Published in final edited form as:
Food Chem. 2014 March 1; 146: 289–298. doi:10.1016/j.foodchem.2013.08.089.

Profiling polyphenols of two diploid strawberry (*Fragaria vesca*) inbred lines using UHPLC-HRMS^n

Jianghao Sun[a], **Xianjin Liu**[b], **Tianbao Yang**[c], **Janet Slovin**[d], and **Pei Chen**[a,*]

[a]U.S. Department of Agriculture, Agricultural Research Service, Beltsville Human Nutrition Research Center, Food Composition and Methods Development Laboratory, Beltsville, MD 20705, United States

[b]Jiangsu Academy of Agricultural Sciences, Institute of Food Safety and Quality, Najing, Jiangsu Province, China

[c]U.S. Department of Agriculture, Agricultural Research Service, Food Quality Laboratory, Beltsville, MD 20705, United States

[d]U.S. Department of Agriculture, Agricultural Research Service, Genetic Improvement of Fruits and Vegetables Laboratory, Beltsville, MD 20705, United States

Abstract

Phenolic compounds in the fruits of two diploid strawberries (*Fragaria vesca* f. semperflorens) inbred lines-Ruegen F7-4 (a red-fruited genotype) and YW5AF7 (a yellow-fruited genotype) were characterised using ultra-high-performance liquid chromatography coupled with tandem high-resolution mass spectrometry (UHPLC-HRMS^n). The changes of anthocyanin composition during fruit development and between Ruegen F7-4 and YW5AF7 were studied. About 67 phenolic compounds, including taxifolin 3-*O*-arabinoside, glycosides of quercetin, kaempferol, cyanidin, pelargonidin, peonidin, ellagic acid derivatives, and other flavonols were identified in these two inbred lines. Compared to the regular octoploid strawberry, unique phenolic compounds were found in *F. vesca* fruits, such as taxifolin 3-*O*-arabinoside (both) and peonidin 3-*O*-malonylglucoside (Ruegen F7-4). The results provide the basis for comparative analysis of polyphenolic compounds in yellow and red diploid strawberries, as well as with the cultivated octoploid strawberries.

Keywords

Strawberry; *Fragaria vesca*; UHPLC; HRMS; Flavonoids; Anthocyanins

1. Introduction

Strawberries are an economically important horticultural crop and much research has been conducted on maximising fruit growth in the field and increasing postharvest fruit quality (Goulas & Manganaris, 2011; Reganold et al., 2010; Shin et al., 2008; Villa-Rojas, Lopez-Malo, & Sosa-Morales, 2011; Wojdylo, Figiel, & Oszmianski, 2009; Yang et al., 2010). In 2011, the worldwide production of strawberries was near 4.6 million tons and the value of

*Corresponding author. Tel.: +1 301 504 8144; fax: +1 301 504 8314. pei.chen@ars.usda.gov (P. Chen).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2013.08.089.

strawberry production in the United States valued at over $2 billion (www.faostats.fao.org). Commercial strawberry *Fragaria* × *ananassa* is an octoploid (2*n* = 8 × = 56) hybrid of two octoploid species, *Fragaria chiloensis* and *Fragaria virginiana*, native to America (Sun & Shi, 2008). *Fragaria vesca* is a widely distributed diploid (2*n* = 2 × = 14) species whose ancestor is believed to be an ancestral genome donor to the octoploid strawberries. The small size of the F. *vesca* plant, its transformability with *Agrobacterium*, its small genome and available genome sequence, and the existing inbred lines support *F. vesca* as a useful reference plant for strawberry {Shulaev, 2011 #11;Slovin, 2009 #12;Kim, 2003 #16263}. Strawberry fruits contain high levels of vitamin C, folate, and phenolic compounds, and are considered to be beneficial to human health. Thus, many studies have been done on the characterisation of these secondary metabolites, their biosynthesis, and their accumulation during fruit development (Bianco et al., 2009; Zhang et al., 2011). The phenolic compounds of regular strawberry fruits, including anthocyanins, proanthocyanindins, flavonols, flavanols, and derivatives of hydroxycinnamic and ellagic acid, are well-studied (Aaby, Ekeberg, & Skrede, 2007; Aaby, Mazur, Nes, & Skrede, 2012; Aaby, Wrolstad, Ekeberg, & Skrede, 2007; Buendia et al., 2010; Hilt et al., 2003; Kelebek & Selli, 2011; Maatta-Riihinen, Kamal-Eldin, & Torronen, 2004). However, there are not much detailed polyphenol content studies on *F. versa*. In a recently published study on *F. versa*, agrimoniin was isolated in the fruit of *F. vesca* and identified as the one of the main ellagitannins (Vrhovsek et al., 2012). High-resolution mass spectrometry (HRMS) has gained popularity in the last few years. HRMS instruments are being used in both quantitative and confirmative food analysis (Kaufmann, 2012). The higher resolution of HRMS provides enough resolving power to calculate the molecular formula for an analyte, so a suggested structure can be confirmed or denied. For example, HRMS can reliably differentiate between a glucosyl ($C_6H_{10}O_5$) and caffeoyl ($C_9H_6O_3$), a rhamnosyl ($C_6H_{10}O_4$) and a coumaroyl ($C_9H_6O_2$), which are commonly seen substituent groups in polyphenols from strawberry while these two pairs of substituent groups exhibit the same mass weights on unit mass spectrometers.

Two inbred lines, *F. vesca*, YW5AF7 and Ruegen F7-4 have yellow fruit with tan achenes and red fruit with red achenes respectively, and were specifically developed for genetic and genomic studies (Kim et al., 2003). To assist the future molecular, genetic, and genomic studies of *F. vesca*, it is necessary to perform a detailed study on the polyphenols in these two diploid lines. Thus an ultra-high-performance liquid chromatography (UHPLC) with diode array detection (DAD) and multi-stage high-resolution mass spectrometry ($HRMS^n$) detection method was established for this purpose, which leads to the identification of 67 phenolic compounds, including taxifolin 3-*O*-arabinoside, glycosides of quercetin, kaempferol, cyanidin, pelargonidin, peonidin, ellagic acid derivatives, and other flavonols.

2. Experimental

2.1. Materials

Diploid strawberry (*F. vesca*) inbred lines YW5AF7 and Ruegen F7-4, as well as octoploid strawberry (*F.* × *ananassa*) cv. Fort Laramie, were grown in a greenhouse with a diurnal rhythm of 16 h light and 8 h darkness following normal cultivation practices. Five fruits (octoploid) and 20 fruits (diploids) were collected from the greenhouse. The fruits at different stages were classified based upon the fruit size and colour of achenes and receptacles. *Green:* small fruit with green achene and receptacle; *turning:* fruit with white receptacles and tan (YW5AF7 and Fort Laramie) or red (F7-4) achenes; *ripe:* ripe fruit with yellow (YW5AF7) or red (F7-4 and Fort Laramie) receptacles (Figure S1). The fruit collection and extraction experiments were repeated at least three times. After harvest, all

the fruits from different stages were washed in water, cut into quarters, immediately frozen in liquid nitrogen and kept at −80 °C for future use.

HPLC-grade methanol, acetonitrile, and formic acid were purchased from Sigma-Aldrich (St. Louis, MO). Pelargonidin 3-*O*-glucoside, cyanidin 3-*O*-glucoside, peonidin 3-*O*-glucoside, ellagic acid, quercetin 3-*O*-glucuronide, quercetin 3-*O*-glucoside, kaempferol 3-*O*-glucuronide and (+)-catechin were purchased from Chromadex Inc. (Irvine, CA).

2.2. Sample preparation

One gram of each freezed-dried sample was homogenised with 50 mL of extraction solution (methanol/water/formic acid; 60:40:1 v/v/v) using an Ultra Turrax T18 Basic Disperser (IKA Werke GmbH & Co, Staufen, Germany) for 1 min on ice followed by sonication in an ultrasound bath for 15 min (Branson 3200; Branson, Danbury, CT). The homogenates were then centrifuged at 5000g for 10 min (IEC clinical centrifuge; IEC, Needham Heights, MA). The upper layer was filtered through a 0.22-μm PTFE filter, transferred into a 2-mL HPLC vial, and 2 μL was injected for UHPLC-HRMS^n analysis.

2.3. The UHPLC-HRMS^n conditions

The UHPLC-HRMS^n system consisted of an LTQ Orbitrap XL MS with an Accela 1250 binary pump, a PAL HTC Accela TMO autosampler, an Accela PDA detector (Thermo Fisher Scientific, San Jose, CA), and an Agilent G1316A column compartment (Agilent, Santa Clara, CA). The separation was carried out on a Hypersil Gold C_{18} column (200 × 2.1 mm, 1.9 μm particle size; Thermo Fisher Scientific, San Jose, CA) with a flow rate of 0.3 mL/min. Mobile phase A was H_2O (0.1% formic acid) and B was acetonitrile (0.1% formic acid). The linear gradient was 4–20% B (v/v) from 0 to 40 min, to 35% B at 60 min, to 100% B at 61 min, and was held at 100% B to 65 min for column washing. The column temperature was set at 60 °C and UV/Vis spectra were recorded between 200 and 700 nm. High-accuracy mass measurements were carried out under both positive and negative ionisation modes. The MS conditions were set as follows: sheath gas at 70 (arbitrary units), auxiliary and sweep gas at 10 (arbitrary units), spray voltage at 4.5 kV for positive ionisation mode and 4 kV for negative ionisation mode, capillary temperature at 250 °C, capillary voltage at 40 V for positive ionisation mode and −50 V for negative ionisation mode, and tube lens at 150 V. For FTMS, the mass range is from *m/z* 100 to 1500 with a resolution of 15,000, AGC target value of 200,000 and 100,000 in full scan and FTMS/MS AGC target at 1e5, isolation width of 1 amu, and max ion injection time of 750 ms; the ion trap settings used were: AGC target value of 30,000 and 10,000 in full scan and MS^n mode, respectively, maximum ion injection time of 200 ms. The most intense ion was selected for the data-dependent scan with normalisation collision energy at 35%.

3. Results and discussions

The basic structures of the above mentioned compounds are shown in Figure S2. Since different classes of phenolic compounds exhibit absorbance maxima at different wavelengths, two wavelengths were selected for real-time monitoring: 280 nm for non-anthocyanin phenolic compounds and 520 nm for anthocyanins. HRMSn detection in both the positive and the negative ionisation modes was used to obtain information on the structural features and the conjugated forms of phenolic compounds. Identification of the phenolic compounds was based on chromatographic behaviour, UV/Vis and mass spectra, accurate mass measurements, consecutive MS^2–MS^4 analyses, and comparison with data in the literature (Buendia et al., 2010; Kelebek & Selli, 2011; Maatta-Riihinen et al., 2004; Mikulic-Petkovsek et al., 2013; Zhang et al., 2011; Zheng, Song, Doncaster, Rowland, & Byers, 2007). Seventy-four compounds, including anthocyanins, dihydroflavonols and

flavonols, flavan-3-ols, proanthocyanidins, and ellagic acid and its derivatives were identified from the two *F. vesca* inbred lines. The basic structures of the abovementioned compounds are shown in Fig. 1 and summarised in Table 1, where the compounds are numbered according to their retention times as shown in typical chromatograms (Fig. 1).

3.1. Identification of anthocyanins in F. vesca var. Ruegen F7-4

Fig. 1(A) shows the HPLC-UV (520 nm) chromatogram of Ruegen F7-4 ripe fruits (stage 3). Previous reports of anthocyanins in strawberries were used to assist in the identification of the anthocyanins in Table 1 in addition to UV and $HRMS^n$ data (Aaby et al., 2007; Buendia et al., 2010; Hilt et al., 2003; Kelebek & Selli, 2011; Zhang et al., 2011).

Peak 20 with M^+ at *m/z* 449.1071 ($C_{21}H_{21}O_{11}$, −1.53 ppm) and a product ion at *m/z* 287 (−162 amu, hexose moiety) was identified as cyanidin 3-*O*-glucoside. Peak **24**, the major peak in Ruegen F7-**4** with M^+ at *m/z* 433.1122 ($C_21H_21O_{10}$, 1.58 ppm) and a major MS^2 product ion at *m/z* 271(−162 amu, hexose moiety), was identified as pelargonidin 3-*O*-glucoside. Peak **30** with M^+ at *m/z* 463.1228 ($C_{22}H_{23}O_{11}$, 1.49 ppm) and a MS^2 product ion at *m/z* 301(−162 amu, hexose moiety) was identified as peonidin 3-*O*-glucoside. Peak **35** with $[M]^+$ at *m/z* 535.1072 ($C_{24}H_{23}O_{14}$, −2.0 ppm) and two major product ions at *m/z* 449 and 287 (−86 amu, and then −162 amu, corresponding to malonyl and glucose moiety, respectively) was identified as cyanidin 3-*O*-malonylglucoside. Peak **41** showed the M^+ ion at *m/z* 519.1126 ($C_{24}H_{23}O_{13}$, −1.38 ppm) and a neutral loss of 248 amu (malonyl-hexosyl residue) for its product ion and was identified as pelargonidin 3-*O*-malonylglucoside. Peak **44** showed the M^+ at *m/z* 549.1226 ($C_{25}H_25O_{14}$, −1.38 ppm) and a neutral loss of 248 amu (malonyl-hexosyl residue) for its product ion and was identified as peonidin 3-*O*-malonylglucoside. Cyanidin and pelargonidin glycosides are commonly found in cultivated strawberry (*F.* × *ananassa* var. Fort Laramie), but peonidin 3-*O*-glucoside and peonidin 3-*O*-malonylglucoside were identified in *F. vesca* var. Ruegen F7-4 for the first time. However, YW5AF7 ripe fruits (stage 3) only contain pelargonidin 3-*O*-glucoside.

3.2. Identification of non-anthocyanin phenolic compounds in Ruegen F7-4 and YW5AF7

Fig. 1 (B) shows the HPLC-UV profiles at 280 nm of Ruegen F7-4 ripe fruits (stage 3). More than sixty non-anthocyanin phenolic compounds were identified. Unlike anthocyanins, the Ruegen F7-4 and YW5AF7 exhibited very similar profiles and all non-anthocyanin phenolic compounds were found in both genotypes (details discussed below). However, the phenolic profiles differed from those of cultivated strawberries previously reported in the literature (Bianco et al., 2009; Buendia et al., 2010; Kelebek & Selli, 2011; Zhang et al., 2011).

3.2.1. Dihydroflavonol and flavonols—Peak **45** exhibited UV/Vis absorption maxima at about 234 and 290 nm. The HRMS gave a deprotonated $[M–H]^–$ ion at *m/z* 435.0321, suggesting the formula of $C_{20}H_{19}O_{11}$ (0.33 ppm). The MS^2 major product ion was *m/z* 303 (−132 amu, pentose), the MS^3 and the MS^4 spectra of peak **45** were consistent with the MS^2 and the MS^3 spectra of taxifolin. Hence this compound was identified as taxifolin 3-*O*-arabinoside, a compound previously reported in cultivated strawberry roots but not in fruits (Ishimaru, Omoto, Asai, Ezaki, & Shimomura, 1995). Peak **29** with *m/z* at 447.0927 ($C_21H_{19}O_{11}$, −1.22 ppm) and a major product ion at *m/z* 285 (−162 amu: hexose) was identified as kaempferol 3-*O*-glucoside. Peak **46** ($C_{21}H_{20}O_{12}$) with $[M–H]^–$ ion at *m/z* 463, a major MS^2 product ion at *m/z* 301, and corresponding MS^3 product ions at *m/z* 151 and 179, was identified as quercetin 3-*O*-glucoside. Similarly, peak 54 ($C_{23}H_{22}O_{13}$) was identified as quercetin-acetyl-hexoside; and peaks **65** and **68** were identified as kaempferol 3-*O*-acetylhexosides.

3.2.2. Flavan-3-ols and proanthocyanidins—(+) Catechin, B type proanthocyanidin dimers, B type proanthocyanidin trimers, and B type proanthocyanidin tetramers were found in these two genetically improved strawberries using $HRMS^n$ data, UV spectral data, and literature reports. Peak **14**, with a deprotonated molecule ion $[M–H]^-$ at *m/z* 289 ($C_{15}H_{13}O_6$) and characteristic MS^2 ions at *m/z* 245, 205, 231, and 179, was identified as (+)-catechin. Peak 13 ($[M–H]^-$ at *m/z* 577.1346, $C_{30}H_{25}O_{12}$, 0.95 ppm, primary MS^2 ion at *m/z* 577, −152 amu *via* a characteristic fragmentation pathway by retro Diels–Alder reaction) was identified as a proanthocyanidin dimer of the B type catechin–catechin (Aaby et al., 2007). Peak **15** was identified as a B type proanthocyanidin trimer. Its $[M–H]^-$ ion was at *m/z* 865.1970 ($C_{45}H_{37}O_{18}$, −1.82 ppm) and MS^2 ions were at *m/z* 695, 739, 713, 577, 425, and 287. The MS^3 ions of *m/z* 695 gave fragment ions at *m/z* 543, 451, 289, and 243. Using the fragment pattern, the sequence of this trimer was epicatechin–epicatechin–epicatechin. Peak **16** had an $[M–H]^-$ ion at *m/z* 1153.2596 ($C_{60}H_{4s9}O_24$, −1.98 ppm) and was tentatively identified as an isomer of a B type proanthocyanidin tetramer according to its characteristic MS^2 ions at *m/z* 865, 1135, 1027, 983, 695, 575 and 407. It was composed of four epicatechin units. Peak **19** had $[M–H]^-$ at *m/z* 561.1393 ($C_{30}H_{25}O_{11}$) and MS^2 ions at *m/z* 289, 543, and 435. The compound was identified as epiafzelechin–epicatechin. The fragmentation pathway of peak **19** was different from that of the B-catechin dimer in strawberries described in a previous study (Aaby et al., 2007). Peak 21 had $[M–H]^-$ at *m/z* 849.2023 ($C_{45}H_{37}O_{17}$), characteristic MS^2 ions at *m/z* 801, 697, 577 (base peak obtained after a loss of 272 amu), 559, 425, 407, and 287. The MS^3 spectra of the MS^2 base peak (*m/z* 577) gave ions at 425, 407, and 289 (−288 amu, epi catechin) and corresponded to B type proanthocyanidin trimer of the type epiafzele-chin−epicatechin−epicatechin (Aaby et al., 2007; Hilt et al., 2003).

3.2.3. Ellagic acid and its Derivatives—Peak **39** had $[M–H]^-$ at *m/z* 300.9983 ($C_{14}H_5O_8$, −0.97 ppm) and MS^2 fragmentation ions at *m/z* 257, 229,185, and 157. It was identified as ellagic acid. The identity was confirmed with an ellagic acid reference standard. The UV/Vis spectra of peaks **30** and **37** suggested glycosylated forms of ellagic acid *(33)*. Peak **30** with $[M–H]^-$ at *m/z* 463 ($C_{20}H_{15}O_{13}$) and the main MS^2 ion at *m/z* 301 (−162, hexose) was tentatively identified as an ellagic acid hexoside. Peak **37** had the $[M–H]^-$ at *m/z* **447** ($C_{19}H_{13}O_{12}$). Its main MS^2 product ion was at *m/z* 301 (MS^3 ions at *m/z* 257, 229, and 185) and corresponded to ellagic acid. Peak **37** was identified as ellagic acid methyl pentoside. A similar compound has been previously reported in strawberries (Aaby et al., 2007).

Peak **62** (*m/z* 461.0725, $C_21H_{17}O_{12}$, −0.107 ppm) is the major compound observed in the HPLC-DAD profiles for both Ruegen and YW5AF7. The maximum UV absorptions were at 243 nm and 375 nm. The characteristic MS^2 product ion at *m/z* 315 (−146 amu, methyl pentose) and its MS^3 product ions at *m/z* 300 and its MS^4 product ions at *m/z* 272, 271, 244 confirmed the identity of methylellagic acid. Hence, Peak **62** was identified as methylellagic acid methyl pentoside. Peaks **57** and **64** had the same *m/z* (461.0725) and exhibited similar fragmentation behaviour to that of peak **62**, except that the relative abundance of product ion at *m/z* 315 was different. These two compounds were tentatively identified as methylellagic acid methyl pentoside isomers. Peaks **61** and 67 had $[M–H]^-$ ions at *m/z* 519 ($C_{23}H_{19}O_{14}$) with the MS^2 product ions corresponding to kaempferol glucoside (*m/z* 315) after the loss of the acetyl and hexosyl moieties (162 + **42** amu). They were identified as methylellagic acid 3-*O*-acetyl-hexosides. Similarly, Peak 56 (*m/z* 447.0563) was identified as methylellagic acid 3-*O*-pentoside. Similar compounds (methylellagic acid-pen-tose conjugates) have been previously reported (Aaby et al., 2007).

Peaks **5, 6, 8, 9,17, 26**, and **36** were identified as ellagitannins. Ellagitannins are hydrolysable tannins, since they are esters of hexahydroxydiphenic acid (HHDP: 6,6’-

dicarbonyl-2,2′,3,3′,4,4′-hexahydroxybiphenyl moiety) and a polyol, usually glucose, and in some cases gallic acid, which are commonly found in strawberries (Aaby et al., 2007; Kelebek & Selli, 2011; Vrhovsek et al., 2012). Typical losses during fragmentation of ellagitannins are galloyl (152 amu), HHDP (302 amu), galloyl-glucose (332 amu), HHDP-glucose (482 amu), and galloyl-HHDP-glucose (634 amu). Recent research found that agrimoniin is one of the most abundant ellagitannins and sanguiin H-6 and lambertianin C are minor compounds in both *F. vesca* and *F. ananassa* D. (Vrhovsek et al., 2012). However, we did not find any of these three compounds in our two strawberry lines. Peak 8 had a $[M–2H]^{2–}$ ion at *m/z* 957.0535 (its isotopic distribution suggests it to be a double-charged ion), implying the formula of $C_{82}H_{52}O_{55}$. Fragmentation of the double-charged ions gave single-charged MS^2 product ions at *m/z* 1557, 1224, 1099, 1096, 943, 930, 451, and 301. Thus Peak **8** has two more oxygen atom substituted in the structures than that of sanguiin H-6/agrimoniin. Similarly, Peak **9** ($C_{82}H_{52}O_{56}$) had three more oxygen atoms substituted in the structure in comparison to literature reports for sanguiin H-6/agrimoniin (Aaby et al., 2007; Vrhovsek et al., 2012).

Peak **5**, with $[M–H]^–$ at *m/z* 783 ($C_{34}H_{23}O_{22}$) and MS^2 fragmentations at *m/z* 481 (−302 amu, loss of HHDP) and 301 (−482 amu, loss of HHDP-glucose) was identified as bis-HHDP-glucose, previously reported in strawberries (Aaby et al., 2007). Peak **36** was identified as galloyl-bis-HHDP-glucose with $[M–H]^–$ at *m/z* 935 and MS^2 product ions at *m/z* 633 −02 amu, loss of HHDP) and 301 (332 amu, loss of galloyl glucose).

Peak **26** had an $[M–H]^–$ at *m/z* 635 ($C_{27}H_{23}O_{18}$), and a major MS^2 product ion at *m/z* 465 (−170 amu, gallic acid). The MS^3 ion of *m/z* 465 was at *m/z* 313 (−152 amu, loss of galloyl unit). The ion at *m/z* 313 could be further fragmented into an MS^4 product ion at *m/z* 169. Peak **26** was identified as tri-galloyl-glucose.

Peak **36** had a $[M–H]^–$ ion at 935.0771, and ions at *m/z 633,m/z* 463 and *m/z* 301 in the MS2 to MS3 specta, indicating the structure of galloyl-bis-HHDP-glucose.

3.2.4. Other compounds—Peak **12** ($C_7H_8O_6$) was identified as benzoic acid with the main MS^2 ion at *m/z* 143 (loss of CO_2). It was reported previously (Russell, Scobbie, Labat, & Duthie, 2009). Peak **32** was identified as a ferulic acid hexose derivative with $[M–H]^–$ at *m/z* **449** and MS^2 ions at *m/z* 355, 329, 287, 269, and 193 (base peak 355) (Aaby et al., 2007). The major MS^3 ion of *m/z* 355 was at *m/z* 193 (loss of a hexose unit). The fragmentation patterns were in agreement with previously published data (Aaby et al., 2007). Possible composition of Peak **32** could be ferulic acid, hexose, and a C_6H_6O group. Peak **1** ($C_{12}H_{22}O_{12}$) was identified as hexosyl-hexose and it is the sugar form that widely exists in fruits and vegetables (Kallio, Hakala, Pel-kkikangas, & Lapvetelainen, 2000). Peaks **2** and **3** ($C_6H_8O_7$) were tentatively identified as citric acid and its isomer (Kelebek & Selli, 2011). Peak **7** ($C_{12}H_{18}O_8$) was tentatively identified as furaneol glucoside, a compound previously reported in detached ripening strawberry fruits (Roscher, Bringmann, Schreier, & Schwab, 1998). Peak 11 ($C_{11}H_{12}O_2N_2$) was identified as tryptophan, which was reported in Chilean strawberry *F.* × *chiloensis* ssp. chiloensis (Cheel et al., 2005).

3.3. The chemical differences of F. vesca Ruegen F7-4 and YW5AF7 and F. × ananassa cv. Fort Laramie

The metabolite profiles of strawberry fruits are strongly affected by developmental, genetic, and environmental factors (Carbone et al., 2009). In previous publications, the difference between wild and cultivated strawberry species in the production of specific volatile terpenoid flavour components was studied using a combination of molecular and biochemical tools. The results suggest that domestication of strawberry has involved

selection for specific alleles in the cultivated species which contribute to a strongly modified flavour profile (Aharoni et al., 2004). In this investigation, we also found a significant difference between wild and cultivated strawberry species as shown by their phenolic profiles. The constituents found in octoploid strawberry *F*. × *ananassa* cv. Fort Laramie are shown in Fig. 2 and Table 2. The two major anthocyanins of Fort Laramie, cyanidin 3-*O*-glucoside and pelargonidin 3-*O*-glucoside, accounted for almost 100% of the total peak area in its HPLC chromatogram at 520 nm; on the other hand, there are many other anthocyanins in diploid strawberry Ruegen F7-4, such as peonidin 3-*O*-glucoside, peonidin 3-*O*-malonylglucoside cyanidin 3-*O*-malonylglucoside, and pelargonidin 3-*O*-malonylglucoside.

The non-anthocyanin polyphenols in Fort Laramie are mainly coumaroyl-glucose, ferulic acid hexose, quercetin, and kaempferol derivatives while in Ruegen F7-4 and YW5AF7, taxifolin 3-*O*-arabi-noside, methylellagic acid rhamnoside, and ellagic acid are the major constituents. Understanding the chemical differences between cultivated strawberry Fort Laramie and wild strawberry Ruegen F7-4/YW5AF7 may help target modification of strawberries for quality improvement.

4. Conclusion

This study was the first chemical investigation to describe the phenolic composition of two diploid inbred lines, Ruegen F7-4 and YW5AF7, which will facilitate genetic and biochemical studies of the enzymes catalysing the biosynthesis of these important compounds. The UHPLC-DAD-HRMS analysis identified the presence of a variety of anthocyanins, dihydroflavonols, flavonols, fla-van-3-ols, proanthocyanidins, free and conjugated forms of ellagic acid, and ellagitannins. A total of 78 phenolic compounds were identified. The results demonstrate the differences in anthocyanin composition in Ruegen F7-4 and cultivated strawberry Fort Laramie are mainly due to peonidin 3-*O*-glucoside and peonidin 3-*O*-malonylglucoside. The identification of phenolic compounds revealed some interesting compounds newly found in strawberry. Taxifolin 3-*O*-arabinoside and methylellagic acid glycosides in Ruegen F7-4 and YW5AF7 were reported in strawberry fruits for the first time. The high diversity of the phenolic com pounds found in wild diploid strawberry samples may have potential beneficial effects for human health. Further studies should be carried out for quantification of major compounds and biochemical and genetic characterisation of biosynthetic pathways.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research is supported by the Agricultural Research Service of the U.S. Department of Agriculture and an Interagency Agreement with the Office of Dietary Supplements (ODS) of the National Institutes of Health (NIH).

References

Aaby K, Ekeberg D, Skrede G. Characterization of phenolic compounds in strawberry (Fragaria x ananassa) fruits by different HPLC detectors and contribution of individual compounds to total antioxidant capacity. Journal of Agriculture and Food Chemistry. 2007a; 55(11):4395–4406.

Aaby K, Mazur S, Nes A, Skrede G. Phenolic compounds in strawberry (Fragaria x ananassa Duch.) fruits: Composition in 27 cultivars and changes during ripening. Food Chemistry. 2012; 132(1):86–97.

Aaby K, Wrolstad RE, Ekeberg D, Skrede G. Polyphenol composition and antioxidant activity in strawberry purees; impact of achene level and storage. Journal of Agriculture and Food Chemistry. 2007b; 55(13):5156–5166.

Aharoni A, Giri AP, Verstappen FW, Bertea CM, Sevenier R, Sun Z, et al. Gain and loss of fruit flavor compounds produced by wild and cultivated strawberry species. Plant Cell. 2004; 16(11):3110–3131. [PubMed: 15522848]

Bianco L, Lopez L, Scalone AG, Di Carli M, Desiderio A, Benvenuto E, et al. Strawberry proteome characterization and its regulation during fruit ripening and in different genotypes. Journal of Proteomics. 2009; 72(4):586–607. [PubMed: 19135558]

Buendia B, Gil MI, Tudela JA, Gady AL, Medina JJ, Soria C, et al. HPLC-MS analysis of proanthocyanidin oligomers and other phenolics in 15 strawberry cultivars. Journal of Agriculture and Food Chemistry. 2010; 58(7):3916–3926.

Carbone F, Preuss A, De Vos RC, D'Amico E, Perrotta G, Bovy AG, et al. Developmental, genetic and environmental factors affect the expression of flavonoid genes, enzymes and metabolites in strawberry fruits. Plant Cell Environment. 2009; 32(8):1117–1131.

Cheel J, Theoduloz C, Rodriguez J, Saud G, Caligari PDS, Schmeda-Hirschmann G. E-cinnamic acid derivatives and phenolics from Chilean strawberry fruits, Fragaria chiloensis ssp chiloensis. Journal of Agriculture and Food Chemistry. 2005; 53(22):8512–8518.

Goulas V, Manganaris GA. The effect of postharvest ripening on strawberry bioactive composition and antioxidant potential. Journal of the Science of Food and Agriculture. 2011; 91(10):1907–1914. [PubMed: 21520448]

Hilt P, Schieber A, Yildirim C, Arnold G, Klaiber I, Conrad J, et al. Detection of phloridzin in strawberries (Fragaria x ananassa Duch.) by HPLC-PDA-MS/MS and NMR spectroscopy. Journal of Agriculture and Food Chemistry. 2003; 51(10):2896–2899.

Ishimaru K, Omoto T, Asai I, Ezaki K, Shimomura K. Taxifolin 3-Arabinoside from Fragaria-X-Ananassa. Phytochemistry. 1995; 40(1):345–347.

Kallio H, Hakala M, Pelkkikangas AM, Lapvetelainen A. Sugars and acids of strawberry varieties. European Food Technology. 2000; 212(1):81–85.

Kaufmann A. The current role of high-resolution mass spectrometry in food analysis. Analytical and Bioanalytical Chemistry. 2012; 403(5):1233–1249. [PubMed: 22179491]

Kelebek H, Selli S. Characterization of Phenolic Compounds in Strawberry Fruits by Rp-Hplc-Dad and Investigation of Their Antioxidant Capacity. Journal of Liquid Chromatography and Related Technologies. 2011; 34(20):2495–2504.

Kim SH, Shin DH, Choi IG, Schulze-Gahmen U, Chen S, Kim R. Structure-based functional inference in structural genomics. Journal of Structural and Functional Genomics. 2003; 4(2–3):129–135. [PubMed: 14649297]

Maatta-Riihinen KR, Kamal-Eldin A, Torronen AR. Identification and quantification of phenolic compounds in berries of Fragaria and Rubus species (family Rosaceae). Journal of Agriculture and Food Chemistry. 2004; 52(20):6178–6187.

Mikulic-Petkovsek M, Schmitzer V, Slatnar A, Weber N, Veberic R, Stampar F, et al. Alteration of the Content of Primary and Secondary Metabolites in Strawberry Fruit by Colletotrichum nymphaeae Infection. Journal of Agriculture and Food Chemistry. 2013; 2:2–19.

Reganold JP, Andrews PK, Reeve JR, Carpenter-Boggs L, Schadt CW, Alldredge JR, et al. Fruit and soil quality of organic and conventional strawberry agroecosystems. PLoS ONE. 2010; 5(9)

Roscher R, Bringmann G, Schreier P, Schwab W. Radiotracer studies on the formation of 2,5-dimethyl-4-hydroxy-3(2H)-furanone in detached ripening strawberry fruits. Journal of Agriculture and Food Chemistry. 1998; 46(4):1488–1493.

Russell WR, Scobbie L, Labat A, Duthie GG. Selective bio-availability of phenolic acids from Scottish strawberries. Molecular Nutrition & Food Research. 2009; 53(Suppl 1):S85–91. [PubMed: 19205002]

Shin Y, Ryu JA, Liu RH, Nock JF, Polar-Cabrera K, Watkins CB. Fruit quality, antioxidant contents and activity, and antiproliferative activity of strawberry fruit stored in elevated CO2 atmospheres. Journal of Food Science. 2008; 73(6):S339–S344. [PubMed: 19241580]

Sun G, Shi C. The overall quality control of Radix scutellariae by capillary electrophoresis fingerprint. Journal of Chromatographic Science. 2008; 46(5):454–460. [PubMed: 18492359]

Villa-Rojas R, Lopez-Malo A, Sosa-Morales ME. Hot water bath treatments assisted by microwave energy to delay postharvest ripening and decay in strawberries (Fragaria x ananassa). Journal of the Science of Food and Agriculture. 2011; 91(12):2265–2270. [PubMed: 21590776]

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. Journal of Agriculture and Food Chemistry. 2012; 60(10):2507–2516.

Wojdylo A, Figiel A, Oszmianski J. Effect of drying methods with the application of vacuum microwaves on the bioactive compounds, color, and antioxidant activity of strawberry fruits. Journal of Agriculture and Food Chemistry. 2009; 57(4):1337–1343.

Yang FM, Li HM, Li F, Xin ZH, Zhao LY, Zheng YH, et al. Effect of nano-packing on preservation quality of fresh strawberry (Fragaria ananassa Duch. cv Fengxiang) during storage at 4 degrees C. Journal of Food Science. 2010; 75(3):C236–240. [PubMed: 20492272]

Zhang J, Wang X, Yu O, Tang J, Gu X, Wan X, et al. Metabolic profiling of strawberry (Fragaria x ananassa Duch.) during fruit development and maturation. Journal of Experimental Botany. 2011; 62(3):1103–1118. [PubMed: 21041374]

Zheng Q, Song J, Doncaster K, Rowland E, Byers DM. Qualitative and quantitative evaluation of protein extraction protocols for apple and strawberry fruit suitable for two-dimensional electrophoresis and mass spectrometry analysis. Journal of Agriculture and Food Chemistry. 2007; 55(5):1663–1673.

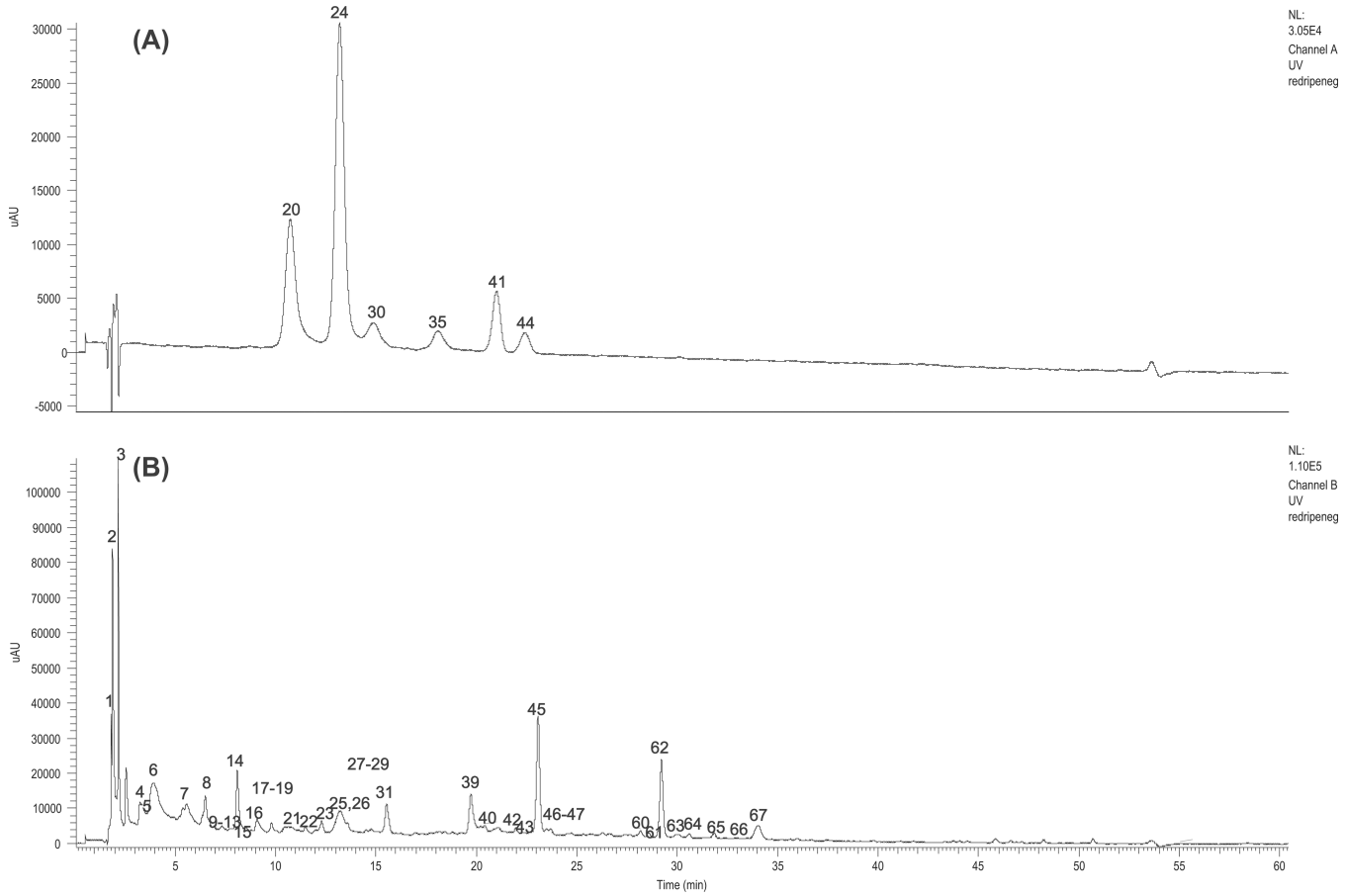

Fig. 1.

The HPLC chromatograms of anthocyanin (A, at 520 nm) and non-anthocyanin polyphenols (A, at 280 nm) from *F* × *vesca* var. Ruegen.

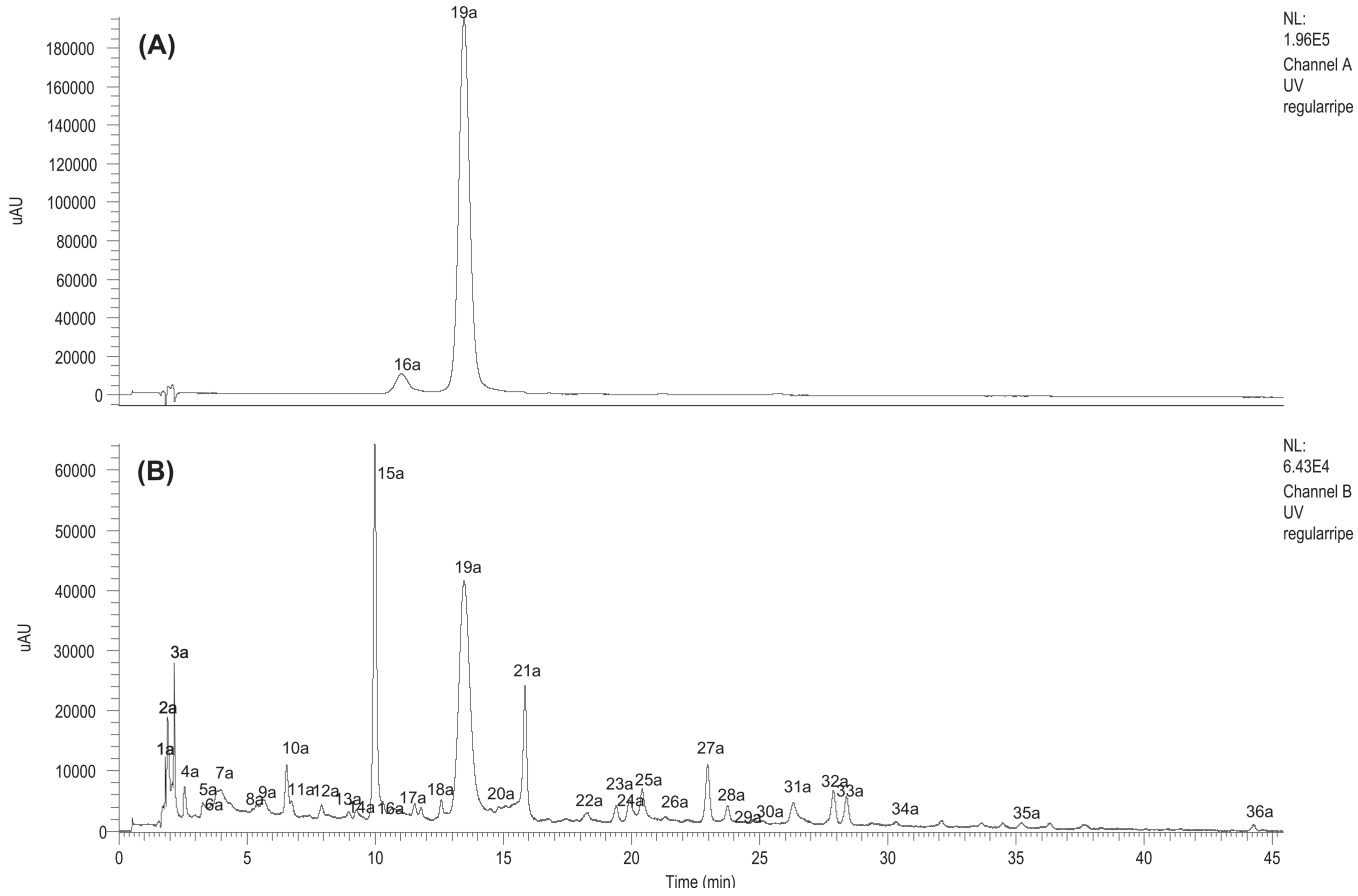

Fig. 2.

The HPLC chromatograms of anthocyanins (A, at 520 nm) and non-anthocyanin polyphenols (B, at 280 nm) from *F* × *ananassa* cv. LAR.

Table 1

Compounds identified from *Fragaria vesca* var. Ruegen and YW5AF7.

Peak no.	t_R(min)	*m/z*	Error (mmu)	Formula	Adduct	MS^2 to MS^4 data	UV λ_{max} (nm)	Tentative identification
1	1.82	341.1083	−0.64	$C_{12}H_{22}O_{12}$	$[M–H]^-$	MS2[341]: 179(100), 161(18), 143(23), 119(13), 113(18), 101(6) MS3[341 → 179]: 161(100), 149(21), 143(97), 131(27), 125(8), 119(45), 113(18), 106(12), 101(8), 89(94), 87(11)MS4[341 → 179 → 161]: 113(100), 101(32), 71(69)	–	Hexosyl hexose
2	1.89	191.0193	−0.41	$C_6H_8O_7$	$[M–H]^-$	MS2[191]: 173(100), 111(64)MS3[191 → 173]: 155(27), 111(100)MS4[191 → 173 → 111]: 67(100)	–	Citric acid
3	2.38	191.0194	−0.33	$C_6H_8O_7$	$[M–H]^-$	MS2[191]: 173(20), 111(100)MS3[191 → 111]: 67(100)	–	Citric acid isomer
4	3.34	629.0411	−0.96	$C_{27}H_{18}O_{18}$	$[M–H]^-$	MS2[629]: 615(31), 613(9), 601(100), 599(9)MS3[629 → 601]: 573(45), 557(16), 529(6), 449(33), 439(6), 431(100), 405(15), 389(7), 387(14), 287(26), 286(13), 261(12)MS4[629 → 601 → 431]: 403(41), 387(62), 371(17), 369(12), 359(8), 343(9), 327(6), 312(6), 299(19), 287(100), 286(94), 285(16), 283(8), 273(10), 271(12), 261(98), 257(8), 245(6), 243(14), 227(9), 225(8), 217(27), 216(10), 215(13), 189(18), 187(7)	–	Gallotannin
5	3.67	783.0665	−1.3	$C_{34}H_{24}O_{22}$	$[M–H]^-$	MS2[783]: 481(31), 301(100), 275(17)MS3[783 → 301]: 301(36), 300(9), 284(39), 257(100), 229(76), 201(13), 185(27)	–	bis-HHDP-glucose
6	3.95	947.0433	0.052	$C_{41}H_{24}O_{27}$	$[M–H]^-$	MS2[947]: 929(100), 901(73), 883(16), 875(8)	–	Unknown ellagitannin
7	5.58	289.0921	−0.761	$C_{12}H_{18}O_8$	$[M–H]^-$	MS2[289]: 161(100), 113(6), 101(8)MS3[289 → 161]: 143(28), 129(22), 125(12), 113(62), 101(100), 99(13), 97(14), 89(6), 85(7), 73(10), 71(29)	275,229	Furaneol hexoside
8	6.34	957.0535	−1.041	$C_{89}H_{48}O_{50}$	$[M– 2H]^{2–}$	MS2[957]: 1557(25), 1224(17), 1099(33), 1096(42), 1026(17), 986(8), 967(17), 943(33), 939(58), 937(25), 934(33), 932(17), 930(100), 928(17), 926(25), 922(17), 917(25), 916(17), 912(25), 911(25), 907(50), 901(17), 899(17), 895(50), 872(25), 842(17), 837(8), 817(8), 814(17), 778(17), 754(25), 739(8), 731(17), 717(8), 655(42), 541(8), 493(8), 469(8), 413(8), 397(17),	–	Unknown ellagitannin
9	6.40	965.0516	−0.428	$C_{89}H_{48}O_{51}$	$[M– 2H]^{2–}$	MS2[965]: 1859(11), 948(16), 947(16), 942(11), 929(16), 921(11), 920(11), 916(21), 903(26), 897(11), 889(11), 887(11), 885(11), 871(21), 852(37), 823(11), 797(11), 795(11), 783(100), 781(16), 764(11), 591(11), 481(11), 479(16), 421(16)	–	Unknown ellagitannin
10	6.50	285.0611	−0.144	$C_{12}H_{14}O_8$	$[M–H]^-$	MS2[285]: 165(7), 153(100), 152(28), 109(9)MS3[285 → 153]: 109(100)	–	1-*O*-protocatechuyl-beta-xylose

Peak no.	t_R(min)	*m/z*	Error (mmu)	Formula	Adduct	MS^2 to MS^4 data	UV λ_{max} (nm)	Tentative identification
11	6.54	203.0822	−0.451	$C_{11}H_{12}O_2N_2$	$[M–H]^-$	MS2[203]: 186(10), 159(100), 142(15), 116(37)MS3[203 → 159]: 144(9), 132(30), 130(56), 129(100), 128(42), 116(58), 115(30)	280, 230	D or L-tryptophan
12	6.84	187.0248	−0.451	$C_7H_8O_6$	$[M–H]^-$	MS2[187]: 143(100)MS3[187 → 143]: 99(100), 85(9)	–	2-methylaconitate or its isomer
13	7.16	577.1346	−0.569	$C_{30}H_{26}O_{12}$	$[M–H]^-$	MS2[577]: 559(16), 451(46), 425(100), 407(59), 299(7), 289(27), 287(11)MS3[577 → 425]: 407(100), 273(6)MS4[577 → 425 → 407]: 389(29), 363(6), 339(14), 297(45), 285(100), 284(11), 283(28), 281(88), 269(6), 256(8), 255(20), 253(17), 243(17)	–	Proanthocyanidin b1 (catechin-catechin)
14	8.11	289.0713	−0.441	$C_{15}H_{14}O_6$	$[M–H]^-$	MS2[289]: 247(7), 245(100), 231(7), 205(33), 179(9)MS3[289 → 245]: 227(27), 217(8), 203(100), 202(7), 188(15), 187(24), 175(9), 161(17)	233, 280, 334	(+)-Catechin*
15	8.75	865.1973	1.096	$C_{45}H_{38}O_{18}$	$[M–H]^-$	MS2[865]: 848(25), 739(55), 713(39), 695(100), 587(22), 577(52), 575(33), 569(9), 557(6), 543(11), 451(19), 449(15), 425(17), 423(6), 413(8), 407(23), 405(7), 395(6), 289(6), 287(16)MS3[865 → 695]: 677(38), 586(8), 585(10), 543(100), 525(23), 451(28), 407(16), 405(33), 391(11), 387(6), 363(22), 299(11), 289(15), 243(33)	–	Proanthocyanidin C1 (catechin trimer)
16	9.09	1153.2596	1.225	$C_{42}H_{58}O_{37}$	$[M–H]^-$	MS2[1153]: 1136(73), 1110(8), 1101(10), 1070(6), 1028(65), 1010(12), 1002(33), 991(6), 984(35), 983(20), 965(6), 917(6), 908(41), 906(8), 865(100), 863(49), 861(10), 857(12), 850(8), 847(14), 846(8), 831(20), 822(6), 814(6), 739(25), 723(6), 713(6), 701(27), 695(16), 694(10), 587(25), 577(22), 575(55), 557(24), 549(8), 533(8), 501(6), 459(6), 457(6), 455(6), 449(12), 423(22), 407(24), 405(10)	233, 271, 360	Proanthocyanidin tetramer
17	9.44	331.1033	−0.305	$C_{14}H_{20}O_9$	$[M–H]^-$	MS2[331]: 313(8), 289(46), 287(7), 271(100), 235(11), 203(9), 169(24), 165(6), 127(10)	233, 271	Galloyl glucose
18	9.57	865.197	−1.517	$C_{45}H_{38}O_{18}$	$[M–H]^-$	MS2[865]: 848(17), 739(62), 720(8), 713(51), 695(100), 587(20), 577(64), 575(38), 557(9), 543(19), 533(6), 525(8), 451(18), 449(15), 425(25), 423(6), 413(9), 407(18), 405(12), 395(9), 363(8), 289(9), 287(27)	233, 271	Proanthocyanidin trimer
19	9.81	561.1398	−0.465	$C_{30}H_{26}O_{11}$	$[M–H]^-$	MS2[561]: 543(42), 435(52), 425(13), 407(17), 289(100), 271(11)MS3[561 → 289]: 247(6), 245(100), 205(33), 203(11), 179(12)	233, 271	Unknown
20	10.74	447.0931	0.952	$C_{21}H_{21}O_{11}^+$	$[M–2H]^-$	MS2[465]: 339(8), 285(100), 241(13)MS3[465 → 285]: 257(9), 243(21), 241(100), 217(19), 199(10), 149(9)	233, 271, 514	Cyanidin 3-*O*-glucoside*
21	11.18	849.2023	−1.353	$C_{45}H_{38}O_{17}$	$[M–H]^-$	MS2[849]: 831(24), 723(100), 697(43), 695(32), 679(19), 577(95), 571(51), 559(78), 553(10), 541(9), 517(6), 451(19), 433(19), 425(22), 407(30), 397(12), 299(7), 289(15), 287(14)	–	Propelargonidin trimer (afz-cat-cat)

Peak no.	t_R(min)	*m/z*	Error (mmu)	Formula	Adduct	MS^2 to MS^4 data	UV λ_{max} (nm)	Tentative identification
22	11.55	631.0565	−0.13	$C_{27}H_{20}O_{18}$	$[M–H]^-$	MS2[631]: 613(12), 451(100)MS3[631 → 451]: 433(79), 423(9), 407(78), 405(28), 395(10), 379(37), 377(10), 367(6), 363(8), 351(100), 337(7), 335(24), 323(21), 311(28), 307(25), 295(14), 285(88), 283(7), 165(8)	215, 233	Castalin or its isomer
23	12.32	331.1026	0.241	$C_{14}H_{20}O_9$	$[M–H]^-$	MS2[331]: 313(22), 312(6), 289(15), 288(6), 271(25), 253(21), 235(33), 211(14), 205(6), 203(32), 193(63), 181(11), 169(64), 161(10), 151(42), 127(100), 125(29), 113(11), 101(9), 97(9)	232, 275	Unknown
24	13.21	431.0979	0.591	$C_{21}H_{21}O_{11}$	$[M–2H]^-$	MS2[431]: 413(10), 387(6), 269(100)MS3[431 → 269]: 241(59), 225(29), 199(12), 197(7), 147(100)	233, 271, 500	Pelargonidin 3-*O*-glucoside*
25	13.83	401.1448	−1.272	$C^{18}H_{26}O_{10}$	[M-H]	MS2[401]: 383(18), 357(8), 356(6), 355(6), 293(9), 269(100), 233(9), 161(20)	–	Apigenin pentose
26	13.90	635.0881	−1.44	$C_{27}H_{24}O_{18}$	$[M–H]^-$	MS2[635]: 465(100)MS3[635 → 465]: 313(100), 295(9), 235(9), 169(19)MS4[635 → 465 → 313]: 295(21), 253(41), 241(31), 223(6), 211(6), 193(17), 169(100), 151(9), 125(15)	233, 270	Trigalloylglucose
27	14.53	525.1964	−0.243	$C_{25}H_{34}O_{12}$	$[M–H]^-$	MS2[525]: 363(100), 345(6), 179(6), 165(10)MS3[525 → 363]: 345(27), 315(19), 239(8), 221(7), 179(32), 165(100)	–	GA8-hexose gibberellin
28	14.74	351.1292	0.577	$C_{14}H_{24}O_{10}$	$[M–H]^-$	MS2[351]: 333(25), 249(100), 231(10), 113(8)MS3[351 → 249]: 231(100), 189(18), 175(18), 157(7), 129(11), 115(18), 113(86), 111(21), 109(7), 99(18), 95(11), 85(29), 83(21), 75(7)	–	Unknown
29	14.87	463.1227	−0.838	$C_{22}H_{23}O_{11}$	$[M–H]^-$	MS2[463]: 301(100), 300(54)MS3[463 → 301]: 301(12), 284(14), 257(100), 229(21), 185(9)	–	Ellagic acid-hexoside
30	14.95	463.0506	−0.188	$C_{20}H_{16}O_{13}$	M^+	MS2[463]: 301(100)MS3[463- > 301]: 286(100)	233, 271, 512	Peonidin 3-*O*-glucoside*
31	15.46	517.1552	0.028	$C_{22}H_{30}O_{14}$	$[M–H]^-$	MS2[517]: 499(10), 471(8), 355(18), 337(46), 295(35), 265(49), 235(55), 193(100), 175(32), 160(14)	–	Unknown
32	15.66	449.1079	0.227	$C_{21}H_{22}O_{11}$	$[M–H]^-$	MS2[449]: 355(100), 329(8), 287(40), 269(28), 193(13)MS3[449 → 355]: 193(100), 192(10), 165(6)MS4[449 → 355 → 193]: 165(100), 137(14)	–	Ferulic acid hexose derivative
33	16.71	371.0977	0.417	$C_{16}H_{20}O_{10}$	$[M–H]^-$	MS2[371]: 249(100)MS3[371 → 249]: 231(87), 175(14), 113(100), 111(8), 103(7), 99(9), 95(11), 85(21)	–	Unknown
34	17.49	585.2184	−0.479	$C_{27}H_{38}O_{14}$	$[M–H]^-$	MS2[585]: 377(100), 329(13)MS3[585 → 377]: 329(100)MS4[585 → 377 → 329]: 314(100), 164(10)	–	Unknown
35	18.07	535.1075	−0.692	$C_{24}H_{23}O_{14}$	M^+	MS2[535]: 287(100)MS3[535- > 287]: 287(100), 269(68), 259(28), 245(11), 241(36), 231(46), 217(6), 216(9), 213(74), 199(12), 189(16), 185(32), 175(35), 163(9), 137(17)	233, 515	Cyanidin 3-*O*-malonylglucoside

Peak no.	t_R(min)	*m/z*	Error (mmu)	Formula	Adduct	MS^2 to MS^4 data	UV λ_{max} (nm)	Tentative identification
36	18.08	935.0771	−1.357	$C_{41}H_{28}O_{26}$	$[M–H]^-$	MS2[935]: 633(100), 301(41)MS3[935 → 633]: 463(8), 301(100)	–	Galloyl bis-hexahydroxydiphenoyl (HHDP)-glucose
37	19.28	433.0408	−0.479	$C_{19}H_{14}O_{12}$	$[M–H]^-$	MS2[433]: 301(100), 300(32)MS3[433 → 301]: 301(49), 284(36), 273(12), 257(100), 229(67), 213(8), 201(6), 185(32)	–	Ellagic acid pentoside
38	19.41	461.2023	−0.505	$C_{21}H_{34}O_{11}$	$[M–H]^-$	MS2[461]: 461(6), 453(9), 443(55), 430(6), 418(9), 417(15), 416(9), 415(30), 400(6), 399(16), 393(6), 376(6), 329(100), 299(10), 293(90), 233(49), 191(185), 161(13), 149(28)	–	Unknown
39	19.72	300.9983	−0.73	$C_{14}H_6O_8$	$[M–H]^-$	MS2[301]: 301(34), 300(13), 284(28), 257(100), 229(64), 201(13), 185(34)MS3[301 → 257]: 229(96), 213(23), 201(11), 185(100), 173(6)	234, 252, 368	Ellagic acid*
40	20.42	447.0564	−0.499	$C_{20}H_{17}O_{12}$	$[M–H]^-$	MS2[447]: 301(100), 300(19)MS3[447 → 301]: 301(15), 284(11), 257(100), 229(29), 185(10)	233, 365	Ellagic acid-methyl pentoside
41	21.07	473.1083	−0.142	$C_{23}H_{23}O_{11}$	$[M–2H]^-$	MS2[473]: 269(100)MS3[473 → 269]: 241(59), 225(62), 224(8), 201(7), 199(9), 147(100)	233, 501	Pelargonidin acetyl hexoside
42	21.89	623.1247	0.414	$C_{27}H_{28}O_{17}$	$[M–H]^-$	MS2[623]: 608(11), 477(46), 476(8), 460(100), 314(11), 313(6)MS3[623 → 460]: 445(10), 314(35), 313(100)MS4[623 → 460 → 313]: 298(100), 285(42), 283(6)	–	Unknown
43	22.26	503.1189	0.498	$C_{24}H_{24}O_{12}$	$[M–H]^-$	MS2[503]: 299(100)MS3[503 → 299]: 284(100), 283(22), 255(23), 240(10), 147(11)	230, 365	Diosmetin acetylhexoside
44	22.43	549.1228	−1.022	$C_{25}H_{25}O_{14}$	M^+	MS2[549]: 301(100)MS3[549- > 301]: 286(100)	233, 515	Peonidin 3-*O*-malonylglucoside
45	23.05	435.0931	−0.145	$C_{20}H_{20}O_{11}$	$[M–H]^-$	MS2[435]: 303(100), 285(34)MS3[435 → 303]: 285(100), 177(11), 125(7)MS4[435 → 303 → 285]: 257(11), 243(17), 241(100), 217(13), 199(23), 175(56)	217, 234, 289	Taxifolin 3-*O*-arabinofuranoside
46	23.50	463.0876	−0.609	$C_{21}H_{20}O_{12}$	$[M–H]^-$	MS2[463]: 301(100), 300(23)MS3[463 → 301]: 283(6), 273(16), 257(15), 229(8), 179(100), 151(63)	233, 364	Quercetin 3-*O*-glucoside*
47	23.61	477.067	−0.444	$C_{21}H_{18}O_{13}$	$[M–H]^-$	MS2[477]: 315(100)MS3[477 → 315]: 300(100)MS4[477 → 315 → 300]: 300(100), 283(6), 272(24), 271(21), 244(53), 243(12), 228(13), 216(17), 200(22)	217, 233, 271	Methylellagic acid hexose
48	23.73	521.2017	−1.115	$C_{26}H_{34}O_{11}$	$[M–H]^-$	MS2[521]: 503(9), 359(100)MS3[521 → 359]: 344(100)MS4[521 → 359 → 344]: 329(34), 328(8), 313(100), 255(16), 203(14), 191(10), 189(52), 173(11), 159(41)	215, 233, 271	Tetramethylellagic acid hexose
49	24.55	491.0831	−0.014	$C_{22}H_{20}O_{13}$	$[M–H]^-$	MS2[491]: 476(20), 328(100), 313(9)MS3[491 → 328]: 313(100)MS4[491 → 328 → 313]: 298(100), 285(54)	215, 233, 280	Dimethylellagic acid hexose
50	24.73	521.2016	−1.235	$C_{26}H_{34}O_{11}$	$[M–H]^-$	MS2[521]: 503(9), 359(100)MS3[521 → 359]: 344(100)MS4[521 → 359 → 344]: 329(32), 328(10), 313(100), 255(16), 203(16), 191(10), 189(45), 173(11), 159(45)	216, 233, 271	Tetramethylellagic acid hexose

Peak no.	t_R(min)	*m/z*	Error (mmu)	Formula	Adduct	MS^2 to MS^4 data	UV λ_{max} (nm)	Tentative identification
51	25.32	709.1252	−0.526	$C_{30}H_{30}O_{20}$	$[M–H]^-$	MS2[709]: 709(7), 691(10), 665(84), 663(9), 625(8), 563(100), 545(13), 519(83), 477(18), 461(8), 447(7), 357(21), 315(46), 301(27), 300(13)	217, 233, 356	Unknown
52	25.60	607.1298	−0.638	$C_{27}H_{28}O_{16}$	$[M–H]^-$	MS2[607]: 461(100)MS3[607 → 461]: 314(100), 299(6)MS4[607 → 461 → 314]: 313(31), 299(97), 286(66), 285(100), 284(21), 283(25)	215, 233	Unknown
53	26.20	567.2076	−0.674	$C_{27}H_{36}O_{13}$	$[M–H]^-$	MS2[567]: 567(10), 558(11), 550(7), 549(14), 545(7), 523(10), 521(40), 499(7), 359(77), 341(100), 329(87)	215, 233	Unknown
54	26.28	505.0983	−0.434	$C_{23}H_{22}O_{13}$	$[M–H]^-$	MS2[505]: 463(22), 301(100), 300(46)MS3[505 → 301]: 283(17), 273(29), 257(10), 229(7), 193(7), 179(100), 151(63)	–	Quercetin acetyl hexoside
55	26.51	327.1234	−0.447	$C_{19}H_{20}O_5$	$[M–H]^-$	MS3[327 → 312]: 295(22), 284(47), 283(30), 281(48), 267(100), 256(12), 253(26), 240(11), 145(30)	–	Unknown
56	26.67	447.0563	−0.599	$C_{20}H_{16}O_{12}$	$[M–H]^-$	MS2[447]: 315(100)MS3[447 → 315]: 300(100)MS4[447 → 315 → 300]: 300(100), 283(12), 272(24), 271(12), 244(69), 243(29), 228(33), 216(47), 200(35), 188(6), 172(10)	–	Methylellagic acid pentose
57	27.34	461.0724	−0.189	$C_{21}H_{18}O_{12}$	$[M–H]^-$	MS2[461]: 328(6), 315(100)MS3[461 → 315]: 300(100)MS4[461 → 315 → 300]: 300(19), 283(59), 272(100), 271(18), 244(39), 228(40), 200(20), 172(17)	–	Methylellagic acid methyl pentose
58	27.68	519.0779	−0.148	$C_{23}H_{20}O_{14}$	$[M–H]^-$	MS2[519]: 315(100)MS3[519 → 315]: 300(100)MS4[519 → 315 → 300]: 300(100), 272(24), 271(18), 244(67), 243(12), 228(12), 216(21), 200(12), 172(6), 151(6)	–	Methylellagic acid acetyl hexose
59	27.88	939.1095	−1.404	$C_{41}H_{32}O_{26}$	$[M–H]^-$	MS2[939]: 787(8), 769(100), 617(9)MS3[939 → 769]: 725(16), 617(100), 601(37), 599(33), 511(7), 465(6), 447(21), 431(11), 429(14), 403(6)MS4[939 → 769 → 617]: 465(100), 447(37), 423(18), 313(8), 295(6), 211(6)	–	Pentagalloyl hexose
60	28.16	447.0932	−0.115	$C_{21}H_{20}O_{11}$	$[M–H]^-$	MS2[447]: 327(18), 285(92), 284(100), 255(14)MS3[447 → 284]: 255(100), 227(13)MS4[447 → 284 → 255]: 255(12), 227(100), 211(57), 183(8), 167(6)	–	Kaempferol 3-*O*-hexoside
61	28.55	519.0779	−0.148	$C_{23}H_{20}O_{14}$	$[M–H]^-$	MS2[519]: 315(100), 300(10)MS3[519 → 315]: 300(100)MS4[519 → 315 → 300]: 300(100), 283(11), 272(27), 271(29), 244(80), 243(17), 228(23), 216(19), 200(34), 172(10)	217, 233, 366	Methylellagic acid acetyl hexoside
62	29.22	461.0718	−0.738	$C_{21}H_{18}O_{12}$	$[M–H]^-$	MS2[461]: 315(100), 314(6)MS3[461 → 315]: 300(100)MS4[461 → 315 → 300]: 300(100), 283(17), 272(36), 271(24), 244(74), 243(21), 228(23), 216(24), 200(39), 172(10)	243, 375	Methylellagic acid rhamnoside
63	30.00	477.1035	−0.399	$C_{22}H_{22}O_{12}$	$[M–H]^-$	MS2[477]: 459(6), 357(23), 315(31), 314(100), 299(6), 285(8), 271(7)MS3[477 → 314]: 299(18), 286(36), 285(100), 271(72), 257(11), 243(24)	–	Methylellagic acid hexose

Peak no.	t_R(min)	*m/z*	Error (mmu)	Formula	Adduct	MS^2 to MS^4 data	UV λ_{max} (nm)	Tentative identification
64	30.56	461.0725	−0.069	$C_{21}H_{18}O_{12}$	$[M–H]^-$	MS2[461]: 315(100)MS3[461 → 315]: 300(100)MS4[461 → 315 → 300]: 300(100), 283(10), 272(17), 271(21), 244(65), 243(18), 228(18), 216(18), 200(24), 172(7)	217, 233, 365	Methylellagic acid hexose
65	31.81	489.1038	−0.029	$C_{23}H_{22}O_{12}$	$[M–H]^-$	MS2[489]: 285(100), 284(7)MS3[489 → 285]: 267(43), 257(100), 256(9), 243(6), 241(29), 240(10), 239(13), 229(49), 223(10), 213(15), 211(9), 199(10), 197(20), 195(9), 163(17)	–	Kaempferol acetylhexoside
66	32.45	341.1393	−0.107	$C_{20}H_{22}O_5$	$[M–H]^-$	MS2[341]: 326(100)MS3[341 → 326]: 311(100)MS4[341 → 326 → 311]: 293(6), 283(100), 267(12), 266(14), 252(7)	–	Unknown
67	34.05	519.1142	−0.214	$C_{24}H_{24}O_{13}$	$[M–H]^-$	MS2[519]: 315(100)MS3[519 → 315]: 300(100), 287(6), 272(6)MS4[519 → 315 → 300]: 272(43), 271(100), 255(66)	215, 233, 329	Methylellagic acid acetyl hexoside

*Confirmed with reference standards, - weak absorbance.

Table 2

Compounds identified from *Fragaria* × *ananassa* cv. Fort Laramie.

Peak no.	t_R (min)	*m/z*	Error (mmu)	Formula	Adduct	MS^2 to MS^4 data	UV λ_{max} (nm)	Possible identification
1a	1.76	341.1083	−0.635	$C_{12}H_{22}O_{11}$	$[M–H]^–$	MS2[341]: 179(100), 161(20), 143(21), 131(7), 119(15), 113(19), 101(6)	–	Hexosyl hexose
2a	1.89	191.0193	−0.406	$C_6H_8O_7$	$[M–H]^–$	MS2[191]: 173(25), 111(100)	–	Citric acid or its isomer
3a	2.11	191.0194	−0.326	$C_6H_8O_7$	$[M–H]^–$	MS2[191]: 173(25), 111(100)	–	Citric acid isomer
4a	2.59	191.0194	−0.286	$C_6H_8O_7$	$[M–H]^–$	MS2[191]: 173(25), 111(100)	–	Citric acid isomer
5a	3.28	865.1965	−2.007	$C_{45}H_{38}O_{18}$	$[M–H]^–$	MS2[865]: 848(11), 739(15), 713(29), 695(100), 587(8), 577(32), 575(28), 543(8), 451(10), 449(10), 425(11), 413(8), 407(12), 405(6), 363(6), 287(13)MS3[865 → 695]: 677(30), 651(11), 586(15), 585(9), 543(100), 525(15), 451(37), 407(15), 405(51), 391(12), 387(7), 363(48), 299(10), 289(27), 243(46)	234	Proanthocyanidin trimer
6a	3.33	865.196	−2.497	$C_{45}H_{38}O_{18}$	$[M–H]^–$	–	–	Proanthocyanidin trimer
7a	3.98	783.0670	−1.685	$C_{34}H_{24}O_{22}$	$[M–H]^–$	MS2[783]: 481(28), 301(100), 275(8)	–	bis-HHDP hexose
8a	5.39	783.0682	−0.465	$C_{34}H_{24}O_{22}$	$[M–H]^–$	MS2[783]: 481(28), 301(100), 275(81)	–	bis-HHDP Hexose
9a	5.62	289.0924	−0.511	$C_{12}H_{18}O_8$	$[M–H]^–$	MS2[289]: 245(6), 161(100), 101(8)MS3[289 → 161]: 143(9), 125(9), 113(61), 101(100), 99(13), 97(11), 89(9), 85(11), 73(7), 71(30)	232, 271	Furaneol hexoside
10a	6.54	285.0614	−0.211	$C_{12}H_{14}O_8$	$[M–H]^–$	MS2[285]: 165(6), 153(100), 152(31), 109(7), 108(6)MS3[285 → 153]: 109(100)	231, 283	Dihydroxybenzoic acid xylose
11a	6.73	951.0743	−0.178	$C_{41}H_{28}O_{27}$	$[M–H]^–$	MS2[951]: 907(100), 783(25)		Geraniin
12a	7.91	865.1976	−0.967	$C_{45}H_{38}O_{18}$	$[M–H]^–$	MS2[865]: 847(21), 739(64), 713(31), 695(100), 677(6), 587(31), 577(57), 575(33), 543(18), 533(7), 451(21), 449(21), 425(21), 423(8), 413(11), 407(26), 405(11), 289(10), 287(17)MS3[865 → 695]: 677(29), 585(6), 543(100), 525(27), 451(49), 407(11), 405(28), 391(7), 387(8), 363(13), 299(9), 289(14), 243(29)	234, 278	Proanthocyanidin trimer
13a	8.98	865.1976	−0.967	$C_{45}H_{38}O_{18}$	$[M–H]^–$	MS2[865]: 847(27), 739(59), 713(49), 695(100), 587(19), 577(63), 575(33), 559(7), 557(6), 543(17), 533(7), 451(22), 449(22), 425(20), 423(8), 413(11), 407(21), 405(10), 395(6), 289(10), 287(18)MS3[865 → 695]: 678(34), 585(6), 543(100), 542(15), 525(28), 451(36), 407(8), 405(26), 391(11), 363(26), 299(9), 289(23), 243(49)	234, 278	Proanthocyanidin trimer
14a	9.25	1153.261	−1.425	$C_{42}H_{58}O_{37}$	$[M–H]^–$	MS2[1153]: 1135(50), 1028(90), 1009(7), 1002(38), 1001(17), 983(85), 982(6), 965(8), 907(24), 865(100), 863(53), 857(7), 847(25), 846(11), 821(8), 739(50), 738(21), 701(32), 695(25), 694(7), 683(9), 588(6), 577(40), 575(55), 569(6), 560(11), 451(11), 449(18), 423(9), 407(26), 405(11), 395(7)	234, 278	Proanthocyanidin tetramer
15a	10.01	325.0929	−1.267	$C_{15}H_{18}O_8$	$[M–H]^–$	MS2[325]: 265(19), 235(7), 205(7), 187(50), 163(90), 145(100), 119(9)MS3[325 → 145]: 117(100)	219, 234, 314	Coumaroyl glucose

Peak no.	t_R (min)	*m/z*	Error (mmu)	Formula	Adduct	MS^2 to MS^4 data	UV λ_{max} (nm)	Possible identification
16a	11.05	449.1074	−0.148	$C_{21}H_{21}O_{11}$	M^+	MS2[449]: 287(100)	276, 428, 500	Cyanidin 3-*O*-glucoside*
17a	11.57	849.2031	−0.563	$C_{45}H_{38}O_{17}$	$[M–H]^–$	MS2[849]: 831(22), 724(90), 723(13), 714(7), 697(34), 695(30), 679(12), 577(100), 571(45), 559(74), 553(15), 543(10), 451(19), 433(14), 425(38), 407(25), 397(11), 289(15), 287(20)	234, 278	Epiafzelechin-(4β → 8)-epicatechin-(4β → 8)-epicatechin
18a	12.61	577.1343	−0.859	$C_{30}H_{26}O_{12}$	$[M–H]^–$	MS2[577]: 559(15), 451(54), 425(100), 407(52), 299(10), 289(26), 287(13)	233, 278	Proanthocyanidin dimer
19a	13.32	433.1125	−0.463	$C_{21}H_{21}O_{10}$	M^+	MS2[433]: 271(100)	234, 276, 428, 499	Pelargonidin 3-*O*-glucoside*
20a	14.87	865.1970	−1.577	$C_{45}H_{38}O_{18}$	$[M–H]^–$	MS2[865]: 847(15), 739(63), 714(34), 713(17), 695(100), 677(6), 587(28), 577(60), 575(29), 569(10), 561(11), 559(8), 543(19), 451(20), 449(12), 425(29), 413(9), 407(33), 405(7), 289(7), 287(18)MS3[865 → 695]: 677(31), 652(7), 569(23), 543(86), 525(49), 451(15), 449(11), 407(100), 405(19), 363(9), 289(12), 243(24)	233, 278	Proanthocyanidin trimer
21a	15.84	449.1089	0.015	$C_{21}H_{22}O_{11}$	$[M–H]^–$	MS2[449]: 355(100), 329(12), 287(36), 269(29), 193(13) MS3[449 → 355]: 193(100), 192(12)	234, 330	Ferulic acid hexose
22a	18.18	935.0782	1.414	$C_{41}H_{28}O_{26}$	$[M–H]^–$	MS2[935]: 633(100), 301(43)MS3[935 → 633]: 463(7), 301(100)	234, 278	Galloyl bis-HHDP Hexose
23a	19.40	433.0406	−0.659	$C_{19}H_{14}O_{12}$	$[M–H]^–$	MS2[433]: 415(6), 301(100), 300(30)MS3[433 → 301]: 301(42), 284(47), 257(100), 229(70), 185(27)	216, 234, 278	Ellagic acid pentoside
24a	19.95	300.9985	−0.45	$C_{14}H_6O_8$	$[M–H]^–$	MS2[301]: 301(43), 300(6), 284(29), 283(20), 273(15), 257(100), 245(9), 229(69), 213(8), 201(9), 185(39)	233, 366	Ellagic acid*
25a	20.44	447.0563	−0.559	$C_{20}H_{16}O_{12}$	$[M–H]^–$	MS2[447]: 301(100), 300(30)MS3[447 → 301]: 301(13), 284(11), 257(100), 229(27), 185(12)	234, 370	Ellagic acid deoxyhexoside
26a	21.31	447.0566	−0.289	$C_{20}H_{16}O_{12}$	$[M–H]^–$	MS2[447]: 301(100), 300(30)MS3[447 → 301]: 301(25), 300(12), 284(14), 283(40), 271(6), 257(100), 255(6), 245(7), 229(50), 185(23)	–	Ellagic acid deoxyhexoside
27a	22.99	477.0673	−0.204	$C_{21}H_{18}O_{13}$	$[M–H]^–$	MS2[477]: 301(100)MS3[477 → 301]: 273(20), 257(15), 179(100), 151(69)MS4[477 → 301 → 179]: 151(100)	216, 233, 350	Quercetin 3-*O*-glucuronide*
28a	23.75	463.088	−0.219	$C_{21}H_{20}O_{12}$	$[M–H]^–$	MS2[463]: 301(100), 300(21)MS3[463 → 301]: 283(8), 273(28), 257(14), 239(6), 193(8), 179(100), 151(75), 107(6)	–	Quercetin 3-glucoside*
29a	24.41	935.0782	−1.354	$C_{41}H_{28}O_{26}$	$[M–H]^–$	MS2[935]: 918(6), 633(100), 463(7), 301(61)	–	Galloyl-bis-HHDP-glucose
30a	24.81	355.1031	−0.395	$C_{16}H_{19}O_9$	$[M–H]^–$	MS2[355]: 337(23), 311(10), 309(100), 207(35), 147(50)	216, 233, 282	Feruloyl hexoside
31a	26.28	934.0712	−0.609	$C_{82}H_{52}O_{54}$	$[M– 2H]^{2–}$	MS2[934]: 1568(100), 1567(9), 1265(25), 1085(26), 916(31), 915(93), 897(84), 783(32), 633(41), 301(86)	233	Agrimoniin
32a	27.89	461.0725	−0.039	$C_{21}H_{18}O_{12}$	$[M–H]^–$	MS2[461]: 285(100)MS3[461 → 285]: 267(42), 257(100), 243(8), 241(23), 240(17), 239(21), 229(41), 223(11), 213(24), 211(10), 199(16), 197(16), 195(7), 185(7), 163(14), 151(8	217, 233, 265, 350	Kaempferol 3-*O*-glucuronide*
33a	28.40	447.0934	0.065	$C_{21}H_{20}O_{11}$	$[M–H]^–$	MS2[447]: 327(23), 301(7), 285(90), 284(100), 255(15)MS3[447 → 284]: 255(100), 227(13)	218, 233, 350	Kaempferol 3-*O*-glucoside

Peak no.	t_R (min)	*m/z*	Error (mmu)	Formula	Adduct	MS^2 to MS^4 data	UV λ_{max} (nm)	Possible identification
34a	30.33	505.0983	–0.494	$C_{23}H_{22}O_{13}$	$[M–H]^-$	MS2[505]: 487(10), 463(25), 343(6), 301(100), 300(53)	–	Quercetin 3-acetylglucoside
35a	32.04	489.1039	0.031	$C_{23}H_{22}O_{12}$	$[M–H]^-$	MS2[489]: 285(100)MS3[489 → 285]: 267(59), 257(100), 256(16), 241(25), 240(12), 239(25), 229(57), 223(12), 213(26), 199(26), 197(21), 195(10), 189(6), 165(6), 163(23)	–	Kaempferol 3-acetylglucoside
36a	44.26	593.1299	−0.124	$C_{30}H_{25}O_{13}$	$[M–H]^-$	MS2[593]: 447(10), 307(6), 285(100)	–	Kaempferol 3-coumaroylglucoside

* Confirmed with reference standards.