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

9.00-9.30	Jean-Luc Le-Quéré - *INRAE-CSGA Dijon* *Twenty years of Direct Injection Mass Spectrometry for aroma research in Dijon*
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

Session 4 | Flavour Complexity and Cooking

Chairs: Fulvio Magni & Caroline Perltier

11.10-11.30	Samo Smrke - *ZHAW School of Life Sciences and Facility Management* *Development of fast-GC PTR-MS method for coffee VOCs analysis*
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*

12.40-14.00 Conference group photo and lunch

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Chairs: Jonathan Beauchamp & Karina Estanol-Gonzalez

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14.15-14.30	Matteo Tonezzer - *University of Cagliari* *PTR-MS as a tool to understand and improve the performance of electronic noses*
14.30-14.45	Andrea Warburton - *University of Otago* *Application of PTR-ToF-MS to monitor development of flavour in sourdough*
14.45-15.05	Paolo Redegalli - *Shimadzu Italia S.r.l.* *Characterization of isoflavones and its metabolites in foods by direct probe ionization mass spectrometer (DPiMS) with high resolution detection*
15.05-15.25	Hansruedi Gygax - *GAS Dortmund* *GC-IMS instruments and their use in food and flavour analysis*

15.25-16.15 Tea break and poster session

Session 6 | Microbial, Fermentation and Modelling

Chairs: Riccardo Flamini & Michele Pedrotti

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16.45-17.05	Vittorio Capozzi - *Institute of Sciences of Food Production - National Research Council of Italy (CNR)* *DIMS techniques and the study on microbial VOCs in food: flavour attributes, fermentation monitoring and emerging trends*
17.05-17.20	Eirini Pegiou - *Wageningen University & Research* *Easy and fast detection of abnormal olive brine fermentation – A showcase of SPOTDETECT.*
17.20-17.40	Caroline Peltier - *INRAE* *Automatic pretreatment and multiblock analysis of flavor release and sensory temporal data simultaneously collected in vivo*
17.40-18.00	Ana Rita Monforte - *AFB INTERNATIONAL* *Modelling the kinetics of flavour formation & relaese as a function of ingredients addition in real food systems*
18.00-18.20	Pietro Franceschi - *Fondazione Edmund Mach* *Mining datasets from untargeted direct analytical methods: a data analyst point of view*
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

Session 7 | Food Spoilage and Off-Flavour

Chairs: Catreinus de Jong & Brian Farneti

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10.00-10.15	Antonella Grosso - *University of Bolzano* *Monitoring autoxidation of vegetable oils by proton transfer reaction mass spectrometry*
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*
10.30-10.45	Davide Papurello - *Turin Polytechnic* *Supporting sustainable energy production by PTR-MS: a review on the work accomplished on biofuel production from food waste to SOFC systems*
10.45-11.05	Rupert Holzinger - *Utrecht University* *Using SI traceable gas standards to improve the accuracy of untargeted PTR-MS measurements*

11.05-11.45 Coffee break and Poster Session

Session 8 | Floral, Biogenics and Phenotyping

Chairs: Rupert Holzinger & Vittorio Capozzi

11.45-12.05	Štefan Matejčík - *Comenius University* *Ion mobility spectrometry detection of plant hormones*
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*
12.40-12.55	Eugenio Aprea - *University of Trento* *Contribution of volatile organic compounds to multifloral honey flavor*
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.26 Venezuelan stingless bee *Tetragonisca angustula* (Latreille, 1811) pot-pollen and cerumen pollen pot Volatile Organic Compound VOC profiles by HS-SPME/GC-MS

Emanuela Betta[1], Ricardo R Contreras[3], Enrique Moreno[4], Silvia RM Pedro[5], Iuliia Khomenko[1], Patricia Vit[2]*

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Summary: Tetragonisca angustula pot-pollen and cerumen pollen pots from Merida, Venezuela were analyzed by HS-SPME/GC-MS. Acetic acid was the major VOC originated from suspected Acetic Acid Bacteria, followed by 2,3-butanediol, propylene glycol, and lower beta phellandrene and furfural. These metabolites confirm the fermenatative nature of fresh pollen processed into pot-pollen by Meliponini.
Keywords: GC-MS, Tetragonisca angustula, pot-pollen

1 Introduction

The great biodiversity of stingless bees reached 605 species worldwide, 474 of then from Neotropical Americas [1]. Stingless bees process honey and pollen in cerumen pots [2]; thus, named pot-honey [3] and pot-pollen [4]. Tetragonisca angustula is the Netropical stingless bee most widespread, from southern Mexico to northern Argentina. The pot-pollen volatiles and cerumen pots were studied here for the first time, as a preliminary approach of their metabolite origins.

2 Experimental

The stingless bee *Tetragonisca angustula* (Latreille, 1811) was identified by Prof. J.M.F. Camargo from Universidade de São Paulo, Ribeirão Preto, SP, Brazil. Pollen pots were collected from the T. angustula nest and submitted to the FEM lab where the pot-pollen was separated from the cerumen of three polen pots. Measurements were conducted using the Headspace-Solid Phase Microextraction/Gas Chromatography-Mass Spectrometry (HS-SPME/GC-MS) technique, following a modified procedure based on the method reported by Wang et al (2019) [5]. Each sample, consisting of 500 mg, was weighed in triplicate and placed in 20 mL glass vials. These vials were hermetically sealed and stored in the autosampler of the GC (CTC combiPAL, CTC Analytics AG, Zwingen, Switzerland) at 20°C until analysis. To achieve equilibration, pot-pollen and cerumen pollen pot samples were maintained at a constant temperature of 40°C for 15 min. Subsequently, a Solid Phase Microextraction (SPME) fiber composed of DVB/CAR/PDMS material (Supelco, Bellefonte, PA, USA) was introduced into the headspace of the vial for 45 minutes. Compound desorption from the SPME fiber occurred at 250°C within the injector port of the GC, which was

interfaced with a mass detector operating in electron ionization (EI) mode (70 eV). The mass detection range spanned from 33 m/z to 350 m/z (GC-MS Clarus500, PerkinElmer, Norwalk CT, USA). Chromatographic separation was conducted using an HP-INNOWax fused silica capillary column (30 m x 0.32-mm inner diameter x 0.5-µm film thickness; Agilent Technologies, Palo Alto, CA, USA). Helium was employed as the carrier gas, flowing constantly at a rate of 1.5 mL/min. The oven temperature was programmed as follows: 40°C (3 min) with a 4°C/min ramp; 210°C (0 min) with a 20°C/min ramp; 250°C (2.5 min). Compound identification relied on mass spectra matching against entries in the NIST/EPA/NIH (NIST 14) and Wiley 7th Mass Spectral Libraries. Linear retention indices (LRI) were determined under identical chromatographic conditions following the injection of a C7–C30 n-alkane series (Supelco).

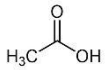
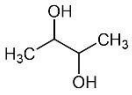
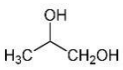
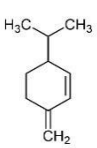
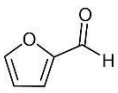
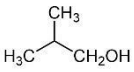
3 Results

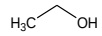
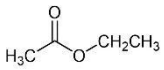
A total of 95 VOCs were identified from different chemical classes such as acids, alcohols, aldehydes, esters, ketones, monoterpenes, oxides, sesquiterpenes, and VOCs of other classes.

Their abundance showed Acetic acid was the major VOC originated from suspected Acetic Acid Bacteria, followed by 2,3-butanediol, propylene glycol, and lower β-phellandrene and furfural. These major metabolites confirm the fermenatable nature of the fresh pollen processed into pot-pollen by Meliponini, evidenced because VOCs indicators were most abundant in pot-pollen than their cerumen container.

Intranest variations of VOCs in each pollen pot showed the importance of plant selection and processing factors such as the botanical origin of the pollen, the inoculated type and quantity of microbes to process them, and the time of processing, among others. See Table 1.

Table 1. *Most abundant metabolites in pot-pollen and its container, the cerumen pot*

Metabolites *descending order of abundance*	**Chemical structures**	**Pot-pollen**	**Cerumen pot**
Acetic acid		50.41	42.27
2,3-Butanediol		23.88	18.60
Propylene glycol		7.08	7.37
β-Phellandrene		9.73	8.53
Furfural		3.70	1.54
2-Methyl-1-propanol		4.47	3.42

Ethanol		3.32	2.39
Ethyl acetate		2.44	1.83

4 Conclusions

The eight most abundant metabolites were presente in pot-pollen and their cerumen pot containers in similar quantities.
The metabolites of microbial origin acetic acid and 2.3-butanediol were 74-60 top abundant in both matrices.

References

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