Journal of Vocational Studies on Applied Research

Optimization of Lemongrass Oil (*Cymbopogon citratus*) Emulsion with the Addition of Cethyl Alcohol as a Natural Cosurfactant

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Abstract - *Lemongrass oil (Cymbopogon citratus) is a plant that produces essential oils as a raw material for fragrance in cosmetics and perfumes. This study aims to determine the optimum conditions for the preparation of Lemongrass oil-wall emulsion (Tween 80 and Cethyl Alcohol) and to determine the effect of the independent variable in the form of the ratio of lemongrass oil-wall to the viscosity produced using the Response Surface Methodology (RSM). The novelty in this research is lemongrass oil with the addition of Tween 80 and Cethyl Alcohol as natural cosurfactants. Tests were carried out with three independent variables, namely stirring time (15, 20 and 25 minutes), stirring speed (20,000, 22,000 and 24,000 rpm) and lemongrass oil-wall ratio (1:3, 1:4, 1:5), which will be designed with the RSM method to obtain optimum operating conditions. Based on the results obtained, the optimal formula for lemongrass oil emulsion is on the 14th running result, which has a pH value of 6.4; density of 0.852; viscosity of 15,450 cP, type M/A, homogeneous and good stability without any change in colour, aroma, and texture. The influence of the lemongrass oil-wall ratio variable, namely the ratio variable, obtained a P-value of 0.000005, which means it is smaller than the value of α = 0.05 so that it can be said that the ratio has a significant effect on the viscosity of the emulsion.*

Keywords: *emulsion; lemongrass oil; response surface methodology*

Received: August 03, 2023 Revised: January 14, 2023 Accepted: February 05, 2024

Doi: http://dx.doi.org/10.14710/jvsar.v5i2.19642
[How to cite this article: Pradasari, A., and Paramita, V. (2023). Optimization of Lemongrass Oil (*Cymbopogon citratus*) Emulsion with the Addition of Cethyl Alcohol as a Natural Cosurfactant. *Journal of Vocational Studies on Applied Research, 5*(2), 44-51. doi: http://dx.doi.org/10.14710/jvsar.v4i2.19642]

INTRODUCTION

Lemongrass oil (*Cymbopogon citratus*) has main ingredients such as citronellal, citral and geraniol. These ingredients can inhibit bacterial activity (Bota et al., 2015). Lemongrass oil has a yellow colour, thick consistency, and a lemon-like aroma.

Now, lemongrass oil has become one of the most well-known oils in the tropics and has been made for generations. Since ancient times, lemongrass oil has been believed to prevent nausea, prevent diarrhoea, relieve headaches and relieve joint pain. In addition, lemongrass oil is also widely used as a raw material in the cosmetic industry, as soap, and as an ionic agent. Ionone is a class of synthetic aromatic compounds which are widely used as fragrances in cosmetics and perfumes. However, lemongrass oil that is used directly generally has limitations. Namely, it can quickly evaporate, which can cause the bioavailability of lemongrass oil to decrease and affect the activity of bacteria (Yumashar et al., 2017). Therefore, efforts are needed to increase the bioavailability and bioactivity of lemongrass oil, in this case, namely the antibacterial activity of the emulsion. Lemongrass oil will later be encapsulated in an emulsion system to reduce evaporation in essential oils (Muzdalifah et al., 2021).

Lemongrass oil emulsion is formulated as an oil-in-water (O/W) emulsion to maintain user comfort during topical use. It is easily rinsed with water and does not leave a sticky impression on the skin.

The emulsifying agent used to manufacture the emulsion will affect its characteristics and stability. A combination of emulsifying agents can be used to achieve the desired Hydrophile-Lipophile Balance (HLB). In interfacial film theory, the presence of a stable interfacial complex condensed film that is formed when a water-soluble emulsifying agent is mixed with a fat-soluble emulsifying agent can form and maintain an emulsion more effectively than using a single emulsifying agent (Laverius, 2011).

In this study, a combination of surfactants and cosurfactants was used, with the reason that this combined emulsifier can lower surface tension more than a single emulsifier so that the emulsion formed will be more stable (Cahyani et al., 2022). The surfactant used is tween 80, where tween 80 has an HLB value of 15, which, with this HLB value, is very suitable for making oil-in-water (O/W) emulsions and cetyl alcohol cosurfactant will act as an increase in interfacial density to strengthen the stability of the emulsion. In addition, cetyl alcohol is a natural co-surfactant, so it is safe for topical use (Setyopratiwi, 2021). Cetyl alcohol is an organic compound that is classified as a fatty alcohol. Fatty alcohols are mixtures of alcohol and fatty acids or oils. Cetyl alcohol is an emollient, emulsifier, thickener and surfactant in various cosmetic and skin care products. This cetyl alcohol can help protect the skin from allergens, bacteria and moisture loss and improve product texture. Previous research stated that Tween 80 had a dominant effect on decreasing the spreadability of the emulsion, while cetyl alcohol had a dominant effect on increasing the viscosity of the emulsion (Shintaningsi et al., 2007).

One of the methods used in making emulsions is the high-energy homogenization method using a homogenizer (Yuliasari et al., 2014). The pH of emulsion preparations must be in the pH range of the skin, namely 4.5-6.5, so that this emulsion is safe for topical use. The pH should not be too acidic. It can irritate and not be too alkaline because it can dry out the skin (Muzdalifah et al., 2021). This study aims to determine the optimum conditions for the preparation of lemongrass oil-wall emulsion (Tween 80 and Cethyl Alcohol) and to determine the effect of the independent variable in the form of the ratio of lemongrass oil-wall to the viscosity produced using the Response Surface Methodology (RSM).

METHODOLOGY

Materials and Tools

The equipment needed in this study was a beaker (Pyrex), measuring cup (Herma), refrigerator, homogenizer (D-500), oven (UN 110), Uv-Vis spectrophotometer (Thermo), dropper pipette, pH meter (AZ 86505), Brookfield viscosimeter (Thermo), pycnometer (Iwaki), digital balance (Pioneer), centrifuge (80-2), glass funnel (Pyrex) and magnetic stirrer (Heidolph). Moreover, the software used is statistics. The materials needed are lemongrass oil, polysorbate 80 (Tween 80), Cetyl Alcohol, aquadest, ethanol 96%, and Methylene blue.

Research Design

In this study, the experiment was designed using the Response Surface Methodology (RSM) with 16 runs and with the independent variables of stirring time, stirring speed and the ratio of lemongrass oil-wall, as shown in Table 1. The walls here are tween 80 and cetyl alcohol. The variables applied in this study were 7.5 ml; 6ml; and 5 ml of lemongrass oil, tween surfactant 80 (HLB = 15), cosurfactant cetyl alcohol (HLB = 11.5), and lemongrass oil-wall ratios 1:3, 1:4, and 1:5.

Table 1. Lemongrass Oil Emulsion Experiment Data

Formulation	Stirring Time (minute)	Stirring Speed (rpm)	Ratio Lemongrass oil : *wall* (v/v)
1	15	20.000	0,25
2	15	20.000	0,16
3	15	24.000	0,25
4	15	24.000	0,16
5	25	20.000	0,25
6	25	20.000	0,16
7	25	24.000	0,25
8	25	24.000	0,16
9	15	22.000	0,20
10	25	22.000	0,20
11	20	20.000	0,20
12	20	24.000	0,20
13	20	22.000	0,25
14	20	22.000	0,16
15	20	22.000	0,20
16	20	22.000	0,20

Emulsion Procedure

The first lemongrass oil emulsion was to make the water phase by mixing aquadest with a water-soluble emulsifier (tween 80) using a magnetic stirrer for 15 minutes. Then make the oil phase by mixing lemongrass oil and melted cetyl alcohol using a magnetic stirrer for 15 minutes. Then both phases were heated to 70ºC. After that, the oil phase was put into the water phase and homogenized using a homogenizer with a predetermined time and speed. After that, tests were carried out in the form of organoleptic analysis, density, viscosity, pH, centrifugation, homogeneity, emulsion type and freeze-thaw analysis.

RESULTS AND DISCUSSION

Stability Analysis and Emulsion Characteristics

Stability analysis using the Freeze-Thaw method to see the stability of the emulsion at extreme temperature changes. This study was carried out for 2 cycles, at 4°C and 40°C for 48 hours (1 cycle counted). After 2 cycles of testing, stable results were obtained; the physical appearance of the preparation did not change, the slightly liquid form, the colour did not change, and no separation occurred, as shown in Figure 1.

As shown in Figure 2, it can be seen from the results of centrifugation at 3000 rpm for 20 minutes; the results obtained from each emulsion preparation were that there was no precipitate or phase separation. The emulsion preparation shows that the emulsion has good stability. In emulsion preparations using tween 80 as a surfactant, it will form a film layer on the droplet surface which can prevent the aggregation of droplets in the dispersing medium so that no precipitation and phase separation occurs in the preparation.

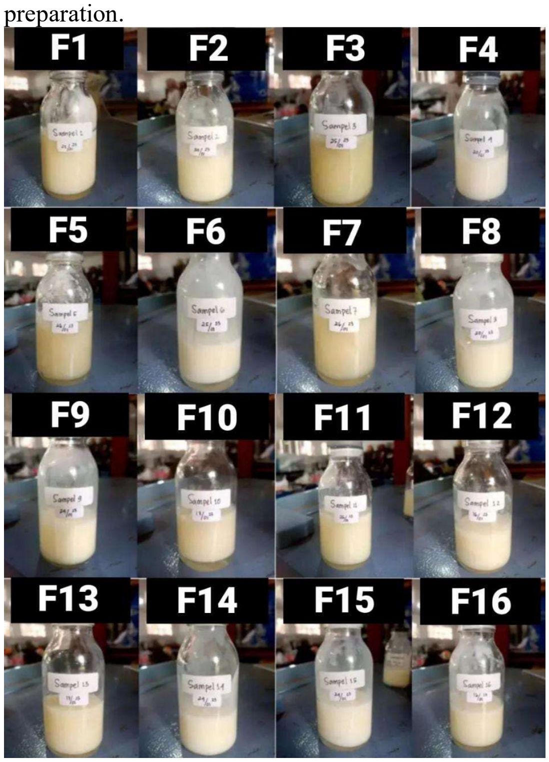


Figure 1. Emulsion after freeze-thaw analysis

According to Handayani & Muhtadi (2013), an emulsion is said to be stable if there are no physical changes such as colour, aroma or texture. The organoleptic test was carried out using the scoring method, where a higher score indicates a better quality of the emulsion preparation.

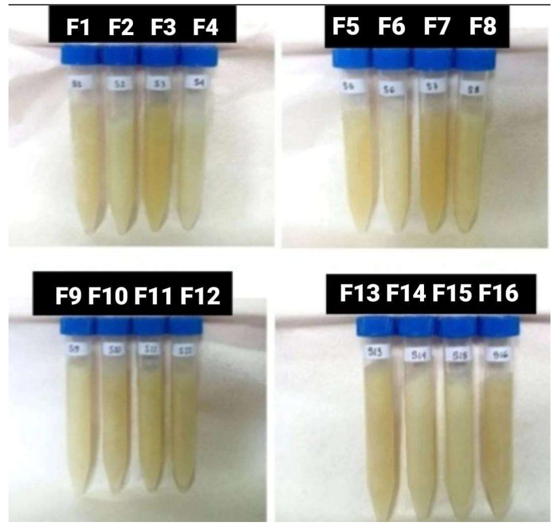


Figure 2. Emulsion after centrifugation analysis

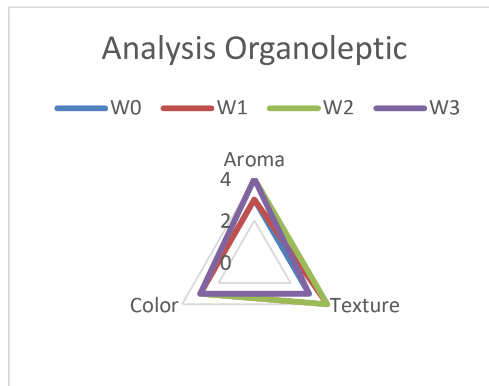


Figure 3. Organoleptic analysis at 0th week to third week at 20 min of stirring time, 22.000 rpm of speed and 0,16 v/v ratio lemongrass oil:wall

The scoring assessment method starts from 1 to 4, and is used to assess the product. Color is defined as 1 = yellow, 2 = yellowish white, 3 = white, and 4 = clear. The aroma was determined as 1 = very rancid aroma, 2 = rancid aroma, 3 = fragrant aroma, and 4 = very fragrant aroma. The texture is determined as 1 = very runny, 2 = runny, 3 = thick, and 4 = very thick. In organoleptic observations of lemongrass oil emulsion for 3 weeks of storage, there was no change in colour, texture or aroma, as shown in Figure 3. There was no significant change in colour, resulting in three different colours, namely white, yellowish white and yellow. This colour difference is due to the large amount of emulsifier (Tween 80) used, where the tween 80 has a clear, slightly yellow appearance so that the emulsion with the use of tween 80 will produce a yellow appearance and the emulsion with the use of tween 80 will produce a white appearance. The odour test produced a distinctive odour of lemongrass oil in the emulsion, and no rancid odour was found in the sixteen samples. Moreover, the texture produced by the emulsion is viscous in the sixteen samples.

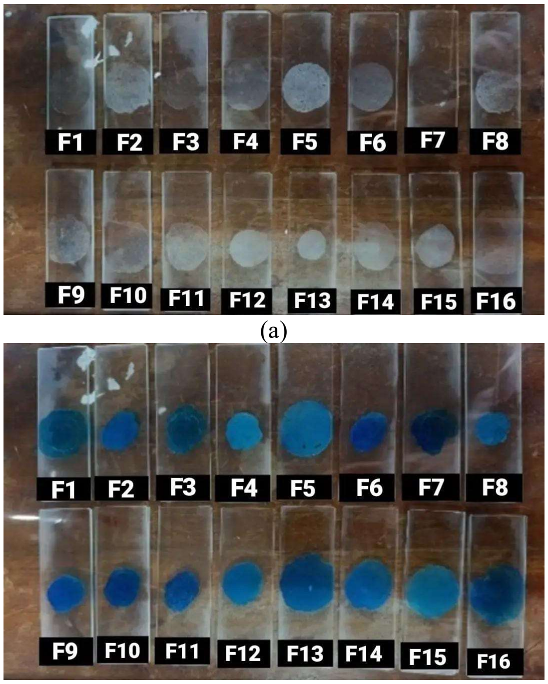
The pH measurement results for the emulsion formula obtained the pH value at week 0 in the range of 6.07-6.49; in the first week in the range of 5.77-6.40; in the second week in the range of 5.68-6.35; and the third week it is in the pH range of 5.45-6.28. The pH obtained has a pH value within the pH range of the skin, so it is safe for topical use, where the pH of the skin is in the range of 4.5-6.5. According to previous research, if the pH is too acidic, it will cause skin irritation; if it is too alkaline, it will cause dry skin. The resulting emulsion pH decreased during storage for 3 weeks at room temperature. The decrease in the pH of this emulsion occurs due to the hydrolysis of oil due to interaction with water to produce free fatty acids. These fatty acids can cause a decrease in pH.

The results of density measurements for 3 weeks at room temperature showed that in the 0th week, it was in the range of values 0.735-1.036 gram/cm^3, in the first week, it was in the 0.789-1.032 gram/cm^3 range, in the second week it was in the value range is 0.788-1.132 gram/cm^3, and in the third week it is in the value range 0.846-1.088 gram/cm^3. Increasing the surfactant concentration in the preparation will increase the density of an emulsion preparation. Moreover, the viscosity measurement results can be seen in Table 2. The greater the concentration of tween 80, the higher the emulsion viscosity value. In addition, the value of the viscosity of an emulsion can be influenced by several factors, including stirring and the proportion of the dispersed phase.

In the homogeneity analysis, which was carried out by applying the emulsion preparation to the object glass and then affixing it to another glass object, the result was that the emulsion was homogeneous because there were no coarse grains or lumps, as shown in Figure 3(a). Then, as can be seen in Figure 3(b), an emulsion-type analysis was carried out by pouring one drop of methylene blue on the surface of the emulsion on a glass object and then stirring with a stirrer. The results were an emulsion type of oil in water (O/W) because the emulsion was evenly dispersed. Moreover, there are no blue spots on the surface.

Table 2. Viscosity Measurement Results

Formulation	Stirring Time (minute)	Stirring Speed (rpm)	Ratio lemongrass oil : *wall* (v/v)	Viscosity (cP)			
				M_0	M_1	M_2	M_3
1	15	20.000	0,25	13800,33	13834	13832,67	13825,33
2	15	20.000	0,16	18001,67	18008,67	18013,67	18016,33
3	15	24.000	0,25	13050	13112,67	13244,33	13256,67
4	15	24.000	0,16	14949,33	14957,33	14960	14961,67
5	25	20.000	0,25	13917,67	13950	13852,67	13853,67
6	25	20.000	0,16	16100,33	16100,33	16104	16105,67
7	25	24.000	0,25	11400,33	11403,33	11408	11414
8	25	24.000	0,16	16250,67	15916,33	16250,67	16252
9	15	22.000	0,20	13950	13951,33	13961	13962,33
10	25	22.000	0,20	13875	13879,67	13883,67	13885
11	20	20.000	0,20	13971,33	13979,33	13979,67	13980,33
12	20	24.000	0,20	14353,33	14460,33	14460,67	14461,33
13	20	22.000	0,25	11852	11858,67	11869,33	11884
14	20	22.000	0,16	15450	15451,33	15452,33	15453,33
15	20	22.000	0,20	14100	14102,67	14103,33	14104,33
16	20	22.000	0,20	14100	14102,67	14103,33	14104,67



(b)

Figure 3. (a) Homogeneity Analysis Results and (b) Emulsion Type

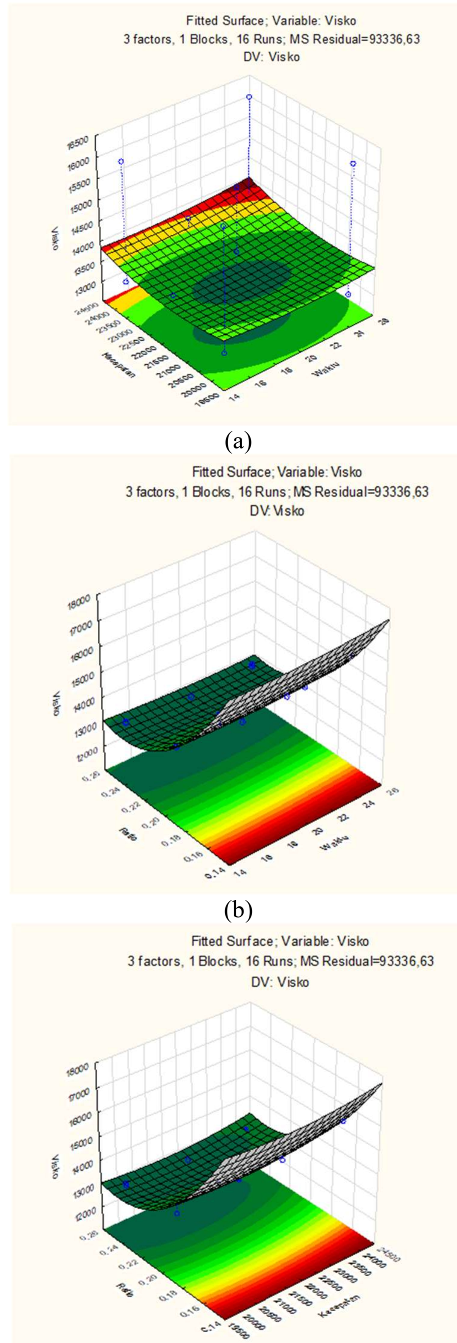
Results of Statistical Analysis using Response Surface Methodology (RSM)

In this research, the analysis using RSM aims to determine the effect of the independent variable in the ratio of lemongrass oil-wall on the resulting viscosity, as well as seek optimization to produce the best response. In this case, the wall combines surfactants (tween 80) and cosurfactants (cetyl alcohol).

According to the response, the surface can be made from the experimental results in Figure 5, which shows the relationship between stirring time, stirring speed and the lemongrass oil-wall ratio. By applying multiple regression analysis to the experimental data, a second-order polynomial equation was obtained to represent the effect of the independent variables on the citronella oil emulsion as follows:

$$Y = 13424{,}73 + 47{,}16X_1 + 118{,}38{X_1}^2 + 176{,}37X_2 + 301{,}38{X_2}^2 - 2969{,}00X_3 + 1746{,}33{X_3}^2 - 64{,}00X_1X_2 - 114{,}27X_1X_3 - 5{,}96X_2X_3$$

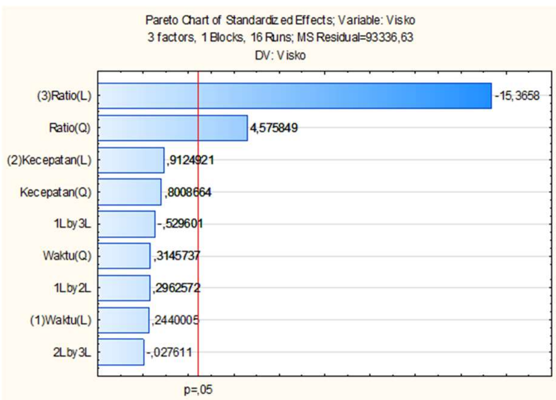
X_1 is the stirring time, X_2 is the stirring speed, and X_3 is the lemongrass oil-wall ratio.



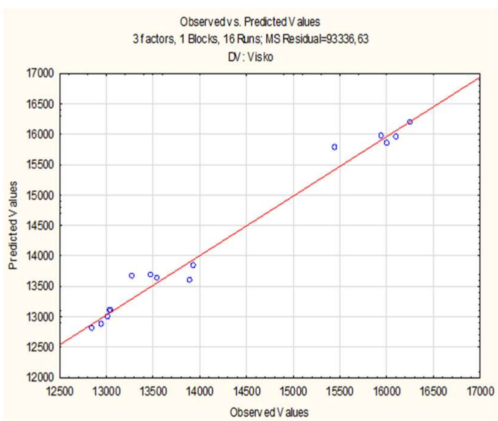
(c)

Figure 5. (a) Surface contour plot of the RSM response to the effect of stirring time and stirring speed on viscosity; (b) Surface contour plot of the RSM response to the effect of stirring time and the ratio of lemongrass oil-wall to viscosity; (c) Surface contour plot of the RSM response to the effect of stirring speed and the ratio of lemongrass oil-wall to viscosity.

The accuracy of the analysis that has been done can be seen from the value of the correlation coefficient R^2. The value of R^2 will provide information about how much influence is exerted by the independent variable or independent variable on the dependent variable or dependent variable. The R-value is always in the range 0-1. If the R^2 value gets closer to 1, then the model can predict responses well (Manurung, 2015). This study produces an R^2 value of 0.97776. This value indicates that 97.776% of the independent variables affect the dependent variable. Other factors influence the other 2.224%.



(a)



(b)

Figure 6. (a) Pareto diagram of the influence of variables on the viscosity value; (b) Comparison of run data with estimated viscosity values

Pareto diagrams can show which variables are most influential in a study presented in Figure 6(a). In the Pareto diagram, it can be seen that the factor that has a significant influence on the viscosity of the resulting emulsion is the lemongrass oil-wall ratio. The higher the citronella-wall oil ratio, the higher the emulsion viscosity value, so the higher the value.

The relationship between the predicted value and the model results obtained from the experiment is presented in Figure 6(b). The plot formed in the figure shows the research data where it is seen that there are deviations at several points from the estimated values, but deviations between these values indicate a relatively good correlation because the resulting research data is close to the linear line of estimated values. The regression coefficient is explained using a Pareto diagram and ANOVA for each influential variable (Paramita et al., 2016).

The response surface model in the analysis of variance (ANOVA) is shown in Table 3. ANOVA is needed to test the significance and adequacy of the model. The F value or Fisher's ratio of variance is a valid statistical measure of how well a factor explains the variation in the average data, and the estimated factor effect is natural, where the more significant the F value, the more uniformity it indicates (Paramita et al., 2016). ANOVA of the regression model shows a significant correlation, as evidenced by the F value of Fisher's test (Fmodel = 259.0539). In addition, the ratio variable obtained a P-value of 0.000005 which means it is smaller than the value of $\alpha = 0.05$, so it can be said that the ratio has a significant effect on the viscosity of the emulsion.

The critical value determines the optimization of the viscosity parameter obtained with the independent variables of stirring time, stirring speed, and lemongrass oil-wall ratio. So that the critical value for viscosity optimization is obtained through saddlepoint RSM analysis with an estimated viscosity.

A value of 12776.47 will be achieved at a stirring time of 21.51 minutes, a stirring speed of 21367.86 rpm, and a lemongrass oil-wall ratio of 0.24 . Based on the critical value experiment that has been carried out, a viscosity of 12776.04 is obtained. The results obtained from the critical value experiment are close to the predicted value of the RSM.

Table 3. Analysis of Variant of Kitchen Lemongrass Oil Emulsion Polynomial Equation Model

	ANOVA; Var.: Visko; R-sqr=,97776; Adj: ,9444 (RSM1) 3 factors, 1 Blocks, 16 Runs; MS Residual=93336,63 DV: Visko				
Factor	SS	Df	MS	F	p
(1)Stirring time (L)	5557	1	5557	0,059	0,815
Stirring time (Q)	9236	1	9236	0,099	0,764
(2)Stirring speed (L)	77716	1	77716	0,833	0,397

Stirring speed (Q)	59865	1	59865	0,641	0,454
(3)Ratio lemongrass oil-wall (L)	22037402	1	22037402	236,107	5×10^{-6}
Ratio lemongrass oil-wall (Q)	1954319	1	1954319	20,938	0,004
1L by 2L	8192	1	8192	0,088	0,777
1L by 3L	26179	1	26179	0,281	0,615
2L by 3L	71	1	71	0,001	0,979
Error	560020	6	93337		
Total SS	25181834	15			259,054

Table 4. Optimum Viscosity Prediction Value on Critical Value of Stirring Time, Stirring Speed and Lemongrass Oil-Wall Ratio

Factor	Minimum Value	Critical Value	Max Value
Stirring time	15,00	21,51	25,00
Stirring speed	20000,00	21367,86	24000,00
Ratio Lemongrass Oil: *Wall*	0,16	0,24	0,25
viscosity approximate value		12776,47	
viscosity validation value		12776,04	

CONCLUSION

In lemongrass oil emulsion preparation with the addition of tween 80 and cetyl alcohol as a surfactant and cosurfactant, the optimal formula for lemongrass oil emulsion was obtained, namely in sample 14 it had a pH value of 6.4; density of 0.852; In viscosity In of In 15,450 In cP In, type M/A, homogeneous and has good stability without any change in colour, aroma, and texture. The influence of the variable ratio of lemongrass oil-wall (tween 80 and cetyl alcohol), namely the ratio variable, obtained a P-value of 0.000005, which means it is smaller than the value of $\alpha = 0.05$ so that it can be said that the ratio has a significant effect on the viscosity of the emulsion. Based on the critical value experiment, a viscosity of 12776.04 was obtained, which is close to the predicted value of RSM.

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