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Comparing Volatile Compounds Among Brands of Argentinian Yerba Mate (*Ilex paraguariensis*) Using High-resolution GC/Q-TOF

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Introduction

Yerba mate, a traditional South American herbal tea is brewed from the leaves and stems of the *Ilex paraguariensis* plant. This beverage contains the stimulant caffeine and other bioactive compounds and has gained a wide popularity worldwide. It had been shown that yerba mate growing conditions are a significant factor in the biochemical composition of the final product (1). During processing, the plant is rapidly heated to deactivate enzymes (blanching), dried and aged, which also affects the aroma and chemical composition of yerba mate. This leads to the obvious question about the complexity of the chemical profile of yerba mate and whether different brands of yerba mate could be distinguished through analytical techniques. In this study, high resolution GC-QTOF data were used to analyze yerba mate extracts of four different brands. In total, 386 compounds were identified of which 76 are newly identified compounds in yerba mate with library match scores of 90 or above.

Experimental

All yerba mate samples were from Argentina. The samples were extracted in three to six replicates using a standard QuEChERS protocol and analyzed using a 7890 GC coupled to the 7250 high-resolution Q-TOF MS in full acquisition mode. The parameters are described in detail in Table 1. Unknowns Analysis tool (of MassHunter Quantitative Analysis Software 10.2)

GC and MS Conditions:	Q-TOF (7250)
GC	7890
Column	30-5MS UI, 15 m, 0.25 mm, 0.25 µm
Inlet	MMI, 4-mm UI liner single taper w wool
Injection volume	1 µL
Injection mode	Splitless
Inlet temperature	280°C
Oven temperature program	50°C for 2 min; 10°C/min to 300°C, 10 min hold
Carrier gas	Helium
Column flow	1.2 mL/min
Transfer line temperature	300°C
Quadrupole temperature	150°C
Source temperature	200°C
Electron energy	70 eV
Emission current	5 µA
Spectral acquisition rate	5 Hz
Mass range	45 to 650 m/z

Table 1. GC/Q-TOF acquisition parameters.

Experimental

was used to perform accurate mass feature finding with SureMass algorithm followed by compound identification with NIST17.L and NIST 20.L libraries (Figure 1). Retention Index and accurate mass information were used to confirm the compound identity. The data were further imported to Mass Profiler Professional (MPP) 15.1 for further statistical analysis and visualization of the data.

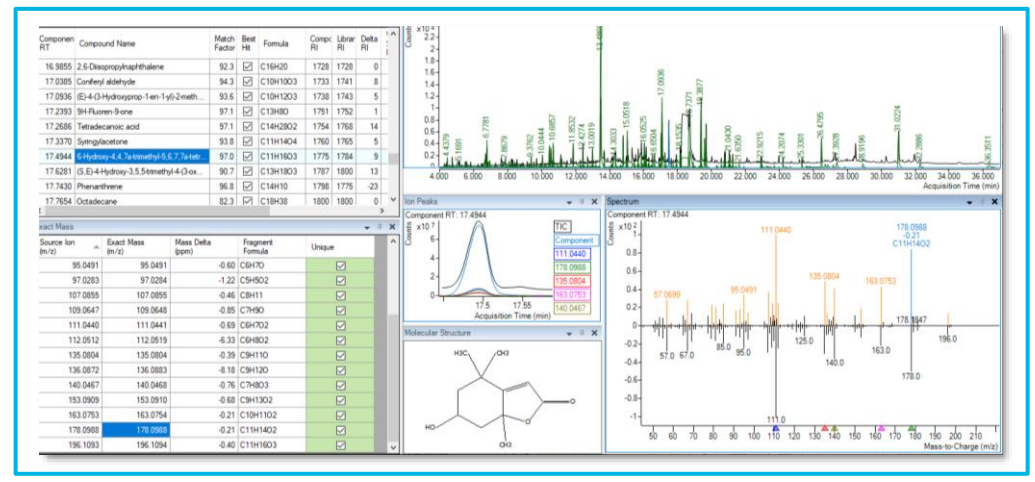

Figure 1. Feature funding, compound identification and verification using Unknowns Analysis. The highlighted compound is 6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one (Loliolide)

Results and Discussion

Yerba Mate Profiling Results

The compounds identified in yerba mate samples from four different brands (designated A, B, C and D here) were grouped based on compound classes (Figure 2). The majority of the identified compounds were phenolic compounds (mostly methoxyphenols) and terpenoids. Of the compounds identified with the library match score >90, seventy six have not been previously reported in yerba mate (Table 2).

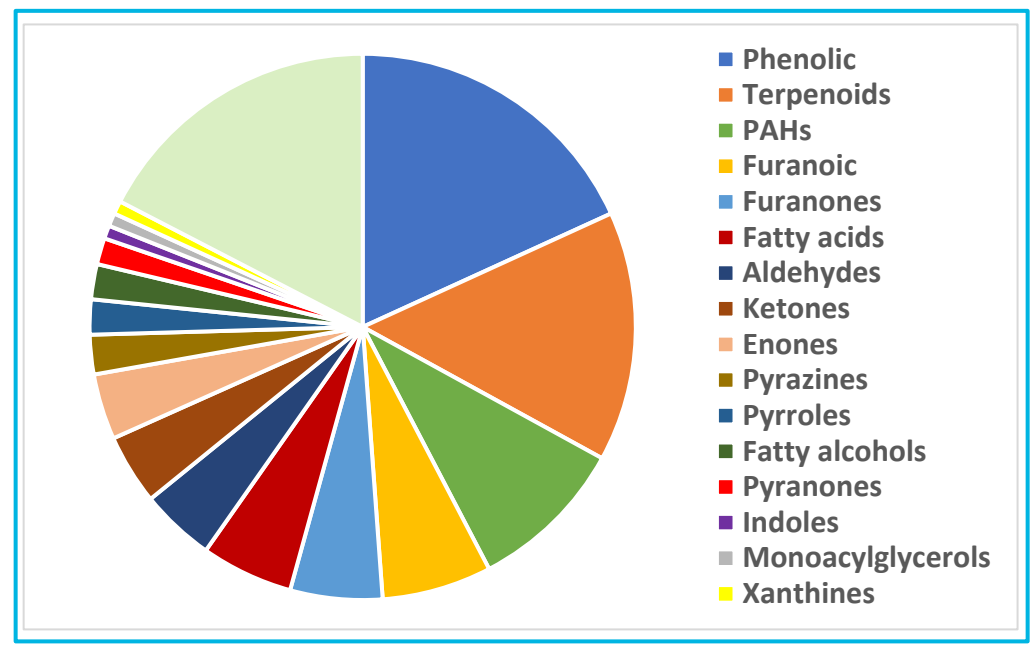

Figure 2. Classes of compounds identified in yerba mate samples

RT	Compound Name	Match Factor	RI
4.310	N-Vinylimidazole	96	796
4.426	2-Ethyl-2-butenal	93	804
4.428	Furfural	99	807
4.723	2-Furanmethanol	99	836
4.969	4-Methyl-1-pentanol	91	861
4.970	5-Methyl-2(3H)-furanone	94	859
5.048	N-Ethylacetamide	91	868
5.165	4-Cyclopentene-1,3-dione	99	880
5.494	3-Methylcyclopentyl acetate	97	908
5.582	2(5H)-Furanone	99	913
5.610	2-Acetylfuran	99	915
5.616	Butyrolactone	93	915
5.676	2,5-Dimethylpyrazine	94	918
5.764	2,3-Dimethylpyrazine	91	923
5.872	2,5-Hexanedione	94	930
6.017	2-Vinylpyrazine	91	939
6.018	5-Methyl-2(5H)-furanone	98	939
6.249	5,5-Dimethyl-2(5H)-furanone	99	952
6.287	2-Pyridinecarboxaldehyde	90	953
6.454	5-Methyl furfural	99	964
6.511	Benzaldehyde	99	967
6.514	3-Hydroxyisovaleric acid	90	968
6.578	2H-Pyran-2-one	91	971
6.661	Hexanoic acid	96	976
6.705	Phenol	98	979
6.760	Isomaltol	90	981
6.776	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	96	983
6.845	6-Methyl-5-hepten-2-one	92	987
6.888	β-Myrcene	91	990
6.930	2H-Pyran-2,6(3H)-dione	97	992
7.051	(E,Z)-2,4-Heptadienal	95	999
7.170	Octanal	91	1006
7.175	N-Methylpyrrole-2-carboxaldehyde	92	1006
7.238	1H-Pyrrole-2-carboxaldehyde	93	1011
7.294	(E,E)-2,4-Heptadienal	91	1014
7.359	Benzyl chloride	94	1018
7.386	α-Terpinene	92	1020
7.459	Maple lactone	93	1024
7.556	o-Cymene	91	1030
7.563	2-Ethyl-1-hexanol	95	1030
7.576	Dimethyl succinate	95	1031
7.586	3,4-Dimethyl-2,5-furandione	93	1031
7.635	2-Acetyl-5-methylfuran	98	1034
7.664	Benzyl alcohol	98	1036
7.680	trans-β-Ocimene	90	1038
7.714	Lavender lactone	92	1039
7.865	α-Ocimene	96	1049
7.939	γ-Caprolactone	90	1053
8.080	2-Acetylpyrrole	99	1062
8.095	γ-Terpinene	95	1062
8.191	Heptanoic acid	93	1068
8.221	(E,E)-3,5-Octadien-2-one	93	1070
8.244	1-Octanol	94	1072
8.258	p-Cresol	95	1072
8.293	cis-Linalool oxide	95	1075
8.315	2,5-Furandicarboxaldehyde	94	1076
8.402	Furyl hydroxymethyl ketone	95	1081
8.545	trans-Linalool oxide (furanoid)	93	1090
8.720	Linalool	96	1101
8.760	6-Methyl-3,5-heptadiene-2-one	93	1103
8.845	Maltol	95	1109
8.932	Phenylethyl Alcohol	93	1114
9.165	Neo-Allo-Ocimene	96	1131
9.375	Pyranone	97	1143
9.430	2,6,6-Trimethyl-2-cyclohexene-1,4-dione	91	1147
9.696	Octanoic acid	94	1164
9.800	1-Nonanol	97	1171
9.920	2,4-Dimethoxybenzaldehyde	92	1178
9.934	5-Hydroxymaltol	91	1179
10.043	Catechol	99	1186
10.125	Naphthalene	91	1192
10.188	Methyl salicylate	97	1196
10.232	L-α-Terpineol	93	1199
10.299	Safranal	94	1203
10.340	Decanal	93	1206
10.449	Coumaran	96	1214
10.538	5-Hydroxymethylfurfural	96	1220
10.687	2-Ethyl-3-methylmaleimide	97	1230
11.003	3-Methylcatechol	93	1252
11.068	2-Methyl-2-vinylmaleimide	94	1257
11.118	Nonanoic acid	95	1260
11.346	(E)-Cinnamaldehyde	93	1276
11.385	4-Methyl-5-thiazolethanol	92	1279
11.424	4-Methylcatechol	93	1281
11.495	Salicylic acid	93	1286
11.613	Indole	96	1294
11.856	4-Vinylguaiacol	97	1312
12.319	Syringol	93	1346
12.430	Eugenol	91	1354
12.468	n-Decanoic acid	94	1357
12.638	Pyrogallol	90	1368
12.705	4-Ethylcatechol	94	1374
12.822	β-Damascenone	91	1383
13.000	Vanillin	99	1396
13.065	1,2-Dihydro-1,4,6-trimethylnaphthalene	95	1401
13.072	6,10-Dimethyl-2-undecanone	91	1402
13.383	α-Ionone	91	1426
13.667	Geranyl acetone	93	1448
13.688	trans-Isoeugenol	95	1449
14.077	Dehydro-β-ionone	94	1480
14.125	Apocynin	97	1484
14.157	β-Ionone-5,6-epoxide	91	1487
14.252	Levoglucosan	94	1494
14.458	Isobutyl 4-hydroxybenzoate	90	1511
14.629	Guaiacylacetone	91	1525
14.799	Dihydroactinidiolide	92	1539
14.967	Isovanillic acid	91	1553
15.048	2,6-Dimethoxy-4-ethenylphenol	97	1560
15.176	1,6,7-Trimethylnaphthalene	90	1570
15.329	Butyrovanillone	94	1583
15.472	Methoxyeugenol	90	1594
15.483	3,4,5-Trimethoxyphenol	93	1596
15.686	3-Hydroxy-β-damascone	92	1613
16.048	Dihydroconiferyl alcohol	95	1645
16.150	Syringaldehyde	95	1654
16.312	Coniferol	92	1668
16.649	trans-4-Propenylsyringol	95	1697
16.726	Indole-3-acetaldehyde	91	1704
16.910	1-Hydroxy-3-(4-hydroxy-3-methoxyphenyl)-2-propanone	90	1721
16.969	Acetosyringone	91	1726
17.037	Coniferyl aldehyde	96	1733
17.089	trans-Coniferyl alcohol	93	1737
17.236	9H-Fluoren-9-one	92	1751
17.266	Tetradecanoic acid	97	1754
17.334	Syringylacetone	93	1760
17.492	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	97	1774
17.742	Phenanthrene	94	1797
17.844	1H-Indole-3-carboxaldehyde	91	1807
18.153	Neophytadiene	96	1837
18.208	Hexahydrofarnesyl acetone	96	1842
18.929	Farnesyl acetone	93	1912
19.377	n-Hexadecanoic acid	98	1957
19.394	Homovanillic acid methyl ester	95	1958
19.590	trans-Sinapaldehyde	94	1980
19.687	trans-Sinapyl alcohol	96	1989
20.293	Verimol K	94	2053
20.577	Fluoranthene	95	2083
20.583	1-Octadecanol	92	2084
20.727	Methyl oleate	90	2099
20.776	γ-Palmitolactone	91	2104
20.804	Pyrene	95	2107
20.815	Phytol	97	2108
21.251	Octadecanoic acid	93	2157
22.013	Tributyl acetylcitrate	96	2243
22.920	4,8,12,16-Tetramethylheptadecan-4-olide	93	2349
23.439	Isopimaric acid	92	2412
23.480	Cyclopenta[cd]pyrene	95	2417
24.207	2-Palmitoylglycerol	95	2508
25.328	(E)-3,3'-Dimethoxy-4,4'-dihydroxystilbene	96	2656
25.778	β-Glyceryl monostearate	91	2717
26.477	Squalene	97	2815
26.700	α-Tocospiro A	91	2847
26.847	α-Tocospiro B	92	2868
27.165	4-(4-Hydroxy-3-methoxystyryl)-2,6-dimethoxyphenol	93	2914
28.916	α-Tocopherol	96	3129
31.014	γ-Sitosterol	96	3320

Table 2. Compounds identified in yerba mate with the library match score >90. Compounds highlighted in green have not been previously reported in yerba mate

Aroma Active Compounds Identified in Yerba Mate

Most prominent groups of aroma active compounds were visualized on a heatmap (Figure 3). The most dramatic trends were observed with phenolic compounds, furanones as well as pyrazines. Phenolic compounds were prevalent in brands A and C, while furanones were most abundant in brands A and D. In brand B these compounds were detected at lower levels, however, pyrazines were found to be generally at higher abundance as compared to A and C.

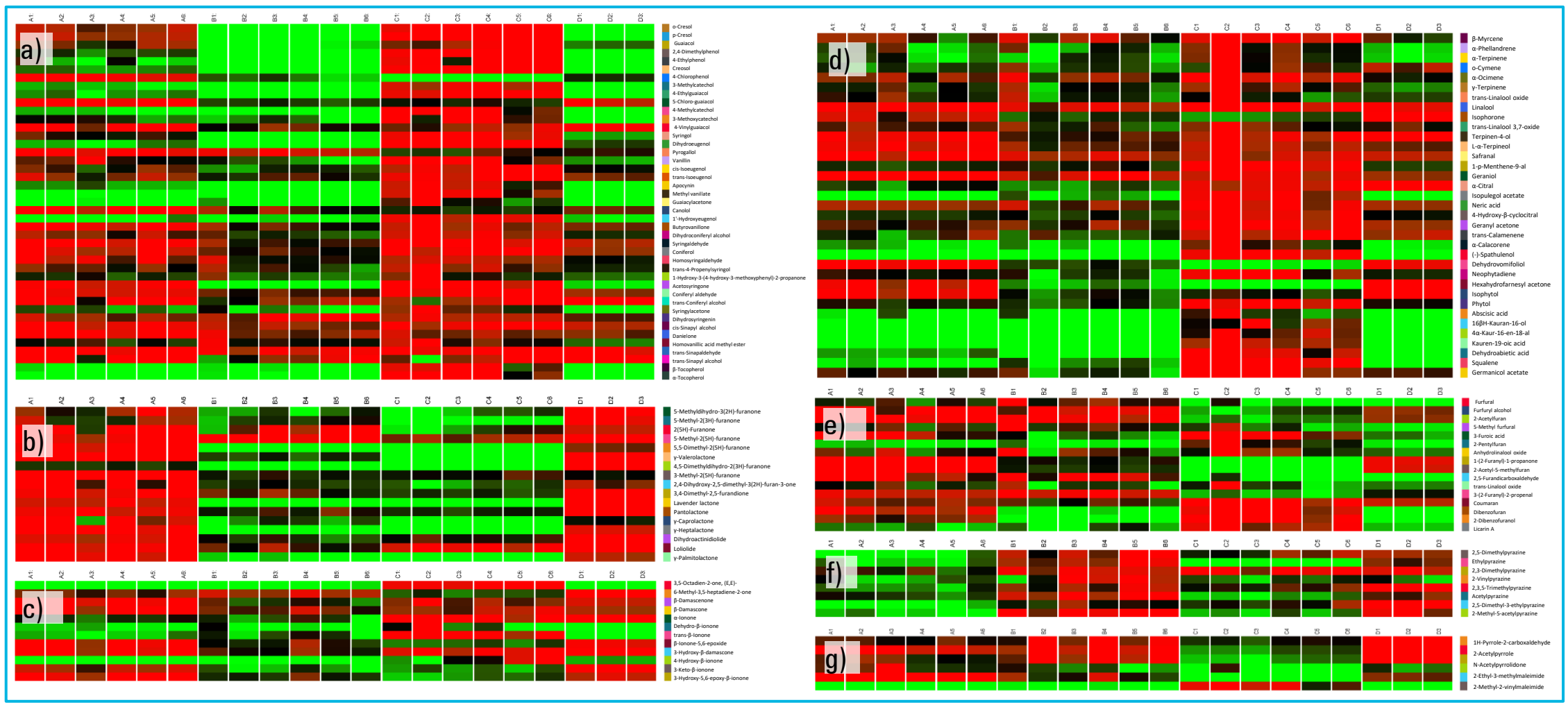

Figure 3. Heatmaps of individual samples from four different brands (A, B, C, and D) of yerba mate grouped by compound class. Selected aroma active compound groups are shown. The raw abundances of compound responses were normalized to an internal standard (triphenyl phosphate). a) methoxyphenols, polyphenols, and other phenolic compounds; b) furanones and benzofuranones; c) enones (including rose ketones) and oxepanes; d) terpenoids; e) furanoic compounds, benzofurans, dibenzofurans and benzofuran flavonoids; f) pyrazines; g) pyrroles

Results and Discussion

Many volatile compounds identified in yerba mate have been previously reported in green and black tea (Table 3).

RT	Compound Name	Previously reported in YM	Reported in GREEN	Reported in BLACK
4.428	Furfural	X		X
4.817	(E)-3-Hexen-1-ol	X	X	X
5.460	(Z)-4-Heptenal	X		X
5.610	2-Acetylfuran	X	X	
5.644	(E,E)-2,4-Hexadienal	X		X
5.676	2,5-Dimethylpyrazine	X	X	
6.454	5-Methyl furfural		X	
6.511	Benzaldehyde	X	X	X
6.661	Hexanoic acid	X		X
6.705	Phenol	X	X	X
6.845	6-Methyl-5-hepten-2-one	X	X	X
6.884	4-Hexenoic acid	X		X
6.888	β-Myrcene	X	X	X
7.051	(E,Z)-2,4-Heptadienal	X	X	
7.144	2,3,5-Trimethylpyrazine		X	
7.170	Octanal	X		X
7.175	N-Methylpyrrole-2-carboxaldehyde		X	
7.238	1H-Pyrrole-2-carboxaldehyde	X		X
7.294	(E,E)-2,4-Heptadienal	X	X	X
7.556	o-Cymene		X	X
7.563	2-Ethyl-1-hexanol	X	X	
7.586	3,4-Dimethyl-2,5-furandione			X
7.617	Limonene	X		X
7.639	2-Acetylpyridine		X	
7.664	Benzyl alcohol	X	X	X
7.680	trans-β-Ocimene	X	X	X
7.730	2,2,6-Trimethylcyclohexanone		X	
8.063	(E)-2-Octenal	X	X	
8.080	2-Acetylpyrrole	X	X	X
8.095	γ-Terpinene	X		X
8.221	(E,E)-3,5-Octadien-2-one	X	X	
8.244	1-Octanol	X	X	
8.258	p-Cresol	X	X	
8.293	cis-Linalool oxide	X	X	X
8.545	trans-Linalool oxide (furanoid)	X	X	
8.605	3,5-Octadien-2-one	X	X	
8.720	Linalool	X	X	X
8.795	Nonanal	X	X	
8.845	Maltol	X	X	
8.932	Phenylethyl Alcohol		X	
9.165	Neo-Allo-Ocimene		X	
9.430	2,6,6-Trimethyl-2-cyclohexene-1,4-dione	X	X	
9.920	2,4-Dimethylbenzaldehyde	X	X	X
10.033	Terpinen-4-ol	X		X
10.188	Methyl salicylate	X	X	X
10.232	L-α-Terpineol	X	X	X
10.299	Safranal	X	X	X
10.340	Decanal	X		X
10.449	Coumaran			X
10.492	2,4-Nonadienal	X		X
10.606	β-Cyclocitral	X	X	X
10.974	Geraniol	X	X	X
11.236	α-Citral			X
11.449	Isopulegol acetate			X
11.495	Salicylic acid			X
11.613	Indole	X	X	
11.856	4-Vinylguaiacol	X	X	
12.434	Syringaldehyde			X
12.574	2-Undecenal	X	X	
12.822	β-Damascenone	X		X
13.000	Vanillin	X		X
13.213	β-Damascone	X	X	
13.383	α-Ionone	X	X	X
13.667	Geranyl acetone	X	X	X
14.077	Dehydro-β-ionone		X	X
14.118	trans-β-Ionone	X	X	X
14.157	β-Ionone-5,6-epoxide	X	X	
14.713	Hexanoic acid, anhydride	X		X
14.779	Dihydroactinidiolide	X	X	X
14.939	α-Calacorene			X
16.485	3-Hydroxy-5,6-epoxy-β-ionone		X	X
18.208	Hexahydrofarnesyl acetone	X	X	
19.377	n-Hexadecanoic acid	X		

Table 3. Compounds identified in yerba mate in this study with the library match score >90 that also have been reported in green and/or black tea

Statistical Analysis and Hierarchical Clustering

Fold Change and statistical analysis (ANOVA) were performed on compounds not included in Figure 3 to select compounds for Hierarchical Clustering. Cluster 1 mostly grouped aldehydes and showed higher levels of these compounds in brand C (Figure 4). Cluster 2 and 5 prevailed by PAHs and steroids, respectively.

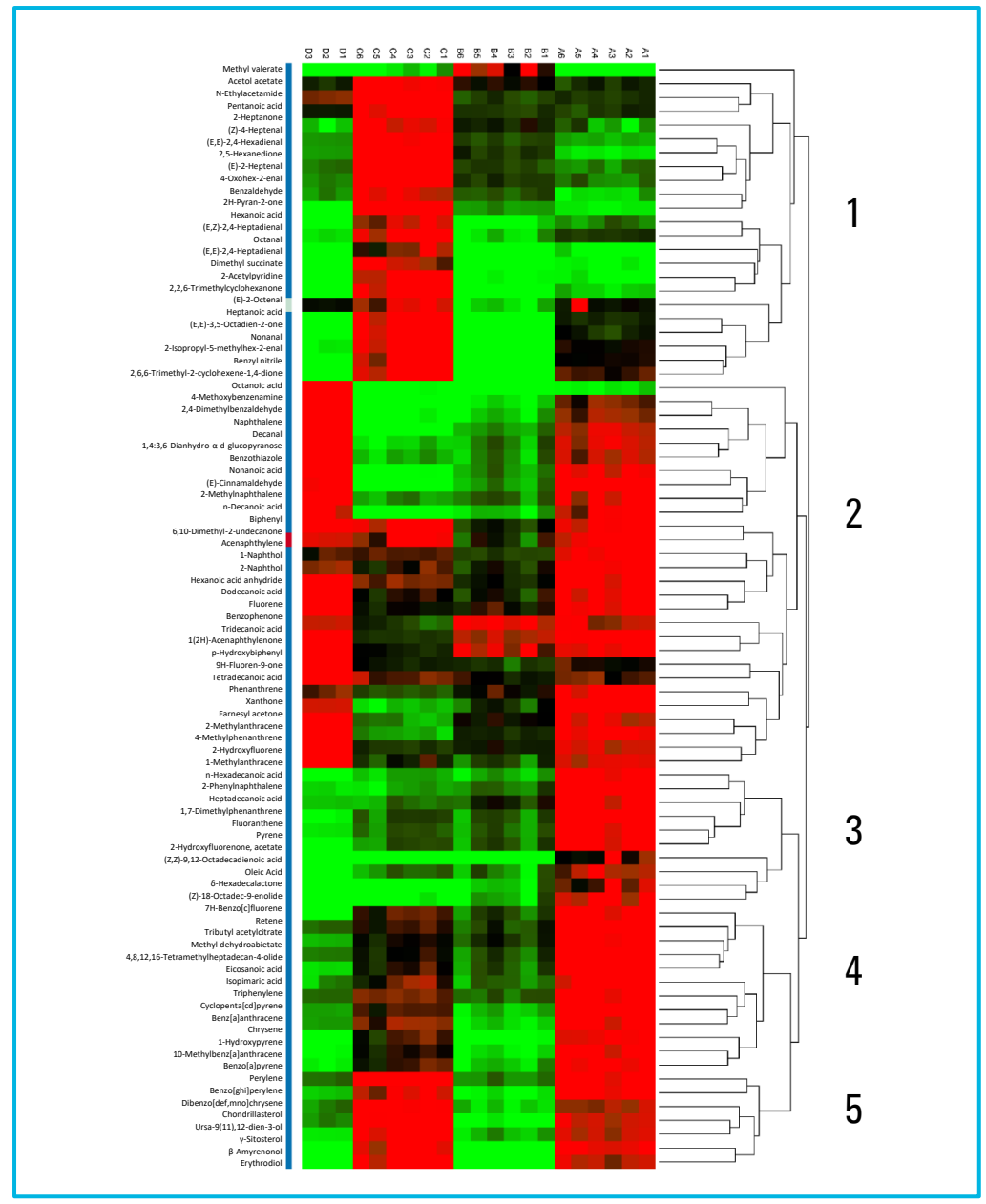

Figure 4. Hierarchical clustering (based on Euclidian distance) on compounds with statistically significant differences (P<0.01) and fold change >2 between the brands of yerba mate

Conclusions

- Profiling of yerba mate extracts of four different brands identified 386 compounds using 7250 GC/Q-TOF
- Heatmap visualization as well as statistical analysis followed by hierarchical clustering identified significant differences between the brands with respect to a variety of compound groups
- The most dramatic trends were observed with phenolic compounds, furanones, pyrazines and PAHs

References

[1] Croge *at al*. Yerba mate: cultivation systems, processing and chemical composition. *Food Sci & Tech*. 2020

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