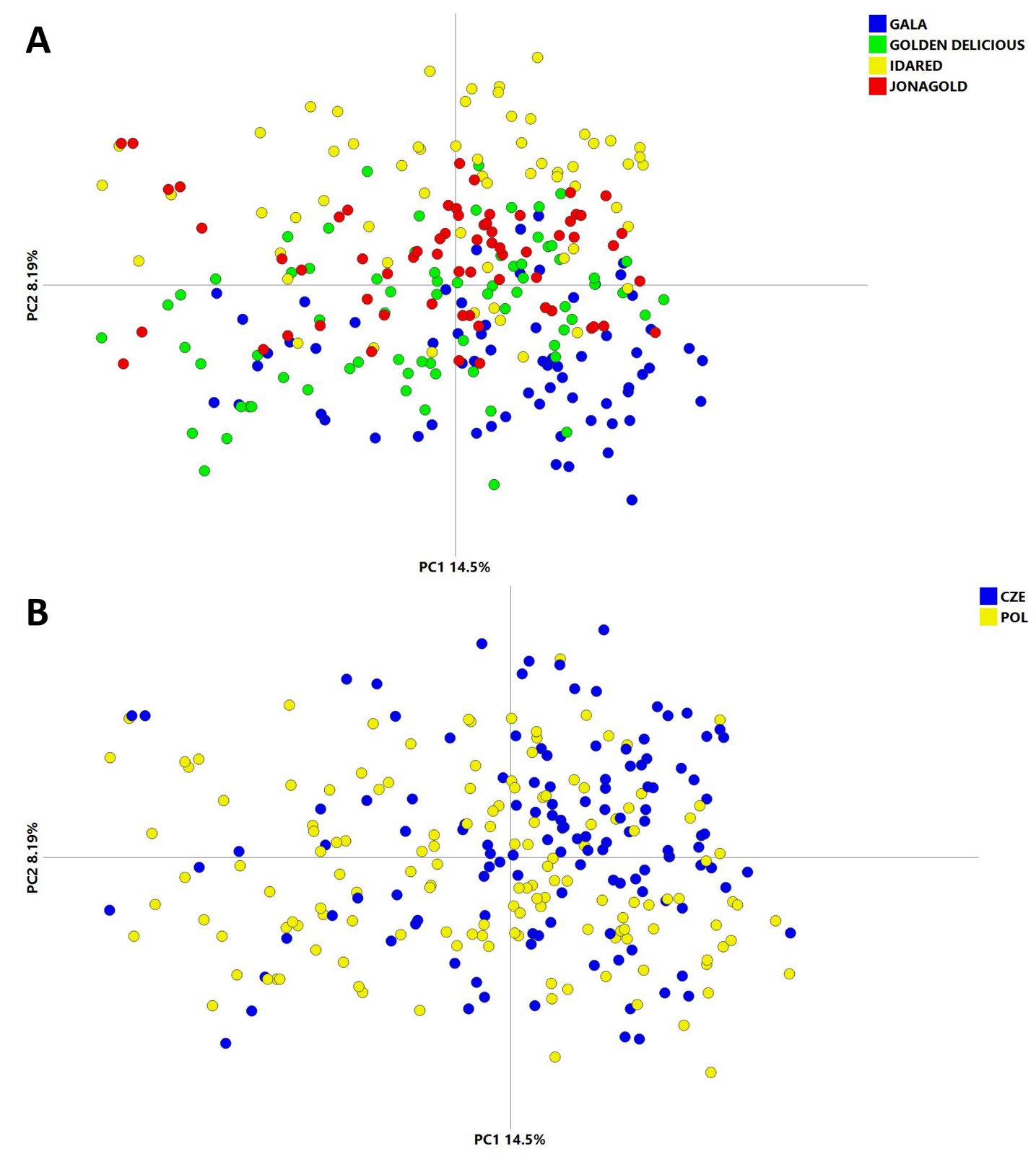
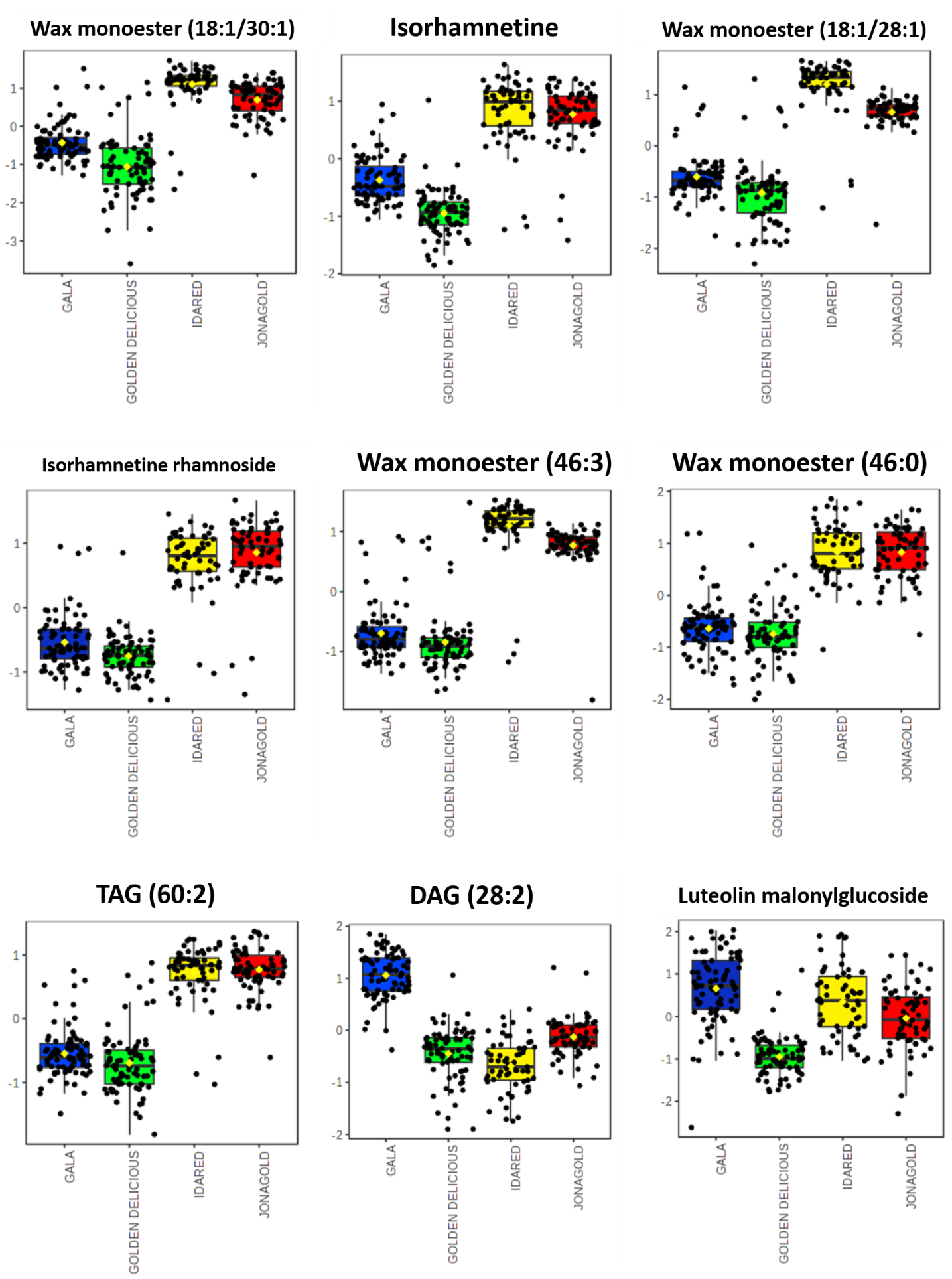
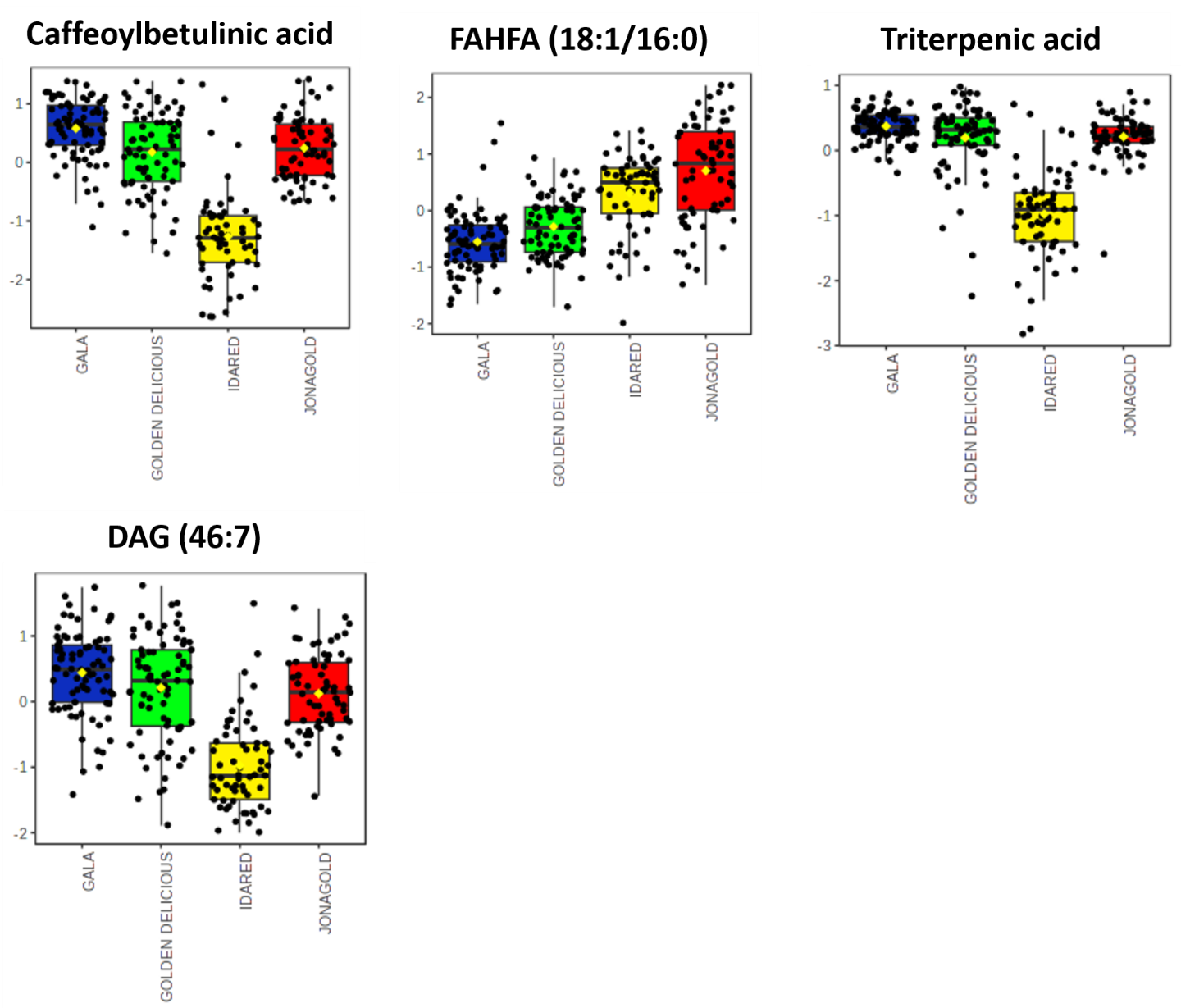


**Figure S1:** Total ion chromatograms (ESI+) showing intensities of compounds extracted by various solvent mixtures



**Figure S2:** PCA Score plots of the complete dataset of ESI- features. Samples are coloured according to their cultivar (A) and geographical origin (*B*).





**Figure S3:** Boxplots of identified markers for classification of apple cultivar.

**Table S1:** Identification of metabolites used for geographical origin classification of apple cultivar ‘Gala’. Markers are in descending order according to the AUC ROC value. The log2 FC value indicates whether the marker is increased in Czech samples (log2 FC > 0) or in Polish samples (log2 FC < 0).

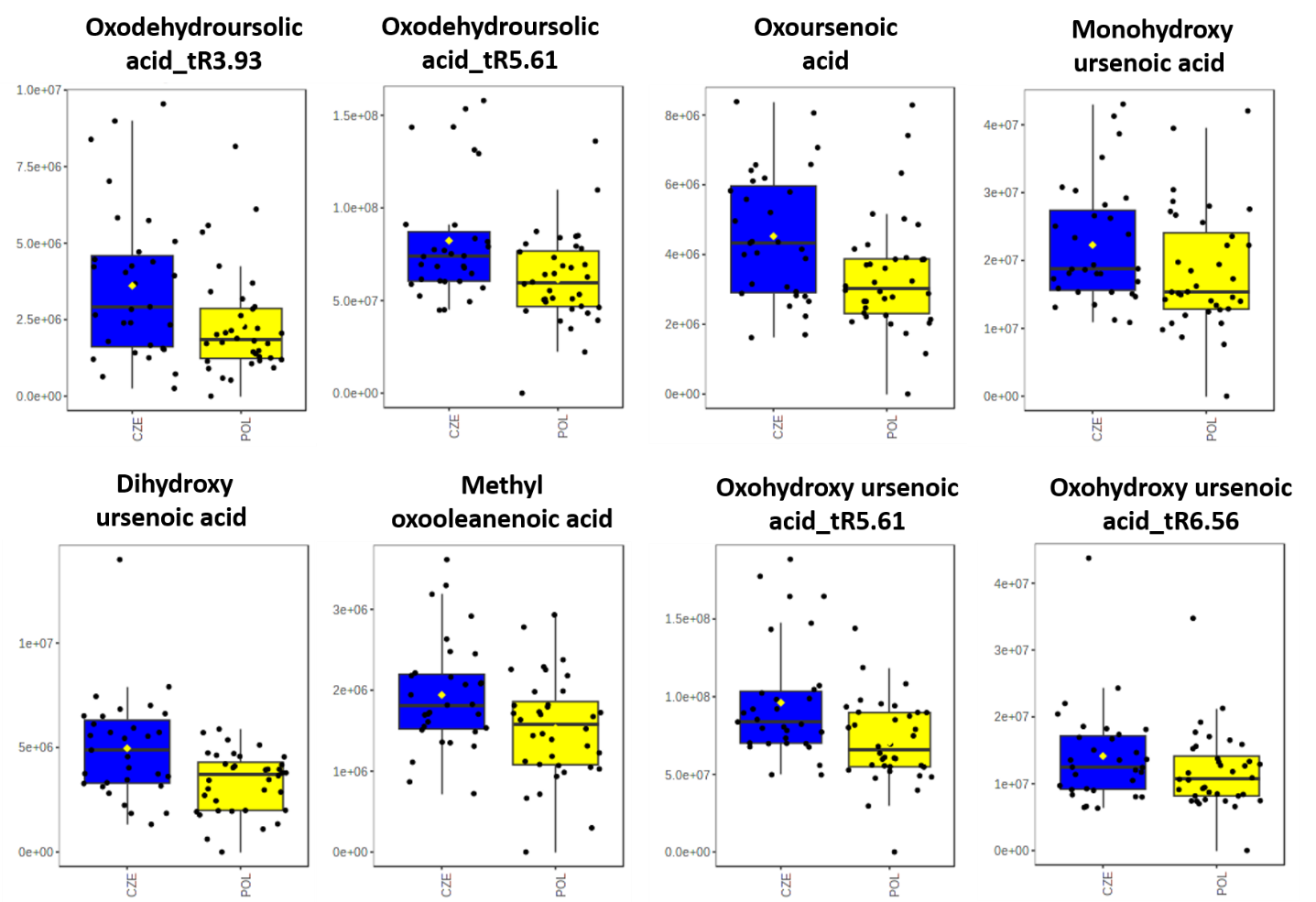
|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Marker ion (m/z) | **Retention time [min]** | **Adduct type** | Elemental formula | **Mass error [ppm]** | **Tentative identification** | **Univariate statistics parameters** | | | **Confidence level** |
| **t-test FDR p-value** | **Log2 FC** | **AUC ROC** |
| 635.6317 | 13.92 | [M+H]+ | C42H82O3 | -3.9 | Hydroxy wax ester (24:0/18:1-O) | 1.90E-08 | 0.78 | 0.91 | 2 |
| 661.6446 | 14.14 | [M+H]+ | C44H84O3 | -8 | Hydroxy wax ester (26:0/18:2-O) | 1.08E-05 | 0.61 | 0.9 | 2 |
| 135.0301 | 1.22 | [M-H]- | C4H8O5 | 5.6 | Trihydroxybutanoic acid | 1.05E-08 | -2.83 | 0.87 | 3 |
| 619.634 | 13.48 | [M+H]+ | C42H82O2 | -8.6 | Wax esters (42:1) | 3.03E-08 | 0.86 | 0.86 | 3 |
| 607.5995 | 13.51 | [M+H]+ | C40H78O3 | -5.6 | Hydroxy wax ester (40:1) | 6.40E-07 | 0.73 | 0.85 | 3 |
| 637.6466 | 13.78 | [M+H]+ | C42H84O3 | -5.1 | Hydroxy wax ester (26:0/16:0-O) | 4.70E-07 | 1.09 | 0.84 | 2 |
| 663.6599 | 14.33 | [M+H]+ | C44H86O3 | -8.5 | Hydroxy wax ester (26:0/18:1-O) | 8.07E-05 | 0.6 | 0.84 | 2 |
| 609.6143 | 13.39 | [M+H]+ | C40H80O3 | -7 | Hydroxy wax ester (24:0/16:0-O) | 1.13E-06 | 1.02 | 0.83 | 2 |
| 521.524 | 14.33 | [M+H]+ | C35H68O2 | -11 | Wax esters (26:0/9:1) | 3.53E-05 | 0.53 | 0.82 | 2 |
| 659.6268 | 13.44 | [M+H]+ | C44H82O3 | -11.2 | Hydroxy wax ester (26:0/18:3-O) | 1.12E-06 | 0.68 | 0.82 | 2 |
| 619.6351 | 13.77 | [M+H]+ | C42H82O2 | -6.8 | Wax esters (26:0/16:1) | 2.26E-06 | 1.04 | 0.81 | 2 |
| 691.6917 | 14.73 | [M+H]+ | C46H90O3 | -7.5 | Hydroxy wax ester (28:0/18:1-O) | 3.51E-04 | 0.5 | 0.81 | 2 |
| 631.5949 | 13.02 | [M+H]+ | C42H78O3 | -12.7 | Hydroxy wax ester (24:0/18:3-O) | 4.68E-05 | 0.73 | 0.81 | 2 |
| 455.3456 | 5.17 | [M+H]+ | C30H46O3 | -15.2 | Oxo ursenoic acid | 6.76E-06 | 0.42 | 0.81 | 3 |
| 615.5967 | 13.74 | [M+H]+ | C42H78O2 | -18.4 | Wax ester (24:0/18:3) | 1.90E-05 | 0.74 | 0.8 | 2 |
| 661.6455 | 12.91 | [M+H]+ | C44H84O3 | -6.7 | Hydroxy wax ester (24:0/18:2-O) | 8.11E-06 | 0.84 | 0.8 | 2 |
| 265.2493 | 9.67 | [M+H]+ | C18H32O | -14.5 | Linolenyl alcohol | 8.69E-06 | -1.12 | 0.79 | 3 |
| 287.2342 | 6.71 | [M+H]+ | C20H30O | -11.5 | Vitamin A | 2.43E-04 | -0.89 | 0.79 | 3 |
| 471.3458 | 5.61 | [M+H]+ | C30H46O4 | -3.5 | Oxohydroxy ursenoic acid | 1.18E-04 | 0.44 | 0.79 | 3 |
| 673.6816 | 14.74 | [M+H]+ | C46H88O2 | -6.9 | Wax ester (28:0/18:2) | 1.19E-04 | 0.43 | 0.79 | 2 |
| 457.3627 | 6.54 | [M+H]+ | C30H48O3 | -11.9 | Monohydroxy ursenoic acid | 4.60E-04 | 0.27 | 0.79 | 3 |
| 549.5176 | 11.68 | [M+H]+ | C36H68O3 | -12.9 | Hydroxy wax ester (36:2) | 7.75E-05 | 0.41 | 0.78 | 3 |
| 263.2368 | 9.13 | [M+H]+ | C18H30O | -2.6 | Farnesylacetol | 9.36E-05 | -1.02 | 0.77 | 3 |
| 318.2987 | 3.84 | [M+H]+ | C18H39NO3 | -6.7 | Phytosphingosine | 3.41E-05 | 0.75 | 0.77 | 3 |
| 453.3358 | 5.61 | [M+H]+ | C30H44O3 | -2.4 | Oxodehydroursolic acid | 2.13E-04 | 0.42 | 0.77 | 3 |
| 469.3625 | 6.73 | [M+H]+ | C31H48O3 | -12.1 | Methyl oxooleanenoic acid | 6.82E-04 | 0.34 | 0.77 | 3 |
| 615.6071 | 13.19 | [M+H]+ | C42H78O2 | -1.5 | Wax ester (24:0/18:3) | 9.98E-05 | 0.87 | 0.77 | 2 |
| 671.6644 | 13.95 | [M+H]+ | C46H86O2 | -9.2 | Wax ester (28:0/18:3) | 5.31E-05 | 0.71 | 0.77 | 2 |
| 673.6794 | 14.02 | [M+H]+ | C46H88O2 | -10.1 | Wax ester (28:0/18:2) | 4.17E-04 | 0.56 | 0.77 | 2 |
| 703.6281 | 15.83 | [M+H]+ | C45H82O5 | 5.7 | DAG (42:3) | 6.02E-05 | 1.13 | 0.77 | 3 |
| 379.0965 | 1.34 | [M+Na]+ | C16H20O9 | -10.6 | Feruloyl glucose | 1.02E-04 | 1.11 | 0.76 | 3 |
| 475.1182 | 1.59 | [M+Na]+ | C21H24O11 | -7.2 | Sieboldin | 8.92E-04 | 0.6 | 0.76 | 3 |
| 525.3529 | 6.44 | [M+Na]+ | C31H50O5 | -5.1 | Methyl ester euscaphic acid | 9.20E-05 | 0.85 | 0.76 | 3 |
| 641.6169 | 15.19 | [M+Na]+ | C42H82O2 | -6.7 | Wax ester (42:1) | 4.38E-05 | 0.35 | 0.76 | 3 |
| 669.6464 | 13.82 | [M+H]+ | C46H84O2 | -12.7 | Wax ester (28:0/18:4) | 3.92E-04 | 0.62 | 0.76 | 2 |
| 683.6628 | 15.97 | [M+Na]+ | C45H88O2 | -7.9 | Wax ester (45:1) | 2.78E-04 | 0.76 | 0.76 | 3 |
| 471.3437 | 6.56 | [M+H]+ | C30H46O4 | -7.9 | Oxohydroxy ursenoic acid | 1.75E-04 | 0.25 | 0.75 | 3 |
| 453.3329 | 3.93 | [M+H]+ | C30H44O3 | -8.8 | Oxodehydroursolic acid | 3.66E-04 | 0.63 | 0.75 | 3 |
| 469.3327 | 5.91 | [M+H]+ | C30H44O4 | 1.9 | Dioxohydroxy ursenoic acid | 4.58E-04 | 0.75 | 0.75 | 3 |
| 633.6124 | 13.74 | [M+H]+ | C42H80O3 | -9.7 | Hydroxy wax ester (24:0/18:2-O) | 2.92E-05 | 0.79 | 0.75 | 3 |

**Table S2:** Identification of metabolites used for geographical origin classification of apple cultivar ‘Golden Delicious’. Markers are in descending order according to the AUC ROC value. The log2 FC value indicates whether the marker is increased in Czech samples (log2 FC > 0) or in Polish samples (log2 FC < 0).

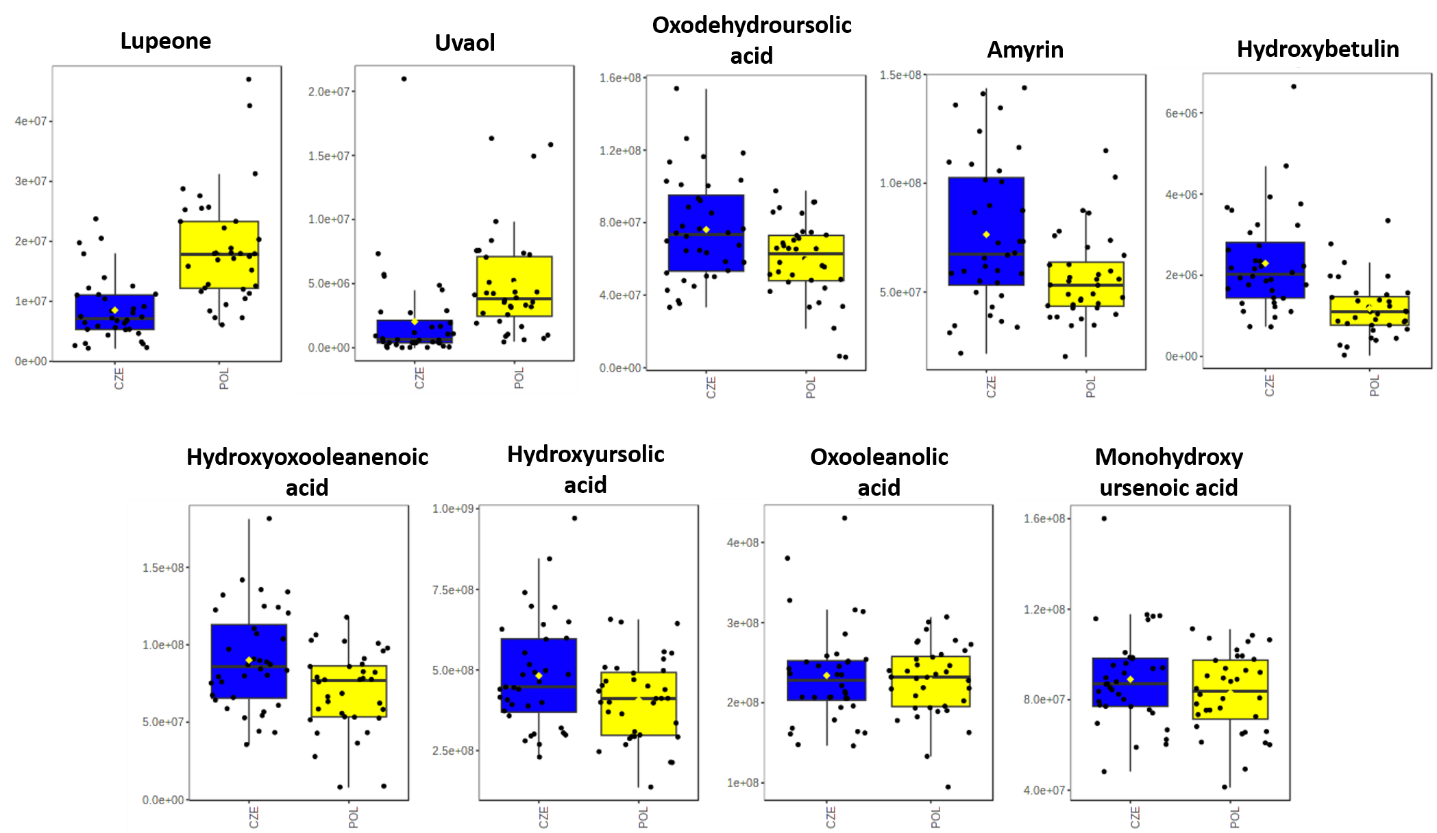
|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Marker ion (m/z) | **Retention time [min]** | **Adduct type** | Elemental formula | **Mass error [ppm]** | **Tentative identification** | **Univariate statistics parameters** | | | **Confidence level** |
| **t-test FDR p-value** | **Log2 FC** | **AUC ROC** |
| 671.6644 | 13.95 | [M+H]+ | C46H86O2 | -9.2 | Wax ester (28:0/18:3) | 1.30E-11 | 0.78 | 0.91 | 2 |
| 661.6455 | 12.91 | [M+H]+ | C44H84O3 | -6.7 | Hydroxy wax ester (24:0/18:2-O) | 5.98E-10 | 1.08 | 0.9 | 2 |
| 661.6455 | 13.58 | [M+H]+ | C44H84O3 | -6.6 | Hydroxy wax ester (24:0/18:2-O) | 2.78E-10 | 1.1 | 0.9 | 2 |
| 659.6295 | 12.78 | [M+H]+ | C44H82O3 | -7.1 | Hydroxy wax ester (26:0/18:3-O) | 4.99E-08 | 0.94 | 0.88 | 2 |
| 691.6893 | 14.19 | [M+H]+ | C46H890O3 | -10.8 | Hydroxy wax ester (28:0/18:1-O) | 2.92E-10 | 0.81 | 0.88 | 2 |
| 631.6307 | 14.14 | [M+H]+ | C43H82O2 | -13.7 | Wax ester (43:2) | 1.50E-08 | 0.68 | 0.87 | 3 |
| 643.6345 | 13.58 | [M+H]+ | C44H82O2 | -7.5 | Wax ester (26:0/18:3) | 4.11E-09 | 1.01 | 0.87 | 3 |
| 459.3801 | 5.89 | [M+H]+ | C30H50O3 | -8 | Hydroxybetulin | 1.91E-07 | 0.95 | 0.86 | 3 |
| 669.5425 | 12.12 | [M+Na]+ | C41H74O5 | -1.4 | DAG (38:3) | 2.96E-08 | 1.3 | 0.86 | 3 |
| 615.6071 | 13.19 | [M+H]+ | C42H78O2 | -1.4 | Wax ester (24:0/18:3) | 1.72E-07 | 1.24 | 0.85 | 2 |
| 647.557 | 12.13 | [M+Na]+ | C39H76O5 | -3.1 | DAG (36:0) | 1.68E-06 | 1.26 | 0.85 | 3 |
| 163.0745 | 10.88 | [M+H]+ | C10H10O2 | -8.9 | Methyl cinnamate | 7.18E-08 | -1.63 | 0.84 | 3 |
| 183.0862 | 1.15 | [M+H]+ | C6H14O6 | -3.6 | Sorbitol | 9.54E-08 | 1.19 | 0.84 | 3 |
| 605.5795 | 11.99 | [M+H]+ | C40H76O3 | -12.8 | Hydroxy wax ester (22:0/18:2-O) | 5.83E-06 | 0.94 | 0.84 | 2 |
| 131.0465 | 1.16 | [M-H]- | C4H8N2O3 | 6.3 | Asparagine | 8.20E-08 | 3.85 | 0.84 | 3 |
| 133.0628 | 12.05 | [M+H]+ | C9H8O | -19.3 | Cinnamaldehyde | 1.06E-06 | 0.67 | 0.83 | 3 |
| 425.3765 | 12.47 | [M+H]+ | C30H48O | -4.4 | Lupeone | 1.90E-07 | -1.13 | 0.83 | 3 |
| 451.4453 | 14.44 | [M+H]+ | C30H58O2 | -13.9 | Triacontenoic acid | 2.38E-06 | -1.73 | 0.83 | 3 |
| 487.4499 | 12.11 | [M+H]+ | C33H58O2 | -3.3 | Wax ester (33:4) | 1.12E-06 | -0.96 | 0.83 | 3 |
| 165.0757 | 1.15 | [M+H]+ | C6H12O5 | -3.9 | Fucose | 6.22E-08 | 1.27 | 0.83 | 3 |
| 659.6288 | 13.76 | [M+H]+ | C44H82O3 | -8.2 | Hydroxy wax ester (26:0/18:3-O) | 2.26E-07 | 0.55 | 0.83 | 2 |
| 663.6599 | 14.33 | [M+H]+ | C44H86O3 | -8.5 | Hydroxy wax ester (26:0/18:1-O) | 6.63E-07 | 0.57 | 0.83 | 2 |
| 665.6752 | 14.16 | [M+H]+ | C44H88O3 | -9 | Hydroxy wax ester (44:0) | 4.72E-07 | 0.64 | 0.83 | 3 |
| 691.6917 | 14.73 | [M+H]+ | C46H890O3 | -7.5 | Hydroxy wax ester (28:0/18:1-O) | 2.32E-06 | 0.27 | 0.83 | 2 |
| 711.585 | 11.77 | [M+Na]+ | C44H80O5 | -7.6 | DAG (41:3) | 5.56E-07 | -1.38 | 0.83 | 3 |
| 445.3968 | 13.46 | [M+H]+ | C30H52O2 | -17.4 | Wax ester (30:4) | 1.43E-06 | -1.57 | 0.82 | 3 |
| 659.6268 | 13.44 | [M+H]+ | C44H82O3 | -11.2 | Hydroxy wax ester (26:0/18:3-O) | 1.87E-06 | 0.44 | 0.82 | 2 |
| 299.0562 | 2.86 | [M-H]- | C16H12O6 | 2.1 | Luteolin methyl ether | 5.75E-07 | -1.82 | 0.82 | 3 |
| 265.2524 | 11.32 | [M+H]+ | C18H32O | -2.6 | Linolenyl alkohol | 3.45E-06 | -1.17 | 0.81 | 3 |
| 457.3661 | 7.28 | [M+H]+ | C30H48O3 | -4.6 | Monohydroxy ursenoic acid | 1.51E-06 | 0.1 | 0.81 | 3 |
| 635.6317 | 13.92 | [M+H]+ | C42H82O3 | -3.9 | Hydroxy wax ester (24:0/18:1-O) | 5.44E-06 | 0.28 | 0.81 | 2 |
| 673.6816 | 14.74 | [M+H]+ | C46H88O2 | -6.9 | Wax ester (28:0/18:2) | 7.60E-06 | 0.2 | 0.81 | 2 |
| 675.5843 | 12.6 | [M+Na]+ | C41H80O5 | -9 | DAG (38:0) | 8.74E-07 | 1.14 | 0.81 | 3 |
| 853.7221 | 15.53 | [M+Na]+ | C53H98O6 | -4.8 | TAG (40:2) | 3.75E-07 | -1.41 | 0.81 | 3 |
| 875.7018 | 14.04 | [M+Na]+ | C55H96O6 | -9.9 | TAG (52:5) | 2.86E-05 | 0.66 | 0.81 | 3 |
| 877.7224 | 15.24 | [M+Na]+ | C55H98O6 | -4.2 | TAG (52:4) | 1.48E-06 | -1.65 | 0.81 | 3 |
| 181.0744 | 1.15 | [M-H]- | C6H14O6 | 17.6 | Galacticol | 5.16E-07 | 0.83 | 0.81 | 3 |
| 263.2368 | 9.13 | [M+H]+ | C18H30O | -2.8 | Farnesylacetol | 1.04E-04 | -1.31 | 0.8 | 3 |
| 133.0642 | 10.42 | [M+H]+ | C9H8O | -8.9 | Cinnamaldehyde | 2.39E-05 | -1.27 | 0.8 | 3 |
| 193.1605 | 9.13 | [M+H]+ | C13H20O | 6.6 | Damascone | 2.07E-06 | -1.37 | 0.8 | 3 |
| 443.3858 | 13.44 | [M+H]+ | C30H50O2 | -7.1 | Uvaol | 7.36E-06 | -1.27 | 0.79 | 3 |
| 471.3458 | 5.61 | [M+H]+ | C30H46O4 | -3.5 | Hydroxyoxooleanenoic acid | 4.97E-05 | 0.37 | 0.79 | 3 |
| 643.6299 | 14.14 | [M+H]+ | C44H82O2 | -14.6 | Wax ester (26:0/18:3) | 1.34E-05 | 0.18 | 0.79 | 2 |
| 427.3927 | 7.83 | [M+H]+ | C30H50O | -3.1 | Amyrin | 2.32E-05 | 0.44 | 0.78 | 3 |
| 445.3971 | 10.15 | [M+H]+ | C30H52O2 | -16.8 | Wax ester (30:4) | 9.40E-03 | -2.15 | 0.78 | 3 |
| 445.3997 | 13.72 | [M+H]+ | C30H52O2 | -10.9 | Wax ester (30:4) | 2.48E-05 | -1.24 | 0.78 | 3 |
| 453.3358 | 5.61 | [M+H]+ | C30H44O3 | -2.4 | Oxodehydroursolic acid | 8.40E-05 | 0.37 | 0.78 | 3 |
| 593.5082 | 10.9 | [M+H]+ | C37H68O5 | -10.6 | DAG (34:2) | 2.11E-05 | -1.63 | 0.78 | 3 |
| 631.5949 | 13.02 | [M+H]+ | C42H78O3 | -12.7 | Hydroxy wax ester (24:0/18:3-O) | 3.94E-05 | 0.65 | 0.78 | 2 |
| 661.6446 | 14.14 | [M+H]+ | C44H84O3 | -8 | Hydroxy wax ester (26:0/18:2-O) | 3.26E-05 | 0.19 | 0.78 | 2 |
| 635.4605 | 10.35 | [M+Na]+ | C39H64O5 | -7.4 | DAG (36:6) | 3.96E-05 | 1.86 | 0.78 | 3 |
| 685.5684 | 13.44 | [M+Na]+ | C42H78O5 | -9.2 | DAG (38:2) | 9.87E-05 | -1.23 | 0.78 | 3 |
| 275.0891 | 1.8 | [M+H]+ | C15H14O5 | -10.4 | Phloretin | 3.23E-05 | 0.43 | 0.77 | 3 |
| 443.3849 | 7.64 | [M+H]+ | C30H50O2 | -9.1 | Wax ester (30:5) | 3.72E-05 | 0.32 | 0.77 | 3 |
| 543.4717 | 12.4 | [M+H]+ | C36H62O3 | -11 | Hydroxy wax ester (36:5) | 2.23E-05 | -1.17 | 0.77 | 3 |
| 609.6143 | 13.39 | [M+H]+ | C40H80O3 | -7 | Hydroxy wax ester (24:0/16:0-O) | 6.61E-05 | 0.55 | 0.77 | 3 |
| 619.6351 | 13.77 | [M+H]+ | C42H82O2 | -6.7 | Wax ester (26:0/16:1) | 1.40E-05 | 0.59 | 0.77 | 2 |
| 755.6085 | 12.28 | [M+H]+ | C43H83N2O6P | 2.4 | SM (20:2) | 2.02E-05 | -1 | 0.77 | 3 |
| 615.4914 | 11.35 | [M+Na]+ | C37H68O5 | -8.2 | DAG (34:2) | 5.68E-05 | 0.63 | 0.77 | 3 |
| 699.5872 | 12.84 | [M+Na]+ | C43H80O5 | -4.6 | DAG (40:2) | 2.73E-04 | 0.7 | 0.77 | 3 |
| 133.0139 | 1.25 | [M-H]- | C4H6O5 | 1.5 | Malic acid | 3.65E-05 | 2.11 | 0.77 | 3 |
| 209.0666 | 1.2 | [M-H]- | C7H14O7 | 2.2 | Heptulose | 5.58E-05 | 1.32 | 0.77 | 3 |
| 447.4142 | 10.22 | [M+H]+ | C30H54O2 | -13.5 | Wax ester (30:3) | 7.10E-05 | -1.29 | 0.76 | 3 |
| 455.3494 | 5.84 | [M+H]+ | C30H46O3 | -6.9 | Oxooleanolic acid | 2.98E-05 | 0.04 | 0.76 | 3 |
| 663.5863 | 12.23 | [M+H]+ | C42H78O5 | -9.7 | DAG (38:2) | 1.47E-04 | -1.35 | 0.76 | 3 |
| 495.3431 | 5.41 | [M+Na]+ | C30H48O4 | -3.9 | Hydroxyursolic acid | 4.54E-05 | 0.24 | 0.76 | 3 |
| 650.5706 | 11.22 | [M+NH4]+ | C40H72O5 | -2.7 | DAG (36:3) | 2.52E-05 | -1.36 | 0.76 | 3 |
| 198.0964 | 1.15 | [M+NH4]+ | C6H12O6 | -6.7 | Mannose | 2.32E-04 | 1.1 | 0.76 | 3 |
| 631.5981 | 13.29 | [M+H]+ | C42H78O3 | -7.7 | Hydroxy wax ester (24:0/18:3-O) | 2.59E-03 | 0.21 | 0.75 | 2 |
| 613.4818 | 8.72 | [M+Na]+ | C37H66O5 | 1.7 | DAG (34:3) | 2.75E-04 | 0.81 | 0.75 | 3 |
| 685.5693 | 10.25 | [M+Na]+ | C42H78O5 | -7.9 | DAG (38:2) | 1.04E-04 | -1.12 | 0.75 | 3 |
| 851.7066 | 13.8 | [M+Na]+ | C53H96O6 | -4.5 | TAG (40:3) | 3.96E-05 | -0.93 | 0.75 | 3 |

**Table S3:** Identification of metabolites used for geographical origin classification of apple cultivar ‘Idared’. Markers are in descending order according to the AUC ROC value. The log2 FC value indicates whether the marker is increased in Czech samples (log2 FC > 0) or in Polish samples (log2 FC < 0).

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Marker ion (m/z) | **Retention time [min]** | **Adduct type** | Elemental formula | **Mass error [ppm]** | **Tentative identification** | **Univariate statistics parameters** | | | **Confidence level** |
| **t-test FDR p-value** | **Log2 FC** | **AUC ROC** |
| 167.0709 | 1.74 | [M+H]+ | C9H10O3 | 0.5 | Ethyl salicylate | 1.37E-05 | -2.82 | 0.82 | 3 |
| 225.0587 | 1.15 | [M+HCOO]- | C6H12O6 | 10.4 | Mannose | 1.04E-05 | 1.25 | 0.82 | 3 |
| 561.1335 | 1.45 | [M+H]+ | C30H24O11 | -11 | Biflavonoid | 1.31E-04 | 1.69 | 0.78 | 3 |
| 209.0666 | 1.2 | [M-H]- | C7H14O7 | 2.2 | Heptulose | 4.21E-05 | 1.18 | 0.78 | 3 |
| 353.0853 | 1.48 | [M-H]- | C16H18O9 | 5.6 | Chlorogenic acid | 3.18E-04 | 1.06 | 0.76 | 3 |
| 131.0465 | 1.16 | [M-H]- | C4H8N2O3 | 6.2 | Asparagine | 4.54E-04 | 1.15 | 0.75 | 3 |



**Figure S4:** Boxplots of markers for classification of geographical origin for cultivar ‘Gala’ identified as triterpenic ursenoic acids and their derivatives and precursors.



**Figure S5:** Boxplots of markers for classification of geographical origin for cultivar ‘Golden Delicious’ identified as triterpenic ursenoic acids and their derivatives and precursors.