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Effect of Hydrophilic- Lipophilic Balance (HLB) Value on the Stability of Cosmetic Lotion Based on Walnut Oil *(Canarium Indicium L.)* Oil-in-Water Emulsion

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Abstract - *Walnut oil is high source of fatty acid especially oleic acid compare to coconut oil. It has 40-70% of oleic acid and contains antioxidants that are suitable as moisturizing agent, anti-inflammatory and good for skin health as a primary ingredient for lotion. This study aims to determine the effect hydrophilic-lipophilic balance (HLB) on the stability of emulsion as a lotion material. The oil-in-water emulsion was formed by emulsification process using Ultra Turrax homogenizer (8000 and 32000 rpm) with addition of tween 80 and span 80 as emulsifiers. Completed randomized design and analysis of variance were used to determine the significant parameters on the stability of emulsion: homogeneity, creaming index, viscosity, density and morphology. Emulsion with the highest stability is sample 8 which is formed at 14.5 HLB value and 32000 rpm homogenizer speed during 5 min. It has complied with SNI-16-4399-1996.*

Keywords: *emulsion, HLB, tween 80, span 80, walnut oil*

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INTRODUCTION

The use of oils from natural ingredients in the cosmetic and pharmaceutical industries has increased continuously (Reiger, 2000). Walnut oil contains rich fatty acids which are good for skin health and usually used as raw materials in the cosmetic industry (Hong et al., 2018). The highest fatty acid contained is oleic acid (40 – 70%) (Leakey et al., 2008). This oleic acid is a moisturizing agent, anti-inflammatory and contains antioxidants that are good for skin health (Mittal et al., 2012).

The potential of walnut oil as a basic ingredient for lotion preparation is processed through an emulsification process (Mitsui, 1997). Emulsions are usually formed by mixing oil phase and water phase with single or combination emulsifiers as stabilizer through the homogenization process (Leyden & Rawlings, 2002). The emulsifiers used in this study are Tween 80 and Span 80. Tween and Span are stable combinations of nonionic emulsifiers with non-irritating properties and suitable for skin (Hou & Papadopoulos, 1997). Stable emulsions can be produced from the use of a single emulsifier or a combination of emulsifiers with HLB values that are close to their needs according to the oil and water phases used (Hong et al., 2018). Calculation of the emulsifiers requirement through the HLB value was discovered by Griffin in 1954 (Jin et al., 2008).

According to Mollet and Grubenmann (2000), hydrophilic-lipophilic balance (HLB) is a value that shows the balance of chemical bonds between hydrophilic and lipophilic groups from each emulsifier. The HLB value can predict the type of emulsion. Emulsion with water-in-oil type produced from 1-8 HLB value and 9-18 HLB value will tend to produce an emulsion with oil-in-water type (Jin et al., 2008). In this study, emulsions of lotion preparations were formed using different ratios of Tween 80 and Span 80 and mixed by Ultra Turrax Homogenizer with

8000 rpm and 32000 rpm homogenizer speed during 2 and 5 min. The homogenization process will reduce surface tension of oil and water droplets to produce emulsion with high stability (Powell & Chauhan, 2016).

The purpose of this research is to observe the physicochemical and morphological properties of the lotion preparation emulsion. The parameters observed during this study included homogeneity, creaming index, viscosity, density and morphology of the lotion emulsion preparation.

METHODOLOGY

Raw materials used for this research are canarium oil, tween 80, span 80, paraffin liquid and aqua dest. The equipments for emulsification process are Ultra Turrax Homogenizer, thermometer, beaker glass, electric stove, stopwatch, measuring cup, pipettes, and digital balance. The fixed variables in this study were canarium oil (150 mL), paraffin liquid (100 mL), aqua dest (320 mL). The variables change are HLB value 10.5 (sample 1 – 4) and 14.5 (sample 5 – 8); homogenizer speed of 8000 rpm and 32000 rpm and homogenization time of 2 and 5 min. This research experiment uses the completely random design (CRD) method to setting the variables change and find the formula of canarium emulsion in the table 1.

Table 1. Formula of canarium emulsion

Sample name	Composition	HLB Value	Homogenizer speed	Homogenization time
S1T1	Tween 80 (17.4 mL); Span 80 (12.6 mL)	10.5	8000 rpm	2 min
S2T2	Tween 80 (17.4 mL); Span 80 (12.6 mL)	10.5	8000 rpm	5 min
S3T1	Tween 80 (17.4 mL); Span 80 (12.6 mL)	10.5	32000 rpm	2 min
S4T2	Tween 80 (17.4 mL); Span 80 (12.6 mL)	10.5	32000 rpm	5 min
S5T1	Tween 80 (28.6 mL); Span 80 (1.4 mL)	14.5	8000 rpm	2 min
S6T2	Tween 80 (28.6 mL); Span 80 (1.4 mL)	14.5	8000 rpm	5 min
S7T1	Tween 80 (28.6 mL); Span 80 (1.4 mL)	14.5	32000 rpm	2 min
S8T2	Tween 80 (28.6 mL); Span 80 (1.4 mL)	14.5	32000 rpm	5 min

Preparation of Canarium oil emulsion

The aqueous phase solution was prepared by dissolving a mixed emulsifier with specified ratio of HLB in 300 mL of distilled water at 60 ℃. The oil mixture (150 mL of Canarium oil and 100 mL of mineral oil) was blended using high speed magnetic stirrer. The O/W emulsion was prepared by dropping the oil phase into the aqueous with continuous stirred under high speed. To complete the emulsification process, the solutions were homogenized with a T 25 digital Ultra-Turrax (IKA Co. Ltd., China) at 8,000 rpm for 2 and 5 min with a 30 s interval between every 1 min of homogenization.

Stability of emulsion

The stability of W/O emulsion were investigated under 75% relative humidity with constant temperature 30 ℃ for 30 days. The homogeneity, creaming index value, viscosity, and physical morphology of emulsion were used to measure the stability of emulsion system.

Measurement of emulsion homogeneity

The homogeneity of emulsion was measured using ratio of gravitational separation during storage time. The phase separation ratio of emulsion could be calculated using Eq. (1):

$$F = \frac{V_u}{V_o} \neq \frac{\emptyset_u H_u}{\emptyset_o H_o} \qquad (1)$$

where F is ratio of phase separation, V is emulsion volume, ∅ is cross-sectional area and H is high of emulsion.

Measurement of creaming index

Creaming index of emulsion was investigated using gravitational separation tests under different relative humidity (RH) conditions at 30 °C for 30 days. Approximately 20 mL of each O/W emulsion was placed in a glass tube and stored at 30 °C under 75% humidity. During intervals storage, the creaming index of emulsion was measured. A 10 mL of emulsion was put into a centrifuge tube, then centrifuged at 4500 rpm for 3 min. The volume of cream formed was measured and percent of creaming index was calculated by Eq.2:

$$Creaming\ index\ (\%) = \frac{H_S}{H_T} x100 \qquad (2)$$

where H_S is the height of a serum layer and H_S is the total height of emulsion.

Measurement of viscosity

The viscosity of emulsion was measured using falling-ball viscosimeter. The value of viscosity was determined according to the Stoke's Law (Eq.3):

$$\eta = \frac{2}{9}\frac{R^2}{v}(\rho_o - \rho_e) \qquad (3)$$

where η is viscosity value of emulsion, v is volume emulsion, R^2 is diameter of falling ball, ρ_o is density of falling ball, and ρ_e is density of emulsion.

Morphology Characterization of Emulsion

Morphology characterization of emulsion was measured using two methods: visual observation and microscopy observation. Visual observation of phase separation in emulsion was carried out using digital high-speed camera 14 MP Vivo Y12. For microscopy observation, an optical microscope Olympus was used. A drop of emulsion was put on a prepared glass slide and examined under a light microscope with 10x magnification.

Statistical analysis

The data were analyzed statistically using Excel 2013, Microsoft office professional plus software. All measurements were done at least 3 replicates. The results are shown as the value of averages ± standard deviations. Three way analysis of variance (ANOVA) with post hoc Duncan (HSD) test was done to identify the significance differences ($p < 0.05$) between data sets.

RESULT AND DISCUSSION

Measurement of emulsion homogeneity

Homogeneity of emulsions was investigated to understand the stability of emulsion from phase separation of emulsions. The results of homogeneity after 30 days of storage time show in Figs. 1 Homogeneity indicated by the appearance of phase separation between water and oil droplet after 30 days storage time in 75% relative humidity (Reiger, 2000). Homogeneity was measured by considering the height of oil and water droplet that formed (Sharp et al., 2016).

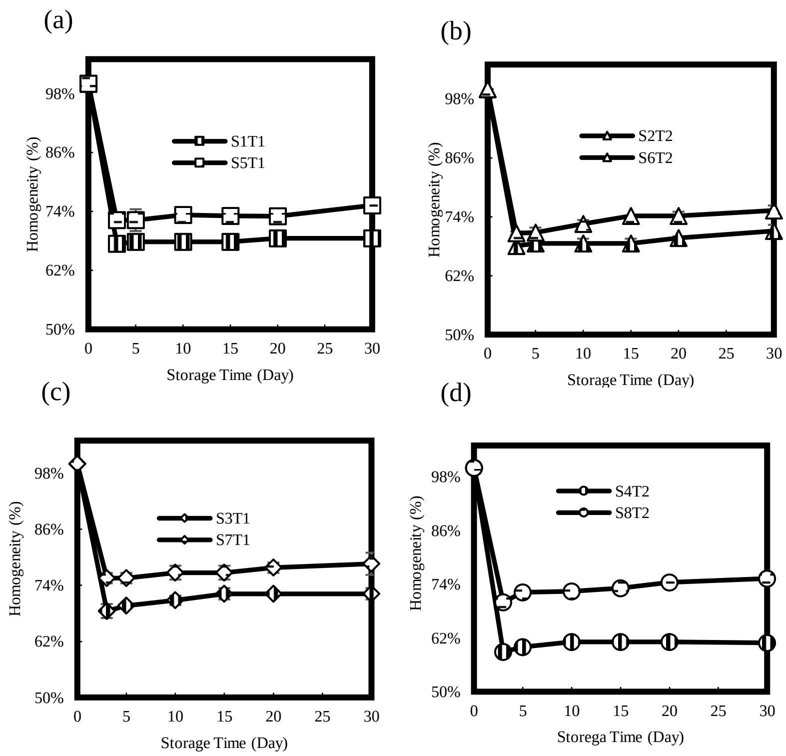

Figure 1. Homogeneity of emulsions (a) sample 1 and 5 with 8000 rpm during 2 min homogenization time, (b) sample 2 and 6 with 8000 rpm during 5 min, (c) sample 3 and 7 with 32000 rpm during 2 min, (d) 32000 rpm during 5 min

Emulsions with 14.5 HLB value (sample 5 – 8) showed the higher homogeneity than 10.5 HLB value (sample 1 – 4). HLB value affect the concentration of emulsifiers used in emulsification (Gadhave, 2014). Ratio of aqueous and oil phase in this emulsification process is 53% (aqueous phase) and 47% (oil phase). Emulsions with higher ratio of aqueous phase stable at 8 – 18 HLB value and need more hydrophilic emulsifier to stable the aqueous phase (Gadhave, 2014). Tween 80 known as hydrophilic emulsifier with 15 of HLB value, so it can bind more water droplets and produce emulsions with smaller phase separations (Jin et al., 2008). Emulsion with 14.5 HLB value with 32000 rpm during 5 min showed the best homogeneity because the intermolecular bonds between dispersed and dispersing phase is tight (Nehme et al., 2021) and the phase separation occurs slowly. The clear phase that formed still contains a lot of dispersed phase even though the phase separation is large (Colucci et al., 2020). Highest homogeneity at 30th storage time is 79% and the lowest 69% of homogeneity.

Measurement of creaming index

The results of creaming index after centrifugation process at 4500 rpm during 3 min show in Figs. 2 Several researchers understand the destabilization mechanism of emulsions after centrifugation process (Anwar et al., 2018). After centrifugation, the cream or serum layer was appeared. Creaming index was measured considering the height of total layer and serum layer as mentioned before. The serum layer rich with oil droplets due to their smaller density (Nehme et al., 2021). Creaming is a form of reversible emulsion instability, the clear layer on the bottom of emulsions can be stabilized again by shaking (Pujiastuti & Kristiani, 2019).

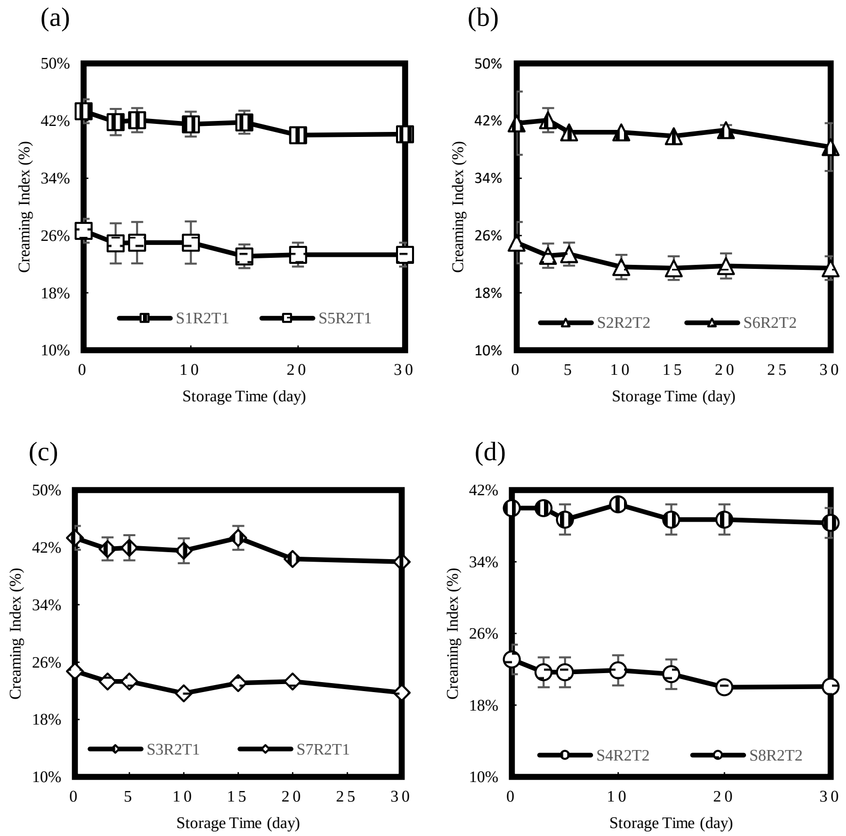

Figure 2. Creaming index of emulsions (a) sample 1 and 5 with 8000 rpm during 2 min homogenization time, (b) sample 2 and 6 with 8000 rpm during 5 min, (c) sample 3 and 7 with 32000 rpm during 2 min, (d) sample 4 and 8 with 32000 rpm during 5 min

The emulsions with 14.5 HLB value and 32000 rpm of homogenizer time during 5 min (S8T2) showed the lowest creaming index and the other emulsions with 10.5 HLB value with 8000 rpm homogenizer time during 2 min in Figs 2 The creaming index can show the stability of emulsion formulation (Anwar et al., 2018). Emulsion with 14.5 HLB value produce the small droplets than the other, especially at 32000 rpm during 5 min. As Stoke's law, the small size of droplets increase the stability and surface tension of emulsion, then decrease the gravitational separation for creaming index (Hong et al., 2018). Emulsions with high stability, decrease the creaming index because the surface tension between dispersed and dispersing phase more tight (Sari & Lestari, 2015). Highest creaming index is 42% at sample 1 (S1T1) and 18% is the lowest one at sample 8 (S8T2).

Measurement of viscosity

Stability of emulsions can investigated by viscosity. Figs. 3 show the results of viscosity that was measured using falling ball viscosity that accordance with Stokes' law at room temperature 30 ℃ with 30 days storage time.

Emulsion with highest HLB value, homogenizer speed and time showed the highest viscosity (S8T2). The aqueous phase used in this emulsion of lotion preparation is larger than the oil phase, therefore an emulsifier with hydrophilic properties needed (Mollet & Grubenmann, 2000). The longer homogenizer time will cause the wider movement of each droplet and increase the collision force then decrease the surface tension between particles and cause the small droplets formed (Jin et al., 2008). The viscosity in each sample decreased due to the coalescence of each molecules that has same properties. In addition, the viscosity also decreases due of heating in the incubator so the molecules have an energy then the molecules move and resulting weak intermolecular interactions, and reduce the viscosity (Ranti, 2017). At the last storage time, there was an increased viscosity although it was not too significant. It's occurs due to the absorption of water to adjust the relative humidity (Mu et al., 2019). The water phase in this lotion preparation was reduce, causing the emulsion viscosity increasing (Ranti, 2017). From the changes in viscosity experienced by the emulsion of this lotion preparation, it can be concluded that the lotion preparation is an Non-Newtonian fluid (Hong et al., 2018). Sample 8 as the most stable emulsions has 25,860.42 cP of viscosity. According to SNI-16-4399-1996 the lotion preparation the range of viscosity is 2,000 – 50,000 cP.

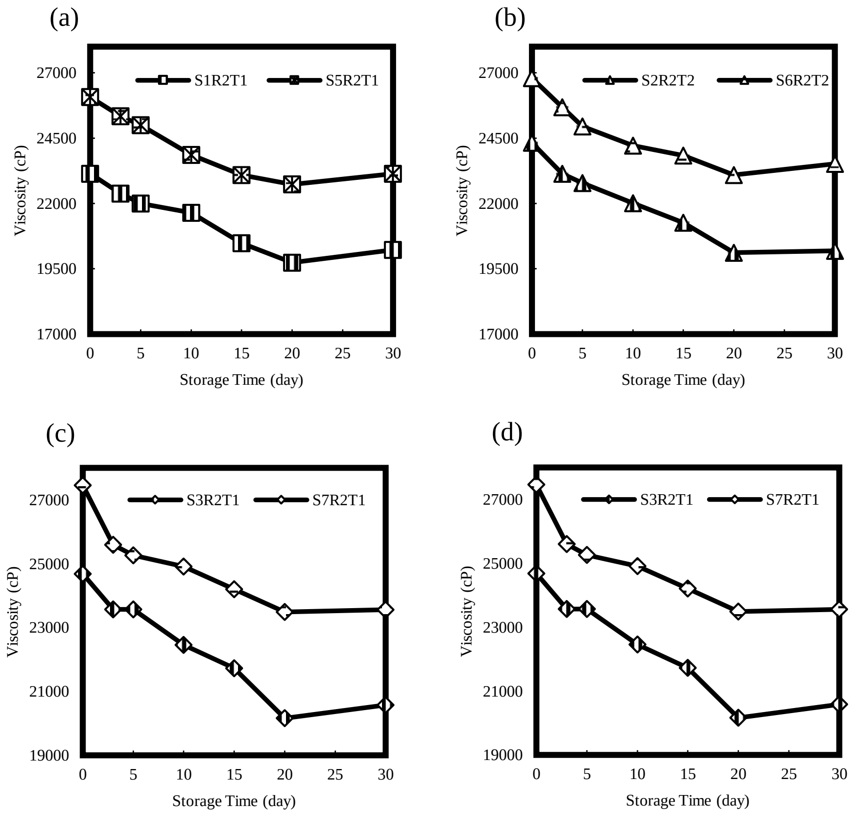

Figure 3. Viscosity of emulsions (a) sample 1 and 5 with 8000 rpm during 2 min homogenization time, (b) sample 2 and 6 with 8000 rpm during 5 min, (c) sample 3 and 7 with 32000 rpm during 2 min, (d) sample 4 and 8 with 32000 rpm during 5 min

Morphology Characterization of Emulsion

Morphology characterization of emulsion was measured using two methods: visual observation and microscopy observation.

Visual Observation

The visual observation of the lotion preparation emulsion was observed the phenomenon of emulsion instability that occurs physically from the lotion preparation emulsion. Physical emulsion was observed on a black box and images were taken using a 12 MP VivoY12 camera during 30 days at room temperature 30 °C. The results of observations based on last storage time showed in Figs. 4.

The homogeneity, creaming index and viscosity in accordance with SNI-16-4399-1996 regarding the quality standard of lotion preparations.

However, the creaming phenomenon which indicated the instability of emulsion began to appear on the 3rd day of observation with 75% RH conditions. The largest phase separation occur in samples with HLB 10.5 using 8000 rpm for 2 minutes. The bonds that formed between the emulsifier and immiscible solutions are weak, causing the increasing of surface tension between the particles and the dispersed phase is separated from the dispersing phase and produces an instability emulsion (Jin et al., 2008). The phase separation that occurs slowly in samples 3, 4 and 8 then produces a large phase separation. However, the clear layer still contains a lot of dispersed phase so that a little cream is produced (Pujiastuti & Kristiani, 2019). The length of storage time affects the movement of the dispersed phase to form a cream layer. The longer the storage time will produced the greater the phase separation (Hou & Papadopoulos, 1997).

Table 2. Standard quality of lotion preparation (SNI-16-4399-1996)

No	Parameters	Unit	Standard
1.	pH	-	4,5 – 8,0
2.	Viscosity	cP	2000 - 50000
3.	Density	g/ml	0,95 – 1,05
4.	Homogeneity	-	Normal
5.	Creaming Index	%	10 - 15

Microscopy Observation

Microscopic observation of lotion preparation emulsion was observed using an Olympus Optical Microscope with a magnification of 10x at room temperature of 30 ℃. Microscopic observations can identify the movement the droplets of dispersed phase which cause creaming and coalescence phenomenon, one of the instability of the emulsion during 30 days storage time. Figs. 5 below show the results of microscopic observations in each condition of relative humidity.

Figs. 5 are the results of microscopic on last time of storage time observations of samples 1 to 8 under the conditions of 75% relative humidity. This observations show that there is a movement of the dispersed phase in the dispersing phase in all interval time of storage (Hong et al., 2018). As long as storage time, the droplets of dispersed phase that containing inside the dispersing phase were reduced. The dispersed phase moves up and form the serum layer as known as creaming (Pujiastuti & Kristiani, 2019). The creaming occurs slowly in samples 3, 4 and 8 then the oil and water droplet still bind each other.

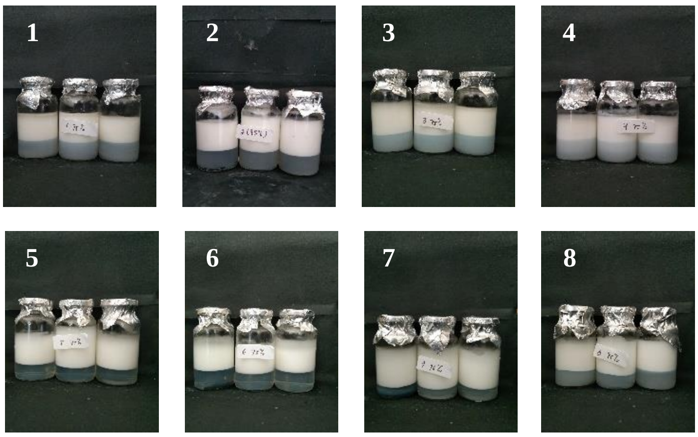

Figure 4. Results of visual observations each sample in 75% RH at 30th storage time

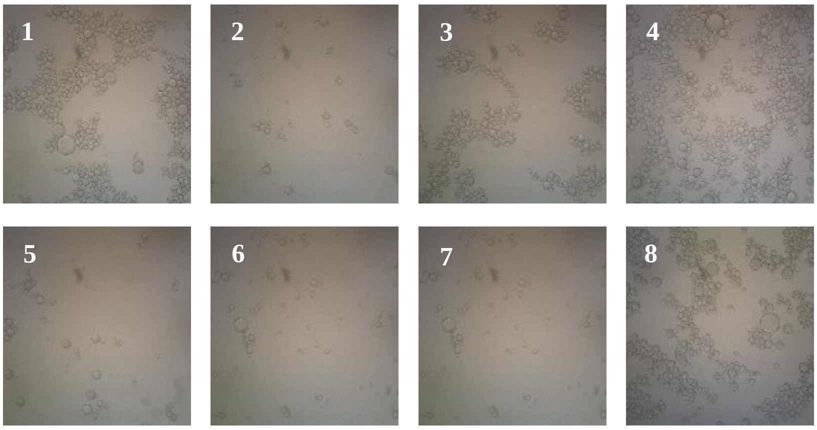

Figure 5. Results of microscopic observations each sample in 75% RH at 30th storage time

Statistical analysis

Significant differences between variables were analyzed using SPSS software using Three way analysis of variance (ANOVA) with post hoc Duncan (HSD) test was done to identify the significance differences ($p < 0.05$) between data sets in measurements of homogeneity, creaming index and viscosity. The results of the significance test of each variable show the significant effect to the stability of emulsions. With the calculated F value for all treatments exceeded the F table and result of the significance shows the 0 value which means that all treatments have a significant effect to the stability of emulsions.

CONCLUSION

In research of emulsification process of walnut oil lotion preparation, all variables have an effect to the stability of emulsions. It confirmed with analysis of variance (ANOVA) with SPSS software and post hoc Duncan resulted that all variables affect the stability of the lotion preparation emulsions. Emulsion with highest stability is sample 8. It formed with 14,5 HLB value, 32000 rpm of homogenizer speed during 5 min. sample 8 has 76% of homogeneity, 18% of creaming index and 25860,42 cP of viscosity. The result of visual and microscopic observation show that phase separation occurs slowly.

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