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Effect of β-cyclodextrin Concentration on the Antioxidant Stability in an Avocado Oil Emulsion-based System

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ABSTRACT

Avocado oil is recognised as a valuable source of vegetable oil, rich in antioxidants and high levels of fatty acids, particularly oleic acid. Oleic acid is commonly utilised as a moisturising agent in cosmetics and as a natural alternative to synthetic chemicals in emulsion-based lotions. This study aimed to investigate the effect of β-cyclodextrin (β-CD) on the antioxidant stability within an avocado oil emulsion system. The emulsion was prepared using a combination of emulsifiers (Tween 80 and Span 80) with a specific HLB ratio and homogenised with a T 25 digital Ultra-Turrax at 15,000 rpm for 10 minutes, including 30-second intervals between homogenisation cycles. The stability of the emulsion was evaluated over 30 days at a constant temperature of 37°C by analysing oil droplet size, viscosity, creaming index, and antioxidant activity. The findings revealed that the stability of the antioxidant in the emulsion system was influenced by the mixed emulsifier's HLB value and the β-CD concentration. The most stable emulsion was achieved with an HLB value of 10.7 and a 1% β-CD concentration. This stabilised emulsion demonstrated significant antioxidant activity, with an IC_{50} value of 44.6 ppm, and showed no signs of phase separation upon visual and microscopic analysis.

Keywords: Avocado oil, β-cyclodextrin, emulsion, stability, surfactant

INTRODUCTION

The avocado tree, *Persea americana* Mill., is a tropical plant known globally for its fruit, which is often used as a culinary component, notably in Indonesia (Orhevba & Jinadu, 2011). The avocado fruit is not

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only consumed as food, but it can also be used in several different preparations. It is well acknowledged by the community for its high content of antioxidants and nutrients, particularly fat (oil), which amounts to 9.8 g per 100 g of fruit flesh (Maitera et al., 2014). In addition, avocado fruit also has a significant number of fatty acids that may be used as a vegetable oil source. The fatty acid composition of this oil includes oleic acid (75.16%), palmitic acid (14.72%), palmitoleic acid (0.19%), stearic acid (0.87%), linolenic acid (3.37%), and linoleic acid (3.57%) (Alkhalaf et al., 2019). Oleic acid, mostly present in avocados, is classified as an unsaturated fatty acid. This fatty acid is exogenous, meaning it is obtained from external sources and is often not endogenously produced by the body. Consequently, oleic acid is often included as a hydrating component in cosmetic products. One of them is used as a chemical alternative in the production of lotions to promote the recycling of natural materials for skincare.

Lotion is a cosmetic product that consists of a blend of water, emollients, emulsifiers, active agents, thickeners, preservatives, and perfumes. The challenge in creating an emulsion lies in the disparity between the liquid and oil phases during the manufacturing process, which cannot be easily blended. To overcome this, the appropriate emulsifier is required to facilitate the formation of the emulsion. Hence, to inhibit the segregation of the oil phase and water phase, cyclodextrin is used as an emulsifying agent. Cyclodextrin has been extensively used in many sectors, including its application in enhancing the stability of perfume oil by protecting it from oxidation and unpleasant odour (Inoue et al., 2010; Ma et al., 2023; Paramita et al., 2022; Shimada et al., 1992). Furthermore, cyclodextrin has the capacity to enhance the stability of emulsions. Cyclodextrin is a cyclic oligosaccharide molecule that has a toroidal shape with a hydrophobic inner surface and a hydrophilic outer section (Xiao et al., 2021). Nevertheless, Hou et al. (2021) indicated that the emulsification efficacy of β-cyclodextrin (β-CD) was insufficient to impede phase separation. Therefore, it is crucial to use supplementary emulsifiers to create a stable emulsion (Hou et al., 2021; Huang et al., 2021; Putri & Ariyanto, 2022; Sahafi et al., 2021). To prevent phase separation in β-CD emulsification, one method is to use Tween 80 and Span 80 as emulsifying agents in the process of emulsion synthesis. Lv et al. (2014) have conducted a study on the use of Tween 80 and Span 80 as emulsifiers in a sunscreen composition including ethanol extracts of avocado leaves. The study findings indicated that the cream preparation's physical qualities, including elasticity, adhesiveness, viscosity, and pH, were influenced by Tween 80 and Span 80. Increasing the amount of Tween 80 leads to improved elasticities and pH levels (Faghmous et al., 2021). Furthermore, the use of Tween 80 and Span 80 in the sunscreen formulation successfully fulfils the desirable physical attributes of a high-quality cream, as determined by the Simplex Lattice Design technique (SLD) (Ghorbanzadeh et al., 2019). Thus, there has been limited research on the efficacy of using cyclodextrin as an emulsifier in emulsion systems.

Therefore, the objective of this research was to examine the impact of β-CD on the durability of the antioxidant in the emulsion system of avocado oil. An emulsion of avocado oil was created by combining a mixture of emulsifiers (Tween 80 and Span 80) in a certain ratio of hydrophilic-lipophilic balance (HLB). The mixture was then homogenised using a T 25 digital Ultra-Turrax at a speed of 15,000 rpm for 10 min, with a 30-second break between each homogenisation cycle. β-CD is a cyclodextrin with a large cavity that can accommodate many molecules. It is more stable and can be quickly isolated from the product without the need for organic solvents due to its extremely low solubility. Meanwhile, Tween 80 and Span 80 are emulsifying agents with non-ionic properties. Each agent has distinct characteristics. Specifically, Tween 80 has a high HLB value and exhibits hydrophobic tendencies, while Span 80 has a low HLB value and exhibits hydrophilic tendencies. Additionally, vitamin E (α-tocopherol acetate) is an antioxidant that plays a crucial role in safeguarding the skin against damage caused by free radicals. The effect of adding β-CD and vitamin E (α-tocopherol acetate) to this emulsion preparation will be determined to assess its impact on the outcomes of the emulsion system.

MATERIALS AND METHODS

Materials

Avocado oil was obtained from PT Darjeeling Semrani Aroma (Bandung, West Java, Indonesia). Beta-cyclodextrin (β-CD) was gifted from Cyclochem Co., Ltd. (Japan). Tween 80 and Span 80 were obtained from CV Indrasari (Semarang, Central Java, Indonesia). α-tocopherol acetate was obtained from Sentana Sempurna (Makassar, South Sulawesi, Indonesia). All chemicals used in this study were of analytical grade.

Methods

Preparation for Avocado Oil Emulsion

The aqueous phase solution was created by dissolving the combined emulsifier with the appropriate hydrophilic-lipophilic balance (HLB) ratio in 200 mL of distilled water at a temperature of 60°C. 140 mL of avocado oil was mixed using a high-speed magnetic stirrer. An oil-in-water emulsion was created by adding the oil phase to water while continuously swirling at a high speed. To achieve emulsification, the solution was homogenised using a T 25 digital Ultra-Turrax (IKA Co. Ltd., China) at 15,000 rpm for 10 min, with a 30-second break after every minute of homogenisation (Hou et al., 2021). The formulation of avocado oil in an emulsion-based system with a specific HLB value is shown in Table 1.

Table 1
Formulation of avocado oil in an emulsion-based formulation with a specific HLB value

Composition	HLB Value	Concentration of β-CD
Tween 80 (87 mL); Span 80 (58 mL)	8.5	0 wt.%
Tween 80 (87 mL); Span 80 (58 mL)	8.5	0.5 wt.%
Tween 80 (87 mL); Span 80 (58 mL)	8.5	1 wt.%
Tween 80 (58 mL); Span 80 (87 mL)	10.7	0 wt.%
Tween 80 (58 mL); Span 80 (87 mL)	10.7	0.5 wt.%
Tween 80 (58 mL); Span 80 (87 mL)	10.7	1 wt.%
Tween 80 (116 mL); Span 80 (29 mL)	12.8	0 wt.%
Tween 80 (116 mL); Span 80 (29 mL)	12.8	0.5 wt.%
Tween 80 (116 mL); Span 80 (29 mL)	12.8	1 wt.%

Emulsion Stability

The stability of O/W emulsion was investigated at a constant temperature of 37°C for 30 days. Viscosity, pH, creaming index value, droplet size, antioxidant activity and physical morphology of the emulsion were used to measure the stability of the emulsion system.

Viscosity Measurement

The viscosity of the emulsion was measured using a Brookfield digital viscometer (Model DV-I + Programmable). Viscosity was evaluated using a No. 64 spindle for approximately 80 mL of sample and then expressed in Cp (centipoise) (Ma et al., 2023).

pH Analysis

Analysis of the degree of acid or pH is carried out based on the Indonesian National Standard method or SNI 06-2413-1991 using the Mettler Toledo Sevencompact S210 pH meter to determine the pH of the emulsion preparation, so that the quality standard can be known to the skin.

Creaming Index Measurement

The creaming index of emulsions was examined by conducting a gravity separation test at a temperature of 37°C for a period of 30 days. Exactly 80 mL of each oil-in-water emulsion was deposited in a glass tube and kept at a temperature of 37°C. The creaming index of the emulsion was tested throughout the storage period. 10 mL of emulsion was placed in a centrifuge tube and then subjected to centrifugation at a speed of 4500 revolutions per minute for a duration of 3 min (Putri & Ariyanto, 2022). The cream's volume was measured, and the creaming index % was computed using Equation 1:

$$Creaming\ index = \frac{HS}{HE} \times 100\% \qquad [1]$$

where HS is the height of the precipitate layer, and HE is the total height of the emulsion.

Droplet Size Measurement

The particle size of the oil droplet was observed using an Olympus optical microscope and then measured using ImageJ software. The droplet diameter data were analysed with Origin software. Where a drop of emulsion was placed on a prepared glass slide and examined under a light microscope with 100x magnification (Xiao et al., 2021).

Antioxidant Activity Measurement

Antioxidant activity can be determined using the DPPH method. A 2 mg quantity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) powder was dissolved in methanol in a 100 mL volumetric flask until the solution reached the limit mark and was agitated to achieve homogeneity. This resulted in a DPPH solution with a concentration of 0.05 mM. The blank solution was prepared by adding 2 mL of methanol to 2 mL of a 0.05 mM DPPH solution and then covering it with aluminium foil. Next, the sample should be agitated and placed in an enclosed space without light for a duration of 30 min. Subsequently, use a UV-Vis spectrophotometer to quantify the solution at a wavelength of 517 nm. By mixing 5 mg of emulsion preparation with 10 mL of methanol until it became uniform, a concentration of 500 ppm was achieved. The solution was prepared using varying concentrations of 10, 20, and 30 parts per million (ppm) by transferring 0.1 mL, 0.2 mL, and 0.3 mL, respectively, into a 10 mL volumetric flask, and then filling the remaining volume with methanol to get a total volume of 10 mL. For each test, 2 mL of the solution was pipetted and then mixed with an equal amount of DPPH. The resulting mixture was then transferred to a test tube and homogenised. The solution was subjected to incubation at a temperature of 37°C for a duration of 30 min, and the measurement of absorbance was performed at a specific wavelength of 517 nm (Hou et al., 2021). The metric used to quantify antioxidant activity was the effective concentration (EC_{50}) or inhibitory concentration (IC_{50}).

$$Q = \frac{A_1 - A_2}{A_1} \times 100\% \qquad [2]$$

where Q is the percentage of inhibition, A_1 is the absorbance of the control, and A_2 is the absorbance of the sample.

Characterisation of Emulsion Morphology

The morphological characterisation of the emulsion was assessed using two methods: eye inspection and microscopic examination. The emulsion's phase separation was visually seen using a high-speed digital camera with a resolution of 64 megapixels, namely the Samsung A52S 5G model. An Olympus optical microscope was used for the purpose of microscopy observation. An emulsion droplet was applied onto a prepared glass slide and viewed using a light microscope at a magnification of 100x.

RESULTS AND DISCUSSION

Effect of β-CD Concentration and HLB Value on the Viscosity of Emulsion Preparation

Viscosity and emulsion stability are tightly interconnected. Table 2 shows the viscosity measurements obtained from a Brookfield Model DV-I Viscometer, using spindle no. 64 and a speed of 4 rpm. The measurements were taken on day 0 and day 30. The investigation revealed that on the first day of preparation (day 0), the viscosity of each sample rose as the β-CD content increased. The sample without β-CD treatment had the lowest recorded value. Following a storage period of 30 days, the viscosity of each sample exhibited a decline, even though each sample underwent a distinct treatment. As a result, water was absorbed by each sample during the storage phase in the incubator. The use of 1% β-CD concentration in the samples showed a beneficial impact on enhancing viscosity. Moreover, it is demonstrated that an HLB of 10.7 is also efficient in preserving the stability of the emulsion. β-CD has a molecular structure that exhibits hydrophilicity on its outer surface and hydrophobicity inside its cavity (Ciobanu et al., 2013). This characteristic makes β-CD suitable for use as an emulsifier in the formulation of emulsions.

Table 2
Viscosity of emulsion at various concentrations of β-CD; 0% β-CD; 0.5% β-CD; 1% β-CD

HLB Value	Concentration of β-CD	Viscosity	
		0-day Storage (cP)	30-day Storage (cP)
8.5	0 wt.%	3050 ± 0.100	2150 ± 0.076
	0.5 wt.%	3200 ± 0.100	2816.7 ± 0.101
	1 wt.%	3350 ± 0.050	2700 ± 0.104
10.7	0 wt.%	26217 ± 0.882	25566.7 ± 1.540
	0.5 wt.%	28980 ± 0.053	25900 ± 1.532
	1 wt.%	33800 ± 0.557	31600 ± 0.671
12.8	0 wt.%	3050 ± 0.161	2966.7 ± 0.060
	0.5 wt.%	3550 ± 0.180	3316.7 ± 0.159
	1 wt.%	4400 ± 0.132	4166.7 ± 0.130

According to research, the addition of β-CD is expected to increase the viscosity of the emulsion preparation (Kibici & Kahveci, 2019).

The results indicate that among all the samples, HLB 10.7 achieves the highest viscosity, which is influenced by the variation in emulsifier composition. According to the table, the HLB 10.7 sample, containing 40% Tween 80 and 60% Span 80, shows a higher viscosity compared to the HLB 8.5 sample (60% Tween 80 and 40% Span 80) and the HLB 12.8 sample (80% Tween 80 and 20% Span 80). The lowest viscosity is observed in the sample with an HLB of 8.5. This demonstrates that the HLB value significantly affects the emulsion's stability during storage. The findings suggest that increasing the proportion of Span 80 relative to Tween 80 can enhance the stability of the emulsion (Suardana et al., 2020). Increasing the concentration of surfactants typically results in a higher apparent viscosity of emulsions. This is because surfactant molecules form structured films at the oil-water interface, which contributes to an increase in viscosity. Sun et al. (2017) also found that increasing the organic/inorganic alkali surfactants raised the apparent viscosity of the O/W emulsion. This increase in apparent viscosity was attributed to the enhanced ionisation of the interfacial active components and the compression of the diffused double layer, with the competition between these factors determining whether the apparent viscosity increased or decreased.

Effect of β-CD Concentration and HLB Value on pH of Emulsion Preparation

The pH value of emulsions may be examined to determine their acidity. This analysis was conducted at regular intervals throughout a 30-day storage period. The findings clearly indicated that the pH values obtained from each sample fall within the acceptable range of 7.08-7.62, as specified by the quality criteria for emulsion, which are outlined in SNI 16-4399-1999. This is due to the component ingredients in emulsion treatments often having a neutral pH range of 6-7. The pH of the emulsion preparation's component ingredients comprises Tween 80 with a pH range of 6-8 and Span 80 with a pH below 8 (Jiang et al., 2024; Lv et al., 2014). The pH of avocado oil is around 7.5 (Flores et al., 2019; Krumreich et al., 2018), whereas β-CD has a pH ranging from 5 to 8 (Paramita et al., 2022). Distilled water has a pH between 6.7 and 7 (Khorolskyi & Kryvoruchko, 2021).

Figure 1 shows the decline in acidity, as shown by the pH values of all samples, after a 30-day storage period. This decline suggested that the emulsion preparation composition underwent structural deterioration (Singh et al., 2019). Increasing the concentration of β-CD may lead to a reduction in the acidity level of the emulsion preparation (Inoue et al., 2010). The results from each sample indicated that the fluctuation of HLB values in emulsion preparations does not have a significant impact on the acidity evaluated by the pH value.

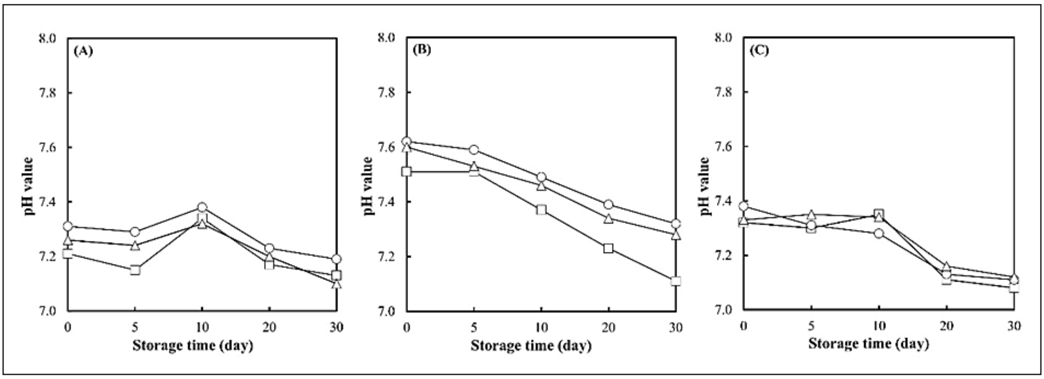

Figure 1. Effect of HLB values on the pH emulsion stability: (a) HLB 8.5; (b) HLB 10.7; (c) HLB 12.8 at different β-CD concentrations: (□) 0 wt.% β-CD; (○) 0.5 wt.% β-CD; (Δ) 1 wt.% β-CD

Effect of β-CD Concentration and HLB value on Creaming Index of Emulsion Preparation

The creaming index value is a measure of the stability of an emulsion, indicating its resistance to separating into distinct layers and its capacity to bind with other particles. The creaming index value of all samples was assessed after 30 days of storage. The findings indicated that the sample with the greatest creaming index percentage was the sample with a hydrophilic-lipophilic balance (HLB) of 8.5, consisting of 60% Tween 80 and 40% Span 80. This was followed by the sample with an HLB of 12.8, including 80% Tween 80 and 20% Span 80. The creaming index suggests that the emulsion preparation is rather unstable. It is proven that the higher the percentage of creaming index in emulsion preparations, the smaller the stability (Whitby et al., 2009).

Emulsions with an HLB of 10.7 consistently exhibited a creaming index value of 0%. This demonstrated that the sample has a high level of stability. The results indicated that there was no difference at the conclusion of the storage period, namely, after 30 days of storage. The addition of a higher concentration of Span 80 leads to the formation of a stable emulsion. This was due to the Span 80 acting as an emulsifier, possessing hydrophobic characteristics that facilitate the binding of the oil phase during the emulsification process (Inoue et al., 2010). An emulsion with high stability will be formed when a larger amount of the oil phase is bound by Span 80. Additionally, the ratio of the cream and clear layer created during the centrifugation process will drop. To reduce the creaming index, the proportion of creaming index created should be decreased (Netto & Jose, 2018; Onuki et al., 2016).

Figure 2 demonstrates that the concentration of β-CD has a favourable impact on the stability of the emulsion. The sample treated with a 1% concentration of β-CD exhibited the lowest creaming index value in comparison to the sample without β-CD and the sample treated with a 0.5% concentration of β-CD. To summarise, the ideal concentration for emulsion formation that demonstrates stability was 1% β-CD with a hydrophilic-lipophilic balance (HLB) of 10.7. There was no significant change in the creaming index of HLB 8.5 and HLB 12.8 samples over the storage period. This reduction is attributed to the occurrence of same-sex coalescence or colloquy, which leads to the separation or creaming of the oil phase and water phase.

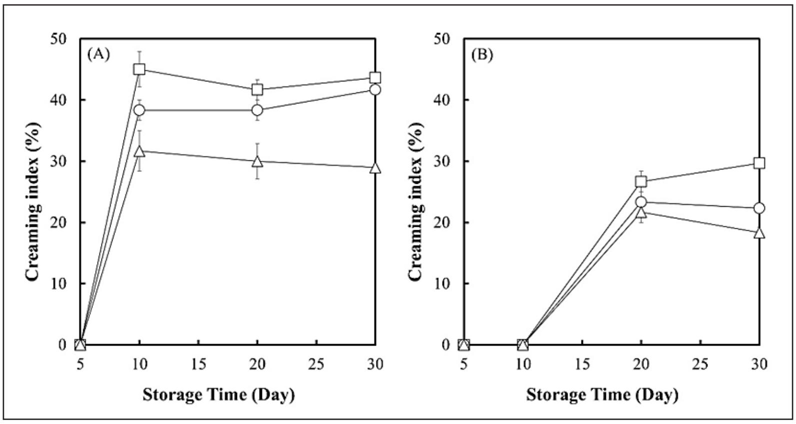

Figure 2. Influence of varying HLB values on the creaming index of the emulsion system: (a) HLB 8.5; (b) HLB 12.8 at different β-CD concentrations: (□) 0 wt.% β-CD; (○) 0.5 wt.% β-CD; (∆) 1 wt.% β-CD

Effect of β-CD Concentration and HLB Value on Droplet Size of Emulsion Preparation

The droplet size of the emulsions was assessed using ImageJ software. On day 0 of the storage period, each sample was examined under an Olympus optical microscope at 100x magnification (Figure 3). Subsequently, the droplet diameter was evaluated to determine the frequency of visible droplet sizes. Observations reveal that samples with HLB 8.5 (60% Tween 80; 40% Span 80) and HLB 12.8 (80% Tween 80; 20% Span 80) exhibit reduced particle sizes in comparison to samples with HLB 10.7 (40% Tween 80; 60% Span 80). This result is consistent with the study conducted by Hou et al. (2021), which shows that higher concentrations of Tween 80 might result in a reduction in particle size. Nevertheless, the HLB 8.5 sample exhibits a little inaccuracy, whereas the HLB 12.8 sample contradicts the earlier evidence. The HLB 12.8 sample has a greater particle size compared to the sample with HLB 8.5. There was a noticeable impact on the size of particles when the concentration of β-CD was altered. This demonstrated the properties of β-CD inclusions that might hinder the merging and/or enlargement of oil droplets (Hou et al., 2021; Inoue et al., 2010; Shimada et al., 1992).

Latest studies have shown that the use of α-tocopherol acetate in emulsion preparation leads to a reduction in particle size. The reason for this is that α-tocopherol acetate has high oil solubility, which may induce turbulence or heightened particle movement, as well as reduce oil surface tension (Sahafi et al., 2021). These circumstances facilitate the diffusion of Tween 80 into the oil particles, leading to the formation of very tiny oil particles. This may facilitate the generation of tiny emulsion particles with enhanced stability against separation (Ahmad et al., 2020).

Effect of Concentration and HLB Value on Antioxidant Activity of Emulsion Preparations

The antioxidant activity of each emulsion stored for 30 days was analysed through a UV-VIS spectrophotometer, observing the absorbance value, and then the IC50 was calculated. IC_{50} (Inhibitory Concentration) is the concentration of the sample solution that can reduce the activity of DPPH by 50%, or is a number that shows the concentration of the sample (ppm) which can inhibit the oxidation process by 50%. It is known that the smaller the IC_{50} value, the higher the antioxidant activity (Sahafi et al., 2021).

Figure 4 indicates that the sample treated with a concentration of 1% β-CD had the lowest IC_{50} value at each storage period, reaching its highest point after 30 days of storage. This demonstrated the efficacy of β-CD in preserving the antioxidant content of the emulsion formulation. The given data indicated that the HLB value did not have a noticeable impact on preserving the antioxidant content. Nevertheless, the sample with HLB 10.7 exhibits a minor discrepancy that contradicts the previously established evidence. It is evident that the sample with an HLB of 10.7 exhibits the lowest IC_{50} value in comparison to the samples with HLB values of 8.5 and 12.8. Incorporating a higher amount of Span 80 led to a rise in emulsion viscosity, preventing the formation of creaming index. Moreover, it effectively inhibited the oxidation process in emulsion preparations, therefore preserving the antioxidant content.

Visual Observation of Emulsion Preparation

The process of visually observing the development of an emulsion showed the occurrence of emulsion instability, which is a physical phenomenon that arises during the preparation of the emulsion. The physical emulsion was visually examined and photographed using a Samsung A52S 5G 64 MP camera over a period of 30 days at a constant temperature of 37°C. The findings obtained by observing the storage time are shown in Figure 5.

Figure 5 illustrates that the sample with an HLB of 8.5, without the addition of β-CD concentration, exhibits the most significant phase separation after 30 days of storage. The solution and emulsifying agent do not mix, resulting in an increase in surface tension between the particles. This causes the dispersed phase to separate from the dispersing phase, leading to an unstable emulsion. Observations indicate that there was no occurrence of phase separation in the sample with an HLB value of 10.7 throughout the 30-day storage period. This demonstrated that the ratio of Tween 80 to Span 80 in terms of volume has an impact on the stability of the resulting emulsion.

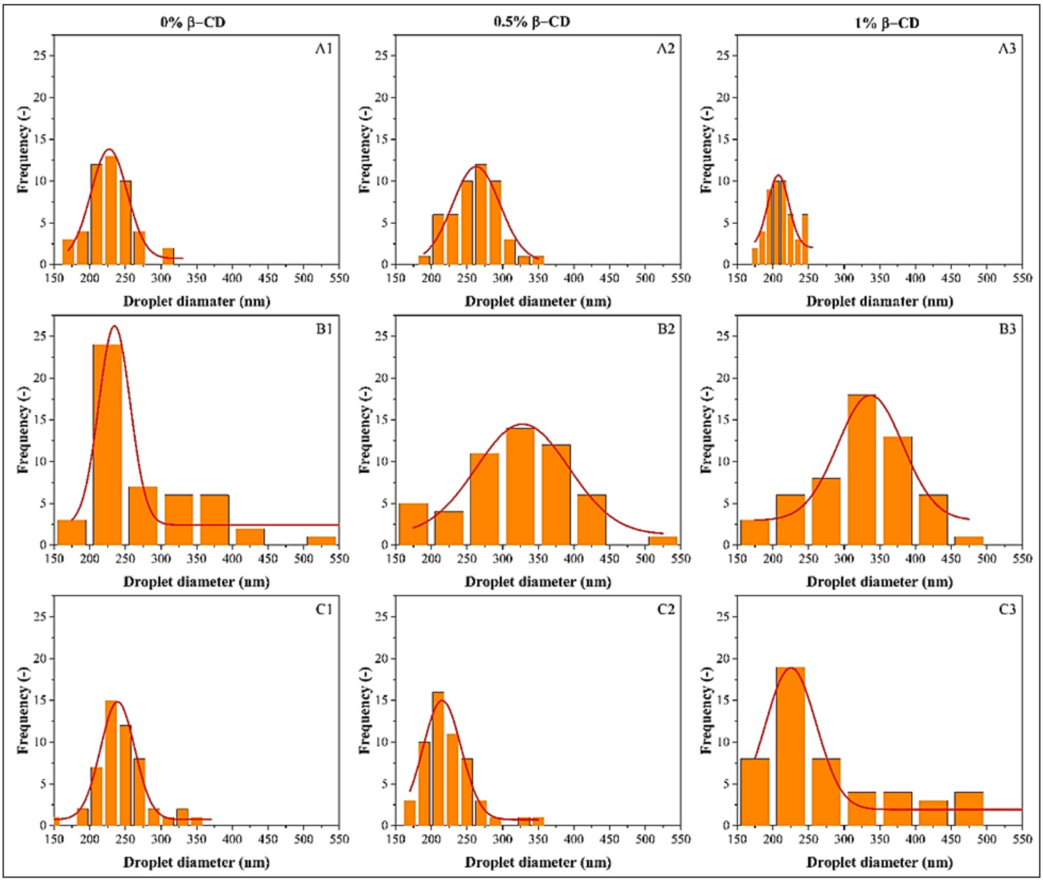

Figure 3. Effect of varying hydrophilic-lipophilic balance (HLB) values on emulsion droplet size: (a) HLB 8.5; (b) HLB 10.7; and (c) HLB 12.8, at different β-cyclodextrin (β-CD) concentrations: (1) 0 wt%; (2) 0.5 wt%; and (3) 1 wt%.

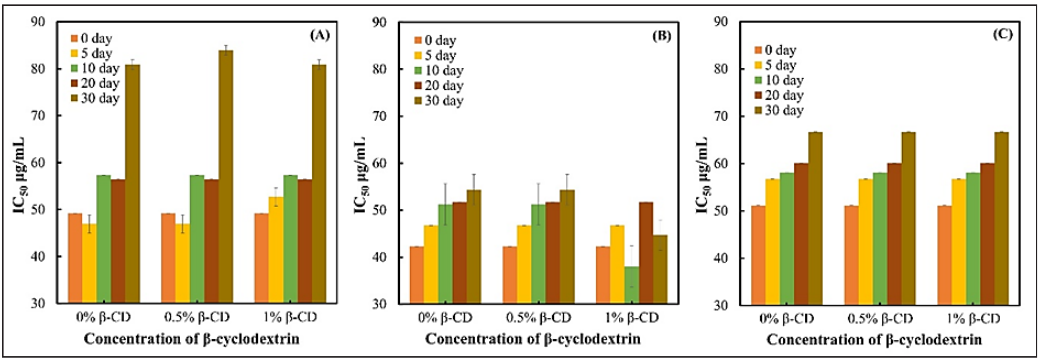

Figure 4. Effect of HLB values on the antioxidant stability of emulsion: (a) HLB 8.5; (b) HLB 10.7; (c) HLB 12

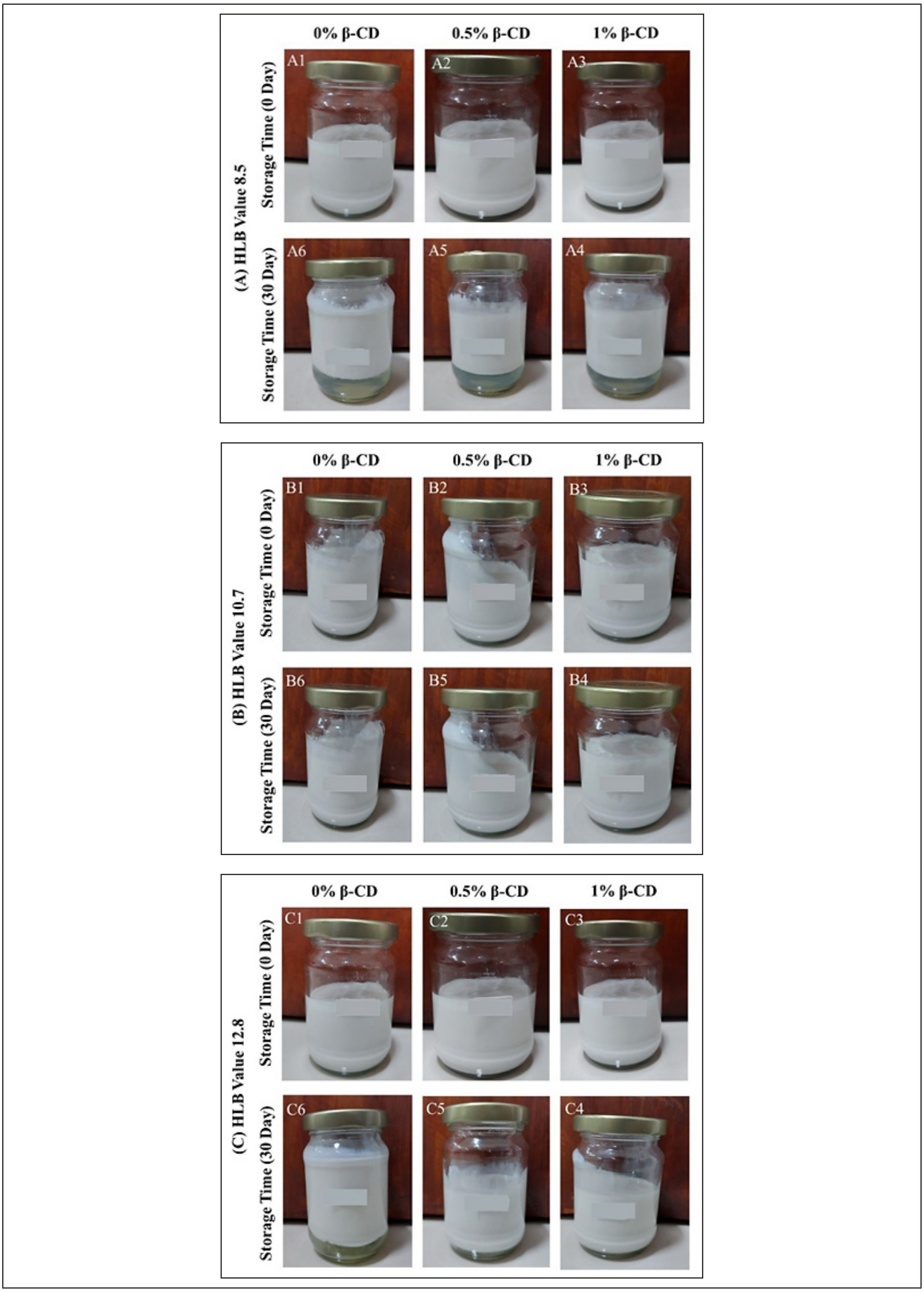

Figure 5. Visual observation of the emulsion system at different HLB values: (a) HLB 8.5; (b) HLB 10.7; (c) HLB 12.8

Microscope Observation of Emulsion Preparation

The emulsion was examined using an Olympus Optical Microscope at a magnification of 100x at 37°C. Microscopic investigations may detect the motion of dispersed phase droplets that lead to creaming and coalescence, which are types of emulsion instability seen after 30 days of storage. Figure 6 shows the findings of microscopic examinations conducted on day 0 and day 30 at regular intervals. Over time, the droplets of the dispersed phase inside the dispersing phase diminish. The phase that was scattered goes in an upward direction and creates a layer called creaming (Putri & Ariyanto, 2022).

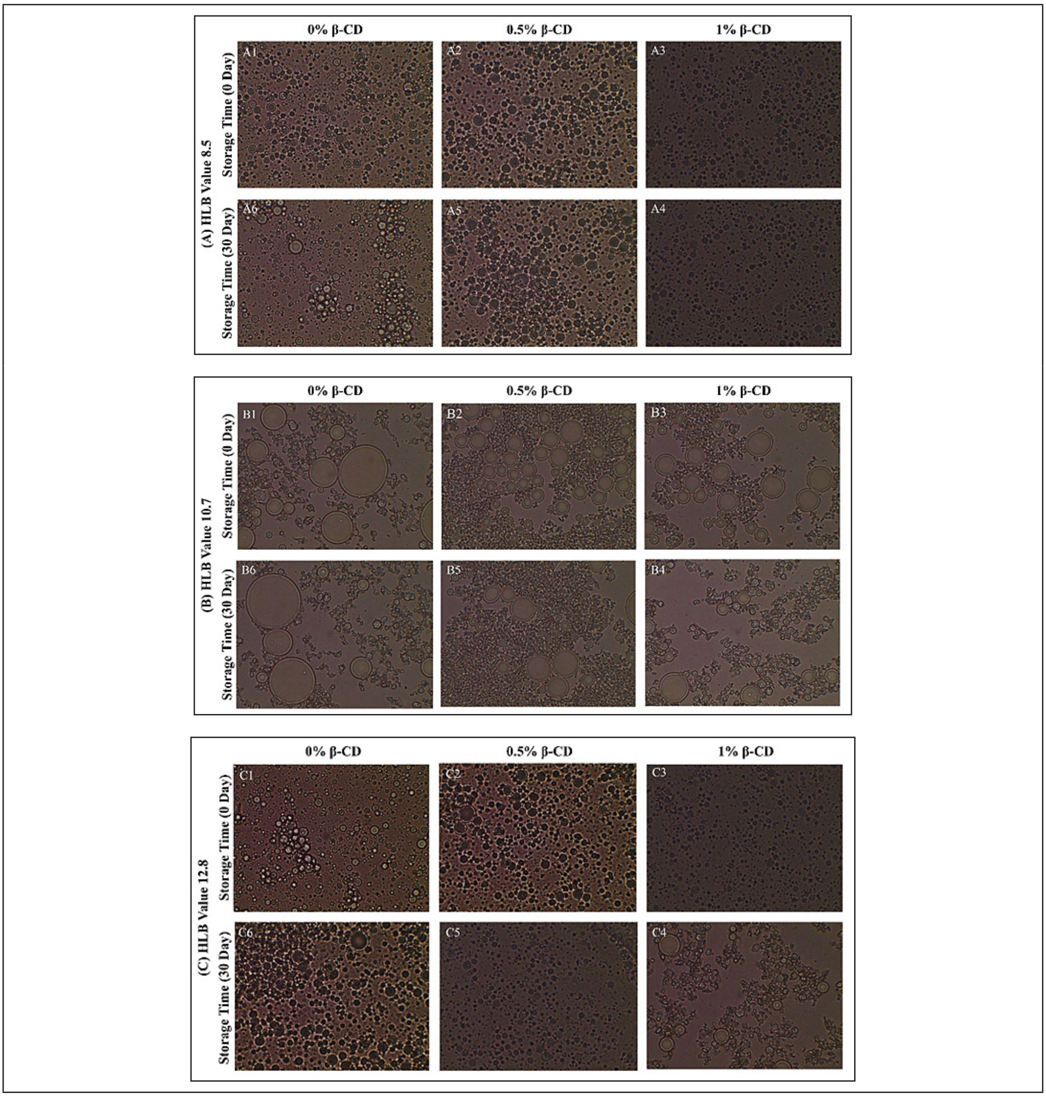

Figure 6. Effect of different HLB values on the morphology properties of emulsion: (a) HLB 8.5; (b) HLB 10.7; (c) HLB 12.8

CONCLUSION

This study investigated the influence of α-tocopherol acetate and β-cyclodextrin (β-CD) on the stability of emulsions in avocado oil lotion. By conducting meticulous experiments, it was shown that the incorporation of these ingredients greatly improved the stability of the emulsion. In this study, the emulsification process of avocado oil lotion preparation with the addition of α-tocopherol acetate and β-CD had a significant effect on emulsion stability. The emulsion with the highest stability was the sample with an HLB value of 10.7 with 1% β-CD concentration. This sample has 0% creaming index, 33800 ± 0.557 cP viscosity, and the lowest IC_{50} value, which proves its high antioxidant activity. Visual and microscopic observation showed no phase separation.

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LIST OF ABBREVIATIONS

β-CD	:	Beta cyclodextrin
DPPH	:	2,2-diphenyl-1-picrylhydrazyl
HLB	:	Hydrophilic-lipophilic balance
IC_{50}	:	Half maximal inhibitory concentration

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