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Optimization of the pH of mosquito repellent from citronella using response surface methodology

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Optimization of the pH of mosquito repellent from citronella using response surface methodology

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Abstract. Citronella is a source of essential oil obtained from steam distillation. The production of citronella oil is quite high; therefore, it can be applied in various fields. One of its applications is as an active substance in mosquito repellent creams. The citronella oil used in this study contained 11.59% citronellol content at a retention time of 16.544 minutes and citronellol content of 14.78% at a retention time of 27.165 minutes using the Gas Chromatography Mass Spectrometry method. In the manufacture of mosquito repellent creams, there are still mismatches in the composition between variables, so there is a need for optimization in this study. Optimization in this study using Response Surface Methodology model Central Composite Design on Statistica 10 software with 19 treatments. The method used was the addition of independent variables, citronella oil (2-4%), stearic acid (10-16%) and cetyl alcohol (4-6%) processed using the RSM-CCD method to determine the optimum pH. Optimization results with RSM at pH resulted in optimum conditions for citronella oil content of 2.1591%, stearic acid 18.045% and cetyl alcohol 6.6818% with a pH of 6.1.

1. Introduction

Aedes aegypti is the main transmitter of four viruses that have the greatest impact on human health, namely the viruses that cause yellow (YFV), dengue (DENV), chikungunya (CHIKV), and Zika fever (ZIKV) [1]. On the other hand, many efforts have been made to prevent this disease, but almost all synthetic mosquito repellents in circulation contain the active ingredient DEET (N, N-diethyl-3-methylbenzamide) which is a relatively dangerous synthetic chemical. In addition, continuous use of DEET can cause toxic reactions in the body [2]. This study aims to find alternative chemical insecticides and determine natural ingredients to make formulations that can be used in the design of new insecticides [3].

The US Environmental Protection Agency (US EPA) has listed the essential oils, lemon and eucalyptus as an insect repellent for application to the skin. These natural products are often used because of their relatively low toxicity dan comparable efficacy [4]. Essential oils are extracted from plants which are potential sources of bioactive natural molecules, one of which is citronella oil.

Citronella oil can be extracted from the leaves and stems of the plant [5]. Not only used in traditional ways as a repellent, fumigant (inhalation poison) or as a fragrance in food and cosmetics [6]. But also, in modern ways such as pharmaceutical, antimicrobial, antiviral, insect repellent, insecticide, anti-inflammatory and anti-allergic industries. The concentration of citronella oil commonly used in the production of insect repellents ranges from 0.05% to 15% either alone or in combination with lavender, clove, or cedar oil [7].

In application as a mosquito repellent, citronella oil is processed in the form of a cream. However, there is still a mismatch in the composition of the cream between the variables. Based on this description, it is necessary to conduct research on the optimization of the composition of the Aedes aegypti mosquito repellent cream. This study is going to determine the effect of independent variables on the resulting optimum pH. This pH measurement aims to determine whether the cream that has been made is acidic or alkaline, while the pH of the skin has criteria of around 4.5-6.5 so it is safe to use and does not irritate the skin [8], [9], [10], [11]. Optimization in this study uses the Response Surface Methodology (RSM) based of Central Composite Design (CCD), which is usually used for experimental design and minimizes the number of experiments for certain factors and levels [12].

2. Method

2.1. Materials

The materials used in this study are steric acid, cetyl alcohol, isopropyl myristate, nipasol, nipagin, glycerines, triethanolamine (TEA), aquades which can be purchased at the Chemical Store Indrasari Semarang and citronella oil is obtained from Kendal Essential Craftsmen.

2.2. Methods

2.2.1. Material preparation

The purified citronella oil was then evaluated by Gas Chromatography-Mass Spectrometry (GC-MS) at the Chemistry Laboratory of the Mathematics and Natural Sciences Department, Universitas Negeri Semarang.

2.2.2. Cream Preparation

Mosquito repellent cream is made as follows, the first solution, stearic acid, cetyl alcohol, nipasol, isopropyl myristate and glycerine was put into a 1000 mL beaker, then melted on a hotplate and stirred until homogeneous. The second solution, TEA, was put into a 50 mL beaker, then dissolved with hot distilled water. The third solution, nipagin, was dissolved with hot aquadest in a 50 mL beaker. In the first solution which had been melted, the second solution in the form of TEA was added and then stirred until it formed a corpus emulsion. The third solution in the form of nipagin is put into a beaker in the first solution little by little and stirred until homogeneous. The remaining distilled water was added to the first solution to 200 g and then stirred until homogeneous. Finally, citronella oil is added to the first solution and stirred until homogeneous.

2.2.3. Research Design

The research design in this paper can be determined the range and level for the RSM design by using a 3-factor variable. The lower level and upper level of the 3 factors are shown in the Table 1.

Table 1. Independent Variable Value Range

Component	Independent Variable	*Range* & Level (%)				
		- α	-1	0	1	+α
X_1	Citronella oil	1.32	2	3	4	4.68
X_2	Stearic acid	7.95	10	13	16	18.05
X_3	Cetyl alcohol	3.32	4	5	6	6.68

The analysis carried out is the evaluation of the content contained in citronella oil using Gas Chromatography-Mass Spectrometry (GC-MS), the pH of the cream that meets skin standards ranging from 4.5 to 6.5 and evaluation of RSM based of CCD to determine the optimal composition. RSM uses 2nd order polynomial regression in equation 1. RSM based of CCD is better suited to the second-order polynomial model [13].

$$Yi = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_{12} + \beta_{13} X_{13} + \beta_{23} X_{23} + \beta_{11} {X_1}^2 + \beta_{22} {X_2}^2 + \beta_{33} {X_3}^2 \quad (1)$$

Where :

Y = response of interest
β_0 = constant coefficient
$\beta_1, \beta_2, \beta_3$ = linear coefficient
$\beta_{12}, \beta_{13}, \beta_{23}$ = coefficient interaction
$\beta_{11}, \beta_{22}, \beta_{33}$ = quadratic coefficient
X_1, X_2 = independent variable

3. Result and Discussion

The results of the GC-MS analysis show that based on Table 2 there are 12 types of compounds with the main components of essential oils in citronella, the citronella content was 11.59% at a retention time of 16.544 minutes. Citronellol content was 14.78% at retention time of 27.165 minutes.

Table 2. Composition of Citronella Oil

Peak Number	Retention Time	%	Compound Name
1	3.730	1.81	Tricyclo [2.2.2.0(2,6)] heptane,1,7,7-trim
2	3.888	4.79	α-Pinene
3	4.491	5.23	Camphene
4	7.093	10.27	D-Limonene
5	16.544	11.59	Citronellal
6	19.164	1.52	β-Pinene
7	24.379	3.01	Carveol
8	26.327	4.19	Citral
9	27.116	2.60	γ-Muurolene
10	27.245	2.37	β-Pinene
11	27.615	14.78	Citronellol
12	30.594	37.84	β-Myrcene
Total		100.00	

Optimization of the influence of variables on determining the optimum pH followed the RSM with an experimental design of CCD. The value of pH Response Observed (RO) and Response Predicted (RP) obtained can be seen in Table 3 with the error calculation obtained in equation 2.

$$\%\, error = \frac{|observed - predicted|}{observed} \times 100\% \quad (2)$$

Table 3. Observed and Predicted pH values

RUN	Value			RO	RP	% Eror
	X_1 (%wt)	X_2 (%wt)	X_3 (%wt)			
1	2.00	10.00	4.00	4.78	5.05	5.65
2	2.00	10.00	6.00	5.86	5.71	2.56
3	2.00	16.00	4.00	6.1	5.67	7.05
4	2.00	16.00	6.00	5.96	5.74	3.69
5	4.00	10.00	4.00	5.88	5.76	2.04
6	4.00	10.00	6.00	5.91	6.00	1.52
7	4.00	16.00	4.00	5.89	5.71	3.06
8	4.00	16.00	6.00	5.96	5.36	10.07
9	1.32	13.00	5.00	5.73	5.87	2.44
10	4.68	13.00	5.00	5.83	6.14	5.32
11	3.00	7.950	5.00	5.89	5.66	3.90
12	3.00	18.05	5.00	4.96	5.64	13.71
13	3.00	13.00	3.32	4.87	4.97	2.05
14	3.00	13.00	6.68	4.88	5.23	7.17
15	3.00	13.00	5.00	4.81	4.87	1.25
16	3.00	13.00	5.00	4.80	4.87	1.46
17	3.00	13.00	5.00	4.92	487	1.02
18	3.00	13.00	5.00	4.95	4.87	1.62
19	3.00	13.00	5.00	4.96	4.87	1.81

The average prediction results are measured using MAPE or Mean Absolute Percentage Error, which is formulated in equation 3 [14,15].

$$MAPE = \sum_{t=1}^{n} \left| \frac{\frac{observed-predicted}{observed}}{n} \right| x\ 100\% \quad (3)$$

MAPE values consist of 4 categories:
<10% = very accurate
10-20% = good
20-50% = reasonable
>50% = inaccurate

Based on equation 3, the MAPE value is 4.07% which shows that the experiments carried out are very accurate. The comparison between observed and predictions values is presented in the form of a graph shown in Figure 1.

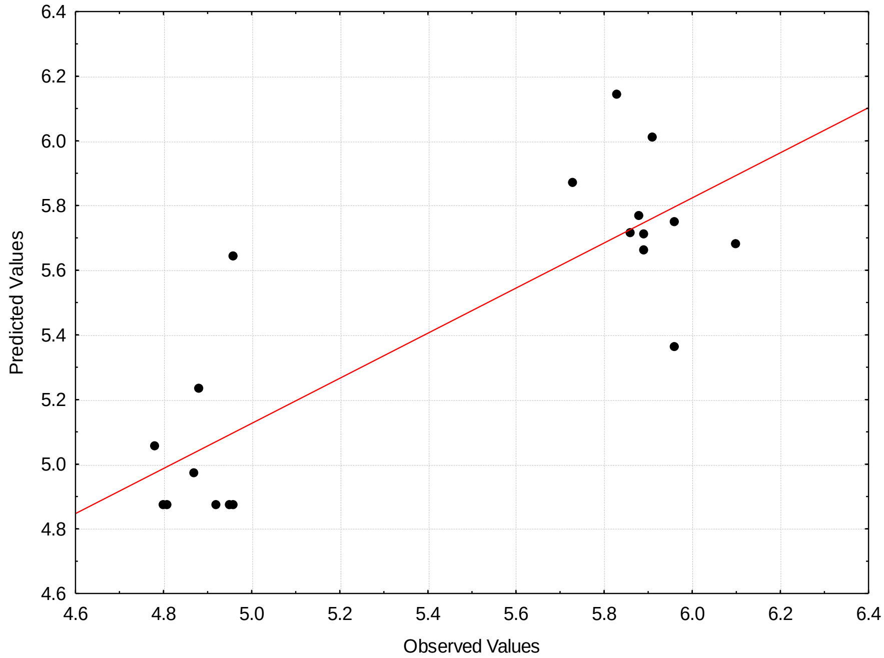


Figure 1. Comparison of Observed and Predictions Values

The results of statistical analysis were carried out by developing mathematical models, analysis of variance, Pareto analysis and model validation. The polynomial equation formed is shown in equation 1. The variables or coefficients obtained by determining the second order polynomial using the Statistica 10 software. Experimental analysis for the regression coefficients is shown in Table 4.

Table 4. Prediction of the Regression Coefficient for a Polynomial of Order 2

Factor	*Effect*	*Std. Err.*	t	P
Mean/Interc	4.872	0.034	141.171	0.000
X_1	0.162	0.041	3.881	0.017
X_1^2	0.803	0.041	19.197	0.000
X_2	-0.012	0.041	-0.294	0.783
X_2^2	0.551	0.041	13.196	0.000
X_3	0.154	0.041	3.701	0.020
X_3^2	0.163	0.041	3.898	0.017
$X_1 X_2$	-0.340	0.054	-6.223	0.003
$X_1 X_3$	-0.210	0.054	-3.843	0.018
$X_2 X_3$	-0.295	0.054	-5.399	0.005
R^2	0.6974	Adj R^2	0.3025	

The values of p and t are used to determine whether or not each term is significant. The influential parameters can be seen from Table 4 where statistically the parameter values significantly affect the response below 0.05. If the p value is below 0.05 then the coefficient price and the more influential on the observations. The parameter for stearic acid (X_2) has a p value of 0.7831, where this value is greater than the p value, so that the stearic acid parameter (X_2) has no significant effect on the optimum pH results. However, the parameter for quadratic stearic acid (X_2^2) affects the optimum pH results because it has a p value of 0.0002.

Pareto diagrams are used to assist in determining the effect of parameters on the process. In the Pareto diagram it can be seen that the stearic acid parameter (X_2) has the smallest t value of -0.294418, so this parameter has no significant effect on the process. The citronella oil parameter (X_1) has the largest t value of 19.197 and crosses the p line, so this parameter has a significant effect.

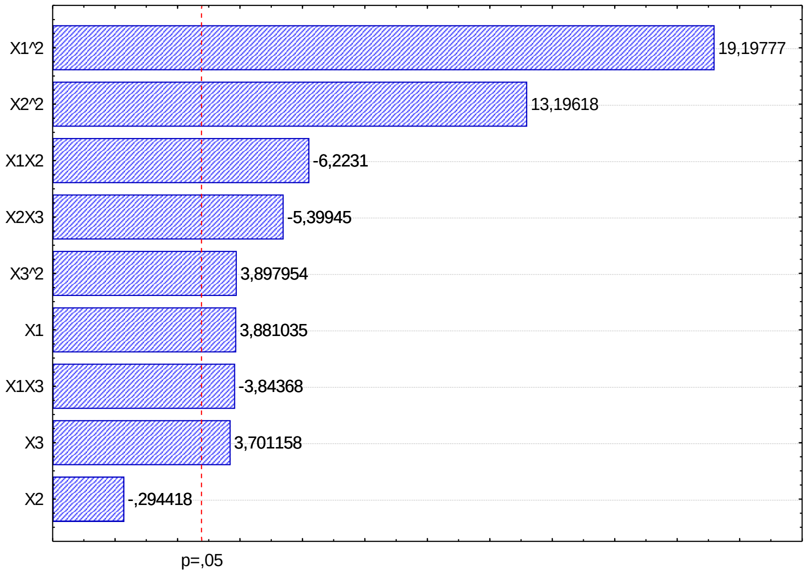


Figure 2. Pareto Chart

Analysis of Variance (ANOVA) of the mathematical model is presented in Table 5. ANOVA can be used to determine whether the independent variable has a significant effect on the dependent variable. The error tolerance limit (α) used is 0.05 or 5%, so the confidence level is (1-0.05) = 95%. For parameters that have an F value greater than the p value, the parameter has a significant effect on the optimum pH.

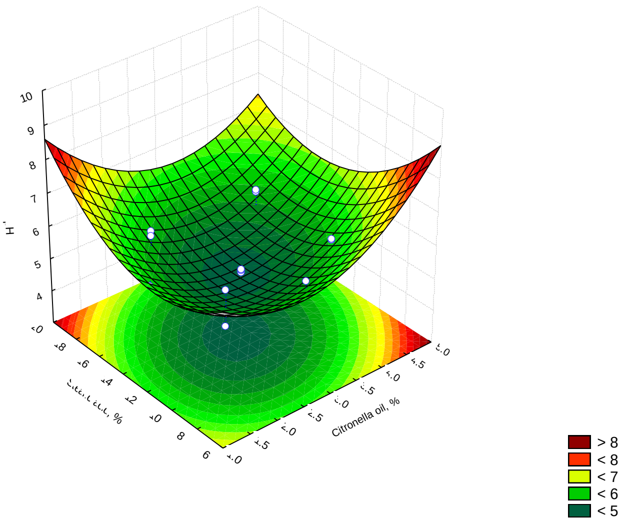
Table 5. Table ANOVA

Factor	SS	Df	MS	F	p
X_1	0.0899	1	0.0899	15.0624	0.0178
X_1^2	2.2002	1	2.2002	368.554	0.0000
X_2	0.0005	1	0.0005	0.08668	0.7830
X_2^2	1.0396	1	1.0396	174.139	0.0001
X_3	0.0817	1	0.0817	13.6985	0.0208
X_3^2	0.0907	1	0.0907	15.1940	0.0175
$X_1 X_2$	0.2312	1	0.2312	38.7269	0.0033
$X_1 X_3$	0.0882	1	0.0882	14.7738	0.0184
$X_2 X_3$	0.1740	1	0.1740	29.1541	0.0056
Lack of Fit	1.5039	5	0.3007	50.3820	0.0010
Pure Error	0.0238	4	0.0059		
Total SS	5.0511	18			
R^2	0.6974	Adj R^2	0.3025		

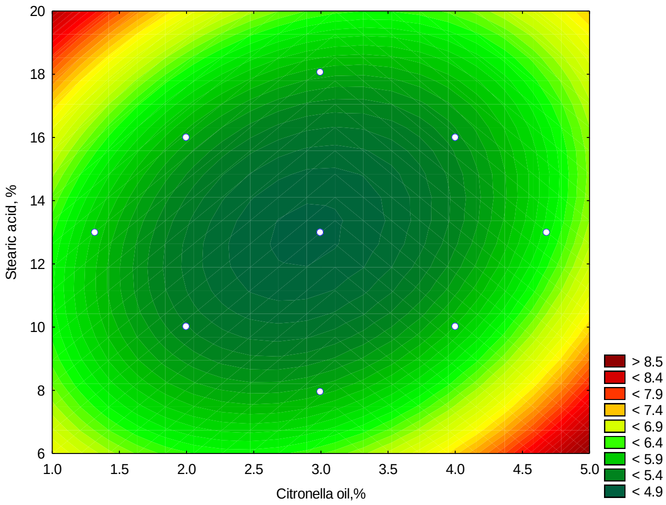
The parameter that had no significant effect was stearic acid (X_2) because the p value was greater than 0.05. The results from Table 5 show the Lack of Fit value (model discrepancy) of 0.00105 or less than the degree of significance (p) 0.05. This shows that overall, the independent variables have an effect on the experiment. From the table above, the R^2 value of 0.69745 means that the effect of citronella oil, stearic acid and cetyl alcohol is 69.745%, while the rest is influenced by other variables that are not included in the model/independent variables.

3.1 Effect of Independent Variables on pH

3.1.1 Effect of Citronella Oil and Stearic Acid on pH



(a)



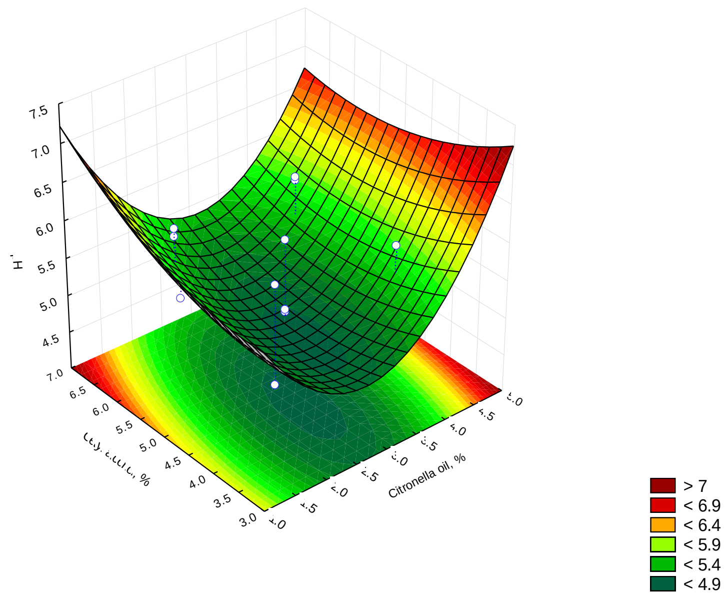
(b)

Figure 3. Effect of Citronella Oil and Stearic Acid on pH a) Surface Plot b) Contour Plot

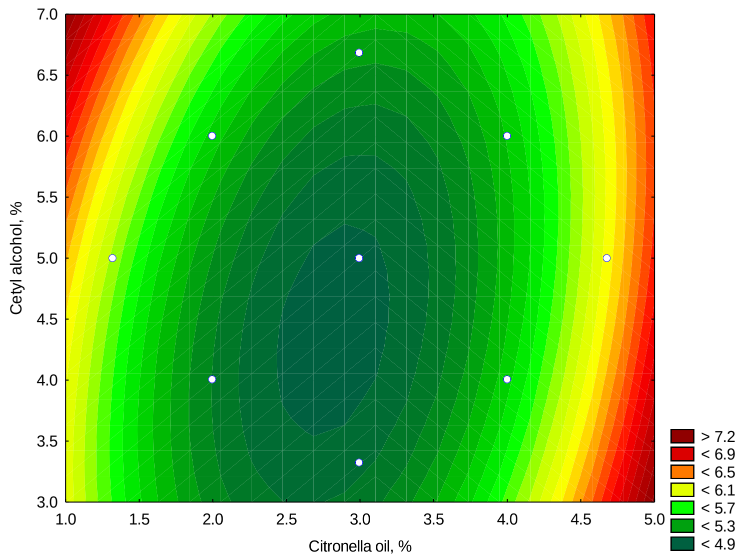
The regression equation model obtained is in equation 4.

$$Z = 11.2410 - 1.5910X_1 + 0.4014{X_1}^2 - 0.6289X_2 + 0.3066{X_2}^2 - 0.0566X_1X_2 \quad (4)$$

3.1.2 Effect of Citronella Oil and Cetyl Alcohol on pH



(a)



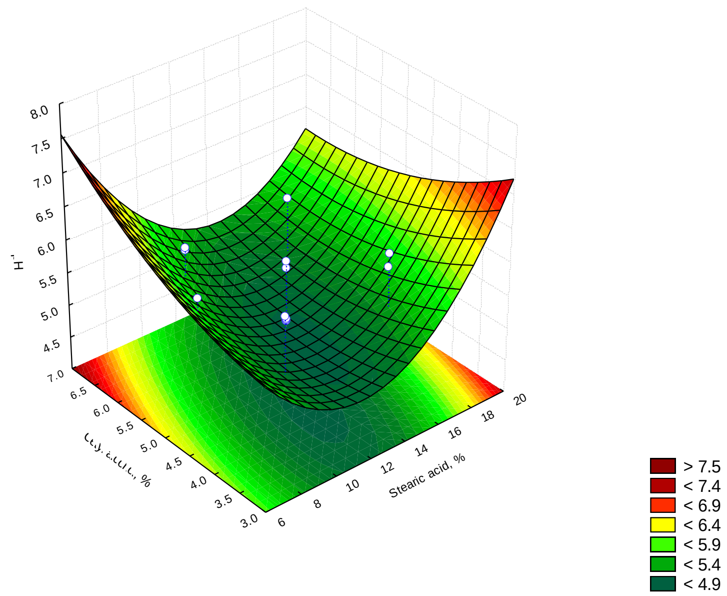
(b)

Figure 4. Effect of Citronella Oil and Cetyl Alcohol on pH a) Surface Plot b) Contour Plot

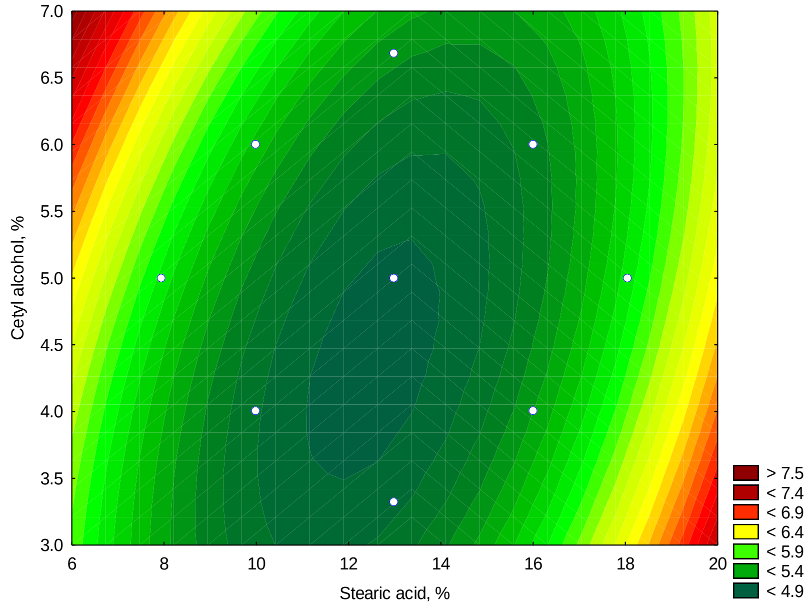
The regression equation model obtained is in equation 5.

$$Z = 8.3183 - 1.8018X_1 + 0.4014{X_1}^2 - 0.8546X_3 + 0.0815{X_3}^2 - 0.105X_1X_3 \quad (5)$$

3.1.3 Effect of Stearic Acid and Cetyl Alcohol on pH



(a)



(b)

Figure 5. Effect of Stearic Acid and Cetyl Alcohol on pH a) Surface Plot b) Contour Plot

The regression equation model obtained is in equation 6.

$$Z = 8.5363 - 0.5532X_2 + 0.3066{X_2}^2 + 0.5313X_3 + 0.0815{X_3}^2 - 0.0491X_2X_3 \quad (6)$$

3.2 Optimum Condition

The optimum condition can be determined by drawing a straight line from the three interactions, between citronella oil, stearic acid and cetyl alcohol on pH. The interaction between variables can be seen in Figure 6.

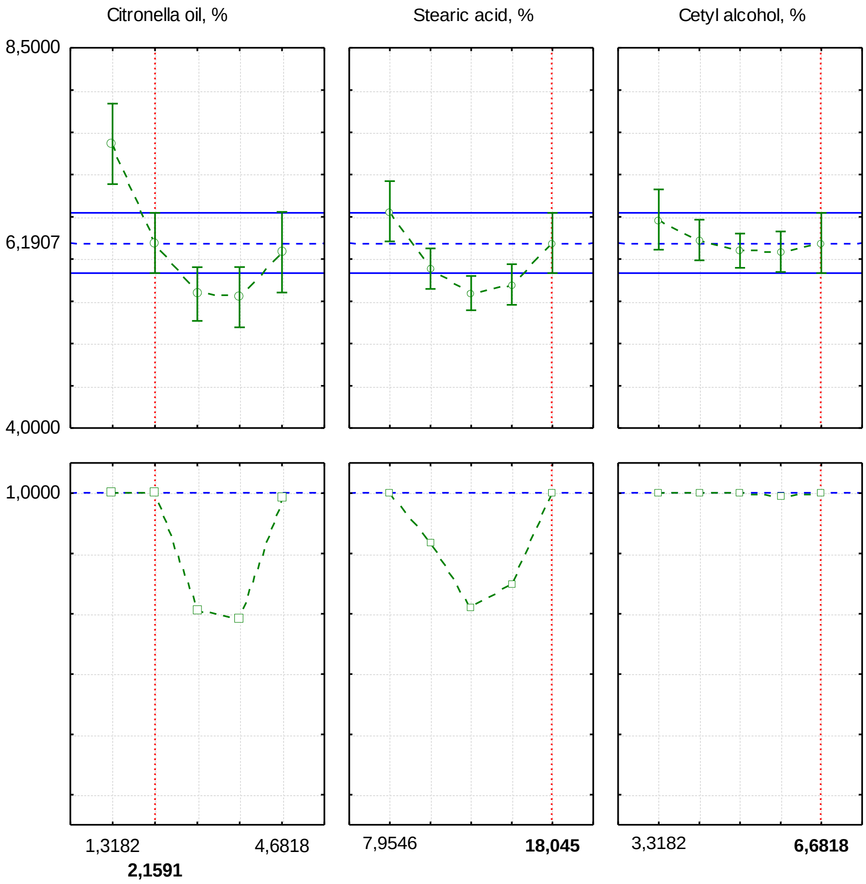


Figure 6. Optimum Process Conditions Against pH

Optimum conditions obtained citronella oil content of 2.1591%, 18.045% stearic acid and 6.6818% cetyl alcohol with a pH of 6.1.

4. Conclusion

Based on the analysis of the optimization results showed that the optimum pH was 6.1 with citronella oil content of 2.1591%, stearic acid of 18.045% and cetyl alcohol of 6.6818%. In the experiment, the effect between independent variables on the pH value will decrease along with the increase in the independent variables, citronella oil content, stearic acid content and cetyl alcohol content. The prediction value using the model from RSM shows the R^2 value of 0.69745 with an error value of 0.30255. Model mathematics polynomial equation:

$$Yi = 4.8724 + 0.1624X_1 + 0.1548X_3 - 0.3400X_{12} - 0.2100X_{13} - 0.2950X_{23} + 0.8030{X_1}^2 + 0.5519{X_2}^2 + 0.1630{X_3}^2$$

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