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Optimization of Microwave-assisted Extraction of Active Components from Chinese Quince using Response Surface Methodology

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Active components in Chinese quince were extracted by using high efficient microwave-assisted extraction. In accordance with central composited design, 20 runs with 3 variables and 5 levels applied for the optimization in response surface methodology system. The extracts were analyzed by spectrophotometeric methods for the total flavonoids content (TFC), total phenolic compound (TPC) and electron donating ability (EDA). The optimum conditions obtained were 44.15% ethanol concentration, 5 min extraction time and 102.1 W microwave power for maximum TFC (477.8 mg RE/100 g), TPC (2249 mg GAE/100 g) and EDA (77.47%) as predicted by overlaid contour plots.

Key words: *central composite design, Chinese quince, microwave-assisted extraction, optimization, response surface methodology*

Chinese quince (*Chaenomeles sinensis*), the only species in the genus Pseudocydonia, is a deciduous or semi-evergreen tree in the family Rosaceae, native to China. Chinese quince fruits are minor crops in the world and not consumed fresh because of their strong acidity, astringency, and hard flesh. Nevertheless, Chinese quince contains higher amounts of phenolic compounds, about 4 times higher than in apple juice [Yasunori *et al*., 2008] and it possesses higher antioxidant ability [Yasunori *et al*., 2006]. The existence of other functional compounds such as flavonoids, dietary fiber and pectins leads to anti-inflammatory activity [Osawa *et al*., 1999], reducing the risk of coronary heart diseases [Hertog *et al*., 1993] and cancer [Knekt *et al*., 1996]. The extraction of these functional compounds has great importance for improvement of human health.

Microwave-assisted extraction (MAE) has been successfully used in recent years for the extraction of functional components from different plant matrices. MAE is based on absorption of microwave energy by molecules of polar chemical compounds and can heat the material homogeneous. Comparing with traditional extraction ways, MAE can considerably reduce not only the extraction time to just few minutes, but also solvent consumption and energy requirements. Importantly, it also has been proved to resulted in high extraction efficiency compared to conventional techniques [Christen and Veuthey, 2001; Guo *et al*., 2001; Hao *et al*., 2002; Alfaro *et al*., 2003; Gao *et al*., 2004; Kerem *et al*., 2005].

In this study MAE parameters for ethanol concentration, extraction time and microwave power were considered because they have importance in the process. The conventional approach usually investigates one variable at a time, which is not only time consuming but also provides less chance to study the interactions between different variables. However, response surface methodology (RSM) can investigate the individual variables and the interactions of the variables simultaneously. The objective of this work was to apply convenient statistical methods to optimize the MAE process variables for the extraction from Chinese quince by evaluating responses of total flavonoids content, total phenolic compound and electron donating ability by using RSM and superimposed contour graphs.

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Abbreviations: EDA, electron donating ability; GAE, gallic acid equivalents; MAE, microwave-assisted extraction; RE, rutin equivalents; TFC, total flavonoids content; TPC, total phenolic compound

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Materials and Methods

Materials. Dried Chinese quince slices without seeds were bought from local Chinese herb market in Daegu, Korea. They were ground to a powder form and screened through 40 size mesh. Powder was stored at 4°C until use. Rutin, gallic acid, 1,1-diphenyl-2- picryhydrazy (DPPH) and Folin-Ciocalteu reagents were of analytical grades and purchased from Sigma Chemicals Company (St. Louis, MO). Sodium nitrite, sodium carbonate, aluminium nitrate, sodium hydroxide and ethanol were purchased from the Duksan Pure Chemical Company (Ansan, Korea).

MAE system. Microdigest 3.6 (Prolabo, Fontenay, France) was used for MAE experiments. It was operated with a focused irradiation process under atmospheric pressure conditions. The emission frequency of the extractor was 2450 MHz. It consisted of control panel for controlling microwave power and time and monitoring temperature. The temperature was measured using a digital Megal 500 gas thermometer (prolabo). The microwave power was linear and adjustable between 0 to 100% which represented microwave power of 0 to 250 W. The extractor was equipped with three 250 mL quartz vessels, a magnetic stirring device and a cool water circulation system using a Graham-type refrigerant column (400 mm length).

Analysis of primary components. The primary components of the extracts from Chinese quince were determined according to AOAC official methods [AOAC, 2003]. The moisture content was determined by drying in an oven at 105°C until constant weight was attained. Crude fat was determined using a Soxhlet system with ether. Ash was determined gravimetrically by heating in furnace at 550°C for 3 h. Vitamin C content was calculated by using ascorbic acid as standard curve with spectrophotometer at 243 nm. Crude fiber was calculated after alkaline/acidic treatments and high temperature incineration. Analytical results showed that moisture of the sample was 10.16%, ash was 13.96%, crude fiber was 11.03%, crude fat was 3.42% and vitamin C content was 611.32 μg/g.

Experimental design. Based on our preliminary work, three MAE variables including ethanol concentration (X_1, % (v/v), ethanol in water), extraction time (X_2, min), microwave power (X_3, W) each at five levels were considered to be the independent variables, the dependent variables were the responses of total flavonoids content, total phenolic compound and electron donating ability. The complete experiment design (Tables 1) was a central composite design (CCD) obtained using statistical system software (SAS). It consisted of 20 experimental runs with six central points. All the experiments were performed in random order.

MAE process. According to CCD (Table 1), 2 g of Chinese quince powder was mixed with 60 mL of solvent at different concentrations and as such 3 different samples were prepared. After stirring on a magnetic stirrer samples were poured into 3 quartz vessels of the extractor. Extractions were performed under different conditions of ethanol concentration (0~ 100%), extraction time (1~9 min) and microwave power (0~160 W). Upon completion of extraction, each sample was filtered using Whatman No.1 filter paper under vacuum and washed 3 times using the same concentration of solvent. The final volume of the filtrate was adjusted to 100 mL in volumetric flask for analysis.

Determination of total flavonoids content. The total flavonoids were measured spectrophotometrically using a modified colorimetric method [Bakar *et al.*, 2009]. Briefly, a 70 μL sample was diluted with 430 μL of distilled water in a 1.5 mL micro tube by addition of 50 μL 5% $NaNO_2$ solution. After keeping for 6 min at room temperature, 50 μL of 10% $Al(NO_3)_3 \cdot 9H_2O$ reagent was added and allowed to stand for another 5 min and 500 μL of 1 N NaOH was added. The absorbance was measured immediately at 510 nm using a spectrophotometer. Rutin was used as a reference for the calibration curve (R^2= 0.9998). The results of total flavonoids content were expressed as mg rutin equivalents per 100 g dry weight (mg RE/100 g).

Determination of total phenolic compounds. The total phenolic compounds were determined according to the Folin Denis Method with slight modification [Lee *et al.*, 2003]. The extract was double diluted and 100 μL of diluted sample was mixed with 50 μL Folin Ciocalteu's reagent and 300 μL 2% Na_2CO_3. After keeping the sample at room temperature for 15 min, 1 mL of water was added before measuring visible absorbance at 725 nm. The calibration curve was obtained using gallic acid and in same way as for sample (R^2=0.999), and the results were expressed as mg of gallic acid equivalents per 100 g of dry weight (mg GAE/100 g).

Determination of electron donating ability. The ability of electron donating of the Chinese quince was determined by using the DPPH radical scavenging assay [Cheng and Yu, 2006]. 100 μL of 12 times diluted sample was mixed with 900 μL analytically pure DPPH dissolved in pure ethanol. After 30 min of storage in the absence of light at room temperature, visible absorption was measured at 520 nm using pure ethanol as blank. The determination of electron donating ability was calculated according to the following equation:

EDA (%)=(1−abosrbance of sample/absorbance of blank)×100

Statistical analysis and optimization. All the experiments were carried out in triplicate and the results were expressed by ±SD. All the response results were analyzed for significance by analysis of variance (ANOVA) and overlaid counter plots were obtained by using statistical analysis system (SAS) software (Version 8.2, SAS Institute, Cary, NC). The level of statistical significance was set at 95% ($p<0.05$). The surface plots were performed using Statistica 6.0 (Statsoft Inc., Tulsa, Oklahoma).

The mean values of the triplicate trials were fit to a second-order polynomial of the following form by the response surface regression procedure (RSREG) in SAS according to central composite design:

$$Y_i=\beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_{11}X_1^2+\beta_{22}X_2^2+\beta_{33}X_3^2+\beta_{12}X_1X_2+\beta_{13}X_1X_3+\beta_{23}X_2X_3 \quad (1)$$

Where Y_i is the predicted responses for TFC, TPC and EDA, β_0 is the estimated coefficient of the fitted response at the center point of the experiment, $\beta_1, \beta_2, \beta_3$ are the linear, $\beta_{11}, \beta_{22}, \beta_{33}$ are the quadratic and $\beta_{12}, \beta_{13}, \beta_{23}$ are the interaction coefficients. The fitted polynomial equations were generated to surface and contour plots so as to visualize the relationship between the response and experimental levels of each factor. Moreover, the usage of overlaid contour plot of each response can interpret the optimum conditions directly.

Results and Discussion

Fitting the model. Multiple regression coefficients were determined by employing least squares technique [Liu et al., 2009] to predict quadratic polynomial models for total flavonoids content (TFC), total phenolic compound (TPC) and electron donating ability (EDA) of Chinese quince extracts. The complete experimental design conditions and the results are listed in Table 1. Each response was evaluated for extracts from Chinese quince under process conditions of variable ethanol concentration (X_1), extraction time (X_2) and microwave

Table 1. Experimental data on total flavonoids content, total phenolic compound, and electron donating ability of the extract of Chinese quince under different conditions of microwave-assisted extraction based on a central composite design for response surface analysis

	Microwave extraction condition			Analytical results		
Runs	Ethanol conc. (%)	Extraction time (min)	Microwave power (w)	TFC[1] (mg/100 g)	TPC[2] (mg GAE/100 g)	EDA (%)
1	25(-1)	3(-1)	40(-1)	277.94±6.99	1819.10±15.92	69.96±0.85
2	25(-1)	3(-1)	120(+1)	361.76±5.90	2254.80±30.26	71.17±1.76
3	25(-1)	7(+1)	40(-1)	230.88±1.94	2024.66±16.60	67.98±0.07
4	25(-1)	7(+1)	120(+1)	358.82±7.01	2291.43±12.45	71.49±1.23
5	75(+1)	3(-1)	40(-1)	291.18±6.99	1441.91±19.09	54.98±0.48
6	75(+1)	3(-1)	120(+1)	317.65±4.59	1736.82±8.74	65.95±0.90
7	75(+1)	7(+1)	40(-1)	222.06±6.59	1712.32±20.40	66.19±0.48
8	75(+1)	7(+1)	120(+1)	294.12±3.70	1712.46±13.40	66.43±0.66
9	0(-2)	5(0)	80(0)	229.41±3.63	1508.17±9.80	56.26±2.00
10	100(+2)	5(0)	80(0)	207.35±4.31	646.69±10.98	24.48±0.25
11	50(0)	1(-2)	80(0)	201.26±5.16	2037.75±16.30	72.25±1.26
12	50(0)	9(+2)	80(0)	149.94±3.32	2233.98±20.40	82.70±0.70
13	50(0)	5(0)	0(-2)	304.21±4.05	1695.86±24.51	67.74±0.07
14	50(0)	5(0)	160(+2)	436.03±4.73	1761.96±23.43	67.90±0.86
15	50(0)	5(0)	80(0)	462.65±5.62	2185.24±48.08	82.06±0.32
16	50(0)	5(0)	80(0)	469.52±5.16	2184.04±8.74	81.94±0.78
17	50(0)	5(0)	80(0)	476.47±4.82	2155.74±11.31	80.34±0.68
18	50(0)	5(0)	80(0)	478.68±4.41	2201.45±2.72	80.78±1.99
19	50(0)	5(0)	80(0)	469.85±2.65	2263.53±3.14	79.62±0.48
20	50(0)	5(0)	80(0)	457.35±0.74	2150.70±5.74	79.78±0.84

All the results were expressed in means of triplicates standard deviation.

[1]The results of total flavonoids content were expressed as mg of rutin equivalents per 100 g dry weight.

[2]The results of total phenolic compound were expressed as mg of gallic acid equivalents per 100 g of dry weight.

Table 2. Regression coefficients of predicted quadratic polynomial models for total flavonoids content, total phenolic compound and electron donating ability of MAE extract of Chinese quince

Coefficient	TFC[1)]	TPC[2)]	EDA[3)]
β_0	-401.885***	334.801	33.339**
linear			
β_1	11.526***	38.434***	1.155**
β_2	173.060***	98.203	2.363
β_3	3.505***	18.636**	0.372**
quadratic			
β_{11}	-0.102***	-0.432***	-0.016***
β_{22}	-18.660***	-1.320	-0.201
β_{33}	-0.016***	-0.067**	-0.002**
Crossproduct			
β_{12}	-0.107	0.010	0.033
β_{13}	-0.014**	-0.051	-0.0008
β_{23}	0.140**	-0.724	-0.013

'***' means p<0.001, '**' means p<0.05,'*'means p<0.01
[1)]Total flavonoids content
[2)]Total phenolic compound
[3)]Electron donating ability

power (X_3). The results of the regression coefficient of predicted quadratic polynomial models for three responses are presented in Table 2. High significance imply that this model have a good linear and quadratic fitness in the experiment [Sereshti *et al*., 2009]. The R^2 values for TFC, TPC and EDA were 0.9929, 0.9690 and 0.9610, respectively. The adjusted R^2 values for TFC, TPC and EDA were 0.9865, 0.9411 and 0.9260, respectively.

Effect of process variables on total flavonoids content. The TFC of extracts extracted under 15 different conditions with 6 central points based on CCD are presented in Table 1. The regression equation in coded levels without insignificant terms was used to calculate the content variation through the response surface analysis as follows:

$$Y_{TFC}=-401.885+11.526X_1+173.060X_2+3.505X_3-0.102X_1X_1-18.660X_2X_2-0.014X_1X_3+0.140X_2X_3-0.016X_3X_3 \quad (2)$$

X_1, X_2 and X_3 in this equation (2) express ethanol concentration, extraction time and microwave power, respectively. The results of analysis of variance (ANOVA) showed that the effect of process variables on TFC of extracts was highly significant (p<0.001) with R^2-value of 0.9929 and adjusted- R^2 value of 0.9865. The estimated stationary point was represented as a maximum point,

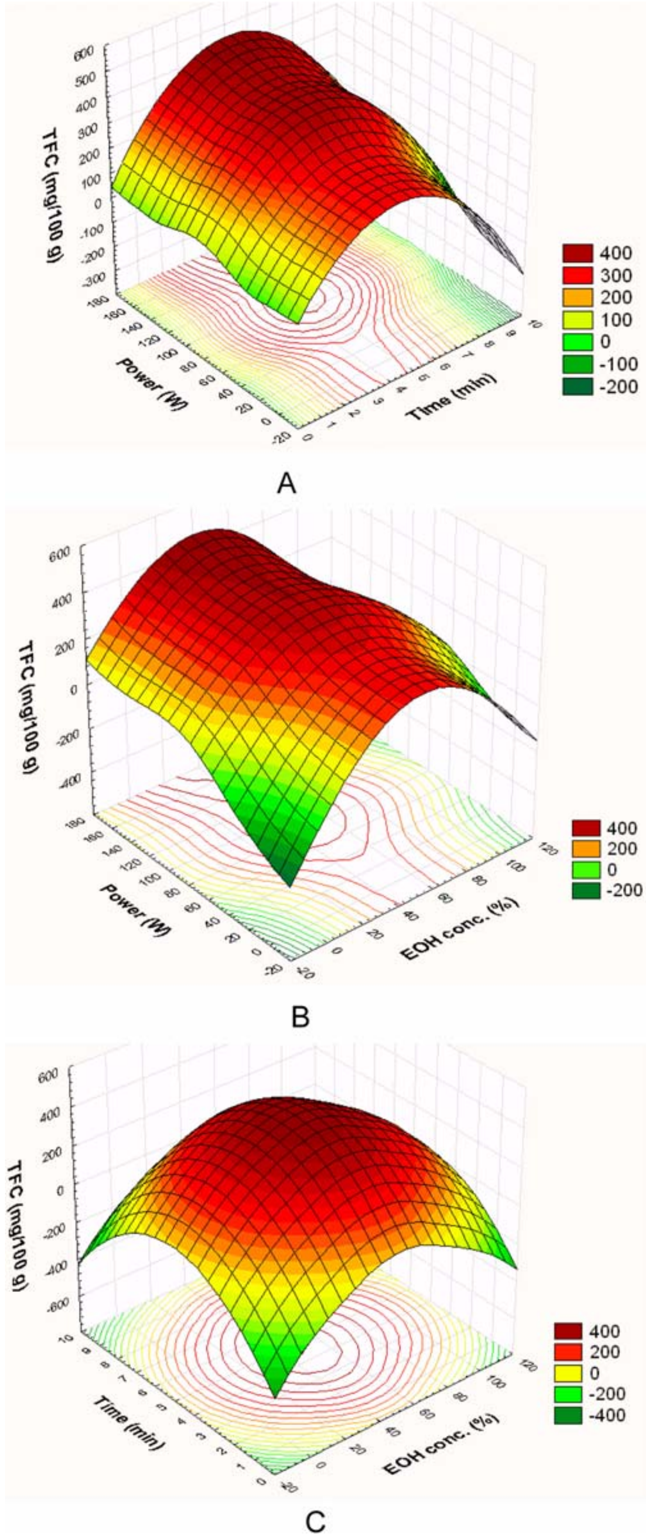

Fig. 1. Response surface plot of the TFC (Y1, mg /100 g) of Chinese quince extracts as (A) a function of extraction time and microwave power at solvent concentration at 50%, (B) a function of solvent concentration and microwave power at extraction time at 5 min and (C) a function of solvent concentration and extraction time at microwave power at 80 W.

and the numerical maximum TFC was estimated to be 480.508±5.069 mg RE/100 g. The optimal extraction conditions to yield this estimated maximum were 46.24% of ethanol concentration, 4.91min of extraction time and 108.84 W of microwave power.

Responses surface plots generated in accordance with equation (2), are shown in Fig. 1. Generally, TFC showed a tendency to increase as ethanol concentration, extraction time increased fast at first, while microwave power have little effect on content change, but Fig. 1B and Fig. 1C indicated that when the extraction time more than 5 min or ethanol concentration above 50%, there was a decline of total flavonoids content, besides pure solvent of ethanol extracted lower amounts total flavonoids content than 50% ethanol has been observed in this research. Similar effects of process variables of ethanol concentration on the extracted component were also reported by [Li *et al*., 2004] in MAE from *Eucommia ulmodies* Oliv. One possible reason for the increased efficiency might be due to the presence of some water, resulting in the increase in swelling of the plant material, which increased the contact surface area between the plant matrix and the solvent.

Effect of process variables on total phenolic compound. TPC of extracts from Chinese quince are presented in Table 1 with highest yield in run 4, which are at least four times more than TPC in apples juice [Yasunori *et al*., 2008]. The following coded level regression equation without insignificant terms was obtained from the response surface analysis in order to predict the variation of TPC in the extract corresponding to the extraction condition.

$$Y_{TPC} = 38.434X_1+18.636X_3-0.432X_1X_1-0.067X_3X_3 \quad (3)$$

R^2 and adjusted- R^2 values of the regression equation (3) for TPC was 0.9690 and 0.9411, respectively. Significance was confirmed at 0.0001. The numerical estimated stationary point in ANOVA is 2339.640± 107.350 mg GAE/100 g at saddle point. The independent variables to show this maximum were 40.48% ethanol concentration, 8.92 min extraction time and 75.96 W microwave power (Table 3).

The response surface plots for TPC are shown in Fig. 2(A, B, C). The TPC was mainly influenced by ethanol concentration and microwave power. Fig. 2A showed that total phenolic compound increased slowly with the increase of the ethanol concentration, however, there was a quick drop of total phenolic yield when ethanol concentration more than 40%. The effect of extraction time was non-significant on TPC of extracts. Generally it is considered that under proper conditions, extract yield increases with increasing extraction time [Wang *et al*., 2008], however considering both of the energy saving and time restraint, extraction for very a prolonged time is not preferable, therefore the extraction was carried out for a maximum of 9 min.

Effect of process variables on electron donating ability. In order to confirm radical scavenging ability in Chinese quince extract, electron donating ability experiment was performed. Table 1 indicates that the examined results on electron donating ability of MAE extracts from Chinese quince were impressive after 12 times diluted. The coded level regression equation without insignificant terms from the response surface analysis for electron donating ability is as follows:

$$Y_{EDA}=33.339+1.155X_1+0.372X_3-0.016X_1X_1-0.002X_3X_3 \quad (4)$$

The regression equation (4) for electron donating ability showed that R^2 and adjusted- R^2 were 0.9610 and 0.9260 respectively. Significance was confirmed at p-value of 0.0001 (Table 3). The numerical estimated maximum electron donating ability was 82.311±1.534% under the conditions of 45.05% ethanol concentration, 7.04 min extraction time and 78.65 W microwave power.

In the response surface plots, as seen in Fig. 3 (A, B, C), it could be noticed that with the increase in microwave power, electron donating ability first increased with microwave power until 80W, after wards it declined. We knew that the temperature of the extraction medium increases with increasing microwave power and the higher extraction temperature is preferable for the

Table 3. Numerical estimated level of extraction condition for the maximum total flavonoids content, total phenolic compound and electron donating ability of the extract from Chinese quince

yn	R^2	adjust-R^2	model *p*-value	x_1[1)]	x_2[2)]	x_3[3)]	max.	Morphology
TFC	0.9929	0.9865	0.0001	46.24	4.91	108.84	480.508±5.069 mg/100 g	maximum
TPC	0.9690	0.9411	0.0001	40.48	8.92	75.96	2339.64±107.350mg GAE/100 g	saddle point
EDA	0.9610	0.9260	0.0001	45.05	7.04	78.65	82.311±1.534%	maximum

[1)]Ethanol concentration (%)
[2)]Extraction time (min)
[3)]Microwave power (W)

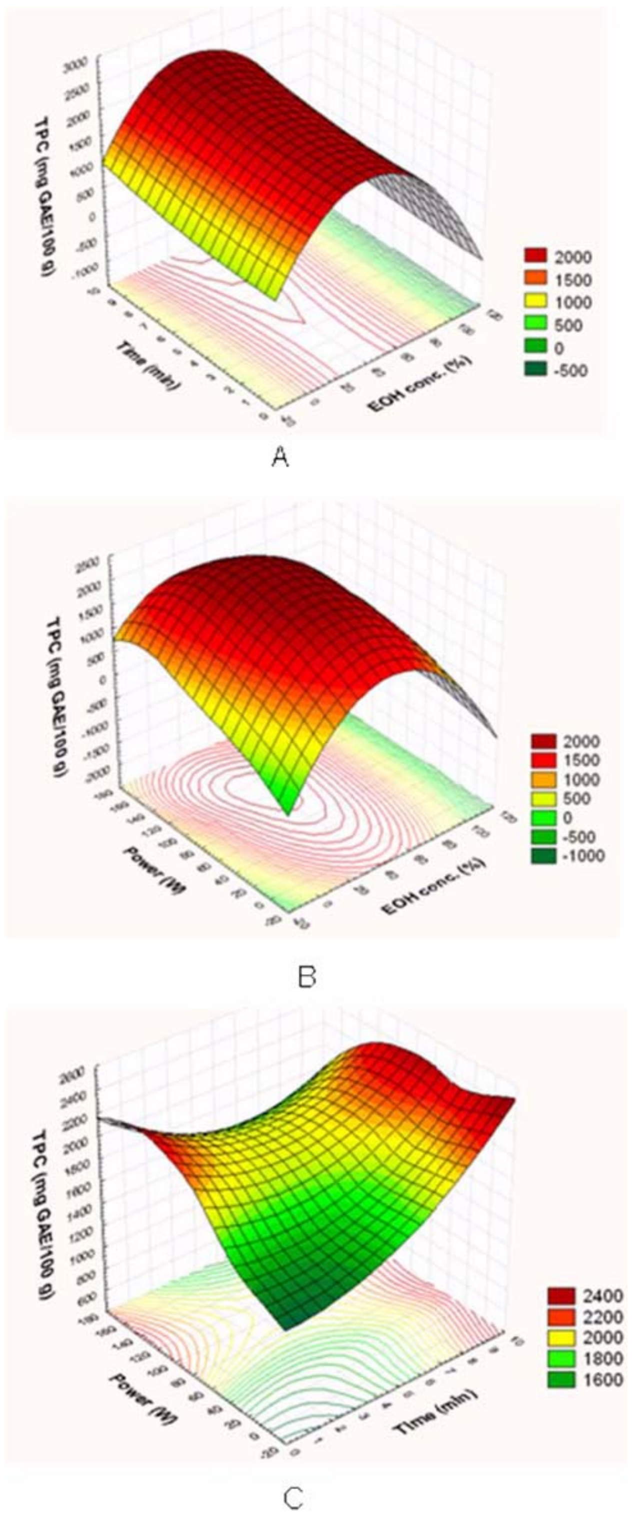

Fig. 2. Response surface plot of the TPC (Y2, mg GAE /100 g) of Chinese quince extracts as (A) a function of solvent concentration and extraction time at microwave power at 80 W, (B) a function of solvent concentration and microwave power at extraction time at 5 min and (C) a function of extraction time and microwave power at solvent concentration at 50%.

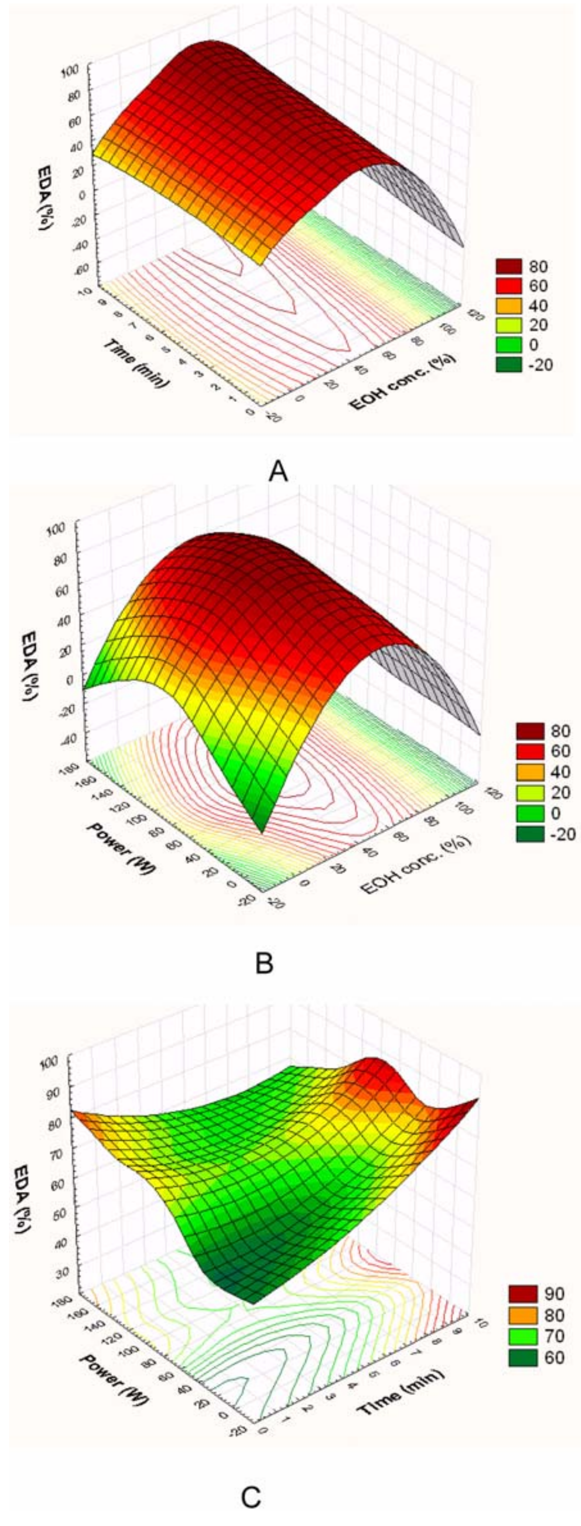

Fig. 3. Response surfaces plot of the EDA(Y3, %) of Chinese quince extracts as (A) a function of solvent concentration and extraction time at microwave power at 80 W, (B) a function of solvent concentration and microwave power at extraction time at 5 min and (C) a function of extraction time and microwave power at solvent concentration at 50%.

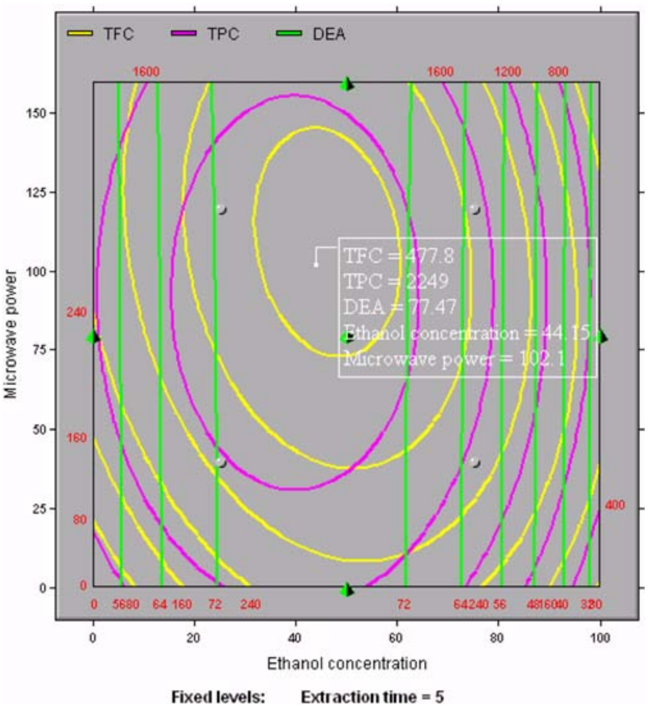

Fig. 4. The overlaid contour plots of TFC, TPC and EDA of microwave-assisted extracts from Chinese quince as effected by solvent concentration and microwave power (extraction time was fixed at 5 min).

extraction because it reduces the extraction time. But, if temperature becomes too high, it may destroy the sensible components for targeted in the extract [Ali *et al.*, 2007].

Optimum extraction conditions for the maximum TFC, TPC and EDA. Although we have obtained the optimum extraction conditions for each response individually. However, in order to predict optimal conditions for three responses simultaneously, the simplest strategy to adopt is visual inspection. The surface can be overlapped to find the experimental region that can satisfy all responses studied [Bezerra *et al.*, 2008]. By analyzing the effects of extraction conditions on the TFC, TPC and EDA, we found that the responses changed substantially with ethanol concentration, extraction time and microwave power. A superimposed contour plot for TFC, TPC and electron donating ability is shown in Fig. 4. As we have mentioned already when extraction time in total flavonoids content more than 5 min resulted in a lost of total flavonoids yield, therefore the superimposed contour plot was fixed at 5 min. Table 3 indicated that ethanol concentration (40~46%) and microwave power (75~108 W) could yield the maximum TFC, TPC and electron donating ability in Chinese quince extract. Eventually, optimal MAE conditions of 5 min extraction time, 44.15% ethanol concentration and 102.1 W microwave power are predicted in Fig. 4, which can result in maximal TFC (477.8 mg RE/100 g), TPC (2249 mg GAE/100 g) and electron donating ability (77.47%) in the extract from Chinese quince.

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