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Response surface modeling and optimization of process parameters for aqueous extraction of pigments from prickly pear (*Opuntia ficus-indica*) fruit

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ABSTRACT

The objectives of this study are to determine the optimum conditions for the aqueous extraction of pigments from prickly pear fruit using a three-level three-factor Box–Behnken design under response surface methodology. Pigments were extracted from prickly pears using water as solvent at different extraction temperature (30–50 °C), time (20–120 min) and mass of fruit (0.5–1.5 g). The experimental data obtained were analyzed by Pareto analysis of variance (ANOVA) and fitted to a second-order polynomial equation using multiple regression analysis. An optimization study using Derringer's desired function methodology was performed and the optimal conditions based on both individual and combinations of all responses (extraction temperature of 40 °C, time of 115 min and mass of 1.44 g) were determined. At this optimum condition, the total betacyanin and betaxanthin content were found to be 13.4354 mg/100 g and 24.2922 mg/100 g with desirability value of 0.917. The experimental values agreed with those predicted values.

1. Introduction

Opuntia spp., the largest genus of the Cactaceae family, includes manifold species and varieties and most known cactus fruits were used for various kinds of food products [1]. Different parts of the *Opuntia ficus-indica* are utilized as fruits and vegetable for human consumption, fodder for cattle, and raw materials for various industries to prepare plywood, soap, dyes, adhesives and glue, pharmaceutical products for treating diabetes and various other disorders, and cosmetics such as shampoo, cream, and body lotions, etc. [2]. In addition to the excellent quality and flavor of the fresh fruit, the young leaves serve both as a vegetable and salad dish and the immature fruit is used to make mock gherkins.

Cactus pear fruit (prickly pear) is a fleshy berry, varying in shape, size, and color and has a consistent number of hard seeds. It is characterized by a high sugar content (12–17%) and low acidity (0.03–0.12%). It has higher vitamin C, potassium, calcium and phosphorous and low sodium [3]. The prickly pears are considered as a rich source of yellow–orange betaxanthins and red–violet betacyanins and the red and purple colored prickly pears contain high amounts of total phenols and purple skinned fruits contain the highest amounts of flavonoids which are responsible for the color of *Opuntia* spp., and having radical-scavenging and reducing properties [4–7]. The betalain content of the cactus pear fruit is found to be having an application in the low acid foods as natural colorants [8]. Cactus pear fruit pulp exhibits a high pH value (5.6–6.5) and total soluble solids content ranging from 11 to 17 °brix and this property making the pulp highly susceptible to microbial spoilage [9–11].

The extraction of bioactive compounds from permeable solid plant materials using solvents constitutes an important step in the manufacture of phytochemical-rich products. The application of this low-cost technology to obtain molecules to be used as food additives or nutraceutical products is an appropriate strategy for the exploitation of some non-timber products found in rain forests such as the fruits of the palm [12]. From the previous findings, many factors such as solvent composition, extraction time, extraction temperature [13], solvent to solid ratio [14] and extraction pressure [15], among others, may significantly influence the extraction efficacy of diverse natural products [16–18].

Response surface methodology (RSM), a powerful mathematical and statistical technique, has been effectively used in testing multiple process factors and their interactive effects [19,20]. RSM can help in investigating the interactive effect of process variables and in building a mathematical model that accurately describes the overall process [21]. Box–Behnken design is a spherical, revolving

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response surface methodology (RSM) design that consists of three interlocking 2^2 factorial design having points, all lying on the surface of a sphere surrounding the center of the design [22]. Several studies have been used successfully the RSM to model and optimize the biochemical and biotechnological processes related to food systems [23–27].

The extraction and purification of phytochemicals from natural sources are needed since these bioactives are often used in the preparation of dietary supplements, nutraceuticals, functional food ingredients and additives, pharmaceutical and cosmetic products [28]. In this study, the effects of various process parameters such as extraction temperature, time and mass of fruit on aqueous extraction of pigments (betacyanin and betaxanthin) from prickly pear fruit were investigated by applying Box–Behnken design under response surface methodology and the optimal conditions were determined to achieve the maximal pigments extraction.

2. Materials and methods

2.1. Fruit

Common name: Indian fig, Tuna and Nopal
English name: Barbary fig and Prickly pear
Botanical name: *Opuntia ficus-indica*
Kingdom: Plantae
Order: Caryophyllales
Family: Cactaceae
Used part: Fruits

The fruit of prickly pears, commonly called cactus fruit, cactus fig and Indian fig or tuna in Spanish, is edible, although it has to be peeled carefully to remove the small spines on the outer skin before consumption. The prickly pear contains betalain, betanin and indicaxanthin, with highest levels in their fruits [29]. The fruit of prickly pears is often used to make candies, jelly, or drinks such as vodka or lemonade and its pulp and juice have been used to treat numerous maladies, such as wounds and inflammations of the digestive and urinary tracts in folk medicine [30]. The aqueous extracts of cactus pear fruits exhibited a marked antioxidant capacity in several in vitro assays, including the oxidation of red blood cell membrane lipids and the oxidation of human LDLs induced by copper and 2,2′-azobis (2-amidinopropane-hydro chloride) [31].

2.2. Fruit preparation and aqueous extraction of pigments

Freshly harvested prickly pear fruits (*Opuntia ficus-indica* – with similar maturity and weight) were used as raw materials and they were procured from the local venders near Erode, TamilNadu, and were stored at 4 °C prior to the experiments. The thick layer or skin and thorns were peeled from the fruits manually and the fruits were washed thoroughly in running tap water to get rid of any impurities adhered to the surface of the fruit and cut into required weight for the extraction of pigments from the fruits. The prickly pear fruit with three different masses (0.5, 1 and 1.5 g) was taken in 250 ml Erlenmeyer flasks. 50 ml of distilled water was used as solvent and it was added to each flask in order to keep the fruit fully immersed in the solvent. Erlenmeyer flask was covered with a plastic wrap during the experiments to prevent evaporation of solvent. The flasks containing fruits along with solvent were incubated at different temperatures (30, 40, 50 °C) and the extracts were taken at different time intervals (20, 70, 120 min). After extraction for a selected time, the mixture was centrifuged at 2268 *g* for 15 min (Remi R-24 Centrifuge, India) and supernatant liquid was collected for the determination of pigments from the prickly pear fruits. Experiments were performed randomized in order to minimize the effects of unexplained variability in the observed responses due to extraneous factors. All the experiments were performed in triplicate and the average value was used for the determination of pigments (betacyanin and betaxanthin) from the prickly pear.

2.3. Total betacyanin and betaxanthin content

A 10 ml of the extracts was mixed with 10 ml of 50% aqueous methanol solution and the solution was agitated (22.7 *g*) at room temperature for 30 min (GeNei SLM-IN-OS-16, India). After they were stirred, the samples were centrifuged at 4731 *g* for 15 min (Remi R-24 Centrifuge, India). Supernatants were filtered through a filter paper (Whatman no. 1, Whatman International Ltd., Maidstone, England), and the extracts obtained were analyzed spectrophotometrically (Shimadzu UV-1800, Kyoto, Japan). Spectrophotometric measurements were performed in triplicate, and the betaxanthin and betacyanin content was calculated [8]. Betaxanthin and betacyanin were assessed as indicaxanthin and betanin equivalents and they were computed by the following equation

$$\mathrm{BS(mg/100\ g)} = \frac{A \times \mathrm{DF} \times \mathrm{MW} \times 100}{\varepsilon \times 1} \quad (1)$$

where BS is betacyanin or betaxanthin, A is the absorption value at the absorption maximum corrected by the absorption at 600 nm, MW is the molecular weight (indicaxanthin = 308 g/mol and betanin = 550 g/mol), ε is the molar extinction coefficient (indicaxanthin = 48,000 L mol^{-1} cm^{-1} and betanin = 60,000 L mol^{-1} cm^{-1}), and 1 is the path length (1 cm) of the cuvette.

2.4. Experimental design

Response surface methodology (RSM) is an empirical statistical modeling technique employed for multiple regression analysis using quantitative data obtained from properly designed experiments to solve multivariate equations simultaneously [32]. RSM is an effective statistical technique for optimizing complex processes. RSM reduces the number of experimental trials required to evaluate multiple parameters and their interactions. In this study RSM was used to optimize and study the effect of independent variables such as extraction temperature, extraction time and mass of the fruits on the extraction of pigments (betacyanin (BC) and betaxanthin (BX)) from the prickly pear fruits. The experiments were established based on a Box–Behnken Design (BBD) with three factors at three levels and each independent variable was coded at three levels between +1, 0 and −1 corresponded to the low level, mid level and high level (Table 1). Coding of the variables was done according to the following equation:

$$x_i = \frac{X_i - X_{cp}}{\Delta X_i} \quad i = 1, 2, 3 \ldots k \quad (2)$$

where x_i, the dimensionless value of an independent variable; X_i, the real value of an independent variable; X_{cp}, the real value of an

Table 1
Independent variables and their levels used for Box–Behnken design.

Variables, unit	Factors	Levels		
	X	−1	0	1
Extraction temperature, Temp (°C)	X_1	30	40	50
Extraction time, Time (min)	X_2	20	70	120
Mass of the fruit, Mass (g)	X_3	0.5	1.0	1.5

independent variable at the center point; and ΔX_i, step change of the real value of the variable i corresponding to a variation of a unit for the dimensionless value of the variable i.

The number of experiments (N) required for the development of BBD is defined as $N = 2k\,(k-1) + C_0$ (where k is number of factors and C_0 is the number of central point). The design included 17 experiments and with 5 central points (used to determine the experimental error). The performance of the process was evaluated by analyzing the responses (Y), which depend on the input factors x_1, x_2..., x_k, and the relationship between the response and the input process parameters is described by

$$Y = f(x_1, x_2 \ldots x_k) + e \tag{3}$$

where f is the real response function the format of which is unknown and e is the error which describes the differentiation.

The behavior of the response surface was investigated for the response function (Y_i) using the second-order polynomial equation. The generalized response surface model is

$$Y = \beta_0 + \sum_{j=1}^{k}\beta_j x_j + \sum_{j=1}^{k}\beta_{jj}x_j^2 + \sum_{i}\sum_{<j=2}^{k}\beta_{ij}x_i x_j + e_i \tag{4}$$

where Y is the response; x_i and x_j are variables (i and j range from 1 to k); β_0 is the model intercept coefficient; β_j, β_{jj} and β_{ij} are interaction coefficients of linear, quadratic and the second-order terms, respectively; k is the number of independent parameters ($k = 3$ in this study); and e_i is the error [33].

2.5. Statistical analysis

The statistical analysis was performed using Design Expert Statistical Software package 8.0.7.1 (Stat Ease Inc., Minneapolis, USA). The experimental data were analyzed using multiple regressions and the significance of regression coefficients was evaluated by F-test. Modeling was started with a quadratic model including linear, squared and interaction terms and the model adequacies were checked in terms of the values of R^2, adjusted R^2 and prediction error sum of squares (PRESS). The significant terms in the model were found by Pareto analysis of variance (ANOVA) for each response and ANOVA tables were generated. The regression coefficients were used to make statistical calculations to generate response surface plots from the regression models.

2.6. Verification of optimized conditions and predictive models

Optimal conditions for the extraction of pigments from prickly pear fruit depend on extraction temperature, time and mass of fruit were obtained by Derringer's desired function methodology. The pigments (betacyanin and betaxanthin) content was determined after extraction under the optimal conditions. The experimental and predicted values were compared in order to determine the validity of the models.

3. Results and discussions

3.1. Box–Behnken analysis

The response surface methodology consists of an empirical modeling technique, which has been used to evaluate the relationship between the experimental and the predicted results [34]. To obtain a proper model for the optimization of the extraction process, the BBD, which is generally the best design for response surface optimization, was selected with three process variables (extraction temperature, extraction time and mass of the fruit) at three levels. Experiments were performed according to the experimental design in order to search the optimum conditions and study the effect of process variables on the extraction of pigments (betacyanin and betaxanthin) from prickly pear fruits. The predicted values were obtained by a model fitting technique using the Design Expert software version 8.0.7.1 and proved to be sufficiently correlated with the observed values. The experimental results and predicted values were exhibited in Table 2.

Fitting of the data to various models (linear, interactive, quadratic and cubic models) was carried out to obtain the regression equations. To decide about the adequacy of models among various models to represent the extraction of pigments from prickly pear fruits, two different tests namely sequential model sum of squares and model summary statistics were carried out in the present study [35], and the results are given in Table 3. The adequacy of model summary output (Table 3) indicates that, the quadratic model is statistically highly significant for the present extraction process. The sequential sum squares showed that the p-value was lower than 0.0001 for quadratic and cubic models. Model summary statistics showed that, quadratic model was found to have maximum "Adjusted R-Squared" and the "Predicted R-Squared" values. Cubic model was not recommended for the

Table 2
Box–Behnken experimental design matrix and experimental responses.

Run	Temperature (°C)	Time (min)	Mass (g)	Betacyanin (mg/100 g)			Betaxanthin (mg/100 g)		
				Y_{exp}	Y_{pre}	% Error	Y_{exp}	Y_{pre}	% Error
1	30	20	1	11.89	11.96	−0.58	18.72	18.83	−0.57
2	30	70	1.5	12.73	12.67	0.44	22.35	22.25	0.44
3	30	120	1	13.04	13.00	0.34	21.77	21.63	0.66
4	50	120	1	13.22	13.15	0.52	22.57	22.47	0.46
5[a]	40	70	1	12.96	12.96	0.00	22.5	22.50	0.00
6[a]	40	70	1	12.96	12.96	0.00	22.5	22.50	0.00
7	40	120	1.5	13.29	13.39	−0.75	24.27	24.51	−1.01
8	50	70	1.5	12.76	12.73	0.24	22	21.86	0.62
9	30	70	0.5	11.36	11.39	−0.28	18.85	18.99	−0.73
10[a]	40	70	1	12.96	12.96	0.00	22.5	22.50	0.00
11	50	20	1	11.86	11.90	−0.37	19.62	19.77	−0.74
12	40	20	1.5	11.54	11.53	0.11	20.72	20.71	0.03
13	50	70	0.5	11.38	11.44	−0.49	21.05	21.15	−0.48
14	40	20	0.5	11.06	10.96	0.90	20.02	19.78	1.21
15[a]	40	70	1	12.96	12.96	0.00	22.5	22.50	0.00
16	40	120	0.5	11.37	11.38	−0.11	21.47	21.48	−0.03
17[a]	40	70	1	12.96	12.96	0.00	22.5	22.50	0.00

[a] Central points (used to determine the experimental error).

Table 3
Adequacy of the model tested.

Source	Sum of squares	DF	Mean square	*F* value	Prob > *F*	Remarks
Sequential model sum of squares for betacyanin						
Mean	2601.53	1	2601.53			
Linear	5.93	3	1.98	6.42	0.0067	
2FI	0.53	3	0.18	0.51	0.6857	
Quadratic	3.43	3	1.14	191.32	<0.0001	Suggested
Cubic	0.04	3	0.01	6.4E+07	<0.0001	Aliased
Residual	0	4	0			
Total	2611.4704	17	153.62			
Sequential model sum of squares for betaxanthin						
Mean	7875.89	1	7875.89			
Linear	24.60	3	8.20	9.24	0.0015	
2FI	2.73	3	0.91	1.03	0.4191	
Quadratic	8.57	3	2.86	83.09	<0.0001	Suggested
Cubic	0.24	3	0.08	6.4E+07	<0.0001	Aliased
Residual	0	4	0			
Total	7912.0307	17	465.41			

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	Remarks
Model summary statistics for betacyanin						
Linear	0.56	0.597	0.504	0.325	6.71	
2FI	0.59	0.650	0.440	−0.096	10.89	
Quadratic	0.08	0.996	0.990	0.933	0.67	Suggested
Cubic	0	1	1	–	+	Aliased
Model summary statistics for betaxanthin						
Linear	0.94	0.681	0.607	0.459	19.56	
2FI	0.94	0.756	0.610	0.276	26.17	
Quadratic	0.19	0.993	0.985	0.893	3.85	Suggested
Cubic	0	1	1	–	+	Aliased

+ case(s) with leverage of 1.0000: PRESS statistic not defined.

Table 4
ANOVA analysis and statistical parameters of the model.

Source	Coefficient estimate	Sum of squares	DF	Standard error	Mean square	*F*-value	*p*-value
Betacyanin							
Model	12.96	9.89	9	0.03	1.10	183.77	<0.0001
X_1	0.03	0.01	1	0.03	0.01	0.84	0.3910
X_2	0.57	2.61	1	0.03	2.61	436.40	<0.0001
X_3	0.64	3.32	1	0.03	3.32	554.20	<0.0001
X_{12}	0.05	0.01	1	0.04	0.01	1.84	0.2167
X_{13}	0.002	0.00	1	0.04	0.00	0.00	0.9503
X_{23}	0.36	0.52	1	0.04	0.52	86.66	<0.0001
X_1^2	−0.11	0.05	1	0.04	0.05	8.13	0.0246
X_2^2	−0.35	0.52	1	0.04	0.52	86.22	<0.0001
X_3^2	−0.80	2.66	1	0.04	2.66	444.85	<0.0001
Residual error		0.04	7		0.01		
Lack of fit		0.04	3		0.01		
Mean	12.37						
C.V. %	0.63						
Adeq. Precision	40.96						
Betaxanthin							
Model	22.50	35.90	9	0.08	3.99	116.04	<0.0001
X_1	0.44	1.58	1	0.07	1.58	45.83	0.0003
X_2	1.38	15.13	1	0.07	15.13	440.00	<0.0001
X_3	0.99	7.90	1	0.07	7.90	229.83	<0.0001
X_{12}	−0.03	0.00	1	0.09	0.00	0.07	0.7952
X_{13}	−0.64	1.63	1	0.09	1.63	47.29	0.0002
X_{23}	0.53	1.10	1	0.09	1.10	32.07	0.0008
X_1^2	−1.19	6.00	1	0.09	6.00	174.55	<0.0001
X_2^2	−0.64	1.70	1	0.09	1.70	49.58	0.0002
X_3^2	−0.24	0.25	1	0.09	0.25	7.28	0.0307
Residual error		0.24	7		0.03		
Lack of Fit		0.24	3		0.08		
Mean	21.52						
C.V. %	0.86						
Adeq. Precision	40.00						

present extraction process as the Box–Behnken matrix has sufficient data to interpret the outcome of the present system [36]. Therefore, quadratic model was chosen for further analysis.

3.2. Fitting of second order polynomial equations and statistical analysis

The empirical relationship between the experimental results obtained on the basis of Box–Behnken experimental design model and the input variables were expressed by a second-order polynomial equation with interaction terms. The final equations obtained in terms of coded factors were given below

$$\begin{aligned}\text{Betacyanin} = {} & 12.96 + 0.025X_1 + 0.57X_2 + 0.645X_3 \\ & + 0.052X_1X_2 + 2.5E - 003X_1X_3 + 0.36X_2X_3 \\ & - 0.11X_1^2 - 0.35X_2^2 - 0.79X_3^2 \end{aligned} \tag{5}$$

$$\begin{aligned}\text{Betaxanthin} = {} & 22.5 + 0.44X_1 + 1.38X_2 + 0.99X_3 + 0.025X_1X_2 \\ & + 0.64X_1X_3 + 0.53X_2X_3 - 1.19X_1^2 - 0.64X_2^2 - 0.24X_3^2 \end{aligned} \tag{6}$$

However, the exploration and optimization of a fitted response surface might produce poor or misleading results [37]. So, it was necessary to check if the model exhibited a good fit or not. The adequacy and fitness of the models were tested by regression analysis and Pareto analysis of variance (ANOVA). The results indicated that the equation adequately represented the actual relationship between the independent variables and responses (Table 4). ANOVA is a statistical technique that subdivides the total variation in a set of data into component parts associated with specific sources of variation for the purpose of testing hypotheses on the parameters of the model [38]. Analysis of variance followed by Fisher's statistical test (*F*-test) was applied to evaluate the significance of each variable. The *F*-value is the ratio of the mean square due to regression to the mean square due to real error and indicates the influence (significance) of each controlled factor on the tested model [34]. The ANOVA results (Table 4) for BC and BX show Fisher *F*-value of 183.77 and 116.04, which implies that the model is significant and higher. The large value of *F* indicates that most of the variation in the response can be explained by the regression equation. The associated *p* value is used to estimate whether *F* is large enough to indicate statistical significance and *p* values lower than 0.05 indicate that the model and the terms are statistically significant [39].

Coefficient of determination (R^2) and adjusted-R^2 were also calculated to check the adequacy and fitness of the model. The R^2 gives the proportion of the total variation in the response predicted by the model, indicating the ratio of the sum of squares due to regression (SSR) to the total sum of squares (SST) [40]. The values of R^2 were calculated to be 0.996 and 0.993 for BC and BX respectively, which imply that 95% of experimental data was compatible. A high R^2 coefficient ensures a satisfactory adjustment of the quadratic model to the experimental data. The use of an adjusted-R^2 is to evaluate the model adequacy and fitness. The adjusted-R^2 value corrects the R^2 value for the sample size and for the number of terms in the model. The value of adjusted-R^2 (0.990 for BC and 0.985 for BX) is also high and advocates a high correlation between the observed and the predicted values. The very small *p*-value (<0.0001) and high coefficient of determination ($R^2 = 0.996$ for BC and $R^2 = 0.993$ for BX) also show that the quadratic polynomial model is significant and sufficient to represent the actual relationship between the response and independent variables.

The coefficient of variation (CV) indicates the relative dispersion of the experimental points from the predictions of the SOP models. As a general rule, the CV should not be greater than 10% and a high CV indicates that variation in the mean value is high and does not satisfactorily develop an adequate response model [41]. The very low value (0.63 and 0.86) of CV clearly representing a very high degree of precision and a good reliability of conducted experiments. Adequate precision measures the signal to noise ratio and compares the range of the predicted values at the design points to the average prediction error. The ratio greater than 4 is desirable and indicates adequate model discrimination [42]. In this work the ratio is found to be >39, which indicates an adequate signal. Therefore, quadratic model can be used to navigate the design space.

3.3. Adequacy of the models

Generally, it is important to confirm the fitted model to make sure that it gives a sufficient approximation to the actual values. Unless the model shows a satisfactory fit, proceeding with an investigation and optimization of the fitted response surface likely gives poor or misleading results [43]. Diagnostic plots such as the predicted versus experimental values (Fig. 1) help us to judge the model satisfactoriness and exhibit the relationship between predicted and experimental values. In this figure, each of the observed values is compared to the predicted value calculated from the model. The data points on this plot lie reasonably close to the straight line and indicate an adequate agreement between the real data and the data obtained from the models. The result suggests that the models used in this research were able to identify operating conditions for selective extraction of pigments from prickly pear fruit.

Data were also analyzed to check the normality of the residuals. Normal probability plot represents the normal distribution of the residuals and the residual gives the difference between the observed value of a response measurement and the value that is fitted under the theorized model, and the small residual value indicates that model prediction is accurate [44]. By constructing a normal probability plot of the residuals, a check was made for the normality assumption as shown in Fig. 2 and the data points on this plot lie reasonably close to a straight line. But, some scatter is expected even with normal data, therefore, it could be concluded that the data was normally distributed.

3.4. Effect of process variables on the extraction of pigments

Pigments from the prickly pear fruit were extracted by the aqueous extraction method and the extraction process was carried out with different extraction temperatures, different extraction times and with different mass of the fruits with an explicit objective of determining optimum extraction conditions. 3D response surface and contour plots were used to represent the effect of process variables on the extraction of pigments from prickly pear fruit. The response surface and contour plots showed the relative effects of any two variables when the remaining variable was kept as constant. The response surface plots estimating the specific

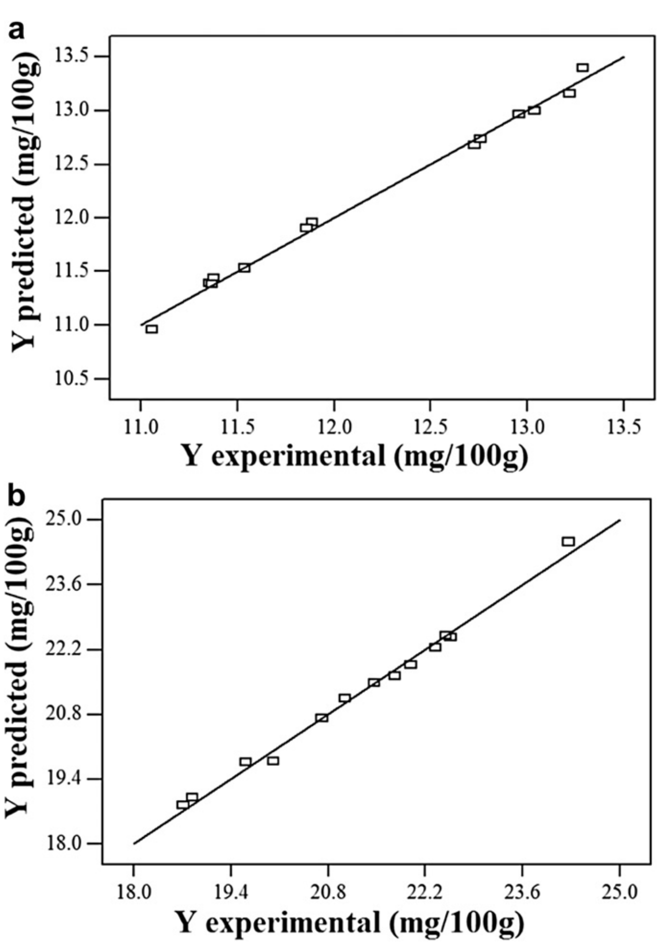

Fig. 1. Comparison between predicted and experimental values of betacyanin (a) and betaxanthin (b).

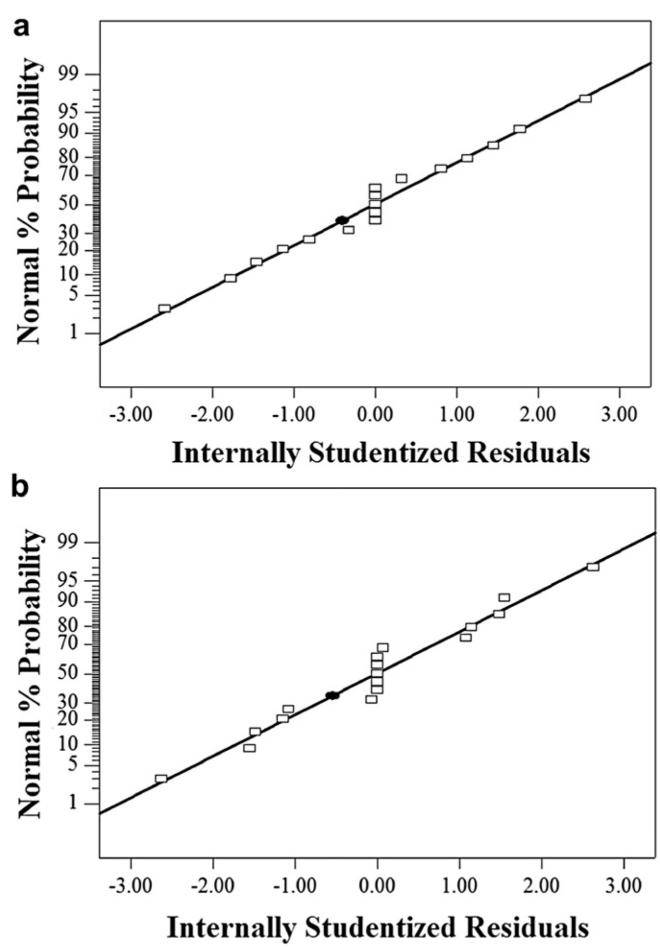

Fig. 2. Normal probability plots of studentized residuals for betacyanin (a) and betaxanthin (b).

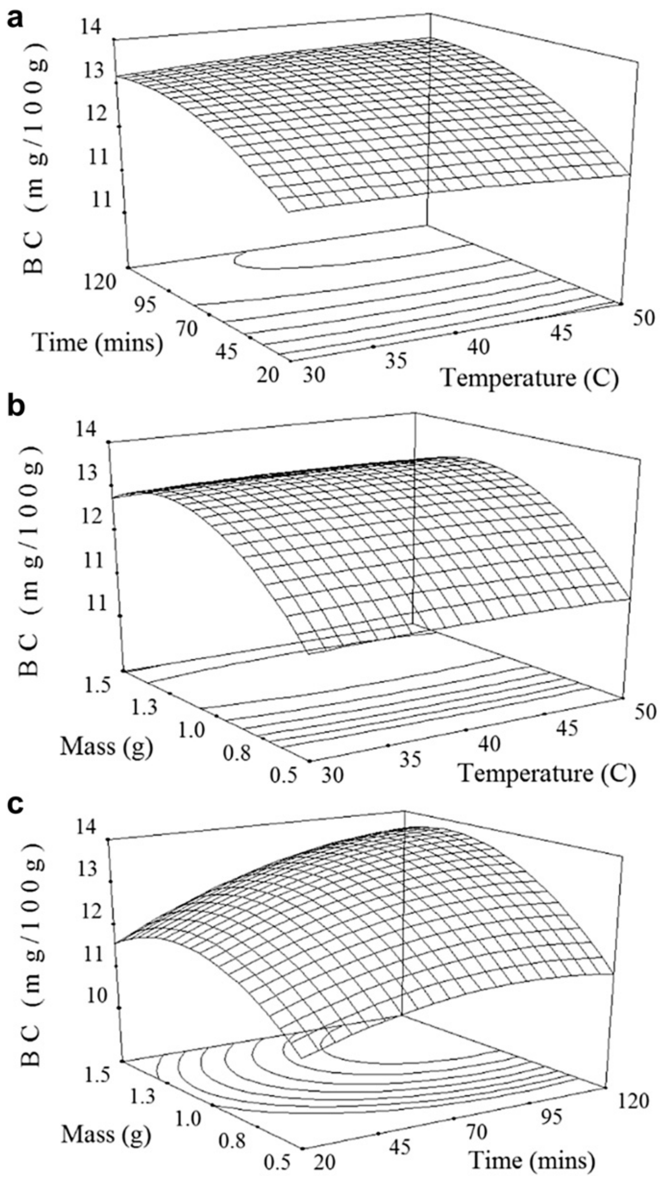

Fig. 3. Response surface plots (3D) showing the effects of variables on the betacyanin yield.

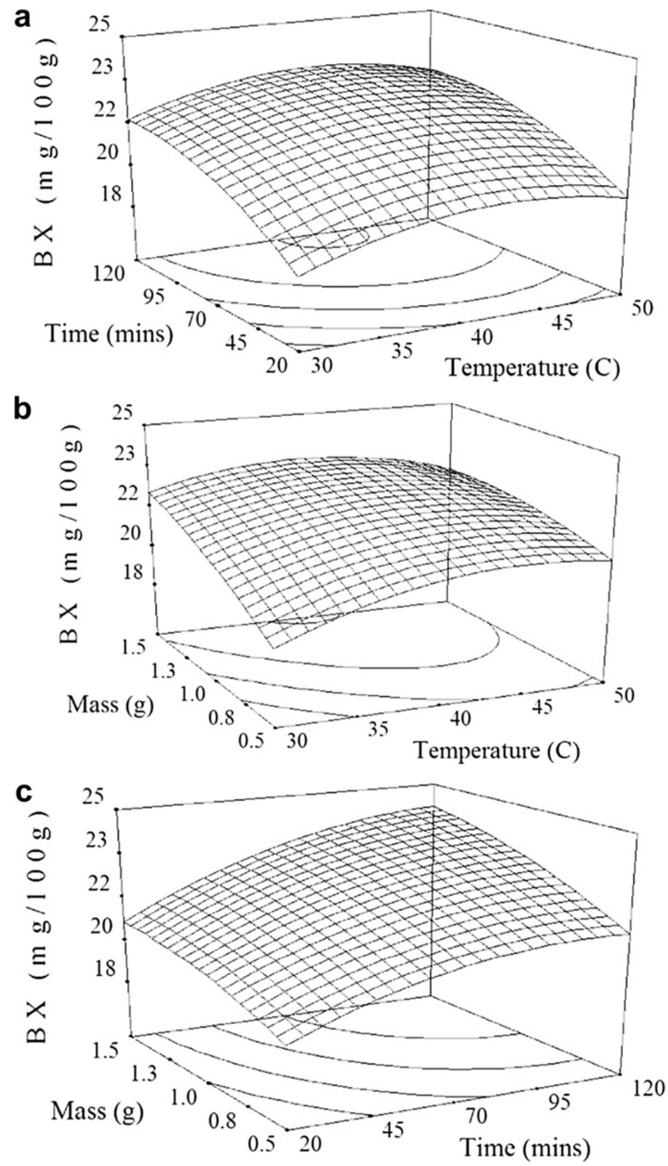

Fig. 4. Response surface plots (3D) showing the effects of variables on the betaxanthin yield.

surface area of pigments extraction versus independent variables are presented in Figs. 3 and 4.

Fig. 3 shows the effects of temperature, time and mass on the extraction of betacyanin pigments from the prickly pear fruit. Extraction time and mass of the fruit were significantly ($p < 0.0001$) affected the total betacyanin yield in both linear and quadratic manners. Both independent variables showed positive effects in linear terms but showed a negative effect on its quadratic terms. The interactive effects between time and mass showed a positive effects and also significant ($p < 0.0001$). Pigments extraction from the fruit depends on both the amount of fruit used for extraction (0.5–1.5 g) as well as the extraction time (20–120 min) (Fig. 3c). Fig. 3a shows that the yield of betacyanin increased with the increasing of extraction temperature and extraction time. Woo et al. [45] showed that, the elevated temperature of 50 °C did not show any effect on the stability of betalain pigments from red dragon fruits. The higher temperature would cause softening of the plant tissue, disrupting the

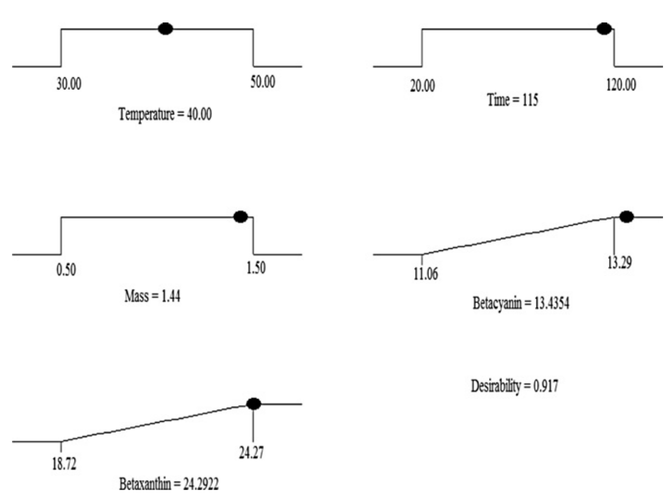

Fig. 5. Desirability ramp for optimization.

Table 5
Predicted and experimental values of the responses at optimum conditions.

Optimal levels of process parameters	Optimized values[a] (predicted values)		Experimental values[b]	
	BC (mg/100 g)	BX (mg/100 g)	BC (mg/100 g)	BX (mg/100 g)
Extraction temperature (°C) = 40 Extraction time (min) = 115 Mass of fruit (g) = 1.44	13.4354	22.2922	13.32 ± 0.15	24.09 ± 0.21

[a] Predicted using response surface quadratic model.
[b] Mean ± standard deviation of triplicate determinations from experiments.

interactions between phenolic compounds and protein or polysaccharides and increasing the solubility of the phenolic compounds, which improves the rate of diffusion, thus giving a higher rate of extraction [46]. The response surface plot (Fig. 3b) demonstrates the interaction effect of the process variables (extraction temperature and mass of fruits) on the betacyanin extraction process. Both the variables have a strong effect on the extraction efficiency and the total amount of betacyanin yield was increased with an increase in both the temperature as well as the mass of fruits used for the extraction. It can be seen that (Table 4) the positive linear effect and negative quadratic effects of time and mass explained the observed nature of the curve.

From Table 4, it may be observed that betaxanthin content depends on the extraction time and mass of the fruit. The linear effect of the extraction time and mass were positive ($p < 0.0001$) whereas its quadratic effect was negative at $p < 0.05$, which resulted in a curvilinear increase in betaxanthin yield. The interactive effect between the extraction time and mass is positive at $p < 0.001$. Fig. 4a shows the effect temperature and time on the extraction of betaxanthin from prickly pear. By increasing the temperature and time, total betaxanthin content is increased significantly. Extraction temperature had a close relationship with the extraction of betaxanthin pigments. So with the increase of temperature from 30 to 50 °C, total betaxanthin yield can be increased with the increase of mass of fruits (Fig. 4b). The betaxanthin content is increased with increasing mass and time, which is probably due to the fact that the solvent can enter into the high mass of cells while more pigments can permeate into the solvent under the higher extraction time and mass of the fruits (Fig. 4c).

3.5. Selection of optimum conditions

An optimum condition for the extraction of pigments from prickly pear was determined to obtain maximum betacyanin content and betaxanthin content. Second order polynomial models obtained in this study were utilized for each response in order to obtain specified optimum conditions. The Derringer's desirability function method was employed to optimize the process variables. This function searches for a combination of factor levels that jointly optimize a set of responses by satisfying the requirements for each response in the design. The optimization is accomplished by: converting each response Y_i ($i = 1, 2, \ldots, m$) into a dimensionless desirability scale that defines a partial desirability function (d_i), combining the individual desirabilities to obtain the composite or global desirability function (D), and finally maximizing the D and identifying the optimal factor settings. The scale of the desirability function ranges between 0 (completely undesirable response) and 1 (fully desired response) [47].

The individual desirabilities (d) for each response are obtained by specifying the goals, i.e., minimize, maximize or target the response, and boundaries required for each one. A weight factor, which defines the shape of the desirability function for each response, is then assigned. Weights must be between 0.1 and 10, with larger weights corresponding to more important responses. A weight factor of 1 was chosen for all individual desirabilities in this work. The "importance" of a goal can be changed in relation to the other goals. It can range from 1 (least importance) to 5 (most important). The default is for all goals to be equally important in a setting of 3.

In order to optimize extraction of pigments from prickly pear the following constraints have taken (1) Extraction temperature (30 °C–50 °C), (2) Extraction time (20–120 min) and (3) Mass of fruit (0.5–1.5 g) respectively, were set for maximum desirability. In numerical optimization, the desired goal was preferred for each variable and response from the menu. The possible goals were: within range (for three independent variables) and maximum (for responses only). Applying the methodology of the desired function, the optimum level of various parameters was obtained and it indicates that extraction temperature of 40 °C, Time of 115 min and mass of 1.44 g give 13.4354 mg/100 g of betacyanin content and 24.2922 mg/100 g of betaxanthin content respectively with overall desirability value of 0.917. Fig. 5 showed a ramp desirability that was developed from optimum points via numerical optimization.

3.6. Verification of optimized conditions and predictive model

The suitability of the model equations for predicting optimum response values was tested under the conditions: extraction temperature 40 °C, Time 115 min and mass of 1.44 g. This set of conditions were determined to be optimum by the RSM optimization approach and to confirm the validity of the optimized conditions; experiments were carried out to compare the experimental results with the predicted values of the responses using the model equation. The experiments were conducted in triplicates and the average values were reported in Table 5. The efficiency on the extraction of pigments (betacyanin and betaxanthin) under these optimum conditions was found to be 13.4354 mg/100 g and 24.2922 mg/100 g, and the experimental value was 13.32 ± 0.15 mg/100 g and 24.09 ± 0.21 mg/100 g. The mean values of the pigment content obtained were compared with the predicted values and indicating the suitability of the developed quadratic models. The percentage deviation of the experimental and theoretical results was found as 0.87 and 0.84% respectively. The pigments obtained through confirmation experiments are within 95% of predicted values. This indicates the suitability of the developed quadratic models and it may be noted that these optimal values are valid within the specified range of process parameters.

4. Conclusion

In this study, the statistical methodology, Box–Behnken Response Surface design is demonstrated to be effective and reliable in finding the optimal conditions for the extraction of pigments from prickly pear fruits. The results showed that, the extraction conditions have significant effects on the extraction of pigments. The response surface plots were used for estimating the interactive effect of three independent variables (extraction temperature, °C; extraction time, min; and mass of fruit, g) on the responses (betacyanin and betaxanthin). Second order polynomial models were developed for predicting betacyanin and betaxanthin content. Analysis of variance showed a high coefficient of determination value (R^2) of 0.996 for betacyanin and 0.993 for betaxanthin ensuring a satisfactory fit of the second-order polynomial regression model with the experimental data. The optimum conditions for extraction of pigments from prickly pear fruit were found to be, extraction temperature of 40 °C, extraction time of 115 min and mass of 1.44 g have been selected to obtain the maximum yield of pigments (betacyanin and betaxanthin) of 13.435 mg/100 g and 22.2922 mg/100 g. Under these optimized conditions the experimental values of pigments agreed closely with the predicted yield.

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