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Compositional Differences of Phenolic Compounds between Black Currant (*Ribes nigrum* L.) Cultivars and Their Response to Latitude and Weather Conditions

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Supporting Information

ABSTRACT: Phenolic compounds in black currants of three Finnish cultivars and their response to growth latitude and weather conditions were analyzed over a six-year period. 'Melalahti' had lower contents of total phenolic compounds (31.4% and 29.2% lower than 'Mortti' and 'Ola', respectively), total anthocyanins (32.6% and 30.5%), and total hydroxycinnamic acid derivatives (23.1% and 23.8%) ($p < 0.05$) and was less affected by growth latitude and weather conditions than 'Mortti' and 'Ola'. However, all the cultivars grown at higher latitude (66°34′ N) had lower contents of total flavonols, total anthocyanins, and total phenolic compounds than those grown at lower latitude (60°23′ N) ($p < 0.05$). The content of total hydroxycinnamic acid conjugates did not vary in 'Melalahti' ($p > 0.05$) but increased as the latitude increased in 'Mortti' and 'Ola' ($p < 0.05$). Temperature and radiation were the major weather variables influencing the composition of phenolic compounds. Delphinidin-3-*O*-glucoside, delphinidin-3-*O*-rutinoside, and myricetin-3-*O*-glucoside content showed positive correlations with temperature and radiation in all three cultivars. The study gives important guidelines for the selection of raw materials and growth sites as well as for the berry cultivation for commercial exploitation of black currant berries.

KEYWORDS: *black currant, cultivar, latitude, phenolic compounds, Ribes nigrum, weather conditions*

INTRODUCTION

Phenolic compounds are a group of the most important and widespread secondary metabolites in the plant kingdom and play a variety of roles in the plant.[1] The majority of phenolic plant secondary metabolites originate from "acetate" (acetyl-coenzyme A) and the $C_6•C_3$ precursors (*p*-coumaric, ferulic, sinapic and caffeic acids) in the so-called "hydroxycinnamate pool" which are derived from α-amino acid L-phenylalanine and/or L-tyrosine.[2] The aromatic amino acids involved in the biosynthesis of phenolic compounds are derived from erythrose-4 phosphate (from the pentose phosphate pathway) and phosphoenolpyruvic acid (from the glycolysis) via the shikimic acid pathway.

Phenolic compounds are readily oxidized and in this context their possible role in the diet upon the amelioration or prevention of major chronic diseases such as cancer and atherosclerosis has received considerable attention during recent years. Their positive contributions to human health have been widely reported.[3−6] Black currants are rich sources of beneficial phenolic compounds, such as anthocyanins, flavonols, flavan-3-ols, and phenolic acids, and are regarded as health-beneficial raw food material worldwide. Black currants are commonly used for producing juice, jam, and syrup in northern Europe. Besides their contribution to nutritional values, the phenolic compounds also influence the appearance and taste of the berries and, therefore, have a final impact on the quality, palatability, and acceptability of food products derived from the berries. The rich purple-black color of black currants is characterized by the anthocyanin profile of the berries.[7] The sweetness and sourness, influencing the sensory properties and consumer acceptance, are determined by the abundance of sugars and acids. Phenolic compounds contribute to astringency and bitterness, which may have a negative impact on the sensory quality and decrease the preference of products by consumers.[8,9] Thus, an investigation of the factors influencing composition of these components is of crucial importance for further enrichment of bioactive components and for improving the sensory qualities of berries by proper cultivation, breeding, and biotechnological methods.

The contents of phenolic compounds in fruits and berries are affected by genetic differences, cultivation techniques, harvesting time, growth locations, and environmental factors.[10−15] Although phenolic compounds have been widely investigated in black currants,[8−12,16] no detailed information concerning the effects of growth latitudes and individual weather parameters on their composition has been reported. Since black currants are mainly grown under contract farming for commercial juice

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processing, it is particularly important to the processors that the essential composition and the sensory properties of the juice remain relatively constant.[17] Therefore, the less the currant cultivar is affected by weather conditions, the more suitable it is to be cultivated for commercial processing. In our previous study, we have found the black currant cultivar 'Melalahti' had higher sugar/acid ratio than cultivars 'Mortti' and 'Ola' and showed relatively constant composition of sugars and acids despite the varying weather conditions. Berries with high contents of sugars and high value of sugar/acid ratio are considered to have pleasant sensory characteristics.[9,18,19] In the present study, the composition of anthocyanins, flavonol glycosides, and hydroxycinnamic acid conjugates in black currant berries of different cultivars were compared, and the effects of growth latitude and weather conditions on the composition of these components were investigated by analyzing berries of three Finnish commercial cultivars grown at two different latitudes in Finland (latitude 60°23′ and 66°34′ N) over a six-year period. This study, together with our previous investigation on the composition of sugars, fruit acids, and ascorbic acid in the same cultivars, complements and provides comprehensive information on the correlations between the qualitative parameters of the berries and the growth latitudes and various weather conditions.

■ MATERIALS AND METHODS

Berry Samples. Three cultivars of black currant (*R. nigrum* L.), 'Mortti', 'Ola', and 'Melalahti', were cultivated in an identical way in Piikkiö, southern Finland (latitude 60°23′ N, longitude 22°33′ E, altitude 5−15 m) and Apukka, northern Finland (66°34′ N, 26°01′ E, 100−105 m) by MTT Agrifood Research Finland.[20] Twelve bushes were planted in four field blocks in May 2002, and no irrigation was applied during the study period. The berries were harvested in quadruplicate from the four field blocks in both southern and northern Finland in six consecutive years from 2005 to 2010. The berries were picked optimally ripe as defined by experienced horticulturists based on color, flavor, and structure of the berries. The harvesting date of each sample is listed in Table 1. The berries were frozen and stored at −20 °C immediately after harvesting until being analyzed. Because of the large amount of samples in this study, three out of four samples harvested in each site and year were selected randomly for the analysis.

Chemicals. Ferulic acid, caffeic acid, and *p*-coumaric acid were purchased from Sigma (St. Louis, MO). Quercetin-3-*O*-rutinoside, quercetin-3-*O*-glucoside, kaempferol-3-*O*-glucoside, delphinidin-3-*O*-glucoside, and cyanidin-3-*O*-rutinoside were purchased from Extrasynthese (Genay, France). Delphinidin-3-*O*-rutinoside and cyanidin-3-*O*-glucoside were purchased from Polyphenols (Sandnes, Norway). The acetonitrile, ethyl acetate, methanol, formic acid, and hydrochloric acid were of HPLC grade or the highest grade available.

Table 1. Harvesting Information of Black Currant (*R. nigrum* L.) Berries

growth site	cultivar		
	'Mortti'	'Ola'	'Melalahti'
Piikkiö, Finland	August 15, 2005	August 19, 2005	August 2, 2005
	August 18, 2006	August 11, 2006	August 11, 2006
	August 1, 2007	August 2, 2007	August 2, 2007
	August 14, 2008	August 7, 2008	August 4, 2008
	August 21, 2009	August 21, 2009	August 4, 2009
	August 17, 2010	August 17, 2010	July 29, 2010
Apukka, Finland	August 31, 2005	August 31, 2005	August 22, 2005
	August 14, 2006	August 16, 2006	August 10, 2006
	August 23, 2007	August 23, 2007	August 23, 2007
	September 5, 2008	September 5, 2008	September 5, 2008
	August 26, 2009	August 26, 2009	August 26, 2009
	August 18, 2010	August 18, 2010	August 18, 2010

Analysis of Anthocyanins. Anthocyanins of black currant berries were analyzed in duplicate according to a method previously applied in our laboratory with slight modifications.[8] About 40 g of berries were thawed in a microwave oven and homogenized with a Bamix mixer (Bamix M133, Switzerland) when half-melted. About 5 g of slurry was weighed accurately and extracted three times consecutively with 15 mL of MeOH/HCl (99:1). After 1.5 min of vigorous mixing, the sample was centrifuged at 3400*g* for 10 min (Beckman model J2-21, Beckman Coulter Inc., Fullerton, CA). The supernatants from three extractions were combined, and the total volume was set to 50 mL with MeOH/HCl (99:1). A portion of 1 mL of sample was taken and filtered through a syringe filter (0.45 μm, VWR International, LLC, PA) for HPLC analysis. A sample of 10 μL was injected into the HPLC-UV system consisting of a DGU-20A$_5$ degasser, an LC-20AB pump, an SIL-20AHT auto sampler, a CTO-10ACVP column oven, an SPD-20A UV detector, and a CBM-20A controller (Shimadzu Corporation, Kyoto, Japan). The samples were analyzed using a Phenomenex Prodigy RP-18 ODS-3 column (250 × 4.60 mm i.d., particle size 5 μm) (Torrance, CA) with a Phenomenex Prodigy precolumn (30 × 4.60 mm i.d., particle size 5 μm). The analysis of anthocyanins was performed using 5% formic acid as solvent A and acetonitrile as solvent B with the following gradient: 0−5 min, 5−10% B; 5−10 min, 10% B; 10−17 min, 10−24% B; 17−22 min, 24−90% B; 22−25 min, 90−5% B; 25−30 min, 5% B. The flow rate of the mobile phase was 1 mL/min. Anthocyanins were detected at 520 nm. Quantitative analysis was carried out using the commercial standards of the four major anthocyanins (delphinidin-3-*O*-glucoside, delphinidin-3-*O*-rutinoside, cyanidin-3-*O*-glucoside, and cyanidin-3-*O*-rutinoside) in black currant as external standards for detected compounds.

Analysis of Flavonols and Hydroxycinnamic Acid Conjugates. Flavonols and hydroxycinnamic acid conjugates were analyzed in duplicate with a modified method of Sandell et al.[8] About 40 g of berries were thawed in a microwave oven and homogenized with a Bamix mixer. The slurry of 5 g was weighed accurately and extracted four times consecutively with 10 mL of ethyl acetate. The samples were mixed vigorously for 1.5 min and centrifuged for 10 min at 3400*g*. The four extracts were combined, and the ethyl acetate was removed using a rotary evaporator. The sample was diluted in 3 mL of MeOH and filtered (0.45 μm) for the HPLC analysis. The analyses were performed using the same Shimadzu HPLC-UV apparatus as described above and using 1% formic acid as solvent A and acetonitrile as solvent B. The eluting gradient program was 0−20 min, 5−30% B; 20−30 min, 30−90% B; 30−35 min, 90−5% B; 35−40 min, 5% B. The flow rate of the mobile phase was 1 mL/min. Flavonols were detected at 360 nm and hydroxycinnamic acid conjugates at 320 nm. Quantitative analysis of flavonols was carried out using quercetin-3-*O*-rutinoside, quercetin-3-*O*-glucoside, and kaempferol-3-*O*-glucoside as the external standards for these compounds and quercetin-3-*O*-glucoside for other flavonol glycosides. Ferulic acid, caffeic acid, and *p*-coumaric acid were used as external standards for quantification of hydroxycinnamic acid conjugates. The total content of phenolic compounds was defined as the sum of anthocyanins, flavonols, and hydroxycinnamic acid conjugates.

Identification of Phenolic Compounds by UV−vis Spectra. The samples of anthocyanins and those of flavonols and hydroxycinnamic acid conjugates of black currants were prepared as described previously and analyzed with a HPLC-DAD system. The system consisted of an LC-10AT pump, an SIL-10A auto sampler, and an SPD-M10AVP diode array detector linked to an SCL-M10AVP data handling station. The chromatographic conditions were as described above. During the analysis, UV absorption spectra were scanned in the range of 200−600 nm. The peaks were identified by comparison of the UV absorption spectra and retention times to those of the reference compounds and the previous data obtained by our laboratory.[8,9]

Table 2. Weather Variables and Their Abbreviations Used in the Study

abbreviations	weather variables	abbreviations	weather variables
Dgs	growth season period (day)	Pjan, Pfeb... Paug, Psep	precipitation in January, Febuary... August, September (mm)
SUMTgs	temperature sum over 5 °C in growth season (°C)	Hgh	average humidity from the start of growth season until the day of harvest (%)
SUMTgh	temperature sum over 5 °C from the start of growth season until the day of harvest (°C)	Hm	average humidity in the last month before harvest (%)
SUMTm	temperature sum over 5 °C in the last month before harvest (°C)	Hw	average humidity in the last week before harvest (%)
HDgh	hot days (temperature >25 °C) from the start of growth season until the day of harvest (day)	Hjan, Hfeb... Haug, Hsep	average humidity in January, Febuary... August, September (%)
HDm	hot days (temperature >25 °C) in the last month before harvest (day)	DH20to30gh	percentage of the days with relative humidity 20–30% from the start of growth season until the day of harvest (%)
Tm	average temperature in the last month before harvest (°C)	DH30to40gh	percentage of the days with relative humidity 30–40% from the start of growth season until the day of harvest (%)
Tw	average temperature in the last week before harvest (°C)	DH40to50gh	percentage of the days with relative humidity 40–50% from the start of growth season until the day of harvest (%)
TDm	mean daily temperature difference in the last month before harvest (°C)	DH50to60gh	percentage of the days with relative humidity 50–60% from the start of growth season until the day of harvest (%)
LTm	average of daily lowest temperature in the last month before harvest (°C)	DH60to70gh	percentage of the days with relative humidity 60–70% from the start of growth season until the day of harvest (%)
HTm	average of daily highest temperature in the last month before harvest (°C)	DH70to80gh	percentage of the days with relative humidity 70–80% from the start of growth season until the day of harvest (%)
Tjan, Tfeb... Taug, Tsep	average temperature in January, Febuary... August, September (°C)	DH80to90gh	percentage of the days with relative humidity 80–90% from the start of growth season until the day of harvest (%)
Rgh	radiation from the start of growth season until the day of harvest (kJ/m^2)	DH90to100gh	percentage of the days with relative humidity 90–100% from the start of growth season until the day of harvest (%)
Rm	radiation during the last month before harvest (kJ/m^2)	DH40to50m	percentage of the days with relative humidity 40–50% in the last month before harvest (%)
Rw	radiation during the last week before harvest (kJ/m^2)	DH50to60m	percentage of the days with relative humidity 50–60% in the last month before harvest (%)
Rjan, Rfeb... Raug, Rsep	radiation in January, Febuary... August, September (kJ/m^2)	DH60to70m	percentage of the days with relative humidity 60–70% in the last month before harvest (%)
Pgh	precipitation from the start of growth season until the day of harvest (mm)	DH70to80m	percentage of the days with relative humidity 70–80% in the last month before harvest (%)
Pm	precipitation in the last month before harvest (mm)	DH80to90m	percentage of the days with relative humidity 80–90% in the last month before harvest (%)
Pw	precipitation in the last week before harvest (mm)	DH90to100m	percentage of the days with relative humidity 90–100% in the last month before harvest (%)

Measurement of Dry Weight. About 5 g of berries were weighed in a watch glass accurately in duplicate and cut with a knife. The residue on the knife was cleaned carefully with distilled water into the watch glass. The berries were dried at 105 °C and weighed accurately upon reaching a constant weight.

Information on Weather Conditions. Data recorded at the weather station in Piikkiö Yltöinen (latitude 60°23′ N, longitude 22°33′ E, altitude 6 m) and Rovaniemi Airport (66°33′ N, 25°50′ E, altitude 195 m) for the years 2005 to 2010 were provided by the Finnish Meteorological Institute (Helsinki, Finland). The weather variables and their abbreviations used in the study are shown in Table 2.

Statistical Analysis. Statistical analyses were performed by using SPSS 16.0.1 (SPSS Inc., Chicago, IL) and Unscrambler 10.1 (Camo Process AS, Oslo, Norway). Differences in the composition between black currant cultivars were investigated by a one-way analysis of variance (ANOVA). Tukey's HSD test for the population with equal variances and Tamhane's test for that with unequal variances were employed to carry out the multiple comparisons of the black currant cultivars at $p < 0.05$. Independent-samples *t*-test was used to investigate the difference between black currants grown at two latitudes. Differences reaching a minimal confidence level of 95% were considered as being statistically significant. Partial least squares - discriminant analysis (PLS-DA) was used to explain the difference between cultivars or locations according to the phenolic contents in berries. Principal component analysis (PCA) and Pearson's correlation coefficients analysis were combined to study the effects of weather conditions on the contents of phenolic compounds and the value of dry weight of each black currant cultivar. The varimax rotation method was applied to maximize the differences among variables in the PCA analysis. Pearson's correlation coefficients analysis was also carried out to investigate the intercorrelation between the metabolites in black currant berries.

RESULTS AND DISCUSSION

Compositional Analysis. Figure 1 shows the HPLC chromatograms of black currant berries of cultivar 'Ola' collected in Apukka, Finland, in 2009. The anthocyanins, flavonol glycosides, and hydroxycinnamic acid conjugates were identified as previously reported in our laboratory.[8,9] Anthocyanins (93.5–95.2% of total phenolic compounds) were the main phenolics in black currant berries (Table 3). Delphinidin-3-*O*-rutinoside, cyanidin-3-*O*-rutinoside, delphinidin-3-*O*-glucoside, and cyanidin-3-*O*-glucoside were unambiguously identified as the four major anthocyanins in black currants according to reference compounds, UV–vis spectra, mass spectra, and the literature.[8,9] Among these anthocyanins, delphinidin-3-*O*-rutinoside (47.7% and 47.5% of total anthocyanins, respectively) was detected to be the most abundant anthocyanin in black currants of 'Mortti' and 'Ola', while cyanidin-3-*O*-rutinoside (47.7% of total anthocyanins) was detected to be the most abundant anthocyanin in 'Melalahti' (Table 3). Among the flavonol glycosides detected, quercetin-3-*O*-glucoside (28.4–30.0% of total flavonol glycosides) was the most abundant compound in all the cultivars studies (Table 4). Quercetin-3-*O*-rutinoside coeluted with myricetin-3-*O*-

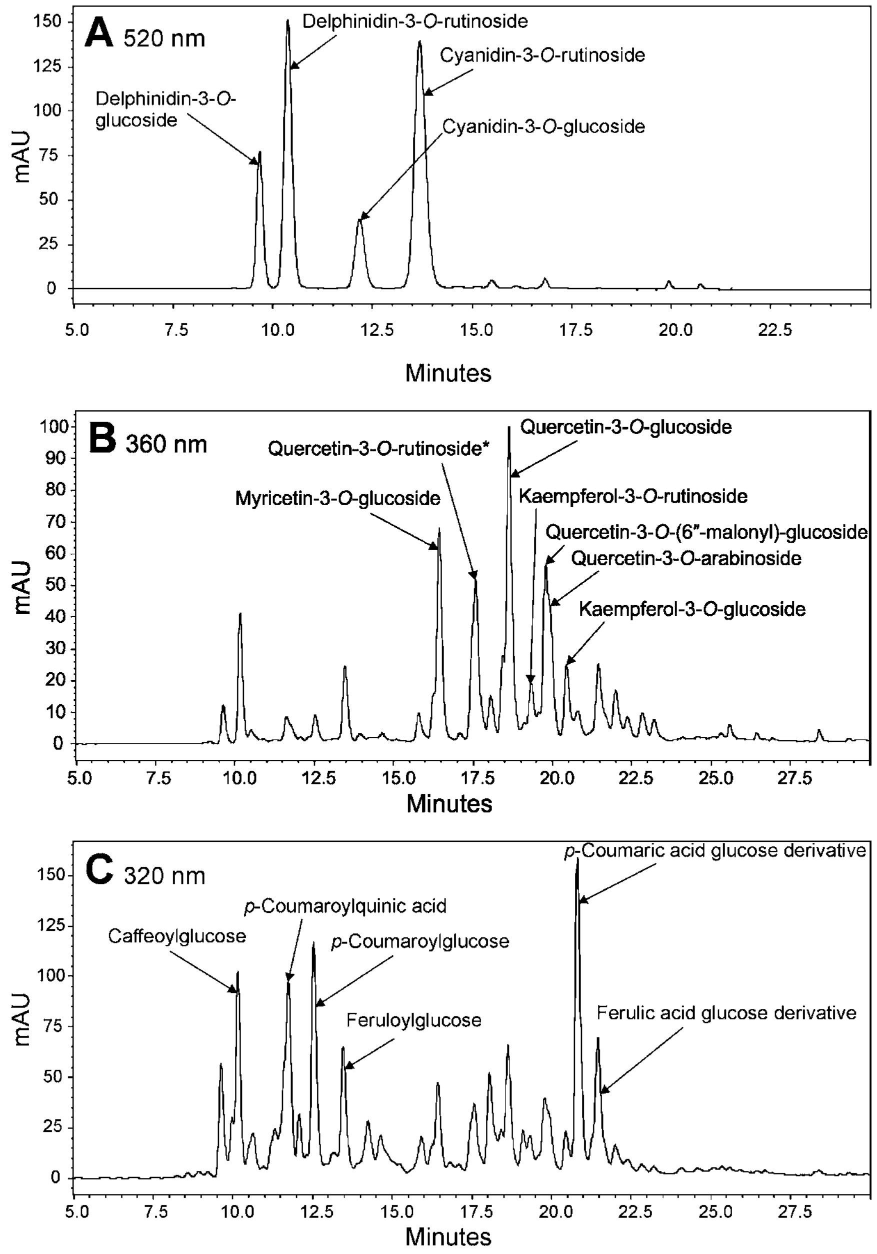

Figure 1. HPLC chromatograms of anthocyanins (A, 520 nm), flavonol glycosides (B, 360 nm), and hydroxycinnamic acid derivatives (C, 320 nm) in black currant berries of cultivar 'Ola' collected from Apukka, Finland in 2009. *, quercetin-3-*O*-rutinoside coeluted with myricetin-3-*O*-arabinoside, myricetin-3-*O*-(6″-malonyl)-glucoside, and auresidin glucoside.

arabinoside, myricetin-3-*O*-(6″-malonyl)-glucoside, and an auresidin glucoside.[8] However, myricetin-3-*O*-arabinoside and auresidin glucoside were only detected in trace amounts. The peaks of quercetin-3-*O*-(6″-malonyl)-glucoside and quercetin-3-*O*-arabinoside overlapped and were quantified as a single peak with an external standard of quercetin-3-*O*-glucoside. A total of nine derivatives of hydroxycinnamic acids were tentatively identified by Laaksonen et al.,[9] among which, six were quantified in our study (Table 5). They are all glucose derivatives of caffeic acid, ferulic acid, and *p*-coumaric acid with an exception of *p*-coumaroylquinic acid detected at a level of 0.84−1.02 mg/100 g of fresh berries. *p*-Coumaric acid derivatives dominated and accounted for 44.5−50.1% of the hydroxycinnamic acid derivatives in the berries of the three cultivars. The dry weight was calculated as the percentage of dried berries on the basis of fresh berries, which varied from 21.16−22.09% among the cultivars (Table 3). The results based both on fresh weight and on dry weight were investigated statistically. Nearly the same trends were observed in the compositional differences of phenolic compounds in black currant berries between different cultivars and growth latitudes, as well as in their compositional response to the varying weather conditions. Therefore, only the results based on the fresh weight are presented in this paper.

Cultivar Comparison. PLS-DA was applied to study the compositional differences between black currant cultivars. The PLS-DA models were based on 105 samples and the classification based on cultivar differentiated 'Melalahti' from 'Mortti' and 'Ola' (R^2 = 86.7% and Q^2 = 84.8 with two factors, Figure 2). 'Mortti' and 'Ola' were poorly separated due to relatively similar phenolic compositions. 'Melalahti' was also clearly different from 'Mortti' and 'Ola' in the composition of sugars and acids, while 'Mortti' and 'Ola' showed similar contents of sugars and acids in our previous study on black currant berries.[20] The loading plot (Figure 2) indicated that the classification was based on the phenolic composition of the berries. 'Melalahti' had significantly lower amount of total phenolic compounds (31.4% and 29.2% lower than 'Mortti' and 'Ola', respectively, $p < 0.05$), total anthocyanins (32.6% and 30.5%, respectively, $p < 0.05$), and total hydroxycinnamic acid derivatives (23.1% and 23.8%, respectively, $p < 0.05$) than 'Mortti' and 'Ola' (Figure 2 and Tables 3 and 5). However, the total flavonol glycosides was located in the middle of 'Melalahti' and 'Mortti'/'Ola' in the loading plot (Figure 2) and showed similar values between all the cultivars investigated ($p > 0.05$, Table 4). The four major anthocyanins were all significantly lower in 'Melalahti' than in the other two cultivars (Figure 2 and Table 3). Black currants are used as raw materials for extraction of anthocyanins as natural pigments. For this purpose, 'Mortti' and 'Ola' would be better choices for high yield of anthocyanin extraction than 'Melalahti'. In view of the flavonol glycosides, most of them showed higher values ($p < 0.05$, Table 4) in 'Melalahti' than in 'Mortti' and 'Ola' with exceptions in content of myricetin-3-O-glucoside and the sum of quercetin-3-O-(6″-malonyl)-glucoside and quercetin-3-O-arabinoside which were located more closer to 'Mortti'/'Ola' and in the middle of the loading plot, respectively (Figure 2). No significant difference ($p > 0.05$) was found in content of caffeoylglucose between the black currant cultivars, while all the other hydroxycinnamic acid derivatives were lower ($p < 0.05$) in 'Melalahti' than in 'Mortti' and 'Ola'. The percentage of dry weight of the berries was statistically at the same level in the three black currant cultivars studied (Table 3).

Quercetin-3-O-rutinoside, kaempferol-3-O-rutinoside, kaempferol-3-O-glucoside, and quercetin-3-O-glucoside among other flavonol glycosides, and hydroxycinnamic acid derivatives were reported to be the compounds contributing to astringency in red currants.[21] The hydroxycinnamic acids are puckering astringent compounds at very low concentration and their ethyl esters were both astringent and bitter in red wine.[22] Berries of 'Mortti' and 'Ola' contained a lower amount of the above flavonoid glycosides but a higher amount of hydroxycinnamic acid conjugates than berries of 'Melalahti'. Thus, it is impossible to predict the levels of astringent and bitter taste of the different black currant cultivars solely based on the compositional information in this study.

Latitude Comparison. PLS-DA was applied to classify the samples by grouping samples belonging to different growth locations. Three separate models (one for each cultivar) were created to explain the differences between southern and northern locations (Figure 3). Attention should be paid that the comparison between latitudes in this study is restricted to Scandinavian areas with high latitudes (above 60° N). Therefore, the application of the current results on berries grown at lower latitudes especially in torrid zone areas should be considered carefully. In the cultivar 'Mortti' (R^2 = 91.5% and Q^2 = 81.1 with six factors, Figure 3A) and 'Ola' (R^2 = 91.7% and Q^2 = 85.7 with five factors, Figure 3B), the classifications in score plots were clear with anthocyanins contributing more to the berries grown in the southern place (Piikkiö) than those grown in the northern place (Apukka), hydroxycinnamic acids vice versa. However, the classification was poorer in 'Melalahti' (R^2 = 72.4% and Q^2 = 56.1 with four factors, Figure 3C) than that in 'Mortti' and 'Ola', which indicated that the difference between phenolic composition of 'Melalahti' grown in different

Table 3. Contents of Anthocyanins, Total Anthocyanins and Total Phenolic Compounds in Black Currant Berries[a]

cultivar	growth place[b]	(mg/100 g fresh berry)						(% fresh berry)
		delphinidin-3-O-glucoside	delphinidin-3-O-rutinoside	cyanidin-3-O-glucoside	cyanidin-3-O-rutinoside	total anthocyanins	total phenolic compounds	dry weight
'Mortti'	S (n = 37)	39.64 ± 6.27 b	194.17 ± 23.02 b	16.68 ± 3.39 a	133.71 ± 21.11 b	384.20 ± 49.47 b	401.28 ± 49.70 b	22.39 ± 1.99 a
	N (n = 36)	28.41 ± 7.27 a	123.59 ± 40.14 a	16.29 ± 2.30 a	114.03 ± 26.79 a	282.32 ± 69.86 a	298.73 ± 69.04 a	21.80 ± 1.73 a
'Ola'	S (n = 34)	38.60 ± 7.36 b	185.99 ± 22.23 b	15.56 ± 3.33 a	126.36 ± 18.19 b	366.51 ± 47.44 b	383.21 ± 48.84 b	22.41 ± 2.55 b
	N (n = 37)	28.98 ± 8.00 a	124.26 ± 45.44 a	16.77 ± 2.18 a	114.02 ± 27.07 a	284.04 ± 76.41 a	300.70 ± 75.57 a	21.01 ± 0.98 a
'Melalahti'	S (n = 36)	16.31 ± 4.62 b	107.71 ± 24.57 b	9.98 ± 2.28 b	108.65 ± 23.23 a	242.64 ± 51.92 b	259.09 ± 52.04 b	21.32 ± 2.62 a
	N (n = 36)	10.13 ± 2.67 a	82.61 ± 19.38 a	8.78 ± 2.49 a	105.86 ± 20.43 a	207.38 ± 37.89 a	222.35 ± 37.52 a	21.00 ± 2.81 a
'Mortti'+ 'Ola' + 'Melalahti'	S(n = 107)	31.46 ± 12.45 b	162.48 ± 45.60 b	14.07 ± 4.23 a	122.94 ± 23.37 b	330.95 ± 80.41 b	347.70 ± 80.93 b	22.04 ± 2.44 b
	N(n = 109)	22.57 ± 10.85 a	110.28 ± 41.39 a	13.97 ± 4.33 a	111.33 ± 25.04 a	258.15 ± 72.64 a	274.17 ± 72.40 a	21.27 ± 2.01 a
'Mortti'	S + N (n = 73)	34.10 ± 8.79 y	159.36 ± 48.07 y	16.49 ± 2.89 y	124.01 ± 25.88 y	333.96 ± 78.91 y	350.71 ± 78.85 y	22.09 ± 1.87 x
'Ola'	S + N (n = 71)	33.59 ± 9.05 y	153.82 ± 47.53 y	16.19 ± 2.84 y	119.93 ± 23.90 y	323.53 ± 76.06 y	340.21 ± 76.06 y	21.69 ± 2.02 x
'Melalahti'	S + N (n = 72)	13.22 ± 4.87 x	95.16 ± 25.35 x	9.38 ± 2.44 x	107.25 ± 21.77 x	225.01 ± 48.49 x	240.72 ± 48.70 x	21.16 ± 2.71 x

[a] Significant differences ($p < 0.05$) between samples grown at different latitudes (every cultivar compared separately) and between samples of different cultivars are marked as a−b and x−y, respectively. [b] S, southern Finland (Piikkiö); N, northern Finland (Apukka).

Table 4. Contents of Flavonol Glycosides in Black Currant Berries[a]

		(mg/100 g fresh berry)						
cultivar	growth place[b]	myricetin-3-*O*-glucoside	qu-rut + my-mal[c]	quercetin-3-*O*-glucoside	kaempferol-3-*O*-rutinoside	qu-mal + qu-ara[d]	kaempferol-3-*O*-glucoside	total flavonol glycosides
'Mortti'	S (n = 37)	2.84 ± 0.64 b	2.33 ± 0.31 b	3.36 ± 0.49 b	0.48 ± 0.08 a	1.15 ± 0.40 a	0.92 ± 0.15 a	11.08 ± 1.39 b
	N (n = 36)	1.84 ± 0.49 a	2.14 ± 0.27 a	2.54 ± 0.48 a	0.48 ± 0.08 a	1.39 ± 0.48 b	0.99 ± 0.38 a	9.37 ± 0.96 a
'Ola'	S (n = 34)	2.83 ± 0.48 b	2.20 ± 0.48 a	3.15 ± 0.62 b	0.45 ± 0.10 a	1.22 ± 0.48 a	0.84 ± 0.10 a	10.68 ± 1.91 b
	N (n = 37)	1.86 ± 0.64 a	2.15 ± 0.30 a	2.63 ± 0.61 a	0.54 ± 0.11 b	1.41 ± 0.42 a	1.03 ± 0.36 b	9.61 ± 1.25 a
'Melalahti'	S (n = 36)	1.63 ± 0.52 b	3.18 ± 0.60 b	3.32 ± 0.71 a	0.57 ± 0.10 a	1.25 ± 0.50 a	1.41 ± 0.25 a	11.37 ± 1.40 b
	N (n = 36)	1.02 ± 0.21 a	2.68 ± 0.45 a	3.10 ± 0.61 a	0.55 ± 0.15 a	1.09 ± 0.28 a	1.59 ± 0.60 a	10.04 ± 1.50 a
'Mortti' + 'Ola' + 'Melalahti'	S (n = 107)	2.43 ± 0.79 b	2.57 ± 0.64 b	3.28 ± 0.61 b	0.50 ± 0.11 a	1.21 ± 0.46 a	1.06 ± 0.31 a	11.05 ± 1.59 b
	N (n = 109)	1.58 ± 0.62 a	2.32 ± 0.43 a	2.76 ± 0.62 a	0.52 ± 0.12 a	1.30 ± 0.43 a	1.20 ± 0.53 b	9.67 ± 1.28 a
'Mortti'	S + N (n = 73)	2.35 ± 0.76 y	2.24 ± 0.31 x	2.96 ± 0.64 xy	0.48 ± 0.08 x	1.27 ± 0.45 x	0.95 ± 0.29 x	10.24 ± 1.47 x
'Ola'	S + N (n = 71)	2.33 ± 0.74 y	2.17 ± 0.39 x	2.88 ± 0.66 x	0.50 ± 0.11 x	1.32 ± 0.46 x	0.94 ± 0.28 x	10.12 ± 1.68 x
'Melalahti'	S + N (n = 72)	1.33 ± 0.50 x	2.93 ± 0.58 y	3.21 ± 0.67 y	0.56 ± 0.13 y	1.17 ± 0.41 x	1.50 ± 0.46 y	10.70 ± 1.59 x

[a] Significant differences ($p < 0.05$) between samples grown at different latitudes (every cultivar compared separately) and between samples of different cultivars are marked as a−b and x−y, respectively. [b] S, southern Finland (Piikkiö); N, northern Finland (Apukka). [c] Concentration sum of quercetin-3-*O*-rutinoside, myricetin-3-*O*-(6″-malonyl)-glucoside, myricetin-3-*O*-arabinoside, and auresidin glucoside. [d] Concentration sum of quercetin-3-*O*-(6″-malonyl)-glucoside and quercetin-3-*O*-arabinoside.

Table 5. Contents of Hydroxycinnamic Acid Conjugates in Black Currant Berries

		(mg/100 g fresh berry)						
cultivar	growth place[b]	caffeoyl-glucose	*p*-coumaroyl-quinic acid	*p*-coumaroyl-glucose	feruloyl-glucose	*p*-coumaric acid glucose derivative	ferulic acid glucose derivative	total hydroxy-cinnamic acids
'Mortti'	S (n = 37)	1.23 ± 0.23 a	0.83 ± 0.13 a	0.83 ± 0.20 a	0.77 ± 0.14 a	1.07 ± 0.19 a	1.26 ± 0.25 a	6.00 ± 0.85 a
	N (n = 36)	1.21 ± 0.17 a	1.14 ± 0.20 b	1.27 ± 0.39 b	0.85 ± 0.18 a	1.32 ± 0.26 b	1.25 ± 0.21 a	7.04 ± 1.15 b
'Ola'	S (n = 34)	1.20 ± 0.22 a	0.91 ± 0.25 a	0.83 ± 0.23 a	0.81 ± 0.17 a	1.09 ± 0.29 a	1.18 ± 0.24 a	6.03 ± 1.22 a
	N (n = 37)	1.28 ± 0.22 a	1.11 ± 0.17 b	1.27 ± 0.27 b	0.83 ± 0.14 a	1.33 ± 0.28 b	1.24 ± 0.21 a	7.06 ± 0.94 b
'Melalahti'	S (n = 36)	1.36 ± 0.28 b	0.81 ± 0.11 a	0.61 ± 0.14 a	0.69 ± 0.13 a	0.79 ± 0.11 a	0.83 ± 0.09 a	5.08 ± 0.61 a
	N (n = 36)	1.24 ± 0.17 a	0.88 ± 0.18 b	0.62 ± 0.19 a	0.65 ± 0.10 a	0.76 ± 0.13 a	0.79 ± 0.18 a	4.93 ± 0.68 a
'Mortti'+ 'Ola' + 'Melalahti'	S (n = 107)	1.27 ± 0.25 a	0.85 ± 0.18 a	0.75 ± 0.22 a	0.76 ± 0.16 a	0.98 ± 0.25 a	1.09 ± 0.28 a	5.70 ± 1.01 a
	N (n = 109)	1.24 ± 0.19 a	1.04 ± 0.21 b	1.05 ± 0.42 b	0.77 ± 0.17 a	1.14 ± 0.36 b	1.09 ± 0.29 a	6.35 ± 1.37 b
'Mortti'	S + N (n = 73)	1.22 ± 0.20 x	0.98 ± 0.23 y	1.05 ± 0.38 y	0.81 ± 0.16 y	1.19 ± 0.26 y	1.26 ± 0.23 y	6.51 ± 1.13 y
'Ola'	S + N (n = 71)	1.24 ± 0.22 x	1.02 ± 0.23 y	1.06 ± 0.33 y	0.82 ± 0.16 y	1.22 ± 0.31 y	1.21 ± 0.23 y	6.56 ± 1.19 y
'Melalahti'	S + N (n = 72)	1.30 ± 0.24 x	0.84 ± 0.15 x	0.61 ± 0.17 x	0.67 ± 0.12 x	0.77 ± 0.12 x	0.81 ± 0.14 x	5.00 ± 0.65 x

[a] Significant differences ($p < 0.05$) between samples grown at different latitudes (every cultivar compared separately) and between samples of different cultivars are marked as a−b and x−y, respectively. [b] S, southern Finland (Piikkiö); N, northern Finland (Apukka).

latitudes was not as big as that in 'Mortti' and 'Ola'. On the basis of the PLS-DA analyses with phenolic composition explaining cultivars or locations, reliable models were created to predict sample origins when providing the composition of phenolic compounds in the unknown samples. However, the predictive validity of growth location by the model in 'Melalahti' is poorer than in 'Mortti' and 'Ola'.

Tables 3−5 give detailed information on the contents of phenolic compounds between black currant berries from southern Finland and northern Finland. The values of total flavonol glycosides, total anthocyanins, and total phenolic compounds, located closer to Piikkiö in the loading plot of PLS-DA (Figure 3), showed decreasing trends as the latitude increased in all the cultivars studied (Tables 3 and 4). However, the content of total hydroxycinnamic acid derivatives showed an increasing trend as the latitude increased in berries of 'Mortti' and 'Ola' but no significant difference in berries of 'Melalahti' (Table 5). The contents of delphinidin-3-*O*-glucoside, delphinidin-3-*O*-rutinoside, and myricetin-3-*O*-glucoside decreased (by 24.9−37.9%, 23.3−36.3%, 34.0−37.3%, respectively, $p < 0.05$) and that of *p*-coumaroylquinic acid increased (by 8.9−37.2%, $p < 0.05$) in all the cultivars as the

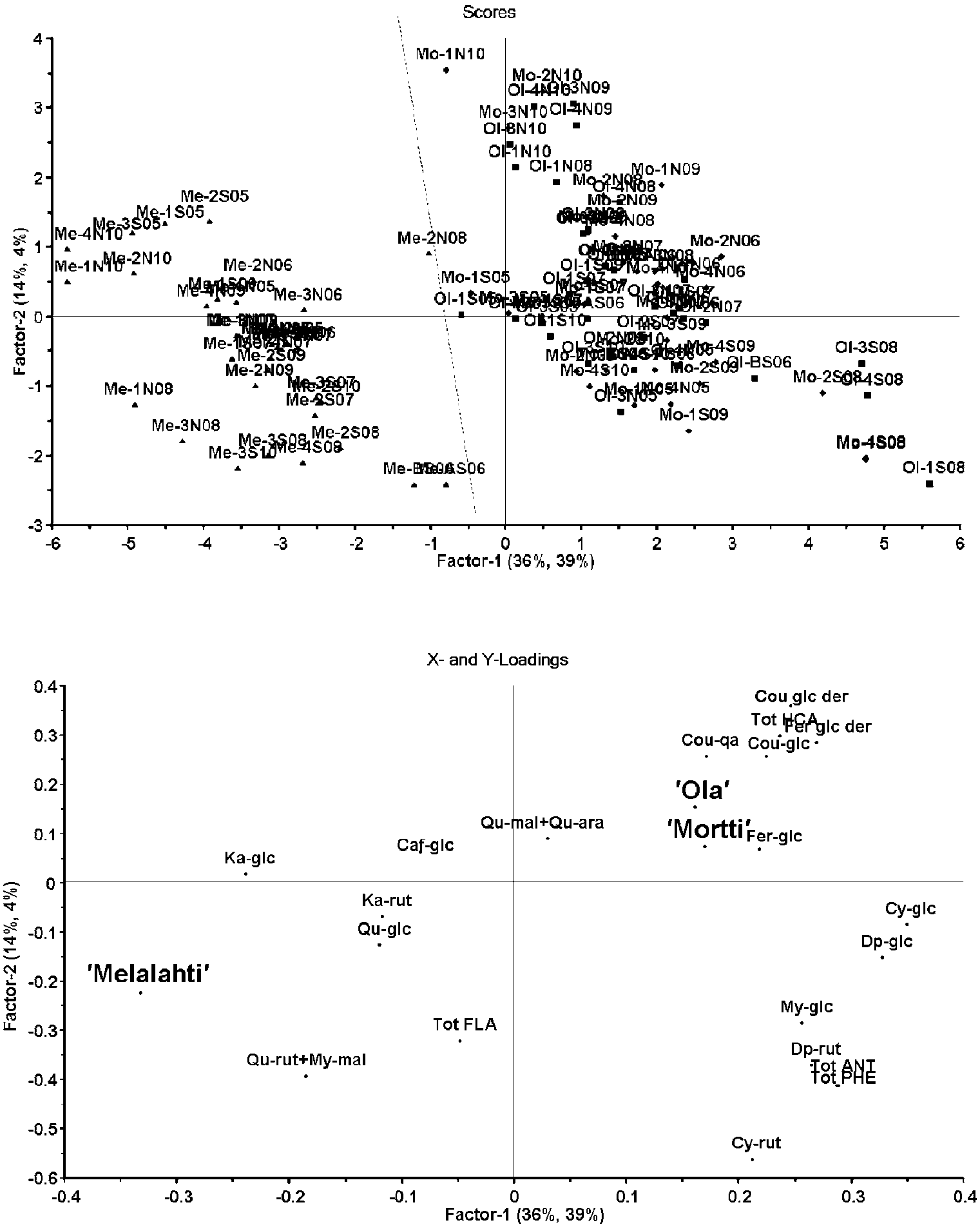

Figure 2. Score and loading plot of PLS-DA model for black currant samples classified according to cultivar. The cultivar 'Mortti' is shown with diamonds (◆), 'Ola' with squares (■), and 'Melalahti' with triangles (▲). Dotted line shows the classification between 'Melalahti' and 'Mortti'/'Ola'. Abbreviations of samples: Mo, 'Mortti'; Ol, 'Ola'; Me, 'Melalahti'; 1, 2, 3, and 4, sample collected from field block 1, 2, 3, and 4, respectively; S, southern Finland; N, northern Finland; 05, 06, 07, 08, 09 and 10, sample collected in year 2005, 2006, 2007, 2008, 2009 and 2010, respectively. Abbreviations of compounds: Dp, delphinidin; Cy, cyanidin; My, myricetin; Qu, quercetin; Ka, kaempferol; glc, glucoside; rut, rutinoside; mal, malonyl glucoside; ara, arabinoside; Caf-glc, caffeoylglucose; Cou-qa, *p*-coumaroylquinic acid; Cou-glc, *p*-coumaroylglucose; Cou glc der, *p*-coumaric acid glucose derivative; Fer-glc, feruloylglucose; Fer glc der, ferulic acid glucose derivative; Tot PHE, total phenolic compounds; Tot ANT, total anthocyanins; Tot FLA, total flavonol glycosides; Tot HCA, total hydroxycinnamic acid conjugates.

latitude increased, while the contents of feruloylglucose and ferulic acid glucose derivative did not change ($p > 0.05$). The other phenolic compounds showed different trends between cultivars in response to the varying latitudes (Tables 4 and 5). 'Mortti' and 'Ola' showed similar varying trends in contents of hydroxycinnamic acid derivatives and anthocyanins in response to the changing latitudes. 'Mortti' and 'Ola' showed higher contents of all the *p*-coumaric acid derivatives but lower content of cyanidin-3-*O*-rutinoside in berries collected in northern Finland than those in southern Finland ($p < 0.05$, Tables 3 and 5). In the case of 'Melalahti', no significant difference between the two latitudes studied was found in sugar conjugates of *p*-coumaric acids and cyanidin-3-*O*-rutinoside. Berries of 'Melalahti' contained lower caffeoylglucose and cyanidin-3-*O*-glucoside in the north than in the south, but no significant difference in these compounds was observed in berries of 'Mortti' and 'Ola' between two latitudes.

In order to have an overview of the latitudinal effects on the composition of phenolic compounds in black currant berries (*R. nigrum* L.), the data of three cultivars were combined and analyzed. The contents of total anthocyanins, total flavonol glycosides, and total phenolic compounds were higher but the

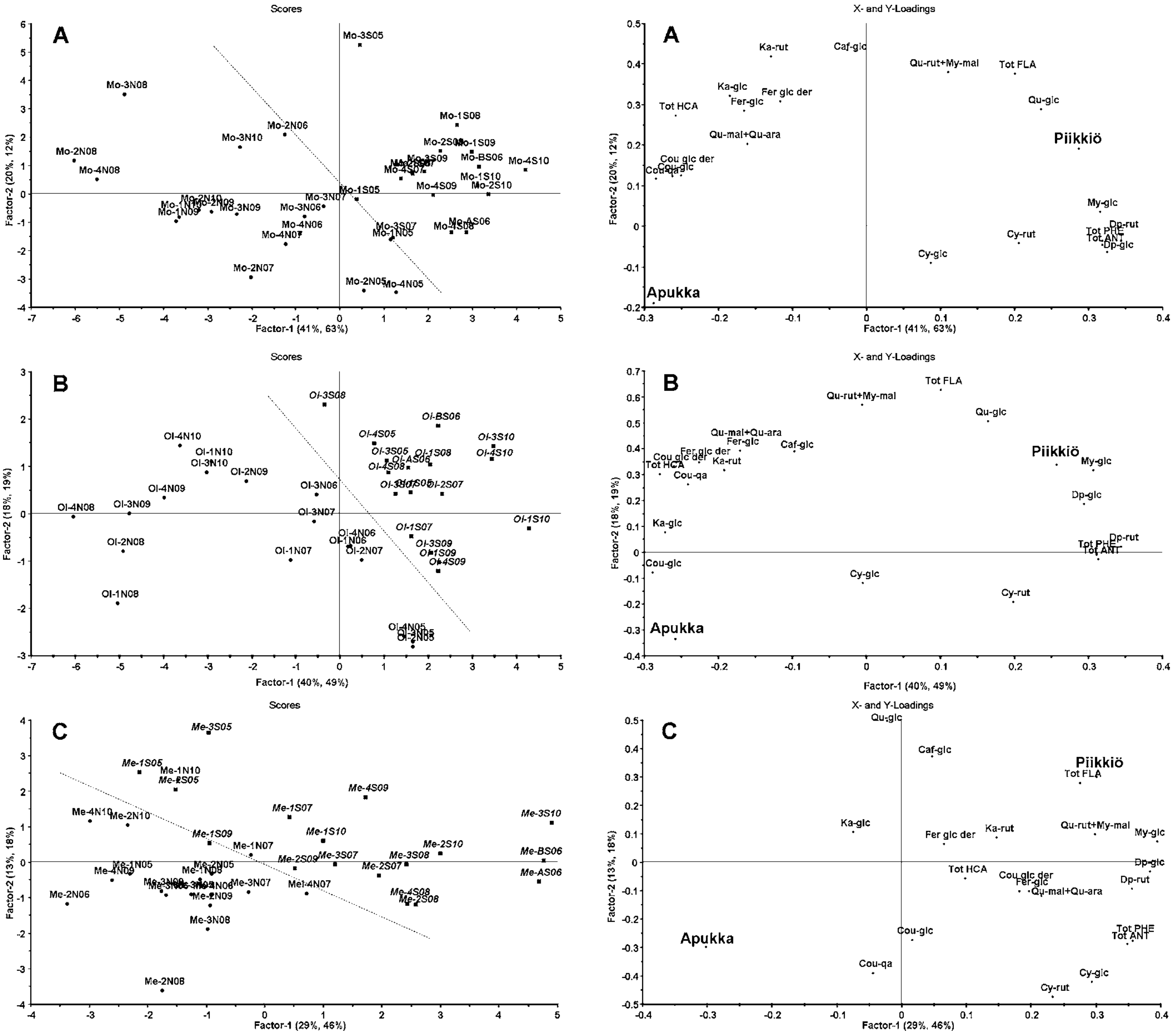

Figure 3. Score and loading plots of PLS-DA models for black currant samples of 'Mortti' (A), 'Ola' (B), and 'Melalahti' (C) classified according to growth locations with (■) for southern Finland and (●) for northern Finland. Dotted lines show the classifications between the south and north locations in each cultivar. Abbreviations of samples and compounds refer to Figure 2.

content of total hydroxycinnamic acids was lower in the berries grown in southern Finland than those grown in northern Finland. The contents of all the anthocyanins in black currants showed a decreasing trend as the latitude increased, although the difference in cyanidin-3-*O*-glucoside content was not significant ($p > 0.05$) between berries grown at the two latitudes. The contents of major flavonol glycosides, myricetin-3-*O*-glucoside, quercetin-3-*O*-glucoside, and the sum of quercetin-3-*O*-rutinoside and myricetin-3-*O*-(6″-malonyl)-glucoside were higher in berries grown at lower latitude than those grown at higher latitude. In contrast, the content of kaempferol-3-*O*-glucoside showed an opposite trend in response to the variation of latitude. In the case of hydroxycinnamic acid derivatives, only the three derivatives of *p*-coumaric acid showed higher contents in the berries grown in northern Finland than those grown in southern Finland. The dry weight of berries grown in southern Finland was statistically but slightly higher (by 3.6%) than berries grown in northern Finland.

Variation in latitude always leads to concomitant changes in climate conditions, such as light intensity and temperature. These factors strongly influence the metabolism and concentrations of metabolites in plants and fruits.[1] Therefore, the effect of latitude on the composition of berry fruits could be explained by both the short-term effect of environmental factors on the metabolism of the plants and the gene evolution resulting from long-term adaption of plants to the environment.[1] A further investigation in this study on the correlations between the weather conditions and the berry composition is necessary for understanding the latitudinal effect on the composition of black currant berries.

Effects of Weather Conditions on Phenolic Compounds. Principal component analysis (PCA) was conducted to give an overview on the compositional response of black currant berries to weather conditions (Figure 4). The monthly

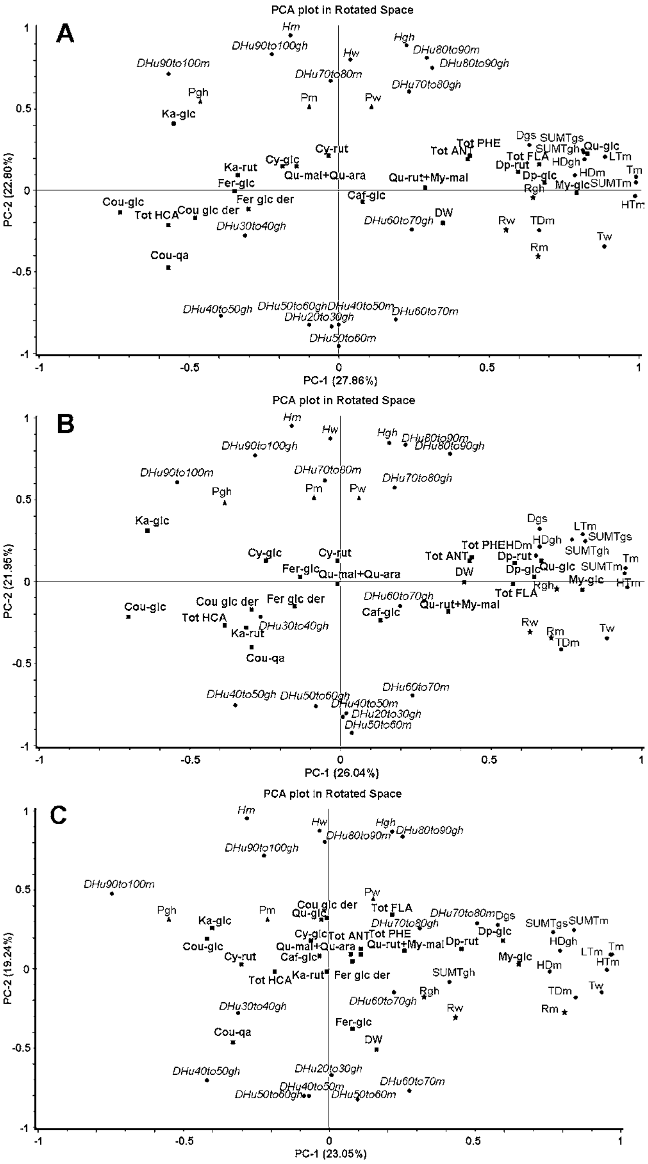

Figure 4. PCA plots of compositional response of phenolic compounds in 'Mortti' (A), 'Ola' (B), and 'Melalahti' (C) to weather conditions. Abbreviations of compounds refer to Figure 2. Abbreviations of weather variables refer to Table 2.

weather variables, including monthly average temperature, monthly radiation, monthly precipitation, and monthly average humidity, were not included in the PCA study. For more information on the effects of individual weather conditions, including the monthly variables, on the composition of phenolic compounds in black currant berries, Pearson's correlation coefficients analysis was applied (Supporting Information).

In the PCA plots, the first two principal components (PCs) explained 50.66, 47.99, and 42.29% of the variance of weather conditions and composition data of 'Mortti', 'Ola', and 'Melalahti', respectively. PC1 (27.86, 26.04 and 23.05% of 'Mortti', 'Ola', and 'Melalahti', respectively) represented the temperature and radiation parameters and separated the components of black currants by their variation in contents in response to temperature and radiation. PC2 (22.80, 21.95, and 19.24% of 'Mortti', 'Ola', and 'Melalahti', respectively) represent precipitation and humidity variables and separated the percentage of the days with various relative humidity by 70%. Hereby, the percentage of the days with a relative humidity above 70% was considered as a high humidity variable and that with a relative humidity below 70% as a low humidity variable. In disagreement with the current study, the study on the effects of weather conditions on contents of sugars and acids in currant berries have separated the low humidity and high humidity variables by the relative humidity of 50%.[20,23] This might be explained by the difference in optimal conditions for biosynthetic reactions of different metabolites in plants. Overall, the components in black currants of all the cultivars were explained more by PC1 than by PC2. This indicated that temperature and radiation may have been major factors influencing the composition of phenolic compounds.

Clear differences between cultivars in the effects of weather conditions on the phenolic compounds of black currant berries were observed by both PCA analysis (Figure 4) and Pearson's correlation coefficient analysis (Supporting Information). Nevertheless, some components showed the same trends in response to varying weather conditions in all the cultivars studied. Among all the phenolic compounds detected in this study, delphinidin-3-*O*-glucoside, delphinidin-3-*O*-rutinoside, and myricetin-3-*O*-glucoside were well explained by PC1 in all three cultivars and showed positive correlations with temperature and radiation variables (Figure 4). An increase in latitude normally appears in concurrence with a decrease in temperature and radiation. This might explain the increase in the accumulation of these glycosides in berries in response to a decrease in the growth latitude. *p*-Coumaroylquinic acid was weakly explained by PC2 and showed positive correlations with low humidity variables (relative humidity <70%) and negative correlations with high humidity variables (relative humidity >70%) (Figure 4). In addition to the general correlations observed via PCA analysis, correlations between components and individual weather parameters, including the monthly weather variables, were also detected via Pearson's correlation coefficients analysis. Some correlations between components and specific weather conditions applied similarly in all the cultivars studied. The precipitation from the start of growth season until the day of harvest (Pgh) correlated negatively with delphinidin-3-*O*-glucoside (correlation coefficients, $r = -0.49$ to -0.51, $p < 0.01$). The concentration of cyanidin-3-*O*-rutinoside correlated negatively with the precipitation in March (Pmar) ($r = -0.50$ to -0.66, $p < 0.01$). The contents of delphinidin-3-*O*-glucoside, delphinidin-3-*O*-rutinoside, and myricetin-3-*O*-glucoside all correlated positively with the average humidity in March and April (Hmar and Hapr) ($r = 0.52-0.83$, $p < 0.01$). The contents of total phenolic compounds and total anthocyanins correlated positively with the temperature sum over 5 °C from the start of growth season until the day of harvest (SUMTgh) ($r = 0.46-0.67$, $p < 0.01$), average temperature in June (Tjun) ($r = 0.55-0.76$, $p < 0.01$), and radiation in February (Rfeb) ($r = 0.51-0.65$, $p < 0.01$) in all the cultivars studied.

Anthocyanin concentration in plants is highly dependent on environmental conditions. Both low temperature and high radiation were reported to enhance anthocyanin accumulation in most of the plants.[24−34] However, Christie et al.[25] reported that although both the 10 and 15 °C stress caused increases in the anthocyanin concentration in maize seedlings, the maize seedlings exposed to temperature of 5 °C or seedlings pretreated at 5 °C then shifted to 25 °C exhibited little change in anthocyanin content compared to the seedlings grown at control temperature of 25 °C. In the present study, the contents of delphinidin glycosides were observed to increase as the temperature and radiation elevated, while the two cyanindin glycoside did not show a consistent and clear change in contents in all the black currant cultivars studied (Figure 4). The positive correlation between total content of anthocyanins and the variables of temperature and radiation were also observed in black currant berries, although the correlations were strong in Mortti, weaker in 'Ola' and the weakest in 'Melalahti'. In contrast to our findings, Borochov-Neori et al. and Schwartz et al. reported an increase in delphinidin 3-glucoside and cyanidin 3-glucoside, as well as total anthocyanin content, in the arils of pomegranates as the temperature decreased.[26,27] It should be mentioned that the natural concurrent changes in radiation and temperature both in their studies[27] and in our study, made it difficult to distinguish clearly the effect of temperature from that of radiation on the composition of fruits and berries. However, anthocyanin production is considered to be photoinduced by light and prohibited by heat.[24−34] Schwartz et al.[27] assumed that the lower anthocyanin content in pomegranates grown under higher temperature and radiation was not due to light intensity, which should have increased anthocyanin biosynthesis, but to the higher temperature leading to a decrease in anthocyanins content. Although most of the plants showed an increase in the amount of anthocyanins with a decrease in temperature, the study on two strawberry cultivars showed an increase in anthocyanin content in response to an increase in temperature.[35] This might implicate the genetic difference of the plants in the regulation of anthocyanin biosynthesis in relation to temperature. Moreover, different responses of anthocyanin content to radiation were reported between different cultivars of grapes (*Vitis vinifera* L.). Low light intensity at veraison restricted accumulation of anthocyanins, especially cyanidin-3-glucoside in Cabernet Sauvignon grapes (*Vitis vinifera* L.).[34] In contrast, Downey et al.[13] did not find significant difference between shaded and exposed bunches in total anthocyanin content in the grape cultivar Shiraz (*Vitis vinifera* L.). In agreement, they observed that the level of expression of the genes encoding UDP glucose: flavonoid-3-*O*-glucosyltransferase (UFGT), one of the key steps in anthocyanin synthesis, did not differ between exposed and shaded fruits. Although no significant difference in total anthocyanins was found between shaded and exposed fruits, Downey et al.[13] reported that the shaded fruits were consistently lower in the proportion of anthocyanins with three oxygen substituents on ring B (glucosides of delphinidin, petunidin and malvidin) and higher in those with two oxygen substituents (glucosides of cyanidin and peonidin) across all the three seasons studied. In our study, the delphinidin derivatives was found to increase as the light intensity increased, but cyanidin derivatives did not vary clearly and consistently in response to the light intensity. As a result, the total content of anthocyanins correlated, more or less, positively with radiation variables. The activity of the flavonoid-3′,5′-hydroxylase (F3′,5′H) and flavonoid-3′-hydroxylase (F3′H) and the expression of the genes encoding these enzymes should be examined to illustrate the biosynthetic cause of the response of anthocyanin composition to the varying radiation. It is worth mentioning that in the studies of Downey et al. and Ristic et al.[13,14] the shading was only applied on the bunch but not the whole bush of the grape vine, whereas the effect of radiation is investigated on the whole bush of black currant in our study. In addition to temperature and radiation, which are considered as the most important factors influencing the concentration of anthocyanins in plants and fruits, water stress also has an effect on the synthesis of anthocyanins.[36,37] This might provide an explanation for the negative correlations discovered between delphinidin-3-*O*-glucoside and Pgh as well as between cyanidin-3-*O*-rutinoside and Pmar.

In this study, the content of myricetin-3-*O*-glucoside in black currant berries of all the cultivars studied presented positive correlations with temperature and radiation variables. Flavones and flavonols generally absorb light at 280−320 nm (UV−B) and function to protect cells from excessive UV−B radiation. Exposure of plants to increased UV−B light has been demonstrated to increase the synthesis of flavones and flavonols.[1] It might be speculated the increased accumulation of myricetin-3-*O*-glucoside in black currant berries might be mainly due to the increase in light intensity. However, the radiation data in this study not only included the UV−B radiation but also the UV−A radiation. This might make the speculation on the correlation between radiation and flavonoids ambiguous. Downey et al.[13] reported a decrease in flavonol accumulation in the skin of Shiraz grape by the practice of bunch shading. They also investigated the expression of the genes encoding flavonol synthase (FLS), the first enzyme in the branch of the flavonoid pathway leading to flavonol biosynthesis. The expression of *VvFLS1* was lower in shaded fruits than in exposed fruits.

Moreover, differences in compositional response of phenolic compounds to the varying environmental conditions between different black currant cultivars were also detected in the present study, showing differences in metabolic and biosynthetic pathway of secondary metabolites in different cultivars of the same species of plant. 'Melalahti' distinguished considerably in the compositional response of phenolic compounds to the variation in weather conditions from 'Mortti' and 'Ola'. From the PCA plot (Figure 4C), the cultivar 'Melalahti' seems to be less affected by weather conditions compared to 'Mortti' and 'Ola' (Figure 4A,B). Besides delphinidin-3-*O*-glucoside, delphinidin-3-*O*-rutinoside, myricetin-3-*O*-glucoside, and *p*-coumaroylquinic acid described above, only dry weight was weakly explained by PC2 and showed positive correlations with low humidity variables and negative correlations with precipitation, average humidity, and high humidity variables in 'Melalahti'. All the other phenolic compounds in 'Melalahti', as located in the center part of the plot, had no general correlations with the weather variables presented on the plot. Nevertheless, individual correlations between components and some specific weather variables were still observed (Supporting Information). In our previous study conducted on the same black currant cultivars, 'Melalahti' contained a higher sugar/acid ratio and its concentration of sensory and quality contributing components, sugars and acids, were hardly influenced by weather conditions. Therefore, it would have been considered as a stable and suitable cultivar for cultivation for commercial processing purpose. However, other factors such as the crop yield of the cultivar and its resistance to diseases should also be considered before introducing it for commercial cultivation.

According to the PCA plots, 'Mortti' and 'Ola' behaved in similar manners in the compositional response of phenolic compounds to the weather conditions with only some minor difference in some compounds (Figure 4A,B). This is in accordance with the results of our previous study,[20] in which 'Mortti' and 'Ola' showed similar responses to weather conditions in the contents of sugars and acids. The results of the two studies might suggest similarity in the metabolism and biosynthetic pathways of the plants and fruits of these two cultivars. In addition to delphinidin-3-*O*-glucoside, delphinidin-3-*O*-rutinoside and myricetin-3-*O*-glucoside, quercetin-3-*O*-glucoside, total flavonol glycosides, total anthocyanins and total phenolic compounds were represented by PC1 and showed positive correlations with temperature and radiation variables in both 'Mortti' and 'Ola' (Figure 4A,B). *p*-Coumaroylglucose in 'Mortti' and 'Ola' was highly represented by PC1 and had negative correlation with temperature and radiation. However, kaempferol-3-*O*-glucoside was weakly explained by PC1 and PC2 in 'Mortti'. Its explanation in 'Ola' by PC1 was comparatively stronger than that in 'Mortti', but the explanation by PC2 was as weak as in 'Mortti'. As a result, generally negative correlations between kaempferol-3-*O*-glucoside and temperature variables were observed in 'Ola'. In 'Mortti', it was found to correlate negatively with all the temperature parameters in the last month before harvest. Kaempferol-3-*O*-glucoside showed negative correlation with the radiation in the last month before harvest (Rm) in both 'Mortti' and 'Ola' ($r = -0.57$ and -0.61, respectively, $p < 0.01$). It also correlated positively with the percentage of the days with a relative humidity of 90−100% in the last month before harvest (DH90to100m) in 'Mortti' and 'Ola', as well as in 'Melalahti' ($r = 0.73$, 0.62 and 0.55, respectively, $p < 0.01$). The other compositional parameters, which are located in the center of PCA plots, were explained neither by PC1 nor by PC2 and had no general correlations with any group of weather conditions indicated by these PCs, although individual correlations between some components and certain weather variables were detected (Supporting Information). Despite these similar responses of 'Mortti' and 'Ola' to weather conditions, different performance of *p*-coumaroylquinic acid, *p*-coumaric acid glucose derivative, and total hydroxycinnamic acids in relation to the variation of temperature variables were detected between 'Mortti' and 'Ola'. These three components were all explained by PC1 and showed negative correlation with temperature variables in 'Mortti'. In contrast, no general correlations between them and temperature parameters were observed in black currants of 'Ola'.

In addition to the different compositional response to weather conditions observed between berries of different cultivars, different varying trends of the components in response to weather conditions at different growth stages were also detected. However, differences were only found in the correlations of the components with the precipitation and humidity variables. For instance, the content of cyanidin-3-*O*-glucoside in 'Mortti' was found to correlate positively with precipitation in June (Pjun) ($r = 0.55$, $p < 0.01$) but negatively with that in March (Pmar) ($r = -0.64$, $p < 0.01$, Supporting Information). Similar findings were observed in our previous study on the effects of weather conditions on the composition of sugars and acids in sea buckthorn berries (*H. rhamnoides* ssp. *mongolica*) and suggested different regulation of the accumulation of the components in the fruits in response to the weather variable during different growth periods.[38] Similar findings were also reported in grapes by Nicholas et al.[39] that high temperature in the postharvest period a year before maturity and from bloom to veraison correlated negatively with the concentrations of anthocyanins, tannins, and iron-reactive phenolics. In contrast, warm temperature from budburst to bloom correlated positively with the concentrations of these components. However, the simultaneous changes in temperature and radiation also existed in their study.

Correlation between Metabolites. Correlations between phenolic metabolites in the berries were observed in the present study. Some of the metabolites showed significant and similar correlations with each other in all three cultivars studied. Delphinidin-3-*O*-glucoside showed strongly positive correlation with delphinidin-3-*O*-rutinoside ($r = 0.91$, 0.89, and 0.86 in 'Mortti', 'Ola', and 'Melalahti' respectively, $p < 0.01$). Cyanidin-3-*O*-rutinoside correlated positively with all the other three anthocyanins in black currant berries ($r = 0.43-0.62$, 0.67−0.75 and 0.70−0.78 for delphinidin-3-*O*-glucoside, delphinidin-3-*O*-rutinoside, and cyanidin-3-*O*-glucoside, respectively, $p < 0.01$). *p*-Coumaroylquinic acid, *p*-coumaroylglucose, and feruloylglucose correlated positively with each other ($r = 0.75-0.83$, 0.65−0.79, and 0.45−0.48 for 'Mortti', 'Ola', and 'Melalahti', respectively, $p < 0.01$). In addition, the glucoside and rutinoside of delphinidin also showed positive correlations ($r = 0.82-0.89$ and 0.75−0.86 respectively, $p < 0.01$) with one of the flavonol glycosides, myricetin-3-*O*-glucoside, in all the cultivars investigated. Correlations between ferulic acid glucose derivative and kaempferol-3-*O*-rutinoside ($r = 0.56-0.62$, $p < 0.01$) and *p*-coumaric acid glucose derivative ($r = 0.63-0.84$, $p < 0.01$) were also detected.

The significant correlations discovered within anthocyanins and within hydroxycinnamic acids are explained by the conversion of these components within anthocyanin pathway and flavonoid pathway, respectively. *p*-Coumaric acid is known to be converted to caffeic acid via hydroxylation with the action of 4-coumarate 3-hydroxylase and further to ferulic acid via *O*-methylation with the function of caffeic/5-hydroxyferulic bispecific *O*-methyltransferase. The phenylalanine-derived hydroxycinnamic acids are products of the shikimic pathway, and their CoA esters are precursors of flavonoids and anthocyanins.[2] This explains the correlations between the hydroxycinnamic acids, flavonoids, and anthocyanins. However, the biosynthetic pathways of the phenolic compounds in plants are extremely complex and regulated by huge number of enzymes. It is difficult to speculate the intercorrelation and conversion steps between two compounds without further physiological and enzymological investigations. This study, with the correlations observed between individual phenolic compounds, provides important support for investigation and elucidation of the metabolism of phenolic compounds in black currants.

In summary, significant differences in the composition of phenolic compounds between black currants of different cultivars were detected. The compositional response of black currant berries to latitude and weather conditions were observed. 'Melalahti' was distinguished from 'Mortti' and 'Ola' by both its composition of phenolic compounds and response to growth latitude and weather conditions. 'Melalahti' had lower contents of total phenolic compounds, total anthocyanins, and total hydroxycinnamic acid conjugates than 'Mortti' and 'Ola'. The total flavonol glycosides did not vary between cultivars. Black currants grown at high latitude in Finland contained lower amounts of total flavonol glycosides, total anthocyanins, and total phenolic compounds than those grown at low latitude in all the cultivars studied. However, total content of hydroxycinnamic acid conjugates did not vary in 'Melalahti' but increased as the latitude increased in 'Mortti' and 'Ola'. The information provided by this study on the differences between cultivars and growth latitudes in phenolic profiles gave useful suggestions on the cultivar selection and growth site selection with special purposes, such as the enrichment of the nutrients or natural pigments. In the study of weather impact on phenolic compounds, temperature and radiation, other than precipitation and humidity, were major factors influencing the composition of phenolic compounds. Compared with those in 'Mortti' and 'Ola', phenolic compounds in 'Melalahti' were less affected by weather conditions. Combined with the results of our previous study on the sugars and acids in black currant berries, 'Melalahti' might be recommended as a satisfactory option for commercial processing purpose than 'Mortti' and 'Ola' because of its high sugar/acid ratio and relatively constant composition despite the annual variation of weather conditions. Nevertheless, delphinidin-3-*O*-glucoside, delphinidin-3-*O*-rutinoside and myricetin-3-*O*-glucoside content showed positive correlations with temperature and radiation, while *p*-coumaroylquinic acid showed positive correlations with low humidity variables (relative humidity <70%) and negative correlations with high humidity variables (relative humidity >70%) in all the three cultivars investigated. This study, with our previous study on sensory determining parameters of black currants, gives important information for agricultural breeding and cultivation of black currants in northern Scandinavia, as well as for the rational utilization of the berries in food industry. This study also provides supporting compositional information on secondary metabolites for basic biochemical and physiological studies of black currants and other plants.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Table of Pearson's correlation coefficients between weather conditions and phenolic compounds in black currant berries. These materials are available free of charge via the Internet at http://pubs.acs.org.

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Notes

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