Società Chimica Italiana
Divisione di Spettrometria di Massa

FONDAZIONE EDMUND MACH

DIFFA23

DIRECT INJECTION FOOD FLAVOUR ANALYTICS

BOOK OF ABSTRACTS

Fondazione Edmund Mach

San Michele all'Adige (TN), Italy

20 - 22 September 2023

1st International Symposium on
Direct Injection Food Flavour Analytics (DIFFA)

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Proceedings of the DIFFA23 - 1st International Symposium on Direct Injection Food Flavour Analytics

Fondazione Edmund Mach – San Michele All'Adige (TN) Italy

20-22 September 2023

This book collects the conference proceedings of the 1st International Symposium on Direct Injection Food Flavour Analytics, held at the Fondazione Edmund Mach from 20th to 22nd September 2023.

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FOREWORD

Volatile organic compounds (VOCs), particularly flavour compounds, represent an invaluable noninvasive metric to follow the multi-faceted journey of food, from the farm to the fork and beyond, such as relating to the human microbiome after consumption or in addressing reduction strategies for food waste. VOCs thereby serve as a direct and swift means of measurement and notably act as a main driver of the perceived quality of food.

Mass spectrometry (MS) is an established yet increasingly pivotal tool in food and beverage characterization with a broad range of applications. When coupled with gas chromatography (GC), it stands as the predominant analytical method for exploring many aspects of food, from safety to traceability and nutritional aspects, and equally facilitates control measures in quality and process monitoring.

Recent remarkable advancements in both technology and methodology have paved the way for highly sensitive, specific, rapid, robust, and validated MS-based techniques that have become indispensable in food science and technology research and application. A subgroup of these technologies has been devised over the past two decades in the form of analytical approaches that enable the analysis of VOCs through direct injection. These methods have gained attention for their rapid, highly sensitive and high-throughput analytical capabilities.

A leading technology in this area is proton transfer reaction-mass spectrometry (PTR-MS), which has driven many innovative applications for direct flavour/food analysis. Commencing 2003, the University of Innsbruck, Austria, has organized a biennial event dedicated specifically to PTR-MS and its applications, including a focused session on food science and technology.

The **1st International Symposium on Direct Injection Food Flavour Analytics (DIFFA23)** was conceived with the backdrop of the PTR-MS conference but with a different aim, namely to embrace a broader community beyond PTR-MS uses, encompassing similar direct injection mass spectrometry (DIMS) technologies, such as atmospheric pressure chemical ionization-mass spectrometry (APCI-MS) and selected ion flow tube-mass spectrometry (SIFT-MS), with a primary emphasis on flavor compounds. It was also not exclusive to MS-based analytical techniques, but welcomed the inclusion of complementary non-MS approaches, such as solid-state sensors, fast gas chromatographic direct approaches and ion mobility spectrometry (IMS), amongst others, to ensure a wider reach and broader engagement. The meeting was established to foster scientific discussions of common interest and facilitate scientific collaborations. This book of abstract highlights the details of the event and contains the contribution summaries of both the oral and poster presentations.

The conference featured one plenary and four keynote lectures delivered by distinguished guests, as well as numerous invited and contributed talks and 25 poster presentations, with 97 attendees from different EU states, the USA, the UK, Israel and New Zealand. The event provided valuable insights into direct injection food/flavour analytics, with reviews from pioneering scientists who played key roles in developing and advancing DIMS methods in its early days, such as Andy Taylor, Patrik Španěl and Jean-Luc Le-Quéré, showcasing both historical developments and recent advancements in analytical performance and novel applications. Topics discussed included nose-space analysis of composite foods, rapid and high-throughput phenotyping, fermentation monitoring, both as an

innovative technological tool and for investigating the human microbiota, advanced data analysis and data mining tools. These are just a few examples of the themes explored during the conference.

Numerous partners contributed to the success of the event: the sponsors, whose engaging presentations and financial support sustained the quality of the meeting and ensured that the conference fees were kept to a minimum, as well as various supporting institutions and patronages. Special thanks go to the Fondazione Edmund Mach (FEM) for its scientific contributions and for hosting the conference at the Research and Innovation Centre, as well as the Division of Mass Spectrometry of the Italian Chemistry Society (DSM-SCI) for their organizational support and creation and hosting of the conference website. The invaluable support from these companies and institutions are further acknowledged through inclusion of their logos on the back cover of this book.

The conference started a fruitful exchange of results, ideas and issues amongst scientists working with direct tools to monitor VOCs in food science and technology, with broad attendance from sensory and applications scientists from academia and industry.

We would like to thank all those who, through their participation and support, made this event possible, which exceeded our most ambitious expectations.

Thank you all, and we look forward to seeing you at the next edition.

On behalf of the Scientific Committee

Franco Biasioli, Jonathan Beauchamp, Pat Silcock

CONFERENCE PROGRAM

20th September 2023

12.30-14.00 Registration and welcome buffet

Conference opening

14.00-14.10	Welcome addresses
	Fulvio Magni - *Società Chimica Italiana-Divisione Spettrometria di Massa*
	Mario Pezzotti - *Fondazione Edmund Mach*
14.10-14.20	Why DIFFA23?
	Franco Biasioli - *Fondazione Edmund Mach*
14.20-15.05	Plenary lecture: *DI-MS – A game changer for flavour research?*
	Andy Taylor - *University of Nottingham*

Session 1 | Unlocking Flavour with DIMS

Chairs: Pat Silcock & Nina Cleve

15.05-15.35	Jonathan Beauchamp - Fraunhofer Institute for Process Engineering and Packaging IVV
	The long and winding road: a flavoursome tale of PTR-MS
15.35-15.55	Graham Eyres - *University of Otago*
	What is Flavour and how can DIMS help untangle the puzzle?
15.55-16.15	Andreas Mauracher - *IONICON*
	Advantages of Next-Gen PTR-ToF instruments for food and flavour sciences

16.15-17.00 Tea break and poster session

Session 2 | DIMS in Health and Wellbeing

Chairs: Donatella Caruso & Eirini Pegiou

17.00-17.20	Josep Rupert - *Wageningen University & Research*
	Signalling volatile compounds in the human gut microbiota: new avenues offered by direct analytical methods.
17.20-17.40	Chris Mayhew - *University of Innsbruck*
	Real-Time Trace Analysis of Breath Volatiles using Proton Transfer Reaction Mass Spectrometry: implications for in-vivo flavour release measurements
17.40-18.00	Enrico Davoli - *Istituto Mario Negri*
	Direct analysis of sex-wellness products using a field deployable MS equipped with a Direct Sampling Atmospheric Pressure (DSAP) source
18.00-18.20	Corrado Di Natale - *University of Rome Tor Vergata*
	Direct injection mass spectrometry and gas sensors: a teacher-pupil relationship
18.20-18.40	Luca Cappellin - *University of Padua*
	Improved compound identification in direct VOC analysis using an EI&CI-TOFMS

19.00 Welcome cocktail - cloister of the monastery and historical cellar

21st September 2023

Session 3 | Linking DIMS Data to Sensory Perception

Chairs: Graham Eyres & Iuliia Khomenko

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9.30-9.50	Catrienus De Jong - *Wageningen University & Research* *Exploring new in vivo and in vitro methods to integrate sensory and instrumental analysis to get insight and improve the flavour of plant-based food products during oral processing and drinking*
9.50-10.10	Markus Stieger - *Wageningen University & Research* *In vivo aroma release and sensory perception of composite foods*
10.10-10.20	Michele Pedrotti - *Wageningen University & Research* *Characterization of plant-based milks by combining sensory analysis with headspace and nose-space direct injection mass spectrometry*
10.20-10.30	Karina Gonzalez-Estanol - *Wageningen University & Research* *In vivo analysis of nose-space concentration by direct injection mass spectrometry to study the effect of chewing rate on aroma release during food consumption*
10.30-10.40	Laura Hill - *University of Nottingham* *Understanding the relationship between lipids, capsaicin and aroma release in confectionery*

10.40-11.10 Coffee break and poster session

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11.30-11.45	Nina Cleve - *Fraunhofer Institute for Process Engineering and Packaging IVV* *Milk matters: Unraveling retronasal aroma release and perception of coffee by combining in vivo nosespace analytics with dynamic sensory methods*
11.45-12.05	Tomasz Majchrzak - *Gdansk University of Technology* *What happens when food goes into oil during deep frying? Monitoring the first minutes of frying using PTR-MS*
12.05-12.20	Gregory Schmauch - *Rational F&E GmbH* *Influence of product quantity, cooking parameter and flow tube pressure on the measurement with Sift-MS in a cooking oven*
12.20-12.40	Vaughan Langford - *Syft Technologies* *Application of SIFT-MS to chemical and sensory screening of packaging materials*

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Chairs: Riccardo Flamini & Michele Pedrotti

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18.20-18.35	Mickael Le Bechec - *Institute of Analytical Sciences and Physico-Chemistry for Environment and Materials (IPREM)* *Volatile fingerprints of food thanks to the untargeted use of SIFT-MS raw data*

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22nd September 2023

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Chairs: Catreinus de Jong & Brian Farneti

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10.15-10.30	Pedro Martinez Noguera - *University of Copenhagen* *Using PTR-ToF-MS to quantify microbial off-flavors geosmin and 2-methylisoborneol in water. Method development, performance assessment and comparison with established GC-MS methods*
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11.05-11.45 Coffee break and Poster Session

Session 8 | Floral, Biogenics and Phenotyping

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12.05-12.25	Brian Farneti - *Fondazione Edmund Mach* *DI-MS as high performing VOC phenotyping tool to support the horticultural production chain management*
12.25-12.40	Alberto Roncone - *Fondazione Edmund Mach* *Validation of gas chromatographic methods for the botanical characterization and authentication of lavender essential oil by stable isotope analysis of its organic volatile compounds*
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12.55-13.15	Daniele Zatta - *University of Padua* *Comparative analysis of VOC purification techniques in complex cooking emission: adsorption, photocatalysis and combined systems.*

13.15-13.30	Closing remarks Fulvio Magni - *Società Chimica Italiana-Divisione Spettrometria di Massa* Franco Biasioli - *Fondazione Edmund Mach*

13.30 Farewell buffet

LIST OF CONTRIBUTIONS

P.03 PTR-ToF-MS as a high sensitivity sensor for online monitoring of Lacto-fermentation in plant-based beverages

Antonia Corvino[1,2]*, Maria Mazzucotelli[1,2], Iuliia Khomenko[1], Vittorio Capozzi[3]

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[2]Center for Agriculture Food Environment C3A, University of Trento, Trento, Italy.
[3]National Research Council of Italy - Institute of Sciences of Food Production (ISPA) c/o CS-DAT, Foggia, Italy.

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Summary: Fermentation is a sustainable process aimed at preserving and enhancing the overall quality of the final product. The advantages offered by this bioprocess can be effectively combined with those provided by PTR-ToF-MS sensor, thereby expediting innovation within this rapidly advancing industry. In this study, PTR-ToF-MS allowed the automatic, real-time, direct, and non-invasive monitoring of *Lactiplantibacillus plantarum* volatilome during the fermentation of different plant-based beverages. Furthermore, PTR-based sensors can facilitate new product development and design, rapidly screening the different matrix- microorganism combinations.
Keywords: PTR-TOF-MS, plant-based beverages, fermentation

1 Introduction

The fermented beverage market has become an important sector in the food industry in the past decade, with various traditional and innovative products and sustainable bio-based solutions. The market demands require a significant diversification of the range of products (e.g. new flavours, plant-based options, ready-to-use products, label cleaning, by-products/waste reuse, and personalised nutrition), and for these reasons, the fermented beverage industry aims to continuously modulate and improve the nutritional, functional, and sensory quality of final products. To meet new consumer needs and enhance acceptability, innovative trends in this area required the exploration of numerous variables, such as changing raw materials, fermentative strategies, exploited microbes, and technological processes [1]. From this perspective, sensor-based approaches can be crucial in ensuring versatile control solutions, accelerating innovation dynamics, and contributing to design strategies to satisfy consumer perception in this dynamic sector [2]. In this context, volatile organic compounds (VOCs), responsible for odour and flavour perception, can be considered interesting targets as biomarkers to track microbial metabolism during fermentation bioprocesses, providing information on the quality of the matrices. Among the sensors that found application to monitor VOCs in the food and beverage sector, DIMS (Direct injection mass spectrometric) techniques have been receiving increasing interest, combining versatility, real- time analysis and good analytical performances [3]. In particular, Proton-transfer-reaction, coupled with Time-of-Flight Mass Spectrometer (PTR-ToF-MS), represents a good model of DIMS technologies in consideration of the relevant sensitivity and accuracy features together with time-saving, non-invasive and eco-friendly analysis [4]. PTR-ToF-MS measurements permit the assessment and monitoring of VOCs *i*) during the fermentation processes to track their evolution and potential reaction kinetics and *ii*) on final products to provide information on possible consumer sensory experience/product quality. This instrumental analytical technique is a green alternative tool for volatilome profiling during food fermentations. In the present work, PTR- ToF-MS was used for the online monitoring of lactic

fermentation in plant-based beverage inoculating, as a single culture, *Lactiplantibacillus plantarum* WCFS1, a well-studied strain belonging to this versatile facultative heterofermentative species of lactic acid bacteria (LAB) found in different matrices and the gastrointestinal tract [5]. This preliminary investigation also aims to develop a PTR-based strategy to build a reference framework for developing and designing fermented beverages.

2 Experimental

Nine plant-based beverages (soy, oat, almond, apple, bergamot, blueberry, carrot, beetroot and tomato) were purchased, and the milk was included as a model beverage of animal origin. Fermentations were performed inoculating at concentrations of 1×10^6 cfu/mL (colony-forming units per milliliter) of *Lactoplantibacillus plantarum* WCFS1 in 3 ml of beverage at 37 °C for 72 h. Each fermentation experiment was carried out by performing three simultaneous independent repetitions for treatment (fermented with *L. plantarum* WCFS1 and non- fermented/uninoculated as control). VOCs produced during fermentation were measured every 4 h by direct injection of the headspace mixture into a commercial PTR-ToF-MS 8000 apparatus (Ionicon Analytik GmbH, Innsbruck, Austria). Measurements were performed in an automated way by using a multipurpose GC automatic sampler (Autosampler, Gerstel GmbH, Mulheim am Ruhr, Germany) as described in [6]. All data detected and recorded by the PTR- ToFMS were processed and analysed using MATLAB R2017a (MathWorks Inc., Natick, MA, USA) and R (R Foundation for Statistical Computing, Vienna, Austria). Principal component analysis, analysis of variance, and Tukey's post-hoc test were performed to spot the differences in the volatile aroma compounds emitted by the beverages.

3 Results

A preliminary data exploration has been made to visualise, through a principal component analysis (PCA), the results of the PTR-ToF-MS analysis of the original beverages as raw materials for the fermentations. The PCA plot in Figure 1 demonstrates that the first and second PCA components together explain 84.68% of the overall variability before fermentation. This plot highlights a clear distinction between milk-like beverages (such as soy, almond, and oat) that form a defined cluster and exhibit pronounced similarity to milk from the juices (bergamot, apple, blueberry, carrot, beetroot, and tomato). Based on these findings, two distinct PCA plots are conducted for the lacto-fermented milk-like beverages (Figure 2) and juices (Figure 3), respectively. In the Figure 2, poor evolution of the uninoculated milks is observed, while it is possible to follow the changes of variability in the time of the inoculated samples. Samples from the different matrices grouped well together, with only small and partial overlapping. In Figure 3, the trend of fermented and non-fermented juices is different, indicating that these two categories of juices have different characteristics and properties, as expected. The clustering patterns of bergamot juice differ from others, as well as apple juice. Additionally, both tomato, carrot, blueberry and beetroot juices exhibit similar trends, with less pronounced clustering by matrices. In the 'juice' experiment, the control/uninoculated samples also show an evolution in the time of VOCs, as the corresponding samples inoculated with *L. plantarum* WCFS1. In this light, it is possible to surmise the development of native microbes during the fermentative process. All the juices were pasteurised. Pasteurisation is a treatment that eliminates the vegetative form of pathogens but not the spore-forming bacteria. Hence, it is possible to suppose that bacterial spores germinate to start a bioprocess in the

uninoculated samples. After the aggregate analysis, tentative identification of the ions and a more detailed study of the evolution of selected peaks associated with different matrices and lactic fermentation was carried out.

4 Conclusion

This study reports a first characterisation of VOCs by means of DIMS in plant-based beverages originating from fruits, vegetables, cereals and legumes. The research underlined the potential of PTR-ToF-MS, combined with tailored data analysis, for the online monitoring of experimental variables associated with lactic acid fermentation in all plant-based beverages. Evidence has highlighted the possibility of assessing differences and similarities in volatile profile by distinguishing various fermented matrices based on their chemical and physical characteristics, as well as the preferred growth substrate for lactic acid bacteria. PTR-ToF-MS can be considered as a driver for new product development and novel foods design, starting from a preliminary evaluation of volatile compounds in raw matrices and in fermented products. The results also provide original findings on some metabolic changes over time when *L. plantarum* grows in plant substrates. Further investigations are needed to understand the genomic pattern associated with volatile production.

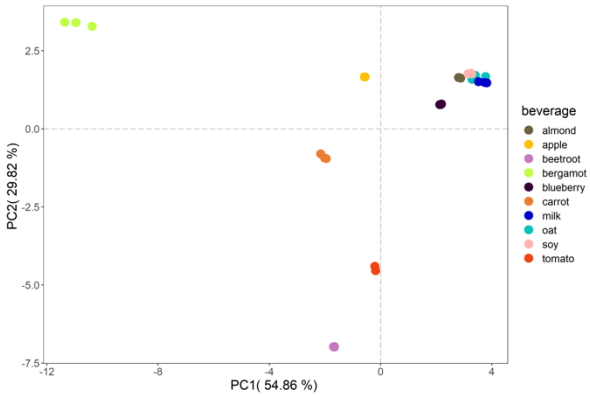

Figure 1. *Score plot of the principal component analysis of VOCs emission before fermentation for each trial tested in this study. Data were logarithmically transformed and centered.*

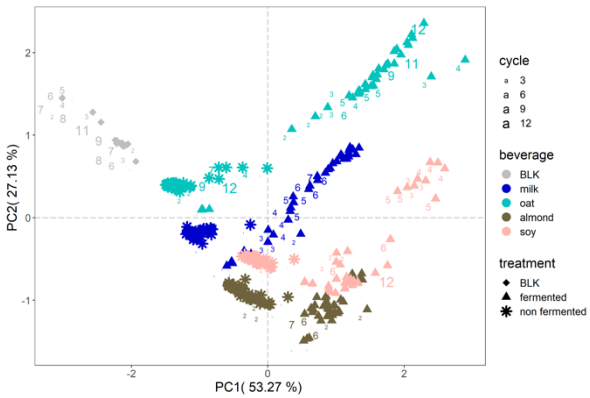

Figure 2. *Score plot of the principal component analysis of VOCs emission from fermented and non-fermented milk – like beverages during 72 h of fermenetation process. Three replicates for beverage were tested in this study. Data were logarithmically transformed and centered.*

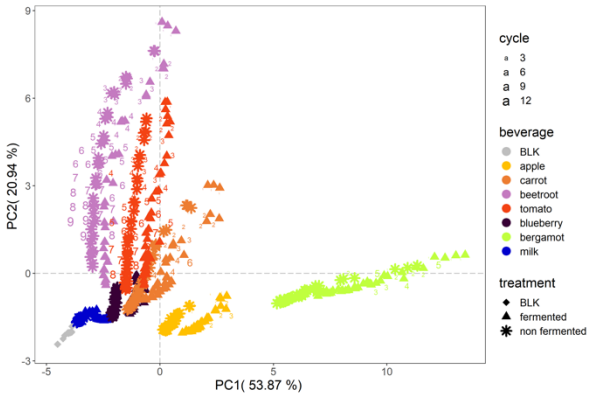

Figure 3. *Score plot of the principal component analysis of VOCs emission from fermented and non-fermented juices during 72 h of fermenetation process. Three replicates for beverage were tested in this study. Data were logarithmically transformed and centered.*

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