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ARTICLE

Optimization of Ultrasound-Assisted Extraction of Phenolic Compounds, Antioxidants, and Anthocyanins from Grape (*Vitis vinifera*) Seeds

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Important functional components from Campbell Early grape seed were extracted by ultrasound-assisted extraction (UAE) technology. The experiments were carried out according to a five level, three variable central composite rotatable design (CCRD). The best possible combinations of ethanol concentration, extraction temperature, and extraction time with the application of ultrasound were obtained for the maximum extraction of phenolic compounds, antioxidant activities, and anthocyanins from grape seed by using response surface methodology (RSM). Process variables had significant effect on the extraction of functional components with extraction time being highly significant for the extraction of phenolics and antioxidants. The optimal conditions obtained by RSM for UAE from grape seed include 53.15% ethanol, 56.03 °C temperature, and 29.03 min time for the maximum total phenolic compounds (5.44 mg GAE/100 mL); 53.06% ethanol, 60.65 °C temperature, and 30.58 min time for the maximum antioxidant activity (12.31 mg/mL); and 52.35% ethanol, 55.13 °C temperature, and 29.49 min time for the maximum total anthocyanins (2.28 mg/mL). Under the above-mentioned conditions, the experimental total phenolics were 5.41 mg GAE/100 mL, antioxidant activity was 12.28 mg/mL, and total anthocyanins were 2.29 mg/mL of the grape seed extract, which is well matched with the predicted values.

KEYWORDS: **Ultrasound-assisted extraction; response surface methods; phenolic compounds; antioxidants; anthocyanins; *Vitis vinifera*; Campbell Early**

INTRODUCTION

Interest in the investigation of active components, especially phenolic compounds, from natural sources such as fruits and vegetables has greatly increased in recent years. The reason for this increased interest is the restricted use of synthetic antioxidants in foods because of the possible undesirable effects on human health (*1*). Grapes (*Vitis vinifera*) are among the most widely consumed fruits, and the demand for grapes and grape products is increasing because of the associated health benefits (*2*). Grapes are rich in phenolic compounds with approximately 75% of grape polyphenols existing in the skin and seeds (*3*). Tons of grape pomace is produced while processing grapes, and the seeds constitute a considerable proportion of it, amounting to 38−52% on a dry matter basis (*4*). Functional ingredients of grape seeds include several flavonoids with a phenolic nature such as monomeric flavanols, dimeric, trimeric, and polymeric procyanidins, and phenolic acids (*5*). The positive physiological effects associated with the consumption of grape and grape derivatives are currently believed to be mainly due to the antiradical and antioxidant properties of the occurring phenolic species (*6*). Grape anthocyanins possess well-known pharmacological properties and strong biological functions such as anti-inflammatory and antioxidant activities (*7*, *8*). These high-quality polyphenolic compounds can be used in different therapeutic procedures with the purpose of free radical neutralization in biological systems (*5*, *9*). The potent antioxidant activity of wine and grape extracts on oxidizing human low-density lipoproteins in vitro correlates significantly to the presence of phenolics (*10*). In addition, various antiplatelet aggregating effects and other potentially disease preventing cellular actions of phenolic compounds have been amply documented (*11*).

Extraction is a very important stage in the isolation, identification, and use of phenolic compounds (*12*). To describe the extraction mechanism in the literature, Fick's second law of diffusion is usually used (*13*). The recovery of these components is commonly performed through a solvent-extraction procedure and the concentration of solvent, time, and temperature are important parameters to be optimized (*14*). In order to seek more environmentally friendly methods, solvent consumption should be decreased, extraction time should be shortened, extraction yield should be increased, and the quality of the extracts should be enhanced. Various novel extraction techniques have been developed for the extraction of nutraceuticals from plants, including ultrasound-assisted extraction, supercritical fluid extraction, microwave-assisted extraction, and solvent extraction (*15*). Among these, ultrasound-assisted extraction (UAE) is an inexpensive, because of low instrumental requirements, simple, and efficient alternative to conventional extraction techniques. The enhancement in extraction obtained by using ultrasound is

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mainly attributed to the effect of acoustic cavitations produced in the solvent by the passage of an ultrasound wave (*16*). Ultrasound also offers a mechanical effect allowing greater penetration of solvent into the sample matrix, increasing the contact surface area between the solid and liquid phase, and as a result, the solute quickly diffuses from the solid phase to the solvent (*17*). The use of higher ultrasonic frequency may enhance OH radical formation; however, the hydrophilic nature of phenolic compounds makes them unavailable for reaction with OH radicals (*18*). Instead, the use of UAE may prevent the possible chemical degradation of targeted compounds due to decreased chemical involvement and reduction in extraction time (*15*).

In this study, UAE parameters such as solvent concentration, extraction temperature, and extraction time were optimized using response surface methodology (RSM), by employing a five level, three variable central composite rotatable design (CCRD), in order to obtain the optimal conditions for the extraction of functional components from the seeds of Campbell Early grapes.

MATERIALS AND METHODS

Materials. Freshly harvested ripened grapes were purchased from a local farm in Kyungbuk province of Korea, and the grape cultivar was identified as Campbell Early. Grapes were excised from the stems and washed. Grapes were manually cut into halves, and the grape seeds were removed with a knife. Grape seeds were oven-dried at 50 °C until the moisture level was constant (6.3% w/w). Dried grape seeds were ground to a powdered form using an electrical grinder and passed through a 0.5 mm sieve. All of the chemicals used were of analytical grade, and they were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Ultrasound-Assisted Extraction from Grape Seeds. A sample of 2 g of powdered grape seeds was kept in a glass flask, and the volume was made to 100 mL with the extraction solvent. Contents were dissolved by using a magnetic stirrer (KMC 130SH; Vision Scientific Co., Ltd., Daegu, Korea) for 5 min. Ultrasound-assisted extraction (UAE) was performed in a sonication water bath (JAC Ultrasonic 2010P; Jinwoo Engineering Co., Ltd., Hwasung, Gyeonggi, Korea) with a useful volume of 10 L. The working frequency and power were fixed at 40 kHz and 250 W, respectively. The temperature and time of extraction was controlled from the panel. After extraction, the flask was immediately cooled to room temperature by using chilled water. The extract was filtered through filter paper #5A under vacuum, and the solution was collected in a volumetric flask. It was then used for the determination of total phenolics compounds, antioxidants, and anthocyanin contents. All of the measurements were carried out in triplicate, and the data reported were means ± SD. Preliminary extraction trials were carried out with water and 25 and 50% ethanol at 30, 40, and 50 °C for 5, 15, and 25 min of extraction time.

Experimental Design. A five level, three variable central composite rotatable design (*19*) was applied to determine the best combinations of extraction variables for the extraction of total phenolic compounds, antioxidants, and anthocyanins from grape seeds. Three independent variables selected for this study were the concentration of solvent, the extraction temperature, and the extraction time (**Table 1**). Preliminary trials showed that the values of responses increased with increasing ethanol concentration, temperature, and time (*14*); therefore, the optimal levels were selected as center points in the designed experiment. The factorial design consisted of eight factorial points, six axial points (two axial points on the axis of each design variable at a distance of 1.68 from the design center), and four center points leading to 18 sets of experiments. Regression analysis was performed on the data of dependent variables obtained by triplicate observations as effected by the extraction conditions and was fitted into an empiric second order polynomial model as shown in the following equation:

$$Y = b_0 + \sum_{i=1}^{5} b_i x_i + \sum_{i=1}^{5} b_{ii} x_i^2 + \sum_{i=1}^{4} \sum_{j=i+1}^{5} b_{ij} x_i x_j \qquad (1)$$

Table 1. Independent Variables and Their Levels Employed in a Central Composite Rotatable Design for Optimization of Grape Seed Extracts

	coded levels				
	−α (−1.68)	−1	0	1	+α (+1.68)
independent variables	natural levels				
ethanol concentration (%)	33	40	50	60	67
extraction temperature (°C)	33	40	50	60	67
extraction time (min)	16	20	25	30	34

where Y is the response variable, and b_0, b_i, b_{ii}, and b_{ij} are regression coefficients of variables for intercept, linear, quadratic, and interaction terms, respectively. x_i and x_j are independent variables.

The responses obtained from the experimental design set (**Table 2**) were subjected to multiple nonlinear regression analysis using the Statistical Analysis System (SAS, version 9.1), to obtain the coefficients of the second polynomial model. The quality of the fit of the polynomial model was expressed by the coefficient of determination R^2, and its statistical significance was checked using an F-test.

Analysis for Total Phenolic Compounds. The total phenolic compounds were analyzed using the Folin−Ciocalteu method with some modifications (*20*). A 200 μL properly diluted sample or a standard solution of varying concentrations were mixed with 400 μL of Folin−Ciocalteu reagent. Deionized water was used for dilution and control. The solution was diluted to a total volume of 4.6 mL using deionized water and then thoroughly mixed. After incubation for 10 min at room temperature, 1 mL of 20% Na_2CO_3 solution was added, then immediately mixed, and incubated for 2 h. The absorbance was read at 765 nm on a spectrophotometer (TU-1800; Human Corporation, Seoul, Korea). Measurements were recorded in triplicate. Gallic acid of 1 mg/mL was used as the standard, and the total phenolic compounds of the samples were expressed in milligram gallic acid equivalent per 100 mL (mg GAE/100 mL).

Determination of Antioxidant Activity. The antioxidant activity of the grape seed extracts was evaluated by the phosphomolybdenum complex method (*21*). Briefly, 0.4 mL of sample solution (100 μL of grape seed extract dissolved in 1 mL of methanol) was combined with 4 mL of reagent solution containing 0.6 M sulfuric acid, 2 mM sodium phosphate, and 4 mM ammonium molybdate. The blank solution contained 4 mL of reagent solution and 1 mL of methanol. Test tubes were capped and placed in hot water for 90 min at 95 °C. After samples were cooled to room temperature, absorbance was measured at 695 nm against a blank. Antioxidant activity was expressed relative to that of ascorbic acid.

Analysis for Total Anthocyanins. Determination of total anthocyanins in grape seed extracts was based on the method described by Iland et al. (*22*) with some modifications. In 1 mL of sample, 10 mL 50% (v/v) ethanol was added, and the sample was centrifuged at 1800*g* for 10 min. Then 200 μL of the centrifuged extract was mixed with 3.8 mL of 1 M HCl and incubated at room temperature for 3 h. The absorbance (A) of acidified diluted extract was measured at 520 nm using 1 M HCl as the blank. Total anthocyanins were calculated as mg/mL of extract solution using the absorbance (B) of a 1% w/v solution of malvidin-3-glucoside as follows.

$$\text{total anthocyanins (mg/mL)} = A \times \text{dilution factor} \times 1000/B$$

Statistical Analysis. All determinations were carried out in triplicate, and the experimental results obtained were expressed as means ± SD. Statistical analysis was performed by using Statistical Analysis System (SAS, version 9.1). Data was analyzed by analysis of variance, and the mean values were considered significantly different when $p < 0.05$. The optimal extraction conditions were estimated through three-dimensional response surface analyses of the three independent variables and each dependent variable.

RESULTS AND DISCUSSION

Modeling of the Extraction Process from Grape Seed. In order to optimize the extraction process with reference to the extraction of phenolic, antioxidant, and anthocyanin components from

Table 2. Experimental Design of the Five-Level, Three-Variable Central Composite and Total Phenols, Antioxidant Activities, and Anthocyanins of Ultrasound-Assisted Grape Seed Extracts

	extraction conditions				analytical results[a]		
test set	X_1, ethanol concentration (%)	X_2, extraction temperature (°C)	X_3, extraction time (min)	yield (%)	total phenols (mg GAE/100 mL)	antioxidant activity (mg/mL)	anthocyanins (mg/mL)
1	40 (−1)	40 (−1)	20 (−1)	10.25 ± 0.25	4.48 ± 0.06	10.08 ± 0.08	1.09 ± 0.09
2	40 (−1)	40 (−1)	30 (+1)	11.55 ± 0.18	4.86 ± 0.03	10.94 ± 0.07	1.50 ± 0.13
3	40 (−1)	60 (+1)	20 (−1)	12.74 ± 0.56	4.72 ± 0.13	10.55 ± 0.12	1.28 ± 0.14
4	40 (−1)	60 (+1)	30 (+1)	18.05 ± 0.48	5.21 ± 0.08	11.67 ± 0.09	1.81 ± 0.08
5	60 (+1)	40 (−1)	20 (−1)	13.52 ± 0.65	4.84 ± 0.10	10.73 ± 0.12	1.40 ± 0.11
6	60 (+1)	40 (−1)	30 (+1)	14.37 ± 0.14	5.03 ± 0.11	11.18 ± 0.10	1.59 ± 0.07
7	60 (+1)	60 (+1)	20 (−1)	27.85 ± 1.22	4.83 ± 0.05	10.70 ± 0.15	1.41 ± 0.13
8	60 (+1)	60 (+1)	30 (+1)	29.44 ± 0.52	5.29 ± 0.04	12.02 ± 0.06	1.98 ± 0.21
9	33 (−1.68)	50 (0)	25 (0)	26.13 ± 0.44	4.69 ± 0.08	10.53 ± 0.14	1.39 ± 0.18
10	67 (+1.68)	50 (0)	25 (0)	24.09 ± 1.22	5.23 ± 0.09	11.69 ± 0.08	1.87 ± 0.05
11	50 (0)	33 (−1.68)	25 (0)	9.39 ± 2.15	4.62 ± 0.12	10.55 ± 0.07	1.31 ± 0.06
12	50 (0)	67 (+1.68)	25 (0)	29.32 ± 0.33	5.20 ± 0.12	11.95 ± 0.11	1.90 ± 0.17
13	50 (0)	50 (0)	16 (−1.68)	17.35 ± 1.84	4.25 ± 0.06	9.84 ± 0.15	0.85 ± 0.08
14	50 (0)	50 (0)	34 (+1.68)	19.4 ± 1.25	5.30 ± 0.08	11.97 ± 0.18	2.27 ± 0.05
15	50 (0)	50 (0)	25 (0)	27.56 ± 0.56	5.25 ± 0.03	11.78 ± 0.24	2.15 ± 0.12
16	50 (0)	50 (0)	25 (0)	27.75 ± 1.21	5.27 ± 0.14	11.69 ± 0.08	2.07 ± 0.02
17	50 (0)	50 (0)	25 (0)	26.95 ± 0.85	5.32 ± 0.07	11.83 ± 0.07	1.99 ± 0.12
18	50 (0)	50 (0)	25 (0)	28.26 ± 0.33	5.26 ± 0.06	11.95 ± 0.13	2.23 ± 0.04

[a] Analytical results are the means ± SD ($n = 3$).

Table 3. Regression Coefficients and Analysis of the Model for Three Response Variables

	coefficients estimated		
coefficient	total phenols	antioxidants	anthocyanins
b_0	−7.141455[b]	−11.413558[c]	−14.056005[b]
b_1	0.151350[b]	0.327603[b]	0.214296[b]
b_2	0.132516[b]	0.201353[c]	0.183255[c]
b_3	0.328528[b]	0.583098[b]	0.389816[b]
b_{11}	−0.001045[b]	−0.002661[b]	−0.001858[b]
b_{22}	−0.001218[b]	−0.002166[b]	−0.001944[b]
b_{33}	−0.006029[a]	−0.011921[a]	−0.007421[b]
b_{12}	−0.000425	−0.000487	−0.000125
b_{13}	−0.000550	0.000510	−0.000450
b_{23}	0.000950	0.002860	0.001250
probability of F value	<0.001	<0.001	<0.001
probability of lack of fit	0.0702	0.0985	0.1604

[a] $p < 0.001$. [b] $p < 0.01$. [c] $p < 0.05$.

grape seed using ultrasound-assisted extraction (UAE), a central composite design was developed as represented in **Table 2**. The experimental values of total phenols, antioxidant activities, and anthocyanins of grape seed extracts at various experimental conditions are also presented in **Table 2**. The results of the analysis of variance, goodness of fit, and the adequacy of the models are summarized in **Table 3**. The data showed a good fit with eq 1, which was statistically acceptable at $p < 0.05$ and adequate with satisfactory R^2 values. The values of coefficients presented in **Table 3** were used in final predictive equations after neglecting nonsignificant terms. On the basis of these equations, three-dimensional plots were constructed to predict the relationships between independent variables and dependent variables.

Effect of Process Variables on Total Phenolics Compounds. The total phenols of grape seed extracts obtained by UAE based on the central composite design are shown in **Table 2**. Multiple regression analysis was performed on the experimental data, and the coefficients of the model were evaluated for significance. The effect of extraction time was highly significant ($p < 0.001$) on the extraction of phenolics, and it was consistent with the findings of Revilla et al. (*23*) who obtained higher yields of phenolics from grape skins when extraction was done for a longer time. The values of the coefficients for total phenols as presented in **Table 3** were used for a final predictive equation neglecting the nonsignificant cross-terms as given below:

$$Y = -7.14145 + 0.15135X_1 + 0.13251X_2 + 0.32852X_3 - 0.00104X_1^2 - 0.00121X_2^2 - 0.00602X_3^2 \quad (2)$$

To determine the optimal levels of variables for the UAE of total phenols from grape seed, three-dimensional surface plots (**Figure 1**) were constructed according to eq 2. Extraction process variables significantly effected ($p < 0.05$) the extraction of total phenols from the seeds of Campbell Early grapes. **Figure 1A** shows the effect of ethanol concentration and extraction time on the content of total phenolic compounds. The total phenolic contents increased slowly with the increase of ethanol concentration at a fixed extraction temperature and nearly reached a peak at the highest ethanol concentration tested. Similarly, the increase in extraction temperature at a fixed ethanol concentration led to a gradual increase in the total phenolic content and reached a maximum at the highest extraction temperature tested. The plot of total phenols as affected by ethanol concentration and extraction time (**Figure 1B**) demonstrates a marked increase in phenolic contents with the increase of ethanol concentration at a fixed extraction time, while an increase in extraction time at a fixed ethanol concentration also led to a marked increase in total phenol contents. A similar linear increase in total phenolic contents with the increase of extraction temperature at a fixed extraction time, while showing an obvious quadratic effect of extraction time, was observed (**Figure 1C**).

Effect of Process Variables on Antioxidant Activity. The mean experimental data showing the extraction of antioxidant components from grape seeds at various UAE conditions are presented in **Table 2**. The highest contents of antioxidants (12.02 mg/mL) were observed in experimental run 8 with 60% ethanol concentration, 60 °C extraction temperature, and 30 min extraction time. The lowest yield of antioxidants (9.84 mg/mL) was observed in experimental run 13 with 50% ethanol concentration, 50 °C extraction temperature, and 16 min extraction time. Statistical analysis revealed that the most relevant variable with $p < 0.001$

was extraction time. The extraction time has this kind of effect on antioxidant activity because increasing the contact time of the solvent with solids may improve the diffusion of the compounds (*24*). The results of multiple regression analysis showed that the antioxidant contents of grape seeds were significantly ($p < 0.05$) affected by the linear and quadratic terms of ethanol concentration, extraction temperature, and extraction time (**Table 3**). The final predictive equation for antioxidant activity of grape seed extract by using significant terms is as follows:

$$Y = -11.413558 + 0.327603X_1 + 0.201353X_2 + 0.583098X_3 - 0.002661X_1^2 - 0.002166X_2^2 - 0.011921X_3^2 \quad (3)$$

As presented in the three-dimensional plots for antioxidant contents (**Figure 2**), the extraction process variables affected the extraction of antioxidants in a way similar to that in the case of total phenolic compounds. This is due to the fact that antioxidant activities of grapes are closely associated with the phenolic compounds (*25*, *26*). Antioxidant activity increased with the increase of ethanol concentration at a fixed temperature and also increased significantly ($p < 0.05$) with the increase of extraction temperature at a fixed ethanol concentration (**Figure 2A**). Similarly, antioxidant value increased with the increase of ethanol concentration at a fixed time, and it rapidly increased with the increase of extraction time at a fixed ethanol concentration as represented in **Figure 2B**. **Figure 2C** shows a linear increase in antioxidant activity with the increase of extraction temperature at fixed time and a similar increase in antioxidant activity with the increase of extraction time at a constant extraction temperature.

Effect of Process Variables on the Anthocyanin Contents. Total anthocyanin contents from grape seeds obtained under various conditions using UAE are presented in the **Table 2**. Experimental data was subjected to regression analysis, and the coefficients of the estimate are presented in **Table 3**. The regression equation was used to calculate the contents' variation through the response surface analysis as follows:

$$Y = -14.056005 + 0.214296X_1 + 0.183255X_2 + 0.389816X_3 - 0.001858X_1^2 - 0.001944X_2^2 - 0.007421X_3^2 \quad (4)$$

On the basis of eq 4, three-dimensional plots to represent the effects of extraction process variables on anthocyanins from grape seeds are presented in **Figure 3**. **Figure 3A** shows the effect of ethanol concentration and extraction temperature on anthocyanin contents. There was a linear increase in total anthocyanin contents with an increase in ethanol concentration at a constant temperature. A similar effect of extraction temperature on grape seed anthocyanins at a fixed ethanol concentration was observed. Similar interactions of ethanol concentration with extraction time (**Figure 3B**) and that of extraction temperature with extraction time (**Figure 3C**) in the UAE of anthocyanins from grape seeds were observed. **Table 3** shows that anthocyanin contents were significantly affected ($p < 0.05$) by linear and quadratic terms of extraction variables. Lapornik et al. (*12*) also found a statistically significant influence of extraction variables on the yields of anthocyanins from red grape marc.

Optimum Conditions for Maximum Extraction from Grape Seeds. The estimated levels of optimum extraction conditions for maximum response of total phenols, antioxidant activities, and anthocyanins of grape seed extracts obtained by UAE are summarized in **Table 4**. The predicted conditions of UAE were 53.15% ethanol concentration, 56.03 °C extraction temperature, and 29.03 min extraction time for the maximum total phenols (5.44 mg GAE/100 mL), 53.06%, 60.65 °C, and 30.58 min for maximum antioxidant activity (12.31 mg/mL), and 52.35%, 55.13 °C, and 29.49 min for maximum anthocyanins (2.28 mg/mL). The R^2 and R^2-adjusted values for total phenols, antioxidants, and anthocyanins as shown in **Table 4** shows that the model had adequately represented the real relationship between the parameters chosen. To compare the predicted results

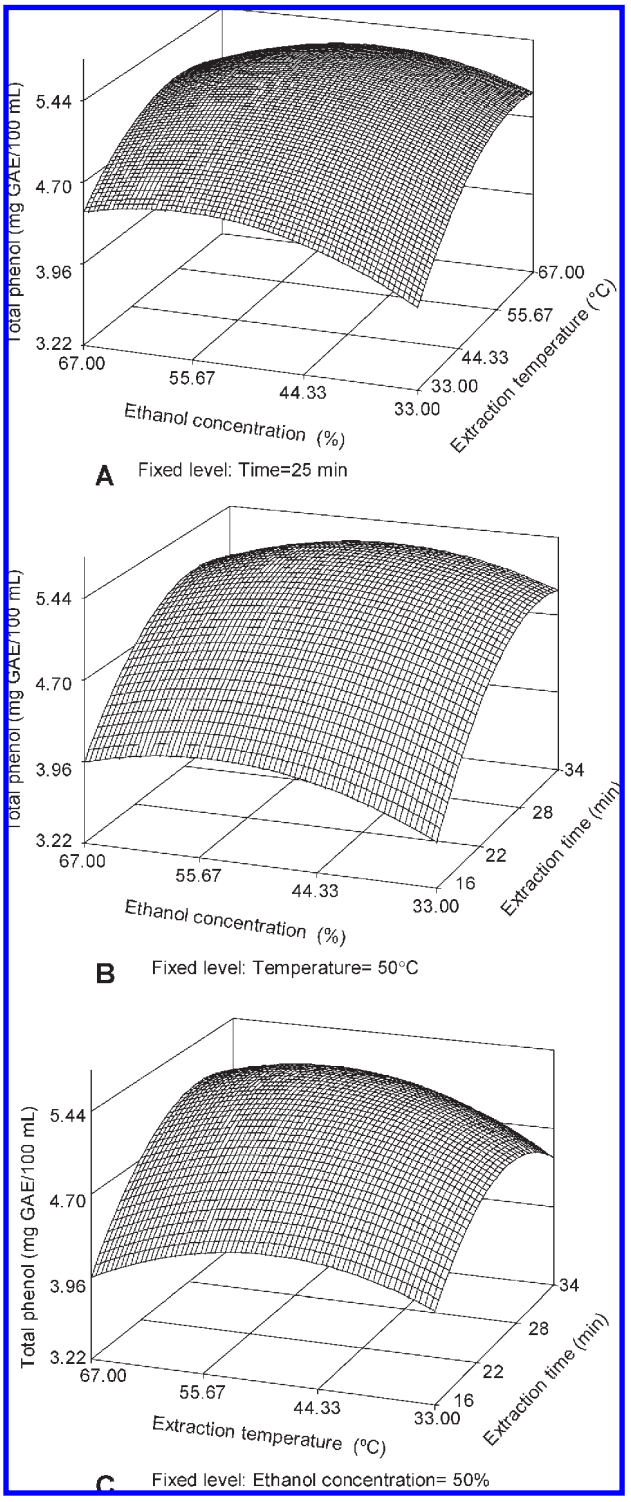

Figure 1. Response surface plots of the total phenolic contents of grape seed extract as affected by ethanol concentration, temperature, and extraction time in ultrasound-assisted extraction. (**A**) Ethanol concentration and temperature (time 25 min); (**B**) ethanol concentration and time (temperature 50 °C); (**C**) temperature and time (ethanol concentration 50%).

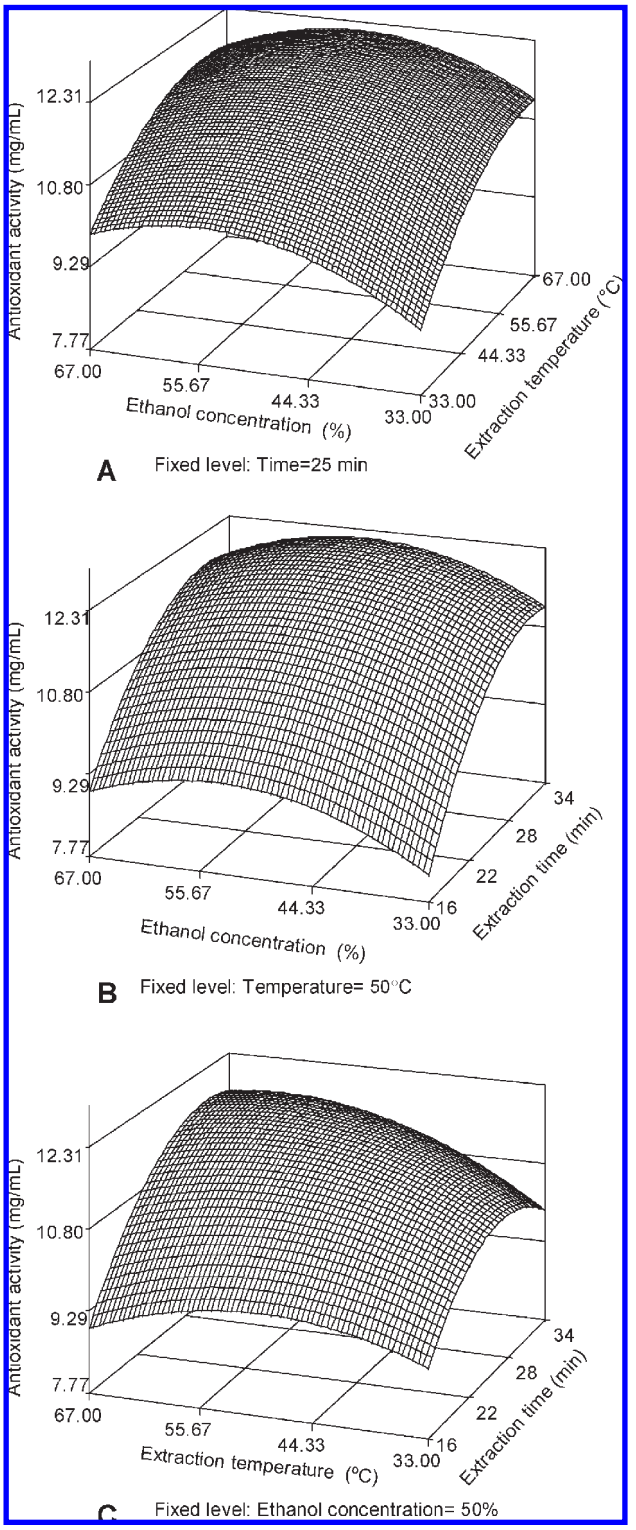

Figure 2. Response surface plots of the antioxidant activity of grape seed extract as affected by ethanol concentration, temperature, and extraction time in ultrasound-assisted extraction. (**A**) Ethanol concentration and temperature (time 25 min); (**B**) ethanol concentration and time (temperature 50 °C); (**C**) temperature and time (ethanol concentration 50%).

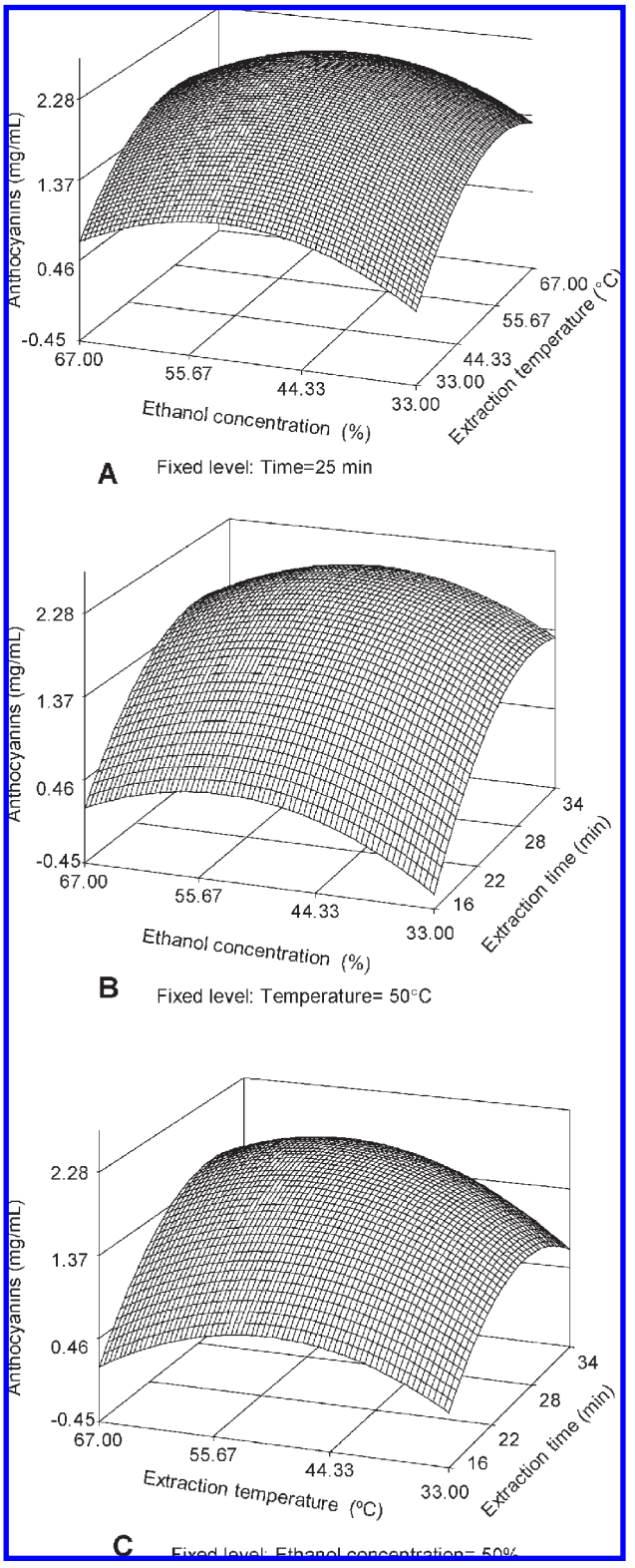

Figure 3. Response surface plots of the anthocyanin contents of grape seed extract as affected by ethanol concentration, temperature, and extraction time in ultrasound-assisted extraction. (**A**) Ethanol concentration and temperature (time 25 min); (**B**) ethanol concentration and time (temperature 50 °C); (**C**) temperature and time (ethanol concentration 50%).

with experimental values, experimental rechecking was performed for each response using the optimum extraction conditions. Mean values of 5.41 mg GAE/100 mL total phenols, 12.28 mg/mL antioxidants, and 2.29 mg/mL anthocyanins obtained from real experiments validated the RSM model. The good correlation between these results confirmed that the response model was adequate in reflecting the expected optimization. The prediction of one set of optimal conditions for three response variables was also done by using the desirability function. A total desirability value of 0.95 was obtained on a scale of 0 to 1, where 0

Table 4. Estimated Optimum Conditions, Predicted, and Experimental Values of Responses under These Conditions

					optimum extraction conditions			maximum value	
response variables	R^2	R^2-adjusted	F-value	p-value	ethanol (%)	temp (°C)	time (min)	estimated	experimental[a]
total phenols (mg GAE/100 mL)	0.9591	0.9305	20.85	0.0001	53.15	56.03	29.03	5.44	5.41 ± 0.152
antioxidant activities (mg/mL)	0.9592	0.9306	20.92	0.0001	53.06	60.65	30.58	12.31	12.28 ± 0.218
anthocyanins (mg/mL)	0.9290	0.8793	11.63	0.0010	52.35	55.13	29.49	2.28	2.29 ± 0.056

[a] Results are the means ± SD (n = 3).

represents a completely undesirable response, and 1 represents the most desirable response. At this desirability, UAE from grape seeds by using 54.8% ethanol, 52.8 °C temperature, and 29.55 min time can yield a maximum of 5.47 mg GAE/100 mL total phenols, 12.22 mg/mL antioxidants, and 2.48 mg/mL anthocyanins.

Solid−liquid extraction is a mass transport phenomenon in which solids contained in a matrix migrate into solvent brought into contact with the matrix. This mass transport phenomenon can be enhanced with changes in diffusion coefficients induced by ultrasounds and extraction temperature (*24*). Solvent concentration and extraction time also play a significant role in the extraction of phenolic compounds from plant materials (*16*). The use of UAE was due to the fact that ultrasound waves break the cells of the vegetal matrix, and the contents of the cells are released into the extraction medium (*27*). Optimization of solvent concentration, time, and temperature is important for the extraction of phenolic compounds from grapes (*14*). Ethanol is preferred as a solvent in the food industry and is regarded as a dietary alcohol (*28*). It has been reported that an increase in the working temperature favors extraction, enhancing both the solubility of the solute and the diffusion coefficient; also, beyond a certain value, phenolic compounds can be denatured (*14*, *29*). Increasing the extraction time from 12 to 24 h was found to have statistically significant effects on the yields of total phenols, anthocyanin contents, and antioxidant activities from red grape pressed marc (*12*). This indicated that the use of UAE for the extraction of total phenols, antioxidants, and anthocyanins was as effective as any other high temperature long time extraction process because it could greatly decrease the extraction time. The efficiency of UAE could be explained by the fact that sonication simultaneously enhanced the hydration and fragmentation process while facilitating the mass transfer of solutes to the extraction solvent (*30*). We may conclude that UAE can improve existing extraction processes and enable new commercial extraction opportunities and processes (*31*). We have observed a strong correlation among the total phenolic contents, antioxidant activities, and anthocyanin levels of the extracts obtained from grape seeds using UAE. Such a correlation between these functional properties has been reported in other fruits and their products, for example, blueberry (*32*) and bayberry juices (*33*). This study also indicates that the Campbell Early grape seed is a good source of important functional components. The extraction variables, particularly extraction time and temperature, strongly influence the UAE of total phenolics, antioxidants, and anthocyanins from grape seeds.

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