

Investigating the quality of Darjeeling teas using comprehensive VOC fingerprints



This study demonstrates the use of immersive sorptive extraction and GC×GC–TOF MS to determine the characteristic flavour compounds in Darjeeling teas. Comparative analysis of different tea types and grades will be shown using streamlined workflows to improve quality assessment.

Introduction

Tea is the one of the most widely consumed beverages in the world. Darjeeling tea, produced in the high elevations of West Bengal, India, is highly sought after and considered to be one of the finest-quality teas in the world. However, the harvest time, climate, soil and manufacturing processes can all impact the aroma characteristics of tea and thus its perceived quality.^[1]

For example, the ‘first flush’ is the harvesting of the first leaves and buds to emerge in spring after the plant’s winter dormancy, which are thought to produce the highest-quality Darjeeling teas, while the more mature ‘second flush’ leaves are picked in May/June and are known to have a fruitier flavour.^[2]

The characteristic flavour of Darjeeling tea is therefore an important criterion in evaluating tea quality and can be measured objectively by analytical instruments such as gas chromatography–mass spectrometry (GC–MS). However, the complex blend of volatiles present in tea poses a significant challenge for conventional 1D GC separations.

Here, we demonstrate the use of automated immersive sorptive extraction coupled with GC×GC–TOF MS for comprehensive characterisation of tea volatiles. The BenchTOF2™ mass spectrometer used in this study not only provides excellent spectral fidelity, for confident matching against commercial libraries, but has the added benefit of Tandem Ionisation® to provide both hard and soft EI data in a single analysis. The complementary soft EI spectra improves chemical selectivity to enhance untargeted ‘discovery’ workflows – as required in quality and authenticity evaluation, where you may not know what compounds are important.

This study shows how the comprehensive data acquired using GC×GC–TOF MS with Tandem Ionisation can be processed in a single, automated workflow to improve discovery of the subtle differences between complex flavour profiles, for fast and efficient quality evaluation.

Experimental

The analytical workflow used in this study is summarised in Figure 1.

Samples: Nine Darjeeling loose leaf teas, as detailed in Table 1, were brewed and sampled using immersive sorptive extraction.

Extraction and enrichment: 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: Prior to desorption, the probes were automatically rinsed with deionised water to remove residual matrix and dried using a stream of nitrogen gas. Probes were desorbed at 260°C (for 10 min) in the inlet of the Centri platform.

GC×GC: INSIGHT® flow modulator (SepSolve Analytical); Modulation period (P_M): 4.8 s.

MS: Instrument: BenchTOF2-TI™ (SepSolve Analytical); Mass range: m/z 25–450; Acquisition rate: 100 Hz in Tandem Ionisation mode at 70 and 14 eV.

Software: Full instrument control and data processing by ChromSpace® with ChromCompare+ (SepSolve Analytical) for untargeted chemometric analysis.

Please contact SepSolve for full analytical parameters.

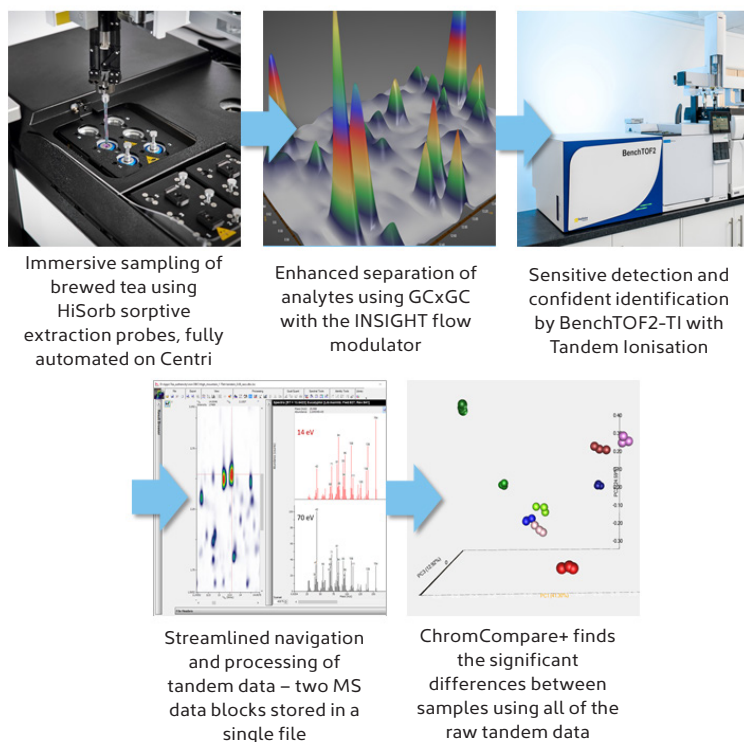


Figure 1

Schematic of the analytical workflow used in this study.

Sample ID	Tea type	Quality
Brand A	Black	First flush
Brand B	Black	First flush
Brand C	White	First flush
Brand D	Black	Second flush
Brand E	Black	First flush
Brand F	Black	Second flush
Brand G	Black	First flush
Brand H	Oolong	Second flush
Brand I	Black	First flush

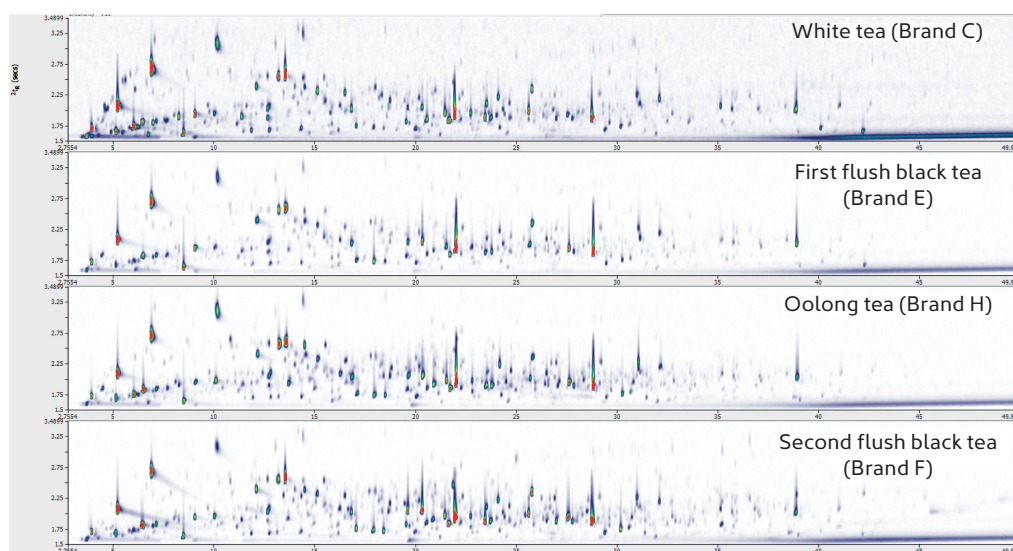
Table 1

Summary of the type and quality of Darjeeling teas analysed in this study.

Results and discussion

In this study, nine Darjeeling tea brands were sampled using immersive sorptive extraction prior to GC×GC–TOF MS. The HiSorb high-capacity sorptive extraction probes not only offer a larger volume of PDMS stationary phase (65 µL) compared to traditional SPME (~0.5 µL) for higher sample loadings, but their robust design also enables immersive sampling – fully automated on the Centri platform. Coupling this high-sensitivity extraction with GC×GC–TOF MS ensures that the wide range of chemical classes present in brewed tea can be efficiently separated and confidently identified.

The power of this approach is evident in Figure 2, with over 300 peaks separated and detected from the analysis of each tea. The GC×GC–TOF MS colour plots show how numerous co-elutions that would have occurred in 1D GC are avoided thanks to the additional separation in the second dimension.

**Figure 2**

GC×GC–TOF MS colour plots for a selection of the Darjeeling teas analysed in this study.

Importantly, each analysis also benefits from Tandem Ionisation to enhance selectivity, as seen in the example in Figure 3 for eucalyptol. The Tandem Ionisation data is opened as a single 'tandem' file, which incorporates both the hard (70 eV) and soft (14 eV) EI data blocks, for streamlined data analysis.

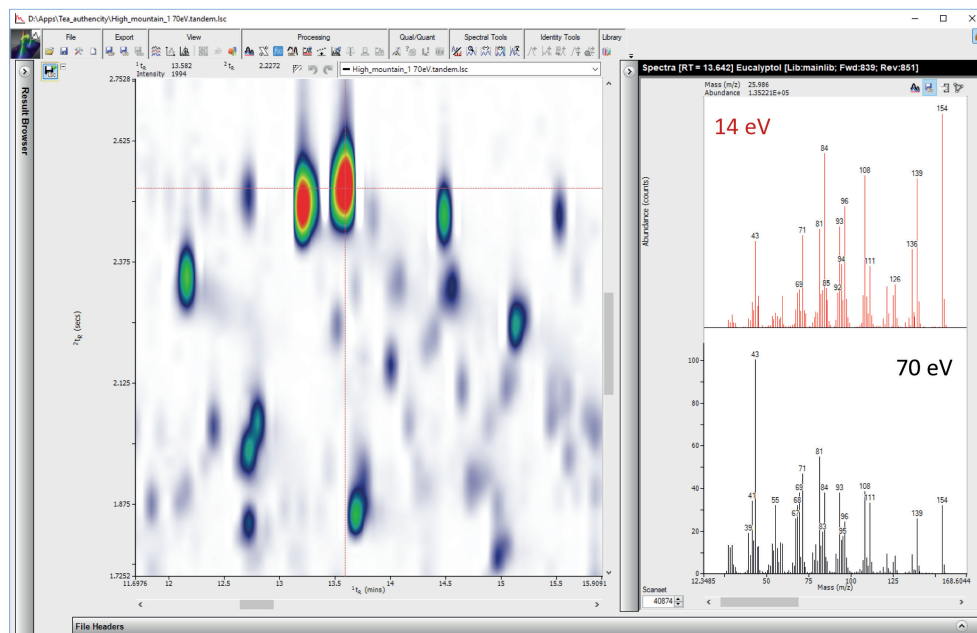


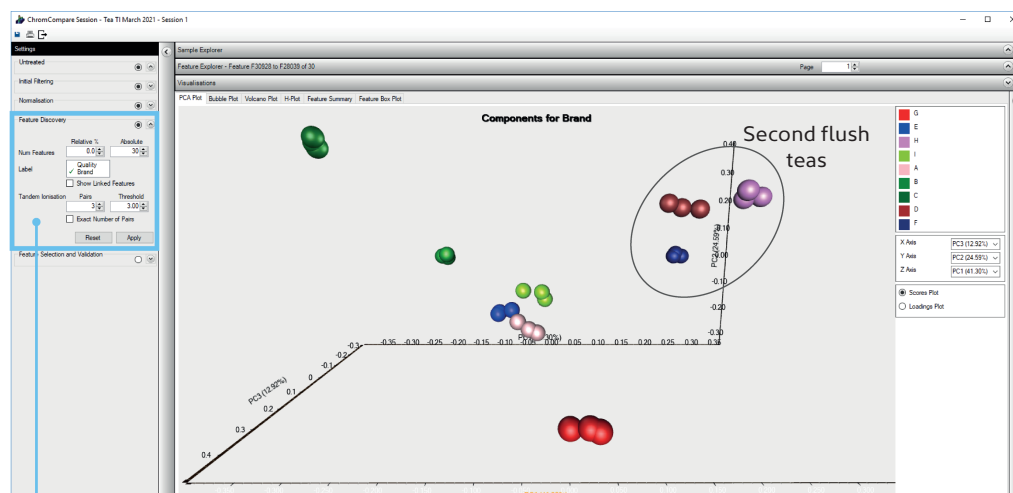
Figure 3

An example 'tandem' data file, containing both hard and soft EI data blocks – enabling complementary spectra to be utilised in a single workflow.

Based on the chromatograms shown in Figure 2, the compositions of the tea samples appear to be very similar, despite the variations in type and quality.

The challenge is therefore to use the comprehensive data obtained by GC×GC–TOF MS and Tandem Ionisation to determine the subtle differences between the VOC profiles and how they relate to perceived flavour and quality.

In this case, untargeted analysis was employed in ChromCompare+ software to utilise all of the Tandem Ionisation data in a single, streamlined workflow and minimise the risk of missing important differences. This approach splits the chromatograms into small sections (or 'tiles') and automatically examines every m/z channel for every chromatogram in the dataset – meaning that time-consuming integration and identification steps are avoided. Finally, during Feature Discovery, the Tandem Ionisation data is used to filter out false positives and improve discovery of true, significant differences, by ensuring that the difference is observed in both the hard and soft EI data.



Tandem Ionisation data is used in a single, automated workflow to reduce false positives and improve discovery of true differences

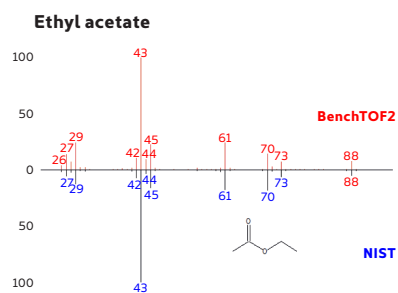
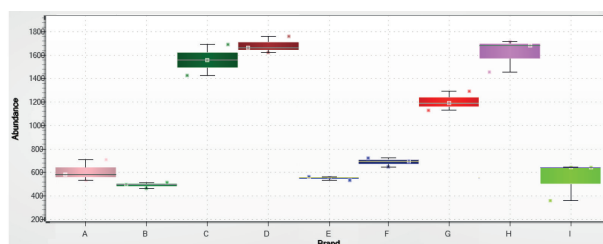
Figure 4

Principal components analysis (PCA) score plot in ChromCompare+ showing the clustering of the nine tea brands after automated untargeted analysis of the Tandem Ionisation data.

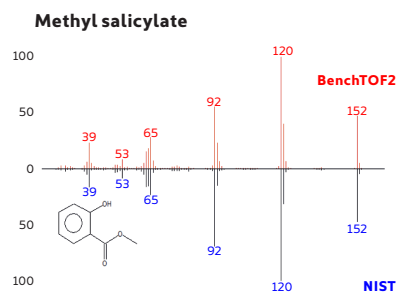
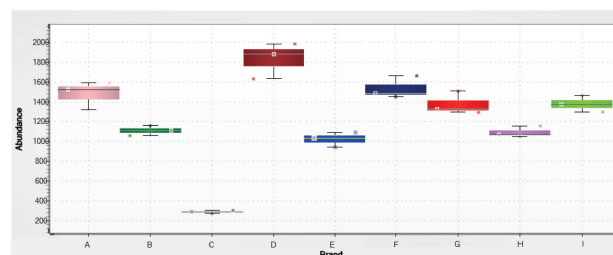
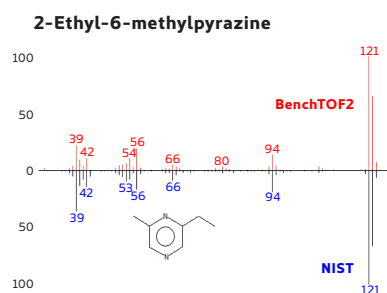
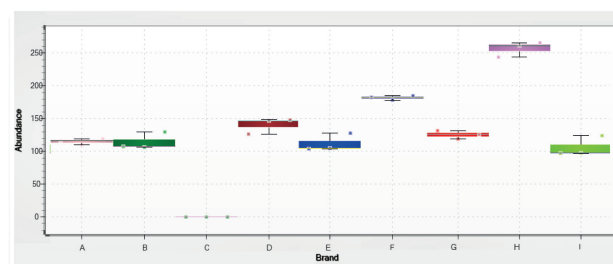
The principal components analysis (PCA) score plot in Figure 4 shows a clear distinction between the brands of tea. The top differentiating features between teas can also be viewed easily in box and whisker plots and were confidently identified using BenchTOF2 spectra (Figure 5).

Most notably:

- ▶ Pyrazines were found in higher levels in the oolong tea (Brand H) and may result in a nutty flavour compared to the other tea samples.
- ▶ Methyl salicylate was found in high levels in all samples except the white tea (Brand C) and is known to impart a sweet, wintergreen flavour.
- ▶ Finally, methyl isobutyl ketone was found to be another possible differentiator of first and second flush teas, as it was found in increased levels in the latter, as well as in the white tea, and is likely to impart a green flavour.

**Figure 5**

Box and whisker plots in ChromCompare+ for three of the top differentiators of tea brand, with the corresponding identification using BenchTOF2 spectra (red) compared against the NIST library (blue).



Interestingly, the three 'second flush' teas cluster together in the PCA plot, despite one of the teas being an oolong tea, while the others are black tea. This indicates that there are significant differences between the VOC profiles of first and second flush teas. To investigate this further, the Feature Discovery tool was also used to uncover the top differentiators of tea quality (Figure 6), i.e., between the first flush (spring harvest) and second flush (May/June harvest) teas.

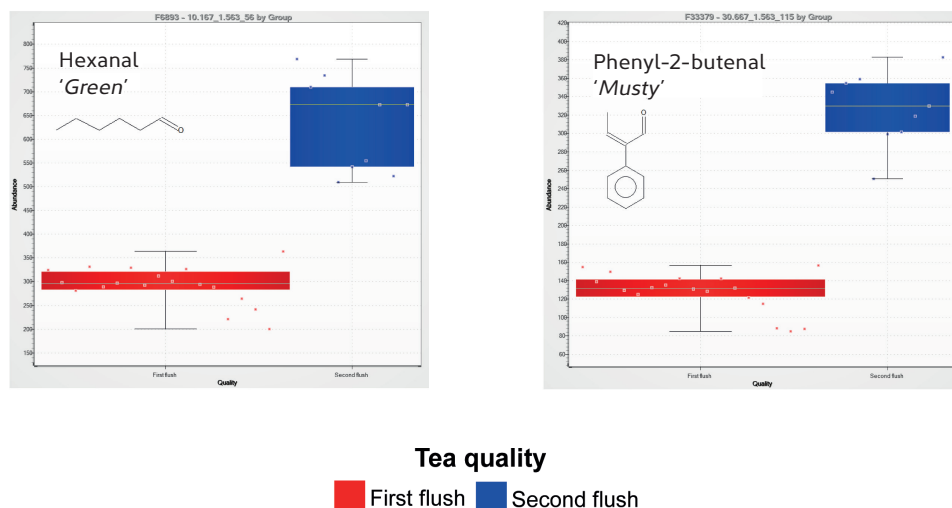


Figure 6

Box and whisker plots in ChromCompare+ representing two key differentiators of tea quality, with feature identities and aroma attributes^[4] provided.

Conclusions

This study has shown an end-to-end workflow for quality and authenticity evaluation of foods and beverages, including:

- ▶ Immersive sampling of a wide range of VOCs and SVOCs using HiSorb high-capacity sorptive extraction probes, fully automated on the Centri platform.
- ▶ Enhanced separation by GC×GC using INSIGHT consumable-free flow modulation to obtain comprehensive aroma profiles.
- ▶ Sensitive detection and excellent spectral fidelity of the BenchTOF2-TI for confident identification of targets and unknowns in a single analysis.
- ▶ Tandem Ionisation adds an extra level of selectivity through complementary hard and soft EI data, with innovative tools to harness the power of both data blocks in a single, streamlined workflow.
- ▶ Fully untargeted data analysis in ChromCompare+ to automatically find the significant differences between tea samples.

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

References

- [1] S. Kang et al., Identification and quantification of key odorants in the world's four most famous black teas, *Food Research International*, 2019, 121: 73–83.
- [2] B. Gohain et al., Understanding Darjeeling tea flavour on a molecular basis, *Plant Molecular Biology*, 2012, 78: 577–597.
- [3] C. Schuh and P. Schieberle, Characterization of the key aroma compounds in the beverage prepared from Darjeeling black tea: quantitative differences between tea leaves and infusion, *Journal of Agricultural and Food Chemistry*, 2006, 54: 916–924.
- [4] The Good Scents Company Information System (search facility), www.thegoodscentscopy.com/search2.html (accessed on 20th September 2020).

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