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**Submission date:** 07-Jan-2022 03:23PM (UTC-0600)

**Submission ID:** 1738674592

**File name:** Naskah\_Rekpros\_RTE.pdf (246.1K)

**Word count:** 3954

**Character count:** 19352

# Extraction of Anthocyanin from Purple Sweet Potato (*Ipomoea batatas* L.) using Deep Eutectic Solvent (DES) based on Citric Acid

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## ABSTRACT

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, solvent has important role. In this research, deep eutectic solvent was used because it is more economical and environmentally friendly. This research will optimize the microwave assisted extraction of anthocyanin content purple sweet potato (*Ipomoea batatas* L.) using 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 response. 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 power microwave of 270 watts for 193 s, with an optimum TAC value of 311.64 mg/100g and an antioxidant value of 44.85%. The use of DES can increase the extraction yield and is environmentally friendly

Keywords: Anthocyanin; Deep eutectic solvent; Extraction; Ipomoeas batatas; Solvent

## 1. Introduction

Purple sweet potato is one of the tubers or roots that is widely grown in Indonesia with the characteristic of the 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 purple sweet potato which have benefits as antioxidants because of the micronutrients which are phytochemical groups from various food ingredients derived in plants. One type of flavonoid that functions as an antioxidant is a natural dye called anthocyanin. Purple sweet potato can be used as a 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 cyaniding (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 was 61.85 mg/100 g, higher than the anthocyanin content in light purple sweet potato, which was 3.51 mg/100 g. Concentrated purple sweet potato has antioxidant activity of 59.25%, greater than 56.64% of light purple sweet potato (Yin et al., 2017; Yuzhi Jiao, 2012).

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 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., 2013a, 2013b; Tomé et al., 2018).

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 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. Chemicals used such as citric acid ( $C_6H_8O_7$ ), ethylene glycol ( $C_2H_6O_2$ ) and other supporting chemicals were obtained from the Chemical Engineering Laboratory of Ahmad Dahlan University.

### 2.2 Procedures

The purple sweet potato was cleaned with water then the cleaned sweet potato is 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 powder, then sieved to 230 mesh. 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 Anthocyanins from Purple Sweet Potato was done by weighing purple sweet potato flour into an Erlenmeyer and add a DES solution with various material to solvent ratio of 1:20, 1:40, 1:60 (w/v). The solution is then extracted using microwave assisted extractor with power variations are 90,180, and 270-Watt, time variations of 60 seconds, 150 seconds 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  $\lambda_{max}$  420 nm, then analyzed for the Total Anthocyanin Content (TAC) by pH differential method.

### 2.3 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. As control of the extraction optimization response 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 <sup>5</sup> was carried out using the Response Surface Methodology. The model of the experimental design for the second-order <sup>12</sup> action is a second-order polynomial model with a 3<sup>k</sup> factorial design, which is presented in equation 1 (Montgomery, 2012).

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

**Table 1.** Experimental design level code and values

Variable	min (-)	mid (0)	max (+)
Ratio (X <sub>1</sub> )	1:20	1:40	1:60
Power (X <sub>2</sub> )	90 Watt	180 Watt	270 Watt
Extraction time (X <sub>3</sub> )	60 s	150 s	240 s

### 3. Results 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. TAC value data based on research results and predictions of mathematical models are presented in Table 2.

**Table 2.** Experiment and Prediction Data of Total Anthocyanin Content (TAC)

Run	Ratio (X <sub>1</sub> )	Power, W (X <sub>2</sub> )	Time, s (X <sub>3</sub> )	TAC experiment (mg/100g)		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

Run	Ratio (X <sub>1</sub> )	Power, W (X <sub>2</sub> )	Time, s (X <sub>3</sub> )	TAC experiment (mg/100g)		TAC prediction
				1	2	
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

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 mathematical equation as follows.

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

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  $F_0$  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. This p value can be said that the model is statistically significant, because it is smaller than the significance level of 0.05. Based on Table 2 of the ANOVA test above, it 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 that is 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 the 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, the graph between the experimental TAC value and the predicted TAC value can be drawn a straight line with an R-square value of 96,18%. This value indicates that 96% of the data can 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 (Agarry and Ogunleye, 2012). As can be seen in Figure 1b, the data point has a position close to the reference line, the scattering of the observed data is minimal compared to the normal data. Therefore, it can be concluded that the data are normally distributed.

**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%

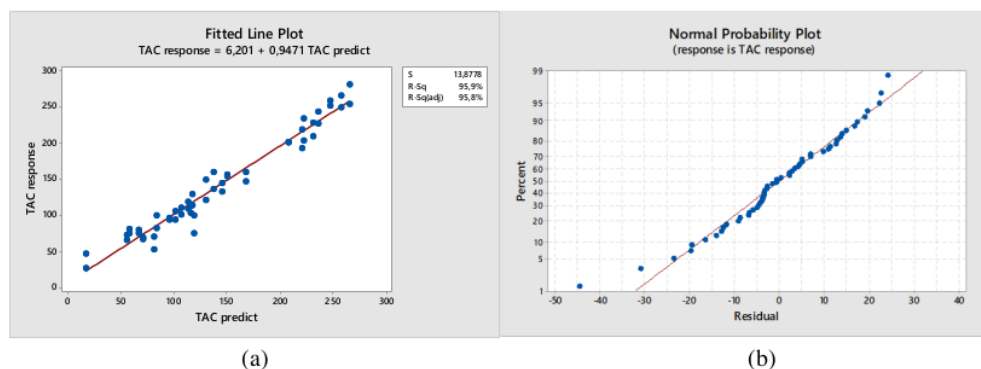
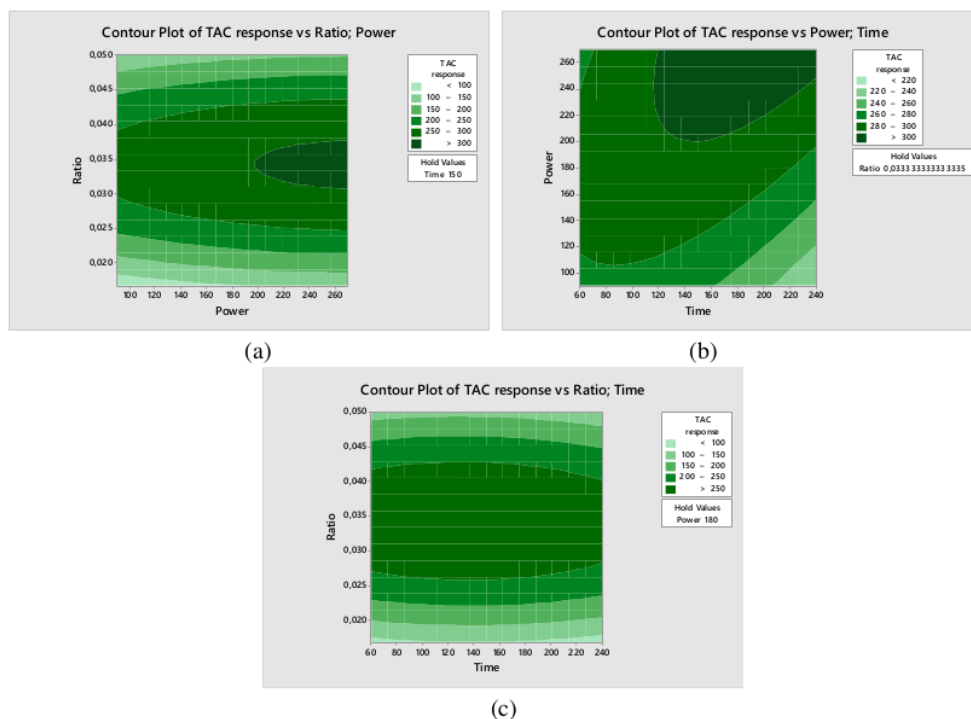


Figure 1. (a) Linear data fitting, and (b) Normal probability of TAC

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.

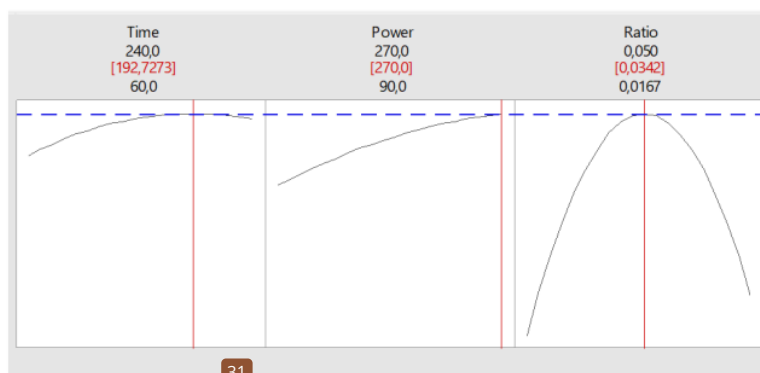
In Figure 2a, a graph of the relationship between the ratio of material to DES and microwave power to the TAC value is presented. It can be seen that microwave power is very influential on the K/S value. The increase in microwave power is closely related to the temperature of the extraction process which 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 that it affected the equilibrium conditions mass transfer of solid-liquid extraction (Duan et al., 2015). Although 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, so they will damage anthocyanin compounds. 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 hydrolysis of the three glycosidic bonds of anthocyanins and produces labile glycones and the opening of the ring causes 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 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 increasing ratio of solids to solution, the extraction yield increases. However, at one point if you add more solids you will decrease the solubility. The increase in anthocyanin extraction yield using DES solvent correlated with its physico-chemical 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 (Kurtulba et al., 2020).



**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

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. Extraction of purple sweet potato anthocyanins using DES solvent obtained optimal results, namely at microwave time is 240 seconds, microwave power is 270 watts and the ratio of the solvent is 1:29 for 193 seconds with TAC of 311.64 mg/100g. The test results of antioxidant levels in anthocyanin extract with DES solvent were 44.85% at optimum conditions.



**Figure 3.** Optimization of Anthocyanin Extraction using RSM

#### 4. Conclusions

In this study, mathematical modeling based on the Response Surface Methodology with a 3<sup>k</sup> factorial experimental design on the second order was presented 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 soaking time with total response values. anthocyanin content (TAC). 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 power microwave of 270 watts for 193 s, with an optimum TAC value of 311.64 mg/100g and an antioxidant value of 44.85%. The use of DES can increase the extraction yield and is environmentally friendly

#### Acknowledgements

Author would like to thank Ahmad Dahlan University Yogyakarta which has provided funding for the PDP scheme research through the Institute for Research and Community Service with contract number PDP-337/SP3/LPPM-UAD/VI/2021 and research facilities at the Process Engineering Laboratory.

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