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## Response surface modeling and optimizing conditions for anthocyanins extraction from purple sweet potato (*Ipomoea batatas* (L.) Lam) grown in Lam Dong province, Vietnam

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**Abstract.** Anthocyanin is increasingly used as a natural and safe coloring agent. In this paper, the extraction of purple sweet potato anthocyanin (PSPAs) was investigated by using response surface methodology (RSM). Different extraction temperatures of solvent ethanol (60 - 70 °C), duration of extraction (35 - 45 min) and solid-liquid ratios (4:1 – 6:1) were selected in order to extract PSPAs. The highest anthocyanin content of 206.019 mg/L of PSPAs was collected at the solid liquid ratio 6:1, extraction time 39.61 min, and temperature 67.38°C. PSPAs yield detailed significant correlation with high F values, low P values (<0.0001), the determination coefficient ( $R^2=0.9986$ ) and a high desirability 93.5%.

### 1. Introduction

Anthocyanins, apart from being recognized as natural plant pigments from the flavonoid family, possess valuable pharmacological properties such as anti-oxidative, anti-inflammatory and antineurodegenerative effects. Despite the ubiquity of the substance in many flowers and fruits of the high plants, it is the purple sweet potato (*Ipomoea batatas* (L.) Lam) that has attracted attention thanks to its high content of anthocyanins, unique color, nutritional value and health benefits. In particular, multiple physiological functions including antimutagenicity, antihyperglycemic effect and free radical-scavenging activity are exhibited from natural purple sweet potato color (PSPC), commonly extracted from purple sweet potatoes [1, 2].

Compared to other variants of sweet potatoes, purple sweet potatoes have high antioxidant activity of PFSP thanks to the high content of anthocyanins in the forms of mono or diacylated cyanidin and peonidin [3]. The analysis of nutraceutical properties of PFSP has revealed that anthocyanins have strong radical scavenging and antimutagenic activities. In addition, when tested on rats, the substances could also reduce blood pressure and prevent carbon tetrachloride-induced liver injury.



Other physiological functions of anthocyanins include anti-inflammatory, antimicrobial, and ultraviolet protection effects [3, 4].

Response surface methodology (RSM) is a collection of statistical techniques and empirical model built devised to examine the impacts of two or more variables (input variables) on a response variable using multiple regression and correlation analysis [5-9]. Through a careful experiment design, the technique aims to optimize a response output variable, influenced by several inputs, in order to attain the best system performance. In this study, extraction temperature, extraction time and solid-liquid ratio are identified as the main factors, and are therefore assumed to be correlated with the yield of anthocyanins extracted from purple sweet potatoes. For each variable, three values, coded as -1, 0 and +1, corresponding to low, intermediate and high value, respectively, were designated. The second order polynomial modal, which related to the selected variables, is shown below:

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i x_i + \sum_{i=1}^3 \beta_{ii} x_i^2 + \sum_{i=1}^2 \sum_{j=i+1}^3 \beta_{ij} x_i x_j \quad (1)$$

Where, Y is a response variable,  $\beta_0$ ,  $\beta_i$ ,  $\beta_{ii}$  and  $\beta_{ij}$  are the regression coefficients of the intercept, linear, quadratic and interaction terms, respectively.  $x_i$  and  $x_j$  are coded values of independent variables affecting the response of Y.

## 2. Material and methods

### 2.1. Sample preparation

PSPAs, rich in anthocyanins, were grown in lam Dong, Viet Nam. The harvested potatoes were washed, cut into cubes (3-5mm), dried by microwave 400W for 30 min, pulverized, and the collected powder was stored in dark black bottles in room temperature for further use. Ethanol (C<sub>2</sub>H<sub>5</sub>OH) is purchased from Sigma Aldrich (US).

For extraction parameter study, 15g of purple sweet potato powder was put in the two neck round bottom flask and was extracted by H<sub>2</sub>O, EtOH40° - EtOH70° (H<sub>2</sub>O:Ethanol = 6:4 to 3:7, mL/mL) solutions, material/solvent ratio: 4:1 - 8:1 (g/mL). The extraction temperature is adjusted to about 30 – 70 (°C) and time of extraction was restricted from 40 to 70 (min). Then, centrifugation took place at 4000 rpm for 15 min. The supernatant was collected and the extract, after being filtered with filter paper, was transferred into plastic bottles for to estimate anthocyanin yield. The pH scanning of supernatant ranges from 400 nm to 700 nm.

### 2.2. Total monometric anthocyanin by the pH-DIFFERENTIAL method

The pH-differential method was used to determine the total monomeric anthocyanin content. Each sample was diluted twice. Sodium acetate (0.4 M) at pH 4.5 and potassium chloride (0.025 M) at pH 1 were used in the first and the second dilution, respectively. At 521nm, absorbance readings of the samples must not exceed 1.2. Following an equilibration period of 15 min, absorbance at 400 and 700 nm of the samples was recorded using a spectrophotometer calibrated with distilled water as the blank. Absorbance was calculated at pH 1.0 and pH 4.5. Calculation of Anthocyanin content (a) follows pH - differential method as follows:

$$a = \frac{A * MW * DF * 10^3}{\epsilon * l} \quad (2)$$

Where: A = (A<sub>521nm</sub> - A<sub>700nm</sub>)<sub>pH1.0</sub> - (A<sub>521nm</sub> - A<sub>700nm</sub>)<sub>pH4.5</sub>. a : the total anthocyanin content (mg); A<sub>521</sub>, A<sub>700</sub>: absorbances at 521 and 700 nm respectively; MW: cyanidin-3-glucoside molecular weight (449.2g/mol); DF: dilution factor;  $\epsilon$ : cyanidin-3-glucoside molar absorptivity ( $\epsilon$  =26900); l : measurement cell path length; 10<sup>3</sup>: conversion factor from g to mg [10].

### 2.3. Single factor investigation

As mentioned before, four contributing factors to the extraction of anthocyanin were concentration of Ethanol solvent (mol/L), time (min), temperature (°C) and solvent/material ratio (mL/g). Therefore, select the optimum condition by testing each of the four alternatives in the selected element.

### 2.4. Optimization of RSM

The extraction experiments were performed by Design Design-Expert® software version 11 (DX11) from Stat-Ease Inc., Minneapolis, Minnesota. Based on the results of single factor experiments, three independent variables were identified: ratio solid/solvent ( $X_1$ ) was 4:1 - 6:1 (mL/g), temperature ( $X_2$ ) at 60-70 (°C), and time on the extraction ( $X_3$ ) was chosen 35-45 (min) to evaluation. Table 1 displayed Total anthocyanin contents (TAC) obtained.

**Table 1.** Independent variables and corresponding levels for RSM model.

Levels	Factor		
	$X_1$ (mL/g)	$X_2$ (°C)	$X_3$ (min)
-1	4	60	35
0	5	65	40
+1	6	70	45

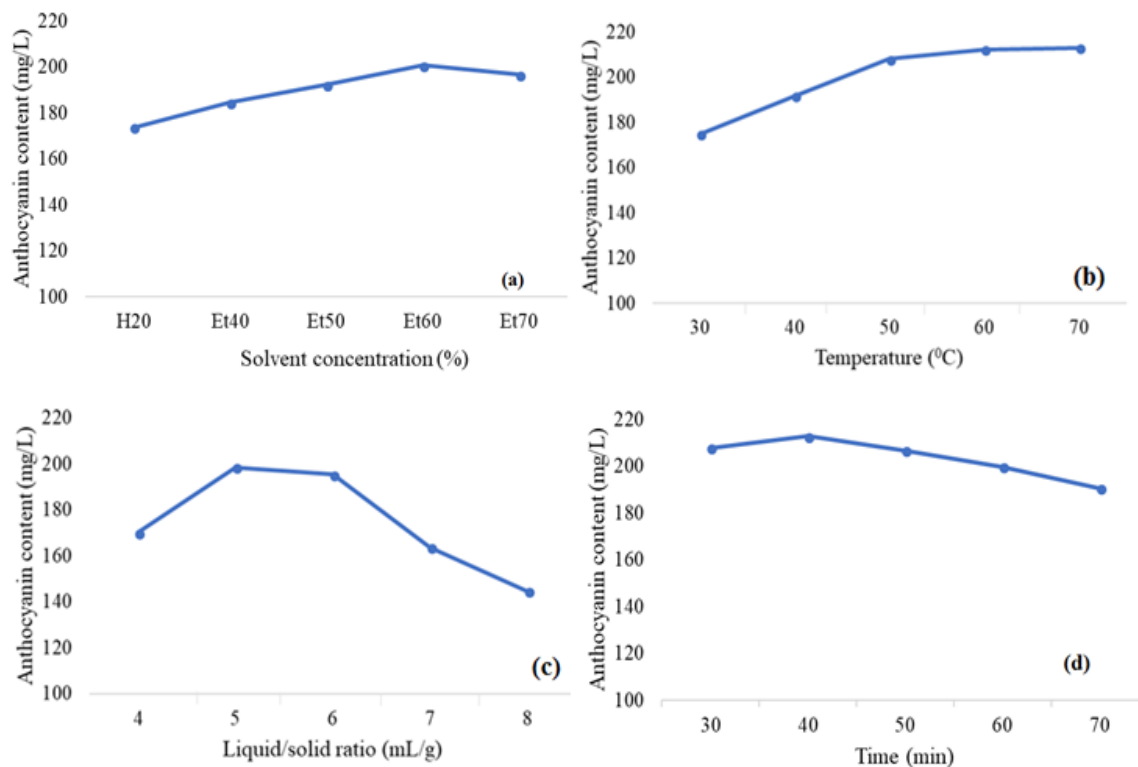
### 2.5. Statistical analysis

Following a RMS design planned by Design-Expert software (Version 11.0), triplicate extraction experiments were performed and data that accrued from the experiments was analyzed with the same software.

## 3. Result and discussion

### 3.1. Single factor investigation

Effects of four individual factors on the anthocyanin yield were summarized in Figure 1. Figure 1(a) showed a rapid rise of anthocyanin content as the concentration of ethanol solution increases (other factors: liquid/solid ratio 5:1, time extraction 40 min, temperature 65°C). However, rising the concentration of the solution from 60% to 70% caused the anthocyanin content to recede. Similar trend could also be interpreted for the impact of temperature, as demonstrated in Figure 1(b). To be specific, anthocyanin yield steadily increases before picking at 60°C (other factors: ethanol 60%, time extraction 40 min, liquid/solid ratio 5:1). The reason for this is that the increase in temperature will promote the phase separation of anthocyanin from purple sweet potato. To achieve the highest performance when extracting, the temperature will be chosen at 60°C. For liquid/solid ratio, Figure 1(c) illustrated that the peak of anthocyanin content is reached at liquid/solid ratio of 5:1. Other anthocyanin yields associating to other ratios were all significantly lower than such threshold (other factors: ethanol 60%, time extraction 40 min, temperature 65°C). The general trend for reaction time in Figure 1(d) was that the longer the reaction time, the higher the produced anthocyanins, with the exception of yields in the period from 30min to 40min, where a mild increase in yield took place (other factors: ethanol 60%, temperature 65°C, liquid/solid ratio 5:1). Herein, concentration of ethanol 60%, extraction time 35-45 min, temperature 60-70°C and reaction time 30~50 min were considered to be optimal in this RSM design.



**Figure 1.** Effects of four different factors on the content of extracted anthocyanin.

### 3.2. Optimization of RSM

The results of 20 experimental runs with the RSM model were completed design and shown in Table 2. The results implied that the yield of anthocyanin would be severely changed as survey parameters change (ratio, temperature, time). In the table, No.1~14 were the factorial experiments, and No.15~20 were the central experiments. The anthocyanin content ranges from 181.826 mg/L to 212.585 mg/L.

**Table 2.** The matrix of observed and predicted values for RSM model.

No	Independent factors			Y (mg/L)		No	Independent factors			Y (mg/L)	
	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	Actual	Predicted		X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	Actual	Predicted
1	4	60	35	197.28	197.256	11	5	56.59	40	190.16	190.159
2	6	60	35	195.45	195.452	12	5	73.41	40	203.27	203.267
3	4	70	35	193.32	193.323	13	5	65	31.59	196.98	196.98
4	6	70	35	198.36	198.358	14	5	65	48.41	202.11	202.115
5	4	60	45	200.44	200.437	15	5	65	40	212.59	212.41
6	6	60	45	181.83	181.826	16	5	65	40	212.53	212.41
7	4	70	45	211.54	211.337	17	5	65	40	212.55	212.41
8	6	70	45	201.26	201.263	18	5	65	40	212.58	212.41
9	3.32	65	40	205.22	205.221	19	5	65	40	211.56	212.41
10	6.68	65	40	194.05	194.049	20	5	65	40	212.57	242.41

The different between actual values and predicted values was not substantial indicating that the results of the experimental experiments have high accuracy. Table 3 show that the factors interacting with yield anthocyanin with significance level  $R^2 = 0.9986$  and confidence level was 93.5%. Overall, the model is significant as demonstrated by the F-value of 814.97. The probability of the model to obtain such value owing to noise is minimal (0.01%). All model terms are also statistically significant as indicated by low p-values. Insignificance of the lack of fit shows that the model fits well with the data, thus no further specification of the model is required. After estimating model coefficients, the following model is obtained:  $Y = 212.41 - 3.24X_1 + 3.76X_2 + 1.40X_3 + 1.92X_1X_2 - 3.99X_2X_3 + 3.92X_1X_3 - 4.59X_1^2 - 5.63X_2^2 - 4.63X_3^2$  (3)

The model is fit with the response variable and independent variables. Therefore, anthocyanin content of 206.019 mg/L was extracted by the optimum parameters as  $X_1 = 6:1$ ,  $X_2 = 67.38$ ,  $X_3 = 39.61$  in order to collect desirability 93.5%, which was completed by DX11 software. Model suitability is accentuated by three statistics, model F-value, P-value of coefficients and the lack of fit. The Model F-value of 918.67 implies that the model is significant as there is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. For p-value of coefficients, the results pointed out that all model terms are significant at 1% confidence level. This signified the unnecessary of inclusion of additional model terms. Insignificance of Lack-of-fit value also indicated reasonable fitness of the data to the resulting model.

**Table 3.** ANOVA data for removal models.

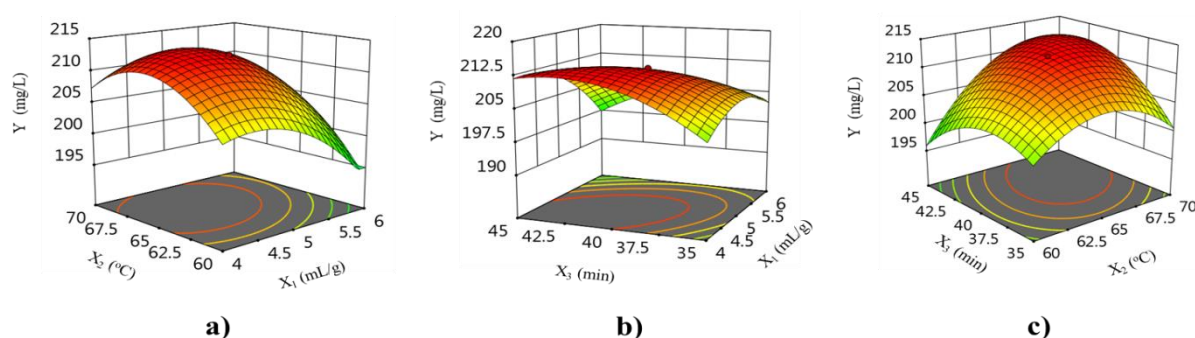
Source	Sum of Squares	Degree of freedom	Mean Square	F-value	Prob. > F	Comment
Model	1537.44	9	170.83	814.97	< 0.0001	SD = 0.4578
A-A	143.32	1	143.32	683.75	< 0.0001	Mean = 202.27
B-B	193.12	1	193.12	921.32	< 0.0001	CV(%) = 0.2263
C-C	26.74	1	26.74	127.58	< 0.0001	$R^2 = 0.9986$
AB	29.55	1	29.55	140.98	< 0.0001	AP = 93.5290
AC	127.33	1	127.33	607.45	< 0.0001	
BC	122.97	1	122.97	586.65	< 0.0001	
A <sup>2</sup>	304.15	1	304.15	1451.03	< 0.0001	
B <sup>2</sup>	456.32	1	456.32	2176.98	< 0.0001	
C <sup>2</sup>	308.27	1	308.27	1470.68	< 0.0001	
Residual	2.10	10	0.2096			
Lack of Fit	1.25	5	0.2499	1.48	0.3399	not significant
Pure Error	0.8467	5	0.1693			

Based on the model estimated by the software, three response surface plots of the anthocyanin yields, corresponding to different combinations of A, B and C, were plotted as in Figure 2. The contour plots represented impact intensity of the interaction variables on the yield. According to the model results, all three interaction terms representing combinations of three factors are significant ( $P < 0.05$ ). The liquid-to-solid ratio positively affects anthocyanin yields. This is explained by the fact that material surface was swelled when more solvent was present leading to more anthocyanin yield. Temperature and extraction time also aided in the thermal accumulation through increased microwave energy and in turn destabilized cell walls, releasing higher amount of anthocyanins to the solution.

From the response surface analysis, optimum process of anthocyanin extraction are identified with the following parameters: temperature 67.38°C, time 39.61 min, and liquid-solid ratio 6:1. The maximum predicted anthocyanin content was 206.019 mg/L. For convenience purposes, we performed the experiment with following parameters to validate the aforementioned optimum process: temperature 65°C, time 40 min, and liquid-solid ratio 5:1. The results is showed in the Table 4. Evidently, the difference between the actual yield and the predicted yield was subtle indicating relative accuracy of data produced by the model to the actual experimental results.

**Table 4.** Results of validating experiment.

	Liquid-solid ratio	Time (min)	Temperature (°C)	Anthocyanin content (mg/L)
Predicted	6:1	39.61	67.38	206.019
Actual	5:1	40	65	212.58



**Figure 2.** 3D model about the influence and interaction of Y with a)  $X_1$  and  $X_2$ , b)  $X_1$  and  $X_3$ , c)  $X_2$  and  $X_3$ .

#### 4. Conclusions

The optimal levels of three factors were founded by single factor investigation (liquid/solid ratio, extraction time, reaction temperature). Following the single factor assays, we conducted 20 experiments planned by RSM to optimize of the extraction process of anthocyanin content. The results of surface response methodology revealed that the optimum combination of process variables for response functions was temperature 67.38°C, time 39.61 min, and liquid-solid ratio 6:1, which showed the highest anthocyanin content of 206.019 mg/L.

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