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Genetic and Environmental Effects on Tannin Composition in Strawberry (*Fragaria* × *ananassa*) Cultivars Grown in Different European Locations

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Supporting Information

ABSTRACT: Strawberry cultivars grown at different locations in Europe showed genotype- and environment-dependent variation in total phenol and anthocyanin contents. This study focused on the compositional diversity of tannins from these cultivars using a high-throughput liquid chromatography−mass spectrometric (LC-MS) technique. Cultivars grown in Germany showed differences in the relative amounts of proanthocyanidins (PACs) and ellagitannins (ETs). Cultivars grown at three different European locations showed differences in their PAC/ET balance. 'Elsanta' grown in Switzerland had higher levels of ET-derived than PAC-derived signals compared to 'Elsanta' grown in Germany and Denmark. The trend to increased ET-derived signals was also noted for 'Clery' grown in Switzerland over Germany and was apparent for 'Korona' grown in Denmark over Germany. The altered ET/PAC balance was confirmed by conventional LC-MS analysis, which highlighted specific changes in composition rather than a general increase in ET components. These findings are discussed with respect to the environmental conditions at the different European locations.

KEYWORDS: *strawberries, ellagitannins, proanthocyanidins, latitude, origin, cultivars, polyphenols*

INTRODUCTION

Although the sensorial and organoleptic properties of strawberries remain key to consumer acceptability, the selection of strawberry cultivars has been increasingly influenced by their content of health beneficial components, which may play a role in the prevention of chronic diseases.[1] Strawberries are commonly ingested in significant amounts and, as such, are an important source of vitamin C and other bioactive compounds, such as polyphenols, that are reported to exhibit beneficial physiological effects.[2] Polyphenols influence strawberry quality, contributing to both sensorial and organoleptic attributes, such as color, flavor, and astringency. The main phenolic classes in strawberries are anthocyanins (responsible for their red color), flavonols, flavanols, hydroxycinnamic derivatives, proanthocyanidins, ellagitannins, and ellagic acid derivatives.[4−6]

Strawberries are the leading soft fruit, with production in the European Union (EU) and global production increasing over the period 1990−2010 by 17 and 77%, to 1.433 amd 4.3667 M tonnes, respectively (FAOSTAT, 2010; http://faostat.fao.org/). As a consequence of their wide availability and popularity, strawberries are a major dietary source of ellagic acid-containing components, ellagitannins, and proanthocyanidins.[7] Over the past few years, interest has been refocused on the tannin components of berries, such as the proanthocyanidins and ellagitannins, due to their high antioxidant potential[5,8] and potential bioactivities relevant to cancer progression,[9,10] vaso-relaxation,[11] and enzyme inhibition relevant to glycemic control[12] or obesity.[13] Strawberries are unique among berries in that they contain substantial amounts of both proanthocyanidins and ellagitannins.[14,15] The polyphenol profiles of strawberries from diverse genetic backgrounds have been studied and reveal substantial cultivar-to-cultivar differences[16−20] including within the tannin components.[5]

This study assesses the variation in polyphenol composition in strawberry cultivars grown in different locations across Europe under comparable agronomic conditions. A previous study confirmed that genotype was the major influence on phenolic composition of strawberry cultivars grown at different latitudes.[21] However, comparison of the same cultivars grown at more northern latitudes revealed lower relative anthocyanin contents than those grown in the south, which suggested that the higher relative total phenol contents were possibly due to increased levels of tannin (proanthocyanidin and ellagitannin) components. This study employs a high-throughput LC-MS technique[22] and focuses on differences in tannin composition between cultivars grown at different locations.

MATERIALS AND METHODS

Plant Material. In 2009, strawberries (*Fragaria* × *ananassa* Duch.) of four different cultivars ('Elsanta', 'Korona', 'Clery', and 'Everest') were grown at an experimental open field in three replications with 40 plants per plot in a randomized order at the Geisenheim Research Center, Germany (D) (49 °N). Fruit of cv. 'Elsanta', produced under comparable growing conditions, that is, open field, plant number, plant density, randomization, etc.), were obtained from two other locations in Europe: University of Copenhagen, Denmark (DK) (55

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Table 1. Total Anthocyanins, Total Phenols, Antioxidant Capacity, and Ascorbic Acid in Four Strawberry Cultivars from Different Locations[a]

cultivar	location	total anthocyanins[b]	total phenols[c]	TEAC[d]	ORAC[e]	ascorbic acid
'Elsanta'	Germany	345 ± 20 cd	1891 ± 41 bcd	24.0 ± 0.4 cd	31.8 ± 2.2 c	980 ± 24 a
'Elsanta'	Switzerland	389 ± 8 c	2312 ± 90 b	30.4 ± 1.9 b	38.0 ± 1.0 b	777 ± 24 b
'Elsanta'	Denmark	266 ± 30 d	3090 ± 259 a	37.7 ± 2.1 a	55.2 ± 1.8 a	853 ± 36 ab
'Clery'	Germany	596 ± 57 b	1844 ± 35 cd	25.6 ± 0.5bcd	35.7 ± 2.5 bc	751 ± 11 b
'Clery'	Switzerland	659 ± 18 ab	2211 ± 83 bc	29.9 ± 0.7 b	35.3 ± 1.2 bc	816 ± 66 b
'Korona'	Germany	726 ± 21 a	1574 ± 36 d	22.9 ± 0.9 d	35.7 ± 2.1 bc	592 ± 63 cd
'Korona'	Denmark	566 ± 16 b	1987 ± 152 bcd	28.2 ± 1.3 bc	38.7 ± 1.7 b	586 ± 41 d
'Everest'	Germany	416 ± 42 c	1931 ± 109 bcd	27.5 ± 2.0 bcd	37.1 ± 0.4 bc	730 ± 23 bc

[a]Data are expressed as the mean ± standard deviation (n = 3). Means within the same column followed by different letters were significantly different at $p \leq 0.01$. [b]Data are expressed as micrograms of pelargonidin 3-glucoside equivalents per gram of fresh weight. [c]Data are expressed as micrograms of gallic acid (GAE) equivalents per gram of fresh weight. [d]Data are expressed as micromoles of Trolox equivalents per gram of fresh weight. [e]Data are expressed as micrograms of pelargonidin 3-glucoside equivalents per gram of fresh weight.

°N), and Agroscope, Changins-Wädenswil, Switzerland (CH) (46 °N). In addition, a second cultivar adapted to the local climate (cv. 'Korona' in DK, cv. 'Clery' in CH) was examined. Therefore, eight samples were examined.

Agronomic procedures (mulching, irrigation, weeding, fertilizer use, etc.) were not standardized but were the commercial standard for each site, and as such the fruit was as close as possible in quality to that available to the consumer in that locale. The temperature during the ripening period was averaged over the 28 days of harvest and was 14.2 °C at the growing site in DK, 16.4 °C in D, and 17.4 °C in CH. Further detailed climatic and agronomical data including radiation, precipitation, yield, and altitude are available in related publications.[21,52] One week after the start of harvest, samples containing ∼500 g of healthy and undamaged ripe fruit were taken. The date of sampling for each location and cultivar is given in Table 1. Each fruit was sliced into quarters. To avoid bias, two counterparts of the fruit quarter were immediately snap-frozen in liquid nitrogen, stored at −28 °C, and pulverized later to a fine powder using a mortar and pestle. The soluble solid content of the thawed powder was measured directly using a digital refractometer (A. Krüss Optotronic GmbH, Hamburg, Germany). For each treatment listed in Table 1, the three biological replicates were extracted separately, and the resultant extracts were analyzed twice as described below (i.e., 24 samples = 3 × 8).

Sample Extraction. Samples were extracted as described previously.[23] For ascorbic acid content, 5.0 g of frozen strawberry powder from each sample was extracted twice with 2% (w/v) oxalic acid. For all other parameters except LC-MS analysis of tannin composition, 5.0 g of powder was extracted twice with 10 mL of 80% (v/v) methanol and made up to a final volume of 25 mL.

Determination of Total Phenols (TP), Oxygen Radical Absorbance Capacity (ORAC), Trolox Equivalent Antioxidant Capacity (TEAC), Ascorbic Acid (AsA), and Total Monomeric Anthocyanin Content (TMA). Ascorbic acid (AsA) concentration was estimated by iodometric titration.[21] TMA concentrations were determined by the pH differential method,[24] and the results were expressed as micrograms pelargonidin-glucoside per gram fresh weight (FW) using a molar absorption coefficient of 15600 L mol^{-1} cm^{-1}. Antioxidant capacity was analyzed using three different assays: total phenol (TP) contents were determined by the Folin−Ciocalteu method[25] using gallic acid (Sigma-Aldrich, Steinheim, Germany) as the reference compound and expressed in micrograms gallic acid equivalents (GAE) per gram FW. The TEAC assay[26] was carried out using 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) with slight modifications. The ABTS radical cation was generated overnight with potassium persulfate to a final absorbance of 0.800 ± 0.050 at 734 nm. Antioxidant capacity was expressed in micromoles Trolox equivalents (TE) per gram FW. The ORAC assay was applied[27] using fluorescein, a black 96-well plate, and a microplate reader (Infinite m200; Tecan, Crailsheim, Germany). Fluorescence was recorded every minute for 90 min at 37 °C, with an excitation wavelength of 485 nm and an emission wavelength of 520 nm. ORAC values were calculated by linear regression of the area under the kinetic curve using Magellan software (Tecan) and expressed as micromoles TE per gram FW.

Extraction Procedure and Sephadex LH-20 Fractionation for Tannin-Rich Fractions. Five grams of frozen strawberry powder was extracted with 5 mL of acetonitrile containing 0.2% formic acid by shaking vigorously for 1 h in the dark and then centrifuged at 4000*g* for 10 min at 4 °C. The supernatant was evaporated by rotary evaporation, and then the dried extracts were dissolved in 2.5 mL of 50% (v/v) ethanol. The procedure utilized the differential binding of tannins to Sephadex LH-20 in aqueous alcohol and aqueous acetone (see http://www.users.muohio.edu/hagermae/). Sephadex LH-20 was swollen (5 g in 50 mL of 70% (v/v) acetone) and then equilibrated in 50% (v/v) ethanol overnight. A slurry was produced by resuspending the settled material in 10 mL of 50% ethanol. For fractionation, a 1 mL aliquot of the Sephadex LH-20 slurry was mixed with 1 mL of dissolved extracts, centrifuged, and washed four times with 1 mL of 50% ethanol. Further washing did not elute further unbound phenolics. The tannin-rich fraction was eluted with 2 × 1.0 mL of 70% acetone. The bound samples routinely had a TPC around 4000 μg GAE/mL. The supernatants were collected, evaporated to dryness, and resuspended in 200 μL of 5% acetonitrile/water containing 0.1% formic acid for further analysis.

Liquid Chromatography−Mass Spectrometry (LC-MS). Samples of resolubilized extracts were analyzed on a LCQ-DECA system, comprising a Surveyor autosampler, pump, and photodiode array (PDA) detector and a ThermoFinnigan mass spectrometer ion trap controlled by Xcalibur software. The PDA detector scanned three discrete channels at 280, 365, and 520 nm. Solvent A was 0.1% formic acid in ultrapure water, and solvent B was 0.1% formic acid in acetonitrile. The mass data were collected in a negative mode scanning over the mass range m/z 80−2000. The MS detector was tuned against ellagic acid in a negative mode. Two scan events were employed in all LC-MS runs; full scan analysis was 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 extracts were analyzed with two methods: an abbreviated chromatography mass spectrometry (ACMS) method, developed previously McDougall et al.,[22] was employed. The ACMS method uses a rapid, partial separation on a short C_{18} column (Synergi; 20 × 2 mm, 2.5 μm; MAX-RP 100A Mercury; Phenomenex Macclesfield, UK).[22] The samples were eluted with a gradient of 98:2 solvent A/B at time = 0 min, 50:50 at time = 3 min, 35:65 at time = 5 min, and 5:95 at time = 6.5 min at a flow rate of 200 μL min^{-1}. The duration of each run was 10 min. Each sample was analyzed in triplicate in a randomized order. The variation in peak area by PDA between replicate ACMS runs was <2% (results not shown).

For detailed investigations, the samples were analyzed using our conventional LC-MS method. This employed a longer column (Synergi Hydro-RP C18 80A; 150 × 4.6 mm, 4 μm; Phenomenex) with a gradient of 95:5 solvent A/B at time = 0 min to 55:45 A/B at time = 30 min. The duration of each run was 45 min with a flow of 400 μL min^{-1}. Each sample was analyzed in duplicate in a randomized order. After

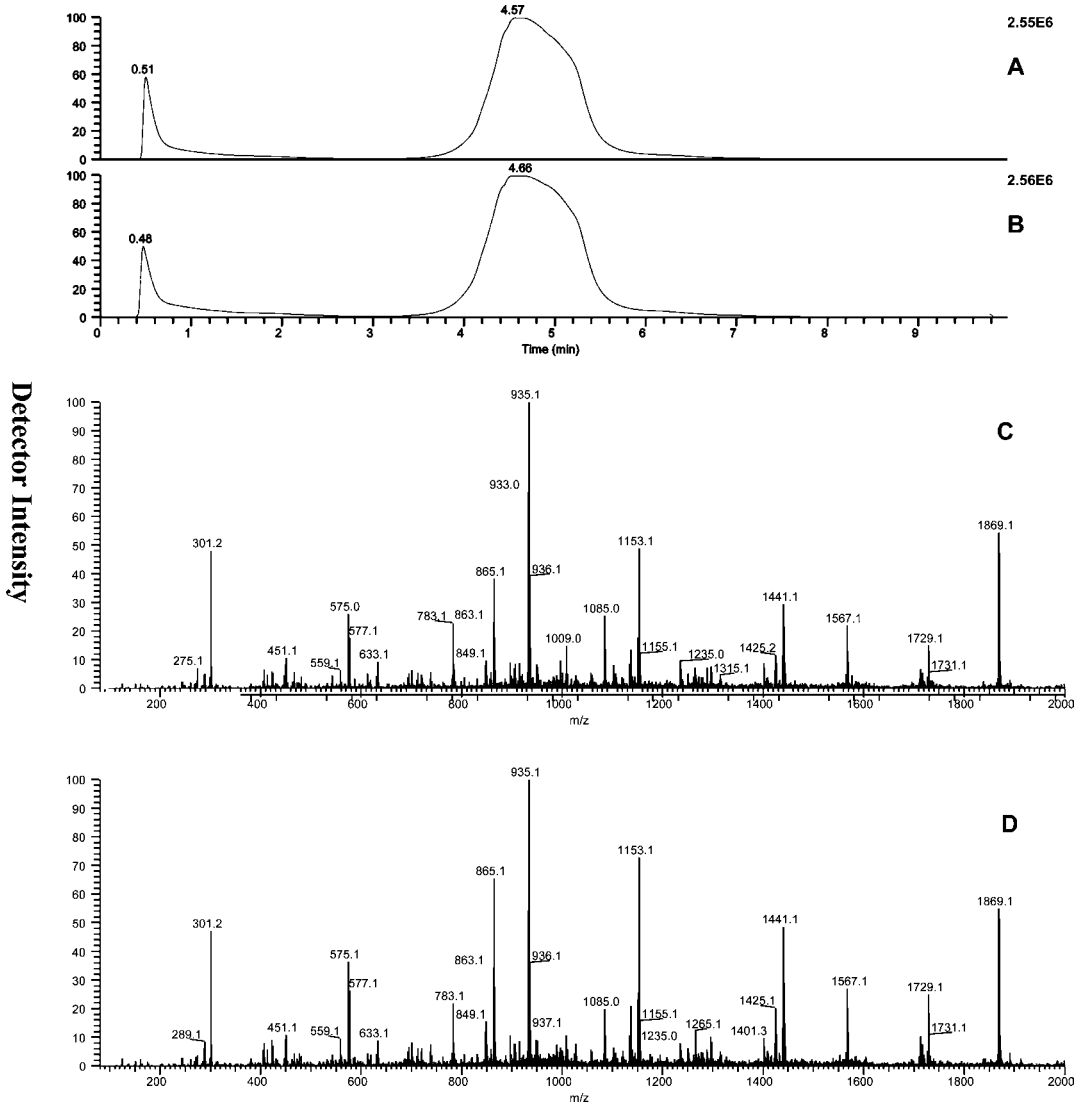

Figure 1. UV chromatograms and MS spectra from the ACMS method on two strawberry tannin-rich samples: (A) UV chromatogram of 'Elsanta' sample from Switzerland; (B) UV chromatogram of 'Elsanta' sample from Denmark (the figure in the top right corner is the full scale deflection of the PDA detector at 280 nm); (C) MS spectra of of 'Elsanta' sample from Switzerland, FSD = 9.9e6; (D) MS spectra of 'Elsanta' sample from Denmark, FSD = 8.6e6.

identification of putative components by their mass spectra, the peak area of the specific m/z value for each molecule was obtained using the extracted ion chromatogram facility of the Xcalibur software. The advantage of this method is that a complete separation of the peaks is not necessary for their quantification. For the proanthocyanidin components, only the percentage distribution was estimated because of the lack of available standards and some overlap of peaks. The ellagitannin components were expressed as ellagic acid equivalents based on m/z peak areas. This approach probably consistently underestimates ellagitannin content, but the results are comparable between samples.

Statistical Analysis. The mass spectrum for each sample of the main included peak over 3−7 min (Figure 1) resulting from the partial separation was exported to a Microsoft Excel data sheet. The data were normalized by dividing each mass intensity (m/z 100−2000) by the total intensity of the combined total masses. A principal component analysis (PCA) was performed using Unit Variance (UV) scaling, which gives equal weight to all masses. This analysis was performed using SIMCA P+ version 12 (Umetrics, Crewe, UK). All other data were analyzed statistically with analysis of variance using SPSS (version 17). Variances highlighted a lack of homogeneity; therefore, Tukey's honestly significant difference (HSD) test was applied with a level of significance of 99%. Results were given as the mean ± standard deviation. Furthermore, the correlation of all results was tested by Spearman.

RESULTS

Total Monomeric Anthocyanin (TMA), Total Phenol Content (TP), and Antioxidant Capacity. There was a large variation in total phenol content (TP) between cultivars and within cultivars grown in different locations (Table 1). For example, 'Elsanta' grown in Denmark had significantly higher TP than 'Elsanta' grown in Germany and Switzerland but, conversely, significantly lower anthocyanin content. The cultivars gave different levels of AsA, with 'Korona' yielding the least, but this component did not vary significantly between growing locations. In fact, the variation in TP between 'Elsanta' grown in the three locales was 1.6-fold, whereas AsA content varied 1.26-fold. There was a large variation in TMA, and this was especially apparent when expressed as percent of TP; for example, 'Korona' (Denmark) had 46% anthocyanin content, whereas 'Elsanta' (Denmark) had 8%. Despite previous results,[21] there was no obvious correlation between latitude of growth and

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Table 2. Spearman Correlations of Total Anthocyanins, Total Phenols, Antioxidant Capacity (TEAC, ORAC), Ascorbic Acid, Sum of Proanthocyanidins (PAC), and Sum of Total Ellagitannins (ET) in Four Strawberry Cultivars Grown at Different Locations

	total anthocyanins	total phenols	TEAC	ORAC	ascorbic acid	sum PAC
total anthocyanins	1					
total phenols	−0.545	1				
TEAC	−0.405	0.923	1			
ORAC	−0.324	0.498	0.568	1		
ascorbic acid	−0.615	0.508	0.330	−0.161	1	
sum PAC (peak area)	−0.783	0.409	0.230	0.245	0.513	1
sum ET (peak area)	0.031	0.439	0.503	0.058	0.280	−0.262

TMA, TP, or indeed the proportion of TA compared to TP in the cultivars.

TP correlated with TEAC ($r = 0.952$) but less well with ORAC ($r = 0.606$), which may reflect the different basis of this antioxidant measurement (Table 2).[28] Interestingly, TP + AsA slightly improved the correlation with TEAC ($r = 0.966$). Correlation between TP by Folins and other antioxidant measurements has been well established in berry samples[29] and may arise because the two methods essentially measure different aspects of redox reactions.[28]

Characterization of Tannin Diversity of Strawberry Samples Using ACMS. An ACMS method, developed previously,[22] was employed to assess the diversity of tannin composition in strawberry cultivars grown in three European locations (Denmark, Germany, and Switzerland). This method uses the MS spectrum (Figure 1C,D) obtained across the retained components from each sample (e.g., RT 3−7 min; Figure 1A,B), which then can be collated and compared using multivariate statistical approaches.

The ACMS spectra of the strawberry tannin extracts were similar to those derived from direct infusion MS (DIMS) methods[30,31] and were dominated by signals characteristic of ETs and PACs. For example, masses at 1869, 1567, 1401, 1265, 1085, 935, 933, 783, 633, 451, and 301 are characteristic of ETs,[5,30,32,33] whereas signals at 1729, 1441, 1425, 1153, 865, 849 577, 575, and 289 are characteristic of procyanidin and propelargonidin PACs.[5,34] Overall, the ACMS method has advantages over DIMS; it is quick and reproducible and reduced problems with ion suppression as the short but steep gradient sufficiently separates and discretely presents the components to the MS detector, leading to higher sensitivity.

PCA plots generated from the ACMS data revealed a distinct separation between the four cultivars grown in Germany (Figure 2A). 'Elsanta' and 'Korona' were clearly separated, mainly on the horizontal axis, from 'Clery' and 'Everest'. The loading plot for this PCA (Figure 2B) suggested that the main differences were in the relative amounts of PACs and ETs in 'Korona' and 'Elsanta' versus 'Clery' and 'Everest', with the former cultivars having higher amounts of signals attributable to PACs (m/z 1729, 1441, 1153, 865, 577, 575, and their isotopic masses; annotated in Figure 2B) and with 'Clery' and 'Everest' having higher amounts of signals characteristic of ETs (m/z 1717, 1085, 935, 933, 783, 633, 301, and their isotopic masses). However, it is notable that certain ET-associated signals (most notably m/z 1401), which can be assigned to lambertianin C-like structures, were not so tightly associated with the positive aspect of PC1 (the aforementioned ET side) and therefore may not have such a strong role in driving this separation.

When the ACMS data were re-examined using orthogonal partial least-squares discriminant analysis (OPLS-DA) to maximize the differences between the cultivars grown in Germany, the same underlying masses were uncovered (see Supporting Information, Figure S1a,b). In addition, separation between cultivars in the vertical axis (especially 'Korona' from

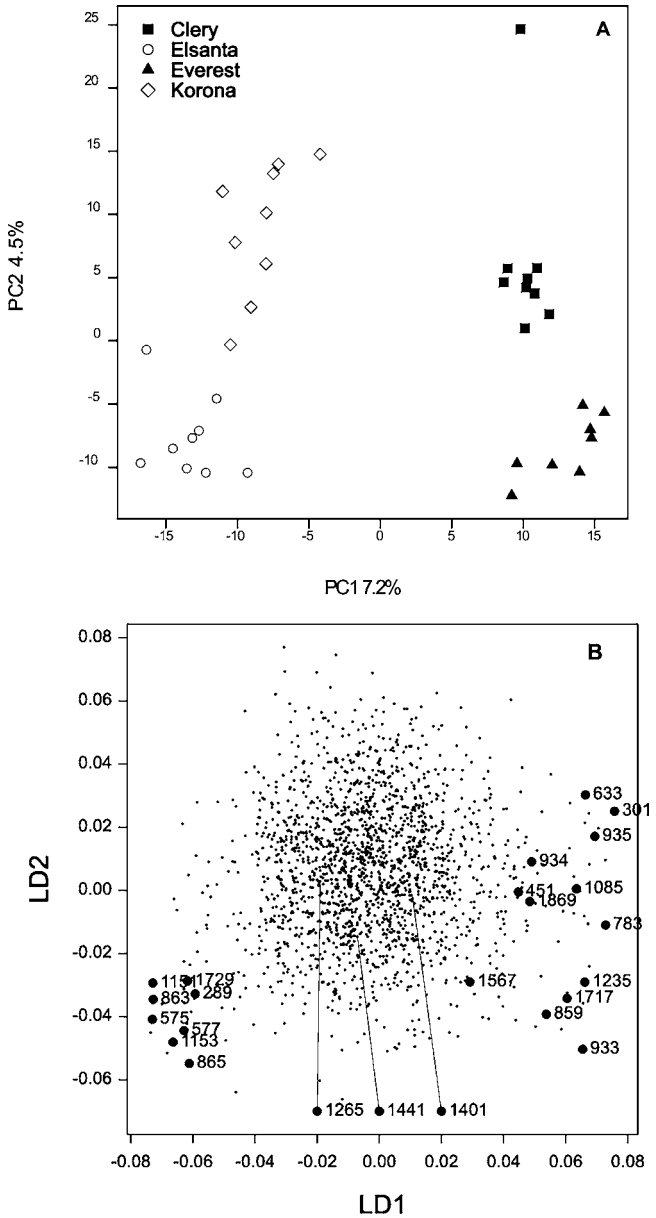

Figure 2. Principal component analysis (PC1 and PC2) of four cultivars, 'Elsanta', 'Clery', 'Korona', and 'Everest', grown at one location (Geisenheim, Germany). Score plot (A) was generated from the samples of the partial separation and loading plot (B) of the mass ions creating the segregation of the observations.

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'Elsanta' and 'Clery' from 'Everest') was associated with the presence of certain masses (m/z 269, 431, 473, and their isotopic masses; results not shown), which are almost certainly due to the carry-over of higher amounts of anthocyanin derivatives (pelargonidin-3-*O*-glucoside, M − H = 431, 269; and pelargonidin-3-*O*-glucoside malonate, M − H = 473, 269; results not shown).

The PCA of the ACMS data from the cultivars grown at different European locations showed another clear separation dependent on location (Figure 3A). It was clear that 'Elsanta' grown in Switzerland separated from those grown in Denmark and Germany along PC1. Similarly, 'Clery' grown in Switzerland separated from 'Clery' grown in Germany, albeit to a lesser extent. In addition, there was a separation between 'Korona' grown in Denmark and 'Korona' grown in Germany driven by both PC1 and PC2.

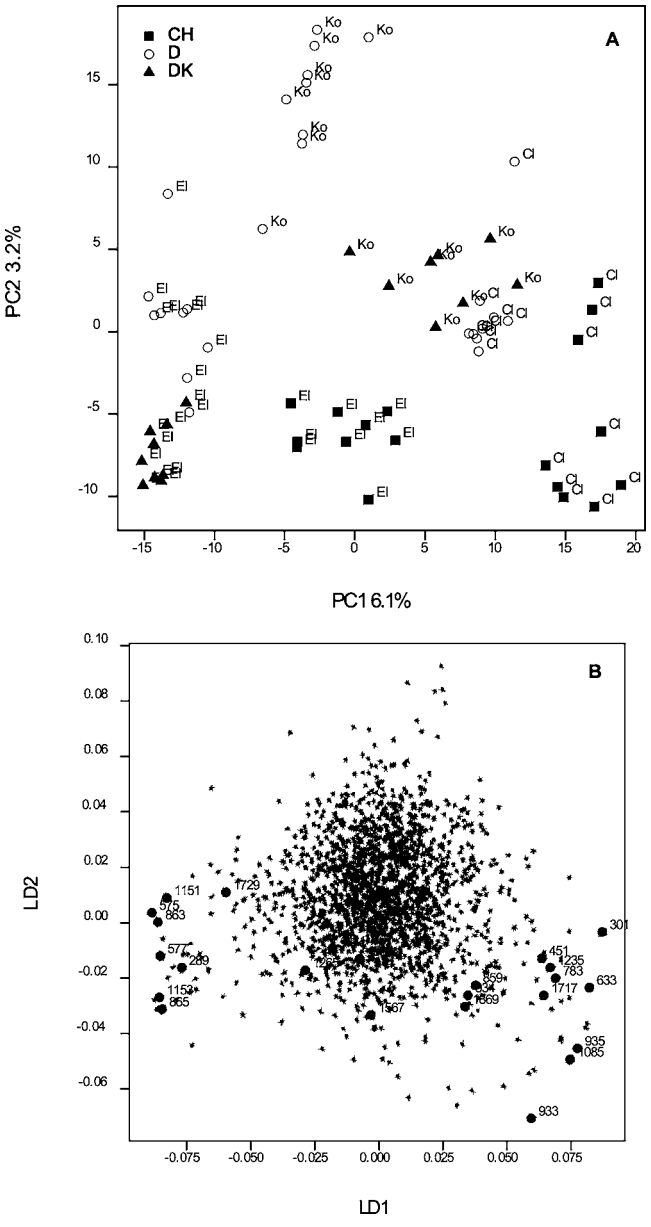

Figure 3. Principal component analysis (PC1 and PC2) of the cultivars 'Elsanta', 'Clery', and 'Korona' grown at three different locations (Denmark, Germany, Switzerland). Score plot (A) was generated from the samples of the partial separation and loading plot (B) of the mass ions creating the segregation of the observations.

An examination of the loading plot (Figure 3B) suggests that, as earlier, the ET-related components were predominantly gathered in the positive region of LD1. Indeed, PCA plots of the separations between individual cultivars grown at the different locations confirmed this trend to higher ET contents at locations outside Germany (results not shown). Nevertheless, the impact of cultivar seems to be stronger than an effect of latitude and growing condition.

In all cases, the PCA explained only a small portion of the variation in the sample sets (i.e., maximum ∼7%). This highlights that the ACMS spectra from the strawberry tannin samples contain many similar signals (within the possible 1920 m/z values of the spectra) and these contribute to considerable "background noise". Indeed, none of the PCA models reached the threshold to be deemed robust ($Q^2 > 0.5$). However, all OPLS-DA models generated from the same data (e.g. Supporting Information, Figure S1) were all robust ($Q^2 > 0.5$). Nevertheless, it is remarkable that obvious and consistent differences within the variable element of ∼7% were noted.

Characterization of Tannin Diversity of Strawberry Samples Using LC-MS. Certain ellagitannin and proanthocyanidin peaks could be assigned using conventional LC-MS (Table 3) and their peak areas assessed (Figures 4 and 5). As noted previously, ET and PAC components do not separate completely on reverse phase C_{18} HPLC,[30] so some of the estimated peak areas may be affected by in-source competition from other partially coeluting components. However, the estimates are carried out on the same basis, and the peak areas are compared between samples. Overall, the trends noted using ACMS were confirmed by the conventional LC-MS results (Tables 4 and 5). The LC-MS results revealed that the main differences between the cultivars were in the amounts of the ET components and that the PAC components did not vary significantly. The marked separation between the cultivars grown in Germany based on ACMS data (Figure 2A) fits with the higher ET content of 'Everest' and 'Clery' over 'Korona' and 'Elsanta' (i.e., 181 and 203 vs 109 and 145, respectively). In addition, it is apparent that only certain ETs were increased in abundance. In 'Elsanta', it was most notable that the amounts of castalagin- and potentillin-like masses increased (m/z 933 and 935; peaks 16 and 17 but not 19) with a significant increase in a cornusiin-B-like ET (m/z 1085; peak 24 but not peak 25). This fits with the suggestion from the ACMS data that there was no overall increase in ET species. Correlations between the LC-MS and ACMS results may be partly obscured by in-source fragmentation in ACMS as, for example, increased signals at m/z 935 may also increase signals at m/z 63, but peak 15 (strictinin) may not have increased in its own right. In addition, doubly charged ions ($[M - H]^{2-}$) may also complicate any correlation. This is illustrated by the "peak" at m/z 934 (peak 19, Figure 5), which largely coelutes with the peak at m/z 1869 (peak 21, agrimoniin, Figure 5) and is due to doubly charged ions ($[M - H]^{2-}$).

Although ETs were increased in 'Clery' grown in Switzerland rather than Germany, the pattern is different with the overall increase (which was higher than in 'Elsanta') being spread across a wider range of ET species. It is notable that 'Korona' grown in Germany had lower levels of ETs than those grown in Denmark. In addition, the changes were subtly different from the other cultivars. This fits with the trend in the ACMS data (Figure 3 A,B), where the separation between these samples was less obvious.

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Table 3. Putative Assignments of Peaks Analyzed by LC-MS

peak	putative component[a]	RT	λ_{max}	m/z [M − H][b]	MS[2c]
1	PAC trimer (EC3)	8.1	250	**865.1**, 574.9, 289.0	695.0, 577.0, 574.9, 451.1, 289.0
2	PAC trimer (EC3)	11.3	245	**865.1**	695.0, 577.0, 407.0
3	PAC trimer (EC2F)	11.6	240	**849.1**	577.0, 558.9, 407.1
4	PAC dimer (EC2)	14.7	240	**577.1**	451.0, 424.9, 406.9, 289.0
5	PAC dimer (EC2)	15.2	240	**577.1**	451.1, 424.9, 406.9, 289.0
6	PAC tetramer (EC4)	15.7	240	**1153.1**	N.D.
7	PAC trimer (EC3)	16.5	245	**865.1**, 577.1	695.0, 577.1, 404.1
8	PAC tetramer (EC4)	18.1	245	**1153.1**	N.D.
9	PAC trimer (EC2F)	18.6	245	**849.1**	577.0, 558.9, 407.1
10	PAC trimer (EC3)	19.2	240	**865.1**, 577.1	451.0, 424.9, 406.9, 289.0
11	PAC pentamer/ tetramer (EC4/5)	19.6	240	**1441.0, 1153.1**	N.D.
12	peduncalagin	12.0	240	**783.1**, 301.1	480.9, 301.1, 275.1
13	peduncalagin	13.0	240	**783.1**, 301.1	480.9, 301.1, 275.1
14	peduncalagin	14.5	245	**783.1**, 301.1	480.9, 301.1, 275.1
15	galloyl-HHDP glucose	15.8	245	**633.1**	463.0, 301.1
16	sanguiin H6 minus galloyl	18.6	245	**1716.9**	1414.8, 1235.1, 1112.9, 932.9, 915.0, 783.1
17	castalagin-like ET	19.5	245	**933.1**	897.0, 631.0, 451.1, 301.1
18	potentillin-like ET	20.0	240	**935.0**	633.0, 480.9, 301.1
19	ET (933 + HHDP)	20.2	245	**1235.0**	933.0, 631.0, 468.9
20	castalagin-like ET	20.9	245	**933.1**	633.0, 451.1, 301.1
21	agrimonin	20.9	240	**1868.9**	1566.9, 1414.9, 1265.1, 1235.0, 933.0, 631.1
22	lambertianin C	21.3	240	**1401.2**	1868.9, 1566.7, 1264.9, 1234.8, 934.9
23	castalagin-like ET	22.5	240	**933.0**	630.9, 451.1, 301.1
24	castalagin-like ET	23.9	245	**933.1**	631.0, 451.1, 301.1
25	cornusiin B-like ET	24.1	245	**1085.0**	897.0, 783.0, 633.1, 451.1
26	cornusiin B-like ET	26.2	240	**1085.0**	897.0, 783.0, 633.1, 451.1

[a]Putative assignments are supported by data from previous papers.[11,30,32,35,37] EC, epicatechin unit; EC3, procyanidin trimer; EC2F, propelargonidin trimer containing one epiafzelchin (F) unit. [b]m/z values in bold were the predominant ions. [c]Underlined m/z values were the main MS[2] fragments. ND, not determined.

A range of peaks were identified with m/z values consistent with proanthocyanidins previously identified in strawberry[4,30] including m/z signals at 577, 865, 1153, and 1441 characteristic of procyanidin dimers, trimers, tetramers, and pentamers.[5,36] Other peaks with m/z signals characteristic of propelargonidin proanthocyanidins (i.e., proanthocyanidins containing at least one (epi)afzelechin unit), for example, at 849, were also evident (Table 2). Signals characteristic of other propelargonidin proanthocyanidins (m/z 833, 1425, etc.) were noted in ACMS spectra, but these more minor components could not be identified as distinct peaks in the conventional LC-MS runs. Strawberry fruit has been found to contain a complex mixture of PACs ranging from dimers, trimers, tetramers, etc., to polymers[5,34] of both procyanidin and propelargonidin types. The mean degree of polymerization has been defined at 6.3.[34]

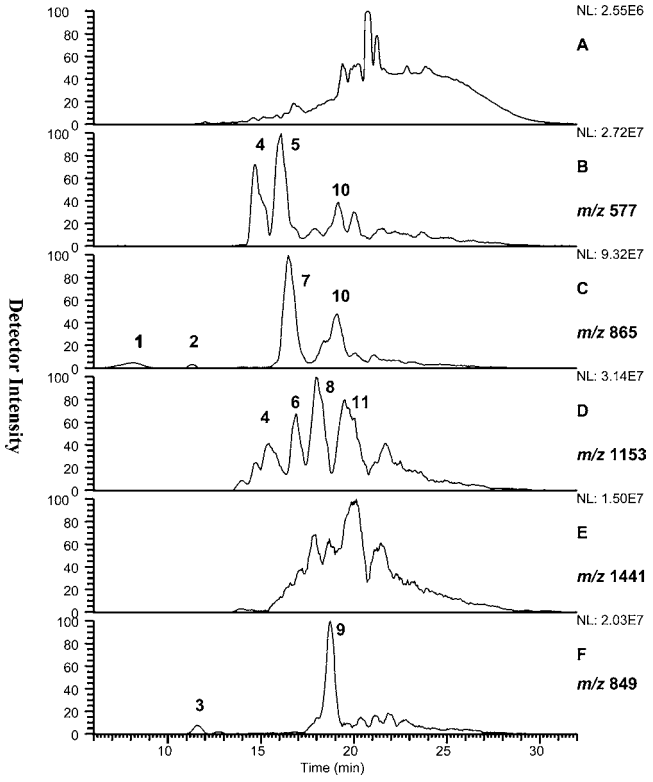

Figure 4. Extracted ion chromatograms of proanthocyanidin components: (A) UV trace at 280 nm; (B) m/z 577; (C) m/z 865; (D) m/z 1153; (E) m/z 1441; (F) m/z 849. Peak numbers refer to Table 4.

RP-HPLC does not completely separate PACs,[5,30] and the use of these MS techniques cannot report on higher polymerized PACs (the limit of detection is 2000 amu). However, analysis of higher molecular weight PACs is feasible, for example, using MALDI-ToF-MS methods to characterize up to 12-mers.[37] However, in this study, the conventional LC-MS approach reported on only up to pentamer PACs (m/z 1441), although signals from putative hexamers (m/z 1729, etc.) were noted in the ACMS spectra. Nevertheless, 11 PAC oligomers were identified and quanitified in the strawberry cultivars (Figure 4; Tables 3 and 5). Interestingly, the detected PAC composition did not vary significantly and was not influenced by either cultivar or origin (Table 5). Differences were observed only in the sum of the total peak area. The sum of detected PACs showed a strong negative correlation to the total anthocyanin content (Table 2). ETs were not correlated to total anthocyanins and only slightly negatively correlated to PACs. The contribution to total phenols and TEAC was comparable for both tannin groups (ETs and PACs).

DISCUSSION

It is well-known that different cultivars of strawberries differ in their phenolic content and composition.[16,19,38] Most obviously, strawberry cultivars may differ in coloration, which may be caused by increased levels of anthocyanins or differences in the proportion of pigmented tissues.[23,39,40] The strong genotypic determinant underlying phenolic content is the basis for breeding efforts to increase the total phenolic content or modulate the phenolic composition, such as increasing (specific) anthocyanin content.[41] However, the effects of interactions between genotype and environment on phenolic content have been less well-defined.[42] Many studies have focused on one

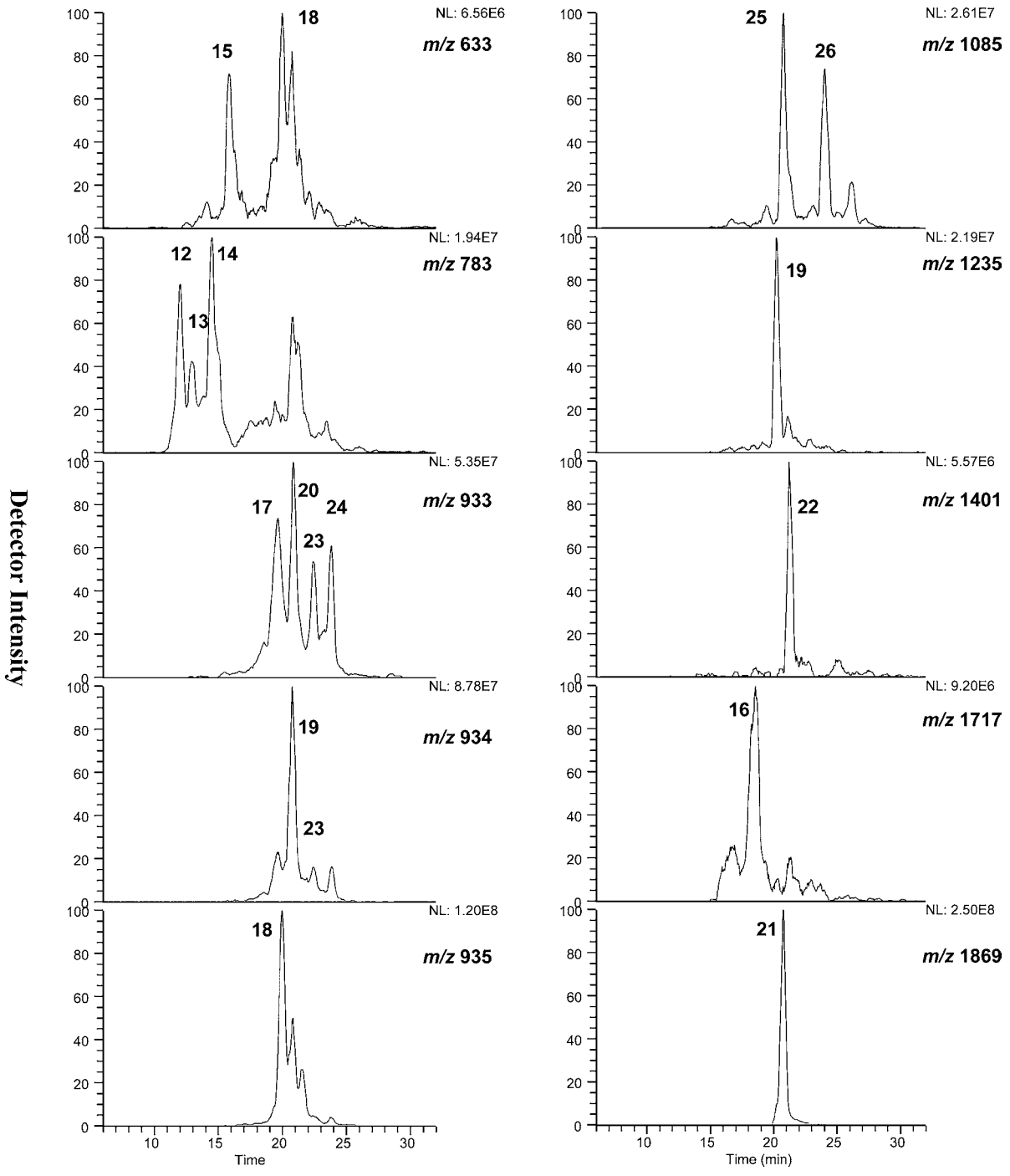

Figure 5. Extracted ion chromatograms of ellagitannin components. M − H *m/z* values are annotated. Peak numbers refer to Table 4.

cultivar under different field conditions or at different locations or have focused on one possible stressor such light quality, temperature, or water supply.[23,43−45] However, others have examined a range of genotypes over a number of growing seasons. For example, there was substantial year-to-year variation in TP and TMA overlying the genotypic ranking of the strawberry cultivars and lines, possibly due to differing growing temperatures between seasons.[46] Another study[47] reported that four strawberry cultivars grown at two locations in Switzerland that differed in altitude (480 and 1060 m above sea level) and other agronomic conditions showed no significant interactions between site and cultivar for TMA or antioxidant capacity and showed the same ranking of genotypes in both locations. However, the yield was significantly decreased at higher altitudes.

The effects of G × E interactions on phenolic composition have been less examined in strawberry. Our study suggested that the tannin compositions of strawberry cultivars grown at different locations differ in their ET/PAC balance. In particular, cultivars grown in Switzerland had higher ET levels than those grown in Germany. In addition, cultivars grown in Denmark had

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Table 4. Ellagitannin Components in Four Strawberry Cultivars from Different Locations

	'Elsanta'			'Clery'		'Korona'		'Everest'
peak	Germany	Switzerland	Denmark	Germany	Switzerland	Germany	Denmark	Germany
12	[a]3.1 ± 0.6 bc	4.0 ± 0.5 b	3.0 ± 0.5 bc	3.7 ± 0.4 b	**7.0 ± 1.2 a**	1.9 ± 0.1 c	2.6 ± 0.8 bc	3.8 ± 0.4 b
13	1.5 ± 0.2 bc	2.0 ± 0.3 b	1.8 ± 0.3 b	1.9 ± 0.4 b	**3.6 ± 0.9 a**	0.8 ± 0.1 c	1.3 ± 0.4 bc	2.0 ± 0.4 b
14	3.2 ± 0.6 bcd	4.2 ± 0.4 b	3.4 ± 0.4 bc	3.8 ± 0.4 bc	**7.1 ± 1.3 a**	2.0 ± 0.2 d	2.6 ± 0.8 cd	4.0 ± 0.5 bc
15	4.4 ± 0.8 bc	6.8 ± 1.0 b	4.5 ± 0.8 bc	6.1 ± 1.1 b	**12.2 ± 2.3 a**	3.5 ± 0.4 c	4.4 ± 0.8 bc	4.4 ± 0.8 bc
16	2.7 ± 0.5 cd	3.4 ± 0.2 cd	4.0 ± 1.3 cd	5.5 ± 0.6 c	**8.5 ± 1 b**	1.5 ± 0.4 d	**4.5 ± 3.2 c**	13.9 ± 1 a
17	9.7 ± 1.2 de	**18.0 ± 1.1 b**	11.6 ± 2.3 cd	9.8 ± 0.8 de	**23.1 ± 2.8 a**	7.4 ± 0.8 e	11.4 ± 2.9 cde	15.3 ± 2.2 bc
18	22.9 ± 4.1 cd	34.5 ± 3.8 cd	25.2 ± 3.5 cd	60.0 ± 4.1 b	**87.7 ± 14.8a**	20.6 ± 2.1 c	**28.7 ± 8.3 d**	35.7 ± 4.9 c
19	3.5 ± 0.5 cd	5.7 ± 1.2 bc	4.9 ± 1 bcd	5.1 ± 0.9 bcd	7.2 ± 0.8 b	3.1 ± 0.3 d	**5.5 ± 2.1 bc**	9.5 ± 0.8 a
20	17.7 ± 4.2 a	18.9 ± 3.3 a	24.3 ± 5.3 a	17.2 ± 3.5 a	19.5 ± 4.3 a	15.4 ± 3.1 a	16.4 ± 3.5 a	16.6 ± 2.5 a
21	44.3 ± 6.3 bc	49.3 ± 4.4 ab	60.5 ± 15.1 a	39.8 ± 4.8 bc	39.5 ± 5.4 bc	31.2 ± 3.7 c	40.7 ± 8 bc	36.9 ± 2.5 bc
22	1.4 ± 0.5 a	1.3 ± 0.4 a	2.4 ± 1.4 a	1.9 ± 0.5 a	2.0 ± 0.3 a	0.8 ± 0.4 a	1.8 ± 0.7 a	2.2 ± 0.7 a
23	3.4 ± 0.7 cd	**6.8 ± 0.6 b**	3.6 ± 1.2 cd	2.8 ± 0.3 cd	**9.4 ± 1.2 a**	2.1 ± 0.3 d	4.2 ± 1.4 cd	4.5 ± 1.2 c
24	3.3 ± 0.4 de	**5.3 ± 0.4 bc**	3.5 ± 0.5 de	6.0 ± 0.4 b	**12.3 ± 1.5 a**	2.4 ± 0.2 d	3.9 ± 0.9 cd	4.1 ± 0.6 cd
25	17.2 ± 2.2 de	**27.0 ± 1.7 bc**	17.1 ± 2.3 de	31.1 ± 2.4 b	**62.5 ± 7.6 a**	12.8 ± 0.9 e	19.4 ± 3.7 cde	22.1 ± 4 cd
26	6.5 ± 0.5 c	9.4 ± 1.5 b	7.1 ± 1.3 bc	8.1 ± 0.7 bc	**16.2 ± 1.6 a**	3.6 ± 0.5 d	5.7 ± 1.4 cd	6.1 ± 1.1 c
sum	144.9 ± 16.7 cd	196.4 ± 42.2 bc	177.1 ± 31.2 bc	202.9 ± 17.1 b	**317.8 ± 42.2 a**	109.0 ± 9.7 d	152.8 ± 33.5 bcd	181.1 ± 17.2 bc

[a]Data are expressed as the mean ± standard deviation ($n = 6$). Means within the same row followed by different letters were significantly different at $p \leq 0.01$ and are highlighted in bold. Data are expressed as micrograms of ellagic acid equivalents per gram of fresh weight.

Table 5. Percentage Distribution of Proanthocyanidin Components in Four Strawberry Cultivars from Different Locations[a]

	'Elsanta'			'Clery'		'Korona'		'Everest'
peak	Germany	Switzerland	Denmark	Germany	Switzerland	Germany	Denmark	Germany
1	1.4 ± 0.2 ab	1.7 ± 0.4 a	1.1 ± 0.3 b	1.9 ± 0.2 a	1.7 ± 0.3 a	1.4 ± 0.1 ab	1.4 ± 0.3 ab	1.9 ± 0.2 a
2	0.3 ± 0.0 a	0.4 ± 0.1 a	0.3 ± 0.1 a	0.5 ± 0.1 a	0.4 ± 0.1 a	0.3 ± 0.0 a	0.4 ± 0.1 a	0.4 ± 0.1 a
3	0.2 ± 0.0 b	0.2 ± 0.1 b	0.1 ± 0.1 b	0.5 ± 0.1 a	0.4 ± 0.1 a	0.3 ± 0.1 b	0.3 ± 0.1 b	0.5 ± 0.1 a
4	1.0 ± 0.1 bc	1.0 ± 0.2 bc	0.9 ± 0.2 bc	1.4 ± 0.2 ab	1.2 ± 0.2 abc	0.8 ± 0.1 c	1.2 ± 0.3 abc	1.5 ± 0.3 a
5	2.2 ± 0.4 a	2 ± 0.2 a	2.6 ± 1.2 a	2.3 ± 0.3 a	2.2 ± 0.6 a	1.7 ± 0.2 a	1.7 ± 0.4 a	2.0 ± 0.4 a
6	10.9 ± 1.0 b	11.8 ± 0.7 ab	11.1 ± 2 ab	13.2 ± 1.6 a	12.2 ± 2.3 ab	9.9 ± 0.9 b	12.3 ± 3.1 ab	13.1 ± 4 a
7	12.4 ± 0.7 abc	13.2 ± 0.4 ab	12.6 ± 0.5 abc	13.6 ± 0.3 a	12.9 ± 0.3 abc	11.7 ± 0.7 c	12.1 ± 1.2 bc	12.6 ± 0.6 abc
8	27.3 ± 1.2 a	26.6 ± 1.3 a	27.3 ± 4.2 a	26.5 ± 1.7 a	25.3 ± 1.2 a	27.2 ± 1 a	25.8 ± 1.8 a	23.5 ± 1.8 a
9	2.1 ± 0.6 b	2.2 ± 0.7 b	2 ± 0.3 b	3.6 ± 0.6 ab	4 ± 0.5 a	2.9 ± 0.6 ab	2.7 ± 1 ab	3.2 ± 1.2 ab
10	6.7 ± 0.5 a	6.5 ± 1.1 a	6.5 ± 1.6 a	6.1 ± 1.2 a	6.6 ± 1.5 a	6.6 ± 1 a	6.5 ± 1.4 a	6.2 ± 1.2 a
11	35.6 ± 2.3 a	34.5 ± 1.1 ab	35.2 ± 3.2 a	30.6 ± 1.2 b	34.5 ± 1.4 ab	37.2 ± 1.5 a	35.7 ± 3 a	35.1 ± 2.1 a
∑ peak area	6.13×10^9 b	4.67×10^9 c	7.65×10^9 a	2.31×10^9 d	2.38×10^9 d	3.17×10^9 d	2.63×10^9 d	3.02×10^9 d
% peak area	100.0	76.2	124.8	38.8	37.7	49.3	42.9	51.7

[a]Data are expressed as the mean ± standard deviation ($n = 6$). Means within the same row followed by different letters were significantly different at $p \leq 0.01$.

higher ET levels than those grown in Germany. It is notable that the ACMS method discriminated between the samples on the basis of cultivar and location and was generally accurate in highlighting the trend toward increased ET content. However, it is clear that the alterations in ET/PAC balance are not a simple matter of latitude and may result from differing climatic conditions or agronomic practices at the different sites Of course, this study is only indicative as the data are from only one year and require further research to tease out influences due to differences in agronomic procedures and local climatic conditions.

One of the main observations of the ACMS study was that the relative levels of signals characteristic of PACs and ETs differed in cultivars grown in different locations in Europe. The LC-MS analysis confirmed these differences in ET/PAC balance and suggested that the levels of PACs in individual cultivars were relatively unaffected by the latitude of growth but that the ET composition was more plastic in response. Previous work has shown significant environmental effects on PAC composition in strawberry cultivars grown in northern and southern Italy,[48] but the differences in composition (i.e., catechin/epicatechin ratio and mDP) were not accompanied by altered total PAC content. However, although this study also indicated higher flavonol content in the northern cultivars, the levels of ellagitannins were not studied.

The reasons for the observed alteration in ET/PAC balance are not known. The effects of latitude on phenolic composition have been discussed in a recent review.[49] A trend toward higher anthocyanin content with elevated growth temperature has been noted in strawberries,[45] but it is difficult to factor out other influences such as differences in rainfall, sunlight, day length, etc. A study of raspberries (cv. 'Glen Ample') grown under tightly controlled conditions where only temperature was altered showed that levels of the main ellagitannins (sanguiin H-6 and

lambertianin C) increased with increasing temperature.[50] These results are in agreement with temperature studies on strawberry, where ETs were lower in fruit grown under cooler conditions.[3] Allowing for considerable cultivar-dependent variation, the ellagitannin levels of the strawberry fruit in our study were generally higher in Switzerland and Denmark thanin Germany. Therefore, the average temperatures during fruit development in Switzerland, Denmark, and Germany (17.4, 14.2, and 16.4 °C, respectively) do not support a simple role for temperature in ET levels in these fruit. Mean fruit weight was increased by lower growth temperature without affecting dry matter.[43,51] Increased fruit size may increase the ratio of flesh to achenes. As the content of ET compounds can be up to 100 times higher in achenes than in flesh,[53] this could reduce ET levels. After consideration, our results may be influenced the effect of temperature through relative achene content or perhaps achene size. ETs differ in their spatial location, and ET composition may differ at the organ level (e.g., many castalagin-like ETs occur mainly in the seeds, whereas agrimoniin-like ETs are mainly located in the receptacle[54]). Therefore, further research is necessary to clarify the occurrence of ET distribution in achenes and flesh and to elucidate the role of temperature.

The effect of environmental stresses on the levels of different polyphenol components must be controlled at the biosynthetic level. ET biosynthesis remains refractory,[55] but ETs are believed to originate from gallic acid, which is formed from shikimate. Proanthocyanidins arise from catechin units, and therefore regulation of their biosynthesis must occur at different levels of the biosynthetic pathway. Indeed, the correlation of anthocyanin content with PAC content but not with ET content suggests differential control of accumulation or biosythesis. Ellagitannins and proanthocyanidins are generally synthesized early in fruit development[48,54] and tend to decline in fresh weight terms during ripening. In contrast to the majority of the ETs, PACs are found both in the flesh and in achenes.[53,54]

ET levels contribute substantially to antioxidative capacity in strawberry[4] and are second only to vitamin C levels. Various studies have suggested that ETs may have potentially important bioactivities.[56,57] Therefore, variation in ET content and composition may be an important criterion for selection of fruits for health benefits. Monitoring phenolic, and tannin, composition of fruit grown under different conditions using high-throughput techniques such as ACMS will help us understand the interplay of genetic or environmental influences to aid in the selection of new cultivars with enhanced qualities for a changing climate.

■ ASSOCIATED CONTENT

Supporting Information

Additional figure. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Hannum, S. M. Potential impact of strawberries on human health: a review of the science. *Crit. Rev. Food Sci.* **2004**, *44*, 1−17.

(2) Seeram, N. P. Berry fruits: compositional elements, biochemical activities, and the impact of their intake on human health, performance, and disease. *J. Agric. Food Chem.* **2008**, *56*, 627−629.

(3) Tomás-Barberán, F. A.; Espin, J. C. Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. *J. Sci. Food Agric.* **2001**, *81*, 853−876.

(4) Aaby, K.; Ekeberg, D.; Skrede, G. Characterization of phenolic compounds in strawberry (*Fragaria* × *ananassa*) fruits by different HPLC detectors and contribution of individual compounds to total antioxidant capacity. *J. Agric. Food Chem.* **2007**, *55*, 4395−4406.

(5) Buendía, B.; Gil, M. I.; Tudela, J. A.; Gady, A. L.; Medina, J. J.; Soria, C.; López, J. M.; Tomás-Barberán, F. A. HPLCMS analysis of proanthocyanidin oligomers and other phenolics in 15 strawberry cultivars. *J. Agric. Food Chem.* **2010**, *58*, 3916−3926.

(6) Seeram, N. P.; Lee, R.; Scheuller, H. S.; Heber, D. Identification of phenolic compounds in strawberries by liquid chromatography electrospray ionization mass spectroscopy. *Food Chem.* **2006**, *97*, 1−11.

(7) Törrönen, R. Sources and health effects of dietary ellagitannins. In *Chemistry and Biology of Ellagitannins: An Underestimated Class of Bioactive Plant Polyphenols*; Quideau, S., Ed.; World Scientific Publishing: Singapore, 2009; pp 298−319.

(8) Beekwilder, J.; Jonker, H.; Meesters, P.; Hall, R. D.; van der Meer, I. M.; Ric de Vos, C. H. Antioxidants in raspberry: on-line analysis links antioxidant activity to a diversity of individual metabolites. *J. Agric. Food Chem.* **2005**, *53*, 3313−3320.

(9) Ross, H. A.; McDougall, G. J.; Stewart, D. Antiproliferative activity is predominantly associated with ellagitannins in raspberry extracts. *Phytochemistry* **2007**, *68*, 218−228.

(10) McDougall, G. J.; Ross, H. A.; Ikeji, M.; Stewart, D. Berry extracts exert different antiproliferative effects against cervical and colon cancer cells grown in vitro. *J. Agric. Food Chem.* **2008**, *56*, 3016−3023.

(11) Mullen, W.; McGinn, J.; Lean, M. E. J.; MacLean, M. R.; Gardner, P.; Duthie, G. G.; Yokota, T.; Crozier, A. Ellagitannins, flavonoids, and other phenolics in red raspberries and their contribution to antioxidant capacity and vasorelaxation properties. *J. Agric. Food Chem.* **2002**, *50*, 5191−5196.

(12) Grussu, D.; Stewart, D.; McDougall, G. J. Berry polyphenols inhibit α-amylase in vitro: identifying active components in rowanberry and raspberry. *J. Agric. Food Chem.* **2011**, *59*, 2324−2331.

(13) McDougall, G. J.; Kulkarni, N. N.; Stewart, D. Berry polyphenols inhibit pancreatic lipase activity in vitro. *Food Chem.* **2009**, *115*, 193−199.

(14) Kähkönen, M. P.; Hopia, A. I.; Heinonen, M. Berry phenolics and their antioxidant activity. *J. Agric. Food Chem.* **2001**, *49*, 4076−4082.

(15) Macheix, J. J.; Fleuriet, A.; Billot, J. *Fruit Phenolics*; CRC Press: Boca Raton, FL, 1990.

(16) Aaby, K.; Mazur, S.; Nes, A.; Skrede, G. Phenolic compounds in strawberry (*Fragaria* × *ananassa* Duch.) fruits: composition in 27 cultivars and changes during ripening. *Food Chem.* **2012**, *132*, 86−97.

(17) Bakker, J.; Bridle, P.; Bellworthy, S. J. Strawberry juice colour: a study of the quantitative and qualitative pigment composition of juices from 39 genotypes. *J. Sci. Food Agric.* **1994**, *64*, 31−37.
(18) Hernanz, D.; Recamales, A. F.; Meléndez-Martínez, A. J.; Gonzalez-Miret, M. L.; Heredia, F. J. Assessment of the differences in the phenolic composition of five strawberry cultivars (*Fragaria* × *ananassa* Duch.) grown in two different soilless systems. *J. Agric. Food Chem.* **2007**, *55*, 1846−1852.
(19) Olsson, M. E.; Ekvall, J.; Gustavsson, K. E.; Nilsson, J.; Pillai, D.; Sjoholm, I.; Svensson, U.; Akesson, B.; Nyman, M. G. L. Antioxidants, low molecular weight carbohydrates, and total antioxidant capacity in strawberries (*Fragaria* × *ananassa*): effects of cultivar, ripening, and storage. *J. Agric. Food Chem.* **2004**, *52*, 2490−2498.
(20) Wang, S. Y. Antioxidant capacity and phenolic content of berry fruits as affected by genotype, preharvest conditions, maturity, and postharvest handling. In *Berry Fruit: Value-Added Products for Health Promotion (Food Science and Technology)*; Zhao, Y., Ed.; Routledge Chapman and Hall: Germany, 2007; pp 147−186.
(21) Josuttis, M.; Carlen, C.; Crespo, P.; Nestby, R.; Toldam-Andersen, T. B.; Dietrich, H.; Krüger, E. A Comparison of bioactive compounds of strawberry fruit from Europe affected by genotype and latitude. *J. Berry Res.* **2012**, *2*, 73−95.
(22) McDougall, G. J.; Martinussen, I.; Junttila, O.; Verrall, S.; Stewart, D. Assessing the influence of genotype and temperature on polyphenol composition in cloudberry (*Rubus chamaemorus* L.) using a novel mass spectrometric method. *J. Agric. Food Chem.* **2011**, *59*, 10860−10868.
(23) Josuttis, M.; Dietrich, H.; Treutter, D.; Will, F.; Linnemannstöns, L.; Krüger, E. Solar UVB response of bioactives in strawberry (*Fragaria* × *ananassa* Duch. L.): a comparison of protected and open-field cultivation. *J. Agric. Food Chem.* **2010**, *58*, 12692−12702.
(24) Giusti, M. M.; Wrolstad, R. E. Characterization and measurement of anthocyanins by UV-visible spectroscopy. In *Current Protocols in Food Analytical Chemistry*; Wrolstad, R. E., Acree, T. E., An, H., Decker, E. A., Penner, M. H., Reid, D. S., Schwartz, S. J., Shoemaker, C. F., Sporns, P., Eds.; Wiley: New York, 2001; p 1.
(25) Singleton, V. L.; Orthofer, R.; Lamuela-Raventós, R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In *Methods in Enzymology Oxidants and Antioxidants Part A, Vol.*, 299; Lester, P., Ed.; Academic Press: San Diego, CA, 1999; pp 152−178.
(26) Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Med.* **1999**, *26*, 1231−1237.
(27) Huang, D.; Ou, B.; Hampsch-Woodill, M.; Flanagan, J. A.; Prior, R. L. R. High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. *J. Agric. Food Chem.* **2002**, *50*, 4437−4444.
(28) Huang, D.; Ou, B.; Prior, R. L. R. The chemistry behind antioxidant capacity assays. *J. Agric. Food Chem.* **2005**, *53*, 1841−1856.
(29) Deighton, N.; Brennan, R.; Finn, C.; Davies, H. V. Antioxidant properties of domesticated and wild *Rubus* species. *J. Sci. Food Agric.* **2000**, *80*, 1307−1313.
(30) McDougall, G. J.; Martinussen, I.; Stewart, D. Towards fruitful metabolomics: high throughput analyses of polyphenol composition in berries using direct infusion mass spectrometry. *J. Chromatogr., B* **2008**, *871*, 362−369.
(31) Stewart, D.; McDougall, G. J.; Sungurtas, J.; Verall, S.; Graham, J.; Martinussen, I. Metabolomic approach to identifying bioactive compounds in berries: advances toward fruit nutritional enhancement. *Mol. Nutr. Food Res.* **2007**, *51*, 645−651.
(32) Gasperotti, M.; Masuero, D.; Vrhovsek, U.; Guella, G.; Mattivi, F. Profiling and accurate quantification of *Rubus* ellagitannins and ellagic acid conjugates using direct UPLC-Q-TOF HDMS and HPLC-DAD analysis. *J. Agric. Food Chem.* **2010**, *58*, 4602−4616.
(33) Mullen, W.; Yokota, T.; Lean, M. E. J.; Crozier, A. Analysis of ellagitannins and conjugates of ellagic acid and quercetin in raspberry fruits by LCMSn. *Phytochemistry* **2003**, *64*, 617−624.
(34) Gu, L.; Kelm, M. A.; Hammerstone, J. F.; Beecher, G. R.; Holden, J.; Haytowitz, D. B.; Prior, R. L. R. Screening of foods containing proanthocyanidins and their structural characterization using LCMS/MS and thiolytic degradation. *J. Agric. Food Chem.* **2003**, *51*, 7513−7521.
(35) Määttä-Riihinen, K. R.; Kamal-Eldin, A.; Törrönen, A. R. Identification and quantification of phenolic compounds in berries of *Fragaria* and *Rubus* species (family Rosaceae). *J. Agric. Food Chem.* **2004**, *52*, 6178−6187.
(36) Gu, L.; Kelm, M. A.; Hammerstone, J. F.; Beecher, G. R.; Holden, J.; Haytowitz, D. B.; Prior, R. L. R. Screening of foods containing proanthocyanidins and their structural characterization using LCMS/MS and thiolytic degradation. *J. Agric. Food Chem.* **2003**, *51*, 7513−7521.
(37) Stringano, E.; Cramer, R.; Hayes, W.; Smith, C.; Gibson, T.; Mueller-Harvey, I. Deciphering the complexity of sainfoin (*Onobrychis viciifolia*) proanthocyanidins by MALDI-TOF mass spectrometry with a judicious choice of isotope patterns and matrices. *Anal. Chem.* **2011**, *83*, 4147−4153.
(38) Tulipani, S.; Mezzetti, B.; Capocasa, F.; Bompadre, S.; Beekwilder, J.; de Vos, C. H. R.; Capanoglu, E.; Bovy, A. G.; Battino, M. Antioxidants, phenolic compounds, and nutritional quality of different strawberry genotypes. *J. Agric. Food Chem.* **2008**, *56*, 696−704.
(39) Capocasa, F.; Scalzo, J.; Mezzetti, B.; Battino, M. Combining quality and antioxidant attributes in the strawberry: the role of genotype. *Food Chem.* **2008**, *111*, 872−878.
(40) Gil, M. I.; Holcroft, D. M.; Kader, A. A. Changes in strawberry anthocyanins and other polyphenols in response to carbon dioxide treatments. *J. Agric. Food Chem.* **1997**, *45*, 1662−1667.
(41) Diamanti, J.; Sguigna, V.; Mezzetti, B.; Faedi, W.; Maltoni, M. L.; Denoyes, B.; Chartier, P.; Petit, A. European small berries genetic resources, GENBERRY: testing a protocol for detecting fruit nutritional quality in EU strawberry germplasm collections. *Acta Hortic.* **2012**, *926*, 33−37.
(42) Davik, J.; Bakken, A. K.; Holte, K.; Blomhoff, R. Effects of genotype and environment on total anti-oxidant capacity and the content of sugars and acids in strawberries (*Fragaria* × *ananassa* Duch.). *J. Hortic. Sci. Biotechnol.* **2006**, *81*, 1057−1063.
(43) Josuttis, M.; Dietrich, H.; Patz, C. D.; Krüger, E. Effects of air and soil temperatures on the chemical composition of fruit and agronomic performance in strawberry (*Fragaria* × *ananassa* Duch.). *J. Hortic. Sci. Biotechnol.* **2011**, *86*, 415−421.
(44) Terry, L. A.; Chope, G. A.; Bordonaba, J. G. Effect of water deficit irrigation and inoculation with *Botrytis cinerea* on strawberry (*Fragaria* × *ananassa*) fruit quality. *J. Agric. Food Chem.* **2007**, *55*, 10812−10819.
(45) Wang, S. Y.; Zheng, W. Effect of plant growth temperature on antioxidant capacity in strawberry. *J. Agric. Food Chem.* **2001**, *49*, 4977−4982.
(46) Tulipani, S.; Marzban, G.; Herndl, A.; Laimer, M.; Mezzetti, B.; Battino, M. Influence of environmental and genetic factors on health-related compounds in strawberry. *Food Chem.* **2011**, *124*, 906−913.
(47) Crespo, P.; Bordonaba, J. G.; Terry, L. A.; Carlen, C. Characterisation of major taste and health-related compounds of four strawberry genotypes grown at different Swiss production sites. *Food Chem.* **2010**, *122*, 16−24.
(48) Carbone, C.; Preuss, A.; De Vos, R. C. H.; D'Amico, E.; Perrotta, G.; Bovy, A. G.; Martens, S.; Rosati, C. Developmental, genetic and environmental factors affect the expression of flavonoid genes, enzymes and metabolites in strawberry fruits. *Plant, Cell Biol.* **2009**, *32*, 1117−1131.
(49) Jaakola, L.; Hohtola, A. Effect of latitude on flavonoid biosynthesis in plants. *Plant, Cell Environ.* **2010**, *33*, 1239−1247.
(50) Remberg, S. F.; Sønsteby, A.; Aaby, K.; Heide, O. M. Influence of postflowering temperature on fruit size and chemical composition of Glen Ample raspberry (*Rubus idaeus* L.). *J. Agric. Food Chem.* **2010**, *58*, 9120−9128.
(51) Wang, S. Y.; Camp, M. J. Temperatures after bloom affect plant growth and fruit quality of strawberry. *Sci. Hortic.* **2000**, *85*, 183−199.
(52) Krüger, E.; Josuttis, M.; Nestby, R.; Toldam-Andersen, T. B.; Carlen, C.; Mezzetti, B. Influence of growing conditions at different

latitudes on strawberry growth performance, yield and quality. *J. Berry Res.* **2012**, *2*, 143−157.
(53) Aaby, K.; Skrede, G.; Wrolstad, R. E. Phenolic composition and antioxidant activities in flesh and achenes of strawberries (*Fragaria* × *ananassa*). *J. Agric. Food Chem.* **2005**, *53*, 4032−4040.
(54) Fait, A.; Hanhineva, K.; Beleggia, R.; Dai, N.; Rogachev, I.; Nikiforova, V. J.; Fernie, A. R.; Aharoni, A. Reconfiguration of the achene and receptacle metabolic networks during strawberry fruit development. *Plant Physiol.* **2008**, *148*, 730−750.
(55) Gross, G. G. Biosynthesis of ellagitannins: old ideas and new solutions. In *Chemistry and Biology of Ellagitannins: An Underestimated Class of Bioactive Plant Polyphenols*; Quideau, S., Ed.; World Scientific Publishing: Singapore, 2009; pp 94−118.
(56) Landete, J. M. Ellagitannins, ellagic acid and their derived metabolites: a review about source, metabolism, functions and health. *Food Res. Int.* **2011**, *44*, 1150−1160.
(57) Vrhovsek, U.; Guella, G.; Gasperotti, M.; Pojer, E.; Zancato, M.; Mattivi, F. Clarifying the identity of the main ellagitannin in the fruit of the strawberry, *Fragaria vesca* and *Fragaria ananassa* Duch. *J. Agric. Food. Chem.* **2012**, *60*, 2507−2516.