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Response Surface Optimization of Critical Extraction Parameters for Anthocyanin from *Solanum melongena*

Arunachalam Thirunavukkarasu[1]* and Rajarathinam Nithya[2]

[1]*Lecturer, Dept. of Biotechnology, PSG College of Technology, Coimbatore, India*
[2]*Asst.Professor, Faculty of Biotechnology, PRIST University, Thanjavur, India*

Abstract

The study investigated the parameter optimization for anthocyanin extraction from egg plant (*Solanum melongena*) with the approach response surface methodology. Extractions were carried out using acid–ethanol with the temperature range (60–90°C), time (30–90 min) and solid–liquid ratio (1:15–1:30). Three level three factor Box-Behnken design was followed to observe the anthocyanin yield for the studied parameters. The maximum yield of anthocyanin was observed at the temperature 76.5°C in 70 min in the ratio of 1:26.

Keywords: Anthocyanin; Egg plant; Response surface methodology; Box-Behnken design

Introduction

Anthocyanins widely exist in naturally occurring pigments of red fruits such as cherries, plums, strawberries, raspberries, blackberries, grapes, red currants and black currants [1]. Also it has been found in the roots, caudexes and leaves as well as flowers and fruits acting as a substitute for synthetic pigments. Anthocyanins are primarily used by humans for its therapeutic purpose such as antineoplastic, radiation-protective, vasotonic, vasoprotective, anti-inflammatory, chemo- and hepatoprotective [2-5]. The skin of eggplants (*Solanum melongena*) contains different kind of anthocyanins and have been extracted and identified [6,7]. However no studies were focussed on optimizing the parameters for anthocyanin extraction from eggplants peel, although other authors have already optimized extraction methods from different anthocyanins of plant sources [8-10]. Hence the present study made an attempt to utilize response surface methodology for developing optimized condtions for anthocyanin extraction from eggplant peel. The prime focus of Response surface methodology (RSM) is to use a sequence of designed experiments to obtain an optimal response. Here the study designed in such a way that differing the three independent variables temperature, time of extraction and solvent-to-solid ratio within a selected range to obtain an optimal response of anthocyanin yield. The basic theoretical and fundamental aspects of RSM have been discussed [11].

Material and Methods

Sample preparation

Solanum melongena were identified and collected locally and washed in running tap water. Then the peel layers with uniform thickness (1 mm) were simply removed using a sharp knife. The layers were finally reduced to 20 mm2 particle size and stored at 20°C as followed by Aldo Todaro et al., [12].

Methods

Peels of eggplant were set into a 50ml Erlenmeyer flask, then added with acid–ethanol (HCl, 1.5 mol/l) with different solid–solvent ratio (1:15–1:30) and placed in thermostatic water bath in the temperature range of (60–90°C) for the time duration (30-90 min), then, centrifuged at 4000 rpm for 15 min. The supernatant was collected and transferred into 50 ml volumetric flask for the determination of anthocyanins yield. About 1 g of the samples was used for each treatment.

Experimental design

RSM was employed to determine the optimum condition for anthocyanin extraction of egg plant. The experimental design and statistical analysis were performed using Stat-Ease software (Design-Expert 6.0.10 Trial, Delaware, USA Echip, 1993). A three-level three-factor Box– Behnken design was chosen to evaluate the combined effect of three independent variables extraction temperature, time and solid–liquid ratio, coded as A1, A2 and A3, respectively. The minimum and maximum values for extraction temperature were set at 60 and 90°C, extraction time between 30 and 90 min and solid–liquid ratio 1:15 and 1:30 Table 1. The response values were anthocyanin pigment yield, L*, C* and H. The complete design consisted of 17 combinations including five replicates of the center point Table 2 [13]. The second-order model is preferred for several reasons that flexible, easy to estimate the parameters [14]. The responses function (η) was partitioned into linear, quadratic and interactive components as followed by Gongjian Fan et al., [15].

$$\eta = \beta 0 + \sum_{i=1}^{n} Bi\ X1 + \sum_{i=1}^{n} Bii\ X2 + \sum_{i=1}^{n} Bij\ Xi\ Xj$$

where β0 is defined as the constant, Bi the linear coefficient, Bii the quadratic coefficient and Bij the cross-product coefficient. Xi and Xj are levels of the independent variables while n equals to the number of the tested factors (n=3). The analysis of variance (ANOVA) tables were generated and the effect and regression coefficients of individual linear, quadratic and interaction terms were determined. The significances of all terms in the polynomial were judged statistically by computing the

***Corresponding authors:** Thirunavukkarasu Arunachalam, Lecturer, Department of Biotechnology, PSG College of Technology, Coimbatore, India, Tel:+91-9952469214; E-mail: thirunaavukarasu@gmail.com

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F-value at a probability (P) of 0.001, 0.01 or 0.05. The regression coefficients were then used to make statistical calculations to generate contour maps from the regression models.

Quantitative analysis

Anthocyanins quantification was done by following the spectrophotometric method of Francis [16]. The molar absorptivity value for the acid–ethanol solvent is 98.2 and it refers to the absorption of a mixture of cranberry anthocyanins in acid–ethanol, measured in a 1-cm cell at 535 nm, at a concentration of 1% (w/v). The total anthocyanins (mg/100 g) were determined by the method proposed by Gongjian Fan et al., [15].

Total anthocyanins (TA) = A530 X dilution factor/98.2.

Statistics

The trials have been carried out in triplicate and all the data were reported as means±SD (Standard Deviation). Student's t-test was performed for the statistical significance at $P<0.01$ or $P<0.001$ level.

Results and Discussion

Statistical analysis

Table 3 summarizes the results of each dependent variable with their coefficients of determination (R2) [16]. The R2 values for anthocyanins yield was 0.9673 indicated that the proposed model was adequate, possessing no significant lack of fit. Since the closer the value of R2 to unity, the better the empirical models fits the actual data. On the other hand, the smaller the value of R2 the less relevance the dependent variables in the model have in explaining the behavior of variations [17]. The probability (P) values of all regression models were less than 0.000, with no lack-of fit.

Independent variable	Units	Symbols	Code levels		
			-1	0	1
Extraction temperature	°C	A1	60	75	90
Time	min	A2	30	60	90
Solid-Liquid ratio	1:X	A3	1:15	1:25	1:35

Table 1: Independent variables and their coded and actual values used for optimization.

Treatment	Independent variables			Dependent variable
	Temp (°C)	Time (min)	Solid-Liquid ratio	Anthocyanin yield (mg/100g)
1	-1	-1	0	110
2	1	-1	0	122
3	-1	1	0	136
4	1	1	0	138
5	-1	0	-1	124
6	1	0	-1	126
7	-1	0	1	120
8	1	0	1	128
9	0	-1	-1	127
10	0	1	-1	135
11	0	-1	1	124
12	0	1	1	138
13	0	0	0	144
14	0	0	0	144
15	0	0	0	142
16	0	0	0	144
17	0	0	0	144

Table 2: Box–Behnken design and experiment data for anthocyanins extraction.

Effects of extraction Temperature, Time and Solid–Liquid Ratio

The effect of the independent variables, Temperature and time on the anthocyanins yield was presented as the contour map Figure 1. As followed by the Table 3, anthocyanins yield mainly depend on the extraction temperature since both linear and quadratic effects were significant at ($P<0.01$) giving an overall curvilinear effect. The figure 1 also shows that the Anthocyanins yield can be increased with the increase of extraction temperature till it reaches 75°C. The linear effect ($P<0.01$) was positive, whereas the quadratic effect ($P<0.01$) was negative, which resulted in a curvilinear increase in Anthocyanins yield for all the extraction times. From this, it is evident that extraction temperature is the prime variable to be considered in the efficiency of extraction. The effect of the independent variables, temperature and S/L ratio on the anthocyanins yield was presented as the contour map Figure 2. The anthocyanins yield depends on solid–liquid ratio where its linear ($P<0.01$) and quadratic ($P<0.01$) effects were significantly affected by pigments concentration. Hence, the overall effect was curvilinear in nature. As the temperature progresses, anthocyanins yield of egg plant was found to increase rapidly till it reaches 75°C. It is evident from the Table 3 that the positive linear and negative quadratic effects of solid–liquid ratio and temperature explained the observed nature of the curve shown in Figure 2. The effect of the independent variables, time and S/L ratio on the anthocyanins yield was presented as the contour map (Figure 3).

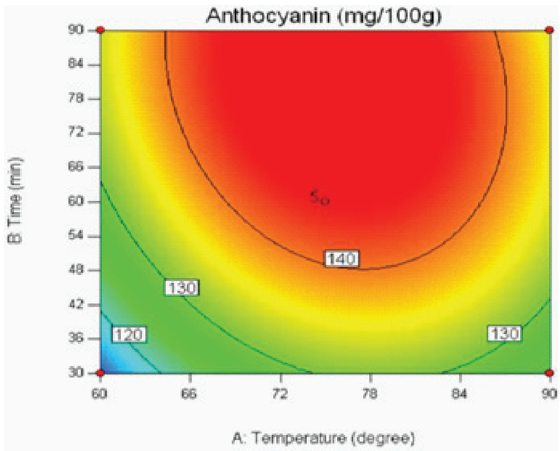

Figure 1: Effects of extraction temperature and time at a constant S/L ratio of 1:25 on the yield of Anthocyanins.

Regression Coefficient	Anthocyanins Yield
β0	143.6
b1	+3.00**
b2	+8.00
b3	+0.25**
b11	-2.50**
b12	+1.50
b22	+1.50
b13	-11.80
b23	-5.30
b33	-7.30**
R2	0.9673
P value	0.0002***

significant at 0.01 level * significant at 0.001 level

Table 3: Regression coefficients, R2, and P values for dependent variable for anthocyanins extraction.

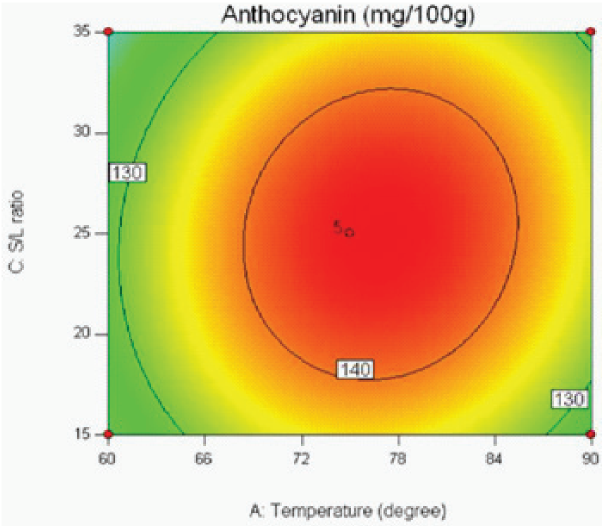

Figure 2: Effects of extraction temperature and S/L ratio at a constant time of 90min on the yield of Anthocyanins.

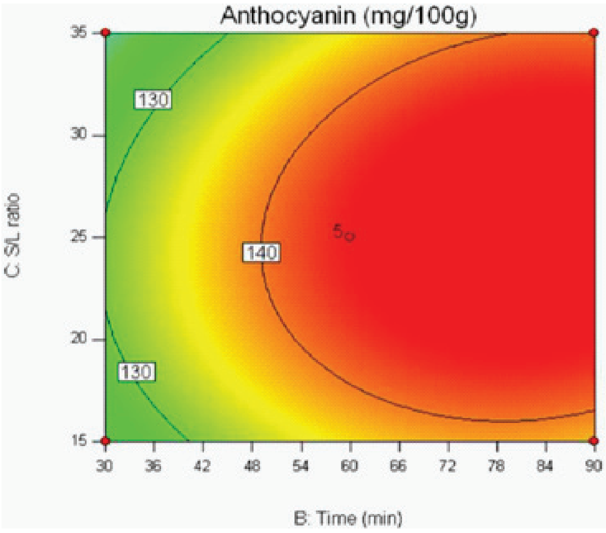

Figure 3: Effects of time and S/L ratio at a constant extraction temperature of 60°C on the yield of Anthocyanins.

The anthocyanins yield depends on solid–liquid ratio where it's linear ($P<0.01$) and quadratic ($P<0.01$) effects were significantly affected by pigments concentration. As the time proceeds, the anthocyanins yield was found to increase rapidly at the beginning but with a slower rate towards the end. It can be seen in Table 3 that the positive linear and negative quadratic effects of time and solid–liquid ratio explained the observed nature of the curve shown in Figure 3.

Optimization

The contour plots show the optimum conditions of the extraction process to anthocyanins yield, respectively. It was noted that the optimum conditions for Anthocyanins yield were slightly different. There are a number of combinations of variables that could give maximum levels of anthocyanins yield. Since the optimum response for each dependent variable did not fall exactly in the same region, the superimposition of all the contour plots obtained was done. Figure 1 shows the superimposed contour plot for optimization of anthocyanins yield. The zone of optimization depicts temperature to be over 76.5°C and solid–liquid ratio 1:26 and time to be maintained at 70 min. During the anthocyanin extraction, the extraction temperature and solid–liquid ratio are important. Therefore, the best combinations of process variables for response functions are found. The process variables for best combination of response function are extraction temperature 76.5°C, time 70 min, and solid–liquid ratio 1:26. The response anthocyanins yield from the optimized conditions was found to be 148 mg/100g.

Conclusions

The parameters extraction temperature, time and solid–liquid ratio for anthocyanins extraction were subjected to second order polynomials to determine their significance in the anthocyanin yield. The optimum conditions for the three independent variables were obtained from the contour plots and the extraction process were carried out to cross-validate their reliability and the yield exactly following at the optimized conditions. The anthocyanin yield from egg plant is estimated to be maximal if the extraction process maintaining the temperature at 76.5°C, time at 70 min, and solid–liquid ratio at 1:26.

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