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# Optimization of aqueous extraction of blue dye from butterfly pea flower

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# Optimization of aqueous extraction of blue dye from butterfly pea flower

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**Abstract.** Dyes are widely used in industries such as textiles, leather, paper, and plastics to colour the final products. People use natural sources such as stem, bark, leaves, roots and flowers to get different colours for dyeing purposes. In addition, natural colorants such as anthocyanins are known for their possible health benefits as dietary antioxidants. The objectives of this study are to extract the blue dye from the butterfly pea flower using maceration method and to determine the optimum condition by response surface methodology. The flowers were dried, grinded and went through the maceration method for the extraction process. Response surface models were developed correlating the extraction yield with three parameters namely residence time, temperature and solid to liquid ratio. The result from this experiment was optimized using response surface methodology to obtain the optimum condition at temperature 54 °C, extraction time 74 minutes and solid-to-liquid ratio of 1:37 to give extraction yield of 45.51 %.

## 1. Introduction

*Clitoria ternatea*, commonly known as butterfly pea is found in great abundance in Malaysia. *Clitoria ternatea* flower is the selected candidate in the studies of floral anthocyanins. *Clitoria ternatea L. Fabaceae* a perennial climber herb, is distributed in tropical countries. It has been implicated to have several medicinal properties. Anthocyanins are considered as antioxidants and their benefits include a risk reduction of cancer, arthritis, heart diseases and strokes [1]. It has several different flower colours; namely dark blue, light blue, mauve, and white. The dark blue colour of *clitoria ternatea* grows widely in Malaysia. The plant bears solitary, axillary papillionaceous bright blue petals flowers with white or light yellow at the centre [2]. Colorants are important ingredients in food, cosmetics, pharmaceuticals, fabrics, paints, toys and many other products. The butterfly pea flower is known to be used as a dye for colouring material in food and herbal drinks [3]. Anthocyanins in *Clitoria ternatea* have been chosen to be extracted to form natural dye. Anthocyanins are colour pigments that provide red, violet and blue colours. They are found in many agricultural products including butterfly pea flower. Generally, the traditional extraction techniques require longer extraction time and have severe risk of thermal degradation of certain bioactive compounds [4]. However, maceration is an inexpensive and simple method without having traces contamination of organic solvent into the targeted products. In addition, extraction of blue dye using maceration method can be optimized by response surface methodology (RSM) to give optimum conditions such as shorter extraction time and lower temperature with higher extraction yield. RSM has been extensively utilized to optimize several parameters in the extraction process. It provides the interactive effects between variables and reduces the required numbers of experimental runs which used to evaluate multiple parameters and their interactions [5].

In the present study, we optimized the maceration conditions for extracting blue dye from butterfly pea flower by response surface design and analyzed the blue dye. Hence, the objectives of this research are to find the optimum extraction condition of the anthocyanin from the butterfly pea flower and to analyzed the blue dye. The research results will provide beneficial information for an efficient extraction of butterfly pea flower by maceration technique.

## 2. Materials and Methods

In this study, the flower samples of dark blue colour of butterfly pea were collected from the locality of Kuantan, Malaysia.

### *2.1. Drying process*

The flowers of butterfly pea were rinsed with distilled water to remove any debris and impurities. The flower samples were dried at 40 °C in an oven overnight to remove water content.

### *2.2. Grinding process*

The dried flowers were grinded by using a mechanical blender to form powdered samples. The grinded samples were kept in a desiccator to prevent exposure to the high humidity from the surroundings. The grinded flowers of the specimen samples were denoted as No.1 until No.16 based on the design of experiment (DOE) by response surface methodology (RSM).

### *2.3. Extraction process*

The dried flower samples were grinded into small particles to enhance mixing with the solvent by increasing the surface area. Maceration technique was used for the extraction of blue dye from the powdered samples. Three main variables were manipulated namely temperature (25-95°C), residence time (40-80 min) and solid to liquid ratio (1:20-1:60) based on the DOE determined by RSM. Distilled water was added as solvent into the samples and heated accordingly. The liquid was strained off but the marc which was the solid residue of this extraction process was pressed to recover large amount of occluded solutions. The obtained strained and the press out liquid were both mixed and separated from impurities by filtration [6]. The liquid was dried in oven at 60 °C overnight to remove water content. The yield percent was calculated by using the dried weight of collected extract divided by the weight of the original sample.

### *2.4. Optimization process*

Response surface methodology (RSM) was utilized to design the required numbers of design of experiment (DOE) and to determine the optimized conditions for the extraction of natural dye from butterfly pea flower. Central composite design (CCD) in the STATISTICA 8.0 of RSM was used to develop a response surface quadratic model for describing the dye extraction process [7]. Full quadratic model of responses as shown in equation (1) was established using the method of least squares.

$$Y = \beta_o + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + \beta_{11}X_{11} + \beta_{22}X_{22} + \beta_{33}X_{33} \quad (1)$$

| | |
|---|---|
| $Y$ | : Predicted response |
| $\beta_o$ | : Intercept coefficient (offset) |
| $\beta_1$, $\beta_2$, $\beta_3$ | : Linear terms |
| $\beta_{11}$, $\beta_{22}$, $\beta_{33}$ | : Quadratic terms |
| $\beta_{12}$, $\beta_{13}$, $\beta_{23}$ | : Interaction terms |
| $X_1$, $X_2$, $X_3$ | : Uncoded independent variables |

## 3. Results and Discussion

The ranges and levels of variables investigated in the research are tabulated in table 1. Three independent variables were studied namely temperature ($X_1$), residence time ($X_2$) and solid to liquid ratio ($X_3$). RSM was utilized to determine the numbers of experimental runs required for the design of experiment (DOE) that produces reliable measurement of the response. The optimization was conducted for single response of extraction yield ($Y_1$).

**Table 1.** Independent variables with range and factor level.

| Factors | Symbol | Range and levels | | | | |
|---|---|---|---|---|---|---|
| | | - α | -1 | 0 | 1 | + α |
| Temperature | $X_1$ | 25 | 40 | 60 | 80 | 95 |
| Time | $X_2$ | 40 | 50 | 60 | 70 | 80 |
| Solid to liquid ratio | $X_3$ | 20 | 30 | 40 | 50 | 60 |

**Table 2.** Design of experiment of three variables and single response.

| Number of RUN | Temperature (°C) $X_1$ | Time (Min) $X_2$ | Solid Liquid Ratio (g/mL), $X_3$ | Extraction Yield (%), $Y_1$ | |
|---|---|---|---|---|---|
| | | | | Observed | Predicted |
| 1 | 95 | 60 | 40 | 42.02 | 40.36 |
| 2 | 80 | 70 | 50 | 33.60 | 37.53 |
| 3 | 60 | 60 | 20 | 34.11 | 31.65 |
| 4 | 60 | 40 | 40 | 40.36 | 38.65 |
| 5 | 40 | 50 | 30 | 36.11 | 33.92 |
| 6 | 40 | 70 | 30 | 23.73 | 28.55 |
| 7 | 60 | 60 | 40 | 50.18 | 45.49 |
| 8 | 80 | 50 | 50 | 42.36 | 38.91 |
| 9 | 80 | 50 | 30 | 40.00 | 45.78 |
| 10 | 40 | 50 | 50 | 42.22 | 45.55 |
| 11 | 25 | 60 | 40 | 40.37 | 39.74 |
| 12 | 80 | 70 | 30 | 38.21 | 35.91 |
| 13 | 60 | 60 | 40 | 41.63 | 45.49 |
| 14 | 60 | 80 | 40 | 32.45 | 31.90 |
| 15 | 40 | 70 | 50 | 53.41 | 48.68 |
| 16 | 60 | 60 | 60 | 43.80 | 44.90 |

Table 2 exhibits the total of 16 runs required for DOE of three variables and single response employed in this experiment. The experimental plan based on central composite design (CCD) consisting of 8 factorial points, 6 axials points and 2 centre points [8]. The overall yields for 16 runs of experiment were observed between 24 and 53%. Experimental run numbered No.15 showed the highest yield of 53.41% at 40 °C, 70 min residence time and solid to liquid ratio of 1:50.

The predictions between the response and independent variables with their interactions were made using a quadratic polynomial equation. The predicted data were fit to following the second order polynomial equation as shown in equation (2).

$$Y_1 = 45.49410+0.35398X_1-3.37500X_2+6.62500X_3-2.25000X_{12}-9.25000X_{13}+4.25000X_{23} -3.55682X_1^2-5.11160X_2^2-3.61160X_3^2 \quad (2)$$

The analysis of variance (ANOVA) shows that the empirical model for extraction yield gives good prediction at 95% confidence level. The significant of each term at 95% level of confidence was determined by *F*-value. Both calculated *F*-values of solid to liquid ratio (6.60) and interaction effect of time and solid to liquid ratio (6.43) were higher than the tabulated *F*-value (4.10) as shown in table 3. Those higher calculated *F*-values could be used to reject the null hypothesis. In addition, the two factors were highly significant to the regression model at 5 % level of significance. The adequacy of the models was justified through analysis of variance (ANOVA) and at least one of the independent variables (solid to liquid ratio) contributes significantly to the model.

**Table 3.** ANOVA results of the quadratic model of extraction yield for *F*-value comparison.

| Factor | Sum of Square (SS) | df | Mean Square (MS) | Calculated *F*-value | Tabulated *F*-value |
|---|---|---|---|---|---|
| (1)Time (Linear) | 0.44 | 1 | 0.44 | 0.02 | 4.10 |
| Time (Quadratic) | 30.36 | 1 | 30.36 | 1.14 | |
| (2)Temperature (Linear) | 45.56 | 1 | 45.56 | 1.71 | |
| Temperature (Quadratic) | 105.11 | 1 | 105.12 | 3.95 | |
| (3) Ratio (Linear) | 175.56 | 1 | 175.56 | 6.60 | |
| Ratio (Quadratic) | 52.47 | 1 | 52.47 | 1.97 | |
| 1(Linear) by 2(Linear) | 10.12 | 1 | 10.12 | 0.38 | |
| 1(Linear) by 3(Linear) | 171.12 | 1 | 171.12 | 6.43 | |
| 2(Linear) by 3(Linear) | 36.12 | 1 | 36.12 | 1.36 | |

The significance of regression coefficient in the regression model is determined by examining its respective *p*-value and *t*-value as shown in figure 1. The smaller *p*-value or greater magnitude of *t*-value of the corresponding coefficient indicates more significant into the model [9]. The linear of liquid to solid ratio has the smallest *p*-value (<0.05) and highest absolute t-values of 2.57. Another significant factor is the interaction effect of time and solid to liquid ratio has also the smallest *p*-value (<0.05) and highest absolute t-values of -2.54. However, the effects of the other factors on extraction yield model are not statistically significant.

Parameters like F-value and R-square are statically obtained values which are used to measure the fitness of the predicted model with the experimentally monitored data. Hence, the goodness of fit of the model is judged from the value of the determination coefficient, $R^2$ which shows the variations of observed values with respect to its mean. The parity plot shows the distribution of predicted values against the observed values of extraction yield as shown in figure 2. The value of *R*-squared of the model was 0.7751%, meaning that 0.7751% of the total variation can be attributed to the three independent variables. In addition, the empirical model should be at least 0.75 to adequately explain most of the variables [10]. The value of $R^2$ is closed to unity representing a complete agreement between the response model and the actual experimental data.

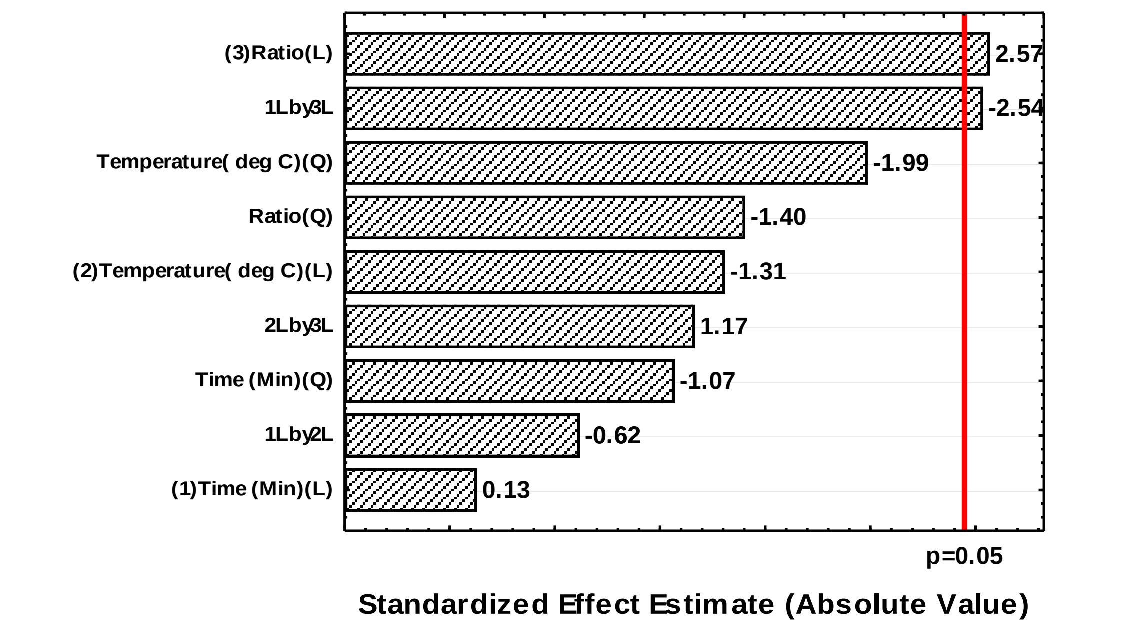


**Figure 1.** Pareto chart describing the effects of variables on the extraction yield.

The three-dimensional (3D) surface of the extraction yield model is shown in figure 3. The 3D response surface is the graphical representations of the regression equation to visualize the interactions of independent variables and the response. Each contour curve represents the infinitive numbers of the combinations of two test variables with the other one maintained at its respective zero level. The maximum predicted value of extraction yield is indicated by the surface confined in the smallest ellipse in the contour diagram. Each line in the contour plot in figure 3a) explains the interaction between temperature and extraction time for fixed liquid to solid ratio. When temperature and extraction time were increased to a certain value, the extraction yield was also increased. The increasing temperature in the solution enhanced the diffusivity and solubility of blue dye [11]. However, degradation of the anthocyanin compound was observed when further increase of temperature [12]. Figure 3b) explains the interaction between solid to liquid ratio and extraction time for fixed temperature. Increasing in both solid to liquid ratio and extraction time enhanced the extraction yield. The extraction yield was increased when the contact surface area of solvent and samples is greater due to the increase of mass transfer [13]. Each line in the contour plot in Figure 3c) explains the interaction between solid to liquid ratio and temperature for fixed time. When time and temperature were increased to a certain value, the extraction yield was also increased. The maximum predicted value of extraction yield is indicated by the surface confined in the smallest ellipse in the contour diagram

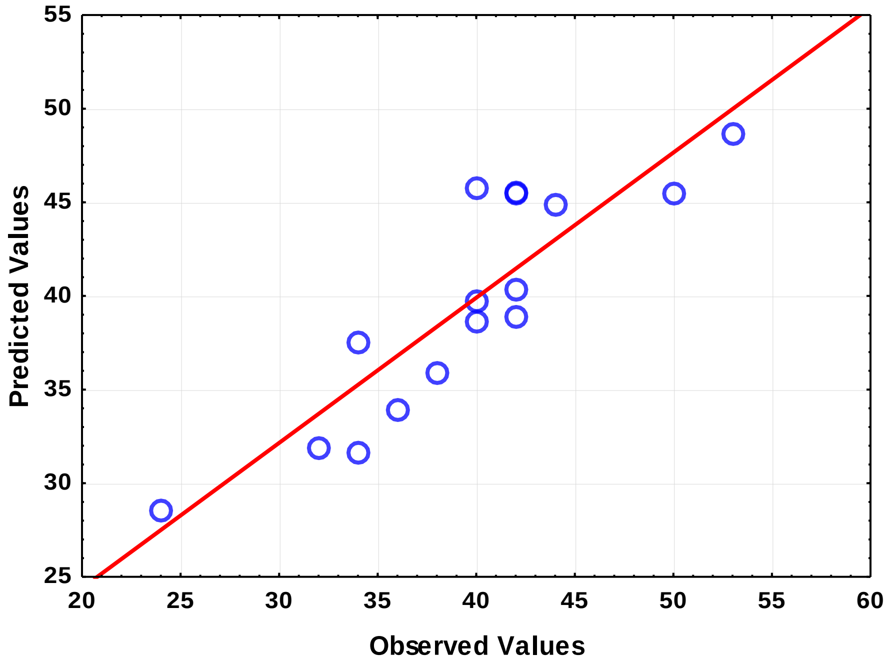


**Figure 2.** Parity plot showing the relation between actual and predicted values for extraction yield.

The data validation was run to ensure the observed experimental data comply with the predicted values. The experiments were replicated 3 times to have an average as shown in table 4. Hence, a small error of 4.55% was closed to zero to show high desirability of the model.

**Table 4.** Data validation between predicted and observed values of the extraction yield.

| Parameters | | | Extraction Yield (%), $Y_1$ | | Error (%) |
|---|---|---|---|---|---|
| $X_1$ | $X_2$ | $X_3$ | Predicted | Observed | |
| 54 | 74 | 37 | 45.51 | 43.44 | 4.55 |

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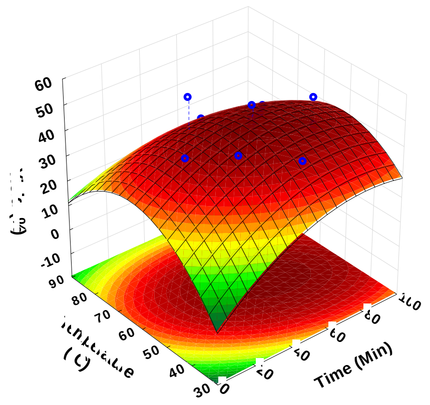
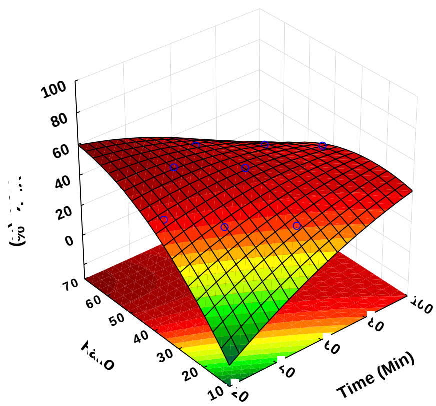
a)




b)




c)

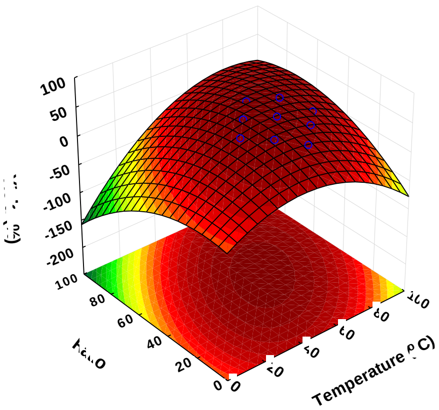



**Figure 3.** Surface plot of the extraction yield as function of a) Temperature and Extraction time, b) Solid to liquid ratio and Extraction time, c) Solid to liquid ratio and temperature.

## 4. Conclusion

Response surface methodology (RSM) was successfully applied to determine the optimum operational conditions for maximum extraction yield of blue dye. The natural dye extraction from Butterfly pea flowers were optimized at temperature 54 °C, extraction time 74 minutes and solid-to-liquid ratio of 1:37 to give extraction yield 45.51 % with an acceptable low error of data validation at 4.55%.

## 5. References

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