

**Modena
7-9 October
2026**

**9th MS
Food Day**



Scientific programme & book of abstracts

SCIENTIFIC PROGRAMME

Book of Abstracts

9th MS FOOD DAY

October 7-9, 2026

Modena - Italy

COMMITTEES**Scientific Committee**

Giuseppe Avellone	<i>Università di Palermo</i>
Giuliana Bianco	<i>Università della Basilicata, Potenza</i>
Franco Biasioli	<i>Fondazione Edmund Mach, S. Michele A/A (TN)</i>
Lucia Bonassisa	<i>BonassisaLab S.p.A., Foggia</i>
Anna Cane	<i>Istituto Nutrizionale Carapelli, Firenze</i>
Roberta Galarini	<i>Ist. Zooprof. Sperim. Umbria e Marche</i>
Renzo Galli	<i>Fileni</i>
Franca Ladogana	<i>Consorzio Tutela Aceto Balsamico di Modena</i>
Luciano Navarini	<i>illycaffè</i>
Michele Suman	<i>Barilla e Università di Parma</i>
Sauro Vittori	<i>Università di Camerino</i>

Organizing Committee

Caterina Durante	<i>Unimore, Modena</i>
Franca Ladogana	<i>Consorzio Tutela Aceto Balsamico di Modena</i>
Rebecca Barbieri	<i>Unimore, Modena</i>
Giorgia Gazzoli	<i>Unimore, Modena</i>
Andrea Mancuso Morini	<i>Consorzio Tutela Aceto Balsamico di Modena</i>
Samuele Pellacani	<i>Unimore, Modena</i>
Lorenzo Strani	<i>Unimore, Modena</i>
Claudia Vatteroni	<i>Parmalat, Sala Baganza (Parma)</i>

ORGANIZED BY



UNDER THE PATRONAGE OF



UNIMORE
UNIVERSITÀ DEGLI STUDI DI
MODENA E REGGIO EMILIA



**CAMERA DI COMMERCIO
MODENA**

SUPPORTING INSTITUTIONS



SPONSORED BY



Agilent Technologies



elementar
EXCELLENCE IN ELEMENTS

LECO
EMPOWERING RESULTS



Thermo
SCIENTIFIC

Waters™

SPONSORED BY



SCIENTIFIC PROGRAMME

Wednesday, October 7th, 2026

- 2:00 p.m. Registration
- 2:30 p.m. Welcome addresses
- Federico Desimoni**
Presidente Consorzio Tutela Aceto Balsamico di Modena, Modena
- Marcello Pinti**
Vice Rector for Tech Transfer, Outreach and Community Engagement, UNIMORE
- Paola Montoro**
University of Salerno, President of the Division of Mass Spectrometry of the Italian Chemical Society
- Gianluca Giorgi**
University of Siena, 9th MS Food Day Chairman
- 3:00 p.m. **Session 1: Volatilomics**
Chairpersons: Luciano Navarini, Carmela Zacometti
- 3:00 p.m. **PL1: The key role of GC×GC-MS in food analysis**
Giorgia Purcaro
Analytical Chemistry, Gembloux Agro-Bio Tech, University of Liège, Belgium
- 3:45 p.m. **OR1: South Tyrolean speck aroma: characterization of volatiles and aroma-active compounds by GC-MS**
Ariana Soledad Poetto, Daniela Hey, Flavia Bianchi, Andrea Moretta, Thomas Winnischhofer, Peter Robatscher
Laboratory of Flavours and Metabolites - Laimburg Research Centre, Auer-Ora, Italy
- 4:00 p.m. **OR2: Volatilomic profiling of balsamic vinegars by HS-SPME-GC×GC-TOFMS: from protected-origin differentiation to maturation assessment**
Allan Polidoro, Sofia Malcangi, Monica Romagnoli, Nicola Marchetti, Flavio A. Franchina
Department of Environmental and Prevention Sciences, University of Ferrara, Ferrara, Italy
- 4:15 p.m. **OR3: Defining the chemical and sensory identity of Italian no- and low-alcohol beers market by HS-SPME-GC×GC-MS and sensory analysis**
Leonardo D'Intino, Gianluca Donadini, Giorgia Spigno, Mario Gabrielli
Dipartimento di Scienze e Tecnologie Alimentari per una filiera agro-alimentare sostenibile (DiSTAS), Università Cattolica del Sacro Cuore, Piacenza (Italy)
- 4:30 p.m. **OR4: From waste to bioactive resource: GC-MS profiling and bioherbicidal potential of *Petroselinum crispum* essential oil**
R. Francolino, R. Gaeta, L. De Martino, V. De Feo
Department of Pharmacy, University of Salerno, Fisciano, SA, Italy

- 4:45 p.m. **OR5: Unveiling chemical fingerprints of aging in balsamic vinegar of Modena (PGI) with GC-IMS**
S. Pellacani, M. Cocchi, G. Gazzoli, F. Martone, R. Marabottini, A. Napolitano, F. Ladogana, F. Desimoni, C. Durante, L. Strani
Dep. of Chemical and Geological Sciences, University of Modena and Reggio Emilia, Modena, Italy
- 5:00 p.m. Poster session (Poster P1-P27), Coffee break
- 5:30 p.m. **Session 2: Stable isotopes & food**
Chairpersons: Roberto Larcher, Giuliana Bianco
- 5:30 p.m. **OR6: Orbitrap HRMS for compound-specific isotope measurements: applications to pharmaceuticals and curcumin**
Alberto Roncone, Domenico Masuero, Roberto Penasa, Silvia Pianezze, Luana Bontempo
Fondazione Edmund Mach, Traceability Lab., San Michele all'Adige (TN), Italy
- 5:45 p.m. **OR7: Application of stable isotope ratio mass spectrometry (IRMS) for verification of geographical origin of egg products: establishment of a French reference dataset**
Michele Suman, Freddy Thomas
Barilla G.R. F.lli SpA, Research, Development & Quality & University of Parma, Parma, Italy
- 6:00 p.m. **OR8: *Griffonia simplicifolia* seed-based supplements: how stable isotope ratio analysis can help in commercial samples authentication**
Silvia Pianezze, Sara Malpaga, Arianna Vanelli, Filomena De Biasio, Matteo Perini
Centro di Trasferimento Tecnologico, Fondazione Edmund Mach, San Michele all'Adige, TN
- 6:15 p.m. **OR9: Authentication of high-value food products using combined GC-MS and GC-IRMS approaches: case studies on lavender and mint essential oils**
Roberto Penasa, Luana Bontempo, Long Chen, Mauro Paolini, Alberto Roncone, Domenico Masuero, Roberto Larcher
Fondazione Edmund Mach, San Michele all'Adige, Trento
- 6:30 p.m. **OR10: From liquid phase to headspace: compound-specific $\delta^{13}\text{C}$ analysis of aceto balsamico tradizionale di modena PDO by IRMS-based techniques**
Veronica D'Eusanio, Mirco Rivi, Andrea Marchetti
Department of Chemical and Geological Sciences, University of Modena and Reggio Emilia, Modena, Italy
- 6:45 p.m. **IP1: High- and low-resolution Agilent GC/MS platforms: technologies applied to the food market**
Marica Beggio
Agilent Italia
- 6:55 p.m. End of session
- 7:00 p.m. Welcome cocktail

Thursday, October 8th, 2026

9:00 a.m. **Session 3: Chemical contaminants**

Chairpersons: Michele Suman, Donatella Caruso

9:00 a.m. **PL2: Mass spectrometry analytical toolbox to tackle the PFASs challenge in food**

Stefan van Leeuwen

Wageningen University & Research (The Netherlands)

9:45 a.m. **OR11: Beyond chromatography: unmasking spice & herbs adulteration via AP-MALDI-MS**

Alessandra Tata, Alessia Di Noi, Andrea Massaro, Chiara Salvitti, Carmela Zacometti, Michele Suman, Federico Pepi, Anna Troiani

Laboratory of Experimental Chemistry, Istituto Zooprofilattico Sperimentale delle Venezie, Vicenza (VI), Italy

10:00 a.m. **OR12: Beyond standard trichothecenes: profiling emerging NX toxins in rice and wheat**

Alessandro Annunziato, Vito D'Ascanio, Daria Carella, Gerline Wiesenberger, Antonia Susca, Miriam Haidukowski, Antonio Moretti

National Research Council (CNR), Institute of Sciences of Food Production (ISPA), Bari, Italy

10:15 a.m. **OR13: Integrated mass spectrometry workflow for PFAS assessment in food contact materials according to the new PPWR requirements**

F. Cozzolino, F. Pratesi, M. Vitulli

Food Contact Center s.r.l

10:30 a.m. **OR14: Determination of phthalates in bottled, purified and reusable bottled water by SPME-GC/MS**

Claudia Lino, David Bongiorno, Serena Indelicato, Giuseppe Avellone

Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche, Università di Palermo, Palermo, Italy

10:45 a.m. **OR15: Development and validation of an analytical procedure based on a simplified μ -QuEChERS extraction for monitoring bisphenols in different honey botanical varieties using UHPLC-MS/MS**

Lorenzo Marino Cerrato, Rosalia López-Ruiz, Roberto Romero-González, Antonia Garrido Frenich, Stefania Albrizio

Department of Pharmacy, School of Medicine and Surgery, University of Naples Federico II, Naples, Italy

11:00 a.m. Poster session (Poster P1-P27), Coffee break

- 11:40 a.m. **Session 4:** Joint session with the Divisione di Chimica degli Alimenti-SCI
Bioactive compounds
Chairpersons: Paola Montoro, Nadia Mulinacci
- 11:40 a.m. **KN: The gas-phase goldmine: volatilomics from cutting edge platforms to real world use**
Philipp Weller, Lukas Bodenbender, Richard Frey, Nabiollah Mobaraki
Technical University of Applied Sciences, Mannheim, Germany
- 12:10 p.m. **OR16: HS-SPME-GC-MS and HPLC-DAD-MS as tools to support the optimization of high-quality flavoured olive oils produced through co-milling: case studies using chili peppers, basil and rosemary**
Lorenzo Cecchi, Carlotta Breschi, Tommaso Ugolini, Erminio Monteleone, Renzo Fusi, Francesca Ieri, Silvio Menghini, Fabio Boncinelli, Maria Bellumori, Marzia Innocenti, Nadia Mulinacci, Bruno Zanon
Department of Agricultural, Food, Environmental, and Forestry Sciences and Technologies (DAGRI) – University of Florence, Firenze, Italy
- 12:25 p.m. **OR17: From cabbage to fermented foods: integrated profiling of bioactive compounds**
Tiziana Nardin, Francesca Martinelli, Roberto Larcher
Fondazione Edmund Mach, Technology Transfer Centre, Food Chemistry, San Michele all'Adige (TN), Italy
- 12:40 p.m. **OR18: Marine by-product valorization: LC-MS/MS Characterization of collagen peptides derived from the edible sea urchin *Paracentrotus lividus***
Tamara Chwojnik, Gijs JC Vreeke, Giovanni A van Kralingen, Matteo Brilli, Stefania Marzorati, Michela Sugni, Jean-Paul Vincken
Department of Environmental Science and Policy, University of Milan, Milan, Italy
- 12:55 p.m. **OR19: Supramolecular solvents for modern food analysis**
Noelia Caballero-Casero, Soledad Rubio
Department of Analytical Chemistry, Institute of Chemistry for Energy and the Environment (IQUEMA), University of Córdoba, Córdoba, Spain
- 1:10 p.m. End of session
- 1:15 p.m. Buffet lunch
- 2:30 p.m. **Session 5: Flavour**
Chairpersons: Franco Biasioli, Caterina Durante
- 2:30 p.m. **PL3: Flavour is not concentration: using dynamic mass spectrometry insights to engineer food perception**
Ian Fisk
International Flavour Research Centre, University of Nottingham
- 3:15 p.m. **OR20: Basil volatilome and quality assessment for high-quality pesto: tracing aroma markers across the production chain**
Giulia Tapparo, Erica Liberto, Gianluca Cucco
Department of Drug Science and Technology, University of Turin, Turin, Italy

- 3:30 p.m. **OR21: Influence of fermentation state and amino acid supplementation on volatile compound production by *Lactiplantibacillus plantarum* in a model fermentation system**
Max Nally, Iuliia Khomenko, Franco Biasioli, Phil Bremer, Pat Silcock, Graham Eyres
Department of Food Science, University of Otago, Dunedin, New Zealand
- 3:45 p.m. **OR22: Pyridine precursors other than trigonelline under coffee roasting conditions**
Valentina Lonzarich, Luciano Navarini
illycaffè S.p.A., Trieste, Italy
- 4:00 p.m. **IP2: Recent advancements in mass spectrometry for food analysis: from sample prep to direct analysis**
Daniela Peroni
SRA Instruments Spa, Cernusco sul Naviglio (MI), Italy
- 4:10 p.m. Poster session (Poster P28-P55), Coffee break
- 4:45 p.m. **Session 6: Targeted & untargeted analysis**
Chairpersons: Sauro Vittori, Chiara Cordero
- 4:45 p.m. **OR23: Soft chemical ionization and ultra fast isomer separation in food analysis using Vocus high-resolution chemical ionisation ion mobility spectrometry time-of-flight (Vocus HR-CI-IMS-TOF)**
Iuliia Khomenko, Andrea Romano, Mehdi Moayed, Jiayi Zhang, Emanuela Betta, Michele Pedrotti, Matteo Tonezzer, Luca Cappellin, Franco Biasioli
Fondazione Edmund Mach, San Michele all'Adige, Italy
- 5:00 p.m. **OR24: A rigorous chemometric workflow for multi-year shelf-life prediction of hazelnuts**
Sara Tanilli, Andrea Caratti, Giuseppe Genova, Alex Fissore, Cristina Casetta, Angelica Fina, Fulvia Trapani, Erica Liberto, Chiara Cordero
Department of Drug Science and Technology, University of Turin, Turin, Italy
- 5:15 p.m. **OR25: The use of the untargeted approach by HPLC-Q-ToF to study the chemical profile of different matrices**
Laura Acquaticci, Simone Angeloni, Massimo Ricciutelli, Giovanni Caprioli
University of Camerino, School of Pharmacy, Chemistry Interdisciplinary Project (ChIP), Camerino, Italy
- 5:30 p.m. **OR26: Exploring the phytochemical profile and biological potential of artichoke petal, leaf and stem extracts**
Lorenza Marinaccio, Eulogio J. Llorent-Martínez, Gokhan Zengin, Angelo Cichelli, Adriano Mollica, Azzurra Stefanucci
Department of Innovative Technologies in Medicine and Dentistry, "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy

- 5:45 p.m. **OR27: A comprehensive characterization of anthocyanins in fungal-resistant red grape varieties by high-resolution mass spectrometry**
M. De Rosso, S. Soligo, E. Piutti, A. Piccinin, D. Piccinin, R. Carraro, L. Tarricone, G. Masi, S. Roccotelli, M. Gardiman, A. Panighel, F. Tarricone, L. Sansone, R. Flamini
Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria (CREA), Conegliano (TV), Italy
- 6:00 p.m. **OR28: Identification of carboxymethylcellulose (CMC) and potassium polyaspartate (KPA) in wine through parallel targeted enzymatic digestion workflows and UHPLC-HRMS**
Francesca Martinelli, Emiliano Fadanelli, Andrea Rosolen, Roberto Larcher, Tiziana Nardin
Fondazione Edmund Mach, San Michele all'Adige (TN), Italy
- 6:15 p.m. **OR29: Integrated untargeted metabolomics workflow enables comprehensive metabolic profiling of *Lepidium sativum* microgreens and vegetative plants**
Francesca Monzillo, Luigi d'Aquino, Vincen Wu, Nicola Zamboni, Sonia Piacente, Paola Montoro
Department of Pharmacy, University of Salerno, Fisciano, Italy
- 6:30 p.m. **OR30: Multiplatform profiling of Maillard reaction products in PEF-treated nuts**
Laura Alessandroni, Albert Sebastià, Emilia Ferrer, Francisco J. Barba, Gianni Sagratini
Chemistry Interdisciplinary Project (ChIP), School of Pharmacy, University of Camerino, Camerino, Italy
- 6:45 p.m. End of session
- 8:30 p.m. Social dinner

Friday, October 9th, 2026

9:00 a.m. **Session 7: AI & metabolomics**

Chairpersons: Linda Monaci, Fabiana Piscitelli

9:00 a.m. **PL4: Chemically grounded AI for untargeted food profiling**

Rasmus Bro

University of Copenhagen, Faculty of Science, Frederiksberg, Denmark

9:45 a.m. **OR31: LC-HRMSMS profiling of bioactive lipids and triterpenoids in pumpkin seeds**

Annunziata Paolillo, Assunta Napolitano, Milena Masullo, Sonia Piacente

Dipartimento di Farmacia, Università degli Studi di Salerno, Fisciano (SA), Italy

10:00 a.m. **OR32: Characterization of pancetta piacentina PDO during ripening through redox markers, fatty acid profiling, and integrated untargeted metabolomics-lipidomics**

Gabriele Rocchetti, Federico Froidi, Giulia Fappani, Michela Errico, Maria Chiara Fontanella, Samantha Sigolo, Aldo Prandini

Department of Animal Science, Food and Nutrition, Università Cattolica del Sacro Cuore, Piacenza, Italy

10:15 a.m. **OR33: Profiling microbial metabolism of verbascoside and olive pomace extract in an *in vitro* fermentation model using a metabolomics approach**

Shaymaa Abdulrazzaq, Rocío García Villalba, Francisco Tomás-Barberán, Stefania Silvi, Polina Makarycheva, Dennis Fiorini, Carlos J. Garcia

School of Science and Technology, University of Camerino, Camerino (MC), Italy

10:30 a.m. **OR34: Formulation, biochemical characterization and shelf-life study of YoAlp® whey based beverage containing local fruits juices**

S. Valentini, M. Merlet, T. Flutto, R. Pramotton, S. Zenato, L. Verneti-Prot

Institut Agricole Régional, Aosta, Italy

10:45 a.m. **OR35: Enhanced MOSH and MOAH screening in fat-rich foods using integrated LC-GC-FID and GC×GC-QToF/FID approaches**

I. Annovazzi, D. Baglio, P. Rovellini, V. Cardenia

Department of Agricultural, Forest and Food Science, University of Turin, Grugliasco (Turin) Italy

11:00 a.m. **IP3: Setting New Standards in PFAS Food Analysis: The AOAC Official Method Developed with Shimadzu LC-MS/MS Technology**

Massimiliano Magna

Shimadzu Italia S.r.l., Milan, Italy

11:10 a.m. **Poster session (Poster P28-P55), Coffee break**

- 11:45 a.m. **Session 8: Primary and specialized metabolites**
Chairpersons: Giuseppe Avellone, Rita Petrucci
- 11:45 a.m. **OR36: Unravelling geographical, seasonal and technological variability in the untargeted metabolomic profile of Parmigiano Reggiano PDO natural whey starter through ASCA-integrated chemometrics**
Pier Paolo Becchi, Rasmus Bro, Valentina Pizzamiglio, Luigi Lucini
Department for Sustainable Food Process, Università Cattolica del Sacro Cuore, Piacenza, Italy
- 12:00 p.m. **OR37: A precursor ion scan-based mass spectrometry workflow for intact glucosinolate characterization in radish microgreens for nutraceutical applications**
Vincenzo Piccolo, Maria Maisto, Adua Marzocchi, Erika Esposito, Emma Mitidieri, Roberta d'Emmanuele di Villa Bianca, Vincenzo Summa, Gian Carlo Tenore
Department of Pharmacy, University of Naples Federico II, Naples, Italy
- 12:15 p.m. **OR38: Enzyme-assisted multidimensional LC-IM-HRMS annotation of acetylated xylo-oligosaccharides generated from commercial xylans**
G. Lupi, A. Fuso, A. Caligiani
University of Parma, Parma, Italy
- 12:30 p.m. **OR39: LC-MS/MS analysis of marker peptides for the detection of various mustard species and rapeseed in foods**
Barbara Prandi, Andrea Faccini, Francesca Lambertini, Tullia Tedeschi
Department of Food and Drug, University of Parma, Parma, Italy
- 12:45 p.m. **OR40: Role of red cabbage anthocyanins in the reaction with AAPH and carotenoid protection**
Claudia Lombroni, Ksenia Morozova, Tiziana Nardin, Roberto Larcher, Matteo Scampicchio
University of Turin, Department of Agricultural, Forest and Food Sciences, Grugliasco (TO), Italy
- 1:00 p.m. Concluding remarks & Arrivederci!!

POSTER COMMUNICATIONS

- P1 From dairy by-product to functional ingredient: valorization of mozzarella stretching water as a source of bioactive peptides through microbial fermentation**
Antonella Lamonaca, Alessia Marzulli, Marianna de Gioia, Loris Pinto, Rosa Pilolli
National Research Council of Italy, Institute of Sciences of Food Production (CNR-ISPA), Bari, Italy
- P2 Data fusion of the non-volatile and volatile metabolome for shelf-life and sensory quality prediction of skim milk powder**
Giulia Gula, Luigi Locuratolo, Franco Rosso, Andrea Peraino, Augusta Caligiani
Food and Drug Department, University of Parma, Parma, Italy
- P3 Aromatic and fatty acid profiling of Aosta Valley milk and fontina PDO cheese: the value of summer alpine pasture**
Thedy Laura, Luca Verneti-Prot, Sabina Valentini
Institut Agricole Régional, Reg. Aosta, Italy
- P4 Comparative metabolite profiling of fourteen aromatic herbs using targeted and untargeted mass spectrometry approaches, combined with chromatographic separation techniques**
Paola Di Matteo, Teresa Bovenzi, Mario Borrelli, Rita Petrucci
Dip. SBAI, Sapienza Università di Roma, Rome, Italy
- P5 Evaluation of Cherry and Datterino tomatoes freshness and variety using a multi-analytical platform: combining PTR-ToF-MS, HS-SPME-GC-MS, and two portable electronic noses**
J. Zhang, I. Khomenko, M. Tonezzer, E. Betta, M. Pedrotti, A. Romano, R. Capuano, A. Catini, C. Di Natale, F. Biasioli
Università di Cagliari, Dipartimento di Scienze Chimiche e Geologiche, Campus di Monserrato, Monserrato, Italy
- P6 HPLC-MS/MS and ¹H-qNMR as complementary techniques for the characterization and quantification of shatavarins in *Asparagus racemosus* L.**
Beatrice Zonfrillo, Maria Bellumori, Mohamad Khatib, Lorenzo Cecchi, Michele Zucherman, Gianluca Bartolucci, Marta Menicatti, Marzia Innocenti, Nadia Mulinacci
Department of Neurofarba, Nutraceutical and Pharmaceutical section, University of Florence, Sesto Fiorentino, Italy
- P7 Effect of *in vitro* gastrointestinal digestion on the metabolic profile of the ancient cultivar “Pericina” pear (*Pyrus communis*) from Picentini Mountains (Salerno, Italy)**
Roberta Cuozzo, Milena Masullo, Sonia Piacente
Dipartimento di Farmacia, Università degli Studi di Salerno, Fisciano (SA), Italy
- P8 High-resolution mass metabolomic profiling for the chemical biodiversity of traditional apulian sweet pepper (*Capsicum annuum* L.) landraces**
Antonino Rizzuti, Emanuela Blanco, Biagia Musio, Vito Gallo, Piero Mastrorilli, Anna Bonasia, Giulia Conversa, Gabriella Sonnante
Department of Civil, Environmental, Land, Construction and Chemistry (DICATECh), Polytechnic University of Bari, Bari, Italy

P9 Characterisation of the volatile fraction of wild food plants before and after fermentation by HS-SPME/GC-MS

Leandra Leto, Benedetta Bottari, Marco Biagi, Francesco Martelli, Federico Bertoldo, Renato Bruni, Martina Cirlini

Department of Food and Drug, University of Parma, Parma, Italy

P10 An ASCA-based investigation of hazelnuts volatilome evolution during storage

Sara Tanilli, Andrea Caratti, Giuseppe Genova, Alex Fissore, Cristina Casetta, Fulvia Trapani, Erica Liberto, Chiara Cordero

Department of Drug Science and Technology, University of Turin, Turin, Italy

P11 Detection of biosynthetic citric acid and sugar addition in Italian tomato passata using liquid chromatography-chemical oxidation-isotope ratio mass spectrometry

Silvia Pianezze, Domenico Masuero, Matteo Perini, Stefania Carpino, Nicola Barilaro, Vincenzo Di Martino, Luana Bontempo

Fondazione Edmund Mach, San Michele all'Adige, Italy

P12 Acrylamide: from bean to cup

Barbara Angelone, Paolo Candotti, Barbara Massardi, Rita Salami, Giulia Lorenzi, Simonetta Menotta, Mara Gasparini

Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini", Brescia, Italy

P13 Linalool in green *Coffea arabica* by HS-SPME/GC-MS and HS/GC-IMS

Gaia Bechis, Valentina Lonzarich, Matteo Bordiga, Luciano Navarini

illycaffè S.p.A., Trieste, Italy

P14 Multi-analytical benchmarking of cold-brew systems: HS-SPME-GC-MS reveals quality evolution during refrigerated storage

Agnese Santanatoglia, Martina Prioretti, Matteo Petrolati, Giovanni Caprioli, Gianni Sagratini

School of Pharmacy (ChIP), University of Camerino, Camerino (MC), Italy

P15 Cellulose-based films from coffee silverskin for sustainable active packaging: the rECOBIOpack project

Chiara Scapuzzi, Tamara Chwojnik, Valeria Romanucci, Luisella Verotta, Luciano Navarini, Paola Crisafulli, Stefania Lupinelli, Giovanni Di Fabio, Daniele Carullo, Stefano Farris, Stefania Marzorati

Department of Environmental Science and Policy, University of Milan, Milan, Italy

P16 Coffee-derived caffeoylquinides rapidly remodel the intestinal endocannabinoidome and improve epithelial barrier function in Caco-2 cells

Fabiana Piscitelli, Marco Allarà, Roberta Verde, Elisabetta De Angelis, Elena Guercia, Javiera Fontecilla-Escobar, Fabio Arturo Iannotti, Luciano Navarini

Consiglio Nazionale delle Ricerche – Istituto di Chimica Biomolecolare (CNR-ICB), Pozzuoli (NA), Italy

P17 Quinolizidine alkaloids in lupin: a case of acute intoxication

Ilaria Prizio, Mariantonietta Peloso, Gaetan Minkoumba Sonfack, Elisabetta Caprai

National Reference Laboratory for natural toxins in food, Food Chemical Department, Izsler, Bologna, Italy

- P18 Capturing toxic tropane alkaloids from *Datura stramonium*, *Datura metel*, *Mandragora autumnalis* in plant based foods via DART-HRMS**
Nicole Cascio, Carmela Zacometti, Francesca Asson, Andrea Massaro, Roberto Stella, Roberta Masin, Giancarlo Biancotto, Alessandra Tata
Istituto Zooprofilattico Sperimentale delle Venezie, Laboratory of Experimental Chemistry, Vicenza, Italy
- P19 Investigating amino acid–sugar interactions for cocoa-like aroma generation in a chickpea flour-based solid food matrix**
Giorgia Mattina, Leonardo Lorenzi, Andrea Francioni, Riccardo Bottiroli, Vincenzo Fogliano, Eugenio Aprea
University of Trento, San Michele all'Adige, Italy
- P20 Advancing peanut aroma analysis through integrated headspace sampling and GC×GC–HRMS**
Andrea Caratti, Angelica Fina, Sara Tanilli, Fulvia Trapani, Erica Liberto, Giuseppe Genova, Cristina Casetta, Carlo Bicchi, Chiara Cordero
Department of Drug Science and Technology, University of Turin, Turin, Italy
- P21 Authentication of the geographical origin of Italian multifloral honey: multi-analytical characterization of the composition and pesticide residues to distinguish the production area**
Francesca Asson, Carmela Zacometti, Andrea Massaro, Fabjola Bilo, Marianna Martinello, Franco Mutinelli, Elisabetta Schievano, Alessandra Tata
Laboratory of Experimental Chemistry, Istituto Zooprofilattico Sperimentale delle Venezie, Vicenza (VI), Italy
- P22 Comprehensive characterization of honey from Kazakhstan, Italy, China and other countries through volatile profiling by GC-MS, polyphenolic profiling by HPLC-MS/MS and quantification of 5-hydroxymethylfurfural by HPLC-DAD**
Gulzhan Khamitova, Laura Acquaticci, Lazzat Karasholakova, Giovanni Caprioli
Research Institute of Biotechnology and Ecology, Zhetysu University named after I. Zhansugurov, Taldykorgan, Kazakhstan
- P23 HS-GC-IMS authenticates the botanical origin of seven types of Italian monofloral honey, annotating the significant volatile compounds by the novel HS- SPME-GC-IMS-MS prototype**
Filippo Panni, Andrea Massaro, Lukas Bodenbender, Carmela Zacometti, Roberto Piro, Angela Felicità Savino, Philipp Weller, Alessandra Tata
Ministero dell'agricoltura, della Sovranità Alimentare e delle Foreste, Dipartimento dell'Ispettorato Centrale della tutela della Qualità e Repressioni Frodi dei prodotti agro-alimentari, ICQRF Laboratory of Perugia, Perugia, PG, Italy
- P24 High-resolution mass spectrometry for varietal authentication of bread wheat**
Lorenzo Lovatti, Felix Bacher, Peter Robatscher, Ksenia Morozova, Matteo Mario Scampicchio
Faculty of Agricultural, Environmental and Food Sciences, Free University of Bozen-Bolzano, Bolzano, Italy
- P25 UPLC–HRMS and ¹H NMR Fingerprinting for the Authentication of Italian Saffron**
Ksenia Morozova, Lucrezia Angeli, Timo D. Stark, Corinna Dawid, Enmanuel Cruz Muñoz, Davide Ballabio, Matteo Scampicchio
Faculty of Agricultural, Environmental and Food Sciences, Free University of Bozen-Bolzano, Bolzano, Italy

- P26 Development of a HS/GC-IMS screening method for honey geographical origin classification**
Giancarlo Quaglia, Cesare Rossini, Matteo Bordiga
Lab Service Analytica s.r.l., Anzola Emilia (Bologna), Italy
- P27 Characterisation of monofloral and polyfloral honey from rural areas of Guatemala and role in the daily nutrition**
Chiara Scapuzzi, Tamara Chwojnik, Luisella Verotta, Maria Pia Macchi, Stefania Marzorati
Department of Environmental Science and Policy, University of Milan, Milan, Italy
- P28 Screening of fruity esters in sparkling wines produced by refermentation in pressurized stainless steel tank (Charmat-Martinotti method)**
A. Panighel, F. Tarricone, M. De Rosso, A. Sparacio, S. Sparla, S. Vincenzi, L. Vettori, R. Flamini
Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria - Centro Ricerca Viticoltura Enologia, Conegliano (TV), Italy
- P29 Impact of a novel copper-free formulation on stilbene biosynthesis in grapevine shoots**
Ghazal Namazzadeh, Laura Alessandroni, Filippo Maggi, Eleonora Spinozzi, Matteo Michelini, Valerio Borzatta, Gianni Sagratini
School of Pharmacy, Chemistry Interdisciplinary Project (ChIP), University of Camerino, Camerino (MC), Italy
- P30 Multi-matrix LC-MS/MS screening of 40 PFAS in edible oils and food contact paper materials**
G. Mosconi, P. Fusari, C. Scagliarini, D. Baglio, P. Rovellini
Innovhub – Stazioni Sperimentali per l'Industria S.r.l., Milan, Italy
- P31 Chemical characterization of olive mill wastewater by untargeted UHPLC-QTOF-MS data-dependent analysis approach**
Simone Angeloni, Laura Alessandroni, Giovanni Caprioli, Gianni Sagratini, Massimo Ricciutelli
Chemistry Interdisciplinary Project (ChIP), School of Pharmacy, University of Camerino, Camerino, Italy
- P32 High-resolution metabolomics uncovers xylem metabolic signatures associated with *Philaenus spumarius* feeding in olive**
Maria Assunta Crescenzi, Erica Tinelli, Vincenzo Verrastro, Daniele Cornara, Paola Montoro
Department of Pharmacy, University of the Study of Salerno, Fisciano, Italy
- P33 Comparison of commercial monovarietal extra virgin olive oil classification methods: targeted profiling with HS-SPME-GC-MS and HPLC-DAD vs untargeted fingerprinting with HS-GC-IMS**
Tommaso Ugolini, Lukas Bodenbender, Nabiollah Mobaraki, Marzia Migliorini, Ilaria Romei, Bruno Zanoni, Lorenzo Cecchi, Philipp Weller
DAGRI –Department of Agricultural, Food, Environmental, and Forestry Sciences and Technologies, University of Florence, Florence, Italy
- P34 Comparative evaluation of green solvents for the extraction of olive leaf phenolics by UHPLC-HRMS/MS-based metabolomics**
Orsolina Vetrano, Maria Assunta Crescenzi, Daniele Sanna, Angela Fadda, Paola Montoro
Department of Pharmacy, University of the Study of Salerno, Fisciano, Italy

P35 Tracking reactive aldehydes in bakery matrices and oven emissions via HS-SPME-GC-MS/MS

Mauro Paolini, Roberto Larcher

Fondazione Edmund Mach (FEM), San Michele all'Adige, Italy

P36 UHPLC-HRMS profiling of botanical extracts for antifungal and bioactive applications in bakery products

Giulia Fappani, Gabriele Rocchetti

Department of Animal Science, Food and Nutrition, Università Cattolica del Sacro Cuore, Piacenza, Italy

P37 An LC-MS/MS method for evaluating cyanotoxins contamination in bivalve mollusks through freshwater-to-marine pathways

S. Mazza, M. Siracusa, S. Bacchiocchi, G. Diomedì, S. Accoroni, A. Tomasini, M. S. Princivalli, A. Baldoni, F. Serenelli, A. Piersanti

Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", Perugia

P38 Levels of 3-monochloropropane-1,2-diol (3-MCPD as sum of free and ester-bound forms) and glycidyl esters (GE) in fish oil: results of five-year monitoring

Valentina Gamba, Elena Pellegrini, Barbara Albanese, Simona Romelli, Mara Gasparini

Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Brescia, Italy

P39 Effects of fishing pressure on the fatty acid composition in red mullet (*Mullus barbatus*) from the central Adriatic sea

Behixhe Ajdini, Federico Girolametti, Silvia Illuminati, Anna Annibaldi, Luca Caracausi, Giacomo Milisenda, Emanuela Fanelli, Cristina Truzzi

Department of Life and Environmental Sciences, Università Politecnica delle Marche, Ancona, Italy

P40 Unravelling aroma modulation in extruded pea protein through carob bean protein incorporation: A comprehensive GC×GC–MS volatilomic study

Faezeh Khoobakht, Francesco Reale, Giorgia Mattina, Silvia Carlin, Panagiotis Arapitsas, Eugenio Aprea

Center Agriculture Food Environment, University of Trento, San Michele all'Adige, Italy

P41 Wildlife: a protein alternative to intensive farming? Food security and environmental bioindicator study

E. Vaccaro, G. Ferrari, V. Armenio, V. Savio, F. Armellino, S. Robetto, B. Moroni, V. Listorti, L. De Paolis, E. Razzuoli, L. Guardone, V. Ciccotelli

Istituto Zooprofilattico Sperimentale Piemonte, Liguria, Valle d'Aosta, Torino, Italy

P42 Zein-based nanoparticles: a green approach to bioactive peptides delivery

Stefania Marzorati, Patrícia Duque-Estrada, Davide Emide, Daniela Maggioni, Thamiris Monteverde Vicentini, Chiara Magni, Chiara Scapuzzi, Rene Lametsch, Iben Lykke Petersen, Mikołaj Dziurzyński, Alessio Scarafoni

Department of Environmental Science and Policy, University of Milan, Italy

P43 Comparison between conventional and functional data analysis of time resolved volatilomics data: a case study of microalgae fermentation with kefir starters

Iuliia Khomenko, Martina Moretton, Emanuela Betta, Antonio Luca Langellotti, Vittorio Capozzi, Vincenzo Fogliano, Franco Biasioli

Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige (TN), Italy

- P44 From insect frass to valuable microalgal biomass: screening species for sustainable omega-3 PUFA production within the ALGINLOOP project**
Behixhe Ajdini, Miles Minio, Federico Girolametti, Lorenzo Massi, Anna Annibaldi, Silvia Illuminati, Alessandra Norici, Cristina Truzzi
Department of Life and Environmental Sciences, Università Politecnica delle Marche, Ancona, Italy
- P45 Real-time PTR–ToF–MS tracking of volatile sulfur compound release in food matrices: the role of whole saliva and the oral bacterial enzyme FnaPatB1**
Fatemeh Izadfar, Michele Pedrotti, Sylvester Holt, Emanuela Betta, Iuliia Khomenko, Flavia Gasperi, Franco Biasioli
Center for Agriculture, Food and Environment (C3A), University of Trento, San Michele all'Adige, Italy
- P46 Chewing on flavour: temporal dynamics of aroma and sweetness in sugar-free gum**
M. Pedrotti, S. van de Laar, L. Menghi, L. Cappellin, G. Rinaudo, M. Roverso, S. Bogialli, S. Pettenuzzo, V. Fogliano, F. Biasioli
Fondazione Edmund Mach, Research and Innovation Centre, San Michele all'Adige, TN, Italy
- P47 Assessment of phthalate migration from reusable plastic water bottles into drinking water**
Claudia Lino, David Bongiorno, Serena Indelicato, Giuseppe Avellone
Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche, Università di Palermo, Palermo, Italy
- P48 Investigation of inorganic elements in insect-based novel foods by ICP-MS**
M. Ciriaci, S. Mazza, F. Griffoni, P. Palombo, M. Siracusa, T. Tavaloni, C. Barola, A. Stramenga, S. Bacchiocchi, A. Piersanti
Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", Ancona-Perugia
- P49 In silico assessment of the potential allergenicity of purple phototrophic bacteria biomass**
Beatrice Aiuto, Cristina Lamberti, Simona Cirrincione, Loredana Tarraran, Antonella Leone, Veronica Lattanzio, Laura Cavallarin
CNR-ISPA Torino, Italy
- P50 Determination of Volatile Organic Compounds (VOCs) released from plastic packaging for dry foods: headspace analysis (HS-GC-MS) and migration studies using Tenax® as food simulant**
Alessandra Como, Carmen Palermo, Diego Centonze, Cristina Nerin
Università di Foggia, Italy
- P51 Land snails as terrestrial sentinel organisms for monitoring heavy metal and phthalate accumulation**
Chiara Marchese, Rosa Avolio, Silvia Olivieri, Roberta Moriano, Paola Brizio, Stefania Squadrone, Maria Cesarina Abete
Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle D'Aosta (IZS PLV), Turin, Italy

P52 Evaluation of SPME fibers and extraction salts for the volatilomic profiling of paprika using a chemometric approach

Alessandra Como, Diego Centonze, Carmen Palermo

Università di Foggia, Foggia, Italy

P53 Effect of Vitis vinifera leaf enrichment on the volatile profile and sensory properties of grape juice

Cristina Matarazzo, Massimo Iorizzo, Livia Malorni, Rosaria Cozzolino

University of Molise, Department of Agriculture, Environmental and Food Sciences, Campobasso, Italy

P54 Geographical authentication of extra virgin olive oil by mid-level fusion of FTIR, NMR and IRMS data

Maria Assunta Acquavia, Francesco Romaniello, Chiara Portesi, Matteo Berruto, Maria Pia Montana Lampo,²Vittorio Capozzi, Giuliano de Seenen, Andrea Mario Rossi

National Metrology Institute of Italy, Matera, Italy

P55 Exploring the aroma profile of fermented mung bean gels using lactic acid bacteria and yeast strains

L. Marchesini, M. Mazzucotelli, E. Betta, I. Khomenko, A. Levante, F. Bot, M. Pedrotti

University of Parma, Parma, Italy

P56 iso CHROM® LC: from honey analysis to a versatile platform for complex food matrices

Pasquale Carlino, Philipp Schodder, Marco Schmidt, Silvia Alfieri, Mike Seed

Elementar, Italy

ABSTRACTS

PLENARY, KEYNOTE, ORAL COMMUNICATIONS

PL1**The key role of GC×GC-MS in food analysis***Giorgia Purcaro*

Analytical Chemistry, Gembloux Agro-Bio Tech, University of Liège, Belgium

Comprehensive two-dimensional gas chromatography (GC×GC), introduced by Phillips in 1991, represents one of the most transformative developments in the field of gas chromatography. Its principal strengths lie in its capacity to achieve simultaneous, highly sensitive targeted and untargeted chemical profiling, enabling an unprecedented level of detail in sample characterization. Additionally, GC×GC can streamline sample preparation workflows and reveal novel chemical insights even in extensively studied matrices.

Although GC×GC is now regarded as a mature analytical technique, its adoption in routine food control laboratories remains limited. Nonetheless, it has demonstrated exceptional value in evaluating the quality, authenticity, and safety of food products. This presentation will highlight several case studies that exemplify the technique's analytical power in food analysis.

GC×GC has proven effective for more many routine applications, including the characterization of complex fatty acid profiles, the assessment of food quality and authenticity—such as in the case of olive oil-, insight in the contamination, as in the case of mineral oil hydrocarbons analysis.

By exploiting either the volatile fraction or the chromatographic fingerprint of minor constituents, GC×GC provides a robust and sensitive platform capable of uncovering critical information related to food quality, safety, and authenticity.

OR1

South Tyrolean speck aroma: characterization of volatiles and aroma-active compounds by GC-MS

Ariana Soledad Poetto,¹ Daniela Hey,¹ Flavia Bianchi,² Andrea Moretta,²
Thomas Winnischhofer,² Peter Robatscher¹

¹ Laboratory of Flavours and Metabolites - Laimburg Research Centre; Laimburg 6, Auer-Ora, Italy

² Meat Products - Laimburg Research Centre; Laimburg 6, Auer-Ora, Italy

Summary: *The aim of this study was to characterize South Tyrolean Speck aroma. The volatile organic compounds (VOCs) were extracted by headspace-solid phase microextraction and analyzed by gas chromatography coupled to mass spectrometry (GC-MS). After identification, based on mass spectra and retention index, the origin and aroma-activity of each VOC was investigated.*

Keywords: South Tyrolean Speck, Aroma, HS-SPME-GC-MS

Introduction

South Tyrolean Speck is a traditional Alpine cured meat product consisting of a lightly smoked and air-dried pork ham, produced and aged in South Tyrol according to PGI specifications. It combines Mediterranean curing practices with Northern European smoking techniques, giving it a characteristic mildly smoky flavour and distinctive sensory profile [1]. Volatile Organic Compounds (VOCs) have a significant role in determining product perception and liking and their formation depends on various factors, such as animal diet, pigmeat muscle enzymatic and physico-chemical modifications, combustion during smoking, addition of salt, spices, and microbial metabolism [2, 3, 4, 5]. VOCs analysis could also be useful for detecting spoilage since improper storage conditions alter the aroma of the product but also serve as quality indicator and vector for production process optimization [4, 6]. In this study, dry-curing, spicing, and smoking have been identified as main process steps involved in aroma formation. Accordingly, the origin of Speck VOCs has been investigated by analysing three different matrices: speck dices, curing spices, and beech wood smoke. VOCs were isolated by headspace-solid phase microextraction (HS-SPME) and the so extracted VOCs were analysed by gas chromatography coupled to mass spectrometry (GC-MS).

Experimental

The analyses were performed on South Tyrolean Speck produced in a pilot plant at the laboratories of the Meat Products Research Group of the Laimburg Research Centre, according to the traditional recipe. Hams were cut in portions and aged for approximately 120 days at 4°C, and finally frozen at -80°C until analysis. GC-MS parameters were set and further optimised based on similar methods for the analysis of dry-cured hams found in the literature [3, 4, 6, 7, 8, 9]. For each of the 24 Speck samples analysed and for the speck spices, 2 g were weight and added with 15 µL of a 1-hexan-D13-ol solution (100 mg/L in water), used as an internal standard. Smoke was produced by slow-burning beech wood chips and juniper berries in a dedicated burning chamber and syringe sampled. For the extraction of VOCs by SPME, a 50/30 µm DVB/CAR/PDMS fiber was used. GC separation was performed on a SLB-5ms fused silica capillary column (30 m x 0.25 mm x 0.25 µm film thickness, Supelco) using a GCMS-QP2010 SE instrument (Shimadzu), employing helium (99,99%) as carrier gas, at a flow of 1 ml/min. A custom temperature program was developed to optimize VOCs chromatographic separation. Electron Ionization at 70 eV and full scan mode were used for MS analysis. Fragmentation pattern and retention index (RI) were used for analytes identification, by matching the experimental results with NIST, FFNSC Shimadzu, and Adams' libraries. Mass spectra similarity was considered if higher than 80% while for RI a deviation of ±20 was accepted.

Results

87 VOCs were annotated. 84 of them were identified according to MS fragmentation pattern and RI, as specified in section 2 (Experimental). As reported in table 1, most VOCs resulted to be aliphatic hydrocarbons, followed by aldehydes, terpenes, and alcohols.

Aliphatic hydrocarbons originate from lipids oxidation [10]. Aldehydes and terpenes resulted to be the most abundant classes of aroma-active compounds. Aldehydes, originating from lipid oxidation, amino acid degradation, and microbial activity, contribute with meaty, fruity, citrus, and onion-like notes.

Terpenes and terpenoids, mainly derived from curing spices but also related to pig feeding, provided herbaceous, pine-like, citrus, floral, and spicy aromas. Despite their low concentrations, these compounds likely play an important role in the characteristic sensory profile of Speck and their presence is appreciated because of their antioxidative and antimicrobial properties [11].

Table 1. Percentage VOCs composition

Chemical group	% VOCs composition
Alkanes and alkenes	78.7 %
Aldehydes	16.4 %
Terpenes	3.0 %
Alcohols	1.8 %
Others	0.02 %

The aromatic hydrocarbons proceed from both plant metabolism and combustion process, and have very different scents, providing clove, vanilla, and woody aromas, but also smoky and phenolic notes [12,13]. Concerning alcohols, even if their role as aroma-active compounds is supposed to be minor compared to aldehydes because of their high perception threshold, they can globally impart an herb-like, woody, and fatty taste [14]. Traces of other VOCs have been also reported, such as ketones and esters, which are thought to be microbial products and are well known for their various hints and floral notes.

Conclusions

In the study a global characterisation of the VOCs of South Tyrolean Speck was carried out by HS- SPME-GC-MS. The same analytical parameters were employed to analyse spices and smoke VOCs, to trace the roots of Speck aroma. Knowing the origin of aroma-active compounds can be helpful for improving product quality by regulating the production process (e.g. production steps duration). The same analytical parameters can be employed to perform microbiological studies on the product to correlate microbial VOCs to specific populations and assess proper storage or to develop an experimental plan to modulate the dry-cured meat microbiota and finally optimize the aroma of the product.

References

1. Colorio S, Precazzini F, Stella M, et al. *Ital J Food Saf.* 2024; 13(4):12630.
2. Karolyi D, Škrlep M, Marušić Radovčić N, et al. *Animals.* 2024; 14(2):286.
3. Górska E, Nowicka K, Jaworska D, Przybylski W, Tambor K. *Asian-Australas J Anim Sci.* 2016; 30(5):720-727.
4. Marušić Radovčić N, Vidaček S, Janči T, Medić H. *Journal of Food Science and Technology* 2016; 53(11):4093-4105.
5. Li P, Bao Z, Wang Y, Su X, Zhou H, Xu B. *Crit Rev Food Sci Nutr.* 2025;56(5):992-1008.
6. Li X, Zhu J, Li C, et al. *Molecules* 2018 23(12):3286.
7. Petričević S, Marušić Radovčić N, Lukić K, Listeš E, Medić H. *Meat Sci.* 2018;137:217-227.
8. Babić K, Strojnik L, Ćirić A, Ogrinc N. *Front Nutr.* 2024; 11:1342417.
9. Saldaña E, Saldarriaga L, Cabrera J, et al. *Food Research International.* 2019, 2019;119:839-849.
10. Huan Y, Zhou G, Zhao G, Xu X, Peng Z. *Meat Sci.* 2005, 71(2):291-299.
11. Šojić B, Milošević S, Savanović D, Zeković Z, Tomović V, Pavlić B. *Molecules* 2023; 28(5):2293.
12. Vazquez T, Tello E, Peterson DG. *Molecules* 2025, Vol 30, Page 720. 2025;30(3):720.
13. Wang H, Chambers E. *Sensory Molecules* 2018 Vol 23, Page 780. 2018;23(4):780.
14. van Gelder K, Forrester T, Akhtar TA. *Planta* 2020;252(1):3-.
15. Sastrohamidjojo H, Fariyatun E. *IOSR Journal of Applied Chemistry* 2016;9(10):105-112.
16. Liu D, Bai L, Feng X, et al. *Meat Sci.* 2020;168.
17. Scognamiglio J, Jones L, Letizia CS, Api AM. *Food and Chemical Toxicology.* 2012; 50(SUPPL. 2):S224-S239.

OR2

Volatilomic profiling of balsamic vinegars by HS-SPME-GC×GC-TOFMS: from protected-origin differentiation to maturation assessment

Allan Polidoro,¹ Sofia Malcangi,² Monica Romagnoli,² Nicola Marchetti,² Flavio A. Franchina²

¹ Department of Environmental and Prevention Sciences, University of Ferrara, Via L. Borsari 46, 44121 Ferrara, Italy

² Department of Chemical, Pharmaceutical, and Agricultural Sciences, University of Ferrara, Via L. Borsari 46, 44121 Ferrara, Italy

Summary: HS-SPME-GC×GC-TOFMS was applied to characterize the volatile profile of protected-origin Italian balsamic vinegars. The workflow enabled differentiation between PDO and PGI products, highlighting chemical features related to processing and aging. The results support the use of volatilomic as a potential tool for authenticity and quality assessment.

Keywords: Balsamic vinegar; comprehensive two-dimensional gas chromatography; volatilomics

Introduction

Balsamic vinegars (BV) from the Emilia-Romagna region are high-value traditional products characterized by complex aroma profiles and protected by European quality schemes. Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI) balsamic vinegars differ substantially in raw materials, processing intensity, and aging regimes. These differences are expected to affect their volatile composition. However, their comprehensive chemical characterization remains analytically challenging due to the matrix's complexity. In this work, headspace solid-phase microextraction combined with comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (HS-SPME-GC×GC-TOFMS) was employed to investigate the volatile composition of PDO and PGI balsamic vinegars and evaluate its potential for product differentiation. The same analytical framework was further applied in a longitudinal study to investigate the evolution of the BV volatile profile during maturation in barrels made from different wood species.

Experimental

In the cross-sectional study, commercial PDO and PGI balsamic vinegars were analyzed alongside pooled quality-control samples and blanks.

Extraction conditions were first assessed using a pooled QC sample by comparing two SPME fiber coatings, PDMS/DVB and DVB/CAR/PDMS, and evaluating the effect of NaCl addition.

The optimized HS-SPME-GC×GC-TOFMS workflow was then applied to the sample set. Data processing included peak detection, chromatographic alignment, blank and artifact removal, statistical filtering, compound annotation, and chemometric analysis. Significant features were selected using the Mann-Whitney U test, followed by false discovery rate correction.

For the longitudinal study, the same workflow was applied to balsamic vinegar samples collected along a traditional maturation sequence from barrels made of oak, chestnut, and cherry wood, allowing the influence of maturation time and barrel material on the volatile profile to be explored.

Results

In the cross-sectional study, multivariate analysis (*Fig. 1*) revealed a clear separation between PDO and PGI samples, indicating that their volatile profiles reflected differences in production and aging. PDO vinegars were enriched in furanoids, lactones, esters, acids, phenols, and alcohols, whereas PGI vinegars showed comparatively higher levels of terpenoids.

These trends are consistent with the greater processing intensity and longer aging of PDO products, as well as the stronger preservation of grape-derived volatile signatures in PGI vinegars.

Mapping volatile features to odor descriptors suggested that PDO vinegars may be associated with sweeter, caramel-like, creamy, balsamic, woody, and floral aromatic attributes, whereas PGI vinegars retain fresher citrus-floral-herbal notes.

In the longitudinal study, preliminary PCA results showed a structured organization of samples along the maturation sequence, with different barrel woods occupying distinct regions of the scores plot.

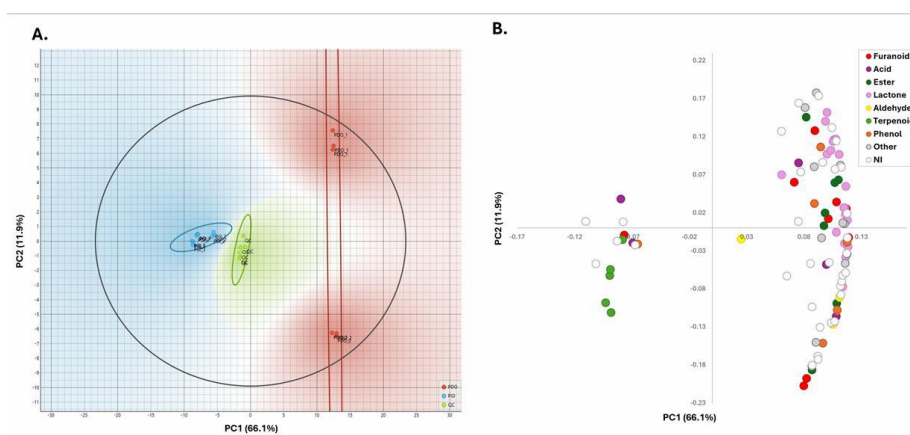


Fig. 1. Principal component analysis (PCA) of balsamic vinegar samples based on volatile compound profiles. (A) Score plot showing PDO, PGI, and quality control (QC) samples with Hotelling's T2 confidence ellipses (95%). (B) Loading plot indicating the contribution of each significant feature to the first two principal components, colored according to chemical class.

Conclusions

The integration of HS-SPME-GC×GC-TOFMS with statistical filtering and multivariate analysis provided a detailed volatilomic fingerprint of BV. The cross-sectional approach enabled differentiation between PDO and PGI samples and highlighted volatile compounds associated with processing and aging. The longitudinal application further suggested that maturation time and barrel wood both contribute to shaping the volatile composition of BV.

Overall, this workflow provides an analytical framework applicable to authenticity assessment, quality control, and the study of process-driven aroma development in traditional high-value food products.

References

1. F. A. Franchina, D. Zanella, L. M. Dubois, J. Focant; *Journal of Separation Science*, 44 (2021), pp 188-210.
2. D. Zanella, J. Focant, F. A. Franchina; *Analytical Science Advances*, 2 (2021), pp 213-224.
3. E. Durán-Guerrero, F. Chinnici, N. Natali, C. Riponi; *Journal of the Science of Food and Agriculture*, 95 (2015), pp 2395-2403.

OR3

Defining the chemical and sensory identity of Italian no- and low-alcohol beers market by HS-SPME-GC×GC-MS and sensory analysis

Leonardo D'Intino, Gianluca Donadini, Giorgia Spigno, Mario Gabrielli

Dipartimento di Scienze e Tecnologie Alimentari per una filiera agro-alimentare sostenibile (DiSTAS),
Università Cattolica del Sacro Cuore, Via Emilia Parmense, 84, Piacenza (Italy)

Summary: *By integrating HS-SPME-GC×GC-MS, physicochemical analysis, sensory profiling, and regularized chemometrics, this study identified ethanol, residual extract, fermentation esters, and wort-derived carbonyls as key drivers of beer perception, highlighting food mass spectrometry as a powerful tool for monitoring quality and guiding process-based optimization of non-alcoholic beers.*

Keywords: *beer, GCxGC-MS, Elastic Net regression*

Introduction

The consumption of non- and low-alcoholic beers (NABs) has increased substantially across Europe, driven by heightened health consciousness and awareness of alcohol-related health risks [1]. However, despite major technological advances in production, brewers continue to face significant challenges in producing NABs that closely match the sensory profiles of conventional beers. While aromatic perception depends by more than 1,000 volatile organic compounds, the links between chemical composition and sensory outcomes remain poorly characterized. This study aimed to bridge this gap by integrating advanced chemical analysis with sensory science and multivariate regression to identify the key chemical drivers of sensory differentiation between regular beers (RBs) and NABs representing more than 80% of the Italian market [2].

Material and Methods

Eight paired commercial beer samples (n=8 NAB-RB pairs) from the Italian market were subjected to a three-stage analytical and statistical framework. Samples are of 3 representative brewing styles: 4 Lager, 3 Pils, 1 Weissbeer.

Chemical analysis comprised physicochemical characterization (alcohol content, pH, total and net extract, bitterness units, acidity) [3] and comprehensive volatile profiling using HS-SPME (DVB/CAR/PDMS) coupled to GCxGC-MS. GCxGC-MS analysis employed a non-polar/polar columns (Rtx-5ms-MS/Stabilwax; 20x5 m) configuration and flux modulation [2, 4]. Detection was performed across 41–360 m/z, at 60 He. Volatiles were identified against authentic standards or by spectral library matching (NIST 2020/Wiley 12; similarity > 80%, Δ LRI<15) and semi-quantified against internal standard (2-Octanol).

Sensory evaluation was conducted using descriptive analysis with 8 gender balanced expert assessors. 15 sensory attributes on a nine-point Likert scale, performed each sample was analysed in duplicate following ISO 8589 guidelines.

Statistical integration employed a three-step chemometric approach. (i) one-way ANOVA on physicochemical, volatile, and linear mixed models (LMMs) on sensory parameters, accounting for assessor-related variability [5]. (ii) exploratory PCAs on physicochemical, volatile, and sensory datasets identified major chemical/perceptual dimensions and firstly identify chemical-sensory associations. (iii) integration through Partial Least Squares (PLS) regression of 15 sensory descriptors as a function of 103 combined physicochemical and volatile parameters. A Clustered Image Map (CIM) was produced to graphically link the obtained PLS correlation between sensory and chemical parameters. To improve the prediction and overcome the limitation of 15 descriptors < 103 variables, an Elastic Net regularized regression combining L1 (LASSO) and L2 (ridge) penalties was applied to identify a sparse, stable subset of chemical drivers, more adequate for multicollinear high-dimensional datasets [6].

Results and Discussion

PC1 and PC2 on physicochemical parameters explained 81.1% variance. Colour and pH discriminated brewing styles on PC1; alcohol and net extract characterized RBs versus NABs on PC2. NABs showed higher total extract due to unfermented residual sugars. The second PCA on volatile compounds explained 48.1% variance. RBs clustered distinctly, rich in fermentation-derived esters, higher alcohols, and sesquiterpenes (64–99% reduction in NABs). NABs showed variable hop-derived terpene profiles, indicating different dealcoholization/aroma enrichment strategies affecting volatile composition [7].

Sensory differences were substantial and multidimensional: RBs demonstrated significantly greater body, perceived alcohol content, maltiness, and solvent-like notes, while NABs were perceived as sweeter, fruitier, richer in wort-like and honey-like notes. Sensory PCA revealed four orthogonal perceptual dimensions:

alcohol-presence/body (PC1; 32.8% variance), bitterness-persistence (PC2; 22.3%), sweetness-wortiness balance (PC3), and beer style-related characteristics (PC4).

The PLS model gained an R^2 of about 1 while Q^2 showed an average of -0.293 , showing severe symptoms of overfitting, as expected with 15 vs 105 predictors. To overcome this limitation, an Elastic Net regression successfully identified a parsimonious ($n=13$) number of chemicals and interpretable predictors, with substantially improved cross-validated performance. These sparse features produced a model with improved prediction, particularly for alcohol-related and sweetness-driven dimensions.

Elastic Net achieved marked performance gains for alcohol and mouthfeel descriptors. Perceived alcohol intensity reached $Q^2 = 0.59$ (compared to $Q^2 = 0.394$ in PLS), while body perception attained $Q^2 = 0.45$ (versus $Q^2 = 0.434$ in PLS). Equally significant improvements emerged for sweetness-related attributes, where PLS demonstrated inadequate or negative predictive capacity: sweetness increased to $Q^2 = 0.44$, honey-like perception to $Q^2 = 0.47$, and wortiness exhibiting the most relevant improvement achieved $Q^2 = 0.53$ ($Q^2 = 0.004$ in PLS).

Ethanol consistently emerged as the dominant predictor for alcohol-related attributes, while total extract proved the major driver of sweetness perception in non-alcoholic beers, reflecting residual sugar content. Fermentative esters (ethyl hexanoate, ethyl octanoate, ethyl decanoate) were systematically selected for alcohol and maltiness descriptors, whereas carbonyl compounds derived from wort (undecanale, 2-undecanone) strongly fitted wortiness perception. The negative association between ethanol and wortiness underscores complete fermentation as an antagonistic factor to unwanted malty notes [8, 9].

Certain descriptors, like phenolic perception ($Q^2 = -0.19$), bitterness, astringency, and sourness remained poorly predictable, reflecting their dependence on style-specific characteristics and complex perceptual interactions beyond linear chemical relationships [10].

Conclusions

While substantial sensory limitations persist in non-alcoholic beers—notably reduced body, diminished fermentative fruitiness, and excessive sweetness and wort-derived notes, some progress has been achieved on style-dependent dimensions through targeted aromatic compensation strategies.

Conversely, fundamental matrix-related parameters, primarily the absence of ethanol and residual sugar accumulation remain limitately improved to NAB production processes. EN regression confirmed ethanol, total extract, and net extract as stable chemical predictors of alcohol intensity and sweetness perception.

These findings establish analytical chemistry and mass spectrometry as valuable, process-linked tools for quality monitoring and improvement in food and beverage manufacturing. The identified chemical drivers (ethanol, residual extract, some volatile markers) transform sensory quality into objective, analytically traceable parameters, enabling robust monitoring and support in the evolution towards new and high quality NABs, and generally tracing and supporting technological advancement in non-alcoholic beer formulation.

References

1. Gliszczyńska-Świgło, A., Klimczak, I., Klensporf-Pawlik, D., Rybicka, I. (2025). *Scientific Reports* 15 (1), 7145.
2. Donadini, G., D'Intino, L., Spigno, G., Gabrielli M. (2026). *Frontiers in Food Science and Technology* 6:1840815.
3. American Society of Brewing Chemists (ASBC). Methods of analysis.
4. Saison, D., De Schutter, D. P., Uyttenhove, B., Delvaux, F., and Delvaux, F. R. (2009). *Food chemistry*, 114(4), 1206-1215.
5. Næs, T., Brockhoff, P. B., Tomic, O. *Statistics for Sensory and Consumer Science*. Wiley 2010.
6. Menéndez, P., Eilers, P., Tikunov, Y., Bovy, A., van Eeuwijk, F. (2012). *Euphytica* 183 (3), 379–387.
7. Piornos, J. A., Koussissi, E., Balagiannis, D. P., Brouwer, E., and Parker, J. K. (2023). *Compr. Rev. Food Sci. Food Saf.* 22 (1), 233–259.
8. Lafontaine, S., Senn, K., Dennenlöh, J., Schubert, C., Knoke, L., Maxminer, J., et al. (2020). *ACS Omega* 5 (36), 23308–23321.
9. Bauwens, J., van Opstaele, F., Eggermont, L., Weiland, F., Jaskula-Goiris, B., De Rouck, G., et al. (2021). *Journal of the Institute of Brewing* 127 (4), 385–405.
10. Müller, M., Gastl, M., Becker, T. (2021). *Journal of the Institute of Brewing*. 127 (3), 262–272.

OR4

From waste to bioactive resource: GC-MS profiling and bioherbicidal potential of *Petroselinum crispum* essential oil

R. Francolino,¹ R. Gaeta,¹ L. De Martino,¹ V. De Feo^{1,2}

¹ Department of Pharmacy, University of Salerno, Fisciano, SA, Italy

² Institute of Food Science, National Research Council (C.N.R.), Avellino, Italy

Summary: The essential oil of *Petroselinum crispum*, agri-food waste, was characterized by GC-MS, revealing 1,3,8-*p*-menthatriene as the main component. Environmental toxicity, assessed via *Artemia salina* bioassay, showed time- and dose-dependent effects. Phytotoxic evaluation identified *Sinapis arvensis* as the most sensitive species, likely due to amylase inhibition and alteration of energy reserves.

Keywords: Essential oil; GC-MS; *Petroselinum crispum*

Introduction

Aromatic plants and their essential oils (EOs) represent a major area of scientific research due to their complex chemical composition and wide spectrum of biological activities, including antimicrobial, antioxidant, insecticidal, and phytotoxic properties [1,2]. In recent decades, growing concern over the environmental and health risks associated with synthetic pesticides and herbicides has spurred increasing interest in plant-derived natural products as safer, biodegradable alternatives [2].

Furthermore, the valorization of plant by-products generated along the agri-food supply chain has emerged as a key strategy to reduce waste and improve resource efficiency [3]. Valorization of food waste through the extraction of bioactive compounds represents a promising approach to optimize resource use and support the circular economy.

Petroselinum crispum (Mill.) Fuss, commonly known as parsley, is a biennial herb of the Apiaceae family widely cultivated for culinary and medicinal purposes throughout Europe and the Mediterranean area. Its EO is characterized by a variable chemical profile depending on chemotype, plant part, geographic origin, and environmental conditions [4,5]. Major constituents reported in the literature include apiol, myristicin, β -phellandrene, and α -pinene, although their relative proportions can differ considerably among studies [4,5]. Although, *P. crispum* EO has been widely investigated for its antioxidant, antibacterial, and antifungal properties [4,5], its phytotoxic and bioherbicidal potential has received limited attention to date.

In the present work, the plant material used for EO extraction was derived entirely from processing waste of *P. crispum*, making this work an applied example of circular bioeconomy principles within the aromatic plant sector.

The aims of this study were to characterize the chemical composition of *P. crispum* EO by GC-MS and to evaluate its antioxidant activity, environmental toxicity (*Artemia salina* bioassay), and phytotoxic effects on two weeds (*Sinapis arvensis* L. and *Lolium multiflorum* Lam.) and two crops (*Raphanus sativus* L. and *Hordeum vulgare* L.), representing both monocot and dicot, assessing morphological parameters and biochemical markers including amylase and catalase activity as indicators of energy reserve mobilization and oxidative stress response, respectively.

Experimental

Fresh plant material (2.50 kg) of *P. crispum*, derived from processing waste of the agri-food supply chain, was subjected to hydrodistillation, yielding 2.53 g of essential oil (0.1% w/w). Chemical composition was determined by GC-MS using an Agilent 6850 Series II instrument equipped with a DB-5 capillary column connected to an Agilent 5973 mass selective detector operating at 70 eV. Compounds were identified by comparing retention indices and mass spectra with authentic standards, commercial libraries (NIST 02, Wiley 275), and published sources; quantitative data were obtained from relative peak areas without correction factors.

Antioxidant activity was assessed using three spectrophotometric assays: DPPH, FRAP, and ABTS, using Trolox as reference standard.

Environmental toxicity was evaluated through an *Artemia salina* lethality bioassay, using potassium dichromate ($K_2Cr_2O_7$) as positive control.

Phytotoxic activity was tested at different doses of EO. For each species, morphological parameters (germination rate, root and hypocotyl length, biomass) and biochemical parameters (total protein content, amylase and catalase specific activity) were measured.

All data are expressed as mean $\pm$ SD of three independent replicates; statistical analysis was performed by Student's t-test and one-way ANOVA followed by Tukey's post hoc test, as appropriate ($p < 0.05$).

Results

GC-MS analysis of *P. crispum* EO identified 20 compounds. The chemical profile was dominated by monoterpene hydrocarbons (77.42%), followed by oxygenated sesquiterpenes (18.85%), sesquiterpene hydrocarbons (3.51%), and oxygenated monoterpenes (0.19%). The three major constituents were 1,3,8-*p*-menthatriene (45.00%), apiol (18.80%), and *p*-mentha-1(7),8-diene (12.49%).

Antioxidant activity, assessed by DPPH, FRAP, and ABTS assays, was low compared to Trolox used as reference standard (DPPH IC₅₀ = 0.72 ± 0.03 mg/mL; FRAP = 0.49 ± 0.17 mmol Fe²⁺ eq/g EO; ABTS = 111.10 ± 22.10 mg TE/g EO).

Environmental toxicity evaluated via *Artemia salina* lethality bioassay showed a dose- and time- dependent response, with LC₅₀ values of 1.108 ± 0.087 mg/mL at 24 h and 0.527 ± 0.074 mg/mL at 48 h. Based on these results, the doses selected for phytotoxic bioassays (125, 250, and 500 µg/mL) were set below the lowest LC₅₀ value, to minimize potential ecotoxicological impact.

Phytotoxic activity showed species-dependent activities across all four species tested. Among monocots, *H. vulgare* displayed the highest sensitivity, with significant inhibition of root growth and biomass at all doses tested. Amylase activity increased significantly at 500 µg/mL, while catalase showed a complex dose-dependent pattern, suggesting a possible perturbation of the antioxidant defense system. *L. multiflorum* showed low sensitivity profile, with significant reductions in hypocotyl and root length mainly at the highest dose. Amylase activity was reduced at all doses, indicating an early enzymatic disruption that precedes morphological effects. Among dicots, *R. sativus* proved highly tolerant, with no statistically significant changes in any morphological parameter across all doses, except root elongation. However, amylase inhibition at 125 µg/mL and increased catalase activity at 250 and 500 µg/mL suggest a mild biochemical response. In contrast, *S. arvensis* was the most sensitive species overall, exhibiting dose- dependent inhibition root and hypocotyl length. Amylase activity was significantly altered at all doses tested, while no significant changes were observed in catalase activity or total protein content.

Conclusions

The EO of *P. crispum* exhibited a distinctive chemical profile dominated by 1,3,8-*p*- menthatriene. While antioxidant properties were limited, the EO displayed relevant phytotoxic activity with selective inhibition toward the weed *S. arvensis*. The significant alteration of amylase activity at all doses tested suggests that interference with seed energy reserve mobilization may represent a key mechanism underlying the observed phytotoxic effect. These findings support the potential of *P. crispum* EO as a natural, low-environmental-impact bioherbicide agent, warranting further investigation for sustainable weed management applications.

References

1. R. Raveau, J. Fontaine, A. Lounès-Hadj Sahraoui; *Foods*, 9 (2020), p. 365.
2. C. Maes, J. Meersmans, L. Lins, S. Bouquillon, M.L. Fauconnier; *International Journal of Molecular Sciences*, 22 (2021), p. 9396.
3. C.V. Berenguer, C. Andrade, J.A. Pereira, R. Perestrelo, J.S. Câmara; *Processes*, 11 (2022), p. 20.
4. G. Nouioura, N. El Hachlafi, H.A. Abuelizz, A.E. Elidrissi, M. Ferioun, N. Soulo, E. Derwich; *Heliyon*, 10 (2024), e28408.
5. H. Zhang, F. Chen, X. Wang, H.Y. Yao; *Food Research International*, 39 (2006), pp. 833–83

OR5

Unveiling chemical fingerprints of aging in balsamic vinegar of Modena (PGI) with GC-IMS

S. Pellacani,¹ M. Cocchi,¹ G. Gazzoli,¹ F. Martone,² R. Marabottini,² A. Napolitano,² F. Ladogana,³ F. Desimoni,³ C. Durante,¹ L. Strani¹

¹ Department of Chemical and Geological Sciences, University of Modena and Reggio Emilia, Modena, Italy

² Ispettorato centrale della tutela della qualità e della repressione frodi dei prodotti agroalimentari – Direzione Generale degli Uffici territoriali e laboratori - Modena Laboratory, Italy

³ Consorzio Tutela Aceto Balsamico di Modena, Via Ganaceto 113, Modena 41121, Italy

Summary: *This work presents the development of a GC–IMS method for characterizing different aged Balsamic Vinegar of Modena PGI. Analytical conditions were optimized using a face-centered central composite design, while GC–IMS signals were processed using PARADiSe to address peak shifts and co-elution issues.*

Keywords: GC-IMS, chemometrics, balsamic vinegar

Introduction

Aceto Balsamico di Modena, a traditional Italian product protected by the PGI designation, is highly valued for its unique sensory profile, which is closely related to its maturation process. According to Regulation (EC) No 583/2009, the product must undergo a minimum aging period of 60 days, while an aging of at least three years allows the “aged” designation. Despite its importance, the chemical changes associated with this process has been only marginally investigated.

Gas Chromatography–Ion Mobility Spectrometry (GC-IMS) has emerged as a promising alternative for volatile fingerprinting of food products. The technique combines rapid chromatographic separation with highly sensitive ion mobility detection, enabling the analysis of volatile organic compounds (VOCs) at trace concentration levels with minimal sample preparation, low operational costs, and reduced environmental impact. Although GC-MS has already demonstrated the ability to discriminate Balsamic Vinegar of Modena PGI samples with different aging levels [1], the potential of GC-IMS for this specific application remains largely unexplored.

Therefore, the aim of this study was to develop a GC-IMS fingerprinting method, combined with chemometric analysis, for the characterization of volatile profiles of Balsamic Vinegar of Modena PGI samples with different aging levels. To address the high degree of data complexity and chromatographic co-elution, GC-IMS data from both blank and sample measurements were processed by PARAFAC2 analysis using the PARADiSe software [2].

Experimental

A total of 60 Balsamic Vinegar of Modena PGI samples were analysed, including 22 aged and 38 non-aged samples. All samples were provided by Consortium for the Protection of Balsamic Vinegar of Modena PGI.

Samples were analysed using an HS-GC-IMS FlavourSpec G.A.S. (Gesellschaft für Analytische Sensorsysteme mbH, Dortmund, Germany) coupled with the PAL-RSL autosampler (CTC Analytics AG, Zwingen, Switzerland). The gas chromatograph was equipped with an MXT-5 capillary column (5% biphenyl, 95% polydimethylsiloxane; 15 m × 0.53 mm i.d., 1 µm film thickness).

The main parameters affecting volatile sampling, namely incubation temperature and time, and sample dilution were optimized using a design of experimental (DoE) approach based on face centered central composite design.

Results

GC-IMS data obtained from the DoE experiments were processed using PARADiSe and subsequently analyzed by principal component analysis (PCA) and multiple linear regression (MLR). Analysis of the response surfaces showed that the highest analytical response was obtained at low dilution levels and high incubation temperatures, whereas incubation time had no significant effect.

Following method optimization, the selected operating conditions were applied to all Balsamic Vinegar of Modena PGI samples. The resulting GC-IMS data were processed using two different data extraction strategies to compare their ability to describe sample variability and characterize the volatile profiles of the analysed vinegars. The first approach was based on peak extraction using VOCal software, whereas the second relied on signal deconvolution using PARADiSe software.

VOCal signal analysis initially identified 136 peak areas. Blank-related signals were subsequently removed, resulting in 89 retained features per sample. For the PARADiSe-based approach, the workflow included down-sampling, ion mobility alignment, cropping, smoothing, baseline correction, interval-based PARAFAC2

decomposition, and feature selection. PARAFAC2 initially resolved 43 chromatographic features. After excluding features associated with blank samples, 35 informative peak areas were retained for subsequent chemometric analysis.

For both data extraction approaches, PCA revealed a clear trend towards sample separation according to ageing level. Non-aged Balsamic Vinegar of Modena PGI samples (60-90 days) were mainly distributed at positive PC1 score values, whereas aged samples (>3 years) were predominantly located at negative PC1 score values. Samples with an intermediate ageing period (1.5-3 years) partially overlapped with the aged group. This pattern is consistent with a progressive modification of the volatile profile with increasing ageing time.

Conclusion

Both workflows successfully captured differences in volatile profiles associated with ageing, enabling the differentiation of samples according to their ageing stage. The obtained results highlight the potential of GC-IMS combined with advanced chemometric processing as a rapid and robust analytical approach for characterising ageing-related differences in Balsamic Vinegar of Modena PGI.

In addition to providing clearer discrimination between non-aged and aged samples, the PARADiSe-based workflow proved more robust to analytical variability, as no evident clustering related to the acquisition sessions was observed and no batch-effect correction was required prior to chemometric analysis.

Acknowledgements

This research was supported by University of Modena and Reggio Emilia - Modena Foundation through FAR Mission Oriented 2024 funds.

References

1. M. Cirlini, A. Caligiani, L. Palla, & G. Palla, *Food Chemistry*, 124 (2011), pp. 1678-1683
2. B. Quintanilla-Casas, R. Bro, J.L. Hinrich, C.L. Davie-Martin, *PROTOCOL* (2023).
<https://doi.org/10.21203/rs.3.pex-2143/v1>.

OR6

Orbitrap HRMS for compound-specific isotope measurements: applications to pharmaceuticals and curcumin

Alberto Roncone, Domenico Masuero, Roberto Penasa, Silvia Pianezze, Luana Bontempo

Fondazione Edmund Mach, Traceability Lab.

Summary: Orbitrap HRMS was evaluated for compound-specific isotope analysis of chloroquine in falsified medicines and curcumin in curcuminoid samples. Carbon and chlorine isotope information was obtained from selected ions and fragments, supporting authentication and sample comparison with limited sample preparation.

Keywords: Orbitrap HRMS; stable isotope analysis; authentication

Introduction

Conventional bulk isotope analysis provides an overall value for the sample and may be influenced by the presence of multiple ingredients. Compound-specific measurements overcome this limitation but often require laborious isolation or purification. Orbitrap high-resolution mass spectrometry offers a complementary approach based on the accurate measurement of monoisotopic ions and their heavy-isotope isotopologues.

Experimental

Two applications were investigated. Falsified antimalarial tablets were first screened by LC–Orbitrap-MS to identify and semi-quantify active pharmaceutical ingredients. Chloroquine-containing samples were then analysed by infusion-Orbitrap-MS, targeting the molecular ion and its ¹³C- and ³⁷Cl-containing isotopologues. Curcuminoid samples of natural, synthetic and unknown origin were analysed by measuring the ¹³C isotopic abundance of intact curcumin and four major product ions generated by MS² fragmentation. In both studies, the areas of the monoisotopic and heavy-isotope ions were extracted, and their ratios were converted into delta values.

Results and discussion

For chloroquine, the combined $\delta^{13}\text{C}$ and $\delta^{37}\text{Cl}$ values revealed at least three groups of samples, suggesting possible differences in production batches, raw materials or manufacturers. For curcumin, $\delta^{13}\text{C}$ values differed significantly between natural and synthetic samples. MS² experiments also provided isotope information from different portions of the curcumin structure, improving discrimination between origins.

Conclusions

Orbitrap HRMS enables compound-specific isotope information to be obtained from selected target molecules with limited sample preparation. The technique combines molecular selectivity, multi-element isotope measurement and fragment-level information, providing a promising complementary tool for authentication, forensic comparison and source investigation.

OR7

Application of stable isotope ratio mass spectrometry (IRMS) for verification of geographical origin of egg products: establishment of a French reference dataset

Michele Suman^{1,2} Freddy Thomas³

¹ Barilla G.R. F.lli SpA, Research, Development & Quality, Sensory and Analytical Food Science, Via Mantova 166, 43122, Parma, Italy

² Department of Food and Drug, University of Parma, Parco Area delle Scienze 27/A, 43124 Parma, Italy

³ Eurofins Analytics France 9, Rue Pierre Adolphe Bobierre, B.P. 42301 F-44323 Nantes Cedex 3, France

Summary: *This research work describes a study using stable isotope ratio mass spectrometry (IRMS) analysis ($\delta^{18}\text{O}$, $\delta^2\text{H}$) to verify the geographical origin of eggs. Based on 88 samples from France, it establishes a reference isotopic database, supporting industrial applications for authenticity, fraud prevention, and supply chain transparency.*

Keywords: *Egg Products, Geographical Origin, Stable Isotope Ratio Mass Spectrometry*

Introduction

The authentication of geographical origin is a critical aspect of food quality assurance, particularly in products associated with strong origin claims. In the egg sector, such claims are increasingly relevant for both regulatory compliance and consumer trust.

Stable isotope analysis has emerged as a powerful method for origin determination.

The isotopic composition of food matrices reflects environmental conditions, including the isotopic signature of local water sources influencing animal metabolism.

In egg products, isotopic ratios of oxygen ($\delta^{18}\text{O}$) and hydrogen ($\delta^2\text{H}$) are primarily linked to the drinking water consumed by hens, making them particularly suitable markers for geographical origin verification.

This study was conducted to support industrial needs for fraud prevention and supply chain transparency. The main objective was to generate a reference isotopic database for French eggs, enabling verification of origin claims within industrial applications.

Experimental

Egg samples were collected from six production sites located in northern and north-western France, representing key production areas.

Sampling covered the period from September 2023 to September 2025, allowing evaluation of both seasonal and interannual variability; the final dataset included 88 samples, collected across multiple months and production batches.

Whole egg samples were homogenized prior to analysis to improve repeatability by combining yolk and albumen fractions.

Stable isotope ratios were measured using Isotope Ratio Mass Spectrometry (IRMS):

- $\delta^{18}\text{O}$ determined via equilibration with CO_2
- $\delta^2\text{H}$ determined via equilibration with H_2

Results

A two-dimensional isotopic model ($\delta^{18}\text{O}$ vs $\delta^2\text{H}$) was constructed to describe the isotopic domain of French egg samples. Measured isotopic values were within relatively narrow ranges.

Absence of a consistent seasonal trend was present across all sites and limited temporal variability relative to overall isotopic range as well.

Relatively homogeneous climatic conditions were found across studied regions.

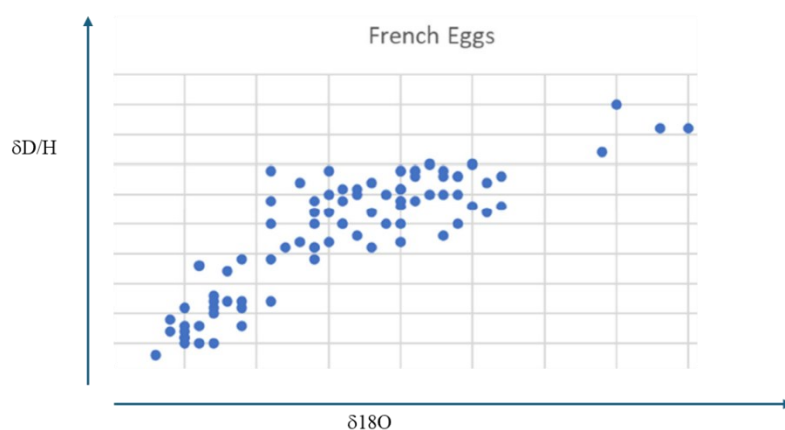


Fig. 1. The figure above gathered all isotopic results. Each sample is represented by its isotopic couple (Delta ^{18}O , D/H).

Conclusions

The methodology was successfully applied to liquid egg products, and the dataset provides a reliable baseline signature of French eggs, exploitable as correspondent reference for authenticity testing.

Future work should address its expansion to additional European countries, integration into routine authentication workflows and long-term monitoring of climatic impacts.

References

1. F. Camin, M. Perini, L. Bontempo, M. Galeotti, E. Tibaldi, E. Piasentier; *Food Chemistry*, 267 (2018), pp. 288-295
2. G. Skrzypek, C.E. Allison, J.K. Böhlke, L. Bontempo, P. Brewer, F. Camin, J.F. Carter, M.M.G. Chartrand, T.B. Coplen, M. Gröning, J.F. Hélie, G. Esquivel-Hernández, R.A. Kraft, D.A. Magdas, J.L. Mann, J. Meija, H.A. Meijer, H. Moossen, N. Ogrinc, M. Perini, A. Possolo, K. Rogers, A. Schimmelmann, A. Shemesh, D.X. Soto, F. Thomas, R. Wielgosz, M.R. Winchester, P.J.H. Yan Z Dunn; *Pure and Applied Chemistry*, 94(11-12) (2022), pp. 1249-1255.
3. P.J.H. Dunn, J.F. Carter, FIRMS Network, *Good Practice Guide for Isotope Ratio Mass Spectrometry*, 2018, 2nd edition.
<https://www.forensicisotopes.org/assets/FIRMS%20Good%20Practice%20Guide%20Second%20Edition.pdf>

OR8

***Griffonia simplicifolia* seed-based supplements: how stable isotope ratio analysis can help in commercial samples authentication**

Silvia Pianezze,¹ *Sara Malpaga*,¹ *Arianna Vanelli*,² *Filomena De Biasio*,² *Matteo Perini*¹

¹ Centro di Trasferimento Tecnologico, Fondazione Edmund Mach, San Michele all'Adige, TN

² EVRA S.r.l. Società Benefit, Lauria (PZ)

Summary: L-5-hydroxytryptophan-based supplements can be obtained either from the natural source seeds of *Griffonia simplicifolia* or it can be produced (bio)synthetically, the latter resulting in a higher profit. Natural products must therefore be protected against economical frauds. Stable isotope ratio analysis can serve this purpose, discriminating effectively between natural and (bio)synthetic products.

Keywords: *Griffonia simplicifolia*, stable isotope ratio analysis, authentication

Introduction

The global trade in seed material from *Griffonia simplicifolia*, a tropical shrub of West Africa, has been boosted in the last few years because of its content of the free amino acid L-5-hydroxytryptophan (5-HTP) [1]. This active ingredient has been interesting consumers since it is a precursor of both serotonin and melatonin. Since *Griffonia simplicifolia* is rich in this amino acid (up to 20%), its cultivation has been increasing in the last years to face consumer's request [2].

On the other hand, L-5-hydroxytryptophan can also be obtained through several synthetic and biosynthetic pathways, which involve cheaper starting materials and result in a more cost-effective procedure than the extraction from the seed [2].

It is well known that where money goes, adulteration follows, and it is also sure that natural supplements have higher prices than (bio)synthetic ones. But when consumers buy a natural-labelled supplement, how can they be 100% sure that it is natural? It is mandatory to have a method to characterise the authentic natural L-5-hydroxytryptophan extracted from *Griffonia simplicifolia*.

In the last decades, stable isotope ratio analysis (SIRA) proved to be a powerful tool for this purpose, figuring as a technique able to discriminate between natural, synthetic and biosynthetic products.

Experimental

In the present work, 2 samples of synthetic 5-HTP, 4 samples of biosynthetic 5-HTP, 15 samples of commercial *Griffonia simplicifolia* extract supplements and 8 samples of *Griffonia simplicifolia* seeds have been considered. To extract 5-HTP from the seeds and from the commercial samples, an ethanol extraction was carried out, followed by purification through preparative HPLC.

Both high-purity and purified samples were therefore analysed through EA-IRMS (Elemental Analysis-Isotope Ratio Mass Spectrometry) to measure both carbon ($\delta^{13}\text{C}$, ‰ vs V-PDB) and nitrogen ($\delta^{15}\text{N}$, ‰ vs Air) isotopic ratios.

Results

The isotopic results for natural, synthetic and biosynthetic 5-HTP are reported in Figure 1. While synthetic and biosynthetic values are quite close to each other in the biplot, the natural samples are statistically different ($p < 0.001$) from the rest of the dataset.

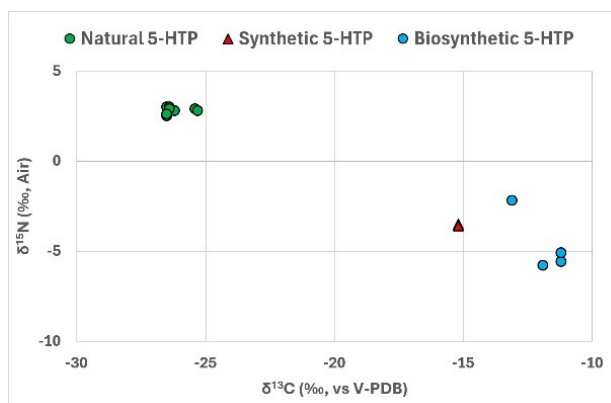


Fig. 2. Nitrogen and carbon isotopic ratios for natural, synthetic and biosynthetic 5-HTP samples.

Since the isotopic ranges for natural and (bio)synthetic 5-HTP are separated by a wide gap, the difference between them is really promising for future commercial samples authentication. If commercial samples values fall inside one of the two ranges, they can be easily labelled as either natural or (bio)synthetic. On the other hand, if for a certain sample the isotopic values range in between the two intervals, it could be due to the addition of (bio)synthetic product to a natural formulate.

Conclusions

SIRA is a promising tool for authenticating *Griffonia simplicifolia* seed supplements, effectively distinguishing natural from (bio)synthetic products through their carbon and nitrogen isotopic ratios.

References

1. P. A. Lemaire R. K. Adosraku; *Phytochemical Analysis*, 13 (2002), pp.333-337
2. M. E. Maffei; *International Journal of Molecular Sciences*, 22, 181 (2021).

OR9

Authentication of high-value food products using combined GC-MS and GC- IRMS approaches: case studies on lavender and mint essential oils

Roberto Penasa, Luana Bontempo, Long Chen Mauro Paolini, Alberto Roncone, Domenico Masuero, Roberto Larcher

Fondazione Edmund Mach, via Mach 1, 38098 San Michele all'Adige, Trento

Summary: Fast GC-MS/MS combined with compound-specific isotope analysis (GC-IRMS) was applied to authenticate lavender and peppermint essential oils and detect adulteration. Volatile profiling, isotopic signatures, and chemometric analysis enabled discrimination of botanical origin and identification of synthetic adulterants, providing a robust analytical strategy for quality control and traceability.

Keywords: Essential oil authentication, Compound-specific isotope analysis (CSIA), GC-IRMS

Authentication of high-value essential oils is essential to protect consumers from fraud and ensure product traceability and quality. In this contribution, the combined use of fast GC-MS/MS and compound-specific isotope ratio analysis (GC-IRMS) is presented through two case studies demonstrating its potential for essential oil authentication and adulteration detection.

The first case study focused on lavender essential oils, which are frequently adulterated with lavandin oil or synthetic compounds.^{1,2} Seventy-nine volatile organic compounds (VOCs) were profiled, while the $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values of 32 compounds were determined in authentic lavender, lavandin, and commercial lavender essential oils. Sparse Partial Least Squares Discriminant Analysis (sPLS-DA) achieved complete discrimination between lavender and lavandin within the investigated dataset, identifying characteristic VOCs together with compound-specific isotopic signatures as robust botanical markers. Isotopic analysis also enabled the detection of adulteration when more than 15% of linalool, linalyl acetate, eucalyptol, or α -terpineol originated from synthetic sources.

The second case study focused on peppermint essential oils produced in Northern Italy and commercial peppermint essential oils available on the Italian market. Their volatile profiles and the $\delta^{13}\text{C}$ and $\delta^2\text{H}$ signatures of menthol and selected minor constituents were compared with those of mint-flavoured commercial products, including candies, chewing gums, mouthwashes, and syrups. The combined chemical and isotopic approach demonstrated its potential as a robust strategy for the authentication of peppermint essential oils and related products.

Overall, these two case studies demonstrate that integrating volatile fingerprinting with compound-specific isotope analysis provides a robust and versatile strategy for determining botanical origin, detecting adulteration, and supporting the authenticity assessment of essential oils and related products.

References

1. P. K. Khatri, M. Paolini, R. Larcher, L. Ziller, D. A. Magdas, O. Marincas, A. Roncone, L. Bontempo, *Food Control*, 154, **2023**, 110002
2. P. K. Khatri, M. Paolini, R. Larcher, L. Ziller, D. A. Magdas, O. Marincas, A. Roncone, L. Bontempo, *Microchemical Journal*, 186, **2023**, 108343

OR10

From liquid phase to headspace: compound-specific $\delta^{13}\text{C}$ analysis of aceto balsamico tradizionale di modena PDO by IRMS-based techniques

Veronica D'Eusanio, Mirco Rivi, Andrea Marchetti

Department of Chemical and Geological Sciences, University of Modena and Reggio Emilia
Via G. Campi 103, 41125 Modena, Italy

Summary: *An integrated LC-IRMS and HS-SPME-GC-C-IRMS approach was applied to characterize Aceto Balsamico Tradizionale di Modena PDO throughout its production chain. Compound-specific $\delta^{13}\text{C}$ analysis revealed isotopic continuity from grapes to aged vinegar and provided new insights into volatile compound evolution during aging, supporting product characterization and process understanding.*

Keywords: *Aceto Balsamico Tradizionale di Modena PDO; LC-IRMS; HS-SPME-GC-C-IRMS*

Introduction

Aceto Balsamico Tradizionale di Modena (ABTM) is a high-value Protected Designation of Origin (PDO) product obtained exclusively from cooked grape must through a complex production process involving alcoholic fermentation, acetic oxidation, and long aging in wooden barrel batteries. During this process, the product undergoes progressive chemical and compositional changes driven by concentration, evaporation, microbial activity, and interactions with wood, resulting in a highly complex matrix [1]. The characterization of ABTM therefore requires analytical approaches capable of following both the origin of the raw material and the transformations occurring from grapes to aged vinegar. In this context, light stable isotope analysis represents a valuable tool to investigate the isotopic continuity along the production chain and to support the authenticity and valorization of this traditional product [2]. This work presents an integrated isotopic study of ABTM by combining compound-specific $\delta^{13}\text{C}$ analysis in the liquid phase with the isotopic characterization of volatile markers directly from the headspace, providing complementary information on the evolution of the product during production and aging.

Materials and Methods

Samples were collected from traditional vinegar producers in the Modena area and included grapes, cooked musts, and aged vinegars from different barrel batteries. The $\delta^{13}\text{C}$ values of glucose, fructose, acetic acid, and glycerol were determined in the liquid phase by LC-IRMS. Volatile compounds, including ethanol, acetic acid, ethyl acetate, and furfural, were analyzed directly from the headspace using HS-SPME-GC-C-IRMS. Preliminary validation tests were performed to verify whether HS-SPME introduced significant isotopic fractionation by comparing gas-phase and liquid-phase measurements. The approach was then applied to ABTM samples aged in single-wood barrel batteries, including mulberry, oak, and chestnut systems.

Results, Discussion and Conclusions

All investigated compounds showed $\delta^{13}\text{C}$ values consistent with C3 plant metabolism, supporting the grape origin and the oenological supply chain of authentic ABTM. The $\delta^{13}\text{C}$ values of glucose and fructose showed a strong isotopic continuity from grapes to cooked must and aged vinegars, including samples collected from barrel batteries. This evidence indicates that no marked carbon isotope fractionation occurs during either must cooking or long-term aging, despite the complex transformations associated with concentration, evaporation, fermentation, and maturation. Fermentation-derived compounds displayed specific isotopic features: acetic acid generally retained the signature of precursor sugars, suggesting negligible fractionation during ethanol oxidation, whereas glycerol was systematically depleted in ^{13}C , reflecting isotope effects associated with its biosynthetic pathway. During aging, acetic acid showed substantial stability or a slight progressive ^{13}C enrichment, likely related to preferential volatilization of lighter isotopologues. HS-SPME-GC-C-IRMS provided $\delta^{13}\text{C}$ values in excellent agreement with liquid-phase data, demonstrating that headspace extraction does not significantly alter isotopic signatures and can be reliably used to study volatile markers in ABTM. Ethanol and ethyl acetate showed wood-dependent differences in occurrence and persistence, while furfural exhibited a progressive ^{13}C enrichment over time, mainly associated with evaporation processes in open barrels. Overall, the combined liquid- and gas-phase isotopic approach provides a comprehensive characterization of ABTM, overcoming analytical limitations related to its dense, sugar-rich matrix. These results demonstrate the potential of light stable isotope analysis as an advanced tool for PDO product characterization, process understanding, authenticity support, and valorization.

References

1. C. Durante, L. Morelli, V. D'Eusano, L. Tassi, A. Marchetti; *Molecules*, 29, 11 (2024), 2647.
2. L. Lancellotti, V. D'Eusano, L. Morelli, E. Truzzi, A. Marchetti, L. Tassi; *Current Research in Food Science*, 10 (2025), 100953.

IP1

High- and low-resolution Agilent GC/MS platforms: technologies applied to the food market

Marica Beggio

Agilent Italia

PL2***Mass Spectrometry Analytical Toolbox to Tackle the PFASs Challenge in Food****Stefan van Leeuwen*

Wageningen University & Research (The Netherlands)

PFAS pollution has evolved to an unprecedented societal challenge. PFASs are found everywhere in our environment and foods, the number of compounds is continuously expanding, and already at low levels exposure may be exceeding the safe levels. Food and drinking water are important vectors for human exposure, and a diversity of mass spectrometric tools are employed to unravel the PFAS complexity in food and at the environment-food interface.

Targeted analysis is employed for the ng/g to pg/g level determination of PFASs in foods and feeds. HRMS with associated data workflows are used for the screening and identification of (yet) unknown PFASs in foods. In this contribution I will discuss the application of these approaches, and how different MS technologies fit in. Both the identification/detection/quantification of legacy PFAS and emerging PFAS will be discussed. In addition, the needs to advance this field further in the future will be addressed.

OR11

Beyond chromatography: unmasking spice & herbs adulteration via AP-MALDI-MS

Alessandra Tata,¹ Alessia Di Noi,² Andrea Massaro,¹ Chiara Salvitti,² Carmela Zacometti,¹ Michele Suman,^{3,4}
Federico Pepi,² Anna Troiani²

¹ Laboratory of Experimental Chemistry, Istituto Zooprofilattico Sperimentale delle Venezie, Viale Fiume, 78, 36100 Vicenza (VI), Italy

² Department of Chemistry and Technology of Drugs, Sapienza University of Rome, P.le Aldo Moro, 5, 00185 Rome, Italy

³ Analytical Food Science, Barilla G. e R. Fratelli S.p.A., Via Mantova, 166, Parma 43122, Italy

⁴ University of Parma, Department of Food and Drug, Viale delle Scienze 27, A-43124, Parma, Italy

Summary: Extraction-free AP-MALDI-MS, coupled to machine learning, allows the detection of economically motivated adulteration in ground black pepper and dried oregano leaves.

Keywords: AP-MALDI-MS, Authentication, Food fraud, Adulteration, Direct analysis, Spices, Oregano.

Introduction

Culinary herbs and spices are among the food commodities most vulnerable to Economically Motivated Adulteration (EMA) due to complex global supply chains and high market value. Fraudulent practices include substitution with lower-value exogenous plant species (e.g., olive, myrtle, or sumac leaves in dried oregano) or dilution with endogenous by-products (e.g., spent pepper or pinheads in ground black pepper). Traditional targeted chromatographic and molecular methods, while robust, are often time-consuming, solvent-intensive, and limited in detecting subtle endogenous adulterations. To address the urgent need for high-throughput screening in industrial quality control and regulatory monitoring, this study presents a direct, non-destructive Atmospheric Pressure Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry (AP-MALDI-MS) untargeted fingerprinting approach coupled with advanced machine learning.

Experimental

Ground black pepper (*Piper nigrum* L., n = 58) and dried oregano leaves (*Origanum* spp., n = 44)—comprising authentic samples, commercial adulterants, and laboratory-prepared mixtures across various concentrations (5–60% w/w)—were evaluated. Sample preparation was minimal, requiring only direct deposition of powdered material (3 mg) with alpha-cyano-4-hydroxycinnamic acid (CHCA) matrix onto a target plate, entirely omitting liquid extractions. Fingerprints were acquired using an AP-MALDI source coupled to a linear ion trap (LTQ-XL) in positive and negative ion modes. Electrospray High-Resolution Mass Spectrometry (ESI-HRMS) on an Orbitrap system was utilized for high-confidence marker annotation. Chemometric workflows including Principal Component Analysis (PCA), Recursive Feature Elimination (RFE), and mid-level data fusion were systematically evaluated. Different machine learning algorithms Random Forest (RF), partial least squared discriminant analysis (PLS-DA) and least absolute shrinkage and selection operator (LASSO) were tested.

Results

Black Pepper: Analyzed in positive ion mode, key diagnostic biomarkers included characteristic monomeric amides (piperine, piperidine, magnoflorine) alongside dimeric amide alkaloids and triacylglycerols characteristic of exogenous seed adulterants. The RF model achieved 85.0% accuracy and 92.3% specificity on test sets.

Oregano: the combination of negative and positive ion-mode data and mid-level data fusion proved superior, isolating key phenolic compounds and flavonoid glycosides. The fused RF classifier demonstrated an overall test accuracy of 85.4% (87.0% in negative mode), successfully detecting leaf adulterants down to 5% substitution levels.

A great effort was made in this study to annotate biomarkers of authenticity and adulteration by high resolution mass spectrometry and MS/MS. It is important to emphasize that prior to employing AP-MALDI coupled with low-resolution mass spectrometry for routine screening, the accurate identification of mass spectral features is crucial to establish reliable biomarkers of authenticity and fraud. Untargeted chemometric workflows rely on selected *m/z* features and are inherently vulnerable to artifactual signals. Because the classification models use a reduced subset of the most discriminating *m/z* values, ensuring that these features carry true biological significance is essential. As a further issue, and regardless of the analytical platform used, the correct attribution of the *m/z* features used in machine learning based approaches could be significantly hindered by various sources of contamination introduced during sample preparation and analysis. These contaminant signals, if not properly identified and excluded from the model building up, may be erroneously interpreted as

biologically relevant features [1].

Without definitive identification confirmation, such interference risks being misclassified as diagnostic biomarkers. In this study, candidate features were validated through accurate m/z measurements and MS/MS spectral matching [2], ensuring that all identified contaminants were excluded prior to model training.

Conclusions

This work demonstrates that direct-powder AP-MALDI-MS combined with machine learning is a powerful, green, and rapid first-line defense against spice fraud. The technique effectively discriminates both exogenous plant substitutes and challenging endogenous waste stream adulterations. By dramatically reducing analysis time and solvent consumption, this analytical framework offers food safety authorities and industry stakeholders a scalable tool for real-time supply chain integrity verification. As the AP-MALDI is a stand-alone source that is a cost-effective alternative to a traditional, dedicated, vacuum-based MALDI source, a future democratizing of the MALDI-MS technologies to food laboratories can be forecasted.

However, moving this method from proof of concept to routine would require: i.) standard operating procedures; ii.) purchase and storage of reference materials; iii.) full traceability of the reference materials; iv.) participation in proficiency tests; v) annual late-stage validation of the classifier with possible integration of new samples to the training set in case of misclassification.

References

1. A. Fiorante, L.A. Ye, A. Tata, T. Kiyota, M. Woolman, F. Talbot, Y. Farahmand, D. Vlaminc, L. Katz, A. Massaro, H. Ginsberg, A. Aman, A. Zarrine-Afsar *Int. J. Mol. Sci.*, 25 (2024), Article 3491, [10.3390/ijms25063491](https://doi.org/10.3390/ijms25063491)
2. E.L. Schymanski, H.P. Singer, J. Slobodnik, I.M. Ipolyi, P. Oswald, M. Krauss, T. Schulze, P. Haglund, T. Letzel, S. Grosse, N.S. Thomaidis, A. Bletsou, C. Zwiener, M. Ibáñez, T. Portolés, R. De Boer, M.J. Reid, M. Onghena, U. Kunkel, W. Schulz, A. Guillon, N. Noyon, G. Leroy, P. Bados, S. Bogialli, D. Stipančev, P. Rostkowski, J. Hollender *Anal. Bioanal. Chem.*, 407 (2015), pp. 6237-6255, [10.1007/s00216-015-8681-7](https://doi.org/10.1007/s00216-015-8681-7)

OR12

Beyond standard trichothecens: profiling emerging NX toxins in rice and wheat

Alessandro Annunziato,¹ Vito D'Ascanio,¹ Daria Carella,¹ Gerline Wiesenberger,^{2,3} Antonia Susca,¹ Miriam Haidukowski,¹ Antonio Moretti¹

¹ National Research Council (CNR), Institute of Sciences of Food Production (ISPA), Bari, Italy

² BOKU University, Department of Agricultural Sciences, Institute of Bioanalytics and Agro Metabolomics, Konrad Lorenz Strasse 20, 3430 Tulln, Austria

^{3c} BOKU University, Department of Agricultural Sciences, Institute of Microbial Genetics, Konrad-Lorenz-Strasse 24, 3430 Tulln, Austria

Summary: This study validates workflows to detect nine trichothecene mycotoxins, emphasizing emerging NX toxins in cereals. Mass spectrometry successfully quantified all analytes, whereas PDA detection missed specific NX toxins. For sample cleanup, Mycosep 227 outperformed OASIS HLB. The research proves mass spectrometry is essential for accurate toxin monitoring in complex matrices.

Keywords: introduce maximum 3 keywords defining the topics of your abstract

Introduction

Fusarium species biosynthesise trichothecene mycotoxins, which contaminate crops and pose significant risks to food safety [1]. Characterised by a tetracyclic 12,13-epoxytrichothec-9-ene core structure, these mycotoxins are classified into type A and type B groups based on the functional group at the C-8 position. Type A compounds, including T-2 and HT-2, possess hydroxyl, ester, or alkyl groups at this position, whereas type B analogues, such as deoxynivalenol (DON) and nivalenol (NIV), feature a ketone group. Although the recently identified NX series toxins constitute a subgroup of type A trichothecenes, they differ structurally due to the absence of the C-8 carbonyl group [2]. To ensure analytical accuracy in food monitoring, there is a critical need for validated methods capable of distinguishing and quantifying these closely related analytes within complex matrices. This study aims to develop and validate two complementary analytical methods for the simultaneous quantification of nine trichothecene mycotoxins: NIV, DON, 3-acetyl-DON (3-AcDON), 15-acetyl-DON (15-AcDON), NX2-M1, NX-2, NX-3, T-2, and HT-2 toxins. Ultimately, the goal is to establish robust, sensitive, reliable, and reproducible workflows to enhance trichothecene detection, support regulatory compliance, and strengthen food safety risk assessments against emerging mycotoxin threats.

Experimental

In this study, the methodology was applied in accordance with the work of D'Ascanio et al. [3]. Specifically, we developed and validated two complementary analytical workflows, single-quadrupole mass spectrometry (UPLC-QDA) and photodiode array detection (UHPLC/PDA), for the simultaneous quantification of nine trichothecenes in rice and wheat matrices, with a special emphasis on NX2-M1, NX-2, and NX-3. For sample preparation, two solid-phase extraction (SPE) cleanup protocols (OASIS® HLB and Mycosep® 227) were systematically compared to evaluate their performance in mitigating matrix effects and optimizing analyte recovery [3].

Results

The UPLC-QDA method achieved reliable quantification of all target analytes, including the NX series. The method's sensitivity is demonstrated by the specific limits of detection (LOD) and quantification (LOQ) reported in Table 1 and Table 2. In addition, accuracy is confirmed by excellent recovery intervals between 70% and 103%, with intra-day accuracy (RSD) stably below 13%. Conversely, the UHPLC/PDA method was unable to detect NX2-M1 and NX-3 due to their poor UV absorption properties. Regarding sample preparation, the evaluation of signal suppression/enhancement (SSE) highlighted significant matrix effects depending on the crop and cleanup protocol, as clearly illustrated in Figure 1. Specifically, Mycosep® 227 demonstrated superior purification efficiency and a remarkable reduction in signal suppression compared to OASIS® HLB, particularly in challenging wheat extracts.

Conclusion

Overall, this study provides a validated and practical framework for monitoring both regulated and emerging trichothecenes. The findings reinforce the necessity of MS-based detection over conventional UV methods for the accurate determination of emerging NX toxins in complex cereal matrices, offering an efficient tool for routine food safety assessment.

Table 1. UPLC-QDA Limit of detection (LOD, S/N=3) and limit of quantification (LOQ, S/N = 10) calculated for DON, NIV, NX2-M1, NX-2, NX-3, 3-AcDON, 15-AcDON, HT-2, T-2 in rice and wheat samples.

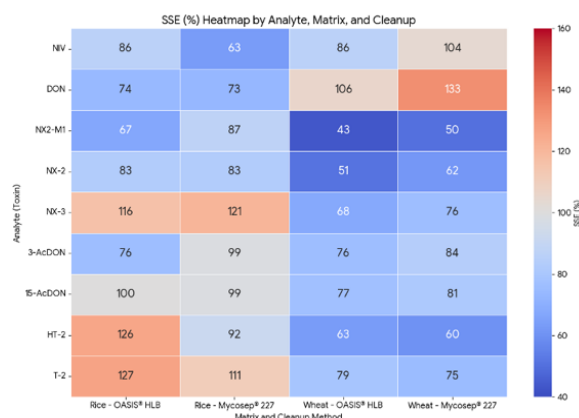
µg toxins/kg matrix OASIS® HLB samples									
	NIV	DON	NX2-M1	NX-2	NX-3	3-AcDON	15-AcDON	HT-2	T-2
LOD	8	2	23	30	28	15	10	25	3
LOQ	26	5	75	100	92	51	32	84	11

µg toxins/kg matrix Mycosep® 227 samples									
	NIV	DON	NX2-M1	NX-2	NX-3	3-AcDON	15-AcDON	HT-2	T-2
LOD	5	1	14	17	34	7	6	18	1
LOQ	17	4	45	55	112	22	20	58	4

Table 2. UHPLC/PDA Limit of detection (LOD, S/N=3) and limit of quantification (LOQ, S/N = 10) calculated for DON, NIV, NX-2, 3-AcDON, 15-AcDON, HT-2, T-2 in rice and wheat samples.

µg toxins/kg matrix OASIS® HLB samples							
	NIV	DON	NX-2	3-AcDON	15-AcDON	HT-2	T-2
LOD	13	16	22	13	20	26	26
LOQ	43	54	73	43	64	87	87

µg toxins/kg matrix Mycosep® 227 samples							
	NIV	DON	NX-2	3-AcDON	15-AcDON	HT-2	T-2
LOD	4	4	18	9	12	11	20
LOQ	13	12	59	31	38	36	66

**Figure 1.** Heatmap of the Signal Suppression/Enhancement (SSE %) for nine trichothecenes (UPLC-QDA) in rice and wheat matrices following two SPE cleanup methods (OASIS® HLB and Mycosep® 227). Blue cells indicate signal suppression (100%). The significant matrix effects observed, with most SSE values falling outside the acceptable 80–120% range (total range 43–133%), mandate the use of matrix-matched calibration for accurate quantification.

References

- Foroud N. A., Baines D., Gagkaeva T. Y., Thakor N., Badea A., Steiner B., Bürstmayr M., & Bürstmayr H. Trichothecenes in cereal grains – An update. *Toxins*, **2019**, *11*: 634.
- Varga E., Wiesenberger G., Hametner C., Ward T.J., Dong Y., Schöfbeck D., McCormick S., Broz K., Stückler R., Schuhmacher R., Krska R., Kistler H.C., Berthiller F. and Adam G. Discovery of a novel type A trichothecene. *Environ Microbiol*, **2015** *17*: 2588-2600.
- D'Ascanio V., Annunziato A., Carella D., Wiesenberger G., Susca A., Haidukowski M., & Moretti, A. Multitoxin Analysis of Trichothecenes, including NX Toxins, in Rice and Wheat. *Applied Food Research*, **2026**, 101709.

OR13

Integrated mass spectrometry workflow for PFAS assessment in food contact materials according to the new PPWR requirements

F. Cozzolino, F. Pratesi, M. Vitulli

Food Contact Center s.r.l

Summary: *An integrated mass spectrometry workflow combining CIC, LC/HRMS, GC/MS, Pyrolysis-GC/MS and TOP Assay was applied to assess PFAS in food contact materials according to the new PPWR requirements. The complementary analytical approaches enabled a comprehensive evaluation of target PFAS, precursors and fluorinated materials, supporting reliable regulatory compliance assessment.*

Keywords: *PFAS; Food Contact Materials; PPWR.*

Introduction

The forthcoming European Packaging and Packaging Waste Regulation (PPWR) introduces a new regulatory framework for the control of per- and polyfluoroalkyl substances (PFAS) in food contact materials (FCMs), establishing three complementary compliance thresholds: 25 µg/kg for individual PFAS, 250 µg/kg for the sum of PFAS after precursor oxidation, and 50 mg F/kg for total fluorine [1,2]. These requirements significantly increase the analytical complexity of PFAS assessment, since no single analytical technique is capable of detecting target PFAS, polymeric fluorinated materials, volatile precursors and unknown fluorinated compounds simultaneously [2,4,5].

Mass spectrometry therefore plays a central role in PFAS analysis, providing complementary information through different analytical approaches. Targeted chromatographic methods enable accurate quantification of regulated PFAS, while high-resolution mass spectrometry supports suspect and non-target screening of unknown fluorinated substances. In parallel, combustion- and pyrolysis-based techniques provide information on total and organic fluorine, complementing chromatographic analyses and supporting the interpretation of PFAS occurrence in complex food contact materials [3–5]. In this work, we present an integrated analytical workflow combining Combustion Ion Chromatography (CIC), Pyrolysis-GC/MS, LC-HRMS, GC-MS and TOP Assay for the comprehensive assessment of PFAS in food contact materials. Rather than proposing an alternative analytical method, the workflow integrates complementary mass spectrometry techniques into a single decision-making strategy supporting regulatory compliance according to the new PPWR requirements.

Experimental

A total of 30 commercial food contact materials (FCMs) representative of different packaging applications were investigated, including baking papers, external paper packaging, flour bags, yoghurt cups, plastic dairy packaging and plastic components used in the food industry. These matrices were selected to cover materials potentially containing intentionally added or non-intentionally added fluorinated substances.

The analytical workflow is summarized in Figure 1. Total fluorine (TF) was determined by Combustion Ion Chromatography (CIC) as an initial screening step. Extractable PFAS were investigated after methanolic extraction using LC-HRMS, enabling

both targeted determination of regulated PFAS and high-resolution suspect/non-target screening. To improve precursor detection, a second aliquot of each extract was subjected to the Total Oxidizable Precursor (TOP) Assay, based on alkaline persulfate oxidation, followed by LC-HRMS analysis of the oxidation products. In parallel, GC-MS was employed for the determination of volatile PFAS, including fluorotelomer alcohols (FTOHs), while Pyrolysis-GC/MS was used to investigate fluoropolymer-related markers and other non-extractable fluorinated materials.

For each sample, the complementary information obtained from the different analytical techniques was integrated into a weight-of-evidence approach, allowing a comprehensive assessment of PFAS occurrence and supporting compliance evaluation according to the new PPWR requirements.

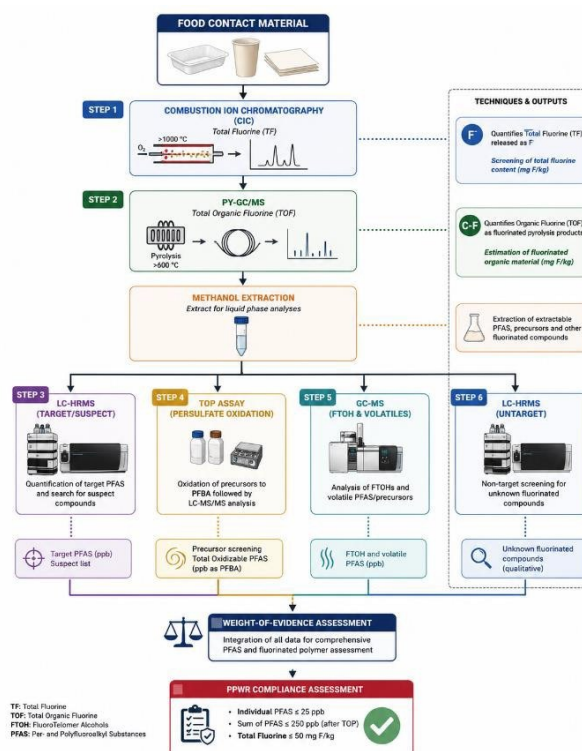


Fig. 1. Protocol of analyses for food packaging samples.

Results and discussion

The integrated analytical workflow provided complementary information on the occurrence of fluorinated compounds in the 30 commercial food contact materials investigated.

Target LC-HRMS analysis did not detect any of the monitored PFAS above the method LOQs (5 µg/kg) in any of the analysed samples, indicating the absence of regulated extractable PFAS. Likewise, GC-MS analysis did not detect fluorotelomer alcohols (4:2, 6:2, 8:2 and 10:2 FTOHs) above the method LOQ (25 µg/kg), excluding the presence of the most common volatile PFAS precursors.

In contrast, Total Fluorine (TF) analysis revealed measurable fluorine in several samples. TF values ranged from 7.9 to 136.2 mg/kg, with one paper-based packaging sample exceeding the 50 mg/kg threshold established by the PPWR. These findings highlighted the need for complementary analytical techniques capable of distinguishing PFAS-derived fluorine from other fluorinated substances.

The TOP Assay significantly extended the analytical coverage. Although all samples were negative by target analysis, oxidation generated detectable PFBA in four samples, demonstrating the presence of oxidizable PFAS precursors that would not have been identified by conventional targeted analysis alone. These results confirm the importance of precursor oxidation for a more comprehensive assessment of fluorinated contaminants in food contact materials. Pyrolysis-GC/MS investigations further contributed to the characterization of fluorinated materials by providing molecular fingerprints of fluoropolymer-related structures and other non-extractable fluorinated species, supplying complementary information to chromatographic analyses. The integration of pyrolysis data with LC-HRMS, GC-MS, CIC and TOP Assay enabled a more complete interpretation of fluorinated substances present in the investigated materials.

Overall, the proposed workflow allowed the discrimination between samples genuinely free of detectable PFAS and samples requiring further investigation because of the presence of fluorinated materials or precursor compounds. The integration of complementary mass spectrometry techniques represents a robust analytical strategy for supporting compliance assessment according to the new PPWR requirement.

Conclusion

The new PPWR requires a paradigm shift in PFAS analysis, moving from single-method determinations to integrated analytical workflows. By combining complementary mass spectrometry techniques with fluorine-based screening approaches, the proposed strategy enables a more comprehensive characterization of fluorinated substances than conventional targeted analysis alone. The workflow provides a practical framework for routine laboratory implementation and for supporting reliable PPWR compliance assessment of food contact materials.

References

1. *European Parliament and Council; Regulation (EU) 2025/40 on Packaging and Packaging Waste (PPWR)*, Official Journal of the European Union, L 2025/40 (2025).
2. OECD; *Reconciling Terminology of the Universe of Per- and Polyfluoroalkyl Substances (PFAS)*, OECD Series on Risk Management, No. 61 (2021).
3. E.F. Houtz, D.L. Sedlak; *Oxidative Conversion as a Means of Detecting Precursors to Perfluoroalkyl Acids in Urban Runoff*, *Environmental Science & Technology*, 46 (2012), pp. 9342–9349.
4. A. Androulakis, N. Alygizakis, E. Bizani, N.S. Thomaidis; *Recent advances in the analysis of per- and polyfluoroalkyl substances (PFAS) through multidimensional and hyphenated techniques*, *Environmental Science: Advances*, 1 (2022), pp. 705– 724.
5. Y. Shen, L. Wang, Y. Ding, S. Liu, Y. Li, Z. Zhou, Y. Liang; *Current Trends in the Analysis of Per- and Polyfluoroalkyl Substances (PFAS): A Review*, *Critical Reviews in Analytical Chemistry*, 54 (2024), pp. 3171–3195.

OR14

Determination of phthalates in bottled, purified and reusable bottle water by SPME-GC/MS

Claudia Lino,¹ David Bongiorno,¹ Serena Indelicato,¹ Giuseppe Avellone^{1,2}

¹ Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche, Università di Palermo, via Archirafi 32, 90123 Palermo (Italy)

² ATeN Center, Università di Palermo, viale delle Scienze, 90128 Palermo (Italy)

Summary: *The SPME-GC/MS method was used to investigate the occurrence of phthalates in drinking water from multiple sources, including bottled mineral water, municipal tap water, purified water, and reusable plastic bottles. Phthalates were detected in all samples, although all concentrations remained below current regulatory limits.*

Keywords: *phthalates, drinking water, SPME-GC/MS*

Introduction

Phthalate esters (PAEs) are a class of synthetic chemicals widely used as plasticisers to enhance the flexibility, durability, and processability of polymeric materials used in food-contact applications and plastic packaging. Due to their widespread industrial use and non-covalent binding to polymer matrices, PAEs can be released into the environment and migrate into food and drinking water during manufacturing, storage or through contact with plastic materials commonly used for food and beverage packaging. Consequently, phthalates are ubiquitous environmental contaminants that represent a potential source of human exposure through dietary intake, and several of them are recognised as endocrine-disrupting chemicals. Increasing evidence has associated chronic exposure to certain phthalates with reproductive and developmental disorders, metabolic dysfunctions and other adverse health outcomes, making their occurrence in drinking water a matter of growing health concern [1]. To protect consumers, the European Food Safety Authority (EFSA) has established tolerable daily intake (TDI) values for several phthalates. The TDI for dibutyl phthalate (DBP), benzyl butyl phthalate (BBP), bis(2-ethylhexyl) phthalate (DEHP), and diisononyl phthalate (DINP) is 50 µg kg⁻¹ body weight day⁻¹ for each molecule, whereas a higher TDI of 150 µg kg⁻¹ body weight day⁻¹ has been established for diisodecyl phthalate (DIDP). These reference values provide the basis for evaluating the potential health risks associated with human exposure to phthalates through drinking water.

Growing attention has recently been devoted to assessing phthalate releases into drinking water from various sources, particularly given the increasing use of recycled plastic materials and reusable bottles as sustainable alternatives to single-use packaging. At the same time, domestic water purification systems are becoming increasingly common, although their effectiveness in removing organic contaminants such as phthalates has not yet been fully investigated.

The present study aimed to develop and validate a rapid, solvent-free solid-phase microextraction coupled with gas chromatography/mass spectrometry (SPME- GC/MS) for the determination of selected phthalates in drinking water. The method was applied to mineral waters bottled in virgin and recycled PET, municipal tap water, purified water obtained from domestic filtration systems, and water stored in reusable plastic bottles. The occurrence of PAEs, their migration behaviour and the potential health risks associated with their ingestion were evaluated through an exposure assessment based on EFSA guidelines.

Experimental

Phthalates were extracted by direct immersion solid-phase microextraction (DI-SPME) using a DVB/CAR/PDMS fiber and analysed by GC/MS. A method was optimised and validated for the determination of phthalates in drinking water samples [2].

The investigated matrices included:

- commercial mineral waters bottled in virgin and recycled PET [3];
- tap waters and purified waters [4];
- water stored in commercially available reusable plastic bottles under simulated consumer-use conditions

Depending on the sample type, different target phthalates were investigated, including DEP, DiBP, DBP, DEHP and other commonly occurring PAEs.

Method validation included evaluation of linearity, LOD, LOQ, precision and accuracy. Human exposure was assessed following EFSA recommendations by calculating estimated daily intake (TDI), risk quotient (RQ) values for adults and toddlers and excess lifetime cancer risk (ELCR).

Results

The analytical method demonstrated good analytical performance, showing high sensitivity, precision and accuracy, together with low LOD and LOQ.

Phthalates were detected in all investigated matrices, although concentrations varied depending on the water source and packaging material.

Mineral waters bottled in virgin and recycled PET contained total phthalate concentrations ranging between 2.2 and 18.96 µg/L. However, considerable variability in phthalate levels was observed among recycled PET bottles, with most samples showing lower concentrations than virgin PET bottles, while one sample exhibited markedly higher levels, suggesting possible contamination during recycling or from the previous use of the plastic. Municipal tap water and purified water samples also contained detectable PAEs. Domestic purification systems reduced the concentration average reduction of approximately 30%, demonstrating their capability to partially remove organic contaminants.

Reusable plastic bottles exhibited the largest variability among samples, with total phthalate concentrations ranging from 0.27 to 9.35 µg/L. Although HDPE and LDPE bottles generally released higher amounts than PP and PETG bottles, bottle-specific characteristics appeared to influence migration more than polymer type alone. Across all studies, DEHP was the predominant phthalate detected in all sample types.

Despite these differences, all measured concentrations remained below the specific migration limits established by current European legislation. Risk assessment indicated estimated TDI and RQ well below tolerable values for both adults and toddlers. Furthermore, ELCR values indicated negligible carcinogenic risk from DEHP in drinking water.

Conclusions

The proposed DI-SPME-GC/MS method represents a rapid, sensitive and environmentally friendly analytical approach for monitoring phthalates in drinking water from multiple sources.

The study confirms the widespread occurrence of phthalates in bottled, tap and purified waters as well as in water stored in reusable plastic bottles. Although detected concentrations were consistently below current regulatory thresholds and no significant health concerns emerged from the exposure assessment, the ubiquitous presence of PAEs highlights the importance of continuous surveillance of drinking water quality. The results further support the implementation of quality control measures for recycled plastic materials, regular maintenance of domestic water purification systems and continued evaluation of reusable food-contact plastics to ensure long-term consumer safety.

References

1. A. Sokołowski, M. Kończak, P. Oleszczuk, Y. Gao, and B. Czech; *Water, Air, and Soil Pollution* 235 (2024): 313.
2. M. M. Alshehri, M. A. Ouladsmene, T. A. Aouak, Z. A. ALOthman, A. Y. B. H. Ahmed; *Chemosphere* 304 (2022): 135214 (2).
3. C. Lino, S. Indelicato, D. Bongiorno, F. D'Agostino, G. Avellone, *Journal of Mass Spectrometry*, 2026; 61:e70089
4. C. Lino, S. Indelicato, D. Bongiorno, F. D'Agostino, S. Indelicato, G. Avellone; *Beverages* 11 (2025): 92.

OR15

Development and validation of an analytical procedure based on a simplified μ -QuEChERS extraction for monitoring bisphenols in different honey botanical varieties using UHPLC-MS/MS

Lorenzo Marino Cerrato,^{1,2} Rosalia López-Ruiz,² Roberto Romero-González,² Antonia Garrido Frenich,² Stefania Albrizio¹

¹ Department of Pharmacy, School of Medicine and Surgery, University of Naples Federico II, Via Domenico Montesano 49, 80131, Naples, Italy

² Research Group "Analytical Chemistry of Contaminants", Department of Chemistry and Physics, Research Centre for Mediterranean Intensive Agrosystems and AgriFood Biotechnology (CIAIMBITAL), University of Almería, Agrifood Campus of International Excellence, ceiA3, E-04120 Almería, Spain

Summary: A simplified μ -QuEChERS method was developed for the determination of thirteen bisphenols in forty commercial honeys, revealing widespread samples contamination with 85% containing at least one bisphenol above LOQ (0.1 μ g/kg). Contamination was significantly associated with plastic-packaging. Risk assessment indicated potential health concerns, highlighting the importance of sensitive monitoring analysis.

Keywords: bisphenols, honey, μ -QuEChERS

Introduction

Bisphenols (BPs) are synthetic compounds widely used in the production of polycarbonate plastics and epoxy resins. Due to their endocrine-disrupting properties and their ubiquitous contamination, increasing attention has been devoted to monitoring BPs occurrence in foods. In 2024, the Commission Regulation (EU) 2024/3190 imposed a total ban on the use of bisphenol A (BPA) and its congeners in food contact materials (FCMs) [1]. Honey represents a challenging matrix for BPs determination because of its complex composition, high sugar content, and viscosity. Therefore, the aim of this study was to develop a sensitive and efficient analytical procedure for the simultaneous determination of thirteen BP residues in honey and to investigate their occurrence in products available on Spanish market.

Experimental

A simplified μ -QuEChERS extraction procedure was optimized for the analysis of thirteen BPs (BPA, BPS, BPF, BPE, BPB, BPC, BPAF, BPZ, BPBP, BPFL, BPG, BPM, and BPP) in honey samples. Briefly, 1 g of honey was extracted with acetonitrile, followed by a salting-out partitioning using NaCl and MgSO₄ and a freezing clean-up step, eliminating the common d-SPE purification prior to the UHPLC-ESI-QqQ-MS/MS analysis, performed in MRM mode using ammonium fluoride (NH₄F) as mobile phase additive. The developed method was then validated according to the SANTE guidelines by the evaluation of linearity, matrix effect (ME%), limit of quantification (LOQ), trueness (RE%), and precision (RSD%) for each residue. The validated procedure was then applied to the analysis of forty honey samples of different botanical varieties, packaged in glass and plastic containers. The dietary exposure and risk assessment were evaluated using the EFSA consumption database and BPA tolerable daily intake (TDI) as toxicological reference value [2].

Results

The developed and validated method demonstrated excellent analytical performance for all the BPs. Good linearities were obtained with determination coefficients (R^2) > 0.99, while matrix effects were limited ($\leq \pm 20\%$) or compensated through matrix- matched calibration curves. Recoveries ranged from 76.1% to 108.9%, with values of repeatability and reproducibility (RSD%) always $\leq 20\%$. LOQ values of 0.1 μ g/kg were achieved for almost all analytes thanks to the use of NH₄F, overcoming the generally scarce BPs ionization efficiency and representing the lowest values reported for BPs determination in honey by LC-MS/MS analysis (Table 1) [3]. When compared to other techniques, this method achieves a well-balanced compromise between analytical performance and environmental sustainability, obtaining an AGREEprep score of 0.36. Method application to commercial products revealed widespread BPs contamination with 85% of samples containing at least one residue above the LOQ level. BPA was the most frequently detected compound (88%) above its LOQ, being quantified in 45% samples and reaching maximum concentration of 3.54 μ g/kg, followed by BPG, BPM, and BPP. Statistical analysis showed significantly higher contamination levels in plastic-packaged honey compared with glass-packaged products for both the number of BPs detected and quantified (Mann–Whitney U test, $p=0.027$ and $p=0.013$), in particular for BPA (Fisher's exact test, $p=0.0036$), suggesting migration from plastic FCMs as a primary source of contamination. Risk assessment for BPA exposure through honey consumption showed potential health risks for vulnerable population groups with a non-carcinogenic hazard quotient (HQ)

> 1. However, when considering BPs cumulative exposure ($\sum$ HQ) by the hazard index (HI), the risk was $\approx$ 3-4 times higher and was extended to other population groups.

Table 1. UHPLC-ESI-QqQ-MS/MS method validation parameters for BPs analysis in honey.

BPs	LOQ ($\mu\text{g/kg}$)	Recovery (%) (n = 5)			Intra-day precision (RSD _r %) (n = 5)			Inter-day precision (RSD _R %) (n = 7)			ME%
		0.1	1	10	0.1	1	10	0.1	1	10	
		($\mu\text{g/kg}$)	($\mu\text{g/kg}$)	($\mu\text{g/kg}$)	($\mu\text{g/kg}$)	($\mu\text{g/kg}$)	($\mu\text{g/kg}$)	($\mu\text{g/kg}$)	($\mu\text{g/kg}$)	($\mu\text{g/kg}$)	
BPA	0.10	92.7	103.6	101.3	6.1	2.7	0.7	10.2	5.2	3.0	9.9
BPS	0.10	96.0	98.7	101.9	2.8	3.7	1.5	4.6	4.2	1.8	-22.8
BPF	0.10	97.3	97.5	97.6	6.5	0.7	0.6	7.2	2.9	1.3	9.1
BPE	0.10	105.4	97.2	101.7	4.3	1.1	0.5	11.8	3.5	2.3	-5.3
BPB	0.10	100.0	103.7	98.3	7.0	2.7	0.5	8.2	3.8	1.3	-22.9
BPC	0.10	96.2	107.4	100.5	4.9	5.8	1.5	6.8	6.7	1.5	6.3
BPAF	0.10	102.0	100.3	102.9	5.8	0.6	1.5	6.7	1.4	2.8	0.1
BPZ	0.10	101.2	94.0	104.5	4.9	2.1	1.9	6.7	4.3	2.6	-11.1
BPBP	1		87.7	97.0		6.1	1.2		10.8	4.6	1.4
BPFL	0.10	106.6	92.4	99.5	3.6	1.8	1.3	4.9	1.9	2.5	0.9
BPG	0.10	87.8	79.7	96.5	3.3	4.9	4.7	7.7	5.3	5.0	1.6
BPM	0.10	108.9	101.0	76.1	0.1	1.2	2.8	0.1	2.1	3.6	14.2
BPP	0.10	77.9	81.6	81.6	3.4	1.8	2.3	6.8	3.4	4.2	12.4

Conclusions

Bisphenols (BPs) are synthetic compounds widely used in the production of polycarbonate plastics and epoxy resins. Due to their endocrine-disrupting properties and their ubiquitous contamination, increasing attention has been devoted to monitoring BPs occurrence in foods. In 2024, the Commission Regulation (EU) 2024/3190 imposed a total ban on the use of bisphenol A (BPA) and its congeners in food contact materials (FCMs) [1]. Honey represents a challenging matrix for BPs determination because of its complex composition, high sugar content, and viscosity. Therefore, the aim of this study was to develop a sensitive and efficient analytical procedure for the simultaneous determination of thirteen BP residues in honey and to investigate their occurrence in products available on Spanish market.

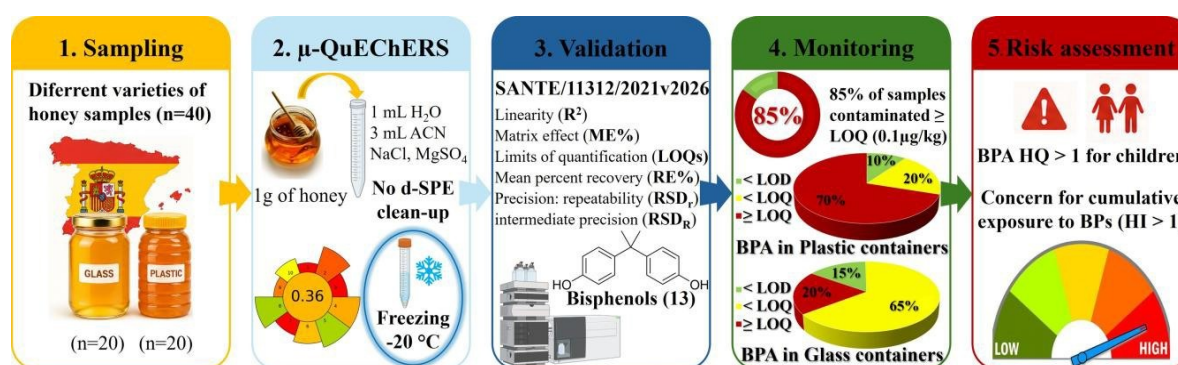


Fig. 2. Graphical representation of the experimental workflow of this study.

References

1. Y. Tang, G. Qin, N. Qian, X. Zeng, R. Li, K. Lai; *Environmental Chemistry and Ecotoxicology*, (7) 2025, 696-705.
2. A. M. Ares, L. Alcaide, J. Bernal, S. Valverde; *Food Research International*, 195 (2024), 114955.
3. B. Martín-Gomez, S. Valverde, J. Bernal, A. M. Ares; *Food Chemistry*, 450 (2024), 139358.

KN1

The gas-phase goldmine: volatilomics from cutting-edge platforms to real-world use

Philipp Weller, Lukas Bodenbender, Richard Frey, Nabiollah Mobaraki

Technical University of Applied Sciences, Mannheim, Germany

Summary: *This presentation will demonstrate modern, untargeted volatilomics strategies from highend to point-of-need in foods and fermentations*

Keywords: *Volatilomics, GC-MS, GC-IMS, Machine Learning*

The term “volatilomics” denotes the systematic characterization of the „volatilome“, the important, yet insufficiently elucidated subset of the volatile fraction of the metabolome, with applications in systems biology as well as in domains, such as biotechnology and food analysis.

In the latter, such methodologies are often subsumed under “foodomic” strategies and have become integral to food authentication and quality assurance. Volatilomics allow to relate volatile organic compounds (VOCs), sampled from the gas phase above a matrix, to defined product attributes; examples include authenticity, quality, and geographical origin. This is particularly important because many aroma-active constituents in foods - such as those in roasted coffee, cocoa, essential oils, or spices [1,2,3] - belong to the VOC fraction and are available in a non-invasive way via headspace analysis. A principal challenge arises from the complexity and sheer diversity of detected compounds, for which the diagnostic value frequently resides not in single analytes but in the aggregate “fingerprint” formed by all detectable species. Interpreting such high-dimensional spectral data necessitates advanced chemometric methods and machine learning, approaches commonly grouped within the broader “omics” paradigm. The integration of these datasets with modern machine learning techniques allows for an optimized information extraction to correlate spectral data in untargeted approaches with abstract parameters, such as quality or authenticity [4].

VOCs also constitute a critical information source in fermentation processes, a core component of modern biotechnology, which feature exceedingly complex gas-phase profiles that remain largely outside current molecule-specific monitoring regimes. The use of these VOC profiles is a truly tempting source of information of the status of fermentation processes – optimal growth or upcoming failure – while at the same time, the sterile barrier is unharmed.

Typical metabolomics approaches and also most volatilomics strategies are based on conventional, mostly MS or HRMS analytical platforms, often with enrichment steps required to meet the sensitivity levels. Consequently, these predominantly laboratory-bound systems are unsuitable for true point-of-need deployment, constraining their practical utility. In this context, gas chromatography coupled to drift tube ion mobility spectrometry (GC–IMS) emerges as a compelling platform to bridge traditional laboratory instrumentation and portable solutions.

The presentation will outline foundational analytical principles, data analysis approaches and show case studies where high-end and benchtop or portable strategies are merged into one coherent volatilomics strategy that allow for the analysis of complex patterns in foods, feeds and fermentations where they are truly needed: at the point of need.

References

1. Bodenbender, L., *et al. Anal Bioanal Chem* (2026). <https://doi.org/10.1007/s00216-026-06415-3>
2. Kiefer, C. *et al. Chemosensors* **2026**, *14*, 34. <https://doi.org/10.3390/chemosensors14020034>
3. Parastar, H. *et al.*, Non-targeted volatilomics for the authentication of saffron by gas chromatography-ion mobility spectrometry and multivariate curve resolution, *Food Chemistry*, Volume 465, Part 2, 2025, 142074, ISSN 0308-8146, <https://doi.org/10.1016/j.foodchem.2024.142074>.
4. Bayat-Afshary, F. *et al. Food Chemistry*, Volume 500, 2026, 147511, ISSN 0308-8146, <https://doi.org/10.1016/j.foodchem.2025.147511>.

OR16

HS-SPME-GC-MS and HPLC-DAD-MS as tools to support the optimization of high-quality flavoured olive oils produced through co-milling: case studies using chili peppers, basil and rosemary

Lorenzo Cecchi,¹ Carlotta Breschi,¹ Tommaso Ugolini,¹ Erminio Monteleone,¹ Renzo Fusi,¹ Francesca Ieri,² Silvio Menghini,¹ Fabio Boncinelli,¹ Maria Bellumori,³ Marzia Innocenti,³ Nadia Mulinacci,³ Bruno Zanoni,¹

¹ Department of Agricultural, Food, Environmental, and Forestry Sciences and Technologies (DAGRI) – University of Florence, Piazzale delle Cascine 18 – 50144 Firenze (Italy)

² National Research Council of Italy (CNR), Institute of Bioscience and BioResources (IBBR), Sesto Fiorentino (Florence) (Italy)

³ Department of Neurofarba, Nutraceutical and Pharmaceutical section, via Ugo Schiff 6, Campus of Sesto Fiorentino, University of Florence (Italy)

Summary: *Chromatographic techniques coupled with mass spectrometry were applied to support the development of high-quality flavoured olive oils produced through co-milling. The integrated characterization of flavouring agents and oils enabled process optimization, comparison with conventional flavouring techniques and the study of compound transfer as a function of flavouring agent concentration*

Keywords: *HS-SPME-GC-MS analysis of VOCs; flavoured olive oils; co-milling techniques; kinetic aromatic transfer*

Introduction

Among the different flavouring techniques currently available, the co-milling of fresh flavouring agents with sound olives has emerged as the most promising approach for producing high-quality flavoured olive oils while preserving the sensory characteristics of fresh plant materials and ensuring excellent shelf-life of at least 18 months [1-3]. The chemical fingerprint of flavouring agents can be exploited to optimize the composition and sensory properties of the resulting flavoured olive oils. This kind of optimization requires analytical tools capable of supporting the selection of suitable flavouring matrices, defining the optimal amount of flavouring agent to be added to olives during milling, and understanding the transfer of aroma- active and bioactive compounds from plant materials into the flavoured oils, also in relationship with their peculiar sensory properties. Within this framework, this study is part of the Co.EVO project and aims to develop an integrated analytical platform combining chromatographic techniques with mass spectrometry, such as HS- SPME-GC-MS and HPLC-DAD-MS, for supporting the rational optimization of flavoured olive oils produced through co-milling. Rather than focusing on a single flavouring agent, the proposed workflow is intended to be applicable to different flavouring agents, allowing the comparison of their chemical characteristics, their behaviour during oil extraction including kinetics of aroma compounds transfer from herbs to oils, and the comparison among the different produced flavoured oils also during their shelf-life.

Experimental

Samples: Three types of flavouring agents were considered: fresh chili peppers, known for their pungency linked to the presence of capsaicinoids, and two aromatic plants like basil and rosemary. Flavoured oils were produced through co-milling and conventional infusion techniques and adding different percentage of flavouring agents to olives and oils, respectively.

Analyses: Fresh flavouring materials, olives, and flavoured olive oils produced through co- milling and infusion were chemically and sensorially characterized to evaluate both the standard parameters for virgin olive oil and typical molecules and sensory attributes linked to the flavouring agents. Concerning liquid chromatography, the integration of different instrument configurations (including DAD, FLD and MSD detectors) were adopted depending on the target molecules linked to the aromatic agent (e.g., capsaicinoids, tocopherols, phenolic compounds). Volatile and terpene compounds were analysed through HS-SPME-GC-MS: a 1- cm 50/30 µm DVB/CAR/PDMS SPME fiber was employed for analytes extraction from the headspace of a 20-ml screw vial with the sample. The analytes were subsequently desorbed at 260°C in a 6890N GC system with a 5975-MS detector (all from Agilent, Palo Alto, CA, USA) and separated in a HP-Innowax capillary column (50m×0.2mm id, 0.4 µm ft). Oven: 2 min at 40°C; to 156°C at 4 °C/min; to 260°C at 10°C/min. MSD worked in scan mode at m/z 29-350 Th, IE energy 70 eV. Peaks identification was performed using commercial standards when available or comparing the linear retention index evaluated analyzing linear alkanes with the NIST08/Wiley98 library. Quantification was carried out using a combination of external standards and internal standards for peak area normalization. This chemical characterization was complemented by sensory evaluation focusing on the typical attributes of both olive oil and the flavoring agents employed. Different data analysis tools were adopted to identify correlations between chemical and sensory data, and flavouring agents and techniques,

and to compare the production techniques in order to identify the best one to produce high-quality flavoured oils also during storage.

Results

HS-SPME-GC-MS highlighted specific volatile profiling of chili peppers, basil and rosemary, allowing identification of 98, 45 and 57 VOCs, respectively, while HPLC-DAD-MS analyses allowed evaluation of relevant bioactive compounds. Characteristic volatile molecules, potentially involved in the flavouring process were identified among the most abundant ones in the volatile profile of each flavouring agent. An example of chromatogram is shown in fig 1 for the volatile fraction of red chili peppers.

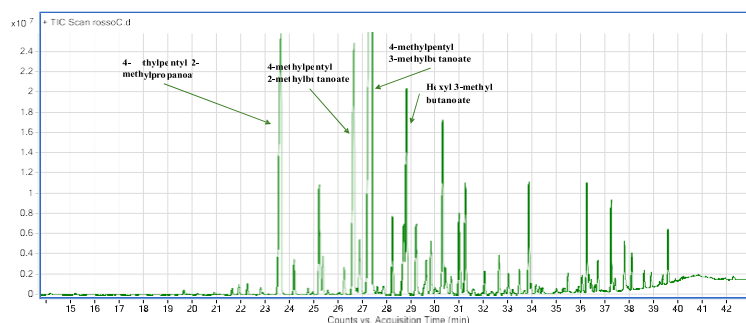


Figure 1. Volatile profile and some characteristics VOCs of red fresh chili peppers.

The corresponding flavoured olive oils produced by co-milling and, in case of chili peppers, also by infusion were also characterized. HS-SPME-GC-MS highlighted distinctive volatile profile for the different oils, allowing the identification of aroma markers potentially involved in the flavouring process, while HPLC-DAD-MS provided information on bioactive compounds relevant to product quality.

Experiments demonstrated that co-milling better preserved and transferred to oils the characteristic volatile compounds of fresh chili peppers than temporary or permanent infusion. The oils produced by co-milling showed improved sensory quality, more fresh aroma profiles, and a good equilibrium among pungency and aroma, maintaining their positive characteristics over 18 months, with no sensory defects.

Production trials were then performed by co-milling to study the kinetics of transfer of VOCs from chili peppers, basil and rosemary to flavoured oils, suggesting slightly different kinetics of transfer. Sensory descriptors correlated with relevant VOCs, phenols and capsaicinoids identified by HS-SPME-GC-MS and HPLC-DAD-MS, respectively. This approach enabled to design production processes with optimal % of flavouring agents to be added to olives to produce flavoured oils with target sensory characteristics. Preliminary sensory results obtained on rosemary-flavoured oils showed concentration-dependent increases in rosemary aroma and taste intensity together with variations in pungency, confirming the importance of integrating instrumental and sensory analyses during process optimization.

Conclusions

The integration of HS-SPME-GC-MS and HPLC-MS-based techniques provides a comprehensive analytical platform for supporting the development of flavoured olive oils produced by co-milling, allowing herbs and oils characterization, optimization of flavour transfer, preservation of desirable aroma characteristics and selection of suitable operating conditions. The progressive extension of the approach from chili peppers to aromatic herbs demonstrates its potential as a versatile strategy for the rational development of a wide range of high-quality flavoured olive oils.

References

1. L. Cecchi, D. Balli, S. Urciuoli, A. Travaglia, B. Zanoni, N. Mulinacci, *Food Chem*, 404 (2023), 134696
2. L. Cecchi, S. Urciuoli, F. Ieri, T. Ugolini, Balli, B. Zanoni, N. Mulinacci, *Food Chem*, 464 (2025), 141817.
3. C. Lazzarini, M. Tura, M. S. Barbieri, E. Valli, A. Bendini, T. Gallina Toschi. *Foods*, 14(4) (2025), 687.

OR17

From cabbage to fermented foods: integrated profiling of bioactive compounds

Tiziana Nardin, Francesca Martinelli, Roberto Larcher

Fondazione Edmund Mach, Technology Transfer Centre, Food Chemistry
Via E. Mach 1, 38098 San Michele all'Adige (TN), Italy

Summary: *Controlled fermentations of savoy, green and red cabbage were investigated by complementary LC-APCI-HRMS, LC-HESI-HRMS and LC-OPA-FLD workflows. Matrix, starter culture and fermentation time affected carotenoid and free amino acid profiles, while untargeted polyphenol and peptide datasets provided complex fingerprints for ongoing annotation of fermentation-related transformations.*

Keywords: *fermented vegetables; high-resolution mass spectrometry; bioactive compounds*

Introduction

Fermented cabbage products are increasingly valued as minimally processed foods and potential sources of bioactive metabolites. Lactic acid fermentation can release, consume or transform amino acids, peptides, polyphenols and carotenoids, but the final profile depends on the plant matrix, microbial starter and process duration [1]. This study combined targeted and untargeted chromatographic workflows to characterize fermented cabbage products and to follow the evolution of bioactive compounds during controlled laboratory fermentations.

Experimental

Nine commercial fermented cabbage products were initially screened. Controlled fermentations were then performed on savoy cabbage, green cabbage and red cabbage using three strategies: spontaneous fermentation, *Lactobacillus plantarum* TH253, and *L. plantarum* TH253 combined with *L. pentosus*. Vegetables were supplemented with 2% (w/w) NaCl and incubated at 20 °C for 10 days, with sampling at T0, T1, T2, T4 and T10. Carotenoids and fat-soluble vitamins were analysed by RP-C30 LC-APCI-HRMS using external calibration. Free amino acids were determined after OPA derivatization by RP-LC-FLD. Polyphenols were extracted with methanol and profiled by LC-HESI-HRMS in positive and negative ionization modes. Water-soluble peptides were investigated by LC-HESI-HRMS over m/z 300-2000, with data-dependent MS/MS acquisition where feasible.

Results and Discussion

The combined platform captured distinct chemical trajectories across matrices and fermentation strategies. Commercial products showed heterogeneous fingerprints, consistent with differences in ingredients and processing. Savoy cabbage displayed the highest quantifiable carotenoid content and the clearest starter-dependent kinetics. The mixed starter produced a marked intermediate increase in the sum of violaxanthin, zeaxanthin, lutein and β -carotene, reaching a maximum at T4, followed by a substantial decline at T10. Green cabbage contained lower carotenoid levels and generally showed progressive losses, whereas most monitored carotenoids in red cabbage remained below the quantification limit. Free amino acids were comparatively stable in savoy cabbage but decreased markedly in green and red cabbage, particularly in inoculated fermentations. Red cabbage showed the highest initial amino-acid pool, and GABA remained especially abundant during spontaneous fermentation. Trends in branched-chain and aromatic amino acids also indicated substantial microbial utilization. Untargeted LC-HRMS analysis of polyphenols and peptides generated a large number of features and complex MS/MS datasets. Alignment, deconvolution and annotation are in progress to define compound families and candidate markers associated with matrix, starter culture and fermentation stage. Because quantitative data are currently expressed on a fresh-weight basis, dry-matter normalization will be included in the final interpretation.

Conclusions

Fermentation did not produce a uniform enrichment of bioactive compounds. Instead, matrix, starter and processing time jointly determined retention, release and consumption. The integrated targeted and untargeted workflow provides a robust basis for identifying fermentation windows that preserve carotenoids and favorable amino-acid profiles, while supporting deeper characterization of polyphenol and peptide transformations.

References

1. J. Karovičová, Z. Kohajdová; *Horticultural Science*, 30 (2003), pp 152-158.

OR18

Marine by-product valorization: LC-MS/MS Characterization of collagen peptides derived from the edible sea urchin *Paracentrotus lividus*

Tamara Chwojnik,¹ Gijs JC Vreeke,² Giovanni A van Kralingen,² Matteo Brilli,³ Stefania Marzorati,¹ Michela Sugni,¹ Jean-Paul Vincken ²

¹ Department of Environmental Science and Policy, University of Milan, Via Celoria 2, 20133, Milan, Italy

² Laboratory of Food Chemistry, Wageningen University, Bornse Weilanden 9, 6708 WG, Wageningen, the Netherlands.

³ Department of Biosciences, University of Milan, Via Celoria 26, 20133, Milan, Italy

Summary: This research compared sea urchin collagen, derived from the restaurant industry by-products (i.e., the peristomial membrane) as alternative to conventional bovine collagen.). The aim was to characterize collagen, by making hydrolysates with different proteases, and analyze these using an LC-MS/MS peptide-mapping approach. The established peptide profile and amino acid composition of sea urchin collagen was significantly different than bovine collagen, showing its potential for valorizing these by-products into high value food ingredients and biomaterials.

Keywords: by-product valorization, sea urchin collagen, LC-MS/MS peptide mapping

Introduction

Collagen is one of the most commercially important proteins, with applications in nutraceuticals, cosmetics, and tissue engineering [1]. While bovine and porcine collagens remain the predominant industrial sources, concerns related to sustainability, religion, and disease transmission have driven the search for alternative collagen sources. In this context, the valorization of seafood-processing by-products as a sustainable source of collagen is gaining increasing attention within a circular economy framework. A relevant example is the Mediterranean sea urchin *Paracentrotus lividus*, whose processing generates large amounts of waste rich in valuable biomolecules, including native fibrillar collagen from the peristomial membrane [2].

As novel collagen sources emerge, it is essential to assess their structural similarity to traditional mammalian collagens. Recent advances in LC-MS/MS peptide mapping have enabled detailed characterization of collagen α -chain composition of non-model organisms [3]. For *P. lividus*, the primary structure of peristomial membrane collagen remains largely uncharacterized due to limited availability of genomic annotation, and direct comparison with bovine collagen is still missing. Therefore, this study applied the peptide mapping workflow developed by Vreeke et al. (2022)[4] to characterize peptides from *P. lividus* collagen hydrolysates and infer collagen α -chain sequences, providing new insights into its molecular composition and structural comparison to commercial collagens.

Experimental

Discards of *P. lividus* (i.e., Aristotle's lanterns) were collected from restaurants in the Milan metropolitan area. The Aristotle's lanterns were dissected, and the peristomial membrane was isolated. A suspension of native collagen was obtained from the peristomial membranes using previously established protocols [2]. Bovine collagen type I was used as a control. Afterwards, the collagens were analyzed for the protein profile (SDS-PAGE), for amino acid profile (using targeted analysis in LC-MS) and for bottom-up proteomics (untargeted peptide mapping with UHPLC-ESI-MS) (Fig. 1).

SDS-PAGE was performed on collagen from both sea urchin and bovine collagen after a 24-hour digestion by pepsin in 0.5 M acetic acid.

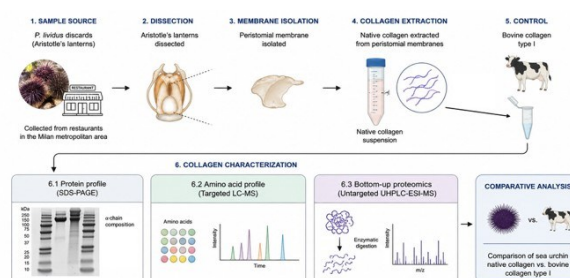


Fig. 1. Experimental workflow

For amino acid composition analysis, sea urchin and bovine collagen were hydrolyzed under various conditions, with norvaline as internal standard. Afterwards, the hydrolysates were derivatized with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate and separated by Vanquish UPLC-PDA (Waters HSS T3 column), then analyzed by Orbitrap Exploris 120 MS. Absolute quantification of all amino acids, including hydroxyproline was performed using calibration curves.

Pepsin-solubilized collagen was subsequently treated with trypsin and chymotrypsin. The resulting hydrolysates were separated with a Waters H-class Acquity UHPLC system using a Waters BEH C18 column. The mass spectra of the peptides were acquired with the Select Series Cyclic IMS (Waters). MS and MS^E fragmentation data were collected. Peptide identification was performed with the peptide mapping package in UNIFI software version 1.8, with settings described in [4]. Hydroxyproline modifications were considered. Peptides were characterized using α -chain sequences from *Bos taurus* (Bovine) and *P. lividus* deposited in the NCBI and UNIPROT database.

Results

SDS-PAGE analysis showed major bands at approximately 120–140 kDa, consistent with collagen α -chain composition. Given that bovine type I collagen consists of α 1 and α 2 heterotrimers, these data suggest a similar type I-like α -chain composition in sea urchin collagen, further supported by peptide mapping.

Amino acid composition confirmed the collagenous nature of the material extracted from the *P. lividus* peristomial membrane, with both sea urchin and bovine collagen showing ~30% glycine, followed by proline and hydroxyproline. While the overall profile was comparable, sea urchin collagen displayed higher levels of aspartic acid, glutamic acid, serine, threonine, and methionine, and lower amounts of alanine, lysine, and phenylalanine relative to bovine collagen. Cysteine and tryptophan were not detected in either sample.

LC-MS/MS analysis showed that trypsin digestion yielded the highest number of peptide identifications, whereas chymotrypsin provided lower sequence coverage. Database searching of tryptic peptides from sea urchin collagen against *P. lividus* sequences (AA29438.1; AAA29440.1) resulted in identification of α 1- and α 2-chain- derived peptides, corresponding to partial sequence coverages of 15% and 22%, respectively. The identified peptides are consistent with collagen- derived Gly-X-Y-rich sequences. Under the same workflow, bovine collagen achieved high sequence coverage, reaching up to 81% for α 1 (P02453) and 51% for α 2 (P02465) chains. Efforts are still ongoing to retrieve better sequence information from the genome for the sea urchin collagen, to increase the number of identified peptides in the chromatograms and to get higher coverage of the sea urchin collagen sequence.

Conclusions

LC-MS/MS peptide mapping enabled the structural characterization of fibrillar collagen from *P. lividus* peristomial membranes, confirming its heterotrimeric organization and revealing conserved motifs and sequence-level differences compared to bovine type I collagen. These findings support its potential as a marine-derived biomaterial source.

References

1. V.F. Alcolea Esinger, D. Lamas, A. Massa; *Environmental Science and Pollution Research*, 32 (2025), pp 16077-16097
2. C. Ferrario, F. Rusconi, A. Pulaj, R. Macchi, P. Landini, M. Paroni, G. Colombo, T. Martinello, L. Melotti, C. Gomiero, M.D. Candia Carnevali, F. Bonasoro, M. Patruno, M. Sugni; *Marine Drugs*, 18 (2020), pp 414
3. M. Tian, C. Xue, Y. Chang, J. Shen, Y. Zhang, Z. Li, Y. Wang; *Food Chemistry*, 316 (2020), pp 126272.
4. G.J.C. Vreeke, W. Lubbers, J.P. Vincken, P.A Wierenga, *Analytical Chimica Acta*, 1201 (2022), pp 339616

OR19

Supramolecular solvents for modern food analysis

Noelia Caballero-Casero, Soledad Rubio

Department of Analytical Chemistry, Institute of Chemistry for Energy and the Environment (IQUEMA),
University of Córdoba, Córdoba, Spain

Summary: Food analysis increasingly demands versatile sample preparation methods capable of handling chemically diverse analytes across complex matrices while simplifying routine workflows. This presentation discusses how supramolecular solvents (SUPRAS) provide sustainable and adaptable extraction platforms for food safety, quality control, authenticity assessment, and recovery of high-value compounds for the food industry.

Keywords: supramolecular solvents, sustainable sample treatment, food analysis

Introduction

Food analysis encompasses a broad range of analytical objectives, including food safety, quality control, authenticity assessment, nutritional characterization, and the recovery of valuable compounds for industrial applications. Regardless of the analytical target, sample preparation remains one of the major bottlenecks due to the enormous diversity of food matrices and the wide range of physicochemical properties exhibited by the compounds of interest.

Conventional extraction methods are often developed for specific analyte classes or matrices, requiring multiple protocols, extensive sample handling, and large volumes of organic solvents. Consequently, there is an increasing demand for versatile sample preparation strategies capable of simplifying analytical workflows while providing broad analyte coverage, effective clean-up, sustainability, and compatibility with modern LC-MS platforms. Supramolecular solvents (SUPRAS) have emerged as promising extraction media owing to their nanostructured organization, tunable physicochemical properties, and multiple interaction mechanisms. These characteristics enable efficient extraction of structurally diverse compounds from complex food matrices using simple, rapid, and environmentally friendly procedures.

Methods

This presentation summarizes recent advances in the development of SUPRAS-based sample preparation platforms for food analysis. The fundamental principles governing SUPRAS formation, their physicochemical properties, and the mechanisms responsible for analyte extraction will be discussed [1]. Particular emphasis will be placed on recent developments aimed at designing versatile extraction methodologies capable of simultaneously extracting compounds with markedly different physicochemical characteristics while providing efficient clean-up and direct compatibility with LC-MS analysis.

Representative applications developed by our research group will be presented to illustrate the versatility of SUPRAS across different food matrices and analytical objectives.

Results

SUPRAS-based methodologies have demonstrated remarkable versatility for the extraction of chemically diverse compounds from both solid and liquid food matrices. Their multiple interaction mechanisms allow efficient recovery of contaminants, natural constituents, and bioactive compounds within a single analytical platform, reducing the need for analyte-specific extraction procedures.

Recent applications include the determination of regulated contaminants, such as mycotoxins, together with emerging contaminants including bisphenols, using both targeted and non-targeted LC-MS approaches [2-4]. Beyond food safety and quality assessment, SUPRAS have also shown considerable potential for the selective extraction of valuable food constituents relevant to ingredient production, functional foods, and circular bioeconomy strategies [5,6]. Overall, SUPRAS simplify routine analytical workflows by integrating extraction and clean-up into a single step, reducing solvent consumption while maintaining high analytical performance.

Conclusions

SUPRAS constitute versatile and sustainable sample preparation platforms capable of addressing diverse analytical challenges in food analysis. Their ability to simplify routine workflows, extract structurally diverse compounds, and accommodate multiple analytical objectives makes them attractive tools for food safety, quality control, authenticity assessment, and the recovery of high-value compounds. These characteristics position SUPRAS as promising alternatives to conventional extraction methodologies for both research and

routine food analysis.

References

1. Rubio, S. *Analytical and Bioanalytical Chemistry* (2020) 412:6037-6058.
2. Saber, A.N. *Journal of Hazardous Materials* (2026) 511:142211
3. Muñiz-Bustamante, L.; Caballero-Casero, N.; Rubio, S. *Food Chemistry* (2025) 490:145092.
4. García-Cansino, L.; Caballero-Casero, N.; García, M.A.; Marina, M.L.; Rubio, S. *Microchimica Acta* (2025) 192:398.
5. Muñiz-Bustamante, L.; Caballero-Casero, N.; Rubio, S. *Applied Food Research* (2026) 6:101644.
6. Muñiz-Bustamante, L.; Caballero-Casero, N.; Rubio, S. *LWT – Food Science and Technology* (2025) 234:118612.

Acknowledgements

The authors acknowledge financial support from MCIU/AEI/10.13039/501100011033/FEDER, UE (grant number PID 2023-149488OB-I00). Dr Caballero-Casero acknowledges the grant RYC2023-045005-I funded by MICIU/AEI/10.13039/501100011033 and by ESF+.

PL3**Flavour is not concentration: using dynamic mass spectrometry insights to engineer food perception**

Ian Fisk

International Flavour Research Centre, University of Nottingham

Food reformulation is commonly approached through changing its composition: reducing salt, sugar, fat or alcohol, or changing the concentration of flavour-active compounds. However, sensory perception is not determined simply by how much of a compound is present in a food, but by how much is released, where and when it is released, and how long that stimulus persists during consumption. We describe this capacity of a food to deliver sensory stimuli as its flavour potential.

Conventional analytical approaches provide powerful measurements of food composition, but often provide only a static snapshot of a highly dynamic process. This presentation will explore how real-time mass spectrometry, supported by saliva characterisation, can measure flavour delivery during consumption and provide mechanistic tools for food reformulation.

A series of case studies will demonstrate how spatial distribution, food structure and phase partitioning can be manipulated to alter taste and aroma delivery independently of total concentration during oral processing. Examples will include controlling sodium delivery through particle and structural design, maintaining flavour perception during salt and sugar reduction, and understanding how changes in food composition and oral physiology influence in-mouth aroma release. Real-time atmospheric pressure chemical ionisation mass spectrometry and ex-vivo saliva characterisation approaches, combined with sensory and physicochemical measurements, enable these dynamic changes in flavour delivery to be directly linked to perception.

Together, these studies demonstrate a shift from measuring what is present to understanding what is delivered. By measuring, predicting and ultimately controlling flavour potential, food reformulation can move towards a mechanism-led approach in which flavour perception becomes an engineerable property of the food.

Food reformulation is commonly approached through changing its composition: reducing salt, sugar, fat or alcohol, or changing the concentration of flavour-active compounds. However, sensory perception is not determined simply by how much of a compound is present in a food, but by how much is released, where and when it is released, and how long that stimulus persists during consumption. We describe this capacity of a food to deliver taste and aroma stimuli during consumption as its flavour potential.

Conventional analytical approaches provide powerful measurements of food composition, but often provide only a static snapshot of a highly dynamic process. This presentation will explore how real-time mass spectrometry, supported by saliva characterisation, can measure flavour delivery during consumption and provide mechanistic tools for food reformulation.

A series of case studies will demonstrate how spatial distribution, food structure and phase partitioning can be manipulated to alter taste and aroma delivery independently of total concentration during oral processing. Examples will include controlling sodium delivery through particle and structural design, maintaining flavour perception during salt and sugar reduction, and understanding how changes in food composition and oral physiology influence in-mouth aroma release. Real-time atmospheric pressure chemical ionisation mass spectrometry, combined with ex-vivo saliva analysis, sensory evaluation and physicochemical measurements, enables these dynamic changes in flavour delivery to be directly linked to perception.

Together, these studies demonstrate a shift from measuring what is present to understanding what is delivered. By measuring, predicting and ultimately controlling flavour potential, food reformulation can move towards a mechanism-led approach in which flavour perception becomes an engineerable property of the food.

OR20

Basil volatilome and quality assessment for high-quality pesto: tracing aroma markers across the production chain

Giulia Tapparo,¹ Erica Liberto,¹ Gianluca Cucco²

¹ Department of Drug Science and Technology, University of Turin, Via Pietro Giuria 9, 10125 Turin, Italy

² F.Ili Saclà S.P.A., Piazza Giovanni Amendola, 2 - 14100 Asti, Italy

Summary: *An HS-SPME-GC-MS workflow characterized the volatile profiles of basil samples from different cultivars, growing areas, and harvest periods over two consecutive growing seasons, and monitored processing effects from fresh basil to pesto. Volatile fingerprinting identified quality markers supporting cultivar selection, food safety, and standardized industrial basil assessment.*

Keywords: *volatilomics; basil authentication; food processing*

Introduction

Sweet basil (*Ocimum basilicum* L.) has experienced a marked increase in global demand, mainly driven by the worldwide popularity of pesto, resulting in expanded cultivation beyond traditional areas such as Liguria, where Protected Designation of Origin (PDO) basil is produced [1,2]. To meet the increasing industrial demand, basil production has intensified through large-scale open-field cultivation systems and multiple harvest cycles within the same season with the aim of maximizing productivity and ensuring a continuous supply of raw material [2].

However, basil cultivation has become increasingly challenged by the spread of several pathogens, particularly downy mildew (*Peronospora belbahrii*), one of the major threats to crop productivity. Moreover, the recent withdrawal of important plant protection products by the European Commission because of toxicological concerns has further complicated disease management strategies [2,3]. Therefore, breeding programmes are continuously developing new disease-resistant cultivars with diverse genetic backgrounds, requiring detailed chemical characterization. Since resistance traits are often introgressed from *Ocimum* accessions with distinct chemotypes, the acquisition of disease resistance may lead to chemotype shifts. This increasing cultivar variability, combined with differences in cultivation environments and agronomic practices, challenges basil chemotype characterization and quality assessment. In particular, monitoring volatile compounds such as estragole, a potentially genotoxic metabolite essentially absent in PDO "Genovese" basil and explicitly flagged by EFSA in basil-derived compound foods, has become increasingly relevant for both food safety and quality control purposes [4]. A further, largely unexplored question concerns how basil aroma characteristics evolve during processing. Blanching, homogenization, and pasteurization can alter the volatile profile, and the retention of cultivar- and origin-related traits in the final pesto product determines the potential of raw material selection as a strategy for quality optimization.

In this context, mass spectrometry-based volatilomics represents a valuable analytical approach for characterizing basil aroma profiles and supporting authentication, traceability, and standardization of raw materials intended for food production. The present work demonstrates how a robust, high-throughput HS-SPME-GC-MS platform coupled with chemometrics can characterize volatile variability across the production chain and identify quality-related markers applicable to industrial basil selection, from cultivation to the final preserved pesto product.

Experimental

Fresh basil leaves from 4 cultivar (G,N,P,Y) were sampled over two consecutive growing seasons (2024–2025) from commercial fields in three Italian production districts (Veneto, Emilia-Romagna, Piemonte), together with a Ligurian PDO-area reference. The experimental design evolved from an initial broad cultivar screening to a targeted assessment of industry-relevant cultivars, evaluated across successive cutting cycles to capture temporal variability under production conditions. Cultivar comparisons were performed within the same field, agronomy and harvest window so that genotype was the only variable.

In parallel, the industrial pesto line was monitored throughout processing, from fresh and blanched basil leaves to semi-finished and pasteurized pesto, using a standardized recipe to isolate the impact of basil variability. The oil phase and blanching water were also characterized to assess their contribution to volatile compound losses and retention across the process.

Volatiles were profiled by automated HS-SPME-GC-MS (DVB/CAR/PDMS fibre, internal standardization, triplicate analyses). Identification combined EI spectral matching against commercial and in-house libraries together with linear retention indices. Data were processed by PCA, hierarchical clustering and heatmaps, with ANOVA on total abundances.

Results

Sixty-two targeted volatile compounds were detected. The volatile profile was qualitatively conserved across cultivars, whereas differences were mainly quantitative, reflecting shifts in the relative abundance of the targeted compounds. Linalool remained the predominant volatile in all cultivars, indicating that the resistant germplasm retained a linalool-dominated profile.

Cultivar had a strong, reproducible effect on aromatic profiles but not on total abundance. PCA separated cultivar P in two independent districts through distinct volatile signatures, with terpinen-4-ol and 1-hexanol contributing to the discrimination and reflecting differences in terpenic and green/herbaceous notes. Cultivar Y was associated with linalool, α -phellandrene and (E)-allo-ocimene, compounds linked to floral, fresh and citrus-like aromatic notes, whereas cultivar N was characterized by 3-octanone, borneol and α -cubebene, contributing to fruity, woody and spicy notes. The fact that cultivar P discriminating markers shifted between districts while its separation remained consistent reveals a genotype $\times$ environment interaction that would remain undetected in single-site studies.

Cultivation area emerged as an important factor shaping aromatic composition, with clustering trends separating Parma from Verona across cultivars. Verona samples showed a higher contribution of terpene-related compounds, particularly from the second cut onward, suggesting a shift toward a different aromatic profile, whereas Parma samples tended to retain fresher aromatic characteristics. The Ligurian Gentile reference was distinguished by higher contributions of eugenol, (E)-isoeugenol and oxygenated monoterpenes, and showed a more consistent clustering pattern. This suggests that cultivation within the PDO area may contribute to a more stable and recognizable aromatic profile, rather than simply generating a different average composition, while the same genotype grown outside the PDO area maintained a detectable, although less pronounced, signature. On a relative-composition basis, eugenol and 1,8-cineole were higher in the Ligurian material compared with Parma and Verona G, whereas estragole showed low relative levels, in agreement with the characteristic PDO-associated volatile profile.

The harvest stage did not show a universal pattern, with effects depending on both cultivar and cultivation area. Nevertheless, two general trends emerged: early harvest events were associated with more terpenic aromatic profiles characterized by resinous/pine-like notes, whereas later harvests showed a greater contribution of woody notes and compounds related to sesquiterpenes. Comparison among harvest events revealed a stronger discriminant capacity of harvest timing than of cultivation area, with samples primarily grouped according to the harvest event. However, when harvest timing and cultivation area were kept constant, cultivar exerted a more pronounced effect than harvest stage.

In the processing, blanching was the most influential operation, with effects depending on both cultivar and cut. Short-chain alcohols and oxygenated monoterpenes decreased, whereas bicyclic monoterpenes and sesquiterpenes increased, while phenylpropanoids were the most stable class. Analysis of the blanching water indicated that volatile changes were largely due to partitioning rather than degradation, with linalool, borneol, eucalyptol, and eugenol recovered in the aqueous phase. Semi-finished product: Mass balance showed that sunflower oil contributed <0.5% of any shared volatile, indicating that the changes reflected chopping and mixing alone. Pesto: cultivar- and cut-specific volatile signatures were retained in the final product despite the formulation, although the relative abundance of individual volatile compounds differed from that in fresh leaves.

Conclusions

HS-SPME-GC-MS volatilomics coupled with chemometrics effectively discriminated basil cultivars, cultivation environments and harvest stages, while confirming that cultivar identity was the main determinant of aroma composition. Processing, particularly blanching, reshaped volatile profiles mainly through compound partitioning rather than degradation, without eliminating cultivar-specific signatures. These results demonstrate that aroma-related quality traits can be monitored from field to final product, providing a robust analytical framework to support cultivar selection, traceability and quality control in industrial pesto production.

References

- 1 Sowmya RS, Warke VG, Mahajan GB, et al. Quality and shelf-life assessment of pesto prepared using herbs cultivated by hydroponics. *International Journal of Gastronomy and Food Science* 2022;30:100608.
- 2 La Placa L, Cornali S, Bertinaria F, et al. Cropping system support in downy mildew control in basil in organic farming: a two-year open field experiment. *Journal of Plant Pathology* 2024;107(1):167–179.
- 3 Commission Implementing Regulation (EU) 2024/1207 of 29 April 2024 on the non-renewal of dimethomorph approval.
- 4 Arsenijević J, Jovanović A, Kovačević N, et al. Basil essential oil: estragole content and chemical profiles. *Alimentaria* 2025;54(1):133–144

OR21

Influence of fermentation state and amino acid supplementation on volatile compound production by *Lactiplantibacillus plantarum* in a model fermentation system

Max Nally,^{1,2} Iuliia Khomenko,² Franco Biasioli,² Phil Bremer,¹ Pat Silcock,¹ Graham Eyres¹

¹ Department of Food Science, University of Otago, Dunedin, New Zealand

²Sensory Quality Unit, Fondazione Edmund Mach, San Michele all'Adige, Italy

Summary: This study investigated the effects of fermentation state and amino acid supplementation on volatile compound production by *Lactiplantibacillus plantarum*. Agarose-based solid-state and liquid fermentations produced distinct volatile profiles, demonstrating that both physical state and nutrient availability influence microbial metabolism and flavour development.

Keywords: Solid-state fermentation; Lactic acid bacteria; PTR-ToF-MS

Introduction

Solid-state fermentation (SSF) is a commonly used method for food production and waste valorization, providing several benefits over submerged fermentation. These include lower energy consumption, reduced production cost and the development of distinct, unique food products [1]. However, the heterogeneous and complex nature of these fermentations has led to limited understanding of the metabolic processes involved with VOC generation in SSF, especially when comparing flavour generation to submerged ferments. Previous work has investigated VOC production by lactic acid bacteria using semi-defined media under a range of fermentation conditions in submerged fermentations (SmF) [2]. However, the influence of fermentation state remains difficult to isolate because conventional SmF and SSF systems differ in both physical properties and nutrient composition. Incorporating agarose into a semi-defined model medium provides a simplified system for investigating the effect of physical state on microbial metabolism.

The objective of this study was to investigate whether fermentation state and amino acid addition influences volatile organic compound (VOC) production by *Lactiplantibacillus plantarum* using a semi-defined model medium.

Experimental

A semi-defined medium was prepared either in liquid form to represent submerged fermentation (SmF) or solidified with 1% (w/v) agarose to simulate solid-state fermentation (SSF). Each medium was prepared either with or without supplementation of a defined amino acid mixture (0.4 g L⁻¹ each). Media was inoculated with *Lactiplantibacillus plantarum* (Fermentis SafSour LP652), with uninoculated controls included for each treatment combination. Fermentations were conducted in 20 mL headspace vials in triplicate and incubated at 25°C for 72 h.

The experimental design comprised of two fermentation states (SmF and SSF), two nutrient conditions (with and without amino acid supplementation) and two inoculation treatments (inoculated and uninoculated), resulting in eight treatment combinations.

Headspace VOCs were monitored at 2 h intervals using proton-transfer-reaction time-of-flight mass spectrometry (PTR-ToF-MS 8000, Ionicon, Innsbruck, Austria) a multipurpose head-space automated sampler (Gerstel GmbH, Mulheim am Ruhr, Germany) and a static headspace module (Ionicon, Innsbruck, Austria), providing high-frequency, real-time analysis of volatile production. Medium pH was measured every 24hrs to confirm fermentation progress. Multivariate statistical analysis was performed using selected mass peaks, followed by targeted analyses to evaluate the effects of fermentation state and amino acid supplementation on VOC production.

Results and Discussion

Clear differences were observed between uninoculated controls and inoculated ferments, demonstrating microbial contribution to VOCs throughout fermentation. Furthermore, principal component analysis (PCA) showed separation between liquid and solid-state ferments, in fermentations supplemented with and without amino acids, indicating that fermentation state influenced the overall VOC profile (Figure 1).

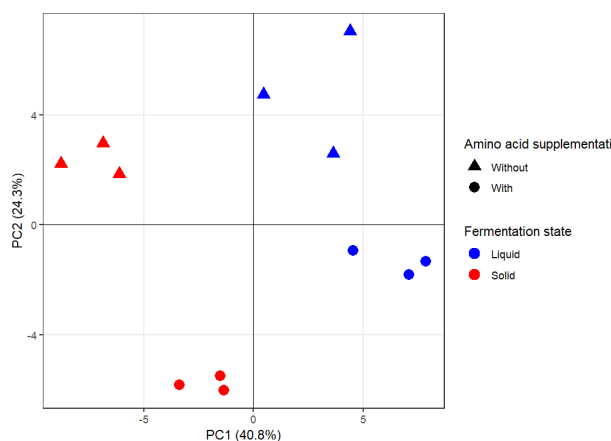


Fig 1. PCA of VOC profiles from inoculated liquid and agarose-solidified fermentations after 70 h. Colours indicate fermentation state and shapes indicate amino acid supplementation.

Several mass peaks tentatively assigned with primary metabolism differed between fermentation states. Ions tentatively associated with acetaldehyde and acetone/propanal (Figure 2) exhibited altered production in agarose-solidified media compared with liquid fermentations. In addition, mass peaks associated with oxygenated C7, C9, C11 and C13 compounds were also affected by fermentation state and amino acid supplementation. These compounds are commonly associated with lipid metabolic pathways and other secondary processes, suggesting that altered diffusion, mass transfer and localized physicochemical conditions within the agarose matrix influenced microbial metabolism.

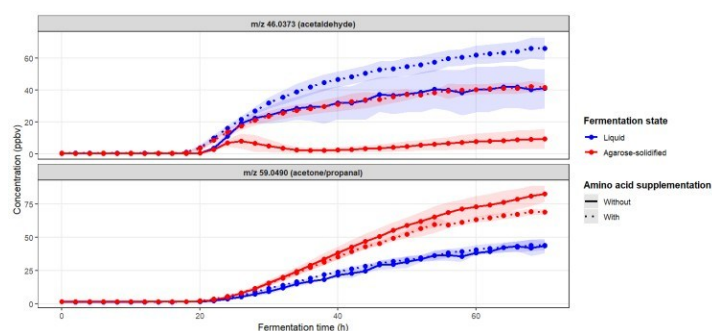


Fig 2. Time-course production of m/z 46.0373 (acetaldehyde) and m/z 59.0490 (acetone/propanal) during fermentation. Colours indicate fermentation state and line type indicates amino acid supplementation.

These findings demonstrate that modification of fermentation state alone was sufficient to change VOC production, supporting the hypothesis that physical environment can independently influence microbial metabolism and flavour generation.

Conclusions

This study demonstrates that agarose-based model systems provide a practical approach for investigating the influence of fermentation state and amino acid addition on microbial volatile production. The observed differences between liquid and solid fermentations indicate that physical state can significantly influence microbial metabolism and VOC generation. Future work will expand this model to include multiple lactic acid bacteria strains, different agarose concentrations and incubation temperatures to further investigate the mechanisms governing flavour development during solid-state fermentation.

References

1. Couto, S. R., & Sanromán, M. Á.: *Journal of Food Engineering*, 76(3) (2006), 291-302.
2. Rajendran, S., Khomenko, I., Silcock, P., Betta, E., Pedrotti, M., Biasioli, F., & Bremer, P; *Fermentation*, 10(6) (2024), 317

OR22

Pyridine precursors other than trigonelline under coffee roasting conditions

Valentina Lonzarich, Luciano Navarini

illycaffè S.p.A., via Flavia 110, 34147 Trieste, Italy

Summary: *Model systems constituted by single amino acids with or without sucrose and thermally treated under different conditions to mimic coffee roasting were characterized by HS-SPME-GC-MS with special attention to pyridine formation. Mechanisms of pyridine formation via the autolysis of several precursors including single amino acids and the role played by Maillard reaction were examined. Considering our experimental findings and the mechanisms reported in the literature, we identified the reaction pathways most likely responsible for pyridine formation in the investigated model systems.*

Keywords: *Pyridine, aroma precursors, amino acids*

Introduction

Pyridine is a six-membered aromatic heterocyclic compound containing one nitrogen atom. In coffee, it is generated during the roasting process and, according to the literature, once the thermal formation begins, its concentration increases continuously with rising temperatures and/or longer roasting times (1, 2, 3).

On the other hand, trigonelline, whose presence in green coffee beans has been identified since 1909 (1), is a N-methylpyridinium salt biogenetically derived from nicotinic acid. During the roasting process, trigonelline undergoes thermal decomposition, producing pyridines, pyrroles and methyl nicotinate. The mechanism of pyridine formation from trigonelline during roasting conditions is well known, although trigonelline is not the only precursor of pyridine in roasted coffee (4). According to literature, pyridine is formed not only by trigonelline pyrolysis but also from proteins, amino acids in the presence of sugars and glycerol under well-defined conditions. (5). Acrolein is a very important intermediate in the formation of pyridine together with other aldehydes. Moreover, acrolein, in addition to being derived from thermal treatment of lipids, carbohydrates and from both Maillard and Strecker reactions, may be produced from amino acids (eg methionine and threonine) and from glycerol. It should be noted that pyridine formation pathways alternative to that from trigonelline have received far less scientific attention.

In the present study, we address this gap by examining in detail precursors other than trigonelline - particularly amino acids - that may contribute to pyridine formation during coffee roasting.

Experimental

Model systems of single amino acids with or without sucrose were prepared in a 20 ml vial closed with a screw cap and heated at 200°C in a thermostatic dry bath (Galli, Italy). Glycerol and ammonium acetate were mixed in a 20 mL vial and thermally treated as reported above, to confirm a possible pathway of pyridine formation. The headspaces of the resulting samples were adsorbed by a car/pdms 75 µm SPME fiber for 10 minutes at 60°C and then inserted into the injector of a GC/MS (GC7890 coupled with a 5977B MS, Agilent, Palo Alto) at 250°C for 10 minutes of desorption. GC was equipped with a ZB-WAX plus column (60m, 0.25 mm I.D. and 0.25 µm film, Phenomenex), temperature program was set at 50°C for 3 minutes, then heated at 4°C/min. to 200°C, followed by the ramp of 30°C/min. to the final temperature of 240°C hold for 5 minutes. MS signals were acquired in full scan mode.

Results

Pyridine formation was observed in almost all samples of single amino acids heated at 200 °C for 20 minutes. The highest observed amounts have been found in lysine model system, followed by methionine and cysteine (see figure 1).

The possible formation mechanisms reported in the literature are consistent with our findings, supporting the idea that lysine may be one of the amino acids most prone to pyridine formation.

1. According to Breitbart and Nawar (6), Lys after α-deamination and decarboxylation is converted into 5-aminopentanal; an intramolecular aldehyde-amine dehydration would produce tetrahydropyridine (fig. 2). Dehydrogenation may then occur to form pyridine, and further hydrogenation would form piperidine. This mechanism may therefore account for the notably high pyridine levels observed in the lysine-containing system if compared to other amino acids.

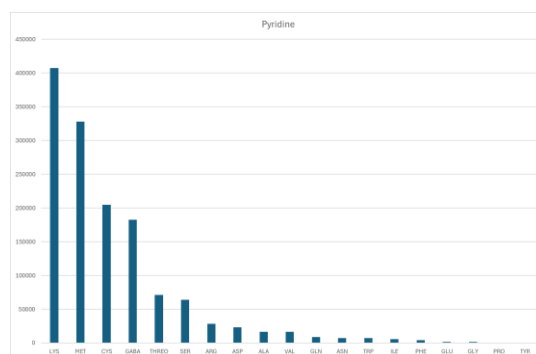


Fig. 1. Area of pyridine peak in model systems with amino acids heated at 200°C for 20 minutes.

2. C₂–C₃ carbonyl compounds formed through the Maillard reaction, and acrolein originating from glycerol thermal degradation, may be involved in pyridine formation. In the glycerol sample heated at 200 °C, both acetaldehyde and acrolein were detected, and acetaldehyde was likewise present in the lysine-containing model system, supporting this possible formation pathway (fig. 3). Moreover, in the ammonia/glycerol system a trace of pyridine was found, even though pyrazines represented by far the most predominant class of compounds.

3. Another mechanism involves methylimine produced from Lys, that can lead to pyridine through reaction with acetaldehyde (fig. 4); acetaldehyde was identified in the samples containing Lys and heated at 200°C; thus, this pathway might be taken into account, although methylimine itself was not detected, as its extreme thermal instability makes it unlikely to be observable under GC–MS conditions.

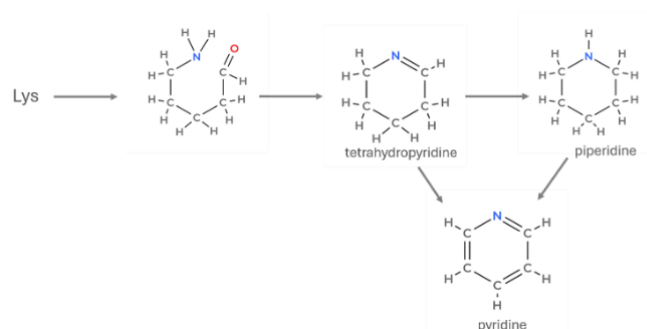


Fig. 2. Proposed mechanism of pyridine formation from lysine.

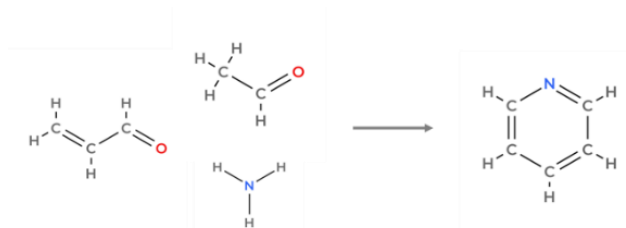


Fig. 3. Proposed mechanism of pyridine formation from acrolein, acetaldehyde and ammonia

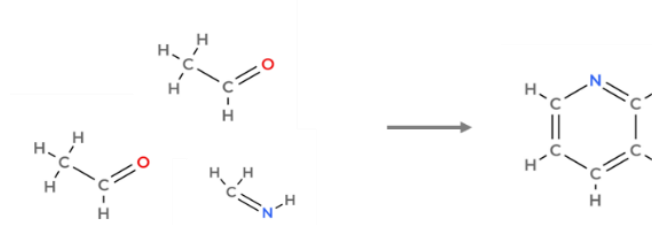


Fig. 4. Proposed mechanism of pyridine formation from acetaldehyde and methylimine.

Conclusions

Model systems of amino acids were thermally treated under different conditions to evaluate the pyridine

formation. Lysine yielded the highest amounts of pyridine, followed by methionine and cysteine. Previously proposed reaction mechanisms have been confirmed considering both the Maillard reaction and the autolysis of N-containing compounds. The present study highlights that trigonelline is not the only pyridine precursors during the coffee roasting process.

References

1. Flament, I., 2001. *Coffee Flavour Chemistry*. Wiley & Sons, New York.
2. Baggenstoss, J., et al. (2008). *J. Agric. Food Chem.* 56(14), 5836-5846.
3. Belgis, M., et al. (2025) *Philippine Journal of Science*, 154(6A), 1495-1506.
4. De Maria et al. (1996). *Food Chemistry*, 55(3), 203-207.
5. Baltes W. and Bochmann G. (1987). *J. Agric. Food Chem.*, Vol. 35, No. 3. 340-346
6. Breitbart, D.J and Nawar, W.W. (1979). *J. Agric. Food Chem.* Vol. 27, No. 3. 511-514

IP2

Recent advancements in mass spectrometry for food analysis: from sample prep to direct analysis

Daniela Peroni

SRA Instruments Spa, Cernusco sul Naviglio (MI), Italy

OR23

Soft chemical ionization and ultra fast isomer separation in food analysis using Vocus high-resolution chemical ionisation ion mobility spectrometry time-of-flight (Vocus HR-CI-IMS-TOF)

Iuliia Khomenko,¹ Andrea Romano,¹ Mehdi Moayed, ^{1, 2} Jiayi Zhang, ^{1, 3, 4} Emanuela Betta,¹ Michele Pedrotti,¹ Matteo Tonzzer,¹ Luca Cappellin,² Franco Biasoli ¹

¹ Fondazione Edmund Mach, San Michele all'Adige, Italy

² Dipartimento di Scienze Chimiche, Università degli studi di Padova, Padua, Italy

³ Dipartimento di Scienza Applicata e Tecnologia (DiSAT), Politecnico di Torino, Turin, Italy

⁴ Dipartimento di Scienze Chimiche e Geologiche, Università di Cagliari, Monserrato, Italy

Summary: *Direct injection mass spectrometry lacks isomer separation. This study evaluates the novel Vocus HR-CI-IMS-TOF platform, combining AIM technology and SLIM-IMS, for real-time food analysis. Three case studies on peach lactones, wine off-flavors, and lipid peroxidation demonstrate enhanced selectivity and high-throughput speed, providing a robust foundation for next-generation online aroma monitoring*

Keywords: *direct injection mass spectrometry, volatile organic compounds, Vocus AIM, SLIM-IMS*

Introduction

In recent decades direct injection mass spectrometry (DIMS) has proved to be a powerful approach for the rapid, real-time analysis of volatile organic compounds (VOCs) in food and agricultural matrices, offering minimal sample preparation and the capacity for high-throughput screening. While DIMS delivers excellent sensitivity, speed, and quantitative performance, its primary limitation is the inability to separate isomers. This can lead to ambiguity in data interpretation, often requiring complementary, time-consuming techniques like gas chromatography for definitive compound identification. To overcome this drawback without compromising DIMS throughput, an ultra-fast separation dimension is required. Recently low-pressure ion mobility spectrometric technology as Structure for Lossless Ion Manipulation (SLIM-IMS) [1] adds a gas-phase separation dimension based on ionic collision cross-section, orthogonal to mass, enabling the separation of isomers in real time without compromising analytical throughput. In this study we performed three case studies in order to evaluate a novel Vocus HR-CI-IMS-ToF instrument (Tofwerk AG, Thun, Switzerland), combining flexible chemical ionisation in both positive and negative modes, including a PTR-MS-like source for the generation of H_3O^+ and NH_4^+ reagent ions, and an AIM source enabling adduct-ion formation chemistry, coupled with ion mobility spectrometry (IMS) and high-resolution time-of-flight detection in food analysis.

Experimental

For all case studies VOCs were monitored using a Vocus High-Resolution Chemical Ionisation Ion Mobility Spectrometry Time-of-Flight (HR-CI-IMS-TOF) mass spectrometer (Tofwerk AG, Thun, Switzerland). As shown in Figure 1 the instrument features a high-resolution time-of-flight mass analyser, providing a mass resolution of 25,000 ($m/\Delta m$). Ion-molecule reactions are carried out within a Vocus AIM (Adduct Ionisation Mechanism) ion-molecule reactor (IMR), which is maintained at a pressure of 50 (± 1) mbar and a temperature of 50°C [2]. The instrument was operated using multiple reagent ion chemistries, including bromide (Br^-), iodide (I^-), acetone-ammonium adducts ($\text{C}_3\text{H}_6\text{O} \cdot \text{NH}_4^+$), acetone dimers ($\text{C}_3\text{H}_6\text{O})_2\text{H}^+$) and benzene cations (C_6H_6^+). For ion mobility-resolved measurements, the Vocus CI-IMS-TOF configuration couples the AIM reactor to a Structures for Lossless Ion Manipulation (SLIM)-based ion mobility spectrometer and a ToF mass spectrometer. This technology allows for extended serpentine paths -such as the 9 m path used here (yellow path in Figure 1) while maintaining a compact instrumentation footprint.

Preliminary and in-depth data extraction and analysis were carried out using TofDaq (Tofwerk AG, version 1.99.1782) and Tofware (Aerodyne Research, Billerica, US, version 4.0.3_IMS) software packages, respectively. Further data analysis and visualization were performed by means of R (R Core Team, R Foundation for Statistical Computing, Vienna, Austria), RStudio Desktop 2025.9.0.387 (Posit Software, PBC, Boston, US), and Python 3.12.13 (Python Software Foundation).

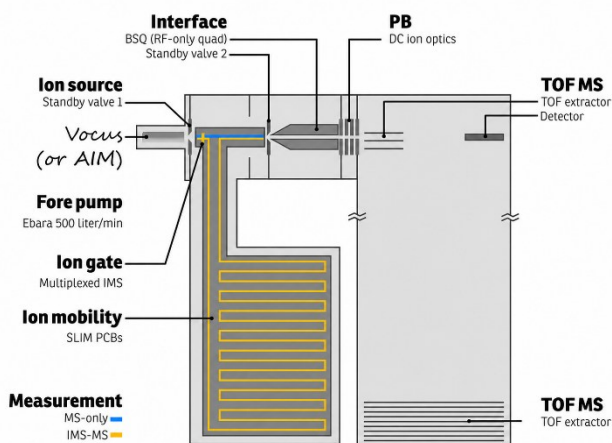


Fig. 1. Schematic representation of the Vocus HR-CI-IMS-TOF instrument

Results

To demonstrate the versatility and analytical potential of the Vocus HR-CI-IMS-TOF this work will demonstrate three case studies where Vocus HR-CI-IMS-TOF was involved into: (i) the real-time separation of structurally related VOC isomers critical to food flavor, such as lactones in peach aroma; (ii) fast and high-throughput monitoring of 4-ethylphenol, a key off-flavour marker in wine, highlighting the system's potential for oenological process control; and (iii) an innovative strategy for investigating lipid peroxidation in food matrices. This latter approach enables the simultaneous detection of radical species, substrate depletion, and oxidation end-products across the full oxidative cascade using a single technique: an approach that may serve as a universal tool for oxidative stability assessment in complex food systems.

Conclusions

This work demonstrates the application of the Vocus HR-CI-IMS-TOF platform – incorporating AIM technology and SLIM-IMS separation – for the soft ionization and detection of food aroma VOCs. This system substantially enhances compound selectivity while retaining the real-time capability and sensitivity of DIMS, opening new analytical avenues in food research. Future perspectives for this workflow include expanding the target panel to additional chemical markers. By bridging the gap between atmospheric instrument science and food technology, this ionization and separation framework provides a robust foundation for the next generation of online aroma analysis.

References

1. Y.M. Ibrahim, A.M. Hamid, L. Deng, S.V.B. Garimella, I.K. Webb, E.S. Baker, R.D. Smith, New frontiers for mass spectrometry based upon structures for lossless ion manipulations, *The Analyst* 142 (2017) pp 1010–1021
2. M. Riva, V. Pospisilova, C. Frege, S. Perrier, P. Bansal, S. Jorga, P. Sturm, J.A. Thornton, U. Rohner, F. Lopez-Hilfiker, Evaluation of a reduced-pressure chemical ion reactor utilizing adduct ionization for the detection of gaseous organic and inorganic species, *Atmospheric Meas. Tech.* 17 (2024) pp 5887–5901

OR24

A rigorous chemometric workflow for multi-year shelf-life prediction of hazelnuts

Sara Tanilli,¹ Andrea Caratti,¹ Giuseppe Genova,² Alex Fissore,² Cristina Casetta,² Angelica Fina,¹ Fulvia Trapani,¹ Erica Liberto,¹ Chiara Cordero¹

¹ Department of Drug Science and Technology, University of Turin, Via Pietro Giuria 9, 10125 Turin, Italy

² Soremartec Italia Srl, Piazzale Ferrero 1, 12051 Alba (Cuneo), Italy

Summary: A GC×GC-based volatilomics approach combined with a rigorous chemometric workflow was employed to predict hazelnut shelf-life across multiple harvesting years. An SVR-based model achieved ~3-month prediction error, demonstrating robust generalizability across years, origins, and storage conditions. Volatiles including aldehydes, alcohols, and fatty acids, were identified as robust aging markers.

Keywords: Hazelnut volatilomics, chemometrics, shelf-life monitoring

Introduction

Shelf-life prediction is a key component of food quality management, supporting quality preservation, waste reduction, and supply chain optimization. In hazelnuts, lipid oxidation and related biochemical processes generate a complex and evolving mixture of volatile compounds during storage, progressively compromising sensory and nutritional quality¹. Accurate shelf-life monitoring is therefore particularly critical. While aldehydes are well-established oxidation markers^{2,3}, expanding the panel of aging indicators may substantially improve shelf-life assessment. Furthermore, volatile profiles are strongly influenced by harvest year and external factors such as geographical origin and storage condition, meaning that predictive models built on single-year datasets are inherently limited in robustness and generalizability.

This study proposes an integrated pipeline combining comprehensive two-dimensional gas chromatography (GC×GC) volatilomics and machine learning to identify robust, multi-year aging markers and develop generalizable shelf-life prediction models for hazelnuts across multiple harvesting years.

Experimental

A total of 624 hazelnut samples from four harvesting years (2020–2023), two geographical origins (Turkey and Italy), and two storage conditions (air and vacuum) were analysed at seven time points over a 24-month storage period.

HS-SPME-GC×GC-MS/FID was employed for the accurate quantification of 43 volatile compounds belonging to six chemical classes: aldehydes, alcohols, ketones, short-chain fatty acids, terpenes, and lactones.

The resulting dataset was processed through a multi-step chemometric workflow in which three harvesting years formed the training set and the fourth was withheld as an independent test set. Exploratory analysis (PCA) was first applied to individual harvesting years to investigate aging trajectories and inter-year variability. Year-specific supervised modelling using Partial Least Squares (PLS) regression then identified relevant aging markers (VIP > 1) for each year. Variables consistently selected across all training set years were retained as consensus markers for the construction of a unified predictive model. Several machine learning algorithms (PLS regression, Random Forest (RF), XGBoost, and Support Vector Regression (SVR)) were compared. Model performance was assessed by Root Mean Square Error (RMSE) in calibration, cross-validation (repeated *k*-fold CV), and external validation on the independent test set.

Results

Statistical analysis revealed marked harvest-year effects: 38 of the 43 quantified volatiles showed significant differences across years at baseline (T₀), underscoring the importance of explicitly accounting for inter-year variability in model development. PCA and PLS applied to individual harvesting years revealed a progressive evolution of the volatilomic profile with storage time, with a pronounced separation emerging from 12 months onward. Representative scores plots illustrating aging trajectories for the 2021 harvest are shown in Figure 1. Year-specific modelling enabled the identification of consensus aging markers shared across training years. The panel of selected markers does not encompass only well-known aldehydes, but it includes also selected alcohols and short-chain fatty acids.

Among the machine learning algorithms evaluated, SVR yielded the best predictive performance. The final model achieved a prediction error of approximately 3 months across calibration, CV, and external validation within a 0-18-month shelf-life window. Seven volatile compounds (hexanal, heptanal, octanal, pentanol, hexanol, octanol, and hexanoic acid) emerged as the most influential and consistent predictors, confirming their potential role as robust, multi-year indicators of hazelnut aging.

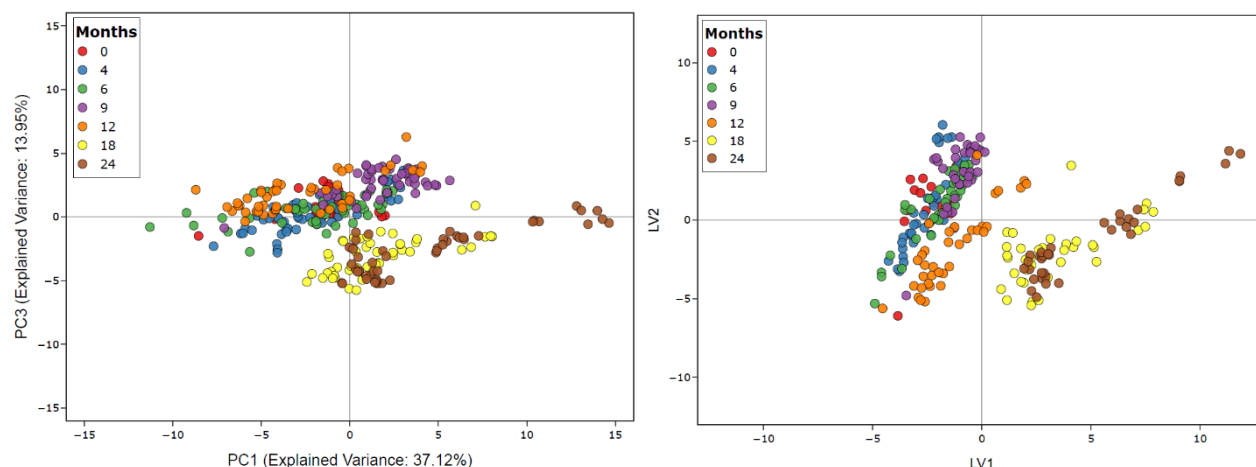


Figure 1. PCA (left) and PLS (right) scores plot showing aging trajectories of samples from year 2021

Conclusions

This study demonstrates that robust, generalizable shelf-life prediction is achievable by combining GC×GC volatilomics with a carefully designed multi-year chemometric strategy. The explicit treatment of harvest-year variability proved essential for developing a robust and generalizable model. The SVR-based model achieved a shelf-life prediction error of approximately three months across a 0-18-month window, representing a practically meaningful level of accuracy for quality management applications. The identified consensus markers, spanning aldehydes, alcohols, and short-chain fatty acids, expand the established panel of hazelnut aging indicators and provide a more comprehensive chemical fingerprint of oxidative deterioration.

Overall, these results support a broader applicability of the proposed pipeline to real-world industrial contexts. The proposed approach could enable a more objective shelf-life monitoring, supporting storage condition optimization, earlier identification of at-risk batches, and thus contributing to more reliable and data-driven quality management strategies in the nut sector.

References

1. Shahidi F. & John J. A., Harris L. editor; *Improving the Safety and Quality of Nuts*, (2013) pp 198-229
2. Squara S., Caratti A., Gavilan F., Bolzoni P., Spigolon N., Genova G., Castello G., Gonzalez M., Cuadros-Rodriguez L., Bicchi C., Cordero C.; *Journal of Food Composition and Analysis*, 114 (2022), Article 104766
3. Kinderlerer J. L. & Johnson S.; *Journal of the Science of Food and Agriculture*, 58 (1992), pp. 89-93
4. Squara S., Caratti A., Fina A., Liberto E., Spigolon N., Genova G., Castello G., Cincera I., Bicchi C., Cordero C.; *Journal of Chromatography A*, 1700 (2023), Article 464041

OR25

The use of the untargeted approach by HPLC-Q-ToF to study the chemical profile of different matrices

L. Acquaticci, S. Angeloni, M. Ricciutelli, G. Caprioli

University of Camerino, School of Pharmacy, Chemistry Interdisciplinary Project (ChIP)

Summary: *In this study, a UHPLC-QToF-MS/MS approach was used to study the chemical profile of acorn flour and river water to characterize and find new interesting molecules. Both automated iterative data-dependent (DDA) and standard full-scan modes were used for the analysis and data were processed using MS-DIAL software.*

Keywords: *untargeted analysis, acorn flour, river water.*

Introduction

Recently, the use of liquid chromatography (HPLC) coupled to mass spectrometry (MS/MS) is continuously increasing due to the high technological improvements made in this field. In fact, the use of targeted analysis to detect and quantify specific compounds has been accompanied by the advent of untargeted analysis. In particular, high resolution mass spectrometry, such as UHPLC-QToF, is used for untargeted metabolomics analysis in different matrices [1,2] or disease diagnosis [3,4]. In this study, a ultra high-performance liquid chromatography-quadrupole time of flight-mass spectrometry (UHPLC-QToF-MS) untargeted approach was used to study the chemical composition of acorn flour and river water in order to characterize and find new interesting compounds.

Experimental

Acorn flour was purchased from Dary Nature (Koryciny, Poland; <https://darynature.pl/>) and it is composed of dried and ground organic acorns of the pedunculate oak (*Q. robur*), as declared in the product's technical specifications, while two water samples were collected in a river of the Marche region, in two different levels. Acorn sample was extracted with 80% frosted methanol and put in the freezer for 1 hour; after that the supernatant was collected, filtered and injected. Water samples were concentrated using an Oasis HLB Cartridge; in particular, 1L of water was dried on a cartridge, washed with methanol and the eluate was dried under nitrogen and the residue was dissolved in 250 µl of methanol.

Samples were analyzed by UHPLC-QTOF in both automated iterative data-dependent (DDA) and standard full-scan modes. Data processing, including peak peaking, alignment, integration, and annotation, was performed using MS-DIAL software. Spectral matching against the NIST 2020 high-resolution tandem mass spectrometry (MS/MS) library was used for compound identification.

Results

The untargeted analysis in acorn flour sample resulted in more than twenty thousands features from the positive and negative acquisitions. A total of 296 molecules were annotated, setting a total identification score cut off at 60%. Among them, 42 compounds had a total identification score higher than 90%. Most of the compounds are gallic acid derivatives, known for their important biological functions, such as radical scavenging, interference with cell signaling pathways or induction of cancer cell apoptosis. The most interesting molecules will be confirmed and quantified by analytical standards to find possible applications in nutraceutical, pharmaceutical and cosmeceutical fields.

A total of 42 compounds were annotated in positive and negative ionization modes with a total identification score cut-off of 70%. Among them, some compounds are natural compounds derived from algae, plants, fungi and bacteria; other compounds are anthropogenic contaminants from cosmetics, detergents, plastics (like erucamide), industries and drugs; fecal and urban biomarkers (like cholesterol derivatives) associated with domestic and livestock wastewater and aquaculture tracers related to feed and trout farms (like daidzein) were found.

Conclusions

The use of UHPLC-QToF coupled to mass spectrometry (UHPLC-QToF-MS/MS) represents a promising analytical tool not only for metabolomic studies but also to characterize food products (such as acorn flour) and to find new emerging contaminants or bioactive compounds in different matrices (such as river water).

References

1. Jian-Fei, X., LIANG, Q. L., Ping, H. U., Yi-Ming, W., Guo-An, L. (2009). Recent trends in strategies and methodologies for metabonomics. *Chinese Journal of Analytical Chemistry*, 37(1), 136-143.
2. Guodong, R., Xiaoxia, L., Weiwei, Z., Wenjun, W., & Jianguo, Z. (2017). Metabolomics reveals variation and correlation among different tissues of olive (*Olea europaea* L.). *Biology Open*, 6(9), 1317-1323.
3. Zhou, X., Liu, L., Lan, X., Cohen, D., Zhang, Y., Ravindran, A. V., ... Xie, P. (2019). Polyunsaturated fatty acids metabolism, purine metabolism and inosine as potential independent diagnostic biomarkers for major depressive disorder in children and adolescents. *Molecular psychiatry*, 24(10), 1478-1488.
4. Trainor, P. J., Yampolskiy, R. V., DeFilippis, A. P. (2018). Wisdom of artificial crowds feature selection in untargeted metabolomics: An application to the development of a blood-based diagnostic test for thrombotic myocardial infarction. *Journal of biomedical informatics*, 81, 53-6

OR26

Exploring the phytochemical profile and biological potential of artichoke petal, leaf and stem extracts

Lorenza Marinaccio,¹ Eulogio J. Llorent-Martínez,² Gokhan Zengin,³ Angelo Cichelli,¹ Adriano Mollica,^{4,5}
Azzurra Stefanucci^{4,5}

¹ Department of Innovative Technologies in Medicine and Dentistry, “G. d’Annunzio” University of Chieti-Pescara, 66100 Chieti, Italy

² Department of Physical and Analytical Chemistry, University of Jaén, Campus Las Lagunillas S/N, E-23071 Jaén, Spain

³ Department of Biology, Selcuk University, 42130, Turkey

⁴ Department of Pharmacy, “G. d’Annunzio” University of Chieti-Pescara, 66100 Chieti, Italy

⁵ SCM Nutraceutici Universitari Srl, Strada degli Oliveti 73, 66100 Chieti, Italy

Summary: *Extracts from Cupello artichoke petals, stems and leaves were analysed using HPLC-MS/MS, revealing caffeoylquinic and dicaffeoylquinic acids as major compounds. UAE and MAE leaf and stem extracts (ethanol:water 70:30) exhibited superior antioxidant capacity in spectrophotometric assays. Additionally, the extracts demonstrated strong tyrosinase inhibitory activity, supporting their potential for nutraceutical applications.*

Keywords: food by-products, antioxidants, HPLC-MS/MS

Introduction

The industrial artichoke processing produces a large amount of waste, approximately 80–85%, which is not suitable for dietary use [1]. This study aimed to valorise Cupello artichoke by-products (Abruzzo, Italy) comparing the phytochemical profiles and biological activities of extracts from the petals (edible part), stems and leaves (by-products).

Experimental

The artichoke extracts were obtained using decoction (DC), ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE). An ethanol-water mixture (70:30, v/v) was used as the extraction solvent for all techniques, with pure ethanol being additionally tested solely for the UAE method. The phytochemical profile was evaluated by HPLC-MS/MS analysis. MS detection was conducted through an Orbitrap IQ-X Tribrid Mass Spectrometer coupled to a Vanquish Neo liquid chromatograph. Electrospray ionization was operated in negative ion mode. HPLC separation was performed using a Luna Omega Polar C18 column (150 x 1.0 mm, 1.6 µm). The antioxidant activity was determined by DPPH, ABTS, CUPRAC, FRAP, metal chelating ability and phosphomolybdenum assays. The enzyme inhibitory effects were evaluated against AChE, BChE, α-glucosidase, α-amylase and tyrosinase through colorimetric assays.

Results

The artichoke extracts (leaf, stem and petal) were obtained using green extraction techniques (UAE and MAE) and a classical one (DC).

The identification of the phytochemical compounds was conducted by HPLC-MS/MS analysis using analytical standards, high-resolution mass spectrometry data, ChemSpider and mzCloud databases, and bibliographic data. Several phenolic acids and flavonoids have been identified or tentatively identified, in particular chlorogenic acid, neochlorogenic acid and dicaffeoylquinic acids.

In terms of biological activities, UAE and MAE of leaves and stems (ethanol-water 70:30, v/v) provided the highest antioxidant capacity, while tyrosinase inhibition was the most pronounced effect among all enzyme assays evaluated.

Conclusion

Cupello artichoke by-products (leaves and stems) displayed strong antioxidant and tyrosinase inhibitory activities, making them promising raw materials for novel nutraceutical formulations.

References

1. M. Francavilla, M. Marone, P. Marasco, F. Contillo, M. Monteleone; *Foods*, 10 (2021), 112.

OR27

A comprehensive characterization of anthocyanins in fungal-resistant red grape varieties by high-resolution mass spectrometry

M. De Rosso,¹ *S. Soligo*,² *E. Piutti*,² *A. Piccinin*,³ *D. Piccinin*,³ *R. Carraro*,¹ *L. Tarricone*,¹
G. Masi,¹ *S. Roccotelli*,¹ *M. Gardiman*,¹ *A. Panighel*,¹ *F. Tarricone*,¹ *L. Sansone*,¹ *R. Flamini*¹

¹ Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria (CREA)
Viale XXVIII Aprile, 26 – 31015 Conegliano (TV), Italy

² Veneto Agricoltura, Agenzia Veneta per l'Innovazione nel Settore Primario
Viale dell'Università, 14 - 35020 Legnaro (PD), Italy

³ Le Carline, Via Carline, 24 - 30020 Pramaggiore (VE), Italy

Summary: *Many fungal-tolerant vine varieties are suitable for production of high-quality wines. UHPLC/QTOF characterization of anthocyanins of nine red grape varieties identified up to 26 anthocyanins. Five varieties had both monoglucosides and diglucosides, four exhibited only monoglucosides. Knowledge of anthocyanin composition is essential in optimizing winemaking and wine refining conditions.*

Keywords: *anthocyanins, fungal disease, resistant grape varieties, UHPLC/QTOF, mass spectrometry*

Introduction

Anthocyanins represent a significant class of natural pigments responsible for the coloration of red berry grapes and wines. Numerous fungus-resistant grape varieties have been produced through successive crossing experiments involving *V. vinifera* and various Asian and American *Vitis* species. Specific genotypes have exhibited considerable tolerance to *P. viticola* and powdery mildew, which are the predominant fungal diseases impacting vines. Generally, these varieties require minimal treatments with low doses of sulfur- and copper-based products and yield grapes that are suitable for production of high-quality wine. The complex composition of skin grape anthocyanins, which imparts color to red grapes and their wines, is primarily governed by genetic factors. In *V. vinifera* varieties, the predominant anthocyanidins are delphinidin (Dp), cyanidin (Cy), petunidin (Pt), peonidin (Pn), and malvidin (Mv) present exclusively as 3-O-monoglucosides together with their acylated derivatives. Instead, resistant hybrids may also contain 3,5-O-diglucoside anthocyanins and their profile is studied to distinguish between *V. vinifera* and hybrid grape varieties.[1]

Experimental

The present study undertakes a thorough UHPLC/QTOF characterization of anthocyanins from fungal-resistant red grape varieties, employing a methodology that has recently been used to investigate anthocyanins also in blueberry and raspberry.[2] For this purpose, various red hybrid grape varieties were analyzed, including Cabernet Volos, Cabernet Cortis, Prior, Roesler, Devico, Pinot Nova, Monarch, Volturnis, and Pinot Kors.

Results

Some of the studied varieties are distinguished by the presence of up to 26 anthocyanins, five of which consist of both monoglucosides and diglucosides, along with their acetyl and *p*-coumaroyl glucoside derivatives. In contrast, four varieties exhibited only monoglucosides. During the oxidative process such as wood barrel aging, the wine color stabilization is affected differently by the two classes of anthocyanins. Diglucosides mainly undergo aglycone oxidation, instead in the case of monoglucosides the formation of pyranoanthocyanins is allowed by the free C5-position and polymerization with flavanols is favoured by lower steric hindrance.[3] The oxidation process, such as during wood barrel aging, influences wine color stabilization differently across the two classes of anthocyanins. Diglucosides primarily undergo aglycone oxidation, whereas in monoglucosides the formation of pyranoanthocyanins is allowed by the free C5-position, and the polymerization with flavanols being enhanced by lower steric hindrance.

Conclusions

Knowledge of anthocyanin composition is essential in optimizing winemaking and wine refining conditions, e.g., skin extraction duration, wood barrel type, size, and time aging, plan the color stabilization techniques may be used (e.g., micro-oxygenation).

References

1. M. De Rosso, L. Tonidandel, R. Larcher, G. Nicolini, V. Ruggeri, A. Dalla Vedova, F. De Marchi, M. Gardiman, R. Flamini; *Analytica Chimica Acta*, 732 (2012), pp 120-129.
2. M. De Rosso, K. Muniyandi, K. Kumar, T. N'gambi, N. Dai, G. Tamir, R. Carraro, L. Tarricone, G. Masi, S. Roccotelli, M. Gardiman, A. Panighel, L. Sansone, I. Maoz, R. Flamini; *Journal of Mass Spectrometry*, 61 (2026), e70030.
3. M. De Rosso, M. Gardiman, R. Carraro, A. Panighel, F. Fagherazzi, L. Sansone, T. Roman, L. Vettori, R. Flamini; *Journal of Agricultural and Food Chemistry*, 72 (2024), pp 7383-7396.

Financial support: Project VITIVOLUTION: Lo sviluppo di tecniche innovative per l'evoluzione del sistema vitivinicolo Veneto. Duration 2025-2027.

OR28

Identification of carboxymethylcellulose (CMC) and potassium polyaspartate (KPA) in wine through parallel targeted enzymatic digestion workflows and UHPLC-HRMS*Francesca Martinelli, Emiliano Fadanelli, Andrea Rosolen, Roberto Larcher, Tiziana Nardin*

Fondazione Edmund Mach, San Michele all'Adige (TN), Italy

Summary: A novel analytical strategy based on parallel enzymatic depolymerization and HILIC-UHPLC-HRMS enables the identification of CMC and KPA in wine. Endo- 1,4 – β glucanase generates specific carboxymethyl-cello-oligosaccharides (DP2–DP6), while Proteinase K produces Asp₂₋₅ oligomers. This approach overcomes conventional matrix interference, providing a robust tool for wine quality control.

Keywords: Carboxymethylcellulose, Potassium Polyaspartate, Wine Tartaric Stabilization, Enzymatic Assays, UHPLC-Orbitrap HRMS

The widespread adoption of protective colloidal polymers, such as sodium carboxymethylcellulose (CMC) [1] and potassium polyaspartate (KPA) [2], has revolutionized tartaric stabilization in modern winemaking by offering sustainable, low-energy alternatives to traditional physical methods [3]. The International Organisation of Vine and Wine (OIV) allows the use of these additives within specified maximum limits and outlines official methods for their quantification [4,5]. However, these methods are time and material consuming, suffer from high limit of detection, lack accuracy and repeatability, and yield results that are highly dependent on operator expertise. On the other hand, conventional chromatographic and spectroscopic techniques often fail to characterize these analytes due to their high molecular weight, structural heterogeneity, and interference from complex native wine matrix components—including grape proteins and structural polysaccharides. In this study, we present two distinct, parallel analytical workflows based on targeted enzymatic depolymerization optimized individually for CMC and KPA, both coupled with high-resolution Orbitrap mass spectrometry (UHPLC-HRMS).

For CMC analysis an incubation with a purified recombinant endo-1,4- β glucanase was carried out, yielding unique fingerprint of carboxymethyl-cello oligosaccharides with a degree of polymerization ranging mainly from DP2 to DP6 and varying degrees of carboxymethylation (Table 1). These diagnostic oligomers are completely absent in native wine glucans, allowing to identify and possibly quantify the addition of oenological CMC. The KPA analysis isolates KPA through cleavage with a proteinase K, depolymerizing the synthetic polyamino acid backbone into characteristic oligomer fragments Asp_n where n = 2-5 (Table 2).

The resulting digests from both parallel assays are subsequently analysed using Chromatographic separation with a Thermo Scientific Ultimate 3000 UHPLC system equipped with an Agilent 300 HILIC (Hydrophilic Interaction Liquid Chromatography) RRHD 1.8 μ m, 2.1 x 100mm column. All the chromatograms were recorded in profile mode through a full MS-data dependent MS/MS experiment employing a Q-Exactive™ hybrid quadrupole-orbitrap mass spectrometer (HQOMS) equipped with heated electrospray ionization (HESI-II) interface. Both methods demonstrated high capability in successfully detecting CMC and KPA additions in both white and red wines, offering a promising, high-throughput tool for quality control, regulatory enforcement, and authenticity testing within the global wine sector.

Table 1. CMC derived carboxymethyl-cello oligosaccharides and relative masses

Oligomer	Chemical formula	Neutral monoisotopic mass (Da)	Ionization (m/z)	[M-H] ⁻
DP2 CM1	C ₁₄ H ₂₄ O ₁₃	400.1217	399.1144	
DP2 CM2	C ₁₆ H ₂₆ O ₁₅	458.1272	457.1199	
DP3 CM1	C ₂₀ H ₃₄ O ₁₈	562.1745	561.1672	
DP3 CM2	C ₂₂ H ₃₆ O ₂₀	620.1800	619.1727	
DP3 CM3	C ₂₄ H ₃₈ O ₂₂	678.1855	677.1782	
DP4 CM1	C ₂₆ H ₄₄ O ₂₃	724.2273	723.2200	
DP4 CM2	C ₂₈ H ₄₆ O ₂₅	782.2328	781.2255	
DP4 CM3	C ₃₀ H ₄₈ O ₂₇	840.2383	839.2310	
DP5 CM2	C ₃₄ H ₅₆ O ₃₀	944.2856	943.2783	
DP5 CM3	C ₃₆ H ₅₈ O ₃₂	1002.2911	1001.2838	
DP6 CM2	C ₄₀ H ₆₆ O ₃₅	1106.3384	1105.3311	
DP6 CM3	C ₄₂ H ₆₈ O ₃₇	1164.3439	1163.3366	

Table 2. KPA derived oligomers and relative masses

Oligomer	Chemical formula	Neutral monoisotopic mass (Da)	Ionization [M-H] ⁻ (m/z)
Asp ₂ (Di-Aspartate)	C ₈ H ₁₂ N ₂ O ₇	248.06447	247.05720
Asp ₃ (Tri-Aspartate)	C ₁₂ H ₁₇ N ₃ O ₁₀	363.09140	362.08412
Asp ₄ (Tetra-Aspartate)	C ₁₆ H ₂₂ N ₄ O ₁₃	478.11834	477.11106
Asp ₅ (Penta-Aspartate)	C ₂₀ H ₂₇ N ₅ O ₁₆	593.14528	592.13800

References

1. A. Bosso, D. Salmaso, E. de Faveri, M. Guaita, D. Franceschi; *Vitis*, 49 (2010), pp 95-99.
2. A. Bosso, S. Motta, L. Panero, M. Petrozziello, A. Asproudi, R. Lopez, M. Guaita; *OENO One*, 54 (2020), pp 15-26.
3. M.P. Martínez-Pérez, A.B. Bautista-Ortín, V. Durant, E. Gómez-Plaza; *Foods*, 9 (2020), p. 1275.
4. International Organisation of Vine and Wine (OIV); International Code of Oenological Practices, Resolutions OIV-OENO 366-2009 & OIV-OENO 543-2016 (2009, 2016).
5. International Organisation of Vine and Wine (OIV); *Compendium of International Methods of Analysis of Wines and Musts*, Methods OIV-MA-AS315-22 (Resolution OIV-OENO 404-2010) & OIV-MA-AS323-11 (Resolution OIV-OENO 619- 2019) (2010, 2019).

OR29

integrated untargeted metabolomics workflow enables comprehensive metabolic profiling of *Lepidium sativum* microgreens and vegetative plants

Francesca Monzillo,¹ Luigi d'Aquino,² Vincen Wu,³ Nicola Zamboni,³ Sonia Piacente,¹ Paola Montoro¹

¹ Department of Pharmacy, University of Salerno, Via Giovanni Paolo II, 84084 Fisciano, Italy

² Italian National Agency for New Technologies Energy and Sustainable Economic Development (ENEA),
Casaccia Research Centre, Via Anguillarese 301
Santa Maria di Galeria, 00060 Roma, Italy

³ Institute of Molecular Systems Biology, ETH Zurich, Otto-Stern-Weg 3, Zurich 8093, Switzerland

Summary: *An innovative untargeted metabolomics workflow combining rapid mixed-mode LC–HRMS/MS, automated data processing, and advanced computational annotation was developed. Applied to *Lepidium sativum*, it revealed extensive developmental metabolic reprogramming and identified rare imidazole alkaloids as a distinctive metabolic signature of the microgreen stage.*

Keywords: *Untargeted metabolomics, Microgreens, Alkaloids.*

Introduction

Untargeted metabolomics relies on robust and efficient analytical workflows capable of addressing the chemical diversity and complexity of food matrices while supporting high-throughput analyses [1]. In this study, a rapid mixed-mode liquid chromatography method, coupled with high-resolution tandem mass spectrometry (LC–HRMS/MS), was integrated with automated data processing to ensure scalability, sensitivity, reproducibility and speed. Metabolite annotation was performed using a complementary multi-platform strategy to enable comprehensive metabolic profiling and improve annotation confidence. The workflow was applied to compare the metabolomes of microgreens and vegetative plants of *Lepidium sativum* (garden cress), a Brassicaceae species of growing nutraceutical interest owing to its rich content of bioactive secondary metabolites, with the aim of investigating development-related metabolic changes. Plants were cultivated under controlled environmental conditions to minimize experimental variability and ensure reproducible metabolic profiles.

Experimental

Plants were cultivated using an innovative fan-lamp cultivation system based on a conventional root substrate, precision lighting and controlled ventilation. Microgreen samples (M) were harvested 9 days after germination, when plants displayed two fully expanded true leaves plus cotyledons, whereas vegetative plants (V) were harvested 28 days after germination.

After collection, fresh tissues were immediately quenched in liquid nitrogen to prevent enzymatic degradation. Metabolites were extracted by ultrasound-assisted extraction (UAE) using ethanol/water (7:3, v/v). Extracts were then dried under a gentle nitrogen stream and reconstituted in methanol/water (7:3, v/v) to a final concentration of 1 mg/mL.

LC–HRMS/MS analyses were performed using an Agilent Infinity 1290 II LC system equipped with a quaternary pump, autosampler, and column oven. Separation was achieved using an Atlantis BEH C18 AX column (1.7 μ m, 2.1 $\times$ 30 mm), integrating reversed-phase and anion-exchange retention mechanisms. The mobile phases consisted of (A) 5 μ M medronic acid in water, (B) 0.1% formic acid in methanol, and (C) 5 mM ammonium formate with 0.1% formic acid in water. The flow rate was maintained at 1.2 mL min⁻¹, with a total chromatographic run time of 4 min. Mass spectrometric detection was performed using a SCIEX ZenoTOF 8600 system equipped with an electrospray ionization source operating in negative mode. Data were acquired in data-dependent acquisition (DDA) mode over an *m/z* range of 60–1000 with a collision energy of 40.

Raw data were processed using the MASter Wizard module, which enables automated LC–MS data processing from raw files to final datasets through intelligent parameter optimization and batch processing. Metabolite annotation was performed using SIRIUS, a Java-based open-source software for small-molecule identification from MS/MS data. The output was further processed using TIMA, which combines chemical and biological context through a meta-scoring system based on spectral similarity and molecular fingerprints, taxonomic distance, structural consistency within clusters, and physicochemical properties. A dedicated Python workflow was used to selectively identify glucosinolates. Finally, multivariate statistical analysis of the full intensity matrix was performed using the MetaboAnalyst web platform to investigate metabolic differences between developmental stages.

Results

The integrated workflow detected 8,493 spectral features, from which 264 metabolites were putatively annotated using the complementary SIRIUS–TIMA annotation strategy. Alkyl glucosinolates represented the predominant class of annotated secondary metabolites, followed by carboxylic acids and their derivatives. The dedicated Python workflow enabled the annotation of six glucosinolates, four of which were assigned a unique structural candidate, whereas the remaining two were annotated as hydroxyglucobrassicin isomers.

Principal component analysis revealed a clear separation between microgreens and vegetative plants, indicating extensive metabolic reprogramming during development. Heatmap visualization and univariate statistical analysis further confirmed a significantly higher accumulation of glucosinolates in microgreens.

Notably, volcano plot analysis revealed that imidazole alkaloids (lepidines), rather than glucosinolates, were the metabolites most strongly driving the separation between developmental stages. Seven lepidines were among the most discriminant metabolites, identifying this poorly explored alkaloid class as a distinctive metabolic signature of *Lepidium sativum* microgreens. Two discriminant features were putatively annotated as structural isomers of Lepidine C based on their MS/MS fragmentation patterns (Fig. 1). The complementary use of SIRIUS and TIMA substantially strengthened confidence in the annotation for this rare class of alkaloids.

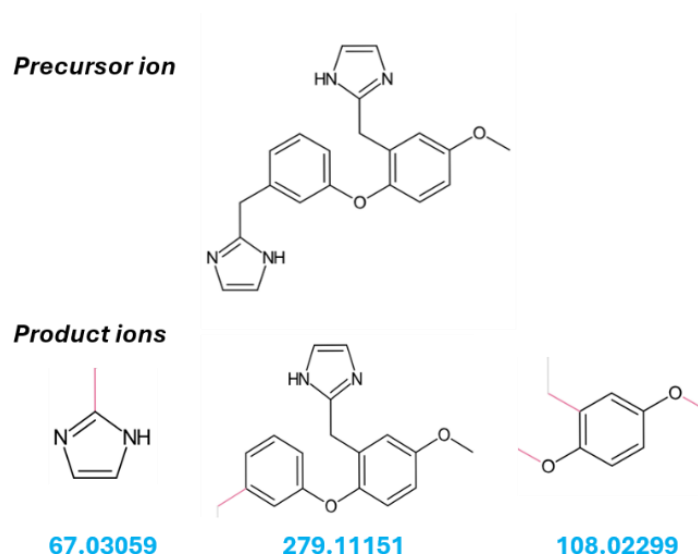


Fig. 3. Lepidine C and its fragmentation pathways

Conclusions

The integrated workflow combines rapid mixed-mode LC–HRMS/MS, automated data processing, and complementary computational annotation into a scalable platform for comprehensive untargeted metabolomics. Applied to *Lepidium sativum*, it not only confirmed the enrichment of glucosinolates during the microgreen stage but also revealed lepidines as key discriminant metabolites of the microgreen stage, expanding the current phytochemical knowledge of this relevant species for nutraceutical use. These findings demonstrate the value of integrated computational annotation strategies for uncovering biologically meaningful metabolites in complex food matrices thereby improving the interpretation of metabolomic datasets and expanding our understanding of food chemical composition.

References

1. Correia, M. S. P., Othman, A., Zamboni, N. The Analyst, 150 (2025), pp 4955–4961.
2. Monzillo, F., Della Mura, B., Matarazzo, C., Crescenzi, M. A., Piacente, S., D'Aquino, L., Cozzolino, R., Montoro, P. Foods, 14 (2025), pp 4148.

OR30

Multiplatform profiling of Maillard reaction products in PEF-treated nuts

Laura Alessandroni,¹ Albert Sebastià,² Emilia Ferrer,² Francisco J. Barba,² Gianni Sagratini¹

¹ Chemistry Interdisciplinary Project (ChIP), School of Pharmacy, University of Camerino
62032 Camerino, Italy

² Research Group in Innovative Technologies for Sustainable Food (ALISOST), Department of Preventive Medicine and Public Health, Food Science, Toxicology and Forensic Medicine, Faculty of Pharmacy and Food Sciences, Universitat de València, Avenida Vicent Andrés Estellés, 22, 46100 Burjassot, València, Spain

Summary: Pulsed Electric Field (PEF) were investigated as a pre-treatment to modulate the formation of Maillard reaction products in roasted nuts. Complementary analytical techniques, including HPLC-DAD, HPLC-MS/MS and GC-MS, were applied to simultaneously evaluate 5-hydroxymethylfurfural, acrylamide and furan derivatives across different nut matrices.

Keywords: Pulsed Electric Field, Maillard reaction products, Roasted nuts

Introduction

Roasting process enhances the sensory quality of nuts but also promotes the formation of specific toxic products generated through the Maillard reaction [1]. Since these compounds originate from related reaction pathways, their simultaneous determination is essential for evaluating the effectiveness of innovative mitigation strategies. Pulsed Electric Fields (PEF) have recently attracted interest as a non-thermal pre-treatment capable of modifying food microstructure and potentially reducing contaminant formation during roasting [2]. The aim of this research work is to investigate the impact of PEF pre-roasting treatment analysing Maillard reaction products in different roasted nuts.

Experimental

Almonds, hazelnuts, and cashews were subjected to PEF pre-treatment at an electric field strength of 3 kV cm⁻¹ and a specific energy input of 300 kJ kg⁻¹. For each trial, 40 g of nuts were immersed in 200 mL of Milli-Q water and treated using a PEF-Cellcrack III system (ELEA, Quakenbrück, Germany) prior to roasting; untreated samples served as controls. Subsequently, the nuts (40 g) were roasted in an electric coffee bean roaster (JIAWANSUN, JMS-209) at 160 °C for 20 min. Not roasted natural samples were analysed as well. 5-HMF was quantified by HPLC-DAD (Infinity 1260, Agilent Technologies), acrylamide by HPLC-MS/MS using an isotopically labelled internal standard (Triple Quadrupole 6420, Agilent Technologies), and volatile furan compounds by GC-MS (8890, Agilent Technologies) according to previously published analytical methods [1]. The obtained analytical data were used to compare contaminant formation in nut matrices among processing conditions through one-way ANOVA test.

Results

Almonds, hazelnuts, and cashews were analysed after roasting. Figure 1 reports the analytes concentration comparison between PEF-treated and untreated samples.

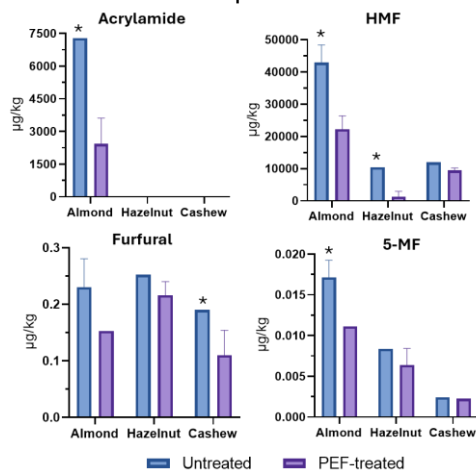


Fig. 1. Concentration of the monitored analytes in PEF-treated compared to untreated samples. * indicates statistically significant difference between PEF-treated and untreated sample of the same matrix.

Acrylamide was detected just in roasted almonds, with statistically higher values in not-PEF-treated samples. Similar trend was registered for HMF in hazelnut and almonds with HMF and for furfural in treated and not-treated cashew.

According to the main focus of this study, after roasting, the data of PEF-treated samples were compared with untreated ones. Figure 2 shows the percentage reduction of analyte concentrations relative to the untreated samples, which were set as 100%. Overall, results clearly indicate that the Maillard reaction product reduction is strongly matrix-dependent.

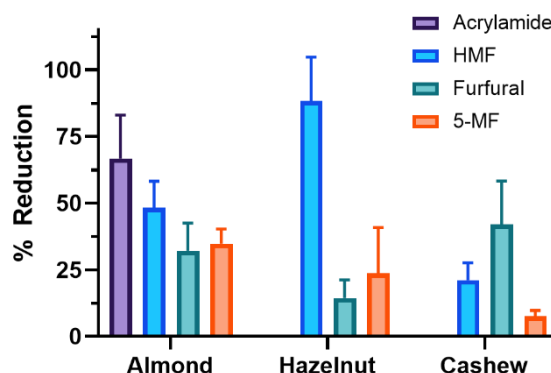


Fig. 2. Quantitative reduction percentage of the monitored analytes in PEF-treated samples after roasting compared to untreated (%)

PEF pre-treatment reduced around the 70% of acrylamide in roasted almonds while the highest reduction was the one of HMF in hazelnut.. No significant differences were observed in the concentrations of the other furanic compounds between PEF-treated and untreated samples. This may be explained by their formation pathways, which are less directly associated with the Maillard reaction and more closely related to storage and oxidative degradation [1].

Conclusions

In this study, PEF treatment emerged to be a possible strategy to reduce Maillard reaction products in roasted nuts, in particular for acrylamide in almonds. Regarding the methods, the integration of complementary chromatographic techniques enables the simultaneous evaluation of chemically diverse structures. This integrated analytical approach provides a comprehensive framework for evaluating the effects of emerging food processing technologies on the formation of Maillard reaction contaminants.

References

1. L. Acquaticci, S. Angeloni, N. Cela, F. Galgano, S. Vittori, G. Caprioli, & N. Condelli; *Food control*, 149 (2023), 109714.
2. J. Li, Y.W. Huang, Y. T. Li, S. Y. Luo, W.Y. Xiong, J.H. Wang, X.A. Zeng, *Food Research International* (2026), 118783.

PL4**Chemically grounded ai for untargeted food profiling***Rasmus Bro*

University of Copenhagen, Faculty of Science, Frederiksberg, Denmark

Untargeted profiling has become an indispensable tool for food science, from understanding flavor chemistry to detecting fraud and ensuring authenticity. Yet the sheer complexity of untargeted GC–MS and LC–MS data often makes data analysis the bottleneck. Over the past three decades, chemometrics has developed as a form of artificial intelligence rooted in chemistry: it provides robust, interpretable ways of transforming complex data into actionable knowledge.

In this lecture I will trace the journey from chemometrics to AI in the context of untargeted profiling in food science. I will show how multi-way methods such as PARAFAC and PARAFAC2 laid the foundation for turning chromatographic “mountains” into resolved chemical profiles, and how these ideas evolved into practical tools such as PARADISE, now widely used to automate the analysis of untargeted GC–MS datasets. Such approaches demonstrate that intelligent data analysis is not only about prediction accuracy, but also about transparency, reproducibility, and scientific trust.

Complex food MS data should not remain an obstacle. With the right AI — chemometrics-driven and chemically grounded — it becomes a driver for innovation.

OR31

LC-HRMSMS profiling of bioactive lipids and triterpenoids in pumpkin seeds

Annunziata Paolillo,^{1,2} Assunta Napolitano,¹ Milena Masullo,¹ Sonia Piacente¹

¹ Dipartimento di Farmacia, Università degli Studi di Salerno, via Giovanni Paolo II n. 132
84084 Fisciano (SA), Italy

² PhD Program in Drug Discovery and Development, Università degli Studi di Salerno, via Giovanni Paolo II
n. 132, 84084 Fisciano, SA, Italy

Summary: Pumpkin seeds (*Cucurbita pepo* var. *styriaca*) are becoming increasingly appreciated as functional foods for their nutritional benefits. LC-HRMSMS analysis of the ethanolic extract revealed various polar and neutral lipids, including sphingolipids. Notably, Cer-(EO)LCBs were identified, representing a real novelty for pumpkin, along with a good number of multiflorane-type triterpenoids.

Keywords: Sphingolipids; triterpenoids; LC-HRMS/MS analysis.

Introduction

In Austria, the special Styrian pumpkin variety (*Cucurbita pepo* var. *styriaca*) has been cultivated for decades and the conditioned green-colored oil obtained from its seeds is particularly renowned for its sensory and optical characteristics. Recent studies have described this oil as a topical, alternative anti-inflammatory agent to treat several inflammatory skin disorders [1]. Not only the oil, also eco-friendly hydroalcoholic pumpkin seed extracts have been associated with beneficial health effects, like the ability to inhibit the proliferation of prostate-, breast- and colon cancer cells but also hyperplastic cells, corroborating the ethnomedical use of pumpkin seeds as accompanying remedy for the treatment of benign prostate hyperplasia [2].

From a chemical point of view, the most studied lipid class for Styrian pumpkin seeds is represented by free fatty acids (FAs), like linoleic (ω -6) and oleic (ω -9) acids. Few information are available on other lipid classes, such as those belonging to the group of polar lipids, e.g. phospholipids, glycolipids, and sphingolipids, that, as well as being natural carriers of essential FAs composing their structures, are gaining growing interest from the scientific community due to their nutritional importance and bioactivities.

This study aims at gaining deep insights that can extend the state of the art on the lipid composition of Styrian pumpkin seeds, including, where possible, unusual subclasses, analyzing its alcoholic extract by a liquid chromatography coupled to high-resolution tandem mass spectrometry (LC-HRMSMS) method. The obtained results highlighted the occurrence of numerous compounds belonging to different classes of polar and neutral lipids, such as phospholipids, sphingolipids, glycolipids, acylglycerols, and oxylipins. Noteworthy, a significant presence of Cer-(EO)LCBs, i.e. Cer- EOS-type ceramides with different long chain base (LCB) and fatty acid composition, was detected, representing a real novelty for pumpkin, along with specialized metabolites such as the multiflorane-type triterpenoids, some of which never described before in pumpkin.

Experimental

Pumpkin seeds were extracted by maceration using ethanol at room temperature in the dark (in triplicate). The ethanolic extracts of the seeds of *Cucurbita pepo* var. *styriaca* were analyzed by LC-ESI/HRMS, using a Thermo Ultimate RS 3000 ultrahigh-performance liquid chromatography (UHPLC) system coupled to a Q-Exactive high-resolution mass spectrometer equipped with a hybrid quadrupole-Orbitrap analyzer and HESI II heated electrospray ionization source, operating in positive and negative ionization modes. Chromatographic separation was performed using a Symmetry 300 C-4 column, a flow rate of 0.2 mL/min, and a mobile phase consisting of 0.1% formic acid in water (v/v) as solvent A and 0.1% formic acid in acetonitrile (v/v) as solvent B. The MS spectra were acquired in a mass range m/z 150-1400 with a resolving power of 70000. To obtain HRMS/MS spectra a "data dependent scan" experiment was performed, in which the first five intense ions in the HRMS spectrum were selected to be subjected to fragmentation, using a normalized collision energy (NCE) of 30 and a resolving power of 17500. Xcalibur software (version 2.2) was used for instrument control, data acquisition, and data analysis.

Results

Some literature reports describe the occurrence in seeds of plants such as sesame and *Camellia oleifera* of a typology of ceramides known as CER-EOS (Ceramide by Esterification of the Omega-hydroxyl group of fatty acid linked on Sphingosine), i.e. ceramides in which the fatty acid *N*-acylated to the LCB sphingosine has an ω - hydroxyl group (hence FA O) involved in the esterification of a second fatty acid (hence FA E) (Fig. 1). Considering this, a similar structural composition for the metabolites identified in Styrian pumpkin seed extract could be suggested. Moreover, the occurrence in the tandem mass spectra of product ions ascribable to

consecutive fragmentations involving both fatty acyl chains confirmed this hypothesis [3]. These findings are noteworthy and, to some extent, could be considered in agreement with the literature evidence describing the seeds oil obtained from Styrian pumpkin variety as a topical anti-inflammatory agent, able to treat several inflammatory skin disorders. In fact, deficiency of ceramides, essential components of the lipid skin barrier, may account, at least in part, for the dysfunction of the skin stratum corneum associated with ageing and others inflammatory skin disorders (e.g. atopic dermatitis, psoriasis, ichthyoses). In agreement with this statement, topical application of ceramides-based preparations as well as dietary ceramides-based supplementations have been proved as treatments able to restore skin-ceramide levels and lipid organization in the stratum corneum, by improving the epidermal barrier functionality in damaged skin, showing positive effects on skin- moisturizing and skin barrier recovery.

Moreover, the analysis of the mass spectrometric data allowed to ascertain the occurrence in the ethanolic extract of the Styrian pumpkin seeds of specialized metabolites belonging to the triterpenoid class. They are characterized by a wide range of biological activities, including anti-inflammatory, anticarcinogenic, antidiabetic, hepatoprotective, antimicrobial, antimycotic, analgesic, immunomodulatory, and cardioprotective [4]. Fragmentation pattern and the literature data allowed to identify the C30 skeleton as that of multiflorane-type triterpenoids (Fig. 2). This finding in the seeds of *C. pepo* var. *styriaca* is remarkable and supports both their medicinal value and nutraceutical use by considering that melanogenesis inhibitory activity, cancer preventing activity, and antioxidant effects have been reported for this chemical class [5].

Conclusions

In conclusion, the results of this study broaden the knowledge on the lipid composition of edible Styrian pumpkin seeds. Particularly interesting is the identification of lipid subclasses such as Cer- EOS-type ceramides. Therefore, this finding suggests a potential role for these edible seeds as dietary supplements of ceramides.

Moreover, it is noteworthy that this analytical method allowed to ascertain the occurrence of specialized metabolites in the seeds, such as the multiflorane-type triterpenoids, some of which never described before in pumpkin.

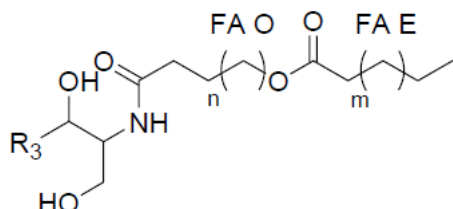


Fig. 1. Structure of CER-(EO)LCBs

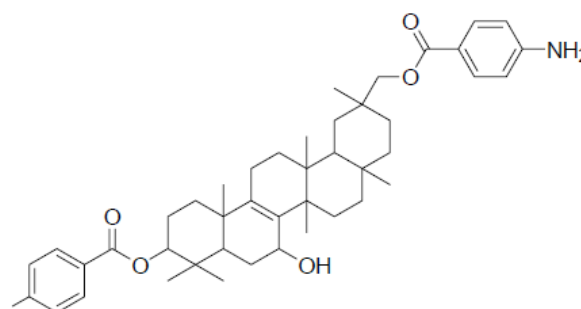


Fig. 2. Multiflorane-type triterpenoid

References

1. G. Fruhwirth, A. Hermetter; *Eur. J. Lipid Sci. Technol.*, 109 (2007), pp 1128–1140.
2. S. Medjakovic, S. Hobiger, K. Ardjomand-Woelkart, F. Bucar, A. Jungbauer; *Fitoterapia*, 110 (2016), pp 150- 156.
3. W. Zeng, X. Liu, Y. Chao, Y. Wu, S. Qiu, B. Lin, R. Liu, R. Tang, S. Wu, Z. Xiao, et al; *Food Chem*, 447 (2024), pp 139046.
4. D. Montesano, G. Rocchetti, P. Putnik, L. Lucini; *Current Opinion in Food Science*, 22 (2018), pp 81-87.
5. G.S. Sandeep; *Studies in Natural Products Chemistry*, 67 (2020), pp 411-461.

OR32

Characterization of Pancetta Piacentina PDO during ripening through redox markers, fatty acid profiling, and integrated untargeted metabolomics-lipidomics

Gabriele Rocchetti, Federico Froidi, Giulia Fappani, Michela Errico, Maria Chiara Fontanella, Samantha Sigolo, Aldo Prandini

¹ Department of Animal Science, Food and Nutrition, Università Cattolica del Sacro Cuore, Piacenza (Italy)

² Department for Sustainable Food Process, Università Cattolica del Sacro Cuore, Piacenza (Italy)

Keywords: *Dry-cured meat; Redox markers; Data Fusion*

Introduction

Pancetta Piacentina Protected Designation of Origin (PDO) is a traditional Italian dry-cured meat product obtained from pork belly and characterized by a high lipid content. During ripening, the product undergoes a series of complex physicochemical and biochemical transformations, including dehydration, proteolysis, lipolysis, and oxidative reactions, which contribute to the development of its characteristic texture, flavor, and overall quality [1]. Among these processes, lipid metabolism plays a central role, as the release and subsequent oxidation of fatty acids generate a wide range of compounds involved in quality development. [2,3]. Recent advances in metabolomics and lipidomics have provided new opportunities to investigate these transformations at the molecular level and to better understand the interactions between metabolic pathways during ripening [4,5]. Therefore, the aim of this study was to characterize the biochemical evolution of Pancetta Piacentina PDO throughout ripening by integrating proximate composition, mineral profile, fatty acid composition, redox-related markers, untargeted metabolomics and lipidomics analyses based on ultra-high-performance liquid chromatography (UHPLC) coupled with high-resolution mass spectrometry (HRMS). In addition, a multi-omics data fusion approach was applied to identify the key molecular drivers associated with product maturation and quality development.

Experimental

Pancetta Piacentina PDO samples were collected from a PDO-certified production plant at five ripening stages (0, 60, 120, 180, and 240 days; n = 6 per stage). Samples were analyzed for proximate composition, mineral profile, fatty acid composition, and redox-related markers, including peroxide value, TBARS, and reduced and oxidized glutathione. To investigate the molecular changes occurring during maturation, untargeted metabolomics and lipidomics analyses were performed using UHPLC coupled to Orbitrap HRMS. Metabolites and lipids were putatively annotated against the FooDB and LipidBlast databases, respectively. The resulting datasets were explored through unsupervised and supervised multivariate statistical analyses, while the integration of metabolomic and lipidomic data was achieved using the DIABLO (Data Integration Analysis for Biomarker Discovery using Latent Variable Approaches for Omics Studies) supervised framework to identify the key biochemical pathways and molecular drivers associated with ripening.

Results

Ripening of Pancetta Piacentina PDO induced significant physicochemical and biochemical modifications, including progressive moisture loss, salt accumulation, and changes in lipid composition. Fatty acid analysis revealed a gradual depletion of polyunsaturated fatty acids, particularly linoleic acid and long-chain n-3 PUFA, consistent with the occurrence of oxidative reactions during maturation. Accordingly, peroxide values and TBARS increased during the early-intermediate ripening stages, while glutathione-based antioxidant defenses were progressively depleted, indicating a marked shift in the redox balance of the product. Untargeted UHPLC-HRMS metabolomics (2316 annotated mass features) and lipidomics (2765 annotated lipid classes) highlighted extensive molecular remodeling throughout ripening, identifying 60 and 240 days as the major biochemical transition points. Long-ripened samples were characterized by the accumulation of lipid oxidation products, amino acid catabolites, bioactive peptides, lysophospholipids, fatty acyl derivatives, glycerolipids, sphingolipids, and sterol-related metabolites, together with a marked depletion of glycerophospholipids, suggesting extensive membrane degradation and phospholipid turnover. Several discriminant compounds associated with proteolysis, redox metabolism, cholesterol transformation, and fatty acid oxidation emerged as biomarkers of advanced ripening. Multi-omics integration using the DIABLO framework demonstrated a very strong correlation between metabolomic and lipidomic datasets ($r = 0.98$), revealing highly coordinated biochemical trajectories linking lipid oxidation, membrane remodeling, fatty acid mobilization, and protein degradation (Fig. 1). Taken together, these findings provide a comprehensive molecular overview of the mechanisms driving quality development and biochemical complexity during long-term ripening of Pancetta

Piacentina PDO.

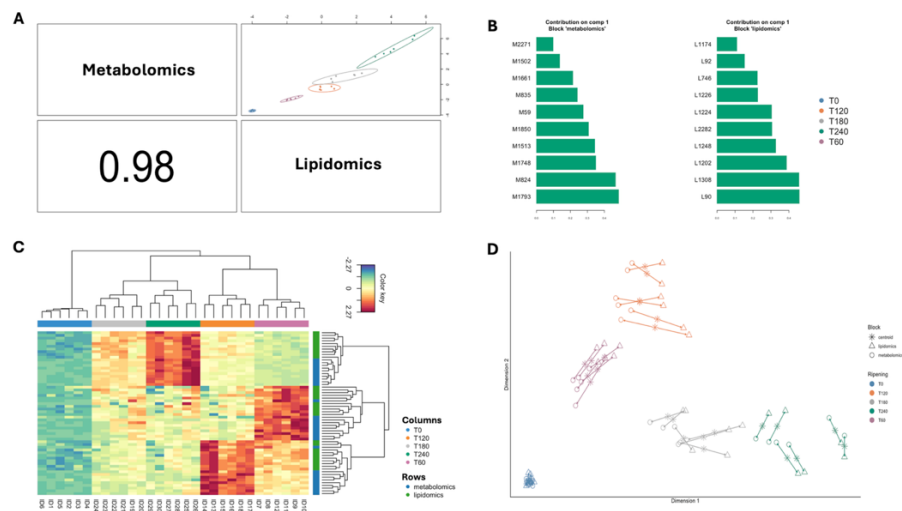


Fig. 4 Multi-omics integration of metabolomic and lipidomic datasets during Pancetta Piacentina PDO ripening using the DIABLO framework (mixOmics).

Conclusions

This study provides a comprehensive overview of the biochemical transformations occurring during the ripening of Pancetta Piacentina PDO by integrating classical compositional analyses with advanced omics approaches. Ripening was characterized by progressive dehydration, modifications in fatty acid composition, and an increase in oxidative processes affecting the most unsaturated lipid fractions. These changes were reflected by the evolution of redox markers, indicating a dynamic oxidative environment accompanied by the depletion of endogenous antioxidant defences. Untargeted metabolomics and lipidomics revealed extensive biochemical remodeling, including the accumulation of oxidation-derived metabolites, peptides associated with proteolysis, and lipid classes related to membrane degradation and lipolysis. The multi-omics integration performed through the DIABLO framework highlighted strong correlations between metabolomic and lipidomic signatures, demonstrating that lipid oxidation, lipid remodeling, and protein degradation are tightly interconnected processes driving the maturation of the product. This integrated approach provides new insights into the molecular mechanisms underlying the ripening of traditional dry-cured meat products and highlights the potential of multi-omics strategies for studying complex food maturation processes.

References

1. M. Busconi, C. Zacconi and G. Scolari. *International Journal of Food Microbiology*, 172 (2014), pp 13-20.
2. R. Domínguez, M. Pateiro, M. Gagaoua, F.J. Barba, W. Zhang and J.M. Lorenzo. *Antioxidants*, 8 (2019), 429.
3. I. Petrova, I.M. Aasen, T. Rustad and T.M. Eikevik. *European Food Research and Technology*, 241 (2015), 587-599.
4. G. Rocchetti, G. Leni, M. Errico, S. Sigolo, V. Lolli, A. Scansani, F. Foldi, A. Rebecchi, A. Caligiani, T. Bertuzzi, L. Lucini and A. Prandini. *Food Chemistry*, 469 (2025), 142612.
5. F. Foldi, G. Rocchetti, G. Leni, G. Giuberti, S. Sigolo and A. Prandini. *Food Bioscience*, 80 (2026), 108969.

OR33

Profiling microbial metabolism of Verbascoside and olive pomace extract in an *in vitro* fermentation model using a metabolomics approach

Shaymaa Abdulrazzaq,¹ Rocío García Villalba,³ Francisco Tomás-Barberán,³ Stefania Silvi,² Polina Makarycheva,² Dennis Fiorini,¹ Carlos J. Garcia³

¹ School of Science and Technology, University of Camerino, Via Madonna delle Carceri 9/B, 62032 Camerino (MC), Italy

² School of Biosciences and Veterinary Medicine, University of Camerino, Via Gentile III da Varano, 62032 Camerino (Italy)

³ Centro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC), Murcia, Spain

Summary: Colonic microbial bioconversion of olive pomace phenolic extract and pure verbascoside were studied by UPLC-ESI-QTOF MS in a static *in vitro* human fecal fermentation model. Eleven metabolites were identified, including a novel amino-acid conjugate, revealing donor-dependent biotransformation and hydroxytyrosol as the prominent metabolite.

Keywords: olive pomace; verbascoside; gut microbiota; metabolomics

Introduction

Olive pomace, the by-product of olive oil production, is rich in bioactive phenolics, of which verbascoside is among the most abundant (Zhao et al., 2022). Phenolic compounds reach the colon largely unabsorbed, where the gut microbiota catabolises them into smaller, often more bioactive metabolites (Catakaya et al., 2020). These microbial-derived metabolites are well known to exert various health-related effects, including antioxidant, anti-inflammatory, and immunomodulatory activities (Cui et al., 2018; Catakaya et al., 2020). Despite documented biotransformation of verbascoside (Cui et al., 2018; Rossi et al., 2024), the full metabolite profiles of verbascoside and complex pomace extracts remain incompletely characterised. This pilot study applied metabolomics to elucidate the colonic microbial bioconversion of a polar Raggia olive pomace extract (EX) and pure verbascoside (VER), with an emphasis on interindividual variability and novel metabolites.

Experimental

A polar phenolic extract from freeze-dried Raggia olive pomace (harvest 2023, Marche, Italy) was prepared via hydroalcoholic extraction (ethanol: water 80:20 v/v) and characterised by HPLC-DAD-ESI-MS/MS. Fecal inocula from five healthy donors were incubated anaerobically in a static *in vitro* model with four treatments: pure verbascoside (VER, 30 µM) and the polar pomace extract (EX, with an amount standardized to deliver approximately 30 µM verbascoside equivalents), alongside two solvent controls. Incubation fluids collected at 0, 8, 24, and 48 hours were subjected to C18 solid-phase extraction followed by untargeted UPLC-ESI-QTOF-MS analysis in negative ion mode. Short-chain fatty acids were also quantified by GC-MS.

Results and Discussion

The polar olive pomace extract showed a rich and complex phenolic profile dominated by phenylethanoid glycosides and secoiridoids with their derivatives, showing verbascoside (371.04 ± 8.65 mg/100 g) and the isomer of oleuropein aglycone 3,4-DHPEA-EA (276.31 ± 1.27 mg/100 g) as the predominant compounds. Untargeted UPLC-QTOF-MS of the 48 hr incubation fluids identified eleven metabolites (M1-M11) present in both treated groups but absent in controls (Figure 1), spanning five chemical classes; all were confirmed by MS/MS. Hydroxytyrosol (M2) emerged as the most stable metabolite across all donors and the only detected phenolic constituent of direct hydrolysis of verbascoside. In the VER-treated group, marked interindividual variability was observed; four donors (V1, V3, V4, V5) showed near-complete verbascoside degradation by 48 hr (high-verbascoside metaboliser phenotype), whereas one donor (V2) retained residual verbascoside (low-verbascoside metaboliser phenotype). The EX-treated group showed more uniform and robust hydroxytyrosol release from free hydroxytyrosol in the extract and microbial hydrolysis of verbascoside and related glycosides. Notably, a novel phenolic acid-amino acid conjugate, hydroxyphenylpropanoyl-leucine (M9), was identified in a donor-restricted manner, representing, to our knowledge, a previously undescribed microbial metabolite in the olive phenolic biotransformation context, yet consistent with the reported capacity of gut bacteria to form amino-acid conjugates. Three mercaptohydroxytyrosol isomers (M6/7/8) and further sulfur-containing conjugates (M11, M10), conceivably from microbial thiol-quinone chemistry, were detected exclusively in treated groups. SCFA production did not differ significantly between treatments and controls over 48 hr; reassuring from a safety perspective, indicating that the phenolic compounds did not adversely affect core microbial growth, yet the short *in vitro* duration was insufficient to produce sustained SCFA changes typically

seen after prolonged *in vivo* exposure. These new findings regarding the microbial metabolism of polyphenols open a new field of research to explore these bioconversions in other phenolic compounds and their bioactivity.

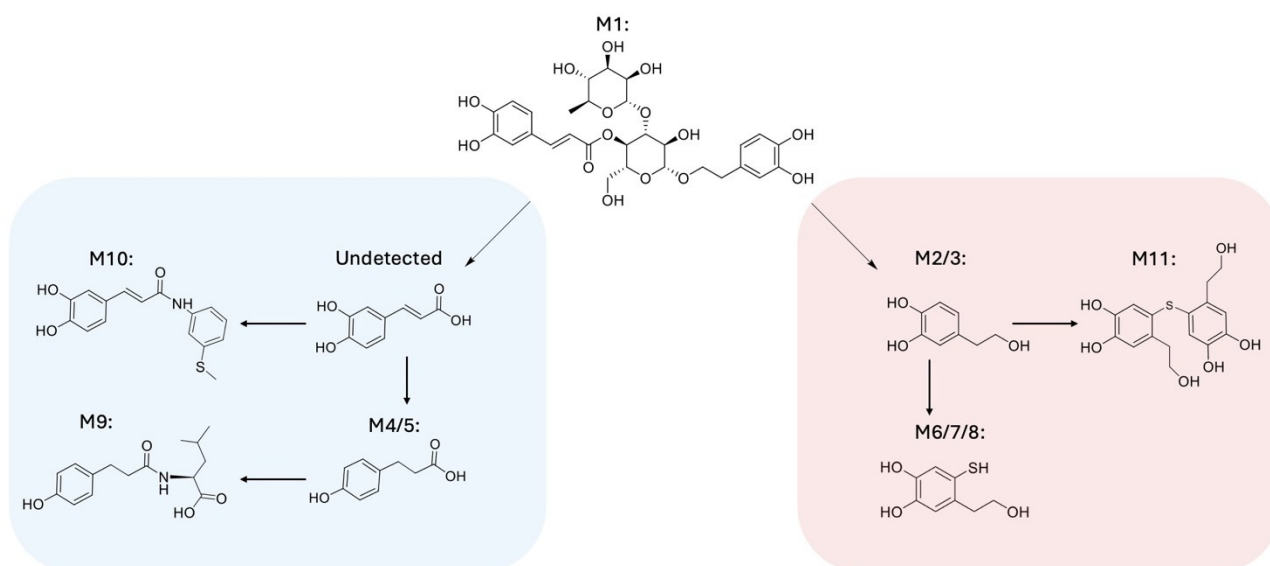


Fig. 1. Schematic representation of the proposed microbial bioconversion pathways of verbascoside (M1), showing the divergent routes yielding hydroxytyrosol isomers (M2/M3), hydroxyphenylpropanoic acid isomers (M4/M5), the novel hydroxyphenylpropanoyl-leucine conjugate (M9), and the sulfur-containing metabolites (M6-M8, M10, M11) detected in this study.

References

1. Q. Cui, Y. Pan, W. Zhang, et al., J. Agric. Food Chem., 66 (2018), pp. 2660-2668.
2. G. Catalkaya, K. Venema, L. Lucini, et al., Food Front., 1 (2020), pp. 109-133.
3. R. Rossi, E. Mainardi, F. Vizzarri, et al., Antioxidants, 13 (2024), 39.
4. H. Zhao, R.J. Avena-Bustillos, S.C. Wang, Foods, 11 (2022), 174.

OR34

Formulation, biochemical characterization and shelf-life study of YoAlp® whey based beverage containing local fruits juices

S. Valentini, M. Merlet, T. Flutto, R. Pramotton, S. Zenato, L. Verneti-Prot

Institut Agricole Régional, Aosta, Italy

Summary: This study evaluates a novel functional beverage made from strained YoAlp® whey and local fruit juices. RP-HPLC-ESI-MS peptidomic profiling identified more than 90 milk-derived peptides, confirming the intact presence of the A2 β -casein health marker beta-casomorphin 9 (BCM-9) and verifying high antioxidant and ACE-inhibitory activities over a 7-day shelf life.

Keywords: Peptidomics, Whey valorization, Liquid chromatography-mass spectrometry.

Introduction

In the last years, consumers have increased their awareness about healthy lifestyle and health promoting foods. Therefore, nowadays foods are not intended only to satisfy hunger and to provide necessary nutrients for humans, but also to prevent nutrition-related diseases and improve consumers' health. These kinds of foods are considered functional foods which are ordinary foods with components or ingredients able to provide specific health effects other than purely nutritional effects. They are not intended as medicines, but they are existing traditional products, intended to be consumed as part of a normal and balanced diet. Among functional foods, dairy-based beverages are very important. The aim of this project was to characterize, through a peptidomic RP-HPLC-ESI-MS and biochemical approach, a new functional beverage starting from YoAlp® whey, a by-product derived from Aosta Valley cattle breed fermented milk and fruit juices typically produced in Aosta Valley. Those two ingredients allow to combine many positive and health promoting nutrients such as polyphenols, antioxidants, vitamins, bioactive peptides as well as the natural probiotic lactic acid bacteria. This product could increase dairies' competitiveness and reduce their environmental impact by creating a sustainable process through the recovery of a common by-product.

Experimental

Functional beverage matrices were formulated by mixing whey obtained from strained YoAlp®, a fermented milk produced using autochthonous Aosta Valley starter cultures (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp *unknown*) with regional raw fruit juices in a 60/40% (v/v) ratio. Peptides characterization of the <10 kDa fraction was executed via an RP-HPLC-ESI-MS method in positive ion mode [1,2]. Peptides identification has been performed using an internal database based on literature data and online databases (BIOPEP/ESI PROT). Each peptide has been also associated to a possible bio-active effect, and the Peptide Ranker online software gave an indication on the probability of each sequence to exert a bioactive effect. Biochemical characterization included an optimized Folin-Ciocalteu assay for total polyphenols after a C18 solid-phase extraction cleanup, DPPH radical scavenging capacity (expressed as Vitamin C Equivalent Antioxidant Capacity, CEAC), and fluorometric kinetic screening for Angiotensin I-Converting Enzyme (ACE) inhibitory activity.

Results

Peptides analysis by LC-MS ESI⁺ on the fraction below 10 kDa of YoAlp® whey and all YoAlp® whey-based beverages detected the presence of more than 90 different peptides. Those peptides were originated from all milk proteins: α s1-casein (α s1-CN), α s2-casein (α s2-CN), k-casein (k-CN), β -lactoglobulin (LGB), α -lactalbumin (LALBA) and from β -casein (β -CN). Bioinformatic scoring verified 35 highly bioactive fragments: 85.29% functioned as ACE inhibitors and 5.88% acted as antioxidants. Peptide Ranker screening categorized 15.52% of mapped entities as high probability bioactives (>75%). Some of these peptides are supposed to resist to the gastric digestion according to literature and YQEPVLGPVRGPFPIIV aminoacidic sequence has been reported to have been found in blood plasma [3,4] where it could exert his bioactivity. Crucially, MS profiling positively confirmed the intact presence of beta-casomorphin BCM-9, sequence YVPFPGPIPN, m/z 551 ([M+H]²⁺, MW 1100), a specific bioactive health marker tracking the β -casein A2 variant characteristic of local alpine cattle breeds, alongside the absolute absence of the deleterious BCM-7 fragment. Whey-based beverages pH discrepancies (pH range from 3.81 to 4.10) modulated starter culture proteolysis and functional yields. The formulation with the higher pH value exhibited superior radical scavenging capacity driven by its massive total phenolic payload. Conversely, the lower-pH formulation unlocked the highest overall functional efficiency, providing the strongest ACE inhibition capacity. Probiotic vitality (>10⁶ CFU/mL for *S. thermophilus*) and strict microbiological safety thresholds were successfully sustained across a 7-day storage shelf life at

4°C.

Conclusions

The presence of bioactive peptides and phenolic compounds can give to the product a possible antiradical power and ACE inhibitory activity which seem to be higher than other similar dairy-based beverages present on the market or under formulation. Furthermore, the marker of allelic variant A2 of β -Casein and the absence of marker of allelic variant A1 of β -Casein variant suggest possible beneficial health effects leading to a lower incidence of cardiovascular disease and type 1 diabetes, and a reduction in cholesterol and triglycerides. Moreover, these beverages contain viable Lactic acid bacteria (*Streptococcus thermophilus*) which can give beneficial effects on the gastrointestinal tract and spoilage microorganisms stay under the regulation threshold up to 7 days of shelf-life at 4°C. In future, in vivo studies should be performed to evaluate the bioavailability of bioactive peptides and phenolic compounds.

References

1. S. Sforza, V. Cavatorta, F. Lambertini, G. Galaverna, A. Dossena, R. Marchelli; Journal of Dairy Science, 95 (2012), pp 3514-3526.
2. C.G. Rizzello, I. Losito, M. Gobbetti, T. Carbonara, M.D. De Bari; Journal of Dairy Science, 88 (2005), pp 2348-2360.
3. D.P. Baptista, M.L. Gigante; J Sci Food Agric., 101(10) (2021), pp 4010-4017
4. L. Sanchez-Rivera, D. Martinez-Maqueda, E. Cruz-Huerta, B. Miralles, I. Recio. Int Food Res J., 63 (2014), pp 1-11.

Acknowledgements and funding

This experimental work was financially supported, through TYPICALP project, by European Union, European Regional Development Fund, Italian State, Swiss Confederation and Cantons co-financed operation, within the framework of the Interreg V-A Italy-Switzerland Cooperation Program.

OR35

Enhanced MOSH and MOAH screening in fat-rich foods using integrated LC-GC-FID and GC×GC-QToF/FID approaches

I. Annovazzi,¹ D. Baglio,² P. Rovellini,² V. Cardenia¹

¹ Department of Agricultural, Forest and Food Science, University of Turin, Largo Paolo Braccini 2, Grugliasco (Turin)

² Innovhub – Stazioni Sperimentali per l'Industria, Via Giuseppe Colombo 83, Milan

Summary: *This work presents a survey of mineral oil hydrocarbons contamination in commercial margarines and butters from 9 European countries. A suitable extracting procedure for high-fat content matrices was applied. Quantification of MOSH and MOAH fractions was conducted using LC-GC-FID, and characterization of the fractions was performed through GC×GC-QToF/FID.*

Keywords: *mineral oil hydrocarbons, fat-rich foods, GC×GC-MS*

Introduction

Lipid-rich matrices are highly susceptible to contamination by mineral oil hydrocarbons (MOH) due to the strong affinity of these lipophilic compounds for the fat phase.

MOH are complex mixtures of chemical compounds derived from crude oil distillation processes and are commonly classified into two main groups: Mineral Oil Saturated Hydrocarbons (MOSH), which include linear, branched, and cyclic saturated hydrocarbons and Mineral Oil Aromatic Hydrocarbons (MOAH), which include aromatic hydrocarbons with one or more alkylated aromatic rings. The main concern is about MOAH because of their potential carcinogenic effects. Conversely, while MOSH are known to accumulate in human tissues, current evaluations suggest they present a lower immediate toxicological risk [1].

Contamination can occur throughout the food supply chain, originating from environmental sources, food-grade lubricants, processing aids, and food contact materials, or it may already be present in the raw materials. Vegetable oils such as rapeseed, sunflower, palm and coconut oils are some of the primary ingredients in margarine formulation and it has been found that, in some cases, they could exhibit MOAH levels exceeding 2.0 mg/kg, which represents the LOQ value for this food category [2].

Moreover, both butter and margarine are heavily consumed worldwide; for instance, annual European consumption peaks at approximately 8 kg per capita for butter in France and 11 kg per capita for margarine in the Netherlands. Given this high dietary intake and the susceptibility of the ingredients, monitoring MOH content in these fat-based products is essential to accurately evaluate consumer exposure.

Experimental

This study provides a comprehensive survey of commercial margarines and butters sourced across Europe. MOSH and MOAH fractions, within the C10–C50 range, were quantified using online high-performance liquid chromatography coupled with gas chromatography and flame ionization detection (LC-GC-FID) according to the ISO 20122:2024 method [3]. To overcome the analytical challenges posed by these complex lipid matrices, additional auxiliary purification steps - specifically alkaline saponification, epoxidation, and ALOX column chromatography - were implemented [4].

Further investigations via comprehensive two-dimensional gas chromatography coupled with high-resolution time-of-flight mass spectrometry and flame ionization detection (GC×GC-QToF/FID) were performed on samples with detectable MOAH levels to confirm MOH contamination [5].

Results

The analytical screening revealed that the analyzed butter samples contained detectable MOSH levels, with concentrations ranging from <

2.0 to approximately 17 mg/kg, while MOAH values were always below the LOQ (< 2.0 mg/kg). Margarine samples exhibited MOSH levels ranging from < 2.0 to approximately 14 mg/kg. Measurable MOAH fractions, slightly above the limit of quantification, were identified in a few samples, especially those with palm or coconut oil as ingredients. In fact, these vegetable oils contain high levels of biogenic lipid classes that can coelute and may be misinterpreted as MOAH.

For further clarification, these samples were analyzed by GC×GC in order to identify the interfering substance classes. The instrument was coupled to two detectors: FID for quantitative analysis and high-resolution MS for identification of compounds by extracting specific ions for each class of substances [6].

This advanced approach successfully recognized endogenous interferences—such as natural olefins, carotenoids, and squalene derivatives—that would cause an overestimation of the MOAH content, while also

providing clearer discrimination between biogenic components and mineral oil contamination [7].

Conclusions

In conclusion, this comprehensive survey highlights the importance of monitoring MOH contamination in heavily consumed fat-based products, such as butter and margarine, across Europe.

While MOSH levels remained within comparable ranges for both matrices, the study underscores that vegetable-based margarine formulations— particularly those containing palm and coconut oils— pose significantly greater analytical challenges than butters, owing to interferences from biogenic lipid classes in the MOAH fraction.

The integration of advanced comprehensive two- dimensional gas chromatography coupled with quadrupole time-of-flight and flame ionization detection (GC×GC-QToF/FID) was essential to identify endogenous interferences, thereby ensuring accurate discrimination between natural biogenic components and actual mineral oil contamination.

Ultimately, these results provide valuable data to improve consumer risk assessments and help food authorities protect public health.

References

1. EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain). *EFSA Journal* 2023; 21(9), 1–143
2. EFSA Panel on Contaminants in the Food Chain (CONTAM); *EFSA Journal* 2012; 10(6):2704. [185 pp.]
3. ISO 20122:2024 “Vegetable oils — Determination of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) with online-coupled high performance liquid chromatography-gas chromatography-flame ionization detection (HPLC-GC-FID) analysis — Method for low limit of quantification”
4. Bratinova S., Robouch P. and Hoekstra E., *Publications Office of the European Union*, Luxembourg, 2023
5. European Union Reference Laboratory for Processing Contaminants (EURL-PC), Montoya-Arroyo, A, Cederberg, TL, Gorska, A, Purcaro, G, Richter, L & Biedermann, M 2025, *European Reference Laboratory for Processing Contaminants (EURL-PC) & DTU National Food Institute*
6. M. Biedermann, K. Grob, *Journal of Chromatography A*, Volume 1375, 2015, Pages 146-153
7. C. Albert, H.-U. Humpf, L. Brühl; *J. Agric. Food Chem.* 24 August 2022; 70 (33): 10337–10348

IP3

Shimadzu

OR36

Unravelling geographical, seasonal and technological variability in the untargeted metabolomic profile of Parmigiano Reggiano PDO natural whey starter through ASCA-integrated chemometrics

Pier Paolo Becchi,¹ Rasmus Bro,² Valentina Pizzamiglio,³ Luigi Lucini

¹ Department for Sustainable Food Process, Università Cattolica del Sacro Cuore, Piacenza, Italy

² Department of Food Science, University of Copenhagen, Frederiksberg, Denmark

³ Consorzio Formaggio Parmigiano Reggiano, Via Kennedy 18, 42124 Reggio Emilia, Italy

Summary: *Untargeted UHPLC-HRMS metabolomics was used to investigate how production altitude, season and the rennet-to-starter ratio used during cheesemaking shape the metabolomic profile of Parmigiano Reggiano natural whey starter. ASCA preprocessing, factor-specific PLS-DA and variable selection improved discrimination, revealed coherent metabolite signatures and underscored the need for independent validation against overfitting.*

Keywords: *Parmigiano Reggiano whey; ASCA-PLS-DA; model validation*

Introduction

Parmigiano Reggiano (PR) natural whey starter samples collected from dairies within the same PDO production chain comply with a common production specification established by the PR cheese Consortium. Nevertheless, substantial intrinsic variability may persist, as the metabolomic composition of Parmigiano Reggiano natural whey starter may vary with seasonality, production altitude, and the relative amounts of rennet and natural whey starter used during cheesemaking. Characterising these overlapping sources of variability is particularly challenging in untargeted food metabolomics because of the high dimensionality of the generated data and the need to manage a large number of samples representative of the entire production area. This complexity requires advanced analytical and chemometric strategies capable of handling sample heterogeneity, separating factor-specific effects and preserving model robustness and interpretability. Principal component analysis (PCA) and hierarchical clustering (HCA) showed extensive class overlap, while partial least squares discriminant analysis (PLS-DA) and orthogonal PLS-DA (OPLS-DA) models did not provide sufficiently robust discrimination. This does not necessarily indicate the absence of meaningful chemical differences, but rather that the effects of individual factors are superimposed within the same matrix. We therefore assessed an ASCA-preprocessed PLS-DA strategy within a validity-by-design framework. Particular attention was given to the risk of "data cheating", defined here as outcome-driven choices in preprocessing, variable selection, class definition or model complexity that improve the apparent fit to the available data without reliable generalisation [2].

Experimental

PR natural whey starter samples were provided by the Parmigiano Reggiano Cheese Consortium. The investigated factors were *altitude* (flatland, hill and mountain production), *seasonality* (winter, spring and summer), and *treatment* (acid, rennet and mixed classes based on the rennet-to-natural whey starter ratio). Sample preparation followed the untargeted metabolomics workflow previously applied to PR milk [1]. Briefly, samples were extracted with acidified acetonitrile, vortexed, sonicated, centrifuged and filtered before analysis. Pooled quality control (QC) samples were injected throughout the sequence to monitor analytical stability. Metabolic profiling was performed using a Vanquish UHPLC system coupled to a Q-Exactive Focus Orbitrap mass spectrometer equipped with a heated electrospray ionisation source operated in positive mode. Chromatographic separation was achieved on a C18 column using a water-acetonitrile gradient [1]. Samples were acquired in full-scan mode, while data-dependent MS/MS spectra were acquired from the QC samples. Raw data were processed in MS-DIAL, and compounds were putatively annotated by matching against FooDB and MoNA, yielding a matrix of 595 mass features.

Data were decomposed by ANOVA-simultaneous component analysis (ASCA) according to seasonality, altitude and whey treatment. Statistical significance was assessed using 1000 permutations. For each factor, the corresponding effect matrix was recombined with the residual matrix before PLS-DA modelling. Samples were divided a priori into calibration and held-out test sets using the Duplex algorithm with a 75:25 split. For the altitude and treatment factors, variable selection based on VIP scores or the selectivity ratio was also evaluated. The resulting models were assessed using calibration, cross-validation and prediction statistics, ROC curves, AUC values and held-out test-set accuracy [3].

Results and Discussion

ASCA identified statistically significant effects for *seasonality*, *altitude* and *treatment* ($p = 0.001$). *Seasonality*

accounted for the largest structured effect (10.03%), followed by *altitude* (2.05%) and *treatment* (1.86%), while *residuals* accounted for the largest variability explained (86.07%), reflecting the intrinsic chemical variability of whey (Table 1).

Table 1. ASCA effects for seasonality, altitude and treatment.

Factor	PCs	Cum Eigen Val	Effect	P-value
Seasonality	2	59.21	10.03	0.0010
Altitude	2	13.26	2.05	0.0010
Treatment	2	13.98	1.86	0.0010
Residuals	—	—	86.07	—

Using the factor-specific effect matrices generated by ASCA, each factor was subsequently analysed separately by PLS-DA after the corresponding dataset had been split into calibration and test sets. For *seasonality*, PLS-DA modelling of the ASCA-derived effect matrix clearly discriminated winter, spring and summer whey samples. The model showed an R^2_{cal} of approximately 0.90, with class-specific R^2_{CV} values of 0.85, 0.68 and 0.76 for winter, spring and summer, respectively.

The model achieved a test accuracy of 97.2%, with 35 samples correctly classified and AUC values above 0.96. Summer and winter samples were mainly separated along LV1, whereas spring samples were distinguished along LV2 (Figure 1).

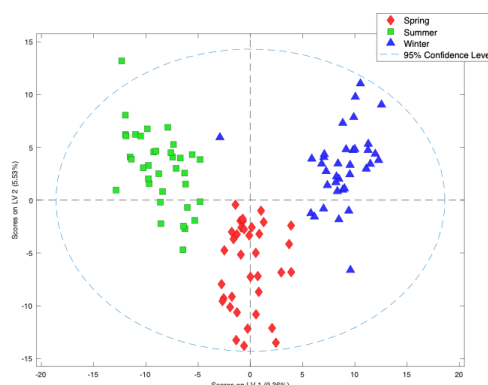


Figure 1. PLS-DA score plot for seasonality, showing the distribution of winter, spring and summer whey samples along the first two latent variables.

Summer whey starter samples were characterised by α -linolenic acid, phosphatidylcholines and sphingomyelins, together with putative plant-derived phenolic compounds, suggesting a stronger contribution from fresh forage and greater lipid unsaturation. Several dipeptides containing phenylalanine, tryptophan, arginine or glutamic acid also contributed to seasonal discrimination.

For *altitude*, the initial three-class PLS-DA model showed limited discrimination and predictive performance. However, grouping hill and mountain samples and comparing them with lowland samples substantially improved classification (Figure 2). The resulting model showed an R^2_{cal} of 0.96 and an R^2_{CV} of 0.81, while its predictive performance on the independent test set was confirmed by an R^2_{pred} of 0.73. Variable selection reduced the matrix to 158 features, and the model achieved 95% test accuracy with AUC values above 0.95. Sixty-four metabolites were identified as discriminant markers. Hill and mountain whey displayed an upland forage signature dominated by omega-3-rich and polyunsaturated di- and triacylglycerols, polar lipids and putative ferulate-related phenylpropanoids. Conversely, propenoylcarnitine and dodecanedioylcarnitine were associated with flatland production and may reflect differences in energy-rich feeding regimens.

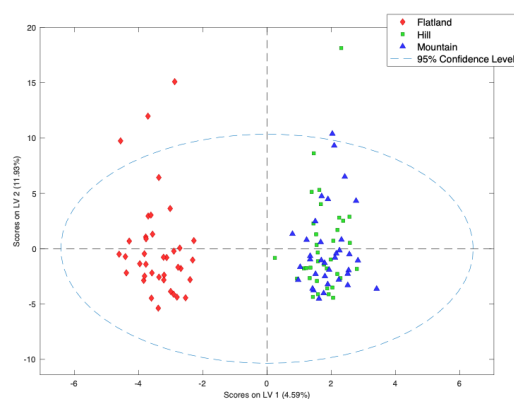


Figure 2. PLS-DA score plot for altitude, comparing lowland whey with grouped hill and mountain samples along LV1 and LV2.

For *treatment*, the three-class model including acid, rennet and mixed whey remained poorly predictive even after variable selection. Better performance was obtained only when the comparison was restricted to “rennet vs acid” whey. The resulting PLS-DA model, built on the orthogonalised dataset, achieved an R^2_{CV} of 0.86 and an R^2_{pred} of 0.743 (Figure 3).

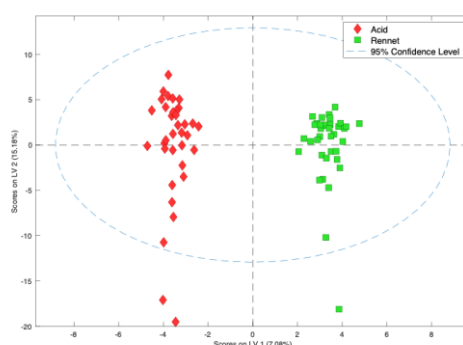


Figure 3. PLS-DA score plot for treatment, comparing acid and rennet whey samples in the orthogonalised dataset along LV1 and LV2.

Seventy-seven metabolites differentiated the two whey types. Acid whey was associated with L-histidine, lysophospholipids and glycerolipids, possibly reflecting pH-dependent partitioning and coagulation kinetics. Rennet whey was characterised by proline, isoleucine, arginylthreonine and guanidinoacetic acid, consistent with differences in soluble nitrogen and peptide fractions.

Conclusions

This study shows that the metabolomic variability of whey collected across the Parmigiano Reggiano PDO area cannot be adequately described by conventional exploratory or directly supervised models alone. ASCA separated the contributions of *seasonality*, *altitude* and *treatment* effects, enabling robust factor-specific PLS-DA modelling. The improved performance obtained after biologically justified class grouping also highlighted the importance of transparent model design. Independent test sets, permutation testing, controlled variable selection and the rejection of poorly predictive models are essential to limit data cheating and obtain interpretable, generalisable results from complex food metabolomics datasets.

References

1. P.P. Becchi, G. Rocchetti, F. Foldi, S. Sigolo, A. Gallo, L. Lucini; *Food Chemistry* 501 (2026), 147637.
2. R. Bro; *Chemometrics and Intelligent Laboratory Systems* 264 (2025), 105461.
3. F. Westad, F. Marini; *Analytica Chimica Acta* 893 (2015), pp 14-24.

OR37

A precursor ion scan-based mass spectrometry workflow for intact glucosinolate characterization in radish microgreens for nutraceutical applications

Vincenzo Piccolo, Maria Maisto, Adua Marzocchi, Erika Esposito, Emma Mitidieri, Roberta d'Emmanuele di Villa Bianca, Vincenzo Summa, Gian Carlo Tenore

Department of Pharmacy, University of Naples Federico II, Via Domenico Montesano 49, 80131, Naples, Italy

Summary: An integrated mass spectrometry approach combining precursor ion scan with quantitative analysis enabled selective screening and characterization of intact glucosinolates in radish microgreens. This workflow supported the identification and standardization of nutraceutical matrices enriched in hydrogen sulfide-releasing phytochemicals for cardiovascular health applications.

Keywords: Precursor ion scan; Glucosinolates; Radish microgreens

Introduction

Glucosinolates (GSLs) are sulfur-containing phytochemicals naturally occurring in Brassicaceae vegetables and have attracted increasing interest as nutraceutical compounds due to their ability to generate hydrogen sulfide (H₂S), an endogenous gasotransmitter that plays a pivotal role in cardiovascular homeostasis by regulating endothelial function, vascular tone and oxidative stress¹. Among Brassicaceae, microgreens have emerged as sustainable plant biofactories and standardized sources of high-value bioactive compounds, including glucosinolates. However, their structural diversity, together with the complexity of plant matrices, makes their comprehensive characterization analytically challenging. Conventional analytical approaches are often based on desulfation procedures, which may alter the native glucosinolate profile and increase sample preparation time. Therefore, robust mass spectrometry-based strategies capable of directly identifying and quantifying intact GSLs are highly desirable. In this study, an integrated HPLC-qQq-MS/MS workflow based on precursor ion scan (PIS) and quantitative tandem mass spectrometry was developed for the comprehensive characterization of intact glucosinolates in hydroponically grown radish microgreens².

Experimental

Seven *Raphanus sativus* cultivars were cultivated under controlled hydroponic conditions and harvested at different developmental stages. Food-grade aqueous and hydroalcoholic extraction protocols were optimized to preserve the native glucosinolate profile and maximize the recovery of bioactive phytochemicals. Intact glucosinolates were quantified in all cultivars by HPLC-qQq-MS/MS operating in multiple reaction monitoring (MRM) mode to identify the glucosinolate-rich cultivar. Based on the quantitative results, Tango was selected for further investigation. Comprehensive characterization of intact glucosinolates was then performed by HPLC-qQq-MS/MS using PIS acquisition mode. Polyphenols and anthocyanins were profiled by HPLC-HESI-MS/MS in data dependent acquisition (DDA) mode, while antioxidant activity was assessed by DPPH, ABTS and FRAP assays. Finally, the H₂S-releasing properties of Tango extracts were evaluated in cell-free and cellular fluorescence assays.

Results

A comparative phytochemical screening was first performed on seven *Raphanus sativus* cultivars harvested at different developmental stages to identify the most promising nutraceutical candidate. Prior to sample analysis, the HPLC-qQq-MS/MS method for intact glucosinolate quantification was thoroughly validated. The method showed excellent linearity over the investigated concentration ranges ($R^2 = 0.9999$ for all analytes), high sensitivity with LOD values ranging from 0.25 ppb to 0.68 ppb and LOQ values between 0.76 ppb and 2.06 ppb, together with excellent analytical stability (QC RSD < 4%) and negligible carry-over ($\leq 1.5 \times 10^{-4}$ %). Precision and accuracy fulfilled international validation criteria, with intra- and inter-day precision generally below 10% CV and bias values within $\pm 10\%$, demonstrating the robustness and reliability of the quantitative workflow. Quantitative HPLC-qQq-MS/MS analysis in MRM mode revealed marked differences in intact glucosinolate accumulation among cultivars and harvesting stages. Tango harvested at day 6 exhibited the highest total glucosinolate content (70.71 mg/g DW), mainly represented by glucoraphasatin (58.98 ± 1.65 mg/g DW) and glucoraphenin (11.73 ± 0.97 mg/g DW), whereas glucoraphanin was detected only at trace levels. Beyond glucosinolates, radish microgreens also represent a valuable source of phenolic compounds and anthocyanins, which substantially contribute to their nutraceutical value. Spectrophotometric assays revealed significant differences among cultivars, with Tango exhibiting the highest total phenolic (17.21 ± 0.67 mg GAE/g DW) and anthocyanin (11.91 ± 0.02 mg cyanidin-3-glucoside equivalents/g DW) contents. The

selected cultivar also displayed remarkable antioxidant activity, with DPPH and ABTS IC₅₀ values of 1.20 and 0.67 mg/mL, respectively, together with the highest FRAP reducing capacity. These findings identified Tango as the most promising cultivar for subsequent phytochemical characterization.

Comprehensive characterization of the selected Tango cultivar was subsequently performed by HPLC-qQq-MS/MS using PIS approach. The optimized PIS strategy, based on the diagnostic fragment ions *m/z* 259, 97 and 96 (Figure 1), enabled the rapid and selective identification of intact glucosinolates directly in complex extracts without prior desulfation, preserving their native molecular structures. Overall, glucoraphasatin, glucoraphenin, glucoraphanin, glucobrassicin and several acylated glucosinolate derivatives were successfully characterized, demonstrating the effectiveness of the proposed workflow for comprehensive glucosinolate profiling. Complementary HPLC-HESI-MS/MS analysis further provided an in-depth characterization of the polyphenolic fraction. Overall, 54 polyphenolic compounds were putatively identified, including hydroxycinnamic acid derivatives, flavonols, flavan-3-ols and cyanidin-based anthocyanins. The polyphenolic profile was particularly enriched in hydroxycinnamic acid derivatives and kaempferol and quercetin glycosides. Anthocyanins were mainly detected as mono-, di- and triglycosylated cyanidin derivatives. Finally, fluorescence-based assays demonstrated a concentration-dependent H₂S release under both cell-free and cellular conditions, confirming the biological relevance of the chemically characterized phytocomplex. Altogether, these findings demonstrate that the integration of quantitative MRM analysis with PIS-based structural characterization provides a powerful analytical workflow for the comprehensive investigation and standardization of glucosinolate-rich food matrices intended for nutraceutical applications.

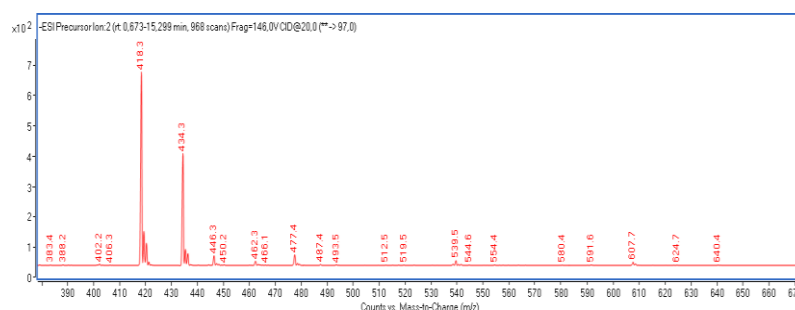


Fig.1. Representative PIS chromatogram monitoring the diagnostic fragment ion *m/z* 97.

Conclusions

An integrated HPLC-qQq-MS/MS workflow combining quantitative MRM and PIS approaches was successfully developed for the comprehensive investigation of intact glucosinolates in radish microgreens. The validated analytical method enabled the reliable comparison of different cultivars, supporting the selection of Tango as the most promising glucosinolate-rich matrix. Subsequent PIS analysis allowed the rapid and selective identification of intact glucosinolates directly in complex plant extracts, while complementary phytochemical and biological investigations confirmed the high nutraceutical value of the selected cultivar. Overall, the proposed mass spectrometry workflow represents a robust analytical platform for the characterization and standardization of glucosinolate-rich food matrices, with potential applications in food quality control and nutraceutical research.

References

1. Demir, K.; Sarikamis, G.; Seyrek, G.C. *Food Chemistry*, 401 (2023), 134088.
2. Maisto, M.; Marzocchi, A.; Piccolo, V.; Esposito, E.; Correale, M.; Mitidieri, E.; Tenore, G.C.; d'Emmanuele, R. *Food Chemistry: X*, 35 (2026), 103816

OR38

Enzyme-assisted multidimensional LC-IM-HRMS annotation of acetylated xylo- oligosaccharides generated from commercial xylans

G. Lupi, A. Fuso, A. Caligiani,

University of Parma, Parma, Italy

Summary: *Controlled enzymatic hydrolysis coupled to LC-IM-HRMS generated diagnostic acetylated xylo-oligosaccharides from xylan substrates. Accurate mass, RT, CCS, enzymatic response and MSP fragmentation were integrated into a curated database for structure-informed characterization of hemicellulose-rich dietary fiber ingredients.*

Keywords: *Food glycomics; Xylo-oligosaccharides; Ion mobility mass spectrometry*

Introduction

The structural characterization of dietary fiber ingredients remains challenging because conventional bulk analyses provide only limited information on molecular architecture. For hemicellulose-rich ingredients, functionality depends on backbone length, substitution degree, acetylation and accessibility to hydrolytic enzymes. In analogy with peptide mapping, controlled enzymatic hydrolysis can generate diagnostic oligosaccharides that retain information from the parent polysaccharide.

This work proposes an enzyme-assisted food- glycomics workflow in which acetylated xylo- oligosaccharides (XOS) are used as molecular fingerprints of xylan domains. The aim is not complete polysaccharide sequencing, but the development of a transferable database to support structure-informed characterization of novel fiber- rich ingredients.

Experimental

Reference xylan substrates, including acetylated and partially acetylated xylans, were hydrolysed using endo-beta-1,4-xylanase and acetyl xylan esterase. Hydrolysates were analysed by UHPLC- TWIMS-HRMS in negative electrospray ionization mode. Feature detection and alignment were performed with Progenesis Q1, and high- energy spectra were exported in MSP format.

A theoretical XOS library was generated and used for accurate-mass filtering. Candidate acetylated XOS were retained when they decreased after esterase treatment in both acetylated substrates. The final curation combined m/z, adduct type, enzymatic response, RT/CCS coherence and Domon-Costello-based fragment assignment.

Results

The initial untargeted table contained about 8000 features. Accurate-mass matching, selection of acetylated candidates, enzymatic filtering and DP- stratified manual curation reduced the feature space to 64 acetylated XOS, covering DP3-DP10 and Ac1-Ac7. DP2 features were excluded because of less coherent chromatographic and ion mobility behaviour.

The curated subset showed ordered physicochemical trends. Retention time increased with DP but decreased with acetylation ($RT = 3.27 + 1.04 \times DP - 1.29 \times Ac$, $R^2 = 0.926$), while CCS increased with both DP and acetylation ($CCS = 167.22 + 16.22 \times DP + 8.91 \times Ac$, $R^2 = 0.982$).

MSP spectra supported the assignments through acetyl-related neutral losses, losses of anhydroxylose units and B/Y, C/Z and A/X-type fragments. These data supported composition- and feature-level annotation, although the exact positions of acetyl groups were not assigned.

The curated database was designed for transfer to new datasets. Candidate matching relies on theoretical m/z, adduct type, RT windows, CCS tolerance, diagnostic fragments and enzyme- response behaviour.

When applied to unknown or industrial fiber-rich ingredients, the database can indicate the presence of xylan domains, acetylated regions, enzyme- accessible substituents and DP/acetylation distributions. In this way, oligosaccharide fingerprints are translated into structural descriptors of the parent polysaccharide.

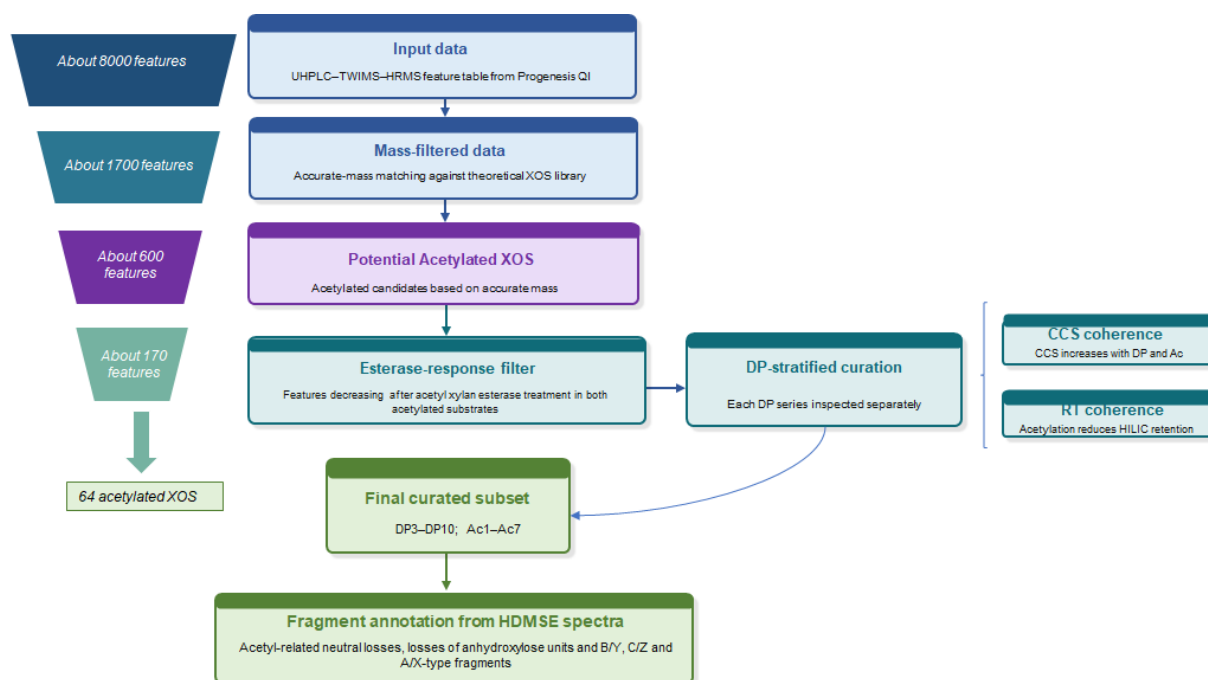


Fig. 1. Workflow for the selection of enzyme-supported acetylated XOS features.

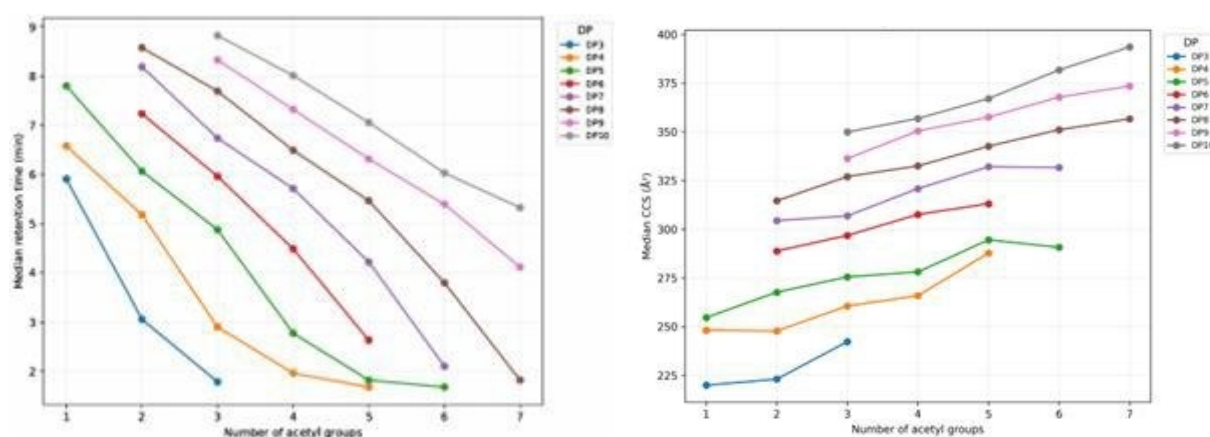


Fig. 2. Relationships between acetylation, retention time and CCS in the curated DP3-DP10 acetylated XOS subset.

Conclusions

The workflow establishes a practical framework for polysaccharide mapping in food glycomics. Controlled enzymatic hydrolysis combined with LC-IM-HRMS and curated databases can support the structure-informed characterization of novel dietary fiber ingredients, bridging the gap between bulk fiber analysis and detailed polysaccharide structural elucidation.

OR39

LC-MS/MS analysis of marker peptides for the detection of various mustard species and rapeseed in foods

Barbara Prandi,¹ Andrea Faccini,² Francesca Lambertini,³ Tullia Tedeschi¹

¹ Department of Food and Drug, University of Parma, Parco Area delle Scienze 27/A, 43124 Parma, Italy

² Interdepartmental Centre for Measurement "G. Casnati", University of Parma, Parco Area delle Scienze 23/A, 43124 Parma, Italy

³ Barilla G. & R. F.lli, Analytical Food Science Research, Via Mantova 166, 43122 Parma, Italy

Summary: *this study developed an LC-MS/MS peptidomic approach for the detection of mustard species and rapeseed in wheat-based matrices. Species-specific marker peptides were identified and validated through SRM analysis in samples contaminated at 1% level. The method showed high selectivity despite the strong sequence homology within the Brassicaceae family, although Indian and black mustard could not be discriminated. Overall, the approach represents a reliable complementary tool to PCR- and ELISA-based methods for allergen detection.*

Keywords: *mustard allergens, LC-MS/MS, marker peptides.*

Introduction

Mustard is widely used in the food industry to produce condiments and is also commonly employed in crop rotation systems. However, mustard is listed among the 14 major food allergens that must be declared according to EU Regulation 1169/2011, and it is responsible for a significant proportion of allergic reactions, including severe cases such as anaphylaxis.

Recent alerts reported in the RASFF portal highlight the recurring issue of mustard contamination in cereal-based products, particularly flour, often due to agricultural practices such as crop rotation or cross-contamination during processing.

Current detection methods, such as PCR and ELISA, may suffer from cross-reactivity issues, especially between closely related species within the *Brassicaceae* family, including rapeseed (*Brassica napus*). Over the past decades, LC-MS/MS has emerged as a powerful tool for food allergen detection, offering high selectivity and specificity and acting as a complementary method to conventional approaches [1].

Therefore, the aim of this study was to develop a peptidomic LC-MS/MS method for the identification of species-specific marker peptides for different mustard species (white, Indian/black, wild) and rapeseed, enabling their selective detection in wheat-based matrices.

Experimental

A total of fourteen samples were analyzed, including pure mustard species (*Sinapis alba*, *Brassica juncea*, *Brassica nigra*, *Sinapis arvensis*), rapeseed (*Brassica napus*), and durum wheat samples, both blank and artificially contaminated at 1% level with individual species or a mixture of *Brassicaceae*.

Proteins were extracted under alkaline conditions, precipitated at the isoelectric point, and quantified fluorometrically.

Subsequently, proteins were reduced, alkylated, and enzymatically digested using trypsin and chymotrypsin to generate peptide mixtures.

Peptides were analyzed by UHPLC/ESI-MS/MS in Full Scan and Selected Reaction Monitoring (SRM) modes, while high-resolution μ HPLC-LTQ-Orbitrap analysis was used for peptide identification and sequence assignment.

ResultsPeptide marker identification

Full Scan analysis allowed the annotation of peptides with molecular weight >800 Da as potential species-specific markers. Many candidate peptides were detected for each species; however, due to the high sequence homology among *Brassicaceae*, only a limited subset of unique peptides could be identified after cross-comparison.

After filtering, the following numbers of unique candidate markers were obtained:

- White mustard: 4 peptides
- Rapeseed: 2 peptides
- Indian/black mustard: 12 peptides
- Wild mustard: 3 peptides

•

Confirmation in contaminated samples

SRM analysis performed on durum wheat samples artificially contaminated at 1% level demonstrated that:

- 4/5 marker peptides were confirmed for white mustard
- 2/2 for rapeseed
- 4/12 for Indian/black mustard
- 2/3 for wild mustard

The peptides showed good detectability even in mixed contamination scenarios, confirming the robustness of the method.

Protein origin and specificity

Several confirmed marker peptides were derived from 11S globulin storage proteins, which are major allergens in these species. These proteins are known to contribute to cross-reactivity phenomena between mustard and rapeseed allergens [2].

Despite high structural similarity between some peptides, species-specific differentiation was achieved through combined SRM transitions. However, it was not possible to discriminate between Indian (*B. juncea*) and black mustard (*B. nigra*), due to their extremely high protein sequence similarity.

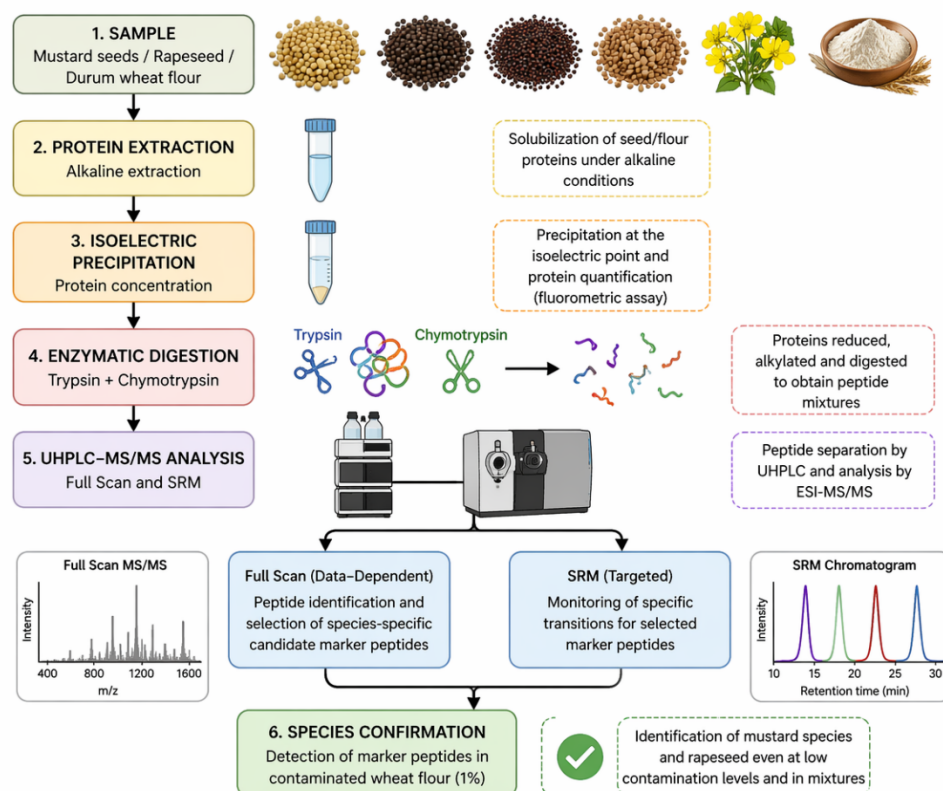


Fig. 1. Schematic workflow of the peptidomic LC-MS/MS approach for mustard detection.

Conclusions

A peptidomic LC-MS/MS method was successfully developed for the detection of mustard species and rapeseed in wheat-based matrices through species-specific marker peptides.

The method demonstrated:

- high selectivity and sensitivity (detection at 1% contamination level)
- ability to detect multiple *Brassicaceae* species simultaneously
- robustness in complex matrices such as flour

Although Indian and black mustard could not be distinguished, the approach represents a valuable complementary tool to PCR and ELISA methods, particularly for confirmatory analysis and improved allergen detection in the food industry.

References

1. M. Bianco, G. Ventura, C. D. Calvano, I. Losito, T. R. I. Cataldi; *Proteomics*, 23 (2023), pp 2200427.
2. A. Fiocchi, L. Dahdah, C. O. Mazzina, Riccardi, V. Fierro; *Asthma Research and Practice*, 2 (2016), pp 13.

OR40

Role of red cabbage anthocyanins the reaction with AAPH and carotenoid protection

Claudia Lombroni^{1,2} *Ksenia Morozova*² *Tiziana Nardin*³ *Roberto Larcher*³ *Matteo Scampicchio*²

¹ University of Turin, Department of Agricultural, Forest and Food Sciences, 10095 Grugliasco (TO), Italy

² Free University of Bozen-Bolzano, Faculty of Agricultural, Environmental and Food Sciences, 39100 Bolzano (BZ), Italy

³ Centro di Trasferimento Tecnologico, Fondazione Edmund Mach, 38010 San Michele all'Adige (TN), Italy

Summary: *This study investigates the antioxidant potential of red cabbage extract through HPLC-HRMS/MS characterization of its anthocyanin profile and evaluation of its radical-scavenging activity. The extract was subsequently assessed for its ability to protect carotenoids against thermal and oxidative degradation, demonstrating its potential as a natural antioxidant system for food applications.*

Keywords: *Anthocyanins, Red Cabbage, High-Resolution Mass Spectrometry*

Introduction

Red cabbage (*Brassica oleracea* var. *capitata* f. *rubra*) is a rich source of acylated anthocyanins, well known for their antioxidant properties [1]. The combination of high-performance liquid chromatography (HPLC) with high-resolution tandem mass spectrometry (HRMS/MS) represents a powerful analytical approach for the characterization of these compounds. Coupling this technique with the 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) assay, a peroxy radical generator at 37 °C and pH 7.4, enables the evaluation of the radical-scavenging reactivity of individual anthocyanins [2]. Beyond their antioxidant activity, red cabbage anthocyanins may also improve the stability of oxidation-sensitive bioactive compounds such as carotenoids, which are susceptible to thermal and oxidative degradation [3]. These findings support the use of red cabbage anthocyanins for clean-label product development in functional food and cosmetics applications.

Experimental

Red cabbage anthocyanins were analyzed using a Dionex Ultimate 3000 HPLC system coupled to a Q Exactive™ Orbitrap high-resolution mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Separation was performed on a Kinetex Biphenyl column (100 × 2.1 mm, 2.6 µm), with DAD detection at 520 nm. The mass spectrometer was operated in positive electrospray ionization (ESI+) mode with a spray voltage of 3.5 kV and capillary temperature of 350 °C. Full-scan spectra were acquired over the *m/z* 100–1500 range at a resolution of 70,000, with an AGC target of 1×10^6 and a maximum injection time of 50 ms. Data-dependent MS/MS acquisition was performed at a resolution of 17,500, using an AGC target of 5×10^5 , maximum injection time of 50 ms, loop count of 5, isolation window of 4.0 *m/z* (offset 1 *m/z*), and normalized collision energy (NCE) of 15%. Carotenoids were analyzed using the same HPLC-Orbitrap platform equipped with an atmospheric pressure chemical ionization (APCI) source operating in positive ionization mode. Chromatographic separation was achieved on a Develosil C30 column (150 × 2.0 mm, 3 µm). Full-scan spectra were acquired over the *m/z* 100–1500 range at a resolution of 70,000, with an AGC target of 3×10^6 and a maximum injection time of 150 ms. Data-dependent MS/MS spectra were acquired at a resolution of 17,500, using an AGC target of 1×10^5 , maximum injection time of 50 ms, loop count of 5, isolation window of 1.0 *m/z*, and NCE of 30%.

Results

Anthocyanins from red cabbage were tentatively identified based on their accurate mass, fragmentation patterns, and retention time. Thirteen cyanidin-derivatives were detected and quantified. For the reaction with AAPH, every 40 minutes and up to 200 minutes, aliquots were taken and immediately injected into the HPLC-HRMS/MS to test the reactivity of single anthocyanins. The degradation of individual anthocyanins was monitored by HPLC, and reaction rates (nM/min) were calculated from peak area decrease over time considering the slope of the best fitting curve of concentration vs time plots. Results of the kinetic study showed that cyanidin- 3-(disinapoyl)-diglucoside-5-glucoside exhibited the fastest degradation showing the highest reactivity toward AAPH-derived radicals under the tested conditions with the radical initiator.

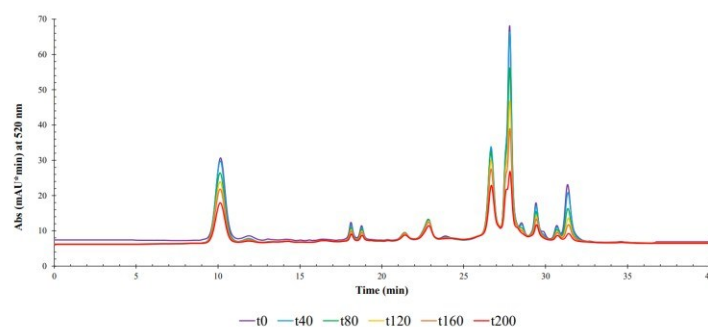


Figure 1. Chromatograms of red cabbage extract measured at 520 nm with HPLC-DAD-ESI-HRMS/MS at time 0 and every 40 minutes up to 200 minutes during the reaction with AAPH radical initiator.

To support the findings from AAPH-induced degradation, the red cabbage extract was further analyzed using high-performance liquid chromatography coupled with diode array and coulometric array detectors (HPLC-DAD-CAD). The CoulArray detector operates by sequentially oxidizing compounds across a fixed range of potentials, producing hydrodynamic voltammograms (HDVs) that plot signal intensity as a function of potential. From these, the half-wave potential ($E_{1/2}$) is derived, reflecting the redox equilibrium between oxidized and reduced forms. Lower $E_{1/2}$ values indicate greater electron-donating capacity, and thus higher antioxidant potential [4]. Correlation between the results obtained from the reaction with AAPH and CAD analysis was evaluated and the Pearson correlation coefficient (r) was determined. The analysis revealed a strong correlation ($|r| > 0.8$) indicating a high degree of consistency between the two datasets [5].

The stability of five carotenoid standards was evaluated under different storage conditions. The experimental design evaluated the effect of temperature (5, 25, and 45 °C), oxygen presence, and anthocyanin addition on carotenoid degradation over 48 h. Anthocyanins significantly improved carotenoid retention under thermal and oxidative stress, with the greatest protective effect observed at 45 °C. PERMANOVA analysis identified the Temperature $\times$ Anthocyanins interaction as the main factor influencing carotenoid stability. The protective effect was further confirmed in *Calendula officinalis* petal extract, where anthocyanin addition significantly increased the retention of all major carotenoids after 72 h at 45 °C, demonstrating the applicability of red cabbage extract as a natural stabilizing system in a complex matrix.

Conclusions

AAPH-based degradation kinetics coupled with HPLC-HRMS/MS provided a comprehensive approach to investigate the antioxidant properties of red cabbage extract. Anthocyanin reactivity was strongly influenced by acylation, glycosylation, and isomeric structure, highlighting the relationship between molecular structure and antioxidant activity. Electrochemical analysis further confirmed these findings, showing that anthocyanins with higher degradation rates exhibited lower half-wave potentials, indicative of greater electron-donating ability. The extract also significantly improved carotenoid stability under thermal and oxidative stress, with the greatest protective effect observed at 45 °C, and this effect was confirmed in *Calendula officinalis* extracts. Overall, these findings highlight the potential of red cabbage extract as a natural antioxidant ingredient for clean-label food applications.

References

1. R. Mattioli, A. Francioso, L. Mosca, P. Silva; *Molecules*, 25 (2020).
2. G.-D. Zhuang, W.-T. Gu, S.-H. Xu, D.-M. Cao, S.-M. Deng, Y.-S. Chen, S.-M. Wang, D. Tang; *Food Chemistry*, 401 (2023).
3. S. Aslam, M. Ahmad, M. Riaz; in M. Zia-UI-Haq, S. Dewanjee, M. Riaz editors, *Carotenoids: Structure and Function in the Human Body*, Springer, Cham, 2021, pp. 171–191.
4. L. Angeli, F. Populin, K. Morozova, Y. Ding, U. Asma, S. Bolchini, A. Cebulj, N. Busatto, F. Costa, G. Ferrentino, M. Scampicchio; *Food Bioscience*, 58 (2024).
5. C. Lombroni, L. Angeli, M. Scampicchio, G. Ferrentino, K. Morozova; *Food Chemistry*, 508 (2026).

POSTER COMMUNICATIONS

P1

From dairy by-product to functional ingredient: valorization of mozzarella stretching water as a source of bioactive peptides through microbial fermentation

Antonella Lamonaca, Alessia Marzulli, Marianna de Gioia, Loris Pinto, Rosa Pilolli*

National Research Council of Italy, Institute of Sciences of Food Production (CNR-ISPA),
via G. Amendola 122/O, 70126, Bari

Summary: Mozzarella stretching water was valorised as a sustainable source of functional metabolites, including bioactive peptides identified by HR-MS/MS. Microbial fermentation with *Lactocaseibacillus* OI20 reshaped its peptidomic profile, generating the most interesting functional profile, enhancing the release, or production, of bioactive sequences with multiple health-promoting properties.

Keywords: dairy by-products, discovery proteomics, bioactive peptides

Introduction

The production of stretched cheeses, such as mozzarella, scamorza provolone and caciocavallo, generates large volumes of cheese stretching water, that is the hot water responsible for casein starching of curd, a nutrient-rich by-product usually disposed despite its high content of residual nutrients and bioactive compounds. In the context of circular economy and sustainable food systems, the valorisation of these streams represents an attractive strategy to reduce environmental impact while promoting alternative sources of high-value formulations for food or biotechnological applications. In addition, microbial fermentation has consolidated its role as transition enhancer of dairy by-products into functional matrices enriched with bioactive compounds. Indeed, the metabolic activity of selected microorganisms can release functional compounds and/or bioactive peptides from milk proteins, with potential antioxidant, antihypertensive, antimicrobial, and anti-inflammatory properties [1].

The aim of this study was to investigate the functional potential of mozzarella stretching water (SW) by untargeted high resolution tandem mass spectrometry (HR-MS/MS) and identify microbial strains able to enhance the release of bioactive peptides during fermentation. Microbial fermentation was carried out using autochthonous lactic acid bacteria; in particular, *Streptococcus thermophilus* ST3 (ST) and *Limosilactobacillus fermentum* T0M1 (LF), previously isolated from cheeses and characterized for their technological properties, and two strains [2], and *Lactococcus lactis* OI1 (LL) and *Lactocaseibacillus paracasei* OI20 (LP), isolated from filtered SW, were included in the study.

Experimental

Stretching waters (SWs) by-products collection and microbial fermentation. SWs were collected during Mozzarella cheese processing made by direct-acidification at the Azinda Casearia Capurso dairy plant, in Apulia region. After fat and cheese particles removal by centrifugation (10,000g, 10 min, 4°C), SW were gauze filtered, sterilized (120°C for 15 min) and cold stored until use. Before strain inoculation, denaturated proteins were removed by centrifugation (10,000g, 10 min, 4°C).

SWs inoculated at 4-5 log cfu mL⁻¹, were fermented as reported in Table 1-

Table 1. Coding of SW samples subjected to different microbial fermentations

<u>Samples (Stretching water. SW)</u>	<u>Coding</u>
SW – CTRL, time 48h	CTRL T48
SW fermented with LF, time 48h,	T48-LF
SW fermented with LL, time 48h,	T48-LL
SW fermented with LP, time 48h,	T48-LP
SW fermented with ST, time 48h,	T48-ST

Sample preparation for LC-MS analysis. 10 mL mozzarella SW was centrifuged (13000g, 10 min), and the supernatant was collected and filtered through a 0.45 µm regenerated cellulose membrane. The filtrate was subjected to ultrafiltration using 3 kDa MWCO centrifugal filters (Amicon® Ultra, 14000g, 30 min) to remove high-molecular-weight components. The resulting <3 kDa fraction was purified by solid-phase extraction (Sep-Pak C18 cartridges) according to the following steps: (i) conditioning with acetonitrile (ii) equilibration with 0.1% formic acid, (iii) sample loading (iv) washing, and (v) elution with acidified acetonitrile/water (80:20v/v, 0.1% formic acid). The purified fraction was dried under nitrogen and reconstituted in mobile phase, achieving a 10-fold concentration factor prior to LC-MS analysis.

Untargeted LC-MS analysis. Untargeted high resolution tandem mass spectrometry (HR-MS/MS) was applied based on data dependent acquisition mode (Full-MS/dd2) on a hybrid quadrupole/orbitrap mass spectrometer (Q-Exactive plus, Thermo Fisher Scientific). Chromatographic separation was accomplished with an Ultimate 3000 UHPLC system (Thermo Fisher Scientific) on an Acclaim PepMap100 C18 column (3 μ m, 1 $\times$ 150 mm) using a binary gradient of water and acetonitrile, both containing 0.1% formic acid, at a flow rate of 60 μ L/min and an injection volume of 10 μ L. Smaller peptides (2–4 amino acids) were identified using Compound Discoverer™ 3.0, whereas longer sequences (5–30 amino acids) were identified using Proteome Discoverer™ 3.0 with the Sequest HT search engine, applying nonspecific cleavage settings against a Bos taurus protein database retrieved from UniProt. The identified peptide sequences were subsequently screened in silico against the BIOPEP-UWM database using exact sequence matching to predict potential biological activities.

Results

Mozzarella SW obtained by direct acidification was subjected to lactic acid bacteria fermentation. All strains increased their cell load of 1-2 orders of magnitude in the SWs after 48 h of incubation, reducing the pH values from 6-7 (control samples) to 4-5. *L. paracasei* OI20 showed a limited acidifying activity in SW.

HR-MS/MS analysis of raw SWs provided a wide spectrum of metabolites with potential functional properties. Among these, focus was placed on peptide sequences deriving from residual milk proteins. To investigate the functional and applicative relevance of the observed peptidomic profile, an in silico bioinformatic analysis was performed. Interestingly, the application of microbial fermentation markedly affected the peptide profile of SWs with differential trends depending on strain and peptide lengths (see Figure 1). Analysis of short peptides (2–4 amino acids) showed that fermentations with LL and especially LP induced substantial changes compared with the non-inoculated control, whereas ST and LF produced minor effects. On the contrary analysis of longer peptides (5-30 amino acids) disclosed wider modifications determined by LL, ST, and LP, respectively, whereas no overall trend was observed after LF fermentation. A pairwise differential analysis was performed on selected representative samples, reporting only statistically significant changes ($|\log_2(\text{Fold Change})| \geq 1$, $p < 0.05$), and correlating the reported differences to biologically relevant sequences according to the BIOPEP-UWM database.

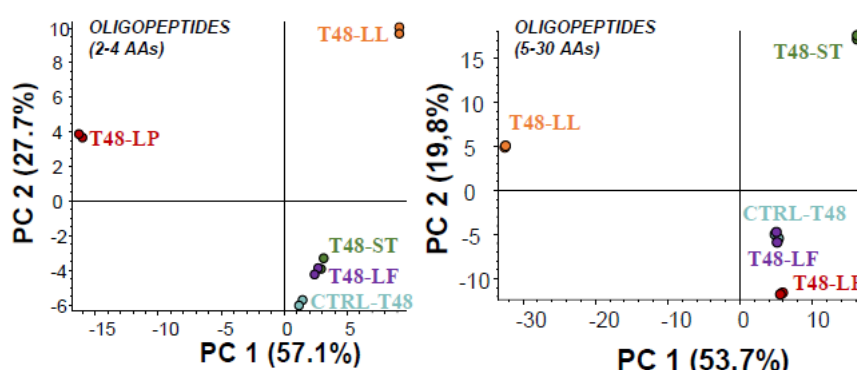


Fig. 1. Principal component analysis (PCA) based on oligopeptides abundances (a.u.) identified in mozzarella SWs samples subjected to different fermentation processes.

Among the tested strains, *L. paracasei* OI20 generated the most favorable profile, increasing the abundance of several peptides matching bioactive sequences. These peptides are associated with antioxidant, ACE-inhibitory, antimicrobial, osteoanabolic, and other health-promoting activities. Such trend was confirmed by replication of the microbial fermentation on three independent production replicates of SW water collected in different days.

Conclusions

Mozzarella SW water represents a sustainable source of health-promoting functional molecules and, in particular, bioactive peptides. Microbial fermentation of directly acidified SW enhanced peptide diversity and bioactive peptide abundance, with *L. paracasei* OI20 emerging as the most promising strain for the valorization of this dairy by-product. The preliminary results obtained in this investigation supported the value of dairy by-products in the development of novel functional formulations for food and nutraceutical applications.

Acknowledgement

This work was supported by the PROGRADE Project “Applications of foodgrade dairy process water” prog. N° F/250010/01-05/X50 funded by the Ministry of Economic Development of Italy

References

1. P. Kashung, T. Karuthapandian Food Production, Processing and Nutrition (2025) 7:6
2. Baruzzi F., et al. 8th International Conference on Microbial Diversity "Microbial diversity for empowering the ecological transition", 23- 26/09/2025, Rome, Italy. BoA pag. O5-3.

P2

Data fusion of the non-volatile and volatile metabolome for shelf-life and sensory quality prediction of skim milk powder

Giulia Gula,¹ Luigi Locuratolo,² Franco Rosso,² Andrea Peraino,² Augusta Caligiani¹

¹ Food and Drug Department, University of Parma, Parco Area delle Scienze 27/A, 43124 Parma, Italy

² Soremartec Italia Srl, Ferrero Group, Piazzale Pietro Ferrero 1, 12051 Alba, CN, Italy

Summary: *The research investigated chemical and sensory changes in skim milk powder during shelf life. The non-volatile and volatile metabolome was characterized by LC- and GC-HRMS, respectively. Mid-level data fusion integrated chemical and sensory data. The project aims to identify rapid, objective quality markers for raw material assessment.*

Keywords: *Skim milk powder; Metabolome; Mid-level data fusion*

Introduction

Skim milk powder (SMP) and whole milk powder (WMP) are widely used in the food manufacturing sector, particularly in confectionery, infant formula, bakery products, beverages, dairy products, and nutritional supplements. According to the OECD–FAO Agricultural Outlook 2025–2034, global production of SMP is expected to increase from about 4.6 million tonnes to 5.3 million tonnes by 2034, highlighting its increasing importance in the global dairy market [1]. Skim milk powder contains less than 1.5% fat (w/w); nevertheless, it remains susceptible to quality deterioration during storage. Changes in temperature, oxygen exposure, humidity, packaging conditions, and storage time can cause a rapid deterioration in milk powder quality. The main reactions that may occur include lipid oxidation, the Maillard reaction, protein modifications, and interactions among these processes [6]. Metabolomics, a branch of systems biology, investigates a wide range of metabolites with diverse chemical properties, providing comprehensive information on the chemical composition of biological systems [3]. Data fusion techniques play a key role in metabolomics, enabling the integration of complementary analytical platforms and enhancing biomarker identification [2]. This study combines LC-HRMS and GC-QTOF-MS to characterize the non-volatile and volatile metabolome, respectively, and investigate chemical changes associated with sensory quality. Mid-level data fusion was subsequently applied to integrate the complementary datasets and assess the ability of metabolomic profiles to predict sensory changes during storage.

Materials and Methods

Skim milk powder samples from three production lots (two suppliers) were analysed at nine shelf-life time points (1, 5, 6, 7, 8, 9, 10, 11, and 12 months), with five analytical replicates per lot at each time point. Before analysis, all samples were stored at –20 °C to preserve their chemical stability.

Sample preparation and extraction

Liquid chromatography analysis :Metabolites were extracted following Rocchetti et al. (2016) with minor modifications. Blank and QC samples were included for quality control [4]. Metabolomic profiling was performed by UHPLC-Q-HRMS (Orbitrap Exploris 120) in Full Scan H-ESI+ mode.

Gas chromatography analysis: A preliminary study was carried out to optimize sample preparation for HS-SPME analysis using hexanal-d12 as internal standard. Different preparation methods were compared, and volatile metabolites were analyzed by HS-SPME/GC-QTOF-MS in Full Scan EI+ mode [5].

Sensory analysis: sensory evaluation was carried out by 45 trained panelists using Quantitative Descriptive Analysis (QDA). A total of 10 sensory descriptors were evaluated, including milk, sweet, salty, sour, cooked, cardboard, grassy, oxidized/rancid, cheese, and animal notes. Samples were evaluated using an intensity scale, and the obtained sensory profile was used for subsequent statistical analysis.

Statistical analysis

Mid-level data fusion (MLDF) is a two-step methodology in which either latent variables or selected features are first extracted independently from each analytical dataset and then concatenated into a single fused matrix for further modelling [2].

In this study, a feature selection approach was adopted. Prior to statistical analysis, all datasets were pre-processed by log transformation [$\log_{10}(x + 1)$] followed by unit variance (UV) scaling. The dataset was then divided into training (70%) and test (30%) sets without splitting technical replicates between subsets.

Since the LC-MS dataset contained more than 3,000 detected features, Variable Importance in Projection (VIP) scores were used to reduce data dimensionality and retain the most informative variables. A cut-off of $VIP \geq 2$ was applied to the LC-MS dataset, reducing the variables from over 3,000 to 30, while a cut-off of $VIP \geq 1$ was applied to the GC-MS dataset, resulting in 18 selected variables. The 48 selected variables were then

concatenated into a single fused dataset and used to develop Partial Least Squares (PLS) models to predict sensory attributes. Model robustness was assessed using this fixed independent test set maintained throughout the workflow and 1,000 permutation tests to exclude chance correlations.

Results

Sensory quality progressively declined during storage, with a pronounced deterioration from the 10th month onward. Initial differences between suppliers became negligible by the end of shelf life.

All PLS models showed good predictive performance and were statistically significant (permutation test, $p < 0.001$). The LC-MS model achieved good calibration and prediction ($Q^2_{CV} = 0.822$; $Q^2_{ext} = 0.678$), while the GC-MS model showed the highest external predictive performance ($R^2_{test} = 0.883$; $Q^2_{ext} = 0.837$) and the lowest prediction error ($RMSE_{test} = 1.38$). The mid-level data fusion model provided the best overall performance, combining the highest cross-validation ability ($Q^2_{CV} = 0.878$), excellent external prediction ($Q^2_{ext} = 0.822$), and the lowest training error ($RMSE_{train} = 0.807$) (Figure 1).

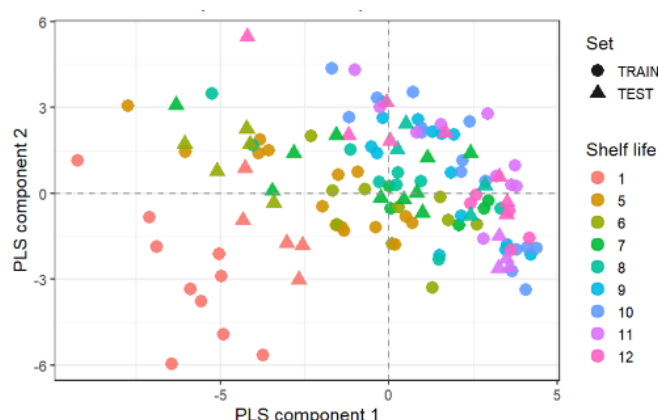


Figure 1. PLS score plot of the final mid-level data fusion model based on the selected VIP variables.

Finally, the robustness of the fused model was confirmed by 1,000 group-wise random train/test splits, which produced very similar performance metrics, demonstrating the stability and reproducibility of the predictive model. The GC-MS correlation network showed that shelf life was associated with several volatile compounds belonging to different chemical classes. Among the most relevant VOCs were aldehydes (pentanal, nonanal, benzaldehyde, 2-methylbutanal and 3-methylbutanal), alcohols (1-pentanol, 1-hexanol, 1-octanol and 1-octen-3-ol), ketones (2-butanone and acetone), together with 2-pentylfuran, dimethyl sulfide and 3-methylbutanoic acid. The prevalence of lipid oxidation products, particularly aldehydes and 2-pentylfuran, indicates that oxidative reactions are major drivers of the volatile changes occurring during storage (Figure 2).

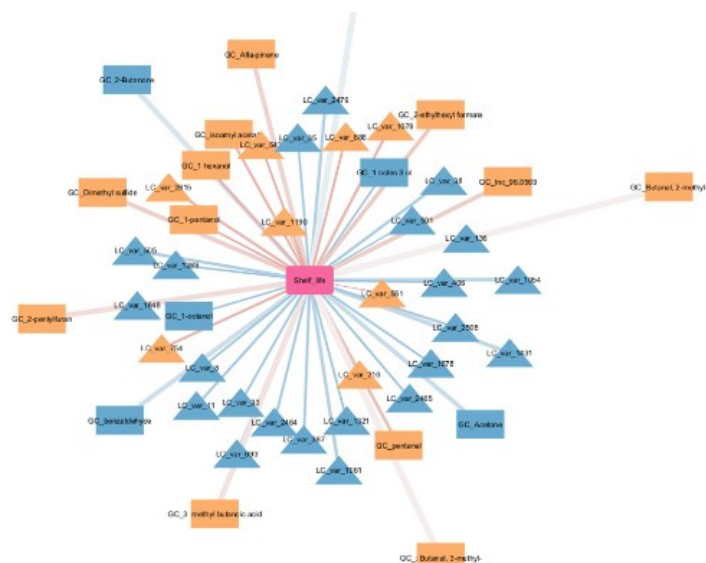


Figure 2. Correlation network between the selected LC-MS and GC-MS VIP variables and shelf life. Edge colour indicates the direction of the correlation (red = positive, blue = negative), while edge thickness reflects the correlation strength. The network highlights metabolites most strongly associated with shelf-life progression.

The predictive performance varied among the sensory attributes. The best results were obtained for sweet ($R^2_{\text{test}} = 0.848$, $Q^2_{\text{ext}} = 0.793$) and cooked ($R^2_{\text{test}} = 0.780$, $Q^2_{\text{ext}} = 0.775$), indicating a strong relationship between the metabolomic profiles and these sensory characteristics. Good predictive performance was also achieved for milk ($R^2_{\text{test}} = 0.864$, $Q^2_{\text{ext}} = 0.587$) and cardboard ($R^2_{\text{test}} = 0.767$, $Q^2_{\text{ext}} = 0.535$). The next step of this work will be the structural annotation and identification of the most relevant LC-MS markers by acquiring MS/MS fragmentation data, allowing the biological interpretation of the variables contributing most to the predictive models.

References

1. OECD/FAO, OECD Publishing, Paris 2025.
2. S. Squara, A. Caratti, A. Fina, E. Liberto, N. Koljančić, I. Špánik, G. Genova, G. Castello, C. Bicchi, A. de Villiers e C. Cordero, Food Research International, p. 114873, 2024.
3. Collins, Annual Review of Analytical Chemistry, 2021.
4. Gabriele Rocchetti, Food Chemistry, vol. 211, p. 287–295, 2016.
5. Cheng, Foods, vol. 10 (9), n. 2061, 2021.
6. G. W. Smithers, In Encyclopedia of Dairy Sciences , vol. 5, pp. 195-212, 2022.

P3

Aromatic and Fatty Acid Profiling of Aosta Valley Milk and Fontina PDO Cheese: The Value of Summer Alpine Pasture

Thedy Laura,¹ Luca Verneti-Prot,¹ Sabina Valentini¹

¹ Institut Agricole Régional, Reg. La Rochère 1/A, 11100 Aosta, Italy

Summary: *This study evaluates the influence of summer alpine pastures on milk and Fontina PDO cheese. Alpine grazing significantly reduced saturated fatty acids, enriched health-promoting fatty acids (CLA, ω 3) and enhanced aromatic complexity, demonstrating how preserving pasture biodiversity is essential to support the unique nutritional and sensory quality of mountain dairy products.*

Keywords: *Fontina PDO cheese, Alpine pasture, Fatty acids & VOCs*

Introduction

Fontina is a traditional Italian semi-hard and semi-cooked PDO cheese produced in the Aosta Valley from Valdostana native cow breed raw milk. Fontina PDO cheeses were ripened for at least 3 months in natural caves. The local dairy value chain relies on seasonal transhumance, shifting from winter indoor feeding to summer grazing on high-altitude alpine pastures. Cattle diet and alpine botanical diversity are crucial in defining the nutritional and organoleptic quality of dairy products. Fresh forage intake positively modulates the lipid fraction, increasing polyunsaturated fatty acids (PUFAs) and conjugated linoleic acid (CLA) [1], while grazing modifies the volatile organic compound fractions, transferring secondary plant metabolites like terpenes directly into the matrix [2]. To comprehensively explore this system, the aim of this work was to evaluate the seasonal transition from winter feeding to summer alpine pasture, characterizing and comparing both the aromatic and fatty acid profiles of raw bulk milk and its processed derivative, Fontina PDO cheese. This approach serves to scientifically validate mountain pastoral practices and protect unique traditional products.

Experimental

Bulk raw milk samples and the corresponding Fontina PDO cheeses at 90 days of ripening were collected over three consecutive years, performing three sampling trials per year. For the summer season, sampling was performed across three different mountain pastures (A1, A2, A3 all located above 2300 m a.s.l.), while winter reference samples were collected from a valley-floor dairy farm. The samples were analysed in duplicate or triplicate depending on the type of determination. The analysis of fatty acid composition from milk and cheese matrices via GC-MS and GC-FID was carried out according to Jiang et al. (1996) [3] and FIL-IDF 182:1999 [4] for the extraction of the lipid component and following Cadiddu et al. (2009) [5] for the qualitative and quantitative determination of the extracted fatty acid methyl esters. The qualitative and semi-quantitative evaluation of the volatile organic compounds (VOCs) profile was carried out using SPME-GC-MS, following Bergamaschi and Bittante (2017) [6] with minor modifications. Subsequently, the alpine cheeses were subjected to hedonic and sensory analysis.

Results

Statistical analysis revealed significant differences ($p < 0.05$, Tukey's HSD test) between winter and summer feeding. Both raw bulk milk and Fontina PDO cheese produced in mountain pastures showed a significant reduction in saturated fatty acids (SFAs) - specifically lauric, myristic, and palmitic acids - coupled with a substantial increase in monounsaturated (MUFAs) and PUFAs. Notably, summer products exhibited exceptionally high levels of conjugated linoleic acid (mostly represented by the bioactive 9c,11t isomer) and ω 3 fatty acids, along with an optimal ω 6/ ω 3 ratio below the recommended dietary threshold. Qualitative analysis identified 40 VOCs in summer milk compared to 30 in winter milk. This richness is directly reflected in the resulting Fontina PDO cheeses in which 53 VOCs were characterized. Terpenes were exclusively detected in summer productions, confirming the direct transfer of secondary plant metabolites from the specific forage species characterizing each pasture into the dairy product. Comparing the three alpine dairy farms, pasture-specific markers emerged. A1 pasture yielded the highest nutritional value, achieving the highest concentrations of CLA (mean value 2.00 g/100 g fat) and the best ω 6/ ω 3 ratio in both milk and cheese (mean value 1.40). Conversely, A3 pasture excelled in providing greater aromatic richness; the summer milk collected in this mountain pasture was highly fragrant, and its final Fontina PDO cheese was significantly enriched in VOCs, particularly alcohols (ethanol and 3-methyl-butanol), esters (ethyl acetate), and terpenes (α -pinene), imparting intense fruity aromatic notes. Hedonic data confirmed that alpine grazing guarantees product typicality and compliance with PDO standards. Sensory analysis identified 12 descriptors characterizing

highland Fontina PDO cheese.

Conclusions

In conclusion, this study confirms that a strict correlation exists between the aromatic and fatty acid composition of the animal diet, milk and dairy products. Therefore, the distinctive profile of alpine milk and cheese fully justifies the practice of summer transhumance. Promoting the maintenance and enhancement of the biodiversity typical of alpine pastures is essential to preserve these unique characteristics, ultimately ensuring the high-quality standards of mountain Fontina PDO cheese that are highly appreciated by consumers.

References

1. S. Buchin, B. Martin, D. Dupont, A. Bonard, C. Achilleos; *Journal of Dairy Research*, 66(4) (1999), pp 579-588.
2. L.M. Battaglini, S. Bosco, V. Malfatto, C. Lussiana, M. Renna; *Scienza e tecnica lattiero-casearia*, 59(6) (2008), pp 487-490.
3. J. Jiang, L. Bjoerck, R. Fondén, M. Emanuelson; *Journal of Dairy Science*, 79 (1996), pp 438-445.
4. International Dairy Federation; *IDF Standard 182*, (1999).
5. A. Cabiddu, G. Molle, M. Decandia, S. Spada, M. Fiori, G. Piredda, M. Addis; *Livestock Science*, 123(2-3) (2009), pp 230-240.
6. M. Bergamaschi, G. Bittante; *Journal of Dairy Science*, 101(5) (2018), pp 3918-3934.

Acknowledgements

This work was financially supported, through TYPICALP project, by European Union, European Regional Development Fund, Italian State, Swiss Confederation and Cantons co-financed operation, within the framework of the Interreg V-A Italy-Switzerland Cooperation Programme.

P4

Comparative metabolite profiling of fourteen aromatic herbs using targeted and untargeted mass spectrometry approaches, combined with chromatographic separation techniques.

Paola Di Matteo, Teresa Bovenzi, Mario Borrelli, Rita Petrucci

Dip. SBAI, Sapienza Università di Roma, Via del Castro Laurenziano 7 – 00161 – Rome, Italy

Summary: *The phytochemical composition of 14 culinary herbs was investigated by HPLC-ESI-MS/MS, ESI-MS/MS, UHPLC-ESI-QTOF, and HS-SPME-GC-MS. A comprehensive metabolite profile was provided for each herb, followed by comparative statistical analyses, evidencing the metabolic variability, discriminant markers, differences and similarities among botanical families and among herbs belonging to the same family.*

Keywords: *Polyphenols targeted analysis; mass spectral fingerprint; HS-SPME-GC-MS volatile profile*

Introduction

Culinary herbs have been widely used since ancient times as food ingredients, thanks to their organoleptic and preservative properties, as well as in traditional medicine, and their application has recently expanded across the food, pharmaceutical and cosmetic industries. Driven by the growing interest in plant-derived bioactive compounds, they have attracted increasing attention as sources of health-promoting compounds, particularly because they can be easily incorporated into the daily diet, within the Mediterranean diet pattern. Properties such as antidiabetic, anti-hypertensive, anti-depression, antioxidant, antimicrobial, anti-inflammatory, and neuroprotective activities [1], among others, are mainly attributed to natural phenolics [2], plant secondary metabolites whose health-promoting effects are widely recognized, as well as to other bioactive compounds.

The purpose of this study was to investigate the phytochemical composition, and provide comparative metabolite profiles of fourteen dried herbs, ready for consumer use. The herbs were provided by the Italian Vitaletti Academy (Italpepe). The selected species included dill, tarragon, chives, marjoram, pennyroyal, sage, thyme, oregano, rosemary, basil, mint, parsley, laurel, lemongrass.

Experimental

Each herb sample was analysed for its polar fraction using integrated mass spectrometry techniques coupled and not with chromatographic separation, and for its volatile fraction using HS-SPME coupled with GC-MS. The polyphenols targeted analysis of the optimized aqueous and hydroalcoholic extracts was carried out by HPLC-ESI-MS operating in SIR mode, using a method previously optimized [3].

The untargeted analysis of the same extracts was carried out by HPLC-ESI-MS/MS in full-scan mode, ESI-MS/MS direct infusion (DI) experiments, and UHPLC-DAD-ESI-QTOF.

The volatile fraction untargeted analysis was carried out by HS-SPME coupled with GC-MS, supported by the NIST library.

Results and Conclusions

The targeted analysis revealed characteristic phenolic profiles, with 28 compounds identified in at least one herb sample, and quantitative patterns strongly dependent on species and extraction solvent. PCA summarized the metabolic variability, and highlighted discriminant markers useful for taxonomic and chemotypic classification.

The integrated untargeted approach by ESI-MS/MS DI, HPLC-ESI-MS/MS, and UHPLC-DAD-ESI-QTOF (Fig.1,2), expanded the chemical characterization to additional classes of not-volatile compounds, such as amino acids, saccharides, organic acids, and other phenolics and glycosides that were species-specific.

The HS-SPME-GC-MS untargeted analysis (Fig. 3) showed distinctive aromatic profiles, and MCA effectively highlighted differences and similarities among botanical families, as well as among herbs belonging to the same family.

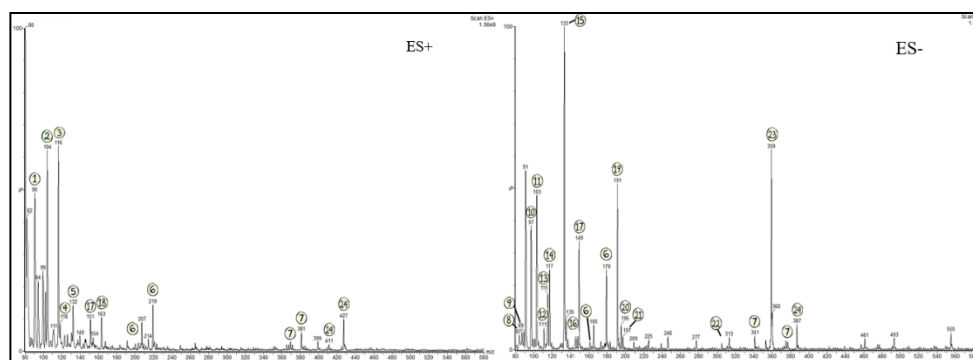


Fig. 5. Sage, mass spectral fingerprint by ESI-MS/MS. Amino acids: Ala **1**, GABA **2**, Pro **3**, Val **4**, Leu/Iso **5**; Saccharides: hexose **6**, dihexose **7**; Organic acids and derivatives: pyruvic a. **8**, lactic a. **9**, phosphoric a. **10**, malonic a. **11**, 2-furoic a. **12**, fumaric a. **13**, succinic a. **14**, malic a. **15**, 2-chetoglutaric a. **16**, tartaric a. **17**, malic a. ethyl ester **18**, citric a. **19**, gluconic a. **20**, β -hydroxy-di-hydrocaffeic a. **21**, sulfo jasmonate **22**, rosmarinic a. **23**, tuberonic a. glucoside **24**.

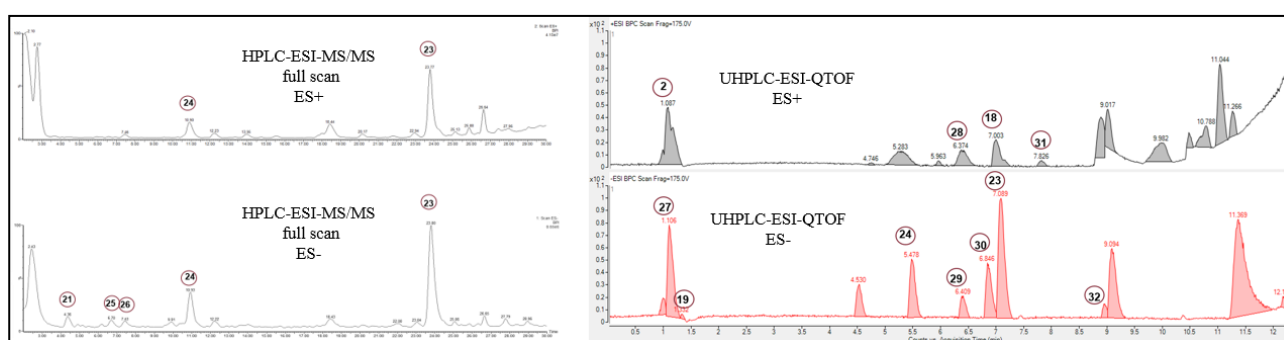


Fig. 6. Sage, untargeted analyses. HPLC-ESI-MS/MS in full scan (left): β -hydroxy-di-hydrocaffeic a. **21**, mono-coumaroyl tartaric a. **25**, mono-feruloyl tartaric a. **26**, tuberonic a. glucoside **24**, rosmarinic a. **23**. UHPLC-ESI-QTOF (right): GABA **2**, malayamycin A **27**, citric a. **19**, tuberonic a. glucoside **24**, Gly-Gly-Arg **28**, scutellarin **29**, salvianolic a. K **30**, citric a. **18**, rosmarinic a. **23**, Lys-Gly-Lys-Gly **31**, N-undecanoyl glycine **32**.

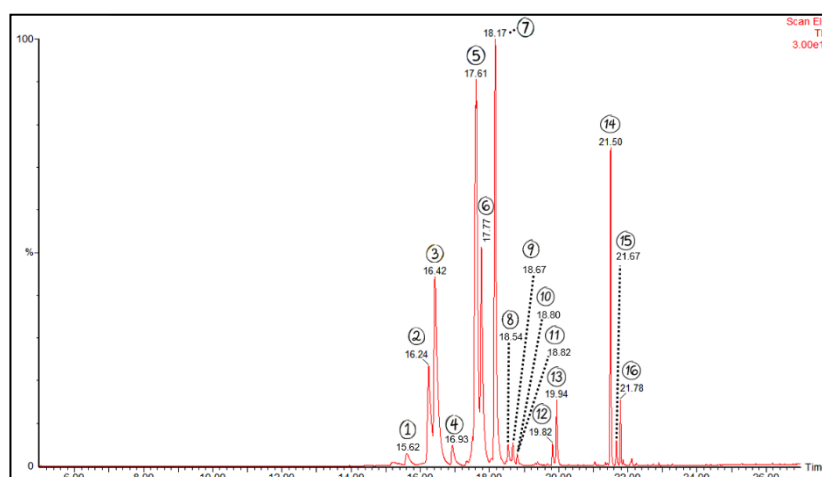


Fig. 7. Sage, untargeted analysis of the volatile fraction by HS-SPME-GC-MS: β -myrcene **1**, o-cimene **2**, eucalyptol **3**, γ -terpinene **4**, isothujone **5**, 3-thujanone **6**, (+)-2-bornanone **7**, (-)-borneol **8**, terpinen-4-ol **9**, α -fenchene hydrate **10**, estragole **11**, anethole **12**, bornyl acetate **13**, β -caryophyllene **14**, aromadendrene **15**, humulene **16**.

References

1. Ali, A. Bashmil, Y.M., Cottrell, J.J., Suleria, H.A.R., Dunshea, F.R. *Antioxidants* 10 (2021) p.1770
2. Petrucci, R., Di Matteo, P., Sobolev, A.P., Liguori, L., Albanese, D., Proietti, N., Bortolami, M., Russo, P. *J Agric Food Chem* 69 (2021) pp. 4816–4826
3. Bortolami, M., Di Matteo, P., Rocco, D., Feroci, M., Petrucci, R. *Molecules* 27 (2022), p. 4962

P5

Evaluation of Cherry and Datterino tomatoes freshness and variety using a multi-analytical platform: combining PTR-ToF-MS, HS-SPME-GC-MS, and two portable electronic noses

J. Zhang,^{1,2,3} I. Khomenko,³ M. Tonezzer,³ E. Betta,³ M. Pedrotti,³ A. Romano,³ R. Capuano,⁴
A. Catini,⁴ C. Di Natale,⁴ F. Biasioli³

¹ Università di Cagliari, Dipartimento di Scienze Chimiche e Geologiche, Campus di Monserrato
09042 Monserrato, Italy

² Politecnico di Torino, Dipartimento di Scienza Applicata e Tecnologia,
Corso Duca Degli Abruzzi 24, 10129 Torino, Italy

³ Fondazione Edmund Mach, Centro Ricerca e Innovazione, 38098 San Michele all'Adige (TN), Italy

⁴ University of Rome Tor Vergata, Dipartimento di Ingegneria Elettronica
Via del Politecnico 1, 00133, Roma, Italy

Summary: This study evaluates the post-harvest freshness kinetics and varietal differentiation of Cherry and Datterino tomatoes using a comprehensive *multi-analytical platform*. By combining the high-resolution capabilities of PTR-ToF-MS and HS-SPME-GC-MS with the rapid tracking of two portable electronic noses (a thermal gradient chemoresistive system and a QMB array), this multi-technique approach successfully monitors freshness evolution (days from harvest) and discriminates between the two tomato varieties, offering a robust and scalable framework for real-time agrifood quality monitoring.

Keywords: Direct injection techniques, Miniaturized electronic thermal nose, Food quality monitoring.

Introduction

Rapid and non-invasive monitoring of agrifood products is critical for ensuring food safety, optimizing supply chain sustainability, and maintaining high quality standards from farm to fork. While traditional analytical methods based on chemical separation are often destructive and time-consuming, direct injection techniques allow for consistent, real-time evaluation without compromising the product [1]. In this context, Proton-Transfer-Reaction Time-of-Flight Mass Spectrometry (PTR-ToF-MS) and Headspace Solid-Phase Microextraction Gas Chromatography-Mass Spectrometry (HS-SPME- GC-MS) represent state-of-the-art analytical techniques for high-sensitivity, non-destructive chemical validation [2]. However, transferring these capabilities to the industrial supply chain requires portable, low-cost alternatives. This study explores the synergy between benchtop mass spectrometry and two innovative, portable electronic nose (E-nose) architectures (a porphyrin-functionalized quartz microbalance (QMB) array and a miniaturized thermal gradient chemoresistor system [3]) to create an integrated framework for quality assessment [4].

Experimental

Two tomato varieties, Cherry and Datterino, were monitored daily for five consecutive days to track freshness degradation and metabolic divergence during their post-harvest. The dynamic headspace analysis was performed using a PTR-ToF-MS 8000 operated in H₃O⁺ mode, capturing mass spectra from m/z 21–400 every second for precise VOC quantification. Simultaneously, HS-SPME- GC-MS was utilized to identify specific odor- active volatile markers, with analytes desorbed at 240°C and separated on a DB-Wax UI column. Headspace gases were evaluated using two distinct portable systems: i) a QMB E-nose featuring with 12 porphyrin-coated sensors (connected in series with the PTR-ToF-MS setup) in Fig.1, and ii) a thermal E-nose (T-nose) based on a five-element chemoresistive array on a single chip, exploiting a thermal gradient to generate unique sensitivity footprints.

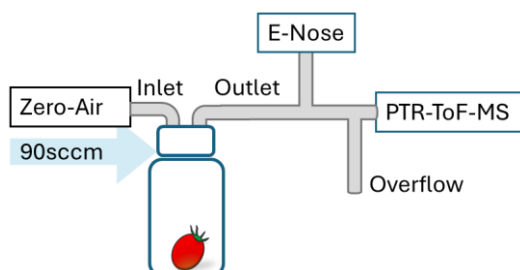


Fig. 1. Schematic diagram of the setup used

Results

The combined multi-analytical approach successfully characterized both the varietal identity and the shelf-life evolution (days post-harvest) of the samples. Mass spectrometry provided the chemical blueprint necessary to interpret and validate the responses of the portable sensors. PTR-ToF-MS established the baseline for tomato ripening and discrimination, identifying key volatile markers such as m/z 83.047 ($C_5H_6OH^+$, t.i. 2-methylfuran), and m/z 33.034 (CH_4OH^+ , t.i. methanol) [5], that drive the discrimination between varieties and track the aging kinetics in Fig.2.

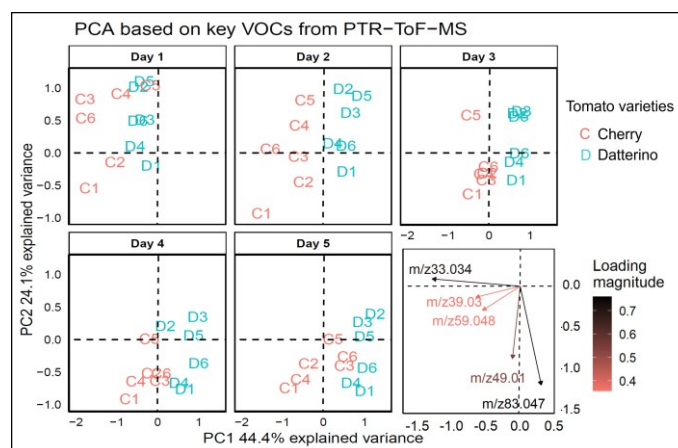


Fig. 2. Temporal evolution of tomato VOC profiles analyzed by PTR-ToF-MS. The first five panels show daily projections of PCA scores for the two varieties; the final panel shows the filtered-out loading vectors.

HS-SPME-GC-MS analysis identified 2- isobutylthiazole and dimethyl disulfide as key compounds distinguishing Cherry from Datterino tomatoes.

Notably, PTR-ToF-MS revealed that biological variability is established at harvest and remains a stable metabolic offset throughout shelf-life. While GC-MS showed superior discriminatory power, the E-noses demonstrated performance levels compatible with industrial requirements when calibrated against these MS benchmarks. The two portable electronic noses successfully mirrored the trends identified by the MS platforms. The miniaturized thermal E-nose demonstrated high sensitivity to the temporal VOC drift, effectively capturing the freshness kinetics over the days post-harvest. The QMB E-nose proved capable of distinguishing the subtle metabolic differences between Cherry and Datterino tomatoes as the post-harvest storage progressed.

Conclusions

This study highlights the powerful synergy between high-resolution mass spectrometry and portable gas-sensing technologies. While PTR- ToF-MS and GC-MS remain the cornerstone for full chemical validation and marker identification, the portable QMB and thermal electronic noses offer a rapid, non-invasive alternative for target applications in food analysis. Ultimately, all four techniques proved successful in assessing tomato variety and tracking freshness kinetics from the day of harvest, paving the way for cost-effective, real-time quality control solutions throughout the agrifood supply chain.

References

1. S. Natarajan, V. Ponnusamy. "Agri-Food Products Quality Assessment Methods". In: "Uddin, M.S., Bansal, J.C. (eds) Computer Vision and Machine Learning in Agriculture", Vol. 2. Algorithms for Intelligent Systems. Springer, Singapore (2022).
2. A.H. Gómez, G. Hu, J. Wang, A.G. Pereira, "Evaluation of tomato maturity by electronic nose", Computers and Electronics in Agriculture.54 (2006), pp 44–52.
3. M. Tonezzer, "Single Nanowire Gas Sensor Able to Distinguish Fish and Meat and Evaluate Their Degree of Freshness", Chemosensors 9 (2021) pp 249.
4. Y. Liu, C. Yang, Q. Wang, J. Zhang, L. Zhang Identification and confirmation of key compounds causing cooked off-flavor in heat-treated tomato juice. Journal of Food Science. (2022) 87(6):2515–2526.
5. B. Farneti, SM. Cristescu, G. Costa, FJM. Harren, EJ. Woltering. Rapid Tomato Volatile Profiling by Using Proton-Transfer Reaction Mass Spectrometry (PTR-MS). Journal of Food Science. (2012) 77(5).

P6

HPLC-MS/MS and ¹H-qNMR as complementary techniques for the characterization and quantification of shatavarins in *Asparagus racemosus* L.

Beatrice Zonfrillo,¹ Maria Bellumori,¹ Mohamad Khatib,¹ Lorenzo Cecchi,² Michele Zucherman,³ Gianluca Bartolucci,¹ Marta Menicatti,¹ Marzia Innocenti,¹ Nadia Mulinacci¹

¹ Department of Neurofarba, Nutraceutical and Pharmaceutical section, University of Florence, via Ugo Schiff 6, 50019, Sesto Fiorentino (Italy)

² Department of Agricultural, Food, Environmental, and Forestry Sciences and Technologies (DAGRI), University of Florence, via Donizetti 6, 50144 Firenze (Italy)

³ Hoefman-laboratoires, 10 Rue Maréchal Joffre, Nice, France

Summary: This study employed complementary HPLC-MS/MS and ¹H-qNMR approaches to characterize and quantify steroidal saponins (shatavarins) in a commercial *Asparagus racemosus* root dry extract. HPLC-MS/MS was used to define the shatavarins fragmentation pattern and to quantify their content. ¹H-NMR provided the organic matter fingerprint and the determination of total saponin content.

Keywords: Shatavarin IV, saponins, NMR, HPLC-MS/MS

Introduction

Commonly known as Shatavari, the roots of *Asparagus racemosus* Willd. (Asparagaceae) are widely used in traditional Indian medicine and, more recently, as a dietary supplement in the USA. In Italy, *A. racemosus* roots are included in the positive list of botanicals issued by the Ministry of Health and are therefore permitted for use in food supplements. The major steroidal saponins, known as shatavarins, are believed to interact with hormonal pathways and exhibit phytoestrogenic activity [1,2]. Saponin-rich root extracts, with Shatavarin IV considered the main bioactive marker compound (Figure 1), have traditionally been used to treat acid reflux and gut inflammation. Patil et al. (2014) reported an HPLC-MS/MS method operating in positive ionization mode for the determination of Shatavarin IV content in crude and commercial samples [3]. More recently, the study of Olom et al. 2017 [4] compared the simultaneous determination of five saponin glycosides in the methanolic extracts of *A. racemosus*, highlighting a better analytical response in negative ionization mode. To the best of our knowledge, no previous study has compared the quantification of shatavarins obtained by HPLC-MS and quantitative ¹H-NMR.

Experimental

Samples: The commercial dry extract of *Asparagus racemosus* roots was purchased from Hoefman-laboratories (France).

Analyses: The extract was dissolved in ethanol/water at pH 3.2 adjusted with formic acid (7:3 v/v) and analyzed using an HPLC-DAD-MS system (Agilent HP 1260L liquid chromatograph equipped with a DAD and a HP 1260 MSD-G6125B). Separation was performed on a Kinetex 2.6 μ m C18 100 Å, 100 x 4.6 mm at a flow rate of 0.6 mL/min. Mobile phase consisted in 0.1% formic acid in water (pH 3.2) (A) and CH₃CN (B). A multi-step linear solvent gradient was applied starting from 95% A: 0-5 min, 95-75% A; 5-25 min, 75-60% A; 25-30 min, 60-12% A; 30-32 min, 12-2% A; 32-35 min, 2% A; 35-37 min, 2-95% A to 95% A in 32 min. Shatavarin IV (Phytolab) was used as external standard for quantitation (EI profile at 885 m/z; linear range 2.2-22 ng, fragmentor 300 V). The remaining saponins were quantified based on their [M-H]⁻ ions according to Olom et al., 2017 [4]. Proton NMR spectra in CD₃OD were acquired after the addition of an appropriate amount of maleic acid as internal standard; quantitation was done according to our previous study [5].

Results

According to the literature, the analysis of complex herbal extracts commonly relies on the use of one or two external standards selected to represent the major constituents of the extract. In *A. racemosus*, the phytochemical profile is characterized by a relatively homogeneous group of glycosylated steroidal saponins, which differ only slightly in their structures. Therefore, Shatavarin IV represents an appropriate external standard for the quantitative estimation of the total saponin content.

HPLC-MS analyses confirmed that Shatavarin I, Dehydroshatavarin I and Shatavarin IV (Figure 1) are the predominant saponins, accounting for more than 80% of the total saponin content (Table 1).

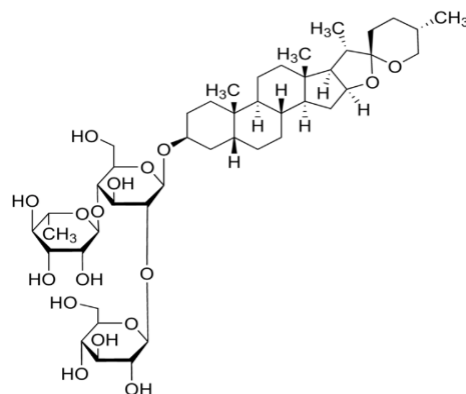


Figure 1. Structure of Shatavarin IV

Interestingly, Dehydroshatavarin I has not been reported in scientific literature as a natural constituent of *A. racemosus* [6], although is reported in some patents concerning the production of commercial extracts of this plant. It can be assumed that this new saponin is an artefact formed during the drying process of the extract. Based on the peculiar structure of Shatavarin I, the loss of one water molecule may readily occur. Indeed, the presence of a tetrahydrofuran ring bearing a hydroxyl group, instead of the typical spiroketal oxygenated ring found in Shatavarin IV, may facilitate dehydration, leading to the formation of a double bond. This hypothesis may explain why Dehydroshatavarin I has been reported only in processed extracts, but not in studies investigating fresh plant material.

The total saponins determined by ¹H-qNMR, calculated using the molecular weight of Shatavarin IV as the reference compound, was approximately two-fold higher than that obtained by HPLC-MS. This difference may partially result from a slight overestimation associated with the selected signals of saponins methyl protons, which resonate in the 0.7-1.4 ppm region where signal overlap may occur. However, this region is generally populated only by signals from lipophilic molecules, whose contribution is expected to be negligible since the plant root of *Asparagus racemosus* contains only trace amounts of lipids. Furthermore, ¹H-qNMR may also enable the quantification of minor saponins in the extract, which were not quantified by HPLC-MS, thereby contributing to the higher total saponin content obtained by NMR.

Table 1: Concentration of Saponins by HPLC-MS and by ¹H-qNMR.

HPLC-MS			
Saponins	[M-H] ⁻	% on DW	RSD
Shatavarin I	1065	12.3	9.5
Dehydro-shatavarin I	1047	8.9	5.5
Dehydro-shatavarin I isomer	1047	1.5	14.7
Shatavarin IV	885	8.0	6.1
Shatavarin V o VI	885	2.0	12.8
Shatavarin IX isomer	901	1.5	29.2
Total saponins		36.2	10.1
q ¹ H-NMR			
		% on DW	RSD
% Purity Shatavarin IV		98%	2.8
Total saponins		82.5	2.2

Conclusions

In conclusion, both HPLC-DAD-MS and ¹H-qNMR analyses indicated that the *Asparagus racemosus* extract is characterized by a high content of saponins and a relatively low abundance of interfering compounds. The predominant compounds were identified as Shatavarin I, Dehydroshatavarin I, and Shatavarin IV. Since all identified analytes belong to the same class of glycosylated steroidal saponins, the extract can be considered phytochemically homogeneous.

A combined use of HPLC-MS and ¹H-qNMR provides complementary information, with HPLC-MS enabling the characterization and-quantification of individual shatavarins and ¹H-qNMR offering an estimate of the overall saponin content.

References

1. Sinha B and Tare H., IJPQA, Volume 15 Issue 1; 531-538; 2024. DOI 10.25258/ijpqa.15.1.80.
2. Pandey A. et al., Phytomedicine Plus 6, 100916- 2026. <https://doi.org/10.1016/j.phyplu.2025.100916>.
3. Patil et al. J. of OAC International Vol. 97, no. 6, 2014 DOI: 10.5740/jaoacint.13-382
4. Onlom C. et al. Natural Product Communications 12 (1), 7-10; 2017. DOI: 10.1177/1934578X1701200103
5. Akande T. et al. Food Chem 2022:375:131813. DOI: 10.1016/j.foodchem.2021.131813.
6. Hayes P. Y. et al. Phytochemistry 69, 796–804; 2008. DOI:10.1016/j.phytochem.2007.09.001

P7

Effect of *in vitro* gastrointestinal digestion on the metabolic profile of the ancient cultivar “Pericina” pear (*Pyrus communis*) from Picentini Mountains (Salerno, Italy)

Roberta Cuozzo,^{1,2} Milena Masullo,¹ Sonia Piacente ¹

¹ Dipartimento di Farmacia, Università degli Studi di Salerno, via Giovanni Paolo II n. 132
84084 Fisciano (SA), Italy

² PhD Program in Drug Discovery and Development, Università degli Studi di Salerno, via Giovanni Paolo II
n. 132, 84084 Fisciano, SA, Italy

Summary: LC-HRMS/MS profiling of the ancient “Pericina” pear revealed phenolics, flavonoids, triterpenes, oxylipins, and glycolipids. Simulated gastric and intestinal digestion caused no significant changes in the metabolic profile, indicating high stability of the main bioactive constituents and supporting the nutritional relevance of this traditional cultivar.

Keywords: *Pyrus communis*, gastrointestinal digestion, LC-HRMS/MS analysis.

Introduction

The “Pericina” pear is an ancient pear variety cultivated since Roman times in the Picentini Mountains, in the province of Salerno. The “Slow Food Piacentini” association and local farmers protect this variety. This pear has an oblong shape, with a long, woody stem. The skin is green at harvest, turning brown when fully ripe. The fruit is very sweet and has a very fine texture. This pear is harvested in late August and September [1]. The pear, one of the oldest crops by humans, represents an important source of biologically active substances and is largely consumed worldwide [2]. The major components of pears are water (approx. 80%), sugar and fructose (approx. 15%), and fiber (approx. 2%). Various active compounds in pears have been identified, such as polyphenols (phenolic acids, flavonoids), triterpenes, and glucosides [3]. The aim of this study was to investigate the chemical profile of this ancient pear variety using LC-HRMS/MS analysis, which allowed the presumed identification of secondary metabolites belonging to the class of cinnamic acid derivatives, phenolic derivatives, flavonoids, triterpenes, glycolipids, and oxylipins. To assess the stability of the identified metabolites, an *in vitro* assay was performed to simulate gastric and intestinal digestion, monitoring the extracts by LC-MS analysis.

Experimental

Fruits of “Pericina” pear were frozen and freeze-dried to remove water. The freeze-dried fruits were extracted with pure ethanol using the conventional maceration process (for 3 days for 3 times). To better visualize the chemical profile of “Pericina” pear fruits, the extract was partitioned with *n*-butanol: water (1:1) to remove free sugars. The butanol fraction of the extract was analyzed by liquid chromatography coupled to electrospray ionization and high-resolution mass spectrometry (QExactive: Quadrupole-Orbitrap Hybrid Mass Spectrometer, Thermo Fischer, Waltham), operating in positive ion mode. LC-MS analysis was performed on a Kinetex 5 µm C18 100Å column (150 × 2.1 mm). The HPLC gradient started at 5% B, after 30 min the percentage of B was 95%, this percentage was maintained for another 5 min before returning to the initial percentage. As described in the literature [4], the following protocol was performed to simulate gastric and enzymatic digestion: -gastric juice (10 ml) was composed of 32 mg of pepsin, 20 mg of NaCl, pH 1.2 (with HCl). 2.5 ml of gastric juice was added to 2.5 ml of extract. The mixture was incubated at 37°C for 4 hours. Every hour, a certain amount of sample was removed and added to the same amount of ice- cold methanol. Each sample was analyzed by LC- HRMS/MS using the same method and conditions as the extract itself. -pancreatic juice consisted of 25 mg of pancreatin in 10 ml of 50 mM potassium phosphate buffer, pH 8, dissolved in water. 2.5 mL of pancreatic juice was added to 2.5 mL of extract. The mixture was incubated at 37°C for 4 hours, following the same procedure described above.

Results

LC-HRMS/MS analysis allowed us to define the chemical profile of “Pericina” pear. Specifically, chromatographic behavior, accurate mass measurements, fragmentation pattern analysis, and comparison with literature data allowed us to identify metabolites belonging to different chemical classes, including carbohydrates and organic acids, such as malic acid; specialized metabolites belonging to different chemical classes, including phenolic derivatives, such as arbutin; hydroxycinnamic acid derivatives; various flavonoids; and numerous pentacyclic triterpenes typical of pears, such as ursolic, pomolic, oleanolic, and tormentic acids; and various oxylipins and complex lipids.

The extracts were subjected to gastric and intestinal digestion and monitored by LC-MS analysis. Analysis of the resulting profiles revealed no differences from the profile of the as-is extract. This is consistent with the stability data reported for the classes of molecules identified in the "Pericina" pear extract. Pentacyclic triterpenes are highly stable in acidic environments [5]; molecules such as chlorogenic acid, for example, are stable at low pH, as is consistent with the literature [6].

Conclusions

In conclusion, LC-HRMS/MS analysis allowed to obtain a complete characterization of the metabolic profile of the ancient cultivar "Pericina", highlighting the presence of numerous classes of bioactive compounds, including phenolic derivatives, flavonoids, pentacyclic triterpenes, oxylipins and complex lipids. Exposure of the extract to simulated gastric and intestinal digestion conditions did not result in significant changes in the metabolic profile, indicating a high stability of the main identified metabolites. These results suggest that the bioactive compounds of "Pericina" pear maintain their chemical integrity during gastrointestinal transit, supporting the nutritional and functional potential of this heirloom pear variety.

References

1. <https://www.fondazione Slow Food.com/it/arca-del-gusto-slow-food/pera-pericina/>
2. E. Azzini, G. Maiani, A. Durazzo, M. S. Foddai, F. Intorre, E. Venneria, V. Forte, S. Lucchetti, R. Ambra, G. Pastore, D. D. Silveri, G. Maiani, A. Polito; *Oxidative Medicine and Cellular Longevity*, 6714103 (2019).
3. S. Y. Hong, E. Lansky, S. S. Kang, M. Yang; *BMC Complement Med Ther*, 21 (2021), p 219.
4. K. Ayda, I. Bouali, S. Belkhir, R. Mokded, S. Smiti, P. Falé, M. E. M. Araújo, M. L. M. Serralheiro; *Pharmaceutical Biology* 55 (2017), pp 101–7.
5. P. L. Falé, C. Ferreira, A. M. Rodrigues, P. Cleto, P. A. Madeira, M. H. Florêncio, M. L. Serralheiro; *J. Med. Plants Res*, 7 (2013), pp 1370-1378.
6. P. Dzubak, M. Hajduch, D. ydra, A. Hustova, M. Kvasnica, D. Biedermann, L. Markova, M. Urbanc, J. Sarek; *Nat. Prod. Rep.*, 23 (2006), pp 394–41.

P8

High-resolution mass metabolomic profiling for the chemical biodiversity of traditional apulian sweet pepper (*Capsicum annuum* L.) landraces

Antonino Rizzuti,¹ Emanuela Blanco,² Biagia Musio,¹ Vito Gallo,¹ Piero Mastrorilli,¹ Anna Bonasia,³ Giulia Conversa,³ Gabriella Sonnante²

¹ Department of Civil, Environmental, Land, Construction and Chemistry (DICATECh), Polytechnic University of Bari, Via Orabona 4, 70125 Bari, Italy

² Institute of Biosciences and Bioresources, National Research Council (CNR-IBBR), Via Amendola 165/A, 70126 Bari, Italy

³ Department of Agriculture, Food, Natural Resources and Engineering (D.A.F.N.E.), University of Foggia, Via Napoli, 25, 71122 Foggia, Italy

Summary: Non-targeted HPLC-high-resolution metabolomics was applied to seven traditional Apulian sweet pepper (*Capsicum annuum* L.) landraces. Distinct cultivar-specific metabolic signatures involving alkaloids, terpenoids, fatty acids, phenolic acids, and flavonoids enabled clear discrimination among accessions. These findings support biodiversity conservation, varietal characterization, food authentication, and the valorisation of local agro-food resources.

Keywords: Sweet pepper landraces; non-targeted metabolomics; high-resolution mass spectrometry

Introduction

Sweet pepper (*Capsicum annuum* L.) is an economically and nutritionally important crop widely cultivated in the Mediterranean basin. Southern Italy preserves many local varieties characterised by distinct morphological, agronomic, and quality-related traits, representing a significant reserve of genetic diversity [1]. As part of the BiodiverSO and BiodiverSO Karpos projects (PSR Puglia 2014–2022, Measure 10.2), several Apulian pepper landraces, including accessions at risk of extinction, were collected and characterised. In this study a non-targeted HPLC-HRMS metabolomics approach was applied to seven Apulian landraces to investigate the cultivar-specific phytochemical signatures. The results of this study will integrate morphological, agronomic and functional traits, providing valuable information for the characterization and valorisation of local pepper biodiversity, and supporting conservation and sustainable exploitation strategies.

Experimental

Local varieties of sweet pepper were grown in an *ex situ* field in Altamura. Standard agronomic procedures were used, in accordance with local practices. Seven local varieties traditionally cultivated in various areas of the Apulia region (southern Italy), were considered. These varieties have diverse morpho-agronomic characteristics, including the colour (red and yellow; see Table 1), which are important commercial traits. Freeze-dried pepper fruits were extracted (80 mg/mL) using a methanol/water/formic acid solution (70:28:2, v/v/v), sonicated, and then centrifuged. The resulting supernatant was analysed by HPLC-HRMS.

Table 1. Local name, colour, and collection site of the seven sweet pepper landraces.

Local Name	Colour	Collection site
Paparule A'core	Red	Gravina in Puglia (BA)
Peperone tondo 'A cumbost'	Red	Anzano di Puglia (FG)
Peperone Tondo Dolce (Papecchia di Gravina)	Red	Gravina in Puglia (BA)
Peperone Giallo a zanne	Yellow	Castellana Grotte (BA)
Peperone Rosso antico	Red	Peschici (FG)
Peperoni Tondi	Red	Presicce (LE)
Peperone Tipo Dolce	Red	Brindisi (BR)

Metabolite profiling was performed using an HPLC system coupled with a MicroTOF-Q II mass spectrometer (Bruker Daltonics) operating in both positive and negative ESI modes. Compound annotation was based on accurate mass, isotopic pattern, and MS/MS fragmentation data, which were then compared against the literature [2] and public databases. The datasets were processed prior to performing multivariate statistical analyses.

Results

The HPLC–HRMS experiments provided a comprehensive overview of the phytochemical classes present in the analysed sweet pepper samples.

Multivariate statistical analysis revealed distinct metabolic signatures associated with specific accessions, enabling their discrimination according to metabolite composition. A three-component PLS-DA model showed satisfactory predictive performance (ESI[−]: $R^2 = 0.9435$ and $Q^2 = 0.7187$ and ESI⁺: $R^2 = 0.9349$ and $Q^2 = 0.7245$), confirming the robustness of cultivar discrimination.

Figure 1 shows the PLS-DA score plots obtained from the metabolomic profiles of freeze-dried pepper samples, analysed in both negative and positive ionisation modes.

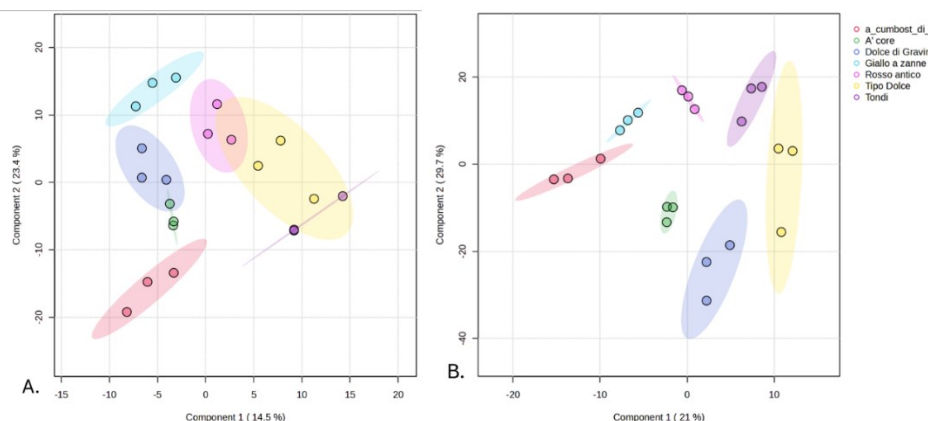


Fig. 8 PLS-DA score plots of freeze-dried sweet pepper samples obtained from LC–HRMS data in negative (A) and positive (B) ionization modes.

The main metabolites identified as discriminatory, as reported in Table 2, belong to the following groups: alkaloids; organic acids, fatty acids and amides; terpenoids; and phenolic acids and flavonoids. Capsaicin and dihydrocapsaicin were consistently enriched in pungent or semi-pungent cultivars such as the *Tondo Dolce di Gravina* and the *Tipo Dolce* pepper. Additionally, metabolites associated with aroma traits, such as loliolide and dihydroactinidiolide as well as antioxidants including vanillic acid glucoside, myricitrin, quercetin 7-rhamnoside, and quercetin diglucoside, were predominantly associated with the *Rosso Antico* and the *Giallo a Zanne* peppers. Overall, the ESI⁺ and ESI[−] dataset clearly resolved cultivar-specific metabolic signatures associated with pungency, aroma, and antioxidant capacity traits which are currently under investigation.

Table 2. Main cultivar-specific metabolic signatures.

Metabolite	Chemical class	m/z VIP
Capsaicin	Alkaloids	306.2071 ^a
Dihydrocapsaicin	Alkaloids	308.2244 ^a
Quinic acid	Organic ac.	191.0532 ^b
Hydroxylinoleic acid	Fatty acids	295.2283 ^b
Hydroxylinolenic acid	Fatty acids	293.2131 ^b
Capsiamide	Fatty amides	270.2798 ^a
Epoxy capsianoside	Terpenoids	677.3396 ^a
Capsianoside V	Terpenoids	513.2707 ^b
Capsianoside I	Terpenoids	659.3293 ^b
Loliolide	Terpenoids	197.1179 ^a
Dihydroactinidiolide	Terpenoids	181.1227 ^a
Vanillic acid glucoside	Phenolic acids	329.0883 ^b
Myricitrin	Flavonoids	303.0511 ^a
Quercetin 7-rhamnoside	Flavonoids	447.0945 ^b
Quercetin diglucoside	Flavonoids	609.1472 ^b

^aESI⁺; ^bESI[−]

Conclusions

Our results demonstrate that HPLC–HRMS metabolomics is a powerful tool for exploring food biodiversity and linking varietal identity to nutritional and functional attributes. The distinct phytochemical profiles identified among Apulian sweet pepper landraces complement their agro-morphological characterisation and highlight their potential for conservation, authentication and sustainable valorization.

References

1. F. Fratianni et. al.; *Antioxidants* 9 (2020):556.
2. J. Que, L. Liu, Y. Gao, Q. Tang, R. Liu, W. Liao, R. Huang, D. Zhang, S. Chen, J. Peng; *Food Chemistry: X* 30 (2025):102905.

P9

Characterization of the volatile fraction of wild food plants before and after fermentation by HS-SPME/GC–MS

Leandra Leto,¹ Benedetta Bottari,¹ Marco Biagi,¹ Francesco Martelli,² Federico Bertoldo,¹ Renato Bruni,¹ Martina Cirlini¹

¹ Department of food and drug, University of Parma, Viale delle scienze 27/A, 43124 Parma, Italy

² Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Via L. Borsari 46, 44121, Ferrara, Italy

Summary: The volatile organic compound (VOC) profile of four different food plants (wild garlic, dandelion, borage, nettle) fermented with distinct LAB strains showed that fermentation substantially modified their aroma profiles.

Keywords: Aroma profile, Gas chromatography, Mass spectrometry

Introduction

Wild food plants constitute a valuable component of the Mediterranean agri-food heritage, contributing to the original formulation of the Mediterranean Diet [1]. Consequently, many alimurgic plants are attracting growing interest as potential ingredients for functional foods and nutraceuticals [2]. In this study, LAB (Lactic Acid Bacteria) fermentation was used as a promising biotechnological approach to enhance the functional and technological value of four different food plants, wild garlic (*Allium ursinum* L.), dandelion (*Taraxacum* spp.), borage (*Borago officinalis* L.) and nettle (*Urtica dioica* L.), by modulating phytochemical composition in terms of volatile profile, reducing unfavourable sensory attributes, through the metabolic activity of LAB [3].

Experimental

Plant materials (leaves, stems and flowers of each species) were fermented in two separate batches with two wild LAB strains belonging to the University of Parma Culture Collection (UPCCO), namely *Lactiplantibacillus plantarum* UPCCO 4265 and *Lacticaseibacillus casei* UPCCO 2240. Plants were fermented at 30 °C in the case of *Lpb. plantarum* and 37°C for *Lcb. casei*. LAB and total aerobic counts were determined by serial dilution and plating on MRS and Plate Count Agar (PCA, Oxoid, Milan, Italy) respectively at the time of inoculation (T0) and after 72 hours (T3) and 7 days (T7) of fermentation.

The volatile profiles of fresh and fermented wild edible plant samples were analysed by headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography–mass spectrometry (GC–MS) [4]. Compounds were identified by comparison with the NIST 14 mass spectral library using a minimum match of 90% and were semi-quantified relative to an internal reference compound (Toluene, 0.5 µg).

Results

Fermentation process markedly modified the VOC profiles of wild garlic, dandelion, borage and nettle with changes depending on both the plant matrix and the bacterial strain. In wild garlic, fermentation promoted the accumulation of characteristic organosulphur compounds while reducing aldehydes, alcohols, and esters associated with fresh, green notes (Fig.1A).

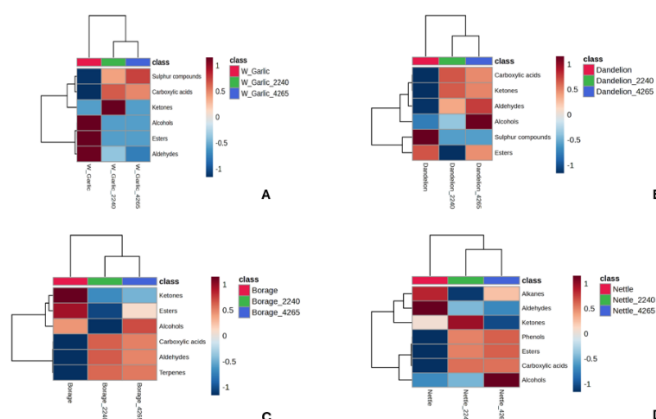


Figure 1. HEATMAP showing differences in the concentrations of the various chemical classes identified in the wild garlic (A), dandelion (B), borage (C) and nettle (D) samples. The values are represented by colour intensity: red indicates high compound intensity, and blue indicates low compound intensity

In dandelion, microbial metabolism increased ketones, aldehydes, alcohols, and carboxylic acids, shifting the aroma towards fermented, buttery, fruity, and floral characteristics (Fig. 1B). In borage, ketones decreased, whereas aldehydes, terpenes, and especially carboxylic acids increased following fermentation (Fig. 1C). In nettle, fermentation reduced green aldehydes while promoting the formation of esters and phenolic compounds, resulting in a more complex, fermented aroma profile (Fig. 1D).

Conclusions

Overall, LAB fermentation reshaped the volatile profiles of all four wild edible plants, promoting the formation of different aroma compounds although the extent of these changes was species and strain dependent. These metabolic transformations highlight the potential of LAB fermentation as a strategy to tailor the sensory characteristics of plant-based foods.

References

1. B. Gori, T. Cossu, H. El Zein, U. Liu, T. Ulian, G. Bacchetta; *Plants, People, Planet* (2025), pp 1–18
2. F. Fantasma, V. Samukha, G. Saviano, M.G. Chini, M. Iorizzi, M.; *Nutraceuticals*, 4(2) (2024), pp 190–231.
3. Q. Zhu, G. Shi, J. Gu, J. Du, J. Guo, Y. Wu, S. Yang, J. Jiang; *Food Bioscience*, 62 (2024).
4. A. Ricci, A. Levante, M. Cirilini, L. Calani, V. Bernini, D. Del Rio, G. Galaverna, E. Neviani, C. Lazzi; *Frontiers Microbiology*, 9 (2018).

P10

An ASCA-based Investigation of hazelnuts volatilome evolution during storage

Sara Tanilli,¹ Andrea Caratti,¹ Giuseppe Genova,² Alex Fissore,² Cristina Casetta,² Fulvia Trapani,¹ Erica Liberto,¹ Chiara Cordero ¹

¹ Department of Drug Science and Technology, University of Turin, Via Pietro Giuria 9, 10125 Turin, Italy

² Soremartec Italia Srl, Piazzale Ferrero 1, 12051 Alba (Cuneo), Italy

Summary: ANOVA-Simultaneous Component Analysis (ASCA) was applied to a GC×GC-based quantitative volatilomic dataset to investigate how origin, cultivar, harvest year, storage condition, and storage time jointly shape hazelnuts quality. Rather than removing confounding factors, ASCA models them explicitly, revealing a lipid-autoxidation signature characterized by aldehydes, alcohols and short-chain fatty acids.

Keywords: Hazelnut volatilomics, ASCA, shelf-life

Introduction

Lipid autooxidation, enzymatic oxidation and microbial processes generate a complex and evolving mixture of volatile compounds during hazelnuts storage, progressively compromising sensory and nutritional quality¹. Investigating shelf-life trends (*i.e.*, how the volatile profiles change during storage) is of great interest for the industry to improve food quality management and supply-chain optimization. A major difficulty in such studies, however, is that volatile profiles are also strongly influenced by external factors such as geographical origin, cultivar, and storage condition, as well as by the climatic and agronomic conditions associated with the harvesting year. Handling such large and complex datasets is of clear interest for industries but advanced chemometric strategies capable of managing this substantial source of variability are required.

ANOVA-Simultaneous Component Analysis (ASCA) is a particularly well-suited approach in this context, as it allows to incorporate the main experimental factors and their interactions into a single multivariate ANOVA-based model that partitions the variance attributable to each factor². The contribution of each experimental factor can thus be assessed and interpreted directly within a single, unified model.

The inferential ASCA-based approach presented here provides a robust counterpart to a different chemometric strategy applied to the same dataset. In that case, harvesting year variability was explicitly removed by subsetting the dataset, and a workflow based on PLS supervised models on single harvesting year was employed to achieve a final robust and generalizable multi-year shelf-life prediction model. Even if a different chemometric strategies was applied, the two approaches converge on several points, mutually supporting and reinforcing their respective conclusions.

Experimental

The dataset included 430 observations, obtained from the analysis of 144 hazelnut samples. Hazelnuts came from 3 harvesting years (2020- 2022), 2 geographical origins (Turkey and Italy), and 2 cultivars per origin. Each sample was stored under 2 storage conditions (5°C under vacuum, 'UV', vs. 5°C atmospheric, 'AA') and sampled at six time points (4, 6, 9, 12, 18, 24 months). Analyses were performed via HS-SPME-GC×GC-MS/FID, with three technical replicates per sample, targeting and accurately quantifying 43 volatile compounds belonging to different chemical classes³.

Chemometric analysis was performed in MATLAB using the MEDA Toolbox (v1.9). Data were log-transformed and autoscaled; technical replicates were averaged after log-transformation to avoid pseudo-replication in the ASCA permutation testing. The model included five main effects (Origin, Cultivar, Year, Storage Type, Storage Time) and six two-way interactions; Cultivar was modelled as a term nested within Origin. Terms significance was assessed by permutation testing (1000 permutations) with Benjamini-Hochberg FDR correction. Loadings bootstrapping was applied to the Storage Time factor to identify which of the compounds contributed significantly to this effect.

Results

The Year×Storage Time interaction emerged as the largest effect in the model (~25% of explainable variance), exceeding the Storage Time main effect itself. This finding provides an independent counterpart to the predictive study's necessity of stratifying the initial dataset by year to prevent this same interaction from confounding storage-related temporal trends.

Storage Time was the second-largest term in the model. Inspection of PCA scores plots showed the temporal trend distributed mainly across PC1 and PC2, with a smaller contribution from PC3 in the separation of the intermediate storage-time points. The PC2 vs. PC3 scores plot (Figure 1) shows a temporal trend from early (4, 6 months), through intermediate (9, 12 months), to late (18, 24 months) time points. Inspection of the

corresponding loadings plot (Figure 1), supported by a loadings bootstrap resampling analysis (1000 resamples), indicates that saturated aldehydes, some short-chain fatty acids, and primary alcohols are the volatiles most consistently increasing at higher storage times. These compound classes closely overlap with the seven markers (hexanal, heptanal, octanal, hexanol, pentanol, octanol, hexanoic acid) identified as most influential in the final SVR model from the parallel predictive workflow.

The Storage Type×Storage Time interaction further shows that UV- and AA-stored trajectories overlapped up to approximately 9 months before diverging. This divergence was more pronounced for AA-stored samples, whose late time points (12, 18, and 24 months) were more separated from the earlier ones compared to UV-stored samples.

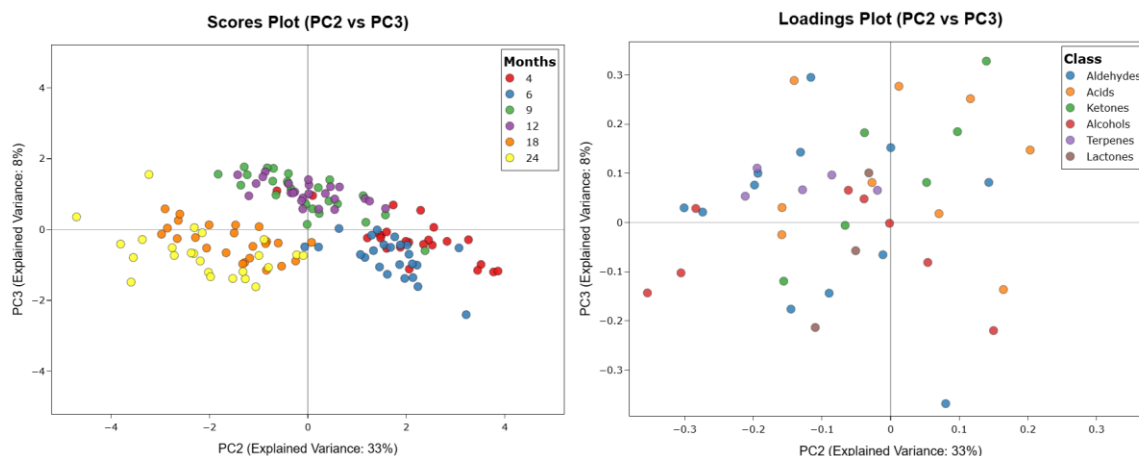


Figure 1. PC2 vs PC3 scores plot (left) and PC1 vs PC3 loadings plot (right) showing temporal trends during storage

Conclusions

Two distinct strategies were applied to the same hazelnut quantitative volatilomic dataset: a predictive workflow based on harvesting year dataset subsetting and supervised models, and an inferential ASCA framework that instead modelled explicitly that variability in a unique model. The two approaches converge on the same qualitative picture of hazelnut aging: storage- driven degradation is dominated by a lipid-oxidation cascade that produces volatiles from different chemical classes, in particular saturated aldehydes, primary alcohols, and fatty acids. Together, the two studies illustrate how prediction- and inference-oriented chemometric strategies, applied in parallel to the same dataset, can support one another and achieve a more complete understanding of a complex dataset.

References

1. Shahidi F. & John J. A., Harris L. editor; *Improving the Safety and Quality of Nuts*, (2013) pp 198-229
2. Smilde, A. K., Jansen, J. J., Hoefsloot, H. C., Lamers, R. J. A., Van Der Greef, J., Timmerman, M. E.; *Bioinformatics*, 21 (2005), pp 3043-3048.
3. Squara S., Caratti A., Fina A., Liberto E., Spigolon N., Genova G., Castello G., Cincera I., Bicchi C., Cordero C.; *Journal of Chromatography A*, 1700 (2023), Article 464041

P11

Detection of biosynthetic citric acid and sugar addition in Italian tomato passata using liquid chromatography-chemical oxidation-isotope ratio mass spectrometry

Silvia Pianezze,¹ Domenico Masuero,¹ Matteo Perini,¹ Stefania Carpino,² Nicola Barilaro,² Vincenzo Di Martino,² Luana Bontempo¹

¹ Fondazione Edmund Mach, San Michele all'Adige, Italy

² Department of Central Inspectorate for Fraud Repression and Quality Protection of the Agri-Food Products and Foodstuffs (ICQRF), MASAF, Italy

Summary: A novel LC-co-IRMS method was developed to detect adulteration in Italian tomato passata. By measuring compound-specific $\delta^{13}\text{C}\%$ values of citric acid, glucose and fructose, the diagnostic ratios RCA/G ($\delta^{13}\text{C}$ Citric acid / $\delta^{13}\text{C}$ Glucose), and RCA/F ($\delta^{13}\text{C}$ Citric acid / $\delta^{13}\text{C}$ Fructose) successfully identified exogenous sugars and biosynthetic citric acid, offering a robust tool against food fraud.

Keywords: Liquid chromatograph-chemical oxidation-isotope ratio mass spectrometry; Tomato passata; Compound specific analysis.

Introduction

According to EU legislation, the addition of citric acid (E330) to tomato sauces is allowed and must be declared in the label, while the addition of exogenous sugars is forbidden. Since tomato is a C3 plant, the addition of C4-derived organic compounds can be detected through isotopic analysis. To the best of our knowledge, no isotopic references for the citric acid naturally present in tomatoes are currently available.

Experimental

A comprehensive dataset including 100 authentic Italian tomato sauces sampled by the ICQRF (Central Inspectorate for Fraud Repression and Quality Protection of Agri-Food Products and Foodstuffs) and 23 commercial market samples was analysed using LC-co-IRMS to simultaneously measure the $\delta^{13}\text{C}\%$ of citric acid, glucose, and fructose. The analytical method was validated by determining the within-day and between-days repeatability on a reference sample. To assess the minimum detectable addition of exogenous citric acid, a selected authentic sample was spiked with increasing amounts of C4-derived citric acid (ranging from 0% to 100%). Furthermore, isotopic difference ratios RCA/G ($\delta^{13}\text{C}$ Citric acid / $\delta^{13}\text{C}$ Glucose), RCA/F ($\delta^{13}\text{C}$ Citric acid / $\delta^{13}\text{C}$ Fructose) and RG/F ($\delta^{13}\text{C}$ Glucose / $\delta^{13}\text{C}$ Fructose) were calculated as diagnostic parameters.

Results

The LC-co-IRMS method allowed for the successful establishment of the official $\delta^{13}\text{C}$ (‰) reference ranges for authentic Italian tomato sauces based on the ICQRF reference dataset. Method validation confirmed high analytical precision across repetitive within-day and between-days measurements. The spiking experiments with C4-derived citric acid (0– 100%) demonstrated high sensitivity, enabling the identification of minimal exogenous additions. The calculated isotopic ratios RCA/G , RCA/F and RG/F provided robust supportive criteria, allowing clear discrimination between authentic products and commercial samples subjected to fraudulent additions of exogenous sugars or industrial citric acid.

Conclusions

In this study, a new approach through LC-co-IRMS was adopted, and the $\delta^{13}\text{C}$ of citric acid, glucose and fructose were measured to detect additions of biosynthetic citric acid or C4 exogenous sugars in Italian tomato passata. The method also demonstrated good repeatability, with intra-day and inter-days standard deviations below 1.1 ‰ for citric acid, glucose and fructose.

P12

Acrylamide: from bean to cup

Barbara Angelone,¹ Paolo Candotti,¹ Barbara Massardi,¹ Rita Salami,¹ Giulia Lorenzi,² Simonetta Menotta,¹ Mara Gasparini¹

¹ Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini" Brescia, Dipartimento sicurezza alimentare e ambientale, Reparto chimica degli alimenti e mangimi (IZSLER)

² Università degli studi di Milano, Facoltà scienze e tecnologie

Summary: *The aim of this study is to evaluate the extraction levels of acrylamide from coffee's beans to three types of beverage: espresso, long and iced coffee. The study was also carried out to assess any differences in exposure based on the type of beverage*

Keywords: *Acrylamide, Coffee, UPLC-MS/MS*

Introduction

Acrylamide is an organic contaminant classified as potentially carcinogenic by the International Agency for Research on Cancer (IARC). Its formation depends on food composition; scientific studies have demonstrated that the presence of asparagine and reducing sugars (such as glucose or fructose), combined with thermal processing (>120 °C), increases acrylamide concentrations in the final product (1,2). Coffee is among the primary foods monitored for acrylamide exposure, with European regulations establishing specific reference levels for roasted coffee (3). This study aims to evaluate acrylamide extraction levels from bean to beverage across three widely consumed coffee types: espresso, long and iced coffee. Consequently, the transfer rate of acrylamide and the potential variations in dietary intake were assessed.

Experimental

Capsule coffee represents a rapid and convenient alternative to traditional home-brewed coffee prepared with a Moka pot. For this study, 54 capsule types from various brands were purchased along with their respective coffee machines. For each coffee type, analyses were performed on both the coffee powder—upon which legislative reference limits are established—and three resulting beverage types: espresso, long and iced coffee.

For each capsule type, the three coffee beverages were prepared following an internal protocol. Key parameters, such as volume, temperature, and operating pressure, were monitored during elution to ensure data comparability.

The acrylamide concentration [µg/kg] and total amount [ng] in both the powder and the three beverage types were determined using an in-house analytical method. This method involved extraction with acetonitrile using QuChERS salts, followed by a dispersive solid-phase extraction (d-SPE) cleanup step. 2 mL aliquot of the purified extract was evaporated to dryness under a nitrogen stream at 40 °C and reconstituted in 0.5 mL of a water:methanol (9:1 v/v) mixture containing 0.1% formic acid. Analytical determination was performed using ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS). An isotopically labeled internal standard (13C-acrylamide) was added at the beginning of the analytical process to monitor method recovery and quantify acrylamide concentrations via isotope dilution. The in-house method, originally developed for coffee powder, was adapted to coffee extract by optimizing the initial sample weight to achieve an analytical sensitivity and to quantify acrylamide concentrations above 10 µg/kg.

Results

Acrylamide concentrations (µg/kg) were determined in all coffee powder samples and in their three corresponding beverage preparations. For all samples analyzed, the acrylamide content in the coffee powder remained below the benchmark levels established by European regulations, as shown in Figure 1. The amounts (ng) of acrylamide extracted by the three brewing methods are reported in Figure 2.

Acrylamide extraction in espresso coffee (20 mL) averaged approximately 78%, with a standard deviation of 15%. In long coffee (50 mL), extraction was essentially complete, reaching about 100% with a standard deviation of 16%. In contrast, cold-brew coffee showed a lower extraction efficiency, with approximately 61% of the acrylamide being extracted and a standard deviation of 11%.

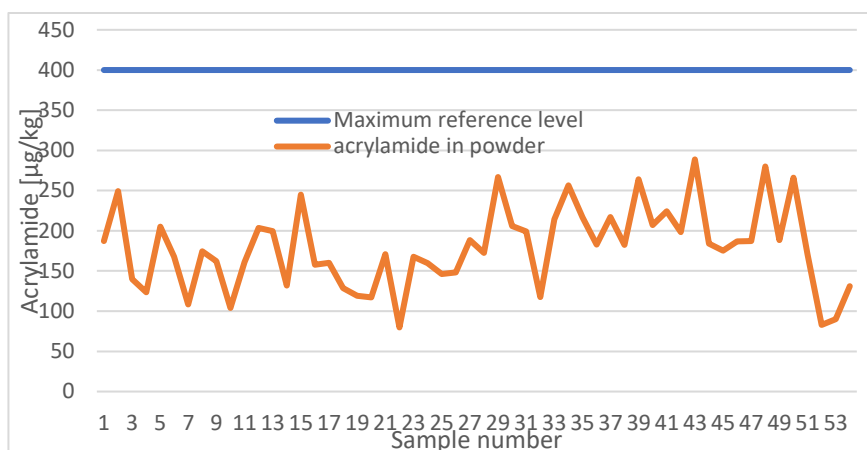


Fig. 9. Acrylamide concentration among 54 coffee samples

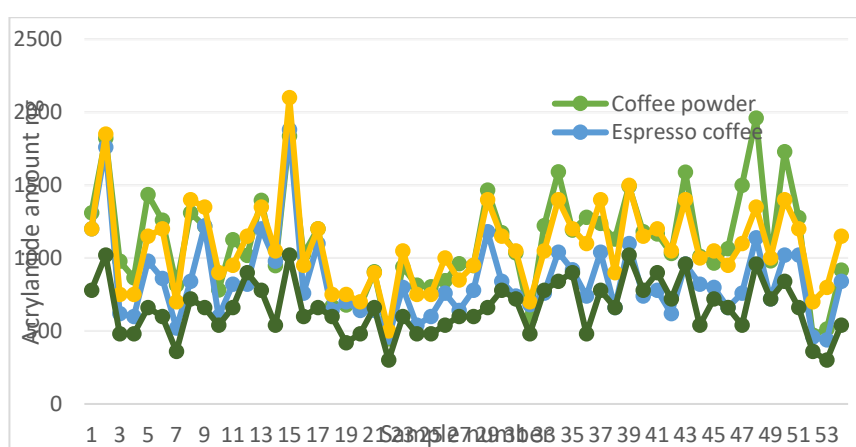


Fig. 2. Acrylamide amount [ng] in beans and in espresso, long and iced coffee

Conclusions

The results of this study suggest that acrylamide amount in coffee powder does not significantly degrade during either hot pressurized extraction or cold brewing. Despite the high-water solubility of this substance, the extraction efficiency appears to be strongly influenced by the brewing conditions. In particular, the larger beverage volume associated with long coffee resulted in nearly quantitative extraction of acrylamide, indicating that almost all the acrylamide present in the coffee powder was transferred to the final beverage.

However, extraction efficiency is not determined only by volume. Temperature and pressure also play a significant role in the extraction process. Indeed, although cold coffee had a larger final volume than long coffee (60 mL vs. 50 mL), it showed the lowest acrylamide extraction rate, suggesting that the lower extraction temperature negatively affects the transfer of acrylamide from the coffee powder to the beverage.

Further statistical analyses will be performed to identify the brewing parameter that has the greatest influence on acrylamide extraction. In addition, spent coffee powder from all preparations will be analyzed to assess the presence of residual acrylamide after extraction, thereby providing additional evidence to support the robustness of data obtained.

References

1. L.F.Bonilla, A. Sandoval-Aldana, L. D.Daza; *Food Chemistry*, 466 (2025), 142247.
2. G.Törös, W. Alibrahem, N K Helu, S. Jevcák, A.Ferroudj, J. Prokisch; *Toxics*, 14 (2026), 110.
3. Commission Regulation (EU) 2017/2158 of 20 November 2017 establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food

Acknowledgement: This study was supported by institutional internal funds of the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER) Autofin_2004001 (IZSLER 01/22) and partially co-funded by the Italian '5 per mille' (5x1000) tax designation fund for sanitary research (Tax code: 00284840170)

P13

Linalool in green *Coffea arabica* by HS-SPME/GC-MS and HS/GC-IMS

Gaia Bechis¹, Valentina Lonzarich¹, Matteo Bordiga,² Luciano Navarini¹

¹ illycaffè S.p.A., via Flavia 110, 34147 Trieste, Italy

² Department of Pharmaceutical Sciences, University of Piemonte Orientale, Novara, Italy

Summary: This preliminary study investigated linalool in green wet-processed *Coffea arabica* samples from two different geographical origins using HS-SPME/GC-MS and HS/GC-IMS. While HS-SPME/GC-MS detected free linalool under all explored experimental conditions, HS/GC-IMS revealed linalool only after acid hydrolysis, indicating that free linalool is present at very low levels and accounts for about 9–10% of total linalool. These results support the use of hydrolytic conditions for reliable linalool detection in green coffee by HS/GC-IMS.

Keywords: Linalool, green coffee, GC-IMS

Introduction

Linalool is one of the key volatile compounds contributing to the aromatic profile of green coffee (1). Although typically present at low concentrations, it is associated with floral and citrus-like notes and may act as an important precursor or marker of coffee quality, varietal differences, and post-harvest processing effects. Linalool can also occur in glycosidically bound forms, where the volatile aglycone is linked to sugar moieties (2). These non-volatile precursors may contribute to the aroma potential of coffee, as linalool can be released during processing, roasting, or enzymatic/hydrolytic reactions, thereby influencing the final sensory profile of the beverage. In green coffee, this monoterpene is generally detected without any particular analytical problems in the headspace using SPME/GC-MS. However, the characterization of green coffee by HS/GC-IMS has evidenced the need for a strategy to detect linalool. In fact, the use of HS/GC-IMS has highlighted that in green coffee the ratio between free linalool and glycosidically bound linalool is very low and it is therefore necessary to invert this ratio in order to be able to detect it without difficulty.

In the present study, we address preliminary this topic by examining green *Coffea arabica* samples from two different geographical origins under both non-hydrolytic and hydrolytic conditions (2) by both HS-SPME/GC-MS and HS/GC-IMS.

Experimental

Samples of green wet-processed *C. arabica* from two different geographical origins (Ethiopia & India) were used. Linalool standard (Sigma-Aldrich, cat. n. W263508, ≥97%, FCC, FG, St. Louis, MO, USA) was used.

In the case of HS-SPME/GC-MS, the headspaces of ground samples (2g; 2g+2g of H₂O; 2g+2g of H₂O+1g of Citric acid) in 20 mL vials were adsorbed by a car/pdms 75 µm SPME fiber for 30 minutes at 60°C and then inserted into the injector of a GC/MS (GC7890 coupled with a 5977B MS, Agilent, Palo Alto) at 250°C for 10 minutes of desorption. GC was equipped with a HP5 UI column: 30 m x 0,250 mm I.D. x 0.25 µm film, (Agilent), temperature program was set at 40°C for 3 minutes, then heated at 5°C/min. to 150°C and 20°C/min. to 200°C, followed by the final temperature of 200°C hold for 2 minutes. MS signals were acquired in full scan and SIM mode simultaneously (selected ions 71, 93, 121 and 136).

In the case of HS/GC-IMS (FlavourSpec, G.A.S., Dortmund, Germany) same samples were analyzed under the following conditions: T IMS: 45°C; T GC: 40°C; GC column: MXT 5 column (15m x 0.53mm x 1 µm); T injection port: 80°C; T transfer line: 90°C - 45°C; T oven: 40°C; T stirrer: 80°C; T syringe: 60°C; Conditioning Time 20 min.

Results

HS-SPME/GC-MS analyses easily detect free linalool in the headspace of green coffee samples under the two non-hydrolytic explored conditions (ground sample and ground sample after water addition) and under the hydrolytic explored condition (ground sample after water and citric acid addition). In both Ethiopian and Indian green coffee samples, the linalool response markedly increased after hydrolysis compared with the non-hydrolytic conditions. This trend indicates that only a minor fraction of linalool is present in the free volatile form in the headspace, whereas most of it occurs as bound precursors that can be released upon hydrolysis. Overall, these findings suggest that free linalool accounts for only a small proportion of the total linalool potentially present in the examined green coffee samples.

Surprisingly, as clearly shown in Figures 1 and 2, analyses by HS/GC-IMS carried out on the two different green coffee samples don't show any signal related to linalool under non-hydrolytic conditions and only under hydrolytic conditions a well resolved linalool spot is evident.

The sensitivity of HS/GC-IMS appears insufficient to detect the low concentration of free linalool present in the headspace of green coffee. To define the linalool quantification range achievable by HS/GC-IMS, several aqueous linalool standard solutions in a wide range of concentrations were analysed. Based on these experimental data a LOD close to 0.315 µg has been determined. Across the investigated range (1–20 µg), the calibration response exhibits a non-linear behaviour, which is characteristic of IMS detection due to reactant ion depletion at higher mass loads. To account for this curvature, the calibration curve was fitted using a logarithmic regression model ($y = 0.5277 \ln(x) + 0.6097$), yielding a good coefficient of determination ($R^2 = 0.9781$).

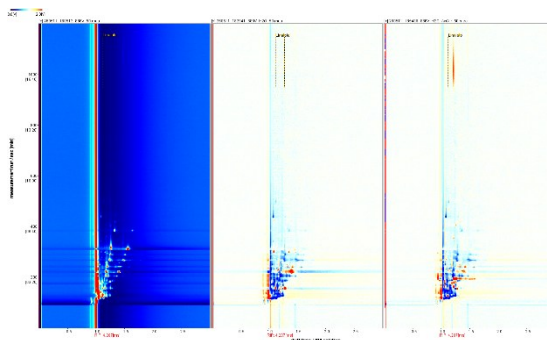


Fig. 1. HS/GC-IMS: *C. arabica* Ethiopia. Left 2g ground sample; Middle 2g+2g H₂O; Right 2g+2g H₂O+1g Citric acid.

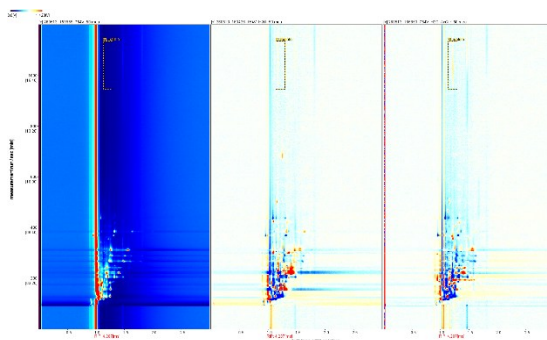


Fig. 2. HS/GC-IMS: *C. arabica* Ethiopia. Left 2g ground sample; Middle 2g+2g H₂O; Right 2g+2g H₂O+1g Citric acid.

Conclusions

The present preliminary investigation has put in evidence that the low free linalool concentration in the headspace of green *C. arabica* is challenging to be detected by HS/GC-IMS. This observation may greatly assist in adopting the proper experimental conditions to perform linalool detection and quantification in green coffee. According to the obtained experimental data, the free linalool in the headspace of green coffee is below 0.315 µg and it represents a small fraction of the total linalool present in green coffee beans.

References

1. Flament, I., 2001. *Coffee Flavour Chemistry*. Wiley & Sons, New York.
2. De Rosso, M., Lonzarich, V., Navarini, L., Flamini, R., 2022, *Current Research in Food Science*, 5, 336-344

P14

Multi-analytical benchmarking of cold-brew systems: HS-SPME-GC-MS reveals quality evolution during refrigerated storage

Agnese Santanatoglia^{1,2}, Martina Prioretti¹, Matteo Petrolati¹, Giovanni Caprioli¹, Gianni Sagratini¹

¹ School of Pharmacy (ChIP), University of Camerino, Via Madonna delle Carceri 9, 62032 Camerino (MC), Italy

² Research and Innovation Coffee Hub (RICH), Via Emilio Betti 1, 62020 Belforte del Chienti, Italy

Summary: Eight cold-brew configurations, each prepared in three independent batches, were assessed by physicochemical measurements, HPLC-DAD, matrix-matched HS-SPME-GC-MS, 12-assessor sensory analysis and microbial enumeration. The integrated design revealed trade-offs among extraction performance, composition, aroma persistence, freshness and hygienic status during refrigerated storage.

Keywords: cold-brew coffee; multi-analytical benchmarking; HS-SPME-GC-MS.

Introduction

Cold-brew systems combine different temperatures, contact times, hydrodynamics, filtration geometries and oxygen exposure, yet extraction performance, chemical composition, aroma, sensory quality and hygiene are usually examined separately. The experimental value of this work is an integrated, independently replicated comparison of eight complete commercial system- protocol configurations under one coffee matrix and brew ratio. This design tests realistic technological trade-offs rather than ranking systems through a single analytical endpoint [1-3].

Experimental

One light-roasted Guatemala honey-process coffee lot was brewed at a constant 85 g/L ratio in eight percolation, immersion or dynamically assisted configurations. Three independent extraction batches per configuration (24 batches) were analysed at T0, 48 h and 7 days at 4 °C using time-specific sealed aliquots. Physicochemical characterisation included roast colour, particle-size distribution, pH, ORP, TDS and mass-based extraction yield. HPLC-DAD quantified caffeine, four caffeoylquinic acids, p-coumaric acid and trans-cinnamic acid. Matrix-matched HS-SPME-GC-MS quantified 13 volatiles (six aldehydes, 2-pentylfuran, linalool, four alcohols and acetoin) by EI-SIM with authentic standards and batch quality controls. A 12-member trained panel evaluated each coded sample twice in separate sessions, while TAMC, presumptive LAB, yeasts and moulds were enumerated on duplicate plates. Technical triplicates were averaged within batch and replicate-level data were analysed using mixed- effects models.

Results and discussion

The analytical platform resolved complementary aspects of performance. Dynamic recirculation system produced the highest TDS (1.81%), caffeine (987.4 mg/L) and summed caffeoylquinic acids (1891.4 mg/L), whereas pulsed percolation system gave the highest mass-based extraction yield (15.68%): beverage concentration and extraction efficiency were therefore not equivalent. All six monitored aldehydes increased from T0 to T7. Enclosed percolation system showed the lowest summed-aldehyde concentration and 2-pentylfuran accumulation and retained the most linalool at day 7; static immersion systems showed the strongest chemical and sensory staling. Volatile trajectories agreed with decreasing freshness and increasing stale and cardboard/papery notes, whereas hygienic- indicator counts produced a different system ranking [4,5]. All HPLC-DAD and GC-MS concentrations were calculated from responses within validated working ranges.

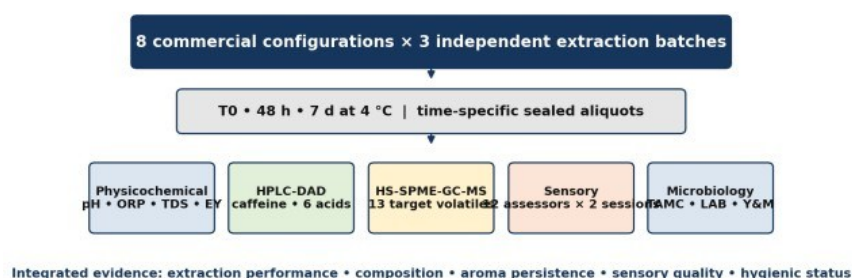


Fig. 1. Replicated multi-analytical design and complementary quality dimensions.

Conclusions

The study provides a replicated system-level framework linking process performance to non-volatile composition, aroma persistence, sensory evolution and hygienic status. Its practical value is to show that no single configuration optimises every quality dimension: extraction yield, beverage concentration, shelf-life aroma and sanitation must be controlled separately but interpreted together. The integrated approach offers firstly in the literature an evidence base for equipment selection and refrigerated shelf-life management, while future work should extend validation to additional coffees and roast levels.

References

1. L. Acquaticci et al.; Food Research International, 187 (2024), 114398.
2. M. Bellumori et al.; LWT, 142 (2021), 111043.
3. J. Heo et al.; Foods, 9 (2020), 1746.
4. S.N. Lopane et al.; Food Science & Nutrition, 12 (2024), pp. 985-996.
5. Y. Wang et al.; LWT, 197 (2024), 115919.

P15

Cellulose-based films from Coffee silverskin for sustainable active packaging: the rECOBIOpack project

Chiara Scapuzzi,¹ Tamara Chwojnik,¹ Valeria Romanucci,² Luisella Verotta,¹ Luciano Navarini,³ Paola Crisafulli,³ Stefania Lupinelli,⁴ Giovanni Di Fabio,² Daniele Carullo,⁵ Stefano Farris,⁵ Stefania Marzorati¹

¹ Department of Environmental Science and Policy, University of Milan, Via Celoria 2, 20133 Milan, Italy

² Department of Chemistry, University of Naples Federico II, Via Cintia 21, 80126 Naples, Italy

³ illycaffè S.p.A., via Flavia 110, Trieste I-34147, Italy

⁴ ILSA S.p.A. Via Quinta Strada 28, 36071 Arzignano, Italy

⁵ Department of Food, Environmental and Nutritional Sciences, Università degli Studi di Milano, Via Celoria 2, 20133 Milan, Italy

Summary: The rECOBIOpack project aims to valorise coffee silverskin, a by-product of coffee roasting, exploiting its peculiar composition to develop an active food packaging material. Cellulose was employed as material backbone, lipids as additives to improve mechanical properties and to reduce gas permeability, and polyphenols to add antioxidant properties.

Keywords: Lipid extract, polyphenols, biobased materials

Introduction

Coffee production generates waste in each step of the value chain, only 1-5% of the coffee cherry ends up in the final cup [1]. Nevertheless, new value could be given to process outputs, reconsidering coffee production in a circular perspective. Coffee silverskin (CS), the thin tegument covering green coffee beans, is the main by-product of coffee roasting. Although usually discarded as a waste, CS has a great potential as functional ingredient due to its chemical composition [2]. Both its lipid fraction and its high content of insoluble dietary fibres represent an opportunity for its valorisation in several fields.

This work presents the development of a functional packaging material valorising lipids, polyphenols and cellulose extracted from CS with the aim of introducing circularity in the coffee value chain to promote more sustainable production and consumption models.

Experimental

CS deriving from a blend of Arabica coffee beans was subjected to supercritical CO₂ extraction at industrial scale (ILSA S.p.A., Italy) to remove the lipidic fraction, and the obtained lipid extract was characterised by GC-MS after derivatisation (both silylation and transesterification were employed) [3]. Afterwards, the phenolic fraction was extracted from the defatted biomass using ultrasound- assisted extraction (UAE) method with a mixture of ethanol/water 7/3 (v/v) for 30 minutes at 80°C. The extract composition was investigated using LC-ESI-TOF. Finally, the remaining biomass was subjected to two steps of purification (alkaline hydrolysis and bleaching) for the recovery of cellulose [4]. The obtained coffee silverskin cellulose was characterised in terms of yield, morphology by scanning electron microscopy (SEM) and purity by solid state ¹³C NMR. Afterwards, cellulose was dispersed in ultrapure water and microfibrillated (GEA Lab Homogenizer PandaPLUS). The obtained suspension was employed for the preparation of cellulose films by solution casting. To improve film properties, the lipidic and polyphenolic extracts obtained from CS were mixed at different amounts (25% and 10%, respectively) into the cellulose suspension. The produced films were characterised in terms of antioxidant properties by ABTS assay, SEM, oxygen transmission rate (OTR), water vapour transmission rate (WVTR) and mechanical properties.

Results

The supercritical CO₂ extraction of lipids at industrial scale was carried out at 50-60 °C and 300 bar. The operational conditions were selected based on lab-scale optimisation [3]. The yield of extraction was 3.4 ± 0.3%. The fatty acid profile presented the predominance of saturated fatty acids compared to unsaturated, with palmitic acid as the most abundant followed by linoleic acid. The fatty acid composition is reported in Fig. 1.

Regarding the extraction of the phenolic fraction, under the selected extraction conditions a yield of 10 ± 0.3% was obtained. Chlorogenic acid and caffeine resulted the main species in the extract.

The treated coffee silverskin biomass was then subjected to cellulose extraction. After this purification step, a yield of cellulose of 28 ± 4% was obtained. The ¹³C NMR result showed the presence of unremoved hemicellulose and lignin at trace concentration. The morphology of cellulose revealed by SEM images showed a fibrous structure, with microfibrils organized into bundles. This structure is lost after homogenization, in fact,

homogenised cellulose suspension present fine microfibrils without significant aggregates.

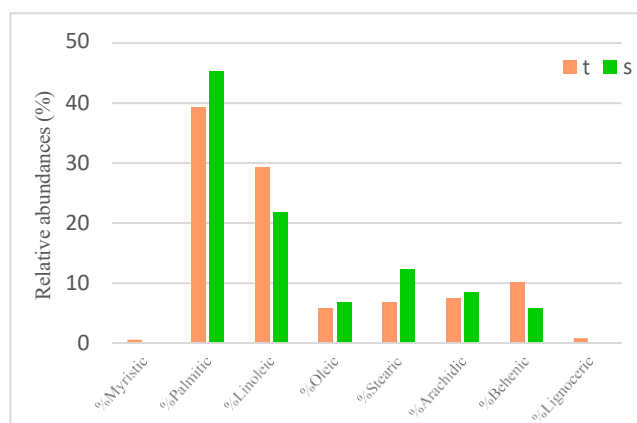


Fig. 1. Relative abundances of fatty acids in the lipidic extract obtained by GC-MS after (t) transesterification and (s) silylation.

The suspension was then employed to produce cellulose films, and they were compared to those obtained in the presence of additives: lipids and phenolic extract. Lipids were added to increase the film flexibility and hydrophobicity. Both the pure cellulose films and the cellulose films in presence of lipids present OTR values lower than $0.2 \text{ cm}^3 \text{ m}^{-2} \text{ day}^{-1}$ at 23°C and 50% RH, moreover in presence of lipids the WVTR decrease from $921 \pm 30 \text{ g m}^{-2} \text{ day}^{-1}$ to $34 \pm 4 \text{ g m}^{-2} \text{ day}^{-1}$ at 23°C and 65% RH. Also, the presence of lipids increases the mechanical properties in terms of Young's modulus, elongation at break, and tensile strength. This result confirms the effectiveness of lipid addition in enhancing barrier properties and improving the films' suitability for food preservation applications.

Regarding the inclusion of the phenolic extract, films incorporating the phenolic-rich extract exhibited rewarding antioxidant activity with EC₅₀ of ABTS assay of about 0.5 mg mL^{-1} .

Conclusions

The results obtained demonstrated the promising application of CS cellulose films for food packaging and the use of biobased and more sustainable solutions in coffee chain.

References

1. International Coffee Organization, *Coffee Development Report 2022-23*, 2024.
2. L. F. Ballesteros, J. A. Teixeira S. I. Mussatto, *Food and Bioprocess Technology*, 7, (2014), pp 3493–3503.
3. C. Scapuzzi, . Chwojnik, L. Verotta, G. Beretta, L. Navarini, S. Lupinelli, S. Marzorati, *Sustainable Chemistry and Pharmacy*, 45, (2025) 102001.
4. C. Rovera, D. Carullo, T. Bellesia, D. Büyüktas, M. Ghaani, E. Caneva, S. Farris, *Frontiers in Sustainable Food Systems*, 6, (2023) 1087867
5. R. Nasti, A. Galeazzi, S. Marzorati, F. Zaccheria, N. Ravasio, G.L. Bozzano, F. Manenti, L. Verotta, *Waste Biomass Valorization*

P16

Coffee-derived caffeoylquinides rapidly remodel the intestinal endocannabinoidome and improve epithelial barrier function in Caco-2 cells

Fabiana Piscitelli,¹ Marco Allarà,¹ Roberta Verde,¹ Elisabetta De Angelis,² Elena Guercia,² Javiera Fontecilla-Escobar,^{1,3} Fabio Arturo Iannotti,¹ Luciano Navarini⁴

¹ Consiglio Nazionale delle Ricerche – Istituto di Chimica Biomolecolare (CNR-ICB), Via C. Flegrei 34, Pozzuoli (NA), Italy

² Aromalab illycaffè spa, Area Science Park, Padriciano 99, 34149 Trieste

³ Cannabinoid and Sperm Biology Laboratory, Institute of Histology and Embryology of Mendoza (IHEM), National Scientific and Technical Research Council (CONICET)

National University of Cuyo, Mendoza, Argentina

⁴ illycaffè spa, via Flavia 110, 34147 Trieste

Summary: *Coffee extracts differentially modulate the intestinal endocannabinoidome and epithelial barrier integrity. LC-MS lipidomics, gene expression and TEER measurements revealed that an extract enriched in caffeoylquinides induced rapid remodeling of bioactive lipids associated with improved barrier function, suggesting a novel mechanism linking coffee phytochemicals with gut homeostasis.*

Keywords: *Coffee; Endocannabinoidome; intestinal barrier*

Introduction

Coffee is one of the richest dietary sources of bioactive phytochemicals, including chlorogenic acids and their roasting-derived derivatives, which have been associated with anti-inflammatory and gut-protective properties [1]. However, the molecular mechanisms underlying these effects remain poorly understood. The endocannabinoidome (eCBome) plays a central role in regulating intestinal permeability, inflammation and epithelial homeostasis [2], yet its modulation by coffee-derived phytochemicals has received little attention. In this study, we investigated whether chemically distinct coffee extracts differentially affect intestinal barrier integrity through modulation of the eCBome.

Experimental

Differentiated Caco-2 monolayers were exposed for 3 h to two coffee extracts (FP1 and FP2) at different concentrations. Barrier integrity was evaluated by transepithelial electrical resistance (TEER). Targeted LC-MS quantified endocannabinoids and related mediators, while RT-qPCR assessed genes involved in eCB metabolism (NAPE-PLD, DAGL α/β , FAAH, MAGL), nuclear receptors (PPAR α/γ) and tight-junction proteins (Occludin and TJP1). FP2 was characterized by a higher abundance of 3-caffeoyl-1,5-quinide (3-CQL) and 4-caffeoyl-1,5-quinide (4-CQL).

Results

FP2 produced the strongest improvement of epithelial barrier function, increasing TEER by approximately 42% after 3 h. This functional response was accompanied by a marked remodeling of the eCBome characterized by reduced AEA levels together with substantial increases in 2-AG, PEA, SEA and 2-DHG. Correlation analysis revealed a negative association between AEA and TEER, whereas 2-AG, PEA and SEA positively correlated with barrier integrity. Interestingly, improved TEER occurred without early transcriptional upregulation of Occludin or TJP1, suggesting that the initial barrier response is mediated by rapid lipid signaling rather than de novo tight-junction gene expression. FAAH expression was markedly reduced in FP2-treated cells, consistent with the accumulation of *N*-acylethanolamines. The greater biological activity of FP2 may be associated with its enrichment in 3-CQL and 4-CQL, although direct mechanistic studies with purified compounds are required.

Conclusions

Our integrated lipidomic, molecular and functional analyses indicate that coffee extracts rapidly remodel the intestinal endocannabinoidome before major transcriptional changes occur in tight-junction genes. The extract enriched in caffeoylquinides (FP2) displayed the strongest barrier-protective effect, suggesting that 3-CQL and 4-CQL may contribute to the modulation of lipid-mediated pathways regulating epithelial homeostasis. These findings identify the intestinal eCBome as a promising mechanistic target linking coffee phytochemicals with gut barrier function and demonstrate the value of targeted LC-MS lipidomics for investigating food–gut molecular interactions.

References

1. M.U. Makiso et al. *Food Sci Nutr*, 2023, 22;12(2):734-764.
2. S. Petrosino et al. *Prog Lipid Res*. 2026, 102:101396.

P17

Quinolizidine alkaloids in lupin: a case of acute intoxication

Ilaria Prizio, Mariantonietta Peloso, Gaetan Minkoumba Sonfack¹, Elisabetta Caprai

National Reference Laboratory for natural toxins in food, Food Chemical Department, Izsler, Bologna, Italy

Summary: An Italian intoxication case highlights the risks of quinolizidine alkaloids in poorly processed lupins. LC-MS/MS analysis confirmed high toxicity in raw seeds, reduced by 98% through proper debittering. The study concludes that harmonized EU maximum levels and detailed labeling instructions are essential to ensure consumer safety in the plant-protein market.

Keywords: Quinolizidine, alkaloids, LC- MS/MS, lupin

Introduction

In March 2026, a case of acute intoxication following the consumption of homemade lupin soup was reported in the Emilia-Romagna region (Italy), highlighting the health risks associated with inadequate domestic processing of lupin seeds. Quinolizidine alkaloids (QAs), naturally occurring secondary metabolites in *Lupinus* species and other *Genisteae* species, are responsible for these toxic effects. Due to their marked bitterness and toxicity, they act as a natural defense mechanism against herbivores, but represent a significant food safety concern. The structural backbone of these alkaloids is the quinolizidine nucleus, consisting of two fused six- membered rings that share a nitrogen atom at a common bridgehead position. Beyond this fundamental scaffold, QAs typically exhibit more complex tricyclic or tetracyclic chemical structures [1].

Their biological synthesis, derived from the amino acid L-lysine, results in over 170 different structures. Among these, lupanine, 13 α -OH- lupanine, sparteine, angustifoline, and lupinine are of particular toxicological importance. The concentration of these alkaloids varies significantly, depending on a complex interaction between plant genotype and environmental factors, such as soil composition and responses to biotic stress. From a food safety perspective, managing QAs represents a crucial challenge as lupin is increasingly utilized as an alternative protein source in bakery products and plant-based substitutes for meat and dairy [2]. The presence of QAs poses a growing risk, particularly when traditional debittering processes (soaking, boiling, and repeated washing) are not properly applied. The primary species used for food and feed include: Andean lupin (*L. mutabilis*); White lupin (*L. albus*); Blue lupin (*L. angustifolius*); Yellow lupin (*L. luteus*), primarily used as raw material for animal feed. The phenotypic distinction between lupin varieties is clearly defined by their QA content: "sweet" varieties with reduced concentrations, typically estimated between 130 and 150 mg/kg (<1000 mg/kg) and "bitter" varieties with extremely high levels, ranging from 40,000 to 80,000 mg/kg (>10,000 mg/kg) [3].

At high concentrations, the product may become inedible; if ingested, it can cause severe poisoning affecting the nervous, circulatory, and digestive systems. Typical symptoms include dizziness, tachycardia, nausea, and loss of motor control, potentially leading to lethal consequences such as cardiac arrest or respiratory paralysis at high dosages.

The current regulatory landscape reflects significant international heterogeneity. While the European Union has not yet established harmonized Maximum Levels (MLs), Food Standards Australia New Zealand (FSANZ) has adopted precautionary limits of 200 mg/kg for lupin derivatives and 5 mg/kg for sparteine in beverages [4]. This European regulatory gap, combined with the growing prevalence of plant- based proteins in modern diets, makes the implementation of a systematic monitoring plan across the supply chain urgent.

Experimental and Results

Following the reported intoxication, the National Reference Laboratory (NRL) analyzed both the consumed soup and the raw lupin seeds. High concentrations of QAs were detected, reaching 2,701.8 mg/kg in the soup and 18,662.6 mg/kg in the raw material, confirming acute exposure.

To investigate the effectiveness of domestic processing, the debittering procedure was experimentally reproduced according to standard instructions, including 24-hour soaking, 3-hour boiling, and repeated washing cycles over 3–4 days. This treatment resulted in a 98% reduction of total QAs, lowering their concentration to 207.1 mg/kg in the final edible product.

The study also demonstrated that alkaloids are extensively transferred into soaking and washing water, confirming that toxicity reduction strictly depends on repeated extraction steps and, critically, on the complete removal of processing water.

Samples were analyzed using a validated in-house LC-MS/MS method. Alkaloids were extracted from 2 g of homogenized sample with 40 mL of methanol/water/formic acid (50:50:0.1, v/v/v). The mixture was shaken for 30 minutes on a rotary tumbler, centrifuged, and an aliquot of the supernatant was diluted with water and

transferred into a 500 µL filter vial. The limits of quantification (LOQ) was 1 mg/kg for lupin seeds [5]. Chromatographic separation was performed on a reverse-phase column, followed by MS/MS detection using an Acquity UPLC I-Class PLUS coupled to a Xevo TQ-XS (Waters). The method allowed the quantification of 14 QAs (albino, anagrine, cytosine, gramine, 13α- hydroxylupanine, lupanine, methylcytosine, multiflorine, sparteine, lupanine, trans-13α- cinnamoyloxylupanine, thermopsine, angustifoline, and epilupanine), ensuring high sensitivity and selectivity [6].

Botanical identification was subsequently confirmed by Sanger sequencing, identifying the sample as *Lupinus albus*. In this type of lupinus, lupanine was the predominant alkaloid, followed by albino, 13α-hydroxylupanine, and multiflorine, while angustifoline and isolupanine were present as secondary components [7].

Conclusions

Although the raw material exhibited inherently high alkaloid levels, the intoxication was primarily caused by inadequate domestic processing. In particular, the retention or reuse of cooking water—identified as a major reservoir of extracted alkaloids—represented a critical failure in risk mitigation.

These findings highlight that improper household preparation can result in residual QAs levels close to or exceeding international safety thresholds, even after treatment.

There is therefore an urgent need for:

1. Harmonized EU regulatory limits for quinolizidine alkaloids in lupin seeds and in lupin-based products.
2. Clear and mandatory labeling, providing precise instructions on debittering procedures to prevent acute intoxication.
3. The promotion and marketing of lupin species with low levels of quinolizidine alkaloids.

References

1. EFSA Panel on Contaminants in the Food Chain (CONTAM); Schrenk D, Bodin L, Chipman JK, Del Mazo J, Grasl-Kraupp B, Hogstrand C, Hoogenboom LR, Leblanc JC, Nebbia CS, Nielsen E, Ntzani E, Petersen A, Sand S, Schwerdtle T, Vlemingckx C, Wallace H, Alexander J, Cottrill B, Dusemund B, Mulder P, Arcella D, Baert K, Cascio C, Steinkellner H, Bignami M. Scientific opinion on the risks for animal and human health related to the presence of quinolizidine alkaloids in feed and food, in particular in lupins and lupin-derived products. EFSA J. 2019 Nov 5;17(11): e05860. doi: 10.2903/j.efsa.2019.5860. PMID: 32626161; PMCID: PMC7008800.
2. Keuth, O., Humpf, H. U., & Fürst, P. (2023). Quinolizidine alkaloids in lupine flour and lupine products from the German retail market and risk assessment of the results regarding human health. Food Additives & Contaminants: Part A, 40(5), 667–674. <https://doi.org/10.1080/19440049.2023.2195954>
3. BfR, 2017. Risk assessment of the occurrence of alkaloids in lupin seeds BfR Opinion No 003/2017, 27 March 2017
4. Australia New Zealand Food Standards Code – Schedule 19 – Maximum levels of contaminants and natural toxicants- F2024C00908 C0613 September 2024- <https://www.legislation.gov.au/F2015L00454/latest/text>
5. EURLMP-method_012 (version 1), Determination of quinolizidine alkaloids in lupin seeds, food products and feed by LC-MS/MS
6. EURL-MP-doc_003 Guidance document on performance criteria (draft May 2024). BfR, 2025. Alkaloids and allergies: current data on health risks from lupin seeds in food. BfR Opinion 035/2025 of 27 September 2025

P18

Capturing toxic tropane alkaloids from *Datura stramonium*, *Datura metel*, *Mandragora autumnalis* in plant based foods via DART-HRMS

Nicole Cascio,¹ Carmela Zacometti,¹ Francesca Asson,¹ Andrea Massaro,¹ Roberto Stella,² Roberta Masin,³ Giancarlo Biancotto,² Alessandra Tata¹

¹ Istituto Zooprofilattico Sperimentale delle Venezie, Laboratory of Experimental Chemistry, Viale Fiume, 78, 36100 Vicenza (VI), Italy

² Istituto Zooprofilattico Sperimentale delle Venezie, SCS2 – Department of Chemistry, viale dell'Università, 10, 35120 Legnaro (PD), Italy

³ University of Padova, Department of Agronomy, Food, Natural Resources, Animals and Environment – DAFNAE viale dell'Università, 16, 35120 Legnaro (PD), Italy

Summary: The study presents DART-HRMS as a rapid screening technique for the identification of tropane alkaloids in plant mixtures. The results demonstrate that this technique is an excellent solution in terms of analysis speed and ease of use, while also highlighting the need for further investigation regarding the matrix effect.

Keywords: DART-HRMS, tropane alkaloids (TAs), food safety

Introduction

In October 2022, a notification published on the Rapid Alert System for Food and Feed (RASFF) platform reported the presence of *Mandragora autumnalis* leaves in spinach-based food products manufactured and marketed in Italy. The notification followed a poisoning incident involving at least 12 individuals, most likely associated with the accidental ingestion of these leaves containing tropane alkaloids (TAs) [1]. Consequently, the need emerged to develop a qualitative analytical method for the screening of TAs and their biosynthetic precursors in plant-based food matrices. Although gas chromatography coupled with mass spectrometry (GC–MS) is currently considered the reference method for TAs determination, it is relatively labor-intensive and therefore not ideally suited to the intended screening purpose. In this context, Direct Analysis in Real Time coupled with High-Resolution Mass Spectrometry (DART-HRMS) represents a valuable alternative for rapid screening. This technique employs a heated gas stream capable of directly desorbing and ionizing low-molecular-weight compounds, providing high selectivity, sensitivity, and rapid analytical performance. This study aims to evaluate the capability of DART-HRMS to capture the presence of toxic tropane alkaloids from *Datura stramonium*, *Datura metel*, *Mandragora autumnalis* in plant based foods.

Experimental

Samples from the toxic botanical species *Datura metel*, *Datura stramonium*, and *Mandragora autumnalis* were grown together with samples from the edible botanical species *Beta vulgaris* var. *cicla*, *Cichorium intybus* L., and *Spinacia oleracea* L. Following homogenization under liquid nitrogen, 0.100 ± 0.020 g of each sample was suspended in methanol (HPLC-grade methanol, 99.9% purity; VWR International, Radnor, USA), mechanically disrupted using a bead disruptor, and subsequently centrifuged at 12,000 × *g* for 5 min at 4°C. The resulting supernatants were collected and directly analyzed by DART-HRMS. To evaluate the sensitivity and effectiveness of the method in detecting TAs, methanolic extracts of the three edible plant species were mixed with methanolic extracts of the three toxic species at five contamination levels: 1%, 5%, 10%, 20%, and 50%. Data acquisition was performed using a DART SVP 100 ion source (IonSense, Sangus, USA) coupled to an Exactive Plus Orbitrap Mass Spectrometer (Thermo Fisher Scientific, Waltham, USA). Five microliters of each extract were individually deposited onto melting point capillary tubes mounted on a custom-built holder connected to the Dip-it® autosampler (IonSense, Sangus, MA, USA). DART source had grid voltage set to 250 V and a gas temperature of 300°C. Mass spectra were acquired at a resolving power of 70,000 FWHM over an *m/z* range of 75–1,125 in positive ion mode [2]. The resulting data were subsequently evaluated using univariate statistical analysis (p-value adj <0.05).

Results

Analysis of the spectra obtained from the extracts of the three toxic botanical species revealed the presence of the alkaloids [Norhygrine+H]⁺ (*m/z* 128.1066) and [Scopolamine+H]⁺ (*m/z* 304.1533), together with their biosynthetic precursors [Dihydroxytropane+H]⁺ (*m/z* 158.1170) and [Tropine+H]⁺ (*m/z* 142.1222), in *Datura metel*. In *Datura stramonium* extracts, the alkaloids [Atropine+H]⁺ (*m/z* 290.1740) and [Scopolamine+H]⁺ (*m/z* 304.1531) were annotated. Finally, *Mandragora autumnalis* extracts contained [Atropine+H]⁺ (*m/z* 290.1742) and [Scopolamine+H]⁺ (*m/z* 304.1535), together with the precursor [Tropine+H]⁺

(m/z 142.1223). As expected, no tropane alkaloids or related precursors were detected in the non-toxic plant matrices *Beta vulgaris* var. *cicla*, *Cichorium intybus* L., and *Spinacia oleracea* L. Spiked samples showed increasing signal intensities of TAs, particularly [Atropine+H]⁺ and [Scopolamine+H]⁺, with increasing contamination levels of *Datura metel* and *Datura stramonium* across the different plant matrices analyzed. Similarly, increasing signal intensities were observed for [Norhygrine+H]⁺ and [Tropine+H]⁺, whereas [Dihydroxytropine+H]⁺ was detectable only at specific contamination levels, depending on the botanical species considered. The alkaloids of *Datura metel* were detectable from the lowest contamination level tested, 1%, in *Beta vulgaris* var. *cicla*, whereas [Atropine+H]⁺ became detectable from 10% contamination in *Cichorium intybus* L., and *Spinacia oleracea* L. In samples contaminated with *Mandragora autumnalis*, [Tropine+H]⁺ was detectable from 10%, while [Atropine+H]⁺ and [Scopolamine+H]⁺ were detectable at ≥10 and 20% contamination levels, reaching 20% in spinach, most likely due to their relatively low concentrations in the spiked samples. For *Datura stramonium*, [Atropine+H]⁺ and [Scopolamine+H]⁺ became clearly discriminative only at contamination levels ranging from 10% to 20% in all edible plant matrices, whereas [Norhygrine+H]⁺ was not detected. The building up and validation of calibration curves were attempted. Differences among plant matrices and toxic species were observed, which may have affected the analytical performance of the method in terms of linearity, precision, and accuracy.

Conclusions

The results demonstrated that DART-HRMS is a promising technique for the rapid screening of multiple samples owing to its high analytical throughput, ease of operation, and minimal sample preparation requirements, making it a valuable tool for the food industry. Nevertheless, the observed matrix effects highlight the need for further studies aimed at optimizing the method and comprehensively evaluating its analytical performance, particularly with respect to accuracy, precision, and its capability to detect tropane alkaloids at very low concentrations.

References

1. RASFF Window, 2022, <https://webgate.ec.europa.eu/rasff-window/screen/notification/574200>
2. Zacometti C, Tata A, Stella R, Leone S, Pallante I, Merenda M, Catania S, Pozzato N, Piro R. DART-HRMS allows the detection of toxic alkaloids in animal autopsy specimens and guides the selection of confirmatory methods in accidental plant poisoning. *Anal Chim Acta*. 2023 Jul 11;1264:341309. doi: 10.1016/j.aca.2023.341309. Epub 2023 May 3. PMID: 37230724.

P19

Investigating amino acid–sugar interactions for cocoa-like aroma generation in a chickpea flour-based solid food matrix

Giorgia Mattina,¹ Leonardo Lorenzi,² Andrea Francioni,² Riccardo Bottiroli,² Vincenzo Fogliano,³ Eugenio Aprea¹

¹ University of Trento, via Mach 1, 38098 San Michele all'Adige, Italy

² Foreverland Food S.r.l. Via Alessandro Volta 37, 70014 Conversano Italy

³ Wageningen University & Research, Bornse Weilanden 9, 6708 Wageningen, The Netherlands

Summary: *This study investigates generating cocoa-like flavors in a chickpea flour matrix by roasting it with specific amino acids and reducing sugars. Using HS-SPME/GC-MS to analyze the volatile compounds, the research aims to leverage Maillard chemistry to guide the design of plant-based, cocoa-inspired food systems.*

Keywords: *Maillard Reaction, Flavor Precursors, Volatile Compounds*

The generation of cocoa-like flavours through controlled Maillard reaction pathways represents a promising approach for developing cocoa-inspired food systems from alternative plant-based matrices. Cocoa aroma development is strongly influenced by the availability and composition of amino acids and sugars precursors generated during fermentation and transformed during roasting. However, in non-cocoa matrices, the contribution of individual precursor to volatile compounds associated to cocoa-like flavours is insufficiently understood.

It is hypothesized that amino acids structures and reducing sugars type will strongly influence the resulting volatile compounds profile, with branched-chain amino acids promoting the formation of alkylpyrazines and Strecker aldehydes, while combined precursor systems may generate a broader and more complex aroma profile.

This study investigates the use of chickpea flour as a solid food matrix to support the Maillard-driven generation of cocoa-like flavour under roasting.. Chickpea flour was selected due to its ability to provide a realistic plant-based environment for precursor incorporation and thermal flavour development. The influence of amino acids precursors commonly associated with cocoa fermentation, including leucine, phenylalanine, alanine, valine, glycine, and their combined systems, will be evaluated in combination with different reducing sugars (fructose and xylose) to determine their contribution to cocoa-like aroma formation.

Following controlled roasting, volatile aroma compounds will be characterized using headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME/GC-MS). Particular attention will be given to key compounds, which contribute to roasted, nutty, and chocolate sensory characteristics.

This work provides insight into the design of cocoa-inspired solid food matrices and the development of plant-based flavour systems through targeted manipulation of Maillard chemistry.

P20

Advancing peanut aroma analysis through integrated headspace sampling and GC×GC–HRMS

Andrea Caratti,¹ Angelica Fina,^{1,2} Sara Tanilli,¹ Fulvia Trapani,¹ Erica Liberto,¹ Giuseppe Genova,² Cristina Casetta,² Carlo Bicchi,¹ Chiara Cordero¹

¹ Department of Drug Science and Technology, University of Turin, Via Pietro Giuria 9, 10125 Turin, Italy

² Soremartec Italia Srl, Piazzale Ferrero 1, 12051 Alba (Cuneo), Italy

Summary: *An integrated analytical workflow combining advanced headspace sampling (SPME Arrow and ITEX) with thermally modulated GC×GC–HRMS was developed to improve the detection and quantification of odor-active compounds in the peanut volatilome. Enhanced chromatographic resolution, accurate-mass selectivity, and increased instrumental sensitivity enabled reliable OAV determination and a more faithful reconstruction of the food chemical odor code, highlighting the potential of advanced hyphenated chromatographic–MS techniques for food aroma characterization.*

Keywords: *Peanuts volatilomics, HRMS, Multidimensional chromatography*

Introduction

The identification and quantification of odor-active volatile compounds are fundamental to food volatilomics and sensomics, where the reconstruction of food aroma relies on odor activity values (OAVs) calculated from accurate concentration data. Because many key odorants occur at concentrations close to or below ng·kg⁻¹, analytical methods must achieve instrumental sensitivity approaching physiological olfactory thresholds while maintaining reliable compound identification in highly complex food matrices. Conventional one-dimensional GC-MS often struggles to meet these requirements due to limited chromatographic resolution and matrix interferences. Comprehensive two-dimensional gas chromatography coupled with high-resolution mass spectrometry (GC×GC–HRMS) represents a powerful analytical platform capable of overcoming these limitations through enhanced separation efficiency and highly selective accurate-mass detection.

Experimental

Roasted peanut (*Arachis hypogaea* L.) was selected as a model food matrix because of its chemically complex volatilome and well-characterized aroma profile. Volatile compounds were extracted from the headspace using complementary high-capacity enrichment techniques, namely SPME Arrow and In-Tube Extraction (ITEX), which provide different extraction efficiencies according to analyte volatility, sorbent affinity and extraction kinetics. Samples were analysed by thermally modulated GC×GC coupled with high-resolution mass spectrometry, exploiting enhanced chromatographic peak capacity together with accurate-mass measurements and high resolving power. To fully exploit the complementarity of the enrichment devices while avoiding multiple independent processing workflows, chromatographic data fusion was applied to integrate the individual extraction profiles into a single information-rich chromatogram for downstream processing and quantitative analysis.

Results

Thermal modulation significantly improved chromatographic performance by compressing chromatographic bands, increasing peak intensity, and reducing co-elution within the complex peanut volatilome. The orthogonal GC×GC separation, combined with HRMS accurate-mass filtering and improved spectral deconvolution, increased confidence in compound annotation and enabled the detection of low-abundance odorants that remained unresolved using conventional one-dimensional approaches. The complementary extraction behaviour of SPME Arrow and ITEX extended volatility coverage and enhanced the recovery of trace odorants occurring at physiologically relevant concentrations, allowing more reliable OAV determination. Chromatographic data fusion further combined the complementary information provided by the different enrichment devices into a single comprehensive chromatographic fingerprint, improving analytical coverage while substantially simplifying data processing and interpretation. A schematic overview of the chromatographic data fusion, is presented in Figure 1.

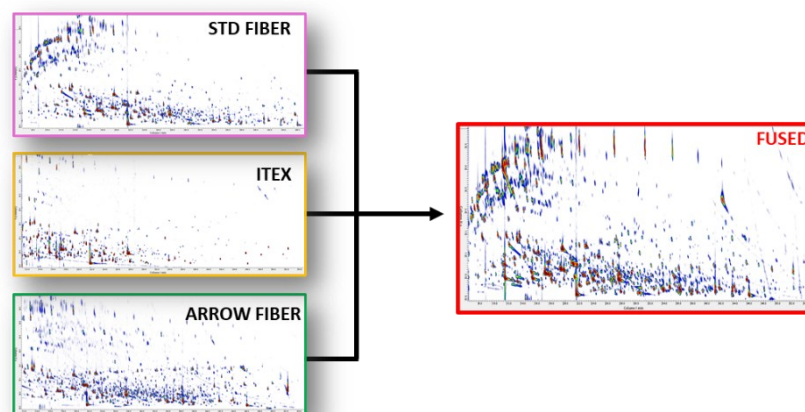


Figure 1. Chromatographic data fusion of complementary headspace extraction techniques. Individual GC×GC chromatograms acquired using SPME fiber, SPME Arrow and ITEX were combined to generate a single fused chromatogram for unified downstream data processing and interpretation.

Conclusions

The integration of advanced headspace sampling, chromatographic data fusion and GC×GC–HRMS provides a robust analytical platform for food odor analysis by combining high chromatographic resolution, accurate-mass selectivity, and enhanced instrumental sensitivity. This multidimensional MS-based workflow enables a more reliable reconstruction of the chemical odor code through improved detection and quantification of odor-active compounds occurring at physiologically relevant concentrations. By merging complementary extraction profiles into a single analytical dataset, the approach also streamlines data processing, making advanced multidimensional food volatilomics more suitable for routine quality assessment and future sensomics-based applications.

References

1. Giddings J. C.; *Journal of Chromatography A*, 703 (1995), pp 3-15

P21

Authentication of the geographical origin of Italian multifloral honey: Multi- analytical characterization of the composition and pesticide residues to distinguish the production area.

Francesca Asson,¹ Carmela Zacometti,¹ Andrea Massaro,¹ Fabjola Bilo,^{2,3} Marianna Martinello,⁴ Franco Mutinelli,⁴ Elisabetta Schievano,¹ Alessandra Tata¹

¹ Laboratory of Experimental Chemistry, Istituto Zooprofilattico Sperimentale delle Venezie, Viale Fiume, 78, 36100 Vicenza (VI), Italy

² Department of Medical and Surgical Specialties, Radiological Sciences, and Public Health, University of Brescia, Viale Europa, 11, 25123, Brescia, Italy

³ INSTM and Chemistry for Technologies Laboratory, Department of Mechanical and Industrial Engineering, University of Brescia, Via Branze 38, 25123, Brescia, Italy

⁴ NRL for Honey Bee Health, Istituto Zooprofilattico Sperimentale delle Venezie, Viale dell'Università 10, 35020 Legnaro, Italy

⁵ Department of Chemistry, University of Padova, Padova, Italy

Summary: *This study presents an integrated multi-analytical strategy for the geographical characterization of Italian multifloral honeys. By combining volatile, elemental, metabolic, pesticide residue, and melissopalynological analyses, the work identifies geographical markers and pollen signatures that support origin authentication and enhance the reliability of honey traceability.*

Keywords: VOCs, DBDI-HRMS, ambient mass spectrometry.

Introduction

Determining the geographical provenience of honey has become a central issue in recent years, since adulteration practices are increasingly widespread and the product price strongly depends on its botanical and geographical origin. For this reason, the European Commission has promoted the development of harmonized analytical methods to assess honey authenticity and to verify compliance with quality specifications. This turned out to be particularly challenging for multifloral honeys, whose chemical composition depends on local biodiversity, environmental factors and agricultural activities [1]. In this work, an integrated multi-analytical approach was applied to characterize Italian multifloral honeys and identify markers associated with their geographical origin. In particular, the 75 honey samples collected from all over the peninsula were analyzed using a combination of analytical technique that include the untargeted profiling of volatile organic compounds (VOCs) via dielectric barrier discharge ionization high-resolution mass spectrometry (DBDI-HRMS), the quantification of sugars and amino acids by quantitative nuclear magnetic resonance (q-NMR), the elemental analysis by total reflection X-ray fluorescence (TXRF), and the targeted assessment of pesticide residues. In parallel, representing the gold standard method for geographical origin determination, several thousand melissopalynological analyses of Italian multifloral honeys collected between 2015 and 2024 were statistically evaluated to identify pollen species characteristic of the Northern, Central, and Southern Italian macro-areas.

Experimental

The DBDI-HRMS technique represents a very sustainable and quick approach for the analysis of VOCs [2]. In particular, DBDI is an ambient ionization source based on the formation of a low- temperature plasma that is produced by discharge between two electrodes separated by a dielectric barrier, and which has been used for the detection of volatiles in a variety of food matrices.

Each honey sample was weighed (2g) and placed in a glass tube that was placed in a thermostat at 40°C for 20 minutes. After the quick preparation, samples were analyzed using a SICRIT source (Plasmion GmbH, Augsburg, Germany) coupled to an Exactive Orbitrap (Thermo Fisher Scientific, Waltham, USA). Samples have been analyzed in duplicate by simply placing the opened glass tube directly in front of the source for ten seconds each. In order to remove the background signal, the overall acquisition lasted 30 seconds: 10 sec with closed vial for background-10 sec with opened vial for acquisition – 10 sec with closed vial for background. The mass range was set to 75-300 Da in positive ion mode. Prior to preprocessing, the repeatability of the two spectral replicates for each honey sample was assessed using cosine similarity.

Details of the methods used for the other techniques have been described by Asson et al. [3]. The data were statistically analyzed by univariate statistical analysis.

Results

All the obtained data were subjected to statistical analysis that revealed clear regional patterns in pollen counts, VOCs, mineral composition and specific sugar and amino acids. This scientifically demonstrated the influence

that local flora, geological background and the environmental conditions have on the final product.

Distinct volatile profiles were obtained from the differently geographically located honeys, indicating that DBDI-HRMS was able to differentiate between Italian macro-regions. In particular, the technique revealed the presence of high concentration of cresol (m/z 137.0960, also known as trimethylphenol), aminoacetophenone (m/z 136.0759) and santene (m/z 123.1168) in multifloral honeys produced in Northern Italy, in accordance with the results of the pollen counts. Methyl-syringate (m/z 213.0753) appeared to be a typical Central and Southern honey marker since it is found in citrus honey and the melissopalynological assessment revealed that these multifloral honeys were distinguished by the high prevalence of citrus pollen.

Q-NMR analysis identified distinctive trends in minor carbohydrates and amino acids.

Specifically, honey from Southern Italy exhibited higher concentrations of proline, leucine, and isoleucine. The amino acid profile likely reflects the predominant botanical origins, particularly citrus and eucalyptus, as confirmed by melissopalynological analysis.

The TXRF analysis enabled the identification of a broad range of elements, among which significantly elevated bromine levels were detected in honey samples originated from central and southern Italy, indicating the probable contribution of the marine aerosol. Similarly, the technique revealed an increment of strontium concentrations going from North to the South of the peninsula.

For what concerns the presence of pesticide residues, it is worth noting that they were generally minimal and largely compliant with regulatory thresholds, confirming the overall high quality of Italian honey. Due to their low occurrence and concentration levels, pesticide residues were not considered suitable variables for the determination of the botanical origin of the samples.

Conclusions

The multi-analytical characterization of multifloral Italian honey conducted in this study allowed to simultaneously evaluate complementary physicochemical parameters that together create a robust geographical fingerprint. However, the application of a full multi-analytical approach is not always feasible due to high costs, long analysis time and the need for specialized instruments and expertise. The development of chemometric models based on a reduced set of the most informative markers discovered in this study could enable reliable geographical classification while minimizing analytical complexity.

References

1. V. Kaškonienė, P.R. Venskutonis, *Comprehensive Reviews in Food Science and Food Safety* 9 (2010) 620–634. <https://doi.org/10.1111/j.1541-4337.2010.00130.x>Digital Object Identifier (DOI)
2. A. Massaro, C. Zacometti, M. Bragolusi, J. Buček, R. Piro, A. Tata, *Food Control* 160 (2024) 110330. <https://doi.org/10.1016/j.foodcont.2024.110330>
3. F. Asson, M. C. Chotzara, C. Zacometti, A. Massaro, F. Bilo, M. Martinello, C. Manzinello, M. Bertola, G. Morbin, R. Piro, L. Piana, F. Mutinelli, L. Borgese, E. Schievano, A. Tata; *Microchemical Journal* (2026) 118662. [10.1016/j.microc.2026.118662](https://doi.org/10.1016/j.microc.2026.118662)

P22

Comprehensive characterization of honey from Kazakhstan, Italy, China and other countries through volatile profiling by GC-MS, polyphenolic profiling by HPLC-MS/MS and quantification of 5-hydroxymethylfurfural by HPLC-DAD

Gulzhan Khamitova,^{1,2} Laura Acquaticci,² Lazzat Karasholakova,¹ Giovanni Caprioli ²

¹ Research Institute of Biotechnology and Ecology, Zhetysu University named after I. Zhansugurov, Zhansugurov street, 187a, 040009, Taldykorgan, Kazakhstan

² School of Pharmacy, University of Camerino, Via Sant'Agostino 1, 62032 Camerino, Italy

Summary: Honey samples from Kazakhstan, Italy, and China were comprehensively characterized using GC-MS, HPLC-QQQ, HPLC-DAD and antioxidant assays. Integrated volatile and polyphenolic profiling revealed distinct geographical signatures, demonstrating the potential of metabolomic approaches for honey authentication, quality assessment, and evaluation of functional properties.

Keywords: Honey; Geographical origin; Food Authentication

Introduction

Honey is a natural food whose chemical composition is strongly influenced by its botanical and geographical origin, making it an important target for authenticity assessment and quality evaluation. This study presents a comprehensive chemical characterization of honey samples collected from Kazakhstan, Italy, China, and other countries by integrating volatile organic compound (VOC) profiling, polyphenolic composition, antioxidant activity, and quantification of 5-hydroxymethylfurfural (5-HMF), a key indicator of honey quality and freshness [1–3].

Experimental

Volatile organic compounds were extracted by headspace solid-phase microextraction (HS-SPME) and analysed using gas chromatography–mass spectrometry (GC–MS). Thirty-six phenolic compounds were quantified by high-performance liquid chromatography coupled with triple quadrupole mass spectrometry (HPLC–QQQ), while 5-hydroxymethylfurfural (5-HMF) was determined by high-performance liquid chromatography with diode-array detection (HPLC–DAD). In addition, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity were evaluated using spectrophotometric assays, including the DPPH radical scavenging assay.

Results

The analytical approach revealed marked differences in the chemical composition of honey samples according to their geographical origin. Distinct VOC fingerprints, comprising aldehydes, alcohols, ketones, terpenes, and aromatic compounds, were identified, while significant variability was observed in polyphenolic composition and antioxidant capacity. Quantification of 5-HMF demonstrated differences in honey quality among samples and confirmed its suitability as an important quality control marker.

Multivariate statistical analysis clearly discriminated honey samples according to their metabolomic profiles, highlighting the complementary discriminatory power of volatile compounds and phenolic constituents for geographical origin classification.

Conclusions

The integration of targeted and untargeted analytical approaches provides a comprehensive strategy for honey authentication, quality assessment, and evaluation of functional properties. The combined analysis of volatile compounds, polyphenols, antioxidant activity, and 5-HMF offers reliable chemical markers for geographical origin discrimination and quality control, contributing to a deeper understanding of honey composition and supporting the development of robust analytical tools for food authenticity.

References

1. Ahmed, S.; Sulaiman, S.A.; Baig, A.A.; Ibrahim, M.; Liaqat, S.; Fatima, S.; Jabeen, S.; Shamim, N.; Othman, N.H. Honey as a Potential Natural Antioxidant Medicine: An Insight into Its Molecular Mechanisms of Action. *Oxid. Med. Cell. Longev.* 2018, 2018, doi:10.1155/2018/8367846.
2. Polak-Śliwińska, M.; Tańska, M. Conventional and Organic Honeys as a Source of Water- and Ethanol-Soluble Molecules with Nutritional and Antioxidant Characteristics. *Molecules* 2021, 26, doi:10.3390/molecules26123746.
3. Hamilton-Amachree, A.; Odokwo, E.O. GC-MS Analysis of the Volatile Constituents and Antioxidant Activity of the Crude Honey Residue from Takum Local Government Area of Taraba State, Nigeria; 2022; Vol. 23.

P23

HS-GC-IMS authenticates the botanical origin of seven types of Italian monofloral honey, annotating the significant volatile compounds by the novel HS- SPME-GC-IMS-MS prototype

*Filippo Panni,¹ Andrea Massaro,² Lukas Bodenbender,³ Carmela Zacometti,²
Roberto Piro,² Angela Felicita Savino,¹ Philipp Weller,³ Alessandra Tata²*

¹ Ministero dell'agricoltura, della Sovranità Alimentare e delle Foreste, Dipartimento dell'Ispettorato Centrale della tutela della Qualità e Repressioni Frodi dei prodotti agro-alimentari, ICQRF Laboratory of Perugia, Via della Madonna Alta, 138 c/d, Perugia, PG, Italy

² Experimental Chemistry Laboratory, Istituto Zooprofilattico Sperimentale delle Venezie, Viale Fiume, 78, 36100 Vicenza, VI, Italy

³ Institute for Instrumental Analysis and Bioanalytics, Technische Hochschule Mannheim, Paul-Wittsack-Str. 10, 68163 Mannheim, Germany

Summary: *The study evaluates the capability of HS-GC-IMS, combined with statistical methods and machine learning, to authenticate the floral origin of 136 Italian monofloral honeys. The results show high classification accuracy and demonstrate the effectiveness of the new HS-SPME-GC-IMS-MS system in identifying the characteristic volatile compounds of honey.*

Keywords: *HS-GC-IMS, Food authentication, HS-SPME-GC-IMS-MS prototype*

Introduction

Monofloral honey, produced mainly from the nectar of a single plant species, possesses distinctive organoleptic, physico-chemical and microscopic characteristics that enhance its commercial value, whilst making it more vulnerable to fraud and mislabelling. In response to the growing prevalence of honey that is adulterated or incorrectly labelled in terms of its botanical and geographical origin, the European Union has updated its legislation on honey (Directive (EU) 2024/1438), introducing measures to strengthen controls and providing for the establishment of a European reference laboratory dedicated to the development of harmonised analytical methods.

Currently, the authentication of botanical origin relies primarily on melissopalynology and sensory analysis. Although effective, both techniques require highly qualified personnel and are characterised by lengthy analysis times and a degree of subjectivity. For this reason, alternative methods have been developed, including physico-chemical analyses and spectroscopic and chromatographic techniques. One of the most promising methods is headspace gas chromatography coupled with ion mobility spectrometry (HS-GC-IMS), which enables the characterisation of the volatile organic compound profile of honey. This technique has already been successfully applied to the authentication of various foods and to the detection of honey adulteration. The present study assessed the effectiveness of HS-GC-IMS, combined with machine learning algorithms, to authenticate the botanical origin of Italian monofloral honeys originating from seven different floral species. The main innovation lies in the use of a fully integrated HS-SPME-GC-IMS-MS prototype, capable of simultaneously acquiring chromatographic, ion mobility and mass spectrometric data within a single workflow, enabling a more accurate characterisation of volatile compounds and offering a new approach to verifying the authenticity of honey.

Experimental

A total of 136 Italian monofloral honey samples, collected during 2022–2023 and representing seven botanical origins (acacia, chestnut, citrus, dandelion, linden, rhododendron, and sunflower), were analyzed. Most samples were provided by the Osservatorio Nazionale Miele, while five official control samples were supplied by the ICQRF Laboratory of Perugia. The botanical origin of the honeys was verified using sensory, physicochemical, and melissopalynological analyses.

Volatile organic compounds (VOCs) were analyzed using Headspace Gas Chromatography–Ion Mobility Spectrometry (HS-GC-IMS) following minimal sample preparation. The most discriminant VOCs were subsequently identified using HS-SPME-GC-IMS-MS, and compound annotation was performed by comparison with the NIST mass spectral library, considering matches of at least 90% as acceptable.

Spectral data were processed in R through filtering, alignment, automatic peak detection, and integration. Principal Component Analysis (PCA) was applied for exploratory data analysis, whereas Partial Least Squares Discriminant Analysis (PLS-DA) was used for supervised classification. To develop a predictive model, the dataset was divided into a training set (107 samples) and an independent test set (29 samples, including the five ICQRF control samples). The model was optimized using five-fold cross-validation and its performance

was evaluated using accuracy, sensitivity, specificity, Cohen's Kappa, and the area under the receiver operating characteristic curve (AUC-ROC).

Results

HS-GC-IMS analysis enabled the generation of characteristic volatile profiles for the different Italian monofloral honeys. The heatmaps revealed distinct signatures for linden, citrus, and dandelion honeys, whereas chestnut, sunflower, rhododendron, and acacia honeys showed some similarities in their volatile compositions.

Exploratory statistical analysis using PCA highlighted a good separation of some groups (citrus, chestnut, linden, and sunflower), while rhododendron, acacia, and dandelion honeys showed greater overlap. However, PLS-DA analysis confirmed the presence of sufficient differences among volatile profiles to allow discrimination of floral origins.

A PLS-DA-based classifier was subsequently developed to authenticate the botanical origin of the honeys. The model showed excellent performance during cross-validation (accuracy 99.07%, AUC 96.73%) and good performance on an independent test set (accuracy 93.10%, AUC 83.33%). The two misclassified samples were likely characterized by the presence of additional nectar and pollen sources, in agreement with sensory evaluations.

The annotation of the main volatile compounds allowed the characterization of each honey type: dandelion honey was rich in nitrile compounds and aromatic molecules; citrus and rhododendron honeys were characterized by aromatic aldehydes and terpenes; chestnut honey showed high levels of nonanal, benzaldehyde, and long-chain aldehydes; acacia honey exhibited high abundances of cis-linalool oxide, benzaldehyde, and linalool; sunflower honey was associated with characteristic alcohols and monoterpenes; and linden honey was characterized by compounds such as acetone, γ -terpinene, terpenols, and aromatic phenols.

Overall, the combination of HS-GC-IMS and multivariate statistical analysis demonstrated a high potential for distinguishing and authenticating the floral origin of monofloral honeys based on their volatile fingerprints.

Conclusions

The research demonstrates that the HS-GC-IMS technique, combined with multivariate statistical analysis, is a rapid, economical, and effective method for classifying monofloral honeys according to their botanical origin and verifying their authenticity. The integrated use of HS-SPME-GC-IMS-MS enabled more accurate identification of characteristic volatile compounds, even in cases of co-elution, thanks to the combination of IMS drift-time separation and MS mass measurements.

The two analytical techniques are complementary: GC-IMS provides comprehensive volatile fingerprints useful for classification, while GC-IMS-MS improves the annotation of VOCs through the NIST libraries. This approach overcomes one of the main limitations of GC-IMS, namely the lack of compounds identification.

The resulting volatilomic profiles could be integrated into food traceability systems, supporting supply-chain controls, regulatory authorities, and consumers in verifying honey origin and authenticity. However, further studies are required to fully validate the method. In conclusion, HS-GC-IMS represents a promising tool for honey authentication and food quality control.

P24

High-resolution mass spectrometry for varietal authentication of bread wheat

Lorenzo Lovatti,^{1,2} Felix Bacher,² Peter Robatscher,² Ksenia Morozova,¹ Matteo Mario Scampicchio¹

¹ Faculty of Agricultural, Environmental and Food Sciences, Free University of Bozen-Bolzano, Piazza Università 1, 39100 Bolzano, Italy

² Laboratory of Flavours and Metabolites, Laimburg Research Centre, Auer, Italy

Summary: *Untargeted HRMS metabolomics is applied to bran and flour of three bread wheat cultivars to define the metabolite markers driving varietal discrimination. ¹H-NMR and FT-NIR are then assessed as rapid screening proxies, and the three blocks are combined by mid-level data fusion.*

Keywords: *untargeted LC-HRMS; Bread Wheat; Cultivar Classification*

Introduction

Bread wheat (*Triticum aestivum* L.) cultivars differ in milling behavior and dough properties [1]. The milling industry therefore needs reliable quality assessment to produce flours and blends with consistent technological quality and defined end- use applications. Routine control relies largely on total protein content, although cultivars with comparable protein content can differ markedly in dough behavior [2]. Cultivar identity may therefore provide complementary information beyond total protein content. Each cultivar is associated with a characteristic, although environmentally modulated, technological profile [1]. Reliable cultivar classification could consequently support the prediction of milling and baking performance.

For this purpose, untargeted metabolomics based on high-resolution mass spectrometry (HRMS) offers high sensitivity, broad metabolite coverage, and structural resolving power, and enables identification of the compounds responsible for cultivar separation. HRMS has already been applied to the discrimination of wheat species and growing locations [3] and to the mapping of metabolite distribution across pearling fractions of bread wheat varieties [4]. Also, ¹H Nuclear Magnetic Resonance (¹H NMR) spectroscopy and Fourier-Transform near-infrared (FT-NIR) spectroscopy are attractive platforms for rapid varietal fingerprinting, and both have already been applied respectively to the separation of bread wheat genotypes [5] and to the discrimination of wheat species in flours [6]. Both techniques, however, have drawbacks: NIR exhibits broad overlapping bands with limited direct interpretability and is affected by physical scattering effects, while ¹H NMR has low sensitivity and limited structural resolution due to peak overlap, especially in complex matrices. HRMS is not subject to these limitations, and its annotations can support the assignment of ¹H NMR signals acquired on the same extract. FT-NIR, for

its part, requires no sample preparation and provides a rapid fingerprint of the intact matrix.

So far, however, these platforms have been applied mostly on their own. ¹H NMR and FT-NIR were only compared for milling fraction classification [7], while ¹H NMR and HRMS were fused, together with gluten protein content, to assess agro- sustainable treatments in durum wheat [8]. The integration of complementary analytical blocks into a single classification model is called data fusion, an approach well established in other food matrices [9,10] but still of limited application to bread wheat. To the best of our knowledge, the three platforms have not yet been combined for cultivar discrimination in bread wheat. So, the objective of this work is a cultivar classification method that combines the sensitivity and structural resolving power of LC-HRMS with the throughput of ¹H NMR and FT-NIR.

In the present work, LC-HRMS untargeted metabolomics is used as the primary analytical platform: (i) to establish, at the compound level, which metabolites separate the cultivars, with accurate mass and MS/MS fragmentation providing molecular formula and structural evidence; and (ii) to supply a chemically interpretable block of the fused classification model in which the three platforms are combined.

Experimental

Three bread wheat cultivars are investigated using eight independent commercial lots per cultivar. Lots are supplied by the milling company at intervals of approximately three to four weeks, thereby incorporating temporal and lot-to-lot variability. For each lot, bran and flour fractions are analyzed separately, with three independent extraction and analytical replicates per fraction, for a total of 144 measurements per analytical platform.

FT-NIR spectra are acquired directly on the milled fractions, without sample preparation, using an integrating sphere (MPA, Bruker Optik, Ettlingen, Germany). Each spectrum is collected with 128 scans at a resolution of 8 cm⁻¹ over the range 14000 to 1200 cm⁻¹.

Samples are first defatted with hexane (1:10, sample-to-solvent ratio), then extracted with 80% aqueous

methanol (2 g sample, 20 mL solvent), and centrifuged to recover the supernatant. A 1 mL aliquot of the supernatant is dried and analyzed directly by LC-HRMS. The remaining supernatant is dried and reconstituted in D₂O containing 25 mM phosphate buffer (pH 7) and 2 mM DSS as internal standard for ¹H NMR. The spectra are acquired on a 400 MHz (JNM-ECZ, JEOL, Tokyo, Japan) instrument. LC-HRMS analysis is performed on an Ultimate 3000 HPLC (Thermo Fisher Scientific, Waltham, USA) coupled to a Q-Exactive Orbitrap mass spectrometer equipped with an ESI source operated in positive mode, using a Luna HILIC column (100 × 2 mm, 3 µm, 200 Å; Phenomenex, Torrance, CA, USA). Chromatographic separation is achieved with 20 mM ammonium formate (pH 3.2) and acetonitrile as mobile phases at a flow rate of 0.4 mL/min. The Orbitrap is operated in full MS/data-dependent MS² mode over an m/z range of 50–750.

Untargeted LC-HRMS data are processed with Compound Discoverer (v. 3.3) and chemometric analyses in R. Each platform is first examined on its own by principal component analysis (PCA) and cross-validated partial least squares discriminant analysis (PLS-DA). A fixed number of PCA scores per platform is then concatenated into a single matrix (mid-level data fusion), to which PLS-DA is applied. Because the PCA scores are extracted without reference to cultivar, class information is used only in the fused model. Validation is by double cross-validation with lot-level splits and by permutation testing.

Expected results

The established ¹H NMR method assigns 12 metabolites by comparison of chemical shifts against the Chenomx reference library, with several signals still to be assigned. Signals for which the library holds no matching compound remain unassigned. Preliminary Orbitrap measurements on the same extracts yield several hundred features, of which 21 are annotated, including those detected by ¹H NMR. Three outcomes are expected. The first is the discriminant capacity of ¹H NMR and FT-NIR measured against that of LC-HRMS, from single- block PCA and PLS-DA models. The second is compound-level confirmation of the metabolites annotated by ¹H NMR: assignment by the comparison of chemical shifts against reference libraries becomes ambiguous wherever signals overlap, and low sensitivity leaves less abundant compounds undetected, whereas accurate mass and MS/MS fragmentation provide independent information on molecular formula and structure. The third is a mid-level fused model, obtained by concatenating the scores extracted from the three platforms.

Conclusions

This approach combines the sensitivity and structural resolving power of HRMS with the throughput and screening capacity of spectroscopic techniques: routine samples can be characterized rapidly using the two spectroscopic platforms, while HRMS identifies which compounds are most important for cultivar classification. Such a workflow would be applicable to varietal control in the milling industry. Future work will have to extend the dataset with Osborne fraction profiling and empirical rheological measurements, in order to connect the molecular profiles to functional and technological quality.

Acknowledgements

The authors thank Rieper S.p.A. for supplying the wheat samples.

References

1. K. El Hassouni, M. Afzal, P. H. G. Boeven et al.; *Scientific Reports*, 15 (2025), 31674.
2. R. Aghagholizadeh, M. Kadivar, M. Nazari, F. Mousavi, M. H. Azizi, M. Zahedi, M. R. Rahiminezhad; *Journal of Cereal Science*, 76 (2017), pp 229–235.
3. T. D. Stark, P. Weiss, L. Friedrich, T. Hofmann; *European Food Research and Technology*, 246 (2020), pp 1617–1626.
4. A. Zhu, Q. Zhou, S. Hu, F. Wang, Z. Tian, X. Hu, H. Liu, D. Jiang, W. Chen; *Food Chemistry*, 369 (2022), 130881.
5. P. R. Shewry, D. I. Corol, H. D. Jones, M. H. Beale, J. L. Ward; *Molecular Nutrition & Food Research*, 61 (2017), 1600807.
6. J. U. Ziegler, M. Leitenberger, C. F. H. Longin, T. Würschum, R. Carle, R. M. Schweiggert; *Journal of Food Composition and Analysis*, 51 (2016), pp 30–36.
7. V. Ramakrishnan, C. D. Ridge, J. Harnly, E. P. Mazzola, D. L. Luthria; *Cereal Chemistry*, 94 (2017), pp 471–479.
8. N. Riboni, E. Cruz Muñoz, C. Muhs, M. Mattarozzi, Caldara, S. Graziano, C. Richter, H. Schwalbe, Marmiroli, D. Ballabio, M. Gulli, M. Careri, F. Bianchi; *Molecules*, 31 (2026), 922.
9. A. Rivera-Pérez, R. Romero-González, A. Garrido Frenich; *Food Chemistry*, 420 (2023), 136156.
10. M. Spiteri, E. Dubin, J. Cotton, M. Poirel, B. Corman, E. Jamin, M. Lees, D. Rutledge; *Analytical and Bioanalytical Chemistry*, 408 (2016), pp 4389–4401.

P25

UPLC–HRMS and ¹H NMR Fingerprinting for the Authentication of Italian Saffron

Ksenia Morozova,¹ Lucrezia Angeli,¹ Timo D. Stark,² Corinna Dawid,² Enmanuel Cruz Muñoz,³ Davide Ballabio,³ Matteo Scampicchio¹

¹ Faculty of Agricultural, Environmental and Food Sciences, Free University of Bozen-Bolzano, Piazza Università 1, 39100 Bolzano, Italy

² Chair of Food Chemistry and Molecular Sensory Science, Technical University of Munich, Lise-Meitner-Strasse 34, 85354 Freising, Germany

³ Milano Chemometrics and QSAR Research Group, Department of Earth and Environmental Sciences, University of Milano-Bicocca, Piazza della Scienza 1, 20126 Milan, Italy

Summary: *Untargeted UPLC-ESI-TOF-MS combined with chemometrics differentiated Italian saffron samples and identified markers associated with commercial quality and adulteration by turmeric and paprika. Complementary ¹H NMR classification detected turmeric addition down to 2.5%, demonstrating the potential of molecular fingerprinting for saffron authenticity assessment.*

Keywords: *high-resolution mass spectrometry; saffron authentication; chemometrics*

Introduction

Saffron, obtained from the dried stigmas of *Crocus sativus* L., is one of the most valuable spices and is therefore particularly vulnerable to adulteration. Its commercial quality is conventionally assessed through the determination of crocins, picrocrocin and safranal. However, targeted measurements may not sufficiently describe complex compositional changes caused by processing, storage or the addition of plant-derived adulterants. Untargeted analytical approaches provide a broader representation of saffron composition and may reveal characteristic markers without requiring their prior selection.

The present work investigated the application of untargeted high-resolution mass spectrometry to differentiate Italian saffron samples, identify molecular indicators of quality and detect adulteration with turmeric and paprika [1]. The MS results were complemented by a subsequent ¹H NMR-chemometric study focused on the detection of turmeric adulteration [2].

Experimental

Saffron samples were obtained from Italian local producers and supermarkets. For MS profiling, freeze-dried samples were extracted with 90% aqueous methanol and analysed by UPLC coupled to electrospray ionisation-travelling-wave ion-mobility time-of-flight mass spectrometry. Data were acquired in positive and negative ionisation modes using simultaneous low- and elevated- collision-energy acquisition (MSE). Chromatographic separation was performed on a BEH C18 column using water and acetonitrile containing 0.1% formic acid.

Raw MS data were aligned, peak-picked and deconvoluted using Progenesis Q1. Features showing significant differences were investigated by principal component analysis (PCA) and orthogonal partial least-squares discriminant analysis (OPLS-DA). Metabolite annotation was based on accurate mass, elemental composition, fragmentation spectra, reference standards and literature data. Saffron samples and a pooled quality-control sample were adulterated with turmeric or paprika at 10, 25 and 50% (w/w).

In the complementary NMR study, 138 extracts comprising pure saffron and samples adulterated with 2.5, 5 and 10% turmeric were analysed by 400 MHz ¹H NMR. Hierarchical partial least-squares discriminant analysis models were evaluated using repeated double validation.

Results

The UPLC-ESI-TOF-MS workflow provided reproducible metabolite fingerprints and differentiated locally produced saffron from several supermarket samples. PCA revealed substantial compositional variability, although no systematic grouping according to geographical origin was observed. This suggests that processing, storage and individual production conditions may influence the saffron metabolome more strongly than geographical provenance alone.

OPLS-DA highlighted several discriminant metabolites. Among them (9S,10S,13S)-Trihydroxy-(11E)-octadecenoic acid was one of the most relevant markers associated with supermarket samples. Oxidised crocins and related saffron metabolites contributed strongly to sample differentiation and were associated with differences in total crocin content and antioxidant properties. These compounds therefore represent potential indicators of saffron degradation and commercial quality. The profiling experiment also revealed metabolites

not previously reported in saffron, illustrating the value of high-resolution MS for exploratory authentication studies.

Adulteration with turmeric and paprika produced clear separation from authentic saffron in the multivariate models, including at the lowest investigated addition level of 10%. For turmeric, the ion at m/z 367.1182 was assigned to cyclocurcumin and increased consistently with adulterant concentration. Saffron-associated markers included quercetin-3-neohesperidoside and 2-O- α -D-glucopyranosyl-L-ascorbic acid.

The complementary ^1H NMR study extended the turmeric investigation to lower adulteration levels. Hierarchical PLS-DA correctly classified 98% of pure saffron samples and 95% of adulterated samples. Turmeric addition was detected down to 2.5% (w/w), with curcuminoid-related resonances, particularly those around 7.56 ppm, contributing to discrimination.

Conclusions

Untargeted UPLC-ESI-TOF-MS provided a rapid and information-rich approach for saffron authentication, allowing the simultaneous evaluation of sample quality, compositional degradation and adulteration. High-resolution fragmentation data enabled the identification of saffron quality markers and adulterant-specific compounds, including cyclocurcumin for turmeric contamination.

The complementary NMR results showed that robust chemometric modelling can further reduce the detectable turmeric concentration to 2.5%. Together, the studies demonstrate that untargeted molecular fingerprinting combined with appropriately validated multivariate analysis can complement conventional saffron quality-control procedures.

References

1. L. Angeli, K. Morozova, C. Dawid, M. Scampicchio, T.D. Stark; *ACS Food Science & Technology*, 4 (2024), pp 1783-1794.
2. L. Angeli, E. Cruz Muñoz, D. Ballabio, K. Morozova, M. Scampicchio; *Food Control*, 179 (2026), 111560.

P26

Development of a HS/GC-IMS Screening Method for Honey Geographical Origin Classification*Giancarlo Quaglia,¹ Cesare Rossini,² Matteo Bordiga³*¹ Department of Drug Science and Technology, University of Torino, Torino, Italy² Lab Service Analytica s.r.l, Via Emilia 51/c, 40011 Anzola Emilia (Bologna), Italy³ Department of Pharmaceutical Sciences, University of Piemonte Orientale, Novara, Italy

Summary: To meet stricter European regulatory requirements for origin transparency, this study evaluated HS/GC-IMS (FlavourSpec®) combined with chemometric analysis as a high-throughput screening method for honey authentication. The approach successfully discriminated international honey samples across different geographical origins (Brazil, China, Chile, and Hungary) and botanical varieties by mapping their unique volatile organic fingerprints. Ultimately, this method serves as a rapid, cost-effective preliminary filter for official and in-house quality controls, effectively isolating suspect samples to protect consumer trust and combat commercial fraud.

Keywords: Honey, GC-IMS, Geographical Origin

Introduction

In recent years, the geographical provenance of honey has become a salient issue for consumers and regulatory authorities, as it exerts a significant influence on the perception of the product's quality, authenticity and economic value. The recent revision of the Honey Directive, as part of the European Union's so-called 'Breakfast Directives', further reinforces this principle. The revision introduces stricter requirements regarding origin labelling, mandating the indication of the countries of harvest and their respective percentages in blends, as well as providing for future harmonized traceability systems and analytical methods to support authenticity verification. These changes underscore the necessity for expeditious, dependable, and economical analytical instruments to ascertain the congruence between the declared and actual origins of honey. At present, the determination of honey's geographical origin is based primarily on melissopalinalological analysis and advanced analytical techniques, such as nuclear magnetic resonance (NMR) spectroscopy, metabolomics using high-resolution mass spectrometry, and multivariate chemometric approaches. Despite the fact that these methodologies deliver high analytical performance, they necessitate costly equipment, highly qualified staff, and analysis times that are not always compatible with large-scale routine testing.

In this context, the use of ion mobility spectrometry (IMS), utilizing FlavourSpec® instrumentation, represents a promising alternative for the development of high-throughput, low-cost screening methods. IMS technology facilitates the expeditious acquisition of a volatile fingerprint of honey, which exhibits a high degree of correlation with its aromatic composition. This composition is, in turn, influenced by the botanical and geographical characteristics of the production area.

The integration of IMS data with multivariate analysis tools and classification algorithms facilitates the effective differentiation of samples from disparate geographical areas, thereby enabling an initial assessment of the product's provenance within a brief timeframe and at a substantially reduced analytical cost when compared to confirmatory methods.

Experimental

Samples of honey from four different geographical origins (Brazil, China, Hungary and Chile) were used. Headspace-gas chromatography-ion mobility spectrometry (HS/GC-IMS) (FlavourSpec®, G.A.S., Dortmund, Germany) was used to assess the volatile composition with an untargeted fingerprinting approach. A 20 mL glass vial was filled with 1.0 g of the sample. Then samples were Then, in splitless mode, a 300 µL headspace sample was automatically delivered through a 50 °C heated syringe. Using an MXT-5 column (15 m × 0.53 mm i.d., 1 µm film thickness; Restek Corporation, Bellefonte, PA, USA), the volatile chemicals were separated at 40 °C. As the carrier gas, 99.999 percent pure nitrogen was employed, and the flow rate program was configured as follows: 2 mL/min for 2 minutes, followed by a 5-minute rise to 150 mL/min and a 3-minute hold. A 3H ionization source ionized the eluted analytes before driving them to a drift tube, which was run at a constant temperature of 45 °C and voltage of 5 kV.

Results

This study reports the volatile organic compound (VOC) analysis of six international honey samples analyzed using HS/GC-IMS. By combining gas chromatographic separation (retention time) with ion mobility drift time separation, GC-IMS generates two-dimensional "chemical fingerprints" of volatile profiles. This technique enables non-destructive, highly sensitive authentication of geographical origin and differentiation between

botanical varieties (Monofloral vs. Polyfloral) without extensive sample preparation. This study aimed to establish an accurate and robust screening method to classify honey's geographical origin for official and internal quality controls. Functioning as a high-throughput preliminary filter, it reduces costs by sending only suspect samples to complex confirmatory testing - ultimately supporting Food Information Directive standards, protecting consumers, and combating commercial honey fraud.

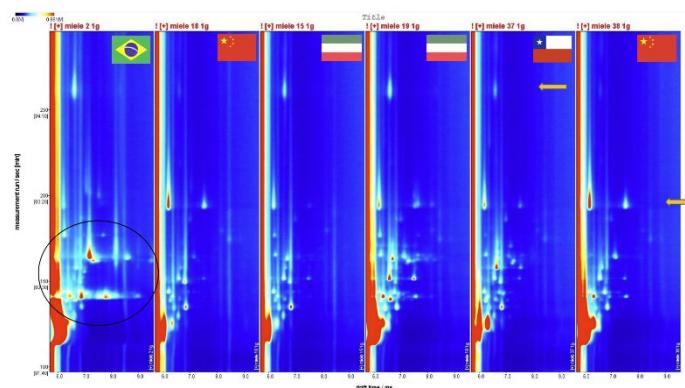


Fig. 1. Two-dimensional chromatographic profiles of different types of honey.

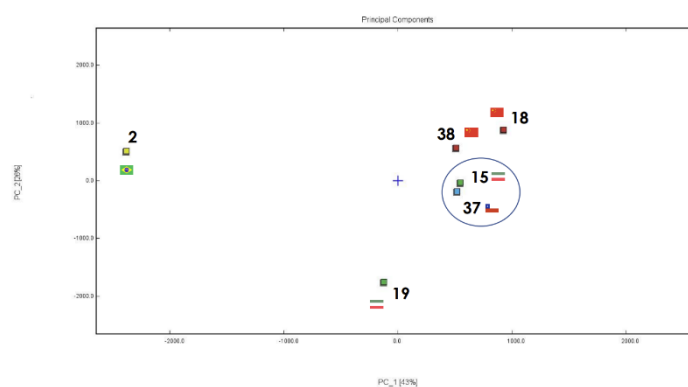


Fig. 2. Principal Component Analysis (PCA) score plot of different honey samples based on volatile profiles

Principal Component Analysis (PCA) explains 69% of total variance (PC1 = 43%, PC2 = 26%) and successfully segregates the samples into four distinct clusters.

Conclusions

This HS/GC-IMS screening approach seamlessly aligns with the evolving European regulatory framework. It effectively separates honeys by both country of origin and floral type, providing a reliable methodology for quality control, traceability, and fraud detection in commercial honey markets. By providing a fast, harmonized analytical tool, it strengthens origin verification and safeguards honey authenticity throughout the entire supply chain.

References.

1. J. Christmann, S. Rohn, P. Weller, Data in Brief 54 (2024) 110532
2. H. Schanzmann, A. L. R. M. Augustini, D. Sanders, M. Dahlheimer, M. Wigger, P.-M. Zech, S. Sielemann, Molecules 2022, 27, 7554

P27

Characterisation of monofloral and polyfloral honey from rural areas of Guatemala and role in the daily nutrition

Chiara Scapuzzi,¹ Tamara Chwojnik,¹ Luisella Verotta,¹ Maria Pia Macchi,² Stefania Marzorati¹

¹ Department of Environmental Science and Policy, University of Milan, Via Celoria 2, 20133 Milan, Italy

² Department of Traditional Medicine, Centro Orientamento Educativo ETS

Via Lazzaroni 8, 20124, Milano, Italy

Summary: *High-quality honey can be a valuable source of nutrients and bioactives. This work aims to characterise 31 Guatemala honey samples produce by local beekeepers regarding physicochemical properties, content of minerals and polyphenols, antioxidant power and presence of pesticides, to highlight the benefits of including honey in the daily diet.*

Keywords: *honey composition, nutritional profile, antioxidant power*

Introduction

Honey is a natural product used for centuries for its nutritional and health promoting effects. It has a complex composition with sugars and water as the main components, and minerals, nitrogen compounds, vitamins and bioactives as minor compounds. However, the specific composition of honey depends on bee species, botanical and geographical origin, season and weather conditions [1]. In this study, thirty-one samples of raw honey (29 polyfloral H1-H29 and 2 monofloral H30-H31) produced in rural areas of Guatemala has been characterised in term of physico-chemical properties, mineral and polyphenol content, antioxidant properties and presence of pesticides. The work is part of the project Alimentación which aims to help fight chronic malnutrition and to promote the achievement of food security in rural and Maya indigenous communities of Guatemala.

Experimental

Honey pH and electrical conductivity (EC) were evaluated following the Harmonised Methods of the International Honey Commission (2009). The mineral content was assessed by ICP-MS after mineralisation by microwave digestion with nitric acid. The content of polyphenol was investigated measuring the absorbance at 450 nm and the Total Phenolic Content (TPC) with a modified Folin- Ciocalteu method [3]. The presence of thirteen polyphenols (gallic acid, chlorogenic acid, ellagic acid, p-cumaric acid, sinapic acid, cinnamic acid, rutin, myricetin, quercetin, apigenin, hesperitin, sakuratinin and pinocembrin) was assessed using a SPE pretreatment with home-made cartridges containing HLB and WCX as sorbent, followed by ESI(-)-UHPLC-TOF detection. The method performances were reported in term of recovery (R%), matrix effect (ME%) and Limit of Detection and Quantitation (LODs and LOQs). Honey antioxidant properties were evaluated using two antioxidant tests: ABTS assay (expressed in term of EC₅₀) and Ferric Reducing Antioxidant Power (FRAP) assay (reported as μM of Fe (II)/g of honey). Finally, the presence of thirteen target pesticides and their metabolites (Chlormequat, Mepiquat, Bentazon, Propamocarb, Thiacloprid, Malathion, Atrazine, Atrazine-desethyl, Chlorpyrifos, Metolaclores, Metolaclo, Terbutylazine, Imidacloprid) was studied using the same pretreatment step of polyphenols and ESI(+)-UHPLC-TOF detection. The figures of merit of the analytical method were evaluated in term of R% and ME%, precision, linearity, LODs and LOQs.

Results

The results obtained for pH (3.1-4.7) and EC (0.25 - 0.78 mS cm⁻¹) are in line with the typical value reported for honey; only one sample, the monofloral avocado honey (H30), presented a high value of EC equal to 1.37 mS cm⁻¹, a data in line with data described elsewhere for this peculiar honey [2]. The mineral content of Guatemala honey samples was investigated considering both essential and nonessential elements. The target chemicals were 5 macrominerals (Na, Ca, K, P and Mg) and 16 microminerals and trace elements (Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Cd, Ba, Pb and Hg). The total mineral content ranges between 431 and 4918 mg kg⁻¹ with avocado honey showing the highest mineral content, in accordance with EC measurements. Among macrominerals, K is the most abundant representing the 79-93% of the total mineral content; the concentration of these elements follows the trend K > Ca, P > Mg > Na. Taking into account the nutrient reference values for healthy populations provided by EFSA and a honey consumption of 20 g day⁻¹, including honey into a healthy diet can contribute to provide essential nutrients, especially for the most fragile population. Regarding microelements and trace elements, is worth noticing the presence of Hg in all the analysed samples (0.6-67 μg kg⁻¹). This trace element, linked with volcanic activity [4], can be considered a geographical marker since Guatemala is a volcanic area.

Regarding polyphenols, the TPC observed for the studied honey samples resulted in the range 58-157 mgGAE kg⁻¹ for the multifloral honey (H1-H29) and the monofloral honey H31, while for avocado honey a content of polyphenolic compounds equal to 483 mgGAE kg⁻¹ was observed. TPC and the absorbance at 450 nm presented a strong correlation ($r = 0.924$). In general, the samples showed a slightly higher content of the target flavonoids compared to phenolic acids. Nevertheless, the content of phenolic acids is definitely higher in avocado honey corresponding to 88% of the target polyphenols. The distribution of the target phenols in the honey samples investigated was reported in Figure 1.

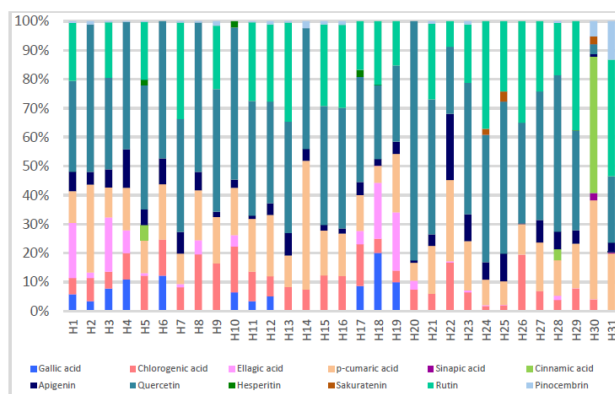


Fig. 1. Relative abundances of the target phenolic acids and flavonoids in the honey samples investigated.

The samples demonstrate good antioxidant activity, in line with literature data. A moderate anticorrelation ($r = -0.577$) was observed between FRAP and ABTS results, suggesting the contribution of different species in the antioxidant response. Finally, the presence of the target pesticide was assessed, and no residuals were detected above the Maximum Residual Level set at 0.05 mg kg⁻¹ [5].

Conclusions

The work presents the characterisation of thirty- one honey samples produced by local beekeepers in rural areas of Guatemala showing that honey serves as source of nutrients and bioactives and can be a valuable product to tackle malnutrition.

References

1. V. Kaskoniene, P. R. Venskutonis, *Comprehensive Reviews in Food Science and Food Safety*; 9 (2010), pp 620-634
2. J. Serra Bonvehia, F. Ventura Collb, J.F. Orantes Bermejo; *Food Chemistry*, 287 (2019), pp214-221
3. G. Berettaa, P. Granataa, M. Ferrerob, M. Oriolia, R. Maffei Facinoa; *Analytica Chimica Acta*, 533 (2005), pp 185-191
4. P. Inaudi, G.M. Sicurella, L. Rivoira, L. Favilli, P. Bracco, S. Bertinetti, O. Abollino, M.C. Bruzzoniti, V. Isaja, A. Giacomino; *Science of the Total Environment*, 993 (2025), 179981
5. Regulation (EU) No 283/2013, Annex 16.1, part A and B

P28

Screening of fruity esters in sparkling wines produced by refermentation in pressurized stainless steel tank (Charmat-Martinotti method)

A. Panighe¹, F. Tarricone,¹ M. De Rosso,¹ A. Sparacio,² S. Sparla,² S. Vincenzi,³
L. Vettori,⁴ R. Flamini¹

¹ Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria - Centro Ricerca Viticoltura Enologia
Viale XXVIII Aprile, 26 – 31015 Conegliano (TV), Italy

² Istituto Regionale del Vino e dell'Olio - Regione Sicilia (IRVO), Via Libertà 66, 90100 Palermo, Italy

³ Interdipartimental Center for Research in Viticulture and Enology (CIRVE)
viale XXVIII Aprile 14, 31015 Conegliano (TV), Italy

⁴ Labor Wine Srls, Susegana, Via Crevada 8/C, 31058 Susegana (TV), Italy

Summary: Influence of second fermentation by Charmat-Martinotti method on the fruity-aroma esters of sparkling wines was studied by GC/MS, as well as PCA and Cluster analysis. Their profile was primarily affected by the first fermentation, while the refermentation had low impact.

Keywords: fruity esters, sparkling wines, Charmat-Martinotti method, GC/MS

Introduction

A "Sparkling wine" is the product with alcoholic content minimum 8.5 % by vol. produced by alcoholic fermentation of fresh grape, grape must, or wine which has CO₂ pressure excess minimum 3 bar at 20 °C in close container which builds up after the bottle is opened creating the signature bubbles (EU Regulation n. 1308/2013/EC).

In Charmat-Martinotti method, a still base wine is added of sugar and yeast, and the second fermentation is carried out into pressurized stainless-steel tanks (autoclave) until the CO₂ pressure reaches 5 bars. In the process, 20-25 g/L of sugar is converted into approximately 1.5% v/v ethanol. Then the wine is cooled at -4 °C for 5–7 days to induce the precipitation of potassium bitartrate excess and, after filtration, the wine is bottled. The entire procedure is conducted within an isobaric system to prevent the CO₂ loss.[1]

The fruity-ester profile of sparkling wine, particularly important for fresh wines such as Prosecco that do not undergo any refinement processes, is affected by yeast metabolism during winemaking. In this study, the effects of the second fermentation carried out using the Charmat-Martinotti method was evaluated.

GC/MS analysis of height main fruity esters including ethyl butanoate, isoamyl acetate, ethyl hexanoate, hexyl acetate, ethyl octanoate, ethyl lactate, ethyl-3-hydroxybutyrate, ethyl decanoate, and phenylethyl acetate, were performed in the base wines (before the refermentation) and final sparkling wines of seven autochthonous Sicilian grape varieties. The effect of Charmat-Martinotti method on the fermentative ester profile was studied by performing statistical multivariate analysis (PCA and Cluster Analysis) of normalized signals of compounds.

Experimental

Fifty-eight base and sparkling wines both white and rosé were produced from four autochthonous Sicilian grape varieties by using different yeast strains. White wines were produced using Zibibbo, Grillo, Catarratto, and Nerello Mascalese grapes, rosé wines using Frappato, Nero d'Avola, and Perricone. Sugar content of all final sparkling wines was adjusted at 10 g/L, except Zibibbo for which a sweet sparkling wine version with sugar content 50 g/L, was produced.

Profiles of fermentative fruity esters were detected by GC/MS analysis using a 6850 gas chromatograph equipped with a fused silica Inno-WAX column (30 m×0.25 mm, 0.25 µm) coupled to a 7693A autosampler and a 5975C mass spectrometer (Agilent Technologies, Santa Clara, MA, USA).

PCA and Cluster Analysis were performed using the software PAST 3.13.[2]

Results

The first two principal components counted 77% of total variability with PC1 56.3% and PC2 20.7%, respectively. PCA and cluster analysis grouped Nero d'Avola samples in the 1st and 2nd quadrant of the diagram, with base wines separated from the wines. The projection of variables shows the acetates are more representative compounds for these wines, such as isoamyl acetate, hexyl acetate, and phenylethyl acetate. Catarratto and Grillo wines fall in the fourth quadrant of diagram. In these samples the most representative are ethyl esters, such as ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl-3-hydroxybutyrate, and ethyl decanoate. In Catarratto, Grillo, Nerello Mascalese, and Zibibbo cluster analysis separated the base wines

from the sparkling wines.

Samples of Frappato, Perricone, and Nerello Mascalese fall in the second and third quadrants. In these wines the more representative esters contributing to the fresh-fruity bouquet were isoamyl acetate and phenylethyl acetate.

Zibibbo wines showed ester contents close to the overall mean data.

Conclusions

The fruity esters in sparkling wines are primarily influenced by the initial fermentation of the base wine, while the secondary fermentation utilizing the Charmat-Martinotti method has a minimal impact on their profile. These observations are consistent with prior research conducted on sparkling wines produced through in-bottle refermentation.[3]

References

1. S. Buxaderas, E. López-Tamames; *Technology and Nutrition*, (2010), pp 553-588.
2. Ø. Hammer, D.A.T. Harper, P.D. Ryan; *Palaeontologia Electronica*, (2001), pp 1-9.
3. G. Nicolini, T. Román, S. Moser, L. Tonidandel, R. Guzzon, L. Raveane, R. Larcher; 8th edizione Enoforum, Arezzo 7–9 maggio 2013, www.infowine.com.

Financial support: Project SAQIRVO: Development of high-quality Sicilian sparkling wines with a strong territorial characterization. Duration 2023-2027.

P29

Impact of a novel copper-free formulation on stilbene biosynthesis in grapevine shoots

Ghazal Namazzadeh,¹ Laura Alessandrini,¹ Filippo Maggi,¹ Eleonora Spinozzi,¹ Matteo Michelini,² Valerio Borzatta,³ Gianni Sagratini¹

¹ School of Pharmacy, Chemistry Interdisciplinary Project (ChIP), University of Camerino, Camerino (MC)

² Natural Development Group Srl, Via Salvatore Quasimodo, Castel Maggiore (BO)

³ Certiquality, Via Gaetano Giardino, Milano MI

Summary: Vine shoots from 18 grape varieties were evaluated under copper-free ND24_55 treatments within the LIFE NaturalAgro project. Optimized UAE conditions enabled *trans*-resveratrol and ϵ -viniferin quantification by HPLC-DAD. Stilbene levels varied strongly by variety and treatment, with Moscatel Graudo and Pinot Meunier showing the highest concentrations.

Keywords: Wine production by-products, copper-free formulation, stilbenes

Introduction

Vine shoots are valuable by-products of viticulture, representing a sustainable source of bioactive polyphenols. Stilbenes, phenolic secondary metabolites, play significant roles in plant protection [1]. Numerous studies suggest that vine shoots can be a rich source of stilbenes, primarily *trans*-resveratrol and ϵ -viniferin, whose levels vary due to intrinsic and extrinsic factors [2]. This study is part of the LIFE "Natural Agro" European project, which aims to bring the concept and practice of sustainable agriculture to grape cultivation within the framework of the circular economy. In this regard, one biomimetic material- based natural Plant Protection Product (PPP) for disease control (ND24_55 at different dosage rates) with %0 copper content were developed and introduced in experimental fields. The impact of this treatment on the *trans*-resveratrol and ϵ -viniferin levels in the final biomass was evaluated. The comparison was between 7 treatments: Control (C), Poltiglia Dispers (P), P+ND24_55 (PT), ND24_55, 2 L/ha (T100), ND24_55, 1.2 L/ha (T60), ND24_55, 4 L/ha (T200), and Industrial Thesis (TA).

Experimental

The quality of the ND24_55 formulation was checked by GC-MS analysis (EI ionization source) of the active ingredient through liquid injection (split 1:20) by using an HP-5MS capillary column (30 m, 250 μ m i.d., 0.25 μ m film thickness) for separation of the analytes. An extraction method was optimized using an Experimental Design tool, with solvent type, extraction time, and matrix/solvent ratio as factors. Extraction yield, *trans*-resveratrol, and ϵ -viniferin concentrations were measured as response variables. Three extraction methods—maceration, Soxhlet, and Ultrasound Assisted Extraction (UAE)—were then explored. A total of 70 grapevine shoots samples of 18 different grape varieties were collected from vineyards in France, Portugal, and Italy, finely ground and freeze-dried. Compound quantification was conducted using High-Performance Liquid Chromatography - Diode Array Detector (HPLC-DAD).

Results

Optimization of extraction. A matrix/solvent ratio of 0.12, 100% ethanol, and 35 minutes of extraction time were identified as the optimal parameters; the UAE method was chosen for its rapid extraction, ease of use, low cost, and environmentally friendly characteristics.

trans-Resveratrol and ϵ -viniferin concentrations ranged from approximately 6.27 ± 0.15 mg/kg in Verdicchio variety treated with T60 to 769.99 ± 8.96 mg/kg freeze-dried (FD) weight in Moscatel Graudo treated with PT, and from 246.53 ± 4.99 mg/kg in Verdicchio variety treated with T60 to 3961.51 ± 21.44 mg/kg FD in Pinot Meunier treated with T60, respectively.

Effect of treatments on *trans*-resveratrol and ϵ -viniferin concentrations

The concentrations of *trans*-resveratrol and ϵ -viniferin varied according to both grape variety and treatment (Fig.2). For *trans*-resveratrol (Fig. 2A), Pinot Meunier showed the highest concentration under T60 treatment, reaching approximately 672 mg/kg dry extract, followed by the control and P treatments. In Merlot, the highest *trans*-resveratrol level was observed under T200, whereas Moscato showed its maximum value under T100. Overall, Pinot Meunier was the most responsive variety for *trans*-resveratrol accumulation, particularly under T60.

For ϵ -viniferin (Fig. 2B), concentrations were markedly higher than *trans*-resveratrol in all varieties and treatments. Pinot Meunier again showed the highest response, with the maximum value recorded under T60, reaching approximately 3962 mg/kg dry extract. High ϵ -viniferin levels were also observed in Pinot Meunier

under P and T200 treatments. In Moscato, the highest ϵ -viniferin concentration was detected under T100, while Merlot showed relatively lower but more stable values across treatments. These results indicate that stilbene accumulation was strongly variety-dependent and that the effect of treatment differed among cultivars.

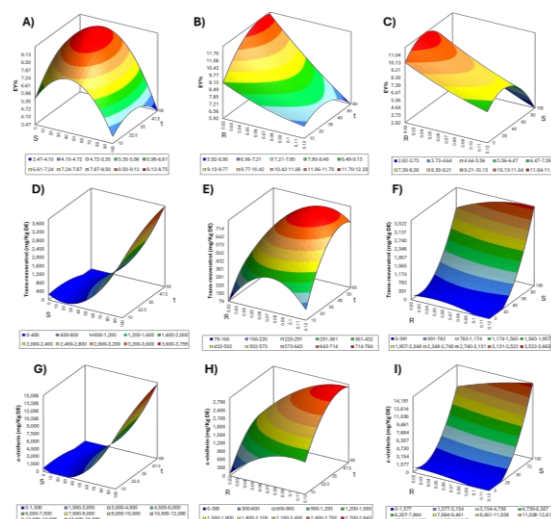


Figure 1. Response surface plots showing the effect of extraction parameters on extraction yield (A, B, C), trans-resveratrol (D, E, F) and ϵ -viniferin (G, H, I). Plots report the data correlation of S vs. t (A, D, G), R vs. t (B, E, H), and R vs. S (C, F, I).

Table 1. Extraction methods comparison. Different letters represent statistically significant differences in concentration values between the three tested methods. (EY: Extraction yield, SLE: Supported liquid extraction, UAE: Ultrasound-assisted extraction)

	SLE	UAE	Soxhlet
EY%	2.11±0.096 a	3.13±0.13 b	3.95±0.10 c
trans-resveratrol (mg/g DE)	4.61±0.46 a	9.50±1.03 b	7.62±0.25 b
ϵ -viniferin (mg/g DE)	16.14±3.05 a	25.66±1.16 b	17.14±1.22 ac

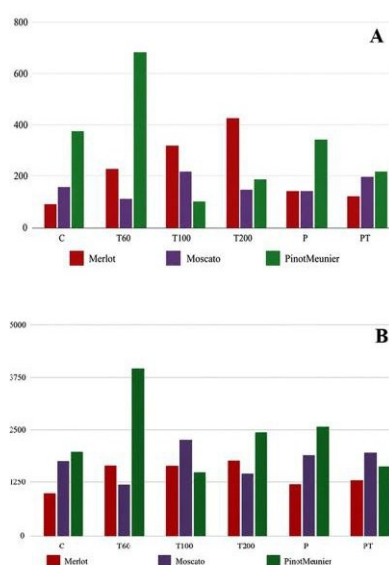


Fig.2. comparison of trans-resveratrol (A) and ϵ -viniferin (B) levels in Merlot, Moscato and Pinot meunier varieties treated with 7 different treatments

Conclusions

Grapevine shoots from 2025 pruning allowed the obtainment of dry extract rich in trans-resveratrol (6 to 800

mg/kg) and ϵ -viniferin (240 to 4000 mg/kg). The highest yields were obtained from Pinot Meunier with 31 mg/kg of *trans*-resveratrol and 169 mg/kg of ϵ -viniferin in freeze-dried matrix. Crucial variables were grape varieties and the time elapsed from pruning to extraction. The ND24_55 treatment influenced the biosynthesis of stilbenes in plants, but the behaviour of each variety was almost unique. Ethanol remained the best solvent to obtain highest concentrations of stilbenes, but, as they are very important for their possible utilization, the possibility to use water will be explored. Further experiment will be performed on selected varieties using water as solvent, according to sustainability, economy, and safety parameters. The water extracts will undergo biological analysis.

References

1. Noviello M, Caputi AF, Squeo G, Paradiso VM, Gambacorta G, Caponio F. *Foods*. (2022), pp 1-11.
2. Sy B, Krisa S, Richard T, Courtois A. *Molecules*. 22 (2023), pp 1-28.

P30

Multi-matrix LC-MS/MS screening of 40 PFAS in edible oils and food contact paper materials

G. Mosconi, P. Fusari, C. Scagliarini, D. Baglio, P. Rovellini

Innovhub – Stazioni Sperimentali per l'Industria S.r.l., Via Giuseppe Colombo 83, 20133 Milan, Italy

Summary: A multi-residue LC-MS/MS screening method was developed for the determination of 40 per- and polyfluoroalkyl substances (PFAS) in edible oils and paper-based food contact materials. Different extraction and clean-up procedures were optimized according to matrix characteristics. The method was applied to commercial samples and evaluated through recovery experiments.

Keywords: PFAS, LC-MS/MS, food contact materials

Introduction

Per- and polyfluoroalkyl substances (PFAS) comprehend a large group of synthetic chemicals extensively employed in industrial processes and consumer products because of their water-, oil- and grease-repellent properties. Their extreme persistence in the environment, together with their potential for bioaccumulation and adverse health effects, has raised increasing concern regarding human exposure through food and food contact materials (FCMs) [1]. Among the various exposure pathways, edible oils and grease-resistant paper-based materials represent challenging matrices due to their physicochemical characteristics and the possible presence of intentionally added fluorinated substances or contaminants originating from manufacturing processes.

Regulatory interest in PFAS has significantly increased in recent years. Commission Recommendation (EU) 2022/1431 encourages the monitoring of PFAS in food [2], while Commission Regulation (EU) 2023/915 establishes maximum levels for selected PFAS in several food categories [3]. Furthermore, Regulation (EC) No. 1935/2004 requires that materials intended to come into contact with food do not transfer their constituents to food in quantities that may endanger human health [4]. The growing demand for analytical methods capable of addressing complex matrices and an extended range of legacy and emerging PFAS has highlighted the need for flexible multi-matrix screening approaches [5,6].

The aim of this study was the development and application of a targeted LC-MS/MS screening method for the determination of 40 PFAS compounds in edible oils and paper-based food contact materials through the implementation of matrix-specific extraction and clean-up procedures.

Experimental

A target list including 40 PFAS compounds belonging to different chemical classes (PFCAs, PFSA, fluorotelomer sulfonates, fluorotelomer carboxylic acids and emerging PFAS) was investigated by LC-MS/MS using an Agilent Infinity 1290 HPLC system coupled to an Agilent Triple Quadrupole 6495 mass spectrometer operating in negative electrospray ionization mode and dynamic multiple reaction monitoring (dMRM). The analytical workflow was developed starting from an adaptation of the EPA Method 1633, tailored to achieve a multi-matrix screening compliance.

For edible oils, sample preparation was based on QuEChERS extraction followed by clean-up using Captiva EMR Food II SPE cartridges. Briefly, 2 g of sample were extracted with acidified acetonitrile after hydration, followed by salt-induced phase separation, SPE purification and solvent evaporation. Extracts were reconstituted in MeOH/H₂O (80/20, v/v) prior to analysis.

For paper and paperboard food contact materials, 2 g of sample were extracted with ethanol at room temperature for 2 h. Aliquots of the extract were concentrated under nitrogen and reconstituted in MeOH/H₂O (80/20, v/v) before LC-MS/MS determination.

Quantification was performed via isotope dilution using a ten-point calibration curve with a set of 14 mass-labelled internal standard (ISTD) to compensate for matrix effects. Procedural blanks, quality control (QC) and fortified samples were included in each analytical batch.

Method performance was preliminarily assessed through recovery experiments carried out on fortified blank samples representative of both matrices. The developed procedure was subsequently applied to commercial samples collected from the market.

Results

The developed workflow enabled the determination of 40 PFAS compounds in matrices characterized by substantially different extraction requirements. Recovery experiments performed on fortified samples demonstrated satisfactory analyte extraction and clean-up efficiency for the majority of target compounds in both matrix types, with recoveries ranging from 70 to 130% for edible oils and from 60 to 120% for paper-

based materials. However, due to severe matrix effects and differences in physicochemical behaviours, critical and outside-range recoveries (<60% or >130%) were observed for specific challenging analytes, particularly fluorotelomer derivatives (e.g., FTSA, FTCA) and ultra- short/long-chain compounds. Despite these extraction limitations, the implementation of isotope dilution successfully compensated for the ionization bias, ensuring the necessary data accuracy for final quantification. In addition, another strategy for improving the accuracy of the method may be to inject the calibration curve more frequently.

Procedural blanks showed no significant contamination above the screening threshold confirming the suitability of the adopted laboratory precautions for PFAS analysis, where background contamination is frequently recognized as a critical issue [6].

The method was applied to 4 edible oil samples and 4 food contact material samples collected from the market. None of the analysed samples showed detectable levels of the investigated PFAS compounds above the method screening limits.

Although no positive findings were observed, the study demonstrated the applicability of a single analytical platform for routine monitoring of multiple PFAS across food and FCM matrices with substantially different physicochemical characteristics, providing adequate selectivity and robustness while minimizing matrix interferences.

Conclusions

A target LC-MS/MS screening method for the determination of 40 PFAS compounds in edible oils and paper-based food contact materials was successfully developed based on an EPA 1633 adaptation.

The adoption of matrix-specific extraction procedures enabled effective analyte recovery from both lipid-rich and cellulosic matrices, while the use of isotope dilution and dMRM detection ensured adequate selectivity for screening purposes. Recovery data highlighted specific challenges related to fluorotelomer and extreme chain-length compounds under multi-matrix conditions; nevertheless, the mass-labelled ISTD approach confirmed its reliability in guaranteeing accurate screening performance. The analysis of commercial samples did not reveal detectable contamination by the investigated PFAS.

The developed methodology represents a useful tool for routine monitoring activities and provides a practical basis for future validation studies and extension to additional food-related matrices.

References

1. EFSA CONTAM Panel. Risk to human health related to the presence of perfluoroalkyl substances in food. *EFSA Journal*, 2020, 18(9), 6223.
2. Commission Recommendation (EU) 2022/1431 of 24 August 2022 on the monitoring of perfluoroalkyl substances in food.
3. Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food.
4. Regulation (EC) No. 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food.
5. OECD. Toward a New Comprehensive Global Database of Per- and Polyfluoroalkyl Substances (PFASs): Summary Report on Updating the OECD PFAS List. OECD Series on Risk Management No. 39, 2021.
6. U.S. EPA. Method 1633: Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids and Tissue Samples by LC-MS/MS. EPA 821-R-24-010, 2024.

P31

Chemical characterization of olive mill wastewater by untargeted UHPLC-QTOF-MS data-dependent analysis approach

Simone Angeloni, Laura Alessandrini, Giovanni Caprioli, Gianni Sagratini, Massimo Ricciutelli

Chemistry Interdisciplinary Project (ChIP), School of Pharmacy, University of Camerino

Summary: Olive mill wastewater (OMWW) is a phytotoxic byproduct of the olive oil production. Different OMWW samples have been chemical characterized by an innovative approach which combined high-resolution mass spectrometry with modern data elaboration generally adopted in metabolomics. This comprehensive metabolites characterization is therefore useful to support a OMWW sustainable valorization.

Keywords: olive mill wastewater, LC-MS, untargeted analysis

Introduction

Olive mill wastewater (OMWW) represents a phytotoxic byproduct of the olive oil production but also a promising source of bioactive compounds. To this end, several studies have already been conducted to explore and enhance its contents and values. [1, 2]. To the best of the authors' knowledge, this is the first research which reports an innovative approach which combined high-resolution mass spectrometry with modern data elaboration generally adopted in metabolomics. Therefore, the aim of the work was to automatically annotate the highest possible number of polar compounds of three OMWW samples from three cultivar by UHPLC-QTOF data-dependent analysis. This approach allows the characterization of a large number of compounds, including those never reported before.

Experimental

The OMWW samples were analyzed by automated iterative UHPLC-QTOF data-dependent analysis together with the classical SCAN modality. Data were elaborated through MS-DIAL software which automatically performed peak-picking (features), alignment, integration, and annotation. The annotation has been carried out by matching with the NIST 2020 high-resolution MS/MS library.

Results

A total of 27,041 and 21,755 aligned features resulted from data elaboration of positive and negative acquisitions, respectively. After data filtration and statistical analysis (one-way ANOVA) a matrix containing 5,000 features (2,500 most-significant features for each polarity) was analyzed and visualized with a PCA and a heatmap with hierarchical clustering. The two OMWW from the Marche region (Sargano di Fermo and multivarietal) were more similar to each other than to the Apulian cultivar, as demonstrated by hierarchical clustering, suggesting a preliminary outcome on the origin discrimination. A total of 364 compounds were annotated with a total identification score cutoff of 60%, reaching a confidence level of 2. Some of these were commonly reported in olive products for example, some iridoids, phenolic compounds, flavonoids, lignans and cinnamic acid derivatives, while other were scarcely reported in these type of matrices such as jaslaceoside B, 8-O-4-hydroxycinnamoylharpagide, verbasoside, melilotoside and cis-melilotoside, picroside III and II, stachydrine and 1-pyrrolidinecarboximidamide among others. Further studies will be necessary to confirm the presence of these compounds in OMWW by using a targeted approach with reference standards. Moreover, the three samples were completely discriminated when PLS-DA was applied to the data matrix of 364 annotated compounds and the annotated compounds with the highest VIP scores were some inositol phosphates, phenolic metabolites and phospholipids [1].

Conclusions

The application of a modern untargeted analytical approach has enabled a thorough screening of metabolites present in olive mill wastewater, leading to the identification of numerous compounds with antioxidant and biological potential, many of which are poorly documented in the olive oil supply chain. The results of this work offer new chemical insights essential not only for rethinking olive mill wastewater as a valuable source of bioactive compounds for the nutraceutical and cosmetic industries, but also for optimizing and developing future purification and detoxification strategies.

References

1. L. Alessandrini, M. Ricciutelli, S. Angeloni, G. Caprioli, G. Sagratini. *Foods*, 14(2025), 4128.
2. P. Paraskeva, E. Diamadopoulos. *Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology*, 81 (2006), pp 1475–1485.

P32

High-resolution metabolomics uncovers xylem metabolic signatures associated with *Philaenus spumarius* feeding in olive

Maria Assunta Crescenzi,^{1,2} Erica Tinelli,² Vincenzo Verrastro,³ Daniele Cornara,² Paola Montoro¹

¹ Department of Pharmacy, University of the Study of Salerno, Via Giovanni Paolo II 132
84084 Fisciano, Italy

² Department of Soil, Plant and Food Science (DiSSPA), University of Bari, Via Amendola 165/A
70126 Bari, Italy

³ CIHEAM-Bari, Via Ceglie 9, 70010 Valenzano (BA), Italy

Summary: Untargeted LC-HRMS metabolomics was applied to investigate olive xylem responses to *Philaenus spumarius* feeding and mechanical wounding. Distinct metabolic signatures discriminated insect-induced defense from wound-related responses, revealing specialized metabolite accumulation during feeding and highlighting high-resolution metabolomics as a powerful tool for studying plant–insect interactions in olive trees.

Keywords: LC-HRMS; Olive xylem sap; Plant–insect interaction

Introduction

The xylem-feeding meadow spittlebug, *Philaenus spumarius*, is the main European vector of the plant pathogen *Xylella fastidiosa*, whose spread has severely affected olive cultivation and extra-virgin olive oil production in Southern Italy. During feeding, the insect penetrates xylem vessels with its stylets, inducing both mechanical damage and plant physiological responses. However, the extent to which the metabolic alterations observed in xylem sap result from the physical injury itself rather than from insect feeding remains largely unexplored [1].

Untargeted metabolomics based on high-resolution mass spectrometry (HRMS) provides a powerful approach for characterizing plant metabolic responses to biotic stress and identifying biomarkers associated with specific plant–insect interactions [2]. In this study, LC-HRMS metabolomics using high-resolution mass spectrometry (HRMS) provides a powerful approach for characterizing plant metabolic responses to biotic stress and for comparing the xylem sap profiles of olive plants exposed to *P. spumarius* feeding, mechanically wounded by sterile water inoculation, and untreated control plants. The aim was to discriminate metabolic changes specifically associated with insect feeding from those induced solely by mechanical penetration.

Experimental

To discriminate metabolic changes associated with insect feeding from those caused by mechanical penetration, olive plants (*Olea europaea* cv. Ogliarola Salentina) at the same phenological stage were injected with 3 pin-pricks of 10 µL of double-distilled water (D). Twenty-four hours after injection, plants were exposed to bacterium-free spittlebugs (DP), whereas untreated plants served as controls (NN). Xylem sap was collected 48 h after the beginning of insect exposure using a Scholander pressure chamber and analysed by untargeted high-resolution mass spectrometry on a Q Exactive Orbitrap platform. Metabolite annotation was performed based on accurate mass measurements, enabling the identification of several chemical classes, including organic acids, amino acids, phenolic compounds, and terpenoids. Multivariate statistical analyses were applied to evaluate treatment-dependent metabolic alterations across sampling times.

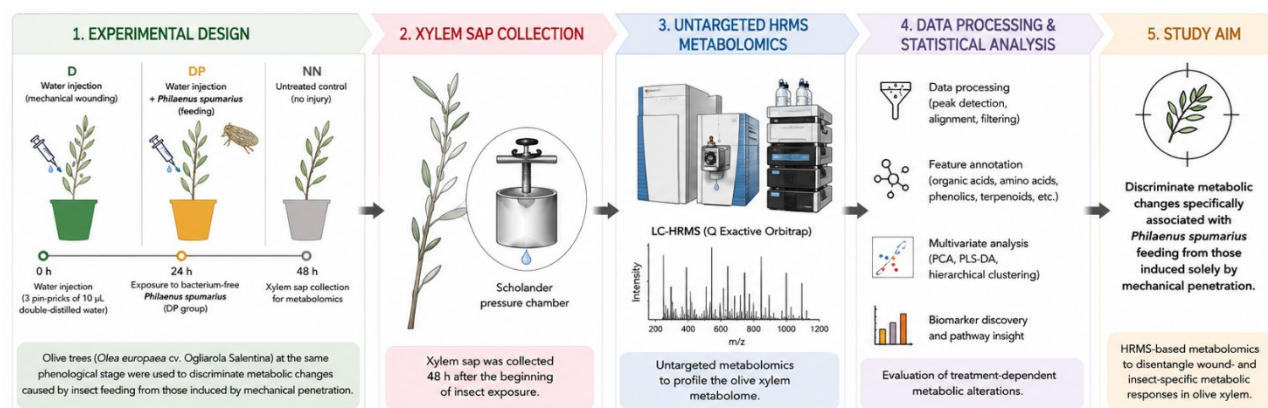


Fig. 10. Schematic overview of the experimental design and untargeted LC-HRMS metabolomics workflow used to compare olive xylem responses to mechanical wounding and *Philaenus spumarius* feeding.

Results

Untargeted LC-HRMS metabolomic profiling revealed distinct metabolic signatures among the three experimental groups. Hierarchical clustering and heatmap analysis clearly discriminated xylem samples from water-injected plants (D), *Philaenus spumarius*-fed plants (DP), and untreated controls (NN). Interestingly, DP samples clustered closer to untreated plants than to water-injected plants, indicating that mechanical wounding alone induced the most pronounced perturbation of the xylem metabolome.

The D group was characterized by the accumulation of metabolites involved in primary metabolism and stress adaptation, including organic acids, amino acids, carbohydrates, and polyols, consistent with a generalized wound response. In contrast, DP samples exhibited increased abundance of specialized metabolites, particularly phenolic compounds and secoiridoids, including oleuropein-related metabolites and hydroxytyrosol derivatives, suggesting activation of defense pathways specifically associated with insect feeding. Untreated plants displayed a characteristic basal metabolic profile enriched in constitutive secoiridoids and glucosidic derivatives.

Conclusions

Overall, the metabolomic analysis demonstrated that the metabolic response elicited by *P. spumarius* feeding differs from that induced by mechanical penetration alone, highlighting HRMS-based metabolomics as an effective tool for disentangling wound- and insect-specific metabolic responses in olive xylem.

References

1. D. Cornara, et al. *Journal of Pests Science*, 97 (4) (2024), pp 1-13.
2. B. Worley, et al. *Current Metabolomics*, 1 (1) (2013), pp 92-107.

P33

Comparison of Commercial Monovarietal Extra Virgin Olive Oil Classification Methods: Targeted Profiling with HS-SPME-GC-MS and HPLC-DAD vs Untargeted Fingerprinting with HS-GC-IMS

Tommaso Ugolini,¹ Lukas Bodenbender,² Nabiollah Mobaraki,² Marzia Migliorini,³ Ilaria Romei,³ Bruno Zanoni,¹ Lorenzo Cecchi,¹ Philipp Weller²

¹ DAGRI – Department of Agricultural, Food, Environmental, and Forestry Sciences and Technologies, University of Florence, via Donizetti 6, 50144 Florence, Italy

² Institut für Instrumentelle Analytik und Bioanalytik, Technische Hochschule Mannheim, Paul Wittsack Strasse 10, 68163 Mannheim, BW, Germany

³ Carapelli Firenze S.p.A., Via Leonardo da Vinci 31, Tavarnelle Val di Pesa, 50028, Florence, Italy

Summary: Phenolic and VOCs profiling of monovarietal EVOOs from four commercial cultivars was used for varietal classification and compared with GC-IMS fingerprint analysis. Both PLS-DA models achieved F-scores > 0.9, with IMS slightly outperforming targeted analysis. Evaluation of VIP scores of the targeted model allowed the identification of cultivar-specific molecular patterns.

Keywords: HS-SPME-GC-MS, Ion Mobility Spectrometry, chemometrics

Introduction

Monovarietal extra virgin olive oil (MEVOO) represents both a high-quality niche product and a large part of modern industrial production. Cultivars Arbequina, Koroneiki, Picual and Coratina are among the most widely cultivated at a global level; despite their wide distribution, a systematic comparison of their chemical and sensory attributes is still lacking. MEVOOs of different cultivars have peculiar chemical characteristics, which influence their sensory profiles and their possible applications within the formulation of commercial blends. Phenolic compounds, terpenes and LOX pathway-derived VOCs are the main molecular classes responsible for the differences in the sensory profiles of EVOO. Classification of MEVOOs according to cultivar using these compounds is a well-established protocol [1,2].

While mass spectrometry remains a unique tool to identify and quantify singular molecules, recent literature has highlighted the possibility of combining fast fingerprinting techniques such as GC-IMS with machine learning algorithms, in order to achieve high throughput screening for classification or authentication purposes [3,4].

In this study, MEVOOs from four of the most widely cultivated olive varieties were analysed with different techniques: targeted analysis with HPLC-DAD (phenolic compounds) and GC-MS (LOX-derived VOCs and terpenes) was used for the detailed profiling of the main chemical parameters that contribute to the sensory profile of MEVOOs and to build a PLS-DA model of classification. GC-IMS was used to analyse the same EVOO samples, and to build another PLS-DA model based on their IMS fingerprint. The performances of the two models were compared in terms of F-scores and classification accuracies.

Experimental

53 Monovarietal EVOO samples (13 Arbequina, 14 Koroneiki, 12 Picual and 13 Coratina) were gathered directly from the producers during the 2025-2026 harvesting seasons. Targeted analyses of LOX VOCs and terpenes were conducted using two different HS-SPME-GC-MS validated methods, both employing multiple internal standard normalization: a total of 175 compounds (including phenols) were quantified, following a comprehensive orthogonal approach of characterization. HS-GC-IMS analysis was conducted with a FlavourSpec (GAS Dortmund) benchtop instrument after optimization of the headspace sampling method with a 5-factor Central Composite Design. Additionally, a THS- GC-IMS-MS instrumental setup was used for simultaneous IMS and MS detection. Analysis of targeted data was performed using RStudio, while EasyIMS software was used for IMS data analysis. In both cases, PLS-DA models were built after VIP filtering (>1) was applied to the variables. F-scores and other figures of merit were calculated based on CV-LOO confusion matrices.

Results

Classification of EVOO according to cultivar is a complicated issue, not only due to the complexity of the lipid matrix, but also because the chemical profile of the oil can be influenced by several confounding factors such as geographical origin, production process, harvest time and storage conditions. In this work commercial oils from different origins were analyzed, aiming to highlight the characteristics of MEVOOs from different cultivars within the actual industrial context. While expert panelists might be able to recognize the oil variety by its sensory nuances, relating them to specific molecules can be challenging; moreover, the sensory profile is often

determined by complex molecular patterns rather than single components. In this context, untargeted fingerprinting methods combined with multivariate data analysis represent an ideal combination as they directly address the patterns while allowing analysts to skip time-consuming operations such as integration of peak areas and external standard calibration.

The confusion matrix in Table 1 was obtained through PLS-DA performed on quantitative data from three different chromatographic methods. The high specificities (>0.95 across all classes) demonstrate the reliability of the model's predictive capacity; however, the figures of merit of the model are lowered by three unclassified samples, yielding an overall F-score of 0.911 and a global accuracy of 0.887.

Table 1. Confusion matrix of the targeted data PLS-DA model after Leave-One-Out Cross Validation

	Arbequina	Coratina	Koroneiki	Picual	None
Arbequina	11	0	0	1	1
Coratina	1	11	0	0	1
Koroneiki	1	0	14	0	0
Picual	0	0	0	11	1
F-score (model):		Global accuracy (model):			
0.911		0.887			

The FlavorSpec HS-GC-IMS instrument is a relatively simple benchtop device: it operates at ambient pressure and requires lower maintenance if compared to mass spectrometers. Table 2 shows the confusion matrix for the fingerprinting data obtained with the IMS detector and analyzed via PLS-DA. The model performed optimally, with 51 samples correctly classified out of a total of 53, and both the F-score and global accuracy surpassing 0.96.

Both analytical methods achieved high degrees of classification; while untargeted GC-IMS analysis provides reliable classification results with short analysis times, classical profiling approaches offer the advantage of a more direct analysis of molecular features, which can be directly visualized after quantitation or semi-quantitation.

Table 2. Confusion matrix of the GC-IMS data PLS-DA model after Leave-One-Out Cross Validation

	Arbequina	Coratina	Koroneiki	Picual
Arbequina	12	0	1	0
Coratina	0	13	0	0
Koroneiki	0	0	15	0
Picual	0	0	1	11
F-score (model):		Global accuracy (model):		
0.964		0.962		

As an example, VIP score analysis from the targeted data model revealed that Coratina cultivar exhibits a significantly higher abundance of sesquiterpenes than the other three varieties, and that C6 acetyl esters are key to discriminating among the four cultivars, as shown in Figure 1.

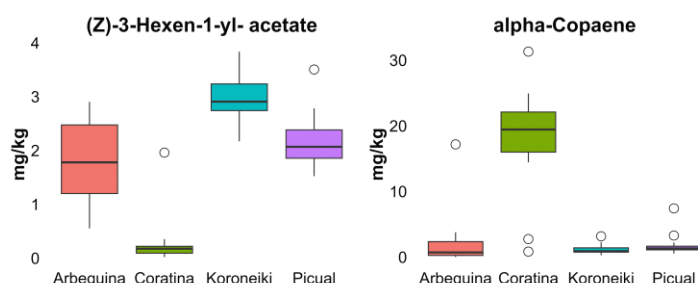


Fig. 1. Boxplots for two features with high VIP scores according to the targeted data PLS-DA model

This kind of information is not readily available after fingerprinting analysis, representing a drawback of this approach. Nevertheless, the present work demonstrates that classification models based on fingerprinting analytical methods can provide accurate and reliable results with relatively short processing times, allowing realtime MEVOO classification.

Conclusions

Chemometric data analysis was used to obtain highly accurate models for EVOO varietal classification. A benchtop fingerprinting technique such as GC-IMS provided comparable (and even higher) classification accuracy with respect to a more complex and time-consuming analytical approach. IMS analysis allows fast and reliable screening and authentication of monovarietal EVOO samples but does not directly provide information to identify and quantitate the discriminating features; for this purpose, mass spectrometry remains irreplaceable.

Additional data collected using simultaneous IMS and MS detection could provide more information on cultivars.

References

1. Ugolini et al. (2026) Journal of Food Composition and Analysis 152 109038
2. Panzani et al (2026) Foods 2026, 15, 519
3. Bayat-Afshary et al. (2026) Food Chemistry 500 147511
4. Gerhardt et al (2019) Food Chemistry 278 720–7

P34

Comparative evaluation of green solvents for the extraction of olive leaf phenolics by UHPLC-HRMS/MS-based metabolomics

Orsolina Vetrano,^{1,2} Maria Assunta Crescenzi,¹ Daniele Sanna,³ Angela Fadda,⁴ Paola Montoro¹

¹ Department of Pharmacy, University of the Study of Salerno, Via Giovanni Paolo II 132
84084 Fisciano, Italy

² PhD Program in Drug Discovery & Development, Pharmacy Department, University of the Study of Salerno, Italy

³ Institute of Biomolecular Chemistry, National Research Council, Traversa La Crucca 3, 07100 Sassari, Italy

⁴ Institute of the Sciences of Food Production, National Research Council, Traversa la Crucca 3, 07100 Sassari, Italy

Summary: *This study compares Natural Deep Eutectic Solvents and biodegradable solvents for the extraction of phenolic compounds from olive leaves using UHPLC-HRMS/MS metabolomics. Although all green solvents showed high extraction efficiency, solvent composition influenced the relative abundance of phenolic subclasses, highlighting differences in extraction selectivity.*

Keywords: *olive leaves, NaDES, biodegradable solvents*

Introduction

The increasing generation of agri-food by-products has stimulated growing interest in sustainable strategies for recovering high-value bioactive compounds within a circular economy framework [1,2]. Among olive-derived residues, olive leaves represent one of the most abundant biomasses generated during pruning and olive oil production. Despite their high content of phenolic compounds, including oleuropein and its derivatives, they are still largely discarded or burned, causing both economic losses and environmental concerns [3]. Their remarkable phytochemical composition makes olive leaves a promising source of natural antioxidants for food, pharmaceutical and cosmetic applications. In this context, the development of environmentally friendly extraction approaches is essential to replace conventional organic solvents. Natural Deep Eutectic Solvents (NaDES) have recently emerged as sustainable extraction media due to their low toxicity, biodegradability and tunable physicochemical properties, while biodegradable solvents such as ethyl lactate have also attracted considerable attention as green alternatives [4,5]. Therefore, this study aimed to compare different NaDES formulations and biodegradable solvents for the extraction of phenolic metabolites from olive leaves through UHPLC-HRMS/MS metabolomics approach coupled with multivariate statistical analysis.

Experimental

Fresh olive leaves were frozen, freeze-dried, and finely ground prior to extraction in order to ensure sample homogeneity and preservation of the native metabolite profile. Natural Deep Eutectic Solvents (NaDES) were prepared according to established procedures [6] using hydrogen bond acceptors and donors in defined molar ratios to obtain stable, homogeneous liquid systems.

Five different NaDES formulations, consisting of choline chloride-based systems combined with organic acids including lactic acid and citric acid and amino acid-based systems including proline-acid combinations, were synthesized and used as extraction media. Their extraction performances were compared with biodegradable conventional solvents, namely ethyl lactate (100%, 90% and 80%, v/v) and ethyl acetate, selected as greener alternatives to traditional organic solvents [4,5].

Extractions were performed under controlled conditions. The resulting extracts were collected and analyzed using a UHPLC system coupled to a QExactive-Orbitrap high-resolution mass spectrometer (Thermo Fisher Scientific). Raw data were processed using Compound Discoverer™ software for peak detection, alignment, and compound annotation. Putative metabolite identification was achieved based on accurate mass measurements, MS/MS fragmentation patterns, database matching, and comparison with data reported in the literature.

Multivariate statistical analyses were then applied, including principal component analysis (PCA) and supervised models such as partial least squares discriminant analysis (PLS-DA).

Results

UHPLC-HRMS/MS analysis confirmed that olive leaves are a rich source of phenolic metabolites, including oleuropein and its derivatives together with flavonoids and phenolic acids. Overall, all investigated green extraction systems efficiently recovered the phenolic metabolome, demonstrating the suitability of NaDESs and biodegradable solvents for the valorization of olive leaf biomass. Multivariate statistical analysis revealed

a clear influence of solvent composition on the extracted metabolomic profile, highlighting differences in the relative abundance of specific phenolic subclasses rather than substantial differences in the overall extraction capability.

These findings suggest that the physicochemical properties of each solvent formulation modulate extraction selectivity, leading to distinct yet complementary metabolomic fingerprints.



Fig. 1. Experimental workflow for the extraction and analysis of olive leaf phenolics.

Conclusions

The metabolomics-based approach proved to be an effective tool for comprehensively evaluating the influence of green solvent formulations on the recovery of olive leaf phenolics. The results confirm that solvent composition affects the qualitative distribution of metabolites more than the overall extraction efficiency, offering valuable information for the rational selection of green solvents in the sustainable valorization of olive by-products.

References

1. Talhaoui N., Taamalli A., Gómez-Caravaca A.M., Fernández-Gutiérrez A., Segura-Carretero A. *Food Research International*, 77 (2015), pp. 92–108.
2. Benincasa C. et al. *Trends in Food Science & Technology*, **143** (2024), 10431.
3. Zeng X. et al. *Frontiers in Nutrition*, **9** (2022), 1008349.
4. Siamandoura P., Tzia C. *Molecules*, **28** (2023), 353.
5. Alañón M.E. et al. (lavori sull'estrazione con NaDES delle foglie di olivo; citazione molto pertinente al tuo studio).
6. Food Chemistry (2024). *Olive tree leaves as a great source of phenolic compounds: Comprehensive profiling of NaDES extracts*. *Food Chemistry*, **456**, 140042.

P35

**Tracking reactive aldehydes in bakery matrices and oven emissions
via HS-SPME-GC-MS/MS**

Mauro Paolini, Roberto Larcher

Fondazione Edmund Mach (FEM), Via E. Mach 1, 38098 San Michele all'Adige, Italy

Keywords: HS-SPME-GC-MS/MS, Reactive aldehydes, Bakery products

The characterization of the volatile profile in bakery products poses an analytical challenge due to the high reactivity and chemical instability of carbonyl compounds. Aldehydes serve as key chemical markers for the Maillard reaction and Strecker degradation in baked goods. However, odour-active compounds such as methional, phenylacetaldehyde, furfural, and branched-chain aldehydes exhibit extremely low olfactory thresholds (down to the ng/L range), requiring highly sensitive and selective quantification methods.

In this context, an analytical protocol is applied to evaluate the potential of antioxidant-rich food by-products in modulating the formation and accumulation of reactive aldehydes during baking. To prevent analyte loss or degradation, the strategy integrates in-situ derivatization using O-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine (PFBHA) with headspace solid-phase microextraction and gas chromatography-tandem mass spectrometry (HS-SPME-GC-MS/MS) in Multiple Reaction Monitoring (MRM) mode. This setup provides the required selectivity to minimize matrix interferences across three distinct analytical fractions: crumb, crust, and the volatile emissions released into the oven air during baking.

By tracking aldehyde levels across the crumb, crust, and baking vapours, this MS-based approach aims to provide a comprehensive quantitative tool to assess aldehyde dynamics and gain insights into how antioxidant by-products influence thermal degradation pathways during baking.

P36

UHPLC-HRMS profiling of botanical extracts for antifungal and bioactive applications in bakery products

Giulia Fappani,¹ Gabriele Rocchetti ¹

¹ Department of Animal Science, Food and Nutrition, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29122 Piacenza, Italy

Summary: This study explored botanical extracts as clean-label antifungal solutions for bread preservation. Clove showed the strongest activity and was tested in free and β -cyclodextrin microencapsulated forms. Although promising for shelf-life extension, sensory drawbacks, especially in the encapsulated formulation, indicate that further optimization is needed before real case bakery application.

Keywords: botanical extracts, antifungal, bioactive compounds

Introduction

Bread and bakery products are highly susceptible to fungal spoilage because of their moisture content and favourable physicochemical conditions. Mould growth during storage reduces product quality and shelf-life, causing sensory defects, food waste, and economic losses [1],[2]. Among the main spoilage fungi, *Penicillium* and *Aspergillus* species are commonly reported in bread and related bakery products [3]. Although preservatives such as calcium propionate are widely used, the increasing demand for clean-label products has promoted the study of natural antifungal alternatives. Botanical extracts are of particular interest because they contain phenolic compounds, terpenoids, and volatile molecules with potential antimicrobial activity. Extracts from clove, cinnamon, thyme, and oregano are rich in compounds such as eugenol, cinnamaldehyde, thymol, and carvacrol, which have been associated with antifungal effects. However, their efficacy in real food products may differ from *in vitro* results because of interactions with the food matrix and possible effects on sensory quality [3],[4]. Recent studies have also suggested that β -cyclodextrin microencapsulation may help improve the stability and release of plant-derived compounds in food applications [5]. Therefore, in this study clove, cinnamon, thyme, and oregano were selected according to their known antifungal potential and their content of bioactive compounds. Extracts were used as natural antifungal ingredients for bread, combining chemical characterization, antifungal screening, and application of the most effective extract in free and microencapsulated form.

Experimental

Firstly, the moisture content of the powdered botanical matrices was determined to calculate extraction yields on the dry matter. Botanicals were extracted (1:10 w/v) using 70% ethanol (v/v) through homogenization and ultrasound-assisted extraction. After centrifugation and filtration, the four extracts were concentrated with rotary evaporator, frozen and lyophilized to obtain dry extracts. Extraction yields were calculated on the initial dry matter. The resulting extracts were characterized through total phenolic content (TPC) determination and UHPLC-HRMS profiling to identify the most represented phenolic classes. Antifungal activity was assessed against *Penicillium chrysogenum* and *Aspergillus sydowii* using potato dextrose agar-inclusion tests at different extract concentrations. This is need to both identify the minimum effective inhibitory concentration (MIC) and minimize any potential sensory impact on the final product. Half of the best-performing extract was then microencapsulated with β -cyclodextrin following the protocol proposed by Liu et al., 2026. Microcapsules were subsequently lyophilized to obtain a dry powder, and the yield was calculated as the ratio between the final dried microcapsule mass and the initial mass of β -cyclodextrin plus extract. The best-performing extract was then included in a pan-bauletto bread. Baking trials included a negative control, a calcium propionate positive control, β -cyclodextrin, free extract, and microencapsulated extract. Then breads were sliced, packed and stored at room temperature for subsequent shelf-life evaluation at day 0 (T0) and after 7 days (T7). During storage, microbiological analyses were performed. Technological quality was monitored through water activity, pH, moisture, texture parameters, and instrumental colour coordinates. In addition, a gustatory-visual-olfactory sensory assessment was performed at T0 and visual-olfactory assessment at T7 to evaluate visual appearance, aroma intensity, and overall acceptability. UHPLC-HRMS-based metabolomic profiling was carried out to study the biochemical evolution of the bread samples and the formation of spoilage-related compounds.

Results

Among the tested botanicals, clove extract at concentration 0.08% showed the strongest antifungal activity, particularly against *P. chrysogenum*, and was selected for application in bread. The β -cyclodextrin

microencapsulated formulation was designed to provide an equivalent dose of active extract while improving the stability of bioactive compounds and favouring their gradual release during shelf-life. The unsupervised HCA (Fig. 1A) performed on untargeted metabolomics dataset of the four extracts clearly distinguished the matrices in two main clusters (oregano and thyme vs. cinnamon and clove) according to their chemical profile similarity. Then phenolics compounds present in clove extract were semi-quantified according to their chemical class. Lignans and other phenolics were the most abundant compounds (127.902 and 54.687 mg/g dry matter, respectively). This result was in line with its expected phytochemical composition, mainly characterized by lignans (e.g. licarin A) and other phenolic compounds (e.g. eugenol), which may contribute to both antifungal and antioxidant effects. Untargeted metabolomics was also applied to the pan-bauletto samples in order to investigate the chemical evolution of the product during shelf-life. This approach allowed the monitoring of biochemical changes linked to formulation and provided preliminary information on the persistence of botanical-derived markers and the possible formation of spoilage-related compounds over shelf-life. The sensory spider plot (Fig. 1B) showed some differences among the five bread formulations. Overall, the β -cyclodextrin sample obtained the highest scores across most attributes, suggesting the best sensory profile among the tested breads. The positive and negative controls received comparable intermediate-to-high scores particularly for alveolation, crumb texture, aroma, and taste. The clove extract bread showed acceptable but lower scores than the controls, suggesting that the direct addition of the extract may alter some sensory notes. The microencapsulated clove extract showed the lowest sensory scores overall. This indicated that the encapsulated formulation may have negatively affected sensory perception or bread structure. In general, the graph suggested that clove extract may contribute to antifungal functionality, but its incorporation, especially in microencapsulated form, needs further optimization to improve sensory quality. In contrast, β -cyclodextrin alone appeared well accepted and did not negatively affect the sensory profile of the bread.

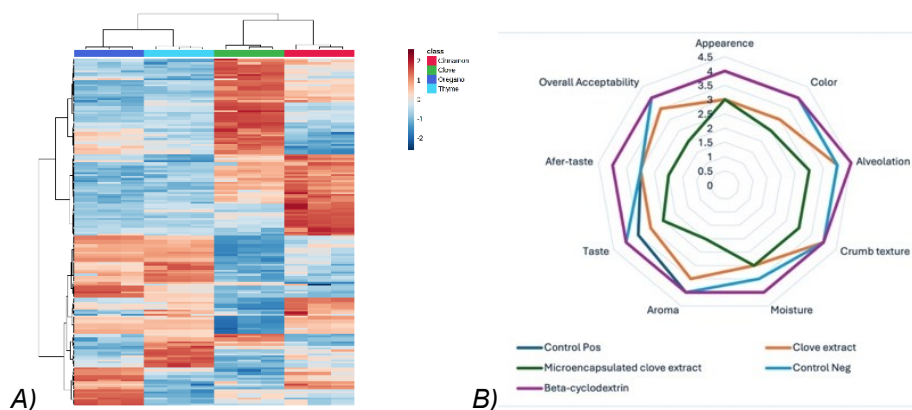


Fig. 11. A) Unsupervised hierarchical cluster analysis (HCA) on the four extracts. B) Bread sensory spider plot

Conclusions

This study provided a preliminary evaluation of selected botanical extracts as natural antifungal ingredients for bread preservation, combining chemical characterization, antifungal screening, microencapsulation, sensory evaluation, and UHPLC-HRMS-based metabolomic profiling. Among the four botanical matrices tested, clove extract showed the most promising antifungal activity related to its phytochemical composition, particularly the presence of lignans (e.g. licarin A) and other phenolic compounds (e.g. eugenol) with known bioactive properties. The integration of the extract (in free and microencapsulated form) into bread-dough highlighted both the potential and the limitations of these substances, including aroma and after-taste modifications, and a lower overall acceptability. Overall, these preliminary results suggest that clove extract may be a promising candidate for the development of clean-label antifungal solutions in bakery products. However, further work is needed to optimize the formulation, improve the sensory impact, and better understand the release and stability of the bioactive compounds during shelf-life.

References

1. F. Islam, F. Saeed, A. Imran, U. Shehzadi, R. Ali, F. Nosheen, A. Chauhan, A. Asghar, M. Ojukwu, *Journal of Food Science and Technology*, 2023
2. D. Gong, D. Prusky, D. Long, Y. Bi, Y. Zhang, *Food Chemistry*, 2024
3. A. Lepoutre, E. Debonne, W. Van Genechten, S. Martini, P. Van Dijck, F. Devlieghere, *Foods*, 2025
4. R. Nemati, M. Gharekhani, S. Tariverdi, H. Hamishehkar, H. Bakhshabadi, *Scientific Reports*, 2025
5. W. Liu, E. Su, *Food Control*, 2026

P37

An LC-MS/MS method for evaluating cyanotoxins contamination in bivalve mollusks through freshwater-to-marine pathways

S. Mazza^{1,2} M. Siracusa,^{1,2} S. Bacchiocchi,^{1,2} G. Diomedì,^{1,2} S. Accoroni,³ A. Tomasini,³ M. S. Princivalli,⁴ A. Baldoni,⁴ F. Serenelli,⁴ A. Piersanti^{1,2}

¹ Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati" - Via G. Salvemini, 1, 06126 Perugia, Italy

² Centro di Referenza Nazionale per il Controllo Microbiologico e Chimico dei Molluschi Bivalvi- Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati" – Via Cupa di Posatora 3, 60131-Ancona, Italy

³ Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Via Brecce Bianche, 60131 Ancona, Italy

⁴ Agenzia Regionale per la Protezione Ambientale - ARPA Marche, Via Ruggeri 5, 60131 Ancona, Italy

Summary: Freshwater cyanobacterial blooms, producers of toxins, may impact marine environments through rivers, representing a potential threat to shellfish. An LC–MS/MS method was implemented to investigate cyanotoxin contamination in field and exposed samples. Although no current risk was identified, future impacts cannot be excluded due to climate change.

Keywords: cyanotoxins, mussels, LC-MS/MS

Introduction

Freshwater cyanobacterial blooms are a well-known concern due to the production of a wide range of toxins such as microcystins (MCs), anatoxins (ATXs), cylindrospermopsins (CYNs) and anabaenopeptins (APs). MCs are the most studied cyanotoxins due to their widespread occurrence, persistence in aquatic ecosystems and well-documented hepatotoxicity. APs are only recently detected therefore their toxicity and presence remain still limited [1].

The most important MCs producers worldwide are *Planktothrix* spp. and *Microcystis* spp.; in Italian basins *P. rubescens* is the most popular species and often co-produces APs [2]. Blooms of *P. rubescens* frequently occurred in Castreccioni Lake, the largest drinking water reservoir in the Marche Region (Italy) [3]. Cyanobacterial blooms through rivers, could potentially impact marine areas, typically characterized by aquaculture farms, leading to a potential accumulation in marine organisms. Although only a few cases of toxin contamination have been reported in the literature [2,4], cyanobacterial blooms could represent an emerging threat for food safety. Trophic transfer experiments to study the potential accumulation of emerging toxins in shellfish have already been reported [5].

In the present study, an LC-MS/MS method for the determination of MCs and APs in shellfish was implemented to investigate the possible contamination in field samples. Moreover, to better understand the capability of mussels to accumulate cyanotoxins, an exposure experiment in laboratory conditions using a *P. rubescens* culture was performed.

Experimental

Analytical method. An LC-MS/MS method for the determination of 9 MCs (MC-RR, MC-LR, MC-LY, MC-LA, MC-LF, MC-LW, MC-YR, [D-Asp3]-MC-RR and [D-Asp3]-MC-LR) and 2 APs (AP-A and AP-B), previously validated in water and cyanobacteria, was implemented for bivalve mollusks. Method performances in terms of limit of quantification (LOQ), accuracy (recovery R%) and reproducibility intra-laboratory (relative standard deviation RSD_R%) were assessed. Validation tests were conducted at the LOQ and 2 typical contamination levels.

Study area and field samples. The study area covered the Castreccioni Lake, connected through Musone River to a marine area (Northern-Central Adriatic Sea).

Thirty-four mussels (*Mytilus galloprovincialis*) and 10 clams (*Chamelea gallina*) were collected between April 2025 and April 2026, from farms (3 mussels and one clams sites) included in official monitoring programs, located near Musone River catchment.

Exposure experiment. *M. galloprovincialis* specimens were obtained from a classified farm and distributed into 2 tanks containing synthetic seawater (50 organisms per 20 L tank): an exposure tank where mussels were fed *P. rubescens* (2.6×10^9 cell/L) isolated from Castreccioni Lake, and a control tank where mussels were fed a non-toxic microalga (*Trentepohlia umbrina*- 2.6×10^9 cell/L as well) for one week.

Results

The validated LC-MS/MS method exhibited optimal sensitivity in terms of LOQ, especially for APs (MCs=3.5µg/kg, APs=2µg/kg) and good performances for the other parameters (R% ranging from 86 to 104%, RSDR% of 22%).

Only two field mussel samples showed detectable AP-A traces (no other MCs or APs were detectable) with concentrations below the LOQ, whereas no contamination was detected in any clam sample. The low contamination reported in molluscs may be due to the absence of cyanobacterial blooms in Castreccioni lake, during the monitoring period (data provided by Regional Environmental Protection Agency), the progressive dilution of *P. rubescens* during transport from the lake to adjacent marine- coastal environments [6] and the distance of the mussel farms from the Musone River mouth (1- 2 NM).

In experimentally exposed mussels, non- detectable traces of [D-Asp3]-MC-RR and [D- Asp3]-MC-LR and low levels of APs (mean values: AP-A=14µg/kg, AP-B=3µg/kg) were found. The toxic profile of *P. rubescens* culture was represented by [D-Asp3]-MC-RR and [D- Asp3]-MC-LR (68%), AP-A (14%) and AP-B

(18%). The concentrations detected in mussels were lower than expected, whereas cyanotoxins were consistently detected in water tanks, supporting the observed low accumulation in the organisms.

The contaminated field samples displayed a toxin profile comparable to that observed in exposed mussels, characterized by a higher abundance of APs compared with MCs, despite the latter being more abundant in *P. rubescens*. This finding suggests a possible toxin-specific accumulation pattern in mussels, which warrants further investigation.

Conclusions

The optimized LC-MS/MS method enables the simultaneous determination of different classes of cyanotoxins, expanding the analytical scope for food matrix analysis. This multi-target approach provided a deeper characterization of the toxin profile, improving the investigation of cyanotoxin accumulation pattern.

The integrated analysis of both field and exposed samples proved to be a reliable approach for evaluating the potential transfer of cyanotoxins from freshwater to marine ecosystems.

Cyanobacterial blooms do not currently pose a significant threat to coastal areas. However, they may become an increasing concern in the future as a result of climate change, which could promote more frequent and intense *P. rubescens* blooms. Consequently, monitoring programs will be needed to assess the risks and support early detection strategies.

References

1. E. Testai et al., *EFSA Supporting Publications*, 13:2 (2016)
2. I. Di Marco Pisciotano et al., *Chemosphere*, 370 (2025)
3. C. Akyol et al., *Environmental Pollution*, 286 : 117535 (2021)
4. R. De Pace et al., *Journal of Ecosystems*, 1 : 374027 (2014)
5. M. E. Giuliani et al., *Marine Drugs*, 17 :10, 595 (2019)
6. E. P. Preece et al., *Harmful Algae*, 61 (2017) pp 31-45

Research funded by the Italian Ministry of Health (Ricerca Finalizzata 2021, RF0012023) grant (GR-2021-12375051)

P38

Levels of 3-monochloropropane-1,2-diol (3-mcpd as sum of free and ester-bound forms) and glycidyl esters (ge) in fish oil: results of five-year monitoring

Valentina Gamba, Elena Pellegrini, Barbara Albanese, Simona Romelli, Mara Gasparini

Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Via Bianchi, 9 – Brescia, Italy

Summary: *In Italy, imported fish oil is primarily used in aquaculture feed production. However, a substantial proportion is also utilized in the manufacture of dietary supplements for human consumption. Following refining and purification, fish oil is incorporated into capsules and other nutraceutical products as a concentrated source of omega-3 fatty acids. This study provides a general overview of the results obtained between 2022 and the first half of 2026 from the analysis of forty fish oil samples (n = 40) for the determination of the process contaminants 3-monochloropropane-1,2-diol (3-MCPD; sum of the free and esterified forms, expressed as 3-MCPD) and glycidyl esters (GE, expressed as glycidol).*

Keywords: *food processing contaminants, 3-MCPD, glycidyl esters, fish oil*

Introduction

3-Monochloropropane-1,2-diol (3-MCPD) and its fatty acid esters, as well as glycidyl esters (GE), may be formed as process contaminants during the refining of vegetable fats and oils [1, 2]. Fish oil and oils from other marine organisms can also contain high levels of these contaminants. GE are believed to be hydrolysed to glycidol, a compound classified as probably carcinogenic to humans (IARC Group 2A). Similarly, MCPD esters are hydrolysed within the gastrointestinal tract. Free 3-MCPD is classified as possibly carcinogenic to humans (IARC Group 2B) [3,4]. Following toxicological evaluations by the European Food Safety Authority (EFSA), the European Commission has established maximum levels (MLs) for MCPD (sum of free and ester-bound forms expressed as 3-MCPD) and GE in edible fats and oils, as well as in infant formula and baby foods [5], and has established performance criteria for analytical methods [6]. In Italy, imported fish oil is primarily used in aquaculture feed production. However, a substantial proportion is also utilized in the manufacture of dietary supplements for human consumption. Following refining and purification, fish oil is incorporated into capsules and other nutraceutical products as a concentrated source of omega-3 fatty acids. In this study, the results obtained from the analysis of forty fish oil samples (n = 40) between 2022 and the first half of 2026 are summarized: samples analyzed between 2022 and 2024 (n = 13) were analyzed by GC-MS, whereas samples analyzed between 2025 and the first half of 2026 (n = 27) were analyzed by GC-MS/MS.

Experimental

Sampling criteria: 3-MCPD and GE quantification. The majority of the samples originated from sampling activities carried out by the Border Control Posts (BCPs), which are the official points where sanitary controls are performed on goods and animals entering the European Union from third countries (non-EU countries). In addition, five samples were commissioned to our laboratory by NLZOH (National Laboratory of Health, Environmental and Food) of Maribor, Slovenia. Each sample was constituted of at least 100 mL.

3-MCPD and GE analysis. Sample preparation consisted of a liquid-liquid extraction of fish oil (100 mg) using ethyl ether for the extraction of free 3-MCPD. For 3-MCPD bound forms and GE two independent assays (A and B) were prepared for each sample. The ester cleavage step was performed by basic hydrolysis, followed by the cleavage termination step through the addition of an acidified sodium chloride solution for the conversion of glycidol into 3-MCPD (assay A) and an acidified chloride-free salt solution (assay B).

Finally, after matrix clean-up with isohexane, and subsequent extraction with a diethyl ether/ethyl acetate (60:40 v/v) mixture, a derivatization step was carried out using a phenylboronic acid (PBA)-saturated diethyl ether solution. Analyses were performed using the GC-MS (from 2022 to 2024) and GC-MS/MS (from 2025 to 2026) equipments described in Table 1.

For samples collected from 2022 to 2024, the quantification of total 3-MCPD (free and bound forms) and glycidyl esters was based on single-point calibration. Quantification was based on the ratio of the analyte signal area to that of the isotopically labelled internal standard (3-MCPD-d₅), calculated from the corresponding ion traces and multiplied by the spiking level of the internal standard in Assay A.

For the determination of total 3-MCPD (free and bound forms), the same approach was applied using the analyte-to-internal-standard signal area ratio obtained from Assay B. Glycidol content was calculated as the difference between the values obtained in Assay A and Assay B.

For samples collected from 2025 to 2026, quantification was based on external calibration curve. The glycidol content was calculated as the difference between the results obtained in Assay A and Assay B. The limit of quantification (LOQ) was 10 µg/kg for 3-MCPD (as sum of free and bound forms) and 80 µg/kg for GE.

Results

From the beginning of 2022 to the first half of 2026, a total of 40 fish oil samples were analysed (Figure 1). 3-MCPD (the sum of free and bound forms) was detected in 21 samples (52.5%), while glycidyl esters (GE) were detected in 19 samples (47.5%). Thirteen samples (32.5%) showed the presence of both 3-MCPD and GE, whereas in 10 samples (25%) neither 3-MCPD nor GE were detected. Taking into account the measurement uncertainty, only one sample (2.5%) exceeded the maximum level of 2500 µg/kg for 3-MCPD whereas no samples exceeded the maximum level of 1000 µg/kg for glycidyl esters (GE).

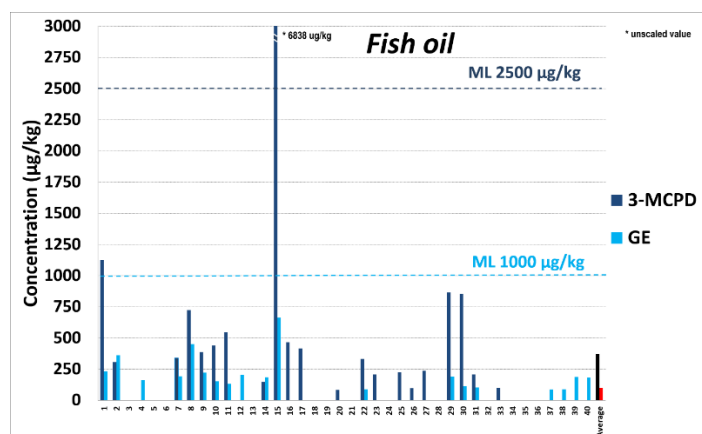


Figure 1. Concentration (µg/kg) of 3-MCPD (dark blue bar) and GE (light blue bar) in 40 fish oil samples. Average value for each graph are shown as a black bar (3-MCPD) and red bar (GE)

Table 1. GC-MS and GC-MS/MS equipment

	GC-MS	GC-MS/MS
Gas-chromatography	6890N (Agilent Technologies)	Intuvo 9000 GC System (Agilent Technologies)
Column	DB-17MS, 30 m x 0.25 mm x 0.25 µm, 50% Phenyl, 50% Dimethylpolysiloxane	Agilent Intuvo DB-17MS (30 m, 0.25mm ID, 0.25 µm film)
Carrier gas	He, 1 ml/min constant flow	He, 1 ml/min constant flow
Temperature programme	85 °C for 0.5 min – 6 °C/min to 150°C, 12°C/min to 180 °C, 25°C/min to 280 °C for 7 min	60 °C for 1 min – 20 °C/min to 160°C, 10 °C/min to 220 °C, 25°C/min to 320 °C for 4 min
Injection temperature	250 °C	250 °C
Injection mode and volume	Splitless, 1 µL	Splitless, 0.2 µL
MS parameters		
Ionisation	Electronic Ionisation	Electronic Ionisation
Operation mode	Selected Ion Monitoring (SIM)	Multiple Reaction Monitoring (MRM)
Monitors Ions	3-MCPD: 147, 196 and 198 3-MCPD-D5: 150, 201 and 203	3-MCPD: 196 > 147 (PQ); 198 > 147 – 3-MCPD-D5: 201 > 150 (PQ); 203 > 150

Conclusions

The present study showed that the process contaminants 3-MCPD and glycidyl esters (GE), were frequently detected in fish oils intended for human consumption. More than half of the samples contained detectable levels of total 3-MCPD, while glycidyl esters (GE) were detected in nearly half of the samples. Nevertheless, only one sample (2.5%) exceeded the maximum regulatory level for total 3-MCPD, whereas no samples exceeded the maximum level established for glycidyl esters. These findings indicate that, although the occurrence of these process contaminants is common in fish oils, non-compliance appears to be infrequent. Continued monitoring remains important to verify compliance with regulatory limits and to assess consumer exposure over time.

References

1. J. Zhang, W. Zhang, Y. Zhang, M. Huang, B. Sun, *Foods* 2021, 10, 2266T.
2. O. Özdikicierler, N. Yüksek, F. Yemişçiouğlu, *La Rivista Italiana delle Sostanze Grasse* 2020, Vol. XCVII, 7-13
3. IARC, 2000. Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 77, 469-486.
4. IARC, 2012. Some Chemicals Present in Industria land Consumer Products, Foods and Drinking-Water, Volume 101, 349-374.
5. Commission Regulation (EC) N. 333/2007 of 28 March 2007, Off. J.Eur. Union L1045 (2024), p.1
6. Commission Regulation (EU) 2023/915 of 25 April 2023 Off. J.Eur. Union L119 (2023), p.103

P39

Effects of fishing pressure on the fatty acid composition in red mullet (*Mullus barbatus*) from the central Adriatic sea

Behixhe Ajdini,¹ Federico Girolametti,¹ Silvia Illuminati,¹ Anna Annibaldi,¹ Luca Caracausi,¹ Giacomo Milisenda,² Emanuela Fanelli,¹ Cristina Truzzi¹

¹ Department of Life and Environmental Sciences, Università Politecnica delle Marche, Via Brecce Bianche, 60131 Ancona, Italy

² Stazione Zoologica Anton Dohrn, Sicily Marine Centre, Department of Integrative Marine Ecology, Lungomare Cristoforo Colombo N. 4521, 90149 Palermo, Italy

Summary: *This research investigates the effects of bottom trawling on the nutritional quality of red mullet (*Mullus barbatus*), focusing on fatty acid composition. The data revealed that the area with high fishing pressure intensity reduces polyunsaturated fatty acids, particularly omega-3 fatty acids. The determination of fatty acid composition was performed by gas chromatography-mass spectrometry.*

Keywords: *Fishing pressure, fatty acid composition, red mullet, Central Adriatic Sea*

Introduction

Bottom trawling is one of the most widespread fishing practices, accounting for approximately 26% of global marine fish catches [1]. However, its persistence at large spatial scales has well-documented impacts on benthic ecosystems, including alterations of community structure, loss of large-bodied infauna, and a shift toward small, disturbance-tolerant taxa [2]. Long-term studies from the Mediterranean and other regions show that bottom trawling degrades seabed structure, reduces sediment heterogeneity, and compromises ecosystem stability and resilience [3,4]. Beyond ecological effects, bottom trawling may also influence the nutritional quality of marine food resources. Fish are a fundamental component of human nutrition, providing high-quality proteins and essential polyunsaturated fatty acids (PUFAs), particularly omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are associated with cardiovascular and anti-inflammatory benefits [5]. While substantial research has addressed links between diet, environment, and nutritional composition in aquaculture systems, far less is known about how environmental degradation and fishing pressure influence the nutraceutical quality of wild-caught species.

In the Mediterranean Sea, the red mullet (*Mullus barbatus*) is one of the most commercially important demersal species closely associated with traditional fishing grounds for trawling. Its diet, trophic position, and muscle composition vary with size, season, environmental conditions, and fishing pressure, making it particularly exposed to habitat disturbance and resource depletion.

This research investigates whether bottom trawling intensity affects the fatty acid composition of *M. barbatus* collected in the coastal waters of the Marche Region in the Adriatic Sea, one of the most intensively exploited regions in the Mediterranean. Specifically, we test the hypothesis that increased fishing pressure reduces omega-3 PUFA content by altering benthic food resources.

Experimental

Study area and sampling design. Sampling was conducted in January 2023 in the coastal waters of the Marche Region in the Adriatic Sea (MSFD monitoring areas), covering three sites characterized by different fishing pressures: (1) Fano–Marotta (low fishing pressure), (2) Senigallia–Falconara Marittima (medium fishing pressure), and (3) Porto Recanati–Civitanova (high fishing pressure). Fishing effort values in each area were based on Vessel Monitoring System (VMS) data. Sampling was performed using a bottom trawl equipped with a 40-mm stretched mesh (20-mm cod-end diamond mesh). In each area, a total of 6 hauls were carried out, and each trawl haul was carried out at a maximum towing speed of 4 knots and a duration of 30 minutes. All *M. barbatus* specimens were isolated and stored at –20 °C until laboratory analyses.

Lipid extraction and fatty acid analysis. Six specimens from each station were selected for fatty acid analysis. Approximately 50 mg dry weight (±0.01) of fish tissue was extracted by microwave-assisted extraction (5 min at 90 °C) (MARS-X, 1500 W, CEM, Mathews, NC, USA), and the mass of extracted lipid was determined gravimetrically. FAs were determined using gas chromatography (Agilent-6890) coupled to an Agilent-5973N quadrupole Mass Selective Detector, after their derivatization to fatty acid methyl esters, using the methyl ester of nonadecanoic acid (19:0, 99.6%, Dr. Ehrenstorfer GmbH, Germany) as internal standard. Analyses were carried out on three aliquots per sample.

Results

Fish collected from areas subjected to different fishing pressures (PC: high, SF: medium, FM: low) showed no

significant differences in total lipid content (mean value: 3.9 ± 0.2 % DW; $p > 0.05$).

In contrast, the fatty acid (FA) composition significantly varied among sampling areas (Fig.1.). While saturated fatty acids (SFAs) remained relatively constant among sites (mean value: 39 ± 0.6 % of total FAs; $p > 0.05$), monounsaturated fatty acids (MUFAs) decreased significantly from the area subjected to high fishing pressure to the low-pressure area (32 ± 3 %, 28 ± 4 %, and 25 ± 3 %, respectively for PC, SF and FM; $p = 0.044$). Conversely, polyunsaturated fatty acids (PUFAs) increased significantly along the same gradient (29 ± 2 %, 34 ± 3 %, and 37 ± 3 %, respectively; $p = 0.023$). Within PUFA classes, the n6 FA differed significantly among sites, with higher contents in fish from SF and FM sites compared with PC ($p = 0.007$). Similarly, total n3 FA increased significantly from PC to FM ($p = 0.049$). Despite these differences, the n3/n6 ratio remained constant across sites (mean value: 3.47 ± 0.05 ; $p > 0.05$). Long-chain n9 FA also differed significantly among groups, decreasing from 19 ± 1 % in PC to 18 ± 2 % in SF and 16 ± 2 % in FM ($p = 0.038$).

At the individual fatty acid level, the health-promoting n3 FA, DHA, increased significantly from 11 % in PC to 13 % in SF, and 15 % in FM ($p = 0.05$), contributing to higher total n3 levels in fish from the low-pressure area. A similar trend was observed for 20:4n-6 ($p = 0.044$), 20:2n-6 ($p = 0.005$), 15:1n-5 ($p = 0.025$), and 20:0 ($p = 0.008$), while an opposite trend was observed for 18:1n-9 ($p = 0.029$), decreasing from PC to FM sites.

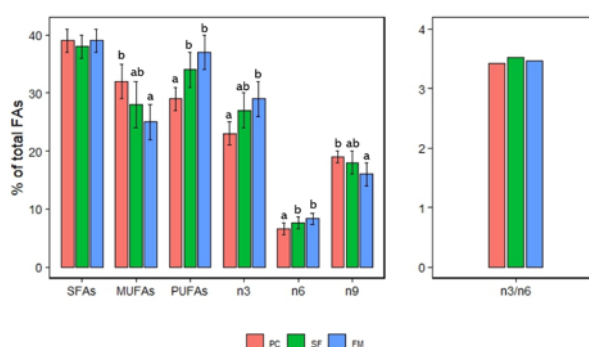


Fig.1. Fatty acids class (% of total FAs) of *Mullus barbatus* specimens collected in PC, SF and FM areas. PC, Porto Recanati-Civitanova; SF, Senigallia-Falconara Marittima; FM, Fano-Marotta; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; n3, omega-3 polyunsaturated fatty acids; n6, omega-6 polyunsaturated fatty acids; n9, omega-9 polyunsaturated fatty acids; n3/n6, omega3/omega6 ratio. Different letters indicate statistically significant differences among sites compared within the same fatty acid class ($p < 0.05$). Values are presented as mean $\pm$ SD ($n=9$).

Conclusions

This study provides the first evidence that bottom trawling intensity is associated with changes in the nutritional quality of *Mullus barbatus*, as reflected by muscle fatty acid composition. Individuals from the high-fishing-pressure area (PC) exhibited lower levels of omega-3 PUFAs than those from the low-pressure site (FM). These findings extend the traditional view of bottom trawling as a driver of benthic ecosystem degradation by highlighting its potential to alter the nutritional quality of commercially important fish species. Incorporating food quality indicators into fisheries assessment frameworks may therefore be relevant for ecosystem-based management approaches in the Mediterranean Sea.

References

1. Food and Agriculture Organization of the United Nations (FAO); The State of World Fisheries and Aquaculture 2022: Towards Blue Transformation (FAO, 2022).
2. J. G. Hiddink et al.; Proceedings of the National Academy of Sciences of the United States of America, 114 (2017), pp. 8301–8306.
3. E. Sañé, J. Martín, P. Puig, A. Palanques; Biogeosciences, 10 (2013), pp. 8093–8108.
4. L. A. Levin, P. K. Dayton; Trends in Ecology & Evolution, 24 (2009), pp. 606–617.
5. E. Weichselbaum, S. Coe, J. Buttriss, S. Stanner; Nutrition Bulletin, 38 (2013), pp. 128–177.

P40

Unravelling aroma modulation in extruded pea protein through carob bean protein incorporation: A comprehensive GC×GC–MS volatilomic study

Faezeh Khoobbakht,¹ Francesco Reale,² Giorgia Mattina,¹ Silvia Carlin,² Panagiotis Arapitsas,² Eugenio Aprea¹

¹ Center Agriculture Food Environment, University of Trento, Via Edmund Mach 1, 38089 San Michele all'Adige, Italy

² Research and Innovation Centre, Fondazione Edmund Mach, Via Edmund Mach 1, 38010, San Michele all'Adige, Trento, Italy

Summary: *Comprehensive two-dimensional gas chromatography–mass spectrometry (GC×GC–MS) enables detailed characterization of complex food volatilomes through superior chromatographic resolution and sensitivity. Applied to extruded pea–carob protein blends, this approach facilitates the identification of key aroma markers and provides valuable insights into flavour modulation in plant-based meat analogues.*

Keywords: GC×GC–MS, Volatile organic compounds (VOCs), plant proteins

Introduction

The growing demand for plant-based meat alternatives (PBMA) has accelerated the development of protein-rich ingredients with improved nutritional and sensory characteristics. Pea is one of the most widely used plant proteins because of its high protein content, balanced amino acid profile, non-GMO origin and functional properties. However, its application remains limited by the presence of undesirable volatile compounds responsible for beany, grassy, and earthy aromas, which can negatively affect consumer acceptance [1].

Extrusion cooking is the leading method for manufacturing texturized vegetable proteins (TVPs) that are used as meat analogues. Apart from structural changes resulting in the modification of texture characteristics, the process of extrusion facilitates chemical reactions such as lipid oxidation, Maillard reactions, and protein denaturation that result in major changes in the VOC composition of the plant proteins. Extrusion is capable of producing both favorable roasted tastes and undesirable flavor compounds [2].

Carob bean protein, an underutilized protein fraction derived from the carob processing industry, has recently emerged as a promising sustainable protein ingredient owing to its favorable functional properties, including high water-holding capacity and emulsifying activity [3]. Beyond its technological functionality, its unique chemical composition may also influence the formation of volatile compounds during extrusion processing. However, little is known about the VOC fingerprint of carob bean protein and its contribution to the volatilome of extruded protein-based systems.

Food aroma is composed of a complex mixture of volatile compounds from multiple chemical classes, making comprehensive characterization challenging using conventional one-dimensional gas chromatography (1D-GC) because of frequent compound co-elution and interference from abundant matrix components. Comprehensive two-dimensional gas chromatography (GC×GC) overcomes these limitations by providing superior separation, sensitivity, and peak capacity, enabling more detailed volatile fingerprinting. Consequently, GC×GC has become a powerful tool for food volatilomics, with applications in aroma profiling, food authentication and classification, quality control, and the analysis of target volatile compounds [4].

Therefore, this study aimed to investigate the effect of partially replacing pea protein with carob bean protein on the volatile profile of extruded protein blends. Comprehensive two-dimensional gas chromatography coupled with mass spectrometry (GC×GC–MS) was used to characterize the VOC profiles of the raw protein ingredients and to elucidate the volatilomic changes occurring during extrusion in both the individual proteins and their blends. The findings provide insight into the potential of carob bean protein as a sustainable, natural ingredient for modulating off-flavor formation in pea protein extrudates.

Experimental

Five protein formulations consisting of pea protein and carob bean protein were prepared at different blending ratios with pea 100% and carob 100% as control samples. Prior to extrusion, the moisture content of each blend was adjusted to the desired level and equilibrated to ensure uniform hydration.

The protein blends were processed using a co-rotating twin-screw extruder under identical extrusion conditions to isolate the effect of formulation on volatile formation. The extrudates were cooled, dried, ground, and stored under controlled conditions until analysis.

VOCs were analyzed by headspace solid-phase microextraction coupled with comprehensive two-dimensional gas chromatography–mass spectrometry HS-SPME–GC×GC–TOFMS (LECO Pegasus BTX-4D). Approximately 2.0 g of homogenized sample was weighed into a 20 mL headspace vial, 2 mL of water was

added. 2-methyl-3-heptanone was used as the internal standard. Samples were equilibrated into the autosampler agitator for 20–30 min, and VOCs were extracted for 30 minutes using a 50/30 µm DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane) SPME fibre. The fibre was thermally desorbed in the GC injector. The separation was performed with an Agilent 8890 gas chromatographer. The 1st GC capillary column was a 30 m × 0.25 mm × 0.25 µm film thickness VF-WAXms (Agilent Technologies), while the 2nd one was a 1.5 m × 0.15 mm × 0.15 µm Rxi 17Sil MS (Restek). Hydrogen was used as the carrier gas. VOCs were identified by comparison of their mass spectra with the NIST mass spectral library and, where available, by comparison of linear retention indices with previous published papers.

Results

At the time of submission of the present abstract the GC analyses are about to begin; thus, data are not available yet. GC×GC–MS analyses will be performed to characterize the volatile profiles of pea protein, carob bean protein, and their extruded blends, and to investigate the effect of partially replacing pea protein with carob bean protein on VOC generation during extrusion. Based on previous studies [2], pea protein is known to contain characteristic volatile compounds associated with beany, grassy, earthy, and mushroom-like off-flavors listed in Table 1, including alcohols (e.g., 1-pentanol, 1-hexanol, 1-octen-3-ol), aldehydes (e.g., hexanal and nonanal), ketones (e.g., 1-octen-3-one), pyrazines, and furans (e.g., 2-pentylfuran), which are mainly formed through lipoxygenase-mediated oxidation of unsaturated fatty acids and lipid autoxidation. Therefore, these compounds will be specifically monitored to determine whether their abundance is altered by the incorporation of carob bean protein.

It is hypothesized that replacing pea protein with carob bean protein will reduce the impact of these characteristic off-flavor compounds, either by decreasing their formation or by promoting the generation of volatile compounds with more desirable roasted, nutty, caramel-like, or cocoa-like aroma characteristics. This hypothesis is supported by preliminary sensory observations in a small focus group, in which most participants perceived the characteristic beany flavor to be less evident in formulations containing carob bean protein. Multivariate statistical analyses of the GC×GC–MS data will be used to identify the volatile markers responsible for the observed differences among formulations and to evaluate the potential of carob bean protein as a natural ingredient for improving the aroma profile of pea protein extrudates.

Table 1. Main beany off- flavor components in pea protein [2].

Compound class	Main compounds	Typical aroma	Origin
Alcohols	1-Pentanol, 1-Hexanol, 1-Heptanol, 1-Octanol, 1-Nonanol, 1-Octen-3-ol, 3-Methyl-1-butanol	Beany, grassy, green, earthy, mushroom-like	Mainly lipoxygenase-mediated oxidation of linoleic/α-linolenic acids; some inherent in peas
Aldehydes	Hexanal, Nonanal	Green, grassy, fatty	Lipoxygenase-catalysed lipid oxidation and autoxidation
Ketones	1-Octen-3-one	Beany, mushroom	Lipid oxidation
Pyrazines	2-Isopropyl-3-methoxypyrazine	Green, grassy, earthy	Naturally occurring in peas
Furanoids	2-Pentylfuran	Earthy, beany	Lipid autoxidation

References

1. Trindler, C., Kopf-Bolanz, K. A., & Denkel, C. (2022). *Food chemistry*, 376, 131892.
2. Lao, Y., Ye, Q., Wang, Y., & Selomulya, C. (2024). *Food Reviews International*, 40(9), 3129–3158.
3. Khoobakht, F., Waleed, A. A., Ferrentino, G., & Aprea, E. (2026). *Food Chemistry*, 149732.
4. Yu, M., Yang, P., Song, H., & Guan, X. (2022). *Journal of Food Composition and Analysis*, 114, 10

P41

Wildlife: A protein alternative to intensive farming? Food security and environmental bioindicator study

E. Vaccaro,¹ G. Ferrari,¹ V. Armenio,¹ V. Savio,¹ F. Armellino,¹ S. Robetto,¹ B. Moroni,¹ V. Listorti,¹ L. De Paolis,¹ E. Razzuoli,¹ L. Guardone,^{1,2} V. Ciccotelli¹

¹ Istituto zooprofilattico sperimentale Piemonte, Liguria, Valle d'Aosta, Via Bologna 148, 10154 Torino, Italy

² Dipartimento di Scienze Veterinarie, Università di Pisa, viale delle Piagge 2, 56124 Pisa, Italy

Summary: Game meat could represent an alternative protein source to intensive livestock farming. In this study we estimated the presence of pesticides in wildlife evaluating both the risk associated with game meat intake and environmental health using wild animals as bioindicators.

Keywords: wildlife, pesticides, bioindicator

Introduction

Game meat is a little-known resource in Italian territory, representing a possible alternative protein source to intensive livestock farming and, when hunting activity is properly regulated, allows a better biodiversity maintenance [1]. On the other hand, environmental contamination by toxic and polluting substances may represent a risk in the consumption of game meat, because of phenomena of bioaccumulation and bioconcentration. In this work we focused on pesticides, also known as plant protection products (PPPs), massively used to protect agricultural crops. The aim of this work was to estimate the presence of pesticides in wildlife evaluating both the risk associated with game meat intake and environmental health using wild animals as bioindicators, since their health reflects soil, water and nourishment quality of the habitat where they live [2].

Experimental

399 samples of liver and muscle of wild animals, collected from hunting and culling trips carried out in the regions of Liguria, Piedmont, and Valle d'Aosta, during the two-year period 2023-2024 were analyzed, as part of project GR-2021-12374932. Animals included roe deer, chamois, wild boar, wolves and foxes. From each sample, 5.0 g $\pm$ 0.1 g were extracted with acetonitrile. After addition of magnesium sulfate, sodium chloride and buffering citrate salts, the mixture was shaken intensively and centrifuged for phase separation. After one-hour freezing-out, an aliquot of the organic phase was cleaned-up by dispersive solid phase extraction (d-SPE) employing primary secondary amine sorbent (PSA), C18 and magnesium sulfate for the removal of residual water. Extracts were then acidified by adding formic acid to improve the storage stability of certain base-sensitive pesticides. The final extract was analyzed in GC-MS/MS Thermofisher Trace 1300 + TQ 8000 EVO with column Zebron ZB-SemiVolatiles (Phenomenex) (30 m x 0.25 mm ID x 0.25 μ m).

Results

Ninety different pesticides were identified in analyzed samples. The liver was the tissue with the highest percentage (66.8%) of samples with at least one PPPs, compared to muscle (44.5%). Piedmont presented a percentage of samples with at least one pesticide residue (60.4%) higher than Liguria (40.0%) and Valle D'Aosta (58.1%), as shown in Table 1.

Table 1. Percentage of samples compared to the total, divided by animal and region, in which GC-MS/MS analysis revealed the presence of at least one pesticide residue.

% samples with pesticide residues	Liguria	Piedmont	Valle d'Aosta	Total
Chamois			56.6%	56.6%
Roe deer		81.8%	66.7%	71.4%
Boar	50.0%	58.3%	69.4%	63.8%
Wolf	11.1%	50.0%		28.1%
Fox	30.0%	54.2%	51.0%	50.0%
Total	40.0%	60.4%	58.1%	55.6%

Wild ruminants showed a higher number of samples with pesticide residues (roe deer 71.4%, chamois 56.6%) than carnivorous (foxes 50%, wolves 28.1%). In some samples, pesticides that are now banned in Europe, such as Desmedipham and Endrin Ketone, were found.

The species with higher percentages of samples with pesticides above MRLs were roe deer (54.5% for Desmedipham, MRL 0.01 mg/kg, range 0.013-2.902 mg/kg), wild boar (25.0% for Bixafen, MRL 0.05 mg/kg, range 0.053-0.091 mg/kg) and chamois (92.3% of livers for Procymidone, MRL 0.01 mg/kg, range 0.016-0.068 mg/kg).

Multivariate PCA analysis [3] was performed on muscle samples to evaluate the distribution of pesticides among the animals and the three regions. The analysis did not reveal any significantly different clusters, although some fox samples from Valle d'Aosta showed a pesticide distribution distinct from the rest of the samples.

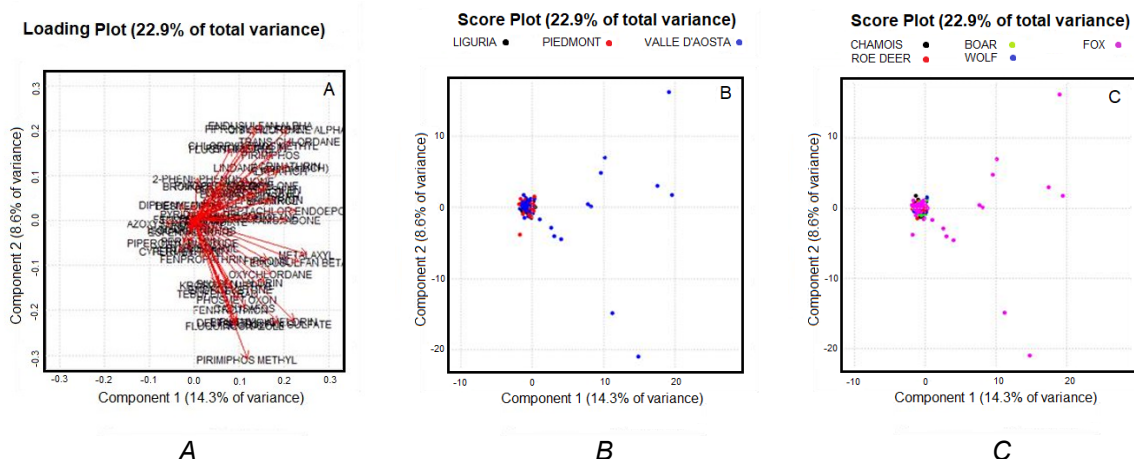


Figure 1. Plots of the multivariate PCA analysis on muscle samples. (A) Loading plot showing the distribution of the analytes in the plane of the first two principal components. (B) Score plot showing the distribution of samples in the plane of the first two principal components, color-coded by the sample's region of origin. (C) Score plot showing the distribution of samples in the plane of the first two principal components, color-coded by animal species.

Conclusions

The results obtained in the present study confirm the suitability of using these bioindicators to evaluate the health of the environment. However, the prevalence of the number of samples from Piedmont containing residues, for all animal species, indicates a possible common exposure through the consumption of water containing traces of pesticides, as highlighted by the SNPA Environmental Report no. 41/2024 [4].

The interspecies differences in distribution highlight how exposure to these substances is driven by diet and behavior: herbivores, showing higher pesticide burdens, have direct exposure through plant consumption, whereas carnivores and saprophytes are exposed indirectly via the food chain by consuming contaminated herbivorous prey [5].

The detection of pesticides whose use has been banned in Europe, such as Desmedipham (an herbicide) and Endrin Ketone (a degradation product of the insecticide endrin), raises the issue of their persistence in the soil, requiring ongoing monitoring of environmental levels to determine possible risks to the health of the ecosystem and humans.

Regarding the food safety of these meats, samples with concentration of pesticides above MRLs are a significant fraction, even exceeding half of the total samples. This raises concern about the risks that consumers of such meats, especially hunters and their families, are exposed to. In conclusion, game meat could represent a more sustainable protein source than factory-farmed meat, but appropriate chemical controls must be carried out to ensure its safety.

References

1. IZS delle Venezie. *Cacciagione un'opportunità nazionale? L'altra medicina* (2021).
2. F. T Tataruch and H. Kierdorf, *Mammals as biomonitors*. Trace Metals and other Contaminants in the Environment, 6 (2003), pp 737-772.
3. R. Leardi, C. Melzi, G. Polotti, CAT (Chemometric Agile Tool), freely downloadable from <http://gruppochemiometria.it/index.php/software>
4. SNPA, *Rapporto nazionale pesticidi nelle acque - dati 2021*, Report ambientali SNPA n. 41/2024.
5. E. Jooste, C.K. Nielsen, D. Chen, *Using terrestrial mammalian carnivores for global contaminant monitoring*. Integrated Environmental Assessment and Management, 10(2) (2014), pp 312–314.

P42

Zein-based nanoparticles: a green approach to bioactive peptides delivery

Stefania Marzorati,¹ Patrícia Duque-Estrada,² Davide Emide,³ Daniela Maggioni,⁴ Thamiris Monteverde Vicentini,¹ Chiara Magni,³ Chiara Scapuzzi,¹ Rene Lametsch,² Iben Lykke Petersen,² Mikołaj Dziurzyński,⁵ Alessio Scarafoni³

¹ Department of Environmental Science and Policy, University of Milan

² Department of Food Science, University of Copenhagen

³ Department of Food, Environmental & Nutritional Sciences (DeFENS), University of Milan

⁴ Department of Chemistry, University of Milan

⁵ Institute of Evolutionary Biology, University of Warsaw

Summary: *BIONanoDELY project develops biodegradable, self-assembling plant protein (zein-based) nanocarriers to protect bioactive cowpea peptides from degradation and improve their stability, bioavailability, and controlled release. This sustainable platform supports applications in human nutrition, providing a safe, environmentally friendly alternative to conventional synthetic and inorganic delivery systems.*

Keywords: *Zein-based nanoparticles; Bioactive peptides; Human health.*

Introduction

The increasing demand for sustainable and efficient solutions for human health has driven significant interest in nanoparticle-based delivery systems for bioactive compounds. Nanoparticles can in fact enhance efficacy by reducing the required dosage of bioactives while protecting them from degradation, thereby improving their stability, bioavailability, and controlled release. However, many conventional nanomaterials raise concerns due to their persistence, potential bioaccumulation, and uncertain long-term health impact. Bio-based carriers offer a safer and more sustainable alternative, as they are biodegradable, non-toxic, and derived from renewable resources. Among these, protein-based nanomaterials are particularly attractive because they naturally degrade into safe byproducts.

The BIONanoDELY project focuses on the development of biodegradable, zein-based nanoparticles as delivery systems for bioactive peptides from cowpea β -vignin, the major glycosylated globulin of cowpea (*Vigna unguiculata* L. Walp) seeds [1]. Bioactive peptides are emerging as promising natural compounds for applications in nutrition due to their antioxidant, antimicrobial [2], anti-inflammatory, and other health-promoting properties [3,4]. However, their practical use is often hindered by poor conformational stability, susceptibility to enzymatic degradation during gastrointestinal digestion, and limited bioavailability. These limitations reduce their efficacy and highlight the need for efficient delivery systems capable of protecting peptides and controlling their release.

Within a 4EU+ University alliance network, the project focuses on optimizing nanoparticle formulation encapsulating bioactive peptides, and assessing biological performance under simulated gastrointestinal conditions.

Experimental

Peptides purification and characterization: Extraction and isolation of raw β -vignin from cowpea seeds was performed as previously described [1]. β -vignin was dissolved (1 mg/mL) in a 50 mM Tris-HCl buffer pH 8, in presence of 100 mM NaCl and 10 mM CaCl₂. The dissolved protein was then digested by stirring it for 1 h at 37 °C with trypsin at a 1:20 ratio (w/v), followed by thermal inactivation. For the identification of peptides obtained after trypsin digestion, a Vanquish UHPLC system coupled to an Orbitrap Exploris 480 mass spectrometer was employed. Samples were first acidified with formic acid to a final concentration of 1% (v/v), and filtered (10 kDa MW cut-off centrifugal filter). Data were processed using Proteome Discoverer software and the cowpea database obtained from Uniprot. PReTP-Stack was used as a tool to predict anti-inflammatory peptides based on stacked ensemble learning [5].

Nanoprecipitation: Zein nanoparticles loaded with β -vignin peptides were prepared using the solvent displacement technique. In detail, 2 mg of zein and 2.2 mg of lyophilized powder (9% purity) (peptides:zein=1:10 by weight) were dissolved in 3 mL of an ethanol:water solution (2:1), introduced in a syringe equipped with a 22G needle, kept in vertical position and dropped into a coagulation bath composed of 5 mL of deionized water. The final suspension was kept under stirring to facilitate ethanol removal at room temperature. Zein nanoparticles formulated in the absence of peptides were prepared and used as a control.

Nanoparticles physico-chemical characterization: samples for Scanning Electron Microscopy (SEM) were prepared by dropping a small aliquot of diluted suspensions on a glass slide, previously fixed on SEM stub

through a biadhesive conductive tape. After drying at room temperature, samples were gold coated (Nanotech Sempreg 2) and observed using a FE-SEM Sigma microscope (ZEISS, Germany).

Dynamic Light Scattering (DLS) analysis was carried out by using a Nanozetasizer (Malvern Instruments) equipped with a 4.0 mW He–Ne laser operating at 633 nm and an avalanche photodiode detector to determine the average hydrodynamic size of the obtained nanoparticles.

Results

Peptides identification by LC-MS. In total, 240 peptides were identified on trypsin digested β -vignin sample; of these, most of the peptides (64%) had a molecular weight ranging between 1000 to 2000 Da, whereas 33% were between 500-1000 Da, showing the efficiency of trypsin digestion in producing short peptides. Only 7% of peptides had a molecular weight between 2000 and 3000 Da, and none were below 500 Da.

Of the 15 most abundant peptides identified by LC-MS, 11 were classified as anti-inflammatory peptides using the bioactivity prediction tool (PreTP-Stack).

Nanoparticles characterization. DLS analysis showed a monomodal size distribution for both formulations. The mean hydrodynamic diameter was 158 ± 51 nm for zein nanoparticles and 160 ± 49 nm for peptides-loaded zein nanoparticles.

SEM micrographs (Fig. 1) showed that both unloaded and peptide-loaded zein nanoparticles exhibited a predominantly spherical morphology. Peptide loading resulted in a more dispersed particle distribution with reduced aggregation compared to unloaded nanoparticles, while the overall particle morphology was preserved.

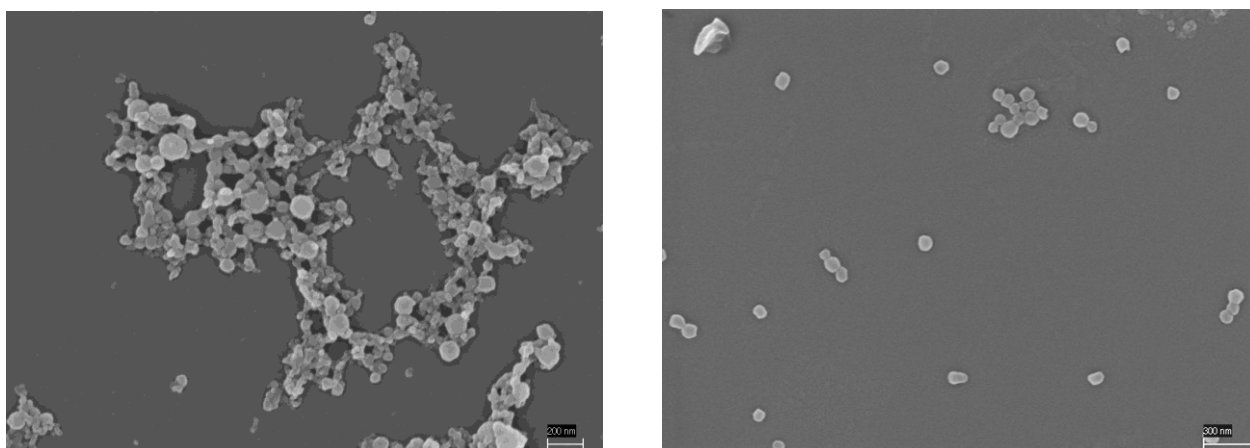


Fig. 1. SEM images of unloaded (A) and peptides-loaded (B) zein-based nanoparticles.

Conclusions

Trypsin digestion efficiently generated short peptides from β -vignin, with most identified peptides having molecular weights between 500 and 2000 Da. Among the 15 most abundant peptides, most were predicted to possess anti-inflammatory activity, making them promising candidates for further investigation. Nanoparticles were characterized by DLS and SEM which confirmed their spherical shape and nano-sized dimensions. Next steps of the project will be to determine whether zein nanoparticles can protect these peptides during gastrointestinal digestion and test their biological activity using intestinal cell models.

References

1. E. de Souza et al., *Bioscience, Biotechnology, and Biochemistry*; 82 (2018), pp 285–291.
2. T. S. Almeida et al., *Technologies*; 10 (2022), pp 89.
3. B. O. Philadelpho et al., *Foods*; 13 (2024), pp 3508.
4. B. O. Philadelpho et al., *Journal of Functional Foods*; 122 (2024), pp 106498.
5. K. Yan et al., *IEEE/ACM Transactions on Computational Biology and Bioinformatics*; 20 (2023), pp 1337–1344.

P43

Comparison between conventional and functional data analysis of time resolved volatilomics data: a case study of microalgae fermentation with kefir starters

Iuliia Khomenko,¹ Martina Moretton,¹ Emanuela Betta,¹ Antonio Luca Langellotti,² Vittorio Capozzi,³ Vincenzo Fogliano,⁴ Franco Biasoli¹

¹ Research and Innovation Centre, Fondazione Edmund Mach, via E. Mach 1, 38098 San Michele all'Adige (TN), Italy

² Centre for Innovation and Development in the Food Industry (CAISIAL), University of Naples Federico II, Via Università 100, 80055 Portici, Italy

³ National Research Council of Italy - Institute of Sciences of Food Production (ISPA) c/o CS-DAT, via Michele Protano, 71121 Foggia, Italy

⁵ Wageningen University, Food Quality and Design Department, Bornse Weiland 9, 6708 WG Wageningen, the Netherlands

Summary: This study evaluated Functional Data Analysis (FDA) against conventional statistics for time-resolved PTR-ToF-MS volatilomics of microalgae-kefir fermentations. Univariate FDA efficiently selected 118 feature peaks while controlling interval-wise error. For multivariate analysis, Multivariate Functional PCA (MFPCA) outperformed conventional PCA by explaining 83.67% of variance and clearly separating sample trajectories.

Keywords: Proton Transfer Reaction Mass Spectrometry, volatile organic compounds

Introduction

Recently, Functional Data Analysis (FDA) has gained increasing attention due to advances in high-throughput, high-frequency data acquisition. Under this paradigm, the analytical focus shifts from individual discrete data points to continuous curves representing the overall trajectory of a sample [1]. While new FDA methodologies have expanded from univariate to multivariate functional domains, their application in metabolomics – and volatilomics in particular – remains scarce. Proton Transfer Reaction Time-of-Flight Mass Spectrometry (PTR-ToF-MS), as a direct-injection technique, enables real-time monitoring of volatile organic compound (VOC) dynamics during biological processes, generating dense time-resolved datasets [2]. In this study, we compare conventional statistical approaches with FDA on a time-resolved volatilomics dataset obtained during water and milk kefir fermentations of three microalgal species.

Experimental

Water and milk kefir starters (Bionova, Villanova sull'Arda, Italy) were utilized to ferment three microalgal species: *Chlorella vulgaris*, *Limnospira platensis* (Spirulina), and *Galdieria sulphuraria*. Fermentations were performed in 20 mL headspace vials containing 1 mL of medium (1% w/v algal load without additional nutrients) in 5 biological replicates and incubated for 48 h in the dark at 27 °C. Uninoculated microalgal suspensions and pure water (5 replicates each) were included as control samples.

Headspace VOC emissions were monitored every 4 h over the 48-h period using a PTR-ToF-MS 8000 instrument (Ionicon Analytik GmbH, Innsbruck, Austria) coupled to a multipurpose headspace autosampler (Gerstel GmbH, Mulheim am Ruhr, Germany) and a SHS module (Ionicon Analytik GmbH). For volatile compound identification, complementary SPME-GC-MS analysis was conducted at 0h and 48h. PTR-ToF-MS dataset preprocessing followed established protocols. Statistical data analysis was conducted in R (RStudio environment) utilizing core workflows alongside specialized packages: tidyverse and purrr for data manipulation, mixOmics for multivariate exploration, and fda, funData, fdatest, and MFPCA for functional data modeling and interval testing.

Results

A total of 342 mass peaks were extracted from the raw PTR-ToF-MS dataset. This volatilomic profile comprised features tracking fermentation kinetics alongside background mass peaks whose trajectories mirrored pure water controls. Visual inspection, pointwise t-tests, and two functional hypothesis testing methods were evaluated for their efficacy in differentiating water from inoculated and uninoculated algal samples across 48 h of fermentation. The Interval Testing Procedure (ITP) with B-spline basis expansion was selected for FDA because it captures local variations in non-periodic fermentation curves while controlling the Interval-Wise Error Rate (IWER). Under this framework, curves were deemed statistically different if any corrected p-value across the time domain fell below 0.05. This local selection approach was contrasted with functional ANOVA, which yields a single global p-value. Following statistical filtering, correlated mass peaks corresponding to

isotopologues and ion clusters were removed, leaving 118 mass peaks for downstream analysis. Both univariate and multivariate analyses on the retained mass peaks highlighted distinct, time-dependent VOC production dynamics. Functional ANOVA was benchmarked against pointwise one-way ANOVA at each time step and classic two-way repeated-measures ANOVA (treating time, alga, and starter as fixed factors). Functional ANOVA effectively overcame the limitations of traditional approaches by modelling the continuous time profile while maintaining statistical rigor.

For multivariate exploration, datasets were centered and unit-variance scaled. Conventional Principal Component Analysis (PCA) applied across all discrete time points captured overall trajectory trends; however, it ignored the autocorrelated structure of VOC emissions, explaining only 43.13% of total variance across the first two components (Fig. 1A). In contrast, Multivariate Functional Principal Component Analysis (MFPCA) captured 83.67% of total variance (PC1: 54.30%, PC2: 29.37%). The resulting score plot (Fig. 1B) mapped each sample as an integrated functional trajectory, revealing clear separation among microalgal species and starter cultures within a low-dimensional functional score space.

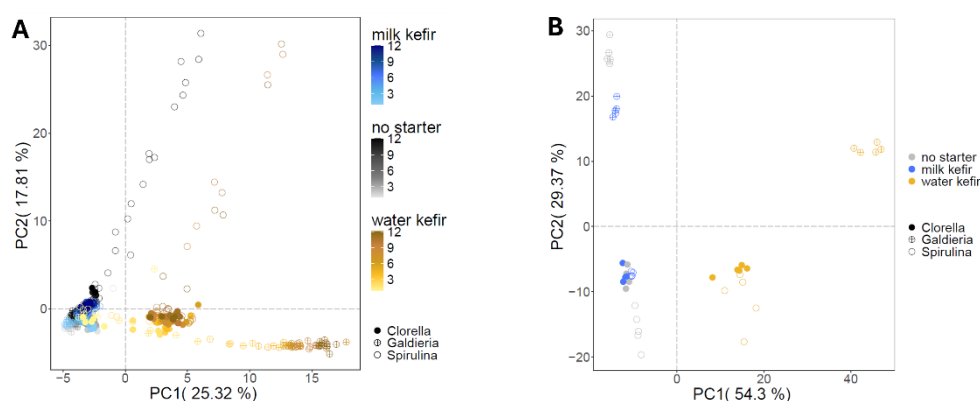


Fig. 2. Scoreplot for PCA (A) and MFPCA (B). The color corresponds to the type of the fermentation and shape point corresponds to the algal type. In the case of PCA the color gradient corresponds to the timeline of the fermentation

Both approaches highlighted substantial volatilomic divergence between water kefir and other treatments along PC1, particularly for *G. sulphuraria*. In conventional PCA, PC2 captured the time evolution of *Spirulina* fermentations, which were enriched in sulfur-containing volatiles toward the end of fermentation. In contrast, MFPCA provided superior resolution, disentangling nuanced interactive effects between *G. sulphuraria* and starter types. These functional shifts were largely driven by volatile alcohols (e.g., ethanol, butanol, methyl butanol) generated during water kefir fermentation – reflecting higher yeast activity relative to milk kefir – with emission kinetics dictated by the microalgal host matrix.

Conclusions

In conclusion, accounting for data continuity is essential when analyzing time-resolved PTR-ToF-MS datasets. Univariate FDA provides a statistically rigorous framework for peak-level feature selection without multiple testing pitfalls. For multivariate analysis, conventional PCA remains useful for visualizing point-by-point temporal progression, whereas MFPCA successfully isolates overall curve behavior, demonstrating clearer separation among microalgal and starter culture profiles.

This study was carried out within the Interconnected Nord-Est Innovation Ecosystem (iNEST) and received funding from the European Union Next-Generation EU (Piano Nazionale di Ripresa e Resilienza (PNRR) – Missione 4 Componente 2, Investimento 1.5 – D.D. 1058 23/06/2022, ECS00000043).

References

1. C. Happ-Kurz; *Journal of Statistical Software*, 93 (2020), doi: 10.18637/jss.v093.i05
2. M. Mazzucotelli, B. Farneti, I. Khomenko, K. Gonzalez-Estanol, M. Pedrotti, M. Fragasso, V. Capozzi, F. Biasioli; *Green Analytical Chemistry*, 3 (2022), 100041

P44

From insect frass to valuable microalgal biomass: screening species for sustainable omega-3 PUFA production within the ALGINLOOP project

Behixhe Ajdini,¹ Miles Minio,¹ Federico Girolametti,¹ Lorenzo Massi,^{1,2} Anna Annibaldi,¹ Silvia Illuminati,¹ Alessandra Norici,¹ Cristina Truzzi¹

¹ Department of Life and Environmental Sciences, Università Politecnica delle Marche, Via Brecce Bianche, 60131 Ancona, Italy

² Department of Environmental Sciences, Informatics and Statistics, Università Ca' Foscari Venezia, Via Torino 155, 30172 Mestre, Italy

Summary: This research explores the use of *Acheta domesticus* frass as an alternative nutrient source for microalgae cultivation within a circular economy approach. Six microalgal species were evaluated for biomass production, lipid content, and omega-3 PUFA profiles to identify the most promising candidates for future dietary supplementation in *A. domesticus* rearing. Fatty acid composition was determined by gas chromatography-mass spectrometry.

Keywords: insect frass, microalgal cultivation, circular bioeconomy, omega-3 PUFA

Introduction

The increasing demand for sustainable food and feed production is driving the need for alternative strategies to generate nutritionally valuable biomass while improving resource efficiency. Microalgae have emerged as a promising sustainable solution due to their high growth rates, ability to adapt to various environmental conditions, and produce valuable compounds, including proteins, lipids, omega-3 polyunsaturated fatty acids (n3 PUFAs), among others [1]. However, their large-scale application remains limited by high cultivation costs, mainly related to nutrient supply and the use of conventional growth media [2]. The valorization of nutrient-rich waste streams as alternative nutrient sources represents a promising approach to the reduction of algal cultivation costs while improving the environmental sustainability of microalgal systems. In this context, insect farming has emerged as an efficient and sustainable source of protein for food and feed applications, due to its lower environmental footprint and reduced resource requirements compared with conventional livestock production [3]. However, insect rearing generates significant amounts of frass, a by-product consisting of insect excreta, residual feed, and cuticle fragments, whose valorisation has mainly focused on agricultural applications such as organic fertilizer [4]. Recent studies have demonstrated the potential of insect frass as an effective nutrient source for *Chlorella vulgaris* growth [5,6]. However, the potential of frass-grown microalgal biomass as a dietary supplement for insect nutrition remains unexplored.

Within the ALGINLOOP project, financed by CARIVERONA (bando Ricerca e Sviluppo 2025, cod. 2025.0064), this study evaluated the use of frass, derived from the rearing of *Acheta domesticus* as an alternative nutrient source for microalgal cultivation. Six microalgal species, including three marines (*Phaeodactylum tricornutum*, *Nannochloropsis gaditana*, and *Tetraselmis suecica*), and three freshwater strains (*Limnospira platensis*, *Chlorella vulgaris*, and *Euglena gracilis*), were selected based on literature data regarding biomass productivity, lipid content, and omega-3 PUFA production potential. The selected species were cultivated in frass-based media and evaluated in terms of biomass productivity, lipid accumulation, and fatty acid composition to identify the most promising candidates with enhanced n3 PUFAs for future dietary supplementation in *A. domesticus* rearing.

Experimental

Microalgal growth. Culture mediums were prepared by adding 0.8 gL⁻¹ autoclaved insect frass to filtered seawater or deionized water for marine and freshwater species, respectively. The mediums were then sonicated (1h) to increase nutrient dissolution. Cultures of *P. tricornutum*, *N. gaditana*, and *T. suecica* grown in the frass-seawater medium and of *L. platensis*, *C. vulgaris*, and *E. gracilis* were set up in three replicates, and cell density was analyzed with a CASY TT Cell Counter (Innovatis AG) or as optical density spectrophotometrically (UV- 1900i, SHIMADZU CORP. Fourier Transform Infrared spectroscopy (FTIR) was then used to assess the ratios between macromolecular pools in the stationary phase

Lipid extraction and fatty acid analysis. Before analysis, microalgae samples were homogenized and freeze-dried until constant weight. Lipid extraction was carried out with the Folch method, and the mass of extracted lipid was determined gravimetrically. FAs were determined using a gas chromatograph (Agilent-7820) coupled to an Agilent-5977N quadrupole Mass Selective Detector, after their derivatization to Fatty Acids Methyl Esters, using the methyl ester of nonadecanoic acid (19:0, 99.6%, Dr. Ehrenstorfer GmbH, Germany) as internal standard. Analyses were carried out on three aliquots per sample.

Results

Microalgal growth. Algal growth varied among species, with *P. tricornutum* showing the highest cell densities among the tested species (Fig.1.). Biomass productivity instead remained similar among species, as it is also dependent on cell size. *C. vulgaris* showed the lowest productivity, while *L. platensis* the highest (Fig.2.) despite showing poor growth in the frass medium as it reached an early stationary phase.

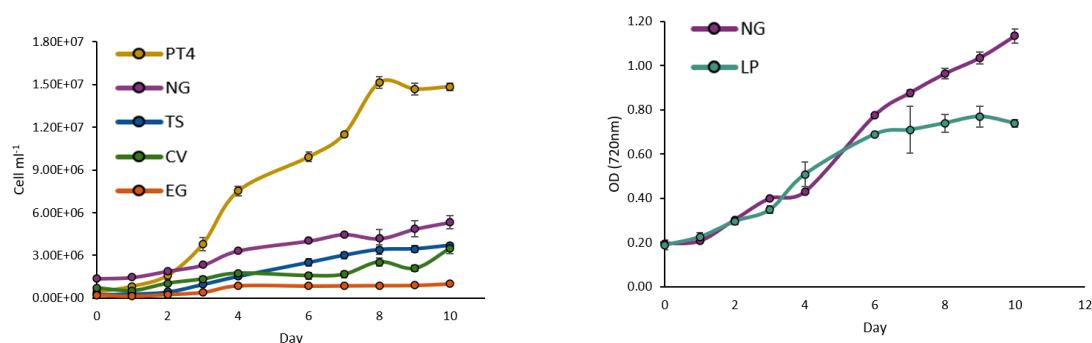


Fig.1. Growth curves of the tested species grown in frass-based medium. The first graph shows the cell densities of *P. tricornutum* (PT), *T. suecica* (TS), *C. vulgaris* (CV) and *E. gracilis* (EG) cultures. In the second graph, growth curves for *N. gaditana* (NG) and *L. platensis* (LP) were measured by their optical density at 720nm.

Total lipid and fatty acid composition. Total lipid content differed considerably among species, ranging from $6.7 \pm 0.9\%$ dry weight (dw) in *L. platensis* to $23.3 \pm 1.5\%$ dw in *E. gracilis*. Intermediate values were observed for *N. gaditana* ($9.5 \pm 1.7\%$ dw), *C. vulgaris* ($12.8 \pm 4.1\%$ dw), *T. suecica* ($12.9 \pm 0.9\%$ dw), and *P. tricornutum* ($14.7 \pm 1.8\%$ dw) (Fig.2.). Regarding the FA composition, preliminary results revealed that the most abundant fatty acids detected in microalgae cultivated on frass-based media were palmitic acid (16:0), stearic acid (18:0), palmitoleic acid (16:1n-7), oleic acid (18:1n-9), linoleic acid (18:2n-6), and α -linolenic acid (18:3n-3). Further investigations will be carried out to assess the content of PUFA, saturated FAs and monounsaturated FAs, with respect to microalgae cultivated on standard medium.

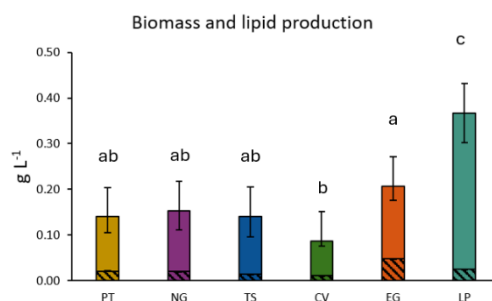


Fig. 2. Biomass and lipid production of the tested species grown in frass medium. The striped portion of the bar represents the lipidic portion of the biomass. *P. tricornutum* (PT), *N. gaditana* (NG), *T. suecica* (TS), *C. vulgaris* (CV), *E. gracilis* (EG), *L. platensis* (LP).

Conclusions

Overall, the preliminary findings suggest that, considering biomass productivity, total lipid and fatty acid composition, microalgae cultivated on frass-based media may represent promising candidates for further investigation as potential dietary supplements for *Acheta domesticus* rearing within a circular production system.

References

1. P. Parmar, R. Kumar, Y. Neha, V. Srivatsan; *Frontiers in Plant Science*, 14 (2023), 1073546.
2. B. Vázquez-Romero, J. A. Perales, et al.; *Science of the Total Environment*, 837 (2022), 155742.
3. B. Ajdini, I. Biancarosa, et al.; *Journal of Insects as Food and Feed*, 10 (2024), pp. 1313–1330.
4. J. Poveda; *Agronomy for Sustainable Development*, 41 (2021), 5.
5. A. Petrucciani, L. Mollo, S. A. Siena, B. Ajdini, et al.; *Bioresource Technology Reports*, 29 (2025), 102339.
6. P. Steinrücken, O. Müller, H. Böpple, D. M. Kleinegris; *Bioresource Technology Reports*, 25 (2024), 101686.

P45

Real-time PTR–ToF–MS tracking of volatile sulfur compound release in food matrices: the role of whole saliva and the oral bacterial enzyme FnaPatB1

Fatemeh Izadfar,^{1,2,3} Michele Pedrotti,² Sylvester Holt,³ Emanuela Betta,² Iuliia Khomenko,² Flavia Gasperi,¹ Franco Biasoli²

¹ Center for Agriculture, Food and Environment (C3A), University of Trento, San Michele all'Adige, Italy

² Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Italy

³ Department of Food Science, University of Copenhagen, Frederiksberg, Denmark

Summary: This study investigates the generation of volatile sulfur compounds from food matrices and cysteine S-conjugates in two complementary experimental systems: microbial C–S lyase activity associated with human whole saliva and the purified enzyme FnaPatB1. Using SPME–GC–MS alongside static and real-time PTR–ToF–MS, baseline profiles and subsequent volatile formation were investigated across four food matrices: aged garlic, shiitake mushroom, enoki mushroom and dried blackcurrant. Static screening revealed significant saliva-associated increases in sulfur-related signals for aged garlic and shiitake, while enoki and blackcurrant showed no comparable changes. Furthermore, real-time PTR–ToF–MS captured the dynamic formation of sulfur-related mass peaks following the addition of purified FnaPatB1 to aged-garlic and shiitake matrices, including signals tentatively assigned to methanethiol and allyl mercaptan in aged garlic. This work provides a framework for real-time tracking of enzyme- and saliva-associated sulfur volatile formation and supports further investigation of how oral microbial enzyme activity may influence sulfur-aroma formation.

Keywords: PTR–ToF–MS; SPME–GC–MS; whole saliva; C–S lyase; cysteine S-conjugates

Introduction

Some foods contain odourless cysteine S-conjugates that can be converted by oral microorganisms into volatile thiols, key contributors to aroma and flavour perception during food consumption [1]. These conjugates can be converted into volatile sulfur compounds by human saliva due to Pyridoxal 5'-phosphate (PLP)-dependent C–S lyase activity associated with the microbial fraction [1,2]. Using S-allyl-L-cysteine (SAC) as a model substrate, Neiers *et al.* demonstrated the microbial formation of volatile allyl-sulfur odorants—such as allyl mercaptan, diallyl sulfide and diallyl disulfide—and identified the enzyme FnaPatB1 from the oral anaerobe *Fusobacterium nucleatum* subsp. *animalis* as one candidate enzyme capable of catalysing this conversion [2]. Beyond its action on SAC, FnaPatB1 exhibits notable substrate promiscuity, demonstrating catalytic activity toward a much broader range of cysteine S-conjugates [2]. However, the total volatile profile generated *in vivo* is more complex, as human saliva harbors a diverse polymicrobial community likely utilizing a cooperative network of multiple enzymes.

The aim of this study is to investigate volatile sulfur compound formation from defined cysteine S-conjugates and selected food matrices in two experimental systems: pooled human whole saliva and purified FnaPatB1. The purified enzyme was included as a defined enzymatic control for whole-saliva C–S lyase activity, while its reaction kinetics were investigated separately using defined cysteine S-conjugates (data not shown). Baseline volatile profiles of the selected food matrices were independently characterized by SPME–GC–MS. In separate static PTR–ToF–MS experiments, saliva-associated changes in sulfur-related headspace ions were assessed by comparing food-only samples with samples incubated with pooled human whole saliva, thereby identifying relevant ion signals for subsequent real-time monitoring. Real-time PTR–MS has previously been used to monitor saliva-driven sulfur volatile formation from a food matrix [3]. In the present study, it is applied to track time-dependent volatile formation after the addition of whole saliva and FnaPatB1 to defined substrates or selected food matrices.

Experimental

Aged garlic, blackcurrant, enoki mushroom and shiitake mushroom were used in dried, powdered form and prepared as aqueous suspensions. Separate aliquots of the food suspensions were analysed by SPME–GC–MS in the absence of saliva to characterize their volatile profiles, following a previously described method [4]. Unstimulated human whole saliva was collected from four donors using the spitting method [5], after they had refrained from eating, drinking and toothbrushing for 2 h. The samples were pooled and used on the day of collection.

For saliva screening, food-suspension aliquots were mixed with pooled saliva at a food suspension:saliva ratio of 1:2. Food-only and saliva-only controls and a water blank were included. Following at least 30 min of incubation in sealed vials at 37 °C, headspace volatiles at the initial sampling point (T0) were measured by

PTR–ToF–MS using H_3O^+ ionization, and signals were expressed in ppbv.

For real-time measurements, selected food matrices in water and buffer were maintained at 37 °C in reaction vials connected to the PTR–ToF–MS under continuous nitrogen flow. After a 5-min baseline, pooled human whole saliva or purified FnaPatB1 was injected, and volatile formation was monitored continuously for another 10 minutes. PLP and the appropriate buffer were included in the purified-enzyme systems.

Results

SPME–GC–MS analysis of the aqueous food suspensions established their baseline sulfur profiles. In terms of sulfur compounds, aged garlic showed several allyl-containing sulfides and polysulfides, including allyl methyl sulfide, diallyl sulfide and diallyl disulfide. Shiitake showed carbon disulfide, dimethyl disulfide and dimethyl trisulfide, together with signals tentatively assigned to 1,2,4-trithiolane and tetrathiane. Dimethyl sulfide was the dominant sulfur peak in blackcurrant, whereas enoki showed only a low dimethyl disulfide peak among the principal sulfur markers.

At T0, 18 of 253 m/z signals in aged garlic and 57 of 205 signals in shiitake were significantly higher in food+saliva than in food-only samples (one-way ANOVA, $p < 0.05$). In aged garlic, the increased signal at m/z 49.026 ($[\text{CH}_4\text{S}+\text{H}]^+$) tentatively identified as methanethiol. In shiitake, increased signals at m/z 76.950 ($[\text{CS}_2+\text{H}]^+$), 124.954 ($[\text{C}_2\text{H}_4\text{S}_3+\text{H}]^+$) and 156.926 ($[\text{C}_2\text{H}_4\text{S}_4+\text{H}]^+$) were consistent with carbon disulfide, 1,2,4-trithiolane and tetrathiane, respectively, which were also detected by the SPME–GC–MS analysis. As representative examples (Fig. 1), m/z 49.026 in aged garlic increased approximately twofold following saliva addition ($p = 0.007$), while m/z 76.950 in shiitake increased approximately 26-fold ($p < 0.001$). Neither enoki nor blackcurrant showed comparable saliva-associated increases in the selected sulfur-related ions.

For aged-garlic samples incubated with purified FnaPatB1, real-time PTR–ToF–MS revealed the formation of the following sulfur-related mass peaks: m/z 49.011 (CH_4SH^+), m/z 75.026 ($\text{C}_3\text{H}_6\text{SH}^+$) and m/z 115.058 ($\text{C}_6\text{H}_{10}\text{SH}^+$), tentatively assigned to methanethiol, allyl mercaptan and diallyl sulfide, respectively. For shiitake mushroom, dynamic measurements revealed the formation of sulfur-containing ions at m/z 63.026 ($\text{C}_2\text{H}_6\text{SH}^+$) and m/z 125.042 ($\text{C}_7\text{H}_8\text{SH}^+$), tentatively assigned to dimethyl sulfide and thioanisole, respectively. These sulfur-containing compounds were formed following the reaction of FnaPatB1 with sulfur-containing precursors present in the aged-garlic and shiitake matrices (data not shown).

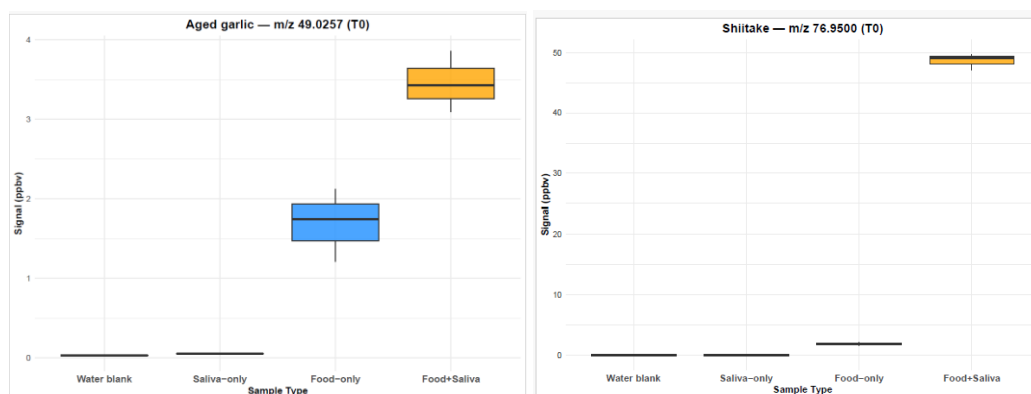


Fig. 1. Boxplots of static PTR–ToF–MS signals at T0 for aged garlic (m/z 49.026) and shiitake (m/z 76.950) across water blank, saliva-only, food-only and food+saliva conditions.

Conclusions

The saliva-associated increases in sulfur-related signals, particularly in aged garlic and shiitake, indicate enhanced volatile sulfur compound formation in the presence of whole saliva and are consistent with microbial C–S lyase activity toward cysteine S-conjugates. No comparable changes were observed in enoki and blackcurrant. PTR–ToF–MS was successfully applied for the first time to monitor in real time sulfur-related volatile formation following the addition of purified FnaPatB1 to both pure substrates (cysteine S-conjugates) and real food matrices (aged-garlic and dried shiitake).

References

1. C. Starkenmann, B. Le Calvé, Y. Niclass, I. Cayeux, S. Beccucci, M. Troccaz; *Journal of Agricultural and Food Chemistry*, 56 (20) (2008), pp 9575–9580, 10.1021/jf801873h.
2. F. Neiers, K. Gourrat, F. Canon, M. Schwartz; *Journal of Agricultural and Food Chemistry*, 70 (32) (2022), pp 9969–9979, 10.1021/acs.jafc.2c01727.
3. D. Frank, U. Piyasiri, N. Archer, J. Jenifer, I. Appelqvist; *Heliyon*, 4 (12) (2018), Article e01045, 10.1016/j.heliyon.2018.e01045.

4. M. Moayedi, M. Pedrotti, I. Khomenko, E. Betta, A. Romano, M. Tonezzer, L. Cappellin, F. Biasioli; *Journal of Fungi*, 12 (7) (2026), Article 477, 10.3390/jof12070477.
5. M. Navazesh; *Annals of the New York Academy of Sciences*, 694 (1993), pp 72–77, 10.1111/j.1749-6632.1993.tb18343.x.

P46

Chewing on flavour: temporal dynamics of aroma and sweetness in sugar-free gum

M. Pedrotti,¹ S. van de Laar,⁶ L. Menghi,² L. Cappellin,³ G. Rinaudo,⁴ M. Roverso,³ S. Bogialli,³ S. Pettenuzzo,³ V. Fogliano,⁵ F. Biasioli¹

¹ Fondazione Edmund Mach, Research and Innovation Centre, Via E. Mach 1, San Michele all'Adige, 38098, TN, Italy

² University of Southern Denmark, Department of Green Technology, Campusvej 55
5230 Odense, Denmark

³ Università degli Studi di Padova, Chemical Science Department, Padova, Italy

⁴ Soremartec Italia srl, Piazzale Ferrero 1, 12051, Alba, CN, Italy

⁵ Wageningen University, Department of Food Quality and Design, P.O. Box 8129,
Wageningen, 6700, EV, the Netherlands

⁶ Independent researcher

Summary: *This study investigated sugar-free chewing gum flavor dynamics using nose-space mass spectrometry, chewing simulators, and dynamic sensory profiling. Increasing aroma concentration boosted volatile release but not sweetness perception. Flavor perception declined faster than actual volatile release, while sweeteners showed compound-specific release kinetics, offering manufacturers formulation strategies to optimize and prolong flavor delivery.*

Keywords: *Flavour perception; Chewing gum; Aroma release; Sweeteners; Proton-transfer-reaction mass-spectrometry; Nose-space analysis*

Introduction

Sugar-free chewing gums (SFG) are prominent confectionery products relying on complex formulations of flavourings and sweeteners to deliver a prolonged consumer experience. Beyond this, SFGs reduce caries increment and lower plaque scores compared to non-chewing controls [1]. Chewing gum is also an ideal model food for exploring the variability in aroma release and perception linked to inter-individual factors [2], as well as product-related characteristics including gum base composition [3], VOCs [4], and the type of sweeteners and polyols used [5]. Although VOCs typically account for only 0.75–3% of the formulation, they play a critical role in determining flavour perception and, consequently, chewing duration and overall liking [6]. While most SFG studies have focused on individual factors, the multimodal interplay between aroma concentration, sweetener release kinetics, and dynamic flavour perception remains poorly understood. Therefore, this study examined how the concentration and release of a “fruity” aroma, as well as the release of different sweeteners from SFG, influence aroma release and flavour perception. Nose-space analysis by Proton Transfer Reaction Mass Spectrometry (PTR-MS) was combined simultaneously with dynamic sensory analysis to characterize *in vivo* aroma release and perception of SFG with five different aroma concentrations. *In vitro* release of sweeteners from one of the samples was investigated by combining an artificial chewing device and ultra high-performance liquid chromatography (UHPLC-HRMS) to evaluate the influence of sweeteners solubility and total sweetener release on flavour perception.

Material and methods

Two experiments utilized tailor-made, uncoated chewing gum samples (gum base, sweeteners, glycerol, citric acid and TF aroma) provided by the industrial partner (Soremartec). The main molecules of TF aroma consisted of ethyl acetate (11%), ethyl butyrate (34%), isobutyl acetate (8%), isoamyl acetate (34%), and vanillin (7%).

In the first experiment, the influences of aroma concentration and physicochemical characteristics of aroma compounds on aroma release and perception were explored. Expert panellists (n = 6) evaluated through dual-attribute discrete time intensity (DTI) the aroma and sweetness intensity of the same chewing gum base containing a fruity aroma (TF: “*tutti frutti*”) in five different concentrations (0.2%, 0.4%, 0.6%, 0.8%, 1.0%). Simultaneously, aroma release was measured by PTR-MS *in vivo* nose-space analysis by using a sampling protocol adapted from a similar study [7]. Samples were tested in triplicates.

In the second experiment, sweeteners release was studied for the sample containing 0.6% TF aroma to evaluate the influence of sweeteners solubility and total sweetener release on aroma perception. Saliva samples were collected using an artificial chewing device, at different time intervals (15, 30, 60, 120, 180, 300 and 420 s). Sorbitol, mannitol, xylitol, maltitol and aspartame were quantified through UHPLC-HRMS in triplicates.

Panel reliability and repeatability were verified through an ANOVA model, following standard procedures for

panel performance evaluation. PTR-MS data were pre-processed and treated according to [2] and to [8]. Sensory and PTR-MS data were then averaged per replicate as a function of Sample and Time and a two-way factorial ANOVA was performed with those as fixed factors, followed by post-hoc tests. Sweeteners release (%) was calculated by multiplying the concentration of each sweetener (in mg/L) by the amount of artificial saliva (15 g) to calculate the release in grams [9] and then dividing by the initial amount of sweetener. The release of each sweetener was calculated for each time interval as follows:

$$R_x = \frac{\Delta S_x}{\Delta t} \quad (1)$$

where ΔS_x represents the difference between two concentrations of a specific sweetener at successive times divided by the time elapsed between the two measurements (Δt). These concentrations in time were then multiplied by their relative sweetness.

Data were analysed in R (v3.6.2) using the *SensoMineR*, *ggplot2*, *ggpubr* and *FactoMineR* packages.

Results

Increasing the TF aroma concentration from 0.2% to 1.0% proportionally raised *in vivo* nose-space volatile concentrations, though a perceptual plateau occurred beyond the 0.4% threshold where perceived intensity ceased to significantly increase. Interestingly, these aroma fluctuations did not alter dynamic sweetness perception, suggesting that cross-modal sweetness enhancement depends on the absolute presence of a congruent aroma rather than minor concentration shifts.

A distinct temporal divergence emerged between sensory and instrumental data: perceived aroma intensity peaked within the first 2 min and fell rapidly, whereas instrumental nose-space analysis indicated a much more gradual decline. This behavior was influenced by compound-specific physics, where low-molecular-weight, hydrophobic esters experienced an early burst peaking at 60 s, while the more polar vanillin exhibited a delayed release peaking at 120 s due to hydrogen-bonding interactions with saliva and the gum base.

Sweeteners followed a distinct biphasic release profile marked by an initial rapid burst during the first 120 s, transitioning into a sustained delivery phase that ultimately released an average of 33% of the total sweetener content after 8 min. The highly water-soluble bulk sweeteners dissolved rapidly upon contact with artificial saliva, with xylitol achieving the highest relative release percentage at 58.4% and sorbitol providing the highest absolute concentration. Conversely, maltitol demonstrated a slower release rate than mannitol due to its higher molecular weight and stronger matrix interactions. High-intensity sweetener dynamics differed significantly, as aspartame exhibited the slowest release kinetics and lowest absolute quantity.

A perceptual incongruity between the physical presence of flavor and its actual perception, particularly after 120 s, was noticed. While perceived sweetness began to decline after 2 min, the calculated relative sweetness in saliva peaked at 3 min, driven by the delayed surge of aspartame.

The premature decline in perceived sweetness is likely driven by central sensory adaptation—where the brain attenuates to a constant stimulus—alongside cross-modal interactions, meaning the rapidly fading aroma perception simultaneously drags down the perceived sweetness.

Conclusions

By coupling PTR-TOF-MS nose-space analysis, DTI sensory evaluation, and *in vitro* UHPLC- HRMS liquid chromatography, this research establishes a comprehensive framework for chewing gum formulation. The insights suggest that confectionery manufacturers should optimize aroma concentrations to maximize the initial sensory burst, while carefully altering sweetener dissolution rates to counteract cognitive adaptation and extend overall flavor longevity.

References

1. D. Ramasubbu, B. Duane; *Evidence-Based Dentistry*, 25 (2) (2024), pp. 89-90, 10.1038/s41432-024-01018-2
2. M. Pedrotti, A. Spaccasassi, F. Biasioli, V. Fogliano; *Food Research International* (2019), 10.1016/j.foodres.2018.12.019
3. M. Alaçam, M. Çinsar, R. Gunes, E. Bölük, D.S. Atik, I. Palabiyik; *Food Chemistry*, 442 (2024), Article 138486, 10.1016/J.FOODCHEM.2024.138486
4. R.V. Potineni, D.G. Peterson; *Journal of Agricultural and Food Chemistry*, 56 (9) (2008), pp. 3260-3267, 10.1021/jf0727847
5. S. Raithore, D.G. Peterson; *Food Chemistry*, 253 (2018), pp. 293-299, 10.1016/J.FOODCHEM.2018.01.123
6. R.W. Hartel, J.H. von Elbe, R. Hofberger (Eds.), *Springer International Publishing* (2018), pp. 393-420, 10.1007/978-3-319-61742-8_14

7. A. van Eck, M. Pedrotti, R. Brouwer, A. Supapong, V. Fogliano, E. Scholten, F. Biasioli, M. Stieger; *Journal of Agricultural and Food Chemistry*, 69 (35) (2021), pp. 10260-10271, 10.1021/acs.jafc.1c02649
8. M. Charles, A. Romano, S. Yener, M. Barnabà, L. Navarini, T.D. Märk, F. Biasioli, F. Gasperi; *Food Research International*, 69 (2015), 10.1016/j.foodres.2014.11.036
9. A.J. Krause, L.S. Henson, G.A. Reineccius; *Flavour and Fragrance Journal*, 26 (1) (2011), pp. 47- 54, 10.1002/ffj.2015

P47

Assessment of phthalate migration from reusable plastic water bottles into drinking water

Claudia Lino,¹ David Bongiorno,¹ Serena Indelicato,¹ Giuseppe Avellone^{1,2}

¹ Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche, Università di Palermo, via Archirafi 32, 90123 Palermo (Italy)

² ATeN Center, Università di Palermo, viale delle Scienze, 90128 Palermo (Italy)

Summary: *This study evaluated phthalate migration from reusable plastic water bottles into drinking water using SPME-GC/MS. DEHP was the predominant phthalate, while considerable variability in phthalate levels was observed among bottle samples. However, all measured phthalate concentrations remained below current regulatory limits.*

Keywords: *phthalates, reusable plastic bottles, SPME-GC/MS*

Introduction

The increasing adoption of reusable plastic water bottles as sustainable alternatives to single-use packaging has significantly changed consumer habits, particularly for daily hydration and sports activities. However, repeated use of these materials may promote chemical migration into beverages due to prolonged contact, mechanical stress, temperature variations, and polymer aging. Among the substances of concern, phthalate esters (PAEs) are widely used as plasticizers to improve polymer flexibility and are recognised as endocrine-disrupting chemicals with potential adverse effects on human health. Since phthalates are not chemically bound to the polymer matrix, they can migrate into aqueous matrices over time, representing a potential source of human exposure [1]. Although European regulations [2] define specific migration limits for phthalates in food-contact materials, limited information is available regarding their release from commercially available reusable water bottles under realistic conditions of use [3]. Therefore, this study aimed to investigate phthalate migration from different reusable plastic water bottles into drinking water using a sensitive and solvent-free DI-SPME-GC/MS analytical approach and to evaluate potential exposure risks associated with their use.

Experimental

Eight commercially available reusable plastic water bottles made of different polymeric materials (LDPE, HDPE, PP, PETG, PCTG, and other mixed materials) were investigated. Bottles were filled with drinking water and stored at room temperature in the dark. Samples were analysed after 6, 12, 24, and 48 h of contact. Phthalates were extracted by direct immersion solid-phase microextraction (DI-SPME) using a DVB/CAR/PDMS fiber and analyzed by GC/MS. Eleven phthalates were investigated, including DEHP, DBP, DiBP, DPP, BBP, DCHP and other higher molecular weight compounds. Migration profiles were evaluated through statistical analyses, including correlation and regression models, while consumer exposure was estimated through risk quotient (RQ) and excess lifetime cancer risk (ELCR) calculations.

Results

The DI-SPME-GC/MS method demonstrated suitable analytical performance, with good linearity, sensitivity, precision, and accuracy for phthalate determination in drinking water samples [4,5]. Eleven phthalates were detected, confirming the migration of these compounds from reusable bottles into the aqueous phase. Total phthalate concentrations ranged from 0.27 to 9.35 µg/L, with substantial variability among samples and contact times. Differences were observed not only in total concentration but also in the qualitative composition of migrating compounds, suggesting the influence of polymer formulation, additive content, manufacturing processes, and possible contributions from ancillary components.

Among the investigated compounds, DEHP was the most frequently detected phthalate and the predominant contributor to total phthalate levels, explaining approximately 63% of the observed variability. HDPE and LDPE bottles exhibited higher average concentrations compared with PP and PETG bottles, although polymer type alone was not sufficient to explain migration behavior. Statistical analysis indicated that contact time did not significantly influence phthalate levels, while differences among bottles approached statistical significance, highlighting the importance of product-specific characteristics.

Despite measurable phthalate migration, all detected concentrations remained below current European regulatory limits. Risk assessment showed that estimated daily intakes were considerably lower than tolerable daily intake values, with RQ values below 1 for both adults and toddlers. Furthermore, calculated ELCR values indicated negligible carcinogenic risk associated with DEHP exposure through drinking water.

Conclusions

This study demonstrates that reusable plastic water bottles can release measurable amounts of phthalates into drinking water under realistic storage conditions. The DI-SPME-GC/MS approach allowed the identification of eleven phthalates, highlighting considerable variability among commercial products in both concentration levels and migration profiles. DEHP was the predominant contaminant and the main contributor to total phthalate variability.

Although migration was observed, all detected concentrations remained below current regulatory limits and risk assessment indicated negligible health risks for adults and toddlers. These findings highlight the importance of considering product-specific characteristics in the evaluation of chemical migration from reusable food-contact materials and support further investigation of long-term use conditions.

References

1. A. Giuliani, M. Zuccarini, A. Cichelli, H. Khan, M. M. Reale; *International Journal of Environmental Research and Public Health* 17 (2020): 5655.
2. European Commission, "Commission Regulation (EU) No 10/2011 of 14 January 2011 on Plastic Materials and Articles Intended to Come Into Contact With Food," *Official Journal of the European Union* L, no. 12 (2011): 1–89.
3. Tisler, S.; Christensen, J.H. *J. Hazard. Mater.* 2022, 429, 128331.
<https://doi.org/10.1016/j.jhazmat.2022.128331>.
4. C. Lino, S. Indelicato, D. Bongiorno, F. D'Agostino, G. Avellone, *Journal of Mass Spectrometry*, 2026; 61:e70089
5. C. Lino, S. Indelicato, D. Bongiorno, F. D'Agostino, S. Indelicato, G. Avellone; *Beverages* 11 (2025): 92.

P48

Investigation of inorganic elements in insect-based novel foods by ICP-MS

M. Ciriaci, S. Mazza, F. Griffoni, P. Palombo, M. Siracusa, T. Tavoroni, C. Barola, A. Stramenga, S. Bacchiocchi, A. Piersanti

Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", Ancona- Perugia, Italy

Summary: Edible insects are promising food sources but may accumulate environmental contaminants. An ICP-MS method was developed for the determination of 30 inorganic elements in insect-based novel foods. Toxic profiles showed negligible Hg concentrations and species-dependent Cd and Ni variability. No significant differences were observed between products intended for feed and food applications.

Keywords: Novel food, ICP-MS, inorganic elements

Introduction

Insects have emerged as potential food source for human and livestock facing the growing interest for alternative sources of high-quality proteins. Insect farming requires, in fact, a small amount of space and water. Edible insects are a good source of proteins, fatty acids, vitamins and minerals, with nutritional profile species-specific [1]. Between June 2021 and January 2023, European Regulations were issued for the authorisation to place on market 6 novel foods for human use, with some restrictions such as maximum levels for lead and cadmium. Four insect species are permitted: *Acheta domesticus* - house cricket, *Alphitobitus diaperinus* - lesser mealworm, *Tenebrio molitor* - mealworm and *Locusta migratoria* - locust [2]. Although data are still limited, edible insects can be vectors of microbiological agents and environmental contaminants (e.g. heavy metals, dioxins, PCBs, mycotoxins) [3]. Attention must also be paid to the allergic properties of foods containing insects, being arthropods, very similar to crustaceans [15]. Therefore, official control plans should consider the potential risks for human health associated with this food.

The aim of the present work was to set up an ICP-MS method to monitor regulated and not regulated inorganic contaminants and assess their nutritional profile in terms of toxic, essential, non-essential and macro-elements. The method was then applied to samples harvested in the European market.

Experimental

Analytical method. Inorganic elements in insect samples were analysed by high-pressure microwave mineralization and ICP-MS, using an already implemented procedure [4]. The method application was extended in order to include up to 30 elements (Al, Sb, Ag, As, Ba, Be, Cd, Ca, Co, Cr, Fe, Mg, Mn, Hg, Mo, Ni, Pb, K, Cu, Se, Na, Sn, Sr, Ti, U, V and Zn), plus B, P, S, 3 elements deemed relevant for the study.

Method validation. Method validation was performed spiking insect flour at the limit of quantification (LOQ) and at other concentration levels to cover the entire measurement field. Performances in terms of accuracy, repeatability and uncertainty were assessed

Sampling. A total of 31 samples (frozen, dried, or powdered whole insects), representing only 3 species authorized for human use, were obtained from both Italian and European e-commerce platforms. *A. domesticus* was readily available in a variety of product, whereas *T. molitor* and *A. diaperinus* were considerably more difficult to source. *L. migratoria* was particularly challenging to obtain, being currently marketed almost only as animal feed ingredient. Although the project was originally designed to include only products intended for human consumption, the limited commercial availability implied that also insects for feed production (19) were included in the monitoring.

Results

The validated ICP-MS method exhibited good performances: recoveries ranging from 70 to 124%, mean intra-laboratory reproducibility of 7% ranging from 2-28, depending on the element and spiking level; and average uncertainty of 20% varying with the considered element and with spiking level. The procedure provides a reliable tool for the simultaneous determination of the 30 different elements in insects and insects derivatives. The method applied to the samples retrieved from the European market showed that most of the elements stood close or below the method LOQs (As, Hg, Ag, Be, Sn, Ti, U, Co, Se, Mo, V, Cr, Sn Sb, B. Cd and Pb concentrations ranged between 0,007-0,11 mg/kg (median: 0,052±0,029) and 0,004-0,68 mg/kg (median: 0,053±0,119) respectively, in some samples of *domesticus*-based products, exceeding the limits set in the regulations authorizing the placing on market of insect based food. The same exceedance was already reported in literature [5]. Insect-based products intended for animal feed showed toxic element levels comparable to those found in product intended for human consumption. Since no specific maximum limits exist for insect-based product those applied to feed in general were used. All contamination levels were below these

limits, indicating a wider safety of products for animal feed. Essential elements (e.g. Zn, Fe, and Cu), together with macro-elements (e.g. Na, K, P, S) were consistently abundant in agreement with literature [1], confirming the insect-based product high nutritional value. *A. domesticus* generally showed higher concentrations of Al, Cd and Pb, whereas *T. molitor* and *A. diaperinus* had lower levels of toxic elements, while maintaining comparable concentrations of minerals (Fig.1). Hg concentrations were negligible in all the species. Anyway, given the limited number of samples available for *T. molitor* and *A. diaperinus* and the species- dependent variability for Cd and Ni, comparisons between the species should be considered very preliminary [6].



Fig.1. Toxic, essential, non-essential and macro-elements levels in the 3 species of insect samples analyzed.

Conclusions

These findings support the implementation of routine monitoring to ensure consumer safety while promoting the sustainable use of insects as alternative protein sources.

References

1. B.A. Rumpold et al., *Innovative Food Science & Emerging Technologies*, 17 (2013), pp 1-11. 2.Reg. EU N° 58/2023, *Off. J. Eur. Union*, L5 (2023).
3. B. M. Kolakowski et al. *Journal of Food Protection*, 84 (2021), pp1575-1581.
4. T. Tavoloni et al., *Food Additives & Contaminants : Part A*, 42:6 (2025), pp 746-757.
5. L.C. Ramos et al., *Foods*, 13:3233 (2024).
6. M. Ciriaci et al., *Food Control*, 178:111505 (2025).

Research funded by the Italian Ministry of Health (Ricerca Corrente 2023, IZS UM 02/23 RC)

P49

In Silico Assessment of the Potential Allergenicity of Purple Phototrophic Bacteria Biomass

Beatrice Aiuto,¹ Cristina Lamberti,¹ Simona Cirrincione,¹ Loredana Tarraran,¹ Antonella Leone,² Veronica Lattanzio,³ Laura Cavallarin¹

¹ CNR-ISPA Torino, Italy

² CNR-ISPA Lecce, Italy

³ CNR-ISPA Bari, Italy

Summary: A DIA-based proteomics workflow was developed to characterize four purple phototrophic bacteria and generate curated protein sequence datasets for in silico allergenicity assessment. Sequence comparisons identified similarities with known plant food allergens, highlighting candidate proteins for further investigation and supporting a weight-of-evidence approach to PPB safety evaluation.

Keywords: Purple phototrophic bacteria; Mass spectrometry; Food safety

Introduction

Purple phototrophic bacteria (PPB) are metabolically versatile microorganisms capable of converting different organic substrates into biomass rich in proteins and bioactive compounds. Within the European Purple4Life project (<https://purple4life.eu>), four PPB species were selected for their potential to produce biomass enriched in coenzyme Q10 and carotenoids. Their high protein content and attractive amino acid profile make PPB promising sustainable ingredients for food and feed applications. However, before their introduction into the food chain, their safety must be thoroughly evaluated, including the potential allergenicity of their protein fraction [1]. This study aimed to establish a proteomics guided workflow based on Data Independent Acquisition (DIA) for the identification of PPB protein groups and the generation of curated sequence datasets for in silico allergenicity assessment.

Experimental

Protein extracts were obtained from the biomass of *Cereibacter sphaeroides*, *Rhodobacter capsulatus*, *Rhodopseudomonas palustris*, and *Rhodospirillum rubrum* and analysed by untargeted liquid chromatography high resolution tandem mass spectrometry (LC-HRMS/MS) using a Q Exactive Orbitrap mass spectrometer. Protein identification was performed against species-specific UniProt databases. DIA-NN reports were processed in R by applying a 1% false discovery rate at peptide and protein group levels (Q.Value and PG.Q.Value ≤ 0.01). Only protein groups supported by at least two unique peptides were retained. The non redundant UniProt accessions associated with the retained protein groups were subsequently matched to combined reviewed and unreviewed species-specific FASTA databases. The corresponding full-length amino acid sequences were retrieved to generate the PPB query datasets. To compare PPB proteins with known allergens and assess their potential allergenic relevance, NCBI protein accession numbers were selected from the subset of plant food allergens available in AllergenOnline [3]. The corresponding sequences were retrieved from NCBI and assembled into a custom reference FASTA database. An R-based workflow generated overlapping 80 amino acid windows from each bacterial protein and compared them with the allergen database using FASTA36. Matches were retained when sequence identity exceeded 35% across the complete 80-amino-acid window [2,3].

Results

The proteomic analysis identified 688 high-confidence protein groups for *C. sphaeroides*, 1250 for *R. capsulatus*, 617 for *R. palustris*, and 1090 for *R. rubrum*. The R-based sequence comparison identified bacterial proteins displaying sequence similarity above the predefined threshold to known plant food allergens. These matches were considered indicators of potential IgE cross-reactivity and were used to prioritize candidate proteins for further evaluation, rather than as evidence that the bacterial proteins are allergenic.

Conclusions

This work provides the analytical foundation for a weight-of-evidence assessment of PPB protein allergenicity. The analysis identified sequence similarities between proteins from the PPB strains and selected plant food allergens, highlighting candidate proteins that will be further investigated through complementary studies. The integration of high-confidence experimental proteomics and bioinformatic sequence comparison represents a new approach methodology consistent with the principles of Next Generation Risk Assessment. The proposed workflow may support hazard identification and provide a transferable strategy for the preliminary safety

evaluation of PPB biomass and other microbial protein ingredients intended for food and feed applications.

References

1. EFSA GMO Panel, Naegeli H., Bresson J.-L., Dalmay T., Dewhurst I.C., Epstein M.M., et al. (2022). Scientific Opinion on development needs for the allergenicity and protein safety assessment of food and feed products derived from biotechnology. *EFSA Journal*, 20(1), 7044. doi: 10.2903/j.efsa.2022.7044.
2. EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens), Turck, D., Bohn, T., Castenmiller, J., et al. (2024). Guidance on the scientific requirements for an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283. *EFSA Journal*, 22(9), e8961.
3. Goodman R.E., Ebisawa M., Ferreira F., Sampson H.A., van Ree R., Vieths S., et al. (2016). AllergenOnline: A peer-reviewed, curated allergen database to assess novel food proteins for potential cross-reactivity. *Molecular Nutrition & Food Research*, 60(5), 1183–1198. doi: 10.1002/mnfr.201500769.

P50

Determination of Volatile Organic Compounds (VOCs) released from plastic packaging for dry foods: headspace analysis (HS-GC-MS) and migration studies using Tenax® as food simulant

Alessandra Como, Carmen Palermo Diego, Centonze, Cristina Nerin

¹ Università di Foggia, Italy

² Universidad de Zaragoza, Spain

Summary: To assess the potential transfer of substances from packaging to food, migration tests were carried out using Tenax®, the official food simulant for dry foods under Regulation (EU) No. 10/2011. Volatile compounds were identified by GC-MS analysis both in headspace with Arrow technique and with direct injection with migration extracts.

Keywords: Volatile Organic Compound (VOCs); Food Contact Material (FCM); Migration

Introduction

Food packaging plays a fundamental role in preserving product quality and extending shelf- life; however, plastic materials in direct contact with food (food contact materials, FCMs) may release residual volatile organic compounds (VOCs) originating from polymerization processes, thermal degradation, or the use of additives and printing inks [1, 2].

In this study, the volatile profile of plastic packaging intended for three dry food matrices of commercial interest was characterized: a meat seasoning (rich in spices and lipids), grated coconut (high in fat) and gluten-free chickpea flour (characterized by a high protein and starch content). To assess the chemical safety of the packaging and the potential transfer of substances from the material to the food, migration tests were carried out on each product. Given the hydrophobic and solid nature of the analysed matrices, Tenax® (poly-2,6-diphenylphenylene oxide) [3], the official dry-food simulant (Simulant E) according to EU Regulation 10/2011 [4], was used.

Volatile compounds from packaging were identified by headspace analysis using Arrow technology coupled to GC-MS, while migration test extracts were analysed by direct injection into GC-MS.

Experimental

VOCs were analysed by HS-SPME/GC-MS using A PAL RSI 85 autosampler Series 2 was used with a gas chromatograph 8890 GC System coupled to a mass spectrometer (5977C GC/ MSD) from Agilent Technologies (California, USA). Chromatographic separation was performed out using a HP-5MS column from Agilent (30 m x 0.25 mm i.d. and 0.25 µm film thickness). The inlet temperature was set at 250°C in splitless mode and ultra inert liners (2 mm id for DVB/CAR/PDMS SPME Arrow injection) were used with a fiber desorption time of 8 min. The oven temperature was as follows: 40°C hold for 3 min, raised to 300°C at 8°C/min, and held for 5 min.

As described in the European Commission (EU) 10/2011 [4], the migration assay is made in contact between the surface of the material employing food simulators. In this way, the samples were in contact with simulant E (tenax, dry foods) for 10 days at 60 °C.

The migration analysis was performed by direct injection into GC-MS of the ethanol extract, obtained after three consecutive extractions with ethanol 95% for 30 min each in an ultrasonic bath. Chromatographic analyses for migration tests were carried out under the same operating conditions as previously described.

For retention index calculation, a series of n- alkanes was injected under the same chromatographic conditions. For the quantitative analysis, standard solutions at appropriate concentration levels were used.

Chromatographic data were processed using MS- DIAL software version 4.9 in combination with the NIST 20 library for peak deconvolution, alignment, and identification.

Results

Qualitative screening by HS-SPME/GC-Arrow identified several volatile compounds of interest in the packaging samples intended for grated coconut, meat seasoning, and chickpea flour, including: diisobutyl phthalate (DIBP), a phthalate-class plasticiser subject to restriction under Regulation (EU) No 10/2011; 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (TXIB), a plasticiser widely used in printing inks and varnishes and a known migrant into packaged foods; and butylated hydroxytoluene (BHT), a volatile antioxidant used to counteract the thermo- oxidative degradation of polymeric matrices.

Specific migration tests, carried out according to Regulation (EU) No 10/2011 using Tenax® as the solid simulant, identified acetyl tributyl citrate (ATBC, CAS 77-90-7, tR 23.0-23.8 min) as the main migrant in all analysed samples (absent in the procedural blank). This citrate-class plasticiser was identified with a high

spectral match quality (82-91%). Its consistent presence across all matrices suggests a common origin from the polymeric layer or the coupling adhesive of the multilayer packaging.

Conclusions

These preliminary findings confirm the release of plasticisers and antioxidants from the packaging materials examined, with ATBC identified as the most relevant and ubiquitous migrant. Although ATBC is generally regarded as a "green" and safer alternative to conventional phthalates, its high migration into dry-food simulants warrants careful monitoring. Completion of the study — through quantification of the identified compounds and comparison with the specific migration limits (SML) set by current legislation — will allow the compliance of the examined packaging to be assessed and will clarify the actual impact of these transfer phenomena on the chemical-toxicological safety and sensory quality of the target dry foods, providing useful indications for optimising higher-barrier packaging solutions.

References

1. J. N. Hahladakis, C. A. Velis, R. Weber, E. Iacovidou, P. Purnell, *Journal of Hazardous Materials*, 344, (2018), pp 179-199.
2. C. Nerín, B. Faust, T. Gude, C. Lesueur, T. Simat, A. Stoermer, E. Van Hoek, O. Peter, *Food additives & contaminants, A*, 39:3, (2022) pp 620-643
3. M. Aznar, C. Domeño, J. Osorio, C. Nerin, *Food Packaging and Shelf Life*, 26, (2020), 100552, pp 1-8
4. Consolidated text: Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food (Text with EEA relevance), Document 02011R0010-20260223, <http://data.europa.eu/eli/reg/2011/10/2026-02-23>

P51

Land snails as terrestrial sentinel organisms for monitoring heavy metal and phthalate accumulation

Chiara Marchese, Rosa Avolio, Silvia Olivieri, Roberta Moriano, Paola Brizio, Stefania Squadrone, Maria Cesarina Abete

Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle D'Aosta (IZS PLV), Turin, Italy

Summary: *Farmed and wild Helix pomatia and Cornu aspersum were investigated as terrestrial sentinel organisms for heavy metals and phthalates. ICP-MS revealed bioaccumulation of essential and non-essential trace elements, while GC-MS/MS detected no phthalates, suggesting physiological purging. Findings support snail-based environmental monitoring and warrant further xenobiotic metabolism research in One Health.*

Keywords: snails, ICP-MS, GC-MS/MS

Introduction

Within a One Health framework, terrestrial snails represent promising sentinel organisms for monitoring environmental contamination due to their limited mobility, sensitivity to environmental changes, and ability to accumulate xenobiotics [1]. *Helix pomatia* and *Cornu aspersum* may provide valuable information on the occurrence of contaminants in terrestrial ecosystems. Recent evidence suggests that microplastics can act as vectors for environmentally and health-relevant contaminants, including metals and phthalates [2]. However, data on the accumulation of these contaminants in the tissues of terrestrial gastropods remain limited and the potential elimination of these contaminants through physiological processes such as purging has been largely unexplored. Addressing these knowledge gaps may strengthen the role of terrestrial snails as bioindicators of environmental contamination.

The aim of this study was to generate new insights into the bioaccumulation potential and possible elimination pathways of heavy metals and phthalates in farmed and wild specimens of *Helix pomatia* and *Cornu aspersum*.

Experimental

Samples of *Helix pomatia* and *Cornu aspersum* were analyzed for trace elements and phthalates determination. Metals were extracted by microwave-assisted acid digestion and quantified using inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7800), calibration curves were prepared from standard solutions of known concentrations. Extraction for Phthalic acid diesters quantification (DMP, DEP, DnBP, BBP, DEHP, and DnOP) was performed by a modified QuEChERS protocol with acidified acetonitrile, followed by dispersive solid-phase extraction (d-SPE) clean-up. Compound identification and quantification were performed by gas chromatography coupled with tandem mass spectrometry (GC-MS/MS; Thermo Scientific TSQ 9610).

Results

Copper, iron, manganese, and zinc exhibited the highest concentrations among essential elements. While specific limits for terrestrial snails do not exist, a few samples exceeded regulatory maximum limits established for similar marine species for cadmium, whereas lead levels remained largely below the threshold. Indium, bismuth, and beryllium remained below the quantification limit across all samples, while some specimens have shown consistent levels of aluminium and barium. Phthalates can serve as chemical tracers for microplastics in environmental matrices; however, preliminary results did not detect them in snail tissues maybe due to potential elimination through physiological processes such as purging.

Conclusions

These preliminary findings demonstrate the viability of using *Helix pomatia* and *Cornu aspersum* to monitor essential and non-essential trace elements. While heavy metal bioaccumulation was clearly observed, the absence of detectable phthalates points to potential clearance via physiological purging, highlighting the need for further investigation into their metabolic processing of organic xenobiotics.

References

1. Gerlach, J., Samways, M., & Pryke, J. (2013). Terrestrial invertebrates as bioindicators: An overview of available taxonomic groups. *Journal of Insect Conservation*, 17(4), 831–850. <https://doi.org/10.1007/s10841-013-9565-9>
2. Yuan, L., Zhou, L., & Li, J. (2024). Effect of microplastics on the allelopathic effects of native and invasive plants on co-occurring invaders. *Frontiers in Plant Science*, 15. <https://doi.org/10.3389/fpls.2024.1425815>

P52

Evaluation of SPME fibers and extraction salts for the volatilomic profiling of paprika using a chemometric approach

Alessandra Como, Diego Centonze, Carmen Palermo

Università di Foggia, Italy

Summary: An HS-SPME/GC-MS method for paprika volatilomics was optimised using chemometric approaches, including Central Composite Design (CCD), to evaluate the extraction capacity of three SPME fibres and different salts. The optimised method was then applied to analyse paprika samples.

Keywords: HS-SPME/GC-MS, salting-out, chemometrics, volatilomics, paprika

Introduction

Solid-phase microextraction (SPME) coupled with gas chromatography-mass spectrometry (GC-MS) is a prominent technique for extracting volatile organic compounds (VOCs) from complex food matrices [1]. However, extraction efficiency strongly depends on multiple variables, primarily the chemistry of the fibre coating and the ionic strength of the sample. To systematically evaluate these and other parameters, multivariate chemometric strategies, such as Central Composite Design (CCD), provide a valuable statistical framework for analytical method development [2]. This study aims to optimise an HS-SPME/GC-MS protocol using a chemometric approach to evaluate various SPME fibres and select the most suitable salting-out agents. The influence of key parameters was assessed in terms of total peak area and the total number of chromatographic peaks, as well as their effects on specific paprika compounds. The method was subsequently applied to a representative set of paprika samples.

Experimental

The optimisation of the HS-SPME/GC-MS method was conducted using a chemometric approach. To evaluate and select the optimal extraction phase, three fibres with different polarities (CAR/PDMS, DVB/PDMS, and DVB/CAR/PDMS) were investigated. A Central Composite Design (CCD) was used to model the extraction efficiency, optimising key variables such as extraction time, temperature, and sample amount. Subsequently, the salting-out effect was evaluated by testing four different salts (KCl, MgSO₄, NaCl, and Na₂SO₄) to assess their impact on the various VOC classes. Chromatographic separation was performed on a J&W HP-INNOWax column (60 m, 0.25 mm, 0.25 µm).

Finally, the optimised analytical procedure was applied to characterise the paprika samples.

Results

The use of CCD models and response surfaces allowed the extraction parameters to be optimised for the three fibres evaluated, highlighting significant interactions between temperature and time, as well as between time and sample quantity (Fig. 1). The method showed high robustness and sensitivity in delineating the volatile profiles of the samples and in identifying different VOCs characteristic of paprika.

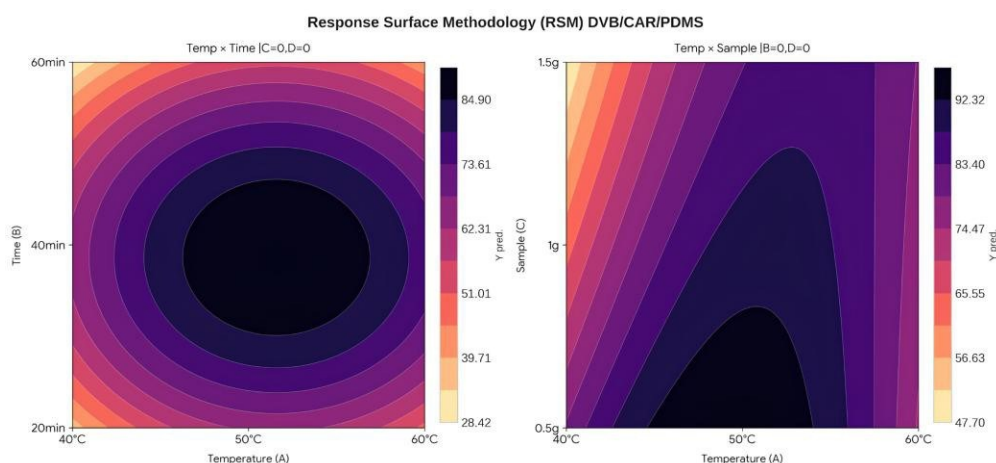


Fig. 1. Response surface plots illustrating the interactive effects of extraction temperature (°C), extraction time (min), and sample amount (g) on the total area of VOCs.

The radar graph (Fig. 2) highlights the differences between SPME fibres: the DVB/CAR/PDMS fibre guarantees the widest and most balanced profile across the different chemical classes, whereas CAR/PDMS and DVB/PDMS show selectivity linked to the polarity of the coating and the class of volatile compounds. A certain complementarity between the types of fibres emerged: DVB/PDMS, for example, preferentially extracted the semi-volatile C12–C18 (e.g. methyl palmitate, methyl tetradecanoate, 5,9-undecadien- 2-one) compared to the other two SPME fibres. The evaluation of different salting-out agents further improved the analysis of paprika volatile compounds.

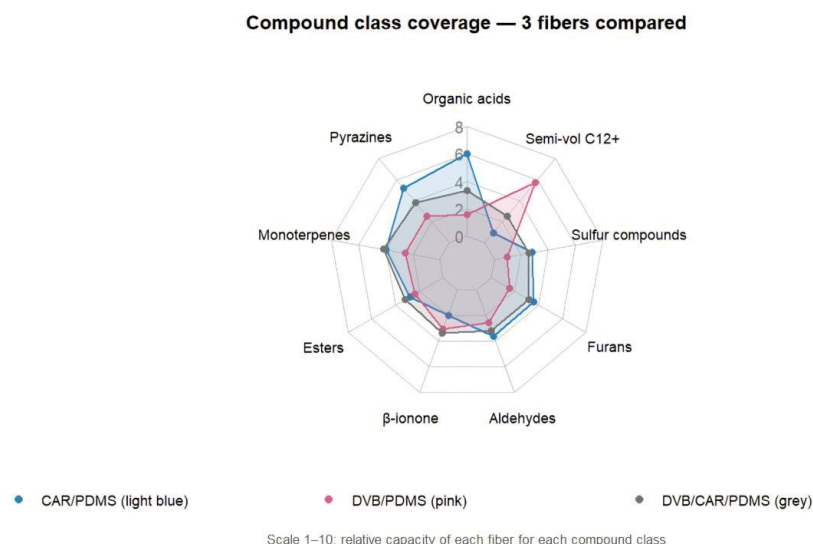


Fig. 2. Comparison of extraction performance across chemical classes of paprika volatile compounds using three SPME fibres.

Conclusions

A chemometric approach enabled the optimisation and validation of an HS-SPME/GC-MS method for paprika analysis. The systematic comparison of fibre coatings and salting-out agents ensured maximum extraction efficiency. The application of this procedure to paprika samples of different origins confirms its analytical validity and potential as a tool for authenticity and traceability studies in spices.

References

1. A. N. Johnson, S. Y. S. S. Adade, J. N. Ekumah, B. A. Kwadzokpui, J. Y. Xu, Q. Chen, *Critical Reviews in Food Science and Nutrition*, 65(30), (2025), pp 7225–7250.
2. Palermo, C.; Mentana, A.; Tomaiuolo, M.; Campaniello, M.; Iammarino, M.; Centonze, D.; Zianni, R., *Foods* 2024, 13, 416.

P53

Effect of *Vitis vinifera* leaf enrichment on the volatile profile and sensory properties of grape juice

Cristina Matarazzo,^{1,2} Massimo Iorizzo,¹ Livia Malorni,² Rosaria Cozzolino ²

¹ University of Molise, Department of Agriculture, Environmental and Food Sciences, Campobasso, Italy

² ISA-CNR, Institute of Food Science, Avellino, Italy

Summary: This study investigated grapevine leaf extract as a sustainable ingredient for functional grape juice. Enriched juice showed significant changes in volatile composition, particularly increased terpenes and C. alcohols. VOC-sensory correlations identified compounds influencing sweetness and sourness. Results demonstrate the potential of grapevine leaves to enhance beverage quality and value.

Keywords: *Vitis vinifera*; Volatile organic compounds (VOCs); HS-SPME/GC-MS

Introduction

Agricultural by-products are increasingly recognised as valuable resources due to their high content of bioactive compounds and functional components. Among these, grapevine leaves remain largely underutilized despite their abundance and high content of bioactive compounds with antioxidant activity. Previous studies have demonstrated that the leaves of *Vitis vinifera* are rich in carotenoids, tocopherols, phytosterols, and polyphenols [1]. However, information on the combined effects of grapevine leaf supplementation on phytochemical composition, volatile profile, sensory properties and storage stability of grape juice remains limited.

In light of this, the aim of the present study was to develop a novel health-promoting beverage by blending grape juice with *V. vinifera* leaf extract. Given the importance of flavour and aroma in determining consumer acceptance of new food products, the Volatile Organic Compounds (VOCs) of the beverages were analysed, followed by a comprehensive sensory evaluation to assess overall consumer appeal.

Experimental

The grape juice and leaves used in this study were obtained from *Vitis vinifera* L. cv. Aglianico. Grape juice enriched with vine leaf extract (GJF) was compared with untreated Aglianico grape juice (GJ) and a commercial grape juice (CGJ).

After 30 days of storage at 4°C, VOCs profiles were determined by HS-SPME/GC-MS. For each analysis, 5 mL of beverage, 2 g of NaCl and 10 µL of 3-octanol as internal standard were placed in a 20 mL headspace vial. Samples were incubated at 40°C for 10 min and volatiles were extracted for 50 min using a DVB/CAR/PDMS fibre. Beverages were analysed in triplicate using a randomized sequence, with blanks included.

To investigate relationships between volatile composition and sensory perception, VOCs and sensory datasets were integrated at the replicate level. Partial Least Squares (PLS) regression was applied to identify the VOCs most relevant for sample discrimination. VOCs with Variable Importance in Projection (VIP) scores > 1.0 were retained for subsequent analysis. Pearson correlation coefficients were then calculated between the selected VOCs and sensory attributes.

Results

HS-SPME/GC-MS analysis identified 63 volatile compounds, including 5 esters, 12 aldehydes, 22 alcohols, 3 ketones, 10 terpenes, 7 acids, 3 furans, and one compound classified as others. Significant differences among samples were observed for all detected VOCs ($p < 0.05$).

Alcohols represented the predominant chemical class in GJ, GJF and CGJ, accounting for approximately 82.3%, 47.5%, and 74.9% of total volatiles, respectively. In GJF, the addition of grapevine leaf extract markedly modified the volatile profile, with a strong increase in terpenes, which represented 34.0% of total VOCs compared with 4.2% and 5.1% in GJ and CGJ, respectively.

Among terpenes, *trans*-geraniol and linalool were particularly characteristic of the enriched grape juice. *Trans*-geraniol represented 24.5% of the total VOCs in GJF, while linalool accounted for 6.6%. Several C6 alcohols were also increased in GJF, contributing to the modification of its aromatic profile.

PLS analysis allowed the identification of the VOCs contributing most to sample discrimination. The integration of VOCs and sensory datasets highlighted specific associations between volatile compounds and sensory attributes. Pearson correlation coefficients (r) were calculated between the 12 VOCs selected by Partial Least Squares (PLS) analysis (Variable Importance in Projection, VIP > 1) and the sensory attributes significantly affected by sample type, as showed in the heatmap (Fig. 1). Sweet odor, sweet taste, and overall sensory

quality were positively associated with octanoic acid, *p*-cresol, linalool oxide, β -fenchyl alcohol, vitispirane, and furfural, whereas sour odor and sour taste were strongly associated with acetic acid, methyl acetate, and 2-methyl-1-butanol [2].

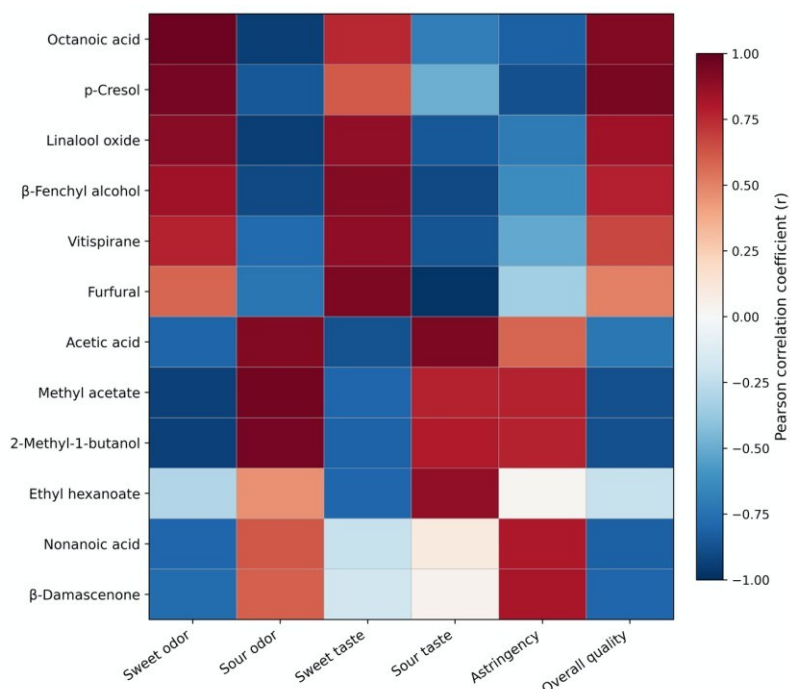


Fig 1. Heatmap exhibit Pearson correlation coefficients (*r*) between the 12 VOCs selected by PLS and the sensory attributes significantly affected by sample type. Red and blue colors indicate positive and negative correlations, respectively, whereas color intensity reflects correlation strength.

Conclusions

The present study demonstrated that *Vitis vinifera* leaf extract can be successfully employed to develop grape-based beverages with enhanced phytochemical composition, antioxidant capacity, and aromatic complexity. Leaf fortification significantly modified the volatile profile, contributing to distinctive sensory characteristics while maintaining satisfactory overall quality and microbiological stability during refrigerated storage. These findings highlight the potential of grapevine leaves, an underutilized viticultural by-product, as a sustainable ingredient for the formulation of functional beverages and value-added grape-derived products within a circular economy framework.

References

1. A. Fratianni, F. Letizia, C. Matarazzo et al.; *European Food Research and Technology*, 252 (2026), pp 252-300.
2. S. Pittari, L. Moio, P. Piombino; *Applied Sciences*, 13 (2023), pp 2165-2169.

P54

Geographical authentication of extra virgin olive oil by mid-level fusion of FTIR, NMR and IRMS data

Maria Assunta Acquavia,¹ Francesco Romaniello,¹ Chiara Portesi,¹ Matteo Berruto,¹ Maria Pia Montana Lampo,² Vittorio Capozzi,³ Giuliano de Seenen,⁴ Andrea Mario Rossi¹

¹ National Metrology Institute of Italy, Via San Rocco, 1, 75100-Matera, Italy

² University of Turin, Italy

³ Institute of Sciences of Food Production, National Research Council of Italy

⁴ BonassisaLab S.p.A

Summary: *Geographical authentication of extra virgin olive oil (EVOO) samples from two Italian region (Apulia and Sicily) was investigated by integrating FTIR, NMR and IRMS data through mid-level data fusion. A leave-one-cultivar-out validation strategy demonstrated that combining complementary spectroscopic and isotopic information improved geographical discrimination, achieving a balanced accuracy of 0.809.*

Keywords: *EVOO; Data fusion; Geographical authentication*

Introduction

Extra virgin olive oil (EVOO) is one of the most valuable agri-food products from both economic and cultural perspectives. Nevertheless, its high market value makes it particularly vulnerable to fraudulent practices, especially concerning authenticity and geographical provenance. The present study aimed to investigate whether the integration of data from three analytical platforms, namely Fourier Transform Infrared spectroscopy (FTIR), Nuclear Magnetic Resonance (NMR), and Isotope Ratio Mass Spectrometry (IRMS), could improve the geographical authentication of Italian EVOOs through mid-level data fusion. Spectroscopic and isotopic techniques provide complementary information on EVOO composition, that can strengthen the assessment of olive oil products' authenticity. Indeed, FTIR provides a rapid global vibrational fingerprint, while NMR spectroscopy describes the molecular and metabolomic profile. On the other hand, IRMS provides isotopic information related to environmental and geographical conditions [1].

Experimental

60 monovarietal EVOO samples were collected from Sicily and Apulia, two major olive oil-producing regions in southern Italy, both associated with EU Protected Designation of Origin (PDO) labels. All samples were characterized by FTIR, NMR and IRMS. FTIR spectra were preprocessed by Savitzky-Golay first derivative and SNV normalization, while NMR spectra were subjected to PQN normalization, FFT-based alignment and 0.01 ppm binning. Isotopic characterization included both bulk oil measurements and compound-specific $\delta^{13}\text{C}$ and $\delta^2\text{H}$ analysis of fatty acids by GC-IRMS. Following pre-processing, all datasets were mean-centered and scaled. Then, Principal Component Analysis (PCA) was used for exploratory analysis and/or dimensionality reduction. The corresponding PCA scores were retained as representative descriptors of the FTIR and NMR data, while IRMS variables were used without preliminary dimensionality reduction. Partial-least-squares discriminant analysis (PLS-DA) was first independently applied to each analytical block and, then, to pairwise mid-level data fused combinations (FTIR+NMR, FTIR+IRMS, and NMR+IRMS) and to the whole dataset (FTIR+NMR+IRMS). A Leave-One-Cultivar-Out (LOCO) approach was used to validate the PLS-DA models, in order to minimize the risk of overestimating predictive performance due to cultivar-related information [2].

Results and Discussion

The results of the supervised analysis showed that the three analytical platforms provided limited cultivar-independent geographical discrimination when considered individually. Balanced accuracies of 0.654, 0.590 and 0.657 were obtained for FTIR, NMR and IRMS-based PLS-DA models, respectively. These results demonstrated that each technique contained information related to geographical origin, although none of the individual fingerprints was enough to provide robust prediction. The integration of complementary analytical information improved model performance. The best performance was achieved through the mid-level data fusion model based on the whole dataset (FTIR+NMR+IRMS), which provided a balanced accuracy of 0.809. A permutation test further supported the statistical significance of the model ($p = 0.000999$).

Conclusions

The results demonstrated that mid-level integration of vibrational, molecular and isotopic fingerprints significantly improves EVOO geographical authentication compared with individual analytical techniques. The use of LOCO validation provides a valuable strategy to ensure model robustness by limiting the influence of

cultivar-specific signatures. The proposed data fusion workflow represents a promising strategy for developing more robust analytical tools for EVOO origin authentication and food traceability. Further studies could include additional production years, geographical areas and independent sample sets to assess the transferability of the proposed approach.

References

1. Karalis, P., Poutouki, A.E., Nikou, T., Halabalaki, M., Proestos, C., Tsakalidou, E., Gougoura, S., Diamantopoulos, G., Tassi, M., Dotsika, E. (2020). *Molecules*, 25, 5816.
 2. Oh, H., Nam, J. H., Park, B. R., Kim, K. M., Kim, H. Y., & Cho, Y. S. (2026). *Foods*, 15(13), 2268.
-

P55

Exploring the aroma profile of fermented mung bean gels using lactic acid bacteria and yeast strains

L. Marchesini,^{1,2} M. Mazzucotelli,² E. Betta,² I. Khomenko,² A. Levante,¹ F. Bot,¹ M. Pedrotti²

¹ University of Parma, Parma, Italy

² Fondazione Edmund Mach, Research and Innovation Centre, Via E. Mach 1,
San Michele all'Adige, 38098, TN, Italy

Summary: Microbial fermentation tailored the volatile profile of a mung bean-based gel. Lactic acid bacteria (LAB) reduced undesirable compounds such as hexanal and enhanced dairy-like notes, particularly in *Streptococcus*-fermented samples, while yeasts generated a broader volatile profile, mainly characterised by esters and carbonyl compounds. These complementary effects highlight fermentation as a promising strategy for aroma improvement in legume-based sources.

Keywords: Mung bean; fermentation; volatile profile

Introduction

The demand for plant-based alternatives has increased in recent years, driven by concerns about animal welfare and environmental sustainability [1, 2]. Pulses are valuable ingredients due to their nutritional profile, but their characteristic beany and earthy off-notes can limit consumer acceptance. These undesirable aromas are mainly associated with volatile organic compounds (VOCs), such as hexanal and 1-hexanol, originating from lipid oxidation [3, 4]. Fermentation may be used to improve aroma by metabolizing undesirable compounds and promoting the formation of more pleasant aroma. This study combined Proton Transfer Reaction Mass Spectrometry (PTR-MS) fingerprinting with Gas Chromatography Mass Spectrometry (GC-MS) characterization to investigate the effect of microbial fermentation on the volatile profile of a mung bean-based gel, focusing on the reduction of undesirable VOCs and the formation of compounds associated with more pleasant dairy-like notes.

Experimental

A total of 40 lactic acid bacteria (LAB) and 13 yeasts were selected from the University of Parma Culture Collection (UPCCO), previously isolated from different food matrices, mainly of dairy origin. The substrate was prepared by mixing a commercial sample of mung bean protein concentrate with distilled water at a 1:7 w/v ratio and pasteurized at 90°C for 35 min. Cultures were then inoculated at a final concentration of 1% v/v and incubated at the optimal growth temperature of the strains. After 48 h of fermentation, pH, microbial counts, and volatile compounds were analysed. On 35 samples, an untargeted PTR-MS approach, using SHS-PTR-ToF-MS (5 replicates for each sample), was used to investigate changes in the overall volatile profile, while HS-SPME- GC-MS (3 replicates for each sample) was employed for compound identification and validate PTR-MS findings.

Results

Among the initial LAB and yeasts, only 19 LAB and 9 yeasts were able to grow in the gel and were therefore analysed. While *Lactocaseibacillus* strains showed the highest growth performance (final pH 4.02 ± 0.07), *Lactobacillus* exhibited the greatest acidification capacity. Among the tested yeasts, *Kluyveromyces* showed the highest growth with a final count of 8.08 ± 0.63 log CFU/ml.

PCA of GC-MS and PTR-MS volatile profiles (Figure 1) revealed consistent patterns, clearly differentiating LAB- and yeast-fermented samples from the unfermented control.

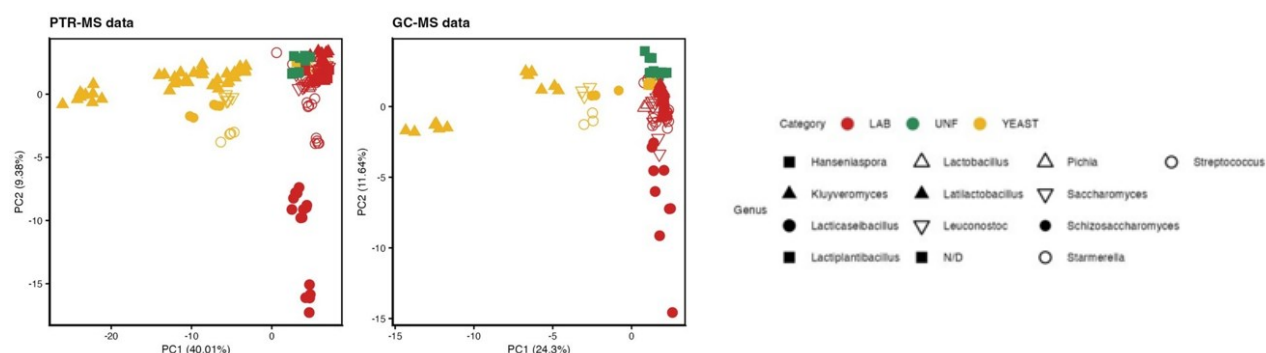


Fig. 1. PCA of PTR-MS data and GC-MS data.

Yeast-fermented samples showed a greater shift from the unfermented profile compared with LAB. The PTR-MS heatmap (data not shown) further confirmed distinct clusters among yeasts, LAB, and unfermented samples, with specific masses characterizing each group. Focusing on key mung bean off-flavour compounds, ANOVA revealed a significant decrease in hexanal (m/z 101.06) in LAB-fermented samples, with genus-dependent differences. Conversely, diacetyl (m/z 87.04), a key dairy-like aroma compound, showed higher levels particularly in samples fermented with *Streptococcus*. Yeasts produced a broader range of VOCs than bacteria, although some strains exhibited profiles closer to those observed for LAB.

Conclusions

LAB fermentation significantly decreased the concentration of undesirable compounds while increasing the content of dairy-like aroma compounds. In contrast, yeasts produced a broader range of VOCs, mainly including esters and carbonyl compounds which may have a positive impact on the sensory properties of the fermented product. The combination of PTR-MS and GC-MS methods allowed to characterize the metabolic profiles of the fermented samples, suggesting that the combined use of LAB and yeasts could be of interest the development of more desirable aroma characteristics.

References

1. Detzel, A., Krüger, M., Busch, M., Blanco-Gutiérrez, I., Varela, C., Manners, R., Bez, J., & Zannini, E. (2022). Life cycle assessment of animal-based foods and plant-based protein-rich alternatives: An environmental perspective. *Journal of the Science of Food and Agriculture*, 102(12), 5098–5110. <https://doi.org/10.1002/jsfa.11417>
2. Grossmann, L., & McClements, D. J. (2021). The science of plant-based foods: Approaches to create nutritious and sustainable plant-based cheese analogs. *Trends in Food Science & Technology*, 118, 207–229. <https://doi.org/10.1016/j.tifs.2021.10.004>
3. Jeske, S., Zannini, E., & Arendt, E. K. (2017). Evaluation of Physicochemical and Glycaemic Properties of Commercial Plant-Based Milk Substitutes. *Plant Foods for Human Nutrition*, 72(1), 26–33. <https://doi.org/10.1007/s11130-016-0583-0>
4. Tangyu, M., Muller, J., Bolten, C. J., & Wittmann, C. (2019). Fermentation of plant-based milk alternatives for improved flavour and nutritional value. *Applied Microbiology and Biotechnology*, 103(23), 9263–9275. <https://doi.org/10.1007/s00253-019-10175-9>

AUTHOR INDEX

Abdulrazzaq S.	OR33	92	Bonasia A.	P8	128
Abete M.C.	P51	215	Boncinelli F.	OR16	56
Accoroni S.	P37	184	Bongiorno D.	OR14,P47	51,207
Acquaticci L.	OR25,P22	76,155	Bontempo L.	OR6,OR9,P11	34,39,135
Acquavia M.A.	P54	220	Bordiga M.	P13,P26	138,162
Aiuto B.	P39,P44,P49	189,199,211	Borrelli M.	P4	119
Albanese B.	P38	186	Borzatta V.	P29	168
Albrizio S.	OR15	53	Bot F.	P55	222
Alessandroni L.	OR30,P29,P31	85,168,173	Bottari B.	P9	131
Allarà M.	P16	144	Bottiroli R.	P19	150
Angeli L.	P25	160	Bovenzi T.	P4	119
Angelone B.	P12	136	Bremer P.	OR21	66
Angeloni S.	OR25,P31	76,173	Breschi C.	OR16	56
Annibaldi A.	P39,P44	189,199	Brilli M.	OR18	59
Annovazzi I.	OR35	96	Brizio P.	P51	215
Annunziato A.	OR12	46	Bro R.	PL4,OR36	87,99
Aprea E.	P19,P40	150,191	Bruni R.	P9	131
Arapitsas P.	P40	191	Caballero-	OR19	61
Armellino F.	P41	193	Casero N.		
Armenio V.	P41	193	Caligiani A.	OR38,P2	104,114
Asson F.	P18,P21	148,153	Candotti P.	P12	136
Avellone G.	OR14,P47	51,207	Capozzi V.	P43, P54	197, 220
Avolio R.	P51	215	Cappellin L.	OR23,P46	72,204
Bacchiocchi S.	P37,P48	184,209	Caprai E.	P17	146
Bacher F.	P24	158	Caprioli G.	OR25,P14,P22,	76,140,155,
Baglio D.	OR35,P30	96,171		P31	173
Baldoni A.	P37	184	Capuano R.	P5	121
Ballabio D.	P25	160	Caracausi L.	P39	189
Barba F. J.	OR30	85	Caratti A.	OR24,P10,P20	74,133,151
Barilaro N.	P11	135	Cardenia V.	OR35	96
Barola C.	P48	209	Carella D.	OR12	46
Bartolucci G.	P6	123	Carlin S.	P40	191
Becchi P. P.	OR36	99	Carpino S.	P11	135
Bechis G.	P13	138	Carraro R.	OR27	79
Beggio M.	IP1	42	Carullo D.	P15	142
Bellumori M.	OR16,P6	56,123	Cascio N.	P18	148
Berruto M.	P54	220	Casetta C.	OR24,P10,P20	74,133,151
Bertoldo F.	P9	131	Catini A.	P5	121
Betta E.	OR23,P5,P43,	72,121,197,	Cavallarin L.	P49	211
	P45,P55	201,222	Cecchi L.	OR16,P6,P33	56,123,176
Biagi M.	P9	131	Centonze D.	P50,P52	213,216
Bianchi F.	OR1	24	Chen L.	OR9	39
Biancotto G.	P18	148	Chwojnik T.	OR18,P14,P27	59,140,164
Biasioli F.	OR21,OR23,P5,	66,72,121,	Ciccotelli V.	P41	193
	P43,P45,P46	197,201,204	Cichelli A.	OR26	78
Bicchi C.	P20	151	Ciriacci M.	P48	209
Bilo F.	P21	153	Cirlini M.	P9	131
Blanco E.	P8	128	Cirrincione S.	P49	211
Bodenbender L.	KN1,P23,P33	55,156,176	Cocchi M.	OR5	32
Bogialli S.	P46	204			

Como A.	P50,P52	213,216	Flamini R.	OR27,P28	79,166
Conversa G.	P8	128	Flutto T.	OR34	94
Cordero C.	OR24,P10,P20	74,133,151	Fogliano V.	P19,P43,P46	150,197,204
Cornara D.	P32	174	Fontanella M.C.	OR32	90
Cozzolino F.	OR13	48	Fontecilla-	P16	144
Cozzolino R.	P53	218	Escobar J.		
Crescenzi M.A.	P32,P34	174,179	Franchina F.A.	OR2	26
Crisafulli P.	P15	142	Francioni A.	P19	150
Cruz Muñoz E.	P25	160	Francolino R.	OR4	30
Cucco G.	OR20	64	Frenich A.G.	OR15	53
Cuozzo R.	P7	126	Frey R.	KN1	55
d'Aquino L.	OR29	83	Froldi F.	OR32	90
D'Ascanio V.	OR12	46	Fusari P.	P30	171
d'Emmanuele di	OR37	102	Fusi R.	OR16	56
Villa Bianca R.			Fuso A.	OR38	104
D'Eusanio V.	OR10	40	Gabrielli M.	OR3	28
D'Intino L.	OR3	28	Gaeta R.	OR4	30
Dawid C.	P25	160	Gallo V.	P8	128
De Angelis E.	P16	144	Gamba V.	P38	186
De Biasio F.	OR8	37	Garcia C. J.	OR33	92
De Feo V.	OR4	30	García-Villalba R.	OR33	92
de Gioia M.	P1	111	Gardiman M.	OR27	79
De Martino L.	OR4	30	Gasparini M.	P12,P38	136,186
De Paolis L.	P41	193	Gasperi F.	P45	201
De Rosso M.	OR27,P28	79,166	Gazzoli G.	OR5	32
de Seenen G.	P54	220	Genova G.	OR24,P10,P20	74,133,151
Desimoni F.	OR5	32	Girolametti F.	P39,P44	189,199
Di Fabio G.	P15	142	Griffoni F.	P48	209
Di Martino V.	P11	135	Guardone L.	P41	193
Di Matteo P.	P4	119	Guercia E.	P16	144
Di Natale C.	P5	121	Gula G.	P2	114
Di Noi A.	OR11	44	Haidukowski M.	OR12	46
Diomedi G.	P37	184	Hey D.	OR1	24
Donadini G.	OR3	28	Holt S.	P45	201
Duque-Estrada P.	P42	195	Iannotti F. A.	P16	144
Durante C.	OR5	32	Ieri F.	OR16	56
Dziurzyński M.	P42	195	Illuminati S.	P39,P44	189,199
Emide D.	P42	195	Indelicato S.	OR14,P47	51,207
Errico M.	OR32	90	Innocenti M.	OR16,P6	56,123
Esposito E.	OR37	102	Iorizzo M.	P53	218
Eyres G.	OR21	66	Izadfar F.	P45	201
Faccini A.	OR39	106	Karasholakova L.	P22	155
Fadanelli E.	OR28	81	Khamitova G.	P22	155
Fadda A.	P34	179	Khatib M.	P6	123
Fanelli E.	P39	189	Khomenko I.	OR21,OR23,	66,72,
Fappani G.	OR32,P36	90,182		P5,P43,P45,	121,197,201,
Farris S.	P15	142		P55	222
Ferrari G.	P41	193	Khoobakht F.	P40	191
Ferrer E.	OR30	85	Ladogana F.	OR5	32
Fina A.	OR24,P20	74,151	Lamberti C.	P49	211
Fiorini D.	OR33	92	Lambertini F.	OR39	106
Fisk I.	PL3	63	Lametsch R.	P42	195
Fissore A.	OR24,P10	74,133	Lamonaca A.	P1	111

Langellotti A.L.	P43	197	Massaro A.	OR11,P18,	44,148,
Larcher R.	OR9,OR17,	39,58,		P21,P23	153,156
	OR28,OR40	81,108,	Massi L.	P44	199
	P35	181	Mastrorilli P.	P8	128
Lattanzio V.	P49	211	Masuolo D.	OR6,OR9,P11	34,39,135
Leone A.	P49	211	Masullo M.	OR31,P7	88,126
Leto L.	P9	131	Matarazzo C.	P53	218
Levante A.	P55	222	Mattina G.	P19,P40	150,191
Liberto E.	OR20,OR24,	64,74,	Mazza S.	P37,P48	184,209
	P10,P20	133,151	Mazzucotelli M.	P55	222
Lino C.	OR14,P47	51,207	Menghi L.	P46	204
Listorti V.	P41	193	Menghini S.	OR16	56
Llorent-Martínez	OR26	78	Menicatti M.	P6	123
E.J.			Menotta S.	P12	136
Locuratolo L.	P2	114	Merlet M.	OR34	94
Lombroni C.	OR40	108	Michelini M.	P29,P33	168,176
Lonzarich V.	OR22,P13	68,138	Milisenda G.	P39	189
López-Ruiz R.	OR15	53	Minio M.	P44	199
Lorenzi G.	P12	136	Minkoumba	P17	146
Lorenzi L.	P19	150	Sonfack G.		
Lovatti L.	P24	158	Mitidieri E.	OR37	102
Lucini L.	OR36	99	Moayed M.	OR23	72
Lupi G.	OR38	104	Mobaraki N.	KN1,P33	55,176
Lupinelli S.	P15	142	Mollica A.	OR26	78
Macchi M.P.	P27	164	Montana Lampo	P54	220
Maggi F.	P29	168	M.P		
Maggioni D.	P42	195	Monteleone E.	OR16	56
Magna M.	IP3	98	Monteverde	P42	195
Magni C.	P42	195	Vicentini T.		
Maisto M.	OR37	102	Montoro P.	OR29,P32,P34	83,174,179
Makarycheva P.	OR33	92	Monzillo F.	OR29	83
Malcangi S.	OR2	26	Moretta A.	OR1	24
Malorni L.	P53	218	Moretti A.	OR12	46
Malpaga S.	OR8	37	Moretton M.	P43	197
Marabottini R.	OR5	32	Moriano R.	P51	215
Marchese C.	P51	215	Moroni B.	P41	193
Marchesini L.	P55	222	Morozova K.	OR40,P24,P25	108,158,160
Marchetti A.	OR10	40	Mosconi G.	P30	171
Marchetti N.	OR2	26	Mulinacci N.	OR16,P6	56,123
Marinaccio L.	OR26	78	Musio B.	P8	128
Marino Cerrato L.	OR15	53	Mutinelli F.	P21	153
Martelli F.	P9	131	Nally M.	OR21	66
Martinelli F.	OR17,OR28	58,81	Namazzadeh G.	P29	168
Martinello M.	P21	153	Napolitano A.	OR5,OR31	32,88
Martone F.	OR5	32	Nardin T.	OR17,OR28,	58,81,
Marzocchi A.	OR37	102		OR40	108
Marzorati S.	OR18,P15,	59,142,	Navarini L.	OR22,P13,	68,138,
	P27,P42	164,195		P15,P16	142,144
Marzulli A.	P1	111	Nerin C.	P50	213
Masi G.	OR27	79	Norici A.	P44	199
Masin R.	P18	148	Olivieri S.	P51	215
Massardi B.	P12	136	Palermo C.	P50,P52	213,216
			Palombo P.	P48	209

Panighel A.	OR27,P28	79,166	Romaniello F.	P54	220
Panni F.	P23	156	Romanucci V.	P14	140
Paolillo A.	OR31	88	Romei I.	P33	176
Paolini M.	OR9,P35	39,181	Romelli S.	P38	186
Pedrotti M.	OR23,P5,P45, P46, P55	72,121,201, 204,222	Romero- González R.	OR15	53
Pellacani S.	OR5	32	Roncone A.	OR6,OR9	34,39
Pellegrini E.	P38	186	Rosolen A.	OR28	81
Peloso M.	P17	146	Rossi A.M.	P54	220
Penasa R.	OR6,OR9	34,39	Rossini C.	P26	162
Pepi F.	OR11	44	Rosso F.	P2	114
Peraino A.	P2	114	Rovellini P.	OR35,P30	96,171
Perini M.	OR8,P11	37,135	Roverso M.	P46	204
Peroni D.	IP2	71	Rubio S.	OR19	61
Petersen I.L.	P42	195	Sagratini G.	OR30,P14, P29,P31	85,140, 168,173
Petrolati M.	P14	140	Salami R.	P12	136
Petrucci R.	P4	119	Salvitti C.	OR11	44
Pettenuzzo S.	P46	204	Sanna D.	P34	179
Piacente S.	OR29,OR31,P7	83,88,126	Sansone L.	OR27	79
Pianezze S.	OR6,OR8,P11	34,37,135	Santanatoglia A.	P14	140
Piccinin A.	OR27	79	Savino A.F.	P23	156
Piccinin D.	OR27	79	Savio V.	P41	193
Piccolo V.	OR37	102	Scagliarini C.	P30	171
Piersanti A.	P37,P48	184,209	Scampicchio M.	OR40,P24,P25	108,158,160
Pilolli R.	P1	111	Scapuzzi C.	P14,P27,P42	140,164,195
Pinto L.	P1	111	Scarafoni A.	P42	195
Piro R.	P23	156	Schievano E.	P21	153
Piscitelli F.	P16	144	Sebastià A.	OR30	85
Piutti E.	OR27	79	Serenelli F.	P37	184
Pizzamiglio V.	OR36	99	Sigolo S.	OR32	90
Poetto A. S.	OR1	24	Silcock P.	OR21	66
Polidoro A.	OR2	26	Silvi St.	OR33	92
Portesi C.	P54	220	Siracusa M.	P37,P48	184,209
Pramotton R.	OR34	94	Soligo S.	OR27	79
Prandi B.	OR39	106	Sonnante G.	P8	128
Prandini A.	OR32	90	Sparacio A.	P28	166
Pratesi F.	OR13	48	Sparla S.	P28	166
Principalli M.S.	P37	184	Spigno G.	OR3	28
Prioretti M.	P14	140	Spinozzi E.	P29	168
Prizio I.	P17	146	Squadrone S.	P51	215
Purcaro G.	PI1	23	Stark T.D.	P25	160
Quaglia G.	P26	162	Stefanucci A.	OR26	78
Razzuoli E.	P41	193	Stella R.	P18	148
Reale F.	P40	191	Stramenga A.	P48	209
Ricciutelli M.	OR25,P31	76,173	Strani L.	OR5	32
Rinaudo G.	P46	204	Sugni M.	OR18	59
Rivi M.	OR10	40	Suman M.	OR7,OR11	35,44
Rizzuti A.	P8	128	Summa V.	OR37	102
Robatscher P.	OR1,P24	24,158	Susca A.	OR12	46
Robetto S.	P41	193	Tanilli S.	OR24,P10,P20	74,133,151
Rocchetti G.	OR32,P36	90,182	Tapparo G.	OR20	64
Roccotelli S.	OR27	79	Tarraran L.	P49	211
Romagnoli M.	OR2,OR23,P5	26,72,121			

Tarricone F.	OR27,P28	79,166
Tarricone L.	OR27	79
Tata A.	OR11,P18, P21,P23	44,148, 153,156
Tavoloni T.	P48	209
Tedeschi T.	OR39	106
Tenore G. C.	OR37	102
Thedy L.	P3	117
Thomas F.	OR7	35
Tinelli E.	P32	174
Tomás-Barberán F.	OR33	92
Tomasini A.	P37	184
Tonezzer M.	OR23,P5	72,121
Trapani F.	OR24,P10,P20	74,133,151
Troiani A.	OR11	44
Truzzi C.	P39,P44	189,199
Ugolini T.	OR16,P33	56,176
Vaccaro E.	P41	193
Valentini S.	OR34,P3	94,117
van de Laar S.	P46	204
van Kralingen G.A	OR18	59
van Leeuwen S.	PL2	43
Vanelli A.	OR8	37
Verde R.	P16	144
Verneti-Prot L.	OR34,P3	94,117
Verotta L.	P14,P27	140,164
Verrastro V.	P32	174
Vetrano O.	P34	179
Vettori L.	P28	166
Vincenzi S.	P28	166
Vincken J.-P.	OR18	59
Vitulli M.	OR13	48
Vreeke G. J.C.	OR18	59
Weller P.	KN1,P23,P33	55,156,176
Wiesenberger G.	OR12	46
Winnischhofer T.	OR1	24
Wu V.	OR29	83
Zacometti C.	OR11,P18, P21,P23	44,148, 153,156
Zamboni N.	OR29	83
Zanoni B.	OR16,P33	56,176
Zenato S.	OR34	94
Zengin G.	OR26	78
Zhang J.	OR23,P5	72,121
Zonfrillo B.	P6	123
Zucherman M.	P6	123