Millis Scientific, Inc

6400 Baltimore National Pike
#201 Baltimore MD 21228
Telephone: 877-844-2635
Email: info@millisscientific.com

Analytical Report

Title	Volatile Organic Compounds Profile by GC-MS in "Dragon Fruit" Flavoring Concentrate
Report No.	021816-025-3
Issue Date	February 18, 2016
Notebook reference	13-02-106
Contributors:	Alexei Gapeev, David Yeh
Quote No.	
Requester	Brendan Woodward Flavorah PO BOX 2624 Woodinville, WA 98072

Specific Aim:

The goal is to determine levels of Acetoin, Diacetyl and Acetylpropionyl with reporting cutoff 5 ppm

Background of the matter

Diacetyl (2,3-butanedione, CAS 431-03-8) is a volatile liquid with intense buttery flavor occurring naturally in diary and fermented foods. It is extensively used as a flavoring agent to impart a buttery flavor.

Acetoin (3-Hydroxy-2-butanone, CAS 513-86-0), Acetylpropionyl (2,3-Pentadione, CAS 600-14-6) are related compounds with somewhat similar custard flavor also used as flavoring agents.

These compounds find use in flavoring compositions designed to impart certain flavors to foodstuffs. They are also common products of fermentation and present in beers, wines and dairy products albeit in low (tens of ppb) levels. When inhaled, these compounds are believed to have deleterious effect on lung function and cause a serious lung disease. Thus it is imperative that no flavoring composition designed to be inhaled has any of the target compounds.

Samples

Sample arrived as somewhat viscous liquid with characteristic odor labeled "**Dragon Fruit**". 4 ml of submitted material was placed in a 40 ml headspace collection vial followed by addition of internal standard (IS). The vial was held at 60C for 4 hours before headspace sampling. Volatile compounds were collected out of headspace with the aid of 1 ml gastight syringe. 0.2 ml of collected headspace was injected at spit ratio of 5.

Experimental:

1. GC conditions:

Injector temperature:	250 C
Initial oven temperature:	40 C
Hold I	2 min
Ramp I	10 C/min
Final temperature I	220 C
Hold II	5 min

2. MS parameters

Ionization and ion polarity	EI+
Scan rate	2 scans/sec
Mass range	35-300 Da
Ion source temperature	150
Transfer line temperature	220C

3. GC-MS analysis. Waters/Micromass Quatro GC mass spectrometer interfaced to a Thermo Electron Trace gas chromatograph was utilized for the analysis. 30M 0.32 mm ID DB-624 column was used to separate components. Carrier gas was helium at 2 ml/min.

4. Data treatment.
   Methylethylketone (MEK) was used as an internal standard. Table 1 lists qualifier and quantifier ions for the target compounds.

RT, min	Compound	Qualifier, m/z	Quantifier, m/z
4.35	Diacetyl	86	43
6.44	Acetylpropionyl	100	43
7.28	Acetoin	88	45

Table 1 Retention times and m/z ratios of the target compounds

Results:

TIC GC-MS chromatogram of the submitted sample is shown in Appendix I, figure 1. Displayed on figure 2 are selected ion chromatograms for ions used to quantify the target compounds. Arrows designate expected retention times (RT) for diacetyl, acetylpropionyl and acetoin elution (4.3,

6.4 and 7.2 min respectively) out of the GC column[1] A green arrow indicates a compound is not present at reporting cutoff and a red arrow indicates that level exceeds the cutoff. "*****" designates internal standard. Table 2 lists target compound levels.

Compound	**Concentration, ppm**
Diacetyl	N/D
Acetylpropionyl	N/D
Acetoin	N/D

Table 2 Sample "Dragon Fruit", target compound levels. Concentration units are ppm or N/D, not detected.

1 Presence of a peak at designated RT does not necessary signify that a target compound detected. If stated as N/D the qualifier ion is not detected.

APPENDIX I

Figure 1. Sample "Dragon Fruit" TIC chromatogram

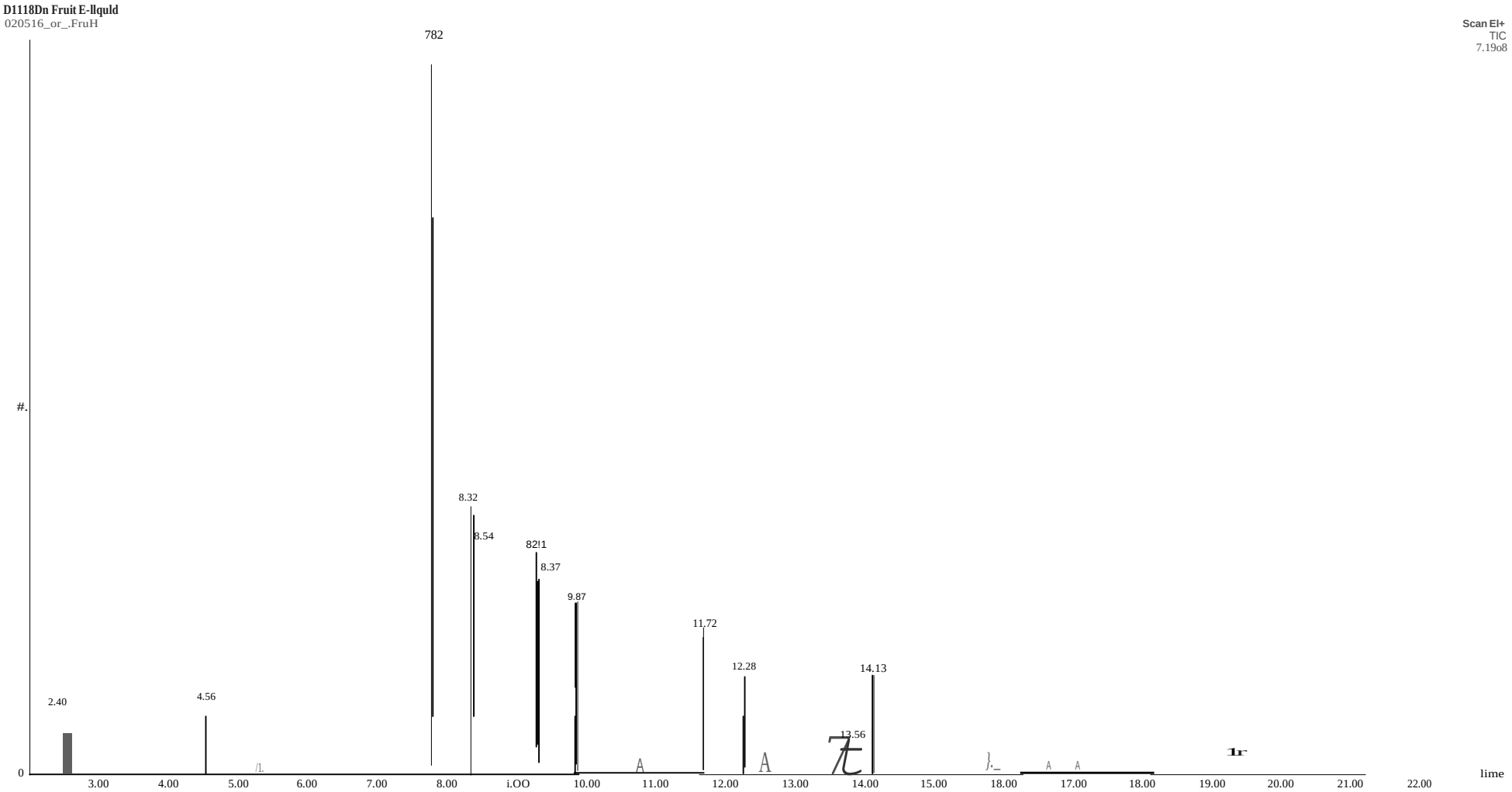

Figure 2. Sample "Dragon Fruit" Selected Ion Chromatograms