TDTS 104

Rapid and sensitive aroma profiling of strawberries using dynamic headspace sampling and TD–GC/TOF MS

Summary

This Application Note describes the fast and highly sensitive detection of trace-level aroma compounds in the headspace gas above fresh strawberries. Sampling is carried out using an easy-to-use microchamber system, with analysis by thermal desorption (TD) and gas chromatography/time-of-flight mass spectrometry (GC/TOF MS). Compound identification is further enhanced by the use of TargetView™ software, allowing rapid screening against a large commercial library and a smaller customised library of sulfur compounds.

Introduction

Food aroma profiles typically contain components over a wide range of concentrations, though very often the most odour-active compounds are only present at trace levels. In such situations all aspects of the sampling and analysis methods need to be optimised for detection and identification of the widest range of analytes.

The aroma of strawberries is one of the most complex of all fruits, and has received a good deal of attention in recent years[1–3], especially from the point of view of identifying the olfactory components that characterise different strawberry varieties. In this Application Note we describe the use of a multi-hyphenated microchamber-based analytical setup that offers the low detection limits necessary for the identification of these key volatiles, while not compromising on the flexibility, speed, and ease-of-use that is critical in food analysis.

Background to the sampling and analysis methodology

Sampling and subsequent analysis of the strawberry samples described here combines four powerful technologies. *Dynamic headspace sampling* flushes the organic vapours from the strawberry onto a sorbent-packed tube, while *thermal desorption* concentrates these vapours and delivers them into the GC in a narrow band of carrier gas for optimum sensitivity. Analysis uses inherently sensitive *time-of-flight mass spectrometry*, and *compound-identification software* speeds up data processing by rapidly and automatically detecting and confirming the identity of headspace components.

Dynamic headspace sampling

Markes' Micro-Chamber/Thermal Extractor™ [4] is a stand-alone sampling accessory for dynamic headspace sampling of organic vapours from a wide variety of materials, including foodstuffs. 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.

The sample is placed in one of the inert-coated microchambers, which can be heated (Figure 1). Air or inert gas passes into the microchamber at a constant flow rate, sweeping the headspace vapours directly onto

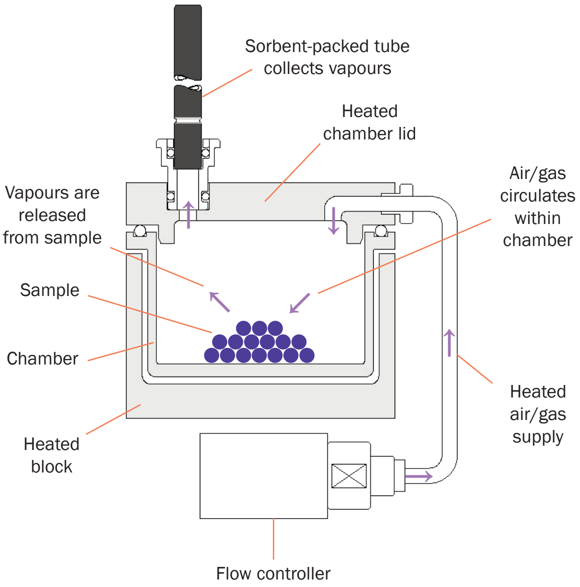


Figure 1: Schematic showing operation of an individual microchamber for sampling organic vapours from samples such as fresh foodstuffs.

a sorbent-packed tube, ready for the thermal desorption stage (see below). This continuous flow results in the collection of large volumes of headspace vapours from across the volatility range, thereby significantly increasing sensitivity and ensuring that the sample is representative of the entire odour/aroma profile under the temperature conditions selected.

Thermal desorption (TD)

Thermal desorption 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 sample matrices – 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 (with its associated limitations of dilution, interferences and manual sample preparation). When used in conjunction with dynamic headspace, TD offers considerable sensitivity advantages for the analysis of VOC profiles from bulk samples such as fresh foodstuffs[5].

In this case, the sorbent tubes used to collect strawberry headspace vapours were analysed using thermal desorption in conjunction with GC/TOF MS. During the two-stage thermal desorption process, the sampled tubes were heated relatively gently in a flow of inert carrier gas. The released components were then transferred to an electrically-cooled smaller 'focusing' trap integral to 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 of the secondary focusing trap 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[6]. This extends the volatility range of compounds that can be sampled.
- The inert flowpath and gentle heating of the tube from ambient temperature makes the system compatible with reactive compounds such as amines and sulfur compounds.
- Samples can be 'split' during desorption (*i.e.* just part of the sample can be transferred to the GC), with the remainder being quantitatively re-collected and re-analysed under the same or different conditions, as required. An example of this is described in the Results and discussion (Section 4).

In this work, we use the TD-100™, Markes' fully automated thermal desorber[7]. This carries up to 100 sorbent tubes, and allows full automation of both sample desorption and re-collection.

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

Time-of-flight mass spectrometry (TOF MS)

The time-of-flight mass spectrometer used in this study, BenchTOF-dx™ [8], is designed specifically for gas chromatography. It offers full spectral information at high sensitivity (equal to that of quadrupole instruments running in selected ion monitoring (SIM) mode), making it ideal for detecting trace-level analytes – both target compounds and unknowns.

BenchTOF-dx produces reference-quality 'classical' EI spectra, which allows matching against commercial databases such as the NIST library. In addition, it acquires 10,000 full-range spectra every second. This provides post-run software packages with the data density needed to reliably deconvolve overlapping peaks and distinguish them from baseline interferences, thus enabling confident identification.

Compound identification software

The advantages of the sampling and analysis setup described here are enhanced by TargetView[9], an easy-to-learn data-processing package that allows accurate and automated identification of trace compounds in complex GC/MS profiles.

TargetView uses sophisticated algorithms to process total ion chromatographic (TIC) data. First it eliminates background interferences and deconvolves co-eluting components into individual analyte peaks. It then applies advanced chemometric techniques to compare the deconvolved spectra against library spectra, thus allowing both target and 'unknown' compounds to be identified confidently, even at trace levels. Application-specific target libraries or general-purpose databases like the full NIST library can be used.

Experimental

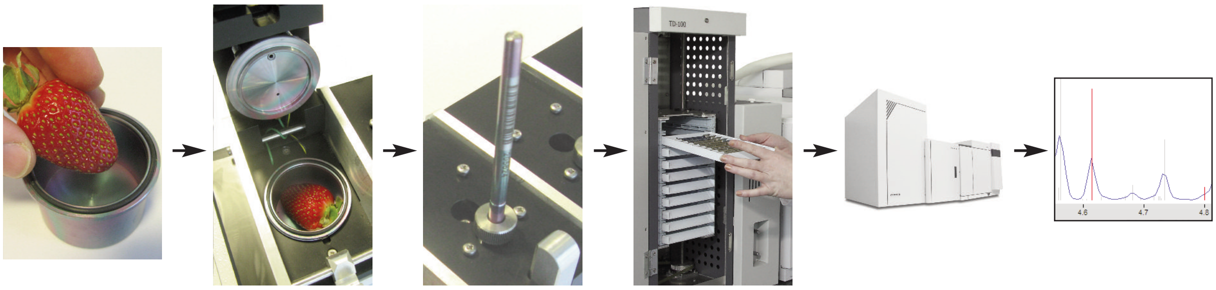
The extraction and analysis process is depicted in Figure 2. Note that the microchamber system easily accommodates whole strawberries, which would not be possible with a standard 20 mL headspace vial.

Sampling:
Instrument: Micro-Chamber/Thermal Extractor (Markes International) (six-chamber version)
Chambers: Inert-coated stainless-steel, 44 mL capacity
Chamber temp.: 40 °C
Equilibration: 50 mL/min of dry nitrogen for 10 min
Sampling flow: 20 min using 50 mL/min dry nitrogen
Headspace volume: 1000 mL sampled
Sorbent tubes: Inert-coated stainless steel packed with:
Quartz wool–Tenax® TA–SulfiCarb™
or
Quartz wool–Tenax TA–Carbograph™ 5TD

TD:
Instrument: TD-100 (Markes International)
Flow path temp.: 160 °C
Focusing trap: Material Emissions trap (Markes International)
Dry-purge: 2 min, 20 mL/min flow
Primary (tube) desorption: 120 °C for 5 min, then 260 °C for 5 min; 40 mL/min trap flow
Pre-trap-fire purge: 2 min; 50 mL/min trap flow; 20 mL/min split flow
Secondary (trap) desorption: Trap low: 25 °C; trap high: 300 °C; heating rate: 24 °C/s; hold time: 5.0 min; split flow: 20 mL/min
TD split: 21:1 outlet split

GC:
Column: Rtx-5MS, 60 m × 0.25 mm × 0.5 µm
Carrier: He, constant flow 1.0 mL/min
Temp. programme: 40 °C (5.0 min), 10 °C/min to 300 °C (5.0 min)
Total run time: 36.0 min

TOF MS:
Instrument: BenchTOF-dx (ALMSCO International)
Ion source: 280 °C
Transfer line: 200 °C
Mass range: 35–350 amu
Data rate: 2 Hz with 5000 spectra per data point
Filament voltage: 1.6 V



Place strawberry in microchamber pot and weigh sample.

Load pot into the Micro-Chamber/ Thermal Extractor.

Attach sorbent tube, pass heated N_2 into the chamber, and collect released vapours.

Load tube into the TD-100 automated thermal desorber and...

...analyse vapours by GC/TOF MS.

Identify compounds using TargetView software.

Figure 2: Flow chart showing the process used to sample and identify the volatile compounds released from strawberries.

Results and discussion

1. Emission profile of a whole strawberry

An example emission profile for a whole strawberry is shown in Figure 3. This chromatogram has been processed by TargetView to remove unwanted background signals, and therefore improve both the identification of trace-level components and the quality of the mass spectra.

To produce a listing of the most significant components in this sample, the options for the ‘all-component’ search in TargetView were adjusted so that it queried the 100 most abundant peaks against the NIST 11 database. The result of this was a list of 76 identified compounds, many being the esters well-recognised as key olfactory components (see Table A1 in the Appendix). Of particular note is the detection of furaneol (peak #47), also known as strawberry furanone, a highly polar and unstable compound with a desirable ‘burnt sugar’ aroma and a sub-ppb odour threshold[10].

The peak area of each analyte was used to calculate an approximate concentration (in nanograms per gram of fruit) in terms of toluene equivalents, based on the peak area obtained for a toluene standard analysed under the same conditions.

2	Ethanol	19	Ethyl butanoate	40	Ethyl hexanoate	59	Octyl acetate
3	Acetone	22	Isopropyl butanoate	41	Hex-3-enyl acetate	62	Octyl butanoate
4	Methyl acetate	25	Hex-3-en-1-ol	42	Hexyl acetate	63	Tetradecane
5	Acetic acid	28	3-Methylbutyl acetate	43	Hex-2-enyl acetate	65	β-Farnesene
7	Ethyl acetate	29	2-Methylbutyl acetate	45	Isopropyl hexanoate	66	Decalactone
9	Butan-1-ol	31	Propyl butanoate	47	Furaneol	69	Butylated hydroxy toluene
10	Benzene	34	Pentyl acetate	50	Linalool	71	Nerolidol
12	Methyl thioacetate	35	Methyl hexanoate	54	Benzyl acetate	72	Octyl hexanoate
15	Methyl butanoate	37	Benzaldehyde	56	Butyl hexanoate	75	Dodecalactone
18	Benzyl methyl ketone	39	Butyl butanoate	58	Dodecane	76	1,2,3,6,7,8,9,10,11,12-Decahydrobenzo[e]pyrene

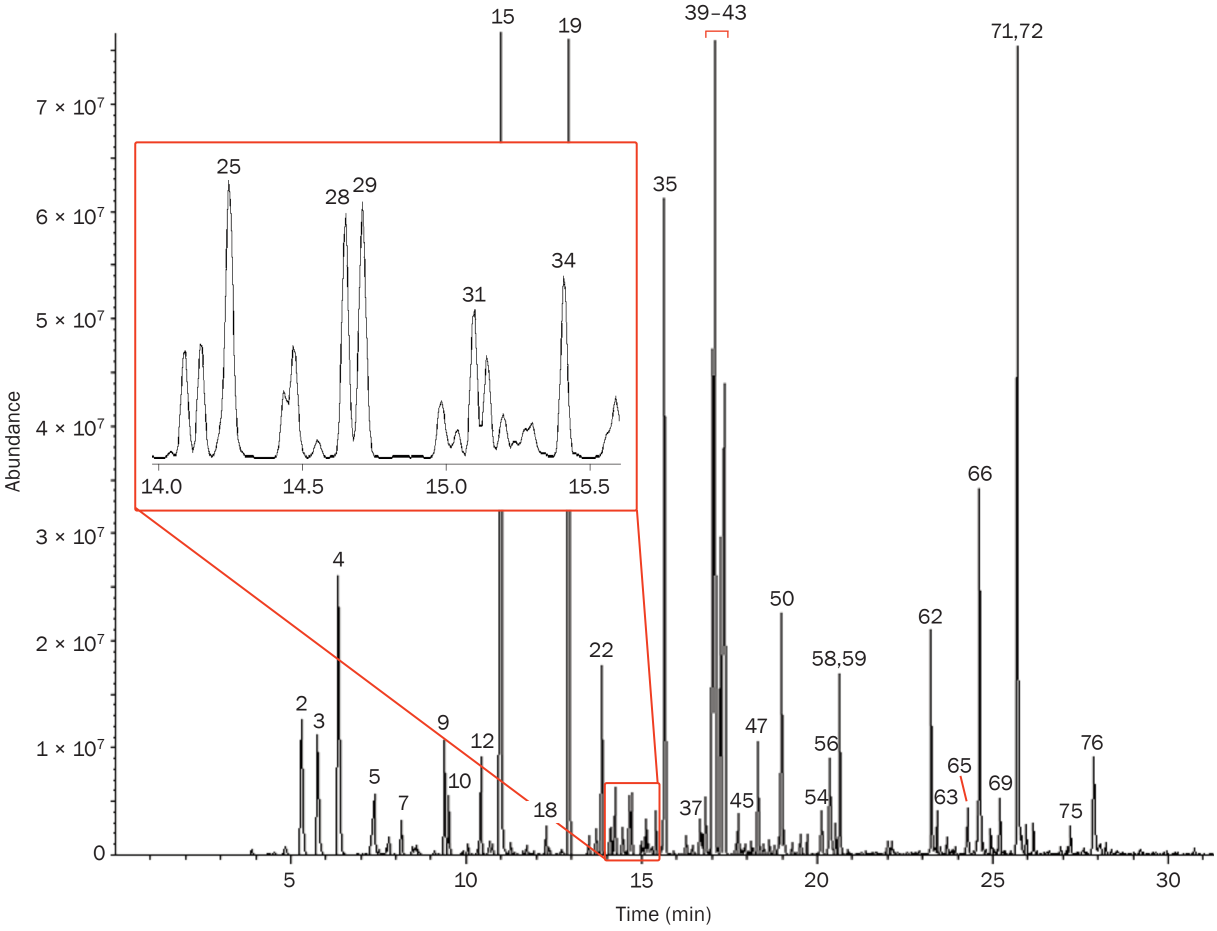


Figure 3: Analysis of the headspace profile of a whole strawberry, sampled onto a Tenax TA–SulfiCarb sorbent tube using the Micro-Chamber/Thermal Extractor, and analysed by GC/TOF MS. The 40 most abundant compounds are labelled (the numbers correlate to those in Table A1 in the Appendix).

2. Comparison of whole and chopped strawberries

Figure 4 shows the increased levels of volatile compounds released when the strawberry is chopped before being placed in the Micro-Chamber/Thermal Extractor.

Processing these two chromatograms against a customised library using TargetView allowed extraction of the deconvolved peak areas for both samples. These values are compared in Figure 5, which highlights the tendency for the higher-boiling components (including furaneol) to be more prevalent in the aroma profile of the chopped strawberry. This data is a good example of the use of the Micro-Chamber/Thermal Extractor for making quick comparisons of similar foodstuffs – such as between different varieties of fruit, foods stored under different conditions, or (as here) samples prepared in different ways.

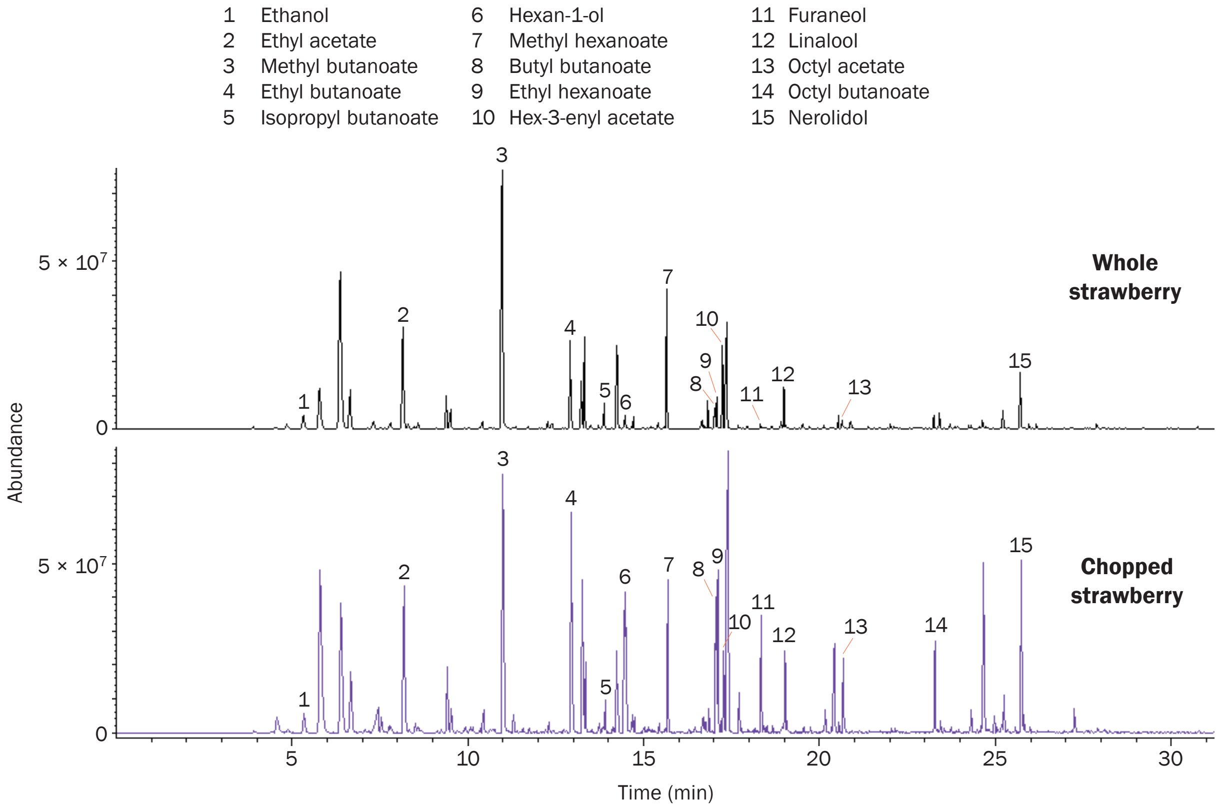


Figure 4: Comparison of the headspace profiles of whole and chopped strawberries sampled onto a Tenax TA–Carbograph 5TD sorbent tube using the Micro-Chamber/Thermal Extractor, and analysed by GC/TOF MS.

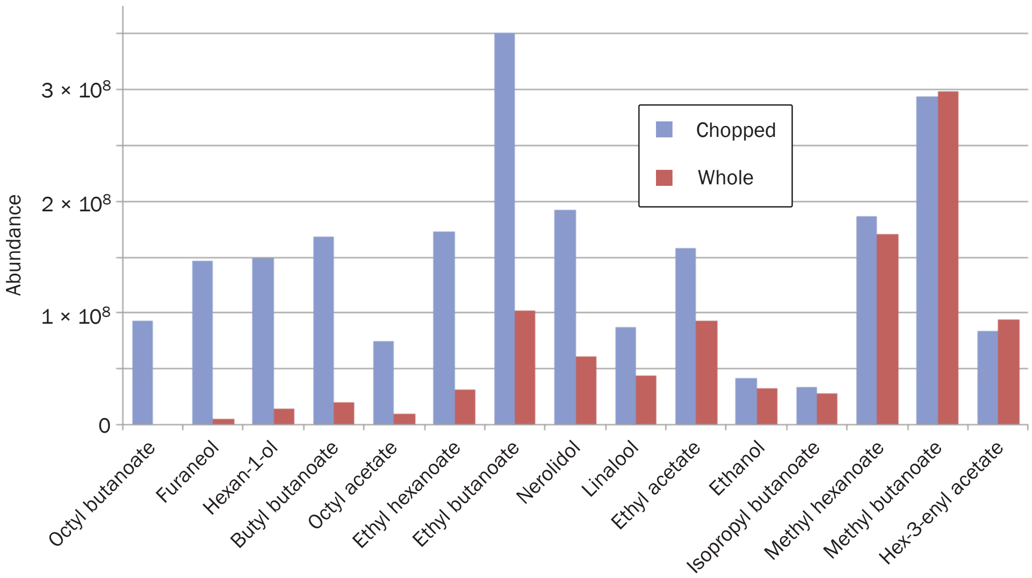


Figure 5: Comparison of peak sums for selected volatiles in the headspace profiles of whole and chopped strawberries shown in Figure 4. Compounds are displayed in order of decreasing [chopped]/[whole] ratio.

3. Detection of sulfur compounds

Low-level sulfur compounds in the aroma profile of strawberries and other foods are known or suspected to be strongly linked to customer perception of quality and flavour[1,2]. However, previous studies into their sensory role have been hampered by the difficulty in detecting and identifying these compounds at very low levels, particularly in complex data sets, where they can be hidden under baseline features or more abundant components.

While the analytical conditions used in this case were selected for compatibility with the widest analyte range and were not optimised for complete recovery of the most reactive sulfur compounds such as thiols, the whole-strawberry data (Figure 3) was nevertheless evaluated against a target library containing 26 sulfur-containing compounds. The results of this search are shown in Table 1.

This search demonstrated the presence of seven sulfur species from the library, including dimethyl disulfide and ethyl (methylthio)acetate, as shown in Figure 6. Cross-checking the spectra of these trace-level components against those in NIST confirmed their identity. While it is interesting that a significant sulfur dioxide peak was detected, this compound is difficult to analyse using conventional GC methods, and is unlikely to be quantitative.

Target compound	Retention time (min)	Match coeff.	Peak sum (TIC)
Sulfur dioxide	4.53	0.941	2 157 741
Carbon disulfide	6.64	0.958	219 426
Methyl thioacetate	10.42	0.926	42 406 848
Dimethyl disulfide	11.72	0.881	3 471 545
Dimethyl sulfone	12.51	0.870	14 638
S-Methyl butanethioate	15.04	0.881	2 187 939
Ethyl (methylthio)acetate	16.93	0.843	458 369

Table 1: Report identifying the sulfur compounds found in the headspace profile of a whole strawberry (Figure 3), following processing against a customised library using TargetView.

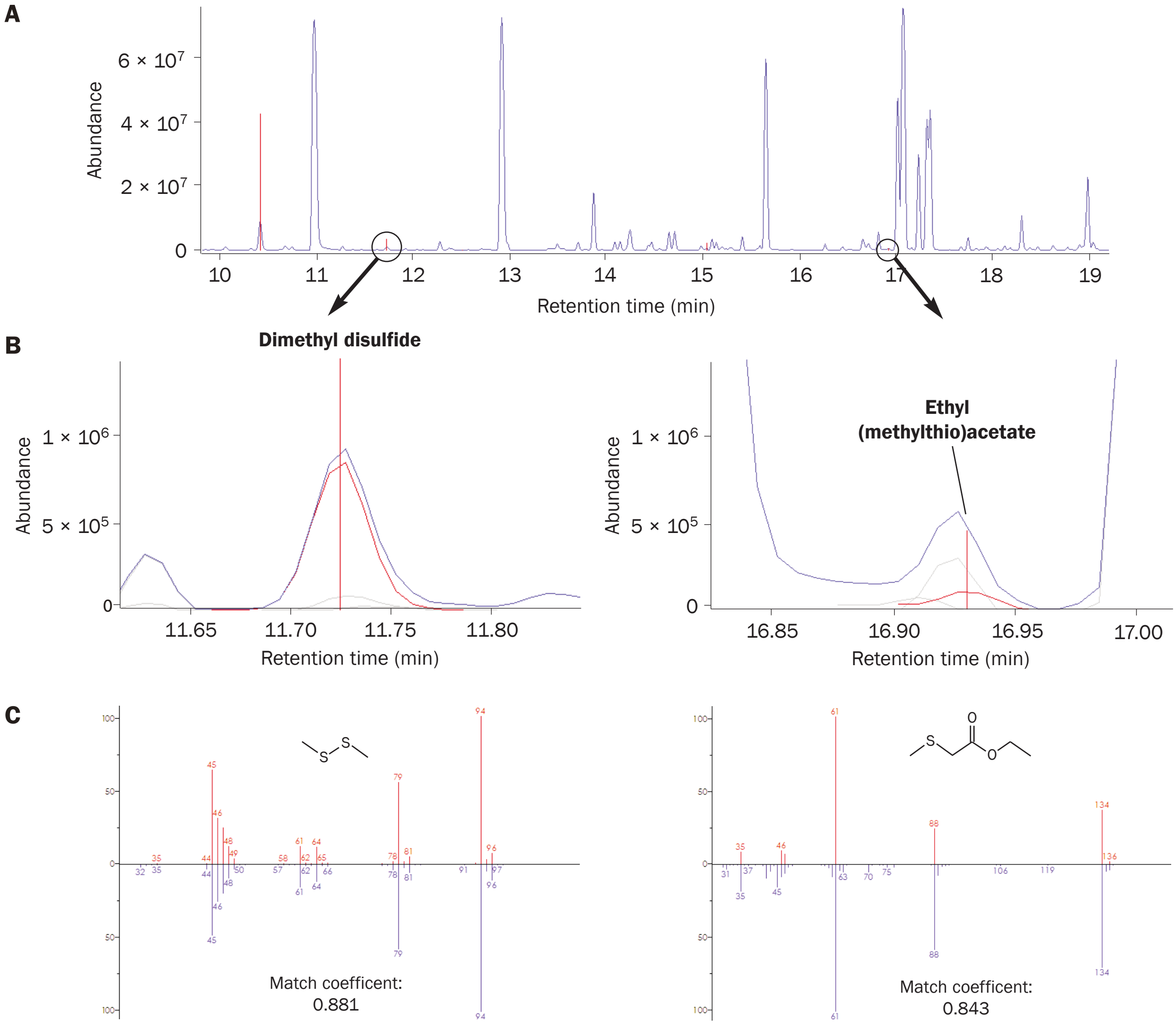


Figure 6: (A, B) Expansions of Figure 3, showing detection of the deconvolved peaks for dimethyl disulfide and ethyl (methylthio)acetate (red traces) alongside co-eluting peaks (grey traces). (C) The corresponding mass spectra (top, red) compared to those in the NIST library (bottom, blue).

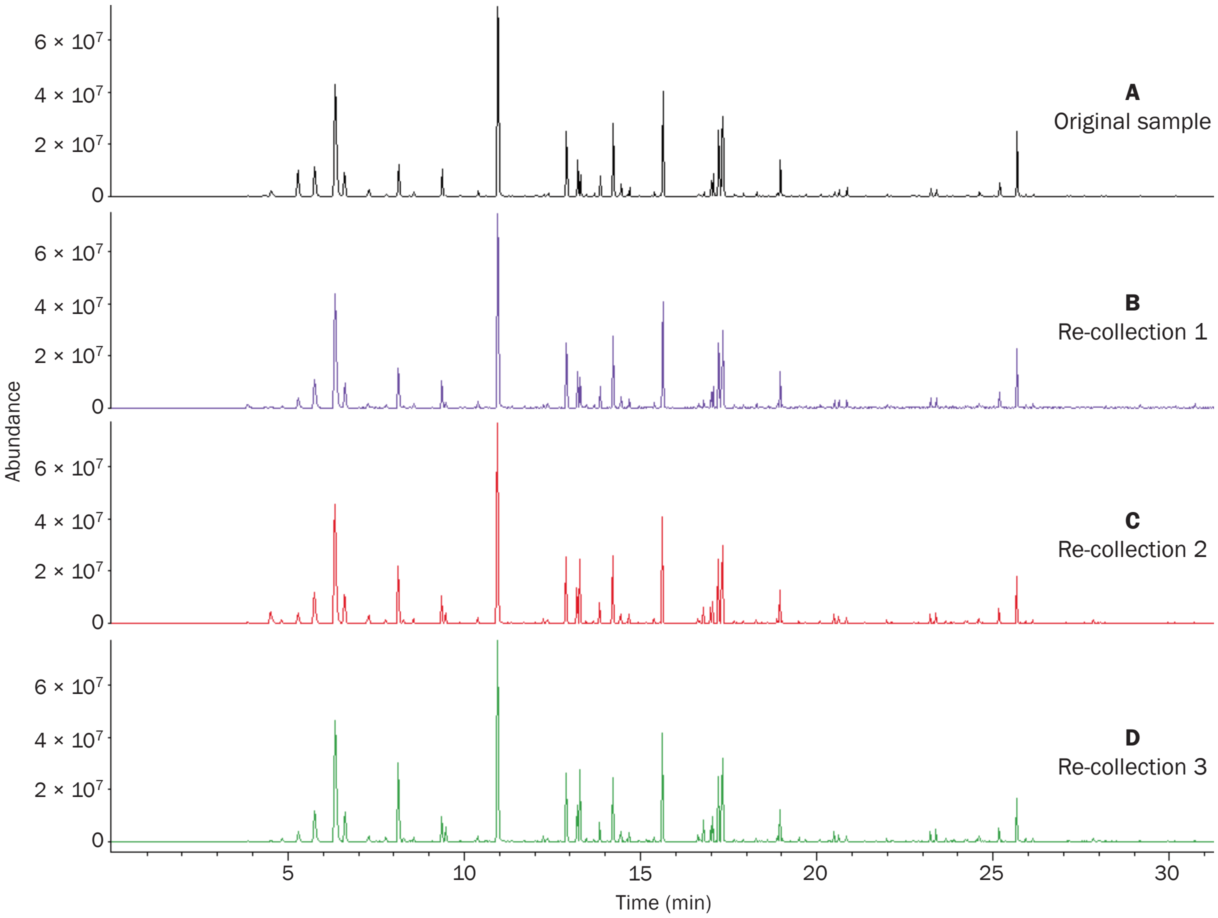


Figure 7: (A) Analysis of the headspace profile of a whole strawberry, sampled onto a Tenax TA–Carbograph 5TD sorbent tube using the Micro-Chamber/Thermal Extractor, and analysed by GC/TOF MS. (B–D) Subsequent analyses following re-collection of the sample onto a Tenax TA–Carbograph 5TD tube.

4. Method validation

Split-flow re-collection and repeat analysis were used to evaluate analyte recovery and method repeatability. This process was repeated three times for the whole-strawberry headspace sample using the TD-100 thermal desorber, and the results are shown in Figure 7. The four chromatograms are visually very similar, indicating good recovery of all major components from the original sample, and the integrity of the analytical process.

Conclusions

In this Application Note, we have shown the ease with which strawberry aroma compounds can be profiled when the inherently sensitive techniques of dynamic headspace extraction, thermal desorption and TOF MS are combined with compound identification using TargetView. This approach has numerous applications – for example, determining the compounds that give rise to off-odours, in formulating products containing strawberries, or studying the differences in volatile profiles between strawberry cultivars/varieties.

Used together, the Micro-Chamber/Thermal Extractor and TD-100 allow rapid and straightforward VOC/SVOC sampling, with their flexibility making for an almost universal sampling and analytical platform for GC-compatible compounds. At the same time, the re-collection capability of the TD-100 makes it easy for the analyst to re-run samples to investigate compound recovery and analytical bias.

Finally, the benefits of TargetView software, with its sophisticated combination of spectral deconvolution and library matching, has been shown to automate and greatly enhance detailed post-run data analysis.

References and notes

1. X. Du, V. Whitaker and R. Rouseff, Changes in strawberry volatile sulfur compounds due to genotype, fruit maturity and sample preparation, *Flavour and Fragrance Journal*, 2012, 27: 398–404.
2. X. Du, M. Song and R. Rouseff, Identification of new strawberry sulfur volatiles and changes during maturation, *Journal of Agricultural and Food Chemistry*, 2011, 59: 1293–1300.
3. J. Dong, Y. Zhang, X. Tang, W. Jin and Z. Han, Differences in volatile ester composition between *Fragaria* × *ananassa* and *F. vesca* and implications for strawberry aroma patterns, *Scientia Horticulturae*, 2013, 150: 47–53.

4. 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 are inert-coated for compatibility with reactive chemicals (*e.g.* odorous compounds), and 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.

5. For examples of the use of dynamic headspace and TD to analyse aroma profiles from bulk materials, see: Markes International Application Notes: TDTS 88 (fruit juices and wine); TDTS 96 (tobacco); TDTS 101 (cheese). See http://www.markes.com/Downloads/Application-notes.aspx.

6. 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 http://www.markes.com/Blog/2012/04/Reverse-logic-Backflush-operation-for-thermal-desorption/ and Application Note TDTS 64.

7. For further details of the TD-100, visit http://www.markes.com/Instrumentation/TD100.aspx.

8. For further details of the BenchTOF-dx, visit http://www.almsco.com/Products/BenchTOF-dx/default.aspx.

9. For more examples of the use of TargetView, visit http://www.almsco.com/Products/Applications.aspx.

10. Y. Chen and L.M. Sidisky, Quantification of 4-hydroxy-2,5-dimethyl-3-furanone in fruit samples using solid-phase microextraction coupled with gas chromatography–mass spectrometry, *Journal of Chromatography A*, 2011, 1218: 6817–6822.

11. See Markes International Application Note TDTS 32 for more information about the analysis of sulfur compounds using TD–GC(MS).

Trademarks

DiffLok™, Micro-Chamber/Thermal Extractor™, SulfiCarb™ and TD-100™ are trademarks of Markes International Ltd, UK.

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Appendix

No.	Compound name	Retention time (min)	Match factor	Peak sum (TIC)	Concentration (ng/g)[a]
1	Isobutene	4.85	929	5 171 958	0.48
2	Ethanol	5.31	940	108 273 359	10.1
3	Acetone	5.77	954	88 110 037	8.23
4	Methyl acetate	6.36	945	181 172 417	16.9
5	Acetic acid	7.39	937	60 241 117	5.63
6	Hexane	7.80	964	8 381 565	0.78
7	Ethyl acetate	8.15	898	15 840 583	1.48
8	Methyl propanoate	8.60	918	4 080 978	0.38
9	Butan-1-ol	9.39	891	52 696 210	4.92
10	Benzene	9.51	940	26 841 797	2.51
11	Pentan-2-one	10.06	898	4 397 158	0.41
12	Methyl thioacetate	10.42	922	42 404 158	3.96
13	Ethyl propanoate	10.68	937	4 631 618	0.43
14	n-Propyl acetate	10.75	927	3 462 729	0.32
15	Methyl butanoate	10.98	891	520 723 683	48.6
16	3-Methylbutan-1-ol	11.27	939	4 323 274	0.40
17	Dimethyl disulfide	11.72	931	3 452 889	0.32
18	Benzyl methyl ketone	12.28	712	10 324 394	0.96
19	Ethyl butanoate	12.92	941	385 430 186	36
20	Methyl pentanoate	13.49	887	5 483 698	0.51
21	Methyl 2-hydroxybutanoate	13.71	859	9 464 464	0.88
22	Isopropyl butanoate	13.87	930	70 329 702	6.57
23	Ethyl 2-methylbutanoic acid	14.09	951	8 480 864	0.79
24	Ethyl 3-methylbutanoate	14.15	924	9 144 115	0.85

Table A1: Major component list of whole strawberry emission profile. [a] Concentrations determined in terms of toluene equivalents by comparison with the peak area of a toluene standard analysed under the same conditions (*Continued on next page*).

No.	Compound name	Retention time (min)	Match factor	Peak sum (TIC)	Concentration (ng/g)[a]
25	Hex-3-en-1-ol	14.25	931	24 037 661	2.25
26	Hex-2-en-1-ol	14.44	849	5 850 983	0.55
27	Hexan-1-ol	14.47	895	9 267 260	0.87
28	3-Methylbutyl acetate	14.65	943	19 095 855	1.78
29	2-Methylbutyl acetate	14.71	940	18 719 139	1.75
30	5-Methylhexan-2-one	14.99	817	3 368 986	0.31
31	Propyl butanoate	15.10	925	11 720 866	1.09
32	Ethyl pentanoate	15.15	909	6 468 384	0.60
33	Styrene	15.20	924	3 223 169	0.30
34	Pentyl acetate	15.41	889	13 327 729	1.24
35	Methyl hexanoate	15.66	919	279 029 740	26.1
36	2-Methylpropyl butanoate	16.27	921	5 807 147	0.54
37	Benzaldehyde	16.66	947	12 868 346	1.20
38	Phenol	16.72	890	7 773 217	0.73
39	Butyl butanoate	17.02	905	197 987 123	18.5
40	Ethyl hexanoate	17.08	917	360 089 117	33.6
41	Hex-3-enyl acetate	17.24	916	122 989 019	11.5
42	Hexyl acetate	17.33	934	266 096 631	24.9
43	Hex-2-enyl acetate	17.36	815	68 809 315	6.43
44	2-Ethylhexan-1-ol	17.68	884	3 476 584	0.32
45	Isopropyl hexanoate	17.75	884	12 125 237	1.13
46	3-Methylbutyl butanoate	18.13	904	4 451 513	0.42
47	Furaneol	18.31	910	36 199 915	3.38
48	Dihydromyrcenol	18.47	871	3 297 492	0.31
49	Acetophenone	18.63	933	4 767 753	0.45
50	Linalool	18.99	906	81 863 418	7.65
51	Nonanal	19.04	729	7 571 481	0.71
52	Methyl octanoate	19.29	886	3 742 245	0.35
53	2-Ethylhexyl acetate	19.71	917	6 038 606	0.56
54	Benzyl acetate	20.13	920	13 876 676	1.30
55	Hex-3-enyl butanoate	20.31	724	3 829 186	0.36
56	Butyl hexanoate	20.36	857	28 479 301	2.66
57	Ethyl octanoate	20.43	875	3 839 019	0.36
58	Dodecane	20.52	889	9 530 542	0.89
59	Octyl acetate	20.65	909	57 258 751	5.35
60	Tridecane	22.02	843	4 274 234	0.40
61	Nonyl acetate	22.12	838	3 705 880	0.35
62	Octyl butanoate	23.25	922	71 266 909	6.66
63	Tetradecane	23.41	890	11 977 368	1.12
64	Biphenyl	23.70	953	6 053 603	0.57
65	β-Farnesene	24.29	718	12 713 145	1.19
66	Decalactone	24.62	937	172 028 704	16.1
67	α-Farnesene	24.94	879	6 914 122	0.65
68	β-Bisabolene	25.15	887	3 148 402	0.29
69	Butylated hydroxy toluene	25.20	895	14 325 161	1.34
70	α-Muurolene	25.22	800	7 044 615	0.66
71	Nerolidol	25.70	917	341 296 175	31.9
72	Octyl hexanoate	25.76	845	14 190 733	1.33
73	Hexadecane	25.95	900	7 833 663	0.73
74	Isobutyl 2,2,4-trimethyl-3-carboxyisopropylpentanoate	26.16	884	8 199 625	0.77
75	Dodecalactone	27.22	833	9 962 960	0.93
76	1,2,3,6,7,8,9,10,11,12-Decahydrobenzo[e]pyrene	27.88	758	62 634 375	5.85

Table A1: (*Continued*).