Unlocking Microbial Aromas: Prioritization of Key Compounds in Food Science using GC-ecTOF

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INTRODUCTION

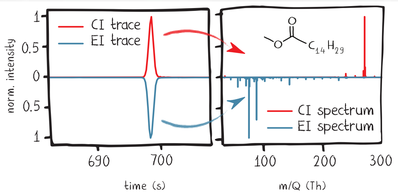
Microbial fermentation represents a key step in the refinement of dairy and dairy-like products such as cheese and yogurt. Their aromatic potential is highly diverse and complex, while being strongly dependent on both the microbial species involved and the cultivation medium. GC-MS remains the gold standard for flavor analysis. However, depending on the sampling and sample preparation strategy, chromatograms are often dominated by non-flavor-related compounds that provide little or no relevant sensory information. As a result, the analysis of such datasets, particularly with regard to unknown compounds, becomes laborious and highly complex. Therefore, an efficient and streamlined prioritization of compounds of interest is essential, ideally already during data acquisition.

The unique capability of the ecTOF to simultaneously acquire high-resolution electron ionization (EI) and chemical ionization (CI) mass spectra enables the transformation of highly complex sample matrices into a focused and actionable set of relevant features. This approach is particularly powerful when selective CI reagents, such as ammonia, are employed. However, the use of ammonia as a CI reagent presents practical challenges for many laboratories. In particular, handling pressurized gas cylinders containing highly corrosive gases introduces significant safety and operational concerns. To address these limitations, the ecTOF incorporates a unique CI reagent control unit that enables the in-situ generation of CI reagents, including ammonia, through the use of safe and easy-to-handle permeation tubes.

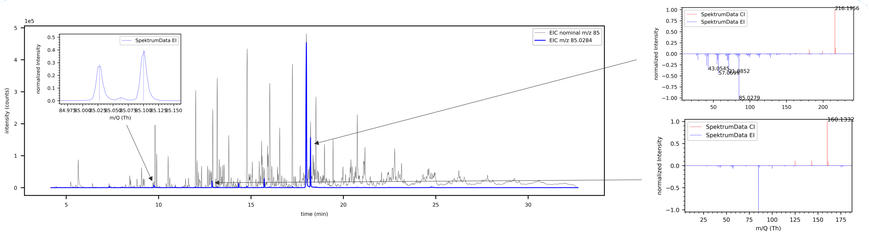
METHOD

Microbial cultures of *Lentilactobacillus plantarum*, *Yarrowia lipolytica*, *Lactococcus lactis* ssp. *lactis*, and *Lactobacillus parafarraginis* were incubated according to the method described by Zia et al. (2019). For fermentation, 20 mL of non-fermented, sterilized grape seed oil were mixed with 80 mL of growth medium and incubated for 24 h at 24 °C. Following fermentation, the mixtures were sterilized at 121 °C for 60 min.

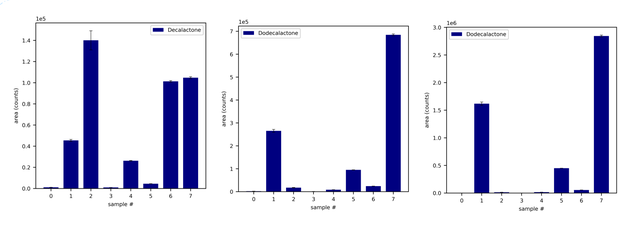
- Aroma compounds from the fermented oil phases were isolated using Solvent-Assisted Flavor Evaporation (SAFE). Subsequently, 1 µL of the extract was injected using a PAL3 autosampler (CTC Analytics AG, Switzerland) coupled to a GC 8500 system and an ecTOF mass spectrometer (Bruker Daltonics, Germany).
- GC injection was performed in splitless mode using an RXI-5 column (30 m × 0.25 mm ID × 0.25 µm film thickness; Restek, Bellefonte, PA, USA). The GC oven temperature program was as follows: initial temperature of 35 °C held for 1 min, followed by a ramp of 10 °C/min to 320 °C and a final hold of 2 min.
- The ecTOF simultaneously operated an electron ionization (EI) source and an HRP chemical ionization (CI) source in parallel. NH_4^+ reagent ions were used for CI measurements. The NH_4^+ ions were generated safely within the mass spectrometer by introducing trace amounts of ammonium hydroxide into the N_2 reagent gas flow of the Helical Resonator Plasma CI source.



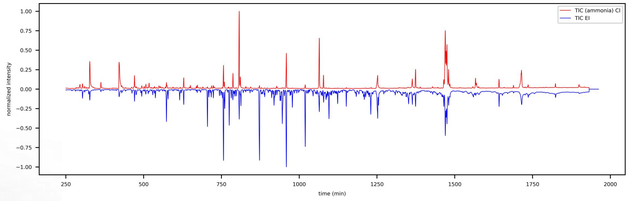
RESULTS



Lactones play an important role in the flavor profile of products such as cheese and were therefore of major interest in this study. Consequently, they were investigated using a suspect-screening approach. Under electron ionization (EI) conditions, lactones exhibit characteristic fragmentation patterns that are highly specific for this compound class. In particular, the dominant fragment ion corresponds to the lactone ring structure itself. Accordingly, six-membered lactones predominantly form the ion $C_4H_5O_2^+$, whereas five-membered lactones mainly generate the fragment ion $C_5H_7O_2^+$. This fragmentation behavior enables a straightforward initial detection of lactones. However, it also results in rather unspecific fragmentation patterns among individual lactone species, making differentiation more challenging. The upper figure shows the extracted ion chromatogram (EIC) of m/z 85.0284, corresponding to the $C_4H_5O_2^+$ fragment, obtained using high-resolution TOF-MS. The high-resolution EIC (HR-EIC) is compared with the nominal mass trace at m/z 85.The nominal mass trace exhibits significant interferences, mainly originating from $C_6H_{13}^+$ ions, which are clearly resolved by high-resolution TOF-MS. The detected lactones can then be readily identified based on their characteristic ring size and molecular sum formula determined by chemical ionization (CI).



The upper figure shows the peak areas of selected compounds detected in the microbial samples. As expected, both the abundance and composition of flavor-active compounds, particularly lactones, strongly depend on the specific microbiome present. Peak areas were determined using the corresponding $[M+H]^+$ or $[M+NH_4]^+$ signals in the mass spectra. Once again, the combination of high-resolution mass spectrometry and selective, highly sensitive chemical ionization (CI) provides a robust platform for accurate peak integration. In comparison to conventional low-resolution GC-MS systems operated solely with EI ionization the likelihood of signal interferences or even complete masking of analyte signals by matrix compounds is significantly reduced



While lactones can be readily filtered based on their characteristic EI fragment ions using high-resolution mass spectrometry, other flavor compounds exhibit a much greater diversity in their EI mass spectra and therefore need to be addressed in a more non-targeted manner. Consequently, an efficient preselection of compounds of interest becomes essential. The use of selective chemical ionization (CI) reagents provides an elegant strategy to reduce the number of detected features, particularly in matrices containing high abundances of completely non-polar compounds such as alkanes. The upper Figure illustrates an ecTOF measurement performed using ammonia CI. Without any filtering, a total of 486 compounds were detected, largely driven by the EI data. Due to the strongly differing ionization efficiencies of non-polar alkanes between EI and ammonia CI, the signal intensities of more polar compounds remain comparatively similar when analyzed with the CI source of the ecTOF. Applying a filter based on the area ratio between EI and CI signals, where only compounds with a ratio below 5 were retained, reduced the number of detected compounds to 130. This corresponds to a reduction to approximately 26% of the original feature count, achieved without applying any additional area-based filtering criteria.

- **The simultaneous EI&CI detection on the ecTOF enables the acquisition of both molecular and NIST library searchable structural information in a single GC run.**
- **High resolution in addition to selective CI reagents spotlights towards the compounds that really matter.**
- **Highly efficient prioritization of features without loosing any depth of information**
- **Clear identification and separation of Matrix, background and analytes even with easy to use 1-dimensional GC due to the unique combination of EI&CI**
- **Safe in-situ generation of ammonia reagent ions compatible with laboratory safety policies.**
- **Robust quantification due to high resolution and integrated CI reagent options**

ecTOF

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The authors with the affiliation TOFWERK and Bruker are employees of a mass spectrometric vendor.

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