	<p>Millis Scientific, Inc</p> <p>6400 Baltimore National Pike #201 Baltimore MD 21228 Telephone: 877-844-2635 Email: info@millisscientific.com</p>
Analytical Report	
Title	Vicinal Diketones Profile in Biscotti Flavor Concentrate via GC-MS
Report No.	032017-13
Issue Date	March 20, 2017
Notebook reference	28-9-01
Contributors:	Alexei Gapeev
Quote No.	
Requester	Flavorah

Specific Aim:

The goal is to determine levels of

- Acetoin, Diacetyl and Acetyl Propionyl with reporting cutoff 5 ppm in Biscotti flavor concentrate.

Table 1 lists target compound levels determined pursuant to the protocol described below.

Sample	Diacetyl	Acetyl Propionyl	Acetoin
Biscotti	N/D	N/D	N/D

Table 1: Target compound levels. Concentration units are ppm or N/D, not detected.

Background of the Matter

Diacetyl (2,3-butanedione, CAS 431-03-8) is a volatile liquid with intense buttery flavor occurring naturally in dairy 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 (typically tens of ppb, diketons; several tens ppm, acetaldehyde) levels. When inhaled, these compounds are believed to have deleterious effect on lung function and cause a serious lung disease. Thus it is imperative to have information on levels of these compounds in flavoring compositions designed to be inhaled.

Samples

Sample arrived as clear liquids of varying viscosity and color. 5 ml of submitted material was placed in 40 ml headspace collection vial followed by addition of internal standards (IS). Volatile compounds were collected out of headspace with the aid of 0.5 ml gastight syringe. 0.2 ml of collected headspace was injected at split ratio of 5.

Experimental:

1. GC conditions:

Injector temperature:	250 C
Initial oven temperature:	40 C
Ramp I	15 C/min
Final temperature I	200 C
Hold II	3 min
2. MS parameters

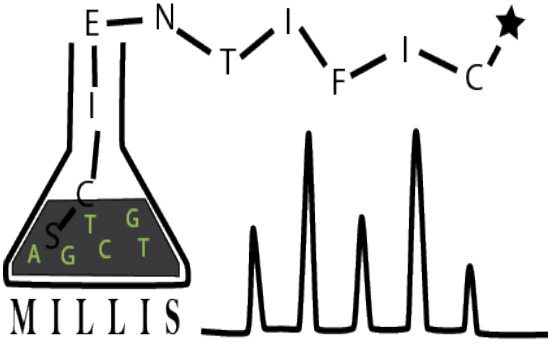
Ionization and ion polarity	EI+
Scan rate	2 scans/sec
Mass range	35-300 Da
Ion source temperature	180
Transfer line temperature	220C
3. GC-MS analysis. Waters/Micromass Quatro GC mass spectrometer interfaced to a Thermo Electron Trace gas chromatograph was utilized for the analysis. 30M 0.32 mm ID DB-624 column was used to separate components. Carrier gas was helium at 2 ml/min.
4. Data treatment.

Isotopically labeled diacetyl (d6), acetylpropionyl (d5) were used as internal standards. Table 1 lists qualifier and quantifier ions for the target compounds. Displayed in Appendix I is the pertinent GC-MS chromatogram.

RT, min	Compound	Qualifier, m/z	Quantifier, m/z
3.4	Diacetyl	43	86
4.8	Acetylpropionyl	43	100
5.5	Acetoin	45	88

Table 2 Retention times and m/z ratios of the target compounds

APPENDIX I

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