 : 5.5	1 : 3	Transparent
8	1 : 6	1 : 3	Transparent
9	1 : 4.5	1 : 3.5	Cloudy
10	1 : 5	1 : 3.5	Slightly cloudy
11	1 : 5.5	1 : 3.5	Transparent
12	1 : 6	1 : 3.5	Transparent
13	1 : 4	1 : 4	Slightly cloudy
14	1 : 5	1 : 4	Slightly cloudy
15	1 : 6	1 : 4	Slightly cloudy

The formulae of 1, 2, 3, and 4 could not form microemulsions system because of insufficient surfactants in the systems. According to Patel *et al*. (2009), the concentration of surfactants must be high enough to provide the number of surfactant molecules needed to stabilize the microdroplets to be produced by an ultralow interfacial tension (< 10^{-3} mN/m). Moreover, the mixed surfactant having low and high HLB value was able to bridge the hydrophilic/lipophilic gap between the water and the oil phases, producing microemulsions with substantial solubilization and ultralow interfacial tension. It was very important to promote the formation of a microemulsion system having a diameter of the droplets in the range of 10-100 nm.

Besides the surfactants having low and high HLB value, we also used surfactant with a medium HLB value. According to Friberg and Chiu (1992), the behavior of the nonionic surfactant depended on the length of its polar chain. With a low number of oxyethylene groups, the nonionic surfactant acted as a powerful cosurfactant and stabilized the lamellar liquid crystal in the system. Thus, by combining these surfactant molecules, the interaction of the surfactant with water and oil provide a more efficient way to bridge the hydrophilic-lipophilic gap between the surfactant and water or oil than using a single surfactant molecule.

According to Hait and Moulik (2002), in w/o microemulsion containing cosurfactant, the water phase is linked with the oil by monolayer at the interface. On the other hand, the cosurfactant essentially partitions between the oil phase and the interfacial region. It may even moderately or weakly partition between the water phase and the interface as well. In this study, the surfactant having a medium HLB value acted as cosurfactant. This ternary system was expected to produce a stable w/o microemulsion.

However, w/o microemulsion can be destabilized by extra addition of oil to make it nonhomogeneous and turbid or cloudy. To satisfy partition requirement there is a depletion of surfactant at the oil-water interface. Higher addition of the surfactant helped to restore the system to a renewed state of thermodynamic equilibrium (De *et al.*, 2010). These phenomena were clearly indicated in formulae 5, 6, 7, 8, 9, 10, 11 and 12. Economically, the use of a large amount of surfactants is considered inefficient. A higher level of surfactant addition may also result in disturbance on sensory properties of the products. Consequently, the transparent systems obtained from the formulae of 5, 6, 7, 8, 11, and 12 were selected and used for further experiment and were coded A, B, C, D, E, and F.

Characteristic of w/o microemulsion

According to Flanagan and Singh (2006), microemulsion characterization can be divided into two main areas, i.e. characterization at the microscopic and macroscopic levels. The viscosity, conductivity, and interfacial tension (Table 2) represented the macroscopic level measurements.

Table 2. Physical characteristic of water-in-virgin coconut oil microemulsions

Formula	Characteristic *)		
	Viscosity (cp)	Conductivity (µs/cm)	Interfacial tension (dyne/cm)
A	107.5 ± 2.5 [a]	0.9 ± 0.0 [a]	22.82 ± 0.38 [d]
B	107.5 ± 6.6 [a]	0.9 ± 0.0 [a]	22.45 ± 0.00 [c]
C	110.0 ± 6.6 [a]	0.9 ± 0.0 [a]	21.67 ± 0.00 [b]
D	111.7 ± 7.6 [a]	0.9 ± 0.0 [a]	21.05 ± 0.62 [a]
E	115.3 ± 5.0 [a]	0.9 ± 0.0 [a]	23.78 ± 0.39 [e]
F	116.7 ± 10.4 [a]	0.9 ± 0.0 [a]	23.40 ± 0.20 [e]

Note: *): average of three replicates
Different letters in the same column indicate significant difference (P<0.05)

Table 2 indicated that all of the microemulsion formulae had no significant (P > 0.05) difference on viscosity and conductivity. However, the interfacial tension significantly (P < 0.05) decreased on increasing the surfactant mixtures ratio. Lim (2006) said that viscosity or conductivity can be used to detect the microemulsion structural changes. Garti *et al.* (2005) reported that viscosity depends largely on the microemulsion structure, i.e., the type and shape of aggregates, concentration and interaction between dispersed particles. The conductivity depends not only on droplet size but also on mean droplet-to-droplet distance or droplet concentration (Bumajdad and Eastoe, 2004). According to García-Río and Hervella (2007), the conductivity of w/o microemulsion is closely related to the rate of mass transfer between droplets. Referring to the said studies, this result indicated that various surfactant mixtures ratio did not impact on microemulsion structure but affected the interaction between water and oil which wasrecognized by interfacial tension measurement.

Interfacial tension is the surface tension caused by intermolecular interactions at the surface separating two immiscible fluids (Rosen, 1989). Under appropriate conditions the microemulsion system is miscible with both the oil and water phases. However, the microemulsion system partitions into three phases, a surfactant-rich phase, a surfactant-rich water phase, and a surfactant-rich oil phase. The surfactant-rich phase is called a middle phase microemulsion. It is in the middle phase microemulsion that a surfactant shows the greatest solubilizing power for both water and oil; here it also gives ultra small values of interfacial tension between oil and water under proper condition. For this reason, increasing surfactant mixtures ratio in this study could result in decreasing interfacial tension. As reported by Cho *et al.* (2008), the surfactant mixtures were more effective in lowering interfacial tension than the individual surfactant. They also reported that lowering interfacial tension was related to higher storage stability.

Stability of w/o microemulsion

The stability of w/o microemulsion can be evaluated by measuring its turbidity changes during storage at room temperature and under accelerated (extreme) condition as shown in Table 3 and Figure 1. The turbidity is the most common way to measure the stability of microemulsion because it's proportional to the average particle diameter (Cho *et al.*, 2008). Thus, the turbidity changes can be used to derive changes in the particle volume resulting from either clustering or growth of the microemulsion droplets, and to obtain information on stability changes.

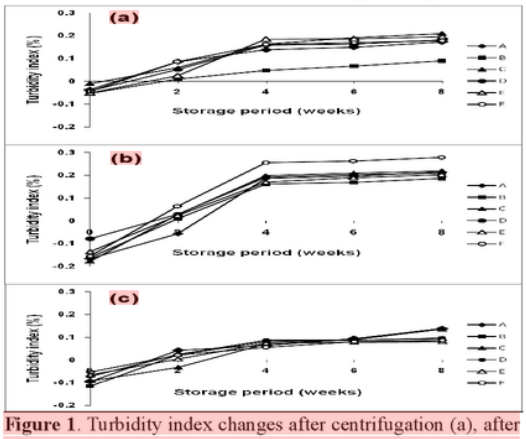

Figure 1. Turbidity index changes after centrifugation (a), after heating at 60ºC (b), and untreated (c) water-in-virgin coconut oil microemulsion formulae A, B, C, D, E, and F during storage at room temperature (30 ± 1ºC)

Table 3. Stability of water-in-virgin coconut oil microemulsion after centrifugation, heating, and storage at room temperature

Formula	Turbidity Index (%)				
	Untreated	After centrifugation	After heating at 60°C	After 1 month storage at room temperature	After 2 months storage at room temperature
A	-0.09±0.01 ^{b}	-0.04±0.01 ^{c}	-0.17±0.00 ^{a}	0.07±0.00 ^{d}	0.14±0.00 ^{e}
B	-0.12±0.01 ^{b}	-0.05±0.01 ^{c}	-0.16±0.00 ^{a}	0.08±0.01 ^{d}	0.09±0.01 ^{e}
C	-0.09±0.01 ^{b}	-0.01±0.00 ^{c}	-0.18±0.01 ^{a}	0.07±0.01 ^{d}	0.14±0.00 ^{e}
D	-0.07±0.00 ^{b}	-0.04±0.01 ^{c}	-0.08±0.01 ^{a}	0.09±0.01 ^{d}	0.10±0.01 ^{e}
E	-0.06±0.00 ^{b}	-0.05±0.00 ^{c}	-0.14±0.00 ^{a}	0.07±0.01 ^{d}	0.08±0.01 ^{e}
F	-0.05±0.00 ^{b}	-0.04±0.01 ^{c}	-0.15±0.00 ^{a}	0.06±0.01 ^{d}	0.09±0.00 ^{e}

Note: *): average of three replicates
Different letters in the same row indicate significant difference (P<0.05)

Table 3 shows that the turbidity decreased after heating, slightly increased after centrifugation, and greatly increased after 1 month storage at room temperature. In this study, the heating test was performed under mild to high temperature (60-105°C) for up to 5 hours and the centrifugation test was conducted at 2,300 g for 15 min. The negative values of turbidity index indicated that the samples have clearer appearance as compared to the reference (distilled water).

This study indicated that at the mild temperature (60°C), all of microemulsions remained stable. They were still transparent after heating, and it was even clearer than that of untreated samples. However, they were unstable when heated at 70°C or higher which resulted in phase separation. The phase separation could be due to the changes in nonionic surfactants structure which are likely to occur during heating. According to Flanagan et al. (2008), the head-group size of nonionic surfactants in particular is affected by changes in temperature, thereby indirectly affecting their ability to solubilize oil. This study confirmed that the water-in-VCO microemulsions were only suitable for food application involving mild heating.

To evaluate the physical stability of microemulsions during storage, all the samples were stored at room temperature (30 ± 1°C) for up to 2 months after heating or centrifugation treatment. Visual observation indicated that all of the microemulsions did not suffer from significant changes in their appearance over the storage period. However, there were slight increases in their turbidity index, especially in the heat treated microemulsions (Figure 1). As explained before, this could be because the nonionic surfactants were affected by heating and resulted in changes in their head-group size (Flanagan *et al.*, 2006). However, the rate of increasing the turbidity index of microemulsions during storage at room temperature was relatively negligible.

According to Li *et al.* (2005), as opposed to the single surfactant, a combined use of surfactants might have provided better surfactants' HLB. As a result, it enhanced the flexibility of the surfactant layer that was formed. It also enhanced the surfactants' ability to partition at higher levels into the water-oil interface, both of which stabilized the microemulsion system. This was the reason for its stabilizing effect on the water-in-VCO microemulsion during storage, even after centrifugation or heating at mild temperature. Although there were significant differences ($p<0.05$) on the turbidity index (Table 3), all of these samples had transparent appearance (Figure 2). According to Cho *et al.* (2008), the microemulsion with transparent appearance and a turbidity value of less than 1% was defined as a stable microemulsion. Thus, all of the microemulsion formulae in this study could be declared as stable microemulsions and are suitable for further food applications.

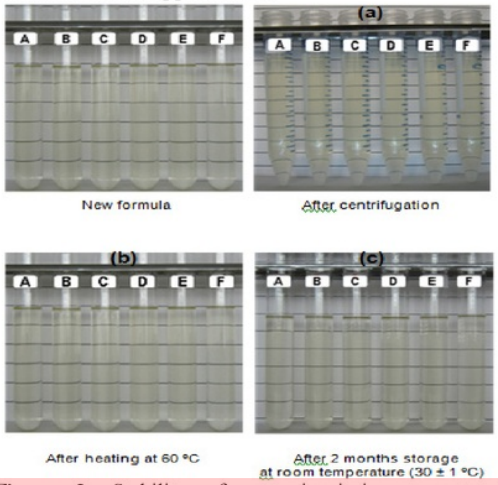

Figure 2. Stability of water-in-virgin coconut oil microemulsion formulae A, B, C, D, E, and F after centrifugation (a), after heating at 60°C (b), and after 2 months storage at room temperature (c)

Conclusion

Stable w/o microemulsions could be obtained by utilizing ternary nonionic surfactants combination with the following proportion: 16.6% of Tween 20, 15.0% of Span 20, and 68.4% of Span 80. Transparent w/o microemulsion can only be formed when the ratio

of water and surfactants was at least 1:4.5 and the ratio of water/surfactants and VCO was not more than 1:3.5. These microemulsions had no significant ($P > 0.05$) difference on their viscosity and conductivity, but their interfacial tension significantly ($P < 0.05$) decreased with the increasing level of surfactant mixtures proportion. All of the microemulsions remained stable during storage at room temperature, but they were not stable when subjected to heating at 70 °C or higher.

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