

3. EXPERIMENTAL RESULT

The chapter will show both the statistical result of the experimental data and the plot of the data in the form of prediction profiler and surface plot. The responses studied in this paper are total phenolic content and antioxidant activity. The result for crude extract yield can be seen in Appendix 1.

The full design of experiment and the result of yield, total phenolic content, and antioxidant activity are shown in Table 1 below. The symbols on 'Pattern' column is explained as follows:

'+' and '-' are factorial points where '+' indicates the highest value of each corresponding factor's factorial point, while '-' indicates the lowest value of each corresponding factor's factorial point. The symbol 'A' and 'a' represent axial points where 'A' indicates the highest value of each corresponding factor's axial point while 'a' indicates the lowest value of each corresponding factor's axial point. The number '0' is used to indicate the middle value of each corresponding factor (i.e. time= 25 minutes, temperature= 50°C, and power= 70 Watt). The left column of the pattern referred to time, the middle column of the pattern referred to temperature, the right column of the pattern referred to power.

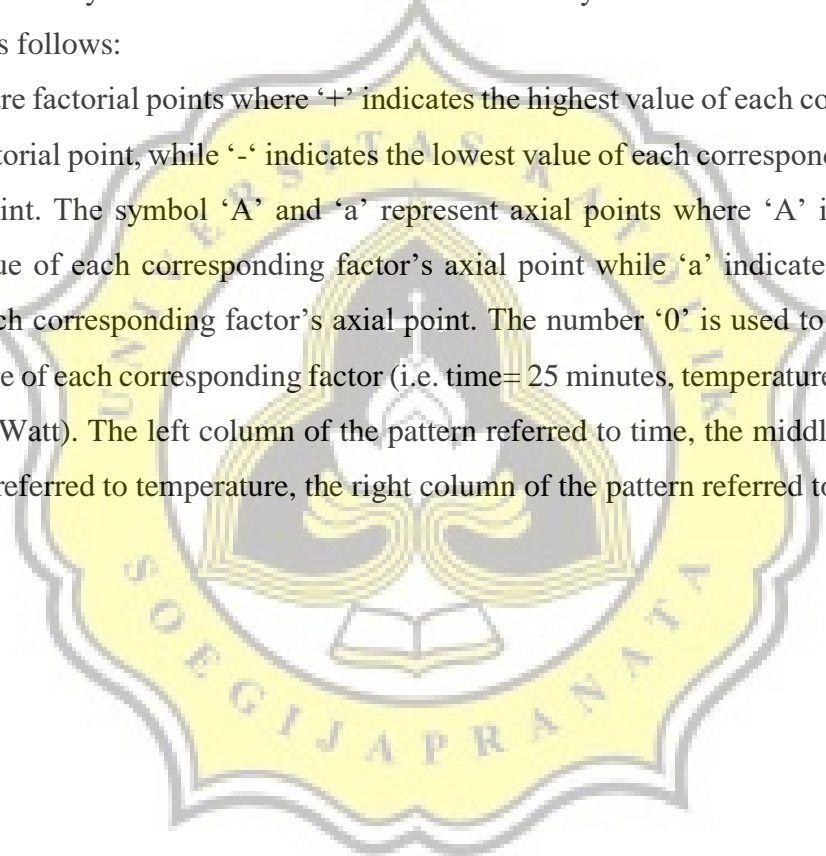


Table 1. Effect of extraction variable of UAE on yield, total phenolic content, and antioxidant activity (% inhibition) in *Piper betel* extract

No.	Pattern	Independent Variables			Dependent Variables	
		Time (minutes)	Temperature (°C)	Power (Watt)	Total Phenol Content (ppm)	Antioxidant Activity (% Inhibition)
1	---	20	45	50	27.65 ± 1.50	18.28 ± 0.42
2	--+	20	45	90	31.44 ± 1.18	17.38 ± 2.63
3	-+-	20	55	50	48.69 ± 1.18	26.91 ± 1.59
4	-++	20	55	90	48.79 ± 1.50	25.97 ± 0.68
5	+--	30	45	50	36.48 ± 0.99	20.16 ± 2.03
6	+ - +	30	45	90	37.38 ± 2.45	22.66 ± 1.32
7	++-	30	55	50	50.48 ± 2.46	29.66 ± 1.76
8	+++	30	55	90	53.10 ± 1.94	31.32 ± 0.96
9	a00	20	50	70	38.54 ± 1.58	21.30 ± 0.65
10	A00	30	50	70	38.19 ± 2.59	23.10 ± 1.94
11	0a0	25	45	70	35.45 ± 1.19	19.59 ± 0.35
12	0A0	25	55	70	53.85 ± 1.37	32.76 ± 1.87
13	00a	25	50	50	40.30 ± 0.61	22.27 ± 0.28
14	00A	25	50	90	38.47 ± 1.84	22.26 ± 0.26
15	000	25	50	70	40.92 ± 1.03	23.49 ± 1.15
16	000	25	50	70	42.38 ± 1.15	23.09 ± 0.93
17	000	25	50	70	43.73 ± 0.60	23.70 ± 1.13
18	000	25	50	70	42.88 ± 0.67	22.16 ± 1.66
19	000	25	50	70	42.51 ± 1.28	23.42 ± 1.78
20	000	25	50	70	41.77 ± 2.06	22.61 ± 2.29

*Numbers show the average ± standard deviation (n=3)

The data from table 1 shows the full design of response surface experiment with independent variables: extraction time, extraction temperature, ultrasound power; and dependent variables: crude extract yield, total phenolic content, and antioxidant activity. Lowest yield was obtained from sample number 15 with response yield of 17.05% while the highest response yield was shown by sample number 8 with yield value of 19.50%. The lowest total phenol content was obtained from sample number 1 with TPC value of 27.65 ± 1.50 ppm gallic acid equivalent while the highest TPC value was obtained from sample number 12 with TPC value of 53.85 ± 1.37 ppm gallic acid equivalent. Correspondingly, sample with highest antioxidant activity, expressed by % inhibition was

sample number 12 with % inhibition value of 32.76 ± 1.87 , while the sample with the lowest antioxidant activity was sample number 2 with % inhibition value of 17.38 ± 2.63 .

In addition to the full design and result of experiment, below is the Effect summary on the experimental result. The effect summary show the p-value of each coefficient. A p-value below 0.05 indicates that the corresponding coefficient is significant in the constructed regression model at 95% confidence level. A p-value under 0.01 means that the corresponding coefficient is significant in the regression model at 99% confidence level.

Table 2. Effect Summary of Response Surface Analysis on experimental results

Source	LogWorth	p-Value
Temperature(45,55)	7,404	0,00000
Time(20,30)	3,296	0,00051
Temperature*Temperature	2,971	0,00107
Time*Time	1,326	0,04719
Time*Temperature	0,894	0,12767
Power*Power	0,789	0,16242
Time*Power	0,707	0,19655
Power(50,90)	0,596	0,25344
Temperature*Power	0,402	0,39636

Table 2 shows the effect summary of experimental analysis. The table shows 9 effects in which 3 are single effects (time, temperature, and power) and the rest of the 6 effects are quadratic effects (temperature², time², power², time*temperature, time*power, and temperature*power). The most significant variable towards the model was temperature with p-value of 0.00000, followed by time with p-value of 0.00051 and then temperature*temperature with p-value of 0.00107, and the last significant coefficient is time*time with p-value of 0.04719. The rest of the coefficients are insignificant in the regression model. The interaction between temperature and power showed the lowest significance with p-value of 0.39636.

3.1. Total Phenolic Content

This sub-chapter will show the result of total phenolic content test. The set of data was obtained by using Folin-Ciocalteu assay. The statistical result comprising lack of fit, summary of fit, ANOVA and surface plot are displayed below in table 3, 4, and 5.

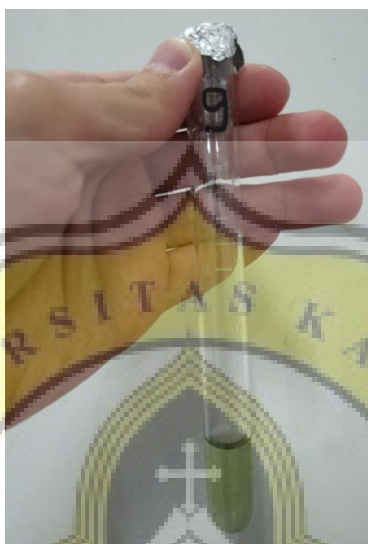


Figure 5. Mixture of sample and Folin Ciocalteu reagent after 2 hours of incubation at room temperature

3.1.1. Predicted Model and Statistical Analysis

Table 3. Lack of Fit of total phenolic content response surface model

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	5	29,529823	5,90596	6,4588
Pure Error	5	4,572024	0,91440	Prob > F
Total Error	10	34,101846		0,0308*
				Max RSq
				0,9948

Table 3 shows the lack of fit test of total phenolic content polynomial regression model. Since the probability of lack of fit is lower than 0.05, the regression model used does not adequately describe the distribution of data from experimental result

Table 4. Summary of Fit of total phenolic content response surface model

RSquare	0,961397
RSquare Adj	0,926655
Root Mean Square Error	1,846669
Mean of Response	41,65049
Observations (or Sum Wgts)	20

Table 4 shows the summary of fit of total phenolic content regression model. The R^2 of the regression model is 0.961397 which means that 96% of the total variations can be explained by the model while only 4% of total variations cannot be explained by the model. The adjusted R^2 is 0.926655 which further confirms that the model is highly significant. The mean of response was 41.65049 with root mean square error of 1.846669.

Table 5. Analysis of Variance of total phenolic content response surface model

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	849,30190	94,3669	27,6721
Error	10	34,10185	3,4102	Prob > F
C. Total	19	883,40374		<,0001*

Table 5 indicates shows that the probability of the regression model is lower than 0.0001 which means that the quadratic model is significant and can be used to optimize extraction factors. Since the model is significant, the regression formula below can be used to predict the value of total phenolic content based on the value of independent variables

$$Y = 41.77 + 2.05(X_1) + 8.65(X_2) + 0.56(X_3) - 1.08(X_1X_2) - 0.48(X_1X_3) - 0.25(X_2X_3) - 2.52(X_1^2) + 3.77(X_2^2) - 1.49(X_3^2)$$

Legend:

Y= Total phenolic content

X_1 = Time

X_2 = Temperature

X_3 = Power

3.1.2. Model Plots

The total phenolic content from 0.025% of sample solution at different ultrasound power: 50 Watt, 70 Watt, and 90 Watt can be seen in figure 6a, 6b, and 6c.

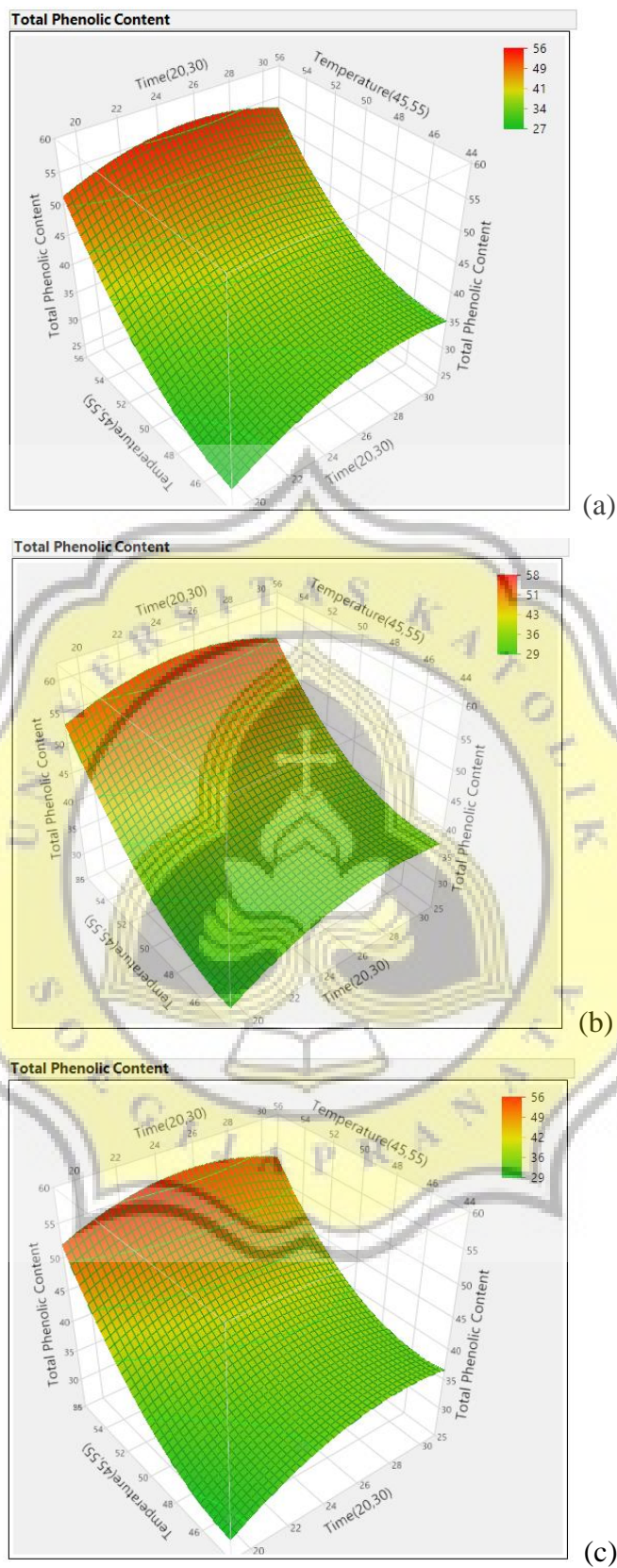


Figure 6. Surface plot of total phenolic content against extraction time and temperature at ultrasound power of 50 Watt (a), 70 Watt (b), 90 Watt (c)

The surface plot above shows the predicted value of total phenolic content over a surface area. The total phenolic content was plotted against time at range 19-31 minutes and temperature at range 44°C-56°C. Figure 6a shows the surface plot at power level of 50 Watt and the lower bound of predicted total phenolic content was shown to be 27 ppm galic acid equivalent and the upper bound was 56 ppm galic acid equivalent. Figure 6b shows the surface plot at power level 70 Watt and the lower bound of predicted total phenolic content was shown to be 29 ppm galic acid equivalent while the upper bound was shown to be 58 ppm galic acid equivalent. Figure 6c shows the surface plot at power level 90 Watt and the lower bound of total phenolic content was shown to be 29 ppm galic acid equivalent while the upper bound was shown to be 56 ppm galic acid equivalent.

3.2. Antioxidant Activity

This sub-chapter will show the result of antioxidant activity test. The set of data was obtained by using DPPH assay. The statistical result comprising lack of fit, summary of fit, ANOVA and surface plot are displayed below in table 6, 7, and 8.

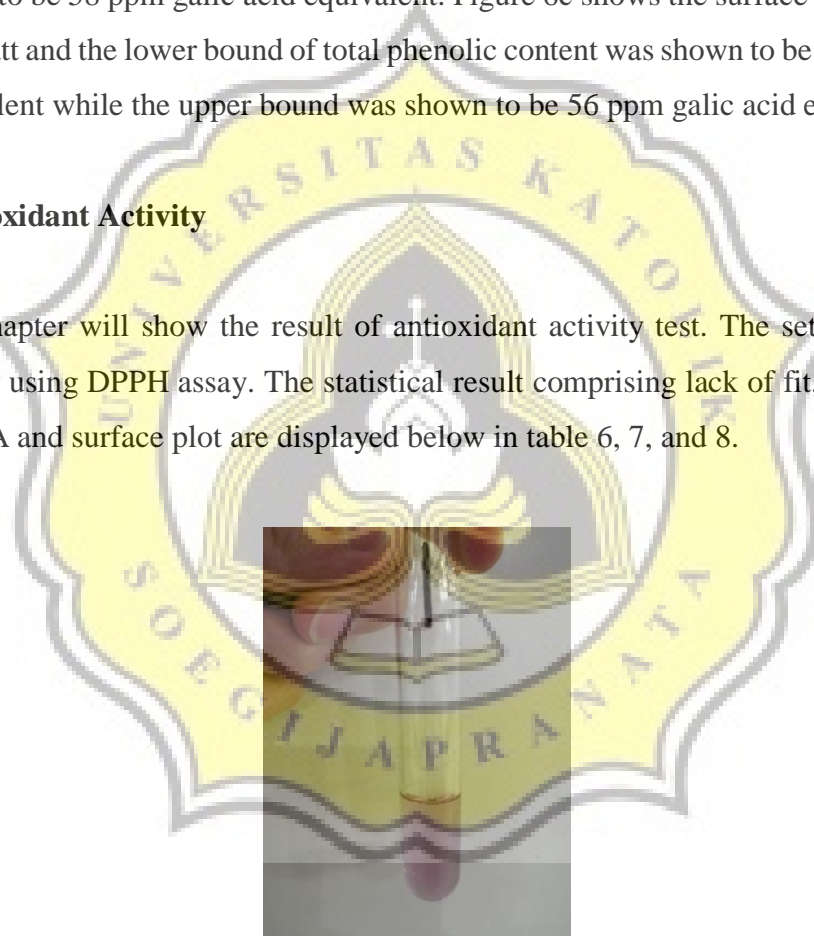


Figure 7. Mixture of sample and DPPH solution after 30 minutes of incubation in dark room at room temperature

3.2.1. Predicted Model and Statistical Analysis

Table 6. Lack of Fit of antioxidant activity response surface model

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	5	9,751926	1,95039	5,6550
Pure Error	5	1,724477	0,34490	Prob > F
Total Error	10	11,476403		0,0401*
				Max RSq
				0,9943

Table 6 shows the lack of fit test of antioxidant activity polynomial regression model. Since the probability of lack of fit is lower than 0.05, the regression model used does not adequately describe the distribution of data from experimental result

Table 7. Summary of Fit of total phenolic content response surface model

RSquare	0,962376
RSquare Adj	0,928514
Root Mean Square Error	1,07128
Mean of Response	23,60422
Observations (or Sum Wgts)	20

Table 7 shows the summary of fit of total phenolic content regression model. The R^2 of the regression model is 0.962376 which means that 96% of the total variations can be explained by the model while only 4% of total variations cannot be explained by the model. The adjusted R^2 is 0.928514 which further confirms that the model is highly significant. The mean of response was 23.690422 with root mean square error of 1.07128.

Table 8. Analysis of variance of antioxidant activity response surface model

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	293,55120	32,6168	28,4208
Error	10	11,47640	1,1476	Prob > F
C. Total	19	305,02760		<,0001*

Table 6 indicates shows that the probability of the regression model is lower than 0.0001 which means that the quadratic model is significant and can be used to optimize extraction

factors. Since the model is significant, the regression formula below can be used to predict the value of antioxidant activity based on the value of independent variables

$$Y = 23.14 + 1.71(X_1) + 4.85(X_2) + 0.41(X_3) + 0.12(X_1X_2) + 0.52(X_1X_3) - 0.34(X_2X_3) - 1.04(X_1^2) + 2.94(X_2^2) - 0.97(X_3^2)$$

Legend:

Y= Antioxidant activity

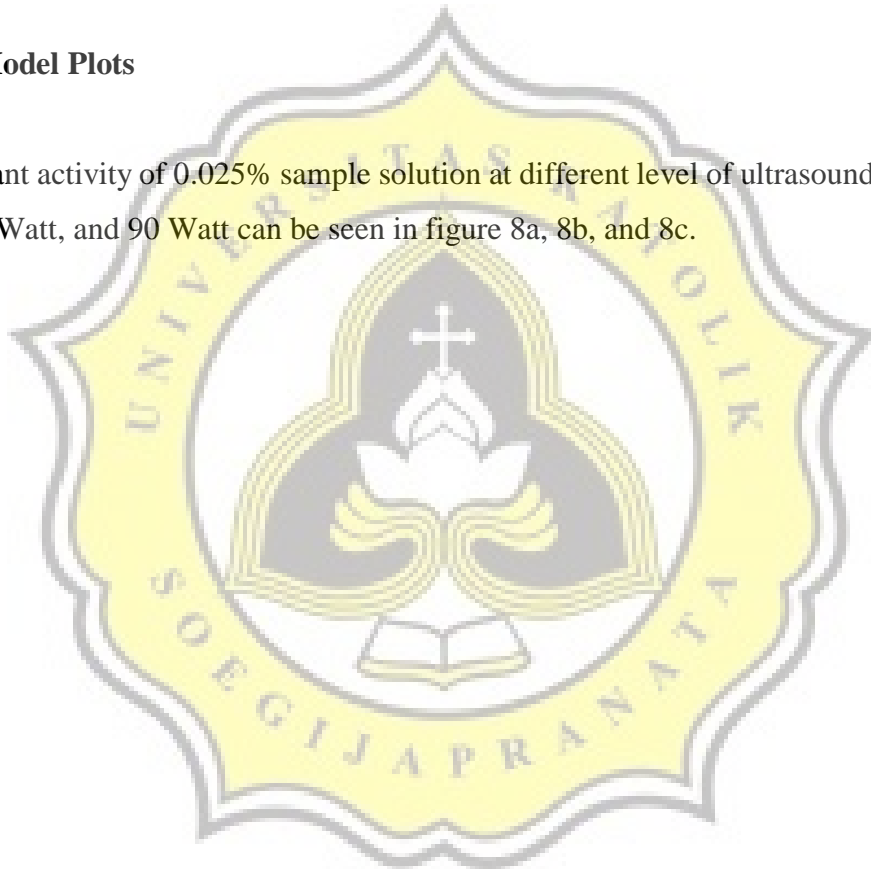
X₁= Time

X₂= Temperature

X₃= Power

3.2.2. Model Plots

Antioxidant activity of 0.025% sample solution at different level of ultrasound power: 50 Watt, 70 Watt, and 90 Watt can be seen in figure 8a, 8b, and 8c.



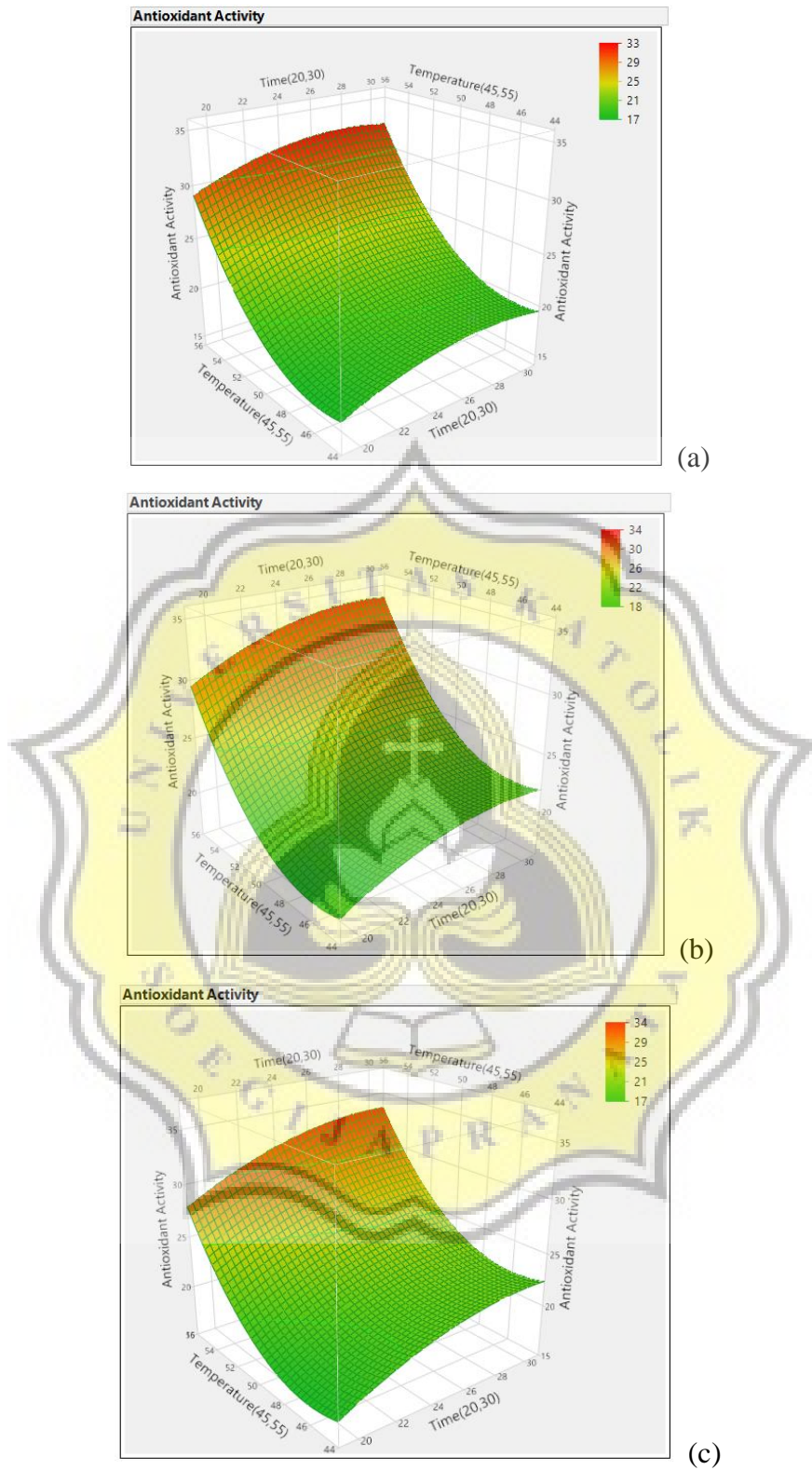


Figure 8. Surface plot of % Inhibition against extraction time and temperature at ultrasound power of 50 Watt (a), 70 Watt (b), 90 Watt (c)

The surface plot above shows the predicted value of antioxidant activity over a surface area. The antioxidant activity (expressed as % Inhibition) was plotted against time at range 19-31 minutes and temperature at range 44°C-56°C. Figure 8a shows the surface plot at power level of 50 Watt and the lower bound of predicted antioxidant activity was shown to be 17% and the upper bound was 3%. Figure 8b shows the surface plot at power level 70 Watt and the lower bound of predicted antioxidant activity was shown to be 18% while the upper bound was shown to be 34%. Figure 8c shows the surface plot at power level 90 Watt and the lower bound of antioxidant activity was shown to be 17% while the upper bound was shown to be 34%.

3.3. Prediction Profiler

The prediction profiler can be used to find the most desired value of the dependent variables (total phenolic content and antioxidant activity) based on the objective of the experiment. Figure 9 shows the prediction profiler when it is set to maximizing response.

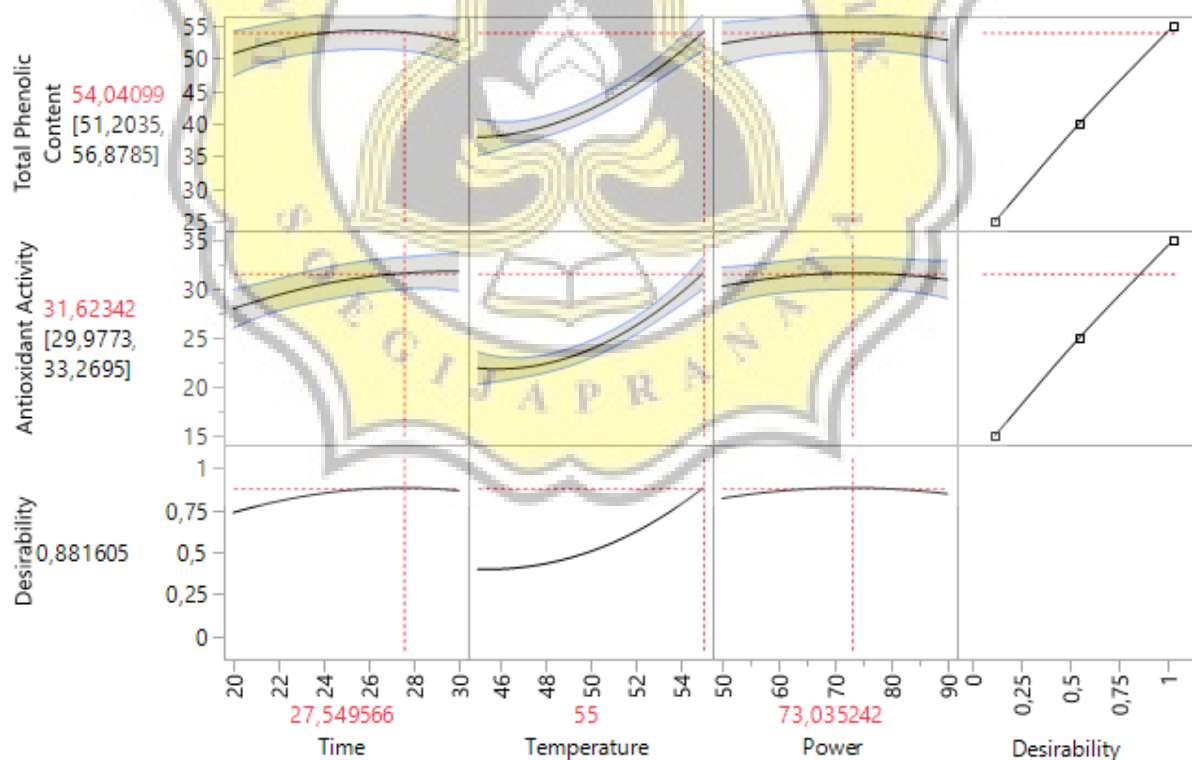


Figure 9. Prediction profiler of three extraction factors on total phenolic content and antioxidant activity

Figure 9 shows the predicted response value when it was set to maximize the value of responses. It can be seen that to achieve maximum desirability of total phenolic content and antioxidant activity, the extraction duration need to be set at 27.55 minutes, the sonication power to be set at 73.04 Watt, and the temperature needs to be set at 55 °C. This set of condition has a desirability value of 0.881605 as shown by the bottom row figure. Since the value is close to 1, this set of conditions fits the purpose of this study which is to maximize the responses: total phenolic content and antioxidant activity.

