White Paper 061
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Exploring the flavour complexity of fish sauce

This white paper investigates the flavour profile of fish sauce using HiSorb™ high-capacity sorptive extraction and GC×GC–TOF MS to gain more detailed insights into sample composition, enabling accurate brand comparisons and faster development of new flavourings.

Introduction

Fish sauce is a condiment commonly used in cooking, particularly in Southeast Asian dishes. It is made by fermenting fish, typically anchovies or other small fish, to produce a liquid with a strong, salty-savoury flavour [1]. Fish sauce adds a complex umami taste to dishes and is a staple ingredient in stir-fries, soups and marinades.

The analysis of volatiles in foods, such as fish sauce, plays a pivotal role in developing the sensory experience and understanding consumer preference. Unfortunately, this is a challenging task due to the complexity of the volatile profiles and the wide range of matrices involved. Additionally, odour thresholds span many orders of magnitude, meaning that sensitive analysis is essential to ensure that trace, yet potent, aroma-active compounds are detected.

Here, HiSorb high-capacity sorptive extraction probes were used to provide a larger volume of sorptive phase (~30 µL) compared to traditional solid-phase microextraction (SPME) (~0.5 µL), allowing higher sample loadings. When combined with trap-based focusing, this provides enhanced sensitivity and improved chromatographic performance. In addition, the strength of the metal probes compared to delicate SPME fibres enables a more robust immersive extraction technique.

However, greater extraction capability often leads to more complex chromatograms. Here, we demonstrate the use of comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC–TOF MS), which offers enhanced separation of complex aroma profiles, allowing confident identification of compounds that would co-elute in conventional 1D GC.

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In this white paper, we show how this advanced analytical technique can provide more detailed insights into flavour profiles, enabling more accurate brand comparisons, a better understanding of consumer preference, and accelerating the development of new flavourings.

Experimental

Samples: Three store-bought brands of fish sauce (herein labelled Brand A, B and C)

Extraction techniques:

All extractions were automated on the Centri® platform (Markes International). Extractions were performed in duplicate using DVB-PDMS-CWR as the sorptive phase.

- Headspace SPME: 5 mL fish sauce in a 20 mL vial, incubated at 50 °C (15 min) and extracted for 40 min
- Headspace HiSorb: 5 mL of fish sauce in a 20 mL vial, incubated at 50 °C (15 min) and extracted for 40 min
- Immersive HiSorb: 8 mL of fish sauce in a 10 mL vial, incubated at 40°C (2 min) and extracted for 60 min

Following headspace or immersive extraction, the HiSorb probes were rinsed, dried and desorbed in a fully automated workflow, with the analyte vapours concentrated on a focusing trap prior to GC–MS injection.

GC×GC: INSIGHT®-Flow modulator; P_M 2.4 s.

TOF MS: BenchTOF2™ mass spectrometer (SepSolve Analytical); m/z 30–300

Software: Full instrument control by ChromSpace®, with data mining and chemometrics in ChromCompare+.

Results and discussion

During method development, each fish sauce was analysed in duplicate using three different sample extraction techniques, namely headspace SPME, headspace sorptive extraction and immersive sorptive extraction. The resulting GC–TOF MS chromatograms for Brand A are provided in Figure 1. The increased capacity of HiSorb sorptive extraction probes provided improved extraction efficiency, with a larger number of components present in these chromatograms.

Semi-volatile higher-molecular-weight compounds, which do not readily partition into the headspace due to their low volatility, were more efficiently extracted by immersing the sorptive phase into the liquid sample, improving the discovery and identification of these components.

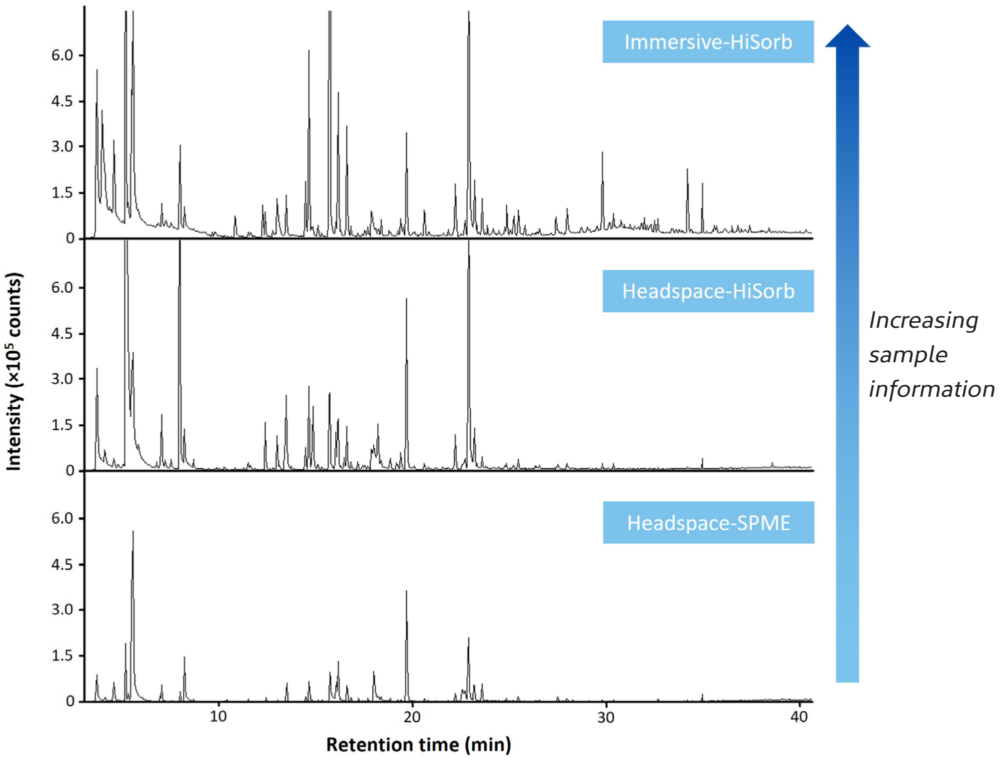


Figure 1

GC–TOF MS chromatograms showing the increase in extraction efficiency of HiSorb high-capacity sorptive extraction compared to SPME.

For example, Figure 2 shows a selection of aroma-active compounds that could only be identified in the samples extracted using immersive sorptive extraction. These compounds are important contributors to the overall aroma and flavour of fish sauce and may have been overlooked using headspace extraction techniques.

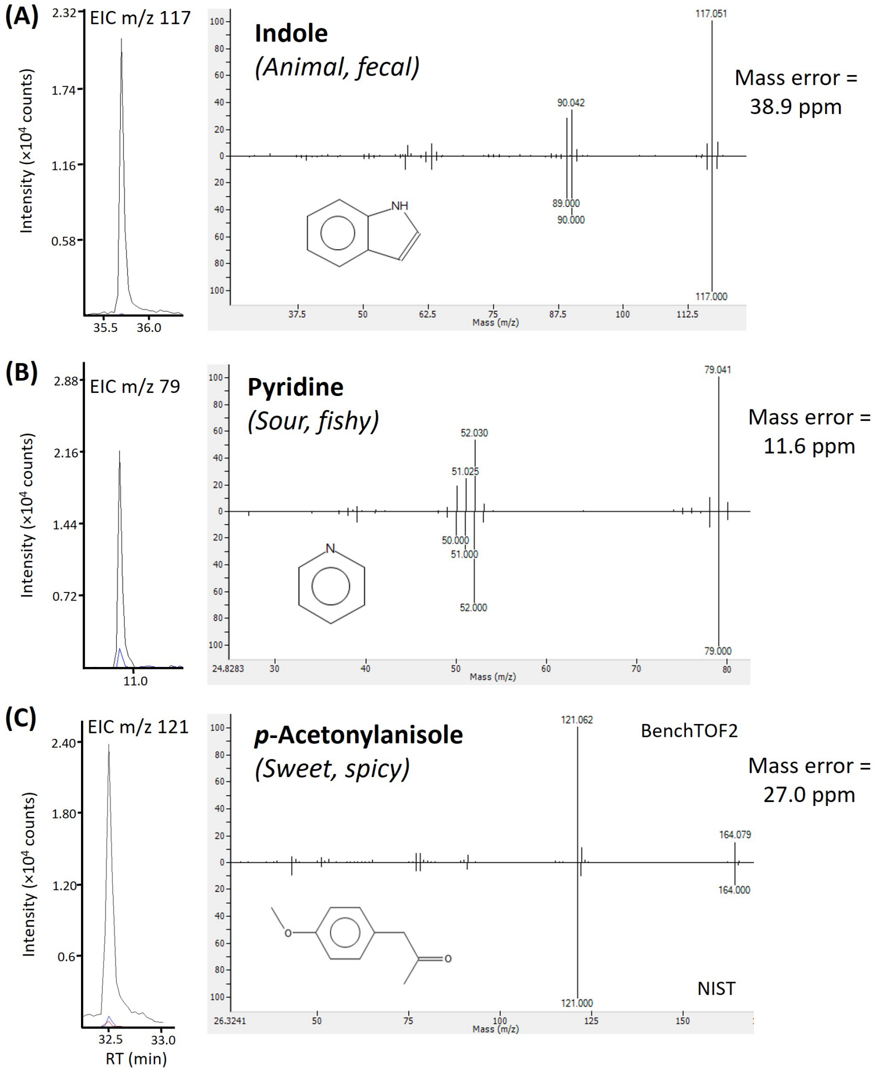
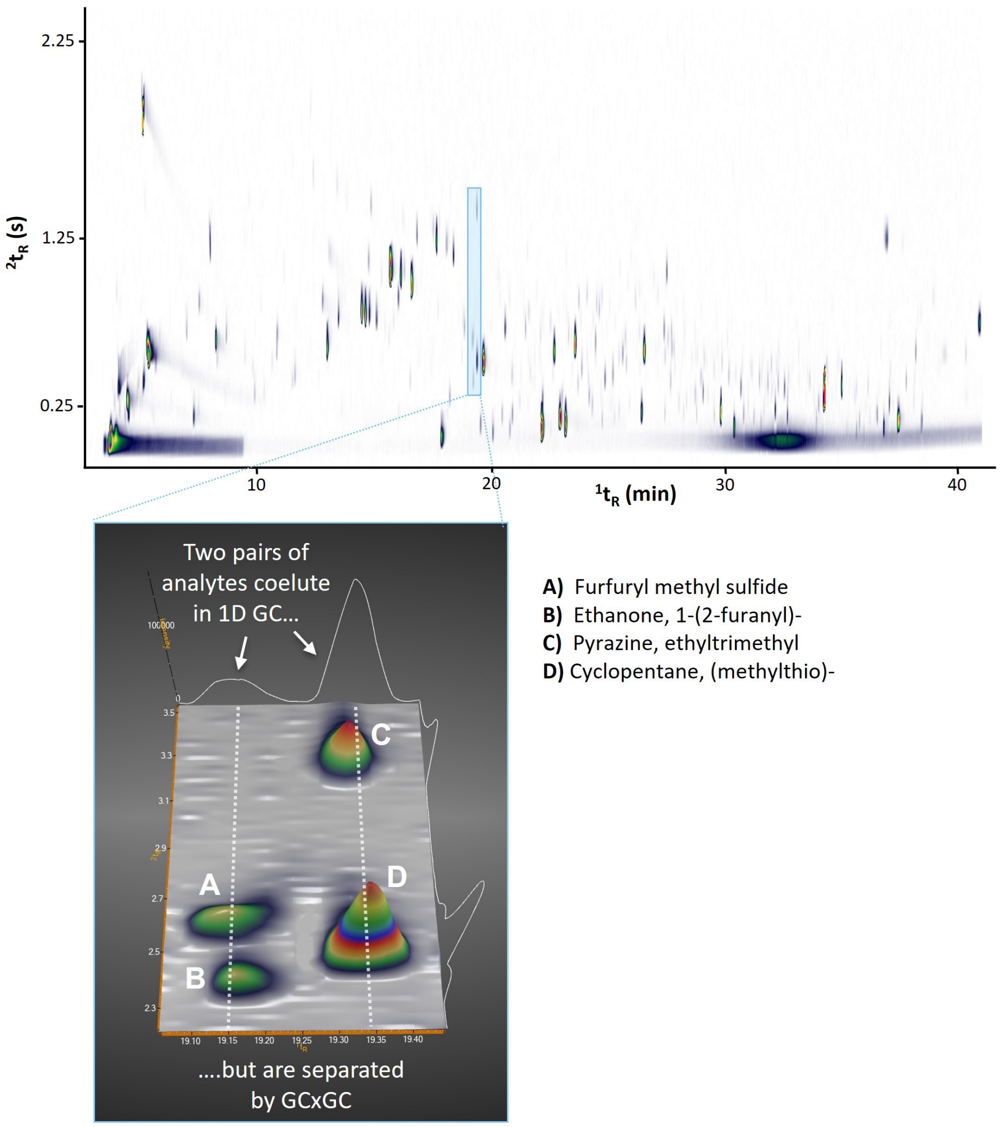


Figure 2

Overlaid EIC chromatograms (left) showing immersive HiSorb (black trace), headspace HiSorb (blue trace) and headspace SPME (red trace) for three flavour compounds in fish sauce that were only identified using immersive HiSorb extraction. Spectral comparisons (right) are provided for BenchTOF2 against the NIST23 library, with all identifications confirmed using the mass accuracy of BenchTOF2 (<50 ppm).

However, with increased extraction efficiency comes increased sample complexity, meaning that traditional 1D GC separations may not resolve all analytes. In such cases, the enhanced separating power of two-dimensional GC (GC×GC) becomes a necessity to resolve co-elutions that would have occurred in 1D GC. Figure 3 provides the GC×GC–TOF MS chromatogram for immersive sorptive extraction of a fish sauce, with over 200 individual peaks detected. The inset shows how two pairs of co-elutions in 1D GC are separated in the second dimension, providing cleaner spectra for more confident identification.



A) Furfuryl methyl sulfide
B) Ethanone, 1-(2-furanyl)-
C) Pyrazine, ethyltrimethyl
D) Cyclopentane, (methylthio)-

Figure 3
GC×GC–TOF MS colour plot for immersive sorptive extraction of fish sauce B, with expanded region showing separation of two pairs of analytes that would have coeluted in 1D.

The next task was to compare the three brands of fish sauce (Figure 4). To do this, a pooled data file was created using the "File Addition" tool in ChromCompare+. Deconvolution was applied to the pooled file to create a comprehensive library of 154 flavour compounds.

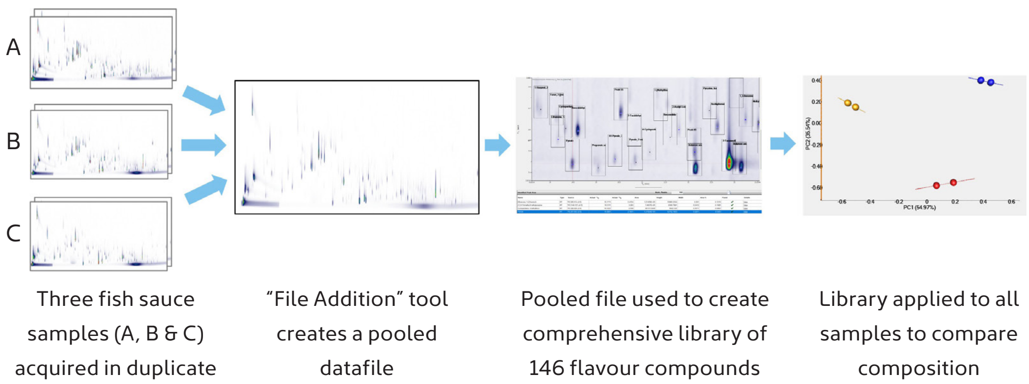


Figure 4
Workflow in ChromCompare+ software for comparative analysis of the flavour profiles.

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This library was then applied to all samples in a sequence, and the peak lists (with EIC peak areas) were automatically collated for easy comparison of composition (Figure 5).

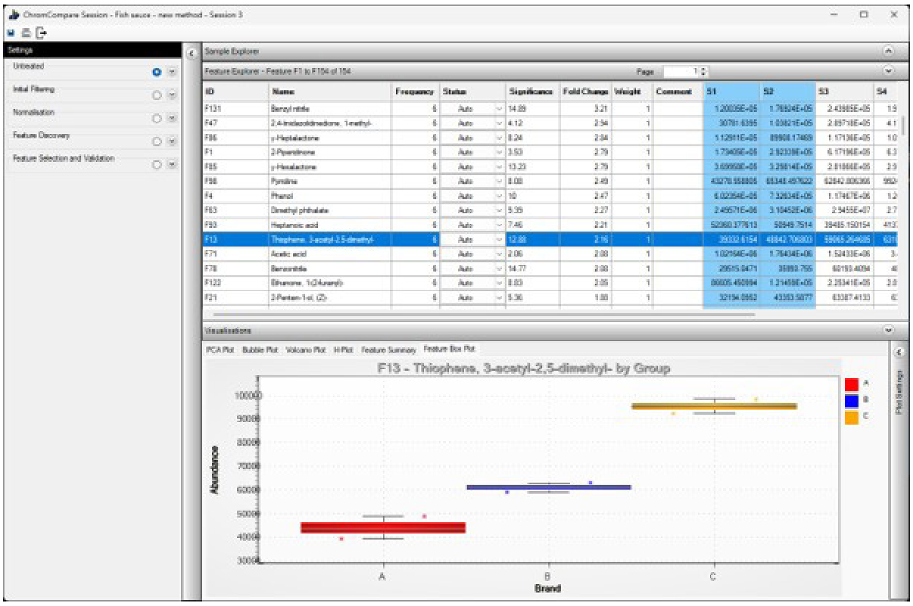
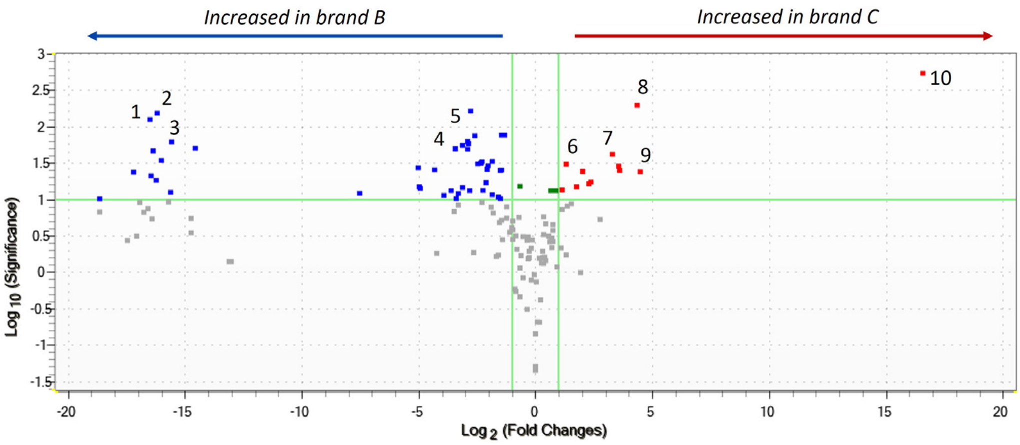


Figure 5

The ChromCompare+ project window showing the simple comparison of volatiles found in the three brands of fish sauce after automated library searching.

The key differentiators of each brand were easily uncovered using simple visualisations, such as the volcano plot in Figure 6, which compares the composition of Brands B and C. The compounds plotted on the left (in blue) are increased in sample B, while those on the right (in red) are increased in sample C. The annotations highlight some of the most significant differences and their flavour characteristics.

This workflow allows comprehensive flavour profiles to be captured and compared to help improve our understanding of consumer preferences and accelerate the development of new flavourings.



1. Anethole (sweet, anise)
2. Pyrazine, 2-propyl- (green, burnt)
3. Benzeneamine, 4-methoxy-
4. Dimethyl disulfide (sulfurous, cabbage)
5. Pyrazine, tetramethyl- (nutty, musty)
6. Hexanoic acid (cheesy, fatty)
7. γ-Nonalactone (coconut, creamy)
8. Phenylethyl alcohol (floral, sweet)
9. 1-Butanol, 3-methyl- (fusel, fermented)
10. 1-Hexanol (green, fruity)

Figure 6

Volcano plot in ChromCompare+ software comparing the composition of fish sauce brands B and C, with a selection of the key differentiators annotated alongside their flavour attributes1 (where known).

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Conclusions

This study has shown that the described GC×GC–TOF MS approach provides comprehensive flavour profiles, specifically:

- Immersive sampling of wide-ranging flavour volatiles using HiSorb high-capacity sorptive extraction probes, fully automated on the Centri platform.
- Enhanced separation by GC×GC using consumable-free INSIGHT-Flow modulation to uncover hidden peaks.
- Sensitive detection and confident identification of analytes using the excellent spectral quality and mass accuracy of the BenchTOF2 mass spectrometer.
- Simplified brand comparisons using automated ChromCompare+ workflows.
- Improved understanding of consumer preferences and accelerated development of new flavourings.

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

References

[1] J. Chen, W. Wang, J. Jin, H. Li, F. Chen, Y. Fei, Y. Wang, Characterization of the flavor profile and dynamic changes in Chinese traditional fish sauce (Yu-lu) based on electronic nose, SPME-GC-MS and HS-GC-IMS, Food Research International, 192, 2024, 114772. https://doi.org/10.1016/j.foodres.2024.114772.

[2] The Good Scents Company Information System (search facility), www.thegoodscentscompany.com/search2.html (accessed on 19th February 2025).

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