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Modeling and optimization of ultrasound-assisted extraction of polysaccharide from *Cucurbita moschata*

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ABSTRACT

Polysaccharides from pumpkin were extracted by ultrasound-assisted extraction technology using four factors at five levels central composite rotatable response surface design (CCRD). On using single factor analysis, process variables such as extraction temperature (50–70 °C), power of ultrasound (50–70 W), time (15–25 min) and solid–liquid ratio (1:10–1:20 g/ml) were selected. Experiments were conducted to evaluate the effects of four independent variables on the maximum extraction yield of polysaccharides. From the experimental data, second order polynomial mathematical model were developed with high coefficient of determination values ($R^2 > 0.96$). From response surface plots, temperature and ultrasound power exhibited independent and interactive effects on the extraction yields. Extraction temperature of 70 °C, ultrasound power of 70 W, time of 23 min and solid–liquid ratio of 1:10 g/ml were determined as optimal conditions with a maximum polysaccharides yield of 16.21%, which was confirmed through the validation of the experiments.

1. Introduction

Polysaccharides from fruits and vegetables have drawn attention of both food producers and consumers due to their physical properties, health-promoting and disease preventing potential and hence have been employed in cosmetic and pharmaceutical products (Willats, Knox, & Mikkelsen, 2006). In recent years, the isolated polysaccharides have been found to play an important role in the biomedical field due to their antioxidant (Guo et al., 2010), immunostimulatory (Sun & Liu, 2009) and antitumor (Zhou, Song, Feng, & Tan, 2011) effects. In the past few years, the pumpkin has received considerable attention because of its nutritional and health protective value, and also its potential in medicinal uses have been explored.

Pumpkin (*Cucurbita moschata*) is an annual herbaceous plant of the family Cucurbitaceae. The fruit of pumpkin is one of the most important vegetables in traditional agricultural systems in the world. The flesh and peel of the fruit represent rich sources of pectin-type dietary fiber and antioxidants (Caili, Huan, & Quanhong, 2006). So far, several beneficial physiological effects, immunological activity and other pharmacological activities such as lipid-lowering, hepatoprotective (Makni et al., 2008), anti-carcinogenic, anti-microbial (Caili, Haijun, Tongyi, Yi, & Quanhong, 2007; Park, Lee, & Kim, 2010) and anti-diabetic properties (Adams et al., 2011; Caili et al., 2006; Xia & Wang, 2006; Yadav, Jain, Tomar, Prasad, & Yadav, 2010) of various pumpkin extracts have been published and its antioxidant activity was reported by Nara, Yamaguchi, Maeda, and Koga (2009) for a water-soluble polysaccharide from the fruit of pumpkin. Pumpkin polysaccharides (PP) are composed of galactose, glucose, arabinose, xylose and glucuronic acid and are water insoluble but organic solvents soluble macromolecular compounds with important biological functions. PP has the biological effects of detoxification, anti-oxidation, reducing blood pressure, reducing blood lipids, lowering cholesterol levels (Yong, Ning, & Liu, 2006), promote the biosynthesis of nucleic acids and proteins, control cell division and differentiation, regulating cell growth and aging, especially for the treatment of diabetes (Zhang, Shen, & Zhu, 2002).

The traditional extraction methods of polysaccharides from plant tissues are maceration, mechanical rabbling and heat reflux. These extraction methods depend largely on energy input and agitation to improve the solubility and mass transfer efficiency of polysaccharides. Usually, the conventional extraction method requires long extraction time and high extraction temperature with low extraction yield, but high-energy consumption (Chen, Li, Liu, Yang, & Li, 2012). Ultrasound in combination with conventional extraction is a potential technique, which is a fully reproducible food process, completed in a shorter time with high reproducibility, reduced processing cost, simplified manipulation and work-up.

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This process gives a higher purity of the final product, eliminates post-treatment of waste water and consumes only a fraction of the time and energy, normally needed for conventional processes (Kim, Chi, & Hong, 2009; Li, Wei, You, & Lydy, 2010; Maran, Manikandan, Thirugnanasambandham, Nivetha, & Dinesh, 2012; Sun, Liu, Chen, Ye, & Yu, 2011). Ultrasound-assisted extraction (UAE) is an ideal extraction method capable of producing high quantities of polysaccharides and is non-destructive with a shorter extraction time.

Previous findings have reported the influence of many independent variables, such as solvent composition, pH, temperature, extraction time, and solid to liquid ratio, on the yields of bioactive compounds which can be extracted from diverse natural products (Bujić-Kojić, Planinic, Srécko, Jakabek, & Seruga, 2009; Cacace & Mazza, 2002, 2003; Pinelo, Rubilar, Sineiro, & Nunez, 2005; Santos, Veggi, & Meireles, 2010; Wettasinghe & Shahidi, 1999). Response Surface Methodology (RSM) is a collection of statistical and mathematical techniques useful for developing, improving and optimizing processes, in which a response of interest is influenced by independent variables, and it generates a mathematical model that describes the chemical process (Prakash Maran, Sivakumar, Sridhar, & Prince Immanuel, 2013) used to find out their optimal values (Triveni, Shamala, & Rastogi, 2001). Several studies on the optimized conditions for the extraction of phenolic compounds from different sources using RSM have been published (Hayouni, Abedrabba, Bouix, & Hamdi, 2007; Pinelo et al., 2005; Pompeu, Silva, & Rogez, 2009; Yang & Zhai, 2010). Hence the objective of the present study is to investigate the individual and interactive effect of UAE process variables such as extraction temperature, power of ultrasound, time and solid–liquid ratio on the extraction yield of polysaccharide from pumpkin and to optimize the processing variables of UAE for the highest yield of pumpkin polysaccharides using central composite rotatable response surface design coupled with Derringer's desired function methodology.

2. Materials and methods

2.1. Pumpkin powder preparation

Freshly harvested pumpkins (*C. moschata*) with similar maturity and weight were purchased from a local fruit orchard near Leeds, UK. The thick layer of the skin and seeds were peeled from the fruits manually and the fruits were washed thoroughly in running tap water to remove any impurities adhered to the surface of the fruit. The washed fruits were dried in the hot air oven (NSW 143, India) at 40 °C until it attains the constant weight. Then the dried samples were pulverized and sifted through a 40-mesh sieve to obtain the powdered samples. The powder (moisture content 8–12% in dry basis) was stored in dark bags and kept in dry environment prior to conduct the experiments.

2.2. UAE of polysaccharide

For the UAE experiments, 10 g of dried pumpkin powder was mixed with an appropriate amount of distilled water in a 500 ml beaker. Experiments were performed using a 20 kHz ultrasonic device (VCX 400, Sonics and Materials, USA and 0–400 W) with a 2.00 cm flat tip probe in a beaker with provisions to set required output power, temperature and time. Ultrasonic generator probe was directly submerged into the suspension and the samples were extracted with continuous ultrasound waves at frequencies of 20 kHz with different levels of power output. An amplitude controller was used to set the desired level of ultrasonic power. Ultrasonic output powers were determined calorimetrically and ranged from 50 to 70 W according to the method described by Li,

Table 1
Central composite rotatable experimental design with results for extraction of polysaccharide yield.

Run order	Blocks	Temperature (°C)	Power of ultrasound (W)	Time (min)	SL ratio (g/ml)	Polysaccharide yield (%)		Residual error	% Error	Actual error
						Experimental	Predicted			
1	1	50	70	15	1:10	9.84	9.59	0.25	2.53	0.25
2	1	60	60	20	1:15	13.58	14.09	−0.51	−3.78	0.51
3	1	50	70	25	1:10	11.99	12.18	−0.19	−1.58	0.19
4	1	50	70	25	1:20	9.42	9.75	−0.33	−3.54	0.33
5	1	70	50	15	1:20	16.02	16.03	−0.01	−0.05	0.01
6	1	70	70	15	1:10	13.67	13.84	−0.17	−1.25	0.17
7	1	60	60	20	1:15	14.45	14.09	0.36	2.47	0.36
8	1	70	70	15	1:20	13.43	13.79	−0.36	−2.68	0.36
9	1	60	60	20	1:15	14.25	14.09	0.16	1.10	0.16
10	1	60	60	20	1:15	14.3	14.09	0.21	1.44	0.21
11	1	70	70	25	1:20	13.15	13.17	−0.02	−0.14	0.02
12	1	70	50	15	1:10	13.61	13.06	0.55	4.01	0.55
13	1	70	70	25	1:10	15.52	16.05	−0.53	−3.38	0.53
14	1	60	60	20	1:15	13.93	14.09	−0.16	−1.18	0.16
15	1	50	50	25	1:10	10.72	10.15	0.57	5.33	0.57
16	1	50	50	15	1:20	13.51	12.77	0.74	5.45	0.74
17	1	70	50	25	1:10	13.49	13.47	0.02	0.16	0.02
18	1	60	60	20	1:15	14.05	14.09	−0.04	−0.31	0.04
19	1	50	50	25	1:20	10.71	10.74	−0.03	−0.25	0.03
20	1	70	50	25	1:20	13.57	13.61	−0.04	−0.27	0.04
21	1	50	50	15	1:10	9.18	9.36	−0.18	−1.96	0.18
22	1	50	70	15	1:20	9.77	9.99	−0.22	−2.25	0.22
23	2	40	60	20	1:15	9.41	9.71	−0.30	−3.17	0.30
24	2	60	40	20	1:15	12.63	13.44	−0.81	−6.38	0.81
25	2	60	60	10	1:15	9.91	10.20	−0.29	−2.92	0.29
26	2	80	60	20	1:15	17.11	16.83	0.28	1.65	0.28
27	2	60	80	20	1:15	14.02	13.23	0.79	5.64	0.79
28	2	60	60	30	1:15	10.64	10.37	0.27	2.57	0.27
29	2	60	60	20	1:05	12.08	12.23	−0.15	−1.28	0.15
30	2	60	60	20	1:25	12.91	12.77	0.14	1.07	0.14

Pordesimo, and Weiss (2004). During the extraction period, temperature was controlled at a desired level within ±1 °C and the experiments were carried out according to Table 1. All the experiments were performed in triplicates and the reported result is the mean of these triplicate measurements.

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 yield of polysaccharide (*Y*) was calculated by the following equation:

$$Y\ (\%) = \frac{w_t}{w_i} \times 100 \tag{1}$$

where w_t is the weight of the crude extract and w_i is the weight of the pumpkin powder.

2.4. Experimental design

Central composite rotatable response surface design (CCRD) was employed to study and optimize the effect of process variables such as extraction temperature (50–70 °C), power of ultrasound (50–70 W), time (15–25 min) and solid–liquid ratio (1:10–1:20 g/ml) on the extraction yield of polysaccharide from pumpkin. The application of statistical experimental design techniques in bioprocess development and its 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 (Maran, Sivakumar, Sridhar, & Thirgananasambandham, 2012). On single factor analysis, process variables and their ranges were selected and independent variables were coded at five levels between −2 and 2. The coding of the variables was done by the following equation (Prakash Maran et al., 2013):

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

where x_i is the dimensionless coded 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 center point; and ΔX_i, step change of the real value of the variable *i*.

In this study, CCRD consists of 16 factorial points, 8 axial points, 6 center points and two blocks. Totally 30 experiments were performed to optimize and study the effect of process variables on the response. The Center point is replicated to find and allow the estimation of experimental error. So the replication of the entire experimental design is not required. It is recommended that six center points have taken in a CCRD with four factors and the total number of experiments (*N*) was calculated by the following equation:

$$N = 2^K + 2K + C_p \tag{3}$$

where *K* is the number of process variable, 2^K is the number of factorial points, 2*K* is the number of the axial points on the axis of each design factor at a distance of $\pm\alpha$ ($\alpha = 2^{K/4} = 2$ for $K = 4$) and C_p is the replicate number of the central point.

In this study, the experimental run was randomized in order to reduce the error arising from the experimental process due to the extraneous factors. A nonlinear regression method was used to fit the second order polynomial (Eq. (4)) to the experimental data and express the mathematical relationship between process variables (X_1, X_2, X_3 and X_4) and the response (*Y*). The generalized form of the second order polynomial equation is shown in Eq. (4).

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

where *Y* is the response; x_i and x_j are variables (*i* and *j* range from 1 to *k*); β_0 is the model intercept coefficient; β_j, β_{jj} and β_{ij} are interaction coefficients of linear, quadratic and the second-order terms, respectively; *k* is the number of independent parameters ($k = 4$ in this study); and e_i is the error (Prakash Maran & Manikandan, 2012). The final mathematical second order polynomial model includes 4 linear terms, 6 two factor interaction terms, 4 squared terms and 1 intercept term.

2.5. Statistical analysis

Design expert 8.0.7.1 statistical software package (Stat-Ease Inc., USA) was used to analyze the experimental data. Multiple regression analysis and Pareto analysis of variance (ANOVA) were used to evaluate the experimental data and the ANOVA table was generated. Significant terms in the model (linear, interactive and quadratic) for the response were found by analysis of variance (ANOVA) and significance was judged by the *F*-statistic value 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), coefficient of variation (CV), determination coefficient (R^2), adjusted determination of coefficient (R_a^2) and predicted determination of coefficient (R_p^2) to reflect the statistical significance of the developed quadratic mathematical model. After fitting the data to the models, the model was used for the construction of three dimensional response surface plots to predict the relationships between independent and dependent variables.

2.6. Total percentage contributions of process variables

The total percentage contributions (P_i) of each individual process variables were calculated based on the regression coefficients obtained from the ANOVA analysis. The following equations were used to find out the P_i of individual process variables as described by Khataee, Fathinia, Aber, and Zarei (2010).

$$P_i = \left(\frac{\beta_i^2}{\sum \beta_i^2} \right) \times 100 \quad (i \neq 0) \tag{5}$$

where β_i is the regression coefficient of individual process variable.

2.7. Determination of optimal conditions

After analyzing the polynomial equation depicting the dependent and independent variables, optimization process was carried out by Derringer's desired function methodology (Derringer & Suich, 1980). 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 1 creates a linear ramp function between the low value and the goal or the high value and the goal. Increased weight (up to 10) moves the result towards the goal and the reduced weight (down to 0.1) creates the opposite effect. Default importance of 3

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	4835.40	1	4835.40			
Linear	76.58	4	19.14	9.23	0.0001	
2FI	20.96	6	3.49	2.15	0.0948	
Quadratic	26.86	4	6.72	25.02	<0.0001	Suggested
Cubic	3.37	8	0.42	4.50	0.0312	Aliased
Residual	0.66	7	0.09			
Total	4963.82	30	165.46			
Lack of fit tests						
Linear	51.36	20	2.57	26.39	0.0009	
2FI	30.40	14	2.17	22.32	0.0015	Suggested
Quadratic	3.54	10	0.35	3.64	0.0834	Aliased
Cubic	0.17	2	0.08	0.87	0.4750	
Pure error	4.87E−01	5	9.73E−02			

Source	Std. dev.	R^2	Adjusted R^2	Predicted R^2	Press	Remarks
Model summary statistics						
Linear	1.44	0.596	0.532	0.416	75.00	
2FI	1.27	0.759	0.633	0.553	57.37	
Quadratic	0.52	0.969	0.939	0.836	21.08	Suggested
Cubic	0.31	0.995	0.979	0.805	25.00	Aliased

was chosen for the response, which can represent the goals to be equally important.

2.8. Verification of the predicted optimized conditions

After optimization, in order to determine the validity of the optimized conditions, triplicate verification 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 in order to find out the accuracy and suitability of the optimized conditions.

3. Results and discussion

3.1. Experimental design (CCRD) analysis

The total number of 30 statistically designed batch experiments were performed for different combinations of the process variables in order to optimize and study the combined effect of independent variables (extraction temperature, power of ultrasound, time and solid–liquid ratio) on the extraction yield of polysaccharides and the results are shown in Table 1, that includes the experimental design, experimental and predicted values of the response. The experimental data was fitted to the various models (linear, interactive (2FI), quadratic and cubic) to obtain regression equation. 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 decide about the adequacy of models among various models to represent the maximum yield of polysaccharide and the results are listed in Table 2.

From Table 2, 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. Therefore the quadratic model incorporating linear, interactive and quadratic terms was chosen to describe the effects of process variables on the extraction of

Table 3
Analysis of variance for the extraction of polysaccharide yield.

Source	Coefficient estimate	Sum of squares	Degree of freedom	Standard error	Mean square	*F* value	*p*-Value
Model	14.09	124.40	14	0.21	8.89	33.11	<0.0001
X_1	1.78	76.04	1	0.11	76.04	283.36	<0.0001
X_2	−0.05	0.06	1	0.11	0.06	0.24	0.6322
X_3	0.04	0.04	1	0.11	0.04	0.16	0.6991
X_4	0.13	0.43	1	0.11	0.43	1.61	0.2239
X_{12}	0.14	0.30	1	0.13	0.30	1.11	0.3094
X_{13}	−0.10	0.15	1	0.13	0.15	0.55	0.4688
X_{14}	−0.11	0.20	1	0.13	0.20	0.75	0.3987
X_{23}	0.45	3.24	1	0.13	3.24	12.07	0.0034
X_{24}	−0.75	9.09	1	0.13	9.09	33.87	<0.0001
X_{34}	−0.71	7.98	1	0.13	7.98	29.74	<0.0001
X_1^2	−0.21	1.17	1	0.10	1.17	4.36	0.0543
X_2^2	−0.19	0.99	1	0.10	0.99	3.70	0.0737
X_3^2	−0.95	24.90	1	0.10	24.90	92.77	<0.0001
X_4^2	−0.40	4.34	1	0.10	4.34	16.17	0.0011
Residual		4.03	15		0.27		
Lack of fit		3.54	10		0.35	3.64	
Pure error		0.487	5		0.10		
Cor total		128.42	29				
Std. dev.	0.52			R^2		0.969	
Mean	12.70			Adjusted R^2		0.939	
C.V.%	4.08			Predicted R^2		0.836	
Press	21.08			Adeq. precision		20.39	

polysaccharide from pumpkin. Furthermore, analysis of variance (ANOVA) was also used to check the adequacy of quadratic model.

3.2. Fitting of second order polynomial equation

By applying multiple regression analysis on the experimental data, the Design-Expert software generated a second-order polynomial equation that can express the relationship between process variables and the response. The final equation obtained in terms of coded factors is given below:

$$\begin{aligned}\text{Yield}\,(\%) = {} & 14.09 + 1.78X_1 - 0.052X_2 + 0.042X_3 + 0.13X_4 \\ & + 0.14X_1X_2 - 0.096X_1X_3 - 0.11X_1X_4 + 0.45X_2X_3 \\ & - 0.75X_2X_4 - 0.71X_3X_4 - 0.21X_1^2 - 0.19X_2^2 - 0.95X_3^2 \\ & - 0.40X_4^2 \end{aligned} \quad (6)$$

where Yield (%) is the polysaccharide yield; X_1, X_2, X_3 and X_4 are the coded values of extraction temperature, power of ultrasound, time and solid–liquid ratio, respectively.

3.3. Statistical analysis

Pareto analysis of variance (ANOVA) and multiple regression analysis were used to analyze the experimental data. The statistical significance of the regression equation was evaluated by the corresponding F and p-values and it is presented in Table 3. The model F and p-value was found to be 33.11 and <0.0001, which indicated that the model was highly statistically significant. The fitness of the model was studied through the lack of fit test. The lack of fit F-value of 3.64 and the associated p-value of 0.0834 was indicated the suitability of the model to predict the variations. The goodness of the fit of the model was evaluated by the determination co-efficient (R^2), adjusted determination co-efficient (R_a^2), predicted determination co-efficient (R_p^2) and co-efficient of variance (CV) and it was listed in Table 3. The R^2 value of the predicted model was 0.969, while R_a^2 value was 0.939, which exhibited the high degree of correlation between the experimental and predicted values. If there are many terms in the models and the sample size is not large enough, R_a^2 may be noticeably smaller than R^2 (Yetilmezsoya, Demirelb, & Vanderbeic, 2009). In our study, the R_a^2 value was found to be smaller and very close to the R^2. The values of R_a^2 and R_p^2 should be approximately within 0.20 of each other, to be in reasonable

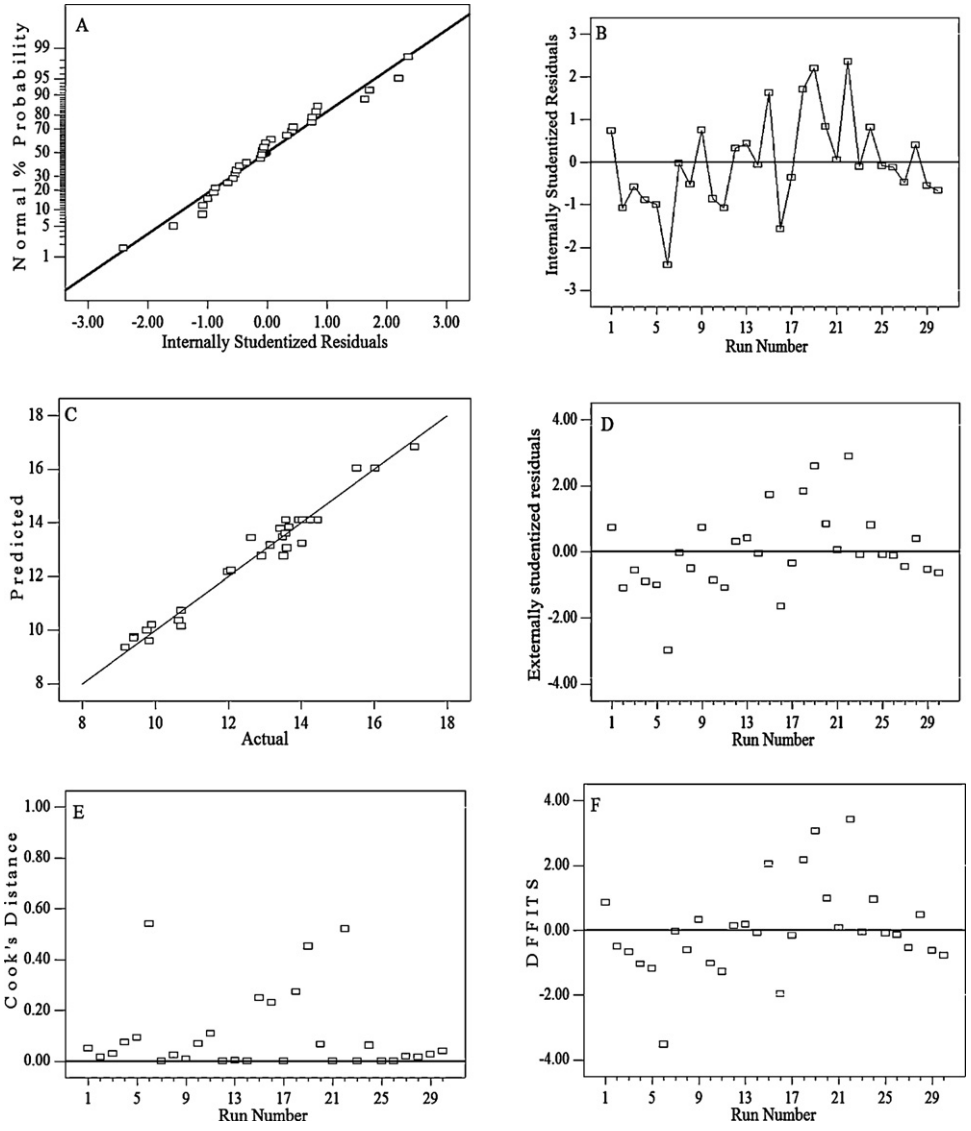

Fig. 1. Diagnostic plots for the model adequacy.

agreement. Otherwise, there may be a problem with either the data or the model (Mourabet et al., 2012). In this study, a high correlation between R_a^2 (0.939) and R_p^2 (0.836) was found and it shows that the form of the model chosen to explain the relationship between the factors and the response is well-correlated. The coefficient of variance (CV) is the ratio of the standard error of the estimate to the average value of the observed response defining by the reproducibility of the model. The low CV (4.08) clearly stated that, the deviations between experimental and predicted values are low, high degree of precision and also showed a good reliability of the conducted experiments. Adequate precision is the measure of signal to noise ratio and greater than 4 of this value is desirable. In this study, the ratio was found to be >20, which indicates an adequate signal and confirm that, the model is significant for this present extraction process. The predicted residual sum of squares (PRESS) value of 21.08 shows how this model fits with each point in the design.

3.4. Diagnostics of model adequacy

Generally, it is important to confirm that the fitted model gives a sufficient approximation to the actual values. Unless the model shows a satisfactory fit, proceeding with an investigation and optimization of the fitted response surface likely gives poor or misleading results. In addition to determination coefficient, the adequacy of the models was also evaluated by the residuals (difference between the observed and the predicted response value) and the influence plots for the experimental data obtained from this study. Residuals are thought as elements of variation, unexplained by the fitted model and then it is expected that they occur according to a normal distribution. Normal probability plots are 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 the variance (Fig. 1A). This can confirm the normal distribution of the data. By constructing internally studentized residuals plot, a check was made to analyze the experimental data to find out the satisfactory fit of the developed models and the plot shows that, all the data points lie within the limits (Fig. 1B).

Diagnostic plots such as predicted versus actual (Fig. 1C) help us to evaluate the model suitability and find out the relationship between predicted and experimental values. The data points on this plot lie reasonably close to the straight line and indicate that an adequate agreement between real data and the data obtained from the models. Fig. 1D showed that, all the data points lie within the limits. Since the Cook's distance values are in the determined range (Fig. 1E), there is no strong evidence of influential observations in the observed data. Difference of fits plot is a measure of the influence of each point on the predicted value. Fig. 1F suggested that, there are no high deviations in the experimental data. Hence, trends observed in Fig. 1 revealed that, no obvious patterns were found and residuals appeared to be randomly scattered.

3.5. Percentage contribution of process variables

The total percentage contributions (P_i) of linear, interactive and quadratic effect of the process variables on the response were calculated based on the regression coefficient obtained from ANOVA. The Pareto analysis gives more significant information to interpret the results. In fact, this analysis calculates the percentage effect of each process variables on the response and it is shown in Fig. 2. As can be seen in this figure, linear effect of extraction temperature (X_1, 56.12%) and quadratic effect of time (X_3^2, 16.08%) produces the main effect on extraction of polysaccharide from pumpkin. This was followed by interactive effect of power + solid–liquid ratio (X_{24}, 10.06%) and time + solid–liquid ratio (X_{34}, 8.84%).

3.6. Influence of process variables

Four factors at five level CCRD was used in this study to investigate the influence of process variables such as extraction temperature, power of ultrasound, time and solid–liquid ratio on the UAE of polysaccharide from pumpkin. To understand the interaction between the independent variables and

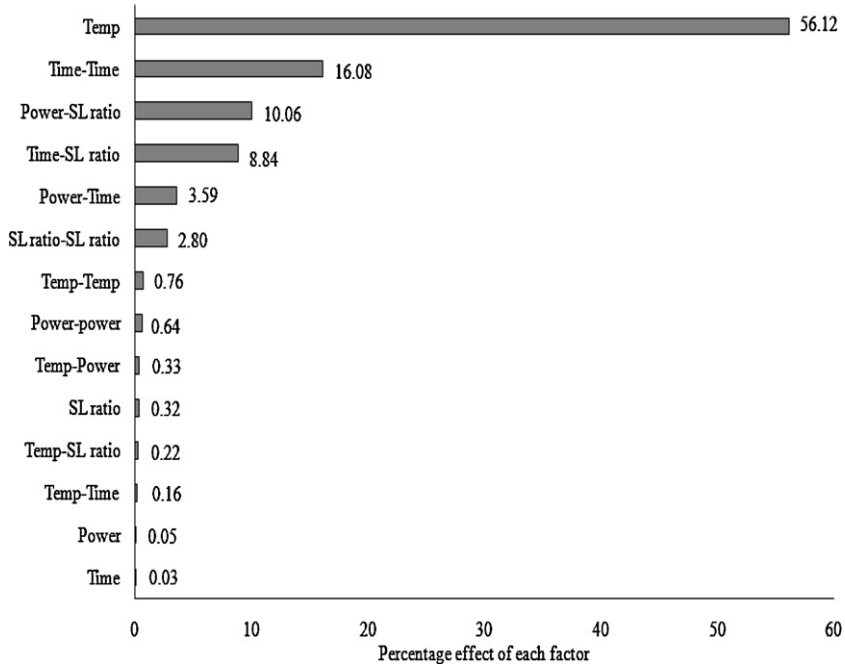

Fig. 2. Detailed schematic diagram showing the percentage contributions of individual process variables.

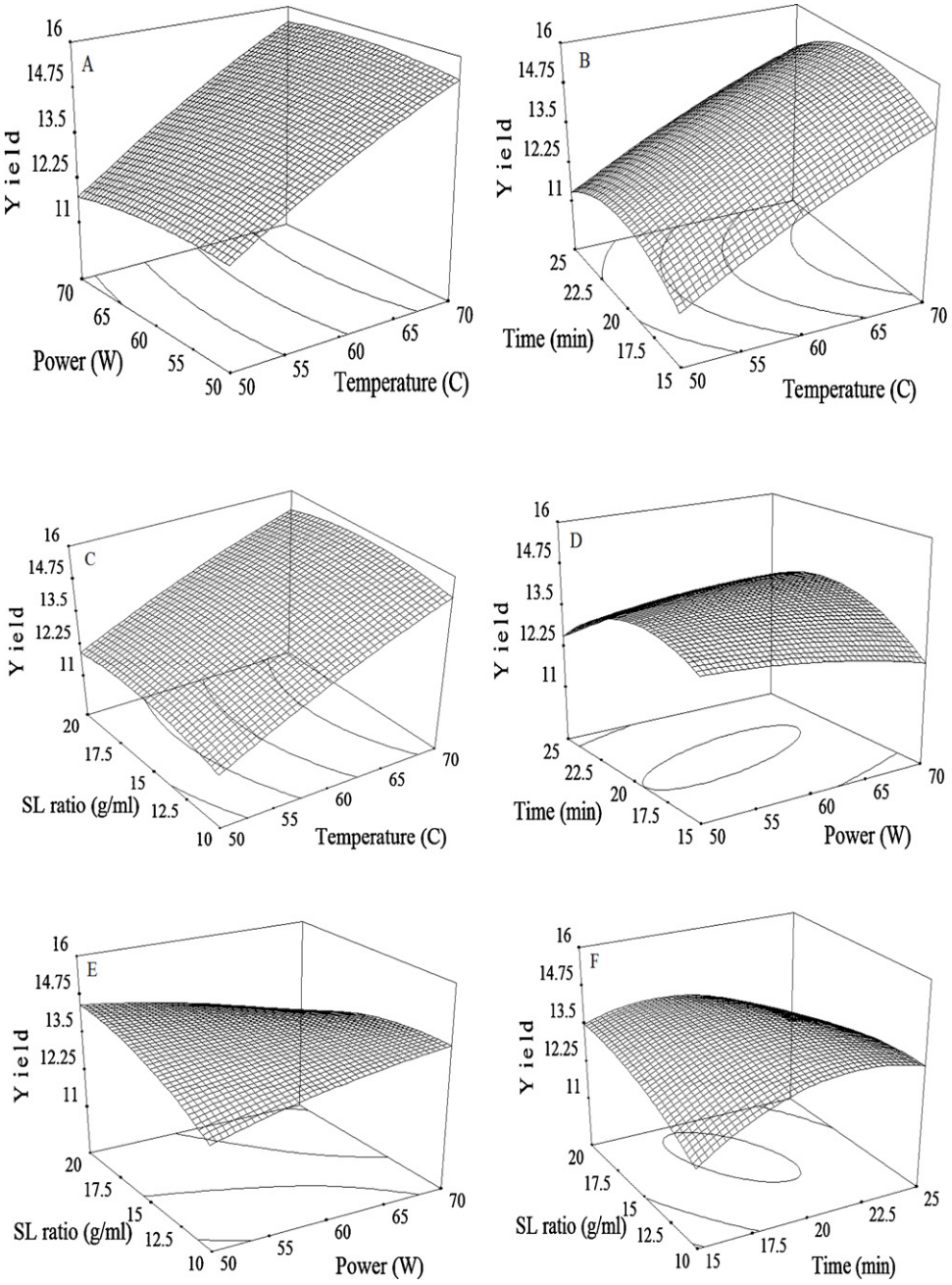

Fig. 3. Response surface plots representing the effect of process conditions on the extraction yield of polysaccharide.

estimate the polysaccharide extraction efficiency over independent variables, three dimensional (3D) response surface plots were plotted from the developed model. In this study, the model has more than two factors. So, the 3D plots have drawn by maintaining two factors at constant level (in turn at its central level), whereas the other two factors were varied in their range 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. *Influence of extraction temperature*

Experiments were carried out to study the effect of temperature over the extraction yield of polysaccharide from pumpkin. From the results, it was observed that, the yield of polysaccharide was increased linearly with increasing temperature from 50 to 70 °C (Fig. 3A–C). Higher temperature can accelerate the molecular movements, conductivity of the solution to the plant material. This effect can increase the solubility and diffusivities of the plant materials in to the solution and increases the extraction yield (Yang et al., 2010). On the other hand, 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 in turn enhanced the mass transfer rate (Toma, Vinatoru, Paniwnyk, & Mason, 2001) and increased the extraction efficiency (Fig. 3A and C). Additionally, solvents have greater capacity to solubilize the analytes at higher temperatures, while surface tension and solvent viscosity decreases with increasing temperature, which will improve sample wetting and matrix penetration, respectively (Xie et al., 2010). As the solvent moves deeper, its area of exposure increases which ends up with higher

extraction efficiency. Based on the results, 70 °C was chosen as the optimum extraction temperature.

3.6.2. Influence of ultrasound power

In this study, the influence of different power of ultrasound (50–70 W) on the extraction yield of polysaccharide was studied. It can be seen that, the yield of polysaccharide was increased linearly with increasing power (Fig. 3A). As the larger amplitude ultrasonic wave passed through the liquid medium, more bubbles were created and collapsed (Quan, Sun, & Qu, 2009). Since the temperature and pressure were very high inside the bubbles and the collapse of bubbles occurred over a very short time, the violent shock wave and high-speed jet were generated which could enhance the penetration of the solvent into the cell tissues and accelerate the intracellular product release into the solvent by disrupting the cell walls (Zhang et al., 2008). Moreover, the violent shock wave and high-speed jet were generated which could enhance the penetration of the solvents in to the cell tissues. This accelerates the intracellular product release into the solvent by disrupting the cell walls via hydration of pectinous material which might lead to the breakup of plant tissue during sonication and also causes the molecules to mix better, thus enhancing the mass transfer rate. However, due to a further increase in the ultrasonic power beyond 70 W, the yield was increased moderately due to presence of hard cell walls of the plant material. Based on the current results, the maximum ultrasonic power 70 W was chosen as the optimum output power.

3.6.3. Influence of time

Time is one of the important factors affecting extraction yield of polysaccharide. Different extraction time would influence the solvent and solid matrix contact. As shown in Fig. 3B, D and F, the extraction yield was increased steadily and reached the maximum at 23 min. This phenomenon could be explained that, all the plant cells will be completely cracked because of acoustic cavitation effects caused by the ultrasonic waves in the earlier period of extraction (Wang, Wu, Dai, Chen, & Shao, 2012). So larger contact area between solvent and material was created and the collapse of the bubbles will promote the interpenetration of the solvent into the plant cells to dissolve most of the polysaccharides present in it and increases the extraction yield. The number of cavitation micro-bubbles created by ultrasound increased with the duration of extraction extended. The asymmetric collapse of micro-bubbles near surfaces was also associated with micro-jets that could scour surfaces and damage substance in solution (Vilkhu, Mawson, Simons, & Bates, 2008). However, when the plant cells ruptured, various compounds such as insoluble substances were also suspended in the extraction liquid, resulting in the lower permeability of the solvent. So the yield was decreased slowly, when the extraction time extended. Thus the optimum extraction time was 23 min.

3.6.4. Influence of solid–liquid ratio

The solvent quantity is also an important factor to the yield of polysaccharide. Therefore, in this study we evaluated its influence on the extraction yield. From the results, it was observed that the extraction yield of polysaccharide was increased with the increasing solid–liquid ratio (Fig. 3C, E and F). 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 favors mass transfer. Too much liquid would not change much of the driving force any more as the limitation to mass transfer is more confined to the solid interior (Zhang et al., 2008). However, the combined effect of solid–liquid ratio (more than 1:10) with the optimum conditions of other process variables (extraction temperature of 70 °C, power of ultrasound of 70 W and time of 23 min) observed in this study would not cause any further increase in the polysaccharide yield. Taking all these factors into consideration, a solid–liquid ratio of 1:10 g/ml was considered as optimal for this present extraction process.

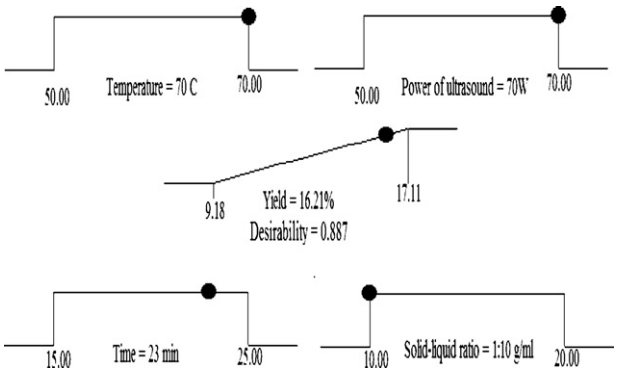

Fig. 4. Desirability ramp for optimization.

3.7. Optimization of extraction parameters and validation of the optimized conditions

According to the CCRD results, the optimal extraction conditions to obtain maximum extraction yield of polysaccharide from pumpkin were determined by Derringer's desired function methodology as follows: extraction temperature of 70 °C, ultrasound power of 70 W, time of 23 min and solid–liquid ratio of 1:10 g/ml. Under these conditions, the predicted extraction yield of polysaccharide was 16.21% with a desirability value of 0.887. A desirability ramp was developed from optimal points via numerical optimization technique (Fig. 4). The suitability of the optimized conditions for predicting the optimum response values was tested using the selected optimal conditions. The maximum predicted yield and experimental yield of pumpkin polysaccharides were given in Table 4. Additional experiments using the predicted optimum conditions for polysaccharides extraction were carried out: extraction temperature of 70 °C, ultrasound power of 70 W, time of 23 min and solid–liquid ratio of 1:10 g/ml, and the model predicted a maximum response of 16.21%. To ensure the predicted result was not bias with the practical value, experiment was performed under the optimal conditions. A mean value of 16.04 ± 0.53% (N = 3) was obtained from the experimental was in agreement with the predicted values obtained from Derringer's desirability function method. The good correlation between these observed results and predicted values indicate the reliability of CCRD incorporate desirability function method and it could be effectively used to optimize the extraction parameters on the maximum extraction yield of polysaccharide.

Table 4
Predicted and experimental value of the response at optimum conditions.

Optimum condition				Polysaccharide yield (%)		Residual error	% Error	Actual error
Temperature (°C)	Power of ultrasound (W)	Time (min)	Solid–liquid ratio (g/ml)	Predicted	Experimental			
70	70	23	1:10	16.21	16.04 ± 0.53	−0.17	−1.06	0.17

4. Conclusions

UAE technology was performed for the polysaccharides extraction from pumpkin in order to increase the extraction yield. Four factors at five levels central composite rotatable design was successfully employed to optimize and study the individual and interactive effect of process variables such as extraction temperature, power of ultrasound, time and solid–liquid ratio on the extraction yield of polysaccharide from pumpkin by UAE. The results indicated that the factors selected in this study had a significant effect on the extraction of polysaccharide. The yield of polysaccharide increased with increasing extraction temperature, ultrasound power and solid–liquid ratio. From the ANOVA results, a high correlation second-order polynomial regression model was developed and this could be employed to optimize polysaccharides extraction from pumpkin by ultrasonic technology. The optimal conditions for UAE were found to be extraction temperature: 70 °C, ultrasound power: 70 W, time: 23 min and solid–liquid ratio: 1:10 g/ml. Under these optimal conditions, the experimental yield of polysaccharides was 16.04 ± 0.53%, which was agreed closely with the predicted yield value (16.21%).

References

Adams, G. G., Imrand, S., Wang, S., Mohammad, A., Kok, S., Gray, D. A., et al. (2011). The hypoglycaemic effect of pumpkins as antidiabetic and functional medicines. *Food Research International*, *44*, 862–867.

Bujić-Kojić, A., Planinic, M., Srécko, T., Jakabek, L., & Seruga, M. (2009). Influence of solvent and temperature on extraction of phenolic compounds from grape seed, antioxidant activity and colour of extract. *International Journal of Food Science and Technology*, *44*, 2394–2401.

Cacace, J. E., & Mazza, G. (2002). Extraction of anthocyanins and other phenolics from black currants with sulfured water. *Journal of Agricultural and Food Chemistry*, *50*, 5939–5946.

Cacace, J. E., & Mazza, G. (2003). Optimization of extraction of anthocyanins from black currants with aqueous ethanol. *Journal of Food Science*, *68*, 240–248.

Caili, F., Haijun, T., Tongyi, C., Yi, L., & Quanhong, L. (2007). Some properties of an acidic protein-bound polysaccharide from the fruit of pumpkin. *Food Chemistry*, *100*, 944–947.

Caili, F., Huan, S., & Quanhong, L. (2006). A review on pharmacological activities and utilization technologies of pumpkin. *Plant Foods for Human Nutrition*, *61*, 73–80.

Chen, R., Li, S., Liu, C., Yang, S., & Li, X. (2012). Ultrasound complex enzymes assisted extraction and biochemical activities of polysaccharides from Epimedium leaves. *Process Biochemistry*, http://dx.doi.org/10.1016/j.procbio.2012.07.022

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

Guo, S. D., Mao, W. J., Han, Y., Zhang, X. H., Yang, C. L., & Chen, Y. (2010). Structural characteristics and antioxidant activities of the extracellular polysaccharides produced by marine bacterium *Edwardsiella tarda*. *Bioresource Technology*, *101*, 4729–4732.

Hayouni, E. A., Abedrabba, M., Bouix, M., & Hamdi, M. (2007). The effects of solvents and extraction method on the phenolic contents and biological activities *in vitro* of Tunisian *Quercus coccifera* L. and *Juniperus phoenicea* L. fruits extracts. *Food Chemistry*, *105*, 1126–1134.

Khataee, A. R., Fathinia, M., Aber, S., & Zarei, M. (2010). Optimization of photocatalytic treatment of dye solution on supported TiO_2 nanoparticles by central composite design: Intermediates identification. *Journal of Hazardous Materials*, *181*, 886–897.

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.

Li, H., Pordesimo, L., & Weiss, J. (2004). High intensity ultrasound-assisted extraction of oil from soybeans. *Food Research International*, *37*, 731–738.

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.

Makni, M., Fetoui, H., Gargouri, N. K., Garoui, E. M., Jaber, H., Makni, J., et al. (2008). Hypolipidemic and hepatoprotective effects of flax and pumpkin seed mixture rich in ω-3 and ω-6 fatty acids in hypercholesterolemic rats. *Food and Chemical Toxicology*, *46*, 3714–3720.

Maran, J. P., Manikandan, S., Thirugnanasambandham, K., Nivetha, C. V., & Dinesh, R. (2012). Box–Behnken design based statistical modeling for ultrasound-assisted extraction of corn silk polysaccharide. *Carbohydrate Polymers*, http://dx.doi.org/10.1016/j.carbpol.2012.09.020

Maran, J. P., Sivakumar, V., Sridhar, R., & Thirgananasambandham, K. (2012). Development of model for barrier and optical properties of tapioca starch based films. *Carbohydrate Polymers*, http://dx.doi.org/10.1016/j.carbpol.2012.09.069

Mourabet, M., ElRhilassi, A., ElBoujaady, H., Bennani-Ziatni, M., ElHamri, 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*, 4402–4441.

Nara, K., Yamaguchi, A., Maeda, N., & Koga, H. (2009). Antioxidative activity of water soluble polysaccharide in pumpkin fruits (*Cucurbita maxima* Duchesne). *Bioscience Biotechnology and Biochemistry*, *73*, 1416–1418.

Park, S. C., Lee, J. R., & Kim, J. Y. (2010). Pr-1, a novel antifungal protein from pumpkin rinds. *Biotechnology Letters*, *32*, 125–130.

Pinelo, M., Rubilar, M., Sineiro, J., & Nunez, M. J. (2005). Effect of solvent, temperature, and solvent-to-solid ration the total phenolic content and antiradical activity of extracts from different components from grape pomace. *Journal of Agriculture and Food Chemistry*, *53*, 2111–2117.

Pompeu, D. R., Silva, E. M., & Rogez, H. (2009). Optimization of the solvent extraction of phenolic antioxidants from fruits of *Euterpe oleracea* using response surface methodology. *Bioresource Technology*, *100*, 6076–6082.

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 Products*, *42*, 159–168.

Quan, C., Sun, Y., & Qu, J. (2009). Ultrasonic extraction of ferulic acid from *Angelica sinensis*. *The Canadian Journal of Chemical Engineering*, *87*, 562–567.

Santos, D. T., Veggi, P. C., & Meireles, A. M. A. (2010). Extraction of antioxidant compounds from jabuticaba (*Myrciaria cauliflora*) skins. Yield, composition and economical evaluation. *Journal of Food Engineering*, *101*, 23–31.

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.

Sun, Y. X., & Liu, J. C. (2009). Purification, structure and immunobiological activity of a water-soluble polysaccharide from the fruiting body of *Pleurotus ostreatus*. *Bioresource Technology*, *100*, 983–986.

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.

Triveni, R., Shamala, T. R., & Rastogi, N. K. (2001). Optimised production and utilization of exopolysaccharide from *Agobacterium radiobacter*. *Process Biochemistry*, *36*, 787–795.

Vilkhu, K., Mawson, R., Simons, L., & Bates, D. (2008). Applications and opportunities for ultrasound assisted extraction in the food industry – A review. *Innovative Food Science & Emerging Technologies*, *9*, 161–169.

Wang, X-S., Wu, Y-F., Dai, S-L., Chen, R., & Shao, Y. (2012). Ultrasound-assisted extraction of geniposide from *Gardenia jasminoides*. *Ultrasonics Sonochemistry*, *19*, 1155–1159.

Wettasinghe, M., & Shahidi, F. (1999). Evening primrose meal: A source of natural antioxidants and scavenger of hydrogen peroxide and oxygen-derived free radicals. *Journal of Agricultural and Food Chemistry*, *47*, 1801–1812.

Willats, W. G. T., Knox, J. P., & Mikkelsen, J. D. (2006). Pectin: New insights into an old polymer are starting to gel. *Trends in Food Science and Technology*, *17*, 97–104.

Xia, T., & Wang, Q. (2006). Antihyperglycemic effect of *Cucurbita ficifolia* fruit extract in streptozotocin-induced diabetic rats. *Fitoterapia*, *77*, 530–533.

Xie, J-H., Xie, M-Y., Shen, M-Y., Nie, S-P., Li, C., & Wang, Y-X. (2010). Optimisation of microwave-assisted extraction of polysaccharides from *Cyclocarya paliurus* (Batal.) Iljinskaja using response surface methodology. *Journal of the Science of Food and Agriculture*, *90*, 1353–1360.

Yadav, M., Jain, S., Tomar, R., Prasad, G. B. K. S., & Yadav, H. (2010). Medicinal and biological potential of pumpkin: An updated review. *Nutrition Research Reviews*, *23*, 184–190.

Yang, L., Cao, Y-L., Jiang, J-G., Lin, Q-S., Chen, J., & Zhu, L. (2010). Response surface optimization of ultrasound-assisted flavonoids extraction from the flower of *Citrus aurantium* L. var. *amara* Engl. *Journal of Separation Science*, *33*, 1349–1355.

Yang, Z., & Zhai, W. (2010). Optimization of microwave-assisted extraction of anthocyanins from purple corn (*Zea mays* L.) cob and identification with HPLC–MS. *Innovative Food Science & Emerging Technologies*, *11*, 470–476.

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.

Yong, M., Ning, H., & Liu, H. (2006). Exploitation and composition of pumpkin powder. *Food Science and Technology*, *6*, 299–301.

Zhang, Y., Shen, X., & Zhu, L. (2002). The recent research development of natural hypoglycemic food-pumpkin. *Food Science and Technology*, *6*, 68–70.

Zhang, Z.-S., Wang, L.-J., Li, D., Jiao, S.-S., Chen, X. D., & Mao, Z.-H. (2008). Ultrasound-assisted extraction of oil from flaxseed. *Separation and Purification Technology*, *62*, 192–198.

Zhou, T., Song, Y. C., Feng, M. Q., & Tan, R. X. (2011). Cloning, expression and biochemical characterization of UDP-glucose 6-dehydrogenase, a key enzyme in the biosynthesis of an anti-tumor polysaccharide from the marine fungus *Phoma herbarum* YS4108. *Process Biochemistry*, *46*, 2263–2268.