Quantitative Headspace Measurement of Volatiles in Dairy Products using Vacuum Assisted Sorbent Extraction (VASE) and GCMS Analysis

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Abstract

The headspace of common dairy products, including cheese and milk, was analyzed utilizing a new sample preparation technique called Vacuum Assisted Sorbent Extraction (VASE). VASE improves the recovery of heavier and more polar volatile compounds in nearly any matrix by GCMS. After introducing a sample into a 20-40mL vial, a cartridge containing 70mg of Tenax is placed into the headspace of the vial using a vacuum tight interface that allows the vial headspace to be evacuated to less than 0.01atm, or at least until the pressure needed to boil an aqueous mixture at 25°C is reached. This results in faster diffusion from the sample/headspace boundary layer to the adsorbent, enhancing the rate of sample extraction. In particular, heavy volatile compounds with low vapor pressures that have little to no response by classical SPME are extracted 10-50x more efficiently. Unlike Dynamic Headspace, which uses an inert gas to sweep the volatiles of a sample through the adsorbent bed to concentrate and trap analytes, VASE is performed statically. VASE allows the sample and headspace to come to an equilibrium in a closed system, causing analytes to diffuse onto and collect at the very front of the adsorbent bed. Consequently, VASE achieves a much better recovery of heavier compounds while reducing the common carryover issues affecting other adsorbent techniques that use flow to push compounds far up into a trap. Once placed under vacuum, the analyst determines the length of extraction time, ranging from minutes to hours, until equilibrium between the sample and headspace is reached to produce complete, reproducible extractions. Dairy products generally have low levels of headspace volatiles. In this study, VASE proceeded for 4-24 hours, with many Sorbent Pens extracting samples simultaneously to simulate conditions of a production laboratory. The increase in sample extraction duration combined with a large phase to sample ratio allows more accurate determination of headspace composition, with reduced matrix affects. Data is presented showing milk and cheese analysis, with recovery of compounds well into the semi-volatiles range.

Introduction

Odors from thousands of volatile and semi-volatile compounds may contribute to distinguishing flavors and taints in dairy products. Headspace compounds can be extracted and analyzed at any phase during production, from the raw ingredients to the final products. Dairy products are typically difficult to analyze using headspace techniques due to the low volatility of many compounds of interest, and the high fat content that creates a high affinity for most organic compounds to the sample matrix. The result is that most headspace techniques do not yield much information, leaving solvent extraction as the only effective technique at seeing low level flavor and odor compounds. However, even solvent extraction has its drawbacks, not the least of which is the substantial amount of labor required.

Figure 1 - The vacuum tight seal allows samples to remain under vacuum after a 30 second evacuation, allowing elevated rates of static diffusion to collect significantly more headspace compounds on the adsorbent than can be collected at atmospheric pressure. Sorbent Pens are all labeled with a barcode sticker. This allows the operator to simply scan each Sorbent Pen into the sequence table to record the its identity and track each Sorbent Pen through its lifetime of hundreds to thousands of extractions and desorptions.

A new headspace technique called VASE, or Vacuum Assisted Sorbent Extraction, greatly enhances sensitivity by placing an adsorbent cartridge, or "Sorbent Pen", directly into the headspace similar to SPME, however a vacuum is then applied on the sample vial "through" the Sorbent Pen using a MicroQT valve at the top of the Pen to greatly increase the extraction efficiency over techniques operating at atmospheric pressure (Figure 1). The Sorbent Pen contains about 100x the amount of sorbent typically found on a SPME fiber, and by using Tenax rather than PDMS, the available surface area is over 1000x greater than a PDMS SPME fiber. This prevents matrix interferences, as the appropriate selection of the sample weight in order to yield proper loading of a capillary column during GCMS analysis should never exceed the capacity of a sorbent device with 70mg of Tenax. This means extractions will be more consistent, and less susceptible to small changes in chemical affinities to the sample matrix.

The vacuum allows recovery of headspace compounds at lower temperatures (4-40°C), preventing changes to any heat sensitive sample matrices being studied. The elimination of solvent extraction can significantly speed up the process of analyzing dairy samples. Furthermore, the reproducibility of VASE sample preparation and GCMS analysis allows very small differences in sample composition to be determined without the need for isotopic dilution. Isotope dilution is very limiting, as it is often not even possible for many compounds of interest.

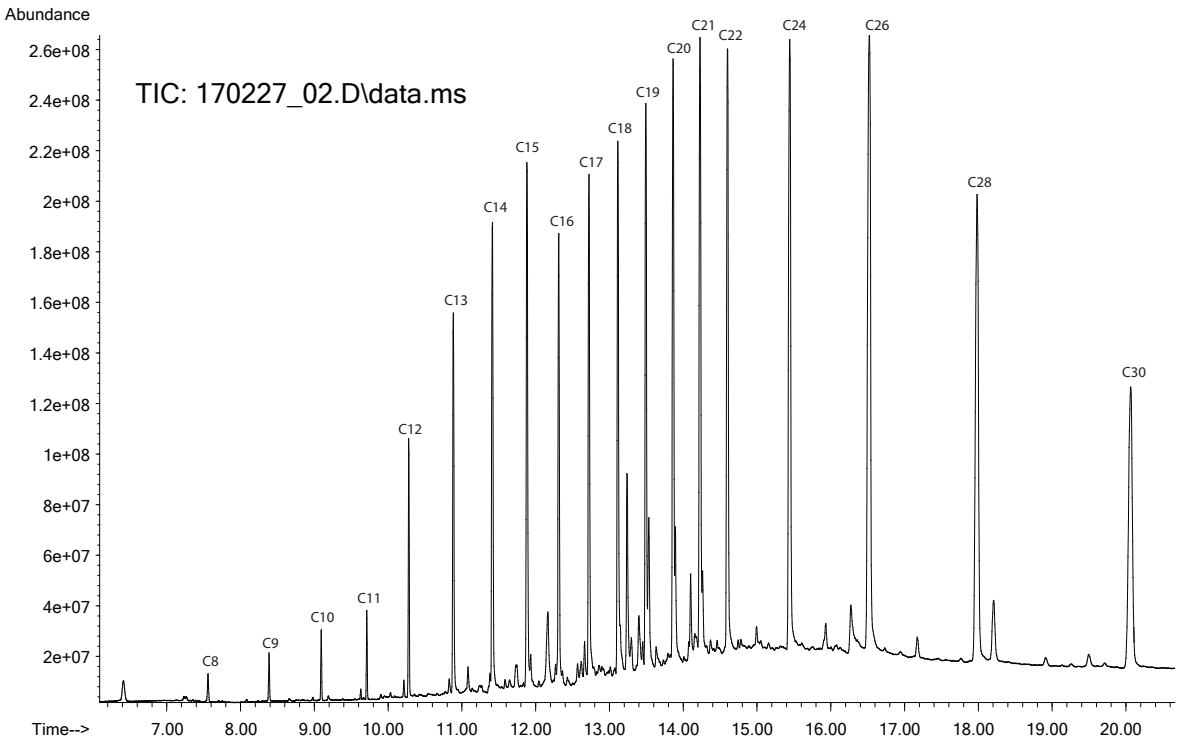


Figure 2 - C8 to C30 Calibration Standard, used to verify proper system operation after installation, including recovery out to C30 prior to sample analysis.

Figure 2 shows a C_8-C_{30} standard being developed to ensure proper recovery from one system to the next. A 1µL aliquot of standard was injected into a 20mL vial, into which the Sorbent Pen was inserted, followed by an overnight extraction at 70° C to obtain recovery out to C30. This standard may also be used to verify proper extraction completeness and reproducibility for new Sorbent Pens after the conditioning step in the 3801 Sorbent Pen Thermal Conditioner (SPTC) (Entech Instruments, Simi Valley, CA) (Figure 3).

Figure 3 - To prepare new Sorbent Pens for extraction, the 3801 Sorbent Pen Thermal Conditioner (SPTC) is used to condition Sorbent Pens prior to use up to 300°C depending on the sorbent type. During the analysis, the Sorbent Pens are both desorbed to the GCMS and thoroughly baked out, eliminating the need for additional thermal conditioning before reuse. However, if a Sorbent Pen was not isolated after extraction or if it contained unusually high concentrations of extracted compounds not completely removed during the previous analysis, the 3801 SPTC can be used for a quick cleanup of the Pen.

Experimental

Two different cheeses, whole milk, and eggnog were included in the study. A cheddar and brie cheese were selected to show reproducibility in the analysis. Whole milk and eggnog were analyzed to show the molecular weight range of chemicals that can be recovered, from light to very heavy.

Cheddar cheese samples were prepared by using a tool created to specifically cut ¾", 1.5g, round disks of cheese. This tool slices each sample to have the same amount of surface area to improve run to run consistency. The cheddar was sliced so the center of the block was used to cut the ¾" cheese disks to avoid any differences possible with using the outer layer of cheese exposed to the packaging. Samples were placed into a respective 40mL vial. After sample introduction, conditioned Sorbent Pens in combination with unique vial liners (Entech Instruments, Simi Valley, CA) were attached to the vial, followed by a 30 second evacuation of the vial through the Sorbent Pen, using a micro seal at the top of the Pen and a dual stage pump capable of achieving a vacuum of <0.01atm.

When a Sorbent Pen is inserted into a sample vial, it seals in the vial liner, which allows the the vacuum to be retained in the vial after removal of the vacuum source, and vacuum levels can be confirmed after sample extraction (Figure 1). Samples were placed into the 5600 Sorbent Pen Extraction System (Entech Instruments). The Sorbent Pen Extraction System, Figure 2, allows temperature control up to 70°C and adjustable agitation (20-300rpm) to further enhance extraction if desired. Brie cheese taken from the center, not in contact with the packaging, was also used, but this time was blended with water to homogenize the sample to improve consistency from sample to sample (1-part cheese to 2-parts DI water). Adding water to the cheese may help diffusion of compounds to the adsorbent under vacuum, as the boiling of water creates agitation. Additionally, by blending the cheese with water, more surface area is exposed for compounds to diffuse from the sample matrix to the adsorbent. Blending food with water also may simulate food being chewed in the mouth. For this study, Brie cheese was blended in a 1:2 ratio with water (70g cheese:140mL water).

Almost 10g of freshly blended sample was weighed and added to a 40mL vial, placed under vacuum, and placed into the 5600 Sorbent Pen Extraction System at 50°C for 3 hours to equilibrate, and then were desorbed and injected directly onto the GC column.

The milk sample was prepared by adding 0.5mL of whole milk into a 20mL vial. A Sorbent Pen was inserted, the vial was placed under vacuum, and the sample was placed into the 5600 SPES at 100rpm agitation for 15 hours at 25°C.

The eggnog sample was prepared by adding 1mL into a 20mL vial, a Sorbent Pen was inserted and the vial was placed under vacuum and extraction occurred in the 5600 SPES at 100rpm for 2 hours at 25°C.

Figure 4 - Sample vials and agitator used to perform VASE. The 5600 Sorbent Pen Extraction System (SPES) provides a convenient way to perform vial extractions using 30-position trays, allowing for significant sample throughput. The sample to be extracted is loaded into the vial, the Sorbent Pen is inserted, a vacuum is created through the MicroQT seal at the top of the Pen, and the sample is loaded onto the tray. The extraction time, temperature, and RPM can be programmed on the 5600 SPES and the vacuum extraction process begins. The Sorbent Pen Extraction System agitates the samples at 30-300 RPM to speed up transfer of volatiles to the headspace, while heating the sample from ambient +4˚C to 70˚C. Extractions can be completed in 1–48 hours depending on the application. Extraction times for this study were 2-15 hours.

Figure 5 - The 5800 Sorbent Pen Desorption Unit (Entech Instruments) was installed on a 7890/5977A, allowing split or splitless desorption of the Sorbent Pens into the GCMS.

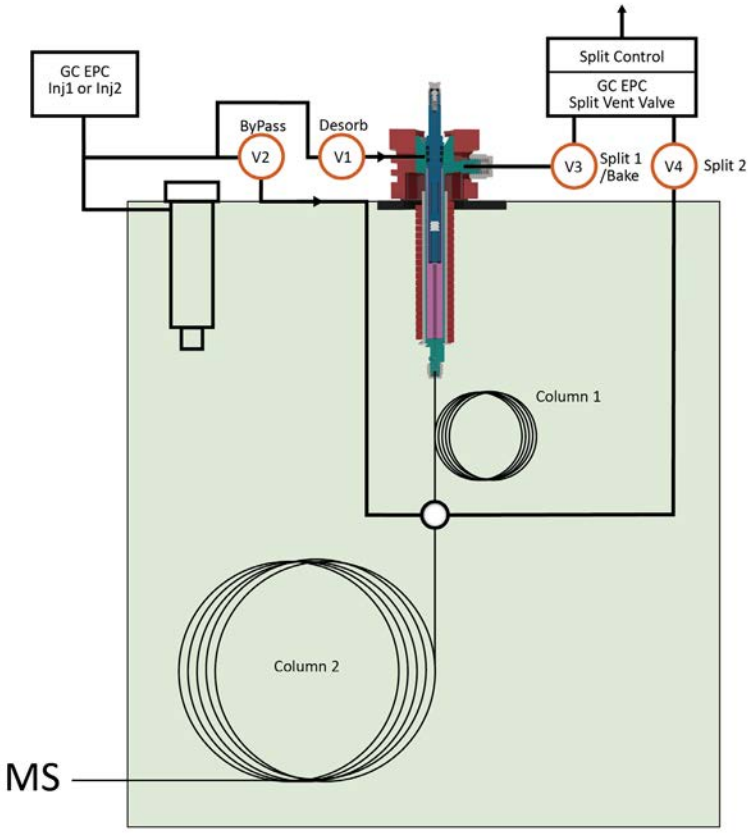
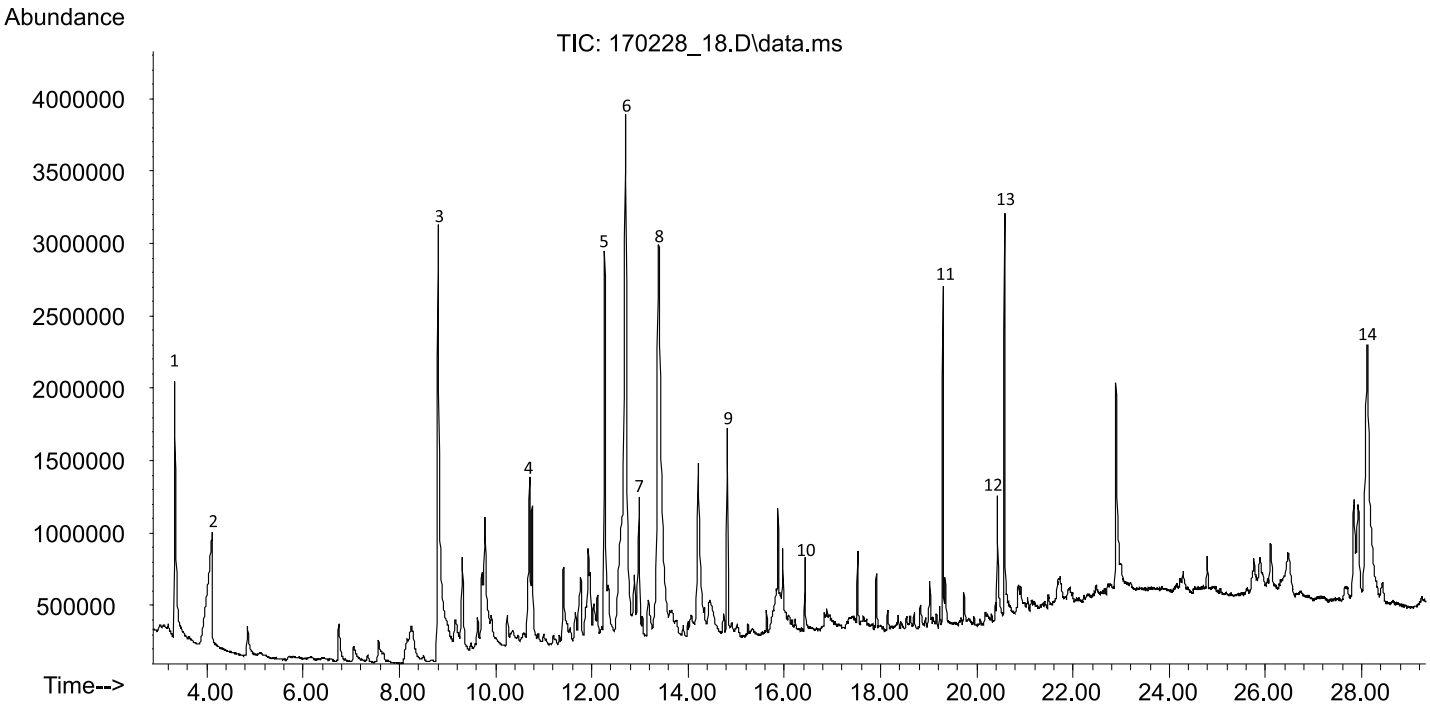


Figure 6 - The 5800 installs into an available GC injection port position. The 5800 provides gas flow control through 4 separate valves. Dairy samples in this study were analyzed split at a 6:1 ratio and in splitless mode. By using a large volume splitless technique where the V3 split valve remains off while the V4 valve turns on, faster desorption and splitless pre-loading of heavier volatiles onto the pre-column occurs.



Peak #	Compound	Retention Time	MW
1	Formic acid	3.331	46
2	Acetic acid	4.114	80
3	2-Furanmethanol	8.806	98
4	Isomaltol	10.707	126
5	Maltol	12.266	126
6	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	12.704	144
7	Benzoic acid	12.884	122
8	5-Hydroxymethylfurfural	13.387	126
9	n-Decanoic acid	14.817	172
10	Dodecanoic acid	6.432	200
11	n-Hexadecanoic acid	19.294	256
12	Oleic Acid	20.428	282
13	Octadecanoic acid	20.573	284
14	Cholesterol	28.116	386

Figure 7 - VASE analysis of milk, showing recovery of over a range of volatile and low volatility compounds out through cholesterol. 15 hour VASE extraction at 25°C, 6:1 split injection.

All samples in this study were analyzed on an Agilent 7890/5977 GCMS (Palo Alto, CA) by thermal desorption of the Sorbent Pen on a 5800 Sorbent Pen Desorption Unit (SPDU) (Entech Instruments) (Figure 5). Carrier gas flow was supplied to it by teeing off of the carrier gas going to the front injector. Operating the front inlet in splitless mode accommodates the variable flow rates to the 5800 and columns when various valves are changing.

The 5800 SPDU was set to a standby temperature of 70°C. Once in place, the SP is desorbed for 5 minutes at 260°C and delivers the sample onto a 5m pre-column (DB1, 5m length, 0.25mm ID, 0.25um, 100% PDMS) with a split vent downstream prior to the primary column to allow higher flow rates during desorption of the cartridges. Following the pre-column and split vent, the compounds flow through the primary column (DB1, 30m length x 0.25mm ID, 0.5µm film) to MS detection. The initial GC temperature was 40° C which after a 5-minute initial hold was ramped to 300°C at 10°C/min. Full scan data was collected from 34-450 amu, with approximately 2.5 scans per second. The NIST Library was used to identify the compounds detected.

Following the 5-minute desorption, the Sorbent Pen remains in the SPDU and is subject to a baking period for 20 minutes at 260°C while the sample is flowing through the GC, and then left at a post bake temperature of 70°C for a few minutes when the run is finished so that the Sorbent Pen and SPDU and cooled back down to idle temperature ready for the next injection.

Alternatively, the 5800 can also provide a classical split injection to increase the rate of injection when analysis of lighter compounds are required. The cheddar and milk samples were desorbed with a classical split injection of 6:1.

Discussion

Figure 7 is a VASE extraction of 0.5mL of milk followed by a 6:1 split injection allowing the analysis of compounds as light as formic acid, and as heavy as cholesterol, demonstrating the wide dynamic range of the VASE technique and especially its ability to recover very low volatility compounds. Figure 8 shows 1mL of eggnog extracted for 2 hours under vacuum, and likewise shows recovery of cholesterol.

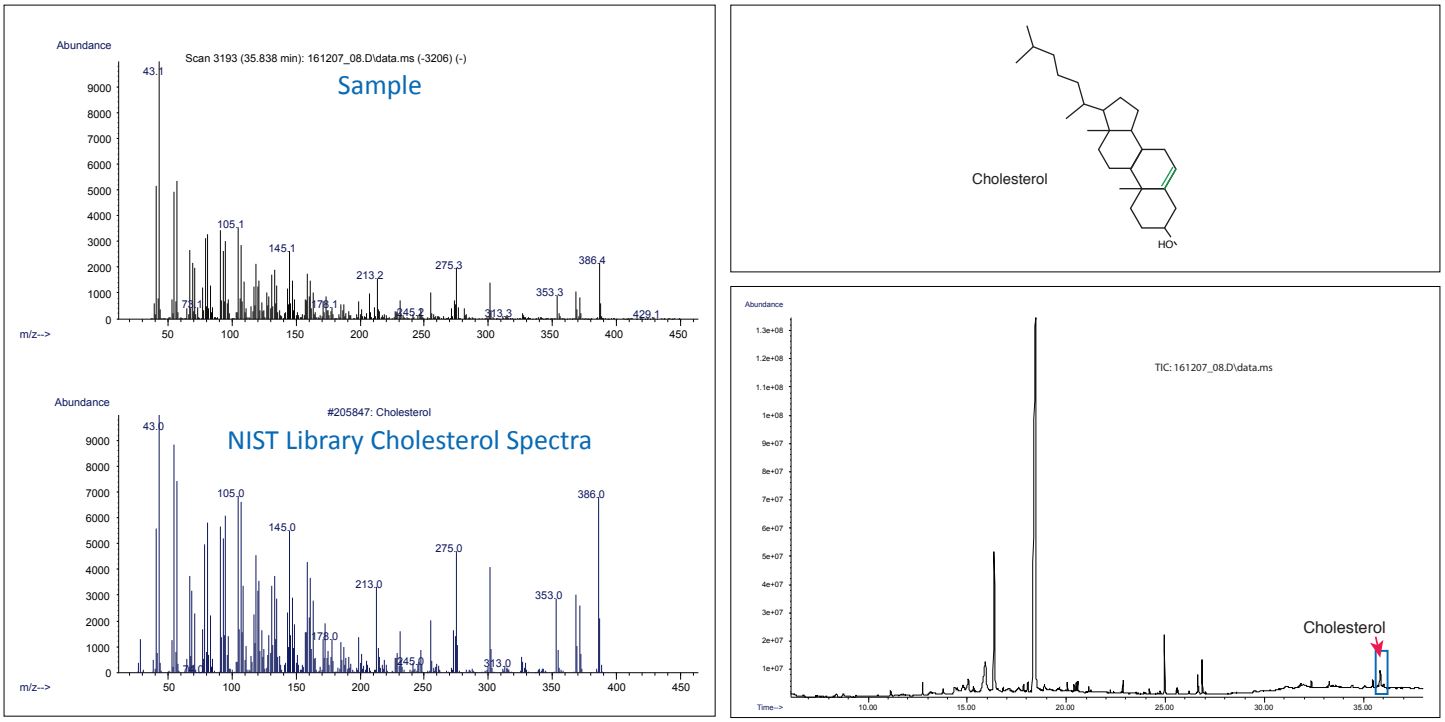
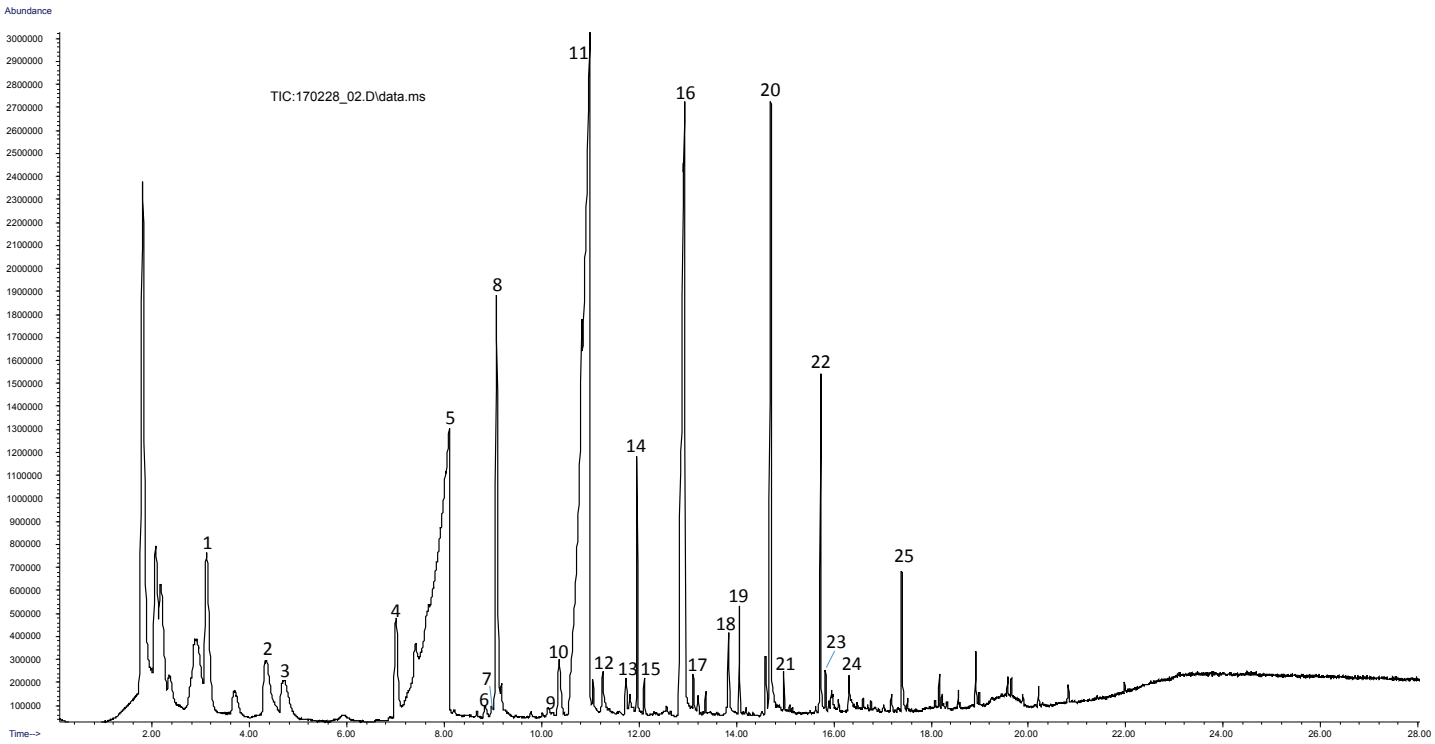


Figure 8 - VASE analysis of 1mL eggnog extracted for 2 hours at 25°C. Divert V4, 5 min Desorb 260°C. Recovery of cholesterol without contacting the matrix is confirmed by the spectral match shown.



Peak #	Compound	Retention Time	MW
1	n-Hexane	3.118	86
2	2-Butanone, 3-methyl-	4.331	86
3	Acetoin	4.694	88
4	2,3-Butanediol	7.003	90
5	Butanoic acid	7.407	88
6	p-Xylene	8.826	106
7	Dimethyl sulfone	8.963	94
8	2-Heptanone	9.064	114
9	Benzaldehyde	10.125	106
10	Dimethyl trisulfide	10.355	126
11	Hexanoic acid	10.815	116
12	Benzeneacetaldehyde	11.253	120
13	2H-Pyran-2-one, tetrahydro-6-methyl-	11.727	114
14	2-Nonanone	11.957	142
15	Nonanal	12.101	142
16	Octanoic acid	12.934	144
17	Octanoic acid, ethyl ester	13.114	172
18	1-Pyrrolidinecarboxaldehyde	13.832	99
19	2-Undecanone	14.055	170
20	n-Decanoic acid	14.702	172
21	Decanoic acid, ethyl ester	14.967	200
22	2H-Pyran-2-one, tetrahydro-6-propyl-	15.722	142
23	2-Tridecanone	15.824	198
24	Dodecanoic acid	16.306	200
25	2H-Pyran-2-one, 6-heptyltetrahydro-	17.392	198

Figure 9 - Analysis of cheddar cheese. Light acids including butanoic acid do not chromatograph well on the non-polar column used, and would be better handled by a polar or intermediate polarity column. Analysis was performed by split injection.

The two types of cheese analyzed were cheddar and brie. Both cheese samples recovered numerous indentifiable compounds, including ketones, fatty acids, lactones, sulfides, esters, terpenes, and aldehydes. With the ability to recover polar acids using VASE, a more appropriate column would be one of at least intermediate polarity, or perhaps even a wax column (Figure 9). Despite this, the duplicate sample preparations on different Sorbent Pens demonstrate the reproducibility of this technique (Figures 10 and 11).

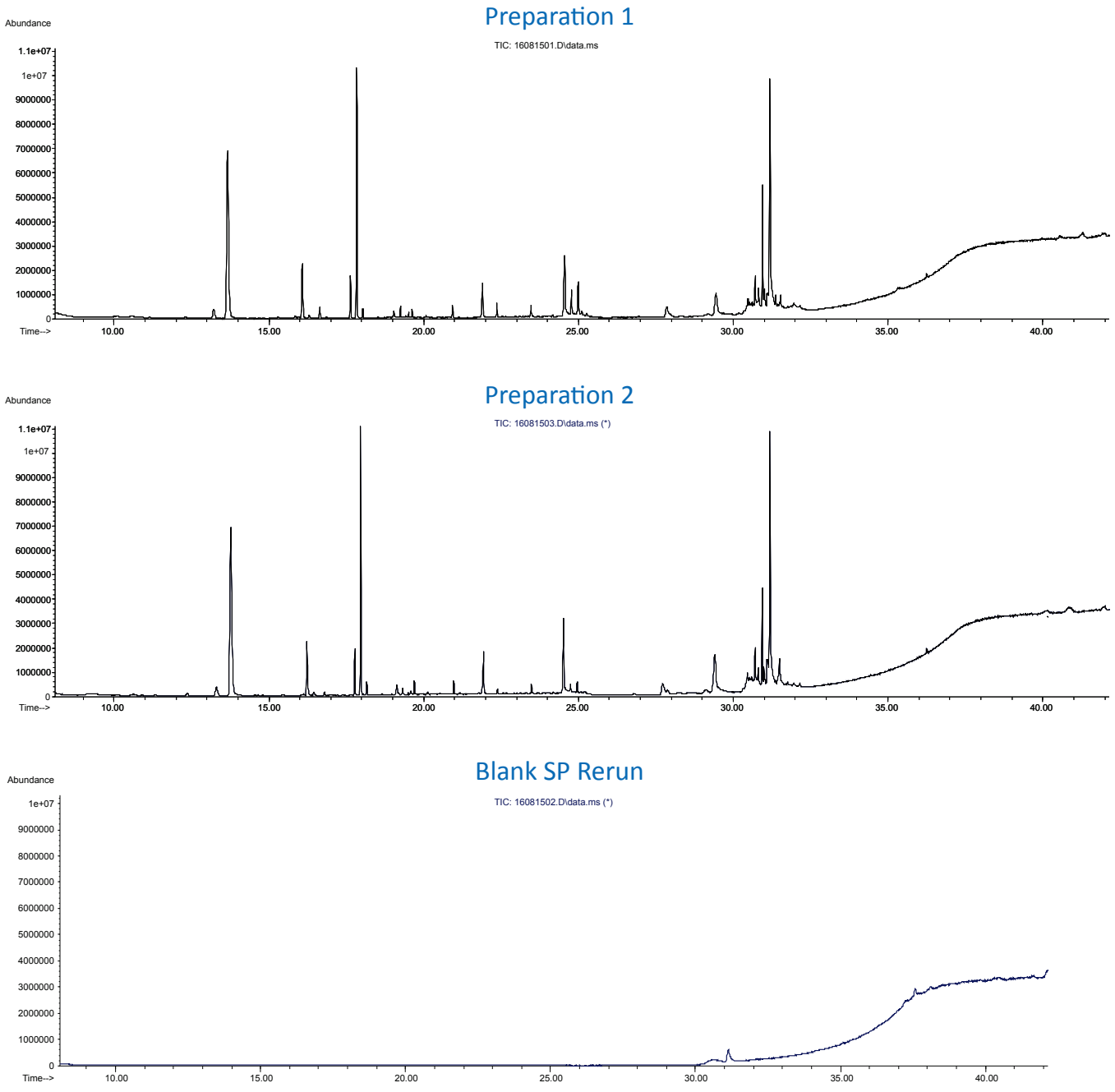


Figure 10 - VASE duplicate Aaalysis of brie cheese. The analysis is very reproducible, shown by scaling the Y Axis the same in both analyses. The slight difference in free fatty acids is believed to be real, based on the difficulty in preparing duplicate cheese samples with identical levels of triglyceride oxidation. The blank run afterwards was very clean.

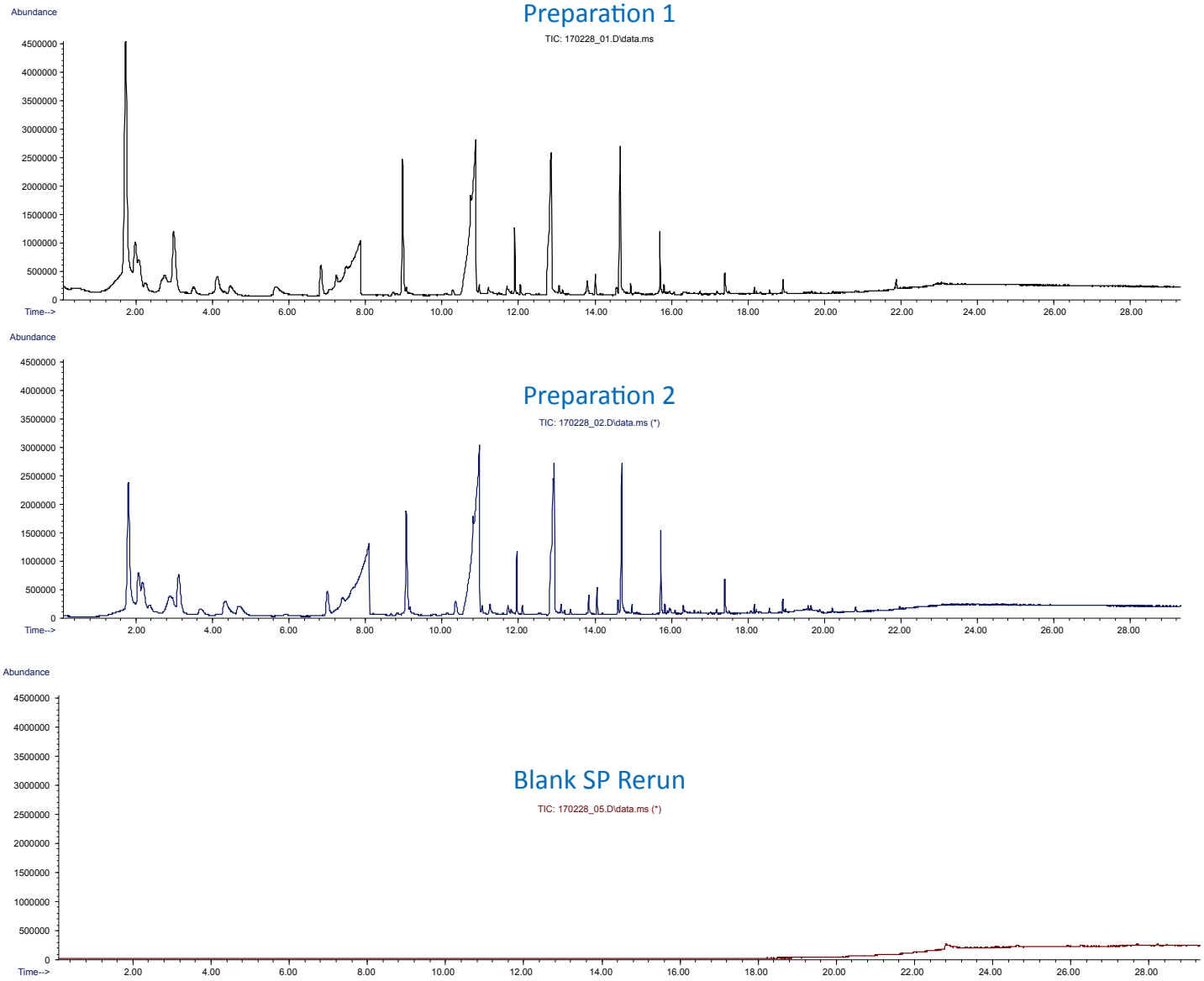


Figure 11 - VASE duplicate analysis of cheddar cheese. Other than a slight difference in the size of the injected air peak at the beginning of the analysis, the intensities and recoveries are very similar. To obtain reproducible analyses, the cheese was sliced to the same thickness, and then 3/4" diameter round samples were made using a 316 stainless tube turned into a "cookie cutter" sample preparation tool to keep surface areas the same.

The Sorbent Pen was also rerun as a blank after the initial cheese sample runs to demonstrate how the desorption and bake-out process completely cleans up the Sorbent Pen and readies it for the next sample extraction. Although both the cheddar and brie chromatograms show large numbers and quantities of compounds, the subsequent blank runs were heavily loaded, the subsequent blanks generated by simply leaving the Sorbent Pen in the 5800 SPDU and pressing START showed absolutely no carryover.

Chemists familiar with packed traps will be surprised that a more thorough clean-up process is not required, as at least a little carryover is virtually guaranteed with dynamic headspace techniques, however, this feature of VASE highlights the significant advantage of performing diffusive sampling under vacuum. No sorbent is packed perfectly within a sorbent tube especially after several cycles of sorbent expansion and contraction, so actively flowing the sample through a trap will cause an effect called "channeling", where the carrier promotes the delivery of even heavy compounds much further into the adsorbent than is possible by diffusive sampling. The further the heavy compounds penetrate the adsorbent, the lower their recoveries will be, and the greater their extent of carryover in subsequent runs. This phenomenon is eliminated when trapping under vacuum by diffusion. Obtaining nearly 100% recovery from the adsorbent, and having essentially zero carryover are two big hurdles in obtaining the perfect headspace extraction technique.

Conclusion and Future Work

Vacuum Assisted Sorbent Extraction has been demonstrated to be effective in recovering light to very heavy volatile compounds from dairy samples, despite low concentrations and high affinity of volatile compounds for the sample matrix. The very reproducible nature of this technique should allow small differences between various dairy products to be apparent as long as the sample is homogenized and preparation from vial to vial remains consistent. Blank runs in between and after duplicate samples show how the Sorbent Pen is completely cleaned up after desorption while the sample is running in the GC, leaving no carryover.

Future work will continue to show what effect vacuum has on the ability to leave dairy products at room temperature without spoilage for longer periods than normal, as there may be microbe growth inhibiting benefits when operating under vacuum. As the vapor pressure of water is reached during evacuation, the boiling of water may directly lower the concentration of living organisms, while the evolving water may chase out any remaining oxygen to eliminate the growth of at least aerobically based organisms.

The results show the extensive range of compounds extracted using the SP and demonstrate its potential as a routine method for examining aroma compounds relating to flavor analysis and contaminants, even in difficult fatty matrices of dairy products. This new technique provides a platform for several potential research projects focusing on characterizing flavor compounds of dairy products during all stages of production, packaging, and storage.

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