Characterization of Phenolic Profiles of Northern European Berries by Capillary Electrophoresis and Determination of their Antioxidant Activity

SILLE EHALA,* MERIKE VAHER, AND MIHKEL KALJURAND

Tallinn University of Technology, Department of Chemistry, Faculty of Science,
Akadeemia tee 15, 12618 Tallinn, Estonia

Berries are known to contain phenolic substances (i.e., flavonoids and phenolic acids), which comprise two large and heterogeneous groups of biologically active nonnutrients. This investigation evaluated the content and profile of the phenolic compounds present in six different berries found in Northern Europe. The latter included bilberry (*Vaccinium myrtillus*), cowberry (*Vaccinium vitis-idaea*), cranberry (*Vaccinium oxycoccus*), strawberry (*Fragaria ananassa*), black currant (*Ribes nigrum*), and red currant (*Ribes rubrum*). The study was focused on two areas. The first involved the extraction and analysis of berries for total phenolic content and determination of their antioxidant activity. The total phenolic level of berries was correlated with their antioxidant activity. Second, the berry extracts were analyzed by capillary electrophoresis to determine the content and profile of selected bioactive compounds. The analytes of interest included *trans*-resveratrol, cinnamic acid, ferulic acid, *p*-coumaric acid, quercetin, and morin.

KEYWORDS: Berries; polyphenols; capillary electrophoresis; antioxidant activity

1. INTRODUCTION

Different studies have shown that the free radicals present in humans cause oxidative damage to different molecules such as lipids, proteins, and nucleic acids and are thus involved in the initiation phase of some degenerative illnesses. As a consequence, those antioxidant compounds that are capable of neutralizing free radicals may play an important role in the prevention of certain diseases, such as cancer and cardiovascular diseases (*1*). The principle function of antioxidants is in delaying the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions by free radicals, and they may reduce oxidative damage to the human body.

Several studies have shown that fruits, including berries, exhibit high antioxidant potential (*2, 3*). Recent works demonstrate that, besides being rich in vitamins and provitamins (L-ascorbic acid, tocopherols, and carotenoids), fruits and vegetables are also one of the most important sources of phenolic compounds in our diets. The phenolic substances can be classified into simple phenols, phenolic acids (cinnamic and benzoic acid derivatives), flavonoids, stilbenes, coumarins, hydrolyzable and condensed tannins, lignans, and lignins. The antioxidant activity of dietary polyphenolics, such as flavonoids and phenolic acids, is considered to be much higher than that of essential vitamins, therefore contributing significantly to the health benefits of fruits (*4*).

A number of methods have been developed and applied for measuring the antioxidant activity and total phenolic content of plant extracts (*5*). However, to separate, identify, and quantify the individual phenolic components that influence antioxidant capacity, chromatographic techniques, especially high-performance liquid chromatography (HPLC), are useful (*6*).

Capillary electrophoresis (CE) is a relatively new separation technique that can offer an alternative to HPLC, the most widely used method for the analysis of phenolic compounds. CE has several advantages over HPLC: (i) very small sample size requirement, (ii) high efficiency due to the nonparabolic fronting, (iii) shorter analysis time, (iv) low cost, and (v) use of no or only a small amount of organic solvent (*4*). In addition to the more commonly used capillary zone electrophoresis (CZE) (*7, 8*), micellar electrokinetic chromatography (*9*) and nonaqueous capillary electrophoresis (*10, 11*) also have been used for the analysis of polyphenols.

The determination of polyphenolic compounds in berry samples usually requires preconcentration procedures prior to the CE analysis. This is because berry matrixes are very complex, and many phenolic compounds are present in them at very low concentrations. Berries contain high amounts of sugar, organic acids, and pectin. Therefore, the solid-phase extraction (SPE) has been rather widely used to separate these compounds and to obtain the desired extract (*12, 13*).

Domestic berries, both wild and cultivated species, are consumed in abundance in Nordic countries. Although some studies have been conducted on these berries in terms of their

* Corresponding author. Telephone: +372 620 4359; fax: +372 620 2828; e-mail: sille@brosven.ee.

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antioxidant capacity and phenolic profile, most studies have been focused on their total phenolic and anthocyanin contents. Thus, considering the growing interest in the potential nutritional properties of fruits and berries, it is relevant to characterize wild and cultivated berries most commonly consumed in Northern Europe. This study concentrated on two aspects of differences in polyphenolic content between six berry species. First, differences in the total phenolic content (TPH) and antioxidant activity between berry species as measured by commonly accepted spectrophotometric assays were examined. Second, this initial analysis was expanded by CE to describe differences in the content and profile of the selected flavonoid, stilbene, and phenolic acid components present in Northern European berries. To decrease the matrix effect and to determine the content of flavonoids and phenolic acids at low concentrations, the SPE treatment was used before the CE analysis.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents.

2,2′-Azinobis-(3-ethyl-benzothiazoline-6-sulfonate) (ABTS), potassium persulfate, ferric chloride, potassium ferricyanide ($K_3[Fe(CN)_6]$), hydrochloric acid, quercetin (3′,4′,5,7-tetrahydroxyflavonol), morin (2′,3,4′,5,7-pentahydroxyflavone), catechin (5,7,3′,4′-tetrahydroxy-flavane), cinnamic acid (3-phenyl-2-propenoic acid), ferulic acid (*trans*-4-hydroxy-3-metoxycinnamic acid), *p*-coumaric acid (*trans*-4-hydroxy-cinnamic acid), caffeic acid (3,4-dihydroxycinnamic acid), gallic acid (3,4,5-trihydroxybenzoic acid), chlorogenic acid (1,3,4,5-tetrahydroxy-cyclohexanecarboxylic acid 3-(3,4-dihydroxycinnamate), *trans*-resveratrol (3,4′,5-trihydroxy-trans-stilbene), and L-ascorbic acid, as well as sodium tetraborate and sodium hydroxide were from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Methanol was purchased from Rathburn Chemicals Ltd. (Walkerburn, Scotland). All chemicals were used without any further purification. The stock solutions of polyphenols were prepared by dissolving these compounds in a CH_3OH/H_2O (70:30) mixture at a concentration of 1 mg/mL; working standard solutions were obtained by diluting the corresponding stock solutions with the same mixture. In preparation of all solutes, deionized water (MilliQ, Millipore S. A. Molsheim, France) was used. All the samples, including standard solutions, were filtered through a 0.45 μM syringe filter before analysis. The chemical structures of the analytes studied are shown in **Figure 1**.

2.2. Sample Preparation.

All berry samples were either purchased from a local grocery store or collected ripe near Tallinn, Estonia at the end of August 2004. The berries were stored in a freezer (-18 °C) until analysis. The species studied included bilberry (*V. myrtillus*), cowberry (*V. vitis-idaea*), cranberry (*V. oxycoccos*), strawberry (*F. ananassa*), black currant (*R. nigrum*), and red currant (*R. rubrum*).

Two different methods were tested for the extraction of berries. The methods used were the microwave-assisted extraction (MAE) and ultrasonic extraction. For analysis, a weighed portion (12.5 g) of frozen berries was squeezed and extracted 3 times by applying microwave power 180 W for 3 min with 25 mL of a C_2H_5OH/H_2O (70:30) mixture. For each berry species, the final amount of the extract obtained was 37.5 mL. After extraction, all berry samples were filtered and kept at $+4$ °C until analyzed.

The ultrasonic extraction was performed with mixtures of CH_3OH/H_2O (50:50), CH_3OH/H_2O (70:30), and C_2H_5OH/H_2O (70:30). To all extraction mixtures, 1% HCl was added. For analysis, a weighed portion (50 g) of frozen berries was squeezed and extracted 3 times in an ultrasonic bath at room temperature for 8 min, with 100 mL of extraction mixture. The extracts were kept for 16 h at $+4$ °C, and then filtered and stored at $+4$ °C until analyzed. In CE measurements, 20 mg of l-ascorbic acid was added to the extraction mixture to reduce the degradation of polyphenols in the extracts over time. For each berry species, the final amount of the extract obtained was 150 mL.

2.3. Total Phenolic Content.

The concentration of total phenols in the extracts was measured according to the Price and Butler method, which is based on the colorization reaction (*14*), using tannic acid as a standard. In short, 250 μL of the berry extract or standard was added to 25 mL of the deionized water and mixed. Then, 3 mL of the ferric chloride ($FeCl_3$) reagent was added, and the mixture again mixed. After 3 min, 3 mL of the potassium ferricyanide ($K_3Fe(CN)_6$) reagent was added with mixing. The samples were incubated at room temperature for a total of 18 min. The absorbance was read at 720 nm using a Jasco V-530 UV–vis spectrophotometer. The results are expressed in milligrams of tannic acid equiv per 100 g of frozen berries (mg of TAE/100 g of FW). The regression equation and the correlation coefficient for the calibration curve are $y = (0.020\pm0.001)x + (0.081\pm0.001)$, $R^2 = 0.985$, where y is the absorption and x the concentration of tannic acid (mg/L).

2.4. Determination of Antioxidant Activity.

To determine the antioxidant activity of berry extracts, an ABTS radical cation decolorization assay was used. The assay was carried out using a Jasco V-530 UV–vis spectrophotometer. This assay has already been widely applied to the determination of the antioxidant capacity of different plant extracts (*15*). It is based on the determination of the relative ability of antioxidants to scavenge a radical cation 2,2′-azinobis-(3-ethyl-benzothiazoline-6-sulfonate) (ABTS•$^+$). The radical was generated through the reaction between ABTS and potassium persulfate (*16*). A stock solution of 7 mM ABTS was prepared in water. To this solution, potassium persulfate (the final concentration 2.45 mM) was added, and the solutions were allowed to react at room temperature in the dark for 12 h. The (ABTS•$^+$) solution was diluted with ethanol to obtain an absorbance of 0.70 $\pm$ 0.02 at 734 nm. The L-ascorbic acid solution and berry extracts were separately mixed with the (ABTS•$^+$) solution and allowed to react until the absorbance reached a plateau (15 min). The antioxidant capacities were determined by comparing the change in absorbance at 734 nm in a reaction mixture containing the berry extract with that containing L-ascorbic acid (AA). The results were expressed as μM AA equiv per 100 g of frozen berries (μM AEAC/100 g of FW).

2.5. Solid-Phase Extraction.

The off-line concentration process before the CE analysis was carried out using 2 g of C-18 cartridges (Mega Bond Elut, Varian). The C-18 cartridge was conditioned with 15 mL of CH_3OH and washed with H_2O. Then, 5 mL of the sample solution, obtained after ultrasonic extraction of berries, was loaded into the cartridge and, using a syringe attached to the outlet, pulled with a low flow rate (3–5 min). After washing the cartridge with H_2O, the phenolic compounds retained were eluted with methanol. The first fraction with a volume of 0.5 mL was introduced for analysis to CE.

2.6. Determination of Individual Polyphenols in Berry Extracts by Capillary Electrophoresis.

2.6.1. Instrumentation.

Capillary electrophoresis analyses were performed using an in-house built CE system equipped with a fused silica capillary (Polymicro Technologies, Phoenix, AZ), 55 cm (effective length 39 cm) × 50 μm i.d., high voltage power supply (Spellmann, Hauppauge, NY), and UV detector (Prince Technologies). The UV detector was coupled to a personal computer. Data acquisition was done by the software written in-house, using a Labview program (National Instruments, Austin, TX). The software recorded the detector signal via an ADAM 4018/4060 interface (Advantech Inc., Taipei, Taiwan).
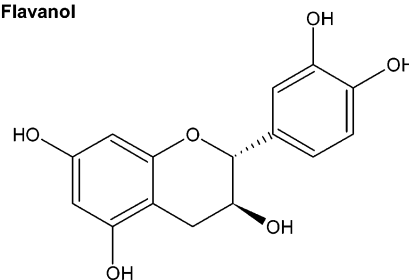
2.6.2. Methodology.

Several research groups have successfully used the borate buffer for the separation of phenolic compounds by capillary electrophoresis (*8, 17*). The separation of polyphenols in CE is based on differences in mass-to-charge ratios of these compounds and on their complex formation with tetraborate molecules when the phenolic compound has *o*-hydroxy groups (*4*). It is known that differences in conductivities between the sample and the separation buffer may cause the widening of the sample zone. Therefore, to avoid the latter and to improve the resolution of analytes, we added 5% of methanol to the separation buffer as the berry extracts also contained methanol. Thus, the CZE separation was performed with 35 mM sodium tetraborate (pH 9.3) containing 5% v/v methanol using an applied voltage of 20 kV at 25 °C. The detection was performed at 210 nm. The standards and samples were injected into the capillary gravitationally at a fixed time of 20 s from

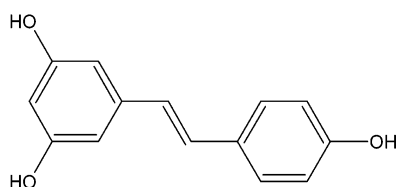**6486** *J. Agric. Food Chem.,* Vol. 53, No. 16, 2005

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Flavonols
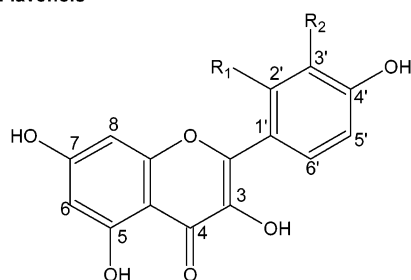
Flavanol

Morin R_1=OH, R_2=H

Quercetin R_1=H, R_2=OH

(+)-Catechin

Cinnamic acid derivatives

Stilbene

trans - Resveratrol
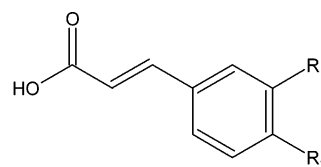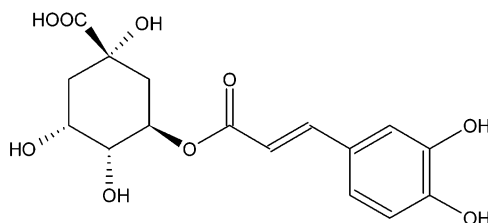
Cinnamic acid

Ferulic acid R_1=OCH$_3$, R_2=OH

p-Coumaric acid R_1=H, R_2=OH

Caffeic acid R_1=R_2=OH

Benzoic acid derivative
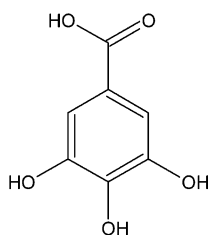
Gallic acid

Chlorogenic acid

Figure 1. Structural formulas of the compounds analyzed.

15 cm. The capillary was conditioned prior to use with 1 M NaOH for 20 min and with H$_2$O for 30 min. After each run, the capillary surface was regenerated by sequential washing with 0.1 M NaOH, H$_2$O, and the separation buffer for 5 min each.

3. RESULTS

3.1. Extraction of Berry Samples.

The yields of flavonoids and phenolic acids by the ultrasonic extraction were higher than by MAE (the results are not shown). The yields of flavonoids and phenolic acids increased with the increasing amount of the methanol in the solvent. The highest yield of polyphenols was achieved by the ultrasonic extraction with 70% methanol, which was chosen for further study. The extracts obtained were used for TPH and antioxidant activity (AC) analysis without further concentration. To study whether the first extraction of flavonoids and polyphenolic acids was exhaustive, the berry samples were extracted for the second time in the same conditions. The concentrations of phenolics determined in both of the extracts were summed, and the amount

of each phenolic in the first extraction mixture was compared to the total. This procedure was performed in triplicate for each berry species. The recoveries varied from 60% for quercetin to about 100% for *trans*-resveratrol, *p*-coumaric acid, and ferulic acid (**Table 1**).

3.2. Total Phenolic Content and Total Antioxidant Capacity of Berries.

The total phenolic content of the berries studied is shown in **Table 2**. It ranged from 10.33 to 43.43 mg of TAE/100 g of FW. Red currant exhibited the lowest total phenolic level (10.33 mg of TAE/100 g of FW), whereas cowberry and bilberry contained the highest levels of total phenolics (35.95 and 43.43 mg of TAE/100 g of FW, respectively).

The relative antioxidant capacities of berries are presented in **Table 2**. Samples of bilberry (1.89 μM/100 g of FW), followed by cowberry (1.76 μM/100 g FW) and black currant (1.27 μM/100 g of FW) had the highest antioxidant capacity on the basis of the weight of the frozen berries.

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Table 1. Recoveries of Quantitatively Determined Polyphenols After the Solvent Extraction

analyte	recovery (%)[a]	R.S.D. (%)
trans-resveratrol	97.8	5.57
cinnamic acid	75.5	6.07
ferulic acid	96.8	4.11
p-coumaric acid	98.5	6.63
quercetin	59.9	6.22
morin	90.9	3.58

[a] To determine the recoveries of individual polyphenols, the berry samples were extracted twice in an ultrasonic bath, and their content in both extracts was determined by CE. Finally, the amount (%) of individual phenolics found in the first extract in relation to their amount in combined extract was determined. *n* = 3.

Table 2. Total Phenolic (TPH) Content and Antioxidant Capacity (AC) of Various Berries[a]

| berries	TPH[b] (mg of TAE/100 g)	AC[c] (μM AEAC/100 g)
red currant	10.33 ± 1.63	0.57 ± 0.06
cranberry	18.08 ± 1.13	0.84 ± 0.03
strawberry	15.74 ± 0.90	0.86 ± 0.10
black currant	25.80 ± 3.59	1.27 ± 0.03
cowberry	35.95 ± 2.02	1.76 ± 0.05
bilberry	43.43 ± 5.83	1.89 ± 0.04

[a] The results are presented as a mean ± standard deviation (SD) for triplicate analysis. [b] TPH results are expressed in tannic acid equivalents (TAE). [c] AC results are expressed in L-ascorbic acid equivalents (AEAC).

3.3. Effect of SPE on Phenolic Profiles.

Kähkönen et al. (*12*) reported that the sugar removal with a Bond Elut C-18 SPE cartridge reduced also the amount of phenolics in berry samples (5−11% reduction of total phenolics was found). The highest losses were among hydroxybenzoic acids and flavanols. Zheng (*13*) also reported the reduction of the antioxidant capacity of the berries after the SPE treatment, claiming that it could be partly attributed to the loss of some water-soluble constituents of berry extracts (e.g., sugars, acids, ascorbic acid, glutathione, and other water-soluble compounds). However, sugars and organic acids showed no antioxidant activity with several methods, and only ascorbic acid and glutathione were shown to exhibit antioxidant activity.

Re et al. (*16*) reported the antioxidant activities for various polyphenols and also for ascorbic acid and glutathione. The antioxidant activities were determined with ABTS•[+] decolorization assay and expressed as Trolox equivalent antioxidant capacity (TEAC). The reported TEAC values for phenolics found in the current study were quercetin 3.10 ± 0.05, *p*-coumaric acid 2.00 ± 0.07, ferulic acid 1.90 ± 0.05, and caffeic acid 0.98 ± 0.06. The TEAC values for ascorbic acid and glutathione were 1.05 ± 0.02 and 1.28 ± 0.04, respectively.

Thus, it may be concluded from the previous information that nonphenolic compounds that are removed during the SPE procedure have a low effect on the antioxidant activity of berries. Moreover, during the SPE process, some of the phenolics are concentrated, but others are removed. Consequently, in our studies, the TPH content and antioxidant activity of berry extracts were determined before the SPE treatment, which, in our opinion, enables characterization of berries more authentically. However, the SPE treatment was used before the CE analysis, to decrease the matrix effect and simplify the electropherograms. The effect of the SPE treatment was tested on a standard mixture of phenolics. It can be seen in **Figure 2** that after the SPE treatment the content of flavanol (±catechin),
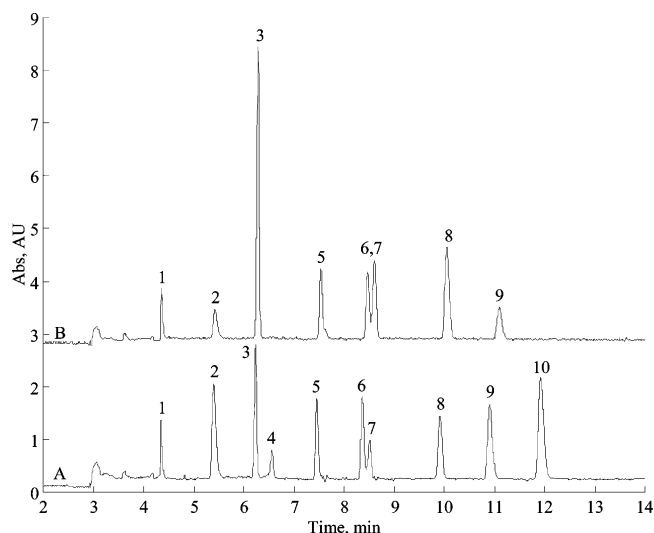
Figure 2. Electropherograms of standard compounds before (**A**) and after SPE (**B**). Peaks: 1, *trans*-resveratrol; 2, (+)-catechin; 3, cinnamic acid; 4, chlorogenic acid; 5, ferulic acid; 6, *p*-coumaric acid; 7, quercetin; 8, morin; 9, caffeic acid; and 10, gallic acid. Experimental conditions: 35 mM sodium tetraborate, pH 9.3, voltage 20 kV, detection wavelength 210 nm.

Table 3. Recoveries of Quantitatively Determined Polyphenols After the SPE Procedure[a]

analyte	recovery (%)	R.S.D. (%)
trans-resveratrol	95.6	3.50
cinnamic acid	221.9	4.94
ferulic acid	92.4	3.80
p-coumaric acid	88.3	3.33
quercetin	236.6	4.95
morin	151.2	3.41

[a] To determine the influence of SPE treatment, the standard mixture of polyphenols was analyzed by CE before and after passing it through a SPE cartridge. Then, the recoveries of individual phenolics were calculated using the peak areas. The concentration of each polyphenol in the standard solution was 5 μg mL^{-1}. *n* = 3.

Table 4. Reproducibility of CZE Method for the Determination of Polyphenols[a]

	R.S.D.[b] (%)			
	peak area		migration time	
analyte	run-to-run	day-to-day	run-to-run	day-to-day
trans-resveratrol	1.96	6.34	2.02	2.32
cinnamic acid	1.67	5.74	1.74	2.60
ferulic acid	1.48	4.95	2.06	2.10
p-coumaric acid	3.19	3.90	2.77	1.89
quercetin	3.68	5.74	2.43	1.85
morin	1.23	6.72	2.02	1.71

[a] The separation was performed with 35 mM borate buffer, pH 9.3 in 55 cm (effective length 39 cm) × 50 μm capillary. [b] *n* = 5.

some of the hydroxycinnamic acids (caffeic and chlorogenic acid) and hydroxybenzoic acid (gallic acid) in the sample mixture was either remarkably reduced or the compounds were completely lost. However, cinnamic acid, quercetin, and morin were concentrated during the SPE process (**Table 3**). SPE proved to be well-suited also for the determination of *trans*-resveratrol, ferulic acid, and *p*-coumaric acid. Recoveries of these compounds varied from 88 to 96% (**Table 3**). Therefore,

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Table 5. Quantitative Results of the Polyphenols Studied by CE

analyte	linear range	$y = b_0 + b_1 c^a$	R^2	LOD	LOQ
trans-resveratrol	0.60–90.0	$b_0 = -0.0038 \pm 1.61 \times 10^{-3}$ $b_1 = 3.4750 \pm 4.03 \times 10^{-2}$	0.9989	0.40	1.32
cinnamic acid	0.40–0.70	$b_0 = -0.0032 \pm 4.17 \times 10^{-3}$ $b_1 = 8.6019 \pm 0.14$	0.9981	0.12	0.42
ferulic acid	0.50–50.0	$b_0 = -0.0066 \pm 8.49 \times 10^{-3}$ $b_1 = 4.0330 \pm 0.37$	0.9982	0.15	0.50
p-coumaric acid	0.60–70.0	$b_0 = -0.0041 \pm 4.62 \times 10^{-3}$ $b_1 = 7.9850 \pm 0.16$	0.9973	0.25	0.85
quercetin	0.60–70.0	$b_0 = -0.0034 \pm 5.54 \times 10^{-3}$ $b_1 = 8.5288 \pm 0.18$	0.9966	0.27	0.90
morin	0.60–90.0	$b_0 = -0.0012 \pm 6.05 \times 10^{-3}$ $b_1 = 8.4565 \pm 0.15$	0.9974	0.30	0.99

[a] b_0, the intercept; b_1, the slope; LOD, the limit of detection; and LOQ, the limit of quantification. LOD and LOQ are in μg mL^{-1}. Experimental conditions as in **Table 4**.

only these compounds were determined quantitatively by CE. All results were corrected for recoveries.

3.4. Determination of Polyphenolic Profiles by Capillary Zone Electrophoresis.

**3.4.1. Performance of the Method.**

To evaluate the precision of the method, the reproducibility of the migration time and peak area were determined for each compound for run-to-run and day-to-day by multiple injections of a single solution of all phenolic compounds (5.0 μg mL^{-1} each analyte). The relative standard deviations (R.S.D.) of the migration times and peak areas obtained are presented in **Table 4**.

For quantitative determination, the calibration graphs were produced from the results obtained by injecting standard solutions in the range of 0.10−90 μg mL^{-1}. Each point of the calibration graph corresponded to the mean value obtained from three independent area measurements. The limits of detection (LOD) and quantification (LOQ) were obtained by multiplying the standard deviation of five measurements of the standard solution (10.0 μg mL^{-1}) by three and ten, respectively. The results of the detection limit and other characteristic parameters for the determination of phenolic compounds are shown in **Table 5**.

3.4.2. Quantitative Determination of Polyphenols in Berry Extracts.

The representative electropherograms of red currant, strawberry, black currant, cranberry, bilberry, and cowberry are presented in **Figure 3**. Individual flavonoids, phenolic acids, and stilbene in the electropherograms obtained were identified by spiking experiments, in which the addition of standards to the sample solutions resulted in an increase of the analyte peak without the appearance of shoulders or split peaks. The compounds of interest were quantified using calibration standards, and the concentrations obtained were verified by the standard addition method. The analytical results for the presence and quantity of the phenolics studied by CE are reported in **Table 5** and are expressed as μg per g of frozen berry extract. All the berries studied, except black currant, contained *trans*-resveratrol at levels of 3.57−30.00 μg/g of FW. Cowberry (30.00 μg/g of FW) had the highest content of *trans*-resveratrol followed by cranberry (19.29 μg/g of FW) and red currant (16.66 μg/g of FW).

The concentration of flavonoids and phenolic acids in the berries studied varied considerably (**Figure 3**). The predominant phenolic acid present in the berries studied was *p*-coumaric acid. Cranberry (20.28 μg/g of FW) and cowberry (17.10 μg/g of FW) had the highest levels of *p*-coumaric acid. However, the
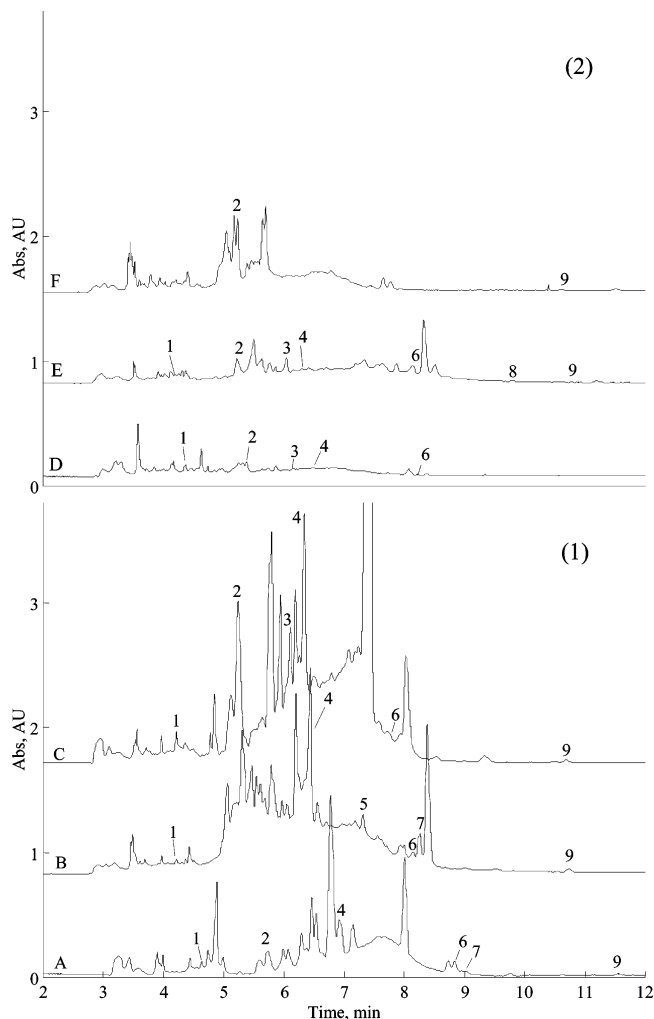
Figure 3. Electropherograms of wild (**1**): A, cranberry; B, bilberry; and C, cowberry and cultivated berries. (**2**): D, red currant; E, strawberry; and F, black currant. The numbered analytes and CZE conditions are as in **Figure 2**.
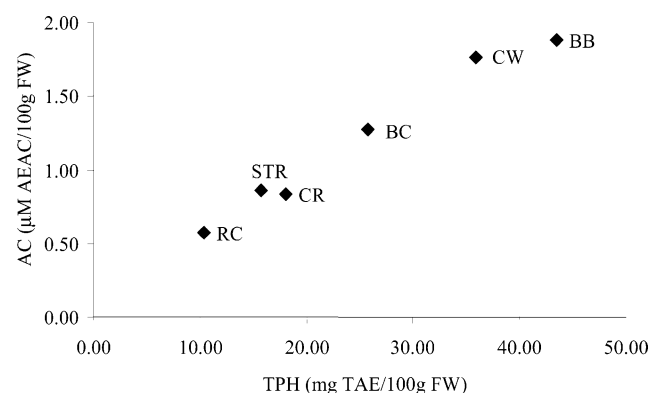
Figure 4. Linear correlation between total phenolic content and antioxidant capacity of berries. AC (antioxidant capacity) and TPH (total phenolic content) are expressed in tannic acid equivalents (TAE) and L-ascorbic acid equivalents (AEAC), respectively. RC, red currant; STR, strawberry; CR, cranberry; BC, black currant; CW, cowberry; and BB, bilberry.

predominant phenolic acid present in the bilberry was ferulic acid (23.01 μg/g of FW), whereas in the remaining five species of berries this compound was not detected. Cowberry, however, contained a substantial amount of cinnamic acid (41.2 μg/g of FW). The latter was also found in strawberry (10.81 μg/g of FW) and red currant (1.09 μg/g of FW).

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Table 6. Stilbene, Flavonoid, and Phenolic Acid Content of Berries[a]

	berries					
analyte (μg/g) of FW	bilberry	cowberry	black currant	cranberry	strawberry	red currant
trans-resveratrol	6.78 ± 0.18	30.00 ± 2.8	N.D.	19.29 ± 1.53	3.57 ± 1.05	15.72 ± 2.05
cinnamic acid	N.D.	41.2 ± 2.36	N.D.	N.D.	10.81 ± 1.36	1.03 ± 0.32
ferulic acid	23.01 ± 1.69	N.D.	N.D.	N.D.	N.D.	N.D.
p-coumaric acid	6.00 ± 0.18	17.1 ± 1.68	N.D.	20.28 ± 1.35	12.48 ± 0.76	1.89 ± 0.51
caffeic acid	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.	N.D.
chlorogenic acid	N.Q.	N.Q.	N.D.	N.Q.	N.Q.	N.Q.
quercetin	12.75 ± 0.70	N.D.	N.D.	5.15 ± 0.40	N.D.	N.D.
morin	N.D.	N.D.	N.D.	N.D.	0.60 ± 0.07	N.D.
catechin	N.D.	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.

[a] The results are presented as a mean ± SD for triplicate analysis. N.D., not detected and N.Q., not quantified. Experimental conditions as in **Table 4**.

The predominant flavonol present in the berries studied was quercetin that was found in bilberry (12.75 μg/g of FW) and cranberry (5.15 μg/g of FW). However, morin was found in strawberry only (0.60 μg/g of FW).

4. DISCUSSION

As seen in **Figure 4**, the total phenolic content is well-correlated to the antiradical measures (TPH vs AC, $R^2 = 0.981$), which is in accordance with previously reported results (*13*). Therefore, it is according to expectations that bilberry and cowberry, which were found to contain the highest TPH levels, would also exhibit the greatest antiradical behavior. Conversely, red currant exhibited the lowest levels of TPH and antiradical capacity.

One aim of this investigation was to compare the TPH and antioxidant capacity of the six species studied to determine the magnitude of the differences between species. The differences between species illustrated by the results (**Figure 4**) are similar to those of Kähkönen (*12*). We also found that bilberry exhibited the highest level of total phenolics followed by cowberry, black currant, strawberry, cranberry, and red currant. The TPH results obtained by Kähkönen (*12*) were, however, given in gallic acid equivalents per 100 g of dry weight, and thus, the particular values are not comparable to the values reported herein. For determination of antioxidant activities of berries, various assays with different reference antioxidants such as trolox, L-ascorbic acid, or α-tocopherol have been used; therefore, the results are difficult to compare. Nevertheless, our results (**Figure 4**) are similar to those reported by Kähkönen (*12*), except for cranberry that, according to mentioned study, had the highest antioxidant capacity followed by bilberry, cowberry, black currant, strawberry, and red currant. According to our results, the antioxidant activity of cranberry was similar to that of strawberry.

The high correlation between TPHs and antioxidant capacities of the berries studied suggests that the antioxidant activity of berries is derived mainly from the content of phenolic compounds in fruits. Therefore, it would be interesting to determine which individual phenolic compounds might contribute to the total antioxidant activity of berries (i.e., to measure the phenolic profiles of different berry species).

The phenolics in berry extracts, separated and identified by using capillary zone electrophoresis (CZE), are presented in **Table 6**. In general, our qualitative and quantitative data for the phenolic content in berries are in accordance with earlier reports (*18−20*). According to Häkkinen et al. (*21*), *p*-coumaric acid, ferulic acid, and quercetin were the most abundant phenolics in bilberries. The same compounds were found in our studies. The high content of chlorogenic acid in bilberry and cowberry (**Figure 3**) was not quantitatively measured. Chloro-
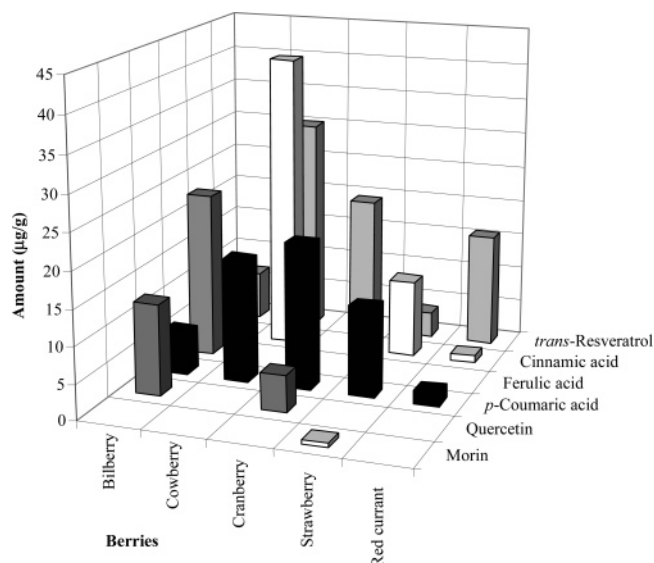
Figure 5. Polyphenolic content of various berries.

genic acid was also detected in cranberry. This is in agreement with the study of Taruscio et al. (*20*), who reported the *Vaccinium* species to contain significant amounts of chlorogenic acid. The main phenolic compounds identified in cranberry were *p*-coumaric acid, *trans*-resveratrol, and quercetin (**Figure 5**). Taruscio et al. (*20*) have also reported high levels of quercetin and *p*-coumaric acid in cranberries. However, the contents of these compounds were reported to be higher as compared to the levels given in this work. These diversities in quercetin content may be due to differences in varieties or cultivars. According to Bilyk (*18*), the variation in flavonol content between six cranberry varieties was large (50−70%). The level of *p*-coumaric acid in cranberries varies, according to different studies, between 0 and 100 μg/g of fresh weight (*13, 20*). According to Häkkinen et al., the main phenolic compound in strawberry is ellagic acid followed by *p*-coumaric acid (*21*). In this study, the concentration of ellagic acid was not determined. The concentration of *p*-coumaric acid was found to be similar to that reported earlier (*22, 23*). Strawberry was the only berry species among those studied in which the flavonol morin was detected. In red currant, an abundant phenolic was *trans*-resveratrol followed by *p*-coumaric and cinnamic acid, but this is not in agreement with the study of Häkkinen et al. who found the content of quercetin to be the highest (*21*). However, the *p*-coumaric acid content determined in this study was similar to that reported by Schuster (*23*). In the study of Justesen (*24*), similarly to cranberry, high levels of quercetin were detected in cowberry. This is not in agreement with our results. In our study, cinnamic acid, *trans*-resveratrol, and *p*-coumaric acid

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were the main phenolics quantified in cowberry. In black currant, none of the phenolics determined quantitatively in other berries was found. However, in black currant, catechin and caffeic acid, which have also been reported by others, were detected (*21, 23*). *trans*-Resveratrol, which was found in high levels in cowberry, cranberry, and red currant, and cinnamic acid, whose content was high in cowberry and strawberry, have not been reported in earlier studies.

As seen from the previous discussion, the contents of flavonoids and phenolic acids in berries vary considerably. The variation in the content of phenolic compounds within one species may be explained by the occurrence of different berry varieties in different countries (*18, 22, 23*) or by different growing conditions (soil nutrients, temperature, light) (*25*). Also, differences in berry ripeness may contribute to the variability of the reported flavonoid and phenolic acid concentrations (*22*).

In summary, bilberries, cowberries, black currants, cranberries, strawberries, and red currants contain large amounts of phenolics and have also a high antioxidant activity. The wild berries have significantly higher antioxidant activities than domestic berries and also a higher content of phenolics (except for cranberry). The CE analysis gave a comprehensive picture of the flavonoids, stilbene, and phenolic acids present in the berries studied. The polyphenolic profiles of berries obtained are the result of analysis of the antiradical components contained in berries and thus may help to provide a better understanding of the health benefits of different berries. The variations in the polyphenolic profile and antioxidant activity of various berries found may be related to their significantly different biological activities. Therefore, more detailed studies are necessary to evaluate the contribution of each phenolic to the total antioxidant activity of berries. This is generally considered to be dependent on their structure and content in berries. However, synergistic interactions between phenolics and some other components of fruits have also been observed to influence the antioxidant activity of fruits.

5. ABBREVIATIONS USED

ABTS, 2,2′-azinobis-(3-ethyl-benzothiazoline-6-sulfonate); AEAC, ascorbic acid equivalents; CE, capillary electrophoresis; CZE, capillary zone electrophoresis; FW, frozen weight; HPLC, high-pressure liquid chromatography; MAE, microwave-assisted extraction; SPE, solid-phase extraction; TAE, tannic acid equivalents; TPH, total phenolics.

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