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Optimization of Ultrasound-Assisted Extraction for Antiradical Activities of Peel and Seed Extracts of Campbell Early Grapes

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Abstract

Central composite design was applied for the ultrasound-assisted extraction from peel and seed of Campbell Early grapes and the extraction processes were optimized for the antiradical activities of the extracts by using response surface methodology. Optimal conditions were 53.45% of ethanol concentration, 45.99°C of extraction temperature and 23.93 min of extraction time for the maximum antiradical activity of grape peel extract (54.98%) and 53.14% of ethanol 56.03°C of temperature and 29.03 min of time for maximum antiradical activity of grape seed extract (90.60%).

Key words: central composite design**,** ultrasound-assisted extraction, grape peel, grape seed, antiradical activity, response surface methodology

Introduction

The interest in the investigation of active components, especially polyphenols, from natural sources (fruits, vegetables, cereals, herbs) has greatly increased in recent years. The main reason for that is restricted use of synthetic antioxidants in foods because of their possible undesirable effects on human health (Jayaprakasha et al., 2003). Grapes are among the most widely consumed fruits in the world. They are rich in polyphenols, with approximately 75% of grape polyphenols existing in the seeds and skin. Grape skin phenols may be classified as cell-wall phenols, non-cell-wall phenols, and phenols associated with the cell nucleus (Pinelo et al., 2006). Functional ingredients of grape seeds include several flavonoids with a phenolic nature, dimeric, trimeric and polymeric procyanidins, and phenolic acids (Yilmaz & Toledo, 2004). 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 (Lurton, 2003). These high-quality polyphenolic compounds can be used in different therapeutic procedures with the purpose of free radical neutralization in biological systems (Heim et al., 2002; Yilmaz & Toledo, 2004). In addition, various anti-inflammatory and anti-platelet aggregating effects and other potentiall disease preventing cellular actions of wine phenolics have been amply documented (Damianaki et al., 2000).

Extraction is a very important stage in isolation, identification and use of phenolic compounds (Baydar et al., 2004; Lapornik et al., 2005; Pekic et al., 1998). To describe extraction mechanism in the literature, Fick's second law of diffusion is usually used (Herodez et al., 2003). 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 (Spigno et al., 2007). Ultrasound, the term used to describe sound waves ranging from 20 kHz to 1 GHz, is usually generated by a transducer which converts mechanical or electrical energy into high frequency vibrations. The enhancement of extraction efficiency using ultrasound is attributed to a phenomenon called cavitation produced in the solvent by the passage of an ultrasonic wave which allows higher penetration of the solvent into the raw plant materials (Sun & Wang, 2008). The objective of our study was to optimize the ultrasonic-assisted extraction conditions of ethanol concentration, extraction temperature and extraction time for antiradical activity of grape peel and seed extracts.

Materials and Methods

Ultrasonic-assisted extraction

Campbell Early grapes purchased from local farmer were

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authenticated as pure cultivar and grapes were excised from the stems and washed. Grapes were manually cut into halves and the grape peels and seeds were separated with a knife. Grape peel and seed were oven dried at 50°C until moisture level was constant. Dried grape peel and seed were ground to a powdered form using an electrical grinder. A sample of 2 g powdered grape peel or grape seed was kept in a glass flask and volume was made 100 mL with extraction solvent (33-67% ethanol). Contents were dissolved by using a magnetic stirrer (KMC 130SH, Vision Scientific Co., Ltd., Daegu, Korea) for 5 min. Extraction was carried out in a sonication water bath (JAC Ultrasonic 2010P, Jinwoo Engineering Co., Ltd., Hwasung, Korea) set a specified temperatures (23-57°C for grape peel and 33-67°C for grape seed) for different time durations (11-29 min for grape peel and 16-34 min for grape seed). The extract was later filtered through filter paper No. 5A and analyzed for the antiradical activity.

Determination of antiradical activity

The free radical activity of the extract was determined by using 1, 1-diphenyl-2-picrylhydrazyl or DPPH (Lee et al., 1998). Briefly, 1 mL solution of the juice extract at a concentration of 100 μL/mL methanol was mixed with 2 mL of 10 mg/L methanolic solution DPPH (Sigma Chemical Co., St. Louis, MO, USA). The mixture was shaken vigorously and allowed to stand at room temperature for 5 min and absorbance was recorded at 517 nm by using a spectrophotometer (TU-1800, Human Corporation, Seoul, Korea). Lower absorbance of the sample indicated the higher free radical scavenging activity. The antiradical activity was expressed as percentage.

Statistical analysis

Response surface methodology (RSM) was used to find optimal ultrasound-assisted extraction conditions from the peel and seed of ‘Campbell Early’ grapes. The extraction experiments were carried out according to central composite designs with 3 factors at 5 levels in each case. Three independent variables selected were concentration of solvent, extraction temperature and extraction time. The effect of each experimental set was observed on the dependent variable which was the antiradical component. The complete experimental design consisted 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 for both extractions. Regression analysis was performed on the data of dependent

Table 1. Experimental design of five-level, three-variable central composite design and total phenols, antioxidant activities and anthocyanins of ultrasonic-assisted grape peel extracts

Test set	Grape peel				Grape seed			
	Extraction conditions			Antiradical activity (%)[a]	Extraction conditions			Antiradical activity (%)[a]
	X_1	X_2	X_3		X_1	X_2	X_3	
1	40	30	15	43.84	40	40	20	74.66
2	40	30	25	48.64	40	40	30	80.99
3	40	50	15	47.24	40	60	20	78.65
4	40	50	25	52.14	40	60	30	86.81
5	60	30	15	48.44	60	40	20	80.65
6	60	30	25	50.34	60	40	30	83.82
7	60	50	15	48.34	60	60	20	80.49
8	60	50	25	53.94	60	60	30	88.15
9	33	40	20	46.94	33	50	25	78.16
10	67	40	20	52.34	67	50	25	87.15
11	50	23	20	46.24	50	33	25	76.99
12	50	57	20	52.04	50	67	25	86.65
13	50	40	11	42.54	50	50	16	70.83
14	50	40	29	53.04	50	50	34	88.31
15	50	40	20	53.54	50	50	25	87.48
16	50	40	20	52.74	50	50	25	87.81
17	50	40	20	53.24	50	50	25	88.64
18	50	40	20	53.64	50	50	25	87.65

$X_{1;}$ Ethanol concentration (%)
$X_{2;}$ Extraction temperature (°C)
$X_{3;}$ Extraction time (min)
[a] Analytical results are means±SD (n=3).

variables obtained by triplicate observations as effected by the extraction conditions. The second regression model of three independent variables and one dependent variable or response was as follows:

$$Y=b_0+b_1X_1+b_2X_2+b_3X_3+b_{12}X_1X_2+b_{13}X_1X_3+ b_{23}X_2X_3+b_{11}X_1^2+b_{22}X_2^2+b_{33}X_3^2 \quad (1)$$

In this model Y is the effective response i.e. the antiradical activity to be extracted and X_1, X_2 and X_3 represent the independent variables of ethanol concentration, extraction temperature and extraction time respectively, b_0 the intercept and b_n shows the regression coefficients. All the analyses were carried out in triplicates and the experimental results obtained were expressed as means±SD. Statistical analyses were performed by using Statistical Analysis System (SAS, version 9.1). Analysis of variance was performed by ANOVA procedure. Mean values were considered significantly different when $P<0.05$. The optimal extraction conditions were estimated through 3-dimensional response surface analysis of three independent and each dependent variable.

Results and Discussion

Modeling of the extraction process from grape peel and seed

To optimize the extraction process with reference to extraction of antiradical components from grape peel and seed under sonication two central composite designs were developed as represented in Table 1. Table 1 also presents the experimental values for the antiradical activities of grape peel and seed extracts at various experimental conditions. The results of analysis of variance, goodness of fit and the

Table 2. Regression coefficients and analysis of the model for antiradical activities of grape peel and seed extracts

Coefficient	Coefficients estimated	
	Grape peel extract	Grape seed extract
b_0	-54.903903[b]	-118.905231[b]
b_1	1.630959[a]	2.519985[b]
b_2	1.281995[b]	2.206388[b]
b_3	3.051869[a]	5.469986[b]
b_{11}	-0.012176[a]	-0.017393[b]
b_{22}	-0.013906[a]	-0.020274[b]
b_{33}	-0.066456[a]	-0.100376[a]
b_{12}	-0.004250	-0.007076
b_{13}	-0.005500	-0.009157
b_{23}	0.009500	0.015818
Probability of *F* value	<0.0001	0.0001

[a] Significant at P < 0.001 and, [b] means P < 0.01

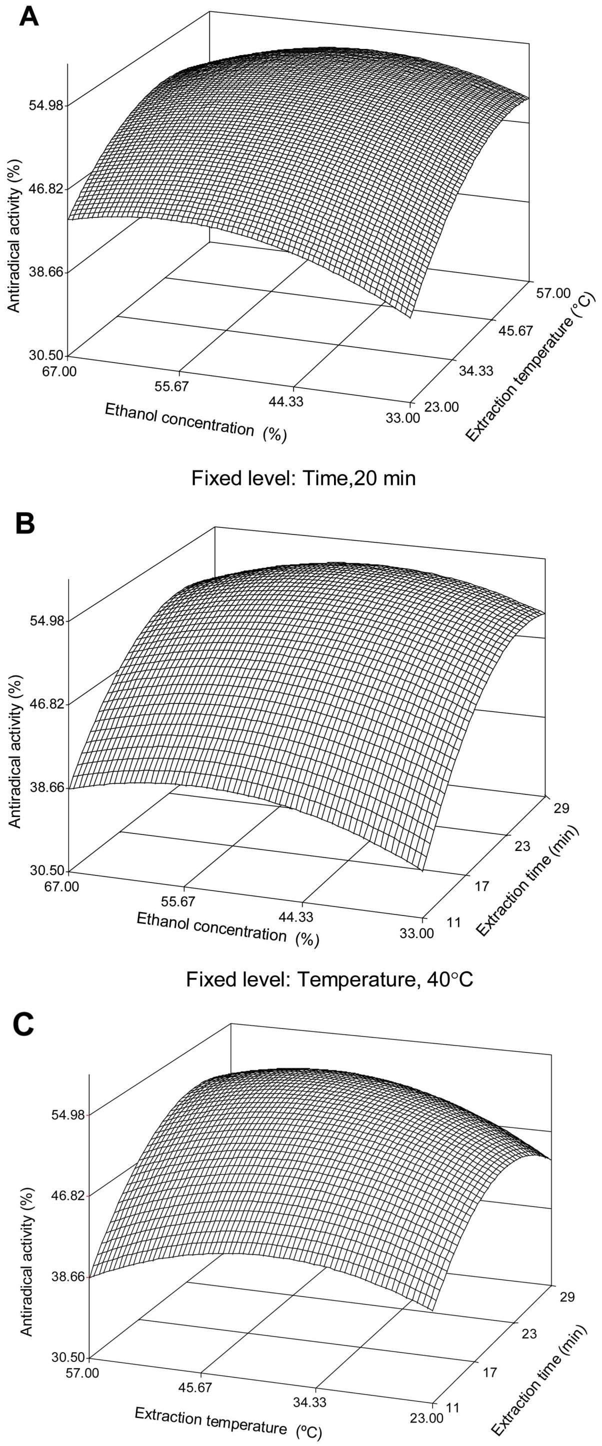

Fig. 1. The response surface plots of the antiradical activity of grape peel extract as affected by ethanol concentration, temperature and extraction time in ultrasonic-assisted extraction. Where (A) is ethanol concentration and temperature (time 20 min), (B) is ethanol concentration and time (temperature 40°C), and (C) is temperature and time (ethanol concentration 50%).

adequacy of the models are summarized in Table 2. The data showed a good fit with the equation 1 which were statistically acceptable at P<0.05 and adequate with satisfactory R^2 values. The full model filled Eq. 1 was made three dimensional and contour plots to predict the relationships between independent variables and the dependent variable.

Effects of process variables on the grape peel antiradical activity

Use of ultrasonication process was due to the fact that ultrasonic waves break the cells of vegetal matrix and the cell contents are released into the extraction medium (Vinatoru et al., 1997). Statistical analysis revealed that independent variables have highly significant effect (P<0.001) on the antiradical activities of grape peel extracts (Table 2). The coefficients of regression as presented in Table 2 were fitted into Eq. 1 to find out the effects of process parameters on the antiradical activity of ultrasonically assisted grape peel extract as follows:

$$Y = -54.903903+1.630959X_1+1.281995X_2+3.051869X_3 -0.012176X_1^2-0.01390X_2^2-0.066456X_3^2 \quad (2)$$

3-dimensional response surface plot were developed as represented in Fig. 1. All the extraction parameters showed signification effects (P<0.05) on the antiradical activity of extracts. A plot of ethanol concentration and extraction temperature (Fig. 1A) shows that the antiradical activity of the extract was higher at higher ethanol concentration. Plotting ethanol concentration against extraction time (Fig. 2B) shows a significant increase of antiradical activity with increasing extraction temperature and a similar effect of extraction time was observed when plotted against extraction temperature (Fig. 1C) as the antiradical activity increased with increasing either time or temperature.

Effects of process variables on the grape seed antiradical activity

The mean experimental data showing the extraction of antiradical components from grape seed at various extraction conditions are presented in Table 1. Statistical analysis revealed that most relevant variable with P<0.001 was extraction time. The extraction time has this kind of effect on the antiradical activity because increasing the contact time of the solvent with solids may improve the diffusion of the compounds (Corrales et al., 2009). The results of multiple regression analysis showed that the antiradical activities of grape seed were significantly ($p < 0.05$) affected by the linear

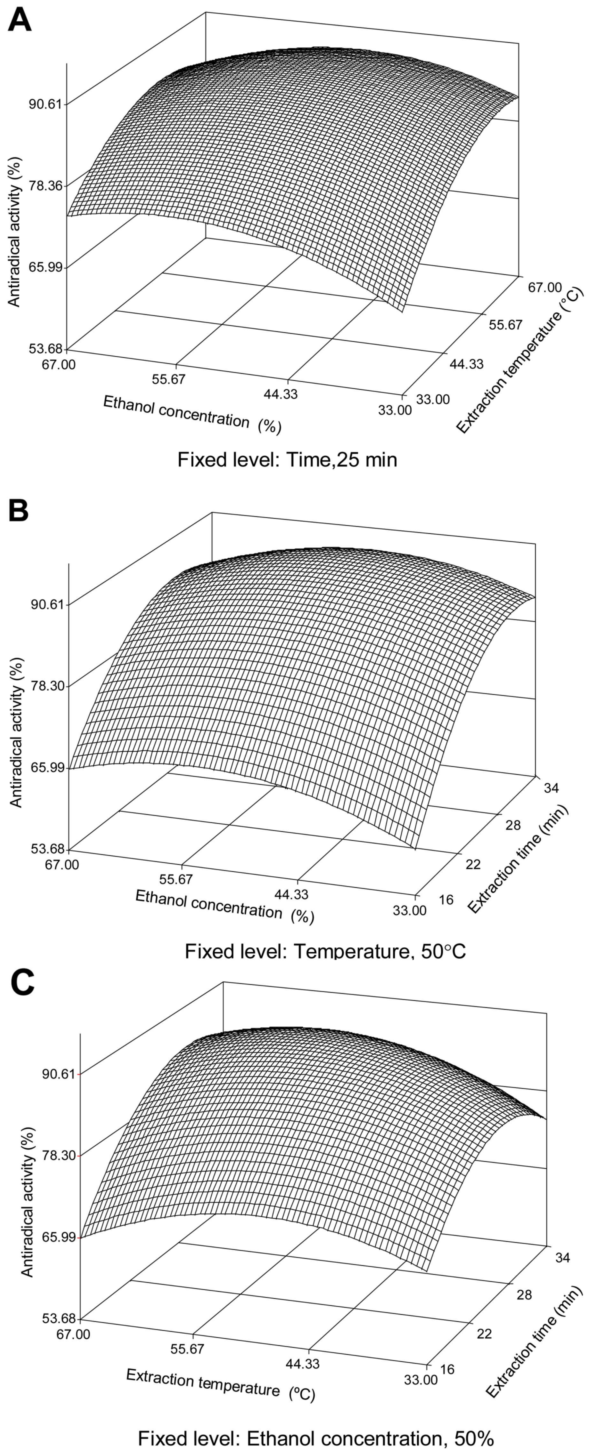

Fig. 2. The response surface plots of the antiradical activity of grape seed extract as affected by ethanol concentration, temperature and extraction time in ultrasound-assisted extraction. Where (A) is ethanol concentration and temperature (time 25 min), (B) is ethanol concentration and time (temperature 50°C), and (C) is temperature and time (ethanol concentration 50%).

Table 3. Estimated optimum conditions, predicted and experimental values of antiradical activity under these conditions

Sample	R^2	*F*-value	*P*-value	Optimum extraction conditions			Maximum antiradical value (%)	
				Ethanol (%)	Temp (°C)	Time (min)	Estimated	Experimental [a]
Grape peel extract	0.9740	33.30	<.0001	53.45	45.99	23.97	54.98	54.86±0.45
Grape seed extract	0.9591	20.85	0.0001	53.14	56.03	29.03	90.60	90.65±0.30

[a] Means± standard deviation (n=3).

and quadratic terms of ethanol concentration, extraction temperature and extraction time (Table 2). The final predictive equation for antioxidant activity of grape seed extract by using significant terms is as follows:

$$Y= -118.905231+2.519985X_1+2.206388X_2+5.469986X_3 -0.017393X_1^2-0.020274X_2^2-0.100376X_3^2 \quad (3)$$

Based on the above equation 3-dimensional surface plots were created to find the effect of independent variables on the antiradical activities of grape seed extracts (Fig. 2). Antiradical activities of grape seed extracts 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 (Fig. 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 Fig. 2B. Fig. 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. We have observed that the extract obtained from grape seed showed higher potential for free radical scavenging as compared with the grape peel. Antiradical activity of grape seed has significant correlation with the total polyphenol content of grape seed extracts as reported earlier (Guendez et al., 2005).

Optimum extraction conditions for maximum antiradical activity in grape peel and seed extracts

The estimated levels of extraction conditions for maximum response of antiradical activities from ultrasonic-assisted grape peel and seed extracts are presented in Table 3. The predicted ultrasonic-assisted extraction conditions were 53.45% ethanol concentration, 45.99°C extraction temperature and 23.93 min extraction time for the maximum antiradical activity of grape peel extract (54.98%), 53.14%, 56.03°C and 29.03 min for maximum antiradical activity of grape seed extract (90.60%). To compare the predicted results with experimental values, experimental rechecking was performed for antiradical activity of each of grape peel and seed using the optimum extraction conditions. Mean values of 54.86% and 90.65% antiradical activities of grape peel and grape seed respectively validated the RSM model. The good correlation between these results confirmed that the response models were adequate in reflecting the expected optimization (Table 3).

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