Research Article

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Chemical composition of ripe fruits of *Rubus chamaemorus* L. grown in different habitats

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Abstract

BACKGROUND: Cloudberry (*Rubus chamaemorus* L.) is one of the most valuable berry-producing plants because of its nutritional properties. The chemical composition and crop yield of ripe fruits of cloudberry grown wild in 10 habitats in northern Finland was analysed over two consecutive summers. For comparison, two clones of cultivated cloudberries were studied as well.

RESULTS: The concentrations of citric and malic acids, α-tocopherol, anthocyanins and β-carotene had notable variations between habitats. In particularly, cloudberries grown on open habitats had higher content of citric acid and less α-tocopherol compared to those grown on shaded sites. In a colder and rainy summer the content of anthocyanins and the unsaturation level of fatty acids were significantly higher than in a warmer and drier summer. Crop yields were higher in the warmer summer, except in open sites where yields were quite equal.

CONCLUSION: Cloudberries grown in open habitats showed notable differences in chemical composition when compared to those grown on shaded sites. Results suggest that the amount of sunlight and temperature levels could be the main factors affecting crop yield and chemical composition of cloudberry. In addition, the amount of rainfall may have an effect on anthocyanin concentrations.

Keywords: *Rubus chamaemorus* L.; cloudberry; chemical composition; crop yield; habitats

INTRODUCTION

Rubus chamaemorus (Rosaceae) is one of the most valuable berry-producing plants because of its nutritional properties and distinctive flavour. Cloudberries are an important dietary source of nutrients such as antioxidant vitamins (C, A and E), fibre, fatty acids and also non-nutrient bioactive phenolic compounds.[1,2] These secondary metabolites, which are mainly ellagitannins with a high content of ellagic acid, have been suggested to exert many beneficial health properties. Cloudberries have more antioxidants than many commonly consumed fruits and vegetables[3] and possess antimicrobial activities against human pathogens,[4,5] most probably brought about by phenolic compounds (mainly ellagitannins) together with organic acids such as malic, citric and benzoic acids. Cloudberries contain more fibres than other berries in general[6] and have a beneficial fatty acid composition: seeds isolated from cloudberries have linoleic, α-linoleic, oleic and palmitic acids amounting to over 95% of all fatty acids.[7,8]

Cloudberries typically inhabit peaty moors, open sphagnum bogs, forested mires, and wet spruce forest within the boreal zone.[9] Plants growing in different habitats express phenotypic variation, such as differences in mean amount of seeds, leaf, and rhizome sizes.[10] For example, cloudberries have been found to have long shoots and large leaves in shady sites, but short shoots and small leaves in open areas. The differences have been attributed to a greater need for large food reserves in competitive and resource-poor environments. Cultivation methods for commercial cloudberry production have been developed in Finland, Norway and North America, but there are still problems with profitability due to the high costs.[11,12]

Despite the fact that cloudberries have been found to express phenotypic variation depending on their habitat, there is a shortage of knowledge pertaining to the chemical composition of cloudberries grown in different habitats. To the best of our knowledge, only the variation in the seed oils of cloudberries has been reported in the literature.[7] Furthermore, no studies are available of the annual variation of cloudberry components or nutritional value of cultivated cloudberries. Therefore, the objectives of this study were to analyse the chemical composition of cloudberries grown in different habitats and the effect of annual variation on the chemical composition of cloudberries grown in the same area in northern Finland. The analyses included both primary and secondary metabolites. Furthermore, the primary metabolites and antioxidants of cultivated cloudberries were analysed and compared to those of wild cloudberries.

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MATERIAL AND METHODS

Plant material

Wild cloudberry samples were collected at 10 sampling sites located in Ulvinsalo Strict Nature Reserve, Kuhmo, eastern Finland (latitude 63°58′21″ N, longitude 30°22′28″ E). Sampling site areas measured 50 m × 50 m, with specified characteristics (Table 1). In each sampling site the entire yield was harvested. The season lasted from mid July to mid August and ripening of fruits varied depending on light and temperature. Harvesting took place on open sites approximately 5–14 days earlier than forested areas, depending on the year. Crop yields were observed for each site in summer 2006 and 2007. Cultivated cloudberries (Fjellgull and Fjordgull) were obtained from a greenhouse located in Kuhmo, Eastern Finland, approximately 40 km from the wild habitat sites. The greenhouse was unheated and the growing substrate was peat at humification stage H2–H4. In the greenhouse, the yield was harvested in June, approximately 4 weeks earlier than in the wild habitat sites. In the greenhouse, the female cultivars Fjellgull and Fjordgull together with the male cultivar Apollo were used.[13,14] Cloudberry fruits were mixed well and stored in a freezer (−20 °C) until analysed. From the frozen fruits (the whole crop), three separate random samples (200 g each) per site were taken in 2006 and one (200 g) random sample per site in 2007 for analysis of chemical composition. The results of year 2006 samples (three replicates) were used to study the differences between habitats, whereas the annual variation was analysed by calculating arithmetic means of all samples collected in a specific year. Therefore the number of replicates in the analysis of annual variation was nine in 2007 (no crop was obtained in site eight) and 30 in 2006.

Laboratory analyses

Frozen samples (200 g) were thawed and homogenized for analysis. The whole set of chemical analyses were performed from the same sample homogenate. Each analysis was repeated at least twice, except in the analysis of dietary fibre, which is very laborious, and in the analysis of fatty acids, where rapeseed oil was used as a control sample to validate the assay in each sample set.

Moisture content of the sample was measured by a gravimetric method.[15]

Table 1. Sampling site description and crop yields of the studied cloudberry populations in 2006 and 2007

Sampling site	Description			Crop yield (kg/ha)	
	Tree stand	Undergrowth	Peat layer depth cm	2006	2007
1	+++	High	60	17	10
2	++	High	150	80	22
3	++	High	80	74	12
4	0	Low	>300	12	16
5	0	Low	>300	35	28
6	++	Low	200	46	10
7	+++	High	200	34	12
8	+	High	150	332	0
9	++	High	100	n.a.	360
10	++	High	100	n.a.	220

Tree stand: 0, open area; +, few small trees; ++, moderate amount of trees; +++, dense forest. n.a., not analysed.

Fat-soluble compounds were extracted from freeze-dried cloudberry samples (1 g) by Soxhlet extraction in hexane and the yield of total fat was determined by a gravimetric method. Fatty acid profile was analysed from samples after conversion of the lipid material to the corresponding methyl esters (FAME). Dried Soxhlet extract was dissolved in toluene (1 mL) and transmethylated with 10% (v/v) borontrifluoride in methanol (100 °C, 60 min). Water (1.6 mL) and hexane (1.6 mL) were added to the cooled sample and mixed well. The organic layer was separated, dried and dissolved in hexane (0.7 mL). FAMEs were analysed using a gas chromatograph (HP6890; Agilent Technologies, Inc., Palo Alto, CA, USA) connected to a mass selective detector (HP5973; Agilent Technologies). The compounds were separated on an HP-FFAP column (25 m, 0.20 mm, 0.30 μm, polyethylene glycol TPA; Agilent Technologies). Samples (1 μL) were injected in a split mode (ratio 30 : 1) at 250 °C and helium (purity 6.0; AGA, Espoo, Finland) was used as a carrier gas. The gas chromatograph oven temperature was held at 60 °C for 2 min, then increased by 15 °C min^{-1} to 190 °C; this was further increased by 2 °C min^{-1} to 210 °C, where it was held for 20 min, and then finally increased again by 10 °C min^{-1} to 220 °C, where it was held for 5 min. Analysis was performed using electron impact (EI) and scan mode. Fatty acids were identified by authentic methylated fatty acid standard (GLC-461, supplied from Nu-chek Prep Inc., Elysian, MN, USA), which contained a mixture of 32 methylated fatty acids ($C_{4:0}$–$C_{24:1}$).

Protein content was determined measuring total nitrogen by the Kjeldahl method[16] and multiplying the obtained nitrogen content by a general nitrogen-to-protein conversion factor of 6.25.

Anthocyanins were extracted from the berries (5 g, fresh weight (fw)) by 0.01% (v/v) HCl in methanol (10 mL) in an ultrasound bath (20 min). Extraction was repeated twice and the three extracts were combined. One millilitre of the extracts was dried under nitrogen and then dissolved in 0.01% HCl water (1 mL) and filtered through a 0.45 μm syringe filter. Anthocyanins were identified and quantified by high-performance liquid chromatography (HPLC) (Agilent 1100 Series HPLC, Agilent Technologies) using a Hyperclone ODS column (2.00 mm × 200 mm, 5 μm; Phenomenex, Torrance, CA, USA) as described in detail earlier.[17]

Total phenolic content was determined by the Folin–Ciocalteu method,[18] in which gallic acid was used as a calibration standard in spectrophotometric (UC-1601; Shimadzu, Kyoto, Japan) measurement.

Vitamin C is very sensitive to light and temperature and oxidizes easily to L-dehydroascorbic acid. Therefore vitamin C was measured as a sum of L-ascorbic acid and L-dehydroascorbic acid. To prevent oxidation and to recover L-dehydroascorbic acid as L-ascorbic acid, dithiothreitol (DTT) treatment was performed for the samples. Each sample was analysed before and after DTT incubation, but only the DTT-treated value was reported, since that gives the total amount of vitamin C and was always higher than the value determined before treatment. The method was modified from previous publications.[19,20] Metaphosphoric acid (10%, 5 mL) was added to the sample (1g, fw) in a volumetric flask (25 mL) and the sample was shaken well. The flask was filled with water and the sample was centrifuged (1808 × *g*) for 5 min. DTT (40 mmol L^{-1}) was added to the filtered extract (1 : 1, v/v), after which the reduction reaction was allowed to proceed in the dark at room temperature overnight. A sample aliquot was then analysed by HPLC (Agilent 1100 Series) connected to a diode array detector (245 nm). An isocratic run was performed with KH_2PO_4 (117.5 mmol L^{-1}) buffer (pH 2.4) using a Zorbax Eclipse XDB-C18 column (5 μm, 4.6 mm × 250 mm, Agilent Technologies).

Quantitative results were calculated using a calibration curve for authentic L-ascorbic acid standard (p.a., Merck KGaA, Darmstadt, Germany).

Citric acid and malic acid were extracted from cloudberries (10 g fw, collected in 2006 and 2007) in 80% ethanol (20 mL) by high-performance disperser (Ultra Turrax, IKA, Staufen, Germany) for 2 min. The extract was centrifuged (1808 × *g*) for 10 min and filtered. Extraction was repeated twice and all three extracts were combined in a 50 mL volumetric flask, which was filled with 80% ethanol. Samples of 1 mL were purified by solid-phase extraction with combined CH(EC) (Isolute, 500 mg, 3 mL; Biotage AB, Norlab Oy, Vantaa, Finland) and SAX (Isolute, 500 mg, 3 mL, Biotage AB, Norlab Oy) columns activated with methanol and water. Columns were eluted with water to remove sugars, after which citric acid and malic acid were eluted with 3 mL of 1 mol L^{-1} hydrochloric acid in a volumetric flask (5 mL), which was filled with water. Samples were filtered and analysed by HPLC (Agilent 1100 Series) connected to a diode array detector (210 nm). The isocratic run was performed with H_2SO_4 (0.00125 mol L^{-1}) using an ORH-801 ion-exclusion column (6.5 mm × 300 mm, Transgenomic, Norlab Oy). Calibration curves were prepared with authentic citric acid standards (p.a., Merck KGaA) and malic acid (purity > 99%, Fluka, Sigma-Aldrich, Buchs, Switzerland).

β-Carotene and α-tocopherol were extracted from cloudberry (5 g, fw) by acetone (15 mL) in an ultrasound bath (4 °C, in darkness). β-Carotene was analysed only in 2006. Samples were extracted twice and three supernatants were combined. α-Tocopherol was analysed from the subsample (1 mL) of the crude acetone extract by HPLC (Agilent 1100) connected to a fluorescence detector (FD), using an Eclipse XDB-C8 (5 µm, 4.6 mm × 150 mm, Agilent Technologies) column with isocratic run (1 mL min^{-1}). The mobile phase consisted of methanol and water (95 : 5, v/v). α-Tocopherol (in 0.5 mL acetone) was determined by FD using 292 nm excitation and 324 nm emission wavelength. Concentrations in samples were calculated by an external standard method using the calibration curve for α-tocopherol (purity 97%; Sigma-Aldrich, Switzerland). β-Carotene was analysed from the acetone extract after concentration and hydrolysis, modified from the previous published method.[21] The extract was evaporated and the sample was dissolved in hexane, washed with water and evaporated to 1 mL. An aliquot of the sample (0.5 mL) was diluted in a methyl tributyl ether (MTBE, 25 mL) and saponified at room temperature overnight (in darkness) with methanolic potassium hydroxide (30% w/v, 1.25 mL). The organic phase was separated, washed three times with 50 mL water, dried in anhydrous sodium sulfate, filtered, and evaporated to dryness. Finally, the dried sample was dissolved in 1 mL acetone for HPLC–diode array detection (DAD) (Agilent 1100) analysis. The HPLC method was identical to the method used for α-tocopherol, except for the detection (DAD at 450 nm). Quantitative results were obtained by an external standard method using the calibration curve of β-carotene (purity 97%; BioChemica, Fluka).

Dietary fibre was analysed by the enzymatic–gravimetric method of AOAC International[22] using an enzyme kit (total dietary fibre, assay kit; Megazyme International Ireland Ltd, Bray, Ireland).

Weather and climate

Weather and climate conditions were obtained from the Finnish Meteorological Institute. Statistics were collected from two weather stations located closest to the sampling sites (Kuhmo and Sotkamo, Oulu province, Finland). Statistics included daily temperatures derived as arithmetic means of eight measured values obtained in 3 h intervals, daily minimum and maximum temperatures that were recorded separately, average monthly temperatures that were calculated as a mean of daily temperatures, monthly sunshine duration and monthly rainfall.

Statistical analyses

Statistical analyses were performed with SPSS Statistics 18. The variations in chemical composition between consecutive years and between open and variously shaded habitats were evaluated using Mann–Whitney *U* and analysis of variance (ANOVA) procedures and the correlations between concentrations of the analytes were measured using non-parametric Spearman's rho correlation test.

RESULTS AND DISCUSSION

Sampling sites

Tree stand, undergrowth and surface geometry affect the availability of light as well as the humidity and temperature conditions of the plants. In this study, sampling sites 4 and 5 represented open sites with low undergrowth and no tree stand, whereas the other sites were shaded by moderate or high frequency of trees and longer undergrowth (Table 1). Sites 2, 3, 9 and 10 had moderate amounts of trees and high undergrowth, whereas sites 1 and 7 were covered with dense forest. Site 6 had moderate amounts of trees and low undergrowth, whereas site 8 had only a few small trees with high undergrowth. Sites 4 and 5 had mainly *Sphagnum* spp. (sphagnum) and other mosses in the undergrowth, in addition to which site 5 had grass. The other sites had taller undergrowth consisting of mosses (including *Sphagnum* spp.), wild grass species, *Ledum palustre* (marsh tea) and other suffrutices (subshrubs) as dominant plant species. Surface geometry in all sites was mainly uneven with natural hummocks, with the exception of sites six and eight, which did not have hummocks, and site 10, which was located on a steep slope. Peat layers were deepest at open sites 4 and 5 (>300 cm) and lowest at sites 1 (60 cm) and 3 (80 cm) (Table 1). At the other sites the peat layers were 100–200 cm deep.

Composition of cloudberries grown in different habitats

Primary metabolites

The dry weights of analysed cloudberries collected from different sampling sites were commonly 170–180 g kg^{-1} (Table 2). The lowest dry weight was in sampling site 8 (150 g kg^{-1}), where also protein and dietary fibre content calculated on a fresh weight basis was lowest: 10 and 63 g kg^{-1}, respectively (Table 2). This is reasonable since dietary fibre is one of the main components contributing to dry weight. The average content of proteins and dietary fibre (fw) were 16 g ± 2 and 73 g ± 7 g kg^{-1}, respectively. Total fat content varied from 4 to 10 g kg^{-1} (relative standard deviation, RSD = 26%) between sampling sites (Table 2). Variation in the contents of other primary metabolites between sampling sites was not high: RSDs of proteins, dietary fibre and dry weight were 16%, 10% and 4%, respectively, when calculated on a fresh weight basis. Calculated on a dry weight basis the RSD for dietary fibre was significantly lower (6%) and a little lower for proteins (14%).

Malic acid and citric acid

There were significant differences in the amount of malic acid and citric acid analysed from different habitats (Table 2). Furthermore, there was a significant negative correlation in the concentrations

of citric acid and malic acid ($P < 0.01$). When citric acid content was low, it was compensated by higher malic acid concentration, contributing to similar total malic and citric acid contents in each growing site (8–12 g kg^{-1}, fw). Malic acid dominated over citric acid in all sampling sites except in sampling sites 4 and 5. Furthermore, the total malic and citric acid contents were higher in these sites (4 and 5) than in other sites. Sampling sites 4 and 5 were the only sites with open populations and with a thicker peat layer (>300 cm) compared to the other sites. The availability of light was higher in the open sites, which could explain the higher citric acid concentration in those sites. In blackcurrant, citric acid accumulated rapidly along with fruit growth and ripening, whereas malic acid content decreased.[23] The levels of analysed citric acid and malic acid contents in cloudberry were comparable to earlier published data: 4 and 8 g kg^{-1}, respectively.[24]

Total phenolics

The main phenolic compound group in cloudberry is ellagitannins and the content of anthocyanins is comparatively low (0.02 g kg^{-1}, fw).[1] Ellagitannin content in cloudberry has been reported to be ~3 g kg^{-1} (fw) in fruits.[25] According to the literature the total ellagic acid content in dried leaves and fresh fruits of cloudberry is 70 g kg^{-1}[26] and 0.6 g kg^{-1},[2] respectively. In our study the total phenolic content in fruits was 2.2–3 g kg^{-1} (fw) and therefore can be assumed to represent mainly the ellagitannin content. Phenolics content did not have significant habitat-related variation (Table 2): the variance within the habitat was higher than between the habitats. Ellagitannins are not as widely studied compounds as most other plant-derived polyphenols and therefore their accumulation and variation are not well known. These results suggest that biosynthesis of ellagitannins could be quite stable in cloudberry. On the other hand, some compounds such as sugars, vitamin C and proteins may interfere with the analysis of total phenolics by the Folin–Ciocalteu method. However, the results of phenolic assay were not altered when sugar was added to the cloudberry sample (data not shown) and the protein and vitamin C contents in samples were similar. Further studies of climate and environmental effects on cloudberry ellagitannins are needed, however, to confirm these conclusions.

Anthocyanins

Cloudberry anthocyanins consist of cyanidine-3-glucoside, cyanidine-3-rutinoside, cyanidin-3-sophoroside and cyanidine-3-(2-glucosylrutinoside).[1] Anthocyanin results are presented as a sum of all detected anthocyanins calculated as cyanidin-3-glucoside equivalents. Our results on anthocyanins (Tables 2 and 3) were closely related to earlier published data: 5–27 *versus* 18–20 mg kg^{-1}, respectively. The anthocyanin content varied between sampling sites in 2006 (Table 2). Extensive variation in the anthocyanin content has been reported also within and between different bilberry populations in Finland.[27] In addition to environment and genotype,[28] fruit size affects the anthocyanin concentration in the plant.[29] Fruit size was not measured in this study.

Vitamin C

Ascorbic acid concentrations between the habitats of wild cloudberry varied from 0.64 to 1.0 g kg^{-1} (Table 2), which is comparable with the results of an earlier publication (0.50–1.50 g kg^{-1}, fw).[30] However, taking into account the deviation within habitats, the difference between habitats was insignificant.

Table 2. Chemical composition of cloudberries ($n = 3$) grown in 10 different habitats in 2006

	Sampling site									
Analytes	1	2	3	4	5	6	7	8	9	10
Proteins (g kg^{-1})	16	17	17	12	17	16	16	10	19	16
SD	*2*	*4*	*4*	*2*	*2*	*1*	*2*	*2*	*3*	*1*
Dietary fibre (g kg^{-1})	72	69	74	64	71	82	84	63	75	76
SD	*5*	*2*	*3*	*10*	*4*	*6*	*11*	*4*	*3*	*5*
Dry weight (g kg^{-1})	173	168	164	166	168	181	178	154	175	169
SD	*2*	*5*	*1*	*10*	*2*	*3*	*5*	*3*	*2*	*3*
Total fat (g kg^{-1})	6.9	9.9	6.9	6.3	4.8	4.4	5.3	5.4	8.1	8.4
SD	*1.0*	*0.1*	*1.8*	*0.4*	*1.2*	*0.0*	*2.3*	*0.6*	*2.3*	*0.5*
Anthocyanins (mg kg^{-1})	16	12	16	11	8	14	6	6	5	8
SD	*1*	*3*	*2*	*3*	*0*	*1*	*1*	*1*	*1*	*0*
β- Carotene (mg kg^{-1})	0.79	0.68	0.90	1.48	0.90	0.90	1.00	2.19	2.43	0.94
SD	*0.07*	*0.20*	*0.28*	*0.02*	*0.26*	*0.16*	*0.07*	*0.48*	*0.41*	*0.13*
Ascorbic acid (g kg^{-1})	0.69	0.82	0.68	0.94	0.64	0.64	0.87	0.74	0.98	1.01
SD	*0.04*	*0.18*	*0.02*	*0.06*	*0.08*	*0.06*	*0.04*	*0.06*	*0.11*	*0.08*
α- Tocopherol (mg kg^{-1})	64	56	60	38	41	49	60	57	59	48
SD	*16*	*23*	*3*	*13*	*7*	*8*	*9*	*24*	*15*	*20*
Phenolics (total) (g kg^{-1})	2.26	2.51	2.83	2.70	3.00	2.50	2.70	2.38	2.48	2.95
SD	*0.37*	*0.44*	*0.19*	*0.49*	*0.43*	*0.15*	*0.25*	*0.27*	*0.20*	*0.33*
Citric acid (g kg^{-1})	4.62	2.64	5.48	7.52	6.48	2.76	3.93	2.12	3.13	3.85
SD	*0.03*	*0.01*	*0.17*	*1.23*	*0.26*	*0.26*	*0.68*	*0.26*	*0.30*	*0.04*
Malic acid (g kg^{-1})	6.01	7.81	6.51	5.50	6.56	9.31	7.34	7.27	9.48	7.46
SD	*0.47*	*0.41*	*0.79*	*0.12*	*0.51*	*0.57*	*0.98*	*0.61*	*0.11*	*0.75*

Table 3. Annual variation in the chemical composition of wild cloudberries collected in 2006 ($n = 30$) and 2007 ($n = 9$) compared to mean concentrations of analytes in cultivated cloudberries ($n = 2–3$)

	Wild cloudberry				Cultivated cloudberry	
Analyte	2006	SD	2007	SD	Mean	SD
Total phenolics (g kg^{-1})	2.6	0.4	2.4	0.5	1.9	0.3
Anthocyanins (mg kg^{-1})	10	4	27	13	14	9
Ascorbic acid (g kg^{-1})	0.80	0.14	0.79	0.15	0.56	0.1
α- Tocopherol (mg kg^{-1})	53	9	47	10	39	1
Citric acid (g kg^{-1})	4.3	1.8	4.5	1.4	4.6	1.4
Malic acid (g kg^{-1})	7.3	1.3	7.0	1.1	7.2	0.3
Proteins (g kg^{-1})	16	2	14	2	15	4
Dry weight (g kg^{-1})	170	4	150	8	150	18
Total fat (g kg^{-1})	7.0	1.0	6.0	1.7	8.0	1.7

Vitamin E

α- Tocopherol content in different sites was comparatively constant (Table 2). The biosynthesis of α-tocopherol in plants is influenced by several different factors, such as seed maturity at harvest, and geographical and climatic conditions.[31] Furthermore, there has been found to be an interaction between tocopherols and other antioxidants such as ascorbic acid and β-carotene.[31] In this study, correlation of α-tocopherol concentrations with those of other antioxidants was not detected. However, statistical analysis showed that α-tocopherol content was lower ($P < 0.05$) in cloudberries grown in open habitats than in other habitats. The α-tocopherol content determined in this study was slightly higher compared to earlier published data of α-tocopherol content in Finnish cloudberry (~30 mg kg^{-1}, fw).[32]

β-Carotene

β- Carotene is the predominant carotenoid in cloudberries,[33] and therefore other cloudberry carotenoids (α-carotene and lutein) were not analysed. Concentration of β-carotene varied considerably (0.68–2.40 mg kg^{-1}, fw) between sampling sites (Table 2). Highest β-carotene levels were found in berries collected from sites 8 and 9. Interestingly, these sites also gave the highest crop yields. High variation in the carotene content has been reported to be found also in other plants, such as in *Cucumis melo* (muskmelon) fruit, *Brassica oleracea* and *Hippophae rhamnoides* (sea buckthorn).[34–36] The concentration levels of β-carotene found in this study are comparable to the earlier reported β-carotene concentration in Finnish cloudberry: 1.40 mg kg^{-1}, fw,[33] although some losses of β-carotene could occur due to hydrolysis of the sample. Hydrolysis was, however, performed to obtain better HPLC chromatograms with less background noise.

Annual variation

Climate and weather

Climatic and weather conditions affect the maturation rate of cloudberries as well as the biosynthesis of the plant's bioactive compounds. Sunshine duration in July was significantly shorter in 2007 (189 h) than in 2006 (280 h), whereas in June 2006 and 2007 the sunshine durations were comparable (about 300 h). The average June temperature was 2 °C higher in 2006 (14.4 °C) than in 2007 (12.3 °C), and the number of warm days with a maximum temperature over 25 °C was 12 in the summer of 2006 and only six in the summer of 2007. Even more striking, the average maximum daily temperature was 3.5 °C higher in July 2006 than in July 2007. Rainfall in July 2007 was over twofold higher than in 2006. In conclusion, the mean temperature of June and July was lower, rainfall was higher and the sunshine duration was shorter in summer 2007 than in 2006. However, climatic data collected from weather stations are only indicative of the climate in the sampling area, owing to probable local differences in weather conditions.

Crop yield

Compared to summer 2006 the crop yields decreased in 2007 in all analysed sampling sites except in site 4, where the yield increased slightly (Table 1). However, in this sampling site the yield was low also in summer 2006. Sampling sites 9 and 10 had high crop yields in 2007, but unfortunately the total crop was not calculated from those sites in 2006. In site 8, the crop yield was very high in summer 2006 (332 kg ha^{-1}), whereas in summer 2007 a crop was not obtained at all. Sampling site 8 in year 2006 and sites 9 and 10 in 2007 had exceptionally high crop yields (over 200 kg ha^{-1}), which might be explained by a favourable microclimate during flowering time. A comparison of sampling site description and crop yields indicates that yields in the open sites (4 and 5) were more equal in both years, whereas in shaded sites the yields were clearly lower in 2007 than in 2006. Crop yields were comparable to the average crop yield reported for wild cloudberry (20–50 kg ha^{-1}).[9]

Chemical composition

Annual variation in the chemical composition of cloudberry was measured as arithmetic mean values of all sampled cloudberries in two consecutive years (Table 3). The contents of both primary and secondary metabolites were surprisingly equal. For example, the mean results of vitamin C analysed in 2006 and 2007 were very consistent (Table 3), especially when compared to studies of other plants.[37] Average water content of cloudberries was 830 ($\pm$10) g kg^{-1} in 2006 and 850 ($\pm$10) g kg^{-1} in 2007, and there were no significant differences between habitats. Annual deviation was insignificant but might be related to the higher rainfall in 2007.

Significant difference in the annual mean values of cloudberries was found only in the content of anthocyanins. The concentration of anthocyanins was higher in summer 2007 than in summer 2006 ($P < 0.05$) (Table 3). It is well known that low temperature increases the anthocyanin content in plant cells.[38] On the other hand, excess water has been reported to cause several-fold increase in flavonoids.[39] Rainfall in July 2007 was two times higher than in July 2006; therefore water excess could possibly increase the stress of the fruit and induce anthocyanin biosynthesis.

The mean amount of total lipids (7 $\pm$ 2 g kg^{-1}, fw) was very consistent in year-to-year comparison (Table 3). However, there was a difference in the fatty acid composition between the samples collected in years 2006 and 2007. Berries collected in 2007 had a higher proportion of linoleic and α-linolenic acids ($P < 0.05$) and a lower proportion of oleic acid ($P < 0.05$) than berries collected in 2006 (Fig. 1). Previous studies have shown that temperature decrease will decrease the degree of saturation of oils in the seed,[7] and in July 2006 the number of warm days and daily maximum temperatures were markedly higher. However, there are several other parameters affecting the seed oil composition in plants, such as maturation level, water deficiency,[40,41] geographical variation,[7] and interaction of latitude, elevation and temperature.[42] Rainfall in July 2007 was twice as high as in July 2006; therefore water

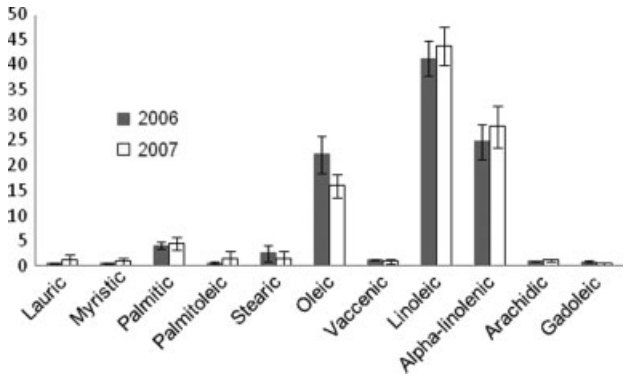

Figure 1. Composition (%) of fatty acids in cloudberries collected in 2006 ($n = 30$) and 2007 ($n = 9$).

excess rather than deficiency and lower temperature could have resulted in the decrease in saturation of fatty acids. Earlier studies on the effect of excess water on oil composition were not found from the literature.

The same profile in malic acid and citric acid concentrations was observed in 2006 and 2007 (results not shown): open sites (4 and 5) differed from other sites (Table 2). This indicates that growing site clearly affected the measured acid concentrations. This is interesting since several factors, especially temperature, affect the amount of acids in the berries. Generally, the amount of total organic acids decreases during the ripening of berries,[43] and warm climate has been found to result in lower acid concentrations in apple and grapes.

Cultivated cloudberry

Protein, water, total fat, malic and citric acid contents in cultivated cloudberry were comparable with wild cloudberries (Table 3). Conversely, the concentrations of total phenolics, α-tocopherol, β-carotene, vitamin C and anthocyanins in cultivated fruits were 75–80% of the concentrations measured from wild cloudberries. This might be due to the isolated growing conditions of cultivated fruits and/or genetic differences between wild and cultivated cloudberries.

CONCLUSIONS

Studied growing sites differed in the level of tree stand and height of undergrowth, which affect the growing conditions of plants, such as availability of sunlight and temperature. Cloudberries grown in habitats having no tree stand and low undergrowth differed from other studied cloudberry populations, whereas differences in cloudberries grown in the areas with low tree stand or dense forest were not found. However, all shaded habitats except one (site 6) had high undergrowth and the results may imply that the undergrowth could contribute notably to shadiness. Two habitats having no tree stand and thicker peat layer of soil were more easily altered by climatic and weather changes. Those two open sites had different concentrations of malic and citric acids than other sites: the total content of acids was higher and citric acid dominated over malic acid in open sites. This can be due to the higher temperature and amount of light in the open sites. Furthermore, the amount of α-tocopherol was lower in the open sites than in shaded sites. Other correlations of the sampling site characteristics on the levels of bioactive compounds were not found in this study. However, the concentrations of anthocyanins and β-carotene varied significantly between habitats, whereas the concentrations of vitamin C were surprisingly consistent even between different years. Extensive variation of β-carotene has been reported in other studies,[34–36] but it has not been possible to explain these observations by any specific characteristics related to habitat. However, this would be an intriguing topic for further studies.

The crop yields in the open sites were more equal in 2006 and 2007 than in other sites. The crop yields in the shaded habitats were higher in 2006 than in 2007 and therefore also the total yields of the studied bioactive compounds (anthocyanins, carotene, tocopherol, total phenolics) were higher in 2006 than in 2007. The concentrations of primary metabolites were consistent and between habitat and annual differences were insignificant, except in the saturation level of fatty acids, whereas secondary metabolites had more variation. The most significant annual differences were found in the saturation level of fatty acids and in the concentrations of anthocyanins. The observed differences were mainly explained by variation in weather conditions in the summers of 2006 and 2007 and they agreed with the previously reported effects of sunlight and temperature on these parameters/metabolite levels.[7,38,39] Significant correlations between primary and secondary metabolites were not found in this study, although the secondary metabolites are biosynthesized from primary metabolites.

Results for cultivated cloudberries suggest that the composition and nutritional value of cultivated cloudberries were comparable to wild cloudberries. The slightly lower amounts of total phenolics (ellagitannins), vitamin C and α-tocopherols detected could be related to the lower stress of plants in the isolated greenhouse environment, resulting in decreased biosynthesis of secondary metabolites protecting plant against stress. However, only two clones of cultivated varieties were analysed, and the genetic variation could likely result in larger variations between different wild populations.

Taken together, this study suggests that the amount of sunlight and temperature levels could be the main factors affecting the crop yield and chemical composition of cloudberry. In addition, the amount of rainfall may have an effect on anthocyanin concentrations. To evaluate more accurately the impact of the environment and climate effects on the composition of cloudberries, a more detailed analysis of sampling sites such as amount of undergrowth, light level and temperature would be needed. Nevertheless, this work provides valuable information on the mean concentrations of nutritional components in cloudberries and their annual and habitat-related variation.

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