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

Tri Nhut Pham1,2, Duy Chinh Nguyen1, Tri Duc Lam1, Pham Van Thinh3, Xuan Tien Le4, Dai Viet Vo Nguyen5, Quang Hieu Vu1,7, Trinh Duy Nguyen1,6, Long Giang Bach1,7,*

1NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam
2Faculty of Chemical Engineering and Food Technology, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam
3Dong Nai Technology University, Bien Hoa City, Dong Nai Province, Viet Nam
4Department of Chemical Engineering, HCMC University of Technology, VNU-HCM, Ho Chi Minh City, Vietnam
5Faculty of Chemical and Natural Resources Engineering, Universiti Malaysia Pahang, Malaysia
6Department of Imaging System Engineering, Pukyong National University, Busan, South Korea
7Department of Chemical Engineering, Pukyong National University, Busan, South Korea
Email:blgiang@ntt.edu.vn

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 (R² = 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) |
|----|---------------------|----------|----|---------------------|----------|
|    | X1      | X2      | X3      | Actual | Predicted | X1 | X2 | X3 | 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 | 119.68 | 13 | 25 | 60 | 53.41 | 45 | 128.7 | 130.31 |
| 4  | 30 | 65 | 40 | 119.75 | 120.74 | 14 | 25 | 60 | 53.41 | 45 | 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  | R² = 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  |                          |
| A²       | 321.95         | 1                 | 321.95      | 41646.26 | < 0.0001  |                          |
| B²       | 174.90         | 1                 | 174.90      | 22625.25 | < 0.0001  |                          |
| C²       | 31.45          | 1                 | 31.45       | 4068.76  | < 0.0001  |                          |
| Residual | 0.0773         | 10                | 0.0077      |          | not significant         |
| 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 R² = 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 - 2.84X₁ + 0.2199X₂ + 0.357X₃ − 3.18X₁X₂ − 0.8073X₂X₃ - 2.64X₁X₃ - 4.74X₁² - 3.5X₂² - 1.49X₃² (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 X₁ = 23:1 (mL/g), X₂ = 60.6 (°C) and X₃ = 46 (min), which was completed by DX11 software.

Based on the model estimated by the software, three response surface plots of the anthocyanin yields, corresponding to different combinations of X₁, X₂ and X₃ 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 (°C), time 46 (min), and liquid-solid ratio 23:1 (mL/g). The maximum predicted anthocyanin content was 132.756 mg/L.

![Figure 1](image1.png) **Figure 1.** The influence and interaction of Y with X₂ and X₁.

![Figure 2](image2.png) **Figure 2.** The influence and interaction of Y with X₁ and X₃.

![Figure 3](image3.png) **Figure 3.** The influence and interaction of Y with X₂ and X₃.

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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