Optimization of the Stability of Nano-emulsion Medium Chain Triglycerides (MCT) using α-Cyclodextrin

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ABSTRACT

This study aims to determine the stability of nano-emulsion synthesized from virgin coconut oil (VCO) using α-cyclodextrin, and lecithin or tween 80 as surfactants. The study procedures included the production of nano-emulsions, examining emulsion type, density, particle size, pH, and zeta potential. The effect of the independent variables on the pH of the product was also examined using the response surface method (RSM). The results obtained 10 nano-emulsion formulas, belonging to the o/w type. The samples typically had a density range of 1.178–1.254 g/mL, with a pH of 5.0–5.5, which was considered safe for the skin. The smallest particle size of 5.495 μm was obtained from formula 6 (60 mL, 16 mL, 18 g, 6 g of water, VCO, cyclodextrin, and tween 80 as surfactant) with a zeta potential of -45.500 to -89.567 mV. Based on these results, formula 6 had the best characteristics, with an optimum pH of 5.5, small particle size, and good stability, as indicated by the zeta potential value.

Keywords: Nano-emulsion; surfactant; virgin coconut oil; zeta potential; response surface methodology

INTRODUCTION

Indonesia is the largest coconut-producing country in the world, with a substantial 31.2% share of the global plantation area of the crop, which is equivalent to 3.86 million ha (Suratinojo, 2014). According to data obtained from the Directorate General of Plantations, Ministry of Agriculture, Indonesia in 2019, several provinces contributed to the large production volume in the country, including Riau, North Celebes, East Java, and Southeast Celebes (DGEC, 2021).

Indonesia's vast expanse of coconut plantations contributes significantly to an annual yield of approximately 18.3 million tons (Mesu and Fangohoi, 2018). However, this is not in accordance with the sales of processed coconut products, such as virgin coconut oil (VCO). This is primarily due to the limited awareness among Indonesians concerning the numerous benefits of VCO, leading to low purchase interest.

In comparison to other vegetable oils, virgin coconut oil is unique due to its distinct properties as it contains approximately 44-52% medium chain

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triglycerides (MCT) (Ardianto and Mutiah, 2018). This sets it apart from the long-chain triglycerides (LCT) that are commonly found in vegetable oils. Several studies have shown that MCTs possess a smaller molecular size, lower melting point, and reduced energy content (8.4 compared to 9.2 kcal/g) (Silalahi, 2020). Consequently, MCTS are readily absorbed and digested by the body, leading to their rapid usage as an energy source, without being stored as body fat (Arpi, 2013). These triglycerides also exhibit remarkable stability compared to LCTs, making them an ideal choice for making nano-emulsions (Fitriani et al., 2016).

Nano-emulsion is an imbalance system that cannot be formed spontaneously, thereby necessitating a surfactant to lower the interfacial tension and facilitate the formation of new drops or droplet emulsion (Wilson et al., 2022). One of the surfactants that is often used in the production of nano-emulsions is tween 80. This is primarily due to its non-toxic properties and non-irritating attributes on the skin (Cheng et al., 2017). The natural surfactant commonly used in the manufacture of nano-emulsions is soy lecithin. In the manufacture of emulsions, combination of surfactants is recommended due to its ability to produce a more stable emulsion. The stability of the product can be further enhanced through the addition of a stabilizer, such as α-cyclodextrin (Sliwa and Girek, 2017). Furthermore, α-cyclodextrin is a non-reducing cyclic saccharide, consisting of 6 glucose units linked through 1-4-glycosidic bonds, which are produced by cyclodextrin glucopyranosyl transferase (Nopiasari et al., 2019).

Cyclodextrins are categorized into three types, namely α, β, and γ. The α-cyclodextrin variant, distinguished by its comparatively smaller size in relation to the β and γ types, exhibits a unique capacity to bind higher amounts of fat in serum (de Miranda et al., 2011, Nopiasari et al., 2019). According to a previous study, its resistance to fat hydrolysis plays a role in preventing damage to the nano-emulsion (Li et al., 2014). Despite its advantageous properties, the use of α-cyclodextrin in the production of nano-emulsions remains relatively uncommon. Therefore, this study aims to optimize the stability of nano-emulsion using α-cyclodextrin as a co-surfactant. The results are expected to serve as the basis for further studies on the production of nano-emulsions using cyclodextrins.

MATERIAL AND METHODS

Materials

The materials used in this study included medium chain triglyceride (MCT) in VCO, α-cyclodextrin, tween 80, lecithin, and distilled water. Furthermore, VCO containing MCT was purchased from a marketplace in CV Ajisaka Kelapa, Malang, Indonesia. α-Cyclodextrin was purchased from Cyclochem Co. Ltd., Japan, Tween 80 was acquired from Kimia Indrasari Semarang, and Lecithin was obtained at Multi Kimia Raya Nusantara.

Nano-emulsion Production

The nano-emulsion was prepared by dissolving alpha-cyclodextrin with a magnetic stirrer at a speed

Table 1. The composition of nano-emulsion medium chain triglycerides (MCT)

Formula	Water (mL)	MCT (mL)	α-Cyclodextrin (g)	Surfactant (g)
Type of surfactant: lecithin				
1	60	20.00	15.00	5.00
2	60	13.33	20.00	6.67
3	40	30.00	22.50	7.50
4	40	20.00	30.00	10.00
5	50	20.00	22.50	7.50
Type of surfactant: tween 80				
6	60	16.00	18.00	6.00
7	40	24.00	27.00	9.00
8	50	25.00	18.75	6.25
9	50	16.67	25.00	8.33
10	50	20.00	22.50	7.50

of 200 rpm for 10 minutes. The alpha-cyclodextrin solution was then homogenized with a surfactant and MCT oil using an ultra turrax homogenizer (IKA T10 basic Ultra Turrax, China) at a speed of 18,000 rpm for 3 minutes.

Density Analysis

Density analysis was measured using a pycnometer and calculated based on Equation (1).

$$\rho = \frac{m_2 - m_1}{V} \quad (1)$$

Where is, ρ = density (g/mL); m_2 = weight of the filled pycnometer (g); m_1 = weight of empty pycnometer (g); and V = pycnometer volume (mL).

Emulsion Type and particle size Analysis

The type of the emulsion was determined by placing the nano-emulsion on an object glass, and adding the methylene blue. Particle size analysis was carried out to determine the type of emulsion based on its droplet size. It was also used to evaluate stability by observing changes in droplet size after being stored in varying temperatures (4 °C and room temperature). The particle size was measured using Horiba Partica LA-960, Japan. The emulsion is classified as nano, micro, and emulsion for the particle size of 1–100 nm, 100–400 nm, and ≥400 nm (Souto et al., 2022).

Zeta Potential Analysis

Zeta potential analysis was carried out to determine the stability of nano-emulsion after being stored at varying temperatures. Emulsions with high zeta potential tended to be more stable compared to those with lower levels. The accumulation of coagulate or flocculate could occur in samples with low zeta potential, leading to poor stability (Lu and Gao, 2010). The zeta potential was measured using the Horiba nano partica SZ-100, Japan.

RESULT AND DISCUSSION

GC-MS on the VCO and Nano-emulsions

The VCO from CV Ajisaka Kelapa, Malang contained MCT (C_6–C_{12}), comprising 0.17% caproic acid, 4.44% caprylic acid, 4.72% capric acid, and 42.67% lauric acid. It also contained LCT, consisting of 20.27% myristic acid, 11.5% palmitic acid, 2.1% linoleic acid, 10.05% oleic acid, 3.89% stearic acid. The result of the GC-MS is presented in Figure 1.

Density Analysis from Nano-emulsion

The results of the density and viscosity analysis of the nano-emulsions is presented in Table 2. Table 2 showed the density and viscosity of nano-emulsion made from VCO, which was analyzed for 3 weeks with a weekly testing time after storage at 4 °C. The nano-emulsions were analyzed using 2 different types of emulsifiers. Furthermore, soy lecithin and tween

Table 2. The result of density and viscosity of nano-emulsions

Formula	Density (g/mL)			
	0	1	2	3
1	1.244	1.247	1.253	1.254
2	1.192	1.196	1.200	1.202
3	1.218	1.221	1.224	1.225
4	1.241	1.244	1.246	1.247
5	1.208	1.210	1.218	1.217
6	1.178	1.179	1.181	1.181
7	1.226	1.228	1.233	1.232
8	1.181	1.185	1.188	1.189
9	1.188	1.192	1.194	1.196
10	1.210	1.217	1.221	1.222

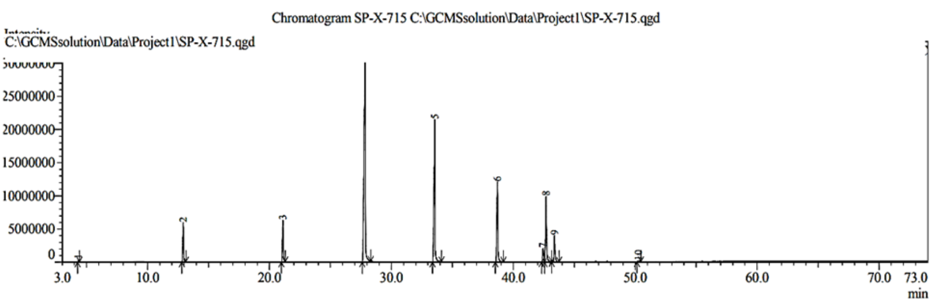

Figure 1. The result of GC MS for identification of triglycerides content in virgin coconut oil

80 served as emulsifiers in formulas 1–5 and 6–10, respectively. The nano-emulsion with soy lecithin and tween 80 produced a density range of 1.192–1.254 g/mL and 1.178–1.233 g/mL, respectively.

The results showed that there was no significant change in the density of each formula. However, the density obtained after storage at 4 °C increased due to the low storage temperature, leading to a decrease in the distance between the particles. At the same volume, the number of particles increased along with the density of the nano-emulsion (Wildan, 2013).

Emulsion Type Test

The emulsion type test was used to determine whether the nano-emulsion produced was o/w or w/o. The determination of the type of emulsion was shown by the distribution of methylene blue. The o/w emulsion is characterized by evenly spread of methylene blue in the nano emulsion. On the other hand, the w/o type of emulsion is described by the formation of blue spots (Rahmawanty and Sari, 2021). The results of the emulsion type test are presented in Figure 2. The result showed that addition of methylene blue to the nano-emulsion resulted in evenly dispersed of nano emulsion, indicating that the emulsion was the oil in water (o/w) type. This was because the emulsifiers in the form of tween 80 and soy lecithin had a high HLB value of 15 and 8, respectively. Emulsifiers with high (hydrophilic) and low HLB were likely to produce o/w and w/o emulsion type, respectively. Furthermore, the value required to form an o/w emulsion was 8–16 and w/o was 4–6 (Syahputri & Patricia, 2019). Soy lecithin was an amphoteric surfactant, with both negative and positive charges (Shamsuri & Jamil, 2020). The results of this study obtained 10 nano-emulsion formulas of the o/w type.

ANALYSIS OF THE EFFECT OF INDEPENDENT VARIABLES ON PH USING THE RESPONSE SURFACE METHOD (RSM)

In this study, the analysis was carried out using RSM to determine the effect of independent variables in the form of solid content and the ratio of MCT-wall to pH on the MCT nano-emulsion produced. It was also performed to achieve optimization, leading to the production of the best response. In this case, the wall was a combination of surfactants, namely tween 80 or lecithin, and alpha-cyclodextrin. The pH results from the 10 formulas are presented in Table 3.

Table 3. The result of pH nano-emulsion

Formula	α-Cyclodextrin (g)	Surfactant (g)	pH (-)
Type of surfactant: lecithin			
1	15.00	5.00	5.4
2	20.00	6.67	5.5
3	22.50	7.50	5.2
4	30.00	10.0	5.3
5	22.50	7.50	5.4
Type of surfactant: tween 80			
6	18.00	6.00	5.5
7	27.00	9.00	5.4
8	18.75	6.25	5.4
9	25.00	8.33	5.5
10	22.50	7.50	5.4

The equations obtained are

$$Y = 4.4952380952381 + 0.027380952380951\ X1 - 0.00035714285714285\ X1X1 + 0.52857142857141\ X2 - 0.14285714285714\ X2X2 + 0.000000000000000399\ X1X2$$

From the equation, X1 is the concentration of SC, X2 is the ratio, and Y is the pH. The equation stated that the pH response increased and was directly proportional to the SC concentration, ratio, and the interaction between SC concentration and ratio, as indicated by a positive constant value. Meanwhile, the pH response decreased along with increasing interaction between SC concentrations, as well as ratios, as indicated by a negative constant value.

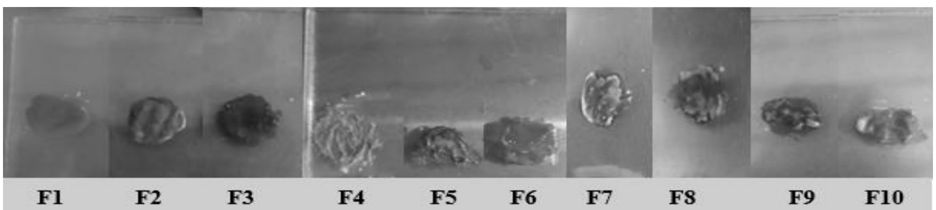

Figure 2. The result of the emulsion type test

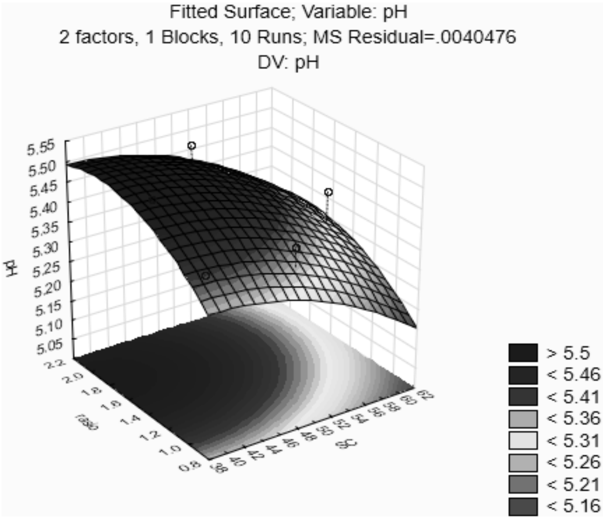

Figure 3. Fitted response surface variable solid content and MCT-wall ratio to pH nano-emulsion

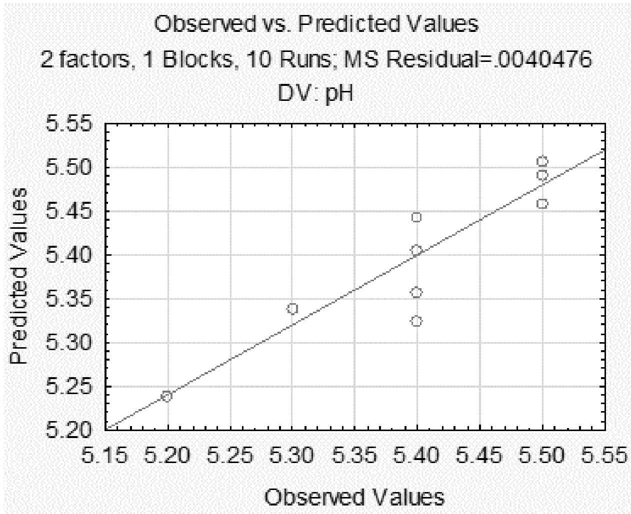

Figure 4. Comparison of experimental data and estimated nano-emulsion pH

The accuracy of the analysis could be seen from the value of the coefficient of determination (R2). The R2 represented the level of influence the independent variable had on the dependent variable, and the value often ranged from 0–1. Values closer to 1 indicated that the model could be considered good at predicting the response. In this analysis, the R2 value was 0.79,

Table 4. Estimation effect data

Factor	Effect	Std. Error
(1) SC (%) (L)	-0.166667	0.051946
SC (%) (Q)	-0.071429	0.083299
(2) rasio (L)	0.100000	0.051946
ratio (Q)	-0.071429	0.083299
1L by 2L	0.000000	0.063621
Average	5.442857	0.038021
R^2	0.79	

Table 5. Analysis of variants of response surface regression for pH nano-emulsions

Factor	SS	Df	MS	F	p
(1) SC (%) (L)	0.041667	1	0.041667	10.29412	0.032637*
SC (%) (Q)	0.002976	1	0.002976	0.73529	0.439512
(2) ratio (L)	0.015000	1	0.015000	3.70588	0.126531
ratio (Q)	0.002976	1	0.002976	0.73529	0.439512
1L by 2L	0.000000	1	0.000000	0.00000	1.000000
Error	0.016190	4	0.004048		
Total SS	0.080000	9			

significant p-value (α<0.05)

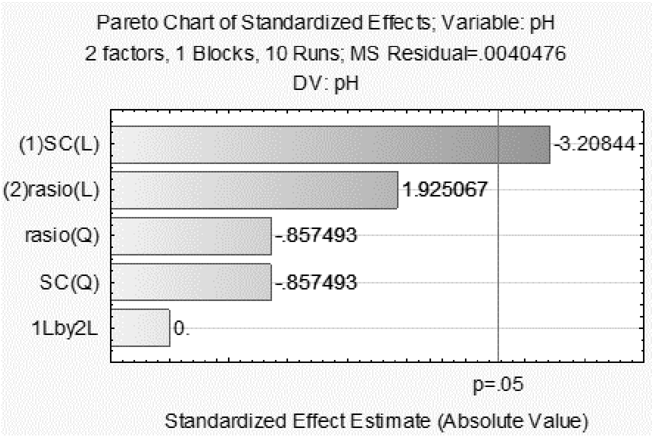

Figure 5. Pareto diagram from the standard effect of independent variables to the dependent variable

indicating that 79% of the independent variables affected the dependent variable. The other 21% were influenced by other factors, which were not included in this study (Nduru et al., 2014). The value of R2 obtained is presented in Table 4.

The next analysis involved evaluating the diagnostic plot (graph plot), which aimed to provide information on whether the model (response surface method) gave poor results, as shown in Figure 4. In Figure 3, the plot data points were distributed around the diagonal line and it could be concluded that the data were normally distributed. This indicated that the model could identify the effect of solid content and ratio on the pH of the nano-emulsion. Furthermore, the analysis of variance was carried out and the results are presented in Table 5. The results obtained were in the form of the P-value of the solid content variable (SC), namely $0.033 < \alpha=0.05$. Based on these findings, SC had a significant effect on the pH of the nano-emulsion.

The ratio variable obtained a p-value of 0.12 and was greater than the value of $\alpha=0.05$, indicating that it had no significant effect on the pH of the nano-emulsion. This could also be proven in the Pareto diagram that was obtained. In a case where the ratio value did not cross the reference line ($\alpha=0.05$), it could be concluded that the ratio did not have a significant effect on the pH of the nano-emulsion. The Pareto diagram obtained is presented in Figure 5.

Based on Figure 5, SC had the highest level of influence on the pH of the nano-emulsion. SC or solid content consisted of materials other than water, such as VCO, tween 80 or lecithin, and alpha-cyclodextrin. According to Mu'awanah et al., the amount of VCO used could affect the pH of the nano-emulsion produced. This was because the VCO contained fatty acids that could affect the pH of the product. An increase in the VCO concentration was expected to cause an increment in the acidity of the nano-emulsion (Mu'awanah et al., 2014). The surfactants used in this study were lecithin and tween 80 with HLB of 8 and 15 respectively. The lower the HLB value, the higher the lipophilicity, thereby leading to lower pH. This could be proven based on the results obtained, where samples with lecithin produced nano-emulsions with a pH range of 5.2–5.5, while those with tween 80 ranged from 5.4–5.5.

Table 6. Prediction value of optimum pH in SC and ratio critical value

Factor	Minimum value	Critical value	Maximum value
SC (%)	40.00000	38.33333	60.00000
Rasio	1.00000	1.85000	2.00000

The optimum yield parameters of the nano-emulsion pH were obtained with variations of SC and MCT-wall ratio based on the critical value. Therefore, the critical value for optimizing the pH of the nano-emulsion was reached at an SC concentration of 38.33333% with

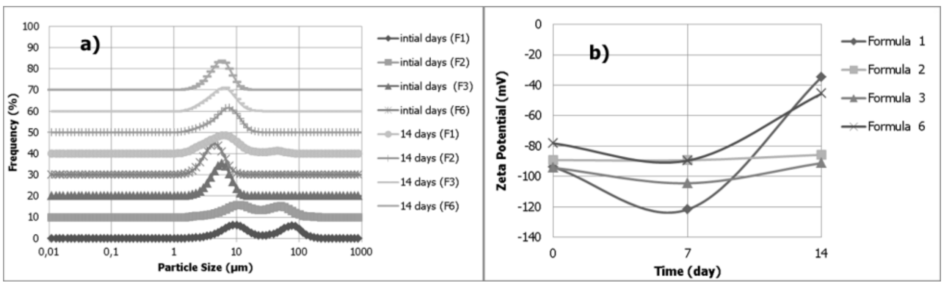

Figure 6. The result of analysis after being stored for 14 days (a) particle size values (b) zeta potential values

an MCT-wall ratio of 1.85000, which had a predicted pH value of 5.508968. When viewed from the predicted pH value obtained, it could be concluded that the best nano-emulsion formulas produced were 2, 6, and 9 with tween 80 or lecithin surfactant.

Particle Size and Zeta Potential Analysis

Particle size and zeta potential analysis was conducted after storage at 4 °C and room temperature for seven days, respectively, and the results are presented in Figure 6.

Figure 6 (a) illustrated the progression of particle size for formulas 1, 2, 3, and 6 after being stored for 14 days. The particle size values decreased in formulas 1 and 2, and there were no significant changes in 3 and 6. Based on the results, formula 6 had the smallest particle size of 5.495 µm. Figure 6 (b) showed that all formulas had good stability with zeta potential values of more than ±30 mV. The highest zeta potential was obtained in formula 3 (-94,233 to -104,433 mV) with the highest amount of surfactant, which was adequate for making it stable (Eid et al., 2013). The negative zeta potential in the results showed that the emulsion had an electrode potential at zero. This was because there was no charge and flocculation in the globules, indicating that the emulsion system was stable. A similar trend was observed in a study by Östbring et al. (2021), who reported that the lowest zeta potential was found at the highest emulsion pH investigation.

CONCLUSION

In conclusion, the current study showed that 10 formulas of nano-emulsion were o/w type emulsion and had a density range of 1.178–1.254 g/mL. The optimization of pH using RSM analysis revealed a value of 5.5, which was obtained in samples 2, 6, and 9 with tween 80 or lecithin as a surfactant. Based on the particle size analysis, formula 6 had the smallest particle size of 5.495 µm, while formula 3 had the highest zeta potential (-94,233 to -104,433 mV). The findings of this study showed that formula 6 had the best characteristics, with an optimum pH of 5.5, small particle size, and good stability, as indicated by the zeta potential value (-45.500 to -89.567 mV).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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