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Optimization of the Process Variables in the Aqueous Extraction of Natural Pigments from *Basella alba* using Box-Behnken Design under Response Surface Methodology

Swamy Gabriela John[1]*, Sangamithra. A[1], Saravanan.S[1], Sivakumar.V[1]

[1]Department of Food Technology, Kongu Engineering College, Perundurai – 638052, TamilNadu, India.

*Corres.author: gabrielafoodtech@gmail.com
Tel.: +91 9500171174; Fax.: +91 4294 220087

Abstract: The aim of the study was to investigate the influence of independent process variables like extraction temperature, time and mass of alba fruits on the aqueous extraction of betalain from *Basella alba*. The optimum conditions for the aqueous extraction of betaxanthin and betacyanin from the fruits were performed using a three-factor and three-level Box-Behnken design (BBD) under response surface methodology. The pigments were extracted from *Basella alba* at temperature (50-70°C), time (30-90 min) and mass of fruit (1-2 g) using water as solvent. The data obtained from the experiments were analyzed by Pareto analysis of variance. Further, the data was fitted to a second-order polynomial equation using multiple regression analysis. The optimal conditions based on both individual and combinations of all responses (extraction temperature – 69.6°C, time – 88.4 min and mass - 2.0 g) were found out.
Keywords - *Basella alba*; Natural colorants; Aqueous extraction; Box-Behnken design; Response surface methodology; Optimization

Introduction

Natural colorants are actively replacing the synthetic ones. Fruits and vegetables are rich sources of natural pigments. *Basella alba* is a wildly cultivated climber. It is a succulent, branched, smooth, twining herbaceous vine, several meters in length. The alba fruit is fleshy, stalkless, ovoid or spherical, 5-6 mm long, and purple when mature. It is a potential source of valuable water-soluble nitrogenous single pigment betalain composed of betacyanin and betaxanthin structural groups[1]. These colorants are free radical scavengers and hence prevent oxidation (oxygen mediated and free radical induced) of biological molecules[2]. As a colorant, they have positive health effects that have made them more popular when compared to the anthocyanins. They have antioxidant, radical-scavenging, antimicrobial and antiviral properties[3].

Response surface methodology (RSM) is an efficient statistical tool that has been successfully used in testing process parameters and their interactive effects[4]. The extraction and purification of betalains are of prime importance as they are one of the potential ingredients used in functional foods. Keeping in view above mentioned facts, aqueous extraction which is a simple, highly efficient and lost-cost method for betalain extraction was employed for the study.

The present research was executed with the clear objective of extracting natural pigment from *Basella alba* fruit by aqueous extraction method and to examine the combined effect of process parameters such as temperature, extraction time and mass of fruit used in the dye extraction process using Box Behnken Design in Response Surface Methodology.

Experimental

Freshly harvested *Basella alba* fruits with similar weight and maturity were used as raw materials and they were procured from Coimbatore, TamilNadu, and were stored at 4°C prior to conducting the experiments. *Basella alba* fruits with three different masses (1, 1.5 and 2 g) were taken in 250 ml Erlenmeyer flasks (conical flask). Approximately 50 ml of distilled water was used as solvent in each flask to keep the pieces fully immersed in the solvent. The flask was roofed with a plastic wrap. The flasks were incubated at 50, 60 and 70°C and the extracts were taken at time intervals 30, 60 and 90 min. After extraction for a selected time, the mixture was centrifuged (Remi R-24 Centrifuge, India) at 1500 rpm for 10 minutes. Experiments were carried out in a randomized order. All the experiments were executed thrice and the average value was used for the determination of betaxanthin and betacyanin from *Basella alba fruit*. . Spectrophotometric measurements (Shimadzu UV-1800, Kyoto, Japan) were performed thrice and the betaxanthin and betacyanin content were calculated. The total quantity of pigment was calculated using the formula

$$Total\ quantity\ of\ pigment\ (mg/100\ g\ of\ fresh\ fruit) = \frac{A\ X\ MW\ X\ V_a\ X\ DF\ X\ 10^2}{\varepsilon\ L\ W_a} \quad (1)$$

where A is the absorption value at the absorption maximum of 480 and 536 nm for betaxanthin and betacyanin respectively, V_a is the total extract volume (mL), DF is the dilution factor, L is the path-length (1 cm) of the cuvette and W_a is the fresh weight of extracting material (g). The molecular weight (MW) and molar extinction coefficient (ε) of betaxanthins (MW=308; ε= 48000 $M^{-1}cm^{-1}$) and betacyanins (MW=550; ε = 60000 $M^{-1}cm^{-1}$) were applied in order to quantify the respective pigments.

Results and Discussion

Table 1: Results of model adequacy tested in the Box-Behnken design

Source	Sum of squares	DF	Mean square	F value	Prob > F	Remarks
Sequential model sum of squares for betaxanthin						
Mean	25315.43	1	25315.43			
Linear	12.18	3	4.06	14.82	0.0002	
2FI	0.75	3	0.25	0.89	0.4814	
Quadratic	2.48	3	0.83	17.57	0.0012	Suggested
Cubic	0.33	3	0.11	6.366E+007	< 0.0001	Aliased
Residual	0.000	4	0.000			
Total	25331.16	17	1490.07			
Sequential model sum of squares for betacyanin						
Mean	4.144E+005	1	4.144E+005			
Linear	18.71	3	6.24	82.96	< 0.0001	
2FI	0.075	3	0.025	0.28	0.8410	
Quadratic	0.80	3	0.27	18.85	0.0010	Suggested
Cubic	0.099	3	0.033	6.366E+007	< 0.0001	Aliased
Residual	0.000	4	0.000			
Total	4.145E+005	17	24380.31			
Source	Std. Dev.	R-squared	Adjusted R-squared	Predicted R-squared	PRESS	
Model summary statistics for betaxanthin						
Linear	0.52	0.7738	0.7216	0.5600	6.92	
2FI	0.53	0.8213	0.7140	0.2209	12.26	
Quadratic	0.22	0.9791	0.9521	0.6648	5.27	Suggested
Cubic	0.000	1.0000	1.0000	+	Aliased	
Model summary statistics for betacyanin						
Linear	0.27	0.9504	0.9389	0.9164	1.65	
2FI	0.30	0.9542	0.9267	0.8491	2.97	
Quadratic	0.12	0.9950	0.9885	0.9192	1.59	Suggested
Cubic	0.000	1.0000	1.0000	+	Aliased	

Sequential model sum of squares and model summary statistics tests were carried out to decide the adequacy of models in the present study[5] and the results are presented in Table 1. From Table 1 it is observed that the quadratic model is highly significant for the extraction of pigments from *Basella alba* fruit. The model summary statistics demonstrated that the quadratic model had maximum R- Squared, Adjusted R-Squared and Predicted R-Squared values. The quadratic model was chosen for further analysis of data. Based on the Box-Behnken experimental design model, the empirical relationship between the input variables and experimental results obtained were expressed by a second-order polynomial equation with interaction terms.

However, the investigation and optimization of a fitted response surface might generate poor or disingenuous results[6]. Therefore, it was essential to ensure the fitness of the model. Regression analysis and Pareto analysis of variance (ANOVA) were used to test the adequacy and fitness of the models. The results in Table 5 point out that the equation sufficiently presented the actual relationship between the input parameters and responses. ANOVA is a statistical technique that subdivides the total variation in a dataset [7]. Coefficient of determination (R^2) was computed to verify the sufficiency and fitness of the model. The values of R^2 were calculated to be 0.9791 and 0.9950 for betaxanthin and betacyanin respectively, which imply that 95% of experimental data was well-suited. The high R^2 coefficient gives a satisfactory adjustment of the quadratic model to the experimental data. The purpose of the adjusted-R^2 is to analyze the model adequacy and fitness. The very low coefficient of variation value (0.56 and 0.076) undoubtedly represents a high degree of precision and reliability of experiments conducted.

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