

Modeling and optimization of supercritical fluid extraction of anthocyanin and phenolic compounds from *Syzygium cumini* fruit pulp

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Abstract Supercritical carbon dioxide extraction (SC-CO₂) of total anthocyanin and phenolic compounds from jamun fruits was investigated using three factors at three levels Box–Behnken response surface design. Experiments were conducted to evaluate the effects of three independent variables (pressure, temperature and co-solvent flow rate) on the maximum extraction yield of anthocyanin and phenolic compounds from jamun fruits. From the experimental data, second order polynomial mathematical models were developed with high coefficient of determination values ($R^2 > 0.98$). From response surface plots, pressure, temperature and co-solvent flow rate exhibited independent and interactive effects on the extraction yields. Pressure of 162 bar, extraction temperature at 50 °C and co-solvent flow rate of 2.0 g/min was identified as optimal conditions. Under these optimal conditions, the experimental value agreed well with the predicted values and indicates the suitability of developed models.

Keywords Supercritical fluid extraction · Phenolic compound · Box-Behnken design · Anthocyanin

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Introduction

Indian blackberry (*Syzygium cumini* L.), commonly known as jamun is a minor and under-utilized tree-fruit of Indian sub-continent. 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 purple coloured jamun fruits are available abundantly during summer season for a short span of time, causing a seasonal glut. The fruits are oblong berries, deep purple or bluish in colour with pinkish pulp, having various medicinal properties and used in Ayurveda as a stomachic, astringent, antiscorbutic, diuretic, antidiabetic, and in chronic diarrhea and enlargement of spleen (Achrekar et al. 1991) including its use as an antimicrobial agent (Migliato 2005). In addition, jamun fruit extract showed anti-proliferative and pro-apoptotic effects against breast cancer cells and showed high antioxidant activity induced by copper acetate in liposomes, (Hassimotto et al. 2005). These beneficial effects are most probably due to the presence of bioactive compounds, such as pigments and phenolic compounds. Anthocyanins often account for much of the phenolic content of these fruits, but flavanols, procyanidins, phenolic acids, and ellagitannins may be the most predominant phenolics in some taxa. Polyphenolic compounds inhibit several oxidative, inflammatory enzymes and have shown antiallergenic, antiviral, antibacterial, antifungal, antitumor, and antihemorrhagic activities (Pietta 2000). Therefore, it is interesting to look for an effective extraction method to extract the valuable bioactive compounds such as anthocyanin and phenolic compounds from jamun fruit.

Supercritical fluid extraction (SFE) with supercritical carbon dioxide (SC-CO₂) has been used for the extraction of compounds from natural products and researchers have paid considerable attention towards various aspects of this process (Lu et al. 2007). These include the isolation phenol and tocopherols from olive leaves (De Lucas et al. 2002), tocopherols from

grape seeds (Bravi et al. 2007), carotenoids, tocopherols, and sitosterols from industrial tomato by-products (Vagi et al. 2006), and valuable compounds from *Citrus junos* seed (Hiro et al. 2008). Products derived from this technology are completely free from harmful residues and without the risk of thermal degradation of processed products. However, to our best knowledge, no publications are available on the extraction of anthocyanins and phenolic compounds from jamun fruits using SC-CO₂ extraction technique. Hence, the objective of this present study is to develop and optimize a SC-CO₂ extraction process that was rapid, simple and easily applicable to the extraction of total anthocyanins and phenolic compounds from jamun fruits. The effects of pressure, temperature and co-solvent flow rate on extraction yield were investigated. Box–Behnken response surface design (BBD) was used to build models between the responses and the independent factors, and also used to optimize the extraction conditions. BBD is a spherical, revolving response surface methodology (RSM) design that consists of three interlocking 2² factorial design having points, all lying on the surface of a sphere surrounding the center of the design (Prakash Maran et al. 2013d) which uses quantitative data from an appropriate experimental design to determine or simultaneously solve multivariate equations.

Materials and methods

Plant materials

Fresh Jamun (*Syzygium cumini*) fruits were procured from local fruit orchards near Chennai, India. The pulp of the fruits were removed manually and washed under running tap water in order to remove the foreign matters adhered to the surface. Then the fruit pulp was dried in a hot air oven at 40 °C until the moisture content was constant (6 % w/w). The dried pulp were pulverized and sieved through a 40-mesh sieve to obtain the powdered form of pulp. The powdered pulp were stored in an airtight dark container to prevent moisture absorption and stored in dry environment prior to the experiments.

Chemicals and reagents

SFE-grade carbon dioxide (purity 99.9 %) supplied in a cylinder with a dip tube was purchased from Chang Chun Gas Inc. (Changchun, China). Cyanidin-3-glucoside and gallic acid (98 % purity) was purchased from Sigma–Aldrich Company, Mumbai. Ethanol and deionized water (HPLC grade) was supplied by southern India scientific corporation (Hong Yan G chemical corporation, China). Potassium chloride, sodium acetate, Folin-Ciocalteu reagent and sodium carbonate were purchased from Loba Chemical Company, Mumbai.

Supercritical carbon dioxide (SC-CO₂) extraction

SC-CO₂ extraction of anthocyanin and phenolic compounds from jamun fruits was performed on a supercritical fluid extractor (SFE) (ABRP200, Pittsburgh, PA, USA) with an extraction volume of 500 ml. Before the experiment was started, the SFE unit was calibrated using standard calibration method prescribed by the equipment supplier. For each experiment, 10 g of powdered fruit pulp sample was mixed with glass wool and loaded in the extractor vessel. After the loaded extractor vessel was assembled, the gas carbon dioxide from CO₂ cylinder was pressurized and introduced in to the extraction vessel, which was filled with samples, with the adjustment of pump (P-50, Thar designs, Inc. Pittsburg, PA, USA). Then, it was pressurized to the desired pressure and heated to the specified temperature in order to reach the supercritical state. Ethanol (99.9 % purity) acted as the co-solvent to increase the polarity of solvent and improve the efficiency of SC-CO₂ extraction of pigment and phenolic compounds from jamun fruit pulp. When the scheduled time was achieved, the extractor vessel was depressurized at ambient temperature and pressure and the extract was separated and collected in collector at ambient temperature and atmospheric pressure. Extracts obtained by SFE at different conditions were analyzed for total anthocyanin and phenolic content.

Estimation of total monomeric anthocyanin content

The total monomeric anthocyanin content (TMAC) was estimated by pH differential method that relies on the structural transformation of anthocyanin chromophore as a function of pH value (Prakash Maran et al. 2013a), which can be measured using a spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). Aliquots (1 ml) of extracts were mixed with potassium chloride (pH 1.0, 0.025 M, 4 ml) and sodium acetate (pH 4.5, 0.4 M, 4 ml) and allowed to equilibrate for 1 h before taking absorbance at 530 and 700 nm, were recorded using a spectrophotometer calibrated with distilled water as the blank. The difference in absorbance was calculated using the following equation:

$$A = (A_{530\text{nm}} - A_{700\text{nm}})_{\text{pH}1.0} - (A_{530\text{nm}} - A_{700\text{nm}})_{\text{pH}4.5} \quad (1)$$

The total monomeric anthocyanin content was calculated by the following equation:

$$\text{TMAC} = \frac{A \times \text{MW} \times \text{DF} \times 1000}{\epsilon \times l} \quad (2)$$

where, A is the absorbance, MW is the molecular weight of cyanidin-3-glucoside (449.2 g/mol), DF is the dilution factor, ϵ is the molar absorptivity of cyanidin-3-glucoside (26,900 L/cm.mol), and l is for a standard 1 cm path length. Total monomeric anthocyanins were reported as milligrams

cyanidin-3-glucoside per 100 g of fruits (mg cyanidin-3-glucoside/100 g).

Analysis of total phenolic content

Total phenolic content (TPC) was measured by Folin-Ciocalteu method as described by Vatai et al. (2009) with minor modifications. 2.5 ml of Folin-Ciocalteu reagent (0.2 mol/l) was mixed with known amount of (0.2 ml) diluted sample and a total volume of 4 ml was made up with deionized water (used for dilution and control). After incubation for 5 min at room temperature, 2.0 ml of sodium carbonate (7.5 g/100 ml) was added, then immediately mixed and incubated at room temperature for 2 h. After the mixture was shaken, the absorbance was measured at 754 nm against a reagent blank (deionized water instead of the sample) with a UV-visible spectrophotometer (Elico, SL 244, India). Gallic acid was used as the standard and results were calculated on the basis of calibration curve of gallic acid and the results were expressed as gallic acid equivalents (mg GAE/100 g).

Identification of different anthocyanin and phenolic compounds by high performance liquid chromatography (HPLC) analysis

The chromatographic analyses were carried out using HPLC equipment (Shimadzu HPLC LC 2010HT, Mumbai), equipped with a pump and a UV detector. Separation was achieved using a Synergi Fusion—RP 80 A reversed phase column (4 μm , 250 \times 4.6 mm, Phenomenex, Torrance, CA) at 33 \pm 1 $^{\circ}\text{C}$. Elution was carried out with a linear gradient and a flow rate of 1 ml/min. The analysis detection was monitored at 530 nm for anthocyanin and 280 nm for polyphenols. The prepared SFE extract was filtered through a 0.22 μm Millex polyethersulphone membrane (Millipore, USA). The filtered sample (10 μl) was injected into the column. An elution gradient consisting of water/acetonitrile/formic acid 87/3/10 (v:v:v) (solvent A) and water/acetonitrile/formic acid 40/50/10 (v:v:v) (solvent B) was used as follows: 0 min 6 % B, 10 min 20 % B, 20 min 40 % B, 25 min 90 % B and 30 min 6 % B. The substances (different anthocyanins and phenolics) were identified by matching their retention times, the UV-vis spectrum and the purity peak against those of standard compounds.

Experimental design

In this study, Box-Behnken response surface experimental design (BBD) with three factors and three levels was chosen to optimize and study the influence of process variables such as pressure (100–200 bar), temperature (40–60 $^{\circ}\text{C}$) and co-solvent (ethanol) flow rate (1–3 g/min) on the maximum extraction yield of TMAC and TPC from jamun fruits. Because

BBD has more efficient than the other experimental designs such as central composite design, three-level full factorial design and Doehlert matrix. Another advantage of the BBD is that it does not contain combinations for which all factors are simultaneously at their highest or lowest levels. So this design is useful in avoiding experiments performed under extreme conditions, for which unsatisfactory results might occur. On the basis single factor experimental analysis, process variables and their ranges were determined and the independent variables was coded at three levels (–1, 0 and +1) for statistical calculation by the method described by Prakash Maran et al. (2013b). After selection of process (independent) variables and their ranges, experiments were established based on a BBD and the complete design consists of 17 experiments with five replicates (used to estimate experimental error) at the centre point. The total number of experiments (N) was calculated from the following equation (Prakash Maran and Manikandan 2012).

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

where, K is number of factors and C_0 is the number of central point.

For predicting the optimal point after performing experiments, a second-order polynomial mathematical equation was fitted to the experimental data to express the relationship between independent variables and responses. For the three factors, the generalized form of second order polynomial equation is

$$Y = \beta_0 + \sum_{j=1}^k \beta_j X_j + \sum_{j=1}^k \beta_{jj} X_j^2 + \sum_i \sum_{<j=2}^k \beta_{ij} X_i X_j + e_i \quad (4)$$

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 (Maran et al. 2013; Prakash Maran et al. 2013g and h).

Statistical analysis

Experimental data was analyzed using the Stat ease Design Expert 8.0.7.1 statistical software (Stat-Ease Inc., Minneapolis, USA). The experimental data was analyzed by multiple regression analysis through the least square method. Pareto analysis of variance (ANOVA) was used to evaluate the observed data and the regression coefficients of linear, quadratic, and interaction terms and their effects were generated and analyzed. All the terms in the model were tested statistically by F-test and verified the significance of the F-values at probability levels ($p \leq 0.05$). The values of determination

coefficient (R^2), adjusted determination coefficient (R^2_{adj}), coefficient of variance (CV %), adequate precision of models and predicted residual sum of squares (PRESS) were obtained to check the quality of the fit polynomial model.

Optimization

Multiple response analysis (Prakash Maran et al. 2013f) using Derringer’s desired function methodology was used to optimize the process conditions of the present study. This numerical Optimization technique will optimize any combination of one or more goals; these may be either process variables or responses. The general approach of desirability function is, first transform the response into a dimensionless individual desirability function (d_i), which varies from 0 to 1 (lowest to highest desirability). The following equation was used to maximize and transform the responses into dimensionless desirability (d_i) value.

$$d_i = \frac{Y_i - Y_{min}}{Y_{max} - Y_{min}} \tag{5}$$

where, Y_i is the obtained response value, Y_{min} is the obtained response minimum value and Y_{max} is the obtained response maximum value.

The overall desirability function (D) is obtained (Eq. 6) from the geometric means of individual desires. The Design expert 8.0.7.1 was used to maximize the D.

$$D = (d_1^{n_1} \times d_2^{n_2} \times d_3^{n_3} \times \dots \times d_k^{n_k})^{1/k} \tag{6}$$

where, d_i is the individual desirability value of the individual response, k is the number of considered responses, and n_i is the weight of each response.

Validation of the optimized conditions

To determine the validity of the optimized conditions, additional triplicate experiments were performed under optimal conditions. The average value of the experiments was compared with the predicted values of the optimized conditions and find out the accuracy and suitability of the optimized conditions and mathematical models.

Results and discussion

Box-Behnken response surface design analysis

In this study, three factors at three levels Box-Behnken response surface design was used to determine the individual and interactive effects of major operating variables (pressure,

temperature and co-solvent flow rate) on maximum extraction efficiency of TMAC and TPC from jamun fruits obtained by SC-CO₂ extraction technique and the results were listed in Table 1. Four models viz., linear, interactive (2FI), quadratic and cubic models were fitted to the experimental data to check whether approximating model would give poor or misleading results. The adequacy of model summary indicates that, linear and interactive (2FI) models were exhibited lower R^2 , adjusted R^2 , predicted R^2 and also high p -values, when compared with quadratic model. Cubic model was found to be aliased (Table 2). So, the quadratic model is found to be the most suitable model for the present extraction process. Adequacy of this model was further justified through analysis of variance (ANOVA).

Development of second order polynomial mathematical models

An empirical relationship between independent variables and responses were expressed by a second-order polynomial equation with interaction terms was fitted between the experimental results obtained on the basis of Box–Behnken experimental design. The final mathematical equation obtained in terms of coded factors is given below

$$\begin{aligned} \text{TMAC} = & 230.91 + 1.22X_1 + 0.34X_2 + 1.66X_3 - 0.09X_1X_2 \\ & + 0.31X_1X_3 - 0.065X_2X_3 - 1.25X_1^2 - 2.45X_2^2 - 1.72X_3^2 \end{aligned} \tag{7}$$

$$\begin{aligned} \text{TPC} = & 1143.87 + 1.09X_1 + 0.036X_2 + 1.41X_3 - 0.09X_1X_2 \\ & + 0.57X_1X_3 - 0.32X_2X_3 - 1.75X_1^2 - 2.94X_2^2 - 1.48X_3^2 \end{aligned} \tag{8}$$

Statistical analysis

The adequacy and fitness of the models were tested by multiple regression analysis through least square method. Pareto analysis of variance (ANOVA) was used to check the significance of the developed models and ANOVA tables were generated, which was shown in Table 3. 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 (Prakash Maran et al. 2013c). As shown in Table 2, ANOVA of regression model demonstrates that the models are highly significant, as it is evident from the high Fisher’s F -test value (1246.31 for TMAC and 50.86 for TPC) with a very low probability value ($p < 0.0001$). R^2 is defined as the ratio of the explained variation to the total variation and is a measurement of the degree of fitness of the models (Prakash Maran et al. 2013d). The values of R^2 were 0.999 for TMAC

Table 1 Box-Behnken experimental design matrix with observed and predicted values of responses

Run order	Pressure (bar)	Temperature (C)	Co-solvent flow rate (g/min)	TMAC (mg/100g)		TPC (mg GAE/100g)	
				Observed	Predicted	Observed	Predicted
1	150	60	3	228.61	228.67	1140.59	1140.51
2	150	50	2	230.91	230.91	1143.87	1143.87
3	100	60	2	226.44	226.42	1138.41	1138.15
4	150	50	2	230.91	230.91	1143.87	1143.87
5	100	50	3	228.11	228.07	1140.07	1140.41
6	200	60	2	228.64	228.68	1139.62	1140.15
7	200	40	2	228.17	228.19	1140.14	1140.40
8	100	50	1	225.27	225.38	1138.26	1138.71
9	150	60	1	225.57	225.48	1138.51	1138.32
10	150	50	2	230.91	230.91	1143.87	1143.87
11	150	40	1	224.74	224.68	1137.68	1137.76
12	150	40	3	228.04	228.13	1141.03	1141.22
13	100	40	2	225.61	225.57	1138.57	1138.04
14	150	50	2	230.91	230.91	1143.87	1143.87
15	200	50	1	227.15	227.19	1140.09	1139.75
16	150	50	2	230.91	230.91	1143.87	1143.87
17	200	50	3	231.24	231.14	1144.16	1143.71

and 0.984 for TPC, which ensures a satisfactory fit of the quadratic models to the experimental data. The value of R^2_{adj} (0.997 for TMAC and 0.966 for TPC) is also high and advocates a high correlation between the observed and predicted values. In this study, the R^2_{pre} of 0.990 for TMAC and 0.859 for TPC is in reasonable agreement with the R^2_{adj} . The low value of residual error (0.053 for TMAC and 1.4 for TPC) shows that, the form of the chosen model to explain the relationship between the factors and response is correct (Prakash Maran et al. 2013e). The very low values of CV % (0.038 for TMAC and 0.039 for TPC) clearly indicated a very high degree of precision and a good reliability of the experimental values (Priya et al. 2013). Adequate precision measures the signal to noise ratio and this

ratio is greater than 4, which is desirable and indicates adequate model discrimination (Prakash Maran et al. 2014a, b, c). In this work, the ratio is found to be >17 for the responses, which indicates an adequate signal.

Effect of process variables

From the developed mathematical model equations (Eqs. 7–8), three dimensional (3D) response surface plots were constructed in this study and it is shown in Fig. 1. Since the regression model has three independent variables, one variable was held at constant at the central level for each plot and the other factors were varied in their range (Prakash Maran et al. 2014a) in order to visualize the relationship

Table 2 Adequacy of the models tested for the responses

Source	Std.Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	Prob > F	Remarks
TMAC							
Linear	1.95	0.413	0.278	0.144	72.17	0.0663	
2FI	2.21	0.419	0.070	-0.442	121.55	0.9926	
Quadratic	0.09	0.999	0.997	0.990	0.84	< 0.0001	Suggested
Cubic	0.00	1.000	1.000	-	+	0.0283	Aliased
TPC							
Linear	2.28	0.274	0.106	-0.084	100.82	0.2299	
2FI	2.57	0.292	-0.133	-0.823	169.58	0.9657	
Quadratic	0.45	0.985	0.966	0.859	22.41	< 0.0001	Suggested
Cubic	0.00	1.000	1.000	-	+	0.0346	Aliased

Table 3 Analysis of variance (anova) for the responses

Source	TMAC				TPC			
	RC	SE	F- value	p-value	RC	SE	F- value	p-value
Model	230.91	0.04	1246.31	< 0.0001	1143.87	0.20	50.86	< 0.0001
X ₁	1.22	0.03	1588.61	< 0.0001	1.09	0.16	47.28	0.0002
X ₂	0.34	0.03	121.33	< 0.0001	-0.04	0.16	0.05	0.8253
X ₃	1.66	0.03	2930.69	< 0.0001	1.41	0.16	79.90	< 0.0001
X ₁₂	-0.09	0.04	4.31	0.0764	-0.09	0.22	0.16	0.6994
X ₁₃	0.31	0.04	52.01	0.0002	0.57	0.22	6.38	0.0395
X ₂₃	-0.06	0.04	2.25	0.1773	-0.32	0.22	2.01	0.1987
X ₁ ²	-1.25	0.04	870.69	< 0.0001	-1.75	0.22	64.16	< 0.0001
X ₂ ²	-2.45	0.04	3361.58	< 0.0001	-2.94	0.22	181.70	< 0.0001
X ₃ ²	-1.72	0.04	1660.90	< 0.0001	-1.48	0.22	46.01	0.0003
Std. Dev.	0.09				0.45			
Mean	228.36				1140.97			
C.V. %	0.038				0.039			
PRESS	0.84				22.41			
Adeq. Precision	97.13				17.815			

RC Regression coefficient, SE Standard error

between independent variables and the responses (Prakash Maran et al. 2014b).

Effect of pressure

Pressure is one of the dominant factors in extraction of pigment and phenolic compounds from jamun fruits by SC-CO₂ extraction process. Figure 1a, b, c and d illustrates the effect of operating pressure on the extraction yield of TMA and TPC. As the pressure increases, two distinguished trends were observed. In the range of 100–162 bar, the extraction yield of TMAC and TPC increases with a mild slope. Further increase in pressure up to 200 bar causes slight decrease in the yield. Increase in pressure could increase the fluid density, decreasing the distance between the molecules by the rupturing effect of pressure and thereby strengthening interactions between the fluid and matrix (Lang and Wai 2001). During rupturing process, the chemical substances within the plant materials are rapidly released into the surrounding extraction solvents, which enhanced the solubility of the solute, mass transfer rate and diffusion of solvent into the plant material and improve the extraction yield (Prasad et al. 2010; Sonsuzer et al. 2004). The decrease in extraction yield with increasing pressure after a certain pressure (162 bar) might be due to the volatility and polarity of extracted analytes and interaction between the other parameters.

Effect of temperature

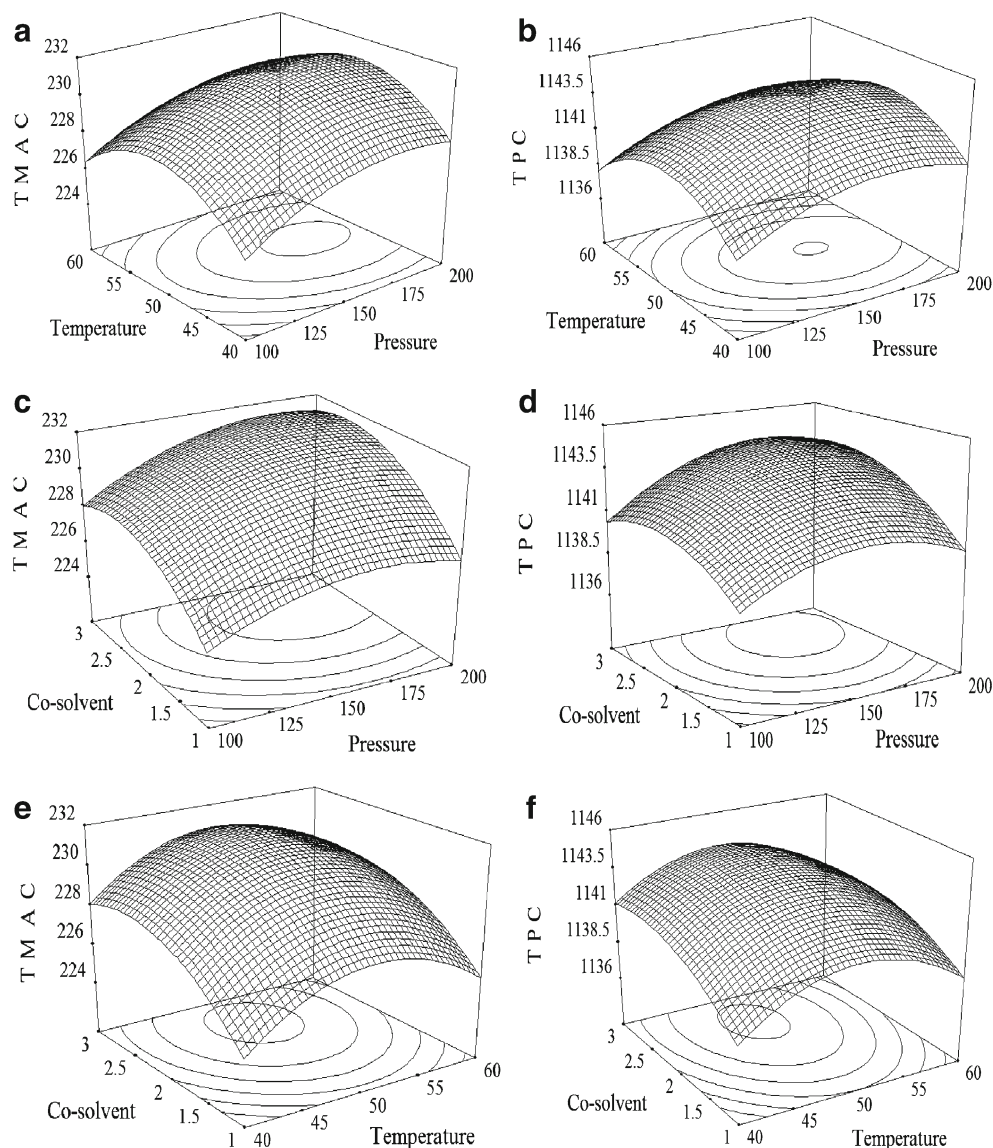
Temperature is the main parameter influencing the physico-chemical properties of ethanol and the compounds to be

extracted, and it has a great influence on the extraction rate, efficiency, and selectivity in SC-CO₂. From the results, it was observed that, the extraction yield of TMAC and TPC was increased when extraction temperature was increased from 40 to 50 °C, but slowly decreases, when the temperature continued to be extended (Fig. 1a, b, e and f). Enhancement of the extraction efficiency can be related to the increased vapor pressures and accelerated thermal desorption of the compounds from the sample matrix (Sheibani and Ghaziaskar 2009). Instead of that, the increasing temperature contributed to damage the particle cell walls, increased the mass transfer rate and as the result, availability of bioactive compounds for extraction was increased (Machmudah et al. 2006). But further increase in temperature resulted in a decrease in the recovery of bioactive contents, due to vaporization or decomposition of volatile components during extraction at higher temperature (Cacace and Mazza 2003) and also due to reduction in the density of CO₂.

Effect of co-solvent

The nature of the solute to be extracted is the critical basis for co-solvent choice (Walsh et al. 1987). The co-solvent improves polarity of the extraction fluid and hence increases the extraction yield. As a result, an increasing co-solvent amount resulted in a higher extraction yield, while the extraction yield reached a maximum when co-solvent amount was up to a certain value (2 g/min), with no further significant improvement (Fig. 1c, d, e and f). This could be explained by the fact that the polar constituents in the plant would be easier to extract with more polar solvent. The addition of ethanol to

Fig. 1 Response surface plots showing effects of process variables (temperature, pressure and co-solvent flow rate) on the responses (TMAC (a, c and e) and TPC (b, d and f))

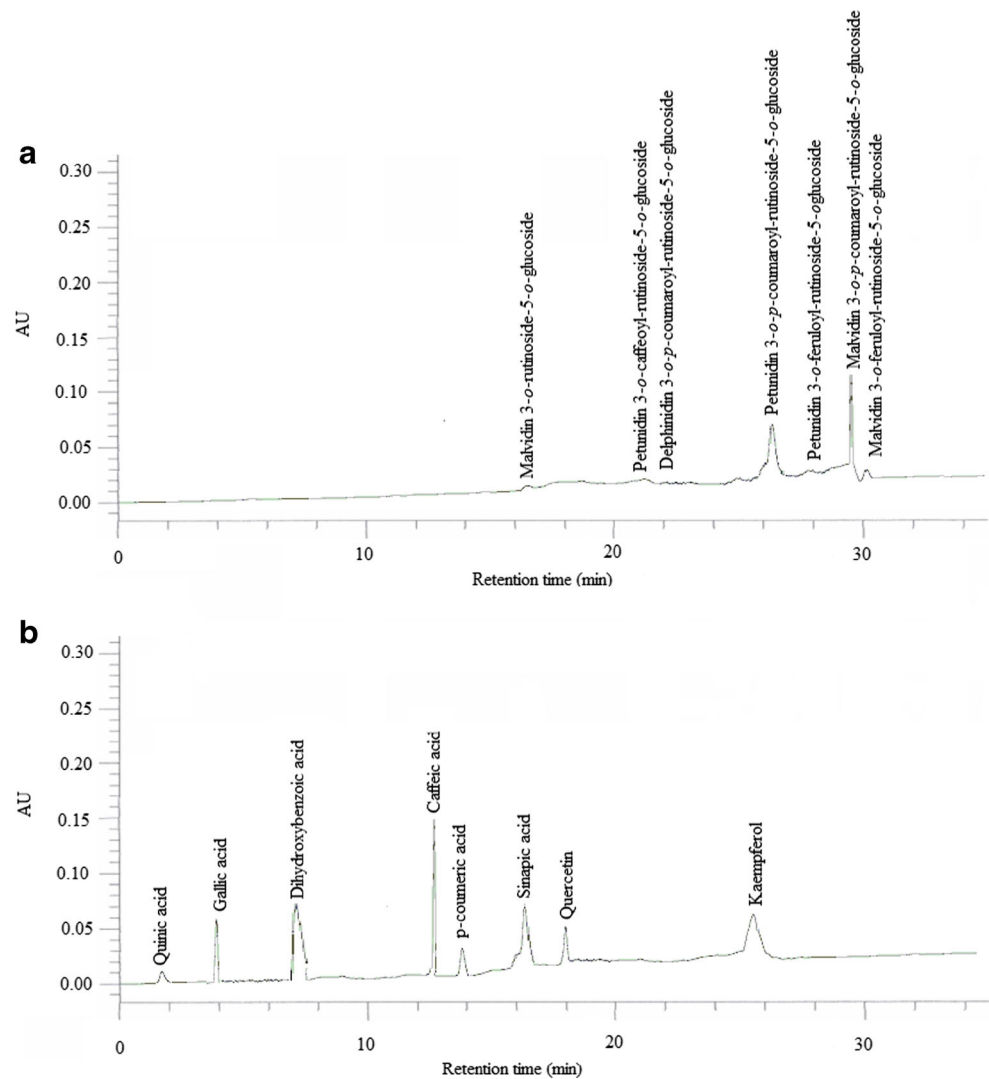


the supercritical solvent seems to provide an enhanced extraction of the pigment and phenolic compounds, as a consequence of the polarity increase in the solvent mixture. As polar co-solvents induced the changes in the structure of the cellular matrix via intra-crystalline, osmotic swelling and break analyte-matrix bindings by competing with polar interactions between matrixes which in turn enhances the solubility of the compounds to be extracted (Bjorklund et al. 1998). These results confirm the affirmation made by Murga et al. (2000), which stated that, due to the fact that ethanol is a solvent capable of hydrogen-bonding and dipole-dipole interactions with phenols, it would likely act as a good co-solvent for the carbon dioxide extraction of phenolic components. Wang et al. (2008) also found a similar result about the effect of co-solvent flow rate on the flavonoid yield from *Pueraria lobata*.

Determination and validation of optimum conditions

Simultaneous optimizations of the multiple responses were carried out using Derringer's desirability function method. Applying the methodology of desired function, the optimum level of various parameters were obtained as an extraction pressure of 162 bar, extraction temperature at 50 °C and co-solvent flow rate of 2.0 g/min. Under these optimum conditions, the predicted extraction yield was TMAC of 231.13 mg/100g and TPC of 1144.032 mg GAE/100 g with a desirability value of 0.927. The experimental efficiency of the extraction of TMAC and TPC under the optimum conditions (mean value of triplicate experiments) was found to be 231.28 ± 0.76 mg/100 g of TMAC and 1143.051 ± 1.58 mg GAE/100 g of TPC respectively. The results obtained through confirmation experiments indicate the suitability of

Fig. 2 HPLC chromatogram of SC-CO₂ extract at optimized condition (pressure of 162 bar, temperature of 50 °C and co-solvent flow rate of 2 g/min) showing different anthocyanins (a) and phenolic compounds (b)



the developed quadratic models and indicate the reliability of BBD coupled desired function methodology.

HPLC analysis and identification of different anthocyanins and phenolic compounds

HPLC analysis was conducted to identify the different anthocyanins and phenolic compounds present in the extract. Retention time of obtained peaks was compared with that of known standards under the same conditions. Figure 2 shows the HPLC chromatogram of the extract from jamun fruit under the optimal SFE conditions. In total, seven different anthocyanins namely malvidin 3-*o*-rutinoside-5-*o*-glucoside, petunidin 3-*o*-caffeoyl-rutinoside-5-*o*-glucoside, delphinidin 3-*o*-*p*-coumaroyl-rutinoside-5-*o*-glucoside, petunidin 3-*o*-*p*-coumaroyl-rutinoside-5-*o*-glucoside, petunidin 3-*o*-feruloyl-rutinoside-5-*o*-glucoside, malvidin 3-*o*-*p*-coumaroyl-rutinoside-5-*o*-glucoside, malvidin 3-*o*-feruloyl-rutinoside-5-*o*-glucoside were identified on the basis of peak area and its retention time

(Fig. 2a). From Fig. 2b, eight different bioactive phenolics including quinic acid, gallic acid, dihydroxybenzoic acid, caffeic acid, *p*-coumeric acid, sinapic acid, quercetin and kaempferol were detected with good separation.

Conclusions

In this study, three factors at three levels BBD was successfully employed to optimize and study the individual and interactive effect of process variables such as pressure, temperature and co-solvent flow rate on the extraction yield of TMAC and TPC from jamun fruits. From the experimental results, second order polynomial mathematical models were developed for the responses with high coefficient of determination (R^2) values (more than 0.98). The optimal conditions to obtain the maximum extraction yield of TMAC and TPC were determined as follows: extraction pressure of 162 bar, extraction temperature of 50 °C and co-solvent flow rate of 2.0 g/min respectively.

Under these conditions, the experimental value (231.28 ± 0.76 mg/100 g for TMAC and 1143.051 ± 1.58 mg GAE/100 g for TPC) was agreed well with the predicted values.

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