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Extraction of Anthocyanins and Other Phenolics from Black Currants with Sulfured Water

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Health benefits of fruits, vegetables, and red wine are attributed to anthocyanins and other phytochemicals. In this research, the extraction of phenolics from black currants was optimized using different SO_2 concentrations (28, 300, 700, 1100, and 1372 ppm), temperatures (6, 20, 40, 60, and 74 °C), and solvent to solid ratios (S/S) (6, 20, 40, 60, and 74 mL/g). Surface response methodology was used to optimize yields of anthocyanins and total phenolics, as well as their antiradical and antioxidant activities. The extraction of phenolics varied with the SO_2 concentration, S/S, and temperature. Maximum yields of total phenolics and anthocyanins were obtained at an SO_2 concentration of 1000−1200 ppm and 19 L of solvent/kg of milled frozen berries. The increase of extraction temperature increased the rate of extraction and, thus, times to reach equilibrium for the extraction of total phenolics and anthocyanins were reduced. However, for the extraction of anthocyanins it is recommended that temperatures of 30−35 °C be used, as higher temperatures will degrade these compounds. Antioxidant activity was affected by all three experimental variables evaluated; however, the main variable affecting it was S/S. The higher the S/S, the lower the antioxidant index.

KEYWORDS: Anthocyanins; phenolics; black currants; sulfur dioxide; flavonols; extraction; antioxidant activity; functional foods; nutraceuticals

INTRODUCTION

The protective effects of fruits and vegetables against coronary heart disease, stroke, and cancer have been attributed to the presence of flavonoids and other phytochemicals, including anthocyanins, flavonols, flavones, and flavanols (*1*, *2*). Flavonoids and other polyphenols have shown a wide range of biological effects including anti-inflammatory, antibacterial, antioxidant, and vasodilating effects (*2*, *3*).

Antioxidant activity of fruits, vegetables, and grain products has been presented (*4*, *5*), as well as the relationship of antioxidant activity with the structure of flavonoids (*6*−*8*). Antioxidant activity has also been related to anthocyanin and total phenolic contents of berries from several genotypes of *Vaccinium* L., *Rubus* L., and *Ribes* L. (*9*, *10*). Berries represent one of the most important sources of phenolic compounds. Anthocyanin contents of berries and other edible plants have been summarized (*11*). Black currant was the second strongest radical scavenger among nine types of berries (*12*). The main black currant anthocyanins are cyanidin 3-glucoside, delphinidin 3-glucoside, cyanidin 3-rutinoside, and delphinidin 3-rutinoside (*11*, *13*). Cyanidin 3-glucoside has been found to be twice as effective as commercially available antioxidants, such as butylated hydroxyanisole (BHA) and α-tocopherol (*7*). Other compounds present in berries, including flavonols and hydroxycinnamic acids, also have an important antioxidant effect. Sweet cherries and blueberries with higher hydroxycinnamates content were efficient inhibitors of food liposome oxidation (*14*). Higher concentrations of hydroxycinnamic acid derivatives, especially caffeic and *p*-coumaric acids (*15*, *16*), and flavonols, such as myricetin, quercetin, and kaempferol, are also present in black currants (*16*−*18*).

Extraction of phytochemicals from permeable solid plant material, using liquid solvents, constitutes an important step in the manufacturing of phytochemicals-rich products. The two fundamental concepts that define this process are equilibrium and mass transfer rate. These phenomena control how much of and how quickly the marker compounds are extracted from the plant tissue. Concentration of a given compound within the plant tissue develops an equilibrium with the concentration that will dissolve into the solvent. The equilibrium is described by the equation

$$m = y_e/x_e \quad (1)$$

where m is the equilibrium distribution ratio or partition

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coefficient, y_e is the weight fraction of a given compound in the extract, and x_e is the weight fraction of the given compound in the dry marc (*19*, *20*). The value of *m* is a characteristic of both the solvent and the compound as well as the temperature. The extraction of anthocyanins from wine pomace (*21*) and from strawberries (*22*) has been studied using different solvents and acidifying conditions. The solvent used for anthocyanin extraction significantly affects the solid−liquid extraction from sunflower (*23*, *24*). Higher anthocyanin extraction from *Vitis vinifera* has been found with increased SO_2 levels (*25*). Gao and Mazza (*23*) reported that the extraction of anthocyanins from sunflower hulls was affected by the type of solvent, and sulfured water performed better than acetic acid and aqueous ethanol. Endeavors using SO_2 water as a solvent seek a reduction of the use of organic solvent as well as the cost of the extraction.

The extraction will also depend on how quickly the compound will dissolve and reach the equilibrium concentration in the liquid. The extraction rate can be increased with an increase of the concentration gradient, a larger diffusion coefficient, or a smaller particle size. Thus, a maximum breakup of sunflower hull (*23*) or a decrease in pomace size of black currant juice press residues (*26*) resulted in higher extraction yields of total phenols and anthocyanins. An increase in temperature and a decrease of the viscosity coefficient significantly increases diffusivities (*27*). However, thermal degradation of anthocyanin pigment extracted with SO_2 water solutions has been reported (*28*). The optimum conditions of temperature and solvent to solid ratio (S/S) for the extraction of phenolics and particularly for extraction of anthocyanins from black currants have thus far not been reported.

Surface response methodology has been successfully used for optimization of anthocyanin yield in the extraction from sunflower hulls (*23*). It has also been used to select the best solvent, temperature, and extraction time to maximize the antioxidant activity of defatted borage meal (*29*). The objective of this work was to optimize the extraction of phenolic compounds from black currants, for yield and antioxidant activity of the extracts with sulfured water using near pilot scale equipment, and mixing conditions that would allow scale-up of the process.

MATERIALS AND METHODS

Preparation of Samples. Individual quick frozen (IQF) black currants, cv. Ben Lomond, from the 2000 production season (M & G Brothers Farm, Abbotsford, BC) were stored frozen, at −36 °C, until used. Berries were dipped in liquid nitrogen and milled with a precooled Wiley mill (model ED-5, Arthur H. Thomas Co., Philadelphia, PA). The mill was set at 520 rpm, with a 6 mm screen and a 2.0 mm blade distance. Particle size distribution was characterized by sieving milled berries at −25 °C with a sieve shaker (model Octagon 200, Endecotts Limited, London, U.K.) and U.S. standard sieves 5 (4.0 mm), 7 (2.8 mm), 10 (2.0 mm), 16 (1.18 mm), 18 (1.0 mm), and 60 (0.25 mm). Sieves were weighed before and after sieving, and the sample weights remaining in each sieve were recorded. Particle size distribution and average size were obtained by plotting sieve mesh size versus the cumulative passing weight percentage using a log-probability plot as shown by Gertenbach (*20*). Milled berries were prepared in a 0 °C cold room and stored at −36 °C in sealed containers flushed with nitrogen gas. Milled berries were extracted within 2 weeks after milling.

Extraction. Milled frozen samples were dispersed in 2.5 L of solvent in an agitated 4 L glass beaker. The solvent consisted of aqueous sulfur dioxide solutions prepared by dissolving sodium metabisulfite ($Na_2S_2O_3$) in water and acidifying to pH 3.8 with acetic acid. The sulfur dioxide concentration, temperature, and weight of berries were varied for each extraction. A 6.35 cm diameter airfoil axial impeller (model A310 Lightnin, Mixing Equipment Co. Inc., Rochester, NY) was used to mix the milled berries in the extraction solvent. The beaker was set in a thermostatic water bath at each desired temperature. Berry samples were calculated from S/S on a dry weight basis (dwb) and added to the SO_2− water solvents when the desired temperature was reached.

During extraction, 3 mL samples of liquid were taken periodically. Extractions were finished when extract and pomace reached equilibrium indicated by no further change in absorbance of the extracts read at 280 or 520 nm. This procedure establishes a difference with previous extraction studies, which have used a constant time (*22*, *26*) or the time as an independent variable (*23*, *29*). The technique used here allowed a more thorough extraction and thus evaluation of the maximum capability of each extraction condition. Besides, the equilibrium time arises as an additional dependent variable. Time to reach equilibrium was obtained from plots of phenolic concentration versus extraction time. Best-fit curves to reach a maximum asymptote and phenolics predicted maximum values were obtained using Sigma Plot software (SPSS Inc., Chicago, IL). Equilibrium times were considered the times when curves reached the predicted maximum value. Partition coefficients *m* were calculated by eq 1. Recoveries *F* (percent) of anthocyanins and total phenolics were calculated using

$$F\ (\%) = (C_{eq}/C_{fb}) \times 100 \qquad (2)$$

where C_{eq} and C_{fb} are the contents of a given marker compound in mg/g of frozen berries on a dwb in the final extract and in the frozen berries, respectively.

Mixing Conditions. The rotational speed was fixed at 1210 rpm to obtain a Reynolds number 10000 and a turbulent regime that ensures approximately constant power (*30*). To eliminate variations from the mixing system employed, the geometry of the vessel and impeller, the position of the shaft and impeller, and the impeller to vessel diameter ratio were fixed for all of the extractions in the experiment. A fluid foil impeller of 6.35 cm and a vessel of 15.6 cm in diameter were selected, and thus the impeller to vessel diameter ratio was 0.41.

The impeller was located at a distance from the bottom of one-fourth of the vessel diameter ($D_t/4$), so that impeller distance from the bottom was 3.9 cm. To obtain uniform circulation and mixing, the solvent volume was 2.5 L; thus, the impeller was adjusted at the recommended position of one-third of the liquid depth above the vessel bottom (*30*). Height changed because the volume increased when berries were incorporated, but all values were in the recommended range of 11.7−23.4 cm. A clamp-mounted 15° angular off-center impeller in a tank with no baffles was used to avoid swirling and vortex formation. Distance from the wall to the impeller was fixed at 4.1 cm.

Analysis. Periodic samples that were taken during extractions and aliquots of final extracts were filtered through 0.45 μm PVDF membrane disks using syringe filter holders. Samples were analyzed for total phenolics, tartaric esters, flavonols, and anthocyanin by absorbance readings at 280, 320, 360, and 520 nm, respectively, using a spectrophotometric method. Standard solutions used included chlorogenic acid, caffeic acid, quercetin, and cyanidin 3-glucoside.

Samples of wet pomace (20 g) and frozen berries (10 g) were extracted in a blender, with 80% ethanol as described previously (*31*). The composition of frozen berries was also determined by the equilibrium method (*19*) in which approximately 4 g samples of milled frozen black currants were soaked with 100 mL of 1100 ppm of SO_2− water solvent and set in an incubator at 20 °C for ∼60 h. Supernatant was filtered through Whatman no. 541 filter paper in a Büchner funnel under vacuum, collected in a 100 mL volumetric flask, and analyzed as described above. Dry matter contents of frozen berries and pomace were determined by drying 2−4 g samples in a vacuum oven at 70 °C for 30 h.

HPLC analysis was carried out using a 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, 4.6 × 250 mm, Agilent Technologies Inc.) preceded by a guard column (Inertsil 5 ODS-2, 5 μm, 30 × 4.6 mm, Phenomenex, Torrance, CA). A gradient solvent system was used with solvent A being formic acid/water (5:95 v/v) and solvent B being methanol. The elution profile had the following proportions (v/v) of

Table 1. Central Composite Experimental Design for Three Variables

run	temp (°C)	S/S[a] (mL/g)	SO_2 concn[b] (ppm)
1	20 (–1)[c]	20 (–1)	300 (–1)
2	20 (–1)	20 (–1)	1100 (+1)
3	20 (–1)	60 (+1)	300 (–1)
4	20 (–1)	60 (+1)	1100 (+1)
5	60 (+1)	20 (–1)	300 (–1)
6	60 (+1)	20 (–1)	1100 (+1)
7	60 (+1)	60 (+1)	300 (–1)
8	60 (+1)	60 (+1)	1100 (+1)
9	6 (–1.68)	40 (0)	700 (0)
10	74 (+1.68)	40 (0)	700 (0)
11	40 (0)	6 (–1.68)	700 (0)
12	40 (0)	74 (+1.68)	700 (0)
13	40 (0)	40 (0)	28 (–1.68)
14	40 (0)	40 (0)	1372 (+1.68)
15	40 (0)	40 (0)	700 (0)
16	40 (0)	40 (0)	700 (0)
17	40 (0)	40 (0)	700 (0)
18	40 (0)	40 (0)	700 (0)

[a] Solvent to solid ratio expressed in mL/g of frozen berries on a dry weight basis (dwb). [b] Calculated as ppm equivalents of sulfur dioxide. [c] Numbers in parentheses are the coded values of variables in the experimental design.

solvent B: 0 min, 10%; 0−30 min, 10−25%; 30−50 min, 25−45%; 50−55 min, 45−100%; 55−60 min, 100%; and 60−65 min, 100−10%. The solvent flow rate was 1.0 mL/ min. Concentrations were calculated using peak areas from standard curves of chlorogenic acid, caffeic acid, quercetin, and cyanidin 3-glucoside at 280, 320, 360, and 525 nm, respectively.

Antioxidant activities of extracts were evaluated at a uniform concentration range as anti-radical efficiency by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method and as antioxidant activity by the β-carotene method described in Fukumoto and Mazza (*7*). Antioxidant activity of extracts was also evaluated by an antioxidant index using the same dilution factor for all extracts, so that concentration differences among extracts were maintained. Thus, absorbance readings at 0 and 90 min from the β-carotene method, of samples lying within a concentration range of 16−150 ppm, were used to calculate antioxidant index as indicated by Wettasinghe and Shahidi (*29*). Antioxidant index was calculated by the ratio between the absorbance at 90 min and the initial absorbance of each sample and expressed as percentage.

Experimental Design. Optimization of extraction was carried out using surface response methodology (*32*). Selected experimental design referred to as central composite design had three factors and five levels. It consisted of 18 runs implemented in random order including four replicates of the center point. Independent variables were SO_2 concentration, temperature, and S/S. Sulfur dioxide concentrations were 28, 300, 700, 1100, and 1372 ppm. Lowest and highest values of S/S and temperature were 6 and 74 (**Table 1**). Phenolic yields, equilibrium times, partition coefficients, antioxidant activity, antiradical activity, and antioxidant indices of extracts were measured.

Data were analyzed using the RSREG, PLOT, and REG procedures of SAS (*33*) (SAS Institute Inc., Cary, NC) and fitted to a second-order polynomial equation to optimize the conditions of extraction. The RSREG was used to estimate the parameters of the model, the contribution of each type of effect (linear, quadratic, and cross-product), the contribution of each factor variable to the model, and the shape of the curve. A goodness-of-fit test of the model was performed with the REG procedure by backward elimination to keep variables significant at the 0.1% level. Response surface plots were obtained using predicted values from the fitted model, by keeping the least effective independent variable fixed at a constant value.

RESULTS AND DISCUSSION

Preparation of frozen black currant particles yielded almost identical particle size distribution and average particle size for three different days berries were milled. Average particle size, corresponding to a passing weight probability of 50% in the plot of particle size distribution, resulted in 1.6 mm for the milling conditions applied in the experiment (**Figure 1**). The obtained uniformity of size and narrow distribution of milled berries ensured no interference of raw material on the results.

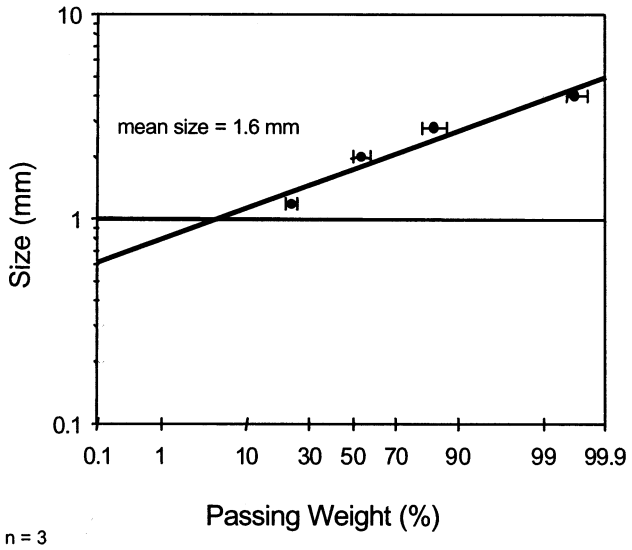

Figure 1. Particle size distribution of frozen black currants milled in a Wiley mill set at 520 rpm, with a 6 mm screen and a 2.1 mm blade distance.

Table 2. Composition of Milled Frozen Black Currants[a]

sample	total phenolics[b]	tartaric esters[c]	flavonols[d]	anthocyanins[e]	dry matter (%)
1[f]	89.4	2.8	2.1	15.8	22.9
2	91	2.7	1.8	14.9	22.1
3	86.3	2.8	1.9	15.2	22.4
av ± SD	88.9 ± 2.4	2.8 ± 0.08	1.9 ± 0.14	15.3 ± 0.44	22.5 ± 0.41
1[g]	21.7	2.2	1.6	15.7	
2	19.5	2	1.3	14.2	
3	18.7	1.9	1.3	13.5	
av ± SD	20.0 ± 1.6	2.0 ± 0.12	1.4 ± 0.17	14.5 ± 1.1	

[a] Phenolic concentrations in mg/g of frozen berries on a dry weight basis (dwb) expressed as equivalents of the following: [b] chlorogenic acid; [c] caffeic acid; [d] quercetin; and [e] cyanidin 3-glucoside. [f,g] Phenolics determined by equilibrium with a 1100 ppm SO_2 water at 100 mL/g solvent to solid ratio at 20 °C for 60 h ([f] calculated from spectrophotometric determination; [g] calculated from HPLC determination).

Anthocyanin content of milled black currant (**Table 2**) was higher than values reported in the literature. However, the same berry samples analyzed for anthocyanins by extraction with ethanol in a blender (data not shown) gave values comparable to values for cv. Ben Lomond reported by Banaszczyk and Pluta (*34*) and Moyer et al. (*10*). Also, water-extracted anthocyanins were within the range attributed to seasonal variations in a six year period for cv. Ben Lomond in Poland (*34*). With the equilibrium method (*19*), extraction time was sufficiently long (60 h) to ensure a complete extraction. Water extraction for such a long time also resulted in a high yield of nonphenolic compounds as indicated by higher absorbance readings at 280 nm than values from ethanolic extractions. Mok and Hettiarachchy (*28*) have reported extraction of non-anthocyanin material with 1000 ppm of SO_2 in water from sunflower hull, and García-Viguera et al. (*22*) reported extraneous materials in water extracts of anthocyanins from strawberry. When samples were injected onto an HPLC to confirm results, values of anthocyanins were close to spectrophotometric values; however,

Table 3. Surface Response for Extraction Yield, Partition Coefficient, and Equilibrium Time of Total Phenolics and Anthocyanins

	total phenolics			anthocyanins		
run	yield[a]	partition coeff	equil time[b]	yield[c]	partition coeff	equil time[b]
1	25.4	0.13	128	12.2	0.25	130
2	32.1	0.18	52	13.4	0.37	55
3	33.3	0.06	68	12.7	0.09	70
4	55.6	0.11	48	13.4	0.12	51
5	23.5	0.12	45	11.4	0.21	32
6	35.6	0.30	15	13.3	0.38	38
7	26.6	0.05	18	13.1	0.08	22
8	39.2	0.31	10	13.7	0.12	22
9	36.4	0.09	135	13.5	0.14	136
10	23.4	0.20	20	9.7	0.11	20
11	23.7	0.47	62	11.6	1.17	80
12	41.3	0.12	29	13.3	0.10	29
13	24.3	0.04	88	11.7	0.08	92
14	40.6	0.30	32	12.5	0.13	40
15	36.9	0.09	40	13.1	0.13	42
16	31.4	0.16	33	12.0	0.13	37
17	32.5	0.07	22	12.8	0.16	46
18	31.0	0.11	40	13.7	0.17	40
model	***[d]	**	***	NS	**	***
linear	***	***	***	*	**	***
quadratic	NS	*	**	NS	**	NS
cross-product	*	NS	NS	NS	NS	NS
R^2	0.928	0.841	0.944	0.630	0.813	0.921
effects						
SO_2 concn	***	**	***	NS	NS	**
temperature	**	NS	***	NS	NS	***
S/S	***	**	*	NS	***	NS

[a] Total phenolic yields in mg/g of frozen berries on a dwb expressed as equivalents of chlorogenic acid. [b] In minutes. [c] Anthocyanin yield in mg/g of frozen berries on a dwb expressed as equivalents of cyanidin 3-glucoside. [d] ***, significant at $p < 0.01$; **, significant at $p < 0.05$; *, significant at $p < 0.1$; NS, not significant.

total phenolics from HPLC were considerably lower. Proteins absorbing at a wavelength close to 280 nm might be interfering in the spectrophotometric determination of berry composition obtained with the equilibrium method as a result of very long extraction times.

The model developed by surface response analysis for total phenolic yield (**Table 3**) was significant at low levels of probabilities ($p < 0.01$), and variability could be very well explained by the model. Surface response models for yields of anthocyanins (**Table 3**), tartaric esters, and flavonols (data not shown) were not statistically significant ($p > 0.1$). Equilibrium times for the extractions of total phenolics and anthocyanins were also highly significant ($p < 0.01$). However, for the antioxidant activity parameters the response varied (**Table 4**). The model was not significant and variability explained was low for antioxidant activity measured by the β-carotene method, but models were significant and variability explained was high for the antiradical activity measured by the DPPH assay and for antioxidant index. Regression coefficients and analysis of variance of the adjusted polynomial second-order models for total phenolic and anthocyanin yields, extraction times, and antioxidant index of extracts are presented in **Table 5**.

With increasing S/S, anthocyanin and total phenolic yields increased and the time to reach constant concentration decreased (**Figure 2**). This effect is more noticeable at low (300 ppm) SO_2 concentration than at high (1100 ppm) SO_2 concentration. An increase in extraction temperature from 20 to 60 °C did not result in a higher anthocyanin extraction but did reduce the time to reach equilibrium. Temperature can affect the extraction of a given compound by modifying the diffusion coefficient or its solubility in the solvent. Increasing the temperature would increase the diffusion coefficient and thus the rate of diffusion leading to a reduction in the extraction time. At 1100 ppm of SO_2 concentration and high S/S (60 mL/g) the increase of extraction temperature from 20 to 60 °C led to a lower anthocyanin extraction yield. This effect can be a consequence of degradation of anthocyanins produced by the high temperature.

Table 4. Surface Response for Antiradical Activity, Antioxidant Activity, and Antioxidant Index

run	antiradical activity[a]	antioxidant activity[b]	antioxidant index (%)
1	−4.02	892	85.8
2	−3.75	694	83.0
3	−3.53	711	58.5
4	−4.30	745	42.8
5	−4.14	937	87.9
6	−4.16	747	87.0
7	−4.99	1331	70.1
8	−6.14	501	70.4
9	−4.43	1189	67.6
10	−5.36	569	80.5
11	−5.21	1142	91.8
12	−5.56	263	51.6
13	−3.59	822	74.2
14	−5.50	416	63.8
15	−4.64	656	70.8
16	−4.75	555	72.1
17	−4.18	369	75.9
18	−4.36	348	70.3
model	*[c]	NS	***
linear	**	NS	***
quadratic	NS	NS	NS
cross-product	NS	NS	***
R^2	0.772	0.627	0.977
effects			
SO_2 concn	NS	NS	**
temperature	*	NS	***
S/S	*	NS	***

[a] Slope coefficient calculated by linear regression in μM DPPH/μM of antioxidant, from total phenolics concentration expressed as chlorogenic acid equivalent. [b] Slope coefficient calculated by linear regression in 10^6 absorbance units/μM of antioxidant, from total phenolics concentration expressed as chlorogenic acid equivalent. [c] ***, significant at $p < 0.01$; **, significant at $p < 0.05$; *, significant at $p < 0.1$; NS, not significant.

Table 5. Regression Coefficients and Analysis of Variance of the Second-Order Polynomial Model Adjusted by Goodness-of-Fit Test for Total Phenolic and Anthocyanin Yields and Equilibrium Times and Antioxidant Index of Black Currant Extracts

	total phenolic coefficients		anthocyanin coefficients		antioxidant
variable[a]	yield	time	yield	time	index coeff
intercept	25.41***	241.5***	12.2***	338.0***	96.83***
X_1	0.014*[c]	−0.16***	0.0012*	−0.29***	
X_2		−4.06**		−6.30***	
X_3		−1.20**		−2.85**	−0.49***
X_1^2		5.1×10^{-5}*		4.5×10^{-5}***	
X_2^2	−0.0015*	0.032***	−0.00054***	0.025**	
X_3^2					
$X_1 \times X_2$	0.0004**			0.0040**	
$X_1 \times X_3$	0.0008***	−0.0012*		0.0031**	−0.00058***
$X_2 \times X_3$			0.00047*	0.044*	
$X_1 \times X_2 \times X_3$	-1.2×10^{-5}**			-5.5×10^{-5}*	9.9×10^{-6}***
model	***	***	**	***	***
R^2	0.945	0.919	0.508	0.946	0.968

[a] Polynomial model $Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{3} \beta_{ij} X_i X_j$ adjusted by backward elimination al the level of 0.1% with the goodness-of-fit test, where X_1 = SO_2 concentration, X_2 = temperature, and X_3 = solvent to solid ratio. [b] NS, not significant ($p > 0.1$). [c] ***, significant at $p < 0.01$; ***, significant at $p < 0.05$; *, significant at $p < 0.1$.

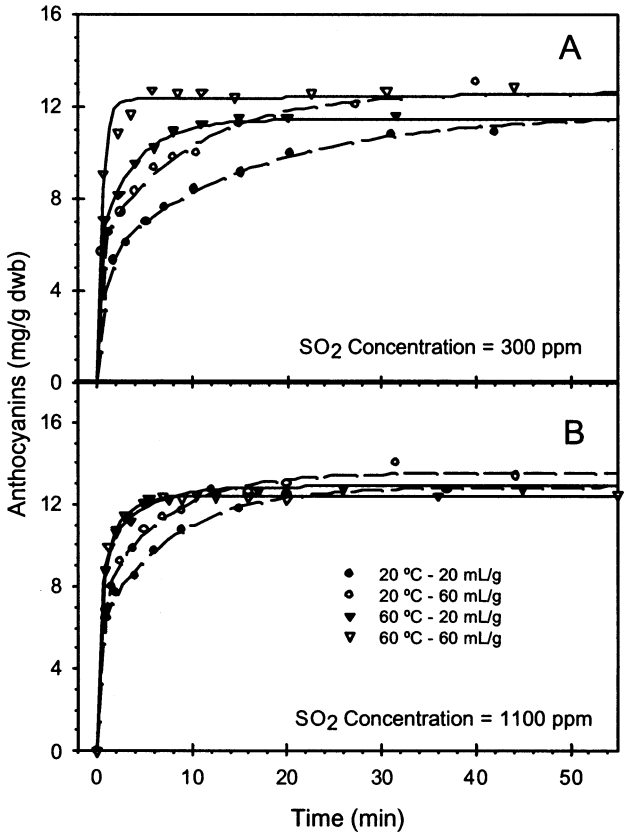

Figure 2. Anthocyanins expressed as cyanidin 3-glucoside equivalents in mg/g dwb of frozen berries extracted with (A) 300 ppm of SO_2 and (B) 1100 ppm of SO_2 from milled frozen black currants at the following temperatures and solvent to solid ratios: ●, 20 °C and 20 mL/g; ○, 20 °C and 60 mL/g; ▼, 60 °C and 20 mL/g; ▽, 60 °C and 60 mL/g.

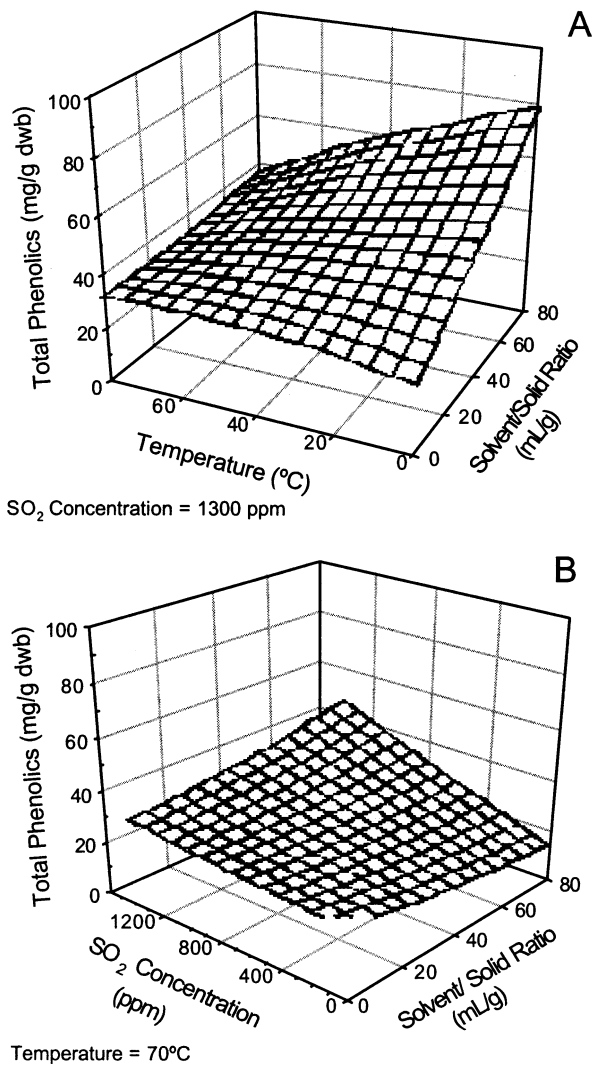

Figure 3. Response surface for the effects of (A) solvent to solid ratio and temperature at a constant SO_2 concentration of 1300 ppm and (B) solvent to solid ratio and SO_2 concentration at a constant temperature of 70 °C on total phenolics expressed as chlorogenic acid equivalents in mg/g dwb of frozen berries.

Total phenolic extraction was affected by the three variables studied, but it was mostly affected by the SO_2 concentration and the S/S. S/S significantly increased the extraction of total phenolics (**Figure 3A**). Total phenolics increased linearly with S/S; however, the slope was steeper at low temperature, increasing from 25 to 78 mg of chlorogenic acid equivalents/g of frozen berries on a dry weight basis. The slower increase of total phenolics at high temperature might be due to the susceptibility of some phenolics to thermal degradation and/or loss of SO_2 from the solvent during extraction at high temperature. A lower effect of temperature on the extraction of total phenolics compared to anthocyanins from lowbush blueberry has been reported by Kalt et al. (*35*). Increasing S/S also resulted in increased total phenolic extraction at high SO_2 concentration, but values were essentially constant at very low SO_2 concentrations (**Figure 3B**). The limiting step of extraction at low SO_2 concentration would be the reduction of the solubility. At high SO_2 concentration solubility would have increased, and then an increase in S/S resulted in a higher extraction of total phenolics.

Total phenolics increased with SO_2 concentration in the whole range of S/S, but the increase was higher at higher S/S (**Figure 3B**). Anthocyanin extraction also increased with increasing SO_2 concentration, but the effect was not statistically significant. The exact mechanism for how SO_2 improves extraction is not known, but interactions leading to improved diffusion through the cell walls and increased solubility have been mentioned as possible reasons (*23*). Higher anthocyanin yields from grapes (*25*) and from sunflower hull (*23*, *28*) have been reported from extractions with higher SO_2 concentration. Extractions of anthocyanins and total phenolics from black currants with 100% water at room temperature have resulted in yields (*9*) similar to those achieved with our lowest SO_2 treatment (28 ppm). Solvent composition changes physical properties such as density and dynamic viscosity that may affect diffusion and the rate of extraction. It also influences the activity coefficient and thus the solubility of a given compound in the solvent. Another physical property of the solvent that may be modified with the solvent composition is the electric permittivity or dielectric constant. This property measures the ability to reduce the interaction of particles with opposite charges and also determine solvation characteristics of a solvent. Reduction of the dielectric constant of a protic solvent (contains relatively mobile protons), such as water ($\epsilon_{H_2O} = 78.5$) into the range of intermediate-behavior solvents such as methanol ($\epsilon_{MeOH} = 32.6$) or ethanol ($\epsilon_{EtOH} = 24.3$) by modifying pressure and temperature has improved extraction of natural products (*36*). The high dielectric constant of water might have been reduced when the SO_2 concentration increased. The dielectric constant of pure liquid SO_2 at 20 °C is 14. A lower dielectric constant reduces the energy required to separate the solvent molecules and allows the solute molecules to enter between them (*37*). From these research results it is suggested

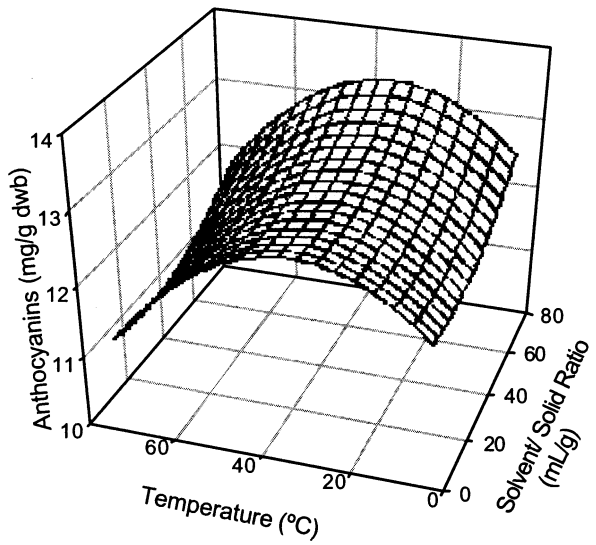

Figure 4. Response surface for the effects of temperature and solvent to solid ratio at a constant SO_2 concentration of 1100 ppm on anthocyanins expressed as cyanidin 3-glucoside equivalents in mg/g dwb of frozen berries.

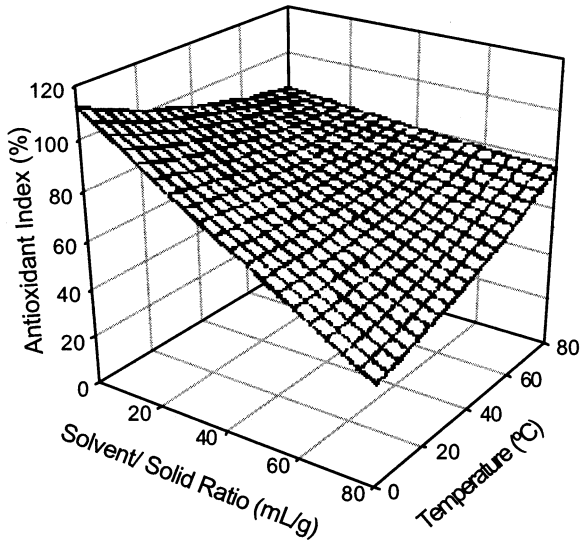

Figure 5. Response surface for the effects of solvent to solid ratio and temperature on antioxidant index of extracts at a constant SO_2 concentration of 26 ppm.

that one mechanism by which SO_2 improves the extraction is by modifying the solvent, thus resulting in increased solubility of phenolic molecules.

Extraction of anthocyanins was affected by S/S and temperature (**Figure 4**), although the effects were not statistically significant. At a constant SO_2 concentration of 1100 ppm, anthocyanin content increased to a maximum at ~40 °C and then decreased with further increase in temperature, independent of the S/S values. Increasing temperature would favor extraction by both increasing solubility of anthocyanins and increasing diffusion coefficient; however, a major effect was to speed extraction by increasing the diffusion coefficient. Reduction of anthocyanin extraction by increasing temperature beyond 35−40 °C can be attributed to anthocyanin degradation at high temperatures. This is in full agreement with the findings of Pifferi and Vaccari (*24*). Both degradation of anthocyanins (*28*) and increased permeability of membranes (*38*) have been attributed to high temperatures, and together these factors would result in confounded effects on anthocyanin extraction. However, the results presented in **Figure 4** appear to indicate that degradation had a greater effect than permeability. An increase in extracted anthocyanins by ~15 times over the initial values was reported during water extraction of anthocyanins at 60 °C from lowbush blueberry (*35*). The results of this study showed that the amounts of anthocyanins extracted at 60 °C were lower than values obtained at 25 °C in the whole range of S/S. Anthocyanins were also affected by S/S. There was an almost linear increase in anthocyanins with the increase of S/S in the range used in the experiment (**Figure 4**). Increase of S/S would favor the extraction of anthocyanins by modifying the concentration gradient, thus increasing the diffusion rate.

Surface response models for partition coefficients and equilibrium times of total phenolics and anthocyanins were statistically significant (**Table 3**). The major effect on the partition coefficients was due to changes in S/S. Equilibrium times for anthocyanins and total phenolics were mainly affected by SO_2 concentration and temperature. The same variables affected equilibrium times in the extraction of phenolics using ethanol−water solvents (*31*).

Response surfaces for the equilibrium time of total phenolics and anthocyanins were similar, and both models showed a large increase in the extraction time when temperature was reduced (plot not shown). A longer extraction time was associated with a lower diffusion rate caused by lower temperatures. Lower SO_2 concentrations also resulted in longer equilibrium times. At higher SO_2 concentrations the favorable effect on diffusion by the SO_2 might have compensated for the reduction of extraction rate caused by the lower temperature preventing a higher increase in extraction time. Minimum extraction times were obtained at an SO_2 concentration of about 900 ppm and 65 °C for anthocyanins at S/S of 10 mL/g and 900 ppm and 60 °C for total phenolics at a S/S of 45 mL/g. Extraction of anthocyanins at a temperature ≤35 °C would allow a minimum time from 40 to 100 min depending on the SO_2 concentration in the range of 1300−200 ppm, respectively. An increase of SO_2 concentration would have reduced the dielectric constant of the solvent and thus reduced solvation of molecules. Extraction with sulfured water resulted in very short extraction times to obtain a high anthocyanin recovery, which varied from approximately 60 to 90%. However, the recovery for total phenolics was considerably lower and ranged from 26 to 62%. This may have been caused by the high absorbance readings at 280 nm attributable to interference of nonphenolic compounds in the determination of composition of berries as mentioned previously. Equilibrium times for anthocyanins varied from 20 to 136 min and those for total phenolics from 10 to 135 min (**Table 3**). Under similar extraction conditions, equilibrium times for extraction with aqueous ethanol were considerably longer (26−328 min) (*31*). Times from 10 to ~60 min were required to reach a complete extraction of anthocyanins from very refined sunflower hulls (0.42−0.85 mm) (*23*). Short equilibrium times indicate a very high extraction rate; however, it should be noted that effects of SO_2 concentration or temperature dictate careful selection of extraction time to maximize yields.

Surface response plots for the antioxidant index had a saddle shape (**Figure 5**). The index was affected by all three experimental variables evaluated. The main variable affecting the index, however, was S/S. The higher the S/S, the lower the antioxidant index. Higher S/S yielded more dilute extracts,

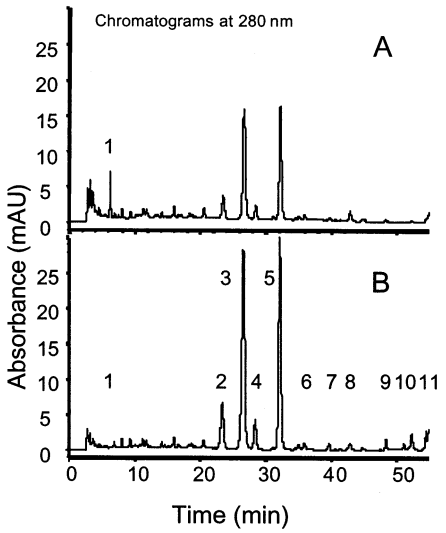

Figure 6. HPLC chromatograms at 280 nm of black currant extracts prepared using a solvent to solid ratio of 40 mL/g dwb of frozen berries and an SO_2 concentration of 700 ppm at (A) 74 °C and (B) 6 °C. Peaks: 1, phenolic acid; 2, delphinidin 3-glucoside; 3, delphinidin 3-rutinoside; 4 cyanidin 3-glucoside, 5, cyanidin 3-rutinoside; 6, 7, 10, and 11, acylated anthocyanins (*31*).

which may explain the lower antioxidant indices. Antioxidant activity as measured with the β-carotene method did not show differences among treatments. However, temperature and S/S affected antiradical activity measured by the DPPH method. The highest antiradical activity was obtained from extractions using a combination of high S/S and temperature. Antiradical activity ranged from −3.5 to −6.1 μM DPPH/μM antioxidant expressed as chlorogenic acid. Antioxidant activity of extracts compared favorably with the activity of well-known antioxidants used as reference such as α-tocopherol (−1.71 μM DPPH/μM) and BHT (−2.75 μM DPPH/μM). Differences in antiradical activity are related to variations in extract compositions. Some compositional changes were observed in samples extracted at various temperatures, and to further investigate this effect, samples from extractions at 6, 40, and 74 °C were analyzed by HPLC.

Phenolic determination by HPLC showed a decrease in anthocyanins with an increase in temperature (**Figure 6**). As seen in **Figure 4**, anthocyanin extraction decreased with the increase in extraction temperature beyond ∼35−40 °C. From the HPLC determination, a decrease in anthocyanins of ∼53% was recorded when samples extracted at 74 °C were compared to samples extracted at 6 °C. Contents of all four major anthocyanins (peak 2, delphinidin 3-glucoside; peak 3, delphinidin 3-rutinoside; peak 4, cyanidin 3-glucoside; and peak 5, cyanidin 3-rutinoside) found in black currant (*11*, *13*, *31*) decreased by the same percentage when the temperature was increased from 6 to 74 °C. Minor anthocyanins (peaks 6, 7, 10, and 11) were present in negligible amounts at 74 °C.

In addition to the reduction in anthocyanins, HPLC chromatograms at 280 (**Figure 6**) and 320 nm of extracts processed at 74 °C showed a decreases of about 48 and 21% of total phenolics and tartaric esters, respectively, as compared to values at 6 °C. A lower number of peaks and reduced peak areas were observed in samples extracted at 74 °C than in samples extracted at 6 °C. An increase of phenolic acids and tartaric ester extraction by effect of the temperature has been found in the extraction of phenolic compounds using aqueous ethanol from black currants (*31*). Thus, at high temperature the extraction of those compounds with ethanol was higher than extraction with sulfured water.

Conclusions. Addition of sodium metabisulfite to water improved the extraction of total phenolics from milled frozen black currants. Maximum yield of phenolics was obtained at an SO_2 concentration of 1000−1200 ppm. Times to reach equilibrium for the extraction of total phenolics and anthocyanins were reduced by increasing the extraction temperature. However, for the extraction of anthocyanins it is recommended that temperatures of 30−35 °C be used, as higher temperatures will degrade these compounds. Increasing the S/S improved total phenolics and anthocyanins extraction, and the maximum yield was obtained with 19 L of solvent/kg of milled frozen berries.

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