Analysis of 2021 Fruit #9 (V211202-454) by GCxGC-MS

Technical Report Prepared for Terpene Belt Processing

One sample of 2021 Fruit #9 was received on Dec. 2, 2021, tagged as V211202-454 then run on GCxGC (after a methanol blank and two Spex standards) using method Terp2D-120 with Batch ID: 20211202AATerp2D.

Results were processed by conducting a dynamic background correction (DBC) and integrating the signals from the mass spec using a deconvolution algorithm with a minimum ion count of 2000 for peaks having a minimum height and area of 10000. Library search was included using the NIST17 database. Analysis of results included categorization of each identified peak into different classes of molecules and calculating the number of peaks and peak area percentages in each class, as well as comparing the peaks retention times with the Spex standards for a more reliable identification.

Integration of 2021 Fruit #9 (V211202-454) using a deconvolution algorithm generated 893 peaks and 183 identified compounds, only 21 of which can be mapped to available terpene standards (from SPEX with 40 terpenes), but these 21 peaks account for 54.95% of the peak areas (which estimate their relative quantities). The other 45.05% of the peak areas consist of 27.97% sesquiterpenes (with 35 compounds identified), 14.50% monoterpenes (with 55 compounds identified), 0.91% esters (with 14 compounds identified), 0.46% other volatile compounds (58 compounds identified) and 1.22% unidentified. *See Table below for more details.*

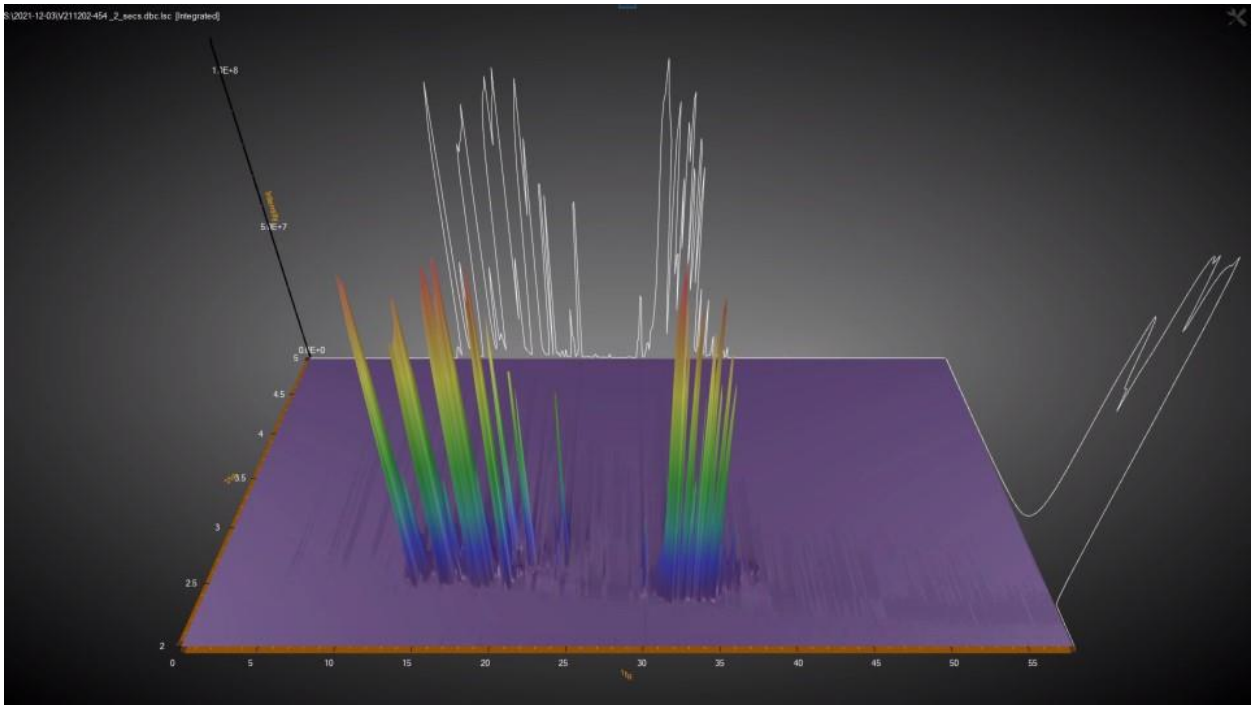

Figure 8. A two-dimensional chromatogram for V211202-454 generated by GCxGC-MS.

Clearly we have detected more peaks than compounds. In addition to the unidentified peaks, the larger number of peaks is due in part to the nature of multidimensional gas chromatography which separates peaks into subpeaks based on further separation by a second column. Some peaks belonging to the same compound may be separated especially if too much of the compound is injected. This was the case for some compounds in this experiment because we wanted to make sure that low level analytes are detected as well. We also use a deconvolution algorithm that resolves coeluting compounds based on their mass spectra. This can further multiply the number of peaks for the same compound, but it also helps identify low level analytes. Indeed, we are excited to see esters in both samples and have listed them at the end of this report. In some cases, multiple peaks are identified as the same compound, which is typical for terpenes which have similar fragmentation patterns. In order to clarify whether these peaks being identified the same are indeed the same compound or separate compounds, we would need to analyze their retention times and other data further. Therefore, we consider the number of compounds identified to be the most conservative estimate of the compounds actually present in the samples.

Analyte Class	2021 Fruit #9		
	No. of compounds	No. of Peaks	Peak Area (%)
Alcohol	9	13	0.05485
Aldehyde	7	11	0.12513
Alkane	3	4	0.01104
Aromatic alcohol	2	2	0.0234
Aromatic hydrocarbon	5	6	0.0979
Cyclic Alcohol			
Cyclic Hydrocarbons	6	8	0.00738
Diterpene	4	27	0.0555
Ester	14	23	0.90597
Fatty acid	2	38	0.05372
Other Hydrocarbons	2	2	0.00051
Ketone	4	6	0.01210
Miscellaneous	14	13	0.02231

Analyte Class	2021 Fruit #9		
	No. of compounds	No. of Peaks	Peak Area (%)
Monoterpene	55	183	14.49588
Sesquiterpene	35	216	27.96974
Standards	21	21	54.94789
Unknown	N/A	320	1.21667
Total	183	893	100.00000

In 2021 Fruit #9, we found 14 esters:

- Benzoic acid, 2-(formylamino)-, methyl ester
- Propanoic acid, 2-methyl-, ethyl ester
- Butanoic acid, ethyl ester
- Butanoic acid, hexyl ester
- Hexanoic acid, 3-hexenyl ester, (Z)-
- 3-Hexen-1-ol, acetate, (Z)-
- Hexanoic acid, hexyl ester
- Butanoic acid, propyl ester
- Butanoic acid, 2-methyl-, ethyl ester
- Butanoic acid, 3-methyl-, ethyl ester
- Hexanoic acid, 2-methylbutyl ester
- Acetic acid, hexyl ester
- Methyl isovalerate
- Hexyl tiglate

In summary, GCxGC-MS provided a very comprehensive analysis of the volatile compounds present in the samples tested. It shows that there are many more compounds in the samples than what the available standards can identify and provides tentative identification for these compounds that characterize their uniqueness and distinguish the samples from each other. Similarities and differences can be further explored in the future.