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Seasonal and yearly variation of total polyphenols, total anthocyanins and ellagic acid in different clones of cloudberry (*Rubus chamaemorus* L.)

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Summary

Cloudberry (*Rubus chamaemorus* L.) is a wild perennial shrub growing on peatland with a circumpolar distribution. The combined berries have a high polyphenol content comprised primarily of ellagitannins. A few commercial cultivars are available, and pre-breeding trials on clonal material from different geographical origins are in progress. The objective of this study was to investigate how the content of polyphenols of four different cloudberry cultivars were affected by harvesting time and climatic variations during a 3-year-period. Plants were grown outside in plots and berries were harvested when mature. Berries were analyzed for total polyphenols and total anthocyanins by spectrophotometer. Total ellagic acid was identified and quantified using HPLC-MS after hydrolysis of the extracts. Results showed that all measured parameters; total anthocyanins, total polyphenols and ellagic acid are strongly influenced by the genetic background. Although low anthocyanin contents were present in all genotypes, they were highly affected by climatic conditions, being highest at low temperatures. However, the content of ellagic acid was less affected by environmental conditions and showed little response to changing temperatures. In conclusion, ellagitannin content was the most dominating polyphenol group observed in this study and was affected by genetics and is therefore a good breeding criterion for increased health benefit of cloudberry.

Keywords: Climatic effects, Cloudberry, Ellagic acid, Gene × environment interaction, Polyphenols, *Rubus chamaemorus*

Abbreviations

EA ellagic acid, dw dryweight, GA gallic acid, GDD growing degree days, TA total anthocyanins, TP total polyphenols

Introduction

Cloudberry (*Rubus chamaemorus* L.) is a dioecious perennial rhizomatous plant, native to the Subarctic regions. Berries have a distinct aromatic flavor and are highly valued in the Nordic countries (THIEM, 2003). In addition, the market and interest in wild berries is increasing, especially in the East Asian region (PAASSILTA et al., 2009; TURTIAINEN and NUUTINEN, 2012). Although most of the commercial harvesting comes from wild stands, four commercial varieties have been released (RAPP and MARTINUSSEN, 2002). Additionally, there is ongoing testing of new cultivars (ULEBERG et al., 2011). Previous breeding strategies used the number of pistils, flowers and shoots as selection criteria (RAPP, 1989). Berries are known to be rich in a variety of bioactive compounds including phytochemicals related to human health benefits (DUTHIE, 2007). Cloudberrries contain a range of bioactive compounds and are especially rich in polyphenols (MARTINUSSEN et al., 2010; JAAKKOLA et al., 2012). The nutritional quality has become a priority for breeding and biotech-

nological strategies, in order to control or to increase the content of specific health-related compounds in fruits and berries (MAZZONI et al., 2015).

Polyphenols have unique physical, chemical, and biological properties (MANACH, 2004) and polyphenol content has been shown to be highly correlated with antioxidant capacity (DOBSON et al., 2012). Total polyphenols in cloudberry has been observed to be affected by genotype (ULEBERG et al., 2011). Ellagitannin occurrence in common food is limited to a few fruit and nut species, and cloudberry is among the fruits with the highest reported levels (KOPONEN et al., 2007) where they are the dominating polyphenol group (HÄKKINEN, 1999; KOPONEN et al., 2007; KYLLI, 2011). Ellagitannins contain ester bonds, which upon hydrolysis give ellagic acid. The gut derivative of ellagic acid have been linked to inhibition of prostate cancer cell growth (SEERAM et al., 2007) and have vascular health benefits (LARROSA et al., 2010). In cloudberry, only a minor part of the total ellagic acid content is found as free ellagic acid. Most ellagitannins are bound and are released upon acid hydrolysis (KYLII, 2011). It has previously been reported that different growing locations and seasons affected the ellagic acid content in cloudberry (LI et al., 2016). Anthocyanins, another group of polyphenols, are sparse in cloudberrries, strongly correlated with berry color, and have higher levels at low temperatures (MARTINUSSEN et al., 2010).

Climatic conditions of high latitude areas, where cloudberrries grow, differ significantly between years, as well as within season, affecting the phenology and quality of the berries. Light conditions vary greatly during the growing season, with midnight sun until late July and a minimum of 12 h daylight until the end of September (BLISS, 1962). Effects of climatic conditions on various quality parameters, including the content of bioactive compounds, have been evaluated in previous cloudberry studies (MARTINUSSEN et al., 2010; JAAKKOLA et al., 2012; LI et al., 2016), as well as other berries including bilberry (ULEBERG et al., 2012; ZORATTI et al., 2014), blueberry (CONNOR et al., 2002) and raspberry (MAZUR et al., 2014). However, phytochemical content varies greatly among genotypes (ANTONNEN et al., 2005; SCALZO et al., 2005; ULEBERG et al., 2011).

By investigating the genetic and environmental effects and their interactions on the polyphenol levels, we will have a better insight into the regulation and variety consistency. The objective of this paper is to investigate how the content of total phenols, total anthocyanins and levels of ellagic acid of four different clones of cloudberry are affected through season and between years.

Materials and methods

Plant material

In 2012, 2013 and 2014, cloudberrries from four different clones ('Fjordgull', 'Fjellgull', '102' and '306') were harvested at Holt in Tromsø 69°39'N 18°57'E. The different clones were grown in two open outdoor benches in separated squares (2 × 2 m), and every clone was represented at random positions in the bench. The two replicated benches consisted of 6 plots, including the female clones 'Fjordgull',

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'Fjellgull', '102' and '306', while the last two plots in each bench consisted of a mixture of three different male clones ('Apollen', 'Apolto' and 'H510'), to ensure sufficient pollination (see Tab. 1 for the geographical origin of the clones). The clones '102' and '306' were included in this study based on their production performances in earlier experiments (ULEBERG et al., 2011; TROST et al., 2013), in addition to the released cultivars 'Fjellgull' and 'Fjordgull'. The plots with male plants were located between the plots with female plants so every plot with a female clone was next to one of the plots with male plants. Data for flowering and harvest were registered per each 2 × 2 m plot. Individual berries were harvested when ripen, weighed and frozen at storage -80 °C and freeze dried before extraction. Depending on the year, the length of the harvest season was approximately one month (Tab. 2). For each year, an early, middle and late harvest date were selected and berries from these dates were analyzed (Tab. 2). The selection criteria for the selected harvesting dates was the earliest and latest date where all clones had mature berries, the middle time was selected as close as possible to the mean of these two dates.

Experimental conditions

Weather data information was downloaded from the weather station at NIBIO Holt, Tromsø. Collected data included average daily temperature as well as daily precipitation. From these data, monthly and total precipitation and monthly and seasonal mean temperatures

Tab. 1: Geographical origin of the different cloudberry (*Rubus chamaemorus* L.) clones and varieties used in the study.

Clone	Origin (County)	Sex
'102'	58°30'N, Aust-Agder	Female
'306'	66°30'N, Nordland	Female
'Fjordgull'	69°06'N, Andøya, Nordland	Female
'Fjellgull'	70°24'N, Ifjordfjellet, Finnmark	Female
'Apollen'	69°06'N, Andøya, Nordland	Male
'Apolto'	69°06'N, Andøya, Nordland	Male
'H510'	69°06'N, Andøya, Nordland	Male

Tab. 2: Harvesting season during 2012-2014.

	First flower	Last flower	Flower season	First harvest	Last harvest	Harvest period	Early	Middle	Late
2012	15.06	28.06	13	01.08	06.09	36	15.08	20.08	28.08
2013	28.05	13.06	16	08.07	16.08	39	15.07	29.07	06.08
2014	04.06	03.07	29	23.07	14.08	22	30.07	04.08	11.08

Tab. 3: Mean monthly and growing season: air temperature, precipitation and growing degree-days (GDD) in Tromsø during 2012-2014.

Month	Mean temperature (°C)			Precipitation (mm)			Growing degree days (GDD)		
	2012	2013	2014	2012	2013	2014	2012	2013	2014
May	4.5	8.7	5.1	107.4	12.0	42.4	30.2	127.1	41.1
June	9.1	11.7	9.1	35.6	52.1	38.4	115.2	201.4	125.0
July	10.9	11.9	15.2	90.1	130.3	30.0	184.0	214.6	314.9
August	9.9	11.9	11.8	29.1	95.0	71.0	151.7	214.5	209.5
September	7.6	10.2	7.9	83.9	40.0	97.4	84.3	157.0	96.8
Total growing	8.4	10.9	9.8	346.1	329.4	279.2	565.4	914.6	787.3

were extracted. Daily, monthly and aggregated growing day degrees above 5 °C were calculated and used for analysis of weather impacts on cloudberry quality (Tab. 3). The berry samples consisted of the pooled berries sampled from a cultivar a given day, never less than two berries. The samples were freeze-dried for minimum 48 h.

Extraction

Freeze-dried powder (100 mg) from ripe harvested cloudberrys were extracted according to TROŠT et al. (2013) with some modifications in 5 ml 80% methanol and vortexed in 1 min and then centrifuged. The supernatant was decanted, and then added 5 ml 80% methanol and vortexed in 1 min before centrifugation.

Total polyphenols

Total phenolic content was determined by the Folin-Ciocalteu method (SINGELTON and ROSSI, 1965) with minor modifications as described by ULEBERG et al. (2011), in which gallic acid was used as a calibration standard in spectrophotometric measurements. Samples (20 µL) was combined with 1.58 mL of dH₂O and 100 µL Folin-Ciocalteu reagent. The mixture was incubated for 5 min at room temperature before adding 300 µL Na₂CO₃ (saturated sodium) carbonate solution. After a 2 h incubation at room temperature, the absorbance of the mixture was measured at 765 nm in a spectrophotometer (SmartSpec Plus, Bio-Rad, Hercules, USA). A gallic acid (GA) reference absorbance curve was subsequently used to calculate the polyphenol content in mg of GA per gram dry weight (dw) sample.

Hydrolysis

Methanolic extracts (2 mL) were transferred to glass tubes and subject to acid hydrolysis by addition of methanol and 37% HCl to 2.5 M at 85 °C for 6 h. Hydrolyzed extracts (1 mL) were diluted with methanol (2 mL), before UHPLC-injections (5 µL).

Ellagic acid

Ellagic acid was identified and quantified using UHPLC-PDA-HR-MS on a Waters Acquity UPLC (Milford, MA, USA), Waters 2996 UV-detector and Waters LCT Premiere time-of-flight MS with elec-

troscopy ionisation. MassLynx version 4.1 (Waters) was used for instrument control and data processing. The extracts were injected on a Waters Acquity ethylene bridged hybrid (BEH) C18 column (2.1 × 100 mm, 1.7 μm) and ellagic acid was separated using a gradient of 5-30% acetonitrile in water (both containing 0.1% formic acid) over 6 min at a flow rate of 0.5 ml/min. The column was kept at 40 °C, and 5 μL of the extracts was injected. The samples were ionized with negative electrospray (ESI-), and data from 150 to 1500 Da were acquired at a scan time of 0.25 s. Capillary and cone voltages were set to -2.8 kV and -50 V, respectively, whereas source and desolvation temperatures were set to 120 °C and 350 °C, respectively. Nitrogen was used as desolvation gas at 650 L/min. The MS was tuned to a resolution of 10,000 (FWHM) and leucine-enkephalin was infused through the reference probe for internal calibration during data acquisition. For quantification of ellagic acid, UV-absorption at 258.2 nm was used. A calibration curve for ellagic acid was made using a commercial standard (Sigma-Aldrich, Darmstadt, Germany).

Ellagic acid eluted at 3.55 min and was identified as a deprotonated species ([M-H]⁻ m/z 300.9983 calculated 300.9990), a deprotonated dimer ([2M-H]⁻ m/z 603.0041 calculated 603.0053) and a deprotonated trimer ([3M-H]⁻ m/z 905.0095 calculated 905.0116). The formation of deprotonated multimers of ellagic acid in the ion source of the MS is concentration dependent; it is therefore difficult to quantify the compounds using MS data. Ellagic acid absorbs very well in the UV-spectrum, and as no other compounds eluting close to ellagic acid (as detected with UV and ESI-), we quantified ellagic acid by integrating the peak at 258.2 nm (± 0.5 nm).

Total anthocyanins

Total anthocyanins were estimated by a pH differential as described by (GIUSTI and WRÖLSTAD, 2001; LEE et al., 2005). Each sample was diluted in a 0.025 M potassium chloride buffer, pH 1.0 and a 0.4 M sodium acetate buffer, pH 4.5. Of these mixtures the λ_{vis}-max (510 nm) and 700 nm (haze) absorbance was measured. The absorbance of the diluted sample then equals (A_{510 nm} - A_{700 nm}) pH 1.0 - (A_{510 nm} - A_{700 nm}) pH 4.5 and the monomeric anthocyanin pigment in mg/L was calculated using (A × MW × DF × 1000)/(ε × l) with MW=449.2 and ε=26,900 defining the major pigment content as cyanidin-3-glucoside. The anthocyanin content for all samples was expressed in mg cyanidin-3-glucoside per gram dw.

Statistical analyses

Data for total polyphenols, total anthocyanins and ellagic acid were analyzed by the GLM procedure of R (R-project) in a model that included the main effects clone/genotype, harvesting time and year and their interactions. Pearson correlation analysis (Minitab 17) was used to indicate potential impact of climate factors (temperature and precipitation) on berry quality. Furthermore, Pearson correlation analysis was carried out to show relationships between the quality parameters total polyphenols, total anthocyanins and ellagic acid.

Results

Plant development

Weather data for the growing seasons of 2012, 2013 and 2014 are summarized (Tab. 3). Heat unit accumulation evident with Growing degree-days (GDD) is presented (Fig. 1). Results illustrated that the time for early-, middle- and late-harvest varied considerably between the three years. The coldest summer, 2012, had a mean temperature of 8.4 °C and 565.4 GDD. The warmest summer was in 2013, the second year of the experiment, with a mean temperature of 10.9 °C

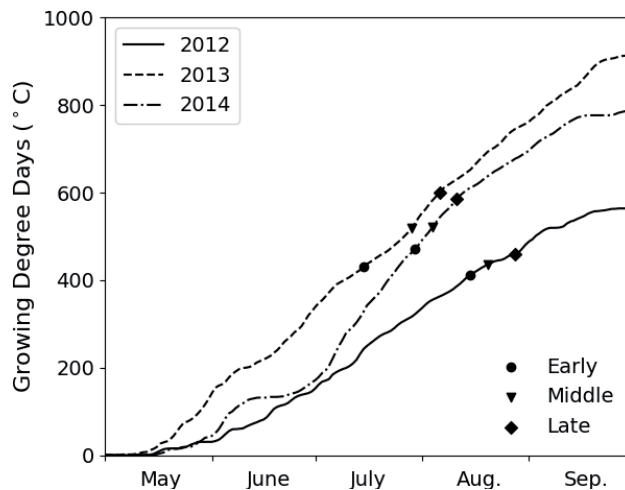


Fig. 1: Development of Growing Degree Days (GDD) through the growing season. Time points for early harvest, middle harvest and late harvest for the harvesting years 2012, 2013 and 2014 are shown.

and 914.6 GDD. In 2014 the mean temperature was 9.8 °C and accumulated GDD was 787.3 (Fig. 1, Tab. 3). Mean temperature during berry ripening, June, July and August, differed from 10.0 °C in 2012 to 11.8 °C in 2013 and 12.0 °C in 2014. Thus, the highest temperature during berry ripening occurred in 2014 while 2013 was the overall warmest summer. Time for first flowering, first and last harvesting and time points for early, middle and late harvest are presented (Tab. 2).

Berries were harvested 2-3 times per week during the harvesting seasons. As '306' and 'Fjellgull' are earlier than 'Fjordgull' and particularly '102', this implies that '306' and 'Fjellgull' produced ripe berries before early harvest and '102' produced ripe berries after late harvest time point. Generally, late-season harvesting involves lower light intensity, shorter day length and lower temperature; compared with early and mid-season especially in 2013 (Fig. 2, Tab. 2).

Total polyphenols

Total polyphenol content was significantly affected by genotype, year and harvest time (Tab. 4 and 5). In the statistical model, 17% of the variation of total polyphenols were assigned to genotype (Tab. 4). 'Fjellgull' (22.99 mg/GA/g dw) was the cultivar with the highest level of total polyphenols while '102' (21.67 mg/GA/g dw) had the second highest level (Fig. 2, Tab. 5). Berries from cultivar '306' (19.18 mg/GA/g dw) had the lowest levels of total polyphenols all years, but not statistically different from 'Fjordgull'. Additionally, approximately 17% of the variation was associated with yearly variation in weather conditions (Tab. 4). Year 2012, with the lowest mean temperature, had significantly higher levels of total polyphenols than 2013 and 2014 (Fig. 1, Tab. 5). All cultivars showed a reduction in levels of total phenols during the three years period of experiment, but the reduction from 2013 to 2014 was not significant (Fig. 2). The yield and mean berry sizes of the plots were higher in 2013 compared to 2012 and 2014 (data not shown). The berries that matured early had the highest levels of total polyphenols (Fig. 2, Tab. 5) and berries from early harvest had significant higher levels of polyphenols than berries harvested late in the harvesting season. Berries harvested in the mid-season were not statistically different from early and late harvested berries. There were no significant interactions between the main effects for total polyphenols. The contents of total polyphenols were negatively correlated with temperature (Tab. 6).

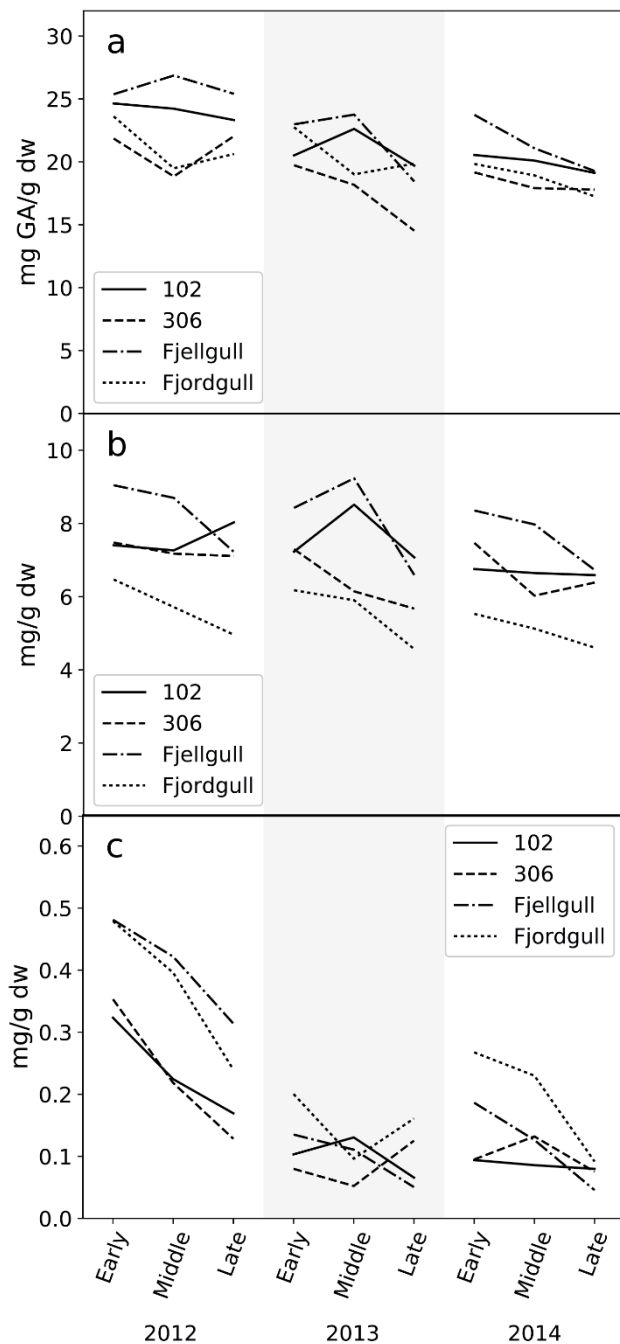


Fig. 2: Yearly (from 2012–2014) and harvesting time variation in the content of a) total anthocyanins (TA) mg/g dry weight (dw) sample; b) total polyphenols (TP) expressed as mg of GA (gallic acid) equivalents per gram dry weight (dw) of sample and c) ellagic acid (EA) mg per gram dw of sample in cloudberry (*Rubus chamaemorus* L.) clones '102', '306' and the commercial varieties 'Fjellgull' and 'Fjordgull'. The growth harvesting season are divided into 'early', 'middle' and 'late'.

Ellagic acid

Ellagic acid content after hydrolysis varied from 8.05 mg/g dw in 'Fjellgull' to 5.44 mg/g dw in 'Fjordgull' (Fig. 2, Tab. 4). Ellagic acid content was significantly affected by genotype (32% of the variation) (Tab. 5). 'Fjordgull', which contained the highest levels of total anthocyanins, had the lowest levels of ellagic acid at all harvest points for all three years. There were also significant effects of year and har-

Tab. 4: Effects of genotype, year and harvesting time and their interactions on the quality parameters total anthocyanin (TA), total polyphenol (TP) and ellagic acid (EA) determined in cloudberry berries. The levels are expressed as mg per g dry weight (dw) of sample, total polyphenols were expressed as mg of GA (Gallic acid) equivalents per gram dw of sample.

	Clone	TP mg/GA/g dw	EA mg/g dw	TA mg/g dw
Genotype	'102'	21.67	7.28	0.14
	'306'	19.18	6.83	0.14
	'Fjellgull'	22.99	8.05	0.21
	'Fjordgull'	20.01	5.44	0.24
Year	2012	23.19	7.22	0.32
	2013	20.51	7.11	0.10
	2014	19.57	6.54	0.12
Harvest time	Early	22.12	7.41	0.24
	Middle	20.97	7.03	0.19
	Late	20.09	6.35	0.13

vest time and interactions between harvesting time and year as well as between cultivar and harvesting time (Tab. 4). Ellagic acid levels were significantly higher in berries collected in early and middle season compared to late season (Fig. 2, Tab. 4). The cultivars varied in response to ellagic acid content throughout the harvest season during the study, indicating that other factors than harvest time may have influenced the seasonal variations. The levels were significantly higher in the coolest year, 2012, than in the warmest year, 2014, while 2013 was not statistically different from the other years (Fig. 2, Tab. 4). Still, ellagic acid content showed a small negative correlation with temperature (Tab. 5).

Total anthocyanins

The highest content of total anthocyanins was observed in berries harvested in 2012, the year with lowest GDD. Additionally, berries harvested early in the 2012 season had the highest level of total anthocyanins. As much as 45% of the variation was linked to the year and harvest time (Tab. 5). The contents of anthocyanins were clearly negative correlated with temperature (Tab. 6). Levels of anthocyanins in 2012 were 70% higher than 2013 and 63% higher than in 2014 (Fig. 2, Tab. 4). All clones had the highest levels in 2012, but the lowest levels for '102' were measured in 2014 while the other clones had the lowest amounts of total anthocyanins in 2013 (Fig. 2, Tab. 4). Additionally, there was an interaction between year and harvesting time (Tab. 5). In 2013 the anthocyanin levels decreased through the harvesting season from early to middle and late. In 2013, levels were highest in the early harvest and in alignment middle and late harvest, while in 2014 the levels were high and leveled in early and middle harvest and lowest in late harvest. Thus, the middle harvest levels differed while the high contents in early season harvesting and low contents in late harvesting were evident all three years. In addition to the environmental conditions, there was also a significant effect of genotype and an interaction between genotype and year. 'Fjordgull' and 'Fjellgull' had significant higher contents than '102' and '306'.

Discussion

Influence of genotype

The present study confirms that genotype significantly influences the content of total polyphenols, ellagitannins and total anthocyanins in cloudberry. This is in alignment with previous studies on

Tab. 5: Percentage of total variation (Sum of squares) ascribed to clone, harvesting time, and year and their interactions on the content of total polyphenols, ellagic acid and total anthocyanins in cloudberry grown during three different years.

	Total polyphenol		Total anthocyanin		Ellagic acid	
	% of variations	p-value	% of variations	p-value	% of variations	p-value
Genotype (G)	17.1	8.3×10^{-11}	10.2	6.0×10^{-9}	32.3	2.0×10^{-16}
Year (Y)	17.2	2.0×10^{-12}	34.4	2.0×10^{-16}	3.4	1.1×10^{-3}
Harvest time (H)	5.7	1.2×10^{-4}	10.1	1.7×10^{-9}	5.7	1.3×10^{-4}
G × Y	1.3	0.24	3.2	3.2×10^{-3}	0.12	0.94
G × H	3.6	0.07	1.6	0.31	5.2	0.01
Y × H	0.6	0.35	1.9	0.01	0.0	0.93

Tab. 6: Correlations (Pearson's) between total anthocyanins, total phenols, ellagic acid, mean temperature and total precipitation during the ripening period, and berry weight.

	TA	TP	EA	Temp	Precipitation
TP	0.548				
EA	0.131	0.629			
Temp	-0.502	-0.434	-0.214		
Precipitation	-0.24	-0.003	0.161	0.062	
Berry weight	0.158	0.363	0.284	-0.141	0.301

20 different cloudberry clones, including the four tested here, which revealed large variation on total polyphenol levels and total anthocyanins (ULEBERG et al., 2011). In raspberry (*Rubus idaeus*) studies, the content of total phenolics, ellagic acid, and total anthocyanins also varied greatly between cultivars (ANTTONEN et al., 2005; BOBINAITE et al., 2012; MAZUR et al., 2014). Additionally, our results showed that genotype had greatest influence on total polyphenols and ellagic acid and less for anthocyanin content, which was more affected by environment. This is in line with findings in black currant (*Ribes nigrum*) (VAGIRI et al., 2013) where the large variation in total anthocyanins was mainly affected by yearly variations and less by genotype while polyphenolics to be more affected by genotype. Likewise, the polyphenol content in blackberry was highly affected by cultivar and less by yearly variations (MILOŠEVIĆ et al., 2012). On the contrary, another study on raspberry (MAZUR et al., 2014), found small yearly variations on the anthocyanin content, however, the temperature difference between the two selected years was smaller than the yearly variations in our study. Cultivar effect on anthocyanins have also been detected in other berry species such as cranberry (*Vaccinium oxycoccos*) (BOROWSKA et al., 2009) and lowbush blueberry (*Vaccinium angustifolium*) (KALT et al., 1996). These results indicate that total polyphenol content is most affected by genotype while total anthocyanins are more controlled by environmental conditions.

Ellagitannins are the major phenolic constituent in cloudberry (KÄHKÖNEN et al., 2001; MÄÄTTÄ-RIIHINEN et al., 2004; KYLLI, 2011) and given this would be an interesting qualitative trait in future plant breeding activities. In this study, there were significant effects of year and harvesting time during the season, yet genotype was the factor with the greatest impact on ellagic acid content. In a Finnish study, ellagic acid content in cloudberry was found to be affected by the location, which could be due both to genetic and environmental differences (HÄKKINEN et al., 1999), while LI et al. (2016) did not find a location effect in four different locations in Atlantic Canada. Our results are consistent with those of ANTTONEN and KARJALAINEN (2005) and BOBINAITE et al. (2012) that described large variations in

ellagic acid content between cultivars of raspberries and ATKINSON et al. (2006) that reported a strong seasonal effect on ellagic acid content in strawberry. MAZUR et al. (2014) reported that genotype was more important for ellagitannin content in raspberry than seasonal variation. Additionally, genotype effect was found to be smaller in red raspberry as compared to blackberry (VRHOVSEK et al., 2008), indicating differences between species. The correlation between total phenolics and ellagic acid suggest that selection for a high total phenolic content will be a good indirect selection criterion for ellagic acid content. In the present study, the differences between the genotypes were less profound than findings from raspberries but still significant for all investigated compounds. Clones '102' and '306' were included in this study based on their performances in earlier experiments (ULEBERG et al., 2011; TROST et al., 2013), in addition to the released cultivars 'Fjellgull' and 'Fjordgull'. Thus, the studied clones are already selected and we expect less variation here than we would in a study with wild material.

Influence of climatic conditions

Total polyphenolic and ellagic acid content were significantly affected by year and harvest time. LI et al. (2016) also found a yearly effect on the total polyphenol content as well as the ellagic acid content in cloudberry collected at four different locations during a two-year period in Atlantic Canada. Total phenols showed a negative correlation with GDD and the highest polyphenol content was observed in the year with the lowest growing temperature, while the levels were reduced the warmest summers (2013 and 2014). Conversely, ellagic acid content was less correlated to temperature, but behaved similar as total phenols through the harvesting season and between years. On the contrary, REMBERG et al. (2010) reported significant higher levels of the analyzed two prevalent ellagitannins, lambertianin C and sanguin HG, in raspberries at increasing temperature. Our results indicate that although ellagitannins are dominating, there are other polyphenols more affected by temperature. In cloudberry, a previous study suggests that in addition to temperature, sunlight is the main factor affecting the chemical composition of cloudberry (JAAKKOLA et al., 2012).

Total anthocyanin levels in the four studied cloudberry clones were strongly affected by year, time of harvesting and climatic conditions as described previously by TRÔST et al. (2013), ULEBERG et al. (2011) and MARTINUSSEN et al. (2010). In the present study, anthocyanin levels were negatively correlated with GDD. The anthocyanin levels were highest in 2012, the coldest summer, while the lowest contents were found in 2013, which had the warmest summer. Findings in the present study thus confirm the results described by MARTINUSSEN et al. (2010) that found enhanced levels of anthocyanins in the cultivar 'Fjellgull' at 9 °C or 12 °C compared to 15 °C and 18 °C. JAAKKOLA et al. (2012) found similar results in cloudberry from different locations over several years; in a cold and rainy summer, the

content of anthocyanins were significantly higher than in a warm and dry summer. Higher anthocyanin levels at low temperatures have been described in various species (CHALKER-SCOTT, 1999), and at high temperatures for others berry species (ULEBERG et al., 2012; JOSUTTIS et al., 2012). The regulation of anthocyanins is found to be a stress response in plants. Depending on the stress conditions production of specific anthocyanins is induced (KOVINICH et al., 2014). In addition to the year and temperature effects, there were also interactions; year by genotype and year by harvesting time (Tab. 3). The sensitivity of anthocyanin accumulation at temperature changes related to different genotypes have also been found to differ significantly in flowers of *Plantago lanceolata* from different genotypes (STILES et al., 2007). In our study, anthocyanin levels decreased from early to late harvesting time. The accumulated GDD (Tab. 2) could explain the reduction in anthocyanin contents that was observed from early to late harvest. In a recent study BARNUUD et al. (2014), using climate-variable-based empirical models to predict quality changes of different cultivars of grapes (*Vitis vinifera*), found that in a warmer climate berry anthocyanins would decline. Dark inhibition of the anthocyanin biosynthesis has been described by ZHANG et al. (2015) who found that light was necessary for anthocyanin production in *Begonia* leaves under low temperature.

Effect of other environmental factors

The variation between years was significant and all genotypes showed a total polyphenolic reduction during the three years period of experiment, even if the reduction from 2013 to 2014 was not significant. The explanation could be weather conditions, as discussed above or other factors as age of the plantlets, total yield or nutritional status in the plots. Cloudberry spread vegetatively so the restricted area of each plot gradually gets more and more populated, increasing the competition for nutrients. Nevertheless, the yields in the plots were highest in 2013 (2421 g) while similar in 2012 and 2014 (1807 g and 1648 g, respectively). Berry yields in cloudberry are highly correlated to the weather conditions during pollination, since insect pollination is necessary for fruit development in the dioecious cloudberry (RAPP and MARTINUSSEN, 2002). The high mean temperature in June 2013 (11.7 °C) compared to 2012 and 2014 (9.1 °C) indicate that the conditions for pollination was better this year, which could explain the observed yield differences. The mean berry weights were similar in 2012 (1.69 g) and 2013 (1.89 g) while significantly lower in 2014 (1.18 g). Even if the berry size was reduced, good berry yields were obtained in 2014, indicating that nutrients were available for fruit growth and development.

Conclusions

To investigate the breeding potential of cloudberry clones with high content of health beneficial compounds total anthocyanins, total polyphenols and ellagic acid content were analyzed in berries harvested from four clones of cloudberry from a three years period. The results illustrated that although climatic effects have strong impact on total anthocyanins, the levels are highly genotype dependent. Although genotype affects the polyphenolic levels in cloudberry, final content was also dependent upon environmental parameters. In addition, results indicated that the different clones respond differently to environmental factors. The presented study expands our knowledge about variation in the content of polyphenols in cloudberry clones and how they are affected by environmental conditions. This knowledge may help for the selection and validation of the clones with the highest health benefit. The strong correlation between total polyphenols and ellagic acid content of cloudberry suggest that selection for a high total phenolic content will be a good indirect selection criterion for ellagic acid content.

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