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Optimization of supercritical fluid extraction of bioactive compounds from grape (*Vitis labrusca* B.) peel by using response surface methodology

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ABSTRACT

Supercritical fluid extraction (SFE) was applied for the extraction of valuable compounds from grape (*Vitis labrusca* B.) peel. Extraction was carried out according to an orthogonal array design (OAD) and independent variables selected were temperature, pressure and modifier concentration. SFE process was optimized by using response surface methodology (RSM) for the extract yield, total phenols, antioxidants and total anthocyanins from grape peel. Effects of extraction temperature and pressure were found to be significant on all responses. Optimal SFE conditions were identified as 45–46 °C temperature, 160–165 kg cm^{-2} pressure and 6–7% ethanol as modifier for maximum extract yield (12.31%), total phenols (2.156 mg GAE/100 mL), antioxidants (1.628 mg/mL) and total anthocyanins (1.176 mg/mL). Experimental values for response variables at these optimal conditions match well with the predicted values. Grape peel extracts obtained by SFE showed more than 93% DPPH radical scavenging activities.

Industrial relevance: This study describes the response surface optimization of supercritical fluid extraction (SFE) process for the enhanced recovery of total phenols, antioxidant and anthocyanins from grape peel. SFE uses CO_2 as supercritical fluid which is environment friendly solvent; allows extraction at lower temperature and the extracts obtained possess higher quality and safety. Industrially, it may be used as a promising technique for the extraction of bioactive compounds from plant materials.

1. Introduction

Grapes are among the most widely consumed fruits. 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, which are bound to polysaccharides by hydrophobic interactions and hydrogen bonds; non-cell-wall phenols, encompassing phenols confined in the vacuoles of plant cells and phenols associated with the cell nucleus (Pinelo, Arnous, & Meyer, 2006). Phenolic compounds can be used in different therapeutic procedures with the purpose of free radical neutralization in biological systems (Heim, Tagliaferro, & Bobilya, 2002; Yilmaz & Toledo, 2004) and inhibition of oxidation of human low-density lipoproteins (Meyer, Yi, Pearson, Waterhouse, & Frankel, 1997). Proanthocyanidins are major polyphenols in the grape skin and include condensed cyanidine-3-glucosides, malvidin-3-glucosides, and peonidine-3-glucosides (Koeppen & Basson, 1966). Grapes anthocyanins possess strong biological functions such as anti-inflammatory and antioxidant activities (Kong, Chia, Goh, Chia, & Brouillard, 2003; Vatai et al., 2008) Positive physiological effects associated with the consumption of grape and grape derivatives are believed to be mainly due to the antiradical and antioxidant properties of phenolic constituents (Lurton, 2003).

Extraction of functional components from plant materials is an important process and various techniques have been studied in this regard (Spigno, Tramelli, & De-Faveri, 2008). Extraction is a major step in the isolation, identification and use of phenolic compounds (Stevigny, Rolle, Valentini, & Zeppa, 2007). Extensive studies are being carried out for the development of extraction processes which are novel and applicable to a variety of bioactive compounds. Supercritical fluid extraction (SFE) with supercritical CO_2 has been used for the extraction from natural products and researchers have paid considerable attention towards various aspects of this process (Lu et al., 2007). Supercritical CO_2 is an inert, non-toxic, environmentally safe solvent and allows extraction at lower temperatures and relatively low pressures. The extracts obtained by SFE are of superior quality as compared to those obtained by conventional organic solvent extraction methods (Friedrich & List, 1982; Gomez, Lopez, & La-Ossa, 1996). SFE extracts are also generally recognized as safe (GRAS) to be used in food products, therefore it may serve as a very promising technology in food processing (King, 2000).

The aim of our study was to optimize SFE variables such as temperature, pressure and concentration of ethanol as modifier for the maximum extract yield, total phenolic compounds, antioxidants

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and total anthocyanins from Campbell Early grape (*Vitis labrusca* B.) peel by using orthogonal array design and response surface methodology.

2. Materials and methods

2.1. Materials

Freshly harvested ripened grapes were purchased from a local farm in Gyeongbuk province of Korea and the grape cultivar was identified as 'Campbell Early'. Grapes were excised from the stems and washed. Peels were manually removed from grape berries and oven dried at 40 °C until the moisture level was constant (5.5% w/w). Dried grape peels were ground to a powdered form using an electrical grinder and passed through a 0.5 mm sieve. All the chemicals used were of analytical grade and they were purchased from Sigma Chemical Co. (St. Louis, MO) and Duksan Pure Chemical Co. (Ansan, Korea).

2.2. Supercritical fluid extraction

Supercritical fluid extraction (SFE) system consisted of CO_2 cylinder, cool water circulator (VTR-620, Jeio Tech., Seoul, Korea), column thermostat (CO-1560, JASCO Corporation, Tokyo, Japan), solvent (CO_2 and modifier) pumps (PU-1580, JASCO), UV/VIS detector (UV-1575, JASCO) and back pressure regulator (880-81, JASCO). 3 g powdered sample of grape peel was kept in the extraction vessel and placed in the column thermostat set at a specific temperature. Pressure was adjusted at the back pressure regulator and solvent pumps. The flow rates for CO_2 and modifier were fixed at 2 mL/min. Once the set temperature and pressure (at solvent pumps and back pressure regulator) were achieved after turning on the injection valve and the system was in equilibrium, the extraction was carried out for 30 min in each experimental run. Extract was collected in a flask connected to the back pressure regulator and stored at −20 °C before further analysis for the extract yield and bioactive components. The percentage extract yield was measured by drying the liquid extract at 105 °C until constant weight of the dried grape peel extract was obtained.

2.3. Experimental design

Orthogonal array design (OAD) was used to arrange SFE experiments for the response surface optimization. OAD is a type of experimental design in which an orthogonal array is used to assign factors to a series of experimental combinations and results can be analyzed using a mathematical procedure (Evangelaras, Kolaiti, & Koukouvinos, 2006; Moore, McKay, & Campbell, 2006). Effects of SFE temperature, pressure and modifier concentration were investigated on the recovery of bioactives from grape peel. An L_{16} (4^5) orthogonal matrix with three factors, each factor containing four levels, was selected to arrange the experiments. Extraction temperatures were 37, 40, 43 and 46 °C; pressures were 140, 150, 160 and 170 kg cm^{-2} and modifiers were 5, 6, 7 and 8% ethanol. The levels for each process variables were selected from a series of preliminary trials without using particular experimental designs. Regression analysis was performed on the data obtained by triplicate analysis for each of the dependent variables. Response surface analysis was also applied on the data from orthogonal array design for modeling and prediction of optimum conditions of SFE for the extract yield, total phenols, antioxidants and total anthocyanins from grape peel.

2.4. Analysis for total phenolic compounds

The total phenolic compounds were analyzed using the Folin Ciocalteu method with some modifications (Singleton & Rossi, 1965). A 200 μL properly diluted extract or a standard solution of varying concentrations was mixed with 400 μL Folin Ciocalteu reagent. The deionized water was used for dilution and control. The solution was diluted to a total volume of 4.6 mL and 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). 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).

2.5. Determination of antioxidant activity

The antioxidant activity of the grape peel extracts was evaluated by the phosphomolybdenum complex method (Prieto, Pineda, & Aguilar, 1999). The assay is based on the reduction of Mo(VI) to Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH (Abdel-Hameed, 2009). In brief, 0.4 mL of sample solution (100 μL of grape peel extract dissolved in 1 mL of methanol) was combined with 4 mL of reagent solution containing 0.6 M sulphuric acid, 2 mM sodium phosphate and 4 mM ammonium molybdate. The blank solution contained 4 mL of reagent solution and the 1 mL of methanol. Test tubes were caped and placed in hot water for 90 min at 95 °C. After samples were cooled to room temperature, the absorbance was measured at 695 nm against blank. Antioxidant activity was expressed relative to that of ascorbic acid.

2.6. Analysis for total anthocyanins

Determination of total anthocyanins in grape peel extracts was based on the method described by Iland, Cynkar, Francis, Williams, & Coombe, (1996) with some modifications. In 1 mL of sample, 10 mL 50% ethanol was added and sample was centrifuged at 1800 g for 10 min. 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 solution. 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:

$$\text{Anthocyanins (mg / mL)} = \text{A} \times \text{Dilution factor} \times 1000 / \text{B}$$

2.7. Determination of antiradical activity

The free radical activity of the grape peel extract was determined by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) (Lee et al., 1998) Briefly, 1 mL solution of the peel extract at a concentration of 100 μL/mL methanol was mixed with 2 mL of 10 mg/L methanolic solution of DPPH. The mixture was shaken vigorously and allowed to stand at room temperature for 5 min and absorbance was recorded at 517 nm by using spectrophotometer. Lower absorbance of the sample indicated the higher free radical scavenging activity. The antiradical activity of the grape peel extract was expressed in percentage.

2.8. Statistical analysis

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

Table 1
Orthogonal array design for supercritical fluid extraction from grape peel and the yield, total phenol, antioxidants and anthocyanin contents of the extracts.

Test set	Supercritical fluid extraction conditions			Analytical results[a]			
	Temperature (°C)	Pressure ($kgcm^{-2}$)	Modifier (% Ethanol)	Extract yield (%)	Total phenols (mg GAE/100 mL)	Antioxidant activity (mg/mL)	Total anthocyanin (mg/mL)
1	37	140	5	6.78 ± 0.74	0.887 ± 0.082	0.476 ± 0.028	0.520 ± 0.028
2	37	150	6	6.89 ± 0.25	0.952 ± 0.091	0.518 ± 0.072	0.580 ± 0.083
3	37	160	7	7.56 ± 0.12	1.265 ± 0.028	0.576 ± 0.009	0.840 ± 0.058
4	37	170	8	8.58 ± 0.45	1.347 ± 0.123	0.725 ± 0.028	0.840 ± 0.054
5	40	140	6	7.29 ± 1.11	1.059 ± 0.072	0.619 ± 0.042	0.540 ± 0.071
6	40	150	5	7.89 ± 0.24	1.286 ± 0.082	0.805 ± 0.071	0.660 ± 0.093
7	40	160	8	8.27 ± 0.33	1.285 ± 0.127	0.825 ± 0.101	0.840 ± 0.073
8	40	170	7	10.78 ± 0.45	1.545 ± 0.214	0.893 ± 0.055	0.925 ± 0.039
9	43	140	7	9.56 ± 0.47	1.193 ± 0.141	0.824 ± 0.081	0.780 ± 0.085
10	43	150	8	10.11 ± 0.39	1.364 ± 0.082	1.115 ± 0.068	0.820 ± 0.077
11	43	160	5	11.55 ± 0.85	1.698 ± 0.082	1.318 ± 0.047	1.080 ± 0.028
12	43	170	6	12.33 ± 0.74	1.838 ± 0.026	1.520 ± 0.172	1.240 ± 0.062
13	46	140	8	10.56 ± 0.69	1.457 ± 0.117	1.245 ± 0.075	1.040 ± 0.033
14	46	150	7	11.33 ± 0.19	1.880 ± 0.092	1.565 ± 0.117	1.125 ± 0.118
15	46	160	6	11.89 ± 0.44	2.225 ± 0.076	1.569 ± 0.072	1.140 ± 0.027
16	46	170	5	13.22 ± 0.59	2.584 ± 0.051	1.878 ± 0.063	1.240 ± 0.076

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

3. Results and discussions

3.1. Modeling of the extraction process from grape peel

The orthogonal array design (OAD), to optimize the supercritical fluid extraction (SFE) of total phenols, antioxidants and total anthocyanins from grape peel, is represented in Table 1. The experimental values of extract yields, total phenols, antioxidant activities and total anthocyanins of grape peel extracts at various experimental conditions are also presented in Table 1. The results of analysis of variance, goodness of fit and the adequacy of the models are summarized in Table 2. The data showed a good fit with $p<0.05$ and adequate with satisfactory R^2 values. The model was used for the construction of three dimensional response surface plots to predict the relationships between independent variables and the dependent variables.

3.2. Effects of process variables on percent extract yield

The percent yields of grape peel extracts obtained by using SFE are presented in Table 1. The regression analysis of the data showed that the extract yield was significantly ($p<0.05$) affected by the extraction temperature and pressure. The relationship of the extract yield and that of extraction temperature and pressure is depicted in Fig. 1 and it was linear with R^2 value of 0.969. An increase in either of temperature and pressure, while the second variable remains constant, results in enhancement of the extract yield. The relationship between process variables and the extract yield is presented in Eq. (1).

$$Y_1 = 10.85033 - 0.93624X_1 - 0.43694X_2 - 1.44027X_3 + 0.00243X_1X_2 + 0.07413X_1X_3 - 0.00826X_2X_3 - 0.00674X_1^2 + 0.00226X_2^2 - 0.04188X_3^2 \quad (1)$$

where Y_1 is the extract yield (%) in grape peel extract, X_1 is the extraction temperature (°C), X_2 the extraction pressure ($kg\,cm^{-2}$) and X_3 the ethanol concentration (%). The equation was based on the data of regression coefficients as presented in Table 2.

Table 2
Regression coefficients and analysis of the model for four response variables.

Coefficient	Coefficients estimated			
	Extract yield	Total phenols	Antioxidants	Anthocyanins
b_0	10.85033	15.67945	8.96062	0.10865
b_1	0.93624	−0.75458[b]	−0.44713	−0.20203
b_2	−0.43694	0.043749	−0.00376	0.03523
b_3	−1.44027	−0.00002	−0.60867	0.04470
b_{11}	−0.00674	0.40256[a]	0.00439	0.00343
b_{22}	0.00226	0.00955	−0.00007	0.00007
b_{33}	−0.04188	−0.00002	0.01894	0.01149
b_{12}	0.00243	0.00123	0.00097	−0.00068
b_{13}	0.07413	−0.01866	0.00711	0.01049
b_{23}	−0.00826	0.00262	0.00027	−0.00218
Probability of F value	<0.001	<0.0001	<0.001	<0.01

[a] $p<0.01$, [b] $p<0.05$.

3.3. Effects of process variables on total phenolic compounds

Total phenolic contents of grape peel extracts obtained by SFE are shown in Table 1. Regression analysis was performed on the experimental data and the coefficients of model were evaluated for significance. The effect of extraction temperature was highly significant ($p<0.001$) on the extraction of phenolic compounds. The effect of CO_2 pressure was also significant ($p<0.05$) while that of ethanol was insignificant. This is because high pressure and temperature increase the solvating power of the CO_2 (Liu et al., 2009). The relationship between total phenols of grape peel extract and main variables is depicted in Fig. 2. Response surface analysis of data in Table 1 demonstrates that the relationship between total phenols and the operating parameters is both linear and quadratic with a good regression coefficient ($R^2 = 0.988$). Eq. (2) shows the relationship between temperature, pressure and ethanol concentration for the extraction of total phenolic compounds.

$$Y_2 = 15.67942 - 0.75458X_1 + 0.04375X_2 - 0.00002X_3 + 0.00123X_1X_2 - 0.01866X_1X_3 + 0.00262X_2X_3 + 0.40256X_1^2 + 0.00955X_2^2 - 0.00002X_3^2 \quad (2)$$

where (Y_2) represents total phenols in grape peel extract. The equation was based on the data of regression coefficients presented in Table 2. Although an increase in temperature favors extraction of phenols by enhancing both the solubility of solute and the diffusion coefficient, it cannot be increased indefinitely; since the denaturation of phenolic compounds may take place at temperatures above 50 °C (Pinelo et al., 2006). SFE allows the extraction of phenolic compounds at lower temperatures hence preserves the quality of the extract.

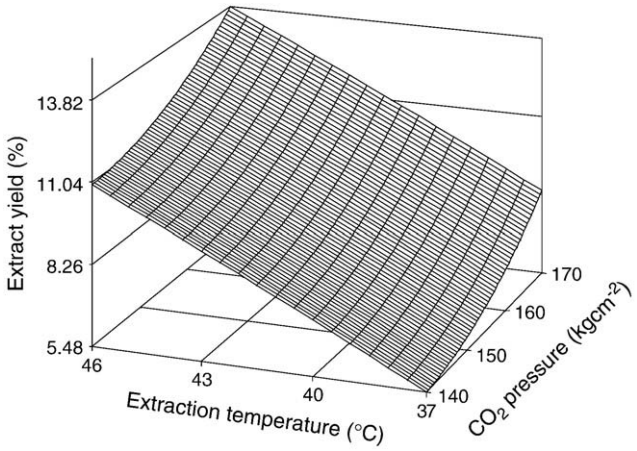

Fig. 1. Response surface analysis for the extract yields during supercritical fluid extraction from grape peel with respect to extraction temperatures and pressures.

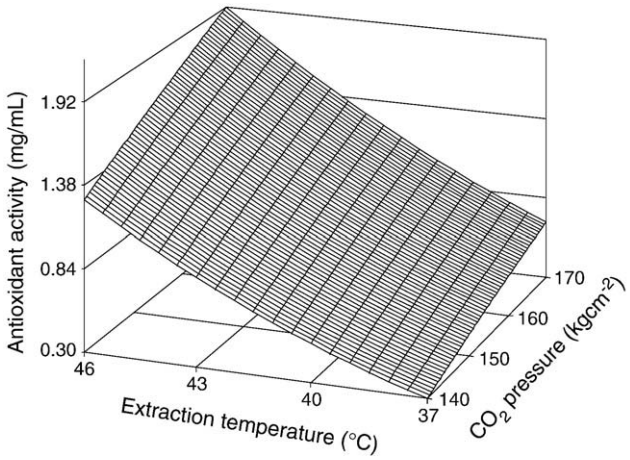

Fig. 3. Response surface analysis for the supercritical fluid extraction of antioxidants from grape peel with respect to extraction temperatures and pressures.

3.4. Effects of process variables on antioxidant compounds

The analytical results of antioxidant compounds in grape peel extracts obtained by SFE are shown in Table 1. The results of regression analysis reflect that the main extraction parameters for antioxidant compounds from grape peel were extraction temperature and pressure, former being highly significant ($p<0.001$). The effect of ethanol concentration was not significant. The relationship between the extraction of antioxidant components, temperature and pressure is depicted in Fig. 3. Antioxidant activities of the grape peel extracts were affected by the linear and quadratic terms of extraction process variables and R^2 value was 0.981. The relationship between extraction variable and antioxidant activities is represented in Eq. (3).

$$Y_3 = 8.96062 - 0.44713X_1 - 0.00376X_2 - 0.60867X_3 + 0.00097X_1X_2 + 0.00711X_1X_3 + 0.00027X_2X_3 + 0.00440X_1^2 - 0.00007X_2^2 + 0.01894X_3^2 \quad (3)$$

Y_3 represents antioxidant activity of the grape peel extract. It can be observed that there is a correlation between total phenols and antioxidant activities of extracts. Such linear correlations between phenolic compounds and antioxidant activities has been observed by various researchers (Cai, Luo, Sun, & Corke, 2004; Kumaran & Karunakaran, 2006).

3.5. Effects of process variables on anthocyanin contents

Total anthocyanin contents of the grape peel extracts are shown in Table 1. Extraction temperature and pressure had significant ($p<0.05$) effects on total anthocyanins and that of ethanol as modifier was not significant. Fig. 4 represents the relationship between total anthocyanin compounds and the extraction temperature and pressure in SFE from grape peel. Unlike total phenols and antioxidants, the effect of process variables on the total anthocyanins was only linear with R^2 value of 0.954. Based on the values of regression coefficients (Table 2), Eq. (4) was developed to present the relationship between total anthocyanins (Y_4) and extraction variables.

$$Y_4 = 0.10865 - 0.20203X_1 + 0.03523X_2 + 0.04470X_3 - 0.00068X_1X_2 + 0.010489X_1X_3 - 0.00218X_2X_3 + 0.00343X_1^2 + 0.00007X_2^2 + 0.01149X_3^2 \quad (4)$$

It has been reported that the extraction of anthocyanins from grapes is enhanced by increasing temperature (Vatai, Skerget, & Knez, 2009). The application of high pressure processing such as SFE not

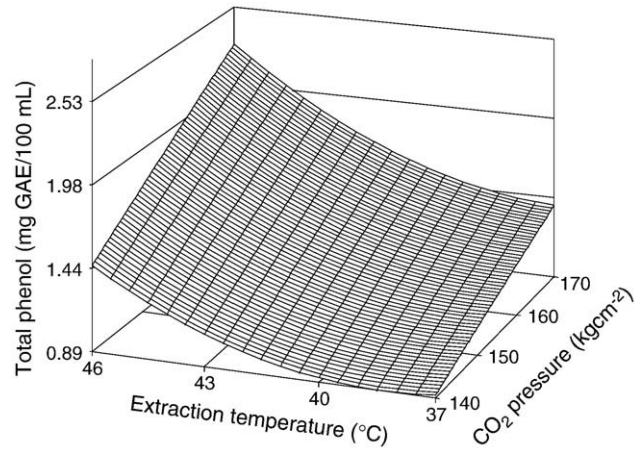

Fig. 2. Response surface analysis for the supercritical fluid extraction of total phenols from grape peel with respect to extraction temperatures and pressures.

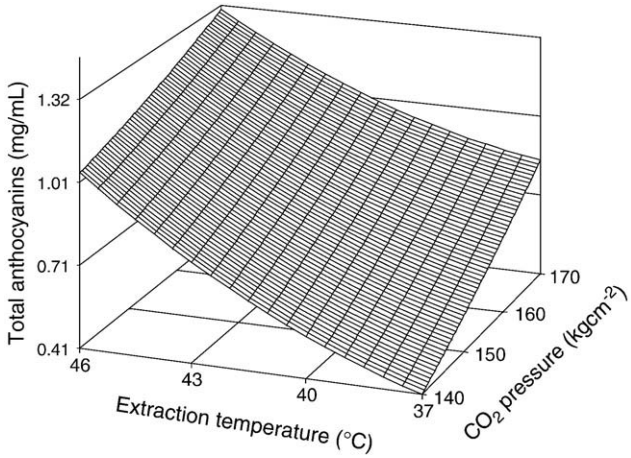

Fig. 4. Response surface analysis for the supercritical fluid extraction of total anthocyanins from grape peel with respect to extraction temperatures and pressures.

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

Response variables	R^2	R^2-adjusted value	F-value	p-value	Optimum SFE conditions			Maximum values	
					Temp (°C)	Pressure ($kgcm^{-2}$)	Ethanol (%)	Estimate	Actual[a]
Extract yield (%)	0.969	0.907	20.68	0.0008	44.31	166.70	6.44	12.31	12.25 ± 1.68
Total phenols (mg GAE/100 mL)	0.988	0.961	53.54	<.0001	45.66	160.1	6.25	2.156	2.215 ± 0.12
Antioxidant activities (mg/mL)	0.981	0.940	33.96	0.0002	45.64	160.8	6.52	1.628	1.652 ± 0.28
Total anthocyanins (mg/mL)	0.954	0.862	13.83	0.0023	45.23	163.3	6.58	1.176	1.172 ± 0.06

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

only increases the quantity of anthocyanins in the extract but also the color stability upon storage (Vatai et al., 2008).

3.6. Optimization of the extraction process

The optimum SFE conditions for the extract yields, total phenols, antioxidant activities and total anthocyanins from grape peel as obtained by using OAD and response surface methodology (RSM) are presented in the Table 3. SFE temperatures of 45–46 °C, extraction pressures in the range of 160–165 $kgcm^{-2}$ and lower concentrations (6–7%) of ethanol as modifier, can result in optimal extract yield (12.31%) total phenols (2.156 mg GAE/100 mL), antioxidant (1.628 mg/mL) and total anthocyanins (1.176 mg/mL) from grape peel. R^2 and R^2-adjusted values are also presented in Table 3. The predicted results matched well with the experimental results obtained using optimum extraction conditions which validated the RSM model with a good correlation. The prediction of one set of optimal conditions for four response variables was also done by using desirability function approach. A total desirability value of 0.87 was obtained on a scale of 0 to 1 where 0 represents a completely undesirable response and 1 represents the most desirable response. At this desirability, SFE from grape peel by using 44.83 °C temperature, 162.1 kg cm^{-2} of pressure and 6.03% ethanol as modifier can results in 12.37% extract yield, 2.161 mg GAE/100 mL total phenols, 1.66 mg/mL antioxidants and 1.27 mg/mL total anthocyanins.

3.7. Antiradical activities of the grape peel extracts

The antiradical activities of the grape peel extracts obtained by using SFE were also assessed using DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging assay. It is quick, reliable and reproducible method to search *in vitro* general antiradical activities of pure compounds as well as plant extracts (Koleva, Van-Beek, Linssen, De-Groot, & Evstatieva, 2002). This method depends on the reduction of purple DPPH to a yellow colored diphenyl picrylhydrazine and the remaining DPPH. The DPPH radical scavenging activities of 16 grape peel extracts, according to OAD as listed in Table 1, are shown in Fig. 5. All the extracts showed higher DPPH radical scavenging activities and the maximum activity (98%) was observed for the extract 8 obtained using SFE temperature of 40 °C and CO_2 pressure of 180 kg cm^{-2}. It has been reported that food materials rich in antioxidants with higher free radical scavenging abilities are protective against certain types of cancer and may also reduce the risk of cardiovascular and cerebrovascular disorders (Miraliakbari & Shahidi, 2008).

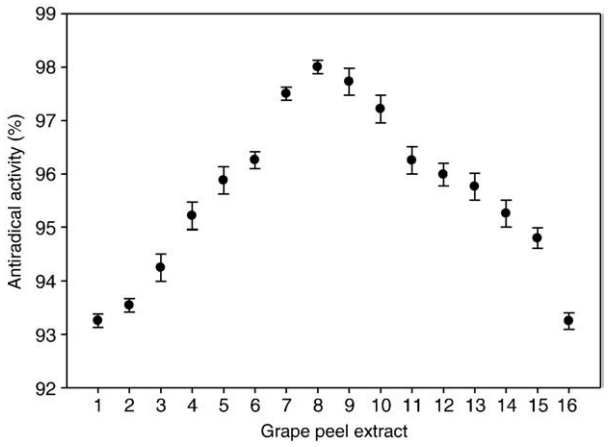

Fig. 5. Antiradical activities of grape peel extracts (1–16 according to OAD) obtained by supercritical fluid extraction. Bars represent standard error of the means ($n = 3$).

Research has been carried out for the optimal extraction of phenolic compounds using SFE and the modifier was 30% ethyl acetate or methanol (Palmal & Taylor, 1999). We used ethanol as modifier in low concentrations and observed that it has non-significant effects on SFE from grape peel. Extraction recovery in SFE is similar or better than solvent extraction methods and it depends on sample matrix, temperature, pressure, and modifier. Using an organic modifier is essential to obtain good recovery, but 10% modifier gives essentially the same recovery as 30% modifier (Seo, Burri, Quan, & Neidlinger, 2005). Modifiers increase the solubility of sample material and the extracted bioactives can be easily recovered from the extraction system. Ethanol is being used as modifier for SFE from plant materials and it is regarded as a safe alcohol for food applications (Ovando, Hernandez, Hernandez, Rodriguez, & Vidal, 2009). Studies have also been carried out on the extraction of oil from grape seed using SFE and parameters such as CO_2 pressure and enzyme treatment of grape seed were studied. The use of cell-wall degrading enzymes and higher pressures of up to 200 bar can significantly improve the extraction yield (Passos, Silva, Silva, Coimbra, & Silva, 2009). The internal mass transport parameters during SFE were also studied using solubility data dealing with extractor vessel with non-negligible void volume (Fiori, 2007). During the extraction of resveratrol from *Vitis inifera* grape peel using SFE, it was observed that the extraction yield was higher at 40 °C temperature, 150 bar pressure, 7.5% ethanol as modifier and 15 min extraction time (Pascual-Martí, Salvador, Chafer, & Berna, 2009). SFE extracts are safer as compared to those prepared by conventional extraction techniques due to the use of carbon dioxide as extraction solvent which is non-toxic and environmental friendly (Friedrich & List, 1982; King, 2000). We can infer that the main process variables of SFE are the extraction temperature and pressure. Grape and grape products have been extensively documented as healthy foods. Extracts obtained from grape peel and seed have been tested to possess anticancer and cancer chemopreventive properties during *in vivo* and *in vitro* studies (Kaur, Agarwal, & Agarwal, 2009). Phenolic compounds contained in these extracts are reported to have beneficial effects on other chronic diseases such as coronary heart disease (Forester & Waterhouse, 2009). These health effects are reported to be due to antiradical and antioxidant properties of phenolics in grapes and grape derivatives (Lurton, 2003).

4. Conclusions

SFE is an effective technology in the recovery of biologically valuable components from grape peel. Extraction process was

significantly affected by extraction temperature and pressure and the effect of modifier concentration was non-significant. This technique can be used as an alternative to conventional organic solvent extraction methods. SFE involves the application of high pressures; therefore extraction is carried out at lower temperatures, which preserve the quality of extracts. By optimizing SFE process variables, yields of total phenols, antioxidants and total anthocyanins can be significantly enhanced. This study also reveals that grape (*V. labrusca*) peel is a good source of phenolic compounds, antioxidants and total anthocyanins. The SFE extracts of grape peel also possess strong radical scavenging activities.

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