ORIGINAL ARTICLE



# Extraction of natural anthocyanin and colors from pulp of jamun fruit

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Abstract In this present study, natural pigment and colors from pulp of jamun fruit were extracted under different extraction conditions such as extraction temperature (40–60  $^{\circ}$ C), time (20-100 min) and solid-liquid ratio (1:10-1:15 g/ml) by aqueous extraction method. Three factors with three levels Box-Behnken response surface design was employed to optimize and investigate the effect of process variables on the responses (total anthocyanin and color). The results were analyzed by Pareto analysis of variance (ANOVA) and second order polynomial models were developed to predict the responses. Optimum extraction conditions for maximizing the extraction yield of total anthocyanin (10.58 mg/100 g) and colors (10618.3 mg/l) were found to be: extraction temperature of 44 °C, extraction time of 93 min and solid-liquid ratio of 1:15 g/ml. Under these conditions, experimental values are closely agreed with predicted values.

Keywords Extraction  $\cdot$  Jamun fruit  $\cdot$  Anthocyanin  $\cdot$  Color  $\cdot$  Box-Behnken design

# Introduction

Pigments are one of the most important external qualities of foods that determine their acceptance by consumers. Currently pigments of various kinds and forms have been used as additives or supplements in food industries, cosmetics, pharmaceuticals, livestock feed and other applications (Boo et al. 2011). Food industry adds pigments to manufactured products

R. Sridhar TamilNadu Pollution Control Board, Salem, TN 635004, India in order to intensify their character. Color is an important factor for appeal, a major criterion of identification, indicator of quality and freshness and is a determinant of consumer acceptability, image, market size and value. In the past, synthetic pigments were used indiscriminately to manipulate food color. However, synthetic pigments are hardly nutrient, and have been blamed for toxic to different extents and some are found to be carcinogenic (Zhang et al. 2006). The use of synthetic food colourants is restricted by legal regulations of both international and national authorities (Masár et al. 1996). Hence, there is a growing demand for eco-friendly/non-toxic colorants, specifically for health sensitive applications such as coloration of foods and dyeing of child textile and leather garments.

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 under-utilized plants (Prakash Maran and Manikandan 2012). Indian blackberry (Syzygium cumini L.), commonly known as jamun fruit and it is an under-utilized minor fruit of the Indian subcontinent. The plant belongs to Myrtaceae family and grows naturally in clayey loamy soil in tropical as well as sub-tropical zones of Indo-gangetic plains. The fruit concentrate of jamun has a very long history of use for various medicinal purposes and currently it has been used for the treatment of chronic diarrhea and other enteric disorders, including its use as an antimicrobial agent (Achrekar et al. 1991; Migliato 2005). These beneficial effects are most probably due to the presence antioxidant properties of anthocyanin pigments. Anthocyanins are considered as potential replacements for synthetic colouring agent because of their bright attractive colors and water solubility (Kong et al. 2003). The incorporation of anthocyanin in to the food systems not only increases the color intensity but

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also increases the medicinal and therapeutic values of the food products.

Development of an economic and efficient conventional extraction method for the extraction of biologically active compounds from jamun fruit provides an opportunity to replace the synthetic colour. From the analysis of lierature it is found that, no publication is reported to the extraction of anthocyanin and colors from jamun fruit. Hence in the present study an attempt has been made to investigate and optimize the individual and the interactive effect of process variables such as extraction temperature, time and solid–liquid ratio on the maximum extraction yield of total anthocyanin and colors from jamun fruit pulp using Box-Behnken response surface design coupled with Derringer's desired function methodology.

# Materials and methods

## Raw materials and chemicals

Freshly harvested jamun fruits (*Syzygium cumini*– 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. Cyanidin-3-glucoside (HPLC grade) was purchased from Sigma chemicals, Mumbai. Potassium chloride (AR grade) and so-dium acetate (AR grade) were supplied by Merck chemicals, Chennai.

#### Aqueous extraction of pigments and colors

Fruits were washed thoroughly in running tap water to get rid of the impurities adhered over its surface. Pulp of the fruits were peeled manually and pulp was dried in the hot air oven at 40°C until it attains constant weight. The dried pulps were ground using a grinder and sieved (150 mesh) to<1 mm particle size. Because, size of the particle is considered as an important variable in an extraction process. Previous studies have shown that, the finest particles results with the highest extraction yield, because of the increase in exchange surface area and decrease in path length travelled by the solute to reach the surface, which in turn helps to reduce the extraction time (Pinelo et al. 2005; Silvaa et al. 2007). Accordingly a dried milled fruit sample (particle size of <1 mm) was used in this study, which would not hinder the experimental work at the filtration step. Aqueous extraction process was performed in a temperature controlled water bath and distilled water was used as a solvent under different aqueous extraction conditions (extraction temperature (40-60 °C), time (20-100 min) and solid-liquid (SL) ratio (1:5-1:15 g/ml). Different solidliquid ratios of samples (1:5, 1:10 and 1:15) were taken in 150 ml Erlenmeyer flasks and it was incubated at different temperature (40, 50 and 60 °C) in a temperature controlled water bath. Extracts were taken at different time intervals (20, 60 and 100 min). During the extraction process, Erlenmeyer flasks were covered with a plastic wrap in order to prevent the evaporation of solvent. After extraction for a selected time, the mixture was centrifuged at 6500 rpm for 15 min (Remi R-24 Centrifuge, India) and supernatant liquid were collected for the determination of pigments and colors from jamun fruits. Experiments were performed randomized in order to minimize the effects of unexplained variability in the observed responses due to extraneous factors. All experiments were done in triplicates and the average values were recorded and are shown in Table 1.

Determination of total monomeric anthocyanin content (TMAC)

pH differential method (Maran et al. 2013a) was used to estimate the total monomeric anthocyanin content (TMAC) of the extracts. The method was based on the anthocyanin structural transformation that occurs when pH changes. Aliquots of the extracts were diluted with pH 1.0 and 4.5 buffers using potassium chloride (0.025 M) and sodium acetate (0.4 M) respectively, and then placed in the dark conditions for 1 h. The absorbance of each solution was recorded using a spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) calibrated with distilled water as the blank at the wavelength of 530 and 700 nm. Difference in absorbance between pH values and wavelengths was calculated using the formula:

$$A = (A_{530nm} - A_{700nm})_{pH1.0} - (A_{530nm} - A_{700nm})_{pH4.5}$$
(1)

The total monomeric anthocyanin content was obtained by

$$TMAC = \frac{A \times MW \times DF \times 1000}{\varepsilon \times 1}$$
(2)

where, A is the absorbance; MW is the molecular weight of cyanidin-3-glucoside (449.2 g mol<sup>-1</sup>); DF is the dilution factor;  $\varepsilon$  is the molar absorptivity of cyanidin-3-glucoside (26,900 L cm<sup>-1</sup> mol<sup>-1</sup>) and 1 is path length.

# Analysis of total color in solution (TCS)

After extraction process, the determination of mass of total color in solution (TCS) was analyzed according to the method described by Sinha et al. (2012). The obtained extracts were filtered using filter paper (What man No.1, England) and filtered extracts were dried in a hot air oven (NSW, India) until all the water gets evaoparated. The dishes were then

Table 1 Un-coded and coded box-behnken experimental design matrix with experimental and predicted response values

Run	Temperature (C)	Time (min)	Solid–liquid ratio (g/ml)	TMAC (mg/100 g)			TCS (mg/l)		
				Y <sub>exp</sub>	Y <sub>pre</sub>	% Error	Y <sub>exp</sub>	Y <sub>pre</sub>	% Error
1	60 (1)	100 (1)	1:10 (0)	8.51	8.56	-0.59	8689.48	8753.05	-0.73
2*	50 (0)	60 (0)	1:10(0)	9.58	9.59	-0.10	9556.37	9554.94	0.01
3	40 (-1)	100 (1)	1:10(0)	8.62	8.74	-1.39	8937.08	8970.95	-0.38
4	40 (-1)	20 (-1)	1:10(0)	7.39	7.34	0.68	7365.2	7301.61	0.86
5*	50 (0)	60 (0)	1:10(0)	9.61	9.59	0.21	9560.28	9554.94	0.06
6	50 (0)	20 (-1)	1:15(1)	9.12	9.16	-0.44	9078.49	9101.32	-0.25
7	60 (1)	60 (0)	1:05 (-1)	8.69	8.68	0.12	8954.23	8913.48	0.46
8	40 (-1)	60 (0)	1:05 (-1)	6.59	6.52	1.06	6551.61	6540.56	0.17
9	60 (1)	60 (0)	1:15(1)	8.34	8.42	-0.96	8422.49	8433.54	-0.13
10	60 (1)	20 (-1)	1:10(0)	8.47	8.36	1.30	8592.39	8558.51	0.39
11	50 (0)	20 (-1)	1:05 (-1)	8.04	8.16	-1.49	8038.37	8113	-0.93
12	50 (0)	100 (1)	1:15(1)	10.56	10.44	1.14	10493	10418.4	0.71
13*	50 (0)	60 (0)	1:10(0)	9.57	9.59	-0.21	9548.18	9554.94	-0.07
14	50 (0)	100 (1)	1:05 (-1)	8.53	8.48	0.59	8682.62	8659.78	0.26
15	40 (-1)	60 (0)	1:15 (1)	9.73	9.74	-0.10	9726.72	9767.46	-0.42

Yexp - Experimental values; Ypre - Predicted values; \* Central points (used to determine the experimental error)

cooled in a desiccator and weighed. The drying, cooling and weighing procedure was repeated to get a constant weight and thus weight of the extract was determined. The weight of the colorant extract obtained per gram of the jamun fruit pulp was then calculated. and the coding was done by the following equation (Prakash Maran et al. 2013a).

$$x_i = \frac{X_i - X_z}{\Delta X_i}$$
  $i = 1, 2, 3.....k$  (4)

# Experimental design

In this study, Box-Behnken response surface experimental design (BBD) with three factors at three levels was used to optimize and investigate the influence of process variables such as extraction temperature (40 - 60 °C), time (20–100 min) and solid–liquid ratio (1:5– 1:15 g/ml) on the TMAC and TCS of the extracts. Process variables and their ranges were determined based on the single factor experimental analysis. After selection of process (independent) variables and their ranges, experiments were established based on a BBD and the complete design consists of 15 experiments with three centre points (used to estimate the experimental error). The total number of experiments was calculated from the following equation (Prakash Maran et al. 2013a).

$$N = 2K(K-1) + C_0 (3)$$

where, K is number of factors and  $C_0$  is the number of central point. For statistical calculations, the process variables were coded at three levels (-1, 0 and +1)

where  $x_i$ , is the dimensionless value of an independent variable;  $X_i$ , the real value of an independent variable;  $X_z$ , the real value of an independent variable at the centre point; and  $\Delta X_i$ , step change of the real value of the variable *i*.

For predicting the optimal point after performing experiments, a second-order polynomial equation was fitted to correlate the relationship between independent variables and responses, which accounts for variations caused by linear, quadratic and interactive effect of the process variables. The mathematical form of second-order polynomial equation is given below

$$Y = \beta_0 + \sum_{j=1}^{k} \beta_j X_j + \sum_{j=1}^{k} \beta_{jj} X_j^2 + \sum_{i=1}^{k} \sum_{j=1}^{k} \beta_{ij} X_i X_j + e_i$$
(5)

where, Y is the response;  $X_i$  and  $X_j$  are variables (i and j range from 1 to k);  $\beta_0$  is the model intercept coefficient;  $\beta_j$ ,  $\beta_{jj}$  and  $\beta_{ij}$  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 (Prakash Maran et al. 2013b).

## Statistical analysis

The experimental data was analyzed by multiple regression analysis through the least square method. Sequential sum of squares and model summary statistics was carried out on the experimental data to evaluate the adequacy of various models (linear, interactive (2FI), quadratic and cubic). Regression coefficients of linear, quadratic, and interaction involved in the model and their effects were analyzed by Pareto analysis of variance (ANOVA) and ANOVA tables were generated. All the terms in the model were tested by student's F-test and significance of the F-values at probability levels ( $p \le 0.05$ ) were analyzed. The experimental data were analysed with various statistical analysis such as sum of squares (SS), determination coefficient (R<sup>2</sup>), adjusted determination of coefficient  $(R_a^2)$ , predicted determination of coefficient  $(R_a^2)$  and coefficient of variation (CV) to reflect the statistical significance of the fitted polynomial equation. All the statistical analyses were done with the help of Stat ease Design Expert 8.0.7.1 statistical software package (Stat-Ease Inc., Minneapolis, USA). After fitting the data to the models, the models were used for the construction of three dimensional (3D) response surface plots to predict the relationships between independent and dependent variables.

## Determination of optimal conditions

After analyzing the polynomial equation depicting the effect of independent variables on the responses, optimization process was carried out by Derringer's desired function methodology (Maran et al. 2013a). This numerical Optimization technique will optimize any combination of one or more goals; these may be either process variables or responses. The possible goals are: maximize, minimize, target, within range, none (for responses only) and set to an exact value (factors only). In this study, goals of the process variables were selected as in a range and the response goals were selected as maximize. A weight factor of 1 was chosen for the response, which can be used to adjust the shape of its particular desirability function. The default value of one creates a linear ramp function between the low value and the goal or the high value and the goal. Default importance of 3 was chosen for the response, which can represent the goals to be equally important.

#### Validation of optimized conditions

After optimization, adequacy of the model equation for predicting the optimum response values was validated under the optimal conditions. Triplicate verification experiments were performed under the optimal conditions and the average value of the experiments was compared with the predicted values of the developed model equations.

# **Results and discussions**

Natural pigments and colors from jamun fruit pulps were extracted by aqueous extraction method and the extraction process was carried out at different extraction temperatures, extraction times and solid–liquid ratio with an explicit objective of studying the effect of process variables and determining the optimum extraction conditions on the extraction yield of pigments and colors. In this study, three factors with three levels BBD was used to evaluate the effect and optimize the process variables on the responses. A total number of 15 batch experiments including three centre points were carried out in triplicates using statistically deigned experiments and the results (means values) are shown in Table 1.

## BBD analysis

The experimental data was analyzed by two different tests namely the sequential model sum of squares and model summary statistics in order to obtain regression models and decide about the adequacy of various models (linear, interactive, quadratic and cubic) to represent the extraction of pigments and color from jamun fruit pulp. The results are listed in Table 2. From the Table 2, it is found that, linear and interactive (2FI) models are exhibited lower R<sup>2</sup>, adjusted R<sup>2</sup>, predicted R<sup>2</sup> and also having high *p*-values, when compared with quadratic model. Cubic model is found to be aliased. Therefore the quadratic model is chosen to describe the effects of process variables on the extraction of natural pigments and colors from jamun fruit pulp. Further more, analysis of variance (ANOVA) is also used to check the adequacy of quadratic model.

### Model fitting

The results obtained from BBD experiments were evaluated by multiple regression analysis method for total extraction of natural pigments and colors from Jamun fruit pulp. An empirical relationship between the response and independent variables has been expressed by a second-order polynomial equation with interaction terms was fitted between the experimental results obtained on the basis of BBD, which will help to predict the extraction efficiency of different sets of combinations of three process variables on the responses. Two empirical models were developed to understand the interactive correlation between the responses and process

Source	Sum of squares	DF	Mean square	F Value	Prob>F	Remarks
Sequential model	sum of squares for total mo	onomeric anthocya	nin content			
Mean	1150.19	1	1150.19			
Linear	5.98	3	1.99	2.78	0.0908	
2FI	3.62	3	1.21	2.27	0.1574	
Quadratic	4.18	3	1.39	91.00	< 0.0001	Suggested
Cubic	0.08	3	0.03	58.23	0.0169	Aliased
Residual	8.67E-04	2	4.33E-04			
Total	1164.05	15	77.60			
Sequential model	sum of squares for total co	lor in solutions				
Mean	1.7E+09	1	1.7E+09			
Linear	6.05E+06	3	2.02E+06	2.99	0.0772	
2FI	4.13E+06	3	1.38E+06	3.35	0.0760	
Quadratic	3.26E+06	3	1.09E+06	207.06	< 0.0001	Suggested
Cubic	2.61E+04	3	8708.34491	228.39	0.0044	Aliased
Residual	76.2580667	2	38.1290333			
Total	1.18E+09	15	7.86E+07			
Source	Std. Dev.	$\mathbb{R}^2$	Adjusted R <sup>2</sup>	Predicted R <sup>2</sup>	PRESS	Remarks
Model summary s	statistics for total monomeri	c anthocyanin con	itent			
Linear	0.85	0.4316	0.4316	0.2765	-0.07	
2FI	0.73	0.6930	0.6930	0.4627	-0.01	
Quadratic	0.12	0.9945	0.9945	0.9845	0.91	Suggested
Cubic	0.02	0.9999	0.9999	-	+	Aliased
Model summary s	statistics for total color in so	olutions				
Linear	820.67	0.4495	0.2994	-0.06	1.42E+07	
2FI	640.44	0.7562	0.5733	0.21	1.06E+07	
Quadratic	72.39	0.9981	0.9945	0.97	4.18E+05	Suggested
Cubic	6.17	0.9992	0.9954	-	+	Aliased

+ Case (s) with leverage of 1.0000: Press statistic not defined

variables. The final model obtained in terms of coded factors is given below

$$TMAC = 9.59 + 0.21X_1 + 0.40X_2 + 0.74X_3$$
(6)  
$$-0.30X_1X_2 - 0.87X_2X_3 + 0.24X_2X_3 -1.03X_1^2 - 0.31X_2^2 - 0.22X_3^2$$

$$TCS = 9554.94 + 259.75X_1 + 465.97X_2 + 686.74X_3$$
(7)  
$$-368.7X_1X_2 - 926.71X_2X_3 + 192.58X_2X_3$$
  
$$-909.14X_1^2 - 249.77X_2^2 - 232.04X_3^2$$

TCS = 0.554.04 + 250.75V + 465.07V + 696.74V

#### Adequacy of models

The adequacy of models was evaluated by constructing diagnostic plots such as predicted versus actual and normal probability plots for the experimental data obtained from this study and it is shown in Fig. 1. From the Figs. 1 a-b, it is observed that, the data points on this plot lie very close to the diagonal line, because residuals for the prediction of each response is minimum and it indicated a good adequate agreement between experimental data and the data predicted by the developed models (Maran et al. 2013b). Normal probability plot is a suitable graphical method for judging residuals normality. The observed residuals are plotted against the expected values, as they lie reasonably close on a straight line and show no deviation of variance (Fig. 1 c-d). This confirms the normal distribution of the observed data and adequacy of the developed models.

#### Statistical analysis

Pareto analysis of variance (ANOVA) and multiple regression analysis were used to analyze the observed data. Significance of the developed model equations was evaluated by their corresponding F and p-values and it is shown in Table 3. The higher model F values (100.05 for TMAC and 284.80





for TCS) and lower *p*-values (p < 0.0001) demonstrated that, the developed model was highly significant. The goodness of fit of the model was evaluated by the determination coefficient ( $\mathbb{R}^2$ ), adjusted determination co-efficient ( $\mathbb{R}^2_a$ ), predicted determination co-efficient  $(R_p^2)$  and co-efficient of variance (CV) and signal to noise ratio (S/N). The high  $R^2$  values (0.9945 for TMAC and 0.9981 for TCS) revealed that, the models are statistically significant and only small variations (0.0055 for TMAC and 0.0019 for TCS) are not explained by the models (Maran et al. 2013c). The value of  $R_p^2$  (0.9125 for TMAC and 0.9689 for TCS) is in reasonable agreement with the value of  $R_a^2$  (0.9845 for TMAC and 0.9945 for TCS) and also shows that the form of the model chosen to explain the relationship between the factors and the response is wellcorrelated (Maran et al. 2013d). Lower CV values (1.41 for TMAC and 0.82 for TCS) clearly stated that, the deviations between experimental and predicted values are low and also showed a high degree of precision (Maran et al. 2013e) and reliability of the conducted experiments (Prakash Maran et al. 2013c). In this present study, the ratio was S/N found to be> 38 (Table 3), which indicates the best fitness of the developed models (Maran et al. 2014).

# Effect of process variables

Three dimensional (3D) response surface plots were plotted from the developed models in order to study the individual and interaction effect among process variables on the responses (Maran et al. 2013f) and also used to determine the optimal condition of each factor for maximum extraction yield of natural pigments and colors of jamun fruits. In this study, the model has more than two factors and hence 3D plots are drawn by maintaining one factor at a constant level (in turn at its central level), whereas the other two factors are varied in their range (Prakash Maran et al. 2013d).

#### Effect of extraction temperature

Experiments were carried out to study the effect of temperature (40, 50 and 60 °C) over the extraction yield of pigments and colors from jamun fruit pulps. From the results, it was observed that, the yield was increased linearly with increasing temperature from 40-50 °C (Fig. 2 a-b and 3 a-b). Increase in temperature up to 45 °C would cause softening of the plant tissue, accelerate the molecular movements and penetration of solvent in to the plant material and enhances the solubility and diffusivities of the pigments and colors into the solvent and increases the extraction yield (Yong et al. 2006). However, temperature beyond  $45^{\circ}$ C resulted in lower recovery of pigment and color, due to heat sensitive anthocyanin pigment.

 
 Table 3
 ANOVA analysis and statistical parameters of the responses

Source	Coefficient estimate	Sum of squares	DF	Mean square	F-value	<i>p</i> -value
total monomeric a	nthocyanin content					
Model	9.59	13.79	9	1.53	100.05	< 0.0001
$X_1$	0.21	0.35	1	0.35	23.04	0.0049
$X_2$	0.40	1.28	1	1.28	83.59	0.0003
X <sub>3</sub>	0.74	4.35	1	4.35	284.15	< 0.0001
X <sub>12</sub>	-0.30	0.35	1	0.35	23.12	0.0048
X <sub>13</sub>	-0.87	3.05	1	3.05	198.85	< 0.0001
X <sub>23</sub>	0.24	0.23	1	0.23	14.73	0.0121
$X_{1}^{2}$	-1.03	3.93	1	3.93	256.84	< 0.0001
$X_{2}^{2}$	-0.31	0.35	1	0.35	22.74	0.0050
$X_{3}^{2}$	-0.22	0.17	1	0.17	11.36	0.0199
Residual error		0.08	5	0.02		
Lack of Fit		0.08	3	0.03	58.23	0.0169
C.V. %	1.41					
S/N ratio	38.80					
total color in solut	ions					
Model	9554.94	1.34E+07	9	1.49E+06	284.80	< 0.0001
$X_1$	259.75	5.40E+05	1	5.40E+05	103.00	0.0002
$X_2$	465.97	1.74E+06	1	1.74E+06	331.48	< 0.0001
X <sub>3</sub>	686.74	3.77E+06	1	3.77E+06	719.98	< 0.0001
X <sub>12</sub>	-368.70	5.44E+05	1	5.44E+05	103.76	0.0002
X <sub>13</sub>	-926.71	3.44E+06	1	3.44E+06	655.54	< 0.0001
X <sub>23</sub>	192.58	1.48E+05	1	1.48E+05	28.31	0.0031
$X_{1}^{2}$	-909.14	3.05E+06	1	3.05E+06	582.38	< 0.0001
$X_{2}^{2}$	-249.77	2.30E+05	1	2.30E+05	43.96	0.0012
$X_{3}^{2}$	-232.04	1.99E+05	1	1.99E+05	37.94	0.0016
Residual error		26201.29	5	5240.259		
Lack of Fit		26125.03	3	8708.345	228.391	0.0044
C.V. %	0.82					
S/N ratio	65.61					

#### Effect of extraction time

Extraction time is one of the important factors for extraction of pigments and colors from plant materials. It is associated with the final concentration of pigments and colors and the efficiency of extraction. From the results, it was found that, the extraction yield was increased rapidly at the initial stages of extraction, but ends with a slower extraction rate. This phenomenon could be explained by that, the mass transfer rate is high at initial stage (Fig. 2b, 3b) due to high concentration gradient during the diffusion of solutes from internal material into the solution, which promotes the transmission of anthocyanin and colors and increases the extraction rate.

#### Effect of solid-liquid ratio

The solvent quantity is also an important factor to influence the extraction yield. From the results, it was observed that, extraction yield was increased with the increasing liquid–solid ratio (Fig. 2 b-c and 3 b-c). Amount of solute required to saturate the liquid increases with increasing quantatity of the liquid and hence extraction of anthocyanin and colour yield are increases with liquid–solid ratio. Similar results were reported during extraction the phenolic compounds from milled berries (Cacace and Mazza 2003), extraction of anthocyanin from red grape pomace (Mantell et al. 2002) and extraction of andrographolide from plants (Wongkittipong et al. 2004).

#### Determination and validation of optimized conditions

Simultaneous optimization of the multiple responses was carried out using Derringer's desired function methodology. This numerical optimization technique evaluates a point that





Fig. 4 Desirability ramp for optimization

maximizes the desirability function (Prakash Maran et al. 2013e). According to BBD results, optimal extraction conditions to obtain the maximum extraction yield of TMAC and TCS from jamun fruit pulp were determined and it is found to be: extraction temperature of 44 °C, extraction time of 93 min and solid-liquid ratio of 1:15 g/ml. Under these conditions, the predicted extraction yield of TAMC and TCS was found to be 10.58 mg/100 g and 10618.3 mg/l with a desirability value of 0.926. A desirability ramp was developed from optimal points via numerical optimization technique (Fig. 4). This set of conditions was determined to be optimum and validated experimentally (Prakash Maran et al. 2013f). Triplicate experiments were performed under the optimized conditions and the mean values ( $10.49\pm0.23$  for TMAC and  $10615.72\pm3.51$ for TCS) obtained from real experiments, demonstrated the validation of the optimized conditions.

### Conclusion

In this study, BBD was employed to study and optimize the process variables such as extraction temperature, time and solid-liquid ratio on the extraction yield of pigment (anthocyanin) and color from jamun fruit pulp. From the results, it was observed that, the extraction conditions have significant effects on the extraction yield of pigments and color. ANOVA showed that the effects of all variables (Extraction temperature, extraction time and solid-liquid ratio) were significant and quadratic models were developed for predicting the responses. Optimum set of the independent variables was obtained by derringer's desired function methodology in order to extract the maximum levels of pigment and color. The optimal conditions were found to be: extraction temperature of 44 °C, extraction time of 93 min and solid-liquid ratio of 1:15 g/ml respectively. Under these optimal conditions, the experimental yield (10.49 $\pm$ 0.23 mg/100 g for TMAC and 10615.72 $\pm$ 

3.51 mg/l for TCS) was closely agreed with the predicted values (10.58 mg/100 g for TMAC and 10618.3 mg/l for TCS).

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