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Extraction of anthocyanins from Butterfly pea (*Clitoria ternatea* L. Flowers) in Southern Vietnam: Response surface modeling for optimization of the operation conditions

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Abstract. In this paper, the Response Surface Methodology (RSM), in conjunction with Central Composite Design (CCD), was used to optimize the extraction of anthocyanins from Butterfly pea (*Clitoria ternatea* L. Flowers) cultivated in Southern Vietnam. The effect of extraction temperatures of solvent ethanol (50-70 °C), duration of extraction (40-50 min) and solid-liquid ratios (20:1–30:1) was measured as independent variables on the total extraction anthocyanins in the response function. The highest anthocyanin content of 132.756 mg/L of butterfly pea anthocyanin was collected at the solid liquid ratio of 23:1, extraction time of 46 min, and temperature 60.6°C. Butterfly pea anthocyanins yield detailed significant correlation with high F values, low P values (<0.0001), and desirable determination coefficient ($R^2 = 0.9994$).

Keywords: Response Surface Methodology, Central Composite Design, Optimizing conditions, Anthocyanins extraction, Butterfly pea

1. Introduction

Clitoria ternatea, commonly known as butterfly pea or blue pea, is found throughout the tropical regions of Vietnam. The plant bears white or blue flowers and grows in wild and also in gardens. It is well known as tropical perennial climber herb from family Fabaceae with slender downy stem [1,2]. Thanks to its anthocyanin compound, the flower could be used as a natural colorant. Anthocyanins in the flower are made up of basic classes and this determines the color. To be specific, pelargonidin is responsible for orange-red color, cyanidin for red hues and delphinidin for lilac to blue hues. Anthocyanins are also widely used as a natural supplements and have been shown to have antiinflammatory activity, anti-oxidant, anticarcinogenic and anti-microbial including anti-candida activities [3-5].



Newly discovered health benefits and applications in the food processing industry has rapidly pushed the demands for anthocyanin. In studies utilizing the single investigation approach, variable was studied individually, therefore failing to control the simultaneous effects of other parameter. As such, number of experimental attempt will be numerous and materials needed are increased. The Response Surface Methodology (RSM) could overcome such limitations by adopting a multivariate system that fits experimental data in a statistical model by a response function [6-10].

Given the huge potential of anthocyanin and the abundance of the substance in Butterfly pea, this study aim to facilitate anthocyanin exploitation in nutraceutical/food industry. In this study, we investigate the total extracted anthocyanins from Butterfly pea (*Clitoria ternatea L.Flowers*) and its experimental parameters. The effect of extraction temperatures of solvent ethanol, duration of extraction, solid-liquid ratios and their interactions was evaluated using a Central Composite Design (CCD) combined with RSM.

2. Material and methods

2.1. Extraction of totals anthocyanins from Butterfly pea

Dried petals of butterfly pea were ground using a commercial grinder and were weighed to 10g, put in the two neck round bottom flask and was extracted by Ethanol 50° solutions. Ethanol (C₂H₅OH) is purchased from Sigma Aldrich (US). Monomeric anthocyanin was calculated as cyanidin-3-glycoside using a pH differential method [11]

First of all, each experimental condition will be tested individually to determine a set of optimal condition. For extraction parameter study, 10g of butterfly pea powder was placed in the two neck round bottom flask as and was extracted by ethanol with concentration at 50. The liquid/solid ratio in this experiment ranges from 10:1 to 30:1 (mL/g) and. The extraction temperature is adjusted from 40 to 80 (°C) and time varies from 15 to 75 (min). Then, centrifugation took place at 4000 rpm for 15 min by high speed centrifuge Model LACE16 (from COLO lab expert). The supernatant was collected and the extract, after being filtered with filter paper, was transferred into plastic bottle for yield estimation. The pH scanning of supernatant ranges from 400 nm to 700 nm.

2.2. Statistical analysis

Response surface analysis conducted by Design-Expert® Version 11 software was employed to determine the optimum result of response affected by three factors (solid/liquid ratio, extraction temperature and extraction time). The analysis results is expressed in the form a statistical model in which anthocyanin content is formulated as a function relating to considered factors. To verify the optimum process, 5 replications of the extraction process at optimum conditions were conducted. The resulting values was then compared to the volume predicted by the model.

Table 1. Independent variables matrix and their encoded levels for RSM model.

Code	Name	Units	Levels				
			- α	-1	0	+1	+ α
A	Water to raw material ratio	mL/g	16.59	20	25	30	33.40
B	Temperature	°C	51.591	55	60	65	68.409
C	Extraction time	Min	36.59	40	45	45	53.41

3. Result and discussion

The results of 20 experimental runs with the RSM model were shown in Table 2. The results implied that the yield of anthocyanin would be severely altered as survey parameters change (ratio, temperature, time). In the table 2, No.1~14 were the factorial experiments, and No.15~20 were the central experiments. The resulting 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 ₁	X ₂	X ₃	Actual	Predicted		X ₁	X ₂	X ₃	Actual	Predicted
1	20	55	40	118.02	113.39	11	25	51.591	45	121.86	127.40
2	30	55	40	120.80	113.94	12	25	68.409	45	122.8	127.95
3	20	65	40	130.332	117.94	13	25	60	36.59	127.31	128.75
4	30	65	40	119.75	119.68	14	25	60	53.41	128.7	130.31
5	20	55	50	125.86	120.74	15	25	60	45	132.26	132.25
6	30	55	50	124.78	121.96	16	25	60	45	132.22	132.25
7	20	65	50	126.99	122.85	17	25	60	45	132.21	132.25
8	30	65	50	113.81	123.83	18	25	60	45	132.22	132.25
9	16.59	60	45	123.77	124.70	19	25	60	45	132.38	132.25
10	33.40	60	45	113.86	125.83	20	25	60	45	132.25	132.25

Table 3 ANOVA data for removal models

Source	Sum of Squares	Degree of freedom	Mean Square	F-value	Prob. > F		Comment
Model	733.66	9	81.52	10544.99	< 0.0001	significant	SD = 0.2024
A-A	117.91	1	117.91	15252.80	< 0.0001		Mean = 125.61
B-B	0.9505	1	0.9505	122.95	0.0025		CV(%) = 0.1611
C-C	2.20	1	2.20	284.00	< 0.0001		R ² = 0.9994
AB	90.20	1	90.20	11667.55	< 0.0001		AP = 130.12%
AC	7.72	1	7.72	998.45	< 0.0001		
BC	52.54	1	52.54	6796.66	< 0.0001		
A ²	321.95	1	321.95	41646.26	< 0.0001		
B ²	174.90	1	174.90	22625.25	< 0.0001		
C ²	31.45	1	31.45	4068.76	< 0.0001		
Residual	0.0773	10	0.0077				
Lack of Fit	0.0578	5	0.0116	2.97	0.1290	not significant	
Pure Error	0.0195	5	0.0039				

The difference between actual values and predicted values was not substantial indicating that the results of the experimental experiments have high accuracy. As indicated by Table 3, determination coefficient $R^2 = 0.9994$ shows a high goodness-of-fit of the predicted values to the real data. Overall, the model is significant as demonstrated by the F-value of 10544.99. The probability of the model to obtain such value owing to noise is minimal (0.016%). 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: $Y = 132.26 - 2.84X_1 + 0.2199X_2 + 0.357X_3 - 3.18X_1X_2 - 0.8073X_2X_3 - 2.64X_1X_3 - 4.74X_1^2 - 3.5X_2^2 - 1.49X_3^2$ (3). The model is fit with the response variable and independent variables. Therefore, anthocyanin content of 132.756 mg/L was extracted by the optimum parameters as $X_1 = 23:1$ (mL/g), $X_2 = 60.6$ ($^{\circ}\text{C}$) and $X_3 = 46$ (min), which was completed by DX11 software.

The overall significance of the model is implied by The Model F-value of 1953.19, suggesting that there is only a 0.01% chance that an F-value this large could occur due to noise. In addition, all model terms are statistically significant suggesting that model reduction is unnecessary. The Lack of Fit F-value of 20.01 implies the Lack of Fit is significant. There is only a 0.25% chance that a Lack of Fit F-value this large could occur due to noise.

Based on the model estimated by the software, three response surface plots of the anthocyanin yields, corresponding to different combinations of X_1 , X_2 and X_3 were plotted as in Fig.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 ($P < 0.05$). From the response surface analysis, optimum process of anthocyanin extraction are identified with the following parameters: temperature 60.6 ($^{\circ}\text{C}$), time 46 (min), and liquid-solid ratio 23:1 (mL/g). The maximum predicted anthocyanin content was 132.756 mg/L.

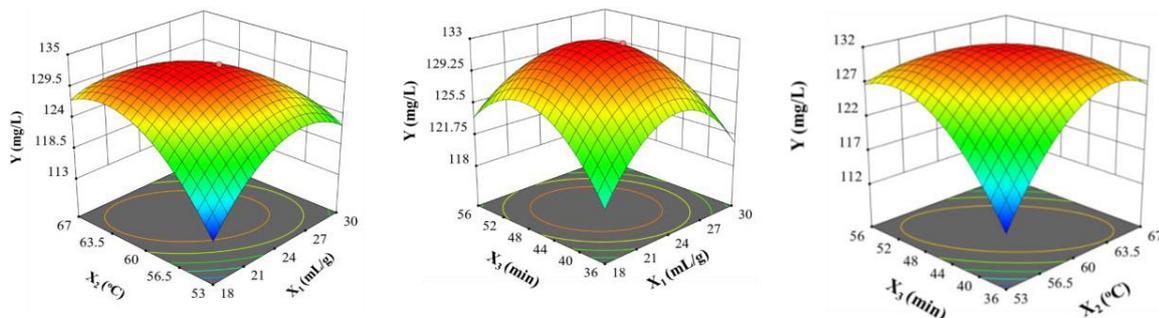


Figure 1. The influence and interaction of Y with X_2 and X_1 .

Figure 2. The influence and interaction of Y with X_1 and X_3 .

Figure 3. The influence and interaction of Y with X_2 and X_3 .

4. Conclusion

We have successfully extracted and optimized of the anthocyanin extraction process from the Butterfly pea (*Clitoria ternatea* L.Flowers) in Southern Vietnam. Response surface methodology (RSM), based on a CCD design, was used. Three-dimensional and contour-response surface plots clearly suggested the presence of impact of the different levels of liquid/solid ratio, extraction time, and reaction temperature on the total anthocyanin content. 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 set of experimental conditions to achieve the highest yield includes temperature 60.6 (°C), time 6 (min), and liquid-solid ratio 23:1 (mL/g), corresponding to the highest anthocyanin content of 132.756 mg/L. Therefore, through the use of RSM, significant factors impacting the extraction yield has been identified, proving a framework for further investigation

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