JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Analysis of Anthocyanin Variation in Wild Populations of Bilberry (*Vaccinium myrtillus* L.) in Finland

ANJA K. LÄTTI,*,† KAISU R. RIIHINEN,‡ AND PIRJO S. KAINULAINEN†

Departments of Environmental Science and Biosciences, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland

The berries of *Vaccinium myrtillus* L. are characterized by 15 anthocyanins. To study the variation in the anthocyanins on a south−north axis of about 1000 km in Finland, the berries from 179 individual bilberry plants in 20 populations were analyzed using an optimized RP-HPLC-DAD method. The mean content of the total anthocyanins was 2878 mg/100 g dry weight. There was extensive variation in the anthocyanin contents within and between the populations, suggesting differences in berry raw material. A significantly lower content of the total anthocyanins was observed in the berries of the southern region compared to those in the central and northern regions. Differences in the proportions of anthocyanins were also observed. The delphinidin glycosides dominated in the northern berries whereas the cyanidin glycosides were most common in the southern ones. Exceptional bilberry individuals were found mainly from eastern Finland with very low amounts of anthocyanidin glucosides. This is the first systematic study to reveal the extremely high variation in the content and distribution of anthocyanins in wild bilberries.

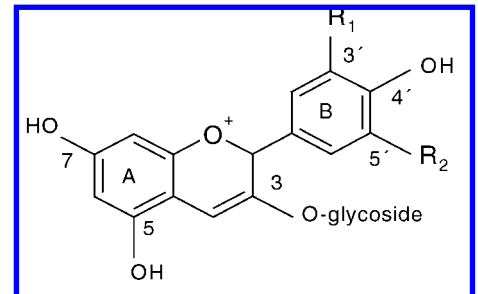
KEYWORDS: Bilberry; *Vaccinium myrtillus*; anthocyanins; population; variation; HPLC-DAD; berry; authenticity

INTRODUCTION

The wild European bilberry (*Vaccinium myrtillus* L.) is one of the most important natural resources in Finland. These small, blue-black, and delicious berries are manually collected from forests for domestic use and for sale to the Finnish berry brokerages. In the past few years there has been growing global interest in bilberry due to its proposed health effects especially for the vision and ocular health (*1*). Most of the research has focused on a group of compounds called anthocyanins since they are considered to be the most pharmacologically active constituents of bilberry (*2*). Several mechanisms of action have been proposed, i.e., stimulation of the regeneration of rhodopsin (*3*), antioxidant activity (*4*), modulation of retinal enzyme activity (*5*), anti-inflammatory properties (*6*), and improved microcirculation (*7*).

The anthocyanin composition of bilberries is characterized by 15 anthocyanidin glycosides in which five anthocyanidins (delphinidin, cyanidin, petunidin, peonidin, malvidin) (**Figure 1**) are combined with three sugars (galactose, glucose, arabinose) (*8, 9*). Anthocyanin profiles have been used in chemotaxonomy and for quality control in the food processing industry (*10*) as plant species (and even subspecies and cultivars) produce characteristic profiles (e.g., refs (*11*) and (*12*)). The content of anthocyanins in bilberries was 60–70% higher than in lowbush (*Vaccinium angustifolium* Aiton) and highbush blueberries (*Vaccinium corymbosum* L.) (*9*). Furthermore, the relative composition of anthocyanins in the berries of these species differs from species to species (*9, 13*).

The content and composition of anthocyanins of the plant individuals are mainly under genetic control although they can



R_1	R_2	Anthocyanidin
OH	OH	Delphinidin (Dp)
OH	H	Cyanidin (Cy)
OCH_3	OH	Petunidin (Pt)
OCH_3	H	Peonidin (Pn)
OCH_3	OCH_3	Malvidin (Mv)

Figure 1. Chemical structures of bilberry anthocyanidins.

* Corresponding author. Tel: +358-17-163244. Fax: +358-17-163230. E-mail:Anja.Latti@uku.fi.
† Department of Environmental Science, University of Kuopio.
‡ Department of Biosciences, University of Kuopio.

10.1021/jf072857m CCC: $40.75
Published on Web 12/12/2007

be affected by environmental factors (e.g., intensity of light, photoperiod, temperature) (*14*). The degree of anthocyanin accumulation is primarily dependent on the light conditions, but a favorable impact of low temperature and a limiting effect of high temperatures have often been reported (e.g., refs (*12*) and (*14*)). Due to these factors, the long summer days in the north and the considerable differences between day and night temperatures should provide ideal conditions for the formation of bilberry anthocyanins.

Variations in the anthocyanins of the Finnish bilberry have not been previously systematically investigated. Some studies have been conducted in order to evaluate the extent of the variation in the anthocyanins of bilberries or blueberries (*9, 15–17*), but there is little information about the variation at the geographical and the population level. The geographical variation in other constituents of berries has been previously studied. Latitudinal differences were found in the contents and composition of the fatty acids of the Finnish cloud- and crowberries (*18*). The geographical origins of bilberry raw material are assumed to be one factor accounting for conflicting results in the clinical trials (*1*); therefore, the detailed and systematic study of bilberry anthocyanins is essential.

The aim of this study was to screen the anthocyanin variation in the berries of *V. myrtillus* growing in 20 populations in Finland on a south−north axis of about 1000 km. An understanding on the anthocyanin profiles would provide valuable information for quality control in the food processing and herbal therapy industry. Future perspectives may lie in breeding and collecting bilberries with high anthocyanin contents or a high proportion of certain biologically active anthocyanins.

MATERIALS AND METHODS

Chemicals. Cyanidin 3-glucoside was purchased from Extrasynthese (Genay, France). Methanol (Laboratory-Scan, Dublin, Ireland) and acetonitrile (J. T. Baker, Deventer, Holland) were of HPLC grade. Formic acid (Riedel-deHaën, Seelze, Germany) was of analytical grade.

Berry Samples. The ripe berries of bilberry (*V. myrtillus*) were handpicked during the summer 2005 at the time periods when they are typically harvested for commercial purposes in Finland in 20 populations at the northern latitudes (60° 21″–68° 34″) which were subdivided into three geographical regions (**Figure 2**, **Table 1**). The forest type according to the Finnish forest classification was mainly *Myrtillus* (MT) (*19*), in which the main tree species is typically spruce or pine. The characteristics of *Myrtillus* type forests are a thick and acidic humus, continuous moss cover, and abundant twig vegetation. Twenty populations were selected on the basis of their geographical distribution. Bushes were randomly selected within the populations, on the precondition that the minimum distance between the studied plants was 10 m in order to ensure that samples would be collected from different genets (*20*). Thus, the berries of one genet represent the berries of a variable sized bilberry patch in a forest. The samples were cooled immediately to below 10 °C and stored at −25 °C before freeze-drying within the next 3 months. Freeze-dried berries were stored in a desiccator at −25 °C.

Extraction. The extractions were performed in duplicate. Freeze-dried berries were ground into a powder and weighed (0.2700 g), and 4 mL of extraction solution was added. The extraction solution consisted of 10% solvent A and 90% solvent B. The solvents used were ACN−MeOH (85:15 v/v) (solvent A) and 8.5% aqueous HCOOH (solvent B). The sample was vortexed (1 min) and then sonicated (10 min), vortexed again, and centrifuged (4500 rpm, 5 min, 4 °C). The filter cake was reextracted three times (3 × 2 mL). The supernatants were combined, and the volume was adjusted to 10 mL. All extracts were filtered through a regenerated cellulose filter (Agilent Technologies) equipped with a glass fiber prefilter (Agilent Technologies) prior to HPLC analysis.

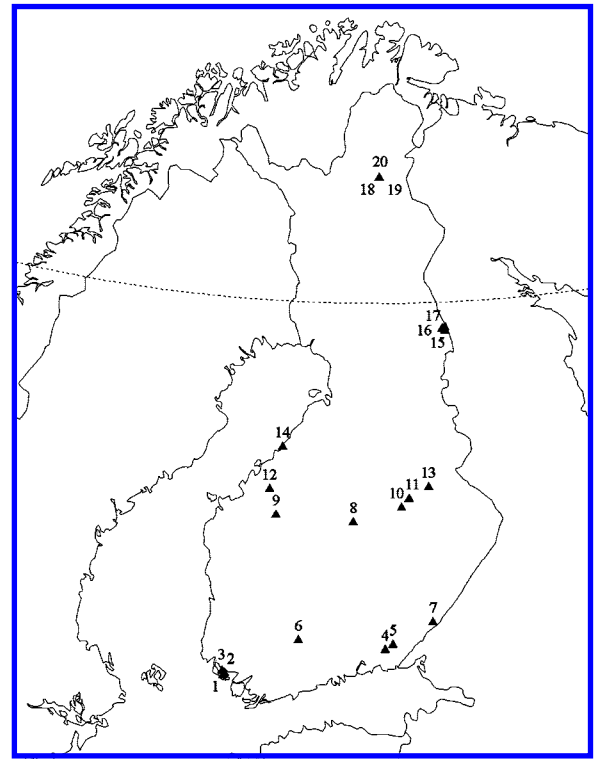


Figure 2. Collecting locations of bilberry samples in Finland on the south−north axis of about 1000 km: Turku (1–3), Luumäki (4, 5), Hämeenlinna (6), Ruokolahti (7), Vesanto (8), Alajärvi (9), Nilsiä (10), Rautavaara (11), Evijärvi (12), Nurmes (13), Himanka (14), Kuusamo (15–17), and Ivalo (18–20).

HPLC Analysis. The chromatographic system consisted of Hewlett-Packard (Waldbronn Analytical Division, Germany) instrument with a quaternary pump, an autosampler (HP 1050), and a photodiode array detector (HP 1040M).

Analytical separation of anthocyanins was carried out using a 150 × 4.6 mm i.d., 5 μm, Phenomenex Gemini C-18 column equipped with a 4 × 3 mm C-18 precolumn with the modified procedure of Buchert et al. (*21*). The gradient program was as follows: 0–2 min, 96–94% B; 4–12 min, 92–91% B; 13–20 min, 91–90% B; 38–46 min, 89% B; 48–52 min, 76–66% B; 55–59 min, 20% B; 61–65 min, 80–96% B. The injection volume was 20 μL, and the equilibration time between the runs was 3 min. The flow of the mobile phase was 1.0 mL/min for 0–4 min, 0.9–0.8 mL/min for 12–13 min, 0.8 mL/min for 13–20 min, 0.85 mL/min for 38–46 min, 0.9–0.95 mL/min for 48–52 min, and 1.0 mL/min for 55–65 min. Anthocyanins were detected at 520 nm.

Identification and Quantification (LC-DAD). Identification was based on retention time, UV spectra, comparison with commercial standards, and the literature (*8*). Duplicate standards (between 2 and 200 μg/mL cyanidin 3-glucoside) were dissolved in solvent A (10%) and solvent B (90%) to generate a six-point external standard calibration curve, the linearity of which was found to be acceptable ($R^2 > 0.998$).

Two quality control standards were analyzed in the beginning and at the end of every run series to control for any possible fluctuations in response. The stability of standard solutions in pH 1.6 (2, 5, 10, 70, 100, 200 μg/mL) was tested, and they proved to be stable for at least 2 months at 9 °C in the dark. The coefficients of variation (percent) of peak areas of six standards between four measurements during 2 months were 2.4–5.4%, on average 3.4%. These values were considered to be acceptable.

The repeatability of retention times was tested (within-day precision) by analyzing standard solution (100 μg/mL) 7 times within 1 day. The coefficient of variation (percent) was 0.3%. The repeatability of the peak area (within-day precision) was studied (20 μL, 100 μg/mL, seven injections) and was found to be 0.3%. The between-day precisions of

Table 1. Total Anthocyanin Contents [mg/100 g dry weight (DW) ± Standard Deviation (SD)] of Bilberry Individuals from 20 Wild Populations in Finland[a]

			anthocyanidin glycosides													
			Dp		Cy		Pt		Pn		Mv		total DW		total FW	
region location	latitude (N)	bushes (genotypes[b])	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
region 1, south																
Turku	60° 21′′	10	537	162	714	196	269	76	194	81	256	79	1971	434	370	78
Turku	60° 23′′	10	481	176	770	289	243	91	205	105	235	103	1934	657	359	96
Turku	60° 25′′	9	541	158	838	208	267	71	202	60	244	77	2092	506	370	94
Kurvila	60° 51′′	9	828	327	737	169	385	148	167	71	371	193	2488	777	359	78
Taavetti	60° 56′′	9	839	227	1014	283	411	107	261	92	401	128	2926	708	421	100
Hämeenlinna	60° 59′′	10	941	428	1090	316	447	180	254	77	432	160	3164	1066	440	107
Ruokolahti	61° 17′′	8	1107	238	887	227	436	102	141	46	340	98	2910	651	350	122
region 2, central																
Vesanto	62° 56′′	9	1061	187	1152	378	468	103	239	113	467	121	3386	795	400	89
Alajärvi	63° 01′′	10	1042	314	977	359	460	127	209	82	423	130	3110	852	408	114
Nilsiä	63° 10′′	9	1205	280	1134	204	490	92	198	50	429	70	3456	442	402	68
Rautavaara	63° 19′′	8	1049	277	939	268	451	104	178	61	400	69	3017	535	424	91
Evijärvi	63° 26′′	9	984	171	1130	405	459	90	279	125	462	120	3313	745	431	102
Nurmes	63° 30′′	9	695	275	875	285	311	107	182	80	300	89	2362	676	400	100
Himanka	64° 08′′	10	1282	420	880	264	522	138	175	51	478	108	3337	834	421	116
region 3, north																
Kuusamo	66° 03′′	8	1264	392	1156	481	566	180	264	154	553	238	3803	1282	498	146
Kuusamo	66° 05′′	7	1264	375	1170	408	529	142	225	87	401	109	3588	915	522	121
Kuusamo	66° 06′′	9	1375	221	1214	159	581	123	240	58	467	136	3877	528	525	64
Ivalo	68° 34′′	9	1012	168	652	144	401	64	116	24	305	63	2486	399	417	78
Ivalo	68° 34′′	9	925	187	757	152	313	40	107	26	200	29	2302	295	371	52
Ivalo	68° 34′′	8	1007	175	726	195	332	61	94	36	205	56	2364	444	353	81
F_{19159}			10.581		3.541		8.075		4.643		7.380		6.546			
P			$p < 0.001$		$p < 0.001$		$p < 0.001$		$p < 0.001$		$p < 0.001$		$p < 0.001$			

[a] The total contents are also expressed as fresh weight (FW). [b] See text: Berry Samples.

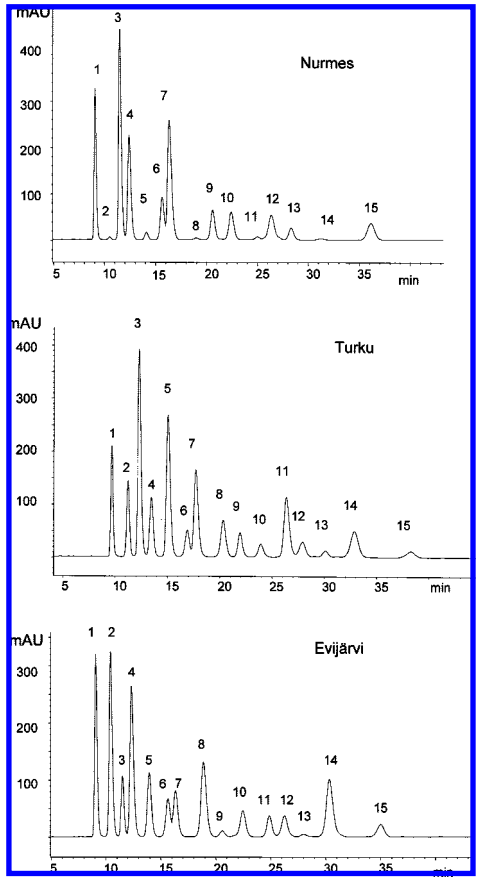


Figure 3. HPLC-DAD chromatograms at 520 nm of three bilberry individuals. Peak identification: 1, delphinidin galactoside; 2, delphinidin glucoside; 3, cyanidin galactoside; 4, delphinidin arabinoside; 5, cyanidin glucoside; 6, petunidin galactoside; 7, cyanidin arabinoside; 8, petunidin glucoside; 9, peonidin galactoside; 10, petunidin arabinoside; 11, peonidin glucoside; 12, malvidin galactoside; 13, peonidin arabinoside; 14, malvidin glucoside; 15, malvidin arabinoside.

the standards (70, 100 μg/mL) were also studied during 3 weeks in 20 runs. The repeatability (CV%) of peak areas was 2.1 and 3.2, respectively. All determined values were considered to be acceptable.

Statistics. The quantitative data were $\log(x + 1)$ transformed, and an arc sin transformation was used for concentrations and relative proportions of anthocyanins before statistical analyses. The variations in anthocyanin levels between populations and regions were evaluated using one-way analysis of variance (ANOVA) and the Tukey HSD procedure. All analyses were performed with SPSS for Windows 14.0 statistical software.

RESULTS AND DISCUSSION

Chromatographic Analyses. The RP-HPLC method provided repeatable and good separation of 15 anthocyanins within 40 min (**Figure 3**). The column Phenomenex Gemini C18 was stable when perfused with 8.5% formic acid. Repeatable quantification of anthocyanins was based on cyanidin 3-glucoside equivalents. This type of quantification underestimates the content of delphinidin glycosides by about 27% as shown by Kähkönen et al. (*22*). The sugar moiety affects the HPLC-DAD online response; e.g., there was a 16% decrease in the content of the cyanidin glycosides if they were quantified as glucoside equivalents instead of using the corresponding glycoside (galactose, arabinose) (*22, 23*). Therefore, the comparison of the contents of bilberry

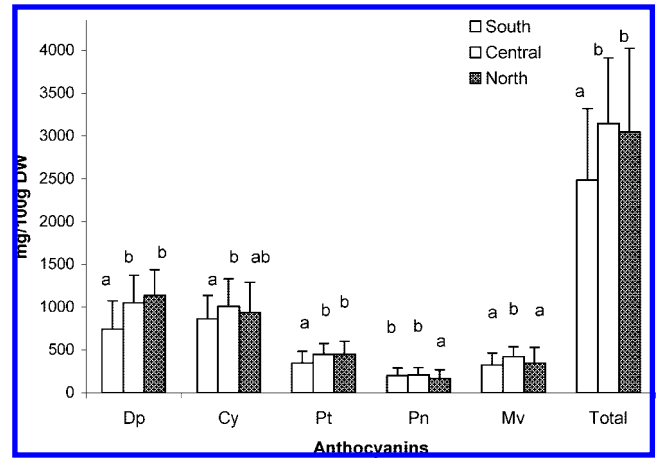


Figure 4. Anthocyanin contents (±standard deviation) in bilberries from three geographical regions (see **Table 1**). Statistically significant differences by Tukey's HSD test ($p < 0.05$) are marked with different letters.

anthocyanidin glycosides to the contents in the literature is complicated owing to analytical differences and/or insufficient data about the origin of the bilberries.

Total Contents of Anthocyanins within and between the Populations. This is the first systematic study on the anthocyanin variation in the berries of bilberry individuals and populations in Finland (**Figure 2**). The results are expressed at the individual, population (**Table 1**), and geographical levels. The average ($n = 179$) content of the anthocyanins was 2878 mg/100 g dry weight (DW). The content was 411 mg/100 g as calculated on a fresh weight basis (FW). The results are in good agreement with those reported in the literature (*22, 24, 25*). The highest total anthocyanin content at the individual level was 5406 (location 15) with the lowest being 1198 (location 1) mg/100 g DW, meaning that there was about a 4.5-fold difference. The variation in the total anthocyanin contents within a population was on average 2.2-fold.

There were statistically significant differences in the total anthocyanin contents between the populations ($n = 20$) (**Table 1**). The total anthocyanin content in the berries from the southern region was significantly lower compared to the central and the northern regions (**Figure 4**). In earlier studies (*11, 22, 26*) there have been only one to three collection locations representing one geographical region. Kähkönen et al. (*22*) reported a lower total anthocyanin content (2657 vs 3090 mg/100 g DW) in bilberries collected from southern (61° 3″ N) than from eastern (63° 3″ N) Finland. The regional differences were also found in Finland in mountain birch (*Betula pubescens* ssp. *czerepanovii*) as the leaves of northern origin contained more proanthocyanidins than the leaves of southern origin (*27*).

The Finnish bilberries contain higher amounts of anthocyanins (**Table 1**) than *Vaccinium ovalifolium* Smith (176–273 mg/100 g FW) (*26*). Previously, *V. ovalifolium* was reported to be the best source of anthocyanins among the other related species in the section *Myrtillus* (*Vaccinium deliciosum* Piper, *Vaccinium membranaceum* Douglas ex Torrey) and in the section *Pyxothamnus* (*Vaccinium ovatum* Pursh) (*26, 28, 29*). Moreover, the berries both in the section *Cyanococcus* (*V. angustifolium*, *Vaccinium ashei* Reade, *V. corymbosum*, *Vaccinium myrtilloides* L.) and in the section *Vaccinium* (*Vaccinium uliginosum* L.) seem to have clearly lower anthocyanin contents than those found in the Finnish bilberry (*9, 11, 13, 17, 25*). The lowbush and highbush

cultivars contained about 100–260 mg of anthocyanins/100 g FW (*13*), which is 24–63% lower than in wild Finnish bilberries according to our average result ($n = 179$).

Composition of Anthocyanins within and between the Populations. The total average ($n = 179$) contents of delphinidin (Dp), cyanidin (Cy), petunidin (Pt), peonidin (Pn), and malvidin (Mv) glycosides were 962, 937, 414, 197, and 368 mg/100 g DW, respectively. On a fresh weight basis, the respective contents were 136, 135, 59, 29, and 52 mg/100 g. In the case of Dp, Cy, Pt, and Mv the average contents (FW) were 28–59% lower in the present study compared to those reported in previous Finnish bilberry studies (*11, 21*). However, the average amount of Pn was at the same level or even 38% higher.

The variations in the amounts of anthocyanins confirm the extensive diversity of the Finnish bilberry anthocyanin profile. There were some bilberry individuals with as high amounts of Mv (>90 mg/100 g FW) as those found in the lowbush (*V. angustifolium*) (*13*), which is rich in Mv (*9, 13*). Furthermore, there were some bilberry individuals with abundant amounts of Pt (>90–100 mg/100 g FW). Pt is a rare anthocyanidin which is typically found in grapes (*Vitis vinifera* L.) but also in bilberries, blueberries, and crowberries (*Empetrum nigrum* L.) (*11, 14*).

Clear geographical variation was observed. The contents of the anthocyanidin glycosides were significantly different between the regions (**Figure 3**). The contents of Dp and its methylated form, Pt, were significantly lower in the southern berries compared to the more northern regions. Cy was significantly more abundant in the bilberries from central Finland than in those from the south, while the content of Pn was significantly lower in northern bilberries compared to those gathered in the south of the country.

Proportions of Anthocyanins within and between the Populations. The total average ($n = 179$) proportions (mean ± standard deviation) of the major bilberry anthocyanins were at the same range, 33 ± 7% for Dp and 33 ± 6% for Cy, in agreement with the other Finnish studies (*11, 21, 22*).

The highest proportions of Dp and Cy were 51% (location 20) and 49% (location 2) at the individual level. The variation between individuals was high, since the lowest proportions were 17% for Dp (location 2) and 18% for Cy (location 12). Between the populations, the proportion of Dp ranged from 25% to 43% and for Cy from 26% to 40%. At the region level (south, central, north) the proportions of Dp were 29%, 33%, and 38% and those of Cy were 36%, 32%, and 31%, respectively.

The total average ($n = 179$) proportions of the less abundant Pt, Pn, and Mv were 14 ± 2%, 7 ± 3%, and 13 ± 3%, which are consistent with values in the literature (*11, 21, 22*). The variations in the proportions of Pt, Pn, and Mv at the individual level were 9–20%, 3–15%, and 6–21%, respectively. The highest proportion of Pn was found from the south in the same bilberry individual with the highest proportion of Cy. Pn is the methylated form of Cy (**Figure 1**). The lowest value of Pn was found in the north in that individual that had the highest proportion of Dp. At the population level, there were variations in the proportions of Pt, Pn, and Mv in the ranges 13–16%, 4–10%, and 4–7%, respectively.

In this study the average ($n = 179$) proportion of Cy was 17% higher and Pt 15% lower than those reported in bilberry of Italian origin (*10*). This finding is in agreement with the study of Martinelli et al. (*30*) in which the Cy glycosides were more abundant in bilberries from northern latitudes (Norway, Sweden) compared to more southern regions (Italy, Romania, Poland).

There are also differences in the proportions of the anthocyanins among related species. *V. ovalifolium* in the section *Myrtillus* had 7–17% and *V. ovatum* in the section *Pyxothamnus* had 34–37% greater (*26*) proportion of Cy in comparison to our average ($n = 179$) value. The clearest distinguishable difference between bilberry and species both in the section *Cyanococcus* and in the section *Vaccinium* was found in the proportion of Mv, which was 14–19% higher in *V. corymbosum* (*9, 26*) and 20–30% higher in *V. uliginosum* (*11, 31*) than in bilberry (13%) in the current study. In contrast, the proportion of Mv was 1–3% but that of Dp was 16–17% higher in *V. uliginosum* (*26*) compared to the average ($n = 179$) values of the bilberries.

These results are interesting, since the anthocyanidin moiety of the anthocyanins has been shown to affect their bioavailability (*32, 33*). However, there is no general rule about which one(s) is/are the most bioavailable.

Sugar Conjugates within and between the Populations. The average ($n = 179$) proportions of sugar conjugates were 32 ± 6% for galactosides, 39 ± 10% for glucosides, and 29 ± 5% for arabinosides, in agreement with other Finnish bilberry studies (*21, 22*). The highest proportion of the galactosides and that of arabinosides in a bilberry individual were 59% (location 10) and 49% (location 13), respectively. The lowest proportions were 25% for galactosides and 20% for arabinosides.

Exceptional Bilberry Individuals. Seven bilberry individuals were exceptional because they displayed a very low proportion (<4%) of anthocyanidin glucosides as shown in **Figure 3**. As far as we are aware, this is the first study to have observed such a divergent anthocyanin profile in bilberry plants. It seems that this kind of variation also exists in the highbush blueberry (*23*). In our opinion, the reason for this variation can be traced to genetic origins. The exceptional individuals mainly originated from eastern Finland (locations 10, 11, 13), but also one from southwestern Finland was identified (location 6). The reduced biosynthesis of anthocyanidin glucosides (6–18%) was found in three individuals from eastern and central Finland (locations 8, 13). In summary, the range of the variation in the glucoside conjugates was wide, since the highest proportion of the glucosides of a bilberry individual was 50% (location 2) and the lowest one 3% (location 10), representing about a 19.5-fold difference.

The proportion of glucosides was 5% higher in the bilberry of French origin (*30*) than the average ($n = 179$) proportion of bilberry in the current study. These kinds of variations in glycosylation are interesting, since the sugar moiety and degree of acylation can influence the absorption of anthocyanins (*32, 33*). In this study, we detected no acylation of anthocyanins, which is thought to reduce their apparent absorption (*33*).

Even though this research revealed significant variability in the anthocyanins between bilberries collected from 20 locations in Finland, it is difficult to determine which of a wide range of factors is responsible for these differences. The observed biochemical variation in the bilberry anthocyanins may be due to genotype differences, the prevalent environmental differences, or a combination of both factors. The bilberry is an extremely plastic species which can adapt to variable environmental conditions without any genetic changes (*34*). However, this study has illustrated the extent of the variations in anthocyanin profiles, which supposedly are mainly genetically determined; i.e., there are genotypes which are known to produce very low amounts of anthocyanidin glucosides. Additionally, the differences in the proportions of Dp and Cy between southern and northern Finland might be of genetic origin, since Castellarin et al. (*35*)

determined that the ratio between delphinidin and cyanidin is largely under genetic control. Presumably, the light and temperature as well as other factors may have caused genetic differentiation in the bilberry populations occurring in different climatic conditions on a south−north axis of about 1000 km. Thus, a long-term study will be necessary in the future to elucidate the significance of these differences within and between populations. In terms of breeding bilberries with a specific desired anthocyanin composition, it does seem that the bilberry germplasm holds great potential.

In conclusion, this research reveals that there is a wide variation in the content and composition of the anthocyanins in the bilberry populations found in Finland. Some exceptional bilberries with very limited biosynthesis of glucoside conjugates were also found. The study provides valuable information for breeding and health effect studies. Furthermore, this research emphasized the need for more sophisticated analytical procedures than spectroscopic methods to assess the anthocyanins in the bilberries or bilberry-based products in clinical trials as well as in quality control of bilberry-containing foodstuffs.

ACKNOWLEDGMENT

We express our gratitude to Pekka and Paula Hyvönen, Kalle Määttä, and Juha Ullgren for gathering the wild berries.

LITERATURE CITED

(1) Canter, P. H.; Ernst, E. Anthocyanosides of *Vaccinium myrtillus* (bilberry) for night vision—a systematic review of placebo-controlled trials. *Surv. Ophthalmol.* **2004**, *49*, 38–50.
(2) Yao, Y.; Vieira, A. Protective activities of *Vaccinium* antioxidants with potential relevance to mitochondrial dysfunction and neurotoxicity. *Neurotoxicity* **2007**, *28*, 93–100.
(3) Matsumoto, H.; Kawamura, S.; Hirayama, M.; Nakamura, Y.; Tachibanaki, S. Stimulatory effect of cyanidin 3-glycosides on the regeneration of rhodopsin. *J. Agric. Food Chem.* **2003**, *51*, 3560–3563.
(4) Milbury, P.; Graf, B.; Curran-Celentano, J.; Blumberg, J. Bilberry (*Vaccinium myrtillus*) anthocyanins modulate heme oxygenase-1 and glutathione S-transferase-pi expression in ARPE-19 cells. *Invest. Ophthalmol. Visual Sci.* **2007**, *48*, 2343–2349.
(5) Jang, Y. P.; Sparrow, J. R.; Zhou, J.; Nakanishi, K. Anthocyanins protect against A2E photooxidation and membrane permeabilization in retinal pigment epithelial cells. *Photochem. Photobiol.* **2005**, *81*, 529–536.
(6) Youdim, K. A.; McDonald, J.; Kalt, W.; Joseph, J. A. Potential role of dietary flavonoids in reducing microvascular endothelium vulnerability to oxidative and inflammatory insults. *J. Nutr. Biochem.* **2002**, *13*, 282–288.
(7) Muth, E. R.; Laurent, J. M.; Jasper, P. The effect of bilberry nutritional supplementation on night visual acuity and contrast sensitivity. *Altern. Med. Rev.* **2000**, *5*, 164–173.
(8) Jaakola, L.; Määttä, K.; Pirttilä, A. M.; Törronen, R.; Kärenlampi, S.; Hohtola, A. Expression of genes involved in anthocyanin biosynthesis in relation to anthocyanin, proanthocyanidin, and flavonol levels during bilberry fruit development. *Plant Physiol.* **2002**, *130*, 729–739.
(9) Kalt, W.; McDonald, J. E.; Ricker, R. D.; Lu, X. Anthocyanin content and profile within and among blueberry species. *Can. J. Plant Sci.* **1999**, *79*, 617–623.
(10) Penman, K. G.; Halstead, C. W.; Matthias, A.; De Voss, J. J.; Stuthe, J. M. U.; Bone, K. M.; Lehmann, R. P. Bilberry adulteration using the food dye amaranth. *J. Agric. Food Chem.* **2006**, *54*, 7378–7382.
(11) Määttä-Riihinen, K. R.; González-Paramás, A. M.; Törrönen, A. R.; Kamal-Eldin, A.; Mattila, P. H. Distribution and contents of phenolic compounds in eighteen Scandinavian berry species. *J. Agric. Food Chem.* **2004**, *52*, 4477–4486.
(12) Ortega-Regules, A.; Romero-Cascales, I.; López-Roca, J.; Ros-García, J.; Gómez-Plaza, E. Anthocyanin fingerprint of grapes: environmental and genetic variations. *J. Sci. Food Agric.* **2006**, *86*, 1460–1467.
(13) Gao, L.; Mazza, G. Quantitation and distribution of simple and acylated anthocyanins and other phenolics in blueberries. *J. Food Sci.* **1994**, *59*, 1057–1059.
(14) Macheix, J.-J.; Fleuriet, A.; Billot, J. *Fruit phenolics* CRC Press: Boca Raton, FL, 1990; pp 1–126.
(15) Connor, A. M.; Luby, J. J.; Tong, C. B. S.; Finn, C. E.; Hancock, J. F. Genotypic and environmental variation in antioxidant activity, total phenolic content, and anthocyanin content among blueberry cultivars. *J. Am. Soc. Hort. Sci.* **2002**, *127*, 89–97.
(16) Kalt, W.; Ryan, D. A. J.; Duy, J. C.; Prior, R. L.; Ehlenfeldt, M. K.; Vander Kloet, S. P. Interspecific variation in anthocyanins, phenolics, and antioxidant capacity among genotypes of highbush and lowbush blueberries (*Vaccinium* section *cyanococcus* spp.). *J. Agric. Food Chem.* **2001**, *49*, 4761–4767.
(17) Prior, R. L.; Cao, G.; Martin, A.; Sofic, E.; McEwen, J.; O'Brien, C.; Lischner, N.; Ehlenfeldt, M.; Kalt, W.; Krewer, G.; Mainland, C. M. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinium* species. *J. Agric. Food Chem.* **1998**, *46*, 2686–2693.
(18) Johansson, A. K.; Kuusisto, P. H.; Laakso, P. H.; Derome, K. K.; Sepponen, P. J.; Katajisto, J. K.; Kallio, H. P. Geographical variations in seed oils from *Rubus chamaemorus* and *Empetrum nigrum*. *Phytochemistry* **1997**, *44*, 1421–1427.
(19) Cajander, A. K. Forest types and their significance. *Acta For. Fenn.* **1949**, *56*, 1–71.
(20) Albert, T.; Raspé, O.; Jacquemart, A. Clonal structure in *Vaccinium myrtillus* L. revealed by RAPD and AFLP markers. *Int. J. Plant Sci.* **2003**, *164*, 649–655.
(21) Buchert, J.; Koponen, J. M.; Suutarinen, M.; Mustranta, A.; Lille, M.; Törrönen, R.; Poutanen, K. Effect of enzyme-aided pressing on anthocyanin yield and profiles in bilberry and blackcurrant juices. *J. Sci. Food Agric.* **2005**, *85*, 2548–2556.
(22) Kähkönen, M. P.; Heinämäki, J.; Ollilainen, V.; Heinonen, M. Berry anthocyanins: isolation, identification and antioxidant activities. *J. Sci. Food Agric.* **2003**, *83*, 1403–1411.
(23) Wang, J.; Kalt, W.; Sporns, P. Comparison between HPLC and MALDI-TOF MS analysis of anthocyanins in highbush blueberries. *J. Agric. Food Chem.* **2000**, *48*, 3330–3335.
(24) Kähkonen, M. P.; Hopia, A. I.; Heinonen, M. Berry phenolics and their antioxidant activity. *J. Agric. Food Chem.* **2001**, *49*, 4076–4082.
(25) Katsube, N.; Iwashita, K.; Tsushida, T.; Yamaki, K.; Kobori, M. Induction of apoptosis in cancer cells by bilberry (*Vaccinium myrtillus*) and the anthocyanins. *J. Agric. Food Chem.* **2003**, *51*, 68–75.
(26) Taruscio, T. G.; Barney, D. L.; Exon, J. Content and profile of flavanoid and phenolic acid compounds in conjunction with the antioxidant capacity for a variety of northwest *Vaccinium* berries. *J. Agric. Food Chem.* **2004**, *52*, 3169–3176.
(27) Karonen, M.; Ossipov, V.; Sinkkonen, J.; Loponen, J.; Haukioja, E.; Pihlaja, K. Quantitative analysis of polymeric proanthocyanidins in birch leaves with normal-phase HPLC. *Phytochem. Anal.* **2006**, *17*, 149–156.
(28) Lee, J.; Finn, C.; Wrolstad, R. Anthocyanin pigment and total phenolic content of three *Vaccinium* species native to the Pacific Northwest of North America. *HortSci.* **2004**, *39*, 959–964.
(29) Lee, J.; Finn, C. E.; Wrolstad, R. E. Comparison of anthocyanin pigment and other phenolic compounds of *Vaccinium membranaceum* and *Vaccinium ovatum* native to the Pacific Northwest of North America. *J. Agric. Food Chem.* **2004**, *52*, 7039–7044.
(30) Martinelli., E. M.; Baj, A.; Bombardelli, E. Computer-aided evaluation of liquid-chromatographic profiles for anthocyanins in *Vaccinium myrtillus* fruits. *Anal. Chim. Acta* **1986**, *191*, 275–281.
(31) Andersen, Ø. M. Anthocyanins in fruits of *Vaccinium uliginosum* L. (bog whortleberry). *J. Food Sci.* **1987**, *52*, 665–666.

(32) McGhie, T. K.; Walton, M. C. The bioavailability and absorption of anthocyanins: towards a better understanding. *Mol. Nutr. Food Res.* **2007**, *51*, 702–713.

(33) Prior, R. L.; Wu, X. Anthocyanins: structural characteristics that result in unique metabolic patterns and biological activities. *Free Radical Res.* **2006**, *40*, 1014–1028.

(34) Vänninen, I.; Laakso, S.; Raatikainen, M.; Vänninen, I.; Raatikainen, M. Geographical variation in the phenology and morphology of bilberry in Finland. *Acta Bot. Fenn.* **1988**, *136*, 49–59.

(35) Castellarin, S. D.; Nonis, A.; Adam-Blondon, A.; Peterlunger, E.; Di Gaspero, G.; Marconi, R.; Paillard, S.; Testolin, R. Colour variation in red grapevines (*Vitis vinifera* L.): genomic organisation, expression of flavonoid 3′-hydroxylase, flavonoid 3′,5′-hydroxylase genes and related metabolite profiling of red cyanidin-/blue delphinidin-based anthocyanins in berry skin. *BMC Genomics* **2006**, *7*, 12–17.

Received for review September 26, 2007. Revised manuscript received November 2, 2007. Accepted November 6, 2007. This work was financially supported by the Finnish Cultural Foundation.

JF072857M