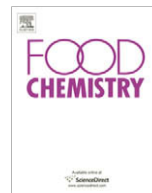
Food Chemistry 119 (2010) 343–348

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



Optimisation of antioxidant activity of grape cane extracts using response surface methodology

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ARTICLE INFO

Article history:
Received 16 April 2008
Received in revised form 4 June 2009
Accepted 17 June 2009

Keywords:
Antioxidant activity
TEAC
$ORAC_{FL}$
Total phenolics
Resveratrol
Bioactives
Extraction
Vitis vinifera
Pinot noir

ABSTRACT

Solid–liquid extraction and response surface methodology were used to optimise conditions for the antioxidant activity of grape cane extracts. The independent processing variables were ethanol concentration, temperature and solvent to solid ratio. Ethanol concentration and temperature significantly affected antioxidant activity measured by the Trolox equivalent antioxidant capacity (TEAC) assay and the oxygen radical absorbance capacity using fluorescein ($ORAC_{FL}$) method ($p \leqslant 0.01$), whereas the solvent to solid ratio did not significantly affect the activity ($p > 0.05$). Antioxidant activity of the extracts, determined by the TEAC assay, varied from 85.6 to 238.6 μmol Trolox equivalents/g of dry sample. $ORAC_{FL}$ values ranged from 308.4 to 1302.7 μmol Trolox equivalents/g of dry sample. Ethanol concentrations of 40.4% and 55.4% were optimal for the highest antioxidant activities measured by the TEAC assay and the $ORAC_{FL}$ method, respectively. The optimal temperature was 83.6 °C. Antioxidant activity correlates with total phenolic content of extracts.

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1. Introduction

Many studies have addressed the possible preventive effects of antioxidants in disease causation and progression. Most published results indicate that diseases including cancer, coronary heart diseases, and aging are associated with oxidative stress caused by an imbalance between oxidants and antioxidants in the body (Ames, 1979; Aruoma, 1998; Baublis, Clydesdale, & Decker, 2000; Halliwell, 1996; Halliwell & Gutteridge, 2007). Food antioxidants have been shown to play an important role in the body's defense system against reactive oxygen species (Aruoma, 1998; Gutteridge & Halliwell, 2000; Halliwell & Gutteridge, 2007; Ou, Huang, Hampsch-Woodill, Flanagan, & Deemer, 2002).

Concurrently, concerns about the safety and adverse effects of synthetic antioxidants in foods have led to increased interest in novel natural sources of antioxidants that could replace synthetic products or find applications as dietary supplements, nutraceuticals, functional food ingredients, food additives, pharmaceuticals and cosmetic products (Balasundram, Sundram, & Samman, 2006; Claudio & Hector, 2000; Goli, Barzegar, & Sahari, 2005; Shahidi & Wanasundara, 1992; Wang, Yang, & Zhang, 2007). To this end, many sources of natural antioxidants have been investigated, including plants and microorganisms (Arai et al., 2002; Bandoniene, Pukalskas, Venskutomis, & Gruzdiene, 2000).

Grape canes (*Vitis vinifera* L.) have been shown to be a rich source of bioactive compounds, including *trans*-resveratrol, *trans*-ε-viniferin and ferulic acid (Karacabey & Mazza, 2008; King, Bomser, & Min, 2006; Püssa, Floren, Kuldkepp, & Raal, 2006, Rayne, Karacabey, & Mazza, 2008) and previously we have shown (Karacabey & Mazza, 2008; Rayne, Karacabey, & Mazza, 2008) that the combination of a 50–70% ethanol/water mixture with a practical temperature (84 °C) is optimal for the extraction of *trans*-resveratrol and *trans*-ε-viniferin from milled grape canes.

This study focuses on the antioxidant activity of milled grape cane extracts and its correlation with the composition of extracts. Antioxidant activity of these and other phenolics has gained increasing importance (Belguendouz, Fremont, & Linard, 1997; Cacace & Mazza, 2003; Castelluccio et al., 1995; Kinsella, Frankel, German, & Kanner, 1993; Lucas-Abellán et al., 2008; Mazza, 2007; Shahidi, McDonald, Chandrasekara, & Zhong, 2008; Shahidi, 2008) and the relationship between antioxidant activity and the structure of phenolic compounds has been described (Burda & Oleszek, 2001; Caruso, Tanski, Villegas-Estrada, & Rossi, 2004;

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doi:10.1016/j.foodchem.2009.06.029

Fukumoto & Mazza, 2000; Rice-Evans, Miller, & Paganga, 1996). Several authors, including Fukumoto and Mazza (2000), Kähkönen, Hopia, and Heinonen (2001), and Moyer, Hummer, Finn, Frei, and Wrolstad (2002), have reported that the antioxidant activity of berries is due primarily to phenolic compounds. In addition, extraction conditions such as extraction temperature, extraction time, solvent composition, and solvent to solid ratio have been found to affect the yields of extracts rich in phenolic compounds (Cacace & Mazza, 2002, 2003; Karacabey & Mazza, 2008; Liyana-Pathirana & Shahidi, 2005).

Several methods have been used to determine antioxidant activity in vitro. The oxygen radical absorbance capacity using fluorescein as the fluorescent probe ($ORAC_{FL}$) and the Trolox equivalent antioxidant capacity assay (TEAC) have been widely used methods to determine the antioxidant capacity of biological samples and foods (Lucas-Abellán et al., 2008; Yoo, Kim, & Lee, 2007). The $ORAC_{FL}$ assay directly measures the hydrophilic chain-breaking antioxidant capacity against the peroxyl radical, which is the most common of the radicals found in the human body (Ou, Hampsch-Woodill, & Prior, 2001; Wang et al., 2004). The TEAC assay is based on the relative ability of hydrogen or electron donation antioxidants to scavenge the ABTS radical cation compared with that of Trolox (Miller, Rice-Evans, Davies, Gopinathan, & Milner, 1993).

Response surface methodology (RSM) is a useful technique for developing, improving, and optimising processes (Cacace & Mazza, 2003; Karacabey & Mazza, 2008; Liyana-Pathirana & Shahidi, 2005; Myers & Montgomery, 2002); thus, the objective of this study was to optimise the antioxidant activity of milled grape cane extracts by solid–liquid extraction using response surface methodology. The correlation between the antioxidant activity of extracts and their total phenolic content was also determined.

2. Materials and methods

2.1. Preparation of sample

Cane samples from the Pinot noir red wine grape variety of *Vitis vinifera* were collected from a private vineyard near Penticton, British Columbia, Canada in February 2007 and freeze-dried. Dried grape canes were ground in a Wiley mill (Thomas®–Wiley® Mill Model ED-5, Arthur H. Thomas Co., Philadelphia, PA, USA), using a 1 mm mesh size screen with 1 mm gap between blades, and then stored in sealed plastic bags in humidity-controlled storage at room temperature until extraction. The average particle diameter (d_p) (255.5 μm) of the milled grape cane was calculated by sieve analysis (Karacabey & Mazza, 2008).

2.2. Extraction

Aqueous ethanol, a safe and efficient solvent for the extraction of bioactives in grape canes (Rayne et al., 2008), was used. Milled dry grape cane samples were dispersed in 2.5 L of aqueous ethanol solvent in an agitated 4 L glass beaker. An airfoil axial impeller with a 63.5 mm diameter (Lightnin model A 310, Mixing Equipment Co. Inc., Rochester, NY, USA) was used for mixing. Mixing conditions were kept constant to avoid a possible effect on the extraction process. The extraction tank was placed in a thermostatic water bath set at the desired temperature and was continuously monitored during the extraction process. The beaker was covered to prevent solvent loss during the extraction. The volume measurements of solvent indicated no significant solvent loss after the extraction was completed. Grape cane samples were mixed with solvents at the desired temperature when the turbulent flow regime was established. Liquid samples were periodically taken from the extractor to determine the equilibrium in terms of mass transfer. Extractions were ended when the extracts and pomace reached an equilibrium of phenolic compounds as indicated by no further change of absorbance readings of liquid samples at 280 and 320 nm, which are the absorbance maximums of total phenolic and stilbene compounds (resveratrol and viniferin), respectively.

2.3. Analysis

To determine free and total phenolics, all extracts were filtered through a 0.2 μm PVDF membrane disc held in a 13 mm diameter syringe filter holder (Chromatographic Specialties Inc., Brockville, ON, Canada) and stored at 4 °C until analysed. LC-DAD analysis was carried out using a high performance liquid chromatograph system (Agilent 1100 series, Agilent Technologies Inc., Palo Alto, CA) equipped with a photodiode array detector, an autosampler, and a control module. Samples of 5 μL were injected onto a reversed-phase C_{18} column (Zorbax SB, 5 μm, 250 × 4.6 mm, ID Agilent Technologies Inc.) preceded by a guard column (5 ODS, 5 μm, 30 × 4.6 mm, ID Phenomenex, Torrance, CA, USA). A gradient solvent system was used with solvent A being phosphoric acid (50 mM) and solvent B being HPLC grade methanol. The elution profile had the following proportions (*v*/*v*) of solvent B: 0 min, 5%; 0–5 min, 5%; 5–51 min, 5–55%; 51–61 min, 55–100%; 61–68 min, 100%; 68–73 min, 100–5%; and 73–83 min, 5%. The solvent flow rate was 0.4 mL/min.

Concentration of total phenolics was calculated using total area of peaks at 280 nm and expressed as an equivalent of *trans*-resveratrol using standard curve of *trans*-resveratrol (Sigma–Aldrich; Oakville, ON, Canada).

2.4. Antioxidant activity

The antioxidant activity of the extracts was measured using the Trolox equivalent antioxidant capacity assay (TEAC) and the oxygen radical absorbance capacity using fluorescein as the fluorescent probe ($ORAC_{FL}$) assay. Both assays have been widely used to determine the antioxidant activity of biological materials and foods. These methods were selected because they are based on different principles, and because they are widely used. The $ORAC_{FL}$ assay directly measures hydrophilic chain-breaking antioxidant capacity against the peroxyl radical which is the most common of the radicals found in the human body. The TEAC assay is based on the inhibition by antioxidants of the absorbance of the radical cation of ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid). These two methods are believed to be adequate to assess the antioxidant capacity of grape cane extracts. The TEAC method was modified from the procedure of Rice-Evans, Miller, and Paganga (1997) and Pellegrini, Re, Yang, and Rice-Evans (1999) for use with microplates (Fukumoto & Mazza, 2000). Briefly, a mixture of 5 mL of 7 mM ABTS (prepared with MilliQ-water) and 88 μL of 140 mM potassium persulphate ($K_2O_8S_2$) (prepared with MilliQ-water) was prepared. This concentrated ABTS reagent was stored in the dark for 12–16 h. On the day of the analysis, 50 μL of Trolox standard (0–50 mg/L) or diluted extract sample (from 0 to 6.79 g/L) was added in triplicate into wells of 96 well-microplates. To each well containing standard or sample, 250 μL of diluted ABTS solution (the mixture of 0.51 mL of concentrated ABTS reagent and 30 mL of 80% MeOH/MilliQ-water) were added to each well of microplate and the plate was read after 5 min using a Spectramax 384 Plus microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 734 nm. A calibration curve was prepared with different concentrations of Trolox calculating reduction of absorbance after 5 min (A734 nm at Conc. 0–A734 nm at Conc. X) corresponding to the Trolox concentration (X). The same procedure was applied for samples at different concentrations. The antioxidant activity

was determined relative to Trolox by dividing the slope of the sample to the slope of standard curve of Trolox. Slopes were calculated for the linear portion of graphs of absorbance versus concentration. The antioxidant activity was expressed as μmol of Trolox equivalents/g of dry sample.

The $ORAC_{FL}$ method was modified from the procedure of Ou et al. (2001) for use with microplates (Fukumoto & Mazza, 2000). Briefly, 87 μM fluorescein stock solution was prepared in 75 mM phosphate buffer at pH 7.4 and stored at 4 °C. On the day of the analysis, 20 μL of the stock solution was diluted to 25 mL with 75 mM phosphate buffer at pH 7.4 to make up 87 nM fluorescein solution and heated to 37 °C in a water bath. A 70 mM of AAPH (2,2′-azobis(2-methylpropionamidine) dihydrochloride) solution was prepared in 75 mM phosphate buffer as the peroxyl radical generator. This solution was also heated up to 37 °C. Trolox standard (0–50 mg/L) was diluted with 75 mM phosphate buffer at pH 7.4. Samples were also diluted with buffer at appropriate dilutions to fit within the standard curve. The wells of an opaque fluorescent plate were filled with 25 μL of sample and standard in triplicate at various concentrations. Wells around the outside edges of the plate were not used. To all wells, 200 μL of 87 nM fluorescein solution were added followed by the addition of 50 μL of 70 mM AAPH solution. The plate was immediately put into a Spectramax Gemini EM microplate fluorometer (Molecular Devices, Sunnyvale, CA, USA) and read. An excitation of 485 nm and emission of 530 nm with a cutoff filter of 515 nm was used and readings were taken every 2 min for 150 min. SoftMax Pro (version 5.3) was used to calculate the area under the curve. A standard curve for Trolox was made from these data. The Trolox equivalent for a sample was found using the standard curve. The mean of three replicates was used to calculate the $ORAC_{FL}$ value for each sample. The results obtained by the $ORAC_{FL}$ method were correlated with the results measured by the TEAC method. Correlation coefficients between antioxidant activities determined by both methods and total phenolics of extracts were determined using SAS v. 9.1.3 (2002–2003) (SAS Ins. Inc., Cary, NC, USA) statistical software.

2.5. Experimental design

Optimisation of extraction conditions for antioxidant activity of grape cane extract by the TEAC method (Z_1), and by the $ORAC_{FL}$ method (Z_2) were carried out using response surface methodology. The independent processing variables were temperature (X_1), solvent to solid ratio (X_2), and ethanol concentration (X_3). A central composite design was selected for optimisation of process variables each at 5 levels with 18 runs including four replicates at the central point. The range and levels of independent variables and code values are presented in Table 1.

Experimental data were analysed using Minitab (Minitab 15.1.0.0.) (Minitab Inc., State College, PA, USA) and SAS v. 9.1.3 (2002–2003) (SAS Ins. Inc., Cary, NC, USA) statistical software and fitted to a second-order polynomial regression model containing the coefficient of linear, quadratic, and two factors interaction effects. The model equation of response (Z) of the three independent variables (X_1, X_2, and X_3) is:

$$Z = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j$$

where Z is the dependent variable, β_0 is the constant coefficient, β_i is the linear coefficient (main effect), β_{ii} is the quadratic coefficient, and β_{ij} is the two factors interaction coefficient.

The response surface graphs of predicted values by models were plotted using Sigma Plot v. 8.02 (2002) (SPSS Inc., Chicago, IL, USA). The values of R^2, adjusted-R^2 of models were evaluated to check the model adequacies.

3. Results and discussion

Antioxidant activity of grape cane extracts determined by the TEAC and the $ORAC_{FL}$ methods varied from 85.6 to 238.6 μmol Trolox equivalents/g of dry sample and from 308.4 to 1302.7 μmol Trolox equivalents/g of dry sample, respectively (Table 1). The surface response analysis for antioxidant activity measured using the TEAC and $ORAC_{FL}$ methods showed that the main effects of ethanol concentration and temperature are statistically significant ($p \leqslant 0.01$ or $p \leqslant 0.001$), whereas solvent to solid ratio was not significant ($p > 0.05$) (Table 2). The significant contribution of ethanol concentration and temperature on total antioxidant activities of wheat and wheat bran has been reported by Liyana-Pathirana and Shahidi (2005). Surface response analysis for antioxidant activity of extracts determined by both methods indicated significant

Table 1
Three-factor, five levels central composite design used for RSM and experimental data of the investigated responses of grape cane extracts.

Standard order[a]	Run order[b]	Factor 1 (X_1) Temperature (°C)	Factor 2 (X_2) Solvent to solid ratio (mL/g)	Factor 3 (X_3) Ethanol concentration (%, v/v)	Response-I TEAC[c]	Response-II $ORAC_{FL}$[c]	Response-III Total phenolic content of extracts[d]
1	4	30 (−1)	50 (−1)	36 (−1)	89.6	407.4	3.80
2	15	70 (1)	50 (−1)	36 (−1)	190.3	1048.6	7.57
3	11	30 (−1)	90 (1)	36 (−1)	91.1	457.8	3.98
4	10	70 (1)	90 (1)	36 (−1)	217.7	999.0	7.97
5	1	30 (−1)	50 (−1)	80 (1)	131.5	1074.0	6.35
6	6	70 (1)	50 (−1)	80 (1)	171.4	1302.7	8.02
7	14	30 (−1)	90 (1)	80 (1)	142.5	963.0	6.16
8	2	70 (1)	90 (1)	80 (1)	184.0	1064.8	7.46
9	3	16.4 (−1.68)	70 (0)	58 (0)	97.5	733.5	5.12
10	9	83.6 (1.68)	70 (0)	58 (0)	238.6	1259.6	8.91
11	16	50 (0)	36.8 (−1.68)	58 (0)	139.0	836.0	6.93
12	5	50 (0)	103.6 (1.68)	58 (0)	147.4	817.8	7.16
13	7	50 (0)	70 (0)	21 (−1.68)	85.6	308.4	3.19
14	18	50 (0)	70 (0)	95 (1.68)	121.3	645.5	6.78
15	12	50 (0)	70 (0)	58 (0)	158.6	994.8	7.22
16	13	50 (0)	70 (0)	58 (0)	160.1	1054.4	7.24
17	17	50 (0)	70 (0)	58 (0)	153.1	906.9	7.29
18	8	50 (0)	70 (0)	58 (0)	146.8	837.6	7.06

[a] Not randomized.
[b] Randomized.
[c] Antioxidant activity expressed as equivalent of μmol Trolox/g of dry sample.
[d] Total phenolic content of grape cane extract as an equivalent of resveratrol (mg resveratrol/g dw).

Table 2
Regression coefficients of predicted models for the investigated responses of grape cane extracts and independent effects of factors.

Variable[a]	TEAC coefficient	$ORAC_{FL}$ coefficient
β_0	-154.731^{**b}	-1180.31^{*}
β_1	2.36^{*}	9.47^{ns}
β_2	$0.245^{ns,c}$	
β_3	5.927^{***}	50.23^{***}
β_{11}	0.02^{*}	0.13^{ns}
β_{22}		
β_{33}	-0.03^{***}	-0.27^{**}
β_{12}		
β_{13}	-0.041^{***}	-0.24^{*}
β_{23}		
Model	***	***
Linear	***	***
Quadratic	***	**
Cross-product	***	*
R^2	0.96	0.87
Adj-R^2	0.94	0.81
Main effects		
Temperature	***	**
Solvent to solid ratio	ns	ns
Ethanol concentration	**	**

[a] Polynomial model $Z = \beta_0 + \sum_{i=1}^{3}\beta_i X_i + \sum_{i=1}^{3}\beta_{ii}X_i^2 + \sum_{i=1}^{2}\sum_{j=i+1}^{3}\beta_{ij}X_iX_j$ adjusted by backward elimination at the level of 0.1% with the lack-of-fit test, where β_0 is the constant coefficient, β_i is the linear coefficient (main effect), β_{ii} is the quadratic coefficient, and β_{ij} is the two factors interaction coefficient.
[b] *, significant at $p \leqslant 0.05$; **, significant at $p \leqslant 0.01$; ***, significant at $p \leqslant 0.001$.
[c] ns, not significant ($p > 0.05$).

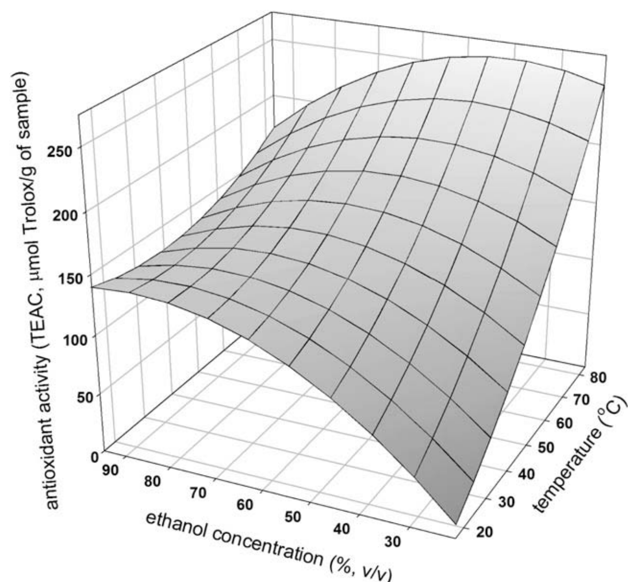

Fig. 1. Response surface of the effects of temperature and ethanol concentration at a constant solvent to solid ratio of 103.5 mL/g on the Trolox equivalent antioxidant capacity of grape cane extracts.

adequacies of the derived models at the 0.001% level of probability, and variability could be explained by the models (Table 2). Regression coefficient and analysis of variance of the adjusted polynomial second-order models for antioxidant activity of grape cane extracts are summarised in Table 2. As shown by an ANOVA of the regression parameters of the surface response analysis of the models, the linear, quadratic, and interaction terms have significant effects ($p \leqslant 0.001$, $p \leqslant 0.01$ or $p \leqslant 0.05$). Control of model parameters, R^2, Adj-R^2, confirmed the model adequacies (Table 2). The lack of fit test at the level of 0.05 indicates no evidence for lack of fit for both of the antioxidant activity models.

The linear increase in antioxidant activity (TEAC method) with increasing temperature was observed at low (<50%, v/v) and moderate (50–60%, v/v) ethanol concentrations, although the effect of temperature almost disappeared at relatively higher ethanol concentrations (>60%, v/v) (Fig. 1). The response surface displayed the curve effect of ethanol concentration (statistically significant second-order term in the model, $p \leqslant 0.05$, Table 2) on the antioxidant activity (TEAC method). The ethanol concentration effect trend changed when moving from 16.4 to 83.6 °C. The increase in antioxidant activity (TEAC) was observed with an increase in ethanol concentration (up to 95%) at lower temperatures. At the highest ethanol concentration (95%), the measured antioxidant activity displayed a small increase when the temperature was raised from 16.4 to 83.6 °C. The strong increase in activity was observed with decreasing ethanol content (up to 40%) at high temperature levels, and a further decrease of ethanol concentration of solvent composition resulted in lower activity (Fig. 1). The highest antioxidant activity of 260.8 μmol Trolox equivalents/g of dry sample (TEAC) was predicted with 40.4% of ethanol–water mixture at 83.6 °C by the fitted model (Table 2), when the solvent to solid ratio was 103.5 mL/g.

Antioxidant activity ($ORAC_{FL}$ method) also increased linearly with increasing temperature level at moderate (50–60%, v/v) and lower (<50%, v/v) ethanol concentrations (Fig. 2). This strong effect of temperature decreased with ascending ethanol concentration up to 70%, and disappeared with a further increase in ethanol concentration (Fig. 2). A curve influence of ethanol concentration on the antioxidant activity ($ORAC_{FL}$ method) of grape cane extracts (Fig. 2) was associated with the significance of second-order term representing ethanol concentration in the fitted model ($p \leqslant 0.05$, Table 2). The antioxidant activity ($ORAC_{FL}$) increased with the increase of ethanol content up to moderate concentrations (50–60%) and decreased with further increase at higher temperatures (>60 °C). The ethanol concentration effect trend changed when moving from higher temperatures to lower ones and the optimum point to obtain higher antioxidant activity ($ORAC_{FL}$) shifted from moderate ethanol content (50–60%) to higher levels (95%) (Fig. 2). The extraction with the ethanol concentration of 55.4% at 83.6 °C resulted in the highest predicted antioxidant activity (1378.7 μmol Trolox equivalents/g of dry sample of grape cane) by $ORAC_{FL}$ irrespective of solvent to solid ratio.

Ethanol concentration and temperature of extraction displayed a similar curve effect and linear effect on the antioxidant activities of wheat and wheat bran extracts, respectively (Liyana-Pathirana & Shahidi, 2005). These results are in agreement with our results as can be seen from the response surfaces of the antioxidant activities of grape cane extracts measured by the TEAC and the $ORAC_{FL}$ methods (Figs. 1 and 2, respectively).

The results indicated that an increase in temperature led to an increase in antioxidant activity (TEAC and $ORAC_{FL}$ methods). This noticeable effect reflects the temperature effect on total phenolic content of the extracts (Table 1).

Ethanol concentration was the most significant extraction factor affecting the antioxidant activity of grape cane extract determined by the TEAC and ORAC methods. This effect may be attributed to the change of solvent polarity with change in ethanol concentration; however, the surface response analysis for antioxidant activities measured by both the TEAC and the $ORAC_{FL}$ methods, indicated that the interaction effect between ethanol concentration and temperature was also significant ($p \leqslant 0.001$ and $p \leqslant 0.05$, respectively) (Table 2). These results indicate that the optimum ethanol concentrations were altered depending on the temperature of the extraction process. At low temperatures, the ethanol concentration of 95% (ethanol/water, v/v) was the optimal, whereas it shifted to 40% and 55% ethanol content (v/v) for TEAC and $ORAC_{FL}$, respectively, at the highest temperature of 83.6 °C (Figs. 1 and 2, respectively). This is likely due to a difference in the composition of the extract which is affected by solvents and

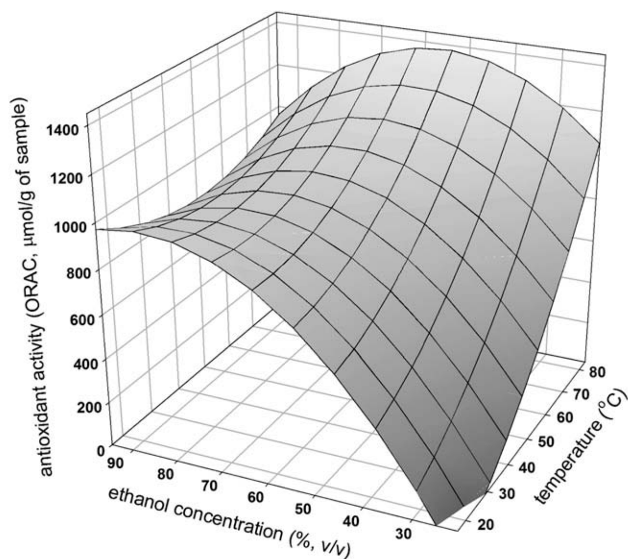

Fig. 2. Response surface of the effects of temperature and ethanol concentration on the oxygen radical absorbance capacity using fluorescein of grape cane extracts.

process temperature. It has been reported that the polarity of solvent used in extraction directly affects not only the quantity of total phenolics, but also the composition and potency of phenolics (Yu, Ahmedna, & Goktepe, 2005). *Trans*-resveratrol, *trans*-ε-viniferin and ferulic acid are the major phenolic compounds of grape cane (Karacabey & Mazza, 2008). Antioxidant capacity of phenolic compounds is determined by the structure of polyphenols (Cao, Sofic, & Prior, 1997; Fukumoto & Mazza, 2000; Lien, Ren, Bui, & Wang, 1999). Differences in the composition of phenolic compounds in extracts due to the use of different solvent compositions may result in the change of the antioxidant capacity of that extract. Solvent dependency of antioxidant capacity may be attributed to structural differences of extracted phenolics. As a result, the composition of the investigated polyphenols, resveratrol and viniferin, varying with ethanol concentration may have a significant effect on the antioxidant activities of extracts.

The antioxidant activity values measured by two methods, TEAC and $ORAC_{FL}$ were positively correlated with total phenolics ($p \leqslant 0.001$, $r = 0.95$ and $p \leqslant 0.001$, $r = 0.89$, respectively). Correlations indicated the strong association between phenolic content and the antioxidant activities of grape cane extracts. Yu et al. (2005) have presented a similar correlation between total antioxidant activity and total phenolic content of peanut skin extracts obtained with water and ethanol. The high correlation between antioxidant activity and total phenolic content of bud and leaf extracts of black currant was also reported by Tabart et al. (2007).

4. Conclusions

The effects of ethanol concentration, temperature, and solvent to solid ratio on antioxidant activity of grape cane extracts were determined by response surface methodology. The maximum predicted activities of 260.8 and 1378.7 μmol Trolox equivalents/g of dry sample measured by the TEAC and $ORAC_{FL}$ methods were obtained with 40.4% and 55.4% of ethanol concentrations, respectively, at the temperature of 84 °C).

Acknowledgements

The authors thank the Agricultural Bioproducts Innovation Program (ABIP) of Agriculture and Agri-Food Canada and the Canadian Biomass Innovation Network program (CBIN) for financial support. Technical assistance provided by Lana Fukumoto, Kim Peddle and David Godfrey is greatly appreciated. Erkan Karacabey thanks Prof. A. Levent Bayındırlı, Prof. Nevzat Artık, and Prof. Sedat Velioğlu for valuable encouragement and support. Erkan Karacabey's research at Agriculture and Agri-Food Canada was supported by T.R. Prime Ministry State Planning Organization (BAP-08-11-DPT2002K120510-GT-4).

References

Ames, B. N. (1979). Identifying environmental chemicals causing mutations and cancer. *Science, 204*, 587–593.

Arai, S., Morinaga, Y., Yoshikawa, T., Ichiishi, E., Kiso, Y., Yamazaki, M., et al. (2002). Recent trends in functional foods science and industry in Japan. *Bioscience, Biotechnology, and Biochemistry, 66*, 2017–2029.

Aruoma, O. I. (1998). Free radicals, oxidative stress, and antioxidants in human health and disease. *Journal of the American Oil Chemists Society, 75*, 199–212.

Balasundram, N., Sundram, K., & Samman, S. (2006). Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chemistry, 99*, 191–203.

Bandoniene, D., Pukalskas, A., Venskutomis, P. R., & Gruzdiene, D. (2000). Preliminary screening of antioxidants of some plants extracts in rapeseed oil. *Food Research International, 23*, 785–791.

Baublis, A. J., Clydesdale, E. M., & Decker, E. A. (2000). Antioxidants in wheat-based breakfast cereals. *Cereal Foods World, 45*, 71–74.

Belguendouz, L., Fremont, L., & Linard, A. (1997). Resveratrol inhibits metal ion-dependent and independent peroxidation of porcine low density lipoproteins. *Biochemical Pharmacology, 53*, 1347–1355.

Burda, S., & Oleszek, W. (2001). Antioxidant and antiradical activities of flavonoids. *Journal of Agricultural and Food Chemistry, 49*, 2774–2779.

Cacace, J. E., & Mazza, G. (2002). Extraction of anthocyanins and other phenolics from black currants with sulfured water. *Journal of Agricultural and Food Chemistry, 50*, 5939–5946.

Cacace, J. E., & Mazza, G. (2003). Optimization of extraction of anthocyanins from black currants with aqueous ethanol. *Journal of Food Science, 68*, 240–248.

Cao, G., Sofic, E., & Prior, R. L. (1997). Antioxidant and prooxidant behavior of flavonoids: Structure-activity relationship. *Free Radical Biology and Medicine, 22*, 749–760.

Caruso, F., Tanski, J., Villegas-Estrada, A., & Rossi, M. (2004). Structural basis for antioxidant activity of *trans*-resveratrol: Ab initio calculations and crystal and molecular structure. *Journal of Agricultural and Food Chemistry, 52*, 7279–7285.

Castelluccio, C., Paganga, G., Melikian, N., Bolwell, G. P., Pridham, J., Sampson, J., et al. (1995). Antioxidant potential of intermediates in phenylpropanoid metabolism in higher plants. *FEBS Letters, 368*, 188–192.

Claudio, C., & Hector, F. (2000). Synthetic antioxidants determination in lard and vegetable oils by the use of voltammetric methods on disk ultramicroelectrode. *Food Research International, 33*, 357–365.

Fukumoto, L. R., & Mazza, G. (2000). Assessing antioxidant and prooxidant activity of phenolic compounds. *Journal of Agricultural and Food Chemistry, 48*, 3597–3604.

Goli, A. H., Barzegar, M., & Sahari, M. A. (2005). Antioxidant activity and total phenolic compounds of pistachio (Pistachia vera) hull extracts. *Food Chemistry, 92*, 521–525.

Gutteridge, J. M. C., & Halliwell, B. (2000). Free radicals and antioxidants in the year 2000 – A historical look to the future. *Annals of the New York Academy of Sciences, 899*, 136–147.

Halliwell, B. (1996). Oxidative stress, nutrition and health. Experimental strategies for optimization of nutritional antioxidant intake in humans. *Free Radical Research, 25*, 57–74.

Halliwell, B., & Gutteridge, J. M. C. (2007). *Free Radicals in Biology and Medicine* (4th ed.). Oxford, UK: Clarendon Press.

Kähkönen, M. P., Hopia, A. I., & Heinonen, M. (2001). Berry phenolics and their antioxidant activity. *Journal of Agricultural and Food Chemistry, 49*, 4076–4082.

Karacabey, E., & Mazza, G. (2008). Optimization of solid–liquid extraction of resveratrol and other phenolic compounds from milled grape canes (*Vitis vinifera*). *Journal of Agricultural and Food Chemistry, 56*, 6318–6325.

King, R. E., Bomser, J. A., & Min, D. B. (2006). Bioactivity of resveratrol. *Comprehensive Food Science and Food Safety, 5*, 65–70.

Kinsella, J. E., Frankel, E., German, B., & Kanner, J. (1993). Possible mechanisms for the protective role of antioxidants in wine and plant foods. *Food Technology, 47*, 85–89.

Lien, E. J., Ren, S., Bui, H., & Wang, R. (1999). Quantitative structure-activity relationship analysis of phenolic antioxidants. *Free Radical Biology and Medicine, 26*, 285–294.

Liyana-Pathirana, C., & Shahidi, F. (2005). Optimization of extraction of phenolic compounds from wheat using response surface methodology. *Food Chemistry, 93*, 47–56.

Lucas-Abellán, C., Mercaderros, M. T., Zafrilla, M. P., Fortea, M. I., Gabaldón, J. A., & Núñez-Delicado, E. (2008). Orac-fluorescein assay to determine the oxygen radical absorbance capacity of resveratrol complexed in cyclodextrins. *Journal of Agricultural and Food Chemistry, 56*, 2254–2259.

Mazza, G. (2007). *Anthocyanins and heart health. Annali dell'Istituto Superiore di Sanita 43*, 369–374.

Miller, N. J., Rice-Evans, C., Davies, M. J., Gopinathan, V., & Milner, A. (1993). A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clinical Science, 84*, 407–412.

Moyer, R. A., Hummer, K. E., Finn, C. E., Frei, B., & Wrolstad, R. E. (2002). Anthocyanins, phenolics and antioxidant capacity in diverse small fruits: Vaccinium, rubus, and ribes. *Journal of Agricultural and Food Chemistry, 50*, 519–525.

Myers, R. H., & Montgomery, D. C. (2002). *Response surface methodology: Process and product optimization using designed experiments*. New York: Wiley.

Ou, B., Hampsch-Woodill, M., & Prior, R. L. (2001). Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of Agricultural and Food Chemistry, 49*, 4619–4626.

Ou, B., Huang, D., Hampsch-Woodill, M., Flanagan, J., & Deemer, E. K. (2002). Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: A comparative study. *Journal of Agricultural and Food Chemistry, 50*, 3122–3128.

Pellegrini, N., Re, R., Yang, M., & Rice-Evans, C. (1999). Screening of dietary carotenoids and carotenoid-rich fruits extract for antioxidant activities applying 2,2′-azinobis (3-ethylenebenzothiazoline-6-sulfonic acid) radical cation decolorization assay. *Methods in Enzymology, 299*, 379–389.

Püssa, T., Floren, J., Kuldkepp, P., & Raal, A. (2006). Survey of grapevine *Vitis vinifera* stem polyphenols by liquid chromatography-diode array detection-tandem mass spectrometry. *Journal of Agricultural and Food Chemistry, 54*, 7488–7494.

Rayne, S., Karacabey, E., & Mazza, G. (2008). Grape cane waste as a source of *trans*-resveratrol and *trans*-viniferin: High-value phytochemicals with medicinal and anti-phytopathogenic applications. *Industrial Crops and Products, 27*, 335–340.

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolics acids. *Free Radical Biology and Medicine, 20*, 933–956.

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science, 2*, 152–159.

Shahidi, F. (in press). Nutraceuticals and functional foods: Whole versus processed foods. *Trends in Food Science and Technology*, 1–12, doi:10.1016/j.tifs.2008.08.004.

Shahidi, F., McDonald, J., Chandrasekara, A., & Zhong, Y. (2008). Phytochemicals of foods, beverages and fruit vinegars: Chemistry and health effects. *Asia Pacific Journal of Clinical Nutrition, 17*, 380–382.

Shahidi, F., & Wanasundara, P. K. J. P. D. (1992). Phenolic antioxidants. *Critical Reviews in Food Science and Nutrition, 32*, 67–103.

Tabart, J., Kevers, C., Sipel, A., Pincemail, J., Defraigne, J. O., & Dommes, J. (2007). Optimisation of extraction of phenolics and antioxidants from black currant leaves and buds and of stability during storage. *Food Chemistry, 105*, 1268–1275.

Wang, C. C., Chu, C. Y., Chu, K. O., Choy, K. W., Khaw, K. S., Rogers, M. S., et al. (2004). Trolox-equivalent antioxidant capacity assay versus oxygen radical absorbance capacity assay in plasma. *Clinical Chemistry, 50*(5), 952–954.

Wang, K. J., Yang, C. R., & Zhang, Y. J. (2007). Phenolic antioxidants from Chinese toon (fresh young leaves and shoots of *Toona sinensis*). *Food Chemistry, 101*, 365–371.

Yoo, K. M., Kim, D. O., & Lee, C. Y. (2007). Evaluation of different methods of antioxidant measurement. *Food Science and Biotechnology, 16*, 177–182.

Yu, J., Ahmedna, M., & Goktepe, I. (2005). Effects of processing methods and extraction solvents on concentration and antioxidant activity of peanut skin phenolics. *Food Chemistry, 90*, 199–206.