Screening of Flavor Volatiles in Exhaled Breath After Tasting Cream Cheese and Fruit Punch Utilizing PDMS Foam Tubes, Thermal Desorption and GC-TOFMS

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Key Words: GC-TOFMS, TDU, Food &Flavors, Deconvolution

1. Introduction

There is much interest in measuring flavor release characteristics of cream cheese after samples have been tasted. The analytical technique under investigation here consists of screening volatiles from exhaled breath utilizing PDMS foam TDU tubes and thermal desorption GC-MS. An example of a method currently used involves masticating the sample, exhaling the volatiles into a glass cylinder, purging the volatiles from the glass cylinder onto an adsorbent trap, followed by thermal desorption GC-MS. The goal is to streamline the current method by eliminating the purge step.

The samples analyzed were a cream cheese base and the same base with approximately twice as much flavoring agent added. A fruit drink sample was also analyzed.

Two days of sample testing were conducted. On the first day, breath sampling was performed by a person chewing cream cheese samples for 5 seconds followed by exhaling breath for 10 seconds into a preconditioned PDMS (polydimethylsiloxane) foam tube. A breath blank sample was analyzed prior to cream cheese testing. The same sampling/extraction conditions were used for breath volatile collection for breath blanks and for breath samples after food tasting. This type of breath blank sampling helps to discriminate between flavor chemicals in the food product and chemicals originating from the sampling equipment (e.g., PDMS foam, desorption tube, connectors) and chemicals in the taster's breath prior to consuming the particular food sample under study. During breath sampling, 100-200 mL of breath was collected on PDMS foam thermal desorption tubes using a breath collection system previously described by the GERSTEL Applications lab (Application GUS-238-JW) with one modification: The restrictive needle on the exit end of the tube was replaced with a flow meter to allow the user to exhale breath at a consistent and measurable flow rate.

The second day of testing involved a different person performing breath sampling. The method was modified slightly. With the second taster, the flow meter was replaced with a Luer needle adapter. Samples were chewed for 5 seconds followed by a 20 second exhalation. On both days of testing, the taster's nose was pinched during chewing and breath exhalation. Also on the second day, samples were analyzed by headspace sorbent extraction (HSSE) using a PDMS-coated stir bar (Twister) to get a better understanding of the chemicals that might be most important to cream cheese flavor.

Results show that the breath sampling method on PDMS foam tubes is simple, fast and capable of detecting major flavor volatiles in cream cheese. However, it does not detect all potentially important flavor compounds.

2. Experimental Conditions

Instrumentation

- GERSTEL MPS 2 Robotic Sampler with TDU option
- GERSTEL CIS 4 Cooled Inlet System with LN2 option
- LECO Pegasus® IV GC-TOFMS

Analysis Conditions

Column:
30 m x 0.32 mm x 0.25 µm DB-5 (J&W Scientific)

Pneumatics

TDU:	Splitless
CIS4:	Glass wool liner, solvent venting with 50 mL/minute

Carrier:
Helium at 1.2 mL/minute constant flow

Temperatures

CIS:	-120°C (4 minutes), 12°C/second to 280°C (3 minutes)
TDU:	30°C, 60°C/minute to 250°C (5 minutes)
GC Oven:	40°C, 15°C/minute to 240°C (10 minutes)

TOFMS Conditions

Ionization:	Electron ionization at 70eV
Source Temperature:	200°C
Stored Mass Range:	40 to 400u
Acquisition Rate:	10 spectra/second

Sample Description

- Cream Cheese Spread: Base
- Cream Cheese Spread: Base with 2 times the added flavoring (2X)
- Fruit Punch Sample

Sample Preparation

Approximately 3 grams of cream cheese sample was chewed with both mouth and nose closed. Between 100 to 200 mL of breath was then exhaled through a conditioned PDMS foam tube for either 10 seconds (Day 1) or 20 seconds (Day 2). To help control exhaled breath flow rate, a flow meter was attached to the exit of the sampling tube (Day 1). On Day 2, the flow meter was replaced by a Luer needle adapter in order to provide a small amount of resistance to flow during breath exhalation. Teflon adapters on each end of the sampling tube were used to attach either the flow meter or adapter to the exit port and a half-inch long silicone tube, which served as a mouthpiece on the inlet side (See Figure 1 from App Note GUS-238-JW, GERSTEL Inc.).

HSSE: 1 gram of sample, 4 mL water, and a micro-stir bar were added to a 20 mL headspace vial. A Twister® was suspended above the sample from a paper clip stuck into the vial's septum. The vial was crimp capped. The volatiles in the sample were extracted at room temperature for 6 hours while the sample was being stirred. Extraction at warmer temperatures and longer times was avoided because of the possibility of microbiological spoilage.

Sample Introduction

PDMS foam tubes were loaded onto the TDU autosampler tray. Samples were thermally desorbed at 250°C in the splitless mode under a 50 mL/minute helium flow for 5 minutes. Analytes were cold trapped in the CIS4 inlet at -120°C on a glass wool plug in the inlet liner. When desorption was complete, analytes were transferred to the GC column in the splitless mode by rapidly heating the CIS4.

3. Results and Discussion

As in previous experiments performed by the GERSTEL Applications lab, condensed moisture in extraction tubes from breath sampling did not lead to inlet freezing during thermal desorption and cryogenic refocusing. Identification of chemicals in chromatograms was greatly facilitated by the use of the LECO Pegasus IV peak deconvolution algorithm.

PDMS degradation compounds were observed in some samples but did not appear to interfere with the analysis. One advantage of the PDMS phase is that its degradation products are easily identified by MS, as they generate characteristic silicone mass fragments. The major flavor volatiles in cream cheese were detected by the breath sampling technique employed. However, results from HSSE experiments showed that not all flavor chemicals were detected by breath sampling. For example, one delta-lactone (retention time 625 seconds), which was detected by HSSE, was not detected by breath sampling, probably because it was present at lower concentrations than the delta-lactone that was detectable by breath analysis (retention time 935 seconds). It was difficult to determine the identities of the delta-lactones since their mass spectra are so similar; identification could be made by retention time comparisons with known lactones.

In Day 2 experiments, a multiple sampling experiment was conducted to test the capacity of the PDMS foam and to see if sensitivity of testing could be improved by performing multiple exhalations into the same tube (result are not shown in figures or tables). Also, it was hoped that the delta-lactone at a retention time of 625 seconds could be detected with this approach.

In these experiments, first a breath blank analysis was performed, and the 2X cream cheese sample was analyzed as previously described. Then the sampling procedure (3 grams of cheese, chewed 5 seconds, exhaled for 20 seconds) was performed three times using the same PDMS foam tube for each of the three exhalations. Results showed that three times as much 2-heptanone and 2-nonanone were collected with three breath exhalations compared to the one breath exhalation. However, the one delta-lactone peak detected in the single breath exhalation procedure was only slightly larger in the three-breath exhalation procedure, and the second delta-lactone was not detected.

In another experiment, the PDMS foam tubes were chilled to approx 5°C prior to exhalation trapping. Results showed that cooling PDMS foam did not improve recovery of flavor volatiles.

4. Conclusions

Results show that the breath sampling method on PDMS foam tubes is simple, fast, and capable of detecting major flavor volatiles in cream cheese. However, it does not detect all potentially important flavor compounds.

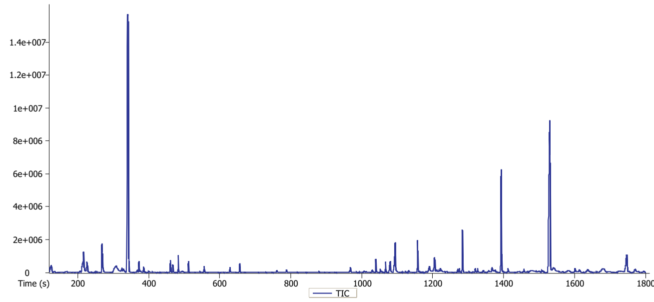

Figure 1. GC-TOFMS chromatogram for a breath blank prior to performing cream cheese tasting.

Table 1. Peak numbers (Peak No), names, retention times in seconds (RT Sec), quantification masses (Quant Mass), and Peak areas (Area) for some of the peaks identified in a breath blank sample (also see Figure 1).

Peak No	Name	RT Sec	Quant Mass	Area
12	Disulfide, bis[1-(methylthio)ethyl]	143	75	23,445
16	Dimethylamine	168	43	280,857
20	Acetic acid	182	45	8,633,828
22	2-Propanone, 1-hydroxy-	194	43	18,169,484
39	1H-Pyrazole, 1,3-dimethyl-	236	96	392,986
49	2(5H)-Furanone	315	55	1,354,369
58	Ethanol, 2,2'-oxybis-	353	45	1,405,843
61	Phenol	359	94	135,277
108	(S)-(+)-2',3'-Dideoxyribonolactone	493	85	1,400,978
112	Benzoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester (PDMS blk)	512	267	1,241,427
151	2-[2-[2-Methoxyethoxy]ethoxy-1,3-dioxalane	880	73	1,057,557
160	n-Decanoic acid	970	60	1,614,683
178	1-Tridecanol	1040	55	2,327,582
185	Tridecanoic acid, methyl ester	1068	74	15,923,889
191	n-Hexadecanoic acid	1097	60	9,236,489
198	Oxybenzone	1133	151	2,504,397
204	1-Hexadecanol	1158	55	1,552,871
218	2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester, isomer1	1205	161	4,105,281
238	2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester, isomer2	1286	161	13,012,555
251	Benzoic acid, octyl ester	1327	105	2,422,656

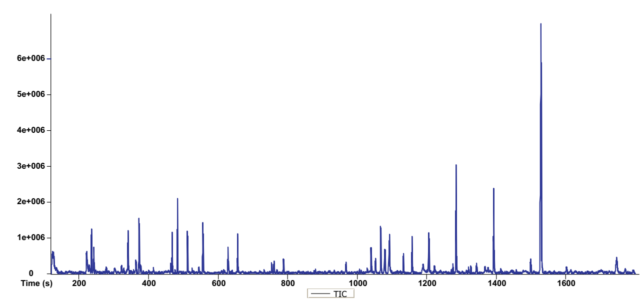

Figure 2. GC-TOFMS chromatogram for breath exhaled after tasting cream cheese base sample.

Table 2. Peak numbers (Peak No), names, retention times in seconds (RT Sec), quantification masses (Quant Mass), and Peak areas (Area) for some of the peaks identified in a breath sample taken after tasting cream cheese (also see Figure 2).

Peak No.	Name	RT Sec	Quant Mass	Area
4	Methanethiol*	128	48	30,102
36	Acetic acid*	236	60	2,538,332
37	2-Propanone, 1-hydroxy-	243	43	12,898,019
48	2-Heptanone*	279	58	1,029,445
54	Butanoic acid*	299	60	129,726
70	1-Hepten-6-one, 2-methyl-	364	43	2,991,887
72	Furan, 2-pentyl- (linoleic acid oxidation product)	368	81	228,114
73	Pentanoic acid	369	60	241,465
78	Octanal (lipid oxidation product)	378	44	747,697
102	2-Nonanone*	457	58	247,275
103	4-Nonenal, (E)-	459	84	64,031
106	Nonanal (lipid oxidation product)	468	41	2,571,092
125	Decanal (lipid oxidation product)	556	41	2,931,820
134	2-Decenal, (Z)-	603	57	106,441
142	2-Octanone	629	58	84,643
159	5,9-Undecadien-2-one, 6,10-dimethyl-	755	43	1,892,821
172	Dodecanoic acid	833	60	128,096
178	a delta lactone*	935	99	147,792
180	Tetradecanoic acid	968	60	887,615
196	n-Hexadecanoic acid	1093	60	3,582,960
200	Oxybenzone	1133	151	1,040,561

**Can be an important flavor compound in cultured dairy products.*

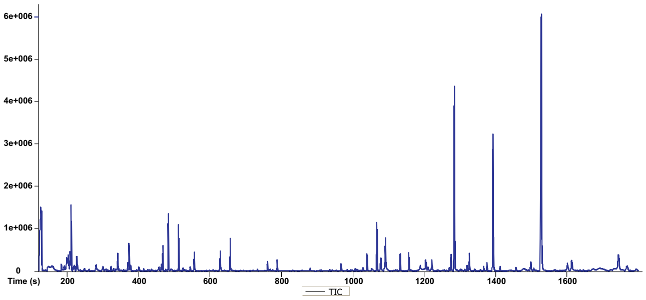

Figure 3. GC-TOFMS chromatogram for breath exhaled after tasting 2X cream cheese sample.

Table 3. Peak numbers (Peak No), names, retention times in seconds (RT Sec), quantification masses (Quant Mass), and Peak areas (Area) for some of the peaks identified in a breath sample taken after tasting 2X cream cheese (also see Figure 3).

Peak No	Name	RT Sec	Quant Mass	Area
15	Ethyl alcohol	130	46	308,339
19	2-Butanone, 3-hydroxy- (common name acetoin)*	134	88	456,079
41	Cyclobutylamine	183	43	1892,301
66	Acetic acid*	211	60	3,010,971
105	2-Heptanone*	281	58	1,097,484
114	Acetaldehyde, methoxy-	308	45	13,427,494
145	Octanal (lipid oxidation product)	378	44	682,580
177	Hexanoic acid*	447	73	18,866
182	2-Nonanone*	457	58	297,297
185	Nonanal (lipid oxidation product)	468	57	1,069,360
212	Octanoic acid*	529	60	79,329
222	Decanal (lipid oxidation product)	556	41	987,429
280	a delta lactone*	935	99	73,160
304	n-Hexadecanoic acid	1092	60	2,884,782

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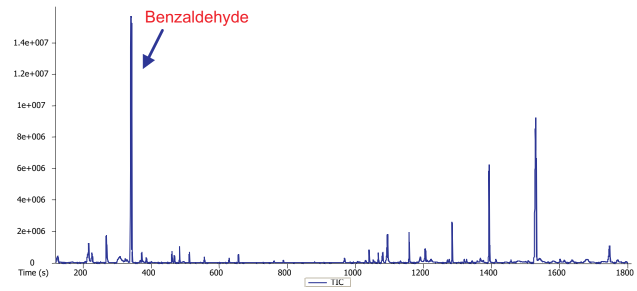

Figure 4. GC-TOFMS chromatogram of breath exhaled after tasting a fruit punch sample.

Table 4. Peak numbers (Peak No), names, retention times in seconds (RT Sec), quantification masses (Quant Mass), and Peak areas (Area) for some of the peaks identified in a breath sample taken after tasting a fruit punch sample (also see Figure 4).

Peak No	Name	RT Sec	Quant Mass	Area
23	Thietane, 3-methyl-	213	46	4,199,343
25	Butanoic acid, ethyl ester	214	44	310,176
27	Acetic acid	217	60	4,062,111
38	Butanoic acid, 3-methyl-, ethyl ester	250	88	207,014
53	Benzaldehyde	340	105	50,915,006
97	Pentanoic acid, 2-methylbutyl ester	469	70	898,868
184	Methyl 6-methyl heptanoate	1181	74	226,697

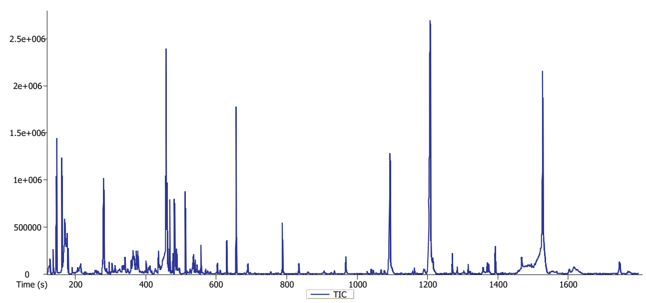

Figure 5. GC-TOFMS chromatogram for a cream cheese extracted using HSSE.

Table 5. Peak numbers (Peak No), names, retention times in seconds (RT Sec), quantification masses (Quant Mass), and Peak areas (Area) for some of the peaks identified in a cream cheese sample extracted using HSSE (also see Figure 5).

Peak No	Name	RT Sec	Quant Mass	Area
13	Acetic acid*	147	45	9,770,459
15	2-Propanone, 1-hydroxy- (fermentation product; common name acetol)*	162	43	10,058,317
17	Propanoic acid*	172	74	333,886
20	2-Butanone, 3-hydroxy- (common name acetoin)*	177	50	6,165
34	Butanoic acid*	215	60	805,120
36	2,3-Butanediol (reduction product of diacetyl and acetoin)	217	45	182,368
54	2-Heptanone*	280	58	5,933,715
57	Heptanal (oxidation product of oleic acid)	289	44	256,134
68	3-Heptanone, 5-methyl-	324	57	150,101
71	2-Heptenal, (E)-	336	41	284,382
74	Benzaldehyde (Strecker aldehyde)	341	105	642,458
90	Phenol	362	94	124,905
93	Pentanoic acid*	366	60	1,974,007
102	Octanal (lipid oxidation product)	377	56	282,449
120	3-Nonanone	408	72	86,101
121	3-Hepten-2-one, (Z)-	410	97	92,163
129	2-Octenal, (E)-	427	70	70,731
147	2-Nonanone*	457	58	8,310,039
152	Sorbic Acid (mold and yeast inhibitor)	463	61	27,233
154	Nonanal (lipid oxidation product)	468	57	1,399,431
166	2,5-Pyrrolidinedione	491	99	103,473
173	2-Decen-1-ol	526	57	112,598
176	Octanoic acid*	536	60	1,884,503
206	a delta lactone*	625	99	36,975
216	n-Decanoic acid	690	60	517,088
217	Butanoic acid, butyl ester	696	89	15,174
236	a delta lactone*	936	99	79,842
238	n-Tetradecanoic acid	969	60	604,888

**Can be an important flavor compound in cultured dairy products.*

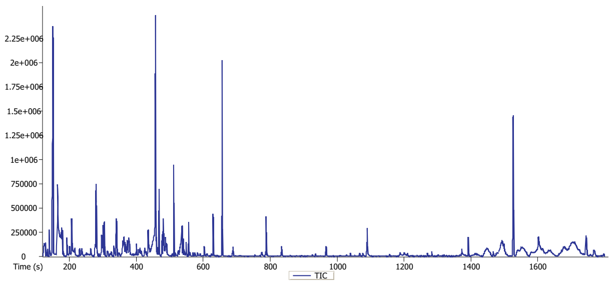

Figure 6. GC-TOFMS chromatogram for the 2X cream cheese extracted using HSSE.

Table 6. Peak numbers (Peak No), names, retention times in seconds (RT Sec), quantification masses (Quant Mass), and Peak areas (Area) for some of the peaks identified in the 2X cream cheese sample extracted using HSSE (also see Figure 6).

Peak No	Name	RT Sec	Quant Mass	Area
22	Methanethiol*	166	78	15,681
23	2-Butanone, 3-hydroxy- (aka, acetoin)*	167	88	155,064
26	Propanoic acid*	176	46	14,951
41	Butanoic acid*	217	60	474,260
53	Butanoic acid, 3-methyl-*	251	60	256,670
57	Butanoic acid, 2-methyl-*	259	74	44,932
61	3-Heptanone	277	57	1,442,901
62	2-Heptanone*	280	58	4,568,804
63	Pentanoic acid*	281	60	77,814
78	Dimethyl sulfone*	309	79	48,487
90	Benzaldehyde (a Strecker aldehyde)	340	74	237,339
116	Octanal (lipid oxidation product)	377	56	255,280
124	Diacetyl sulphide*	391	43	362,293
134	3-Nonanone	408	72	87,065
147	6-Hepten-3-one, 4-methyl-	426	60	165,919
151	Acetophenone	435	74	69,012
170	2-Nonanone*	457	58	8,833,493
173	Sorbic Acid (mold and yeast inhibitor)	458	97	413,476
196	Indole*	500	117	14,396
207	Octanoic acid*	537	60	3,350,373
216	Octanoic acid, ethyl ester	549	88	328,064
238	a delta lactone*	625	99	49,669
269	a delta lactone*	936	99	71,889

**Can be an important flavor compound in cultured dairy products.*

5. Lactones in Cream Cheese by an HSSE Technique*

The Importance of Peak Deconvolution for Accurate Peak Area Determination of Lactones

*1 gram of cream cheese sample (not previously analyzed) and4 mL water were added to a 20 mL extraction vial. Sample was stirred for 6 hr with a Twister PDMS bar attached to a paper clip in the headspace of the vial. Figures 7-9 illustrate the importance of peak deconvolution for accurate peak area determinations of lactones. Because mass spectra of delta-lactones are so similar, lactone standards should be tested individually to confirm identities of the lactones reported below.

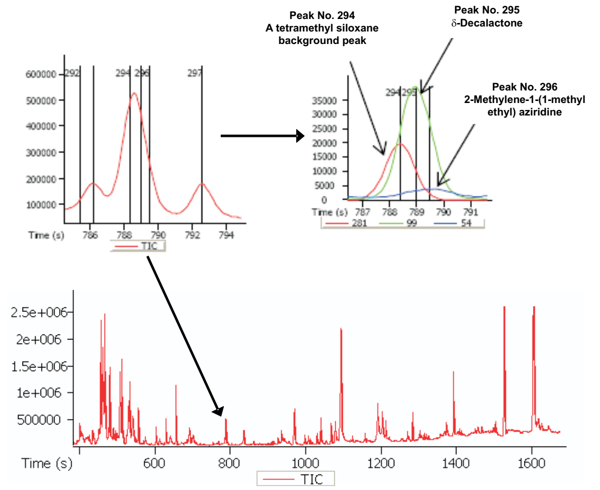

Figure 7. GC-TOFMS chromatogram of cream cheese by HSSE. A delta lactone (tentative identification is δ-decalactone) is the peak eluting at 789 seconds and is examined in detail above. Note the deconvolution of the lactone from the coeluting peaks.

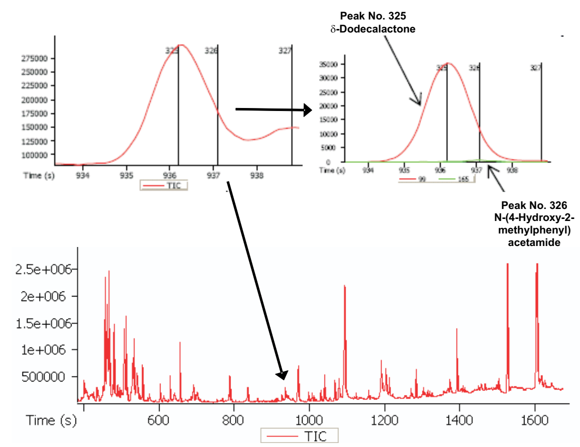

Figure 8. GC-TOFMS chromatogram of cream cheese by HSSE. Another delta lactone (tentative identification is δ-dodecalactone) is the peak eluting at 937 seconds and is examined in detail above.

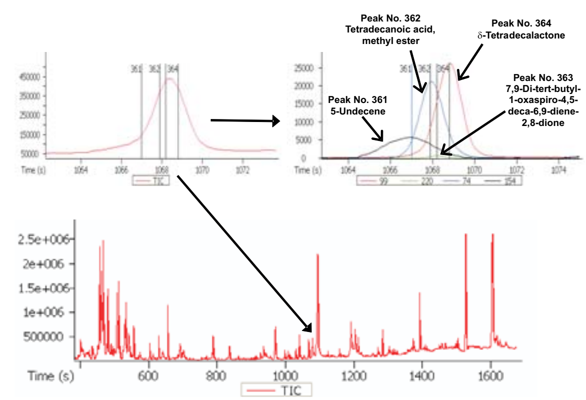

Figure 9. GC-TOFMS chromatogram of cream cheese by HSSE. Another delta lactone (tentative identification is δ-tetradecalactone) is the peak eluting at 1069 seconds and is examined in detail above. Note again the deconvolution of the lactone from coeluting peaks.

Note: Another sample of cream cheese was tested by a slightly different HSSE technique that was more sensitive than the HSSE used in Figures 7-9. With the more sensitive technique, 15 grams of cream cheese was placed in a 250 mL Erlenmeyer flask, heated to 55°C and extracted for 2 hrs with two PDMS Twisters positioned in the headspace of the flask. Five delta-lactones and one gamma-lactone were detected in the sample with this technique.

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