**Supplementary Information**

**Flavonoid biosynthesis is differentially altered in detached and attached ripening bilberries in response to spectral light quality**

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**Number of figures: 2**

**Number of tables: 4**



**Figure 1.** Supplemental blue (460 nm), red (660 nm) and far-red (735nm) light treatments provided for bilberry bushes and detached berries with Heliospectra LED lamps alongside control (400-700 nm). Relative intensities of light spectra from the treatments were expressed as arbitrary units (AU).



**Figure 2.** Effect of light spectral treatment on gene expression of a major flavonoid biosynthetic gene and a regulatory gene in bilberry leaves, Chalcone synthase (*VmCHS*) **(A)**, R2R3-MYB transcription factor (*VmMYBPA1.1*) **(B)**. The expression levels are normalized to the reference gene *VmGAPDH* (Glyceraldehyde 3-phosphate dehydrogenase). Error bars represents ±SE of three biological replicates and significant differences between control and light treatments were analyzed by comparison of means using student’s t-test (indicated in asterisks\*) with p-value ≤ 0.05.

**Supplementary Table 1.** List of primers used in qRT-PCR analysis.

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| **Gene** | **Forward primer sequence** |  **Reverse primer sequence** |
| *VmCHS* | CCAAGGCCATCAAGGAATG | TGATACATCATGAGTCGCTTCAC |
| *VmF3'H* | TTCTTCGACACCCGAAAGTC | TCGAACCCTTTGGAATGAAG |
| *VmF3'5'H* | GATTGCGTGGATGGACTTACA | AAATCTGGGTTCCCTTTACGC |
| *VmDFR* | GAAGTGATCAAGCCGACGAT | ATCCAAGTCGCTCCAGTTGT |
| *VmANS* | GCAACTCTTCTACGAGGGCAAA | CCTGTGGAGAATGCTCTTGCAC |
| *VmUFGT* | CATCCAAACCCTGTTCCCATCC | TCATCCCTGCCTTCAAGCTCTC |
| *VmMYBA1* | CTCGACCACAAACCTTGTCCA | GCCTCCTCATTTGATCCGTCA |
| *VmMYBPA1.1* | GGACATTCAACGCCAATCTGGT | CGGCAAAGGAATCCAACTGAAG |
| *VmCOP1* | TGAGAAATGTCAGCCAACCA | CTCTAAATGTGCGCAGTGGA |
| *VmHY5* | GGGAGGAAGTAAGGTCCAAATG | TATAGGGTTACCGGGAGGAATG |
| *VmGAPDH* | CAAACTGTCTTGCCCCACTT | CAGGCAACACCTTACCAACA |
| *VmActin* | TTCCCTGGGATTGCTGATAG | GGTCTTGGCAATCCACATCT |

**Supplementary Table 2**. Concentration of different classes of anthocyanin compounds (mg g-1 DW) quantified by LC-MS in fully ripe bilberries at the end of spectral light treatment from both attached and detached berries.

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|  |  **Attached berries**  **Control (W) Control (D) Far-Red Red Blue** |  **Detached berries** **Control (W) Control (D) Far-Red Red Blue** |
| **DEL\_GLU****DEL\_GAL****DEL\_ARA**Total\_DEL |

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
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| --- | --- | --- | --- | --- |
| 2.7±0.15a |  1.5±0.31a | 2.0±0.15a | 3.8±0.06a |  2.2±0.71a |
| 2.7±0.13ab | 1.2±0.27b | 1.9±0.16b | 5.4±0.23a  | 1.4±1.03b |
| 4.0±0.18ab | 1.5±0.23b | 2.1±0.26b | 6.0±0.66a | 1.3±1.14b |
|  **9.55** |  **4.28** |  **6.16** |  **15.29** |  **5.12** |

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| --- | --- | --- | --- | --- |
| 1.7±0.32a | 1.7±0.20a | 1.7±0.10a | 2.1±0.37a | 3.9±1.30a |
| 1.6±0.24a | 1.5±0.17a | 1.6±0.09a | 2.0±0.33a | 3.8±1.22a |
| 4.1±0.56a | 2.5±0.46a | 3.5±0.03a | 5.0±0.46a | 5.0±0.61a |
| **7.63** | **5.81** | **6.87** | **9.24** | **12.90** |

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| **CYA\_GLU****CYA\_GAL****CYA\_ARA**Total\_CYA**MAL-GLU****MAL-GAL****MAL-ARA**Total\_MAL |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| 2.4±0.12a | 1.9±0.30a | 1.9±0.26a | 1.7±0.19a | 2.4±0.81a |
| 2.3±0.10a | 1.4±0.27a | 1.5±0.21a | 2.3±0.30a | 1.4±1.33a |
| 2.0±0.04a | 2.0±0.31a | 1.5±0.07a | 2.4±0.14a | 1.1±0.95a |
| **6.90** | **5.46** | **5.07** | **6.53** | **5.07** |
| 3.2±0.25a | 2.1±0.40a | 2.7±0.14a | 2.7±0.13a | 2.0±0.66a |
| 1.1±0.14a | 0.47±0.10a | 0.79±0.10a | 1.2±0.70a | 0.6±0.31a |
| 1.2±0.12a | 0.69±0.07a | 1.0±0.21a | 1.3±0.09a | 0.5±0.22a |
| **5.63** |  **3.37** | **4.60** | **5.40** | **3.18** |

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| --- | --- | --- | --- | --- |
| 1.5±0.16a | 2.9±0.31a | 1.9±0.15a | 1.3±0.16a | 2.9±0.78a |
| 1.2±0.07a | 2.2±0.24a | 1.6±0.09a | 1.1±0.11a | 2.6±0.68a |
| 2.3±0.26a |  2.8±0.50.a | 2.4±0.08a | 2.5±0.37a | 3.3±0.29a |
| **5.13** | **8.10** | **6.05** | **5.05** | **8.91** |
| 2.7±0.37a | 3.9±0.43a | 3.6±0.48a | 3.2±0.53a | 5.4±1.42a |
| 0.9±0.09b | 1.2±0.16ab | 1.4±0.20ab | 1.1±0.15b | 2.2±0.49a |
| 2.9±0.28a | 2.5±0.53a | 3.8±0.23a | 3.4±0.49a | 3.5±0.46a |
| **6.68** | **7.74** | **8.92** | **7.84** | **11.32** |

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| **PEO-GLU****PEO-GAL****PEO-ARA**Total\_PEO**PET-GLU****PET-GAL****PET-ARA**Total\_PET |

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
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| --- | --- | --- | --- | --- |
| 1.5±0.15a | 1.4±0.32a | 1.3±0.25a | 0.7±0.07a | 1.4±0.51a |
| 0.3±0.04a | 0.2±0.07a | 0.2±0.05a | 0.2±0.01a | 0.2±0.18a |
| 0.2±0.03a | 0.3±0.02a | 0.2±0.05a | 0.2±0.02a | 0.1±0.10a |
|  **2.19** |  **2.08** | **1.83** |  **1.29** |  **1.87** |
| 2.8±0.16a | 1.2±0.25a | 1.9±0.23a | 2.8±0.27a | 2.1±0.64a |
| 1.2±0.05ab | 0.4±0.08b | 0.7±0.09ab | 1.7±0.15a | 0.6±0.39b |
| 1.5±0.08ab | 0.5±0.05b | 0.8±0.11b | 2.1±0.23a | 0.6±0.39b |
| **5.55** | **2.23** | **3.55** | **6.73** |  **3.41** |

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| 1.2±0.17b | 3.0±0.31a | 1.9±0.24ab | 1.2±0.15b | 2.5±0.40ab |
| 0.2±0.02b | 0.6±0.10a | 0.4±0.06ab | 0.2±0.03b | 0.6±0.10ab |
| 0.5±0.06b | 1.0±0.13a | 0.9±0.06ab | 0.6±0.06b | 0.8±0.12ab |
| **2.02** | **4.83** | **3.38** | **2.13** | **3.99** |
| 2.0±0.3a | 1.8±0.24a | 2.0±0.25a | 2.1±0.31a | 3.6±1.02a |
| 0.7±0.10a | 0.6±0.08a | 0.7±0.07a | 0.8±0.10a | 1.6±0.46a |
| 2.0±0.19ab | 1.3±0.22b | 1.9±0.04ab | 2.4±0.22a | 2.2±0.14ab |
| **4.76** | **3.90** | **4.77** | **5.42** | **7.56** |

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1 The amounts are expressed in average of three biological replicates ± SE. Different letters indicate significant difference from pairwise comparison by ANOVA followed by Tukey’s post-hoc test (*p*-value ≤ 0.05).

2 DEL-Delphinidins, CYA-Cyanidins, MAL-Malvidins, PEO-Peonidins, PET-Petunidins; GLU-Glucoside, GAL-Galactoside, ARA-Arabinoside.

**Supplementary Table 3**. Concentration polyphenolic compounds (mg g-1 DW) quantified by LC-MS in fully ripe bilberries at the end of spectral light treatment from both experimental setups.

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|  |  **Attached berries****Control (W) Control (D) Far-Red Red Blue** |  **Detached berries**  **Control (W) Control (D) Far-Red Red Blue** |
| **Myr-3-glu****Que-3-ara****Que-3-rha****Syr-3-gal****Syr-3-glu****Lar-3-gal****Lar-3-glu****Que-3-gal****Que-3-glu****Total Flavonols****1S-3R****Caff-4-glu****5Caff-shik****Chlor acid****Z-Chloro****Neo-chlor****p-tran.cou****Epicatech****Catechin****Procya B1****Procya B2****Procya C1****Gallocatec****Nandin A****Leuco-cyanidin** |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| 0.05±0.005b | 0.02±0.004c | 0.07±0.004ab | 0.09±0.004a |  0.05±0.008bc |
| 0.14±0.004a | 0.13±0.029a | 0.16±0.001a | 0.20±0.023a | 0.11±0.013a |
| 0.07±0.016a | 0.01±0.007c | 0.02±0.001bc | 0.05±0.004ab | 0.07±0.009a |
| 0.10±0.012a | 0.07±0.001a | 0.07±0.009a | 0.06±0.004a | 0.10±0.017a |
| 0.01±0.002a | 0.006±0.003a | 0.01±0.002a | 0.01±0.0006a | 0.01±0.002a |
|  0.11±0.002ab | 0.05±0.007b | 0.06±0.005b | 0.10±0.006ab | 0.13±0-019a |
| 0.02±0.001ab | 0.01±0.0006b | 0.02±0.001ab | 0.03±0.0005a | 0.01±0.003b |
| 0.47±0.01a | 0.52±0.08a | 0.53±0.041a | 0.64±0.082a |  0.37±0.11a |
| 0.82±0.031a | 0.85±0.17a | 1.09±0.01a | 1.18±0.065a |  0.77±0.03a |
| **1.8372** | **1.7025** | **2.0817** | **2.4021** | **1.6617** |
|   1.25±0.08a |  1.59±0.25a |  1.31±0.10a | 1.70±0.08a |  2.15±0.15a |
|  1.28±0.21b |  1.78±0.32ab |  2.75±0.17a | 0.99±0.08b |  1.28±0.32b |
|  0.13±0.0bc |  0.11±0.0bc |  0.27±0.02a |  0.06±0.001c |  0.17±0.021b |
|  1.90±0.07a |  2.84±0.67a |  2.76±0.16a | 1.74±0.037a |  3.03±0.20a |
|  0.12±0.02a |  0.25±0.07a |  0.27±0.00a |  0.1±0.006a |  0.14±0.04a |
|  0.0±0.00a |  0.05±0.02a |  5.5±0.00a | 0.004±0.00a |  0.07±0.0a |
|  10.49±0.65ab |  4.93±0.77c |  12.6±1.0a | 7.60±0.76bc |  8.61±1.0abc |
|  0.56±0.1a |  0.96±0.21a |  1.0±0.01a | 0.76±0.03a |  0.53±0.02a |
|  0.00±0.0b |  0.01±0.0ab |  0.01±0.00ab | 0.04±0.00a |  0.01±0b |
|  0.00a |  0.0±0.0a |  0.03±0.00a | 0.01±0.01a |  0.007±0a |
|  0.88±0.15b |  1.41±0.28ab |  1.8±0.04a | 1.23±0.04ab |  1.0±0.32ab |
|  0.35±0.06b |  0.59±0.16ab |  0.83±0.03a | 0.48±0.00ab |  0.26±0.15b |
|  0.01±0.0b |  0.00b |  0.00±0.0b | 0.08±0.00a |  0.01±0b |
|  0.66±0.06a |  1.0±0.1a |  0.61±0.0a | 0.40±0.02a |  0.44±0.05a |
|  0.22±0.04a |  0.28±0.10a |  0.34±0.01a  | 0.51±0.00a  |  0. 26±0.23a |

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| 0.04±0.004b | 0.03±0.002b | 0.06±0.002ab | 0.04±0.002ab |  0.07±0.013a |
| 0.16±0.001a | 0.16±0.023a | 0.20±0.028a | 0.19±0.009a | 0.16±0.002a |
| 0.02±0.0012a | 0.03±0.005a | 0.03±0.007a | 0.03±0.004a | 0.02±0.008a |
| 0.06±0.002c | 0.19±0.025a | 0.13±0.001ab | 0.07±0.011bc | 0.16±0.005a |
| 0.01±0.001c | 0.01±0.004bc | 0.02±0.000ab | 0.01±0.002c | 0.02±0.002a |
| 0.04±0.004b | 0.09±0.011ab | 0.06±0.006ab | 0.04±0.006ab | 0.10±0.026a |
| 0.01±0.002b | 0.01±0.001b | 0.02±0.000ab | 0.01±0.001b | 0.02±0.005a |
| 0.46±0.050a | 0.51±0.071a | 0.60±0.07a | 0.52±0.033a | 0.48±0.033a |
| 1.45±0.09ab | 1.07±0.021b | 1.30±0.07ab | 1.59±0.063a |  1.10±0.111b |
|  **2.2884**  |  **2.1275** |  **2.4605** |  **2.5483** |  **2.1773** |
|   3.36±0.07a | 2.86±0.08abc | 1.81±0.14c | 3.24±0.50ab |  2.10±0.43bc |
|  0.68±0.0b | 1.97±0.34a | 1.24±0.03ab |  0.67±0.01b |  0.52±0.08b |
|  0.21±0.0a |  0.13±0.01ab | 0.14±0.03ab | 0.21±0.005a |  0.09±0.01b |
|  3.70±0.3ab | 4.23±0.031s | 2.65±0.04ab | 3.68±0.69ab |  2.01±0.70b |
|  0.31±0.0ab | 0.43±0.01a | 0.25±0.01ab | 0.42±0.08a |  0.19±0.06b  |
|  0.0 8±0.0a | 0.07±0.01a | 0.04±0.00a | 0.08±0.01a |  0.05±0.0a |
|  12.52±1.47a | 7.37±1.0b | 8.54±0.23ab | 11.9±1.38ab |  7.11±0.92b  |
|  1.10±0.04a | 1.0±0.07a | 0.74±0.02a | 1.19±0.02a |  0.74±0.04a |
|  0.03±0.00a | 0.01±0.00a | 0.01±0.00a | 0.03±0.00a |  0.02±0.0a |
|  0.05±0.00a | 0.02±0.00a | 0.03±0.00a | 0.05±0.00a |  0.02±0.01a |
|  1.90±0.05a | 1.76±0.49a | 1.35±0.03a | 1.85±0.05a |  1.20±0.25a |
|  0.86±0.02a | 0.62±0.06a | 0.55±0.00a | 0.77±0.05a |  0.45±0.11a |
|  0.02±0.0ab |  0.00c | 0.005±0.00bc | 0.04±0.00a |  0.03±0.00a |
|  0.91±0.12b | 1.9±0.41a | 1.03±0.03b | 1.13±0.16ab |  0.70±0.06b |
|  0.39±0.05a | 0.41±0.02a | 0.33±0.03a | 0.43±0.03a |  0.50±0.08a |
|  |  |  |  |  |

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1 The amounts are expressed in average of three replicates ± SE. Different letters indicate significant difference from pairwise comparison by ANOVA followed by Tukey’s post-hoc test (p-value ≤ 0.05).

2 Que-Quercetin, Mye-Myricetin, Lar-Laricitrin, Syr-Syringetin; glu-glucoside/glucuronide, rha-rhamnoside, gal-galactoside, ara-arabinopyranoside. 1S-3R-(1S,3R)-3-(beta-D-glucopyranosyloxy)-1-methylbutyl (2E)-3-(4-hydroxyphenyl) prop-2-enoate), Caff-4-glu-(E)-caffeoyl 4-glucoside, 5caff-shik-5-O-caffeoylshikimic-acid, Chlor acid/Z-Chloro/Neo-chlor-(Z)/neo/Chlorogenic acid, p-tran.cou-p-trans-coumaroyl monotropein, Catechin/Epicatech/Gallocatc -Epicatechin/Gallocatachin, Procya B1/B2/C2 - Procyanidin B1/B2/C2, Nandin-A-Nandinaside A

**Supplementary Table 4**. Details of analytes measured using LC-MS.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Compound** | **CAS** | **Exact Mass** | **Formula** | **ID\_ConfidenceA** | **RT (min)** | **EquivalenceB** |
| ***Anthocyanins*** |  |  |  |  |  |  |
| cyanidin 3-arabinoside | 27214-72-8 | 419.0973 | C20H19O10 | 2 | 6.9 | cyanidin 3-glucoside |
| cyanidin 3-galactoside | 142506-26-1 | 449.1078 | C21H21O11 | 1 | 5.55 | cyanidin 3-glucoside |
| cyanidin 3-glucoside | 7084-24-4 | 449.1078 | C21H21O11 | 1 | 6.28 | cyanidin 3-glucoside |
| delphinidin 3-arabinoside | 324533-67-7 | 435.0922 | C20H19O11 | 2 | 5.79 | cyanidin 3-glucoside |
| delphinidin 3-galactoside | 28500-00-7 | 465.1028 | C21H21O12 | 2 | 4.5 | cyanidin 3-glucoside |
| delphinidin 3-glucoside | 50986-17-9 | 465.1028 | C21H21O12 | 2 | 5.08 | cyanidin 3-glucoside |
| malvidin 3-arabinoside | 863107-21-5 | 463.1235 | C22H23O11 | 2 | 10.48 | cyanidin 3-glucoside |
| malvidin 3-galactoside | 104880-34-4 | 493.1341 | C23H25O12 | 2 | 9.07 | cyanidin 3-glucoside |
| malvidin 3-glucoside | 7228-78-6 | 493.1341 | C23H25O12 | 1 | 9.71 | cyanidin 3-glucoside |
| peonidin 3-arabinoside | 27214-74-0 | 433.1129 | C21H21O10 | 2 | 9.37 | cyanidin 3-glucoside |
| peonidin 3-galactoside | 260256-26-6 | 463.1235 | C22H23O11 | 2 | 7.94 | cyanidin 3-glucoside |
| peonidin 3-glucoside | 68795-37-9 | 463.1235 | C22H23O11 | 2 | 8.7 | cyanidin 3-glucoside |
| petunidin 3-arabinoside | 749848-37-1 | 449.1078 | C21H20O11 | 2 | 8.2 | cyanidin 3-glucoside |
| petunidin 3-galactoside | 260256-23-3 | 479.119 | C22H23O12 | 2 | 6.84 | cyanidin 3-glucoside |
| petunidin 3-glucoside | 6988-81-4 | 479.119 | C22H23O12 | 2 | 7.47 | cyanidin 3-glucoside |
| ***Polyphenols*** |  |  |  |  |  |  |
| (1S,3R)-3-(beta-D-glucopyranosyloxy)-1-methylbutyl (2E)-3-(4-hydroxyphenyl)prop-2-enoate | 1206159-19-4 | 412.1733 | C20H28O9 | 3 | 6.03 | chlorogenic acid |
| (E)-caffeoyl 4-glucoside | 147511-61-3 | 342.0951 | C15H18O9 | 2 | 2.5 | chlorogenic acid |
| 5-O-caffeoylshikimic acid | 73263-62-4 | 336.0845 | C16H16O8 | 2 | 3.69 | chlorogenic acid |
| catechin | 154-24-4 | 290.079 | C15H14O6 | 1 | 2.69 | catechin |
| chlorogenic acid | 327-97-9 | 354.0951 | C16H18O9 | 1 | 2.74 | chlorogenic acid |
| (Z)-chlorogenic acid | 15016-60-1 | 354.0951 | C16H18O9 | 2 | 3.37 | chlorogenic acid |
| epicatechin | 490-49-0 | 290.079 | C15H14O6 | 1 | 3.39 | epicatechin |
| gallocatechin | 970-73-0 | 306.0739 | C15H14O7 | 2 | 1.45 | epicatechin |
| laricitrin 3-galactoside | 93219-26-2 | 494.106 | C22H22O13 | 2 | 4.84 | quercetin 3-galactoside |
| laricitrin 3-glucuronide | 1026666-46-5 | 508.0853 | C22H20O14 | 2 | 4.85 | quercetin 3-galactoside |
| leucocyanidin | 480-17-1 | 306.074 | C15H14O7 | 2 | 2.23 | epicatechin |
| myricetin 3-galactoside | 15648-86-9 | 480.0904 | C21H20O13 | 2 | 4.1 | quercetin 3-galactoside |
| myricetin 3-glucoside | 19833-12-6 | 480.0904 | C21H20O13 | 2 | 4.18 | quercetin 3-galactoside |
| myricetin 3-glucuronide | 77363-65-6 | 494.0696 | C21H18O14 | 2 | 4.12 | quercetin 3-galactoside |
| nandinaside A | 1813517-24-6 | 446.1213 | C22H22O10 | 3 | 6.27 | epicatechin |
| neochlorogenic acid | 202650-88-2 | 354.0951 | C16H18O9 | 1 | 1.7 | chlorogenic acid |
| p-trans-coumaroyl monotropein | 870785-25-4 | 536.153 | C25H28O13 | 2 | 4.7 | chlorogenic acid |
| procyanidin B1 | 20315-25-7 | 578.1424 | C30H26O12 | 1 | 2.11 | procyanidin B2 |
| procyanidin B2 | 29106-49-8 | 578.1424 | C30H26O12 | 1 | 3.08 | procyanidin B2 |
| procyanidin C1 | 37064-30-5 | 866.2058 | C45H38O18 | 2 | 3.71 | procyanidin B2 |
| quercetin 3-arabinopyranoside | 22255-13-6 | 434.0849 | C20H18O11 | 2 | 5.18 | quercetin 3-galactoside |
| quercetin 3-galactoside | 482-36-0 | 464.0955 | C21H20O12 | 1 | 4.7 | quercetin 3-galactoside |
| quercetin 3-glucuronide | 22688-79-5 | 478.0747 | C21H18O13 | 2 | 4.79 | quercetin 3-galactoside |
| quercetin 3-rhamnoside | 522-12-3 | 448.1006 | C21H20O11 | 1 | 5.37 | quercetin 3-galactoside |
| syringetin 3-galactoside | 55025-56-4 | 508.1217 | C23H24O13 | 2 | 5.44 | quercetin 3-galactoside |
| syringetin 3-glucuronide | 1094607-09-6 | 522.101 | C23H22O14 | 2 | 5.54 | quercetin 3-galactoside |

 A Identification confidence: 1 = authentic standard; 2 = accurate mass and supporting information; 3 = accurate and limited supporting information

B Authentic standards were not available for the quantitation of all analytes. In these cases, quantitation was achieved using a chemically similar compound and concentrations are reported as equivalence.