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Optimization of Ultrasound Assisted Extraction (UAE) Conditions on Vitamin C from Roselle Flower

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Abstract— Roselle plant (Hibiscus sabdariffa L.) has many benefits. Vitamin C is an antioxidant in which the human body required. It contains a high amount of vitamin C. However, vitamin C is susceptible to high temperatures. Proper extraction treatment conditions produce optimum vitamin C. This study aims to determine the optimization of vitamin C in rosella flowers. This research uses UAE with Qsonica type – Q500 (500 W, 20 kHz). Extraction treatment conditions control amplitude, time and amount of solvent. The research method used is a laboratory experiment with the RSM (Response Surface Methodology) type CCD (Central Composite Design) approach. The research method was an experimental laboratory using a CCD (Central Composite Design) via RSM (Response Surface Methodology) with an amplitude level from 20% to 50%, a time level from 10 minutes to 20 minutes, and a ratio of the amount of solvent from 140 mL to 260 mL by weight. The results showed that the length of time and the amount of solvent had a significant effect, while the amplitude had no significant effect. The significant quadratic model at p value 0.05 and R2 0.9221 with optimum UAE conditions in the treatment combination of 50 percent amplitude, 20 minutes of time, and 140 ml of solvent resulted in an optimum vitamin C content of 38.76 mg/100g. This research can be used as a reference to determine the value of vitamin C with different amplitude, time, and amount of solvent from the research data.

Keywords— Optimization; roselle flower; RSM; UAE; vitamin C.

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I. INTRODUCTION

Indonesia is various kinds of biodiversity. The roselle plant *(Hibiscus sabdariffa L.)* is one example of biodiversity that can be utilized. According to [1] and [2], the roselle plant has numerous advantages. The Roselle plant's fruit, flower petals, and leaves can be eaten. The plant roselle part of the most used is the flower petal. Flower petals contain flavonoids formed from anthocyanins, known as antioxidants [3-6]. Other nutrients present in roselle include *calcium, niacin, riboflavin*, and *iron,* all of which are abundant. Protein, crude fiber, sodium, vitamin C, and vitamin A are also contained in red roselle petals [7]. Compared to fruits like oranges, apples, papaya, and guava, roselle has a high vitamin A and C content. Vitamin C's content increases the human body's resistance to disease attacks [8],[9]. Vitamin C is an antioxidant needed to maintain collagen [10]. Collagen can reduce skin wrinkles and maintain immunity from infection and allergies. These anthocyanins and vitamin C are essential in warding off free radicals that can cause degenerative diseases [11]. Vitamin C, or ascorbic acid, is an organic compound that the body cannot synthesize. Vitamin C is derived from food to meet the human body's needs. Vitamin C could be used as a supplement in medications, foods, and functional beverages [1],[2],[8].

The extraction process can obtain vitamin C in rosella flower petals. The extraction is carried out at a low temperature. Due to the essence of vitamin C, which is readily oxidized at high temperatures, low temperatures are used [12-14]. Maceration is a popular method of extraction. The maceration process takes a long time and could be more efficient regarding solvent consumption. The research was carried out using *ultrasound-assisted extraction (UAE)*. The UAE is extracted with the help of ultrasonic waves [15]. Ultrasound technology is growing in various scientific fields [16-19]. The extraction process in the UAE does not cause high temperatures and is efficient in mass transfer, energy, efficient use of solvents, and high productivity in a fast time [20]. The performance of ultrasonic extraction with a particular amplitude can cause cavitation on both the plant cell wall and membrane [21]. This effect impacts better solvent penetration to the cell membrane, thereby increasing the mass transfer rate in the tissue and the transfer of active compounds from the cell to the solvent. The magnitude of the amplitude value in the UAE depends on the shape and the difference in probe diameter between the driven and transmitter parts. According to [22-24], factors that affect the ability of ultrasonic to cause cavitation effects in the extraction process include frequency, solvent viscosity, temperature, surface pressure and vapor pressure, amplitude intensity, and time. Particle size is a parameter that affects extraction [25]. Meanwhile, time and amplitude are essential factors in the extraction of the UAE [19]. Research conducted by [26] and [27] states that extraction time affects the results. Extraction time and the amount of solvent used affect the anthocyanin results [28], [29]. According to the literature, optimizing the UAE extraction conditions is important to extract the maximum amount of vitamin C from roselle petals. The RSM approach is one of the design approaches that can be used to assess the optimal conditions of a sample.

RSM is a collection of mathematical and statistical techniques used in modeling and analyzing problems in responses that are influenced by several variables and aims to optimize these responses [30]. RSM is an effective method for optimization with interactions between variables that affect the desired results. RSM describes the most favorable conditions of a study [31]. The advantage of using RSM is the lack of combination units, replication, and time required [32]. Based on research conducted [33], it is stated that optimization with various treatment combinations with RSM can produce the optimum conditions. The optimization process was determined by the response goals to be met, which can be either maximization or minimization. The study aims to achieve optimum vitamin C levels. Thus, the optimization carried out for the response is maximization. The research objective was to determine the optimum UEA condition using a combination of three variables: amplitude, time, and the amount of solvent in the solution.

II. Materials and Methods

A. Materials

The research material used as a sample was 60 mesh of dried red roselle flower petal powder (Hibiscus sabdariffa Linn) obtained from Malang, Lowokwaru, East Java. Additional ingredients were 96% food-grade ethanol as a solvent, distilled water, starch and I2, and materials used to analyze vitamin C levels.

B. Equipment

Equipment used a glass beaker (Pyrex), a biuret (Pyrex 50 ml), a cup, a glass funnel (Herma), a desiccator, an Erlenmeyer (Herma), a measuring cup (Herma), pH paper, filter paper (Whatman 42), and an oven. (Memmert), pipette (Herma), pycnometer (Pyrex 1ml), Rotary Vacuum Evaporator (Heidolph p/n 562–01300–00), infrared thermometer (GM300), analytical balance (Adventure–pro, accuracy 0.0001 g), technical scale (Boeco r–300, accuracy 0.01 g), and Ultrasonic processor (Qsonica–Q500, 500 W, 20kHz). Ultrasonic processor with a power rating specification of 500 watts; frequency 20 KHz, voltage 110V, 50/60Hz.

C. Methods

The research method used is a laboratory experimental method using the RSM type CCD. *The* number of treatments (running) research is determined by the data processing software application Design Expert 11. This design combined three independent variables: amplitude, time, and the amount of solvent in the solution. The dependent variable or response is the content of vitamin C. Each variable has a maximum value (-) and a maximum limit (+). Based on the CCD-type RSM method, the experimental design of each variable in this study is shown in Table 1.

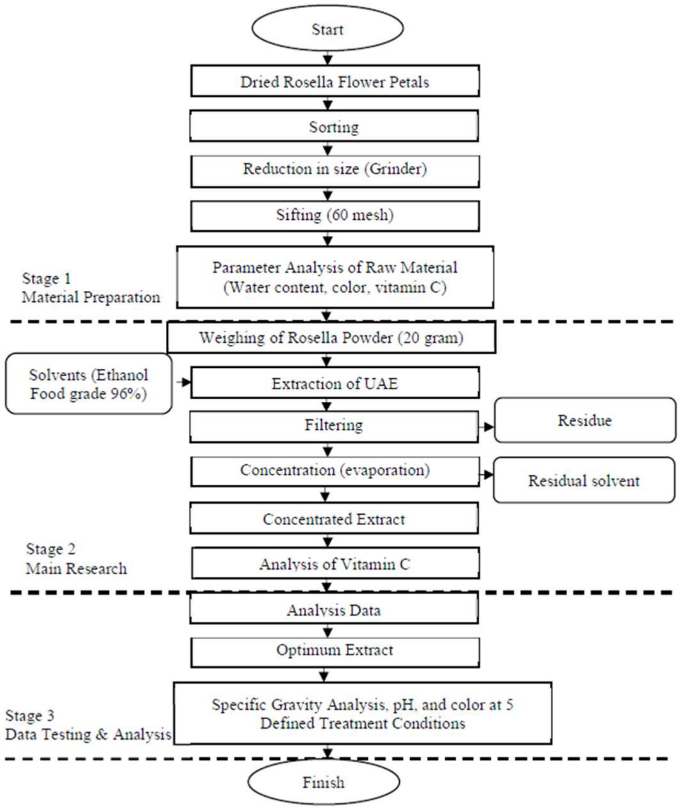

Fig. 1 Research Process Diagram

TABLE I
LEVEL AND CODE OF INDEPENDENT VARIABLES

Independent Variable	**–α**	**-1**	**0**	**1**	**+α**
Amplitude (%)	20	20	35	50	60
Time (minute)	6.6	10	15	20	23.4
Solvent Amount (mL)	99.1	140	200	260	300.9

Based on the number of factors and the CCD type level with Design Expert 11 experimental designs in the study, there were 20 treatments (run) consisting of 6 replications of the central point, 8 maximum and variable minimum limits (factorial point), and 6 treatments above. The maximum point and below the variable minimum point (axial point).

After getting the number of treatment combinations (run), then extraction was carried out at each run using 20g of roselle flower petals using 96% food grade ethanol solvent based on a combination of variables, the filtering process of the extraction results, the filtrate concentration process (solvent reduction), the analysis of vitamin C extract, Analysis of models and validation of optimization results as well as analysis of supporting parameters such as testing for specific gravity, color and pH were carried out at 5 extraction treatment conditions, namely the optimum extraction conditions which produced extracts with maximum vitamin C content, and treatment conditions that produced extracts with vitamin C levels. the highest, the treatment conditions that produced extracts with moderate vitamin C levels, the treatment conditions that produced the extracts with the lowest vitamin C levels, and the extracts with control treatment (maceration). The research process diagram is presented in Fig. 1.

II. Results and Discussion

A. Characteristic Analysis of Raw Material

Analysis of the characteristics of raw materials includes analysis of the water content, levels of vitamin C, and color [34-36]. Water content was determined using the thermogravimetric method. The measured water content obtained from the Malang area was 11.09 ± 0.73%. According to [37], simplicial was below 10% (moisture dry basis). Color testing of dried roselle petals using Color Flex. Color testing yields values for L, a *, b *, C, and Hue. The color test results are seen in Table 2.

TABLE II
The Color Test Results

Raw Material	Color Parameter					Color
	L*	a*	b*	C	H	
Dried Roselle Flower Petals	45.62	24.34	10.97	26.69	24.20	*Red*

Based on these results, it can be said that the raw material for roselle flower petals is yellow. In addition to the L *, a *, and b * values, the Chroma and Hue values are also obtained [38]. Chroma is the degree of color intensity that shows the purity of a color. The higher the C value obtained, the lower the color intensity will be. The Hue value is a value that represents the dominant wavelength. Hue value is the value obtained from the a * and b * values. The Hue value obtained is then adjusted to the chromaticity color range area. Based on the data obtained, the Hue value of the raw material for roselle flower petals is 24.20. Based on these results, it can be said that the raw material for roselle flower petals in this study is included in the chromaticity red range. Testing of vitamin C as raw material for dried roselle petals aims to analyze changes in vitamin C levels before and after extraction. Testing for vitamin C using iodometric titration (3 gr of powder roselle petals in Duplo). The analysis results of vitamin C dried roselle flower petals were 74.5 ± 1.69 mg/100g.

B. UAE Conditions

Ultrasonic-assisted extraction uses high-frequency sound waves. The research frequency control is 20 kHz. Extraction of dried roselle flower petal powder each 20 gr, then dissolved in food-grade ethanol solvent according to treatment. The ultrasonic waves are streamed through the ultrasound processor probe. Each process will experience a different temperature increase, depending on the treatment combination between the amplitude, time, and the amount of solvent used. Based on the results obtained, the greater the extraction time used, the higher the increase in temperature. This can be seen in Figure 2, which shows the temperature increase during the extraction process.

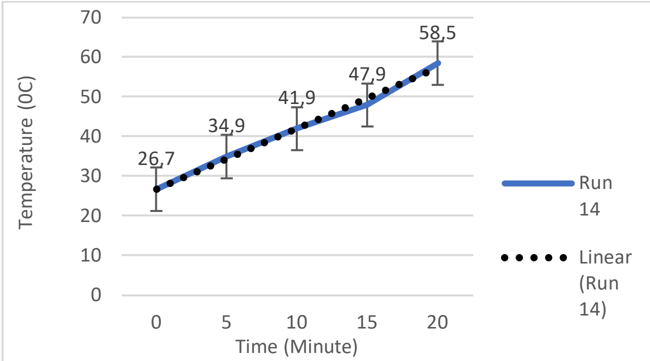

Fig. 2 Temperature –Time Profiles in Run 14

The increase in extraction temperature is also influenced by the magnitude of the amplitude used, the higher the % amplitude, the higher the temperature increase. The increase in temperature can occur due to the greater energy used as the % amplitude increases. Apart from being influenced by amplitude and time, the increase in extraction temperature is also influenced by the amount of solvent, solvent causes a rise in temperature.

C. Optimization Analysis of Extraction Conditions: An Analysis of Treatment and Response Optimization

The results of vitamin C observations for each treatment combination are presented in Table 3.

TABLE III
Results of Vitamin C for Each Combination

Run	Amplitude (%)	Time (Minute)	Solvent (mL)	Vitamin C (mg/100g)
1	50	20	260	29.33
2	35	15	200	24.92
3	60	15	200	39.59
4	35	15	200	27.12
5	35	15	300.9	19.06
6	35	15	200	21.27
7	35	15	99.1	35.93
8	35	23.4	200	31.53
9	50	10	140	26.40
10	35	6.6	200	21.26
11	20	10	140	34.46
12	20	20	260	24.93
13	35	15	200	24.93
14	50	20	140	37.38
15	20	10	260	24.19
16	35	15	200	28.60
17	50	10	260	21.26
18	35	15	200	24.93
19	20	20	140	35.93
20	20	15	200	31.53

Table 3 shows that the resulting vitamin C has the highest value of 37.38 mg/100 gr compared to vitamin C, which is the raw material of 74.05 mg/100g. The small amount of vitamin C extract is suspected that there was a decrease in vitamin C during the extract-making stage. According to [12], contact with air, heat, and metals will easily damage vitamin C so levels will decrease. Moreover, [39] stated that the effect of extraction on the levels of vitamin C roselle syrup, vitamin C in roselle syrup extracted without heating, was higher than the vitamin C content of roselle syrup extracted by heating.

1) *Analysis of Variance (ANOVA)*

The influence of variables and the response of vitamin C to roselle petal extract on the quadratic ANOVA model is shown in Table 4.

TABLE IV
ANOVA FOR QUADRATIC MODEL

Source	*Sum of Squares*	Df	*Mean Squares*	*F-value*	*p-value*	
Model	633.71	9	70.41	13.16	0,0002	*Significant*
A-amplitude	1.25	1	1.25	0.2335	0.6393	
B-time	108.57	1	108.57	20.29	0.0011	
C-Solvent	288.89	1	288.89	53.99	< 0.0001	
AB	35.43	1	35.43	6.62	0.0277	
AC	8.16	1	8.16	1.53	0.2450	
BC	1.66	1	1.66	0.3104	0.5897	
A^2	180.19	1	180.19	33.68	0.0002	
B^2	0.2579	1	0.2579	0.0482	0.8306	
C^2	0.9609	1	0.9609	0.1796	0.6807	
Residual	53.50	10	5.35			
Lack of Fit	22.63	5	4.53	0.7329	0.6293	*Not significant*
Pure Error	30.87	5	6.17			
Cor Total	687.21	19				
R^2	0.9221					
Adjusted R^2	0.8521					
Predicted R^2	0.6570					

Based on Table 4, the quadratic model has a greater R^2 value than the other models, which is 0.9221, with an adjusted R^2 value of 0.8521 and a predicted R^2 of 0.6570. The value of R^2 shows the magnitude of the combination of the independent variables that affect the response value. The R^2 value is close to number one, so the model is getting better. The adjusted R^2 and predicted R^2 values have a difference of 0.1951. The difference between adjusted R^2 and predicted R^2 $\leqslant$ 0.2 has a good value. Based on the difference value obtained, it can be said that the model obtained can model the data well. Based on ANOVA, the model has a p-value $\leqslant 0.05$, equal to 0.0002. The p-value $\leqslant 0.05$ indicates that the model is obtained significantly. The significance of the model is by the 95% confidence level, which means it is significant if the value is below 0.05 ($\alpha = 5\%$).

The ANOVA results showed that the variables that had a significant effect on vitamin C were time (B), the amount of solvent (C), the interaction between amplitude and time (AB), and the quadratic of the amplitude (A^2), the value of each variable was $p \leqslant 0.05$. Conversely, the variables that did not significantly affect vitamin C consisted of amplitude (A), the interaction between amplitude and amount of solvent (AC), the interaction between time and the amount of solvent (BC), the quadratic of time (B^2), and the quadratic of the amount of solvent (C^2)), the value of each variable is $p \geqslant 0.05$. The lack of fit value shows that it is insignificant, $p \geqslant 0.05$, which is 0.6293. The insignificant lack of fit indicates the suitability of the resulting model. The lack of fitness, which is insignificant, is the condition that a model is considered good. The mathematical model of the CCD-type RSM experimental design was as follows:

$$Y = 77.37346 - 2.06905A + 0.045935B - 0.121733C + 0.028060AB + 0.001122AC - 0.001519BC + 0.020012A^2 - 0.005338B^2 + 0.000071C^2$$

Information:
Y = Vitamin C response
A = Amplitude
B = Time
C = Amount of Solvent

The equation can be used as a reference to determine the value of vitamin C if you use the independent variable amplitude, time, and the amount of solvent that is different from the research data. Mathematical model equations show that vitamin C increases with time, the interaction between amplitude and time, the interaction between amplitude and amount of solvent, quadratic amplitude, and quadratic amount of solvent. A positive constant value indicates this.

The increase in vitamin C with increasing time is thought to increase the time the solvent contacts the ultrasonic waves used to extract more vitamin C. This is due to the research of [28], which stated that the longer the extraction time with the UAE, the higher the anthocyanin levels. Furthermore, vitamin C will increase with increasing amplitude and time. It is assumed that the magnitude of the amplitude can have a good cavitation effect on the sample. According to [19], large amplitudes can cause a good cavitation effect on plant cell walls and membranes. The effect of cavitation impacts better solvent penetration, thereby increasing the mass transfer rate in the tissue and the transfer of active compounds from the cell to the solvent [21]. According to [18], [26], [27], [29], time is one of the most critical factors in the UAE extraction process because it affects the large number of bioactive components extracted. The effect of the interaction between amplitude and time variables on the response to vitamin C can also be seen in Fig. 3.

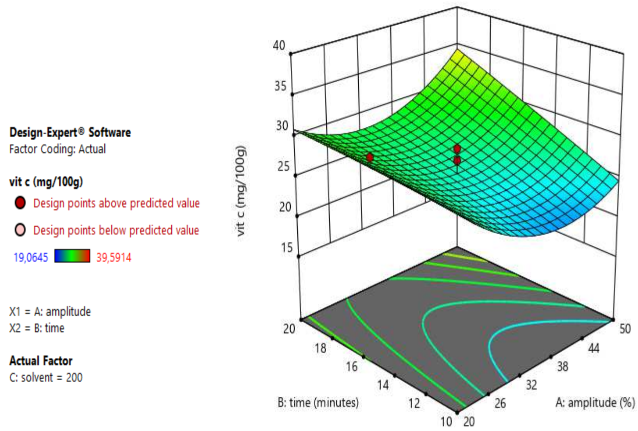

Fig. 3 Interaction of Time and Amplitude on Vitamin C

Fig. 3 represents the time relation to vitamin C increases in a straight line. The amplitude of vitamin C is like an inverted parabolic line, at the midpoint, vitamin C decreases. On the other hand, the interaction between the amplitude and time of vitamin C is like a parabolic line, at the midpoint, vitamin C increases. The longer the time, the vitamin C produced will increase. This shows that the quadratic of time is significant for vitamin C.

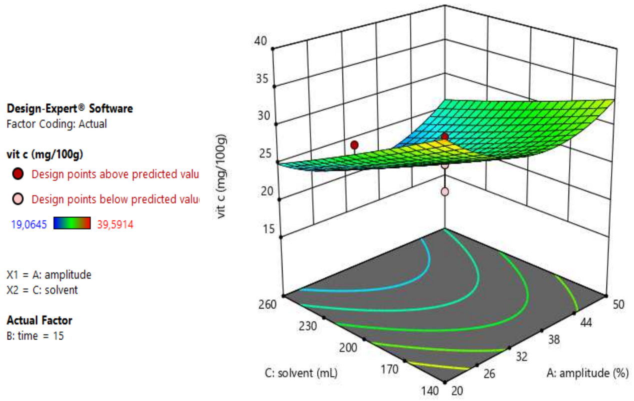

Fig. 4 Interaction of Amount of Solvent and Amplitude to Vitamin C

Fig. 4 showed the amount of solvent to vitamin C increasing as a straight line up. The amplitude for vitamin C and the interaction between the amount of solvent and the amplitude for vitamin C such as a reverse parabolic line, at the midpoint of vitamin C decreased. The inverted parabola shape gives insignificant relation. According to [38], UAE is an extraction that effectively reduces the amount of solvent required. The amplitude in the UAE can increase the temperature to degrade vitamin C [12],[29], vitamin C will be easily damaged by heat contact so that it decreases. However, vitamin C will increase as the amplitude of use increases. This indicates that the amplitude is insignificant for vitamin C. Whereas, if the amplitude is squared, the amplitude is significant for vitamin C. The decrease in vitamin C can be caused by the increase in the quadratic of the time variable, if the time used is squared, the longer the solution is exposed to heat, causing a decrease in vitamin C. decreased vitamin C.

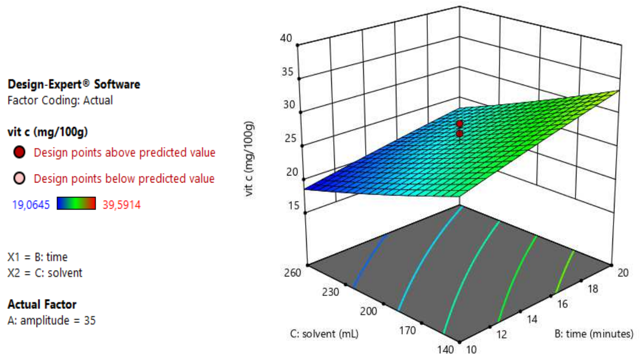

Fig. 5 Interaction of Amount of Solvent and Time on Vitamin C

Fig. 5 The greater the amount of solvent, which causes a decrease in vitamin C. The amount of solvent causes the distribution of vitamin C to be wider so that the resulting vitamin C is low. This can be seen in Fig. 4 and 5 which show a decrease in vitamin C as the number of solvents increases. Based on these results, the amount of solvent is significant to vitamin C. In addition, the increased interaction between time and the amount of solvent can reduce vitamin C, where the longer the time and the more the amount of solvent used, the lower the vitamin C will decrease. This can happen because, for a long time, the resulting temperature will increase, and with a high amount of solvent, the distribution of vitamin C in the solution is wider so that the resulting vitamin C is low. However, when seen in Figures 3 and 5, vitamin C will increase as the extraction time is used. The interaction between the time variable and the amount of solvent does not have a significant effect on the response to vitamin C.

2) Optimization results and model validation

Based on the optimization results, the RSM provides several solutions. The solution chosen is the solution with the highest desirability value. The solution for the optimum conditions chosen is a solution with a 50% amplitude treatment, 20 minutes time, and the amount of solvent 140 mL with a desirability value of 0.959. The solution of this optimum condition shows that the extraction treatment with an amplitude of 50%, time of 20 minutes, and the amount of solvent 140 mL produces roselle petal extract which has characteristics that match the optimization target of 95.9%, with a prediction of vitamin C levels of 38.76 mg. / 100g. The results of optimization and desirability (optimization targets) can be described in the form of a contour which can be seen in Fig. 6 and Fig. 7

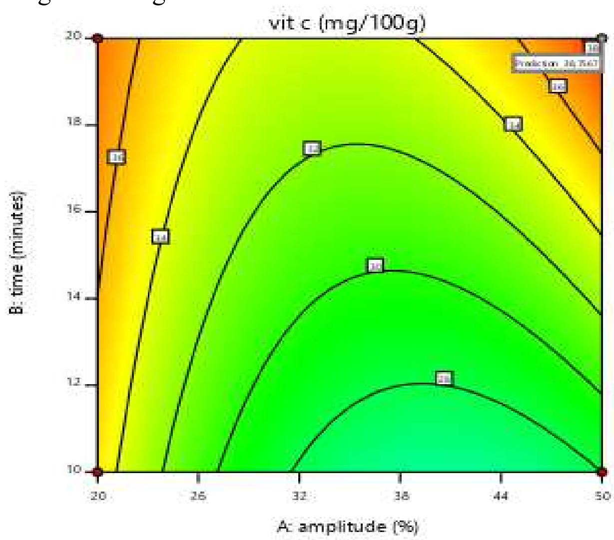

Fig. 6 Contour graph of optimum condition

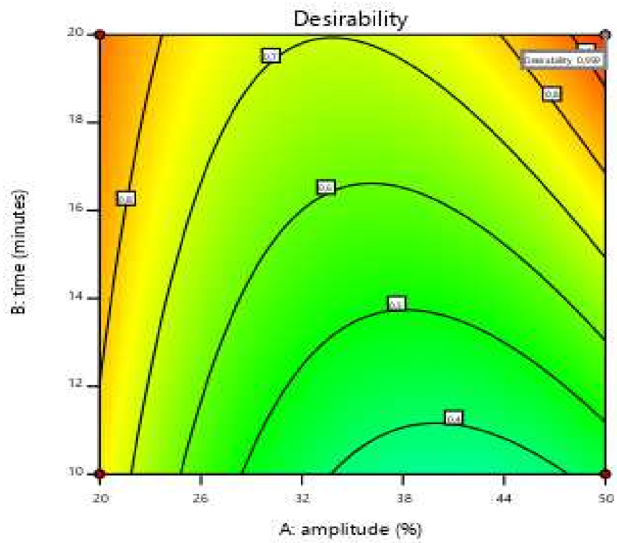

Fig. 7 Contour graph of desirability value of optimum condition

Furthermore, model validation was carried out to determine the accuracy of the mathematical model obtained. Model validation was comparing actual vitamin C levels with predicted vitamin C levels. The actual vitamin C was obtained from the research results. The results of the model validation obtained are in Table 5.

TABLE V
VALIDATION

Run	Amplitude (%)	Time (minute)	Amount of Solven (mL)	Vitamin C mg/100g)	Validation (%)
Predicted	50	20	140	38.76	96.44
Actual	50	20	140	37.38	

Based on the data in Table 5, it can be observed that there was a significant difference between the actual research vitamin C and the optimization prediction using the CCD type RSM. The accuracy of the validation results obtained from the CCD type RSM model was 96.44%. In other words, the CCD type RSM model obtained is good to be used as a reference for the extraction process using the UAE.

D. Characteristics of Roselle Flower Petal Extract

Testing the characteristics of the roselle petal extract was carried out on 5 treatment conditions, namely the treatment of the optimum extraction conditions which resulted in the maximum vitamin C levels (run 14), treatment with the highest vitamin C levels (run 3), treatment with moderate vitamin C levels (run 4), treatment with the lowest vitamin C levels (run 5), and control treatment (maceration).

1) Total Yield:

The total yield was the ratio between the mass of roselle petal extract produced and the mass of roselle flower petals as raw material. The total yield of roselle petal extract can be seen in Fig. 8.

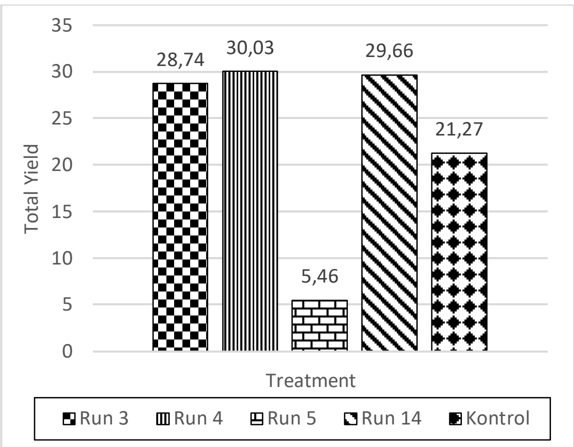

Fig. 8 Total Yield of Rosella Flower Petals Extract

Fig. 8, it can be seen that the total yield produced by each treatment is different. The highest total yield of 30.03% was produced by run 4 with an amplitude of 35%, time of 15 minutes, and solvent 200 mL. Meanwhile, the lowest total yield of 5.46% was produced by running 5 with 35% amplitude treatment, 15 minutes, and 300.9 mL of solvent. The total yield produced by run 3 with 60% amplitude treatment, 15 minutes, and 200 mL solvent was 28.74%. The total yield produced by run 14 (optimum) with 50% amplitude treatment, 20 minutes, and 140 mL solvent was 29.66%. In addition, the total yield produced by the control treatment was 21.27%. Based on the highest and lowest total yield data results, treatment with the same amplitude and time with different amounts of solvents produced different yields. According to [39], UAE proved to be a very effective extraction method to reduce the amount of solvent required. Based on these data, treatment with a smaller amount of solvent resulted in a greater total yield than a larger amount. When viewed from the results obtained in run 3 and run 4, treatment with the same time and amount of solvent with different amplitudes produces different yields, where the total yield decreases with increasing amplitude. This is presumably due to treatment with a large amplitude producing a smaller extract mass than treatment with a small amplitude, so the total yield is less. According to [22], increasing the sonication intensity (sonication intensity is proportional to the square of the amplitude) will increase the sonochemical process, but this is limited by the ultrasonic energy that enters the system.

Based on the data above, the total yield produced by run 14 (optimum) is greater when compared to the control treatment (maceration). The combination of amplitude, time, and amount of solvent treatment affects the total yield produced. From the resulting data compared to the control treatment, the use of UAE tends to produce a higher total yield. According to [19], ultrasonic extraction with a certain amplitude can cause a good cavitation effect on plant cell walls and membranes. This effect leads to better solvent penetration thereby increasing the mass transfer rate in the tissue and the transfer of active compounds from the cell to the solvent. Thus, the use of UAE causes the compounds in the roselle petals to be extracted more.

2) Specific gravity

Specific gravity is used to describe the extract's purity level. Specific gravity is the ratio of the mass of a substance to the mass of water at the same volume and temperature. The value of the specific gravity of the extract can be seen in Table 6.

TABLE VI
SPECIFIC GRAVITY OF ROSELLA FLOWER PETALS EXTRACT

Run	Treatment	Specific Gravity
3	Amplitude 60%, time15 minute, solvent 200 mL	0.93
4	Amplitude 35%, time 15-minute, solvent 200 mL	0.88
5	Amplitude 35%, time 15 minutes, solvent 300,9 mL	0.85
14	Amplitude 50%, time 20 minute, Solvent140 mL	0.94
Control	Hot maceration	0.91

Based on the data in Table 6, the weight of the extract obtained has a greater value than the density of ethanol, which was 0.798 g/mL. This can be caused by the compounds in the roselle petals being bound by the ethanol solvent used. The weight of the type of extract produced by each treatment is different. From the results obtained, the specific gravity of roselle petal extract <1. This result is smaller than the specific gravity of water, so it can be said that the physical form of the roselle petal extract is liquid and does not sink in water [29].

3) Color

Color testing is done with a tool called Color Flex. This tool is connected to the software so that the results obtained can be seen through the device. Color testing using this tool can generate values for L*, a*, b*, Chroma, and Hue. The results of the color appearance test of the extract can be seen in Table 7.

TABLE VII
COLOR TESTING DATA OF ROSELLA FLOWER PETALS EXTRACT

Run	Color Parameter					Chromaticity
	L*	a*	b*	C	H	
3	1.65	5.70	0.84	5.76	8.38	Red Purple
4	1.78	4.82	0.73	4.88	8.56	Red Purple
5	1.62	8.58	1.88	8.78	12.40	Red Purple
14	1.73	6.88	1.48	7.04	12.16	Red Purple
Control	0.71	3.60	0.69	3.66	10.94	Red Purple

Based on these results, it can be said that the extract obtained is red. The highest a* value was found in running 5 with an amplitude treatment of 35%, 15 minutes of time, and a solvent of 300.9 mL. The value of b* is a value that represents a mixture of blue and yellow. Moreover [37] stated, b* values from 0 to 70 represent yellow, while b * values from -70 to 0 represent blue. Based on the data in Table 10, the b * value in the roselle petal extract produced by each treatment was positive, ranging from 0.69 to 1.88. Based on these results, it can be said that the extract obtained is yellow. The highest b * value was found at running 5 with an amplitude treatment of 35%, time of 15 minutes, and a solvent of 300.9 mL. In addition to the L *, a *, and b * values, the Chroma and Hue values are also obtained. Chroma is the degree of intensity of a color that shows the purity of a color, whether it tends to be dominant (pure) or dirty (grayish). The higher the C value obtained, the lower the color intensity. Based on the data in Table 10, the C value of the rosella petal extracts produced for each treatment ranged from 3.66 to 8.78. The highest C value is found in running 5 with 35% amplitude treatment, 15 minutes, and 300.9 mL solvent. The Hue value is a value that represents the dominant wavelength. The Hue value is obtained from the a* and b* values. The Hue value obtained was then adjusted to the chromaticity color range. Based on the data obtained, the Hue value of the roselle petal extracts produced in each treatment ranged from 8.38 to 12.40. Based on these results, it can be said that the extract obtained in this study belongs to the chromaticity range of *red purple*.

4) pH Value

The pH test on the roselle petal extract was carried out to determine the acidity level of the extract obtained. Normal pH has a value of 7. Whereas if the pH value <7 indicates acidic properties and if the pH value> 7 indicates alkaline properties. The pH value of the roselle petal extract in the analyzed extraction treatment can be seen in Fig. 10.

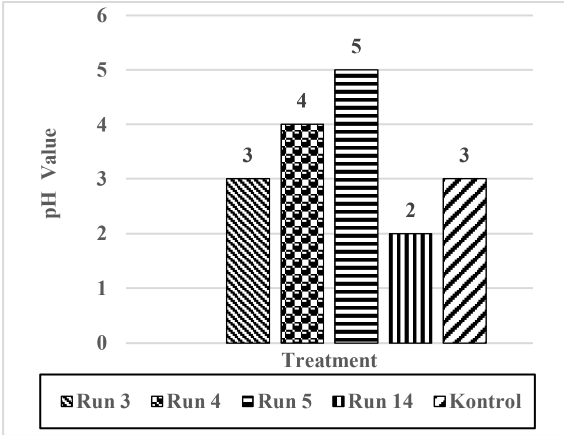

Fig. 9 pH value of Roselle Flower Petal Extracts

Based on the data in Fig. 9, that the resulting roselle petal extract has an acidity level ranging from 2 to 5. This indicates that the extract is in acidic condition. The highest pH value was produced by run 5 (35% amplitude, 15 minutes time, and 300.9 mL solvent) with the lowest vitamin C content. Meanwhile, the lowest pH value is generated by run 14 (50% amplitude, 20 minutes time, and 140 mL solvent), which is the optimum treatment to produce vitamin C. Based on these results indicate that the pH value will increase along with the decrease in vitamin C. related to ascorbic acid in roselle identifies the content of vitamin C. This is related to research conducted by [24], which states that the pH value in *Kelubi* fruit extract increases along with the decrease in vitamin C.

IV. CONCLUSIONS

The resulting model was significant with a quadratic form at p-value <0.05 and R2 0.9221, with the optimum UAE condition in the combination of 50% amplitude treatment, 20 minutes, and 140 ml of solvent, resulting in a maximum vitamin C level of 38,7567mg/100g based on the highest desirability value of 0.959. The accuracy of the validation results obtained from the CCD-type RSM model is 96.44%. In other words, the CCD-type RSM model obtained is good enough to be used as a reference for the extraction process using the UAE. The variables that significantly affect the response of vitamin C consist of time (B), the amount of solvent (C), the interaction between amplitude and time (AB), and the quadratic of the amplitude (A^2), while the variables that do not significantly affect the response of vitamin C consist of from the amplitude (A), the interaction between the amplitude and the amount of solvent (AC), the interaction between time and the amount of solvent (BC), the quadratic of the time (B^2), and the quadratic of the amount of solvent (C^2); and The mathematical models obtained from the RSM experimental design were:

$$Y = 77.37346 - 2.06905A + 0.045935B - 0.121733C + 0.028060AB + 0.001122AC - 0.001519BC + 0.020012A^2 - 0.005338B^2 + 0.000071C^2$$

This model could be applied as a reference to determine the value of vitamin C levels that can be generated if using the independent variable amplitude, time, and the amount of solvent that is different from the research data carried out.

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REFERENCES

[1] J. I. Mwasiagi and T. Phologolo, “Growing and Uses of Hibiscus sabdariffa L. (Roselle): A Literature Survey,” Roselle, pp. 43–52, 2021, doi: 10.1016/b978-0-323-85213-5.00014-7.

[2] B. B. Mohamed, “Roselle (Hibiscus sabdariffa L.) in Sudan: Production and Uses,” Roselle, pp. 121–127, 2021, doi: 10.1016/b978-0-323-85213-5.00018-4.

[3] P.-J. Tsai, J. McIntosh, P. Pearce, B. Camden, and B. R. Jordan, “Anthocyanin and antioxidant capacity in Roselle (Hibiscus Sabdariffa L.) extract,” *Food Research International*, vol. 35, no. 4, pp. 351–356, Jan. 2002, doi: 10.1016/s0963-9969(01)00129-6.

[4] H. Xiaowei et al., “Conventional and rapid methods for measurement of total bioactive components and antioxidant activity in Hibiscus sabdariffa,” Roselle (Hibiscus sabdariffa), pp. 199–214, 2021, doi:10.1016/b978-0-12-822100-6.00011-2.

[5] H. Z. Abeda et al., “Production and enhancement of anthocyanin in callus line of Roselle (Hibiscus sabdariffa L.),” *Int. J. of Recent Bio.Tech*., vol. 2, no.1, pp. 45–56, 2014.

[6] I. Borrás-Linares et al., “Characterization of phenolic compounds, anthocyanidin, antioxidant and antimicrobial activity of 25 varieties of Mexican Roselle (Hibiscus sabdariffa),” *Industrial Crops and Products*, vol. 69, pp. 385–394, Jul. 2015, doi:10.1016/j.indcrop.2015.02.053.

[7] A. A. Mariod, H. E. Tahir, and G. K. Mahunu, “Composition of Hibiscus sabdariffa calyx, pigments, vitamins,” Roselle (Hibiscus sabdariffa), pp. 69–75, 2021, doi: 10.1016/b978-0-12-822100-6.00002-1.

[8] T. S. Jamini and A. K. M. A. Islam, “Roselle (Hibiscus sabdariffa L.): Nutraceutical and Pharmaceutical Significance,” Roselle, pp. 103–119, 2021, doi: 10.1016/b978-0-323-85213-5.00001-9.

[9] Lubis M, Siregar GA, Bangun H, Ilyas S. The effect of roselle flower petals extract (Hibiscus sabdariffa Linn.) on reducing inflammation in dextran sodium sulfateinduced colitis. Med Glas (Zenica). 2020 Aug 1;17(2):395-401. doi: 10.17392/1095-20.

[10] Sarima, R. I. Astuti, and A. Meryandini, “Modulation of Aging in Yeast Saccharomyces cerevisiae by Roselle Petal Extract (Hibiscus sabdariffa L.),” *American Journal of Biochemistry and Biotechnology*, vol. 15, no. 1, pp. 23–32, Jan. 2019, doi: 10.3844/ajbbsp.2019.23.32.

[11] J. A. Murillo Pulgarín, L. F. García Bermejo, and A. Carrasquero Durán, “A fast and simple FIA-chemiluminescence method for the evaluation of Roselle flowers as scavenger of the free radicals generated by UV irradiated antibiotics,” *Journal of Pharmaceutical and Biomedical Analysis*, vol. 164, pp. 630–635, Feb. 2019, doi: 10.1016/j.jpba.2018.11.004.

[12] H. Setyawati and M. Ali Mustofa, “Analisis Kadar Vitamin C Kelopak Rosella (Hibiscus sabdariffa L.) Muda dan Tua yang dikoleksi Dari Berbagai Ketinggian Tempat yang Berbeda,” *Biogenesis: Jurnal Ilmiah Biologi*, vol. 5, no. 2, pp. 99–103, Dec. 2017, doi: 10.24252/bio.v5i2.3945.

[13] T. C. Tham et al., “Impacts of different drying strategies on drying characteristics, the retention of bio-active ingredient and colour changes of dried Roselle,” *Chinese Journal of Chemical Engineering*, vol. 26, no. 2, pp. 303–316, Feb. 2018, doi: 10.1016/j.cjche.2017.05.011.

[14] J. P. Gweyi-Onyango, M. Osei-Kwarteng, and G. K. Mahunu, “Measurement and maintenance of Hibiscus sabdariffa quality,” Roselle (Hibiscus sabdariffa), pp. 47–67, 2021, doi: 10.1016/b978-0-12-822100-6.00008-2.

[15] M.S. Triyastuti and M. Djaeni, "Improvement of anthocyanin production process from rosella flower petals with ultrasound assisted extraction," *J. Eng*, vol. 40, no. 2, pp. 115-121, 2019.

[16] S. Majhi, “Applications of ultrasound in total synthesis of bioactive natural products: A promising green tool,” *Ultrasonics Sonochemistry*, vol. 77, p. 105665, Sep. 2021, doi: 10.1016/j.ultsonch.2021.105665.

[17] Q. Jiang, M. Zhang, and B. Xu, “Application of ultrasonic technology in postharvested fruits and vegetables storage: A review,” *Ultrasonics Sonochemistry*, vol. 69, p. 105261, Dec. 2020, doi: 10.1016/j.ultsonch.2020.105261.

[18] M. Sholihah, “Application of Ultrasonic Waves to Increase Extraction Yield and Antioxidant Effectiveness of Mangosteen Peels,” *J. of Agricul. Eng*., vol 5, no. 2, pp. 161-168, 2017.

[19] F. Chemat, Zill-e-Huma, and M. K. Khan, “Applications of ultrasound in food technology: Processing, preservation and extraction,” *Ultrasonics Sonochemistry*, vol. 18, no. 4, pp. 813–835, Jul. 2011, doi: 10.1016/j.ultsonch.2010.11.023.

[20] A. M. Idrovo Encalada et al., “High-power ultrasound pretreatment for efficient extraction of fractions enriched in pectins and antioxidants from discarded carrots (Daucus carota L.),” *Journal of Food Engineering*, vol. 256, pp. 28–36, Sep. 2019, doi: 10.1016/j.jfoodeng.2019.03.007.

[21] T. Yamamoto and S. V. Komarov, “Enhancement of oscillation amplitude of cavitation bubble due to acoustic wake effect in multibubble environment,” *Ultrasonics Sonochemistry*, vol. 78, p. 105734, Oct. 2021, doi: 10.1016/j.ultsonch.2021.105734.

[22] S.Wardiyati, "Utilization of ultrasonics in the field of chemistry," Tangerang: *Research and Dev. Center for Science and Tech. of BATAN Materials*, 2004.

[23] J. Pinela et al., “Optimization of heat- and ultrasound-assisted extraction of anthocyanins from Hibiscus sabdariffa calyces for natural food colorants,” *Food Chemistry*, vol. 275, pp. 309–321, Mar. 2019, doi: 10.1016/j.foodchem.2018.09.118.

[24] T. Guo, C. Wan, F. Huang, C. Wei, and X. Xiang, “Process optimization and characterization of arachidonic acid oil degumming using ultrasound-assisted enzymatic method,” *Ultrasonics Sonochemistry*, vol. 78, p. 105720, Oct. 2021, doi: 10.1016/j.ultsonch.2021.105720.

[25] S.I. Rahmawati, S. Hidayatulloh & M. Suprayatmi, “Phycocyanin extraction from spirulina plantesis as biopigment and antioxidant,” *J. Agri*., vol. 8, no.1, pp. 36-45, 2017.

[26] S.Rosalinda, et al., “Separation Optimization Using Different Speed and Time of Centrifuge in Garut Regency Local Varieties Corn Starch Extraction ”*Teknotan J*., 13 (1). 2019.

[27] Desniorita, N. Nazir, - Novelina, and K. Sayuti, “Sustainable Design of Biorefinery Processes on Cocoa Pod: Optimization of Pectin Extraction Process with Variations of pH, Temperature, and Time,” *International Journal on Advanced Science, Engineering and Information Technology*, vol. 9, no. 6, pp. 2104–2113, Dec. 2019, doi: 10.18517/ijaseit.9.6.10670.

[28] M. Djaeni, et al., "Extraction of anthocyanins from the petals of rosella (hibiscus sabdariffa l.) using ultrasonic assistance", overview of antioxidant activity,” *J. of Food Tech. App*., vol. 6, no. 3, 2017.

[29] S.Rosalinda, et all.,”Optimization of ultrasonication extraction conditions on vitamin C pomegranate (punica granatum L.) using surface response,” *J. Agricul. & Biosystem Eng. Scie.*, vol.9, no.2, pp.143-158, 2021

[30] D.C. Montgomery, “*Design and Analysis of Experiments,” 5th Edition,* John Wiley & Sons, Inc., 2001, New York.

[31] H.A. Oramahi, “Usage theory and applications RSM (Response Surface Methodology), “ Yogyakarta: Ardana Media, 2008

[32] A. Maksum, & S.M.P. Ike, “Optimization of microwaves assisted extraction of phenolic compounds from rosella (Hibiscus sabdariffa) Petals,” *J. Agrin*., vol. 21, no.2, 2017.

[33] J.-S. Yang, T.-H. Mu, and M.-M. Ma, “Optimization of ultrasound-microwave assisted acid extraction of pectin from potato pulp by response surface methodology and its characterization,” *Food Chemistry*, vol. 289, pp. 351–359, Aug. 2019, doi: 10.1016/j.foodchem.2019.03.027.

[34] A. A. Sundarraj, R. Thottiam Vasudevan, and G. Sriramulu, “Optimized extraction and characterization of pectin from jackfruit (Artocarpus integer) wastes using response surface methodology,” *International Journal of Biological Macromolecules*, vol. 106, pp. 698–703, Jan. 2018, doi: 10.1016/j.ijbiomac.2017.08.065.

[35] V. S. Astianto, M.Sri and I G. Ayu, “effect of type of solvent and drying temperature on characteristics of extracts in Kelubi (Eliodoxa Conferta), ”*J. of Agro-Indus. Eng. and Manag*., vol. 5, no. 3, pp. 35-44, 2017.

[36] Z. Raji, F. Khodaiyan, K. Rezaei, H. Kiani, and S. S. Hosseini, “Extraction optimization and physicochemical properties of pectin from melon peel,” *International Journal of Biological Macromolecules*, vol. 98, pp. 709–716, May 2017, doi: 10.1016/j.ijbiomac.2017.01.146.

[37] BPOM RI. Food and Drug Supervisory Agency of the Republic of Indonesia, "Regulation of the head of the food and drug administration of the Republic of Indonesia number 14 of 2014 concerning quality requirements for traditional medicine", Jakarta. 2014.

[38] Suyatma. “Hunter color diagram (literature review),” *Scien. Res. J. of Agricul. Tech.*, Bogor Agricultural University, 2009.

[39] U. Mukaromah, S.S. Hetty, and A. Siti, “Vitamin C levels, physical quality, ph and organoleptic quality of Rosella syrup based on extraction method, ” J. *of Food and Nutrition*, vol.1, no.1, 2010.

[40] J. Wang, Y.-M. Zhao, Y.-T. Tian, C.-L. Yan, and C.-Y. Guo, “Ultrasound-Assisted Extraction of Total Phenolic Compounds fromInula helenium,” *The Scientific World Journal*, vol. 2013, pp. 1–5, 2013, doi: 10.1155/2013/157527.