Electronic Supplementary Material (ESI) for Molecular Omics.


# Electronic Supplementary Information

## Food Authentication: Truffle Species Classification by non-targeted Lipidomics Analyzes using Mass Spectrometry assisted by Ion Mobility Separation

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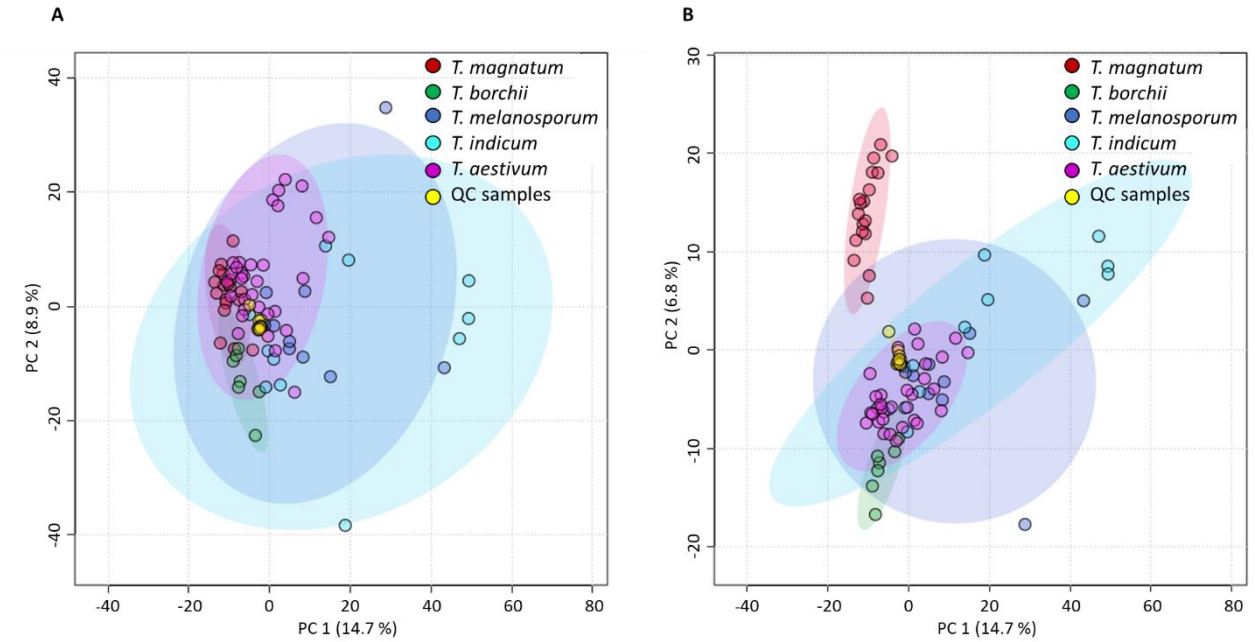


**Figure S1.** PCA scores plot of all measurements including QC samples. A) PC 1 *vs.* PC 2 and B) PC 1 *vs*. PC 3).

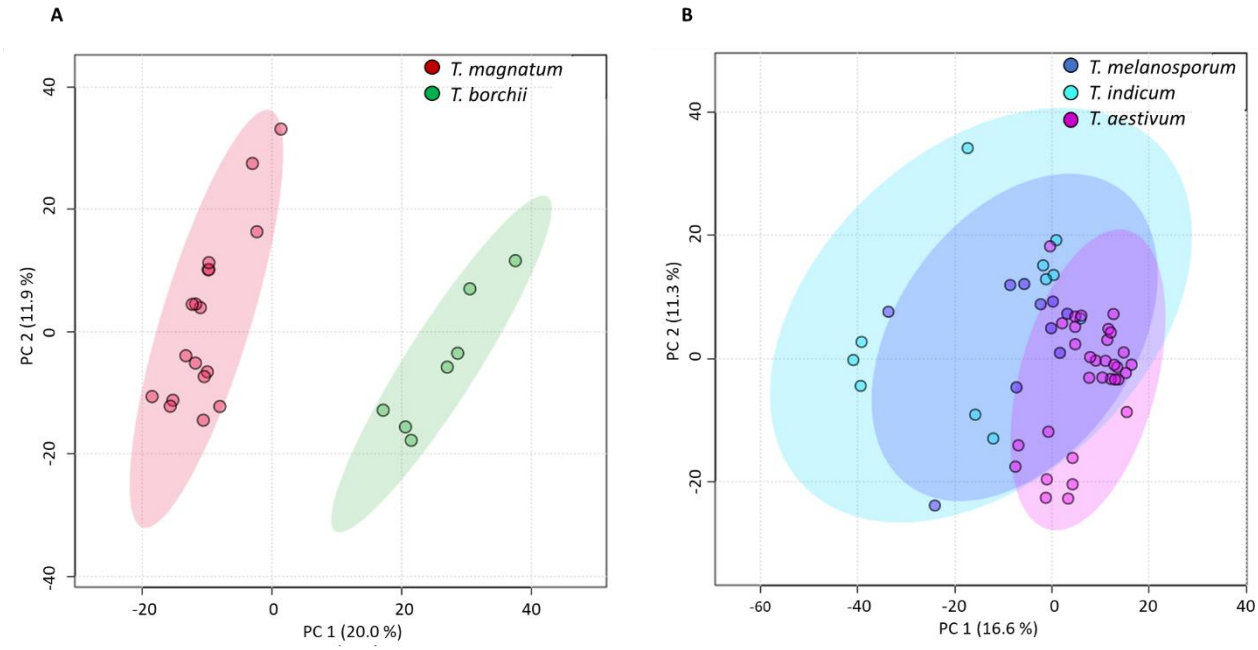


**Figure S2.** PCA scores plot of all detected features. A) White truffle varieties. B) Black truffle varieties.

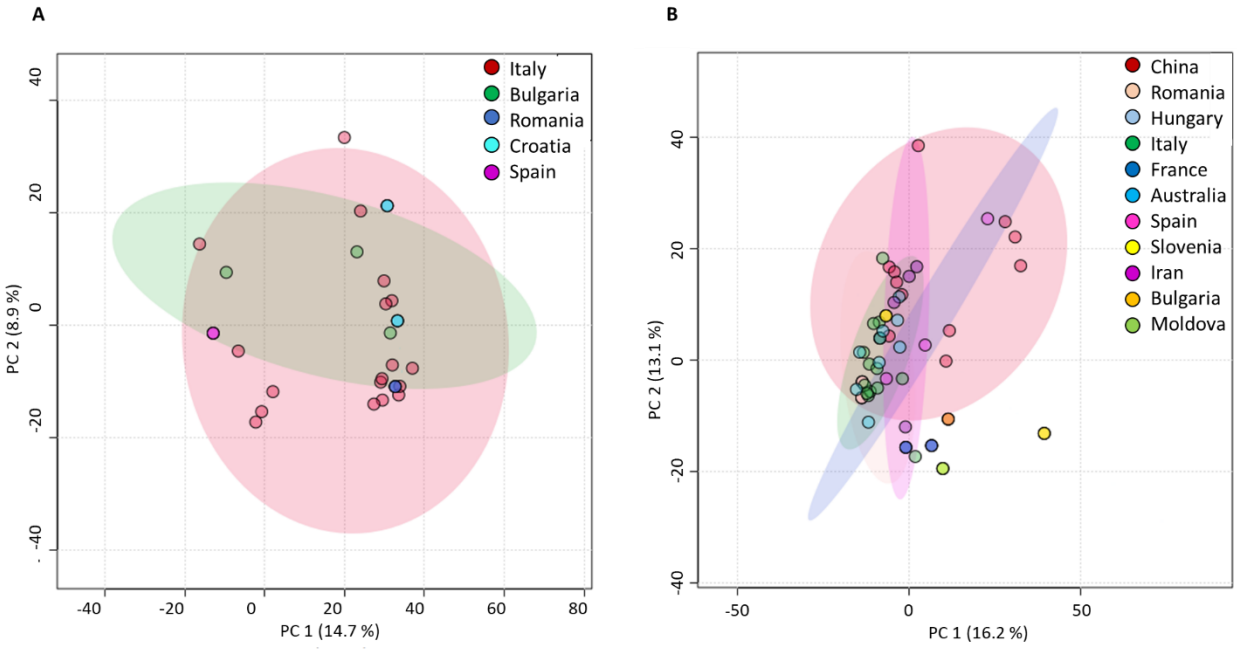


**Figure S3.** PCA scores plot of all detected features. A) White truffle varieties colored according to their geographical origin. B) Black truffle varieties colored according to their geographical origin.

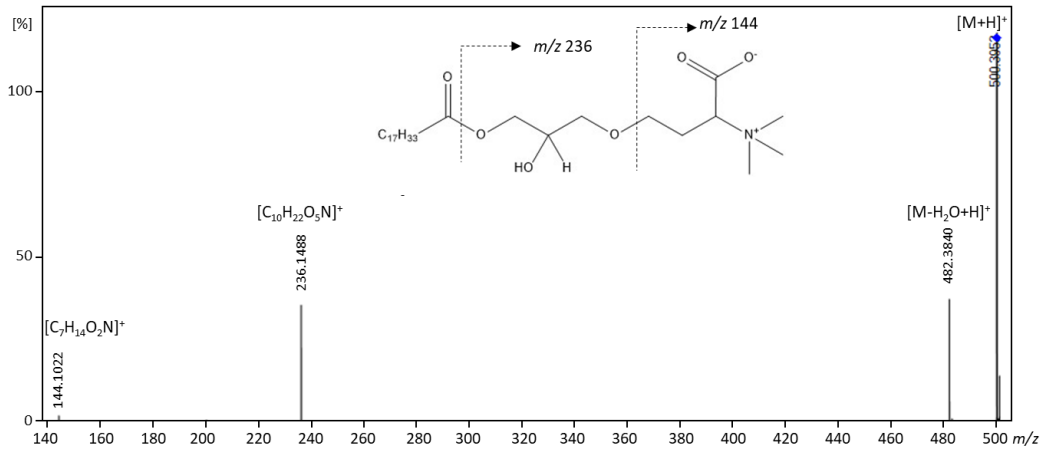


**Figure S4.** MS/MS spectrum of LDGTS (18:1).

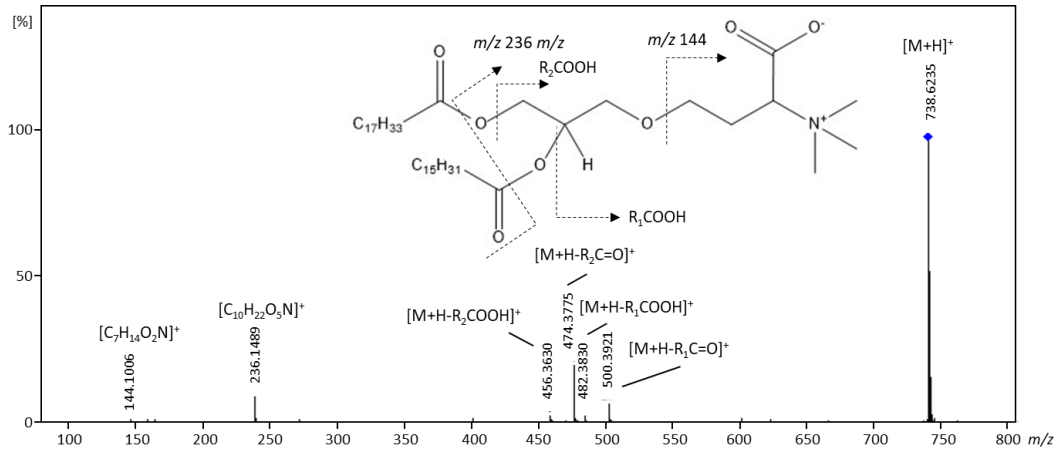


**Figure S5.** MS/MS spectrum of DGTS (34:1).

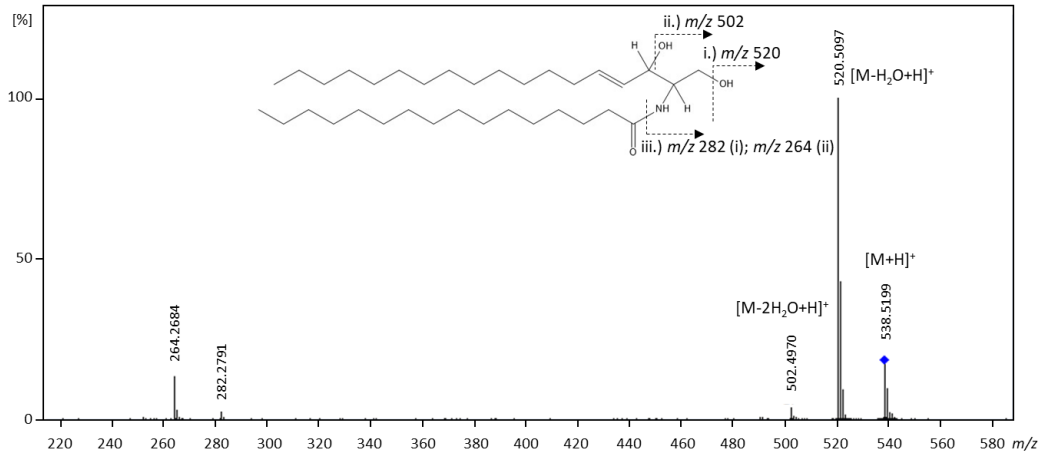


**Figure S6.** MS/MS spectrum of Cer (34:1;O2)

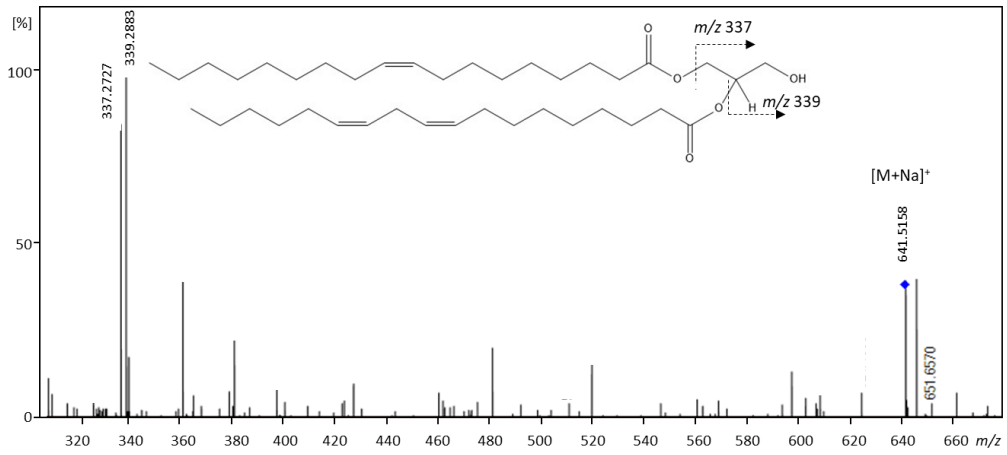


**Figure S7.** MS/MS spectrum of DG (36:3).

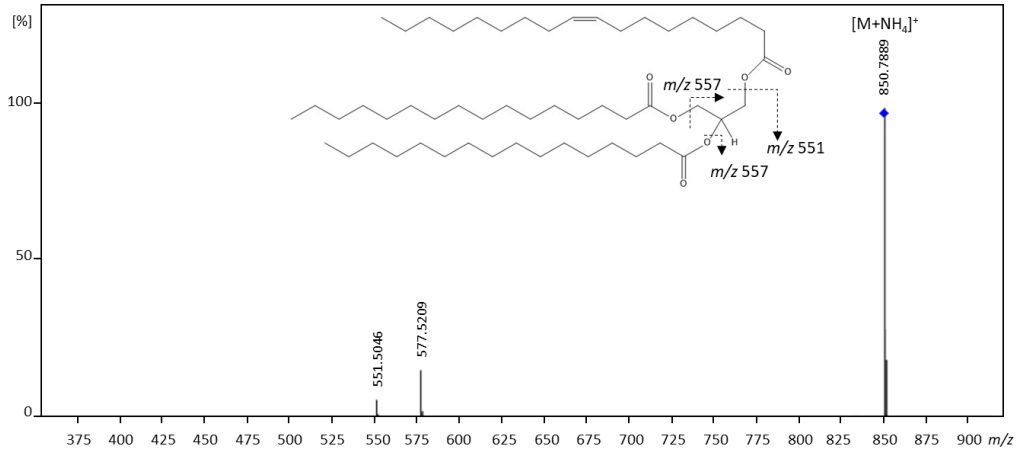


**Figure S8.** MS/MS spectrum of TG (50:1)

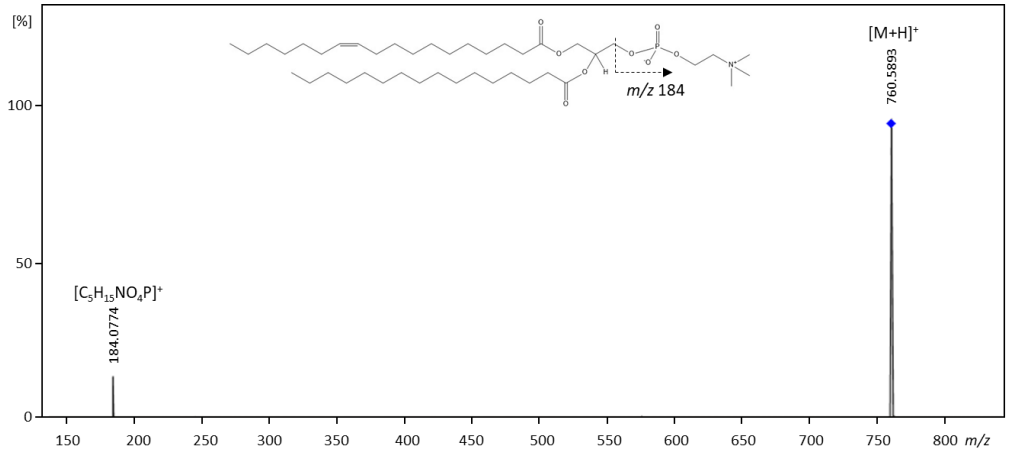


**Figure S9.** MS/MS spectrum of PC (34:1).

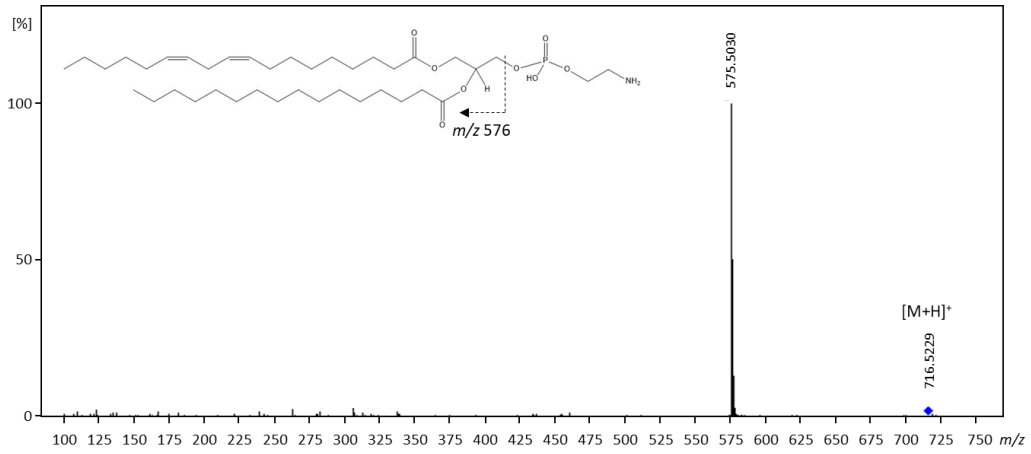


**Figure S10.** MS/MS spectrum of PE (34:2).

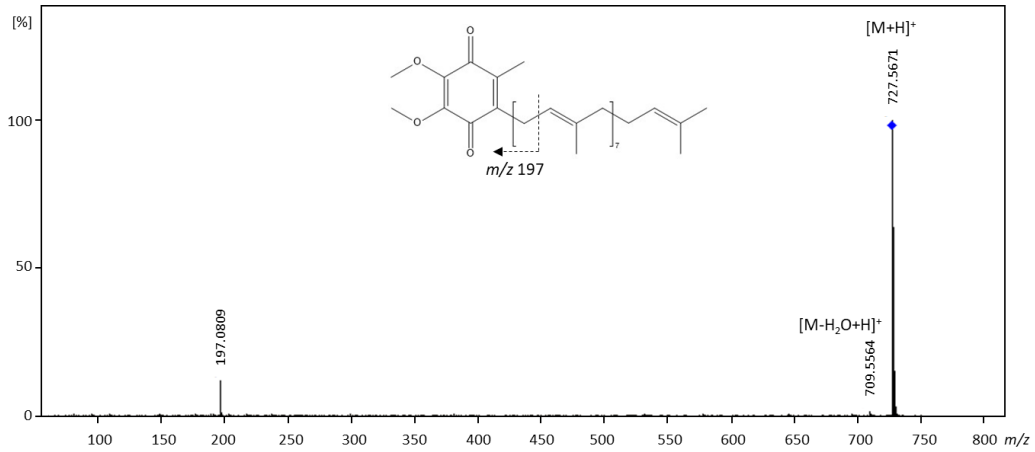


**Figure S11.** MS/MS spectrum of Coenzyme Q8.

**Table S1.** Identified key metabolites of the white truffle samples with their LC-IM-MS data, which show a dependency due to different origins.

| Tentative compound | Proposed formula | RT [min] | Adduct | m/z calculated [Da] | m/z measured [Da] | Error [ppm] | Relevant fragments | Match Score Total (Lipid Annotator) | CCS value measured [Å²] | CCS value calculated [Å²] | Delta CCS [%] | FDR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Betaine diradylglycerols** | | | | | | | | | | | | |
| LDGTS (18:1) | $C_{28}H_{53}NO_6$ | 4.5 | $[M+H]^+$ | 500.3946 | 500.3953 | 1.47 | 482.38; 236.15; 144.10 | 90.14 | 228.6 | no entry | no entry | 2.4E-7 |
| LDGTS (18:2) | $C_{28}H_{51}NO_6$ | 4.2 | $[M+H]^+$ | 498.3789 | 498.3788 | -0.23 | 480.37; 236.15; 144.10 | 95.41 | 224.5 | no entry | no entry | 2.9E-9 |
| DGTS (34:1) | $C_{44}H_{83}NO_7$ | 9.3 | $[M+H]^+$ | 738.6242 | 738.6245 | 0.37 | 474.38; 236.15; 144.10 | 75.27 | 286.4 | no entry | no entry | 5.0E-21 |
| DGTS (34:2) | $C_{44}H_{81}NO_7$ | 8.5 | $[M+H]^+$ | 736.6086 | 736.6081 | -0.65 | 236.15 | 75.09 | 283.6 | no entry | no entry | 3.5E-18 |
| DGTS (36:2) | $C_{46}H_{85}NO_7$ | 9.4 | $[M+H]^+$ | 764.6399 | 764.6392 | -0.89 | 500.40; 236.15; 144.10 | 74.71 | 291.2 | no entry | no entry | 9.3E-10 |
| DGTS (36:3) | $C_{46}H_{83}NO_7$ | 8.6 | $[M+H]^+$ | 762.6242 | 762.6245 | 0.35 | 500.40; 482.38; 236.15; 144.10 | 74.85 | 288.7 | no entry | no entry | 2.6E-15 |
| DGTS (36:4) | $C_{46}H_{81}NO_7$ | 7.7 | $[M+H]^+$ | 760.6086 | 760.6103 | 2.26 | 498.38; 480.37; 236.15; 144.10 | 75.06 | 285.9 | no entry | no entry | 8.3E-15 |
| **Ceramides** | | | | | | | | | | | | |
| Cer (34:0;O3) | $C_{34}H_{69}NO_4$ | 9.5 | $[M+H]^+$ | 556.5299 | 556.5322 | 4.08 | 538.52; 520.50 | / | 257.5 | 259.6 | 0.8 | 5.1E-6 |
| Cer (36:3;O3) | $C_{36}H_{67}NO_4$ | 9.6 | $[M+H]^+$ | 578.5143 | 578.5142 | -0.15 | 560.50 | / | 256.5 | no entry | no entry | 6.3E-7 |
| HexCer (35:3;O2) | $C_{41}H_{75}NO_8$ | 7.6 | $[M+H]^+$ | 710.5565 | 710.5564 | -0.20 | 548.50; 530.49 | 86.50 | 278.3 | no entry | no entry | 6.2E-6 |
| **Glycerolipids** | | | | | | | | | | | | |
| DG (36:3) | $C_{39}H_{70}O_5$ | 10.4 | $[M+H]^+$ | 619.5296 | 619.5285 | 1.78 | 337.27 | / | 261.8 | 262.3 | 0.2 | 1.1E-6 |
| DG (36:3) | $C_{39}H_{70}O_5$ | 10.4 | $[M+NH_4]^+$ | 636.5562 | 636.5561 | -0.08 | 339.29; 337.27 | 88.10 | 261.4 | 263.6 | 0.8 | 3.2E-7 |
| DG (36:3) | $C_{39}H_{70}O_5$ | 10.5 | $[M+Na]^+$ | 641.5115 | 641.5119 | 0.57 | 339.29; 337.27 | / | 261.8 | 264.0 | 0.8 | 2.9E-6 |
| TG (50:1) | $C_{53}H_{100}O_6$ | 18.6 | $[M+NH_4]^+$ | 850.7858 | 850.7889 | 3.7 | 577.52; 551.50 | 90.19 | 317.1 | 315.7 | -0.4 | 5.1E-10 |
| TG (50:1) | $C_{53}H_{100}O_6$ | 18.6 | $[M+Na]^+$ | 855.7412 | 855.7410 | -0.25 | / | 78.44 | 315.4 | 316.1 | 0.2 | 6.8E-9 |
| TG (50:2) | $C_{53}H_{98}O_6$ | 17.9 | $[M+NH_4]^+$ | 848.7702 | 848.7707 | 0.64 | 577.52; 575.50; 549.49 | 88.45 | 315.0 | 313.4 | -0.5 | 1.2E-10 |
| TG (50:2) | $C_{53}H_{98}O_6$ | 17.9 | $[M+Na]^+$ | 853.7256 | 853.7259 | 0.41 | / | 79.87 | 314.1 | 313.8 | -0.1 | 4.5E-9 |
| TG (52:2) | $C_{55}H_{102}O_6$ | 18.6 | $[M+NH_4]^+$ | 876.8015 | 876.8055 | 4.7 | 603.53; 577.52 | 87.96 | 322.0 | 319.3 | -0.8 | 2.9E-6 |
| TG (52:2) | $C_{55}H_{102}O_6$ | 18.7 | $[M+Na]^+$ | 881.7569 | 881.7547 | -2.52 | 577.52 | 78.42 | 321.1 | 319.6 | -0.5 | 1.1E-6 |
| TG (54:1) | $C_{57}H_{108}O_6$ | 19.3 | $[M+Na]^+$ | 911.8038 | 911.8038 | -0.01 | 607.56; 605.55 | 75.82 | 327.6 | 327.3 | -0.1 | 3.0E-7 |
| TG (54:2) | $C_{57}H_{106}O_6$ | 20.0 | $[M+NH_4]^+$ | 904.8328 | 904.8360 | 3.65 | 605.55; 603.54 | 90.54 | 327.9 | 325.1 | -0.9 | 2.9E-6 |
| TG (54:3) | $C_{57}H_{104}O_6$ | 19.2 | $[M+NH_4]^+$ | 902.8171 | 902.8193 | 2.47 | 605.55; 603.54; 601.52 | 87.73 | 326.4 | 324.1 | -0.7 | 2.1E-7 |
| TG (57:4) | $C_{60}H_{108}O_6$ | 19.2 | $[M+Na]^+$ | 947.8038 | 947.8043 | 0.53 | 645.58; 599.50 | 69.05 | 330.3 | 331.8 | -0.5 | 1.2E-5 |
| **Phospholipids** | | | | | | | | | | | | |
| PC (32:1) | $C_{40}H_{78}NO_8P$ | 8.0 | $[M+H]^+$ | 732.5538 | 732.5567 | 3.99 | 184.07 | 92.58 | 281.3 | 281.9 | 0.2 | 1.5E-6 |
| PC (34:1) | $C_{42}H_{82}NO_8P$ | 9.2 | $[M+H]^+$ | 760.5851 | 760.5893 | 5.55 | 184.07 | 96.67 | 287.1 | 288.1-288.2 | 0.4 | 1.2E-17 |
| PC (34:2) | $C_{42}H_{80}NO_8P$ | 8.3 | $[M+H]^+$ | 758.5694 | 758.5707 | 1.67 | 184.07 | 94.00 | 284.6 | 281.4-287.6 | -1.1-1.03 | 3.1E-7 |
| PC (35:2) | $C_{43}H_{82}NO_8P$ | 8.5 | $[M+H]^+$ | 772.5851 | 772.5847 | -0.49 | 184.07 | 85.18 | 287.9 | 287.9-289.1 | 0.0-0.4 | 9.1E-7 |
| PC (35:4) | $C_{43}H_{78}NO_8P$ | 7.3 | $[M+H]^+$ | 768.5538 | 768.5529 | -1.15 | 184.07 | 97.33 | 283.5 | 284.2-286.3 | 0.3-1.0 | 6.5E-6 |
| PC (35:6) | $C_{43}H_{74}NO_8P$ | 8.5 | $[M+H]^+$ | 764.5225 | 764.5206 | -2.46 | 184.07 | / | 282.0 | 281.2-282.0 | -0.3-0.0 | 1.5E-7 |
| PC (36:5) | $C_{44}H_{78}NO_8P$ | 8.1 | $[M+H]^+$ | 780.5538 | 780.5507 | -3.95 | 184.07 | 86.41 | 288.0 | 286.2-288.4 | -0.6-0.1 | 1.1E-5 |
| PC (37:4) | $C_{45}H_{82}NO_8P$ | 8.0 | $[M+H]^+$ | 796.5851 | 796.5841 | -1.23 | 184.07 | 87.55 | 290.8 | 290.7-292.8 | 0.0-0.6 | 8.9E-7 |

**Table S1.** Continued.

| Tentative compound | Proposed formula | RT [min] | Adduct | m/z calculated [Da] | m/z measured [Da] | Error [ppm] | Relevant fragments | Match Score Total (Lipid Annotator) | CCS value measured [Å²] | CCS value calculated [Å²] | Delta CCS [%] | FDR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PC (37:5) | $C_{45}H_{80}NO_8P$ | 8.7 | $[M+H]^+$ | 794.5694 | 794.5656 | -4.83 | 184.07 | 65.20 | 291.4 | 289.8-291.1 | -0.5-0.0 | 7.9E-7 |
| PC (38:4) | $C_{46}H_{84}NO_8P$ | 8.4 | $[M+H]^+$ | 810.6007 | 810.5999 | -1.03 | 184.07 | 97.67 | 292.4 | 285.5-296.4 | -2.4-1.3 | 1.2E-7 |
| PC (40:7) | $C_{48}H_{82}NO_8P$ | 8.5 | $[M+H]^+$ | 832.5851 | 832.5818 | -3.95 | 184.07 | / | 294.9 | 295.4-296.8 | 0.2-0.6 | 1.0E-7 |
| PE (34:2) | $C_{39}H_{74}NO_8P$ | 8.2 | $[M+H]^+$ | 716.5225 | 716.5229 | 0.59 | 575.50 | 85.94 | 273.1 | 273.2-275.5 | 0.0-0.9 | 3.2E-7 |
| PE (36:3) | $C_{41}H_{76}NO_8P$ | 8.3 | $[M+H]^+$ | 742.5381 | 742.5382 | 0.09 | 601.52 | 80.53 | 276.4 | 278.1-280.2 | 0.6-1.4 | 1.7E-6 |
| **Other** | | | | | | | | | | | | |
| Coenzyme Q8 | $C_{49}H_{74}O_4$ | 12.4 | $[M+Na]^+$ | 749.5479 | 749.5480 | 0.09 | / | / | 276.2 | 277.5 | 0.5 | 6.5E-6 |
| Ergosteryl glucoside | $C_{34}H_{54}O_6$ | 5.9 | $[M+Na]^+$ | 581.3813 | 581.3817 | 0.79 | 203.05 | / | 262.6 | no entry | no entry | 2.4E-6 |

Abbreviations: Cer, ceramide; DG, diacylglycerol; DGTS, diacylglyceryl-O-4'-(N,N,N-trimethyl) homoserine; FDR; false discovery rate; LDGTS, lyso-diacylglyceryl-O-4'-(N,N,N-trimethyl) homoserine; PC, glycerophosphocholine; PE, glycerophosphoethanolamine; TG, triacylglycerol.

Some compounds could not be assigned automatically using the Lipid Annotator software from Agilent because they were not present in the databases or because MS/MS spectra could not be acquired in all-ion fragmentation mode. In such cases, the software cannot carry out any evaluation. These spectra were recorded in targeted MS/MS mode and evaluated manually, which is why no match scores were given.

**Table S2.** Identified key metabolites of the black truffle samples with their LC-IM-MS data, which show a dependency due to different origins.

| Tentative compound | Proposed formula | RT [min] | Adduct | m/z calculated [Da] | m/z measured [Da] | Error [ppm] | Relevant fragments | Match Score Total (Lipid Annotator) | CCS value measured [Å²] | CCS value calculated [Å²] | Delta CCS [%] | FDR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Betaine diradylglycerols** | | | | | | | | | | | | |
| DGTS (34:1) | $C_{44}H_{83}NO_7$ | 9.5 | $[M+H]^+$ | 738.6242 | 738.6233 | -1.26 | 236.15 | 75.27 | 286.2 | no entry | no entry | 5.5E-13 |
| DGTS (34:2) | $C_{44}H_{81}NO_7$ | 8.7 | $[M+H]^+$ | 736.6086 | 736.6101 | 2.07 | 263.15; 144.10 | 75.09 | 283.7 | no entry | no entry | 1.4E-15 |
| DGTS (36:1) | $C_{46}H_{87}NO_7$ | 10.4 | $[M+H]^+$ | 766.6555 | 766.6579 | 3.09 | 236.15; 144.10 | 73.59 | 292.8 | no entry | no entry | 7.1E-7 |
| DGTS (36:2) | $C_{46}H_{85}NO_7$ | 9.4 | $[M+H]^+$ | 764.6399 | 764.6417 | 2.38 | 236.15; 144.10 | 74.71 | 291.2 | no entry | no entry | 3.2E-7 |
| DGTS (36:3) | $C_{46}H_{83}NO_7$ | 8.5 | $[M+H]^+$ | 762.6242 | 762.6263 | 2.72 | 500.40; 236.15; 144.10 | 74.85 | 288.6 | no entry | no entry | 5.2E-8 |
| DGTS (36:4) I | $C_{46}H_{81}NO_7$ | 8.0 | $[M+H]^+$ | 760.6086 | 760.6108 | 2.92 | 498.38; 480.37; 236.15; 144.10 | 75.06 | 286.6 | no entry | no entry | 4.0E-7 |
| DGTS (36:4) II | $C_{46}H_{81}NO_7$ | 9.3 | $[M+H]^+$ | 760.6086 | 760.6082 | -0.50 | 236.15 | / | 287.5 | no entry | no entry | 1.3E-17 |
| **Ceramides** | | | | | | | | | | | | |
| Cer (34:1;O2) | $C_{34}H_{67}NO_3$ | 9.3 | $[M-H_2O+H]^+$ | 520.5088 | 520.5070 | -3.48 | 502.49; 264.27 | / | 252.5 | no entry | no entry | 2.5E-11 |
| Cer (34:1;O2) | $C_{34}H_{67}NO_3$ | 9.4 | $[M+H]^+$ | 538.5194 | 538.5199 | 0.98 | 520.50; 502.50; 282.28; 264.27 | / | 254.1 | 256.2 | 0.8 | 4.0E-10 |
| Cer (34:2;O2) | $C_{34}H_{65}NO_3$ | 8.9 | $[M+H]^+$ | 536.5037 | 536.5040 | 0.52 | 518.50; 282.28; 264.27 | / | 255.7 | 248.9-252.2 | -2.7-1.4 | 2.2E-8 |
| Cer (34:2;O2) | $C_{34}H_{65}NO_3$ | 8.8 | $[M+Na]^+$ | 558.4857 | 558.4884 | 5.11 | 540.53; 264.27 | / | 248.7 | 250.7-253.9 | 0.8-2.1 | 3.9E-9 |
| Cer (34:3;O) | $C_{34}H_{63}NO_2$ | 8.9 | $[M+H]^+$ | 518.4932 | 518.4927 | -0.88 | / | / | 250.1 | no entry | no entry | 8.6E-9 |
| **Phospholipids** | | | | | | | | | | | | |
| PC (34:2) | $C_{42}H_{80}NO_8P$ | 8.3 | $[M+H]^+$ | 758.5694 | 758.5717 | 2.99 | 184.07 | 94.00 | 284.6 | 281.4-287.6 | -1.1-1.0 | 1.9E-16 |
| PC (36:1) | $C_{44}H_{86}NO_8P$ | 10.1 | $[M+H]^+$ | 788.6164 | 788.6192 | 3.58 | 184.07 | 91.24 | 293.8 | 294.2-294.6 | 0.1-0.3 | 5.5E-7 |
| PC (36:2) | $C_{44}H_{84}NO_8P$ | 9.3 | $[M+H]^+$ | 786.6007 | 786.6020 | 1.61 | 184.07 | 95.39 | 291.2 | 291.4-296.2 | 0.1-1.7 | 1.4E-9 |
| PC (36:3) | $C_{44}H_{82}NO_8P$ | 8.4 | $[M+H]^+$ | 784.5851 | 784.5872 | 2.70 | 184.07 | 97.86 | 289.1 | 289.8-291.7 | 0.2-0.9 | 2.6E-9 |
| PE (37:7) | $C_{42}H_{70}NO_8P$ | 13.0 | $[M+NH_4]^+$ | 765.5177 | 765.5190 | 1.70 | / | / | 277.7 | 274.1-274.8 | -1.2-1.0 | 8.9E-19 |
| PE (39:8) | $C_{44}H_{72}NO_8P$ | 8.3 | $[M+H]^+$ | 774.5068 | 774.5064 | -0.56 | / | / | 281.1 | 278.1 | -1.1 | 1.2E-6 |
| PE (O-37:2) | $C_{42}H_{82}NO_7P$ | 13.0 | $[M+H]^+$ | 744.5902 | 744.5938 | 4.80 | / | / | 278.8 | 282.1 | 1.2 | 1.4E-12 |
| PG (41:2) | $C_{47}H_{89}O_{10}P$ | 12.3 | $[M+H]^+$ | 845.6266 | 845.6229 | -4.39 | / | / | 326.5 | no entry | no entry | 2.7E-12 |
| PS (38:2) | $C_{44}H_{82}NO_{10}P$ | 10.5 | $[M+NH_4]^+$ | 833.6015 | 833.6060 | 5.57 | / | / | 290.8 | 291.7-292.9 | 0.3-0.7 | 8.6E-9 |
| **Other** | | | | | | | | | | | | |
| Coenzyme Q8 | $C_{49}H_{74}O_4$ | 12.7 | $[M+H]^+$ | 727.5660 | 727.5671 | 1.53 | 197.08 | / | 273.2 | 273.9 | 0.3 | 1.8E-13 |
| Coenzyme Q8 | $C_{49}H_{74}O_4$ | 12.7 | $[M+Na]^+$ | 749.5479 | 749.5488 | 1.19 | / | / | 276.5 | 277.5 | 0.4 | 1.3E-18 |

Abbreviations: Cer, ceramide; DGTS, diacylglyceryl-O-4'-(N,N,N-trimethyl) homoserine; FDR; false discovery rate; PC, glycerophosphocholine; PE, glycerophosphoethanolamine; PG, glycerophosphoglycerol; PS, glycerophosphoserines.

Some compounds could not be assigned automatically using the Lipid Annotator software from Agilent because they were not present in the databases or because MS/MS spectra could not be acquired in all-ion fragmentation mode. In such cases, the software cannot carry out any evaluation. These spectra were recorded in targeted MS/MS mode and evaluated manually, which is why no match scores were given.