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Towards fruitful metabolomics: High throughput analyses of polyphenol composition in berries using direct infusion mass spectrometry☆

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ABSTRACT

Tannin-enriched extracts from raspberry, cloudberry and strawberry were analysed by liquid chromatography–mass spectrometric (LC–MS) techniques. The raspberry and cloudberry extracts contained a similar mixture of identifiable ellagitannin components and ellagic acid. However, the strawberry extract contained a complex mixture of ellagitannin and proanthocyanidin components that could not be adequately resolved to allow identification of individual peaks. Nevertheless, the negative ESI-MS spectra obtained by direct infusion mass spectrometric (DIMS) analysis described the diversity of these samples. For example, the predominance of signals associated with Lambertianin C in cloudberry and Sanguiin H6 in raspberry tannin extracts could be discerned and the diversity of signals from procyanidin and propelargonidin oligomers could be identified in the strawberry extract. The dose response for the main ellagitannin-derived signals in the raspberry tannin sample revealed a saturation effect probably due to ion suppression effects in the ion trap spectrometer. Nevertheless, DIMS spectra of whole berry extracts described qualitative differences in ellagitannin-derived peaks in raspberry, cloudberry and strawberry samples. In addition, positive mode DIMS spectra illustrated qualitative differences in the anthocyanin composition of berries of progeny from a raspberry breeding population that had been previously analysed by LC–MS. This suggests that DIMS could be applied to rapidly assess differences in polyphenol content, especially in large sample sets such as the progeny from breeding programmes.

1. Introduction

The agricultural policies of the developed world have meant that, for these countries at least, food is plentiful but changing eating patterns have seen an increase in the consumption of ready-made meals and food elevated with respect to sugar and fat [1]. The knock-on effect of this is evident in the rapidly increasing level of obesity in the western world with 20% of males and 25% of females now classified as obese in the United States [2]. Associated with this and the related dietary shift are increases in the incidence of degenerative diseases such as atherosclerosis, some cancers etc. [3]. Fundamental, clinical and epidemiological research into the basal causes and consequences of these diseases has highlighted the gross and specific benefits of including a significant level of fruit and vegetable within the diet. Indeed, this broad evidence has translated through to inform governmental policy with the UK five-a-day and the Swedish half-a-kilo-a-day programme clear examples of this [4].

This accretion of supportive evidence for the association between fruit and vegetable consumption and improved general health and well being, and reduced incidence of degenerative diseases and conditions has led to many more fundamental studies into the phytochemical components driving these effects. The direct effect of plant-derived vitamins on human health has already been comprehensively covered elsewhere [5–9] but it is the other components, specifically the polyphenolics, that are currently under intense focus with respect to their bioefficacy [10–16].

Polyphenols are ubiquitous in plants and, with respect to dietary plant sources, particularly prevalent in many fruit species and in particular in the common soft fruit such as strawberries, blackcurrant, raspberries etc. [17–21]. Within these common fruit, the polyphenols encompass the anthocyanins, flavanols, flavanals, flavanones, isoflavones, cinnamic acids, phenolic acids, catechins, proanthocyanidins and ellagitannins (Fig. 1): a wide diversity of chemical structures when the subsequent substitution by sugars,

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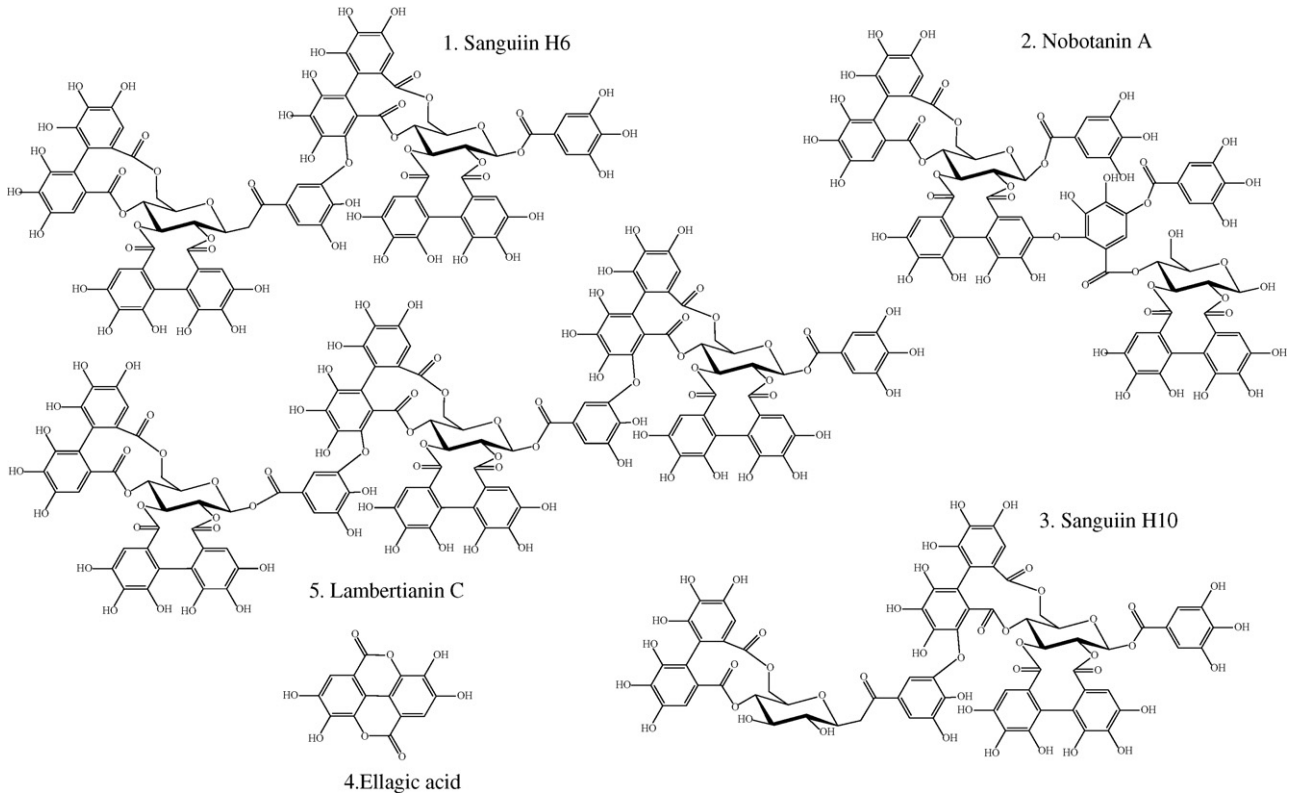

Fig. 1. Selected ellagitannin structures.

acyl, etc. groups are also included [22–24]. As a result of the putative potent health benefits of these compounds, crop (including fruit) breeding has undergone a paradigm shift with the adoption of global metabolite screening rapidly becoming an integral part of the process [25–27]. Indeed, the diversity of the chemical structures describing these chemotypes means that it is only over the last few years with technological advances, particularly in MS detection and deconvolution, that this approach has been used in earnest [28–30].

As part of our research aims, we have explored the uses and advantages of broad screen LC–MS metabolite profiling in select soft fruits. Coupled with appropriate genomics research effort, this should facilitate a reduction in the time taken to enhance both the nutritional and organoleptic characteristics of a target crop.

2. Experimental

2.1. Plant material and extraction

Cloudberries (*Rubus chamaemorus* L.) were obtained from Dr Harri Kokko, University of Kuopio, strawberries (*Fragaria ananassa* cv. Elsanta) were purchased from a supermarket in Dundee and raspberries (*Rubus idaeus* L. cv. Glen Ample) were obtained from farmers around Dundee. Berries were also sampled from the segregating raspberry progeny of a cross between the European cv. Glen Moy and the North American cv. Latham grown in two different field locations as described previously [27].

The fruits were extracted and polyphenol-rich fractions obtained by the methods described previously [16]. Briefly, frozen fruit was homogenised in a Waring Blender (6 × 20 s at full power) using an equal volume to weight of ice-cold 0.2% (v/v) acetic acid in water. The extract was filtered through a glass sinter and applied to C18 solid phase extraction units (Strata C18-E, GIGA units, Phenomenex Ltd., UK) pre-washed in 0.2% (v/v) acetic acid in acetonitrile then pre-equilibrated in 0.2% (v/v) acetic acid in water. Unbound material, which contained the free sugars, organic acids and vitamin C, was discarded. After extensive washes with 0.2% (v/v) acetic acid in water, the polyphenol-enriched bound extracts were eluted with acetonitrile. The C18-bound extracts were evaporated to dryness in a Speed-Vac (Thermo Fisher, Basingstoke, UK). Samples from the raspberry cross progeny were obtained by a similar procedure [27].

Tannin-rich fractions were obtained by sorption to Sephadex LH-20 in aqueous ethanol and selective elution with aqueous acetone using an established method (Tannins Handbook kindly made available at www.users.muohio.edu/hagermae/tannin.pdf). Briefly, a column of Sephadex LH-20 was washed in 80% (v/v) ethanol/water then 50% (v/v) acetone/water before being equilibrated with three bed volumes of 80% ethanol. The berry C18 extracts were dissolved in 80% ethanol, applied to the column and the run-through plus a bed volume of 80% ethanol collected as the unbound fraction. With strawberries and raspberries, this material was bright red as it contained the bulk of the anthocyanins. The column was washed with three bed volumes of 80% ethanol. The bound fraction was eluted with three bed volumes of 50% acetone. The unbound and bound fractions were evaporated to near dryness then stored frozen. All data come from single extraction of each fruit sample with, at least, triplicate technical replicates of individual assays.

2.2. Anthocyanin and phenol assays

The total anthocyanin concentration was estimated by a pH differential absorbance method [31]. The absorbance value was related to anthocyanin content using the molar extinction coefficient calculated for cyanidin-3-*O*-glucoside (purchased

from ExtraSynthese Ltd., Genay, France). Phenol content was measured using a modified Folin–Ciocalteau method [31]. Phenol contents were estimated from a standard curve of gallic acid.

2.3. Liquid chromatography–mass spectrometry (LC–MS)

Samples (containing 20 μg gallic acid equivalents by Folin assay) were analysed on a LCQ–DECA system, comprising Surveyor autosampler, pump and photo diode array detector (PDAD) and a ThermoFinnigan mass spectrometer iontrap controlled by the XCALIBUR software (version 1.4, ThermoFinnigan, Hemel Hempstead, UK). The PDAD scanned three discrete channels at 280, 365 and 520 nm. Solvent A was 0.1% formic acid in ultra pure water and solvent B 0.1% formic acid in acetonitrile. Samples were eluted with a gradient of 95:5 solvent A:B at time = 0 min to 65:35 A:B at t = 60 min at a flow rate of 400 μl/min on a C18 column (Synergi Hydro C18 with polar end capping, 4.6 mm × 150 mm, Phenomenex Ltd., Macclesfield, UK). The LCQ–DECA LC–MS was fitted with an electrospray ionization interface and the samples were analysed in negative ion mode. There were two scan events; full scan analysis followed by data dependent MS/MS of the most intense ions. The data dependant MS/MS used collision energies (source voltage) of 45% in wideband activation mode. The MS detector was tuned against ellagic acid for negative mode and cyanidin glucoside for positive mode.

2.4. Direct infusion mass spectroscopy (DIMS)

The direct infusion MS (DIMS) technique was also carried out on the LCQ–DECA system. The sample was injected into the LC–MS mobile phase (200 μl/min of 50% acetonitrile containing 0.1% (v/v) formic acid) and then directly into the ESI source. ESI-MS spectra (positive or negative mode) were acquired from m/z 80–2000 for 2 min and each sample was followed by three blank injections (solvent only) to ensure no carry over between samples. The following parameters were used: capillary temperature: 275 °C, capillary voltage: 20 V, spray voltage: 5 kV, tube lens: −5 V, sheath gas: 70 arbitrary units and auxiliary gas: 15 arbitrary units. Triplicate injections were made in a randomized fashion and the peak heights of the relevant spectral peaks averaged. The peaks heights in blank spectra were subtracted.

3. Results and discussion

3.1. Analysis of tannin-rich extracts from berries

Ellagitannins are effective antioxidants and have been shown to have biological activities, such as anti-cancer properties, in their own right [16] but also as the dietary source of ellagic acid [32]. Tannin-rich extracts from raspberry, cloudberry and strawberry were prepared by adsorption to lipophilic Sephadex LH-20, analysed for phenol and anthocyanin content and were effectively free of anthocyanins. In this study, the composition of tannin-rich fractions was studied using LC–MS and the possibility of using rapid high through-put DIMS techniques to link mass spectral properties to metabolite diversity was examined.

The separation achieved by our standard HPLC method for berry polyphenols [33] was adequate to display the differences between the cloudberry and raspberry components. Slightly better separation of raspberry and cloudberry components could be achieved by a shorter but more complex gradient method but this did not afford any improvement in the separation of the components in the strawberry sample. As could be expected from their close taxonomic relationship, cloudberry and raspberry tannin extracts were

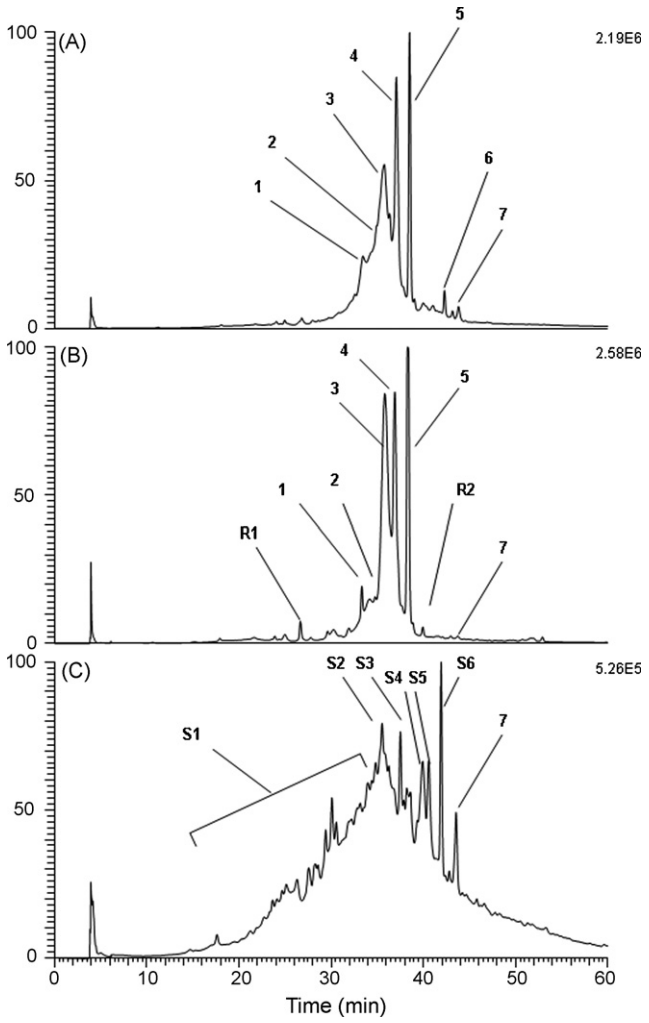

Fig. 2. UV traces of berry tannin samples. Part (A) is the cloudberry tannin extract, part (B) is the raspberry tannin extract and part (C) is the strawberry tannin extract. All show trace at A280. The peak numbers refer to Table 1. The full scale deflection is given in the top right corner of each trace.

similar, differing only in the qualitative amounts of certain components (Fig. 2, compare traces a and b; Table 1). The raspberry and cloudberry samples were predominantly composed of isomers of Sanguiin H6, Sanguiin H10 and Lambertinian C and ellagic acid [33–35] with cloudberry having comparably larger amounts of Lambertianin C. This component gives a characteristic doubly charged m/z signal at 1401.1 (true mass of 2804) which yields singly charged MS^2 fragment ions of 1868.9 and 1566.9, which appear anomalously larger than the apparent mass at 1401.1 [34,35]. It should be noted that the ellagitannin components produce many characteristic m/z signals as a result of in-source fragmentation of the main ion, which are often also present as MS^2 fragments. It is also possible that some of the peaks assigned as Sanguiin H6-like could be doubly charged ions representative of Lambertianin D (true mass 3740) but this ellagitannin component has not been identified out with blackberry [35]. Very minor amounts of two pedunculagin isomers (m/z 783.1; MS^2 481.1, 301.2) could be detected early in the gradient (at 17.7 and 24.1 min) in all three samples.

The strawberry sample contained a more complex mixture of ellagitannin and proanthocyanidin components (Fig. 2C; Table 1) with some peaks distinguishable in the smear between RT 18 and 40 min. Indeed, tannin-enriched fractions from lingonberry were

Table 1
Putative identities of peaks in LC–MS analysis of tannin samples

Peak	Min	PDA	*m/z* [M–H]	MS^2	Putative identity
1	33.45	275	**1567.1**, 934.2, 783.3, 633.1, 301.2	1264.9, 1234.9, 1102.8, 932.9, 897.0	Sanguiin H6
2	34.93	275	**1401.3**, 1868.8, 1566.9, 934.2, 633.1, 301.2	1866.9, 1566.9, 1250.2, 1234.9, 897.1	Lambertianin C
3	35.76	275	**1868.9**, 1566.9, 1234.9, **934.2**, 633.2, 301.2	1566.8, 1234.9, 933.0	Sanguiin H10
4	37.08	275	**1401.3**, 1868.8, 1566.9, 934.2, 633.1, 301.2	1866.9, 1566.9, 1250.2, 1234.9, 897.1	Lambertianin C
5	38.52	275	**1868.9**, 1566.9, 1234.9, **934.2**, 633.2, 301.2	1566.8, 1234.9, 933.0	Sanguiin H10
6	42.30	275	1868.9, **935.1**, 783.0, 301.2	897.8, 633.0, 301.3	Ellagitannin
7	43.82	370	**301.2**, 275.1	257.1	Ellagic acid
R1	26.67	275	1567.1, **783.1**, 301.2	1102.3, 933.0, 633.0	Sanguiin H6-like ellagitannin
R2	38.86	275	**1718.9**, 933.0, 859.2, 633.1, 301.2	1416.7, 1102.7, 1084.9, 782.9	Nobotannin A-like ellagitannin
S1	18 - 35	250-300	Not resolved	Not resolved	Mixture of ellagitannins and proanthocyanidins
S2	35.53	275	**935.0**, 301.2	633.0, 451.2, 301.2	Potentillin/casuarictin-like ellagitannin
S3	37.51	275	**935.0**, 301.2	633.0, 451.2, 301.2	Potentillin/casuarictin-like ellagitannin
S4	39.93	275	1868.8, **934.2**, 783.1, 301.2	896.9, 633.0, 451.0, 301.2	Sanguiin H10-like ellagitannin
S5	40.60	275	1868.8, **934.2**, 783.1, 301.2	896.9, 633.0, 451.0, 301.2	Sanguiin H10-like ellagitannin
S6	41.93	275	**1868.8**, 935.1, 783.0, 301.2	1566.9, 1264.8, 1084.9, 935.0, 897.1	Sanguiin H10-like ellagitannin

Bold *m/z* values were predominant ions and were selected for fragmentation. Underlined *m/z* values were the major MS^2 products. Components assigned as e.g. Sanguiin H10-like have similar *m/z* spectra but yield different MS^2 spectra to the authentic components.

also poorly resolved on LC–MS [36] and berry proanthocyanidins are difficult to separate using reverse phase methods so many workers have employed normal phase methods [37].

The ellagitannins from strawberry were structurally different from those in cloudberry and raspberry with a predominance of *m/zm/z* values at 935.1, suggestive of casuricitin-like and/or potentillin-like (galloyl diHHDP glucose) structures [35,38]. Certain peaks could be resolved with *m/zm/z* values consistent with proanthocyanidins previously identified in strawberry [39–41]. For example, *m/zm/z* signals at 577.1, 865.1, 1153.1, 1441.1 and 1729.0 are characteristic of procyanidin dimers, trimers, tetramers and pentamers [37] with fragmentation patterns consistent with previous studies (e.g. *m/z* 577 assigned to (epi)catechin–(epi)catechin; MS^2 of 533.0, 451.0, 424.9, 407.0 and 289.1; underlined values are the dominant fragments). In addition, *m/z* signals characteristic of propelargonidin proanthocyanidins (i.e. proanthocyanidins containing at least one (epi)afzelechin unit and (epi)catechin units) at 561.0, 833.0, 849.1, 1121.1, 1137.0, 1409.0 and 1425.0 could also be distinguished, although these were not well resolved into distinct peaks, possibly due to the presence of isomeric forms. These masses gave fragment ions (e.g. *m/z* 561 assigned to (epi)afzelechin–(epi)catechin; MS^2 of 435.1, 425.1, 407.0, 381.0, 329.0, 289.1 and 271.1) as reported previously [37].

The negative mode DIMS spectra effectively described the structural diversity of the three tannin samples (Fig. 3). In particular, the predominance of the Lambertianin C derived *m/z* signal at 1401.3 was apparent in the cloudberry spectrum (Fig. 3A). In fact, the Lambertinian C content of the raspberry sample was estimated at $15.3 \pm 3.3\%$ of the cloudberry sample content (by comparing the peak areas of three, presumably isomeric, peaks at *m/z* 1401 in three replicate samples) whereas the similar figure estimated by DIMS was $26.7 \pm 3.1\%$. This difference suggests that Lambertianin C is over-estimated by DIMS, possibly due to ionisation effects.

The strawberry sample yielded lower intensity signals from the ellagitannin components (301.2, 783.1, 935.1, 1568.9 and 1868.7) than the cloudberry or raspberry spectra (compare Fig. 3A–C). The strawberry extract contained a notably more intense signal at *m/z* 935.1 reflecting the higher amount of potentillin/casuricitin structures in strawberry. In addition, the signals at *m/z* 577.1, 865.1, 1153.1 and 1729.2 putatively assigned to procyanidin dimers, trimers, tetramers and pentamers (Fig. 3C) and propelargonidin oligomer-derived *m/z* signals at 561.0, 833.0, 849.1, 1121.1, 1137.0, 1409.0 and 1425.0 (Fig. 3) were also apparent in the strawberry DIMS spectra. However, the signals at 561.0 and 833.0 are obscured by other signals but the triple signals differing by 16 a.m.u. are high-

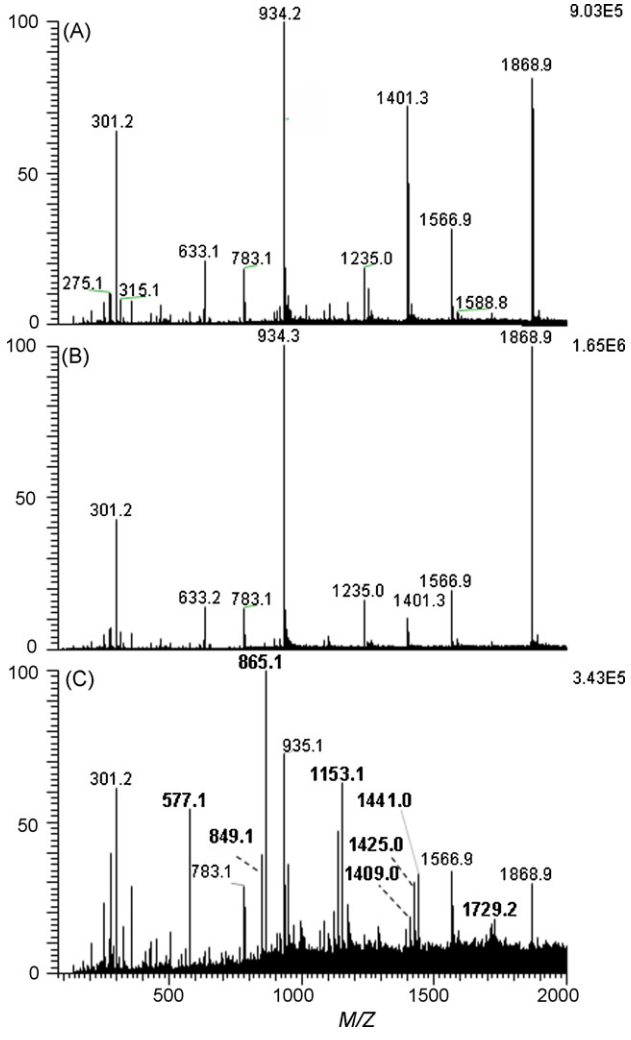

Fig. 3. DIMS Mass spectra of tannin samples. Part (A) is the DIMS spectra derived from the cloudberry tannin extract, part (B) is the DIMS spectra derived from the raspberry tannin extract and part (C) is the DIMS spectra derived from the strawberry tannin extract. The full scale deflection is given in the top right corner of each trace. The peaks annotated in bold (C) refer to the proanthocyanidin masses discussed in the text.

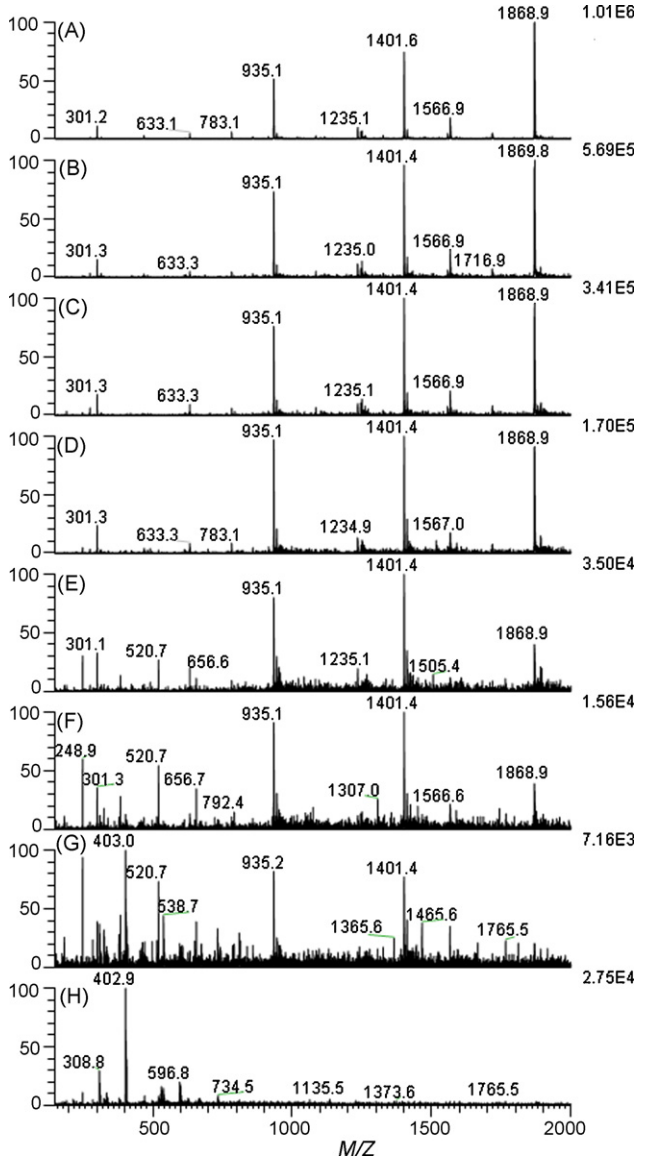

Fig. 4. DIMS spectra of dilutions of raspberry tannin extract. Part (A) denotes the ESI-MS spectra of the raspberry tannin sample at 10 μg injection; (B) 5 μg injection; (C) 2 μg injection; (D) 1 μg injection; (E) 200 ng injection; (F) 100 ng injection; (G) 50 ng injection; (H) blank. The full scale deflection is given in the top right corner of each trace.

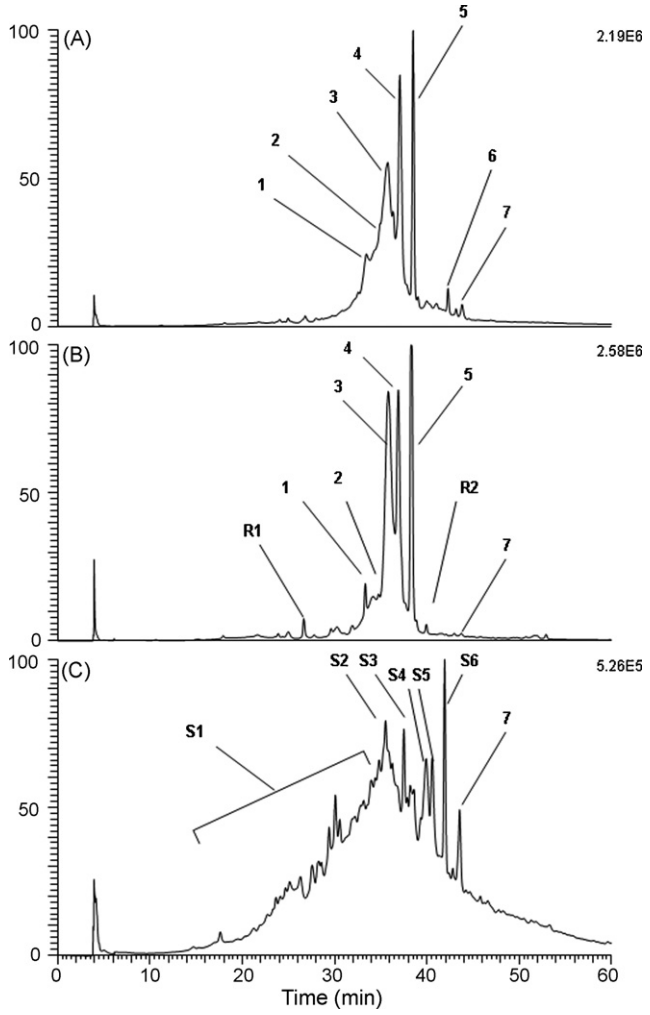

Fig. 5. MS Response curve for raspberry tannin extract. Each value is the average of three replicate samples ± standard error.

lighted at 1409.0, 1425.0 and 1441.1 and the triplet at 1121.1, 1137.0 and 1153.1 are also present.

Putative ellagitannin *m*/*z* signals could be detected at the equivalent of 50 ng phenol content in DIMS spectra of diluted raspberry tannin extract (Fig. 4A–H). The detector response was saturable with a greater response at lower amounts than at higher amounts of sample (Fig. 5). This signal dampening may be caused by ionization effects especially relevant to the type of ion trap MS used. Because the ion trap MS operates by capturing a set amount of ions, suppression of ionisation can readily occur and is likely to be more severe at higher concentrations. In addition, components which ionise more effectively will exert a higher exclusive pressure when present at higher concentrations. Nevertheless, the standard curve, although not linear, may be sufficiently robust to provide a means to quantify changes in ellagitannin content by DIMS.

DIMS spectra of whole raspberry and cloudberry extracts yielded characteristic ellagitannin signals (Fig. 6A and B) and ellagitannin-derived signals were present in strawberry extracts (Fig. 6C) but at lower levels that reflect the lower content of ellagitannins in strawberry [42,43]. Because some MS signals can arise due to in-source fragmentation of larger ellagitannin components, it is probably only valid to compare the DIMS signals at *m*/*z* 1401 and 1869 as indicators of Lambertianin C and Sanguiin H6 content. As the amount of ellagitannins applied is low, only the linear part of the standard curve is required (Fig. 5). The content of ellagitannins in the cloudberry extract was estimated at 0.81–1.02 μg per injection of 10 μg gallic acid equivalents of phenol content using the Sanguiin H6 signal for the lower value and the Lambertinian C signal for the higher value. Therefore, ellagitannin content was estimated at ~20% of total polyphenol content, which is low for cloudberry [42] but the use of only two *m*/*z* values underestimates content and the estimate should include contributions made by other signals. On this basis, the raspberry extract contained 56.2% and the strawberry sample 28.2% of the cloudberry Sanguiin H6 signal (*m*/*z* 1869). The raspberry extract contained 80% of the cloudberry Lambertianin C signal but the strawberry extract, as seen above, contained no detectable Lambertianin C. The apparently high content of Sanguiin H6 in strawberry seems anomalous as the peak looks comparably small but the spectral intensity is higher for this particular sample (Fig. 6C). This rapid DIMS method requires to be correlated and validated against other more exhaustive and time-consuming methods for analysing ellagitannin content and structure [44].

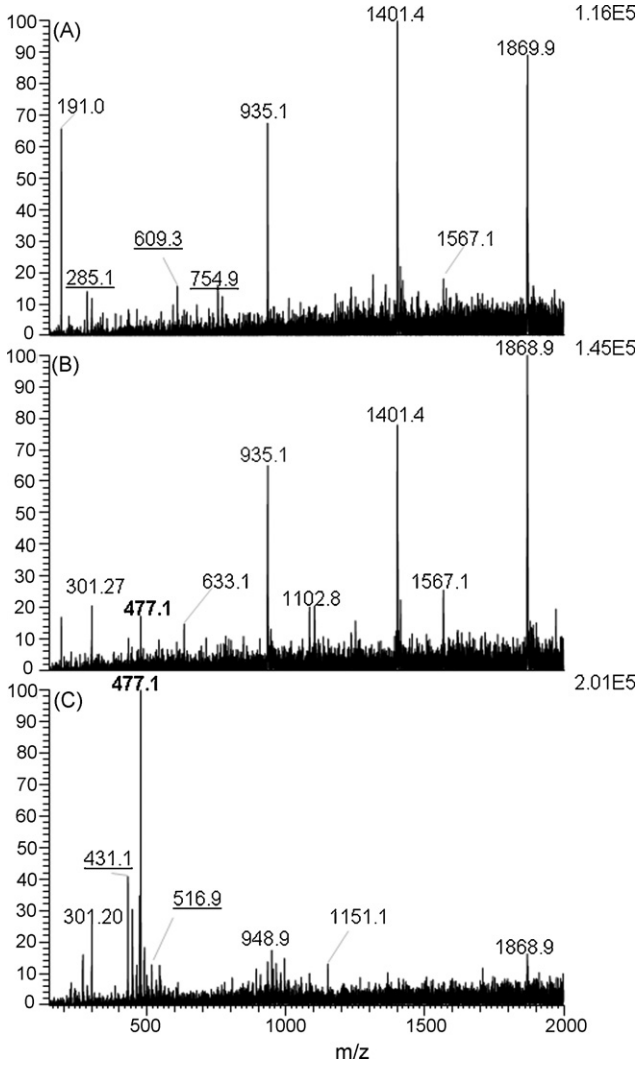

Fig. 6. DIMS spectra of raspberry, cloudberry and strawberry extracts part (A) is DIMS spectra of the whole raspberry extract; part (B) is DIMS spectra of the whole cloudberry extract; part (C) is DIMS spectra of the whole strawberry extract. Underlined masses are from anthocyanin components and those in bold represent a mass derived from quercetin glucuronide.

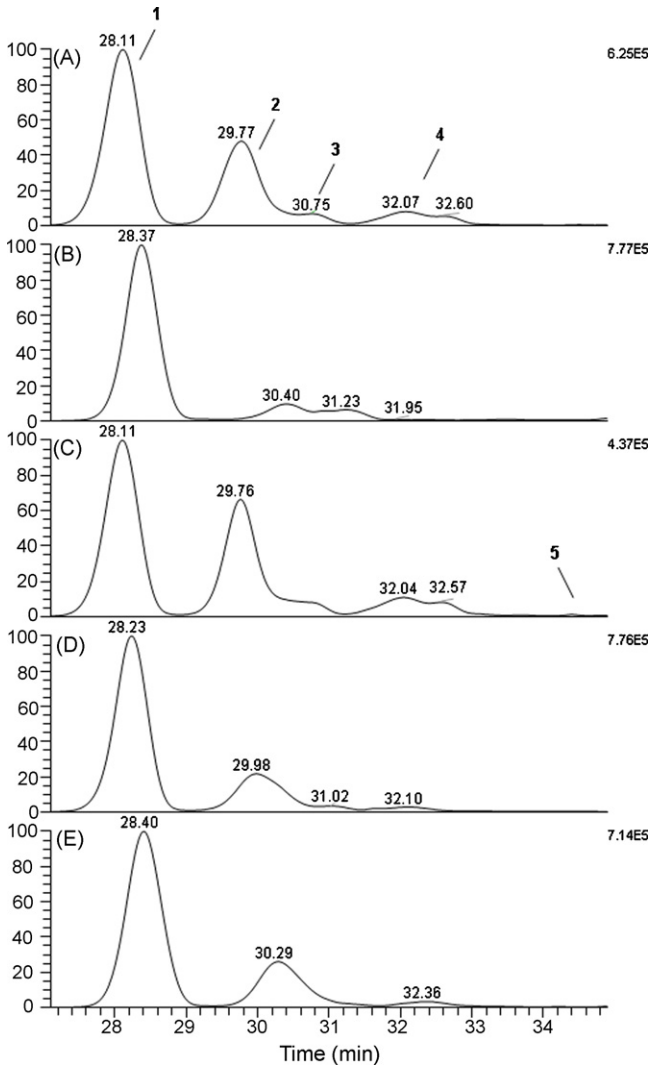

Fig. 7. LC–MS traces of anthocyanins from raspberry progeny. Part (A) is the trace at 520 nm of line 280 from H-field, part (B) is the trace at 520 nm of line 48 from H-field, part (C) is the trace at 520 nm of line 48 from B-field, part (D) is the trace at 520 nm of line 11 from H-field and part (E) is the trace at 520 nm of line 11 from B-field. The full scale deflection is given in the top right corner of each trace. Peaks 1–5 are discussed in the text.

In whole berry extracts, other signals are present that reflect the high anthocyanin content of raspberry [at *m*/*z* values of 609.2, 593.3, 754.9, 285.1 for cyanidin sophoroside, cyanidin rutinoside, cyanidin glucosyl rutinoside and the cyanidin aglycone produced by in-source fragmentation [33]] and strawberry [431.0, 516.8 and 269.1 for pelargonidin glucoside, pelargonidin malonylglucoside and pelargonidin aglycone produced by in-source fragmentation [40], even though it is generally accepted that anthocyanins ionize more readily and dominate positive mode DIMS spectra. Anthocyanin-related signals were absent from cloudberry, which reflects low or negligible anthocyanin content [42,43]. The strong signal at *m*/*z* 477.1 in strawberry and cloudberry arises from quercetin glucuronide, which like many flavonols, ionizes well in negative mode [45] and may dominate in negative mode DIMS.

3.2. Analysis of anthocyanin content in raspberry progeny

LC–MS analysis revealed considerable variation in anthocyanin composition between different raspberry progeny and in different field locations. Although only five peaks at 520 nm could be discerned (Fig. 7), eight anthocyanins could be detected in varying amounts by searching the MS data at specific characteristic *m*/*z* values (as described by McDougall et al. [33]). The major components were cyanidin-3-*O*-sophoroside (peak 1) and cyanidin-3-*O*-(2G)-glucosylrutinoside (peak 2) with smaller amounts of cyanidin-3-*O*-glucoside and pelargonidin-3-*O*-sophoroside (peak 3), cyanidin-3-*O*-rutinoside, pelargonidin-3-*O*-(2G)-glucosylrutinoside and pelargonidin-3-*O*-glucoside (peak 4) and pelargonidin-3-*O*-rutinoside (peak 5). The order of abundance was generally cyanidin-3-*O*-sophoroside > cyanidin-3-*O*-(2G)-glucosylrutinoside > cyanidin-3-*O*-glucoside > cyanidin-3-*O*-rutinoside > pelargonidin-3-*O*-sophoroside > pelargonidin-3-*O*-(2G)-glucosylrutinoside > pelargonidin-3-*O*-glucoside > pelargonidin-3-*O*-rutinoside with 20–50-fold differences in abundance between the most and the least abundant. For example, peak 1 eluting at 29.7 mins (identified as cyanidin-3-*O*-(2G)-glucosylrutinoside; *m*/*z* assigned to 757.1, MS^2 = 611.1 and **287.2**) was more abundant in line 280 > line 11 > line 48 in field H (compare Fig. 7A and B). However, berries from line 48 grown in field location B had much higher levels of this anthocyanin whereas line 11 had similar levels in both field locations (compare Fig. 7D and E).

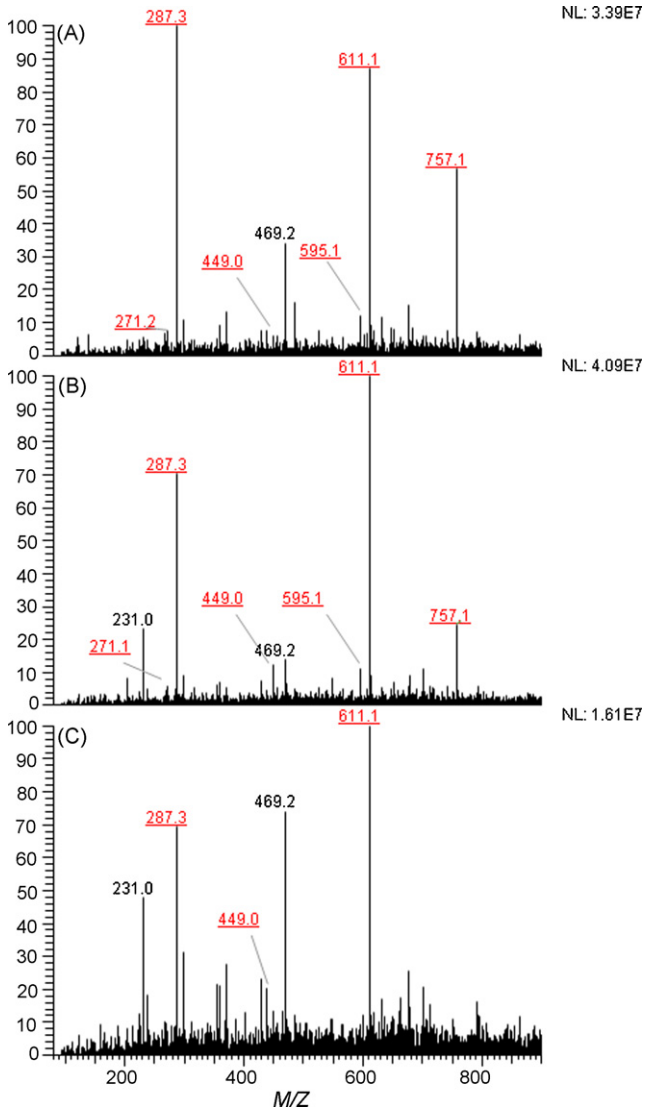

Fig. 8. Positive mode DIMS spectra of raspberry progeny samples. Part (A) is the DIMS spectra line 280 from H-field, part (B) is the DIMS spectra of line 11 from H-field and part (C) is the DIMS spectra line 48 from H-field. The full scale deflection is given in the top right corner of each trace. Underlined masses are from anthocyanin components.

Positive mode DIMS spectra of the same lines from field H also showed this qualitative variation in the *m*/*z* 757.1 signal, assigned to cyanidin-3-*O*-(2G)-glucosylrutinoside (Fig. 8). However, although qualitative differences in anthocyanin composition were described by DIMS, quantification of anthocyanins by LC–MS and DIMS does not concur. If DIMS was quantitatively accurate then the ratios of the amount of anthocyanin detected by LC–MS (μg cyanidin glucoside equivalents) over the peak height for each specific anthocyanin signal in DIMS (e.g. the anthocyanin response factor) should be similar for each sample. In fact, this ratio is much higher for cyanidin-3-*O*-(2G)-glucosylrutinoside in line H11 which suggests sample dependent responses (Table 2).

These sample dependent differences may be caused by ionization effects and, in particular, it is possible that other ionizing or non-ionizing components in samples influence ionization of the target components. It is notable that the FSD for DIMS spectra of line H48 was considerably lower than for lines H280 and H11 (Fig. 8) even though the FSD of the LC–MS traces for the lines were similar (Fig. 7), which may indicate ion suppression.

Table 2
Comparison of anthocyanin response for DIMS and LC–MS

Line	Anthocyanin response factor (DIMS peak height/μg)		
	CySoph	CyGRut	CyGlc
H280	1.66E+06	1.66E+06	1.08E+06
H11	2.50E+06	4.20E+06	2.06E+06
H48	1.20E+06	1.61E+07	2.18E+06

The extent of in-source fragmentation may be different for different anthocyanin structures. Indeed, in our experience, certain anthocyanins give higher levels of aglycone and intermediate fragmentation products than others. In-source fragmentation of certain anthocyanins will affect the apparent levels of other putative anthocyanin signals (e.g. cyanidin-3-*O*-(2G)-glucosylrutinoside (*m*/*z* 757.1) yields an in-source fragment at *m*/*z* 611.1 and both cyanidin-3-*O*-sophoroside (*m*/*z* 611.1) and cyanidin-3-*O*-rutinoside (*m*/*z* 595.2) yield in source fragments of 449.1). However, it is difficult to see how sample dependent differences in quantification could occur unless in source fragmentation is also subject to ion suppression.

These results illustrate that DIMS methods can be used to qualitatively describe the phytochemical diversity of berry polyphenols. The method has certain drawbacks (e.g. linearity of response, in-source fragmentation and predominance of certain readily ionisable components) but these can be overcome by characterisation of their influence through scrupulous regimes and the use of standards. DIMS is most useful as a means to rapidly assess variations in key ionisable polyphenol components resulting from specific treatments such as processing methods, cooking or gastrointestinal digestion. DIMS can probably only be employed semi-quantitatively as ion suppression effects cannot be fully ascertained in different samples and the analytical time required to run sufficient standards for full quantification of all component classes defeats the purpose of rapid analysis. Nevertheless, DIMS provides a rapid, high through-put, automatable and scaleable screen to assess phytochemical diversity which is particularly suited for screening large numbers of samples required for successful breeding programs. The scale of the data obtained, which can easily reach $>10^6$ MS data points, requires the use standard and more sophisticated multivariate data handling techniques such a principal component analysis [27] and random forest approaches [46]. Of course, DIMS yields more relevant information if the phytochemical composition of the samples has been well defined at the mass spectral level and is therefore well suited for studies of berry progeny. However, if novel MS signals are detected then these progeny can be "back analysed" by LC–MS.

Finally, if this approach is applied to defined breeding populations with suitable genetic maps, the inheritance of key polyphenol components, such as anthocyanins, can be rapidly dissected [27]. As seen above, DIMS could also be applied to germplasm selections that differ in their year or location of growth to assess phytochemical plasticity associated with genetic–environmental interactions or indeed the effects of particular agronomic treatments.

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