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Original Article

Extraction Optimization of Phenolic Antioxidants from microwave treated *Nelumbo nucifera* Seed Flour

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ABSTRACT

Background: *Nelumbo nucifera* seeds are medicinally important due to the presence of phytochemicals with strong antioxidant potential. Extraction of these phytochemicals has been a major problem faced by the researchers. **Objective:** The purpose of the study was to optimize the process conditions for extraction of phenolic antioxidants from *N. nucifera* seed. **Methods:** Process variables for microwave assisted extraction of phenolic antioxidants from *N. nucifera* seed flour were optimized by response surface methodology using a multi-factorial central composite design based on five levels of each of four input variables including X1: particle size (in terms of mesh No.), X2: microwave treatment period (MTP), X3: solvent concentration (SC) and X4: extraction period (EP). Total extract yield was calculated as Total extractable components (TEC) and the extracts were analyzed for total phenolic content (TPC) and total antioxidant activity (TAOA). **Results:** Data analysis showed a significant increase ($p<0.05$) in TEC, TPC and TAOA in response to increase in the levels of selected factors. Optimum levels of extraction variables to achieve maximum level of response parameters were found to be: 125.96, 2.45 min, 55.21% and 3.87 h for TEC, 61.73, 1.66 min, 80.08% and 4.98 h for TPC and 60.89, 2.37 min, 44.98% and 1.123 h for TAOA. **Conclusion:** The extraction of phenolic antioxidants from N. nucifera seed flour is significantly increased in response to an increase in mesh No. (decrease in particle size), MTP, SC and EP but up to a certain limit. The decrease in extraction yield and antioxidant activity at higher levels of studied factors may be attributed to the possible decomposition of phenolic compounds due to prolonged duration of microwave heating and prolonged extraction time.

Key words: *Nelumbo nucifera* seed flour, Microwave assisted extraction, Antioxidant activity, Response surface methodology, Central composite design, Phenolic content.

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INTRODUCTION

Increasing demand of natural antioxidants as compared to synthetic antioxidants in the field of food and pharmaceutical industries in last two decade has attracted the attentions of researchers to explore new, cheap, reliable, effective and frequently available sources of natural antioxidants. Plant materials, particularly the underutilized vegetative crops and waste biomass from food processing industries, have been proved to be valuable sources of natural antioxidants which would be advantageous over synthetic ones to overcome the problems of oxidative damage to live stock and human health.[1-3] Phenolic compounds are generally considered as the non nutritional components present in plant foods which are taken in diet and play surprising role in protection against oxidative damage. These compounds, owing to their redox potential, free radical scavenging capacity, hydrogen donating ability, reducing and metal ion chelating properties, possess strong antioxidant characteristics and reduce the oxidative stress induced by imbalanced generation and removal of reactive oxygen and nitrogen species.[4,1,5-7] Various studies have been reported which have proved that plant phytochemicals are potent antioxidants and show wide biological activities including antidiabetic, anticancer, antiproliferative antiobesity and antimicrobial activity.[8-10]

Increase in the extraction yield and recovery of phenolic compounds from plant biomass at economical costs is the major concern of the day in bioprocess technology. Several studies have been reported regarding the method development and optimization of extraction of bioactive phenolic compounds from plant matrices. Particle size of plant material, extraction temperature, nature and concentration of extracting solvent and extraction period are some important factors which significantly affect the extraction process of phenolic compounds from plant tissues. Several techniques such as ultrasonication, microwave irradiation, gamma irradiation, super critical fluid extraction and soxhelt extraction have been employed to assist the extraction procedure and increase the extraction yield.[11-14] Solvent extraction has been found to be cost saving and easy to handle method of phenolic extraction. Solvent polarity is important factor for the selective extraction of bioactive compounds of a particular nature. Among the series of polar and nonpolar organic solvents hydroalcoholic solvent combinations are commonly used for extraction of phenolic compounds.[15-18] It is, however, still demanding to explore new and more efficient extraction methods and optimize the existing methods to increase the bioavailability, extraction yield and recovery of phenolic antioxidants from underutilized food materials and waste biomass.

Nelumbo nucifera, commonly known as lotus is an aquatic plant mainly found in China and subcontinent. Leaves, seeds and rhizome of *N. nucifera* are good source of nutritional and medicinal components.[19-22] Previously, studies have been reported on the extraction and antioxidant properties of phenolic compounds from *N. nucifera* rhizome, seeds and leaves. Seed extracts have been found to be rich in a variety of naturally occurring phenolic compounds which possess certain biological activities including antioxidant, antiproliferative, antiarrythmia, anti-inflammatory, anticancer, antiobesity, hypolipidemic, hepatoprotective, and immu-

nomodulating properties.[10,23-25] Any progress in research on extraction optimization of phenolic compounds from *N. nucifera* would contribute significantly in making it a valuable competitor in food and pharmaceutical industry.

Microwave treatment has been found to affect the extraction yield and antioxidant properties of plant phytochemicals.[26,27] Microwave assisted extraction of bioactive compounds from plant material is frequently used method of the day to increase the extraction yield. The microwave irradiation and extraction of phenolic compounds from various plant materials have been optimized using different methods.[12,27-29] In present study the effect of four factors including particle size of flour, microwave treatment period, solvent concentration and extraction period on extract yield and antioxidant activity of phenolic compounds from *N. nucifera* seed flour was optimized using response surface methodology (RSM). RSM is a statistical technique which has got preference over conventional method of varying one parameter keeping others constant for use in multifactor optimization procedures. It has been proved to be the suitable methodology for optimization of multivariate processes because it neglects the inter-parameter effect on the optimal value.[30,31] It has been extensively used in various fields of research to create response-surface models for optimization and prediction of changes in response variables as a function of changes in input variables.[13,18,28,32]

MATERIALS AND METHODS

Dry mature seeds of *N. nucifera* were purchased from local market, cleaned and seed coat was removed manually. Seed plumule was ground in an electric grinder at low speed (1000 rpm) to minimize the temperature fluctuations beyond 35°C. The flour thus obtained was packed in air tight glass bottles and stored in dark at standard laboratory conditions until further processing.

Experimental design

The cumulative effect of four input variables including particle size (in terms of sieve mesh No.), microwave treatment period (MTP), solvent concentration (SC) and extraction time (EP) on extraction yield in terms of total extractable components (TEC), total phenolic content (TPC) content and Trolox equivalent total antioxidant activity (TAOA) of *N. nucifera* flour was optimized by response surface methodology (RSM) using a four factor five level rotatable central composite design (CCD). The coded levels of the selected variables were calculated as:

$$X_i = \left[\frac{\xi_i - \xi_i^-}{S_i} \right] \quad i = 1, 2, \ldots k$$

where X_i is the coded value of an independent variable. ξ_i is the specific location of independent variable, ξ_i^- is the center point and S_i is the scale factor *i.e.* the difference between different levels of variables. The actual and coded levels of input variables are presented in Table 1.

The relationship of total extractable components, total phenolic content and total antioxidant activity of *N. nucifera* seed flour against the Mesh No., MTP, SC and EP was determined by developing a second order polynomial quadratic model. The suggested model finds the levels of input variables in region of optimal response. The study was done in phases based on CCD.

Sieving

The flour was sieved successively through micro screens of mesh No. 60, 80, 100, 120 and 140 meshes to obtain different levels of particle size. The distribution of particle size was done on the basis of sieve mesh No. As the particle size is inversely proportional to sieve mesh No., a gradual decrease in particle size is observed with a respective increase in mesh No. The range of particle size of flour obtained from selected sieves was as under (Sigma Aldrich 2014):

Sieve mesh No.	Particle size (μm)
60	178-250
80	150-177
100	126-149
120	106-125
140	<106

Microwave treatment of flour

The flour of various particle sizes (50g each) as obtained from the selected sieves was processed for microwave treatment at various levels of treatment period as selected by CCD. Microwave treatment was carried out in glass beakers using LG MH2043DRY WT 20L Microwave Oven. The operating conditions were selected as radiation intensity: at medium low power (200 W), sample mass per load: 5g, treatment duration: as selected by experimental design. The treatment was discontinued after each min for 30 sec and the flour was mixed thoroughly to avoid the burning of flour.

Extraction

The untreated flour (5 g) of *N. nucifera* seed at random particle size ranging from 60-120 mesh No. was extracted in 80% aqueous methanol (solid to solvent ratio 1:20 w/v) for 5 h. TEC were calculated as g/100 g dry wt.:

TEC (g/100 g dry wt) = (Weight of extract/Weight of sample) × 100

The microwave treated flour (5 g) at various levels of particle size was extracted in aqueous methanol (solid to solvent ratio 1:20 w/v) at various levels of methanol concentration (% v/v) and extraction period as selected by CCD. The extracts were evaporated to dryness at 25±5°C under vacuum, weighed and TEC were calculated as above.

The crude methanolic extracts were stored at 4 ± 1°C in air tight sterile laboratory environment to minimize the chances of microbial contamination and growth throughout the study period. The samples were also protected from direct sunlight exposure throughout the study period in order to minimize the chances of photo-oxidation of antioxidant compounds.

Phytochemical and antioxidant analysis

TPC in crude methanolic extract (10 mg/100 ml) was estimated by previously described Folin-Ciacolate method.[33] TPC was calculated as g gallic acid equivalent/100 g dry wt using a linear regression equation obtained from standard curve of gallic acid ($R^2 = 0.9843$):

TPC (g/100 g dry wt.) = Abs.at 720 nm/18.284

TAOA of extracts was determined by Phosphomolybdenum assay using the reported method (34). TAOA was calculated as Trolox equivalent g/100 g dry wt. using a linear regression equation obtained from standard curve of Trolox (R^2=0.9795):

TAOA (g/100 g dry wt.) = Abs.at 695 nm/6.576

Statistical analysis

The data was statistically analyzed by one way analysis of variance (ANOVA) using second order polynomial quadratic response surface model. The optimum levels of response variable as a function of input variables were predicted by creating polynomial regression equations.

Table 1: Actual and coded levels of selected input variables

Variable	Actual Levels	Equation for coding	Coded levels
X_1: Particle size (Mesh No.)	60, 80, 100, 120, 140	X_1 = (Mesh No. – 100)/20	-2, -1, 0, 1, 2
X_2: Microwave treatment period (min)	0.5, 1, 1.5, 2, 2.5	X_2 = (Microwave treatment period – 1.5)/0.5)	-2, -1, 0, 1, 2
X_3: Solvent concentration (%)	20, 40, 60, 80, 100	X_3 = (Solvent concentration – 60)/20	-2, -1, 0, 1, 2
X_4: Extraction period (h)	1, 2, 3, 4, 5	X_3 = (Extraction period – 3)/1	-2, -1, 0, 1, 2

which consists of 30 experimental points with nf = 16 factorial points, na = 8 axial point and nc = 6 centre points.

The generalized polynomial regression model for prediction of variation in response variables is given as:

$$Yi = \beta_0 + \sum_{i=1}^{4}\beta_i X_i + \sum_{i=1}^{4}\beta_{ii} X_i^2 + \sum\sum_{i<j=1}^{4}\beta_{ij} X_{ij}$$

where *Yi* is the predicted response, *βo* is the regression coefficient for main, *βi* for linear, *βii* for quadratic and *βij* for interaction effect of input variables *Xi and Xj*.

The polynomial regression equation was used to obtain the predicted values of response variables which were plotted against the experimental ones to check the adequacy and validity of the suggested response-surface models. The degree of scatter of data points in response surface model, fairness of fit of regression equation and significance of estimated regression coefficient for each response were assessed by determining the coefficient of determination (R^2), adjusted coefficient of determination (R^2adj) and lack of fit (*F*-value) at a probability $p \leq 0.05$ respectively. Coefficient of variation (*CV*) and adequate precision were determined to check the reliability and precision of experiments. For graphical expression of variability in responses as a function of input variables, the three-dimensional plots were constructed between response and independent variables. The optimum levels of input variables in a region of optimal responses were found by numerical optimization of data at maximum desirability. The development of experimental design, data analysis and optimization procedure was carried out using statistical software, Design Expert 10.0 (Stat-Ease, Inc.).

RESULTS

In an initial experiment phenolic antioxidants of native *N. nucifera* seed flour were extracted in 80% methanol for 5 h and analyzed for TEC, TPC and Trolox equivalent TAOA. TEC, TPA and TAOA were found to be 15.90 ± 2.23, 0.68 ± 0.14 and 8.32 ± 1.67 g/100 g respectively (Table 2). In a subsequent experiment the experimental conditions for extraction of phenolic antioxidants from microwave treated flour of *N. nucifera* seed were optimized using RSM. The experimental values of TEC, TPA and TAOA of extracts obtained at random levels of extraction variables as selected by experimental design are presented in Table 3. TEC, TPC and TAOA ranged from 10.79 to 22.47, 0.18 to 0.99 and 5.11 to 16.01 with mean ± standard deviation 17.47 ± 1.61, 0.55 ± 0.09 and 10.35 ± 1.45 g/100 g dry wt. respectively. The results obtained at various combinations of extraction variables were found to be statistically different ($p<0.05$).

Table 2: TEC, TPC and TAOA of crude methanolic extracts of native *N. nucifera* seed flour

	Experimental value (g/100 g dry wt.)
TEC	15.90 ± 2.23
TPA (gallic acid equivalent)	0.68 ± 0.14
TAOA (Trolox equivalent)	8.32 ± 1.67

Response surface analysis and optimization of results

The experimental values of TEC, TPA and TAOA were used to optimize the extraction of phenolic antioxidants and their antioxidant activity at selected levels of process variables. The main, quadratic and interaction effects of particle size, microwave treatment period, solvent concentration and extraction period on TEC, TPC and TAOA of seed flour as obtained by analysis of variance (ANOVA) are given in Table 4. For graphical representation of the main, quadratic and interaction effects of Mesh No., MTP, SC and EP on TEC, TPC and TAOA of *N. nucifera* seed flour, three dimensional (3D) response surface plots were drawn (Figure 1A-F, Figure 2A-F, Figure 3A-F respectively).

The relationship between the input variables and responses including TEC, TPC and TAOA was found by following polynomial regression equations obtained from response surface model:

$$\begin{aligned}&\text{TEC}(\text{g}/100\text{g dw})\\&= 20.708 - 0.344X_1 - 2.078X_2 + 0.169X_3 + 3.616X_4 + 7.648E^{-004}X_1^2\\&-0.671X_2^2 - 1.507E^{-003}X_3^2 - 0.789X_4^2 + 0.067X_1X_2 + 1.033E^{-003}X_1X_3\\&+9.156E^{-003}X_1X_4 - 0.019X_2X_3 + 0.771X_2X_4 + 5.313E^{-004}X_3X_4\end{aligned}$$

$$\begin{aligned}&\text{TPA}(\text{g}/100\text{g dw})\\&= 1.012 - 0.031X_1 + 0.736X_2 + 6.271E^{-003}X_4 - 7.083E^{-003}X_4\\&+1.831E^{-004}X_1^2 + 0.053X_2^2 + 5.495E^{-005}X_3^2 + 0.012X_4^2 - 5.188E^{-003}X_1X_2\\&+2.031E^{-005}X_1X_3 - 2.183E^{-004}X_1X_4 - 3.563E^{-003}X_2X_3 - 0.0488X_2X_4\\&+4.063E^{-004}X_3X_4\end{aligned}$$

$$\begin{aligned}&\text{TAOA}(\text{g}/100\text{g dw})\\&= 1.311 - 0.288X_1 + 19.069X_2 + 0.209X_3 + 4.073X_4 + 2.164E^{-003}X_1^2\\&-1.782X_2^2 + 9.924E^{-003}X_3^2 + 0.246X_4^2 - 0.059X_1X_2 - 1.933E^{-003}X_1X_2\\&+0.03X_1X_4 - 0.044X_2X_3 - 1.434X_2X_4 + 9.094E^{-003}X_3X_4\end{aligned}$$

These equations were used for the prediction of response values at each of the combinations of input variables selected by experimental design. To test the applicability of the model, the predicted values of TEC, TPC and TAOA were plotted against the experimental values (Figure 4A-C).

The numerical optimization of process variables was carried out by keeping the independent variables in range of levels selected by CCD and desirability of response variables was selected at their maximum (Figure 5A-C). Optimum level of particle size in terms of sieve mesh No., microwave treatment period, solvent concentration and extraction period to achieve desired prediction of response parameters (TEC: 24.25, TPC: 1.067, TAOA: 16.14 g/100g dry wt.) were found to be: 125.96, 2.45 min, 55.21% and 3.87 h, 61.73, 1.66 min, 80.08% and 4.98 h and 60.89, 2.37 min, 44.98% and 1.123 h respectively.

DISCUSSION

Plants are rich source of phenolic compounds which possess antioxidant potential. *N. nucifera* seeds are good source of phenolics and other

Table 3: Experimental values of TEC, TPC and TAOA of crude methanolic extracts of *N. nucifera* seed flour at selected levels of input variables by CCD

Std.	Runs	X_1 Mesh No.	X_2 MTP (min)	X_3 SC (%)	X_4 EP (h)	TEC (g/100 g dry wt.)	TPC (g/100 g dry wt.)	TAOA (%)
28	1*	100	1.5	60	3	18.47	0.45	9.50
27	2*	100	1.5	60	3	18.47	0.45	9.50
16	3	120	2.0	80	4	20.84	0.57	10.97
17	4	60	1.5	60	3	18.47	0.83	8.69
9	5	80	1.0	40	4	16.07	0.35	7.02
11	6	80	2.0	40	4	19.09	0.42	9.27
15	7	80	2.0	80	4	20.50	0.84	12.68
20	8	100	2.5	60	3	22.47	0.64	9.10
6	9	120	1.0	80	2	16.07	0.84	10.38
22	10	100	1.5	100	3	19.52	0.96	14.91
8	11	120	2.0	80	2	19.15	0.63	10.59
7	12	80	2.0	80	2	18.57	0.88	15.19
29	13*	100	1.5	60	3	18.47	0.45	9.50
21	14	100	1.5	20	3	11.96	0.18	6.04
26	15*	100	1.5	60	3	18.47	0.45	9.50
25	16*	100	1.5	60	3	18.47	0.45	9.50
12	17	120	2.0	40	4	18.61	0.27	9.81
1	18	80	1.0	40	2	17.01	0.35	7.68
10	19	120	1.0	40	4	13.38	0.28	11.06
19	20	100	0.5	60	3	12.49	0.43	5.11
18	21	140	1.5	60	3	20.28	0.72	16.01
5	22	80	1.0	80	2	17.98	0.70	12.18
13	23	80	1.0	80	4	16.10	0.79	13.70
14	24	120	1.0	80	4	17.84	0.99	15.48
24	25	100	1.5	60	5	19.20	0.42	10.94
23	26	100	1.5	60	1	10.79	0.64	8.80
2	27	120	1.0	40	2	11.99	0.25	8.25
30	28*	100	1.5	60	3	18.47	0.45	9.50
4	29	120	2.0	40	2	18.57	0.36	10.27
3	30	80	2.0	40	2	16.24	0.35	9.38
Mean ± STD						17.47±1.61	0.55±0.09	10.35±1.45

*Center point: Mean value of six parallel replicates was repeated at each of center point.

compounds which show strong antioxidant activity.[10] Various extracts of *N. nucifera* seeds have been found to contain 0.56-20.12 g gallic acid equivalent/100 g dry wt.[35] The present results indicate that a valuable amount of crude methanolic extract with significant value of phenolic compounds and antioxidant activity is obtained from native and microwave treated *N. nucifera* seeds flour. Present results for TPC covered the range: 0.313-0.469 g catechin equivalent/100 g dry wt. reported earlier in hydro alcoholic extracts of seed pods[36], 33.59 ± 0.30 and 30.9-55.03 mg gallic acid equivalent/g extract reported in methanolic extracts of *N. nucifera* seeds.[37,38]

Response surface analysis and optimization of results

The data were statistically analyzed by applying response surface models to find the levels of four input factors in the optimal region of extraction yield and antioxidant activity of phenolic antioxidants of *N. nucifera* seed flour. The significance and adequacy of the response surface model were measured in terms of *F*-value (lack of fit) and probability value at 5% significance level ($p \leq 0.05$). The variation in corresponding variables with relatively larger *F*-values and smaller *p*-values ($p<0.05$) were considered more significant. The measurement of *F*-value and *p*-values for main effects indicated that the model is significant for TEC ($F=5.31$, $p=0.0013$), TPC ($F=11.69$, $p=<0.0001$) and TAOA ($F=5.58$, $p=<0.001$). The values of coefficients of estimate (18.47, 0.45 and 9.50) with positive signs suggest that each of the studied parameter is increased in response to increase in the levels of extraction variables. However, mixed response for linear, quadratic and interaction terms was shown by each parameter against the input variables. Mesh No. showed non-significant linear effect on each response and a significant positive quadratic effect on TPC and TAOA. MTP showed a significant linear negative effect on TEC and non-significant quadratic effects on each response. A significant linear

Table 4: **Response surface analysis of data showing main, linear, interaction and quadratic terms and regression coefficients**

Source	TEC				TPC				TAOA			
	Sum of Squares	Std. Error	F-Value	p-value*	Sum of Squares	Std. Error	F-Value	p-value	Sum of Squares	Std. Error	F-Value	p-value
β0-Model	192.45	0.66	5.31	0.0013	1.37	0.037	11.69	< 0.0001	172.82	0.61	5.58	0.0010
β1-Mesh No. (mesh)	0.093	0.33	0.036	0.8526	0.021	0.019	2.52	0.1336	8.58	0.30	3.88	0.0676
β2-MTP (min)	84.71	0.33	32.75	< 0.0001	$1.50E^{-003}$	0.019	0.18	0.6773	4.50	0.30	2.03	0.1743
β3-SC (%)	40.59	0.33	15.69	0.0013	1.11	0.019	133.39	< 0.0001	88.82	0.30	40.16	< 0.0001
β4-EP (h)	23.34	0.33	9.02	0.0089	$3.50E^{-003}$	0.019	0.42	0.5269	4.47	0.30	2.02	0.1758
β12	7.09	0.40	2.74	0.1186	0.043	0.023	5.16	0.0383	5.61	0.37	2.53	0.1322
β13	2.73	0.40	1.06	0.3205	$1.06E^{-003}$	0.023	0.13	0.7270	9.56	0.37	4.32	0.0551
β14	0.54	0.40	0.21	0.6553	$5.06E^{-004}$	0.023	0.061	0.8088	5.75	0.37	2.60	0.1278
β23	0.56	0.40	0.22	0.6488	0.020	0.023	2.43	0.1397	3.09	0.37	1.40	0.2557
β 24	2.38	0.40	0.92	0.3528	$9.51E^{-003}$	0.023	1.14	0.3028	8.22	0.37	3.72	0.0730
β34	$1.806E^{-003}$	0.40	$6.982E^{-004}$	0.9793	$1.06E^{-003}$	0.023	0.13	0.7270	0.53	0.37	0.24	0.6318
β12	2.57	0.31	0.99	0.3350	0.15	0.017	17.62	0.0008	20.56	0.28	9.29	0.0081
β22	0.77	0.31	0.30	0.5928	$4.80E^{-003}$	0.017	0.57	0.4601	5.44	0.28	2.46	0.1375
β32	9.97	0.31	3.85	0.0685	0.013	0.017	1.59	0.2270	4.32	0.28	1.95	0.1825
β42	17.08	0.31	6.60	0.0214	$3.94E^{-003}$	0.017	0.47	0.5028	1.66	0.28	0.75	0.4003
Lack of Fit	38.80				0.13				33.18			
Pure Error	0.000				0.000				0.000			
R^2		0.8322				0.9162				0.8389		
Adj. R^2		0.6756				0.8380				0.6886		
CV (%)**		9.21				16.73				14.37		
AP*		9.544				13.341				9.032		

*$p \leq 0.05$ indicates the significant variation in response

**CV: coefficient of variance, RC: Regression coefficient, AP: Adequate precision.

positive effect of SC was observed on each response. EP showed significant linear positive and quadratic negative effect on TEC only. The interaction effect of each factor was found to be non-significant on each response. Three dimensional response surface plots clearly represented the main, quadratic and interaction effects of extraction variables on TEC, TPC and TAOA of *N. nucifera* seed flour (Figure 1A-F, Figure 2A-F, Figure 3A-F respectively).

The variability of the model in the observed response values was determined by correlation coefficient (R^2). A value of R^2 closer to unity gives better prediction of the response and high significance of the model. The calculated values of R^2 indicated that more than 83% of the variability in TEC (R^2=*0.8322*), 91% in TPC (R^2=*0.9160*) and 83% in TAOA (R^2=*0.8389*) of *N. nucifera* as a function of selected variables could be explained by the suggested model. The values of adjusted R^2 (*TEC: 0.6756, TPC: 0.8380, TAOA: 0.6886*) also advocate the significance of the model. Coefficient of variation (CV) is a measure standard deviation as a percentage of the mean. A relatively low value of *CV* (9.21-16.73) suggests a better precision and reliability of the experiments and claims a good reproducibility at suggested optimum levels of variables. Adequate precision measures the signal to noise ratio. A ratio greater than 4 indicates an adequate signal to navigate the design space. The high value of adequate precision (9.032-13.341) for the studied parameters suggests a better precision and reliability of the experiments.

The polynomial regression equations were yielded by RSM in order to show an empirical relationship between the response and input variables. These equations include the coefficient for intercept, linear, interaction and quadratic effects. The influence of each factor on the response is shown by the sign and magnitude of the main effect. The response surface analysis indicated that the relationship between extraction variables and studied parameters of *N. nucifera* seed flour could be explained significantly by second order polynomial regression models.

The linear plot of predicted values of response variables against the experimental ones (Figure 4A-C) showed a good agreement with high values of correlation coefficients (*R2=0.8322-0.9153*). The higher values

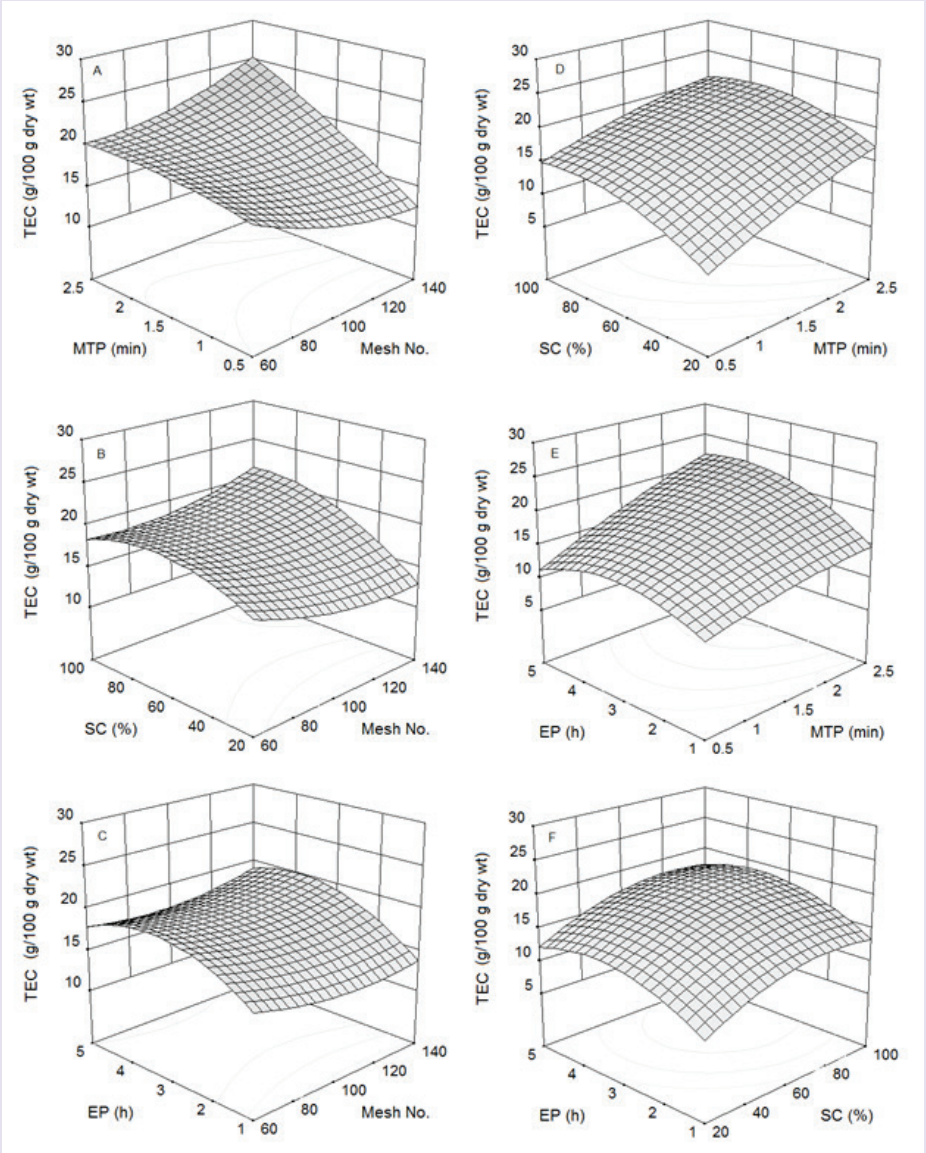

Figure 1A-F: 3D response surface plots of TEC of *N. nucifera* seed flour at selected levels of input variables.

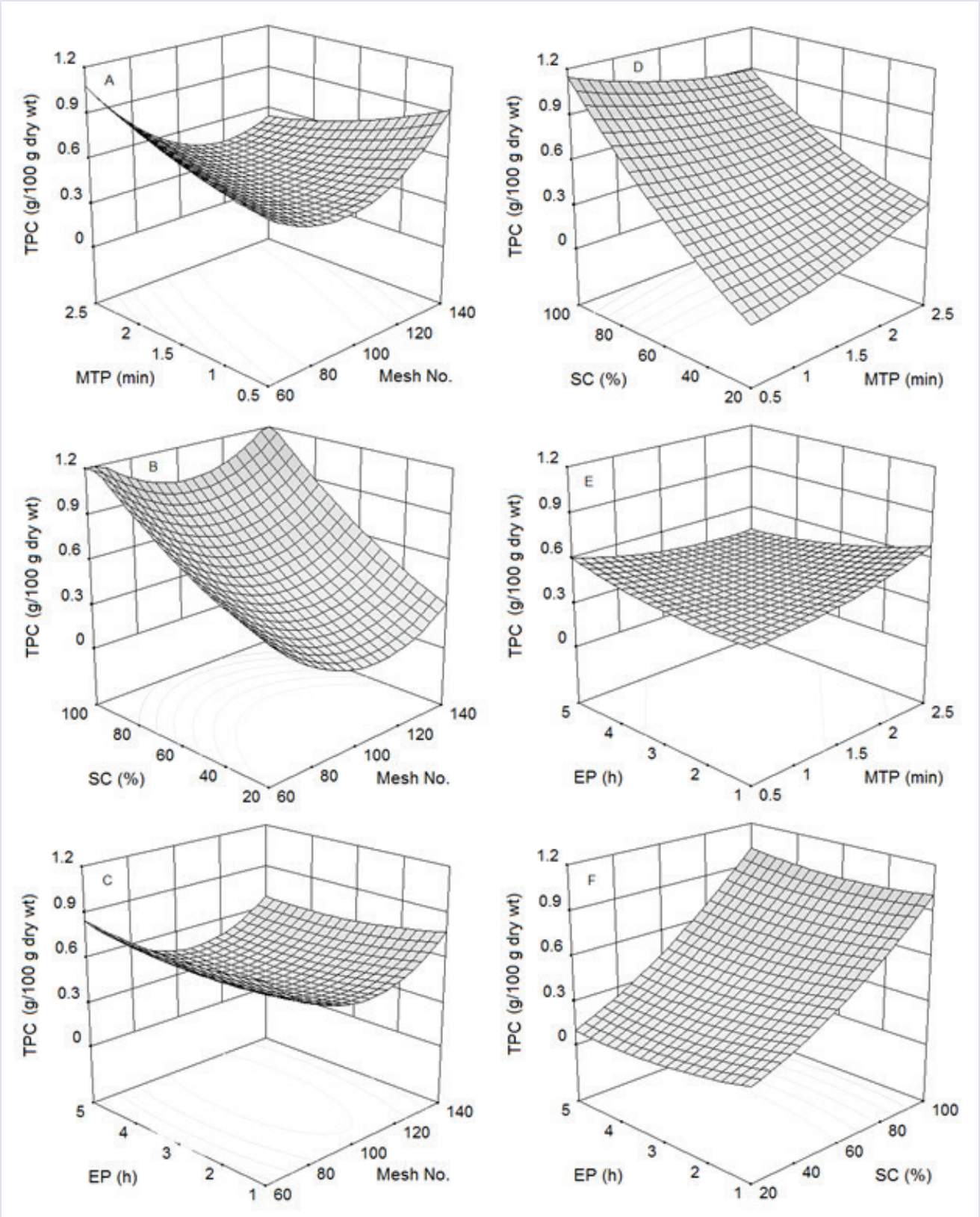

Figure 2A-F: 3D response surface plots of TPC of *N. nucifera* seed flour at selected levels of input variables.

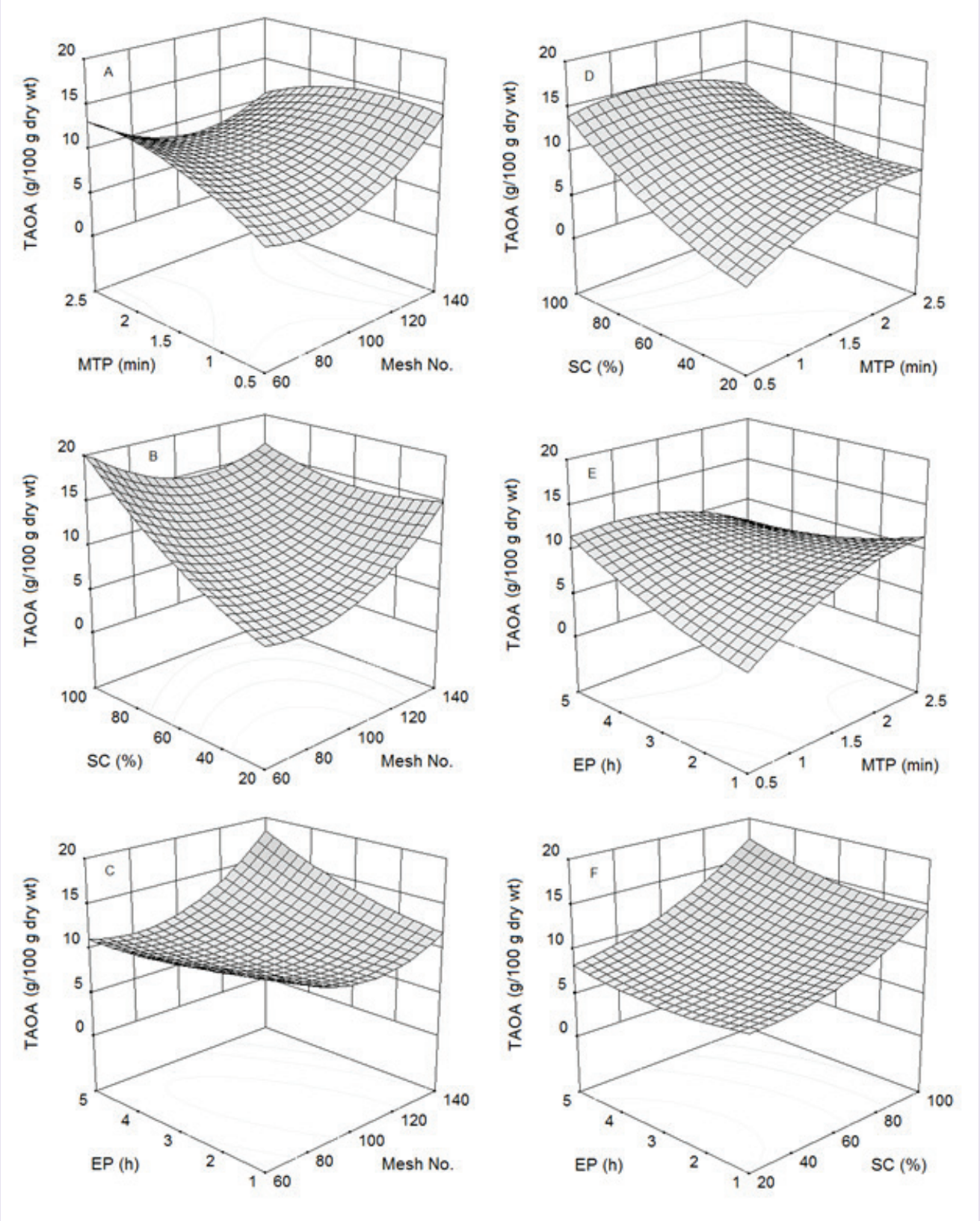

Figure 3A-F: 3D response surface plots of TAOA of *N. nucifera* seed flour at selected levels of input variables.

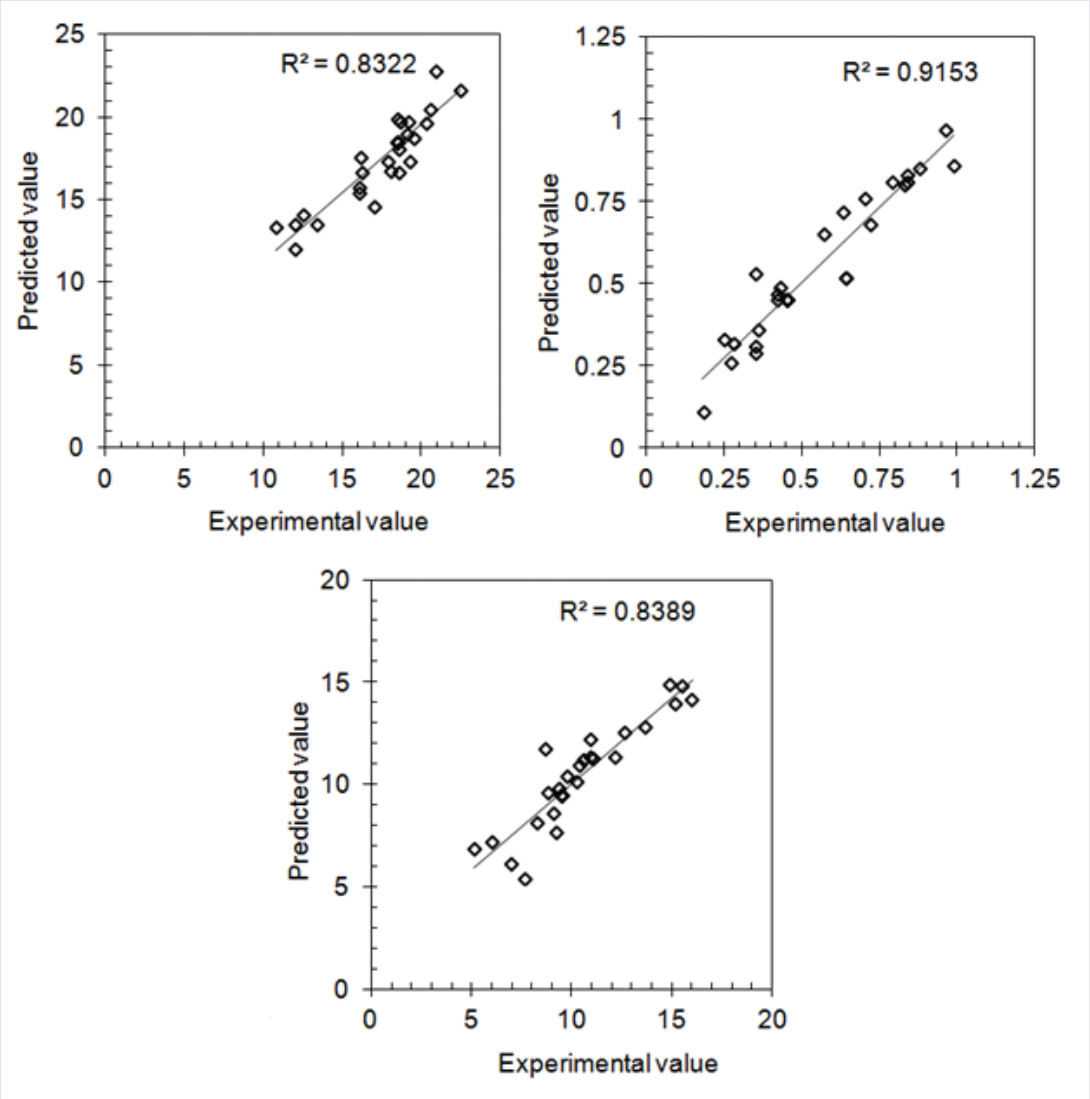

Figure 4A-C: Agreement between experimental values and predicted values of TEC, TPC and TAOA of *N. nucifera* seed flour.

of R^2 prove the applicability of proposed model with good accuracy to study the effect of Mesh No., MTP, SC and EP on extract yield, of phenolic cntent and antioxidant properties of *N. nucifera* seed flour.

The numerical optimization of process variables showed that maximum extraction yield (24.25 g/100 g dry wt.) could be achieved at particle size in terms of sieve Mesh No.: 125.96, MTP: 2.45 min, SC: 55.21% and EP 3.87 h (Figure 5A). Optimum level of extraction variables to achieve desired prediction of TPC (1.067 g/100g dry wt.) and TAOA (16.14 g/100g dry wt.) were found to be 61.73, 1.66 min, 80.08% and 4.98 h and 60.89, 2.37 min, 44.98% and 1.123 h respectively.

CONCLUSION

It is clear from RSM results that extraction of phenolic antioxidants from *N. nucifera* seed flour significantly increased in response to an increase in Mesh No. (decrease in particle size), MTP, SC and EP but up to a certain limit. An increase in the level of studied factors beyond the optimum values suggested by the applied statistical model results in a decrease in the response values. The decrease in extraction yield and antioxidant activity at higher levels of studied factors may be attributed to the possible decomposition of phenolic compounds due to prolonged duration of microwave heating, high methanol/water ratio and prolonged extraction time. The data would be significant contribution to the research in pharmaceutical and food science regarding the extraction of phenolic antioxidants.

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CONFLICT OF INTEREST

The research project was not funded by any funding agency and collaborated with any other institute. Therefore, there is no conflict of interest regarding this research work.

Criteria

Name	Goal	Lower limit	Upper limit
Mesh No.	is in range	60	140
TP (min)	is in range	0.5	2.5
SC (%)	is in range	20	100
EP (h)	is in range	1	5
TEC (g/100g dry wt)	maximize	10.79	22.47
TPA (g/100g dry wt)	maximize	0.18	0.99
TAOA (g/100g dry wt)	maximize	5.11	16.01

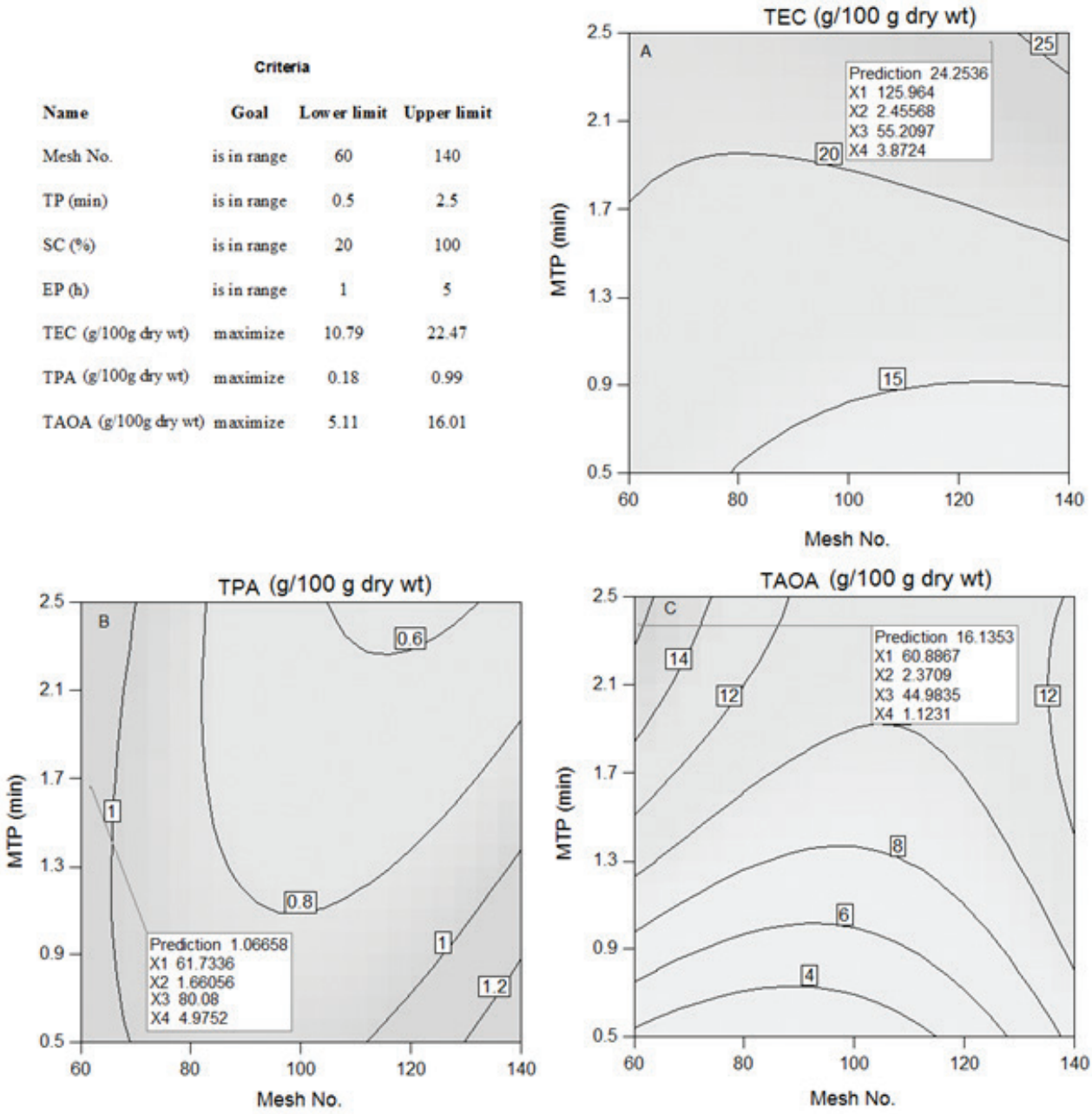

Figure 5A-D: Selection criteria and optimum levels of independent variables at maximum prediction of response.

ABBREVIATIONS USED

AP: Adequate precision; **CCD:** Central composite design; **CV:** Coefficient of variance; **EP:** Extraction period; **MTP:** Microwave treatment period; **RC:** Regression coefficient; **RSM:** Response surface methodology; **SC:** Solvent concentration; **TAOA:** Total antioxidant activity; **TEC:** Total extractable components; **TPC:** Total phenolic content.

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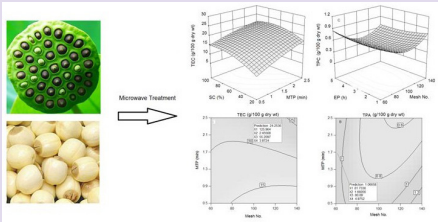
PICTORIAL ABSTRACT



SUMMARY

- Extraction parameters of phenolic antioxidants from *Nelumbo nucifera* seed flour were optimized.
- Studied factors significantly affected the response parameters.
- Particle size, microwave treatment, solvent concentration and extraction period significantly affect the extraction of phenolic antioxidants from *Nelumbo nucifera* seed flour.
- Microwave treatment for 1.66-2.45 min was found to be effective for extraction.

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