Aroma profiling of hops and beer using high-capacity sorptive extraction with GC×GC–FID/TOF MS/SCD

This study demonstrates the use of a multi-hyphenated analytical system to provide flexible yet comprehensive aroma profiling of hops and beer, as well as detection of trace odour taints, on a single, fully-automated platform.

Introduction

Beer contains hundreds of organic ingredients, and the combination of these results in its unique aroma and flavour. Many of the aroma-active compounds come from hops, and monoterpenes (C_{10}) and sesquiterpenes (C_{15}) in particular provide much of the characteristic 'bitterness' of the finished beer.[1]

However, these compounds can span many orders of magnitude in concentration and may have very low odour thresholds, so robust and highly sensitive analysis is needed to assess the quality of the hops before brewing commences.

Traditionally, solid-phase micro-extraction (SPME) has been used to sample volatile organic compounds (VOCs) from foods and beverages. Although SPME is fast and simple, it can suffer from limited sensitivity and poor reproducibility, and can be prone to capillary effects that limit fiber lifetime when immersive sampling is used.

High-capacity sorptive extraction can tackle these issues, by providing a larger volume of PDMS stationary phase (65 µL compared to ~0.5 µL for SPME) that results in higher sample loadings. The probes are also robust and easily rinsed free of matrix for trouble-free immersive sampling of beer. Used in conjunction with secondary refocusing, this approach offers excellent sensitivity, as well as the ability to re-collect a portion of the sample for repeat analysis in a fully automated workflow, using the Centri® platform from Markes International.

However, even when benefitting from the advantages of such systems, the aroma profiles of hops and beer are often highly complex. As a result, higher-loading components can frequently mask important compounds (such as sulfur species that cause undesirable odour taints even at trace levels).

Comprehensive two-dimensional gas chromatography (GC×GC) coupled with time-of-flight mass spectrometry (TOF MS) is an excellent choice to tackle these challenges, with the enhanced separation capacity of GC×GC complementing the ability of TOF MS to provide confident identification of targets and unknowns.

In this study, we apply flow-modulated GC×GC within a completely cryogen-free configuration that allows parallel detection by three methods, all in a single run:

► Flame ionisation detection (FID) for robust quantitation of the high-loading terpenes.

► TOF MS for confident identification of aroma-active species.

► Sulfur chemiluminescence detection (SCD) for highly specific sulfur detection.

As we will show, the result of using this setup is confident but affordable aroma profiling, illustrated here with both hops and beer.

Experimental

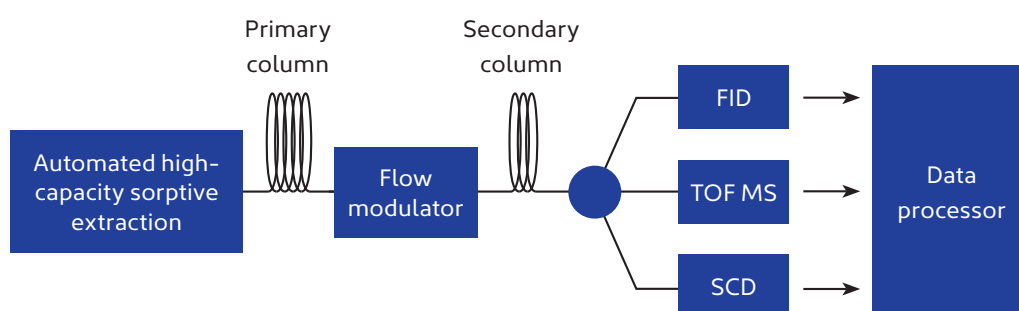The experimental workflow is shown in Figure 1.

Figure 1

Schematic and photograph of the analytical system used in this study.

Samples: (A) American hops ('Citra', 'Mosaic' and 'Amarillo') (2 g); (B) American pale ale (10 mL). Samples were placed in a 20 mL headspace vial.

Sampling and preconcentration: Instrument: Centri® (Markes International). Sampler: Inert-coated stainless steel HiSorb™ PDMS probe (Markes International). Equilibration time: 60 min; Temperature: 35°C; Agitation: 400 rpm. HiSorb probes were desorbed at 260°C (for 10 min) in the inlet of the Centri platform.

GC×GC: Flow modulator: INSIGHT® (SepSolve Analytical); P_M: 5 s. Parallel detection was configured using a three-way splitter.

FID: H_2 flow: 30 mL/min; Air flow: 300 mL/min; Temperature: 300°C.

TOF MS: Instrument: BenchTOF-Select™; Mass range: m/z 35–600.

SCD: Burner temperature: 800°C; Base temperature: 250°C; Oxidiser flow: 11.5 mL/min; Upper H_2 flow: 38 mL/min; Lower H_2 flow: 8 mL/min.

Software: Full instrument control and data processing of FID, TOF MS and SCD data using ChromSpace® (SepSolve Analytical).

Please contact SepSolve for full analytical parameters.

Results and discussion

1. Screening by FID

The monoterpenes and sesquiterpenes in beer are often found at concentrations many orders of magnitude greater than other components, making it a challenge to quantify them in a single GC–MS run while also investigating trace-level species. Using GC×GC with parallel detection allows FID to be employed to capture these high-loading species.

Table 2 lists the major terpenes found in the three hop varieties, and this data is shown graphically in Figure 2. These eight components alone make up ~70% of the total volatiles present in each case, and β-myrcene was present at levels at least two orders of magnitude greater than the other terpenes shown. On the other hand, β-farnesene was found in much lower abundance, and not detected in 'Mosaic' at all.

Compound	Composition (%)			Aroma[2]
	Amarillo	**Mosaic**	**Citra**	
β–Myrcene	88.01	95.95	96.67	Spicy
β–Pinene	3.39	0.30	0.38	Herbal
Limonene	3.00	1.85	1.52	Citrus
α–Humulene	2.62	0.85	0.46	Woody
β–Caryophyllene	1.20	0.38	0.39	Spicy
α–Pinene	1.02	0.56	0.44	Herbal
Linalool	0.47	0.11	0.13	Floral
β–Farnesene	0.28	0.00	0.01	Woody

Table 2

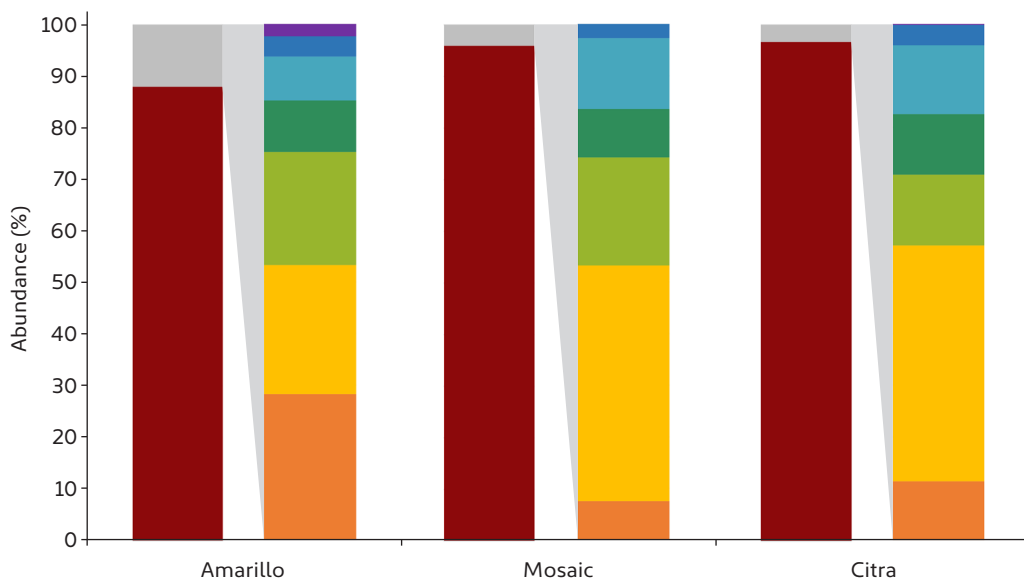Percentage composition of the key mono- and sesquiterpenes in the three hop varieties.

2. Confident identification by TOF MS

To investigate the remaining aroma-active species, the GC×GC–TOF MS data was examined. Figure 3 shows an expansion of a region of high complexity in the profile of 'Citra', demonstrating the enhanced separation achieved, and how this avoids the co-elution that would have occurred if 1D GC had been used. The associated spectra (Figure 4) show the 'quadrupole-like' data generated by BenchTOF™, producing excellent matches to the spectra in reference libraries.
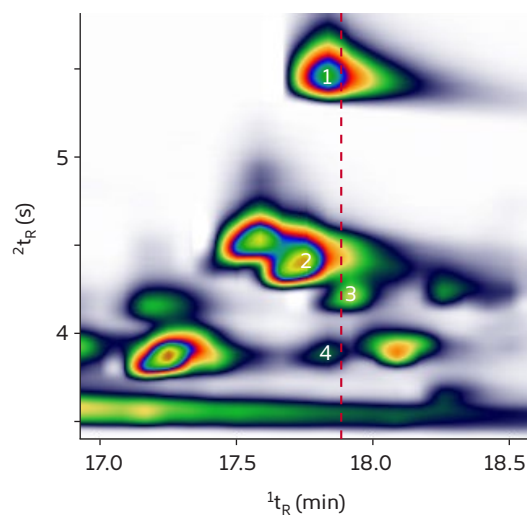
1: Linalool

2: Perillene

3: 1,1-Dimethyl-4-methylenehexahydro-4H-cyclopenta[c]furan

4: 2-Methylbutyl 2-methylbutanoate

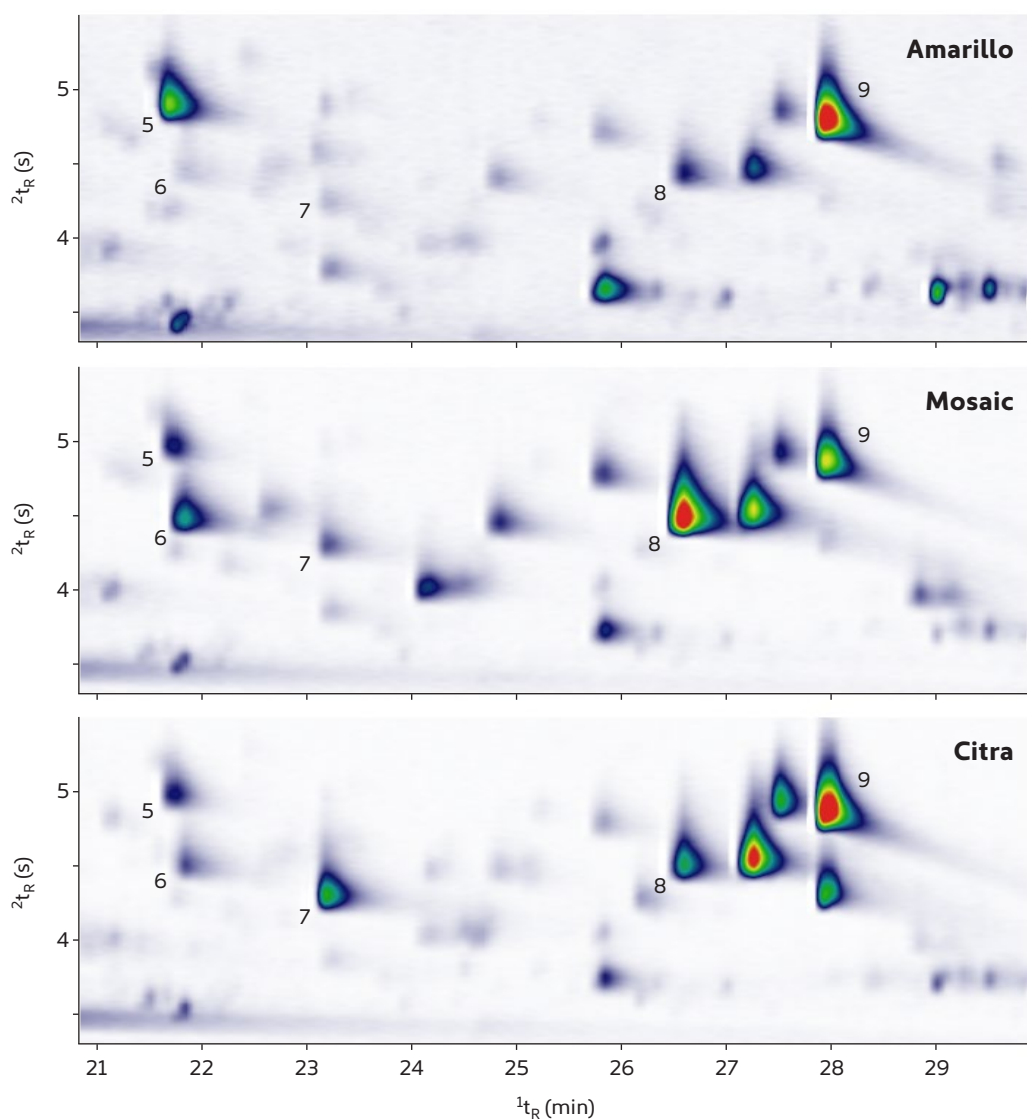The enhanced separation also enables differences between complex aroma profiles to be spotted more readily (Figure 5).

Figure 5

GC×GC–TOF MS colour plots for a region of the headspace aroma profile of the three hop varieties. Compounds showing major differences in abundance are indicated, with their aromas.[2]

5 Neral
   Sweet, citrus, lemon
6 Decan-2-one
   Orange, floral
7 Methyl nonanoate
   Sweet, fruity, pear
8 Undecan-2-one
   Sweet, fruity
9 Methyl geranate
   Green, fruity, waxy

3. Sensitive sulfur detection by SCD

Sulfur chemiluminescence detection (SCD) provides highly selective and sensitive analysis of sulfur species, which often cause undesirable odour taints in food and beverages. Figure 6 shows the relative abundances of sulfur compounds detected in the three hop varieties. Dimethyl disulfide ("sulfurous, vegetable, cabbage, onion"[2]) and dimethyl sulfide ("sulfurous, onion, sweet"[2]) were found in highest abundances, while 'Amarillo' had the lowest overall content of sulfur compounds.
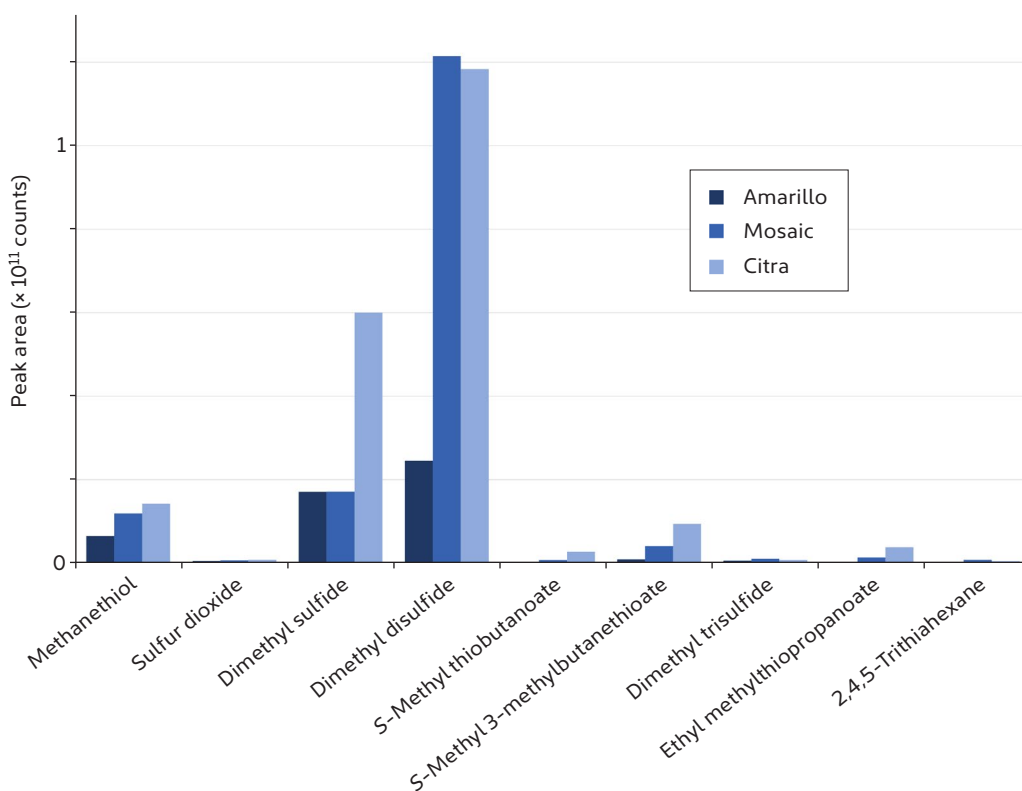

Figure 6
SCD peak areas for sulfur compounds detected in the three hop varieties.

To further demonstrate the power of parallel detection by SCD, immersive sorptive extraction was performed on an American pale ale, with the HiSorb™ probes being rinsed free of matrix in the automated wash/dry station of Centri®.

As shown in Figure 7, the SCD data helped to locate the trace-level sulfur species in the TOF MS data, where they may otherwise have been overlooked, or hidden by higher-loading peaks.

Of particular importance is the identification of 3-methylbut-2-ene-1-thiol (#10, 3-MBT), which is well-known to cause an undesirable 'lightstruck' or 'skunky' character in beer.[3] With flavour thresholds in the low-ppt range, it is important to have access to highly-sensitive instrumentation in order to detect this potential flavour taint.
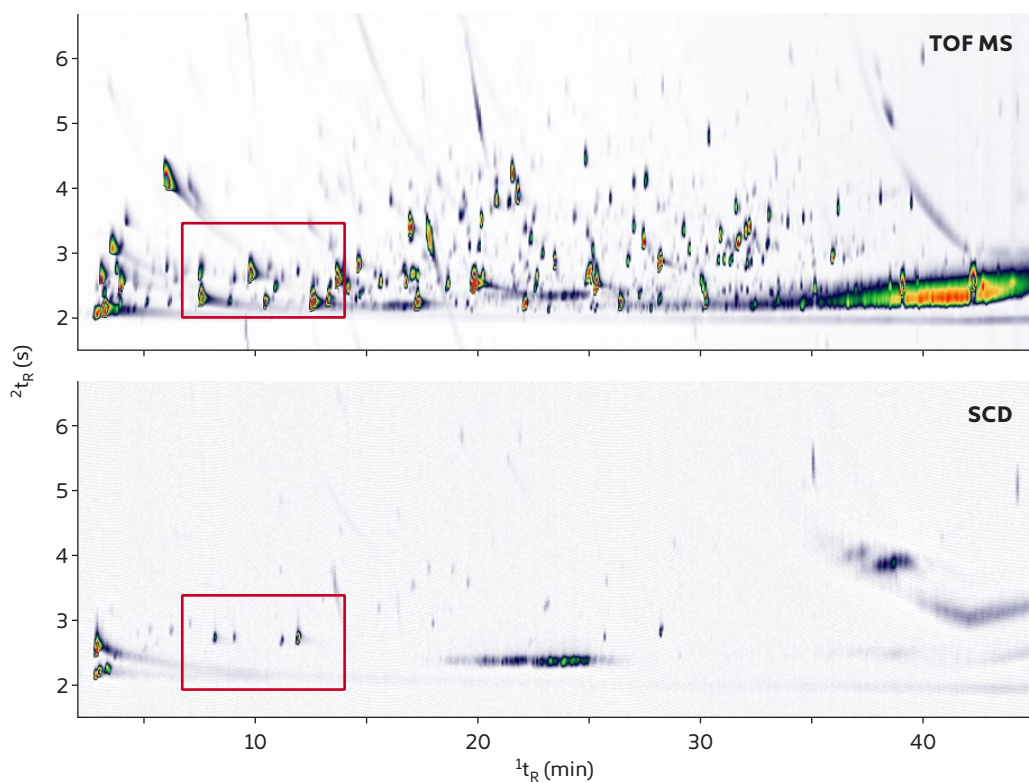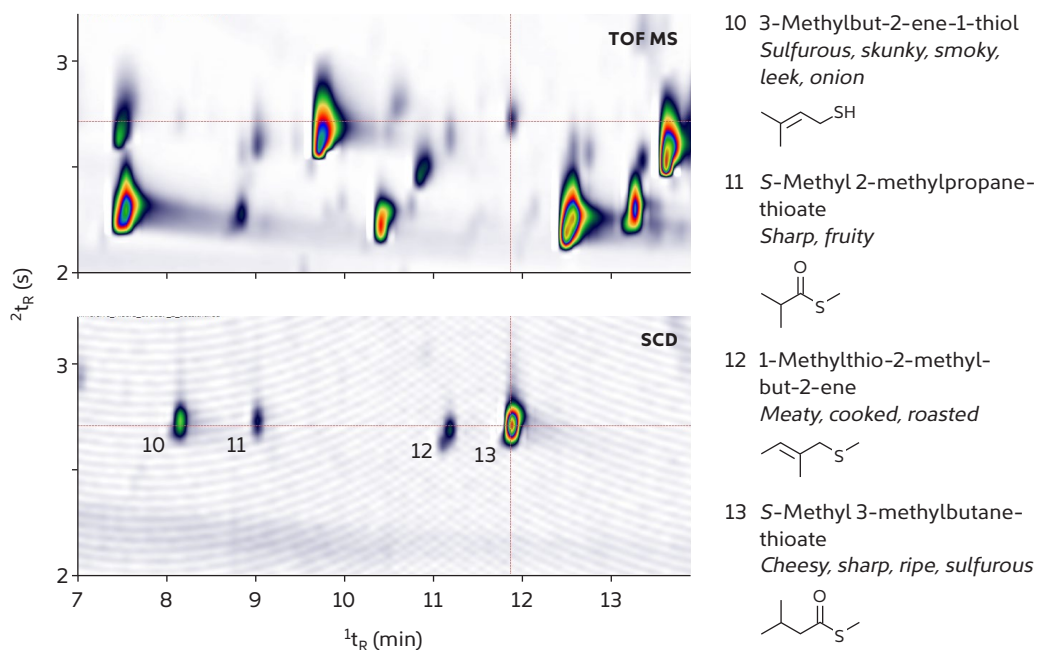
10 3-Methylbut-2-ene-1-thiol
Sulfurous, skunky, smoky, leek, onion

11 *S*-Methyl 2-methylpropane-thioate
Sharp, fruity

12 1-Methylthio-2-methyl-but-2-ene
Meaty, cooked, roasted

13 *S*-Methyl 3-methylbutane-thioate
Cheesy, sharp, ripe, sulfurous

The BenchTOF™ mass spectrometer used in this study provides highly sensitive detection, which in conjunction with its reference-quality spectra allows these sulfur compounds to be targeted (using guidance from the SCD data) and identified confidently. The same features also enable it to screen the entire aroma profile, and in the pale ale over 600 peaks were detected.

Conclusions

This study has shown that high-capacity sorptive extraction in conjunction with GC×GC–FID/TOF MS/SCD provides:

► Robust and sensitive sampling (in both headspace and immersive modes) using HiSorb™ high-capacity sorptive extraction probes, automated on the Centri® platform.

► Cryogen-free, solvent-free preconcentration and INSIGHT® consumable-free flow modulation, for major cost savings.

► Comprehensive aroma profiles for beverages and their raw materials (e.g. beer and hops), with confident identification using BenchTOF™.

► Highly sensitive screening for odour taints caused by trace sulfur species.

► Fully-automated workflows for unattended operation.

► Full instrument control and simple, unified data processing in ChromSpace® software.

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

References

[1] J.L. Gonçalves et al., A powerful methodological approach combining headspace solid phase microextraction, mass spectrometry and multivariate analysis for profiling the volatile metabolomic pattern of beer starting raw materials, *Food Chemistry*, 2014, 160: 266–280, http://doi.org/10.1016/j.foodchem.2014.03.065.

[2] The Good Scents Company Information System (search facility), www.thegoodscentscompany.com/search2.html (accessed on 8th March 2019).

[3] G. Lermusieau and S. Collin, Volatile sulfur compounds in hops and residual concentrations in beer – A review, *Journal of the American Society of Brewing Chemists*, 2003, 61: 109–113, http://doi.org/10.1094/ASBCJ-61-0109.