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Microemulsion Characteristics of Oil-in-Water with Surfactants as α-tocopherol Carriers

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Abstract

Microemulsions are relatively stable carrier agents for bioactive components. This study aimed to determine the characteristics of oil-in-water (O/W) microemulsions as carriers of α-tocopherol compounds in variations of α-tocopherol concentration, pH, and dilution. The O/W microemulsion was made from a mixture of surfactants (Tween 80, Tween 20, and Span 80) with virgin coconut oil (VCO) in a ratio of 85:15 with a total volume of 5 mL added with 10 mL of distilled water. The research used a randomized block design (RBD). The data were analyzed using ANOVA and continued with Tukey's test and regression analysis. Treatments in microemulsion were the addition of α-tocopherol with concentrations of: 0.25, 1.50, 1.75 and 2.0%. Observations were made on the turbidity index values, appearance, droplet diameter, and microemulsion stability. In addition, observations were made on the stability of the microemulsion at variations of pH (4.5, 5.5, 6.5), and dilution (10, 50, and 100 times). The results showed that the microemulsion was able to carry α-tocopherol compounds up to a concentration of 2% with a turbidity value reaching 0.1036 ± 0.0033% with a transparent appearance. A mixture of three surfactants (Tween 80, Tween 20 and Span 80) has the ability to increase droplet stability. The α-tocopherol microemulsion had a droplet diameter below 30 nm and stayed stable against the effects of centrifugation at pH 4.5 with dilutions up to 50 times and remained stable upto pH 6.5 with dilutions between 50 – 100 times.

Keywords: Microemulsion characteristics, Concentration of α-tocopherol, Surfactant, pH and dilution

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Introduction

Macroemulsions have unstable thermodynamics, weak kinetic stability, and large droplet sizes in the range of 1-100 nm. This causes the bioavailability of bioactive compounds to be ineffective and easily damaged. Microemulsion is an alternative to increase the bioavailability of antioxidants or bioactive compounds [1]. The IUPAC defines a micro-emulsion as a dispersion made up of water, oil, and surfactant which is an isotropic system and thermodynamically stable in domain diameter varying approximately from 1 to 100 nm, typically 10 to 50 nm [2].

Microemulsions have stability thermodynamically and they also have kinetic stability [3,4]. Microemulsion technology has been widely applied to the food, pharmaceutical, nutritional, and cosmetic industries because of its transparency, easy preparation, and better stability [1, 5, 6]. In addition, the nanoscopic size can make better absorption into cells because microemulsions have the ability to overcome solubility and stability problems which can be used for phytotherapeutics, nutraceuticals, and food additives [7, 8]. Bergonzi et al. [9] stated that curcumin added to a microemulsion showed more stable bioavailability during storage.

The stability of microemulsions is very complex and is influenced by the oil phase and type of surfactant, temperature, pH, centrifugation, and dilution [10, 11]. Non-ionic surfactants Tween 80, Tween 20, and Span 80 are food-grade surfactants. Making microemulsions from food-grade surfactants and oil phase (VCO) is an O/W microemulsion product that is safe to use [4]. Permana and Suhendra [12] found an O/W microemulsion formulation that had high stability of the three non-ionic surfactants at a VCO concentration of 7.5%. Microemulsion (O/W) using the three surfactants (Tween 80, Tween 20, and Span 80) and the VCO disperse phase had stability at hydrophilic-lipophilic balance (HLB) 11, 12, 13, and 14, while the highest stability was at HLB 13 and kept stable during 7 weeks of storage [13]. Ningrum and Budhiyanti [14] found a stable microemulsion with a surfactant and water ratio of 20:80. Three types of surfactants were used, namely Tween 80: Span 80: Tween 20 with a ratio = 92: 5.5: 2.5 (v/v) and 87: 5.5: 7.5 (v/v). The oil phase used was *Ulva lactusa* fatty acid extract of 3000 ppm.

Tocopherol is a strong radical chain-breaking antioxidant. It can protect cells from oxidative damage caused by free radicals generated in cells in response to metabolic processes in cells [15]. Tocopherol donates a hydrogen atom to the 6-hydroxy group on its chromium ring to a lipid peroxy radical. The rate of reaction of α-tocopherol with lipid peroxy radicals is 107 $M^{-1}s^{-1}$ [16, 17] and 105–106 times faster than that of unsaturated lipids with lipid peroxy radical antibacterial activity, and anti-inflammatory activity [16].

The capacity of microemulsions as carriers of bioactive compounds is influenced by surfactants, oil phase, and water phase. In O/W microemulsions, bioactive compounds are usually present in the oil phase. O/W microemulsions having small droplet sizes (<100 nm) tend to have a large capacity as a carrier for bioactive compound α-tocopherol. Suhendra et al. [18] investigated O/W microemulsions as a carrier system for the bioactive compound fucoxanthin. The surfactants used consist of three types, namely Tween 80, Tween 20, and Span 80, while the oil phase used was VCO. The capacity of the microemulsion as a carrier for fucoxanthin was 12 ppm. Vitamin E (α-tocopherol) is a bioactive antioxidant compound dissolved in oil, so the carrier was in the oil phase. Yuwanti et al. [19] used a combination of three non-ionic surfactants, they are Tween 80, Span 80, and Span 40 with a proportion of 90:3.33:6.67 (%w/w). The resulting microemulsion was capable of carrying 5,000 ppm of α-tocopherol. However, the O/W microemulsion capacity of the three surfactants (Tween 80, Tween 20, and Span 80) with VCO oil phase as carriers of α-tocopherol has not been investigated. The aim of this study is to obtain the O/W microemulsion capacity of three surfactants (Tween 80, Tween 20, and Span 80) with VCO oil phase as a carrier for α-tocopherol compounds and their microemulsion characteristics.

Materials and Methods

Materials

The materials used for this research include Tween 80, Tween 20, Span 80, α-tocopherol, Na_2HPO_4, and NaH_2PO_4 purchased from Merck, Darmstadt, Germany, distilled water (Water One), and VCO obtained from the home industry in Denpasar.

Instruments

The instruments used were Particle Size Analyzer (Horiba Scientific Nano Particle Analyzer SZ-100) Japan, a Hot plate magnetic stirrer (Companion HP-300, USA), a Centrifuge (Clements GS 150, Australia), Analytical balance (Shimadzu AUW 220, Japan) and micropipette (Soccorex, Switzerland), UV-Vis Spectrophotometer (Thermo Fisher Scientific-Evolution 201, USA).

Methods

Experiment design

This study used a simple randomized block design (RBD) with α-tocopherol concentrations of 0, 0.25, 0.5, 0.75, 1, 1.25, 1.50, 1.75, and 2%. The treatments were grouped into two manufacturing time groups.

Microemulsion O/W preparation

The O/W microemulsion is a mixture of VCO, surfactant, and distilled water. The method of the preparation was as follows. A mixture of surfactant Tween 80: Tween 20: Span 80 with a ratio of 89.5: 5: 5.5 (v/v) was made. The surfactant was mixed with VCO in a ratio of 15:85 with a total volume of 5 mL. It was then added dropwise with 10 mL of distilled water at a temperature of 70 ±5 °C in a Hot plate. The surfactant was regulated by HLB = 14. The microemulsion formed was observed visually to determine the formation of a transparent solution. A microemulsion is stable if there is no gel formed and when it is shaken for 1 min, it is not cloudy and remains transparent. Furthermore, the formation of O/W microemulsion stability was determined. An O/W microemulsion was called stable if the turbidity index was less than 1%. Microemulsion turbidity index was measured with a UV-VIS spectrometer at a wavelength of 502 nm with the formula: turbidity index x cuvette length = 2.303 x absorbance.

Preparation of α-tocopherol microemulsion capacity

α-tocopherol microemulsion was made by dissolving α-tocopherol in VCO with a concentration of 0, 0.25, 0.5, 0.75, 1, 1.25, 1.50, 1.75, and 2%. The surfactant and water were then added dropwise at 70±5 °C. Furthermore, the stability of the α-tocopherol microemulsion formed was analyzed based on its appearance and turbidity index.

Microemulsion stability test against centrifugation

To test the stability of the microemulsion against centrifugation was carried out by taking a sample of the microemulsion (10 mL) and centrifuging it at 4000 rpm, with a rotor diameter of 14 cm for 30 min. Microemulsion turbidity index was measured with a UV-VIS spectrometer at a wavelength of 502 nm with the formula: turbidity index x cuvette length = 2.303 x absorbance.

Diameter distribution of microemulsion droplets

The droplet diameter distribution was measured using Horiba Scientific Nano Particle Analyzer SZ-100. Measurement condition: Temperature: 25.0 °C, Diluent Name: Water, Refractive Index: 1.3328, Viscosity: 0.8852 cp, Scattering Intensity: 17934 cps.

Microemulsion stability test against pH and dilution

The microemulsion stability tests due to the influence of pH and dilution were carried out by diluting the microemulsion 10, 50 and 100 times using water with buffer at pH 4.5, 5.5, and pH 6.5. The solution was then measured using a pH meter. The electrode was calibrated with a pH standard first, and then, the value was recorded. The pH measurements were carried out at room temperature. Furthermore, the solution was tested for the turbidity index value.

Data analysis

The data obtained were analyzed for variance (ANOVA) and continued with Tukey's test using the SPSS 25.0 program. The relationship between α-tocopherol concentration and turbidity was plotted to form a graph and regression analysis was carried out.

Results and Discussion

Turbidity index value of α-tocopherol microemulsion

The average value of the turbidity index (%) of the O/W microemulsion at various concentrations of α-tocopherol compounds is shown in Table 1. The results of variance (Table 1) showed that the average value of the turbidity index was very significantly different ($P<0.01$) from the replenishment of several concentrations of α-tocopherol compounds. Fig.1 shows that the form of the regression equation was quadratic $y= 0.0112x^2 + 0.0012x + 0.0562$ with a determination value (R^2) of 0.9978.

Table 1 shows that the concentration of α-tocopherol increases, so the value of the turbidity index tends to increase. The concentration of α-tocopherol increases the possibility of the droplets formed on the O/W microemulsion to enlarge [1]. The droplets formed on the O/W microemulsion tend to increase the turbidity index value [2]. The addition of α-tocopherol compounds up to 0.75% has the same turbidity index value. The turbidity index increases after the addition of a 1% concentration of α-tocopherol. When it is added with α-tocopherol compounds to a concentration of 2%, the turbidity index value is still below 1%. Turbidity index values below 1% indicate the formation of O/W microemulsion with a transparent appearance [4, 17].

Table 1. **The average value of the turbidity index of O/W microemulsion at various concentrations of α-tocopherol.**

Concentration (%)	Average value of turbidity index (%)	Appearance
0.00	$0.057±0.001^{f}$	Transparent
0.25	$0.056±0.002^{f}$	Transparent
0.50	$0.059±0.002^{f}$	Transparent
0.75	$0.063±0.002^{ef}$	Transparent
1.00	$0.070±0.005^{de}$	Transparent
1.25	$0.075±0.002^{cd}$	Transparent
1.50	$0.083±0.003^{bc}$	Transparent
1.75	$0.092±0.003^{b}$	Transparent
2.00	$0.104±0.003^{a}$	Transparent

Note: The same letters in the same column indicate that they are not significantly different ($P <0.05$)

Fig. 1 shows that the form of the regression equation was quadratic $y= 0.0112x^2 + 0.0012x + 0.0562$ with a termination value (R^2) of 0.9978. This shows that the addition of α-tocopherol causes the turbidity index value to increase quadratically. Increasing too large addition of the concentration of α-tocopherol causes the droplet to increase quadratic, so the size can cause the microemulsion to be unstable [16]. The value of the α-tocopherol microemulsion turbidity index below 1% indicates that the microemulsion has the ability as a carrier system for α-tocopherol compounds up to a concentration of 2%.

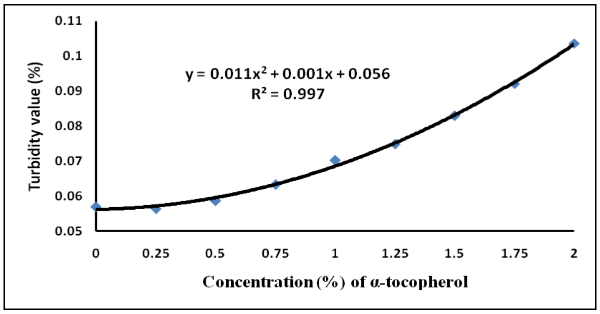

Figure 1. **The regression equation of the turbidity index value of O/W microemulsion on the addition of different concentrations of α-tocopherol.**

pH value of α-tocopherol microemulsion

The average pH value of the O/W microemulsion when added with several concentrations of α-tocopherol compounds is shown in Table 2. The results of the variance showed that the average pH value was not different ($P > 0.05$) when added with several concentrations of α-tocopherol compounds.

Table 2 shows that the pH value of the O/W microemulsion does not differ from the increasing α-tocopherol concentrations. This is because the structure of α-tocopherol does not have a compound group that causes a change in pH. The surfactants used are non-ionic, so the addition of active compounds tends to have a neutral pH into the microemulsion and it does not affect the degree of acidity [1, 16]. The pH range of α-tocopherol in the concentration of 0 – 2% was 4.4 – 4.6. The pH range for cosmetics is between 4.5 and 6.5 [18].

Table 2. **The average pH value of the O/W microemulsion at various α-tocopherol concentrations.**

Concentration (%)	Average of pH
0.00	4.75±0.071[a]
0.25	4.60±0.141[a]
0.50	4.55±0.071[a]
0.75	4.45±0.071[a]
1.00	4.45±0.071[a]
1.25	4.40±0.141[a]
1.50	4.50±0.141[a]
1.75	4.55±0.071[a]
2.00	4.45±0.071[a]

Note: The same letters in the same column indicate that they are not significantly different ($P < 0.05$)

Stability of α-tocopherol microemulsion against centrifugation

The average value of the turbidity index (%) of α-tocopherol microemulsion after centrifugation (4000 rpm, 30 min) is shown in Table 3. The results of variance (Table 3) showed that the average value of the turbidity index was very significantly different ($P < 0.01$) by adding several concentrations of α-tocopherol compounds.

The higher the α-tocopherol concentration, the higher the droplet size was formed. The increase in the size of the droplet diameter causes the turbidity index to increase, but up to 2% α-tocopherol concentration the microemulsion is still stable. It is indicated by a turbidity index value below 1%.

Table 3. **Average turbidity index of α-tocopherol microemulsion after centrifugation (4000 rpm, 30 minutes).**

Concentration (%)	Average of Turbidity index (%)
0.00	0.059±0.001[e]
0.25	0.063±0.001[d]
0.50	0.067±0.001[c]
0.75	0.075±0.001[b]
1.00	0.068±0.001[c]
1.25	0.061±0.001[de]
1.50	0.055±0.001[f]
1.75	0.086±0.001[a]
2.00	0.084±0.001[a]

Note: The same letters in the same column indicate that they are not significantly different ($P < 0.05$)

Table 3 shows that the centrifugation test at high speed tends to cause the emulsion formed to separate, if the droplet size has a large enough diameter. This is due to the large molecular weight of the droplets, resulting in coalescence between droplets and the instability of the emulsion formed [3, 16]. This is different if the droplet diameter is very small, the droplets formed are stable and strong, so that centrifugation does not have a significant effect [2].

The α-tocopherol microemulsion has high stability against centrifugation, possibly because the mixture of surfactant, VCO, and water is completely homogenized. This is confirmed by the microemulsion which has a droplet diameter below 30 nm, so that the filling of α-tocopherol up to 2% does not affect the stability of the α-tocopherol microemulsion formed. The ability of the microemulsion found as a carrier for α-tocopherol was higher compared to the results of Yuwanti et al. [19] which was only 5,000 ppm or 0.5 percent. Meanwhile, other information shows that microemulsions from a mixture of surfactants (Tween 80 and propylene glycol, 5:1) with isopropyl myristate and water (ratio 50:40:10 respectively) supplemented with vitamin A (concentration 0.05% w/w) and/or vitamin E (concentration 0.1% w/w) and stirred in a magnetic stirrer for 30 min showed physical stability [20].

The O/W microemulsion droplet diameter distribution

Fig. 2 shows the diameter distribution of O/W microemulsion droplets. Diameter distribution was measured using a Particle Size Analyzer (PSA). O/W microemulsion droplets have a diameter below 30 nm. This indicates that the emulsion formed is an O/W microemulsion.

The diameter size distribution was 20.2 nm (22.2%), 21.5 nm (28.8%), 23.2 nm (23.5%), 25 nm (14.8%), 27 nm (7.5%), and 29 nm (3%). The droplets formed have a diameter of less than 30 nm and have a transparent appearance. This indicates that the emulsion is in the form of a microemulsion. The microemulsion has kinetic and thermodynamic stability.

Gupta et al. [1] reported that the droplet sizes for nanoemulsions were in the range of 20-500 nm and for microemulsions in the range of 10-100 nm. Nanoemulsions are thermodynamically unstable. This is different from thermodynamically stable microemulsions [7]. Microemulsion and nanoemulsion are emulsions that have kinetic stability in the size range of 100 nm. Microemulsion has stability on heating, but nanoemulsion is not resistant to heating above 100 °C [4, 18].

Praça et al. [20] reported that vitamin E microemulsion at a concentration of 0.1% (w/w) had a particle size of 256.7 nm. The microemulsion was obtained from a mixture of surfactant: co-surfactant (Tween 80 and propylene glycol) at a ratio of 5:1 with Isopropyl myristate as the oil phase. A microemulsion mixture of three non-ionic surfactants (Tween 80, tween 20, and Span 80) with VCO was able to carry up to 2% vitamin E with a particle size below 30 nm. Meanwhile, Rahman et al. [21] found out that a microemulsion from a mixture of tween 80, oleic acid, and water in a ratio of 5:5:90 had a transparent appearance and an average particle size of 32.6 ± 3.6 nm. This shows that the difference in surfactant formula and oil phase as a vitamin E carrier system influences the ability to carry vitamin E [22]. Microemulsion based on the regression equation has an ability as a vitamin E carrier system of 9%.

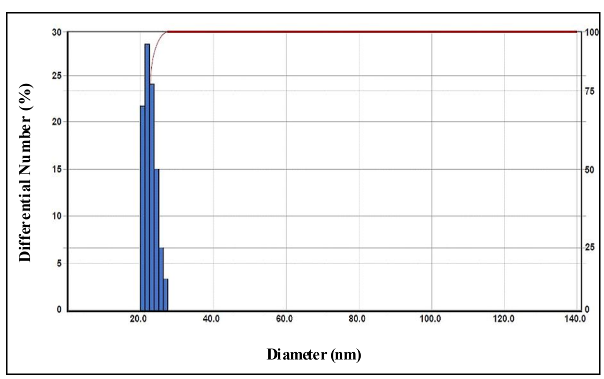

Figure 2. The diameter distribution of O/W microemulsion droplets

The stability of α-tocopherol microemulsion at different pH and dilutions

Fig. 3a shows the stability of α-tocopherol microemulsion at pH 4.5 with dilutions of 10, 50, and 100 times. The changes in dilution caused differences in the stability of microemulsions in carrying α-tocopherol. The increase in dilution causes a decrease in surfactant concentration and results in a decrease in the turbidity index value. Increasing the dilution of α-tocopherol microemulsion at pH 4.5 decreases its turbidity index. This is supported by the opinion that reducing the surfactant concentration causes the surfactant to form micelles and to release into its monomer form [23, 24]. This shows that the α-tocopherol microemulsion was able to maintain its stability.

Fig. 3b shows the stability of α-tocopherol microemulsion at pH 5.5 with dilutions of 10, 50, and 100 times. The changes in dilution cause differences in the stability of microemulsions in carrying α-tocopherol. Dilution up to 100 times causes a sharp decrease in the turbidity index value, which allows the α-tocopherol microemulsion to become unstable. Meanwhile, diluents up to 50 times cause a slow decline in the turbidity index value. This allows the α-tocopherol microemulsion to be able to maintain its stability. This is in accordance with the opinion that the turbidity index of microemulsions at a certain pH tends to decrease with increasing water concentration [20, 25].

Fig. 3c shows the stability of α-tocopherol microemulsion at pH 6.5 with dilutions of 10, 50, and 100 times. The changes in dilution cause differences in the stability of microemulsions in carrying α-tocopherol. Dilution 50 times at pH 6.5 makes the turbidity index value decrease quite sharply. The turbidity index of α-tocopherol microemulsion at pH 6.5 decreased with the increasing dilution or increasing water concentration. This causes the α-tocopherol microemulsion to become unstable. The turbidity index of microemulsions at a certain pH tends to decrease with increasing dilution [23, 26].

Kanike et al. [27] explained that the characteristics of nonionic oil/water microemulsions were strongly influenced by the changes in the surfactant-co-surfactant-oil ratio with a solvent with a hydrophobic charge. This affects the microstructure of the microemulsion due to the changes in water dilution and oil concentration [28, 29].

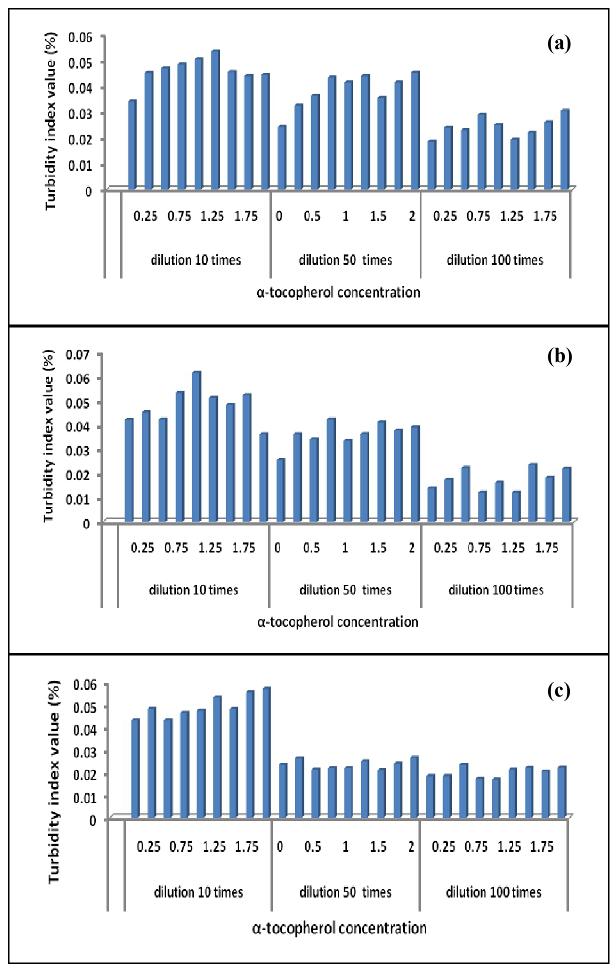

Figure 3. **The stability of α-tocopherol microemulsion at (a) pH 4.5, (b) pH 5.5, and (c) pH 6.5 with dilutions of 10, 50, and 100 times.**

Conclusion

The microemulsion is able to carry α-tocopherol compounds up to a concentration of 2% with a turbidity value reaching 0.1036 ± 0.0033% with a transparent appearance. A mixture of three surfactants (Tween 80, Tween 20 and Span 80) has the ability to increase droplet stability. The α-tocopherol microemulsion has a droplet diameter below 30 nm and remains stable against the effects of centrifugation at pH 4.5 with dilutions up to 50 times and is still quite stable at 4.5 – 6.5 with dilutions between 50 – 100 times.

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Conflict of Interest

There is no conflict of interest among the authors or with other parties.

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