



Application Note 273

Aroma and flavour analysis of ground beef using fully automated headspace-trap and HiSorb™ sorptive extraction

This application note describes the headspace analysis of ground beef samples using Centri®, a fully automated, multi-mode extraction and enrichment platform. Two sampling modes are compared – static headspace-trap and high-capacity sorptive extraction (HiSorb) – to assess the volatile organic compounds (VOCs) that contribute to the aroma and flavour of ground beef as well as give an insight into other compounds of concern that cause a negative impact on human health.

Introduction

Analysis of VOCs that contribute to the aroma and flavour of a food product is important for quality control. The results can indicate whether a product is spoiled and reveal the source of this spoilage, such as contamination from packaging.

In this study, ground beef samples were analysed by gas chromatography-mass spectrometry (GC-MS). Sample preparation was carried out by both static headspace-trap and high-capacity sorptive extraction on Centri.

The key enabling innovation at the heart of Centri is a multi-sorbent-bed focusing trap in the sample flow path, which enables a wide range of analytes to be retained and enriched during sample introduction, and a 'backflush' injection (where the carrier gas flow reverses during trap desorption) ensures that the analytes are efficiently released to the GC(-MS). These have benefits for dealing with the high-moisture-content samples under investigation in this study:

- Large volume preconcentration (LVP): Volumes up to 5 mL in a single headspace extraction (automated using standard syringes) are preconcentrated on the trap prior to GC injection, meaning a greater quantity of each analyte is introduced to the analytical system for detection, enhancing the sensitivity.
- Water management: By selecting relatively hydrophobic sorbents in the focusing trap, combined with ambient trapping temperatures, water that is introduced by the sample extract can be purged away from the analytical system before injection to the GC(-MS). This leads to an improvement in chromatographic performance and can extend column and detector lifetimes.

The multi-mode extraction approach provides complementary data for comparative analysis, enabling food scientists to maximise their understanding of the VOC aroma and flavour

profiles of their products, leading to better decision making through product development to quality control.

Experimental

Sample preparation: Ground beef (27% fat) obtained from a supermarket. 5 g placed into standard 20 mL vials for extraction of headspace.

Extraction and enrichment:

Instrument: Centri (Markes International).

Headspace-trap

Extraction volume: 5 mL
 Incubation: 70°C (30 min)
 Injection: 250°C (2 min)

Headspace HiSorb

Probe: Short-length, inert, PDMS (H1-AXABC)
 Incubation: 70°C (20 min)
 Probe desorption: 250°C (10 min, 50 mL/min)
 Flow path: 180°C
 Focusing trap: Material emissions (U-T12ME-2S)
 Trap purge: 50 mL/min (2 min)
 Trap desorption: 30°C to 300°C (5 min)
 Split ratio: 20:1

GC:

Column type: DB-624ms™, 60 m × 0.32 mm × 1.8 µm
 Column flow: Helium, 0.5 mL/min (constant flow)
 Oven program: 35°C (5 min), 7°C/min to 200°C (2 min)

Quadrupole MS:

Source: 280°C
 Transfer line: 260°C
 Scan mode: m/z 35–300

Background to Centri and HiSor

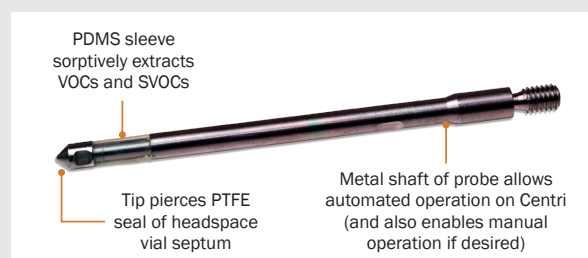
The **Centri** system from Markes International is the first GC-MS sample extraction and enrichment platform to offer high-sensitivity unattended sampling and preconcentration of VOCs and SVOCs in solid, liquid and gaseous samples.

Centri allows full automation of sampling using HiSor high-capacity sorptive extraction, headspace (-trap), SPME (-trap), and tube-based thermal desorption. Leading robotics and analyte-trapping technologies are used to improve sample throughput and maximise sensitivity for a range of applications.

In addition, Centri allows samples from any injection mode to be split and re-collected onto clean sorbent tubes, avoiding the need to repeat lengthy sample extraction procedures and improving security for valuable samples, amongst many other benefits.



The **HiSor** capability deployed in this study involves use of robust metal probes fitted with a section of high-capacity sorbent polymer, to extract and enrich compounds from liquids and solids. Samples contained in standard 20 mL or 10 mL vials are loaded onto Centri, and the HiSor probe inserted into the vial for either immersive or headspace extraction. The probe is then automatically washed, dried and desorbed, with the analyte vapours concentrated on the Centri focusing trap prior to GC-MS injection.



For more on Centri and HiSor, visit www.markes.com.

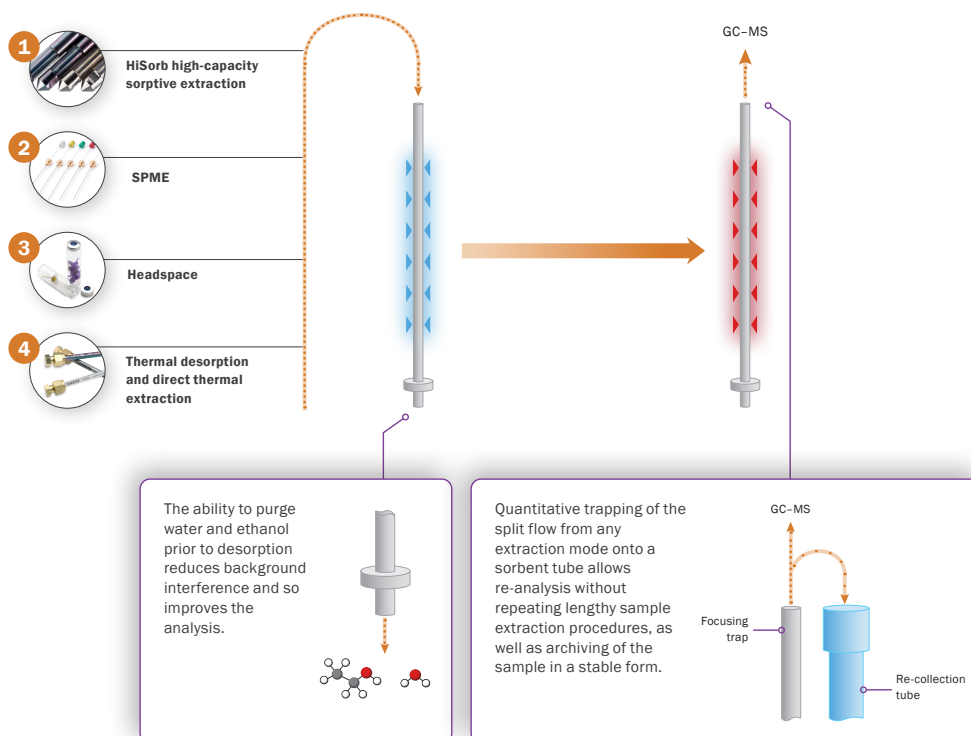
ANALYTE TRAPPING ON CENTRI *(optional for headspace and SPME)*

FIRST STAGE: Sample focusing

Samples collected using one of the four extraction modes are preconcentrated on Centri's cryogen-free sorbent-packed focusing trap.

SECOND STAGE: Trap desorption

The trap is heated rapidly to desorb the analytes and inject them into the GC-MS in a narrow band.



Results and discussion

Ground beef profile

The total ion chromatogram (TIC) profiles for the two headspace extraction modes are compared in Figure 1. The upper trace represents high-capacity sorptive extraction using HiSorb and the lower, inverted trace shows results achieved using headspace-trap. ChromSpace® GC-MS software (SepSolve, UK) was used to dynamically remove unwanted background noise, which improved baseline stability, and to deconvolve co-eluting analyte peaks into individual peaks for enhanced compound identification.

In this study, a broad range of VOCs was identified using the complementary techniques, ranging from very volatile components such as acetaldehyde (#3) to semi-volatile organic compounds (SVOCs) such as the plasticiser diethyl phthalate (#60). The numbered peaks in Figure 1 are identified in Table 1, which provides a comprehensive list of 61 compounds identified with a NIST match factor (MF) > 750. Also supplied are their odour characteristics and the individual peak area % values of the total TIC area in each extraction mode. Where a compound was detected but with a MF < 750, no area % is shown (referenced with a **X**) and the actual MF value is shown in red. VOCs that were not detected are shown as ND.

Many of the compounds detected, including alcohols, esters, ketones and certain aldehydes, provide positive aroma responses, which are well known and have been documented for these compounds. However, the compounds with the largest responses using headspace-trap – hexanal (#33) and acetone (#10) – provide negative aroma responses. An increase in hexanal (which causes a rancid aroma) indicates a deterioration in the quality of raw beef, so the response in this analysis may indicate that the product has been stored for too long. The reason for the high acetone content is difficult to determine: it could be related to animal feed or it could be a metabolism by-product or a result of bacterial activity within the end-product. The SVOC plasticiser diethyl phthalate is absent from the headspace-trap profile, but the HiSorb technique provided a better recovery. This high response also indicates a potential problem with storage or contact with packaging, which would have been missed had headspace-trap alone been used (and is discussed further in the next section).

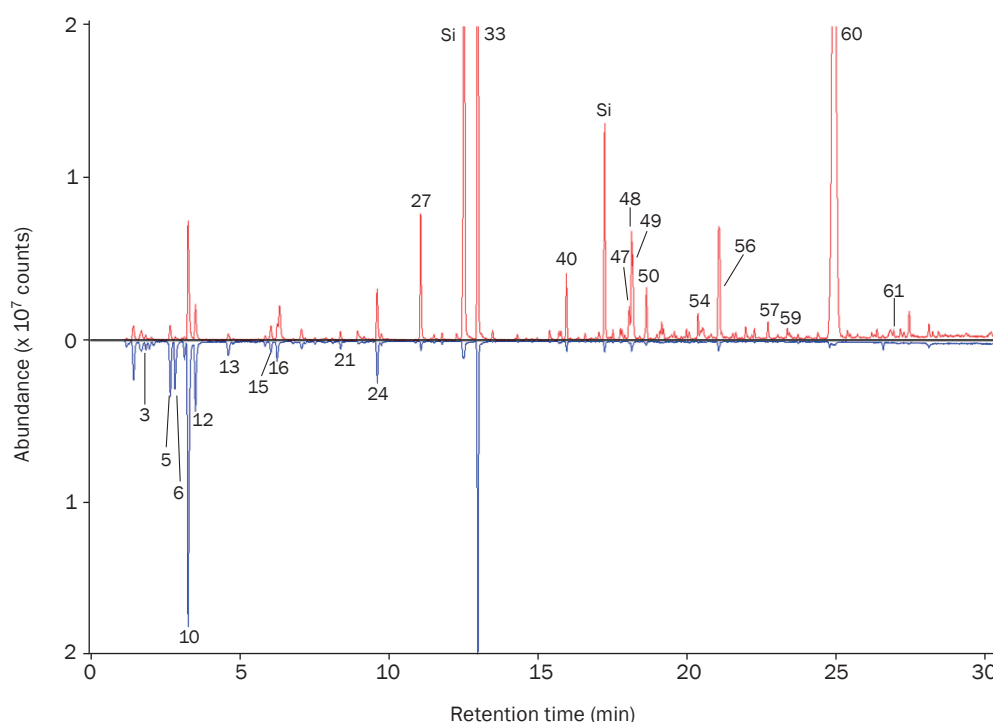


Figure 1: Aroma profiles of ground beef samples (5 g) using HiSorb (upper trace) and headspace-trap (lower, inverted trace). Major compounds are numbered (Table 1). “Si” = known siloxane compounds originating from the HiSorb sorptive phase.

Negative (aroma) impact VOC/SVOCs

In addition to the high hexanal response, other indicators of spoilage were identified in the meat sample; for example acetoin, ethyl benzene, propyl benzene, styrene, 3-methyl butanoic acid and acetic acid, which have been identified as potential markers for salmon spoilage.¹ Acetoin is typically associated with the presence of 2,3-butanedione (commonly referred to as diacetyl, #15), which was also identified in the ground beef sample using both techniques. Bacterial spoilage is a prime causative for the presence of these compounds in meat, and *Leuconostoc gasicomitatum* and *Brochothrix Thermosphacta* are potential candidates for this.²

DEP (#60) showed a very high response but was only identified using the sorptive extraction approach, demonstrating the clear advantage of HiSorb for these types of semi-volatile compounds. Although DEP has a low log $K_{o/w}$ value (2.65),³ the large PDMS phase on the HiSorb probe enables a more efficient extraction from the headspace in the vial during sampling. This compound is associated with food packaging materials and, with long contact exposure times, can migrate into the food source, which is a concern because it is a known endocrine (hormone) disruptor.⁴

Moreover, sulfur compounds that could affect the overall aroma, for example methanethiol (#4), dimethyl sulfide (#11) and dimethyl disulfide (#26), were all present in the sample; these were only detected using headspace-trap. As their concentrations increase, they become an indicator of spoilage.

In summary, the presence of DEP, hexanal, acetoin and diacetyl may indicate that the meat product has been in contact with its packaging for a long period of time and some bacterial activity has occurred, producing spoilage off-odours.

Finally, it is worth mentioning the toxic BTX components benzene (#18), toluene (#29) and xylene (#34), which are typically present in food as a result of the animal food source inhaling or ingesting the compounds from airborne vehicle emissions. These compounds are of concern because of their negative impact on human health.

Peak no.	RT	Compound	CAS#	Odour notes	% TIC	% TIC	NIST MF
					HS-trap	HiSorb	HS/HiSorb
1	1.73	Sulfur dioxide	7446-09-5	Pungent	1.41	X	860/735
2	1.92	Butane	106-97-8		0.61	X	858/578
3	2.03	Acetaldehyde	75-07-0	Fruity	0.80	X	865
4	2.17	Methanethiol	74-93-1	Rotten cabbage	0.53	X	893
5	2.73	Pentane	109-66-0	Gasoline	6.20	0.44	925/896
6	2.89	Ethanol	64-17-5	Fruity, ethereal	5.55	0.11	932/779
7	3.13	2-Propenal	107-02-8	Burnt fat (breakdown of glycerol upon cooking)	ND	0.06	856
8	3.21	Trimethylene oxide	503-30-0		1.77	0.11	908/798
9	3.26	2-Methoxyethanol	109-86-4		0.003	ND	813
10	3.33	Acetone	67-64-1	Solvent, apple	31.17	3.92	968/937
11	3.38	Dimethyl sulfide	75-18-3	Vegetable, cabbage	1.38	X	897/682
12	3.57	Isopropyl alcohol	67-63-0	Alcohol, woody	8.16	1.21	964/958
13	4.67	2-Methylpropanal	78-84-2	Pungent, fruity	1.88	0.24	868/818
14	5.91	Butanal	123-72-8	Pungent, musty	0.55	0.17	911/861
15	6.10	2,3-Butanedione	431-03-8	Burnt fat associated with meat spoilage bacterium <i>Brochothrix thermosphacta</i>	1.35	0.52	853/920
16	6.31	2-Butanone	78-93-3	Buttery, sweet, fruity	2.31	0.57	934/884
17	7.58	3-Methylhexane	589-34-4		0.32	0.07	843/874
18	7.84	Benzene	71-43-2	Sweet	0.08	X	769/684
19	8.17	3-Methylbutanal	590-86-3	Malty	0.21	X	802/722
20	8.25	2,3,3-Trimethylcyclobutanone	28290-01-9		0.04	ND	754
21	8.44	Heptane	142-82-5	Gasoline, associated with meat spoilage	0.79	0.26	884/886
22	9.04	Acetic acid	64-19-7	Vinegar, additive to meat	0.36	0.39	822/895
23	9.08	2-Ethylfuran	3208-16-0	Sweet burnt chemical	0.08	ND	823
24	9.66	Pentanal	110-62-3	Pungent	3.77	1.43	925/912
25	9.80	2,3-Pentanedione	600-14-6	Buttery fermented	0.44	0.19	860/753

Table 1: The top 61 compounds tentatively identified in the ground beef samples with NIST match factors (MFs) >750.

Key: ND = not detected, X = peak detected, failed NIST MF (values in red). (Continued on next page.)

Peak no.	RT	Compound	CAS#	Odour notes	% TIC	% TIC	NIST MF
					HS-trap	HiSorb	HS/HiSorb
26	10.95	Dimethyl disulfide	624-92-0	Breakdown product, oxidation product of methanethiol, garlic/ asparagus-like odour	0.18	X	855/522
27	11.13	Acetoin	513-86-0	Buttery	0.89	3.29	843/861
28	11.24	2-Methylbutanenitrile	18936-17-9		ND	0.12	783
29	11.46	Toluene	108-88-3	Sweet	0.06	0.05	860/874
30	11.57	3-Methylbutanenitrile	625-28-5		ND	0.12	783
31	11.83	Octane	111-65-9	Gasoline	0.28	0.17	886/907
32	12.53	1-Pentanol	71-41-0	Apricot	1.51	1.78	914/927
33	13.03	Hexanal	66-25-1	Green, grassy, fatty – a measure of rancidity in beef	24.34	22.07	946/951
34	14.66	<i>o</i> -Xylene	95-47-6	Sweet	0.03	0.05	785/893
35	14.85	Nonane	111-84-2	Gasoline	ND	0.02	765
36	15.02	(<i>E</i>)-2-Hexenal	6728-26-3	Green, fatty, cheesy	ND	0.06	810
37	15.44	1-Hexanol	111-27-3	Herbal, oily, pungent	ND	0.27	852
38	15.75	Tribromoacetic acid	75-96-7	Medicinal – haloacid associated with decontamination	ND	0.20	852
39	15.81	2-Heptanone	110-43-0	Cheesy	0.11	0.21	787/791
40	16.01	Heptanal	111-71-7	Green, fatty, oily	0.77	1.46	890/912
41	16.63	3-Methyloctane	2216-33-3		0.10	0.16	835/799
42	16.83	1,1'-Oxybisoctane	629-82-3		ND	0.04	770
43	17.09	1-Butoxy-2-propanol	5131-66-8	Ether-like	ND	0.25	797
44	17.56	Decane	124-18-5	Gasoline	ND	0.22	873
45	17.81	2-Pentylfuran	3777-69-3	Metallic, green, earthy	ND	0.25	830
46	17.86	(<i>E</i>)-2-Heptenal	18829-55-5	Green, sweet, apple	X	0.24	706/760
47	18.10	Benzaldehyde	100-52-7	Almond	0.23	0.67	833/895
48	18.18	2-Methyl-3-octanone	923-28-4		0.84	2.72	811/799
49	18.23	1-Octen-3-ol	3391-86-4	Mushroom, earthy, green	ND	1.47	835
50	18.68	Octanal	124-13-0	Citrus, green	0.40	1.26	821/918
51	19.19	Hexanoic acid	142-62-1	Sweaty	ND	0.39	849
52	19.25	2,6-Dimethyloctane	2051-30-1		0.16	0.25	750/781
53	19.52	2-Ethyl-1-hexanol	104-76-7	Citrus, fresh, floral	ND	0.08	830
54	20.41	(<i>E</i>)-2-Octenal	2548-87-0	Fatty, green	ND	0.54	823
55	20.56	(<i>E</i>)-2-Octen-1-ol	18409-17-1	Green, citrus, fatty	0.12	0.38	770
56	21.13	Nonanal	124-19-6	Waxy, orange peel	0.22	1.78	761/894
57	22.75	(<i>E</i>)-2-Nonenal	18829-56-6	Soapy, fatty, green	ND	0.48	845
58	23.09	Benzyl nitrile	140-29-4		ND	0.10	834
59	23.39	Decanal	112-31-2	Orange, citrus	ND	0.22	820
60	24.96	Diethyl phthalate	84-66-2	Plasticiser	ND	48.85	944
61	26.96	2-Undecenal	2463-77-6	Fruity, orange peel	ND	0.14	805

Table 1: The top 61 compounds tentatively identified in the ground beef samples with NIST match factors (MFs) >750.
Key: ND = not detected, **X** = peak detected, failed NIST MF (values in red). (Continued from previous page.)

Extraction efficiency

Comparing the efficiencies of the two headspace extraction techniques, Figure 1 shows a noticeable difference in the degree of VOC/SVOC recovery achieved. Headspace-trap has a higher level of recovery of the more volatile early eluting compounds compared to sorptive extraction. This is because the polydimethylsiloxane (PDMS) phase loading on the HiSorb probe is less selective for the early eluting compounds. However, it is worth noting that several of these compounds were detected with sorptive extraction but with lower recoveries that did not provide sufficiently high NIST-quality statistics.

The enhanced headspace-trap recovery can also be related to the sample volume used. In this work, headspace volumes of 5 mL were taken from each vial. The preconcentration step on the trap allows refocusing of the sample components, which are then injected into the GC in a narrower band of vapour, so that these large volumes can be accommodated without affecting the chromatographic performance.

At approximately 10 minutes, there is a change in both TIC profiles, with headspace-trap recovery diminishing and sorptive extraction increasing, the latter providing higher sensitivity for the less volatile compounds. This is due to both the high sampling capacity of the HiSorb probe (with a PDMS volume of 65 µL) and partitioning of these compounds into the PDMS phase, compared to partitioning into the headspace gas phase for syringe-based extraction.

High moisture levels in meat samples can affect chromatographic performance and detection. To reduce this effect, hydrophobic sorbents are used in the focusing trap to minimise water retention during the preconcentration step. In addition, a purge step, in which ambient-temperature carrier gas is passed through the trap and away from the analytical system prior to heating, reduces the residual water before GC-MS injection, improving results and extending column and detector lifetime.

Conclusion

These results demonstrate the capability of Centri to provide enhanced sample characterisation of aroma and flavour compounds from a beef product using a combination of sample extraction modes. The complementary techniques enable a more comprehensive sample profile to be generated, as shown here where the more volatile species (such as methanethiol) were only detected using headspace-trap, and less volatile compounds (such as diethyl phthalate) were identified using high-capacity sorptive extraction. The results indicate a product that is beyond a usable consumption date, potentially suffering from bacterial spoilage and contamination from polymeric packaging material.

The extraction techniques described here can be applied to a broad range of both solid and liquid food products. Centri provides a highly flexible and automated platform for sample characterisation to discover important aroma and flavour VOCs, as well as an insight into other compounds of concern that cause a negative impact on human health. The inclusion of a focusing trap enables larger than normal headspace volumes to be enriched for increased sensitivity, without

compromising chromatographic performance. The fully automated HiSorb technique provides a robust sampling option, and when combined with a large PDMS sorptive phase, enables both VOCs and SVOCs to be efficiently analysed in a single analysis to aid in quality control of food products.

References

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