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Anthocyanin and Flavonol Variation in Bog Bilberries (*Vaccinium uliginosum* L.) in Finland

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The flavonoids, anthocyanins and flavonols, in bog bilberries (*Vaccinium uliginosum* L.) were studied from 15 populations in Finland on a south−north axis of ∼1000 km. Four anthocyanidin xylosides and 14 flavonol glycosides were tentatively identified by means of HPLC−ESI-MS. Twenty-five major flavonoids were quantified by HPLC−DAD. The averages (±standard deviation) in the contents of anthocyanins and flavonols were 1425 ± 398 and 1133 ± 290 mg/100 g of dry weight, respectively. The most abundant anthocyanidin was malvidin, followed by delphinidin, petunidin, cyanidin, and peonidin. Quercetin was the major flavonol, followed by myricetin, laricitrin, syringetin, and isorhamnetin. Anthocyanins were mostly glucosides, whereas flavonols were mainly conjugated to galactose. The anthocyanin content in the berries from the south was the lowest. The delphinidin content was the highest but the proportion of malvidin the lowest in the north. The total flavonol content and the level of myricetin and quercetin were the highest in the north.

KEYWORDS: ***Vaccinium uliginosum*; anthocyanins; flavonols; HPLC−DAD−ESI-MS; authenticity**

INTRODUCTION

Bog bilberry (*Vaccinium uliginosum* L.) is a deciduous shrub growing mainly in the boreal regions of North America, Europe, and Asia (*1*). This genetically and morphologically very variable species (*1*−*3*) has blue-colored berries that have been traditionally consumed in Finland, Canada (*4*), Japan (*5*), and Poland (*6*). The berries, a good source of vitamin C, are eaten fresh or processed into jams, jellies, or liqueurs (*2*, *4*, *5*).

Bog bilberries contain an abundance of flavonoids, anthocyanins, and flavonols (*7*, *8*). The anthocyanins are galactosides, glucosides, and arabinosides of delphinidin, cyanidin, petunidin, peonidin, and malvidin (**Figure 1**) (*7*). The flavonol glycosides (**Figure 1**) are found as galactosides of myricetin, quercetin, and syringetin, glucosides of quercetin and syringetin, and rhamnosides and arabinosides of quercetin (*5*, *8*), but quantitative data are available for only the myricetin and quercetin aglycons (*8*−*11*). Anthocyanins are generally extracted with acid (pH <2) in aqueous organic solvents by repeated maceration of crushed or ground plant material (*12*, *13*). Flavonols were previously analyzed after acid hydrolysis as aglycons from *Vaccinium* berries (e.g., refs *9*−*11* and *14*−*16*), although there are reports about flavonol conjugates after solvent extraction (*8*, *17*, *19*).

The content of flavonols in the Western diet is estimated as being rather low, in contrast to anthocyanins (*20*). Flavonoids have been suggested to have numerous health benefits, including protection from cardiovascular disease and cancer (e.g., refs *21*−*23*). Most of the effects are supposed to derive from the antioxidant properties of these compounds as well as their ability to modulate many cellular enzyme functions (*21*−*23*). These activities are governed by the number, positions, and types of substitutions in the A, B, and C rings in the chemical structure (**Figure 1**) (*21*, *24*). The multiple hydroxyl groups endow the molecule with substantial antioxidant, chelating, and prooxidant activities (*21*, *25*).

Wild berry stands are mixtures of genotypes of the same plant species which differ markedly in their abilities to produce flavonoids. The primarily genetically determined composition of these compounds is also affected by environmental factors such as light, temperature, and drought (*13*, *26*). The solar radiation increased the contents of cyanidin 3-glucoside and quercetin in the leaves of *Vaccinium myrtillus* (*27*). Moreover, exposure to UV light induced the biosynthesis of flavonols with higher hydroxylation levels (*26*). The increase in the contents of flavonoids and phenolic acids in leaves has been proposed to be a direct response to oxidative stress produced due to the excess light (*28*). An indirect response may be triggered by low temperatures, which limit photosynthesis and thus increase oxidative stress (*28*).

The continuous light during the growing season with low night temperatures in the north is thought to increase the production of secondary compounds also in berries. Previously, we found that the anthocyanin content and the content of the hydroxylated anthocyanidin, delphinidin, were significantly lower in bilberries (*V. myrtillus*) from the south compared to their counterparts from central and northern Finland (*18*). Geographical variation of the content of flavonols in berries has not been previously systematically investigated. Our objective was to screen the anthocyanin and flavonol variation in bog bilberries from 15 populations in Finland on a south−north axis of ∼1000 km between the

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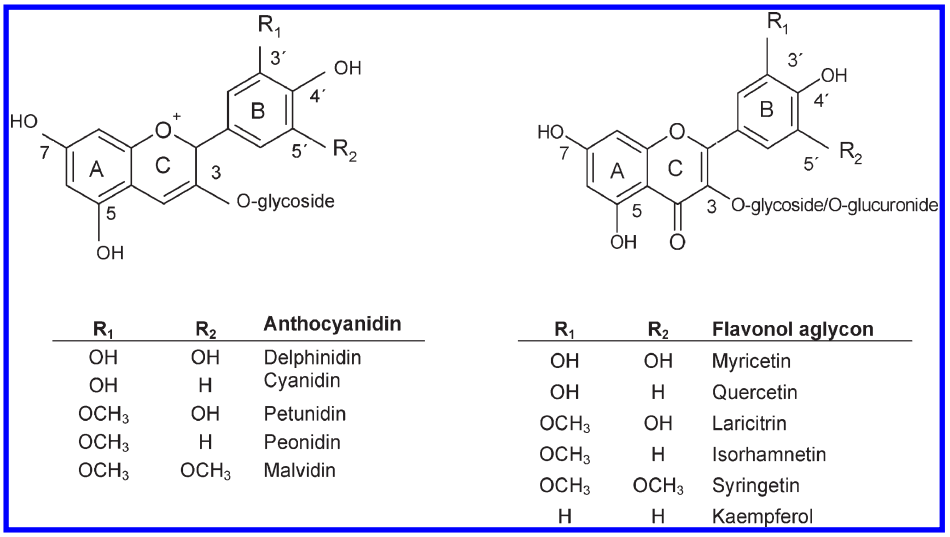

R_1	R_2	Anthocyanidin
OH	OH	Delphinidin
OH	H	Cyanidin
OCH_3	OH	Petunidin
OCH_3	H	Peonidin
OCH_3	OCH_3	Malvidin

R_1	R_2	Flavonol aglycon
OH	OH	Myricetin
OH	H	Quercetin
OCH_3	OH	Laricitrin
OCH_3	H	Isorhamnetin
OCH_3	OCH_3	Syringetin
H	H	Kaempferol

Figure 1. Chemical structures of anthocyanidins and flavonol aglycons found in the berries of *V. uliginosum*.

Table 1. Anthocyanin Contents as Cyanidin 3-Glucoside Equivalents [milligrams per 100 g of dry weight (DW) ± standard deviation (SD)] in Bog Bilberries from 15 Wild Populations in Finland[a]

			delphinidin		cyanidin		petunidin		peonidin		malvidin		unknown		total DW		total FW	
location	latitude (N)	no. of bushes (genotypes[b])	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
region 1 (south)																		
Turku	60° 23′′	9	291	76	62	21	215	50	48	17	566	67	18	5	1200	161	173	28
Janakkala	60° 53′′	9	314	134	92	86	207	72	48	40	403	71	13	4	1077	362	155	51
Savitaipale	61° 08′′	9	290	50	75	51	218	36	68	51	646	132	21	5	1319	256	175	42
Vammala	61° 18′′	8	401	169	83	35	261	91	41	17	467	96	15	4	1269	354	153	47
Ruokolahti	61° 25′′	10	317	90	57	20	235	54	43	15	559	123	19	8	1229	248	170	38
region 2 (central)																		
Vesanto	62° 56′′	9	380	121	103	62	277	71	70	32	613	108	22	3	1464	302	195	37
Lapua	63° 00′′	8	368	156	95	50	251	90	65	26	626	222	22	8	1426	502	175	54
Keitele	63° 13′′	10	427	170	87	48	308	113	67	35	762	188	24	8	1676	518	226	71
Lieksa	63° 16′′	9	355	111	58	26	262	77	47	21	562	106	23	5	1307	310	190	51
Juuka	63° 20′′	10	435	117	134	144	299	71	85	82	657	139	23	7	1633	368	224	53
Lieksa	63° 23′′	10	430	187	132	67	294	110	89	56	696	200	24	6	1665	509	245	71
Valtimo	63° 49′′	10	464	108	104	44	297	56	55	26	575	149	18	7	1513	332	208	57
region 3 (north)																		
Kuusamo	66° 03′′	8	503	147	149	42	307	72	73	35	548	113	17	5	1596	316	220	44
Kuusamo	66° 06′′	9	558	156	133	43	336	70	63	10	559	146	18	5	1667	367	206	48
Ivalo	68° 34′′	9	459	141	128	42	259	81	52	22	357	139	12	7	1268	404	177	49
$F_{14,122}$			3.171		2.204		2.297		1.435		5.206		3.879		2.674			
p			<0.001		0.011		0.008		ns[c]		<0.001		<0.001		0.002			

[a] The total contents are also expressed as fresh weight (FW). The variations between 15 populations were evaluated using one-way analysis of variance (ANOVA) and the Tukey HSD procedure. [b] See Berry Samples. [c] Not significant.

latitudes of 60° 23′′ N and 68° 34′′ N. Moreover, the identities of all flavonoids were studied by combining data obtained by DAD and electrospray ionization MS after separation by RP-HPLC. The information obtained will be useful in breeding programs and in authenticity studies. Furthermore, it can be utilized in the herbal therapy industry and in the development of various functional food products.

MATERIALS AND METHODS

Chemicals. Commercial standards of cyanidin 3-glucoside, quercetin 3-glucoside, syringetin 3-galactoside, and syringetin 3-glucoside were purchased from Extrasynthese (Genay, France). Methanol (Laboratory-Scan, Dublin, Ireland) and acetonitrile (J. T. Baker, Deventer, The Netherlands) were of HPLC grade. Formic acid (Riedel-deHaën, Seelze, Germany) was of analytical grade.

Berry Samples. The ripe berries of bog bilberry (*V. uliginosum*) were handpicked from 15 populations in August 2005 in Finland (20−300 m a.s.l.) at latitudes from 60° 23′′ N to 68° 34′′ N (south−north axis of ∼1000 km) which were subdivided into three geographical regions (**Tables 1** and **2**). The populations were selected from the same regions as previously described (*18*). Bushes were randomly selected within the populations, on the precondition that the distance between the studied plants was more than 10 m to ensure that samples would be collected from different genets. The samples (n = 137) were cooled immediately to < 10 °C and stored at −25 °C before being freeze-dried within the next 3 months. Freeze-dried berries were stored in a desiccator at −25 °C and analyzed in random order over a period of 11 months (2006−2007).

Extraction. Freeze-dried berries were ground into a powder and weighed (0.2700 g). The duplicate extractions were conducted as described in our previous studies (*18*, *29*).

HPLC−DAD. The anthocyanins of the samples were separated, identified, and quantified by RP-HPLC similarly, as described in the previous studies (*18*, *29*). The same chromatographic conditions were used for separation of the flavonols. The gradient program was composed of two mobile phases, MeCN/MeOH (85:15) (A) and 8.5% aqueous

Table 2. Flavonol Contents as Quercetin 3-Glucoside Equivalents [milligrams per 100 g of dry weight (DW) ± standard deviation (SD)] in Bog Bilberries from 15 Wild Populations in Finland[a]

			myricetin		quercetin		laricitrin		isorhamnetin		syringetin		total DW		total FW	
location	latitude (N)	no. of bushes (genotypes[b])	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
region 1 (south)																
Turku	60° 23′′	9	361	57	507	99	67	16	16	7	47	17	999	151	144	30
Janakkala	60° 53′′	9	377	85	556	124	57	27	15	11	45	40	1050	110	151	15
Savitaipale	61° 08′′	9	288	88	358	103	60	22	18	12	59	27	783	123	104	20
Vammala	61° 18′′	8	393	90	711	256	47	10	13	13	28	18	1192	284	144	40
Ruokolahti	61° 25′′	10	357	65	556	138	64	19	17	9	45	21	1038	115	144	19
region 2 (central)																
Vesanto	62° 56′′	9	446	159	640	175	89	37	25	17	70	47	1271	365	169	46
Lapua	63° 00′′	8	412	112	540	125	68	22	18	8	53	26	1092	169	135	15
Keitele	63° 13′′	10	379	86	598	143	71	12	25	11	61	19	1134	137	153	18
Lieksa	63° 16′′	9	372	168	530	208	60	28	18	11	38	19	1018	388	148	59
Juuka	63° 20′′	10	380	97	598	152	63	22	17	11	47	22	1106	181	152	27
Lieksa	63° 23′′	10	382	183	543	132	64	18	21	12	51	16	1062	244	157	37
Valtimo	63° 49′′	10	412	143	642	200	81	15	27	15	73	25	1235	327	170	51
region 3 (north)																
Kuusamo	66° 03′′	8	443	119	744	170	51	19	14	13	28	18	1279	196	176	25
Kuusamo	66° 06′′	9	428	149	832	283	49	18	18	13	29	15	1356	376	169	54
Ivalo	68° 34′′	9	545	192	745	283	68	16	13	16	37	13	1409	399	197	46
$F_{14,122}$			1.694		4.013		2.665		1.443		3.786		3.860			
p			ns[c]		<0.001		0.002		ns[c]		<0.001		<0.001			

[a] The total contents are also expressed as fresh weight (FW). The variations between 15 populations were evaluated using one-way analysis of variance (ANOVA) and the Tukey HSD procedure. [b] See Berry Samples. [c] Non significant.

HCOOH (B). Quantification of flavonols was conducted using the DAD chromatograms obtained at 360 nm, by means of a five-point external standard calibration curve, which was generated by dissolving the duplicate quercetin 3-glucoside standards (between 2 and 120 μg/mL) in solvent A (10%) and solvent B (90%). The linearity of the curve was acceptable ($R^2 > 0.9996$).

HPLC−ESI-MS. The compounds were tentatively identified by UV−vis spectra, elution order, ESI-MS fragmentation patterns, and literature (*5*, *8*, *18*, *19*, *29*). The HPLC−ESI-MS system and conditions used were the same as those described previously (*29*). The data were analyzed with Finnigan Xcalibur version 1.4 SR1 (Thermo Fischer Scientific Inc., Waltham, MA).

Statistical Analyses. The variations in anthocyanin and flavonol levels between populations and regions were evaluated using one-way analysis of variance (ANOVA) and the Tukey HSD procedure. Before analyses, the data were checked for normality and homogeneity of variance, and when necessary, the values were transformed to satisfy the assumptions of ANOVA. All analyses were performed with SPSS for Windows 16.0 (SPSS Inc., Chicago, IL) statistical software.

RESULTS AND DISCUSSION

In this study, the anthocyanins and flavonol glycosides in the berries of *V. uliginosum* were simultaneously extracted for optimized analysis via RP-HPLC−DAD. Previously, flavonoids were analyzed after hydrolysis to aglycons (*9*−*11*, *15*, *16*), and separate gradient elution programs were used for flavonols and anthocyanins (*8*, *10*). Analysis of aglycons simplifies the HPLC−DAD chromatogram, but essential information about the native forms of flavonoids and part of the content may be lost (*9*, *30*). To achieve a more precise comparison, the values from the literature were converted to the cyanidin 3-glucoside and quercetin 3-glucoside equivalents presented herein.

Identification of Anthocyanins and Flavonols. As far as we are aware, this is the first time that kaempferol and isorhamnetin aglycons were identified in bog bilberries (*V. uliginosum*). Moreover, this is the first report about the tentative identification of four anthocyanidin xylosides and 14 flavonol glycosides. Individual anthocyanin and flavonol glycosides detected and quantified by means of HPLC−DAD were designated with numbers (1−25), and the new minor flavonoids subsequently identified via HPLC−ESI-MS analyses were coded with letters (A−O) (**Table 3**). The tentative identification of anthocyanins was done as described previously and was supported with our published data for other related *Vaccinium* species analyzed with the same RP-HPLC program (*18*, *29*) by comparison of the elution order, visible maxima, and MS fragmentation patterns of the separated anthocyanin peaks (**Table 3**). The peaks assigned as flavonol glycosides exhibited typical UV−vis spectra with shapes and characteristic absorption maxima in the region of 354−358 nm. The conjugates of myricetin, quercetin, laricitrin, kaempferol, isorhamnetin, and syringetin were further identified on the basis of MS data, elution order, literature, and available standards of quercetin glucosides and syringetin galactosides and glucosides (**Table 3**).

Peak **22** was quercetin pentoside according to the MS fragmentation pattern and was tentatively named arabinoside (**Table 3**), with the support of previous NMR studies on bog bilberries (*V. uliginosum*) (*5*). The pentoside of myricetin was tentatively identified as myricetin arabinoside (peak **16**) on the basis of elution order (*19*) and MS data (**Table 3**). The presence of the aglycons of laricitrin and syringetin in bog bilberries (*V. uliginosum*) has recently been confirmed by NMR studies (*24*), and via the same technique, the galactosides and glucosides of syringetin were also found and confirmed (*5*). The minor pentosides of laricitrin, isorhamnetin, and syringetin (peaks **F**, **I**, and **J**, respectively) were further named as arabinosides on the basis of the findings of myricetin and quercetin arabinosides.

Glucuronides of myricetin (peak **D**), quercetin (peak **20**), and laricitrin and isorhamnetin (peaks **G** and **M**, respectively) were more complicated to identify, but a reliable way was found with careful reading of both DAD and ESI-MS spectra. The glucuronide conjugate always eluted after the corresponding glucose derivatives as previously reported (*19*). However, at the milder acid concentration used in the HPLC−ESI-MS run (1% HCOOH) compared to HPLC−DAD (8.5% HCOOH), not only were the retention times longer but the retention order was

Table 3. Characterization of Bog Bilberry (*V. uliginosum*) Anthocyanins and Flavonols (bold) by HPLC−DAD and ESI-MS Detection Using the Positive Ionization Mode

compound[a]	UV−vis maximum (nm)	M^{+b} $[M+H]^+$	MS^2	MS^3	tentative identification
1	526	465	303 (100)	303 (100), 257 (34)	delphinidin galactoside
2	526	465	303 (100)	303 (100), 257 (34)	delphinidin glucoside
3	518	449	287 (100)	287 (100)	cyanidin galactoside
4	526	435	303 (100)	303 (100), 257 (36)	delphinidin arabinoside
5	518	449	287 (100)	287 (100)	cyanidin glucoside[d]
6	526	479	317 (100)	302 (100), 317 (8)	petunidin galactoside
7	518	419	287 (100)	287 (100)	cyanidin arabinoside
8	526	479	317 (100)	302 (100), 317 (10)	petunidin glucoside
9	518	463	301 (100)	286 (100), 301 (28)	peonidin galactoside
A	nq[c]	435	303 (100)	257 (100), 303 (60), 229 (29)	delphinidin xyloside (*29*)
10	526	449	317 (100)	302 (100), 317 (8)	petunidin arabinoside
11	518	463	301 (100)	286 (100)	peonidin glucoside
12	358	**481**	319 (100)	273 (100), 301 (40), 165 (34), 245 (32), 153 (28)	**myricetin galactoside** (*19*)
B	nq[c]	**481**	319 (100)	273 (100), 301 (40), 245 (34), 165 (32), 263 (28)	**myricetin glucoside** (*19*)
13	526	493	331 (100)	299 (100), 315 (94), 287 (66), 270 (52), 179 (16)	malvidin galactoside
C	nq[c]	419	287 (100)		cyanidin xyloside (*29*)
14	518	433	301 (100)	286 (100), 301 (26)	peonidin arabinoside
15	526	493	331 (100)	315 (100), 299 (98), 287 (74), 270 (54), 331 (42)	malvidin glucoside
D	nq[c]	**495**	319 (100)	273 (100), 301 (40), 165 (38), 245 (34)	**myricetin glucuronide**
16	358	**451**	319 (100)	273 (100), 301 (44), 165 (36), 245 (34), 153 (28)	**myricetin arabinoside**
17	526	463	331 (100)	315 (100), 299 (96), 287 (68), 270 (54), 331 (38)	malvidin arabinoside
18	526	449	317 (100)	302 (100), 317 (12)	petunidin xyloside (*29*)
19	354	**465**	303 (100)	303 (100), 257 (86), 229 (64), 285 (54), 165 (46)	**quercetin galactoside**
E	nq[c]	**465**	303 (100)	257 (100), 229 (70), 285 (52), 165 (44), 247 (30)	**quercetin glucoside**[d]
20	354	**479**	303 (100)	257 (100), 229 (70), 285 (56), 165 (54), 247 (30)	**quercetin glucuronide**
21	358	**495**	333 (100)	303 (100), 257 (86), 229 (68), 285 (54), 165 (48)	**laricitrin galactoside**
F	nq[c]	**495**	333 (100)		**laricitrin glucoside**
G	nq[c]	**509**	333 (100)	318 (100), 277 (54), 301 (42), 273 (36), 165 (32)	**laricitrin glucuronide**
22	354	**435**	303 (100)	303 (100), 257 (82), 229 (60), 285 (48), 165 (44)	**quercetin arabinoside** (*5*)
23	530	433	301 (100)	286 (100), 301 (28)	peonidin xyloside (*29*)
		463	331 (100)	315 (100), 299 (96), 287 (68), 270 (56), 331 (38)	malvidin xyloside (*29*)
H	nq[c]	**449**	287 (100)	241 (100), 287 (72), 165 (70), 213 (64)	**kaempferol hexoside**
I	nq[c]	**465**	333 (100)	318 (100), 277 (58), 301 (56), 273 (38), 165 (28)	**laricitrin arabinoside**
J	nq[c]	**493**	303 (100)	257 (100), 229 (74), 285 (64), 165 (48), 247 (34)	**quercetin derivative**
24	355	**479**	317 (100)	302 (100), 285 (38)	**isorhamnetin galactoside**
K		**479**	317 (100)		**isorhamnetin glucoside**
25	358	**509**	347 (100)	153 (90), 287 (78), 332 (54), 315 (52), 165 (26)	**syringetin galactoside**[d] (*5*)
L		**509**	347 (100)		**syringetin glucoside**[d] (*5*)
M	nq[c]	**493**	317 (100)	302 (100), 285 (38)	**isorhamnetin glucuronide**
N	nq[c]	**449**	317 (100)	302 (100), 285 (38)	**isorhamnetin arabinoside**
O	nq[c]	**479**	347 (100)	291 (100), 153 (82), 287 (80), 315 (50), 332 (50)	**syringetin arabinoside**

[a] Compounds detected and quantified by means of HPLC−DAD are designated with numbers (1−25), and those detected and tentatively identified by HPLC−ESI-MS are designated with letters (A−O). [b] M^+ is for molecular weight of positively charged anthocyanins, and $[M+H]^+$ is for protonated molecules of flavonol glycosides. [c] Not quantified. [d] The identifications were checked by respective standards. The literature cited (in parentheses) confirmed the identification.

rearranged; e.g., quercetin glucuronide (peak **20**) eluted after laricitrin galactoside (peak **21**). A similar pH-dependent behavior of acidic flavonol glucuronides has been reported previously (*31*). The elution order of the flavonol aglycons (myricetin, quercetin, laricitrin, kaempferol, isorhamnetin, and syringetin) in the RP column was the same as in the other studies (*31*, *32*) as was the elution order of the flavonol glycosides (galactose, glucose, glucuronic acid, and arabinose) (*19*).

Contents of Anthocyanins. The results are expressed at the individual, population, and geographical (**Table 1** and **Figure 2**) levels. The average ($n = 137$) content [±standard deviation (SD)] of the anthocyanins was 1425 ± 398 mg/100 g of dry weight (DW). This corresponds to 194 ± 56 mg/100 g on a fresh weight (FW) basis, which is in the same range as values previously published (*7*, *33*, *34*). There were statistically significant differences in the anthocyanin contents between the populations ($n = 15$), but in the case of peonidins, the differences were not significant (**Table 1**). The anthocyanin content in the berries from the southern region was significantly lower compared to that in the berries from the central and northern regions (**Figure 2**), in accordance with our previous study on *V. myrtillus* from 20 wild populations in Finland (*18*). The average ($n = 137$) contents (±SD) of delphinidin, cyanidin, petunidin, peonidin, malvidin, and one unknown, anthocyanidin glycosides were 400 ± 148, 99 ± 65, 269 ± 82, 61 ± 39, 576 ± 168, and 19 ± 7 mg/100 g of DW, respectively (54 ± 20, 14 ± 9, 37 ± 11, 8 ± 5, 78 ± 24, and 3 ± 1 mg/100 g of FW, respectively). The contents of the anthocyanidin glycosides were significantly different between the regions (**Figure 2**). The contents of the two nonmethylated anthocyanidins, delphinidin and cyanidin, were significantly higher in the north than in other parts of the country. The content of the methylated anthocyanidin, malvidin, was significantly lower in northern than in central Finland, as in *V. myrtillus* (*18*). These differences between regions (south, central, and north) may reflect the adaptation to different climates, especially the amount of light.

Proportions of Anthocyanins. The average ($n = 137$) proportions (±SD) of the major *V. uliginosum* anthocyanins were 28 ± 5% for delphinidin and 41 ± 8% for malvidin. The average ($n = 137$) proportions of the less abundant cyanidin, petunidin, and

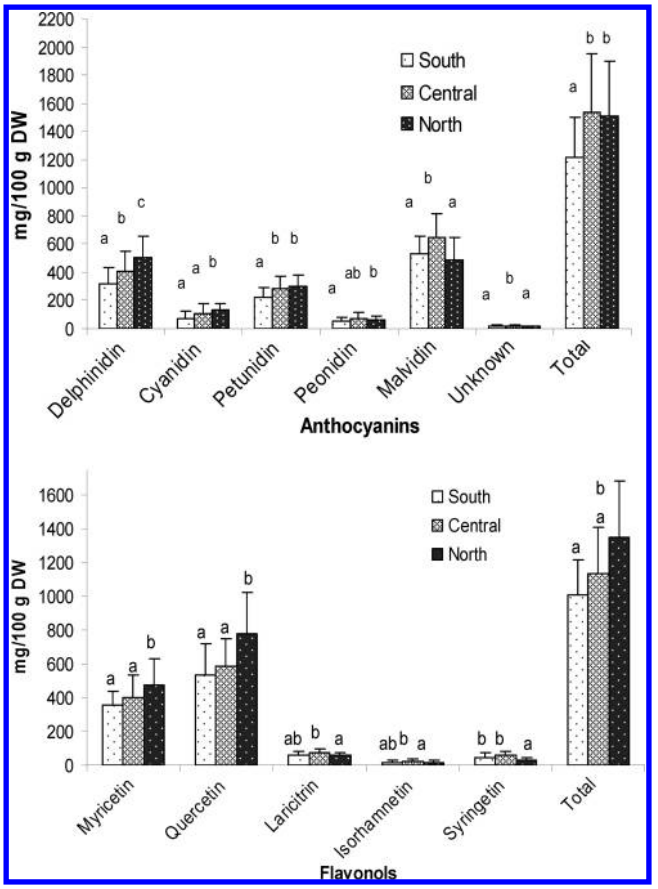

Figure 2. Anthocyanin and flavonol contents (±standard deviation) in bog bilberries (*V. uliginosum*) from three geographical regions (see **Tables 1** and **2**). Statistically significant differences by Tukey's HSD test ($p < 0.05$) are marked with different letters.

peonidin were 7 ± 3, 19 ± 2, and 4 ± 2%, respectively. These results are in the same ranges reported for *V. uliginosum* berries from Nordic countries (*7*, *8*, *34*), but the higher proportion of delphinidin (mean of 48%) and lower values of malvidin (mean of 15%) were reported for populations ($n = 2$) of the northwestern United States (*11*).

Sugar Conjugates of Anthocyani(di)ns. The average ($n = 137$) proportions (±SD) of sugar conjugates (19 ± 7 for galactosides, 59 ± 9 for glucosides, 20 ± 4 for arabinosides, and 2 ± 1 for xylosides) were in accordance with a previous study (*7*). Two bog bilberry individuals from south and northeastern Finland (Ruokolahti and Ivalo) exhibited an exceptionally low proportion of anthocyanidin glucosides (< 10%), with no detectable xylosides by HPLC−DAD (**Table 3**, peaks 18 and 23) and weak signals in ESI-MS detection. Individual plants with a very low proportion of anthocyanidin glucosides were found also in bilberries (*V. myrtillus*) (*18*).

Contents of Flavonols. The average ($n = 137$) content (±SD) of the flavonols was 1133 ± 290 mg/100 g of DW (154 ± 40 mg/100 g of FW), a value in good agreement with the literature (*8*, *33*). There were statistically significant differences in the flavonol contents between the populations ($n = 15$) (**Table 2**). The flavonol contents in the berries from the southern and central regions were significantly lower than those in berries gathered in the north (**Figure 2**). The flavonol contents in *V. uliginosum* analyzed with the hydrolysis method (*9*, *10*) were clearly lower (28−48 mg/100 g of FW as quercetin 3-glucoside equivalents) than the lowest flavonol content at the population level (**Table 2**) in this study. The average ($n = 137$) contents (±SD) of myricetin, quercetin, laricitrin, isorhamnetin, and syringetin glycosides were 397 ± 132, 605 ± 205, 64 ± 23, 19 ± 12, and 48 ± 27 mg/100 g of DW, respectively (54 ± 19, 82 ± 27, 9 ± 3, 3 ± 2, and 7 ± 4 mg/100 g of FW, respectively).

Quercetin has been reported to be more abundant than myricetin in the Nordic berries of *V. uliginosum* (*8*−*10*), and generally, this was also found in our study, though myricetin did predominate in 17 plant individuals. Berries of *V. uliginosum* of the northwestern United States contained more myricetin than quercetin (*11*). Quercetin was the major flavonol in blueberries (*Vaccinium angustifolium* × *Vaccinium corymbosum*, *V. corymbosum*, *Vaccinium deliciosum*, *Vaccinium membranaceum*, *Vaccinium ovalifolium*, and *Vaccinium ovatum*) and bilberries (*V. myrtillus*) in sections *Cyanococcus*, *Myrtillus*, and *Pyxothamnus* (*11*, *16*, *17*, *35*), although slightly more myricetin than quercetin has been detected in some *Vaccinium ashei* cultivars (section *Cyanococcus*) (*16*).

Quercetin is found in most of the berries (*8*, *15*). Myricetin is more rare but does occur as a minor flavonol along with the more common quercetin in the berries of *Hippophaë rhamnoides* L., *Ribes nigrum* L. (green currant), *Ribes* × *pallidum* Otto and Dietr., *Ripes uva-crispa* L., and *Vaccinium oxycoccos* L. (*8*). Laricitrin, isorhamnetin, and syringetin glycosides are less frequently detected flavonols, but all of these minor flavonols have been previously identified in grapes (*31*, *32*). Both laricitrin and isorhamnetin were identified in bilberries (*V. myrtillus*) (*19*), and isorhamnetin was identified in the berries of *Vaccinium macrocarpon* Ait., *R. uva-crispa* L., and *H. rhamnoides* (*8*, *36*).

There were significant regional differences in the contents of flavonol glycosides (**Figure 2**); i.e., there were more myricetin and quercetin glycosides in the berries of *V. uliginosum* from the north than from the other regions. Stark et al. (*37*) also found that the concentrations of quercetin derivatives were significantly higher in the leaves of white birch (*Betula pubescens* Ehrh.) from northern Finnish populations compared to their more southern counterparts. The higher contents of more hydroxylated myricetin and quercetin derivatives in the north may be a consequence of the adaptation to northern climatic factors. Even though the hydroxylation does not affect the light absorbing properties of the compounds, it does affect the antioxidant activity (*26*).

Proportions of Flavonols. The average ($n = 137$) proportions (±SD) of the major bog bilberry flavonols were 35 ± 7% for myricetin and 53 ± 9% for quercetin, and for the minor flavonols, laricitrin, isorhamnetin, and syringetin, the proportions were 6 ± 2, 2 ± 1, and 5 ± 3%, respectively. Between the populations, the proportion of myricetin was in the range of 32−39%; for quercetin, it was 46−61%.

Conjugates of Flavonols. This was the first quantitative study of flavonol conjugates in *V. uliginosum* berries. The average ($n = 137$) proportions (±SD) of the major sugar conjugates were 81 ± 3% for galactosides and 17 ± 2% for arabinosides. The flavonol glucuronides accounted for 2 ± 1%. The minor glucoside conjugates were detected only by HPLC−ESI-MS and thus could not be quantified by HPLC−DAD.

Approximately one-quarter (22%) of the bog bilberry samples contained greater amounts of flavonols than anthocyanins, although the average ($n = 137$) anthocyanin content was higher. Previously, the anthocyanins have been regarded as the major phenolic class in the berries of *V. uliginosum* (*8*, *33*). This information about *V. uliginosum* genotypes with a high capacity for biosynthesizing flavonols could be useful for breeding purposes.

The berries of *V. uliginosum* are rich sources of both anthocyanins and flavonols. In comparison to the available literature on other *Vaccinium* berries, bog bilberries have a distinctive flavonol and anthocyanidin profile. Thus, it seems that their

flavonoid profile could be used to differentiate them from the berries from sections *Hemimyrtillus* (*Vaccinium arctostaphylos*), *Myrtillus* (*V. deliciosum*, *V. membranaceum*, *V. myrtillus*, and *V. ovalifolium*), and *Pyxothamnus* (*V. ovatum*) (e.g., refs *11*, *16*, *18*, *29*, and *38*).

The anthocyanin contents were significantly higher in berries from the central and northern regions than in berries from the south (**Figure 2**), as in our previous study (*18*), even though an exceptionally low content was found from the most northern population (Ivalo) (**Table 1**). The low contents were mainly due to the reduced amounts of malvidin. The reason for this phenomenon will need to be studied with cloned material of southern and northern origin. On the other hand, the flavonol contents at the population level rose as one moved north, being the highest in the most northern location (Ivalo). Rieger et al. (*39*) observed decreasing amounts of anthocyanins and increasing levels of flavonols with an increase in altitude. However, in our study, the differences between the altitudes of studied northern locations were only ~120 m. These findings support the possibility that flavonoids contribute to plant protection but via distinctly different defense mechanisms, which are various and complex (*40*).

The presence of two new flavonol aglycons, as well as the tentative identification of 18 flavonoid glycosides in bog bilberries (*V. uliginosum*), is reported here for the first time. This is also the first report about the quantification and geographical variation of flavonol glycosides in bog bilberries. Despite the significant variation between the populations and individuals, the evaluation of the results suggested that northern climatic conditions favor the biosynthesis of anthocyanins and flavonols in berries, especially those more hydroxylated derivatives, which have been shown to have the greatest antioxidant capacity in vitro.

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