TDTS 101

Rapid aroma profiling of cheese using a Micro-Chamber/Thermal Extractor with TD–GC/MS analysis

Summary

This Application Note describes the benefits of using Markes' Micro-Chamber/Thermal Extractor™ in conjunction with thermal desorption (TD) and GC/MS to analyse the aroma profiles of cheese. Various cheeses are examined and compared, and it is demonstrated how this 'multi-hyphenated' technique allows rapid yet powerful assessment of the VOCs released.

Introduction

Hundreds of varieties of cheese are enjoyed by people around the world. The nature of cheese varies considerably, and the subtle differences in aroma, flavour and texture all add to its appeal. Various factors are involved in the nature of the final product, including the type of animal, its diet, processing of the milk, the cheese culture, and the ageing conditions. In addition, natural flavourings and moulds may be introduced to impart a distinctive character to some cheeses.

The annual value of cheese to the global economy runs into many billions of dollars, and manufacturers expend a great deal of effort in ensuring their product is of a consistently high quality. Rigorous attention is paid to the ingredients, and the appearance, texture, taste and aroma of the final product.

The aroma profile is an important part of the consumer experience of cheese, and investigations have shown that a range of compounds combine to create their distinctive odours. These range from the relatively mild to the extremely pungent, and in most instances are dependent on the maturity of the cheese. This presents analysts with a substantial challenge when wishing to identify key aroma components, many of which are present at trace levels and have low odour thresholds relative to more abundant components such as fatty acids.

Here we show how Markes' thermal desorption sampling technology can be used to characterise the aroma profile from a range of cheese samples, with analysis both of desirable compounds as well as off-odours. The method also enables the identification of changes in the composition over time, providing useful data relating to shelf life and product safety.

Background to the sampling methodology

The sampling methodology used for the analysis of the cheese samples described here combines two powerful techniques:

Dynamic headspace sampling, to flush the organic vapours from the cheese onto a sorbent-packed tube, followed by *thermal desorption* to concentrate these vapours into a narrow band of carrier gas, suitable for introduction into a GC/MS system. These two techniques are described in more detail below.

(1) Dynamic headspace sampling

Markes' **Micro-Chamber/Thermal Extractor**[1] is a stand-alone sampling accessory for dynamic headspace sampling of organic vapours from a wide variety of materials, including foodstuffs (Figure 1).

Operation is simple, with short sampling times (typically <60 minutes) and the capability to analyse up to four or six samples at once, depending on the model chosen[1].

Figure 1: The Micro-Chamber/Thermal Extractor with cheese samples in place.

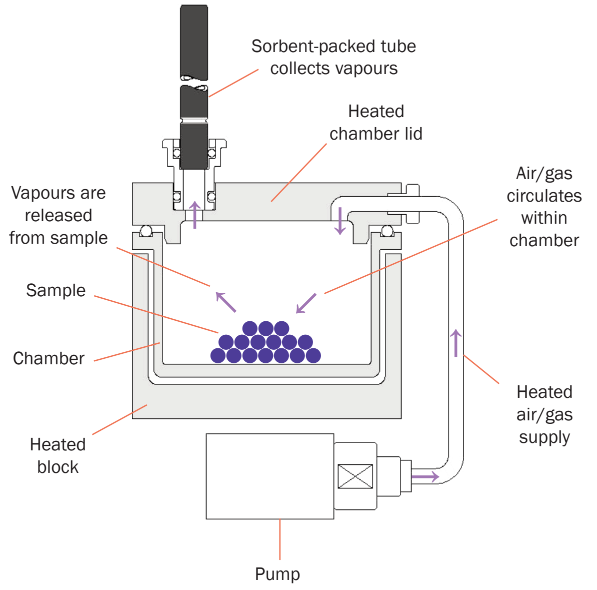


Figure 2: Schematic showing operation of an individual micro-chamber for sampling organic vapours from bulk samples.

The sample is placed in one of the inert-coated heated micro-chambers (Figure 2). Air or inert gas passes into the micro-chamber at a constant flow rate, sweeping the headspace vapours directly onto a sorbent-packed tube, ready for the thermal desorption stage (see below).

This dynamic headspace process is compatible with compounds over a wide volatility and concentration range, enabling a complete aroma profile to be determined.

In this Application Note we use the Micro-Chamber/Thermal Extractor to sample the headspace of various cheeses, and show how its flexibility and ease-of-use makes it an appealing alternative to methods such as solid-phase micro-extraction (SPME), distillation and solvent extraction.

(2) Thermal desorption

Thermal desorption (TD) is a versatile 'front-end' technology for GC and GC/MS that is applicable to the analysis of volatile and semi-volatile organic compounds (VOCs and SVOCs) in a wide range of samples – gases, liquids and solids. It combines pre-concentration, desorption/extraction and GC injection into one sensitive and fully automated operation that removes the need for solvent extraction and associated dilution.

In this case, each sorbent tube used to collect cheese aroma constituents was thermally desorbed by heating it in a flow of inert gas using a Markes desorber. The released components were transferred to an electrically-cooled smaller 'focusing' trap integrated within the TD system. After completion of the primary (tube) desorption stage, the focusing trap was itself desorbed, very quickly, by heating it rapidly in a reverse ('backflush') flow of carrier gas to transfer/inject the organic compounds into the capillary GC analytical column. This two-stage desorption process optimises concentration enhancement and produces narrow chromatographic peaks, thus optimising sensitivity.

Other advantages of Markes' TD systems that make them particularly suitable and convenient for challenging odour profiling applications like this include:

- Electrical (Peltier) cooling dispenses with the need for liquid cryogen, resulting in substantial savings of time and money. It also facilitates precise temperature control and water management
- The 'backflush' operation of the sorbent tube and focusing trap allows multiple sorbents to be used in series[2]. This extends the volatility range of compounds that can be sampled
- The inert flowpath and the ability to heat the tube from ambient temperature makes the system compatible with difficult analytes such as amines and sulfur compounds
- Samples can be 'split' (*i.e.* just part of the sample can be transferred to the GC) and the remainder collected and re-analysed under the same or different conditions, as required. An example of this is described in Section 3 (page 5).

The **UNITY 2**™ thermal desorber[3] is the cornerstone of Markes' TD systems. In this work, we use its fully automated analogue, the **TD-100**™ (Figure 3)[4]. This carries up to 100 sorbent tubes, and allows full automation of both sample desorption and re-collection.

Figure 3: The TD-100 automated thermal desorber, showing sampling trays.

The TD-100 harnesses Markes' patented DiffLok™ caps to protect sample tubes. These caps prevent analyte loss and stop ingress of contaminants from the laboratory environment. However, at the same time they allow tubes to be processed automatically without the complications of cap removal and replacement.

For more information on the Micro-Chamber/Thermal Extractor or thermal desorption, visit our website or contact our specialists at enquiries@markes.com.

Experimental

The aroma profiles of samples of the following cheeses were examined:

- Full-fat Welsh Cheddar (extra-mature)
- Low-fat Cheddar (mild)
- Comte (a hard cheese made with unpasteurised cow's milk)
- Emmental (a medium-hard cheese)
- Channel Island Brie (a soft cheese).

Sampling was carried out using the Micro-Chamber/Thermal Extractor, with analysis by TD–GC/MS. Experimental conditions are described below.

Micro-Chamber/Thermal Extractor (6-chamber model, max. 120°C):

Sample:	5 g cheese, grated
Chamber temp.:	40°C
Chamber flow:	50 mL/min dry nitrogen
Equilibration time:	20 min
Sampling time:	20 min

TD (TD-100):

Flow path temp.:	160°C
Sorbent tube:	Quartz wool–Tenax® TA–Carbograph™ 5TD (part number C3-AAXX-5304)
Focusing trap:	Material emissions (part number U-T12ME-2S)
Dry-purge:	2 min, 20 mL/min flow to split
Primary (tube) desorption:	1st stage: 150°C for 5 min, 50 mL/min trap flow, no split flow; 2nd stage: 300°C for 5 min, 50 mL/min trap flow, no split flow
Secondary (trap) desorption:	Trap low: 30°C, trap high: 300°C, heating rate: 24°C/s, hold time: 5.0 min, split flow 50 mL/min (high split), 5 mL/min (low split)
Pre-trap fire purge:	2 min, 50 mL/min trap flow, 50 mL/min split flow (high split), 5 mL/min split flow (low split)
Overall TD split:	51:1 (high split), 6:1 (low split)

GC:

Column:	HP-Innowax, 30 m × 0.25 mm × 0.25 µm
Column flow:	Constant flow 1.0 mL/min (initial *P* = 6.4 psi)
Temp. programme:	40°C (2.0 min), 5°C/min to 180°C, 20°C/min to 260°C (6.0 min)
Total run time:	36.0 min
Carrier gas:	He
GC inlet temp.:	160°C

MS (BenchTOF-dx™, ALMSCO International):

Ion source:	260°C
Transfer line:	265°C
Mass range:	33–350 amu
Data rate:	2 Hz

Spectra were matched against those in the NIST database with the help of TargetView™ software (ALMSCO International)[5]. This software package for GC/MS aids the identification of compounds in complex chromatograms, by removing baseline interferences, deconvolving overlapping peaks, library matching, and producing a simple report.

Three points should be noted regarding the experimental setup:

- A chamber temperature of 40°C was chosen for heating the sample. The VOCs released at this temperature give a good representation of the aroma of the cheese when in the mouth – known to contribute substantially to sensory perception
- The Innowax column was chosen to provide optimum chromatography for the polar compounds expected to be present in these samples
- The BenchTOF-dx time-of-flight mass spectrometer[6] from ALMSCO International was used in these experiments. Although TOF instruments for GC have been widely viewed as large, expensive and inaccessible to the analyst, BenchTOF-dx is an affordable solution for both routine monitoring situations and research-level applications. Specifically, it provides:
  - – Sensitivity at similar levels to that of quadrupole instruments run in selected ion monitoring (SIM) mode, but across the full spectral range (m/z 1–1500)
  - – Sub-unit mass resolution, allowing selective elimination of interferences and enhancing the detection of trace analytes in complex matrices
  - – Reference-quality spectra, which aid matching against target libraries.

Results and discussion

1. Analysis of the VOC profile of full-fat Cheddar

A chromatogram of a sample of full-fat Cheddar analysed under high-split conditions is shown in Figure 4, with the high-abundance compounds and some representative trace-level compounds labelled[7]. The profile is clean and free from background interference, reflecting the suitability of the sampling/analysis conditions and the removal of baseline interferences by TargetView software. In particular, the combination of TD and the Innowax GC column gives good peak shapes even for those analytes that can be notoriously difficult to chromatograph, such as carboxylic (fatty) acids.

The lower trace in Figure 4 shows the results of a subsequent run of the same sorbent tube, demonstrating minimal levels of carryover – an inherent feature of Markes' TD systems.

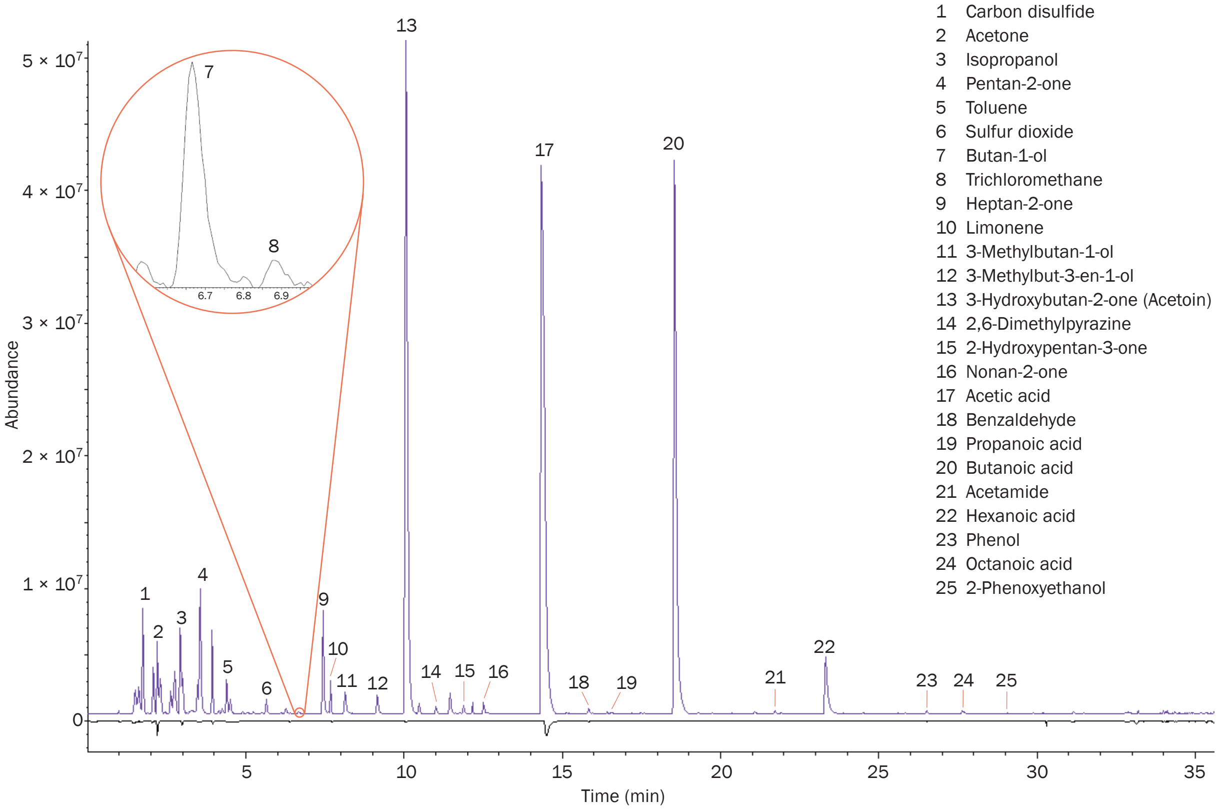
Fatty acids are a predominant feature of the full-fat cheddar in Figure 4. The most abundant compound in the profile, acetic acid (#17), would be expected to provide a vinegary character to the aroma, while butanoic acid (#20) and hexanoic acid (#22) would impart a more rancid note. All three are well-known to be major Cheddar odorants.

Another predominant compound in this sample was 3-hydroxybutan-2-one or acetoin (#13). Generally, this has a pleasant buttery odour, but also has been reported to impart sour milk/herbaceous/truffle notes.

Other ketones were also present at significant levels – pentan-2-one (#4), with an orange peel/sweet/fruity contribution, heptan-2-one (#9), known to contribute various notes (including Gorgonzola and blue cheese), and nonan-2-one (#16), reported to provide aromas of Gorgonzola/hot milk.

Pyrazines are important contributors to Cheddar odour/flavour, typically providing a roasted/earthy note, with 2,6-dimethylpyrazine (#14) being found in this sample.

TD–GC/MS has the ability to provide a wide dynamic range, even in one run. An example of this is the detection of trichloromethane (#8), which has an abundance ~0.04% that of acetic acid. This has been noted previously to impart a 'hay' odour to Cheddar.



1	Carbon disulfide
2	Acetone
3	Isopropanol
4	Pentan-2-one
5	Toluene
6	Sulfur dioxide
7	Butan-1-ol
8	Trichloromethane
9	Heptan-2-one
10	Limonene
11	3-Methylbutan-1-ol
12	3-Methylbut-3-en-1-ol
13	3-Hydroxybutan-2-one (Acetoin)
14	2,6-Dimethylpyrazine
15	2-Hydroxypentan-3-one
16	Nonan-2-one
17	Acetic acid
18	Benzaldehyde
19	Propanoic acid
20	Butanoic acid
21	Acetamide
22	Hexanoic acid
23	Phenol
24	Octanoic acid
25	2-Phenoxyethanol

Figure 4: Analysis of the VOC profile of full-fat (extra-mature) Cheddar, with high-abundance compounds and some representative trace-level compounds labelled. Blue line: Sampling using the Micro-Chamber/Thermal Extractor, with analysis by TD–GC/MS under high-split conditions. Black line (inverted) – Subsequent run of the same sorbent tube, showing low levels of retained analytes.

2. Comparison of different cheeses

One of the benefits of the Micro-Chamber/Thermal Extractor is that several samples can be run under identical conditions quickly and easily.

To obtain the chromatograms shown in Figure 5, vapours from four cheeses were collected simultaneously from adjacent chambers, and then run under identical TD–GC/MS conditions. This allows the VOC profiles to be compared easily, while the sizes of the peaks give a good approximation of the relative abundances of the individual components.

Particular points of note for these samples are:

- The similar profiles of the low-fat and full-fat Cheddar, with the main differences being in peak intensity
- The presence of carbon disulfide (#2) in all four samples, along with trace levels of hydrogen sulfide (#1), dimethyl disulfide (#4) and dimethyl sulfone (#14) in the Comte. This shows that the inert flow path of Markes' thermal desorption systems can handle labile sulfur compounds, which are difficult to sample reliably using less inert TD systems or other extraction techniques
- The presence of large amounts of propanoic acid (#9) and 3-methylbutanoic acid (#12) in the odour profiles of the Emmental and Comte, with the added presence of 2-methylpropanoic acid (#10) in the latter. Fatty acids, both straight-chain and branched, are key contributors to the aromas of various cheeses
- The presence of 3-methylbutan-1-ol (#6) in all four samples. This compound, which is known to confer a pleasant aroma of fresh cheese, was most abundant in the Comte and Emmental
- In the Emmental, the relatively large amounts of two alcohols – propan-1-ol (#3) and pentan-2-ol (#5). The respective 'sweet' and 'fresh' notes that have been reported for these components may contribute to the distinctive aroma of this cheese.

3. Analysing high- and trace-level components in a single run (High/Low analysis)

It is well-known that trace-level aroma components can have a very low odour threshold, and therefore a disproportionately large effect on consumer perception of odour. This presents a problem for the gas chromatographer – to measure trace-level and high-concentration compounds in the same sample.

One approach is to introduce a concentrated sample to the GC/MS system. This would enhance the response of trace-level constituents, but the more abundant

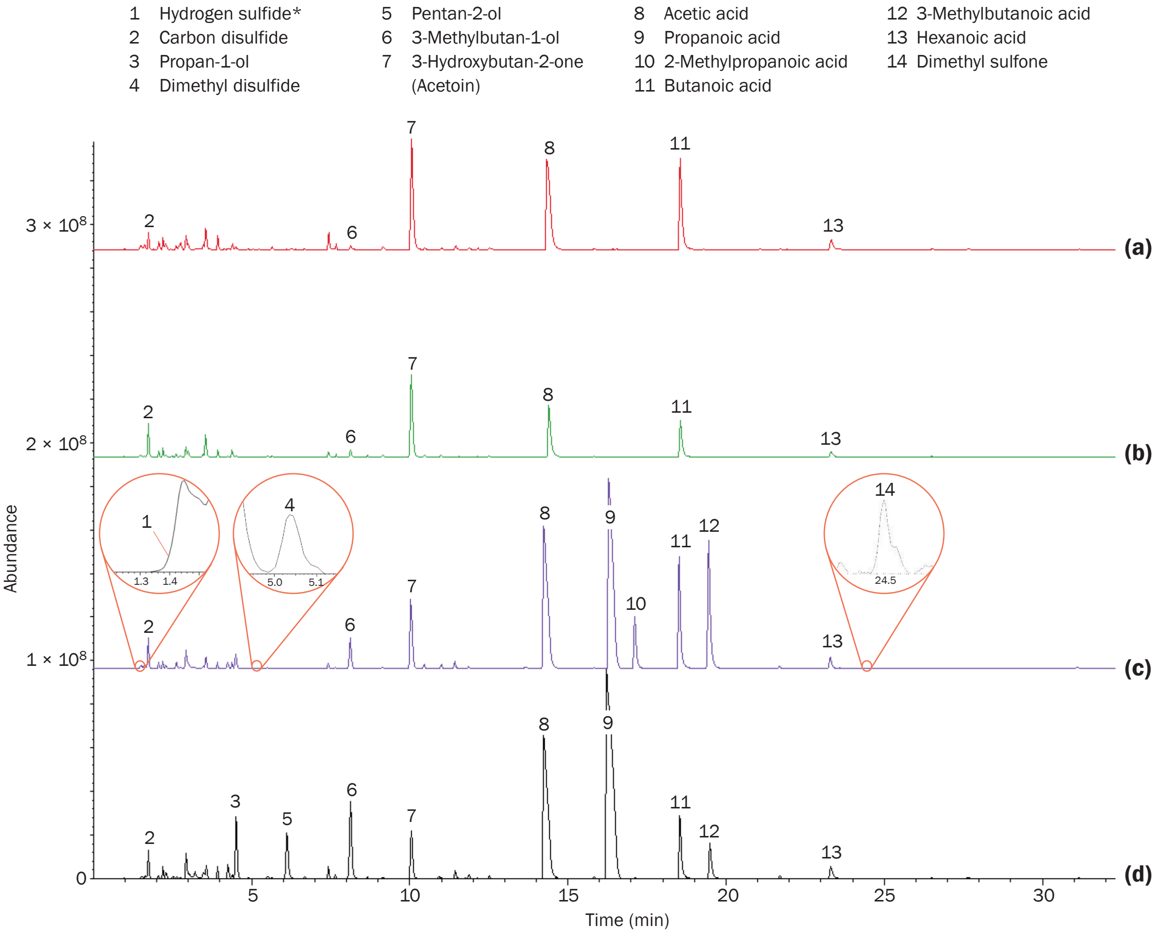


Figure 5: Analysis of the VOC profiles of (a) full-fat (extra-mature) Cheddar, (b) low-fat Cheddar, (c) Comte and (d) Emmental, with major compounds and some sulfur compounds labelled (insets). Sampling was carried out in parallel using the Micro-Chamber/Thermal Extractor, with analysis by TD–GC/MS analysis under high-split conditions.
*** Non-quantitative under these sampling and analytical conditions (see Application Note TDTS 32 for more information).**

constituents may then exceed the capacity of a highly resolving capillary column, resulting in column (and potentially detector) overload.

To avoid this problem and the need to run two separate extracted samples, Markes' re-collection technology can be used to run a single sample twice. First, the sample is desorbed using a 'high split' – *i.e.* a small volume is sent to the GC, allowing the high-concentration components to be analysed without overloading the analytical system. The remainder is re-collected onto a fresh sorbent tube, and then desorbed as before but using a 'low split' – *i.e.* sending a higher proportion of the sample to the GC. This allows the trace-level components to be quantified.

An example of the power of this High/Low technique is shown in Figure 6 for the full-fat Cheddar. The high-split and low-split runs of a single sample were obtained using automated sample re-collection. In this case an initial assessment of the component concentrations on-tube indicated that they extended from microgram levels for the fatty acids to sub-nanogram levels for the trace-level

1 Heptane
2 Carbon disulfide
3 Furan
4 Acetone
5 2-Methylbutanal
6 3-Methylbutanal
7 Propan-2-ol
8 Sulfur dioxide
9 Pentan-2-one
10 Butane-2,3-dione
11 Butan-2-ol
12 Toluene
13 But-2-enal
14 Propan-1-ol
15 Pentane-2,3-dione
16 Dimethyl disulfide
17 Hexan-2-one
18 Hexanal
19 2-Methylpropan-1-ol
20 Butanenitrile
21 2-Methylthiophene
22 Ethylbenzene
23 Pentan-2-ol
24 *p*-Xylene
25 Butan-1-ol
26 1-Ethoxypropan-2-ol
27 Heptan-2-one
28 Limonene
29 3-Methylbutan-1-ol
30 3-Methylbut-3-en-1-ol
31 Styrene
32 3-Hydroxybutan-2-one
33 1-Hydroxypropan-2-one
34 2-Methylbut-2-en-1-ol
35 2,6-Dimethylpyrazine
36 Hexan-1-ol
37 2-Hydroxypentan-3-one
38 Nonan-2-one
39 Trimethylpyrazine
40 2-Butoxyethanol
41 Acetic acid
42 Benzofuran
43 Benzaldehyde
44 2-Methylpropanoic acid
45 γ-Valerolactone
46 Butanoic acid
47 Acetophenone
48 2-Furanmethanol
49 Naphthalene
50 Pentanoic acid
51 5*H*-Furan-2-one
52 Acetamide
53 Hexanoic acid
54 Dimethyl sulfone
55 Phenylethyl alcohol
56 Biphenyl
57 Diphenylmethane
58 Phenol
59 Octanoic acid
60 3-Phenylthiophene
61 4-Methylphenol
62 2,6-Diisopropylnaphthalene
63 Nonanoic acid
64 Caprolactam
65 n-Decanoic acid
66 2,4-Bis(1,1-dimethyl-ethyl)phenol
67 4-Methylpent-3-enoic acid
68 Benzoic acid
69 *o*-Hydroxybiphenyl

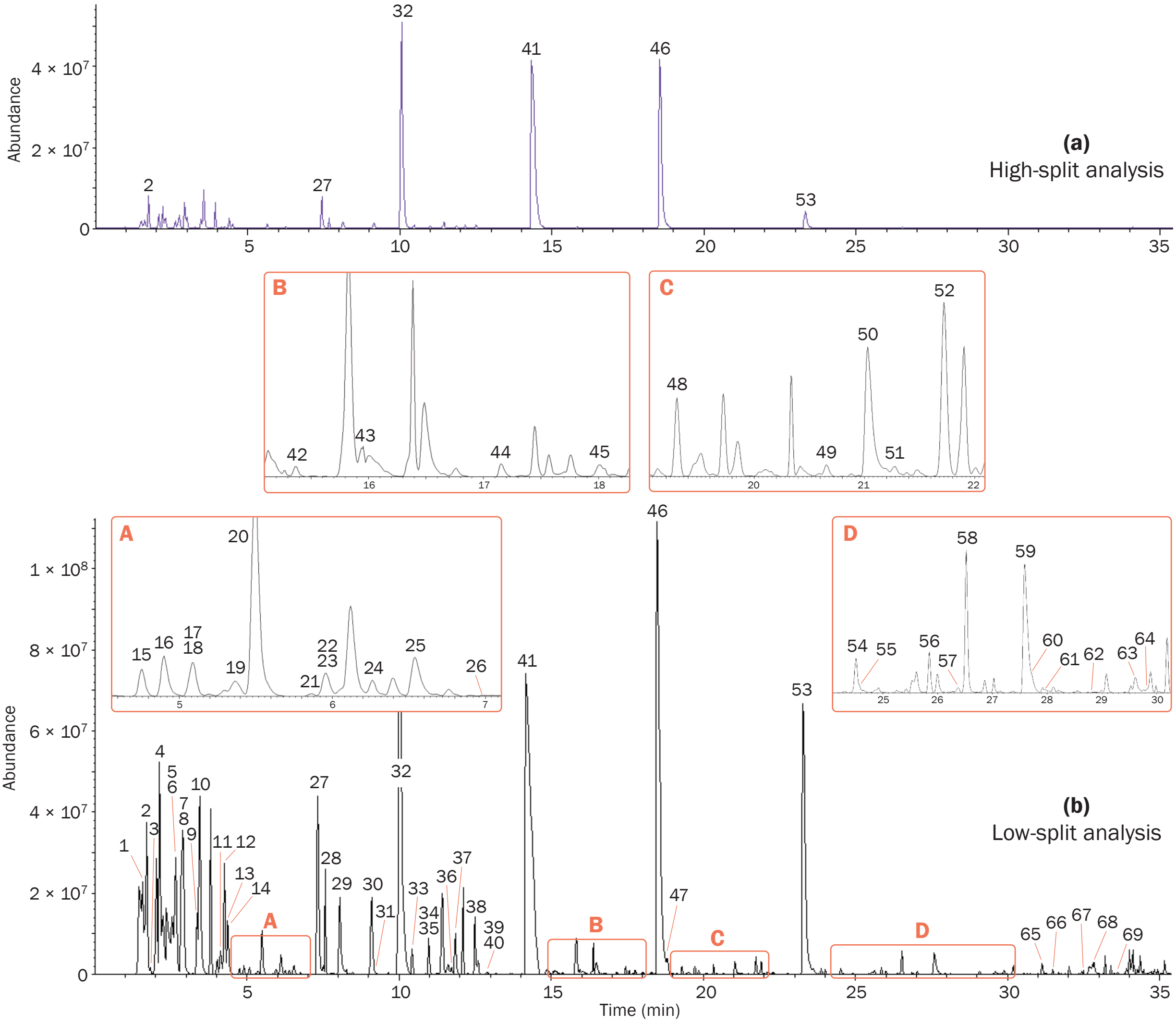


Figure 6: Analysis of the VOC profile of full-fat (extra-mature) Cheddar. Sampling was carried out using the Micro-Chamber/Thermal Extractor, with analysis by TD–GC/MS under (a) high-split (51:1) conditions, to provide an indication of the quantities of high-concentration components, and (b) low-split (6:1) conditions, to aid identification of trace-level components. Using the power of TargetView software, a wide range of these have been identified here, with many (but by no means all) being labelled in insets A–D. See Table A1 (in the Appendix) for associated data.

components. Identification of all compounds, including the trace-level analytes, was straightforward due to the ability of TargetView software to remove background interferences and separate contributions from overlapping components.

This capability is shown to good effect by the case of the trace-level components dimethyl sulfone (#54) and phenylethyl alcohol (#55) from Figure 6, which overlap considerably. Figure 7 shows an expansion of the relevant region generated using TargetView, illustrating that despite this overlap, the individual components of the peak in the total ion chromatogram can be 'deconvolved'.

Identification of these peaks was achieved by comparing the spectra derived from the deconvolved peaks against those in the NIST library. The similarity of these spectra can be seen in Figure 7, and this is confirmed by the good spectral matching coefficients of 0.91 for dimethyl sulfone and 0.80 for phenylethyl alcohol.

The quality of these matches is due to the ability of the mass spectrometer – the BenchTOF-dx time-of-flight instrument from ALMSCO International[6] – to produce reference-quality spectra, even for trace-level components in complex mixtures.

The BenchTOF-dx also enhanced the analysis of these challenging samples by virtue of its sensitivity and linear dynamic range, which allowed compounds over a wide concentration range to be detected.

It is also worth noting that High/Low analysis can be completely automated using the TD-100 autosampler[4], allowing multiple methods with significantly different split ratios to be run in one sequence, speeding up method development and extending the dynamic range by at least an order of magnitude.

An additional feature of the chromatograms obtained in the High/Low analysis of the full-fat Cheddar sample (Figure 6) is the range of analyte volatilities. In this case, we identified both acetone (#4) and *o*-hydroxybiphenyl (#69), a C_{12} phenol with b.p. ~290°C.

The fact that such a wide range of analytes can be collected in a single sampling is because both the sorbent tubes and focusing traps can be packed with multiple sorbents with different retention properties. This in turn is only possible because of the 'backflush' operation of Markes' TD systems[2]. In this case, we have chosen Tenax TA (an inert, general-purpose, weak porous polymer sorbent, retaining n-C_7 to n-C_{30}) and Carbograph 5TD (a medium/strong graphitised carbon black, retaining n-$C_{3/4}$ to n-$C_{6/7}$), giving a sorbent combination that can handle the full range of analytes expected to be present in the VOC profile of cheese. More information on sorbent selection can be found in Application Note TDTS 5.

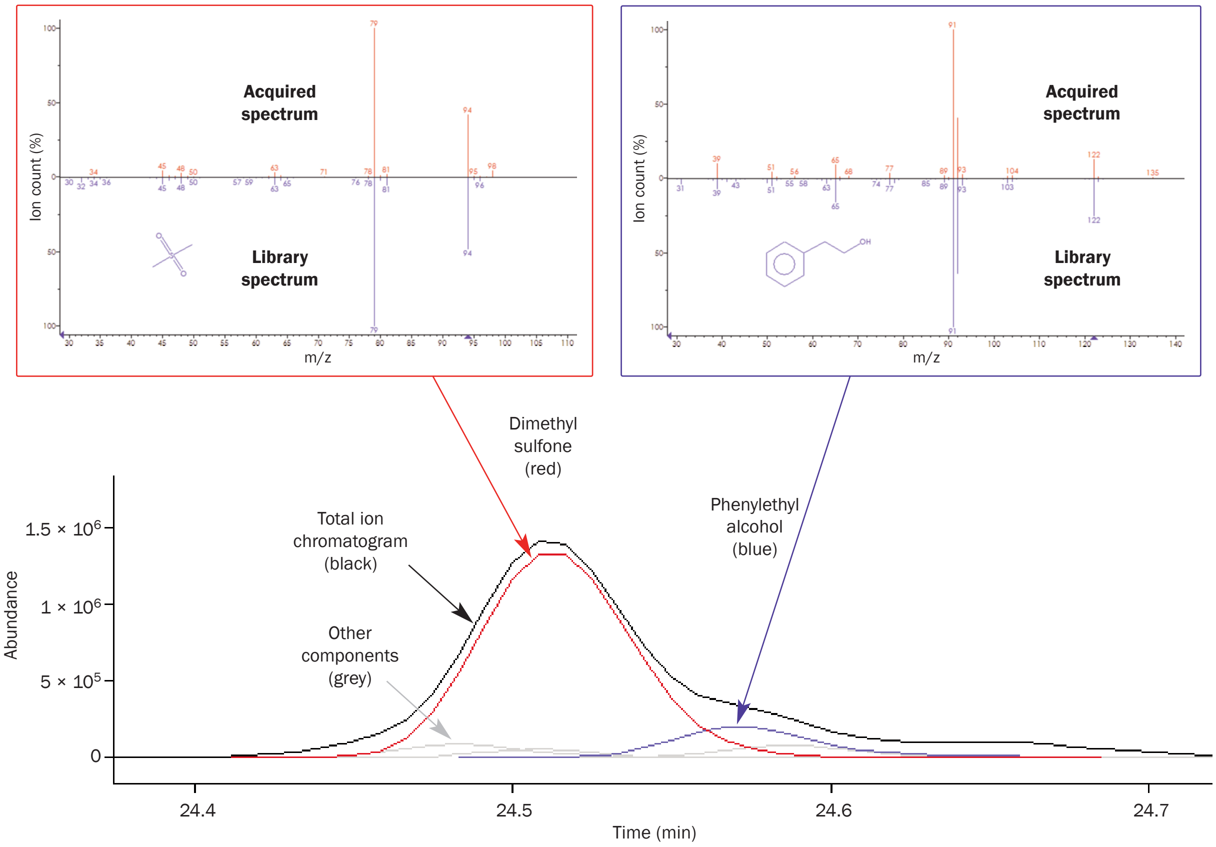


Figure 7: The deconvolution facility of TargetView, illustrated using dimethyl sulfone (#54) and phenylethyl alcohol (#55) from the full-fat Cheddar sample. The spectra acquired on the BenchTOF-dx are an excellent match for the NIST library spectra, despite the fact that the peaks overlap in the total ion chromatogram.

4. Comparison of fresh and aged cheese samples

A final aspect of cheese analysis that is easy to carry out using the Micro-Chamber/Thermal Extractor is to monitor how the profile of an individual sample changes with time. This may be useful, for example, in understanding what happens to the perceived odour of cheese during storage, or how long it takes for key volatiles to be released when cheese is brought to room temperature after being refrigerated.

To demonstrate this, the VOC profile of Channel Island Brie was analysed, and the analysis repeated after 5 days at 40°C in the same chamber using the same sampling/GC conditions (Figure 8). Nitrogen gas was used to purge the chamber, to see how the sample responded to an oxygen-free atmosphere. There is clearly a substantial change, with a loss of more volatile components, and a large increase in the proportion of fatty acids.

In short, the flexibility of the Micro-Chamber/Thermal Extractor setup allows a variety of experimental regimes to be run.

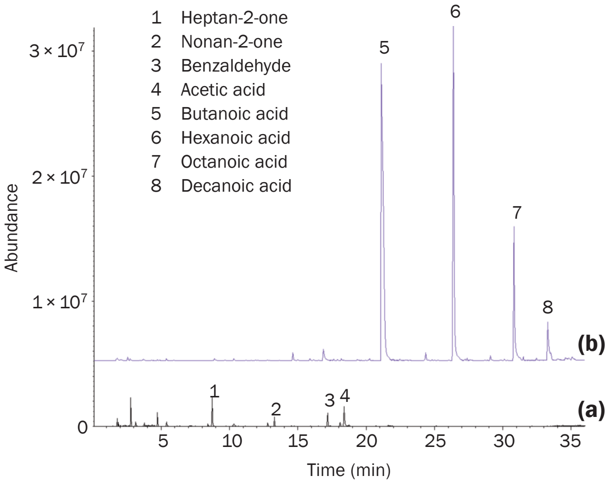


Figure 8: Analysis of the VOC profiles of Channel Island Brie, (a) as-received, and (b) after 5 days at 40°C. Sampling was carried out using the Micro-Chamber/Thermal Extractor, with analysis by TD–GC/MS.

Conclusions

In this Application Note we have shown how the Micro-Chamber/Thermal Extractor allows rapid and straightforward VOC/SVOC sampling from a range of cheese samples. In conjunction with TD–GC/MS, a wealth of information is provided that allows users to identify key components, compare samples side-by-side, and assess changes in the VOC profile with time.

The flexibility of this 'multi-hyphenated' technique makes it suitable for a wide range of sampling situations – from initial screening of 'unknown' samples to in-depth analysis of samples for quantitation of trace-level components. The sampling conditions, sorbents and GC/MS setup can all be optimised for the purpose, enabling high-quality analysis to be achieved whatever the sample.

Complementing the analytical performance of the instrumentation is the performance of the BenchTOF-dx mass spectrometer and the power of TargetView software, allowing the maximum amount of information to be retrieved from chromatograms with minimum effort.

References and notes

1. Two models of the Micro-Chamber/Thermal Extractor are available: one with six 44 mL chambers and a maximum temperature of 120 °C, and the other with four 114 mL chambers and a maximum temperature of 250 °C. Both models can use air or inert gas. Please visit http://www.markes.com/Instrumentation/Micro-ChamberThermal-Extractor-CTE.aspx for further information and to download the brochure.
2. The backflush operation of sorbent tubes and focusing traps means that analytes enter and leave the tube or trap at the same end – the end with the weakest sorbents. This ensures that low-volatility 'sticky' analytes are retained on the weakest possible sorbent, so that when the gas flow is reversed, they desorb easily. For further details see Application Note TDTS 64.
3. For more details on the UNITY 2 thermal desorber, please visit http://www.markes.com/Instrumentation/UNITY.aspx.
4. For further details of the TD-100, visit http://www.markes.com/Instrumentation/TD100.aspx.
5. For more examples of the use of TargetView, visit http://www.almsco.com/Products/Applications.aspx.
6. For more information on BenchTOF-dx, visit http://www.almsco.com/Products/Benchtof.aspx to download the brochure.
7. The following review was found to be a useful source of information about analytes found in a range of cheeses: P.M.G. Curioni and J.O. Bosset, Key odorants in various cheese types as determined by gas chromatography–olfactometry, *International Dairy Journal*, 2002, 959–984.

Trademarks

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Applications were performed under the stated analytical conditions. Operation under different conditions, or with incompatible sample matrices, may impact the performance shown.

Appendix

Peak no.	Target compound	CAS No.	Retention time (min)	Matching coefficient	Peak sum (TIC)	Peak sum (quant ion)	Extracted ion	Area (EIC) (%)	Area (TIC) (%)
1	Heptane	142-82-5	1.566	0.883	253514819	61473041	43	2.013	2.725
2	Carbon disulfide	75-15-0	1.703	0.864	310605404	170551844	76	5.585	3.339
3	Furan	110-00-9	1.920	0.873	1661395	121958	68	0.004	0.018
4	Acetone	67-64-1	2.024	0.844	138438421	25648425	58	0.840	1.488
5	2-Methylbutanal	96-17-3	2.617	0.814	72026813	14594996	41	0.478	0.774
6	3-Methylbutanal	590-86-3	2.660	0.829	224741012	17946563	58	0.588	2.416
7	Propan-2-ol	67-63-0	2.847	0.869	237857843	109502297	45	3.586	2.557
8	Sulfur dioxide	7446-09-5	2.917	0.869	55396971	30717897	64	1.006	0.596
9	Pentan-2-one	107-87-9	3.353	0.858	79908412	7226590	86	0.237	0.859
10	Butane-2,3-dione	431-03-8	3.447	0.901	395034145	262570741	43	8.599	4.247
11	Butan-2-ol	78-92-2	4.128	0.884	38109270	20168477	45	0.660	0.410
12	Toluene	108-88-3	4.262	0.934	186129167	73714520	91	2.414	2.001
13	But-2-enal	4170-30-3	4.366	0.890	84767785	18051139	70	0.591	0.911
14	Propan-1-ol	71-23-8	4.384	0.763	4594760	959250	42	0.031	0.049
15	Pentane-2,3-dione	600-14-6	4.757	0.772	6507986	4318544	43	0.141	0.070
16	Dimethyl disulfide	624-92-0	4.901	0.876	13060756	4913751	94	0.161	0.140
17	Hexan-2-one	591-78-6	5.076	0.867	4553818	998514	58	0.033	0.049
18	Hexanal	66-25-1	5.093	0.819	7514774	2067865	44	0.068	0.081
19	2-Methylpropan-1-ol	78-83-1	5.368	0.843	5552579	1380277	41	0.045	0.060
20	Butanenitrile	109-74-0	5.495	0.920	80399853	57469514	41	1.882	0.864
21	2-Methylthiophene	554-14-3	5.861	0.872	683069	460599	97	0.015	0.007
22	Ethylbenzene	100-41-4	5.948	0.896	4795695	2813702	91	0.092	0.052
23	Pentan-2-ol	6032-29-7	5.971	0.812	3525218	17305	71	0.001	0.038
24	*p*-Xylene	106-42-3	6.264	0.919	4870508	2128731	91	0.070	0.052
25	Butan-1-ol	71-36-3	6.541	0.835	16666602	5201147	56	0.170	0.179

Table A1: List of compounds identified from the full-fat Cheddar sample shown in Figure 6, run with a low split for optimum detection of trace-level components (*continued on next page*).

Peak no.	Target compound	CAS No.	Retention time (min)	Matching coefficient	Peak sum (TIC)	Peak sum (quant ion)	Extracted ion	Area (EIC) (%)	Area (TIC) (%)
26	1-Ethoxypropan-2-ol	1569-02-4	6.983	0.786	214503	77731	59	0.003	0.002
27	Heptan-2-one	110-43-0	7.328	0.895	389719203	90214022	58	2.954	4.190
28	Limonene	138-86-3	7.509	0.902	10981522	2888355	68	0.095	0.118
29	3-Methylbutan-1-ol	123-51-3	8.055	0.890	166785071	32709369	55	1.071	1.793
30	3-Methylbut-3-en-1-ol	763-32-6	9.093	0.894	147270736	25554727	56	0.837	1.583
31	Styrene	100-42-5	9.130	0.810	19279878	5396858	104	0.177	0.207
32	3-Hydroxybutan-2-one	513-86-0	10.050	0.860	1369220500	605780018	45	19.838	14.720
33	1-Hydroxypropan-2-one	116-09-6	10.431	0.896	68141730	56690291	43	1.856	0.733
34	2-Methylbut-2-en-1-ol	4675-87-0	10.969	0.854	45141149	12879110	71	0.422	0.485
35	2,6-Dimethylpyrazine	108-50-9	10.984	0.851	20260545	5398816	108	0.177	0.218
36	Hexan-1-ol	111-27-3	11.733	0.792	8623777	2759494	56	0.090	0.093
37	2-Hydroxypentan-3-one	5704-20-1	11.849	0.824	73555962	14211059	57	0.465	0.791
38	Nonan-2-one	821-55-6	12.494	0.887	99612842	30849662	58	1.010	1.071
39	Trimethylpyrazine	14667-55-1	12.886	0.845	3900299	1494522	42	0.049	0.042
40	2-Butoxyethanol	111-76-2	12.934	0.762	597718	240875	57	0.008	0.006
41	Acetic acid	64-19-7	14.171	0.844	2022790505	504596574	60	16.524	21.746
42	Benzofuran	271-89-6	15.369	0.882	2498788	1224921	118	0.040	0.027
43	Benzaldehyde	100-52-7	15.829	0.896	58729364	14141432	105	0.463	0.631
44	2-Methylpropanoic acid	79-31-2	17.155	0.873	3181059	1381267	43	0.045	0.034
45	γ-Valerolactone	108-29-2	17.991	0.765	1036025	213999	85	0.007	0.011
46	Butanoic acid	107-92-6	18.473	0.894	1624127220	443289208	60	14.517	17.460
47	Acetophenone	98-86-2	18.821	0.879	18490865	6458562	105	0.212	0.199
48	2-Furanmethanol	98-00-0	19.298	0.878	10689364	1796995	98	0.059	0.115
49	Naphthalene	91-20-3	20.658	0.910	1834613	1184386	128	0.039	0.020
50	Pentanoic acid	109-52-4	21.031	0.901	28662094	12359678	60	0.405	0.308
51	5*H*-Furan-2-one	497-23-4	21.272	0.868	1513465	251925	84	0.008	0.016
52	Acetamide	60-35-5	21.721	0.796	29874695	8543803	59	0.280	0.321
53	Hexanoic acid	142-62-1	23.278	0.852	662378791	219553750	60	7.190	7.121
54	Dimethyl sulfone	67-71-0	24.512	0.906	9361487	5466798	79	0.179	0.101
55	Phenylethyl alcohol	60-12-8	24.572	0.797	1487159	606673	91	0.020	0.016
56	Biphenyl	92-52-4	25.851	0.906	10601587	5227605	154	0.171	0.114
57	Diphenylmethane	101-81-5	26.380	0.858	1261067	309779	168	0.010	0.014
58	Phenol	108-95-2	26.523	0.914	38788397	16793765	94	0.550	0.417
59	Octanoic acid	124-07-2	27.589	0.863	67911844	16475462	60	0.540	0.730
60	3-Phenylthiophene	2404-87-7	27.727	0.769	2537612	563913	160	0.018	0.027
61	4-Methylphenol	106-44-5	27.978	0.866	541690	177900	107	0.006	0.006
62	2,6-Diisopropylnaphthalene	24157-81-1	28.823	0.806	149137	42344	197	0.001	0.002
63	Nonanoic acid	112-05-0	29.605	0.869	5542305	1156816	60	0.038	0.060
64	Caprolactam	105-60-2	29.824	0.816	518213	110423	113	0.004	0.006
65	n-Decanoic acid	334-48-5	31.128	0.781	16270589	3089040	60	0.101	0.175
66	2,4-Bis(1,1-dimethylethyl)phenol	96-76-4	31.489	0.878	4519248	2411386	191	0.079	0.049
67	4-Methylpent-3-enoic acid	504-85-8	32.440	0.762	2096548	148534	43	0.005	0.023
68	Benzoic acid	65-85-0	32.808	0.860	18965217	3458389	122	0.113	0.204
69	o-Hydroxybiphenyl	90-43-7	33.626	0.806	1451295	460638	170	0.015	0.016

Table A1 (*continued from previous page*).