Reverse engineering the aroma of plant-based meat substitutes

This study describes the use of headspace sorptive extraction with GC×GC–TOF MS to compare the volatile profiles of ground meat and plant-based substitutes.

Introduction

Plant-based meat substitute products have increased in popularity in recent years, and a goal for food producers is to create a product that mimics the sensory experience of cooked meats.

However, ground meat may release hundreds of volatile organic compounds (VOCs) during cooking, many of which are odour-active and cover a wide range of chemical classes; therefore, capturing a comprehensive VOC profile to reverse engineer the aroma is a challenging task.

Firstly, high sensitivity is required to ensure that trace compounds are detected because even a trace amount of a compound with a low odour threshold can have a large impact on the overall aroma. Here, HiSorb™ high-capacity sorptive extraction probes were used to provide a larger volume of polydimethylsiloxane (PDMS) stationary phase (65 μL) compared to traditional solid-phase microextraction (SPME) (~0.5 μL) for higher sample loadings. When used in combination with the Centri® sample extraction and enrichment platform, HiSorb offers fully automated sorptive extraction with trap-based focusing for optimal sensitivity and improved chromatographic performance.

Secondly, the diverse range of chemical classes requires advanced separations to resolve co-elutions and provide confident identification of the analytes present. Comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC–TOF MS) can tackle this challenge by coupling two columns of different selectivity to separate the analytes based on two different chemical properties (e.g., volatility and polarity).

Finally, sophisticated data analysis software is required to quickly identify subtle differences between aroma profiles. In this case, an automated untargeted comparison was performed using the ChromCompare+ chemometrics platform to reduce the risk of missing important differences and minimise manual processing steps.

In this study, we demonstrate the performance of the workflow described to compare the aroma profiles of ground meat and plant-based substitutes from different manufacturers.

Experimental

Samples: Three ground meat products and three plant-based substitutes were obtained from different food manufacturers. Headspace sorptive extraction was performed on 4 g of each product in triplicate.

Extraction: Instrument: Centri® (Markes International); Sampler: Inert-coated stainless steel HiSorb™ PDMS probe (Markes International). Temperature: 80°C; Sampling time: 60 min; Agitation: 300 rpm. HiSorb probes were desorbed at 250°C (for 15 min).

GC×GC: Modulator: INSIGHT® flow modulator (SepSolve Analytical); Modulation period (P_M): 4 s.

MS: BenchTOF-Select™ time-of-flight MS; Ionisation energy: 70 eV; Mass range: m/z 35–450.

Software: Full instrument control by ChromSpace®, with chemometric data analysis in ChromCompare+ (SepSolve Analytical).

Please contact SepSolve for full analytical parameters.

Results and discussion

Comprehensive aroma profiles for the six products were obtained using headspace sorptive extraction coupled with GC×GC–TOF MS (Figure 1). The complexity of the VOC profiles is apparent, with hundreds of peaks visible in the TIC surface charts, spanning a wide range in volatility, from volatiles such as acetaldehyde and carbon disulfide to semi-volatiles such as 2-undecanone and butylated hydroxytoluene.
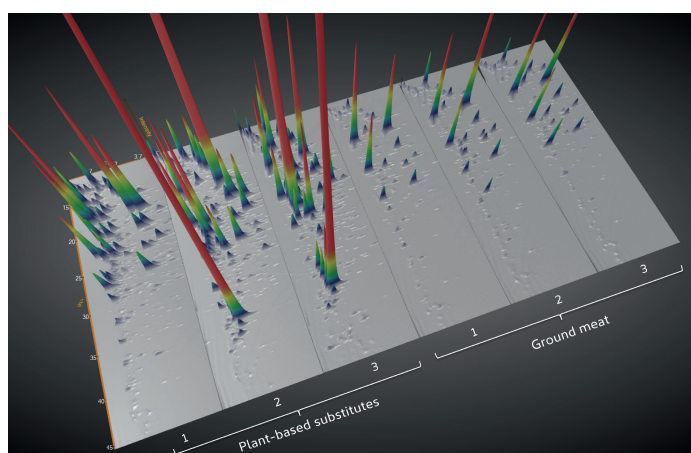

Figure 1

GC×GC–TOF MS surface charts of the ground beef and plant-based substitutes included in this study.

Figure 2 clearly demonstrates the increased peak capacity provided by GC×GC–TOF MS to separate and identify the wide-ranging chemical classes present in the aroma profile of a plant-based substitute. Additionally, GC×GC produces structured chromatograms where, due to the complementary nature of the 1D and 2D stationary phases, compound classes tend to elute in bands, aiding the identification of unknowns. In this case, an increase in second dimension retention time is correlated with an increase in compound polarity.
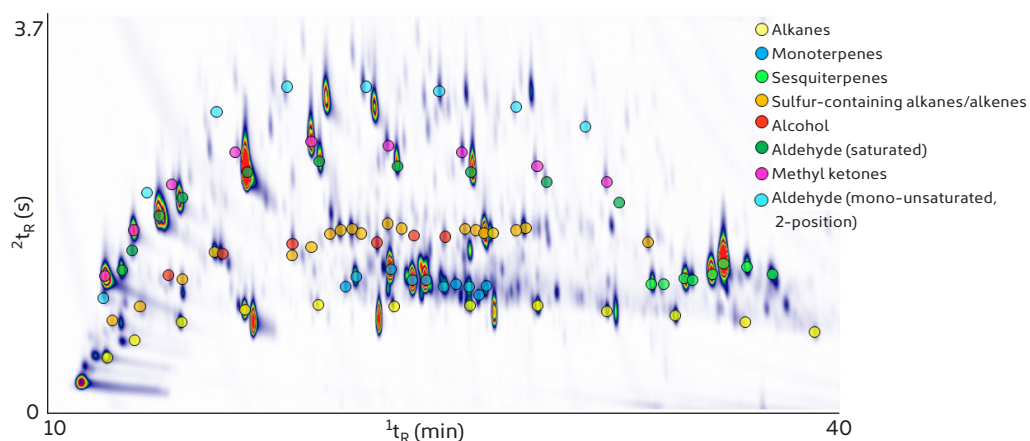

- Alkanes
- Monoterpenes
- Sesquiterpenes
- Sulfur-containing alkanes/alkenes
- Alcohol
- Aldehyde (saturated)
- Methyl ketones
- Aldehyde (mono-unsaturated, 2-position)

Figure 2

GC×GC–TOF MS colour plot showing the enhanced separation of chemical classes in the aroma profile for plant-based substitute #3.

An enhanced region of the chromatogram is shown in Figure 3, where numerous analytes that would have co-eluted in a 1D GC separation have been fully resolved, providing clean spectra for confident identification against commercial libraries such as NIST. Many of the identified compounds are odour-active, meaning that GC×GC–TOF MS provides greater detail on the aroma profile – a necessity when performing reverse engineering.
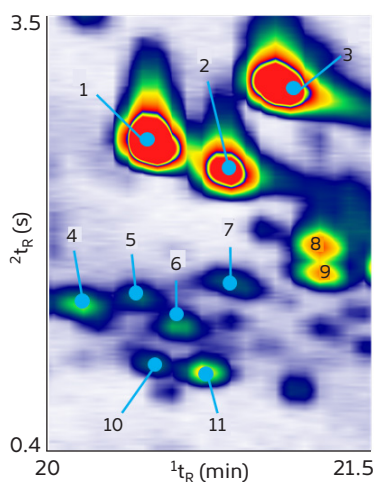
Label	Analyte name	MF	Aroma descriptor[1]
1	2-Heptanone	886	Fruity, spicy, sweet, herbal, woody
2	Heptanal	880	Green
3	Methional	908	Vegetable
4	p-Xylene	853	N/A
5	3,4-Dimethyl thiophene	880	Savory roasted onion
6	2-n-Butyl furan	840	Fruity, winey, sweet, spicy
7	Styrene	936	N/A
8	2,5-Dimethyl pyrazine	925	Nutty, peanut, earthy, powdery, roasted cocoa
9	2,4-Dimethyl thiophene	880	N/A
10	1-Nonene	905	N/A
11	Nonane	897	N/A

Figure 3

GC×GC–TOF MS colour plot (left) showing separation of analytes that would have co-eluted in an equivalent 1D analysis and the corresponding identifications (right) from screening BenchTOF spectra against the NIST 20 library.

However, the remaining challenge was to distill these complex chromatograms into meaningful results, which had previously meant hours of analysts' time spent organising and manually reviewing peak tables. With hundreds of peaks in each chromatogram, the important differentiating odorants are not obvious and comparing large sample batches can be difficult and time-consuming. To tackle this challenge, we used ChromCompare+, a novel chemometrics platform for untargeted data analysis.

ChromCompare+ automatically aligns and compares multiple chromatograms to discover significant differences between samples classes. In this study, the raw data was chromatographically aligned and then imported directly into ChromCompare+ using an untargeted workflow. In this approach, the chromatograms are not integrated and all the raw data is utilised, so there is a reduced risk of missing important trace differences and the analyst need not have any prior knowledge of the sample composition to perform the processing.

In this case, the untargeted workflow yielded over 480,000 analytical features across the dataset. To reduce the dataset and eliminate redundant features, the Feature Discovery tool was used to automatically identify the top 100 differences between the sample classes. The resulting principal components analysis (PCA) score plot (Figure 4) shows clear separation of the ground meat and the plant-based substitute samples along the first principal component (PC1, 57.16% of variance).
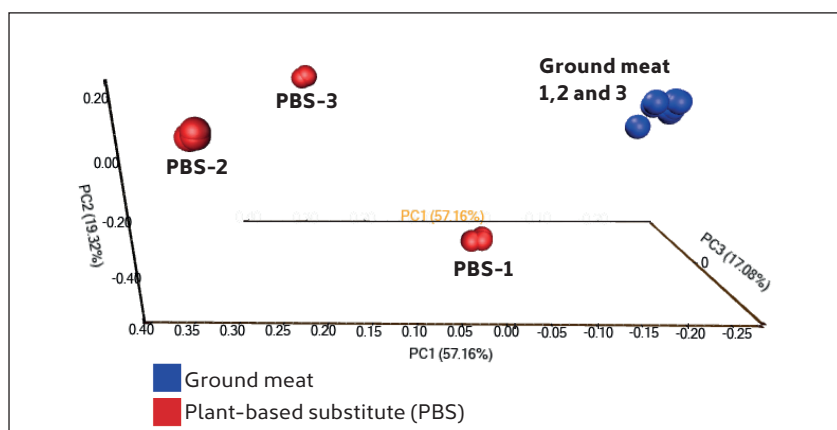

Ground meat
Plant-based substitute (PBS)

Figure 4

Principal components analysis (PCA) score plot in ChromCompare+, showing four distinct clusters for the ground meat samples (blue) and the three plant-based substitutes (red).

The three ground meat samples cluster tightly on the scores plot, indicating that all three products share similar VOC profiles. In contrast, the plant-based substitutes (PBS) possess unique VOC profiles and all three products form distinct clusters, with PBS-1 found to be most similar to the ground meat products. It is also worth noting that the triplicate samples are all in tight clusters, indicating high repeatability of the analytical method.

The Feature Discovery tool identified a number of compounds that were elevated in all of the plant-based substitutes, relative to the ground meat products. For example, methyl propyl disulfide and 3-ethyl-2,5-dimethyl pyrazine were elevated in the plant-based substitutes (Figure 5), both of which are odour-active, associated with alliaceous and nutty aromas, respectively.[1]
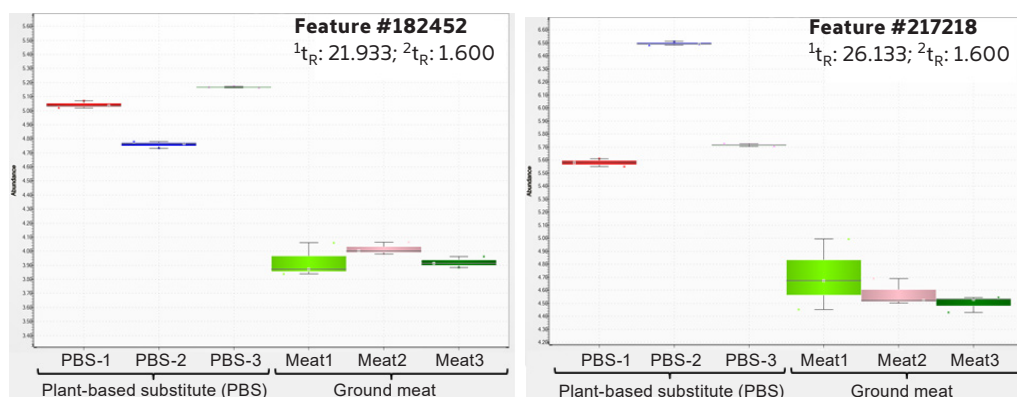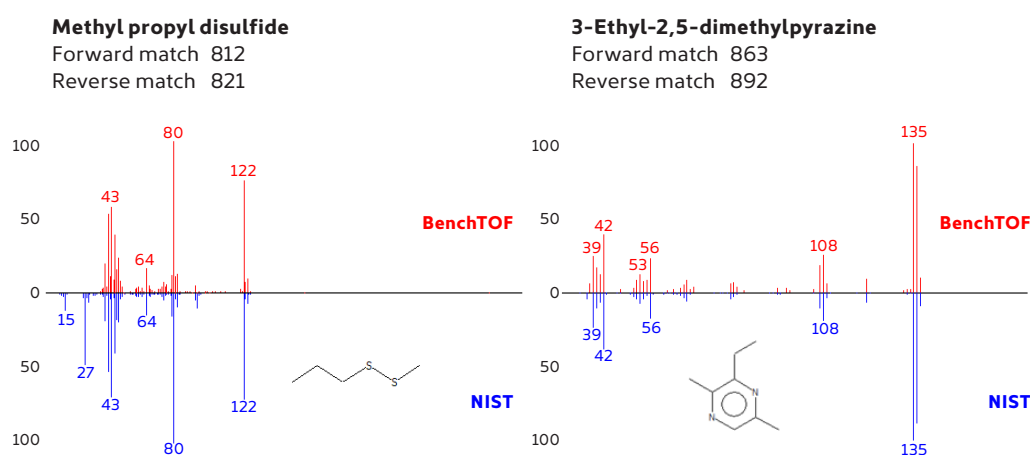
Feature #182452
^1t_R: 21.933; ^2t_R: 1.600

Feature #217218
^1t_R: 26.133; ^2t_R: 1.600

Methyl propyl disulfide
Forward match 812
Reverse match 821

3-Ethyl-2,5-dimethylpyrazine
Forward match 863
Reverse match 892

The PCA loadings were also investigated in ChromCompare+, revealing further key differentiators between the sample classes, as displayed in the box plots in Figure 6. The PBS-2 and PBS-3 samples were primarily distinguished from PBS-1 and the ground meat samples due to increased levels of sulfur species, such as methyl propyl trisulfide. Both of these plant-based substitutes list garlic powder among their ingredients, while PBS-1 does not, meaning it could be a potential source of these additional sulfur compounds.

On the other hand, sample PBS-1 was distinguished from the other samples by PC2 (19.32% of variance), primarily due to increased levels of oxygenated compounds, such as pulegone and dihydro-2-methyl-3(2H)-furanone, which give minty and bready aromas, respectively.[1]

Finally, the ground meat samples were shown to be depleted of terpenes, such as limonene and 3-carene, relative to the plant-based substitutes. This was not surprising, considering that terpenes are plant metabolites.
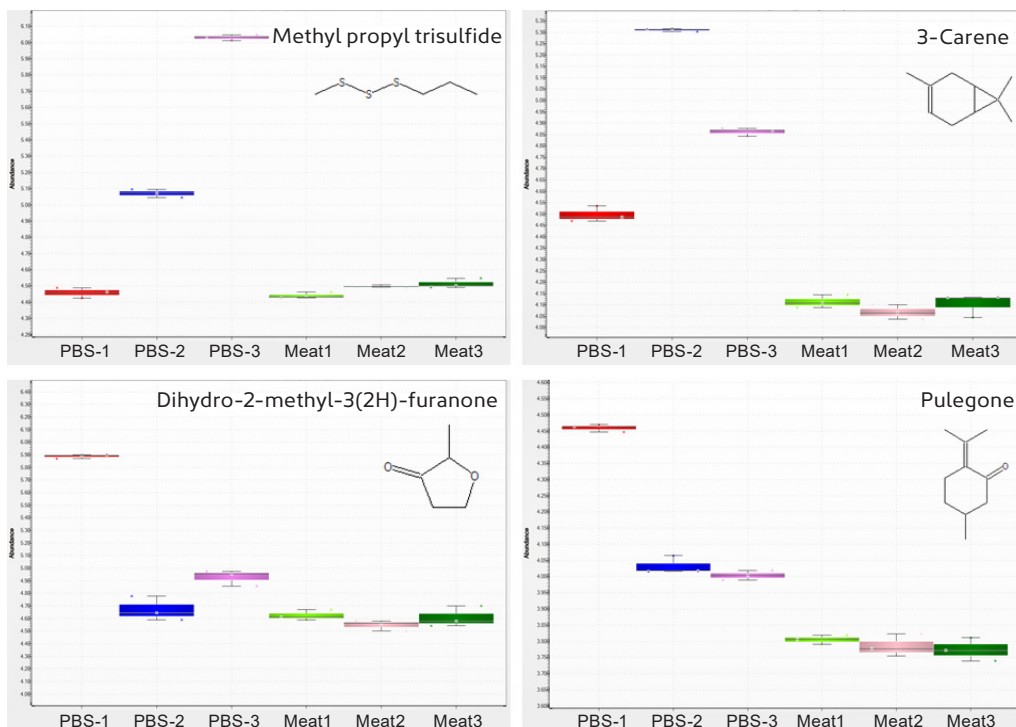
Conclusions

This study has shown that high-capacity sorptive extraction in conjunction with GC×GC–TOF MS and powerful chemometrics software provides comprehensive VOC profiling to aid reverse engineering of aroma, specifically:

- ► Fully automated sampling of VOCs and SVOCs using HiSorb high-capacity sorptive extraction probes.
- ► Cost savings through cryogen-free, solvent-free preconcentration and INSIGHT consumable-free flow modulation.
- ► Enhanced separation of GC×GC gains greater insight into sample composition.
- ► Confident identification of key quality markers using BenchTOF mass spectrometers.
- ► Time-saving untargeted workflows in ChromCompare+ automatically uncover the significant differences between complex aroma profiles.

For more information on this application, or any of the techniques or products used, please contact SepSolve.

References

[1] The Good Scents Company Information System (search facility), www.thegoodscentscompany.com/search2.html (accessed on 16th December 2020).

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