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Box–Behnken design based statistical modeling for ultrasound-assisted extraction of corn silk polysaccharide

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ABSTRACT

In this study, ultrasound assisted extraction (UAE) conditions on the yield of polysaccharide from corn silk were studied using three factors, three level Box–Behnken response surface design. Process parameters, which affect the efficiency of UAE such as extraction temperature (40–60 °C), time (10–30 min) and solid–liquid ratio (1:10–1:30 g/ml) were investigated. The results showed that, the extraction conditions have significant effects on extraction yield of polysaccharide. The obtained experimental data were fitted to a second-order polynomial equation using multiple regression analysis with high coefficient of determination value (R^2) of 0.994. An optimization study using Derringer's desired function methodology was performed and the optimal conditions based on both individual and combinations of all independent variables (extraction temperature of 56 °C, time of 17 min and solid–liquid ratio of 1:20 g/ml) were determined with maximum polysaccharide yield of 6.06%, which was confirmed through validation experiments.

1. Introduction

Today foods are not intended to only satisfy hunger, but also to prevent nutrition-related diseases and improve physical and mental well-being. There has been significant development in the field of food and nutrition, still plants plays the role of major raw materials for treating various ailments of human being. Corn silk (*Zea mays* L.) is a traditional herb that has been widely used for treatment of diseases (Maksimovic, Malencic, & Kovacevic, 2005) such as immune enhancement (Kim, Choi, & Choi, 2004), resistance to insect attacks (Guevara, Perez-Amador, Zuniga, & Snook, 2000), anti-proliferative effects on human cancer cell lines (Habtemariam, 1998), anti-diabetic activity (Rau, Wurglics, Dingermann, Abdel-Tawab, & Schubert-Zsilavecz, 2006) and antioxidant activity (El-Ghorab, El-Massry, & Shibamoto, 2007) due to the presence of anthocyanins, *p*-coumaric acid, vanillic acid, protocatechuic acid, derivatives of hesperidin and quercetin, and bound hydroxycinnamic acid forms composed of *p*-coumaric and ferulic acid (Ebrahimzadeh, Pourmorad, & Hafezi, 2008). Polysaccharides from corn silk possess significant antioxidant activities (Maksimovic & Kovacevic, 2003) which could lead to weight loss (Du, Xu, & Gao, 2007) and improve gastrointestinal movement (Du & Xu, 2007).

Consumer demands for healthier foods with functional properties, as well as the strong evidence provided for possible toxicity of synthetic additives, have shifted research interest in extraction of bioactive compounds from plants. Ultrasound in combination with conventional extraction is a potential technique, which is a fully reproducible food processes, completed in shorter time with high reproducibility, reduced processing cost, simplified manipulation and work-up, gave higher purity of the final product, eliminated post-treatment of waste water and consuming only a fraction of the time and energy normally needed for conventional processes (Kim, Chi, & Hong, 2009; Li, Wei, You, & Lydy, 2010; Sun, Liu, Chen, Ye, & Yu, 2011). The acoustic cavitation in ultrasound assisted extraction (UAE) causes disruption of the cell walls (Toma, Vinatoru, Paniwnyk, & Mason, 2001), reduction of the particle size and enhancement over the contact between solvents and targeted compounds (Rostagno, Palma, & Barroso, 2003). Therefore, UAE provides increased extraction yield, increased rate of extraction, reduced extraction time and higher processing throughput along with the advantage of usage of reduced temperature and solvent volume (Luque-García & Luque de Castro, 2003) which is very useful for the extraction of heat labile compounds (Huang, Xue, Niu, Jia, & Wang, 2009; Vinatoru, 2001). From the literature, it was found that UAE has been investigated to extract polysaccharides from various plant materials (Li, Fan, Ding, & Ding, 2007; Yang et al., 2008)

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Table 1
Coded and uncoded Box–Behnken design with the observed and predicted data.

Run order	Extraction temperature (X_1, C)	Extraction time (X_2, min)	Solid–liquid ratio (X_3, g/ml)	Polysaccharide yield (%)		Residual error	%Error	Absolute error
				Observed	Predicted			
1	60 (1)	10 (−1)	1:20 (0)	5.73	5.72	0.01	0.17	0.01
2	40 (−1)	10 (−1)	1:20 (0)	3.91	3.97	−0.06	−1.53	0.06
3	50 (0)	30 (1)	1:30 (1)	5.91	5.99	−0.08	−1.35	0.08
4	50 (0)	10 (−1)	1:30 (1)	5.24	5.26	−0.02	−0.38	0.02
5	60 (1)	20 (0)	1:10 (−1)	5.31	5.4	−0.09	−1.69	0.09
6	40 (−1)	30 (1)	1:20 (0)	4.77	4.78	−0.01	−0.21	0.01
7	40 (−1)	20 (0)	1:30 (1)	4.85	4.77	0.08	1.65	0.08
8	50 (0)	30 (1)	1:10 (−1)	4.97	4.95	0.02	0.40	0.02
9	50 (0)	10 (−1)	1:10 (−1)	5.14	5.06	0.08	1.56	0.08
10	50 (0)	20 (0)	1:20 (0)	5.97	5.91	0.06	1.01	0.06
11	50 (0)	20 (0)	1:20 (0)	5.86	5.91	−0.05	−0.85	0.05
12	40 (−1)	20 (0)	1:10 (−1)	4.03	4.05	−0.02	−0.50	0.02
13	50 (0)	20 (0)	1:20 (0)	5.89	5.91	−0.02	−0.34	0.02
14	50 (0)	20 (0)	1:20 (0)	5.94	5.91	0.03	0.51	0.03
15	50 (0)	20 (0)	1:20 (0)	5.87	5.91	−0.04	−0.68	0.04
16	60 (1)	30 (1)	1:20 (0)	5.59	5.53	0.06	1.07	0.06
17	60 (1)	20 (0)	1:30 (1)	5.94	5.92	0.02	0.34	0.02

and it resulted with significantly reduced the extraction time and increasing maximum extraction yield of targeted compounds (Lee & Lin, 2007), as compared to conventional methods.

Moreover UAE gives many advantages, but none of the investigations involve the use of ultrasound on extraction of polysaccharide from corn silk. So, development of an economic and efficient UAE technique for extraction of biologically active polysaccharides from corn silk is an emerging interest in the biomedical area and also creates a novel opportunities to exploit the various valuable properties of corn silk polysaccharide. Hence the main objective of this work is to study and optimize the effect of UAE process variables such as extraction temperature, extraction time and solid–liquid ratio on the extractive yield of polysaccharide (YP) from corn silk using Box–Behnken response surface design.

2. Materials and methods

2.1. Plant materials

Dried corn silk samples were purchased from a local shop near Chennai, India, pulverized and sifted through a 40-mesh sieve to obtain the powdered samples. The powder (moisture content 12–14%) was stored in dark bags and kept in dry environment prior to the experiments.

2.2. UAE of polysaccharide

UAE was performed according to the method described by Ying, Han, and Li (2011) in an ultrasonic device (Powersonic, Korea) working at a frequency of 40 kHz, input power of 250 W and heating power of 500 W, equipped with digital time and temperature controller. About 10 g of ground powder was mixed with an appropriate amount of distilled water. From the preliminary experiments, the working frequency of 40 kHz and the input power of 250 W were selected and experiments were carried out according to Table 1.

2.3. Determination of polysaccharide yield

After extraction, the extracts were centrifuged at 2600 × g for 15 min (Remi R-24 Centrifuge, India) and filtered through a filter paper (Whatman no. 1, England). The obtained extracts were concentrated with a rotary evaporator (Büchi, UK) at 60 °C under vacuum. The remaining solution was mixed with four volumes of 95% (v/v) ethanol for 48 h at 4 °C and centrifuged again to collect the precipitate as the crude extract, which was freeze dried at −40 °C under vacuum and ground to powder. The percentage YP (%) was calculated by the following equation (Zhao et al., 2011)

$$YP(\%) = \frac{\text{weight of crude extract (g)}}{\text{weight of corn silk powder (g)}} \times 100 \quad (1)$$

2.4. Experimental design

Box–Behnken design (BBD) is an independent, rotatable quadratic design with no embedded factorial or fractional factorial points where the variable combinations are at the midpoints of the edges of the variable space and at the center (Prakash Maran, Sivakumar, Sridhar, & Prince Immanuel, 2013). In the present study, by employing the Box–Behnken statistical experiment design and the RSM, the effects of the three independent variables (extraction temperature of 40–60 °C, time of 10–30 min and solid–liquid ratio of 1:10–1:30 g/ml) on the response (YP) was investigated and to determine the optimal conditions were determined to maximize the percent yield of polysaccharide from corn silk. The application of statistical experimental design techniques in bioprocess development and optimization can result in enhanced product yields, closer conformance of the process output or response to target requirements and reduced process variability, development time and cost. On single factor analysis, independent variables and their ranges were selected. Experiments were established based on a BBD with three factors at three levels and each independent variable were coded at three levels between −1, 0 and +1. The coding of the variables was done by the following equation (Prakash Maran & Manikandan, 2012):

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

where x_i, is the dimensionless value of an independent variable; X_i is the real value of an independent variable; X_z is the real value of an independent variable at the center point; and ΔX_i is the 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 experimental design consists of 17 experiments with five center points (in order to allow the estimation of pure error) and allows calculations of the response function at intermediate levels and enables estimation of the system performance at any experimental point within the studied range (Hamed & Sakr, 2003). The total number of experiments (N) was calculated as follows:

$$N = K^2 + K + C_p \quad (3)$$

where K is the factor number and C_p is the replicate number of the central point (Edrissi, Asl, & Madjidi, 2008).

All the experiments were done in triplicate and the average YP obtained was taken as the response (Table 1). A non-linear regression method was used to fit the second order polynomial (Eq. (4)) to the experimental data and to identify the relevant model terms. Considering all the linear terms, square terms and interaction items, the quadratic response model can be described as

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

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 (Prakash Maran & Manikandan, 2012).

2.5. Statistical analysis

Multiple regression analysis and Pareto analysis of variance (ANOVA) were conducted for fitting the mathematical model using Design Expert software (Version 8.0.7.1, Stat-Ease Inc., USA). The modeling was started with a quadratic model including linear, squared, and interaction terms. Significant terms in the model for each response were found by analysis of variance (ANOVA) and significance was judged by the F-statistic calculated from the data. The experimental data was evaluated with various descriptive statistical analysis such as p value, F value, degrees of freedom (DF), sum of squares (SS), mean sum of squares (MSS), coefficient variation (CV), absolute average relative deviation (AARD), determination coefficient (R^2), adjusted determination of coefficient (R_a^2), correlation coefficient (R), Mallow's C_p statistic, Durbin–Watson (DW) statistic, chi-square (χ^2) test and bias to reflect the statistical significance of the developed quadratic mathematical model. All the calculations of descriptive analysis were carried out in the Microsoft Excel® 2003 as ODBC (Open Database Connectivity) data source running under windows. After fitting the data to the models, the generated data were used for plotting response surfaces and contour plots.

2.6. Percentage contributions of process variables

Based on the sum of squares obtained from the ANOVA, the percentage contributions (PC) for each individual process variables were calculated by the following equations described by Yetilmezsoya, Demirelb, and Vanderbeic (2009).

$$\mathrm{TPC}_i = \frac{\sum_{i=1}^{n} SS_i}{\sum_{i=1}^{n}\sum_{i=1}^{n} SS_i + SS_{ii} + SS_{ij}} \times 100 \quad (5)$$

$$\mathrm{TPC}_{ii} = \frac{\sum_{i=1}^{n} SS_{ii}}{\sum_{i=1}^{n}\sum_{j=1}^{n} SS_i + SS_{ii} + SS_{ij}} \times 100 \quad (6)$$

$$\mathrm{TPC}_{ij} = \frac{\sum_{i=1}^{n}\sum_{i=1}^{n} SS_{ij}}{\sum_{i=1}^{n}\sum_{j=1}^{n} SS_i + SS_{ii} + SS_{ij}} \times 100 \quad (7)$$

where TPC_i, TPC_{ij} and TPC_{ii} are total percentage contributions (TPC) of linear, interactive and quadratic terms; SS_i, SS_{ij} and SS_{ii} are the computed sum of squares (SS) for linear, interactive and quadratic terms respectively.

2.7. Optimization by Derringer's desired function methodology

After the results were obtained, Derringer's desired function methodology (Derringer & Suich, 1980) was performed to evaluate the optimal operating conditions to yield maximum amount of polysaccharide from corn silk by UAE. The general approach of desirability function is to first transform the response into a dimensionless individual desirability function (d_i) that varies from 0 to 1 (lowest to highest desirability). From the geometric means of different individual d_i values, overall desirability function (G) was obtained by combining the individual desirability values.

$$G = (d_1 \times d_2 \times d_3 \times \cdots \times d_n)^{1/n} \quad (8)$$

where d_i indicates the desirability of the response and n is the number of responses in the measure. If any of the responses is beyond the desirability, then overall function will be turned into zero. It can be extended to

$$G = \left(d_1^{\alpha_1} \times d_2^{\alpha_2} \times d_3^{\alpha_3} \times \cdots \times d_n^{1/n}\right)^{1/n}, \quad 0 \le \alpha_i \le 1 (i = 1, 2, 3, \ldots, n), \quad \alpha_1 + \alpha_2 + \cdots + \alpha_n = 1 \quad (9)$$

where d_i indicates the desirability of the different responses Y_i ($i = 1, 2, 3, \ldots, n$) and i represents the importance of responses. So, maximum overall desirability function G depends on the α_i (importance) value (Mourabet et al., 2012). For optimization of any response, the goal must have a low and high value. In this study, the goals were assigned between low and high value. The following desirability function criterion was used to obtain maximum yield of polysaccharide from corn silk.

$d_i = 0$ if response < low value.
$0 \le d_i \le 1$ as response varies from low to high.
$d_i = 0$ if response > high value.

A weight factor of 1 and default importance of 3 was chosen for the individual desirability of the response, which defines the shape and represent the goals to be equally important for the response.

2.8. Verification of the predicted optimized conditions

To determine the validity of the developed mathematical model equation, triplicate experiments were performed under the optimal conditions as predicted by the model. The average value of the experiments was compared with the predicted values of the developed model and find out the accuracy and suitability of the developed model.

3. Results and discussion

3.1. BBD analysis

In order to study the combined effect of independent variables (extraction temperature, time and solid–liquid ratio) on the extraction YP, experiments were performed for different combinations of the physical parameters using statistically designed experiments and the results are shown in Table 1, that includes the design and the experimental and predicted values. Model adequacy checking was performed on the experimental data to determine whether the approximating model would give poor or misleading results. Four high degree polynomial models viz., linear, interactive (2FI), quadratic and cubic models were fitted to the experimental data. Three different tests namely the sequential model sum of squares, lack of fit tests and model summary statistics were carried out in this study to conclude about the adequacy of models among various models to represent the maximum YP and the results are exhibited in Table 2.

Table 2
Sequential model fitting for the yield of polysaccharide.

Source	Sum of squares	DF	Mean square	F value	Prob > F	Remarks
Sequential model sum of squares						
Mean	486.26	1	486.26			
Linear	4.10	3	1.37	6.07	0.0082	
2FI	0.44	3	0.15	0.58	0.6395	
Quadratic	2.44	3	0.81	126.45	<0.0001	Suggested
Cubic	0.04	3	0.01	5.41	0.0683	Aliased
Residual	0.01	4	0.00	–	–	
Total	493.28	17	29.02	–	–	
Lack of fit tests						
Linear	2.92	9	0.32	145.26	0.0001	
2FI	2.48	6	0.41	185.35	<0.0001	Suggested
Quadratic	0.04	3	0.01	5.41	0.0683	Aliased
Cubic	0.01	4	0.00	–	–	
Pure error	8.92E−03	4	2.23E−03	–	–	
Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	Press	Remarks
Model summary statistics						
Linear	0.47	0.5836	0.4875	0.3432	4.61	
2FI	0.50	0.6456	0.4330	0.0722	6.52	
Quadratic	0.08	0.9936	0.9853	0.9156	0.59	Suggested
Cubic	0.05	0.9987	0.9949	–	–	Aliased

From Table 2, Quadratic model was found to have maximum R^2, adjusted R^2, predicted R^2 and also exhibited low p-values. The quadratic model is found to be the most suitable model for the extraction of polysaccharide from corn silk. The adequacy of model was further justified through analysis of variance (ANOVA).

3.2. Fitting of second order polynomial equation

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

$$\text{Yield }(\%) = 5.91 + 0.63X_1 + 0.15X_2 + 0.31X_3 - 0.25X_1X_2 - 0.047X_1X_3 + 0.21X_2X_3 - 0.59X_1^2 - 0.31X_2^2 - 0.28X_3^2 \quad (10)$$

3.3. Statistical analysis

The experimental data was analyzed by Pareto analysis of variance (ANOVA) and the significance of the regression coefficients were evaluated by their corresponding p-values is presented in Table 3. From the p-values of each model terms, it could be concluded that three linear coefficients (X_1, X_2 and X_3), three quadratic coefficients (X_1^2, X_2^2 and X_3^2) and two interactive coefficients (X_1X_3 and X_2X_3) were significant and indicate the pattern of the interactions between the variables. The model F-value of 120.35 indicated that the model was significant at $p < 0.0001$. The lack of fit F-value of 5.41 and the associated p-value of 0.0638 was insignificant due to relative pure error. The goodness of the fit of the model was evaluated by the determination co-efficient (R^2), correlation coefficient (R) and adjusted determination co-efficient (R_a^2) and co-efficient of variance (CV) and it was listed in Table 4. The determination coefficient ($R^2 = 0.994$) value of the quadratic regression model, indicating that only 0.0060% of the total variations was not explained by the model. The value of the adjusted determination coefficient ($R_a^2 = 0.985$) is very high and confirmed that the model was highly significant. If there are many terms in the models and the sample size is not enough large, the R_a^2 may be noticeably smaller than R^2 (Yetilmezsoya et al., 2009). In our study, the R_a^2 value was found to be very close to the R^2. Moreover, a very high value of the correlation coefficient ($R = 0.997$) exhibited an excellent correlation between the experimental and predicted response values. The low CV (1.50) clearly indicated that the deviations between experimental and predicted values are low and it not only showed a high degree of precision but also had a good deal of reliability

Table 3
ANOVA of the regression model for the prediction of polysaccharide yield.

Source	Coefficient estimate	Sum of squares	Degree of freedom	Standard error	Mean square	F value	p-Value
Model	5.91	6.98	9	0.04	0.78	120.35	<0.0001
X_1	0.63	3.14	1	0.03	3.14	487.03	<0.0001
X_2	0.15	0.19	1	0.03	0.19	28.88	0.0010
X_3	0.31	0.78	1	0.03	0.78	120.30	<0.0001
X_{12}	−0.25	0.25	1	0.04	0.25	38.81	0.0004
X_{13}	−0.05	0.01	1	0.04	0.01	1.40	0.2752
X_{23}	0.21	0.18	1	0.04	0.18	27.38	0.0012
X_1^2	−0.59	1.49	1	0.04	1.49	230.80	<0.0001
X_2^2	−0.31	0.41	1	0.04	0.41	63.52	<0.0001
X_3^2	−0.28	0.33	1	0.04	0.33	50.97	0.0002
Residual		0.05	7		0.01		
Lack of fit		0.04	3		0.01	5.41	0.0683
Pure error		0.009	4		0.002		
Cor total		7.02	16				
Adeq. prec.	32.77						

Table 4
Detailed descriptive statistical regression analysis for the developed quadratic model.

Descriptive statistics	Calculation	Regression results for YP
Sum of residuals	$\sum_{i=1}^{n}(Y_0 - Y_p)$	−3.00E−02
Average residuals	$\frac{1}{n}\sum_{i=1}^{n}(Y_0 - Y_p)$	−1.76E−03
Residual or error sum of squares (absolute)	$SS_E = \sum_{i=1}^{n}(Y_0 - Y_p)^2$	0.045
Residual or error sum of squares (relative)	$(SS_E)_R = \frac{\sum_{i=1}^{n}(Y_0-Y_p)^2}{\sigma_i^2}$	0.045
Error variance of the estimate (MSS_E)	$\partial^2 = \frac{SS_E}{n-p}$	0.0065
Standard error of the estimate (S_E)	$\partial = S_E = \sqrt{\frac{SS_E}{n-p}}$	0.080
Absolute average deviation (AARD)	$AARD = \frac{1}{n}\sum_{i=1}^{n}\frac{\lvert Y_o-Y_p\rvert}{Y_o}$	0.008
Determination coefficient (R^2)	$R^2 = \frac{\sum_{i=1}^{n}(Y_p-\bar{Y}_p)^2}{\sum_{i=1}^{n}(Y_p-\bar{Y}_p)^2+\sum_{i=1}^{n}(Y_o-Y_p)^2}$	0.994
Correlation coefficient (R)	$R = \sqrt{\frac{\sum_{i=1}^{n}(Y_p-\bar{Y}_p)^2}{\sum_{i=1}^{n}(Y_p-\bar{Y}_p)^2+\sum_{i=1}^{n}(Y_o-Y_p)^2}}$	0.997
Adjusted determination coefficient (R_a^2)	$R_a^2 = 1 - \left[(1-R^2)\left(\frac{n-1}{n-k-1}\right)\right]$	0.985
Coefficient of variance (CV)	$CV = \left(\frac{\sqrt{MSS_E}}{\bar{Y}_o} \times 100\right)$	1.50
Durbin–Watson statistic	$DW = \frac{\sum_{i=2}^{n}(e_i-e_{i-1})^2}{\sum_{i=1}^{n}e_i^2}$	1.51
Mallow's C_p statistic	$C_p = \left(\frac{SS_E}{MSS_E}\right) + 2p - n$	10
Chi-square (χ^2) test	$\chi^2_{cal} = \frac{\sum_{i=1}^{n}(O_i-E_i)^2}{E_i}$	0.009
Bias	$Bias = \exp\left(\frac{1}{n}\sum_{i=1}^{n}\ln\left(\frac{Y_o}{Y_p}\right)\right)$	1.00

in conducted experiments. Adequate precision is greater than 4 is desirable and the ratio was found to be >32, which indicates an adequate signal and confirm that, this model is significant for this extraction process.

The autocorrelation or correlation between errors in the model and linear association between adjacent residuals was evaluated by Durbin–Watson (DW) static method. The range of DW statistic varies between 0 and 4. The DW value below 2 indicates positive correlation and above 2 indicates negative correlation (Khajeh, 2011). Our study shows that, the DW value of 1.51 is close to 2, indicating the good of fit of the model. Mallow's C_p statistic can be used to determine how many terms can be omitted from the model. For a response surface model including all terms, $C_p = p$, where p is the number of parameters or variables in the regression model including the interaction term. For response surface model to omitted terms $C_p \sim p$ shows a good model with little bias, and $C_p \leq p$ shows a very good prediction model. The goal is to remove terms from the response surface model until a minimum C_p value near p is obtained. If $C_p > p$, this shows that too many terms have been removed or some remaining terms are not necessary (Dawson & Martinez-Dawson, 1998). In this study Mallow's C_p statistic ($C_p = 10$) shows the third condition ($C_p < p$ and $p = 10$ including $\beta_0, \beta_1, \ldots, \beta_3^2$), indicating a very good prediction model, as similarly reported by Yetilmezsoya et al. (2009).

The significant difference between the experimental and predicted values was also checked by the chi-square (χ^2) test. The calculated χ^2 value (0.009) was found to be less than the tabulated value (26.296), exhibiting that there was no significant difference between predicted and experimental data. Bias is an estimator used to find out the normal distribution of errors between experimental and predicted value. In our study, bias value of 1 indicted that, the errors are normally distributed and shows the good fit of the model.

3.4. Diagnostics of model adequacy

Model adequacy checking was performed to determine whether the approximating model would give poor or misleading results. Fig. 1 shows the residual and the influence plots for the experimental data obtained from this study. Raw residuals, which cannot be explained by the model, represent the deviations between experimental and predicted values. For these, *Shapiro–Wilk (W)* normality test was carried out and normality test gave a significant value of *W* statistics ($W = 0.943$, $p = 0.0004$), indicating model predict very well for extractive YP from corn silk. The predicted values obtained were quite close to the experimental values, and the points of all predicted and experimental response values fall very close to the 45° line (Fig. 1a), indicating that the model developed was successful in capturing the correlation between the process variables on the response. Fig. 1b shows the normal% probability plot of residuals for response was normally distributed, as they lie reasonably close on a straight line and shows no deviation of the variance.

The good fit of the model was analyzed by constructing the internally studentized residuals versus experimental runs and

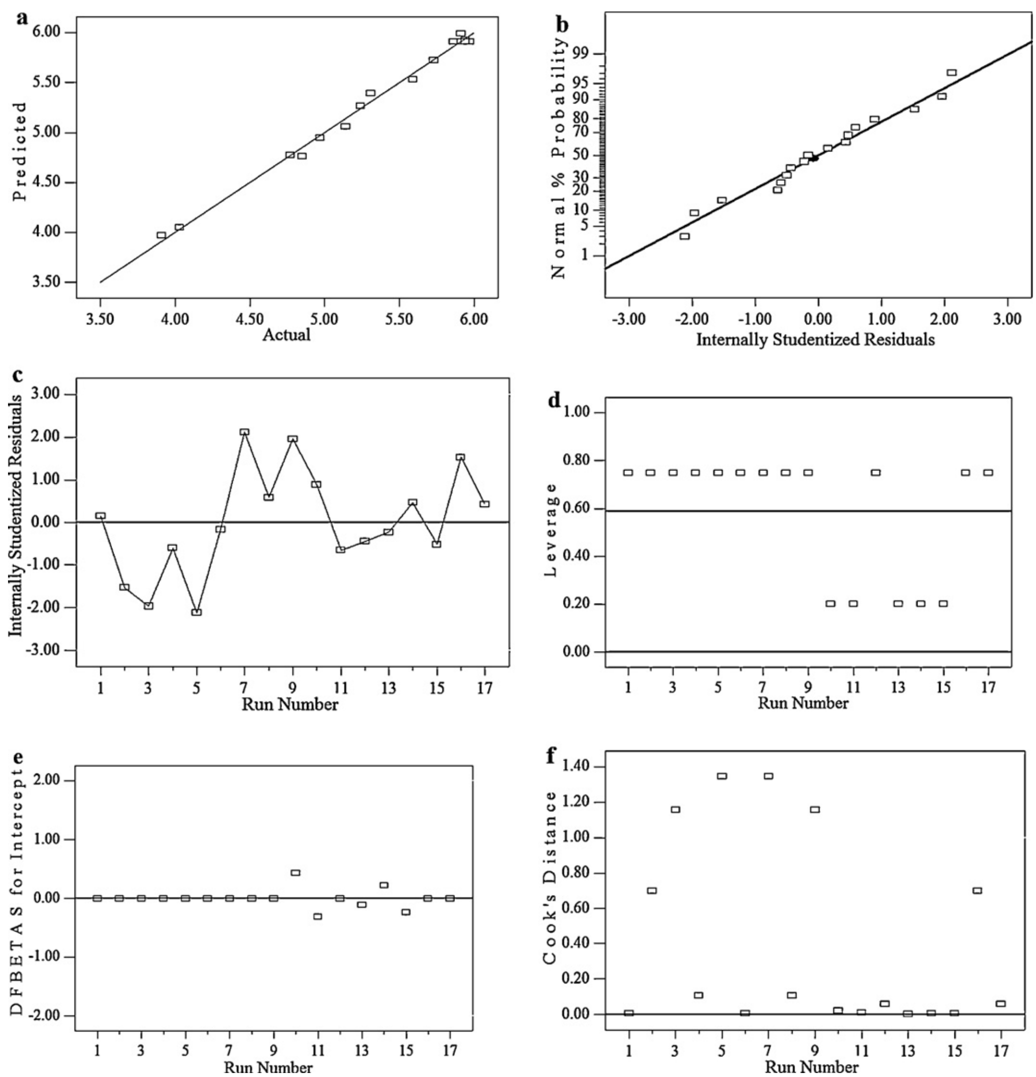

Fig. 1. Diagnostic plots for the model adequacy.

shows that all the data points lay within the limits (Fig. 1c). Since all leverage values were less than 1 (Fig. 1d), there are no outliers or unexpected errors in the model. However, difference in beta values plot (Fig. 1e) showed no undue influence of any observation on any of the regression coefficients. Since the Cook's distance values are in the determined range (Fig. 1f), there is no strong evidence of influential observations in experimental data. Hence, no obvious patterns were found in the analysis of model and indicated the accuracy of the developed model.

3.5. Percentage contribution of process variables

Based on the sum of squares obtained from the ANOVA, the percentage contributions (PC) for each individual process variables were calculated and detailed schematic figure showing the percentage contributions of process variables on the response is shown in Fig. 2. The linear terms showed the highest level of contribution (60.62%) on the TPC compared with the other terms and this was followed by the quadratic terms (32.89%). Among the all terms, the interactive terms exhibited the lowest level of significance (6.49%) and did not showed a large effect in prediction of the YP. Hence, TPC values proved that, the linear independent variables have a direct relationship on the dependent variable.

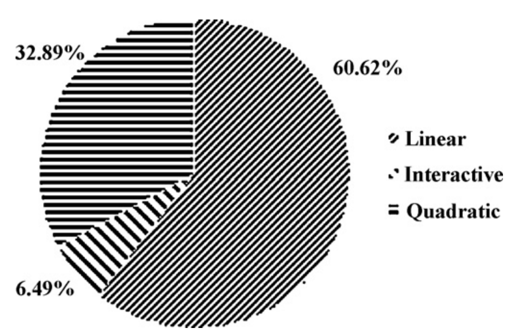

Fig. 2. Detailed schematic diagram showing the percentage contributions of process variables.

3.6. Effect of process variables on the YP

Three factors at three levels BBD were used in this study to investigate the influence of process variables such as extraction temperature, extraction time, and solid–liquid ratio on the UAE of polysaccharide from corn silk. From the developed model, the three dimensional response surface and contour plots were constructed to illustrate the main and interactive effects of independent variables on a response variable. These graphs are easy to understand and drawn by maintaining two factors constant (in turn at its

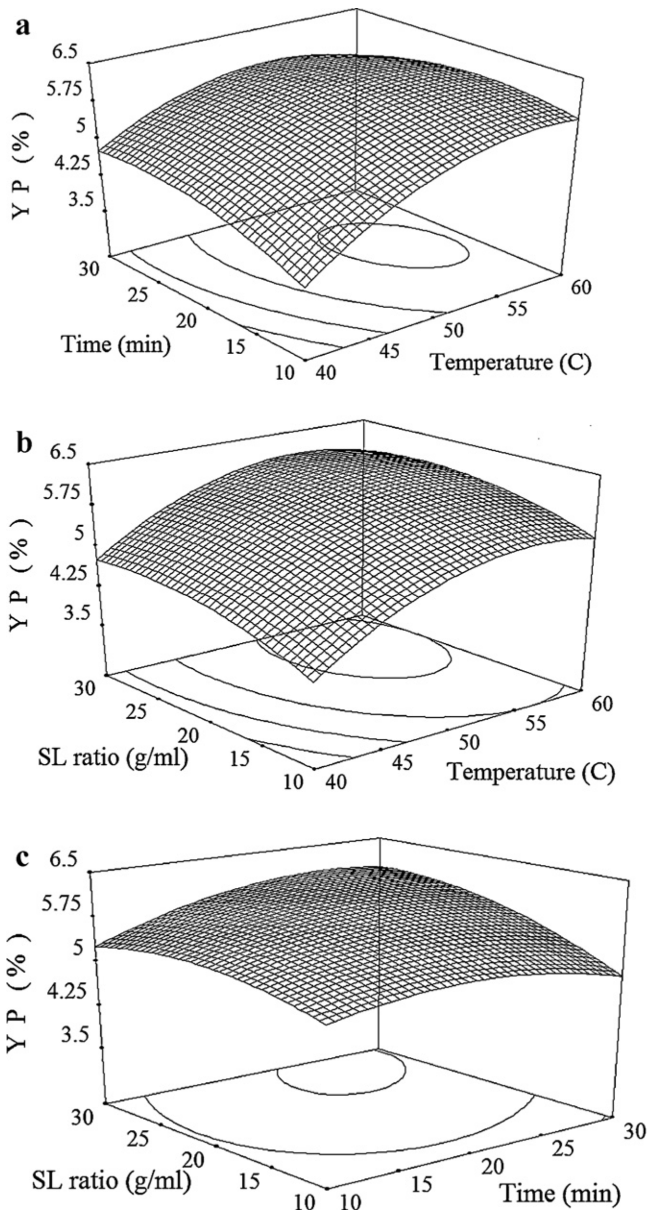

Fig. 3. Response surface plots representing the effect of process conditions on the YP.

central level) whereas varying the other two factors in order to understand their main and interactive effects on the dependent variables. It is also used to locate the optimum conditions.

3.6.1. Effect of extraction temperature

Studies were conducted to evaluate the effect of temperature over the YP. From the results, it can be concluded that extraction temperature exhibits a positive linear and negative quadratic effect (Table 3) on YP. Temperature has a greater influence on the cavitation threshold, which is responsible for acoustic cavitation and also results in the formation of cavitational nucleus. The influence of relative greater force ruptured and erupted the formed cavitational nucleus and disrupted the cell tissues during extraction, which will in turn enhance mass transfer (Toma et al., 2001) increased the extraction efficiency (Fig. 3a and b). When temperature is increased from 40 to 60 °C, viscosity and density of the extracts were decreases, which facilitate the penetration of solvent deeper into sample matrix (Chen et al., 2012). As solvent moves deeper, its area of exposure increases which ends up with higher extraction efficiency.

3.6.2. Effect of extraction time

The YP was increased when extraction time was increased from 10 to 20 min but slowly decreases, when the duration continued to be extended with higher solid–liquid ratio (Fig. 3c). Most of the polysaccharide from the tissues gets released at the early period of extraction, because ultrasound facilitates the disruption of corn silk's cell wall, which enhances both the solubility and release of polysaccharide to the exterior solvent. However, longer extraction time with ultrasound treatment might induce the degradation of polysaccharide (Ying et al., 2011) and decreased the yield, because of continual asymmetric collapse of micro-bubbles. The results are in agreement with Rostagno, Palma, and Barroso, 2007 and Ying et al. (2011), who clearly indicated that 20 min of sonication time was sufficient enough to extract phenolics from soy beverages and polysaccharide from mulberry leaves.

3.6.3. Effect of solid–liquid ratio

From Fig. 3b and c, it is clear that YP increases with increasing solid–liquid ratio ranges from 1:10 to 1:20 (g/ml). Higher concentration of solvent–liquid ratio enhances the efficiency of extraction by creating a concentration difference between the interior plant cell and the exterior solvent, which in turn augments the mass transfer rate and ends up with the increase in extraction efficiency. The physical effects such as liquid circulation and turbulence produced by cavitation help in increasing the contact surface area between the solvent and targeted compounds by permitting greater penetration of solvent into the sample matrix (Romdhane

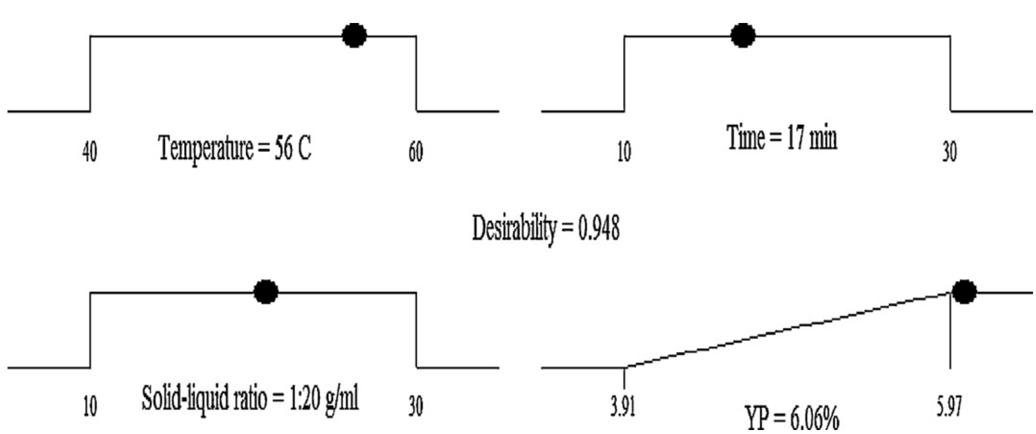

Fig. 4. Desirability ramp for optimization.

& Gourdon, 2002) without causing any significant decomposition of the solvent (Toma et al., 2001) and thus increased the extraction efficiency. However, a high ratio of water to raw material prolonged the distance of diffusion toward the interior tissues. Thus, the extraction efficiency was increased slowly when the solid–liquid ratio increased from 1:20 to 1:30 (g/ml).

3.7. Optimization and verification of the model

Derringer's desired function methodology was employed to optimize the extraction process conditions on the maximum extractive YP from corn silk as follows: extraction temperature of 56 °C, extraction time of 17 min and solid–liquid ratio of 1:20. Under these conditions, the predicted YP was 6.06% with a desirability value of 0.948. A desirability ramp was developed from optimal points via numerical optimization technique (Fig. 4).

For their validation of the optimum conditions, triplicate confirmatory experiments were carried out under the optimized conditions and the average polysaccharide yield was $6.02 \pm 0.02\%$. The results are closely related with the data obtained from optimization analysis using desirability functions, indicating Box–Behnken design incorporate with desirability function could be effectively used to optimize the extraction parameters on the YP.

4. Conclusions

Box–Behnken response surface design was successfully employed to optimize and study the individual and interactive effect of process variables such as extraction temperature, time and solid–liquid ratio on the YP from corn silk by UAE. The results indicated that the application of ultrasound during extraction and the process variables had a significant effect on the maximum yield of polysaccharide from corn silk. Model summary statistics showed that, developed model was adequate and precise with the experimental data. Analysis of variance showed a high coefficient of determination value (R^2) of 0.994 for ensuring a satisfactory fit of the developed second-order polynomial regression model with the experimental data. The optimum conditions were found to be, extraction temperature of 56 °C, extraction time of 17 min and solid–liquid ratio of 1:20 and the predicted maximum yield of polysaccharide is 6.06%. Under these optimized conditions the experimental values of polysaccharide agreed closely with the predicted yield.

References

Box, G. E. P., & Behnken, D. W. (1960). Some new three level designs for the study of quantitative variables. *Technometrics*, *2*, 455–475.

Chen, W., Huang, Y., Qi, J., Tang, M., Zheng, Y., Zhao, S., et al. (2012). Optimization of ultrasound-assisted extraction of phenolic compounds from areca husk. *Journal of Food Processing and Preservation*, http://dx.doi.org/10.1111/j.1745-4549.2012.00748.x

Dawson, P. L., & Martinez-Dawson, R. R. (1998). Using response surface analysis to optimize the quality of ultrapasteurized liquid whole egg. *Poultry Science*, *77*, 468–474.

Derringer, G., & Suich, R. (1980). Simultaneous optimization of several response variables. *Journal of Quality Technology*, *12*(4), 214–219.

Du, J., & Xu, Q. T. (2007). A study on mechanisms of stigma maydis polysaccharide on weight loss in experimental animals. *Zhongguo Yaolixue Tongbao*, *23*, 816–820.

Du, J., Xu, Q. T., & Gao, X. H. (2007). Effects of stigma maydis polysaccharide on gastrointestinal movement. *Zhongguo Zhong Yao Za Zhi*, *32*, 1203–1206.

Ebrahimzadeh, M. A., Pourmorad, F., & Hafezi, S. (2008). Antioxidant activities of Iranian corn silk. *Turkish Journal of Biology*, *32*(1), 43–49.

Edrissi, M., Asl, N. R., & Madjidi, M. (2008). Interaction of mefenamic acid with cobalt (II) ions in aqueous media: Evaluation via classic and response surface methods. *Turkish Journal of Chemistry*, *32*, 505–519.

El-Ghorab, A., El-Massry, K. F., & Shibamoto, T. (2007). Chemical composition of the volatile extract and antioxidant activities of the volatile and nonvolatile extracts of Egyptian corn silk (*Zea mays* L.). *Journal of Agricultural and Food Chemistry*, *55*(22), 9124–9127.

Guevara, P., Perez-Amador, M. C., Zuniga, B., & Snook, M. (2000). Flavones in corn silks and resistance to insect attacks. *Phyton-International Journal of Experimental Botany*, *69*, 151–156.

Habtemariam, S. (1998). Extract of corn silk (stigma of *Zea mays*) inhibits the tumour necrosis factor-alpha- and bacterial lipopolysaccharide-induced cell adhesion and ICAM-1 expression. *Planta Medica*, *64*(4), 314–318.

Hamed, E., & Sakr, A. (2003). Application of multiple response optimization technique to extended release formulations design. *Journal of Controlled Release*, *73*(2–3), 329–338.

Huang, W., Xue, A., Niu, H., Jia, Z., & Wang, J. (2009). Optimised ultrasonic-assisted extraction of flavonoids form Folium eucommaiae and evaluation of antioxidant activity in multi-test systems in vitro. *Food Chemistry*, *114*, 1147–1154.

Khajeh, M. (2011). Response surface modelling of lead pre-concentration from food samples by miniaturised homogenous liquid–liquid solvent extraction: Box–Behnken design. *Food Chemistry*, *129*(40), 1832–1838.

Kim, H. J., Chi, M. H., & Hong, I. K. (2009). Effect of ultrasound irradiation on solvent extraction process. *Journal of Industrial and Engineering Chemistry*, *15*(6), 919–926.

Kim, K. A., Choi, S. K., & Choi, H. S. (2004). Corn silk induces nitric oxide synthase in murine macrophages. *Experimental and Molecular Medicine*, *36*(6), 545–550.

Lee, M. H., & Lin, C. C. (2007). Comparison of techniques for extraction of isoflavones from the root of radix puerariae: Ultrasonic and pressurized solvent extractions. *Food Chemistry*, *105*, 223–228.

Li, H., Wei, Y., You, J., & Lydy, M. J. (2010). Analysis of sediment-associated insecticides using ultrasound assisted microwave extraction and gas chromatography–mass spectrometry. *Talanta*, *83*(1), 171–177.

Li, J. W., Fan, L. P., Ding, S. D., & Ding, X. L. (2007). Nutritional composition of five cultivars of Chinese jujube. *Food Chemistry*, *103*, 454–460.

Luque-García, J. L., & Luque de Castro, M. D. (2003). Ultrasound: A powerful tool for leaching. *Trends in Analytical Chemistry*, *22*, 41–47.

Maksimovic, Z., Malencic, D., & Kovacevic, N. (2005). Polyphenol contents and antioxidant activity of Maydis stigma extracts. *Bioresource Technology*, *96*(8), 873–877.

Maksimovic, Z. A., & Kovacevic, N. (2003). Preliminary assay on the antioxidative activity of Maydis stigma extracts. *Fitoterapia*, *74*(1–2), 144–147.

Mourabet, M., El Rhilassi, A., El Boujaady, H., Bennani-Ziatni, M., El Hamri, R., & Taitai, A. (2012). Removal of fluoride from aqueous solution by adsorption on Apatitic tricalcium phosphate using Box–Behnken design and desirability function. *Applied Surface Science*, *258*, 4402–4410.

Prakash Maran, J., & Manikandan, S. (2012). Response surface modeling and optimization of process parameters for aqueous extraction of pigments from prickly pear (*opuntia ficus-indica*) fruit. *Dyes and Pigments*, *95*, 465–472.

Prakash Maran, J., Sivakumar, V., Sridhar, R., & Prince Immanuel, V. (2013). Development of model for mechanical properties of tapioca starch based films. *Industrial Crops and Products*, *42*, 159–168.

Rau, O., Wurglics, M., Dingermann, T., Abdel-Tawab, M., & Schubert-Zsilavecz, M. (2006). Screening of herbal extracts for activation of the human peroxisome proliferator-activated receptor. *Pharmazie*, *61*(11), 952–956.

Romdhane, M., & Gourdon, C. (2002). Investigation in solid–liquid extraction: Influence of ultrasound. *Chemical Engineering Journal*, *87*, 11–19.

Rostagno, M. A., Palma, M., & Barroso, C. G. (2003). Ultrasound-assisted extraction of soy isoflavones. *Journal of Chromatography A*, *1012*, 119–128.

Rostagno, M. A., Palma, M., & Barroso, C. G. (2007). Ultrasound-assisted extraction of isoflavones from soy beverages blended with fruit juices. *Analytica Chimica Acta*, *597*, 265–272.

Sun, Y. D., Liu, D., Chen, J., Ye, X., & Yu, D. (2011). Effects of different factors of ultrasound treatment on the extraction yield of the all-trans-β-carotene from citrus peels. *Ultrasonics Sonochemistry*, *18*(1), 243–249.

Toma, M., Vinatoru, M., Paniwnyk, L., & Mason, T. J. (2001). Investigation of the effects of ultrasound on vegetal tissues during solvent extraction. *Ultrasonics Sonochemistry*, *8*, 137–142.

Vinatoru, M. (2001). An overview of the ultrasonically assisted extraction of bioactive principles from herbs. *Ultrasonics Sonochemistry*, *8*, 303–313.

Yang, C. X., He, N., Ling, X. P., Ye, M. L., Zhang, C. X., & Shao, W. Y. (2008). The isolation and characterization of polysaccharides from longan pulp. *Separation and Purification Technology*, *63*, 226–230.

Yetilmezsoya, K., Demirelb, S., & Vanderbeic, R. J. (2009). Response surface modeling of Pb (II) removal from aqueous solution by *Pistacia vera* L.: Box–Behnken experimental design. *Journal of Hazardous Materials*, *171*(1–3), 551–562.

Ying, Z., Han, X., & Li, J. (2011). Ultrasound-assisted extraction of polysaccharides from mulberry leaves. *Food Chemistry*, *127*, 1273–1279.

Zhao, W., Yu, Z., Liu, J., Yu, Y., Yin, Y., Lina, S., et al. (2011). Optimized extraction of polysaccharides from corn silk by pulsed electric field and response surface quadratic design. *Journal of Science, Food and Agriculture*, *91*, 2201–2209.