RESEARCH ARTICLE

Microwave assisted extraction of anthocyanin from purple sweet potato (Ipomoea batatas L.) using deep eutectic solvent (DES) based on citric acid

Rachma Tia Evitasari[1*], Hana Rofiqoh[1], Putri Alfina Damayanti[1], Firda Mahira Alfiata Chusna[1]

[1]Department of Chemical Engineering, Faculty of Industrial Technology, Universitas Ahmad Dahlan Kampus IV UAD, Jalan Jend. Ahmad Yani, Tamanan, Banguntapan, 55191 Bantul

OBJECTIVES Purple sweet potato (*Ipomoea batatas L.*) is one of the tubers or roots that is widely grown in Indonesia that contain high antioxidants. One type of flavonoid that functions as an antioxidant is a natural dye called anthocyanin. In the process of extraction of anthocyanin, the solvent has an important role. METHODS In this research, the deep eutectic solvent was used because it is more economical and environmentally friendly. This research will optimize the microwave assisted extraction of anthocyanin content in purple sweet potato (*Ipomoea batatas L.*) using a deep eutectic solvent with the variation of material to solvent ratio, microwave power, and extraction time. Optimization is done by using the statistical method Response Surface Method (RSM) with total anthocyanin content as a response. RESULTS The optimum operating conditions for the extraction of anthocyanins from purple sweet potato using the deep eutectic solvent were obtained at solid to solvent ratio of 1:29 at microwave power of 270 watts for 193 s, with an optimum TAC value of 311.64 mg/L and an antioxidant value of 44.85%. CONCLUSIONS The use of DES can increase the extraction yield and is environmentally friendly.

KEYWORDS anthocyanin; deep eutectic solvent; extraction; Ipomoea batatas; solvent

1. INTRODUCTION

Purple sweet potato is one of the tubers or roots that is widely grown in Indonesia with the characteristic of overall color being purple. Purple sweet potato (*Ipomoea batatas L.*) was first developed in Japan, this introduction has varieties with many advantages compared to local purple sweet potatoes from Gunung Kawi and Samarinda (Afandy et al. 2017; Gras et al. 2017).

Purple sweet potato or *Ipomoea batatas L.* is one of the plants that contain high antioxidants. There are many types of flavonoids contained in the purple sweet potato , which have benefits as antioxidants because of the micronutrients, which are phytochemical groups from various food ingredients derived from plants. One type of flavonoids that function as an antioxidant is a natural dye called anthocyanin. Purple sweet potato can be used as healthy processed food to reduce the risk that it can damage cells due to the presence of radical compounds (Gras et al. 2017; Liu et al. 2019; Montilla et al. 2010). The food industry is currently developing quite rapidly and can spur the use of synthetic dyes which, if consumed, will have a very dangerous impact and are not good for health. Examples of content in synthetic dyes are heavy metals such as tin, iron, and aluminum. The high risk of using synthetic colors in food has developed other alternatives using natural ingredients such as purple sweet potato.

Natural dyes in purple sweet potato are anthocyanins (Li et al. 2019). Anthocyanin is a natural dye that is widely distributed in plants such as flowers, tubers, vegetables, and fruits. Anthocyanin is a water-soluble pigment that can be found in leaves, flowers, fruits, stems, and roots. The types of anthocyanins that are commonly found in nature are cyanidin (Cy), delphinidin (Dp), petunidin (Pt), peonidin (Pn), pelargonidin (Pg) and melvidin (Mv). Indonesia is a country that has a tropical climate and abundant natural resources, one of which is the productivity of purple sweet potato which reaches 1.9 million tons/year. The anthocyanin content in dark purple sweet potato is 61.85 mg/100 g, higher than the anthocyanin content in light purple sweet potato, which is 3.51 mg/100 g. Concentrated purple sweet potato has an antioxidant activity of 59.25%, greater than 56.64% of light pur-

ple sweet potato (Yin et al. 2017).

Deep eutectic solvent (DES) or natural deep eutectic solvent (NADES) is a mixture of natural compounds, i.e. organic acids and bases, drying organisms upon entering an aging state for long-term survival, e.g. seeds, in resurrection plants and lichens, as a medium in biosynthesis of water-insoluble compounds as well as for the transportation and storage of water-insoluble metabolites (Owczarek et al. 2016). DES can be defined as a mixture of two or more components, either liquid or solid with a high melting point lowering composition into one liquid at room temperature. DES consists of organic compounds that function as hydrogen bond donors (HBD) such as amines, sugars, alcohols, and carboxylic acids mixed with quaternary ammonium salts (Dai et al. 2013; Tomé et al. 2018).

Various extraction methods can be used, including maceration, solid-liquid extraction in a three-neck flask, supercritical CO_2 extraction, ultrasonic extraction, microwave extraction, and high-pressure extraction. Several studies have been conducted on the extraction of anthocyanins from purple sweet potatoes. Truong et al carried out the extraction of purple sweet potatoes with a pressurized liquid extraction method using a solvent mixture of water, methanol, and acetic acid to produce the optimum extract at a temperature of 80-100°C (Truong et al. 2012). Research on the extraction of anthocyanins from purple sweet potatoes was also carried out by Huang using an ultrasonic-assisted extraction method and polyethylene glycol solvent. From this study, it was found that the optimum extraction conditions at a ratio of 42 mL/g, 83% PEG concentration, the ultrasonic temperature of 64 °C and a sonication time of 80 minutes, resulted in the best extraction of anthocyanins and phenolics (Huang et al. 2019).

This research uses purple sweet potato as the basic ingredient because the high anthocyanin content can be used as a natural dye. Natural dyes from purple sweet potato were chosen because they are safe if used in the long term. This research will optimize the extraction of anthocyanin content in purple sweet potato (*Ipomoea batatas L.*) using a deep eutectic solvent. Optimization is done by using the statistical method Response Surface Method (RSM). The analysis used is the analysis of anthocyanin content and antioxidant content.

2. RESEARCH METHODOLOGY

2.1 Materials

The material used in this study was purple sweet potato (*Ipomoea batatas L.*) from the market in the Yogyakarta area. For the deep eutectic solvent, citric acid, $C_6H_8O_7$ (Merck, pro analyst), and ethylene glycol, $C_2H_6O_2$ (Merck, pro analyst) were used. For analysis of anthocyanin content, sodium acetate (Supelco, Merck), potassium chloride (Emsure, Merck), and hydrochloric acid (Merck) were used.

2.2 Methods

The purple sweet potato was washed with water then the clean sweet potato was grated and then steamed for 15 minutes. After that, it was dried using an oven with a temperature of 120° C for 4 hours. The dried sweet potato was mashed using a blender until it became a purple sweet potato flour pow-

der, then sieved to 230 mesh. The deep eutectic solvent was made by reacting citric acid and ethylene glycol with a mole ratio of 1:4 in a flask. The temperature in the reaction was kept at 80°C. Extraction of anthocyanin from Purple Sweet Potato was done by weighing purple sweet potato flour into an Erlenmeyer and adding a DES solution with various material-to-solvent ratios of 1:20, 1:40, and 1:60 (w/v). The solution was then extracted using microwave-assisted extractor with power variations are 90, 180, and 270-Watt, and time variations of 60, 150, and 240 seconds. The anthocyanin extract was then filtered using filter paper and then put into a centrifuge. The resulting extract was tested for absorbance using UV Vis Spectrophotometer at λ_{max} 420 nm, then the total anthocyanin content (TAC) was determined by pH differential method according to Lee et al. (2005).

2.2.1 Design experiment

In the process of extracting anthocyanins from purple sweet potatoes using a deep eutectic solvent, there are 3 variables used, namely the ratio of material to solvent, microwave power, and extraction time. The control of the extraction, the optimization response is the absorbance value (A). Based on the three independent variables that influence this research, the experimental design is presented in Table 1. The optimization of the microwave assisted anthocyanin extraction process using DES from each independent variable to the response variable was carried out using the Response Surface Methodology in Minitab16. The model of the experimental design for the second-order reaction is a second-order polynomial model with a 3^k factorial design, which is presented in equation 1.

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum \sum_{i<j=2}^{k} \beta_{ij} X_i X_j + \varepsilon \quad (1)$$

3. RESULT AND DISCUSSION

This research was conducted based on the cube factorial design to optimize the predicted value of the Total Anthocyanin Content (TAC) parameter on the effect of the ratio of ingredients to deep eutectic solvent on the extraction yield. The TAC value was observed on independent variables that had an effect, namely the ratio of material to solvent, power, and extraction time. Mathematical modeling is used to get the optimal value of TAC. The TAC value data based on research results and predictions of mathematical models are presented in Table 2.

The mathematical equation that relates the optimal value of Total Anthocyanin Content (TAC) to the independent variables (ratio of material to solvent, power, and extraction time) is in the form of a second-order polynomial mathema-

TABLE 1. Experimental design level code and values.

Variable	min (-)	mid (0)	max (+)
Ratio (X_1)	1:20	1:40	1:60
Power (X_2)	90 Watt	180 Watt	270 Watt
Extraction time (X_3)	60 s	150 s	240 s

TABLE 2. Experiment and prediction data of Total Anthocyanin Content (TAC).

Run	Ratio (X1)	Power, W (X2)	Time, s (X3)	TAC experiment (mg/L)		TAC prediction
				1	2	
1	1:20	90	60	128.58	113.83	117.363
2	1:40	90	60	217.94	191.77	220.736
3	1:60	90	60	79.73	75.12	67.415
4	1:20	180	60	148.86	120.29	130.033
5	1:40	180	60	226.63	242.53	236.145
6	1:60	180	60	99.08	81.57	83.737
7	1:20	270	60	118.30	109.22	113.717
8	1:40	270	60	233.81	202.97	222.567
9	1:60	270	60	66.82	68.52	71.073
10	1:20	90	150	93.55	105.54	101.436
11	1:40	90	150	200.34	200.48	208.035
12	1:60	90	150	65.90	72.91	55.790
13	1:20	180	150	136.10	159.00	137.920
14	1:40	180	150	251.18	257.64	247.258
15	1:60	180	150	96.32	93.55	95.925
16	1:20	270	150	132.13	144.25	145.417
17	1:40	270	150	248.42	265.01	257.494
18	1:60	270	150	109.73	100.65	107.075
19	1:20	90	240	80.65	74.47	58.520
20	1:40	90	240	146.10	159.64	168.345
21	1:60	90	240	46.54	26.54	17.176
22	1:20	180	240	74.19	98.94	118.817
23	1:40	180	240	208.78	227.44	231.382
24	1:60	180	240	52.07	70.51	81.125
25	1:20	270	240	155.31	153.47	150.129
26	1:40	270	240	253.87	280.24	265.432
27	1:60	270	240	103.27	111.99	116.088

tical equation as follows.

$$TAC = -517.3 + 44464\,X_1 + 0.290\,X_2 + 0.123\,X_3 \\ - 643335\,X_1{}^2 - 0.001206\,X_2{}^2 - 0.002112\,X_3{}^2 \\ - 0.83\,X_1X_2 + 0.002734\,X_2X_3 - 1.43\,X_1X_3 \quad (2)$$

Statistical analysis with ANOVA was carried out to see the suitability of the model with the experimental data. In ANOVA, each variation component is analyzed for its specificity to the model parameter test. The values of p and f indicate the parameters that have a significant effect on the equation of the mathematical model. The results of the ANOVA analysis are shown in Table 3. As shown in Table 3, the p-value for the proposed mathematical equation model is 0. Based on this p-value, it can be said that the model is statistically significant as it is smaller than the significance level of

0.05. Table 2 of the ANOVA test shows a significant p-value of the influence of the variable. Almost all of the p-values obtained are less than the specified significance number, which is 0.05. This means that in order 2, there is only one variable that has no effect which is a product of time and ratio with a significance value of 0.454. From the Lack of Fit test on the model, the p-value is less than 0.05, which means it is significant or has a significant effect on the absorbance response. The individual variables, time, power, and ratio parameters also have p-values less than 0.05 so it can be said that all of the individual variables are significant to the model. The suitability of the predicted value with the actual experimental value also needs to be considered for the validation of the mathematical model used. It can be seen from Figure 1a, that the graph between the experimental TAC value and the predicted TAC value can be drawn as a straight line with an R-square value of 96.18%. This value indicates that 96% of the data can
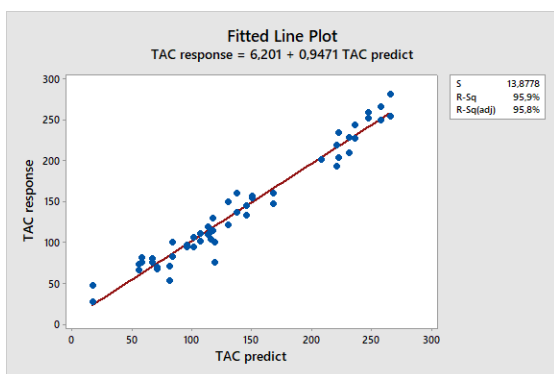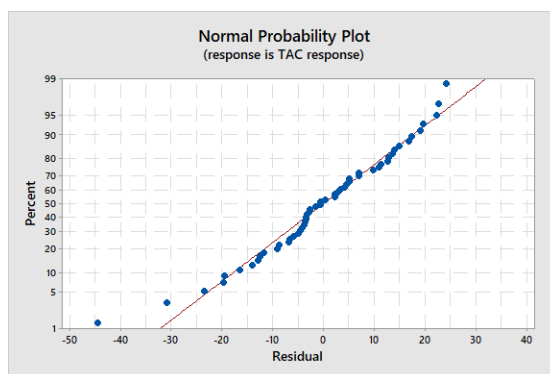
FIGURE 1. (a) Linear data fitting, and (b) normal probability of TAC.

TABLE 3. ANOVA statistical analysis.

Source	DF	Adj. sum of square	Adj. mean square	f-value	p - value
Model	9	233535	25948	123.07	0.000
Time	1	1230	1230	5.83	0.020
Power	1	15895	15895	75.39	0.000
Ratio	1	14637	14637	69.42	0.000
Time*Time	1	3511	3511	16.65	0.000
Power*Power	1	1146	1146	5.43	0.024
Ratio*Ratio	1	198981	198981	943.74	0.000
Time*Power	1	11772	11772	55.83	0.000
Time*Ratio	1	120	120	0.57	0.454
Power*Ratio	1	41	41	0.19	0.663
R-square 96.18%					

be represented by the model that has been compiled. Figure 1b is a normal probability graph showing the normal distribution of the data, other variables affecting the response, or deviations in the data. The accurate model is indicated by a small residual value. As can be seen in Figure 1b, the data point has a position close to the reference line, and the scattering of the observed data is minimal compared to the normal data. Therefore, it can be concluded that the data are normally distributed.

In this study, the optimum value of Total Anthocyanin Content (TAC) was sought based on the independent variables of DES material and solvent ratio, microwave power, and extraction time. The graph showing the effect of independent variables on the TAC value is shown in Figure 2.

In Figure 2a, a graph of the relationship between the ratio of material to DES and microwave power to the TAC value is presented. It can be seen that microwave power is very influential on the K/S value. The increase in microwave power is closely related to the temperature of the extraction process which has a positive effect on the TAC value. Temperature is very influential on the extraction process, with increasing molecular activity of the material so that the extraction yield increases (Bueno et al. 2012; Castañeda-Ovando et al. 2009). Increasing the temperature significantly increased the extraction yield of anthocyanins expressed as TAC so it affected the equilibrium conditions and mass transfer of solid-liquid extraction (Duan et al. 2015). Although the high temperature has a good effect on increasing the extraction yield, the increase in temperature must be considered because anthocyanin compounds are not thermally stable. Figure 2b shows a graph of the relationship between microwave power and time of the extraction process to the value of TAC. With

the increasing power of the microwave and the longer the extraction process, the TAC value will increase. However, it should be noted that anthocyanins will change color when exposed to high temperatures. This is due to the hydrolysis of the three glycosidic bonds of anthocyanins, producing labile aglycones and the opening of the ring, which cause the formation of a colorless carbinol group (Cavalcanti et al. 2011; Huang et al. 2019; Kim et al. 2012).

In Figure 2c, a graph of the relationship between the ratio of material to DES and extraction time to the TAC value can be seen. It can be seen that on the effect of extraction time, the relative TC value is not too affected. Meanwhile, on the effect of the ratio of material and DES, with an increasing ratio of solids to solution, the extraction yield increases. However, at one point if more solids were added, the solubility will decrease. The increase in anthocyanin extraction yield using DES solvent correlates with its physicochemical properties, including hydrogen bond interactions, polarity, and pH (Duan et al. 2016; Kalhor and Ghandi 2019). DES proved to be a suitable green extraction solvent to selectively and efficiently extract bioactive compounds such as anthocyanins from purple sweet potatoes (Kurtulbaş et al. 2022). Compared to the conventional methods of anthocyanins from purple sweet potatoes shown in Table 4, extraction in microwave assisted and DES were excellent.

Optimization using the Response Surface Method can provide better optimization results by paying attention to the effect and significance of a variable on the desired response. In this study, the effect of each extraction operating condition such as the ratio of material and solvent, microwave power, and extraction process time were analyzed individually to produce the optimum value, presented in Figure 3. Ex-
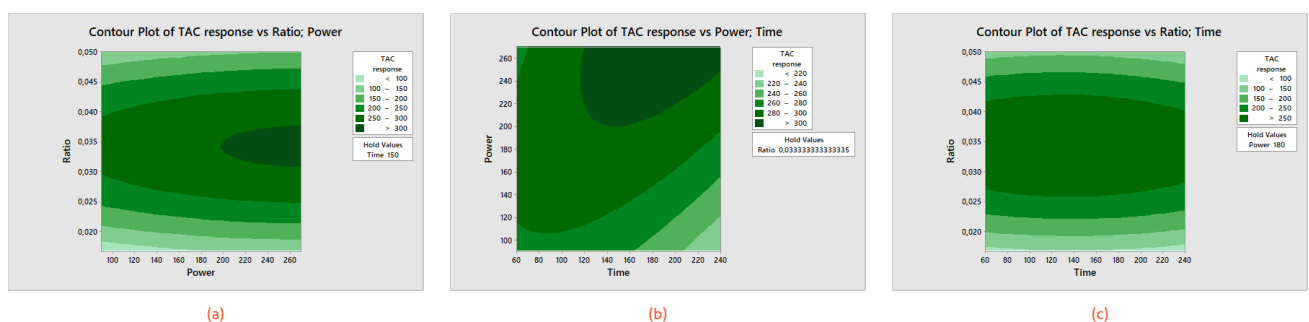

(a)   (b)   (c)

FIGURE 2. The contour graph shows the influence of individual variables on the TAC, (a) ratio and power, (b) ratio and time, and (c) power and time.
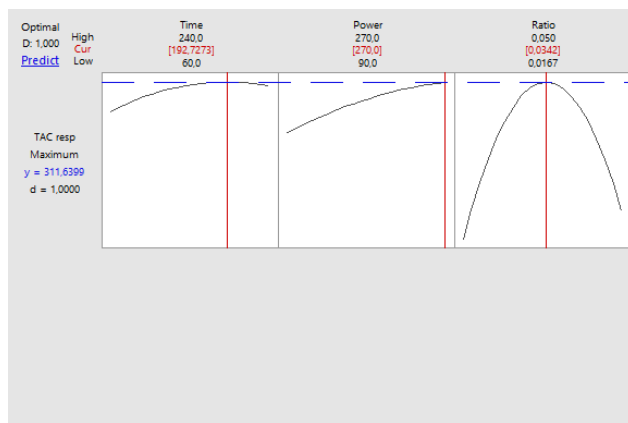
FIGURE 3. Optimization of anthocyanin extraction using RSM.

traction of purple sweet potato anthocyanins using DES solvent obtained optimal results, namely at microwave time of 240 seconds, microwave power of 270 watts and the ratio of the solvent of 1:29 for 193 seconds with TAC of 311.64 mg/L. The test results of antioxidant levels in anthocyanin extract with DES solvent were 44.85% at optimum conditions.

The right part of Figure 3 shows the interaction between the ratio of solvent and DES to the total anthocyanin content. As shown in the graph, the lower solvent ratio and higher concentration of purple sweet potato will decrease the total anthocyanin content. The higher the solute percentage, the less polar the solution and the lower the anthocyanin solubility. Cacace and Mazza (2003) reported that, above the ethanol percentage, the extraction efficiency decreased with increasing ethanol concentration to about 50%, regardless of the solvent percentage of the extract from the berries, which was also tracked in our study.

4. CONCLUSIONS

In this study, mathematical modeling based on the Response Surface Method with a 3^k factorial experimental design on the second order was proven to represent research data on the extraction of anthocyanins from purple sweet potatoes using a deep eutectic solvent with variations in the ratio of material to solvent, microwave power, and staining time with total response values. The optimum operating conditions for the extraction of anthocyanins from purple sweet potato using a deep eutectic solvent were obtained at a solid to solvent ratio of 1:29 at a microwave power of 270 watts for 193 s, with an optimum TAC value of 311.64 mg/L and an antioxidant value of 44.85%. The use of DES can increase the extraction yield and is environmentally friendly.

TABLE 4. Results of conventional extraction of Ipomoea batatas L.

Method/Solvent	TAC	Reference
Maceration	11.02 mg/L	Armanzah and Hendrawati (2016)
Extraction in Subcritical Water	0.474 mg/g	Yudiono (2011)
HCl-Methanol Solvent Extraction	3.78 mg/g	Mahmudatussa'adah et al. (2014)

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