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Aroma Analysis of Cooked Ground Beef and Plant-Based Meat using Automated and Optimized Dynamic Headspace Extraction with Dry Purge Function

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Keywords

Aroma Analysis, Ground Beef, Plant-Based Meat, Dynamic Headspace, Thermal Desorption, PTV Inlet, Cooled Injection System, Gas Chromatography-Mass Spectrometry

Abstract

Aroma analysis is of paramount importance in the plant-based meat industry. The ability to replicate flavors and mimic real meat is vital in appealing to consumers. To capture the aroma profiles of cooked meat, analysts may often resort to equilibrium-based headspace extraction approaches such as headspace-solid phase microextraction (HS-SPME) instead of dynamic purge approaches like dynamic headspace (DHS) sampling, despite the potential of the latter for higher recovery and sensitivity. The concern about using DHS for cooked meat or other food with considerable water content arises from the risk of moisture build-up in the sorbent tube potentially disrupting the gas chromatography-mass spectrometry (GC-MS) analysis. This study aims to showcase how the GERSTEL LabWorks Platform with automated DHS option and its unique dry purge feature, can effectively address this issue using both cooked ground beef and plant-based meat as examples. Additionally, a workflow for optimizing DHS settings relevant to this study is presented. The aroma profiles of cooked ground beef and plant-based meat are analyzed and compared.

Introduction

Food aroma analysis involves the scientific exploration of volatile compounds to understand the complex interactions that create the characteristic aromas and flavors of different food products. This knowledge is crucial for enhancing food quality, developing new products, and providing consumers with an enjoyable culinary experience. Hence, extraction techniques such as static headspace (SHS) sampling, headspace-solid phase microextraction (HS-SPME), dynamic headspace (DHS) sampling, and purge and trap (P&T) are commonly used in conjunction with gas chromatography-mass spectrometry (GC-MS) to study food aroma profiles [1,2].

The growing plant-based meat market exemplifies the importance of aroma analysis. Studies have shown that flavor and texture are key for consumer acceptance [3]. Obtaining the aroma profiles of cooked meat is therefore critical to product developers as it helps them replicate authentic meaty flavors to create convincing meat alternatives.

DHS is a powerful technique that uses a non-equilibrium approach to detect analytes at ultra-trace levels as opposed to equilibrium-based approaches, for instance SHS or HS-SPME. DHS involves using a controlled inert gas flow to continuously purge the

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headspace of a sample for a specific period of time while trapping the purged analytes on an appropriate sorbent (e.g., Tenax TA). The DHS technique involves purging of the sample headspace, while the similar purge and trap (P&T) technique is based on purging the liquid phase with a gas stream. Both DHS and P&T lead to moisture build-up in the sorbent tube/trap when extracting samples that contain water [1]. If the moisture from the trap is introduced to the GC-MS system, undesirable effects, such as interference with the chromatographic analysis and damage to the MS detector, can arise [4]. Meat has a natural water content of about 75% [5]. Only a fraction of that water, up to 10%, is lost during cooking, leaving a substantial amount still in the meat [6]. Therefore, among the extraction techniques mentioned earlier, DHS and P&T are perhaps the least popular options for analyzing cooked meat or any other food with significant water content.

Figure 1: GERSTEL Dynamic Headspace – DHS option.

The GERSTEL DHS option (Figure 1) allows users to customize the parameters for incubation (time, temperature, and agitation) and trapping (gas volume, flow, and temperature), thereby enabling a fully automated extraction process for all samples. A pre-purging option is available if needed. In addition to basic sampling, the GERSTEL automated DHS option can carry out sophisticated techniques such as Full Evaporation Dynamic Headspace (FED-HS) [7] and Multi-volatile method (MVM) [8]. These methods are specifically designed for uniform enrichment and higher sensitivity of small-volume aqueous samples. For larger sample sizes where complete vaporization is not feasible, a selectable dry purge function (Figure 2), unique to the GERSTEL automated DHS option, can be utilized to resolve the moisture issue in the sorbent tube after the trapping phase and prior to GC-MS analysis. The MAESTRO software also includes an in-built Water Vapor Calculator which helps users to find optimal settings for the drying phase.

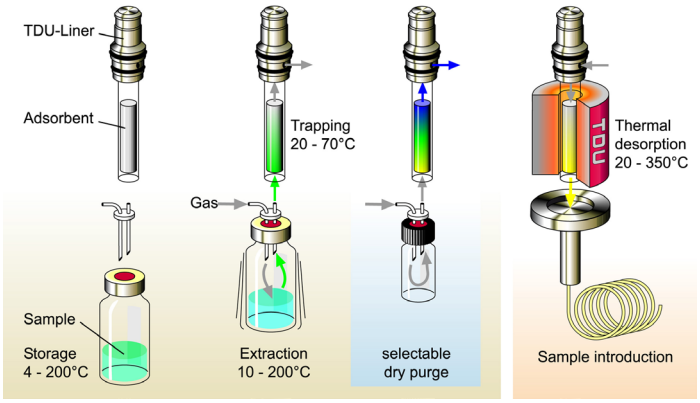

Figure 2: Schematic diagram of automated DHS sampling process.

This preliminary study demonstrates how the GERSTEL automated DHS option, and its dry purge function can be used to extract aroma compounds from cooked ground beef and plant-based meat, both of which have high water content. The water content in cooked ground beef is about 55 – 60% [5], while that of plant-based meat varies from 50 to 80% [9]. This application note also describes a workflow for optimizing the DHS settings for cooked ground beef. The optimal settings are then applied to cooked plant-based meat, and their aroma profiles are compared.

Experimental

Sample Preparation

Raw ground beef and two brands of plant-based meat (PBM), X and Y, were purchased from a local supermarket.

2 g samples of each food type were weighed into separate 10-mL vials with screw neck. The vials were placed in a pre-heated oven to cook at 160 °C for 3 minutes. The cooked samples were then left to rest at room temperature for 5 minutes before loading onto the sample tray for subsequent automated DHS-TD-GC-MS analysis.

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Settings for DHS optimization

The DHS parameters were modified in the window as shown in Figure 3.

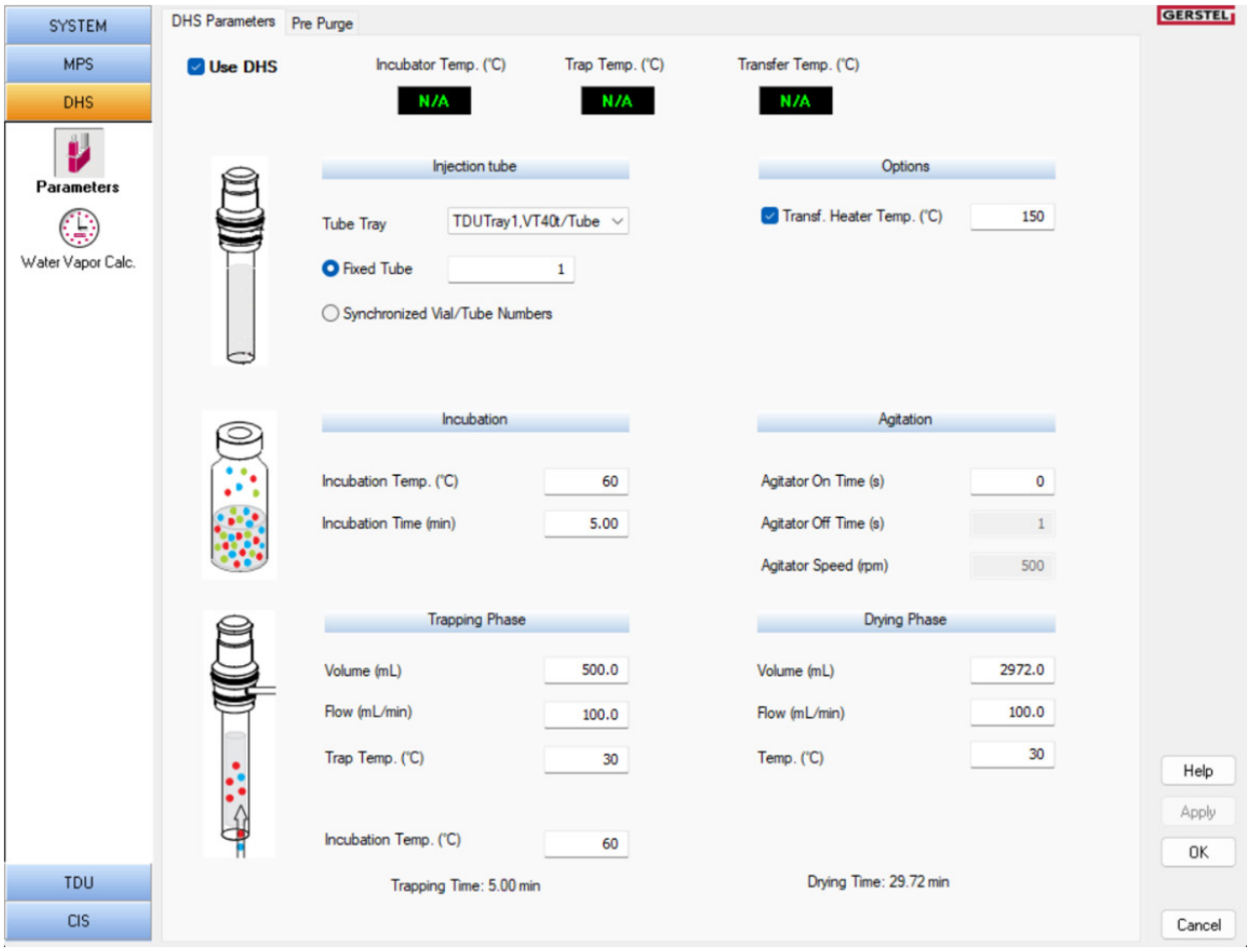

Figure 3: DHS method parameter window. Optimized settings as shown.

The cooked sample was incubated for 5 minutes at 60 °C, which is the recommended serving temperature for cooked meat [10]. This mimics the conditions for aroma perception experienced by a diner when the food is served. The compounds in the headspace were then transferred to and collected on a Tenax TA sorbent trap. Tenax TA was selected as it has high affinity for volatile and semi-volatile compounds but low affinity for water [11]. After the trapping phase, the sorbent tube was dried in the DHS at 30 °C using 2972 mL of nitrogen gas at 100 mL/min, which is the highest recommended flow rate for Tenax TA according to specifications.

The drying phase gas volume was determined using the Water Vapor Calculator, as illustrated in Figure 4. A detailed explanation on the use of the Water Vapor Calculator can be found in the DHS Method Reference, a supplementary document by the authors of this application note. Essentially, the required drying phase gas volume is influenced by three factors – sample incubation temperature, trapping phase gas volume for sampling, and drying phase temperature. Based on primary findings (data not shown), a safety margin correction factor of 0.7 was applied to ensure complete drying of the tube and an acceptable analysis throughput.

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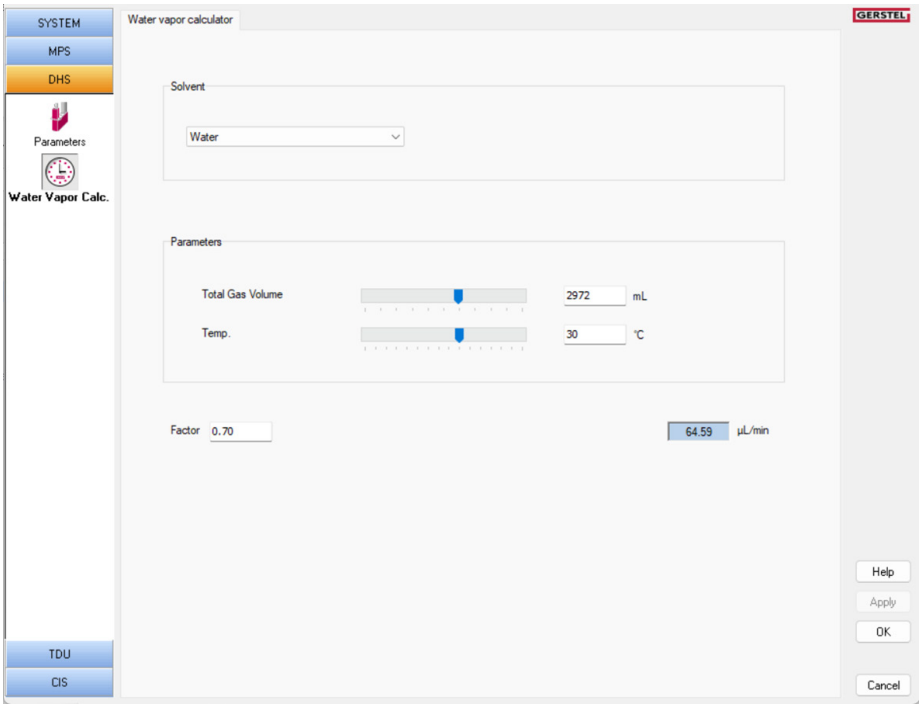

Figure 4: Use of the Water Vapor Calculator to find the gas volume required for the drying phase.

Instrumentation

GERSTEL LabWorks Platform with Dynamic Headspace (DHS) option and Cryostatic Cooling Device (CCD 2) on Agilent 7890 GC/5977B MSD.

Analysis Conditions LabWorks Platform

DHS

Sorbent Trap	Tenax TA (pre-conditioned)
Incubation	60 °C (5 min)
Transfer Heater	150 °C
Trap Volume	500 mL
Trap Flow	100 mL/min
Trap Temp	30 °C
Dry Volume	2972 mL
Dry Flow	100 mL/min
Dry Temp	30 °C

TDU

Desorption Mode	Splitless
Desorption Flow	50 mL/min
Temp	30 °C (0.01 min); 60 °C/min to 230 °C (2 min)
Transfer Temp	240 °C

CIS

Liner	Tenax TA (pre-conditioned)
Pneumatics Mode	Solvent-venting
Split Ratio	1:20
Temp	20 °C (0.01 min), 10 °C/s to 230 °C (5 min)

Analysis Conditions GC Agilent 7890

Column	30 m DB-WAX (Agilent), d_i = 0.25 mm, d_f = 0.25 µm
Pneumatics	He; P_i = 109.38 kPa Constant flow; 1.25 mL/min
Oven	50 °C (7 min), 3 °C/min to 180 °C (0 min), 10 °C/min to 230 min (5 min)

Analysis Conditions MSD Agilent 5977B

Scan	29 to 350 amu

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Results and Discussion

With reference to previous studies on cooked beef [4,12], a total of 24 odor-active compounds were identified in the cooked ground beef samples used in this experiment (Table 1). Five key aroma compounds that are characteristic of cooked beef [1,4], namely 2-methylbutanal, hexanal, nonanal, 1-octen-3-ol and methional, were selected to illustrate the trends at each optimization step.

Table 1: Analyte information.

No.	Analyte	B.P. [a] [°C]	Ret. Time [min]	Frag. Ions [b] [m/z]
1	Ethyl Acetate	77.1	6.34	43, 61, 70
2	2-Butanone	80.0	6.63	43, 72, 57
3	2-Methylbutanal	90.0 – 93.0	7.02	44, 58, 71
4	2,3-Butanedione	88.0	8.59	43, 86, 42
5	Pentanal	103.0	8.78	58, 57, 44
6	Toluene	111.0	11.03	91, 92, 65
7	2,3-Pentanedione	109.9	11.77	57, 100, 43
8	Hexanal	131.0	12.88	56, 57, 44
9	Heptanal	153.0	17.78	70, 96, 81
10	2-Pentylfuran	169.7	19.99	81, 82, 138
11	1-Pentanol	138.0	21.10	55, 42, 70
12	Styrene	146.0	21.18	104, 103, 78
13	Acetoin	148.0	22.64	45, 43, 88
14	Octanal	171.0	22.84	84, 82, 81
15	1-Hexanol	157.0	25.97	56, 43, 55
16	Nonanal	191.0	27.66	57, 56, 98
17	1-Octen-3-ol	175.0	30.18	57, 43, 72
18	Methional	165.5	30.40	104, 48, 76
19	1-Heptanol	176.0	30.54	70, 56, 55
20	2-Ethyl-1-Hexanol	186.0	31.98	57, 83, 70
21	Benzaldehyde	178.7	33.27	106, 105, 77
22	1-Octanol	196.0	34.83	56, 55, 84
23	Phenylacetaldehyde	195.0	38.08	91, 92, 120
24	2-Acetylthiazole	212.5	38.27	127, 99, 112

[a] at 760 mmHg. Data sources: PubChem (https://pubchem.ncbi.nlm.nih.gov) and ChemSpider (https://www.chemspider.com)
[b] The underlined fragment ion was used as quantifier

DHS Optimization

The sequence for optimization was determined based on tests done prior to this work.

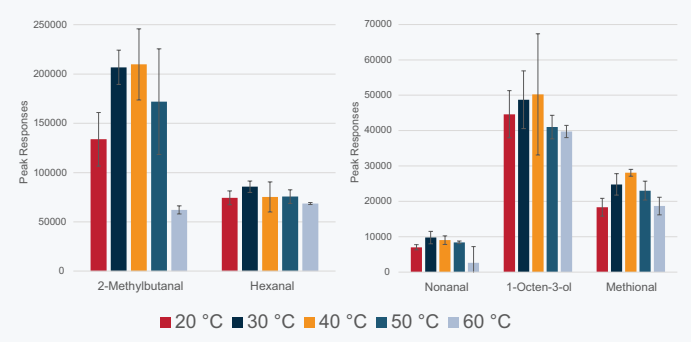

Figure 5: Comparison of peak responses of selected analytes among different trap temperatures in DHS. Conditions: Incubation, 60 °C, 5 min; Trapping Phase, 300 mL, 10 mL/min; Drying Phase, 3067 mL, 100 mL/min, 20 °C. Error bars show the standard deviations (n=3).

The impact of varying trap temperatures (20 – 60 °C) on the adsorption efficiency of analytes on Tenax TA during the trapping phase was first assessed. It was postulated that increasing the trap temperature would reduce the retention of compounds, particularly of those with higher volatility. It was initially observed in Figure 5 that compounds were retained with higher recovery on Tenax TA as the trap temperature was increased from 20 °C to either 30 °C or 40 °C. In preliminary experiments, condensation was observed in the sorbent region of the tube when the trap temperature was set below the incubation temperature. This could negatively impact analyte adsorption efficiency. With increasing trap temperature, the amount of condensation was reduced, in turn improving recovery of the aroma compounds on the sorbent. However, a further increase in trap temperature reduced the analyte breakthrough volumes leading to a decline in peak responses. This was notably significant for the more volatile compounds, for instance 2-methylbutanal. Most detected aroma compounds displayed a similar trend, with either 30 °C or 40 °C yielding higher responses. A trap temperature of 30 °C was consequently identified as the optimal setting due to the better overall repeat ability achieved.

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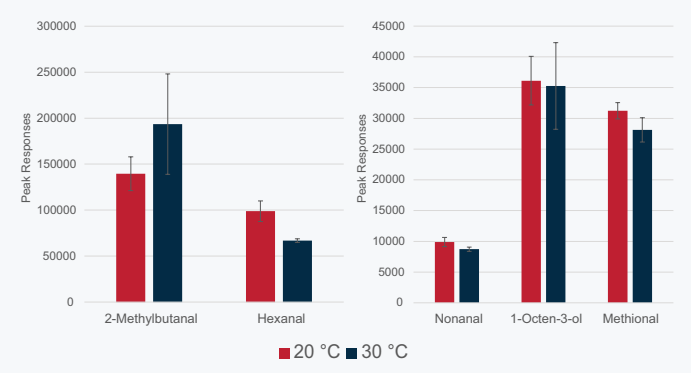

Figure 6: Comparison of peak responses of selected analytes among different drying phase temperatures in DHS. Conditions: Incubation, 60 °C, 5 min; Trapping Phase, 300 mL, 10 mL/min, 30 °C; Drying Phase, 100 mL/min. Error bars show the standard deviations (n=3).

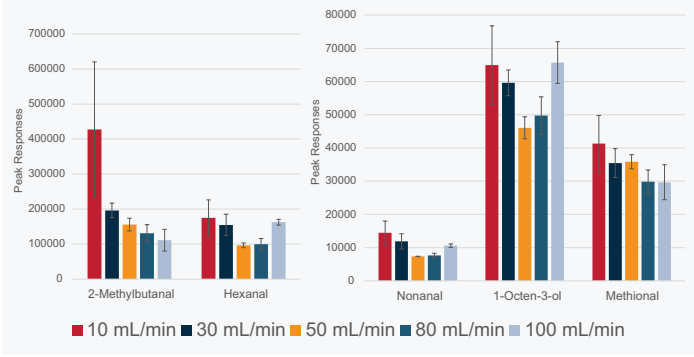

Figure 7: Comparison of peak responses of selected analytes among different trapping phase gas flow rates in DHS. Conditions: Incubation, 60 °C, 5 min; Trapping Phase 300 mL, 30 °C; Drying Phase, 1783 mL, 100 mL/min, 30 °C. Error bars show the standard deviations (n=3).

Different drying phase temperatures were next applied to determine the effect on compound retention on the Tenax TA sorbent. It was found in initial studies that a drying phase temperature of either 20 °C or 30 °C is sufficient to effectively dry the sorbent tube, allowing for subsequent GC-MS analysis without significant moisture effects. In this work, a drying phase temperature of 20 °C required a drying phase gas volume of 3067 mL, corresponding to a duration of 30.67 minutes. A drying phase temperature of 30 °C reduced the gas volume required to 1783 mL, which would take 17.83 minutes. An increase in the drying phase temperature was expected to lead to a greater amount of volatile aroma compounds breaking through the sorbent bed. However, only a slight decline in peak responses was observed at a higher drying phase temperature (Figure 6) for the chosen representative compounds, excluding 2-methylbutanal. In fact, compounds of higher volatility, like 2-methylbutanal, showed weaker responses when subjected to a lower drying phase temperature. The loss could be attributed to the larger drying phase gas volume causing the more volatile compounds to break through more readily. Generally, more than half of the aroma compounds detected in the cooked ground beef samples showed improved, though not significantly higher, peak responses when drying at 30 °C compared with drying at 20 °C. The required duration for the drying phase was also notably reduced by at least 40%, enabling more efficient sample analysis. Therefore, 30 °C was established to be the optimal value for the drying phase.

Thirdly, the effect of the trapping phase gas flow rate on aroma compound recovery on the Tenax TA was investigated. It was presumed that a higher gas flow rate would transport the analytes from the headspace to the Tenax TA more effectively. However, it was observed that better peak responses were achieved at lower trapping phase gas flow rates, albeit with poorer repeatability (Figure 7). This could be due to the fact that the cooked ground beef samples remained incubated at an elevated temperature of 60 °C throughout the extended trapping phase duration, leading to continuous release of aroma compounds from the sample and thus obtaining higher recovery. Nonetheless, cooked food is not typically kept at the same temperature when served or consumed. Hence, to capture a more accurate representation of the aroma profile of cooked ground beef as experienced by a diner, a high trapping phase gas flow rate of 100 mL/min with the shorter sampling period was favored.

Given the minimal changes in peak responses observed overall in this study, it was inferred that the gas flow rate during the trapping phase had only a minor impact on the analyte adsorption efficiency.

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Finally, a range of trapping phase gas volumes from 300 mL to 800 mL were examined to determine the impact on the amount of aroma compounds transferred to and retained on the sorbent tube. As previously discussed, the trapping phase gas volume directly affects the drying phase gas volume. The required drying phase gas volumes were calculated with the Water Vapor Calculator corresponding to the trapping phase volumes used. The results and the respective durations of each phase are outlined in Table 2.

Table 2: Different trapping phase gas volumes, their corresponding drying phase gas volumes, and the associated durations for both trapping and drying. Conditions: Incubation temp, 60 °C; Drying phase temp, 30 °C.

	Trapping Phase			Drying Phase		
	Volume [mL]	Flow [mL/min]	Time [min]	Volume [mL]	Flow [mL/min]	Time [min]
1	300	100	3.00	1783	100	17.83
2	400	100	4.00	2378	100	23.78
3	500	100	5.00	2972	100	29.72
4	600	100	6.00	3566	100	35.66
5	700	100	7.00	4161	100	41.61
6	800	100	8.00	4755	100	47.55

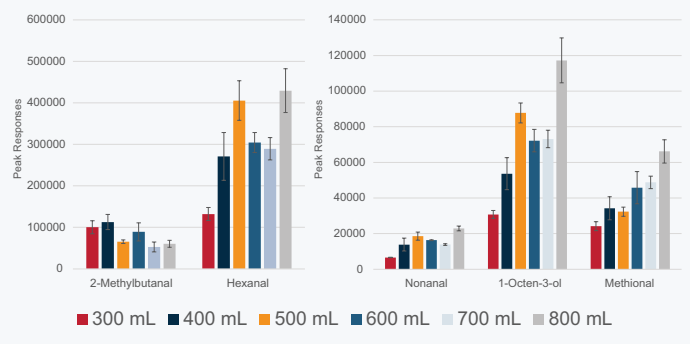

Figure 8: Comparison of peak responses of selected analytes at different trapping phase gas volumes in DHS. Conditions: Incubation, 60 °C, 5 min; Trapping Phase, 100 mL/min, 30 °C; Drying Phase, 100 mL/min, 30 °C. Error bars show the standard deviations (n=3).

Generally, for most of the aroma compounds (as represented by hexanal, nonanal and 1-octen-3-ol in Figure 8), a rise in peak responses was seen when increasing the trapping phase gas volume to 500 mL. This outcome was anticipated since the larger volume should transport more analytes from the sample headspace to the sorbent. However, when increasing the volume further from 500 mL to 800 mL, increased variability in peak responses was observed across the range of analytes. This suggested another influencing factor. As the trapping phase gas volume during DHS sampling was increased, the drying phase gas volume required increased as well. The larger volume of inert gas passing through the Tenax TA sorbent bed during the drying phase would result in analyte breakthrough and loss, explaining the observed fluctuations in peak responses at higher trapping and drying phase gas volumes.

The majority of aroma compounds detected in the cooked ground beef samples showed only small differences in peak responses between trapping phase gas volumes of 500 mL and 800 mL. Furthermore, the repeatability for all compounds generally improved when the volume was 500 mL or larger and 500 mL was chosen as the optimal setting. This volume not only ensured good repeatability but also shortened the DHS extraction process, resulting in higher analysis throughput.

Optimized DHS application to PBM and Comparison of Aroma Profiles

Two brands of PBM, Brand X and Y, were analyzed in this work. They underwent the same cooking procedure and were analyzed with the optimized DHS settings.

The aroma compounds detected were compiled and marked to indicate their presence in cooked ground beef and both brands of PBM in the list shown (Table 3). Analytes found in these PBM samples aligned with the findings from other studies on simulated beef flavor [13] and plant-based substitutes [14,15]. A stacked view of the total ion chromatograms (TICs) obtained for each cooked sample is presented in Figure 9. Some peaks, like 1-pentanol and styrene, were observed to overlap in the TICs of cooked ground beef and PBM X. To identify and integrate all analyte peaks, deconvolution of the mass spectra was carried out, followed by the use of extracted ion chromatograms. If further separation of the peaks is required, the offline GC-heartcutting technique can be performed with the aid of the GERSTEL Olfactory Detection Port (ODP 4). As demonstrated in Application Note 223 [16], co-eluting compounds can be trapped on a sorbent tube, then desorbed and analyzed on a separate TD-GC-MS system using a column of different polarity.

Within-day repeatability was determined with three replicates to evaluate the precision of the optimized methodology. For the majority of the compounds detected in each type of cooked sam-

ple, relative standard deviations (RSDs) of less than 20% were achieved. For the aroma compounds in cooked ground beef, the average RSD was 13.1%. An average RSD of 17.3% was observed across all 31 compounds in PBM X; for the 26 identified analytes in PBM Y, an average RSD of 14.5% was achieved. In summary, the repeatability of the automated DHS extraction procedure with the additional dry purging step proved to be quite satisfactory.

Table 3: Aroma compounds detected in cooked ground beef and plant-based meat, and their odor descriptions.

No.	Analyte	Odor Descriptions [a]	Ground Beef	PBM X	PBM Y
1	Ethyl Acetate	Caramel, sweet	X	X	
2	2-Butanone	Chemical, burnt, choco-late	X		X
3	2-Methylbutanal	Pungent, sweet, roasty	X	X	X
4	2-Ethylfuran	Acid, sour, whey butter-like		X	
5	2,3-Butanedione	Sweet, buttery	X		
6	Pentanal	Almond, malt, pungent	X	X	X
7	Toluene	Sweet, pungent	X	X	X
8	2,3-Pentane dione	Buttery, fruity, lemon-like	X		
9	Hexanal	Green, fatty, fresh	X	X	X
10	Diallyl Sulfide	Sulfurous, onion, garlic			X
11	2-Heptanone	Citrus, grape-fruit		X	X
12	Heptanal	Fruity, fatty, rancid	X	X	X
13	D-Limonene	Citrus, orange		X	
14	2-Pentylfuran	Green bean, butter	X	X	X
15	1-Pentanol	Fuel oil, bal-samic	X	X	X
16	Styrene	Penetrating odor, sweet	X	X	
17	Methylpyrazine	Nutty, roasted		X	X
18	Acetoin	Buttery, creamy, sweet	X	X	X

Table 3 (cont.): Aroma compounds detected in cooked ground beef and plant-based meat, and their odor descriptions.

No.	Analyte	Odor Descriptions [a]	Ground Beef	PBM X	PBM Y
19	Octanal	Citrus, fatty, soapy	X	X	
20	2,5-Dimethyl pyrazine	Fried rice, pop-corn, pungent, green		X	X
21	1-Hexanol	Woody, cut grass, chemi-cal-winey	X	X	X
22	2-Nonanone	Hot milk, soap, green		X	
23	Nonanal	Sweet, floral, waxy	X	X	X
24	2-Ethyl-5-methylpyrazine	Fruity, sweet, pungent			X
25	Trimethyl pyrazine	Nutty, coffee		X	X
26	E-2-Octenal	Aldehyde, green, floral		X	X
27	1-Octen-3-ol	Mushroom	X	X	X
28	Methional	Cooked potato, meat broth	X		
29	1-Heptanol	Woody, winey, herb	X		
30	Furfural	Bready, sweet		X	X
31	Diallyl Disulfide	Onion, garlic, metallic			X
32	2-Ethenyl-6-methyl pyrazine	Cooked rice, coffee-like, smoky		X	
33	2-Ethyl-1-hexanol	Resin, flower, green	X		X
34	E,E,2,4-Hepta-dienal	Aldehyde, green, spicy,		X	
35	Benzaldehyde	Almond	X	X	X
36	1-Octanol	Fatty, citrus, walnut	X		
37	Phenylacet aldehyde	Sweet, fruity, honey	X		X
38	2-Acetylthiazole	Roasted	X	X	X
39	2-Furan methanol	Bready, sweet		X	X

Table 3 (cont.): Aroma compounds detected in cooked ground beef and plant-based meat, and their odor descriptions.

No.	Analyte	Odor Descriptions [a]	Ground Beef	PBM X	PBM Y
40	E,E,2,4-Deca dienal	Sweet, rubbery, plastic		X	
41	Maltol	Sweet, caramel, cotton candy		X	
42	Furaneol	Roasted al-monds, sweet		X	

[a] Adapted from literatures [1,4,12,13], and The Good Scents Company Information System (the-goodscentscompany.com)

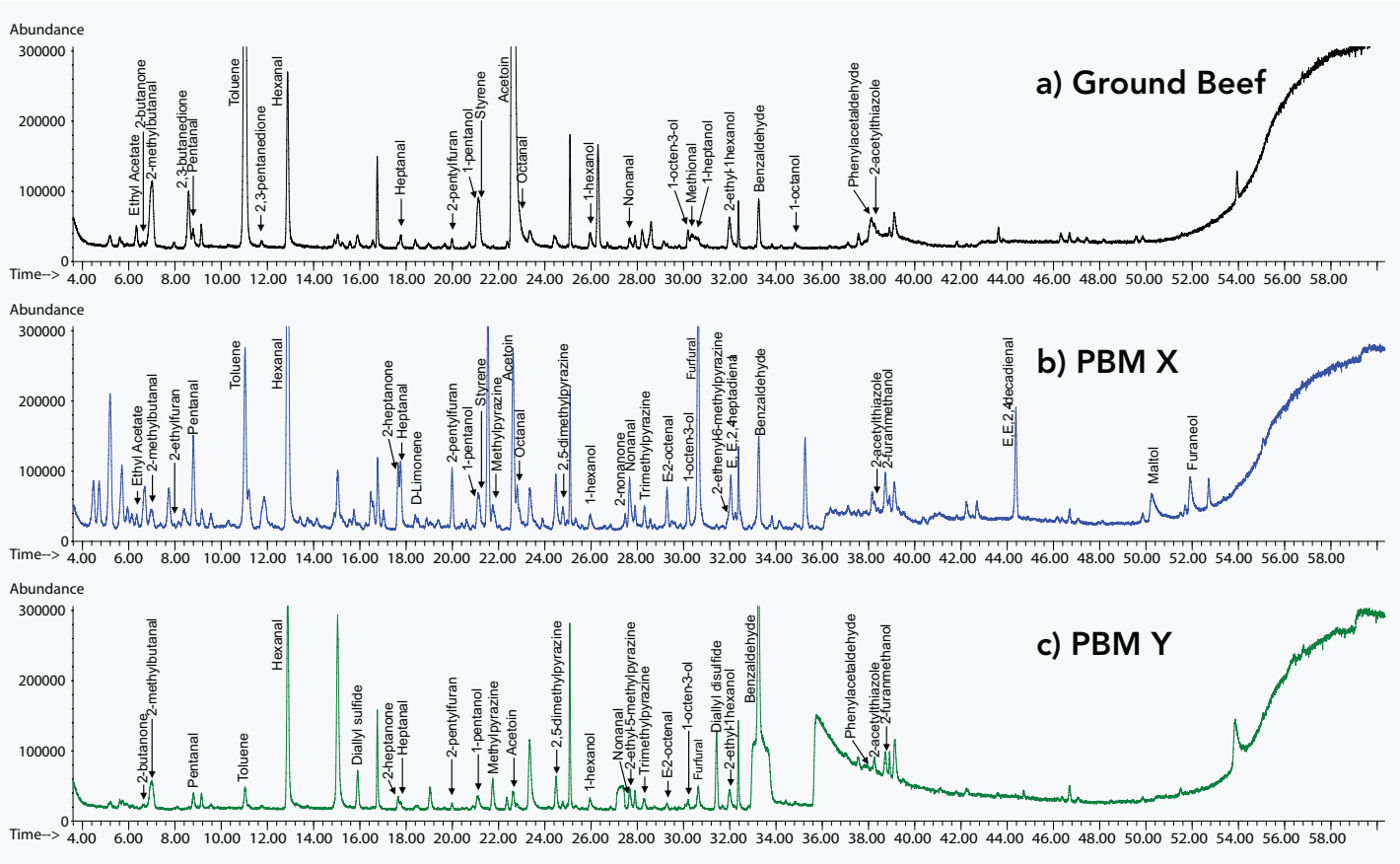

Figure 9: Stacked view of TICs for each type of cooked sample. a) Ground Beef, b) PBM X, and c) PBM Y. The TICs only display labelled analyte peaks that have been reported in other studies on cooked beef and its plant-based substitutes [4,12,13,14,15].

Conclusions

This work is a preliminary investigation that examines the feasibility of using DHS for GC-MS analysis of samples with a high water content. The GERSTEL LabWorks Platform with DHS option, with its unique dry purge feature, was demonstrated to be a highly effective tool for analyzing foods with substantial water content, like cooked ground beef and plant-based meat alternatives. With the appropriate settings, the dry purge function aids in removing moisture from the sorbent trap, preventing potential complications from impacting results and GC-MS system stability. In addition to food aroma analysis, the insights gained from this study can enable the use of the GERSTEL DHS option and its dry purge function for other applications, such as the determination of fragrances and/or off-odors in consumer products and cosmetics, as well as for environmental samples.

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