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# Assessing the Influence of Genotype and Temperature on Polyphenol Composition in Cloudberry (*Rubus chamaemorus* L.) Using a Novel Mass Spectrometric Method

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Supporting Information

**ABSTRACT:** A high-throughput abbreviated liquid chromatography mass spectrometric (ACMS) method was used to assess the relative influence of genotype and temperature on polyphenol composition in cloudberries. Principal component analysis (PCA) plots of the collated ACMS data showed a separation between crosses based on their female parents (Nyby or Fjellgull). Crosses with Nyby as the female parent had higher relative levels of masses assignable to certain ellagitannin derivatives. Crosses with Fjellgull had higher levels of distinctive masses assignable to quercetin derivatives (including a hydroxy-3-methylglutaroyl hexose derivative not previously identified in cloudberry) and anthocyanin derivatives. There was also a separation between samples grown at lower and higher temperatures, which was driven by *m*/*z* signals associated with ellagitannins and notably a major component, sanguiin H-6. Therefore, abbreviated MS techniques can discern genetic and/or environmental influences in polyphenol composition and can quickly assess quality in breeding programmes or in response to environmental changes.

**KEYWORDS:** Cloudberry, metabolomics, ACMS, LCMS, polyphenol, anthocyanin, ellagitannin, quality, health

## INTRODUCTION

Cloudberry (*Rubus chamaemorus* L.) is a unisexual circumpolar species, which is collected from the wild for domestic and commercial use in Fennoscandia. It has a long history of use[1] eaten fresh or frozen in desserts and in a variety of processed formats, such as jams, wines, liqueur, sweets and syrups, and comfitures. The unique combination of color, taste, and aroma has also driven several efforts to domesticate cloudberry.[2] These ongoing efforts to maximize, exploit, and grow cloudberry, while maintaining quality, have been concentrated in the northern latitudes, principally Canada,[3] Norway, and Finland, and have included studies focusing on cultivation,[4] breeding,[2,5] and biodiversity.[6]

Besides its organoleptic aspects, cloudberry is a good source of selected nutrients and human health beneficial compounds. For example, it is a rich source of vitamin C (~0.8 mg/g fresh weight[7]) particularly in comparison to its more common sister species, raspberry, *Rubus ideaus* L. (~0.2 mg/g fresh weight[7]). Furthermore, it is a good source of micro- and macronutrients such as Fe, Cu, Mn, Zn, Mg, K, Ca, and P.[8] More recently, however, it has been the polyphenol complement and diversity that has attracted the majority of research interest, particularly ellagitannins (ETs) and ellagic acid.[9] Polyphenols have been shown to exhibit potential benefits in model, ex vivo, and clinical interventions targeted at several areas of human health such as cancer progression,[10,11] antibacterial properties (e.g., ref 12), obesity and calorific intake,[13] and aging.[14] Although the precise mechanisms of the biological activities are still unclear, it is clear that the in vitro antioxidative effects of cloudberry are mainly due to ETs,[9] and these components have been implicated in many of the above potential health benefits. In addition, this property has been exploited in the food sector by using cloudberry extracts as natural antioxidants to retard the development of meat rancidity.[15] Clearly, any future strategies to expand the use of cloudberry, particularly in the high return, human health, and functional food/ingredient/nutraceutical sector, will need to pay close attention to any factors that impact deleteriously on the polyphenol complement.

The antioxidative activities and the chemical composition of berries are affected both by genotype and environment (e.g. refs 16−18). Kähkönen et al.[9] reported significant differences in the contents of anthocyanins, flavonols, and ETs in cloudberries collected at various locations in Finland, but genetic and environmental influences were confounded and could not be separated.

In a recent study with a female clone and a hermaphroditic clone of cloudberry, we have shown that low temperature, 9−12 °C, was favorable for berry size.[19] Furthermore, at the level of polyphenol classes, there was a trend to enhanced levels of total anthocyanins with decreasing temperature, but temperature

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did not have a consistent significant effect on the level of total phenols. Differences in anthocyanin levels between the female genotypes were highly significant, but no consistent effect of pollen parent was detected.[19]

In this study, we have undertaken compositional analysis using a novel high-throughput liquid chromatography−mass spectrometry (LC-MS) technique to determine the effects of temperature and genotype on changes in polyphenol composition and content, a crucial and emergent quality trait in cloudberry.

## MATERIALS AND METHODS

**Plant Material and Growth Conditions.** Clonally propagated plants of three cloudberry cultivars (*R. chamaemorus* L.) were used, the female cultivar Fjellgull and the male cultivar Apollen, from the Norwegian Institute for Agricultural and Environmental Research (Bioforsk Nord Holt), and the hermaphrodite cultivar Nyby, from Laukaa Research Station, Finnish Agricultural Research Organization. Plants were grown in the phytotron of the University of Tromsø (69° 39′ N lat.) for one season at 18 °C in natural 24 h photoperiod. As a preparation for cold treatment, plants were exposed for 6 weeks to a 12 h photoperiod at 6 °C, after which they were stored at 4 °C for 6 weeks. The experiment was started October 10, 2007. All of the plants had abundant and normal flowering. Plants were grown under controlled temperature treatments (9, 12, 15, and 18 °C; variation ±0.5 °C) in 24 h photoperiod. Daylight was supplemented with cool white fluorescent tubes giving the minimum of 150 $\mu$mol cm$^{-2}$ s$^{-1}$ PAR. The humidity was regulated to ensure a 0.5 MPa water vapor deficit. Plants were watered daily and fertilized with a complete nutrient solution once a week. The experiment was started October 10, 2007 (for further details, see ref 19).

**Treatments.** The treatments were as follows. For Fjellgull: (1) control, no pollination treatment; (2) pollination with pollen from Apollen (F × A); (3) pollination with pollen from Nyby (F × N); and (4) one application of 5 $\mu$g of gibberellic acid (GA) dissolved in ethanol and given as a 10 $\mu$L microdrop to the carpels (F × GA). For pollination, carpels were rubbed with stamens from two or three flowers of pollen cultivar. For Nyby: (5) control, no assisted pollination (N × self); (6) assisted self-pollination (N × N), pollen was applied to flowers with a brush, repeated over 2 days; and (7) application of 5 $\mu$g of GA as above (N × GA).

Most of these treatments were carried out at all four temperature conditions, and the number of treated flowers varied from 11 to 36. The control samples were only set up at 9 °C.

Flowers for the treatments were tagged randomly, and when possible, all of the treatments were applied daily. Therefore, one single plant could have several treatments. Flowers were not isolated, but none of the control, unpollinated flowers of Fjellgull, or the emasculated and nonpollinated flowers of Nyby developed any fruit, indicating that no unintended pollination took place. Fruit developed in the selfed Nyby control. No indications of any carry-over effects between flowers on the same plant were observed. For example, in plants where several of the flowers were treated with GA, untreated and unpollinated flowers did not show any development.

**Harvest and Extraction.** Ripe berries (which were soft and easily detached from the sepals/pedicle) were harvested daily. For each treatment and temperature combination (apart from the F × GA treatment at 18 °C, which did not provide enough material), six intact berries were obtained, frozen, and stored individually at −80 °C prior to extraction, and three replicate extractions were made using two berries. These berries were weighed and homogenized in a hand-held glass tissue homogenizer with PTFE pestle (product FB56705, Fisher Scientific, Loughborough, United Kingdom) in acetonitrile containing 1% acetic acid in a 1:1 (fruit weight: solvent volume) ratio. Therefore, each treatment had three biological replicates, and the average sample size was 3.72 g. Seeds, covered by hard endocarp, were not crushed during the extraction.

**LC-MS Analysis.** Samples were analyzed on a LCQ-DECA system, comprising Surveyor autosampler, pump, and photo diode array detector (PDAD) and a Thermo mass spectrometer iontrap controlled by the Xcalibur software (Thermo Scientific Ltd., United Kingdom) as described previously[20] with minor modifications. The PDA detector scanned three discrete channels at 280, 365, and 520 nm. Solvent A was 0.1% formic acid in ultra pure water, and solvent B was 0.1% formic acid in acetonitrile.

The high-throughput abbreviated liquid chromatography mass spectrometric (ACMS) method used a short C18 column (Synergi MAX-RP 100A Mercury (20 mm × 2 mm, 2.5 $\mu$m) (Phenomenex Ltd., Macclesfield, United Kingdom) at a flow rate of 200 $\mu$L/min. The gradient was as follows: $t$ = 0, 98:2 solvent A:B; $t$ = 1.5 min, 98:2 solvent A:B; $t$ = 3, 50:50 solvent A:B; and $t$ = 5 min, 35:65 A:B; and $t$ = 6.5 min 5:95 A:B; $t$ = 7, 98:2 A:B; and $t$ = 10, 98:2 A:B. The duration of each run was 10 min, and the injection volume was 10 $\mu$L.

The LCQ-Deca LC-MS was fitted with an electrospray ionization (ESI) interface, and each sample was analyzed in positive and negative ion mode. The MS was tuned against cyanidin-3-*O*-glucoside for positive mode and quercetin-3-*O*-glucoside (Extrasynthese, France) for negative mode. There were two scan events: full scan analysis followed by data-dependent MS/MS of the most intense ions using collision energies of 45% source voltage (set at 3 kV). The capillary temperature was set at 250 °C, with sheath gas at 60 psi and auxiliary gas at 15 psi. The triplicate extracts from each treatment/temperature were run as triplicate technical replicates in a statistically validated randomized fashion. Standards (cyanidin-3-*O*-glucoside for positive mode and quercetin-3-*O*-glucoside for negative mode) and blanks were run every 10 samples through the sequence both to ensure that sample-to-sample carry over was minimal and to monitor MS sensitivity. The limit of detection was <5 ng/injection for both standards, and the method was linear in response from 5 to 500 ng. The accuracy of the method was defined by the accuracy of the injector system and was assessed to be <2% of mean using the PDA peak areas of the in-run standards. All data shown are from the negative mode runs, as the positive mode spectra were dominated by signals from the anthocyanins, even though these were present at low levels.

The composition of selected samples was also analyzed using a standard longer LCMS method, which has been reported previously.[13] Briefly, samples were applied to a C18 column (Synergi Hydro C18 with polar end-capping, 4.6 mm × 150 mm, Phenomenex Ltd., UK) and eluted using a linear gradient of 5% acetonitrile (0.1% formic acid) to 40% acetonitrile (0.1% formic acid) over 30 min at a rate of 400 $\mu$L/min. This method used MS conditions analogous to those described above.

**LTQ Orbitrap LCMS.** In addition to the above standard LC-MS analysis, a second system, the LTQ Orbitrap XL LC-MS, fitted with an Acella 600 Pump, Acella PDA, and Acella autosampler, was used for accurate mass spectrometry to assist structural characterization and identification. The Orbitrap MS analyzed selected samples in positive and negative ion modes. There were two scan events: FTMS full scan (80−2000) analysis followed by data-dependent MS/MS of the most intense ions using normalized collision energy of 35%. The Orbitrap MS provides the facility to measure accurate mass data to four decimal places. The capillary temperature was set at 300 °C, with sheath gas at 40 psi and auxiliary gas at 5 psi. Samples (10 $\mu$L) were applied to a C18 column (Synergi Hydro C18 with polar end-capping, 2 mm × 150 mm, Phenomenex Ltd.) and eluted using a linear gradient of 5% acetonitrile (0.1% formic acid) to 40% acetonitrile (0.1% formic acid) over 30 min at a rate of 200 $\mu$L/min. Exact mass data were used to assign potential structural formulas using the resident Xcalibur QualBrowser software (Thermo, United Kingdom).

**Statistical Analyses.** Full scale MS spectra ($m/z$ 80−2000) were acquired across the separation zone ($t$ = 1.5−7.0 min) for each sample using the ACMS method. These spectra were exported into Excel

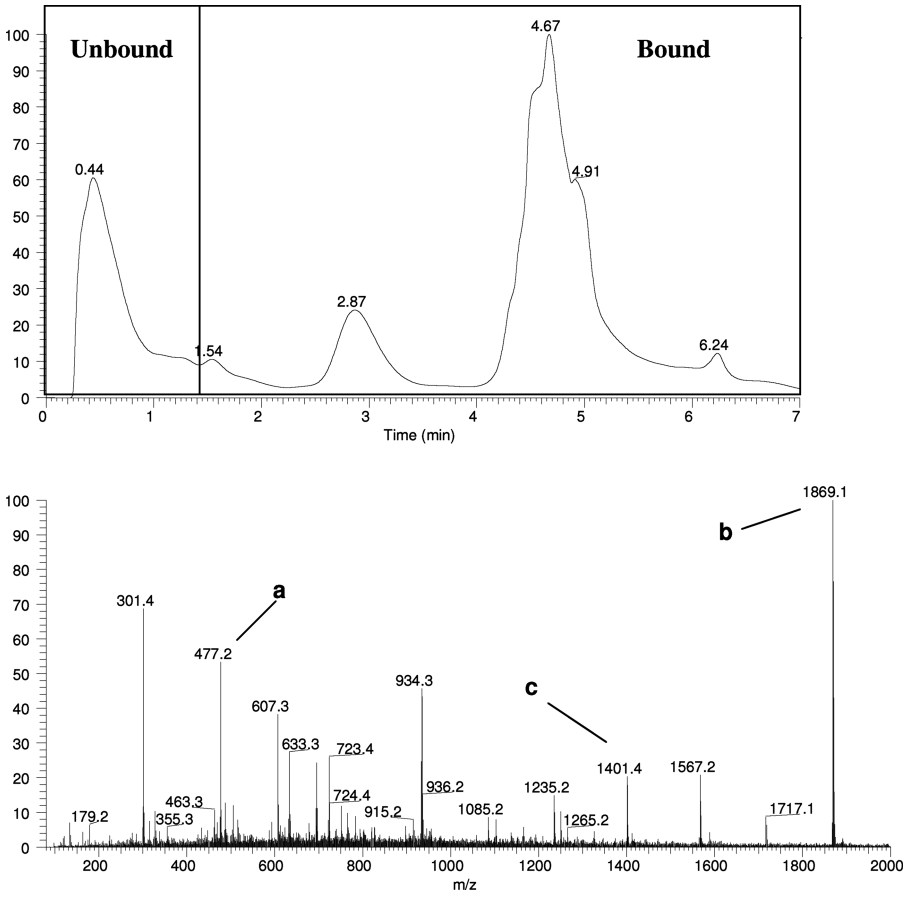


**Figure 1.** Derivation of mass spectra using the ACMS technique. The top panel shows the UV trace at 280 nm of a sample obtained using the ACMS technique. The unbound and bound materials are illustrated. The bottom panel shows the MS spectra obtained from the bound material (RT 1.5−7.0 min). Signals denoted by a−c are discussed in the text. The figures in the top right corners represent the full scale deflections.

spreadsheets using the "export spectra to clipboard" (exact mass) option in the Xcalibur 2.07 software (Thermo Fisher Scientific). Only results from the negative mode spectra were used in this study.

Using techniques developed for direct injection mass spectrometry,[20] the spectral data for each sample were normalized against the total ion response and then collated and subjected to multivariate analysis techniques in SIMCA-P+ (version 12.0.1.0, UMETRICS AB). Principal components analysis (PCA) was performed in the first instance, and the MS data were subsequently subjected to a supervised analysis using hypotheses suggested by the PCA plots. Orthogonal partial least squares (OPLS) analyses were performed in SIMCA-P+ where X (input data from samples) was used to construct a model of Y (containing qualitative values, such as parentage or temperature) that maximized the covariance between the X variables and the Y variables.

## RESULTS AND DISCUSSION

As part of ongoing investigations[17−19] into the potential impact of abiotic stressors, such as environment and climate change, on soft fruit quality, we have focused on the relative influence that genotype (parentage) or temperature (environment) have on polyphenol content and diversity in cloudberry. To achieve this, we employed a high-throughput ACMS method. This method is a refinement of our previous approach to polyphenol analysis using direct infusion mass spectrometric (DIMS) analysis[20] but included a rapid chromatographic separation on a short reversed phase high-performance liquid chromatography (HPLC) column. This has a number of advantages: First, although the method takes longer (10 vs 1 min for DIMS), the multiple blanks used in DIMS between samples are not required. In addition, the method effectively carries out solid phase extraction on the column thereby reducing preanalysis steps. The method was reproducible producing near identical chromatographs for replicate samples many hours apart in the randomized injection order (e.g., reproducibility of bound peak area was >95%). Second, as the various metabolites are partly separated, they are presented to the iontrap MS detector in discrete packages that are more sensitively detected than in the all-at-once DIMS method.[20] This also reduces ion suppression effects in the MS spectra that hindered the utility and uptake of DIMS methods.[20,21] Lastly, although the metabolites were rarely separated into discrete unique peaks, their elution and abundance can be tracked in the chromatographs by their $m/z$ or PDA properties (see the Supporting Information, Figures S1 and S2). This can be particularly useful to double-check individual components, which are identified as being significantly different between treatments. For example, the anthocyanin content of the samples was assessed by measuring the total peak areas of bound material in traces at 520 nm for each sample. The trends in anthocyanin content captured by this method correlated with total anthocyanin content, as measured previously,[19] in a linear fashion ($R^2$ value = 0.84; Supporting Information, Figure S3).

The phenolic composition of cloudberry is dominated by ETs and flavonols with varying but low amounts of anthocyanins.[9,22]

The MS spectra are effectively similar to those generated previously,[20] and the main $m/z$ signals can be assigned to known components such as quercetin-3-*O*-glucuronide (Figure 1a; $m/z$ = 477), sanguiin H-6 (Figure 1b; $m/z$ = 1869), and lambertinian C (Figure 1c; $m/z$ = 1401). Unlike DIMS, ACMS allows for confirmation of some assignments using MS/MS data (e.g., MS/MS for quercetin-3-*O*-glucuronide at 301). We also confirmed these putative MS assignments using full length LC-MS analysis of certain samples (results not shown).

Principal component analysis (PCA) plots derived from the collated mass spectral data showed a clear trend that separated crosses based on the female partner (Figure 2A) on the first component (PC[1] vs PC[2]). Crosses with Nyby as the female partner broadly separated to the left with those with Fjellgull as the female partner to the right in this PCA figure. To assist interpretation of the derivation of this segregation, the corresponding PCA loadings plot with the masses identified is shown in Figure 2B. In all cases, the PCA analysis only explained a small portion of the variation in the sample sets, from approximately 8−10%. This highlights that the ACMS spectra from the cloudberry samples contain many similar signals, and as a result, none of the PCA models reached the threshold value to be deemed robust ($Q^2$ > 0.5). However, it is interesting that differences within the variable element of ∼10% were noted. To support hypotheses gained from PCA analyses, the data were classified on the basis of observation (i.e., female parent) and reanalyzed using OPLS, which clarifies segregation by dividing the systematic variation between components into related and unrelated (or orthogonal) and thereby generates models that are "cleaner" and easier to interpret.[23] In all cases, the derived OPLS models were robust ($Q^2$ > 0.5). In this first case, the derived OPLS model maximized separation between the two female parents on the first and second components, and the loading plots indicate that the same masses as identified by the PCA analysis were causing this separation (see the Supporting Information, Figure S4A,B).

The crosses with Nyby as female parent had higher relative levels of a range of masses assignable to ET and ellagic acid derivatives ($m/z$ 1717, 935, 933, 633, 315, 301, etc. and associated signals from their natural abundance isotopes). However, crosses based on Fjellgull as the female parent have higher levels of distinctive masses at 477, 505, and 607 (Figure 2B). The differences in apparent abundance of these masses were confirmed by determining their $m/z$ peak areas within the ACMS traces (results not shown).

The separation based on more abundant ET-derived masses for the Nyby female crosses may be influenced by their lower anthocyanin content.[19] However, it is intriguing that the major ET mass in the spectra $m/z$ = 1869 (attributable to sanguiin H-6) is not a driving feature. Along with the appearance of particular $m/z$ signals at 1717, which has been ascribed to a minor ET in *Rubus* species,[24] this suggests that there are more subtle differences in ET composition and structure between Nyby and Fjellgull. Indeed, the enhanced signal at 315 may be related to methyl ellagic acid derivatives.[25] However, care must be taken as certain $m/z$ signals can arise from in-source fragmentation of larger ET molecules or indeed from doubly charged ions ($[M-H]^{2-}$). Furthermore, it was also notable that the separating masses for Nyby contained a signal at $m/z$ = 121, confirmed as benzoic acid using a standard.[22]

The distinctive masses driving the segregation of the Fjellgull female parent derived samples were at $m/z$ = 477, 505, and 607.

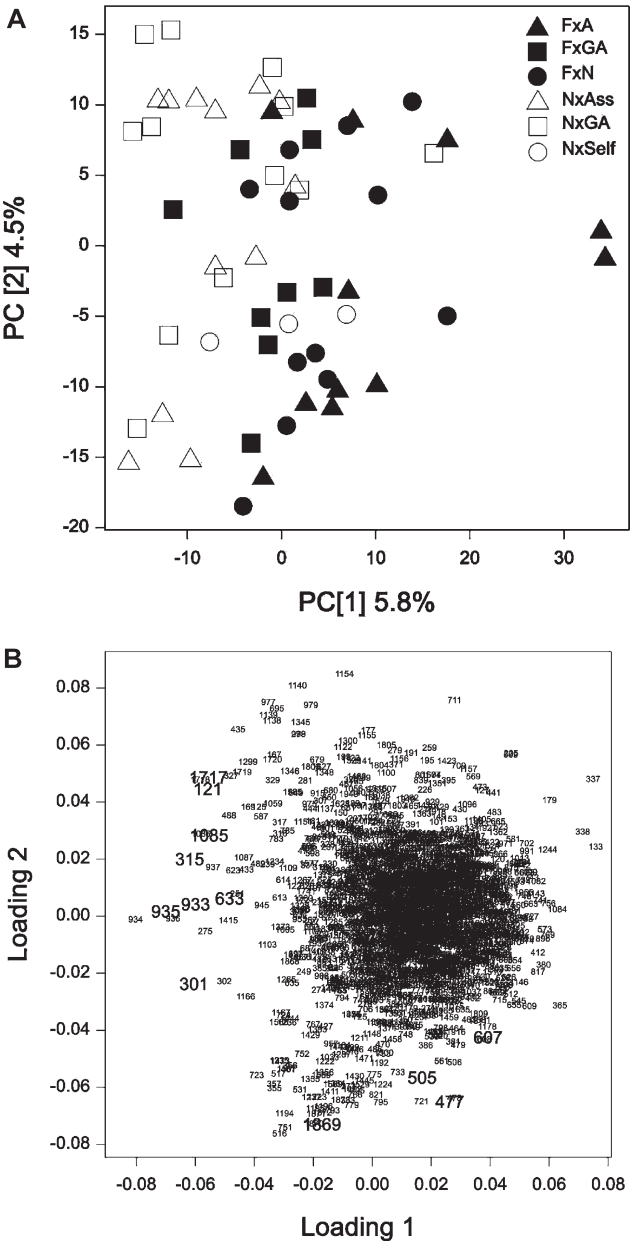


**Figure 2.** (A) PCA plot showing separation of cloudberry parents. The plot shows the first component (PC[1]) against the second component (PC[2]) and accounts for 10.3% of the variation in the data set. (B) Loadings plot for the PCA showing masses that drive separation of the parents. The masses highlighted in large type and bold are discussed in the text.

The $m/z$ signal at 477 gave a MS/MS of 301 and can be identified as quercetin-3-*O*-glucuronide (e.g., see refs 25 and 26). The $m/z$ at 505 gave MS/MS fragmentation products of 463 and 301. This pattern is suggestive of an acetylated quercetin hexose [301 (aglycone) + hexose (162) + 42 (acetyl)], but compounds with these $m/z$ properties in other species of *Rubus*[26] have been attributed to quercetin glucoside malonate conjugates.

The $m/z$ signal at 607 gave a MS/MS of 545, 505, 463, and 301 (Figure 3). This fragmentation can be explained as a neutral loss of 144 to a quercetin hexose, which has not been reported previously in cloudberry. However, this neutral loss has been reported for 3-hydroxy-3-methylglutaroyl (HMG) derivatives of

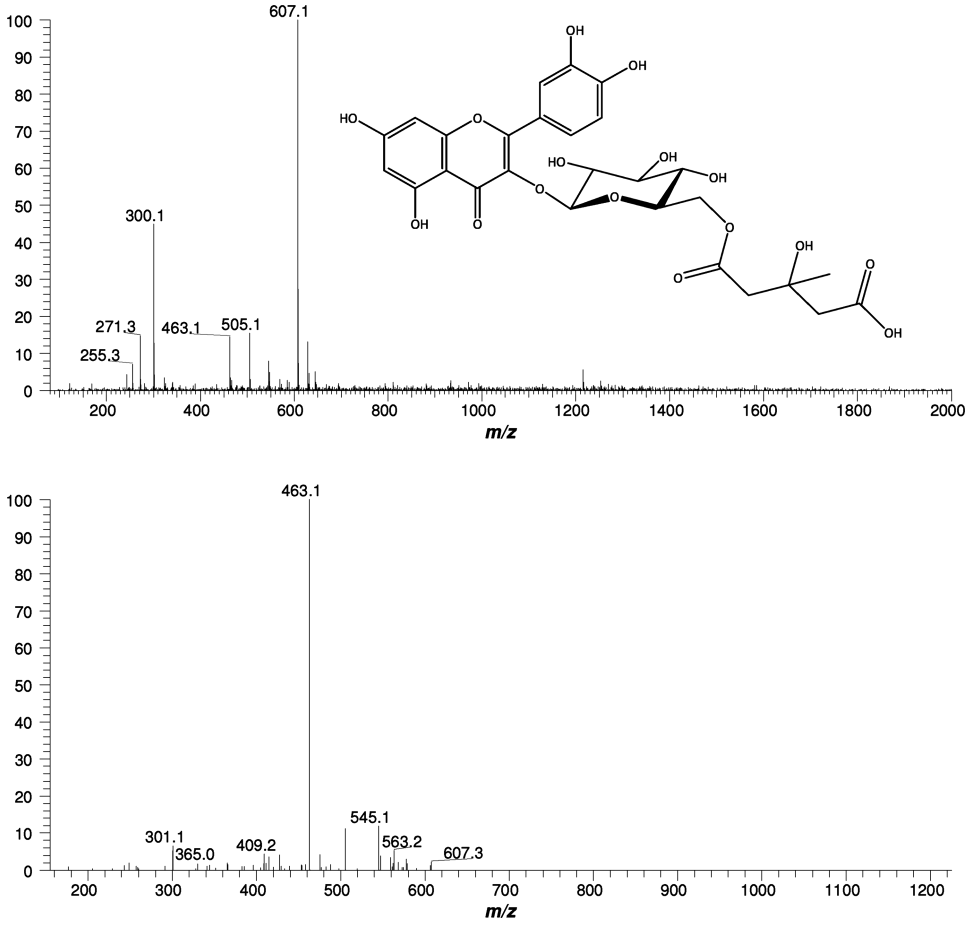


**Figure 3.** Mass spectral properties of unknown compound with $m/z$ = 607. The upper panel shows the full MS spectra (80−2000 $m/z$) for this component. The lower panel shows the MS/MS spectra. The insert shows a possible structure of the putative component as quercetin 3-*O*-[6″-(3-hydroxy-3-methylglutaroyl)-*β*-glucoside].

quercetin rhamnoside in lingonberry[27] and HMG derivatives of quercetin galactosides in blackberry.[28] Furthermore, the reproducible presence of a component with $m/z$ 607 was confirmed by LC-MS analysis of extracts prepared from a range of samples from other wild cloudberry clones (results not shown) collected in test plots at Bioforsk Nord Holt. The component eluted after quercetin-3-*O*-glucuronide with a PDA maximum around 355 nm and MS/MS properties as described above. In addition, this component was enriched in cloudberry subfractions prepared by procedures (e.g., Sephadex LH-20 fractionation[13] or acidification and partition into ethyl acetate[29]) known to enrich flavonols. Exact mass MS data obtained using the Orbitrap MS yielded a $m/z$ value of 607.12907 for the unknown component, which gave a best fit predicted molecular formula of $C_{27}H_{27}O_{16}$ at a Δ ppm value of −0.463 (whereas data from the M + H spectra gave an exact mass of 609.15013, which yields $C_{27}H_{29}O_{16}$ with Δ ppm < 10). These findings match with the molecular formula for a quercetin HMG glycoside structure (Figure 3 insert). The same procedure also correctly predicted the molecular formulas for benzoic acid, quercetin glucuronide, and *p*-coumaric acid peaks within the same samples. The $m/z$ 607 component yielded the same MS/MS products as before. Further work is required to confirm the identity of this component.

A PCA plot of the first and fourth component (PC[1] and PC[4]) revealed another clear discrimination between Fjellgull and Nyby (Figure 4A). The masses driving this separation (Figure 4B) for Fjellgull types revealed a dominance of $m/z$ values characteristic of anthocyanins found in cloudberry (593, 447, 285, and their natural abundance isotopic masses) that may be derived from cyanidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside, and in-source fragmentation to cyanidin, respectively.[20,22] Considering the difference in anthocyanin content between these parents,[19] this separation is not unexpected.

Examination of the PCA plot between the second and the third component (PC[2] and PC[3]) of the ACMS data revealed a separation based on temperature (Figure 5A). There was a general trend of separation based on temperature between the lower (9 and 12 °C) and the higher (15 and 18 °C) temperatures, but there was a marked separation between samples grown at 9 °C and the others. OPLS models constructed from the same data set showed this separation between samples grown at 9 °C and those grown at 12 °C but could not separate those grown at 15 and 18 °C. Therefore, the OPLS model (Supporting Information Figure S5A) was based on a three-class model (i.e., 18 + 15, 12, and 9 °C), and this clearly showed separation between these classes.

The masses that drive this separation are mainly those associated with ETs (e.g., 1869, 1567, and 1235 and their natural abundance isotope masses), and it is notable that the dominant $m/z$ signal at 1869 in the MS spectra is one of the main drivers in this

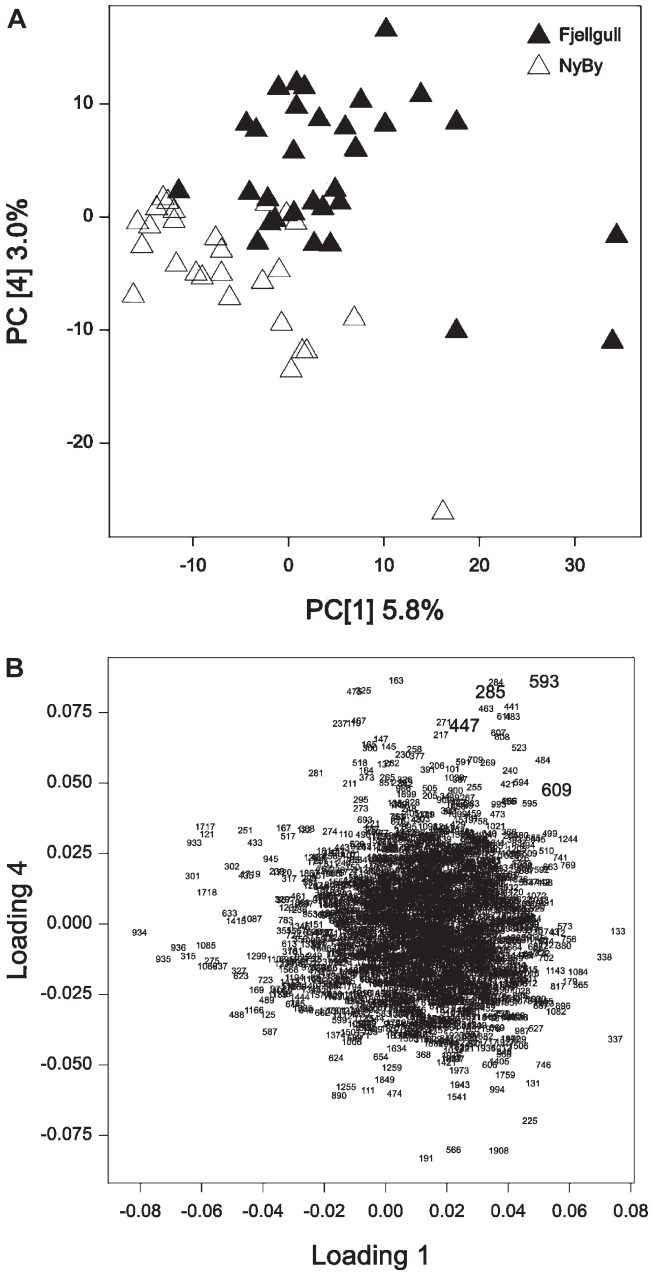


**Figure 4.** (A) PCA plot showing separation of Fjellgull and Nyby parents. The plot shows the first component (PC[1]) against the fourth component (PC[4]) and accounts for 8.8% of the variation in the data set. (B) Loadings plot for the PCA showing masses that drive separation of the parents. The masses highlighted in large type and bold are discussed in the text.

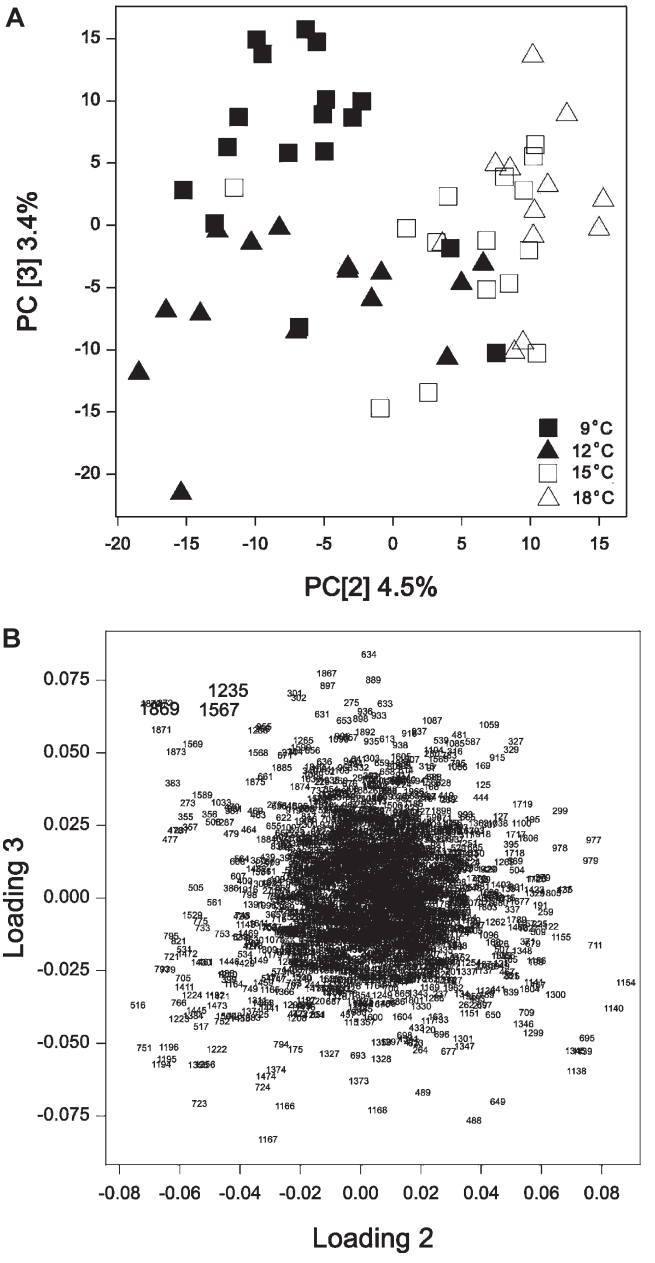


**Figure 5.** (A) PCA plot showing separation on the basis of temperature. The plot shows the second component (PC[2]) against the third component (PC[3]) and accounts for 7.9% of the variation in the data set. (B) Loadings plot showing masses driving separation on basis of temperature. The masses highlighted in large type and bold are discussed in the text.

separation (Figure 5B). The other ET-associated signals at 1567 and 1235 may arise from in-source fragmentation of $m/z$ 1869 or other ET molecules. However, it is also notable that the signal at 1401, which has often been assigned as the $[M - H]^{2-}$ ion characteristic of lambertinian C type ETs,[20,22,24−26,30,31] was not represented in this set. In fact, it is found near the center of the PCA plot, which suggests that not all ET components are equally affected by temperature. The loadings plot from the OPLS analysis (Supporting Information, Figure S5B) confirmed that the same underlying masses drove the temperature-dependent separation (see the Supporting Information, Figure S5B).

Overall, these studies suggest that fruit with the cultivar Nyby as the female parent had higher concentrations of certain ETs and benzoic acid (Figure 2B) than those with Fjellgull as the female parent. The low coloration of Nyby was reflected in lower total anthocyanin contents[19] and consequently in lower intensities of $m/z$ signals attributable to anthocyanin components (Figure 4B). As well as indicating subtle differences in ET composition between the Nyby and the Fjellgull offspring, the ACMS technique also highlighted the presence of a novel flavonol component (putatively identified as a quercetin-HMG hexose derivative) in cloudberry, which was enriched in the

Fjellgull-derived crosses. This component was confirmed as a minor component by further LC-MS studies (results not shown), but it was probably highlighted by its high signal intensity in negative mode ESI. This emphasizes an advantage and disadvantage of the ACMS approach. For example, benzoic acid levels were suggested to be higher in Nyby-derived crosses, but the relatively low ESI intensity of this component made it less apparent. It is clear that care needs to be taken when generating and/or challenging hypotheses using ACMS approaches, and in this case, we supported our ACMS-based observations with targeted standard LC-MS approaches.

Overlaid on these differences related to genetic factors were differences associated with growth temperature. The main difference observed was between cloudberry fruit grown at 9 °C and the other temperatures (Figure 5A). The main masses underlying this segregation were those attributable to a major ET component in cloudberry fruit, sanguiin H-6 ($m/z$ = 1869[25,30,31]) along with a trend to higher flavonol content (attributable masses of $m/z$ 477, 505, 607, etc.). This was not due to the berries grown at 9 °C having the highest phenol content.[19] It is known that total phenol and total anthocyanin contents vary from season to season within specific cultivars, often with wide variations in growth temperature (e.g., ref 32).

However, comparably little is known about the effects of growth temperature on phenolic content and composition in berries as few studies have addressed this issue with sufficient rigor to prevent confounding by other environmental conditions (e.g., light intensity, day length, and cultivar type). A recent review has elegantly outlined some general theories about the effect of temperature on phenolic composition (see ref 33). For example, trends toward higher anthocyanin content with elevated growth temperature have been noted in strawberries[34] and apple skins,[35] but the opposite trend noted for grape skins (e.g., ref 36). However, Wang and Zheng[34] also showed that flavonol and phenolic acid accumulation was also higher at higher temperatures. Rieger et al.[37] explained decreases in anthocyanin content in berries of *Vaccinium myrtillus* grown at high altitude as an effect of lower growth temperature.

A recent study of raspberries (cv. Glen Ample) grown under controlled conditions[38] at different temperatures (12, 18, and 24 °C) indicated that levels of the main ETs (sanguiin H-6 and lambertianin C) increased with increasing temperature. This appears to contradict our findings with the related cloudberry. This work also indicated that the levels of the two dominant anthocyanins, cyanidin-3-*O*-glucosyl rutinoside and cyanidin-3-*O*-sophoroside, increased and decreased, respectively, with increasing temperature. The increases in ET content were related to decreased fresh weight of the berries grown at 24 °C presumably due to decreased water uptake, and as a result, ET content on a dry weight basis may not have been significantly different. Temperature also affected berry size in cloudberry. Total berry weight was highest in Fjellgull over Nyby-derived berries, and the largest berries were found at 12 and 9 °C.[19]

The apparent contradictions in the relationship between ET content and temperature in this study with those of Remberg et al.[38] may result from differences in growth conditions. First, the raspberry plants in Remberg's study were placed outdoor in plastic tunnels until a relatively late stage in berry development, and the different temperature treatments applied may not greatly affect the polyphenol composition. Second, the plants were given varying natural light treatments (from 18 to 12 h/day), whereas this study used a 24 h daylight regime, which may provide more photosynthate for polyphenol biosynthesis.

In addition, this study used whole berries, but the seeds were not broken by the homogenization method, whereas Remberg et al.[38] could have crushed and extracted the ET-rich seeds. Also, there may be an effect of growth temperature on seed size with berries at 9 °C having the largest seeds.[19]

This focused study has revealed interplay between genetic and environmental control on polyphenolic content and composition. The consensus regarding climate change is that Northern Europe will experience increased periods of temperature extremes and precipitation (e.g., ref 39). To ensure that soft fruit industries can remain viable through these changes, we must be able to predict how climate change will impact on crop development, quality, and ultimately economic sustainability. In addition, the market and consumer perception of berries has changed with increased expectation of health-promoting benefits beyond nutritive value.[40,41] As a result, fluctuations in polyphenol composition are central to fruit quality and consumer acceptability, and polyphenols are increasingly becoming targeted in breeding programs.[42] Approaches, such as the ACMS technique employed in this study, which combine high-throughput and data richness, will provide evidence for variety development and understanding of the intricacies of genetic or environmental influences on polyphenol composition. To this end, such techniques are being used to examine the inheritance of polyphenol composition in related *Rubus* species, such as raspberry, which have more established genetic resources.[17,18]

## ■ ASSOCIATED CONTENT

**Ⓢ Supporting Information.** Figures showing sample traces for cloudberry sample, mining for different [M − H] values, correlation between colorimetric measurement of total anthocyanin content and anthocyanin content measured by ACMS, OPLS-PCA plots showing separation based on female parent and temperature, and scores plot for OPLS-PCA plots showing separation forced on female parent and based on temperature. This material is available free of charge via the Internet at http://pubs.acs.org.

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