Effect of Surfactant Addition on The Physico-Chemical Properties and Stability of Virgin Coconut Oil Nanoemulsions

Pengaruh Penambahan Surfaktan terhadap Sifat Fisikokimia dan Stabilitas Emulsi VCO

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ABSTRACT

Virgin coconut oil (VCO) is high quality coconut oil and categorized as the healthiest oil and functional foods. Based on these benefits, the development of a VCO in emulsion product might increase the human consumption of coconut oil because consumers dislike the only taste of pure VCO. The aim of this study was to develop the water compatible form of VCO through nano-emulsification. The effect of different types and amounts of surfactants (Tween 80 and Span 80) on the physio-chemical characteristic of emulsion containing VCO was investigated. VCO based emulsions were prepared with the aid of Ultra-Turrax homogenizer. Emulsions were developed by adding and mixing VCO with surfactants. The ratio of Tween 80 and Span 80 used were 0:10, 2.5:7.5, 5:5, 7.5:2.5, and 10:0. The droplet size of nanoemulsions consisting of Tween 80:Span 80 (0:10; 2.5:7.5, 5:5, 7.5:2.5, and 10:0) were 1.343, 0.606, 0.829, 1.439, and 2.506µm, respectively. Based on the TEM analysis and polydispersity index (PDI) >0.5 showed the oil droplets are in not uniform shape, indicating a unstable emulsion. VCO emulsion with ratio Tween 80:Span 80 (0:10) obtained a homogeneous emulsion (stable) compare than that of others and w/o type emulsion. The stability of emulsion is evaluated by turbidity measurement using UV-VIS spectrophotometer with wavelength 502 nm. A combination of treatments (ambient condition, thermal treatmant (40°C), and centrifuge) of VCO emulsion has resulted on thermal treat, the turbidity measured from the emulsion was higher than the other emulsion, reflecting the presence of the smaller droplets in this emulsion.

Keywords: Nanoemulsion, Ultra-Turrax Homogenizer, Tween 80, Span 80

ABSTRAK

Virgin Coconut Oil adalah minyak kelapa kualitas tinggi dan dikategorikan sebagai minyak paling sehat dan pangan fungsional. Berdasarkan manfaatnya, VCO dikembangkan dalam produk emulsi yang dapat meningkatkan konsumsi minyak kelapa karena sebagian konsumen tidak menyukai rasa VCO. Tujuan dari penelitian ini adalah mengembangkan bentuk VCO yang kompatibel dengan air melalui nano-emulsification. Emulsi dibuat dengan menambahkan VCO dan surfaktan (Tween 80 dan Span 80). Rasio Tween 80 dan Span 80, yaitu 0:10; 2.5:7.5; 5:5; 7.5:2.5 dan 10:0. Ukuran droplet dari sampel nanoemulsi yang diperoleh masing-masing adalah 1,343; 0,606; 0,829; 1,439 dan 2,506µm. Berdasarkan hasil analisis TEM dan indeks polidispersitas (PDI) >0,5 menunjukkan droplet minyak tidak seragam yang mengindikasi emulsi tidak stabil. Emulsi VCO dengan rasio Tween 80:Span 80 (0:10) diperoleh emulsi yang homogen (stabil) dibandingkan sampel lain dan termasuk tipe emulsi w/o. Stabilitas emulsi dievaluasi dengan mengukur turbiditas emulsi menggunakan spektrofotometer UV-Vis pada panjang gelombang 502 nm dengan perlakuan penyimpanan pada suhu ruang, suhu tinggi (40°C) dan sentrifus. Emulsi VCO dengan penyimpanan suhu tinggi (40°C) memiliki nilai turbiditas yang tinggi, mengindikasikan droplet minyak dalam emulsi lebih kecil dibandingkan sampel lain.

Kata kunci: Nanoemulsi, Ultra-Turrax Homogenizer, Tween 80, Span 80
INTRODUCTION

Presently, public awareness on functional food is increasing and it is expected that virgin coconut oil (VCO) to used in food functional applications (Ng Siou et al. 2014). Virgin coconut oil (VCO) is high quality coconut oil and categorized as the healthiest oil and functional foods (Marina et al. 2009). VCO is a unique group of oil products, characterized by the high content of medium chain triglycerides (MCT) C6-C12, especially the lauric acid (Silalahi, 2020). VCO has many health benefits such as reduce levels of total cholesterol, triglyceride, fosfolipid, and low density lipoprotein (LDL), also increase levels of high density lipoprotein (HDL) known as good cholesterol (Srivastava et al. 2013).

Medium chain triglyceride (MCT), especially lauric acid gets converted in the human system into monolaurin, the best that mother’s milk has and monolaurin are found only in coconut oil other than mother’s milk (Silalahi, 2020). Monolaurin can be digested rapidly for use as energy and improve the absorption of other nutrients such as minerals, vitamins, and amino acids (Kern et al. 2000). VCO can strengthen the body’s immune system, triglycerides of VCO are hydrolyzed into monoglycerides and free fatty acids, which have antibacterial, antifungal, antiviral, hypoglycemic activities, prevent obesity, induce insulin sensitivity and hence decrease the incidence or prevent diabetes (Silalahi 2020). Lauric acid preventing various cardiovascular diseases risks such as high blood pressure or atherosclerosis by lowering total cholesterol to a good cholesterol level (HDL) and hence decrease LDL:HDL ratio (Mikolajczak 2017). Based on these benefits, the development of a VCO in emulsion product might increase the human consumption of coconut oil because consumers dislike the only taste of pure VCO (Khor et al. 2014).

Emulsion are dispersants of one liquid in another immiscible liquid (Gani and Benjakul 2018). The stability of emulsions is the most important parameters for the shelf life of the food products (Li and Xiang, 2019). The emulsions have a small particle size (nano size), high stability, large interfacial area, and low interfacial tension (Dizaj 2013).

Nanoemulsions are primarily produced either by high-energy emulsification (e.g., high-pressure homogenization) consume significant energy (~10^8-10^10 Wkg^-1) to make small droplets or by low energy emulsification (using physicochemical properties of the components) to make small without consuming significant energy (~10^3 Wkg^-1) (Bernardi et al. 2011; Gupta et al. 2016). Nanoemulsions usually have better stability to particle aggregation and gravitational separation due to their small droplet sizes (McClements and Rao 2011; Ahmed et al. 2012). The purpose of this study was to determine the effects of type and amount of surfactants on stability, physical, and chemical properties of nanoemulsions prepared from virgin coconut oil by using Ultra-Turrax homogenizer (low-energy method) including observation through droplet size, morphology particle, emulsion stability, viscosity, color test, and pH measurements.

MATERIALS AND METHODS

This research was conducted in the Laboratory of Indonesian Center for Agricultural Post Harvest Research and Development on the period of August-December 2020. The material used are virgin coconut oil from Palu Tall coconut variety was obtained from Indonesian Palmae Crops Research Institute (IPCRI) Manado with Direct Micro Expelling (DME), Tween 80 and span 80 (pharma grade), and mineral water.

Preparation of virgin coconut oil emulsions

Emulsions based on VCO were prepared with the aid of Ultra-Turrax homogenizer. Emulsions were prepared by mixing VCO and surfactants (Tween 80 and Span 80). The formula of emulsion VCO is shown in Table 1. HLB (Hydrophilic Lipophilic Balance) value of Tween 80 and Span 80 is 15 and 4.5. VCO used is 15% (b/b) with total weight is 100 g.
Effect of Surfactant Addition on The Physico-Chemical Properties and Stability of Virgin Coconut Oil Nanoemulsions (Linda Trivana, et al.)

Table 1. The formula of emulsion VCO

| Composition | Sample | 1  | 2  | 3  | 4  | 5  |
|-------------|--------|----|----|----|----|----|
| Tween 80    |        | 0  | 2.5| 5  | 2.5| 10 |
| Span 80     |        | 10 | 7.5| 5  | 7.5| 0  |
| VCO         |        | 15 | 15 | 15 | 15 | 15 |
| Aquades     |        | 75 | 75 | 75 | 75 | 75 |

Emulsions were prepared by mixing VCO as oil phase, water as water phase, and surfactants (Tween 80 and Span 80). Furthermore, the mixture was heated at temperature of 65°C while stirring with a stirrer speed of 700 rpm using a hot plate magnetic stirrer for 15 minutes, and then homogenized with Ultra-Turrax homogenizer (IKA T25 digital ULTRA-TURRAX) at a speed of 11000 rpm for 10 minutes.

Fatty acid analysis

Analysis of Fatty acid in VCO by Gas Chromatography with column CP-SIL-88 (colom length: 30 m, diameter: 0.32 mm), Flame Ionization Detector (FID), injector and detector temperature on 230°C, Initial column temperature is 120°C and then programmed to be 200°C with temperature gradient 8°C/minute, and nitrogen gas pressure of 3 kg/cm².

Evaluation of particle size and polydispersed index

Each sample emulsion was diluted in distilled water. An aliquot of sample was measured in a range of 0.1-1000 µm for the average oil droplet size and poly dispersity index (PDI) by Particle Size Analyzer. A PDI < 0.1 was considered to be a good quality, while the values of 1 were considered to be poor quality samples. All of the measurements were performed under standard conditions of dilution and temperature, and they were executed in 3 replications (triplicate).

Evaluation a drop of micro-emulsion by TEM

Transmission Electron Microscope (TEM) was employed for microscopic evaluation of a drop of emulsion. Evaluation of a drop of micro-emulsion was applied on carbon coated grid with 2% uranium acetate and it was left for 30 sec. The dried coated grid was taken on a slide and after placing the cover slip, it was observed under TEM, operated at 60-80kV at different magnifications.

Determination of the emulsion type

Determination of the emulsion type was done following a dilution method. The sample was dissolved in the water phase (1:100) and oil phase (1:100). An emulsion is categorized as O/W type when it is completely dissolved in the water phase, while it is W/O type if the sample completely dissolves in the oil phase (Yuliani et al. 2016).

Colour measurement

The L*, a*, and b* colour system values of the emulsion samples were measured using a Minolta Colorimeter CR-300 (AMTAST). In this colour system, L* represents the lightness, and a* and b* are the colour coordinates, whereby +a represents the red coordinate, -a is the green coordinate, +b is the yellow coordinate, and -b is the blue coordinate. As the values of a* and b* rise, the colour becomes more saturated or chromatic, but these values approach zero for neutral colours (white, grey, or black) (Ng Siou et al 2014). The colour measurements of each emulsion sample were performed in triplicate.

Evaluation of pH value

The pH of nanoemulsions was evaluated by pH meter. The measurement at 0, 3, 5, and 7 days was run in triplicate.

Evaluation of viscosity

The viscosity of each sample was determined using a Brookfield viscometer (HAAKE VISCOTESTER Thermo Scientific). All of the observations were performed at 25°C with spindel number L1 and speed 3 rpm. The measurement at 0; 3; 5; and 7 days.
Stability test of emulsion

Stable emulsion samples were tested for stability by centrifuge, thermal treatment and ambient condition test according to Ariviani et al. (2015). Centrifuge test was performed at a speed of 10000 rpm for 30 min to separate coconut cream and aqueous phases. The thermal treatment at 60°C for 5 hour. Freshly VCO emulsion was left in the room for 7, 14, 21, and 28 days then observed turbidity using spectrophotometer at wavelength of 502 nm. The turbidity is measured by following the equation (Fletcher and Suhling 1998):

\[
\text{Turbidity (\%)} = \frac{2.303 \times \text{Absorbance}}{\text{cuvette width}} \times 100\%
\]

RESULTS AND DISCUSSION

Fatty acid analysis

The composition of fatty acid in VCO DME shown Table 2. The fatty acids component were identified from the chromatograms and standardcurves of commercial standard mixture of FAME. Based Table 2, the dominant fatty acid found in VCO DME was lauric acid (47083 mg/100g). It has known that VCO is rich in saturated fatty acid due to its high content of lauric acid. The other saturated fatty acids such as myristic acid was the second highest concentration of fatty acid (16678 mg/100g), followed by caprylic acid (8583 mg/100g), palmitic acid (8481 mg/100g), capric acid (6447 mg/100g), oleic acid (5589 mg/100g), and stearic acid (mg/100g).

Evaluation of Particle size and poly dispersity index

Nanoemulsions were prepared using the low energy method. The oil and surfactant were mixed together, and then simply added to the water using slow stirring. When two phase are brought into contact, some of the component that is partially miscible with both phases will move from its original location in one phase into the other phase. The underlying mechanism is the movement of the water-dispersible substance from the oil phase to the water phase, leading to spontaneous oil droplet formation (McClemets and Rao 2011). The result of homogenized with Ultra-Turrax Homogenization shown in Table 3.

| Fatty Acid Component       | Concentration (%) |
|---------------------------|-------------------|
| A. Asam lemak Jenuh       |                   |
| Caprylic Acid (C8:0)       | 8.583             |
| Asam Kaprilat (C8:0)       |                   |
| Capric Acid (C10:0)        | 6.447             |
| Asam Kaprat (C10:0)        |                   |
| Lauric Acid (C12:0)        | 47.083            |
| Asam Laurat (C12:0)        |                   |
| Myristic Acid (C14:0)      |                   |
| Asam Miristat (C14:0)      | 16.678            |
| Palmitic Acid (C16:0)      | 8.481             |
| Asam Palmitat (C16:0)      |                   |
| Stearic Acid (C18:0)       | 4.197             |
| Asam Stearat (C18:0)       |                   |
| Arachidic Acid (C20:0)     | 0.073             |
| Asam Arahidat (C20:0)      |                   |
| Dodecanonic Acid (C22:0)   | nd                |
| Asam Dodekanoat (C22:0)    |                   |
| Total Saturated Fatty Acid |                   |
| Total Asam Lemak Jenuh     | 91.542            |
| B. Unsaturated Fatty Acid  |                   |
| Myristoleic Acid (C14:1)   | nd                |
| Asam Miristoleat (C14:1)   |                   |
| Palmitoleic Acid (C16:1)   | 0.007             |
| Asam Palmitoneat (C16:1)   |                   |
| Oleic Acid (C18:1)         | 5.589             |
| Asam Oleat (C18:1)         |                   |
| Linoleic Acid (C18:2)      | 1.096             |
| Asam Linoleat (C18:2)      |                   |
| α-Linolenic Acid (C18:3)   | nd                |
| Asam α-linolenat (C18:3)   |                   |
| Eicosanoic Acid (C20:1)    | 0.028             |
| Asam Ekikosanoat (C20:1)   |                   |
| Arachidonic Acid (C20:4)   | nd                |
| Asam Arabinodonat (C20:4)  |                   |
| EPA (C20:5)                | nd                |
| EPA (C20:5)                |                   |
| DHA (C20:6)                | nd                |
| DHA (C20:6)                |                   |
| Total Unsaturated Fatty Acid|                   |
| Total Asam Lemak Tak Jenuh | 6.710             |
| B. Unknown Fatty Acid      |                   |
| B. Asam Lemak Tidak Dikenal| 0.262             |
| D. Total Fatty Acid        |                   |
| D. Total Asam Lemak        | 98.514            |

Note: nd: not detected
Keterangan: nd: tidak terdeteksi

Table 2. Fatty acids profile of VCO DME
Tabel 2. Profil asam lemak VCO DME
### Table 3. Evaluation of particle size, PDI, emulsion type, and appearance VCO emulsion

| Treatment | Analysis parameter | Emulsion type | Appearance |
|-----------|-------------------|---------------|------------|
| Tween 80  | Tween 80 Span 80  | Particle Size (µm) | PDI value | Tipe emulsi | Penampakan |
| 0         | 0 10              | 1.343±0.105   | 0.848±0.264 | O/W         | Homogenous |
| 2.5       | 7.5               | 0.606±0.009   | 0.990±0.016 | O/W         | 2 layers   |
| 5         | 5                 | 0.829±0.055   | 0.762±0.280 | O/W         | 2 layers   |
| 7.5       | 2.5               | 1.439±0.015   | 1.000±0.000 | O/W         | 2 layers   |
| 10        | 0                 | 2.506±0.011   | 0.804±0.186 | O/W         | 2 layers   |

Type of surfactant has an impact on emulsion properties. Table 3 shows effect surfactant addition on homogeneous system of emulsions. The results suggested that the surfactant effect of Tween 80:Span 80 (0:10) was much greater than the others. VCO emulsion with surfactant Tween 80:Span 80 (0:10) has a homogeneous. Emulsion’s instability is evidenced...
by creaming occurs at VCO emulsion (not homogenous). Creaming may be defined as the upward movement of dispersed globules to form a thick layer at the surface of the emulsion (Madaan et al. 2014). A number of factors may contribute to creaming stability of the emulsions. First, the creaming velocity of a lipid droplet increases in emulsions containing larger droplets than those containing smaller ones. Second, creaming may occur due to insufficient emulsifier in the emulsion system will cause phase separation so the presence of non adsorbed surfactant micelles between droplets resulting flocculation leading to rapid creaming (Yang et al. 2012).

VCO emulsion sample using Tween 80 obtained not homogeneous emulsion which was formed in 2 layers because Tween 80 is hydrophilic (HLB 15) and insoluble in oil (Prieto and Calvo 2013). While, VCO emulsion sample with only Span 80 has a homogeneous emulsion, because Span 80 have HLB 4.3 is surfactant lipophilic. Span 80 is emulsifier type water in oil (w/o) with an oil-soluble hydrophobic group and insoluble in water but can dispersed in hot and cold water (Mahdi et al. 2011). Several factors were identified to cause stability of emulsion were kinds and concentration of surfactant, emulsification techniques, and water contents in emulsification process (Suryadi et al. 2019).

The Polidispersity Index (PDI) and particle size are other measurements in nanoparticle characterization were analyzed using Particle Size Analysis (PSA). Polydispersity Index (PDI) describes the level of uniformity in a system, the smaller the PDI value, the more uniform the distribution of particles in a monodispersion system (Luo et al. 2007). The small PDI value (which is close to 0) shows that the droplet size is increasingly uniform and homogeneous (Ariviani et al. 2015). The size and polydispersity (PDI) of nanoemulsions can affect properties such as particle stability, rheology, appearance, colour, texture, and shelf life (Bernadi et al. 2011).

**Effect of type surfactant on colour emulsion**

Type of surfactant has impact on emulsion properties (Pengo et al. 2013). The measurement of color obtained values like \( L^* \) (achromatic colour) represents the parameter of brightness (lightness) with a value range from 0-100 (indicating dark to light). The value of \( a^* \) and \( b^* \) (chromatic colour), \( a^* \) value (negative) indicates the colour to green and \( a^* \) (positive) to red with range 0-60. The value of \( b^* \) (negative) indicates the colour to blue and \( b^* \) (positive) to yellow. \( C^* \) value is the value of colour sharpness. The higher the \( C^* \) value, the clearer the colour and if the \( C^* \) value is small, the colour will fade. The Hue value is expressed in a degree of hue (\(^\circ\)Hue), it represents the visual colour seen (Safitri dan Adawiyah 2009). Table 4 shows the colours of VCO emulsion.

### Table 4. The colours of VCO emulsion

| Ratio       | L*     | a*     | b*     | C*     | Hue |
|-------------|--------|--------|--------|--------|-----|
| Tween 80:  | 27.30±0.13d | -7.47±0.18a | 1.62±0.16a | 7.50±1.04a | 167.31±1.04b |
| Span 80     |        |        |        |        |     |
| Rasio       |        |        |        |        |     |
| Tween 80:  | 27.30±0.13d | -7.47±0.18a | 1.62±0.16a | 7.50±1.04a | 167.31±1.04b |
| Span 80     |        |        |        |        |     |
| 0:10        | 27.30±0.13d | -7.47±0.18a | 1.62±0.16a | 7.50±1.04a | 167.31±1.04b |
| 2:5:7.5     | 27.25±0.08d | -7.22±0.32a | 1.52±0.16a | 7.38±0.34a | 168.10±0.86b |
| 5:5         | 23.94±0.18a | -7.47±0.58a | 2.33±0.41b | 7.82±0.67a | 162.75±1.63a |
| 7.5:2.5     | 23.70±0.33b | -7.17±0.20a | 2.47±0.36b | 7.59±0.24a | 160.95±2.51a |
| 10:0        | 24.02±0.03c | -7.49±0.22a | 2.24±0.20b | 7.82±0.24a | 163.33±1.20a |

Note: Numbers followed by the different letter at the same column are significantly different at 5% of DMRT

*Keterangan: angka yang diikuti huruf berbeda pada kolom yang sama berbeda nyata pada taraf uji DMRT 5%*
Based on Table 4, no significant difference at \( a^* \) and \( C^* \) value for all treatment. While, \( b^* \) and Hue value at Tween 80:Span 80 (0:10) and (2.5:7.5) significant difference on (5:5); (7.5:2.5), and (10:0). The \( L^* \) value at sample Tween 80:Span 80 (0:10) and (2.5:7.5) no significant difference but significant different (5:5); (7.5:2.5), and (10:0). This might because Span 80 (HLB 4.3) soluble in VCO and can form a more stable emulsion compared than samples with higher Tween 80 (HLB 15) which forms unstable emulsion.

**Effect of storage time on pH emulsion**

Monitoring the pH value is important for determining the stability of the emulsions because pH changes indicate the occurrence of chemical reactions that can compromise the quality of the final product. Effect of storage time on pH emulsion shown in Figure 1. Freshly VCO emulsion has pH 7.88-8.50 and decreases the longer it is stored. A decrease in pH due to the hydrolysis of fatty acid esters (ion H\(^+\) of VCO dissociated) into free fatty acid degradation products (Bernardi et al. 2011), increase the level of free fatty acids (FFA) in the VCO.

The change in pH of the emulsion during storage indicates that the emulsion is less stable during storage. Based on Figure 1, treatment Tween 80:Span 80 (5:5) and (10:0) has high initial pH value but experienced a sharp drop in pH compare than the others. This shows that sample with ratio Tween 80:Span 80 respectively 5:5 and 10:0 more unstable (phase separation) than the other samples which shows a sharp decrease in pH.

**Effect storage time on viscosity emulsion**

The initial viscosity of each treatment ratio Tween 80:Span 80 (0:10; 2.5:7.5; 5:5; 7.5:2.5; 10:0) respectively 97.5 cp, 97.4 cp, 97.4 cp, 97.8 cp, and 98.0 cp. The viscosity of the emulsion can be affected by the droplet size. Emulsions with large droplet sizes (Table 2) have high viscosity values (McClements and Rao, 2011). Effect of storage time on viscosity emulsion shown in Figure 2. Viscosity values for 8 days at five sample of VCO emulsion tended to decreasing. The faster viscosity value was changing closely related to emulsion stability (Suryadi et al. 2019). The most constant of viscosity at treatment Tween 80:Span 80 (0:10), because the surfactant system able to maintain emulsion stability at Tween 80:Span 80 (0:10). The emulsion could retard the free motion of the oil droplets, thereby delaying creaming, flocculation, and coalescence of the oil droplets (Tesch and Schubert, 2002).

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**Figure 1.** The effect of storage time on pH emulsion

*Gambar 1. Pengaruh waktu penyimpanan terhadap pH emulsi*
The viscosity decreases, it means that the emulsion is dilute which causes the dispersed phase to move easily to the dispersing medium so that larger globules or particles are formed. The decrease in the apparent viscosity of the emulsions with time at a constant shear was most likely due to the deformation and/or reorganisation of oil droplet flocs that had previously aggregated due to weak forced (Ng Siou et al. 2014).

**Evaluation of emulsion type in VCO emulsion**

The emulsion type was determined by dilution method. Emulsion type was determined by observing what happened when a drop of each emulsion was added to volume of either VCO or pure water. Drops of W/O emulsion are dispersed in oil and remained as drops in water (Von 2009). The all treatment include is O/W type cause completely dispersed in the water and drops in oil (Figure 3). The emulsion type can also be determined by the amount of each substance. The emulsion of W/O type, it means that the amount of water is less than oil so that water acts as a dispersant agent and oil as a dispersing agent. Whereas, type O/W the amount of oil as a dispersant is less than the amount of water as a dispersing agent.

**Figure 2. The effect storage time on viscosity emulsion**

*Gambar 2. Pengaruh waktu penyimpanan terhadap viskositas emulsi*

The all treatment include is O/W type cause completely dispersed in the water and drops in oil (Figure 3). The emulsion type can also be determined by the amount of each substance. The emulsion of W/O type, it means that the amount of water is less than oil so that water acts as a dispersant agent and oil as a dispersing agent. Whereas, type O/W the amount of oil as a dispersant is less than the amount of water as a dispersing agent.
Stability test of emulsion

Emulsion with 100% Span 80 has a homogeneous or not separate emulsion, then the emulsion will be tested for emulsion stability. The stability of an emulsion is concerned with the maintenance of internal phase dispersion in the external phase without effective changes in both phases (the same number and size of dispersed droplets in dispersion medium) (Alam et al., 2015). The stability of emulsions against droplet aggregation and flocculation was determined by turbidity measurement using UV-VIS spectrophotometer with wavelength 502 nm. The principle of this method is based on the fact that the size of partially coalesced fat droplets is bigger than individual droplets that are not partial coalesced, which in turn make the coalesced droplets have less total surface area to scatter light, hence less absorbance value reading (Marshall et al., 2003). The turbidity of emulsions has been shown to be inversely related to the oil droplet size, i.e., the lower the turbidity, the bigger the mean particle diameter or flocculation (Aoki et al., 2005).

The absorbance of diluted emulsions was measured at 502 nm by UV-visible spectrophotometer. Emulsion sample was placed in test tube then left to stand for 7, 14, 21, and 28 days at ambient condition and also observation by centrifuge method with speed of 10000 rpm for 30 min and thermal treatment at 60°C for 5 hour. Trends of absorbance value plotted in linear equation. The most stable emulsion has linear equation with slope closely to zero. It means there is no change in the emulsion system at particle scope (Suryadi et al., 2019). Fig. 4 shows the trend of absorbance ratio depending of time.

![Figure 4. The absorbance of diluted emulsion on storage time](image)

| Treatment       | Turbidity (%) | Turbidity (%) |
|-----------------|---------------|---------------|
|                 | 0  | 7  | 14 | 21 | 28 |
| Ambient condition | 1.213±0.0084 | 0.4681±0.0009 | 0.1278±0.0008 | 0.0642±0.00013 | 0.0215±0.00017 |
| Centrifuge      | 0.2814±0.0040 | 0.2229±0.0014 | 0.0621±0.0021 | 0.0154±0.0004 | 0.0079±0.0009 |
| Thermal treat   | 1.3413±0.0017 | 0.5541±0.0016 | 0.1323±0.0025 | 0.0676±0.0009 | 0.0246±0.0010 |

Table 5. Turbidity of emulsion on accelerated stability test
Tabel 5. Turbiditas emulsi pada uji stabilitas dipercepat
The results show that as the absorbance of absorbance values decreases with increasing storage time. Decreasing of absorbance value to time caused by particles in emulsion form the bigger aggregate and creaming occurs. Small particle were remained in emulsion phase and causing the turbidity value smaller because the bigger aggregate has settled and undetectable at same point in UV-Vis Spectrophotometry (Suryadi et al. 2019). The absorbance of emulsions changes inversely with the particle size of suspended particle in emulsions (Reddy Fogler, 1981). The turbidity of emulsion during storage time (days) shown in Table 5.

The turbidity usually decreases with increasing mean particle diameter which may be attributed to the creaming of those droplets if this occurs during storage (Aoki et al., 2005; Ogawa et al., 2004). At thermal treat, the turbidity measured from the emulsion was higher than the other emulsion, reflecting the presence of the smaller droplets in this emulsion. The turbidity of the other emulsions was measured to be low, indicating the larger particle size present in the emulsions based on the turbidity measurement. The slope value of emulsion expresses in Table 6. Minus (-) value of slopes describes the trend of decreasing turbidity during storage (Suryadi et al. 2019). From the slope value, it can conclude that emulsion with thermal treat is the rate of reduction in turbidity is slower than centrifuge and ambient condition. Large droplet size of emulsions causes emulsion stability decreasing because easier to coalescence and form the creaming (Suryadi et al. 2019).

### Evaluation a drop of micro-emulsion by TEM

Emulsions are not thermodynamically stable and undergo destabilization through various phenomena, including droplet flocculation and coalescence. Microscopic structure of VCO emulsion is shown in Fig. 5. Based a Fig. 5(a), where it can be observed that the oil droplets are in not uniform shape, indicating a unstable emulsion. Fig. 5(b) shows the microstructure of VCO emulsion has been flocculation occurs. Flocculation is a phenomenon where oil droplets dispersed in emulsions become clustered without losing their original shape but with maintaining their individual identity, thus can be reversed into their original droplets (Goff and Hartel 2013). The flocculation would increase the rate of creaming (Madaan et al. 2014). Fig. 5(c) shows the oil droplets coalesced and formed bigger droplets. Coalescence is the complete fusion of droplets leads to a decrease in the number of droplet and the ultimate separation of the two immiscible phases (Madaan et al. 2014).

**Figure 5. Morphology of droplet VCO emulsion with 100% Span 80 (a); flocculation (b); coalescence (c).**

**Gambar 5. Morfologi droplet VCO dengan 100% Span 80 (a); flokulasi (b); koalesens (c).**

**CONCLUSIONS**
Effect of surfactant addition on VCO emulsion obtained was homogenous emulsion, physico-chemical properties and stability of emulsions. Emulsion with Span 80 as a surfactant at the concentration of 10% (b/b) obtained homogenous emulsion compare than that of others and the type emulsion is w/o. This emulsions has good stability with an appropriate pH value and increased viscosity during storage. The particle size and PDI value of emulsion can be fixed by the time and speed of Ultra-Turrax Homogenizer, use the other mechanical mixing as High Pressure Homogenizer or Ultrasound, and the amount and type of emulsifier. Stable emulsion samples were tested for stability by centrifuge, thermal treatment and room temperature test. The stability of emulsions against droplet aggregation and flocculation was determined by turbidity measurement. At thermal treat, the turbidity measured from the emulsion was higher than the other emulsion, reflecting the presence of the smaller droplets in this emulsion and the smallest slope value, it can conclude that emulsion with thermal treat is the rate of reduction in turbidity is slower than centrifuge and ambient condition.

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REFERENCES

Ahmed, K., Li, Y., McClements, D.J., Xiao, H. 2012. Nanoemulsion and emulsion based delivery systems for curcumin: encapsulation and release properties. Food Chemistry Journal 132: 799-807.

Alam, M.S., Ali, M.S., Ala, M.I., Anwar, T., Asafhi, M.M. 2015. Stability testing of beclomethasone dipropionate nanoemulsion. Tropical Journal of Pharmaceutical Research 14(1):15-20

Aoki, T., Decker, E.A., McClements, D.J. 2005. Influence of environmental stresses on stability of o/w emulsions containing droplets stabilized by multilayered membranes produced by a layer-layer electrostatic deposition technique. Food Hydrocolloids 19(2): 209-220.

Bernardi, D.S., Pereira, T.A., Maciel, N.R., Bortoloto, J., Viera, G.S., Oliveira, G.C., Rocha-Filho, P.A. 2011. Formation and stability of oil-in-water nanoemulsions containing rice bran oil: in vitro and in vivo assessments. Journal of Nanobiotechnology 9(1): 1-9.

Ariviani, S., Raharjo, S., Anggrahini, S., Naruki, S. 2015. Formulasi dan stabilitas mikroemulsii o/w dengan metode emulsifikasi spontan menggunakan VCO dan minyak ssawit sebagai fase minyak: pengaruh rasio surfaktan-minyak. Agritech 35(1): 27-34.

Bernadi, D.S., Pereira, T.A., Maciel, N.R., Bortoloto, J., Viera, G.S., Oliveira, G.C., Rocha-Filho, P.A. 2011. Formation and stability of oil-in-water nanoemulsions containing rice bran oil: in vitro and in vivo assessments. Journal of Nanobiotechnology 9(1):1-9

Dizaj, S.M. 2013. Preparation and study of vitamin A palmitate microemulsion drug delivery system and investigation of co-surfactant effect. Journal of Nanostructure in Chemistry 3(1):1-6.

Gupta, A., Eral, H.B., Hatton, T.A., Doyle, P.S. 2016. Nanoemulsions: formation, properties, and application. Royal Society of Chemistry Journal 12: 2826-2841.

Mahdi, E.S., Sakeena, M.H., Abdulkarim, M.F., Abdullah, G.Z., Sattar, M.A., Noor, A.M. 2011. Effect of surfactant and surfactant blends on pseudoternary phase diagram behavior of newly synthesized palm kernel oil esters. Drug Design, Development and Therapy Journal 5:311-323.

Marshall, R.T., Goff, H.D., Hartel, R.W. 2003. Ice Cream. 6th ed. New York: Kluwer Academic/Plenum Publishers. 371p.

McClements, .D., Rao, J. 2011. Food-grade nanoemulsions: formulation, fabrication, properties, performance, biological fate, and potential toxicity. Critical Review in Food Science and Nutrition 51(4): 285-330.

Meybodi, N.M., Mohammadifar, M.A., Farhoodi, M., Skytte, J.L., Abdolmaleki, K. 2017. Physical stability of oil in water emulsions in the presence of gamma irradiated gum tragacanth. Journal of Dispersion Science and Technology. 38(6): 909-916.

Mikolajczak, N. 2017. Coconut oil in human diet nutrition value and potential health benefits. Journal of Education, Health, and Sport 7(9): 307-319.
Ng Siou, P., Lai Oi, M., Abas, F., Lim, H.K., Tan, C.P. 2014. Stability of a concentrated oil-in-water emulsion model prepared using palm olein-based diacylglycerol/virgin coconut oil blends: Effects of the rheological properties, droplet size distribution and microstructure. *Food Research International* 64: 919-930.

Ogawa, S., Decker, E.A., McClements, D.J. 2004 Production and characterization of o/w emulsions containing droplets stabilized by lecithin-chitosan-pectin multilayered membranes. *Journal of Agricultural and Food Chemistry* 52(11): 3595-3600.

Pengon, S., Chinatangkul, N., Limmatvapirat, C., Limmatvapirat, S. 2018. The effect of surfactant on the physical properties of coconut oil nanoemulsions. *Asian Journal of Pharmaceutical Sciences* 2(5): 1-15.

Prieto, C., Calvo, L. 2013. Performance of the biocompatible surfactant tween 80, for the formation of microemulsions suitable for new pharmaceutical processing. *Journal of Applied Chemistry* 1-10.

Reddy, S.R., Fogler, H.S. 1981. Emulsion stability: determination from turbidity. *Journal of Colloid and Interface Science* 79(1): 101-104.

Silalahi, J. 2020. Nutritional values and health protective properties of coconut oil. *Indonesian Journal of Pharmaceutical and Clinic Research* 3(2): 1-12.

Srivastava, Y., Semwal, A.D., Swamy, M.S.L. 2013. Hypcholesterimic effects of cold and hot extracted virgin coconut oil (VCO) in comparison to commercial coconut oil: evidence from a male wistar albino rat model. *Food Sci. Biotechnol* 22(6): 1501-1508.

Suryadi, J., Winardi, S. dan Nurtono, T. 2019. The effect of water contents to diesel fuel-water emulsion fuel stability. *The Journal for Technology and Science* 30(2): 2088-2033.

Yang, Y., Marshall-Breton, C., Leser, M.E., Sher, A.A., McClements, D.J. 2012. Fabrication of ultrafine edible emulsions: comparison of high-energy and low-energy homogenization methods. *Food Hydrocolloids Journal* 29(2): 398-406.

Yuliani, S.H., Hartini, M., Stephanie, Pudyastuti, B., Istyastono, P. 2016. Comparison of physical stability properties of pomegranate seed oil nanoemulsion dosage forms with long-chain triglyceride and medium-chain triglycerides as the oil phase. *Traditional Medicine Journal* 21(2): 93-98.