White Paper 016
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Aroma profiling of pet food using high-capacity sorptive extraction and TD–GC×GC–TOF MS

This study demonstrates the use of the INSIGHT flow modulator for TD–GC×GC–TOF MS aroma profiling of meat-based cat food. We show that the enhanced separation provided by GC×GC, combined with sensitive, high-capacity sorptive extraction, ensures that trace off-odour components can be reliably separated and detected.

Introduction

Pet food manufacturers place great importance on the aroma of their products, as off-odours may be unappealing to both the pets and their owners. Confident identification of the volatile organic compounds (VOCs) from pet food can help these companies better understand the factors governing the release of pleasant and unpleasant aromas.

VOC aroma profiles are typically analysed by solid-phase micro-extraction (SPME), which although a fast and simple technique, can be limited in terms of sample capacity, reproducibility and sensitivity. An alternative to SPME is high-capacity probe-based sorptive extraction, which results in higher sample loadings because of the large volume of PDMS phase. Typically, a SPME fiber has a sorbent volume of just 0.5 µL, while HiSorb sorptive extraction probes contain 65 µL of sorbent. When used in conjunction with secondary refocusing by thermal desorption (TD), the result is greater sensitivity across a wide analyte range.

Further analytical benefits can be achieved for analyte separation and detection, by using comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC–TOF MS). The enhanced separation capacity of this approach is ideal for handling the complex aroma samples of pet food, because it allows the entire composition to be screened in a single analysis, with confident identification of compounds that would ordinarily co-elute.

In this study, we demonstrate the value of high-capacity sorptive extraction with TD–GC×GC–TOF MS to investigate the volatile compounds present in the headspace of two brands of cat food, and discuss how these compounds might influence their aroma profiles. In this case the GC×GC method employs INSIGHT™

reverse-fill/flush flow modulation, lowering running costs and avoiding the logistical issues associated with liquid cryogen.

For more information on GC×GC please read our white paper.

Experimental

Sample preparation: Tinned meat-based cat food (6 g) was placed in a 20 mL headspace vial (Figure 1).

Headspace sorptive extraction: PDMS sampler: Inert HiSorb™ probe (Markes International); Agitation: 500 rpm. Temp.: 60°C; Equilibration time: 20 min; Sampling time: 15 min.

TD: Instrument: TD100-xr™ (Markes International); Focusing trap: 'General-purpose'. HiSorb probes were inserted into empty inert-coated stainless-steel TD tubes.

GC×GC: Flow modulator: INSIGHT™ (SepSolve Analytical); P_M: 3.0 s.

TOF MS: Instrument: BenchTOF-HD™; Mass range: m/z 45–500.

Software: ChromSpace® GC×GC software for full instrument control and data processing.

Please contact SepSolve for full analytical parameters.

Figure 1

Cat food being sampled in a headspace vial using a HiSorb probe. The pointed tip of the probe pierces the PTFE seal of the headspace vial septum, simplifying operation.

Results and discussion

1. Overall component separation

GC×GC–TOF MS surface charts for the two brands of cat food are displayed in Figure 2, and show excellent separation across the analyte profile.

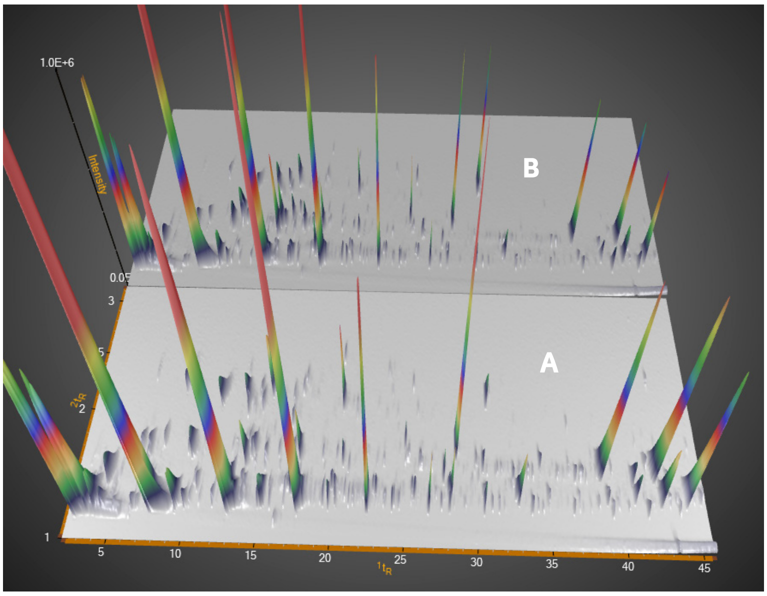


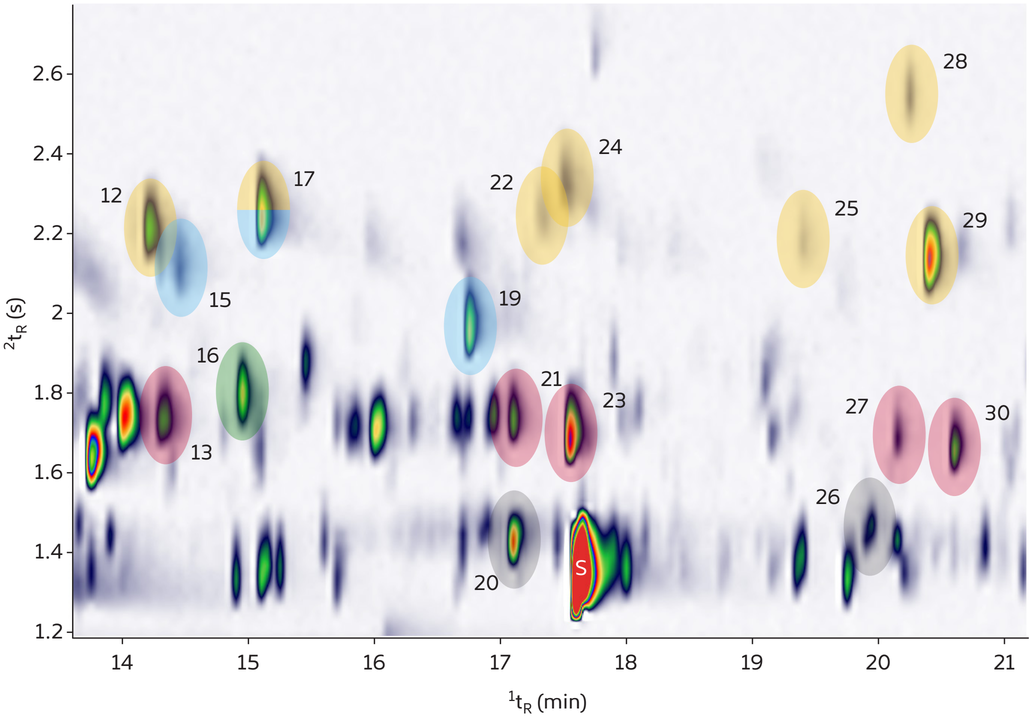
Figure 2

GC×GC–TOF MS surface plots of two brands of cat food using headspace sampling by HiSorb probes, viewed in ChromSpace.

2. Separation of co-eluting components

The enhanced separation achieved by INSIGHT GC×GC is clearly seen in Figure 3, in which peaks that would co-elute in conventional GC are separated in the second dimension. This is particularly valuable for compounds with low odour thresholds, which despite being detectable by GC–olfactometry, may be overlooked or misidentified due to co-elutions with high-loading peaks.

For example, in Figure 3 we can see that the small peak due to 2-acetylthiophene (#24) would be obscured both by nonanal (#23) and a large siloxane interference. 2-Acetylthiophene has an undesirable ‘sulfurous’ aroma[1] and an odour threshold of just 0.08 ppb,[2] making confident identification at trace levels important. As well as being separated from these components with GC×GC, 2-acetylthiophene is confidently identified by comparison against the NIST 17 database (Figure 4), as is the related odorous compound 2-acetyl-3-methylthiophene (#25, with a ‘phenolic’ aroma[1]).

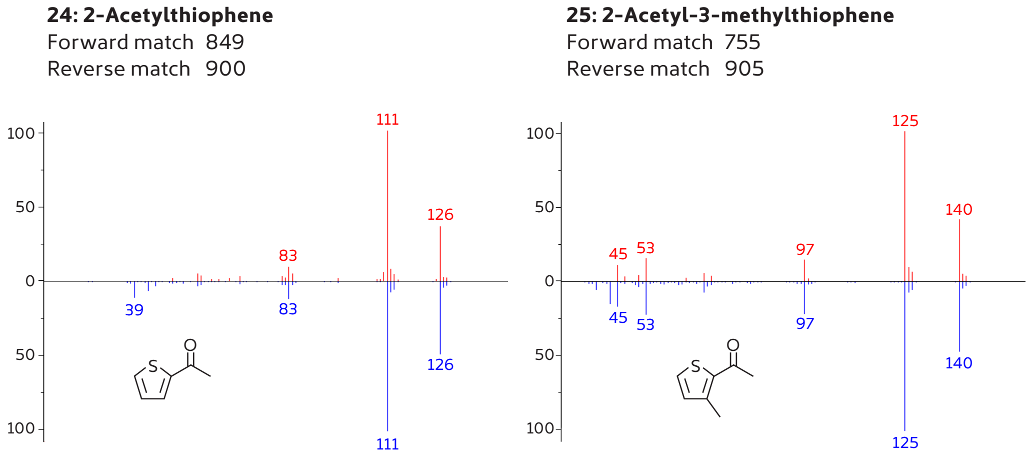


12	2-Methyltetrahydro-thiophen-3-one
13	Octanal
15	2-Ethyl-3-methylpyrazine
16	1,2,4-Trimethylbenzene
17	2-Acetylthiazole
19	2,5-Dimethyl-3-ethylpyrazine
20	Undecane
21	Nonan-2-one
22	5-Methyl-2-formylthiophene
23	Nonanal
24	2-Acetylthiophene
25	2-Acetyl-3-methylthiophene
26	Dodec-1-ene
27	Decan-2-one
28	1,2-Dithian-4-one
29	Kahweofuran
30	Decanal

🟡 Sulfur-containing heterocycles
🔵 Nitrogen-containing heterocycles
🟢 Aromatic hydrocarbons
🔴 Aliphatic ketones and aldehydes
⚪ Alkanes and alkenes

Figure 3

Enhanced separation of a range of compound classes in the headspace aroma profile of cat food (sample B) by GC×GC–TOF MS.
S = Siloxane.


24: 2-Acetylthiophene
Forward match 849
Reverse match 900

25: 2-Acetyl-3-methylthiophene
Forward match 755
Reverse match 905

Figure 4

BenchTOF spectra (top, red) and NIST 17 spectra (bottom, blue) for two sulfur heterocycles identified in Figure 2.

3. Identification of aroma-active compounds

Figure 5 and Table 1 provide an overview of the key compounds identified. As expected for a meat-based pet food, a number of compounds were found to contribute 'meaty' or 'nutty/roasted' aromas.

Some points of interest include:

- Tetrahydrothiophen-3-one (#8, 'garlic, meaty') was only present in sample A, while its alkylated derivative, 2-methyltetrahydrothiophen-3-one (#12, 'sulfurous, fruity, berry'), was only found in sample B.
- Pyrazines (#1, 5, 7, 15, 19, often described as 'nutty') had similar relative abundances in both samples.
- A number of compounds present are reported to have 'sulfurous' or 'animal' aromas, and could be perceived as off-odours. Dimethyl disulfide (#2, 'sulfurous, vegetable, onion') was found in sample A only.
- The ketones heptan-2-one (#6, 'cheesy, fruity') and octan-2-one (#11, 'earthy, weedy') were present in greater abundance in sample B.

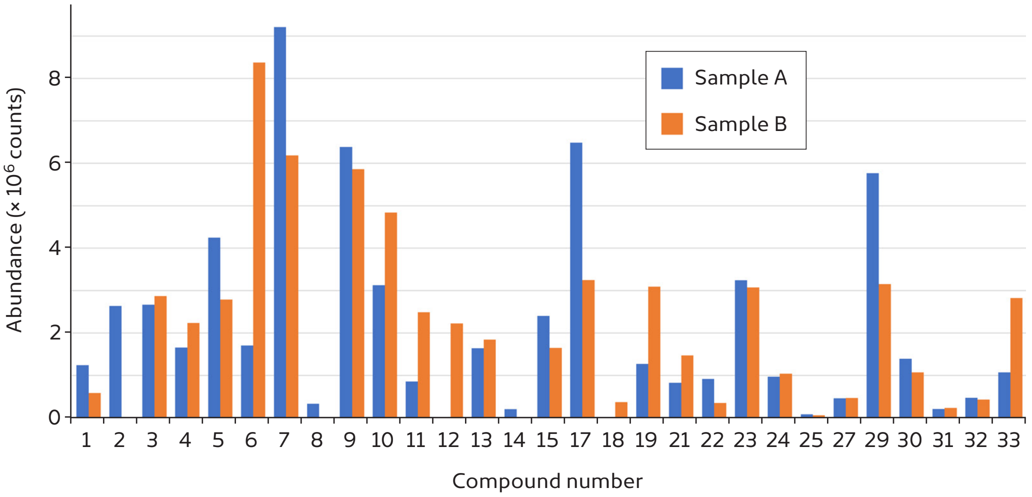


Figure 5

Comparison of the aroma profiles of cat food samples A and B. Compound identities are listed in Table 1.

Table 1

Compounds identified in the pet food headspace likely to contribute to the aroma.

No.	Compound	^1t_R (min)	^2t_R (s)	Peak area (× 10^5 counts)		Reported aroma[1]
				Sample A	Sample B	
1	Pyrazine	6.156	2.340	12.4	5.87	Pungent, sweetcorn, roasted hazelnut
2	Dimethyl disulfide	6.213	1.728	26.4	–	Sulfurous, vegetable, onion
3	2-Methylthiophene	6.877	1.700	26.7	28.7	Sulfurous, alliaceous, onion
4	Hexanal	7.752	1.790	16.6	22.4	Fresh, green, fatty
5	Methylpyrazine	8.600	2.336	42.5	27.9	Nutty, cocoa, roasted
6	Heptan-2-one	10.561	1.832	17.1	83.8	Cheesy, fruity, spicy, sweet, herbal
7	2,6-Dimethylpyrazine	11.450	2.169	92.1	61.9	Cocoa, roasted nuts
8	Tetrahydrothiophen-3-one	13.117	2.366	3.34	–	Garlic, meaty, green, vegetable
9	Benzaldehyde	13.372	2.133	63.8	58.6	Fruity, sharp, sweet, bitter
10	2-Pentylfuran	13.781	1.638	31.3	48.4	Fruity, green, earthy
11	Octan-2-one	13.877	1.778	8.56	24.9	Earthy, weedy, natural
12	2-Methyltetrahydrothiophen-3-one	14.253	2.200	–	22.3	Sulfurous, fruity, berry
13	Octanal	14.355	1.729	16.4	18.4	Aldehydic, waxy, citrus, orange peel
14	Δ^3-Carene	14.383	1.595	2.07	–	Sweet, citrus, terpenic
15	2-Ethyl-3-methylpyrazine	14.497	2.099	24.0	16.5	Nutty, peanut, musty
17	2-Acetylthiazole	15.172	2.228	64.9	32.5	Toasted, nutty, bready
18	Acetophenone	16.683	2.176	–	3.72	Floral, sweet, pungent
19	2,5-Dimethyl-3-ethylpyrazine	16.813	1.961	12.7	31.0	Potato, cocoa, roasted, nutty
21	Nonan-2-one	17.149	1.731	8.31	14.7	Fruity, fresh, sweet, green, weedy
22	5-Methyl-2-formylthiophene	17.359	2.229	9.23	3.49	Bitter, sweet, almond
23	Nonanal	17.566	1.689	32.5	30.8	Waxy, aldehydic, rose, fresh
24	2-Acetylthiophene	17.567	2.305	9.73	10.5	Sulfurous, nutty
25	2-Acetyl-3-methylthiophene	19.393	2.181	0.87	0.64	Phenolic, wintergreen
27	Decan-2-one	20.177	1.689	4.65	4.69	Orange, floral, fatty, peach
29	Kahweofuran (2,3-Dihydro-6-methyl-thieno[2,3-c]furan)	20.433	2.135	57.7	31.5	Sulfurous, smoky, roasted
30	Decanal	20.631	1.656	13.9	10.8	Sweet, aldehydic, waxy, orange peel
31	Undecanal	23.483	1.642	2.11	2.34	Waxy, soapy, floral, aldehydic
32	Indole	23.729	2.388	4.76	4.34	Animal, floral, mothball, fecal
33	α-Ionene	24.998	1.806	10.7	28.3	Sweet, woody, floral, violet

Conclusions

In this study, we have shown that the combination of high-capacity sorptive extraction with TD pre-concentration and INSIGHT GC×GC–TOF MS analysis is a powerful approach to characterising the complex headspace aroma profiles of pet food.

The high-capacity sorptive extraction probes used in this study are robust and reusable, minimising the cost per sample while allowing unattended sampling. As well as being applicable to headspace or immersive sampling, they are compatible with a wide range of chemical classes, with improved sensitivity over SPME due to the substantially larger (>100×) volume of sorbent.

Thermal desorption of the probes using instrumentation from Markes International offers the additional advantage of avoiding the need for duplicate samples to be taken, because of the ability to quantitatively re-collect a portion of the sample onto a clean sorbent tube for repeat analysis.

Crucial for this application is the need for a high degree of chromatographic resolution, in order to generate the clean mass spectra needed to confidently identify odour taints. This is an inherent advantage of GC×GC–TOF MS with INSIGHT flow modulation, which also further reduces running costs by avoiding the use of cryogen.

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 15 October 2017).

[2] D.S. Mottram, Meat (Chapter 5), in: *Volatiles in Food*, H. Maarse (ed.), CRC Press, 1991.