Optimization of Solid−Liquid Extraction of Resveratrol and Other Phenolic Compounds from Milled Grape Canes (*Vitis vinifera*)

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Optimization of the solid−liquid extraction conditions for *trans*-resveratrol, *trans*-ϵ-viniferin, ferulic acid, and total phenolics from milled grape canes has been investigated. The temperature and ethanol concentration were found to be major process variables for all responses, whereas the solvent to solid ratio was found not to be significant for any of the responses studied. The yields of *trans*-resveratrol, *trans*-ϵ-viniferin, and total phenolics increased with increasing temperature. Maximum yields of *trans*-resveratrol (4.25 mg/g dw), *trans*-ϵ-viniferin (2.03 mg/g), and total phenolics (9.28 mg/g dw) were predicted from the combination of a moderate ethanol concentration (50−70%) and the highest temperature (83.6 °C), whereas an ethanol concentration of 35% at the lowest temperature studied (16.4 °C) was optimal for the extraction of ferulic acid (1.05 mg/g dw). Effective diffusivity values of resveratrol in the solid phase, D_{eff} for different extraction conditions, were calculated by fitting the experimental results to a model derived from the Fick's second law. Effective diffusivity of resveratrol in the solid phase varied from 3.1×10^{-13} to 26.6×10^{-13} m^2 s^{-1} with changing extraction conditions. The increase in effective diffusivity of resveratrol was observed with increasing temperature, and the highest predicted level was obtained when using 54% ethanol/water mixture at 83.6 °C. The increase in ethanol concentration exhibited the favorable effect up to 50−55%, thereafter effective diffusivity decreased with a further increase in concentration.

KEYWORDS: Solid−liquid extraction; stilbene compounds; phenolics; bioactives; ferulic acid; nutraceuticals; effective diffusivity; mass transfer; *Vitis vinifera*; Pinot noir

INTRODUCTION

Grape canes (*Vitis vinifera*), a byproduct of the wine industry, may have considerable potential as a source of bioactive compounds, especially resveratrol and other phenolic compounds (*1*). Resveratrol (*trans*-3-5-4′-trihydroxy stilbene) is a polyphenol demonstrated to elicit a broad spectrum of biological effects including antioxidant capacity, cardioprotection, anticancer activity, anti-inflammatory effects, and estrogenic/antiestrogenic properties (*2–5*). Likewise, *trans*-ϵ-viniferin, a dimer of *trans*-resveratrol, has been demonstrated to be toxic to fungal parasites (*6*) and has been found to have inhibitory effects on human cytochrome P450 enzymes (*7*) and antioxidant activity in aqueous and nonaqueous mediums (*8*). Ferulic acid (4-hydroxy-3-methoxycinnamic acid), another abundant phenolic compound in grape canes, has received much attention in the study of Chinese medicine since it was found to be one of the effective components in Chinese medicinal herbs such as *Angelica sinensis*, *Cimicifuga heracleifolia*, and *Lignsticum chuangxiong* (*9*). The reported physiological functions of ferulic acid and its derivatives include antioxidant activity, cholesterol-lowering activity, antimicrobial and anti-inflammatory activity, and anticancer effects (*10–13*). Thus, the extraction and purification of bioactive compounds from natural sources have become important for the utilization of phytochemicals in the preparation of dietary supplements or nutraceuticals, functional food ingredients, and additives to food, pharmaceutical, and cosmetic products (*14*).

Many factors such as extraction temperature, extraction time, solvent composition, and solvent to solid ratio can influence the extraction process (*14–17*). Also, different variables can impact the process differently, and there may be interactions of analyzed factors. Thus, statistical analysis is often used to optimize the extraction of bioactives. Response surface meth-

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odology (RSM), which enables evaluation of the effects of independent process variables and their interactions on dependent variables, is a package of statistical and mathematical techniques employed for developing, improving, and optimizing processes (*18*).

The interaction between the solute-containing particle and the solvent during the extraction process includes a series of phenomenological steps. During the extraction of plant components, diffusion mass transfer of a solute in the solid phase is usually the rate-controlling step (*19, 20*). Thus, the modeling of effective diffusivity in this step is essential to explain this phenomenon in mathematical terms.

The objective of this study was to optimize the solid−liquid extraction of the major phenolics present in grape canes by RSM. Mass transfer of *trans*-resveratrol from milled grape cane particles by solid−liquid extraction in an agitated vessel was also studied, and effective diffusivities in the solid phase were determined.

MATERIALS AND METHODS

Preparation of Sample. Grape cane samples of the *V. viniferia* variety Pinot noir (one of the most renowned red grapes in the world) were collected from a private vineyard, near Penticton, B.C., Canada, in February, 2007, and dried in a freeze drier. Dried grape canes were ground in a Wiley mill (Thomas−Wiley Mill model ED-5, Arthur H. Thomas Co., Philadelphia, PA), using a screen having a 1 mm mesh size and 1 mm gap between blades, and then stored in sealed plastic bags in a humidity-controlled storage room at 23−25 °C until extraction. The average particle diameter (d_p) (255.5 μm) of milled grape cane was calculated by sieve analysis (*15*).

Extraction. Ground samples were extracted in an agitated 4 L glass beaker of 15.3 cm i.d. with 2.5 L of solvent for optimization experiment. Ethanol/water mixtures (v/v) at different concentrations were used as solvents. An airfoil axial impeller (Lightnin model A 310, Mixing Equipment Co. Inc., Rochester, NY) with a 6.35 cm diameter was used for mixing. The extraction tank was set in a thermostatic water bath set at the desired temperature. A cap was attached on the top of the beaker to avoid solvent loss, condensing the vaporized solvent during the extraction. Shape factors were adjusted as reported by Cacace and Mazza (*16*) to ensure uniform mixing and to minimize their effect on mass transfer. Grape cane samples were mixed with solvents after desired temperature and flow regime were built up. 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 equilibrium, indicated by no further change of absorbance readings of liquid samples at 280 and 320 nm.

Mixing Conditions. The axial impeller was located at a distance of $d_t/4$ above the tank bottom and at a 15° angle in the tank without baffles. The mixing rate was adjusted in the range of 1150−1750 rpm for dispersion of particles in solvents and for rapid initial mixing of liquid reactants (*21*). The rotational speed, N, was fixed at 1250 rpm to ensure that the flow was in the turbulent regime (Re > 10^4), avoiding variation of the effect of the rotational speed on mass transfer of a given compound.

The solvent height in the tank was kept constant at 14 cm so that the vessel solvent−height to diameter ratio was 0.92. The geometry of the vessel and impeller, shape factors, rotational speed, and location of shaft and impeller were fixed to avoid variation in the mixing system for all extractions carried in this study.

As a reference method, solvent extraction using an ethanol/water 80:20 (v/v) solution was carried out for 30 min at 60 °C with gentle stirring (*22*). The solvent to solid ratio was 125 mL/g.

Analysis. For the determination of free phenolics and total phenolics, all extracts were filtered through a 0.2 μm PVDF membrane disk held in 13 mm diameter syringe filter holders (Chromatographic Specialties Inc., Brockville, Ontario, Canada) and stored in a refrigerator at 4 °C until their analysis. Liquid chromatography-diode array detection analysis was carried out using a liquid chromatograph system (Agilent 1100 series, Agilent Technologies Inc., Palo Alto, CA) equipped with

Table 1. Three-Factor, Five-Level Central Composite Design Used for RSM

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)
1	4	30 (−1)	50 (−1)	36 (−1)
2	15	70 (1)	50 (−1)	36 (−1)
3	11	30 (−1)	90 (1)	36 (−1)
4	10	70 (1)	90 (1)	36 (−1)
5	1	30 (−1)	50 (−1)	80 (1)
6	6	70 (1)	50 (−1)	80 (1)
7	14	30 (−1)	90 (1)	80 (1)
8	2	70 (1)	90 (1)	80 (1)
9	3	16.4 (−1.68)	70 (0)	58 (0)
10	9	83.6 (1.68)	70 (0)	58 (0)
11	16	50 (0)	36.8 (−1.68)	58 (0)
12	5	50 (0)	103.6 (1.68)	58 (0)
13	7	50 (0)	70 (0)	21 (−1.68)
14	18	50 (0)	70 (0)	95 (1.68)
15	12	50 (0)	70 (0)	58 (0)
16	13	50 (0)	70 (0)	58 (0)
17	17	50 (0)	70 (0)	58 (0)
18	8	50 (0)	70 (0)	58 (0)

[a] Not randomized. [b] Randomized.

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 mm × 4.6 mm, ID, Agilent Technologies Inc.) preceded by a guard column (Inertsil 5 ODS, 5 μm, 30 × 4.6 mm, ID Phenomenex, Torrance, CA). A gradient solvent system was used with solvent A being phosphoric acid (50 mM) and solvent B being pure methanol at high-performance liquid chromatography (HPLC) grade. 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.

trans-Resveratrol (detection at 320 nm) and ferulic acid (detection at 320 nm) were analyzed qualitatively by comparing their retention times and UV spectras with authentic standards (Sigma-Aldrich; Oakville, ON, Canada). Concentrations of resveratrol and ferulic acid were calculated using their peak areas and standard curves. ϵ-Viniferin (detection at 320 nm) was identified by comparing its UV spectra to those reported in the literature (*23*). ϵ-Viniferin was quantified by assuming an identical molar extinction coefficient with *trans*-resveratrol, as described previously (*24*). The concentration of total phenolics was calculated using the total area of peaks at 280 nm and expressed as an equivalent of *trans*-resveratrol.

Experimental Design. Optimization of extraction conditions for yields of *trans*-resveratrol (Z_1), *trans*-ϵ-viniferin (Z_2), ferulic acid (Z_3), total phenolics (Z_4) and effective diffusivity of *trans*-resveratrol (Z_5) were carried out using RSM. The independent variables were temperature (X_1), solvent to solid ratio (X_2), and ethanol concentration (X_3). A central composite design was selected for the optimization of process variables each at five levels with 18 runs including four replicates at the central point. The range and levels of independent variables and coded values are presented in **Table 1**.

Experimental data were analyzed using Minitab (Minitab 15.1.0.0.) and SAS v. 9.1.3 (2002−2003) (SAS Ins. Inc., NC) 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

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

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). The
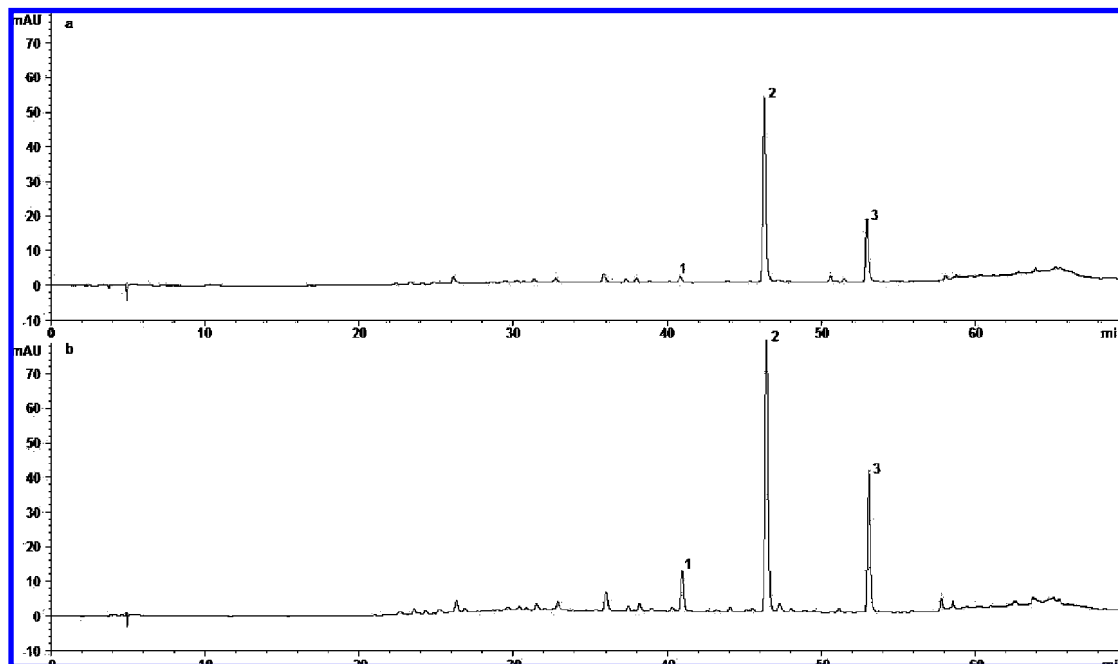
Figure 1. HPLC chromatograms (280 nm) of extracts obtained by a reference method (*21*) (**a**) and by the solid−liquid extraction method carried out using 58% ethanol/water (v/v) solution (70 mL/g) at 50 °C (**b**). Phenolic compounds **1**, **2**, and **3** are ferulic acid, *trans*-resveratrol, and *trans*-ε-viniferin, respectively.

values of R^2, adjusted-R^2, predicted-R^2, and prediction error sum of squares (PRESS) of models were evaluated to check the model adequacies (*18*).

Mathematical Considerations. Convection, molecular diffusion, and eddy diffusion are the different mechanisms involved in solute transfer in solid−liquid extraction. However, generally, it is diffusion due to random molecular motion in a solid that dominates the extraction (*20*). Convection and eddy diffusion are fast as compared to molecular diffusion, and in the leaching of foods, diffusion in the solid is usually the rate-controlling step (*19, 20*). The extent of control is indicated by the Biot number defined by Schwartzberg and Chao (*19*):

$$Bi = \frac{k_c ma}{D_{eff}} \quad (2)$$

where k_c is the external mass transfer coefficient in the extract, a is the characteristic dimension of the solid, D_{eff} is the diffusivity in the solid, and m is the partition coefficients for a solvent, and it was calculated using the following equation for each extraction trial:

$$m = \frac{y_e}{x_{dm}} \quad (3)$$

where y_e is weight fraction of a given compound in the liquid phase at equilibrium, whereas x_{dm} is the weight fraction of the same compound in dry solid phase at equilibrium, and it was calculated from the mass balance (eq 4) (*20*):

$$x_{dm} = \frac{x_m - (1 - DM_p) \cdot y_e}{DM_p} \quad (4)$$

where x_m and y_e are the weight fractions of the marker in the wet residue and liquid extract, respectively. The factor DM_p is the weight fraction of dry matter in the wet residue.

The extraction process for grape cane samples can be assumed to progress under the following conditions:

• The solid sample material is spherical particles with a radius of a, the characteristic dimension of a solid.
• The solvent in the extractor is well-mixed.
• There is no interaction between the diffusion of a given compound and other compounds in the sample.
• The diffusivity of a given compound in the solid phase is constant.

• The initial given compound concentration in the solid phase is uniform.
• The controlling stage of extraction is the diffusion of a given compound in the solid phase.
• There is no chemical reaction.

The extraction of a phenolic compound from the spherical plant particles was assumed to obey Fick's second law (*14, 19, 20, 25*), and to determine the values of τ corresponding to experimental dimensionless extract concentration (Y), the analytical solution of Fick's law for finite volume ratio (α), and very short time (τ), the method described by Schwartzberg, and Chao (*19*) was used.

Fick's number (τ) is a dimensionless time defined by

$$\tau = \frac{D_{eff}t}{a^2} \quad (5)$$

The value of D_{eff} was evaluated by multiplying the slope of the model predicted by linear regression of τ vs time, t by a^2.

RESULTS AND DISCUSSION

Target phenolic compounds (*trans*-resveratrol, *trans*-ε-viniferin, and ferulic acid) contributed more than 70% of the total phenolic content of grape cane extracts. **Figure 1** shows the comparison of HPLC chromatograms (280 nm) of extracts obtained by a reference method (*21*) and by our solid−liquid extraction method carried out using a 58% ethanol/water (v/v) solution (70 mL/g) at 50 °C. *trans*-Resveratrol, *trans*-ε-viniferin, ferulic acid, and total phenolics of grape cane extracts produced by the solid−liquid method varied from 1.3 to 4.1 mg/g dw, from 0.8 to 2 mg/g dw, from 0.2 to 1 mg/g dw, and from 3.2 to 8.9 mg/g dw, respectively (**Table 2**). *trans*-Resveratrol, *trans*-ε-viniferin, ferulic acid, and total phenolics levels from the analysis of the extract obtained by the reference method were 3.85 ± 0.02, 1.25 ± 0.03, 0.16 ± 0.001, and 6.54 ± 0.09 mg/g dw, respectively. The yield of stilbene compounds from grape cane achieved during optimization trials ranged from 34 to 106% for resveratrol and from 64 to 160% for viniferin. Recently, Püssa et al. (*26*) reported that stems of three frost-hardy grapevine varieties [Hasaine (Hasansky) sladki, Zilga, and

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Table 2. Experimental Data of the Investigated Responses of Grape Cane Extracts under Different Extraction Conditions Shown in **Table 1** and Independent Effects of Factors

standard order	*trans*-resveratrol yield[a]	*trans*-ε-viniferin yield[a]	ferulic acid yield[a]	total phenolics yield[b]	*trans*-resveratrol diffusivity,[c] $D_{eff} \times 10^{13}$
1	1.59	0.88	0.95	3.80	6.33
2	3.66	1.73	0.19	7.57	15.79
3	1.71	0.93	0.95	3.98	5.24
4	3.78	1.76	0.21	7.97	16.22
5	3.19	1.15	0.56	6.35	5.79
6	3.93	2.00	0.20	8.02	17.97
7	3.22	1.18	0.43	6.16	6.23
8	3.91	1.78	0.16	7.46	17.66
9	2.41	1.02	0.90	5.12	3.12
10	4.06	1.87	0.17	8.91	26.61
11	3.14	1.90	0.73	6.93	15.52
12	3.02	1.68	0.82	7.16	13.97
13	1.29	0.83	0.64	3.19	13.76
14	3.74	1.64	0.15	6.78	5.77
15	3.17	1.70	0.73	7.22	14.67
16	3.12	1.72	0.77	7.24	13.98
17	3.22	1.67	0.70	7.29	13.53
18	3.08	1.66	0.87	7.06	13.53
main effects temperature	e	e	e	e	e
solvent to solid ratio	NS	NS	NS	NS	NS
ethanol concentration	e	d	e	e	NS

[a] In mg/g dw (dried weight). [b] Total phenolics yield in mg/g dw of grape cane expressed as equivalents of resveratrol. [c] In $m^2 \, s^{-1}$. NS, not significant. [d] Significant at $p \leq 0.05$. [e] Significant at $p \leq 0.01$.

Yubilei Novgoroda] from Estonia contain 1.1−3.2 mg/g dw of *trans*-resveratrol and 0.3−1.7 mg/g dw of ε-viniferin. The *trans*-resveratrol and *trans*-ε-viniferin concentrations in our material compare favorably with this available data set. In red wines, the concentration of *trans*-resveratrol has been found to be in the range of 0.2−7.7 mg/L (*24, 27–30*), and grape skins contain 0.01−0.2 mg/g resveratrol (*21, 30*). Romero-Pérez et al. (*21*) reported the *trans*-resveratrol content of skins in the range of 11.0−47.6 μg/g dw for white grapes (Parellada, Macabeo, Chardonnay, and Xarelâlo) and 17.3−39.4 μg/g dw for red grapes (Carinñena, Cabernet Sauvignon, and Merlot). *trans*-Resveratrol yields in our study are 10−400 times higher as compared to published values for grape skins. Cultivar, climatic differences, and/or disease pressure are the effective factors on the synthesis of stilbenes. Resveratrol yields obtained from different parts of grape vines may be attributed to the sensitivity of the different parts of grape vine to these factors. Li et al. (*32*) have reported the difference in the response of resveratrol synthesis in grape skin and grape seeds being associated with the variable sensitivity of different parts of grape vine to climate change.

The effective diffusivity of resveratrol in the solid phase, D_{eff}, was also calculated by fitting the equilibrium concentrations of resveratrol in to the governing equation derived from Ficks' second law for diffusion. Results ranged from 3.12×10^{-13} to $26.61 \times 10^{-13} \, m^2 \, s^{-1}$ and are presented in **Table 2**. The surface response analysis for *trans*-resveratrol, *trans*-ε-viniferin, ferulic acid, and total phenolics indicated that temperature was the most effective independent variable ($p \leq 0.01$) (**Table 2**). The ethanol concentration was also a significant factor for the yield of all phenolic compounds assayed and for total phenolics ($p \leq 0.05$ or $p \leq 0.01$). However, for the effective diffusivity of resveratrol, surface response analysis showed that the ethanol concentration was not statistically significant (**Table 2**). The solvent to solid ratio was found not to be a significant factor for all

responses ($p > 0.05$) (**Table 2**). Models developed by surface response analysis for *trans*-resveratrol, *trans*-ε-viniferin, ferulic acid, total phenolics yields, and effective diffusivity of resveratrol were significant at the level of 0.001% of probabilities, and variability could be explained by the models (**Table 3**). Regression coefficients and analysis of variance of the adjusted polynomial second-order models for resveratrol, viniferin, ferulic acid, total phenolics, and effective diffusivity of resveratrol are summarized in **Table 3**. An analysis of variance of the regression parameters of the surface response analysis of models for resveratrol, viniferin, ferulic acid, total phenolics yields, and effective diffusivity of resveratrol in the solid phase indicated the existence of the significant effects of linear and quadratic terms ($p \leq 0.001$ or $p \leq 0.01$) except linear terms of the yield of ferulic acid ($p > 0.05$). Interaction terms were only significant in the models for resveratrol, ferulic acid, and total phenolics yields ($p \leq 0.001$ and $p \leq 0.01$) (**Table 3**). Control of other model parameters, Adj-R^2, Pred-R^2, and PRESS indicated that model adequacies were good (**Table 3**).

The solvent to solid ratio in the studied range of 36.8−103.6 mL/g was not significant for resveratrol, viniferin, ferulic acid, and total phenolics yields. Cacace and Mazza (*14*) reported that the solubility and equilibrium constants are modified by the main effect of the solvent to solid ratio varying from 6 to 74 mL/g, and extraction results in a higher yield being maximum at the highest solvent to solid ratio. However, in the present study, no influence of solvent to solid ratio was observed. This may be attributed to the relatively high value of the lowest solvent to solid ratio selected in this study and/or the significant difference in the physicochemical structure of the milled grape cane, used in this study, vs berries.

Effect of Temperature. Total phenolics yield increased with increasing temperature (**Figures 2** and **3**). The increase in yield of total phenolics with temperature was practically linear at the lower and moderate ethanol concentrations, but the temperature effect almost disappeared with a further increase in ethanol concentration (**Figure 3**). **Figure 3** also shows an interaction of temperature and ethanol concentration, particularly at lower temperature and dilute ethanol solutions. The combined effect of temperature and ethanol concentration on the yield of *trans*-resveratrol and *trans*-ε-viniferin is clearly shown in **Figure 4A,B**. At lower ethanol concentrations, a linear temperature effect on resveratrol yield was observed; however, when approaching the higher concentration level, a change due to the temperature nearly disappeared (**Figure 4A**). The yield of viniferin was mainly influenced by temperature. The arch shape temperature effect was observed in the whole range of ethanol concentrations, but it was more apparent at lower concentration levels (**Figure 4B** and **Table 2**). Unlike resveratrol and viniferin, ferulic acid yield increased with the decrease of temperature (**Table 2**). Change due to temperature was strong and almost linear at lower ethanol concentration levels, whereas a weak curve effect was seen when approaching higher ethanol concentrations (plot not shown).

An increase of temperature favors extraction by enhancing the solubility of the compounds extracted. The solubility is assosiated with the property of the mixture (the activity coefficient) and the properties of the solute (entropy of fusion and melting point). Solubility increases with high temperature and low melting point. The solubility is called ideal when the activity coefficient is equal to 1. The temperature dependence of solid solubility is determined not only by the ideal solubility but also by changes in the activity coefficient with temperature (*33*). By contrast, in the present study, a decrease in the yield

Table 3. Regression Coefficients of Predicted Models for the Investigated Responses of Grape Cane Extracts

	coefficient				
variable[a]	*trans*-resveratrol yield	*trans*-ϵ-viniferin yield	ferulic acid yield	total phenolics yield	effective diffusivity of *trans*-resveratrol ($\times 10^{13}$)
β_0	−3.3968[c,e]	−1.4052[e]	0.7664[c]	−7.2617[e]	−11.4029[c]
β_1	0.0753[e]	0.0422[e]	−0.0015 (NS)	0.1416[e]	0.3060[e]
β_2	0.0001 (NS)[b]		−0.00002 (NS)	0.0008 (NS)	−0.0114 (NS)
β_3	0.1113[e]	0.0496[e]	0.0173[c]	0.2826[e]	0.4042[d]
β_{11}		−0.0003[c]	−0.0003[e]		
β_{22}					
β_{33}	−0.0004[d]	−0.0004[e]	−0.0003[e]	−0.0015[e]	−0.0038[d]
β_{12}					
β_{13}	−0.0008[e]		0.0002[d]	−0.0014[e]	
β_{23}					
model	e	e	e	e	e
linear	e	e	NS	e	e
quadratic	d	e	e	e	d
cross-product	e		d	e	
R^2	0.94	0.92	0.95	0.97	0.92
Adj-R^2	0.92	0.90	0.93	0.95	0.90
Pred-R^2	0.81	0.75	0.84	0.88	0.74
PRESS	2.21	0.64	0.3	4.8	157.6

[a] Polynomial model $Z = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j$ 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] NS, not significant ($p > 0.05$). [c] Significant at $p \leq 0.05$. [d] Significant at $p \leq 0.01$. [e] Significant at $p \leq 0.001$.
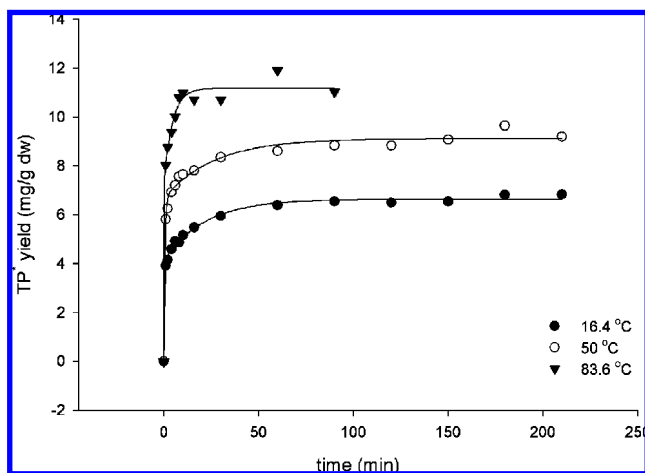

Figure 2. Effect of temperature on the extraction of total phenolics from milled grape canes; TP*, total phenolics as an equivalent of resveratrol.
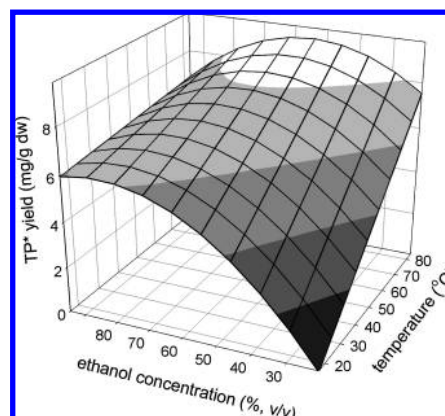

Figure 3. Response surfaces for the effects of temperature and ethanol concentration at a constant solvent to solid ratio of 103.6 mL/g on the yield of total phenolics from grape cane. TP*, total phenolics as an equivalent of resveratrol.

of ferulic acid with increasing temperature was observed. A high temperature may also degrade temperature-sensitive molecules and accelerate oxidation. Thus, the lower yields for ferulic acid may be due to oxidation and degradation reactions favored by the higher extraction temperature (*34, 35*).

Effect of Ethanol Concentration. There was almost a linear increase in yield of resveratrol with increasing ethanol concentration at low temperature levels, whereas the response surface for yield of resveratrol displayed the insignificant curve effect of ethanol concentration at higher temperature levels (**Figure 4A**). The highest yield of *trans*-resveratrol of 4.25 mg/g dw was predicted using the ethanol solution of 58% at the highest temperature of 83.6 °C being independent of solvent to solid ratio. A noticeable curve effect of ethanol concentration, being optimum in the range of 65−70%, on the yield of viniferin was observed in the whole range of temperature (**Figure 4B**). The highest yield was achieved at the highest temperature. An ethanol solution of 68% was found to produce the maximum predicted yield of *trans*-ϵ-viniferin of 2.03 mg/g dw irrespective of the solvent to solid ratio. The ethanol concentration displayed

curve influence on ferulic acid yield in the range of temperatures studied (plot not shown). Using aqueous ethanol (35%), a maximum yield of 1.05 mg/g dw was calculated at 16.4 °C using a fitted model for ferulic acid (**Table 3**).

Modification in extraction solvent by mixing different solvents over a limited compositional range can enhance the solubility of solute. The change in the physical properties of the solvents such as density, dynamic viscosity, and dielectric constant is attributed to ethanol concentration. The solubility of compounds, thus, can be modified by a change in ethanol concentration, and this can affect the extractions of given compounds. A lower solubility of compound in water is associated with the energy required to overcome the attraction between the water molecules. This energy due to the attraction between the partial charges of the water dipoles is important when a much weaker interaction with covalent molecules is considered. The energy required to break the configuration of water molecules becomes dominant for nonpolar covalent molecules, and this could affect the extraction of a given compound having low solubility in water (*15, 16, 33, 36*). The yields of resveratrol and viniferin,
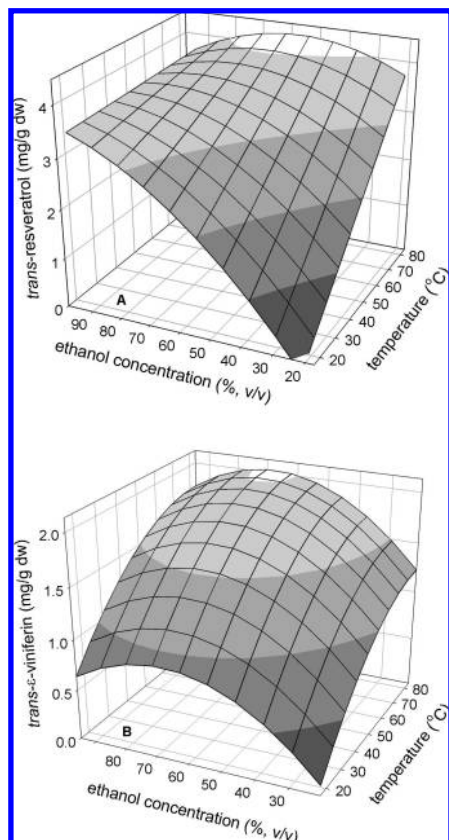
Figure 4. Response surfaces for the effects of temperature and ethanol concentration at constant critical values of solvent to solid ratio levels of 103.6 mL/g for *trans*-resveratrol yield (**A**) and 70 mL/g for *trans*-ε-viniferin yield (**B**) from grape cane.

having lower polarity, are compatible with the above-mentioned phenomenon by showing higher solubility in less polar solvents (moderate and concentrated aqueous ethanol solution) as compared to dilute ethanol solutions. Ferulic acid, however, exhibited a higher solubility in slightly moderate ethanol concentration (30−40%), which can be attributed to being a more polar phenolic compound than the stilbene compounds. Phenolic acids with a carboxylic group and an hydrophobic glycosilated benzene ring may be considered as covalent polar molecules (*33*). The effect of ethanol concentration on the yield of total phenolics (**Figure 3**) was very similar to that for resveratrol (**Figure 4A**). This similarity can be attributed to resveratrol, having the highest contribution to the total phenolics yield. The yield of total phenolics increased with an increase in ethanol content up to moderate concentration levels (50−60%) and decreased with further increase irrespective of the solvent to solid ratio and temperature studied (**Figure 3**). This trend is clear at higher temperature levels; however, the change in yield with ethanol concentration from dilute to moderate levels is stronger than the change observed between moderate and higher concentrations at lower temperature (**Figure 3**). The maximum yield was predicted from a combination of an ethanol concentration of 55%, solvent to solid ratio of 103.6 mL/g, and the highest temperature of 83.6 °C. The reason for the optimal ethanol concentration in the range of 50−70% for the extraction of both stilbene compounds, resveratrol and viniferin, can be attributed to the fact that these compounds have similar polarities and thus show closer solubility in the same ethanol concentration. Total phenolics yield results indicated optimal ethanol concentration nearly in the same range as resveratrol and
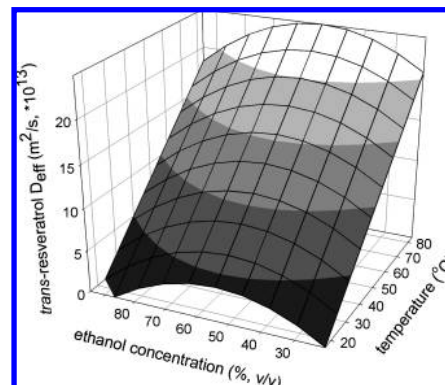

Figure 5. Response surfaces for the effects of temperature and ethanol concentration at a constant solvent to solid ratio of 36.8 mL/g on the effective diffusivity of *trans*-resveratrol.
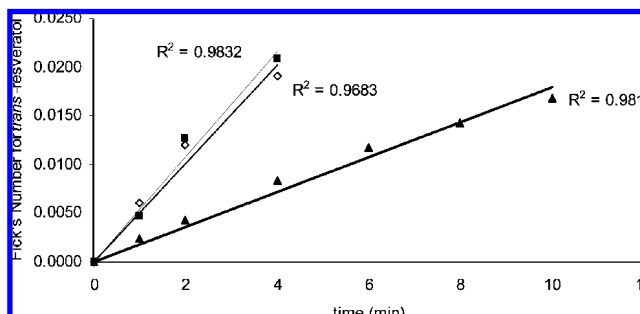

Figure 6. Change of Fick's Number for *trans*-resveratrol at 21 (◇), 58 (■), and 95% (▲) at 50 °C and at the solvent solid ratio of 70 mL/g.
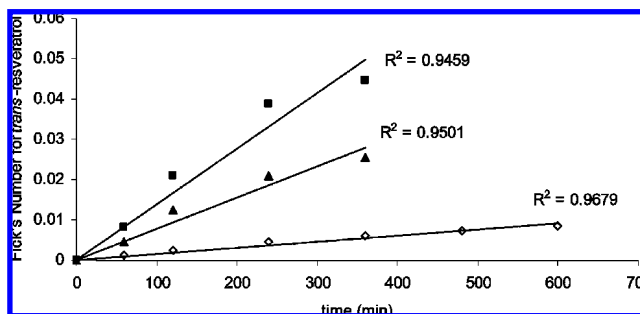

Figure 7. Change of Fick's Number for *trans*-resveratrol at 16.4 (◇), 50 (▲), and 83.6 °C (■) with 58% ethanol solution at the solvent solid ratio of 70 mL/g.

viniferin, and this indicates that the stilbene compounds are the major phenolic group contributing to the total phenolics content of grape cane (**Table 2**).

Effective Diffusivity. Effective diffusivity of *trans*-resveratrol in the solid phase of grape cane extraction varied from 3.12×10^{-13} to 26.61×10^{-13}, effective diffusivity unit of m^2 s^{-1}. The surface response of effective diffusivity of resveratrol showed a saddle shape (**Figure 5**). An increase in the effective diffusivity was observed from 21% ethanol−water mixture up to the range of 55−60%; thereafter, diffusivity of *trans*-resveratrol decreased with a further increase in the ethanol concentration, as can be seen from **Figure 5** and from the slope of the models predicted by linear regression of τ (Fick's number) vs time in **Figure 6**. The predicted maximum effective diffu-sivity of resveratrol in the solid phase (24.6×10^{-13} m^2 s^{-1}) was achieved using an ethanol concentration of 54% at the highest temperature (83.6 °C) and the lowest solvent to solid ratio (36.8 mL/g) studied. A change in ethanol concentration enhances the diffusion of given compounds in the solid phase

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by altering the solvent properties such as density, dynamic viscosity, surface tension, and dielectric constant (*20, 33*). **Figure 6** also shows that the change in effective diffusivity achieved with varying ethanol concentration from 58 to 95% was higher as compared to the change achieved when using an ethanol concentration in the range between 21 and 58%. This difference may be associated with the alteration in the solid matrix. The solvent polarity increases with the water content of the ethanol solution. Extraction with dilute ethanol solution may cause the dissolution of other compounds such as structural polysaccharides, resulting in pore size enlargement. These changes in the solid matrix may have enhanced the penetration of the solvent into the solid matrix and resulted in a higher extraction rate.

An increase in temperature resulted in an increase in effective diffusivity (**Figures 5** and **7**). The linear temperature effect on effective diffusivity was seen in the whole range of ethanol concentration and reflects the effect of temperature on resveratrol yield (**Figure 4A**). Temperature influence can be attributed to improvement of mass transfer and solute−solid interactions. An increase in temperature produces efficient penetration of the solvent in the solid phase, reducing viscosities of solvents and enhancing the diffusivity of solute in the solid and the liquid phases (*20*). From another point of view, the temperature effect on diffusivity can be associated with an increase in the internal energy of the molecules and thus their mobility and a reduction of the dynamic viscosity coefficient. Thus, a higher extraction rate and hence shorter extraction time are achieved (*14*).

Sineiro et al. (*37*) have reported the effective diffusivity values for polyphenols on sunflower press cake extraction varying from 2.21×10^{-13} to 6.13×10^{-13} m^2 s^{-1}. These results were comparable to those obtained in our study. Antioxidants extraction from grape byproducts (skins, seeds, and stems) using ethanol as a solvent also displayed similar effective diffusivity values ranging from 1.33×10^{-13} to 10.55×10^{-13} m^2 s^{-1} (*38*). The effective diffusivity values for anthocynanins and total phenolics from milled berries being between 1.2×10^{-11} and 25×10^{-11} m^2 s^{-1} for aqueous ethanol extraction (*14*) were higher than our results. This difference may be attributed to the differences in the structure of samples studied. The structure of grape canes is obviously much more rigid than that of berries.

In conclusion, the investigation and optimization of the effects of temperature, solvent to solid ratio, and ethanol concentration on the extraction of resveratrol, viniferin, ferulic acid, and total phenolics from milled grape canes revealed that the combination of a moderate ethanol concentration (50−70% ethanol/water mixture) and a practical temperature (83.6 °C) were optimal conditions for the extraction of the high-value phytochemicals *trans*-resveratrol, *trans*-ϵ-viniferin, and other phenolics.

ABBREVIATIONS USED

a, characteristic dimension of the solid (radius of solid particle) (m); d_p, diameter of solid particle (m); d_t, vessel diameter (m); D_{eff}, effective diffusivity in the solid (m^2 s^{-1}); DM_p, weight fraction of dry matter in the wet pomace; k_c, the external mass transfer coefficient in the extract (m s^{-1}); *m*, partition coefficient; *N*, rotational speed of agitator (rps); *T*, absolute temperature (K); *t*, extraction time (s); x_{dm}, weight fraction of a given compound in dry solid phase at equilibrium; x_m, weight fraction of a given compound in wet solid phase at equilibrium; *Y*, dimensionless extract concentration; y_e, weight fraction of a given compound in the liquid phase at equilibrium; α, volume ratio; dimensionless numbers: *Bi*, Biot number; τ, Fick's number.

LITERATURE CITED

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

(2) King, R. E.; Bomser, J. A.; Min, D. B. Bioactivity of resveratrol. *Compr. Food Sci. Food Saf.* **2006**, *5*, 65–70.

(3) Wolter, F.; Stein, J. Biological activities of resveratrol and its analogs. *Drugs Future* **2002**, *27*, 949–959.

(4) Roemer, K.; Mahyar-Roemer, M. The basis for the chemopreventive action of resveratrol. *Drugs Today* **2002**, *38*, 571–580.

(5) Bhat, K. P. L.; Kosmeder, J. W.; Pezzuto, J. M. Biological effects of resveratrol. *Antioxid. Redox Signaling* **2001**, *3*, 1041–1064.

(6) Langcake, P. Disease resistance of *Vitis* spp. and the production of the stress metabolites resveratrol, ϵ-viniferin, α-viniferin and pterostilbene. *Physiol. Plant Pathol.* **1981**, *18*, 213–226.

(7) Piver, B.; Berthou, F.; Dreano, Y.; Lucas, D. Differential inhibition of human cytochrome P450 enzymes by ϵ-viniferin, the dimer of resveratrol: Comparison with resveratrol and polyphenols from alcoholized beverages. *Life Sci.* **2003**, *73*, 1199–1213.

(8) Privat, C.; Telo, J. P.; Bernardes-Genisson, V.; Vieira, A.; Souchard, J. P.; Nepveu, F. Antioxidant properties of ϵ-viniferin as compared to stilbene derivatives in aqueous and nonaqueous media. *J. Agric. Food Chem.* **2002**, *50*, 1213–1217.

(9) Sakai, S.; Kawamata, H.; Kogure, T.; Mantani, N.; Terasawa, K.; Umatake, M.; Ochiai, H. Inhibitory effect of ferulic acid and isoferulic acid on the production of macrophage inflammatory protein-2 in response to respiratory syncytial virus infection in RAW264.7 cells. *Mediators Inflammation* **1999**, *8*, 173–175.

(10) Ou, S.; Kwok, K. Ferulic acid: pharmaceutical functions, preparation and applications in foods. *J. Sci. Food Agric.* **2004**, *84*, 1261–1269.

(11) Ketsawatsakul, U.; Whiteman, M.; Halliwell, B. A reevaluation of the peroxynitrite scavenging activity of some dietary phenolics. *Biochem. Biophys. Res. Commun.* **2000**, *279*, 692–699.

(12) Lo, H. H.; Chung, J. G. The effects of plant phenolics, caffeic acid, chlorogenic acid and ferulic acid on arylamine*N*-acetyltransferase activities in human gastrointestinal microflora. *Anticancer Res.* **1999**, *19*, 133–139.

(13) Mori, H.; Kawabata, K.; Yoshimi, N.; Tanaka, T.; Murakami, T.; Okada, T.; Murai, H. Chemopreventive effects of ferulic acid on oral and rice germ on large bowel carcinogenesis. *Anticancer Res.* **1999**, *19*, 3775–3778.

(14) Cacace, J. E.; Mazza, G. Mass transfer process during extraction of phenolic compounds from milled berries. *J. Food Eng.* **2003**, *59*, 379–389.

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

(16) Cacace, J. E.; Mazza, G. Extraction of anthocyanins and other phenolics from black currants with sulfured water. *J. Agric. Food Chem.* **2002**, *50*, 5939–5946.

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

(18) Myers, R. H.; Montgomery, D. C. *Response Surface Methodology: Process and Product Optimization Using Designed Experiments*, 2nd ed.; Wiley: New York, 2002.

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J. Agric. Food Chem., Vol. 56, No. 15, 2008   **6325**

(19) Schwartzberg, H. G.; Chao, R. Y. Solute diffusivities in leaching processes. *Food Technol.* **1982**, *36*, 73–86.

(20) Gertenbach, D. D. Solid−liquid extraction technologies for manufacturing nutraceuticals. In *Functional Foods: Biochemical and Processing Aspects*; Shi, J., Mazza, G., Le Maguer, M. Eds.; Crc Press: New York, 2002; Vol. 2, pp 331−366.

(21) Perry, R. H.; Greene, D. W.; Maloney, J. O. Section 18. In *Perry's Chemical Engineers' Handbook*; McGraw Hill: New York, 1997; pp 5−17.

(22) Romero-Pérez, A. I.; Lamuela-Raventós, R. M.; Andrés-Lacueva, C.; Carmen de la Torre-Boronat, M. Method for the quantitative extraction of resveratrol and piceid isomers in grape berry skins. Effect of powdery mildew on the stilbene content. *J. Agric. Food Chem.* **2001**, *49*, 210–215.

(23) Jean-Denis, J. B.; Pezet, R.; Tabacchi, R. Rapid analysis of stilbenes and derivatives from downy mildew-infected grapevine leaves by liquid chromatography-atmospheric pressure photoionisation mass spectrometry. *J. Chromatogr. A* **2006**, *1112*, 263–268.

(24) Adrian, M.; Jeandet, P.; Breuil, A. C.; Levite, D.; Debord, S.; Bessis, R. Assay of resveratrol and derivative stilbenes in wines by direct injection high performance liquid chromatography. *Am. J. Enol. Vitic.* **2000**, *51* (1), 37–41.

(25) Franco, D.; Sineiro, J.; Pinelo, M.; Nuñez, M. J. Ethanolic extraction of *Rosa rubiginosa* soluble substances: Oil solubility equilibra and kinetic studies. *J. Food Eng.* **2007**, *79*, 150–157.

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

(27) Pezet, R.; Gindro, K.; Viret, O.; Spring, J. L. Glycosylation and oxidative dimerization of resveratrol are respectively associated to sensitivity and resistance of grapevine cultivars to downy mildew. *Physiol. Mol. Plant Pathol.* **2004**, *65*, 297–303.

(28) Abril, M.; Negueruela, A. I.; Pérez, C.; Juan, T.; Estopañán, G. Preliminary study of resveratrol content in Aragón red and rosé wines. *Food Chem.* **2005**, *92*, 729–736.

(29) Lamuela-Raventós, R. M.; Waterhouse, A. L. Occurrence of resveratrol in selected California wines by a new HPLC method. *J. Agric. Food Chem.* **1993**, *41* (4), 521–3.

(30) Dong, Z. Molecular mechanism of the chemopreventive effect of resveratrol. *Mutat. Res.* **2003**, *523/524*, 145–150.

(31) Navarro, S.; León, M.; Roca-Pérez, L.; Boluda, R.; García-Ferriz, L.; Pérez-Bermúdez, P.; Gavidia, I. Characterisation of Bobal and Crujidera grape cultivars, in comparison with Tempranillo and Cabernet Sauvignon: Evolution of leaf macronutrients and berry composition during grape ripening. *Food Chem.* **2008**, *108*, 182–190.

(32) Li, X.; Wu, B.; Wang, L.; Li, S. Extractable amounts of *trans*-resveratrol in seed and berry skin in *Vitis* evaluated at the germplasm level. *J. Agric. Food Chem.* **2006**, *54*, 8804–8811.

(33) Frank, T. C.; Downey, J. R.; Gupta, S. K. Quickly screen solvent for organic solids. *Chem. Eng. Prog.* **1999**, (Dec), 41–46.

(34) Mazzoleni, V.; Caldentey, P.; Silva, A. Phenolic compounds in cork used for production of wine stoppers as affected by storage and boiling of cork slabs. *Am. J. Enol. Vitic.* **1998**, *49*, 6–10.

(35) Maga, J. A. The flavor chemistry of wood smoke. *Food Rev. Int.* **1987**, *3*, 139–183.

(36) Mackay, K. M.; Mackay, R. A. Solution chemistry. *Introduction to Modern Inorganic Chemistry*, 3rd ed.; International Textbook: London, United Kingdom, 1981; pp 86−103.

(37) Sineiro, J.; Dominguez, H.; Nuñez, M. J.; Lema, J. M. Ethanol extraction of polyphenols in an immersion extractor. Effect of pulsing flow. *J. Am. Oil Chem. Soc.* **1996**, *73*, 1121–1125.

(38) Pinelo, M.; Sineiro, J.; Nuñez, M. J. Mass transfer during continuous solid-liquid extraction of antioxidants from grape byproducts. *J. Food Eng.* **2006**, *77*, 57–63.