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

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Abstract

The headspace analysis of some dairy products has been evaluated using a new sample preparation technique called “Vacuum Assisted Sorbent Extraction” (VASE) that improves the recovery of heavier and more polar volatile compounds. 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 0.01 atm, or at least until the pressure needed to boil an aqueous mixture at 25 deg C. 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 that pushes volates from a sample through the adsorbent bed, VASE vacuum extraction is done statically which deposits the sample much closer to the entrance of the adsorbent bed, providing much better recovery of heavier compounds, and far less carryover. Once placed under vacuum, the extraction is allowed to proceed for between minutes to many hours, or even up to 24 hours, to effect complete, reproducible extractions. In the analysis of dairy products where headspace volatiles are generally at low concentrations, VASE extraction is allowed to proceed for 4-24 hours, with many Sorbent Pens collecting in parallel to allow many samples to be prepared simultaneously to accommodate the 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

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 of most organic compounds to the sample matrix. Most headspace techniques typically 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.

A new headspace technique call VASE, or Vacuum Assisted Sorbent Extraction, greatly enhances sensitivity by placing an adsorbent cartridge, or “Sorbent Pen”, right into the headspace like SPME, but then a vacuum is exerted on the sample vial “through” the Sorbent Pen using a micro seal at the top of the Pen to greatly increase the extraction efficiency over techniques operating at atmospheric pressure. 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 to yield properly loading of a capillary column during GCMS analysis should never exceed the capacity of a sorbent device with 70mg of Tenax. This means that 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 deg C), preventing changes to any heat sensitive sample matrices being studied. The elimination of solvent extraction can significally speed up the process of analyzing dairy samples, and 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, which is very limiting and often times not even possible for most compounds of interest.

Experimental

Two different cheeses, whole milk, and eggnog were included in the study. A chedder and Brie cheese were selected to show reproducibility in the analysis, and whole milk and eggnog were analyzed to show the molecular weight range of chemicals that can be recovered, from light to very heavy. Between 2-5 g of each were prepared into 20 or 40mL vials. After sample introduction, pre-conditioned Sorbent Pens in combination with special vial liners (Entech Instruments, Simi Valley) 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 <0.01 atm. The Sorbent Pen makes a seal to the liner that allows the vacuum to be retained in the vial after removal of the vacuum source, and vacuum levels can be confirmed after sample extraction. Samples were placed in an agitator that can be heated up to 70 deg C, but extractions for this study were conducted at 25 deg C. After 4-16 hours of extraction, the Sorbent Pens were removed Analysis was performed by thermal desorption of the Sorbent Pens on a 5800 SPDU (Entech Instruments) to deliver the sample onto a 5m pre-column (0.25mm ID, 0.25um, 100% PDMS) with a split vent down stream prior to the primary column to allow higher flow rates during desorption of the cartridges. Alternatively, the 5800 can also provide a classical split injection to increase the rate of injection when analysis of lighter compounds are required. Analyses were performed on an Agilent 7890/5977 GCMS (Palo Alto, CA) using a 30m, 0.25mm ID, DB1 column with 0.5um film thickness. The initial GC temperature was 40 deg C which after a 5 minute initial hold was ramped to 300 deg C at 10 deg/min. Full scan data was collected from 34-450 amu, with approximately 2.5 scans per second.

Figure 1 - Sample vials and Agitator used to perform Vacuum Assisted Sorbent Extraction. Extraction times were 4-16 hours. Extraction of up to 30 samples at a time allow for significant sample throughput.

Figure 2 - 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 extract on the adsorbent than can be performed at atmospheric pressure.

Figure 3 - A 3801 Sorbent Pen Conditioner (Entech Instruments) was used to initially condition the Sorbent Pens. During the analysis, the Sorbent Pens are desorbed to the GCMS and thoroughly baked out, eliminating the need for additional thermal conditioning before reuse.

Figure 4 - 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.

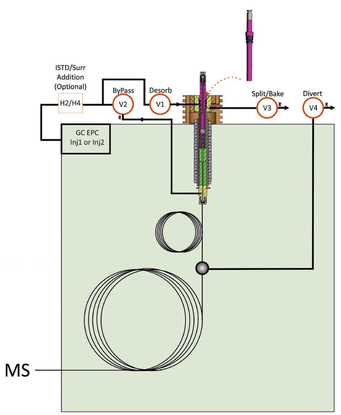

Figure 5 - The 5800 installs into an available GC injection port position, providing gas flow control through 4 separate valves. Dairy samples in this study were analyzed both splitless at 6:1, and by using a large volume splitless technique where the V3 split valve remains off, while the V4 valve turns on to allow faster desorption and splitless pre-loading of heavier volatiles onto the pre-column.

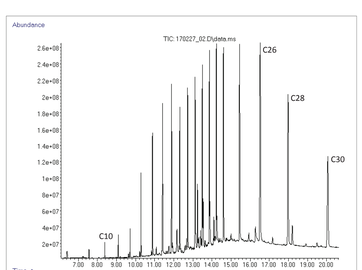

Figure 6 - C8 to C30 Calibration Standard, used to verify proper system operation, including recovery out to C30 prior to sample analysis.

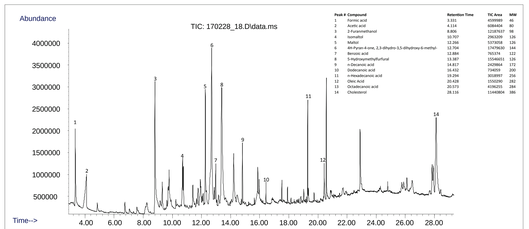

Figure 7 - VASE analysis of Milk, showing recovery of volatile and low volatility compounds out through Cholesterol. 15 hour VASE extraction at 25C, then 50C for 1 hour, 6:1 split injection

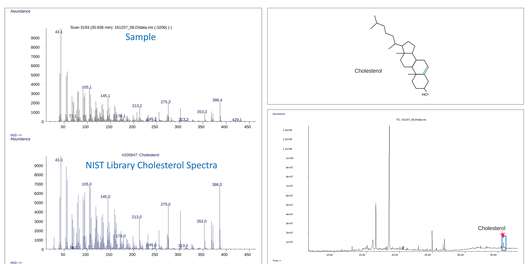

Figure 8 - VASE analysis of 1mL Eggnog extracted for 2 hours at 25. Divert V4, 5 min Desorb 260C. Recovery of Cholesterol without contacting the matrix is confirmed by the spectral match shown.

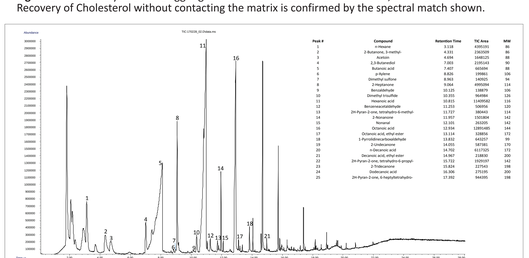

Figure 9 - Analysis of Cheddar Cheese. Light acids including Buteric Acid do not chromatograph well on the non-polar column used, and would be better handled by a polar or intermediate polarity column. Analysis was performed by split injection rather

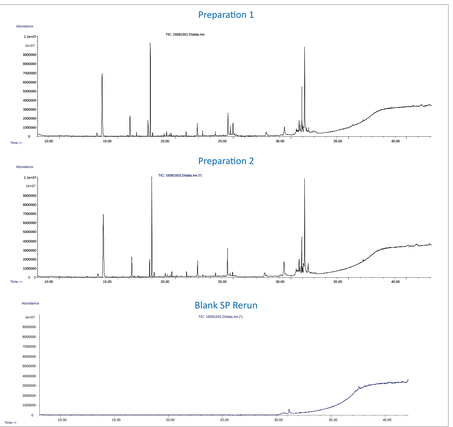

Figure 10 - VASE Duplicate Analysis of Brie Cheese. The analysis is very reproducible, shown by scaling the Y Axis the same in both analyses. The slight difference in free fatty acids is believed to be real, based on the difficulty in preparing duplicate cheese samples with identical levels of triglyceride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

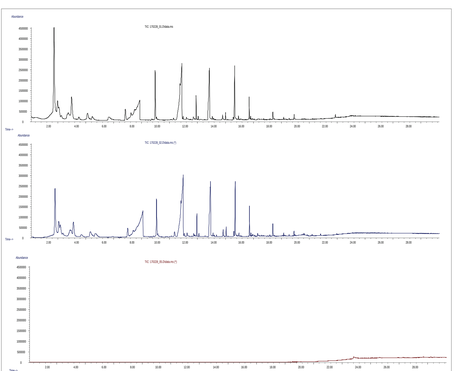

Figure 11 - VASE Duplicate Analysis of Cheddar Cheese. Other than a slight difference in the size of the injected air peak at the beginning of the analysis, the intensities and recoveries are very similar. To obtain reproducible analyses, the cheese was sliced to the same thickness, and then 3/4” diameter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

Discussion

Figure 6 shows a C8-C30 standard that is being developed to ensure proper recovery from one system to the next. A 1ul aliquot of the standard was injected into a 20mL vial, into which the Sorbent Pen was inserted, followed by an overnight extraction at 70 deg C to obtain recovery out to C30. Figure 7 is a VASE extraction of 1mL 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 1g of eggnog extracted for 4 hours under vacuum, and likewise shows recovery of Cholesterol. Two types of cheese were analyzed; Cheddar and Brie. A special tool was created to present a perfect 3/4” round disk of cheese to a 40mL vial to improve run to run consistency. 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. Although both the Cheddar and Brie cheese runs were heavily loaded, the subsequent blanks generated by simply leaving the Sorbent Pen in the 5800 Desorber and pressing START showed absolutely no carryover. Chemists familiar with thermal desorption will find this difficult to understand, as at least a little carryover is virtually guaranteed with dynamic headspace techniques, but this is the difference between how the sample deposits onto the adsorbent when performing diffusive sampling under vacuum. No sorbent is packed perfectly within a sorbent tube especially have 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

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 sample presentation within the vial is consistent. Work will continue to see 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.