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ARTICLE

Variation in the Anthocyanin Concentration of Wild Populations of Crowberries (*Empetrum nigrum* L subsp. *hermaphroditum*)

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Crowberry (*Empetrum nigrum* L.) is a relatively under-utilized wild berry that occurs widely throughout the northern hemisphere such as in Canada, Eurasia, and northern Europe. In this work, the anthocyanins of crowberries were analyzed from four geographically distinct crowberry populations in Finland using HPLC-DAD and HPLC-ESI/MS/MS. A total number of 15 anthocyanins were detected; 15 (11 structure elucidated) in all samples in order to profile-specific anthocyanin compositions throughout Finland. The major anthocyanin found in the samples collected from central and eastern Finland was delphinidin 3-galactoside accounting for more than 24% of the total anthocyanin content, while the cyanidin 3-galactoside was the major anthocyanin in the northernmost and in the western samples. Significant variation in the concentrations of different anthocyanins between and within crowberry populations were found suggesting that the synthesis of anthocyanins is modified by site-specific environmental conditions. The suitability of the crowberries as a potential source of health-promoting ingredients for incorporation into pharmaceutical and food industrial products is highlighted in this work due to the diverse anthocyanin profile.

KEYWORDS: Crowberry; *Empetrum nigrum*; HPLC; mass spectrometry; anthocyanins; population; variation

INTRODUCTION

Berries contain impressive amounts of vitamin C and a diverse range of phenolic compounds with potential health properties (*1*). Therefore, berry-derived ingredients are increasingly being incorporated into many nutraceutical, cosmetic, and functional food products all over the world.

Berries are particularly rich sources of anthocyanins (*2*). The compounds occur in plants in 3- or 3,5-glycosylated forms of anthocyanidins (aglycones), often linked with glucose, galactose, arabinose, rhamnose, xylose, or fructose. Cyanidin is the predominant anthocyanidin, though delphinidin, peonidin, pelargonidin, petunidin, and malvidin are present in various concentrations in different berries. Anthocyanins are generally sensitive to degradation and stable only at low pH. Glycosylation increases the structural stability and water solubility of anthocyanins (*3*).

Anthocyanin-rich foods have been demonstrated to confer beneficial protective function against certain cancers (*4*), cardiovascular diseases (*5*), type II diabetes (*6*, *7*), obesity (*8*), and age-related macular degeneration (*9*). A significant increase in the plasma anthocyanin concentration and antioxidant capacity has been detected following the consumption of anthocyanin-rich juice (*10*) suggesting that part of the beneficial roles of anthocyanins may be related to their free-radical scavenging function. However, many potential health promoting properties may be independent of antioxidant activity, and anthocyanins may directly or indirectly exert their health effects by affecting key signal transduction processes (*11*, *12*).

The average per capita anthocyanin consumption ranges generally from 10 to 100 mg/day. It has been estimated that in Finland 82 mg and in USA 12.5 mg of anthocyanins are consumed daily (*2*). The consumption levels are relatively low in terms of their potential bioactivities since only minor amounts of the ingested anthocyanins are absorbed into the human body (*13*). Therefore, the food processing industry needs further information about potential anthocyanin rich plants that can be used in different functional food products.

Because of their high and diverse anthocyanin content, wild berries are an interesting new ingredient source to be incorporated into diverse products in the field of functional and wellness industry all over the world. There is emerging evidence (*3*, *14*, *15*) for beneficial health properties for wild berries such as bilberries

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(*Vaccinium myrtillus* L.), bog whortleberries (*Vaccinium uliginosum* L.), and lingonberries (*Vaccinium vitis-idaea*), i.e., there is a solid scientific basis for product development. Crowberry (*Empetrum nigrum* L.) is a relatively unused wild berry with great potential applications in the food, cosmetic, and pharmaceutical industries. Crowberries occur widely in the northern hemisphere such as in Canada, Greenland, Eurasia, Islands of the Northwest Territories, continental Northwest Territories, and northern Europe. Crowberries contain a wide range of phenolic compounds (*16*) and a very high amount of anthocyanins, which resemble bilberries in their concentration and structural features (*17*, *18*). The antioxidant capacity of berries, as determined by the FRAP assay, is very high. Crowberry extract was found to restore the cellular antioxidant enzyme activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and heme oxygenase-1 (HO-1), which were reduced by H_2O_2 treatment (*19*), suggesting that crowberry extract can protect cells against H_2O_2-induced cell damage via antioxidant properties by scavenging ROS and enhancing antioxidant enzyme activities.

Crowberry is a relatively under used wild berry today, but it may hold great future potential applications in the food, cosmetic, and pharmaceutical industries due to its high and diverse phenolic content. However, although crowberries occur widely in the northern hemisphere, little is known about the concentration of bioactive compounds in wild populations over different localities. This study was designed to provide new information on the anthocyanin variation in wild populations in geographically distant areas in Finland.

MATERIALS AND METHODS

Berry Samples. Samples were collected during one week in August 2009 from semidry pine forests. Populations were selected from different parts of Finland: Heinävesi from the eastern, Tervo in central Finland, Kaustinen in western, and Utsjoki in northern Finland (**Figure 1**). Distances between locations ranged from 120 to 900 km. In the samples from Tervo, Heinävesi, and Utsjoki, one sample was collected to represent one population. From the location Kaustinen, seven samples were collected from the same population to analyze anthocyanin variation within the same population. Each sample was collected by hand from an area with a diameter of five meters. Distances between sample areas in Kaustinen were 10 m. Sample sizes were in the ranges of 20−100 g depending on the yield in each particular sample area. All of the samples were immediately cooled to +4 °C and frozen to −18 °C in 24 h.

Anthocyanin Extraction. About 15 g of berries were homogenized at +4 °C using a domestic food processor, and three subsamples weighing 3.5 g were taken from the homogenate for extraction. Fruits were extracted by shaking vigorously for 20 min with 20 mL of 70% aqueous acetone containing 1% of formic acid. The supernatant was separated by centrifugation (3000*g*, 8 min), and the extraction was repeated twice with 15 mL of the solvent. Acetone was evaporated from the combined extract using a rotary evaporator at room temperature, and the volume was adjusted to 50 mL with 1% formic acid. Anthocyanin content was analyzed immediately after extraction.

Anthocyanin Analysis. Extracts were centrifuged (13 000*g*, 5 min) and filtered through 0.45 μm syringe filters (Agilent Technologies, Germany) before high-performance liquid chromatography (HPLC) analysis. HP 1090 series HPLC (Agilent Technologies, Palo Alto, CA) equipped with a diode array detector was used. Controlling and data evaluation were done with HP Chemstation rev. A 10.02 software. Anthocyanins were separated in a 250 mm × 4.6 mm i.d., 5 μm XTerra Phenyl column (Waters, Ireland). Gradient elution was used with 5% v/v formic acid (A) and acetonitrile (B). The column temperature was set to 35 °C, and the flow rate was 1 mL/min. The gradient program was as follows: 0−5 min, 2% of B; 5−30 min, 2−13% of B; 30−35 min, 13−100% of B; 35−37 min, 100% of B; 37−42 min 100−2% B followed by 8 min equilibrium time. The injection volume was 25 μL and detection at a wavelength of 520 nm. Quantification was based on peak areas, and cyanidin 3-*O*-galactoside (Sigma-Aldrich, Fluka Analytical, USA) was used as a standard compound. Single analyses of each extract were conducted, and the results are given as mg/g of fresh weight of crowberries.

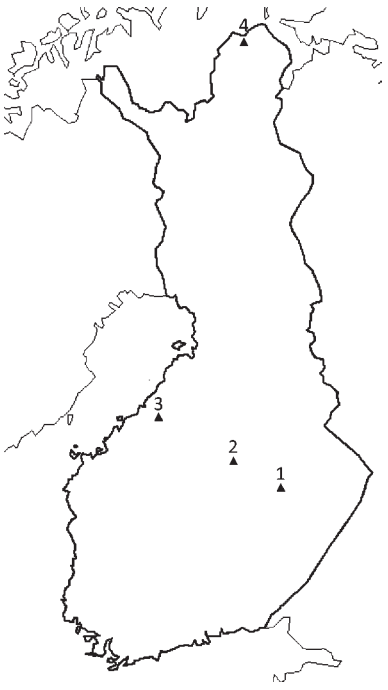
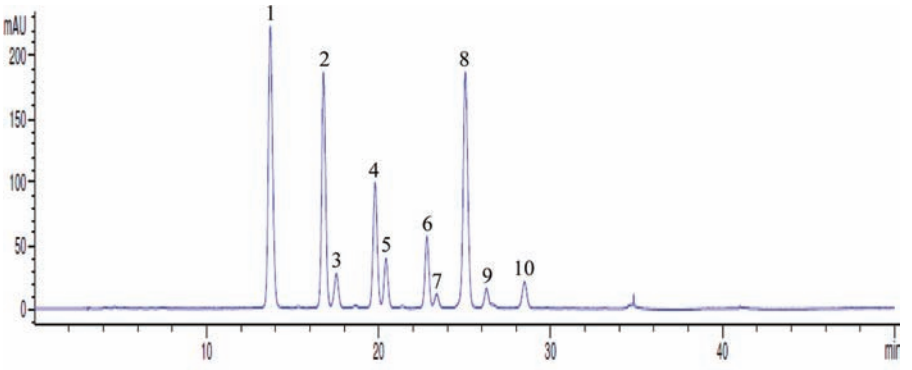


Figure 1. Map of Finland with the locations of crowberry samples: Heinävesi (1), Tervo (2), Kaustinen (3), and Utsjoki (4). From locations Heinävesi, Tervo, and Utsjoki one sample was collected to represent one population, whereas seven samples were collected from the location Kaustinen to reveal the variations in anthocyanins within a population. The distance between Heinävesi and Utsjoki is about 900 km.

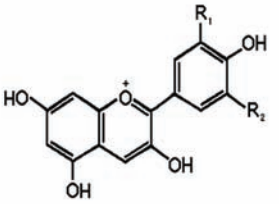
Anthocyanin Characterization. Anthocyanins were characterized using HPLC-ESI/MS/MS (Thermo LTQ ion trap). HPLC system consisted of a Finnigan Surveyor MS pump and a Finnigan autosampler (Thermo Electron, USA) with the column described in the Anthocyanin Analysis section. In the MS analyses, the column temperature was set to 30 °C and the injection volume to 20 μL; otherwise, HPLC was carried out as described above. The mass analysis system consisted of a Finnigan LTQ linear ion trap spectrometer equipped with Finnigan ion mode operating in a positive mode (Thermo Electron, USA). Instrument conditions: nitrogen sheath gas flow, 30 arbitrary units; spray voltage, 3.8 kV; capillary temperature, 250 °C; capillary voltage, 33 V; and tube lens voltage, 80 V. In the first MS, ions in the range of m/z 280−700 were measured. In the second MS, the most intense ions from the MS spectrum were selected to the collision induced dissociation, and the expected fragment ions from parent ions m/z 465, 449, 435, 479, 419, 463, 493, and 433 reported by Ogawa et al. (*17*) were measured. Data acquisition was conducted by using Xcalibur 1.4 SR1 software.

Validation. The validation of the extraction method was based on extracted total phenolics (*20*). In the HPLC analysis, the repeatabilities ranged from 3.0 to 5.4% RSD (relative standard deviation) (n = 3) for different crowberry anthocyanins. System suitability was 1.3% RSD (n = 5) for total anthocyanins.

Statistics. Statistical analyses were done using SPSS for Windows, release 14.0 (SPSS Inc., Chicago, IL). Differences in anthocyanin contents between samples were evaluated with one-way analysis of variance (ANOVA), and multiple comparison was done using the Games-Howell test. Differences at the level $P < 0.05$ were considered to be significant. Anthocyanin contents of crowberry samples were analyzed by hierarchial cluster analysis (HCA), which is a suitable method for small quantities of data, using the average between-groups linkage method and squared Euclidean distance interval measurement. The average between-groups linkage method reveals the dissimilarities between two clusters according to the maximum of all the possible distances between cases in different clusters. In this case, HCA clustered samples by their anthocyanin contents forming relatively homogeneous groups and compared clusters as pairs including



Peak	RT[a] (min)	Anthocyanins	$[M]^+$	Fragment ion $[M]^+$
1	13.6	delphinidin 3-galactoside	465	303
2	16.7	cyanidin 3-galactoside	449	287
3	17.4	delphinidin 3-arabinoside	435	303
4	19.7	petunidin 3-galactoside	479	317
5	20.3	cyanidin 3-arabinoside	419	287
6	22.7	peonidin 3-galactoside	463	301
7	23.3	petunidin 3-arabinoside	449	317
8	25.0	peonidin 3-glucoside	463	301
		malvidin 3-galactoside	493	331
9	26.2	peonidin 3-arabinoside	433	301
10	28.4	malvidin 3-arabinoside	463	331



Delphinidin: $R_1 = OH$, $R_2 = OH$
Cyanidin: $R_1 = OH$, $R_2 = H$
Petunidin: $R_1 = OH$, $R_2 = OCH_3$
Peonidin: $R_1 = OCH_3$ $R_2 = H$
Malvidin: $R_1 = OCH_3$, $R_2 = OCH_3$

Figure 2. Top: typical HPLC chromatogram of crowberry anthocyanins detected at 520 nm. Bottom left: characterized anthocyanins from the crowberry extract with HPLC-MS/MS. Bottom right: aglycone structures of characterized anthocyanins. [a] indicates the retention time.

different samples of each cluster resulting in average distances between clusters. A dendrogram was used to visualize the results of the HCA analysis.

RESULTS AND DISCUSSION

Anthocyanin Profile of Crowberries. Fourteen anthocyanin peaks were detected from the crowberry extracts. Ten peaks accounting for ~99% of the total content of anthocyanins were selected for the quantification and identification on the basis of the fact that their peak areas were suitable for accurate quantification (**Figure 2**). The total number of detected anthocyanins was 15 (11 characterized) in all samples in order to undertake a location-dependent anthocyanin profile analysis throughout Finland.

The major anthocyanin found in the samples collected from central and eastern Finland, Tervo and Heinävesi, was delphinidin 3-galactoside accounting for more than 24% of the total anthocyanin content. Delphinidin 3-galactoside is one of the main anthocyanins also in bilberries (*21*). Cyanidin 3-galactoside was the major anthocyanin in the northernmost samples from Utsjoki and in the western samples from Kaustinen. Cyanidin 3-galactoside is a major anthocyanin also in lingonberries and is present at moderate levels in bilberries (*21*).

Peak number 8 (**Figure 2**) consisted of two different anthocyanins, peonidin 3-glucoside and malvidin 3-galactoside, which were the second most common anthocyanins in crowberries from samples collected from Tervo, Heinävesi, and Kaustinen. Peonidin 3-glucoside and malvidin 3-galactoside can be also found in bilberries (*21*), but the exact amounts of these anthocyanins were not determined from the crowberries due to insufficient peak separation.

Relatively high concentrations of petunidin 3-galactoside and low amounts of petunidin 3-arabinoside were detected in crowberries, while peonidin 3-galactoside and peonidin 3-arabinoside levels in crowberries occurred at moderate levels compared to that in other berries (*16*, *18*). Crowberries represent a great potential as a new source of peonidins along with bilberries and bog whortleberries (*16*, *22*).

Cyanidin 3-arabinoside is a common anthocyanin in berries, and it was also detected in low amounts in crowberries. Compared to crowberries, cyanidin 3-arabinoside proportions with respect to the total anthocyanin contents of lingonberries (~18%) and bilberries (~12%) are notable. Similarly, malvidin 3-arabinoside was detected in low amounts in crowberries as it is found in bilberries (*21*). Delphinidin 3-arabinoside, the major anthocyanin in bilberries (*21*), was found only in trace amounts in crowberries.

Variation between Crowberry Populations. Significant divergence in anthocyanin contents was found between crowberry populations (**Table 1**). The total amount of anthocyanins was approximately 1.7-fold (273 mg) higher in samples collected from western Finland, Kaustinen, compared to the population with the lowest anthocyanin content found in samples collected from central Finland, Tervo. Interestingly, the most similar total anthocyanin contents were found in the populations collected from the most distant areas, samples from Heinävesi (eastern Finland) and Utsjoki (northern Finland, with the distance between the two populations of 900 km), while geographically the closest populations, Heinävesi and Tervo (distance 120 km), showed ~1.3-fold (130 mg) difference in favor of the eastern Finland population.

Significant variation was also found in individual anthocyanin contents. The highest variation in anthocyanin concentrations

Table 1. Content (mg/100g) of Anthocyanins in Different Crowberry Populations[a]

	Tervo[b]	Heinävesi[b]	Utsjoki[b]	Kaustinen[c]
cyanidin 3-arabinoside	17.0 ± 4.9 (6)	22.4 ± 4.2 (6)	32.6 ± 1.8 (6)	35.5 ± 1.7 (6)
cyanidin 3-galactoside	79.4 ± 4.6 (3)	105.0 ± 2.8 (3)	161.5 ± 2.1 (1)	182.1 ± 1.3 (1)
delphinidin 3-arabinoside	12.7 ± 4.9 (7)	16.1 ± 4.3 (7)	15.4 ± 3.6 (7)	15.0 ± 1.9 (8)
delphinidin 3-galactoside	106.3 ± 4.27 (1)	132.1 ± 2.6 (1)	132.7 ± 2.4 (2)	132.8 ± 1.5 (3)
malvidin 3-arabinoside	10.64 ± 4.0 (8)	14.7 ± 4.5 (8)	10.7 ± 2.0 (9)	14.90 ± 2.13 (9)
peonidin 3-arabinoside	6.8 ± 3.0 (9)	10.1 ± 4.1 (9)	12.0 ± 0.9 (8)	18.5 ± 1.8 (7)
peonidin 3-galactoside	24.1 ± 4.8 (5)	34.2 ± 1.1 (5)	44.0 ± 2.1 (5)	69.7 ± 1.6 (4)
peonidin 3-glucoside/malvidin 3-galactoside	94.7 ± 4.5 (2)	131.6 ± 3.6 (2)	94.2 ± 1.9 (3)	141.4 ± 1.7 (2)
petunidin 3-arabinoside	4.9 ± 5.4 (10)	6.5 ± 2.6 (10)	5.8 ± 4.1 (10)	6.3 ± 3.5 (10)
petunidin 3-galactoside	45.3 ± 4.5 (4)	58.8 ± 3.5 (4)	53.1 ± 2.4 (4)	58.6 ± 1.5 (5)
total	401.8 ± 4.5	531.5 ± 3.1	562.0 ± 2.1	674.8 ± 2.7

[a] Results are given as averages ± RSD. The number in parentheses indicates the rank in the column (population). [b] $n = 3$. [c] $n = 21$.

Table 2. Contents (mg/100g) of Anthocyanins in the Crowberry Population from Kaustinen[a]

	Kaustinen 1	Kaustinen 2	Kaustinen 3	Kaustinen 4	Kaustinen 5	Kaustinen 6	Kaustinen 7
cyanidin 3-arabinoside	29.1 ± 3.5 (6)	32.3 ± 2.4 (6)	30.5 ± 0.4 (6)	39.4 ± 0.3 (6)	42.2 ± 0.9 (6)	37.1 ± 2.7 (6)	37.7 ± 2.1 (6)
cyanidin 3-galactoside	160.0 ± 1.6 (1)	159.1 ± 1.8 (1)	156.5 ± 1.0 (1)	202.0 ± 0.5 (1)	204.0 ± 0.6 (1)	199.5 ± 1.9 (1)	193.7 ± 1.7 (1)
delphinidin 3-arabinoside	10.2 ± 4.3 (9)	17.2 ± 2.3 (7)	12.8 ± 0.4 (9)	16.9 ± 0.7 (9)	14.9 ± 0.9 (9)	16.2 ± 3.1 (9)	16.7 ± 1.8 (8)
delphinidin 3-galactoside	92.6 ± 1.4 (3)	152.6 ± 2.0 (2)	112.6 ± 1.1 (3)	151.2 ± 0.9 (3)	123.1 ± 0.7 (3)	154.0 ± 2.3 (3)	143.4 ± 1.4 (2)
malvidin 3-arabinoside	11.9 ± 4.2 (8)	11.4 ± 1.5 (9)	14.5 ± 1.7 (8)	17.0 ± 0.6 (8)	16.6 ± 1.6 (8)	17.2 ± 2.1 (8)	15.7 ± 3.3 (9)
peonidin 3-arabinoside	16.9 ± 2.3 (7)	11.6 ± 1.0 (8)	16.6 ± 1.5 (7)	21.3 ± 0.3 (7)	25.4 ± 1.6 (7)	20.2 ± 1.6 (7)	17.2 ± 4.6 (7)
peonidin 3-galactoside	72.2 ± 2.3 (4)	42.2 ± 2.7 (5)	62.6 ± 1.1 (4)	78.3 ± 0.5 (4)	89.1 ± 0.4 (4)	78.7 ± 1.6 (4)	64.8 ± 2.4 (4)
peonidin 3-glucoside/malvidin 3-galactoside	122.5 ± 2.4 (2)	103.7 ± 3.1 (3)	137.2 ± 0.3 (2)	163.3 ± 0.6 (2)	153.0 ± 0.8 (2)	169.1 ± 1.9 (2)	140.6 ± 2.8 (3)
petunidin 3-arabinoside	4.6 ± 3.9 (10)	6.1 ± 4.9 (10)	5.7 ± 4.8 (10)	7.7 ± 2.1 (10)	6.7 ± 1.7 (10)	7.3 ± 3.1 (10)	6.4 ± 3.8 (10)
petunidin 3-galactoside	44.4 ± 2.6 (5)	57.8 ± 2.3 (4)	52.4 ± 0.6 (5)	68.7 ± 0.4 (5)	55.0 ± 0.5 (5)	68.9 ± 2.6 (5)	62.7 ± 1.6 (5)
total	564.4 ± 1.9	594.0 ± 2.2	601.4 ± 0.6	765.8 ± 0.5	730.0 ± 0.5	768.2 ± 2.0	698.9 ± 2.0

[a] The numbers after Kaustinen indicate the different samples analyzed in random order. Results are given as averages ± RSD. The number in parentheses indicates the rank in the column (sample). $n = 3$ for each sample.

was in cyanidin 3-galactoside, peonidin 3-galactoside, and malvidin 3-galactoside/peonidin 3-glucoside contents, which differed significantly between all four populations. The contents of delphinidin 3-galactoside and petunidin 3-galactoside contents varied significantly between samples collected from Tervo and other populations. The cyanidin 3-arabinoside content differed widely between populations except for those samples collected from western Finland (Kaustinen) and northern Finland (Utsjoki), which most resembled each other with respect to individual anthocyanins. Malvidin 3-galactoside contents varied significantly between Tervo and Heinävesi, Tervo and Kaustinen, Heinävesi and Kaustinen, and Heinävesi and Utsjoki. The most extensive variation in the content of malvidin-3-arabinoside was detected between populations collected from Tervo and Heinävesi, Tervo and Kaustinen, Heinävesi and Utsjoki, and Utsjoki and Kaustinen. Interestingly, delphinidin 3-arabinoside and petunidin 3-galactoside seemed to be conserved among anthocyanins in crowberries throughout Finland, although the anthocyanin content of bilberries revealed a statistical difference in a similar study (*23*).

Data from bilberries (*23*) offer another explanation for the differences in phenolic concentrations between southern and northern Finland. The total phenolic concentrations were higher in warm than cold summers, and the temperature also explained the altitudinal variation (*23*). However, it was also found by Laine and Henttonen (*24*) that the nitrogen concentration was low in warm summers compared with that in cold summers. Support for the proposal that temperature may affect phenolic concentrations via the nutrient interaction has emerged from recent molecular studies. Flavonoid accumulation was found to be induced by exposing plants for 1 week to nitrogen depletion at 10 °C, leading to high levels of anthocyanins and 3-glucoside-7-rhamnosides, 3,7-dirhamnosides, and 3-rutinoside-7-rhamnosides of kaempferol and quercetin (*25*). There are some transcription factors which are important for the nutrient depletion responses; these belong to the MYB family (PAP1, PAP2, and GL3, responsible for pigment production), and PAP1/2 appears to stimulate gross activation of the flavonoid pathway in response to diverse abiotic stress factors (*26*).

Interestingly, in crowberries, the sample from the geographically distant region (northern Finland, Utsjoki) contained a similar level of anthocyanins as that found in samples from western Finland (Kaustinen). Both of these locations are known to be susceptible to frost attack, which may explain the similarities in the anthocyanin levels in these regions. In Norway spruce, the total phenolic concentrations have more than doubled during the course of cold hardening (*27*). Petunia plants, recovering from a chilling injury following 3 weeks of cold pretreatment, demonstrated an increase in the total phenolics suggesting that antioxidant protection may be involved in cold adaptation (*28*). Interestingly, the antioxidant capacity was only moderately related to chilling tolerance, indicating that factors other than antioxidant containing phenolics may also play a role in the chilling tolerance observed in petunia plants (*28*). Recently, cold treatment for 12 h in *Lepidium sativum* L., induced a strong elevation in the total antioxidant capacity (TAC), and enzymatic activities (peroxidases and catalase) were activated, evidence of metabolic and transcriptional changes during the first 12 h of cold treatment (*29*). Thus, frost stress may have a major impact on crowberries in those areas exceptionally sensitive to summer frost, and changes in phenolic compounds may reflect adaption to stress situations.

Variation within Crowberry Populations. In the Kaustinen region, seven individual crowberry samples were collected from seven areas within a diameter of 5 m (**Table 2**). Distances between

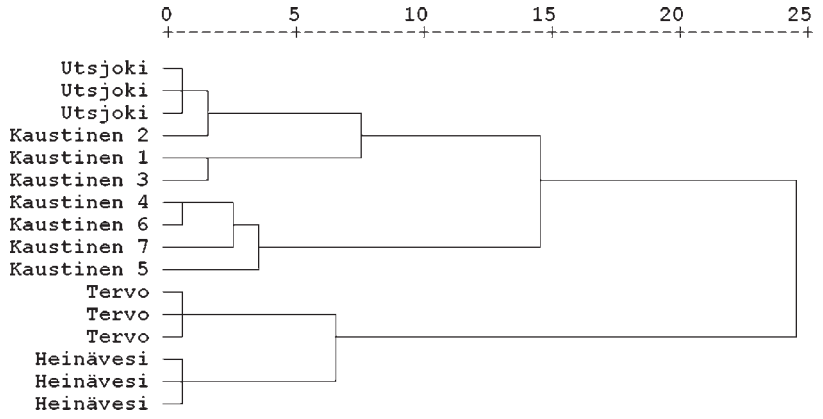


Figure 3. Dendrogram of hierarchial cluster analysis of the anthocyanin contents of crowberries. Samples of locations are expressed as the averages of three subsamples. The scale bar on top indicates the squared Euclidean distance.

sample areas were 10 m. The highest variation of the total anthocyanin content was ~1.4-fold (204 mg). With respect to the individual anthocyanin level, only petunidin 3-arabinoside did not reveal any significant difference between samples with respect to the variation found between populations. Peonidin 3-arabinoside, malvidin 3-galactoside/peonidin 3-glucoside, and peonidin 3-galactoside displayed the most extensive variation throughout the population. There was a high variation in concentrations of delphinidin 3-galactoside, cyanidin 3-galactoside, petunidin 3-galactoside, and cyanidin 3-arabinoside with significant differences in at least half of the samples. Malvidin 3-arabinoside and delphinidin 3-arabinoside showed a low variation within the population. Similar levels of variation were detected between the populations. Consequently, with regard to the levels of variation found in all samples, our study suggests that variations were at the same levels as that found between the large geographic distance (from 10 m to 900 km).

Hierarchial Cluster Analysis. Hierarchial cluster analysis was conducted to evaluate and illustrate the differences of anthocyanin contents between and within crowberry populations (**Figure 3**). The content and composition of anthocyanins seem to be controlled more by genetic rather than environmental factors according to the HCA dendrogram. HCA clearly showed parallel heterogeneity in the anthocyanin content within and between populations. Samples from the same population cannot be directly inserted into the same cluster, and one cannot attribute the same origin of samples compared to when one only examines their anthocyanin levels.

In conclusion, a significant variation in anthocyanin contents between different crowberry populations and within populations was found, and this should be taken into consideration in experimental layouts in population studies. Crowberries with high concentrations of anthocyanins can be found in local populations independently of their latitudinal location. Our study also emphasizes the potential of crowberries as a source of anthocyanins; this plant has a considerable content of potentially bioavailable anthocyanins such as peonidin 3-glucoside/-galactoside and cyanidin 3-galactoside (*30*, *31*). The suitability of crowberries as a potential raw material for health-promoting ingredients in pharmaceutical and food industrial products is highlighted in this work due to the diverse anthocyanin profile.

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