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FULL PAPER

Optimization of vitamin c extraction of roselle petals (*Hibiscus sabdariffa*) assisted with microwaves using response surface methodology

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Abstract

Vitamin C contained in roselle petals acts as an antioxidant that provides health benefits. In order to make it easier to use, vitamin C needs to be extracted out of the roselle petal cells in the form of extracted raw roselle juice. The commonly used conventional method has disadvantages that can damage heat-sensitive compounds such as vitamin C. An alternative extraction method used was microwave-assisted extraction. The success of this method in extracting vitamin C was influenced by a few factors, such as the ratio of the roselle petals-water, microwave power, and extraction time. The purpose of this study was to determine the optimum conditions for microwave-assisted extraction of vitamin C in roselle extract and to determine the physical and chemical characteristics of roselle extract produced in the optimum conditions. This study utilized response surface methodology (RSM) in a central composite design (CCD). The optimized factors included the roselle petals-water ratio (5 - 10%), microwave power (150 - 350 W), and extraction time (3 - 7 mins). The parameter response tested in this study was vitamin C. While the extract produced under optimum conditions was also characterized by the total anthocyanin content, total phenols, antioxidant activity, pH value and colour (L, a* and b*). The results showed that the recommended optimum conditions were a 5% roselle petals-water ratio, 250 W of microwave power, and 6.09 mins of extraction time. The verification results show that this optimum condition produced vitamin C higher than the conventional method. The characteristics of roselle extract produced by microwave-assisted extraction at optimum conditions were vitamin C at 9.973±0.508 mg/g, total anthocyanins at 1.771±0.124 mg/100 g, phenol at 30.848±1;899 m/g, IC_{50} at 6.699±0.627 ppm, pH at 2.133±0.057, colour with an L value of 31.033±0.404, a* value of 1.066±0.155, and b* value of 18.100±0.458.

1. Introduction

Ascorbic acid (Vitamin C) is one of the most important active substances which humans should consume in sufficient amounts together with food. Recent epidemiological studies have associated the natural antioxidant properties of vitamin C with possible health benefits in the prevention of the development of coronary heart disease and anticancer activities (Bai *et al.*, 2015). Recently, vitamin C has been widely recommended as an immune-boosting supplement during the COVID-19 pandemic.

In line with increasing public knowledge and awareness of health, natural antioxidants are increasingly in demand. One plant that has the potential to be used is roselle (*Hibiscus sabdariffa* L). Roselle does not just taste good but it also contains large amounts of vitamin C (Purbowati *et al.,* 2020). According to Babajide *et al.* (2004), roselle petals contain several vitamins and minerals such as vitamin C, calcium, niacin, riboflavin, and flavonoids which play a role in the body's cell

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rejuvenation process. The content of vitamin C in roselle petals per 100 grams is 260-280 mg. According to Purbowati *et al*. (2016), vitamin C, total phenol, anthocyanin, and yield of microwave-assisted extraction respectively were 10.74±0,14 mg/g, 23.77±0.25 mg/g, 14.80±0.08 mg/g, and 22.09±3.3%. Maksum and Purbowati (2017), reported that the extraction of roselle using the MAE method with an extraction time of 7 mins, a roselle weight of 10 g, and a power of 400 W produced 74.8 mg/100 mg of Vitamin C. The optimized extraction parameters were determined at a soaking time of 48 mins, the solid-solvent ratio of 44% and the extraction temperature of 55°C produced 7.8 mg/100 g L -ascorbic acid (Peter *et al.,* 2014).

It is known that vitamin C inhibits oxidation processes and can be used as an antioxidant. Purbowati *et al.* (2015) reported that the antioxidant activity in rosella flowers is not only caused by phenol compounds but is also determined by the amount of vitamin C. Therefore, technological processes used for extraction have an impact on the amount of vitamin C. Most of it was lost during the extraction process. Extracting vitamin C using conventional extraction methods was time-consuming and less efficient, and according to Hapsari *et al*. (2021), extraction of roselle by maceration method with sample-solvent ratio 1:12.5 took an extraction time of 72 hrs, while the methanol solvent yielded 27.3% of the extract, total phenolic compounds of 29.2 mg GAE/g, total flavonoid compounds of 36.7 mg QE/g, and DPPH scavenging activity of 78%. Whereas Kurtulbaş *et al.* (2020) reported that extraction by the MAE method with power 550 W, time 180 s, DES in 50% water solvent produced total phenolic compounds of 31.90 mg GAE/g, total anthocyanin compounds of 3.00 mg Cy-3-GE/g and DPPH scavenging activity of 95.89%. An alternative extraction method that can be done is the Microwave-Assisted Extraction (MAE) method.

MAE had several advantages, such as lower extraction temperature and shorter extraction time compared to the conventional one, thereby reducing the thermolabile compounds (Wei *et al*., 2009). Water is a suitable solvent for MAE because water has a dielectric coefficient of 78.3 throughout it can absorb extraction microwaves well (Markom, 2007).

Solvent volume, material weight, extraction time, and microwave power will interact to influence the yield and activity of bioactive compounds produced in the microwave-assisted extraction process (Mandal *et al*., 2007). The solvent-raw material ratio affects the ability of the solvent to dissolve the compound from the material until the solution reaches its saturation point. Microwave power and extraction time affect the amount of heat energy received by vitamin C. These three factors need to be known to optimize extraction conditions during microwave-assisted extraction. Aulia (2018), reported that the extraction of soursop leaves in total phenol and antioxidant response obtained the optimum point from the extraction time of 9 mins 84 s with a solvent ratio of 25.19 with the raw material in the form of dried soursop leaf powder as much as 25 g.

Based on the description above, the objectives to be achieved in this study were to determine the optimum conditions for microwave-assisted extraction to the response of vitamin C content in roselle extract, as well as to know the physical and chemical characteristics of roselle extract produced in optimum conditions.

2. Materials and methods

2.1 Equipment

The tools used to make roselle powder include cabinet dryers, grinders, 80 mesh sieves, and microwaves (Electrolux EMM 2007X) for extraction. While the tools for analysis include a UV-Vis spectrophotometer, centrifuge, colour reader, rotary evaporator, water bath, vortex, pH meter, Erlenmeyer flask, measuring flask, measuring cup, beaker glass, test tube, drop pipette, measuring pipette, analytical balance, paper, filter, funnel, plastic cup, metal spatula, aluminium foil.

2.2 Material

The materials used were roselle flowers, while the materials for analysis included NaCl, NaOH, HCl, Na_2CO_3, 95% ethanol, methanol, DPPH, Folin-Ciocalteu, starch, iodine, and distilled water.

2.3 Experimental design

This study used the Response Surface Method using the Central Composite Design (CCD). The factors observed were the roselle petals-water ratio (5, 10, 15% w/v) (Chumsri, *et al*., 2008), microwave power (150, 200, and 250 W) (Purbowati *et al*., 2015), and extraction time (3, 5, and 7 mins) (Purbowati *et al*., 2015).

2.4 Preparation of roselle powder

The preparation was conducted according to the method of Purbowati *et al.* (2019). The roselle petals were sorted and then dried in a cabinet dryer at a temperature of 50ºC for 6 hrs, after drying they were ground into powder form. It was then sieved with an 80 mesh sieve.

2.5 Optimization

Starting from the determination of the independent variable to determine the maximum-minimum value range which was then inputted into the Design-Expert program using the Central Composite Design. Twenty treatment combinations obtained were analysed for vitamin C. The program will determine the optimization based on the input response measurement data, thus providing recommendations for some optimal combinations according to the program. The most optimal response was chosen with the highest desirability value.

2.6 Extraction

Approximately 10 g of the dried roselle petals were ground for 1 min using a grinder and sieved in a 60 mesh sieve. The extraction was done in 100 mL distilled water, microwave power extraction 250 W for 5 mins. The microwave used for extraction was Electrolux EMM 2007X. The slurry was radiated in a microwave oven at regular intervals (one-min radiation and two mins off) to ensure the temperature did not rise above the boiling point (Purbowati *et al.*, 2016). Roselle extract was filtered and concentrated with a vacuum evaporator at 70°C, 44 cmHg and blowing with N_2 to ensure the solvent evaporate.

2.7 Vitamin C analysis

The optimum conditions of the extraction process are determined based on the vitamin C response as measured by (Jacob, 1999). Standardization of sodium thiosulfate solution, this standardization was done by pipetting 5.00 mL of KIO_3 solution + 10 mL of 10 % KI solution + 10 mL of dilute H_2SO_4 into a conical flask. The liberated I_2 was titrated against $NS_2S_2O_3$ solution from a burette until a faint yellow colour was reached. Then 10 drops of the starch solution were added and the titration was continued until the blue-black colour of the starch-triiodide complex just disappeared. The titration was repeated until concordant results were obtained. The normality of the sodium thiosulfate solution was calculated. For the titration of the standard solution of ascorbic acid, 5.00 mL of the sample solution was pipetted and the steps for the standardization of sodium thiosulfate solution were repeated. The concentration of the ascorbic acid was calculated from the relation, Milliequivalent of sodium iodate-milliequivalent of sodium thiosulfate = milliequivalent of ascorbic acid. Titration of samples, 5.00 mL of the treated sample was pipetted into a conical flask and the steps were repeated as with standard ascorbic acid. The data resulting from these measurements were processed by the Design Expert to determine the optimum conditions.

2.8 Data analysis

The results of vitamin C data were analysed using RSM. The optimization design was carried out using CCD (Central Composite Design) and analysed using the Response Surface Methodology. The equation with a single expected response (Y) was:

$$Y = \beta 0 + \beta ixi + \beta iixi2 + \beta ij\ xi\ xj + €$$

Where 0 was the value of the expected response at the centre of the design being developed, namely (0, 0, 0). i, ii, and ij were linear, quadratic, and cross-product regression model constants, respectively.

2.9 Verification

The optimum conditions were obtained by running the program of Central Composite Design with the highest response to vitamin C. After the optimum condition was chosen, the verification experiments were conducted 3 times and each replication will be followed by physical and chemical characterization, colour (Soewarno, 1990), total anthocyanins (Fuleki and Francis, 1968), total phenol (Bray and Thorpe, 1954), IC_{50} (Sheikh *et al.*, 2009 with modification), pH (Suwetja, 2007). The verification results obtained were then compared with the response variable values predicted by RSM.

The results of the verification were carried out also by comparing the characteristics of the roselle extract between microwave-assisted extraction and extraction using conventional heating. This conventional one was carried out in the optimum conditions for microwave-assisted extraction on a water bath device.

3. Results and discussion

3.1 Optimization

The determination of the model to predict the relationship between treatment and response was based on the data in Table 1, which is the basis for determining model selection. The data from the optimization were tested in the Analysis of Variance (ANOVA) to find a suitable equation model that relates the solvent-solvent ratio, microwave power, and extraction time with the vitamin C content as the response in this study. ANOVA results from the extraction optimization process in these studies can be seen in Table 2.

Based on the results of the ANOVA test, the optimization results of roselle extraction with vitamin C response were obtained with a significant quadratic model because it had a p-value of <0.0001. These showed that the regression model can represent the independent variables in influencing vitamin C. Supported by the insignificant Lack of Fit value because it had a value of 0.5040 (p-value> 0.05) which means that there was a fit of the model with the response data of vitamin C The model used can be described by the

Table 1. Central Composite Design (CCD) of research factor

Run	Factor 1 A: roselle petals-water ratio (%)	Factor 2 B: power microwave (Watt)	Factor 3 C:time extraction (mins)	Response Vitamin C (mg/g)
1	10.00	250.00	5.00	7.04
2	15.00	150.00	7.00	4.106667
3	10.00	250.00	5.00	7.92
4	5.00	150.00	3.00	7.04
5	5,00	350.00	7.00	8.8
6	15.00	350.00	3.00	4.106667
7	15.00	150.00	3.00	4.693333
8	10.00	250.00	5.00	7.92
9	15.00	350.00	7.00	3.52
10	5.00	350.00	3.00	7.04
11	5.00	150.00	7.00	8.8
12	10.00	250.00	5.00	7.92
13	10.00	250.00	1.64	5.28
14	10.00	100.00	5.00	6.16
15	10.00	250.00	5.00	7.92
16	1.59	250.00	5.00	11.06918
17	10.00	250.00	8.36	6.16
18	18.49	250.00	5.00	5.235262
19	10.00	250.00	5.00	7.92
20	10.00	418.18	5.00	4.4

Table 2. Analysis of variance

Source	Sum of Squares	df	Mean Square	F-Value	p-value Prob > F	
Block	0.24	2	0.12			
Model	71.95	9	7.99	58.63	< 0.0001	significant
A-roselle petals-water ratio	46.00	1	46.00	337.39	< 0.0001	
B-microwave power	1.25	1	1.25	9.17	0.0163	
C-extraction time	1.07	1	1.07	7.86	0.023	
AB	0.17	1	0.17	1.26	0.2938	
AC	2.75	1	2.75	20.19	0.002	
BC	0.00	1	0.00	0.00	1.0000	
A^2	0.07	1	0.07	0.51	0.4965	
B^2	12.89	1	12.89	94.55	< 0.0001	
C^2	9.00	1	9.00	66.01	< 0.0001	
Residual	1.09	8	0.14			
Lack of Fit	0.70	5	0.14	1.09	0.504	not significant
Pure Error	0.39	3	0.13			
Cor Total	73.28	19				

equation $Y = 7.77 - 1.84A - 0.30B + 0.28C - 0.15AB - 0.59AC + 0.000BC + 0.069A^2 - 0.95B^2 - 0.79C^2$.

3.2 Effect of roselle petals-water ratio, microwave power and extraction time to the total of vitamin C in crude roselle extract

Based on Figure 1, it was known that microwave power has a significant effect on the total vitamin c of roselle crude extract with a significance value of 0.0163 less than 0.05. According to Kusuma and Mahfud (2015), the microwave will break the cell walls of the material. An increase in microwave power can cause damage to the cell wall more quickly and the solvent will easily diffuse into the material to dissolve the compound. The higher the extraction temperature, the wider the cell pores are, making it easier for the solvent to enter cell-matrix and extract the compound (Wuryantoro and Susanto, 2014). However, too high a temperature causes a compound with a certain degree of resistance to temperature to be decomposed into its components. According to Pakaya (2014)' this vitamin C was easily damaged by the oxidation process, especially when exposed to heat. The results of previous studies by Kadakal *et al.* (2018), ascorbic acid is very unstable at 95°C with a k value of 15×10^{-3}.

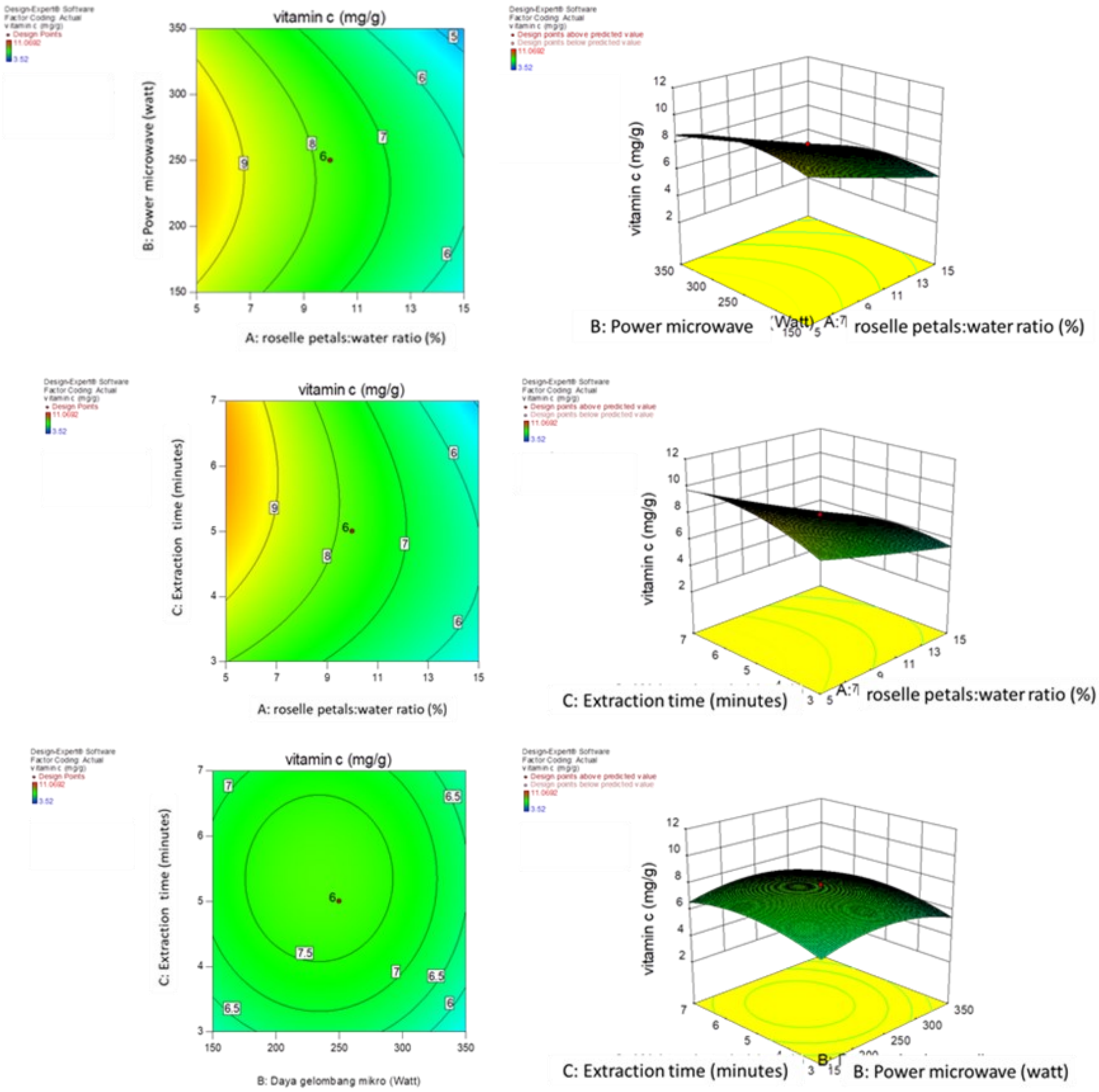

Figure 1. Surface response from the optimization between treatment and responses

Figure 1 also show that the roselle petal-water ratio has a significant effect on the total vitamin C of roselle crude extract with a significance value of 0.0001. The roselle petals-water ratio affected the amount of surface area the solvent could contact. According to Gertenbach (2002), the greater the roselle petals-water ratio would increase the concentration of dissolved solutes on the surface of the particles, thus increasing the concentration gradient inside and on the surface of the solid particles.

When the extraction time increases, the solvent has more opportunity to come into contact with the material, making the solvent diffused more into the pores of the material to dissolve vitamin C. The extracted material components would continue to increase until the solution became saturated. After passing the saturation point of the solution, there would be no increase in the extraction yield with the addition of solvent (Siregar, 2005). Figure 1 shows that extraction time also has a significant effect on the total vitamin C of roselle crude extract with a significance value of 0.0230. Winata (2015)’ agreed the longer the extraction time, the greater the amount of material extracted because the contact time between the material and the solvent is getting longer, and the results would increase to the saturation point of the solution.

The next step after getting the equation in modelling is to determine the level of importance of each optimization variable. This was done to get the desired response (desirability) in the setting of criteria, as in Table 3. Based on the Design Expert v.10 program/ application of the modelling used for optimization and the criteria for the level of importance, the program provides the optimum combination of conditions described by the response surface (Response Surface). Referring to the 3D contour in Figure 1, the optimum point was indicated by a red dot at the top of the saddle. The optimum conditions were obtained at a roselle petals -water ratio of 5%, microwave power of 250 W, and an extraction time of 6.09 mins with a desirability value of 0.920. The desirability value indicates that the optimum conditions for roselle extraction in accordance with the determination of the criteria of interest for optimization are 92.0%, which means that the optimum value was suitable because the desirability value was close to 1.

3.3 Verification

Table 3. Solution

No	Roselle petals-water ratio	Microwave power	Extraction time	vitamin C	StdErr (vitamin C)	Desirability	
1	5	250	6.099	9.916	0.178	0.92	Selected

Based on the results of verification at optimum conditions, it was known that the actual data falls within the Prediction Interval (PI) range. It was shown that the optimum condition was obtained by a combination of a roselle petals-water ratio of 5%, microwave power of 250 W, and an extraction time of 6.09 mins which will produce 9.91 mg/g of vitamin C. A previous study by Purbowati (2014), extracted roselle using ethanol as a solvent, and obtained optimum conditions at the extraction time of 4.91 mins, 78.36% ethanol and 250 W microwave power with vitamin C of 10.74±0.14 mg/g. The amount of vitamin C extracted (Wang *et al.* 2010) performed on mulberry leaf extraction with MAE, resulted at 255 W for 5 mins with a yield of 8.37 mg/g.

Table 4 shows that extraction using MAE had higher characteristics than conventional extraction. During MAE, heat transfer occurs from the material to the solvent in the MAE and was distributed volumetrically throughout the irradiated sample. In this phenomenon, the heat was created in the microwave absorbent agent and transferred to the extraction medium. The similarity of the two transport phenomena, which are heat and mass transfer, makes the MAE extraction rate higher than conventional solvent extraction (Taghvaei *et al.*, 2014). This could prove that microwave-assisted extraction could provide the advantage of faster mass transfer, leading to a shorter extraction time to obtain optimum results. According to Amir *et al.* (2016), the energy in the extraction process using the MAE method goes directly to the specific target sample reducing the heat that was lost to the environment. Also, the bioactive compounds in the material were easier to dissolve because the heating process takes place in the closed system.

Vitamin C produced from the extraction of the MAE method was higher than that of the conventional method, which was 9.973 mg/g. This is in line with the higher total anthocyanin content and lower pH in the MAE method. According to Harris and Karmas, (1989), the stability of ascorbic acid (vitamin C) will increase with decreasing pH value. In addition, Wong *et al.* (2002) also agreed that in acidic conditions or if the pH approaches pH 1, more anthocyanin pigments are in the form of flavour cations which are red and stable.

Table 4. Characteristics of roselle petals extract under optimum conditions with MAE and conventional

Characteristics	MAE	Conventional
Vitamin C (mg/g)	9.973±0.508	8.213±0.508
Total anthocyanin (mg/g)	1.771±0.124	0.901±0.184
Total Phenol (mg/ 100 g)	30.848±1.899	10.705±0.490
IC_{50} (ppm)	6.699±0.627	10.170±1.128
pH	2.133±0.057	2.466±0.057
Colour		
L	31.033±0.404	27.533±0.568
a*	1.066±0.155	0.667±0.115
b*	18.100±0.458	16.866±0.665

4. Conclusion

This research recommends that the optimum conditions for vitamin C extraction in roselle were obtained at a roselle petal-water ratio of 5%, microwave power of 250 W, and an extraction time of 6.09 mins. This optimum condition is expected to extract 9.91 mg/g of vitamin C. The verification results show that this optimum condition produces vitamin C higher than the conventional method. The characteristic of roselle extract produced by microwave-assisted extraction at optimum conditions, having vitamin C of 9.973±0.508 mg/g, total anthocyanins of 1.771±0.124 mg/100 g, phenol of 30.848±1.899 mg/g, IC_{50} of 6.699±0.627 ppm, pH of 2.133±0.057, colour with L* value of 31.033±0.404, a* value of 1.066±0.155, b* value of 18.100±0.458.

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