# AGRICULTURAL AND FOOD CHEMISTRY

# Effects of Latitude and Weather Conditions on Phenolic Compounds in Currant (*Ribes* spp.) Cultivars

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**Supporting Information** 

**ABSTRACT:** Effects of growth latitude and weather conditions on phenolic compounds of currants (*Ribes* spp.) were investigated. The berries of red currant cultivar 'Red Dutch', white currant 'White Dutch', and green currant 'Vertti' were collected in seven consecutive years from two growth sites (south and north) with a latitudinal distance of 690 km. The contents of hydroxycinnamic acid conjugates and flavonol glycosides in 'Vertti' were higher than those in 'White Dutch' by 8 and 5 times, respectively, and by 50 and 3 times than those in 'Red Dutch', respectively. The total content of phenolic compounds was 10-19% higher in the north than in the south (p < 0.05). In 'Red Dutch', anthocyanins were 12% richer in berries from the north compared with those from the south (p < 0.05). The total content of hydroxycinnamic acid conjugates in 'Vertti' and 'White Dutch' from the north was 30% higher than those from the south (p < 0.05). High radiation and temperature were associated with low contents of the major phenolic compounds in all the cultivars studied. High humidity correlated with low levels of hydroxycinnamic acid conjugates in green and white currants.

**KEYWORDS:** air humidity, anthocyanins, flavonol glycosides, hydroxycinnamic acid conjugates, growth site, precipitation, radiation, Ribes sp., temperature

# INTRODUCTION

Currants (Ribes spp.), of the family Grossulariaceae, are berries commonly cultivated in home gardens and commercial plantations in Europe. They are traditionally consumed as juices, jams, jellies, and syrups. The berries have also been used for berry wine production and as a food ingredient and coloring extract. As a result, both the sensory properties and the nutritional value are important for consumer acceptance and for the commercial value of the berries and berry products. Sugars and acids, together with phenolic compounds, influence the taste of berries and berry products.<sup>1-5</sup> Anthocyanins are the main colorants contributing to the blue and red colors of black and red currants and therefore can be used as food colorants.<sup>6-8</sup> In addition to their contribution to appearance and taste of the berries, anthocyanins and the other phenolic compounds have been widely reported for their antioxidative, anticancer, and anti-inflammatory properties and for their potential in dietary management of various diseases such as hypertension, osteoporosis, and cardiovascular diseases.<sup>9-13</sup>

Therefore, the concentration and variation of the phenolic compounds contributing to sensory properties and bioactivity of foods are of special interest in the view of consumer acceptance and the industrial utilization of berries as food and food ingredients. Genetic differences, cultivation methods, harvesting time, growth places, and environmental conditions are among the factors influencing the composition and quality of berries.<sup>7,14–20</sup> In our previous studies conducted on black currants,<sup>19,20</sup> clear differences in composition were observed between berries of different cultivars and between berries

grown at different latitudes. Significant correlation between quality parameters and weather conditions were detected, and the correlation differed among black currant cultivars.

In the current study, berries of a green currant cultivar 'Vertti', a red currant cultivar 'Red Dutch', and a white currant cultivar 'White Dutch' were collected from southern Finland and northern Finland over seven consecutive years to investigate the compositional differences of phenolic compounds between different cultivars and between berries grown at different latitudes (60°23' N vs 66°34' N). Effects of weather conditions (temperature, radiation, relative humidity, and precipitation) on phenolic composition were studied. The results complement the previous study on sugars and acids of the same cultivars<sup>18</sup> and give comprehensive information on biosynthetic and compositional response of the berries to the varying growth latitude and weather conditions. In addition, compared to the high levels of anthocyanins in black and red currants, green and white currants, which evolved from black and red currants, respectively, do not contain anthocyanin pigments,<sup>15,21</sup> indicating different metabolic pathways between these genetically closely related currant cultivars. This study together with the previous study on black currants<sup>20</sup> investigated and compared the influence of environmental factors on the biosynthesis of phenolic compounds in currants with

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closely related genetic background but different phenolic and metabolic profiles, i.e., containing or lacking of anthocyanin biosynthetic pathways. The study provides important and comparative information for biochemical and physiological studies on the metabolism and metabolic regulation of currants or plants with different flavonoid biosynthetic pathways. Moreover, the correlation of phenolic compounds within each cultivar was also studied.

#### MATERIALS AND METHODS

Berry Samples. The berries were hand-picked from bushes of the green currant (Ribes nigrum L.) cultivar 'Vertti', the red currant (Ribes rubrum L.) cultivar 'Red Dutch', and the white currant (Ribes rubrum L.) cultivar 'White Dutch' (henceforth 'Vertti', 'Red Dutch', and 'White Dutch', respectively) at the research fields of MTT Agrifood Research Finland in southern Finland (Piikkiö, latitude 60°23' N, longitude 22°33' E, altitude 5-15 m) and northern Finland (Apukka, 66°34' N, 26°01' E, 100-105 m) during the years 2005-2011. All the bushes were cultivated in identical ways in the south and in the north.<sup>18</sup> The berries were harvested in quadruplicate from four field blocks (each block consisted of three bushes) in each location in order to minimize plant-to-plant variation. The berries were picked optimally ripe as defined by experienced horticulturists based on the color, flavor, and structure of the berries. The harvesting dates of the sample are listed in Supporting Information Table 1. The berries were frozen and stored at -20 °C immediately after harvesting until being analyzed.

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

Analysis of Anthocyanins. Anthocyanins of red currant berries were analyzed in duplicate according to a method previously applied in our laboratory with slight modifications.<sup>20</sup> About 40 g of berries were thawed in a microwave oven and homogenized with a Bamix mixer (Bamix M133, Switzerland) when half-melted. About 7 g of slurry was weighted accurately and extracted and diluted to a final volume of 50 mL with MeOH/HCl (99:1). After filtration, a sample of 20  $\mu$ L was injected into the HPLC-DAD system consisting of a GT-154 vacuum degasser, two LC-10AT pumps, an SIL-10A autosampler, a CTO-10A column oven, and an SPD-M10AVP diode array detector linked to an SCL-M10AVP data handling station (Shimadzu Corporation, Kyoto, Japan). The system was operated using the LCsolution Workstation software. The samples were analyzed using a Phenomenex Luna C18(2) 100A column (250 mm  $\times$  4.60 mm i.d., particle size 5  $\mu$ m) (Torrance, CA) with a Phenomenex analytical guard cartridge system (Torrance, CA). The analysis of anthocyanins was performed using 5% formic acid as solvent A and acetonitrile as solvent B with the modified procedure of Lätti et al.<sup>22</sup> The gradient program was as the following: 0-2 min, 4-6% B; 2-4 min, 6-8% B; 4-12 min, 8-9% B; 12-20 min, 9-10% B; 20-25 min, 10-10.3% B; 25-35 min, 10.3-40% B; 35-41 min, 40-80% B; 41-43 min, 80-20% B; 43-46 min, 20-4% B; 46-49 min, 4% B. The flow rate of the mobile phase was set as follows: 0-4 min, 1 mL/min; 4-12 min, 1-0.9 mL/min; 12-13 min, 0.9-0.8 mL/min; 13-20 min, 0.8 mL/min; 20-25 min, 0.8-0.81 mL/min; 25-35 min, 0.81-0.95 mL/min; 35-37 min, 0.95-1 mL/min; 37-49 min, 1 mL/min. Anthocyanins were detected at 520 nm and quantified with the calibration curve of cyanidin-3-O-rutinoside as an external standard.

Analysis of Flavonol Glycosides and Hydroxycinnamic Acid Conjugates. Flavonol glycosides and hydroxycinnamic acid conjugates were analyzed in duplicate according to the method described earlier with some modification.<sup>20</sup> About 40 g of berries were thawed in a microwave oven and homogenized with a Bamix mixer when halfmelted. The slurry of 10 g was weighted accurately and extracted four times with 10 mL of ethyl acetate. The four extracts were combined, and the ethyl acetate was removed by rotary evaporation. The extract was then dissolved in 2 mL of MeOH and filtered for the HPLC analysis. A sample of 10  $\mu$ L was injected into the HPLC-DAD system as described above, and the analyses were performed as described earlier.<sup>20</sup> Flavonol glycosides were detected at 360 nm and hydro-xycinnamic acid conjugates at 320 nm. Quantitative analysis of flavonol glycosides was carried out using quercetin-3-*O*-rutinoside, quercetin-3-*O*-glucoside, kaempferol-3-*O*-rutinoside, and kaempferol-3-*O*-glucoside as the corresponding 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 corresponding hydroxycinnamic acid conjugates. The total content of phenolic compounds was calculated as the sum of anthocyanins, flavonol glycosides, and hydroxycinnamic acid conjugates.

Identification of Phenolic Compounds. The peaks of anthocyanins, flavonols, and hydroxycinnamic acid conjugates were identified according to UV-vis spectra, retention times, reference compounds, and mass spectra, as well as literature data.<sup>2,4,14,15,21,23,2</sup> UV absorption spectra in the range of 200-600 nm were obtained during the HPLC-DAD analyses. The anthocyanin extracts of berries of 'Red Dutch' for HPLC-MS analyses were prepared as described above. The extracts of flavonol glycosides and hydroxycinnamic acid conjugates were concentrated for HPLC-MS analyses, to five times the concentration of samples for HPLC-DAD analyses. The chromatographic conditions were the same as in the quantitative analysis, and the LC-MS system used was an Acquity Ultra Performance LC (Waters, Milford, MA) interfaced to a Waters Quattro Premier quadruple mass spectrometer. ESI-MS analyses for phenolic compounds were performed according to the method previously applied in black currant analysis in our laboratory.<sup>2</sup> Data were acquired over a mass range of m/z 250-800 for the analysis of anthocyanins and m/z 100–1000 for flavonol glycosides and hydroxycinnamic acid conjugates. Samples of flavonol glycosides and hydroxycinnamic acid conjugates of black currants were also prepared and analyzed with HPLC-DAD and HPLC-MS to give comparable information for the identification because green currant and black currant belong to the same species (R. nigrum), while red currant and white currant are both R. rubrum in family Grossulariaceae.

**Measurement of Dry Weight.** About 5 g of berries from each sample were weighed in a watch glass accurately in duplicate and cut with a knife. The residue on the knife was flushed carefully with distilled water into the watch glass. The berries were dried at 105  $^{\circ}$ C and weighed accurately upon reaching a constant weight. The dry weight was calculated as the percentage of dried berries of fresh berries.

**Information on Weather Conditions.** Data recorded at the weather station in Yltöinen, Piikkiö (latitude  $60^{\circ}23'$  N, longitude  $22^{\circ}33'$  E, altitude 6 m) and Rovaniemi Airport ( $66^{\circ}33'$  N,  $25^{\circ}50'$  E, 195 m) for the years 2005–2011 were provided by the Finnish Meteorological Institute (Helsinki, Finland). The weather variables and corresponding abbreviations used in this publication are shown in Table 1.

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 different 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 currant cultivars at p < 0.05. Independent-samples t-test was used to investigate the difference between currant berries of the same cultivar 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. To get a more complete view on cultivar comparison, earlier published information of phenolic compounds in black currants 'Mortti' and 'Melalahti',<sup>20</sup> cultivated in the same experimental sites in 2005-2010, were included in the PLS-DA. Cultivars 'Ola' and 'Mortti' had similar phenolic profiles.<sup>20</sup> Thus only one of them was

Table	1.	Weather	Variables	and	Their	Abbreviations	Used	in	the	Study
										/

abbreviations	weather variables	abbreviations	weather variables
DTas	growth sesson period with temperature over $5^{\circ}C$ (day)	Dw	nrecipitation in the last week before harvest (mm)
SUMTes	temperature sum over 5 °C in growth season (°C)	Pian. Pfeb	precipitation in January, February, August, September (mm)
0011190		Paug, Psep	
SUMTgh	temperature sum over 5 $^{\circ}\mathrm{C}$ from the start of growth season until the day of harvest ( $^{\circ}\mathrm{C})$	Hgh	average humidity from the start of growth season until the day of harvest $(\%)$
SUMTm	temperature sum over 5 $^{\circ}\mathrm{C}$ in the last month before harvest ( $^{\circ}\mathrm{C})$	Hm	average humidity in the last month before harvest $(\%)$
HDTgh	hot days (temperature >25 $^{\circ}C)$ from the start of growth season until the day of harvest (day)	Hw	average humidity in the last week before harvest $(\%)$
HDTm	hot days (temperature >25 $^{\circ}\mathrm{C})$ in the last month before harvest (day)	Hjan, Hfeb Haug, Hsep	average humidity in January, February August, September (%)
Tm	average temperature in the last month before harvest (°C)	DH20to30gh	percentage of the days with relative humidity 20–30% from the start of growth season until the day of harvest $(\%)$
Tw	average temperature in the last week before harvest (°C) $% \left( {{\left( {{_{\rm{C}}} \right)} \right)_{\rm{C}}} \right)$	DH30to40gh	percentage of the days with relative humidity 30–40% from the start of growth season until the day of harvest $(\%)$
TDm	mean daily temperature difference in the last month before harvest (°C)	DH40to50gh	percentage of the days with relative humidity 40–50% from the start of growth season until the day of harvest $(\%)$
MiniTm	minimum temperature 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 $(\%)$
MaxiTm	maximum 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 $(\%)$
HTm	average of daily highest temperature in the last month before harvest ( $^{\circ}C)$	DH80to90gh	percentage of the days with relative humidity 80–90% from the start of growth season until the day of harvest $(\%)$
Tjan, Tfeb Taug, Tsep	average temperature in January, February August, September (°C)	DH90to100gh	percentage of the days with relative humidity 90–100% from the start of growth season until the day of harvest $(\%)$
Rgh	radiation from the start of growth season until the day of harvest $\left(kJ/m^2\right)$	DH40to50m	percentage of the days with relative humidity 40–50% in the last month before harvest $(\%)$
Rm	radiation during the last month before harvest $\left(kJ/m^2\right)$	DH50to60m	percentage of the days with relative humidity $50-60\%$ in the last month before harvest (%)
Rw	radiation during the last week before harvest $\left(kJ/m^2\right)$	DH60to70m	percentage of the days with relative humidity 60–70% in the last month before harvest $(\%)$
Rjan, Rfeb Raug, Rsep	radiation in January, February August, September $\left(kJ/m^2\right)$	DH70to80m	percentage of the days with relative humidity 70–80% in the last month before harvest $(\%)$
Pgh	precipitation from the start of growth season until the day of harvest (mm)	DH80to90m	percentage of the days with relative humidity $80{-}90\%$ in the last month before harvest $(\%)$
Pm	precipitation in the last month before harvest (mm)	DH90to100m	percentage of the days with relative humidity 90–100% in the last month before harvest (%)

included. Principal component analysis (PCA) and Pearson's correlation coefficients analysis were applied to study the effects of weather conditions on the contents of phenolic compounds and the value of dry weight of each currant cultivar. The weather variables of radiation in January, precipitation in January, February, March, and April, and humidity in January, February, March, and April, were not included in the PCA because of the existence of missing values in some investigating years. Pearson's correlation coefficients analysis was also applied to investigate the intercorrelations between metabolites.

# RESULTS AND DISCUSSION

**Chromatographic Profiles.** Figure 1 shows the HPLC-DAD chromatograms of different groups of phenolic compounds in the green currant cultivar 'Vertti', the white currant cultivar 'White Dutch', and the red currant cultivar 'Red Dutch' recorded at 320, 360, and 520 nm. Identification of the compounds was based on the chromatographic and mass spectral characteristics, reference compounds, and the literatures.<sup>2,4,14,15,21,23,24</sup> The chromatographic profile of flavonols and hydroxycinnamic acid conjugates of green currant 'Vertti' is close to that of black currant (*Ribes nigrum* L.) analyzed in this study and those reported previously.<sup>20</sup>

Eight hydroxycinnamic acid conjugates were tentatively identified in berries of 'Vertti', four in 'White Dutch', and four in 'Red Dutch' (Figure 1). The compounds have earlier been identified also in black currant variety 'Mortti'.<sup>4</sup>

hydroxycinnamic acid conjugates, *p*-coumaric acid derivatives dominated and accounted for 70%, 87%, and 56% of the hydroxycinnamic acid derivatives in 'Vertti', 'White Dutch', and 'Red Dutch', respectively (Figure 1 and Table 2). Seven flavonol glycosides were identified in 'Vertti', five in 'White Dutch', and five in 'Red Dutch' (Table 2, Figure 1). These compounds were all detected in black currants,<sup>2,20</sup> although the proportions naturally differ. Unlike our earlier findings in black currant,<sup>2</sup> myricetin-3-O-arabinoside was not

Caffeoylglucose in 'White Dutch' and 'Red Dutch' was not

quantified because of coelution with unknown compounds. For

the same reason, p-coumaroylglucose in 'Red Dutch' was not

taken into account in quantitative analysis. One caffeic acid

glucose derivative was detected only in berries of 'Vertti', but

also it coeluted with a p-coumaric acid derivative. Among the

detected in any of the samples of this study. The MS spectra suggested coelution of myricetin-3-*O*-(6"-malonyl)-glucoside and an auresidin glucoside with quercetin-3-*O*-rutinoside in 'Vertti'.<sup>2,14,15,23</sup> In 'Red Dutch', only quercetin-3-*O*-rutinoside and myricetin-3-*O*-(6"-malonyl)-glucoside were detected, and the berries of 'White Dutch' exhibited a simple mass spectrum of quercetin-3-*O*-rutinoside. In the study of Määttä et al.,<sup>15</sup> the content of myricetin-3-*O*-(6"-malonyl)-glucoside was much lower than that of quercetin-3-*O*-rutinoside in green and red currants. The overlapping peak was considered to consist



**Figure 1.** HPLC chromatograms of hydroxycinnamic acid conjugates (320 nm), flavonols (360 nm), and anthocyanins (520 nm) in green currant cultivar 'Vertti' collected from Apukka, Finland, in 2005 (A), white currant cultivar 'White Dutch' collected from Piikkiö, Finland, in 2008 (B), and red currant cultivar 'Red Dutch' collected from Piikkiö, Finland, in 2010 (C). Abbreviations of compounds refer to Table 2. \*, caffeic acid glucose derivative coeluted with *p*-coumaric acid derivative. \*\*, quercetin-3-O-rutinoside coeluted with myricetin-3-O-(6"-malonyl)-glucoside and auresidin glucoside in berries of 'Vertti', and with myricetin-3-O-(6"-malonyl)-glucoside in berries of 'Red Dutch'. \*\*\*, quantification was not applied because of the coelution with unknown compounds.

mainly of and quantified as quercetin-3-*O*-rutinoside, which has to be taken into consideration while comparing the different currant cultivars.

Nevertheless, quercetin glycosides existed as the major flavonol glycosides in all the cultivars. This is in accordance with the findings of Määttä et al.<sup>15,17</sup> Quercetin glycosides accounted for 76%, 68%, and 100% of the total flavonol glycosides in 'Vertti', 'White Dutch', and 'Red Duch', respectively. Trace amount of myricetin-3-O-glucoside, coeluting with an unknown compound, was detected but not quantified in 'Red Dutch'. Malonylglucosides of flavonols were not detected in 'White Dutch'. Dihydroflavonols are direct precursors of flavonols. The conversions between dihydrokaempferol and dihydroquercetin and between dihydrokeampferol and dihydromyricetin are catalyzed by flavonoid-3'-hydroxylase (F3'H) and flavonoid-3',5'-hydroxylase (F3',5'H), respectively.<sup>25</sup> It would be worthwhile to investigate the levels of expression of the genes encoding these two enzymes to illustrate the cause of different proportions of these flavonol glycosides in currant cultivars.

Free quercetin aglycon with m/z 303 was detected in trace amounts in all the samples based on UV spectrum and retention time of the reference compound (Figure 1). Quercetin has earlier been reported in the enzyme-treated black currant juice at higher levels than in the fresh, nontreated juice.<sup>2,4,20</sup>

Neither berries of 'Vertti' nor berries of 'White Dutch' contained any anthocyanins. Out of seven anthocyanins

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Table 2. (	Contents of P.	henolic C	ompounds	in Currant (	(Ribes spp.)	Berries <sup>a</sup>								
							n)	ng/100 g fresh be	(yrite)					
cultivar	growth place <sup>b</sup> (	caffeoyl- glucose (Caf-glc)	<i>p</i> -coumaroyl- quinic acid (Cou-qa)	<i>p</i> -coumaroyl- glucose (Cou-glc)	feruloyl- glucose (Fer-glc)	caffeic acid glucose derivative (Caf glc der)	<i>p</i> -coumaric acid glucose derivative (Cou glc der)	ferulic acid glucose derivative (Fer glc der)	myricetin-3- O-glucoside (My-glc)	quercetin-3- O-rutinoside <sup>c</sup> (Qu-rut)	quercetin-3- O-glucoside (Qu-glc)	kaempferol-3- O-rutinoside (Ka-rut)	quercetin-3- 0-(6"- malonyl)- glucoside (Qu-mal)	kaempferol-3- O-glucoside (Ka-glc)
'Vertti'	S + N = 108 $(n = 108)$	7 ± 0.35	$0.40 \pm 0.13 c$	$6.23 \pm 1.97$	$0.59 \pm 0.10$ c	$0.51\pm0.07~\mathrm{b}$	$1.25 \pm 0.17$ b	$0.42 \pm 0.09 \text{ b}$	$0.39 \pm 0.10$	$2.76 \pm 0.69$	2.32 ± 0.56 c	$0.76 \pm 0.19 \text{ c}$	$1.69 \pm 0.59 c$	$0.94 \pm 0.18 \text{ c}$
'White Dutch'	S + N na $(n = 98)$		$0.11 \pm 0.02$ a	$0.97 \pm 0.29$	$0.16 \pm 0.04 \text{ b}$	nd a	nd a	nd a	$0.07 \pm 0.01$	$0.66 \pm 0.27$	$0.29 \pm 0.16$ a	$0.30 \pm 0.08 \text{ b}$	nd a	$0.08 \pm 0.03 \text{ b}$
'Red Dutch'	$S + N \qquad na \\ (n = 100)$		$0.13 \pm 0.03 \text{ b}$	na	$0.10 \pm 0.02$ a	nd a	nd a	nd a	na	$0.79 \pm 0.39$	$0.56 \pm 0.31$ b	nd a	$1.00 \pm 0.56 \text{ b}$	nd a
'Vertti'	S(n = 52) 1.9	$4 \pm 0.36 \text{ x}$	$0.43 \pm 0.08 \text{ y}$	$4.83 \pm 1.50 \text{ x}$	$0.58 \pm 0.09 \text{ x}$	$0.52 \pm 0.07 \text{ y}$	$1.26 \pm 0.17 \text{ x}$	$0.44 \pm 0.07 \text{ y}$	$0.40 \pm 0.12 \text{ x}$	$2.81 \pm 0.79 \text{ x}$	$2.54 \pm 0.45 \text{ y}$	$0.72 \pm 0.23 \text{ x}$	$1.52 \pm 0.67 \text{ x}$	$0.93 \pm 0.20 \text{ x}$
'White Dutch'	N $(n = 50)$ 1.9 S $(n = 52)$ na	9 ± 0.33 x	$0.3/ \pm 0.16 \text{ x}$ $0.12 \pm 0.02 \text{ y}$	$7.52 \pm 1.57$ y $0.82 \pm 0.29$ x	$0.59 \pm 0.10 \text{ x}$ $0.15 \pm 0.04 \text{ x}$	u.49 ± u.u/ x nd	x /1.0 ± 62.1 nd	nd = 0.39 ± 0.11 x	$0.39 \pm 0.07 x$ $0.07 \pm 0.01 x$	$2./1 \pm 0.34 x$ $0.64 \pm 0.34 x$	2.12 ± 0.59 x 0.27 ± 0.16 x	$0.80 \pm 0.13 \text{ y}$ $0.30 \pm 0.10 \text{ x}$	1.85 ± 0.44 y nd	0.08 ± 0.03 x 0.08 ± 0.03 x
	N ( $n = 46$ ) na		$0.10 \pm 0.01 \text{ x}$	$1.14\pm0.18~\mathrm{y}$	$0.18\pm0.04~\mathrm{y}$	pu	pu	pu	$0.08\pm0.01~\mathrm{y}$	$0.69 \pm 0.17 \text{ x}$	$0.32 \pm 0.15 \text{ x}$	$0.30 \pm 0.06 \text{ x}$	pu	$0.08 \pm 0.02 \text{ x}$
'Red Dutch'	S $(n = 46)$ na		$0.14 \pm 0.03 \text{ y}$	na	$0.09 \pm 0.02 x$	pu	pu	pu	na	$0.89 \pm 0.47 \text{ y}$	$0.61 \pm 0.41 \text{ x}$	pu	$1.04 \pm 0.71 \text{ x}$	pu
	N ( $n = 54$ ) na		$0.12 \pm 0.03 \text{ x}$	na	$0.10 \pm 0.02 \text{ y}$	nd	pu	nd	na	$0.71 \pm 0.29 \text{ x}$	$0.51 \pm 0.17 \text{ x}$	pu	$0.96 \pm 0.40 \text{ x}$	nd
							(mg/	100 g fresh berry.						
cultivar	$\operatorname{growth}_{\operatorname{place}^b}$	0 4	yanidin-3- <i>O</i> - sophoroside (Cy-soph)	cyanidin-3-0- glucosyl- rutinoside (Cy-glc-rut)	cyanidin- sambubio (Cy-sam	3- <i>O</i> - cyanic 3- <i>O</i> - xyl side rutii (Cy-)	lin-3- <i>O</i> - osyl- cy noside i	ranidin-3-0- rutinoside (Cy-rut)	total hydroxy- cinnamic acid conjugates (Tot HCA)	total flavonol glycosides (Tot FLA)	tota anthocya (Tot A)	l tota unins cou VT) (T	l phenolic mpounds ot PHE)	(% fresh berry) dry weight
'Vertti'	S + N (n = 10)	08) n	id a	nd a	nd a	nd a	pu	ła	11.35 ± 2.17 c	8.88 ± 1.88 c	: nd a	20.2	3 ± 3.13 b	19.89 ± 1.55 c
'White Dutch'	S + N (n = 9)	8) n	ld a	nd a	nd a	nd a	nd	l a	$1.25 \pm 0.34 \text{ b}$	1.40 ± 0.52 a	n nd a	2.65	± 0.75 a	$17.20 \pm 1.17 \text{ b}$
'Red Dutch'	S + N (n = 10)	00) 1.	.94 ± 0.42 b	$5.28 \pm 1.42$ b	$3.04 \pm 1.9$	94 b 9.38 ±	± 3.16 b 2.4	$41 \pm 1.03 \text{ b}$	0.23 ± 0.04 a	$2.35 \pm 1.23 \text{ b}$	, 27.06 ± 4	1.63 b 29.6	3 ± 5.00 c	15.49 ± 1.31 a
'Vertti'	S(n = 52)	u	d	pu	pu	pu	nd	1	10.01 ± 1.84 x	$8.92 \pm 2.13 x$	t nd	18.9.	2 ± 3.27 x	19.60 ± 1.13 x
	N (n = 56)	u	p	pu	nd	pu	nd	Ŧ	$12.61 \pm 1.64 \text{ y}$	$8.84 \pm 1.64 x$	t nd	21.4	4 ± 2.47 y	20.15 ± 1.83 x
White	S $(n = 52)$	u	р	pu	pu	pu	ná	ł	$1.08 \pm 0.34 \text{ x}$	$1.35 \pm 0.62 \text{ x}$	r nd	2.43	± 0.92 x	$17.54 \pm 1.25$ y
Dutch	N ( $n = 46$ )	u	р	pu	pu	pu	ná	Ŧ	$1.43 \pm 0.22 \text{ y}$	$1.47 \pm 0.38 \text{ x}$	t nd	2.90	± 0.36 y	$16.81 \pm 0.93 \text{ x}$
'Red Dutch'	S ( $n = 46$ )	1	.83 ± 0.40 x	$5.07 \pm 1.67 \text{ x}$	t 6.62 ± 1.4	06 x 9.15 <u>⊣</u>	± 4.00 x 2.7	73 ± 1.30 y	$0.23 \pm 0.04 \text{ x}$	$2.54 \pm 1.57 \text{ x}$	t 25.41 ± ₄	4.74 x 28.1	7 ± 5.34 x	$16.21 \pm 1.34 \text{ y}$
	N(n = 54)	2	.02 ± 0.43 y	5.46 ± 1.15 x	t 9.26 ± 1.4	67 y 9.58 <u>±</u>	± 2.23 x 2.i	14 ± 0.62 x	$0.23 \pm 0.05 \text{ x}$	$2.19 \pm 0.82 \text{ x}$	t 28.47 ± 4	4.06 y 30.8	8 ± 4.37 y	14.88 ± 0.93 x
<sup>a</sup> Significant na, not ana glucoside aı	: difference $(p < ly z > ly z < ly $	0.05) betw of overlappi coside in b	reen samples ing peaks; no verries of 'Ver	of different cul 1, not detected rtti', and with :	ltivars and bet 1. <sup>b</sup> S, souther myricetin-3- <i>C</i>	ween samples n Finland (Pi )-(6"-malonyl)	. grown at diff iikkiö); N, no )-glucoside in	erent latitudes orthern Finland berries of 'Reu	(every cultivar 1 (Apukka). <sup>c</sup> C d Dutch'.	compared sep; Quercetin-3- <i>O</i> -1	arately) are m utinoside coe	larked as a–c sluted with m	and x—y, resp yricetin-3- <i>O</i> -(	ectively. Key: 6″-malonyl)-

reported in various cultivars of red currants,<sup>8,16,24,26,27</sup> five cyanidin glycosides were detected in 'Red Dutch' in this study. In elution order, they were identified as cyanidin-3-*O*sophoroside, cyanidin-3-*O*-glucosylrutinoside, cyanidin-3-*O*sambubioside, cyanidin-3-*O*-xylosylrutinoside, and cyanidin-3-*O*-rutinoside. Among them, cyanidin-3-*O*-xylosylrutinoside (35% of total anthocyanins) and cyanidin-3-*O*-sambubioside (30%) were the two most abundant anthocyanins. Wu et al.<sup>24</sup> reported in a red currant cultivar 'Red Lake' delphinidin-3-*O*sambubioside at a level of 0.10 mg/100 g fresh weight. This was found neither in our samples nor in the other cultivars investigated.<sup>7,8,16,26,27</sup>

**Cultivar Comparison.** The phenolic composition and dry weight of the berries of three cultivars grown at two latitudes in Finland are listed in Table 2. Berries of 'Vertti' had the highest value of dry weight while those of 'Red Dutch' the lowest (p < 0.05). The contents of phenolic compounds based on both fresh weight and dry weight of the berries were analyzed statistically, and analogous patterns were detected on the compositional differences between cultivars. Furthermore, compositional response of currant berries to the latitude and weather conditions based on fresh weight and dry weight showed, again, analogous trends. Thus, only the results based on fresh weight are presented.

Among the three currant cultivars studied, green currant 'Vertti' contained the highest amounts of total hydroxycinnamic acid conjugates and total flavonol glycosides, being 9.1 and 6.3 times, and 50 and 3.8 times, of the amounts in 'White Dutch' and 'Red Dutch', respectively (p < 0.05, Table 2). Nevertheless, red currant 'Red Dutch', due to its high content of anthocyanins, contained the highest level of total phenolic compounds.

To investigate the compositional difference in phenolic compounds between cultivars within the same species, PLS-DA was applied on the compositional data of 'Red Dutch' and 'White Dutch' for *R. rubrum* and on data of 'Vertti' and black currant cultivars 'Mortti' and 'Melalahti'<sup>20</sup> in an earlier investigation for R. nigrum, respectively. PLS-DA classified the samples, based on their phenolic composition, by grouping the samples belonging to different cultivars (Figure 2). In the PLS-DA loading plots of both R. rubrum and R. nigrum, the total flavonol glycosides and total anthocyanins, as well as total phenolic compounds, were located close to the red and black currants and far away from those of green and white currants, while the situation of the total hydroxycinnamic acid conjugates was vice versa. Therefore, green currant and white currant were clearly separated from black currant ( $R^2 = 0.85$  and  $Q^2 = 0.83$ ) with 2 factors, Figure 2A) and red currant ( $R^2 = 0.97$  and  $Q^2$  = 0.97 with 2 factors, Figure 2B), respectively, by the lack of anthocyanins and lower contents of total flavonol glycosides and total phenolic compounds but higher content of total hydroxycinnamic acid conjugates. This might have been due to the lower activity of chalcone synthase (CHS) or lower expression of the genes encoding CHS in green and white currants than in black and red currants. CHS is an important enzyme catalyzing the first committed step from the 'hydroxycinnamic acid' pool to the flavonoid pathway.<sup>28</sup> The next metabolites in the pathway, dihydroflavonols, are converted to flavonols by the action of flavonol synthase (FLS), whereas dihydroflavonol 4-reductase (DFR) and anthocyanidin synthase (ANS) utilize dihydroflavonols for the formation of anthocyanidins and proanthocyanidins. Study on the availability and activity of the key enzymes, CHS, FLS, DFR, and ANS, might provide new insight into the biochemical mechanism

explaining the different distribution of flavonols and anthocyanins in the currant cultivars.  $^{25,29}\,$ 

**Latitude Comparison.** As shown in Table 2, berries of the three cultivars showed analogous responses to varying latitude in contents of total phenolic compounds. Berries grown at the higher latitude contained higher amount of total phenolic compounds (by 10-19%, p < 0.05) than those grown at the lower latitude.

PLS-DA was applied to create predictive models for the green, white, and red currants investigated to differentiate samples collected from different locations by their phenolic composition. As a result, berries of 'Vertti' ( $R^2 = 0.72$  and  $Q^2 = 0.58$  with 3 factors), 'White Dutch' ( $R^2 = 0.82$  and  $Q^2 = 0.78$  with 3 factors), and 'Red Dutch' ( $R^2 = 0.68$  and  $Q^2 = 0.57$  with 5 factors) collected in southern Finland were clearly separated from those in northern Finland (Figure 3).

Hydroxycinnamic acid conjugates are a major group of phenolic compounds in green and white currants. The total contents of these compounds were much higher in berries from the north than in those from the south in 'Vertti' (by 26%, p < 0.05) and in 'White Dutch' (by 32%, p < 0.05) (Table 2). In accordance, coumaroylglucose, the most abundant hydroxycinnamic acid conjugate in these two cultivars was more abundant (by 56% for 'Vertti', and by 39% for 'White Dutch', p < 0.05, Table 2) in Apukka than in Piikkiö. As a minor group of phenolic compounds in berries of 'Red Dutch', the total content of hydroxycinnamic acid conjugates did not differ between berries from different latitudes (p > 0.05). Hydroxycinnamic acid derivatives and flavonol glycosides contribute to astringency and bitterness of berries.<sup>2,4,5,30,31</sup> Thus, the possible influence of growth location on the content of hydroxycinnamic acid conjugates and the sensory properties of the berries should be taken into account when selecting growth sites and agricultural practices for the cultivation of berries.

Unlike hydroxycinnamic acid conjugates, flavonol glycosides are the least influenced by the growth latitude among the phenolic compounds. However, latitude seemed to have some opposite effects on the content of quercetin-3-O-glucoside and quercetin-3-O-(6"-malonyl)-glucoside in 'Vertti', which might have been a result of increased conversion of the former compound to the latter in north.

The major group of phenolic compounds in berries of 'Red Dutch', anthocyanins, was more abundant (by 12%, p < 0.05, Table 2) in berries from northern Finland than those from southern Finland. In black currants, the situation was vice versa,<sup>20</sup> which indicated species-specific differences in the biosynthetic pathways and their responses to the environmental variations. The content of cyanidin-3-O-sambubioside, one of the most abundant anthocyanins, was higher by 40% in berries of red currant from the north compared with those from the south (p < 0.05). Cyanidin-3-O-sophoroside showed the same trend even though the difference between the two latitudes (10% higher in berries grown in the north than those in the south, p < 0.05) was lower compared to that of cyanidin-3-O-sambubioside (Table 2). In this study, the content of cyanidin-3-O-rutinoside decreased (by 22%, p < 0.05, Table 2) as latitude increased, accompanied by increase in the levels of cyanidin-3-O-glucosylrutinoside (by 8%, p > 0.05) and cyanidin-3-O-xylosylrutinoside (by 5%, p > 0.05). This might be an indication of an increased conversion of cyanidin-3-O-rutinoside to cyanidin-3-O-glucosylrutinoside and cyanidin-3-O-xylosylrutinoside at the higher latitude.

Effects of Weather Conditions on Berry Composition. Clear annual variations were recognized in levels of phenolic



Figure 2. Loading plots of PLS-DA model for currant samples of species *R. nigrum* (A) and *R. rubrum* (B) classified according to cultivar based on the phenolic composition. Abbreviations of compounds refer to Table 2.

compounds and their response to changes in latitude, suggesting the significant impact of weather conditions on the composition of the berries (Supporting Information Tables 2–4). PCA was applied to investigate the overall correlations between the contents of phenolic compounds and the weather variables in different currant cultivars (Figure 4). The closer the weather parameters and the compositional parameters are located to each other in the plot, the stronger positive correlations are suggested between them. In opposition, the parameters located centrosymmetrically far away from each other are likely to have negative correlations. More detailed information on individual correlations between weather variables and various phenolic compounds are presented by Pearson's correlation coefficients in Supporting Information Table 5.

Influence of Humidity on Phenolic Compounds in Currant Berries. In both green currant 'Vertti' and white currant 'White Dutch', the contents of the major hydroxycinnamic acid, p-coumaroylglucose, and of total hydroxycinnamic acid conjugates positively associated with low humidity variables but negatively with high humidity variables, i.e., percentage of days with relative humidity below 70% (for low humidity variables) and above 70% (for high humidity variables), respectively, during the growth season until the day of harvest and in the last month before harvest (Figure 4A). In 'Vertti', Caffeic acid glucose derivative and p-coumaric acid glucose derivative are located oppositely to caffeoylglucose and p-coumaroylglucose in the PCA plot and showed opposite correlations with humidity variables (Figure 4A). This indicates that high humidity favors the biosynthesis of the formers but inhibits that of the latters. All of these indicated the significance of relative humidity on the biosynthesis of hydroxycinnamic acid conjugates in green and white currants. However, the



Figure 3. Loading plots of PLS-DA models for currant samples (one for each cultivar) classified according to growth location based on the phenolic composition. Abbreviations of compounds refer to Table 2.

Article



Figure 4. continued



Figure 4. continued



Figure 4. continued



**Figure 4.** PCA plots of the correlations between phenolic compounds and humidity variables (A), radiation variables (B), temperature variables (C), and precipitation variables (D) in 'Vertti', 'White Dutch', and 'Red Dutch'. Abbreviations of weather variables refer to Table 1 and those of compounds to Table 2.

samples in this study were naturally grown in the test field and exposed to a combination of varying environmental factors.

Thus, the coinfluence of factors other than relative humidity on the biosynthesis of metabolites in the fruits should be considered carefully. Positive associations were observed between relative humidity, radiation, and temperature variables in the years studied, and this makes it difficult to completely distinguish the effects of humidity from those of temperature and radiation.

Flavonol glycosides were another major group of phenolic compounds in green and white currants, but compared with hydroxycinnamic acid conjugates, the contents of flavonol glycosides in the berries of green and white currants seemed to be less dependent on humidity variables (Figure 4A), although some correlations were found between the levels of some compounds and certain humidity variables (Supporting Information Table 5). The total content of phenolic compounds in green and white currants associated positively with low humidity variables during growth season, mostly due to the response of hydroxycinnamic acid conjugates to the humidity variables.

In 'Red Dutch', the contents of anthocyanins and other phenolic compounds (hydroxycinnamic acid conjugates and flavonol glycosides) were poorly explained by PC1, indicating little correlation with humidity variables (Figure 4A). As a result, no clear effects of humidity on the total content of phenolic compounds were seen in red currant.

Only a little information is available on the effects of relative humidity on the biosynthesis and the contents of phenolic compounds in fruits and berries. Dannehl et al.<sup>32</sup> reported an increase in total content of phenolic compounds in tomatoes grown in the green house with a combined application of a high pressure fog system and CO<sub>2</sub> enrichment, leading to increases in the levels of mean temperature, relative humidity, and CO<sub>2</sub> concentrations. It could, however, not be clearly determined which variable had the major impact.<sup>32</sup> Low air humidity may affects the accumulation of secondary metabolites by enhancement of the leaf transpiration and reduction of the surface temperature of the leaves, which alters the rates of photosynthesis and photorespiration and thus the photosynthetic assimilates supply for secondary metabolism.<sup>33</sup>

Influence of Radiation and Temperature on Phenolic *Compounds in Currant Berries.* Both the radiation (Figure 4B) and temperature (Figure 4C) variables showed negative association with the contents of major hydroxycinnamic acid conjugate (p-coumaroylglucose) and total hydroxycinnamic acid conjugates in green and white currants and with the content of major anthocyanin, cyanidin-3-O-sambubioside, in red currant. Thus it suggested negative impact of high radiation and high temperature during the growth season on the accumulation of major phenolic compounds in the berries, possibly due to the reduction of biosynthesis and/or increased degradation of these compounds. In contrast, regardless of some individual associations found by Pearson's correlation coefficient analysis (Supporting Information Table 5), the contents of flavonol glycosides showed less dependence on radiation and temperature variables compared with the two other groups of phenolic compounds studied (Figure 4B,C).

Flavones and flavonols generally absorb light at shorter wavelengths and function to protect cells from excessive UV-B radiation (280–320 nm).<sup>33</sup> Anthocyanins, in addition to their important roles in attracting animals for pollination and seed dispersal,<sup>33</sup> are also important as feeding deterrents and as protection against damage from UV irradiation.<sup>25</sup> As a result, plants appear to adapt to high UV radiation by increasing the accumulation of these flavonoids.<sup>11,33,34</sup> Hydroxycinnamic acids constitute the so-called 'hydroxycinnamic pool', the principal source of these flavonoids.<sup>33</sup> Therefore, the increased rates of

transcription of genes encoding phenylalanine ammonia lyase (PAL), the key enzyme for their biosynthesis, induced by UV irradiation in parsley cells was explained by their involvement in the synthesis of compounds related to UV resistance.<sup>35</sup> This is in contradiction with our findings, where the major phenolic compounds, hydroxycinnamic acids in green and white currants and anthocyanins in red currant, showed consistently inhibited accumulations under high radiation. Similar to the findings of ours, Ibrahim et al..<sup>36</sup> reported a negative correlation between total flavonoids and the irradiation level in Labisia pumila var. alata. Through the correlation study between production of total flavonoids and phenolics with photosynthesis, maximum efficiency of photosystem II (Fv/Fm) and electron transfer rate (Fm/Fo), they suggested the up-regulation of carbon-based secondary metabolites (CBSM) under reduced photoinhibition at low light levels. In addition, they also found that the increase in the production of CBSM was due to the high phenylalanine ammonialyase activity under low light conditions.<sup>36</sup> Moreover, the concurrent changes in temperature in our study might influence the response of flavonoid biosynthesis to radiation levels.

The accumulation of anthocyanins was reported to be induced by both low temperature and high radiation in most of the plants.<sup>37–40</sup> In our study, the concurrent changes in temperature and radiation and their strong and positive correlations with each other made it difficult to distinguish the effects of temperature from those of radiation on the content of anthocyanins in berries of 'Red Dutch'. In agreement with the negative association of cyanidin-3-*O*-sambubioside with the temperature and radiation variables observed in this study, the contents of delphinidin 3-glucoside and cyanidin 3-glucoside in the arils of pomegranates were reported to correlate negatively with temperature.<sup>40,41</sup> The concurrent changes in radiation and temperature also occurred in these studies. Schwartz et al.<sup>41</sup> assumed that temperature had more pronounced effects on the content of anthocyanin in pomegranates.

Moreover, when comparing our results with other published information, it is worth noticing that both of our experimental fields are located quite far north, between 60 and  $70^{\circ}$  of the northern latitude. The declination of sun is low, but the day-length during the growth season is longer than areas further south. This may be one cause of the selective metabolic differences in phenolic compounds in various published experiments.

Influence of Precipitation on Phenolic Compounds in Currant Berries. Unlike the situation in temperature and radiation variables, the PCA plots in Figure 4D do not show clear correlation between the contents of phenolic compounds and most precipitation variables, suggesting clearly less dependence of these compounds on precipitation. Pearson's correlation coefficient analysis detected some correlation between phenolic compounds and certain precipitation variables (Supporting Information Table 5). High precipitation in March was associated with lower contents of anthocyanins in 'Red Dutch' and lower levels of flavonol glycosides and hydroxycinnamic acid conjugates in 'White Dutch'. High precipitation in September may lead to a decrease in contents of hydroxycinnamic acid conjugates in 'Vertti'.

Weather Conditions As Explanatory Factors for Variation between Growth Locations and Years. Principle component analysis showed overall lower humidity, lower radiation, and lower temperatures during the growth season in the North (Apukka) than in the South (Piikkiö) (Figure 4A–C). These differences in the weather conditions were the major factors contributing to the higher contents of phenolic compounds found in the berries grown in the north compared with those of the same cultivars grown in the south. There was no clear difference in precipitation between the two growth locations (Figure 4D). Therefore, precipitation variables may not have been among the major factors explaining the difference in the phenolic content between the berries cultivated in the different locations.

Also varying weather conditions provide explanation for the annual variations observed in the content of phenolic compounds. Taking as an example the green currant cultivar 'Vertti' grown in the southern Finland, the berries collected in 2010 and 2011 had the lowest contents of total hydroxycinnamic acids (7.6 and 7.9 vs 10.0-12.2 mg/100 g) among berries from all the harvesting years (Supporting Information Table 2). On the contrary, the berries harvested in 2008 contained highest level of total hydroxycinnamic acid conjugates among the years studied (12.2 vs 7.6-11.1 mg/100 g, Supporting Information Table 2). In accordance, year 2010 and 2011 were characterized with overall higher values of humidity variables, while year 2008 the lowest values of humidity variables in Piikkiö (Supporting Information Figure 2). Further, the year 2008 was also differentiated from the years 2010 and 2011 by lower values of temperature sum during the growth season until harvest and the temperature parameters during summer (Supporting Information Figure 2). These differences in temperature and humidity variables are likely the factors explaining the highest contents of total hydroxycinnamic acids in berries of 2008, and the lowest contents in berries of 2010 and 2011 from Piikkiö. Moreover, the findings may also indicate the variation of the major phenolic compounds in green currants is attributed more to the variation of temperature and air humidity than to that of radiation. In the northern Finland, year 2006 is clearly distinguished from the other years by lower humidity. In accordance, the berries of 'Vertti' collected in 2006 stood out from the samples of other years by higher contents of total hydroxycinnamic acids conjugates (14.6 vs 10.2-13.8 mg/100 g). However, although year 2008 in Apukka is distinguished from the other years by the higher values of humidity variables (Supporting Information Figure 2), the hydroxycinnamic acid conjugates did not presented the lowest values among the berries collected in different years as expected. This might have been due to the coinfluence of the low temperature in 2008 on the accumulation of these compounds.

**Correlation between Components.** *p*-Coumaric acid is first synthesized via the phenylpropanoid pathway and then converted to caffeic acid, ferulic acid by *p*-coumarate 3-hydroxylase (C3H), and caffeic acid *O*-methyltransferase (COMT).<sup>42,43</sup> Positive correlations between *p*-coumaroylquinic acid and feruloylglucose were detected in all the three cultivars (r = 0.44-0.64, p < 0.05). In addition, significant correlations between other hydroxycinnamic acid derivatives were also observed in different cultivars.

4-Coumaroyl CoA derived from phenylpropanoid pathway functions as one of the substrates in the flavonoid biosynthetic pathways.<sup>44</sup> This might provide an explanation on the positive correlations between kaempferol-3-*O*-rutinoside and *p*-coumaroylglucose (r = 0.42 and 0.49 in 'Vertti' and 'White Dutch', respectively, p < 0.01) and between kaempferol-3-*O*-rutinoside and feruloylglucose (r = 0.56 and 0.37, respectively, p < 0.01) presented in 'Vertti' and 'White Dutch'. In berries of 'Vertti', positive correlations between kaempferol-3-*O*-glucoside and *p*-coumaroylquinic acid (r = 0.49, p < 0.01) and between

ka<br/>empferol-3-O-glucoside and feruloyl<br/>glucose (r = 0.75, p < 0.01) were also detected.

In view of the flavonoid pathway, dihydrokaempferol is first converted from naringenin via hydroxylation by flavonone 3-hydroxylase (F3H) and can be converted further to dihydroquercetin and dihydromyricetin via the action of F3'H and F3',5'H. Flavonol glycosides are, then, converted from dihydroflavonols by flavonol synthase (FLS), rhamnosyl transferase (RT), and UDP-glucosyltransferase (FGT).<sup>44</sup> In accordance, we found that myricetin glycosides, quercetin glycosides, and kaempferol glycosides correlated positively with each other in 'White Dutch' (r = 0.31-0.92, p < 0.01). These flavonol glycosides also showed positive correlation with each other in 'Vertti' (r = 0.36 - 0.83, p < 0.01), with exception of the glucosides of myricetin, quercetin, and kaempferol. In addition, the higher contents of quercetin glycosides compared to myricetin glycosides in both 'Vertti' and 'White Dutch' indicated the availability and activity of F3'H might be much higher than F3',5'H. Moreover, quercetin glycosides showed strong and positive correlations (r = 0.68 - 0.95, p < 0.05) with each other in all the cultivars except of the correlation between quercetin-3-O-glucoside and quercetin-3-O-(6"-malonyl)glucoside in 'Vertti', which was too weak (r = 0.22, p <0.05). The two kaempferol glycosides detected, kaempferol-3-O-rutinoside and kaempferol-3-O-glucoside, also correlated strongly and positively with each other in 'Vertti' and 'White Dutch' (r = 0.77 and 0.87, respectively, p < 0.05).

Cyanidin is derived from dihydroquercetin in the flavonoid biosynthetic pathway.<sup>25</sup> In our study, cyanidin-3-*O*-glucosylrutinoside showed positive correlation with all the quercetin glycosides (r = 0.45-0.59, p < 0.05) while cyanidin-3-*O*-rutinoside correlated with quercetin-3-*O*-rutinoside.

## ASSOCIATED CONTENT

#### Supporting Information

Tables of harvesting information of currant samples, phenolic composition of berry samples collected from different growth locations and years, and Pearson's correlation coefficients between weather conditions and phenolic compounds in currant berries. Figure of PCA plots of the differences in weather conditions between years in each growth site. This material is available free of charge via the Internet at http:// pubs.acs.org.

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#### Notes

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