GCMS Inlet for Detection and Characterization of “Aroma Significant Compounds" in Foods and Beverages

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Abstract
A new headspace technique for GC-MS analysis of food and beverage aroma compounds is presented that specifically targets only those aroma compounds that are significant at specific temperatures. Sample is placed into vials ranging from 40cc to 1000cc and placed under vacuum to facilitate extraction of aroma compounds with temperatures from ambient to 150 degrees centigrade. Vacuum extraction reaches all surfaces of the sample so that no channeling will occur as can happen with Purge & Trap. Sample headspace can be transferred to the GC-MS by either loop injection or through a 3 stage preconcentrator that can concentrate as much as 1000cc, providing the ideal inlet for first performing GC/O (olfactory detection), and then injecting a much larger volume to obtain enough signal in the MS to obtain a reliable spectrum. The entire aliquot of headspace is concentrated and separated from interfering matrix compounds without changing the distribution of light and heavy compounds. Data is presented showing the process of identifying the elution times of aroma compounds using small volume injections, followed by preconcentrating 100- 500cc of sample to properly identify which compounds are responsible for creating the aromas in the unconcentrated headspace.

Introduction
Identifying Aroma Significant Compounds (ASCs) in foods and beverages by GCMS has the following challenges:

1. Elution Times of Aroma Significant Compounds must be identified using an unconcentrated injection of sample headspace

2. Additional headspace injections must be performed with much larger headspace volumes to allow detection of ASCs by the MS detector

3. The matrix has to be removed or otherwise managed to prevent chromatographic and MS interference when performing Large Volume Static Headspace (LVSH) analysis.

4. Light to heavy headspace compounds must be equally represented

5. All classes of compounds contributing to the aroma must be recovered

Figure 1 Vacuum tight vials ranging from 40 to 1000mLs utilize a septum-less interface that typically yields leak rates of less than 2 x 10-8 cc/sec, making them appropriate for both vacuum extraction and Large Volume Static Headspace techniques.

Figure 2 Bottle-Vac samplers can be used for both gas-phase and liquid/ solid sampling. Liquid samples with minimal suspended particulates can be injected directly through the Micro QT septum-less interface into a pre-evacuated bottle using a blunt end 16 gauge needle.

Aroma Significant Compounds

All compounds that are not already saturating the olfactory senses (eg - H2O and O2) have a smell or odor when concentrations become high enough. For most compounds found in a typical, non- concentrated headspace, concentrations are below the odor threshold. It is important to present a non-concentrated, representative sample of headspace to the GCMS, while splitting at the end of the column to a "sniff port" so that only the compounds contributing "significantly" are identified.

Large Volume Injection to the MS

While a 1-2cc injection is ideal for olfactory detection, the human nose is many times more sensitive than today's mass spectrometers for most important aroma-producing compounds. Volumesupto1000ccmustbeconcentratedand injected to be able to "see" by MS what the human nose is able to detect in just 1-2cc of headspace.

Matrix Management

Along with the aroma compounds, the headspace above foods and beverages contains a matrix at PPM to % levels that must be removed to avoid chromatographic distortion and MS interferences. These may include water, CO2, and ethanol, as well as elimination of the air background.

Equal Representation of Entire Boiling Range

Loop injection typically injects a non-fractionated headspace sample, but many other "concentrating" techniques discriminate against either the light or heavy end compounds (SPME). A large volume inlet system must perform the preconcentration and matrix management without loss of the light or heavy compounds.

All Classes of Compounds Represented

Many aroma compounds are thermally labile, so utilizing a strong adsorbent technique (or in some cases any adsorbent) to show a C2 to C30 recovery of alkanes may totally "miss" the most important aroma compounds in the headspace. These may include primary amines and ammonia, fatty acids, phosphorous and sulfur-containing compounds.

A new inlet system is presented that provides software selection between 3 different headspace sample preparation techniques, from direct on-column cryo-focusing for the most thermally labile compounds, 3-stage trapping for sophisticated matrix management, and initial trapping just inches away from the column using Dean Switching to allow recoveries out to beyond C25. Quantitative delivery of 2cc to 1000cc of headspace allows olfactory or MS detection utilizing an open split interface to accommodate a sniff port for ASC detection. Silonite coated tubing is used throughout to bring the inertness level of the GC to the sample

preparation system. Matrix compounds, including ethanol, CO2, air, and water, are eliminated using either dry purging, Extended Cold Trap Dehydration (ECTD), or Microscale Purge and Trap (MP&T). Data will be presented on several foods, showing the process by which all significant aroma compounds are identified.

Experimental

Food and beverage samples were prepared in 500mL Pulsed Vacuum Extraction Headspace (PVEH) vials (Entech Instruments, Inc, Simi Valley, CA), which were quickly evacuated and allowed to equilibrate for 30 minutes under vacuum prior to refilling with UHP grade nitrogen to a slight positive pressure (3-5 psig). A 7500A Robotic Headspace Autosampler (Entech Instruments) was used to deliver headspace samples at ambient to 150oC to the 7101AR Preconcentrator (Entech). The design of the Micro-QT septum-less vial interface allows a Silonite coated, 1/16" transfer line to be introduced into the sample headspace, providing a completely fused silica lined flow path. The 7500's heated Silonite transfer line was introduced into a 7101AR configured with both a loop and a Direct GC valve that allowed transfer of the sample to the following 3 locations:

1. 3-stage trapping for management of water, EtOH, CO2, and fixed gases.

2. SV Trap (Semi-Volatiles Trap) mounted on top of a 7890GC just inches from the column.

3. Directly to the on-column focuser for direct LN2 focusing of 5 to 30cc.

The 3-stage technique was developed for trace Environmental monitoring, but has been applied successfully to food and flavor analysis. The second two preconcentration techniques were recently developed specifically to extend the range of recoveries out to C25, while also being able to recovery thermally labile compounds (Direct on-Column Focusing), such as primary amines and ammonia.

For this study, Extended Cold Trap Dehydration was used to look at bananas, strawberries, oranges, and apple juice. A small volume of 2-10cc is used along with olfactory detection to determine where Aroma Significant Compounds elute. Larger volumes of 100-500cc were then used to obtain MS spectra for identification of peaks eluting at the specific retention times where aromas were detected. Although Dean Switching could be used to repeat this procedure using a confirmatory column to better identify the aromas when co-elutions occur, this was not conducted for this study. Bananas were specifically chosen to allow determination of the lag time between the olfactory detection of amyl acetate at the sniff port, vs. the peak centroid in the MS. This time differences was expected to be a constant. During trapping, a first stage was cooled to -40oC, while a second Tenax trap cooled to -40oC collected the sample. The first stage was then heated to 10oC, and an additional 20cc of helium was purged through this trap over to the cold Tenax trap to complete the transfer. The cold Tenax trap was back desorbed to a focusing trap, which was flash heated onto a 60m, 0.32mmID, 1um film column in a 7890 GC (Agilent, Palo Alto, CA). The column was held at 35oC for 5 minutes, then heated at 10oC/min to 240oC for a 5 min hold. The 5975 MS (Agilent) was scanned initially from 29-160 for the first 6 minutes to include light volatiles (H2S, etc), then from 33-270 amu, at 3scans/sec.

A single analysis was also performed on strawberries using the SV Trap mounted just inches from the GC column to look at the higher molecular weight compounds and to determine whether the 3-stage ECTD technique was missing any high boiling compounds. Olfactory detection was not performed using the SV trap for this study.

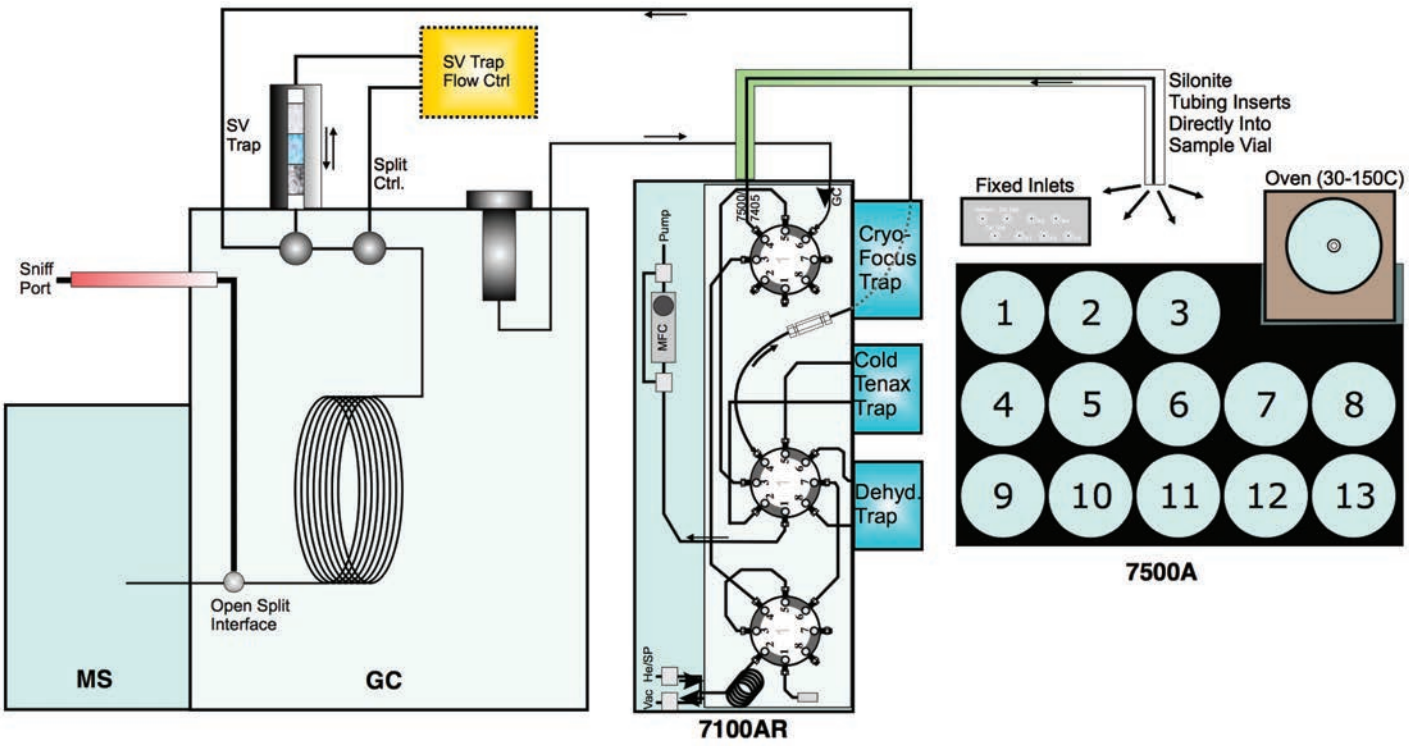

Figure 3 The 7500A/7100AR GCMS inlet system automates the analysis of gaseous samples in Silonite Minicans, or headspace analysis in Bottle-Vac or Pulsed Vacuum Extraction Vials. Three sample preparation techniques are under software control, including Cold Trap Dehydration, Direct Cryo-Focusing (TICs), and SV Trap operation (CWA's).

Discussion

Figures 4-13 show the data collected for the four fruits analyzed. Banana was run first to determine the time difference between amyl acetate detection at the sniff port and the MS. Applying this to the other fruits to determine where the Aroma Significant Compounds eluted was straightforward. Aromas detected during the low volume injections are shown with the blue arrows. As expected, no peaks are visible at some of the aroma elution times when only 10cc are injected. The selection of the volume to injection when performing olfactory detection is up to the analyst, but no more than 10cc should be used to avoid detecting compounds that are normally below odor threshold in the natural headspace. In this case, 10cc was probably bringing out aromas that are below normal odor thresholds, as some of the light alcohols that were detected are probably not contributing to the overall aroma in the normal headspace.

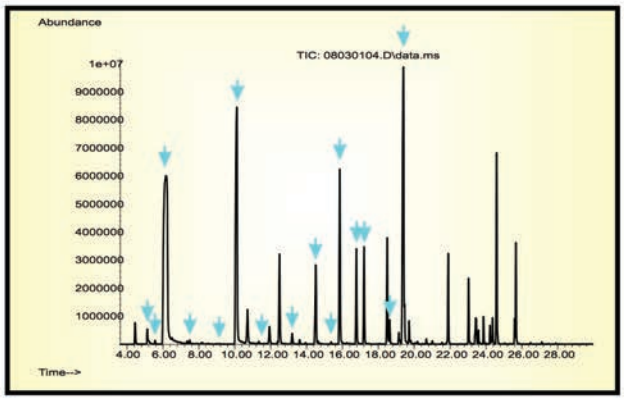

Figure 4 10cc headspace, 50g Banana, ECTD, Olfactory aroma detection

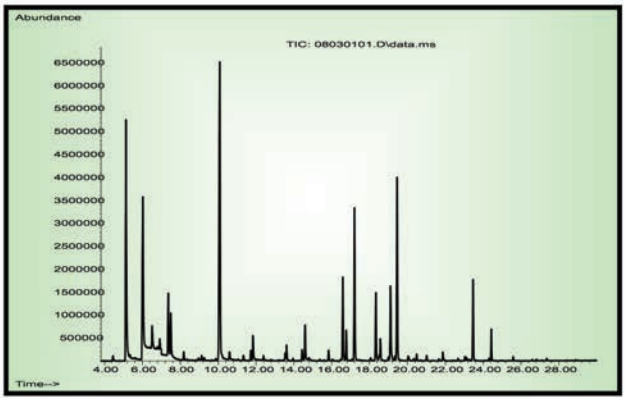

Figure 7 350cc Headspace, 50g Apple Juice, ECTD

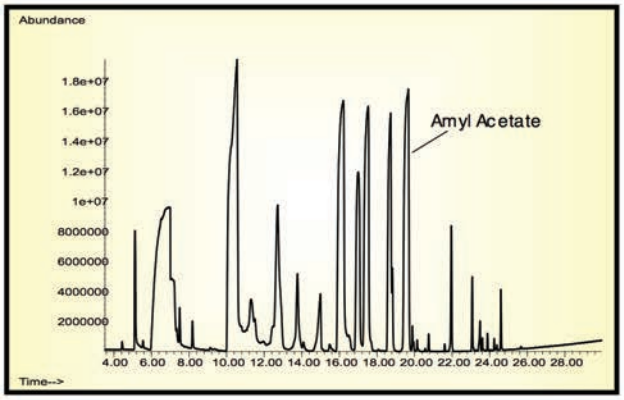

Figure 5 100cc Headspace, 50g banana, ECTD

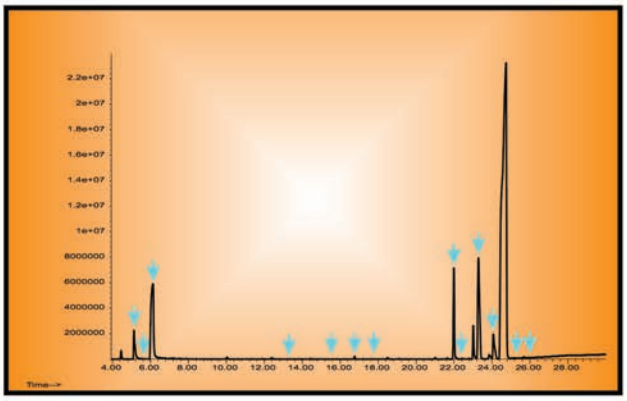

Figure 8 10cc Headspace, 50g Fresh Cut Orange, ECTD

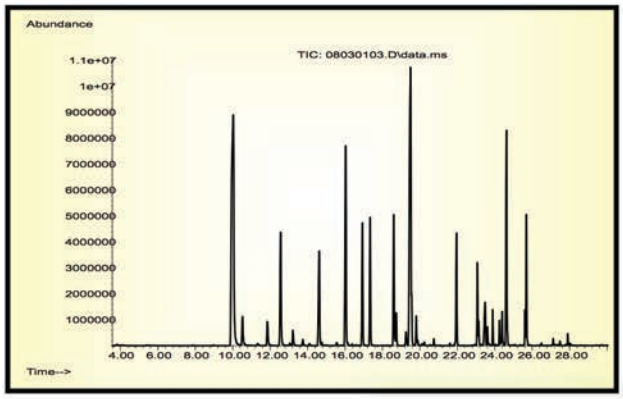

Figure 6 100cc Headspace, 50g banana, SV Trap, 5:1 Split

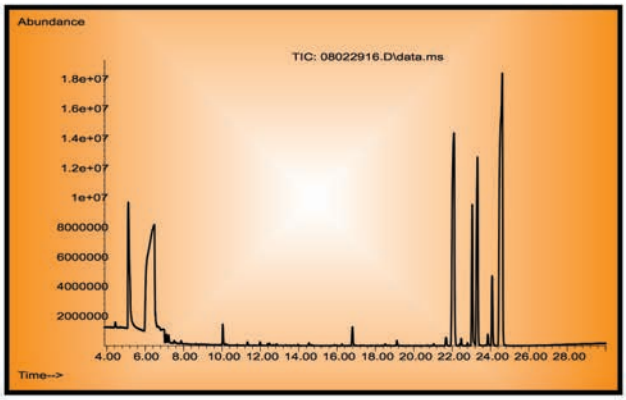

Figure 9 100cc Headspace, 50g Fresh Cut Orange, ECTD

The strawberry and banana samples were also run using the SV trap. This technique will show compounds in the headspace out to C25. However, the Tenax trap is operated at 5-10oC higher than the sample to prevent water accumulation, and the loss of the light ends are apparent. Included in these light ends is methyl mercaptan, visible as the second peak in the TIC chromatogram in **Figure 11**. ECTD has also been demonstrated to recover H2S, another important flavor compound. Although a taller amyl acetate peak is apparent in the SV chromatogram for bananas, analysis of these fruits at room temperature indicates that the heavier SVOCs just are not present at these temperatures. Some of the other differences in the appearances of the ECTD and SV Trap runs is due to slightly overloaded peaks in the splitless ECTD analysis, as compared to the SV Trap where a 5:1 split is typically used to assist in recovery of C18-C25 compounds. This is affecting the relative peak heights, but not the ratio of the peak areas. Due to the split, **Figure 12** is actually showing 20cc on-column for the SV Trap run. A larger sample size of 500cc or more can always be used to make up for this added split.

Depending on the complexity of the headspace, this technique can be repeated using a confirmatory column. The mass spectra can be compared at the time where the odor was detected (after adjusting for the time difference between olfactory and MS detection), and the spectra can be background subtracted for those peaks not found in both runs. Using Dean Switching options that are available on the 7890/5975 GCMS, switching between both columns can be under method control.

Conclusion

A flexible approach has been presented for the analysis of aroma compounds in foods and beverages by GCMS. Several different matrix management techniques are available, and optimization of compound class recovery is possible using 3 different sample introduction techniques. Analysis of small volumes to obtain the elution times of significant aroma compounds, followed by the preconcentration of larger volumes to obtain MS spectra has been confirmed. Extended Cold Trap Dehydration allowed recovery of all Aroma Significant Compounds from fruit samples at room temperature, as utilization of the SVOC approach (GC mounted SV Trap) did not show an increase of heavier boiling compounds. Other studies have shown that heated sample analysis can benefit by the use of the SV Trap.

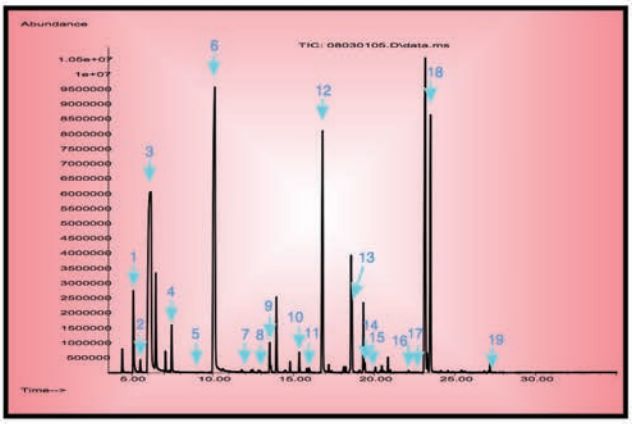

Figure 10 10cc Headspace, 50g strawberries, ECTD,

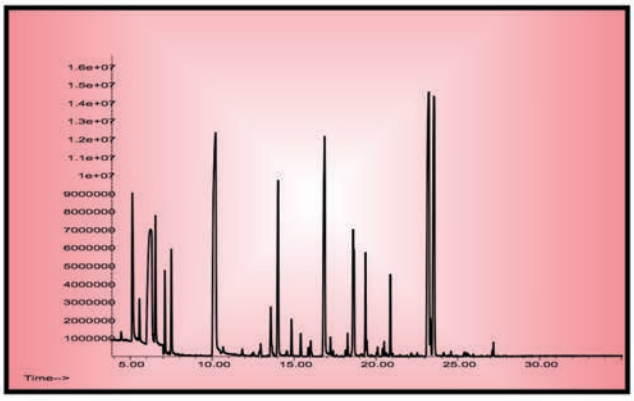

Figure 11 100cc Headspace, 50g strawberries, ECTD

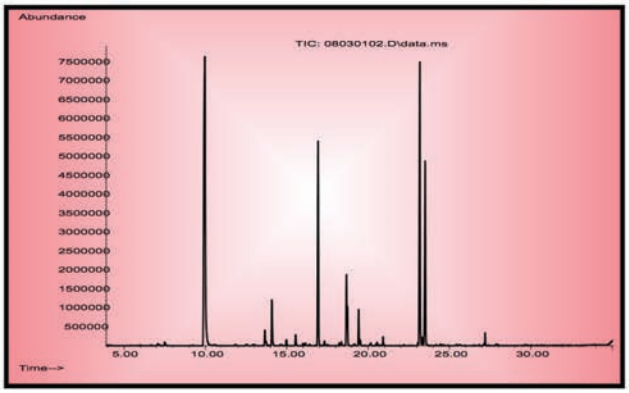

Figure 12 100cc Headspace, 50g strawberries, SV Trap, 5:1 split

Peak #	Retention Time (min)	Tentative ID	Aroma / Odor
1	5.2	Methanol	Alcohol
2	5.6	Methyl Mercaptan	Sulfur, pungent
3	6.1	Ethanol	Alcohol
4	7.4	Dimethyl Sulfide	Sulfur, sour
5	9.0	Diacetyl	Butterscotch
6	10.1	Ethyl Acetate	Sweet, moldy
7	12.0	2-Propenal	Faint butterscotch
8	13.0	Propanethiol-s-oxide	Sour, sulfur
9	13.6	Valeric Acid	Sweet, moldy
10	15.4	Caproic Acid Isomer	Butterscotch (Aldehyde)
11	16.0	Caproic Acid	Sour, putrid
12	16.9	Ethyl Butyrate	Sweet, sour
13	18.7	Isobutyl Propionate	Sweet, strawberry
14	19.5	$C_{10}H_{14}O_2$	Sweet, caramel
15	19.9	Heptanoic Acid	Sour
16	22.2	Ethyl Caproate	Sweet
17	22.7	$C_6H_{10}O_4$	Sour, mild sulfur
18	23.4	$C_8H_{14}O_2$	Sweet, strawberry
19	27.3	Isoamyl Isovalerate	Sweet, sour

Figure 13 100CC Strawberry Headspace, ECTD, Tentative IDs based on Elution times of aromas found during the 10cc volume analysis

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